

FACTORS AFFECTING DENITRIFICATION IN HEADWATER PRAIRIE STREAMS

by

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Abstract

Human-induced stressors such as increased nitrogen (N) loadings, altered watershed land-use, and biodiversity losses are a few of the numerous threats to aquatic systems. Prairie streams experience natural disturbances, such as flooding and desiccation, which may alter responses to anthropogenic stressors. Denitrification, the dissimilatory reduction of NO_3^- to N gas (N_2O or N_2), is the only permanent form of N removal from terrestrial or aquatic ecosystems, and is important in mitigating N pollution to streams and downstream waters. Little is known about the relationships between denitrification and riparian prairie vegetation or large consumers. In the first chapter, I used outdoor mesocosms to determine the impact of a grazing minnow, *Camptostoma anomalum*, on structural and functional responses of prairie streams to a simulated flood, focusing on denitrification. In terrestrial ecosystems, grazing can stimulate denitrification, but this has not been studied in streams. Ammonium (NH_4^+) enrichments, used to simulate fish excretion, alleviated N limitations on denitrification. Both fish and NH_4^+ affected algal biomass accrual, but only fish affected algal filament lengths and particulate organic matter. In a second experiment, I examined the impact of woody vegetation expansion, a primary threat to tallgrass prairie, on riparian and benthic denitrification. Expansion of woody vegetation in these grasslands is due primarily to altered fire regimes, which historically inhibited woody vegetation growth. To determine the effect of woody vegetation expansion on benthic and riparian denitrification, woody vegetation was removed from the riparian zone of a grazed and an ungrazed watershed. Both soil and benthic denitrification rates from this removal buffer were compared to rates in grassy or woody riparian zones. Riparian soil denitrification was highly seasonal, with greatest rates occurring during early spring, and rates being low throughout the remainder of the year. Benthic denitrification was also temporally variable but did not exhibit seasonal trends, suggesting benthic denitrification is driven by factors other than water temperature. Removal of woody vegetation stimulated soil and benthic denitrification rates over rates found in naturally vegetated riparian zones. Elevated N loadings will continue to affect aquatic ecosystems, and these effects may be exacerbated by biodiversity losses or changing riparian vegetation.

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CHAPTER 1 - Introduction

Natural ecosystems are experiencing increasing anthropogenic impacts as the global human population continues to expand (Vitousek et al. 1997*a*). Increasing nitrogen (N) loadings (Vitousek et al. 1997*b*, Galloway et al. 2004), watershed land-use changes, and decreasing biodiversity (Dudgeon et al. 2006) are but a few of the anthropogenic pressures affecting streams. In addition to anthropogenic stressors, prairie streams are subject to frequent natural abiotic disturbances, such as flooding and desiccation (Matthews 1988). These anthropogenic and natural stressors can have large impacts on stream ecosystem functions, such as nutrient retention and primary production. Headwater streams are especially important in terms of nutrient retention, due to high microbial activity in the water-sediment interface (benthic) zone (Peterson et al. 2001), the high ratio of benthic surface area to water column volume (Alexander et al. 2000), and the fact that the majority of the length of river networks is made up of small (1st or 2nd order) streams (Alexander et al. 2000, Alexander et al. 2007).

Although N is often a limiting nutrient, it is also considered one of the most significant stressors in aquatic environments (USEPA 2002) due to anthropogenic loadings causing deleterious ecological and economic effects (Carpenter et al. 1998, Dodds et al. 2009). The efficiency of N processing and retention in headwater streams decreases as N loadings increase, and these systems eventually become N saturated (Mulholland et al. 2008). Therefore, reducing N inputs into streams is of great importance. Terrestrial-aquatic transition (riparian) zones are capable of processing a large amount of N before it reaches aquatic systems, and are often manipulated in management to reduce nutrient pollution from non-point sources. Nitrate (NO₃⁻), the most soluble form of nitrogen, is primarily retained or removed from ecosystems by the

processes of vegetative uptake (short-term retention) and denitrification – the dissimilatory reduction of NO_3^- to nitrous oxide (N_2O) or dinitrogen gas (N_2) (Diebel et al. 1994). Although vegetation can assimilate a large amount of NO_3^- , assimilation only temporarily stores N until decomposition of vegetation. Contrary to vegetative uptake, denitrification constitutes a permanent removal of N from the ecosystem to the atmosphere.

Watershed land management can have dramatic effects on the structure and function of riparian zones and streams. Riparian zones in tallgrass prairie ecosystems of the Great Plains were historically dominated by herbaceous, C_4 grass species (e.g., *Andropogon gerardii*), whereas gallery forests were limited to riparian zones of higher order streams (Knight et al. 1994, Dodds et al. 2004). There has been a dramatic expansion of woody vegetation throughout tallgrass prairie region following the European colonization of the Great Plains, and this expansion has been most intense in riparian zones (Knight et al. 1994, Dodds et al. 2004, Briggs et al. 2005). The full effect of this woody vegetation expansion is unknown, but woody expansion may cause dramatic changes in nutrient cycling and biodiversity of the ecosystem as a whole.

Decreasing biodiversity and invasive species also threaten to impact aquatic ecosystem functioning. A recent meta-analysis showed that increases in species richness lead to increasing biomass/standing stock of the next highest trophic level, and that this relationship is more pronounced in aquatic than terrestrial ecosystems (Cardinale et al. 2006). As biodiversity losses increase, the potential exists for a loss of organismal functional guilds (e.g., grazers, decomposers, N-fixers, etc.). Algivorous fishes represent an important functional guild in headwater prairie streams, as they have been shown to alter algal communities, particulate organic matter size, and gross primary productivity (Power et al. 1985, Gelwick and Matthews

1992, Bertrand and Gido 2007). Macroconsumers such as algivorous fishes can also alter the recovery trajectories of prairie streams in response to desiccation (Murdock et al. 2010) and flooding (Bertrand et al. 2009), but more data regarding their effects on different ecosystem functions (e.g., N transformations, GPP, CR) are needed.

The major questions addressed in this thesis are: 1) how does a key macroconsumer, *Campostoma anomalum*, affect the structural (i.e., algal community structure, invertebrate community, particulate size) and functional (i.e., gross primary productivity, community respiration, denitrification) recovery of experimental streams in response to a simulated flood, and 2) how woody vegetation expansion affected denitrification in riparian and benthic zones of pristine tallgrass prairie headwater streams. For the first chapter I used large, outdoor mesocosms to study the impact of an algivorous minnow, *Campostoma anomalum*, on recovery of ecosystem structure and function following a flood. This study was novel in the fact that it is, to my knowledge, the first to analyze the impacts of fish on denitrification in a lotic (flowing) ecosystem. In the second chapter, I measured riparian and benthic denitrification at two watersheds, each of which had three sites delineated by riparian vegetation type – naturally grassy, woody vegetation encroached, and a site which had its woody vegetation removed. This is the first study, to my knowledge, to analyze the impact of forestation of historically non-forested systems (via woody expansion) on denitrification, as opposed to the large number of studies that have analyzed the effect of restoring historically forested systems.

CHAPTER 2 - Influence of grazing minnows (*Campostoma anomalum*) on structural and functional recovery of stream mesocosms following a scouring flood

ABSTRACT

Anthropogenic nitrogen loadings and perturbations of macroconsumer communities impair the ecological and economic values of streams. While organisms are adapted to natural regimes of flooding and desiccation, how these anthropogenic and natural disturbances interact is poorly understood. We used large outdoor mesocosms to study the impact of *Campostoma anomalum*, a common prairie headwater stream minnow, and NH_4^+ additions (to simulate fish excretion) on the recovery of ecosystem structure and function following a flood. Fish and NH_4^+ additions decreased particulate organic matter size, increased invertebrate biomass, differentially altered filamentous algal structure, and increased algal biomass accrual rates. Treatments also altered the response of denitrifiers to various nutrient and energy amendments, and denitrification rates decreased following the recovery of mesocosms. Altered algal community structure, coupled with minimal change in ecosystem metabolism, suggest algal communities are functionally redundant and resilient to moderate grazing pressure. Temporal changes in denitrification were likely caused by increasing hyporheic DO concentrations, leading to potentially less anoxic microsites for the production of denitrification enzymes. To the authors' knowledge, this is the first study of the impacts of grazing on benthic denitrification in lotic systems.

INTRODUCTION

Increased nitrogen (N) loadings into aquatic ecosystems, primarily due to anthropogenic activities, will continue to rise along with the global human population (Vitousek et al. 1997a, Galloway et al. 2004) causing deleterious ecological and economic impacts (Carpenter et al. 1998, Dodds et al. 2009). Headwater stream N cycling has received increased attention in recent years due to the ability of these streams to process N and prevent it from moving downstream (Alexander et al. 2000, Peterson et al. 2001, Mulholland et al. 2008). The high biological activity associated with the benthic zone of headwater streams, along with increased sediment-water contact in shallow streams (Peterson et al. 2001), and the high proportion of water and N originating from headwaters (Alexander et al. 2007), leads to headwater streams being important natural filters for processing increased N loadings.

Although fishes have long been known to impact ecosystem structure, limnologists have only recently established that fishes can also have strong impacts on stream ecosystem function (e.g., Vanni in press) and many studies of trophic cascades only measure ecosystem structure, not function (i.e. relative biomass, not material and energy fluxes; as seen in review by Duffy et al. 2007 which used biomass as its response variable). Biogeochemical cycling - an important stream ecosystem function - controls nutrient retention (Mulholland et al 2008). Little is known about how fishes alter nutrient retention and loss via denitrification in streams, which is increasingly important with increasing pressures on aquatic biodiversity due to anthropogenic stressors (Dudgeon et al. 2006).

Specific biogeochemical processes are rarely mechanistically linked to fishes (but see Persson and Svensson 2006). Studies examining the relationship between freshwater consumers (e.g., fish), increasing nutrient levels, and biogeochemical processes could establish the

importance of consumers on fundamental ecosystem processes, an effect often claimed, but rarely specifically demonstrated (Wetzel 2001). Recycling of nutrients by fishes (e.g., McIntyre et al. 2007, McIntyre et al. 2008) and other aquatic consumers (e.g., gastropods, Liess and Hillebrand 2006; mussels, Bracken 2004) can influence ecosystem function (e.g., migratory Pacific salmon, reviewed by Janetski et al. 2009), but aside from these specific cases, the general effects of higher trophic level organisms on ecosystem processes such as nutrient cycling and ecosystem metabolism are only beginning to be understood (Vanni in press).

Algivorous minnows strongly influence ecosystem structure, while impacts on ecosystem function are more variable in prairie streams. The most consistent impact of grazing minnows across studies has been a reduction in algal filament lengths, in natural systems (Power et al. 1985, Gelwick and Matthews 1992, Bertrand and Gido 2007) and mesocosms (Bertrand and Gido 2007, Bengtson et al. 2008). Grazing minnows can also reduce algal biomass, alter invertebrate community structure and decrease particulate organic matter size (Gelwick and Matthews 1992, Bertrand and Gido 2007, Bengtson et al. 2008). Evidence for grazing minnows altering ecosystem function is more variable. Gelwick and Matthews (1992) found grazing by central stonerollers (*Camptostoma anomalum*) increased net primary productivity (NPP) in natural pools, and Murdock et al. (2010) found that macroconsumer (fishes, crayfish, and tadpoles) exclusion in a natural stream led to significantly higher community respiration (CR) and NH_4^+ uptake. Studies using southern redbelly dace (*Phoxinus erythrogaster*) found no change in NPP or gross primary productivity (GPP) in either experimental mesocosms or natural systems (Bertrand and Gido 2007, Bengtson et al. 2008). Grazing can increase nutrient cycling rates and denitrification in terrestrial systems directly via excretion (e.g., Monaghan and Barraclough 1993) and indirectly via increased litter quantity and quality (Hobbs 1996), but to

the authors' knowledge, no previous study has attempted to determine the impact of grazers on benthic denitrification.

Natural abiotic disturbances such as flooding and desiccation cause intermittent prairie headwater streams to be in an almost permanent state of succession (Dodds et al. 2004). Recovery of the algal community within these prairie streams occurs rapidly, with algal biomass returning to pre-flood levels within 3 weeks of a scouring flood event (Dodds et al. 1996a). Nutrients and primary production strongly affect ecosystem recovery (Dodds et al. 1996a), but ecosystem recovery trajectories can also be influenced by consumers (Bertrand et al. 2009, Murdock et al. 2010). Short-term recovery of the algal community is especially influenced by consumers, whereas the final state of an ecosystem is less dependent on the presence of macroconsumers (Murdock et al. 2010). In contrast, little, if anything, is known about the impact macroconsumers have on biogeochemical processes, specifically denitrification, over short-term recovery periods.

We designed an experiment to study the impacts of *C. anomalum* on ecosystem structure and function following a simulated flood. In addition to previously studied measures of structure and function (see Bertrand and Gido 2007, Murdock et al. 2010) we analyzed the impacts of fish on denitrification and used NH_4^+ additions to separate the effects of fish excretion from those of grazing on ecosystem function. *Campostoma* and NH_4^+ were added to experimental streams to determine if effects seen in the fish treatments were caused by increased NH_4^+ concentrations due to N mineralization (excretion), or by physical grazing effects. We hypothesized that *C. anomalum* would change ecosystem structure by reducing algal filament length, decreasing mean particulate organic matter (POM) size and altering invertebrate community structure. Ammonium additions were predicted to alter both ecosystem structure (i.e., increased algal

filament length and biomass) and function (i.e., increased GPP, NPP, and benthic denitrification). We predicted that increased N availability from excretion and the alteration of particle size caused by *C. anomulum* additions would have a subsequent positive effect on denitrification.

METHODS

Description of experimental streams and experimental design

Nineteen large outdoor experimental mesocosms at the Konza Prairie Biological Station (KPBS), located approximately 10 km southeast of Manhattan, KS, USA, were used to test the effects of *C. anomulum* and NH_4^+ additions on ecosystem structure and function. Each stream consisted of a 2.54 m² circular pool (mean depth = 0.5 m) downstream of a 0.84 m² rectangular riffle (mean depth = 0.15 m) (see Matthews et al. 2006 for detailed stream design). Polyvinyl chloride tubes (1 m x 2.5 cm i.d.) were placed vertically within the substrata of each stream pool, with the open bottom of the tube ~0.5m below the surface of the substrata, to measure hyporheic dissolved oxygen (DO). Water was supplied to each stream from a groundwater spring similar to those that feed the natural prairie streams on KPBS at a rate of ~1.0 L min⁻¹. Water was re-circulated in each stream using electric trolling motors at a constant rate of ~10 L s⁻¹ to simulate natural currents. Benthic substrata were a mixture of cobble, pebble, gravel, and fine sediment collected from a local quarry.

