NITROGEN POLLUTION AND PRODUCTIVITY IN BIOENERGY GRASSLANDS: DO DIVERSITY AND FERTILIZER MANAGEMENT MATTER?

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

(Environment and Resources)

at the

UNIVERSITY OF WISCONSIN-MADISON

2013

ACKNOWLEDGEMENTS

As I reach the end of this stage of my life's journey, there are many who deserve acknowledgement and thanks. Thank you to my husband Phillip for his unending support. The laughter and love you give when the stresses of field work and writing get the best of me have carried me through. Thanks to my family – the camping, hiking, star gazing, and cow pie stomping gave me a love for the natural world that has shaped my academic career and my life goals. Thank you, also, to my friends for offering a haven of adventure and fun when I most needed an escape from scholarship.

Thank you to my advisors, Dr. Chris Kucharik and Dr. Randy Jackson, as well as the DOE Great Lakes Bioenergy Research Center, the DOE OBP Office of Energy Efficiency and Renewable Energy, the UW College of Agriculture and Life Sciences Interdisciplinary Hatch Grant, Anita Thompson and the UW Department of Biological Systems Engineering Multi-State Hatch Grant, the UW Department of Agronomy, and the D.C. Smith Family for project funding. Thank you to my graduate committee – Dr. Chris Kucharik, Dr. Randy Jackson, and Dr. Jack Kloppenburg – for your support and contributions to my graduate success and for the time you have dedicated to this endeavor.

Thanks to my labmates – there are so many of you and I am grateful to the assistance and unique insights each of you has given me regarding field work, writing, statistics, ecology, and life. Thank you for your generosity of time and deep thought in support of my research. Thanks, too, for the fun times. Thank you especially to Mike Cruse and Gregg Sanford. Mike, you are a rock in the field. No matter what needed to be done, through blazing sun and pouring rain, you helped me to get it done with a cheerful, positive attitude – thank you. Gregg, you've been with me since the beginning of my life as an ecologist and introduced me to the joys and struggles of field research. I am ever grateful for your sage agronomic, scientific, and life advice and thank you, too, for putting up with my trampling in the name of science.

None of this work could have been done without my advisors. Randy, thank you for providing me with support and insight as I delved into a new area of ecology. Though I might not admit it at the time, I am grateful for your hard questions for they have made me a stronger scientist. Thanks Chris, for seeing my potential as a field researcher and bringing me into your lab family. With your help, I have grown both as a scientist and as an individual. You have supported me in research when I needed it most - not many advisors would willingly sieve soils – while giving me the freedom to explore the realm of science outreach. I owe much of my success to you. Thank you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure **Page**

RESEARCH BRIEF

Productivity and nitrogen retention tradeoffs in bioenergy grasslands

There may be a tradeoff between biomass production and nitrogen retention in perennial grasslands managed for biofuels. A study by UW-Madison researchers with the Great Lakes Bioenergy Research Center (GLBRC) found that fertilized switchgrass had higher productivity but also higher nitrogen pollution than more diverse perennial grasslands.

Nitrogen losses, often high in agriculture because of nitrogen fertilizer application, have both economic and environmental consequences. At $350-800 per ton, nitrogen fertilizer is an expensive input, yet most crops cannot use the majority of applied nitrogen before it is lost from the ecosystem. Crops with higher nitrogen use efficiency will reduce nitrogen losses and improve farmer profits.

The environment is also negatively affected by excess nitrogen. Nitrous oxide, a greenhouse gas which holds heat in the atmosphere approximately 300 times better than carbon dioxide, may be emitted at higher rates from soils with too much nitrogen. Nitrate leaching, especially problematic in fertilized cropland, causes phytoplankton blooms in aquatic ecosystems which can lead to oxygen depletion and eventually “dead-zones” such as is found in the Gulf of Mexico. Additionally, nitrate can contaminate drinking water supplies leading to fatal methemoglobinemia or “blue baby syndrome”.

Most biofuel in the United States is produced from corn grain; however, corn typically requires large nitrogen fertilizer inputs and corn grain biofuels compete with other important uses for corn, such as human food and animal feed. Perennial grassland crops may someday be managed as an alternative source of biofuel that requires less fertilizer inputs and reduces competition with food. The diversity and types of plant species present in a grassland in addition to fertilizer management may affect rates of nitrogen loss as well as aboveground biomass production.

At the UW-Madison Arlington Agricultural Research Station, researchers compared nitrous oxide emissions, potential nitrate leaching, and biomass production from fertilized (50 lbs N/acre/year) and unfertilized plots of three perennial grassland bioenergy crops: monoculture switchgrass, a native grasses mix, and a native prairie mix. Common species in the native grasses crop were Canada wildrye, big bluestem, and switchgrass, and Canada wildrye, prairie coneflower, wild bergamot, and Kentucky bluegrass were common to the prairie crop. Crops were seeded in 2008 and measurements were performed during the 2011 and 2012 field seasons.

Nitrogen losses were highest in fertilized switchgrass, but the effect of diversity was unclear

Fertilized switchgrass had up to 7 times higher nitrous oxide emissions and up to 6 times higher potential nitrate leaching than the fertilized polycultures – native grasses and prairie. Because polycultures are more diverse, they may contain species that can access different nitrogen reserves in the soil; this is compared to a monoculture in which one species can only access a single reserve. Greater access to nitrogen reserves will reduce soil nitrogen in polycultures. This may reduce nitrogen losses because less nitrogen is available to be lost. Due to simple probability, diverse crops are also more likely to contain a plant species that has high nitrogen uptake, again reducing the amount of nitrogen left in the soil.

Unfertilized switchgrass also had higher nitrous oxide emissions than the unfertilized polycultures, which again may be attributed to diversity. Interestingly, nitrogen losses were approximately the same in the native grasses and prairie crops even though prairie was more diverse and contained different species. Thus, a certain level of diversity may be necessary to reduce nitrogen losses, but a further increase in diversity may have no effect.

In the native grasses crop, greater amounts of switchgrass and other C4 photosynthetic pathway grasses (including big bluestem and Indian grass) were correlated with a reduction in potential nitrate leaching. C4 grasses have extensive root systems and are highly productive and so are likely to have enhanced uptake of soil nitrate. Inclusion of these grasses in a cropping system may be important in retaining nitrogen and enhancing economic efficiency.

Fertilizer increased N losses in switchgrass but had minimal effect in polycultures

Fertilizer application is expected to increase nitrogen losses by adding excess nitrogen to the ecosystem. Following this expectation, fertilized switchgrass had higher nitrogen losses than unfertilized switchgrass; however, the polycultures showed only a small increase in nitrous oxide emissions and no increase in potential nitrate leaching when fertilized. The more diverse crops may better utilize excess nitrogen and reduce nitrogen pollution when fertilized.

Higher diversity was linked to lower productivity

The most diverse crop, the native prairie, had the lowest productivity, while monoculture switchgrass had the highest productivity. Because the switchgrass cultivar used in this study (Cave in Rock) was partly chosen for its relatively high biomass production, the crop's high productivity in monoculture was not surprising. Likewise, the native grasses crop included a variety of productive C4 grass species and had higher productivity than the more diverse prairie crop. Even within the polyculture crops, plots with higher diversity had lower biomass production. On these productive soils, higher total grass cover and inclusion of highly productive species (e.g. switchgrass, big bluestem, Canada wildrye) may increase polyculture biomass.

Fertilizer increased productivity in the polycultures, but had no effect on switchgrass. The lack of fertilizer effect in switchgrass may be due to early-season weed encroachment and subsequent herbicide application in the fertilized switchgrass plots in 2012. Likely due to summer drought, switchgrass did not readily re-colonize after herbicide application leading to patchy growth and low biomass production. Weed encroachment was minimal in unfertilized switchgrass as well as in fertilized and unfertilized native grasses and prairie plots. These results emphasize the importance of biodiversity in resisting weed invasion. Though fertilizer was applied at the same time to the polycultures, early-season native species were already established, preventing the growth of weed species.

Tradeoffs

Land managers will have to decide whether the differences in nitrous oxide emissions and potential nitrate leaching among these crops are ecologically and economically significant. Nitrous oxide emissions estimates can be converted to carbon dioxide equivalents ~~emissions~~ based on how well these gases trap heat in the atmosphere. The difference in nitrous oxide emissions between fertilized switchgrass and unfertilized prairie was equivalent to 587 lbs of carbon dioxide per acre. About half an acre of U.S. forest growth would be required to offset this carbon release. The maximum contaminant level for nitrate in drinking water, as determined by the EPA, is 10 mg per liter. Fertilized switchgrass would potentially leach 1.71 mg nitrate per liter more than fertilized prairie. These levels of nitrogen pollution are in exchange for increases in productivity of 2.6 tons per acre and 1.5 tons per acre for fertilized switchgrass compared to unfertilized prairie and fertilized prairie, respectively.

This study shows that diverse bioenergy crops can help reduce nitrogen pollution especially when fertilizer is used, but there may be a tradeoff with aboveground biomass production. Future work will need to study these tradeoffs on less fertile soils, as bioenergy crops may be relegated to marginal lands to minimize competition with food crops.

CHAPTER 1

Sustainability tradeoffs: Fertilized switchgrass has high productivity and high nitrogen losses compared to more diverse bioenergy grassland crops

ABSTRACT

Nitrogen (N) losses including nitrous oxide (N_2O) emissions and nitrate (NO_3^-) leaching are a major source of pollution from agricultural ecosystems. Perennial grasslands may someday be managed as agricultural systems to produce cellulosic biofuel; however, little is known about how production and management of grassland crops will affect N losses or aboveground productivity. We investigated the effects of perennial grassland bioenergy system type, fertilizer management regime, and plant species diversity and composition on N_2O emissions, potential NO_3^- leaching, and productivity in southern Wisconsin. The cropping systems we compared represent a range of plant diversity and included fertilized and unfertilized monoculture switchgrass, mixed native grasses, and native prairie mix. We found significantly higher N losses in from fertilized switchgrass compared to all other crop × fertilizer treatments. Although N losses were reduced in fertilized polyculture versus fertilized monoculture, there was generally ? no reduction in N losses between the more and less diverse polycultures. Fertilizer significantly increased N_2O emissions and potential NO_3^- leaching in monoculture switchgrass; however, fertilizer application only marginally increased N_2O losses and had no effect on potential leaching in the polycultures. Productivity was highest in monoculture switchgrass and decreased with increased species diversity. Fertilizer increased polyculture productivity but had no effect in switchgrass. Species composition rather than species diversity was generally more important in reducing N losses and

? a better predictor

improving productivity. As policy makers and land managers make decisions about the types of cellulosic biofuels to be grown and how they will be managed, it is imperative that they consider impacts on both productivity and N losses in addition to effects on carbon sequestration.

Abbreviations: EISA, Energy Independence and Security Act of 2007; GLBRC, Great Lakes Bioenergy Research Center; HDPE, high-density polyethylene; NH_4^+, ammonium; NO_3^-, nitrate; N, nitrogen; N_2O, nitrous oxide; WFPS, water filled pore space.

INTRODUCTION

(cite)

The primary forms of nitrogen (N) pollution from temperate-zone agroecosystems are nitrous oxide (N_2O) emissions and N leaching as nitrate (NO_3^-) and ammonium (NH_4^+). Nitrous oxide, released from the soil into the atmosphere during denitrification or nitrification by soil microbes, is a potent greenhouse gas. Though currently found in small quantities in the atmosphere, the global warming potential of N_2O is ~300 times greater than that of carbon dioxide, making it an important driver of climate change (Forster et al., 2007). In the United States, agricultural soil management is attributed with 69.3% of the total emissions of N_2O (cite). This includes management practices that add or lead to greater release of mineral N to the soil, such as synthetic and manure fertilizer application, production of N-fixing crops and forages, manure deposition by livestock, retention of crop residues, irrigation, and tillage practices, among others. Other major sources of N_2O emissions are stationary fuel combustion, fuel combustion in motor vehicles, manure management, and nitric acid production (EPA, 2013a). Additionally, N_2O catalytically destroys stratospheric ozone and is the dominant ozone-depleting substance emitted today (Ravishankara et al., 2009).

Because anionic exchange capacity is typically low in temperate soils, NO_3^- is only loosely held by positive charges on soil surfaces and as a result, is easily lost to surface- and ground-water by leaching. As an essential plant nutrient, NO_3^- can cause phytoplankton blooms in aquatic ecosystems, which contribute to the severe oxygen depletion, or hypoxia, that is creating an average 15,650-km^2 "dead zone" in the Gulf of Mexico (Rabalais et al., 2001; Mississippi River/Gulf of Mexico Watershed Nutrient

Task Force, 2009). Nitrate in groundwater can cause fatal methemoglobinemia "blue baby syndrome" if it contaminates drinking water supplies. The cation NH_4^+ is more need a transition tightly held by negatively charged soil particles in most temperate soils minimizing leaching of this nutrient, however, it may be converted to NO_3^- by soil microbes adding to the potential for N loss. Additionally, soil NO_3^- and NH_4^+ concentrations may be linked to N_2O emissions (Snyder et al., 2009). Aside from environmental concerns, N loss via N_2O emissions or NO_3^- leaching can increase costs for agricultural producers as greater nutrient loss will require higher inputs of expensive fertilizers to maintain high yields.

The high losses of nitrogen from agricultural systems stem largely from widespread use of N fertilizer in crop production. Crops are unable to fully utilize the large pulses of N typically applied in spring to summer row crops in the central U.S. In corn, the most widely grown crop in the U.S., about 37% of applied N fertilizer is taken up by the crop (Cassman et al., 2002). The remaining pool of N is available for loss as N_2O or through NO_3^- leaching. In the past decade, corn production has increasingly been used to fuel the growing bioethanol industry, and in three of the past four years, more corn was used for ethanol than for any other single use (USDA Economic Research Service, 2013). To meet the mandate of the Energy Independence and Security Act of 2007 (EISA), 36 billion gallons of biofuels must be produced by 2022; however, only 15 billion gallons can be derived from corn starch and 16 billion gallons must be from cellulosic biofuels (United States Congress, 2007). Perennial grasslands are being investigated as a potential source of cellulosic biofuel.

Perennial grasses are expected to require less fertilizer and contribute less to N pollution than corn ethanol because of higher N-use efficiency, lower N demand, nutrient retranslocation to roots prior to harvest, and year-round cover of soils (Costello et al., 2009; Robertson et al., 2011; Smith et al., 2013). To date, studies of perennial grassland systems have generally focused on aboveground productivity and its relationship with plant species diversity. Highly productive bioenergy crops will be necessary to meet the mandates of the EISA and more productive grasslands may sequester more carbon in root biomass (Tilman et al., 2006). Typically, studies of plant diversity-productivity responses in grasslands have utilized small, intensively managed, experimental assemblages of plant species and have found positive diversity-productivity relationships (Naeem et al., 1994; Tilman et al., 1997, 2006; Symstad et al., 1998; van Ruijven and Berendse, 2005; Weigelt et al., 2009). This positive relationship is usually explained by one of two mechanisms. First, the complementarity effect which states that a more diverse communities will access and utilize resources more completely through niche differentiation which may lead to higher productivity (Loreau and Hector, 2001; Hooper et al., 2005). Another possible explanation is the selection effect. More diverse communities, are more likely to include a species that is functionally important, in this case, highly productive (Loreau and Hector, 2001; Hooper et al., 2005). expand

explain

Though the use of experimental assemblages allows for a rigorous analysis of many different combinations and monocultures of species, it is unlikely this level of management would be practical across hundreds of acres and over decades of management. Additionally, these studies often compare productivity from diverse mixtures to average monoculture productivity, but an agricultural producer is unlikely to grow a low-productivity species in monoculture. Thus, when considering likely bioenergy cropping systems, it may be more appropriate to compare only high-productivity species in monoculture to diverse mixtures. In a meta-analysis of 44 manipulative experiments, Cardinale et al. (2007) found that only 12% of diverse polycultures had greater biomass than their single most productive species. Unlike these manipulative studies, in an observational study across 34 unmanaged conservation grassland sites, higher diversity grasslands had both lower biomass production and a lower ethanol yield per unit biomass compared with less diverse grasslands (Adler et al., 2009). If perennial grasslands are to be managed for biofuels, more information will be needed to determine how crop type and management affect productivity.

Though it is expected that diversity and composition may affect productivity of a perennial grassland crop, little is known about how diversity and composition might affect N losses. wordsmith We know of only a few studies of the relationships between diversity and composition and N_2O emissions. Using experimental assemblages, Niklaus et al. (2006) found a negative correlation between species diversity and N_2O emissions. However, as with the many diversity-productivity studies, experimental assemblages do not represent typical field management and it will be necessary to determine emissions under real field conditions and management (cites?). Focusing on species composition, Epstein et al. (1998) found significantly lower N_2O emissions in C3/C4 grass mixtures when compared to C3-only plots on clay soils in one measurement year, but no differences among plant community types on sandy clay loam. Thus the effect of plant species composition may not be consistent across soil types and interannual variability may be high.

no ¶

Smith et al. (2013) studied bioenergy crops under typical field management and found that fertilized switchgrass emitted more N_2O than unfertilized prairie, though the difference was not significant. It may be that this difference in emissions was solely due to fertilizer application rather than plant community differences; therefore, a comparison of switchgrass and prairie under similar nitrogen fertilizer regimes is needed to determine the effect of crop type on N_2O emissions.

The effects of biodiversity on soil inorganic N concentrations and NO_3^- leaching have been mixed. Several studies have found reduced soil nitrate pools with higher plant species richness and functional group diversity in experimental assemblages (Tilman et al., 1996; Palmborg et al., 2005; Oelmann et al., 2007). Hooper and Vitousek (1998), on the other hand, found no reduction in NO_3^- leaching with increasing diversity. Instead, they found functional group identity explained more of the variance in leaching losses than did richness alone. In particular, legume presence has been found to increase N losses (Scherer-Lorenzen et al., 2003; Palmborg et al., 2005). On field-scale plots, Smith et al. (2013) found no significant difference in NO_3^- leaching between fertilized switchgrass and unfertilized prairie plots. As with crop productivity and N_2O emissions, a better understanding of the effect of diversity and composition on NO_3^- leaching under typical field management is needed.

