OCCURRENCE AND GENERATION OF NITRITE IN GROUND AND SURFACE WATERS IN AN AGRICULTURAL WATERSHED

Emily Stanley

2011

OCCURRENCE AND GENERATION OF NITRITE IN GROUND AND SURFACE WATERS IN AN AGRICULTURAL WATERSHED

Emily Stanley
Center for Limnology
University of Wisconsin-Madison

TABLE OF CONTENTS

LIST OF FIGURES

PROJECT SUMMARY

Title: Occurrence and Generation of Nitrite in Ground and Surface Waters in an Agricultural Watershed

Project I.D.: WR07R003

Investigator(s): PI- Emily Stanley, Professor, Center for Limnology, University of Wisconsin Madison

PA/RA- David Bylsma, Nelson Institute for Environmental Studies, University of Wisconsin Madison, and Stephen Powers, Center for Limnology, University of Wisconsin Madison

Period of Contract: July 1 2007-June 30 2010

Background/Need: Approximately 70% of the population in Wisconsin relies on groundwater as a drinking water source, and 10% of the State's private wells have nitrate (NO_3^-) concentrations that exceed the EPA's maximum contaminant level of 10 mg/L. Nitrite (NO_2^-) may be formed as a bi-product of NO_3^- enrichment, and prior research revealed its presence in surface waters of agriculturally dominated areas of the State. This observation leads to a compelling need to determine if NO_2^- is also present in Wisconsin ground waters and to identify the sources and pathways of NO_2^- generation in surface waters. By examining NO_2^- formation and distribution, we addressed multiple UWS groundwater research priorities, including: (1) transport of pollutants in groundwater; (2) impact of agricultural practices on groundwater quality; and (3) interactions of groundwater and surface water including chemical transformations in the hyporheic zone and influence of groundwater discharge on water quality.

Objectives: The goal of this project was to address the question: What conditions lead to the accumulation of nitrite (NO_2^-) in surface water environments in an agricultural watershed? This overarching question was addressed via three specific questions:

Q1. Is NO_2^- present in groundwater in N-rich areas of southern Wisconsin?
Q2. When and where is NO_2^- present along groundwater flow paths?
Q3. What processes and conditions are responsible for surface water NO_2^- accumulation?

Methods: Research activities were divided into three elements: (1) monthly surface and groundwater sampling to determine spatial and temporal patterns of NO_2^- occurrence at the East Branch Pecatonica River (Iowa Co, WI); (2) surveys of groundwater and springs in Mud Branch (Lafayette Co.) and Big Spring (Adams Co.) to determine if NO_2^- was present in groundwater in other agricultural streams in the State; and (3) laboratory and field experiments to identify possible pathways and conditions favoring NO_2^- production.

Results and Discussion: Assessment of nutrient chemistry in the East Branch Pecatonica River and its surrounding groundwater revealed a consistent pattern of highest NO_2^- concentrations occurring in surface waters but not groundwater. Streamwater concentrations varied over time,

iv

but were often highest during warm summer months. The groundwater nitrogen pool was, as expected, dominated by NO_3^-. NO_2^- was often at or below detection limits across all wells, suggesting that NO_2^- generation occurs either in the stream channel or as NO_3^--rich groundwater discharges to the surface environment. This same pattern of NO_3^- presence/NO_2^- absence in groundwater and presence of both forms of N in surface water was consistent with observations from other N-rich streams in southern Wisconsin.

More detailed sampling of stream bed sediments in the East Branch Pecatonica revealed erratic vertical concentration profiles, but notably, NO_2^- was often present in hyporheic sediments. Laboratory experiments confirmed the subsequent prediction that stream bed sediments were capable of generating NO_2^-. These experiments also revealed that the dominant pathway of NO_2^- generation was reduction of NO_3^- under low oxygen conditions rather than oxidation of NH_4^+. Rates of NO_2^- generation increased as a function of the initial NO_3^- concentration up to ca. 5 mg N/L before reaching a plateau rate of production. Rates also varied among sediment types; gravel size classes had a limited capacity to generate NO_2^- in these lab experiments, while silty, organic-rich sediments supported high production rates.

