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Denitrification and Nitrous Oxide Emissions in Annual Croplands, Perennial Grass Buffers, and Restored Perennial Grasslands

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Inclusion of perennial vegetation filter strips (PFSs) in the toeslope of annual cropland watersheds can decrease NO_3^-–N losses to ground and surface waters. Although PFSs are similar to riparian buffers, the processes responsible for NO_3^-–N removal from PFSs are not well understood. Our objectives were to (i) determine the importance of denitrification as a sink for NO_3^-–N loss from PFSs and (ii) evaluate how PFSs alter the biophysical processes that affect the relative importance of N_2O and N_2 emissions. To address our objectives, we used a coupled field laboratory approach with experimental watersheds that included the following treatments: (i) PFSs covering the bottom 10% of the watershed and an annual corn–soybean crop rotation covering the remaining upslope 90% (PFS); (ii) 100% corn–soybean rotation (CORN); and (iii) 19-yr-old 100% restored native grassland (RNG). In situ N_2O flux rates and laboratory $N_2O/(N_2 + N_2O)$ ratios were highest in CORN watersheds followed by PFS and RNG watersheds. In contrast, potentially mineralizable C and denitrification enzyme activity (DEA) were highest in PFS and RNG watersheds and lowest in CORN watersheds. Furthermore, there was a negative correlation between $N_2O/(N_2 + N_2O)$ ratio and DEA. In the laboratory, N_2 fluxes were highest in PFS followed by RNG and CORN. These results indicate that PFS watersheds support greater total denitrification while emitting less N_2O than croplands. Greater potentially mineralizable C in PFS and RNG suggest C availability is an important factor affecting more complete denitrification. These results suggest PFSs function similar to riparian buffers and have potential to reduce NO_3^-–N losses from annual croplands by denitrification to N_2.

Abbreviations: CORN, corn–soybean rotation; DEA, denitrification enzyme activity; DNRA, dissimilatory nitrate reduction to ammonium; NSNWR, Neal Smith National Wildlife Refuge; PFSs, perennial vegetation filter strips; PVC, polyvinyl chloride; RNG, restored native grassland; SOM, soil organic matter; TN, total N; TOC, total soil organic C; VWC, volumetric water content; WFPS, water-filled pore space.

Grassland to cropland conversion has increased N losses to air and water resources. Croplands are a major source of NO_3^-–N flux to rivers and estuaries (Howarth et al., 2012), and agricultural soil management is the leading anthropogenic source of N_2O to the atmosphere (Smith et al., 2007). Many strategies have been developed to mitigate these N losses. Some prominent options include drainage water management (Gilliam et al., 1979), catch (cover) crops (Thorup-Kristensen et al., 2003), and perennial vegetation riparian buffers (Lowrance et al., 1984; Peterjohn and Correll, 1984; Hill, 1996; Hefting et al., 2005).

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A recent variation of perennial vegetation riparian buffers is small-scale incorporation of PFSs in the toeslope portion of cultivated watersheds where the water table approaches the soil surface. This practice has effectively reduced soil erosion and dissolved N losses from upslope croplands (Helmers et al., 2005). For example, Zhou et al. (2010) found a significant decrease in NO_3^-–N concentrations in the vadose zone and groundwater after incorporation of PFSs in corn–soybean cropping systems that are the subject of this study. Others have found that PFSs reduce NO_3^-–N concentrations in surface runoff (Lin et al., 2007; Ryder and Fares, 2008).

Reduced NO_3^-–N concentrations after PFS incorporation into row crop systems can result from a number of processes including microbial N immobilization in soil organic matter (SOM), dissimilatory nitrate reduction to ammonium (DNRA), plant uptake, or denitrification (Matheson et al., 2002; Hefting et al., 2005). Microbial NO_3^-–N immobilization and transfer to SOM is unlikely to be a significant NO_3^-–N sink in cropland riparian buffers (Groffman et al., 1996). In the watersheds that are the subject of this study, less than 10% of isotopically labeled spring-applied $^{15}NO_3^-$–N was recovered in SOM in the subsequent fall (Mitchell et al., 2014). Although DNRA may be an important NO_3^-–N sink (Fazzolari et al., 1998), DNRA represents a very short-term sink because the NH_4^+ product is subject to nitrification. Plant uptake can be a significant NO_3^-–N sink in the riparian zone (Peterjohn and Correll, 1984; Hefting et al., 2005; Harrison et al., 2012), but plants represent a relatively finite N pool that can be rapidly remineralized after senescence (Reddy and Patrick, 1984).

In contrast to these relatively small or short-term NO_3^-–N sinks, denitrification is thought to be the major process responsible for NO_3^-–N removal when plant uptake is low (Haycock and Pinay, 1993). However, denitrification can produce several N gas products including the largely inert gas N_2 as well as the greenhouse gas N_2O. Thus, there is significant concern that riparian buffers and PFSs may increase N_2O losses while decreasing NO_3^-–N losses, thereby trading a water quality problem for a greenhouse gas problem (Prather et al., 1995). Indeed, in a riparian zone, Hefting et al. (2006) found increased N_2O emissions in locations with high NO_3^-–N removal efficiency.

Nevertheless, there are number of factors that affect denitrification rates and N gas product ratios (i.e., N_2O/N_2). Water, through its effect on O_2 diffusion into the soil and N gas diffusion out of the soil, is well known to affect denitrification rate and N_2O/N_2 product ratios. As gas diffusion and O_2 availability decreases, denitrification rate increases and the N_2O/N_2 product ratio decreases (Davidson et al., 2000). The availability of potentially mineralizable C and NO_3^-–N can also affect denitrification rate and N_2O/N_2 product ratio (Firestone and Davidson, 1989). Potentially mineralizable C can promote denitrification by increasing O_2 consumption (anaerobicity) and substrate availability (when C is limiting). Moreover, high potentially mineralizable C/NO_3^-–N ratios favor a low N_2O/N_2 denitrification product ratio (Firestone and Davidson, 1989). Consistent with these concepts, N_2O fluxes typically decrease with increasing soil C/N ratio (Klemedtsson et al., 2005).

Relative to croplands, riparian buffers, and PFSs can alter these biophysical controls on denitrification in many ways. Perennial grasses can affect the water balance by increasing infiltration, soil water holding capacity, and evapotranspiration (Hickman et al., 2010). Perennial grasses can also affect denitrification substrate availability (by both reducing NO_3^-–N and increasing C) and O_2 availability by increasing potentially mineralizable carbon (Pérez-Suárez et al., 2014). Given the many ways in which PFSs can alter biophysical controls on denitrification, as well as the relatively recent implementation of PFSs compared with riparian buffers, there is a critical need for the evaluation of mechanisms responsible for NO_3^-–N removal and N gas production from PFSs.

Using a paired watershed study in Iowa, we investigated the effects of PFSs on NO_3^-–N removal, N_2O emissions, and denitrification. Our study included (i) three watersheds with PFSs covering the bottom 10% of the watershed and corn covering the remaining 90% of the watershed (PFS), (ii) three watersheds with 100% corn (CORN), and (iii) two watersheds with 19-yr-old 100% RNG (Fig. 1). Using comparisons of measurements in perennial filter strip portions of PFS and similar locations in CORN and RNG watersheds, our main objectives were to (i) determine if PFSs are a larger source of N_2O than CORN, (ii) determine if potential denitrification is greater in PFS than CORN, and (iii) quantify the ability of PFS to support complete denitrification of NO_3^-–N to N_2. Preliminary data demonstrated that PFS installation significantly reduced vadose zone and groundwater NO_3^-–N concentrations (Zhou et al., 2010). Accordingly, we hypothesized that total denitrification is greater, but the N_2O/N_2 ratio of denitrification gas products is lower in PFS and RNG watersheds than CORN.

