Nitrous oxide emissions during establishment of eight alternative cellulosic bioenergy cropping systems in the North Central United States

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Abstract

Greenhouse gas (GHG) emissions from soils are a key sustainability metric of cropping systems. During crop establishment, disruptive land-use change is known to be a critical, but under reported period, for determining GHG emissions. We measured soil N2O emissions and potential environmental drivers of these fluxes from a three-year establishment-phase bioenergy cropping systems experiment replicated in southcentral Wisconsin (ARL) and southwestern Michigan (KBS). Cropping systems treatments were annual monocultures (continuous corn, corn–soybean–canola rotation), perennial monocultures (switchgrass, miscanthus, and poplar), and perennial polycultures (native grass mixture, early successional community, and restored prairie) all grown using best management practices specific to the system. Cumulative three-year N2O emissions from annuals were 142% higher than from perennials, with fertilized perennials 190% higher than unfertilized perennials. Emissions ranged from 3.1 to 19.1 kg N2O-N ha⁻¹ yr⁻¹ for the annuals with continuous corn > corn–soybean–canola rotation and 1.1 to 6.3 kg N2O-N ha⁻¹ yr⁻¹ for perennials. Nitrous oxide peak fluxes typically were associated with precipitation events that closely followed fertilization. Bayesian modeling of N2O fluxes based on measured environmental factors explained 33% of variability across all systems. Models trained on single systems performed well in most monocultures (e.g., R² = 0.52 for poplar) but notably worse in polycultures (e.g., R² = 0.17 for early successional, R² = 0.06 for restored prairie), indicating that simulation models that include N2O emissions should be parameterized specific to particular plant communities. Our results indicate that perennial bioenergy crops in their establishment phase emit less N2O than annual crops, especially when not fertilized. These findings should be considered further alongside yield and other metrics contributing to important ecosystem services.

Keywords: Bayesian model averaging, cellulosic biofuels, corn, greenhouse gas, miscanthus, poplar, restored prairie, switchgrass

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Introduction

Nitrous oxide (N₂O) is a potent greenhouse gas (GHG) and the main contribution to radiative forcing in the atmosphere by agriculture (Robertson et al., 2000). For first generation biofuels derived from edible oils and starches, N₂O emissions from feedstock production comprise a substantial proportion of their total carbon footprint (Gelfand et al., 2011). The development of second-generation biofuels derived from cellulosic materials offers the potential to substantially reduce N₂O emissions associated with feedstock production (Sanderson & Adler, 2008; Smith et al., 2013). As bioenergy cropping system viability is considered, the greenhouse gas emissions of these systems will be a key component of sustainability evaluation (Reay et al., 2012).

Nitrous oxide emitted from soils is primarily the product of microbially driven nitrification and denitrification. These processes are influenced by a broad range of environmental factors including temperature, oxygen availability, rates of microbial activity, and the availability of nitrogen substrates (Robertson & Groffman, 2015). The effect of these factors can depend on soil-specific properties (Henault et al., 2005) including the composition of the microbial community (Cavigelli & Robertson, 2001),
while their relative importance may differ among cropping systems (Dechow & Freibauer, 2011). Numerous process-based models have been developed in an attempt to account for these contextual effects within a generalized framework, but they are rarely calibrated to the actively managed and harvested perennial cropping systems that have emerged as leading candidates for second-generation biofuel feedstocks (Chen et al., 2008). More broadly, the dynamics governing N\textsubscript{2}O emissions in perennial cropping systems managed for biofuel feedstock production are poorly represented in the literature (but see Nikiema et al., 2011, 2012; Palmer et al., 2014).

While systems based on polycultures and perennial species are anticipated to emit less N\textsubscript{2}O than conventional agricultural cropping systems, this reduction is likely to be contingent on previous land-use and conversion methodology, phase and length of establishment, soil type, and management of inputs and production processes. Land conversion to cropping systems either through extensification or intensification of open land systems such as pasture is known to significantly increase soil organic carbon (SOC) loss (Adler et al., 2007; Zenone et al., 2011; Sanford et al., 2012). Vegetation removal and cultivation may also affect the N cycle, especially during conversion and establishment (Bouwman et al., 2010; Gelfand et al., 2011; Nikiema et al., 2012; Ruan & Robertson, 2013) and lead to significant nitrogen loss through leaching and gaseous emissions (Robertson et al., 2012; Smith et al., 2013). We must improve our understanding of N\textsubscript{2}O emissions of likely biofuel feedstock systems to help ensure that expansion of bioenergy production generates expected societal benefits (Robertson et al., 2008; Dale et al., 2011, 2014).

We compared the establishment-phase N\textsubscript{2}O emissions of annual monocultures of continuous corn and corn–soybean–canola rotations; perennial monocultures of switchgrass, miscanthus, and hybrid poplar; and perennial polycultures of early successional species, native grasses, and native prairie species. Our results cover the 2- to 4-year period following planting over which many perennial crops attain ‘full capacity’ biomass production (McLaughlin & Adams Kszos, 2005; Anderson-Teixeira et al., 2013). Our aims were to (i) provide a direct comparison of the aggregate N\textsubscript{2}O emissions from a broad range of feedstock production systems; (ii) characterize the effects of location and year on N\textsubscript{2}O emissions from these cropping systems; and (iii) evaluate cropping system impacts on relationships among N\textsubscript{2}O fluxes and environmental factors.