Streams were filled for one week prior to the initiation of each experiment, allowing algal and macroinvertebrate communities to colonize each stream. After this colonization period, stream substrata and walls were scoured with groundwater using a pressure sprayer; the streams were then drained and immediately re-filled. Scouring removed the majority of organic matter

accumulated over the one-week colonization period, homogenized biomass of algal and macroinvertebrate communities across streams, and acted as a flood for these mesocosms. Following scouring, plastic mesh baskets (5.5 x 10 x 10 cm, 2 x 1.25 cm mesh) were filled with dry rocks and placed flush with the stream bottom. Twelve baskets were randomly placed in each of the stream pools and six baskets were placed in each of the riffles. Within 24 hr of scouring, ten streams were randomly selected to receive fish, while four were selected for NH_4^+ additions and five were designated controls. *Campostoma* were added at two different densities (approximately natural field density and 2x natural densities, Franssen et al. 2006), but preliminary analyses showed no density effect. Therefore, all streams receiving *Campostoma* additions were designated as a fish treatment (n=10). An NH_4^+ nutrient enrichment treatment (to stimulate fish excretion; n=4), and a no-fish, no NH_4^+ -N, control (CONT; n=5) were also employed. Initially, different levels of NH_4^+ enrichments were added to each stream (attempted to enrich streams to 4, 8, 16, and 32 x background [NH_4^+]) continuously using a peristaltic pump. Amendments were a combination of NH_4Cl and 0.1% HCl in a 20 L bucket. HCl was in the amendments to inhibit growth and nitrification within the amendment bucket. This HCl addition had a negligible effect on pH, due to high dilution, high alkalinity spring water, and the CaCO_3 composition of the benthic substrata. The 8x NH_4^+ enrichment rate was approximately the same as calculated N excretion rates for average *Campostoma* treatment density (unpublished data). Due to similar responses across the nutrient enriched streams (no significant regressions were found using enrichment level as the predictor variable, data not shown) the four NH_4^+ enrichments were grouped into a single treatment.

Ecosystem structure

Variables related to ecosystem structure were measured on days 14 and 27 of the experiment. Prior to disturbing the streams, water samples were collected in 60 mL acid-washed bottles to measure $[\text{NH}_4^+]$ and $[\text{NO}_2^- + \text{NO}_3^-]$. Samples were placed on ice immediately after collection and stored frozen until analysis. Analyses were performed on an OI Analytical Flow Solution IV autoanalyzer using the indolphenol blue method to measure $[\text{NH}_4^+]$ and the cadmium reduction method to measure $[\text{NO}_2^- + \text{NO}_3^-]$ (APHA 1998). Hyporheic DO was measured every hour from pre-dawn to solar noon on days 14 and 31 of the experiment (to coincide with ecosystem metabolism measurements). A handheld DO probe (YSI Model 550-A, Yellow Springs Instruments, Yellow Springs, OH) was submerged as deep as possible in open-bottom PVC tubes installed at the beginning of the experiment for hyporheic DO measurements and allowed to equilibrate for 2 min before taking a measurement.

Algal filament lengths were measured using a meter stick in each stream along three transects in the riffle (three points per transect) and three transects in the pool (11 total points). The longest filament found in the appropriate location along each transect was measured. Both mean filament length and filament length variability (standard deviation within a stream) were calculated for each stream. Algal biomass (as chl *a*) was measured from three randomly selected pebbles in the riffles and five from the pools. Chlorophyll *a* was extracted from pebbles via submersion in 95% ethanol at 78°C (Sartory and Grobbelaar 1984). Rock surface area was determined using Sigmascan Pro (version 5; Hearne Scientific Software Pty. Ltd., Melbourne, Australia), allowing expression of algal biomass on an areal basis (i.e. chlorophyll density was based on projectional area on the stream bottom, not total surface area of each rock).

Particulate organic matter (POM) and macroinvertebrate communities were sampled by randomly collecting two plastic mesh baskets from the pool and one from the riffle. Substrata from the baskets were dumped into a 20 L bucket filled with 5 L of stream water. The solution was agitated by hand until a homogenous slurry was formed. A 500-mL sub-sample of this slurry was collected for analysis of POM and preserved with formalin. Following the POM sub-sample, the remaining slurry was filtered through a 250- μm sieve to collect macroinvertebrates, which were preserved with formalin.

The POM sub-sample was separated into three size-classes by running the slurry through a series of filters ($\geq 516 \mu\text{m}$ = coarse particulate organic matter (CPOM); $98 - 515 \mu\text{m}$ = medium particulate organic matter (MPOM); $0.7 - 98 \mu\text{m}$ = fine particulate organic matter (FPOM)). Each size fraction was then dried (60°C , ≥ 48 h), weighed, combusted in a muffle furnace (470°C , 6 h), weighed, re-wetted (to return water stored in clays lost due to volatilization), dried (60°C , ≥ 48 h) and weighed to determine ash-free dry mass (AFDM) of each size fraction.

Macroinvertebrates were counted and identified to family (Dipterans) or order using a dissecting microscope. Lengths were taken of each macroinvertebrate and length-biomass relationships were used to determine total biomass of each macroinvertebrate taxon (Benke et al. 1999).

Ecosystem function

Ecosystem function variables were measured on days 14 and 31 of the experiment. Whole-stream metabolism was measured using an open-system single station approach. A handheld dissolved oxygen (DO) probe (YSI Model 550-A, Yellow Springs Instruments, Yellow Springs, OH) measured DO at the bottom of each riffle approximately every h from pre-dawn to solar noon (1 pm CST) during sunny days. Night-time DO consumption was assumed to be

constant; therefore the pre-dawn measurement was used to calculate respiration for each stream. Turbulence-induced aeration was assumed to be similar across all streams due to similar stream morphology, inflow, and re-circulation rates. The reaeration coefficient ($k = 0.432 \text{ d}^{-1}$) used to model whole-stream metabolism was measured previously in these mesocosms (Murdock et al. 2010). Algal biomass accrual ($\text{mg Chl } a \text{ m}^{-2} \text{ d}^{-1}$) was calculated as the temporal change in algal biomass (i.e., for the second sampling date, accrual was calculated by subtracting algal biomass of sample one from that of the second sampling date, and scaled on a daily basis).

Benthic denitrification was measured on samples from four mesh baskets from each stream pool on each date. Substrata from each basket were randomly assigned to a specific amendment (see below) and stored at 4°C until incubations began (within 24 h of sampling). The acetylene-inhibition method was used to determine denitrification rates (Smith and Tiedje 1979, Groffman et al. 1999, see Bernot et al. 2003, Groffman et al. 2006 for discussion of limitations and benefits of the method).

Approximately 100 g of benthic substrata and 100 mL of amendment solution were added to a 475 mL glass jar with a sealed metal top (Mason jar) equipped with a rubber septum to allow gas sampling. Each jar received 1 mM chloramphenicol to inhibit the generation of new enzymes (Brock 1961, Smith and Tiedje 1979), and one of four amendments to assess NO_3^- and carbon (C) limitations: deionized water (-N-C), 5 mM dextrose (-N+C), 20 mM KNO_3 (+N-C), or 5 mM dextrose and 20 mM KNO_3 (+N+C) (all of these are final concentrations). Following the addition of the amendment solution, jars were made anoxic by three cycles of three minutes evacuation to a 700 mm Hg vacuum and one minute flushing with N_2 gas. Once anoxia was induced, 20 mL of CaC_2 generated acetylene (approximately 10% of the headspace volume) were added to the jars. Samples were then incubated at 125 rpm on a rotary shaker table for 90

min. Gas samples (5 mL) were collected at 30 and 90 min and stored in 4 mL pre-evacuated BD vacutainer vials (BD, Franklin Lakes, NJ, USA) until analysis by gas chromatography (within 48 hrs). Bunsen coefficient corrections were used to calculate total N₂O produced in the glass jars. Areal *in situ* denitrification rates were calculated by converting the un-amended denitrification rates to an areal basis using total particulate organic matter measurements.

Statistical analysis

A blocked repeated-measures analysis of variance (rmANOVA) was performed to test effects of *C. anomulum* and/or NH₄⁺ additions on ecosystem structure and function. Visual differences among rows of experimental streams were observed throughout the experiment, potentially due to previous experiments or differences in light availability; therefore the rmANOVA was blocked by stream row. Ecosystem structure and function measurements (see above) were the dependent variables, individual streams were the repeated measures, while treatment (CONT, NH₄, or FISH) was the explanatory variables. Temporal differences in a response variable were analyzed using paired t-tests. Particulate organic matter size and algal biomass data were used in two separate analyses; therefore p-values were subsequently Bonferroni corrected for these analyses. To determine the effects of fish and NH₄⁺ on denitrification and its response to various amendments, a blocked, two-way rmANOVA was used, with stream row as the block, denitrification rate as the response variable, and treatment and incubation amendment (un-amended, +C, +N, or +N+C) as the explanatory variables. Data were checked for normality and homogeneity of variance. Kendall's tau was used to determine relationships between un-amended denitrification rates and particulate organic matter size classes. Denitrification data did not meet the assumption of normality, and were therefore logarithmically transformed prior to analysis. All other data met the assumptions of ANOVA.

All statistical analyses were performed in SPSS (version 11.0, Chicago, IL, USA). Differences between treatments are reported as treatment mean \pm one SE, and temporal differences are reported as sampling date grand mean (all treatment mean) \pm one SE.

RESULTS

Ecosystem structure

Water Chemistry

As expected, both $\text{NH}_4^+\text{-N}$ ($p < 0.001$) and NO_3^-N ($p = 0.077$) concentrations differed among treatments, with concentrations being increased in streams receiving the $\text{NH}_4^+\text{-N}$ addition (Table 2.1). $\text{NH}_4^+\text{-N}$ decreased in the streams over time ($p = 0.014$). Presence of *Campostoma* did not significantly affect inorganic N in the water column. Hyporheic DO was not affected by treatment, but increased temporally from the first ($2.84 \pm 0.14 \text{ mg O}_2 \text{ L}^{-1}$) to the second sampling date ($5.27 \pm 0.15 \text{ mg O}_2 \text{ L}^{-1}$; $p < 0.001$; Table 2.1).

Filament length and algal biomass

Neither mean filament length nor filament length variability in riffles was affected by fish or nutrients. Although riffle filament length and variability was similar across treatments on the first sampling date, on the second sampling date there was a potential trend of decreased filament length in the fish treatments and an increase in the nutrient treatments (Fig 2.1a). Mean filament length was significantly impacted by treatment ($p = 0.065$), with streams containing fish having shorter algal filaments (Fig 2.1b). Pool filaments were generally longer and more variable than riffle filaments (Fig 2.1). Algal biomass (measured as chl *a*) was not significantly impacted by fish or nutrient amendments in either the pools or the riffles, but was generally greater in pools ($11.59 \pm 0.96 \mu\text{g cm}^{-2}$) than riffles ($9.26 \pm 0.64 \mu\text{g cm}^{-2}$; Table 2.1).

POM Size fractions

Total particulate organic matter (POM), medium particulate organic matter (MPOM), and coarse particulate organic matter (CPOM) did not differ among treatments (Table 2.1). Fine particulate organic matter (FPOM) was significantly affected by treatment ($p=0.011$; Table 2.1), with the NH_4^+ enriched streams having more FPOM than either the fish or control streams. Proportional FPOM was not affected by treatment, whereas proportional MPOM ($p=0.040$) was greater and proportional CPOM ($p=0.032$) was less in streams which contained grazers compared to streams which received nutrients; proportional distributions of POM in control streams did not differ from either of the other treatments (Fig 2.2). Temporally, total FPOM did not change, while total MPOM, CPOM, and POM increased from the first to the second sampling ($p<0.025$). Proportional MPOM did not change temporally, whereas proportional FPOM decreased ($p=0.002$) while proportional CPOM increased temporally ($p=0.002$; Fig 2.2).

Invertebrates

Invertebrate biomass increased significantly from the first ($353 \pm 47.5 \text{ g DM m}^{-2}$) to the second sampling date ($1030 \pm 128 \text{ g DM m}^{-2}$; $p<0.001$) but was not affected by either $\text{NH}_4^+\text{-N}$ addition or fish (Table 2.1). This temporal increase was largely due to an increase in *Chironomidae* - the dominant taxon found in the streams - biomass, which increased over time ($297 \pm 46.0 \text{ g DM m}^{-2}$ on week 2 to $797 \pm 116 \text{ g DM m}^{-2}$ on week 4; $p<0.001$). Unlike total invertebrate biomass, *Chironomidae* density was affected by treatment ($p=0.017$), with densities being greater in NH_4^+ enriched streams than fish or control streams (Table 2.1). On average, 84.1 % of the entire invertebrate biomass was made up of *Chironomidae* on the first sampling date and 77.3 % on the second sampling. Although not significant, there was a trend for fish to decrease *Chironomidae* biomass compared to the control.

Ecosystem function

Denitrification

Denitrification rates were significantly affected by different amendments ($p=0.055$), but not by the different treatments ($p=0.529$). No interaction terms were significant. Based on Tukey's HSD post-hoc analysis, un-amended rates did not differ from C amended rates; whereas N and N+C amended incubations expressed greater rates than un-amended incubations but did not differ from C amended rates (Fig 2.3). There were no significant additive or super-additive effects seen in response to fully amended compared to only C or only N amended incubations. Although never significant, un-amended rates were more related to FPOM than CPOM on both sampling dates (week 2 $\tau = 0.222$ for FPOM and 0.116 for CPOM, week 4 $\tau = 0.151$ for FPOM and 0.098 for CPOM). Areal denitrification rates were not significantly affected by either treatment or sampling date (Fig 2.4).

Differing temporal trends were seen within the treatments ($p=0.057$). Both the control and fish treatments showed a temporal decrease in C-amended denitrification, while the NH_4^+ treated streams exhibited temporal increases (Fig 2.3). These trends suggest that the NH_4^+ amended streams become more C limited with time, while the control and fish treatments are increasingly limited by some other factor. Nitrate limitations changed temporally ($p<0.001$) with N-amended denitrification rates decreasing in both the control and fish treatments, but not changing in the NH_4^+ treatments (Fig 2.3). The NH_4^+ effect was driven by the first sampling date: the NH_4^+ enriched systems had lower N-amended denitrification rates ($1.29 \pm 0.71 \text{ ng N g}^{-1} \text{ DM h}^{-1}$) than either the control ($6.64 \pm 2.16 \text{ ng N g}^{-1} \text{ DM h}^{-1}$) or the fish ($5.73 \pm 1.64 \text{ ng N g}^{-1} \text{ DM h}^{-1}$) treatments on the first sampling date, suggesting that the treatments without NH_4^+ fertilization were more limited by N availability, especially early in the colonization of these

systems. After four weeks, the control and fish treatment denitrification rates were less N limited, and exhibited similar rates to those of the NH_4^+ amended systems. Fully amended denitrification rates also differed temporally ($p < 0.001$), with a decrease in rates being seen from the first sampling date ($6.42 \pm 0.98 \text{ ng N g}^{-1} \text{ DM h}^{-1}$) to the second ($1.20 \pm 0.33 \text{ ng N g}^{-1} \text{ DM h}^{-1}$). This suggests that following a flood, denitrification is limited by C, nitrate, or co-limited, but becomes increasingly limited by some other factor (i.e., oxygen or microbial abundance) with following a flood.

