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Direct and indirect effects of central stoneroller (*Campostoma anomalum*) on mesocosm recovery following a flood: can macroconsumers affect denitrification?

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Abstract. Anthropogenic N loadings and perturbations of macroconsumer communities impair ecological and economic services provided by streams. Organisms are adapted to natural disturbances, such as flooding and desiccation, but how anthropogenic and natural disturbances interact is poorly understood. We used large outdoor mesocosms to study the effect 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, highlighting the potential for *Campostoma* (and other macroconsumers) to affect denitrification. *Campostoma* and NH_4^+ treatments differentially affected particulate organic matter size and filamentous algal structure. Ecosystem structure responded differently to mesocosm treatment over time, a result suggesting that grazers or NH_4^+-N availability may be especially important during early recovery periods. The presence of *Campostoma* did not influence denitrification, but NH_4^+ additions altered the response of denitrifiers to nutrient and energy amendments, and denitrification rates decreased following the recovery of mesocosms. Temporal changes in denitrification probably were caused by increasing hyporheic dissolved O_2 concentrations, which led to potentially fewer anoxic microsites for production of denitrification enzymes. Our study shows that grazers affect the recovery of ecosystem structure, but denitrification in the context of these prairie-stream mesocosms appears to be unaffected by *Campostoma*.

Key words: *Campostoma*, grazer, prairie streams, flood, recovery, denitrification, ecosystem function, mesocosm.

Increased N loadings into aquatic ecosystems, primarily from anthropogenic activities, will continue as the global human population expands (Vitousek et al. 1997, Galloway et al. 2004) and will cause deleterious ecological and economic effects (Carpenter et al. 1998, Dodds et al. 2009) on stream ecosystems. Headwater-stream N cycling has received increased attention in recent years because of 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). Denitrification is the predominant long-term form of N removal from terrestrial or aquatic ecosystems. The high biological activity associated with the benthic zone of headwater streams, increased sediment–water contact in these shallow streams (Peterson et al. 2001), and the high proportion of water and N originating from headwaters (Alexander et al. 2007) make headwater streams important natural filters for processing increased N loadings.

Fishes affect ecosystem structure, but stream ecologists have only recently established that fishes also can affect stream ecosystem function (McIntyre et al. 2008, Knoll et al. 2009, Vanni 2010). In many studies of the effects of consumers (e.g., grazing fishes) on ecosystems, only structural but not function were measured (i.e., relative biomass, not material and energy fluxes; e.g., Cardinale et al. 2006 used biomass as the response variable and did not take productivity into account in a meta-analysis), even though multiple pathways exist for consumers to influence both structure *and* function. Grazing fishes

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have the potential to affect stream N removal, an important ecosystem function, by increasing N supply (Hillebrand et al. 2002), altering algal nutrient demand (Flecker et al. 2002), and reducing particulate organic matter (POM) size (Bertrand and Gido 2007). Reduced particle size directly increased denitrification in aquifer microcosms (Dodds et al. 1996b). All of these mechanisms can affect denitrification, and therefore, stream N removal, but no empirical studies have been done on the effect of fishes on N loss via denitrification in streams. Any effects of grazing fishes on denitrification are increasingly important because of the rising pressure on aquatic ecosystems caused by anthropogenic stressors, such as N pollution and decreasing biodiversity (Galloway et al. 2004, Dudgeon et al. 2006).

Specific biogeochemical processes rarely are linked mechanistically to fishes (but see Persson and Svensson 2006). Examination of the relationship between freshwater consumers, increasing N levels, and biogeochemical processes is needed to establish the influence of consumers on fundamental ecosystem processes, an effect often claimed, but rarely demonstrated (Wetzel 2001). Recycling of nutrients by fishes (e.g., McIntyre et al. 2007, 2008, Knoll et al. 2009) and other aquatic consumers (e.g., gastropods: Liess and Hillebrand 2006, mussels: Bracken 2004) can influence ecosystem function. For example, excretion by shrimps in Puerto Rican streams accounted for up to 20% of total stream NH_4^+-N uptake (Benstead et al. 2010). These specific studies provide insight into the general effects of higher-trophic-level organisms on ecosystem processes, but the number of empirical studies is small, and the roles of consumers affecting ecosystem function are only beginning to be understood (Vanni 2010).

Grazers can influence primary producers directly (via consumption) and indirectly (via nutrient recycling). The influence of grazers on primary producer biomass is well studied, but measurements of ecosystem function are lacking (see Hillebrand 2002 for a meta-analysis). Previous investigators have not separated the relative importance of grazer effects caused by consumption vs nutrient recycling (but see Knoll et al. 2009). Both pathways can elicit responses in algal biomass and stoichiometry. Consumption negatively affects producers, whereas nutrient recycling elicits a positive response (Knoll et al. 2009). Grazers can increase nutrient supply to (Hillebrand and Kahlert 2001) and influence nutrient demand of (Flecker et al. 2002) primary producers. Grazer stoichiometry also influences the relative effect of N remineralization on producers (Knoll et al. 2009), highlighting the importance of species-specific studies.

Much of the research on the effects of grazers on stream ecosystem structure and function has been done in prairie streams. Algivorous minnows—a ubiquitous fish guild in midwestern USA prairie streams—strongly influence ecosystem structure, whereas effects on ecosystem function are more variable (Gido et al. 2010). The most consistent effect of grazing minnows in prairie streams has been to reduce the length of algal filaments in streams (Power et al. 1985, Gelwick and Matthews 1992, Bertrand and Gido 2007) and mesocosms (Bertrand and Gido 2007, Bengtson et al. 2008). Grazing minnows also can reduce algal biomass and decrease POM size (Gelwick and Matthews 1992, Bertrand and Gido 2007, Bengtson et al. 2008).

Evidence that macroconsumers alter prairie stream ecosystem functions is more variable. Gelwick and Matthews (1992) found that grazing by central stonerollers (*Campostoma anomalum*) increased net primary productivity in natural pools, and Murdock et al. (2010) found that exclusion of macroconsumers (fishes, crayfish, and tadpoles) in a natural stream led to significantly higher community respiration (CR) and NH_4^+-N uptake. Investigators using southern redbelly dace (*Phoxinus erythrogaster*), a functionally similar species to *C. anomalum*, found no change in net or gross primary productivity (GPP) in either experimental mesocosms or natural systems (Bertrand and Gido 2007, Bengtson et al. 2008).

