Floodplain restoration enhances denitrification and reach-scale nitrogen removal in an agricultural stream

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Abstract.   Streams of the agricultural Midwest, USA, export large quantities of nitrogen, which impairs downstream water quality, most notably in the Gulf of Mexico. The two-stage ditch is a novel restoration practice, in which floodplains are constructed alongside channelized ditches. During high flows, water flows across the floodplains, increasing benthic surface area and stream water residence time, as well as the potential for nitrogen removal via denitrification. To determine two-stage ditch nitrogen removal efficacy, we measured denitrification rates in the channel and on the floodplains of a two-stage ditch in north-central Indiana for one year before and two years after restoration. We found that instream rates were similar before and after the restoration, and they were influenced by surface water NO_3^- concentration and sediment organic matter content. Denitrification rates were lower on the constructed floodplains and were predicted by soil exchangeable NO_3^- concentration. Using storm flow simulations, we found that two-stage ditch restoration contributed significantly to NO_3^- removal during storm events, but because of the high NO_3^- loads at our study site, <10% of the NO_3^- load was removed under all storm flow scenarios. The highest percentage of NO_3^- removal occurred at the lowest loads; therefore, the two-stage ditch's effectiveness at reducing downstream N loading will be maximized when the practice is coupled with efforts to reduce N inputs from adjacent fields.

Key words: agriculture; denitrification; floodplain; stream restoration; Tippecanoe River, north-central Indiana, USA; two-stage ditch.

Introduction

Anthropogenic activities have doubled the input of reactive nitrogen (N) onto landscapes across the globe, which has increased N loading to freshwater and marine systems (Vitousek et al. 1997). In the agricultural Midwest, USA, extensive N fertilization and artificial drainage has resulted in high N loading rates to agricultural streams: ~20 million hectares of agricultural soils in the Mississippi River Basin are drained with subsurface tile drains, which discharge to incised streams or ditches (Osborne and Wiley 1988, Johnson et al. 1997, Galloway et al. 2003). Channelization and tile drainage ensure rapid conveyance of water from crop fields, which improves agricultural production, but reduces water contact time with soils and sediments, and ultimately reduces N retention on fields (Randall et al. 1997, Royer et al. 2006). The export of excess N from the agricultural Midwest has been linked to coastal

hypoxia in the Gulf of Mexico (Rabalais et al. 2002), as well as locally contaminated drinking water (Fan and Steinberg 1996) and loss of freshwater biodiversity (Carpenter et al. 1998). Best management practices (BMPs), which improve biological N removal in agricultural streams, while simultaneously meeting the drainage needs of the agricultural community, may improve downstream water quality without loss of agricultural function.

Agricultural areas in the Midwest typically have low relief and poor drainage. As a result, artificial subsurface drainage (i.e., tile drains) is necessary for crop growth to occur. In addition, many streams are managed by county-level drainage commissions who use dredging to create trapezoid-shaped stream channels. These channels are effective at quickly moving water downstream, but often are subject to bank slumping and may require dredging to maintain their shape. An alternative management strategy to the trapezoidal channel is the two-stage ditch, in which floodplains are restored alongside the stream channel (Fig. 1D and E; Powell et al. 2007). In this design, tile drains flow directly onto the floodplains. During high flows, water spreads onto the floodplains and slows down, depositing fine sediments. The floodplains also decrease shear stress, which results in greater channel stability and decreased erosion (Powell et al. 2007). The two-stage ditch design mimics
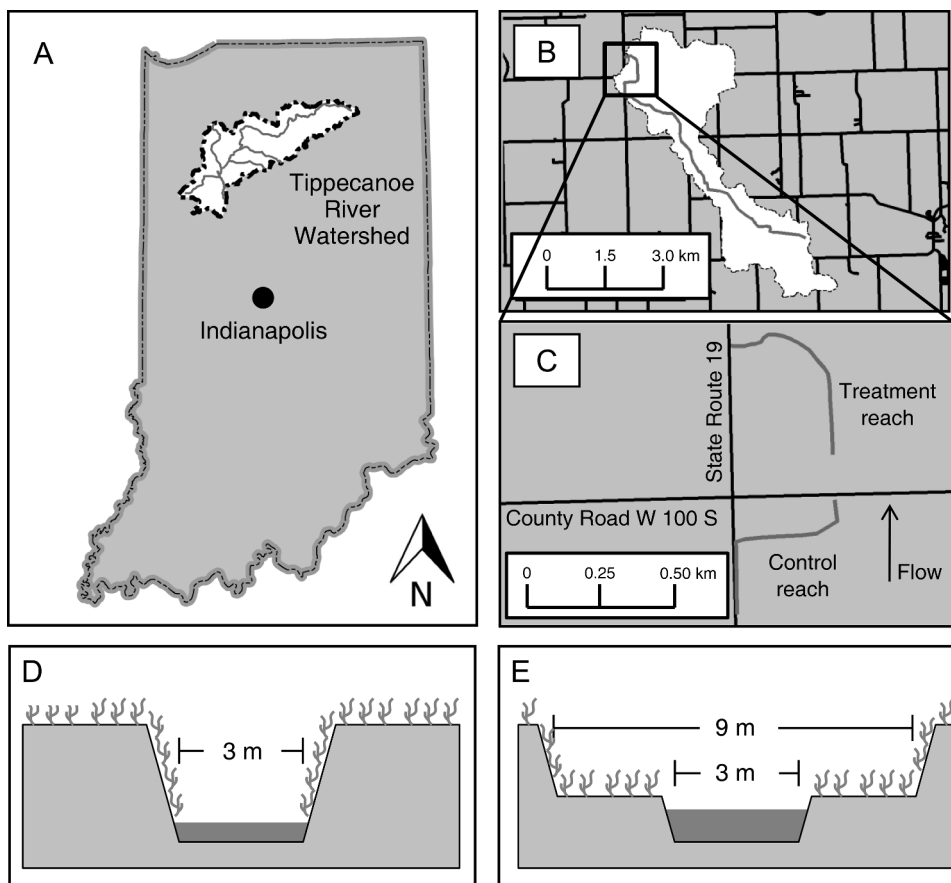
Fig. 1. (A) Location of the Tippecanoe River watershed within Indiana, USA. (B) Shatto Ditch watershed, with the study area denoted by the black box. (C) The control and treatment reach of Shatto Ditch. Note that the control reach is upstream of the treatment reach. (D) Cross section of a trapezoidal stream channel. (E) Cross section of the two-stage ditch restoration, which was completed on the treatment reach. The base flow channel is left intact, and floodplains are excavated on both sides of the base flow channel.

natural fluvial processes; an unmaintained, channelized stream typically forms small floodplains ("naturalized" floodplains) as a result of bank slumping, undercutting, and sediment deposition (Landwehr and Rhoads 2003). The floodplains of these tile-drained, agriculturally influenced channels are typically inundated numerous times each year during storm flows (Landwehr and Rhoads 2003, Kallio 2010b). In addition to improving channel stability, the two-stage ditch may enhance N removal by increasing water residence time and providing additional bioreactive surface area for transformations of nitrogen, including denitrification. Through heterotrophic microbial denitrification, nitrate (NO_3^-) is converted to nitrogenous gases in the presence of organic carbon and anoxic conditions in the sediments (Knowles 1982). Thus, denitrification results in a permanent removal of N from the system (Galloway et al. 2003).

Floodplain soils may be better suited for microbial denitrification than upland fields or riparian grass buffer strips, particularly in the tile-drained landscape of the agricultural Midwest. Floodplain soils are closer to the water table and hydrologically connected to surface water, resulting in higher soil moisture and redox conditions suitable for denitrification (Kaushal et al. 2008, Gift et al. 2010). In tile-drained landscapes, high-NO_3^- groundwater is shunted through underground drainage pipes, which minimizes its interaction with soils and limits the denitrification potential of buffer strips (Fennessy and Cronk 1997, Ducros and Joyce 2003). Furthermore, plant matter is often removed from buffer strips and crop lands each year during harvest, which decreases organic carbon availability and may further limit the denitrification potential of buffer strips (Hedin et al. 1998).

Natural floodplains have been found to be generally effective at N removal, but have mainly been studied in mid-order rivers (Pinay et al. 1993, Hoffmann et al. 1998, Van Der Lee et al. 2004, Schilling and Jacobson 2009). Additionally, floodplain restorations have been largely examined in the context of hydrologic reconnection with existing floodplains, where success has been

mixed. In one case, denitrification rates remained unchanged post-restoration (Orr et al. 2007). In other studies, hydrologic reconnection with floodplains increased N removal rates by creating favorable redox conditions, increasing delivery of NO_3^- to soils with high denitrification potential, and increasing floodplain water residence time (Groffman and Crawford 2003, Sheibley et al. 2006, Kaushal et al. 2008).

In contrast to previous floodplain restoration studies, the two-stage ditch results in the creation of a novel floodplain habitat. As a result, it is unclear how this new habitat will function. Organic carbon is potentially limiting, because soil removal during floodplain excavation may reveal inorganic, formerly subsurface substrates. Microbial populations could also be potentially limiting; we do not know if denitrifiers will be present in freshly excavated soil, or how long it will take for them to colonize. Finally, although two-stage restoration is not a direct manipulation of the stream channel, over time it can result in channel narrowing (Powell et al. 2007), which could increase water velocity for flows below the floodplain elevation, and potentially change stream sediment characteristics and resultant instream N removal processes.

