Groundwater Research Report
WR08R003

NITRATE TOXICITY AND NITRATE REMOVAL IN A CENTRAL PLAIN SAND STREAM

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WR08R003: Nitrate toxicity and nitrate removal in a Central Sand Plain stream
Project Completion Report

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PROJECT SUMMARY

Title: **Nitrate toxicity and nitrate removal in a Central Sand Plain stream**

Project I.D. **WR08R003**

Investigators: Principal Investigator- **Dr. Robert S. Stelzer**, Associate Professor, Department of Biology and Microbiology, University of Wisconsin Oshkosh
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Period of Contract: 7/1/2008-6/30/2010

Background/need: In many regions in Wisconsin, and throughout the world, ground water is elevated in nitrate concentration. Although the threats of high groundwater nitrate (> 10 mg NO_3-N/L) to human health are well understood, much less is known about effects on animals in groundwater dominated habitats such as the sediments of gaining streams. Shallow ground water associated with streams in the Central Sand Plains of Wisconsin has nitrate concentrations as high as 100 mg NO_3-N/L. In these systems infaunal (sediment dwelling) invertebrates, such as amphipods, are exposed to nitrate concentrations that exceed those known to cause lethal and sublethal toxic effects in a variety of animals (fishes, amphibians, aquatic invertebrates). There is also a large need to determine how nitrate is processed in Wisconsin ecosystems that receive high amounts of nitrate in groundwater. In addition to nitrate toxicity, this project also addressed removal of nitrate, and denitrification in particular, in a Wisconsin stream ecosystem that receives groundwater with high nitrate concentration.

Objectives: The main objectives of the research project were: 1) to assess the lethal and sublethal effects of elevated nitrate concentrations in shallow ground water on aquatic infaunal invertebrates in the Central Sand Plains of Wisconsin, and 2) to determine how groundwater nitrate processing and profiles changed with sediment depth in a sand plains stream.

Methods: To meet the first objective, *Gammarus pseudolimnaeus* amphipods were exposed to seven different nitrate concentrations in the laboratory and lethal and sublethal effects of this exposure were assessed. The nitrate treatments were each replicated 40 times. Amphipods were held in individual microcosms in incubators. Water and food (conditioned red maple leaves) were replenished every two and four days, respectively. Sublethal effects measured included growth rates, egestion rates, and molting. To meet the second objective, sediment cores were collected during summer, fall, and spring from Emmons Creek in Central Wisconsin, and sectioned. Denitrification rates were measured in the laboratory using the acetylene block method. Piezometer nests and porewater samplers (peepers) were used to determine fine-scale gradients in groundwater nitrate concentration in the sediments of Emmons Creek.

Results and Discussion: Nitrate concentration did not affect mortality, egestion rate, molting, and C:N ratio of amphipods. Amphipod growth decreased slightly with increasing nitrate concentration based on the results of a linear regression but a 1-way ANOVA suggested that mean growth rates were not different among nitrate treatments. Denitrification rates were higher in shallow core sediments. However, core sections deeper than 5 cm accounted for 68 % of the

total integrated denitrification rates, on average. The nitrate profiles were also consistent with nitrate loss from groundwater in deep stream sediments.

Conclusions/Implications/Recommendations: Elevated nitrate concentration did not have lethal effects on amphipods. There was no evidence of effects of elevated nitrate concentration on egestion rate, molting or C:N. However, our results suggested that elevated nitrate concentration may have weak negative effects on amphipod growth rates. We think that additional research is needed on the sublethal effects of elevated nitrate concentration on aquatic invertebrates, particularly for those taxa that have demonstrated sensitivity to other chemical stressors. The results from the biogeochemical portion of the study suggest that not accounting for denitrification of groundwater nitrate in deeper sediments could lead to underestimates of nitrate removal in streams. If nitrate processing in deep stream sediments is widespread, this may have implications for regional and global models of nitrogen transport and retention. Our results emphasize the importance of healthy intact sediments for groundwater nitrate removal in nitrate-contaminated stream ecosystems. If stream sediments become degraded because of toxin exposure or physical removal (e.g. dredging) ecosystem services they provide, such as nitrate removal, may be compromised.