We investigated the effects of perennial grassland bioenergy system type, fertilizer management regime, and plant species diversity and composition on N_2O emissions, potential NO_3^- leaching, and aboveground productivity. The cropping systems we compared represented a range of plant diversity and included fertilized and unfertilized monoculture switchgrass (*Panicum virgatum* L.), mixed native grasses, and native prairie mix, as well as unreplicated restored prairie and giant miscanthus (*Miscanthus* × *giganteus* J.M. Greef & Deuter ex Hodkinson & Renvoize) reference plots.

METHODS

Field sites

All measurements were performed at the Great Lakes Bioenergy Research Center’s (GLBRC) Biofuels Cropping Systems Trial site at the Arlington Agricultural Research Station (Arlington, WI, 43°17'N, -89°22'W) during the 2011 and 2012 field seasons. Soils at this site are classified as Plano silt loams (Fine-silty, mixed, superactive, mesic typic Argiudolls). These are highly productive soils formed under the Empire Prairie and part of the Prairie-Savanna ecotone. Average corn grain yield on these soils is 8.61 Mg ha^{-1} and 9.40 Mg ha^{-1} for a corn-soybean rotation (Posner et al., 2008). Mean annual air temperature is 6.8°C and mean annual precipitation is 869 mm (1981-2010, National Climate Data Center).

The cropping systems trial, established in 2008, consists of 10 potential cellulosic bioenergy cropping systems in a randomized, complete block design consisting of 5 blocks. For this experiment, we studied three perennial grass-based cropping systems (hereafter termed “crop”) of varying levels of biodiversity—monoculture ‘Cave-in-Rock’ switchgrass (*Panicum virgatum* L.), mixed native grasses, and high-diversity native prairie—replicated across three randomly chosen blocks (Table 1). Each plot was split into a main-plot (43×9 m) and a micro-plot (43×4.5 m) with differing N fertilizer regimens. Each year, maintenance levels of N fertilizer (56 kg N ha^{-1} as ammonium nitrate) were added to the main plot of the switchgrass and mixed native grasses treatments and the micro-plots of the native prairie systems. No fertilizer was applied to the switchgrass and native grasses micro-plots nor to the prairie main plots. During this study, fertilizer application occurred on 27 May 2011 and 11 May 2012. All treatments were no-till managed and all management used field-scale equipment.

Six 1.5 × 1.5-m quadrats were delineated in each plot with 3 quadrats placed in each of the main-plot and the micro-plot. The three-block design resulted in 9 quadrats per crop × fertilizer combination. A reference set of 9 quadrats were delineated at a restored prairie (established in 1976) 4 km to the north of the cropping systems trial. This restored prairie is located on the same Plano silt loam soil series as the cropping systems trial. It is unfertilized and periodically burned though it had not been burned for 3 years prior to this study. These quadrats were used as a reference example of an unmanaged grassland. In 2012, an additional 6 quadrats were delineated in a plot of fertilized (56 kg N ha^{-1}) giant Miscanthus (*Miscanthus* × *giganteus* J.M. Greef & Deuter ex Hodkinson & Renvoize) at the GLBRC biofuels cropping system trial. These quadrats provided an additional monoculture for reference.

Nitrous oxide emissions

Soil N_2O fluxes were measured twice monthly from 9 May - 14 September 2011 and 7 May - 6 September 2012. This included one measurement prior to fertilization in each fertilized quadrat. N_2O emissions were measured using a closed, non-through-flow trace gas flux chamber method (Livingston and Hutchinson, 1995). A 27.15-cm diameter stainless steel, open-bottomed chamber was inserted 5.5 cm into the soil in each quadrat. Chambers had an effective height of 17.3 cm from the soil surface and volume of 0.01 m^3. Chambers were fitted with a 2-mm diameter vent and vent tube located on the chamber wall approximately 14 cm from the soil surface to avoid pressure perturbations during chamber closure and gas sampling (Hutchinson and Livingston, 2001). In an effort to limit disturbance to the sampling area, trampling was minimized during the installation of the chambers and during each sampling period. Plants growing within the chambers were clipped flush with the chamber top at least 24 h before measurement to allow the lid to be closed.

On each measurement date, a white, high-density polyethylene (HDPE) lid with a butyl rubber syringe port was clamped onto each chamber to allow gas concentration to increase for the purposes of estimating a gas flux. Using a 30-mL Luer-Lok™ syringe (BD, Franklin Lakes, NJ, USA) fitted with a polycarbonate stopcock (Cole-Parmer, Vernon Hills, IL, USA) and 23-gauge needle (BD, Franklin Lakes, NJ, USA), gas was extracted from the chamber headspace at 0, 10, 20, and 30 min after the lid was clamped on the chamber. Chamber headspace was not mixed prior to sampling, because mixing may cause pressure perturbations and/or excess dilution of headspace gas (Parkin and

Venterea, 2010). As recommended in a review of gas flux chamber methods by Rochette and Eriksen-Hamel (2008), glass 5.9-mL Exetainer® vials (Labco Ltd., Buckinghamshire, UK) were used. Vials were overcharged with 10-mL gas samples to guard against sample contamination and facilitate subsequent removal of the gas sample for analysis (Parkin and Venterea, 2010). Concurrently with field measurements of chamber gas samples, ambient air and field standards (400 ppm CO_2, 1 ppm N_2O, and 1 ppm CH_4) were loaded into vials to assess background GHG concentrations and potential storage degradation in the period between sampling and analysis. Samples were transported back to a UW-Madison lab for analysis by gas chromatography with a flame ionization detector (CH_4), a 15 mCi ^{63}Ni electron capture detector (N_2O) (Agilent 7890A GC System, Santa Clara, CA, USA) and infra-red gas analyzer for CO_2 (LI-COR LI-820 CO_2 Analyzer, Lincoln, Nebraska, USA). The gas chromatograph was calibrated before each analysis using 15- 25 laboratory standards (400 ppm CO_2, 1 ppm N_2O, and 1 ppm CH_4). Air temperature at time of deployment, obtained from an on-site weather station (30-min continuous data), was used to correct for temperature differences between gas sampling and lab analysis based on the perfect gas law (Rochette and Hutchinson, 2005).

The HMR method was used to estimate the increase in gas concentration over time, or flux (Pedersen et al., 2010). The HMR method applies a non-linear model when possible (revised from Hutchinson and Mosier, 1981) but can also automatically detect
appropriate?
when a linear regression should be applied or when data should be characterized as having a zero-flux value (for details see Pedersen et al. 2010). The HMR approach was implemented in R (version 2.15.2, R, 2012). The HMR default flux calculations were

used except in the case of outliers. Outliers were determined by comparing HMR default flux calculations to fluxes calculated by linear regression. Outliers were visually checked using the HMR algorithm and also checked against concurrently measured CO_2 concentrations to determine whether HMR, linear regression, or no flux was most appropriate. Linear interpolation between measurement dates was used to estimate total N loss via soil N_2O emissions for the length of the measurement period. Fertilizer-induced emissions, or emission factors, as used by the IPCC, were calculated as the N_2O-N loss from a crop with N fertilizer application minus the N_2O-N loss from the same crop without fertilizer and expressed as a percentage of the N applied as fertilizer (De Klein et al., 2006; Stehfest and Bouwman, 2006). Arithmetic means were used for emission factor calculations.

Because soil environmental conditions are correlated with flux rates (Rustad et al., 2000; Dobbie and Smith, 2003), soil temperature and moisture were measured to determine whether differences in these conditions were occurring and whether these environmental differences were affecting diversity-flux relationships. Concurrently with N_2O flux measurements, 10-cm soil temperatures were obtained using a hand-held temperature probe (HANNA Instruments, Inc., Woonsocket, RI, USA). Three measurements were taken within 25 cm of each chamber and measurements from all sampling dates were averaged to obtain a season average soil temperature for each quadrat. Similarly, within each quadrat, three measurements of volumetric water content (VWC) (m^3m^{-3}) in the surface 6-cm of soil were measured using a Theta Probe soil moisture sensor (Dynamax, Inc., Houston, TX, USA) and averaged across all measurement dates to obtain a single season average VWC value for each quadrat. Using VWC, bulk densities for each plot (measured in 2008 for the surface 10-cm), and a standard particle density of 2.65 g cm^{-3}, soil water filled pore space (WFPS) was calculated for each quadrat.

Soil inorganic nitrogen

biomass

Soil samples were taken near peak plant productivity in late July/early August of each year. Three soil samples at a depth of 50 to 80 cm were removed from each quadrat and combined giving one sample per quadrat. These samples were used to estimate potential NO_3^- leaching. Globally, more than 80% of root biomass is found in the surface 30 cm of temperate grasslands soils (Jackson et al., 1996). Additionally, in remnant and restored Wisconsin mesic prairies representative of the larger region, Kucharik et al. (2006) found 88- to 92% of fine root biomass to 50 cm was contained in the top 30 cm. Thus, it is expected that very little nitrate that has leached beyond 50 cm will be taken up by roots. Samples were homogenized with a 2-mm sieve and plant and animal materials and rocks were removed. Ten-gram subsamples were weighed out for immediate (within 48 h of field sampling) inorganic N extraction in 2 *M* KCl (Robertson, Coleman et al. 1999). Extracts were frozen prior to colorimetric NO_3^- (according to USEPA Method 353.2, O-I-Analytical Method #2648) determination on a O-I-Analytical FS 3100 flow injection analyzer (O-I-Analytical, College Station, TX). Soil subsamples were weighed before and after drying to obtain soil gravimetric water content to calculate inorganic N on a mass basis.

Plant diversity, composition, and aboveground productivity

Plant species richness and composition were measured near peak plant productivity, 15-23 August 2011 and 16-20 July 2012. The earlier measurement dates in 2012 were a result of ~~much above~~ average March temperatures and summer drought.
that were well above
Plant species richness and composition were measured within each 1.5-m^2 quadrat using the point-intercept method (Elzinga et al., 1998). Each quadrat was divided by 7 horizontal and 7 vertical lines forming 49 intersections where a rod was dropped and every plant species intercepted by the rod was counted, though each species was counted only once per intercept. The number of hits divided by the total possible hits (49) resulted in an estimation of percent cover for each species within the quadrat. Because multiple species could be counted at each point, total percent cover could total greater than 100% (Elzinga et al., 1998). After all field measurements were completed (23-30 September 2011 and 21-27 August 2012), all aboveground biomass was hand-harvested to ground level from each quadrat, dried, and weighed to estimate aboveground productivity. in

Statistical analysis

Likelihood ratio tests of competing linear mixed effects models (R version 2.15.2, R, 2012) were used to compare N-loss pathways, aboveground productivity, and soil temperature and WFPS among crop and fertilizer treatments. This method was also used to test for plant species diversity and composition effects on these response variables. Nitrous oxide emission rates and potential NO_3^- leaching data were log-transformed to normalize distributions prior to analysis. We accounted for year-to-year variability, variability among blocks within each year, and variability between the fertilized and unfertilized split-plots within each block by modeling them as nested random effects. We tested for unequal variances among crop and fertilizer treatments by comparing models with separate variance terms for each treatment combination to models with pooled error variances. To test significances of fixed effects, saturated models were first constructed. For crop and fertilizer treatment effects, least-squares means were used to determine which treatments were most similar. These treatments were grouped and ANOVA was used to compare to the saturated model. If models were not significantly different ($p>0.05$), the simpler model (combined treatments) was used. The effects of diversity and composition factors were tested by comparing models with and without each diversity or composition factor. If the models were significantly different ($p<0.05$), the diversity or composition factor was considered to have a significant effect on the response variable. Correlation coefficients among all diversity and composition factors were also calculated (Microsoft Office Excel 2007).

RESULTS

We found a significant crop × fertilizer effect on total N_2O emissions (Fig. 1) where fertilized switchgrass was 4.6 and 6.9 times higher than fertilized prairie and fertilized mixed native grasses, respectively. Fertilized native grasses and prairie were more similar though prairie was significantly higher. Unfertilized switchgrass was also higher than unfertilized native grasses and prairie, and these polycultures were not significantly different from each other. Though unreplicated, Miscanthus had emissions most similar to unfertilized switchgrass. The restored prairie had low emissions, similar to the other unfertilized polycultures. Fertilizer increased N_2O emissions in all crops though the effect was greatest in switchgrass with 3 times higher emissions when fertilized. Fertilizer-induced emissions were 1.9%, 0.14%, and 0.39% of the fertilizer N applied for switchgrass, native grasses, and prairie, respectively.

There was also a significant crop × fertilizer effect on average potential NO_3^- leaching (Fig. 2). In particular, fertilized switchgrass had up to 6 times higher potential leaching than the fertilized polyculture crops. Fertilized prairie had the lowest potential leaching even compared to the unfertilized crops. All three unfertilized crops were not significantly different from one another. Miscanthus was similar to fertilized switchgrass, and the restored prairie had lower potential leaching than all other crop/fertilizer treatments. Potential NO_3^- leaching was significantly higher in fertilized versus unfertilized switchgrass, but there was no effect of fertilizer in the polycultures.

There was a significant crop × fertilizer effect on aboveground productivity as well (Fig. 3). Fertilized and unfertilized switchgrass had the highest productivity followed by native grasses and prairie. Miscanthus had even higher productivity than switchgrass, while the restored prairie had the lowest productivity. Fertilizer increased productivity in the polycultures, but did not increase switchgrass productivity.

Average 10 cm soil temperatures exhibited a crop x fertilizer interaction (Table 2). Unfertilized native grasses and unfertilized prairie were not significantly different from one another. Likewise, fertilized and unfertilized switchgrass, fertilized native grasses, and fertilized prairie did not have significantly different soil temperatures.

However unfertilized native grasses and prairie had a significantly higher soil temperature (0.37-0.68°C) than fertilized and unfertilized switchgrass and fertilized native grasses and prairie. Soil water filled pore space (WFPS) differed by crop and by fertilizer treatment but there was no crop x fertilizer interaction (Table 2). Switchgrass had the highest WFPS followed by prairie and native grasses. Unfertilized quadrats had higher WFPS on average than fertilized quadrats, with a difference of 1.47%.

Mean values for several diversity and composition measures and the species with the highest cover for each crop are shown in Table 1. We found no effect of diversity and composition on N_2O emissions from native grasses and prairie plots. We also found no effect of diversity and composition when we analyzed unfertilized native grasses and unfertilized prairie together. In native grasses, C4 grass cover and switchgrass cover explained a significant amount of variability in potential NO_3^- leaching (Fig. 4). As switchgrass or C4 cover increased, there was a decrease in potential leaching. There was no effect of diversity and composition on potential NO_3^- leaching in prairie. Similarly, when unfertilized native grasses and prairie were combined, there was no effect of diversity or composition on potential NO_3^- leaching. Within native grasses quadrats, species richness explained a significant amount of the variability in aboveground productivity (Fig. 5). As species richness increased, productivity decreased in native grasses quadrats. C4 grass cover and total grass cover were also significantly positively correlated with productivity, but only within unfertilized native grasses (Fig. 6). Within prairie, Shannon's diversity index was significantly negatively correlated with productivity, while C3 grass cover was significantly positively correlated with productivity (Fig. 7).

C4 grass cover and switchgrass cover were highly positively correlated (r=0.80, Table 3). This was the highest coefficient of correlation for C4 grass cover. Percent C4 grass cover was also strongly correlated with total grass cover (r=0.71). Shannon's diversity index and C3 grass cover had a correlation coefficient of 0.60.

DISCUSSION

N losses were highest in fertilized switchgrass, but the effect of diversity was unclear

Fertilized monoculture switchgrass had substantially higher N_2O emissions and potential NO_3^- leaching than the fertilized polyculture crops, native grasses and prairie. Through complementary resource use, the native grasses and prairie crops may have taken up a greater amount of soil N, resulting in lower N losses from the system. Complementarity here? The selection effect may also have played a role, as the polyculture crops may have contained a plant species that had relatively high rates of nitrogen uptake, thus reducing N losses from the system. any indication of this? Several studies have found reduced rooting zone nitrate with increased grassland diversity (Tilman et al., 1996; Palmborg et al., 2005; Oelmann et al., 2007) and soil inorganic N concentrations are considered a major driver of N_2O emissions and NO_3^- leaching (Di and Cameron, 2002; De Klein et al., 2006).

Higher soil water filled pore space (WFPS) in switchgrass may also have increased rates of nitrification and denitrification and thus, N_2O emissions (Mosier et al., 1996; Epstein et al., 1998; Dobbie and Smith, 2003). However, higher WFPS in unfertilized prairie compared to unfertilized native grasses without concurrently higher emissions suggests WFPS may not have significantly impacted emissions in these crops. Because optimum conditions for N_2O production occur at upwards of 60% WFPS (Skiba and Smith, 2000), the small differences in WFPS we observed may not have been sufficient to alter emissions. Higher soil temperatures can also lead to higher N losses by enhancing soil microbial activity (Mosier et al., 1996; Skiba and Smith, 2000; Smith et al., 2011; Sun et al., 2013). However, soil temperature does not appear to have played a major role in increasing N losses in these crops. Though soil temperatures were higher in unfertilized native grasses and prairie compared to unfertilized switchgrass, potential NO_3^- leaching did not differ significantly and N_2O emissions were actually lower in the polycultures.