Conclusions/Implications/Recommendations: Collectively, these surveys and experiments suggest that NO_2^- presence in agricultural streams is the result of elevated NO_3^- concentrations, and that its generation is favored under warm, low-oxygen, N-rich conditions in silty stream bed sediments. Discharge of NO_3^- rich groundwater into silty hyporheic habitats appears to be a common configuration favoring NO_2^- accumulation in the surface water environment. This result has both positive and negative implications. On the positive side, presence of NO_2^- indicates active nitrogen cycling in these N-rich streams, and its presence is consistent with occurrence of denitrification (i.e., microbial removal of NO_3^- from the aquatic environment), and field experiments indicate that NO_2^- turnover is rapid. On the negative side, NO_2^- generation puts a solute into circulation that is known to have chronic effects on sensitive aquatic biota at relatively low concentrations. Highest concentrations during warm summer months may add to the stress of warmer temperatures on organisms such as cool water fish species. Further, NO_2^- generation appears to be favored within the thick layers of silty sediments that are often pervasive in many agricultural streams of southern Wisconsin. Removal of these sediments during stream restoration could have the potential to reduce occurrence of NO_2^-. A critical future research avenue will be to unequivocally determine if the reduction pathway that is associated with NO_2^- accumulation is in fact denitrification, as we suspect, or an alternative pathway (such as dissimilatory nitrate reduction to ammonium) as some researchers have hypothesized.

INTRODUCTION

There is an abundance of information demonstrating that a widespread consequence of agricultural land use is enrichment of ground- and surface waters with nitrogen (N). Fertilizer N is applied to farm fields in a variety of forms, but generally accumulates as nitrate (NO_3^-) in aquatic environments. A survey of western and southern Wisconsin revealed that the occurrence of high NO_3^- concentrations in streams in agricultural watersheds is also accompanied by the presence of nitrite (NO_2^-) during summer baseflow conditions (Stanley and Maxted 2008). This form of N was present at low absolute concentrations and made up a small percent of the total N pool (~0.5-5%), but it nonetheless occurred at environmentally significant levels at many sites. For example, the European Union NO_2^- limit for waters supporting salmonids is 3 µg N/L (Kelso et al. 1997), a concentration that was exceeded at all sites with >40% agriculture in this survey. Thus, the presence of NO_2^- in many Wisconsin streams- particularly those capable of supporting cold- and coolwater fishes that are often sensitive to pollutants- is a worrisome observation. Further, if NO_2^- also accompanies NO_3^- in the groundwater environment, it could conceivably pose human health risks for drinking water wells tapping into N-enriched aquifers. The goal of this research project was to determine if NO_2^- is present in groundwater or if it is generated in streams following the discharge of NO_3^- rich groundwater by addressing the question: *What conditions lead to the accumulation of nitrite (NO_2^-) in surface water environments in an agricultural watershed?*

Nitrite can be considered a 'gateway molecule' in the N cycle, as many transformations involve NO_2^- production or consumption as an intermediate step. These transformations include the well-studied processes of nitrification and denitrification, as well as other pathways that are less well understood, but may nonetheless be extremely important in freshwater systems (e.g., dissimilatory nitrate reduction to ammonium, or DNRA; Burgin and Hamilton 2007). At the coarsest scale, presence of NO_2^- may result either from oxidation of ammonium (NH_4^+) or the reduction of nitrate (NO_3^-), and examples of NO_2^- generation via either pathway have been reported in aquatic environments (e.g., Kelso et al. 1997, Stief et al. 2002, Smith et al. 2006). Our goal was to determine which of these two pathways may be more common. To do so, we considered these two pathways to be alternative hypotheses explaining NO_2^- presence in N-rich streams (Fig.1).