The two factors that will determine two-stage ditch efficacy for reach-scale N removal are areal denitrification rates and water residence time. In general, N removal rates increase with residence time, because there is more time for surface water to interact with sediment denitrifiers before the water is exported downstream (Seitzinger et al. 2006). Floodplain restoration increases water residence time during floodplain inundation, because the water spreads out over a greater area and thus velocities decline. In addition, high-nitrate stream water is exposed to soil denitrifiers in restored floodplains. We predict that these interacting factors will increase reach-scale N removal in the two-stage ditch.

Two-stage restoration is a novel BMP that allows drainage while maintaining channel stability. It has the potential to improve biological N removal rates, as well, but its efficacy in that regard has not been evaluated. The objective of this research was to determine if floodplain restoration via the two-stage ditch promotes microbial denitrification at the stream reach scale. Results from this study identify controls on both instream and floodplain denitrification rates, estimate the efficiency of NO_3^- removal relative to stream water NO_3^- loads, and evaluate the potential role of the two-stage ditch in the management of N loading in agricultural landscapes.

MATERIALS AND METHODS

Site description

We conducted our study in Shatto Ditch, which is a tributary of the Tippecanoe River in north-central Indiana, USA (Fig. 1). Shatto is a first-order stream, and base flow discharge ranges from 12 to 174 L/s. The surrounding landscape is primarily tile-drained row crop agriculture in a maize–soy bean rotation. The stream is surrounded by a 3 m wide grass buffer strip that is periodically mowed or burned. Shatto Ditch has historically been maintained as a drainage ditch with steep slopes and an incised, trapezoid-shaped channel. As a result, it has a flashy hydrograph and high concentrations of dissolved inorganic nutrients. The stream bed is primarily a mix of fine benthic organic matter (FBOM) and sand, although gravel is present after spring snowmelt, particularly in the upstream portion of the reach.

Experimental design

We employed a before–after–control–impact (BACI) design for our study (Stewart-Oaten et al. 1986) by establishing a 600-m downstream treatment reach, paired with a 600-m upstream control reach, with a 50-m buffer located between the reaches. We measured denitrification rates on sediments collected every two months in both reaches for 13 months prior to the two-stage restoration (September 2006–October 2007). After the restoration, we collected instream and floodplain denitrification data for two years (December 2008– November 2009). A summary of the field measurements is presented in Table 1.

Floodplains in the treatment reach of Shatto Ditch were restored in November 2007. To determine floodplain height relative to the stream bottom, we measured the height of naturally formed floodplains, where they existed. Using a backhoe, the side slopes were excavated on each side of the stream to the desired depth (mean = 0.4 m from stream bottom in the center of the channel to the floodplain; Kallio 2010a), creating a new 3 m wide floodplain (Fig. 1D and E). The stream channel had an average width of 2.7 m; thus, the two-stage ditch restoration more than tripled the stream corridor area.

Field sampling of stream sediments and floodplain soils

Every two months, we collected sand and FBOM from the stream channel, to a depth of 2 cm, at evenly spaced transects (25 m apart), using a 3.6 cm diameter polyvinyl chloride (PVC) corer. We collected sufficient sediments for five replicate denitrification assays for each substrate type from each reach, including nutrient limitation assays (see the next section, *Laboratory denitrification assays on sediments and soils*). In April and June of 2009, the control reach contained primarily sand and gravel (66% sand, 16% gravel in April; and 58% sand, 23% gravel in June), so we sampled gravel instead of FBOM. During each sampling, we also characterized the sediment every 10 cm along each transect, and used these habitat data to determine the proportion of the channel containing each substrate type, as well as average channel width and stream channel area (average width multiplied by channel length).

After the two-stage restoration, we also collected floodplain soil cores for denitrification assays. At five

TABLE 1.   Summary of measurements made in this study at Shatto Ditch watershed, Indiana, USA.

Sample location	Sediment depth (cm)	Explanatory variables	Sampling frequency	Substrate type	Slurry conditions	Experimental reach†
Stream channel	0–2	stream water NO_3^-, sediment organic matter	pre- and post-restoration, every 2 months ($n = 19$)	fine benthic organic matter (FBOM), sand	stream water added	CTL, TRT
Floodplains	0–15, in 5 cm increments	soil-exchangeable NO_3^-, soil organic matter, soil gravimetric water content	post-restoration, every 2 months ($n = 10$)	floodplain soils	ambient conditions or stream water added	TRT

  † Abbreviations are: CTL, control reach; TRT, treatment reach.

randomly chosen sites, we collected two adjacent soil cores, each 15 cm long, with a PVC corer. These cores almost always penetrated the water table. We extruded the cores onto a plastic tray, and cut them into 5-cm sections.

Laboratory denitrification assays on sediments and soils

Within 24 h of stream sediment or floodplain soil collection, we estimated microbial denitrification rates using the redox-optimized chloramphenicol-amended acetylene block technique (Smith and Tiedje 1979, Royer et al. 2004, Arango et al. 2007). Acetylene (C_2H_2) blocks the microbial conversion of N_2O to N_2, allowing N_2O, which is more easily measured, to accumulate in assay bottles. Chloramphenicol is an antibiotic that blocks the production of de novo denitrifying enzymes (Brock 1961), and reduces the bottle effects of laboratory denitrification assays by limiting their response to ideal redox conditions (Smith and Tiedje 1979). When chloramphenicol is added, denitrification rates measured with the acetylene block technique are comparable to other methods, particularly under short-term incubations (Bernot et al. 2003). We did not artificially elevate nutrient concentrations in the assay bottles, but instead used only ambient stream water for incubations. We feel this methodological approach is suitable for the unconsolidated sediments and high-NO_3^- water (range: 2.4–9.8 mg NO_3^--N/L) in our study stream (Royer et al. 2004, Inwood et al. 2005, Arango et al. 2007). These redox-optimized, ambient nutrient incubations are easily replicated over space and time and do not require the addition of large quantities of isotopically enriched $^{15}NO_3^-$.

For each sediment replicate, we homogenized the sample and subsampled 5 mL for analysis of dry mass (DM) and ash free dry mass (AFDM). We placed 25 mL of sediment in a 125-mL glass bottle with a rubber septum cap, and added 45 mL of stream water, along with 5 mL of 3.1 mmol/L chloramphenicol, to achieve a final slurry concentration of 0.21 mmol/L chloramphenicol (Bruesewitz et al. 2008). We cut each soil core in half, and used one half each for denitrification measurements and nutrient extraction. Because the soils were generally too moist for sieving, we used a metal spatula to cut the core into small pieces before placing in the assay bottles, which allowed C_2H_2 to more easily diffuse into the soils (Groffman et al. 1999). For each pair of adjacent cores, we incubated one without stream water during the denitrification assay, and one with stream water added to simulate floodplain conditions during storm flows. Although stream water NO_3^- and organic carbon concentrations may differ between storm flows and the base flow conditions under which we collected the assay water (e.g., Royer et al. 2004, Vidon et al. 2008), the stream water amendments nonetheless provide an indication of whether floodplain denitrification responded to inundation, although the magnitude of the response may only be an approximation. If the floodplains happened to be naturally inundated on the sampling date (which occurred in February 2008), we added stream water to all samples, and collected cores at 10 randomly chosen sites, instead of pairs at five sites. We added chloramphenicol to all bottles, as above.

We sealed all bottles with septum caps and sparged with ultra-high purity N_2 for 5 min, shaking periodically to remove oxygen. After sparging, we vented the bottles to return them to ambient atmospheric pressure, and then added 15 mL of pure C_2H_2, for a 10% headspace concentration of C_2H_2. We generated C_2H_2 by mixing calcium carbide with deionized water (Arango and Tank 2008).

We incubated each assay bottle for 4 h and took five headspace samples over that time period, each approximately 1 h apart at 0.25, 1.25, 2.25, 3.25, and 4.25 h. We shook each bottle for several seconds prior to sampling to equilibrate the gases in the headspace and the slurry, then removed 5 mL of gas with a syringe and injected the gas into pre-evacuated 3-mL serum vials with rubber septa (Wheaton, Millville, New Jersey, USA). We maintained constant pressure in the assay bottles by injecting 5 mL of 10% C_2H_2 (balance of N_2) after sampling. We incubated all bottles at room temperature to minimize variability among substrates and reaches.

To determine if stream sediment denitrification was limited by the availability of NO_3^- or dissolved organic carbon (DOC) in stream water, we completed nutrient limitation assays on most sampling dates. In these

assays, we added NO_3^- (to 1.1 mg/L above background) and glucose (to 2.7 mg/L above background) singly and in combination, creating three additional treatments: $+NO_3^-$, +DOC, and $+NO_3^-$ +DOC. We prepared these samples as described in the previous three paragraphs, but used a nutrient-amended chloramphenicol solution (NO_3^- as KNO_3 [10 mg NO_3^--N/L], and DOC as glucose [24 mg C/L]) (Royer et al. 2004, Bruesewitz et al. 2009). We had three replicates of each nutrient treatment for each substrate and stream reach. We completed these assays only on stream sediments, not on floodplain soils, due to time and equipment limitations.

