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ECOSYSTEMS

Woody Vegetation Removal Stimulates Riparian and Benthic Denitrification in Tallgrass Prairie

Alexander J. Reisinger,[1,2]* John M. Blair,[1] Charles W. Rice,[3] and Walter K. Dodds[1]

[1]*Division of Biology, Kansas State University, Manhattan, Kansas 66506, USA;* [2]*Present address: Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana 46556, USA;* [3]*Department of Agronomy, Kansas State University, Manhattan, Kansas 66506, USA*

ABSTRACT

Expansion of woody vegetation into areas that were historically grass-dominated is a significant contemporary threat to grasslands, including native tallgrass prairie ecosystems of the Midwestern United States. In tallgrass prairie, much of this woody expansion is concentrated in riparian zones with potential impacts on biogeochemical processes there. Although the effects of woody riparian vegetation on denitrification in both riparian soils and streams have been well studied in naturally wooded ecosystems, less is known about the impacts of woody vegetation encroachment in ecosystems that were historically dominated by herbaceous vegetation. Here, we analyze the effect of afforestation and subsequent woody plant removal on riparian and benthic denitrification. Denitrification rates in riparian soil and selected benthic compartments were measured seasonally in naturally grass-dominated riparian zones, woody encroached riparian zones, and riparian zones with woody vegetation removed in two separate watersheds. Riparian soil denitrification was highly seasonal, with the greatest rates in early spring. Benthic denitrification also exhibited high temporal variability, but no seasonality. Soil denitrification rates were greatest in riparian zones where woody vegetation was removed. Additionally, concentrations of nitrate, carbon, and soil moisture (indicative of potential anoxia) were greatest in wood removal soils. Differences in the presence and abundance of benthic compartments reflected riparian vegetation, and may have indirectly affected denitrification in streams. Riparian soil denitrification increased with soil water content and NO_3^-. Management of tallgrass prairies that includes removal of woody vegetation encroaching on riparian areas may alter biogeochemical cycling by increasing nitrogen removed via denitrification while the restored riparian zones return to a natural grass-dominated state.

Key words: woody encroachment; denitrification; riparian vegetation; nitrogen removal; prairie streams; tallgrass prairie.

INTRODUCTION

Tallgrass prairie is one of the most endangered ecosystems in North America, with areal declines from the pre-industrial to the modern era estimated between 82 and 99% (Samson and Knopf 1994). Primary threats to remaining tallgrass prairie include

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**Corresponding author; e-mail:* areisin1@nd.edu

landscape fragmentation and the encroachment of native woody vegetation (Briggs and others 2005). Woody vegetation encroachment into grasslands is a widespread phenomenon, and can be driven by climate change, elevated atmospheric carbon dioxide concentration, increased nitrogen (N) deposition, altered grazing pressure, and changes in fire regimes (that is, frequency and intensity of fire; Briggs and others 2005). Woody vegetation encroachment occurs throughout the prairie landscape, but is especially intense in riparian zones of headwater prairie streams that were historically open canopied with grass-dominated riparian vegetation (Dodds and others 2004). Forests have expanded upstream, transforming naturally grass-dominated headwater riparian areas into riparian forests (Knight and others 1994; Briggs and others 2005), fundamentally changing the unique character of prairie streams. As grasslands are the dominant vegetation type over large areas of the earth (Dodds 1997), it is important to understand how contemporary land-cover changes, such as shifts in the plant community, may alter the ecology and biogeochemistry of streams draining these ecosystems.

Excess N is a major stressor of aquatic ecosystems (United States Environmental Protection Agency (USEPA) 2002), where increased N loading (Vitousek and others 1997) has numerous ecological and economic impacts (Carpenter and others 1998; Dodds and others 2009). Nitrogen is an especially important pollutant in aquatic ecosystems surrounded by agriculture where a loss of native riparian cover and increased fertilizer application has altered stream water chemistry (Johnson and others 1997). Conversion of grassy vegetation to agricultural, urban, or woody riparian zones can dramatically alter both riparian and stream environments (Johnson and others 1997; Lyons and others 2000). These alterations can indirectly affect N retention in headwater streams, with afforestation of native grassy riparian zones increasing allochthonous carbon (C) inputs (as leaf litter) to the streams, altering C and N cycling in riparian soils (Claessens and others 2010).

Riparian soils are biogeochemical hotspots due to the high availability of C, N, water, and spatially and temporally variable redox potentials (McClain and others 2003). These zones can retain or remove substantial quantities of N, particularly in small streams (Dodds and Oakes 2006). In addition to riparian soils, headwater streams also have the ability to retain a large amount of N entering the system due to high benthic surface area: water volume and active benthic microbial communities (Peterson and others 2001; Mulholland and others 2008). Denitrification, the dissimilatory reduction of nitrate (NO_3^-) to N gas (nitrous oxide, N_2O and dinitrogen, N_2), is a major nitrogen removal mechanism in both riparian (Pinay and others 1993; Hill 1996) and benthic (Mulholland and others 2008) zones of streams. Because of the favorable conditions for denitrification common in riparian and benthic zones, these transition zones are vital for protecting downstream ecosystems from N pollution. To reduce effects of N pollution, management should focus on N retention in riparian zones.

A common management practice for reducing N loading into headwater streams is riparian restoration. This can include restoration of hydrological connection between riparian soil and streams (for example, Kaushal and others 2008), restoration of degraded floodplains (for example, Orr and others 2007), or establishment of riparian buffer zones (for example, Pinay and others 1993; Hill 1996). Restored riparian zones (buffers) retain sediment (Dillaha and others 1989), reduce N concentrations in surface and groundwater flow paths (Dillaha and others 1989), and other nutrients (that is, phosphorus) and organic contaminants (Vidon and others 2010), thus improving water quality. Restoration of native riparian vegetation often implies creating riparian forests, even in regions that were historically devoid of trees. For example, the 1996 US ‘‘Farm Bill’’ required land managers to plant trees in riparian buffers to qualify for monetary assistance (National Resource Conservation Service (NRCS) 1997). Both grassy and woody riparian buffers can provide substantial, albeit different, benefits to water quality (Lyons and others 2000); woody buffers generally increase N retention and reduce surface flow velocity, whereas grassy buffers are more suited for reducing erosion and controlling phosphorus pollution (Lyons and others 2000).

Although NO_3^- concentrations are generally low in unimpacted grasslands, denitrification can play a significant role in the N cycle of tallgrass prairie (Groffman and others 1993). This study evaluates both the effect of a contemporary threat to grassland prairies on denitrification and suggests potential mechanisms for effects of different vegetation types on riparian denitrification in general. We focus on the effect of woody encroachment, and its subsequent removal, on riparian and benthic denitrification in prairie streams. We also assessed potential seasonal variation in denitrification rates, driven by temperature, precipitation, and variable substrate supply (Groffman and others 1993) and which may differentially influence forest and grassland processes. We predicted that: (1) riparian denitrification would exhibit seasonal variability, with the greatest rates in the spring due to the combination of increased temperature and precipitation, (2) denitrification would differ among riparian vegetation types (Lyons and others 2000), and (3) denitrification in the benthic zone of streams would be dependent upon the benthic substrata present in the system (for example, filamentous algae, sediment, root wads, or leaf packs). These predictions were based upon: (1) the combination of relatively warm temperatures, high soil moisture, and the greatest supply of carbon and nitrate during the spring in this tallgrass prairie (for example, Groffman and others 1993; Turner and others 1997) stimulating spring denitrification, (2) effects of vegetation types (grass versus woody) on soil chemistry (Lyons and others 2000), and (3) prior studies showing that different types of benthic substrata are capable of supplying labile carbon at differential rates (Cross and others 2005; Ishida and others 2008) and have different potentials for anoxic microzones (Kemp and Dodds 2001a).