Specific predictions can be generated about changes or conditions that would occur if NO_2^- is generated via NH_4^+ oxidation (Hypothesis 1) versus NO_3^- reduction (Hypothesis 2), including the likely location of each process and other forms of N that would be present or be generated or consumed along with NO_2^- production (Fig. 1). We tested these predictions through (1) monthly surface and groundwater sampling to determine spatial and temporal patterns of NO_2^- occurrence at the East Branch Pecatonica River (Iowa Co, WI) in 2007-2008; and (2) surveys of groundwater and springs in Mud Branch (Lafayette Co.) and Big Spring (Adams Co.) to determine the occurrence of NO_2^- in groundwater in other agricultural streams in Wisconsin; and (3) field and laboratory experiments to identify possible pathways and conditions favoring NO_2^- production.

1

Hypothesis 1:
NO_2^- from NH_4^+ oxidation
(O_2 present)

Hypothesis 2:
NO_2^- from NO_3^- reduction
(O_2 absent)

NO_2^- present in groundwater
NO_2^- produced from $+NH_4^+$, $+O_2$
NO_2^- production accompanied by:
    $-NH_4^+$ disappearance
    $-NO_3^-$ generation

NO_2^- present in stream sediments
NO_2^- produced from $+NO_3^-$, $-O_2$
NO_2^- production accompanied by:
    $-NO_3^-$ disappearance

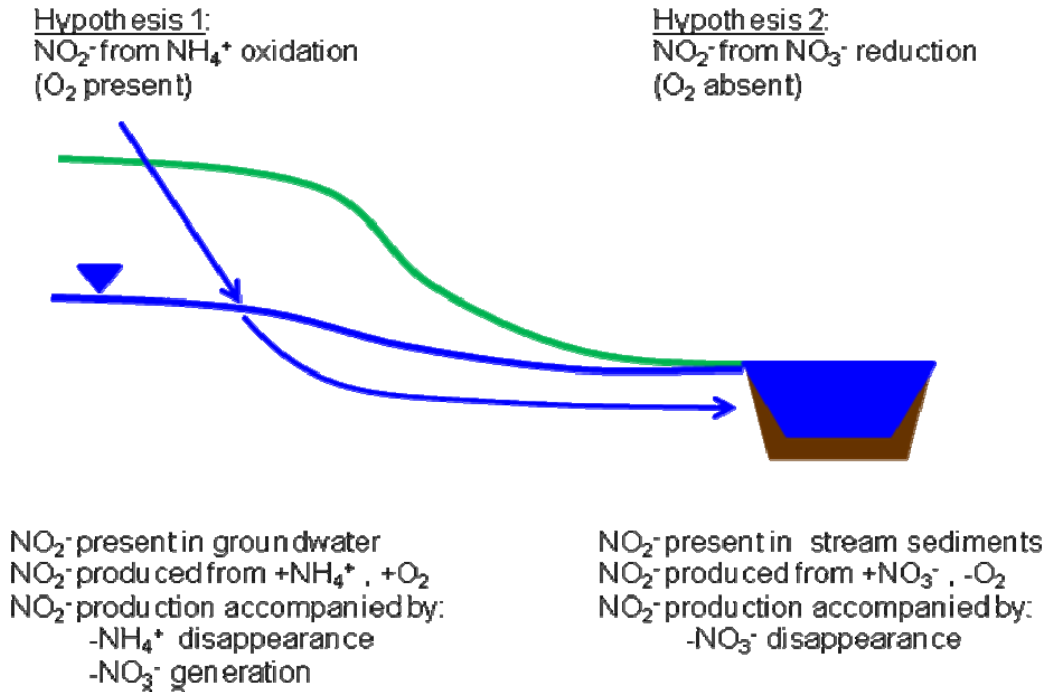Fig. 1. Alternative pathways of NO_2^- production in the hillslope/stream environment. Arrows indicate direction of groundwater flow from the hillslope to the stream channel. Blue (dark) line denotes the position of the water table. If NO_2^- is generated by oxidation of NH_4^+, the process is expected to occur in oxygenated soil or groundwater environments. Controlled experiments would yield high NO_2^- when sediments are aerated and given ample amounts of NH_4^+; NO_3^- would be generated along with NO_2^- (i.e., the process of nitrification). If NO_2^- is generated by reduction of NO_3^-, then NO_2^- should be present in fine sediments and saturated soils which are typically anoxic, and sediments supplied with NO_3^- following O_2 removal should generate NO_2^- as the NO_3^- is reduced (disappears).