Algal biomass accrual

Based on rmANOVA, algal biomass accrual ($\text{mg Chl } a \text{ m}^{-2} \text{ d}^{-1}$) was not significantly affected by $\text{NH}_4^+\text{-N}$ or fish. The lack of a significant result was caused by the fish and $\text{NH}_4^+\text{-N}$ treatments having high algal accrual rates on the first date, and rates that were not different from zero on the second sampling date, while the control streams had intermediate rates of algal biomass accrual on both sampling dates (Fig 2.5). These trends were consistent for both riffle (Fig 2.5a) and pool algal biomass accrual (Fig 2.5b). The greater rates over the first sampling period in NH_4^+ and fish treatments suggests that these treatments stimulated algal community development compared to the control, leading to equilibrium being reached more quickly (i.e., rates not differing from zero over the second sampling date).

Net ecosystem metabolism

Gross primary production (GPP) was marginally impacted by either treatment ($p = 0.090$; Fig 2.6a), with the NH_4^+ enriched streams having greater GPP than streams which contained fish. The three treatments expressed similar GPP rates on the first sampling date, but the NH_4^+ streams had higher GPP ($3.33 \pm 0.60 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$) than either the control ($2.53 \pm 0.35 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$) or fish ($2.03 \pm 0.26 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$) treatments on the second sampling date (Fig 2.6a).

Community respiration (CR) also was significantly affected by treatment ($p=0.023$; Fig 2.6b), with NH_4^+ enriched streams having greater absolute (more negative) rates of CR than streams with fish. Similar trends were seen in CR as GPP, with NH_4^+ -N amended streams exhibiting apparently higher CR on the second sampling date ($-5.16 \pm 1.01 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$) than either the control ($-3.95 \pm 0.58 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$) or the fish ($-3.03 \pm 0.31 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$) treatments (Fig 2.6b). Interestingly, GPP and CR increased from the first to the second sampling in the NH_4^+ treatments but decreased in the fish and control treatments. Overall NEP was also affected by treatment ($p=0.017$; Fig 2.6c), with NH_4^+ amended streams expressing more negative values of NEP than the fish treatments. Control streams did not differ from either of the other treatments in any metabolism measurement. Both GPP and CR decreased significantly from the first to the second sampling date ($p<0.001$), but a greater decrease in CR than GPP led to streams being less heterotrophic (less negative NEP) on the second sampling date (Fig 2.6c).

DISCUSSION

Our results are consistent with the growing evidence that grazing minnows alter ecosystem structure, mainly by decreasing algal filament lengths, but also by altering POM size. Fish significantly reduced filament length and variability in pools, whereas there was no apparent fish effect on riffle filaments. This is likely because the filaments in control riffles rarely exceeded 5 cm, which is typically the maximum length of a grazed filament (personal observation), suggesting the lack of filament growth in control riffles overshadowed any grazer effect. Reduced filament length may lead to a reduction in algal biomass and GPP, but this was not the case in the present study. Grazing minnows are known to reduce algal filament length (Power et al. 1985, Liess and Hillebrand 2004, Bertrand and Gido 2007), but this reduced filament length is often coupled with an increase in diatoms and cyanobacteria (Power et al.

1985, Gelwick and Matthews 1992, Murdock et al. 2010), negating any algal biomass or GPP responses to grazing.

Factors controlling ecosystem function

Altered ecosystem structure caused by grazing does not appear to greatly alter the function of these streams (i.e., minimal change in GPP, CR, or denitrification). There are several possible reasons for this lack of a functional response. First, the presence of grazers may select against filamentous algae and for a non-filamentous benthic community that is less susceptible to grazing (Power et al. 1985, Liess and Hillebrand 2004, Murdock et al. 2010), or the algal production may become more efficient (e.g., greater rates of production per unit algal biomass). The lack of a grazer impact on GPP, CR, or algal biomass suggests that the algal community is able to withstand grazer disturbances via functional redundancy (i.e., shifts in algal communities allow for similar ecosystem functions in response to grazers; Power et al. 1985), and actually benefit from grazers based on algal biomass accrual. Functional redundancy has been a long-standing idea in biodiversity studies (e.g. Walker 1992, Naeem and Li 1997), but most have focused on redundancies in higher trophic level organisms (e.g., O'Connor and Crowe 2005, McIntyre et al. 2007) rather than producers. To further understand impacts of grazing on stream ecosystem function (specifically GPP and CR), more experiments studying functional redundancies among primary producers are needed.

Fish decreased the size of POM in streams, with fish treatments having higher proportional FPOM and lower MPOM and CPOM. These trends are consistent with a previous study using *Phoxinus* in the same experimental stream complex, which showed a reduction in CPOM and an increase in FPOM due to *Phoxinus* grazing (Bertrand and Gido 2007). The NH_4^+ treatment did not affect relative POM sizes, suggesting that any change in ecosystem function

due to altered particle size caused by fish are directly attributable to grazing or mechanical effects, not N mineralization. Reduced sizes of POM could increase C and N cycling, due to increased surface area for microbial decomposition of POM. Any change in this decomposition should be evident in community respiration data, but fish did not affect CR, suggesting that the altered POM sizes had minimal affect on C and N cycling.

Trophic state in aquatic systems is a function of both heterotrophic and autotrophic activity (Dodds and Cole 2007). Denitrification rates were not significantly affected by treatment, but there were differences seen in carbon or nitrate limitations, with control and fish treatments showing temporal reductions in denitrification rates in response to carbon amendments, whereas the NH_4^+ enriched streams were more carbon limited on the second sampling date. Denitrification rates on the first sampling date in the NH_4^+ enriched streams showed co-limitation by both carbon and nitrate (Fig 2.3c). There was no evidence for co-limitation in either the control or fish treatments. Surprisingly, fully amended incubations in fish and control treatments showed similar denitrification rates to either the nitrate or carbon amended treatments (Fig 2.3a, b). There is no obvious reason for this lack of carbon, nitrate, or co-limitation, but this may suggest that DO is inhibiting denitrification. Ammonium amendments increased absolute NEP (more negative), generally due to increased (more negative) CR, whereas grazer presence had no effect. Therefore, any grazer effect on primary productivity is likely to be due to N mineralization. It is possible that this increase in oxygen consumption (CR) could be due to nitrification of the added NH_4^+ -N, and not aerobic respiration. Nutrient mineralization by fish can be great relative to ecosystem need (Vanni et al. 2002, McIntyre et al. 2008), suggesting an interaction between N mineralization and primary production is possible, if not probable. Grazer control of primary productivity via mineralization has been seen previously,

with bullhead minnows increasing nutrient translocation to sediments, causing increased benthic primary productivity (Hargrave 2006). Differences between N mineralization and physical grazing effects on ecosystem structure and function are difficult to tease apart and are likely both important in the current study.

This is the only study, to our knowledge, to analyze the effect of grazers on denitrification and hyporheic DO in a lotic system. The apparent inverse relationship between hyporheic DO and denitrification is consistent with the fact that oxygen is a more efficient electron acceptor for carbon oxidation than nitrate. Hyporheic DO was not significantly influenced by either fish or NH_4^+ , suggesting the increase in DO may simply be a result of the ecosystem reaching equilibrium, with the flood potential increasing total organic matter throughout the hyporheic zone, leading to greater respiration and thus decreased DO.

A previous study of the effects of macroconsumers on ecosystem recovery from drought found short-term ecosystem recovery to be affected by macroconsumers, but the final state of the system was independent of macroconsumer presence (Murdock et al. 2010). These findings are congruent with our measurements of total algal biomass and algal biomass accrual. No differences were found in the final amount of algal biomass in these mesocosms, but accrual was more rapid in both fish and ammonium treatments than the control, suggesting that the short-term recovery of the algal community is affected by consumer N mineralization, whereas the final state of the ecosystem is independent of consumers.

Recovery from flood in prairie streams

Headwater prairie streams are subject to frequent flooding and desiccation, creating a near-constant state of succession in these systems (Dodds et al. 1996a, Dodds et al. 2004). The recovery from these disturbances is rapid (algal biomass reaches pre-flood levels within 3

weeks), making them ideal systems for studying ecosystem recovery trajectories (Matthews 1988, Dodds et al. 1996a, Murdock et al. 2010). The use of large, outdoor mesocosms to study ecosystem recovery allows for mechanistic manipulations on a manageable scale, providing future questions to be examined by performing large-scale manipulations of natural systems.

Beginning with the seminal paper on temporal succession in lotic systems by Fisher et al. (1982), recovery trajectories from floods and droughts have been of primary interest for aquatic ecologists. Natural prairie headwater streams generally rely on algal productivity, receive relatively less allochthonous C than forested streams (Dodds et al. 2004), are highly N retentive (Dodds et al. 1996b, Dodds et al. 2000, Kemp and Dodds 2001), and are subjected to frequent disturbances (i.e., desiccation and scouring floods). These factors make prairie streams ideal for studying short-term recovery trajectories of stream ecosystems. Our results show that *Camptostoma* increases the recovery rate of algal biomass, likely due to N mineralization, and that benthic denitrification may be highest immediately following a flood, provided that there is sufficient NO_3^- . As the ecosystem recovers, denitrification becomes limited by other factors, such as oxygen, labile C, or the microbial community.

Algal communities of these prairies streams recover quickly from disturbances, but less is known about microbial responses to floods in prairie streams. Based on microbial recovery from flooding in a desert southwest stream (Holmes et al. 1998), algal recovery is more rapid than that of the microbial community. However, transplanted substrata adjusted to local nitrification rates in 6 days in Kings Creek (Kemp and Dodds 2002), suggesting rapid microbial colonization following disturbance in prairie streams. Low denitrification rates and the lack of a grazer impact on denitrification are possibly due to a mostly oxic sediment, as seen in the higher correlation between un-amended denitrification and FPOM (compared to CPOM), which is consistent with

greater rates of denitrification found in aquifer microcosms with finer particle sizes (Dodds et al. 1996c).

Although our results showed minimal grazer impact on algal biomass, GPP, and CR, a recent meta-analysis on the impacts of herbivory on primary production found a significant increase in benthic primary producer biomass in response to herbivore exclusion across 32 studies (Gruner et al. 2008). Differences between the current study and the general results of herbivore exclusion could be due to the lack of invertivorous fishes in the current study, allowing invertebrate grazers to substitute for *Campostoma* grazing (Bertrand et al. 2009), or there could be an actual functional difference in the response of prairie stream primary producers to grazing compared to benthic primary producers in other systems. Based on the consistency of our results with previous studies prairie stream studies (Gido et al. in press), we believe that grazing minnows indeed impact the structure of prairie streams, but have less of an impact on the functional response (as GPP, CR, or denitrification) to this altered ecosystem structure. Nitrogen limitation appears to have a stronger influence on prairie stream ecosystem function than grazing by *Campostoma*.

CONCLUSIONS

The results of this study confirm that grazing minnows reduce algal filament lengths and alter POM size. These structural changes failed to drastically alter GPP, CR or algal biomass, but fish and NH_4^+ -N both increased algal biomass accrual. Furthermore, NH_4^+ amendments alleviated N limitation of denitrification, whereas grazers had minimal affect on denitrification. The modest changes in ecosystem function may be due to redundancy in the algal community (e.g., an increase in diatoms to compensate for a reduction in filamentous algae; Power et al. 1985), or an increase in available nutrients due to both enhanced N mineralization and sediment

disturbance. Recovery of the algal community from scouring floods appears to be more rapid in the presence of grazers, apparently due to N mineralization. Grazing did not have any apparent effects on the hyporheic redox conditions, but further studies should be performed to determine what mechanisms cause this increase in DO following a flood. Future studies of the role of grazing and flooding and its effects on ecosystem function, especially denitrification, should be performed in natural field settings using exclosures to confirm these interactions hold true in the field.

TABLES AND FIGURES

Table 2.1 Mean inorganic N concentrations, particulate organic matter size fractions, proportional POM, invertebrate biomass, and gross primary productivity from three different treatments (CONT = control, FISH = fish added, NH4+ = NH₄⁺-N amended streams) from two sampling dates (Week 2 and Week 4). Numbers in parentheses denote one standard error.

