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Nitrogen fertilization challenges the climate benefit of cellulosic biofuels

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Abstract

Cellulosic biofuels are intended to improve future energy and climate security. Nitrogen (N) fertilizer is commonly recommended to stimulate yields but can increase losses of the greenhouse gas nitrous oxide (N_2O) and other forms of reactive N, including nitrate. We measured soil N_2O emissions and nitrate leaching along a switchgrass (*Panicum virgatum*) high resolution N-fertilizer gradient for three years post-establishment. Results revealed an exponential increase in annual N_2O emissions that each year became stronger ($R^2 > 0.9$, $P < 0.001$) and deviated further from the fixed percentage assumed for IPCC Tier 1 emission factors. Concomitantly, switchgrass yields became less responsive each year to N fertilizer. Nitrate leaching (and calculated indirect N_2O emissions) also increased exponentially in response to N inputs, but neither methane (CH_4) uptake nor soil organic carbon changed detectably. Overall, N fertilizer inputs at rates greater than crop need curtailed the climate benefit of ethanol production almost two-fold, from a maximum mitigation capacity of -5.71 ± 0.22 Mg $CO_2e\ ha^{-1}\ yr^{-1}$ in switchgrass fertilized at 56 kg N ha^{-1} to only -2.97 ± 0.18 Mg $CO_2e\ ha^{-1}\ yr^{-1}$ in switchgrass fertilized at 196 kg N ha^{-1}. Minimizing N fertilizer use will be an important strategy for fully realizing the climate benefits of cellulosic biofuel production.

1. Introduction

The global production of biofuels has increased dramatically in response to calls for greater energy security and climate change mitigation. In the US, legislation mandates production of 136 billion liters of ethanol biofuel by 2022 with a growing fraction from cellulosic sources (US Congress 2007). Cellulosic biofuels offer the potential for greater environmental benefits compared to grain based biofuels (Tilman *et al* 2006, Robertson *et al* 2008, 2011). Switchgrass (*Panicum virgatum*), a perennial grass native to North America, is among the most promising cellulosic biofuel crops due to its ability to grow on marginal and erosive lands, sequester soil carbon (Liska and Cassman 2008), reduce nitrogen (N) leaching (Smith

et al 2013), and be grown with relatively little fossil fuel input (McLaughlin and Adams Kszos 2005).

Switchgrass is often considered an inherently N-thrifty plant, especially when managed for biomass production (Parrish and Fike 2005). Nevertheless, multiple studies have documented a productivity response to added N, with most reporting maximum yields at N rates between 56 and 202 kg N ha^{-1} (Vogel *et al* 2002, Mulkey *et al* 2006, Mooney *et al* 2009, Nikièma *et al* 2011). In a recent on-farm experiment (Schmer *et al* 2008), farmers fertilized switchgrass at rates up to 212 kg N ha^{-1}.

Although N fertilizer can increase biomass production, added N increases the greenhouse gas (GHG) contributions of biofuel production substantially: not only through the production, transportation, and

distribution of the fertilizer itself, but also through fertilizer-induced microbial emissions of nitrous oxide (N_2O), a GHG with a global warming potential $\sim$300 times that of carbon dioxide (CO_2) (Solomon *et al* 2007) and a major cause of stratospheric ozone depletion (Portmann *et al* 2012). Moreover, fertilizer N lost to the environment as nitrate (NO_3^-) leads to indirect emissions of N_2O elsewhere in downstream surface waters (Beaulieu *et al* 2011) as well as to degraded water quality (Robertson and Vitousek 2009). Additionally, well-aerated soils are a globally significant sink for atmospheric methane (CH_4), and ammonium (NH_4^+) from N fertilizers can competitively inhibit microbial CH_4 oxidation in soils (Gulledge and Schimel 1998, Le Mer and Roger 2001).

Earlier studies have noted the potential for N fertilizer inputs to substantially reduce and even eliminate the climate benefit of food crops grown for biofuels (Crutzen *et al* 2007, Mosier *et al* 2009, Smith *et al* 2012). While Erisman *et al* (2010) further noted that this is unlikely to be the case for purpose-grown cellulosic crops because of their lower N fertilizer needs, perennial nature, and higher C:N ratios in harvested biomass, this has not yet been empirically tested.

Here we test the potential for N fertilization to significantly reduce the climate change mitigation benefit of cellulosic biofuels. We present results from a 3 yr experiment to investigate direct and indirect N_2O emissions, CH_4 uptake, NO_3^- leaching, soil organic carbon (SOC) accumulation, and biomass production in recently established switchgrass under eight different N fertilizer rates. Our analysis allows an evaluation of the impact of N fertilization on the net GHG balance of switchgrass grown as a cellulosic biofuel feedstock.

2. Methods

The experiment was conducted at a site in southwest Michigan USA, in the northeastern portion of the US Corn Belt. The Switchgrass N Rate Experiment is part of the Great Lakes Bioenergy Research Center (GLBRC) and located at the Kellogg Biological Station Long-term Ecological Research Site (www.lter.kbs. msu.edu; 42°23′ N, 85°22′ W, elevation 284 m asl). Precipitation averages 1005 mm yr^{-1} with an average snowfall of $\sim$1.3 m. Mean annual temperature is 10.1 °C ranging from a monthly mean of -3.8 °C in January to 22.9 °C in July (NCDC 2013). Soils are mesic Typic Hapludalfs of Kalamazoo loam developed on glacial outwash (Robertson and Hamilton 2015). Prior to establishing the experiments, soil pH (0–25 cm depth) was 7.47 ± 0.04 (mean ± standard error, $n = 12$ plots), bulk density (BD) was 1.24 ± 0.04 g cm^{-3}, total N was 1.25 ± 0.09 g kg^{-1} soil, and SOC was 10.2 ± 0.74 g kg^{-1} soil (http://data.sustainability.glbrc.org/).