The influence of grazing fishes is especially important in intermittent headwater prairie streams where natural abiotic disturbances, such as flooding and desiccation, cause these streams to be in an almost permanent state of succession (Dodds et al. 2004). Grazers influence algal community composition and standing crop during succession in desert streams, but the degree of effect depends on the grazing species (Peterson and Grimm 1992). In Kings Creek, a highly studied prairie stream, return times of disturbance are short, and $>⅓$ of the scouring floods occur within 12 d of each other (Bertrand et al. 2009). Recovery of the algal community in these prairie streams also occurs rapidly. Algal biomass returns to preflood levels within 3 wk of a scouring flood event (Dodds et al. 1996a). Nutrients and primary production strongly affect recovery of these streams (Dodds et al. 1996a), but recovery trajectories also are influenced by macroconsumers (Bertrand et al. 2009, Murdock et al. 2010). Short-term recovery of the algal community is especially influenced by macroconsumers, whereas the final state of an ecosystem is less dependent on the presence of macroconsumers (Murdock et al. 2010). However, relatively little is known about the effect of macroconsumers on

biogeochemical processes, specifically denitrification, over short-term recovery periods.

We designed an experiment to study the effects of *C. anomalum* on ecosystem structure and function in experimental mesocosms following a simulated flood. We analyzed the effects of grazing on denitrification in addition to previously studied measures of structure and function (see Bertrand and Gido 2007, Murdock et al. 2010). *Campostoma* and NH_4^+ were added to separate experimental streams to determine if effects seen in fish treatments were caused by NH_4^+ remineralization or by other grazing effects (e.g., ingestion and physical disturbance). We hypothesized that *C. anomalum* would change ecosystem structure by reducing algal filament length and altering POM size and that NH_4^+ additions would alter both ecosystem structure (i.e., increased algal filament length and biomass) and function (i.e., increased GPP, community respiration [CR], and benthic denitrification). We predicted that increased N availability from excretion by *C. anomalum* 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 (KBPS), ~10 km southeast of Manhattan, Kansas (USA), were used to test the effects of *C. anomalum* and NH_4^+ additions on ecosystem structure and function. Each mesocosm consisted of a 2.54-m^2 circular pool (mean depth = 0.5 m) downstream of a 0.84-m^2 rectangular riffle (mean depth = 0.15 m) (see Matthews et al. 2006 for details of mesocosm design). Polyvinyl chloride (PVC) tubes (1 m × 2.5 cm inner diameter) were placed vertically in the substratum of each pool with the open bottom of the tube ~0.5 m below the surface of the substratum to measure hyporheic dissolved O_2 (DO). Water was supplied to each mesocosm from a groundwater spring similar to those that feed the natural prairie streams on KPBS (input rate ≈ 1.0 L/min) and was recirculated by electric trolling motors at a constant rate of ~10 L/s to simulate natural currents. Benthic substrata were the same as natural substrata in the region and were a mixture of cobble, pebble, gravel, and fine sediment collected from a local quarry.

Mesocosms were filled 1 wk before initiation of each experiment to allow colonization by algal and macroinvertebrate communities. After this colonization period, mesocosm substrata and walls were scoured with a pressure sprayer filled with groundwater. The mesocosms were then drained and immediately refilled. Scouring removed most organic matter accumulated over the 1-wk colonization period, homogenized biomass of algal and macroinvertebrate communities across mesocosms, and had effects similar to scouring floods in nearby streams (Bertrand et al. 2009). After scouring, plastic-mesh baskets (5.5 × 10 × 10 cm, 2 × 1.25-cm mesh) were filled with dry rocks and placed flush with the mesocosm bottom for later measures of ecosystem structure and function. Twelve baskets were randomly placed in each mesocosm pool, and 6 baskets were placed in each riffle.

Within 24 h of scouring, 10 mesocosms were randomly selected to receive fish, 4 were selected for NH_4^+ additions, and 5 were designated controls. *Campostoma* were added at 2 different densities (approximately natural field density and 2× natural densities; Franssen et al. 2006). No differences were seen in any response variables between the 2 treatments (data not shown), therefore all streams receiving *Campostoma* additions were designated as a fish treatment (FISH; $n = 10$). Different levels of NH_4^+-N were added to each stream (target enrichment levels = 4, 8, 16, and 32× background NH_4^+) continuously with a peristaltic pump. Amendments were a combination of NH_4Cl and 0.1% HCl and were held in 20-L buckets. Hydrochloric acid was included in the amendments to inhibit growth and nitrification in the bucket. This HCl addition had a negligible effect on pH within the mesocosms because of high dilution, high-alkalinity spring water, and the $CaCO_3$ composition of the benthic substrata. The 8× NH_4^+ enrichment rate was approximately the same as the calculated N excretion rates for average *Campostoma* treatment density (AJR, unpublished data). Because of similar responses across the NH_4^+-enriched streams (no significant regressions were found using enrichment level as a predictor variable; data not shown) the 4 NH_4^+ enrichments were grouped into a single treatment (NH4+). Definitive statements cannot be made regarding the effect of varying levels of NH_4^+ enrichment (because of lack of replication), but effects of NH_4^+-amended vs unamended streams can be tested statistically (i.e., NH_4^+ is a categorical variable in subsequent analyses).

Ecosystem structure

Variables related to ecosystem structure were measured on days 14 (week 2) and 27 (week 4) of the experiment. Before disturbing the streams, water samples were collected in 60-mL acid-washed bottles to measure [NH_4^+] and [$NO_2^- + NO_3^-$]. Samples were placed on ice immediately after collection and stored frozen until analysis. Analyses were done on an OI

Analytical Flow Solution IV autoanalyzer (OI Analytical, College Station, Texas) using the indophenol blue method to measure [NH_4^+] and the Cd-reduction method to measure [NO_2^- + NO_3^-] (APHA 1998). Hyporheic DO was measured every hour from predawn to solar noon on days 14 (week 2) and 31 (week 4) of the experiment (to coincide with ecosystem metabolism measurements). A handheld DO probe (YSI Model 550-A; Yellow Springs Instruments, Yellow Springs, Ohio) was submerged as deeply as possible in the PVC tubes installed at the beginning of the experiment and allowed to equilibrate for 2 min before measuring hyporheic DO.