Related Publications:

Stelzer, R.S. and B.L. Joachim. 2010. Effects of elevated nitrate concentration on mortality, growth, and egestion rates of Gammarus pseudolimnaeus amphipods. Archives of Environmental Contamination and Toxicology 58: 694-699. DOI 10.1007/s00244-009-9384-x

Key words: nitrate, groundwater, toxicity, denitrification, sediments, streams, sand plains, biogeochemistry, amphipods

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PROJECT COMPLETION REPORT for WR08R003: Nitrate toxicity and nitrate removal in a Central Sand Plain stream

PART I: Lethal and nonlethal effects of elevated groundwater nitrate concentration on *Gammarus pseudolimnaeus* amphipods.

Introduction- In watersheds with extensive agricultural activity surface water nitrate concentration is frequently 10 mg NO_3-N/L or higher (Royer et al. 2004) and ground water upwelling to streams can exceed 20 mg NO_3-N/L (Kraft and Stites 2003). Nitrate can cause acute and chronic toxicity in a variety of aquatic animal species at environmental concentrations (Camargo and Alonso 2006). Although several studies (Camargo et al. 2005, McGurk et al. 2006) indicate that nitrate concentrations as high as 30 and exceeding 100 mg NO_3-N/L do not cause substantial mortality (LC50) in several species of aquatic animals, other studies (Smith et al. 2005) have shown that a variety of species of amphibians, freshwater fishes, aquatic insect larvae, and freshwater crustaceans experience lethal effects of nitrate at concentrations as low as 8 to 30 mg NO_3-N/L. Most studies of nitrate toxicity in aquatic animals have focused on lethal effects of nitrate exposure. However, several studies have shown that elevated nitrate concentration can have sublethal effects on aquatic animals, including alterations of growth rate, development, and reproduction (e.g. McGurk et al. 2006). Sublethal effects of nitrate on aquatic animals can occur at much lower concentrations than lethal effects. *Gammarus* amphipods are widely distributed and locally abundant in low to mid-gradient streams and rivers. No previous study to our knowledge has examined sublethal aspects of nitrate toxicity in amphipods. In the Central Sand Ridges Ecoregion of Wisconsin *Gammarus pseudolimnaeus* is commonly found associated with fine sediment in streams with porewater nitrate concentration as high as 30 mg NO_3-N/L (Stelzer, unpublished data). Thus, nitrate concentrations that *Gammarus pseudolimnaeus* are exposed to exceed concentrations known to cause lethal and sublethal affects in a variety of aquatic animals including invertebrates (Camargo et al. 2005). We conducted a laboratory experiment in which we exposed *Gammarus pseudolimnaeus* to elevated nitrate concentration. The objectives of our study were:

1) to determine if *Gammarus* experiences acute nitrate toxicity at environmentally realistic nitrate concentrations,

2) to assess the effects of elevated nitrate concentration on somatic growth, molting, and egestion rates of *Gammarus*.

Procedures and Methods-*Gammarus pseudolimnaeus* were exposed individually to one of seven nitrate concentration treatments (target concentrations were 0.5, 4, 8, 16, 32, 64, and 128 mg NO_3-N/L) for 21 days in microcosms (Table 1). Each treatment was randomly assigned to microcosms and replicated 40 times, for a total of 280 experimental units. *Gammarus* were collected in July 2008 from Emmons Creek in Portage County, WI. Surface water and pore water nitrate concentration near the collection location range from 2.0 to 2.6 and 0.2 to 3.6 mg NO_3-N/L, respectively. Microcosms were housed in a Fisher Isotemp Model 307C incubator set at 15 °C, near the average temperature of pore water in Emmons Creek. Nitrate treatment solutions were prepared every two days using water collected weekly from Emmons Creek at the outflow of Fountain Lake. Amphipods received 90 ml of fresh treatment solution every other day. The presence of amphipod mortalities and exuviae (evidence of molting) were noted each time water was replenished in the microcosms. Amphipods were fed conditioned red maple

Table 1. Chemical and biological attributes (means ± SD) of the nitrate treatment groups. *N* = the number of replicates based on *Gammarus* that survived the duration of the growth experiment. There were five replicates, through time, for measured nitrate, specific conductivity and chloride, and three replicates for pH.