To determine carbon quality, we also measured the carbon dioxide (CO_2) concentration in the gas samples (see next paragraph) and calculated the ratio of CO_2 production to N_2O production (in μmol/h) on a subset of sampling dates. A higher ratio corresponds to lower carbon quality; it requires more carbon to be oxidized per mole of NO_3^- reduced (Pfenning and McMahon 1996). In using this index, we assume that other anaerobic heterotrophic processes (e.g., sulfate reduction) are equal among streams, substrates, and sampling dates. In addition, we only used this index when NO_3^- was not limiting (i.e., on floodplain samples that were incubated with surface water, and instream samples that were not NO_3^- limited, as determined by the nutrient limitation assays).

All gas analysis was completed on a Varian CP-3800 gas chromatograph (Varian, Walnut Creek, California, USA), equipped with an electron capture detector (ECD), a thermal conductivity detector (TCD), a Haye SepQ column (AllTech, Deerfield, Illinois, USA), a valve to vent water and C_2H_2 away from the detector, and a CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland). The injector temperature was set at 50°C, the column oven at 50°C, the ECD at 300°C, and the TCD at 120°C. We used the ECD to measure N_2O concentration and the TCD to measure CO_2 concentration. The carrier gases were ultra-high purity N_2 and ultra-high purity helium, respectively. We applied Bunsen coefficients to account for gas dissolved in the slurry (Inwood et al. 2005), then plotted N_2O and CO_2 mass vs. time and used the slope of the best-fit linear regression line to determine production rates. We scaled the N_2O production rate (units: μg N_2O-N/h) to the amount of dry mass (DM), ash free dry mass (AFDM), and stream area (m^2) in the assay bottle. We also calculated habitat-weighted denitrification rates by weighting areal denitrification rates by the proportion of the reach containing each substrate (units: μg N_2O-N·m^{-2}·h^{-1}). We used the habitat-weighted, areal denitrification rates (denoted U_{dstr} [denitrification in stream]) in our calculations of reach-scale NO_3^- removal (see *Reach-scale NO_3^- removal calculations*).

Temperature experiment

To determine the effect of incubating samples at room temperature, we did two temperature experiments. We collected sand and FBOM from the treatment reach in October 2007, when the stream temperature was 14°C, and February 2008, when the stream temperature was 1°C. On each sampling date, we measured denitrification rates as described in the previous section, but incubated five replicate samples each at 0°C, 6°C, 15°C, and 22°C. We plotted temperature vs. denitrification rate (μg N_2O-N/h) and fitted a simple linear regression (SLR) to the resulting data to determine the temperature correction. The distribution of stream temperatures from our regular denitrification sampling dates (taken every two months) was bi-modal (cold ranged from 0°C to 7°C, and warm ranged from 10°C to 19°C), and we applied the temperature correction from February to the cold dates and the October correction to the warm dates. To apply the correction, we multiplied the slope of the best-fit regression line by the difference between the stream temperature and the incubation temperature, and subtracted that value from the N_2O production rate (units: μg N_2O-N/h). We then scaled the N_2O production rate by DM, AFDM, and area, as described in the previous section, and compared the temperature-corrected rates to the room temperature rates.

Ancillary physicochemical variables

We measured a suite of physicochemical variables, including surface water chemistry, floodplain soil chemistry, and stream discharge. We collected surface water samples on every sampling date by filtering 60 mL of stream water (glass fiber filters,1-μm nominal pore size; Pall, Ann Arbor, Michigan, USA) into acid-washed, stream water-rinsed, high-density polyethylene bottles and freezing for subsequent analysis in the laboratory. We analyzed surface water and soil extractions (see next paragraph) for NO_3^--N on a Lachat QC8500 Flow Injection Autoanalyzer (Lachat Instruments, Loveland, Colorado, USA) using the cadmium reduction method (APHA 1995).

We extracted soil nutrients from all floodplain samples within 3 d of field collection. Before the extractions, soil samples were kept in closed sample containers at 4°C. In these extractions, we added 40 mL of 2 mol/L potassium chloride (KCl) to 4 g of soil at field moisture, agitated the samples at 100 rpm on a shaker table for 1 h, filtered, and then froze the supernatant for later analysis (SSSA 1996). Extractable nutrient concentrations were expressed per unit dry mass.

To estimate continuous stream discharge, we deployed capacitance meters (Odyssey, Christchurch, New Zealand), which recorded stream stage every 20 min. We then created a stage-discharge relationship by measuring stream discharge directly at a range of flow conditions (Gore 2006). On several dates, we released a 20-L slug of saturated sodium chloride or rhodamine dye solution at the upstream end of both the control and treatment reaches (Gordon et al. 1992). We placed a Hydrolab Minisonde (Hach, Loveland, Colorado, USA) at the

downstream end of the reach and recorded conductivity or fluorescence. We calculated discharge as

$$Q = VC / \int (C_t - C_b) \, dt \qquad (1)$$

where Q is discharge (L/s), V is volume of dye or salt solution released, C is the concentration of dye or conductivity of NaCl release solution, C_t is the stream water fluorescence or conductivity at the downstream monitoring station at time t, and C_b is the background fluorescence or conductivity in the stream. We calculated average velocity as the time from release to peak fluorescence or conductivity. On the date of each slug release, we also measured the wetted width at 23 evenly spaced transects along the length of the reach. We then calculated average depth over the reach as

$$z = Q / (vw) \qquad (2)$$

where z is average depth, v is mean velocity, and w is mean width. To estimate continuous average depth, we developed a relationship between measured average depth and capacitance meter stage. To account for time periods when the capacitance meters failed, we also created a relationship between discharge measured with slug releases and the nearby Eel River USGS gauge (*available online*).[6]

In addition, we used the stage data to determine the frequency of bank-full events. Bank-full events occur when water flows out of the main channel and onto the floodplains, whereas ditch-full events occur when water flows out of the ditch and onto the field.

Reach-scale NO$_3^-$ removal calculations

We calculated reach-scale N removal with an application of stream nutrient spiraling metrics (Newbold et al. 1981), using calculations from Royer et al. (2004). First, we calculated the uptake velocity of NO$_3^-$ as a result of denitrification ($V_{f,dn}$) by dividing NO$_3^-$ uptake via denitrification (U_{dstr}) by stream water NO$_3^-$ concentration (C, mg NO$_3^-$-N/m^3). We used the habitat-weighted denitrification rate for U, as described previously in the section *Laboratory denitrification assays on sediments and soils*. Next, we calculated the metric $-k$ (percentage per day), which is the NO$_3^-$ loss rate attributed to denitrification (Alexander et al. 2000), according to

$$-k = (V_{f,dn}) / z. \qquad (3)$$

In addition, we calculated denitrification uptake length ($S_{w,dn}$), which is the average distance traveled by a molecule of NO$_3^-$ before being denitrified:

$$S_{w,dn} = (vz) / V_{f,dn}. \qquad (4)$$

The floodplains were inundated on only one sampling date (February 2008). For all other dates, the floodplains were not inundated, and were therefore unlikely

to influence $-k$ or $S_{w,dn}$. To estimate the influence of the constructed floodplains when they were inundated, we simulated nutrient spiraling metrics for a trapezoidal and a two-stage ditch under a range of flow conditions. In both simulations, we varied Q from 174 L/s (the lowest Q under water starts to flow onto the floodplains) to 1600 L/s (ditch-full flow) while holding U and C constant. Stream width was set equal to the width of the trapezoidal channel and two-stage channels (i.e., active stream channel plus floodplains), 3 m and 9 m width, respectively. Mean depth was calculated from our empirical relationship between discharge and depth (see the previous section). Water velocity was calculated as

$$v = Q / (wz). \qquad (5)$$

NO$_3^-$ removal rate was set equal to the median habitat-weighted areal denitrification rate supported by the stream sediments (U_{dstr}) in 2008 and 2009. In the two-stage ditch simulation, U_{dn} was calculated as the median habitat-weighted areal denitrification rate supported by the stream sediments and floodplain soils (U_{ddit}; [denitrification in ditch]) in 2008 and 2009. We calculated $-k$ and S_w for both simulations as described in the previous paragraph.

In addition to the reach-scale NO$_3^-$ removal estimated with nutrient spiraling metrics (described above in the first paragraph of this section), we also estimated NO$_3^-$ removal with simple areal scaling. This approach is commonly used in lentic, wetland, and floodplain systems and when flow conditions in lotic systems are not known (Smith et al. 2000, Poe et al. 2003, David et al. 2006, Sheibley et al. 2006). This technique allowed us to scale our removal estimates to the watershed level, and to examine the importance of flow in load reduction calculations. Under base flow conditions, we calculated NO$_3^-$ removal as the habitat-weighted areal denitrification rate in the stream (U_{dstr}) multiplied by stream area. When the floodplains are inundated, we estimated NO$_3^-$ removal as the habitat-weighted areal denitrification rate in both the stream and the floodplains (U_{ddit}), multiplied by ditch area.