Switchgrass (variety Cave-in-Rock) was planted at a seeding rate of 7.84 kg ha^{-1} on 11 July, 2008, after tillage to a depth of 25 cm. Plots were established on land that had been in alfalfa, corn, and occasional soybean production for preceding decades. Eight fertilization treatments (0, 28, 56, 84, 112, 140, 168, and 196 kg N ha^{-1}) were established in switchgrass plots (4.5 × 6 m) arranged in a randomized complete block design with four replicate blocks, for a total of 32 experimental plots. Nitrogen fertilizer was applied once per year in 2009–2011: granular 46% urea was broadcasted on 17 June, 2009, and liquid 28% urea ammonium nitrate was sprayed on 10 May, 2010, and 16 May, 2011. Biomass was harvested in late fall annually on each plot using a John Deere 7330 tractor with a plot harvester (Wintersteiger Inc., Salt Lake City, UT) and HarvestMaster HM800 Plot Harvest Data System (Juniper Systems Inc., Logan, UT). Dry matter percentage was determined by oven-drying subsamples from harvested plots at 50 °C until a constant weight. Harvest height was $\sim$10 cm.

2.1. N_2O and CH_4 sampling

N_2O and CH_4 fluxes were measured using a static chamber—gas chromatography approach (Ruan and Robertson 2013) from May to December in 2009–2011. We measured fluxes 2–3 times per week during the growing season to capture the temporal dynamics of N_2O and CH_4 fluxes as influenced by fertilization and precipitation, and then measured fluxes every 2 weeks after mid-September. A vented chamber (28 cm diameter × 26 cm height) equipped with a detachable lid and septum was installed in each treatment plot for a total of 32 chambers. Chamber bases were inserted into the soil $\sim$5 cm for the duration of the study. Vegetation inside (but not surrounding) chambers was clipped to maintain plant heights lower than chamber heights. During flux samplings, chambers were tightly sealed with the lid and then headspace gas samples were collected four times with a 10 ml syringe at approximately 15 min intervals. Samples were stored over-pressurized in 5.6 ml glass vials (Labco Ltd, High Wycombe, UK). Gases were analyzed within three days by gas chromatography (Hewlett Packard 5890 Series II, Rolling Meadows, IL, USA). Gases were separated on a Porapak Q column (1.8 m, 80/100 mesh) at 80 °C; CH_4 was analyzed with a flame ionization detector at 300 °C and N_2O was analyzed with a ^{63}Ni electron capture detector at 350 °C.

2.2. Soil water-filled pore space (WFPS %), inorganic N, and NO_3^- leaching

At each gas sampling event we measured soil temperature, gravimetric water content, and inorganic N (NH_4^+ and NO_3^-) concentrations at 0–25 cm depth. Soil gravimetric water content (GWC, g water g^{-1} dry soil) was determined by oven-drying soil at 60 °C for 48 h until constant mass. Soil BD was measured three

times during each growing season using a fixed-volume soil core (123 cm^3) for each treatment plot. WFPS% was calculated as

$$\% \text{ WFPS} = 100\% \times \frac{\text{GWC (g g}^{-1}) \ \times \text{BD (g cm}^{-3})}{\text{soil porosity}},$$

where soil porosity $= 1 - [\text{BD (g cm}^{-3})/\text{particle density (g cm}^{-3})]$. Soil particle density was assumed to be the standard 2.65 g cm^{-3}.

For measuring NH_4^+ and NO_3^-, three 10 g soil samples (4 mm sieved) were extracted with 100 ml of 1 M KCl. Filtrates from soil extracts were analyzed colorimetrically on a Flow Solution IV autoanalyzer (OI Analytical, College Station, TX, USA).

Nitrate leaching below the root zone (1.0 m depth) was determined by measuring concentrations in soil pore water and then multiplying concentrations by downward water percolation (drainage) from the overlying soil. The study site has no detectable overland runoff because of its highly permeable soils. Soil pore water was sampled at weekly to fortnightly intervals (except when the ground was frozen) using low-tension porous ceramic cup samplers (Eijkelkamp Agrisearch Equipment, California, USA) installed at a 45° angle from the soil surface. The collected and filtered (1 μm nominal pore size; Pall A/E) water samples were analyzed for NO_3^- using a Dionex 600 ion chromatograph. Previous work at this site (Syswerda *et al* 2012) has shown that NO_3^- dominates N leaching with negligible leaching of NH_4^+ or dissolved organic N.

Percolation of water from the root zone was modeled at a daily time step using the systems approach for land use sustainability model well-calibrated for KBS soils (Basso and Ritchie 2015), which accounts for management practices, water balance, soil organic matter change, nitrogen and phosphorus dynamics, heat balance, and plant growth and development. The soil water balance module is based on CERES models (Ritchie *et al* 1998) with revisions for infiltration, soil water export (Suleiman and Ritchie 2004), evaporation (Suleiman and Ritchie 2003), and runoff. Daily leaching losses of nitrate were estimated from modeled water percolation plus linear interpolation of the measured nitrate concentrations.

2.3. SOC sampling

One intact soil core (7.6 cm diameter × 100 cm depth) was taken from each of the 32 experimental plots in June 2008 and May 2013 with a hydraulic sampler (Geoprobe model 540MT, Salina, KS). Each core was then cut into three profile segments: 0–25 cm (to represent the Ap layer), 25–50 cm (E layer), and 50–100 cm (Bt layer) (Syswerda *et al* 2011). Each segment was sieved (4 mm), oven-dried, and weighed for BD. Dry soil samples were then finely ground in a roller mill and three 10 mg samples were analyzed for C using a Costech elemental combustion system