Target Nitrate Concentration (NO_3-N/L)	Measured Nitrate Concentration (NO_3-N/L)	*N*	Initial *Gammarus* blotted wet mass (mg)	Specific Conductivity ($\mu S\ cm^{-1}$)	pH	Chloride (mg/L)
Control	0.36±0.09	36	3.63 ± 0.69	291 ± 4	8.56 ± 0.05	4.70±0.17
4	4.46±0.14	33	3.59 ± 0.78	321 ± 6	8.57 ± 0.03	4.46±0.42
8	8.21±0.06	37	3.48 ± 0.72	352 ± 6	8.57 ± 0.05	4.25±0.16
16	16.13±0.37	38	3.70 ± 0.70	414 ± 7	8.58 ± 0.05	4.28±0.14
32	33.01±0.70	38	3.44 ± 0.63	540 ± 9	8.57 ± 0.04	4.26±0.14
64	64.08±1.34	33	3.34 ± 0.65	776 ± 15	8.57 ± 0.03	4.78±0.24
128	126.53±1.84	37	3.60 ± 0.74	1241 ± 16	8.55 ± 0.03	5.22±0.26

(*Acer rubrum*) leaves every fourth day. Eleven milligrams (dry mass) of leaf material, as 1.3 cm diameter disks, were given to each amphipod at the beginning of the experiment. The amount of food was increased to 16.5 and then 22 mg towards the end of the experiment to account for increased food demand for the growing amphipods. Blotted wet mass of amphipods was measured to 0.01 mg initially and after 21 days with a Mettler Toledo MX5 ultra micro balance. Instantaneous growth rate was determined as $\ln(M_t/M_o)/t$ where M_t and M_o were the masses of an amphipod at time t and the start of the interval. Egestion rate was measured at 15 °C after final amphipod mass was determined. Amphipods were allowed to feed for 24 to 32 hours. At the end of the incubation, the uneaten leaf material was removed and the water in each microcosm was vacuum filtered onto pre-weighed, 25 mm Whatman GF/F filters. Filters were stored at -20 °C, dried at 60 °C and then reweighed to determine the dry mass of egested material.

Results and Discussion- The measured nitrate concentrations were similar to the target nitrate concentrations for all of the treatments (Table 1). Mortality rates of *Gammarus* were low during the experiment. Of 280 amphipods, 252 survived the 21 day growth experiment. There was no effect of nitrate concentration on amphipod mortality (Table 2; G = 6.67, P

Table 2. Percent mortality, average number of molts per individual *Gammarus*, molting frequency (number of individuals who molted) and percent of individuals who molted (*N* as in Table 1). Molting data are only based on individuals that survived the duration of the growth experiment.

Nitrate Treatment (NO_3-N/L)	Mortality (%)	Observed Molts		
		Average	Frequency	Percent
Control	10	0.8	24	67
4	17.5	0.7	18	54
8	7.5	0.9	27	73
16	5	0.7	22	58
32	5	0.7	21	55
64	17.5	1.1	25	76
128	7.5	0.8	25	67

> 0.25). Because of the low amount of mortality and lack of treatment effects we were not able to calculate LC10 or LC50 for Gammarus. Nitrate concentration did not affect molting frequency (Table 2, G = 4.17, P > 0.50). There was no difference in mean instantaneous growth rate of *Gammarus* among the nitrate treatments, (Fig. 1A, F-ratio = 1.85, P = 0.0901). A linear regression of *Gammarus* instantaneous growth rate on measured nitrate concentration was statistically significant (P = 0.049, y = -0.00003 x + 0.0248). However, the r2 of 0.015 revealed that a negligible amount of the variation in *Gammarus* growth rate was explained by nitrate concentration. The grand mean egestion rate of 0.262 mg feces (dry) per mg amphipod wet mass per day suggested that *Gammarus* consumed, on average, over 100% of its body weight each day (based on a 3.88 wet mass:dry mass ratio of *Gammarus*, determined empirically). Egestion rate, scaled to *Gammarus* blotted wet mass, did not differ among the nitrate treatments (Fig 1B, F-ratio = 1.66, P = 0.132). Per-capita egestion rate did also not differ among treatments (F-ratio = 1.211, P = 0.301). The C:N ratio of *Gammarus* was not affected by nitrate concentration (Fig 1C, F-ratio = 0.137, P = 0.991).