METHODS

Site Description

We performed this study on two separate branches of King's Creek, located entirely within Konza Prairie Biological Station—a 3,487-ha native tallgrass prairie jointly owned by The Nature Conservancy and Kansas State University. Extensive descriptions of the King's Creek watershed have been published previously (for example, Dodds and others 2000; Kemp and Dodds 2002). Sampling sites were located in two experimental sub-watersheds of King's Creek: K2A, an ungrazed watershed (67 ha) on the north branch of King's Creek that is burned every 2 years, and N04D, a watershed (137 ha) on the south branch of King's Creek that is burned every 4 years and grazed by native American bison (*Bison bison*). In general, bison behave differently from cattle in that they spend little time in riparian zones (unpublished GPS collar data; A Joern and D. Larson, personal communication) and prefer upland areas. Bison tend to concentrate their impact near stream channels on specific crossing trails (Fritz and Dodds 1999) and crossings did not occur in areas that were sampled for denitrification. From here on, these watersheds will be referred to as grazed (N04D) and ungrazed (K2A). Soils at both sites are classified in the Ivan Silt Loam series. Study sites on each watershed were selected to include three reaches based on riparian vegetation: a naturally (native) grass-dominated riparian reach with an open canopy, a woody vegetation reach with a closed canopy, and a reach which had woody vegetation removed prior to the initiation of the study and subsequently had an open canopy (Riley and Dodds 2012). Aerial photographs of the treatment reaches are published in Riley and Dodds (2012). In the grazed watershed, the sampled reaches were ordered, from upstream to downstream: removal, closed canopy, and grassy canopy. In the ungrazed watershed, the order from upstream to downstream was: grassy, closed canopy, and removal. Differentially ordering treatments in the two watersheds reduced the potential for confounding upstream–downstream effects to influence statistical analyses.

For the woody vegetation removal treatment, we cleared all woody plants from immediately adjacent to, and within 30 m away from, the stream channel in December 2007 by a combination of brush cutting for smaller plants and using chainsaws to remove larger woody plants. Woody debris was subsequently removed from the riparian zone. Treatments were maintained by brush cutting during each subsequent winter. The ungrazed watershed was burned in the spring of 2008 and 2010, whereas the grazed watershed was burned in the spring of 2009. Ungrazed, grass-dominated areas generally burn intensely and completely, whereas burning is often patchy and incomplete in wooded riparian areas, or areas that are heavily grazed. Because the removal treatments were imposed in a 30-m wide zone, we focused on the riparian zone within 30 m of the stream at all study sites. These riparian soils were rarely saturated (only during times of high precipitation) as the topography was relatively steep and flooding did not inundate the riparian zones in the areas of study. Although the depth to groundwater was probably not below the deepest roots of the plants, it was generally well below the depth of the soil cores taken in this study. Bank heights were generally about a half meter on these sinuous stream channels that were dominated by limestone cobble and characterized by riffle pool sequences typical of streams of this size (typical channel widths during periods of flow of 2–3 m) in moderately rough topography.

Naturally grassy riparian zones in both the watersheds were dominated by big bluestem (*Andropogon gerardii*) and Indian grass (*Sorghastrum nutans*). Western ragweed (*Ambrosia psilotachya*), along with several other perennial forbs were located throughout the grassy riparian zone, whereas small patches of rough-leaved dogwood (*Cornus drummondii*) and other woody shrubs were confined to stream banks. Vegetation in the woody riparian zones of the two watersheds differed, with the woody riparian zone at the grazed watershed being dominated by American elm (*Ulmus americana*) and honey locust (*Gleditsia triacanthos*), whereas the woody riparian zone at the ungrazed watershed was dominated by bur oak (*Quercus macrocarpa*) and chinkapin oak (*Quercus muehlenbergii*). Both the woody reaches had diverse understories comprised of multiple species of grasses and forbs. Woody removal zones were distinct from other vegetation zones due to a lack of big bluestem and Indian grass, but prior to removal the removal reaches had vegetation similar to the woody riparian zones. The removal riparian zone at the grazed watershed was composed primarily of Japanese brome (*Bromus japonicus*), western ragweed, and dogwood patches, whereas the ungrazed removal reach consisted of more woodland understory species, such as Virginia creeper (*Parthenocissus quinquefolia*), buckbrush (*Andrachne phyllanthoides*), and black snakeroot (*Sanicula canadensis*).

We selected a 30-m transect perpendicular to the stream at each vegetation treatment, attempting to keep topography similar across transects. Ten sampling points were evenly spaced across the 30-m transect, leading to 10 unique soil samples from each vegetation type each sampling date. In the first year of the study (2009), sampling was performed seasonally (April, June, July, and October). In March and April of 2010, five samples were collected at transect mid-points (15 m) to determine interannual variability of denitrification.

Field Collection

On each sampling date, intact soil cores (collected in 4 × 20 cm sharpened polyvinyl chloride pipe with butyl-rubber septa placed 2 cm below unsharpened end of pipe) were collected from the top 15 cm of soil at each sampling point. Cores were then sealed on the bottom with a rubber stopper, and maintained intact for later assays of actual denitrification. Along with each intact core, three bulk soil samples were collected from the top 15 cm of soil using an Oakfield corer (2 × 15 cm; Oakfield Apparatus, Inc., Oakfield, Wisconsin, USA). All soil samples were stored in a cooler on ice until returned to the laboratory, where they were stored at 4°C until incubation. Bulk soil samples were pooled to provide one bulk sample per sampling location, and homogenized (4-mm-mesh sieve size). Soils were returned to room temperature prior to denitrification incubations (see below), which occurred within 24 h of sampling.

Benthic sampling was performed within 1 week of riparian sampling in April, June, July, and October of 2009, with an additional sampling date in January of 2010, which did not include riparian sampling due to frozen soil. Prior to collecting biotic compartments for benthic denitrification, stream water chemistry samples were collected from the downstream end of each reach in acid-washed 60-mL bottles. Reaches were then surveyed to identify benthic compartments present; compartments were collected in triplicate on each sampling date and included sediment, leaf packs, grass root wads, and filamentous algae (not all compartments were present within each reach on each sampling date). Sediment samples were collected to a depth of 5 cm using a circular metal sleeve (4 cm diameter × 5 cm long), filamentous algae were collected by removing all algae from a 225-cm^2 area that was selected by visually identifying algal mats, and grab samples were used to collect grass root wads and leaf packs. Sediment was the only compartment present in all reaches on each sampling date, whereas the other compartments were present on the majority of sampling dates, but not at all reaches.

Denitrification Incubations

The acetylene-inhibition method was used to measure denitrification (Smith and Tiedje 1979; Groffman and others 1999). This method was selected due to the low cost, the ability to process a large number of samples, and the ease of comparison across studies. For riparian denitrification, both potential and actual denitrification rates were measured (see below); only potential denitrification was measured for benthic compartments. Problems with this method include the inhibition of nitrification by acetylene removing the potentially coupled nitrification–denitrification pathway, leading to an underestimation of denitrification rates (see Bernot and others 2003; Groffman and others 2006).

The static-core technique was used to measure actual denitrification rate in riparian soil cores (Robertson and others 1987; Groffman and others 1999). Following field collection, intact soil cores were allowed to reach room temperature and both ends were sealed with rubber stoppers. Ten milliliters of acetylene, generated via reaction of CaC_2 with deionized H_2O, were added to each core (~10% of the headspace volume). As this technique attempts to simulate in situ rates, O_2 was not removed from the headspace within the core. Core headspace was pumped repeatedly with a 60-mL syringe to ensure complete mixing. Five milliliters of gas samples were taken at 2 and 6 h and transferred to 4-mL pre-evacuated BD-vacutainer vials (BD, Franklin Lakes, New Jersey, USA). Volume removed did not appear to affect headspace pressure, with 5-mL removal equating to 8% of the total headspace in the core. Any effect on headspace pressure, however, would dilute N_2O in the core thus making our estimates of N_2O accumulation (and therefore, denitrification) conservative. Prior to gas sample collection, core headspace was re-homogenized by repeated pumping with a 60-mL syringe.