(Costech Analytical Technologies, Valencia, California).
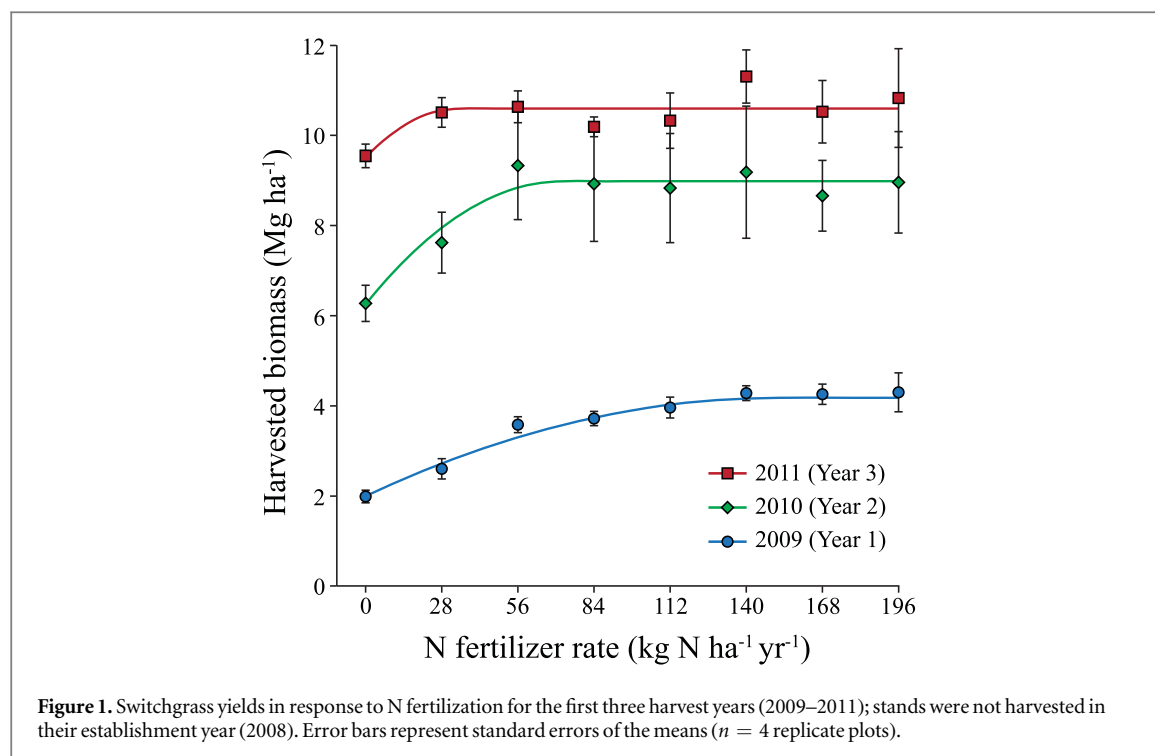
2.4. Net GHG balance

To estimate the global warming impact (GWI) of GHG fluxes, we multiplied fluxes of CH_4 and N_2O by their 100 yr horizon global warming potential factors of 25 and 298, respectively, to yield CO_2 equivalents (CO_2e) (Solomon *et al* 2007).

We assumed that all CO_2 taken up from the atmosphere as net primary production by switchgrass was stored in harvested biomass and SOC. SOC change in CO_2e was calculated as the product of the difference of SOC (Mg ha^{-1} yr^{-1}) over the 4 yr study and the conversion factor of C to CO_2 (44/12).

Fossil fuel offset credit (Mg CO_2e ha^{-1} yr^{-1}) is defined as the avoided CO_2 emissions due to the displacement of fossil fuel use by biofuels during production, transportation, distribution, combustion, and coproducts allocation (Plevin 2009). Avoided CO_2e emissions were calculated from a comparison of life cycle analyses of petroleum gasoline versus ethanol from switchgrass. Gasoline releases 94 g CO_2e per MJ petroleum gasoline produced, distributed, and combusted (Farrell *et al* 2006, Wang *et al* 2012). Net CO_2e emissions per MJ of switchgrass ethanol were calculated as the product of net CO_2e emissions (Mg CO_2e ha^{-1}) from ethanol production, transportation, distribution and combustion and the total energy equivalent of biomass yield (MJ ha^{-1}). Net CO_2e emissions were calculated using the GREET model (Huo *et al* 2009) to calculate fossil fuel offset credits for the fossil fuel CO_2 emissions that would be displaced by the production of both ethanol and biorefinery coproducts (Farrell *et al* 2006, Gelfand *et al* 2013), with all farming inputs equal to 0. Farming inputs were calculated separately using actual values from the study site as presented in table S3. Total energy equivalent (MJ ha^{-1}) was calculated as the product of harvestable dry-weight biomass (Mg ha^{-1}), biorefinery ethanol yield (380 l Mg^{-1} biomass) (Schmer *et al* 2008, Gelfand *et al* 2011) and ethanol energy content (21.1 MJ l^{-1}; low heating value) (Gelfand *et al* 2011, 2013). Finally, the fossil fuel offset credit (Mg CO_2e ha^{-1}) was calculated as the product of the CO_2e difference from life cycle analyses of petroleum gasoline and ethanol from switchgrass (g CO_2e MJ^{-1}) and the ethanol energy content.

2.5. Data analysis

Cumulative fluxes of gases over annual periods were calculated by linear interpolation of daily fluxes between sample days. Data were analyzed using the PROC MIXED procedure in SAS 9.2 (SAS Institute, Cary, NC, USA). Treatment means were compared for significance using t-tests at $\alpha = 0.05$ level. The relationships between daily N_2O emissions and environmental factors such as soil temperature, soil

Figure 1. Switchgrass yields in response to N fertilization for the first three harvest years (2009–2011); stands were not harvested in their establishment year (2008). Error bars represent standard errors of the means ($n = 4$ replicate plots).

moisture, and soil total N were assessed by multiple linear regressions (stepwise) using PROC REG. Quadratic-plateau curves for switchgrass yields versus N fertilization rates were calculated using PROC NLIN, with switchgrass yields at successive fertilizer rates weighted by the inverse of its rank order along the N gradient (1/1 to 1/8). To determine the relationship between annual N_2O emissions or leached nitrogen and N fertilization rate we performed exponential regression using PROC NLIN and linear regression using PRPC REG. Likelihood ratio-based R^2-values, the Akaike information criterion (AIC) and the Bayesian information criterion (BIC), were calculated for both linear and exponential models. Higher values of R^2 and lower values of AIC and BIC indicated better models.

