Published December 1, 2014

Journal of Environmental Quality TECHNICAL REPORTS

LANDSCAPE AND WATERSHED PROCESSES

Comparing Nitrate Sink Strength in Perennial Filter Strips at Toeslopes of Cropland Watersheds

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Abstract

Integration of perennial filter strips (PFS) into the toeslopes of agricultural watersheds may decrease downstream nitrate (NO_3) losses. However, long-term NO_3 removal depends on the relative importance of several NO_3 sinks in the PFS. Plant biomass and labile soil organic matter (SOM) are temporary NO_3 sinks, while stable SOM is a long-term, but potentially finite, NO_3 sink. In contrast, denitrification is a permanent NO_3 sink. We investigated the relative importance of these NO_3 sinks in PFS at the toeslope of row crop watersheds in Iowa. Using 25- × 30-cm in situ mesocosms, we added $^{15}NO_3$ to PFS soils and quantified $^{15}NO_3$–N recovery in plant biomass and SOM after one growing season. Further, we compared $^{15}NO_3$–N recovery in particulate (relatively labile) and mineral-associated (relatively stable) SOM in mesocosms with and without growing perennial vegetation. To determine the potential importance of denitrification, we compared denitrification enzyme activity in soils from paired watersheds with and without PFS. Transfer of $^{15}NO_3$–N into labile and stable SOM pools was rapid and initially independent of growing vegetation. However, SOM and plant biomass were both relatively minor NO_3 sinks, accounting for <30% of $^{15}NO_3$–N inputs. Denitrification enzyme activity data indicated that dissolved organic carbon derived from perennial vegetation increased potential denitrifier activity in PFS soils compared with row crop soils. Together, these results constrain SOM and plant biomass as NO_3 sinks and indicate that denitrification was the most important NO_3 sink in perennial filter strips over one growing season.

Agroecosystems can lose substantial quantities of nitrate (NO_3) to ground and surface waters (Howarth et al., 2012). In the Upper Mississippi River Basin, maize (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] croplands are the greatest source of NO_3 delivery to groundwater and streams, which is a major contributor to eutrophication in the Gulf of Mexico (Alexander et al., 2008; David et al., 2010). As a result, a variety of management strategies have been developed to decrease dissolved NO_3 losses from these systems.

Nitrate delivery to aquatic systems can be decreased by establishing perennial vegetation in agricultural watersheds. Buffers of woody or herbaceous perennial vegetation in riparian zones have been shown to decrease NO_3 delivery to streams when NO_3 is hydrologically transported through biologically active soil layers in the buffers (Hill, 1996; Mayer et al., 2007). Similarly, perennial filter strips (PFS) may be established above the riparian zone within the toeslopes of agricultural watersheds that drain to intermittent first-order streams. This type of vegetation buffer was initially designed to reduce soil erosion (Dillaha et al., 1989; Helmers et al., 2012) but also has been shown to decrease subsurface NO_3 concentrations and thus potentially NO_3 leaching (Zhou et al., 2010).

Perennial filter strips can remove NO_3 from subsurface flow via three sinks: (i) denitrification, (ii) uptake by vegetation, and (iii) transfer to soil organic matter (SOM). However, these NO_3 sinks differ in long-term effectiveness and the relative importance of the three sinks is not well understood (Martin et al., 1999; Mayer et al., 2007). Denitrification has been found or inferred to be the dominant NO_3 sink in many riparian vegetation buffers (Martin et al., 1999), and thus may be important in toeslope PFS as well. During denitrification, NO_3 is converted to gaseous N during heterotrophic respiration of soil organic carbon (SOC) under conditions of high soil moisture. Denitrification can be

J. Environ. Qual.
doi:10.2134/jeq2014.05.0201
Supplemental material is available online for this article.
Received 6 May 2014.
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Abbreviations: AGBM, aboveground biomass; CRBM, course root biomass; DEA, denitrification enzyme activity; DOC, dissolved organic carbon; FRBM, fine root biomass; MAOM, mineral-associated organic matter; MAOM-C, mineral-associated organic matter carbon; MAOM-N, mineral-associated organic matter nitrogen; OM, organic matter; PFS, perennial filter strip; POM, particulate organic matter; POM-N, particulate organic matter nitrogen; PVC, polyvinyl chloride; SOC, soil organic carbon; SOM, soil organic matter.

stimulated by inputs of labile SOC derived from perennial vegetation, especially when the water table is near the surface (Hill, 1996; Schade et al., 2001). Denitrification permanently removes NO_3 from soils. Thus, if denitrification is the dominant NO_3 sink in PFS, these buffers should decrease NO_3 losses indefinitely as long as NO_3 inputs do not exceed potential denitrification rates (Martin et al., 1999; Mayer et al., 2007).

Nevertheless, NO_3 uptake by vegetation can be the most important NO_3 sink in some perennial buffer systems (e.g., Bedard-Haughn et al., 2004; Hefting et al., 2005). The strength of this NO_3 sink varies in magnitude with vegetation type, management, and environmental factors (Bedard-Haughn et al., 2005; Hefting et al., 2005). Harvest of aboveground plant biomass can create a permanent sink for NO_3–N inputs. However, unharvested biomass is necessarily a temporary NO_3 sink since decomposition transfers plant biomass N to SOM or inorganic N pools (Hill, 1996; Hefting et al., 2005).

Though previous research has focused on denitrification and plant biomass as NO_3 sinks, SOM is also a potentially important NO_3 sink in PFS. Indeed, SOM has been found to be the largest sink for inorganic N inputs in many terrestrial ecosystems (e.g., Magill et al., 2000; Kaye et al., 2002). Nitrate N can be incorporated into SOM through plant uptake and subsequent decomposition or directly through microbial and abiotic immobilization processes (Davidson et al., 1990; Stark and Hart, 1997; Fitzhugh et al., 2003; Fricks et al., 2009).