PROCEDURES AND METHODS

Study Sites- The East Branch of the Pecatonica River (EBP) is located in the Driftless region of southwest Wisconsin (Iowa County) and passes through a narrow valley constrained on both sides by steep hillsides. Typical of many streams in this part of the State, the EBP is extremely N-rich, with NO_3-N concentrations in excess of 5 mg/L throughout the year. Land use in the basin is dominated by agriculture, including extensive row cropping in the valley; however, there are also large tracts of land being managed for conservation purposes, and our primary study area was subject to restoration overseen by The Nature Conservancy in 2006. The goal of the restoration was to remove the accumulated layer of anthropogenic soil from the valley as well as the woody riparian vegetation that had become established on this soil layer (Booth et al. 2009). As a consequence of these management activities, extensive deposits of silty stream bed sediments were lost, exposing coarser sand and gravel substrates. However, several areas in the study reach retained silty sediments despite the restoration, resulting in a heterogeneous composition of stream bed sediments.

Multiple piezometers and instruments were installed by Eric Booth and Stephen Loheide for an affiliated GCC project (WR07R005), allowing us to monitor groundwater chemistry at several locations in the valley adjacent to the channel. Additional site and instrumentation information is available in Booth and Loheide (2010). We also opportunistically collected samples from tile drainages along a stream reach adjacent to a corn field.

To determine if patterns observed at EBP were representative of other N-rich agricultural streams, groundwater and surface water samples were also collected for N determination from Mud Branch (Lafayette Co.) and Big Spring (Adams Co.) for analysis of all inorganic N fractions (NH_4^+, NO_2^-, and NO_3^-). Wells at these sites were already present, and had historically been used (and in some cases continued to be used) for drinking water purposes for private land owners, and were situated within 10-50 m of the stream.

Objective 1: Spatial and temporal patterns of N at EBP- Ground- and stream water samples were collected monthly starting in 2007 from EBP, although groundwater sampling was not possible in January-March because of freezing. Four pairs of piezometers were sampled, and each pair consisted of a shallow piezometer that ended within a silty alluvial soil layer (typically 40-50 cm) and a deep piezometer (ca. 60-90 cm) that sampled water from the underlying Holocene gravel/sand stratum. All samples were collected in acid-washed bottles using a Geopump peristaltic pump equipped with an in-line filter (0.4 μm cellulose acetate membrane). Bottles were placed on ice and transported to the lab for analysis. Each sample was divided into 2 subsamples; the first sub-sample was frozen for later determination of NO_3-N and NH_4-N, and NO_2-N was determined within 24 h using the second subsample. NO_2-N was measured colorimetrically after addition of sulfanilamide and dihydrochloride (APHA 1998) using a Beckman DU-640 UV/VIS spectrophotometer (Beckman-Coulter, Fullerton, California, USA). NO_3-N and NH_4-N were determined on an Astoria Pacific Instrument nutrient autoanalyzer following protocols for the North Temperate Lakes Long-Term Ecological Research program (NTL-LTER; http://lter.limnology.wisc.edu).