Algal filament lengths were measured with a meter stick in each stream along 3 transects in the riffle and 3 transects in the pool. The longest filament growing on a pebble intersected by a transect was measured. Three filaments were measured per transect to give 9 measurements in the riffle and 9 in the pool of each mesocosm (Bertrand and Gido 2007, Bengtson et al. 2008, Murdock et al. 2010). Maximum filament length was used to determine the greatest attainable biomass under the treatment conditions while minimally disturbing the benthic community and to remain consistent with earlier studies (Bertrand and Gido 2007). Mean filament length was calculated for each stream pool and riffle separately (i.e., the mean longest filament in a riffle or pool was the response variable). Total algal biomass (as chlorophyll *a* [chl *a*]) was measured from 3 haphazardly selected pebbles in riffles and 5 in 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), which allowed expression of algal biomass on an areal basis (i.e., chl *a* was based on rock area in contact with the water column, not total surface area of each rock).

POM was sampled by randomly collecting 2 plastic-mesh baskets from the pool and 1 from the riffle. Baskets were removed as gently as possible from the stream, and substrata from the baskets were emptied 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 subsample of this slurry was collected for analysis of POM and preserved with formalin. This subsample was separated into 3 POM size classes by running the slurry through a series of filters ($\geq$516 μm = coarse POM [CPOM], 98–515 μm = medium POM [MPOM], 0.7–98 μm = fine POM [FPOM]). Each size fraction was then dried (60°C, $\geq$48 h), weighed, combusted in a muffle furnace (470°C, 6 h), weighed, rewetted (to return water stored in clays lost from volatilization), dried (60°C, $\geq$48 h) and weighed to determine ash-free dry mass (AFDM) of each size fraction. POM size classes were expressed as a proportional amount by calculating the ratio of a specific size class (e.g., CPOM) to total POM (FPOM + MPOM + CPOM).

Ecosystem function

Ecosystem function variables were measured on days 14 (week 2) and 31 (week 4) of the experiment (different from ecosystem structure measurements because skies were overcast on day 27). Whole-stream metabolism was measured using an open-system, single-station approach. A handheld DO probe (YSI Model 550-A) was used to measure DO at the downstream end of each riffle approximately every h from predawn to solar noon (Bengtson et al. 2008). Night-time DO consumption was assumed to be constant. Therefore, O_2 consumption between the predawn measurement and the dawn measurement (before producers began photosynthesizing) was used to calculate respiration for each stream. Turbulence-induced aeration was assumed to be similar across all streams because stream morphology, inflow, and recirculation rates were similar. The reaeration coefficient (k = 0.432/d) used to model whole-stream metabolism was measured previously in these mesocosms (Murdock et al. 2010). Measurements of 24-h diurnal DO trends taken with logging sensors verified that measurements made from predawn to solar noon led to very similar estimates of whole-mesocosm metabolism (data not shown). Algal biomass accrual (μg chl *a* cm^{-2} d^{-1}; rate of recovery of algal biomass) was calculated as the temporal change in algal biomass (i.e., for the 2nd sampling date, accrual was calculated by subtracting algal biomass on the 1st sampling date from that on the 2nd sampling date) and scaled on a daily basis.

Benthic denitrification was measured on samples from 4 mesh baskets from each mesocosm pool on each date. Substrata from each basket (a mixture of rocks initially placed in the baskets, and silts and organic matter deposited during the in-stream incubation) were randomly assigned to a specific amendment (i.e., each amendment was done on a subsample of an individual basket; 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 1 of 4 amendments to assess NO_3^- and 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 performing 3 cycles of 3-min evacuation to a 700-mm-Hg vacuum and 1-min flushing with N_2 gas. Once anoxia was induced, 20 mL of CaC_2-generated acetylene (~10% of the headspace volume) were added to the jars. Samples were 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, New Jersey) until analysis by gas chromatography (within 48 h). Bunsen coefficient corrections were used to account for N_2O dissolved in solution, and total N_2O produced in the jars was subsequently calculated.

Statistical analysis

Blocked repeated-measures analysis of variance (rmANOVA) was used to test the effects of *C. anomalum* and NH_4^+ additions on ecosystem structure and function. Visual differences among experimental stream rows, which differed in antecedent conditions, were observed throughout the experiment. Therefore, the rmANOVA was blocked by stream row. NH_4^+, NO_3^-, hyporheic DO, algal filament lengths, algal biomass accrual (chl *a*), and whole-stream metabolism measures were the response variables, individual streams were the repeated measures, and stream treatment (i.e., control [CONT], NH4+, or FISH) was the explanatory variable. For dependent variables collected separately from riffle and pool habitats (filament lengths, algal biomass, algal accrual), separate analyses were done for each habitat type. Significant time × treatment interactions were assumed to indicate that treatments differentially affected the recovery of the response variable. If no significant interaction term was found, significant temporal effects were assumed to represent recovery of the ecosystem affecting the response variable regardless of treatment. Significant treatment effects in the absence of a significant interaction indicated that FISH or NH4+ affected the response variable regardless of the recovery state of the mesocosms.

Because POM size classes were proportional amounts of total POM, multivariate analysis of variance (MANOVA) with Wilks' lambda as the test statistic was used to determine the effect of treatment and time (explanatory variables), blocked by stream row, on proportional size classes of POM. Significant factors identified by repeated-measures ANOVA or MANOVA were analyzed using Tukey's Honestly Significant Difference (HSD) test.

A blocked, 2-way rmANOVA was used to determine the effects of FISH and NH4+ on denitrification and its response to various amendments. Stream row was the block, denitrification rate was the response, and treatment and amendment (−N−C, −N+C, +N−C, or +N+C) were the explanatory factors. Kendall's τ was used to identify relationships between −N−C denitrification rates and POM size classes. Denitrification data did not meet the assumption of normality and were logarithmically transformed prior to analysis. All other data met ANOVA assumptions. All statistical analyses were performed in SPSS (version 19.0; SPSS, Chicago, Illinois).