While a fraction of SOM is relatively labile, the majority is relatively stable (e.g., Kaye et al., 2002; von Lutzow et al., 2007; Brown et al., 2014). Particulate organic matter (POM) is considered to be a relatively labile SOM pool, while mineral-associated organic matter (MAOM) is chemically adsorbed to silt and clay particles and protected from mineralization in the long term (Hassink, 1997; Six et al., 2002). However, the amount of SOM that can be stabilized in MAOM is limited (Hassink, 1997; Feng et al., 2012), and after the MAOM pool saturates, additional SOM inputs are potentially available for mineralization (Stewart et al., 2007). This concept extends to inorganic N inputs; as SOM accumulates, transfer of inorganic N to MAOM decreases and inorganic N inputs are instead transferred to labile pools such as POM (Castellano et al., 2012). Therefore, if SOM is the dominant NO_3 sink in PFS soils, some NO_3–N may be transferred to MAOM and retained in the long term; however, this pool would eventually saturate, and further NO_3 inputs would instead be transferred to labile pools and potentially lost from the soil in the long term.

Thus, if plant biomass and SOM are the major NO_3 sinks in PFS soils, PFS may decrease watershed NO_3 losses only in the short term. In contrast, if denitrification is the major NO_3 sink, PFS are expected to decrease watershed NO_3 losses indefinitely. The objective of this study was to evaluate the NO_3 sink strength of SOM, plant biomass, and denitrification in toeslope PFS in an agricultural landscape in Iowa over one growing season. We used $^{15}NO_3$ tracer to quantify the NO_3 sink strengths of plant biomass and SOM and measured denitrification enzyme activity (DEA) to assess the strength of the denitrification sink. Previous data from our study site had showed substantial organic N accumulation in PFS soils (Perez-Suarez et al., 2014). Thus, we hypothesized that soil organic matter is the most important sink for subsurface NO_3 inputs to these PFS.

Materials and Methods

Study Site

This study was conducted at an experimental site within the Neal Smith National Wildlife Refuge in Jasper County, IA (41°33′ N, 93°16′ W). This site was established primarily to assess the ability of toeslope perennial filter strips to improve water quality (Helmers et al., 2012). Soils at this site are mostly classified as Ladoga (fine, smectitic, mesic Mollic Hapludalf) or Otley (fine, smectitic, mesic Oxyaquic Argiudolls) soil series. The site contains three pairs of experimental watersheds (Fig. 1), ranging in size from 0.5 to 3.2 ha. All watersheds had been managed as unfertilized brome grass (*Bromus* sp.) for at least 10 yr before 2006. In 2006 and 2007, the watersheds were mulch-tilled. The following treatments were begun in 2007; in each pair, one watershed had a perennial filter strip planted in the lowest 10% of the area and a no-till maize–soybean rotation (soybean in 2007) established in the remaining 90%, while the other watershed had a no-till maize–soybean rotation established in 100% of the area (Fig. 1). Anhydrous ammonia was applied to maize at 134 and 186 kg N ha^{-1} in 2008 and 2010, respectively, while no N fertilizer was applied to soybeans. Soybean was the crop planted in 2011. Biomass in the PFS (>15-cm height) was harvested and removed on 30 Oct. 2010 and 18 Nov. 2011 to maintain productivity. By 2011, 90% of the vegetation in the PFS was perennial grasses and forbs (Hirsh et al., 2013; Perez-Suarez et al., 2014). Further details about the site and treatment establishment are given in Zhou et al. (2010).

Subsurface Nitrate Concentrations

To measure the effect of PFS on subsurface NO_3 concentrations, porous cup suction lysimeters (Model 1920F1L24, Soilmoisture Equipment Corp.) and shallow groundwater wells were installed at the site in 2004. Lysimeters and wells were installed in the toeslope and upslope positions in each watershed (Fig. 1). Further details about lysimeter and well installment are given in Zhou et al. (2010). During 2011, lysimeter water (1-m depth) and groundwater samples were collected monthly from April to October, and groundwater

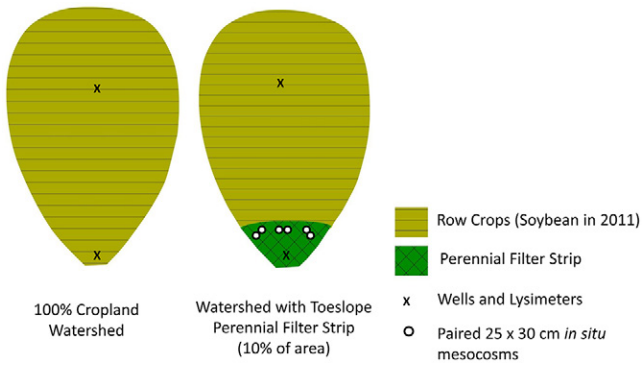

Fig. 1. Conceptual design of paired watersheds: one watershed managed as 100% cropland, the other with a perennial filter strip covering the lowest 10% (toeslope) of the watershed area. Black x shows approximate locations of shallow groundwater wells and tension lysimeters in toeslope and upslope positions in the paired watersheds. White circles show approximate locations of paired 25 × 30 cm in situ mesocosms for $^{15}NO_3$ addition within the perennial filter strip. Actual watershed dimensions and locations are given in Zhou et al. (2010).

depths were measured using a submersible level transmitter in the wells (Keller America, Inc.). Nitrate concentrations in groundwater and lysimeter samples were measured with a Quickchem 2000 Automated Ion Analyzer flow injection system with a 0.2 mg L^{-1} detection limit (Lachat Instruments).