Potential denitrification from riparian and benthic samples was measured using bulk soil (or benthic compartment) samples and the denitrification enzyme activity (DEA) assay (Smith and Tiedje 1979; Groffman and others 1999). Either 25 g of homogenized bulk riparian soil or 25 g of a specific benthic compartment (wet weight), and 25 mL of media (20 mM KNO_3, 5 mM dextrose, 1 mM chloramphenicol, final concentrations) were added to an acid-washed 150-mL Erlenmeyer flask. Nitrate and dextrose were added to alleviate nitrate and energy limitations, whereas chloramphenicol was added to inhibit de novo synthesis of denitrification enzymes and reduce bottle effects (Brock 1961; Smith and Tiedje 1979). Flasks were sealed with butyl-rubber stoppers and subjected to three cycles of evacuation (3 min) and flushing with N_2 (1 min) to induce anoxia, shaking intermittently to insure headspace homogeneity. Once anoxic, 10 mL of C_2H_2, generated as above, were added to each flask. Flasks were incubated for 90 min on a rotary shaker table at 125 rpm. Five milliliters gas samples were taken at 30 and 90 min and stored in 4-mL BD-vacutainer vials; shorter incubations were used for DEA because rates were expected to be greater than actual denitrification incubations. After all incubations were completed, gas samples were analyzed for N_2O using electron-capture gas chromatography (within 72 h of field collection) on a Shimadzu GC-14A equipped with a Poropak Q (80/100 mesh, 0.318 cm diameter × 74.5 cm) column and an electron-capture detector (injection temperature = 100°C, column temperature = 65°C, detector temperature = 320°C, with a 95% Ar: 5% CH_4 carrier gas at flow rate of 30 $mL \cdot min^{-1}$). Actual denitrification rates were temperature corrected ($Q_{10} = 2.0$; Stanford and others 1975) using field-temperatures at the time of collection to provide an estimate of in situ rates; DEA rates were corrected for N_2O dissolved in solution using Bunsen-coefficient corrections.

Ancillary Data

Bulk density was calculated for each static core and used to express rates on an areal basis. Soil inorganic nitrogen (NH_4^+ and $NO_2^- + NO_3^-$) was extracted from bulk soil samples using 2 M KCl (5:1 KCl v:soil v). The extract was analyzed on an OI Analytical Flow Solution IV using the indophenol-blue method (NH_4^+-N) and the cadmium-reduction method ($NO_2^- + NO_3^-$-N) (American Public Health Association (APHA) 1998); October samples (except the grassy and woody reaches of the ungrazed watershed) were contaminated with NH_4^+ during the extraction process, and therefore NH_4^+-N concentrations are unavailable for four of the six sites in October. Soil water content was determined by drying all remaining bulk soil at 60°C for at least 48 h. Dried soil was ground into a fine powder using a ball mill (8000D Dual Mixer/Mill, SPEX CentiPrep, Metuchen, New Jersey, USA) and analyzed for total carbon (TC) and total nitrogen using a Carlo Erba NA 1500 Analyzer (Carlo Erba, Milano, Italy). Stream water chemistry samples were analyzed for NH_4^+-N and $NO_2^- + NO_3^-$-N as detailed by Dodds (2003).

Statistical Analysis

Preliminary analyses revealed distance from the stream to be unrelated to riparian denitrification (non-significant simple linear regressions, $P > 0.1$, data not shown); therefore, distance was removed from subsequent analyses. Blocked two-way analysis of variance (ANOVA) was used to determine the impact of riparian vegetation on potential and actual denitrification rate of riparian soils. Watershed (grazed or ungrazed) and riparian vegetation (grass, wood, or removal) were the explanatory variables, and sampling date blocked the analyses. Watershed was used as an explanatory variable to explore the potential impact of confounding grazing and fire regime effects. Ancillary data were analyzed in the same way. Of the four benthic compartments found throughout the study, only sediment was found at every reach on every sampling date. Because of this, the relatively high areal cover of sediment in all habitats (visual observation, not quantified, but consistent with prior studies in the same stream; for example, Dodds and others 2000), and the fact that sediment was expected to directly reflect riparian inputs regardless of whether sediment originated from grass or woody riparian sources, the impact of riparian vegetation on benthic denitrification was analyzed using a blocked two-way ANOVA, with riparian vegetation and watershed as the explanatory variables, sampling date as the blocking factor, and potential denitrification rate of sediment as the response variable. Differences among potential denitrification of benthic compartments were determined using a blocked one-way ANOVA, with compartment as the explanatory variable and sampling date as the blocking factor. All data exhibited unequal variance, and were therefore $\log(x + 1)$ transformed prior to analysis. Tukey's HSD was used to perform post hoc comparisons of significant variables. Additionally, Pearson's r was used to determine correlations between potential drivers of denitrification (NO_3^-–N, total C, soil water content) and actual and potential denitrification. Data are expressed as annual mean ± SE unless otherwise noted.

RESULTS

Soil and Water Parameters

Extractable soil NH_4^+ was affected by watershed (blocked two-way ANOVA: $F_{1,228} = 4.227$, $P = 0.041$), riparian vegetation (blocked two-way ANOVA: $F_{2,228} = 7.375$, $P = 0.001$), and sampling date (blocked two-way ANOVA: $F_{4,228} = 86.6$, $P < 0.001$). Ammonium was greater at the ungrazed than the grazed watershed, and was lower in removal and woody riparian soils than grassy riparian soils (Tukey's HSD: $P < 0.05$). Ammonium concentration was greater in the early spring than the summer (Tukey's HSD: $P < 0.05$; Figure 1A). Extractable soil NO_3^- did not differ between watersheds (blocked two-way ANOVA: $F_{1,287} = 0.151$, $P = 0.70$), but was significantly influenced by riparian vegetation (blocked two-way ANOVA: $F_{2,287} = 52.9$, $P < 0.001$) and sampling date (blocked two-way ANOVA: $F_{5,287} = 5.594$, $P < 0.001$), with woody removal soils having the greatest NO_3^- concentration, whereas the grassy riparian soils had the least NO_3^- (Tukey's HSD: $P < 0.05$; Figure 1B); early summer samples (June and July) had greater amounts of NO_3^- than early spring or fall samples (Tukey's HSD: $P < 0.05$; Table 1). Significant differences in total soil N were seen between watersheds (blocked two-way ANOVA: $F_{1,289} = 28.7$, $P < 0.001$) and vegetation types (blocked two-way ANOVA: $F_{2,289} = 33.6$, $P < 0.001$; Tukey's HSD: $P < 0.05$; Table 1), with total N being greater at the grazed than the ungrazed watershed. Grassy riparian soils had lower total N than woody or removal riparian zones (Table 1). Soil water content differed among watersheds (blocked two-way ANOVA: $F_{1,289} = 90.2$, $P < 0.001$), vegetation types (blocked two-way ANOVA: $F_{2,289} = 7.3$, $P = 0.001$), and sampling dates (blocked two-way ANOVA: $F_{5,289} = 13.2$, $P < 0.001$). The grazed watershed had lower mean

