IMPACTS OF BIOFUEL CROPS ON GREENHOUSE GAS EMISSIONS FROM AGRICULTURAL ECOSYSTEMS

By

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ABSTRACT

IMPACTS OF BIOFUEL CROPS ON GREENHOUSE GAS EMISSIONS FROM AGRICULTURAL ECOSYSTEMS

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Biofuels are intended to improve future energy and climate security. However, greenhouse gases (GHGs) can be produced from soil during biofuel cultivation and thus offset the biofuel climate mitigation effect. In this dissertation, I examine three major knowledge gaps with respect to GHG fluxes in biofuel landscapes: fertilization, tillage practices and climate change feedbacks.

In Chapter 2, I measured nitrous oxide (N_2O), methane (CH_4), and nitrate (NO_3^-) fluxes along a switchgrass (*Panicum virgatum*) nitrogen (N) input gradient for three years to reveal an exponential increase in annual N_2O emissions that each year post-establishment became stronger ($R^2 > 0.9$, $P < 0.001$) even as the biomass N response diminished. Nitrate leaching also increased exponentially across the N gradient. Net greenhouse gas benefits were consequently curtailed up to ~50% at N fertilizer rates above the crop requirement. Fertilization above the crop requirement substantially challenges the climate mitigation benefit of switchgrass and presumably other cellulosic biofuel crops.

In Chapters 3 and 4, I measured GHG balances in portions of three 9-21 ha USDA Conservation Reserve Program (CRP) grassland fields in Michigan converted to conventional tillage (CT) or NT soybean production, and compared these fields to a fourth field left in grassland for reference in 2009. For the initial 201-day conversion period, average daily N_2O emissions were significantly different in the order: CT > NT > reference. Similarly, soil CO_2 emissions in CT were 1.2 times those in NT and 3.1 times those in the unconverted CRP reference field. All treatments were minor sinks for CH_4 with no significant differences among treatments. Including foregone mitigation, I conclude that NT management can reduce GHG costs by ~60% compared to CT during initial CRP conversion. In chapter 4, I studied Conservation Reserve Program (CRP) grasslands converted to corn, prairie, and switchgrass following an initial soybean year with and without conventional tillage (CT) in 2010 and 2011. Over two years I found that after conversion to CT the soils emitted twice as much N_2O and 20% more CO_2 compared to no-till (NT) conversion. Net ecosystem exchange of CO_2 was strongly positive for all CT treatments and negligible (prairie) or negative (corn and switchgrass) for NT treatments. CH_4 oxidation was unaffected by treatment. Overall, my results show that, by reducing N_2O and CO_2 emissions, NT practices can reduce the global warming impact of grassland conversion compared to CT.

In chapter 5, I investigated the winter response of N_2O emissions to changes in snowdepth in a corn-soybean-wheat cropping system for three winters using an automated chamber system. In treatments where snow was removed, N_2O emissions were 69% higher than in ambient controls and 95% higher than in double-snow treatments ($P<0.001$). The proportion of annual N_2O emissions represented by wintertime fluxes consequently increased by ~46%. Higher fluxes coincided with a greater number of freeze-thaw cycles that destroyed macroaggregates and increased inorganic nitrogen availability. Future winters with less snow can thus be expected to accelerate N_2O emissions from agriculture, creating the potential for a positive climate change feedback.

Dedicated to my parents and grandparents,
who have been always supportive and encouraging…

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CHAPTER 1: DISSERTATION INTRODUCTION

OVERVIEW

IPCC (2007a) reported that global mean surface temperatures have increased 0.74 ˚C from 1906 to 2005 and predicted future temperature rises of 1.1-6.4 ˚C by 2100. The increase is largely attributed to the rise in the atmospheric greenhouse gases (GHGs) carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O). Concentrations of CO_2 in the atmosphere have increased from 280 ppm in 1750 to 391 ppm in 2011, CH_4 concentrations have increased from 715 ppb to 1803 ppb, and N_2O concentrations have increased from 270 ppb to 324 ppb (IPCC, 2013).

Carbon dioxide is the most important GHG. About two thirds of anthropogenic CO_2 emissions are from fossil fuel burning and about one third is from land use change (IPCC, 2007a). Agricultural soil CO_2 emissions are usually included in the land use, land use change and forestry sector (Singh, 2009). Soil CO_2 emissions are integrated processes representing autotrophic root respiration and heterotrophic microbial respiration during litter and soil organic matter decomposition (Braig and Tupek, 2010). Although CO_2 exchange between the atmosphere and agricultural lands is approximately balanced (with CO_2 emissions $\sim 0.04 \times 10^{15}$ g CO_2/yr only) (IPCC, 2007b), agriculturally-related practices such as deforestation and soil tillage can release stored carbon back to the atmosphere as CO_2. Soil contains 1500 Pg (1 Pg=1Gt=10^{15} g) of organic carbon, roughly three times the amount of carbon in vegetation and twice the amount in the atmosphere (Singh, 2009).

Atmospheric CH_4 is emitted from anthropogenic and natural sources. Anthropogenic sources include enteric fermentation by cattle and other ruminants, rice cultivation, biomass burning, coal mining and wastewater treatment and landfills. Natural sources of methane include wetlands and termites. The major CH_4 sinks are oxidation by OH in the troposphere, biological CH_4 oxidation in unsaturated soils, and loss to the stratosphere (IPCC, 2007a). About 5-20% of total global CH_4 emissions are from saturated soils but CH_4 oxidation in well-aerated soils is an important sink (5%, globally) for atmospheric CH_4. Generally, the atmospheric CH_4 sink is of nearly the same magnitude (581 Tg CH_4 yr^{-1}) as the annual increase (582 Tg CH_4 yr^{-1}) (IPCC, 2007a).

Nitrous oxide is produced by anthropogenic sources that include cultivated soils, animal waste, biomass burning and industry and by natural sources that include soils supporting natural vegetation as well as ocean water. Of the anthropogenic sources of N_2O emissions, agricultural soil emissions account for about 60% of the total. In soil, N_2O is mainly produced by two microbial processes: denitrification and nitrification. Denitrification occurs under anaerobic conditions when soil NO_3^- is reduced to NO, N_2O and finally to N_2 by anaerobic denitrifiers. Nitrification occurs under aerobic conditions when soil NH_4^+ is oxidized to NO_2^- (nitrite) and then to NO_3^-. Intermediary compounds formed during nitrification can decompose to gaseous N_2O and as well, some nitrifiers can denitrify NO_2^- to N_2O (Robertson and Groffman, 2007).

Generally, agriculture contributes 10-12% of total global GHG emissions (IPCC, 2007a). In view of the importance of agricultural soils in global climate change, a wide range of methods have been proposed for mitigating GHG emissions from agriculture. These include improving crop land management (e.g., nutrient use, tillage, residue and water management), improving water and rice management, and reducing land use change (e.g., conversion of cropland to grassland) (IPCC, 2007b). Production of biofuels from agricultural feedstocks (e.g., crop residues, dung, and purposefully grown energy crops) is another option for reducing GHG emissions by substituting in energy sources contemporary biomass carbon for fossil fuels. Biofuels are carbon-based energy sources in which plants capture solar energy and then their biomass is burned for fuel either directly or following transformation to liquid form (Parrish et al., 2005). The production and use of biofuels has increased rapidly, and the International Energy Agency (IEA) predicts that the consumption of biofuels will rise from 1% today to 4% - 7% of road transport fuels in 2030 (IEA, 2006). In the U.S, the Energy Independence and Security Act of 2007 (EISA) mandates an increase in ethanol biofuel production to 36 billion gallons by 2022: 15 billion gallons from corn-based ethanol and 21 billion from cellulosic or other non-corn-based sources. Not only can biofuels alleviate a reliance on fossil fuel for energy, but also they can help to mitigate GHG emissions and thereby slow global warming: when biofuels are burned, they release only CO_2 recently captured by photosynthesis during crop growth, so the CO_2 added to the atmosphere was recently taken up by photosynthesis of the crop. In contrast, burning fossil fuel releases the carbon sequestered by dead plants and animals millions of years ago. Therefore, fossil fuels are considered “carbon positive” but biofuels are called “carbon neutral” (Abbasi et al., 2010) or “GHG neutral”.

However, biofuels are not 100% GHG neutral. Biofuel systems comprise three general sub-systems: 1) a crop cultivation system to provide feedstock, 2) a biorefinery to convert feedstock to utilizable fuels, and 3) a co-products subsystem to utilize by-products (Liska et al., 2008). Biofuel crop production requires fossil fuel for cultivation, planting and harvest, and requires chemical inputs derived from fossil fuels, such as fertilizer and pesticide. After harvest, transportation and conversion to liquid fuels also requires energy. The extent to which fossil fuels are required for these tasks will reduce the degree to which use of a biofuel offsets the avoided fossil fuel CO_2 emissions.

Additionally, GHGs can be produced from soil during biofuel cultivation. CO_2 is released when soil carbon is oxidized following cultivation, CH_4 uptake (oxidation) is reduced when unmanaged lands are returned to cultivation, and N_2O is released when soils are cultivated or fertilized (Ruan and Robertson, 2013). Particularly a problem is N_2O, which has a global warming potential ~300 times that of CO_2 and ~15 times that of CH_4 (IPCC, 2007a). Energy crops need nitrogen (N) fertilizer for their growth, which enhance soil N_2O emissions due to more available NH_4^+ and NO_3^- for nitrification and denitrification, respectively. Crutzen et al. (2008) suggested that there may be no GHG mitigation using biofuels when large fertilization-induced soil N_2O emissions are properly considered in the full life cycle assessment of corn ethanol production. Crutzen et al. (2008) also showed that biofuel crops with less N demand have more favorable climate impacts.

The United Nations Framework Convention on Climate Change (2006) recommends a constant emission factor of 1% to calculate direct N_2O emission for all agricultural N inputs, which is based on the assumption that 1% of fertilizer N is transformed to N_2O during cultivation. This method does not take environmental factors and crop differences into account. For example, there are few studies about N_2O emissions in dedicated perennial cellulosic biofuel crops (Nikièma et al., 2011). Moreover, there is a debate about the relationship between N fertilizer application and N_2O emissions. Some studies have found a linear relationship between N inputs and soil N_2O emissions (Helgason et al, 2005; Liu et al., 2005; Mosier et al., 2006; Halvorson et al., 2008), but few studies (Gao et al., 2013) have used the three or more fertilization rates necessary to detect a significant nonlinear response. Studies with more fertilization rates have reported a non-linear relationship between N input and N_2O emissions, possibly due to greater N availability for microbes after plant N demand is satisfied (McSwiney and Robertson, 2005; Ma et al., 2010; Hoben et al., 2011). If so, the contribution of soil N_2O to the whole GHG balance will have been underestimated because the linear relationship will estimate lower N_2O emissions than will the non-linear relationship. Therefore, the contribution of soil N_2O to the whole GHG balance during biofuel crop production might be underestimated by the use of the linear equation.

Additionally, higher prices for corn (*Zea mays* L.) and expanded biofuel production are expected to induce farmers to return their CRP land to grain production (Du et al., 2008; Secchi et al., 2009). Land use change is known to release large quantities of GHGs, both N_2O and CO_2, and to diminish CH_4 oxidation (Robertson et al. 2000; Tilman et al., 2006; Searchinger et al., 2008). CRP conversion to no-till cropland results in large GHG fluxes including a 4-fold increase in N_2O (Gelfand et al., 2011). CRP conversion to tilled cropland would be expected to release even more GHG than conversion to no-till cropland but this is still unknown. Failing to consider all GHG emissions during conversion will overestimate the reduction of GHG emissions by biofuels.

Finally, when I think about climate change caused by increasing GHG emissions, I also need to consider the impacts of feedbacks of climate change on GHG emissions. Snow cover provides an example. IPCC (2007a) reported a decrease in snow cover from 1966 to 2005 in the northern hemisphere. Snow insulates the soil surface and thereby reduces freeze-thaw events that can destroy soil aggregation and release GHGs (Sharma et al., 2006). Snow also keeps direct insolation from warming the surface layers where much of the GHG production occurs (IPCC, 2007b). Climate change thus has the potential to increase freeze-thaw cycles during winter due to warm and more variable temperatures and a thinner snowpack. Some studies have reported that more freeze-thaw cycles can cause more N_2O loss (Koponen et al., 2004; Groffman et al., 2006;

Maljanen et al., 2010). Most of these studies, however, used controlled laboratory conditions (van Bochove et al., 2000; Koponen et al., 2004; Zhu et al., 2009) or sparsely sampled static chambers in situ (Dorsch et al., 2004; Groffman et al., 2006; Maljanen et al., 2010) to assess the impacts of freeze and thaw cycles caused by reduced snow cover on N_2O emissions. However, it can be difficult to extrapolate laboratory results to the field. Moreover, N_2O emissions caused by freeze and thaw cycles are usually strong but short-lived, and it can be difficult to capture all emissions after melting. Additionally, melting ground is vulnerable to compaction by trampling for collecting samples by hand.

OBJECTIVES

There are, then, at least three major knowledge gaps with respect to GHG fluxes in biofuel crop production systems: 1) fertilization; 2) tillage practices; and 3) climate change feedback. Therefore, my objectives in this dissertation are 1) to evaluate the GHG response of a cellulosic biomass crop to N fertilization (Chapter 2); 2) to evaluate the GHG cost of tilling a long-term CRP soil and to quantify the degree to which no-till can prevent GHG emissions following conversion from CRP lands (Chapters 3 and 4); and 3) to evaluate whether changes in snowfall can increase N_2O emissions from biofuel crop soils using a near-continuous automated chamber flux system (Chapter 5).

CHAPTER 2: NITROGEN FERTILIZATION CHALLENGES THE CLIMATE BENEFITS OF CELLULOSIC BIOFUELS

ABSTRACT

Cellulosic biofuels are intended to improve future energy and climate security. Nitrogen (N) fertilization can stimulate biomass production but increases reactive N loss. We measured nitrous oxide (N_2O), methane (CH_4), and nitrate (NO_3^-) fluxes along a switchgrass (*Panicum virgatum*) N input gradient for three years to reveal an exponential increase in annual N_2O emissions that each year post-establishment became stronger ($R^2>0.9$, $P<0.001$) even as the biomass N response diminished. Nitrate leaching also increased exponentially across the N gradient. Net greenhouse gas benefits were consequently curtailed up to ~50% at N fertilizer rates above the crop requirement. Fertilization above the crop requirement substantially challenges the climate mitigation benefit of switchgrass and presumably other cellulosic biofuel crops.

INTRODUCTION

The global production and use of biofuels has increased dramatically in response to calls for greater energy security and climate change mitigation. In the U.S, legislation mandates ~136 billion liters of ethanol biofuel by 2022 with a growing fraction from cellulosic sources (EISA, 2007). Cellulosic biofuels offer the potential for greater environmental benefits compared to grain based biofuels. Switchgrass, a perennial grass native to North America, is among the most promising cellulosic biofuel crops (Vogel et al., 2002) due to its ability to grow on marginal and erosive lands, sequester soil carbon (Liska et al., 2008), reduce N leaching (Smith et al., 2013) and be grown with relatively little fossil fuel input (McLaughlin et al., 2005).

Switchgrass is often considered an inherently N-thrifty plant, especially when managed for biomass production (Parrish and Fike, 2005). Nitrogen fertilization rates vary widely with most studies reporting maximum small-plot yields at rates between 56 and 202 kg N ha^{-1} (Vogel et al., 2002; Mulkey et al., 2006; Mooney et al., 2009; Nikiema et al., 2011). In a recent on-farm experiment (Schmer et al. 2008), farmers fertilized switchgrass at rates from 0 to 212 kg N ha^{-1} with a mean application rate of 74 kg N ha^{-1}.

Although N fertilizer can increase biomass production and thus create additional ethanol or other liquid transportation fuel, it also increases the greenhouse gas (GHG) contributions of its production substantially: not only through the production, transportation, and distribution of the fertilizer itself, but also through fertilizer-induced microbial emissions of N_2O, a GHG with a global warming potential ~300 times that of CO_2 (IPCC, 2007a) and the major driver of stratospheric ozone depletion (Portmann et al., 2012). Moreover, fertilizer lost to the environment as nitrate leads to indirect emissions of N_2O elsewhere in the landscape as well as contributes to other environmental problems (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 et al., 1998; Le Mer et al., 2001).

Fertilization thus has the potential to substantially attenuate the climate change mitigation potential of cellulosic biofuels. Here I present results from a 3-yr study of direct and indirect N_2O emissions, CH_4 uptake, NO_3^- leaching, soil organic carbon accumulation, and biomass production in recently established switchgrass under different N fertilizer rates to evaluate the impact of N fertilization on the net GHG balance of switchgrass grown as a cellulosic biofuel feedstock.

MATERIALS AND METHODS

Site description and experimental design

My study site was located at the Great Lakes Bioenergy Research Center (GLBRC) intensive site of the Kellogg Biological Station Long-term Ecological Research Site in southwestern Michigan (42˚23' N, 85 ˚22' W, elevation 284 m). Precipitation averages 1,027 mm y^{-1} with an average snowfall of ~1.4 m. Mean annual temperature is 9.9 °C ranging from a monthly mean of -4.2 °C in January to 22.8 °C in July (Robertson and Hamilton 2014). Soils are mesic Typic Hapludalfs of Kalamazoo loam developed on glacial outwash. Prior to establishing the experiments, soil pH (0-25 cm depth) was 7.47± 0.04 (mean ± standard error, n=12), bulk density was 1.24±0.04 g cm^{-3}, total N was 1.25±0.09 g kg^{-1} soil, and soil organic carbon 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 July 11, 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}) consisted of switchgrass plots (4.5 × 6 m) arranged in a randomized complete block design with four replicate blocks. Nitrogen fertilizer was applied only once per year between 2009 to 2011: granular 46% urea was broadcasted on June 17, 2009, and liquid 28% Urea Ammonium Nitrate (UAN) was sprayed on May 10, 2010, and May 16, 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 Systems (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 ~10 cm.

Nitrous oxide and CH_4 sampling

Nitrous oxide and CH_4 fluxes were measured using a static chamber – gas chromatography approach as described by Ruan and Robertson (2013) from May to December in 2009-2011. I measured fluxes 2 to 3 times per week during the growing season to capture the temporal dynamics of N_2O and CH_4 fluxes 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 ~5cm for the duration of the study. Vegetation inside 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-minute 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, and 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.

Soil water-filled pore space (WFPS %), inorganic nitrogen and nitrate leaching

At each gas sampling event I measured soil temperature, gravimetric water content, and inorganic N [ammonium (NH_4^+) and nitrate (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 hours until constant mass. Soil bulk density (BD) was measured three times at each growing season using a fixed-volume core (123 cm^3) for each treatment plot. Water-filled pore space (WFPS %) was calculated as

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

where soil porosity = 1- [BD (g cm^{-3}) / particle density (g cm^{-3})]. Soil particle density was assumed to be 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 almost no 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-micrometer nominal pore size; Pall A/E) water samples were analyzed for NO_3^- using a Dionex 600 ion chromatograph. Previous work at this site has shown that NO_3^- dominates N leaching with relatively little leaching of NH_4^+ or dissolved organic N. Nitrate export from the root zone was modeled at a daily time step using the Systems Approach for Land Use Sustainability (SALUS) model (Basso et al. 2006, Ritchie and Basso 2008), which accounts for management practices, water balance, soil organic matter change, N and phosphorus dynamics, heat balance, and plant growth and development. The soil water balance module is based on CERES models (Ritchie 1998) with revision for infiltration, soil water export (Suleiman and Ritchie 2004), evaporation (Suleiman and Ritchie 2003), and runoff.

Soil organic carbon (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 bulk density. 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).

Net greenhouse gas balance

To estimate the global warming impact of greenhouse gas fluxes, I 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)(IPCC, 2007a).

I assumed that all CO_2 taken up from the atmosphere via 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 vs. 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 (Greenhouse Gases, Regulated Emissions, and Energy use in Transportation) model with all farming input equal to 0. 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., 2006; Gelfand et al., 2011) and ethanol energy content (21.1 MJ L^{-1}; low heating value) (Gelfand et al., 2011; Gelfand et al., 2013). Finally, 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.

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 α=0.05 level. The relationships between daily N_2O emissions and environmental factors such as soil temperature, soil moisture, and soil total N were assessed by multiple linear regressions (stepwise) using PROC REG. To determine the relationship between annual N_2O fluxes or leached N and N fertilization rate I 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.

RESULTS AND DISCUSSION

Switchgrass (variety Cave-in-Rock) was planted in tilled plots in 2008 and N fertilizer was applied annually at 8 rates (0-196 kg N ha^{-1}) in early summer during 2009-2011. Switchgrass yields were responsive to N fertilizer in 2009, but were less responsive in 2010 and 2011 (Figure 2.1) 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, 72 kg N ha^{-1} in 2010, and 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).

I measured N_2O and CH_4 fluxes using static chambers (Ruan and Robertson, 2013) 2-3 times per week through the growing season and bi-weekly at other times (see Supplementary Information for details). I observed an increase in annual N_2O emissions with increasing N fertilization rates in each year (Figure 2.2). From 2009 to 2011, mean daily N_2O emissions ranged from 1.28±0.14 to 25.8±1.9 g N ha^{-1} d^{-1} for the low (0 kg N ha^{-1}) and high (196 kg N ha^{-1}) fertilization treatments, respectively (Figure 2.3). 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 (mg N kg^{-1}) and % soil water-filled pore space (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 fluxes were highly dependent on WFPS (Figure 2.4).

The Akaike information criterion (AIC) and the Bayesian information criterion (BIC)

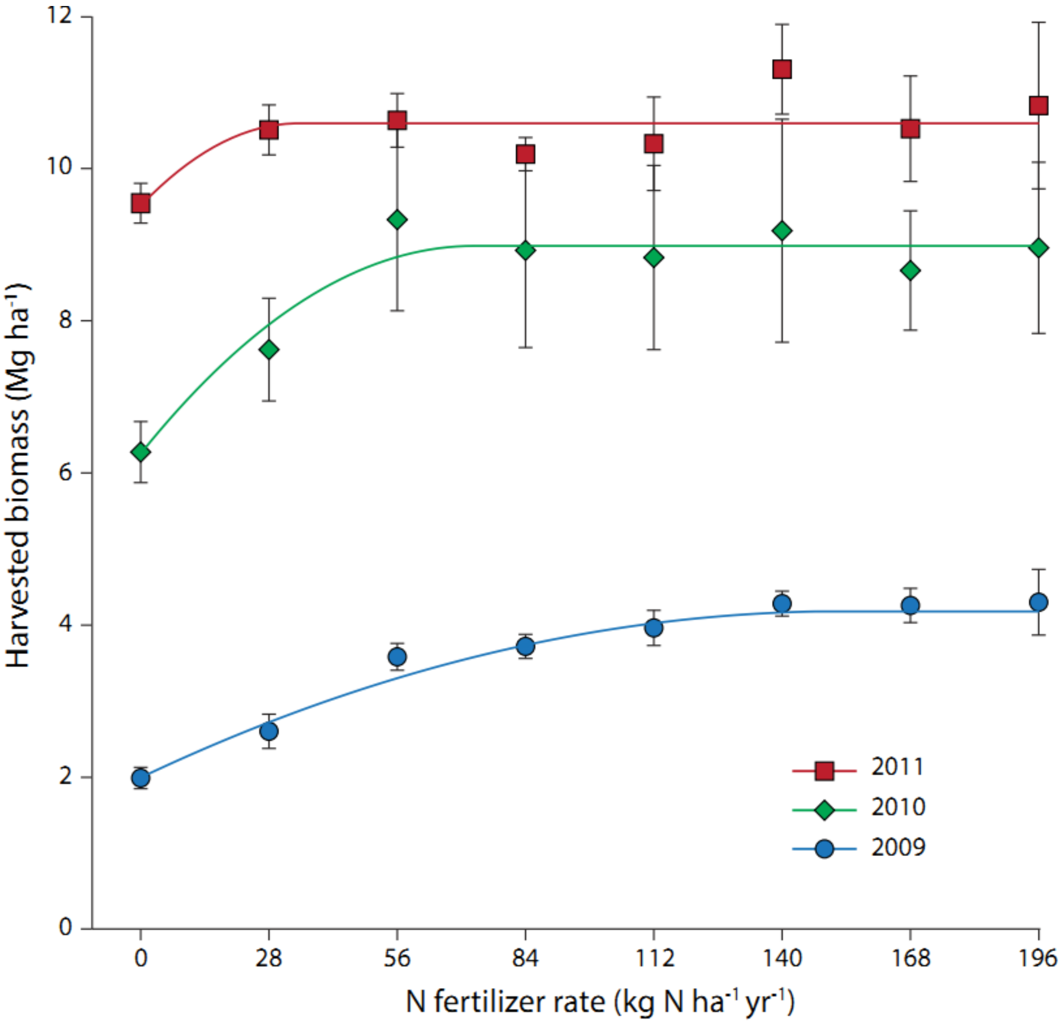

Figure 2.1. Switchgrass yields in response to N fertilization for 2009-2011; stands were established in 2008. Error bars represent standard errors of the means (n=4 replicate plots). For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.

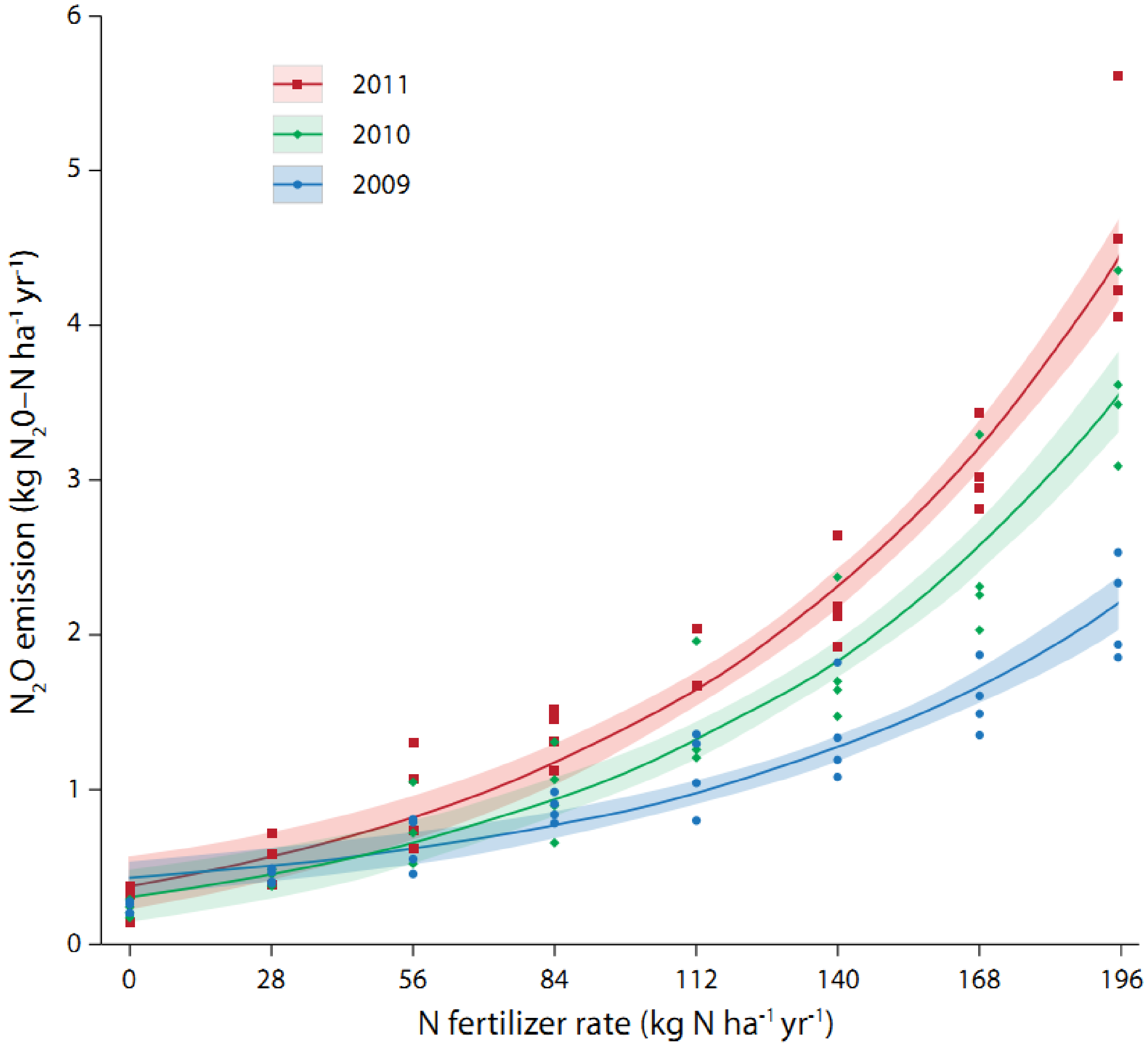

Figure 2.2. Exponentially increasing annual N_2O emission in response to increasing N fertilization rates for 2009-2011 ($P<0.001$, bands represent 95% confidence intervals).