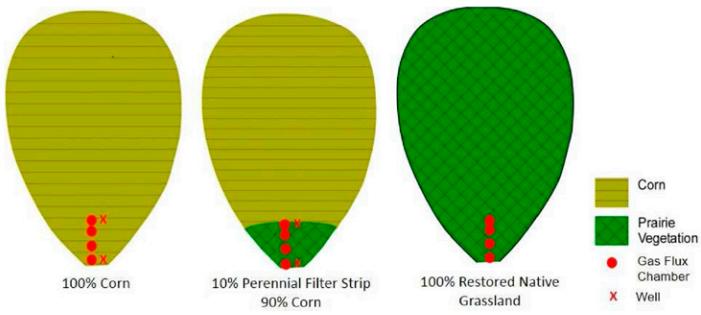

Fig. 1. Conceptual design of watersheds.

MATERIALS AND METHODS

Site Description

Watersheds were located at the Neal Smith National Wildlife Refuge in Jasper County, Iowa (NSNWR) (41°33′ N, 93°16′ W). The refuge includes annual crop production and restored native grassland. The NSNWR is located on the southern Iowa drift plain that contains steep rolling hills of Wisconsin-age loess on pre-Illinoian glacial till (Prior, 1991). Average annual precipitation is 850 mm, most of which occurs in rain events from May to July. Most soils are highly

erodible and classified as Ladoga (Mollic Hapludalf) or Otley (Oxyaquic Argiudolls) soil series with 5 to 14% slopes (Nestrud and Worster, 1979; Soil Survey Staff, 2003).

Experimental Design

Eight small zero order (intermittent in hydrological outflow) watersheds in an unbalanced incomplete block design were distributed across three blocks. Blocks are termed Basswood, Interim, and Orbweaver. The size of the watersheds varied from 0.5 to 3.2 ha, with average slopes ranging from 6.1 to 10.5% (Table 1). Interim and Orbweaver blocks each contained three watersheds with the following treatments: 100% annual crop rotation of corn and soybeans (CORN); 10% perennial filter strip at the toeslope position with 90% annual crop rotation of corn and soybeans upslope (PFS); and 100% perennial restored native grassland vegetation (RNG). Basswood contained only CORN and PFS treatments (Table 1). The lack of an RNG watershed in the Basswood block reduced our statistical power, but the RNGs, provided a valuable reference condition that simulated a native prairie ecosystem of the Midwestern United States. Although the PFS always covered the lowest 10% of the watershed (by area), the size and width of the PFS varied from 27 to 41 m based on the size and shape of the watershed. In this study, only the presence of PFS at each watershed position was considered as a treatment, while the area of PFS was not considered as a treatment because Zhou et al. (2010) did not find a significant effect of PFS area on soil or water quality. Details of watershed treatment establishment were reported by Zhou et al. (2010). In the year of this study (2012), corn was the crop in rotation and anhydrous ammonia was knifed into the row crop areas at a rate of 157 kg N ha^{-1} on 27 Mar. 2012. Grain yield was measured with a combine monitor and converted to aboveground standing biomass at senescence with the standard 0.5 harvest index. Details of the conversion method are provided by Brown et al. (2014). In all other years when corn was the crop in the rotation, the same N fertilizer rate was applied. No fertilizer is added to the 10% of the PFS watersheds maintained in perennial vegetation. In PFS perennial vegetation strips, aboveground standing biomass at senescence was measured by clipping in three randomly placed 1 m^2 quadrats. Destructive aboveground biomass clipping was not permitted in the RNG. The RNG was managed with annual spring burning, which is typical for native grassland management in the region. Although the 10% PFS establishment was similar to the RNG, it was mowed and harvested rather than burned because of management constraints.

Pretreatment Data

Before the spring of 2007, PFS and CORN watersheds were maintained in bromegrass (*Bromus* L.) for at least 10 yr without fertilizer application. In the spring of 2007, all watersheds were tilled, and PFS and CORN treatments were installed. From 2005 to 2010, 0 to 15 cm of total soil N and organic C decreased in the bottom 10% of the CORN watersheds by approximately 20% (Pérez-Suárez et al., 2014). Pretreatment soil and vegetation data

Table 1. Watershed description and experimental design.

Watershed	Size, ha	Slope, %	Treatment
Basswood-1	0.53	7.5	10% perennial filter strip
Basswood-6	0.84	10.5	100% annual crops
Interim-2	3.19	6.1	10% perennial filter strip
Interim-3	0.73	9.3	100% annual crops
Interim†	–	–	100% restored native grassland
Orbweaver-1	1.18	10.3	10% perennial filter strip
Orbweaver-3	1.24	6.6	100% annual crops
Prairie†	–	–	100% restored native grassland

† Watershed areas of 100% restored native grasslands are unknown, but fall within the range of other watersheds included in this study.

are not available for the PFS and RNG watersheds. However, data from similar watersheds at the research site demonstrate PFS increased total soil N and organic C by increasing net primary productivity relative to annual crops, retaining eroding soil from upslope locations, and minimizing soil erosion from within the perennial vegetation strips (Helmers et al., 2012; Pérez-Suárez et al., 2014). Data from these watersheds suggest aboveground productivity is similar between PFS perennial vegetation strips and RNG, but C4 grasses rather than C3 grasses are more dominant in RNG watersheds (Pérez-Suárez et al., 2014).

In 2005, lysimeters and groundwater wells were installed at "upslope" and toeslope" landscape positions in watersheds that received PFS and CORN treatments in 2007 (Fig. 1). In PFS watersheds, the upslope wells and lysimeters were located where the interface of crops and perennial vegetation would occur after the onset of cropping in 2006. In CORN watersheds, the bottom 10% of the watershed was identified with a procedure identical to that used for identifying the bottom 10% of PFS watersheds, and the upslope wells and lysimeters were located where the interface between crops and perennial vegetation would occur if a 10% PFS were to be inserted in the CORN watersheds. Toeslope wells and lysimeters were located at the bottommost position of the watershed. To better represent the bottom 10% of each watershed in our measurements, two additional slope positions, sideslope and downslope between the upslope and toeslope, were identified. Note that hydrological flow in these watersheds is intermittent, and no stream channel exists because of cultivation operations in the CORN watersheds and growth of perennial vegetation in the PFS and RNG watersheds. Comparing pre- and post-treatment data from lysimeters and wells, Zhou et al. (2010) reported that vadose zone and groundwater NO_3^-–N concentrations remained low in PFS watersheds (<2.0 mg NO_3^-–N L^{-1}) after conversion from bromegrass, but conversion from bromegrass significantly increased vadose zone and groundwater NO_3^-–N concentrations in CORN watersheds (>10 mg NO_3^-–N L^{-1}).

Zhou et al. (2010) demonstrated lateral groundwater flow through the bottom 10% of watersheds based on higher water table depth above mean sea level in upslope compared with toeslope wells in all watersheds from 2005 to 2008. During our study period (March–October 2012), the groundwater table depth was measured monthly using a submersible level transmitter (Keller America, Newport News, VA). Groundwater samples were also

drawn each month from each well. Water samples were analyzed for NO_3^-–N concentrations on a Quickchem 2000 Automated Ion Analyzer flow injection system with a 0.2 mg L^{-1} detection limit (Lachat Instruments, Milwaukee, WI).

