Nitrogen sources and sinks in Iowa soils: biogeochemical links between carbon inputs, nitrate leaching, and nitrous oxide emissions

by

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ABSTRACT

Nitrogen (N) lost from agricultural soils in the forms of nitrate (NO_3) and nitrous oxide (N_2O) have become major environmental concerns. Because N cycling is coupled with organic carbon (C) cycling, management practices that influence soil organic C inputs and cycling may affect reactive N losses. Management practices have been proposed to reduce N losses, including perennial vegetation buffers (PVB) and overwintering non-legume cover crops. However, the effects of these practices on N losses depend on the biogeochemical interactions between soil N and C cycling. This thesis presents investigations of the effects of these management practices on NO_3 and N_2O losses from row crop systems in Iowa, USA. In PVBs, soil organic matter and plant biomass acted as sinks for NO_3 inputs. However, denitrification, stimulated by organic C inputs from perennial vegetation, appeared to be the most important NO_3 sink. These results indicate that integration of perennial vegetation into agricultural landscapes can return substantial amounts of N to the atmosphere and decrease watershed NO_3 losses in the long term. The effects of cover crops on N_2O emissions were found to vary with N fertilizer rate, and cover crops increased N_2O emissions at an economical N rate. These results indicate that overwintering cover crops should not be expected to consistently decrease N_2O emissions from agricultural soils, even when they do decrease NO_3 availability for denitrification. In row crop systems with PVBs and cover crops, mineralizable C inputs to soils are a key factor influencing the biogeochemical N transformations that lead to N retention or losses. Considering the interactions between C and N cycling in agricultural soils is necessary to understand and predict the effects of management practices on environmental N losses.

CHAPTER 1. GENERAL INTRODUCTION

Introduction

Nitrogen (N) cycling plays a central role in agricultural production and global change. Nitrogen fertilizer is a key factor sustaining the high yields of modern agricultural systems (Cassman et al., 2002). However, loss of various forms of reactive N from agricultural ecosystems has had serious environmental consequences (Robertson and Vitousek, 2009). Globally, human activity has greatly accelerated N cycling, largely due to high inputs of N to agricultural systems; resultant N losses have had great effects on the biosphere (Vitousek et al., 1997; Galloway et al., 2008). Management of N in agricultural systems to sustain productivity while decreasing reactive N losses therefore presents a great challenge (Robertson and Vitousek, 2009).

Maize-based agroecosystems are a major land cover in the United States, accounting for over one-third of cropland (NASS, 2011). Maize-soybean agroecosystems, predominant in the upper Midwestern USA, are characterized by high crop productivity, providing more than one-third of global corn and soybean production (Dobermann and Cassman, 2002). However, substantial N losses also occur from these systems (Vitousek et al., 2009). Notably, nitrate (NO_3) losses from this cropping system have led to contamination of rivers and streams (Hatfield et al., 2009). Nitrate originating from maize-soybean agriculture has been identified as the leading cause of the annual hypoxic zone in the Gulf of Mexico (Alexander et al., 2008; David et al., 2010).

In addition to dissolved NO_3 losses to aquatic ecosystems, emissions of the greenhouse gas nitrous oxide (N_2O) are an environmental concern due to the contribution of N_2O to radiative forcing in the atmosphere (Smith et al., 2007). Agriculture is currently the dominant cause of increasing atmospheric N_2O concentrations (Reay et al., 2012). Though maize-based agricultural systems can also be sources of carbon dioxide and methane, N_2O contributes the most to the total global warming potential of these systems (Robertson et al., 2000; Mosier et al., 2005; Adviento-Borbe et al., 2007). Models of N_2O emissions from agricultural soils have identified the upper Midwestern US as a major source of this greenhouse gas (USEPA, 2010).

There is potential to reduce N losses from agroecosystems by re-coupling nitrogen inputs to biological processes (Drinkwater and Snapp, 2007). Management changes beyond modification of N fertilizer rate and timing are likely necessary to substantially decrease reactive N losses (Hatfield et al., 2009). Since N cycling processes in soil are closely coupled to soil organic carbon (SOC) (Booth et al., 2005), the effectiveness of practices meant to reduce N losses depends in part on the links between N cycling and soil C inputs and cycling (Drinkwater and Snapp, 2007). Among the practices proposed to decrease reactive N losses from croplands are increasing land cover by perennial vegetation (Schulte et al., 2006) and replacing winter fallow periods with cover crops (Thorup-Kristensen et al., 2003).

Thesis Organization

This thesis presents studies of two management practices aimed to reduce reactive N losses from soils in maize-soybean agroecosystems in Iowa, USA. These studies focus on N losses as NO_3 (Chapter 2) and as N_2O (Chapter 3) in relation to management practices proposed to reduce these losses. Both studies investigate organic C inputs and cycling as key factors controlling reactive N losses from these soils. The overall conclusions from these studies are synthesized and summarized in Chapter 4.

The study presented in Chapter 2 investigates the NO_3 sink strength of soils managed in perennial vegetation within row crop landscapes. While these perennial vegetation buffers (PVB) have been shown to decrease NO_3 losses to downstream ecosystems, the effectiveness of this ecosystem service depends on the strength of various biogeochemical sinks for NO_3 entering these soils in subsurface flow. Though previous studies of PVB have focused on plant uptake and denitrification, soil organic matter (SOM) may acts as a major NO_3 sink as well, since SOM is the largest sink for N in terrestrial ecosystems (Aber et al., 1998). This study links ecosystem nutrient retention theory (Vitousek and Reiners, 1975) to SOM saturation theory (Six et al., 2002) and applies these principles to a management practice meant to improve environmental quality.

The study presented in Chapter 3 investigates the effect of overwintering non-legume cover crops in a maize-soybean rotation on emissions of N_2O from the soil surface. While studies have proposed that cover crops decrease soil N_2O emissions, this effect is not consistent.

Chapter 3 presents evidence that the inconsistent effects of cover crops on N_2O emissions may be influenced by mineral N availability, as influenced by N fertilizer rate. This study draws from previous work examining the interaction between soil mineralizable C and NO_3 availability as controls on N_2O emissions. This study provides evidence that expectations of cover crops to decrease N_2O emissions from the soil may be confounded by the complex interactive effects of cover crops and N management, as well as climate and other factors, on N_2O emissions.

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CHAPTER 2. NITRATE SINKS IN AGRICULTURAL PERENNIAL VEGETATION BUFFERS

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Abstract

Nitrate (NO_3) leaching from agricultural watersheds is a major cause of pollution in aquatic ecosystems. Integration of perennial vegetation into agricultural watersheds may decrease NO_3 losses to surface and groundwaters. However, the long-term effectiveness of this ecosystem service depends on the relative importance of several NO_3 sinks. Vegetation and labile soil organic matter (SOM) are temporary sinks for NO_3. In contrast, stable SOM is a long-term, but potentially finite, NO_3 sink, while gaseous loss through denitrification is a permanent sink. We investigated the relative importance of NO_3 sinks in perennial vegetation buffers that were integrated into agricultural watersheds in Iowa, USA, and had been shown to decrease soil solution NO_3 concentrations up to 100%. Using a $^{15}NO_3$ tracer, we quantified NO_3-N recovery in vegetation and SOM sinks after one growing season while comparing NO_3-N recovery in SOM with and without growing perennial vegetation. We also compared

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potential denitrification enzyme activity in soils from paired watersheds with and without perennial vegetation buffers. Assimilation of NO_3-N into labile and stable SOM pools was rapid and initially independent of vegetation; however, the presence of vegetation increased retention of NO_3-N in SOM by the end of the growing season. Nevertheless, NO_3-N recovery in SOM and vegetation accounted for <20% of NO_3-N inputs. Denitrification enzyme data indicated that labile C inputs from perennial vegetation increased denitrifier activity in soils under perennial vegetation. Together, these results indicate that denitrification may be a more important sink for NO_3 inputs than vegetation and SOM in perennial buffers. Carbon inputs from perennial vegetation appear to be important for NO_3 retention in stable SOM and loss by denitrification.

Keywords: soil organic matter; nitrogen retention; nitrate leaching; denitrification

Abbreviations: PVB, perennial vegetation buffers; NO_3, nitrate; SOM, soil organic matter; SOC, soil organic carbon; SON, soil organic nitrogen; AGBM, aboveground biomass; FRBM, fine root biomass; CRBM, course root biomass; TSEN, total salt-extractable nitrogen; SEOC, salt-extractable organic carbon; DOC, dissolved organic carbon; DEA, denitrification enzyme assay

Introduction

Nutrient loss from agricultural land has become a leading source of ground and surface water pollution in the United States (Carpenter et al., 1998). In the Upper Mississippi River Basin,

maize (*Zea mays* L.)-soybean [*Glycine max* L. (Merr.)] croplands are the major source of nitrate (NO_3) contamination of streams which is responsible for the annual hypoxic zone in the Gulf of Mexico (Alexander et al., 2008; David et al., 2010). Contamination of drinking water by agriculturally-derived NO_3 also poses risks to human health (Townsend et al., 2003).

Perennial vegetation buffers (PVB) in agricultural landscapes can reduce NO_3 loads to downstream ecosystems by removing NO_3 from runoff (Hill et al., 1996; Mayer et al., 2007). However, PVB can remove NO_3 from agricultural runoff by various mechanisms, including uptake by vegetation, storage in soil organic matter (SOM), and gaseous loss through denitrification. From the perspective of reducing dissolved NO_3 losses, these processes differ in their long-term effectiveness (Schade and Lewis, 2006). According to nutrient retention theory, ecosystems receiving chronic nitrogen (N) inputs have a limited capacity to retain these inputs in biological and organic matter sinks. After this capacity is reached, N outputs in the form of gaseous and dissolved losses are equivalent to N inputs (Vitousek and Reiners, 1975; Aber et al., 1989). Thus, if plant biomass and SOM are the major sinks for NO_3 inputs in PVB, reductions in watershed NO_3 losses observed after PVB implementation may not continue in the long-term. In contrast, gaseous loss of NO_3 through denitrification represents a more permanent sink for NO_3 inputs (Martin et al., 1999; Mayer et al., 2007).

Denitrification has been found or inferred to be the dominant sink for agriculturally-derived NO_3 in many PVB (Vought et al., 1994; Verchot et al., 1997; Martin et al., 1999), including those in which plant demand has been saturated (Hanson et al., 1994a, 1994b). Availability

of mineralizable soil organic carbon (SOC) beneath perennial vegetation can stimulate denitrification (Schade et al., 2001; Baker and Vervier, 2004). During denitrification, NO_3-N is lost from the ecosystem as gaseous N species. Thus, if denitrification is the principal sink for NO_3 inputs to PVB, reductions in NO_3 loads to downstream systems would be expected to continue in the long term.

The magnitude of NO_3 uptake by vegetation varies with type and management, but can be the major factor reducing NO_3 losses in some PVB systems (Hefting et al., 2005). However, plant biomass is necessarily a temporary sink for NO_3-N; biomass-N does not accumulate indefinitely, but instead ultimately either is mineralized or becomes soil organic N (SON) (Vitousek and Reiners 1975). While N mineralized from plant biomass can be readily lost from the soil, SON deriving from biomass may or may not be retained in the long term. Nevertheless, harvest of aboveground biomass can provide a permanent sink for N inputs to PVB.

Though previous research has focused on denitrification and plant biomass, incorporation of NO_3-N into SOM is potentially an important sink for NO_3 inputs into PVB. Soil organic matter is the largest N sink in terrestrial ecosystems (Aber et al., 1998). Furthermore, since inorganic N transformations in soil correlate closely with SOC (Booth et al., 2005), SOM potentially plays a central role in NO_3-N removal from agricultural runoff passing through PVB. Nitrate-N can be incorporated into SOM through plant uptake and subsequent litter decomposition or directly through microbial and abiotic mechanisms (Barrett and Burke, 2002; Dell et al., 2005).

While a fraction of SOM is available for microbial mineralization, the remainder is relatively stable in the long term. Particulate organic matter (POM), composed of large compounds derived from plant biomass, is considered to be potentially mineralizable by microorganisms (Six et al., 2002). In contrast, SOM that is physico-chemically bound to silt and clay particles, known as mineral-associated organic matter (MAOM), is considered to be relatively resistant to microbial mineralization (Six et al., 2002; Kleber et al., 2007). The amount of SOM that can be protected from mineralization as MAOM is limited and a function of the silt + clay content of the soil (Feng et al., 2012). After the soil MAOM pool reaches capacity (saturates), additional SOM inputs would not be physico-chemically protected from mineralization (Hassink, 1997; Stewart et al., 2007). In this case, as SOM accumulates and N sinks in MAOM saturate, further N inputs would not be protected from re-mineralization and loss (Castellano et al., 2012). Therefore, if SOM is the principal sink for NO_3-N inputs to PVB, decreases in watershed NO_3 losses may not continue in the long term due to saturation of stable SOM and active cycling of N retained in mineralizable SOM.

