Hydrologic connectivity increases denitrification in the hyporheic zone and restored floodplains of an agricultural stream

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[1] Stream ecotones, specifically the lateral floodplain and subsurface hyporheic zone, can be important sites for nitrogen (N) removal via denitrification, but their role in streams with constructed floodplains has not been examined. We studied denitrification in the hyporheic zone and floodplains of an agriculturally influenced headwater stream in Indiana, USA, that had floodplains added as part of a "two-stage ditch" restoration project. To examine the potential for N removal in the hyporheic zone, we seasonally measured denitrification rates and nitrate concentrations by depth into the stream sediments. We found that nitrate concentration and denitrification rates declined with depth into the hyporheic zone, but denitrification was still measureable to a depth of at least 20 cm. We also measured denitrification rates on the restored floodplains over the course of a flood (pre, during, and post-inundation), and also compared denitrification rates between vegetated and non-vegetated areas of the floodplain. We found that floodplain denitrification rates increased over the course of a floodplain inundation event, and that the presence of surface water increased denitrification rates when vegetation was present. Stream ecotones in midwestern, agriculturally influenced streams have substantial potential for N removal via denitrification, particularly when they are hydrologically connected with high-nitrate surface water.

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1. Introduction

[2] Human activities have doubled the availability of reactive nitrogen (N) on Earth [*Vitousek et al.*, 1997; *Galloway et al.*, 2003], with numerous consequences for freshwater ecosystems, including contaminated drinking water [*Fan and Steinberg*, 1996; *Ward et al.*, 1996], loss of freshwater biodiversity [*Carpenter et al.*, 1998], and periodic but recurring coastal hypoxic zones [*Diaz and Rosenberg*, 2008; *Rabalais et al.*, 2002]. Nitrogen loading to the Gulf of Mexico largely comes from the agricultural Midwest [*Alexander et al.*, 2008], where fertilizer inputs and artificial drainage facilitate the movement of excess N downstream. In much of the Midwest, subsurface tile drains rapidly convey water off fields and into channelized ditches [*Osborne and Wiley*, 1988]. Artificial drainage improves crop yields, but it also minimizes opportunities for biological processing and N removal prior to downstream export [*Randall et al.*, 1997; *Royer et al.*, 2006]. Enhancing biological N removal, while maintaining crop yields, can simultaneously maintain the economic function of the landscape and minimize negative environmental influences on downstream ecosystems.

[3] Denitrification is the microbial conversion of nitrate (NO_3^-) to nitrogenous gases [*Knowles*, 1982], and it permanently removes NO_3^- from the ecosystem [*Galloway et al.*, 2003]. Thus, maximizing denitrification, in either riparian or stream habitats, could improve downstream water quality by preventing the export of excess N. Denitrification occurs in the presence of NO_3^-, organic carbon, and anoxia, and as a result, denitrification rates are primarily controlled by the availability of these factors [*Arango et al.*, 2007; *Christensen et al.*, 1990; *Mulholland et al.*, 2009], which are in turn influenced by flow conditions, underlying geology, and land use characteristics [e.g., *Osborne and Wiley*, 1988; *Stanley and Boulton*, 1995]. In general, midwestern agricultural streams support high denitrification rates [*Inwood et al.*, 2005; *Roley et al.*, 2012; *Royer et al.*, 2004], but these systems are often saturated with NO_3^- from ongoing agricultural runoff. As a result, they are only able to denitrify a small portion of the NO_3^- load [*Bernot et al.*, 2006; *Mulholland et al.*, 2009; *Mulholland et al.*, 2008]. Therefore, in order for denitrification to reduce N exports from agricultural streams, N removal efficiencies (i.e., uptake relative to load) must be improved.

[4] Stream ecotones (i.e., the subsurface hyporheic zone and the lateral floodplain) can be hot spots for biogeochemical

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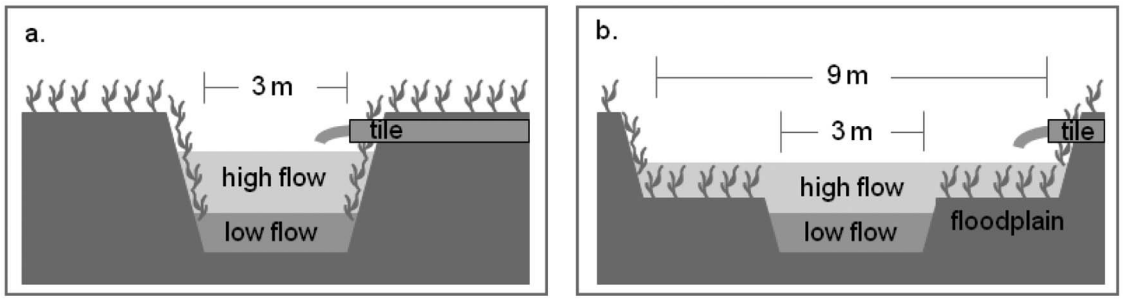

Figure 1. The two-stage ditch design: (a) Trapezoidal channel, prior to floodplain restoration. Note the tile drain entering the ditch, the steep sides, and the lack of floodplain. (b) Two-stage ditch. The dark gray represents water levels during base flow, and the light gray represents water levels during stormflow.

transformations, including denitrification [*Groffman et al.*, 2009; *Naiman and Décamps*, 1997; *Ocampo et al.*, 2006], but their role in N removal has received little study in agricultural streams with restored floodplains. We define the hyporheic zone as sediments deeper than 4 cm; sediments that do not directly interact with surface water. In rivers, substantial denitrification can occur in the hyporheic zone [*Fischer et al.*, 2005; *Hill et al.*, 2000; *Jones and Holmes*, 1996; *Puckett et al.*, 2008], but rates are often directly limited by NO_3^-, redox conditions, and electron donors [*Hill et al.*, 2000; *Puckett et al.*, 2008], and indirectly limited by flow conditions; groundwater–surface water interactions can influence NO_3^- delivery and redox conditions [*Predick and Stanley*, 2010]. Sampling for denitrification in small streams is typically limited to the surficial sediments, in the top 2–5 cm below the stream sediment interface [e.g., *Arango et al.*, 2007; *Christensen et al.*, 1990; *Royer et al.*, 2004], but results from the few studies in fine-grained, low-gradient streams suggest that denitrification can occur in the hyporheic zone, as well [*Inwood et al.*, 2007; *Stelzer et al.*, 2011]. Thus, typical stream sampling for denitrification potential may have underestimated reach-scale N removal, if denitrification occurs below 5 cm depth into the sediments [*Stelzer et al.*, 2011].

[5] In addition to the hyporheic ecotone, floodplains and riparian areas can remove groundwater or stream water NO_3^- via denitrification [*Forshay and Stanley*, 2005; *Pinay et al.*, 1993]. However, channelized agricultural streams typically have a trapezoidal channel where floodplains have been eliminated, and this channel morphology is maintained through periodic dredging (Figure 1a) [*Landwehr and Rhoads*, 2003]. The restoration of floodplains adjacent to channelized, agricultural streams is becoming more common in management circles and is known as "two-stage ditch" management (Figure 1b) [*Powell et al.*, 2007]. The two-stage channel increases water residence time and bioreactive stream surface area during floodplain inundation, which in turn increases the potential for reach-scale N removal via denitrification [*Roley et al.*, 2012]. The floodplains of a two-stage ditch are typically inundated numerous times per year [*Kallio*, 2010; *Roley et al.*, 2012], resulting in multiple opportunities for enhanced N removal during rain events, which is when most N export occurs [*Royer et al.*, 2006]. Reach-scale N removal during storms is strongly influenced by floodplain denitrification rates, because during inundation, the majority of stream surface area is floodplain. Therefore, identifying direct and indirect controls on floodplain denitrification rates is important for estimating the effectiveness of floodplains (restored or natural) on reach-scale N removal, thereby informing best management practices (BMPs) and providing insights into floodplain functioning, in general.

[6] Direct controls on floodplain denitrification rates include NO_3^- concentration [*Gift et al.*, 2010; *Pinay et al.*, 1993], soil organic matter content [*Gift et al.*, 2010; *Groffman and Crawford*, 2003; *Sheibley et al.*, 2006], and soil moisture, via its influence on redox conditions [*Groffman and Crawford*, 2003; *Groffman et al.*, 1992; *Machefert and Dise*, 2004]. Indirect controls on floodplain denitrification potentially include duration of inundation and floodplain vegetation cover and type (e.g., riparian grasses versus wetland plants). Denitrification generally increases with NO_3^- concentration in both floodplains and streams [*Gift et al.*, 2010; *Mulholland et al.*, 2008; *Pinay et al.*, 1993], so the influx of high- NO_3^- stream water could increase denitrification rates during floods. Furthermore, over the course of an inundation event, soil redox conditions become more suitable for denitrification [*Ensign et al.*, 2008], potentially increasing rates as microbes synthesize new denitrifying enzymes in response to changing environmental conditions [*Brock*, 1961]. However, some previous studies have shown no relationship between floodplain denitrification rates and NO_3^- availability [*Watson et al.*, 2010; *Orr et al.*, 2007], and *Roley et al.* [2012] found that the addition of stream water usually had no effect on denitrification rates in floodplain soils. Therefore, further experimentation is necessary to understand how floodplain soil denitrification responds to inundation in agriculturally influenced streams.