An interaction between switchgrass and the microbial community by a mechanism other than competition for nitrogen may also be affecting N losses. For example, Mao et al. (2013) demonstrated that cultivation of perennial grassland bioenergy crops significantly changed the abundance and diversity of ammonia-oxidizing, N-fixing, and denitrifying organisms in soils compared with corn. Further investigation into the impact of bioenergy grassland crops on the microbial community may be necessary to better understand the differences in N losses we observed among crops.

are occurring...

awkward

Like the fertilized crops, unfertilized switchgrass had higher N_2O emissions than unfertilized polycultures; however, it did not have significantly higher potential NO_3^- leaching. Again, the lower emissions in the polycultures may be due to complementarity, the selection effect, or a change in the microbial community. Importantly, there were consistently higher N_2O emissions from switchgrass regardless of fertilizer management indicating an effect of crop type.

Although N losses were reduced in fertilized polyculture versus fertilized monoculture, there was generally no reduction in N losses between the more and less diverse polycultures. Simply converting from a monoculture to a polyculture may have reduced N losses regardless of precise particular species richness and composition. However, switchgrass quadrats were rarely total monocultures in our study. Though mean switchgrass cover was 92% in switchgrass quadrats, weed pressure raised mean species richness to 3.6 species per quadrat and the crop sometimes had two species with greater than 50% cover each. Instead, there may be a tipping point in species diversity. Perhaps, N losses are reduced when a plant community reaches a particular level of diversity, but beyond that point there is no effect of diversity on emissions. This is supported by Niklaus et al. (2006) who found reduced N_2O emissions in diverse grasslands compared to monocultures, but found no further reduction in emissions as diversity increased from six to nine species. This is also consistent with our finding that, within the polyculture native grasses or prairie crops, species diversity had no significant relationship with N_2O emissions or potential NO_3^- leaching. However, species composition did play a role in potential leaching. In the native grasses crop, switchgrass and C4 grass cover were really ?

significantly negatively correlated with potential NO_3^- leaching. Because switchgrass and C4 grasses have extensive fine root systems, low root turnover, and are highly productive, uptake of root zone NO_3^- was likely enhanced by increased cover of these grasses (Craine et al., 2002).

We were surprised to find higher N_2O emissions from the fertilized prairie compared to the less diverse fertilized native grasses crop. Higher emissions may have been induced by the presence of N-fixing legumes in the prairie (Niklaus et al., 2006) or by higher WFPS; however, unfertilized prairie, which both contained legumes and had higher WFPS, did not have higher emissions than unfertilized native grasses. Instead, a reduction in C4 grass cover in the prairie versus the native grasses might explain the higher emissions. Nitrogen uptake by C4 grasses may be especially important when soil N is high from fertilization. Fertilized prairie also had the lowest potential nitrate leaching which may be linked to its higher N_2O emissions (Brumme et al., 1999). Higher N_2O emissions may have reduced soil N so that less NO_3^- was lost below the root zone.

Although potential NO_3^- leaching in fertilized Miscanthus was similar to fertilized switchgrass, interestingly, N_2O emissions from Miscanthus were more similar to unfertilized switchgrass and fertilized polycultures than to fertilized switchgrass. This indicates monocultures may not all behave in the same manner. Instead, the high N_2O emissions from fertilized switchgrass may be an effect of the switchgrass species itself on nitrogen cycling rather than the result of low diversity alone. As other species are added to the community, the effects of switchgrass on nitrogen cycling may be reduced through complementarity or through a simple reduction in the amount of switchgrass present. Importantly, Miscanthus data was collected only in 2012 when summer drought reduced soil moisture, potentially leading to reduced fluxes. Also, fewer Miscanthus quadrats were measured and there were no replicates. Therefore, although these data suggest a difference between monocultures, further study of N losses in Miscanthus, switchgrass, and other potential bioenergy crop monocultures is necessary. As expected, the restored prairie site had very low N losses, most similar to the unfertilized polycultures. This suggests that polyculture grassland crops, when left unfertilized, may behave similarly to a natural grassland ecosystem.

Fertilizer increased N losses in switchgrass but had minimal effect in polycultures

Fertilizer significantly increased N_2O emissions and potential NO_3^- leaching in monoculture switchgrass; however, fertilizer application only marginally increased N_2O losses and had no effect on potential leaching in the polycultures. This difference between switchgrass and the polycultures is emphasized by the emission factors. The N_2O emission factor for switchgrass (fertilized with 56 kg N ha^{-1}) was 1.9%, substantially higher than the IPCC suggested default of 1% (De Klein et al., 2006). However, the emission factors for fertilized native grasses and prairie (0.14% and 0.39%, respectively) were much lower than the default. Perhaps through complementarity or the selection effect, the polycultures appear to have significantly reduced the impact of fertilizer on N losses. These results also emphasize that emission factors are dependent on crop type and management and, if possible, more accurate emission factors should be used when determining N losses from managed systems. Additionally, these emission factors are based on a single rate of fertilizer application, but N_2O emission factors may not be constant and instead dependent on rates of N input (Kim et al., 2013).

Though the effect of fertilizer on N losses was reduced in polycultures, N_2O emissions were still higher in fertilized plots. In general, N_2O emissions will increase with fertilization if a) microbes out compete plants in consumption of available N or b) fertilization rates exceed optimal N plan uptake rates (Kim et al., 2013). Because the field site in this study had naturally fertile soils, the addition of fertilizer may have exceeded plant need and led to increased emissions, regardless of crop type. In the literature, the relationship between N fertilizer treatment and emissions in grasslands is unclear. For example, Mosier et al. (1991) found higher N_2O emissions in fertilized pasture treatments, while Nikièma et al. (2011) found no impact of fertilizer on N_2O emissions in switchgrass. Therefore, although we found an increase in emissions from all fertilized crops, this relationship is likely dependent on soil type, climate, weather, and other factors.

More diverse plots were less productive

~~Higher diversity was linked to lower productivity~~

Contrary to manipulative studies (Naeem et al., 1994; Tilman et al., 1997, 2006; Symstad et al., 1998; van Ruijven and Berendse, 2005; Weigelt et al., 2009) and in agreement with the findings of Adler et al. (2009), we found reduced aboveground productivity as diversity increased. Because the switchgrass cultivar used in this study was partly chosen for its relatively high productivity, we were not surprised by its high productivity in monoculture (Casler and Boe, 2003). Likewise, the native grasses crop included a variety of productive C4 grass species and had higher productivity than the more diverse prairie crop. The restored prairie followed the trend, with low productivity and diversity similar to the bioenergy polycultures (mean 8.4 species per quadrat). Within both the native grasses and the prairie crops, diversity was negatively correlated with productivity. Therefore, both within crops and among crops, increased diversity was linked with lower aboveground productivity. The fertile soils of this study site may have affected the relationship between productivity and biodiversity. When nutrients are more limiting, such as in the marginal soils of some manipulative biodiversity-productivity studies, complementary resource use may play a more essential role in plant community productivity than it did in our study. Additionally, studies using experimental assemblages of plant species often compare the productivity of diverse systems to an average productivity of monoculture systems, rather than to the most productive monoculture. In an agricultural system, such as those studied here, it is more relevant to compare only to the most productive monocultures, as these are the crops that will likely be chosen by a producer. Though this study is not as rigorous a test of diversity as the manipulative studies, our results better represent the true effects of crop type under typical agricultural management.

communities?

Though species diversity negatively affected productivity, several species compositions improved productivity. Productivity of unfertilized native grasses had a significant positive relationship with C4 grass cover and total grass cover, both of which are highly correlated with one another. Therefore, at this site, crop productivity may be increased through the inclusion of more C4 grasses. Because switchgrass cover was not significant, it seems that any C4 grass, not just switchgrass, will improve productivity. This relationship between C4 grass cover and productivity was probably significant in the unfertilized rather than fertilized native grasses crop because productivity is more reliant on N-use efficiency when N is more limiting and C4 grasses have particularly high N-use efficiency (Brown, 1978). In the prairie crop, productivity had a significant positive relationship with C3 grass cover which was likely driven by the abundance of the productive C3 grass, *Elymus canadensis* L. (Canada wildrye).

As expected, fertilizer application increased productivity in native grasses and prairie. This is supported by several studies showing increased aboveground productivity with fertilizer application in grasslands (Heggenstaller et al., 2009; Weigelt et al., 2009; Wullschleger et al., 2010). In our study, fertilizer did not increase switchgrass productivity which is likely due to relatively low fertilized switchgrass productivity in 2012. Weed encroachment into the fertilized switchgrass plots was high in 2012, probably due to the unseasonably warm spring and the nitrogen boost the early-season weeds received from the fertilizer application. Switchgrass was unable to re-colonize the area after herbicide application leading to patchy growth and low aboveground productivity. Weed encroachment was minimal in unfertilized switchgrass as well as fertilized and unfertilized native grasses and prairie plots. These results emphasize the importance of biodiversity in resisting weed invasion. Though fertilizer was applied at the same time to the polycultures, early-season native species were already established, preventing the growth of weed species.

Land managers will have to decide whether the differences in N_2O emissions and potential NO_3^- leaching among these treatments are ecologically and economically significant. The difference in N_2O emissions between fertilized switchgrass and unfertilized prairie over the course of the measurement period was 2.12 kg N_2O ha^{-1} or 658 kg CO_2-eq ha^{-1}. This is equivalent to the amount of carbon sequestered annually by 0.22 ha of U.S. forest (EPA, 2013b). The difference in potential NO_3^- leaching between fertilized switchgrass and fertilized prairie was 1.20 μg-N g dry weight $soil^{-1}$. If we assume all of this N below the root zone will be leached (likely an overestimation), fertilized switchgrass would leach 1.71 mg L^{-1} more than fertilized prairie. The maximum contaminant level for nitrate in drinking water, as determined by the EPA, is 10 mg L^{-1} (EPA, 2009). These N losses are in exchange for increases in productivity of 5.9 Mg ha^{-1} and 3.4 Mg ha^{-1} for fertilized switchgrass compared to unfertilized prairie and fertilized prairie, respectively.

In addition to land management, these results have implications for ecosystem modeling. Ecosystem models, such as DAYCENT, Agro-IBIS, APEX, and EPIC are used to scale-up from the field to a regional-, national-, or global-scale in order to determine the broader impacts of crops and management on productivity, the greenhouse gas balance of the system, climate change, and other environmental effects (Norman et al., 1997; Parton et al., 2001; Kucharik, 2003; Gassman et al., 2010; Izaurralde et al., 2012). Currently, these models are unable to account for the differences we observed in N losses and fertilizer effects among these perennial grassland crops. These data, and other similar field datasets, will be necessary to improve ecosystem models and calculate more accurate estimates for greenhouse gas budgets and environmental impacts.

CONCLUSIONS

We found significant differences in N losses and aboveground productivity in the perennial grassland bioenergy systems studied here. In particular, fertilized switchgrass, with high N losses and high productivity, demonstrated a tradeoff between ecological and economic sustainability. Fortunately, unfertilized switchgrass may provide a good compromise, with N losses more similar to the polycultures and, at least over 2 years, no loss in productivity compared to fertilized switchgrass. Other monocultures, such as Miscanthus, may have different effects on N losses, and a field scale study comparing various monocultures to more diverse systems is necessary. Unlike in many previous studies, biodiversity reduced aboveground productivity. Instead, species composition, including C4 grass cover in the native grasses crop and C3 grass cover in the prairie crop, was correlated with higher productivity. More diverse crops did have lower N losses though species diversity per se was not important within polycultures. Again, species composition, particularly switchgrass and C4 grass cover, was important in reducing potential NO_3^- leaching. These results highlight the importance of species composition, rather than solely diversity, in determining ecosystem function. Importantly, more diverse crops better utilized fertilizer and minimized its effects on N losses while still gaining a boost in productivity. Thus, at least in polycultures, fertilizer may be a worthwhile investment. The results of this study are from a fertile site. To minimize competition with food crops, bioenergy crops may be relegated to marginal lands. Therefore, it will also be necessary to determine productivity of these grassland crops and their effects on N losses when grown on less fertile soils. As policy makers and land managers make decisions about the types of cellulosic biofuels to be grown and how they will be managed, it is imperative that they consider impacts on both productivity and N losses in addition to effects on carbon sequestration.

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TABLES

Table 1: Species planted, realized mean species diversity and composition, and most common species present (% cover > 15%) for switchgrass, native grasses, and prairie crops.

Crop	Species planted	Realized species diversity and composition								
		Species richness	Simpson's diversity index	Shannon's diversity index	Functional richness	% C3 grass cover	% C4 grass cover	% non-legume forb cover	% legume cover	Most common species (% cover)
Switchgrass	*Panicum virgatum* L.	3.6 ± 0.31	1.9 ± 0.10	0.73 ± 0.07	1.9 ± .13	5.0 ± 1.8	127.2 ± 8.2	11.9 ± 3.1	0	*Panicum virgatum* L. (92%), *Setaria faberi* Herrm. (28%)
Native grasses	*Panicum virgatum* L., *Elymus canadensis* L., *Schizachyrium scoparium* (Michx.) Nash, *Andropogon gerardii* Vitman, *Sorghastrum nutans* L.	6.0 ± 0.33	3.4 ± 0.17	1.4 ± 0.05	2.6 ± 0.08	72.7 ± 5.0	103.2 ± 7.9	6.8 ± 1.9	0	*Elymus canadensis* L. (61%), *Andropogon gerardii* Vitman (45%), *Panicum virgatum* L. (31%)
Prairie	*Panicum virgatum* L., *Elymus canadensis* L., *Schizachyrium scoparium* (Michx.) Nash, *Andropogon gerardii* Vitman, *Sorghastrum nutans* L., *Koeleria cristata* (Ledeb.) Schult., *Desmodium canadense* (L.) DC., *Lespedeza capitata* Michx., *Baptisia alba* (L.) Vent. var. *macrophylla* (Larisey) Isely, *Rudbeckia hirta* L., *Anemone canadensis* L., *Asclepias tuberose* L., *Monarda fistulosa* L., *Silphium perfoliatum* L., *Ratibida pinnata* (Vent.) Barnhart, *Solidago rigida* L., *Solidago speciosa* Nutt., *Aster novae-angliae* (L.) G.L. Nesom	10.9 ± 0.29	5.3 ± 0.23	1.9 ± 0.04	3.7 ± 0.09	90.2 ± 3.8	21.1 ± 3.7	86.5 ± 4.1	10.4 ± 2.4	*Elymus canadensis* L. (60%), *Ratibida pinnata* (Vent.) Barnhart (40%), *Monarda fistulosa* L. (21%), *Poa pratensis* L. (17%)

Table 2: Mean 10 cm soil temperatures and 0-10 cm soil water filled pore space (WFPS) for each crop and fertilizer treatment for the measurement period (May-September) across 2011 and 2012. Given are arithmetic means ± standard error. Lowercase letters indicate significant temperature differences among crop and fertilizer treatments ($p<0.05$). Uppercase letters indicate significant WFPS differences among crops. Greek letters indicate significant WFPS differences among fertilizer treatments.

	Fertilizer treatment	10 cm soil temperature	0-10 cm % water-filled pore space (WFPS)
Switchgrass	Fertilized	20.08 ± 0.22^{a}	38.16 ± 0.60^{A}
	Unfertilized	20.06 ± 0.22^{a}	
Native grasses	Fertilized	20.06 ± 0.22^{a}	34.83 ± 0.46^{B}
	Unfertilized	20.44 ± 0.25^{b}	
Prairie	Fertilized	20.03 ± 0.21^{a}	37.47 ± 0.54^{C}
	Unfertilized	20.71 ± 0.21^{b}	
All fertilized crops		-	$36.09 \pm 0.49^{\alpha}$
All unfertilized crops		-	$37.56 \pm 0.45^{\beta}$

Table 3: Correlation matrix of all diversity and composition factors tested. Correlations between diversity and composition factors that had significant relationships with response variables are bolded.