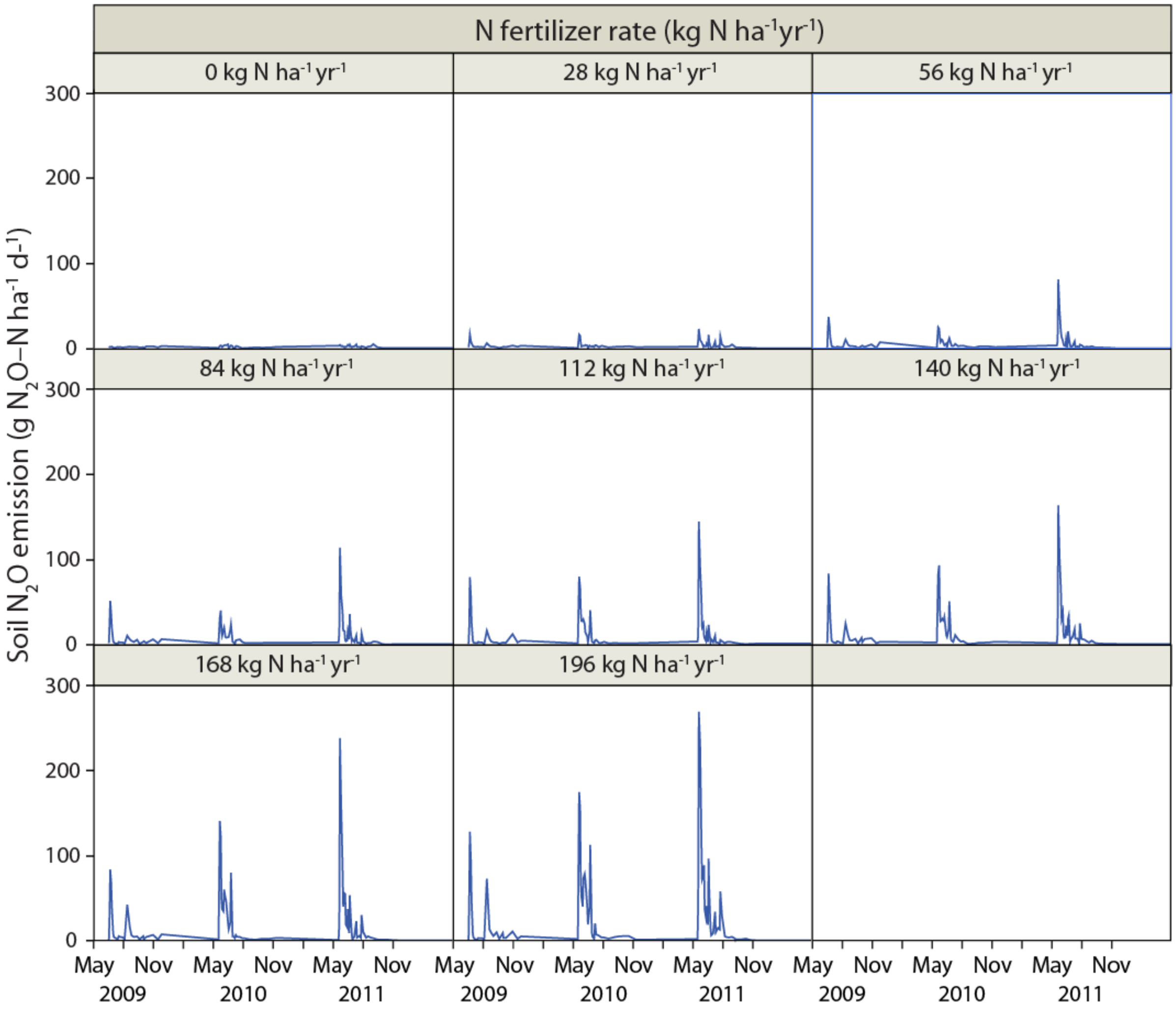

Figure 2.3. Mean daily N_2O emissions May to December during 2009 – 2011 across the eight fertilization treatments. Error bars omitted for clarity (n=4 replicate chambers per treatment).

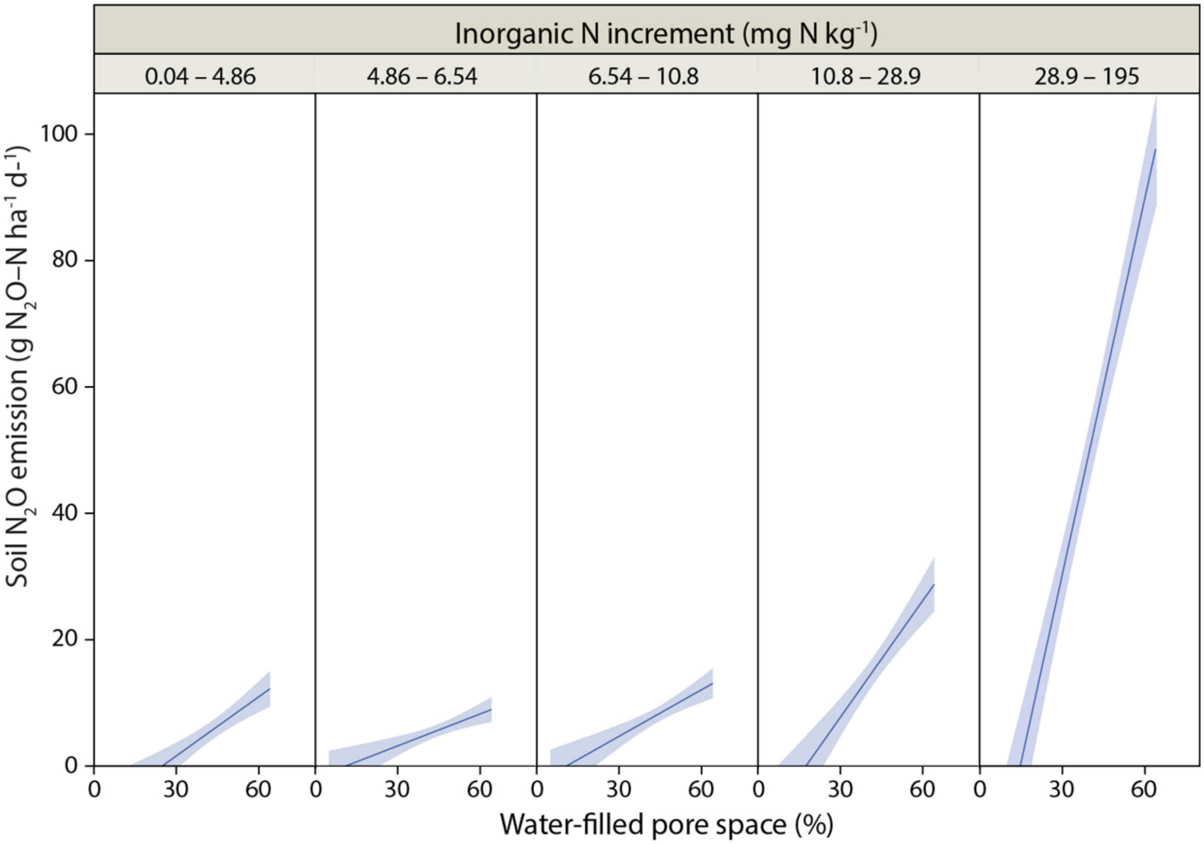

Figure 2.4. Relationship of daily N_2O emission to soil water content (water-filled pore space) at 0–25 cm depth across several levels of soil inorganic nitrogen (KCl-extractable NO_3^- + NH_4^+). Bands show 95% confidence intervals around linear regression fits to the data (data points not shown); all fits are significant at α=0.05.

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 A.1). Additionally, R^2 values for the exponential model (R^2=0.90-0.94) were consistently higher than for the linear model (R^2=0.84-0.88) (Table A.1).

Soil water was collected from lysimeters at 1-m depth in each plot to measure nitrate concentrations at biweekly intervals during the growing the season and at 2-3 month intervals in winter. Water drainage (percolation out of the root zone) was modeled using the Systems Approach for Land Use Sustainability (SALUS) model, which accounts for infiltration inputs, soil water holding capacity, and evapotranspiration losses of soil water (Basso et al., 2006). Modeled drainage was 275, 399 and 515 mm yr^{-1} in 2009, 2010 and 2011, respectively, representing 37, 39 and 46% of annual precipitation. Annual nitrate leaching rates ranged from 2.65±1.29 kg N ha^{-1} yr^{-1} for unfertilized switchgrass to 56.0±2.65 kg N ha^{-1} yr^{-1} for switchgrass fertilized at 196 kg N ha^{-1}, and also increased exponentially (Table A.2) in response to increasing N inputs with no significant difference in the increase among years (Figure 2.5).

The exponential increases in N_2O emissions and nitrate leaching are likely due to surplus soil N at levels above which plant N demands are met, resulting in more available NO_3^- for the microbial 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 (IPCC, 2006) varies with N fertilizer rate instead of being constant at 1% across all rates, as currently assumed, in agreement with other recent studies for annual crops (McSwiney and Robertson, 2005; Hoben et al., 2011). In this study the emission factor increased from 0.6 to 2.1% across the range of added N (Figure 2.6). Using a constant emission factor would have

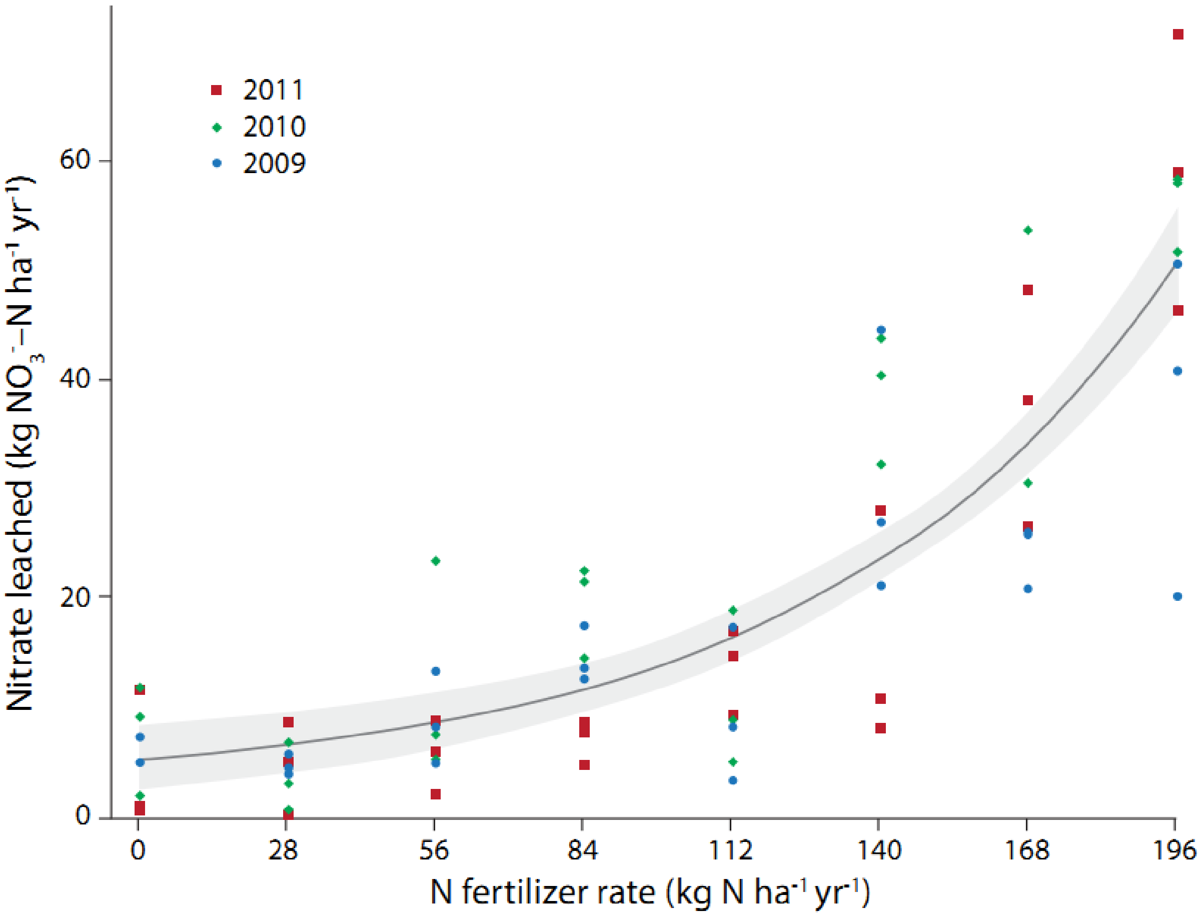

Figure 2.5. Exponentially increasing annual nitrate leaching in response to increasing N fertilization rates for 2009-2011 (R^2=0.74, $P<0.0001$, band represents 95% confidence interval).

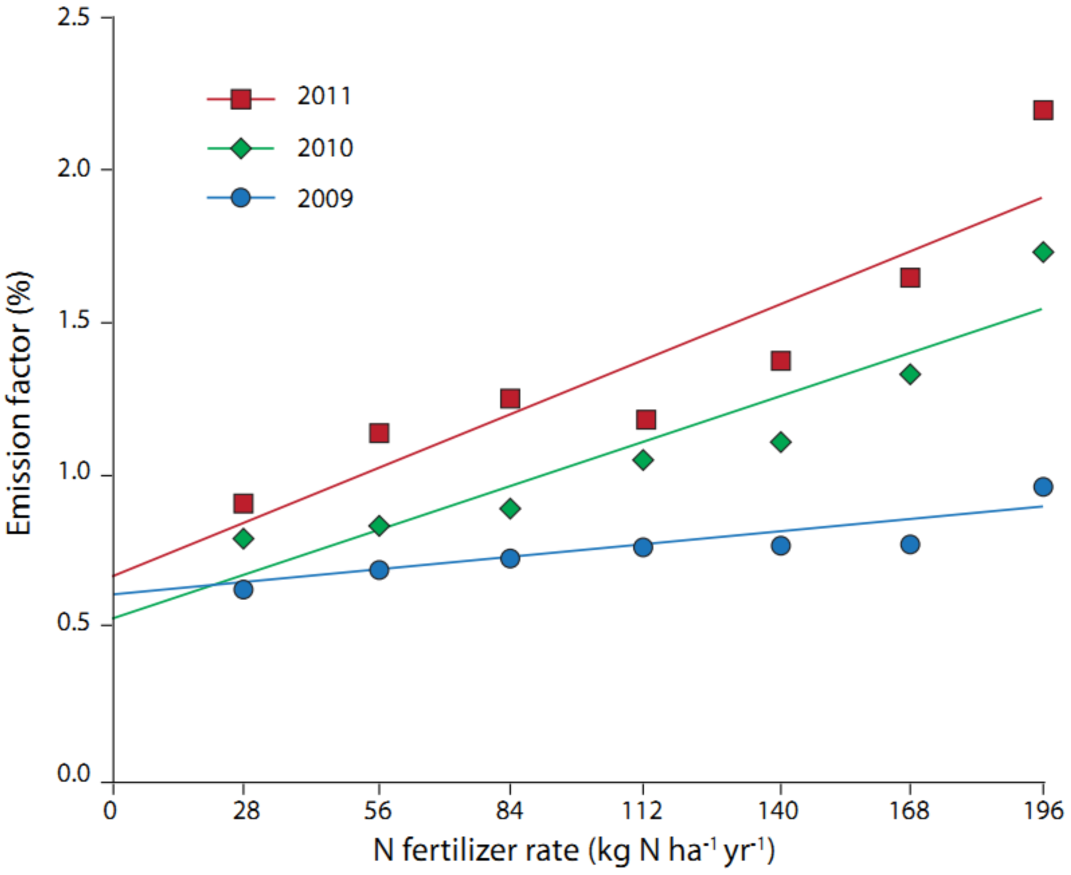

Figure 2.6. Relationships between soil N_2O emission factors (% of N fertilizer input that was ultimately emitted as N_2O) and N fertilization rates for 2009- 2011, including linear regression fits.

underestimated 3-year N_2O fluxes by 30% at lower levels of N fertilizer 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 8 fertilizer levels (Figure 2.7). There were no significant 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. I found no significant soil organic carbon (SOC) accumulation in any of my N treatments over the 3 year study period (Figure 2.8), although spatial variability makes it difficult to observe SOC change in fewer than 10 years at my site (Syswerda et al., 2011). Additionally, SOC loss upon conversion to a biofuel cropping system (e.g. Gelfand et al. 2011) can delay the appearance of significant net SOC gain.

I combined my 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; Gelfand et al., 2013) to estimate the overall GHG balances in units of CO_2 equivalents (CO_2e) for each of my N treatments (Table A.3). Measurements of NO_3^- loss allow us to include a major portion of indirect N_2O production, missing from most empirical GHG assessments. Estimated indirect N_2O emissions from the loss of leached NO_3^- ranged from 22.1±7.83 for unfertilized switchgrass to 157±12.3 kg CO_2e ha^{-1} yr^{-1} for switchgrass fertilized at 196 kg N ha^{-1}. I additionally used the Greenhouse Gases, Regulated Emissions, and Energy use in Transportation (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 (see methods in Supplementary Information) (Gelfand et al., 2011; Gelfand et al., 2013). Three-year average fossil fuel offsets for my eight N treatments ranged from 4.84 ±0.12 Mg CO_2e ha^{-1} yr^{-1} in my unfertilized treatment to 6.42 ±0.38 Mg CO_2e ha^{-1} yr^{-1} in my treatment with the highest yield per

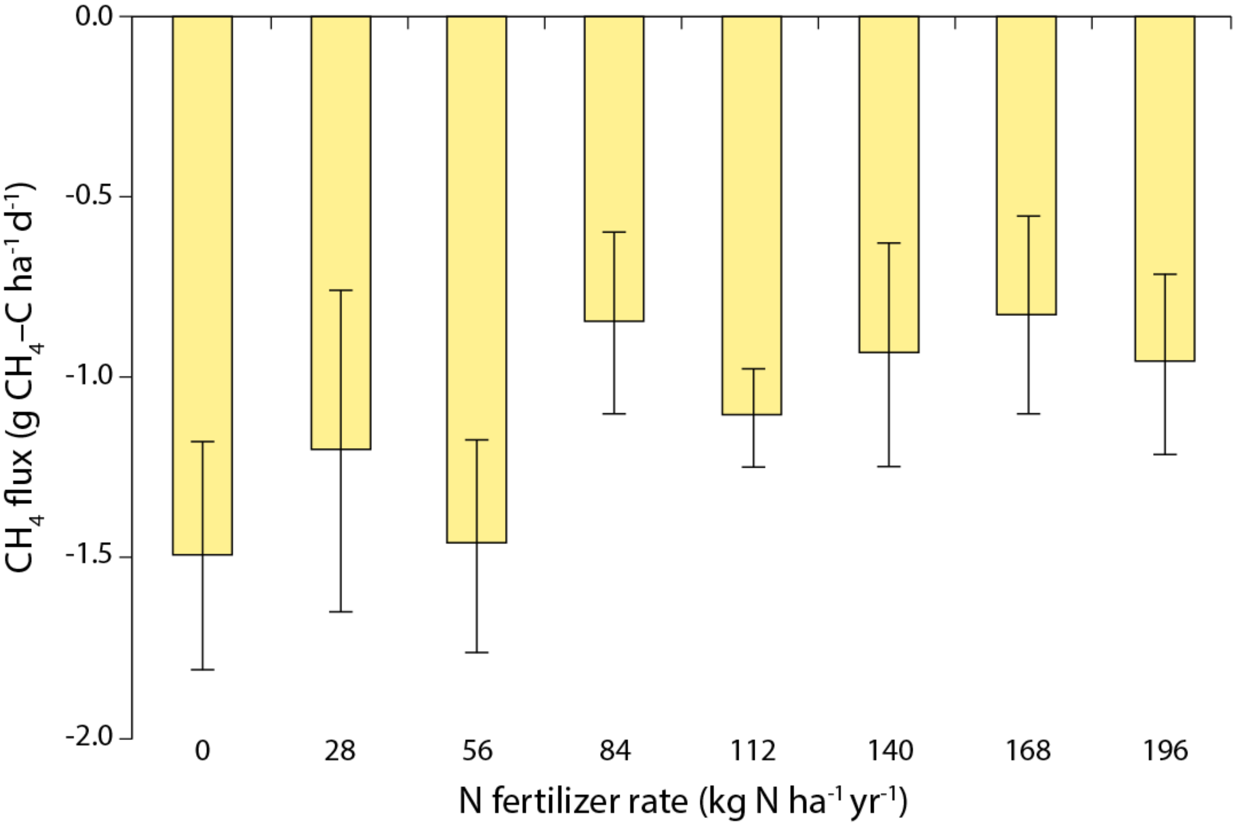

Figure 2.7. Average daily CH_4 uptake rates in each switchgrass N fertilization treatment for May to December in 2009-2011. Error bars represent standard errors of the mean (n=4 replicate plots). There are no significant differences among treatments (α=0.05).

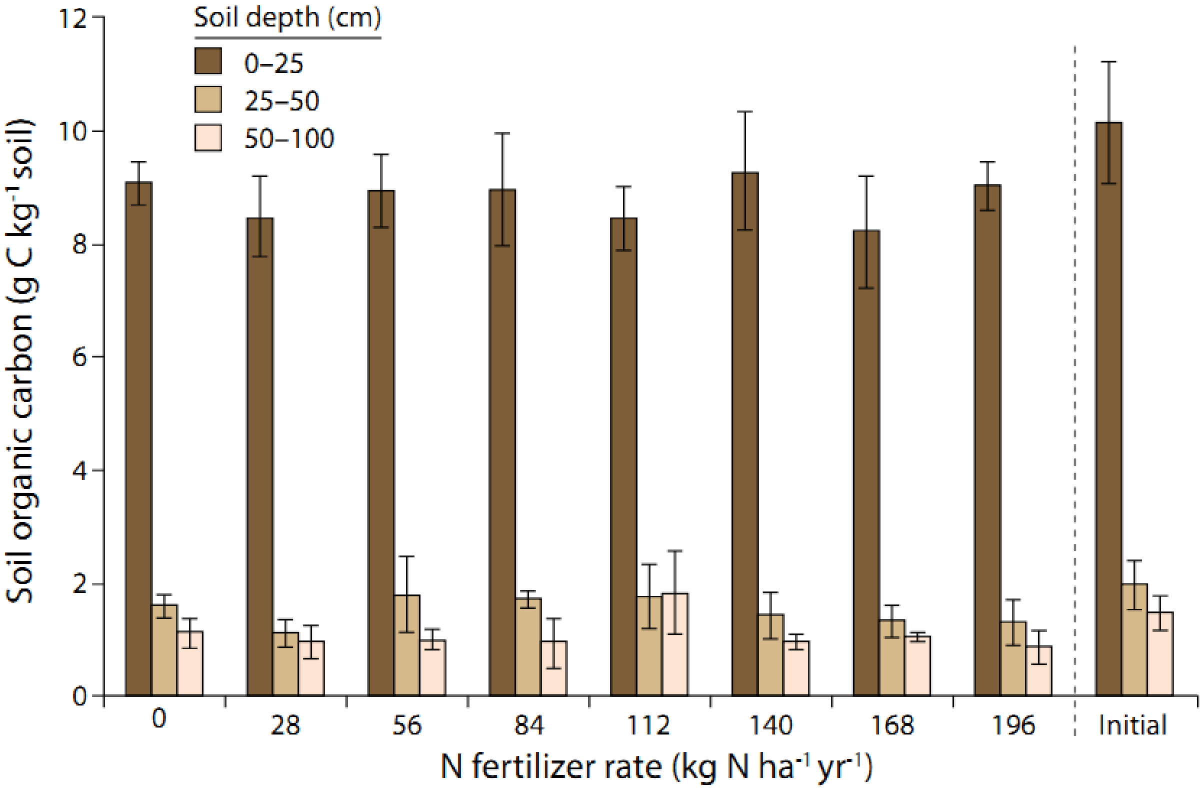

Figure 2.8. Soil carbon concentrations at depths of 0-25, 25-50, and 50-100 cm across the N fertilization gradient. Error bars represent standard errors based on n=4 replicate plots. Bars to the left of the dashed line represent samples taken in 2012 versus those taken at the outset of the experiment in 2008 (to the right side of dashed line). There are no significant differences among treatments (α=0.05).

unit N added (56 kg N ha^{-1}) (Figure 2.9a). Including all GHG sources and credits in the GHG balances, each N treatment shows net climate change mitigation (i.e., negative CO_2e balances) ranging from -4.64±0.12 Mg CO_2e ha^{-1} yr^{-1} in the unfertilized treatment to -5.71±0.22 in the treatment fertilized at 56 kg N ha^{-1} (Figure 2.9b). At fertilization rates above 56 kg N ha^{-1} the mitigation effect decreases consistently 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 2.9b).

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 major obstacle to 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 nil: some varieties of switchgrass are known to be unresponsive to fertilizer N (Christian et al., 2002), presumably because of a combination of high N use efficiency and the presence of other N acquisition mechanisms, possibly including biological N fixation.

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 N as conservatively as possible will be needed to meet the climate benefit claims of this emerging industry (Robertson et al., 2008). Incentives would have the additional advantage of reducing unnecessary N pollution of ground- and surface waters and lowering the cost of biofuel crop production.

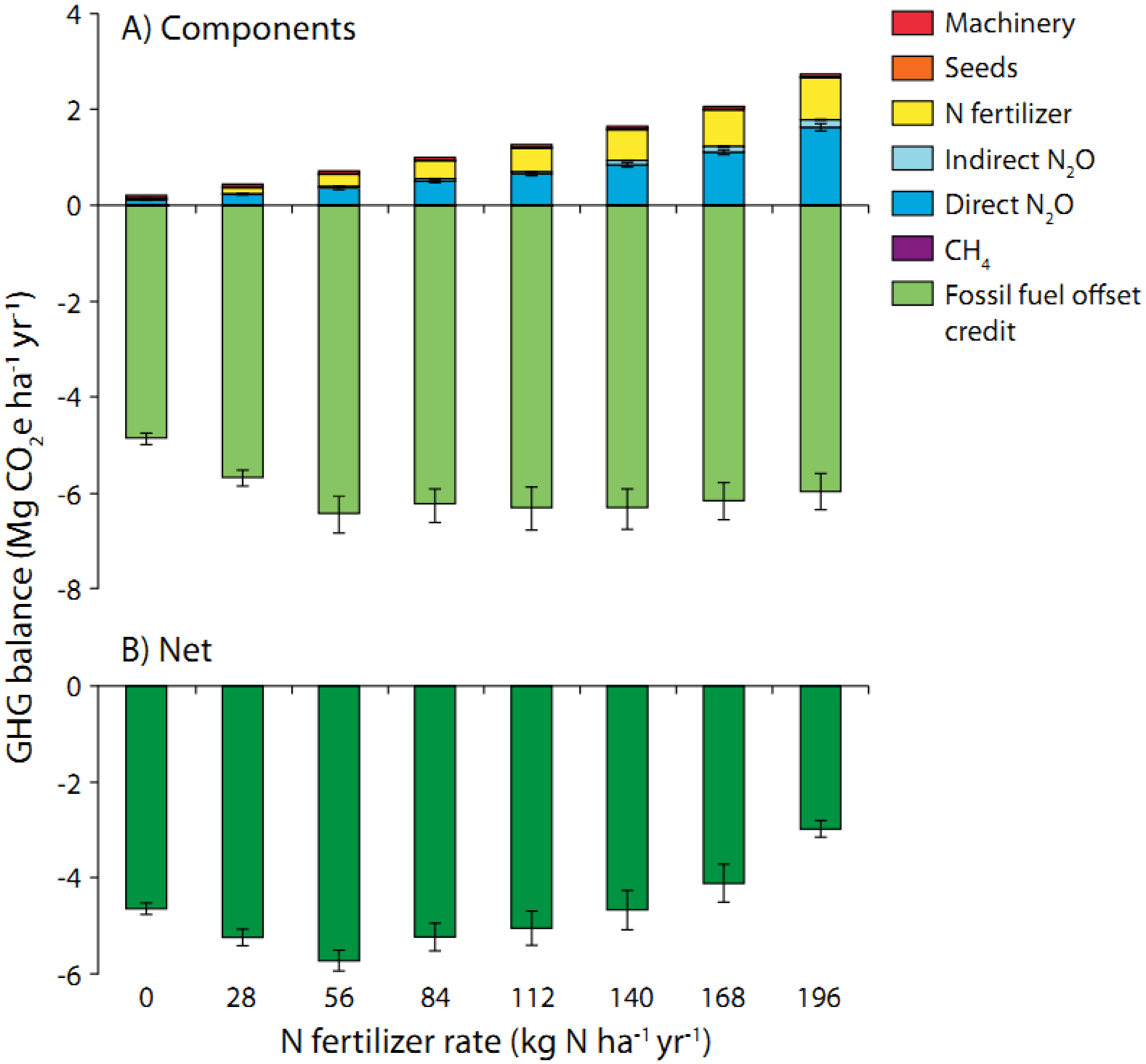

Figure 2.9. Annual greenhouse gas (GHG) balances for switchgrass production across the gradient of N fertilization rates. A) Component GHG sources and the resultant fossil fuel offset credits for displacement of fossil fuel by biofuel; B) the net GHG balance. GHG emissions from agricultural inputs include farm machinery, switchgrass seed production, and N fertilizer production, transportation and distribution. Indirect N_2O emissions represent N_2O produced off-site by leached nitrate. Direct N_2O and CH_4 flux rates are from field measurements during 2009-2011. CH_4 uptake rates were negligible and are not visible in graph. Error bars represent standard errors based on n=4 replicate plots.

CHAPTER 3[1]: INITIAL NITROUS OXIDE, CARBON DIOXIDE AND METHANE COSTS OF CONVERTING CONSERVATION RESERVE PROGRAM GRASSLAND TO ROW CROPS UNDER NO-TILL VS. CONVENTIONAL TILLAGE

ABSTRACT

Around 4.4 million ha of land in USDA Conservation Reserve Program (CRP) contracts will expire between 2013 and 2018 and some will likely return to crop production. No-till (NT) management offers the potential to reduce the global warming costs of CO_2, CH_4 and N_2O emissions during CRP conversion but to date there have been no CRP conversion tillage comparisons. In 2009, I converted portions of three 9-21 ha CRP fields in Michigan to conventional tillage (CT) or NT soybean production and reserved a fourth field for reference. Both CO_2 and N_2O fluxes increased following herbicide application in all converted fields, but in the CT treatment substantial and immediate N_2O and CO_2 fluxes occurred after tillage. For the initial 201-day conversion period, average daily N_2O fluxes (g N_2O-N ha^{-1} d^{-1}) were significantly different in the order: CT (47.5±6.31, n=6) >> NT (16.7±2.45, n=6) >>reference (2.51±0.73, n=4). Similarly, soil CO_2 fluxes in CT were 1.2 times those in NT and 3.1 times those in the unconverted CRP reference field. All treatments were minor sinks for CH_4 (-0.69±0.42 to -1.86±0.37 g CH_4-C ha^{-1} d^{-1}) with no significant differences among treatments. The positive Global Warming Impact (GWI) of converted soybean fields under both CT (11.5 Mg CO_2e ha^{-1}) and NT (2.87 Mg CO_2e ha^{-1}) was in contrast to the negative GWI of the unconverted reference field (-3.5 Mg CO_2e ha^{-1}) with on-going GHG mitigation. N_2O contributed 39.3% and 55.0% of

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[1] This chapter has been published in Global Change Biology. Ruan, L., and G. P. Robertson. 2013. Initial nitrous oxide, carbon dioxide, and methane costs of converting conservation reserve program grassland to row crops under no-till vs. conventional tillage. Global Change Biology 19:2478–2489.

the GWI under CT and NT systems with the remainder contributed by CO_2 (60.7% and 45.0%, respectively). Including foregone mitigation, I conclude that NT management can reduce GHG costs by ~60% compared to CT during initial CRP conversion.