Statistical analysis

In accordance with the BACI design, we subtracted the control reach denitrification rate from the treatment reach denitrification rate for each sampling date, and used a t test to determine if the relationship between the control and treatment reaches had changed after the floodplain restoration (Stewart-Oaten et al. 1986). We also used BACI analysis to test for changes in reach-scale NO$_3^-$ removal, $-k$, $V_{f,dn}$, and $S_{w,dn}$. We used repeated-measures analysis of variance (RM ANOVA) to determine if there were differences in denitrification rates between substrates or reaches. To determine if instream denitrification was nutrient limited, we performed a two-way ANOVA on the data from the nutrient limitation assays (Tank and Dodds 2003).

We used simple linear regression to determine relationships between denitrification rates and physicochemical variables. For instream denitrification, these physicochemical variables included surface water NO_3^- concentration and sediment organic matter content. For floodplain denitrification, we examined soil exchangeable NO_3^- concentration, soil gravimetric water content, and soil organic matter content.

We used analysis of covariance (ANCOVA) to determine if the dependence of the simulated storm flow NO_3^- removal metrics ($-k$ and $S_{w,dn}$,) on Q was different in the trapezoidal vs. two-stage ditch.

We performed most statistical analyses with SYSTAT 12 (SYSTAT Software 2007), and considered $P < 0.05$ to be significant. To meet the assumptions of parametric statistics, we tested all data for normality with the Shapiro-Wilk test ($P > 0.05$) and log-transformed the data when necessary. When normality could not be achieved with these transformations, we rank-transformed the data (Iman and Conover 1979). The nonparametric Kendall's Tau test was applied to non-normal data sets with points below the detection limit (Helsel 2005) using R version 2.11.1 and version 1.5–3 of the NADA package (R Core Development Team 2010).

Results

Floodplain inundation

Bank-full events (floodplain inundation), occurred an average of 12 times a year, although there was strong interannual variability in the timing and duration of these events. In 2008, the floodplains were inundated eight separate times, for a total of 29 days. In 2009, the floodplains were inundated 16 separate times, for a total of 132 days. The median duration of each inundation event was 2.5 days, but in the winter and spring of 2009, they were inundated continuously for 50 days. Prior to restoration, there were 756 m^2 of naturally formed floodplains, and after restoration, there were 3520 m^2 of floodplains. Because the restored floodplains were constructed at the same height as the naturally formed floodplains, restoration did not increase bank-full channel capacity, nor did it increase the frequency of bank-full events (Kallio 2010a); rather, floodplain restoration increased stream surface area during bank-full events. In addition, floodplain restoration increased average ditch-full cross-sectional area from 8 m^2 to 12.5 m^2. Ditch-full events occur when water flows out of the ditch and onto the fields, so by increasing ditch-full area, we decreased the frequency of ditch-full events.

Sediment denitrification rates

The restoration had no effect on instream denitrification rates; the difference between the upstream control and downstream restoration reaches remained consistent across years (t test of differences, $P > 0.75$). In general, we found that denitrification rates were higher on FBOM compared to sand, particularly when denitrification was expressed per gram DM (Fig. 2).

Expressing denitrification per gram AFDM (i.e., accounting for differences in organic matter content of the sediment) dampened the differences among substrates, but rates were still higher on FBOM, regardless of the way denitrification was expressed (e.g., g/AFDM, g/DM, or m^{-2}; RM ANOVA, $P < 0.002$ for all tests; Fig. 2C and D). We found seasonal patterns in instream sediment denitrification rates, with the highest rates measured in the late winter and early spring, and the lowest rates measured in late summer and fall (RM ANOVA, $P < 0.002$; Fig. 2). Denitrification rates were 1.5 to 2 times higher in the treatment reach than the control reach on both sand and FBOM, when expressed per gram DM and per m^2. Rates were 1.3 times higher in the treatment reach on sand when denitrification was expressed per gram AFDM (RM ANOVAs, $P < 0.002$ for all tests; Fig. 2). Over the course of the three-year study, we also found interannual variation in denitrification rates, regardless of how they were expressed, with the lower rates occurring in the last year of our study (RM ANOVA, $P < 0.03$ for all tests; Fig. 2).

Warming stream sediments during laboratory incubations resulted in an increase in denitrification rates for all treatments, with the exception of sand during the winter (stream temperature = 1°C; Fig. 3). Under cool stream temperatures (e.g., winter samples), correcting for incubation temperature resulted in a 40% decrease in denitrification rates on FBOM (from an average of 0.63 to 0.38 µg N_2O-N·[g DM$]^{-1}$·h^{-1}), and a 0% decrease on sand. Under warm stream temperatures (e.g., summer samples), correcting for temperature resulted in a 10% decrease in denitrification rates on FBOM (from an average of 0.41 to 0.37 µg N_2O-N·[g DM$]^{-1}$·h^{-1}), and a 30% decrease in denitrification rates on sand (from an average of 0.13 to 0.09 µg N_2O-N·[g DM$]^{-1}$·h^{-1}). However, even when denitrification rates were corrected for incubation temperature, the relationship between reaches remained unchanged (i.e., the t test of differences was still not statistically significant; Fig. 2).

Sediment denitrification rates on FBOM were predicted by surface water NO_3^- concentrations (SLR with log-transformation, $r^2 = 0.35$, $P < 0.001$; Fig. 4A), and secondarily predicted by sediment organic matter content (SLR with x- and y-axis rank-transformation, $r^2 = 0.12$, $P < 0.001$; Fig. 5B). Denitrification rates on sand were predicted by sediment organic matter content (SLR with y-axis log-transformation, $r^2 = 0.23$, P p < 0.001; Fig. 5A), but not by surface water NO_3^- concentration (SLR, $r^2 < 0.01$, $P > 0.1$).

The nutrient limitation assays demonstrated that the addition of glucose and NO_3^- significantly increased denitrification rates on only four of 15 sampling dates. Both reaches and substrates were nutrient limited in October 2007, concurrent with low sediment organic matter and water column NO_3^-, while on the other dates, nutrient limitation occurred in a single reach and substrate, and did not follow a consistent pattern (Table 2).
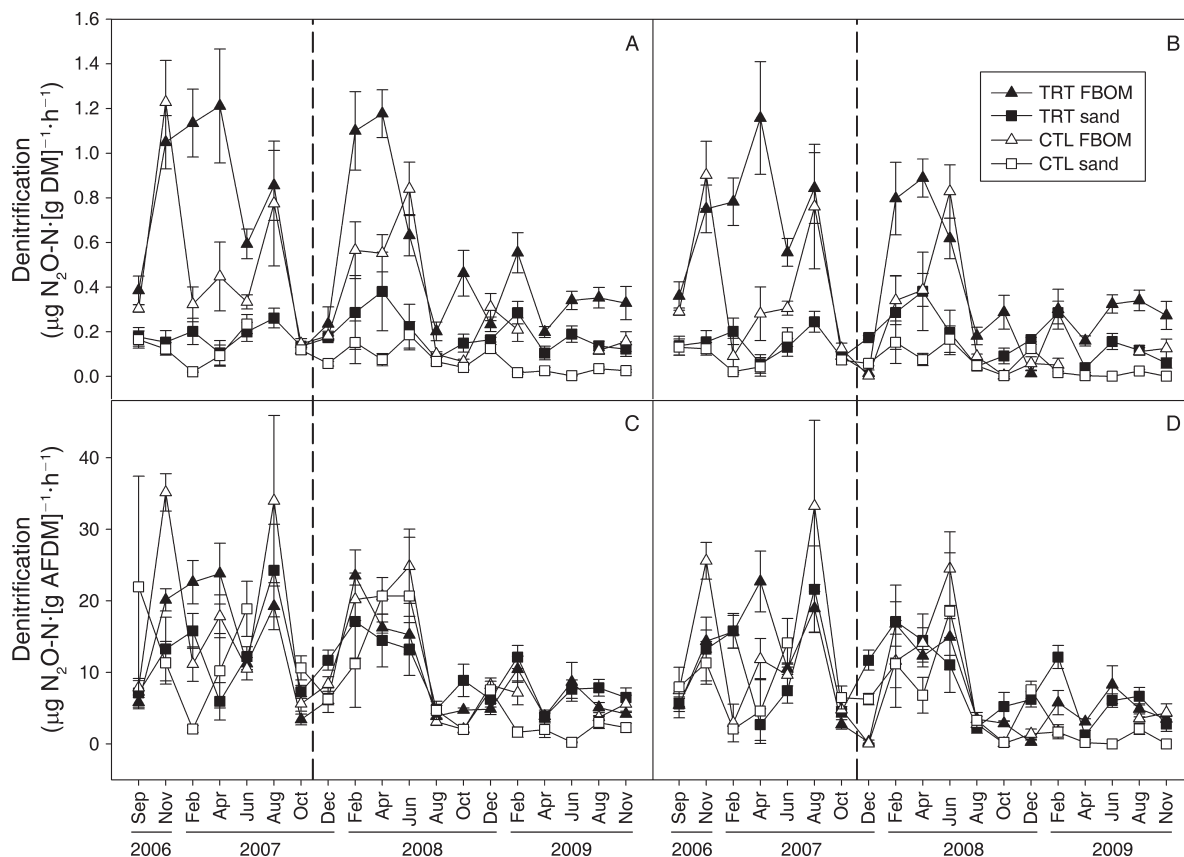
Fig. 2. Denitrification rates in the control (CTL) and treatment (TRT) reaches, on the dominant stream substrate types (sand and fine benthic organic matter [FBOM]). Denitrification is expressed (A) per gram of dry mass (DM), without temperature correction; (B) per gram of DM, corrected for temperature; (C) per gram ash-free dry mass (AFDM), without temperature correction; and (D) per gram AFDM, corrected for temperature. The error bars indicate $\pm$SE, and the vertical dashed lines indicate the date of the two-stage restoration. Instream denitrification rates exhibited seasonal and interannual variation, but rates were consistently higher in the TRT reach and on FBOM. Correcting for temperature results in slightly lower denitrification rates, but the seasonal patterns remain the same.