The objective of this study was to evaluate the NO_3-N sink strength of plant biomass, SOM, and denitrification in PVB integrated into an agricultural landscape in Iowa, USA. We used an isotope tracer to quantify the NO_3 sink strengths of plant biomass and SOM. Relatively few previous studies have used this method in agricultural PVB (Matheson et al., 2002; Bedard-Haughn et al., 2004), and to our knowledge, no previous studies have used this method in the Midwestern region of the USA. Preliminary data has shown that, since implementation, SON concentrations in these PVB have increased by 50-200% (Pérez-

Suárez, *unpublished data*), showing accumulation of N inputs in SOM. Based on these preliminary results, we have hypothesized that SOM is the most important sink for NO_3 in this system.

Methods

Field Site and Experimental Design

This study was conducted at an experimental site within the Neal Smith National Wildlife Refuge in Jasper County, IA, USA (41°33′ N, 93°16′ W). This site was established to evaluate PVB as a conservation practice to mitigate soil erosion and NO_3 loss (Zhou et al., 2010). At this site, three pairs of experimental watersheds were established. All watersheds had been managed as unfertilized brome (*Bromus* sp.) for at least 10 yrs prior to 2006. Watersheds were tilled with a mulch tiller in fall 2006 and spring 2007. The following treatments were begun in 2007: perennial vegetation buffers were planted in July 2007 in the lowest 10% of the area of one watershed in each pair, while the remaining 90% of those watersheds and 100% of the area of the other watershed in each pair was managed as a no-till maize-soybean rotation (soybean in 2007). The vegetation community of PVBs at the time of this study was 90% perennial (Hirsh, 2012). Anhydrous ammonia was applied to maize at 134 and 186 kg N ha^{-1} in 2008 and 2010, respectively. Biomass (>15 cm in height) has been harvested and removed from the PVB in late October or November annually since 2010. Compared to the equivalent landscape position in watersheds managed entirely as row crops, solution NO_3 concentrations were decreased by PVB establishment in vadose zone (50-

100%) and shallow groundwater (60-80%) (Zhou et al., 2010). Though subsurface flow rates could not be quantified at the site, they can be inferred to be lower in watersheds with PVB since evapotranspiration in PVB is expected to be greater than in row crops. Thus, though NO_3-N loads from these watersheds could not be calculated, loads from watersheds with PVB are inferred to be decreased compared to from watersheds entirely in row crops.

The NO_3-N sink strengths of PVB plant biomass and SOM were measured in the field using a procedure adapted from Dell et al. (2005). Three pairs of polyvinyl chloride (PVC) cores (25 cm diameter × 30 cm height, open on both ends) were pushed completely into the soil in each PVB. The pairs of PVC cores were installed at random locations within 5 m of the uphill edge of each PVB; this placement was chosen based on the assumption that N retention processes would be most important close to the interface between cropland and perennial vegetation, as found in similar studies of ecosystems receiving hydrologic N inputs (Lowrance et al., 1992; Haycock and Pinay, 1993). Since Zhou et al. (2010) had shown decreased NO_3 losses in watersheds with PVB compared to watersheds without, cores were not installed in watersheds without PVB. Instead, the focus of this study was to evaluate the strength of sinks within the PVB. Perennial vegetation emerged in all PVC cores after installation.

On 11 May 2011, one PVC core from each pair was randomly selected for vegetation removal. Vegetation in these cores (hereafter "de-vegetated cores") was cut to ground level. Glyphosate [*N*-(phosphonomethyl)glycine] solution (20% in water) was brushed onto cut vegetation with a paintbrush. The cut above-ground biomass was then placed on the soil

surface within the cores. Perennial plants did not re-emerge in de-vegetated cores for the remainder of the growing season, indicating that herbicide application was effective. Weed seedlings were removed manually from the de-vegetated cores periodically throughout the growing season. Vegetation in the other core from each pair (hereafter "vegetated cores") was allowed to grow undisturbed throughout the growing season.

Isotope Addition and Initial Soil Data

On 15 June 2011, 1 L of 3.63 m*M* KNO_3 (54.5 mg NO_3-N / L) at 98 atom-% ^{15}N was injected into each PVC core. This was performed during period of high precipitation (Figure 1) when water tables in the PVB were near the surface of the soil. Injections were made using 40 cm side-port needles, evenly distributing solution between 30 cm depth and the soil surface (Hart et al., 1994). Twenty injections were made per core, and were evenly distributed over the surface area of the cores to the extent possible. Points of injection were marked at the soil surface. Solution injections were equivalent to 11.1 kg N per ha and 20.4 mm of rainfall, and thus simulated realistic hydrologic NO_3 and water inputs. Soil solution NO_3-N concentrations ranged from 10-30 ppm, within the range of soil solution concentrations measured upslope of these PVB and presumably in subsurface flow entering these PVB (Zhou et al., 2010).

Soil samples (2 cm diameter to 30 cm depth) were collected on the day of isotope addition to determine initial soil NO_3 concentrations in the PVC cores. Samples were taken from outside of vegetated cores but inside of de-vegetated cores before isotope addition. Subsamples were

extracted in 2*M* KCl (5:1 extract: soil ratio). Extracts were filtered and frozen until analysis. Soil NO_3-N + NO_2-N (hereafter NO_3-N) concentrations were determined by colorimetric analysis using the Griess-Ilosvay reaction with VCl_3 as a reducing agent (Hood-Nowotny et al., 2010). Soil gravimetric water content (VWC) to 30 cm depth was determined by drying subsamples of fresh soil at 105 °C. Bulk density (BD) for the watershed soils were taken from Gutierrez-Lopez (2012). Extract NO_3-N concentrations and BD values were used to calculate the total mass of NO_3-N in each core, and VWC data were converted to WFPS using these BD values.

Soil Collection

To measure recovery of ^{15}N in SOM pools 7 d after addition, soil cores (2 cm diameter to 30 cm depth) were collected from points of isotope injection within the PVC cores on 22 June 2011. To measure recovery of ^{15}N in SON pools over the full growing season, the PVC cores and all soil inside were collected on 29 (Watershed 1) and 31 Oct 2011 (Watersheds 2 and 3), which corresponded to 136 and 138 d after isotope addition (hereafter 137 d). After sampling on both days, soil was stored at 4 °C until processing.

Salt-extractable N and Microbial Biomass

Within 1 week of each soil collection, a sequential extraction procedure adapted from Holmes et al. (2003) was performed on 40 g subsamples of fresh soil to remove extractable C and N and microbial biomass. Soils were sieved to 2 mm, extracted in 0.5*M* K_2SO_4 (5:1

extract: soil ratio), and left to settle overnight at 4 °C. Extracts were filtered over pre-leached Whatman 42 filter papers. Extract total N concentrations were measured with a TOC-L CPN (Shimadzu, Kyoto, Japan) to determine and salt-extractable N, which includes NO_3-N, NH_4-N, and salt-extractable organic N (SEON). Extract NO_3-N concentrations were determined as described above, and extract NH_4-N concentrations were determined with the Berthelot reaction (Hood-Nowotny et al., 2010). Enrichment of salt-extractable N with ^{15}N was not determined.

To extract microbial biomass, extracted soils were fumigated with chloroform ($CHCl_3$) using a direct fumigation procedure adapted from Witt et al. (2000) and Perakis and Hedin (2001). A 4.5 mL aliquot of $CHCl_3$ was pipetted directly onto each soil in the high-density polyethylene (HDPE) bottles. Bottles were immediately agitated by hand to spread the soil slurry across the bottle surface and maximize penetration of $CHCl_3$ into the soil pores. Fumigated soils and blanks were kept at room temperature in the dark for 5 d. After fumigation was complete, soils were subjected to vacuum venting to vaporize $CHCl_3$. Soils were then extracted in 0.5*M* K_2SO_4 as described above. Enrichment of microbial biomass N with ^{15}N was not determined. Extracted soils were left to air-dry under the fume hood; once dry, they were stored at room temperature until further analysis.

Soil Organic Matter Analyses

Retention of ^{15}N in POM and MAOM at 7 d and 137 d was measured in soils that had been sequentially extracted and air-dried. To separate POM and MAOM, subsamples were placed

in 0.08 *M* Na hexametaphosphate solution (4:1 solution: soil ratio) and shaken on a reciprocal shaker for ~20 hrs. Soil slurries were then emptied onto a 0.53 µm sieve and rinsed until all silt + clay + MAOM had washed through, with only sand + POM remaining on the sieve. Both fractions were dried at 65 °C. Subsamples (20-60 mg) of dried soil fractions were sent for analysis at the Stable Isotope Facility at University of California, Davis, CA, USA. Total C and N content and ^{15}N enrichment of the soil fractions were determined with an elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the Stable Isotope Facility at University of California, Davis, CA, USA.

Plant Biomass Collection and Analysis

Plant aboveground biomass (AGBM) from within the vegetated cores was harvested 137 d after isotope addition. Aboveground biomass was immediately set at 35 °C, subsequently oven-dried at 60 °C for 4 days, and pulverized. Rhizomes, root mats, and roots >2 mm in diameter that would not be represented by random sampling (hereafter "course roots") were collected from air-dried soil from the cores collected at 137 d. Course roots were washed in 0.01 *M* $CaCl_2$ three times for 5 minutes to remove mineral N from root surfaces, oven-dried at 60 °C for 48 hrs, and weighed. Subsamples (~800 g) of air-dried bulk soil were collected for fine root analyses. Soils were placed in mesh containers (0.28 mm pore size) and washed in an elutriator to remove silt, clay, and fine sand. Fine roots and non-root litter were floated out of the remaining course sand, and non-root litter was removed manually. Fine roots were re-washed to remove remaining soil particles, dried at 60 °C for 48 hrs, and then pulverized.

Subsamples (3-5 mg) of pulverized AGBM, course root biomass (CRBM), and fine root biomass (FRBM) were sent for determination of total C and N and enrichment by ^{15}N at the Stable Isotope Facility at the University of California, Davis, as described above.

Denitrification Enzyme Assay

Soil samples (2 cm diameter to 30 cm depth) for denitrification enzyme assay (DEA) were collected on 25 June 2011. Soils were collected from the proximity of the PVC cores and from the equivalent topographic position in adjacent watersheds managed entirely as row crops (soybean in 2011). Soil NO_3-N concentrations at time of sampling were determined by extracting in 2*M* KCl, as described above. To determine soil dissolved organic C (DOC) concentrations, fresh soil subsamples were extracted in 0.01 *M* $CaCl_2$ (2:1 extract: soil ratio), shaken orbitally at 160 rpm for 12 min, centrifuged at 2200 rpm for 15 min, and filtered to 0.45 µm. Extract C concentrations were measured by a Shimadzu TOC-L CPN.

Denitrification enzyme assay (DEA) was performed on fresh soil subsamples. The DEA protocol was based on Tiedje (1994). Soils were placed in 310 mL glass bottles and saturated in solution of 1 m*M* glucose ($C_6H_{12}O_6$) and KNO_3. Soil slurries were flushed with He, evacuated repeatedly, and received 30 mL of acetylene (C_2H_2) gas. Soil slurries were then shaken to maintain gas equilibrium with the bottle headspaces. Gas samples were taken from the bottle headspaces at 0, 30, 60 and 90 min. Nitrous oxide (N_2O) concentrations in gas samples were determined with a gas chromatograph with an electron capture detector at

325°C. Gas species separation was accomplished with stainless steel columns packed with Haysep D and maintained at 50°C, using N_2 as carrier gas. Flux rates of N_2O from soil slurries were considered to represent denitrification enzyme potential. Only linear portions of fluxes were included in rate calculations (Tiedje, 1994).

Statistical Analyses

Statistical analyses were performed using PROC GLM in SAS (SAS Institute Inc., Cary, NC, USA). Watersheds were considered blocks; time of collection and vegetation treatment (for the PVC core soil data) and vegetation type (perennial vs. row crop) for the DEA data were considered fixed categorical factors.

Results

Soil Mineral N and Organic Matter

Initial soil NO_3-N concentrations in de-vegetated cores were lower than in vegetated cores (p = 0.007) and more variable (Table 1). Soil water contents were high (0.70-1.0 cm^3 cm^{-3} WFPS to 30 cm depth) on the day of and 7 d after isotope addition, and did not differ between vegetation treatments. Soil NH_4-N and NO_3-N concentrations were greater in de-vegetated than in vegetated cores at both sampling times (Table 2).

Soil POM-N concentration increased between 7 d and 137 d, but there was an interactive effect of vegetation removal with time (Figure 2). Soil POM-N was greater in de-vegetated than vegetated cores at 7 d; however, POM-N increased from 7 d to 137 d in vegetated cores, but not in de-vegetated cores. Carbon: nitrogen ratio of POM decreased between 7 d and 137 d, but did not differ between vegetation treatments (Figure 2). Soil MAOM-N and MAOM-C concentrations increased from 7 d to 137 d across vegetation treatments, but there was no effect of vegetation treatment (Figure 3). Across vegetation treatments, MAOM C:N ratio increased from 7 d (mean=10.26) to 137 d (mean=10.40; $p = 0.055$).

Greater percentages of tracer $^{15}NO_3$-N were recovered as MAOM-N than POM-N both 7 and 137 d after $^{15}NO_3$ addition (Figure 4). Percentage of $^{15}NO_3$-N recovered as POM-N and MAOM-N did not differ between vegetation treatments at 7 d. However, at 137 d, greater percentages of $^{15}NO_3$-N were recovered as POM-N and MAOM-N in vegetated than in de-vegetated cores (though this interaction was not significant for MAOM-N) (Figure 4). Percent of ^{15}N tracer recovered in POM + MAOM ranged from 3.6 to 10.1% (Figure 5).