3. Results and discussion

Switchgrass yields were responsive to N fertilizer in 2009, but were less responsive in 2010 and 2011 (figure 1). Based on a quadratic plateau model ($R^2_{2009} = 0.86$, $P < 0.01$; $R^2_{2010} = 0.69$, $P < 0.01$; $R^2_{2011} = 0.21$, $P < 0.05$), maximum yields of 4.2, 8.9 and 10.6 $Mg\,ha^{-1}\,yr^{-1}$ occurred at 147 $kg\,N\,ha^{-1}$ in 2009, at 72 $kg\,N\,ha^{-1}$ in 2010, and at only 34 $kg\,N\,ha^{-1}$ in 2011, respectively. Yields for 2010 and 2011 are consistent with average regional yields of 8.7 ± 4.2 $Mg\,ha^{-1}\,yr^{-1}$ for post-establishment switchgrass (Wullschleger *et al* 2010).

We observed an exponential increase in annual N_2O emissions with increasing N fertilization rates in each year (figure 2). The AIC and the BIC values were consistently lower for the exponential model for each

study year and as well for all 3 years together: for individual years AIC and BIC values for the linear model were 52%–121% and 76%–173% higher than those for the exponential model, respectively (table S1). Additionally, R^2 values for the exponential model (0.90–0.94) were consistently higher than were R^2 values for the linear model (0.84–0.88) (table S1).

From 2009 to 2011, mean daily N_2O emissions ranged from 1.28 ± 0.14 $g\,N\,ha^{-1}\,d^{-1}$ in the low (0 $kg\,N\,ha^{-1}$) fertilization treatment to 25.8 ± 1.9 g $N\,ha^{-1}\,d^{-1}$ in the high (196 $kg\,N\,ha^{-1}$) fertilization treatment (figure S1). The maximum daily N_2O emission was 270 ± 25 $g\,N\,ha^{-1}\,d^{-1}$ in the highest N treatment and the minimum daily emission was undetectable in treatments that received less than 56 $kg\,N\,ha^{-1}\,yr^{-1}$. Most of the fertilizer-associated N_2O emissions occurred within 40 days following fertilization, coincident with soil wetting by rainfall. N_2O emissions were strongly correlated with soil inorganic N concentrations ($mg\,N\,kg^{-1}$) and % soil WFPS (N_2O emission $= -34.8 + 0.83 \times$ inorganic N $+ 81.9 \times$ WFPS, $R^2 = 0.48$, $n = 2112$, $P < 0.001$). At all soil inorganic N levels, N_2O emisions were highly dependent on WFPS (figure S2).

Modeled soil water drainage was 275, 399 and 515 $mm\,yr^{-1}$ in 2009, 2010 and 2011, respectively, representing 37%, 39% and 46% of annual precipitation. Annual NO_3^- leaching rates ranged from 2.65 ± 1.29 $kg\,N\,ha^{-1}\,yr^{-1}$ for unfertilized switchgrass to 56.0 ± 2.7 $kg\,N\,ha^{-1}\,yr^{-1}$ for switchgrass fertilized at 196 $kg\,N\,ha^{-1}$, and also increased exponentially (table S2) in response to increasing N inputs, with no significant difference in the increase among years (figure 3).
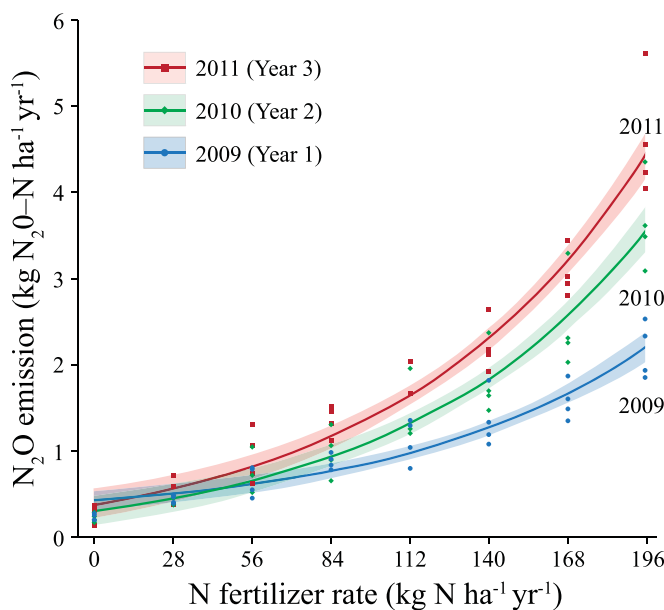
Figure 2. Exponentially increasing annual N_2O emission in response to increasing N fertilization rates for the first three harvest years (2009–2011) ($P < 0.001$, bands represent 95% confidence intervals).
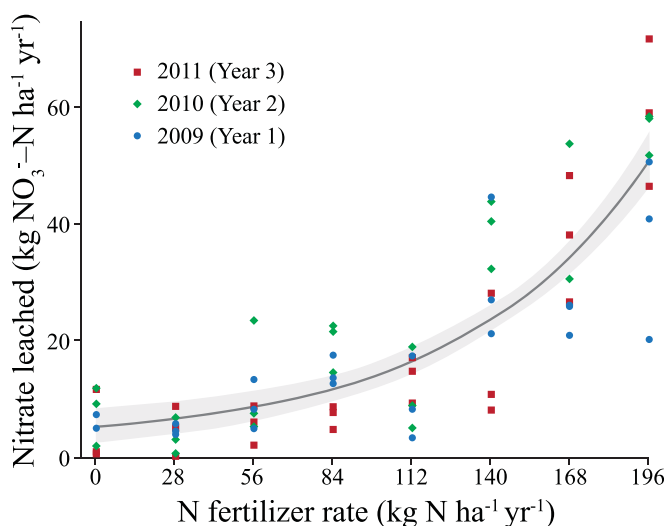

Figure 3. Exponentially increasing annual NO_3^- leaching in response to increasing N fertilization rates for the first three harvest years (2009–2011) ($R^2 = 0.74$, $P < 0.0001$, band represents 95% confidence intervals).