**Materials and methods**

One study site was located in southwestern Michigan at Michigan State University’s W.K. Kellogg Biological Station (KBS) in Hickory Corners, MI (42° 23' 47" N, 85° 22' 26" W and 288 m asl), and another site was located in southcentral Wisconsin, USA, at the University of Wisconsin’s Arlington Agricultural Research Station (ARL) in Arlington, WI (43° 17' 45" N, 89° 22' 48" W and 315 m asl). Mean annual air temperature at KBS is 9.9 °C, and annual precipitation is 1027 mm (MSCO, 2013). Soils are well-drained Kalamazoo loam (fine-loamy, mixed, semi-active, mesic Typic Hapludalfs with soil C < 15 g kg\textsuperscript{−1}, N ≤ 0.13 g kg\textsuperscript{−1}) developed over glacial outwash (Crum & Collins, 1995). At ARL, mean annual temperature and precipitation are 6.9 °C and 869 mm, respectively (NWS, 2013). Soils at the site are classified as Plano silt loam (fine-silty, mixed, superactive, mesic Typic Argiudolls with soil C > 20 g kg\textsuperscript{−1}, N ≥ 0.19 g kg\textsuperscript{−1}) developed over glacial till (Jokela et al., 2011). Experiments at both sites were established in spring 2008 in a randomized complete block design. Ten treatments (eight cropping systems including each phase of the three-phase corn–soybean–canola rotation) were represented in five blocks of 30 × 40 m plots at each location, for a total of 100 plots. The systems under study were (i) continuous no-till corn (Zea mays L.); (ii) corn–soybean (Glycine max [L.] Merr.-canola (Brassica napus L.) rotation with all 3 phases represented; (iii) monoculture switchgrass (Panicum virgatum L.); (iv) monoculture miscanthus (Miscanthus x giganteus); (v) hybrid poplar (Populus nigra x P. maximowiczii ‘NM6′) on a 6-year coppicing rotation; (vi) a mixture of five native grass species; (vii) a mixture of 18 native prairie species; and (viii) an early successional community defined by the pre-existing seed bank and novel recruitment with no management other than fertilizer application and harvest. Of note, miscanthus at ARL suffered >95% mortality over the 2008–2009 winter and was subsequently replanted in May 2010. With the exception of miscanthus at KBS and the early successional community at both sites, perennial systems did not receive N fertilizer in 2009, while poplar at both sites received N fertilizer in 2010 only (full crop and management details are given in Table S1).

**Estimating nitrous oxide emissions**

When soils were consistently >0 °C, N\textsubscript{2}O fluxes were measured biweekly with additional sampling to characterize episodic events (i.e., fertilizer application and precipitation events) using vented static chambers. All measurements were made between 1000 and 1600 h local time. Cylindrical chamber bases of 28.5 cm diameter were inserted ~5 cm below the soil surface. With the chamber lid installed, the chamber had an effective headspace volume of ~10 l (~17 cm height). Lids had a septum for gas extraction and a 2-mm diameter vent and vent tube to allow for chamber pressure equilibration. Headspace gas from within the chambers was extracted immediately following lid placement with a 30-ml nylon syringe and a 23-gauge needle. Three subsequent extractions were made at 20-min intervals over a 60-min period. Glass 5.9-ml Exetainer vials (Labco Limited, Buckinghamshire, UK) were flushed with 20 ml of extracted sample and then overpressurized with 10 ml of sample to avoid contamination and facilitate analysis. At each sampling event, field standards (1 ppm N\textsubscript{2}O, 1 ppm CH\textsubscript{4}, and 400 ppm CO\textsubscript{2}) and ambient air were loaded into vials to assess
potential sample loss prior to analysis. Sample CO2, N2O, and CH4 concentrations were determined by gas chromatography using an infrared gas analyzer (IRGA, LiCor 820, Lincoln, NE, USA) for CO2, an electron capture detector (micro-ECD, Agilent 7890A GC System, Santa Clara, CA, USA) for N2O, and a flame ionization detector (FID, Agilent 7890A) for CH4.

Visual inspection of CO2 accumulation curves identified samples with lost pressure or other measurement problems, for which fluxes were discarded (~2% of total measured fluxes). Remaining fluxes were analyzed with the nls package (v0.3.1, Pedersen, 2011) in the R statistical environment (v3.0.3, R Core Team, 2014) to fit gas concentrations against time with a nonlinear model (Hutchinson & Mosier, 1981), a linear regression, and a null flux based on root mean squared error minimization. Of the 4139 flux estimates, a nonlinear model was used for the 691 (16.7%) fluxes where the 95% confidence interval for the nonlinear estimate excluded the corresponding linear estimate. In all other cases, including the case of a null flux, the linear flux estimate was used. This estimate was used to calculate the aggregate flux for the day it was sampled (daily flux) by assuming the estimate was the average flux during that day. Annual fluxes were calculated by integrating the linear interpolation of daily fluxes over one calendar year (Smith & Dobbie, 2001). Cumulative three-year emissions were then calculated for each experimental plot by summing the aggregated annual emissions from the three study years within a plot.

Assessing soil environmental variables
Concurrent with trace gas sampling, soil volumetric water content (VWC) (m3 m–3) and soil temperature (°C) were measured within 1 m of the chamber with a time domain reflectometer (Checktemp 1C, Hanna Instruments, Smithfield, RI, USA) for CO2, an electron capture detector (micro-ECD, Agilent 7890A GC System, Santa Clara, CA, USA) for N2O, and a flame ionization detector (FID, Agilent 7890A) for CH4.