In Situ Nitrous Oxide Gas Flux Measurements

Nitrous oxide flux measurements were performed between 9:00 and 13:00 h from 27 Mar. to 15 Oct. 2012 (the portion of the year when average daily soil temperature was >10°C). Gas flux measurements were performed approximately every other week with greater sampling frequency in the spring and after precipitation events. Our measurement frequency was designed to determine if there was a land use treatment effect on the mean rate of N_2O emissions, not to determine the cumulative amount of N_2O emissions from each land use. A vented, static closed chamber was used to measure gas flux. The chamber consisted of two parts: a polyvinyl chloride (PVC) ring (inner diameter of 25.2 cm and a height of 9.1 cm.) served as a base and was covered with a PVC lid (inner diameter of 25.2 cm and a height of 11.2 cm.). To reduce the effect of radiative heating during gas sampling, thermal insulation tape was added to the outside of PVC chambers. Four gas flux measurement positions on the landscape were selected (upslope, sideslope, downslope and toeslope; Fig. 1). One week before the start of gas flux measurements, one chamber was installed at each location in PFS and RNG watersheds while two chambers were installed in CORN watersheds to account for the systematic heterogeneity (crop row and interrow). Although one chamber per landscape location (no subreplication) limited our ability to identify a landscape effect, it standardized the location of chambers in proportion to the watershed area. The chambers were installed to a depth of 5 cm and only moved at the time of fertilizer application and crop planting. During gas measurements, the PVC base was sealed to the PVC chamber over a period of 30 min. Four gas samples drawn at 0, 10, 20, and 30 min with a 10-mL polypropylene syringe were injected into evacuated glass serum vials and brought to the laboratory for gas analysis within 72 h.

Nitrous oxide was analyzed on a gas chromatograph (Agilent 7890), which was operated with an electron capture detector at 350°C for N_2O detection. 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. Gas emission rates were calculated from the increase in N_2O concentration over time and these relationships were best fit with a linear model. Soil temperature and volumetric water content (VWC) were measured at 5-cm depth near each chamber on each sampling date. Soil temperature was measured with a digital thermometer (±0.5°C), and soil water content was measured via the dielectric constant with a Dynamax SM150 (Houston, TX) soil moisture sensor (±3% VWC). Volumetric water content data were converted to water-filled pore space (WFPS) using bulk density data from each site.

Soil Physical and Chemical Analysis

To determine soil NO_3^-–N and NH_4^+–N concentrations during gas flux measurements, two soil sample cores (2-cm diameter to 10-cm depth) were collected within 25 cm of each chamber. Soil samples were extracted in 2 M KCl (5:1 solution: soil ratio). Extracts were filtered through preleached Whatman 1 filter paper and frozen until analysis. Extract (NO_3 + NO_2)-N (hereafter NO_3^-–N) and NH_4^+–N concentrations were measured using the Griess–Ilosvay reaction with vanadium(III) chloride as a reducing agent and the Berthelot reaction, respectively (Hood-Nowotny et al., 2010). Soil pH was measured in 1:5 soil/water solution of fresh soil samples. Particle size was determined with the micropipette method (Miller and Miller, 1987). Total C and N were determined by dry combustion elemental analysis.

Denitrification Enzyme Activity

Denitrification enzyme activity was measured from soils collected at each gas flux location (Fig. 1) at five depth increments: 0 to 20, 20 to 40, 40 to 60, 60 to 80, and 80 to 100 cm in September 2012. Soils were brought to the laboratory and kept at 4°C for 3 d until performance of the DEA assay as described by Smith and Tiedje (1979). During the DEA assay, gas samples were taken at 0, 30, 60, 90, and 120 min, stored in evacuated glass vials, and analyzed for N_2O using gas chromatography as described above.

Two phases with an increase in N_2O concentration in headspace over time were found. Rates obtained from soils incubated during Phase I reflected DEA of the existing bacterial enzymes rather than Phase II, which reflects the period of bacterial growth (Smith and Tiedje, 1979). Denitrification rates were calculated as the rate of N_2O accumulation in the headspace in Phase I (between 0 and 60 min) of incubation.

Denitrification Measurements

We measured denitrification using an intact core incubation with (i) ^{15}N isotopic labeling (Mosier and Klemedtsson, 1994) and (ii) acetylene inhibition methods (Ryden et al., 1987). We observed an exponential decrease in DEA with depth (results) so these experiments were limited to surface soils (0–15 cm). Soils were collected in 20- by 4.1-cm butyrate soil cores from each in situ gas sampling location (one soil core per chamber). Power analyses based on in situ N_2O flux data indicated that one core per location would be sufficient to detect a treatment effect, while one core per landscape location provided little power to detect a landscape effect. After collection, the butyrate sleeves were fitted with rubber stoppers at each end. The samples were kept in dark at 4°C for 3 d until processed. Before denitrification measurements, cores were saturated overnight by wrapping cheese cloth around base of core and putting the base of the cores in 2 cm of deionized water. The cores and tub of water were maintained at 4°C to minimize microbial activity during the wetting phase. Subsequently, the cores were allowed to come to room temperature (~24°C) and drain to field capacity. Cores were then weighed so that the water content of cores could be

kept constant through all denitrification measurements. The internal gas volume of each core was measured using a pressure transducer (Parkin et al., 1984).

Three denitrification measurements were consecutively made on each soil core. In the first set of denitrification measurements (here afterward as No C_2H_2), the ^{15}N gas flux technique was accomplished by adding 2 mL of enriched $^{15}NO_3^-$–N (0.5 mg per mL or 1 mg $^{15}NO_3^-$–N per core) solution in each soil core through 20-cm side port needles. The solution was injected throughout each core to achieve uniform ^{15}N labeling. Water-filled pore space adjustments were not considered because the soil cores were assumed at field capacity as stated above. Labeling for $^{15}NO_3^-$–N resulted in 9 to 96% atom percent enrichment in cores with lower values in nitrate-rich soils sampled from the CORN watersheds and higher values in relatively nitrate-poor soils sampled from the PFS and RNG watersheds. The standardized 1-mg $^{15}NO_3^-$–N addition to each core was chosen based on nearby soil NO_3^-–N concentrations at the time of soil core sampling and resulted in final NO_3^-–N concentrations that were within the observed range during field measurements of N_2O flux. After NO_3^-–N addition, CORN treatment concentrations ranged from 8.7 to 49.5 mg NO_3^-–N kg soil^{-1}, while field observations during N_2O measurements ranged from 0.11 to 300 mg NO_3^-–N kg soil^{-1} with a mean concentration of 22.9 mg NO_3^-–N kg soil^{-1}; RNG treatment concentrations ranged from 4.7 to 8.2 mg NO_3^-–N kg soil^{-1}, while field observations during N_2O measurements ranged from 0.03 to 15.2 mg NO_3^-–N kg soil^{-1} with a mean concentration of 1.7 mg NO_3^-–N kg soil^{-1}; PFS treatment concentrations ranged from 5.1 to 40 mg NO_3^-–N kg soil^{-1}, while field observations during N_2O measurements ranged from 0.05 to 96 mg NO_3^-–N kg soil^{-1} with a mean concentration of 8.6 mg NO_3^-–N kg soil^{-1}.

After enrichment, the cores were sealed with rubber stoppers and incubated for 45.5 h. Gas samples were drawn from the headspace at 0, 4, 21.5, and 45.5 h to ensure a linear increase of N_2O with time; after 0, 4, and 21.5 h samples, 20 mL of laboratory air was added to each core to replace the gas sample. Samples were injected into separate evacuated 10-mL glass serum vials for N_2O and $^{15}N_2$ analyses. After this set of measurements, cores were left open to remove accumulated N_2O, and stoppers were changed for the next set of measurements.

In the second set of measurements (hereafter "Low C_2H_2"), 11 mL of 65 Pa C_2H_2 concentration was added to each soil core with gas volume of 119 mL to yield a final C_2H_2 concentration of approximately 6 Pa which is known to inhibit nitrifier activity and thus N_2O production from nitrification (Mosier, 1980). Cores were then sealed with stoppers and incubated for 29 h. Gas samples were drawn from the headspace at four sampling points (0, 5, 22, and 29 h). Before collecting gas samples at each sampling point, 11 mL of low C_2H_2 was added to each core to replace the withdrawn sample and return the C_2H_2 concentration to 6 Pa. The added air in core was mixed by repeated pumping with a 60-mL syringe, and then 11 mL of gas samples were taken for N_2O in 6-mL evacuated glass serum vials. After this set of measurements, cores were left open to remove accumulated N_2O, and stoppers were changed for the next set of measurements.