	Species richness	Log (species richness)	Simpson's diversity index	Shannon's diversity index	Exp (Shannon's diversity)	Functional richness	% switchgrass	% C4 grass	% C3 grass	% non-legume forb	% legume	% grass	% forb	Total % cover
Species richness	1	0.9659	0.8977	0.9257	0.9506	0.8503	-0.6559	-0.5774	0.5942	0.7781	0.5142	-0.2247	0.8056	0.6040
Log (species richness)		1	0.8567	0.9458	0.9062	0.8470	-0.6635	-0.5121	0.6087	0.7233	0.4288	-0.1189	0.7387	0.6407
Simpson's diversity index			1	0.9419	0.9846	0.7618	-0.5690	-0.3854	0.5140	0.6880	0.5430	-0.0346	0.7307	0.7216
Shannon's diversity index				1	0.9585	0.7910	-0.6539	-0.4103	**0.6023**	0.6815	0.4462	0.0172	0.7048	0.7444
Exp(Shannon's diversity index)					1	0.7996	-0.6225	-0.4747	0.5654	0.7228	0.5316	-0.1091	0.7595	0.6761
Functional richness						1	-0.6408	-0.6088	0.5487	0.7297	0.4866	-0.3133	0.7564	0.4711
% switchgrass							1	**0.8008**	-0.8963	-0.5496	-0.3244	0.2406	-0.5610	-0.3486
% C4 grass								1	-0.6998	-0.6526	-0.3618	**0.7125**	-0.6613	0.0145
% C3 grass									1	0.4210	0.2088	0.0026	0.4215	0.4392
% non-legume forb										1	0.4025	-0.5001	0.9819	0.5190
% legume											1	-0.3015	0.5687	0.2896
% grass												1	-0.5117	0.4517
% forb													1	0.5263
Total % cover														1

FIGURES

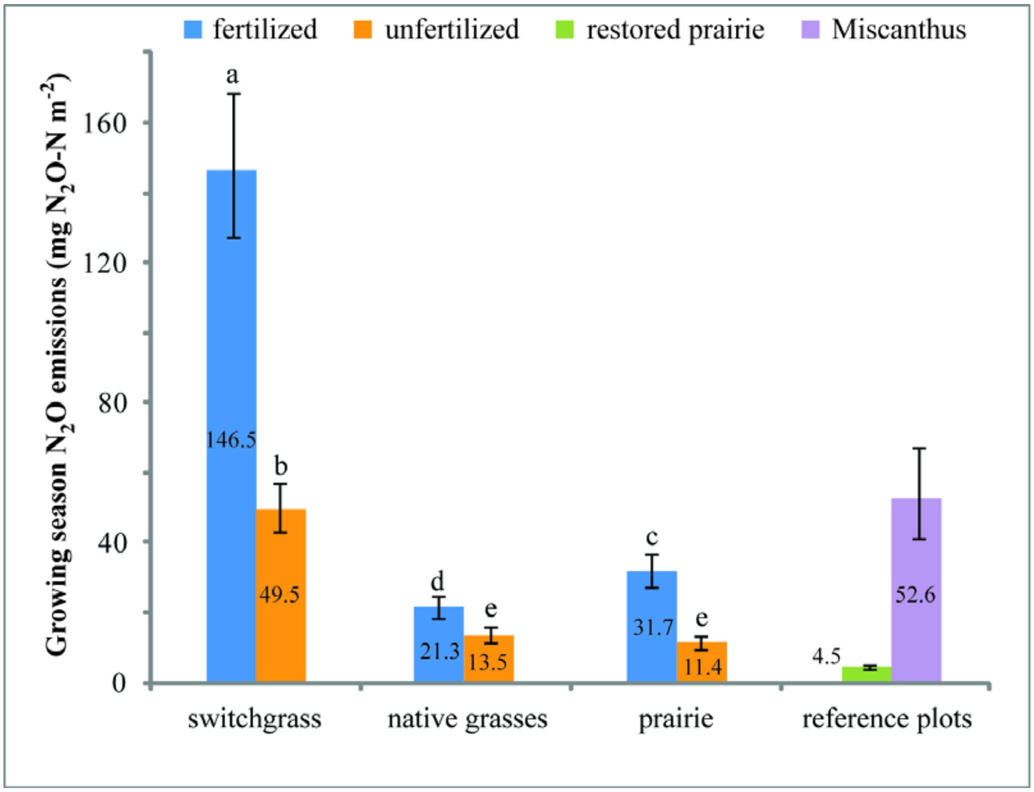

Figure 1: Average total N_2O emissions for the measurement period (May-September) across 2011 and 2012 for each crop and fertilizer treatment. Given are back-transformed geometric means ± standard error. Letters indicate significant differences among treatments ($p<0.05$). The reference plots were not included in the statistical analysis.

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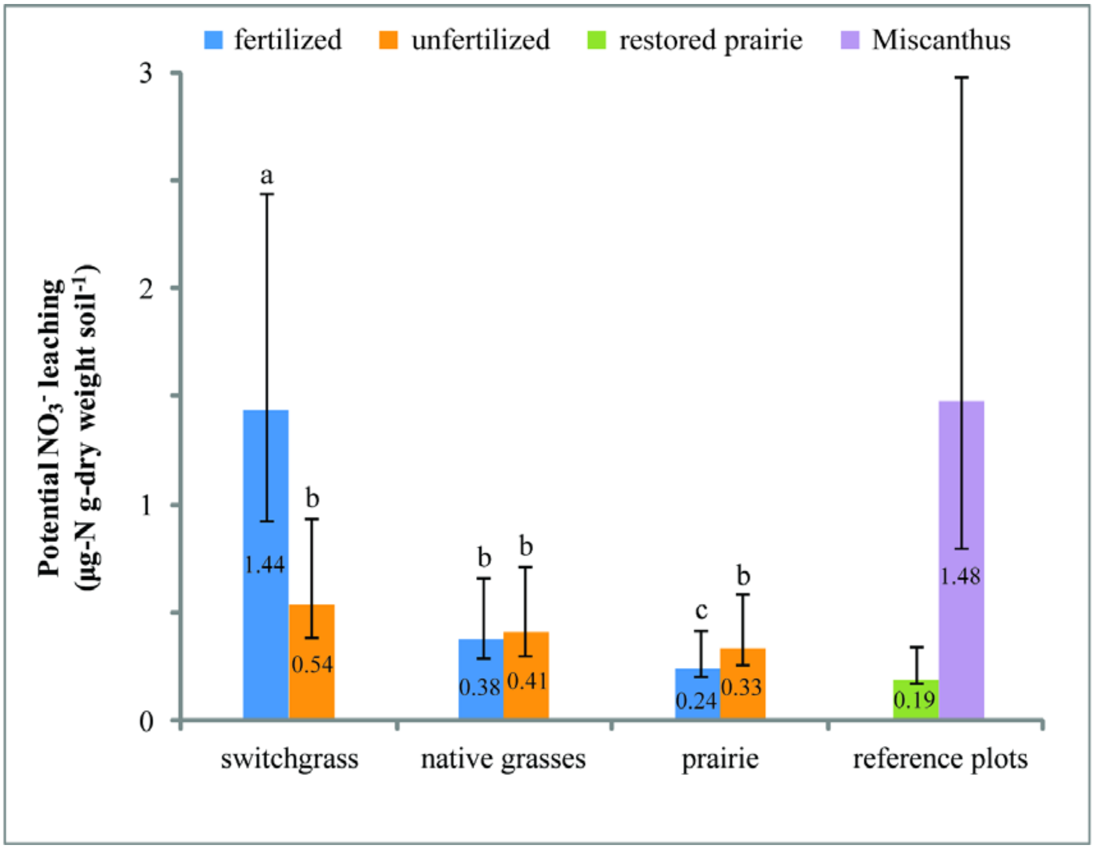

Figure 2: Average potential NO_3^- leaching for each crop and fertilizer treatment across 2011 and 2012. Given are back-transformed geometric means $\pm$ standard error. Letters indicate significant differences among treatments ($p<0.05$). The reference plots were not included in the statistical analysis.

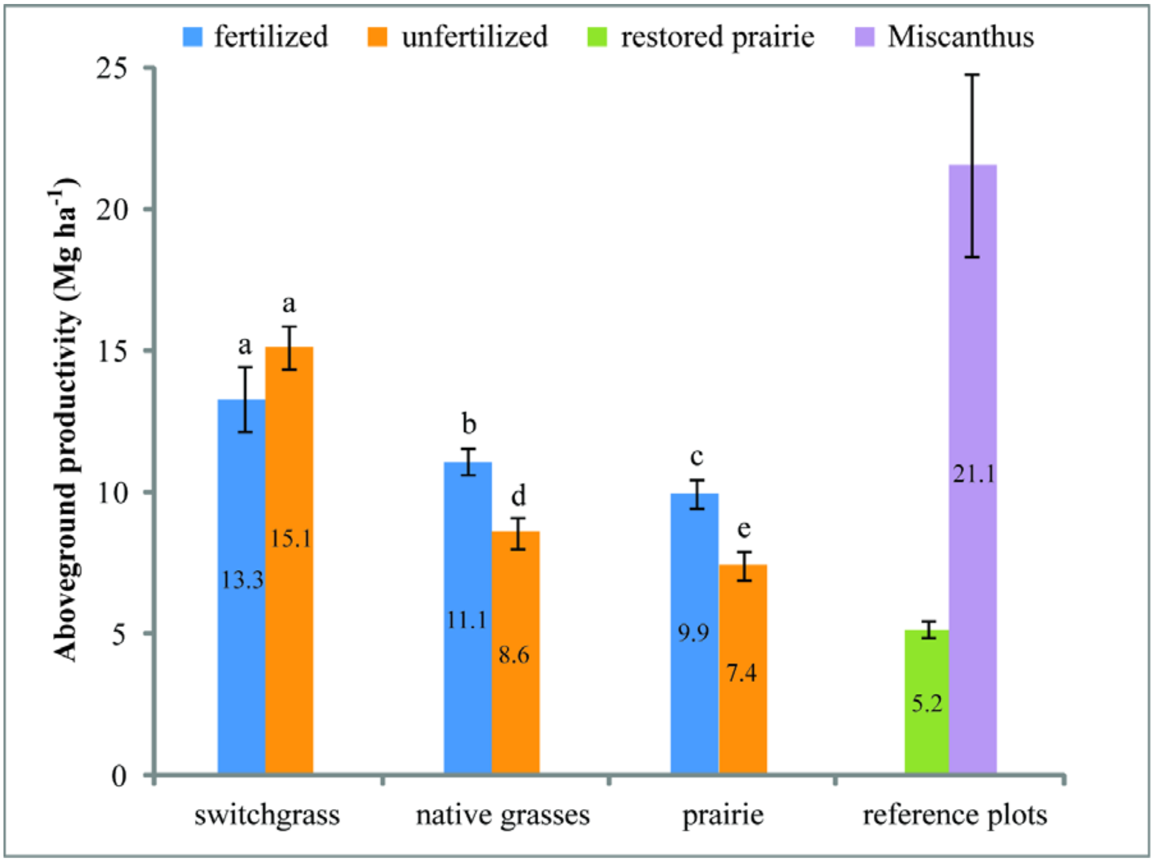

Figure 3: Average aboveground productivity for each crop and fertilizer treatment across 2011 and 2012. Given are arithmetic means ± standard error. Letters indicate significant differences among treatments ($p<0.05$). The reference plots were not included in the statistical analysis.

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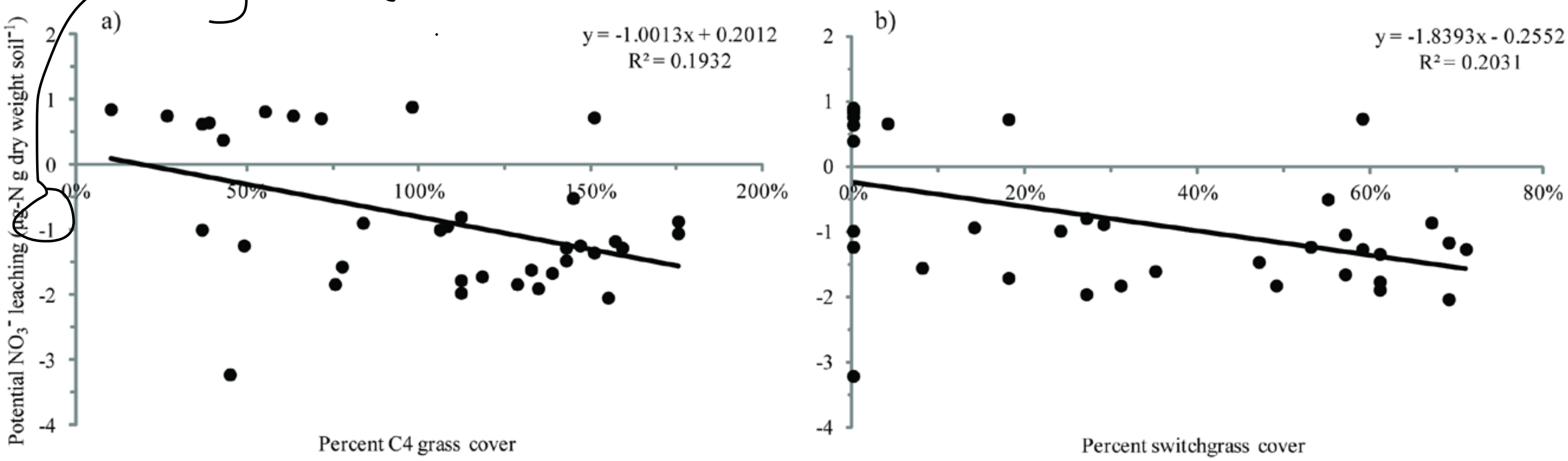

Figure 4: Based on model comparison ($p<0.05$), significant relationships between (a) C4 grass cover and (b) switchgrass cover and potential NO_3^- leaching for native grasses quadrats.

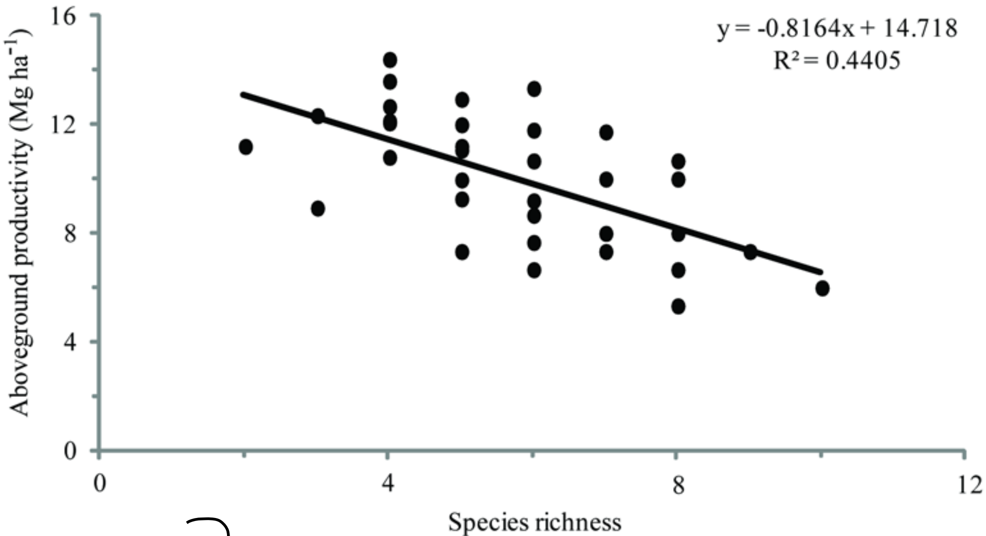

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Figure 5: Based on model comparison (p<0.05), significant relationship between species richness and aboveground productivity for native grasses quadrats.

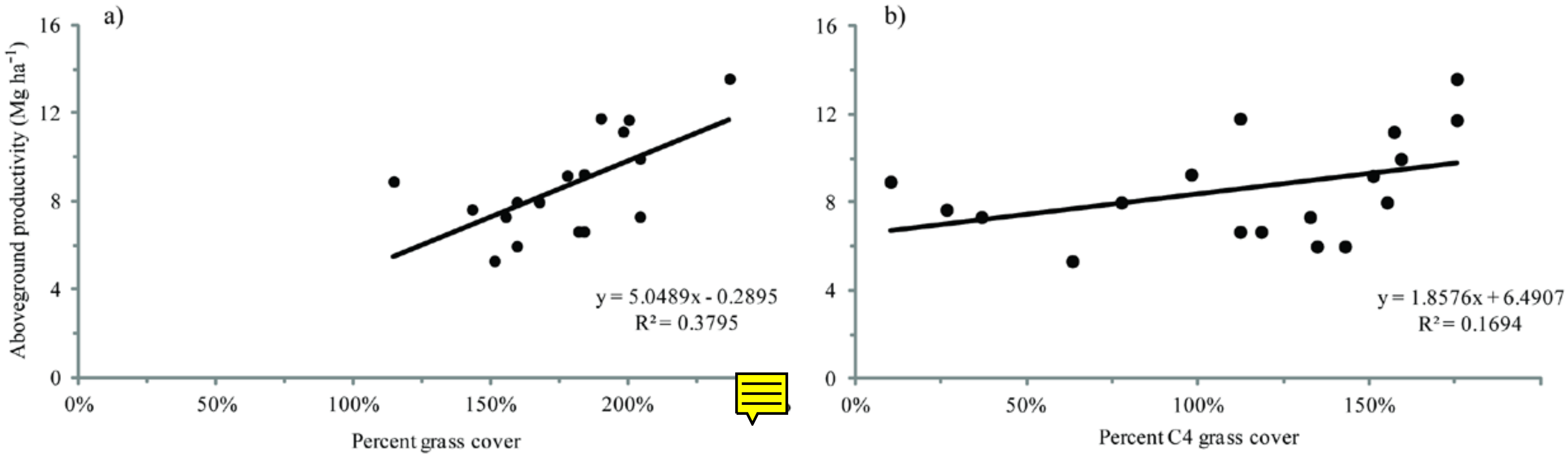

Figure 6: Based on model comparison ($p<0.05$), significant relationships between (a) total grass cover and (b) C4 grass cover and aboveground productivity for unfertilized native grasses quadrats.

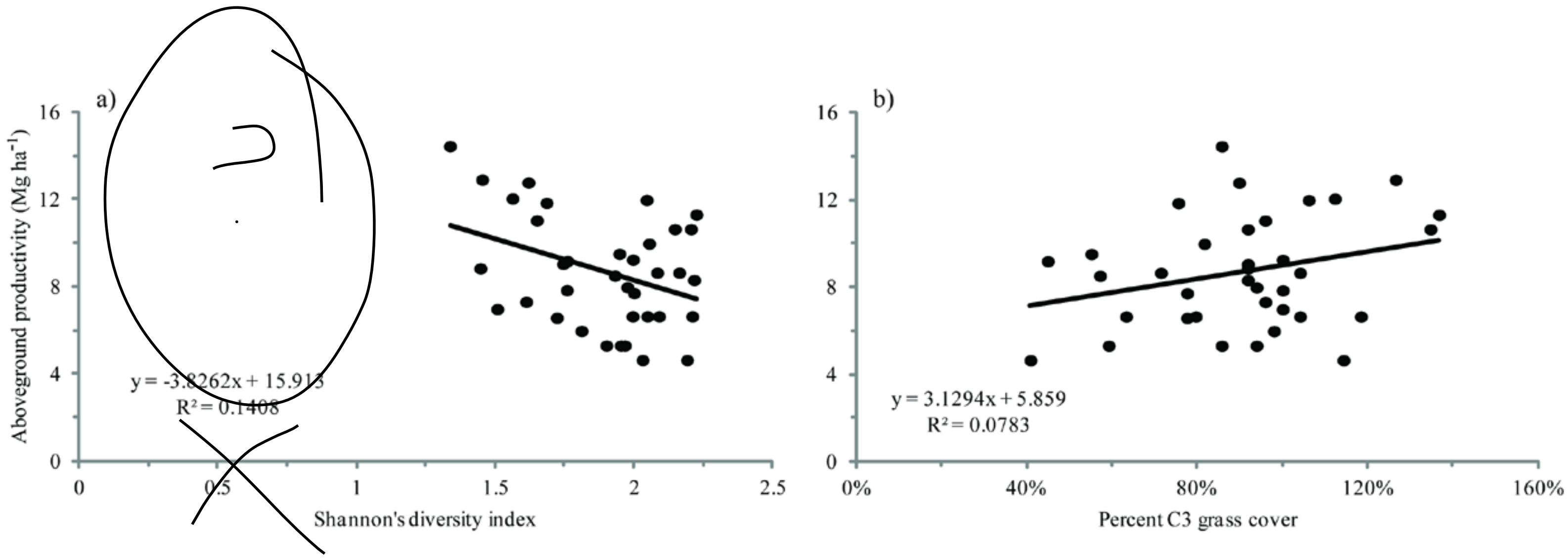

Figure 7: Based on model comparison ($p<0.05$), significant relationships between (a) Shannon's diversity index and (b) C3 grass cover and aboveground productivity for prairie quadrats.