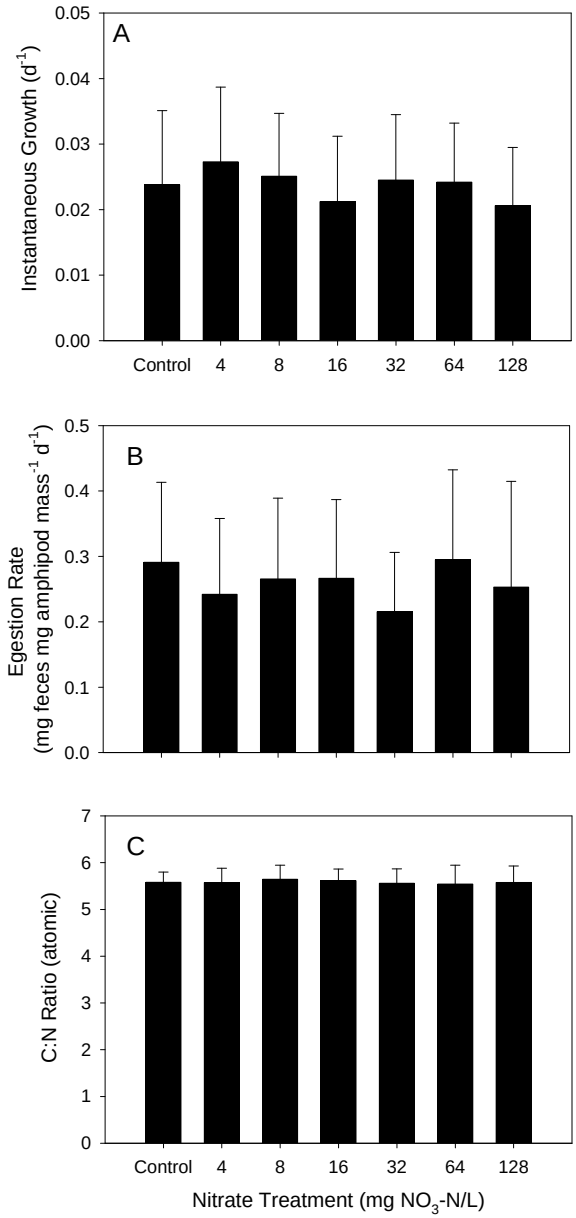

Figure 1. *Gammarus pseudolimnaeus* mean (+ SD) instantaneous growth rate (A), egestion rate (dry mass of feces per amphipod wet mass per day) (B), and C:N ratio (C) based on exposure to seven nitrate treatments.

Camargo et al. (2005) showed that two other species in the Gammaridae, *Echinogammarus echinosetosus* and *Eulimnogammarus toletanus*, were much more sensitive (higher mortality) to elevated nitrate concentrations than our results with *Gammarus pseudolimnaeus*. Our results are consistent with several other studies documenting no effect of elevated nitrate concentration on mortality in aquatic invertebrates, expect at high nitrate concentrations (> 100 mg NO_3-N/L), unlikely to be encountered by aquatic organisms in most natural environments (e.g. Corrao et al. 2006). The studies by Camargo and Ward (1992, 1995) and Camargo et al. (2005) are the only studies to our knowledge that demonstrated lethal effects of elevated nitrate in aquatic invertebrates at concentrations likely to be encountered in surface water or pore water in aquatic ecosystems. If *Gammarus* did not increase nitrate uptake when environmental nitrate concentration was elevated, then this could

have led to the weak effects of nitrate concentration on *Gammarus* performance and lack of mortality response. Although we didn't measure nitrate uptake by *Gammarus*, we used tissue C:N ratio as an indirect measure of nitrate uptake. If nitrate uptake by *Gammarus* increased with increasing nitrate concentration, then the C:N of *Gammarus* would be expected to decrease. The lack of a nitrate concentration effect on Gammarus C:N ratio suggests that elevated nitrate concentration did not affect nitrate uptake.

Scott and Crunkilton (2000) is the only other study of nitrate toxicity in aquatic invertebrates, to our knowledge, that reported data on sublethal effects. The authors found that the lowest-observed effect concentration (LOEC) of nitrate on *Ceriodaphnia* neonate production had a mean of 42.6 mg NO3-N/L and was as low as 14 mg NO3-N/L in individual trials. No study to our knowledge has reported data on sublethal effects of elevated nitrate concentration for benthic invertebrates. Exposure history of *Gammarus* to nitrate may have played a role in our results. We collected *Gammarus* from Emmons Creek, a stream with elevated nitrate concentration relative to streams in the same region prior to widespread human impact (Smith et al. 2003). It is possible that the *Gammarus* population in this stream was acclimated or selected to tolerate high nitrate concentrations, and thus was less sensitive to elevated nitrate concentration than populations from low-nitrate ecosystems.