In third set of measurements (hereafter "High C_2H_2"), 11 mL of 100% C_2H_2 gas was added in each soil core of gas volume 119 mL to achieve final concentration of approximately 10 kPa to inhibit the activity of nitrous oxide reductase (Ryden et al., 1987). After sealing with stoppers, the cores were incubated for 28 h. Gas samples were drawn from the head space at four sampling points (0, 5, 23, and 28 h). Before collecting gas samples, 11 mL of C_2H_2 was added to each core, mixed by repeated pumping with a 60-mL syringe, and then 11 mL of gas samples were drawn for N_2O in 6-mL evacuated glass serum vials. The three denitrification measurements were completed in 9 d, and subsequently the soil was air dried and analyzed for residual total ^{15}N by using a Fisons NA 1500 Series 2 Elemental Analyzer coupled with a Finnigan Delta V mass spectrometer (Thermo Electron, Bremen, Germany). Although C availability is expected to decrease during the incubation, the high water content of soil cores was expected to minimize heterotrophic respiration. By repeating measurements on the same cores, we minimized spatial variability but increased temporal variability. During the three sets of denitrification measurements, N_2O gas production rates were calculated from the increase in N_2O gas concentration from the initial three points, while the last sampling point was discarded.

We measured $^{15}N_2$ and $^{15}N_2O$ by using a Finnigan GasBench II (Thermo Electron) interfaced to the mass spectrometer. The analytical precision is 0.2 delta for N_2 and N_2O measurements. Gas chromatography was used to measure total N_2O. In acetylene inhibition methods, N_2O production was measured with linear model based on the increase in concentration over time (accounting for the dilution of laboratory air that was injected to maintain headspace pressure). The following equations from Mosier and Klemedtsson (1994) were used to calculate N_2 flux from $^{15}N_2$ measurements using the first (time 0) and third sampling point (time 21.5 h):

$$\Delta r = (^{29}N_2/^{28}N_2)\,\text{sample} - (^{29}N_2/^{28}N_2)\,\text{reference} \quad [1]$$

$$\Delta \acute{r} = (^{30}N_2/^{28}N_2 + ^{29}N_2)\,\text{sample} - (^{30}N_2/^{28}N_2 + ^{29}N_2)\,\text{reference} \quad [2]$$

$$^{15}X_N = 2.015\,(\Delta \acute{r}/\Delta r)/\{1 + [2.015(\Delta \acute{r}/\Delta r)]\} \quad [3]$$

$$d = \Delta \acute{r}/(^{15}X_N)_2 \quad [4]$$

$$\text{Total denitrification N gas evolved from the soil into the core} = \text{total } N_2 \text{ in the core volume} \times d \quad [5]$$

$$N_2\ \text{flux} = \Delta C/A\,t \quad [6]$$

where "sample" is an air sample collected within the core at a time t after addition of $^{15}NO_3$–N, "reference" is an ambient laboratory air sample at the time of the experiment, ($^{29}N_2/^{28}N_2$) and ($^{30}N_2/^{28}N_2$ + $^{29}N_2$) are ion current ratios determined by the mass spectrometer, $^{15}X_N$ is mole fraction of ^{15}N in the soil NO_3^- pool, d is the fraction of total N gas in the soil core attrib-

Table 2. Mean (± standard error) groundwater depth and NO_3^-–N concentration.†

	CORN			PFS		
	Groundwater height above mean sea level*	Groundwater NO_3^-–N^{NS}	Soil NO_3^-–N*	Groundwater height above mean sea level^{NS}	Groundwater NO_3^-–N*	Soil NO_3^-–N*
	m	mg L^{-1}	mg kg^{-1}	m	mg L^{-1}	mg kg^{-1}
Upslope	298.9 ± 4.03	4.63 ± 0.57	26.94 ± 4.16	298.4 ± 2.09	3.78 ± 1.08	9.75 ± 1.32
Toeslope	289.4 ± 4.87	5.05 ± 1.79	12.54 ± 1.60	289.4 ± 6.00	1.58 ± 0.79	6.13 ± 1.77

† Data represent mean values of six sampling dates from April to September 2012. Mean (± standard error) NO_3^-–N concentration in surface soils (0–10-cm depth) represent mean values of 14 sampline dates; data represent mean values of 14 sampling dates from March to October 2012. Treatments include 100% annual row crop (CORN) and 10% perennial filter strip (PFS) watersheds at Neal Smith National Wildlife Refuge (N = 3). The Restored native grassland did not contain wells. See Fig. 1 for sample locations. Probability from paired t test between upslope and toeslope; *P < 0.01: ^{NS}, Not significant.

utable to denitrification, A is soil surface area in the soil core, t is the time when core was sealed, and ΔC is the change in concentration of $^{30}N_2$ and $^{29}N_2$ in the soil core during time t.

By using data from the acetylene inhibition methods, we calculated N_2 production as the difference in N_2O production between cores with "No C_2H_2" and "High C_2H_2" (Ryden et al., 1979). Similarly, $N_2O/(N_2 + N_2O)$ was calculated from estimates of N_2O produced from intact cores with No C_2H_2 and High C_2H_2. Estimates of N_2O production from denitrification were calculated as N_2O flux with Low C_2H_2 because nitrification was inhibited (Mosier, 1980). We calculated N_2O production from nitrification by taking the difference of N_2O rates in soil cores with No C_2H_2 and Low C_2H_2. However, values were extremely low, and slightly negative values were common suggesting little to no N_2O production from nitrification, as would be expected at 100% water holding capacity (Zhu et al., 2013).

Carbon Mineralization

Potentially mineralizable C (Cmin) was determined from soil samples collected during September 2012 for the denitrification measurements. Ten grams of soil was incubated in 120-mL bottles during a 5-d incubation at 25°C. Headspace CO_2 concentration was quantified daily using an LI-7000 infrared gas analyzer (LI-COR, Lincoln, NE). Carbon dioxide emissions were calculated by linear interpolation and numerical integration between sample times.

Statistical Analysis

All data were tested for normality and homogeneity of variance. If needed, data were log transformed before statistical analysis. In CORN watersheds, in situ measurements of N_2O fluxes, NO_3^-–N, and NH_4^+–N were calculated by taking the average of data from row and inter-row because there were no significant differences between these locations. For in situ field measurements, the effects of treatment, time, landscape, treatment × time, treatment × landscape, and treatment × time × landscape were evaluated for soil N_2O fluxes, temperature, moisture, and NO_3^-–N and NH_4^+–N concentration with a repeated measures analysis of variance (ANOVA) model with fixed effects of treatment, time, landscape, and their interactions and block as a random effect (SAS Institute, Cary, NC). We did not calculate cumulative N_2O flux from the 14 in situ measurements because our goal was not to determine the cumulative amount of N_2O emissions from each land use treatment. For laboratory measurements including DEA, denitrification, Cmin, and soil properties, the same ANOVA model was used but without repeated measures. Significant main effects were assessed with Tukey's range test for mean separation. Groundwater height above sea level, groundwater NO_3^-–N, and soil NO_3^-–N mean values from upslope and toeslope locations were compared using paired t tests.

RESULTS

Groundwater and NO_3^-–N Dynamics

Groundwater height above mean sea level decreased from upslope to toeslope wells (Table 2). Mean groundwater NO_3^-–N concentrations in the CORN did not change from upslope and toeslope wells; however, mean groundwater NO_3^-–N concentration in the PFS significantly decreased from the upslope to toeslope wells. A decrease in soil NO_3^-–N (2 M KCl extractions) from upslope to toeslope was observed in both CORN and PFS (Fig. 2 and Table 2).