The exponential increases in N_2O emissions and NO_3^- leaching are likely due to surplus soil N at levels above which plant N demands are met, resulting in more available N for the nitrifiers and denitrifiers that produce N_2O, as well as for leaching. The exponential increase for N_2O also implies that the N_2O emission factor used for most national GHG inventories (De Klein *et al* 2006) varies with N fertilizer rate, in agreement with other recent studies for annual crops but yet untested for perennial crops (Shcherbak *et al* 2014). In this study the emission factor increased from 0.6% to 2.1% across the range of added N (figure 4). A constant emission factor, as called for by IPCC Tier 1 methodology (De Klein *et al* 2006), would underestimate 3

year N_2O emissions by 30% at lower levels of N fertilizer and up to 107% at higher levels.

Mean daily CH_4 uptake rates ranged from -1.49 ± 0.31 to -0.82 ± 0.27 g CH_4-C ha^{-1} d^{-1} across all eight fertilizer levels (figure S3). There were no significant N treatment differences detected ($P > 0.1$), although mean CH_4 uptake rates in highly fertilized soils (>56 kg N added ha^{-1}) were only 55%–74% of those in the unfertilized treatment. We also found no significant SOC accumulation in any of our N treatments over the 3 year study period (figure S4). Likely this is due to lost soil C on conversion of the field to switchgrass in 2008, although spatial variability makes it difficult to detect SOC change in fewer than
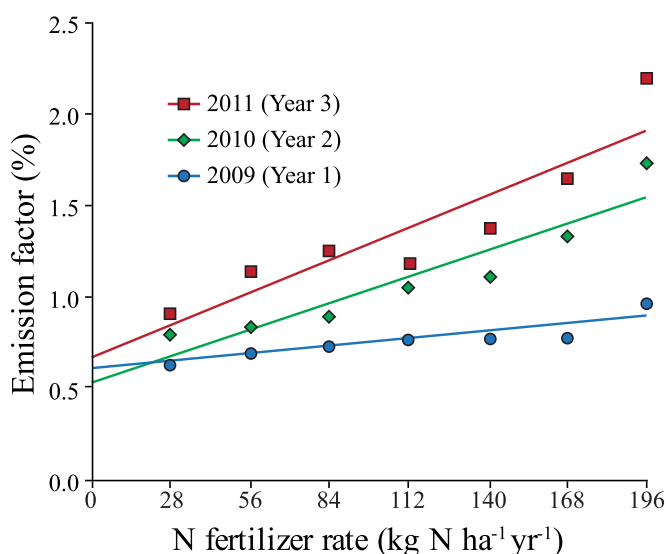
Figure 4. Relationships between soil N_2O emission factors (% of N fertilizer input that was ultimately emitted as N_2O) and N fertilization rates for the first three harvest years (2009–2011), including linear regression fits.
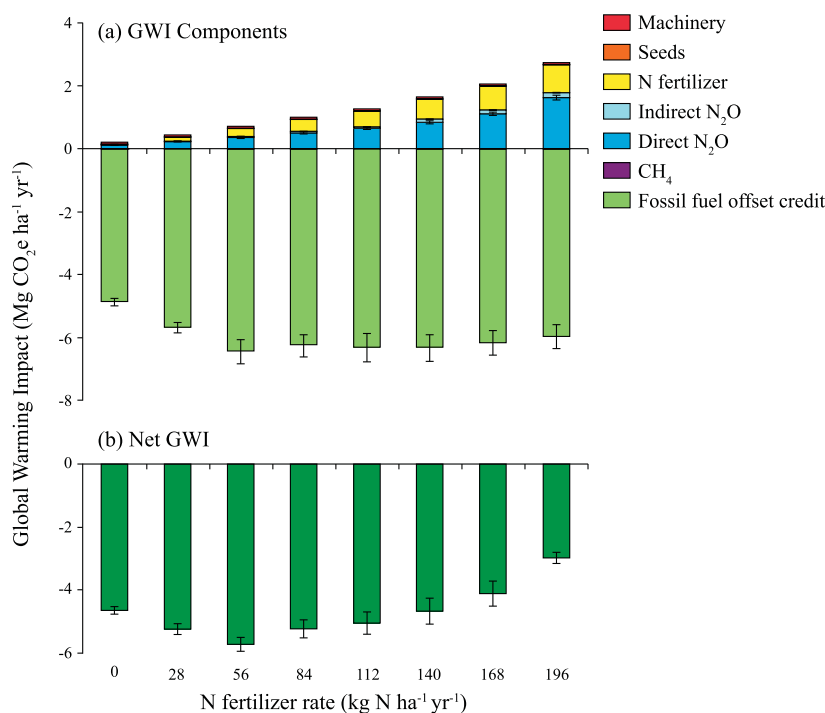

Figure 5. Annual global warming impacts (GWI; based on overall GHG balances) for switchgrass production across the N fertilizer gradient. (a) GWI components including fossil fuel offset credits for displacement of gasoline by biofuel; (b) net GWI. GHG emissions from agricultural inputs include farm machinery, switchgrass seed production, and N fertilizer production, transportation and distribution. Indirect N_2O emissions represent N_2O produced off-site by leached NO_3^-. Direct N_2O and CH_4 fluxes are from field measurements during 2009–2011. CH_4 uptake rates were negligible and are not visible in the graph. Error bars represent standard errors based on $n = 4$ replicate plots.