Conclusions and Recommendations- Nitrate concentration did not affect mortality, egestion rate, molting, and C:N ratio of amphipods. Amphipod growth decreased slightly with increasing nitrate concentration based on the results of a linear regression but a 1-way ANOVA suggested that mean growth rates were not different among nitrate treatments. Collecting organisms from ecosystems with low background nitrate concentration, as some other investigators have done, is suggested for future experiments on nitrate toxicity. We think that there is need for more research on the sublethal effects of elevated nitrate concentration in additional invertebrate taxa, particularly those species of benthic invertebrates that are known to be sensitive to other types of chemical stressors, to determine the prevalence of nitrate toxicity in benthic habitats of aquatic ecosystems.

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PART II: Denitrification and groundwater nitrate profiles in a sands plains stream with elevated groundwater nitrate concentration

Introduction-

Humans have dramatically altered the nitrogen cycle during the past several decades, doubling the amount of fixed nitrogen worldwide. These changes have resulted in increases in the concentration and fluxes of available nitrogen in rivers and increases in the concentrations of available nitrogen in groundwater in many parts of the world, including Wisconsin (Saad 2008). Elevated nitrate in groundwater has implications for human health (Kross et al. 1992) and contributes to nitrogen loading in river and lakes where groundwater discharges to surface water.

Because the supplies of available nitrogen to ecosystems have been increasing and are projected to continue to increase, there is growing interest in processes that can retain or remove available nitrogen in streams and rivers (Mulholland et al. 2008). Processes contributing to nitrate retention in streams include assimilatory uptake by autotrophs and by heterotrophic microbes (e.g. Stelzer et al. 2003) and dissimilatory uptake, including denitrification, by microbes. It is well known that processes in riparian zones (e.g. Hedin et al. 1998), in hyporheic zones (where groundwater and surface water mix) (Hill and Lymburner 1998) and in the surface water of streams and rivers (Mulholland et al. 2008) can retain and remove substantial amounts of available nitrogen. Much less is known about the role of deep sediments beneath the stream channel (below the hyporheic zone) in nitrogen processing. Many studies of nitrogen processing in streams do not include deep sediments. For example, most studies of denitrification in streams only include denitrification measurements from surficial sediments (cores less than 5 cm deep) (e.g. Herrman et al. 2008). In groundwater-fed streams groundwater typically passes through substantial quantities of sediment before discharging to the stream. Previous studies have suggested that available nitrogen is retained along upwelling flow paths in deep sediments (Puckett et al. 2008, Stelzer et al. 2010). However, most previous studies have not included process-oriented measurements in deep sediments or have not included the fine-scale vertical profiles of available nitrogen necessary to infer where nitrogen retention occurs in deep sediments.

Our main objective was to determine how groundwater nitrate processing and profiles changed with sediment depth in a sand plains stream in Central Wisconsin that receives groundwater with elevated nitrate concentration.

Procedures and Methods-

Denitrification rates and nitrate profiles were determined in summer, fall, and spring in Emmons Creek in Portage Co., Wisconsin. Six piezometer nests, each consisting of 6 piezometers positioned at different depths, were placed in upwelling locations along a 700-m study reach (Fig. 2). During each season eight sediment cores (up to 35 cm in length) were collected, sectioned into 5 cm sub-cores, and subjected to acetylene block denitrification assays in the laboratory (Richardson et al. 2004). Incubations were carried out in a Fisher Isotemp Model 307C incubator set to the ambient temperature of groundwater at the time of core collection. Groundwater was pumped from piezometers adjacent to the sampling location of each sediment core for used in the incubations. Twenty-five ml of sediment, 20 ml of groundwater, and 5 ml of chloramphenicol solution was added to glass canning jars (246 ml) fitted with grey butyl septa. Immediately after addition of 20 ml of acetylene, jars were placed on a shaker (Innova Model 2000) set at 175 rpm in the incubator. Head space gas was sampled with a 5 ml syringe at ca. 30-min intervals during the ca. 90-min incubations and was immediately transferred to evacuated 2 ml serum vials. Within three weeks of the incubations, nitrous oxide (N_2O) concentration in the vials was measured on a Hewlett-Packard Model 5890 gas chromatograph fitted with a 63Ni electron capture detector (ECD) at the Upper Midwest Environmental Sciences Center (UMESC) in La Crosse, WI. Denitrification rate was calculated as the rate of N_2O production during the incubations. Samples from groundwater used in the incubations were analyzed for nitrate concentration. Subsamples of sediments from each core section will be analyzed for organic matter content, bulk density, and sediment grain size fractions using standard methods.