INTRODUCTION

The USDA Conservation Reserve Program (CRP) builds contracts with agricultural landowners in the U.S. to retire highly erodible and environmentally sensitive cropland and pasture into perennial vegetation for periods ≥10 years. The program, established by the Food Security Act of 1985, is designed to reduce soil erosion, improve water and air quality, enhance wildlife populations, and sequester carbon in soil and biomass. In 2007, as many as ~15 million ha were enrolled, representing ~9% of total U.S cropland (ERS, 2011; FSA, 2012). Since then, enrolled land had decreased to ~12 million ha in 2012, and an additional ~4.4 million ha of land are in CRP contracts that will expire between 2013 and 2018 (FSA, 2012). Higher prices for corn (*Zea mays* L.) and other crops and expanded biofuel production are expected to induce farmers to return CRP land to grain production (Du *et al.*, 2008; Secchi *et al.*, 2009). Many environmental benefits may subsequently be lost. Of particular concern are changes to greenhouse gas (GHG) emissions – fluxes of CO_2, nitrous oxide (N_2O) and methane (CH_4) during and after conversion (CAST, 2011).

Grassland conversion to crop production can accelerate both soil C and nitrogen (N) cycles, and results in significant GHG emissions. In particular, land conversion practices such as plowing can enhance soil organic matter oxidation, nitrification, and denitrification and substantially increase CO_2 and N_2O emissions (Pinto *et al.*, 2004; Grandy and Robertson, 2006a; Nikièma *et al.*, 2012). No-till (NT) offers the potential to attenuate such increases but to date there have been no GHG comparisons of NT and conventional tillage (CT) during CRP conversion.

The effects of tillage on soil carbon are well-known. Plowing mixes crop residues with the soil, increases the aeration of surface soil and reduces soil aggregation, all of which enhances organic matter decomposition and CO_2 release (Haas *et al*., 1957; Buyanovsky and Wagner, 1998; Grandy and Robertson, 2006b; Regina and Alakukku, 2010). In contrast, the soil under NT is left undisturbed. More stable aggregates under NT protect soil organic carbon (SOC) from microbial decomposition and allow SOC storage (Six *et al*., 2000). Dolan *et al*. (2006), for example, reported that NT managed soil contained over 30% more SOC than CT soils to 20cm after 23 years of NT. Syswerda *et al*. (2011) reported ~11% higher SOC to 1 m depth under NT than CT after 12 years of NT. West and Post (2002) used a global database of 67 long term agricultural experiments to estimate that conversion from CT to NT can annually sequester 48 ± 13 g C m^{-2} yr^{-1} in surface horizons. There is little evidence for statistically different changes at deeper depths (Kravchenko and Robertson, 2011). Following CRP conversion, Follett *et al*. (2009) reported no SOC change (0-30 cm depth) within 6.5 years after conversion of CRP grasslands to NT corn in Nebraska. Anken *et al*. (2004), however, reported that SOC (0-20cm depth) decreased under both NT and CT similarly in Switzerland for the first 7 years after conversion of a10 year-old grassland to maize-winter wheat production.

Effects of CT on soil N_2O emissions compared to NT are still in debate. Agricultural soil N_2O emissions account for about 60% of global total anthropogenic N_2O production (IPCC, 2007a) due to two microbial processes: denitrification and nitrification (Robertson and Groffman, 2007). Theoretically, NT can strongly affect both of these processes through effects on soil water, carbon, pore space, and soil N concentrations. In practice, some studies have shown higher N_2O

emissions from NT than CT (e.g. Baggs *et al*., 2003; Rochette *et al*., 2008), with higher rates in NT mostly attributed to restricted soil aeration due to higher water content, which is conducive to denitrification. However, others have found lower emissions in NT than CT, attributed to improved soil structure and lower soil temperatures (e.g. Chatskikh and Olesen, 2007; Ussiri *et al*., 2009). Still others have found no difference between NT and CT (e.g. Robertson *et al*., 2000; Choudhary *et al*., 2002; Boeckx *et al*., 2011).

Methane oxidation is also affected by agricultural management. CH_4 oxidation by methanotrophic bacteria in well-aerated soils is an important sink (5%, globally) for atmospheric CH_4 (IPCC, 2007a). In theory, a less disturbed soil structure and improved gas diffusion in NT should enhance the CH_4 oxidation capacity of methanotrophic bacteria relative to CT (Six *et al*., 2004; Ussiri *et al*., 2009). However, studies to date have reported no significant NT effects on CH_4 oxidation rates (Robertson *et al*., 2000; Jacinthe and Lal, 2005).

In an earlier study Gelfand *et al*. (2011) reported that the conversion of CRP land to NT soybean production released significant amounts of CO_2 and N_2O and had little effect on CH_4 oxidation rates. Here I extend their results to examine the impact of CT practices on GHG fluxes during conversion. Specifically, I hypothesize that for the CRP conversion year, NT relative to CT will 1) attenuate N_2O emissions, 2) reduce C loss, and 3) avoid the loss of CH_4 oxidation. Further, I evaluate the relative importance of each flux to the overall GHG cost of CRP conversion.

MATERIALS AND METHODS

Site description

My experimental fields were located at the Great Lakes Bioenergy Research Center

(GLBRC) scale-up field at the Marshall Farm of the Kellogg Biological Station (KBS) Long-term Ecological Research (LTER) site in southwest Michigan (42° 26′ N, 85°19′ W, elevation 288 m). Annual precipitation is ~890 mm with about half falling as snow, and the mean annual temperature is 9.7 °C. I conducted experiments in 4 separate fields enrolled in the CRP for 22 years beginning in 1987, when all fields were planted to the C3 grass smooth brome (*Bromus inermis* Leyss). Fields were 9-21 ha in size and within 1.8 km of one another. In 2009, three fields were converted to soybean (*Glycine max)* production. No fertilizers were applied although ammonium sulfate (0.33 kg N ha^{-1}) was added to glyphosate as a surfactant. The fourth was reserved as a reference field unconverted.

Soils in all fields are mesic Typic Hapludalfs of 3 intermixed series: Boyer (loamy sand), Kalamazoo (fine-loamy) and Oshtemo (coarse-loamy) developed on glacial outwash. Prior to conversion, there were no significant differences among key soil properties including soil C and N contents, bulk density and soil texture among the 4 CRP fields (Table 3.1) (http://data.sustainability.glbrc.org/).

Experimental design and treatments

I established 2 replicated NT and CT plots in each of the 3 converted fields. I also randomly identified 4 replicate plots in the reference field. Treatment plots were 36 m by 9 m arranged in a randomized complete block design for a total of 16 plots (3 fields × 2 treatments × 2 replicate plots plus 1 reference field × 4 replicate plots). Brome grass was killed at the converted fields on May 5, 2009, with glyphosate (N-phosphonomethyl, Syngenta, Greensboro, NC, USA) at a concentration of 2.85 kg ha^{-1} and killed grass residue was left in place. CT plots were tilled (25 cm deep) using a chisel plow and secondary tillage for leveling the surface on

Table 3.1 Soil physical and chemical properties (0-25cm) of the four CRP grassland fields prior to conversion. Means within columns marked with the same letters are not significantly different ($P<0.05$).

	pH	Bulk density ($g\ cm^{-3}$)	Nitrogen ($g\ kg^{-1}$)	Carbon ($g\ kg^{-1}$)	Sand ($g\ kg^{-1}$ soil)	Silt ($g\ kg^{-1}$ soil)	Soil texture
Field 1	6.4^{a}	1.41^{a}	2.10^{a}	22.8^{a}	664^{a}	256^{a}	Sandy loam
Field 2	6.7^{a}	1.34^{a}	1.81^{a}	20.6^{a}	697^{a}	245^{a}	Sandy loam
Field 3	6.3^{a}	1.42^{a}	1.72^{a}	19.9^{a}	688^{a}	264^{a}	Sandy loam
Reference	6.2^{a}	1.41^{a}	2.07^{a}	22.5^{a}	595^{a}	328^{a}	Sandy loam

June 8. NT plots were left untilled, as was the remainder of each converted field. Soybeans (Pioneer 92M91) were planted on June 9 in all converted fields at a seeding rate of 355,680 seeds ha^{-1} using a no-till planter. The reference field was left undisturbed.

Gas and soil measurement protocols

CO_2, CH_4 and N_2O flux measurements were made using a static chamber approach as described by Hoben *et al*. (2011) between May 7 and November 24, 2009. I measured fluxes 1 to 2 times a week during the growing season to capture the temporal dynamics of gas fluxes influenced by different management activities, and then measured fluxes every 2 weeks after mid-September. Two chambers were installed in each treatment plot of the converted fields and one chamber was installed in each of 4 reference plots for a total of 36 chambers. Each chamber (28 cm diameter × 26 cm height) was equipped with a removable lid and septum. Chamber bases were embedded 5cm into the soil for the duration of the study except during farm operations (tillage, soybean planting, and harvest), when chambers were removed from plots in the converted fields and replaced in the same spot within 2 hours afterwards.

For flux measurements, chamber lids were attached and headspace gas samples (10 mL) were collected four times with a 10mL syringe from each chamber at intervals of approximately 15 minutes. Samples were stored overpressurized in 5.6 mL glass vials (Labco Ltd., High Wycombe, UK). Vials were returned to the laboratory where contents were analyzed by gas chromatography (Hewlett Packard 5890 Series II, Rolling Meadows, IL, USA) usually within 12 hours of collection. Gases were separated on a Porapak Q column (1.8 m, 80/100 mesh) at 80 ˚C. CO_2 was analyzed using an infrared gas absorption analyzer (LI-820 CO_2 analyzer, LI-COR, Lincoln, NE, USA); 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.

I also calculated the net ecosystem exchange (NEE) of CO_2 at each field using data from Zenone *et al*. (2011) as reported on the KBS LTER website: http://lter.kbs.msu.edu/datatables/198. Four 3m tall eddy covariance towers were located in the center of each field. The eddy-covariance system included a LI-7500 open-path infrared gas analyzer (IRGA) (Li-Cor Biosciences, Lincoln, NE, USA), a CSAT3 three-dimensional sonic anemometer (Campbell Scientific Inc., Logan, UT, USA), and a CR5000 data logger (Campbell Scientific Inc.). The effective measurement radius of each eddy covariance tower was approximately 200 m and every 30-min NEE was calculated as the covariance of vertical wind speed and the concentration of CO_2 as described in Zenone *et al*. (2011). Individual treatment plots (CT, NT and reference) were outside the effective range of the towers such that NEE measurements were for NT soybeans (converted fields) or unconverted smooth brome grass (reference field). I calculated the NEE for CT soybean as NEE for NT soybean plus the difference I measured in soil CO_2 fluxes between CT and NT treatments. This assumes that both CT and NT soybean treatments removed the same amount of CO_2 from the atmosphere through photosynthesis as confirmed by similar yields for CT and NT treatments, and that CO_2 fluxes from plant and herbivore respiration were similar for each treatment.

To estimate the global warming impact of conversion attributable to changes in CO_2, CH_4 and N_2O fluxes, I multiplied fluxes of each gas by its global warming potential (GWP) to yield CO_2 equivalents (Mg CO_2e ha^{-1}). For CO_2, CH_4 and N_2O fluxes I used the IPCC 100-y horizon GWP factors of 1, 25 and 298, respectively (IPCC, 2007a).

At each gas sampling event, I measured soil temperature, gravimetric water content, ammonium (NH_4^+) and nitrate (NO_3^-) concentrations, bulk density, and water-filled pore space (WFPS%). Four 2.5 cm diameter cores (0-25 cm depth) were randomly collected within and between plant rows from each treatment plot. One core was then oven-dried to constant weight at 60 ˚C for 48 hours to obtain gravimetric soil moisture (g water/ g dry soil). The remaining 3 cores were composited and sieved to 4 mm. Three 10 g subsamples were then each extracted with 100 mL of 1 M KCl. Soil extracts were shaken by hand for 1 min, equilibrated overnight, reshaken and settled for 2 hours before filtering through a 1-mm glass fiber syringe filter. Filtrates were stored in 7 ml polyethylene vials and frozen until analysis for NH_4^+ and NO_3^-at a later date. Both analyses were performed on a Flow Solution IV continuous flow analyzer (OI Analytical, College Station, TX, USA) using colorimetric techniques.

Ion exchange resin strips were also used to estimate NH_4^+ and NO_3^- availability (Qian and Schoenau 1995). Two pairs of anion and cation strips (2.5 cm × 10 cm × 0.62 mm thick) (GE Power & Water, Trevose, PA, USA) were buried directly into the soil at each treatment plot. After 37 days, strips for each plot were collected and put into a 237 mL polyethylene cup. I added 35 mL of 2.0 M KCl per resin strip to each cup (i.e. 140 mL for 4 strips) and cups were then shaken at 40 rpm for 1 h on an orbital shaker (IKA KS 501, Wilmington, NC, USA). A 5 mL extract was stored in a 7 mL polyethylene vial and frozen until analysis for NH_4^+ and NO_3^- as above.

Soil bulk density (BD) (0-25 cm depth) was measured on May 20, June 10, and Nov 20, 2009 using a fixed-volume core (123 cm^3) for each treatment plot. Water-filled pore space (WFPS %) was calculated as

$$\text{WFPS\%} = \frac{\text{Gravimetric water content (\%, g / g)} \times \text{BD (g cm}^{-3}\text{)}}{\text{[water density (1 g cm}^{-3}\text{)} \times \text{soil porosity (\%)]} \times 100\%}$$

Where soil porosity = 1- BD (g cm^{-3}) / particle density (g cm^{-3}). Particle density of the soil was assumed to be 2.65 g cm^{-3}.

Data analysis

Cumulative fluxes of gases were calculated by linear interpolation of daily fluxes between sample days in 2009. Data were analyzed using the PROC MIXED procedure in SAS 9.1 (SAS Institute, Cary, NC, USA). When comparing differences between CT and NT treatments, the experiment was analyzed as a randomized complete block design with the field as a blocking factor. Plots within the fields subjected to CT and NT treatments were used as experimental units for testing treatment effects. For comparisons between CT and the reference treatment or NT and the reference treatment the experimental unit was the field. To determine the relationship between daily fluxes (CO_2, N_2O and CH_4) and environmental factors such as soil temperature, gravimetric soil moisture, and soil total N, I performed multiple linear regressions (stepwise) using PROC REG and nonlinear regression using PROC NLIN. Normality of the residuals and homogeneity of variance assumptions were checked using stem-and-leaf box and normal probability plots of the residuals, and Levene's test. Data were not transformed prior to analysis. Treatment means were compared for significance using t-tests or Tukey's test at $\alpha=0.05$ level.

RESULTS

Weather, bulk density and WFPS

Air temperature, precipitation and soil moisture are shown in Figure 3.1. Mean daily air temperature was 15.4 ˚C for the study period of May 3 to November 24, 2009, ranging between 2.8 ˚C and 26.9 ˚C. Cumulative precipitation was 443 mm with a drought period from July 1 to August 7, during which time no precipitation >2 mm occurred.

Soil bulk density (0-25 cm depth) in the CT treatment decreased from 1.51±0.01 to 1.32±0.02 g cm^{-3} after tillage operations on June 8 and gradually increased back to 1.49±0.04 g

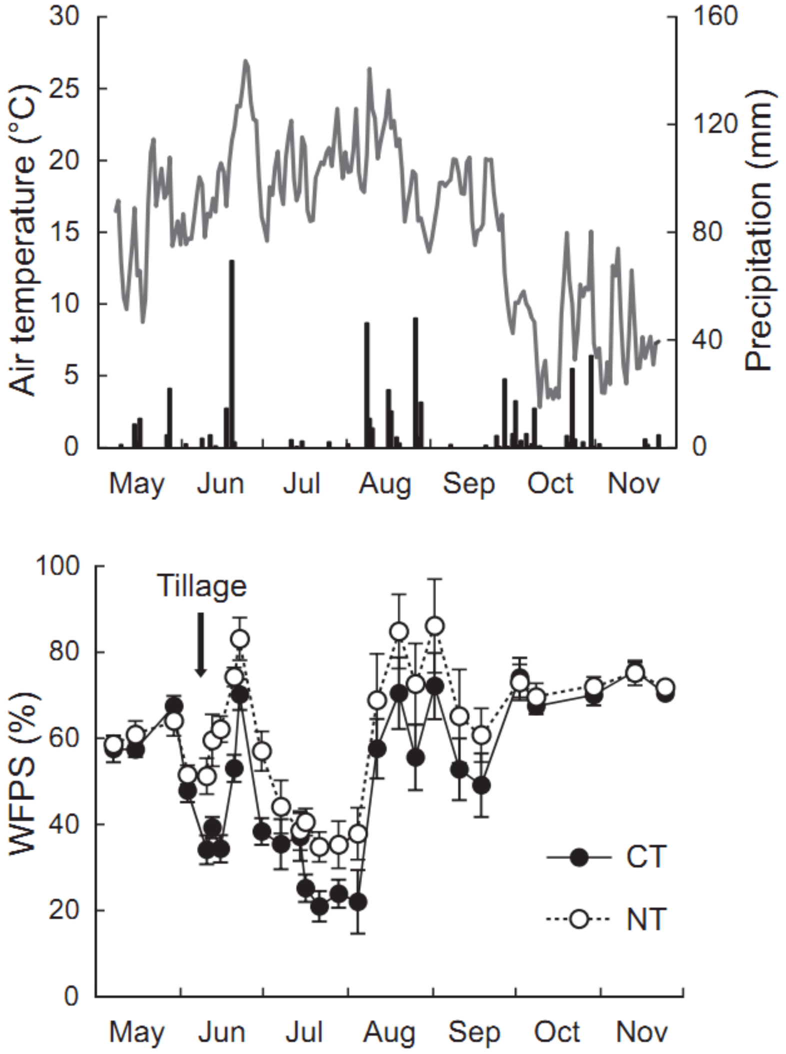

Figure 3. 1 Precipitation and air temperatures (top) and water-filled pore space (WFPS %) to 25 cm depth (bottom) under CT and NT soybeans in 2009. Arrow indicates tillage date in the CT treatment.

cm^{-3} by the end of the season. On the other hand, bulk density in NT and reference treatments stayed stable over the study period at 1.51±0.01 g cm^{-3}.

WFPS% varied between 21.0 and 86.1% with highest values in June and August and lowest values in July. No significant differences were found before June 8 (tillage date in CT) between CT and NT treatments. During the 2 months after June 8, average WFPS% in NT was significantly higher than in CT (52%±0.04 vs. 36%±0.03, respectively, $P<0.05$). Over June 17-19, 83 mm precipitation occurred and WFPS% under both treatments reached a peak. After a 64 mm precipitation event on August 8, there were no significant differences in soil water content between CT and NT for the remainder of the study.

Soil N_2O fluxes

High N_2O fluxes occurred immediately after CT tillage on June 8, and ranged from 196 to 1192 g N_2O-N ha^{-1} d^{-1} among the 3 converted fields. In contrast, on the same date NT fluxes ranged from 10.6 to 63.6 g N_2O-N ha^{-1} d^{-1} among fields, and in the reference field fluxes ranged from 1.58 to 5.92 g N_2O-N ha^{-1} d^{-1} (Figure 3.2). Tillage-induced fluxes persisted for 30 to 40 days. Other two relatively large peaks occurred at the converted fields on June 20-22 and August 11 after rainfall events. Subsequently, significant fluxes took place mostly when WFPS in the 0-25 cm depth was greater than 60%. N_2O emissions from the reference field remained at low levels (<7.21 g N_2O-N ha^{-1} d^{-1}) even after substantial rainfall. After August 25, N_2O fluxes were low in all fields, coincident with less available soil N (Figure 3.3) and lower air temperatures beginning in mid-September (Figure 3.1). Soil temperature and WFPS% showed a positive correlation with daily N_2O fluxes but the correlation was not significant ($P>0.05$). Overall, for the May 7 to November 24 period, mean daily N_2O emissions under CT were 2.8 times those of NT (47.5 ±6.31 vs. 16.7 ±2.45 g N_2O-N ha^{-1} d^{-1}; $P<0.01$) and in both CT and NT treatments rates

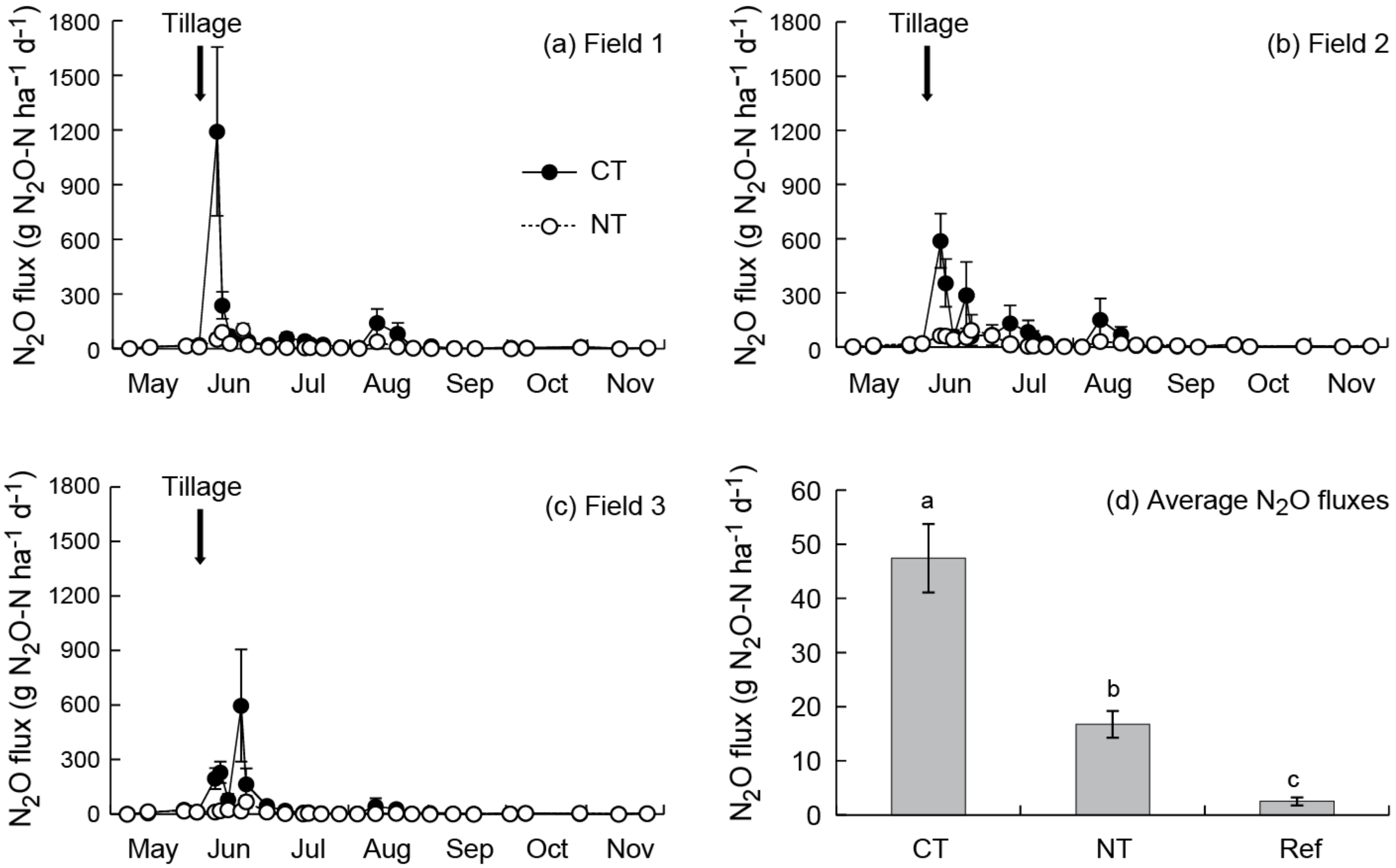

Figure 3.2. Daily N_2O fluxes by treatment (CT vs. NT soybean) in each of 3 CRP fields (panel a-c) for May 7 to November 24, 2009. Error bars represent standard errors of N_2O emissions based on n=2 replicate plots. Arrows indicate tillage date. Panel (d) shows average N_2O fluxes for CT and NT treatments (n=6 replicate plots) and an unconverted CRP reference field (Ref) (n=4 replicate plots). Treatments marked with different letters are significantly different from one another ($p<0.01$).

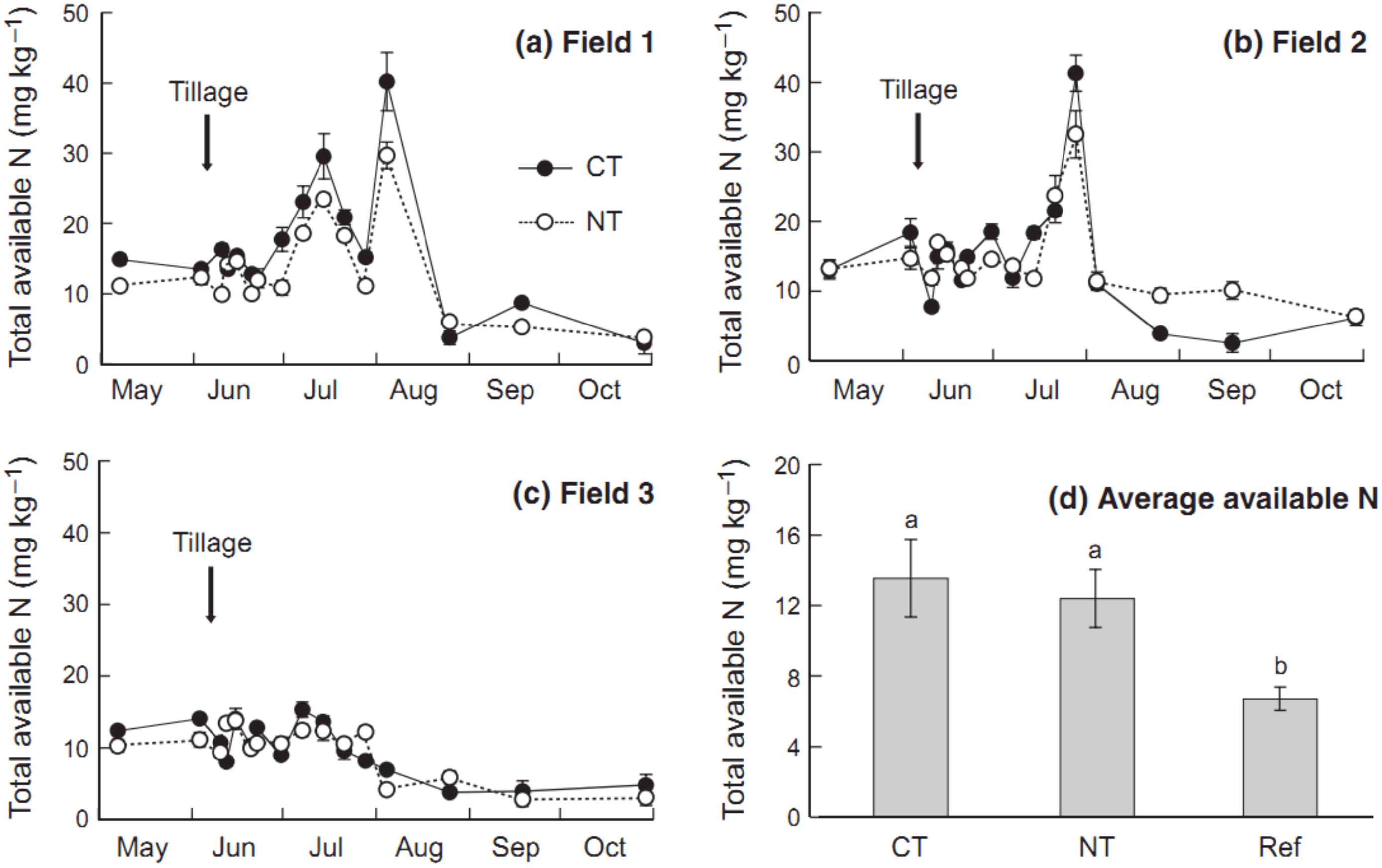

Figure 3.3. Seasonal dynamics of soil inorganic N pools to 25cm depth (NH_4^+-N plus NO_3^--N) measured in soil cores under CT and NT soybeans in 2009 (panel a-c). Error bars represent standard errors of total inorganic N based on n=2 replicate plots. Arrows indicate tillage date. Panel (d) shows mean soil inorganic N in CT and NT treatments (n=6 replicate plots) and an unconverted CRP reference field (Ref) (n=4 replicate plots) over the study period. Treatments marked with same letters are not significantly different from one another ($p<0.05$).

were substantially higher than in the reference field (2.51 ±0.73 g N_2O-N ha^{-1} d^{-1}; $P<0.01$) (Figure 3.2d).

Soil CO_2 fluxes

Soil CO_2 fluxes (chamber measurements) showed a seasonal trend in all treatments with high emissions through the growing season and lower emissions after October (Figure 3.4), coincident with lower air temperatures (Figure 3.1). After herbicide application at the converted fields on May 5, chamber-based CO_2 fluxes increased sharply and reached a peak on May 29 before tillage started. Immediately following CT tillage on June 8, average CT CO_2 fluxes on June 8 ranged from 72.2-140 kg CO_2-C ha^{-1} d^{-1}, compared to 29.6-43.7 kg CO_2-C ha^{-1} d^{-1} in the NT treatments (Figure 3.4). High fluxes associated with tillage lasted ~20 days, during which daily fluxes ranged from 0.12 to 168 kg CO_2-C ha^{-1} d^{-1}. Overall, mean CO_2 fluxes under CT were 1.2 times those of NT (50.7 ±2.50 vs. 43.0 ±1.43 g kg CO_2-C ha^{-1} d^{-1}; $P<0.05$) and were 3.1 times those from the reference (16.3±2.36 kg CO_2-C ha^{-1} d^{-1}; $P<0.05$) (Figure 3.4d). When only comparing the first 30 days after tillage, the CT treatment emitted 2.0 times higher CO_2 fluxes than did the NT treatment.