[7] In addition to the influence of subsurface soil conditions, vegetation may also influence floodplain denitrification through several pathways which include: (1) evapotranspiration can lower the water table and aerate the soil, creating oxidizing conditions not conducive to denitrification [*Schilling and Jacobson*, 2009]; (2) plants can take up NO_3^- and assimilate it into their tissues, making it unavailable for denitrification [*Pinay et al.*, 1993]; or (3) alternatively, roots could provide a source of labile organic carbon, thereby enhancing floodplain denitrification rates [*Pinay et al.*, 1993; *Gift et al.*, 2010]. The two-stage ditch floodplains are somewhat unique, however, as they typically harbor herbaceous vegetation, rather than trees, and they flood regularly throughout the year. As a result, vegetation combined with frequent floodplain

Table 1. Summary of the Experiments Completed in This Study

Experiment	Sampling Dates	Sampling Depth	Treatments[a]	Substrates/Habitat[b]	Explanatory Variables[c]
1 Stream Sediment Depth	Fall 2008; winter, spring, and summer 2009	≥20 cm, in 4 cm intervals	+SW	sand, FBOM	PW NO_3^- sediment OM
	September 2008	≥20 cm, in 4 cm intervals	+SW, +PW	sand	PW NO_3^- sediment OM
2 Floodplain Inundation	Base flow: Pre-flood and post-flood (1 date)	0–5 cm	Dry, +SW, +SW+C, +SW+N, +SW+N+C	FS	soil OM soil exchangeable NO_3^-
	Stormflow: Days 1, 2, and 4 of a floodplain inundation	0–5 cm	+SW, +SW+C, +SW+N, +SW+N+C	FS	soil OM soil exchangeable NO_3^-
3 Floodplain Vegetation	Winter 2007; summer and fall 2008; summer 2009; spring and fall 2010	0–5 cm, 5–10 cm	Dry, +SW	Veg FS, Non-Veg FS	soil OM soil exchangeable NO_3^- CQI soil moisture
	Spring 2010	0–5 cm, 5–10 cm	Dry, +SW, +SW+C, +SW+N, +SW+N+C	Veg FS, Non-Veg FS, Roots FS	soil OM soil exchangeable NO_3^- CQI soil moisture
	Summer 2009	0–5 cm, 5–10 cm	Dry, +SW	6 vegetation categories	soil OM soil exchangeable NO_3^- soil moisture

[a]Experimental treatments include: Dry = no water added to soils during assay, +SW = surface water added, +PW = pore water added, +C = glucose amendment, +N = nitrate amendment, +N+C = nitrate and glucose amendments.
[b]Abbreviations are as follows: FBOM = fine benthic organic matter, FS = floodplain soils, Veg FS = floodplain soils with vegetation, Non-Veg FS = floodplain soils without above- or below-ground vegetation (which occurred naturally or through experimental manipulation), Roots FS = floodplain soils with below-ground vegetation only.
[c]Abbreviations are as follows: PW NO_3^- = pore water nitrate, OM = organic matter, CQI = carbon quality index (CO_2 production/N_2O production).

inundation may interact to create unique conditions for denitrification that deserve further study.

[8] We completed a series of experiments that identified controls on denitrification rates in the hyporheic zone and restored floodplains of the two-stage ditch. In doing so, we determined if hydrological connection to existing and newly constructed ecotones would enhance N removal via denitrification. Specifically, we addressed the following research questions: (1) When and where does N removal via denitrification occur in the hyporheic zone?; (2) Does floodplain inundation increase denitrification rates?; and (3) Are floodplain denitrification rates influenced by the presence and type of vegetation?

2. Methods

2.1. Site Description

[9] We conducted all denitrification experiments at Shatto Ditch, an agriculturally influenced stream in north-central Indiana, USA. More than 80% of its catchment is tile-drained, row-crop agriculture, and Shatto Ditch has historically been managed for the purpose of draining surrounding fields. Like many ditches, it had a trapezoidal channel maintained through periodic dredging, a resultant flashy hydrograph, and consistently high concentrations of dissolved inorganic nitrogen (NO_3^- concentrations ranged from 1.6 to 11 mg N L^{-1}).

[10] In November 2007, we restored lateral floodplains along a 600 m reach of Shatto Ditch, using the management strategy known as the two-stage ditch [*Powell et al.*, 2007]. On average, the base flow channel width measures 2.7 m, and the restored floodplains are 3 m wide on both sides of the stream (Figure 1b) with a mean floodplain height of 0.4 m, as measured from the stream bottom at the center of the channel to the bottom of the floodplain. After the floodplains were constructed, a native seed mix, including *Schizachyrium scoparium*, *Elymus canadensis*, *Rudbeckia hirta*, *Solidago rigida*, *Carex frankii*, *Carex lurida*, *Carex trichocarpa*, *Juncus effusus*, and *Eupatorium perfoliatum*, was scattered on the floodplains to promote vegetative growth, floodplain stability, and native biodiversity. Although native species established on some parts of the floodplain, a common invasive, *Phalaris arundinacea* (reed canary grass), is the dominant species in many places. In low spots on the floodplain, wetland plants (*Typha angustifolia*, *Juncus effusus*, and *Leersia oryzoides*) have also established.

[11] To assess the efficacy and controls of N removal in the ecotones of this restored agricultural stream, we conducted 3 denitrification experiments. We measured denitrification seasonally in the hyporheic zone, over the course of a floodplain inundation event, and seasonally in vegetated and non-vegetated floodplain plots (Table 1). First, we describe the denitrification assay, in general, and then the specific details of each experiment.

2.2. Denitrification Assays

[12] For all of the denitrification measurements (field sampling methods for sediments/soils are described below), we used the redox-optimized, chloramphenicol-amended, acetylene block technique [*Smith and Tiedje*, 1979; *Royer et al.*, 2004; *Inwood et al.*, 2005; *Roley et al.*, 2012]. Acetylene (C_2H_2) was used to block the conversion of nitrous oxide (N_2O) to molecular nitrogen (N_2), allowing the easily measured N_2O to accumulate in assay bottles. The addition of chloramphenicol prevents the de novo synthesis of denitrifying enzymes [*Brock*, 1961], and limits the "bottle effects" of laboratory assays [*Bernot et al.*, 2003; *Smith and Tiedje*, 1979; *Tiedje et al.*, 1989]. This technique is useful for experimental work, because it allows for the simultaneous measurement of numerous replicates over both space and time [*Arango et al.*, 2007; *Bruesewitz et al.*, 2009; *Findlay et al.*, 2011; *Inwood et al.*, 2005, 2007].

[13] For each sample replicate, we placed 25 mL of sediment or soil into a 156 mL glass media bottle, equipped with a rubber septum cap. We added 45 mL of unfiltered stream water and 5 mL of 3.1 mM chloramphenicol, to achieve a final slurry concentration of 0.21 mM chloramphenicol [*Bruesewitz et al.*, 2009; *Roley et al.*, 2012]. Next, we sealed all bottles with septum caps and sparged with ultra-high purity N_2 gas for 5 min, swirling regularly to remove oxygen. We vented each bottle to return it to atmospheric pressure, and then added 15 mL of C_2H_2 gas to achieve a headspace concentration of 10% C_2H_2. We created the C_2H_2 gas in the laboratory, by adding deionized water to calcium carbide [*Arango and Tank*, 2008].

[14] We incubated the assay bottles at room temperature for 4 h, taking a sample approximately every hour (samples at 0.25, 1.25, 2.25, 3.25, and 4.25 h). We shook each bottle prior to sampling, removed 5 mL of headspace with a syringe, and injected the sample into an evacuated 3 mL serum vial that was capped with a rubber septum (Wheaton, Millville, NJ). We maintained pressure inside each assay bottle by injecting 5 mL of 10% C_2H_2 (balance of N_2) after each sampling period.

[15] We measured the N_2O and CO_2 concentrations in the serum vials, and used the N_2O concentrations to calculate denitrification rates (see following paragraph), and the CO_2 concentrations to calculate the carbon quality index (CQI; see below). We measured both N_2O and CO_2 with a Varian CP-3800 gas chromatograph, equipped with a thermal conductivity detector (TCD), an electron capture detector (ECD) (Varian, Inc, Walnut Creek, CA), a Haye SepQ column (AllTech, Deerfield, IL), a valve to vent water and C_2H_2 away from the detectors, and a CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland). The injector was set at 50°C, the oven at 50°C, the TCD at 120°C, and the ECD at 300°C. The ECD was used to measure N_2O, and the carrier gas was ultra-high purity N_2. The TCD was used to measure CO_2, and the carrier gas was ultra-high purity helium.

[16] To calculate denitrification rates, we first accounted for gases dissolved in the slurry by applying Bunsen coefficients [*Inwood et al.*, 2005]. Next, we plotted N_2O production over time, and took the slope of the best fit linear regression to get a production rate (units: μg N_2O-N h^{-1}). We scaled the N_2O production rates by dividing by grams of dry mass (units: μg N_2O-N g DM^{-1} h^{-1}), grams of ash-free dry mass (units: μg N_2O-N g $AFDM^{-1}$ h^{-1}), and stream surface area (units: μg N_2O-N g m^{-2} h^{-1}) represented by each replicate assay bottle.

[17] To determine if denitrification was limited by NO_3^- or organic carbon, we completed nutrient limitation assays during some of our experiments. In these denitrification assays, we added carbon and nitrate singly and in combination, creating three additional treatments: +C, +N, and +C +N. We prepared these samples as described in the previous paragraphs, except that we used nutrient-amended chloramphenicol to assay bottles as follows: +N treatments with NO_3^- as KNO_3^- (10 mg NO_3^--N L^{-1}), and + C treatments with dissolved organic carbon (DOC) added as glucose (24 mg C L^{-1}) [*Royer et al.*, 2004; *Bruesewitz et al.*, 2009].

[18] On all dates, except the summer 2009 vegetation assays, we calculated the CQI as the CO_2:N_2O production ratio. The CQI can be interpreted as the following: a higher ratio corresponds to lower carbon quality, because it requires more carbon to be oxidized per mole of NO_3^- reduced [*Pfenning and McMahon*, 1997]. In using the CQI, we assumed that other processes that produced CO_2 (e.g., sulfate reduction, carbonate dissolution) were equal among treatments and sampling dates. We suggest that this is a reasonable assumption because the redox states and pH of all bottles were similarly poised, as a result of all being sparged in the same way and having the same surface water added. In addition, we only used the CQI when NO_3^- was presumably not limiting; that is, on samples that were incubated with surface water. We calculated CO_2 and N_2O production in μmol h^{-1}, using Bunsen coefficients and the best fit linear regression, as described above.