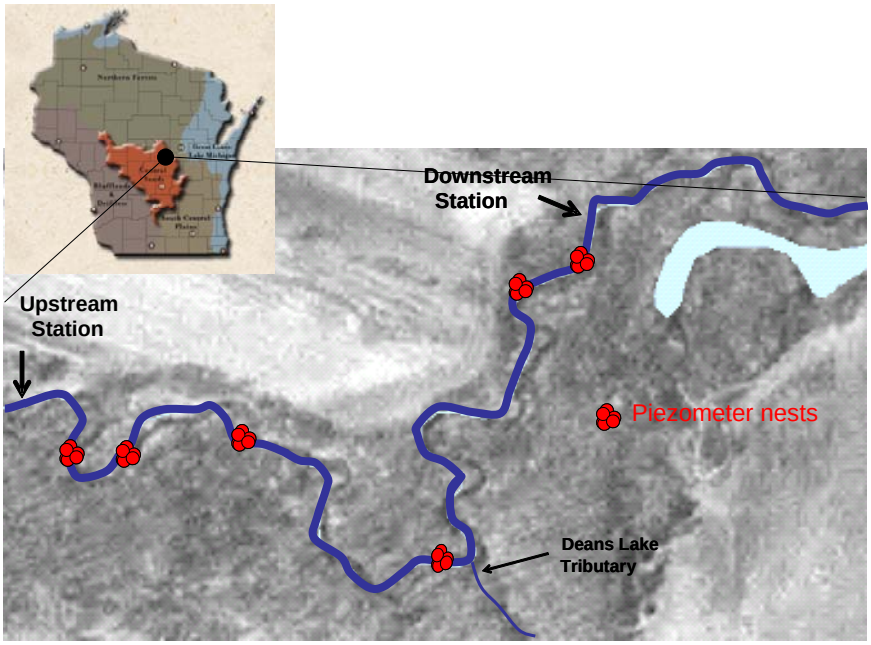

Figure 2. Study reach of Emmons Creek with piezometer nest locations indicated

Modified Pore Water Hesslein Samplers (Peepers) were used to determine groundwater nitrate and chloride profiles in the top 30 cm of sediment, at ca. 1 cm spatial resolution. Peeper samples and those collected from the piezometers were used to produce nitrate and chloride profiles for the top 60-70 cm of stream sediment. Groundwater chloride profiles from each peeper deployment were used to calculate expected nitrate concentrations in groundwater based on the assumption of conservative behavior. Comparisons of actual nitrate concentrations to expected nitrate concentrations were be used to determine the extent of nitrate retention or uptake in the sediments. Nitrate and chloride concentrations were measured using a Dionex ICS-1000 ion

chromatograph equipped with an IonPac AS14A column. Groundwater dissolved oxygen was measured routinely in the sediments using an oxygen microelectrode (Microelectrodes, Inc.). Groundwater temperature was measured with a hand-held thermometer.

Results and Discussion-

Denitrification rates were highest in the top 5 cm of sediments and decreased with sediment depth (Table 3). Denitrification rates below 5 cm accounted for 68 % of the total denitrification rate, on average, in a sediment core. It is likely that higher amounts of particulate organic matter in the shallower sediments (data forthcoming) accounted for the higher rates of denitrification at this depth. Most previous studies of denitrification in stream sediments were based on shallow (<5 cm deep) sediment cores. Our results suggest that not accounting for denitrification occurring in deeper sediments could lead to underestimates of denitrification in streams, which has implications for balancing watershed and global nitrogen budgets.