Soil Properties and Field Measurements

Soil physical properties were not significantly different among treatments. However, total soil organic carbon (TOC), total N, and C/N ratio were significantly higher in PFS and RNG than CORN (Table 3). In CORN and PFS watersheds, average corn aboveground standing biomass at senescence was 14.6 Mg dry matter ha^{-1} (grain + residue). Perennial vegetation aboveground standing biomass at senescence in the perennial filter strips was 6.6 Mg dry matter ha^{-1}.

Across all field measurements, mean soil WFPS did not differ among the treatments (Table 4), but within treatments WFPS increased from upslope to toeslope in PFS and decreased from upslope to toeslope in CORN (Table S1). Mean soil temperature across all measurement dates decreased among treatments in the order of CORN > RNG > PFS (Table 3 and Table 4). Across all measurements, mean soil NH_4^+–N and NO_3^-–N concentrations were highest in CORN followed by PFS and RNG (Fig. 2).

Across all dates, mean in situ soil N_2O flux rate was highest in CORN but did not differ among PFS and RNG (Fig. 3). On 12 of 14 measurement dates, N_2O flux in CORN was greater than PFS and RNG (Fig. 3). There was a treatment by landscape position interaction on N_2O (Table 4). Within treatments, N_2O fluxes in PFS decreased from upslope to toeslope. In contrast, N_2O fluxes in CORN increased from upslope to toeslope (Fig. 3). There was no effect of landscape position on N_2O fluxes in RNG.

Denitrification Enzyme Activity

Across all treatments, DEA was highest in the surface soil (20-cm depth) and exponentially decreased with increasing soil depth (Fig. 4). As a proportion of total DEA from 0 to 100 cm, 69.6% occurred from 0 to 20 cm, 15.0% from 20 to 40 cm, 6.4% from 40 to 60 cm, 5.8% from 60 to 80 cm, and 3.3% from 80 to 100 cm. Landscape position did not affect DEA (Table 5). Opposite in situ N_2O fluxes, DEA was higher in RNG and PFS than CORN (Fig. 4). There was a positive linear correlation between TOC and the natural logarithm of DEA (R = +0.48, P = 0.04; Fig. 5) as well as NH_4^+–N and DEA (R = +0.66, P < 0.01; Fig. 5). There was a negative linear correlation between NO_3^-–N and the natural logarithm of DEA (R = −0.71, P < 0.01; Fig. 5).

Denitrification: C_2H_2 Technique

Across treatments, there was no consistent pattern between denitrification and landscape position. However, we caution that our analysis of landscape position was limited because of a lack of statistical power. Thus, we focus on the treatment effect and correlations among DEA, NO_3^-–N concentration, potentially mineralizable C, and denitrification.

Total denitrification rates (N_2 + N_2O), measured in intact cores, were not different among the treatments (Fig. 6 and Table 5), and soil NO_3^-–N concentrations and total denitrification rates were not correlated. However, across treatments, N_2O production from denitrification did not show the same pattern as total denitrification; N_2 production from denitrification was highest in PFS followed by CORN and RNG (Fig. 6). Moreover, the pattern of N_2O production from denitrification was opposite the pattern of DEA and potentially mineralizable C (Fig. 4 vs. Fig. 6 and Fig. 7).

The $N_2O/(N_2 + N_2O)$ ratio was significantly lower in PFS than RNG and CORN (Fig. 6). This was also opposite the pattern of DEA and potentially mineralizable C. There was a negative correlation between $N_2O/(N_2 + N_2O)$ and the natural logarithm of DEA (Fig. 8) and a positive exponential relationship between $N_2O/(N_2 + N_2O)$ and NO_3^-–N (Fig. 8).

Denitrification: ^{15}N Technique

Total N_2 ($^{15}N_2$ + $^{14}N_2$) production was highest in PFS followed by RNG and CORN (Fig. 9). Across treatments, the pattern in total N_2 production was coincident with DEA and potentially mineralizable C, but opposite several N_2O measurements including N_2O fluxes measured in situ, N_2O production during C_2H_2 inhibition, and N_2O production during the ^{15}N tracing (Fig. 3, 6, and 9). Residual ^{15}N remaining in the intact soil cores after incubation exhibited the opposite pattern of $^{15}N_2$ fluxes and was higher in CORN than PFS or RNG (Fig. 10). Denitrification measurements with C_2H_2 method were approximately double those measured with the ^{15}N method.

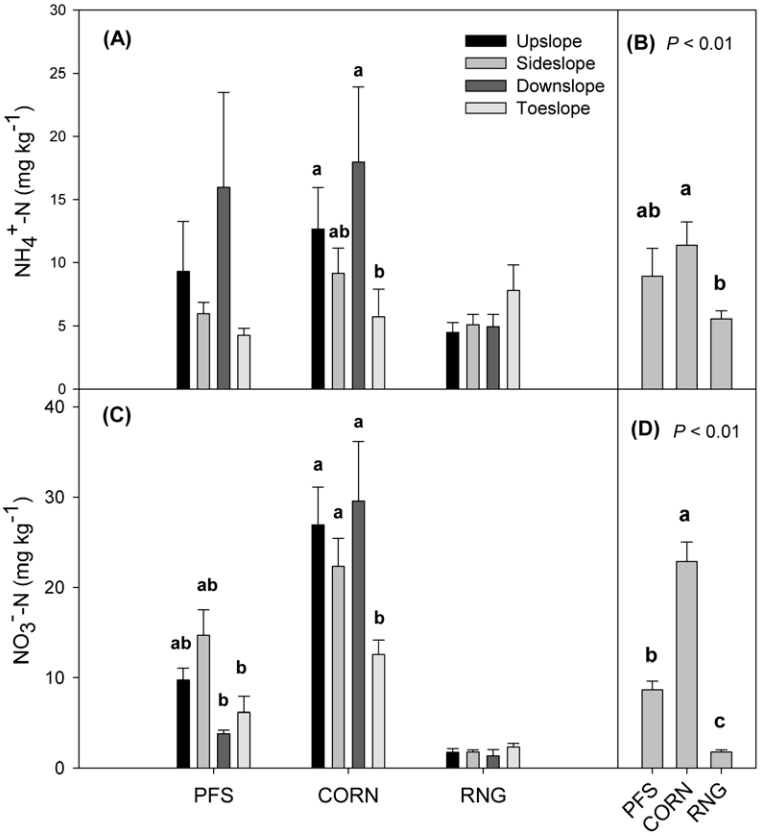

Fig. 2. In situ soil (a) NH_4^+–N and (c) NO_3^-–N contents as determined at four landscape positions (upslope, sideslope, downslope, and toeslope) at 10-cm depth in each treatment during the growing season (March to September) of 2012. Error bars indicate standard error of the mean (n = 28–42). Different letters indicate significant differences among landscape positions within each treatment as determined by Tukey's test. Mean in situ soil NH_4^+–N and NO_3^-–N contents across landscape positions are also shown in Fig. 2b and 2d, respectively, where different letters indicate significant treatment differences determined after a significant main effect of treatment in repeated measures ANOVA. PFS, three watersheds with perennial vegetation filter strips covering the bottom 10% of the watershed and corn covering the remaining 90% of the watershed; CORN, three watersheds with 100% corn; RNG, two watersheds with 19-yr-old 100% restored native grassland.