Plant Biomass and Unrecovered ^{15}N

Tracer ^{15}N was recovered in AGBM, CRBM, and FRBM in all vegetated cores (Figure 5). Total % of ^{15}N tracer in plant biomass ranged from 4.2 to 19.5% (Figure 5). Percent ^{15}N recovered in AGBM was more variable than percent recovered in roots, and correlated positively with AGBM-N and negatively with AGBM C:N (data not shown). Similar trends were not found for percent ^{15}N recovered in roots. Total percent of tracer ^{15}N unrecovered in

plant biomass and SOM pools ranged from 70.4 to 92% (Figure 5). Percent of tracer ^{15}N unrecovered correlated negatively with % recovered in AGBM (R^2 = 0.84; data not shown).

Denitrification Enzyme Assay

Soil NO_3-N concentrations were greater in row crop than PVB soils from equivalent landscape positions (Figure 6). However, denitrification enzyme potential showed the opposite trend, with DEA >50% higher in PVB compared to row crop soils, though the difference was not statistically significant (Figure 6). Water-filled pore space and DOC concentrations were greater in prairie than soybean soils (Figure 6).

Discussion

Retention of N in SOM

Previous data had shown contrasting soil C and N dynamics in watersheds with and without PVB. At the toeslope landscape position, organic C and N had increased and C:N had decreased in PVB soils since implementation, while organic N had decreased and C had not changed in row crop soils since conversion from brome (Pérez-Suárez, *unpublished data*). These data indicated that organic N had been accumulating in PVB soils but not row crop soils since treatment implementation, suggesting that SON could be a potentially significant sink for N inputs to these watersheds.

In this study, both POM-N and MAOM-N increased between mid-June and late October in cores with growing vegetation, indicating that these soils had accumulated organic N. However, in de-vegetated cores, only MAOM-N increased over this time period. Incorporation of $^{15}NO_3$-N into POM and MAOM occurred rapidly (within 7 d of input) and independently of vegetation treatment. Similarly, Matheson et al. (2002) observed $^{15}NO_3$-N incorporation into SOM within 24 d not to differ between planted and unplanted PVB soils. However, in these soils, vegetation did affect longer-term recovery of $^{15}NO_3$-N in SOM pools. In de-vegetated cores, amount of tracer ^{15}N recovered in POM and MAOM did not differ between 7 d and 137 d after addition. In contrast, the presence of growing perennial vegetation increased recovery of ^{15}N in both SOM pools at 137 d compared to 7 d, though this result was only statistically significant for POM. These results indicate that, while incorporation of NO_3-N into both POM and MAOM occurred in these soils independently of plant growth, plant growth increased retention of NO_3-N in both pools over the course of the growing season.

However, while these results show that NO_3-N inputs were retained in SOM, and this retention was enhanced by plant growth, SOM was nevertheless a relatively minor sink for NO_3-N inputs. The amount of ^{15}N recovered in SOM pools of vegetated cores after 137 d represented <10% of $^{15}NO_3$-N inputs (Figure 5). Matheson et al. (2002) also found SOM to be a minor sink for NO_3-N in PVB soils.

Retention of N in Plant Biomass

In this study, AGBM at the end of the growing season was the most variable sink for $^{15}NO_3$-N inputs, while relatively consistent amounts were recovered in roots. Because the amount of ^{15}N retained in SOM and roots were relatively consistent, recovery in AGBM correlated closely and negatively with the amount of ^{15}N that was unrecovered. However, similarly to SOM, plant biomass at 137 d represented a minor sink for NO_3-N inputs; total ^{15}N recovered in plant biomass represented <20% of $^{15}NO_3$-N inputs, similar to results found by Matheson et al. (2002). Since only 3-7% of ^{15}N was recovered in AGBM at 137 d (Figure 5), late-fall harvest of AGBM from these watersheds is not likely to provide a major sink for NO_3-N inputs. Total uptake of ^{15}N by vegetation was not determined in this study, and may represent a greater percentage of ^{15}N inputs. Plant biomass represents a temporary pool for N inputs, and N retained in herbaceous plant biomass would be expected to either mineralize or become SON on the scale of years to decades (Hefting et al., 2005). Further studies are needed to determine the long-term fate of N retained in plant biomass pools.

Unrecovered N and Denitrification as an N Sink

The majority of ^{15}NO-N inputs to these soils were not recovered in SOM or plant biomass. The amount of $^{15}NO_3$ leached out of the PVC cores (to depths >30 cm) could not be determined in this study. However, since subsurface NO_3 losses from watersheds with PVB have been substantially decreased compared to watersheds without PVB (Zhou et al., 2010), subsurface leaching appears not to be a major loss pathway for NO_3 in these watersheds. Therefore, even if substantial amounts of $^{15}NO_3$ had been lost from the soils within the PVC

cores by leaching, whole-watershed data indicate that the majority of this $^{15}NO_3$ would not have been lost from the PVB in subsurface flow.

The lack of recovery of ^{15}N in SOM and plant biomass and demonstrated decrease in subsurface dissolved NO_3 losses from these watersheds together indicate that denitrification is the major sink for NO_3 inputs to these PVB. Denitrification has been found to be the major NO_3 sink in many riparian PVB (e.g. Martin et al., 1999; Matheson et al., 2002). If denitrification had accounted for 100% of unrecovered $^{15}NO_3$-N inputs in this study, it would be equivalent to 7.3-9.6 kg NO_3-N denitrified ha^{-1}, which is within observed denitrification rates in herbaceous PVB during summer months (Hefting et al., 2003, 2004) as well as other PVB systems (e.g. Hanson et al., 1994b).

Recovery of ^{15}N in salt-extractable N and microbial biomass were not determined. Concentrations of extractable N measured in the cores at 7 indicate that this pool could have accounted for as much as 30-100% of the unrecovered $^{15}NO_3$-N at this time (data not shown). However, mineral and extractable organic N forms represent a very short-term sink for N inputs, and any ^{15}N recovered in these pools would be expected to enter another sink or be lost from the system on a short time scale. Microbial biomass has been shown to not function as a major sink for N inputs in PVB (Hanson et al., 1994a).

The results from the DEA showed that, despite substantially lower NO_3 concentrations, greater mean denitrification enzyme potentials were observed in PVB soils than row crop soils in each watershed, indicating that greater percentages of soil NO_3 are denitrified in PVB

than row crop soils. Greater denitrification enzyme potential corresponded to greater DOC concentrations, as well as slightly but consistently greater WFPS in PVB than row crop soils. Given that WFPS were high (>0.70 cm^3 cm^{-3}) in both vegetation types at the time of soil collection, greater availability of DOC substrate for denitrification more likely accounted for the difference in denitrification enzyme potential. Data from the DEA procedure cannot be used to quantify denitrification rates in the field, and, in this study, were only collected during one part of the season. Thus, further research is needed to verify the role of denitrification in decreasing NO_3 loss in subsurface flow from these watersheds. Additionally, since denitrification can produce nitrous oxide (N_2O), denitrification in PVB can potentially change an aquatic pollutant to an air pollutant (Verhoeven et al., 2006; Stevens and Quinton, 2009). Further research is needed to determine the composition of gaseous N (N_2O / N_2) produced by denitrification in these PVB.

Conclusion

These results indicate that denitrification is the major sink for NO_3-N in PVB. While NO_3-N inputs are retained in SOM and plant biomass, the magnitudes of these sinks are small. Since denitrification can remove NO_3 from the soil indefinitely under favorable conditions, these results indicate that initial reductions in subsurface NO_3 losses observed in these watersheds will likely continue in the long term. Future research is needed to determine the nature of gaseous N products and whether or not similar results would be observed in comparable sites with varying hydrological and topographic features.

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Table 2.1. Soil NO_3-N concentrations by watershed and vegetation treatment immediately before and immediately following isotope addition. Values in parentheses are standard deviations of 3 replicates.

Watershed	Vegetation Treatment	mg NO_3-N kg^{-1} soil (before)	mg NO_3-N kg^{-1} soil (after)
1	Vegetated	0.69 (0.32)	3.45 (0.32)
	De-vegetated	2.83 (1.75)	4.58 (1.75)
2	Vegetated	0.45 (0.20)	3.33 (0.20)
	De-vegetated	1.94 (2.57)	4.82 (2.57)
3	Vegetated	0.38 (0.25)	3.25 (0.25)
	De-vegetated	3.99 (4.26)	6.86 (4.26)

Table 2.2. Soil NH_4-N and NO_3-N concentrations by watershed and vegetation treatment 7 and 137 days after isotope addition. Values in parentheses are standard deviations of 3 replicates.

Watershed	Vegetation Treatment	mg NH_4-N kg^{-1} soil		mg NO_3-N kg^{-1} soil	
		7 d	137 d	7 d	137 d
1	Vegetated	1.10 (0.09)	2.27 (0.56)	0.56 (0.40)	0.49 (0.51)
	De-vegetated	1.89 (0.99)	2.99 (0.56)	1.83 (1.70)	4.10 (1.47)
2	Vegetated	0.76 (0.65)	1.34 (0.99)	0.61 (0.40)	2.68 (3.03)
	De-vegetated	2.31 (1.72)	3.95 (1.78)	1.31 (0.62)	5.22 (1.59)
3	Vegetated	0.78 (0.68)	5.45 (0.23)	0.51 (0.25)	0.97 (0.80)
	De-vegetated	2.43 (1.46)	5.55 (1.61)	2.27 (1.74)	2.33 (2.33)

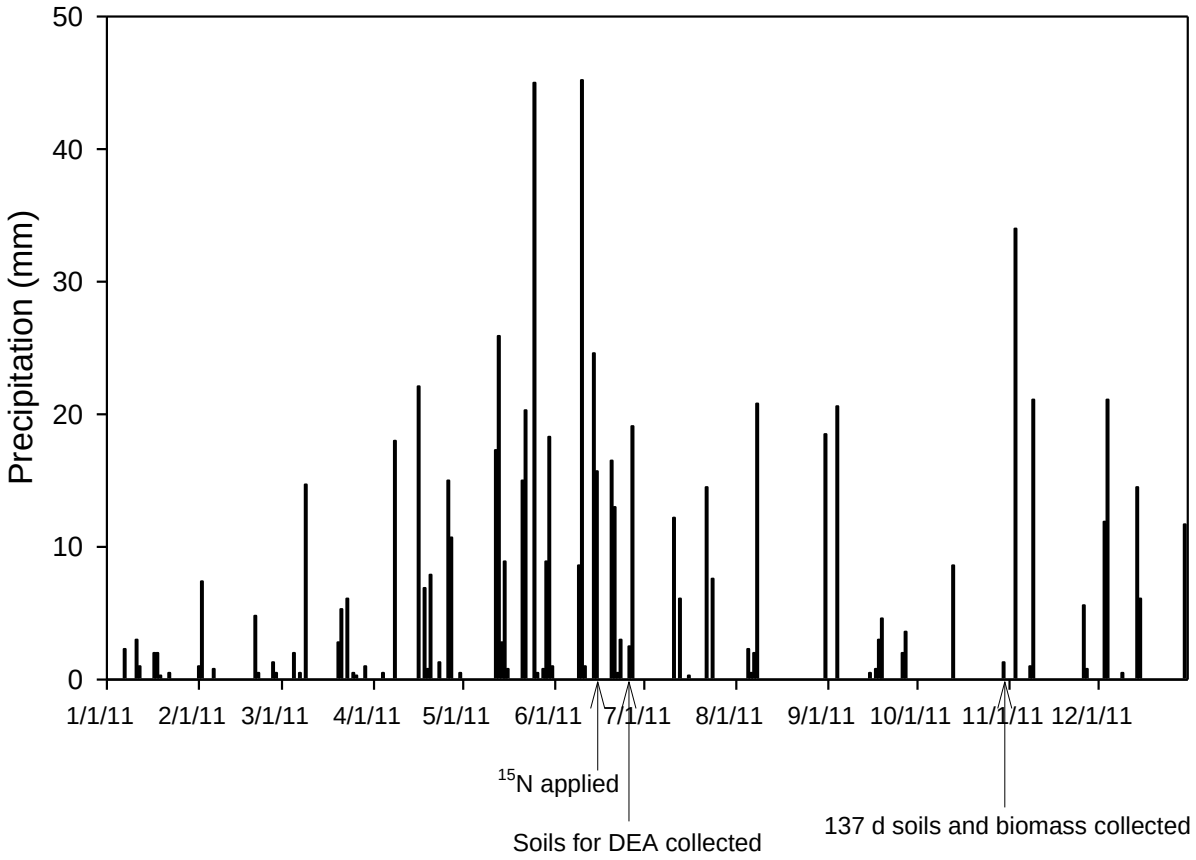

Figure 2.1. Precipitation at the research site in 2011, showing dates for ^{15}N application (June 15), collection of soils for DEA (June 25), and final collection of soils and biomass in the cores (October 29 and 31).