Soil denitrification rates on floodplains

Floodplain denitrification rates from the top 5 cm of soil ranged from 0.02 to 6.7 mg N_2O-N·m^{-2}·h^{-1}, and were significantly lower than instream habitat-weighted rates in the treatment reach (RM ANOVA, $P < 0.002$), which ranged from 3.2 to 20.3 mg N_2O-N·m^{-2} h^{-1}. Under ambient conditions, the highest rates occurred in the top 5 cm of the floodplains, but denitrification was observed to 15 cm depth in nearly all cores (Fig. 6). On average, denitrification in the top 5 cm represented 56% $\pm$ 6% (mean $\pm$ SE) of denitrification measured in the 15 cm core, and this did not change seasonally (RM ANOVA, $P > 0.5$).

Mean soil exchangeable NO_3^- from the floodplain soils was 2.6 $\pm$ 0.3 µg NO_3^--N/g soil, with a range of below detection (3 µg/L) to 33.7 µg NO_3^--N/g soil. Soil exchangeable NO_3^- was correlated with floodplain soil denitrification rates (Kendall's tau, $\tau = 0.34$, $P < 0.001$; Fig. 7), but denitrification in floodplain soils was measurable even when soil exchangeable NO_3^- was below detection. Surface water NO_3^- concentration did

not predict denitrification in laboratory assay samples experimentally saturated with stream water (SLR, $r^2 = 0.02$, $P > 0.1$). In fact, the addition of stream water to floodplain soils in the laboratory stimulated denitrification only on the last sampling date, in November 2009 (t test, $P < 0.05$; Fig. 6). In addition, the soil denitrification rates measured during naturally flooded conditions (February 2008) were within the range measured under laboratory inundations (Fig. 6).

Soil organic matter content averaged 7.8% $\pm$ 0.3% (range: 0.7–32%), and was a weak predictor of soil denitrification when surface water was not added (SLR, $r^2 = 0.06$, $P < 0.02$). Soil gravimetric water content from floodplains averaged 42% $\pm$ 1%, but was quite variable in space (range: 13–67%) and was not a significant predictor of floodplain denitrification rates (SLR, $r^2 = 0.01$, $P > 0.3$). The carbon quality index, calculated as the molar ratio of CO_2 production rate to N_2O production rate, was significantly higher on the floodplains compared to stream sediments (RM ANOVA, $P < 0.001$; Fig. 8).

Nitrogen removal via denitrification

Base flow NO_3^- removal rate $(-k)$ within the stream channel averaged 15 ± 1 percentage per day, with a range of 5% to 29% (Fig. 9A), and did not change post-floodplain construction, relative to the control reach (t test of differences, $P > 0.2$; Fig. 9A). In addition, NO_3^- uptake velocity via denitrification ($V_{f,dn}$) did not change post-construction (t test of differences, $P > 0.6$), and neither did the variables used to calculate $V_{f,dn}$ (denitrification and NO_3^- concentration; see *Methods: Reach-scale NO_3^- removal calculations*). Finally, the NO_3^- uptake length ($S_{w,dn}$) in the stream channel at base flow did not change post-restoration (t test of differences, $P > 0.16$), and fell within the range measured in agricultural stream channels reported in previous studies (Table 3; Royer et al. 2004, Mulholland et al. 2009).

In contrast to base flow conditions, under simulated storm conditions that would result in the inundation of floodplains, NO_3^- loss $(-k)$ was typically higher in the two-stage ditch than the trapezoidal channel (Fig. 9B). As stream flows increased, $-k$ decreased, because NO_3^- load increases with discharge. However, the rate of decrease in $-k$ with increasing Q was lower in the two-stage compared to the trapezoidal channel (ANCOVA of 2008 data, $P < 0.01$; ANCOVA of 2009 data, $P < 0.03$; Fig. 9B); therefore, the two-stage ditch dampens storm flow NO_3^- export. Similarly, the denitrification uptake length ($S_{w,dn}$) during storm flow was always lower in the two-stage, compared to the trapezoidal, channel, indicating that NO_3^- is denitrified closer to its point of entry into the stream channel and does not travel as far when the two-stage ditch is present. Therefore, although the two-stage ditch had minimal influence on water column NO_3^- removal during base flow, it did reduce storm flow NO_3^- export.

Estimates of NO_3^- loss with the areal scaling method were lower than with nutrient spiraling, but exhibited the same patterns. Base flow NO_3^- removal averaged $1.3\% \pm 0.2\%$ per day (range: 0.3–2.3%) with areal scaling, compared to 15 ± 1 percentage per day (range: 5–29%) calculated with nutrient spiraling. Under simulated storm flows, NO_3^- loss averaged $0.5\% \pm 0.09\%$ per day (range: 0.3–0.8%) with areal scaling and $6.1\% \pm 0.4\%$ per day (range: 5.0–7.8%) with nutrient spiraling.

DISCUSSION

We completed a multiyear assessment of a novel, reach-scale stream restoration technique in a midwestern agricultural stream. This sort of analysis is rare but necessary for proper evaluation of stream restorations (Bernhardt et al. 2005, Palmer et al. 2007), and provides important insights into the utility of the two-stage ditch restoration practice. Our statistical design (BACI) was critical for separating the influence of interannual variation from the influence of the two-stage ditch, because there were pre-existing differences between the control and treatment reaches, as well as interannual variation in denitrification rates.
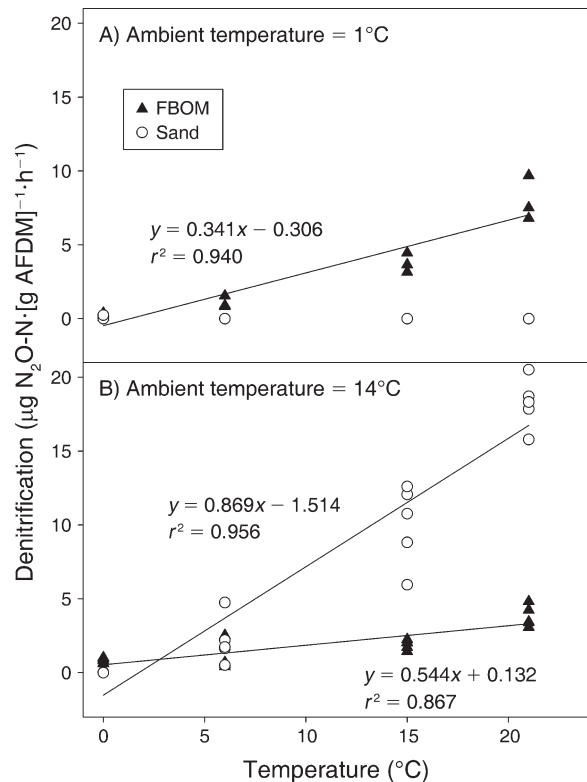

FIG. 3. The effect of laboratory incubation temperature on denitrification rates supported by sand and FBOM, under (A) cool and (B) warm ambient stream water temperatures. Regression equations are displayed only when the regression was statistically significant ($P < 0.5$). The effect of temperature varied by season and substrate.

We observed large interannual variation in floodplain inundation frequency and duration. Greater NO_3^- export generally occurs during high-precipitation years than during low-precipitation years (Royer et al. 2006), but high precipitation will also result in increased floodplain–water column contact time. As a result, we expect that the influence of the two-stage ditch on NO_3^- export will vary substantially from year to year, and that the greatest relative reduction in NO_3^- export will occur during wet years.