Particle density, assumed to be 2.65 g cm–3, was determined by deionized water to remove visible soil and then extracted in 2 M KCl. Extracted strips were regenerated with 0.5 M HCl and 0.5 M NaHCO3 prior to next use. For the years 2010 and 2011, soil cores to 15 cm depth were taken concurrently with N2O measurements to estimate soil inorganic nitrogen (N) pools. A 10 g wet-weight subsample was weighed out for immediate inorganic N extraction in 2 M KCl following Robertson et al. (1999). Potassium chloride extracts were stored in 20-mL polyethylene scintillation vials frozen at ~20 °C prior to analysis. Colorimetric determination of extracts for ammonium (NH4+) (USEPA method-Pub# 27200110) and nitrate (NO3–) (USEPA method-Pub# 27190110) was performed on a Flow Solution 3100 segmented flow injection analyzer (OI Analytical, College Station, TX, USA).

Data analysis
Emissions were analyzed with linear mixed-effect models using the nlme package (v3.1, Pinheiro et al., 2013) in the R statistical environment (v3.1.1, R Core Team, 2014). Nitrous oxide emissions were summed over three calendar years; sums were log-transformed prior to analysis to approximate normally distributed data. Models were constructed to analyze response variables as a function of the fixed effects of treatment (cropping system) and site, accounting for the random effect of block nested within site (site/block). Models were improved by allowing for distinct variances among cropping systems, sites, or both, and evaluated with likelihood ratio tests. With the variance structure in place, significant fixed effects and interactions were determined by sequentially collapsing treatment levels and comparing subsequent models with likelihood ratio tests. This process continued iteratively until none of the remaining groups could be collapsed. Annual emissions considered year as an additional potential component of the variance structure and used crop rather than cropping system as a factor and each combination of year and site was analyzed separately. Variance structure optimization and assessment of treatment differences were conducted as described above.

Bayesian model averaging (BMA) was used to evaluate relationships between measured environmental variables and daily N2O fluxes for cropping system level (Hoeting et al., 1999; Marojà et al., 2009). We conducted the model averaging process using the bic.glm function of the R package BMA (v 3.16.2.2, Raftery et al., 2013). Because N2O fluxes are seldom normally distributed, and negative fluxes are biologically relevant (Schlesinger, 2013), we used a hyperbolic arcsine transformation (Burbidge et al., 2013) on daily flux data prior to analysis. The maximal model was defined as soil temperature, WFPS, NO3–, NH4+, year, and site, as well as all second-order interactions among these terms. Because we used a different method for estimating inorganic N in 2009, we only used 2010 and 2011 data. Of the 2657 data points in these two years, 2176 (82%) included all environmental measures. Annual emissions recalculated from this subset were highly correlated to those obtained from the full dataset (R2 = 0.90), although the range of values was slightly greater. We trained the model on the full dataset and also subsets of the data by cropping system to generate system-specific models. We then used environmental data from the full dataset to evaluate the capacity of models trained...
from a given system to predict emissions from other systems or from the full dataset.

Statistical analyses are discussed in greater detail in Appendix S1.

Results

Cumulative nitrous oxide emissions over the 3-year establishment phase

Cropping systems at the Wisconsin research station (ARL) emitted 23% more N₂O than their Michigan (KBS) counterparts. The only systems for which this pattern did not hold were continuous corn and miscanthus, where emissions were not significantly different between the sites, and the native grasses, where KBS had higher cumulative emissions (Fig. 1). Note that while we did not have miscanthus data from ARL in 2009 because of winter kill, cumulative 2010–2011 emissions were similar at both sites. Across both sites, N₂O emissions relative to aboveground yield of continuous corn were slightly greater than the rotation (0.88), switchgrass (0.67), and early successional community (0.79). The emissions relative to yield in native grasses were just under half that of continuous corn (0.43), while the restored prairie (0.18), miscanthus (0.15), and poplar (0.14) were the lowest.

Annual nitrous oxide emissions by year and site

We analyzed annual N₂O emissions separately by year and site (Fig. 2) due to a significant site × year × cropping system interaction ($P < 0.001$). For 2009 treatment comparisons, model selection indicated that continuous corn and miscanthus at KBS were not significantly different and had the highest emissions, followed by the rotational phase of corn and poplar; all other systems had emissions that were not significantly different from each other (Fig. 2a). The treatments responded differently at ARL where the corn phase of the rotation had more than twice the emissions of continuous corn. After continuous corn and the corn phase of the rotation, there were no differences among other systems with the exception of restored prairie, which had the lowest emissions (Fig. 2b).

The general patterns of emissions from treatments in 2010 were similar at both sites. Continuous corn and the corn phase of the rotation had the highest emissions at both KBS and ARL (Fig. 2c,d). Most systems at KBS had relatively low emissions (Fig. 2c), similar to emissions from native grass mix and restored prairie at ARL (Fig. 2d). With the exception of continuous corn, which was 21% higher at KBS, average emissions at ARL were 12% higher from annual systems, and 68% higher from perennial systems than the respective treatments at KBS.