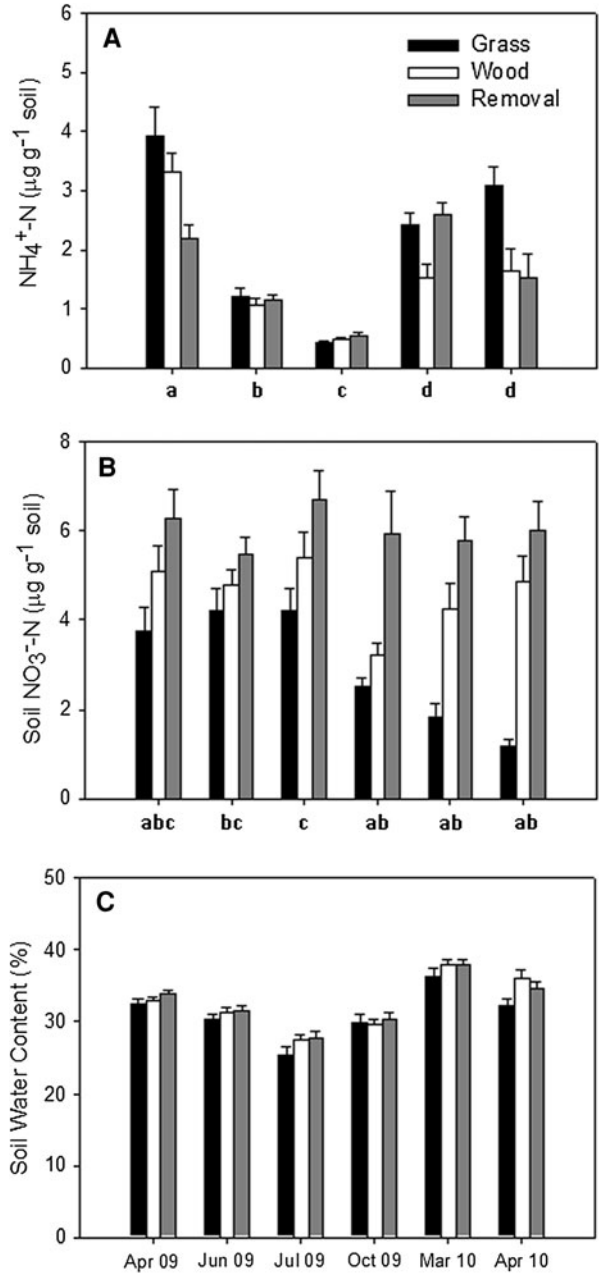

Figure 1. Riparian soil NH_4^+–N (**A**), NO_3^-–N (**B**), and soil water content (**C**) for three riparian vegetation treatments (*black* grassy, *white* woody, *gray* wood removed) for all sampling dates. *Letters* below dates for **A** and **B** denote significant differences at the $\alpha = 0.05$ level among sampling dates. For **C**, all sampling dates were significantly different. Each vegetation treatment was significantly different at the $\alpha = 0.05$ level for (**B**), whereas wood and removal treatments did not differ in (**A**) or (**C**).

soil water content (29.75 ± 0.39%) than the ungrazed watershed (33.0 ± 0.3%), and soils of grassy riparian zones were drier throughout than soils of woody or removal vegetation (Tukey's HSD: $P < 0.05$; Figure 1C).

TC differed by riparian vegetation type (blocked two-way ANOVA: $F_{2,289} = 48.1$, $P < 0.001$) but did not differ among watersheds or sampling dates. Soils under grassy riparian zones had less TC (35.3 ± 0.4 $\mathrm{mg \cdot g^{-1}}$) than woody riparian soils (39.8 ± 0.4 $\mathrm{mg \cdot g^{-1}}$), which had less TC than the removal soils (41.8 ± 0.6 $\mathrm{mg \cdot g^{-1}}$; Tukey's HSD: $P < 0.05$; Table 1). Soil carbon-to-nitrogen ratios (C:N) were greater in the ungrazed (12.8 ± 0.2) than the grazed watershed (11.7 ± 0.1; blocked two-way ANOVA: $F_{1,289} = 21.3$, $P < 0.001$) and were significantly affected by riparian vegetation (blocked two-way ANOVA: $F_{2,289} = 9.9$, $P < 0.001$). Removal riparian soils had a higher mean C:N (12.9 ± 0.3) than woody riparian zones (11.7 ± 0.1; Tukey's HSD: $P < 0.05$). No statistical tests were run on water chemistry due to low replication, but no obvious trends were evident in stream water NO_3^- or NH_4^+; values were generally similar in reaches with differing riparian vegetation types and watersheds (Figure 2).

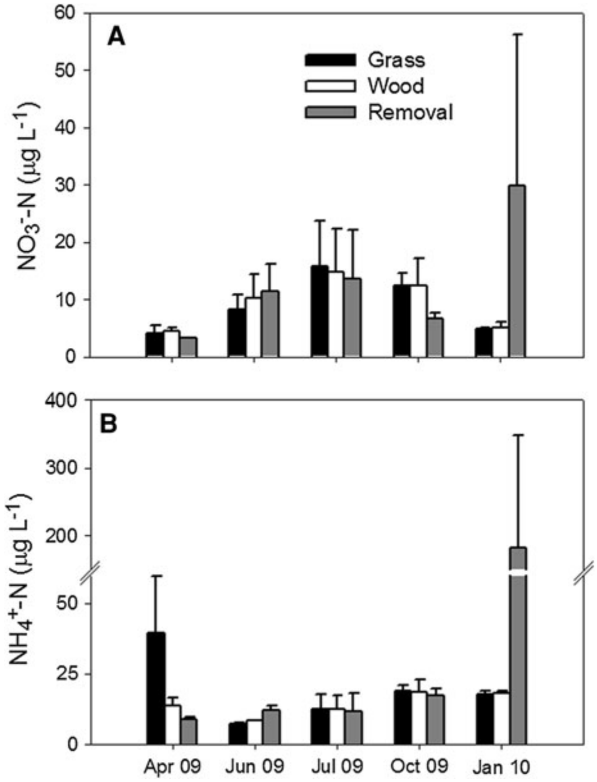

Figure 2. Stream water NO_3^-–N (**A**) and NH_4^+–N (**B**) for three riparian vegetation treatments (*black* grassy, *white* woody, *gray* wood removed) averaged across two watersheds for all sampling dates.

Riparian Soil Potential Denitrification

Potential denitrification rates of riparian soils differed significantly among sampling dates (blocked two-way ANOVA: $F_{5,289} = 60.0$, $P < 0.001$) and riparian vegetation (blocked two-way ANOVA: $F_{2,289} = 3.2$, $P = 0.044$), but not watershed (Figure 3), and there was a significant interaction between riparian vegetation and watershed (blocked two-way ANOVA: $F_{2,\,289} = 4.4$, $P = 0.013$; Figure 3). Post-hoc analyses revealed early spring to be the season with greatest potential denitrification, with April of 2009 samples exhibiting the greatest potential rate, followed by April of 2010 (Tukey's HSD: $P < 0.05$; Figure 3). Due to the interaction between vegetation and watershed, we can only say that the effect of treatment differed between watersheds. However, the lack of a significant watershed-specific effect, coupled with the general trend for the removal and woody riparian zones to exhibit greater denitrification than the grassy zone (Figure 3), suggests that riparian vegetation did influence DEA. Nitrate and soil water content were significantly correlated with riparian soil DEA, whereas total soil C was not (Table 2).

Table 1. Annual Means of Site-Specific Soil Nitrogen, Carbon and C:N

Site	TN ($\mathrm{mg \cdot g^{-1}}$ soil)	TC ($\mathrm{mg \cdot g^{-1}}$ soil)	C:N
Ungrazed grass	2.82 (0.04)[1]A[2]	35.51 (0.65)A	12.71 (0.34)B
Ungrazed woody	3.23 (0.08)BC	38.70 (0.75)B	11.90 (0.11)AB
Ungrazed removal	3.08 (0.08)AB	41.21 (0.94)BC	13.74 (0.49)C
Grazed grass	2.95 (0.07)A	34.76 (0.68)A	11.85(0.13)AB
Grazed woody	3.57 (0.04)D	40.92 (0.39)BC	11.47 (0.05)A
Grazed removal	3.58 (0.09)CD	42.34 (0.72)C	12.02(0.21)AB

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[1] *Mean (SE).*
[2] *Letters denote Tukey's HSD groupings within columns.*

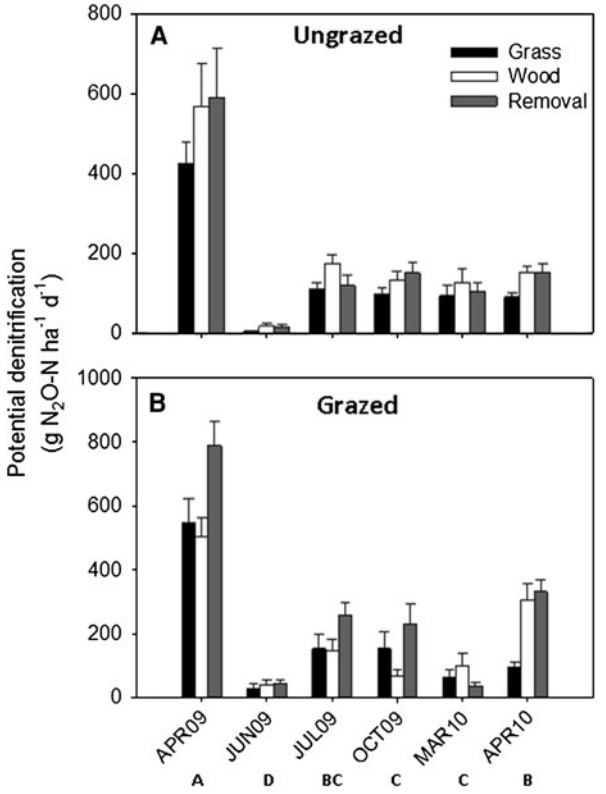

Figure 3. Riparian soil potential denitrification (as DEA) for three riparian treatments (*black* grass, *white* wood, *gray* wood removed) at **A** the ungrazed watershed and **B** the grazed watershed over six sampling dates. *Letters* indicate significant differences at the $\alpha = 0.05$ level among sampling date. *Note* Statistical tests were run on $\log(x + 1)$ transformed data. *Error bars* 1SE.