2.3. Experiment 1: Denitrification by Depth in Stream Sediments

[19] To determine where and when denitrification was occurring in the hyporheic zone, we seasonally measured denitrification and pore water NO_3^- concentration with depth (Table 1). On each of four sampling dates, we retrieved pore water samples and sediment samples from the two dominant substrates in the stream channel: sand and fine benthic organic matter (FBOM).

[20] We obtained the NO_3^- depth profile by deploying multichambered equilibrium dialysis samplers, i.e., peepers [*Hesslein*, 1976, *Teasdale et al.*, 1995]. Our peepers were constructed from a solid piece of clear plastic, punctuated with 19 or 27 wells (1 cm height × 5 cm width × 3 cm depth), located 1 cm apart. We filled the peeper wells with deionized water and attached a Biodyne nylon membrane (0.2 μm pore size, Pall, Ann Arbor, MI) while submersed in deionized water, taking care not to introduce any air bubbles. We placed the peepers in a large zippered plastic bag filled with deionized water, and bubbled N_2 gas into the bag to remove dissolved oxygen [*Teasdale et al.*, 1995]. We then deployed the peepers vertically in the streambed, so that two or three wells were located above the sediment, and the remaining wells extended into the sediments, to a depth of 50 cm (27-well peepers) or 34 cm (19-well peepers). We placed 7 total peepers each season, spaced at approximately even intervals along the reach, with at least 2 peepers each in sand and FBOM. We left them in place for at least 2 weeks to allow equilibration with the pore water [*Webster et al.*, 1998]. Upon retrieval of each peeper from the streambed, we washed off any remaining sediment with deionized water, and then removed the water from each well by puncturing the membrane with a needle, and drawing the water out with a syringe. We placed the water samples on ice for transport to the laboratory, after which we froze the water samples for later analysis.

[21] On each date of peeper retrieval, we also collected sediment cores for denitrification assays. Using a PVC corer, we retrieved one core next to each peeper, and each sediment core was at least 20 cm deep. In the field, we extruded the core onto a plastic tray, sliced it at 4 cm intervals, and returned the core sections to the laboratory. We used 4 cm intervals because it corresponded to the well depths on the peepers and allowed us to obtain sufficient sediment for denitrification. We then measured denitrification rates on each core section using the denitrification assay described above. We used stream surface water for the sediment slurries, because there was not sufficient volume in the peeper

wells for both water chemistry analyses and denitrification assays.

[22] In addition to the denitrification rates obtained from individual sediment samples, we also calculated the rate of decline in denitrification with sediment depth (−k). For each season and substrate, we plotted the natural logarithm of denitrification rates versus depth and calculated −k as the slope. This calculation is similar to a decay coefficient commonly used in decomposition studies [*Benfield*, 2006]. In a typical peeper profile, NO_3^- declined rapidly, and then remained relatively constant at a low concentration. In many of the profiles, NO_3^- concentrations declined too rapidly to calculate slope (i.e., the decline occurred in the first 1 or 2 wells below the sediment-water interface). As a result, we did not calculate −k for NO_3^-, but instead compared the depth at which the decline stopped, and the concentration below the initial decline.

[23] Ideally, we would use pore water, instead of surface water, in our denitrification assays; however, there was not sufficient volume in the peepers to do so. To determine if denitrification rates were influenced by the use of stream surface water, we conducted an experiment comparing denitrification rates on sediments incubated with pore water versus surface water. In September 2008, we deployed all the peepers within 1 m^2 and pooled the water from wells at corresponding depths to obtain a single depth profile. We used the pooled water samples for both the denitrification assay and water chemistry analysis. We obtained two sediment cores, sliced into 4 cm sections, and combined and homogenized the corresponding sections. For each section, we placed 25 mL of sediment each in two bottles. We added stream surface water to one bottle and pore water from the corresponding depth to the other, and then measured denitrification rates as described in the previous section.

2.4. Experiment 2: Floodplain Denitrification Rates in Response to a Storm Inundation Sequence

[24] To determine if floodplain denitrification rates changed over the course of a floodplain inundation event, we collected floodplain soil samples prior to, during, and after a 5 May 2010 storm that caused floodplain inundation. At 5 evenly spaced sites, we collected 15 sediment cores, each 5 cm long, with a metal soil corer (diameter = 1.8 cm). In the lab, we homogenized the cores from within each site, and placed 45 mL of soil into each of 4 assay bottles, for measuring denitrification and nutrient limitation. We completed the denitrification and nutrient limitation assays 3 times over a four day inundation, as well as 2 weeks before and 1 month after the flood, during base flow conditions (no water on the floodplains). During base flow conditions, we added an additional treatment: to one set of bottles, we did not add any surface water ("Dry"), in order to determine soil denitrification rates when floodplains were not inundated. Each time, we measured denitrification rates within 16 h of field collection of soil samples.

2.5. Experiment 3: The Influence of Vegetation on Floodplain Denitrification Rates

[25] To determine the influence of vegetation on floodplain denitrification rates, we conducted 6 denitrification assays, during winter 2007; summer of 2008; summer of 2009; and spring and fall of 2010, on vegetated and non-vegetated plots along the restored floodplains in Shatto Ditch. For the vegetated plots, we chose areas in the floodplain in which plants had fully established. In December 2007 and July 2008, we compared vegetated areas with areas that had not yet vegetated after the restoration ("unvegetated"). As vegetation colonized the floodplains, there were fewer places completely devoid of vegetation, and these areas seemed to have atypical soil characteristics for the site. Rather than compare vegetated plots to those with uncharacteristic soils, we established plots in which we removed all aboveground and most of the below-ground biomass ("de-vegetated"), and covered the plot with shade cloth to prevent vegetative regrowth. After removing the vegetation, we waited at least two weeks before sampling floodplain soils for denitrification assays. During summer 2009, and spring and fall 2010, we also established plots in which we removed only the aboveground biomass, and left the belowground roots intact. In 2009, we sampled both de-vegetated plots and unvegetated plots, and also further investigated the influence of different types of floodplain vegetation (Table 1). In doing so, we established and sampled 6 different plot types: (1) Invasive, where *Phalaris arundinacea* (reed canary grass) had established; (2) Native, with native forbs and grasses, including *Elymus canadensis* (Canada wild rye), *Carex frankii* (bristly cattail sedge), *Rudbeckia hirta* (black-eyed Susan), and *Eupatorium perfoliatum* (common boneset) had established; (3) Wetland, where common wetland species, including *Typha angustifolia*, (narrow-leaved cattail), *Juncus effusus* (common rush), and *Leersia oryzoides* (rice cutgrass) had established; (4) De-vegetated, where we manually removed aboveground and below-ground vegetation; (5) Roots, where we manually removed aboveground biomass, leaving only roots; and (6) Unvegetated, where vegetation had not established after the restoration.

[26] Because plot size and number varied across experiments, and depending on floodplain conditions, we summarize these details in Table 2 for reference. For example, in December 2007, we did not establish plots, because the vegetation existed only in a narrow strip along the edge of the stream. In July 2009, we established just one plot per vegetation treatment, because we included some unique habitats that only existed in a limited area of the floodplain. In contrast, in Spring and Fall 2010, we established 5 plots of each type.

[27] We sampled for denitrification with a PVC soil corer, as described previously. We extruded the core onto a plastic tray, and sliced each core into two 5-cm sections (0–5 cm and 5–10 cm). On all sample dates, we assessed the response of soils to surface water inundation. To do so, we collected pairs of cores immediately adjacent to each other, and incubated one core with stream water, and the other at ambient soil moisture. On all dates and for all experimental treatments, we collected 5 replicate samples for denitrification assays.

2.6. Ancillary Physicochemical Variables

[28] On all sampling dates, we measured soil organic matter content, soil gravimetric water content, and surface water and soil exchangeable NO_3^- concentrations. We measured organic matter content by drying a soil subsample for ≥48 hrs at 60°C, or until constant mass, and then combusting for 2 h at 550°C. Gravimetric water content was measured as

Table 2. Description of Plots Used in Vegetation Experiments

Sampling Date	Vegetation Treatment[a]	Plot Size (m^2)	Number of Plots per Vegetation Treatment	Number of Replicates per Experimental Treatment[b]
Winter 2007	Veg, Un-Veg	NA[c]	NA[c]	5
Summer 2008, Fall 2008	Veg, Un-Veg	1	1	5
Fall 2008	Veg, De-Veg	1	1	5
Summer 2009	De-Veg, Un-Veg, Roots, Natives, RCG, Wetland	0.5	1	5
Spring 2010, Fall 2010	Veg, De-Veg, Roots	0.5	5	5

[a]Abbreviations are as follows: Veg = vegetation present, Un-Veg = vegetation not established, De-Veg = above- and below-ground vegetation removed, Roots = aboveground vegetation removed, Natives = a mix of native plants established, RCG = reed canary grass established, Wetland = wetland plants established. See text for species.

[b]Experimental treatments include depth and laboratory nutrient additions, as described in Table 1. We used the same number of replicates in all treatments.

[c]NA = not applicable. In Winter 2007, vegetation was only present in a thin strip along the edge of the stream, and so we sampled at evenly spaced points along the stream, rather than establishing plots.

the difference between soil mass immediately after collection, and soil mass after drying for ≥1 week at 60°C. We collected surface water samples by filtering 60 mL of stream water through glass fiber filters (1 μm nominal pore size, Pall, Ann Arbor, Mich.) into acid-washed, filtered stream water-rinsed, high-density polyethylene bottles, transported them to the lab on ice, and froze them for later analysis. We extracted soil NO_3^- by adding 40 mL of 2M KCl to 4 g of soil at field moisture, placing on a shaker table for 1 h at 100 rpm, and filtering and freezing the supernatant [*Soil Science Society of America*, 1996]. We later analyzed the extracted soil samples, the peeper samples, and the surface water samples for NO_3^- on a Lachat Flow Injection Autoanalyzer (Lachat Instruments, Loveland, Colo.), using the cadmium reduction method [*American Public Health Association*, 1995].