Results

Ecosystem structure

Water chemistry.—Significant time × treatment interactions were seen for both NH_4^+-N (rmANOVA, $p < 0.001$) and NO_3^--N (rmANOVA, $p = 0.028$), suggesting NH_4^+-N and NO_3^--N responded differently to treatments over time (Tables 1, 2). *Campostoma* did not significantly affect inorganic N in the water column. Differences in NH_4^+-N were seen only in the NH4+ mesocosms (Table 1). Similar to inorganic N, the relationship between hyporheic DO and mesocosm treatment differed between the 2 sampling dates as indicated by a significant interaction between time and treatment (rmANOVA, $p = 0.034$; Table 2). NH4+ mesocosms had the lowest DO on the 1st sampling date and the highest DO on the 2nd date (Table 1). Hyporheic DO after 2 wk was less than DO after 4 wk across all treatments (Table 1).

Filament length and algal biomass.—The response of filament lengths to treatments differed over time. A significant interaction term was observed for riffle filaments (rmANOVA, $p = 0.025$), and a marginally significant interaction was observed for pool filaments (rmANOVA, $p = 0.078$; Table 2). Filaments in riffles and pools decreased in FISH mesocosms and increased in NH4+ mesocosms from the 1st to the 2nd sampling date (Fig. 1A, B). A marginally significant treatment effect was observed for pool filaments (rmANOVA, $p = 0.065$). Maximum filament lengths were greater in pools than riffles (paired *t*-test, $p < 0.001$; Fig. 1A, B), and filaments in pools were shorter in FISH mesocosms than in CONT or NH4+ mesocosms (Tukey's HSD, $\alpha = 0.1$; Fig. 1B). Algal biomass accrual (mea-

TABLE 1. Mean (SE) inorganic N concentrations, total particulate organic matter, algal biomass, and hyporheic dissolved O_2 from 3 treatments (CONT = control, FISH = fish, NH4+ = NH_4^+-amended streams) across 2 sampling dates (weeks 2 and 4). * indicates differences between sampling dates ($p < 0.05$). Values with the same lower-case letters are not significantly different ($p > 0.10$) among treatments.

	CONT		FISH		NH4+	
Variable	2	4	2	4	2	4
NH_4^+-N* (μg/L)	10.90 (0.84)[a]	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)	22.12 (6.39)	13.87 (6.63)	48.32 (21.91)	30.87 (8.75)	247.99 (161.01)	56.07 (42.42)
Riffle algal biomass* (μg chl *a*/cm^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 (μg chl *a*/cm^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 dissolved O_2* (mg/L)	3.08 (0.28)	5.11 (0.09)	2.96 (0.11)	5.14 (0.15)	2.26 (0.40)	5.78 (0.60)

sured as chl *a*) was not significantly affected by treatment, time, or an interaction between these 2 factors ($p > 0.05$ for all cases; Table 2), but generally was greater in pools (11.59 ± 0.96 μg/cm^2) than riffles (9.26 ± 0.64 μg/cm^2; Table 1).

POM size fractions.—Proportional POM size classes were significantly affected by treatment (MANOVA, $p = 0.015$), but did not differ over time (MANOVA, $p = 0.126$; Fig. 2A, B). Tests of between-subjects effects revealed that proportional FPOM did not differ among treatments (MANOVA, $p > 0.1$), whereas proportional MPOM and CPOM did (MANOVA, $p = 0.013$ and 0.004, respectively). Proportions of different size classes of organic matter were similar between CONT and FISH and CONT and NH4+ mesocosms, whereas proportional MPOM was greater in FISH than in NH4+ mesocosms and proportional CPOM was

TABLE 2. Results of repeated-measures analysis of variance of ecosystem structural and functional responses in study mesocosms. Boldface indicates significant ($p < 0.05$) effect.

Response	Factor	df	*F*	*p*
NH_4^+-N	Time	1,13	101.18	**<0.001**
	Treatment	2,13	30.32	**<0.001**
	Time × treatment	2,13	59.91	**<0.001**
NO_3^--N	Time	1,13	7.53	**0.017**
	Treatment	2,13	3.14	0.077
	Time × treatment	2,13	4.79	**0.028**
Hyporheic dissolved O_2	Time	1,13	137.03	**<0.001**
	Treatment	2,13	0.05	0.952
	Time × treatment	2,13	4.41	**0.034**
Riffle filament length	Time	1,13	1.92	0.190
	Treatment	2,13	1.78	0.209
	Time × treatment	2,13	5.0	**0.025**
Pool filament length	Time	1,13	1.20	0.294
	Treatment	2,13	3.40	0.065
	Time × treatment	2,13	3.12	0.078
Riffle chlorophyll *a*	Time	1,13	2.41	0.145
	Treatment	2,13	1.21	0.328
	Time × treatment	2,13	1.24	0.320
Pool chlorophyll *a*	Time	1,13	0.02	0.882
	Treatment	2,13	1.12	0.355
	Time × treatment	2,13	0.41	0.669
Gross primary production	Time	1,13	0.07	0.801
	Treatment	2,13	2.92	0.090
	Time × treatment	2,13	4.58	**0.031**
Community respiration	Time	1,13	0.28	0.605
	Treatment	2,13	3.44	**0.023**
	Time × treatment	2,13	2.05	0.168