Table 3. Denitrification rates from Emmons Creek sediments Summarized over three different sampling dates

Core Section (cm)	Denitrification Rate ($mg\ N_2O\text{-}N\ cm^{-2}\ hr^{-1}$) Mean	SD	n
0-5	1.63	0.92	24
5-10	1.30	0.92	24
10-15	1.08	0.85	24
15-20	0.59	0.65	21
20-25	0.46	0.63	13

The chloride profiles suggest that groundwater upwelled through the sediments at the peeper and piezometer nest locations and that the zone of groundwater-surface water mixing was very shallow. Most nitrate profiles (e.g. Fig. 3, Fig. 4) indicated substantial nitrate retention at sediment depths below the groundwater-surface water mixing zone while a minority of profiles (e.g. Fig. 5) were indicative of less nitrate retention. When concentrations of solutes from the peepers were expressed as a percentage of deep groundwater concentrations, it is apparent that nitrate in the shallower groundwater was reduced to about 20 to 40 % of that in the deep groundwater (Fig. 6). The denitrification rates and nitrate profile results are both consistent with substantial nitrate processing below the interface where groundwater and surface water mix in Emmons Creek. This result suggests that nitrate processing can be important in deep stream sediments.

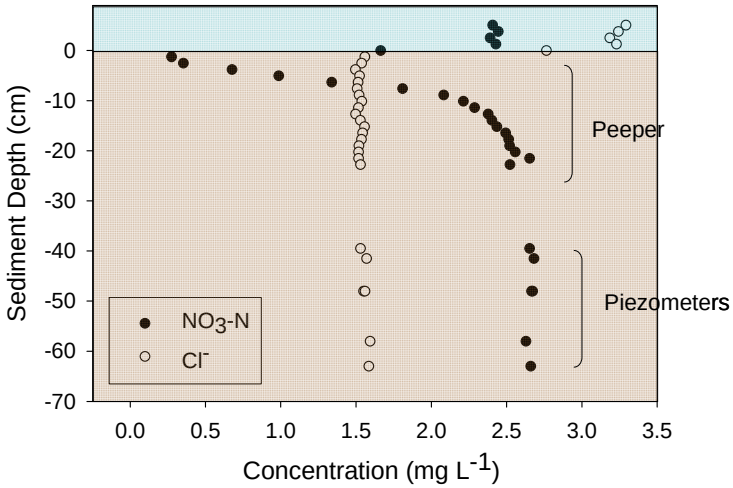

Fig. 3. Groundwater nitrate and chloride profiles from Emmons Creek at position 609 m in October of 2009

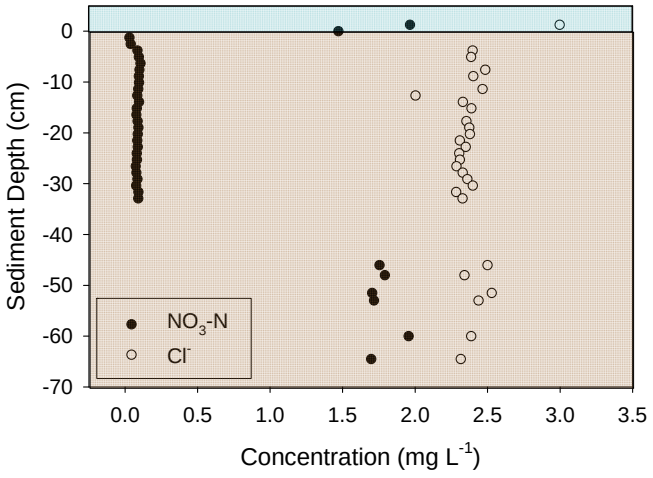

Fig. 4. Groundwater Nitrate and chloride profiles from Emmons Creek at position 23 m in July of 2009

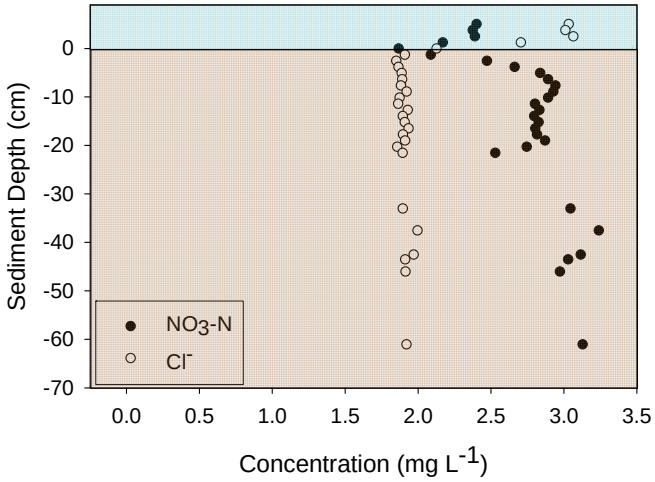

Fig. 5. Groundwater nitrate and chloride profiles from Emmons Creek at position 333 m in October of 2009