Controls on denitrification rates

The restoration of floodplains did not compromise instream denitrification rates, which remained high throughout the three-year study period (Fig. 2), indicating that the NO_3^- removal capacity of the stream channel was maintained. In contrast, there is some evidence that floodplain construction can influence instream sediment characteristics over the long term (i.e., years): The main stream channel tends to narrow, which increases water velocities (Powell et al. 2007), and can result in the removal of lighter FBOM particles, changing the stream bed to predominantly sand bottom. Since sand supports lower denitrification rates than
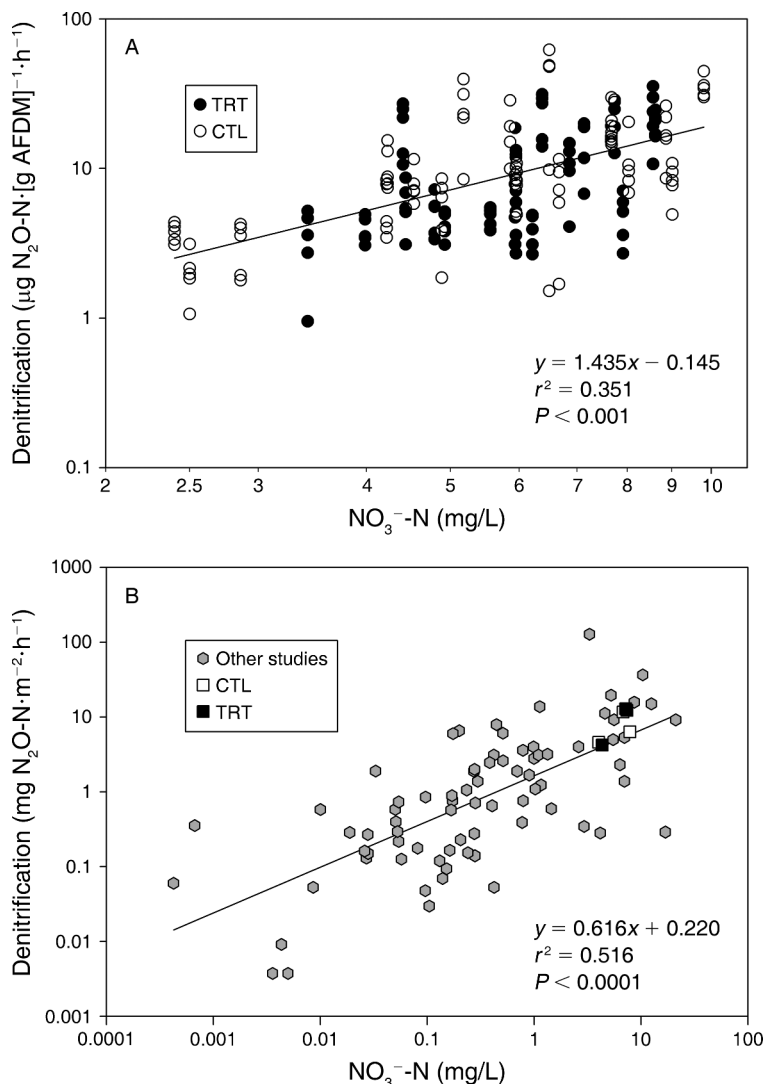
FIG. 4.   (A) Relationship between instream denitrification rates on fine benthic organic matter (FBOM) and stream water NO_3^- concentration. Each data point represents the denitrification rates measured in one of five replicate samples collected on each sampling date. The relationship between instream denitrification rates and stream water NO_3^- was not significant in sand. (B) Whole-stream, habitat-weighted denitrification rates and stream water NO_3^- concentrations in Shatto Ditch, compared with previously published data from other systems. Denitrification rates were measured with many methods, including whole-stream $^{15}NO_3^-$ releases, membrane inlet mass spectrometry (MIMS), in situ C_2H_2 block chambers, and laboratory C_2H_2 slurries (Triska and Oremland 1981, Duff et al. 1984, Christensen et al. 1990, Nielsen et al. 1990, García-Ruiz et al. 1998, Pattinson et al. 1998, Bernhardt and Likens 2002, Kemp and Dodds 2002, Böhlke et al. 2004, Laursen and Seitzinger 2004, Mulholland et al. 2004, 2008, Royer et al. 2004, Schaller et al. 2004, Inwood et al. 2005, Pribyl et al. 2005, Arango et al. 2007). Each point in Shatto represents the seasonal average (winter, spring, and summer/fall) within the control or treatment reach. Note that some of the Shatto Ditch points overlap. Both denitrification rates and stream water NO_3^- concentrations in Shatto Ditch were among the highest reported in the literature. Note the log–log scale plots.

FBOM, the result would likely be lower reach-scale denitrification rates. However, we did not observe a decrease in FBOM throughout our study; thus, if this is a trend, it may take longer than two years post-construction to become apparent.

Previous research has demonstrated that sediment microbial denitrification rates are best predicted by stream water NO_3^- concentrations (Pina-Ochoa and Alvarez-Cobelas 2006, Mulholland et al. 2009), and

results from this study are consistent with these prior results (Fig. 4B). Yet agricultural streams may also represent unique cases in that NO_3^- concentrations routinely reach a threshold beyond which denitrification is no longer NO_3^- limited (Inwood et al. 2005, Wall et al. 2005). The exact value of this threshold varies among systems and is likely related to sediment quality and availability of alternative electron acceptors. Stream water NO_3^- concentrations in this study apparently
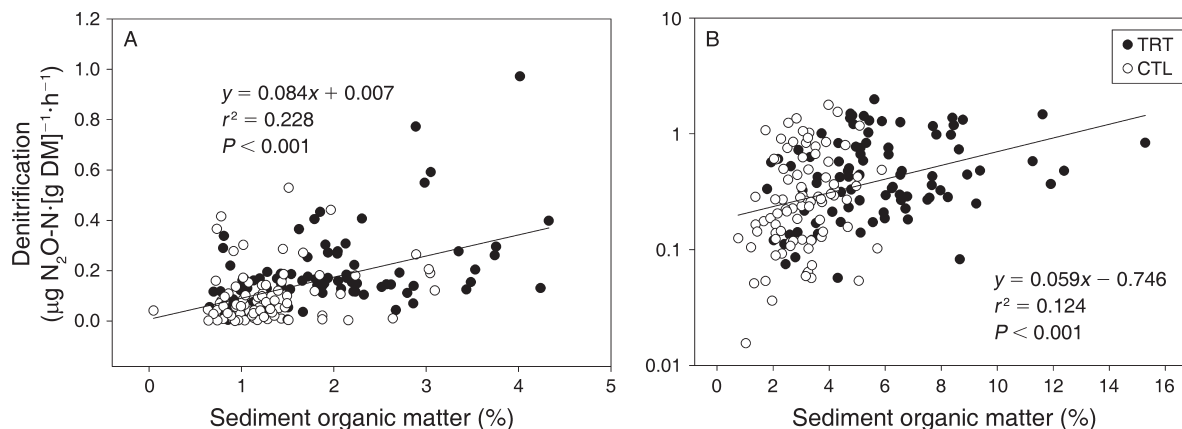
Fig. 5. Relationship between sediment organic matter content and denitrification rate on (A) sand and (B) fine benthic organic matter (FBOM) with log scale for denitrification.

exceeded the threshold value for denitrification supported by sand, as evidenced by the lack of relationship between denitrification on sand and surface water NO_3^- concentration. The threshold value was apparently not exceeded on FBOM, probably as a result of high carbon availability (Arango et al. 2007) and increased surface area for microbial colonization of the finer particles associated with FBOM (Inwood et al. 2007).

The response of denitrifiers to temperature varied by substrate and season. Under cold ambient stream temperatures, warming had no effect on denitrification rates on sand, but slightly stimulated denitrification on FBOM (Fig. 3). Under warm ambient stream temperatures, the effect of temperature was more pronounced (Fig. 3), but because the stream temperature was closer to the incubation temperature, correcting for the difference had only a small effect on denitrification rates. Thus, the combined adjustments during both cold and warm sampling resulted in somewhat dampened seasonal trends in denitrification rates, yet seasonal

patterns remained apparent (Fig. 2). In summary, seasonal changes in surface water NO_3^- concentration and sediment organic matter content are responsible for much of the variability in denitrification rates.

In contrast to stream sediments, denitrification rates in restored floodplains are consistently controlled by soil organic matter content (Groffman and Crawford 2003, Sheibley et al. 2006, Orr et al. 2007, Gift et al. 2010), although the strength of the relationship varies substantially. Previously published relationships between denitrification and other soil variables are less consistent; soil nitrogen, soil moisture, and root biomass all predicted denitrification to some degree in some systems (Sheibley et al. 2006, Gift et al. 2010). In this study, floodplain denitrification rates were controlled by soil-exchangeable NO_3^- (Fig. 7) and weakly controlled by soil organic matter content. Floodplain denitrification rates were less predictable than instream rates, but floodplains are more spatially and temporally variable than streams (Naiman et al. 1988, Hedin et al. 1998).

TABLE 2. Denitrification rates (mean ± SE) per treatment on dates and substrates in which nutrient limitation occurred.