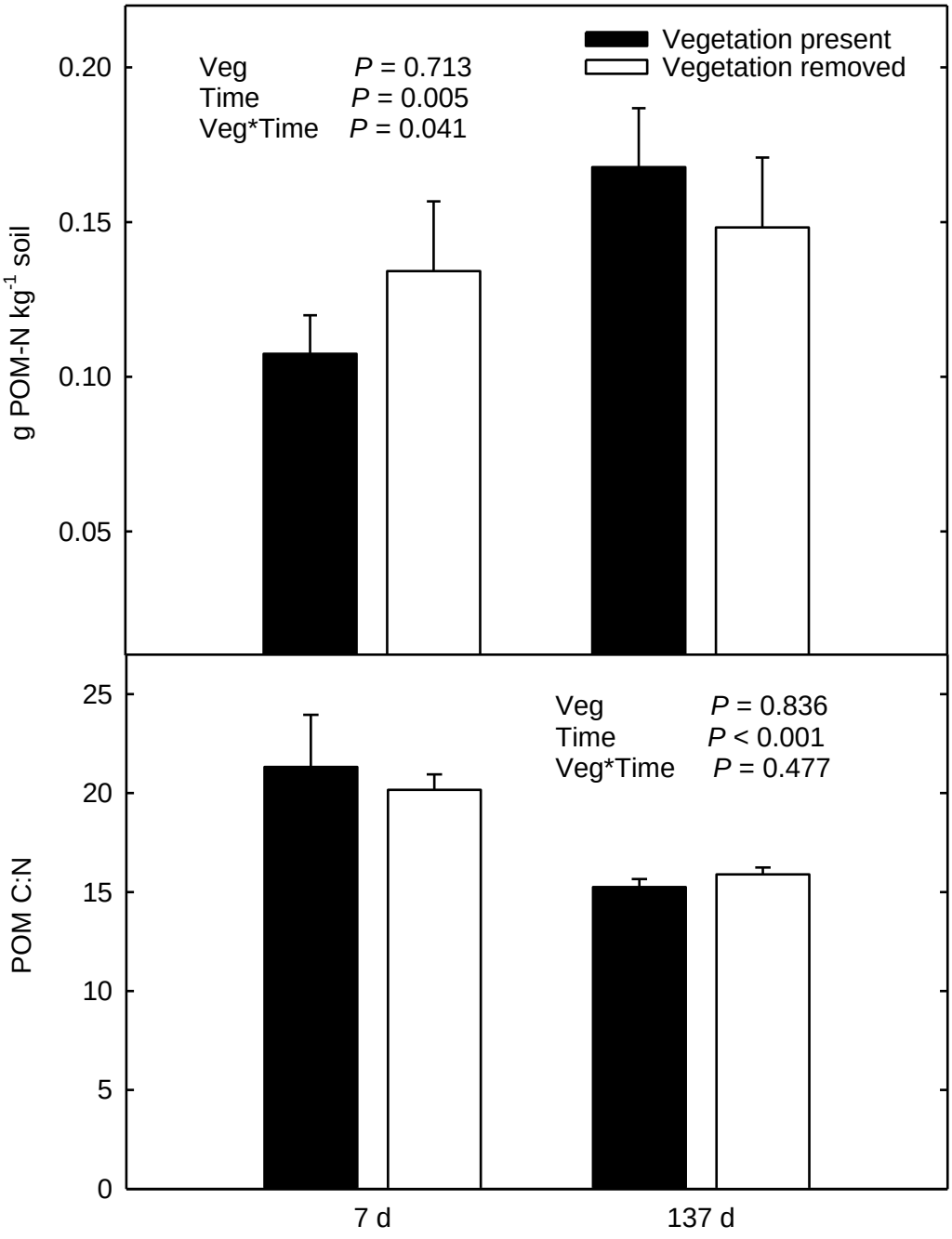

Figure 2.2. Soil particulate organic matter nitrogen (POM-N) concentrations and carbon: nitrogen ratio (POM C:N) by vegetation treatment and time after isotope addition (7 and 137 d), with analysis of variance results. Error bars show standard error of means of 9 replicates.

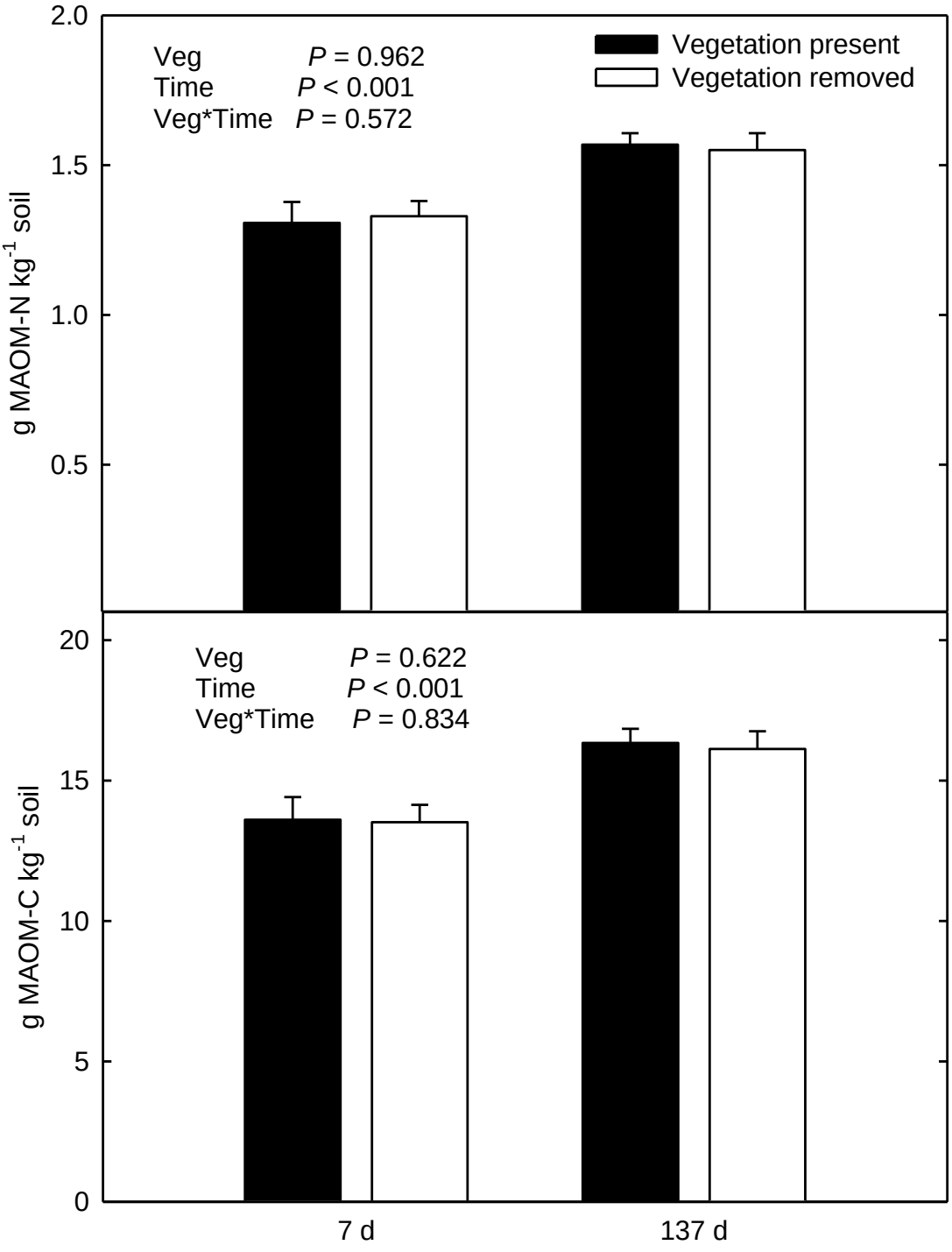

Figure 2.3. Soil mineral-associated organic matter nitrogen (MAOM-N) and carbon (MAOM-C) concentrations by vegetation treatment and time after isotope addition (7 and 137 d), with analysis of variance results. Error bars show standard error of means of 9 replicates.

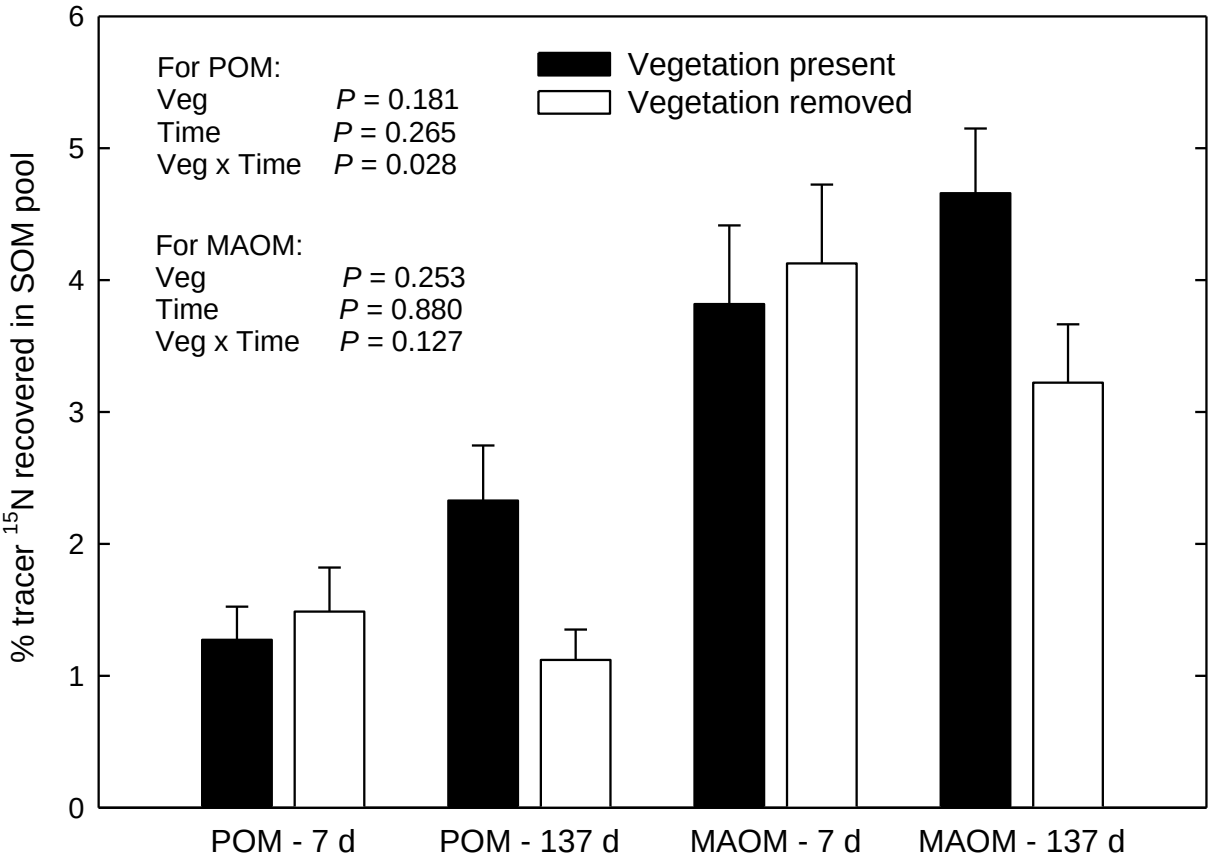

Figure 2.4. Percent recovery of ^{15}N tracer in particulate organic matter (POM) and mineral-associated organic matter (MAOM) by vegetation treatment and time after isotope addition (7 and 137 d), with analysis of variance results shown for POM and MAOM. Error bars show standard errors of means of 9 replicates.