Soil CH_4 fluxes

CH_4 fluxes oscillated in all fields between net emission and net uptake without a discernable seasonal trend. Mean daily CH_4 fluxes were low, ranging from -6.4 to 4.5 g CH_4-C d^{-1} (Figure 3.5). Over the entire study period all treatments exhibited net CH_4 uptake but no significant treatment differences were detected. Although mean CH_4 oxidation rates were 1.7 times higher under NT than under CT (-1.86±0.37 vs. -0.69±0.42 g CH_4-C ha^{-1} d^{-1}, respectively), the difference was not statistically significant ($p=0.06$). Reference field fluxes also were not

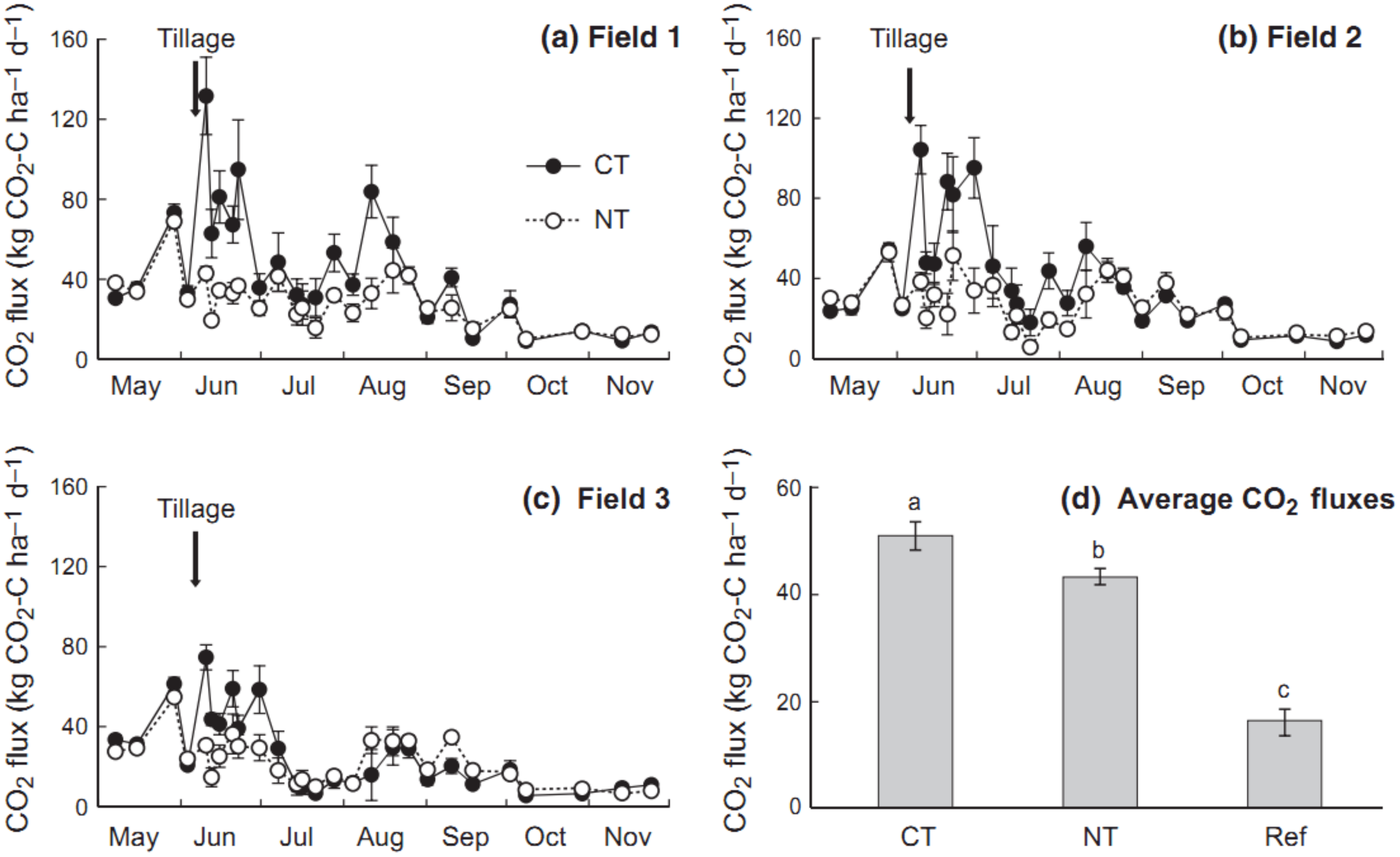

Figure 3.4. Daily CO_2 fluxes by treatment (CT vs. NT soybean) in each of 3 CRP fields (panel a-c) for May 7 to November 24, 2009. Error bars represent standard errors of CO_2 emissions based on n=2 replicate plots. Arrows indicate tillage date. Panel (d) shows average CO_2 fluxes for CT and NT treatments (n=6 replicate plots) and an unconverted CRP reference field (Ref) (n=4 replicate plots). Treatments marked with different letters are significantly different from one another ($p<0.05$).

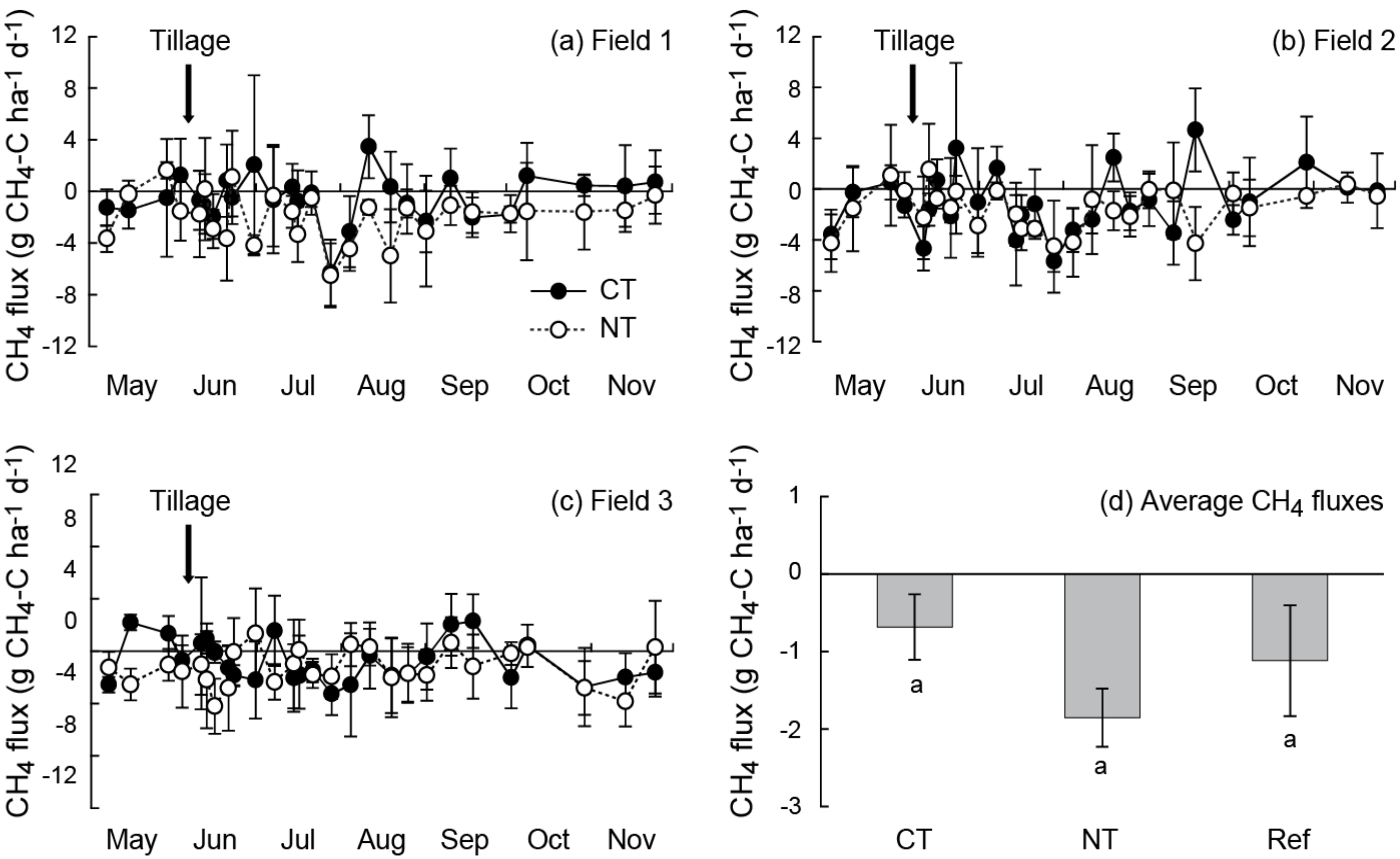

Figure 3.5. Daily CH_4 fluxes by treatment (CT vs. NT soybean) in each of 3 CRP fields (panel a-c) for May 7 to November 24, 2009. Error bars represent standard errors of CH_4 emissions based on n=2 replicate plots. Arrows indicate tillage date. Panel (d) shows average CH_4 fluxes for CT and NT treatments (n=6 replicate plots) and an unconverted CRP reference field (Ref) (n=4 replicate plots). Treatments marked with different letters are significantly different from one another ($p<0.05$).

significantly different from those in the CT treatment (p=0.32), although uptake in CT soils was only 60% of that in the reference field.

Grain Yield

Soybean grain yield in individual plots ranged from 2.0 to 2.5 Mg ha^{-1}. The overall comparison of mean soybean grain yield showed no significant differences between CT (2.4±0.18 Mg ha^{-1}) and NT (2.3±0.14 Mg ha^{-1}) treatments.

Global Warming Impact (GWI)

As noted earlier, NEE for NT soybean was measured directly as for the reference field. Since there were no significant yield differences between CT and NT treatments, NEE for CT soybean was calculated as the sum of NEE for NT soybean plus the difference between CT and NT soil CO_2 fluxes, i.e. 10.7±1.37, 6.61±2.02, and 3.66±1.32 Mg CO_2e ha^{-1} for the 3 converted fields over the study period.

Over my 201-day study period, then, GWIs were 11.5, 2.87 and -3.50 Mg CO_2e ha^{-1} under CT, NT and reference treatments, respectively (Figure 3.6). Both CT and NT soybean had positive GWIs, with the GWI of CT soybean approximately 2.6 times that of NT soybean (Figure 3.6).

Soil inorganic nitrogen

Resin strip results (Figure 3.7) showed that tillage greatly increased soil N availability for at least the first month following plowing. Over the 37 day period beginning June 8, strips under CT accumulated 4.8 times more total inorganic N, mostly as NO_3^-, than did strips under NT (60.0 vs. 12.4 μg N cm^{-2}). Over the following month, this difference began to diminish (70.4 vs. 45.7 μg N cm^{-2}, P>0.05) and there were no discernable differences later in the season. Daily N_2O

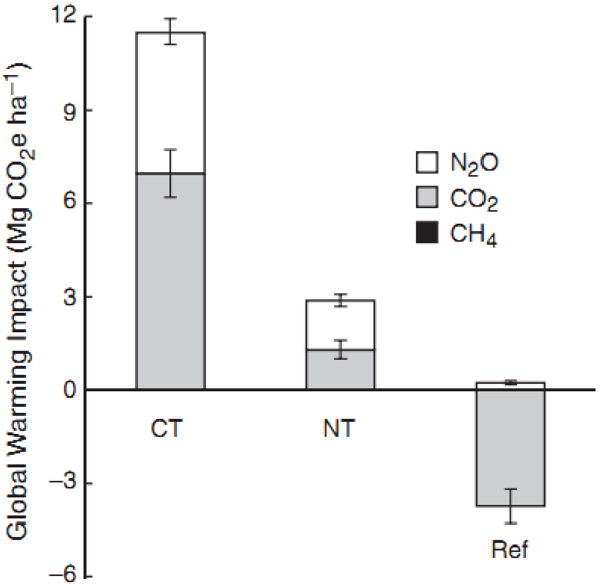

Figure 3.6. The global warming impact (GWI) of individual greenhouse gases for CT soybean, NT soybean and the unconverted CRP reference field (Ref) for May 7 to November 24, 2009. Methane values were negligible and are not visible in graph. For N_2O and CH_4, error bars represent standard errors based on n=6 replicate plots of CT and NT and n=4 replicate plots for the reference plots; for the net ecosystem exchange (NEE) of CO_2, error bars represent standard errors based on n=6 replicate plots of CT and n=3 of NT and n=1 for reference fields.

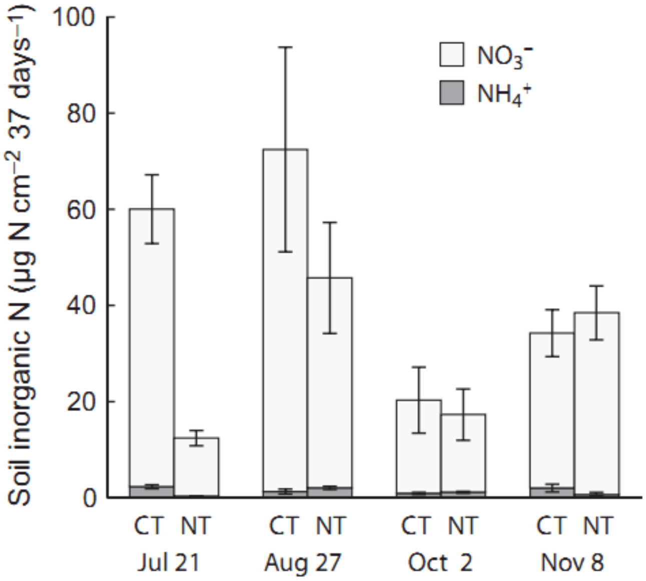

Figure 3.7. Seasonal dynamics of soil inorganic nitrogen (NH_4^+-N + NO_3^--N) measured with cation (NH_4^+) and anion (NO_3^-) resin strips under CT and NT soybeans. Error bars represent standard errors of NH_4^+-N and NO_3^--N based on n=6 replicate plots. Resin strips were buried (0-15 cm depth) for ~37 days since tillage (June 8, 2009) and then replaced with new pairs 3 times during the season.

fluxes showed a positive relationship with total available N: N_2O fluxes=34.8× Exp (0.36×available N) (R^2=0.19, $P<0.01$).

In contrast, by the soil-KCl extraction method, soil inorganic N concentrations (0-25cm depth) (Figure 3.3) were significantly higher under CT than NT (16.2 vs. 10.0 mg kg^{-1}, $P<0.05$) in only one field for the first day following tillage and overall results showed no consistent differences (Figure 3.3d). Mean total inorganic N concentrations in reference fields were significantly lower than those in the CT and NT treatments ($P<0.05$) (Figure 3.3d). Soil inorganic N concentrations showed a seasonal trend in both treatments with high concentrations through the growing season and lower concentrations after September. Among different fields, soil inorganic N concentrations ranged from a high of 21.6 mg kg^{-1} on June 28 to low values of 3.8-5.1 mg kg^{-1} after September.

DISCUSSION

The conversion of my CRP grasslands to row crops resulted in a substantial GHG release that differed by tillage practice. The most remarkable difference between CT and NT management during conversion was in N_2O fluxes. I found immediate and substantial tillage-induced N_2O emissions under CT that exceeded the CO_2-equivalent loss of soil C over the 201-day study period. Total N_2O emissions under converted CT soybean were 2.1-fold higher than under converted NT soybean and 18.8-fold higher than under unconverted smooth brome grass (reference field). The magnitude of CT N_2O emissions exceeded those of fertilizer-induced N_2O fluxes in the same area (Robertson *et al.*, 2000; Hoben *et al.*, 2011). Even with NT practices, however, CRP conversion still caused large N_2O emissions, with fluxes under NT 5.3 times higher than under unconverted reference.

Soil CO_2 emissions under CT were also significantly higher than those under NT and reference treatments. Cumulative NEE of CO_2 under CT were 2.2-fold higher than those under NT over the study period. The converted fields under both CT and CT were carbon sources under both CT and NT, whereas the unconverted reference treatment was a net carbon sink. All treatments were a small sink for atmospheric CH_4. However, changes in CH_4 oxidation rates did not contribute significantly to the GWI of conversion compared with N_2O and CO_2. Overall, N_2O accounted for 39.3% of the net GWI of conversion under CT and 55.0% under NT with the remainder contributed by CO_2 (60.7% and 45.0%, respectively), excluding the CO_2 costs of herbicide and fuel, which were negligible (Gelfand et al., 2011).

N_2O emissions

N_2O fluxes increased 18- to 55-fold immediately on the first day after tillage operations in all CT treatments. Over the study period, mean daily CT N_2O emissions (47.5±6.3 g N_2O-N ha^{-1} d^{-1}) were relatively higher than those reported for fertilized annual crops at a nearby site (3.35±0.30 g N_2O-N ha^{-1} d^{-1}) (Robertson *et al.*, 2000) and for heavily fertilized crops elsewhere in Michigan (25.8 g N_2O-N ha^{-1} d^{-1}from corn fertilized at 225 kg N ha^{-1}) (Hoben *et al.*, 2011). Similar substantial amounts of N_2O emissions following tillage have been reported for other studies where unmanaged vegetation has been converted to cropland. For example, Grandy and Robertson (2006a) reported a 3.1-7.7 fold increase in N_2O emissions after plowing long-term undisturbed grassland over a 3-year period. Nikiema *et al.* (2012) reported high N_2O fluxes of 57.2 and 41.8 g N_2O-N ha^{-1} d^{-1} after converting heavily manured pastureland (200 kg N ha^{-1}yr^{-1}) to poplar and willow production, respectively. Possible reasons for high N_2O emissions could be increased production of available N and C after soil organic matter (SOM) mineralization (Grandy and Robertson, 2006a) and increased substrate supply to nitrification and denitrification after the incorporation of residues into the soil (Piva *et al.*, 2012). In contrast, daily N_2O fluxes under NT also continuously increased from 1.93±0.75 to 66.7±16.0 g N_2O-N ha^{-1} d^{-1} for the first 45 days after herbicide application but overall rates were approximately one third of those from under CT. Available C and N from decomposed dead grass and roots are likely reasons.

In the unfertilized fields studied here, available N could be one of the most important driving factors for N_2O emissions. Accelerated N mineralization from SOM and incorporated residue after tillage can increase available N and thus enhance nitrification and denitrification. Resin strip measurements indicate that for the 37 day period after CT tillage, soil NO_3^--N and NH_4^+-N concentrations under CT (57.7±7.16 and 2.30±0.41μg N cm^{-2}) were substantially higher than those under NT (12.1±1.59 and 0.31±0.08 μg N cm^{-2}), respectively. Daily N_2O fluxes were strongly correlated with total available N from resin strip measurements (N_2O fluxes=34.8×Exp (0.36×available N), R^2=0.19, P<0.01). However, NO_3^--N and NH_4^+-N concentrations in soil cores showed no consistent differences between CT and NT. This is likely because soil KCl extractions measure only the soil available N pool size. This pool can be rapidly utilized by microbes and plants or leached out of the soil so that it cannot be detected accurately, especially when the N pool size is small. In contrast, ion exchange strips measure both the soil available N pool and the flux of N ions through the mineral pool (Bowatte *et al.*, 2008). In my study the resin strips provided the more interpretable results.

Soil N_2O fluxes were also likely affected by available soil carbon (Dalal *et al.*, 2003; Wang *et al.*, 2011). Firstly, killed and incorporated brome grass, in conjunction with dead roots, provided heterotrophic denitrifiers with more available carbon and as well will have increased O_2 demand. CO_2 as an end product of decomposition indicated the extent to which dead brome grass was decomposed. Especially during the period between herbicide application (May 5) and tillage operations in the CT treatment (June 8), soil CO_2 emissions were 5.7 times, those of emissions from the unconverted reference field, indicating that more decomposition took place in the herbicide applied fields than in the reference field. In addition, the old CRP land had accumulated relatively high amounts of SOC, which has a potential to provide more available carbon for N_2O production due to SOC decomposition after tillage. Compared to SOC at nearby LTER experimental sites (Syswerda *et al*., 2011), SOC concentrations in my studied fields (21.3±0.8 g C kg^{-1} soil) prior to the conversion were comparatively higher than annual crops under CT (10. 4±3.4 g C kg^{-1} soil) and NT (11.5±0.4 g C kg^{-1} soil) and close to deciduous forest levels (24.0±3.4 g C kg^{-1}) for 0-20 cm depth. In addition, enhanced soil organic matter decomposition will consume oxygen and create localized anaerobic conditions favoring denitrification (Wang *et al*., 2011).

Soil N_2O fluxes are also affected by soil water content. Two relatively larger N_2O peaks occurred after rainfall in my study when WFPS% was >60%. The possible reason is that rainfall events create anaerobic conditions, which can stimulate N_2O emissions from denitrification. This finding has been reported by many studies (e.g. Elder and Lai 2008, Wang *et al*., 2011). However, in my study overall N_2O fluxes showed no significant correlation with soil moisture ($P>0.05$). Wet soil conditions did not necessarily give rise to high N_2O emission. For example, soil N_2O fluxes in the reference field remained low and stable through the whole study period even after considerable rainfall. Additionally, I observed low emissions of N_2O at all fields after September even when WFPS% was larger than 60% following rainfall. For both cases this indicates that N_2O production was likely restricted by other more limiting factors such as available N or low temperature.

The comparison between NT and CT N_2O fluxes has been widely studied and it is still difficult to generalize. Six *et al*. (2004) analyzed 44 comparisons of N_2O emissions under CT and NT globally and found higher N_2O emissions in the first 10 years of NT than CT and thereafter similar or lower N_2O emissions under NT. They argued that increased soil water content under NT promoted denitrification and thus enhanced N_2O production in the first 10 years. A more recent study using a meta-analysis of 239 direct comparisons between CT and NT/reduced tillage (Van Kessel *et al*., 2013) found no N_2O emissions differences. However, in my study, CRP land with its long-term no till history and high SOC may provide a special case. My results suggest that adopting NT practices can significantly reduce N_2O emissions compared to CT but NT management cannot eliminate the cost of N_2O emissions during CRP conversion.

CO_2 emissions

Soil CO_2 emissions under both CT and NT soybeans were significantly higher than in unconverted reference fields ($P<0.05$). Two possible reasons are 1) decomposition of dead grass and roots in the soil and 2) accelerated SOM decomposition after tillage. Additionally, soil CO_2 emissions in CT soybean were higher than emissions in NT soybean ($P<0.05$). Similar results have been reported in many studies (e.g. Grandy and Robertson, 2006a; Chatskikh and Olesen, 2007; Alluvione *et al*., 2009). Tillage enhanced SOC decomposition and thus increased CO_2 release to the atmosphere.

Soil CO_2 fluxes can be governed by soil temperature, moisture, and other factors. Multiple Linear regressions of soil CO_2 fluxes with soil temperature and WFPS% showed no significant correlation between CO_2 fluxes and WFPS%, although WFPS% might have affected CO_2 emission at some specific times during the drought period in July with its relatively low emissions. On the other hand, a positive relationship was found between soil CO_2 fluxes and soil temperature: soil CO_2 fluxes$=11.5 \times \mathrm{Exp}\ (0.07\times$ soil temperature), $R^2=0.21$, $P<0.01$).

Exponentially increased soil CO_2 fluxes with rising temperature have been reported by many studies (e.g. Lloyd and Taylor, 1994; Reichstein and Beer, 2008; Almaraz *et al*., 2009).

The NEE of CO_2 fluxes for CT soybeans was more than twice that for NT soybeans, and the converted fields under both CT and NT were net sources for CO_2. This is because carbon released from the decomposition of grass residue and SOC exceeded the carbon uptake from photosynthesis in converted fields. On the contrary, the unconverted reference field was a net sink for atmospheric CO_2.

CH_4 emissions

The range of daily CH_4 fluxes (-6.4 to 4.5 g CH_4-C ha^{-1} d^{-1}) I observed were similar to CH_4 fluxes of -1.80±0.06 g CH_4-C ha^{-1} d^{-1} for cropland in Michigan (Robertson *et al*., 2000). All fields were net sinks for CH_4, although some other studies found cropland under CT could be a small net source (Alluvione *et al*., 2009; Ussiri *et al*., 2009). Fluxes in CO_2 equivalents were negligible compared with CO_2 and N_2O fluxes, which had generally been reported for other upland cropping systems (Robertson *et al*., 2000; Wang *et al*., 2011).

No statistically significant differences in CH_4 oxidation rates were found among any treatments, although oxidation rates in CT were 62.9% and 38.8% lower than those in the NT and reference treatments, respectively. Similar results of no differences between CT and NT systems have been reported in some studies for sites nearby (Robertson *et al*., 2000; Suwanwaree and Robertson, 2005). However, other studies reported higher oxidation rates in NT than CT or uptake in NT but net emissions in CT (Ussiri *et al*., 2009). They attributed this to undisturbed soil structure and greater gas diffusion under NT. Another possible reason was that increased mineralization after tillage enhanced NH_4^+ production, and NH_4^+ could competitively inhibited CH_4 oxidation. Additionally, I found no significant difference in CH_4 oxidation before and after conversion of CRP land, although some studies have found the CH_4 oxidation rates of a grassland were reduced by 75% after only 8 months of conversion to CT cropland (Ball *et al.*, 1999) or higher CH_4 oxidation rates in midsuccessional grassland than cropland (Robertson *et al.*, 2000). It seems likely that CH_4 oxidation rates had not increased under 20 years of CRP brome grass sufficiently to be significantly re-suppressed by cropping.

CH_4 oxidation rates can also be regulated by soil water content and soil temperature. CH_4 oxidation rates were found negatively correlated with soil water content in some studies, probably due to limited CH_4 diffusion in the wet soil (Del Grosso *et al.*, 2000; Khalil and Baggs, 2005). However, CH_4 oxidation may be inhibited in dry soils (Khalil and Baggs, 2005). In my study, no apparent seasonal CH_4 flux patterns were observed. I found CH_4 fluxes were not significantly related with either WFPS% or soil temperature in any treatments, although other studies have shown CH_4 flux from NT to be negatively correlated with soil temperature (Ussiri *et al.*, 2009).

Global Warming Impact (GWI)

Over the study period (201 days), the GWI of converted soybean fields was 11.5 and 2.87 Mg CO_2e ha^{-1} for CT and NT operations, respectively, whereas the GWI of the unconverted CRP reference field was -3.5 Mg CO_2e ha^{-1} (Figure 3.6). The positive GWI of the converted fields indicates net GHG emissions to the atmosphere, while the negative GWI in the reference field indicates on-going GHG mitigation. The possibility that increased N_2O emissions might offset the enhanced soil carbon sequestration in NT systems has been a concern for adopting NT practices (Six *et al.*, 2002, Li *et al.*, 2005), but this was not the case in my study. NT played an important role in reducing GWI compared to CT, by significantly decreasing N_2O emissions and reducing SOC loss.

The CT system exhibited a net positive GWI of 11.5 Mg CO_2e ha^{-1}. In this system, about 39.3% of the GWI was contributed by N_2O production (4.52 Mg CO_2e ha^{-1}) even in the absence of synthetic N fertilizer additions. SOC loss as indicated by net CO_2 emissions contributed the remainder (60.7% or 6.98 Mg CO_2e ha^{-1}). For the NT system, net GWI was 2.87 Mg CO_2e ha^{-1}, about 55.0% of which was contributed by N_2O production (1.57 Mg CO_2e ha^{-1}) with the remaining 45% from CO_2 emissions (1.30 Mg CO_2e ha^{-1}). The contribution of CH_4 oxidation was negligible (<0.1%) under both CT and NT systems.

In contrast to converted fields, the unconverted reference fields showed a net mitigation potential of -3.50 Mg CO_2e ha^{-1} due to very low rates of N_2O production and a net uptake of CO_2.

The net mitigation potential for the unconverted reference fields indicates that the conversion of CRP land not only increases the emissions of GHGs, but also causes the loss of the CRP land's net GHG mitigation ability: 3.5 Mg CO_2e ha^{-1} mitigation would have happened had no conversion occurred. This foregone mitigation capacity must be added to the post-conversion GHG fluxes to provide a total net GWI (Gelfand *et al.*, 2011). This yields a total initial cost of 6.4 Mg CO_2e ha^{-1} for NT and 15.0 Mg CO_2e ha^{-1} for CT soybean. Thus, NT can reduce GHG costs by ~60% as compared to CT.

Robertson *et al.* (2000) calculated for a nearby site under the same soil series that NT practices sequestered 30 g C m^{-2} yr^{-1}. Based on this rate, CRP conversion by CT rather than NT cost ~8 years of NT carbon sequestration with a single tillage event.

Over time, this additional cost will change depending on future management. If planted perennial biofuel crops (no tillage and no N fertilization), the plowed soils will stop losing and begin re-accumulating soil carbon and N_2O fluxes will likely be low. In contrast, if planted annual grain crops that are plowed and fertilized every year, soil carbon will continue to be lost until the soil equilibrates (to ~10.4 g C kg^{-1} soil from annual crops under CT at the nearby KBS LTER site). N_2O production differences due to CT and NT will likely diminish (Van Kessel *et al*., 2013) but N_2O fluxes will continue to be high due to N fertilization (Hoben *et al*., 2011).

CONCLUSIONS

1. Conversion of CRP land substantially increased N_2O production. Tillage-induced fluxes were observed immediately and persisted for 30 to 40 days. Overall, mean daily N_2O emissions under CT were 2.8 times that of those under NT, and in both treatments, rates were substantially higher than in the reference field.
2. Conversion of CRP land also substantially enhanced soil CO_2 production. Tillage-induced fluxes were observed immediately and lasted for 20 days. Mean soil CO_2 fluxes under CT were 1.2 times those of NT and 2.6 times those of the unconverted CRP reference field.
3. CH_4 oxidation rates on a CO_2 equivalent basis were negligible compared to N_2O and CO_2 fluxes. All treatments were net sinks for atmospheric CH_4 but conversion under both CT and NT practices did not significantly change the oxidation rates relative to the unconverted reference.
4. The positive GWI of converted soybean fields under CT and NT indicates net GHG emissions to the atmosphere, whereas the negative GWI of the unconverted CRP reference field indicates on-going GHG mitigation. N_2O contributions to the conversion-induced GWI were higher than for CO_2 under both CT and NT systems.
5. NT can significantly reduce by ~60% but not eliminate the GHG emissions costs of CRP conversion as compared to CT.

CHAPTER 4: GREENHOUSE GAS EMISSIONS FROM TILLAGE DURING CONVERSION OF CONSERVATION RESERVE PROGRAM GRASSLANDS TO BIOFUELS

ABSTRACT

Expanding biofuel production is expected to accelerate the conversion of grassland to meet biomass feedstock needs. Greenhouse gas production during conversion jeopardizes ensuing climate benefits, but most research to date has focused only on conversion to annual crops and only following tillage. I studied conversion of Conservation Reserve Program (CRP) grassland to corn, prairie, and switchgrass following an initial soybean year with and without conventional tillage (CT). Over two years I found that CT conversion produced twice as much nitrous oxide (N_2O) and 20% more soil CO_2 emission compared to no-till (NT) conversion. Net ecosystem exchange of CO_2 was strongly positive for all CT treatments and negligible (prairie) or negative (corn and switchgrass) for NT. Methane oxidation was unaffected by treatment. Overall, my results show that, by reducing N_2O and whole ecosystem CO_2 emissions, NT practices can reduce the global warming impact of grassland conversion compared to CT.

INTRODUCTION

Biofuels have been proposed as an option for reducing greenhouse gas (GHG) emissions. In the U.S, the Energy Independence and Security Act of 2007 (EISA) mandates an increase in ethanol biofuel production to 36 billion gallons by 2022: 15 billion gallons from corn (*Zea mays* L.)-based ethanol and 21 billion from cellulosic or other non-corn-based ethanol sources. Expanding biofuel crop production will correspondingly increase demand for land, and together with high prices for corn is expected to induce the further conversion of grasslands such as those in the USDA Conservation Reserve Program (CRP) back to crop production (Secchi et al. 2009, Wright and Wimberly, 2013).