10 years in many soils (Kravchenko and Robertson 2011), including ours (Syswerda *et al* 2011).

We combined our field measurements with published carbon costs for agricultural inputs (Robertson *et al* 2000, Farrell *et al* 2006, Schmer *et al* 2008, Gelfand *et al* 2011, 2013) to estimate overall GWI in units of CO_2 equivalents (CO_2e) for each of our N treatments (table S3). Measurements of NO_3^- loss allow us to include a major portion of indirect N_2O production, missing from most empirical GWI assessments. Estimated indirect N_2O emissions from the loss of leached NO_3^- ranged from 22.1 ± 7.8 for unfertilized switchgrass to 157 ± 12.3 kg CO_2e ha^{-1} yr^{-1} for switchgrass fertilized at 196 kg N ha^{-1}. Calculated 3 year averages of fossil fuel offsets for our eight N treatments ranged from -4.84 ± 0.12 Mg CO_2e ha^{-1} yr^{-1} in our

unfertilized treatment to -6.42 ± 0.38 Mg CO_2e ha^{-1} yr^{-1} in our treatment with the highest yield per unit N added (56 kg N ha^{-1}) (figure 5(a)).

Including all GHG sources and credits in the GWI analysis, each N treatment shows net climate change mitigation (i.e., negative net CO_2e), with maximum net mitigation capacities as high as -5.71 ± 0.22 Mg CO_2e ha^{-1} yr^{-1} in the treatment fertilized at 56 kg N ha^{-1} (figure 5(b)). However, at fertilization rates above 56 kg N ha^{-1} net mitigation decreased monotonically with each increment of added N to only -2.97 ± 0.18 Mg CO_2e ha^{-1} yr^{-1} for the 196 kg N ha^{-1} treatment (figure 5(b)).

Greater N_2O emissions in N fertilized compared to non-fertilized switchgrass has been noted in Nova Scotia Canada (Wile *et al* 2014), Nebraska USA (Schmer *et al* 2012), northern Michigan USA (Nikièma *et al* 2011), and southern Michigan and Wisconsin USA (Oates *et al* 2016). Two studies Nikièma *et al* (2011) and Wile *et al* (2014) included three fertilizer rates, and while N_2O responses were in some site years consistent with an exponential response, three rates are insufficient to statistically test for nonlinearity. We are not familiar with any N leaching studies that have tested the effects of fertilizer rates on nitrate loss in switchgrass or any other perennial biofuel crop.

That the mitigation potential of switchgrass fertilized at high N rates is only about half of its mitigation potential at yield-optimizing N rates points to a significant challenge for realizing the environmental potential of cellulosic biofuels. Knowledge of and careful management for crop N needs appear to be crucial. In many cases, such as for the maturing switchgrass crops in this study, fertilizer needs may be close to nil: some varieties of switchgrass are known to be unresponsive to fertilizer N (Christian *et al* 2002), presumably because of a high N use efficiency and/or the presence of other N acquisition mechanisms, possibly including biological N fixation.

4. Conclusions

Breeding for low N needs, and then fertilizing only as needed—if at all—to meet these needs will be an important strategy for meeting the full climate mitigation benefits of cellulosic biofuels. In the meantime, incentives to grow N-conserving crop varieties and to apply as little fertilizer N as necessary will be needed to meet the climate benefit claims of this emerging industry (Robertson *et al* 2008). Incentives to reduce N fertilizer use would have an additional advantage of reducing unnecessary N pollution of ground- and surface waters and lowering the cost of biofuel crop production.

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Supplementary data for "Nitrogen fertilization challenges the climate benefit of cellulosic biofuels"

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Contains:

Supplementary tables S1-S3

Supplementary references

Supplementary figures S1-S4

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Supplementary tables

Table S1. Model comparisons for the response of annual N_2O emissions to N fertilization rate. AIC = Akaike information criterion and BIC = Bayesian information criterion. In this comparison the best model has the lowest criteria; % AIC and % BIC represent the percentage increases for the linear models compared with the better exponential models that are indicated with "√".

Year	Model	R^2	AIC	% AIC	BIC	% BIC
2009	Linear	0.88	-3.02	52%	1.37	173%
	Exponential	0.90	-6.3	√	-1.87	√
2010	Linear	0.84	43.7	90%	48.1	76%
	Exponential	0.91	23.0	√	27.4	√
2011	Linear	0.86	54.0	121%	58.4	103%
	Exponential	0.94	24.4	√	28.7	√
All 3 yr	Linear	0.73	176	13.5%	184	12.9%
	Exponential	0.75	155	√	163	√

Table S2. Model comparisons for the response of annual N leaching to N fertilization rate. See Table S1 legend for further information.

Year	Model	R^2	AIC	% AIC	BIC	% BIC
2009	Linear	0.60	174	1%	178	1%
	Exponential	0.65	172	√	176	√
2010	Linear	0.73	182	4%	185	4%
	Exponential	0.80	175	√	178	√
2011	Linear	0.65	190	15%	194	14%
	Exponential	0.87	166	√	170	√
All 3 yr	Linear	0.64	547	4%	554	4%
	Exponential	0.74	525	√	532	√

Table S3. Estimation of CO_2-equivalent emissions associated with switchgrass for cellulosic biofuel crop production. GWI = Global warming impact.

GWI component[a]	Mg CO_2e ha^{-1} yr^{-1}	Data source
Seeds[b]	0.014	See footnote b
Planting	0.013	Gelfand *et al* 2013
N fertilizer production[c]	$4.5 \times X \times 0.001$	Robertson *et al* 2000
N fertilizer application	0.026	Gelfand *et al* 2013
Harvesting (baling)	0.019	Gelfand *et al* 2013
Direct N_2O emissions	Field data	This study
Indirect N_2O emissions[d]	Leaching N × 0.75%	This study and IPCC 2006 N_2O indirect emission factor (De Klein *et al* 2006*)*
CH_4 uptake	Field data	This study
SOC change	Field data	This study
Fossil fuel offset credits[e]	Field data and GREET	This study

[a] Phosphorus and potassium fertilizers were not applied in our study, nor were herbicides, insecticides or lime.