CHAPTER 2

Comparison of two chamber methods for measuring soil trace-gas fluxes in bioenergy cropping systems

ABSTRACT

Greenhouse gas emissions from soils are often measured using trace-gas flux chamber techniques without a standardized protocol, raising concerns about measurement accuracy and consistency. To address this, we compared measurements from non-steady-state non-through-flow (NTF) chambers with a non-steady-state through-flow (TF) chamber system in three bioenergy cropping systems located in Wisconsin. Additionally, we investigated the effects of NTF flux calculation method and deployment time on flux measurements. In all cropping systems, when NTF chambers were deployed for 60 min and a linear (LR) flux calculation was used, soil CO_2 and N_2O fluxes were, on average, 18% and 12% lower, respectively, than fluxes measured with a 15 min deployment. Fluxes calculated with the HMR method, a hybrid of non-linear and linear approaches, showed no deployment time effects for CO_2 and N_2O and produced 27-32% higher CO_2 fluxes and 28-33% higher N_2O fluxes in all crops than the LR approach with 60 min deployment. Across all crops, CO_2 fluxes measured with the TF chamber system were higher by 24.4 to 84.9 mg CO_2-C m^{-2} h^{-1}, than fluxes measured with NTF chambers using either flux calculation method. These results suggest NTF chamber deployment time should be shortened if the LR approach is used though detection limits should be considered, and the HMR approach may be more appropriate when long deployment times are necessary. Significant differences in absolute flux values with different

chamber types highlight the need for significant effort in determining the accuracy of methods or alternative flux measurement technologies.

Abbreviations: GC, gas chromatography; GHG, greenhouse gas; GLBRC, Great Lakes Bioenergy Research Center; HDPE, high-density polyethylene; IRGA, infrared gas analyzer; NTF, non-through-flow non-steady-state chamber; PVC, polyvinyl chloride; SD, standard deviation; TF, through-flow non-steady-state chamber; VWC, volumetric water content.

INTRODUCTION

Soil surface emissions of CO_2 and N_2O are commonly measured using trace-gas flux chamber techniques; however, there is no established standard chamber type or measurement protocol, raising concerns about measurement accuracy and reliability (Rochette and Eriksen-Hamel, 2008). Trace-gas flux chambers can be categorized as steady-state through-flow, non-steady-state through-flow, or non-steady-state non-through-flow (Livingston and Hutchinson, 1995). Data collected using all three chamber types can be found in recent literature (Powers et al., 2010; Singh et al., 2010; Samuelson and Whitaker, 2012). In addition to this variability in chamber type, variability in chamber design, measurement protocol, and flux calculation methods are also significant. In a meta-analysis by Rochette and Eriksen-Hamel (2008) of 356 studies using non-steady-state non-through-flow chambers to measure soil N_2O flux, large variation in chamber size and design, deployment duration, and number of samples taken during deployment was reported, as well as the types of data analyses used. Two of the most commonly used chamber types are the non-steady-state non-through-flow chamber (NTF) and the non-steady-state through-flow chamber (TF).

A NTF chamber, sometimes called a closed static chamber, is one in which gas concentration is allowed to increase within a closed chamber headspace. Discrete measurements of gas concentration determined by gas chromatography (GC) are regressed against time to determine the rate of increase or flux of the gas. A NTF chamber and GC can be readily used to quantify the concentrations of CO_2, N_2O, and methane (CH_4). More than 95% of field measured N_2O fluxes reported in the literature were obtained using NTF chambers (Rochette and Bertrand, 2008); thus most of our knowledge of N_2O dynamics is from NTF chambers. NTF chambers are also widely used to measure CO_2 fluxes because they are inexpensive, can measure multiple gases simultaneously, and long deployment times allow for rotational sampling of several chambers at once.

The TF chamber system (also known as a closed dynamic chamber) is a widely used method of measuring soil CO_2 flux and commercial systems are available (e.g. LI-COR LI-6400, LI-COR, Inc., Lincoln, NE, USA). The TF system cycles air through the chamber, out to an infrared gas analyzer (IRGA), and back into the chamber, measuring flux while the chamber gas concentrations are near ambient. The TF system is often portable, allowing for greater spatial diversity in sampling sites, but is significantly more expensive than the typical NTF chamber.

It is well recognized that non-steady-state chambers can alter gas fluxes from their undisturbed values (Healy et al., 1996; Conen and Smith, 2000; Rochette and Hutchinson, 2005). Because diffusion rate, and thus gas flux, is proportional to the concentration gradient between the soil surface and the chamber headspace, alteration of the concentration gradient over time will affect flux, which can then become time dependent. When a non-steady-state chamber is closed, gas accumulation within the chamber headspace decreases the concentration gradient leading to a suppression of soil gas fluxes compared to what the rates would be in a natural setting. Because NTF chambers are often closed for up to 60 min during a complete measurement cycle, studies have demonstrated substantial underestimations in CO_2 and N_2O fluxes with these chambers (Rayment, 2000; Pumpanen et al., 2004). There is also evidence from laboratory experiments and numerical models that increasing chamber deployment time results in lower gas fluxes due to ever increasing chamber gas concentrations (Bekku et al., 1995; Gao and Yates, 1998; Venterea, 2010).

Linear regression (LR) remains the most widely used method for calculating fluxes with NTF chambers, though it is widely recognized that this may underestimate fluxes and that non-linear models are needed to account for flux suppression within chambers, especially when long deployment times are used (Kutzbach et al., 2007; Kroon et al., 2008; Venterea et al., 2009). However, non-linear models may in some cases be over-parameterized or estimate large fluxes from sites with little or no gas flux by failing to account for random variation in concentration data. The recently developed HMR approach attempts to account for these possible errors by applying a revised Hutchinson & Mosier (HM) non-linear model (Hutchinson and Mosier, 1981) when possible but can also automatically detect when LR should be applied or when data should be characterized as having a zero flux value. A more thorough discussion of the HMR method can be found in Pederson et al. (2010). Relatively few studies have reported the effect of varying deployment times on fluxes in an experimental field setting, and there is still no widely accepted standard protocol for deployment duration or flux calculation method.

Both the NTF and the TF chambers are non-steady-state systems, but the TF system is better able to minimize the impacts of an altered concentration gradient between the soil and chamber headspace. By calculating flux while the chamber air is near ambient atmospheric CO_2, the natural concentration gradient should be minimally affected, preventing flux suppression. Additionally, the TF system is able to make multiple flux measurements in $\leq$5 min, depending on the magnitude of fluxes, which further minimizes the increase in headspace CO_2 concentration. Unlike semi-permanent and often larger NTF chambers, the short measurement time and portability of the TF system allows for many measurements across a study area to capture the inherent spatial variability of soil CO_2 fluxes (Davidson et al., 2002). Furthermore, the TF chamber system provides instantaneous flux data allowing the researcher to identify and fix measurement errors while in the field. Errors in NTF chamber measurements are often not discovered until after samples have been brought back to the laboratory and analyzed with the GC.

The TF chamber is not without possible measurement errors. Because the flux measurement centers around a user-entered ambient CO_2 concentration, accurate determination of this ambient concentration is necessary for a reliable flux measurement (Martin et al., 2004). Also, chamber closure, though short, is still likely to alter the concentration gradient (Widen and Lindroth, 2003). Studies using laboratory calibration systems with various “soil” substrates have measured both under- and over-estimations of fluxes with the LI-COR LI-6400 or its predecessor (LI-6200), both TF systems. (Widen and Lindroth, 2003; Martin et al., 2004; Pumpanen et al., 2004). However, these calibration methods are still being perfected and results may not be completely reliable. Additionally, unlike the NTF chamber method, the LI-6400 TF system is only able to measure CO_2 fluxes and cannot currently provide measurements for N_2O or CH_4.

Because gas fluxes cannot be controlled in the field, others have compared various chamber systems to validate measurements. To our knowledge, only two studies have compared the LI-6400 (or LI-6200) TF system to NTF chamber CO_2 flux measurements in the field (Norman et al., 1997; Sullivan et al., 2010). These studies found NTF chambers using LR flux estimation measured considerably lower fluxes than the LI-6400/LI-6200 systems probably largely due to the much longer deployment times of the NTF chambers. We are aware of no work comparing NTF fluxes estimated with the HMR model to fluxes measured with a TF system.

The objectives of this study were to determine the effect of NTF chamber deployment time on CO_2 and N_2O fluxes, to compare LR and HMR calculated fluxes at an array of deployment times, and to determine the degree of difference in CO_2 fluxes measured with a NTF chamber versus the TF system. We investigated fluxes from both chamber types in three bioenergy cropping systems in south-central Wisconsin:

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continuous corn (*Zea maize*), switchgrass (*Panicum virgatum*), and hybrid poplar (*Populus nigra x populus maximowiczii*). We used both a linear model and the HMR approach to estimate CO_2 and N_2O fluxes from the NTF system. Based on work by Venterea et al. (2009), we also examined whether flux measurement variability differed among deployment times. Though we expected to see the algebraic sign of the flux

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discrepancy between the NTF and TF chamber types to be similar to that found by Norman et al. (1997) and Sullivan et al. (2010), the magnitude of this discrepancy had not previously been studied in managed, agricultural systems in the Midwest. We focused on testing three hypotheses: (i) independent of cropping system, CO_2 and N_2O fluxes estimated with the LR approach would decrease as chamber deployment time increased, due to increasing headspace concentrations; (ii) NTF fluxes estimated with HMR vs. LR would exhibit higher correlation with TF chamber fluxes; (iii) at short deployment times, the LR and HMR methods would produce similar flux estimates.

METHODS

Study site

All measurements were performed at the Great Lakes Bioenergy Research Center's (GLBRC) Biofuels Trial site at the Arlington Agricultural Research Station, Arlington, WI (43°17'N, -89°22'W). Mean annual air temperature is 6.8°C and mean annual precipitation is 869mm. The site, established in 2008, consists of 10 potential bioenergy cropping systems in a randomized, complete block design. We studied three replicates each of monoculture switchgrass (*Panicum virgatum*), continuous corn (*Zea maize*), and hybrid poplar (*Populus nigra x populus maximowiczii*). These three cropping systems represent a range of perenniality and management intensity. Both the TF and the NTF chamber systems are currently being used in these cropping systems at this site to measure soil trace-gas emissions in support of assessing the net greenhouse gas (GHG) flux of each agroecosystem. The corn system was managed no-till and had 72 to 202 kg N ha^{-1} of fertilizer, based on University of Wisconsin best management practices, applied each year beginning in 2008. Corn rows ran north-south and were spaced 76 cm (30 in). Switchgrass was fertilized (56kg N ha^{-1}) in 2010 and 2011, and poplar was fertilized (86kg N ha^{-1}) only in 2010. Poplar rows ran north-south and were spaced 1.5 m (5 ft). Plot size was 43x27m and the soil throughout the research site is classified as a Plano silt loam (Fine-silty, mixed, superactive, mesic typic Argiudolls).

Trace gas flux measurement protocol

In each replicated study plot, gas flux measurements were taken with a non-steady-state non-through-flow chamber (NTF) and a non-steady-state through-flow (TF), LI-6400 portable photosynthesis system with LI-6400-09 soil CO_2 flux chamber (LI-COR, Inc., Lincoln, NE, USA) within the same hour to minimize effects of changing soil temperatures and soil moisture. Plots within each replicate were measured within a 2-3 hour period. All measurements were typically completed between 0900-1600 local time and all plots were measured on the same day when possible. Measurements were performed in 2011 on May 16 and May 20 (due to inclement weather, two replicates were completed on May 16 and one on May 20), May 31, June 30, July 15, and Aug. 8 and Aug. 9 (due to inclement weather, two replicates were completed on Aug. 8 and one on Aug. 9). Differences in the measurements split between dates due to inclement weather did not impact our results. The specific measurement protocol for each chamber method is highlighted below.

Non-steady-state, non-through-flow chamber

We inserted one 27.15 cm diameter stainless steel chamber 5.5 cm into the soil near the south end of each replicated plot on May 9, 2011. Chambers had an effective height of 17.3 cm from the soil surface and volume of 0.01 m^3. Chambers were fitted with a 2 mm diameter vent and vent tube located on the chamber wall approximately 14 cm from the soil surface to avoid pressure perturbations during chamber closure and gas sampling (Hutchinson and Livingston, 2001). The sample areas were first hand clipped to ground level and vegetation was removed to facilitate chamber installation. In corn and hybrid poplar, the chamber was placed between planted rows. In an effort to limit disturbance to the sampling area, trampling was minimized during the installation of the chambers and during each sampling period. One NTF chamber was placed in each replicate plot, giving a total of 3 chambers per crop treatment (switchgrass, continuous corn, hybrid poplar) on each measurement date. This resulted in a total of 15 measurements per crop treatment for the season (May-August). These data were used for all analyses.

On each measurement date, a white, high-density polyethylene (HDPE) lid with a butyl rubber syringe port was clamped onto each chamber to allow gas concentration to increase for the purposes of estimating a gas flux. Using a 30 mL Luer-Lok™ syringe (BD, Franklin Lakes, NJ, USA) fitted with a polycarbonate stopcock (Cole-Parmer, Vernon Hills, IL, USA) and 23-gauge needle (BD, Franklin Lakes, NJ, USA), gas was extracted from the chamber headspace at 0, 5, 10, 15, 20, 40, and 60 minutes after the lid was clamped on the chamber. Chamber headspace was not mixed prior to sampling, as mixing may cause pressure perturbations and/or excess dilution of headspace gas (Parkin and Venterea, 2010). As recommended in a review of gas flux chamber methods by Rochette and Eriksen-Hamel (2008), glass 5.9 mL Exetainer® vials (Labco Ltd., Buckinghamshire, UK) were used. Vials were overcharged with 10mL gas samples to guard against sample contamination and facilitate subsequent removal of the gas sample for analysis (Parkin and Venterea, 2010). Concurrently with field measurements of chamber gas samples, ambient air and field standards (400 ppm CO_2, 1 ppm N_2O, and 1 ppm CH_4) were loaded into vials to assess background GHG concentrations and potential storage degradation in the period between sampling and analysis. Samples were transported back to UW-Madison for analysis by gas chromatography with a flame ionization detector (CH_4), a 15 mCi ^{63}Ni electron capture detector (N_2O) (Agilent 7890A GC System, Santa Clara, CA, USA), and infra-red gas analyzer for CO_2 (LI-COR LI-820 CO_2 Analyzer, Lincoln, Nebraska, USA). The gas chromatograph was calibrated before each analysis using 15- 25 laboratory standards (400 ppm CO_2, 1 ppm N_2O, and 1 ppm CH_4). Average coefficients of variation across all measurement dates for these standards were 0.023 for CO_2 and 0.014 for N_2O. Air temperature at time of deployment, obtained from an on-site weather station (30 minute continuous data), was used to correct for temperature differences between gas sampling and lab analysis based on the perfect gas law (Rochette and Hutchinson, 2005).

To analyze differences in gas fluxes for varying deployment times, a linear regression (LR) and the HMR flux estimation method were separately applied to each of the following measurement time periods: 0-15 min, 0-20 min, 0-40 min, and 0-60 min. Four sampling points were used for each flux calculation, regardless of deployment time.

For example, for the 0-15 min deployment time, samples at 0, 5, 10, and 15 min were used, while at 60 min, samples at 0, 20, 40, and 60 min were used. Deployment times of less than 15 min were not tested because it has been recommended that a minimum of four discrete samples during the deployment period should be used to ensure the most accurate calculation of fluxes and four samples are necessary for the HMR calculation (Rochette and Eriksen-Hamel, 2008; Pedersen et al., 2010) For the LR approach, the slope of each CO_2 regression was tested to determine whether it was statistically different from zero with an $r^2>0.95$. If the CO_2 measurements were accepted, the N_2O measurements from those samples were also assumed to be acceptable. For consistency when comparing flux estimation methods, only measurement series accepted in the LR approach were calculated using the HMR approach. The HMR approach was implemented using the R algorithm (R version 2.15.2, R, 2012) and the default recommendation (i.e. 'non-linear', 'linear', or 'no flux') was used.

Positive and negative detection limits for the LR flux calculation method and the HMR method (when a non-linear model was applied) were determined using the procedure presented by Parkin et al. (2012) which is based on flux calculation method, analytical precision, chamber deployment time, and ambient concentration of the gas. Detection limits were determined for CO_2 and N_2O fluxes under 15, 20, 40, and 60 min chamber deployment times.