2.7. Statistical Analyses

[29] For Experiment 1: Stream Sediment Depth (Table 1), we determined the pore water NO_3^- concentration after the decline, and the depth at which the NO_3^- decline stopped. We compared all of these metrics, by season and substrate, with a repeated-measures analysis of variance (RM ANOVA), in which the factor was substrate. We compared the rates of decline of denitrification with depth ($-$k) with an analysis of covariance (ANCOVA), in which the factor was season or substrate and the covariate was depth.

[30] For the base flow samples (i.e., collected when the floodplains were not inundated) in Experiment 2: Floodplain Inundation, we used a one-way ANOVA, with Tukey's post-hoc test, to determine if denitrification rates on floodplain soils increased with the addition of surface water, NO_3^-, and glucose. During floodplain inundation, when the "Dry" treatment was eliminated, we used a two-way ANOVA to determine if nutrient limitation occurred [*Tank and Dodds*, 2003]. We completed these analyses within individual dates, because our intent was to see how frequently nutrient limitation occurred, rather than if denitrification rates were changing over time.

[31] For Experiment 3: Floodplain Vegetation (Table 1), we used a three-way RM ANOVA to analyze differences in denitrification rates between vegetated and non-vegetated plots, and the factors were depth, vegetation, and surface water. We also used RM ANOVA to compare soil organic matter content, CQI, and soil moisture between vegetated and non-vegetated plots. We used a three-way ANOVA (factors: depth, surface water, and plot type) to compare denitrification rates among vegetation types in Summer 2009 (Table 1).

[32] To assess relationships between denitrification rates and explanatory variables (soil and sediment organic matter content, soil gravimetric water content, pore water NO_3^- concentration), we used simple linear regression (SLR). We also used SLR to determine if there were changes in denitrification rates over the course of the floodplain inundation.

[33] We tested all data for normality with the Shapiro-Wilk test ($p > 0.05$) and for homogeneity of variances with Levene's test ($p > 0.05$). When data did not meet parametric assumptions, we transformed accordingly. We were able to successfully transform and meet assumptions for all data used in ANOVAs but not for all SLRs. When the transformed data did not meet the statistical assumptions for regression, we rank-transformed the data and completed the regression on the ranks [*Iman and Conover*, 1979]. All of the statistical tests described in the previous paragraphs were completed with SYSTAT 12 (SYSTAT Software, Chicago, IL).

[34] Because many of our soil NO_3^- concentrations were below detection and had a non-normal distribution, we used the non-parametric Kendall's tau to determine correlations between denitrification and soil NO_3^- concentrations. We used the Peto-Prentice test of differences to compare NO_3^- concentrations among plot types [*Helsel*, 2005]. For both of these tests, we used R version 2.11.1 (The R Foundation for Statistical Computing) and version 1.5–3 of the Non-Detects and Data Analysis (NADA) package.

3. Results

3.1. Experiment 1: Denitrification by Depth in Stream Sediments

[35] Within each stream sediment depth profile, pore water NO_3^- concentration declined rapidly with depth, and then remained relatively constant (changing <10 μg L^{-1} to the bottom of the profile) at a concentration that was <0.01 times that of the surface water concentration (Figure 2). On average, pore water NO_3^- concentrations declined rapidly until they reached a mean depth of 7 ± 1 cm (mean ± SE) beneath the sediment surface, reaching an average minimum concentration of 23 ± 3 μg NO_3^--N L^{-1} (mean ± SE), although the minimum concentration varied by season (RM ANOVA, $p < 0.05$);

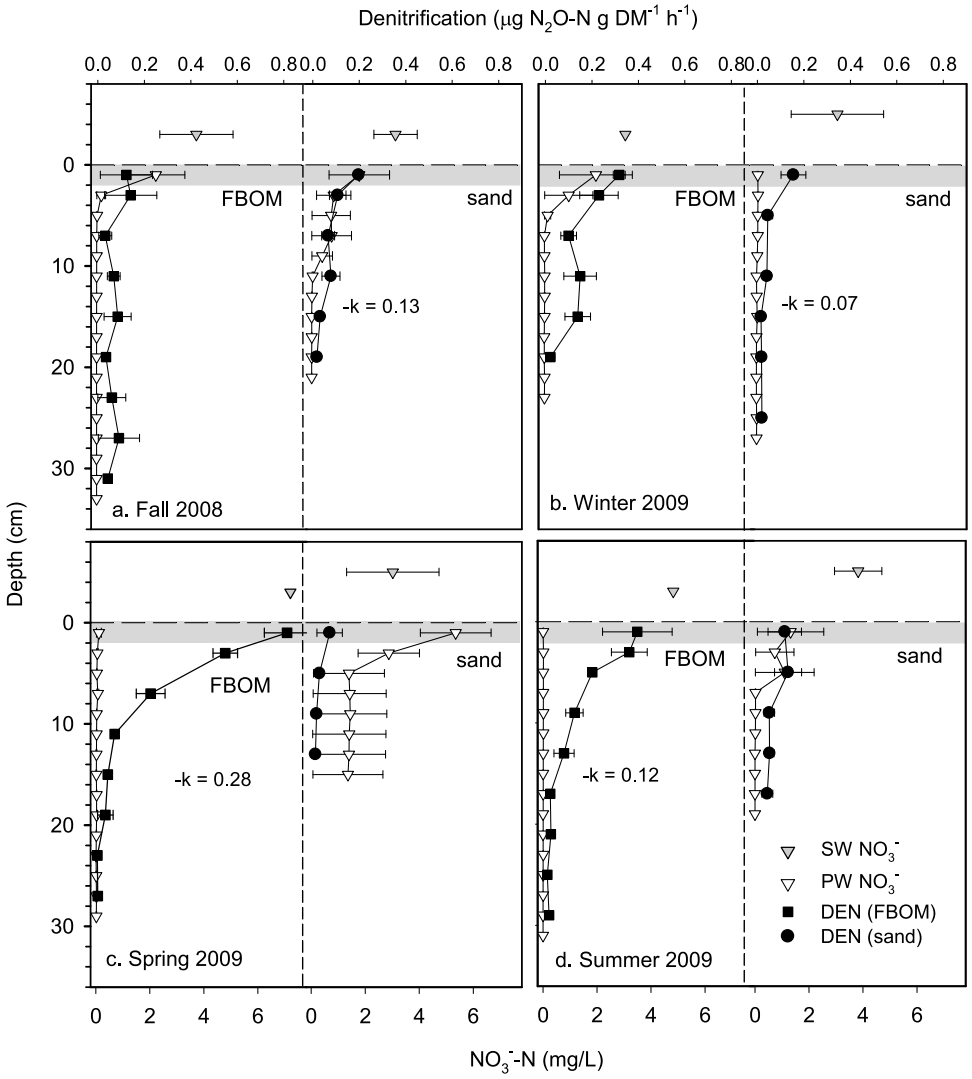

Figure 2. Denitrification and NO_3^- concentration by depth in (a) Fall 2008, (b) Winter 2009, (c) Spring 2009, and (d) Summer 2009. The left half of each panel shows denitrification rates on FBOM, and the right half on sand. The dashed horizontal line indicates the sediment-water interface, and the shaded area indicates the top 4 cm of sediment, the area sampled in a typical denitrification assay. Error bars represent the standard error of the mean. The rate of decline in denitrification with depth (−k) is shown when there was a statistically significant decline. SW = surface water, PW = pore water, sampled from the peepers, DEN = denitrification, and FBOM = fine benthic organic matter.

for example, in the summer, NO_3^- was below detection in most samples deeper than 2.5 cm.

[36] Sediment denitrification was measurable in all seasons, and in nearly all cores and depth strata. In general, denitrification occurred to a stream sediment depth of at least 20 cm in both sand and FBOM habitats. For simplicity, we report all denitrification data here as μg N_2O-N g dry mass $(DM)^{-1}$ h^{-1}, but have included the rates expressed as μg N_2O-N g ash-free dry mass $(AFDM)^{-1}$ h^{-1} and mg N_2O-N m^{-2} h^{-1} in the auxiliary materials.[1] Denitrification rates in the surface sediments (0–4 cm below sediment-water interface) averaged 0.22 ± 0.05 μg N_2O-N g DM^{-1} h^{-1} (mean ± SE), and ranged from 0.002 to 0.91 μg N_2O-N g DM^{-1} h^{-1}. Deeper subsurface denitrification rates (from 4 to 20 cm) averaged 0.9 ± 0.01 μg N_2O-N g DM^{-1} h^{-1} (mean ± SE), and ranged from 0.001 to 0.6 μg N_2O-N g DM^{-1} h^{-1}. The top stratum (0–4 cm) accounted for 30% of total core denitrification (i.e., combined denitrification of all strata, 0–20 cm), on average. The relative contribution of surface sediments ranged from 1 to 90% and did not vary significantly by season or sediment type (RM ANOVA, $p > 0.2$).

[37] Although denitrification rates were generally highest in the surface sediments, we did not always find an exponential decline in denitrification rates with depth (i.e., −k was not always statistically significant). Specifically, there was no significant decline in denitrification with depth in the fall, on FBOM (Figure 2a); in the winter, on FBOM (Figure 2b); in the spring, on sand (Figure 2c); or in the summer, on sand (Figure 2d; SLR, $p > 0.1$ for all regressions).

[1] Auxiliary materials are available at ftp://ftp.agu.org/apend/jg/2012JG001950.