Week	CONT		FISH		NH4+	
	2	4	2	4	2	4
NH ₄ ⁺ -N * ¹ (µg L ⁻¹)	10.90 (0.84) a ²	9.38 (2.12) a	12.60 (0.84) a	8.95 (0.90) a	32.41 (8.64) b	20.89 (7.88) b
NO ₃ ⁻ -N (µg L ⁻¹)	22.12 (6.39) a	13.87 (6.63) a	48.32 (21.91) a	30.87 (8.75) a	247.99 (161.01) b	56.07 (42.42) b
FPOM (mg AFDM m ⁻²)	0.0034 (0.0002) a	0.0047 (0.0002) a	0.0039 (0.0003) a	0.0033 (0.0005) a	0.0044 (0.0004) b	0.0075 (0.0014) b
MPOM * (mg AFDM m ⁻²)	0.0032 (0.0011)	0.0043 (0.0010)	0.0028 (0.0003)	0.0037 (0.0008)	0.0031 (0.0005)	0.0053 (0.0014)
CPOM * (mg AFDM m ⁻²)	0.0043 (0.0035)	0.0077 (0.0031)	0.00045 (0.0002)	0.0043 (0.0032)	0.0034 (0.0015)	0.0125 (0.0062)
POM * (mg AFDM m ⁻²)	0.0111 (0.0047)	0.0167 (0.0042)	0.0072 (0.0006)	0.0114 (0.0041)	0.0109 (0.0016)	0.0253 (0.0072)
Invertebrate Biomass * (mg m ⁻²)	209.5 (58.9)	485.3 (99.0)	263.6 (47.2)	860.2 (203.9)	610.4 (56.7)	1309.1 (273.3)
<i>Chironomidae</i> Biomass * (mg m ⁻²)	177.5 (60.9) a	446.3 (92.6) a	188.1 (26.7) a	627.4 (149.0) a	561.4 (74.3) b	1247.3 (284.3) b
Riffle Algal Biomass * (mg Chl <i>a</i> m ⁻²)	5.45 (0.71)	10.29 (1.43)	9.40 (1.42)	9.76 (1.20)	9.78 (3.15)	10.66 (0.99)
Pool Algal Biomass (mg Chl <i>a</i> m ⁻²)	8.32 (1.83)	11.38 (2.25)	11.68 (1.29)	11.78 (2.59)	14.41 (4.03)	12.39 (2.71)
Hyporheic DO * (mg L ⁻¹)	3.08 (0.28)	5.11 (0.09)	2.96 (0.11)	5.14 (0.15)	2.26 (0.40)	5.78 (0.60)

¹ Differences between sampling dates (p<0.05) indicated by *

² Different lower case letters indicate significant (p<0.10) differences among treatments

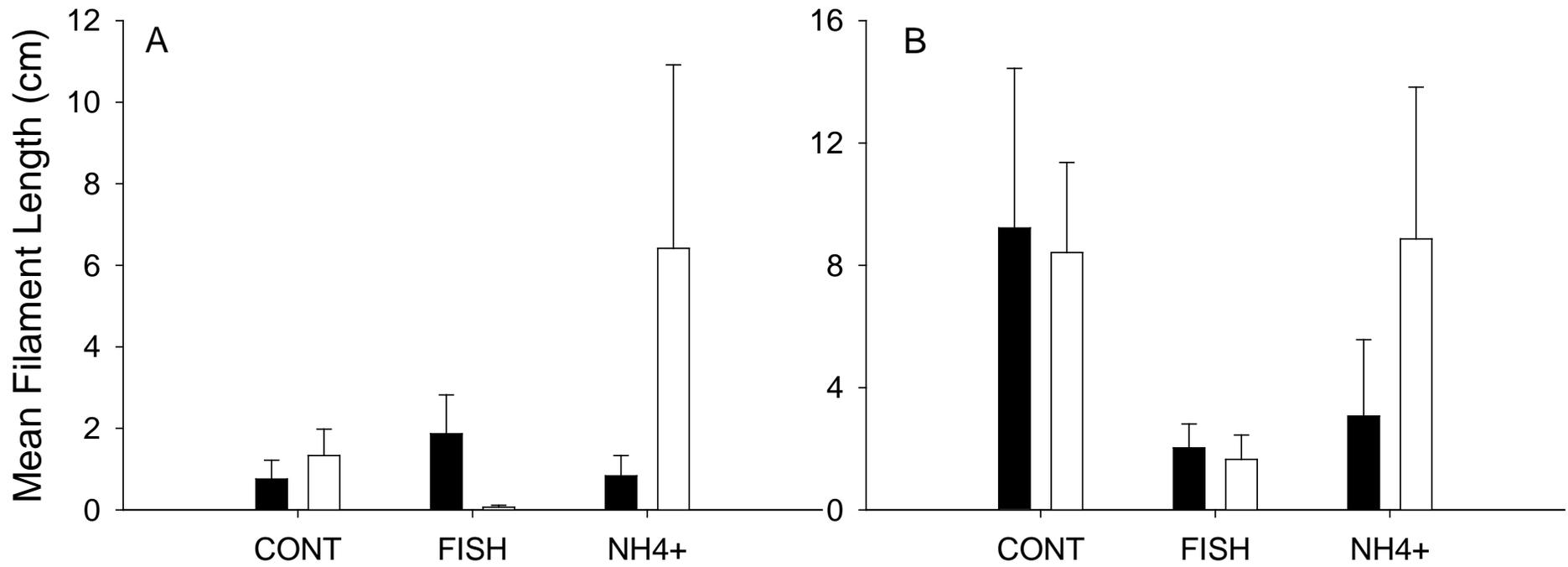


Figure 2.1 Mean filament lengths in experimental riffles (A) and pools (B) from control (CONT; n=5), fish (FISH; n=10), and NH₄⁺-N (NH4+; n=4) treatments on the day 14 (black bars) and day 28 (white bars) sampling dates. Error bars are ± 1 SE.

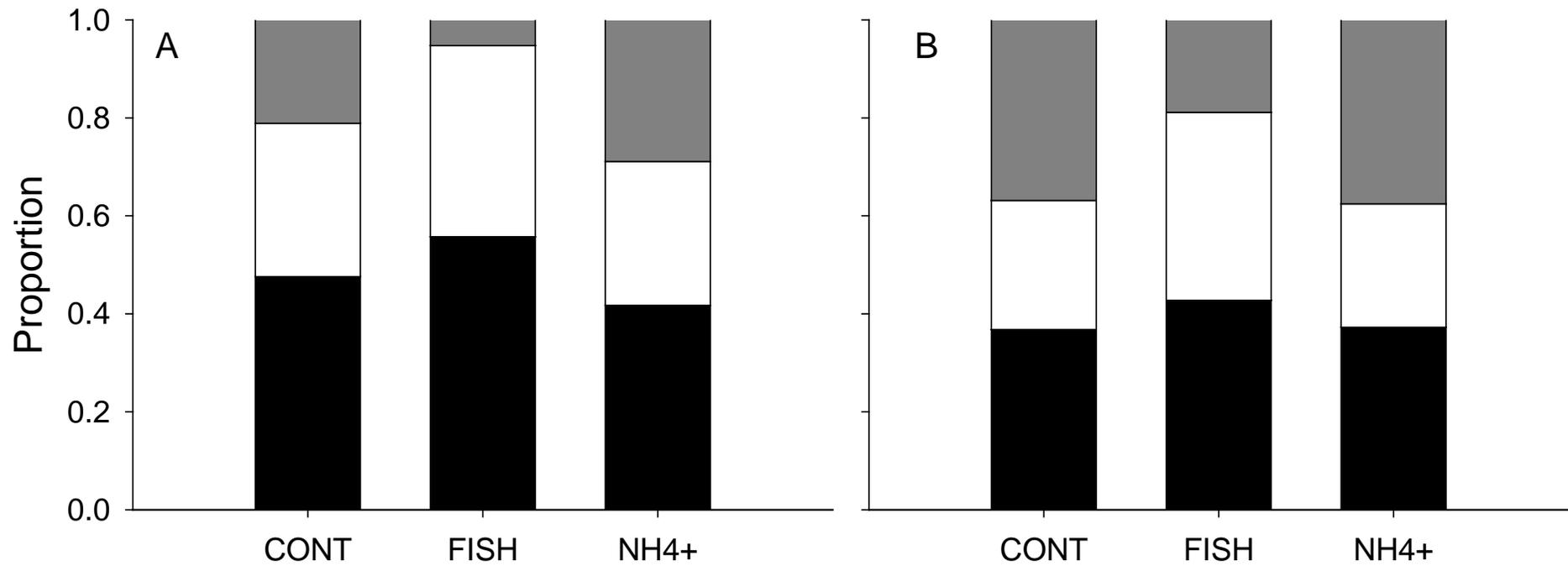


Figure 2.2 Proportional amounts of FPOM (black), MPOM (white), and CPOM (gray) from control (CONT), fish (FISH), and NH₄⁺-N (NH₄⁺) treatments collected on days 14 (A) and 27 (B) of the experiment.

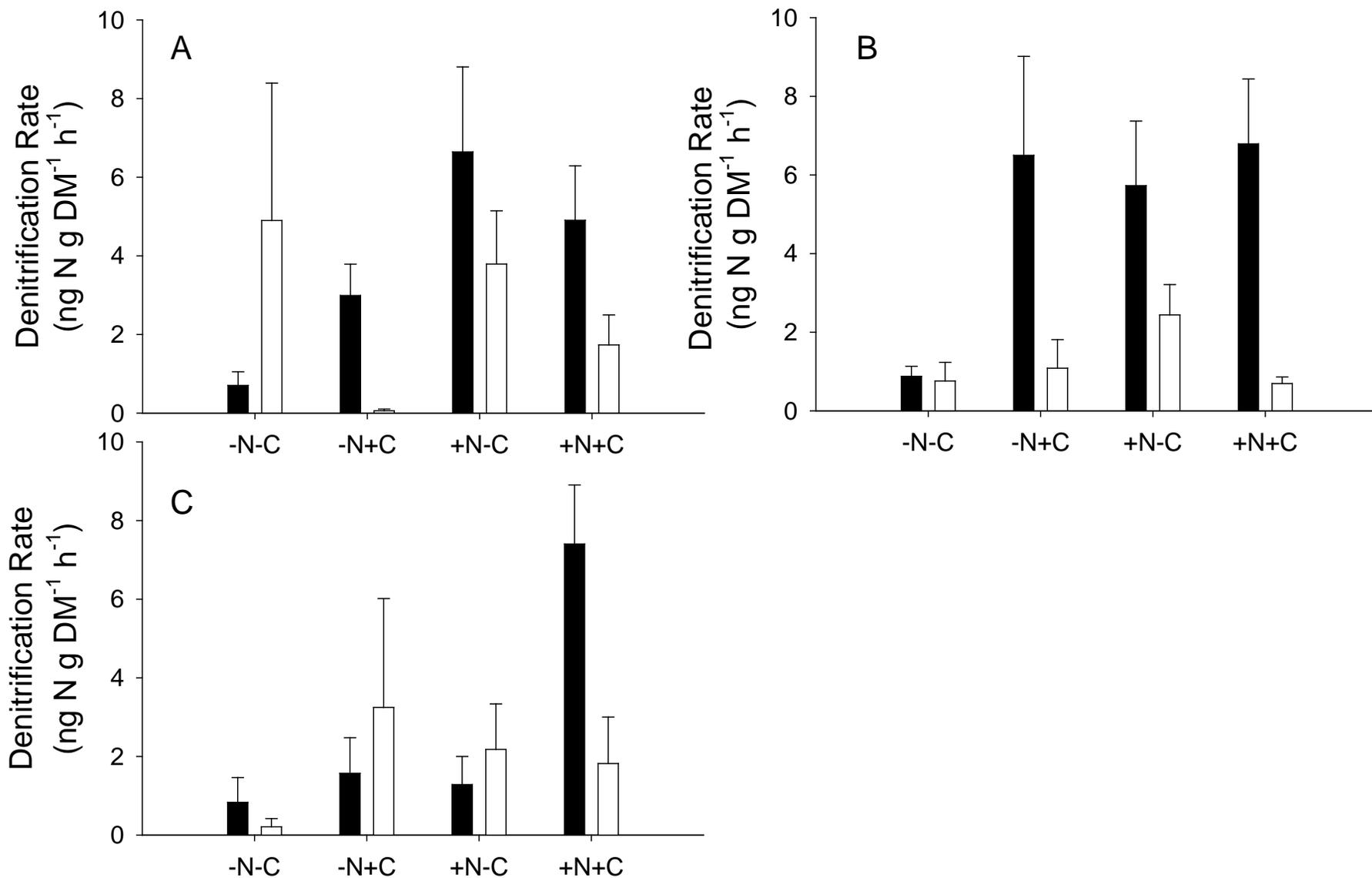
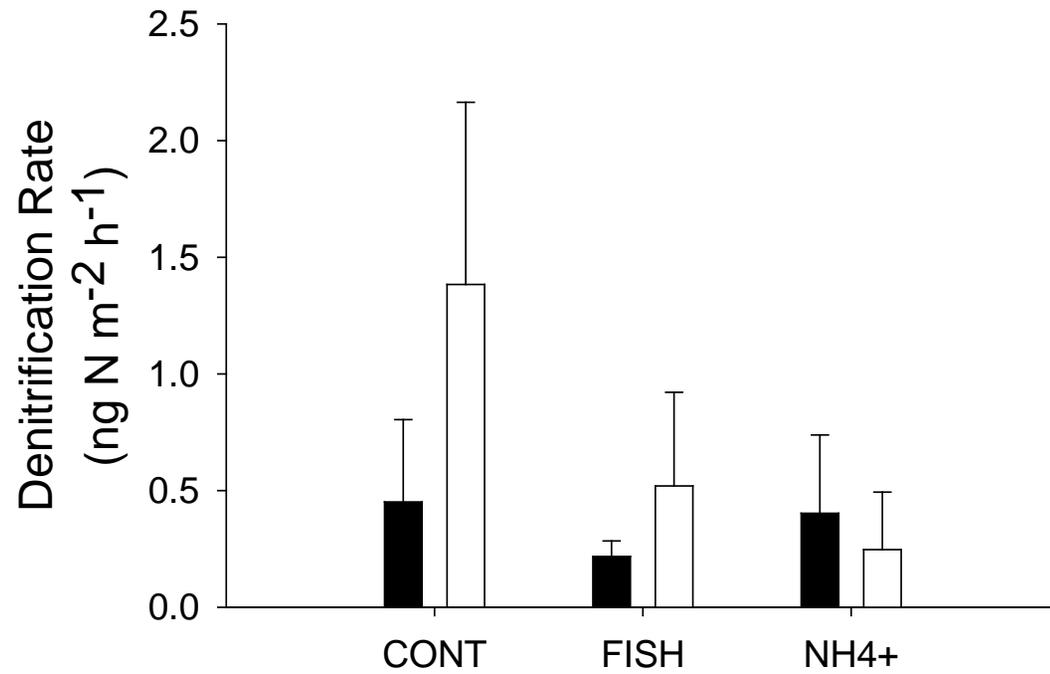


Figure 2.3 Response of denitrification rates to different amendments in control (A), fish amended (B), and NH_4^+ amended (C) streams. Rates from unamended (-N-C), dextrose amended (-N+C), nitrate amended (+N-C), and fully amended (+N+C) incubations on day 16 (black) and day 31 (white) are shown. Error bars are ± 1 SE.