Objective 2: Presence of NO_2^- in groundwater of other N-rich streams: Surface and groundwater samples were collected during repeated summertime surveys at Big Spring (BS) and Mud Branch (MB). Samples were collected, filtered, and analyzed for all inorganic N fractions using the same methods as at EBP.

Objective 3: Sediment experiments to determine NO_2^- production pathways- Three sets of experiments were performed to determine how and where NO_2^- was being generated (Experiment 1 and 3) and if the oxidative or reductive pathway was likely responsible for NO_2^- generation (Experiment 2).

Experiment 1- Because of the presence of both fine and densely-packed sediments, conventional hyporheic sampling using wells or piezometers was not possible in most sections of the EBP study reach. Instead, we collected 7 sediment cores as a means of assessing vertical distribution of inorganic N forms in the stream bed. Samples were intended to capture the range of bed sediment types, from fine organic silts to small gravel. A clear plastic tube (2.54 cm ID) was slowly pushed into the bed as far as possible, then sealed at both ends and transported to the laboratory for processing. Sediment cores varied from 10 to 20 cm, depending on sediment size

and depth to refusal. In the lab, plastic sleeves were cut longitudinally to expose the sediments, which were then cut into 2-3 cm slices. Each subsample was placed in a 125 mL beaker and combined with 50 mL milli-Q H_2O, sealed, shaken vigorously for 30 sec, then allowed to settle for 15 minutes before being filtered through a Whatman GF/F filter for NO_2-N determination. Sediments were dried and weighed, and vertical profiles were described as g water-extractable N per g dry sediment.

Experiment 2- Five replicate sediment samples were collected randomly from the EBP study reach and refrigerated until sediment assays were performed, typically 24-48 h later. Each replicate was divided in half and each half was randomly assigned to one of the two major treatments. The first treatment was intended to promote NO_2^- formation via the oxidative pathway (test of Hypothesis 1). Approximately 30-40 g of wet sediment was placed into a container and amended with 100 mL water enriched with NH_4^+ and mixed. Five enrichment levels were made using a certified NH_4^+ standard solution to achieve final concentrations of 0, 0.5, 1, 2.5, or 5 mg NH_4-N/L. After removing an initial sample for later inorganic N analyses, slurries were aerated using an aquarium aerator and incubated at room temperature for 2 h before taking a final water sample. The second treatment was intended to promote NO_2^- formation via reduction (test of Hypothesis 2), and thus amendments included 0, 0.5, 2, 5, or 10 mg NO_3-N/L, followed by sparging with N_2 gas for 5 min to deoxygenate the sediment slurries. Samples were sealed during incubation to prevent oxygenation. Initial and final samples were filtered through an 0.7 μm GF/F filter and analyzed for the different inorganic N fractions as described above. Sediments were dried and weighed and rates were expressed as NO_2-N production per g sediment per h.

Experiment 3- We evaluated the effects of sediment texture by collecting 5 replicate sediment samples from areas dominated by gravel, silt, or deposits that were composed of a mixture of the two size classes ("mixed"). Approximately 40 g of wet sediments were placed into a jar and amended with 100 mL of unfiltered stream water. The control treatment used for this experiment was unfiltered stream water without any sediments. No effort was made to either oxygenate or deoxygenate samples, although jars were sealed during the incubation. Five subsamples were taken from each replicate at 0, 15, 30 60, and 120 minutes to document the time course of NO_2^- generation as well as assessing effects of sediment texture. Water samples were filtered and processed as described above.