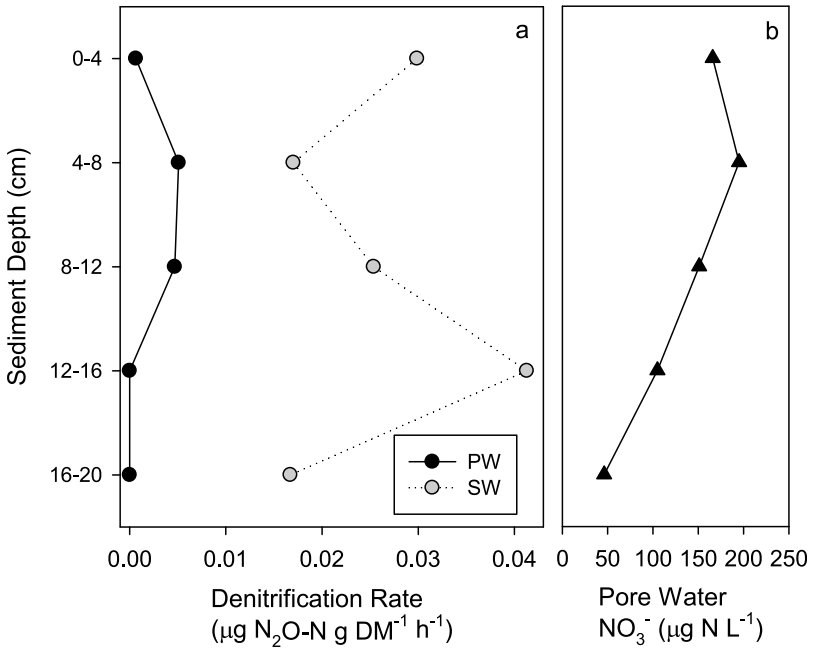

Figure 3. (a) Denitrification rates by depth in fall 2008, when sediments were incubated with pore water (PW, dark circles) and with surface water (SW, gray circles). (b) Sediment pore water NO_3^- concentrations. Surface water NO_3^- concentration was 1600 μg NO_3^--N L^{-1}.

When −k was significant, the decline in denitrification varied by season and substrate type (ANCOVA, $p < 0.001$; Figure 2), with the most rapid decline occurring in the spring, on FBOM (Figure 2c; −k = 0.28), followed by summer, on FBOM (Figure 2d; −k = 0.12); Fall, on sand (Figure 2a; −k = 0.13); and winter, on sand (Figure 2b; −k = 0.07). When denitrification data were pooled by sediment type (i.e., sand or FBOM), sediment denitrification rates were most strongly associated with sediment organic matter content in sand (SLR, $r^2 = 0.264$, $p < 0.001$, data not shown), but there were no significant relationships between denitrification and hypothesized predictor variables (i.e., sediment organic matter content, pore water NO_3^- concentration) in FBOM.

[38] Sediment denitrification rates were significantly higher when stream surface water, rather than pore water from peepers, was used in the denitrification assay (paired t-test, $p < 0.04$, Figure 3a); surface water NO_3^- concentration was 1600 μg NO_3^--N L^{-1}, whereas pore water NO_3^- concentrations were all <200 μg NO_3^--N L^{-1} (Figure 3b). The effect was particularly pronounced at the surface, where denitrification rates in sediments incubated with surface water were 2–46 times higher than denitrification rates in sediments incubated with pore water. In addition, denitrification was measurable at least 8 cm deeper into the cores when sediments were incubated with surface water (Figure 3).

3.2. Experiment 2: Floodplain Denitrification Rates in Response to a Storm Inundation Sequence

[39] During base flow conditions (no water on the floodplains), in April and July 2010, denitrification was stimulated by the addition of high-nitrate stream water, which had concentrations of 7.9 mg NO_3^--N L^{-1} and 4.2 mg NO_3^--N L^-, respectively (ANOVA, then Tukey's post-hoc, pairwise comparison of Dry and +SW, $p < 0.02$ on both dates, Figure 4a). The addition of stream water apparently alleviated nutrient limitation of denitrification; the additional amendments of labile carbon as glucose (+SW +C treatment), NO_3^- (+SW +N treatment), and both combined (+SW +N+C treatment) did not further stimulate denitrification (ANOVA, then Tukey's post-hoc, all pairwise comparisons of +SW, +SW +N, +SW+C, and +SW+N+C $p > 0.6$ for both dates and all nutrients; Figure 4a).

[40] In general, denitrification rates on floodplain soils increased over the course of the 4 day inundation event that occurred in May 2010 (SLR, $r^2 = 0.629$, $p < 0.001$), and surface water NO_3^- concentrations remained high throughout the sequence (11.0 mg N L^{-1} on Day 1, 8.8 mg N L^{-1} on Day 2, and 8.6 mg N L^{-1} on Day 4). During the floodplain inundation, nutrient limitation only resumed on Day 4, when stream surface water had mostly retreated and only standing pools of remnant stream water remained (Figure 4b). At this point, denitrification was co-limited by NO_3^- and C (two-way ANOVA, $p < 0.05$ for both nutrients, Figure 4b); the addition of NO_3^- (+N) increased denitrification rates by 28%, +C increased by 12%, and +N+C increased rates by 55%.

3.3. Experiment 3: The Influence of Vegetation on Floodplain Denitrification Rates

[41] We hypothesized that the presence of vegetation on the floodplains would increase soil organic matter content and the carbon quality index (CQI), and decrease soil moisture and soil NO_3^-, thereby influencing major controlling variables of soil denitrification. We found that soil exchangeable NO_3^- concentrations were significantly higher in both de-vegetated and unvegetated plots than in vegetated plots (Peto-Prentice test of differences, $p < 0.01$), and averaged 2.49 ± 0.38 μg NO_3^--N g $soil^{-1}$ (mean ± SE), and 1.23 ± 0.21 μg NO_3^--N g

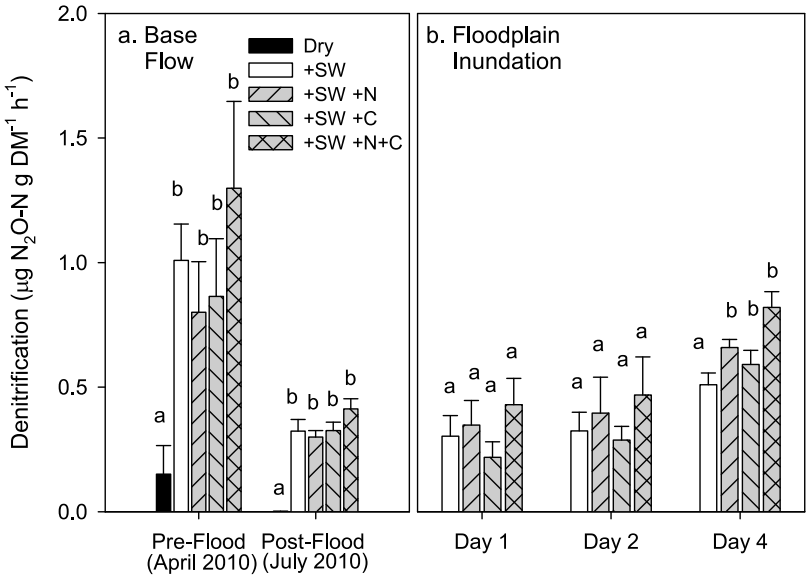

Figure 4. Floodplain denitrification rates during (a) base flow and (b) over the course of a May 2010 floodplain inundation event. Error bars represent the standard error of the mean. Dry = incubated under field soil moisture, +SW = incubated with surface water, +SW +N = incubated with surface water and NO_3^- amendment, +SW +C = incubated with surface water and glucose amendment, +SW +N+C = incubated with surface water, NO_3^-, and glucose amendments. Note that the Post-Flood, Dry treatment mean is too low (0.0017 ± 0.0007 SE) to appear on the figure, and note that the Dry treatment was eliminated from the samples taken during the flood (Figure 4b). Treatments were compared within each sample date using a one-way ANOVA with Tukey's post-hoc (Figure 4a) and using a two-way ANOVA (Figure 4b) [*Tank and Dodds*, 2003]. Letters above the bars indicate which treatments are statistically different from one another ($p < 0.01$) within that sample date.

$soil^{-1}$ (mean ± SE), respectively. In contrast, soil organic matter content was higher in vegetated than in de-vegetated plots (two-way RM ANOVA, $p < 0.03$), but higher in unvegetated plots (i.e., plots where vegetation had not established) than in vegetated plots (two-way RM ANOVA, $p < 0.001$). Soil organic matter content was higher at the surface (0–5 cm) than in deeper soils (5–10 cm) (two-way RM ANOVA, $p < 0.001$). Neither the CQI nor gravimetric soil moisture content were significantly different between vegetated and non-vegetated plots (RM ANOVA, p for both tests >0.29). Thus, the herbaceous vegetation on the two-stage floodplains appeared to decrease soil NO_3^- availability and have a variable effect on soil organic matter content, but it had no influence on soil moisture or CQI.

[42] We also measured floodplain soil denitrification rates in vegetated and non-vegetated plots through time, and compared them with respect to soil depth and the presence or absence of surface water (mimicking floodplain inundation). In general, we found that soil denitrification rates were higher in shallow surface soils (0–5 cm, Figure 5a) than at depth (5–10 cm, Figure 5b) (three-way RM ANOVA, $p < 0.0001$), but that the effects of vegetation and inundation did not change with depth (depth × vegetation, depth × inundation, and depth × inundation × vegetation interaction terms for three-way ANOVA, $p > 0.08$). Overall, soil denitrification rates were not different between the vegetated and non-vegetated plots (three-way RM ANOVA, $p > 0.1$), but they exhibited different patterns when inundated with surface water (three-way RM ANOVA, inundation × vegetation interaction term, $p < 0.002$). Under base flow conditions (i.e., no surface water added), denitrification rates were generally higher in non-vegetated plots (both de-vegetated and unvegetated) than in vegetated plots; whereas under simulated floodplain inundation (i.e., surface water added to soils), soil denitrification rates were generally higher in vegetated plots than non-vegetated (Figure 5). In fact, in vegetated plots, the addition of surface water increased denitrification rates on all but one sample date, and on average, denitrification was 68 times higher in the presence of surface water. In the non-vegetated plots, adding surface water increased denitrification rates on just one date, during Spring 2010, when it was 15 times higher (Figures 6 and 7).