Riparian Soil Actual Denitrification

Actual denitrification rates of riparian soils differed among sampling dates (blocked two-way ANOVA: $F_{5,289} = 137.4$, $P < 0.001$), riparian vegetation (blocked two-way ANOVA: $F_{2,289} = 31.0$, $P < 0.001$), and watershed (blocked two-way ANOVA: $F_{1,289} = 3.9$, $P = 0.05$; Figure 4). There was also a significant interaction between watershed and vegetation (blocked two-way ANOVA: $F_{2,\ 289} = 5.2$, $P = 0.006$; Figure 4). With the exception of April 2009, average denitrification rate was similar between watersheds (Figure 4). Denitrification was generally greatest at the beginning of the growing season, with April 2009 having the greatest rate, followed by April 2010 and June 2009, which had greater rates than October 2009, July 2009, and March 2010 (Tukey's HSD: $P < 0.05$; Figure 4A). The interaction between watershed and vegetation again suggests treatment effects of vegetation type differed between watersheds, although the general pattern was that removal soils exhibited greater rates (90.1 ± 17.7 g N ha^{-1} day^{-1}) than woody soils (66.9 ± 19.2 g N ha^{-1} day^{-1}), which were, in turn, greater than in grassy riparian soils (45.8 ± 11.9 g N ha^{-1} day^{-1}; Figure 4). Similar to riparian potential denitrification, nitrate and soil water content were both significantly correlated to actual denitrification in riparian soils, whereas TC and actual denitrification were not correlated (Table 2).

Benthic Potential Denitrification

Potential denitrification in sediments was significantly affected by watershed (blocked two-way ANOVA: $F_{1,79} = 31.8$, $P < 0.001$), riparian vegetation (blocked two-way ANOVA: $F_{2,79} = 8.6$, $P < 0.001$), and sampling date (blocked two-way ANOVA: $F_{4,79} = 9.8$, $P < 0.001$). There was a significant interaction between watershed and riparian vegetations (blocked two-way ANOVA: $F_{2,79} = 7.9$, $P = 0.001$; Figure 5). Potential denitrification of sediment was temporally variable, but there was no obvious seasonal effect (Figure 5). Potential denitrification was lower in reaches with grassy (0.06 ± 0.02 µg N g DM^{-1} hour^{-1}) or woody (0.03 ± 0.01 µg N g DM^{-1} hour^{-1}) riparian vegetation than potential denitrification of sediment in removal reaches (0.10 ± 0.02 µg N g DM^{-1} hour^{-1}; Figure 5B), but this effect was dependent upon watershed (Figure 5).

Potential denitrification rates in leaf packs, grass root wads, and filamentous algae were not affected by riparian vegetation (blocked two-way ANOVAs: $P > 0.05$, data not shown), but standing stocks

Table 2. Pearson's *r* Correlations Between Riparian Denitrification and Potential Drivers

	NO_3^-–N		TC		SWC	
	r	*P*	*r*	*P*	*r*	*P*
Actual denitrification	**0.189**	**0.001**	0.076	0.189	**0.196**	**0.001**
Potential denitrification	**0.137**	**0.018**	0.082	0.156	**0.182**	**0.002**

Significant correlations denoted in bold.

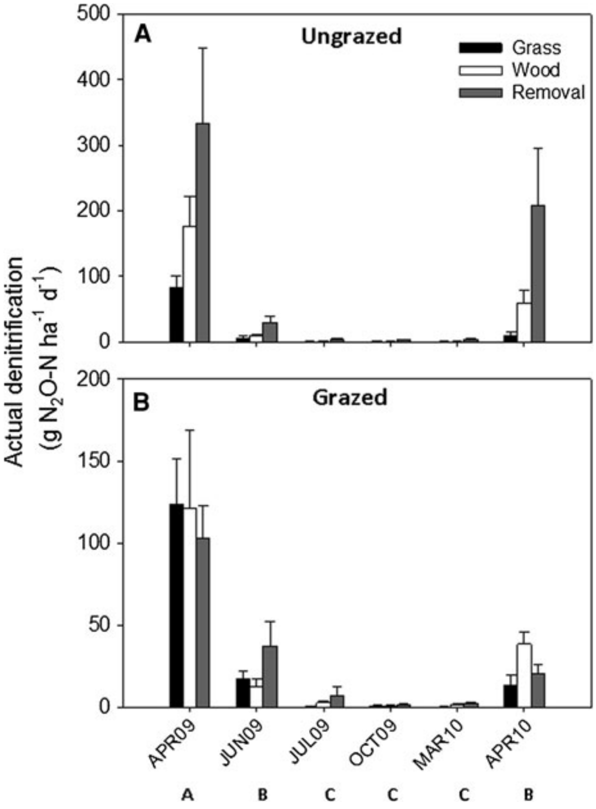

Figure 4. Riparian soil actual denitrification for three riparian treatments (*black* grass, *white* wood, *gray* wood removed) at **A** the ungrazed watershed and **B** the grazed watershed over six sampling dates. *Letters* indicate significant differences at the $\alpha = 0.05$ level among sampling date. *Note* Statistical tests were run on $\log(x + 1)$ transformed data. *Error bars* 1SE.

varied; filamentous algae (Riley and Dodds 2012) and root wads (personal observation) were rarely found in reaches with woody riparian vegetation. Significant differences in potential rates were seen among compartments (blocked one-way ANOVA: $F_{3,208} = 35.5$, $P < 0.001$), with filamentous algae (0.49 ± 0.09 μg N g DM^{-1} $hour^{-1}$) and grass root wads (0.44 ± 0.07 μg N g DM^{-1} $hour^{-1}$) exhibiting greater potential rates than leaf packs (0.15 ± 0.02 μg N g^{-1} DM $hour^{-1}$) or sediment (0.06 ± 0.01 μg N g DM^{-1} $hour^{-1}$; Figure 6). Benthic compartment rates also varied temporally (blocked one-way ANOVA: $F_{4,208} = 11.5$, $P < 0.001$), but no obvious seasonality was evident.

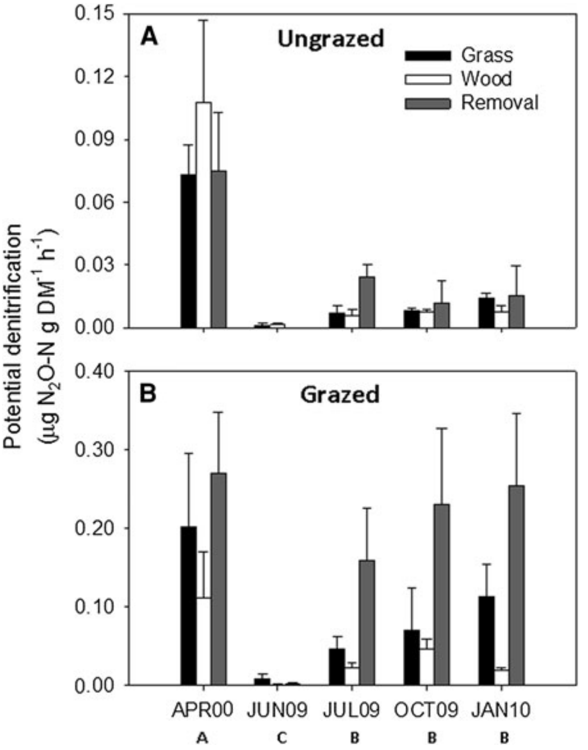

Figure 5. Mean sediment potential denitrification (as DEA) for three riparian treatments (*black* grass, *white* wood, *gray* wood removed) at **A** the ungrazed watershed and **B** the grazed watershed over five sampling dates. *Letters* indicate significant differences at the $\alpha = 0.05$ level among sampling date. *Note* Statistical tests were run on $\log(x + 1)$ transformed data. *Error bars* 1SE.