Fig. 1 Cumulative 2009–2011 N₂O fluxes from 8 bioenergy cropping systems grown at Arlington Agricultural Research Station, WI (ARL) and Kellogg Biological Station, MI (KBS). Values presented are geometric means with $n = 5$ at ARL and $n = 4$ at KBS. Phases of a corn–soybean–canola rotation are separated by the contribution of each specific rotation phase in ascending chronological order; analysis was conducted on the summed fluxes. Bars sharing a letter can be grouped during stepwise factor level collapse without significantly reducing the explanatory power of the model ($P > 0.05$). Miscanthus bars and letters correspond to 2010–2011 fluxes; for KBS, the dotted line and corresponding letter show the contribution of 2009 flux.
Emissions in 2011 differed from the patterns observed in previous years. At KBS, the highest emissions were from the native grasses, followed by continuous corn, which grouped with the switchgrass, miscanthus, and early successional community systems. Poplar, restored prairie, and the soybean phase of the rotation responded with the lowest emissions (Fig. 2e). At ARL, the switchgrass monoculture deviated from prior patterns and together with continuous corn averaged 34% higher emissions than the average across all phases of the rotation, 54% higher than miscanthus, poplar, and early successional community, and 82% higher than the average of the native grass mix and restored prairie systems (Fig. 2f).

**(Environmental predictors of daily nitrous oxide flux**

ARL and KBS differed substantially in their soil moisture; water-filled pore space (WFPS) values at ARL were almost universally higher than at KBS (Table 1). Nevertheless, seasonal patterns within sites were largely similar, with reduced summer WFPS in 2009 and 2011, but sustained WFPS during the wet summer in 2010 (Fig. S1). Median NO$_3^-$ and NH$_4^+$, as measured by resin strips, were similar at both sites although the range of values for both species observed at ARL was greater. Extractable values of NH$_4^+$ were very similar at KBS and ARL for both 2010 and 2011, but the range of values for extractable NO$_3^-$ was again greater at ARL (Table 1).

The timings of precipitation events, fertilizer applications, and N$_2$O flux measurements varied among sites and years, with sharp increases in daily N$_2$O fluxes tending to occur when these events synchronized (Fig. 3). In 2011 at KBS, for instance, fertilization of most perennial crops was followed in rapid succession by a 10-mm precipitation event and a very large N$_2$O flux (Fig. 3f); aggregate annual emissions from perennial systems were very high at KBS that year (Fig. 2e). During the late spring and early summer, systems receiving no N fertilization tended to show limited changes in their emissions. By contrast, fluxes from corn systems varied over several orders of magnitude. N fertilization in corn (blue arrows, Fig. 3) preceded a sharp increase in N$_2$O emission; the sole exception occurred in 2011 at ARL, when there were no precipitation events between fertilization and the next flux measurement (Fig. 3e). The canola rotational phase and most perennial systems

![Fig. 2 Geometric mean of annual nitrous oxide emissions from the Biofuels Cropping System Experiment for the periods of 2009, 2010, and 2011. Left panels (a, c, and e) show results from KBS, n = 4, and right panels (b, d, and f) show results from ARL, n = 5. See Fig. 1 legend for further information.](image-url)
were fertilized earlier in the year and at a lower rate than corn (Table S1; gray arrows, Fig. 3). The range of emissions from these systems tended to be lower than for corn, as were the emission increases following fertilization events. Our data are too limited to broadly infer a generalized relationship between precipitation amounts and subsequent \( \text{N}_2\text{O} \) fluxes, but we note that in all cases where \( \text{N}_2\text{O} \) flux increased sharply after fertilization there was at least one precipitation event of 10 mm or more between

| Year | Site | Soil temp (°C) | WFPS (%) | Resin strip (µg N cm\(^{-2}\) day\(^{-1}\)) | Soil pool (µg N g\(^{-1}\) soil) |
|------|------|---------------|----------|----------------------------------------|---------------------------------|
| 2009 | KBS  | 12.5 (3.0–22.0) | 20 (7–29) | 0.010 (0.002–0.071) | 0.38 (0.03–3.68) |
|      | ARL  | 13.7 (1.1–23.3) | 71 (40–89) | 0.008 (0.001–0.149) | 0.39 (0.04–5.59) |
| 2010 | KBS  | 14.0 (5.4–21.0) | 20 (13–27) | 2.9 (0.7–12.6) | 2.6 (1.5–5.3) |
|      | ARL  | 15.5 (2.9–22.8) | 72 (53–93) | 3.7 (1.9–17.4) | 5.3 (0.9–56.3) |
| 2011 | KBS  | 15.0 (4.0–23.5) | 22 (9–31) | 1.7 (0.7–7.7) | 2.3 (1.3–4.4) |
|      | ARL  | 15.0 (–0.4–25.0) | 67 (37–83) | 1.7 (0.9–7.7) | 2.7 (0.4–25.2) |

Median values are presented, with 5th and 95th percentile values in parentheses. Sites were Arlington Agricultural Research Station, WI (ARL) and Kellogg Biological Research Station, MI (KBS). WFPS is water-filled pore space.
fertilization and flux measurement, whereas among the cases where fertilization was not followed by a large flux increase, there was only one instance where a precipitation event of more than 10 mm was observed prior to flux measurement (Fig. 3a, Group A).