Non-steady-state through-flow chamber

We used the commercially available LI-6400 portable photosynthesis system attached to a LI-6400-09 soil CO_2 flux chamber (LI-COR, Inc., Lincoln, NE, USA) as a representative non-steady-state through-flow (TF) chamber. The LI-6400 system was factory calibrated during the previous winter season as recommended by LI-COR, Inc., and the LI-6400 infrared gas analyzer zero and span (e.g., calibration with a gas of known concentration) were also periodically checked in the laboratory during the measurement season. We tested two spatially distinct methods of TF chamber deployment. The first, denoted here as the “point method”, consisted of using four polyvinyl chloride (PVC) collars (10 cm diameter, 6 cm height; inserted to a 3 cm soil depth) located approximately 1m from the NTF chamber in each cardinal direction. In the corn and hybrid poplar, two collars were placed in-row and two collars between-rows. Point method collars were typically not moved throughout the season except when field equipment activities required it. The point method was expected to most closely represent the GHG fluxes measured within the NTF chamber due to the close spatial proximity of the four collars. The second TF chamber method, denoted here as the “transect method”, consisted of 10 collars randomly positioned and spaced along a north-south transect through the middle of each replicate plot. In corn and hybrid poplar, five, randomly chosen collars were placed in-row and five collars were inserted between-rows. This is the method currently used at the GLBRC site for carbon balance studies, and it aims to account for spatial variability within the plot. Transect collars were moved approximately every four weeks to limit the collar effects on the soil environment.

The LI-6400-09 soil chamber, after placed on the soil collar, had an effective height from the soil surface of 15 cm and approximate system volume of 1200 cm^3. The LI-6400 soil chamber contains a pressure equilibration tube and the headspace within the chamber is thoroughly mixed via a perforated manifold (LI-COR, 2004). After the chamber was placed on each PVC measurement collar, soda lime was used to draw the CO_2 concentration 10 μmol mol^{-1} below ambient atmospheric CO_2 concentration, and automated flux measurements were collected as the concentration increased to 10 μmol mol^{-1} above ambient CO_2. This measurement sequence was automatically repeated three times during a chamber deployment period of approximately 5 min for each measurement collar. Following each measurement, the LI-6400 software fit the intermediate flux data with a linear regression to determine the CO_2 flux at the ambient CO_2 concentration. This procedure provided three flux measurements for each collar for a total of 12 flux measurements for the point method (4 collars) and 30 measurements for the transect method (10 collars) in each plot. The mean of these 12 and 30 flux measurements provided plot level estimates of CO_2 flux for the point and transect method, respectively. These plot level means, 3 per crop treatment on each measurement date for a total of 15 flux measurements per crop for the season, were used for all analyses.

Soil environmental conditions

Because soil environmental conditions are correlated with flux rates (Rustad et al., 2000; Dobbie and Smith, 2003), soil temperature and moisture were measured to verify that differences in these conditions were not confounding differences in gas fluxes among chamber methods. Concurrently with gas flux measurements, 10 cm soil temperatures were obtained using a hand-held temperature probe (HANNA Instruments, Inc., Woonsocket, RI, USA) for the NTF chambers. A 10 cm soil temperature probe attachment for the LI-6400 system was used with the LI-6400-09 soil chamber. The mean of three temperature measurements from around each NTF chamber was used to obtain an average soil temperature for each chamber on each measurement date. Three temperature measurements were taken near each of the four and ten TF collars for the point and transect methods, respectively. These 12 and 30 measurements were averaged to give mean soil temperatures in each plot, at each measurement date, for the point and transect methods, respectively. Volumetric water content (VWC) (m^3m^{-3}) in the 0-6cm soil surface layer was measured using a theta probe soil moisture sensor (Dynamax, Inc., Houston, TX, USA). Again, the mean of three measurements from around each NTF chamber was used to obtain an average VWC for each chamber. Three VWC measurements were taken around each of the 4 and 10 TF collars for the point and transect methods, respectively. These 12 and 30 measurements were averaged to give mean VWC in each plot, at each measurement date, for the point and transect methods, respectively.

Statistical analysis

All statistical analyses were performed using PROC MIXED in the SAS v9.3 software package (SAS Institute, Inc., Cary, NC, USA). On a given day, all flux measurements from a plot were collected either as part of a single deployment (deployment time comparison and LR vs. HMR) or within an hour of each other (NTF vs. TF) to minimize diurnal variability as a confounding factor. We accounted for both diurnal and day-to-day variability by modeling our observations as repeated measurements of a plot on a specific day. Seasonal means (May-August) were calculated and compared using this model, and the Tukey-Kramer method was used to correct for multiple comparisons. Standard deviations of gas fluxes were similarly tested to compare measurement variability among methods. Soil volumetric water content and soil temperature were also compared. A value of $p<0.05$ was considered significant for all analyses.

RESULTS

Impact of NTF deployment time on gas fluxes

Examples of field collected CO_2 and N_2O concentration data over time of deployment are shown in Figures 1a and 1b, respectively. These figures are representative of the output from the HMR approach and illustrate both linear and non-linear fits to the gas concentration data. Deployment time had a significant effect on the magnitude of CO_2 flux estimates when the LR approach was used with the NTF chambers. When all treatments were averaged across all measurement dates, CO_2 fluxes were similar at 15 min and 20 min deployment times, but as deployment time increased past 20 min, CO_2 fluxes decreased significantly (Fig. 2a). Overall, CO_2 fluxes across all treatments and all measurement dates for 40 min and 60 min deployment times were 11% and 18% lower, respectively, than flux rates calculated for the 15 min deployment time. Carbon dioxide fluxes were highest in switchgrass and lowest in hybrid poplar, and the crop x deployment time interaction was marginally insignificant ($p=0.0588$) (Fig. 2b). A scatter plot of all 15 min/LR fluxes versus the paired 60 min/LR fluxes showed a significant and positive linear relationship (Fig. 3). Therefore, the relationship between fluxes with these two deployment times was independent of flux magnitude.

In all cropping systems, when the HMR approach was used to calculate NTF chamber fluxes, deployment time had no effect on CO_2 flux estimates (Fig. 2a and 2c). With a 60 min deployment, 95% of flux calculations required a non-linear model and 5% a linear model. With a 15 min deployment time, 40% required a non-linear model and the remainder were calculated with a linear regression. There were no significant differences in standard deviations of CO_2 fluxes among deployment times for both LR and HMR methods.

N_2O fluxes averaged across all treatments and measurement dates also exhibited declining values as deployment time exceeded 20 min when the LR approach was used (Fig. 4a). The average fluxes across all treatments were 5% and 12% lower at the 40 min and 60 min deployment times, respectively, compared to the 15 min deployment. There was a marginally insignificant ($p=0.0675$) crop x deployment time effect on N_2O fluxes calculated with LR (Fig. 4b). The corn agroecosystems had the highest N_2O fluxes and the poplar systems the lowest. As with CO_2, there was a significant linear relationship between all 15 min/LR N_2O fluxes versus the paired 60 min/LR fluxes (Fig. 5). Again, the relationship between fluxes with these two deployment times did not change with flux magnitude.

Regardless of cropping treatment, N_2O fluxes did not differ by deployment time using the HMR method (Fig. 4a and 4c). With a 60 min deployment, 76% of fluxes were calculated with a non-linear model and 21% with a linear model (the remainder were calculated as no flux). With a 15 min deployment, 48% were calculated with a non-linear approach, 45% with a linear model, and the remainder were calculated as no flux. Standard deviations of N_2O fluxes did not significantly differ among deployment times for the LR or the HMR method.

Positive and negative CO_2 and N_2O detection limits for the HMR and LR approaches at 15-60 min deployment times are given in Table 1. Detection limits decreased with longer deployment times with approximately 4 times larger limits with a 15 min versus a 60 min deployment time. For both gases at all deployment times, detection limits for the HMR method were approximately six times larger than detection limits for the LR method.

Comparison of NTF and TF CO_2 flux measurements

Soil CO_2 fluxes were compared among the two NTF deployment time extremes, 15 min and 60 min, using both LR and HMR methods, and the two TF measurement methods, point and transect. There were significant differences among all six methods (Fig. 6). Transect method fluxes were significantly higher than point method fluxes. HMR calculated fluxes with 15 and 60 min deployment were 27-32% higher than LR calculated fluxes with a 60 min deployment time, but HMR fluxes were not significantly different from LR fluxes with a 15 min deployment time. All four NTF chamber methods resulted in significantly lower fluxes than the two TF methods. NTF fluxes were more similar to TF point fluxes than TF transect fluxes. These relationships among methods were consistent across all crops. A scatter plot of all TF/transect CO_2 fluxes versus the paired NTF/60 min/LR fluxes showed a significant linear relationship (Fig. 7). Thus, the relationship between fluxes with these two methods did not appear to change as a function of flux magnitudes. Standard deviations for CO_2 fluxes did not differ significantly among the six methods.

Comparison of LR and HMR N_2O flux measurements

Soil N_2O fluxes were compared among the 15 min and 60 min LR and 15 min and 60 min HMR methods (Fig. 8). There was no significant crop x method effect. Across all crops, on average, the HMR method using either deployment time resulted in 28-33% higher fluxes than the LR/60 min deployment combination. HMR N_2O fluxes were not significantly different from LR/15 min fluxes. The Tukey-Kramer method, used here, corrects for the increased probability of making a type I error induced by multiple comparisons. More comparisons lead to a more conservative estimate of significance. Due to the conservative nature of the Tukey-Kramer analysis, 15 min and 60 min LR N_2O fluxes were not significantly different when analyzed with the HMR means. However, when analyzed separately from the HMR data, these fluxes were significantly different with similar results to those shown in Figure 4a.

Soil environmental conditions

Mean soil temperature and VWC for each flux measurement method on each measurement date and season average soil temperature and VWC for each method are shown in Tables 2 and 3. Season average soil environmental conditions were compared among the NTF chamber method, the transect method, and the point method. Soil volumetric water content did not differ significantly among the three methods. There were significant differences in soil temperatures between the NTF chamber method and the two TF methods, but they were small. Seasonal average soil temperatures with the NTF chamber method were 0.30 and 0.39°C higher than with the TF point and transect methods, respectively.

DISCUSSION

Impacts of NTF deployment time and flux calculation method on trace gas fluxes

As hypothesized, when LR was used for flux estimation we found a significant suppression of trace gas fluxes with longer chamber deployment time. Carbon dioxide fluxes were suppressed by 9-21% in all cropping systems when deployment times were 40 min or longer (Fig. 2a). No suppression was detected between 15 min and 20 min deployment times. This may be explained by the calculated detection limits (Table 1) which indicate the change in headspace concentration may have been too small over the 5 min period to detect a significant difference in fluxes. It may also be that significant suppression did not occur before 20 min, but this is unlikely based on studies showing immediate flux suppression at chamber closure (Healy et al., 1996; Conen and Smith, 2000). Assuming the average seasonal fluxes shown in Fig. 2b, a deployment time of 60 min versus 15 min using the LR approach would underestimate carbon losses by approximately 1187, 705, and 494 kg CO_2-C ha^{-1} or 21%, 17%, and 14% over the course of the season (May-August) in switchgrass, corn, and hybrid poplar, respectively. Interestingly, we found that flux magnitude had no impact on the relationship between deployment time and flux (Fig. 3). This is contrary to a study by Norman, et al. (1997) in which a NTF chamber measured similar CO_2 fluxes at 10 min and 30 min when fluxes were low (<86 mg CO_2–C m^{-2} h^{-1}); however, with increasing soil CO_2 fluxes, the 30 min sampling time resulted in lower flux estimates.

Similarly, N_2O fluxes calculated with the LR approach were suppressed with chamber closure of 40 min or longer (Fig. 4a). Assuming the average seasonal flux in Figure 4b, chamber deployment of 60 min versus 15 min would underestimate nitrogen losses by approximately 0.39 kg N_2O-N ha^{-1} or 23% in corn over the course of the season if the LR method is used. Our results agree with others who found CO_2 flux suppression after only 20-30 min of NTF chamber deployment using laboratory measurement systems (Bekku et al., 1995; Pumpanen et al., 2004). Likewise, Venterea (2010) demonstrated decreasing CO_2 and N_2O fluxes after 20-30 min with field NTF chambers at two Minnesota sites under corn production.

Though the crop x deployment time effect on N_2O fluxes was not statistically significant (p=0.0675), visually there appeared to be differences in deployment time effects among crops (Fig. 4b). Additional replication may have improved detection of a crop x deployment time effect. Poplar, which had the lowest fluxes, showed the least amount of suppression while corn, with the highest fluxes, seemed to show the greatest flux suppression. However, a regression of 15 min versus 60 min LR N_2O fluxes showed a strong linear relationship demonstrating that at both high and low fluxes the relationship between deployment times was the same (Fig. 5). Therefore, the apparent differences in the deployment time effect among crops may be due to some variable related to crop-type that we did not measure.

When the HMR method was used to calculate CO_2 and N_2O fluxes, there were no deployment time effects in any crop (Fig. 2a and 4a). With a 60 min deployment time, nearly all CO_2 fluxes (95%) and the majority of N_2O fluxes (76%) required a non-linear calculation method. This emphasizes the inadequacy of applying a linear regression to gas measurements obtained from long chamber deployment times. At 15 min, gas concentrations are more likely to follow a linear trajectory with 60% of CO_2 fluxes and 45% of N_2O fluxes calculated by linear regression with the HMR approach. The HMR approach appears to effectively correct for, when necessary, flux suppression resulting from extended deployment time.

Although both CO_2 and N_2O detection limits are quite large for the HMR approach (Table 1), it is important to note that these detection limits are for non-linear calculations only. The HMR approach applies a linear regression when appropriate and so these fluxes will have detection limits equal to the LR approach's limits. In order to remain consistent across deployment times, flux calculations were based on four sampling points for all deployment times. However, more sampling points will enhance

HMR model accuracy and reduce detection limits. This is especially important with longer deployment times when non-linear fluxes are more likely.

Unlike Venterea et al. (2009), we found no significant differences in the variability of flux measurements among deployment times for either flux calculation method. Short chamber deployment times resulted in flux measurements as precise as measurements from long deployment times. Thus, at least to 15 min, shortened deployment times offered equally precise measurements compared to longer deployment times. However, this does not take into account detection limits which play a greater role at short deployment times and with low fluxes (Table 1). For example, at a 20 min deployment, the detection limit value is 4.9% of the CO_2 flux in poplar. For N_2O fluxes, the detection limit value at 20 min is 12.8% of the flux in poplar.

When LR and HMR CO_2 fluxes were directly compared, the 15 and 60 min HMR fluxes were significantly larger than the 60 min LR fluxes in all crops (Fig. 6). Because the LR approach does not account for flux suppression over time, it underestimated fluxes compared to the HMR approach. Assuming the average seasonal fluxes in Figure 6, chamber deployment of 60 min calculated with LR would underestimate carbon loss by 968-1144 kg CO_2-C ha^{-1} or 27-32% over the season, compared to fluxes using the HMR method. With a 15 min deployment time, LR calculated fluxes were not significantly different from HMR fluxes. This suggests that when chamber deployment time is shortened, the flux rate becomes more linear, and the LR approach may provide equivalent fluxes to the HMR method. This is supported by the increased use of a linear model by the HMR approach as deployment time decreased. Venterea et al. (2009) also found improved linearity of simulated fluxes with decreased deployment time.

Similar to the CO_2 flux results, N_2O fluxes were significantly different when calculated with the HMR approach compared to the fluxes calculated with the LR method and a 60 min deployment time. Given the average seasonal fluxes across all crops shown in Figure 8, the 60 min/LR calculated fluxes would underestimate nitrogen loss by 0.26-0.32 kg N_2O -N ha^{-1} or 27-32% over the course of the season compared to HMR fluxes. Again, similar to the CO_2 flux results, HMR N_2O fluxes did not differ from LR fluxes using a 15 min deployment time. Therefore, at short deployment times, both flux calculation methods may give similar outputs.

These results suggest that regardless of crop type or flux magnitude, CO_2 and N_2O flux suppression increases with longer deployment times. However, the degree of N_2O suppression may be dependent on some crop type related factor we did not study. If longer deployment times are necessary due to labor constraints, LR flux calculations may be improved by removing later time points in measurements where suppression is observed. However, at least 3, and preferably 4, sample time points should remain for the calculation. It is also important to consider soil structure when choosing a deployment time. In loose, well aerated soils with low soil CO_2 or N_2O concentrations, the gas concentration gradient between soil and chamber headspace will be more sensitive to increases in headspace gas concentrations than in less porous, compacted soils with high soil gas concentrations (Welles et al., 2001). Likewise, chamber performance will be better in fine-textured compared with sandy soil and wet compared with dry soil (Rochette and Hutchinson, 2005). Therefore, coarse textured, sandy soils may require even shorter deployment times than those used in this study on fine textured, silt loam soils. Of course, with shorter deployment times, detection limits are reduced and this must also be taken into consideration when choosing a deployment time.

These results also indicate that the HMR approach may be effective for calculating fluxes for 15-60 min deployment times in a variety of cropping systems. Especially if long deployment times are necessary due to labor limitations, it may be preferable to utilize a flux estimation approach that incorporates non-linear models, such as HMR, as opposed to solely a linear method. Even N_2O fluxes, which were nearly 10,000 times smaller than CO_2 fluxes, showed a large degree of non-linearity, especially with long deployment times. It is important to note that measurement sensitivity may be lower for HMR when a non-linear calculation is used compared to the LR approach. If fluxes are low or deployment time is too brief, HMR may not provide accurate measurement calculations. Instead, LR may be a better option.