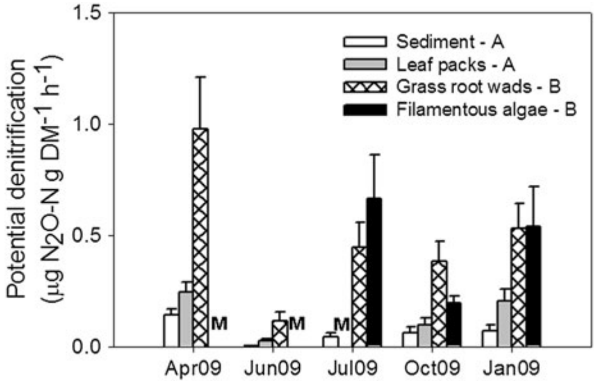

Figure 6. Benthic potential denitrification (as DEA) averaged over four benthic compartments (*white* sediment, *gray* leaf packs, *hatched* grass root wads, *black* filamentous algae) for five sampling dates. *Letters* in the legend indicate differences at the $\alpha = 0.05$ level among benthic compartments. *M* compartments that were not sampled during a specific sampling date. *Error bars* 1SE.

DISCUSSION

As predicted, denitrification rates in riparian soils exhibited seasonality, with greatest denitrification occurring in the spring. Additionally, the riparian vegetation treatments affected benthic denitrification, though specific mechanisms underlying these changes will require further study, and the removal of vegetation stimulating denitrification may only be a transient effect. Finally, benthic denitrification was affected differently by different benthic compartments, with compartments known to provide greater amounts of labile carbon (for example, grass roots, filamentous algae; Cross and others 2005; Ishida and others 2008) exhibiting the greatest rates of denitrification. A larger scale study with increased replication would be required to verify that mechanisms suggested by these results (see ''Woody vegetation removal stimulates riparian denitrification'') affect denitrification.

Spatial and Temporal Variability of Denitrification

Denitrification in riparian soils varied temporally, with both potential and actual denitrification rates being at least three times greater in April 2009 than any other sampling date. Higher denitrification in April may be due to the majority of annual rainfall in tallgrass prairies occurring in the spring, which, when coupled with low plant uptake and high N mineralization (for example, Blair 1997) leads to a pulse of NO_3^- entering riparian soils from uplands and increased anoxic microsites within the soil (Groffman and others 1993; Blair 1997). Additionally, due to the phenology and physiology of the dominant C_4 plant community, plant activity and nutrient uptake is low during the early growing season, allowing increased access to NO_3^- for denitrifiers (Groffman and others 1993). These factors, coupled with increasing temperatures, may allow increased microbial activity that contributed to the high rate in April 2009. Denitrification in April 2010 was not as high as the previous year, but was greater than other months sampled, suggesting that April 2009 was not an anomaly. Upland and hillslope soil denitrification in this tallgrass prairie exhibited similar seasonal variability as the current study, with greatest actual and potential denitrification occurring in April and May (Groffman and others 1993). Interestingly, denitrification potential in riparian soils in our study was comparable to rates measured by Groffman and others (1993), but measures of actual denitrification were 2–5 times greater in the riparian zone than upland or hillslope sites (Groffman and others 1993). This suggests that denitrifier communities may be similar in these different topographic positions, but physicochemical factors (for example, NO_3^-, labile C, or redox) are limiting in upland areas.

Actual riparian soil denitrification varied among watersheds (Figure 4), with the grazed watershed exhibiting greater denitrification overall than the ungrazed watershed, but this effect was related to riparian vegetation. Grazing can decrease N losses due to fire due to a reduction in aboveground stocks of N (Hobbs and others 1991) and increase soil N cycling rates both directly, via enhanced N availability as a result of urine or dung deposition, and indirectly by altering plant litter quantity and quality (Hobbs 1996). Introduction of bison increases total nitrogen concentrations in streams draining the grazed watershed (Kemp and Dodds 2001b), and grazing by bison stimulates upland N cycling on Konza by increasing net N mineralization and nitrification at grazed sites compared to ungrazed sites (Johnson and Matchett 2001). Intensive grazing can also increase DEA in annually burned, grazed soils compared to annually burned, ungrazed soils (Groffman and others 1993). Grazing can both increase intrasystem N cycling rates and reduce N losses due to fire, which can be a major pathway of N loss in ungrazed tallgrass prairie (Turner and others 1997). These previously documented effects can potentially explain the increase in denitrification rates at the grazed watershed.

Similar to riparian soil denitrification, benthic DEA exhibited high temporal variability, but unlike riparian zones, there was no seasonality. Benthic patterns differed from general patterns reported in the literature, as denitrification in aquatic systems is generally greatest during the summer months due to increased water temperatures (Piña-Ochoa and Álvarez-Cobelas 2006). However, a study of 18 agricultural and urban streams found the greatest rates during the winter, with NO_3^- and labile C inputs, not temperature, controlling denitrification (Arango and Tank 2008). Water column NO_3^- and potential denitrification were both greater at the grazed than the ungrazed watershed, but there were no clear seasonal patterns for DEA, suggesting that something other than temperature, such as C availability, controlled benthic DEA in the current study. Our denitrification results mirrored measurements of whole-stream metabolism made by Riley and Dodds (2012) for these same reaches in that interannual and seasonal variability was considerable in these same reaches.

Woody Vegetation Removal Stimulates Riparian Denitrification

The factors that can promote denitrification (more NO_3^-, labile C, and anoxia as indicated by soil water content) were all greater in woody and removal riparian soils than grassy riparian soils (Figure 1A; Table 1). These differences in denitrification-promoting factors likely caused the observed differences in actual and potential riparian soil denitrification. Rates of soil denitrification were greater in the woody vegetation removal treatment relative to either intact woody vegetation or naturally grassy riparian areas. Though we lack pretreatment data, we suggest that woody vegetation (and its subsequent removal) stimulated soil denitrification for the following reasons. Soil redox conditions are directly related to soil water content, which was increased by the removal of woody vegetation (Figure 1C). There are multiple potential mechanisms for different soil C under differing riparian vegetation. For example, soil C may be elevated under woody vegetation due to mycorrhizal symbioses (Rygiewicz and Anderson 1994), root exudation (Kuzyakov and Domanski 2000), or increased litter fall. In the removal zones, soil C may be elevated due to enhanced root decomposition, causing increased dissolved C in the soil (removal zones). Enhanced root decomposition in removal soils may also be the mechanism for elevated soil NO_3^- concentrations in these woody removal riparian soils (Fornara and others 2009).

Studies of woody vegetation in riparian areas generally assess the impact of restoration of naturally occurring woody vegetation. Our study is unique because riparian woody vegetation is often not a natural condition along headwater streams in tallgrass prairie. Restoration of woody riparian zones reduces stream water NO_3^- concentrations (Newbold and others 2010), increases total N retention (Osborne and Kovacic 1993; Haycock and Pinay 1993), and increases uptake of nutrients by vegetation (Lyons and others 2000). These effects have also been shown in woody encroached soils (Norris and others 2007). We found the greatest rates of denitrification in wood removal riparian zones, but this may only be a transient effect as riparian vegetation transitions from woody to grassy or result from mechanical disturbance of vegetation removal.