Bayesian model averaging of environmental predictors

Models trained on specific systems varied greatly in their capacity to explain variation in both their training data and the full data (Table 2). Specifically, poplar, miscanthus, and systems containing corn were relatively well modeled while the polycultures (native grasses, early successional community, and restored prairie) were not. There were no environmental factors that substantially contributed to model fits across all systems (Table S2). There were also substantial differences in how well models trained on a given system predicted fluxes from other systems (Table S3). This relationship was not reciprocal. For example, the model based on data from native grass system predicted fluxes from poplar system better than the model based on poplar data predicted fluxes from native grasses.

We tested how effectively models trained on specific systems captured the temporal dynamics of fluxes from their own and other systems (Fig. 4). Flux dynamics at ARL were better modeled than those from KBS, where the models failed to predict major emission events (Fig. 4d–f). Despite the high correlation between predictions from the corn and native grass-based models ($R^2 = 0.58$), actual values from the native grass model were systematically lower than those from the corn model. The switchgrass-based model was inconsistent in its relationship to the other two models, sometimes tracking the corn model (Fig. 4b,d) and at other times the native grass model (Fig. 4f). Overall, each model’s performance was poor on systems other than the one on which it had been trained.

Table 2  Bayesian model averaged posterior probabilities of inclusion for environmental factors used to predict N$_2$O fluxes

| Factor                  | Corn     | Miscanthus | Native grasses | Early successional community | Poplar | Restored Prairie | Switchgrass |
|-------------------------|----------|------------|----------------|-------------------------------|--------|------------------|-------------|
| Site                    | 0.61     | 0.53       | 0.60           | 0.25                          | 1.00   | 0.03             | 0.17        |
| Year ± Site             | 0.00     | 0.01       | 0.01           | 0.04                          | 0.02   | 0.19             | 0.12        |
| NH$_4^+$ ± Site         | 0.81     | 0.13       | 0.01           | 0.04                          | 0.09   | 0.05             | 0.90        |
| NO$_3^-$ ± Site         | 0.95     | 0.53       | 0.54           | 0.53                          | 0.04   | 0.12             | 0.04        |
| Soil temperature (ST)   | 0.00     | 0.23       | 0.23           | 0.24                          | 0.04   | 0.01             | 0.14        |
| Site × Year             | 0.00     | 0.02       | 0.05           | 0.03                          | 0.01   | 0.04             | 0.02        |
| Site × NH$_4^+$         | 1.00     | 0.02       | 0.04           | 0.32                          | 0.01   | 0.06             | 0.96        |
| Site × NO$_3^-$         | 1.00     | 0.02       | 0.04           | 0.32                          | 0.01   | 0.06             | 0.96        |
| Site × ST               | 0.00     | 0.02       | 0.02           | 0.08                          | 0.05   | 0.02             | 0.02        |
| Site × WFPS$_C$         | 0.00     | 0.02       | 0.02           | 0.08                          | 0.03   | 0.02             | 0.02        |
| Year × NH$_4^+$         | 0.00     | 0.02       | 0.02           | 0.01                          | 0.04   | 0.02             | 0.02        |
| Year × NO$_3^-$         | 0.00     | 0.02       | 0.02           | 0.01                          | 0.04   | 0.02             | 0.02        |
| Year × ST               | 0.00     | 0.02       | 0.02           | 0.01                          | 0.04   | 0.02             | 0.02        |
| Year × WFPS$_C$         | 0.00     | 0.02       | 0.02           | 0.01                          | 0.04   | 0.02             | 0.02        |
| NH$_4^+$ × NO$_3^-$     | 0.00     | 0.02       | 0.02           | 0.01                          | 0.04   | 0.02             | 0.02        |
| NH$_4^+$ × ST           | 0.00     | 0.02       | 0.02           | 0.01                          | 0.04   | 0.02             | 0.02        |
| NH$_4^+$ × WFPS$_C$      | 0.00     | 0.02       | 0.02           | 0.01                          | 0.04   | 0.02             | 0.02        |
| NO$_3^-$ × ST           | 0.00     | 0.02       | 0.02           | 0.01                          | 0.04   | 0.02             | 0.02        |
| NO$_3^-$ × WFPS$_C$      | 0.00     | 0.02       | 0.02           | 0.01                          | 0.04   | 0.02             | 0.02        |
| ST × WFPS$_C$           | 0.00     | 0.02       | 0.02           | 0.01                          | 0.04   | 0.02             | 0.02        |
| Training $R^2$          | 0.33     | 0.22       | 0.27           | 0.24                          | 0.22   | 0.02             | 0.20        |
| Full data $R^2$         | 0.33     | 0.22       | 0.27           | 0.24                          | 0.22   | 0.02             | 0.20        |

Models were trained on N$_2$O flux data obtained from individual cropping systems. NH$_4^+$ and NO$_3^-$ concentrations were log-transformed for analysis. The two sites were Arlington Agricultural Research Station (ARL) and Kellogg Biological Research Station (KBS). WFPS$_C$ is water-filled pore space scaled and centered separately for each site. See Table S2 for factor coefficients and probabilities for all cropping systems.
Cropping systems that included corn were consistently higher in N\textsubscript{2}O emissions for any given site year, notwithstanding a high degree of interannual variability. With few exceptions, N\textsubscript{2}O emissions from fertilized perennial systems were much lower than annual systems, while those from unfertilized restored prairie were lowest overall.