[b] Calculation for seed production (Mg CO_2e ha^{-1} yr^{-1}):
  Switchgrass seeds production energy, 43.8 MJ kg^{-1}, was based on the analysis of Schmer *et al.* (2008). In order to convert energy (MJ kg^{-1} yr^{-1}) to carbon emissions (Mg CO_2e ha^{-1}), we assumed that energy used in seed production consisted of a 50, 20, and 30% mix of fuel oil, natural gas, and electricity, respectively (Börjesson 1996). We also used energy conversion factors of 0.094 kg CO_2e MJ^{-1} (Farrell *et al* 2006), 0.056 kg CO_2e MJ^{-1} (Farrell *et al* 2006), and 0.21 kg CO_2e MJ^{-1} (EPA 2014) for the conversion of gasoline, natural gas and electricity to carbon emissions, respectively. As a result:
  Fuel: 43.8 MJ kg^{-1} × 50% × 0.094 kg CO_2e MJ^{-1} = 2.06 kg CO_2e kg^{-1}
  Natural Gas: 43.8 MJ kg^{-1} × 20% × 0.056 kg CO_2e MJ^{-1} = 0.49 kg CO_2e kg^{-1}
  Electricity: 43.8 MJ kg^{-1} × 30% × 0.21 kg CO_2e MJ^{-1} = 2.73 kg CO_2e kg^{-1}
  Total carbon emissions (kg CO_2e kg^{-1}) = Fuel + natural gas + electricity = 5.28 kg CO_2e kg^{-1}
  The seeding rate in our study is 7.84 kg ha^{-1}. Therefore,
  Total carbon emissions (Mg CO_2e ha^{-1}) = 5.28 kg CO_2e kg^{-1} × 7.84 kg ha^{-1}× 0.001 Mg kg^{-1} = 0.04 Mg CO_2e ha^{-1}
  Since it is a perennial grass, switchgrass does not need to be seeded annually. Therefore, we averaged this value over the study period (3 yr), i.e., 0.014 Mg CO_2e ha^{-1} yr^{-1}.

[c] Based on Robertson et al. (2000), 4.5 kg CO_2e released per kg of N produced and transported to field crops; X is the N fertilization rate (kg N ha^{-1} yr^{-1}); 0.001 is the conversion factor from kg to Mg.

[d] Leaching N data from the field experiment in this study.

[e] GREET (Huo *et al* 2009) was used to calculate fossil fuel offset credits for different yields from each N treatment, with all farming inputs set to 0 as noted in Methods. Offset credits include co-product credits, which for lignocellulosic biomass includes lignin, used to generate electricity.

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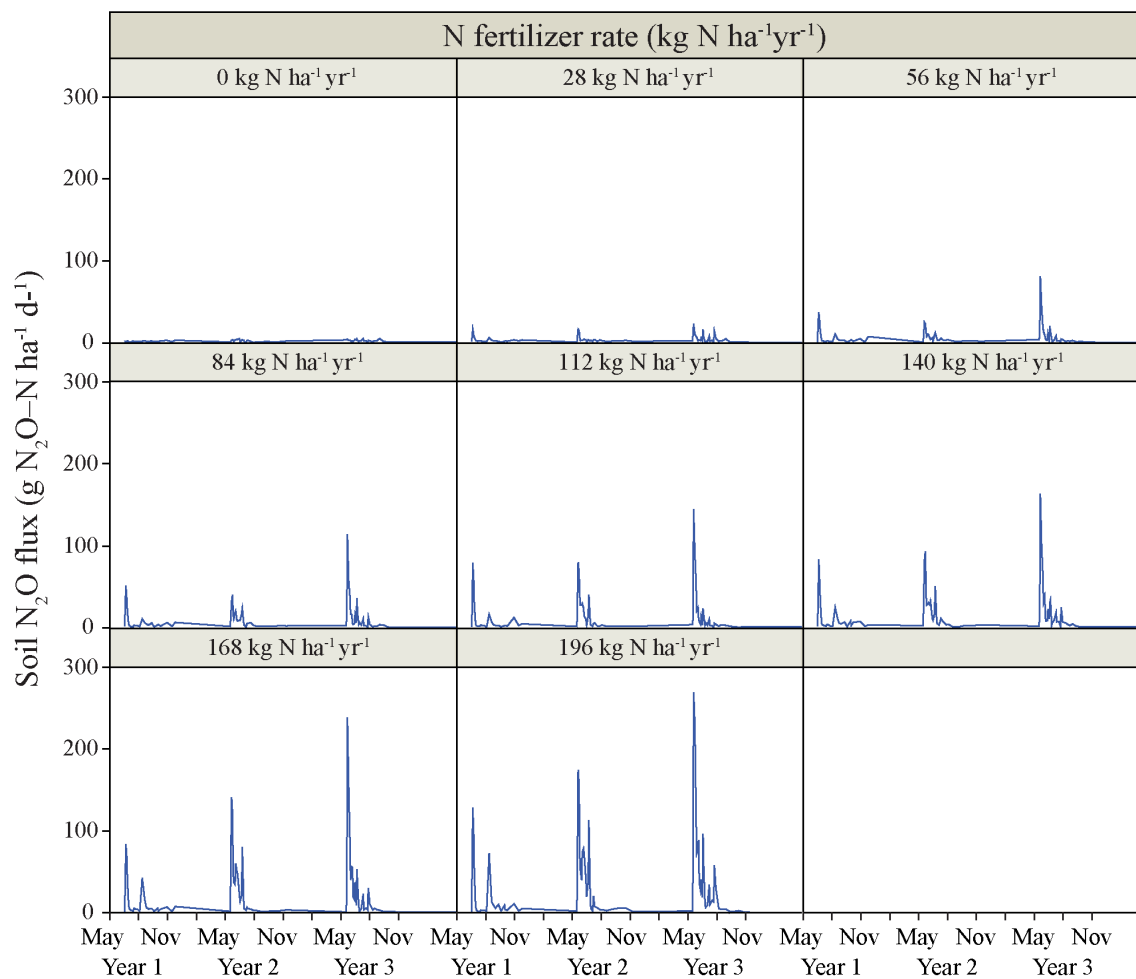
Supplementary figures