2.4 Areal unamended denitrification rates from three treatments (CONT, FISH, NH4+) on day 14 (black) and day 31 (white). Error bars are ± 1 SE

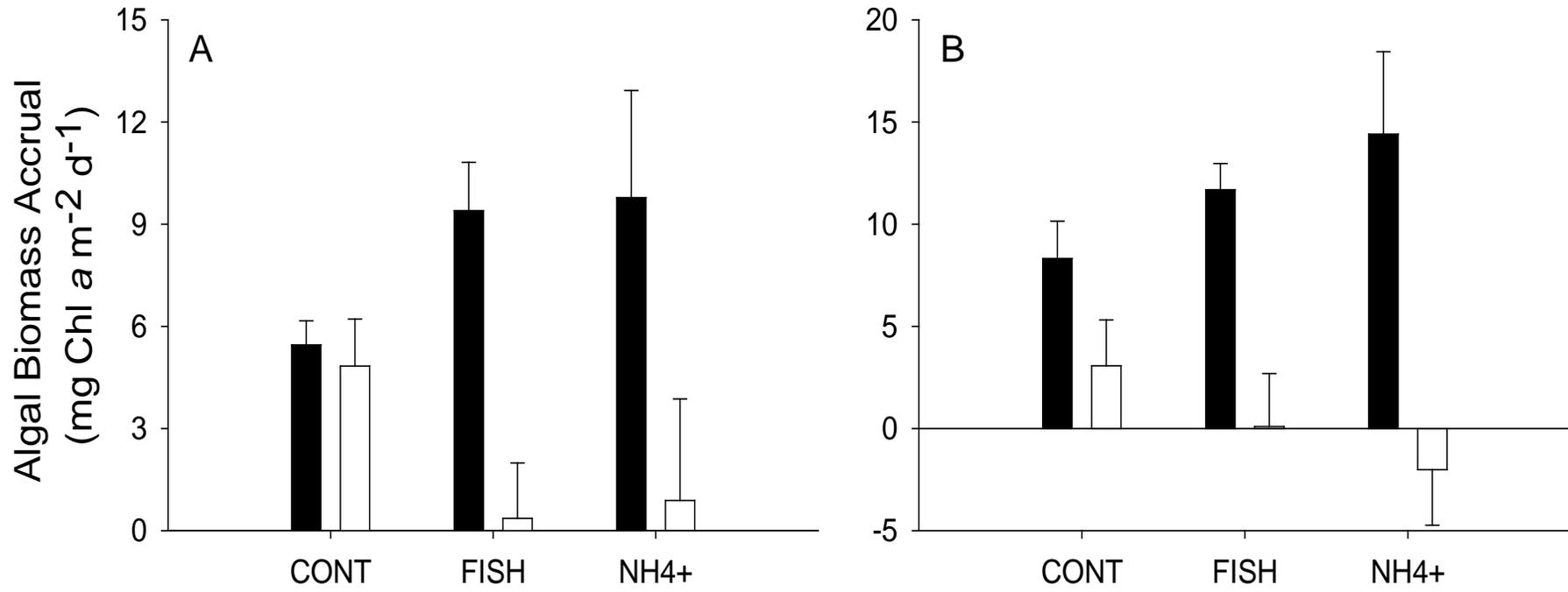


Figure 2.5 Algal biomass accrual in riffles (A) and pools (B) on day 14 (black) and day 27 (white) from control (CONT), fish (FISH), and NH₄⁺-N (NH4+) treatments. Error bars are ± 1 SE.

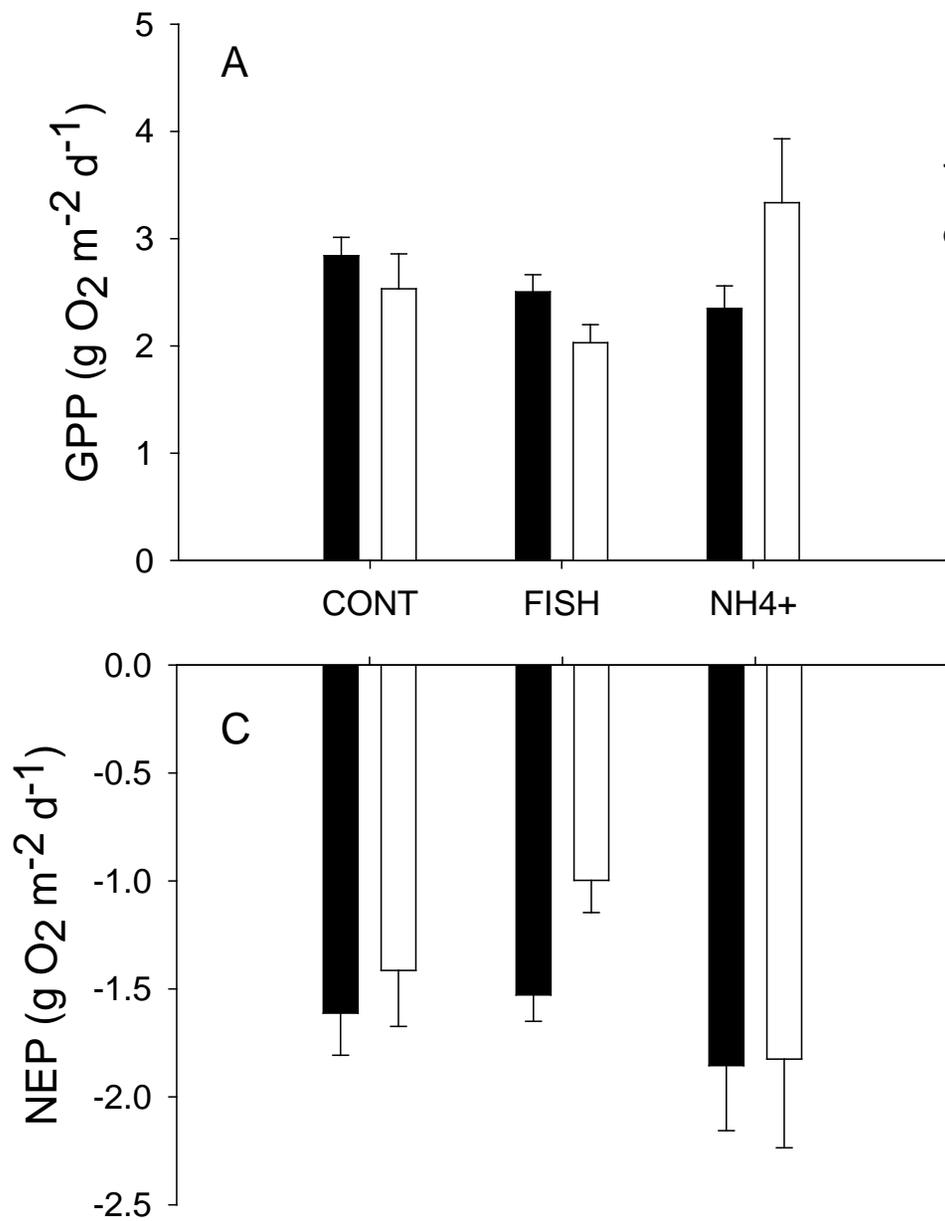


Figure 2.6 Gross primary productivity, community respiration, and net ecosystem production from control (CONT), fish (FISH), and $\text{NH}_4^+\text{-N}$ (NH_4^+) treatments measured on day 14 (black) and day 31 (white) of the experiment. Error bars are ± 1 SE

CHAPTER 3 - Encroaching riparian woody vegetation and its subsequent removal from prairie streams: influence on denitrification associated with riparian soils and stream bottoms

ABSTRACT

Expansion of native woody vegetation, especially in riparian zones, is a primary threat to tallgrass prairie ecosystems of the Midwestern United States. The relationship of woody riparian vegetation to denitrification of both riparian soils and benthic biofilms is well-studied, but typically in natural woody ecosystems. Here we analyze the effect of forestation, via woody encroachment, and its subsequent removal (restoration), on denitrification. Denitrification in riparian soil and benthic compartments (sediment, leaf packs, grass root wads, and filamentous algae) were measured at two separate watersheds, one ungrazed and one grazed by native bison, among three riparian vegetation types: natural grassy vegetation with an open canopy, woody encroached with a closed canopy, and woody vegetation removed in the winter of 2007 with an open canopy. Soil and benthic denitrification were measured seasonally using the acetylene inhibition technique. Riparian soil denitrification was highly seasonal, with greatest rates in the early spring. Benthic denitrification also exhibited temporal variability, but there was no obvious seasonality. Removal of woody vegetation stimulated both riparian soil and benthic sediment denitrification due to increased nitrate, carbon, and potential anoxia. Riparian vegetation also indirectly affected benthic denitrification by changing the compartments present in the streams (replacement of filamentous algae and root wads by leaf packs in reaches with woody riparian vegetation). Differences were seen among watersheds, with the grazed watershed exhibiting greater rates of denitrification. Riparian soil denitrification was related to seasonal changes in

soil water content and spatial patterns of soil carbon. Carbon content was also related to differences in benthic sediment denitrification among reaches of different riparian vegetation.

INTRODUCTION

Land cover and land use alterations continue to impact the natural state of ecosystems (Vitousek et al. 1997*a*). Tallgrass prairies are one of the most endangered ecosystems in North America, with areal declines from the pre-industrial to the modern area estimated between 82-99%, exceeding declines reported for any other major North American ecosystem (Samson and Knopf 1994). Currently, the primary threats to remaining tallgrass prairies of North America are landscape fragmentation and expansion of native woody vegetation (Briggs et al. 2005). Potential drivers of woody vegetation encroachment include climate change, atmospheric carbon dioxide concentration, increased nitrogen deposition, altered grazing pressure, and changes in fire regimes (i.e., frequency and intensity of fire; Briggs et al., 2005). Woody vegetation encroachment occurs throughout the entire prairie ecosystem, but is especially intense in riparian zones which were historically dominated by open-canopied grassy riparian zones (Dodds et al. 2004); forests are moving upstream, transforming these naturally grassy headwater riparian areas to gallery forests (Knight et al. 1994, Briggs et al. 2005) and fundamentally changing the unique character of prairie streams. As grasslands are the natural vegetation type over large areas of the earth (Dodds 1997), it is important to understand fundamental changes to the system that might alter the biogeochemistry and ecology of streams draining these ecosystems.

Riparian zones typically display high rates of biogeochemical activity related to high availability of organic carbon (C), nitrogen (N), and existence of spatially and temporally variable redox (McClain et al. 2003). These zones can be correlated with substantial retention or removal of nutrients, particularly in low order streams (Dodds and Oakes 2006). High benthic surface area to water volume ratios inherent in headwater streams and active benthic microbial communities mean that a substantial proportion of nutrients entering headwater streams are never

transported downstream (Alexander et al. 2000, Peterson et al. 2001, and Mulholland et al. 2008). Denitrification, the dissimilatory reduction of NO_3^- -N to N gas (N_2O and N_2), is a primary form of nitrogen removal in both riparian (Hill et al. 1996) and benthic (Mulholland et al. 2008) zones of streams. Because of the favorable conditions for denitrification common in riparian and benthic zones of headwater streams, these transition-zones are important for protecting downstream ecosystems from N pollution. However, efficiency of N removal decreases as N loadings increase and small streams lose their ability to remove N as they become saturated (O'Brien et al. 2007, Mulholland et al. 2008). Therefore effective management of riparian vegetation to encourage denitrification could be highly beneficial to controlling downstream nitrogen pollution.

Restored riparian zones (buffers) can trap more sediment (Dillaha et al. 1989), reduce nitrogen concentrations in surface and groundwater flow paths (Jacobs and Gilliam 1985, Dillaha et al. 1989), and retain other nutrients (i.e., phosphorus) and organic contaminants (Vidon et al. 2010). Restoration of native riparian vegetation often implies creating riparian forests. For example, the 1996 U.S. "Farm Bill" required land managers to plant trees in riparian buffers in order to qualify for monetary assistance (NRCS 1997). Grassy riparian buffers also provide substantial benefits (Lyons et al. 2000). Woody buffers are generally thought to have greater N retention and reduce surface flow velocity, while grassy buffers are more suited for reducing erosion and controlling phosphorus pollution (Lyons et al. 2000).

Nitrogen is a key stressor in aquatic ecosystems (USEPA 2002) where increased N loadings (Vitousek et al. 1997b, Herridge et al. 2008), can have numerous ecological and economic impacts (Carpenter et al. 1998, Dodds et al. 2009). Nitrogen is an especially important pollutant in agricultural systems where a loss of native riparian cover and fertilization impact

stream water chemistry (Johnson et al. 1997). Historically, native riparian vegetation was primarily grasses in a majority of the Midwest, where tallgrass prairies dominated (Knight et al. 1994) and conversion of these grassy riparian zones to either agricultural, urban, or woody riparian zones can have dramatic effects on both riparian and stream conditions (Lyons et al. 2000). These alterations can indirectly affect N retention in headwater streams, while forestation of native grassy riparian zones increases allochthonous carbon (C) inputs (as leaf litter), which alters C and N cycling (Claessens et al. 2010).

This study was designed to determine the impact of woody vegetation encroachment and its subsequent removal on riparian and benthic denitrification in a pristine tallgrass prairie. Seasonal variation (i.e., temperature and precipitation), which is extreme in tallgrass prairie ecosystems (Groffman et al. 1993) and differentially influences woody and grassland processes, was also studied. We hypothesized that: (1) riparian denitrification would exhibit seasonal variability, with the greatest rates seen in the spring, (2) riparian denitrification would be affected by vegetation type, with woody vegetation having greater rates than grassy vegetation, and (3) benthic denitrification would be affected by riparian vegetation due to changes in benthic compartments present in the system (e.g., increased leaf packs in woody vegetation reaches) and altered nutrient and energy inputs. This is the first study, to the authors' knowledge, to analyze the influence of forestation via woody encroachment, and its subsequent restoration, on historically non-forested systems on denitrification.

METHODS

Site description

This study was performed on two 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 (e.g., Dodds et al. 2000, Kemp and Dodds 2002). Sampling sites were located in two experimental sub-watersheds of King's Creek: K2A, an ungrazed watershed on the north branch of King's Creek that is burned every two years, and N04D, a watershed on the south branch of King's Creek that is burned every four years and grazed by native American bison (*Bison bison*). Soils at both sites are classified in the Ivan Silt Loam series. Each watershed was separated into three reaches based on riparian vegetation: a naturally grassy 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. Woody vegetation was removed from the riparian areas (up to 30 m away from the stream) in December 2007 by a combination of mowing and using chainsaws to remove larger woody plants, and maintained by mowing each subsequent winter. K2A was burned in the spring of 2008 and 2010, whereas N04D was burned in the spring of 2009.

Naturally grassy riparian zones at both 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. Woody vegetation seen at the two watersheds differed, with the woody riparian zone at N04D being dominated by American elm (*Ulmus americana*) and honey locust (*Gleditsia triacanthos*), whereas the woody riparian zone at K2A was dominated by bur oak (*Quercus macrocarpa*) and chinkapin oak (*Quercus muehlenbergii*). Both woody reaches had diverse understories comprised of multiple species of grasses and forbs. Woody vegetation removal zones were distinct from other vegetation zones due to a lack of big

bluestem and Indian grass. The removal reach at N04D was comprised primarily of Japanese brome (*Bromus japonicus*), western ragweed, and dogwood patches, whereas the removal reach at K2A consisted of more woodland understory species, such as Virginia creeper (*Parthenocissus quinquefolia*), buckbrush (*Andrachne phyllanthoides*), and black snakeroot (*Sanicula canadensis*).

At each riparian vegetation treatment a 30 m transect perpendicular to the stream was selected, attempting to keep topography similar across all sites. Ten sampling points were evenly spaced across the 30 m transect, leading to 10 unique samples from each vegetation type at each watershed on 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 the mid-point of the original transects (15 m) to determine annual variability of denitrification.