Mesocosm $^{15}NO_3$ Addition Experiment

Installation and Vegetation Removal

The NO_3 sink strength (defined as the percentage of NO_3 inputs recovered in a given sink) of PFS plant biomass and SOM were measured in the field using an isotopic tracer in situ mesocosm procedure adapted from Dell et al. (2005). In March 2011, three pairs of polyvinyl chloride (PVC) cores (25-cm diameter × 30-cm height, open on both ends) were completely pushed into the soil in each PFS to serve as in situ mesocosms. The pairs of PVC cores were installed at random locations within 5 m of the upslope edge of each PFS and near the interface with the cultivated portions of the watersheds (Fig. 1). This placement was chosen based the assumption that N retention processes would be most important close to the interface between cropland and perennial vegetation as has been observed in many studies (e.g., Cooper, 1990; Lowrance, 1992; Hill, 1996) and subsequently confirmed at this site (Javed Iqbal, personal communication, 2014). Perennial vegetation was not substantially disturbed by mesocosm installation. Mesocosms were not installed in 100% cropland watersheds because the focus of this study was to evaluate the strength of NO_3 sinks within the PFS.

On 11 May 2011, one mesocosm from each pair was randomly selected for vegetation removal. Vegetation in these mesocosms (hereafter *devegetated mesocosms*) was cut to ground level. Glyphosate [*N*-(phosphonomethyl)glycine] solution (20% in water) was brushed onto cut vegetation with a paintbrush, and the cut aboveground biomass (AGBM) was then placed on the soil surface within the mesocosms. Perennial plants did not re-emerge in devegetated mesocosms for the remainder of the growing season, indicating that herbicide application was effective. Weed seedlings were removed manually from the devegetated mesocosms periodically throughout the growing season. Vegetation in the other mesocosm from each pair (hereafter *vegetated mesocosms*) was allowed to grow undisturbed throughout the growing season.

$^{15}NO_3$–N Addition

On 15 June 2011, 1 L of 3.63 mM KNO_3 (54.5 mg NO_3–N L^{-1}) at 98 atom % ^{15}N was injected into the soil in each mesocosm. Enriched $^{15}NO_3$ was applied during a period of high precipitation (Supplemental Fig. S1) when soil water-filled pore space was high (0.80–0.90 cm^3 cm^{-3} to 30-cm depth). This timing was chosen to simulate hydrologic NO_3 inputs from the cropland, which are expected to be greatest during periods of high precipitation. 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 per core were evenly distributed throughout each mesocosm. Solution injections were equivalent to 20.4 mm of rainfall and thus did not represent excessive water inputs. Based on soil moisture measurements, soil solution $^{15}NO_3$–N concentrations after injection ranged from 7 to 11 mg L^{-1}. These concentrations are well within the range of vadose zone NO_3–N concentrations measured upslope of these PFS in the cropland portions of the watersheds (Zhou et al., 2010) and thus, presumably, within the range of NO_3–N concentrations in subsurface flow entering these PFS.

Soil Organic Matter Collection and Analysis

To determine short-term recovery of $^{15}NO_3$–N in SOM pools, soil cores (2-cm diameter to 30-cm depth) were collected from within the mesocosms 7 d after $^{15}NO_3$ addition. To determine recovery of $^{15}NO_3$–N in SOM over the full growing season, the entire mesocosms were collected 137 d after $^{15}NO_3$ addition. At both times, collected soil was stored at 4°C until processing.

To remove inorganic N, extractable organic matter (OM), and microbial biomass, a sequential extraction procedure adapted from Holmes et al. (2003) was performed on the soils. Subsamples of 40 g fresh soil were sieved to 2 mm and shaken in 0.5 M K_2SO_4 (5:1 extract:soil ratio) to remove inorganic N and extractable organic matter. Microbial biomass was subsequently extracted from these soils by a direct chloroform ($CHCl_3$) 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 and soils were shaken to maximize $CHCl_3$ penetration into the soil pores. Fumigated soils were kept at room temperature in the dark for 5 d; after this, soils were subjected to vacuum venting to vaporize $CHCl_3$ then extracted by shaking in 0.5 M K_2SO_4. Recovery of $^{15}NO_3$–N in inorganic N, extractable OM, and microbial biomass was not determined because these represent very short-term N pools (e.g., Davidson et al., 1990; Zogg et al., 2000; Perakis and Hedin, 2001). Any $^{15}NO_3$–N transferred to these pools would be expected to enter another sink or be lost from the system on a short time scale.

Retention of $^{15}NO_3$–N in POM and MAOM was measured in soils collected at 7 and 137 d that had been sequentially extracted to remove inorganic N, extractable SOM, and microbial biomass as described above. 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 h. 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 (Moran et al., 2006). Both fractions were dried at 65°C. Total C and N content and ^{15}N enrichment of the soil fractions were determined with an elemental analyzer (Elementar Analysensysteme GmbH) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd.) at the Stable Isotope Facility at University of California, Davis. Separate soil samples were collected from outside of the mesocosms to determine natural abundance of ^{15}N in SOM pools; these samples were processed following the same protocol but separately to avoid ^{15}N contamination.

Plant Biomass Collection and Analysis

Plant AGBM from within the vegetated mesocosms was harvested on 30 Oct. 2011, 137 d after $^{15}NO_3$ addition. During collection, AGBM was separated by height (>15 cm and <15 cm) to determine percentage of AGBM that would be harvested with current site management. Both fractions of AGBM were oven-dried at 60°C for 4 d, weighed, and pulverized. Rhizomes, root mats, and roots >2 mm in diameter (hereafter, course root biomass [CRBM]) were collected manually from the

air-dried soil from the mesocosms collected at 137 d. Course roots were washed in 0.01 M $CaCl_2$ three times for 5 min to remove inorganic N from root surfaces, oven-dried at 60°C for 48 h, weighed, and pulverized. Subsamples (~800 g) of air-dried bulk soil were collected for fine root biomass (FRBM) analyses. Soils were placed in mesh containers (0.28-mm pore size) and washed in an elutriator. Fine roots and nonroot litter were floated out of the remaining course sand, and nonroot litter was removed manually. Fine roots were rewashed to remove remaining soil particles, dried at 60°C for 48 h, and then pulverized. Subsamples (3–5 mg) of pulverized AGBM, CRBM, and 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. Biomass from outside of the mesocosms was also collected to determine natural abundance of ^{15}N in AGBM, CRBM, and FRBM pools, and was processed following the same protocol, but separately to avoid contamination.