RESULTS AND DISCUSSION

Objective 1: Spatial and temporal patterns of N at EBP and Objective 2: Presence of NO_2^- in groundwater of other N-rich streams- Routine monthly sampling of stream water and 4 pairs of wells revealed that surface and groundwater N was, as expected, dominated by NO_3^- in EBP. Streamwater NO_2^- concentrations varied over time, but were highest during warm summer months (Fig. 2). There was no relationship between stream water NO_2^- and NO_3^- concentrations, but NO_2^- and NH_4^+ were highly correlated (r = 0.75), in part due to 2 dates when NH_4^+ and NO_2^- concentrations spiked simultaneously.
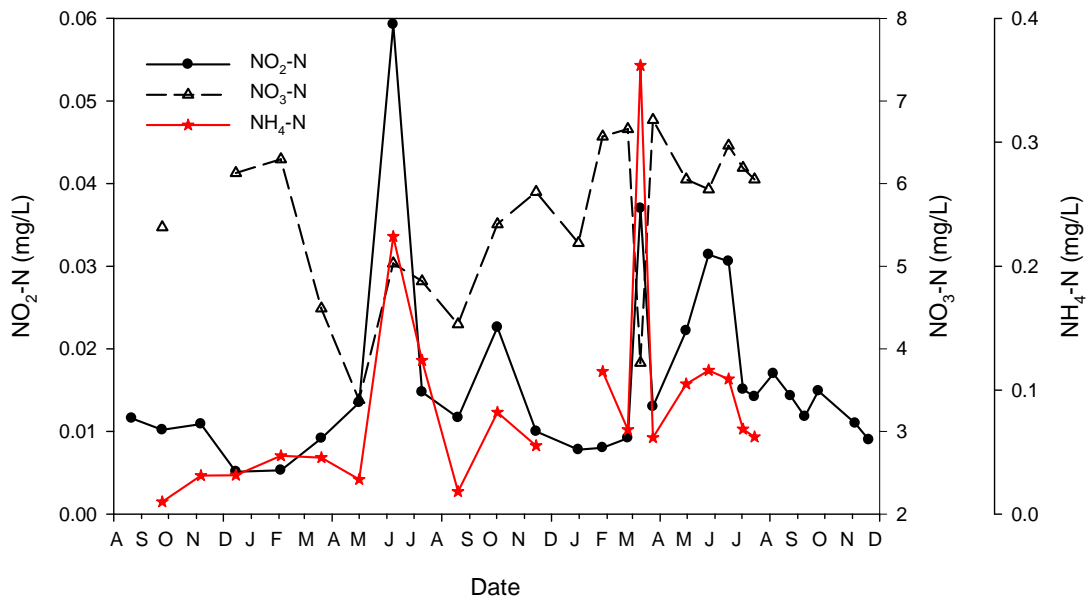
Fig. 2 Surface water concentrations of NO_2-N, NO_3-N and NH_4-N fractions in EBP. Notice that each inorganic N fraction has its own axis and scale.

Only minor temporal variation was observed in most piezometers and NO_3^- concentrations were typically an order of magnitude higher than both NH_4^+ and NO_2^-. NO_2^- concentrations were often near or below detection for most piezometer/date combinations, although one piezometer (W06-F) showed moderate NO_2^- levels (Fig. 2), reflecting occasional high relative concentrations (0.03-0.06 mg/L) interspersed among dates when levels were typically less than 0.010 mg/L. NO_2^- was also extremely low in opportunistically collected samples from a tile discharge draining an upstream cornfield at EBP. Consistent with this pattern, groundwater NO_2^- concentrations were lower than stream water at Mud Branch and below detection limits at Big Spring (Fig. 3). Results of these surveys provide strong evidence against a groundwater source and the nitrification pathway of NO_2^- generation (i.e., Hypothesis 1; see Fig.1).