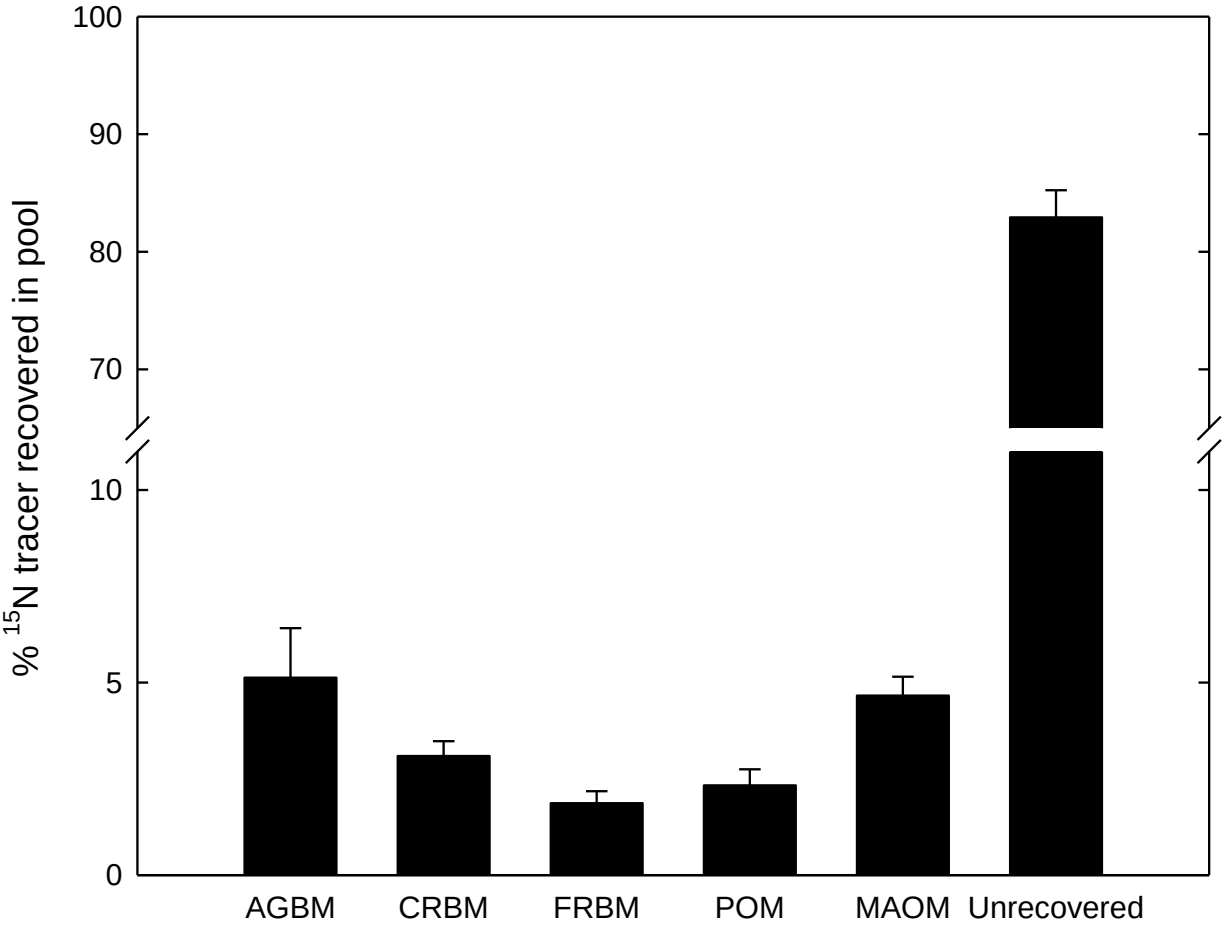

Figure 2.5. Percent of ^{15}N tracer recovered in aboveground biomass (AGBM), course root biomass (CRBM), fine root biomass (FRBM), particulate organic matter (POM), mineral-associated organic matter (MAOM), and unrecovered in vegetated cores 137 days after ^{15}N addition. Note break and change of scale in the y-axis. Error bars show standard errors of means of 9 replicates.

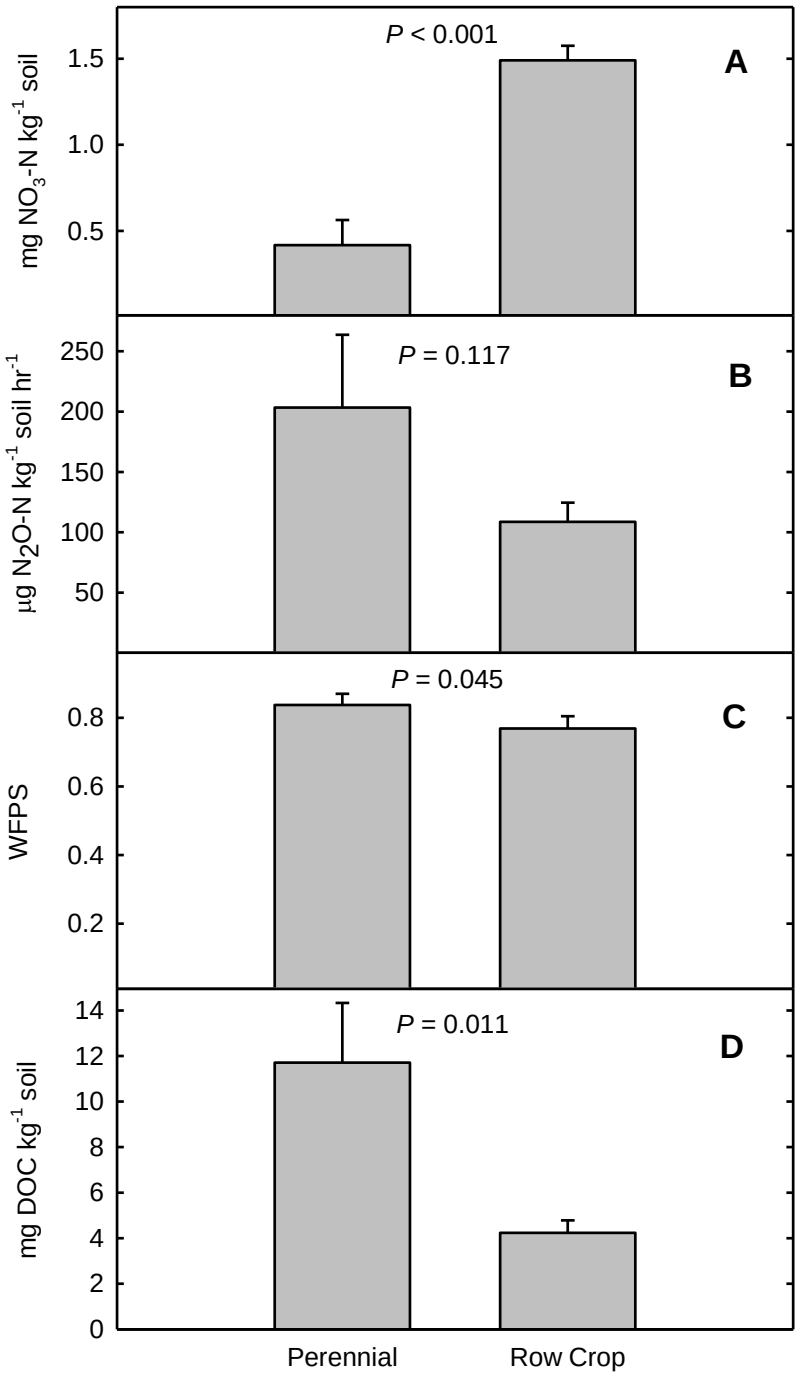

Figure 2.6. Comparison of A) NO_3-N concentrations, B) denitrification enzyme potential, C) water-filled pore space (WFPS), and D) dissolved organic carbon (DOC) concentrations in soils from equivalent topographic positions in perennial vegetation buffers and row crops (soybean) collected 25 June 2011. Analysis of variance p-values are for comparisons between vegetation types. Error bars show standard errors of means of 9 replicates.

CHAPTER 3. COVER CROP EFFECTS ON NITROUS OXIDE EMISSIONS FROM A MAIZE-BASED CROPPING SYSTEM: INTERACTION OF CARBON AND NITROGEN INPUTS

A paper submitted to the journal *Soil Biology and Biochemistry*

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Abstract

Nitrous oxide (N_2O) emissions from cultivated soils often increase with N fertilizer inputs and soil nitrate (NO_3) concentrations. The growth of cover crops from fall to spring can reduce soil NO_3 concentrations and has been promoted as a strategy to decrease N_2O emissions. However, mineralizable carbon (C) availability can be a more important control on N_2O emissions than NO_3 in inorganic N-rich soils, and cover crop residue provides a mineralizable C input. We measured the effect of a winter rye (*Secale cereale* L.) cover crop on soil N_2O emissions from a maize (*Zea mays* L.)-based cropping system treated with three N fertilizer rates (0, 135, and 225 kg N ha^{-1}) at a site in Iowa, USA. In addition, we conducted a laboratory incubation to determine if N_2O emissions from soils from these treatments were limited by mineralizable C or NO_3. The rye cover crop decreased soil NO_3 concentrations at all N rates. Although the cover crop decreased N_2O emissions at 0 kg fertilizer N ha^{-1}, it increased N_2O emissions from fertilized treatments. Results from

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laboratory incubations were consistent with field data; N_2O emissions from fertilized treatments did not increase with added NO_3, but did increase with added glucose. These results indicate that mineralizable C inputs can limit N_2O emissions and that C inputs from the rye cover crop likely increased N_2O emissions from fertilized soils. Mineralizable C availability should be considered in future evaluations of cover crop effects on N_2O emissions, particularly as cover crops are evaluated as a potential strategy to mitigate agricultural N_2O emissions.

Keywords: Nitrous oxide, cover crops, nitrate, mineralizable carbon, denitrification

Introduction

Atmospheric concentration of the potent greenhouse gas nitrous oxide (N_2O) has increased 40-50% since pre-industrial times as a consequence of human activity (Smith et al., 2007). Agricultural soils are the largest anthropogenic source of N_2O (Reay et al., 2012). The microbial processes nitrification and denitrification, as well as abiotic processes, produce N_2O in soils (Bremner, 1997). In cultivated soils, denitrification tends to be the dominant process producing N_2O (Ostrom et al., 2010). In denitrification, NO_3 is reduced to N_2O during organic carbon (C) oxidation in the absence of oxygen (O_2). Nitrous oxide can be further reduced to N_2 before diffusing from the soil; however, high NO_3 availability inhibits N_2O reduction (Blackmer and Bremner, 1978; Firestone and Davidson, 1989). Thus, emission of denitrification-derived N_2O is controlled by anaerobicity, the availability NO_3

and mineralizable C substrates, and the amount of N_2O reduced to N_2 before leaving the soil (Firestone and Davidson, 1989).

Laboratory studies of denitrification (Myrold and Tiedje, 1985; Lalisse-Grundmann et al., 1988) and field studies of N_2O emissions (Sehy et al., 2003; van Groenigen et al., 2004; Adviento-Borbe et al., 2007) have shown that these processes are not necessarily limited by NO_3 concentration in fertilized soils. In soils with high NO_3 concentrations and low O_2 availability, mineralizable C availability can control denitrification (Burford and Bremner, 1975; Bijay-Singh et al., 1988) and mineralizable C availability has been shown to limit N_2O emission from NO_3-rich (30 - 100 mg NO_3-N kg^{-1} soil) cultivated soils (e.g. Weier et al., 1993; McKenney et al., 1995; Gillam et al., 2008), even with high soil organic C content (Sainz Rozas et al., 2001; Sánchez-Martín et al., 2008).

Overwintering non-legume cover crops can decrease NO_3 concentrations in agricultural soils through N uptake during growth and immobilization during residue decomposition (Thorup-Kristensen, 2003). By decreasing the soil NO_3 pool, these cover crops can also decrease soil N_2O emissions (Baggs et al., 2000). However, some studies have found that cover crops increase (Petersen et al., 2011) or have no consistent effect on N_2O emissions (e.g. Jarecki et al., 2009; Smith et al., 2011). When cover crops are killed shortly before N application, mineralizable C from their residue may stimulate denitrification and N_2O emissions (Sarkodie-Addo et al., 2003; Petersen et al., 2011).

Together, inconsistent effects of cover crops on N_2O emissions and evidence for C limitation of agricultural N_2O emissions indicate that cover crop effects on the availability of C as well as NO_3 can affect N_2O emissions. An improved understanding of the relationship between cover crops and N_2O emissions is particularly important as cover crops are increasingly promoted as a greenhouse gas mitigation strategy (Eagle and Olander, 2012). The goal of this study was to determine how cover crop C inputs interact with soil NO_3 to affect N_2O emissions in a maize-soybean [*Glycine max* (L.) Merr.] crop rotation in Iowa, USA. We used a coupled field and laboratory experimental approach. In the field, we measured the effects of three N fertilizer rates and a rye cover crop on soil N_2O emissions in the maize phase of the rotation. In the laboratory, we incubated soils from the field experiment with glucose and NO_3 additions to determine biochemical limitations on N_2O emissions as related to field observations.

Methods

Field Study

A field study was conducted at the Iowa State University Agricultural Engineering and Agronomy Ames Research Farm in Boone County, Iowa, USA (42.02N, 93.77W). Long-term mean annual temperature and precipitation at this location are 9.4 °C and 872 mm yr^{-1}. The soil at this site is Clarion loam series (fine-loamy, mixed, superactive, mesic Typic Hapludolls), with pH 6.4 in water (1:1 soil: water ratio), 2.4% total C, and 0.2% total N. The site was managed as a no-till maize-soybean rotation and treated in a split-plot design with

four replicates. Presence or absence of a winter rye cover crop was the main plot and N fertilizer rate for maize was the sub-plot. Of the six N rates at this site, those included in this study were 0, 135, and 225 kg N ha^{-1} (hereafter N0, N135, and N225). No N was applied to soybean. Cover crop treatments were begun in fall 2008 and N rate treatments in spring 2009. Individual plot size was 6.1 m (8 maize rows) × 15.2 m length. The experimental site for this study was primarily established to better determine the economical optimum N rate (EONR) for maize following soybean with and without a rye cover crop. The long-term optimal N rate for this rotation in Iowa is 152 kg N ha^{-1} at a fertilizer to grain price ratio of 0.1, according to the Corn Nitrogen Rate Calculator (Iowa State Univ. Agronomy Extension, 2012), a widely-used resource for N fertilizer recommendations in the USA (Sawyer et al., 2006; Robertson and Vitousek, 2009).