Previous work has demonstrated that rate of N fertilizer application has a strong influence on N\textsubscript{2}O emissions from both annual and perennial systems (Dobbie et al., 1999; Millar et al., 2010). The percentage of applied fertilizer emitted as N\textsubscript{2}O varies with site conditions and management, but, in general, N\textsubscript{2}O emissions are highest where inorganic N is readily available. This is reflected in emissions from the continuous corn system which received -3x the rate of N (160 kg ha\textsuperscript{-1}) as the fertilized perennial systems (56 kg ha\textsuperscript{-1}). Other factors such as crop rotation (i.e., crop diversity) can influence N\textsubscript{2}O emission rates. Crop rotation is often associated with reduced N demand which results in reduced N inputs and N\textsubscript{2}O emissions (Drury et al., 2008; Osterholz et al., 2014). The effect of crop rotation in our study was less clear with cumulative emissions being highly dependent on climatic conditions during the corn phase, indicating a strong interaction between crop phase and year.

Cumulative N\textsubscript{2}O emissions can be driven by short-duration, high-intensity flux events (Molodovskaya et al., 2012). These events typically occur when coincidence of fertilization and precipitation results in limited soil oxygen and readily available reactive N (Dobbie & Smith, 2003; Castellano et al., 2010; Matthews et al., 2010). In general, we observed this pattern, where nearly concurrent fertilization and precipitation events resulted in substantial emissions spikes; of all the fertilized systems, this effect was most clearly observed in corn while emissions for crops grown in the absence of fertilizer were almost invariant throughout this period at ARL and were only slightly variable at KBS.

The difference in cumulative N\textsubscript{2}O emissions between ARL and KBS is likely driven by soil properties. Soils at the two sites differed in both their order and texture, with ARL soils consisting of fine textured mollisols and KBS a coarser-textured alfisol. Of the two soil orders, mollisols are typically associated with higher carbon and nutrient contents, consistent with the higher carbon and inorganic N values recorded at ARL. Given that both sites received comparable precipitation, greater WFPS at ARL appears to stem from greater water-holding capacity emerging from high soil organic matter and finer soil texture. We thus attribute the consistently higher emissions at ARL to soil moisture-driven increases in anaerobic microsite abundance and longevity (Bouwman, 1996) coupled with greater N and C availability. Our findings are consistent with studies in both agricultural and wildland ecosystems that have linked finer soil texture and greater soil carbon
availability to increased N\textsubscript{2}O emissions (Bouwman et al., 2002; Stehfest & Bouwman, 2006). While the restored prairie system was not fertilized at either site, greater soil C at ARL suggests potential for rapidly mineralizing N, and this coupled with higher WFPS, may have facilitated the slightly higher fluxes observed at ARL. In contrast, between-site differences in the poplar system were at least partially attributable to an infestation of the ARL plots with the fungal leaf pathogen Marssonina (Marssonina populi (Lib.) Magnus). The infestation peaked in mid-August 2010 leading to complete defoliation by 15 September; this likely reduced plant N uptake in the only year that N fertilizer was applied to poplar, leaving more N available for microbial conversion and loss as N\textsubscript{2}O.

Cropping systems based on perennial species require multiple years to become fully established (Parrish & Fike, 2005). This development is most evident in the delay in attaining maximum yields, but N-cycling processes may also change during this period (Smith et al., 2013; Lesur et al., 2014). During the period of this study, N\textsubscript{2}O emissions per unit aboveground yield were much lower in the perennial systems. It is likely this ratio will improve as the systems come into full production phase and as farmers become more efficient, both in harvest timing and mechanical efficiency, at harvesting perennial biomass. Perennial systems also produce greater biomass belowground which over time will likely improve soil organic matter and site fertility. While our study was not structured to explicitly explore the effects of the establishment period, it is still a potentially relevant contextual element for interpreting results from the perennial cropping systems. Our results were largely comparable to studies of perennial systems similarly conducted over the establishment phase (Hernandez-Ramirez et al., 2009; Smith et al., 2013). However, given the high influence of interannual variability on our results, it is clear that long-term studies will be required to bound this variability and determine whether establishment-phase N\textsubscript{2}O emissions are representative of established perennial cropping systems.

A consideration for our results is that our study lacked measurements during winter (December–February), which could have resulted in underestimation of N\textsubscript{2}O emissions. Substantial N loss can occur during this fallow period, especially in conventionally managed annual row crops when bare soil is subjected to freeze–thaw events (Johnson et al., 2010). Soil N is susceptible to denitrification during this period, especially for systems where manure has been applied after the primary crop has been harvested (Parkin et al., 2006), or where vegetative cover is not present during winter (McSwiney et al., 2010). However, only our annual cropping systems had significant bare soil during winter, and as discussed above, N\textsubscript{2}O emissions in these systems were dominated by relatively brief spikes following fertilizer events. Nitrogen was not applied in the fall to any of our cropping systems so we expect that winter measurements would have negligible influence on the magnitude and comparison of N\textsubscript{2}O emissions.

The Bayesian averaged models we analyzed in detail suggested alternative cropping systems would produce different N\textsubscript{2}O fluxes under a given set of environmental conditions. Our statistical models based on emissions measured from our various cropping systems had very distinct parameterizations, even when the systems were as similar as continuous and rotational corn. Previous studies have similarly found that key environmental predictors of N\textsubscript{2}O emissions vary among systems (Dechow & Freibauer, 2011; Imer et al., 2013). The BMA based on switchgrass, for instance, frequently predicted substantially higher emissions than the model based on native grasses, implying that at a given soil moisture, soil temperature, and inorganic N concentration, switchgrass and native grasses would have different N\textsubscript{2}O emissions. Cropping systems may differ not only in their effect on environmental parameters (e.g., through crop species differences in N uptake and water use), but also in their response to these parameters. The role of plant community composition and diversity in determining trace gas fluxes from soil has received little attention (but see Hoefl et al., 2012).