DISCUSSION

Groundwater and NO_3^-–N Dynamics

Groundwater height above mean sea level decreased from upslope to toeslope wells, providing evidence for lateral water flow through the bottom 10% of CORN and PFS watersheds (Table 2). Moreover, the relatively steep slopes (6–10%) of the study wa-

Table 3. Mean (± standard error) of selected soil properties of watersheds from 0- to 15-cm soil depth at Neal Smith National Wildlife Refuge.†

Property	PFS	CORN	RNG
Total organic carbon, %	2.99 ± 0.17a	2.32 ± 0.09b	2.78 ± 0.16a
Total nitrogen, %	0.26 ± 0.0a	0.22 ± 0.01b	0.24 ± 0.01ab
C/N ratio	11.32 ± 0.37ab	10.53 ± 0.08b	11.71 ± 0.28a
Bulk density, g cm^{-3}	1.17 ± 0.04a	1.22 ± 0.03a	1.18 ± 0.02a
pH	6.38 ± 0.18b	6.12 ± 0.11b	6.91 ± 0.08a
Sand, %	8.06 ± 3.00a	9.89 ± 3.57a	9.53 ± 1.84a
Silt, %	25.89 ± 1.17a	24.21 ± 1.38a	22.39 ± 1.31a
Clay, %	66.06 ± 2.61a	65.90 ± 2.34a	68.08 ± 1.78a
Soil temperature, °C	17.00 ± 0.39b	18.11 ± 0.40a	17.73 ± 0.45ab
Water-filled pore space, %	54.34 ± 1.55a	55.19 ± 1.27a	53.68 ± 1.68a

† Same letters within a row indicate insignificant differences (P > 0.05). PFS, perrenial filter strip; RNG, restored native grassland.

Table 4. Probability values (repeated measured ANOVA) of the impact of treatment, landscape, and treatment × landscape interaction on in situ N_2O fluxes and parameters measured during the growing season (March to September) of 2012 (14 sample dates).

Factor	N_2O	Soil temperature	Water-filled pore space	NH_4^+–N	NO_3^-–N
Treatment	<0.01	<0.01	0.26	<0.01	<0.01
Landscape	0.95	0.33	0.64	0.01	<0.01
Treatment × landscape	0.02	0.44	<0.01	0.02	0.02

tersheds (which are not tilled) likely promote lateral hydrological flow paths (Zhu and Lin, 2009), and lateral flow can promote NO_3^-–N transport downslope (Castellano et al., 2013). The coincident decrease in groundwater levels and NO_3^-–N concentrations from upslope to toeslope wells in the PFS suggests PFS create a NO_3^-–N sink during lateral flow through these systems. Consistent with this interpretation of 2012 data presented herein, Zhou et al. (2010) concluded that lateral flow through PFS led to NO_3^-–N removal from 2005 to 2008 in the watersheds that are the subject of this study.

In Situ Nitrous Oxide Fluxes

Relative to CORN, consistently low N_2O fluxes in PFS that were similar to RNG (Fig. 3) could reflect not only the lack of direct fertilizer application to the PFS portion of the watershed, but also (i) fewer N_2O emissions due to nitrification, (ii) differences in corn vs. perennial vegetation N sinks, and (iii) greater N_2O reduction (i.e., more complete denitrification). Differences in nitrification N_2O emissions could partially explain the differences because CORN watersheds received 157 kg ha^{-1} of anhydrous ammonia fertilizer. Nevertheless, during the course of field measurements (March–September), mean soil NH_4^+–N concentration was similar among CORN and PFS despite the large NH_4^+–N addition to CORN, indicating greater N mineralization in PFS due to higher SOC and TN. These data suggest reduction differences in N_2O fluxes are more likely due to differences in NO_3^-–N rather than NH_4^+–N substrate.

Although N inputs to the lower 10% of CORN and PFS watersheds differ, so do vegetation N sinks. Total aboveground dry matter production of 14.6 Mg ha^{-1} in CORN vs. 6.6 Mg ha^{-1} in the perennial vegetation portion of PFS suggest that corn is a greater N sink than perennial vegetation. However, the temporal pattern of N uptake differs among these plants so that corn N uptake is concentrated in the summer, whereas prairie vegetation N uptake begins in the early spring (Abendroth et al., 2011; Pérez-Suárez et al., 2014). The lack of spring N demand in CORN watersheds may help to explain why treatment differences in N_2O flux were greatest in the spring and decreased over time (Fig. 3).

Potentially mineralizable C may also be an important factor affecting treatment differences in N_2O flux, particularly in the early spring when vegetation productivity was low in all treatments and CORN N_2O emissions were approximately 100% greater than PFS. Across treatments, patterns of N_2O fluxes and $N_2O/(N_2O + N_2)$ ratios were opposite potentially mineralizable C, suggesting differences in C availability may affect the magnitude of N_2O fluxes and the $N_2O/(N_2O + N_2)$ ratio (Fig. 3 and 6). Low mineralizable C availability can limit the reduction of NO_3^-–N to N_2O and subsequently N_2O to N_2 (e.g., Parsons et al., 1993; Mitchell et al., 2013). Accordingly, greater

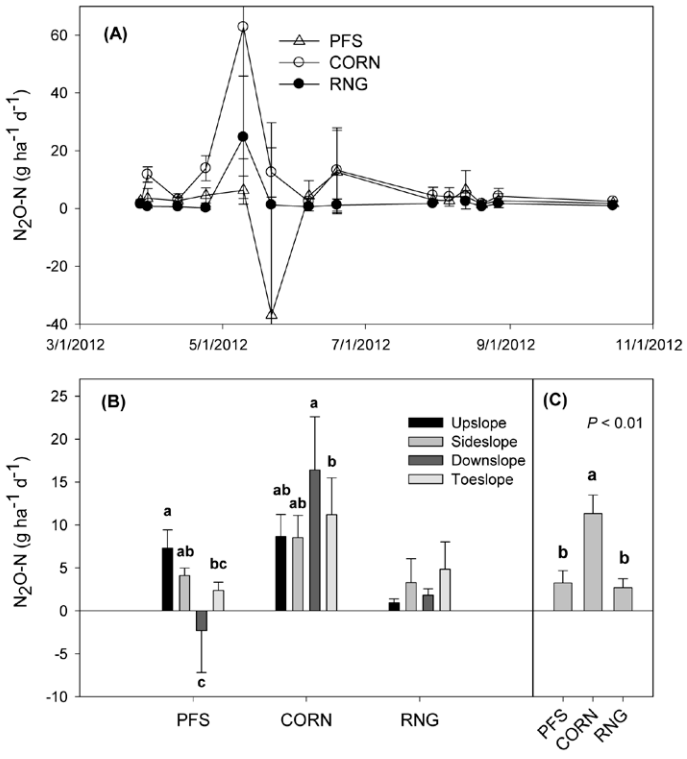

Fig. 3. (a) In situ soil N_2O–N fluxes as determined during the growing season (March to September) of 2012 and (b) mean N_2O–N fluxes at four landscape positions (upslope, sideslope, downslope and toeslope) in each treatment. Error bars indicate standard error of the mean. Different letters indicate significant differences among landscape positions within each treatment as determined by Tukey's test. (c) Mean soil N_2O–N flux rates across landscape positions within treatments. Different letters indicate significant treatment differences determined after a significant main effect of treatment in repeated measures ANOVA.

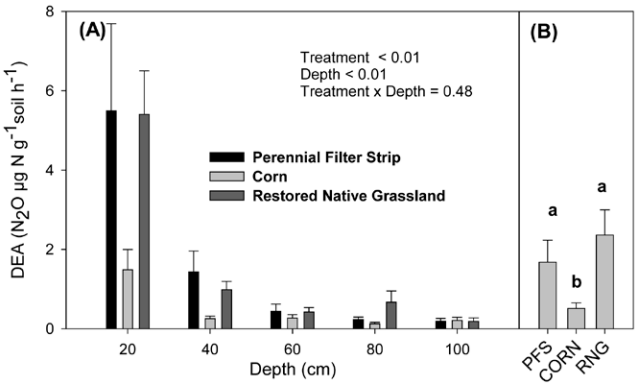

Fig. 4. (a) Denitrification enzyme activity (DEA) measured from 0- to 100-cm depth in three treatments. Analysis of variance results ($P > F$) for treatment, depth, and treatment × depth are shown. (b) Denitrification enzyme activity measured at 0- to 15-cm depth in three treatments. Different letters indicate significant differences among treatments as determined by Tukey's test after a significant main effect of treatment ($P < 0.01$). Vertical error bars indicate standard error of the mean (n = 8–12).