Rye was drill-seeded after soybean harvest on 5 Oct 2010 at 70 kg seed ha^{-1} and killed with glyphosate [*N*-(phosphonomethyl)glycine] on 2 May 2011. Rye aboveground biomass was sampled before glyphosate application using a 0.09 m^2 frame at six random locations per replicate. Since soil NO_3 concentrations following 2010 soybean harvest did not differ between N rates, rye biomass was pooled from all N rates per replicate. Samples were dried at 60 ºC, ground to pass a 2 mm sieve, and sub-sampled for total C analysis by dry combustion (LECO CHN-2000 analyzer, LECO Corp., St. Joseph, MI, USA). Maize was planted on 10 May 2011 at 76-cm row spacing with the planter equipped with residue cleaners and no-till coulters. The N fertilizer was urea ammonium nitrate (UAN, 32% N), side-dressed in bands to 15 cm depth between every other crop row on 19 May 2011. Nitrogen fertilizer was therefore highly concentrated in a small soil zone, as is typical for

injected N applied to maize. Maize grain was machine harvested on 5 Oct 2011 from the center four rows of each plot, with yield adjusted to 155 g kg^{-1} moisture.

Soil surface N_2O fluxes were measured approximately fortnightly from 11 Apr to 3 Oct 2011 using the static chamber method (Parkin and Venterea, 2010). Polyvinyl chloride (PVC) rings (25 cm diameter x 10 cm height) were placed in the plots to 5 cm depth. Two rings were installed in each plot in the following configuration: one ring directly over the N fertilizer band, the second ring overlapping the adjacent crop row and the next inter-row space (which received no N fertilizer). A vented PVC lid (25 cm diameter x 5 cm height) was used for a total chamber volume of 0.0049 m^3. Change in N_2O concentration inside the chambers was analyzed *in situ* with a 1412 Infrared Photoacoustic Gas Monitoring System (Innova Air Tech Instruments, Ballerup, Denmark) (Iqbal et al., 2012). Fluxes were measured between 08:00 and 14:00 hrs to obtain flux rates proximate to the 24 hr period. Fluxes were measured in all 6 treatments in one replicate each day of measurement, with the treatment order randomized each day. During each measurement, N_2O concentration in the chamber was measured every 2 min over 14 min for a total of eight measurements including time zero. All eight measurements were used to calculate flux rates with a linear model. Flux rates with R^2 <0.65 were considered zero. Flux rates from the two rings were weighted proportional to the area of the plot they represented. Cumulative N_2O emissions from the period of measurement were calculated by linear interpolation and numerical integration between sample times.

During each gas flux measurement, soil temperature at 5 cm depth was measured with a thermometer and volumetric water content (VWC) to 5 cm was measured with a TH300 theta probe (Dynamax Inc., Houston, TX, USA). Since soil bulk density did not vary with time or treatment over the period of measurement, VWC data were converted to water-filled pore space (WFPS) using the average bulk density (1.09 g cm^{-3}) for the site. One soil sample (2 cm diameter to 10 cm depth) was collected during each gas flux measurement corresponding to each gas flux ring location. A soil subsample was extracted in 2 *M* KCl (5:1 solution: soil ratio) by shaking for 1 hr at 180 rpm. Extracts were filtered through pre-leached Whatman 1 filter paper and frozen until analysis. Extract (NO_3 + NO_2) (hereafter NO_3) and NH_4 concentrations were measured in microplates using the Griess-Ilosvay reaction with VCl_3 as a reducing agent and the Berthelot reaction, respectively (Hood-Nowotny et al., 2010). Soil mineral N concentrations corresponding to the two gas flux rings were weighted proportional to the area of the plot they represented.

Incubation Study

Soil cores (2 cm diameter to 10 cm depth) were collected from the N fertilizer bands in all field treatments between 13 and 16 June 2011, during a period of high N_2O flux rates. Soils were air-dried (~25 °C) upon returning to the lab and sieved to 4 mm. Initial NO_3 and NH_4 concentrations were determined in a subsample of air-dried soil using the methods described above.

Subsamples of 15 g air-dried soil from each field treatment were placed in 120 mL bottles. In a pilot study, soil water content of 90% water-holding capacity (WHC), or ~0.80 cm^3 cm^{-3} WFPS, was determined to maximize N_2O emissions from these soils (data not shown). Soils were brought to this water content with one of 4 treatment solutions: de-ionized water, 1 m*M* KNO_3 in water, 1 m*M* glucose ($C_6H_{12}O_6$) in water, or 1 m*M* of both KNO_3 and glucose in water. Concentrations of KNO_3 and glucose in treatment solutions were based on the denitrification enzyme assay protocol (Tiedje 1994). Treatment solutions were applied factorially to soils from each field treatment from each replicate. Soils were then incubated in the dark at ~22 °C with bottles open to laboratory air. Soils were maintained at 90% WHC by periodic re-application of the treatment solutions, though initial application accounted for 91% of water, NO_3, and glucose added. Total additions over the 10 day incubation were 7.8 mg NO_3-N and 40.3 mg glucose-C kg^{-1} soil.

Nitrous oxide emissions from the incubating soils were measured 1, 4, and 10 d after initial treatment solution application. Air samples were collected from the bottle headspaces at 0 and 30 min after sealing the bottles with rubber septa and stored in pre-evacuated Exetainer® vials (Labco Ltd., Lampeter, Ceredigion, UK). An equal volume of air was added to each bottle immediately after removing the 0 min samples to maintain headspace pressure. Concentrations of N_2O in the vials were measured with a gas chromatograph (Agilent 7890, Santa Clara, CA, USA) operated with an electron capture detector at 350°C. Gas species separation was accomplished with stainless steel columns packed with Porapak Q, 80/100 mesh and maintained at 85°C. Carrier gas was 10% CH_4 and 90% Ar. Carbon dioxide production in the incubating soils was measured more frequently during the 10 day

incubation using a LI-7000 infrared gas analyzer (LI-COR, Omaha, NE, USA). Cumulative N_2O and CO_2 emissions were calculated by linear interpolation and numerical integration between sample times.

Statistical Analyses

All cumulative N_2O emission and soil NO_3 and NH_4 concentration data were log-transformed before analysis to meet the assumptions of normality and homogeneity of residuals. Soil temperature, moisture, and NO_3 and NH_4 concentration data from the field study were analyzed as repeated measures. All data were analyzed using PROC GLM in Statistical Analysis Software package (SAS Institute Inc., Cary, NC, USA).

Results

Field Study

Soil temperatures were highest in N0 but did not vary between the other N rates or cover crop treatments. Water-filled pore space did not differ between cover crop treatments or N rates. The rye cover crop had no effect on soil NH_4 concentrations (data not shown) but decreased NO_3 concentrations at all three N rates (Figure 1). Soil NO_3 concentrations were greater at N225 than N135 (Figure 1). Rye produced 160-345 kg C ha^{-1} in aboveground biomass. Maize yields were greater at N225 than N135 in both cover crop treatments (Table 1).

Cover crop and N rate treatments interacted to affect N_2O emissions (Figure 2). The rye cover crop decreased cumulative N_2O emissions at N0, increased emissions at N135, and had no effect at N225. Without the cover crop, N_2O emissions were greater at N225 than N135. However, with the cover crop, emissions did not differ between these N rates. The fertilizer bands were the dominant source of N_2O from the fertilized treatments at this site despite the small portion of the plot area they covered. On average, fertilizer bands accounted for 18, 65, and 70% of total plot N_2O emissions at N0, N135, and N225, respectively.

Incubation

Initial NO_3 concentrations were lower in soils with the cover crop than without cover crop across all N fertilizer rates (Figure 3). Initial NO_3 in incubated soils were greater in soils from N225 than N135 and lowest in soils from N0 (Figure 3). Across NO_3 addition and glucose addition treatments, cumulative CO_2 emission from incubating soils did not differ between N fertilizer rate and cover crop treatments. Average CO_2 emissions from incubating soils over 10 days were 222.3 mg CO_2-C kg^{-1} soil from soils with glucose added and 195.0 mg CO_2-C kg^{-1} soil from soils without glucose added. In contrast, N fertilizer rate (field treatment) had a main effect on cumulative N_2O emissions from the incubated soils (Table 2). Cover crop and NO_3 addition treatments did not have main effects on N_2O emissions from incubating soils; however, there was an interaction between N fertilizer rate and NO_3 addition (Table 2). Across cover crop and glucose addition treatments, NO_3 addition increased N_2O emission from N0 soils but not N135 and N225 soils (Figure 4). In contrast,

there was a main effect of glucose addition on N_2O emission, as well as an interaction between cover crop, N fertilizer rate, and glucose addition treatments (Table 2, Figure 5). In soils from N0, glucose addition increased cumulative N_2O emission only in soils without cover crop. In soils from N135, glucose addition increased N_2O emissions in soils from both cover crop treatments. In soils from N225, glucose addition increased N_2O emissions in soils with cover crop but not without cover crop (Figure 5).

Discussion

Field N_2O Emissions

Nitrogen fertilizer is a major control on N_2O emissions from cultivated soils (Stehfest and Bouwman, 2006). Emissions of N_2O have been found to increase with N fertilizer rate both linearly (e.g. Mosier et al., 2006; Smith et al., 2011) and non-linearly (e.g. McSwiney and Robertson, 2005; Stehfest and Bouwman, 2006). However, other studies have not found consistent increases in N_2O emissions with N fertilizer rate (e.g. Sehy et al., 2003; van Groenigen et al., 2004; Adviento-Borbe et al., 2007). In this study, N_2O emissions were greater in fertilized treatments than N0, but the relationship between N_2O emission and N rate was not consistent between cover crop treatments. N_2O emissions were greater at N225 than N135 without the rye cover crop, but did not differ with the rye cover crop, despite greater soil NO_3 concentrations in N225 than N135 across cover crop treatments (Figure 1 and 2).

Since non-legume cover crops can decrease soil NO_3 concentrations though plant uptake and microbial immobilization during residue decomposition, they have potential to decrease N_2O emissions (Baggs et al., 2000; McSwiney et al., 2010). However, the rye cover crop in this study only decreased N_2O emissions at N0, despite decreasing soil NO_3 concentrations at all N rates (Figure 1 and 2). In contrast, the cover crop in this study increased N_2O emissions at N135. Although the cover crop had no consistent effect on N_2O emissions at N225, variability at N225 with cover crop was exceptionally high (Figure 2). These results indicate that N_2O emissions were not limited by soil NO_3 concentration in the fertilized treatments, even with a decreased NO_3 pool with the cover crop. Since soil moisture and temperature did not differ between cover crop treatments, the increase in N_2O emission with the rye cover crop at N135 cannot be explained by cover crop effects on soil temperature, moisture, or mineral N concentrations.

Limitation of N_2O emission by available C may partially explain the inconsistent relationships between N_2O emissions, N fertilizer rate, and mineral N concentrations. Cover crop residue inputs to NO_3-rich soils provide mineralizable C substrate that can stimulate N_2O production (McKenney et al., 1995), especially when the cover crop kill coincides with N fertilizer application (Sarkodie-Addo et al., 2003; Petersen et al., 2011). In this study, the rye cover crop provided 160-345 kg C ha^{-1} in aboveground residue, in addition to root residue (not quantified in this study), 17 days before N application. A portion of this C was likely still available for microbial mineralization following N application. Thus, C inputs from cover crop residue may have influenced the increased N_2O emissions with the cover crop at N135.

Incubation Treatments and Limitation on N_2O Emission

In the incubation, N_2O emission was limited by NO_3 availability in soils from N0 and by mineralizable C availability in soils from N135 and N225. This result agrees with previous studies that have demonstrated that denitrification and N_2O emission are limited by NO_3 availability in low-NO_3 soils and by mineralizable C availability in high-NO_3 soils (Weier et al., 1993; Gillam et al., 2008). Although initial NO_3 concentrations were lower in fertilized soils with the rye cover crop than without, NO_3 addition to these soils during incubation did not increase N_2O emissions (Table 2, Figure 3 and 4). In contrast, glucose addition did increase N_2O emissions from fertilized soils (Table 2, Figure 5), demonstrating that mineralizable C inputs, such as those from cover crop residue, can stimulate N_2O emissions. While C mineralization in the incubating soils did not differ between cover crop treatments, these data may not reflect C availability in the field since soils were air-dried and re-wetted for incubation, which changes C availability (Bijay-Singh et al., 1988). In incubating soils from N225 without cover crop, glucose additions had no effect on N_2O emissions, in contrast to other fertilized treatments (Figure 5). High NO_3 concentrations in soils from this treatment may have inhibited denitrification (Lalisse-Grundmann et al., 1988; Weier et al., 1993).

Since some N_2O produced was likely reduced to N_2 before diffusing from the soil, N_2O emission data did not represent total denitrification in incubating soils. Some studies have found decreased N_2O emissions with glucose additions to soils incubating at high water content, attributed to greater reduction of N_2O to N_2 with increased C availability (e.g. Miller

et al., 2008; Sánchez-Martín et al., 2008; Andersen and Petersen, 2009). In this experiment, substantial reduction of N_2O to N_2 was likely inhibited by the high NO_3 concentrations in the fertilized soils (Blackmer and Bremner, 1978). Because more electrons are accepted by N in the reduction of NO_3 to N_2O (4 electrons) than the reduction of N_2O to N_2 (1 electron), the mass of glucose added may not have provided sufficient demand for electron acceptors to decrease N_2O emissions. In addition to denitrification, nitrification could have produced N_2O in the incubating soils. However, substantial production of N_2O by nitrification was unlikely given the high WFPS at which soils were incubated (Firestone and Davidson, 1989).