Conversion of CRP and other uncropped grasslands back to production can lead to carbon (C) loss, as modeled for conversion to annual biofuel crops by Fargione et al. (2008) and as measured for conversion to no-till (NT) crops by Gelfand et al. (2011). Fargione et al. (2008) estimated a C debt of 134 Mg CO_2 equivalents (CO_2e) ha^{-1} during grassland conversion to corn. Gelfand et al. (2011) reported an initial C debt of 10.6 CO_2e ha^{-1} during the first year conversion of CRP grassland to NT soybeans (*Glycine max* (L.) Merr.), and Zenone et al. (2013) reported a 3-year debt of 18.1 CO_2e ha^{-1} for subsequent conversion to NT corn and 14.2 CO_2e ha^{-1} for subsequent conversion to NT switchgrass and native prairie.

Still missing, however, are measured GHG costs for converting CRP grasslands using conventional tillage (CT), which is today the most common means for clearing grassland for cultivation. I know from recent work (Ruan and Robertson, 2013) that chisel plowing can cause 2-3 times more C debt than NT during the first year of conversion due to substantial nitrous oxide (N_2O) emissions, accelerated soil organic matter (SOM) decomposition, and foregone C sequestration. Unresolved, however, is the persistence of this effect in subsequent years: whether the C debt continues to mount or can begin to be repaid.

In addition to SOM decomposition and N_2O release, grassland conversion can also affect soil methane (CH_4) oxidation. Aerated soils are a globally important sink for atmospheric CH_4 because of CH_4 oxidation by methanotrophic bacteria (Dalal et al., 2008). Oxidation is substantially reduced by cultivation (Ball et al., 1999; Del Grosso et al., 2000) but can partially recover on conversion to grassland (Levine et al., 2011). Methane oxidation that would have occurred in the future but will not occur upon converting grassland back to cropland might thus be another source of C debt – here called foregone CH_4 oxidation.

In addition to tillage, choice of biofuel crop can also substantially affect the resulting GHG balance. There are well known differences in environmental impacts between annual grain-based and perennial cellulosic crops (Farrell et al., 2006; Fargione et al., 2010) including those related to C debt, net GHG balance, and fertilizer N loss; less is known about to what extent perennial grasses might offset the GHG costs of tillage production.

Here I report the results of an experiment intended to quantify the initial tillage-induced costs of biofuel crop establishment. On land just converted from grassland to no-till soybean I provide a net GHG balance for the first two years of corn, native prairie, or switchgrass establishment under conventional vs. NT management. I hypothesize that continued NT management will substantially lower the net GHG costs associated with establishment by reducing N_2O and CO_2 emissions and by avoiding the loss of CH_4 oxidation as compared to conventional tillage, regardless of biofuel crop types.

MATERIALS AND METHODS

Site description

Four experimental fields (9-21 ha each) were established at the Kellogg Biological Station's Great Lakes Bioenergy Research Center (GLBRC) Marshall Farm site in southwestern Michigan (42^{o} 26' N, 85^{o}19' W, elevation 288 m). Annual precipitation averages 1,027 mm with an average snowfall of ~1.4 m. Mean annual temperature is 9.9 °C ranging from a monthly mean of -4.2 °C in January to 22.8 °C in July (Robertson and Hamilton 2014). Soils developed on glacial outwash and in all four fields are mesic Typic Hapludalfs of three intermixed series: Boyer (loamy sand), Kalamazoo (fine-loamy) and Oshtemo (coarse-loamy).

All four fields were in agricultural row crops and then enrolled in CRP with smooth brome (*Bromus inermis* Leyss) planted in 1987 (Zenone et al., 2013). In 2009 the brome grass in three of the fields was killed with glyphosate prior to planting NT soybeans, which were harvested in fall 2009. The fourth field was reserved as a reference site, unconverted. Soil C and N contents were lower than in the unconverted reference field (Table 4.1).

Experimental design and treatments

In Spring 2010 each of the converted fields was assigned to either corn (*Zea mays*), prairie (mainly *Elymus canadensis*, *Schizachyrium scoparium*, *Sorghastrum nutans, Rudbeckia hirta*, and *R. triloba*), or switchgrass (*Panicum virgatum*) and I established 3 replicate NT and CT plots (20 m × 5m) in each field. The remainder of each field was managed without tillage (NT). I also randomly identified four replicate plots to sample in the reference field, for an overall total of 22 plots (3 fields × 2 treatments × 3 replicate plots] + [1 reference field × 4 replicate plots]).

In the corn field, primary tillage in the three CT plots consisted of chisel plowing (25 cm deep) with secondary tillage performed with a disc harrow. Tillage took place on April 28, 2010, and again on May 6, 2011. NT plots were left untilled. Corn (Dekalb DK-52) was planted at a density of 69,000 seeds ha^{-1} in 70-cm row widths using a no-till planter on April 29, 2010, and on May, 12, 2011. In 2010 liquid Urea Ammonium Nitrate (UAN; 28%) was injected at a rate of 32 kg N ha^{-1} on 29 April and then side-dressed at a rate of 114 kg N ha^{-1} on 9 June. In 2011, 34 kg N ha^{-1} was injected as UAN on 12 May and then another 137 kg N ha^{-1} was side-dressed on 21 June. In both years, herbicide [Lumax (5.9 L ha^{-1}), Atrazine 4L (0.78 L ha^{-1}), Honcho Plus (2.4 L ha^{-1}), and $(NH_4)_2SO_4$ (0.92 kg ha^{-1})] was applied 1-2 weeks after planting. Corn was harvested in late October, 2010 and early November, 2011.

In the prairie field, a mix of native prairie species with oats (*Avena sativa*) as a first-year nurse crop was planted on June 8, 2010. Conventional-till plots were plowed once on June 7, 2010, as in the corn field and the NT plots were left untilled. No N fertilizers were applied, and the field was harvested for the first time in late October, 2011.

In the switchgrass field, switchgrass was planted with oats on June 8, 2010. Conventional-till plots were plowed once on June 7, 2010, as in the corn field and the NT plots were left untilled. N fertilizer (UAN) was applied on July 7, 2011, at a rate of 56 kg N ha^{-1}. Switchgrass was first harvested in late October, 2011.

Trace gas fluxes and net greenhouse gas balance

Gas fluxes were determined using a static chamber approach (Ruan and Robertson, 2013). Before sampling, three stainless steel chambers (28 cm diameter × 30 cm high) were permanently installed to a soil depth of 5 cm in each treatment plot in the non-reference fields, and one chamber was installed in each of the 4 reference field plots, for a total of 58 chambers. Gas sampling was generally performed 1-2 times per week during the growing season and then every other week thereafter.

For each 1-hr gas sampling period each chamber was tightly covered with a lid and its headspace sampled four times with a 10 mL syringe. Gas samples (10 mL) were transferred to 5.6 mL glass vials (Labco Ltd., High Wycombe, UK) that had been pre-flushed with 10 mL of headspace taken immediately prior to the sample. Within 24 hr of collection, CO_2 was analyzed using an infrared gas absorption analyzer (LI-820 CO_2 analyzer, LI-COR, Lincoln, NE, USA). N_2O and CH_4 were analyzed by gas chromatography (Hewlett Packard 5890 Series II, Rolling Meadows, IL, USA), for which gases were separated on a Porapak Q column (1.8 m, 80/100 mesh) at 80 ˚C. N_2O was detected with a ^{63}Ni electron capture detector at 350 ˚C and CH_4 was detected with a flame ionization detector at 300 ˚C.

I interpolated daily gas fluxes between sampling times to estimate annual fluxes of N_2O, CO_2, and CH_4. Global warming impact (GWI) was calculated by multiplying fluxes of each gas by its global warming potential: 1 for CO_2, 25 for CH_4, and 298 for N_2O (IPCC, 2007a) to yield CO_2e.

To calculate the total GHG balance for each treatment I incorporated data on the net ecosystem exchange (NEE) of CO_2 measured with eddy covariance towers in the center of each field from Zenone *et al*. (2013). The eddy-covariance system included a LI-7500 open-path infrared gas analyzer (IRGA) (Li-Cor Biosciences, Lincoln, NE, USA), a CSAT3 three-dimensional sonic anemometer (Campbell Scientific Inc., Logan, UT, USA), and a CR5000 data logger (Campbell Scientific Inc.). The effective measurement radius of each tower was approximately 200 m and every 30-min NEE was calculated as the covariance of vertical wind speed and the concentration of CO_2 as described in Zenone *et al*. (2013). I estimated the NEE for CT treatments as the sum of NEE for NT treatments plus the difference in chamber measurements of CO_2 fluxes between CT and NT treatments as described in Ruan and Robertson (2013). This method assumes that CT and NT treatments in each field captured the same amount of CO_2 via photosynthesis (as indicated by similar yields for CT and NT treatments), and that CO_2 fluxes from plant and herbivore respiration were likewise similar.

In addition, I also calculated fossil fuel offset credits (Mg CO_2e ha^{-1} yr^{-1}) as avoided CO_2 emissions due to the displacement of fossil fuel use by biofuels, considering the production, transportation, distribution, combustion and coproducts allocation according to Plevin (2009). Avoided CO_2e emissions were calculated from life cycle analyses of the difference in CO_2e emissions between petroleum gasoline vs. ethanol from dry corn grain and grass. Then the fossil fuel offset credit was calculated as:

$$\text{Fossil fuel offset credit (Mg CO}_2\text{e ha}^{-1}\text{ yr}^{-1}) = e\ \frac{\text{g CO}_2\text{e}}{\text{MJ}} \times f\ \frac{\text{MJ}}{\text{ha yr}} \times \frac{10^{-6}\text{ Mg}}{1\text{ g}} \qquad \text{(Eq. 1)}$$

Where: e (g CO_2e MJ^{-1} yr^{-1}) is the difference in CO_2e emissions from life cycle analyses of petroleum gasoline and ethanol from dry corn grain or grass. Gasoline releases 94 g CO_2e per MJ gasoline produced, distributed, and combusted (Farrell et al, 2006; Wang et al., 2012). Net CO_2e emissions per MJ of dry corn grain and grass ethanol were calculated using the GREET (Greenhouse gases, Regulated Emissions, and Energy use in Transportation) model (Huo et al., 2009) with all farming inputs set to 0. f is the total ethanol energy equivalent from biomass, estimated from harvested biomass as:

$$f\,(\text{MJ ha}^{-1}\text{ yr}^{-1}) = w\ \frac{\text{Mg Dry biomass}}{\text{ha yr}} \times c\,\frac{\text{L fuel}}{\text{Mg dry biomass}} \times d\ \frac{\text{MJ energy}}{\text{L fuel}} \qquad \text{(Eq.2)}$$

Where: w is the harvested dry biomass (Mg ha^{-1} yr^{-1}); c is the conversion factor for cellulosic biomass to ethanol (430 L bioethanol Mg^{-1} dry corn grain and 380 L bioethanol Mg^{-1} dry grass biomass) (Schmer et al., 2006; Gelfand et al., 2011); and d is ethanol energy content (21.1 MJ L^{-1}) (lower heating value) (Gelfand et al., 2011, 2013).

The net GHG balance was then calculated as the CO_2e sum of directly measured field GHG fluxes and indirectly calculated agricultural inputs (Appendix Table B.1) less carbon offset credits.

Soil sampling

I measured soil temperature, gravimetric water content, and ammonium (NH_4^+) and nitrate (NO_3^-) concentrations at each gas sampling event. Soil gravimetric water content (g water/ g dry soil) for the 0-25 cm soil layer was determined by oven-drying soils at 60 ˚C for 48 hours. For measuring NH_4^+ and NO_3^-, three 2.5 cm diameter cores (0-25 cm depth) randomly collected within each treatment plot were composited and passed through a 4 mm sieve. Three 10 g subsamples were then each extracted with 100 mL of 1 M KCl. Filtrates from soil extracts were analyzed on a Flow Solution IV colorimetric analyzer (OI Analytical, College Station, TX, USA).

Soil bulk density (BD) (0-25 cm depth) was measured three times each in 2010 and 2011 using a fixed-volume core (123 cm^3) for each treatment plot. Water-filled pore space (WFPS %) was then calculated as

$$\text{WFPS\%} = \frac{\text{Gravimetric water content (\%, g / g)} \times \text{BD (g cm}^{-3}\text{)}}{\text{[water density (1 g cm}^{-3}\text{)} \times \text{soil porosity (\%)]} \times 100\%}$$

Where soil porosity = 1- BD (g cm^{-3}) / particle density (g cm^{-3}). Particle density was assumed to be 2.65 g cm^{-3}.

Data analysis

Treatment differences were analyzed using one-way ANOVA in SAS 9.2 (SAS Institute, Cary, NC, USA). Treatment means were compared for significance using t-tests at the α=0.05 significance level. Multiple linear regressions (stepwise) between daily gas fluxes and influencing factors were performed in PROC REG and nonlinear regressions in PROC NLIN. Normality of the residuals and homogeneity of variance assumptions were checked using stem-and-leaf box and normal probability plots of the residuals, and Levene's test. Data were not transformed prior to analysis.

RESULTS

Soil N_2O fluxes and inorganic N

In CT corn during 2010, N_2O fluxes increased immediately after tillage on April 28, when fluxes were 56.6±13.7 (mean ± standard error) g N_2O-N ha^{-1} d^{-1} as compared to 3.05±0.79 g N_2O-N ha^{-1} d^{-1} in NT corn (Figure 4.1a). For the 42 days between plowing and side-dress N application, average daily N_2O fluxes were substantially higher in CT vs. NT (105±20.7 vs. 21.5±7.3 g N_2O-N ha^{-1} d^{-1}, respectively, $P<0.05$). After side-dressing, N_2O fluxes remained high for ~15 days in both CT and NT treatments (63.2±7.34 and 67.0±7.40 g N_2O-N ha^{-1} d^{-1}, respectively). In both treatments the three largest fluxes occurred on 14 May, 3 June, and 16 June, after rainfall events elevated soil moisture to > 50% WFPS. After mid-July, N_2O fluxes were low in both CT and NT treatments regardless of WFPS.

In CT corn during 2011, soil N_2O fluxes also increased immediately after tillage, to 19.7±3.79 g N_2O-N ha^{-1} d^{-1} on 4 May as compared to 1.16±0.41 g N_2O-N ha^{-1} d^{-1} in the NT treatment. Following N fertilizer added at planting on 12 May, by 16 May N_2O fluxes increased to 62.1±5.0 and 61.2±25.1 g N_2O-N ha^{-1} d^{-1}, respectively, in the CT and NT treatments. After side-dressing on 21 June, N_2O fluxes increased again to 219±31.9 and 232±49.7 g N_2O-N ha^{-1} d^{-1}, respectively. Additional peaks occurred in both treatments on 12 July, 3 August, and 18 August after rainfall events elevated soil moisture to > 60% WFPS.

In prairie and switchgrass fields during 2010, soil N_2O fluxes were low in April and May in both CT and NT treatments. In CT plots N_2O fluxes increased immediately after tillage on 7 June to 241±98.8 and 195±102 g N_2O-N ha^{-1} d^{-1} in prairie and switchgrass fields, respectively, as compared to 5.9±2.1 and 10.8±5.8 g N_2O-N ha^{-1} d^{-1} in the respective NT plots (Figure 4.1b, 4.1c). Tillage-induced fluxes lasted for 20 to 30 days. Other large fluxes occurred in only CT treatment on 16 June and 23 June after rainfall events.

In prairie fields during 2011, tillage did not occur and N_2O fluxes remained low (<6.43 g N_2O-N ha^{-1} d^{-1}) in both CT and NT treatments throughout the year (Figure 4.1b). In switchgrass

Table 4.1 Soil properties (0-25cm depth) of the study fields (mean ±standard error, n=10). SOC = Soil Organic Carbon; N = nitrogen.

Crop	SOC ($g\ kg^{-1}$ soil)	Total N ($g\ kg^{-1}$soil)	Bulk density ($g\ cm^{-3}$)	pH
Corn	18.3±1.00	1.67±0.09	1.62±0.01	5.8±0.09
Prairie	16.1±1.38	1.49±0.12	1.42±0.03	6.1±0.17
Switchgrass	17.1±1.03	1.43±0.09	1.55±0.01	5.8±0.06
Reference	21.2±1.31	1.96±0.12	1.50±0.03	6.1±0.04

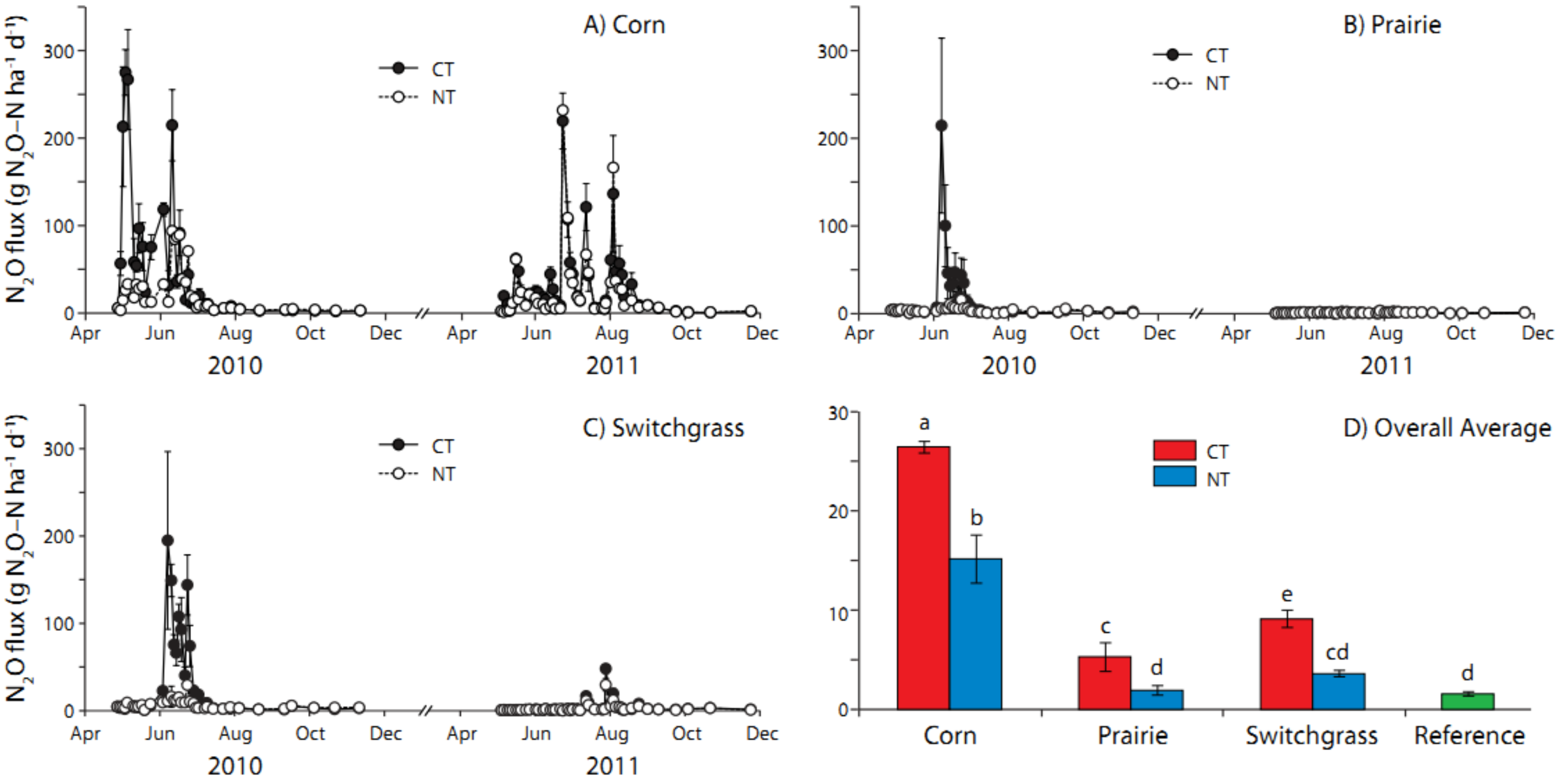

Figure 4.1. Daily N_2O fluxes by treatment (CT vs. NT) in corn, prairie, and switchgrass fields, respectively (panels a-c) in 2010 and 2011. Error bars represent standard errors of mean N_2O emissions based on n=3 replicate plots. Panel (d) shows average N_2O fluxes for CT and NT in corn, prairie and switchgrass fields (n=3 replicate plots) and an unconverted CRP reference field (n=4 replicate plots). Treatments marked with different letters for each crop are significantly different from one another (P<0.05).

fields, fluxes in both CT and NT treatments responded to the 7 July fertilizer application and on 28 July reached 48.0±4.91 and 29.5±6.9 g N_2O-N ha^{-1} d^{-1} in CT and NT treatments, respectively. Two additional peaks occurred in both treatments on 3 August and 24 August after rainfall events. N_2O fluxes diminished and stayed low after September (Figure 4.1c).

N_2O fluxes from the reference field were low in both 2010 and 2011 (<8.21 g N_2O-N ha^{-1} d^{-1}) regardless of rainfall events.

Overall, for the 2-year study period, N_2O emissions in corn were significantly higher than those in prairie, switchgrass, and the reference field ($P<0.05$) (Figure 4.1d). Switchgrass emitted more N_2O under CT than did CT prairie, but no difference was found between prairie and switchgrass under NT. Mean daily N_2O emissions in CT were 1.75, 2.67 and 2.51 times those of NT in corn, prairie and switchgrass fields, respectively (Figure 4.1d). For both CT and NT treatments, emissions were significantly higher than in the reference field (26.4±0.57 in CT corn, 5.30±1.46 in CT prairie, 3.65±0.31 in CT switchgrass vs. 15.1±2.38 in NT corn, 1.98±0.50 in NT prairie, 9.17±0.85 in NT switchgrass vs.1.66±0.20 g N_2O-N ha^{-1} d^{-1} in the reference; $P<0.05$) (Figure 4.1d).

In 2010, average soil inorganic N contents were significantly higher in CT than in NT treatments ($P<0.05$) in all 3 fields. In 2011, soil inorganic N differences were significant only in the corn field (Table 4.2).

Average daily N_2O fluxes in each year were strongly correlated with soil inorganic N across all treatments ($R^2=0.63$, $P<0.01$) (Figure 4.2). Daily soil moisture (%WFPS) also showed a significant but extremely weak positive correlation with daily N_2O fluxes ($R^2=0.01$, $P<0.001$).

Soil CO_2 fluxes

Across all fields in 2010 and 2011, chamber-based soil CO_2 fluxes generally

Table 4.2 Average soil inorganic nitrogen (0-25 cm depth) of the study fields during April-December in 2010 and 2011 (mean ±standard error, n=3). Treatments marked with different letters within each crop are significantly different from one another (α=0.05).

Crop type	Tillage type	2010	2011
Corn	CT	15.7±1.34^{a}	33.9±7.38^{a}
	NT	9.14±0.90^{b}	16.1±3.22^{b}
Prairie	CT	8.90±0.54^{a}	5.30±0.14^{c}
	NT	7.26±0.09^{b}	5.46±0.28^{c}
Switchgrass	CT	7.96±0.15^{a}	6.35±0.38^{c}
	NT	6.33±0.06^{b}	6.45±0.10^{c}
Reference		4.93±0.15^{c}	5.51±0.16^{c}

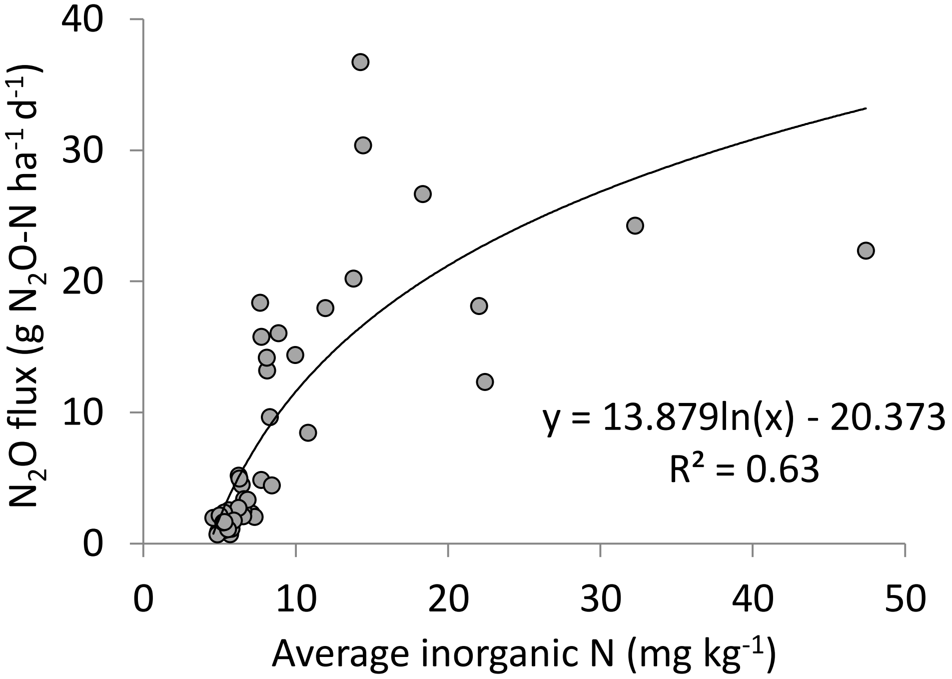

Figure 4.2. Relationship of average daily N_2O emission rates across all treatments (n=44) to mean soil inorganic nitrogen (0-25 cm) in 2010 and 2011.

demonstrated a seasonal trend with higher emissions from June-August and lower emissions before May and after October (Figure 4.3), coincident with the seasonal trend in air temperatures (Appendix Figure B.1). In 2010, immediately after CT tillage on 28 April in corn fields and 7 June in prairie and switchgrass fields, soil CO_2 fluxes increased to 97.4±16.6, 105.1±2.8, and 86.6±2.0 kg CO_2-C ha^{-1} d^{-1}, respectively, as compared to respective NT fluxes of 27.0±3.3, 49.1±6.5, and 32.7±0.9 kg CO_2-C ha^{-1} d^{-1} (Figure 4.3). Tillage-induced CO_2 fluxes persisted ~30-40 days. In 2011, tillage-induced fluxes in corn persisted only for ~7 days (average 58.6±7.27 kg CO_2-C ha^{-1} d^{-1} under CT vs. 30.0±2.07 kg CO_2-C ha^{-1} d^{-1} under NT). In contrast, there were no treatment effects on CO_2 emissions in the prairie or switchgrass fields (Figure 4.3a-c).

Over the 2-year study period, all cropped fields released more soil CO_2 than the reference field ($P<0.05$) (Figure 4.3d). Soil CO_2 emissions under CT corn were higher than those in CT prairie and switchgrass but no difference was found under NT across crops. Mean soil CO_2 fluxes in the CT treatment increased by 27.2%, 13.8% and 12.2% in the corn, prairie, and switchgrass fields, respectively, as compared to the NT treatment (48.4±1.3 vs. 39.3±0.7 kg CO_2-C ha^{-1} d^{-1} in corn; 44.7±1.5 vs. 39.3±1.4 kg CO_2-C ha^{-1} d^{-1} in prairie; 43.7±0.9 vs. 39.0±0.3 kg CO_2-C ha^{-1} d^{-1} in switchgrass fields, $p<0.05$). Across all non-reference fields, CT fluxes were 29-43% higher and NT fluxes were averagely ~15% higher than those in the reference field (33.9±2.85 kg CO_2-C ha^{-1} d^{-1}; $p<0.05$).

Daily soil CO_2 fluxes were positively correlated with soil temperature: soil CO_2 fluxes = $15.9 \times e^{0.06 \times \text{soil temperature}}$ ($R^2=0.49$, $P<0.001$) (Figure 4.4). I found no significant relationship between daily CO_2 fluxes and soil moisture (WFPS %).

Soil CH_4 fluxes

During 2010 and 2011, all fields exhibited both positive and negative daily CH_4 fluxes

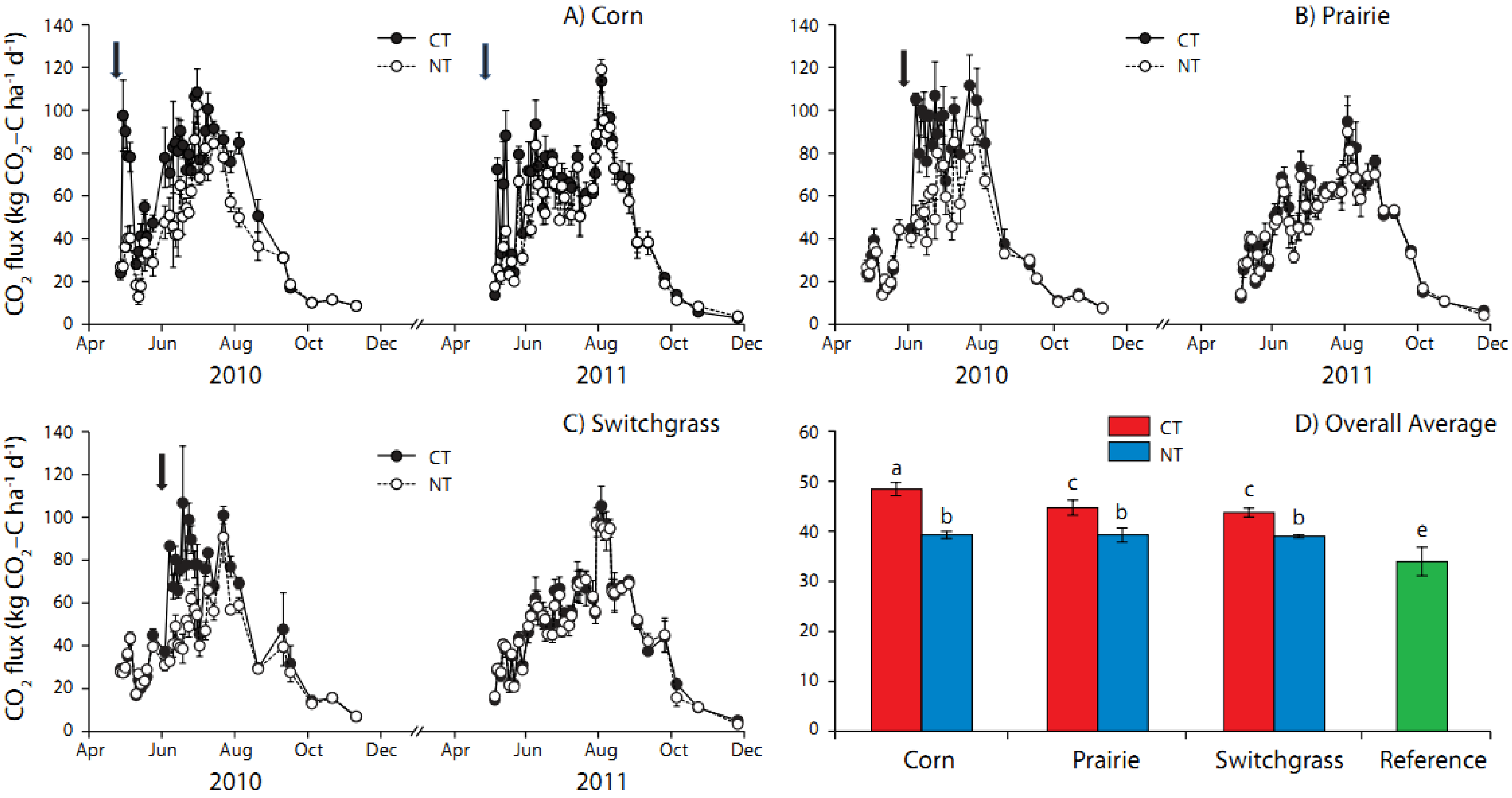

Figure 4.3. Daily CO_2 fluxes by treatment (CT vs. NT) in corn, prairie and switchgrass fields, respectively (panel a-c) in 2010 and 2011. Error bars represent standard errors of mean CO_2 emissions based on n=3 replicate plots. Panel (d) shows average CO_2 fluxes for CT and NT in corn, prairie, and switchgrass fields (n=3 replicate plots) and an unconverted CRP reference field (n=4 replicate plots). Treatments marked with different letters within each crop are significantly different from one another (P<0.05).