Objective 3: Sediment experiments to determine conditions and pathways of NO_2^- production-Experiment 1- Because surveys of near-stream wells, seeps, and tile drains demonstrated that NO_2^- concentrations were consistently lower than in stream water and often below detection limits, we concluded that NO_2^- generation was likely occurring within the stream channel, and in particular, within stream bed sediments. This first experiment was intended to validate this conclusion by determining if NO_2^- was in fact present in the benthic/hyporheic environment by extracting inorganic N from sediments. Vertical profiles of NO_2^- were erratic, but demonstrated the presence of this intermediate ion in all samples (Fig. 4), indicating active NO_2^- production in EBP stream bed sediments.
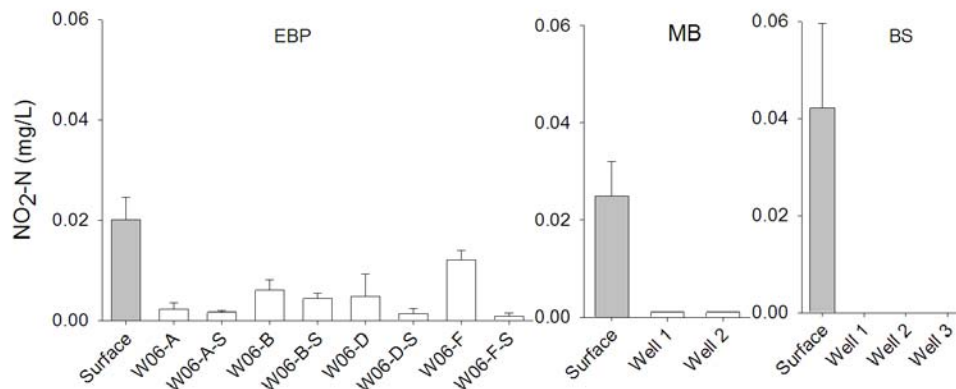
5

Fig. 3. Average NO_2-N concentrations (+1 SE) in stream water (grey bar) and ground water (open bars) in East Branch Pecatonica River (EBP), Mud Branch (MB) and Big Spring (BS) Wisconsin. EBP values represent means from monthly samples; values at MB and BS are from 3-5 summertime surveys of wells and stream water.

Experiment 2- Strong differences in NO_2^- generation were apparent between experimental treatments intended to promote its formation via either an oxidative or reductive pathway (Fig. 5). For the $+NH_4/+O_2$ treatment, NO_2^- concentrations at time 0 (i.e., NO_2^- initially present in the sediments) declined within 30 min then remained consistently low throughout the remainder of the incubation. We interpret this initial decline as NO_2^- oxidation to NO_3^-, and once any initial NO_2^- was converted, no further build-up was apparent. Nitrification is a 2-step process in which NH_4^+ is first converted to NO_2^- and then to NO_3^-. The second step (NO_2^- to NO_3^-) is thermodynamically more efficient, making NH_4^+ conversion to NO_2^- the rate-limiting step in this process. Thus, if nitrification was occurring in these sediments, then it would appear that NO_2^- build-up was prevented by its rapid conversion to NO_3^-. We observed a decline in added NH_4^+ over the course of the experiment, consistent with nitrification; however, we failed to detect a measurable increase in NO_3^- (results not shown), leaving some degree of uncertainty regarding the processing of the added NH_4^+. NH_4^+ may have been sorbed onto sediment surfaces (Triska et al. 1994) and not subjected to additional transformation. Alternatively, we cannot dismiss the possibility that aeration of the water overlying the sediments was not sufficient to oxygenate the entire sediment layer that settled on the bottom of assay containers. If some sediment anoxia existed during the incubation, then any NO_3^- produced could have been subject to denitrification, consistent with the observation of low NO_3^- concentrations at the end of the 2 h period.

Addition of NO_3^- and removal of O_2 from sediment slurries had a strong positive effect on NO_2^- generation (Fig. 5). Rates of NO_2^- production increased as a function of added NO_3^- concentration up to 5 mg NO_3-N/L before reaching an asymptote. At the same time, added NO_3^- was depleted from all addition levels, resulting in uniformly low final concentrations. We also observed consistent positive, but small, increases in NH_4^+ concentrations (0.1-0.2 mg NH_4-N/L). These results suggest that most added NO_3^- was subject to denitrification, with a minor fraction