Although the presence of woody vegetation, and its subsequent removal, increased denitrification rates, NO_3^- concentrations were lower in grassy riparian soils, suggesting that other mechanisms may enhance overall nitrate retention and/or removal in grassy riparian zones. One potential mechanism is the ability of grass-dominated areas to produce abundant fine litter that leads to intense and relatively complete combustion during fire (Knapp and Seastedt 1986). Following a fire, much of the aboveground grass biomass and litter is volatilized, including N, whereas $<50\%$ of aboveground woody biomass generally burns (Kaufmann and others 1994). Thus, much of the N sequestered in aboveground portions of grasses is volatilized during fire, reducing the soil N pool compared to woody vegetation (Kaufmann and others 1994; Turner and others 1997). In addition, fire favors the dominance of C_4 grass species with high nitrogen use efficiency, and leads to greater inputs of root material with a large C:N, thereby promoting enhanced immobilization of inorganic soil N (Blair 1997; Dell and others 2005). Therefore, the natural state of grassy riparian vegetation, maintained by periodic burning, may reduce N inputs into headwater streams as much as woody vegetation, but due to different mechanisms.

Woody Vegetation Alters Benthic Denitrification

Riparian vegetation also affected benthic DEA, both directly with the removal of wood stimulating sediment denitrification, and indirectly by altering the benthic compartments present in the system. Removal of riparian woody vegetation increased sediment DEA above rates seen in sediment from reaches with either grassy or woody riparian zones (Figure 5). Riparian soils in the removal reaches, which may enter the stream during storm events, had higher TC and TN than natural riparian soils (Table 1). Arango and Tank (2008) showed that denitrification in anthropogenically impacted streams is related to sediment C. This relationship holds true in sediment of various depths and sizes (Inwood and others 2007) and across streams of variable NO_3^- concentrations (Arango and others 2007). The consistency of this relationship, coupled with the increased C content of woody removal riparian soils, suggests increased sediment C is a likely mechanism for stimulated denitrification seen in removal sediments.

Riparian vegetation also indirectly affected benthic denitrification by altering the compartments present in the benthic zone. Filamentous algae and grass root wads expressed consistently greater DEA rates than leaf packs or sediments (Figure 6), but were only found in reaches with open canopies (grassy or removal reaches). The removal of woody vegetation increased filamentous algal biomass, with a negative relationship seen between canopy cover and filamentous algal biomass (Riley and Dodds 2012). Potential denitrification rates are generally higher on periphyton than sediments (Ishida and others 2008; this study), potentially due to exudation of photosynthates (Heffernan and Cohen 2010) or increased habitat complexity and surface area for denitrifying bacteria. The lack of filamentous algae or grass root wads in reaches with woody riparian vegetation suggests that woody vegetation encroachment indirectly inhibits denitrification by excluding grasses from rooting in the stream channel and reducing light inputs, which limits filamentous algal growth (Riley and Dodds 2012).

CONCLUSIONS

Soil denitrification in riparian zones of Konza Prairie was highly seasonal, with the majority of denitrification occurring in the early spring. These temporal patterns are similar to those published previously for uplands and hillslopes (Groffman and others 1993). Benthic denitrification was also temporally variable, but exhibited no distinct seasonality, suggesting factors other than water temperature are controlling benthic denitrification. Grazing by bison and changes in riparian vegetation probably both affected riparian soil and benthic sediment denitrification; potential and actual denitrification rates were greater in the grazed watershed, and the removal of woody vegetation was associated with higher denitrification. Although the current study design allows only tentative conclusions regarding the effects of woody vegetation encroachment on denitrification due to limited replication and potential confounding effects of fire and grazing, the effect of riparian vegetation on denitrification in these two watersheds suggests a potentially important mechanism for riparian management in both pristine and impacted systems for increased N removal prior to its entry into aquatic systems.

Woody vegetation encroachment is a primary threat to remaining tallgrass prairie streams and may impact the ability of these systems to respond to increased N deposition in the future. Expansion of forests upstream may increase riparian denitrification while reducing benthic denitrification due to an alteration of compartments present in the benthic zone. Removal of woody vegetation apparently stimulated soil denitrification to levels greater than rates present in either woody or grassy riparian zones, but this could be a short-term impact until the removal reach returns to a stable grassland community. Mechanisms that may account for this stimulation of denitrification include increased anoxia due to reduced evapotranspiration, increased labile C and N in the soil due to root decomposition, and less competition between plants and microbes for NO_3^-. Although denitrification was greater in woody and removal riparian soils than grassy soils, NO_3^- concentration and TN in grassy riparian soils was lower than in other treatments, suggesting grasses may be better at overall N retention/removal in tallgrass prairie riparian zones. We suggest that any benefits provided by increased denitrification rates in woody riparian zones may be counteracted by other terrestrial (that is, vegetative uptake and subsequent volatilization during fire events) and aquatic (that is, increased benthic denitrification via alternative benthic compartments present in the stream) N removal mechanisms. Therefore, management practices to reduce woody vegetation encroachment and secure the existence of endangered tallgrass prairies probably lead to minimal functional loss in terms of altering N retention.

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REFERENCES

American Public Health Association (APHA). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, DC.

Arango CP, Tank JL. 2008. Land use influences the spatiotemporal controls on nitrification and denitrification in headwater streams. J N Am Benthol Soc 27:90–107.

Arango CP, Tank JL, Schaller JL, Royer TV, Bernot MJ, David MB. 2007. Benthic organic carbon influences denitrification in streams with high nitrate concentration. Freshw Biol 52:1210–22.

Bernot MJ, Dodds WK, Gardner WS, McCarthy MJ, Sobolev D, Tank JL. 2003. Comparing denitrification estimates for a Texas estuary by using acetylene inhibition and membrane inlet mass spectrometry. Appl Environ Microbiol 69:5950–6.

Blair JM. 1997. Fire, N availability, and plant response in grasslands: a test of the transient maxima hypothesis. Ecology 78:2359–68.

Brock TD. 1961. Chloramphenicol. Bacteriol Rev 25:32–48.

Briggs JM, Knapp AK, Blair JM, Heisler JL, Hoch GA, Lett MS, McCarron JK. 2005. An ecosystem in transition: causes and consequences of the conversion of mesic grassland to shrubland. Bioscience 55:243–54.

Carpenter SR, Caraco NF, Correll DL, Howarth RW, Sharpley AN, Smith VH. 1998. Nonpoint pollution of surface waters with phosphorous and nitrogen. Ecol Appl 8:559–68.

Claessens L, Tague CL, Groffman PM, Melack JM. 2010. Longitudinal and seasonal variation of stream N uptake in an urbanizing watershed: effect of organic matter, stream size, transient storage and debris dams. Biogeochemistry 98:45–62.

Cross WF, Benstead JP, Frost PC, Thomas SA. 2005. Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. Freshw Biol 50:1895–912.

Dell CJ, Williams MA, Rice CW. 2005. Partitioning of nitrogen over five growing seasons in tallgrass prairie. Ecology 86:1280–7.

Dillaha TA, Reneau RB, Mostaghimi S, Lee D. 1989. Vegetative filter strips for agricultural nonpoint source pollution control. Trans Am Soc Agric Eng 32:513–19.

Dodds WK. 1997. Distribution of runoff and rivers related to vegetative characteristics, latitude, and slope: a global perspective. J N Am Benthol Soc 16:162–8.

Dodds WK. 2003. Misuse of inorganic N and soluble reactive P concentrations to indicate nutrient status of surface waters. J N Am Benthol Soc 22:171–81.

Dodds WK, Oakes RM. 2006. Controls on nutrients across a prairie stream watershed: land use and riparian cover effects. Environ Manage 37:634–46.

Dodds WK, Evans-White MA, Gerlanc NM, Gray L, Gudder DA, Kemp MJ, Lopez AL, Stagliano D, Strauss EA, Tank JL, Whiles MR, Wollheim WM. 2000. Quantification of the nitrogen cycle in a prairie stream. Ecosystems 3:574–89.

Dodds WK, Gido K, Whiles MR, Fritz KM, Matthews WJ. 2004. Life on the edge: the ecology of Great Plains prairie streams. Bioscience 54:205–16.

Dodds WK, Bouska WW, Eitzmann JL, Pilger TJ, Pitts KL, Riley AJ, Schloesser JT, Thornbrugh DJ. 2009. Eutrophication of US Freshwaters: analysis of potential economic damages. Environ Sci Technol 43:12–19.