Conclusions and Recommendations-

Although denitrification rates were higher in shallow core sediments, core sections deeper than 5 cm accounted for 68 % of the total denitrification rates per core, on average. The nitrate profiles were also consistent with the notion of nitrate loss in deep stream sediments and probably are more reflective of *in situ* conditions in the sediments than the lab-based denitrification rate measurements. Our results suggest that not accounting for denitrification of groundwater nitrate in deeper sediments could lead to underestimates of nitrate removal in streams. If nitrate processing in deep stream sediments is widespread, this may have implications for regional and global models of nitrogen transport and retention. Many other investigators have highlighted the importance of stream sediments as locations with high biogeochemical activity. Our results emphasize the importance of healthy intact sediments for nitrate removal in nitrate-contaminated streams. If stream sediments become degraded because of chemical contamination or physical removal (e.g. dredging) ecosystem services they provide, such as nitrate removal, may be compromised.

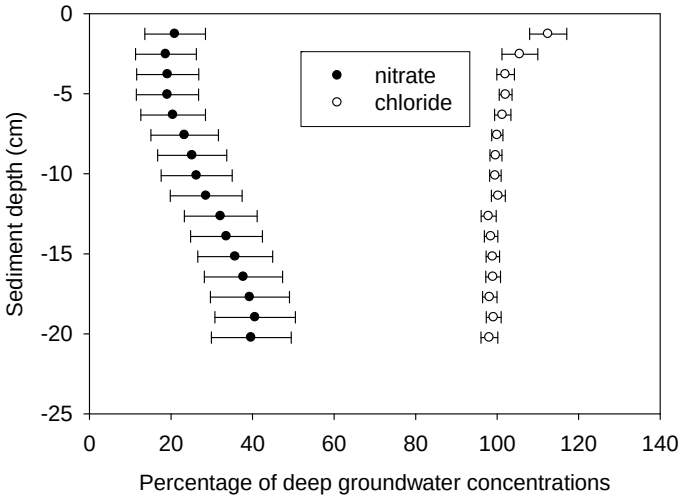

Figure 6. Grand mean (+ SE) nitrate and chloride concentrations in the shallow groundwater (peepers) of Emmons Creek expressed as a percentage of deep groundwater solute concentrations (piezometers) from July and October of 2009 and May of 2010.

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APPENDIX A:

Journal articles

Stelzer, R.S. and B.L. Joachim. 2010. Effects of elevated nitrate concentration on mortality, growth, and egestion rates of Gammarus pseudolimnaeus amphipods. Archives of Environmental Contamination and Toxicology 58: 694-699. DOI 10.1007/s00244-009-9384-x

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Stelzer, R.S., L.A. Bartsch, W.B. Richardson, and E. Strauss. 2010. The dark side of the hyporheic: nitrogen processing and profiles in deep stream sediments. Abstract of an oral presentation at the joint meeting of the North American Benthological Society and the American Society of Limnology and Oceanography. Santa Fe, New Mexico.

Stelzer, R.S., L.A. Bartsch, W.B. Richardson, and E. Strauss. 2010. Nitrate processing below the hyporheic zone in a sand plains stream. Abstract of an oral presentation at American Water Resources Association (Wisconsin Section) Annual Meeting, Madison, WI.

Stelzer, R.S., B.L. Joachim, S.L. Eggert and M.A. Muldoon. 2009. Effects of elevated nitrate concentration on mortality, growth, and egestion rates of *Gammarus pseudolimnaeus* amphipods. Abstract of a poster presentation at the annual meeting of the North American Benthological Society. Grand Rapids, MI.

Stelzer, R.S., B.L. Joachim, S.L. Eggert and M.A. Muldoon. 2009. Effects of elevated nitrate concentration on mortality, growth, and egestion rates of *Gammarus pseudolimnaeus* amphipods. Abstract of a poster presentation at American Water Resources Association (Wisconsin Section) Annual Meeting. Stevens Point, WI.