Cover Crop Effects on N_2O Emissions

The use of cover crops has been proposed to reduce N_2O emissions from cropping systems, attributed to their ability to reduce mineral N availability for N_2O-producing processes (McSwiney et al., 2010; Eagle and Olander, 2012). However, the effects of cover crops on factors influencing N_2O emissions are complex and interact with climate and management practices. When N fertilizer is broadcast and incorporated, N immobilization by cover crops may reduce mineral N concentrations sufficiently to reduce N_2O emissions. In contrast, in this study, fertilizer was applied in concentrated bands, coincident with high rainfall and soil moisture (data not shown). Due to the high mineral N availability in the bands, N immobilization in cover crop residue may have been insufficient to decrease N_2O emissions (Jarecki et al., 2009). At the same time, given the favorable conditions for denitrification in the bands soon after N application, C substrate provided by cover crop residue likely stimulated N_2O emissions. Though not observed in this study, cover crops can also decrease

soil moisture by evapotranspiration (Thorup-Kristensen et al., 2003), which may decrease N_2O emissions in systems where N_2O emissions are limited by O_2 diffusion.

Given the complex effects of cover crops on the factors influencing N_2O emissions, and the interactions of these factors with climate and management practices, general expectations that cover crops decrease N_2O emissions from agricultural soils (e.g. Eagle and Olander, 2012) may be unfounded. However, N_2O emissions from the soil surface do not represent the total effect of cover crops on N_2O emissions or global warming potential of the cropping system. Non-legume cover crops, such as rye, can decrease NO_3 leaching losses to aquatic systems (Tonitto et al., 2006). Since models of agricultural N_2O emissions estimate a percentage of leached NO_3 to be emitted as N_2O downstream (Smith et al., 2007), cover crops may decrease total N_2O emissions by decreasing NO_3 leaching (Snyder et al., 2009). Cover crops also affect the soil C balance, both by providing an input of C and by affecting crop yield (Thorup-Kristensen et al., 2003; Eagle and Olander, 2012).

Conclusion

Despite reducing soil NO_3 concentrations at a range of N fertilizer rates, a rye cover crop preceding maize only decreased N_2O emissions in unfertilized treatments, but increased N_2O emissions at an N rate close to the economic optimum. The degree of mineral N immobilization by over-wintering cover crops may not be sufficient to reduce soil N_2O emissions, while the input of mineralizable C in cover crop residue following N application may stimulate N_2O emissions. The effects of cover crops on multiple factors controlling N_2O

emissions, including C availability, should be considered in evaluations of their net effect on N_2O emissions from agricultural soils.

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Table 3.1. Maize grain yields (at 155 g kg^{-1} moisture) at the field site harvested 5 Oct 2011.

N rate	No rye cover crop	With rye cover crop
kg N ha^{-1}	- - - - - - - - - - Mg ha^{-1} - - - - - - - - - -	
0	5.18 (0.39)†	3.22 (0.33)
135	9.83 (0.28)	9.63 (0.44)
225	10.51 (0.59)	10.37 (0.86)

† Values in parentheses are standard deviations.

Table 3.2. Analysis of variance of cumulative N_2O produced over 10 days in incubating soils. Cumulative emission data were log-transformed before statistical analysis.

Treatment	*P* > *F*
Cover Crop	0.209
N Fertilizer Rate	0.062
Cover Crop × N Fertilizer Rate	0.136
NO_3 Addition	0.608
Glucose Addition	0.071
Cover Crop × NO_3 Addition	0.662
N Fertilizer Rate × NO_3 Addition	0.008
Cover Crop × N Fertilizer Rate × NO_3 Addition	0.677
Cover Crop × Glucose Addition	0.721
N Fertilizer Rate × Glucose Addition	0.140
Cover Crop × N Fertilizer Rate × Glucose Addition	0.004
NO_3 Addition × Glucose Addition	0.167
Cover Crop × NO_3 Addition × Glucose Addition	0.305
N Fertilizer Rate × NO_3 Addition × Glucose Addition	0.136
Cover Crop × N Fertilizer Rate × NO_3 Addition × Glucose Addition	0.512

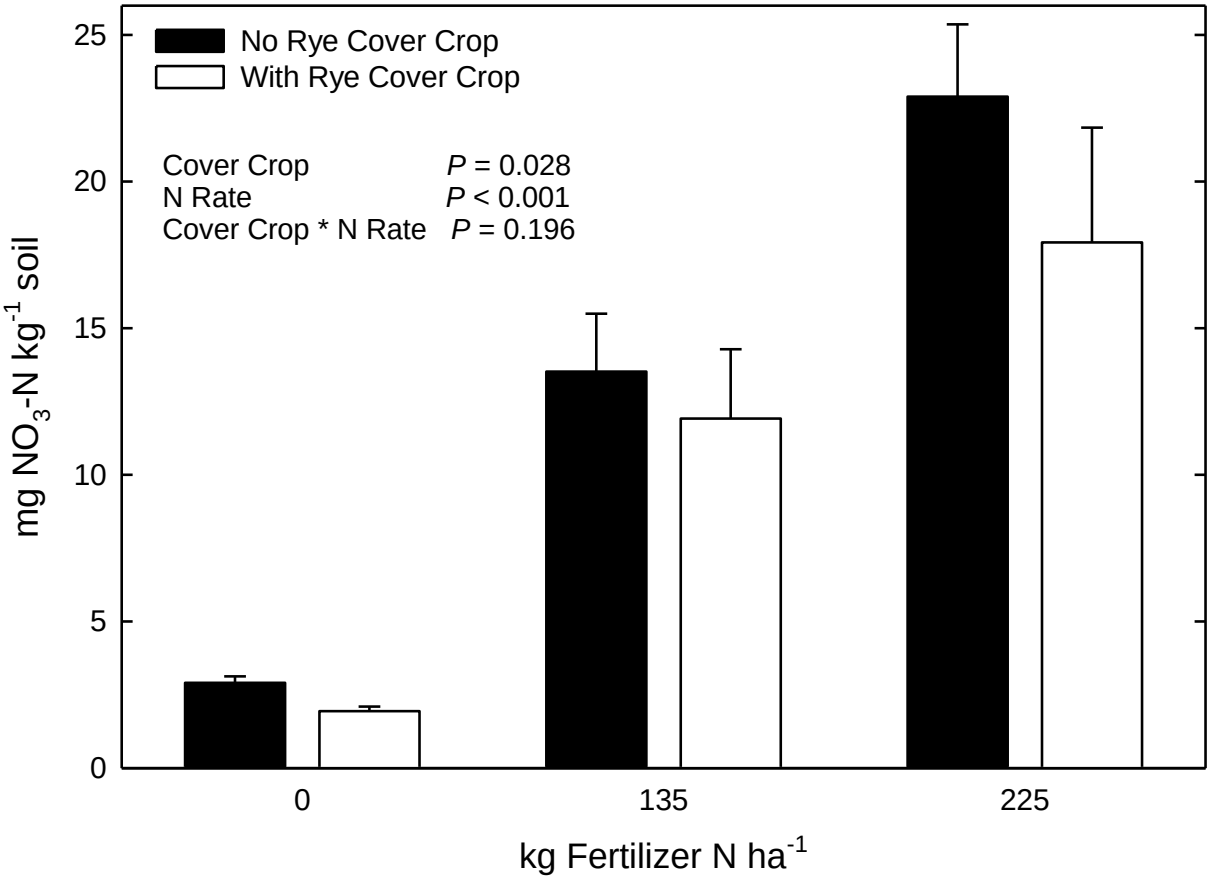

Figure 3.1. Mean soil NO_3-N concentrations over the period of field gas flux measurements (11 Apr - 3 Oct 2011) with one-way analysis of variance results. Data were log-transformed for statistical analysis. Error bars show standard errors of means of 4 replicates.

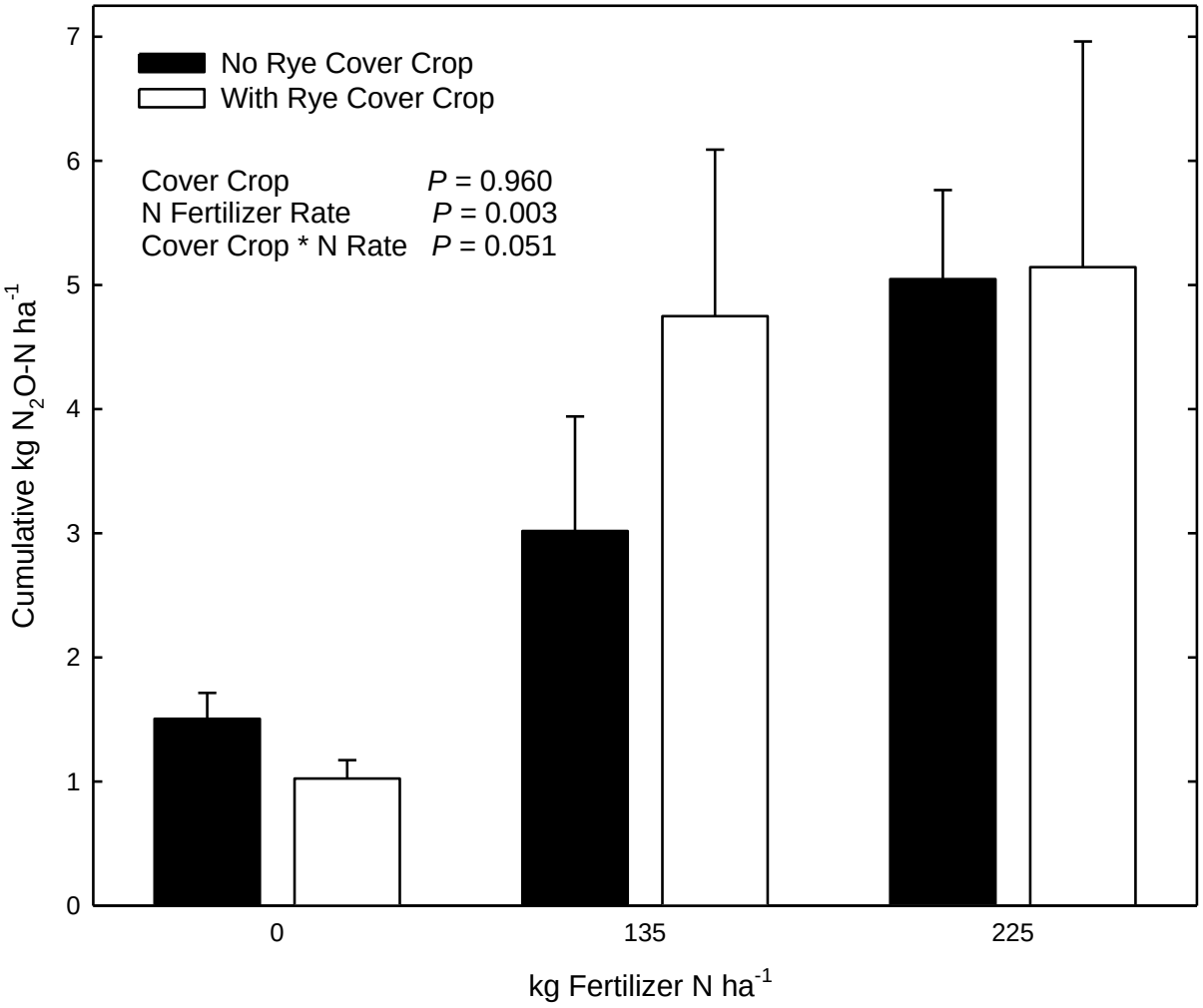

Figure 3.2. Cumulative N_2O-N emissions from the field study (11 Apr - 3 Oct 2011) with one-way analysis of variance results. Data were log-transformed for statistical analysis. Error bars show standard errors of means of 4 replicates.