Isotope Calculations

Percentages of $^{15}NO_3$–N tracer recovered in plant biomass and SOM pools were calculated based on the masses of ^{15}N, masses of total N, and natural abundance F ^{15}N (defined as [mg ^{15}N]/[mg ^{15}N + mg ^{14}N]) measured for each pool. Equal assimilation of added $^{15}NO_3$–N and native NO_3–N (i.e., negligible fractionation) was assumed for all pools. The following equation was used to calculate percentage $^{15}NO_3$–N tracer recovered in each pool for each mesocosm:

% $^{15}NO_3$–N tracer recovered = [(mesocosm F ^{15}N in pool–natural abundance F ^{15}N in pool) × total mg N in pool/mesocosm]/(54.5 mg $^{15}NO_3$–N tracer added/mesocosm) × 100%

Denitrification Enzyme Activity

Soil samples (2-cm diameter to 30-cm depth) were collected from the paired watersheds on 25 June 2011 for DEA assay. Soils were collected from the proximity of the mesocosms in the PFS and from the equivalent toeslope topographic positions in 100% cropland watersheds (soybean in 2011). To determine soil NO_3 concentrations, subsamples of fresh soil 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). 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 1350 *g* for 15 min, and filtered to 0.45 μm. Extracted C concentrations were measured with a TOC-L CPN (Shimadzu).

Denitrification enzyme activity assay was performed on subsamples of fresh soil. The DEA assay protocol was based on Tiedje (1994). Soils were placed in 310-mL glass bottles and saturated in solution of 1 mM 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 32°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 potential DEA. Only linear portions of fluxes were included in rate calculations (Tiedje, 1994).

Statistical Analyses

Analyses of variance were performed using PROC GLM in SAS (SAS Institute, 2013). For the mesocosm soil data, time of collection was considered a random factor and vegetation treatment (vegetated vs. devegetated) was considered a fixed categorical factor. For the subsurface NO_3 concentration and DEA datasets, vegetation type (perennial vs. row crop) was considered a fixed factor, while sampling time was considered to be a random factor for the subsurface NO_3 dataset. Watershed pairs were considered blocks for all datasets. Correlations between NO_3 recovery in plant biomass and SOM sinks were found using R software (R Core Team, 2014).

Results

Lysimeters and Groundwater Wells

Water tables in the experimental watersheds reached 0 to 1 m below the surface in all PFS during periods of high precipitation in 2011 (Supplemental Fig. S2). During 2011, NO_3–N concentrations were significantly decreased in toeslope PFS compared with the toeslopes of the 100% cropland watersheds in lysimeter samples from 1-m depth (Fig. 2) and shallow groundwater samples (data not shown).

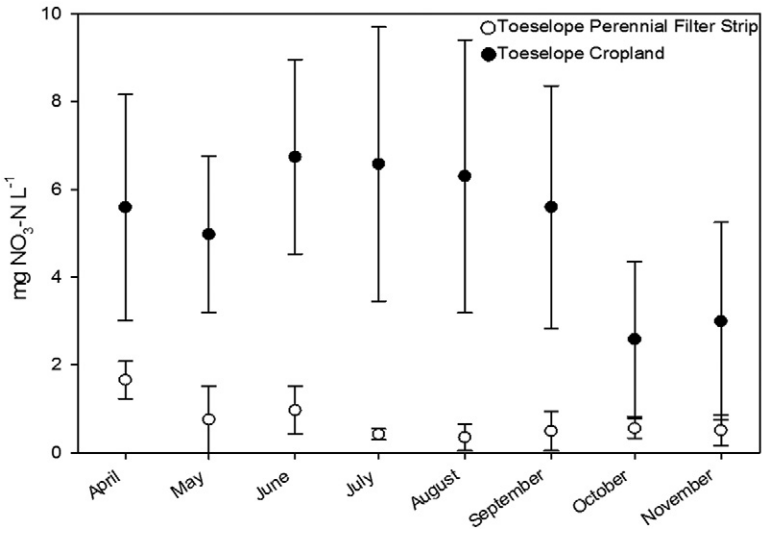

Fig. 2. Nitrate N concentrations in vadose zone solution collected from toeslope porous cup tension lysimeters at 1-m depth from watersheds managed as 100% row crops (black circles) or with toeslope perennial filter strips (white circles) in 2011. Error bars show standard errors of means of three replicates. Vadose zone NO_3–N concentrations differed significantly between vegetation types (P < 0.001).

Soil Organic Matter

Soil POM-N concentrations increased between 7 and 137 d, but there was an interactive effect of vegetation removal with time (Fig. 3). At 7 d, POM-N concentrations were greater in devegetated than vegetated mesocosms, but POM-N concentrations increased from 7 to 137 d in vegetated mesocosms but not in devegetated mesocosms. Soil POM-C concentrations did not differ between vegetation treatments and did not change between sampling times (data not shown). Carbon/nitrogen ratio of POM decreased between 7 and 137 d, but did not differ between vegetation treatments (Fig. 3). Soil MAOM-N and MAOM-C concentrations increased from 7 to 137 d across vegetation treatments, but there was no effect of vegetation treatment (Fig. 4). Carbon/nitrogen ratio of MAOM did not differ between vegetation treatments and did not change between sampling times (data not shown).