Table 5. Probability values (two way ANOVA) of the impact of treatment, landscape, and treatment × landscape interaction on parameters measured in the laboratory.†

			Acetylene inhibition experiment				^{15}N tracer experiment		
Factor	DEA	Cmin	N_2O‡	N_2	$N_2 + N_2O$	$N_2O/(N_2 + N_2O)$	$^{15}N_2$	N_2O§	Residual ^{15}N
Treatment	<0.01	<0.01	0.28	0.04	0.35	0.05	0.04	0.10	<0.01
Landscape	0.83	0.20	<0.01	0.73	0.52	0.32	0.87	0.04	0.38
Treatment × landscape	0.96	0.28	0.46	0.78	0.76	0.59	0.65	0.46	0.30

† DEA, denitrification enzyme activity; Cmin, potentially mineralizable carbon.

‡ Denitrified N_2O from the acetylene inhibition experiment.

§ Total N_2O from the ^{15}N tracer experiment.

organic C availability in PFS could have resulted in greater N_2O reduction to N_2. This result is consistent with the C_2H_2 and ^{15}N laboratory measurements that indicated similar total denitrification across treatments but lower N_2O production in PFS. In RNG, high C availability and low NO_3^-–N availability due to the lack of upslope N fertilization and accompanying NO_3^-–N runoff may have contributed to low N_2O flux.

Denitrification, Denitrification Enzyme Activity, and the $N_2O/(N_2 + N_2O)$ Ratio

The DEA assay evaluates denitrification potential by providing nonlimiting conditions for a short time period. Our results indicated that despite lower NO_3^-–N concentrations, soils in PFS and RNG had greater denitrification potential than those in CORN. These results are consistent with higher DEA in riparian buffers compared with crop fields (Groffman et al., 1993; Kim, 2008).

Consistent with previous work (Davis et al., 2008), we also observed a positive correlation between TOC and DEA as well as NH_4^+–N and DEA. The positive relationship between NH_4^+–N and DEA could be due to (i) NH_4^+–N regulation of NO_3^-–N consumption and production (Davis et al., 2008) or (ii) it can be an indirect relationship resulting positive correlations among TOC, NH_4^+–N, potentially mineralizable C, and N mineralization (Schomberg et al., 2009; Haddad et al., 2013).

Opposite patterns of in situ N_2O fluxes and DEA across the watershed treatments led us to pursue denitrification measurements. Despite higher NO_3^-–N concentrations and in situ N_2O emissions, less N_2 production was found in CORN than PFS soils. This pattern was consistent with lower DEA, lower potentially mineralizable C, and lower TOC in CORN compared with PFS watersheds. A number of factors control the production and consumption of N_2O and N_2 through the denitrification process. The complete reduction of NO_3^-–N to N_2 through denitrification is controlled by the relative availability of oxidant (oxidized N) and reductant (most commonly organic C). If oxidant availability greatly exceeds reductant availability, then the oxidant may be incompletely utilized; that is, N_2O rather than N_2 will be produced (Firestone and Davidson, 1989).

Lower NO_3^-–N concentrations, higher organic C, and higher DEA in PFS might have stimulated the reduction of N_2O to N_2, whereas higher NO_3^-–N availability and lower C availability in CORN likely limited N_2O reduction (Fig. 2, 3, and 6). Across all treatments, DEA was negatively associated with $N_2O/(N_2 + N_2O)$ while NO_3^-–N was positively associated with $N_2O/(N_2 + N_2O)$ (Fig. 8). Similar to our results, Hill et al. (2000) observed a significant increase in denitrification after in situ additions of labile C in a riparian zone. With regard to denitrification, these results suggest perennial filter strips function similarly to riparian buffer zones (Lowrance et al., 1984; Matheson et al., 2002) and wetlands (Harrison et al., 2012).

Consistent with previous research, results from the ^{15}N and C_2H_2 denitrification measurements were well correlated (Myrold, 1990). Although the C_2H_2 method yielded denitrification rates that were approximately double those measured with ^{15}N (~2.5 vs. 5.0 mg N g^{-1} soil h^{-1}), these results are reasonably close given inherently high variability in these methods. Denitrification is well known to be highly spatially and temporally variable (Parkin, 1987; Groffman et al., 2009). In our laboratory analyses we could not eliminate both sources of variation because of sample number constraints, so we decided to minimize spatial variation (by conducting the ^{15}N and C_2H_2 analyses consecutively on identical, intact soil cores). Interestingly, the C_2H_2 analyses yielded higher denitrification rates despite the fact that they were conducted after the ^{15}N analyses without any substrate addition. This result suggests substrate availability was not limiting denitrification. Although NO_3^-–N addition can stimulate denitrification and increase the $N_2O/(N_2 + N_2O)$

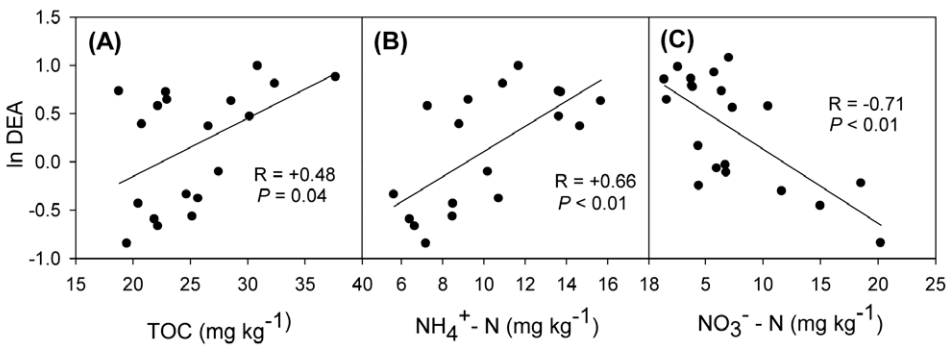

Fig. 5. Relationships between the natural logarithm of denitrification enzyme activity (ln DEA) and (a) total organic carbon (TOC mg kg^{-1}), (b) Soil NH_4^+–N (mg kg^{-1}), and (c) Soil NO_3^-–N concentrations (mg kg^{-1}).

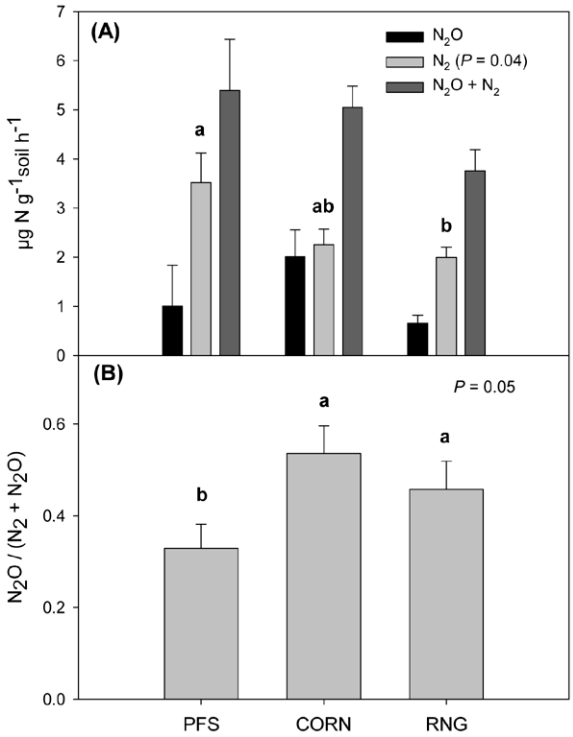

Fig. 6. Results from the acetylene inhibition experiments (see method details in Methods): (a) Denitrified N_2O, N_2, and $N_2O + N_2$ and (b) $N_2O/(N_2 + N_2O)$ ratio. Vertical error bars indicate standard error of the mean (n = 8–12). Analysis of variance results ($P > F$) for the treatment main effect are shown. Different letters indicate significant differences among treatments as determined by Tukey's test.