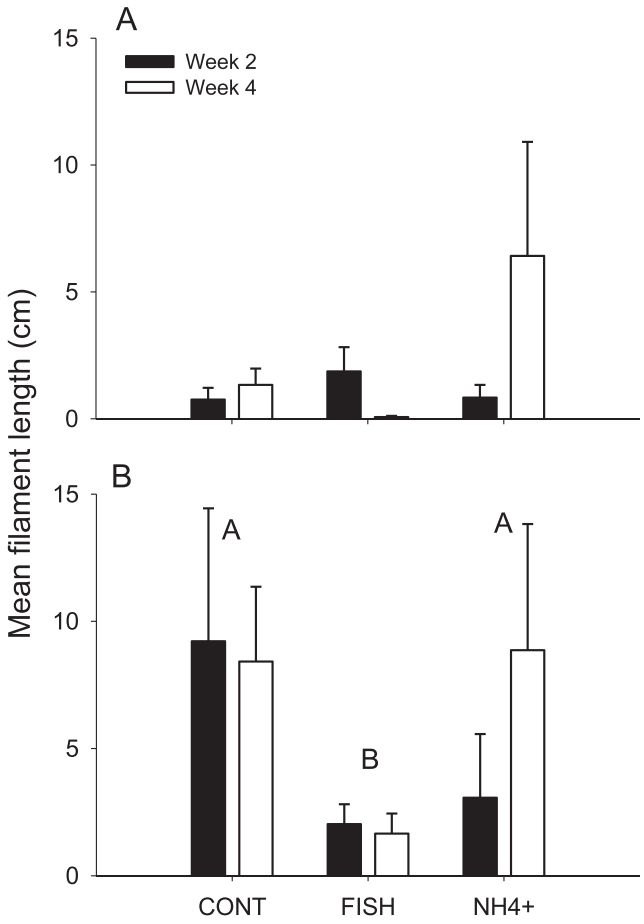

FIG. 1. Mean (±1 SE) filament lengths in experimental riffles (A) and pools (B) from control (CONT; $n = 5$), *Campostoma* (FISH; $n = 10$), and NH_4^+-N (NH4+; $n = 4$) treatments on weeks 2 and 4. Treatment bars with the same letters are not significantly different ($p > 0.1$).

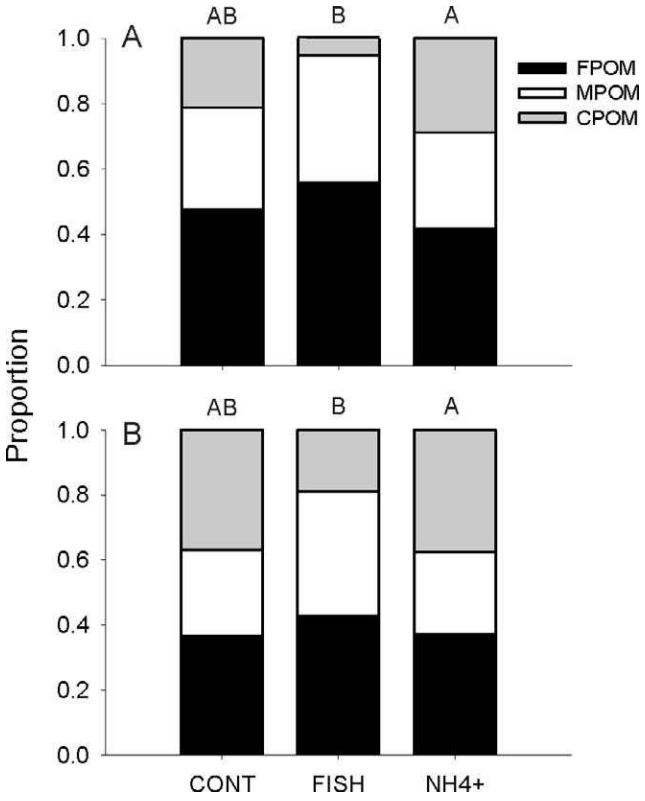

FIG. 2. Proportional amounts of fine particulate organic matter (FPOM), medium particulate organic matter (MPOM), and coarse particulate organic matter (CPOM) in control (CONT), *Campostoma* (FISH), and NH_4^+-N (NH4+) treatments collected on weeks 2 (A) and 4 (B) of the experiment. Data from the 2 sample dates were analyzed together, but are presented separately for ease of interpretation. Treatment bars with the same letters do not have significantly different POM size-class structure.

lower in FISH than in NH4+ mesocosms (Tukey's HSD; Fig. 2A, B). The proportion of smaller particles was greater in FISH than in NH4+ mesocosms (Tukey's HSD).

Ecosystem function

Denitrification.—The response of denitrifiers to various amendments differed temporally (rmANOVA, time × amendment interaction, $p = 0.017$). No significant treatment, treatment × time, or treatment × amendment effects were found (Table 3). Rates were lower in −N−C than in +N−C and +N+C amendments, but did not differ from rates in −N+C ammendments (Tukey's HSD, $\alpha = 0.05$; Fig. 3A–C). Because of the significant time × amendment interaction, a separate rmANOVA was run for each amendment to determine those that differed temporally. The response of denitrification to −N+C and +N+C amendments differed significantly between the 2 sampling dates ($p = 0.036$, $p = 0.001$, respectively), but rates did not differ between dates in −N−C and +N−C amendments (both $p > 0.05$; Fig. 3A–C, Table 3). Kendall's τ tests revealed no significant

TABLE 3. Results of 2-way repeated-measures analysis of variance of denitrification rates from 3 treatments (control, *Campostoma*, NH_4^+) and 4 nutrient amendments (−N−C, +N−C, −N+C, +N+C). Boldface indicates significant ($p < 0.05$) effect.

Effect	df	*F*	*p*
Time	1,61	13.033	**0.001**
Treatment	2,61	0.52	0.768
Amendment	3,61	6.318	**0.001**
Time × treatment	2,61	1.372	0.261
Time × amendment	3,61	3.678	**0.017**
Time × treatment × amendment	6,61	1.05	0.403