Greater percentages of $^{15}NO_3$–N tracer were recovered in MAOM (~4%) than POM (1–2%) at both 7 and 137 d (Fig. 5). Percentages of $^{15}NO_3$–N tracer recovered in POM and MAOM did not differ between vegetation treatments at 7 d. However, at 137 d, greater percentages of $^{15}NO_3$–N were recovered in POM and MAOM in vegetated than in devegetated mesocosms (though this interaction was not significant for MAOM) (Fig. 5). Total percentage of $^{15}NO_3$–N tracer recovered in SOM (POM + MAOM) in vegetated mesocosms ranged from 3 to 10% (Fig. 6).

Plant Biomass and Unrecovered ^{15}N

In all vegetated mesocosms, $^{15}NO_3$–N tracer was recovered in AGBM, CRBM, and FRBM (Fig. 6). Total percentages of $^{15}NO_3$–N tracer recovered in plant biomass ranged from 4 to 20% (Fig. 6). The percentage of $^{15}NO_3$–N tracer recovered in AGBM that would be harvested under current management (>15-cm height, harvested at the end of the growing season) ranged from 1 to 7%.

Across all vegetated mesocosms, total percentage of $^{15}NO_3$–N tracer unrecovered in plant biomass and SOM pools ranged from 70 to 92% (Fig. 6). Percentage of $^{15}NO_3$–N unrecovered correlated negatively with percentage recovered in total plant biomass (Pearson's correlation, $R = -0.94$, $P < 0.001$) and with percentage recovered in AGBM (Pearson's correlation, $R = -0.92$, $P < 0.001$). In contrast, percentage $^{15}NO_3$–N unrecovered was unrelated to percentage recovered in POM, MAOM, and total SOM in vegetated mesocosms.

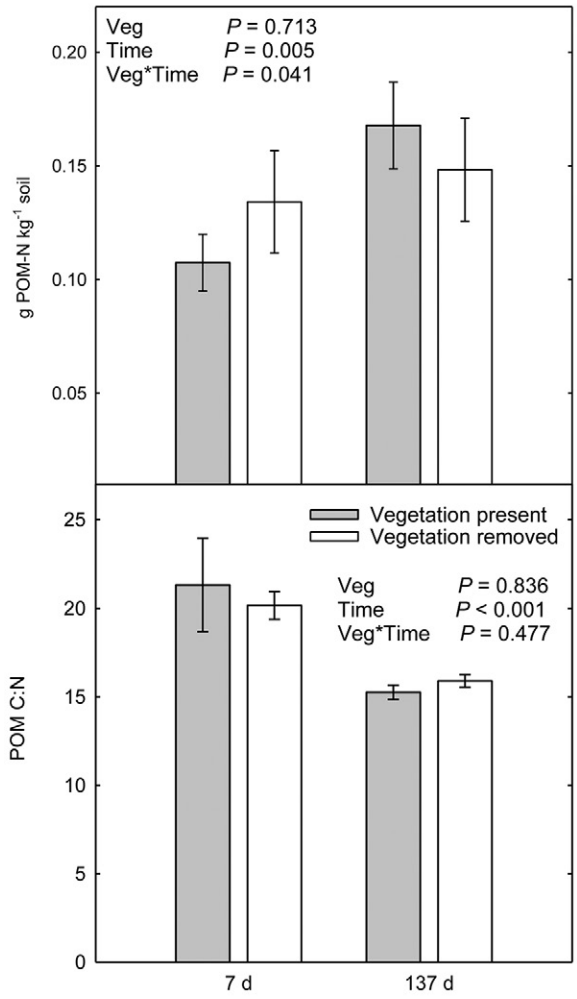

Fig. 3. Particulate organic matter nitrogen (POM-N) concentrations and carbon:nitrogen ratio (POM C:N) in mesocosm soils by vegetation treatment and time after $^{15}NO_3$ addition (7 and 137 d) with analysis of variance results. Error bars show standard error of means of nine replicates.

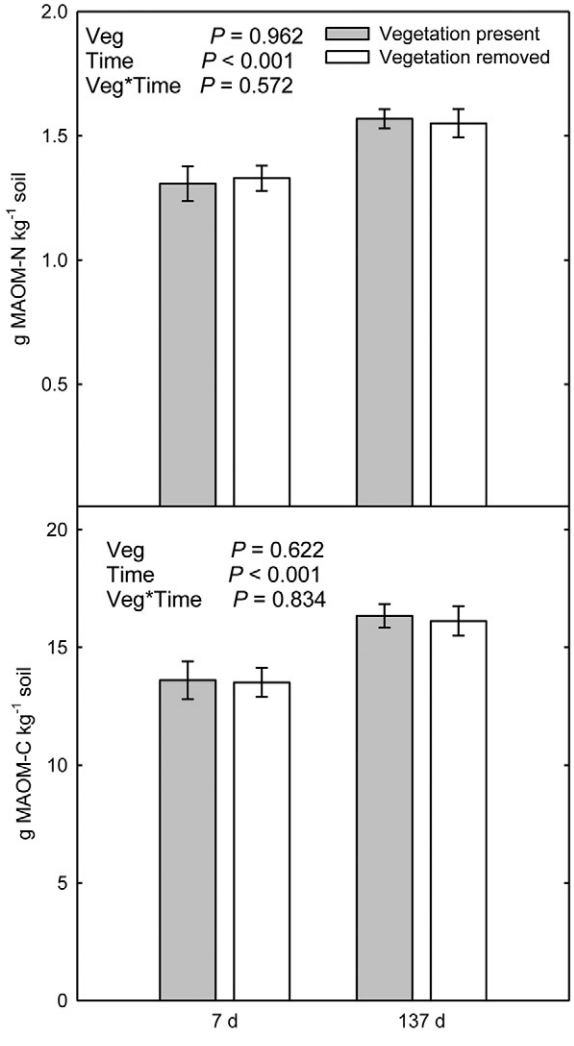

Fig. 4. Mineral-associated organic matter nitrogen (MAOM-N) and carbon (MAOM-C) concentrations in mesocosm soils by vegetation treatment and time after $^{15}NO_3$ addition (7 and 137 d) with analysis of variance results. Error bars show standard error of means of nine replicates.