Comparison of NTF and TF CO_2 flux measurements

Soil CO_2 fluxes measured with the TF chamber system were considerably higher than fluxes measured using 15 min and 60 min deployment times with the NTF chamber (Fig. 6). This was the case for both LR and HMR calculated fluxes. These results support laboratory and field comparisons in the literature (Norman et al., 1997; Pumpanen et al., 2004; Sullivan et al., 2010). Norman et al. (1997) calculated adjustment factors of 1.30

and 1.45 to bring two NTF/LR chamber fluxes into agreement with LI-6200 fluxes (note: the LI-COR LI-6200 was the predecessor to the LI-6400). Measurements by Sullivan et al. (2010) would require an adjustment factor of 1.43-2.32. Similarly, to bring our 15 min NTF chamber flux into agreement with the TF transect flux would require an adjustment factor of 1.40 and 1.29 for LR and HMR calculated fluxes, respectively. The soil type, climate, and vegetation of the region studied here differs considerably from the boreal forests and Inceptisol and glacial clay soils of the Norman et al. study and the Arizona ponderosa pine forests and “monsoon” type precipitation pattern of the Sullivan et al. study, yet we found a similar relationship between NTF and TF fluxes. Assuming the average seasonal flux across all crops (Fig. 6), this discrepancy in flux measurements between the NTF/15 min chamber and the TF transect method would lead to a 1733 and a 1361 kg CO_2-C ha^{-1} difference in measured carbon loss over the course of the measurement period (May-August) for LR and HMR calculated fluxes, respectively. The relationship between the two most disparate methods, NTF/60 min/LR and TF/transect, showed a generally linear relationship (Fig. 7). Thus, regardless of flux magnitude, there appears to be a consistent impact of method on CO_2 flux measurements.

Because the TF chamber was typically closed $\leq$5 min and its through-flow method maintained headspace concentrations within 10 μmol mol^{-1} below ambient to 10μmol mol^{-1} above ambient, CO_2 buildup within the chamber was extremely limited. This is in contrast to the NTF chamber which required a longer deployment time and allowed headspace CO_2 concentrations to buildup 1.3-11.8 times higher than ambient depending on deployment time. The HMR approach appears to account for some of this flux suppression, but it is possible that suppression occurs immediately upon chamber closure or within the first 15 min of deployment.

Although flux readings are made around ambient CO_2 concentrations in the TF system, it is possible the scrubbing of CO_2 below ambient caused a flush of CO_2 from the soil and led to the higher fluxes with this system. It seems unlikely, though, that this minimal decrease in CO_2 concentration caused the large discrepancy between the two chamber types. Ambient readings are measured with the TF system before the chamber is closed and then entered into the system by the user. Therefore, it is also possible the ambient readings have some error due to, for example, the presence of humans breathing around the equipment or variation in CO_2 concentrations over short distances caused by plant canopy microclimates. Additionally, fluxes with the TF chamber are measured quickly after chamber closure when disturbance to the soil environment is likely maximum. Slight soil disturbance and pressure changes from the placement of the gas chamber on the collar may have resulted in an unnatural increase in fluxes. Lastly, the insertion depth of the TF collar was 3 cm compared to the 5.5 cm for the NTF chamber. An insufficient chamber insertion depth will allow for lateral gas diffusion and may result in lower gas concentrations within the chamber (Livingston et al., 2006). However, because deployment time was less than 5 min with the TF system, lateral gas diffusion is assumed to be minimal (Hutchinson and Livingston, 2001; Rochette and Eriksen-Hamel, 2008).

Plant material within the chambers may also affect gas fluxes. Plant photosynthesis should not impact fluxes because the chambers are closed and no photosynthetically active radiation (PAR) exists inside. Dark respiration, though, may increase CO_2 flux within the chamber. Plant matter may also decrease the true volume of the chamber causing flux calculations to be inaccurately high. Other experiments using the NTF chambers were ongoing during this study; thus, plant material was cut from within the NTF chambers only at chamber insertion and was not cut through the remainder of the season. This would only pose an issue in switchgrass plots, as NTF chambers in the row-crops, corn and hybrid poplar, were placed between the rows and did not have any plant matter growing within them. TF collars were placed over bare soil. Similar relationships between the NTF chamber CO_2 fluxes and the TF CO_2 fluxes in all three crops indicate plant matter had little effect on NTF fluxes in switchgrass. As expected, soil CO_2 fluxes with the TF point method better matched NTF fluxes than did fluxes with the transect method. This may be due to the close spatial orientation of the point measurements around the NTF chamber. Although their measurements were more similar, TF point fluxes were still significantly different from the NTF measurements.

Carbon dioxide fluxes were consistently lower when measured with the TF point method versus the TF transect method. Though we expected there to be differences between methods due to the improved ability of the transect to capture spatial variability, the consistency of those differences cannot be explained with any confidence. There were no differences in soil temperature or moisture between point and transect methods to cause the flux differences. We tested whether a north-to-south decrease in fluxes with the point method only capturing lower fluxes at the south end of the plot was occurring; however, we found no such trend. We also investigated whether the monthly movement of the transect collars was better minimizing the effect of the collars on the soil environment; however, some point collars were also moved periodically due to field equipment activities and no clear trend was found. Our best hypothesis is that there may be higher levels of trampling and compaction near the point collars because of their positioning around the NTF chambers. Compaction may influence soil permeability or induce pressure gradients that could alter transport pathways or induce mass flow into or away from the chamber (Livingston and Hutchinson, 1995). Based on these findings, a portion of the difference between the NTF chambers and TF transect measurements may be due to some environmental disparity. This may also partly explain why the TF point fluxes were more similar to the NTF fluxes than were the TF transect fluxes.

Importantly, because we found no crop x method effect on CO_2 fluxes, the type of chamber used should not matter in studies of relative differences in fluxes between treatments. However, those aiming to determine gas flux magnitudes should be aware of the effects of chamber type and flux calculation method on these numbers.

Soil temperature differences among chamber methods

We found soil temperatures to be significantly higher with the NTF chamber method compared with the two TF methods (Table 2). This slightly higher temperature (0.3°C and 0.39°C higher compared to the point and transect methods, respectively) may be partly due to in-row and between-row soil temperature differences in the row crops.

The NTF chambers could only be placed between rows due to their size, while the smaller collars used in the TF methods allowed for half of the collars to be placed in-row and half between rows. In-row collars had slightly lower temperatures, particularly in late May through July when the plant canopy shaded the soil in-row but had not completely closed to shade between-row. The lower in-row temperatures lowered the mean temperatures for the TF method compared with temperatures with the NTF chamber method.

We would expect that higher soil temperature would result in higher CO_2 efflux due to an increased rate of plant root and microbial metabolism (Lloyd and Taylor, 1994). Thus, the differences in fluxes we found between the NTF chamber and the TF chamber methods may be underestimated. Accounting for the soil temperature differences, the NTF chamber may actually measure even lower fluxes compared with the TF chamber methods than we calculated here.

CONCLUSIONS

Though trace-gas flux chambers have been used for decades, there are still no standardized best practice chamber methods. Regardless of crop type or flux magnitude, we found considerable suppression of CO_2 fluxes as deployment time increased past 20 min, likely caused by increasing headspace gas concentrations. N_2O fluxes were also suppressed as deployment time increased with the LR method, though there may be some crop type effect and this should be investigated further. The lack of effect of flux magnitude on the degree of flux suppression is somewhat surprising and should be investigated further. Though we were not able to determine it in our study, it is possible significant suppression occurred before 20 min, especially when fluxes were high. Further work should be done to determine the degree of this potential suppression. We also found that the HMR method successfully modeled CO_2 and N_2O flux suppression occurring between 15 and 60 min of chamber deployment. At shorter deployment times both LR and HMR flux calculation methods may be acceptable; however, non-linearity increased with increased deployment time and HMR may be more appropriate with long deployment times. We used four sampling points for all flux calculations; however, additional sampling points will likely improve accuracy and reduce detection limits. The degree to which sampling point quantity and timing (e.g. 0, 5, 10, 40 min vs. 0, 20, 30, 40 min) affect flux calculations is unknown and warrants further investigation. No matter the calculation used, detection limits must be considered, especially if fluxes are low.

We cannot say definitively with this study whether NTF chambers underestimated and/or TF chambers overestimated trace gas fluxes. However, these results suggest that, without a standard chamber methodology, comparisons among studies using different chamber types, field methods, or flux calculation methods should continue to be scrutinized. Not only do unreliable flux measurements make comparisons among studies nearly impossible, they affect the accuracy of carbon and nitrogen budgets, global change models, and emissions inventories. Because bioenergy crops are being explored as a way to reduce GHG emissions compared to fossil fuels, accurate estimates of GHG emissions in these crops are necessary to determine their effectiveness. If either NTF or TF chambers continue to be used to collect data for these purposes, further effort is needed to determine the absolute accuracy of flux measurements with each method. Additionally, the adoption of alternative flux measurement technologies that allow for more detailed time series or continuous N_2O concentration measurements in the field is likely necessary. Quantum cascade lasers and photoacoustic methods are two such technologies currently being explored (Guimbaud et al., 2011; Iqbal et al., 2013). These methods collect real-time flux rates, measuring more gas samples in a shorter period of time. In the mean time, it may be necessary relegate the use of trace-gas flux chambers to studies of relative treatment differences where absolute flux values are not necessary.

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TABLES

Table 1: Calculated NTF chamber detection limits for CO_2 and N_2O fluxes calculated with the linear regression (LR) or HMR approach under 15, 20, 40, and 60 min chamber deployment times. HMR detection limits are for fluxes calculated with the non-linear approach only.

Deployment time (min)	Detection limits			
	CO_2 (mg CO_2-C m^{-2} h^{-1})		N_2O (µg N_2O-N m^{-2} h^{-1})	
	LR	HMR	LR	HMR
15	± 8.05	± 48.04	± 3.74	± 22.35
20	± 6.10	± 36.42	± 2.84	± 16.94
40	± 3.06	± 18.25	± 1.42	± 8.49
60	± 2.02	± 12.06	± 0.94	± 5.61

Table 2: Mean soil temperature ± standard deviation (SD) for each chamber method on each measurement date. Includes measurements from all crop treatments (switchgrass, continuous corn, hybrid poplar). Season mean is the mean of all temperature measurements for each chamber method.

Chamber method	Soil temperature (mean ± 1 SD) (°C)							Season mean
	May 16†	May 20‡	May 31	June 30	July 15	August 8†	August 9‡	
NTF	10.44 ± 2.34	14.29 ± 1.78	20.12 ± 1.51	19.63 ± 1.40	20.13 ± 0.80	20.87 ± 0.31	20.89 ± 0.90	18.50 ± 3.79
Point	10.90 ± 1.60	14.20 ± 1.48	19.24 ± 1.68	19.16 ± 1.16	19.83 ± 0.72	20.91 ± 0.25	20.52 ± 0.36	18.20 ± 3.45
Transect	10.34 ± 1.00	13.87 ± 0.78	19.06 ± 1.71	19.29 ± 0.97	19.89 ± 0.65	20.86 ± 0.19	20.68 ± 0.37	18.11 ± 3.60

†Only 2 replicates of each crop measured.
‡Only 1 replicate of each crop measured.

Table 3: Mean volumetric water content (VWC) ± standard deviation (SD) for each chamber method on each measurement date. Includes measurements from all crop treatments (switchgrass, continuous corn, hybrid poplar). Season mean is the mean of all VWC measurements for each chamber method.

Chamber method	Soil volumetric water content (VWC) (mean ± 1 SD) ($m^3 m^{-3}$)							Season mean
	May 16†	May 20‡	May 31	June 30	July 15	August 8†	August 9‡	
NTF	27.73 ± 2.15	25.34 ± 1.14	27.14 ± 3.41	24.34 ± 2.95	20.71 ± 3.98	12.34 ± 1.72	10.08 ± 3.19	22.14 ± 6.49
Point	27.07 ± 2.64	26.01 ± 1.36	26.58 ± 3.58	24.04 ± 3.80	19.85 ± 3.54	13.08 ± 1.80	10.91 ± 2.99	21.91 ± 6.23
Transect	26.79 ± 2.40	27.15 ± 0.29	26.97 ± 3.99	24.19 ± 2.70	20.29 ± 2.74	14.96 ± 1.39	12.67 ± 3.47	22.51 ± 5.57

†Only 2 replicates of each crop measured.

‡Only 1 replicate of each crop measured.

FIGURES

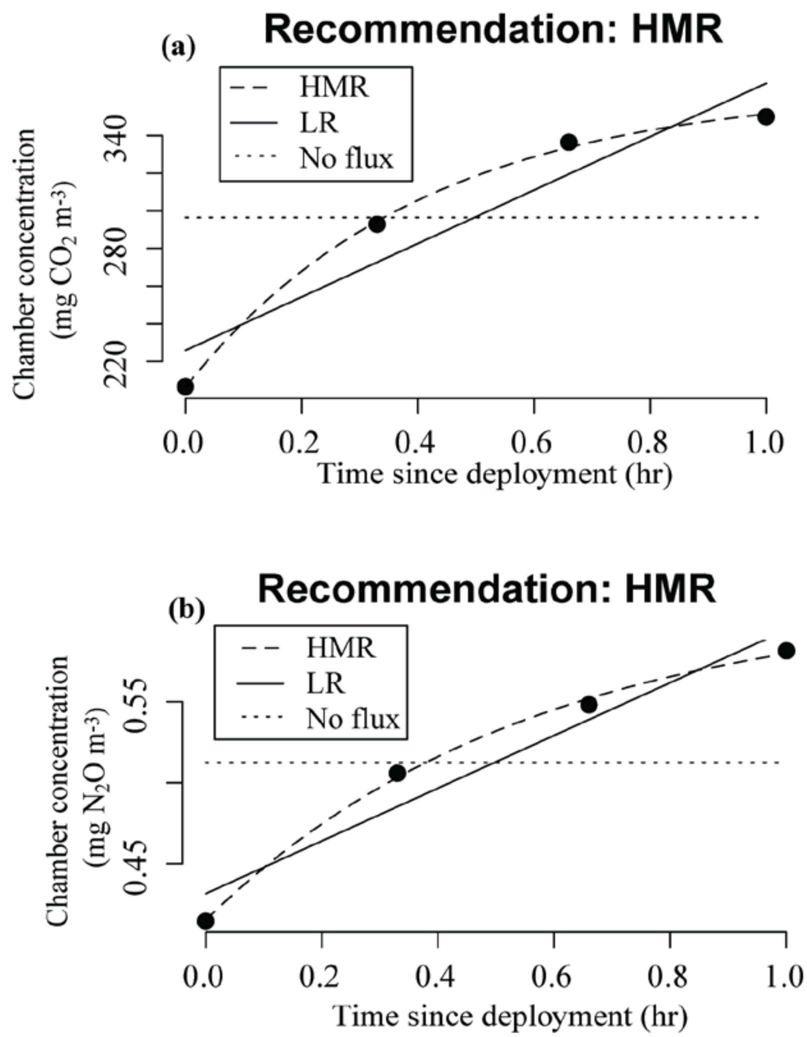

Figure 1: Representative examples of (a) CO_2 and (b) N_2O concentration data over time of chamber deployment. Output is from the R algorithm of the HMR method. Linear (LR) and HMR model fits are shown.

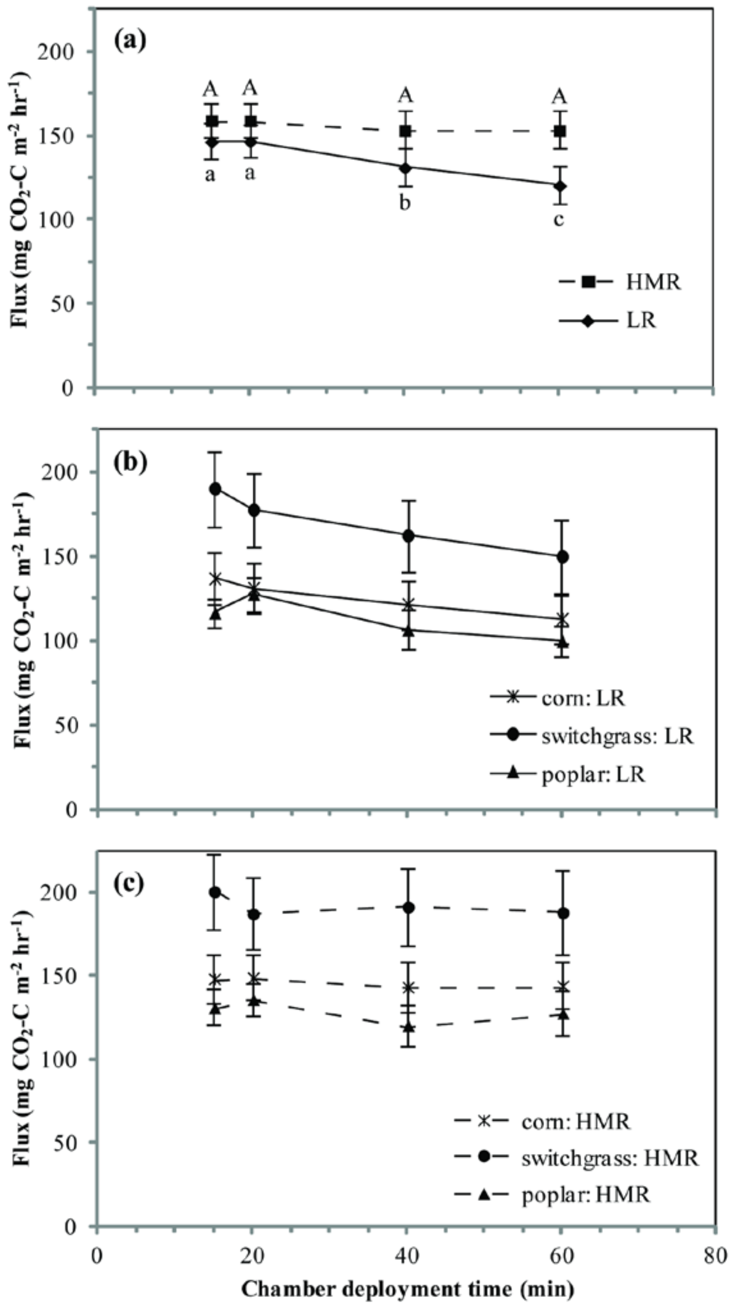

Figure 2: CO_2 fluxes with chamber deployment time for (a) all crops (switchgrass, corn, hybrid poplar) using both linear (LR) and HMR flux calculation methods, (b) each crop individually using LR approach, and (c) each crop individually using HMR approach. Given are seasonal (May-August) means $\pm$ standard error. Letters indicate significant differences among deployment times ($p<0.05$, Tukey-Kramer test). Because there were no significant crop x deployment time interactions, significance letters are not shown in (b) and (c).