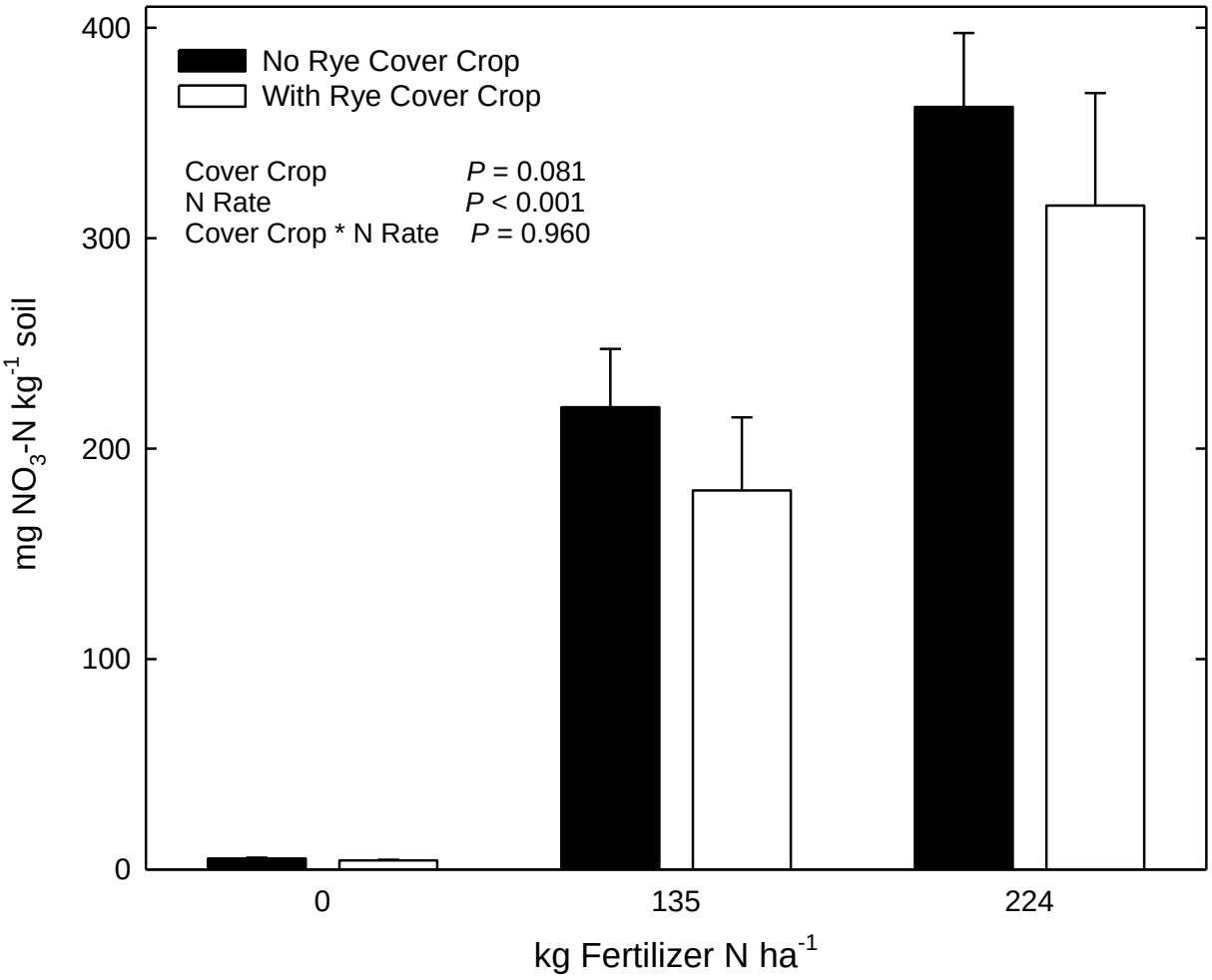

Figure 3.3. Initial NO_3-N concentrations in incubating soils collected from the fertilizer bands of the field site 13-16 June 2011 with one-way analysis of variance results. Data were log-transformed for statistical analysis. Error bars show standard errors of means of 4 replicates.

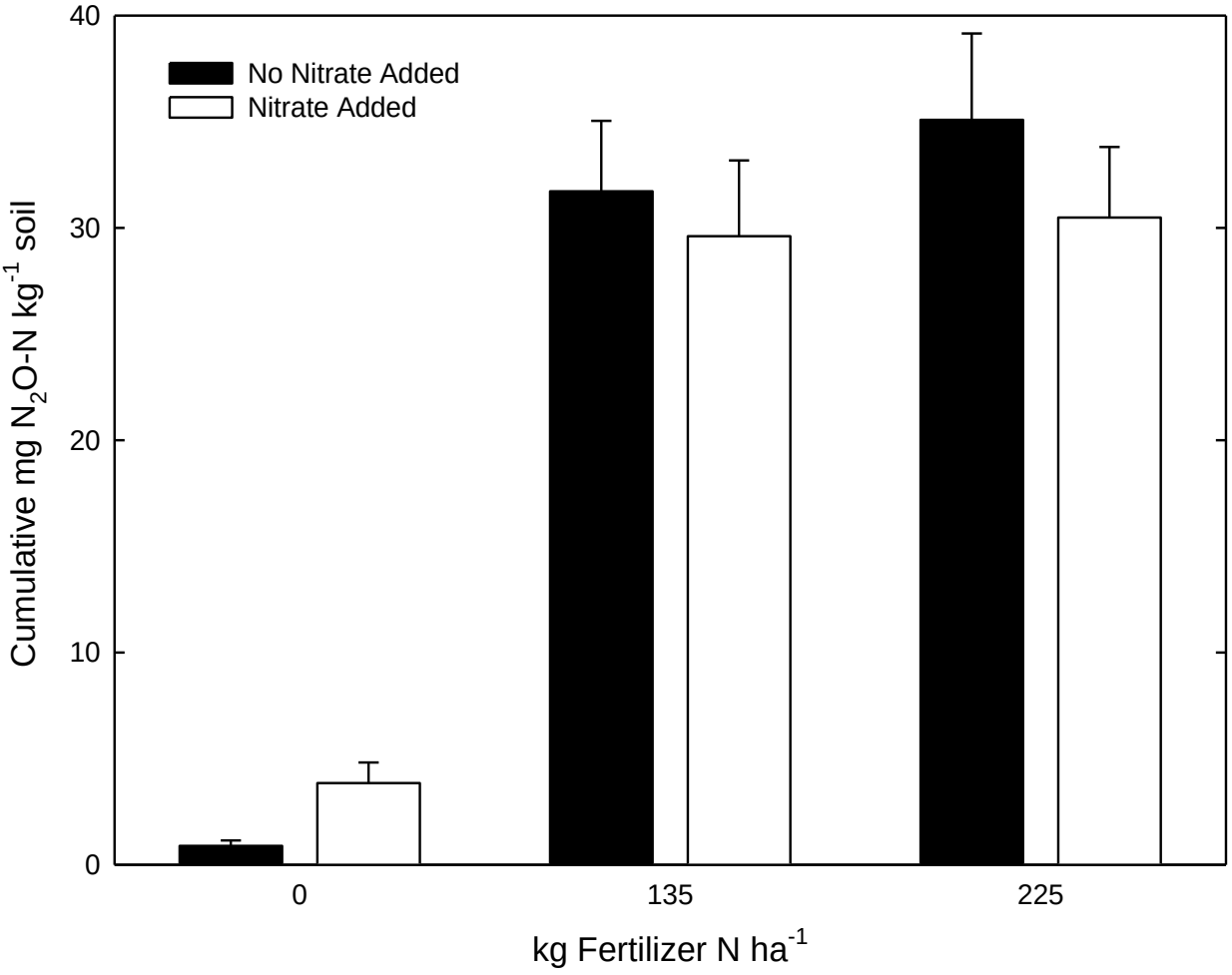

Figure 3.4. Cumulative N_2O-N emissions over 10 days from soils collected from the fertilizer bands of the field site and incubated at 0.8 cm^3 cm^{-3} WFPS, showing the effect of NO_3 addition at each N fertilizer rate across cover crop and glucose addition treatments. Error bars show standard errors of means of 4 replicates.

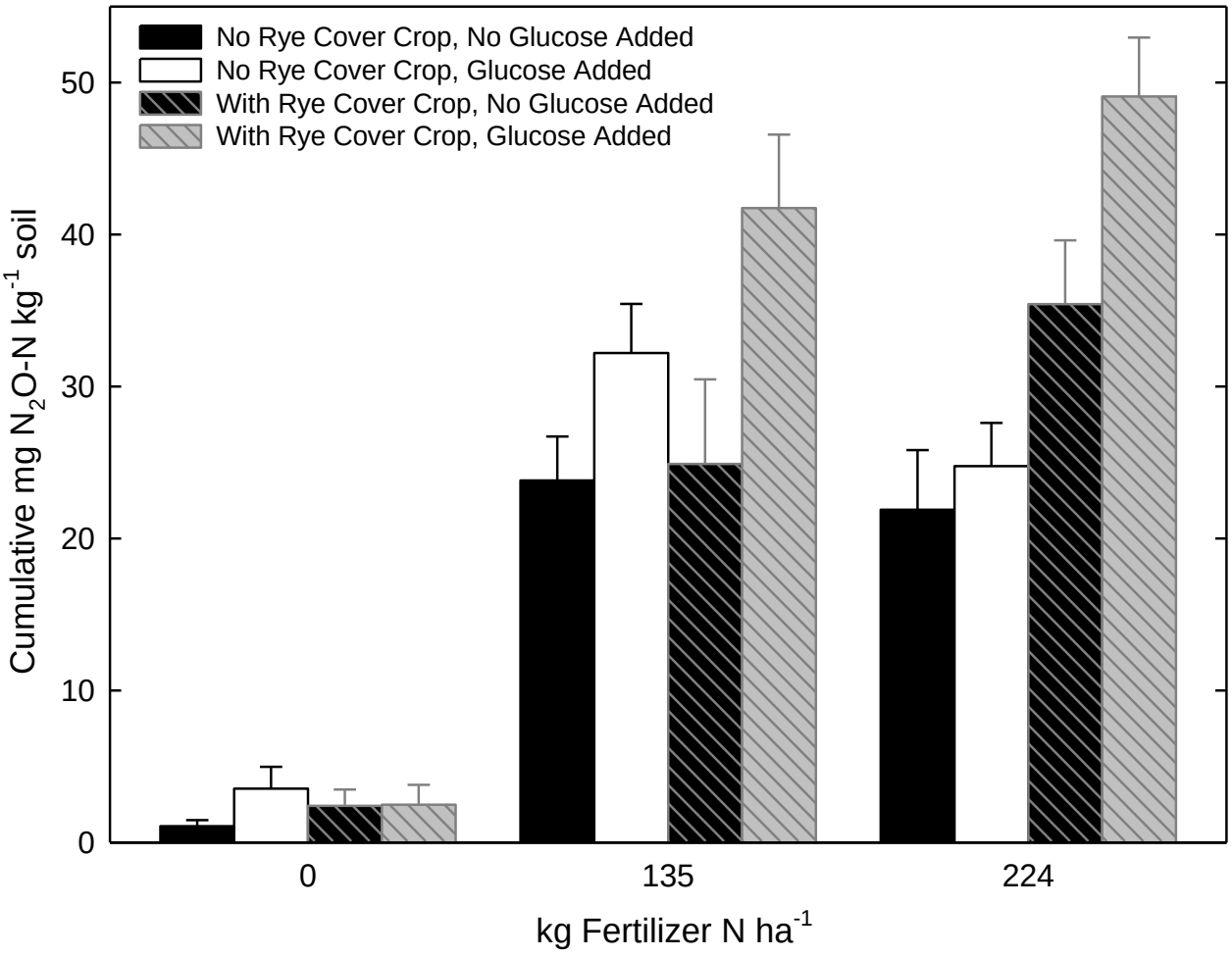

Figure 3.5. Cumulative N_2O-N emissions over 10 days from soils collected from the fertilizer bands of the field site and incubated at 0.8 cm^3 cm^{-3} WFPS, showing the effect of glucose addition at each cover crop × N fertilizer rate combination. Error bars show standard errors of means of 4 replicates.

CHAPTER 4. GENERAL CONCLUSIONS

Since N and C cycles are coupled in agroecosystems, management practices meant to reduce loss of N forms must take into account the central of role of organic C inputs and cycling in agricultural soils. The studies presented in this thesis provide two examples of how soil C inputs influence loss of reactive N forms (NO_3 and N_2O). These studies combine insights from the fields of ecosystem ecology and soil biology and biochemistry and apply these insights to evaluate the impact of agricultural management practices on environmental quality.

In Chapter 2, denitrification was identified as the most important sink for NO_3 inputs into a perennial vegetation buffer (PVB) ecosystem. This result contrasted with the hypothesis, based on preliminary data and previous studies of ecosystem N retention, that soil organic matter would be the most important NO_3 sink. While the literature on NO_3 retention has focused on riparian buffer systems such as wetlands and riparian forests, the site of this study provides a unique opportunity to investigate the ability of PVBs integrated into row crop landscapes to decrease NO_3 losses to streams. Despite the differences in hydrology and ecology between this system and most riparian systems, denitrification has been identified as the principal NO_3 sink in both systems. Because NO_3-N is lost from the ecosystem during denitrification, these results indicate that PVB will remain effective as NO_3 sinks in the long term. These results also indicate that organic C inputs from perennial vegetation are a key factor increasing denitrification in PVB soils compared to row crop soils, leading to decreased NO_3 leaching losses. Further research is needed to determine if these results would

be consistent across varying hydrological and geological conditions, as well as to quantify N_2O emissions produced by denitrification in these systems.

In Chapter 3, an overwintering non-legume cover crop was found to have inconsistent effects on N_2O emissions from the soil surface across a wide range of N fertilizer rates. This study provided an example of a case in which a cover crop increased N_2O emissions from fertilized soil, in contrast to previous studies which have proposed cover crops as a practice to decrease N_2O emissions. Furthermore, in this study, mineralizable C inputs from cover crop residue were identified as a likely factor influencing increased N_2O emissions from fertilized soils. These results agree with many studies showing limitation of N_2O emissions by mineralizable C availability in fertilized soils with high NO_3 availability. Further studies are needed to determine the full effect of cover crops on N_2O emissions to the atmosphere, since decreases in NO_3 leaching with a cover crop may lead to less N_2O emissions downstream. Nevertheless, this study adds to a body of evidence that cover crop effects on N_2O emissions from cropping system soils should not be expected to be consistent across management practices, climatic conditions, and other factors.

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