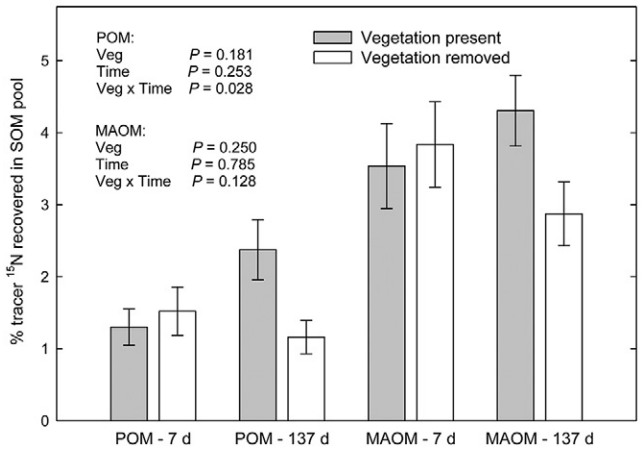

Fig. 5. Percentage of $^{15}NO_3$–N tracer recovered in particulate organic matter (POM) and mineral-associated organic matter (MAOM) in mesocosm soils by vegetation treatment and time after $^{15}NO_3$ addition (7 and 137 d) with separate analysis of variance results shown for POM and MAOM. Error bars show standard errors of means of nine replicates.

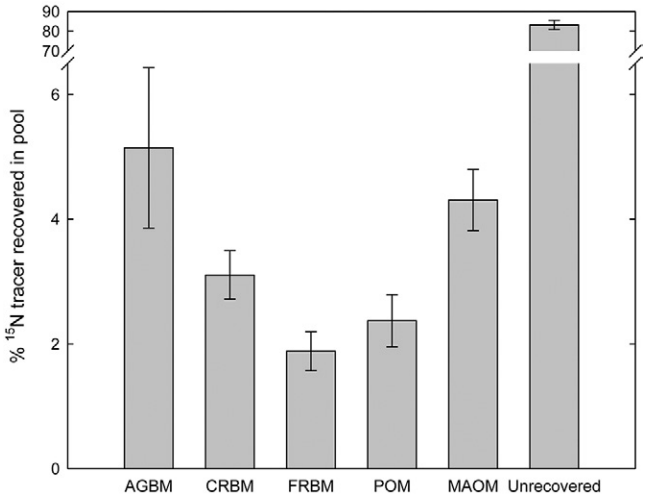

Fig. 6. Percentage of $^{15}NO_3$–N tracer recovered in aboveground biomass (AGBM), course root biomass (CRBM), fine root biomass (FRBM), particulate organic matter (POM), mineral-associated organic matter (MAOM), and unrecovered in vegetated cores 137 d after $^{15}NO_3$ addition. Note break and change of scale in the *y* axis. Error bars show standard errors of means of nine replicates.

Denitrification Enzyme Activity

Soil NO_3 concentrations were greater in cropland than PFS soils sampled for DEA (Fig. 7). However, DOC concentrations and potential DEA showed the opposite trend; DOC concentrations were approximately 300% greater and DEA was approximately 50% greater in PFS soils compared with cropland soils (though the difference was only statistically significant for DOC concentrations) (Fig. 7). Moreover, water-filled pore space was greater in PFS than cropland soils (Fig. 7).

Discussion

While perennial filter strips may decrease subsurface NO_3 losses from agricultural watersheds, their long-term effectiveness depends on the relative importance of soil organic matter, plant biomass, and denitrification as NO_3 sinks. In this study, we used $^{15}NO_3$ tracer to quantify the importance of SOM and

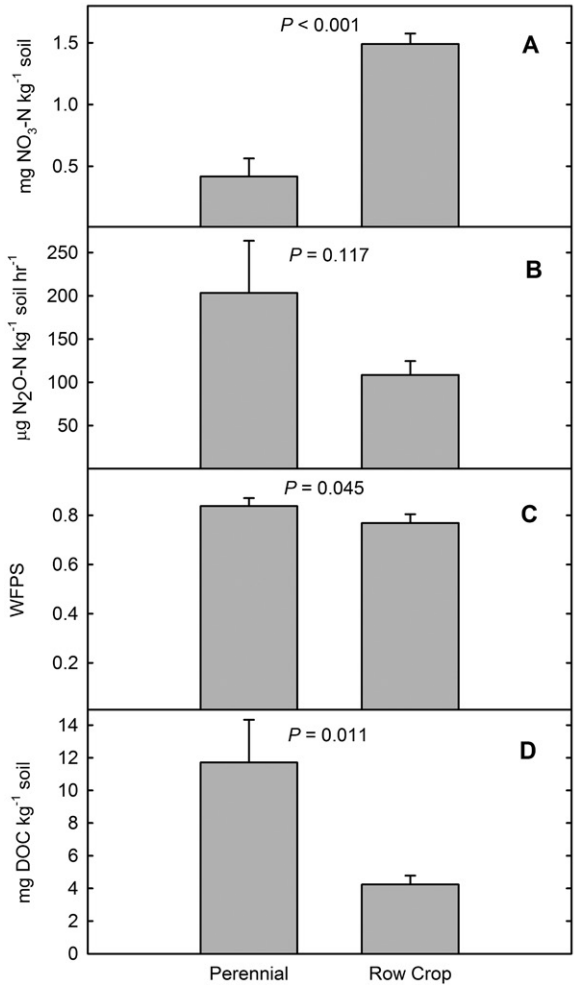

Fig. 7. Comparison of (A) NO_3–N concentrations, (B) N_2O production during denitrification enzyme activity assay, (C) water-filled pore space (WFPS), and (D) dissolved organic carbon (DOC) concentrations in soils from toeslopes of paired watersheds with toeslope perennial filter strips and watersheds managed as 100% cropland (soybean in 2011) collected 25 June 2011. Analysis of variance p-values are for comparisons between vegetation types (perennial filter strip vs. soybean). Error bars show standard errors of means of 9 replicates.

plant biomass as NO_3 sinks, as well as the relative importance of labile (POM) and stable (MAOM) SOM sinks, over one growing season. Relatively few studies have used this method in herbaceous PFS in agricultural settings (Bedard-Haughn et al., 2004, 2005; Davis et al., 2006; Zhao et al., 2009) and, to our knowledge, none in the Midwest region of the United States where PFSs are increasingly promoted as a management practice to decrease NO_3 pollution (Zhou et al., 2014).