Field collection

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

Benthic sampling was performed within one week of riparian sampling in April, June, July, and October, with an additional sampling date in January, which was excluded from riparian sampling due to frozen soil. Prior to collecting compartments for benthic denitrification, stream water chemistry samples were collected from the bottom of each reach in acid-washed 60 mL bottles. Reaches were then surveyed for all different compartments present; compartments were collected in triplicate on each sampling date and included sediment, leaf packs, root wads, and filamentous algae. Sediment samples were collected to a depth of 5 cm using a circular metal sleeve (4 cm diameter x 5 cm long), filamentous algae was collected by removing all algae from a 225 cm² area that was selected by visually identifying algal mats, grass root wads and leaf packs were collected via grab sampling.

Denitrification incubations

The acetylene-inhibition method was used to measure denitrification rates (Smith and Tiedje 1979, Groffman et al. 1999). Problems with this method can include the inhibition of nitrification by acetylene producing artificially low nitrate concentrations, leading to an underestimation of denitrification rates (see Bernot et al. 2003, Groffman et al. 2006 for reviews). However, this method was selected due to the relatively low cost, the ability to process a large number of samples in a short time, 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.

The static-core technique was used to measure riparian actual denitrification (Robertson et al. 1987, Groffman et al. 1999). Following field collection of static cores, cores were allowed to reach room temperature, and both ends were sealed with rubber stoppers. Ten mL of acetylene, generated via reaction of CaC₂ with deionized H₂O to ensure purity, were added to

each core (~10% of the headspace volume). Core headspace was then pumped repeatedly with a 60 mL syringe to ensure complete mixing of the headspace. Five mL gas samples were taken at two and six hrs (the linear phase of denitrification) and transferred to 4-mL pre-evacuated BD-vacutainer vials (BD, Franklin Lakes, NJ, USA). Prior to gas sample collection, core headspace was re-homogenized by pumping with a 60-mL syringe.

Potential denitrification from riparian and benthic samples was measured using the denitrification enzyme activity (DEA) assay (Smith and Tiedje 1979, Groffman et al. 1999). Either 25 g of homogenized bulk soil or 25 g of specific benthic compartment (wet weight), and 25 mL of media (20 mM KNO₃, 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 nutrient and energy limitations, while 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 then subjected to three cycles of evacuation (3 min) and flushing with N₂ (1 min) to induce anoxia. Once anoxic, 10 mL C₂H₂, generated as above, were added to each flask. Flasks were then incubated for 90 min on a rotary shaker table at 125 rpm. Five mL gas samples were taken at 30 and 90 min and stored in 4-mL BD-vacutainer vials. After all incubations were completed, gas samples were analyzed for N₂O 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) x 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% CH₄ carrier gas at flow rate of 30 mL min⁻¹). Actual denitrification rates were temperature corrected ($Q_{10} = 2.0$; Stanford et al. 1975) to provide a

measure of field rates; DEA rates were corrected for N₂O dissolved in solution using Bunsen coefficient corrections.

Ancillary data

Bulk density was calculated for each static core and was used to express riparian denitrification rates on an areal basis. Soil inorganic nitrogen (both NH₄⁺-N and NO₂⁻ + NO₃⁻-N) was extracted from bulk soil samples using 2 M KCl (5:1 KCl v: soil v). The extracted solution was then analyzed on an OI Analytical Flow Solution IV using the indophenol blue method (NH₄⁺-N) and the cadmium reduction method (NO₂⁻ + NO₃⁻-N) (APHA 1998); October samples (except the grassy and woody reaches of K2A) were contaminated with NH₄⁺ during the extraction, and therefore NH₄⁺-N and total inorganic N values 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 48 h. Dried soil was then ground into a fine powder using a ball-mill (8000D Dual Mixer/Mill, SPEX CentiPrep, Metuchen, NJ, USA), and analyzed for total carbon and total nitrogen using a Carlo Erba NA 1500 Analyzer (Carlo Erba, Milano, Italy). Stream water chemistry samples were analyzed for NH₄⁺-N and NO₂⁻ + NO₃⁻-N using the same methodology as the KCl extracts.

Statistical Analysis

Preliminary analyses revealed distance from the stream to be unrelated to riparian denitrification (insignificant 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 land management on riparian potential and actual denitrification. Watershed (K2A or N04D) and riparian vegetation (grass, wood, or removal) were the explanatory variables, and the analyses were blocked by sampling date. 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 relatively high areal cover in all habitats,, and the fact that sediment was expected to directly reflect riparian inputs regardless of if they 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 rates of sediment as the response variable.

Differences among potential denitrification rates 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. Data are expressed in the text as annual means \pm SE, unless otherwise noted.

RESULTS

Soil and water parameters

Soil water content differed among watersheds ($p < 0.001$), vegetation type ($p = 0.001$), and sampling date ($p < 0.001$; Table 3.1). N04D had lower soil water content (29.75 ± 0.39 %) than K2A (33.0 ± 0.3 %); soils of grassy riparian zones were drier throughout the than woody or removal soils ($p < 0.001$; Table 3.1). Extractable soil NO_3^- was significantly related to riparian vegetation, with woody removal soils having greatest NO_3^- and grassy riparian soils had the least NO_3^- ($p < 0.001$; Table 3.1). Extractable NO_3^- did not differ between watersheds, but there was temporal variation, with the early summer samples (June and July) having greater amounts of NO_3^- than early spring or fall samples ($p < 0.001$; Table 3.1). Extractable soil NH_4^+ was affected by riparian vegetation ($p = 0.001$), watershed ($p = 0.041$), and sampling date ($p < 0.001$). NH_4^+ was

greater at K2A than N04D, and was similarly low in the removal and woody riparian soils compared to the grassy riparian soils. Ammonium concentration was greater in the early spring than the summer; fall samples were excluded from analysis due to contamination of some samples (Table 3.1). Significant differences between watersheds were seen in total N, which was significantly greater at N04D than K2A ($p < 0.001$). Grassy riparian zone soils had lower total N than woody or removal riparian zones, ($p < 0.001$; Table 3.1). Total C differed among soils below riparian vegetation ($p < 0.001$), with soils under grassy riparian zones having less TC ($35.3 \pm 0.4 \text{ mg g}^{-1}$) than woody zones ($39.8 \pm 0.4 \text{ mg g}^{-1}$), which had less TC than the removal riparian soils ($41.8 \pm 0.6 \text{ mg g}^{-1}$; Table 3.1). Soil carbon to nitrogen ratios were greater in K2A (12.8 ± 0.2) than N04D (11.7 ± 0.1 ; $p < 0.001$) and removal riparian soils had a greater mean C:N (12.9 ± 0.3) than woody riparian zones (11.7 ± 0.1 ; $p < 0.001$); C:N of grassy riparian soils (12.4 ± 0.2) did not differ from either removal or woody riparian zones. Stream water NO_3^- and NH_4^+ exhibited no obvious trends, and values were generally similar in reaches with differing riparian vegetation types and watersheds (Table 3.2). No statistical tests were run on water chemistry due to low replication.

Riparian soil potential denitrification

Riparian soil potential denitrification rates were significantly impacted by sampling date ($p < 0.001$; Fig 3.1a) and marginally affected by riparian vegetation ($p = 0.078$; Fig 3.1b), but not watershed (Fig 3.1a). There was a significant interaction between riparian vegetation and watershed ($p = 0.015$; Table 3.3). Post-hoc analyses revealed early spring to be the season with greatest denitrification, with April of 2009 samples expressing the greatest rates, followed by April of 2010 (Fig 3.1a). The grazed watershed (N04D) had higher rates than the un-grazed watershed (K2A) across the most sampling dates (Fig 3.1a), but there was no statistical

difference between the watersheds ($p=0.290$). Rates were significantly greater in the removal riparian soils than the grassy riparian soils, while the woody riparian soils did not differ from either riparian vegetation type (Fig 3.1b).

Riparian soil actual denitrification

Actual soil denitrification rates were significantly related to sampling date ($p<0.0001$; Fig 3.2a), riparian vegetation ($p<0.0001$; Fig 3.2b), and marginally to watershed ($p=0.068$; Fig 3.2a). There was also a significant interaction between watershed and vegetation ($p=0.003$; Table 3.3). Soils with riparian wood removal exhibited greater rates ($90.1 \pm 17.7 \text{ g N ha}^{-1} \text{ d}^{-1}$) than where wood remained ($66.9 \pm 19.2 \text{ g N ha}^{-1} \text{ d}^{-1}$), which in turn had greater rates than grassy riparian soils ($45.8 \pm 11.9 \text{ g N ha}^{-1} \text{ d}^{-1}$; Fig 3.2b). Average annual rates were higher at the grazed ($77.6 \pm 15.5 \text{ g N ha}^{-1} \text{ d}^{-1}$) than the ungrazed watershed ($58.1 \pm 11.3 \text{ g N ha}^{-1} \text{ d}^{-1}$), but this apparent difference was driven by April 2009, as rates were similar among watersheds on all other sampling dates (Fig 3.2a). Rates were generally greatest at the beginning of the growing season, with April 2009 having the greatest rates, followed by April 2010 and June 2009, which had higher rates than October 2009, July 2009, and March 2010 (Fig 3.2a).

Benthic potential denitrification

Potential denitrification rates of sediment were significantly affected by watershed ($p<0.001$), riparian vegetation ($p<0.001$), and sampling date ($p<0.001$). There was a significant interaction between watershed and riparian vegetation ($p=0.001$; Table 3.3). Temporal variability was seen in potential denitrification of sediment, but there was no obvious seasonal effect (Fig 3.3a). Rates were higher at N04D ($0.10 \pm 0.02 \text{ } \mu\text{g N g DM}^{-1} \text{ h}^{-1}$) than K2A ($0.02 \pm 0.01 \text{ } \mu\text{g N g DM}^{-1} \text{ h}^{-1}$; Fig 3.3a). Potential denitrification rates of sediment in reaches with grassy ($0.06 \pm$

0.02 $\mu\text{g N g DM}^{-1} \text{ h}^{-1}$) or woody ($0.03 \pm 0.01 \mu\text{g N g DM}^{-1} \text{ h}^{-1}$) riparian vegetation were lower than rates of sediment in the removal reaches ($0.10 \pm 0.02 \mu\text{g N g DM}^{-1} \text{ h}^{-1}$; Fig 3.3b).

Sediment was the only benthic compartment where rates (but not standing stocks) were expected to be impacted by riparian vegetation, and this was indeed the case. Rates in leaf packs, grass root wads, and filamentous algae were not affected by riparian vegetation, but standing stocks varied; grass root wads and filamentous algae were rarely found in reaches with woody riparian vegetation (A. Riley, personal communication). Significant differences in rates were seen among compartments ($p < 0.001$; Table 3.3), with filamentous algae ($0.49 \pm 0.09 \mu\text{g N g DM}^{-1} \text{ h}^{-1}$) and grass root wads ($0.44 \pm 0.07 \mu\text{g N g DM}^{-1} \text{ h}^{-1}$) exhibiting greater potential rates than leaf packs ($0.15 \pm 0.02 \mu\text{g N g}^{-1} \text{ DM h}^{-1}$) or sediment ($0.06 \pm 0.01 \mu\text{g N g DM}^{-1} \text{ h}^{-1}$; Fig 3.4).

DISCUSSION

Spatial and temporal variability of denitrification

Denitrification was highly variable temporally, as expected. In riparian soils, both potential and actual denitrification rates were at least three times higher in April 09 than any other sampling date. The majority of annual rainfall in tallgrass prairies occurs in the spring, providing a pulse of highly soluble NO_3^- and increasing anoxic microsites within the soil. Additionally, due to the physiology of the C_4 dominated plant community, plant activity is low during the early growing season, allowing increased access to NO_3^- for denitrifiers (Groffman et al. 1993). These factors, coupled with the warming temperatures allowing for increased microbial activity, explain the extremely high rates seen in April 2009. Rates in April 2010 were not as high as in the previous year, but they were generally greater than other months sampled, suggesting that the rates in April 2009 were 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 rates occurring in April and May (Groffman et al. 1993). This previous study, along with the current one, show minimal contribution of summer denitrification to annual denitrification flux, as denitrification is likely limited by either NO_3^- (ungrazed watersheds) or water (grazed watersheds) during summer months.

Riparian soil actual denitrification varied significantly among watersheds (Fig 3.2a), with the grazed watershed (N04D) having significantly greater rates overall than the ungrazed watershed (K2A). There was also a trend for the grazed watershed to have greater potential rates than the ungrazed watershed, but this trend was statistically insignificant (Fig 3.1a). Grazing can decrease N losses due to fire (Hobbs et al. 1991), and increase N cycling rates both directly via increasing N availability as urine or dung and indirectly by altering plant litter quantity and quality (Hobbs 1996). Grazing stimulated upland N cycling on KPBS by increasing net N-mineralization and nitrification at grazed sites compared to ungrazed sites (Johnson and Matchett 2001). Intensive grazing can increase potential denitrification in annually burned, grazed soils compared to annually burned, ungrazed soils (Groffman et al. 1993), and in intensively grazed upland soils compared to lightly grazed soils (Le Roux et al. 2003). Contrary to our results, grazing was previously thought to have minimal impact on potential denitrification in lowland soils (Le Roux et al. 2003). Increased N cycling rates induced by grazing may be responsible for the significant increase in denitrification rates at N04D.

We found temporal variability of benthic potential rates but no seasonality. A meta-analysis of denitrification studies from a variety of aquatic ecosystems found that rates in aquatic systems including, but not limited to streams, are generally greatest during the summer months, due to high water temperatures (Piña-Ochoa and Álvarez-Cobelas 2006), whereas a separate

study of 18 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 N04D than K2A, but there was no clear relationship between DEA and temperature, suggesting that something other than temperature, such as NO_3^- or C availability, controlled benthic DEA in the current study.

Woody vegetation removal stimulates riparian denitrification

The factors that promote denitrification (NO_3^- , labile C, and anoxia) were all present in higher amounts in the woody and removal riparian zones than the grassy riparian zones (Table 3.1). These different amounts of denitrification-promoting factors likely cause differences in actual and potential riparian soil denitrification. Removal of woody vegetation stimulated actual and potential soil denitrification rates compared to naturally grassy riparian zones and also led to higher rates of actual denitrification compared to woody riparian zones. Soil redox conditions are directly related to soil water content, and the removal of woody vegetation increased soil water content (Table 3.1). Soil C was also significantly affected by riparian vegetation, with woody vegetation, and its removal, increasing soil C, with woody vegetation increasing C inputs (as leaf litter), and the removal of woody vegetation likely enhancing root decomposition, causing increased dissolved C in the soil. The removal of woody vegetation increased both soil water content, a surrogate for anoxia, and soil C; alleviations of these two limiting factors of denitrification led to greater rates being seen in the removal riparian soils than the woody or grassy riparian soils.