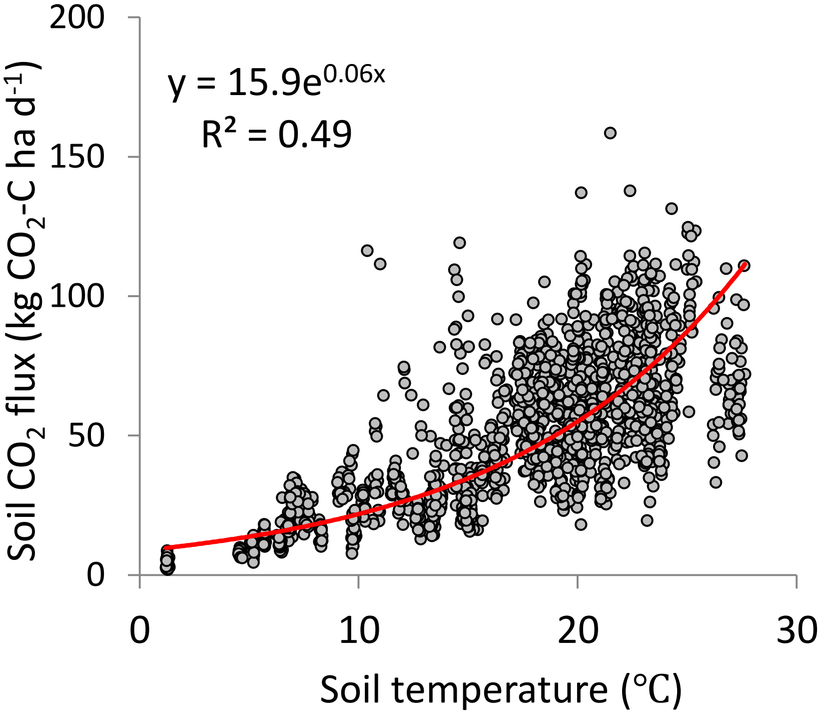

Figure 4.4. Relationships of daily CO_2 fluxes across all treatments (n=1528) to soil temperature (0-25 cm) in 2010 and 2011.

without consistent seasonal patterns or significant treatments effect. Fluxes were generally low, with most rates ranging from -4.13 to 4.27 g CH_4-C d^{-1} ha^{-1} (Figure 4.5a-c). Average daily CH_4 fluxes were negative in all treatments, indicating net CH_4 oxidation or uptake (Figure 4.5d). Mean CH_4 oxidation rates under NT were greater by 132% in corn and 45.9% in prairie, respectively, as compared to those under CT (-0.72±0.11 vs. -0.31±0.17 g CH_4-C d^{-1} ha^{-1} in corn; -1.37±0.20 vs. -0.94±0.44 g CH_4-C d^{-1} ha^{-1} in prairie), but the differences were not statistically significant ($P>0.10$). Likewise, mean CH_4 oxidation rates in CT corn, prairie and switchgrass fields were only 16.4%, 50.3% and 43.7% of those in the reference field, but these differences were also not statistically significant (Figure 4.5d). No measured soil or environmental factor was found to correlate significantly with CH_4 fluxes.

Biomass Yields

Over the 2-year study period, no significant differences in mean annual dry biomass yields were found between CT and NT treatments across any of the three cropped fields (respectively, 11.5±0.4 vs. 11.3±0.6 Mg ha^{-1} for corn; 4.72±0.67 vs. 5.73±0.63 Mg ha^{-1} for prairie; and 5.74±0.24 vs. 6.38±0.22 Mg ha^{-1} for switchgrass, $p>0.05$) (Figure 4.6). Between the two perennial grasses, prairie yields under CT and NT were 82% and 90% of those under CT and NT in switchgrass, respectively, although the treatment difference were not statistically different ($P>0.1$).

Global Warming Impact (GWI)

The mean annual NEE of CO_2 (May-December) for CT corn, prairie, and switchgrass fields all exhibited positive values (net C emissions) from soil (3.91±0.49, 4.57±0.19 and 0.50±0.87 Mg CO_2e ha^{-1}, respectively) (Figure 4.7a). In contrast, mean NEE of CO_2 for NT corn,

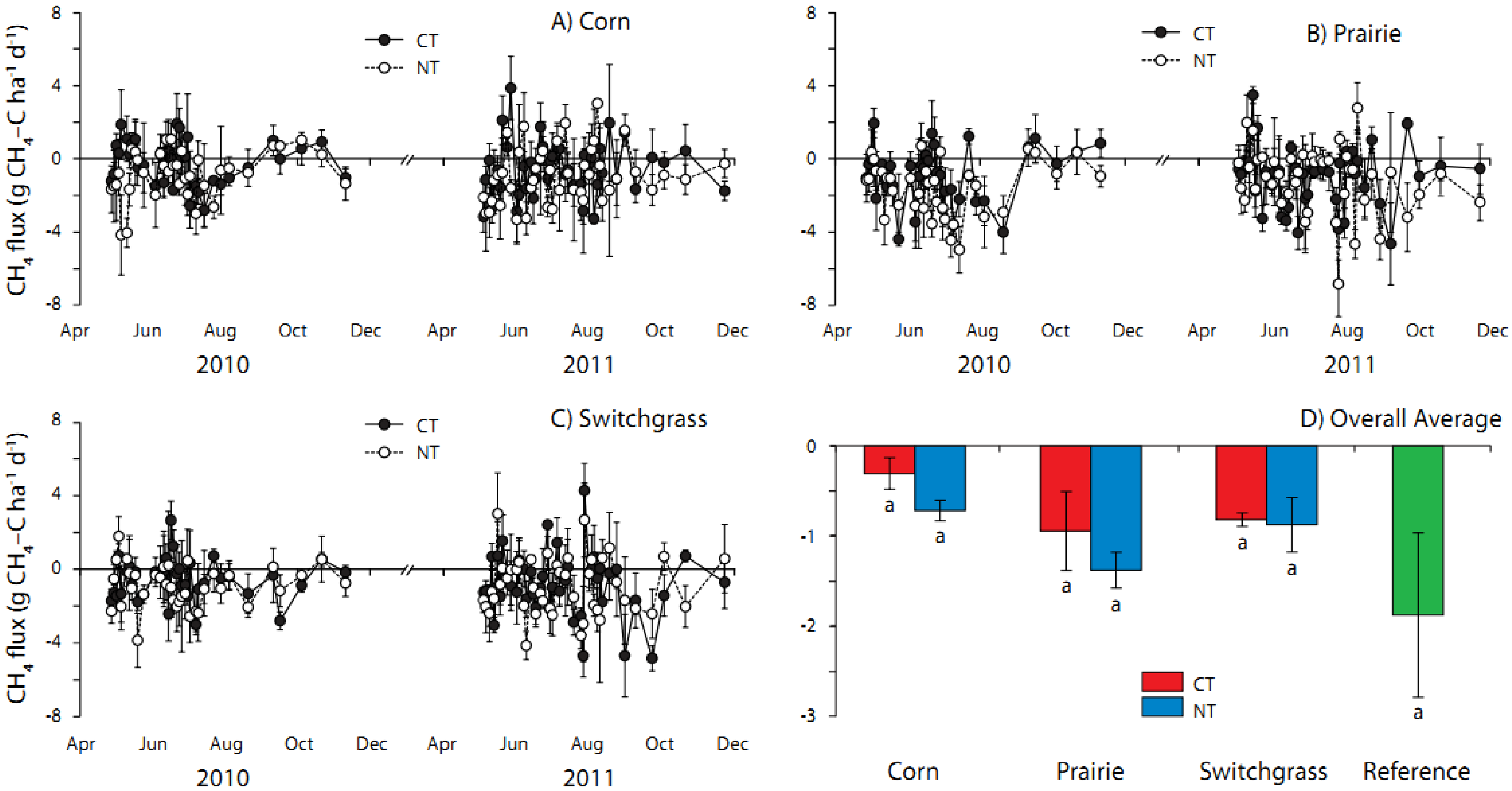

Figure 4.5. Daily CH$_4$ fluxes by treatment (CT vs. NT) in corn, prairie and switchgrass fields, respectively (panel a-c) in 2010 and 2011. Error bars represent standard errors of mean CH$_4$ emissions based on n=3 replicate plots. Panel (d) shows average CH$_4$ fluxes for CT and NT in corn, prairie and switchgrass fields (n=3 replicate plots) and an unconverted CRP reference field (n=4 replicate plots). Treatments marked with different letters within each crop are significantly different from one another (P<0.05).

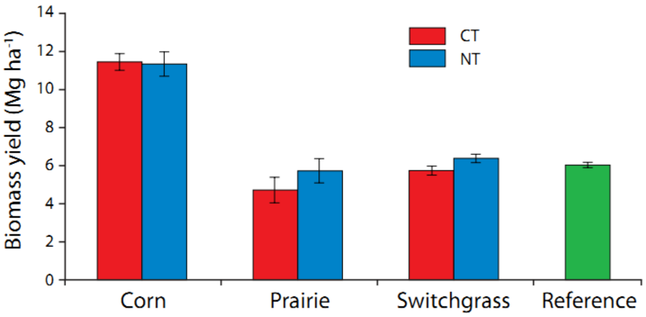

Figure 4.6. Mean dry biomass aboveground biomass production (grain yield for corn) under CT and NT in 2010 and 2011 (Mg ha^{-1}). Error bars represent standard errors based on n=3 replicate plots. No significant difference of yields across all treatments ($P>0.05$).

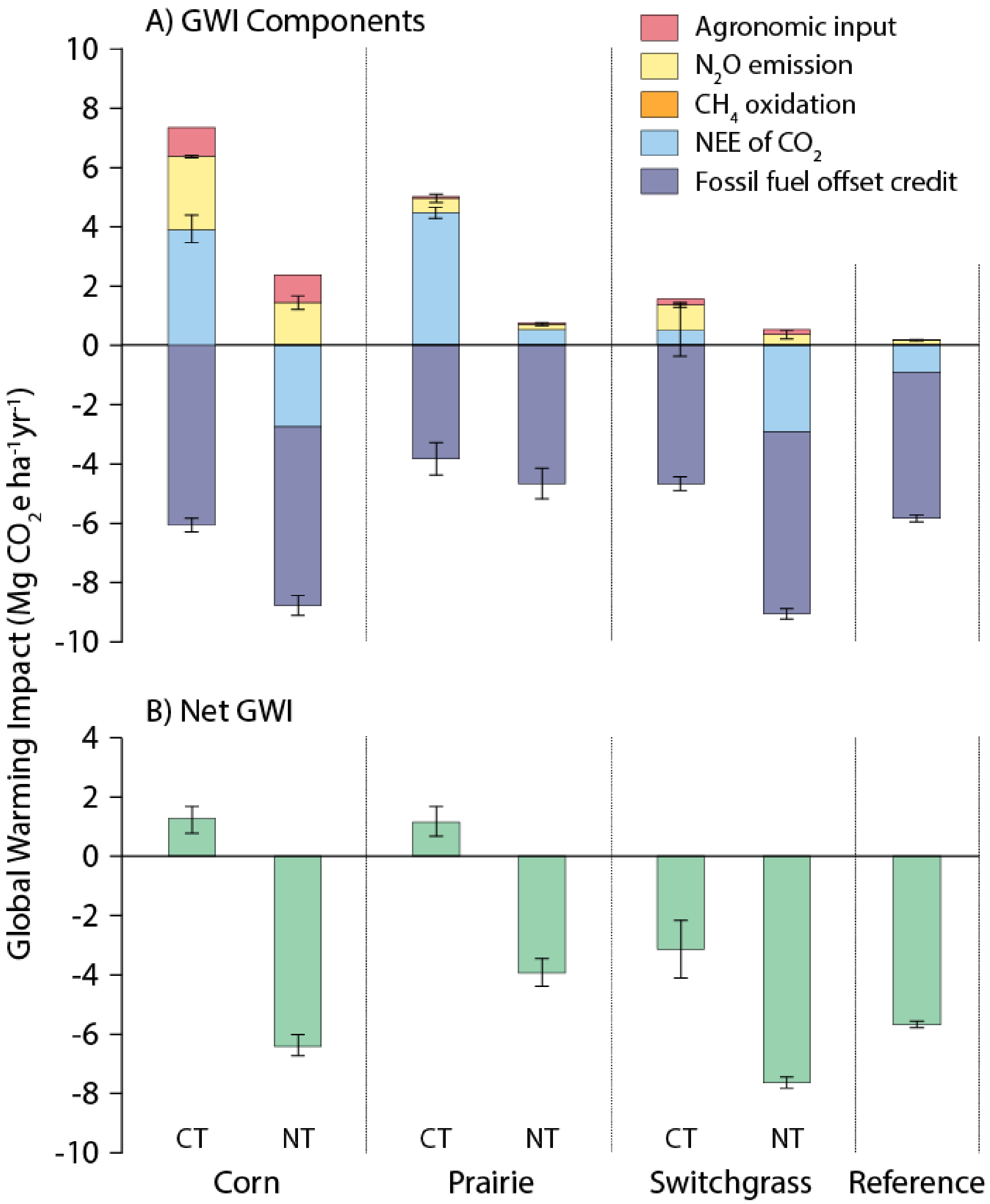

Figure 4.7. Annual GHG balances of biofuel crop production in CT, NT and reference sites. a) detailed GHG balance; b) net GHG balance. See Appendix Table B.1 for GHG emissions from agriculture inputs. N_2O and CH_4 represent mean in-situ measurements in 2010 and 2011. CH_4 oxidation rates are negligible and not visible in graph. Error bars represent standard errors based on n=3 replicate plots (no error bars for agricultural input).

prairie, and switchgrass fields exhibited negative (net C uptake) or very low positive values (-2.76, 0.51 and -2.94 Mg CO_2e ha^{-1}, respectively).

The CO_2e associated with N_2O emissions were higher in corn (2.48±0.05 Mg CO_2e ha^{-1} under CT and 1.43±0.22 Mg CO_2e ha^{-1} under NT) than prairie (0.49±0.14 Mg CO_2e ha^{-1} under CT and 0.18±0.05 Mg CO_2e ha^{-1} under NT), switchgrass (0.85±0.08 Mg CO_2e ha^{-1} under CT and 0.34±0.14 Mg CO_2e ha^{-1} under NT) and reference fields (0.16±0.02 Mg CO_2e ha^{-1}) (Figure 4.7a).

Methane oxidation contributions to the GHG balance across all fields were negligible, on the order of −0.002±0.001 to −0.01±0.006 Mg CO_2e ha^{-1} (Figure 4.7a). CO_2e emissions from agricultural inputs (Table B.1) were similar between CT and NT treatments but higher in corn than in prairie and switchgrass (Figure 4.7a).

Fossil fuel offset credits of 58.6 and 102.0 g CO_2e MJ^{-1} were calculated for avoided fossil fuel C emissions due to the use of biofuels from dry corn grain and grass, respectively. This results in fossil fuel offset credits for corn of -6.09±0.23 under CT and -6.03±0.34 Mg CO_2e ha^{-1} yr^{-1} under NT; for prairie credits of -3.85±0.55 under CT and -4.68±0.52 Mg CO_2e ha^{-1} yr^{-1} under NT; for switchgrass credits of -4.69±0.19 under CT and -5.22±0.18 Mg CO_2e ha^{-1} yr^{-1} under NT; and for the reference field credits of -4.93±0.12 Mg CO_2e ha^{-1} yr^{-1}(were the reference field harvested for biofuel production). Fossil fuel offset credits did not differ by tillage treatment in any field (Figure 4.7a).

In general, annual net GWIs under CT were significantly higher than those under NT in corn, prairie, and switchgrass (for corn 1.26±0.45 under CT vs. -6.42±0.36 Mg CO_2e ha^{-1} yr^{-1} under NT; for prairie 1.14±0.50 under CT vs. -3.94±0.47 Mg CO_2e ha^{-1} yr^{-1} under NT; for switchgrass -3.15±0.97 under CT vs. -7.65±0.19 Mg CO_2e ha^{-1} yr^{-1} under NT) (Figure 4.7b). Both CT corn and CT prairie had positive GWIs, and all GWIs under CT were significantly higher than those from the reference field (-5.69±0.11 Mg CO_2e ha^{-1} yr^{-1}). GWIs under NT corn were not different from the reference field. In contrast, GWIs under NT prairie were significantly lower than the reference but GWIs under NT switchgrass were higher than the reference ($P<0.05$) (Figure 4.7b).

DISCUSSION

My results support the hypothesis that NT management can mitigate N_2O and CO_2 emissions compared to CT management during CRP grassland conversion to biofuel production. I found no significant tillage effect on CH_4 oxidation. The beneficial effect of NT was especially pronounced for conversion to corn, where the GHG cost was 6 times greater for CT than for NT conversion (1.26±0.45 vs. -6.42±0.36 Mg CO_2e ha^{-1}). Even for cellulosic crops, however, the benefit was substantial – NT conversion provided 1-4 times greater mitigation than did CT conversion. Crop differences were similarly dramatic (Figure 4.7b): under NT, the cellulosic crops had a negative GHG impact of 61% (prairie) and 119% (switchgrass) of that of corn; under CT cellulosic crops had a positive GHG impact of 90% (prairie) and -250% (switchgrass) of that of corn. The largest differences were between CT corn (1.26±0.45 Mg CO_2e ha^{-1}) and NT switchgrass (-7.65±0.19 Mg CO_2e ha^{-1}) over the two years of this study.

The main reasons for the NT benefit are lower N_2O and CO_2 emissions since differences in CH_4 oxidation were negligible. N_2O emissions in CT corn, prairie, and switchgrass fields were 1.75, 2.67 and 2.51 times those under NT, respectively. Differences were higher in year 1 than in year 2. CO_2 emissions from soil were always lower and NEE was always negative or only slightly positive under NT: NT crops were either a net C sink (NT corn and NT switchgrass) or net C loss was minor (prairie). In contrast, all CT crops expressed a net C loss with a positive

NEE of CO_2.

N_2O fluxes. During the first year, N_2O emissions under CT management in all converted fields increased substantially compared to those under NT management (Figure 4.1d). Especially for the first 30 days after tillage, N_2O emissions under CT were 5-7 times of those under NT. Similar results have been reported previously based on shorter term studies (Nikiema et al., 2012; Ruan and Robertson, 2013).

The likely reason for the pulse is that enhanced SOM decomposition under CT increased the availability of inorganic N (NH_4^+ and NO_3^-) (Table 4.2), which stimulated nitrification and denitrification and thus N_2O production. Unlike the one-year studies (Nikiema et al., 2012; Ruan and Robertson, 2013), in the second year I found fewer differences in N_2O emissions between CT and NT. In corn, N_2O emissions under CT were 2.6 times that of NT in the first year (31.5±2.97 g N_2O-N ha^{-1} d^{-1} in CT vs.13.6±2.51 in NT) but only 1.3 times in the second year (21.8±1.82 g N_2O-N ha^{-1} d^{-1} in CT vs.17.0±1.98 in NT). In prairie and switchgrass, the first year difference in N_2O production between CT and NT disappeared in the second year. This is likely because the pulse of substrate that occurred upon tillage the first year had diminished by the second year. Based on CT vs. NT differences in long-term cropping systems nearby (Robertson et al. 2000), I expect N_2O emission differences between CT and NT corn will also eventually diminish and disappear (Ruan and Robertson, 2013).

Over the study period, that corn produced more N_2O than perennial crops in both NT and CT treatments can be attributed to its higher available soil N (Table 4.2), a significant predictor of N_2O fluxes (Figure 4.2).

Biofuel life cycle analyses (Plevin, 2009) typically use the IPCC Tier 1 emission factor (IPCC, 2006) to estimate direct N_2O emissions. Tier 1 methodologies apply a constant emission factor (EF) of 1% of fertilizer inputs to estimate direct N_2O emission in crops without residue return, without regard for tillage or other management factors. Thus EF-estimated N_2O emissions for corn would be the same as for CT and NT, in this study 1.59 kg N_2O-N ha^{-1} yr^{-1} on average. For switchgrass, EF-estimated N_2O would be 0.28 kg N_2O-N ha^{-1} yr^{-1}, and for prairie, 0 kg N_2O-N ha^{-1} yr^{-1} since prairie was unfertilized. In all cases life cycle analysis based on the EF approach would have substantially underestimated the actual contribution of N_2O emissions to the overall GHG balance, which ranged from 1.05 kg N_2O-N ha^{-1} yr^{-1} for unfertilized CT prairie to 5.29 kg N_2O-N ha^{-1} yr^{-1} for fertilized CT corn. In contrast, calculated EFs were 4.2% and 1.6% for CT and NT corn, respectively, for the first year and 2.4% and 1.8% for the second year: during the first year over 4 times more N_2O under CT corn was released than estimated by the IPCC's 1% EF. Using solely N fertilizer rate to estimate N_2O emissions can thus substantially underestimate the importance of N_2O emissions to the overall GHG balance.

CO_2 fluxes. My results showed significantly higher soil CO_2 emissions under CT than NT over the study period (Figure 4.3d). Tillage destroys soil aggregates and exposes soil C previously protected against decomposition and thus results in increased soil CO_2 emissions following CT operations. Similar results have been observed by other studies (e.g. Grandy & Robertson, 2006a; Almarz et al., 2009; Ruan & Robertson, 2013). As for N_2O, in the second year I found smaller differences between CT and NT CO_2 emissions in corn, and no difference was detected in prairie and switchgrass, although for the entire 2-year study period there is a significant difference. Other studies have also shown bigger differences in soil CO_2 emissions between CT and NT in the first year than in subsequent years (Almarz et al., 2009). Most likely soil active C is lost quickly from the top 25 cm after tillage during the the first year so that less easily decomposed carbon was left for the second year.

Soil CO_2 fluxes can also be affected by environmental factors such as soil temperature and moisture (Reichstein and Beer, 2008; Almarz et al., 2009). This 2-year study showed an exponential increase in soil CO_2 fluxes with increasing soil temperature, consistent with reports by others (e.g. Reichstein and Beer, 2008; Ruan and Robertson, 2013). There was no significant correlation between CO_2 and WFPS%, however.

The positive NEE of CO_2 fluxes for all CT treatments indicates net C loss to the atmosphere. NEE of CO_2 under CT prairie was even higher than under CT corn. One possible reason is that corn grain yields were ~ 2.2 times that of prairie biomass and higher C uptake from photosynthesis can offset the carbon loss from intensive tillage. In contrast to CT prairie, CT switchgrass received the same tillage management but C loss was only 11% of CT prairie. This may be because of higher root production in switchgrass than in prairie, which would mean more C uptake in switchgrass (Zenone et al., 2013).

In contrast, both corn and switchgrass under NT (-2.76 and -2.95 Mg CO_2e ha^{-1}, respectively) were a net C sink. Although some C was lost from the NT prairie system, losses were 88% less than those under CT prairie. Net loss means that C released from soil C decomposition exceeds the C uptake from photosynthesis. Because total photosynthesis is unaffected by tillage (biomass production was the same under both CT and NT for all crops), lower or more negative NEE indicates less soil C loss. NT, then, protected soil C so that less C was released under NT management. In addition, both corn and switchgrass under NT showed even higher C uptake than did the unconverted CRP reference field (-0.92 Mg CO_2e ha^{-1}).

CH_4 oxidation. Well-aerated soils can be a small sink for atmospheric CH_4 and previous work has shown that both tillage (Hütsch 1998; Ballet al., 1999; Ussiri et al., 2009) and N fertilization (Gulledge et al., 1998; Suwanwaree and Robertson, 2005; Chu et al., 2007) can affect soil CH_4 oxidation rates. Over the 2-yr study period, all fields were net sinks for CH_4 (Figure 4.5d). My recorded daily CH_4 fluxes in cropland (ranging from -0.31±0.17 to -1.37±0.20 g CH_4-C ha^{-1} d^{-1}) were close to the average CH_4 oxidation rates of -1.80±0.06 g CH_4-C ha^{-1} d^{-1} at nearby crop sites in Michigan (Robertson et al., 2000). However, no significant differences in CH_4 oxidation rates were found among treatments, although rates under CT were 43%, 69% and 94% of those under NT in corn, prairie and switchgrass, respectively, and rates under corn were only 33% and 38% of those under prairie or switchgrass, respectively.

In addition, I found no statistically significant differences before and after CRP grassland conversion, although average rates under converted sites were only 45 % (16% for CT corn to 73% for NT prairie) of those under unconverted CRP grassland. Similarly, Robertson et al. (2000) and Suwanwaree & Robertson (2005) also reported no differences in CH_4 oxidation between CT and NT treatments or between fertilized and non-fertilized treatments for sites nearby. In contrast, Ussiri et al. (2009) reported that NT soil was a sink while CT soil was a source of CH_4 due to undisturbed soil structure and greater gas diffusion under NT. Moreover, ammonium-based N fertilization has been reported to competitively inhibit CH_4 oxidation in other studies (Suwanwaree and Robertson, 2005; Chu et al., 2007). In general, CH_4 fluxes were negligible compared with N_2O and CO_2 fluxes, which are consistent with findings elsewhere nearby (Robertson et al., 2000; Gelfand et al., 2009; Ruan and Robertson, 2013).

Global warming impact. Negative GWIs (Figure 4.7b) indicate net climate change mitigation; GHG benefits include those from fossil fuel offset credits based on the use of biofuel in place of gasoline. During the first two years of biofuel crop establishment, my results show that by reducing N_2O and whole ecosystem C loss, NT practices, with an average GWI of -6.01 Mg CO_2e ha^{-1} yr^{-1} across all crops, can substantially reduce the GWI of grassland conversion compared to CT, with an average GWI of -0.25 Mg CO_2e ha^{-1} yr^{-1}.

Regardless of tillage management – whether CT or NT – and regardless of crop, N_2O emissions are of similar GWI importance as system C loss during grassland conversion. Across all cropped fields, N_2O emissions in terms of CO_2e were ~50% of the net ecosystem C loss.

Furthermore, the net GWIs of NT corn showed no significant difference from those in unconverted CRP reference field. The negative GWIs of NT switchgrass were even lower than those in the reference. This suggests that NT conversion can repay the equal or more C debt than the direct use of existing CRP grasslands for cellulosic feedstock production after C debt during soybean transition year (Gelfand et al., 2011; Ruan and Robertson, 2013).

That NT practices regardless of crop type can substantially reduce that GHG impact of GWIs during CRP grassland conversion, which suggests that conversion, when it occurs, should be conducted without tillage. The C debt continues to increase over the two years after conversion in corn and prairie under CT. In contrast, the C debt begins to be repaid in corn and prairie under NT and switchgrass under both CT and NT for the first two establishment years. If the rationale for governments to promote cellulosic biofuel crops is partly to reduce GHG emissions, policies should be designed to encourage adoption of NT practices.

CHAPTER 5: WARMING-INDUCED FREEZE-THAW CYCLES ACCELERATE N_2O EMISSIONS FROM AGRICULTURAL SOILS

ABSTRACT

Snow cover has decreased in many regions as global surface temperatures have increased. Reduced snow cover can affect soil temperatures and freeze-thaw cycles and thus soil carbon and nitrogen dynamics, including, perhaps, soil nitrous oxide (N_2O) fluxes. I investigated the winter response of N_2O emission to manipulations of snow depth in a corn-soybean-wheat cropping system in Michigan (USA) for three winters using an automated chamber system. In treatments where snow was removed, N_2O emissions were 69% higher than in ambient controls and 95% higher than in double-snow treatments ($P<0.001$). The proportion of annual N_2O emissions represented by winter fluxes consequently increased by ~46%. Higher fluxes coincided with a greater number of freeze-thaw cycles that destroyed macroaggregates and increased inorganic nitrogen availability. Future winters with less snow can thus be expected to accelerate N_2O fluxes from agriculture, creating the potential for a positive feedback cycle as the climate warms.

INTRODUCTION

With increasing global surface temperatures snow cover has decreased globally; in the northern hemisphere snow cover has decreased in every winter month except November and December from 1966 to 2005 (IPCC, 2007a). Snow is an effective insulator, so the reduced snow cover will enhance soil freezing and increase the depth of frost and, coupled with warmer winters, also increase the frequency of soil freeze-thaw cycles (Schurmann et al., 2002). Additionally, more extreme weather events may cause more frequent mid-winter thaws (Henry, 2007).

Freeze-thaw cycles can strongly impact soil carbon (C) and nitrogen (N) dynamics, including the production of nitrous oxide (N_2O), a greenhouse gas with ~300 times the global warming potential of carbon dioxide (CO_2). Agricultural soils account for ~60% of anthropogenic N_2O emissions worldwide (IPCC, 2007a). Laboratory studies (van Bochove et al., 2000; Koponen et al., 2004; Zhu et al., 2009) corroborated by short-term field studies (Groffman et al., 2006, Maljanen et al., 2007; 2009; Maljanen et al., 2010; Duran et al., 2013) have established that pulses of N_2O can be emitted from frozen soil just after thawing, attributed to: 1) release of physically trapped N_2O (Burton and Beauchamp, 1994); 2) enhanced microbial activity on release of dissolved organic C from aggregate disruption (Sharma et al., 2006); and 3) anaerobic conditions induced by thawing and consequent soil water saturation, which are conducive to denitrification (Kim et al., 2012). Extrapolation of laboratory and short-term field measurements to season-long in-situ estimates are problematic because of the strongly pulsed nature of thaw-induced fluxes and the rapidity with which freeze-thaw events happen in the field. Moreover, no studies have experimentally manipulated snow cover to infer the effect of winter warming on N_2O emissions in agricultural soils, which are likely to be more sensitive than grassland and forest soils (Maljanen et al., 2007; 2009; Groffman et al., 2006) due to a lack of winter vegetation cover. Freeze-thaw induced emissions during early spring have been estimated to be responsible for as much as 65% of annual N_2O emissions in agricultural soils based on weekly sampling at MN, USA (Johnson et al., 2010).