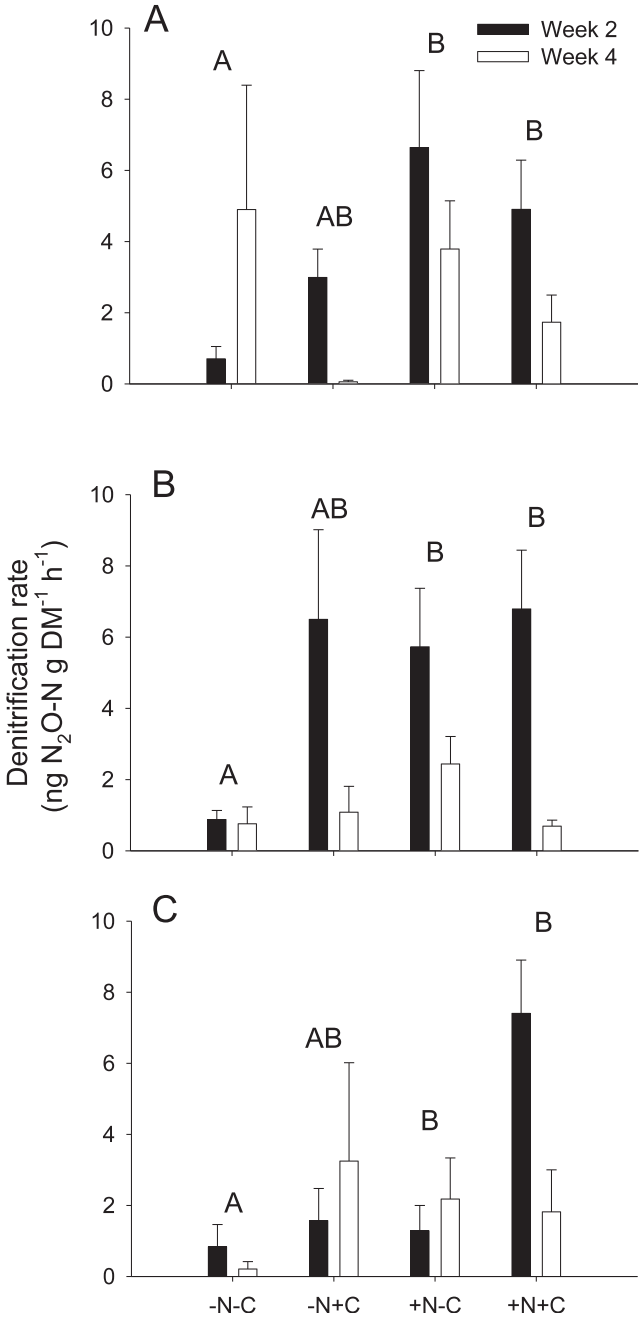

FIG. 3. Mean (±1 SE) denitrification rates in control (A), *Campostoma* (B), and NH_4^+-amended mesocosms (C) from unamended (−N−C), dextrose amended (−N+C), NO_3^--amended (+N−C), and fully amended (+N+C) incubations on weeks 2 and 4. Treatment bars with the same letters are not significantly different among amendments based on repeated measures analysis of variance across all treatments ($p > 0.1$).

relationships between denitrification and any POM size class.

Algal biomass accrual.—Algal biomass accrual was not differentially affected by treatments over time, but

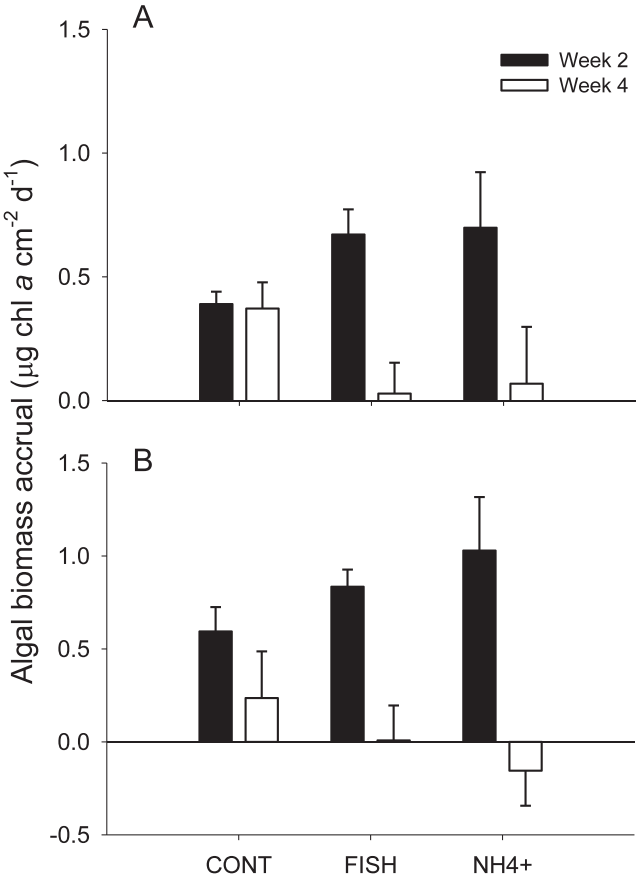

FIG. 4. Mean (±1 SE) algal biomass accrual in riffles (A) and pools (B) on weeks 2 and 4 in control (CONT), *Campostoma* (FISH), and NH_4^+-N (NH4+) treatments.

algal accrual did differ between the 2 sampling periods in both riffles and pools (rmANOVA, $p = 0.018$ and 0.002, respectively; Fig. 4A, B) across all treatments. Cumulative means across treatments decreased from the 1st to the 2nd sampling period in both riffles (from 0.603 to 0.127 μg cm^{-2} d^{-1}) and pools (from 0.812 to 0.033 μg cm^{-2} d^{-1}).

Net ecosystem metabolism.—A time × treatment interaction was found for GPP (rmANOVA, $p = 0.031$). This effect probably was driven by the large but delayed increase in GPP in the NH4+ mesocosms (Fig. 5A). CR was not affected by this time × treatment interaction (Table 2, Fig. 5B). However, CR was significantly affected by treatment (rmANOVA, $p = 0.023$). No significant temporal effects were found for CR. The lack of a significant time × treatment interaction for CR suggests that, throughout the study, the NH4+ mesocosms had significantly greater rates of CR than FISH mesocosms (Tukey's HSD, $p < 0.05$). Both GPP and CR increased from the 1st to the 2nd sampling date in the NH4+ mesocosms but decreased in FISH and CONT mesocosms (Fig. 5A, B).