Studies of woody vegetation in riparian areas are generally aimed towards the impact of removal or restoration of naturally occurring woody vegetation. This study is unique because we analyzed riparian woody vegetation as an unnatural condition. Restoration of woody riparian

zones can reduce stream water NO_3^- concentrations (Newbold et al. 2010), increase total N retention (Osborne and Kovacic 1993, Haycock and Pinay 1993), and increase uptake of nutrients by vegetation (Lyons et al. 2000). Encroachment of woody vegetation in prairie ecosystems appears to have the same effect on denitrification and nitrogen retention as these previous studies have indicated (Norris et al. 2007). The removal of encroaching woody vegetation could restore the nitrogen dynamics of these riparian zones to that of the natural state seen in the grassy riparian zones, and the stimulation of denitrification in the current study may only be a transient effect. Also, the presence of woody vegetation, and its subsequent removal, increased denitrification rates, but NO_3^- concentrations were lower in grassy riparian soils. This suggests that other mechanisms, such as nitrate assimilation by grasses and subsequent volatilization during fire events, may enhance overall nitrate storage and removal by grassy riparian zones compared to woody riparian zones.

Woody vegetation alters benthic denitrification

Riparian vegetation also affected benthic DEA, both directly, with removal of wood stimulating sediment denitrification, and indirectly, by altering the benthic compartments present in the system. Removal of woody vegetation from the riparian zone increased benthic sediment DEA above rates seen in sediment from reaches with either grassy or woody riparian zones (Fig 3.3b). Riparian soils in the removal reaches had higher TC and TN than natural riparian soils (Table 3.1). The removal of woody vegetation also increased the amount of FPOM and VFPOM (Vandermyde and Whiles unpublished data), which increases both the C content and potential for anoxic microsites in the benthos. Arango and Tank (2008) showed that sediment denitrification was higher in anthropogenically impacted streams, with a significant positive relationship between sediment C and denitrification. This relationship between sediment C and denitrification

holds true in sediment of various depths and sizes (Inwood et al. 2007) and across streams of low and high NO_3^- concentrations (Arango et al. 2007). The consistency of this relationship, coupled with the increased C content of woody removal riparian soils, suggests that increased sediment C could increase denitrification rates in sediment from reaches with riparian woody vegetation removed.

Riparian vegetation also had indirect impacts on benthic denitrification by altering the compartments present in the benthos. Filamentous algae and grass root wads expressed consistently greater DEA rates than leaf packs or sediments (Fig. 3.4), and were only found in reaches with open canopies (grassy or removal reaches). Potential denitrification rates are generally higher on periphyton than sediments (this study, Ishida et al. 2008), 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 benthos and reducing light inputs, which limits filamentous algal growth.

CONCLUSIONS

Soil denitrification in riparian zones of Konza Prairie is highly seasonal, with the vast majority of denitrification occurring in the early spring and minimal rates found throughout the remainder of the year. These patterns are similar to those published previously for uplands and hillslopes. Benthic denitrification is also temporally variable, but there is less seasonality, suggesting some factor other than water temperature is controlling benthic denitrification. Grazing by bison and changes in riparian vegetation both affected riparian soil and benthic

sediment denitrification; rates were higher in the grazed watershed and the removal of woody vegetation stimulated denitrification.

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 gallery forests upstream leads to increased riparian denitrification, but potentially reduced benthic denitrification due to an alteration of compartments present in the benthos. Removal of woody vegetation 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 stabile grassland community. Mechanisms likely for this increase include increased anoxia due to reduced evapotranspiration, increased labile C in the soil due to root decomposition, and less competition between plant and microbes for NO_3^- . Although denitrification rates were greater in woody and removal riparian soils than grassy soils, nitrate concentrations in grassy riparian soils were lower than other treatments, suggesting grasses may be better at overall nitrate retention/removal in tallgrass prairie riparian zones. We recommend that any benefits provided by increased denitrification rates in woody riparian zones are greatly outweighed by the possibility of losing one of North America's most endangered ecosystems.

TABLES AND FIGURES

Table 3.1 Mean (SE) values of soil parameters under different riparian vegetation types (Grass, Wood, Removal) or watersheds (K2A or N04D). Note: statistical tests were performed on log(x+1) transformed data.

		Apr 09	Jun 09	Jul 09	Oct 09	Mar 10	Apr 10
NO ₃ ⁻ -N (μg g ⁻¹)	Grass a ¹	3.75 (0.53) ABC ²	4.19 (0.51) BC	4.19 (0.53) C	2.51 (0.20) AB	1.84 (0.29) AB	1.18 (0.16) A
	Wood b	5.10 (0.55) ABC	4.78 (0.36) BC	5.40 (0.56) C	3.23 (0.25) AB	4.26 (0.56) AB	4.86 (0.56) A
	Removal c	6.28 (0.64) ABC	5.48 (0.37) BC	6.71 (0.62) C	5.95 (0.93) AB	5.77 (0.54) AB	6.02 (0.65) A
	K2A	5.55 (0.60) ABC	4.56 (0.37) BC	5.53 (0.52) C	3.59 (0.31) AB	4.55 (0.64) AB	4.99 (0.80) A
	N04D	4.53 (0.37) ABC	5.08 (0.32) BC	5.33 (0.48) C	4.20 (0.68) AB	3.54 (0.66) AB	3.05 (0.81) A
NH ₄ ⁺ -N (μg g ⁻¹)	Grass a	3.92 (0.49) D	1.20 (0.16) B	0.42 (0.04) A	- ³	2.41 (0.22) C	3.09 (0.30) C
	Wood b	3.31 (0.33) D	1.07 (0.12) B	0.48 (0.04) A	-	1.52 (0.22) C	1.63 (0.37) C
	Removal b	2.18 (0.25) D	1.16 (0.07) B	0.55 (0.04) A	-	2.60 (0.19) C	1.52 (0.40) C
	K2A * ⁴	3.44 (0.35) D	1.09 (0.11) B	0.53 (0.04) A	-	2.84 (0.95) C	2.21 (0.28) C
	N04D *	2.84 (0.29) D	1.19 (0.09) B	0.44 (0.03) A	-	1.55 (0.95) C	1.95 (0.28) C
TN (mg g ⁻¹)	Grass a	2.94 (0.09)	3.04 (0.07)	2.78 (0.09)	2.99 (0.09)	2.62 (0.08)	2.59 (0.09)
	Wood b	3.11 (0.10)	3.44 (0.09)	3.23 (0.06)	3.35 (0.09)	3.78 (0.17)	3.91 (0.21)
	Removal b	3.33 (0.13)	3.44 (0.16)	3.03 (0.14)	3.20 (1.53)	3.41 (0.19)	3.63 (0.25)
	K2A *	2.83 (0.06)	3.09 (0.09)	3.02 (0.08)	2.96 (0.09)	3.29 (0.13)	3.46 (0.17)
	N04D *	3.42 (0.09)	3.52 (0.09)	2.75 (0.09)	3.40 (0.10)	3.25 (0.16)	3.29 (0.18)

Table 3.1 Continued

		Apr 09	Jun 09	Jul 09	Oct 09	Mar 10	Apr 10
TC (mg g ⁻¹)	Grass a	35.73 (1.06)	36.33 (0.98)	34.42 (1.30)	36.36 (1.00)	32.19 (0.88)	32.06 (0.64)
	Wood b	36.58 (0.98)	39.66 (0.84)	39.10 (0.73)	38.93 (0.85)	42.76 (1.65)	44.58 (1.82)
	Removal c	40.70 (1.28)	42.45 (1.47)	41.78 (1.66)	41.40 (1.53)	41.11 (1.66)	43.14 (2.13)
	K2A	36.27 (1.02)	38.79 (1.14)	39.03 (1.05)	38.11 (1.10)	38.97 (1.30)	41.35 (1.87)
	N04D	39.07 (0.88)	40.16 (0.88)	32.17 (1.18)	39.69 (0.91)	38.40 (1.56)	38.50 (1.54)
C:N	Grass b	12.31 (0.49)	12.03 (0.43)	12.52 (0.56)	12.28 (0.39)	12.28 (0.15)	12.48 (0.30)
	Wood a	11.81 (0.16)	11.59 (0.13)	12.15 (0.20)	11.67 (0.14)	11.31 (0.19)	11.42 (0.34)
	Removal c	12.46 (0.56)	12.71 (0.71)	14.17 (0.77)	13.49 (0.19)	12.06 (0.23)	11.91 (0.37)
	K2A *	12.92 (0.46)	12.76 (0.53)	13.16 (0.51)	13.14 (0.58)	11.88 (0.15)	12.02 (0.15)
	N04D *	11.47 (0.10)	11.46 (0.13)	11.71 (0.15)	11.81 (0.24)	11.88 (0.15)	11.86 (0.26)
SWC (%)	Grass a	32.58 (0.50) C	30.31 (68) B	25.34 (1.30) A	29.84 (1.21) B	36.38 (1.05) D	32.24 (0.97) C
	Wood b	32.96 (0.58) C	31.39 (0.48) B	27.56 (0.72) A	29.59 (0.75) B	37.88 (0.84) D	36.13 (1.15) C
	Removal b	33.96 (0.52) C	31.64 (0.64) B	27.79 (0.84) A	30.43 (0.78) B	37.87 (0.75) D	34.51 (1.07) C
	K2A *	32.30 (0.41) C	32.62 (0.43) B	29.84 (0.62) A	31.83 (0.75) B	39.39 (0.53) D	37.82 (0.76) C
	N04D *	34.02 (0.43) C	29.61 (0.41) B	23.96 (0.62) A	28.07 (0.60) B	35.36 (0.65) D	30.77 (0.95)

¹Lower case letters following riparian vegetation treatments indicate significant differences among treatments (p=0.05)

²Capital letters following watersheds indicate significant differences among watersheds (p=0.05)

³Dashes denote data that were contaminated prior to analysis, and thus data is not available

Table 3.2 Mean (SE) stream water NO₃⁻-N and NH₄⁺-N concentrations among riparian vegetation treatments and watersheds across five sampling dates.

		Apr09	Jun09	Jul09	Oct09	Jan10
NO ₃ ⁻ -N (µg L ⁻¹)	Grass	4.21 (1.24)	8.34 (2.57)	15.90 (7.78)	12.38 (2.27)	4.88 (0.28)
	Wood	4.43 (0.78)	10.29 (4.20)	14.87 (7.46)	12.44 (4.73)	5.04 (1.10)
	Removal	3.32 (0.03)	11.48 (4.70)	13.72 (8.35)	6.71 (0.91)	29.84 (26.46)
	K2A	3.32 (0.19)	6.21 (0.30)	6.97 (0.83)	11.63 (2.86)	3.97 (0.35)
	N04D	4.65 (0.68)	13.86 (1.55)	22.69 (17.75)	9.38 (2.69)	22.53 (16.89)
NH ₄ ⁺ -N (µg L ⁻¹)	Grass	39.39 (20.00)	7.40 (0.19)	12.35 (5.49)	18.93 (2.01)	17.84 (1.00)
	Wood	13.57 (3.07)	8.42 (0.12)	12.45 (4.98)	18.53 (4.41)	18.06 (1.01)
	Removal	8.79 (0.71)	11.87 (1.67)	11.77 (6.20)	17.48 (2.29)	182.16 (165.32)
	K2A	25.99 (16.72)	9.68 (1.95)	6.63 (0.56)	21.22 (0.93)	16.91 (0.07)
	N04D	15.18 (2.95)	8.78 (0.76)	17.75 (0.16)	15.41 (0.81)	128.46 (109.51)

Table 3.3 ANOVA tables for riparian soil actual and potential denitrification, benthic sediment potential denitrification, and benthic compartment specific potential denitrification. WS=watershed, VEG=riparian vegetation type, BENT=benthic compartment.

	Factor	df	F	p
<i>Riparian soil actual denitrification</i>				
	MONTH	5	142.06	<0.001
	WS	1	3.36	0.068
	VEG	2	29.54	<0.001
	WS*VEG	2	6.11	0.0025
<i>Riparian soil potential denitrification</i>				
	MONTH	5	60.26	<0.0001
	WS	1	0.63	NS
	VEG	2	2.58	0.078
	WS*VEG	2	4.26	0.015
<i>Benthic sediment potential denitrification</i>				
	MONTH	4	85.133	<0.001
	WS	1	31.805	<0.001
	VEG	2	8.574	<0.001
	WS*VEG	2	7.907	0.001
<i>Benthic compartment potential denitrification</i>				
	MONTH	4	263.78	<0.001
	BENT	3	11.547	<0.001

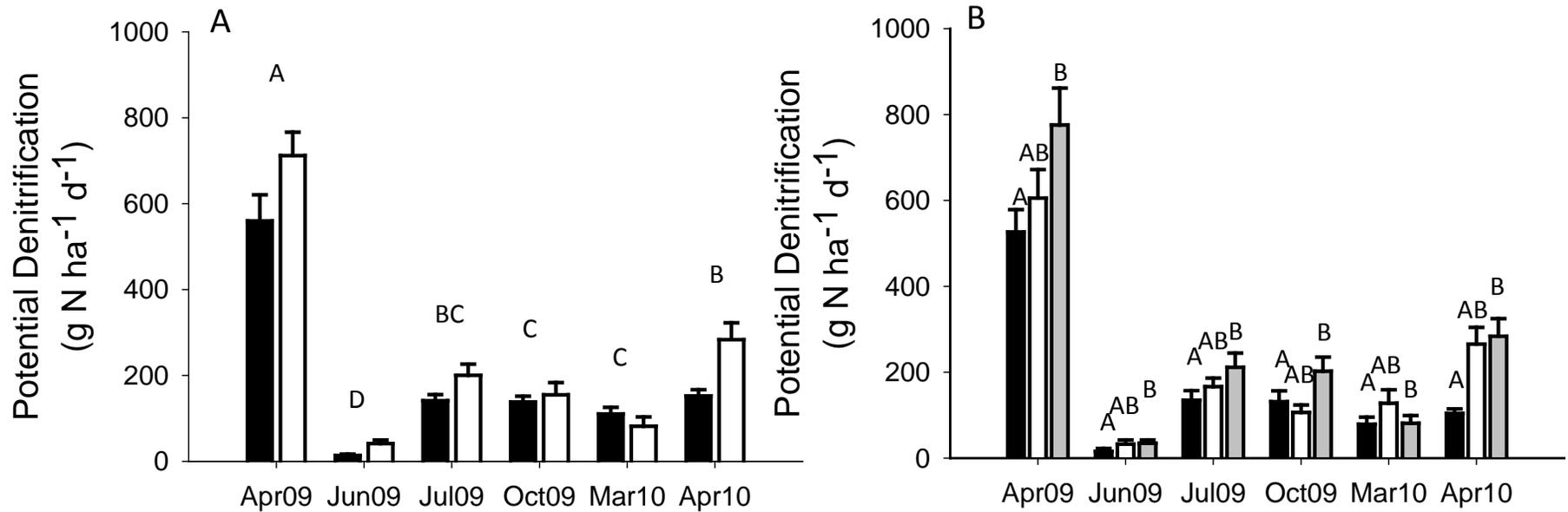


Figure 3.1 Riparian soil potential denitrification (as DEA) averaged over (A) two watersheds (black = K2A, white = N04D) for six sampling dates, and (B) three riparian vegetation treatments (black = grassy, white = woody, gray = wood removed) for six sampling dates. Letters above the bars indicate significant differences at the 0.05 level among (A) sampling date (using pooled vegetation data and watersheds) and (B) riparian vegetation (using pooled sampling dates and watersheds). Note: statistical tests were run on log(x+1) transformed data. Error bars are SE.