[43] To further explore this differential response of soil denitrification to surface water inundation in the presence and absence of vegetation, in Spring 2010, we also measured nutrient limitation in plots with vegetation, plots in which all vegetation was removed, and plots with only below-ground vegetation (roots) remaining (Table 1). First, we examined the effect of stream surface water addition (NO_3^- concentration = 4.4 mg NO_3^--N L^{-1}) on denitrification and found that in the top stratum (0–5 cm), the addition of surface water significantly increased soil denitrification rates in all three plot types (ANOVA with Tukey's post-hoc, $p < 0.01$ for pair-wise comparison of Dry and +SW, Figure 6a). The magnitude of effect differed by plot type: when inundated, denitrification rates were 15 times higher in the de-vegetated plot, 11 times higher in the roots plot, and 107 times higher in the vegetated plot, compared to denitrification rates at ambient soil moisture (Figure 6a). Additional amendments of NO_3^- (+N) and glucose (+C) did not further increase soil

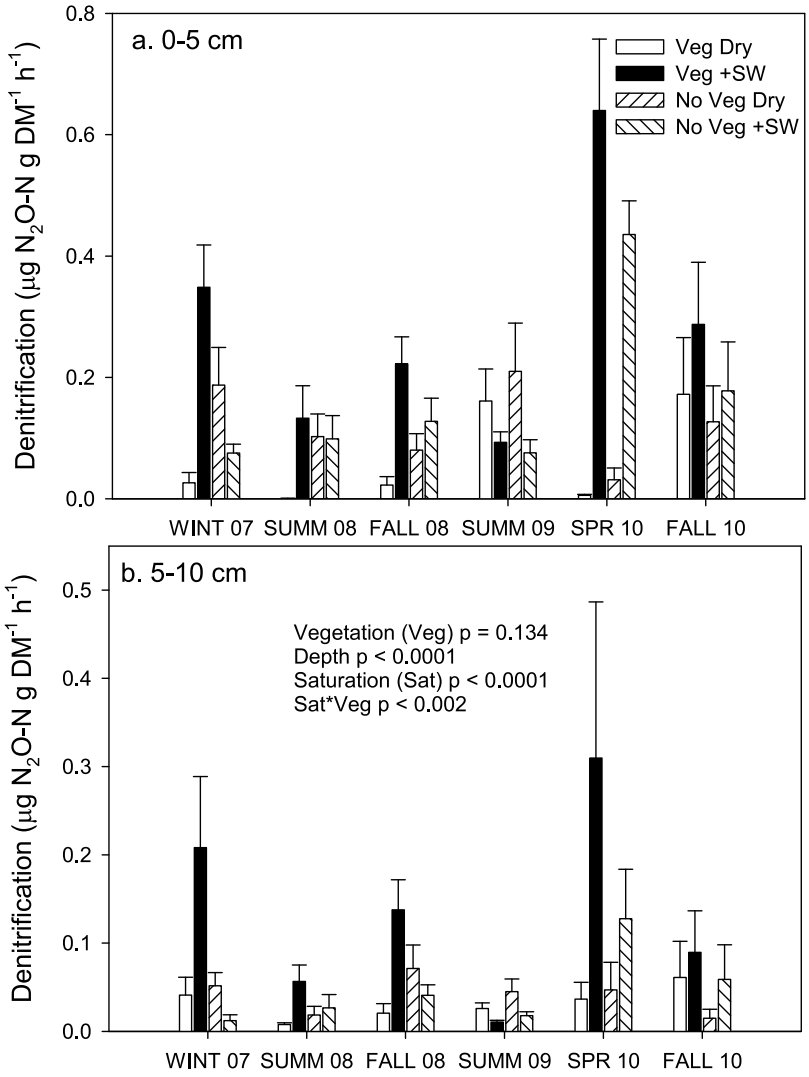

Figure 5. Floodplain soil denitrification rates in vegetated and non-vegetated plots under field moisture (Dry) and when inundated with surface water (+SW) in (a) the top layer of soil (0–5 cm), and (b) in the 5–10 cm layer of soil. Error bars represent the standard error of the mean. Results of the three-way RM ANOVA, excluding nonsignificant interactions (i.e., depth × saturation, depth × vegetation, and depth × saturation × vegetation), are included in Figure 5a.

denitrification rates in any of the plot types (ANOVA with Tukey's post-hoc, $p > 0.8$ for all pairwise comparisons of +SW, +SW+N, +SW+C, and +SW+N+C, Figure 6a). In contrast to the 0–5 cm stratum, neither the addition of surface water nor any nutrient amendments stimulated soil denitrification rates at depth (5–10 cm) in any of the plot types (ANOVA, $p > 0.2$ for all plots, Figure 6b). Thus, as in the floodplain inundation experiment (Experiment 2), nutrient limitation in the topsoil stratum was alleviated by surface water.

[44] We also hypothesized that the influence of vegetation may be mediated by species identity, so we compared soil denitrification in floodplain plots with different species of vegetation (Table 1). Floodplain soil denitrification rates varied with plot type (three-way ANOVA, $p < 0.001$, Figure 7), but pair-wise comparisons revealed that most of these differences were attributable to the unique habitats (i.e., wetland plants and naturally unvegetated soils). More specifically, soil denitrification rates in the natural, unmanipulated plots (i.e., reed canary grass and natives) and the experimentally manipulated plots (i.e., roots and de-vegetated) were all equivalent to one another (Tukey's post-hoc, $p > 0.8$ for all comparisons, Figure 7), and significantly higher than rates in the rarer habitats (i.e., wetland plants and unvegetated soils, Tukey's post-hoc, $p < 0.001$for all comparisons, Figure 7). Denitrification rates in the wetlands and unvegetated plots were not significantly different from one another (Tukey's post-hoc, $p > 0.1$).

[45] As in other experiments, the addition of surface water significantly changed denitrification rates (three-way ANOVA, $p < 0.001$); however, the magnitude and direction of the response varied with vegetation type (vegetation × inundation interaction term, $p < 0.001$). Specifically, in the wetland plot, denitrification rates were 2–4 orders of magnitude higher with surface water, whereas in other plots,

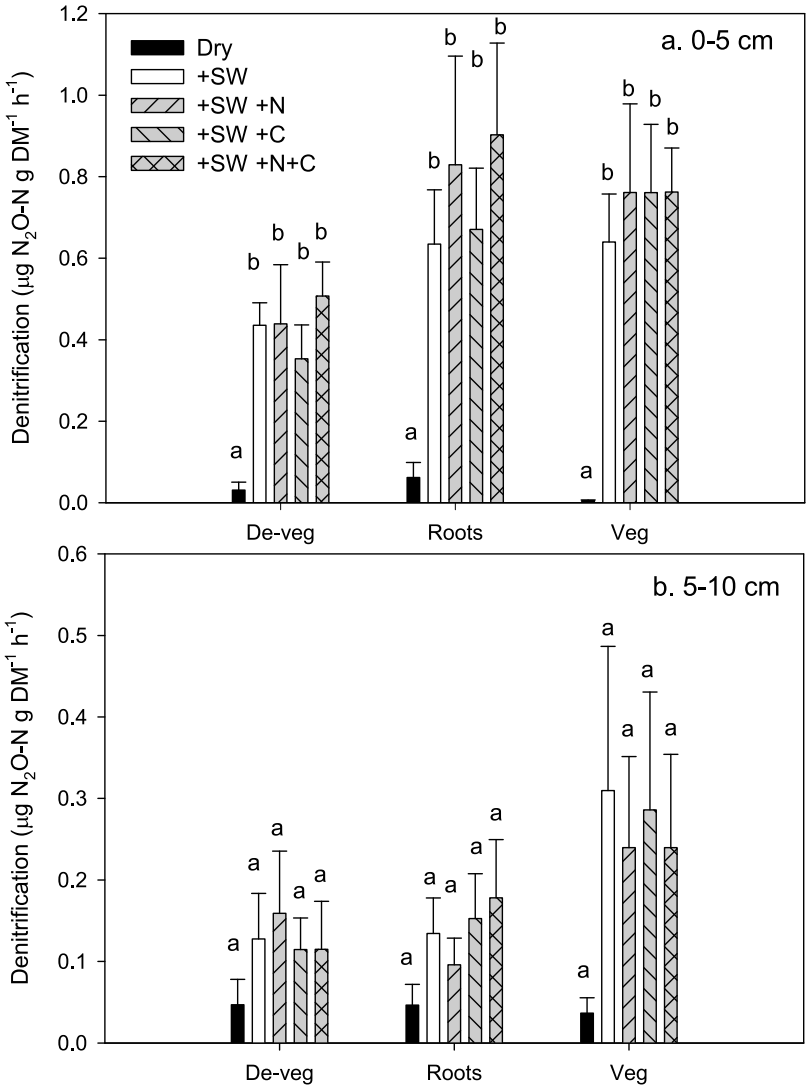

Figure 6. The effect of inundation and nutrient additions on floodplain soil denitrification in de-vegetated ("De-veg"), vegetated ("Veg"), and plots with below-ground vegetation only ("Roots"). Dry = incubated under field moisture, +SW = incubated with surface water, +SW +N = incubated with surface water and nitrogen amendment, +SW +C = incubated with surface water and glucose amendment, +SW +N+C = incubated with surface water, nitrate, and glucose amendment. A one-way ANOVA with Tukey's post-hoc was conducted within each plot and depth. Letters above the bars indicate which treatments are statistically different from one another ($p < 0.01$) within that plot type. Error bars represent the standard error of the mean.

denitrification rates were lower or not different when surface water was present (Figure 7).