Date and reach	Substrate	Denitrification rate (μg N_2O-N·[g $DM]^{-1} \cdot h^{-1}$)†			
		0	N	C	NC
2007					
Oct CTL	sand	0.12 ± 0.02	0.16 ± 0.02	**0.24 ± 0.02**	0.25 ± 0.06
Oct CTL	FBOM	0.15 ± 0.02	**0.24 ± 0.04**	**0.27 ± 0.06**	**0.39 ± 0.03**
Oct TRT	sand	0.13 ± 0.01	**0.16 ± 0.01**	0.17 ± 0.02	**0.25 ± 0.01**
Oct TRT	FBOM	0.12 ± 0.01	**0.17 ± 0.03**	0.14 ± 0.02	**0.38 ± 0.07**
2008					
Feb TRT	FBOM	1.10 ± 0.18	**2.06 ± 0.15**	1.11 ± 0.08	1.94 ± 0.20
Apr TRT	FBOM	1.18 ± 0.11	**1.81 ± 0.15**	1.30 ± 0.20	1.84 ± 0.20
2009					
Aug CTL	FBOM	0.12 ± 0.02	**0.17 ± 0.02**	0.09 ± 0.01	0.15 ± 0.01

Notes: Abbreviations are: CTL, control reach; TRT, treatment reach; and FBOM, fine benthic organic matter. Numbers in boldface indicate significant ($P < 0.05$) nutrient limitation, according to a two-way ANOVA from Tank and Dodds (2003).

† Treatments are: 0, control, no nutrient amendments; N, nitrate added, as KNO_3^-; C, carbon added, as glucose; and NC, nitrate and carbon added.
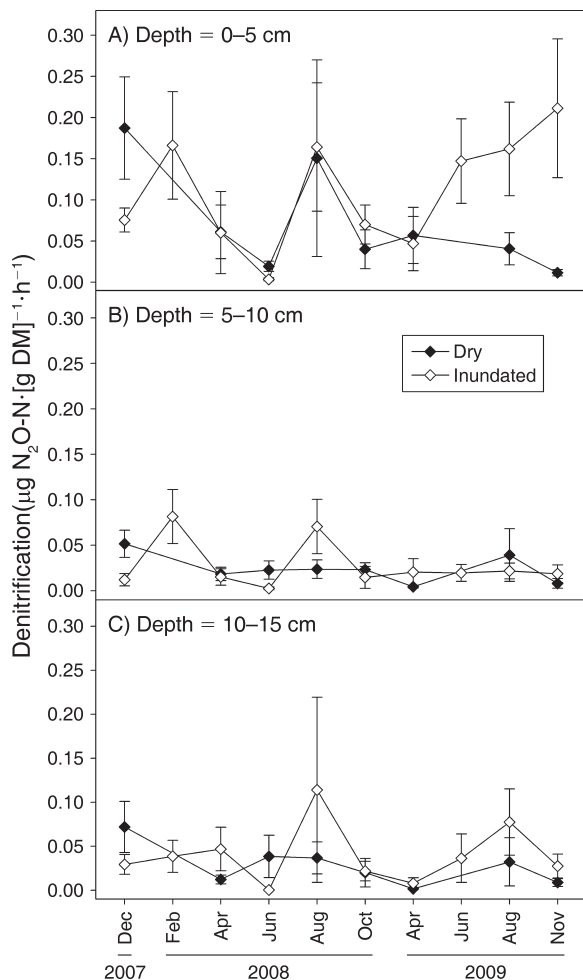
FIG. 6. Post-restoration floodplain denitrification rates by stratum and incubation type. Samples were either incubated with surface water added (Inundated) or at field moisture, without any surface water added (Dry). Error bars are standard error. The highest denitrification rates occurred in the top 5 cm, although denitrification was measurable to 15 cm. The addition of stream water stimulated denitrification rates only on the final sampling period.

Post-restoration, soil denitrification was measurable on the floodplains almost immediately after construction and did not change significantly after the first sampling date (Fig. 6), suggesting that denitrifying microbes were either already present or colonized rapidly. Denitrification rates were consistently lower on floodplain soils compared to stream sediments, especially FBOM. This may be due, in part, to differences in carbon quality (Fig. 8), but may be also explained by differences in oxygen availability or in the microbial community colonizing particles. Floodplain soil denitrification did not respond to the addition of stream water, perhaps because (1) the microbes on floodplain soils were not limited by NO_3^-, (2) the addition of chloramphenicol did not allow the microbes to take advantage of the

influx of NO_3^-, or (3) carbon on the floodplains was not of sufficiently high quality.

Contribution of the two-stage restoration to nitrogen removal

We found no change in base flow NO_3^- removal, because the construction of stream-side floodplains did not affect instream denitrification rates (Figs. 2 and 9A). However, our estimates of base flow NO_3^- removal are conservative, because they assume that floodplains do not contribute to stream water NO_3^- removal unless they are inundated. At Shatto Ditch, the floodplains were created from a grass buffer strip, and the surrounding fields are drained with subsurface tiles, which convey high-NO_3^- groundwater from fields directly to the stream, allowing minimal opportunity for N processing (Fennessy and Cronk 1997, Ducros and Joyce 2003). In contrast, the floodplains are a part of the stream corridor and close to the water table. As a result, the soils are more saturated and have more anoxic microsites, which are necessary for denitrification. In addition, the floodplains receive NO_3^- inputs from tile drains, periodic surface water inundations, soil nitrification, and hyporheic flow. Therefore, the constructed floodplains support higher denitrification rates than the buffer strip (M. L. Stephen, *unpublished data*), likely reducing the NO_3^- load entering the stream.

In our calculations, we only used denitrification rates from the surface layer (0–2 cm in the stream, 0–5 cm on the floodplains) because (1) this method allows direct comparison to previous stream studies (e.g., Christensen et al. 1990, Royer et al. 2004, Arango et al. 2007), and (2) we used the rates to estimate nutrient spiraling metrics. Nutrient spiraling only accounts for the surface sediments, because they interact directly with surface water. We found that denitrification rates decreased with depth in the floodplain soils, but the top layer only accounted for 56% of total denitrification, indicating that there is substantial N removal potential at depth.
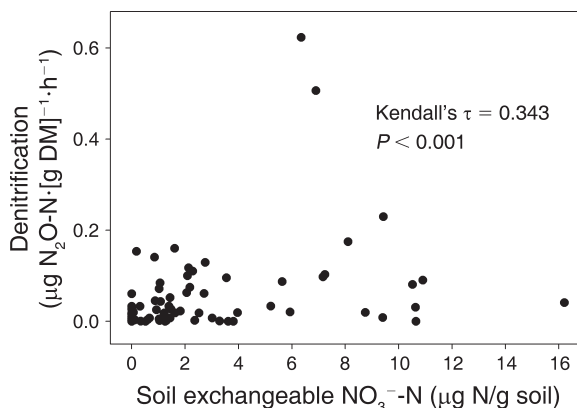

FIG. 7. Relationship between denitrification rates on floodplain soils (incubated without the addition of stream water) and soil exchangeable NO_3^- concentration.
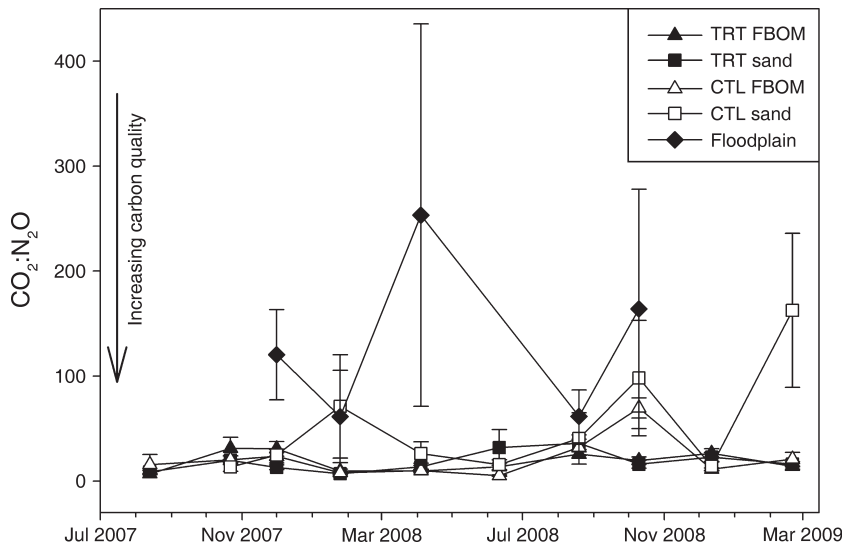
FIG. 8.   Ratio of carbon dioxide (CO_2) to nitrous oxide (N_2O) sediment production rates during denitrification assays, an index of carbon quality, through time. Data are grouped by reach type (i.e., treatment [TRT] or control [CTL]) and sediment type (i.e., fine benthic organic matter [FBOM] or sand). Floodplain denitrification rates were from samples incubated with stream water.

Similarly, we found that the top 2 cm of stream sediment supported 44% of the denitrification observed throughout a 20 cm core (S. S. Roley, *unpublished data*), suggesting that our estimates of reach-scale denitrification are conservative because they don't account for denitrification activity on deeper material.