Previous data from this site had shown substantial organic N accumulation in the toeslope PFS; between 2005 (before PFS establishment) and 2010, soil N increased up to 100% in the PFS (Perez-Suarez et al., 2014). Based on this evidence, we had hypothesized that SOM is the most important sink for subsurface NO_3 inputs to the PFS. However, in contrast to this hypothesis, we found SOM to be a minor NO_3 sink along with plant biomass. Instead, our results indicate that denitrification, which is the only NO_3 sink that does not saturate in the long term, was the most important NO_3 sink in these perennial vegetation strips over the timeframe of this study.

Subsurface Nitrate Removal by Perennial Filter Strips

This study and previous studies have provided strong evidence that the PFS at this research site are decreasing subsurface NO_3 losses from agricultural watersheds. Water table heights above mean sea level are greater at the upslope than toeslope positions in the watersheds at this site (Zhou et al., 2010), confirming that lateral subsurface flow occurs. While the PFS are located above the riparian zone, the water tables nonetheless reach within 0 to 0.5 m of the surface during periods of high precipitation, both during the year of this study and in previous years (Zhou et al., 2010). Together these data indicate that subsurface flow from the cultivated portions of the watersheds passes through the soil profiles of the PFS during periods of high precipitation.

During 2011, subsurface NO_3 concentrations were greatly decreased in toeslope PFS compared with toeslopes of 100% cropland watersheds in previous years (Zhou et al., 2010). The percentage decreases in subsurface NO_3 (up to 95% for lysimeter samples) were far greater than the percentage of watershed area removed from cultivation (10%) by PFS establishment. Decreased subsurface NO_3 concentrations in PFS can be caused by NO_3 retention in sinks or dilution of NO_3 in subsurface flow. While rates of subsurface dilution could not be calculated, we can infer that dilution did not occur in PFS relative to cropland because precipitation and hydrology did not differ, while evapotranspiration rates are greater from perennial vegetation than row crops (Hickman et al., 2010). Thus, it can be inferred that NO_3 entering PFS in subsurface flow is being removed by some combination of transfer to SOM, plant uptake, and denitrification.

Soil Organic Matter as Nitrate Sink

Between mid-June and late October, soil MAOM and POM-N increased in vegetated PFS mesocosms. During this time period, soil MAOM increased equally in devegetated and vegetated PFS mesocosms, confirming that new MAOM was derived from decomposition rather than plant growth (Hassink, 1997). Incorporation of $^{15}NO_3$–N tracer into POM and MAOM occurred within 7 d of addition and, on this time scale, was not affected by the presence of vegetation. Rapid (minutes to days) incorporation of NO_3–N inputs into SOM independent of plant uptake has been observed many times in forest soils and has been attributed to microbial assimilation (e.g., Stark and Hart, 1997; Zogg et al., 2000). Abiotic processes may also play a role in such rapid incorporation of NO_3 into SOM (Fitzhugh et al., 2003; Fricks et al., 2009). Similar to our study, Matheson et al. (2002) observed that rapid incorporation of $^{15}NO_3$–N into SOM did not differ between planted and unplanted PFS mesocosms.

In contrast to short-term recovery, plant growth did affect recovery of $^{15}NO_3$–N in SOM at the end of the growing season. Recovery in both POM and MAOM increased between 7 and 137 d in vegetated mesocosms, but not in devegetated mesocosms, though this difference was only statistically significant for POM. Plant growth may have affected $^{15}NO_3$–N transfer to SOM through uptake and subsequent litter deposition, incorporation into organic compounds derived from root exudates, or other effects of plant growth on microbial activity and SOM cycling.

Nevertheless, both MAOM and POM were relatively minor NO_3 sinks, together containing <10% of $^{15}NO_3$–N inputs to vegetated mesocosms at the end of the growing season. Thus, despite the substantial accumulation of N in these soils since PFS establishment (Perez-Suarez et al., 2014), we reject our hypothesis that SOM was the most important sink for subsurface NO_3 inputs, at least during the timeframe of our study. It is possible that SOM had retained more NO_3 inputs in the years immediately after PFS establishment. Additionally, SOM was sampled after only one growing season, and it is likely that ^{15}N retained in plant biomass during this growing season would eventually enter SOM on the scale of years or decades. However, the amount of $^{15}NO_3$–N retained in plant biomass was also quite constrained, as discussed below. Finally, it is possible that a fraction of $^{15}NO_3$–N inputs entered soluble SOM and subsequently leached from the soil. Further studies are needed to determine the magnitude of organic N leaching from these PFS and its contribution to watershed N loss.

Plant Biomass as Nitrate Sink

In vegetated mesocosms, AGBM was the largest directly measured sink for $^{15}NO_3$–N inputs at the end of the growing season, while root biomass was a smaller but less variable sink. Because recovery of $^{15}NO_3$–N in SOM and roots was relatively consistent between vegetated mesocosms, recovery in AGBM correlated closely and negatively with the amount of $^{15}NO_3$–N not recovered in any measured sink (unrecovered). However, similarly to SOM, plant biomass at the end of the growing season represented a minor NO_3 sink; total recovery in plant biomass represented 5 to 20% of $^{15}NO_3$–N inputs. Previous studies of herbaceous vegetation buffers have found plant uptake to vary greatly in importance as a NO_3 sink depending on vegetation type and management (Hefting et al., 2005; Zhao et al., 2009), ranging from a minor sink (Matheson et al., 2002; Davis et al., 2006) to the most important sink (Bedard-Haughn et al., 2004).