[46] Denitrification rates were higher in the shallow surface soil samples (0–5 cm) than samples from the 5–10 cm stratum (three-way ANOVA, $p < 0.001$), but the magnitude of difference varied by vegetative plot type (vegetation × depth interaction term, $p < 0.001$). In other ways, the deeper soil samples reflected the patterns at the surface soils; specifically, the response to surface water inundation did not change by depth (inundation × depth interaction term, $p > 0.3$), nor did the interaction between plot type and surface water addition (inundation × depth × vegetation interaction, $p > 0.08$). Overall, plant species identity appeared to have minimal influence on floodplain denitrification rates; most of the differences occurred in plots that were rare for the site (i.e., wetland and unvegetated).

[47] Our hypothesized predictor variables only weakly explained variation in floodplain soil denitrification rates. Under base flow conditions, soil exchangeable NO_3^- was significantly correlated with denitrification rates (Kendall's tau, tau = 0.32, $p < 0.001$, data not shown). When soils were inundated, soil organic matter content was a weak predictor of denitrification rates (rank transformed, $r^2 = 0.136$, $p < 0.001$, data not shown). Thus, it appears that soil NO_3^- availability influences denitrification under base flow conditions, while soil organic matter content influences

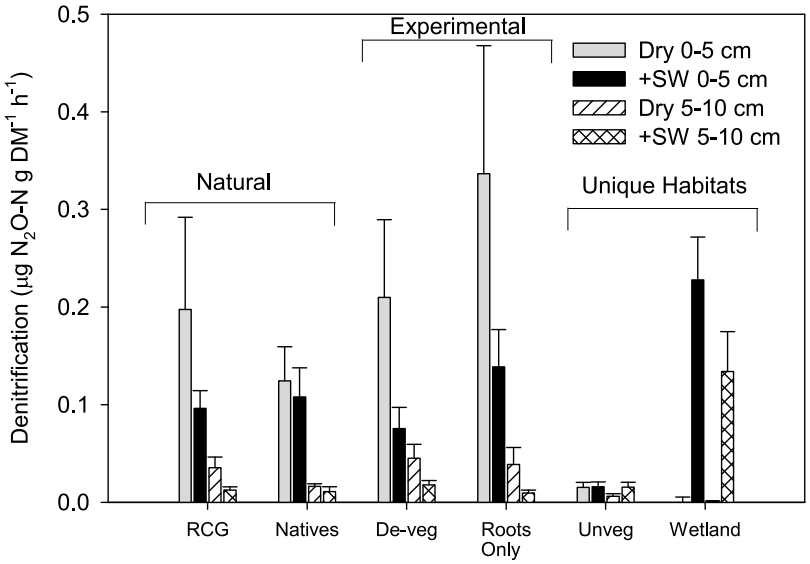

Figure 7. Floodplain soil denitrification rates in plots with different overlying vegetation. Natural = plots that were not manipulated, Experimental = plots that were manipulated, Unique Habitats = plots that were not manipulated and were representative of a small portion of the floodplain, RCG = reed canary grass, Natives = native grasses and forbs, De-veg = above- and below-ground biomass removed, Roots Only = aboveground biomass removed, Unveg = no vegetation present, Wetland = wetland plants present. Error bars represent the standard error of the mean.

denitrification during floodplain inundation, when surface water provides abundant NO_3^-.

4. Discussion

[48] We conducted a multiexperiment study to examine controls on the nitrogen biogeochemistry of a novel stream system: an agricultural stream where floodplains were constructed to restore ecosystem services, including N removal. Specifically, we completed three experiments that addressed the N removal potential, as well as the direct and indirect controls on denitrification rates in the subsurface, stream channel hyporheic zone, and in the restored floodplain in a two-stage ditch. We found that the hyporheic and floodplain ecotones in an agricultural stream have substantial denitrification potential, particularly when connected with high-NO_3^- surface water.

4.1. Sediment Denitrification in the Stream Hyporheic Zone

[49] Stream sediment denitrification rates generally decreased with depth into the hyporheic zone, which we considered to be sediments below 4 cm depth. On average, the surface layer accounted for 30% of core-integrated total denitrification, indicating substantial denitrification potential in the hyporheic zone. Our denitrification rates fell within the range previously observed in small, agriculturally influenced streams: they were somewhat lower, with a lower proportion of total core-integrated denitrification occurring at the surface (0–5 cm) than in a headwater stream in Michigan [*Inwood et al.*, 2007]; yet were higher at the surface, and had a similar proportion of total core-integrated denitrification occurring at the surface than in a third-order stream in Wisconsin [*Stelzer et al.*, 2011]. Sediment organic matter content was a significant predictor of denitrification, in sand, which is consistent with previous research in the hyporheic sediments of larger rivers: denitrification rates were often correlated with the availability of electron donors, such as organic carbon [*Fischer et al.*, 2005; *Hill et al.*, 2000; *Puckett et al.*, 2008] and rates generally decreased with depth [*Fischer et al.*, 2005]. In contrast, we found no significant predictors of denitrification in FBOM, probably because there was abundant organic carbon.

[50] The effectiveness of the stream hyporheic zone as an N sink, specifically via denitrification, depends upon hydrology, as well. For example, NO_3^- must be able to reach the subsurface sediments in the hyporheic zone and be retained long enough (e.g., via longer subsurface flowpaths) for appreciable NO_3^- to be removed [*Puckett et al.*, 2008]. At Shatto Ditch, the hyporheic zone had high potential for N removal via denitrification, but it is unclear how readily NO_3^- reaches this zone; subsurface NO_3^- concentrations were low, possibly due to a lack of exchange between surface water and pore water, or because denitrification rapidly removed NO_3^- from the pore water. In addition, we found no relationship between pore water NO_3^- concentration and sediment denitrification rate, which may be evidence for rapid denitrification. It may also be an artifact of our study design, as we were unable to use pore water in most of our assays because of a lack of volume in the peeper wells. When we did use pore water, we found that rates were lower than denitrification rates measured with surface water (Figure 3), but were still higher than those reported by *Stelzer et al.* [2011], who measured subsurface denitrification with pore water obtained from piezometer nests. Although our experiments were not designed to quantify hyporheic connectivity or subsurface water residence time, our experimental data suggest that there is potential for

substantial subsurface denitrification in agriculturally influenced streams.

4.2. Soil Denitrification During Floodplain Inundation

[51] When surface water was added to floodplain soils, either during a natural flood or experimentally in the lab, denitrification rates were immediately higher. In the time series experiment, denitrification rates continued to increase over the course of a flood, and nutrient limitation did not occur until the fourth day of floodplain inundation (Figure 4b). The initial positive response to surface water inundation was probably a response to the influx of NO_3^-. The subsequent increase through time and eventual NO_3^- and glucose limitation may be the result of increased microbial enzyme synthesis, in response to changing redox conditions [*Brock*, 1961, *Ensign et al.*, 2008], which resulted in biological demand that exceeded supply. The experimental results from Shatto Ditch suggest that restored floodplains possess tremendous N removal potential during floods, but as previous work has shown, floodplain denitrification potential can be limited by a lack of connection with high-NO_3^- groundwater or surface water [*Groffman and Crawford*, 2003; *Kaushal et al.*, 2008; *Machefert and Dise*, 2004]. In the agricultural Midwest, tile drainage and ongoing stream channelization limit soil-water contact time [*Ducros and Joyce*, 2003; *Fennessy and Cronk*, 1997], but the two-stage ditch offers a management strategy in which floodplain reconnection enhances N removal, especially during high stream flows.

4.3. The Influence of Vegetation on Denitrification in Floodplain Soils

[52] Vegetation growing on floodplain soils appeared to reduce soil NO_3^- availability, presumably through plant assimilatory N uptake and/or an increase in soil microbial demand. However, the presence of vegetation did not influence floodplain soil water content, or carbon quality, as represented by the CQI, and its influence on soil organic matter content is somewhat unclear (it was highest in unvegetated, intermediate in vegetated, and lowest in de-vegetated plots). Other studies have reported that plant evapotranspiration can lower the water table of forested floodplains [*Pinay et al.*, 1993, *Schilling and Jacobson*, 2009], but we did not see that effect, probably because the herbaceous vegetation found in Shatto Ditch's floodplains does not require as much water as trees [*Lyons et al.*, 2000]. In addition, these restored, headwater floodplains are inundated more frequently than typical forested river floodplains, and any biological influence on soil moisture may be overwhelmed by hydrology.

[53] Soil denitrification rates increased when surface water was added to soils from vegetated plots, while soils from unvegetated plots did not respond to surface water additions, and de-vegetated plots responded on only one sampling date, and the magnitude of increase was relatively less than in the soils from vegetated plots (Figure 5). Similarly, in bimonthly soil samples collected across the floodplains (without regard to overlying vegetation species or density), denitrification rates did not increase in response to surface water addition until November 2009, two years after floodplain construction [*Roley et al.*, 2012]. It appears that as the restored floodplains age and vegetate, they are better able to denitrify during floodplain inundation.