Fornara DA, Tilman D, Hobbie SE. 2009. Linkages between plant functional composition, fine root processes and potential soil N mineralization rates. J Ecol 97:48–56.

Fritz KM, Dodds WK. 1999. The effects of bison crossings on the macroinvertebrate community in a tallgrass prairie stream. Am Midl Nat 141:253–65.

Groffman PM, Rice CW, Tiedje JM. 1993. Denitrification in a tallgrass prairie landscape. Ecology 74:855–62.

Groffman PM, Holland E, Myrold DD, Robertson GP, Zou X. 1999. Denitrification. In: Robertson GP, Bledsoe CS, Coleman DC, Sollines P, Eds. Standard soil methods for long term ecological research. New York: Oxford University Press. p 272–88.

Groffman PM, Altabet MA, Böhlke JK, Butterbach-Bahl K, David MB, Firestone MK, Giblin AE, Kana TM, Nielsen LP, Voytek MA. 2006. Methods for measuring denitrification: diverse approaches to a difficult problem. Ecol Appl 16:2091–122.

Haycock NE, Pinay G. 1993. Groundwater nitrate dynamics in grass and poplar vegetated riparian buffer strips during the winter. J Environ Qual 22:273–8.

Heffernan JB, Cohen MJ. 2010. Direct and indirect coupling of primary production and diel nitrate dynamics in a subtropical spring-fed river. Limnol Oceanogr 55:677–88.

Hill AR. 1996. Nitrate removal in stream riparian zones. J Environ Qual 25:743–55.

Hobbs NT. 1996. Modification of ecosystems by ungulates. J Wildl Manage 60:695–713.

Hobbs NT, Schimel DS, Owensby CE, Ojima DS. 1991. Fire and grazing in the tallgrass prairie: contingent effects on nitrogen budgets. Ecology 72:1374–82.

Inwood SE, Tank JL, Bernot MJ. 2007. Factors controlling sediment denitrification in Midwestern streams of varying land use. Microb Ecol 53:247–58.

Ishida CK, Arnon S, Peterson CG, Kelly JJ, Gray KA. 2008. Influence of algal community structure on denitrification rates in periphyton cultivated on artificial substrata. Microb Ecol 56:140–52.

Johnson LC, Matchett JR. 2001. Fire and grazing regulate belowground processes in tallgrass prairie. Ecol 82:3377–89.

Johnson LB, Richards C, Host GE, Arthur JW. 1997. Landscape influences on water chemistry in Midwestern stream ecosystems. Freshw Biol 37:193–208.

Kaufmann JB, Cumming DL, Ward DE. 1994. Relationships of fire, biomass and nutrient dynamics along a vegetation gradient in the Brazilian Cerrado. J Ecol 82:519–31.

Kaushal SS, Groffman PM, Mayer PM, Striz E, Gold AJ. 2008. Effects of stream restoration on denitrification in an urbanizing watershed. Ecol Appl 18:789–804.

Kemp MJ, Dodds WK. 2001a. Centimeter-scale patterns in dissolved oxygen and nitrification rates in a prairie stream. J N Am Benthol Soc 20:347–57.

Kemp MJ, Dodds WK. 2001b. Spatial and temporal patterns of nitrogen concentrations in pristine and agriculturally-influenced prairie streams. Biogeochemistry 53:125–41.

Kemp MJ, Dodds WK. 2002. Comparisons of nitrification and denitrification in prairie and agriculturally influenced streams. Ecol Appl 12:998–1009.

Knapp AK, Seastedt TR. 1986. Detritus accumulation limits productivity of tallgrass prairie. Bioscience 36:662–8.

Knight CL, Briggs JM, Nellis MD. 1994. Expansion of gallery forest on Konza Prairie Research Natural Area, Kansas, USA. Landscape Ecol 9:117–25.

Kuzyakov Y, Domanski G. 2000. Carbon input by plants into the soil. Review. J Plant Nutr Soil Sci 163:421–31.

Lyons J, Trimble SW, Paine LK. 2000. Grass versus trees: managing riparian areas to benefit streams of central North America. J Am Water Resour Assoc 36:919–30.

McClain ME, Boyer EW, Dent CL, Gergel SE, Grimm NB, Groffman PM, Hart SC, Harvey JW, Johnston CA, Mayorga E, McDowell WH, Pinay G. 2003. Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems 6:301–12.

Mulholland PJ, Helton AM, Poole GC, Hall RO, Hamilton SK, Peterson BJ, Tank JL, Ashkenas LR, Cooper LW, Dahm CN, Dodds WK, Findlay SEG, Gregory SV, Grimm NB, Johnson SL, McDowell WH, Meyer JL, Valett HM, Webster JR, Arango CP, Beaulieu JJ, Bernot MJ, Burgin AJ, Crenshaw CL, Johnson LT, Niederlehner BR, O'Brien JM, Potter JD, Sheibley RW, Sobota DJ, Thomas SM. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature 452:202–5.

Newbold JD, Herbert S, Sweeney BW, Kiry P, Alberts SJ. 2010. Water quality functions of a 15-year-old riparian forest buffer system1. J Am Water Resour Assoc 46:299–310.

Norris MD, Blair JM, Johnson LC. 2007. Altered ecosystem nitrogen dynamics as a consequence of land cover change in tallgrass prairie. Am Midl Nat 158:432–45.

NRCS (National Resource Conservation Service). 1997. Riparian forest buffer. Conservation Practices Sheet 391. US Department of Agriculture, Washington, DC.

Orr CH, Stanley EH, Wilson KA, Finlay JC. 2007. Effects of restoration and reflooding on soil denitrification in a leveed Midwestern floodplain. Ecol Appl 17:2365–76.

Osborne LL, Kovacic DA. 1993. Riparian vegetated buffer strips in water-quality restoration and stream management. Freshw Biol 29:243–58.

Peterson BJ, Wollheim WM, Mulholland PJ, Webster JR, Meyer JL, Tank JL, Marti E, Bowden WB, Valett HM, Hershey AE, McDowell WH, Dodds WK, Hamilton SK, Gregory S, Morrall DD. 2001. Control of nitrogen export from watersheds by headwater streams. Science 292:86–90.

Piña-Ochoa E, Álvarez-Cobelas M. 2006. Denitrification in aquatic environments: a cross-system analysis. Biogeochemistry 81:111–30.

Pinay G, Roques L, Fabre A. 1993. Spatial and temporal patterns of denitrification in a riparian forest. J Appl Ecol 30:581–91.

Riley AJ, Dodds WK. 2012. The expansion of woody riparian vegetation, and subsequent stream restoration, influences the metabolism of prairie streams. Freshw Biol 57:1138–50.

Robertson GP, Vitousek PM, Matson PA, Tiedje JM. 1987. Denitrification in a clearcut Loblolly pine (*Pinus taeda* L.) plantation in the southeastern US. Plant Soil 97:119–29.

Rygiewicz PT, Anderson CP. 1994. Mycorrhizae alter quality and quantity of carbon allocated below ground. Nature 369:58–60.

Samson F, Knopf F. 1994. Prairie conservation in North-America. Bioscience 44:418–21.

Smith MS, Tiedje JM. 1979. Phases of denitrification following oxygen depletion in soil. Soil Biol Biochem 11:261–7.

Stanford G, Dzienia S, Vander Pol RA. 1975. Effect of temperature on denitrification rate in soils. Soil Sci Soc Am J 39:867–70.

Turner CL, Blair JM, Schartz RJ, Neel JC. 1997. Soil N and plant responses to fire, topography, and supplemental N in tallgrass prairie. Ecology 78:1832–43.

USEPA (United States Environmental Protection Agency). 2002. National water quality inventory: 2000 Report. EPA/841/R-01/001. Office of Water, Washington, DC.

Vidon P, Allan C, Burns D, Duval TP, Gurwick N, Inamdar S, Lowrance R, Okay J, Scott D, Sebestyen S. 2010. Hot spots and hot moments in riparian zones: potential for improved water quality management 1. J Am Water Resour Assoc 46:278–98.

Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman DG. 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecol Appl 7:737–50.