Plant biomass represents a temporary pool for N inputs unless biomass is harvested and removed from the watersheds. Given that <7% of $^{15}NO_3$–N tracer was recovered in the harvestable portion of AGBM at the end of the growing season, late-fall biomass harvest as practiced at this site is unlikely to provide a major NO_3 sink. Nitrogen in herbaceous plant biomass that is not harvested should enter SOM or mineralize on the scale of years to decades (Hefting et al., 2005).

Unrecovered ^{15}N and the Denitrification Nitrate Sink

Since the majority of ^{15}NO-N inputs to vegetated PFS soils were not recovered in SOM or plant biomass over one growing season, $^{15}NO_3$–N must have been removed from the mesocosms by some combination of denitrification and subsurface leaching. Since NO_3 concentrations in subsurface flow were substantially reduced (up to 95%) by PFS establishment, subsurface NO_3 leaching was not a major loss pathway in these PFS. Though organic N leaching may have removed some ^{15}N inputs, denitrification was most likely the major NO_3 sink. Denitrification has been determined to be the greatest NO_3 sink in many riparian vegetation buffers (Martin et al., 1999; Mayer et al., 2007), and the high water tables in these PFS during periods of high precipitation likely provided similarly favorable conditions for denitrification. If denitrification had accounted for 100% of unrecovered $^{15}NO_3$–N inputs to these 25- by 30-cm mesocosms, it would have been equivalent to 7 to 10 kg NO_3–N

denitrified ha^{-1}, well within denitrification rates observed in herbaceous perennial vegetation buffers receiving subsurface NO_3 inputs (Cooper, 1990; Lowrance et al., 1995; Clement et al., 2002; Hefting et al., 2003, 2004).

Results from the DEA assay showed that PFS soils had greater mean potential DEA than cropland soils despite substantially lower NO_3 concentrations, indicating that greater percentages of NO_3 were potentially lost to denitrification in PFS soils than cropland soils. While DEA data represent a potential and cannot be used to quantify denitrification rates in the field, these results nonetheless provide insight into the factors influencing denitrifier activity in the field. Greater DEA in PFS soils corresponded to greater DOC concentrations as well as slightly greater water-filled pore space compared with cropland soils. However, water-filled pore space was high (>0.70 cm^3 cm^{-3}) in both vegetation types at the time of soil collection. Thus, rather than a chronic difference in soil moisture, greater DOC availability is a more likely explanation for the greater DEA in PFS soils. Previous studies have identified C inputs from perennial vegetation to be a key factor in stimulating denitrification in soils receiving hydrologic NO_3 inputs (Schade et al., 2001; Davis et al., 2007). Chronic C inputs from vegetation can sustain high denitrification potentials in these soils, so that denitrification is limited by NO_3 availability (Cooper, 1990; Lowrance, 1992; Schipper et al., 1993) and thus can rapidly remove hydrologic NO_3 inputs.

This study provides an example of denitrification removing NO_3 from agricultural PFS soils over one growing season at one site. However, the role of denitrification as a NO_3 sink in similar settings elsewhere could vary greatly with climate, subsurface hydrology, vegetation type, soil type, and other factors (Martin et al., 1999). In addition, the importance of denitrification at this site could vary over longer timescales with weather, management, and other factors that influence denitrification rates. Furthermore, since the greenhouse gas nitrous oxide (N_2O) is one potential product of denitrification, conditions favorable to denitrification in PFS have the potential to change an aquatic pollutant to an air pollutant (Hefting et al., 2003; Stevens and Quinton, 2009). Further research is needed to determine the composition of gaseous N (N_2O/N_2) produced by denitrification in PFS and to determine how PFS can be managed to decrease both NO_3 leaching and N_2O emissions from agricultural watersheds.

Conclusions

We compared the NO_3 sink strengths of denitrification, soil organic matter, and plant biomass in perennial filter strips established within agricultural watersheds. Our results indicate that denitrification was the major NO_3 sink in these filter strips. While NO_3 inputs were retained in soil organic matter and plant biomass, these sinks were small in magnitude, and most NO_3 inputs were unrecovered. Dissolved organic C inputs from perennial vegetation likely provided substrate for denitrification in PFS soils. Denitrification can remove NO_3 from subsurface flow indefinitely as long as rates of NO_3 input do not exceed rates of potential denitrification. Thus, these results indicate that observed reductions in subsurface NO_3 losses from these watersheds will likely continue in the long term. As perennial filter strips are promoted as a management practice in row crop landscapes, further research is needed to determine whether these results could be replicated long term in PFS with differing climate, hydrology, pedology, and ecology.

Acknowledgments

The authors thank the following individuals for their assistance: Pauline Drobney, Carl Pedersen, Chris Witte, David Sundberg, Danielle Wilson, Teresita Chua, and Kirsten Hofmockel. Funding for the research site was provided by the Leopold Center for Sustainable Agriculture, Iowa State University College of Agriculture and Life Sciences, USDA Forest Service Northern Research Station, the Iowa Department of Agriculture and Land Stewardship, and the USDA–AFRI Managed Ecosystems program under proposal number 2011-00512. This research is part of a regional collaborative project supported by USDA–NIFA Award no. 2011-68002-30190, Cropping Systems Coordinated Agricultural Project (CAP): Climate Change, Mitigation, and Adaptation in Corn-based Cropping Systems. Project Web site: sustainablecorn.org.

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