Given our results, there may be a significant gap in our ability to account for how plant community composition influences the response of N\textsubscript{2}O fluxes to environmental drivers. Single-species monocultures have typically been used to model the broader category of herbaceous biomass crops (Surendran Nair et al., 2012), with switchgrass used as a model for exploring the properties and environmental responses of bioenergy crops (Lewandowski et al., 2003; Tulbure et al., 2012). The cropping system specificity we observed in the response of N\textsubscript{2}O fluxes to environmental parameters suggests it may be risky to rely on model systems to predict the behaviors of perennial and polycultural biomass cropping systems, particularly with the potential for high variability during the establishment phase.

In summary, across years with highly variable climate, N\textsubscript{2}O emissions were consistently higher from annual than perennial cropping systems. Under particular conditions, namely rainfall following fertilizer, emissions from corn dwarfed all other systems. N\textsubscript{2}O emissions were consistently low for unfertilized restored prairie harvested for biomass. Perennial cropping systems on highly productive mollisols had higher N\textsubscript{2}O emissions than the same systems growing on moderately productive alfisols. Finally, N\textsubscript{2}O flux responses to environmental conditions during establishment were
not generalizable across cropping systems, indicating that use of model systems, especially for perennials and polycultures, should be performed cautiously.

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References

Adler PR, Del Grosso SJ, Parton WJ (2007) Life-cycle assessment of net greenhouse-gas flux for bioenergy cropping systems. *Ecological Applications*, 17, 675–691.

Anderson-Teixeira KJ, Masters MD, Black CK, ZerI M, Hussain MZ, Bernacchi CJ, DeLucia EH (2013) Altered belowground carbon cycling following land-use change to perennial bioenergy crops. *Ecosystems*, 16, 508–520.

Bouwman AF (1996) Direct emission of nitrous oxide from agricultural soils. *Nutrient Cycling in Agroecosystems*, 46, 53–70.

Bouwman AF, Boumans LJM, Batjes NH (2002) Emissions of N2O and NO from fertilized fields: summary of available measurement data. *Global Biogeochemical Cycles*, 16, 6–16.

Bouwman AF, Van Grinsven JJM, Eickhout B (2010) Consequences of the cultivation of energy crops for the global nitrogen cycle. *Ecological Applications*, 20, 101–109.

Burbidge JB, Magee L, Robb AL (2013) Alternative transformations to handle extreme values of the dependent variable. *Journal of the American Statistical Association*, 83, 123–127.

Castellano MJ, Schmidt JP, Kaye JP, Walker C, Graham CB, Lin H, Dell CJ (2010) Hydrological and biogeochemical controls on the timing and magnitude of nitrous oxide flux across an agricultural landscape. *Global Change Biology*, 16, 2711–2720.

Cavigelli MA, Robertson GP (2011) Role of denitrifier diversity in rates of nitrous oxide consumption in a terrestrial ecosystem. *Soil Biology and Biochemistry*, 33, 297–310.

Chen D, Li Y, Grace P, Mosier AR (2008) N2O emissions from agricultural lands: a synthesis of simulation approaches. *Plant and Soil*, 309, 169–189.

Crum JR, Collins HP (1995) KBS Soil. Kellogg Biological Station Long-Term Ecological Research, Michigan State University, Hickory Corners, MI. Available at: http://txr.kbs.msu.edu/research/site-description-and-maps/site-description (accessed 19 September 2014).

Dale VH, Kline KL, Wright LL, Perluck RD, Downing M, Graham RL (2011) Interactions among bioenergy feedstock choices, landscape dynamics, and land use. *Ecological Applications*, 21, 1059–1054.

Dale BE, Anderson JE, Brown RC et al. (2014) Take a closer look: biofuels can support environmental, economic and social goals. *Environmental science and Technology*, 48, 7200–7203.

Dechow R, Freibauer A (2011) Assessment of German nitrous oxide emissions using empirical modelling approaches. *Nutrient Cycling in Agroecosystems*, 91, 235–254.

Dobbe K, Smith K (2003) Nitrous oxide emission factors for agricultural soils in Great Britain: the impact of soil water-filled pore space and other controlling variables. *Global Change Biology*, 9, 204–218.

Dobbe KE, McTaggart IP, Smith KA (1999) Nitrous oxide emissions from intensive agricultural systems: variations between crops and seasons, key driving variables, and mean emission factors. *Journal of Geophysical Research*, 104, 26891.

Drury CF, Yang XM, Reynolds WD, McLaughlin NB (2008) Nitrous oxide and carbon dioxide emissions from monoculture and rotational cropping of corn, soybean and winter wheat. *Canadian Journal of Soil Science*, 88, 163–174.

Gelfand I, Zenone T, Jaretta P, Chen J, Hamilton SK (2011) Carbon debt of Conservation Reserve Program (CRP) grasslands converted to bioenergy production. *Proceedings of the National Academy of Sciences*, 108, 13864–13869.