Here I report the results of a snow manipulation experiment designed to evaluate how future changes in snow cover will affect soil N_2O fluxes in agricultural soils, using an automated sampling system that captures fluxes at a sub-daily resolution. I hypothesize that i) snow reduction will increase soil freeze-thaw cycles, which will ii) increase N_2O emissions throughout the winter due to iii) the destruction of soil aggregates. Snow addition is hypothesized to have the opposite effects.

MATERIALS AND METHODS

Site description

During the 2011-2013 winters (December 2010 to March 2013) I measured N_2O emission in an agricultural field in southwest Michigan (USA). The field was located at the Kellogg Biological Station (KBS) Long-Term Ecological Research (LTER) site (42° 24' N, 85°24' W, elevation 288 m). Soils are co-mingled Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs) and Oshtemo (coarse-loamy, mixed, mesic Typic Hapludalfs) loams developed on glacial outwash. Annual precipitation averages 1,027 mm with an average snowfall of ~1.4 m. Mean annual temperature is 9.9 °C ranging from a monthly mean of -4.2 °C in January to 22.8 °C in July (Robertson and Hamilton 2014). Figure C.1-3 shows the increasing winter temperature and decreasing number of snow cover days in the past 63 years at KBS.

Experimental design and treatments

During the growing season, snow treatment plots were managed as a corn (*Zea mays* L.) – soybean (*Glycine max*) – winter wheat (*Triticum* spp.) rotation following regional norms. All plots received conventional chemical inputs including pre- and post-emergence herbicide, N, and other fertilizers according to regional best management practices and integrated pest management procedures. Nitrogen was applied to corn (on average, 168 kg N ha^{-1}), to wheat (84 kg N ha^{-1}) and to soybeans (7 kg N ha^{-1}). Plots were managed without tillage and without cover crops. The experiment was a completely randomized design with three snow treatments (ambient, no snow, and double snow): in the no-snow treatment, snow was removed completely after each snow event; in the double-snow treatment, snow was added to make up twice ambient levels. Each treatment had four replicates for a total of twelve 4×4 m plots.

Nitrous oxide (N_2O) Emission

Winter N_2O fluxes were measured in each plot with an automated flux chamber system. Each chamber (50 cm × 50 cm × 38 cm). Chamber bases were embedded 5 cm into the soil for the duration of the study. When the treatment snow depth was higher than chamber height, extensions were installed in all treatments and then removed following snowmelt to maximize the sensitivity of chamber measurements. Each chamber was sampled four times per day at six-hour intervals. During sampling the chamber lid was closed and headspace samples were pumped to a gas chromatograph located in a nearby trailer. During non-sampling time, the chamber lid was open and snow depth was maintained carefully in each treatment. N_2O concentrations were measured four times from each chamber at intervals of approximately 30 minutes. Three standards were injected at the beginning and end of each sampling period. The system also collected an air sample from each chamber prior to chamber closure. Gas samples were directly analyzed by gas chromatography (SRI 8610C with custom sample acquisition, Torrance, CA, USA). Samples were separated on a Restek packed HS-Q (3.7m, 60/80 mesh) column and then N_2O was analyzed with a ^{63}Ni electron capture detector at 350 °C.

Soil temperature at 0-5 cm depth was measured every 30 minutes using HOBO pendant temperature data loggers (Onset Computer corporation, Inc., Pocasset, MA, USA) installed in pairs in each plot. Air temperature was recorded at a weather station approximately 100 m from the study site. In addition, to calculate changes in the proportion of wintertime to annual N_2O emissions I obtained growing season N_2O emissions data from measurements of non-automated static chambers at four nearby plots with the same soil properties and agricultural management.

Soil inorganic nitrogen

Ammonium (NH_4^+) and nitrate (NO_3^-) availability were estimated using in-situ ion exchange resin strips to minimize sampling disturbance (Qian & Schoenau, 1995). Three pairs of anion and cation resin strips (2.5×10×0.62 mm thick; GE Power & Water, Trevose, PA, USA) were buried directly into soil in each treatment plot one day before the experiment commenced each winter and left in place for the season. After collection 35 ml of 2.0 M KCl per resin strip were added to a polyethylene cup that was then shaken for 1 h on an orbital shaker (IKA KS 501, Wilmington, NC, USA) at 40 rpm. A 5 mL extract was then analyzed for NH_4^+ and NO_3^- on a continuous flow analyzer (Flow Solution IV, OI Analytical, College Station, TX, USA) using colorimetric techniques.

Water-stable aggregate distribution

Soil aggregate distributions were determined before and after each winter season using water-stable aggregate distribution methods (Elliott, 1986; Grandy & Robertson, 2006b). On each sample date, five 12-cm diameter soil cores (0-10 cm depth) were taken from each treatment plot, sieved through an 8-mm sieve and air dried at 25 °C. Three 50-g air-dried subsamples from each plot were then wet-sieved in water through a series of 2000, 250, and 53 µm sieves to obtain 4 size fractions: 2000 to 8000 µm (large macroaggregates), 250 to 2000 µm (small macroaggregates), 53 to 250 µm (microaggregates), and <53 µm (silt and clay particles). Before wet-sieving, soils were submerged in water on the surface of the 2000-µm sieve for 5 minutes. Then soils were sieved under water into a stainless steel pan by moving up and down over 2 minutes with a stroke length of 3 cm for 50 strokes. Soils remaining on the sieve were oven-dried at 60 °C for 48 h. Soils passing the 2000 µm sieve and remaining in the pan were then wet-sieved through the 250-mm (50 strokes) and 53-mm (30 strokes) sieves. Sand content was determined by shaking soil from each of the >53 µm size classes in sodium hexametaphosphate (0.5%) for 48 h on a rotary shaker at 190 rpm and then sieving through a 53-µm screen. The mean weight diameter (MWD) of sand-free aggregates was calculated as the sum of products of the mean diameter of each size fraction and the proportion of the total dry sample weight (Van Bavel, 1949).

Data analysis

I took one week before the first snow as a starting point and one week after the last snow as the ending point for each winter's experimental period. Cumulative N_2O fluxes over the period were calculated by linear interpolation of hourly fluxes between sample events. Statistical analysis was conducted in SAS 9.1 (SAS Institute, Cary, NC, USA). Treatment means were compared using one-way ANOVA with LSD in Proc Mixed at $\alpha=0.05$ level. Linear regression between cumulative N_2O fluxes and soil total available N was conducted in PROC REG. Normality of the residuals and homogeneity of variance assumptions were checked using stem-and-leaf box and normal probability plots of the residuals, and Levene's test.

RESULTS

Snow depth, soil temperature, and soil N_2O fluxes

Total snowfall was 942, 767 and 959 mm for the 2010-2011, 2011-2012 and 2012-2013 winter seasons, respectively, which were lower than the average 1376 mm snowfall for the past 60 years (Figure C.1). Likewise, the total number of days with snow cover for the three winters were 58, 33, and 43 days, respectively (Figure 5.1a, 5.2a and 5.3a), lower than the 60-year average of 66 days per winter (Figure C.3).

Over the three winters, average air temperature was warmest in the second (2011-2012) season (-0.20 °C for the sampling period), compared to -3.57 and -2.27 °C in the first and the third seasons, respectively. Average soil temperature at 5-cm depth in the no-snow treatment was colder than in the ambient and double-snow treatments for all three winters ($P<0.05$) and also experienced more freezing degree hours ($P<0.05$) and freeze-thaw cycles ($P<0.05$; Table 5.1). Soil temperatures under the double-snow treatment appeared to fluctuate less than in the other treatments, while soil temperatures in the no-snow treatment warmed more quickly in response to increased air temperature (Figures 5.1b, 5.2b and 5.3b).

Hourly soil N_2O fluxes ranged from undetectable to 132±21 μg N_2O-N m^{-2} h^{-1} during the three winters. N_2O fluxes in the no-snow treatment fluctuated more than those in the ambient and double-snow treatment (Figure 5.1c). High fluxes occurred mostly with the onset of warm periods when soil temperature increased to above 0 °C. For instance, soil temperature stayed below 0 °C on December 30, 2010, and increased to > 4.8 °C across all snow treatments on January 1, 2011. During these two days, N_2O fluxes reached their seasonal peaks across all treatments (Figure 5.1c). These high fluxes persisted for a few hours to 1-2 days.

In all three winter seasons, soil N_2O emissions were significantly higher in the no-snow treatment than in the ambient and double-snow treatments, whereas there were no significant differences between ambient and double-snow treatments (Figure 5.4). On average, over all three winters N_2O emissions in the no-snow treatment (9.19±0.61 μg N_2O-N m^{-2} h^{-1}) were 69% and 95% higher than in the ambient (5.43±0.31 μg N_2O-N m^{-2} h^{-1}) and double snow (4.71± 0.17 μg N_2O-N m^{-2} h^{-1}) treatments ($P<0.001$).

Snow removal significantly increased ($P<0.05$) the seasonal importance of wintertime

Table 5.1. Wintertime soil temperatures (0-5 cm depth) and N_2O fluxes. Data were collected from snow treatments (no snow, ambient and double snow) between December to March each year. Freezing hours refer to the total time soil temperature was below 0 °C (percent of total winter hours in parentheses). Mean soil temperatures (mean±s.e), freezing hours, and freeze and thaw cycles that are significantly different from one another within years ($P<0.05$) are noted with different letters within columns.

Winter Year	Snow treatment	Mean soil temperature (°C)	Freezing hours (% of total hours)	Freeze-thaw cycles (n)
2010-2011	No snow	-0.76(±0.07)^{a}	1878(82.6%)^{a}	49^{a}
	Ambient	-0.13(±0.05)^{b}	1534(69.8%)^{b}	27^{b}
	Double snow	-0.09(±0.05)^{b}	1416(70.1%)^{b}	24^{b}
2011-2012	No snow	0.66(±0.04)^{a}	485(23.3%)^{a}	37^{a}
	Ambient	0.89(±0.06)^{b}	146(7.1%)^{b}	12^{b}
	Double snow	1.00(±0.11)^{b}	74(3.6%)^{b}	9^{b}
2012-2013	No snow	-0.51(±0.09)^{a}	1514(65.1 %)^{a}	48^{a}
	Ambient	-0.29(±0.03)^{b}	1318(56.0%)^{b}	28^{b}
	Double snow	-0.19(±0.05)^{b}	1142(49.0%)^{b}	26^{b}

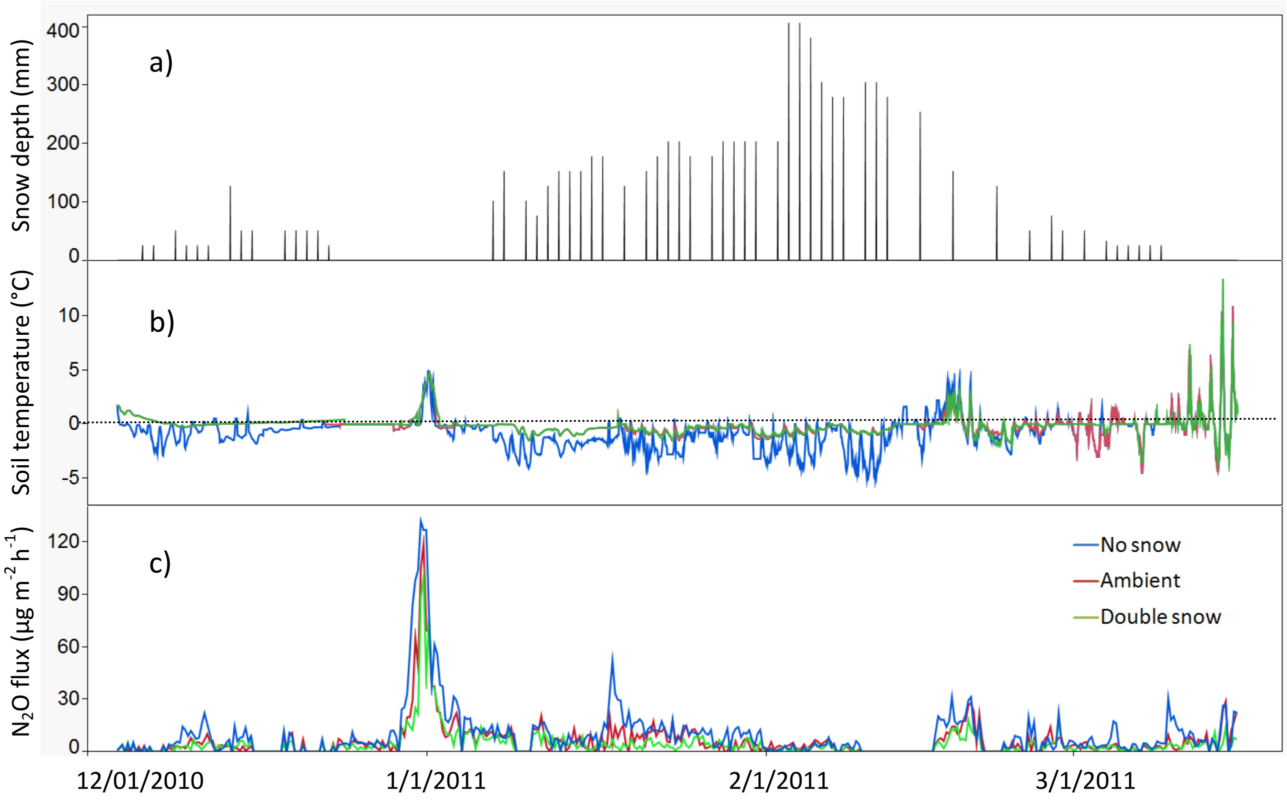

Figure 5.1. Ambient snow depth (a), mean soil temperature at 5-cm depth (b) and daily soil N_2O fluxes (c) for the first of the three winters studied (December 3, 2010, to March 15, 2011). Four replicates for soil temperature and N_2O fluxes in each treatment. Error bars omitted for clarity for soil temperature and N_2O fluxes.

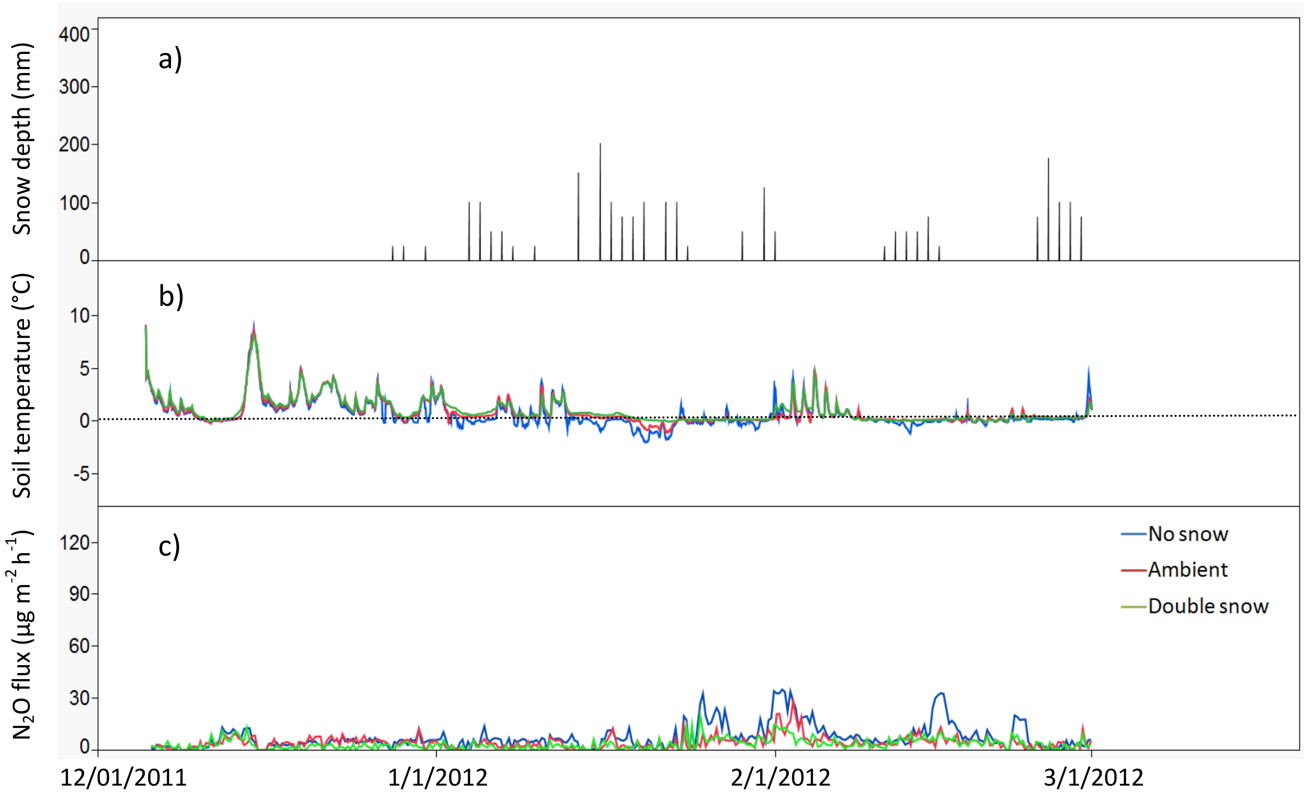

Figure 5.2. Ambient snow depth (a), mean soil temperature at 5-cm depth (b) and daily soil N_2O fluxes (c) for the second of the three winters studied (December 5, 2011, to March 1, 2012). See Figure 5.1 legend for further information.

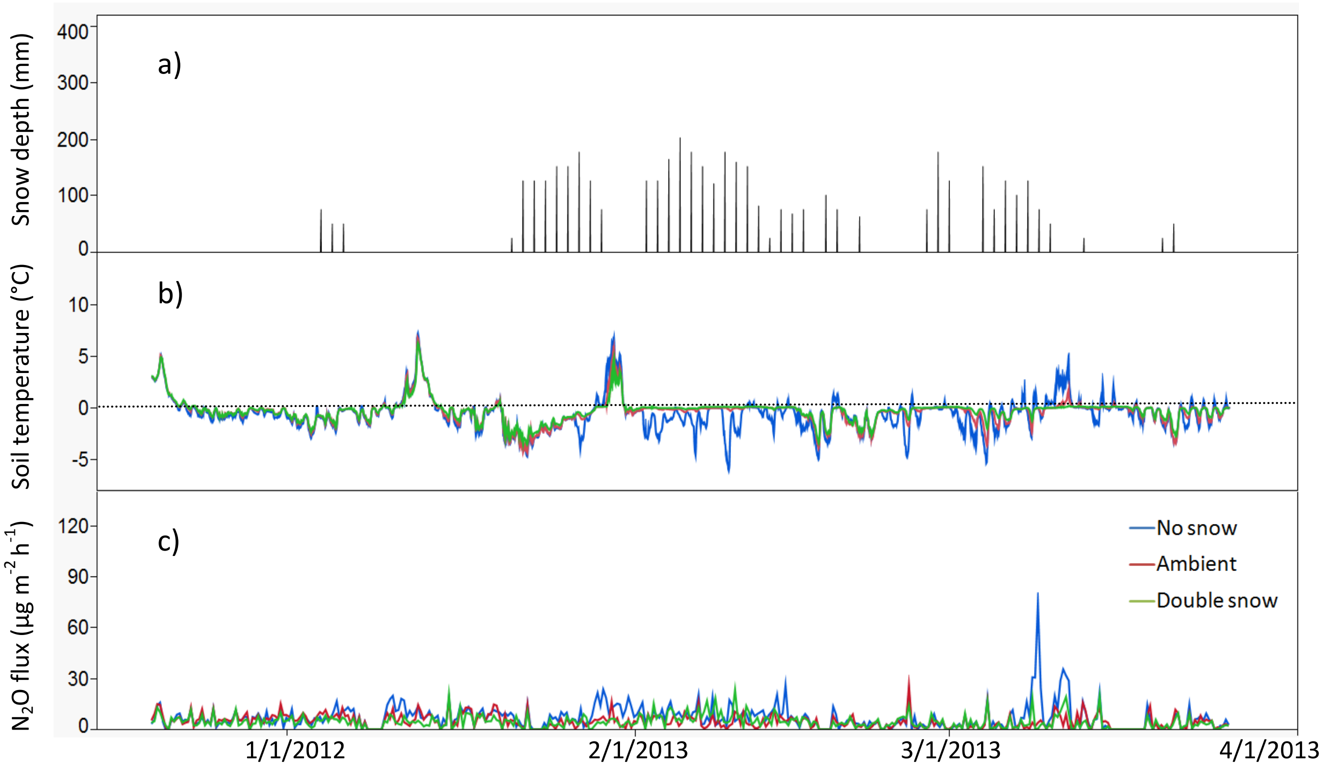

Figure 5.3. Ambient snow depth (a), mean soil temperature at 5-cm depth (b) and daily soil N_2O fluxes (c) for the third of the three winters studied (December 19, 2012, to March 25, 2013). See Figure 5.1 legend for further information.

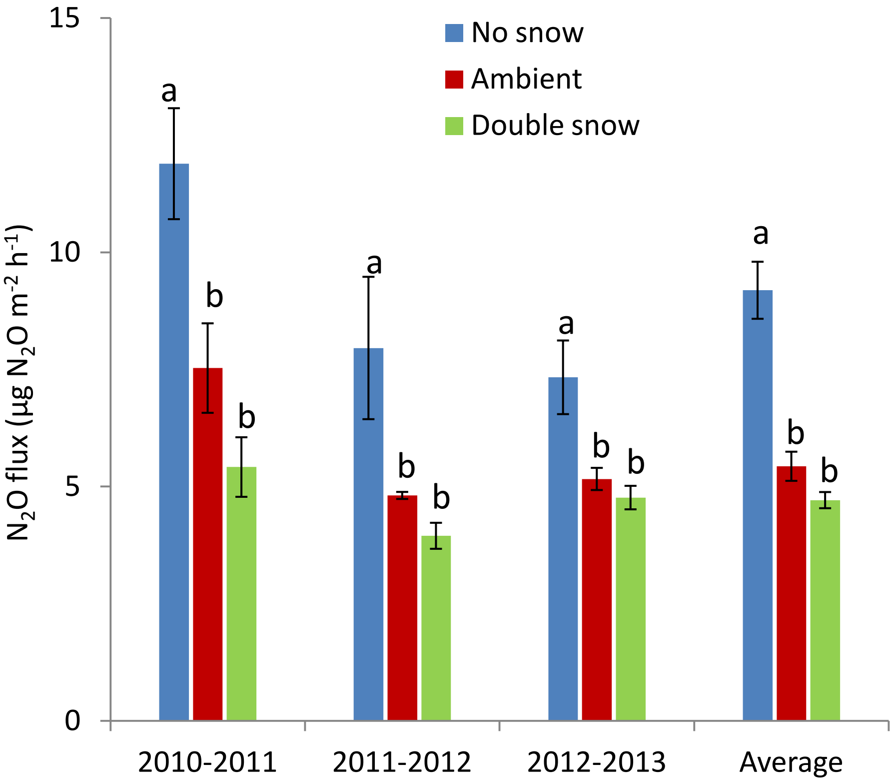

Figure 5.4. Soil N_2O emissions in different snow treatments for three winter seasons. Error bars represent standard errors based on n=4 replicate plots. Treatments within a season marked with different letters are significantly different from one another ($P<0.05$).

N_2O emissions regardless of annual crop type (Figure C.4). During the 2010-2011 wheat year, wintertime fluxes in the no-snow treatment were 17.6±1.46% of total annual fluxes, as compared to 12.1±1.36% for the ambient and 9.01±0.93% for the double-snow treatments. During the 2011-2012 corn year, wintertime fluxes were 8.16±1.43% in the no-snow treatment as compared to 5.13±0.08% for the ambient and 4.25±0.18% for the double-snow treatments. For the 2012-2013 soybean year, the proportion of wintertime snow was 18.9±1.65% for the no-snow treatment, 14.2±0.56% for the ambient treatment, and 13.2±1.36% for the double-snow treatment. Overall, snow removal increased the wintertime proportion of annual N_2O fluxes by 46% compared to ambient and by 77% compared to double snow. There was no significant difference between the ambient and double-snow treatment.

Soil aggregation

Before each winter experiment commenced, there were no significant differences in any of the four aggregate size fractions among snow treatments (Figure C.5). At the onset of the experiment in all three winters, the 2000-8000 μm and 250-2000 μm (macroaggregate) fractions were on average ~ 0.7 g g^{-1} soil and the 53-250 μm and <53 μm (microaggregates) fractions were ~0.3 g g^{-1}. At winter's end, soil macroaggregates in the no-snow treatment had declined significantly ($P<0.05$) by 38% to 0.44 g g^{-1} on average as compared to pre-winter soils, whereas the microaggregate fraction increased by 98% to 0.56 g g^{-1} (Figure 5.5). Soil aggregate size did not change in the ambient and double-snow treatment significantly, although macroaggregates declined 9% and microaggregates increased 28% in the ambient treatment. In addition, the mean weight diameter (MWD) of sand free aggregates was significantly ($P<0.05$) lower in the no-snow treatment than in the ambient and double-snow treatments for all three winters (Figure 5.6).

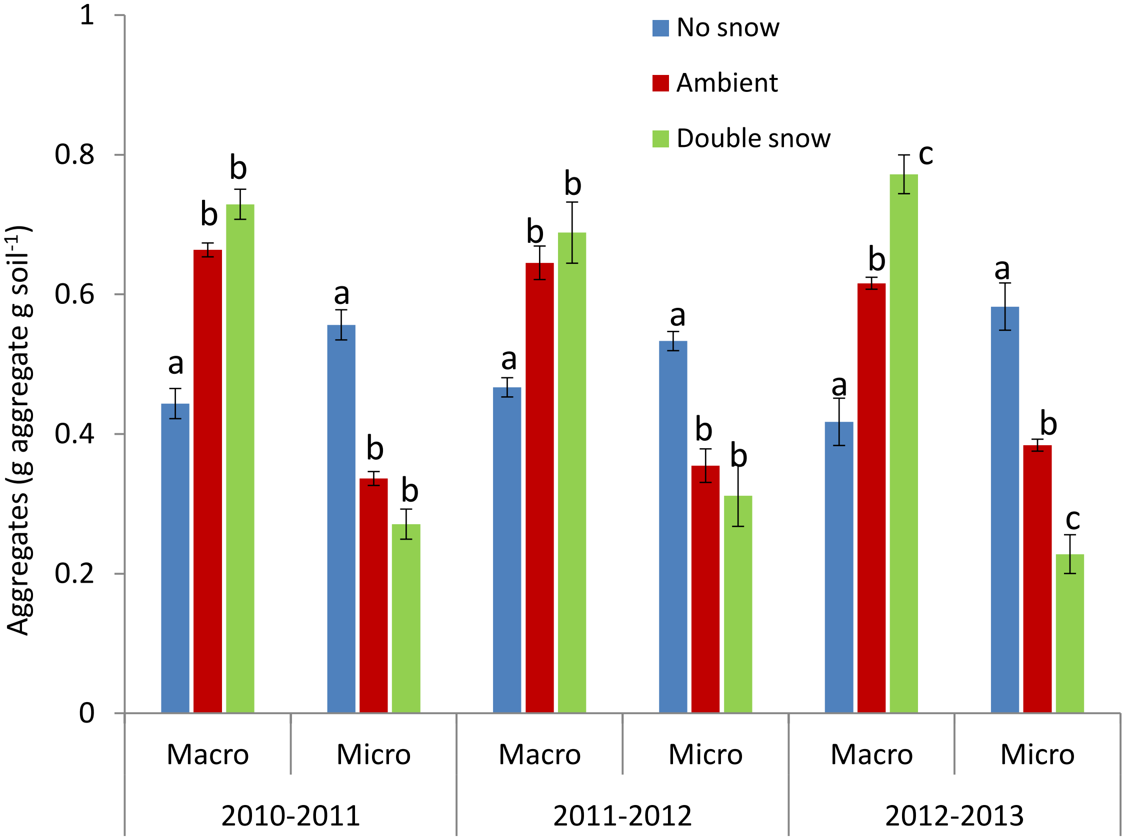

Figure 5.5. Distribution of surface soil (0-10 cm depth) aggregates among size fractions in different snow treatments for three winter seasons. Macroaggregates (Macro) include the 2000-8000 μm and 250-2000 μm fractions; microaggregates (Micro) includes the 53-250 μm and <53 μm fractions. Treatments within a size fraction marked with different letters are significantly different from one another ($P<0.05$).

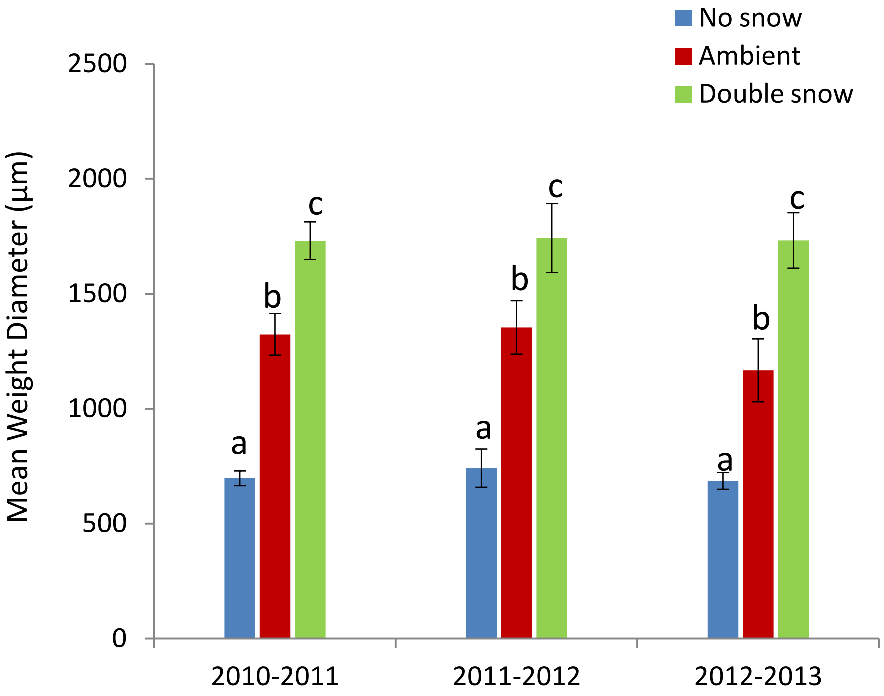

Figure 5.6. Mean weight diameter (MWD) of sand-free surface soil (0-10 cm depth) aggregates. Error bars represent standard errors based on n=4 replicate plots. Treatments within a season marked with different letters are significantly different from one another ($P<0.05$).