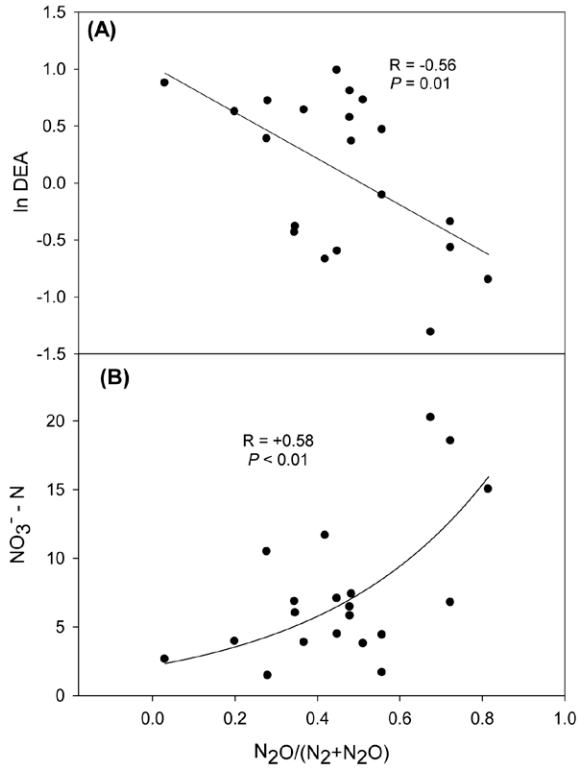

Fig. 8. Relationships between $N_2O/(N_2 + N_2O)$ ratio and (a) the ln DEA and (b) NO_3^-–N contents.

ratio, we note that our $^{15}NO_3^-$–N additions were proportionally largest (relative to background NO_3^-–N) in the RNG treatments which had the lowest $N_2O/(N_2 + N_2O)$ ratio and proportionally smallest in the CORN treatments which had the highest $N_2O/(N_2 + N_2O)$ ratio and lower denitrification rates.

CONCLUSIONS

Our data show that perennial filter strips can decrease NO_3^-–N concentrations in near-surface groundwater without increasing N_2O losses to the atmosphere. High potentially mineralizable C coupled with lateral flow of NO_3^-–N from upslope cropland through downslope PFS likely creates a favorable environment for denitrification. Together, laboratory experiments and field observations suggest greater C availability in PFS than

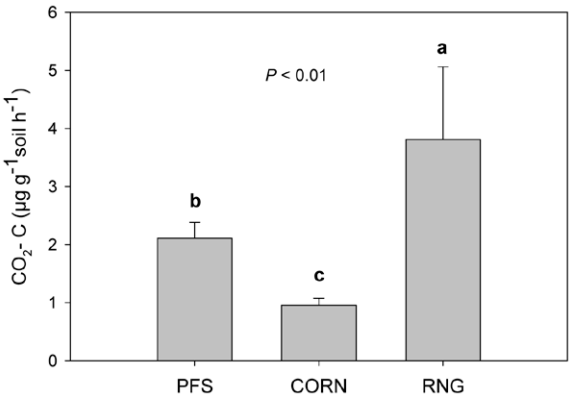

Fig. 7. Potentially mineralizable carbon as determined from a 5-d laboratory incubation during September 2012. Vertical error bars indicate standard error of the mean (n = 8–12). Analysis of variance result ($P > F$) for the treatment main effect is shown. Different letters indicate significant differences among treatments as determined by Tukey's test.

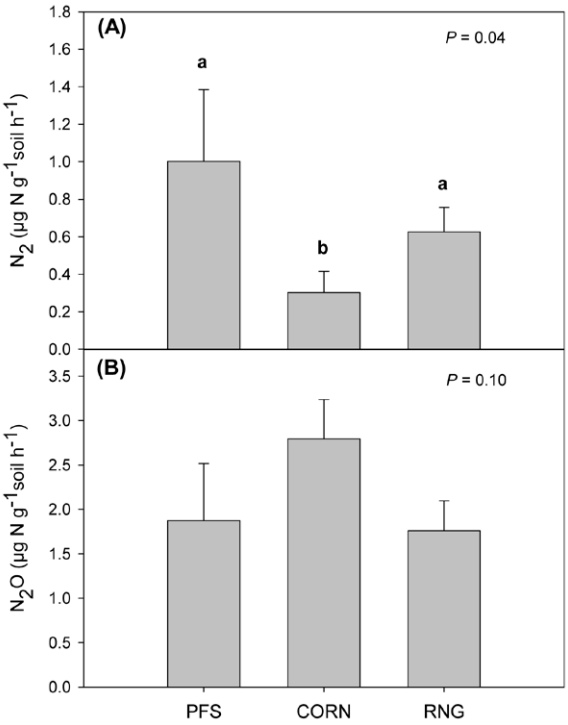

Fig. 9. Result from (a) the ^{15}N tracer experiment: N_2 fluxes and (b) N_2O fluxes. Vertical error bars indicate standard error of the mean (n = 8–12). Analysis of variance results ($P > F$) for treatment main effect are shown. Different letters indicate significant differences among treatments.

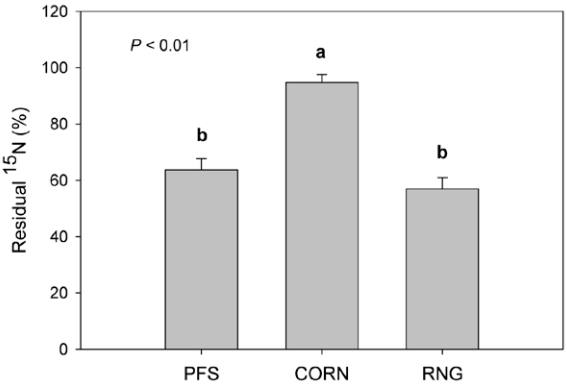

Fig. 10. The percentage of ^{15}N remaining in intact soil cores after 9 d of enrichment. Analysis of variance results ($P > F$) for treatment main effect are shown. Different letters indicate significant differences among treatments as determined by Tukey's test. Vertical error bars indicate standard error of the mean (n = 8–12).

CORN leads to more complete denitrification of NO_3^-–N to N_2. Perennial vegetation filter strips can enhance potentially mineralizable C through three processes: (i) PFSs trap sediment that is eroded from upslope organic C-rich surface soils; (ii) PFSs minimize erosion within the PFS buffer; and (iii) perennial grasses produce more belowground net primary productivity than the corn–soybean rotation crop system (Helmers et al., 2012; Pérez-Suárez et al., 2014).

Lower residual ^{15}N in laboratory-incubated cores (Fig. 10) and lower groundwater NO_3^-–N concentration (Table 2) in PFS vs. CORN watersheds confirmed that PFSs promote greater NO_3^-–N removal efficiency than croplands. Furthermore, relatively low $N_2O/(N_2 + N_2O)$ ratios in PFS suggest that these systems may have a higher nitrate removal capacity than the load to which they are subjected because reduction in the $N_2O/(N_2 + N_2O)$ ratio might be expected before an increase in NO_3^-–N is transport through PFS because of lower total denitrification. Although the amount of NO_3^-–N that can be removed by PFSs is affected by complex interactions among hydrology, vegetation, and soil processes, it appears that the area of PFSs in this study (10% of the watershed) is sufficient to remove large amounts of NO_3 runoff.

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SUPPLEMENTAL MATERIAL

Supplementary information associated with this article includes in situ water filled pore space (%) and soil temperature (°C) as determined across landscape positions in perennial filter strip, 100% annual row crop, and restored native grassland during the growing season of 2012.

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