Hennault C, Bizoard F, Laville P, Gabrielle B, Nicoulaut B, Germon JC, Cellier P (2005) Predicting in situ soil N2O emission using NOE algorithm and soil database. *Global Change Biology*, 11, 115–127.

Hernandez-Ramirez G, Bredero SM, Smith DR, Van Scyoc CE (2009) Greenhouse gas fluxes in an eastern Corn Belt soil: weather, nitrogen source, and rotation. *Journal of Environmental Quality*, 38, 841–854.

Hoefl I, Steude K, Wragge N, Veldkamp E (2012) Response of nitrogen oxide emissions to grazer species and plant species composition in temperate agricultural grassland. *Agriculture, Ecosystems and Environment*, 151, 34–43.

Hoeting JA, Madigan D, Raftery AE, Volinsky CT (1999) Bayesian model averaging: a tutorial. *Statistical Science*, 14, 382–417.

Hutchinson GL, Mosier AR (1981) Improved soil cover method for field measurement of nitrous oxide fluxes. *Soil Science Society of America Journal*, 45, 311.

Imer D, Merbold L, Eguigter W, Buchmann N (2013) Temporal and spatial variations of soil CO2, CH4 and N2O fluxes at three differently managed grasslands. *Biogeosciences*, 10, 5933–5945.

Johnson JM, Archer D, Barbour N (2010) Greenhouse gas emission from contrasting management scenarios in the northern Corn Belt. *Soil Science Society of America Journal*, 74, 396–406.

Jokela W, Posner J, Hedtcke J, Balser T, Read H (2011) Midwest cropping system effects on soil properties and on a soil quality index. *Agronomy Journal*, 103, 1552–1562.

Lesur C, Bazot M, Bio-Beri F, Mary B, Jefroy M-H, Loize C (2014) Assessing nitrate leaching during the three-first years of *Miscanthus* x *giganteus* from on-farm measurements and modeling. *GCB Bioenergy*, 6, 439–449.

Lewandowski I, Scurlock JMO, Lindwall E, Christou M (2003) The development and current status of perennial rhizomatus grasses as energy crops in the US and Europe. *Biomass and Bioenergy*, 25, 335–361.

Maroja LS, Andres JA, Walters JR, Harrison RG (2009) Multiple barriers to gene exchange in a field cricket hybrid zone. *Biological Journal of the Linnean Society*, 97, 390–402.

Matthews RA, Chadwick DR, Retter AL, Blackwell MSA, Yamulki S (2010) Nitrous oxide emissions from small-scale farmland features of UK livestock farming systems. *Agriculture, Ecosystems and Environment*, 136, 192–198.

McLaughlin SB, Adams Kaziou P (2005) Development of switchgrass (*Panicum virgatum*) as a bioenergy feedstock in the United States. *Biomass and Bioenergy*, 28, 515–535.

McSweeney CP, Snapp SS, Gentry LE (2010) Use of N immobilization to tighten the N cycle in conventional agroecosystems. *Ecological Applications*, 20, 648–662.

Millar N, Robertson GP, Grace PR, Gehl RJ, Hoben JP (2010) Nitrogen fertilizer management for nitrous oxide (N2O) mitigation in intensive corn (maize) production: an emissions reduction protocol for US Midwest agriculture. *Mitigation and Adaptation Strategies for Global Change*, 15, 185–204.

Molodovskaya M, Singurthy O, Richards BK, Warland J, Johnson MS, Steenhuis TS (2012) Temporal variability of nitrous oxide from fertilized croplands: hot moment analysis. *Soil Science Society of America Journal*, 76, 1728.

MISCO (2013) Michigan State Climatologist’s Office: 27 year summary of average values for Gull Lake (3504) 1981–2010, Available at: http://climate.geo.msu.edu/climate_ml/stat/stations/3504/1981-2010%20annual%20summary.pdf (accessed 2 April 2014).

Nikiema P, Rothstein DE, Min DI-H, Kang CJ (2011) Nitrogen fertilizer application of switchgrass increases biomass yield and improves net greenhouse gas balance in northern Michigan, USA. *Biomass and Bioenergy*, 35, 4366–4376.

Nikiema P, Rothstein DE, Miller RO (2012) Initial greenhouse gas emissions and nitrogen leaching losses associated with converting pastureland to short-rotation woody bioenergy crops in northern Michigan, USA. *Biomass and Bioenergy*, 39, 413–426.

NWS (2013) National Weather Service: Wisconsin 30 year average temperature and precipitation 1981–2010, Available at: www.crh.noaa.gov/images/mks/climate/avg_30_year_precip.png and www.crh.noaa.gov/images/mks/climate/avg_30_year_temp.png (accessed 2 April 2014).

Osterholz WR, Kuchark J, Hedtcke J, Posner J (2014) Seasonal nitrous oxide and methane fluxes from grain- and forage-based production systems in Wisconsin, USA. *Journal of Environment Quality*, 43, 1833–1843.

Palmer MM, Forrester JA, Rothstein DE, Miedendorf DJ (2014) Conversion of open lands to short-rotation woody biomass crops: site variability affects nitrogen cycling and N2O fluxes in the US Northern Lakes. *GCB Bioenergy*, 6, 450–464.

Parkin TB, Kaspar TC, Singer JW (2006) Cover crop effects on the fate of N following soil application of swine manure. *Plant and Soil*, 269, 141–152.