Storm flow NO_3^- removal was higher in the two-stage ditch than the trapezoidal channel (Fig. 9B), indicating that the two-stage ditch can reduce NO_3^- loads during high flows, which is when agricultural streams export the most NO_3^- (Royer et al. 2006). The magnitude of the increase was strongly influenced by the denitrification rate in the floodplains, because when the ditch was fully flooded, the majority of benthic surface area was

floodplain soils. On an annual basis, the influence of the floodplains also depended upon the length of time they were inundated. In 2008, floodplain inundation was minimal (29 days), and the floodplains contributed only 12% of the annual ditch N removal (Table 4). In contrast, in 2009, the floodplains contributed 47% of the annual ditch N removal, both because of extended inundation and a higher median denitrification rate (Table 4). The success of two-stage ditches at retaining N will depend upon the quality of floodplain substrate for denitrification, and the elevation of the restored floodplains, which controls inundation frequency.

Denitrification uptake length ($S_{w,dn}$) was shorter in the two-stage ditch than in the trapezoidal channel
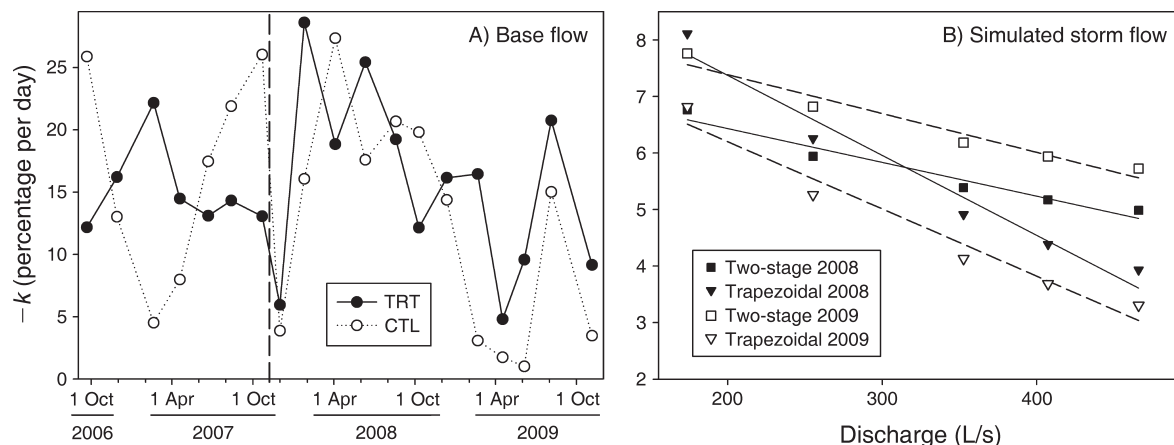

FIG. 9.   Percentage of the NO_3^- load removed per day ($-k$) (A) under base flow conditions and (B) under simulated storm flow conditions. (A) The dotted line indicates the date of two-stage construction. (B) Simulated storm flow was calculated with the median denitrification rates from 2008 and 2009. There was a significant difference, by ANCOVA, in the slopes of the regression lines for 2008 data (solid lines, $P < 0.01$) and 2009 data (dashed lines, $P < 0.05$).

Table 3. Denitrification uptake length ($S_{w,dn}$) in this study and other studies of agriculturally influenced streams.

Source	$S_{w,dn}$ (km)	
	Lowest	Highest
Mulholland et al. (2009)	1	184
Royer et al. (2004)	8	>200
This study		
Base flow	21	152
Two-stage storm flow	42	218
Storm flow (no two-stage)	56	320

(Table 3); on average, NO_3^- in the water column of a two-stage ditch does not travel as far prior to denitrification as does NO_3^- from a trapezoidal channel. Shatto Ditch is a high-NO_3^- stream, even for an agriculturally influenced system (NO_3^- concentration ranged from 2–9 mg NO_3^--N/L), so our estimates of two-stage ditch potential are likely conservative: In a lower NO_3^- system, higher NO_3^- removal efficiencies may be achieved.

Although floodplain construction via the two-stage ditch dampens storm flow NO_3^- export, at least 90% of the load was still exported in all storm flow scenarios (Fig. 9B). Under base flow conditions, maximum NO_3^- removal was 28%, which occurred when the NO_3^- load was relatively low (41 kg/d). Across a gradient of land use, stream N removal efficiencies in a multi-biome study of 72 headwater streams varied from 0.5% to 100% (Mulholland et al. 2008, 2009), with the highest removal efficiencies relative to N loading occurring under the lowest NO_3^- loads. Thus, even though two-stage ditch restoration enhanced reach-scale N removal at Shatto Ditch, it is unlikely to substantially reduce NO_3^- loads unless landscape management practices that reduce stream NO_3^- inputs from upland fields are also adopted (Craig et al. 2008).

Two-stage ditch influence at the sub-watershed scale

All of our reach-scale calculations of NO_3^- removal were based on our 600-m study reach, but additional NO_3^- removal benefit may be achieved by increasing the length of restored floodplains. The floodplains along the 600-m treatment reach in this study occupy <0.04% (0.4 ha) of the upstream watershed area (1036 ha) and ~7% of the total drainage network length (Fig. 1B). To examine the potential for widespread implementation of two-stage ditches to reduce NO_3^- loads, we developed a model that integrates drainage network, channel morphology, and hydrology data with measured denitrification rates for the stream sediments and floodplain soils, as well as the additional stream surface area created during storms (J. D. Witter, *unpublished method*). This method accounts for changes in surface area, but not water residence time, so it results in lower NO_3^- removal estimates than those calculated with the nutrient spiraling method. Nonetheless, it offers an estimate of the relative increase in NO_3^- removal.

To estimate NO_3^- removal at a sub-watershed scale, we evaluated several scenarios which varied the percentage of the drainage network in two-stage ditch treatment (7%, 50%, and 100% of the drainage network) and applied the median denitrification rates from 2008 and 2009 (Table 4) to estimate annual NO_3^- load reductions if the length of two-stage ditch were extended beyond this experiment's 600-m treatment. Denitrification in the stream channel was simulated for the entire year and estimates of floodplain denitrification were limited to days when the floodplains were inundated with surface water. Results of the analysis suggest that if all of the stream length in the Shatto Ditch sub-watershed were placed in two-stage, the addition of the floodplains would be capable of removing 10–11% of the annual NO_3^- load, compared to 6–9% of the annual load when only 600 m is restored (Table 4).

Comparison to other best management practices

Floodplains can form naturally in unmaintained ditches, particularly when the channel is wider than necessary (Landwehr and Rhoads 2003), but their formation occurs slowly over a long period of channel instability. In addition, surface area is limited to the original channel width (i.e., no surface area is added). In contrast, the construction of floodplains associated with the two-stage ditch restoration results in immediate improvement in channel stability and an increase in bioreactive surface area. Microbial denitrification occurs on soils in both constructed and naturally formed floodplains (Powell and Bouchard 2010), but we believe that the larger surface area and immediate benefits

Table 4. (a) Annual load reductions achieved with two-stage ditch and (b) potential load reductions achieved by extending the two-stage ditch.

a) Data used for scaling up:

Scenario description	2008	2009
Median instream denitrification†	9.5	5.9
Median floodplain denitrification†	3.1	6.1
Duration of floodplain inundation (d)	29	132
Annual instream N removal‡	162	100
Annual floodplain N removal§	21	89

b) Results of the scaling:

Two-stage extent		Total network load reduction (%)	
Length (km)	Percentage of watershed	2008	2009
0.6	7	8.9	5.9
4.1	50	9.5	7.9
8.4	100	10.0	10.3

Notes: Model estimates using the median channel and floodplain denitrification rates are shown. Note that two-stage ditch effectiveness increases with ditch length, and is strongly influenced by denitrification rate and duration of inundation.

† Denitrification rate in mg N·m^{-2}·h^{-1}.

‡ N removal via denitrification, in kg N/yr.

§ N removal via denitrification, when floodplains are inundated, in kg N/yr.

associated with two-stage restoration make it preferable to naturally formed floodplains.

Another widely recommended BMP is constructed wetlands (Mitsch et al. 2001), whose soils typically have redox conditions that are ideal for denitrification, and whose relatively long water residence time can result in high N removal efficiencies (Mitsch et al. 2005, Borin and Tocchetto 2007). However, wetlands are less effective during storm flows, when their water holding capacity is overwhelmed (Kovacic et al. 2000, 2006). Wetlands and two-stage ditches are potentially complementary BMPs, with wetlands intercepting base flow tile drain water, and two-stage ditches accommodating storm flow and increasing NO_3^- removal rates during floodplain inundation. Further investigation into optimal complementary placement of these surface water BMPs is warranted.

The N removal efficiencies of wetlands, streams, and two-stage ditches generally decrease with N load (Mitsch and Gosselink 2000, Mulholland et al. 2008, 2009). Agriculturally influenced streams (including Shatto Ditch) are notoriously high in NO_3^- (Osborne and Wiley 1988, Johnson et al. 1997, Stanley and Maxted 2008), and as a result, we do not expect that two-stage ditches or constructed wetlands will completely alleviate downstream N loading problems. Instead, we recommend that instream practices, including two-stage ditches, should be implemented in concert with landscape management practices, such as cover crops and precision fertilizer application, which reduce N export from fields to surface waters (Dinnes et al. 2002). As our data have shown, the two-stage ditch has the potential to increase NO_3^- removal rates in stream reaches via denitrification, but must be considered part of a suite of complementary surface water and landscape management practices, in which N removal is enhanced at multiple landscape locations.

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