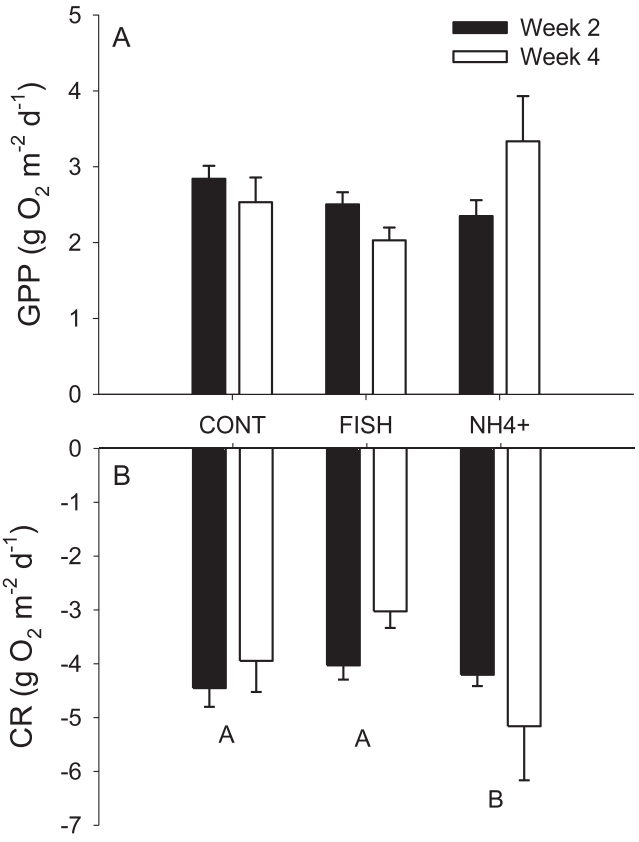

FIG. 5. Mean (±1 SE) gross primary productivity (GPP) and community respiration (CR) from control (CONT), *Campostoma* (FISH), and NH_4^+-N (NH4+) treatments on weeks 2 and 4 of the experiment. Treatment bars with the same letters are not significantly different ($p > 0.05$).

Discussion

Our study is one of the first to separate the effects of grazing caused by N remineralization from other grazing mechanisms (but see Knoll et al. 2009), and it adds to the growing body of evidence that macroconsumers shape prairie stream recovery (Bertrand et al. 2009, Murdock et al. 2010). In addition, ours is the first study to analyze the effect of a macroconsumer on denitrification in a lotic system. POM size was smaller in FISH mesocosms than in NH4+ mesocosms, and NH_4^+ amendments increased mesocosm CR throughout the study. Grazer and NH_4^+ additions also affected recovery trajectories (evident as a significant time × treatment interaction) of ecosystem structure (NH_4^+-N concentration, NO_3^--N concentration, hyporheic DO, riffle filament length) and function (GPP). The significant treatment and interaction terms for numerous response metrics showed that grazers can affect ecosystem structure and function, but the importance of grazers is context dependent (Vanni 2010) and detection of these effects is variable over time.

We attempted to simulate excretion rates with NH_4^+ amendments, but NH4+ mesocosms had greater NH_4^+-N concentrations than FISH mesocosms. This result might have been a consequence of how NH_4^+ was added (in the water column in NH4+ mesocosms vs near the benthic zone in FISH mesocosms), but a more likely explanation is that effects of *Campostoma* grazing offset effects of fertilizer excretion. *Campostoma* excretion also includes P, which could have increased the efficiency of uptake of NH_4^+ from the water column. However, *Campostoma* did not stimulate primary production. Thus, our treatments probably did not mimic fish excretion exactly, and more mechanistic studies, including simulation of the stoichiometry of excretion and the mechanical aspects of grazing, might be beneficial. In our view, the absence of response to fish excretion in the presence of a response to a comparable N addition is an interesting result that warrants further study.

Denitrification rates were not affected by *Campostoma*, but substrate limitations on denitrification varied over time. Variable hyporheic DO concentrations over this short-term study coupled with the significant amendment × time effects on denitrification suggest that the response of denitrification to scouring floods should be studied in more detail. The effects of grazers on ecosystem function were variable, but our results are consistent with previous evidence that grazing minnows can alter ecosystem structure (Gido et al. 2010), mainly by decreasing algal filament lengths (Fig. 1B), but also by altering POM size (Fig. 2A, B).

Factors controlling ecosystem function

Altered ecosystem structure caused by grazing did not appear to alter the function of these streams greatly (i.e., no treatment effect on GPP or denitrification, but a significant time × treatment interaction for GPP). Several possible explanations exist for this lack of a functional response. First, the presence of grazers may select against filamentous algae and for a nonfilamentous 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). We did not analyze changes in the algal community and, therefore, cannot speculate on the importance of functional redundancy of primary producers in these experimental streams. In addition, grazers can influence not only algal biomass, but also the quantity and quality of C produced and excreted by biomass. Thus,

grazers can affect the lability of the energy source available for denitrification. Experiments designed specifically to analyze the importance of functional redundancy among primary producers and effects of grazers on C quality are needed to provide further understanding of the effects of grazing on stream ecosystem metabolism.

Grazing decreased the size of POM in our mesocosms compared to NH_4^+ amendments. FISH mesocosms had increased proportional MPOM and decreased proportional CPOM relative to NH4+ mesocosms (Fig. 2A, B). These trends are consistent with results of a study with *Phoxinus erythrogaster* in the same experimental stream complex, in which grazing by *P. erythrogaster* reduced CPOM and increased FPOM (Bertrand and Gido 2007, Gido et al. 2010). Particle sizes in FISH and NH4+ mesocosms did not differ from those in CONT mesocosms, but the reduced particle size in FISH mesocosms suggests that changes in ecosystem function may be attributable to direct grazing or other mechanical effects (e.g., sloppy feeding or bioturbation of the sediment) and not N remineralization. Reduced POM size has the potential to increase denitrification (Dodds et al. 1996b) and C and N cycling via increased surface area for microbial decomposition of POM. Any change in this decomposition should be evident in CR data. However, *Campostoma* did not affect CR, and POM was not related to denitrification.

Substrate and energy limitations on denitrification were highly variable among mesocosms. Unamended denitrification rates were not significantly affected by treatment, but responses to amendments differed. In these mesocosms, denitrification was N limited, as evidenced by significantly higher rates found in +N−C and +N+C incubations compared to rates in unamended incubations. Moreover, denitrification rates were greater early in the recovery period. Responses to C decreased in CONT and FISH mesocosms, whereas responses to C increased in NH4+ mesocosms as recovery progressed. In CONT mesocosms, denitrification rates were greater in −N−C incubations than in +N or +C incubations (although all results were highly variable; Fig. 3A). No obvious reason exists for this lack of C, NO_3^-, or colimitation, but in situ DO might limit denitrifier biomass (i.e., favor aerobic respiration over denitrification).