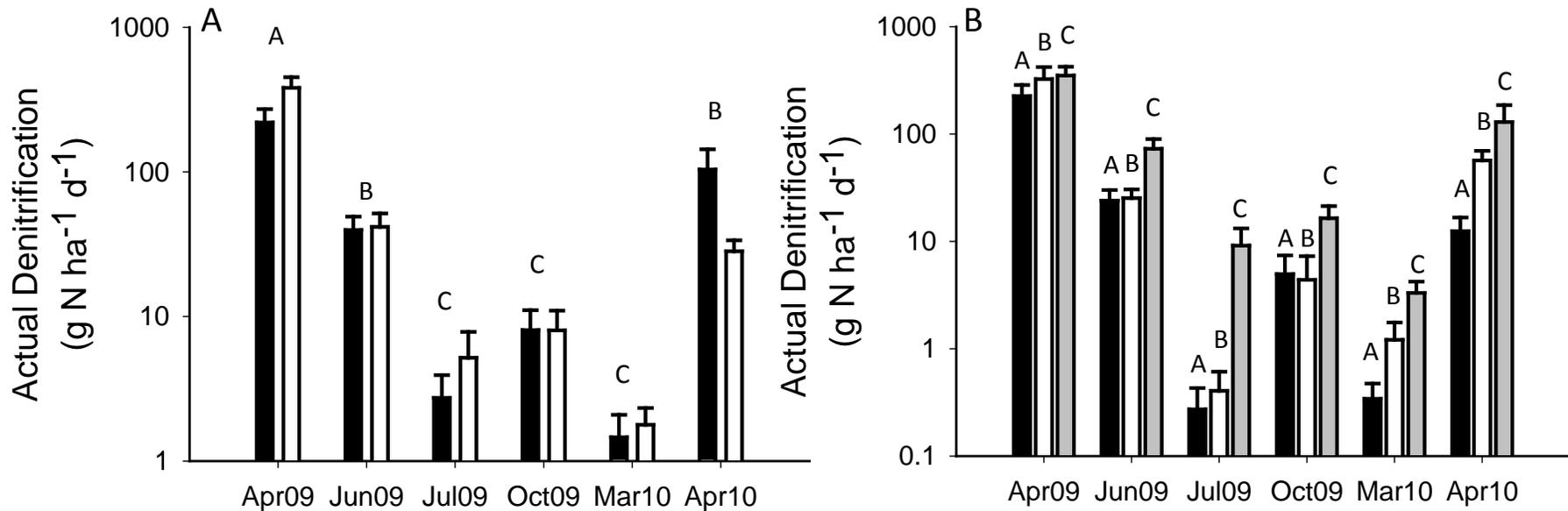


Figure 3.2 Riparian soil actual denitrification averaged over (A) two watersheds (black = K2A, white = N04D) for six sampling dates, and (B) three riparian vegetation treatments (black = grassy, white = woody, gray = wood removed) for six sampling dates. Letters above the bars indicate significant differences at the 0.05 level among (A) sampling date (using data pooled across watersheds and riparian vegetation types) and (B) riparian vegetation (using data pooled across sampling dates and watersheds). Note the logarithmic scale. Error bars are SE.

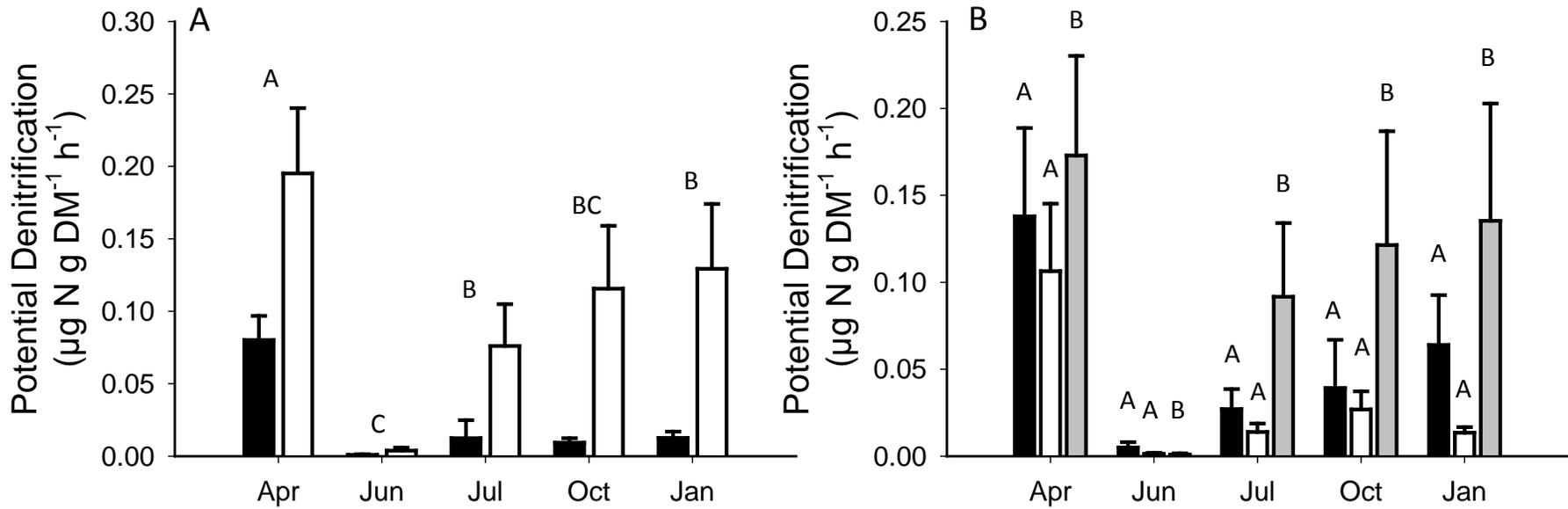


Figure 3.3 Mean sediment potential denitrification (as DEA) averaged over (A) two watersheds (black=K2A, white=N04D) for five sampling dates, and (B) three riparian vegetation types (black = grassy, white = woody, gray = wood removed) for five sampling dates. Letters above the bars indicate significant differences at the 0.05 level among (A) sampling date (using data pooled across watersheds and riparian vegetation types) and (B) riparian vegetation (using data pooled across sampling dates and watersheds). Note: statistical tests were run on $\log(x+1)$ transformed data. Error bars are SE.

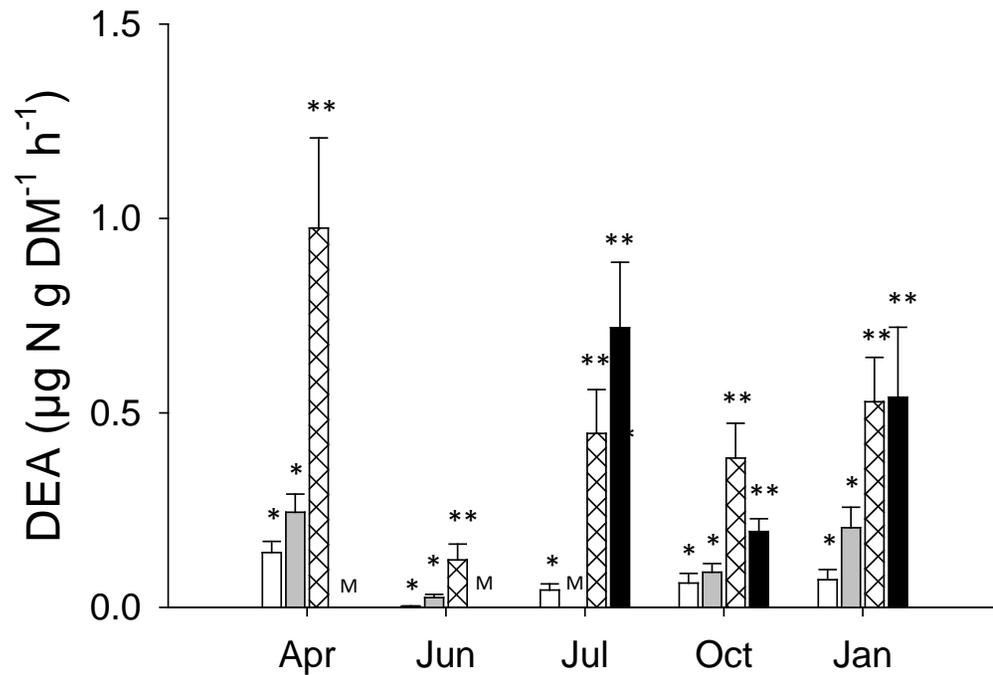


Figure 3.4 Benthic potential denitrification (as DEA) averaged over four benthic compartments (white = sediment, gray = leaf packs, hatched = grass root wads, black = filamentous algae) for five sampling dates. Asterisks above bars indicate significant differences at the 0.05 level among benthic compartments (using data pooled across sampling dates, riparian vegetation types, and watersheds). M indicates compartments that were not sampled during a specific sampling date.

CHAPTER 4 - Summary and Conclusions

Increased nitrogen loadings, primarily due to human activities, have multiple negative effects on aquatic ecosystems. Riparian and benthic zones of headwater streams process a large amount of nitrogen before it reaches downstream ecosystems, but the ability of these transition zones to process and retain nitrogen is threatened by altered watershed land use and biodiversity losses. This thesis analyzed how nitrogen processing, specifically denitrification, would be affected by altered riparian vegetation, and how a common algivorous minnow affects recovery trajectories of ecosystem structure and function (including denitrification) from a simulated flood.

The first chapter explored the role of a prairie headwater minnow in controlling ecosystem structural and functional recovery from a flood, and the potential for indirect effects of these fish caused by NH_4^+ excretion. Either *Campostoma anomalum* or NH_4^+ was added to large outdoor mesocosms, which simulated a natural headwater prairie stream. Ecosystem structure and function was then assessed for four weeks following a simulated flood. Fish altered the structure of the ecosystem by reducing algal filament lengths and altering particulate organic matter size. Ammonium enrichment altered the limiting factors of denitrification, reducing N limitation while increasing C limitation. Temporal decreases in denitrification were apparently caused by increasing dissolved oxygen (DO) in the hyporheic zone of these mesocosms, inhibiting the production of new denitrification enzymes. However, hyporheic DO was not affected by either the fish or NH_4^+ treatments. Algal biomass accrual over the study period was affected by both the presence of fish and NH_4^+ amendments, with algal biomass reaching equilibrium within two weeks, compared to four weeks in control streams. Results from this study suggest that macroconsumers increase the short-term recovery of the algal community of

prairie streams via mineralization of N, but the final state of the ecosystem is independent of macroconsumers.

The second chapter considered the impact of native woody vegetation expansion on riparian and benthic denitrification rates. Actual and potential rates of denitrification were measured in riparian soils at two sites (one grazed, one ungrazed) among three vegetation types (grassy, woody, and woody vegetation removed in the winter of 2007) at each site; potential denitrification was also measured for all biotic compartments found along the bottom of the stream at each of these sites (i.e., sediment, leaf packs, grass root wads, and filamentous algae). The removal of woody vegetation stimulated riparian soil denitrification rates and potential denitrification rates of benthic sediment. Riparian vegetation indirectly affected benthic potential denitrification by altering the compartments present in the benthos, which had significantly different potential rates. Grass root wads and filamentous algae, which were only present in reaches with open canopies (grassy and wood-removal riparian vegetation), had significantly higher rates than sediment (found in all reaches) and leaf packs (more abundant in reaches with woody riparian vegetation). Denitrification rates were also highly variable temporally; a strong seasonal effect was seen in riparian soil denitrification, with greatest rates found in the early spring, whereas benthic denitrification did not exhibit a seasonal effect, although it was also highly variable temporally, suggesting that benthic denitrification is driven by something other than temperature (e.g., NO_3^- or carbon supply). Removal of woody vegetation stimulated both riparian soil and benthic denitrification rates, but this effect is likely only temporary as the riparian vegetation returns to a grass-dominated vegetative community.

The two studies presented in this thesis showed that (1) the presence of a grazing minnow alters the short-term recovery trajectories of prairie stream mesocosms and affects denitrification,

and (2) woody vegetation expansion alters riparian and benthic denitrification, but additional studies are needed to draw broader conclusions. Alterations of the overall consumer community (not just one species of fish) on benthic denitrification should be analyzed using both mesocosm experiments to provide easily manipulated communities, and consumer exclosures in natural prairie streams. Whereas the presence of one fish species alters ecosystem recovery, the presence of a full suite of consumers may create more interactive effects. The effects of woody vegetation expansion on other aspects of stream ecosystem structure and function are currently being analyzed by other projects (Vandermyde and Whiles unpublished data, Riley and Dodds unpublished data), but these studies are not assessing biogeochemical changes due to woody expansion. More quantitative studies of stream N processing need to be performed to determine whether the semi-quantitative changes in benthic denitrification are confirmed (e.g., a ^{15}N release to measure nutrient spiraling, nitrification, and denitrification). Additionally, both studies of this thesis were performed in a pristine tallgrass prairie, but the effects of woody encroachment and macroconsumers may differ in more impacted (i.e., agricultural, urban, more N-rich) watersheds.

Proper management of prairie ecosystems requires managers to make decisions based upon a multiple trade-offs. For instance, woody expansion is one of the primary threats to the remaining tallgrass prairie ecosystems, but increasing N deposition (and multiple other anthropogenic stressors) also imperils these prairies. If managers are attempting to increase N retention of riparian zones, encouraging woody vegetation expansion may be an appropriate choice due to greater denitrification rates seen in woody riparian zones. However, increasing woody expansion threatens to permanently and completely remove the remaining tallgrass prairies of North America; therefore, any benefits of woody vegetation in terms of N retention are overshadowed by the possible extinction of a unique ecosystem and I recommend removal

and inhibition of woody vegetation expansion are of primary managerial importance to preserve tallgrass prairies.

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