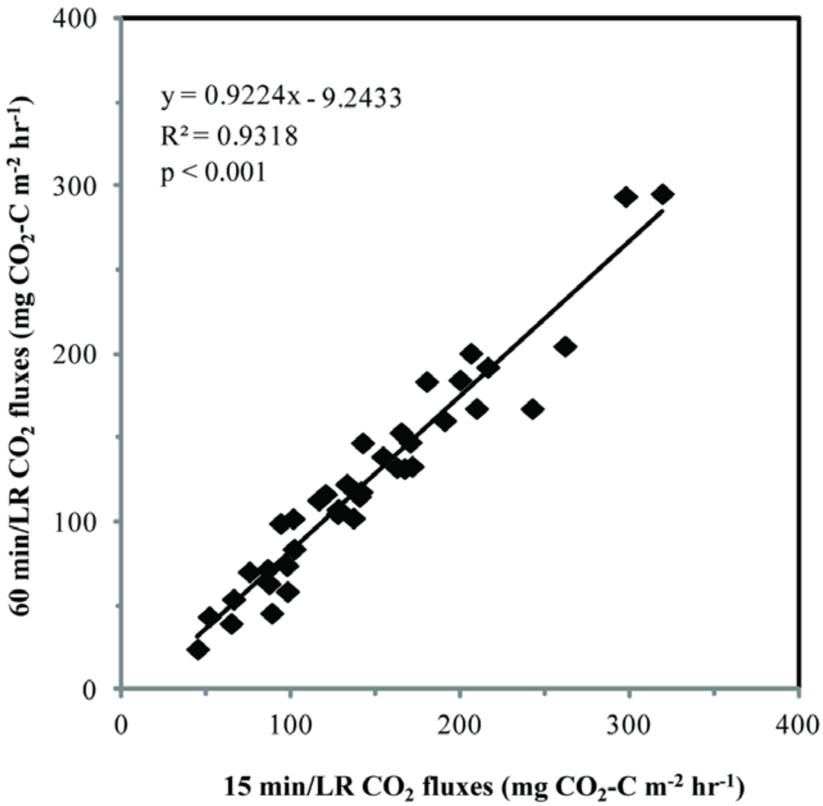

Figure 3: Scatter plot of 15 min/LR CO_2 fluxes versus the paired 60 min/LR CO_2 fluxes from all plots and all measurement dates. A linear trendline, equation of that line, R^2 value, and p-value are shown.

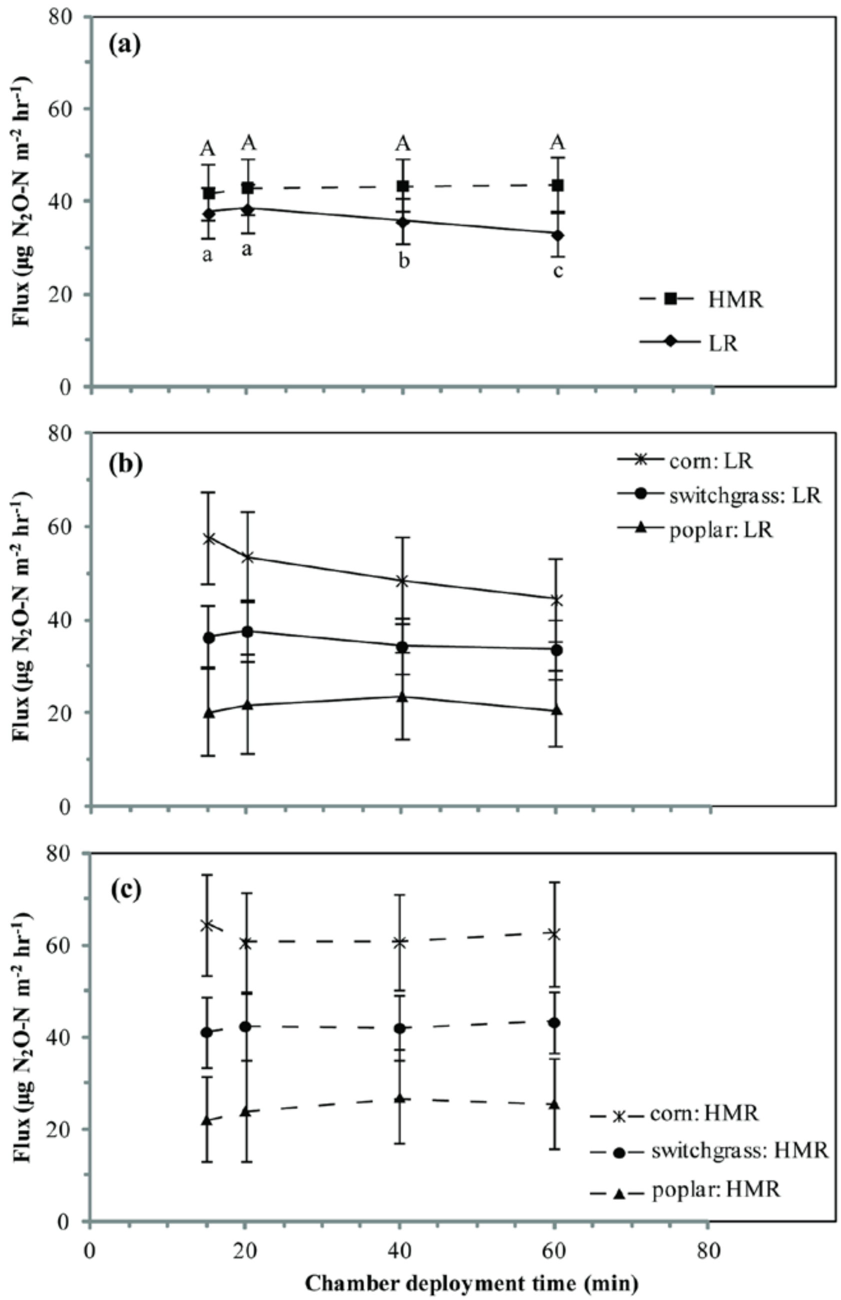

Figure 4: N_2O fluxes with chamber deployment time for (a) all crops (switchgrass, corn, hybrid poplar) using both linear (LR) and HMR flux calculation methods, (b) each crop individually using LR approach, and (c) each crop individually using HMR approach. Given are seasonal (May-August) means $\pm$ standard error. Letters indicate significant differences among deployment times ($p<0.05$, Tukey-Kramer test). Because there were no significant crop x deployment time interactions, significance letters are not shown in (b) and (c).

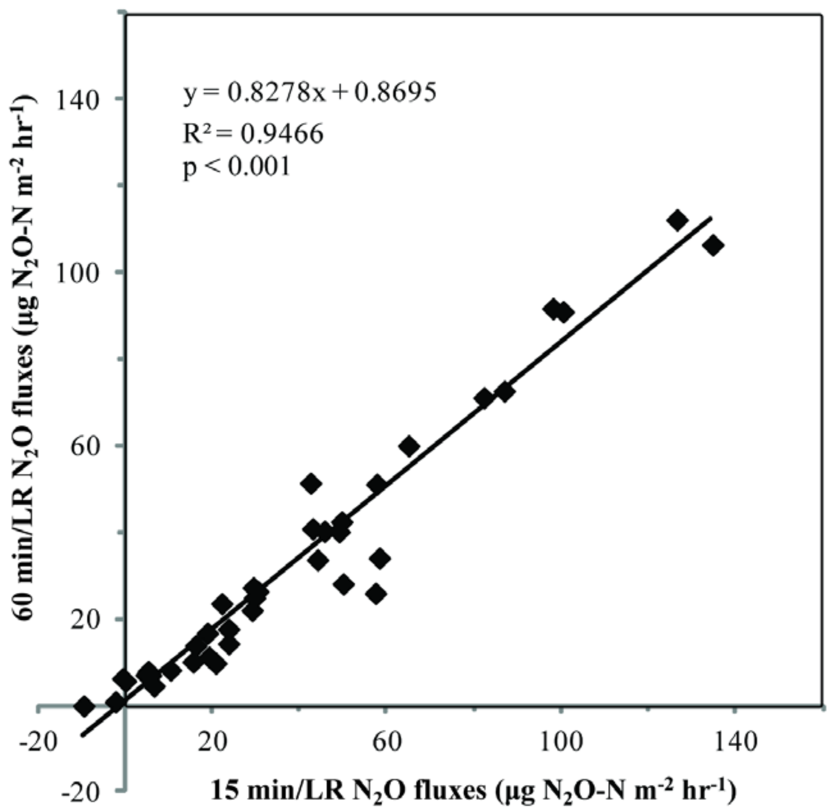

Figure 5: Scatter plot of 15 min/LR N_2O fluxes versus the paired 60 min/LR N_2O fluxes from all plots and all measurement dates. A linear trendline, equation of that line, R^2 value, and p-value are shown.

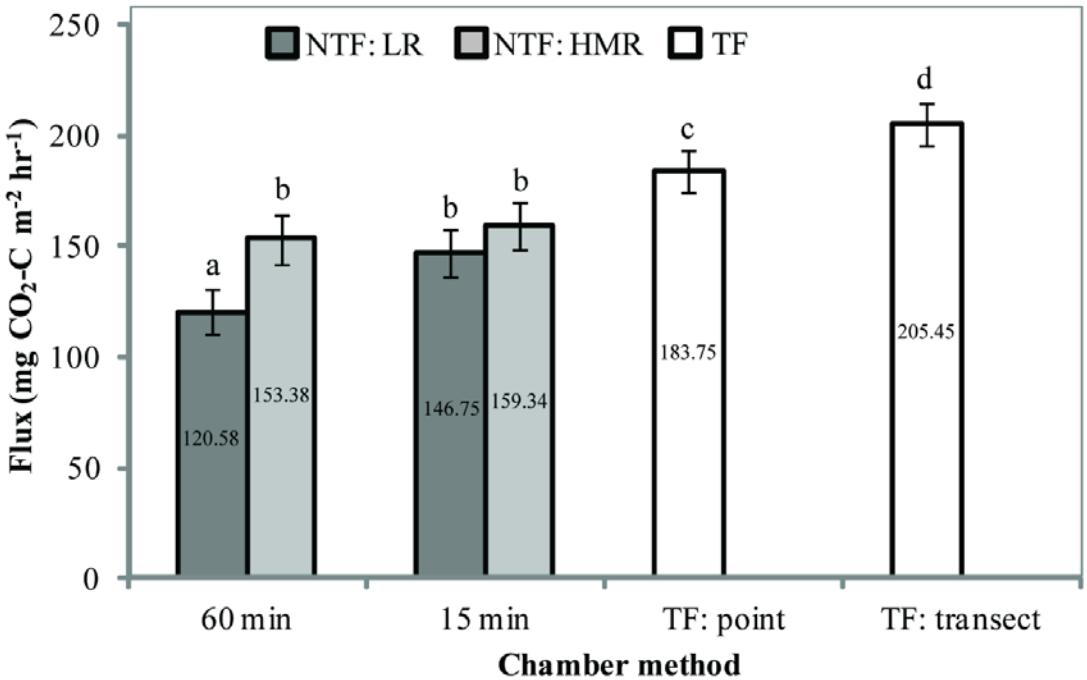

Figure 6: CO_2 fluxes by chamber method for all crops (switchgrass, corn, hybrid poplar). Given are season (May-August) means $\pm$ standard error. Non-through-flow non-steady-state chambers (NTF) were analyzed with 60 min and 15 min deployment times and with linear (LR) and HMR flux calculation methods. Through-flow non-steady-state chambers (TF) were measured using 4 collars around the NTF chamber (point) and 10 collars along a transect (transect). Letters indicate significant differences among methods ($p<0.05$, Tukey-Kramer test). There was no crop x method interaction.

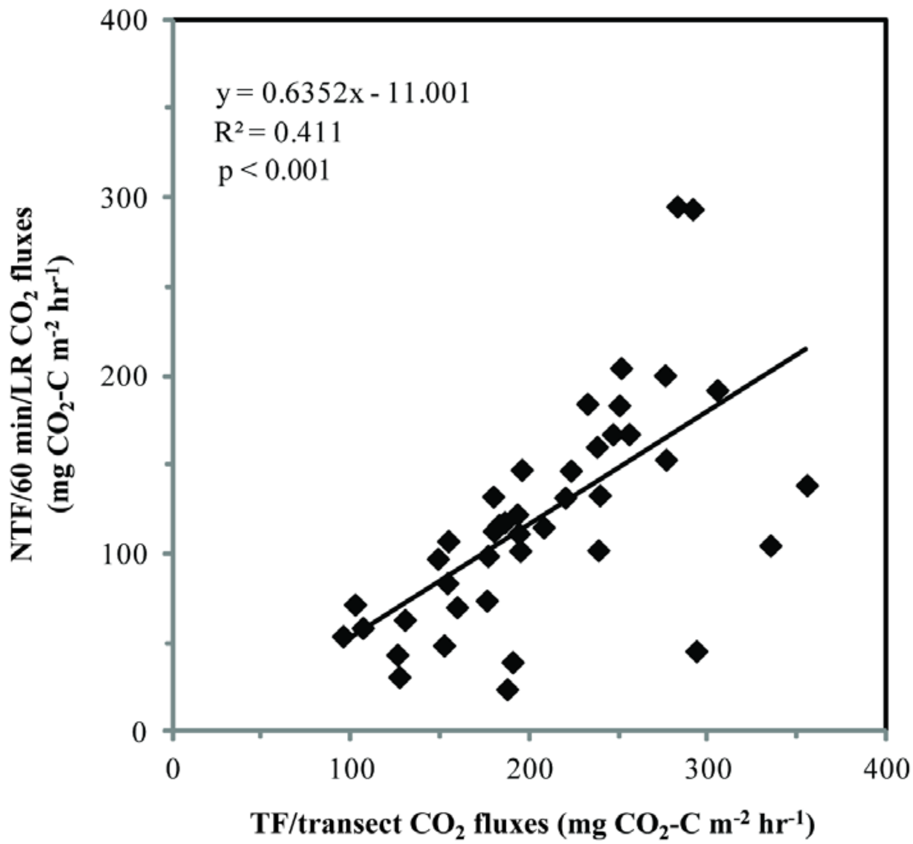

Figure 7: Scatter plot of CO_2 fluxes from all plots and all measurement dates measured with the non-steady-state through-flow (TF) chamber and transect method versus the paired fluxes measured with the non-steady-state non-through-flow (NTF) 60 min deployment time and linear regression method (LR). A linear trendline, equation of that line, R^2 value, and p-value are shown.

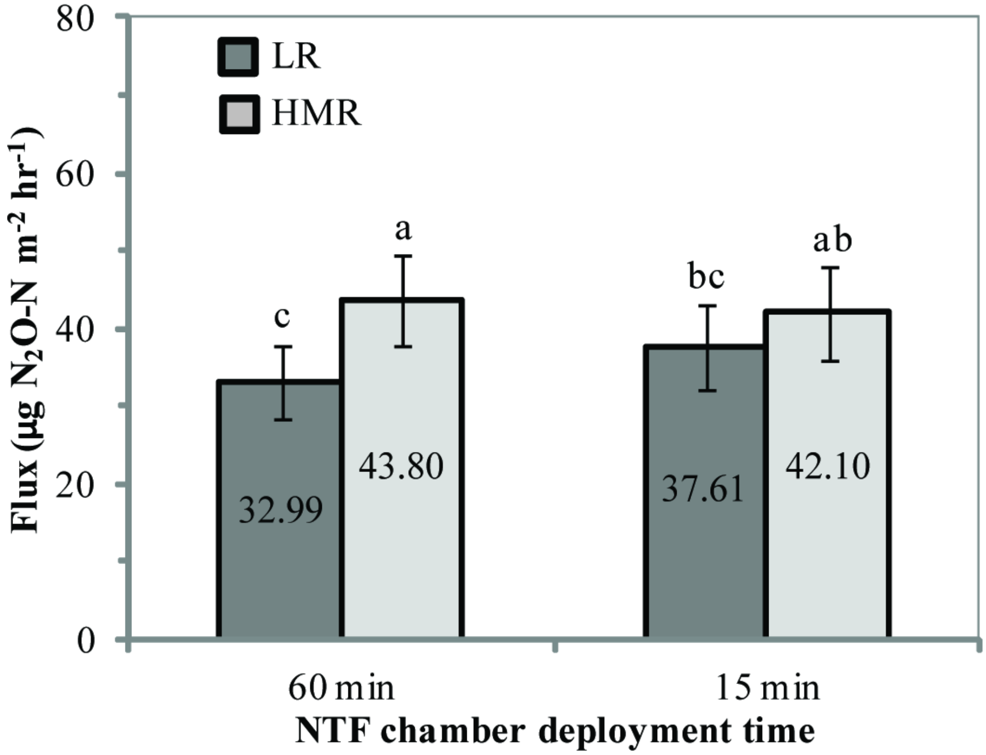

Figure 8: N_2O fluxes by flux calculation method, linear (LR) and HMR, at 15 min and 60 min deployment times. Given are season (May-August) means $\pm$ standard error. Letters indicate significant differences among methods ($p<0.05$, Tukey-Kramer test).