Denitrification rates were not affected by treatment, but CR was higher in NH4+ mesocosms than in FISH mesocosms, a result indicating that the effect of N remineralization by grazers on CR was negated by some other aspect of grazing. The increase in O_2 consumption (CR) could have been a consequence of nitrification of the added NH_4^+-N rather than of aerobic respiration. For nitrification to account for the increase in CR, a nitrification rate = ~300 mg NH_4^+-N m^{-2} d^{-1} would have been required. In a previous study of nitrification in Konza prairie streams, rates were as high as 180 mg NH_4^+-N m^{-2} d^{-1} in nutrient-enriched portions of a nearby stream in NH_4^+-N-amended incubations (Kemp and Dodds 2002). Assuming rates in the mesocosms are similar to those in natural prairie streams, a substantial portion, but not all, of the O_2 consumption seen in the NH_4^+-N-enriched stream could have been the result of nitrification. In addition, N remineralization by fishes has been linked to increased primary production (Hargrave 2006) and algal biomass (Knoll et al. 2009). Therefore, both autotrophic and heterotrophic processes probably are responsible for increased CR in NH4+ mesocosms. N remineralization by fish can be large relative to ecosystem need (Vanni 2002, McIntyre et al. 2008). Thus, an interaction between N remineralization and primary production is possible, if not probable.

To our knowledge, we are the first investigators to publish a study of the effect of grazers on denitrification and hyporheic DO in a lotic system. *Campostoma* did not affect denitrification, but interesting temporal trends were evident in denitrification. The inverse relationship between hyporheic DO and denitrification is consistent with the fact that O_2 is a more efficient electron acceptor for C oxidation than NO_3^-. Thus, DO inhibits denitrification. The recovery trajectory of hyporheic DO differed among treatments over time. The simulated flood probably flushed organic matter into the hyporheic zone (Olsen and Townsend 2005), leading to greater hyporheic respiration over the 1st sampling period. The significant time × treatment interaction indicates that *Campostoma* can affect the recovery of hyporheic DO and potentially could affect hyporheic biogeochemical processes. However, this was not the case in our study.

Recovery from flood in prairie streams

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 across a variety of ecosystems. 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. 2000, Kemp and Dodds 2001, O'Brien et al. 2007), and are subjected to frequent disturbances (i.e.,

desiccation and scouring floods; Dodds et al. 2004). These factors make prairie streams ideal for studying short-term recovery trajectories of stream ecosystems. Algal biomass standing stock and accrual rates were not affected by *Campostoma* or NH_4^+ amendments in our study. These results contradict the results of a previous study of the effects of macroconsumers on recovery from drought. In that study, macroconsumer presence affected the temporal recovery of algal biomass, but the final state of the system was independent of macroconsumer presence or absence (Murdock et al. 2010).

In prairie streams, algal communities recover quickly from disturbances (Dodds et al. 1996a, Murdock et al. 2010), but less is known about microbial responses to floods. Results of a study of microbial recovery from flooding in a southwestern USA desert stream (Holmes et al. 1998) indicate that algal recovery is more rapid than heterotrophic microbial recovery. However, transplanted substrata adjusted to local nitrification rates within 6 d in a prairie stream (Kemp and Dodds 2002), a result suggesting rapid microbial colonization following disturbance in prairie streams. Rapid microbial colonization coupled with decreased hyporheic DO could account for the greater denitrification rates found during the 1st sampling period. As recovery progressed, organic matter flushed into the sediment via the initial flood was respired, leading to reduced hyporheic DO (Olsen and Townsend 2005). Once the initial pulse of organic matter was respired, hyporheic DO recovered, depressing denitrification during the 2nd sampling period.

Our results show minimal grazer effect on algal biomass, GPP, and CR, but in a recent meta-analysis on the effects of herbivory on primary production, Gruner et al. (2008) found a significant increase in benthic primary producer biomass in response to herbivore exclosure across 32 studies. The trends of our results are consistent with, but less pronounced than, results of previous studies of natural and mesocosm prairie streams (Gido et al. 2010). Therefore, we think that grazing minnows typically affect the structure of prairie streams. However, effects of grazing minnows on the function of these prairie stream mesocosms were much less apparent and presumably is highly context dependent (Vanni 2010). For example, top-down grazer effects on primary producers would be expected in systems not limited by nutrients (Rosemond et al. 1993). We think that the function of the ecosystem is resilient to grazing pressure because these streams are adapted to low nutrient concentrations and frequent disturbances (Dodds et al. 2004).

We found minimal effects of grazers on ecosystem function, but grazers can affect ecosystem function in less disturbance-prone systems. A model of grazer effects on algal biomass suggests that grazers maintain algae at low levels under steady-state conditions, but increasing disturbance (flood) frequency leads to algal blooms (Rutherford et al. 2000). Responses to macroconsumers also might be stronger in ecosystems with abundant, diverse fish communities (McIntyre et al. 2008). In our study, grazers failed to alleviate N limitation, which has a stronger influence than *Campostoma* grazing on ecosystem function in prairie streams.

In conclusion, grazing *Campostoma* and NH_4^+ amendments affected the recovery trajectories of most measures of ecosystem structure in our study. Ecosystem function was much more variable, and *Campostoma* had no effect on denitrification rates or stream metabolism. These mesocosms were recovering from a scouring flood, so flood effects could have negated any *Campostoma* effects on denitrification. Grazers and NH_4^+ were important regulators of structural recovery in these prairie stream mesocosms, but regulation of structural recovery did not necessarily translate to recovery of ecosystem function. We expected pronounced grazer effects in the controlled experimental conditions of our study, but ecosystem function was not affected by grazers. Thus, grazers can have significant effects on ecosystem function (e.g., McIntyre et al. 2008, Knoll et al. 2009), but this assumption should not be accepted a priori.

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