[54] We expected that vegetation would increase the soil organic matter content, which would allow denitrifiers to take advantage of the additional NO_3^- provided in surface water, and indeed, the vegetated plots had more organic matter than the de-vegetated plots. However, shortly after floodplain restoration, the near-channel, vegetated zone had the same amount of organic matter as the unvegetated floodplain [*Roley et al.*, 2012], and unvegetated plots had more organic matter than vegetated and de-vegetated plots in the following two summers. Furthermore, soil organic matter content did not increase through time, at least over two years, as the floodplains became fully vegetated. We measured the carbon quality index (CQI), to see if the organic matter in vegetated plots was of higher quality than in de-vegetated and unvegetated plots, but it was not different among plot types, nor did it change through time. Another potential explanation is that the presence of roots increased soil porosity, allowing surface water NO_3^- to penetrate more of the soil profile and stimulate denitrification. However, we expect that our denitrification methods would obscure this effect, because we used slurries, which do not keep the soil structure intact. Therefore, some other, unmeasured, change in the floodplain soils must have occurred that allowed the entire floodplain to respond to surface water, as the vegetated plots did throughout the experiment. This change could be some other component of soil carbon, or a change in the microbial community colonizing the floodplain soils. The direct mechanism remains elusive; however, our data do suggest that as constructed floodplains age and become fully vegetated, soil denitrification rates increase in response to floodplain inundation.

[55] The type of vegetation, be it functional (wetland versus perennial grasses and forbs) or species-specific (e.g., reed canary grass versus a suite of natives), did not appear to influence soil denitrification rates in floodplains. We had predicted that the highly productive, but invasive, reed canary grass would have high assimilatory NO_3^- and water demands, while the deep-rooted natives would facilitate denitrification in the deeper soils by providing organic matter and facilitating the movement of high-NO_3^- surface water, but our experimental results did not support this (Figure 7). Thus, while establishing native plants may help promote local biodiversity, it is not likely to influence the efficacy of soil denitrification in floodplains.

[56] Interestingly, the experimental plot with wetland vegetation responded most strongly to the addition of surface water, and had the highest soil denitrification rates in the 5–10 cm layer (Figure 7). This is consistent with previous research showing that soils that support wetland vegetation also support high denitrification rates. For example, *Xue et al.* [1999] found that denitrification rates ranged from 2.0 to 11.8 mg N m^{-2} h^{-1} in an Illinois wetland receiving agricultural tile-drainwater, and *Poe et al.* [2003] found that rates ranged from 0.7 to 9.24 mg N m^{-2} h^{-1} in a wetland receiving runoff from row crop agriculture. In our study, the average denitrification rate in the wetland plot was within that range: when inundated with surface water in the lab, rates were 6.77 $\pm$ 1.37 mg N m^{-2} h^{-1} (mean $\pm$ SE) at the surface (0–5 cm), and 3.35 $\pm$ 1.06 mg N m^{-2} h^{-1} (mean $\pm$ SE) in the 5–10 cm stratum. In fact, when the floodplains were inundated naturally during a

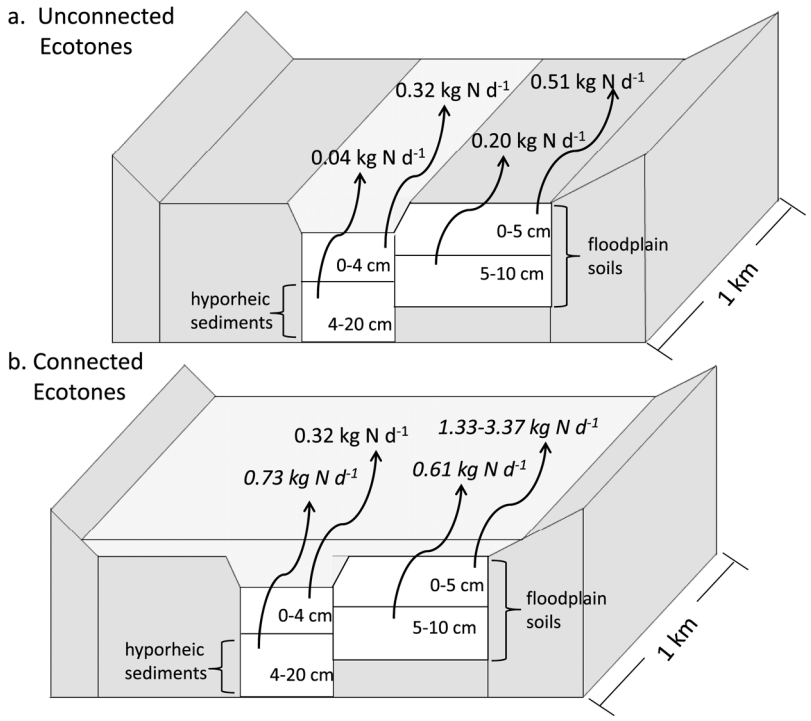

Figure 8. N removal via denitrification over 1 km of stream, partitioned into stream surface sediments, stream hyporheic sediments, and floodplain soils, (a) when stream water only contacts surface sediments, and (b) when stream water is in contact with floodplains and hyporheic sediments (i.e., during bankfull flows and when sub-surface flowpaths facilitate exchange between the surface water and hyporheic zone). Dashed horizontal lines indicate the 0–5 cm and 5–10 cm strata in the floodplains. Note: this figure is not to scale.

storm, denitrification rates were within or above the range of wetland denitrification rates; the mean rate during floodplain inundation was 9.2 $\pm$ 1.3 mg N m^{-2} h^{-1} (mean $\pm$ SE), and average denitrification rates on the last day of the floodplain inundation experiment reached 23.4 $\pm$ 3.37 mg N m^{-2} h^{-1} (Figure 4). Thus, when the floodplains are inundated, denitrification rates are comparable or even higher than those in wetlands. Constructed wetlands have been recommended as a N-removal strategy in the Mississippi River Basin [*Mitsch et al.*, 2001], and our data suggest that stream ecotones can be as effective when they are hydrologically connected to surface water. Thus, floodplain restoration and wetland construction may be complementary BMPs, with wetlands intercepting and denitrifying base flow tile drainwater, while floodplains denitrify during floodplain inundation events, when high flows can overwhelm a wetland's water-holding capacity [*Kovacic et al.*, 2000; *Kovacic et al.*, 2006].

4.4. Role of Stream Ecotones in N Removal

[57] Our study suggests that both the hyporheic zone and floodplains have high denitrification potential in a restored agricultural stream. As in other studies, this potential is limited by hydrology; high-NO_3^- water must reach these sites in order for N to be removed [*Ducros and Joyce*, 2003; *Groffman and Crawford*, 2003; *Kaushal et al.*, 2008]. In some upwelling zones, high-NO_3^- groundwater can reach the hyporheic zone and be denitrified [*Stelzer et al.*, 2011], but in other systems, impermeable clay layers can limit upwelling and armored stream bottoms can limit downwelling into the hyporheic zone [*Brunke and Gonser*, 1997] thereby creating a physical barrier for hydrologic exchange. In contrast, floodplains in midwestern, channelized streams flood frequently [*Landwehr and Rhoads*, 2003; *Powell et al.*, 2006]; in Shatto Ditch, they were inundated 12 times per year, on average, and denitrification rates were enhanced during inundation, particularly on fully vegetated floodplains. Although we did not quantify surface water-groundwater exchange at Shatto Ditch, presumably exchange occurred; in spring, fall, and winter, pore water NO_3^- was measurable, but low, throughout the peeper profile. In sandy-bottomed agricultural streams, the subsurface hyporheic zone may contribute substantial denitrification, as these streams are likely to contain particles that are large enough to allow exchange and yet still have sufficient organic matter to support denitrification.

[58] We estimated the reach-scale effect of stream ecotones on N removal with a back-of-the-envelope calculation. We multiplied the average areal denitrification rate from each of our experiments by the area of each habitat in 1 km of two-stage ditch, with the dimensions of Shatto Ditch (Figure 8). We completed these calculations under two scenarios: (1) surface water directly contacts stream surface sediments only (i.e., when floodplains are not inundated and there is no hydrologic connection between surface water and the hyporheic zone), and (2) surface water contacts the hyporheic zone and floodplains soils (i.e., floodplains are inundated and there is a strong surface water-hyporheic zone connection). We found that under base flow conditions at

Shatto Ditch, the stream sediments alone removed 0.32 kg N d^{-1} in 1 km of ditch (Figure 8a). The addition of unconnected ecotones resulted in total reach-scale N removal that is over 3 times higher (1.05 kg N d^{-1} Figure 8a), and the addition of hydrologically connected ecotones resulted in total reach-scale N removal that is 9 to 15 times higher (2.99 kg N d^{-1} on the first day of inundation to 5.03 kg N d^{-1} on the last day of inundation, Figure 8b). In comparison, average base flow NO_3^- load in Shatto Ditch was 23 kg N d^{-1}, and average stormflow NO_3^- load was 201 kg N d^{-1}. This translated to removal of 1% of the load by unconnected ecotones (i.e., surficial stream sediments only) during base flow, 5% of load when ecotones were connected during base flow, and 2% of load when ecotones were connected during stormflow. Despite these low removal efficiencies, our N removal rates are comparable to previous studies. For example, the total N removal at base flow for stream sediments alone fell within the range reported in a multistream study by *Mulholland et al.* [2009], while the N removal with ecotones included at base flow is higher than the range reported by *Mulholland et al.* [2009]. Also, we note that our removal rates are generally lower than those reported by *Böhlke et al.* [2004], whose rates are among the highest reported by tracer studies. Finally, the proportion of total stream NO_3^- removed via denitrification is within the range expected, given the high NO_3^- concentrations in this stream [*Mulholland et al.*, 2008].

[59] Stream ecologists have long known that the hyporheic zone is intimately linked with the surface water [*Boulton et al.*, 1998], and a recent study demonstrated that floodplains retain N during seasonal floods [*Forshay and Stanley*, 2005]. Through a series of experiments, our study in Shatto Ditch demonstrated that these patterns also hold in agriculturally influenced streams where denitrification occurred in two important ecotones: the stream channel hyporheic zone and the lateral restored floodplains. Most notably, our study demonstrated that hydrologic connection, which can be achieved through floodplain restoration, enhances N removal in high-nitrate agricultural streams. In fact, during floodplain inundation, N removal rates in constructed floodplains can approach those of constructed wetlands, which suggests that hydrologic connection is a potentially useful strategy for N retention and removal in agricultural landscapes.

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