Figure S1. Mean daily N_2O emissions May to December for the first three harvest years (2009-2011) following establishment in 2008 across the eight fertilization treatments. Error bars omitted for clarity (n=4 replicate chambers per treatment).
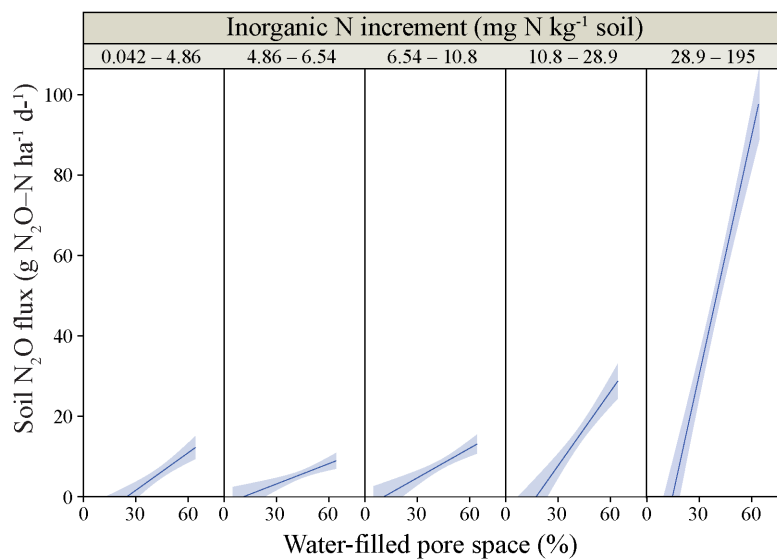
Figure S2. Relationship of daily N_2O emission to soil water content (water-filled pore space) at 0–25 cm depth across several levels of soil inorganic nitrogen (KCl-extractable NO_3^- + NH_4^+) during the first three harvest years (2009-2011). Bands show 95% confidence intervals around linear regression fits to the data (data points not shown); all fits are significant at α=0.05.
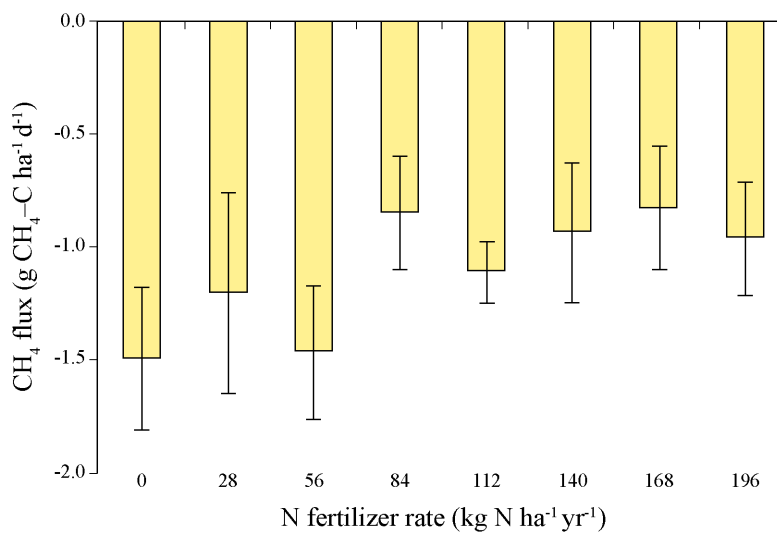

Figure S3. Average daily CH_4 uptake rates in each switchgrass N fertilization treatment for May to December for the first three harvest years (2009-2011). Error bars represent standard errors of the mean (n=4 replicate plots). There are no significant differences among treatments (α=0.05).
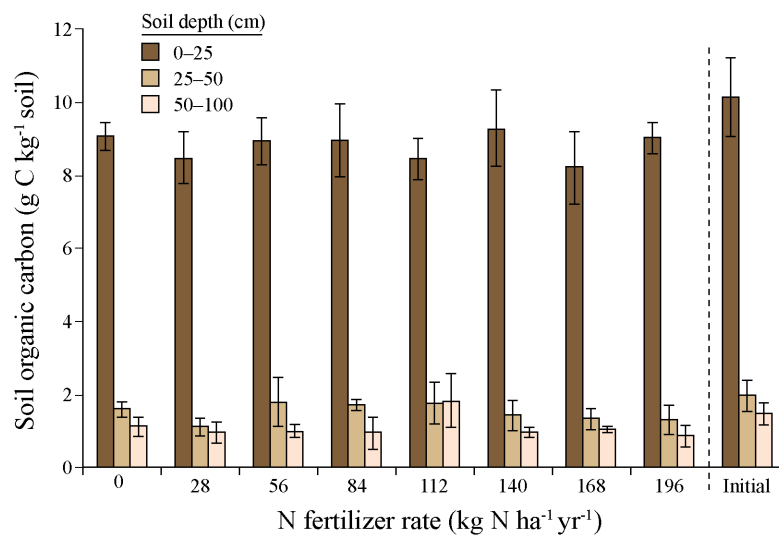
Figure S4. Soil carbon concentrations at depths of 0-25, 25-50, and 50-100 cm across the N fertilization gradient. Error bars represent standard errors based on n=4 replicate plots. Bars to the left of the dashed line represent samples taken in 2012 versus those taken at the outset of the experiment in 2008 (to the right side of dashed line). There are no significant differences among treatments (α=0.05).