Soil inorganic nitrogen

Snow removal significantly increased both soil NH_4^+ and NO_3^- availability over the winter ((Figure 5.7; $P<0.05$). Specifically, resin strip NO_3^- concentrations in the no-snow treatment (70.3±3.7 $\mu g\ cm^{-2}$) were 22% and 45% higher than NO_3^- concentrations in the ambient (57.8±2.8 $\mu g\ cm^{-2}$) and double snow (48.5±5.1 $\mu g\ cm^{-2}$) treatments. Resin strip NH_4^+ concentrations were very low (<7.8 $\mu g\ cm^{-2}$) compared to NO_3^- concentrations, but NH_4^+ concentrations in the no-snow treatment were also significantly higher than NH_4^+ concentrations in the other treatments.

Soil inorganic N concentrations explained 37% of mean cumulative N_2O fluxes (Figure 5.8). N_2O fluxes showed a positive linear relationship with the sum of NH_4^+ and NO_3^- resin concentrations (available inorganic N): N_2O fluxes = 1.40 × available N + 61.5 ($R^2=0.37$, $P<0.001$).

DISCUSSION

Results strongly support the hypothesis that reduced snow cover can increase N_2O emissions as a result of intermittent wintertime soil warming that increases the frequency of soil freeze-thaw cycles. On average, across all three winters, snow removal significantly stimulated N_2O emissions by 69% relative to ambient conditions and by 95% relative to double-snow conditions. Fluxes were highly episodic, lasting for a period of hours to days following intermittent soil freeze-thaw cycles, which occurred 70-300% more frequently in the no-snow treatments than in the ambient and double-snow treatments, respectively. Snow removal resulted in a 46% increase in the seasonal importance of wintertime fluxes, which under ambient snow conditions were 12% of total annual fluxes for the wheat year, 5% for the corn year, and 10% for

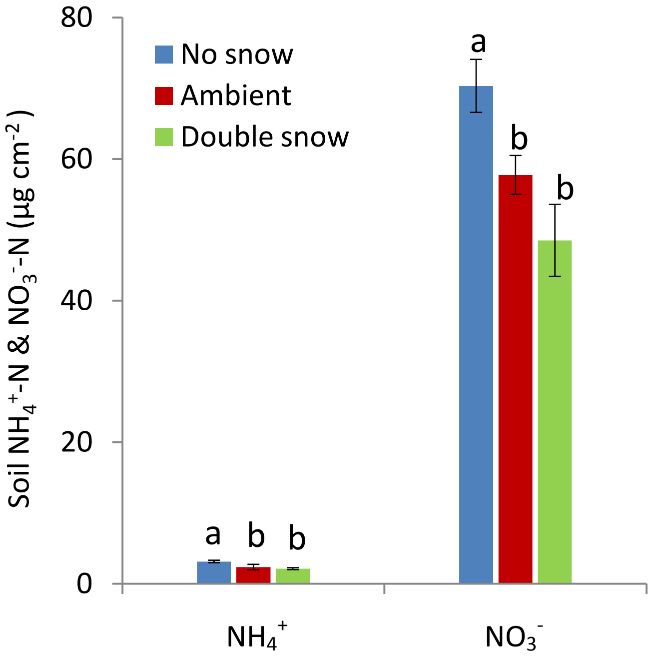

Figure 5.7. Soil inorganic nitrogen (NH_4^+-N + NO_3^--N) (0-10 cm depth) measured with cation and anion resin strips. Bars represent three-winter means ± standard errors based on n=4 replicate plots. Treatments marked with different letters within groups are significantly different from one another ($P<0.05$).

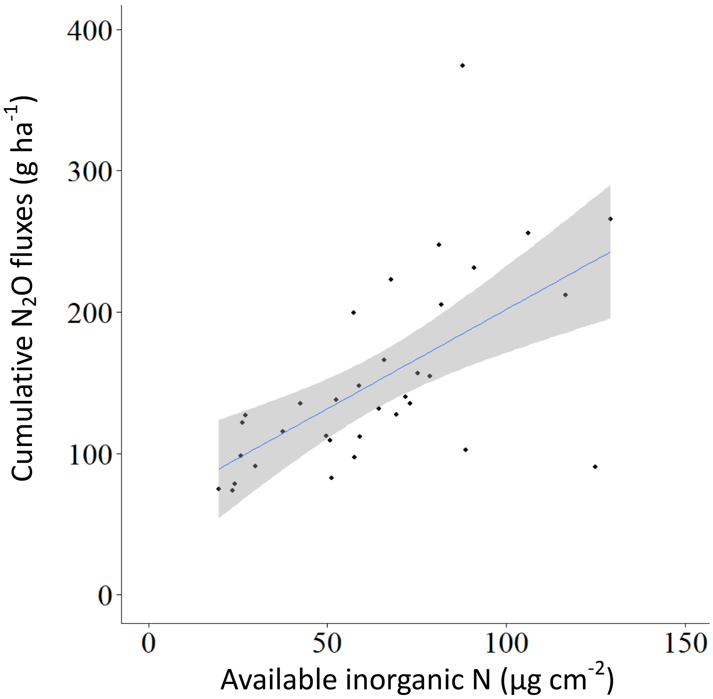

Figure 5.8. Relationship of cumulative N_2O fluxes for all treatments for the three winters (n=36) to soil inorganic nitrogen measured with resin strips (0-10 cm depth) (R^2=0.37, P<0.001).

the soybean year.

Inferential evidence suggests that emissions increased as a result of macroaggregate breakup and enhanced inorganic N availability. In the no-snow treatment soil macroaggregates declined 31% on average relative to ambient conditions, whereas the number of microaggregates increased by 56%. Likewise, soil NO_3^- concentrations were 22% higher in the no-snow treatment than in the ambient, and 45% higher than in the double-snow treatment.

Research in forests and grassland sites has also shown that reduced snow cover can increase N_2O fluxes. Both Groffman et al. (2006) and Maljanen et al. (2010) showed that decreased snow cover increased cumulative N_2O fluxes by ~100% in northern hardwood and boreal upland forests, respectively. A similar trend was also shown for urban (Duran et al., 2013) and boreal (Maljanen et al., 2007; 2009) grasslands. The present study shows that north temperate annual croplands, with their lower soil organic matter content and more exposed wintertime soils, are also affected by reduced snow cover and consequent increased frequency of freeze-thaw cycles.

Although with global climate change less snowfall is predicted for most regions, in some areas snowfall may increase (IPCC, 2007a). The double-snow treatment informs this possibility as well as the historical case in places like southwest Michigan, where, for example, average annual snowfall was 55% higher during the past 60 years than during the past three years. Consistent with increased N_2O emissions under conditions of less snow cover, results here showed that doubled snow cover diminished soil N_2O emissions by 15% relative to ambient, though not significantly (P=0.24).

My estimate that wintertime N_2O fluxes contribute 5-14% of annual N_2O fluxes at this site is in contrast to Johnson et al. (2010), who estimated that wintertime N_2O emissions accounted for up to 65% of total annual emissions in alfalfa fields. The lower estimate in the present study is based on a more frequent sampling interval (4 times per day) vs. their biweekly sampling, which, based on the episodic nature of fluxes observed in the present study, suggests that their results could be biased. The range of 5% for corn to 14% for soybeans in this study is likely related to growing-season fertilizer use. Corn with its high N fertilizer requirements (160 kg N ha^{-1} yr^{-1} in my study) produced more N_2O during the non-winter period than less fertilized wheat (56 kg N ha^{-1} yr^{-1}) and soybean (7 kg N ha^{-1} yr^{-1}). The potential for a substantial increase in the importance of wintertime fluxes with less snow (up to 46%), coupled with the inference that there have been already some historical shifts (to perhaps 15% in this study), suggests a reassessment of the common assumption that wintertime fluxes are negligible in northern climates.

Elevated N_2O emissions in the no-snow treatment can be attributed to several factors. First, increased freezing time enhances the mortality rate for microbes and fine roots, resulting in the release of labile organic carbon and N into the soil (Groffman et al., 2001; Tierney et al., 2001). Snow removal decreased soil temperatures and increased freezing time in all 3 winters: the no-snow treatment had, on average, 283 more hours below 0 ºC than did the ambient treatment. Conversely, the double-snow treatment had 132 fewer hours below 0 ºC. Loss of snow-cover insulation resulted in freezing period differences that likely caused substrate availability differences among snow treatments. Heterotrophic denitrification, a dominant source of N_2O in these soils (Ostrom et al. 2010), is strongly affected by substrate availability (Robertson and Groffman 2007), especially during winter when thawed soils are saturated and largely anaerobic.

Second, the physical disruption of soil aggregates due to more freeze-thaw cycles where snow is absent may release previously protected organic matter to microbial attack, also resulting in substrate availability differences among snow treatments. In this study, soils with no snow experienced twice the number of freeze-thaw cycles as ambient snow treatments. The greater number of freeze-thaw cycles substantially reduced the number of macroaggregates – by 38% in the no-snow treatment. Similarly, Cecillon et al. (2009) found that soil macroaggregates in the Ah horizons of French alpine soils were diminished by 25% in colder plots with freeze-thaw events as compared to warmer frost-free plots. Edwards (2013) reported a 28% average decrease in larger aggregates (4750-9500 µm) together with a 33% increase in smaller aggregates (<500 µm) in arable soils of the Atlantic coast of Canada following multiple freeze-thaw cycles in the lab. On the other hand, Steinwig et al. (2010) showed no aggregate size fraction changes in a snow removal treatment at the Hubbard Brook forest, NH, USA and they suggested that high water content, high organic matter, and relatively slow rates of freezing can minimize structural disruption by freeze-thaw cycles.

Although the ambient and double-snow treatments also experienced freeze-thaw cycles, soil aggregate distributions appeared not to change much (Figure 5.5, Figure C.5). The resilience of soil macroaggregate content in the present study was also remarkable. By the start of the following winter, the number of macroaggregates in the no-snow treatment had recovered to the same level as the prior year despite their reduction over winter.

Third, and likely related to increased freezing times and macroaggregate breakup, snow removal resulted in a greater availability of soil inorganic N, which can provide NH_4^+ for nitrification and NO_3^- for denitrification and thus increase N_2O production. Soil NO_3^- concentrations were especially elevated in the no-snow treatment. Inorganic N availability was assessed here with resin exchange strips, which measure both the static soil N pool and the N ions that flux through the mineral pool (Bowatte et al., 2008), and thus can more readily represent temporally variable N availability than more typical soil N extraction methods. This may explain the difference between these results and those of Groffman et al. (2006), who did not find a snow removal effect on inorganic N availability.

These results provide a strong argument for using automated chambers with high sampling frequency (multiple times per day) to investigate highly variable N_2O fluxes such as those that occur following mid-winter thaws. Automated chambers have an advantage over manual chamber methods especially in winter. First, they reduce soil disturbance, especially compaction caused by trampling from manual sampling. Soil compaction can reduce porosity and increase water-filled pore space (WFPS), which in turn limits oxygen diffusion rates and results in an anaerobic state favorable for denitrification (van Groenigen et al., 2005; Ball et al., 2008). Second, they can more precisely estimate total winter N_2O emissions with a sub-daily sampling frequency that captures episodic events. For example, in this study an N_2O peak of 118±34 μg N_2O-N m^{-2} h^{-1} was observed on December 31, 2010, in the ambient snow treatment after thawing. Two weeks later (on January 14, 2011), which is a commonly reported interval for N_2O sampling (Groffman et al., 2006; Johnson et al., 2010), the measured flux was 4.60±4.31μg N_2O-N m^{-2} h^{-1}. Linear interpolation between these sampling dates provides a cumulative flux estimate of 207±53 g N_2O-N ha^{-2} for the period, compared to 62.0±7.9 g N_2O-N ha^{-2} estimated by my sub-daily measurements. Using the two-week interval inappropriately increases the wintertime N_2O contribution to the annual budget from 12.1±1.4% to 22.4±2.2%. On the other hand, underestimation could as easily have been the case had other days been sampled, since most fluxes remained high for only 2-48 hours. Parkin (2008) found that the deviation of cumulative N_2O flux increased as the sampling interval increased, and that sampling every 21 d can yield estimates ranging from +60% to -40% of the actual cumulative N_2O flux in a Iowa chisel-plowed corn/soybean field.

The presence of a positive feedback to climate change, whereby reduced snowfall and warmer wintertime temperatures create more wintertime N_2O emissions that then further warm the atmosphere, will tend to further magnify the impact of agricultural N on global N_2O fluxes. These findings provide more incentive to curb agricultural soil N_2O emissions, which already are thought to be responsible for 60% of global anthropogenic emissions. More conservative N management that reduces the availability of surplus reactive N in soil is one strategy for combatting accelerated wintertime fluxes. Another is encouraging winter cover crops and maintaining crop residues that can trap and retain snow. Cover crops have the additional benefits of scavenging residual inorganic N and favoring macroaggregate formation.

CONCLUSIONS

1. Average soil temperature at 5-cm depth in the no-snow treatment was colder than in the ambient and double-snow treatments for all 3 winters and the no-snow treatment experienced more freezing degree hours and freeze-thaw cycles.
2. Soil N_2O emissions during the winter were 69% and 95% higher in the no-snow treatment than in the ambient and double-snow treatments, respectively.
3. Snow removal increased the proportion of annual N_2O emissions that occur in the winter by ~46% as compared to ambient snow levels.
4. Snow removal led to the destruction of macroaggregates and increased soil inorganic N availability compared to ambient and double-snow treatments.
5. As the climate warms, soil N_2O fluxes will be greater in future winters with less snow cover

due to a combination of more freeze-thaw cycles, the breakup of soil aggregates, and higher soil N availability.

APPENDICES

APPENDIX A

Table A.1. 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	0.9	-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 A.2. 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 A.3. Estimation of CO_2-equivalent emissions associated with switchgrass for cellulosic biofuel crop production

GHG emission category[1]	Mg CO_2e ha^{-1} yr^{-1}	Data source
Seeds[2]	0.014	Schmer et al., 2008; Börjesson, 1996; Farrell et al., 2006; EPA, 2007
Planting	0.013	Gelfand et al. (2013)
Nitrogen fertilizer [3]	4.5 × X × 0.001	Robertson et al. (2000)
Fertilizer application	0.026	Gelfand et al. (2013)
Harvesting (bailing)	0.019	Gelfand et al. (2013)
Direct N_2O emissions [4]	Field data	This study
Indirect N_2O emissions [4]	Leaching N × 0.75%	This study and IPCC 2006 emission factor
CH_4 uptake [4]	Field data	This study
SOC change [4]	Field data	This study

Note:

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

[2] 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}), I used an assumption that energy used in seed production consisted of a 50, 20, and 30%

Table A.3 (cont'd)

mix of fuel oil, natural gas, and electricity, respectively (Börjesson, 1996). I 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} (U.S EPA, 2011) 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 my 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, I averaged this value over the study period (3 yr), i.e., 0.014 Mg CO_2e ha^{-1} yr^{-1}.

[3] 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.

[4] Data from the field experiment in this study.

APPENDIX B

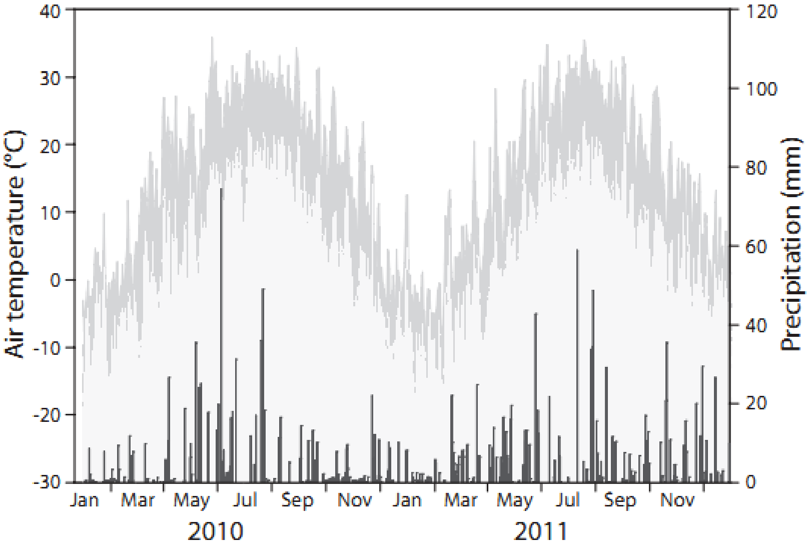

Figure B.1. Air temperature (˚C, minimum - maximum; continuous band) and precipitation (mm; vertical bars) in 2010 and 2011.

Table B.1. Agricultural inputs (Mg CO_2e ha^{-1} yr^{-1}) during corn, switchgrass and prairie production. For comparison, the Reference site is included as though it had been harvested.

Crop	Tillage	Chisel Plow(1)	N Fertilizer(2)	Fertilizer Application(3)	Herbicide(4)	Herbicide Application(4)	Seeds(3)	Planting(3)	Harvesting(1)
Corn	CT	0.026	0.73575	0.026	0.056	0.005	0.0788	0.013	0.03
Corn	NT	-	0.73575	0.026	0.056	0.005	0.0788	0.013	0.03
Prairie	CT	0.013	-	-	-	-	0.02	0.0065	0.019
Prairie	NT	-	-	-	-	-	0.02	0.0065	0.019
Switchgrass	CT	0.013	0.126	0.013	-	-	0.02	0.0065	0.019
Switchgrass	NT	-	0.126	0.013	-	-	0.02	0.0065	0.019
Reference	-	-	-	-	-	-	-	-	0.019

1) Lal, R. 2004. Carbon emission from farm operations. Environment international 30:981–990.

2) Robertson, G. P., E. A. Paul, and R. R. Harwood. 2000. Greenhouse gases in intensive agriculture: Contributions of individual gases to the radiative forcing of the atmosphere. Science 289:1922–1925.

3) West, T. O., and G. Marland. 2002. A synthesis of carbon sequestration, carbon emissions, and net carbon flux in agriculture: comparing tillage practices in the United States. Agriculture, Ecosystems & Environment 91:217–232.

Table B.1 (cont'd)

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APPENDIX C

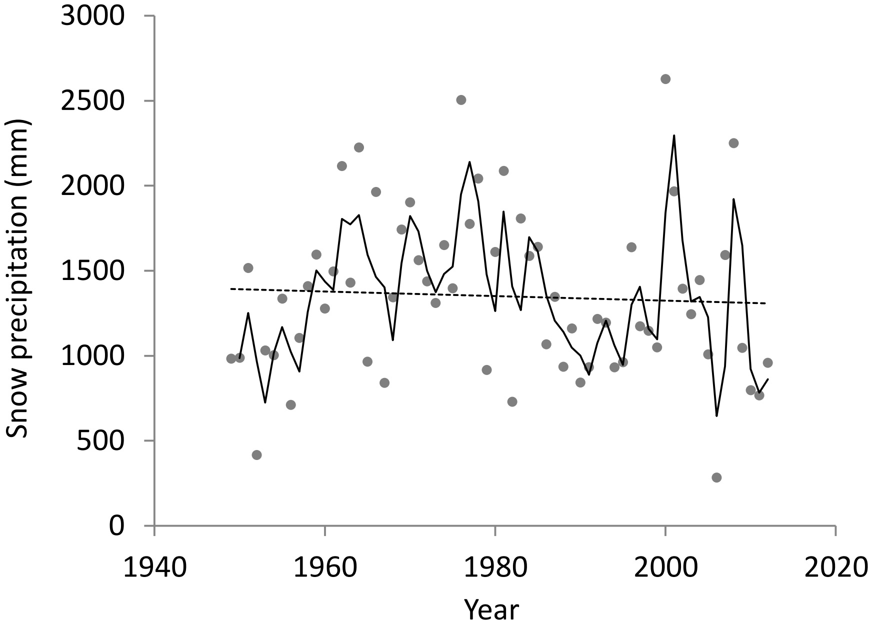

Figure C.1. Snowfall (mm) at the Kellogg Biological Station for 1949-2013. Snowfall was summed from early November to late April of the following year. Data source: Kellogg Biological Station National Weather Service Station (http://lter.kbs.msu.edu/datatables/31).

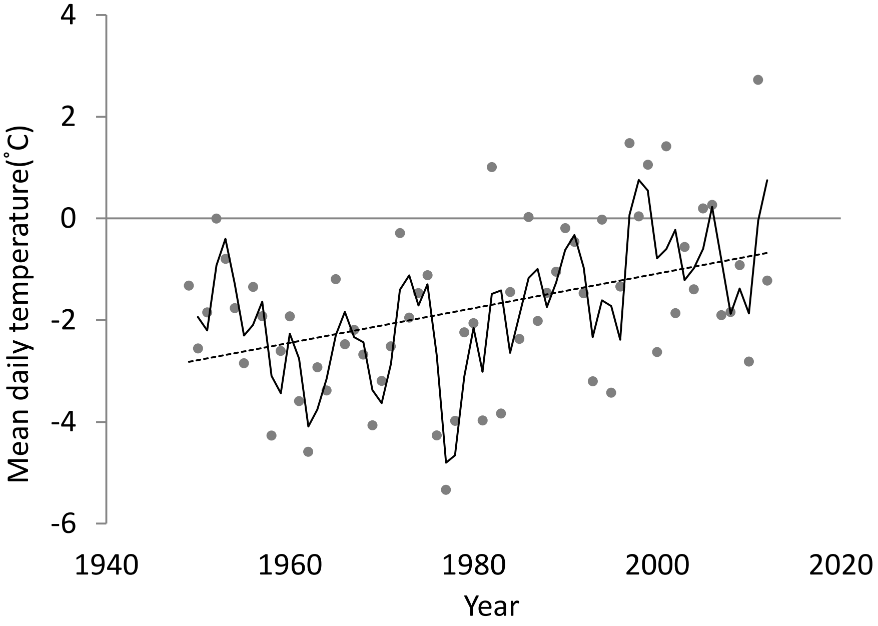

Figure C.2. Mean daily air temperature at Kellogg Biological Station during 1949-2013 winters (December to March). Data source: Kellogg Biological Station National Weather Service Station (http://lter.kbs.msu.edu/datatables/31).

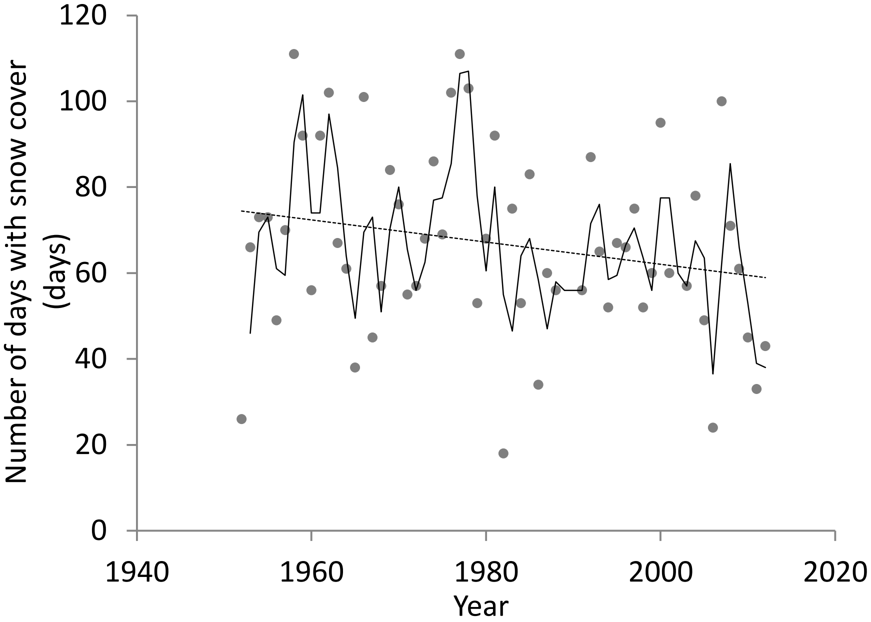

Figure C.3. Number of days with snow cover at Kellogg Biological Station during 1949-2013 winters (December to March). Data source: Kellogg Biological Station National Weather Service Station (http://lter.kbs.msu.edu/datatables/31).

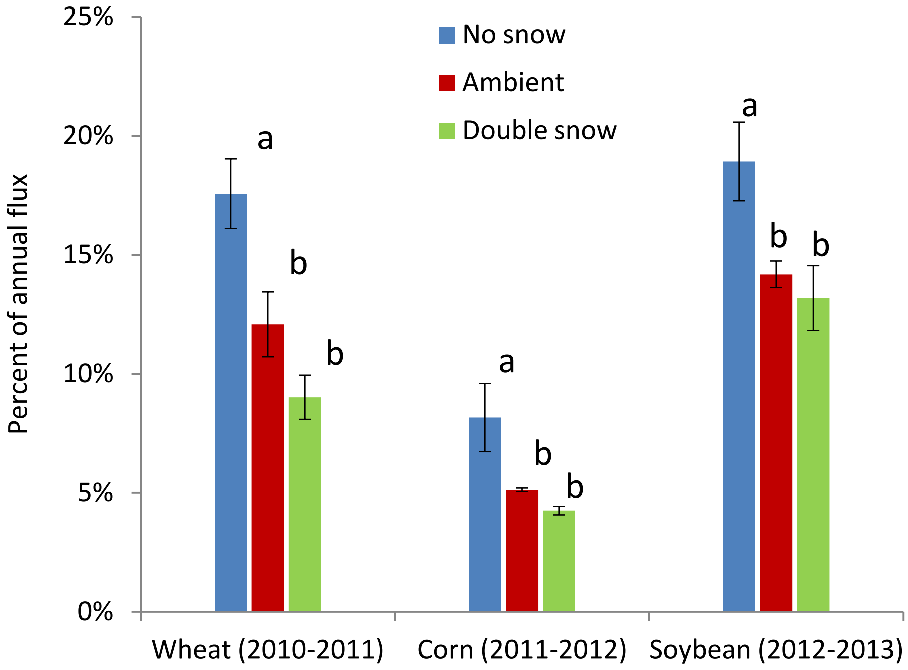

Figure C.4. Proportion of annual N_2O emissions represented by wintertime fluxes. Error bars represent standard errors based on n=4 replicate plots. Treatments within a season marked with different letters are significantly different from one another ($P<0.05$).

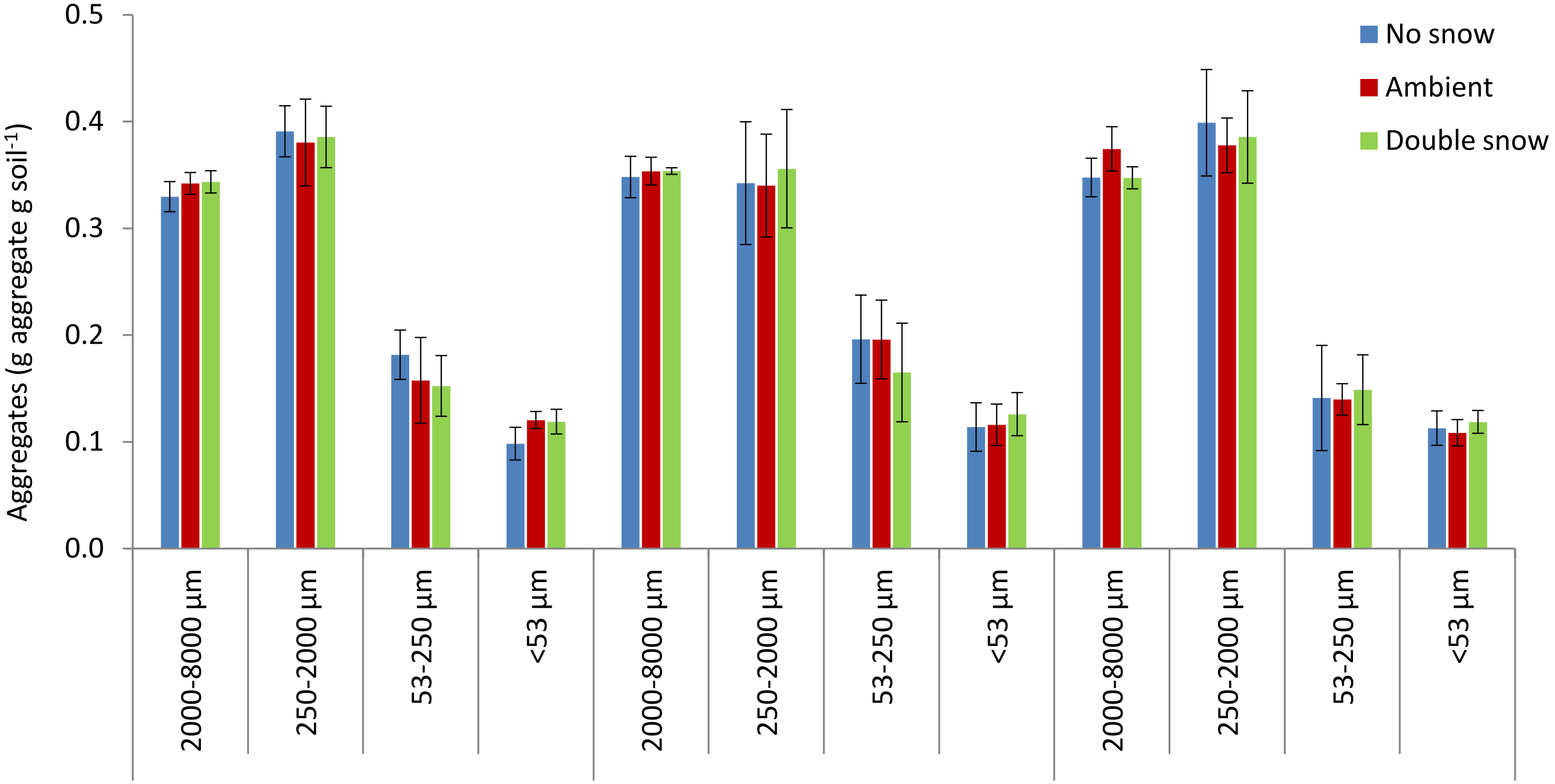

Figure C.5. The distribution of surface soil aggregates (0-10 cm depth) in four aggregate size classes prior to sampling each winter of the experiment. Error bars represent standard errors based on n=4 replicate plots. There were no significant differences ($P<0.05$) among treatments within any size class, hence no letters are shown to denote significant differences.

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