6

μg NO$_2$-N / g sed

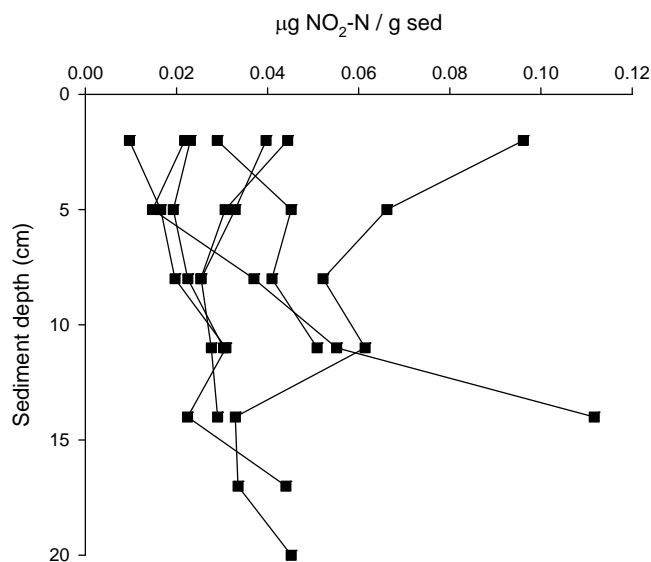Fig. 4. Vertical profiles of NO$_2$-N extracted from 7 sediment cores collected from the bed of East Branch Pecatonica River.

(at most) of the added N getting converted to NH$_4^+$ via DNRA or related process. Thus, this experiment suggests that NO$_2^-$ is predominantly generated as a bi-product or a measurable transition step associated with sedimentary denitrification in the EBP.

That NO$_3^-$ reduction was apparently the overwhelming source of NO$_2^-$ was an unexpected result given that nitrification has been identified as the dominant source in some other study systems (e.g., Smith et al. 1997, Chen et al. 2010). And even if it was not the main pathway, some contribution from nitrification was expected based on results from a detailed process-based study using paired ^{15}N and [18]O tracers and combinations of isotopic enrichments in an agricultural stream in Indiana (Böhlke et al. 2007). These investigators estimated that while most NO$_2^-$ production was attributable to NO$_3^-$ reduction, as much as 30% apparently resulted from nitrification.

Experiment 3- Production rates of NO$_2^-$ were high and increased steadily over time in fine silty EBP sediments (Fig. 6). In contrast, we observed virtually no NO$_2^-$ accumulation in gravel or mixed sediment classes, indicating that silty sediment deposits are hot spots of NO$_2^-$ production in EBP. Differences among sediment categories began to emerge within 30 min, and were distinct within 60 min, suggesting rapid N processing within silt.

As expected from the results of Experiment 2, NO$_3^-$ in the water overlying silt and mixed sediments declined over the 2 h incubation, although NO$_3^-$ changes in gravel treatments were not significantly different from controls. However, in contrast to Experiment 2, we saw significant rates of NH$_4^+$ accumulation in the silt treatment (Fig. 6). This NH$_4^+$ could have been released from the sediments as a result of agitation during the experiment, or, alternatively, could be indicative of an different pathway of NO$_3^-$ reduction, namely dissimilatory nitrate reduction to ammonium (DNRA). Burgin and Hamilton (2007) have argued that reduction of NO$_3^-$ to NH$_4^+$ is in fact widespread in wetland sediments, and may have led to an overestimation of the capacity of these and similar ecosystems to remove excess N via denitrification. It is difficult to determine which process dominates in the EBP; absence of NH$_4^+$ production coupled with disappearance of added NO$_3^-$ in Experiment 2 provides strong evidence for denitrification, while NH$_4^+$ production/NO$_3^-$ disappearance in Experiment 3 points to DNRA. Similarly, simultaneous peaks in NH$_4^+$ and NO$_2$- in surface water (Fig. 1) suggest some coupling in the production of these two N fractions. Which process dominates under what circumstances remains to be
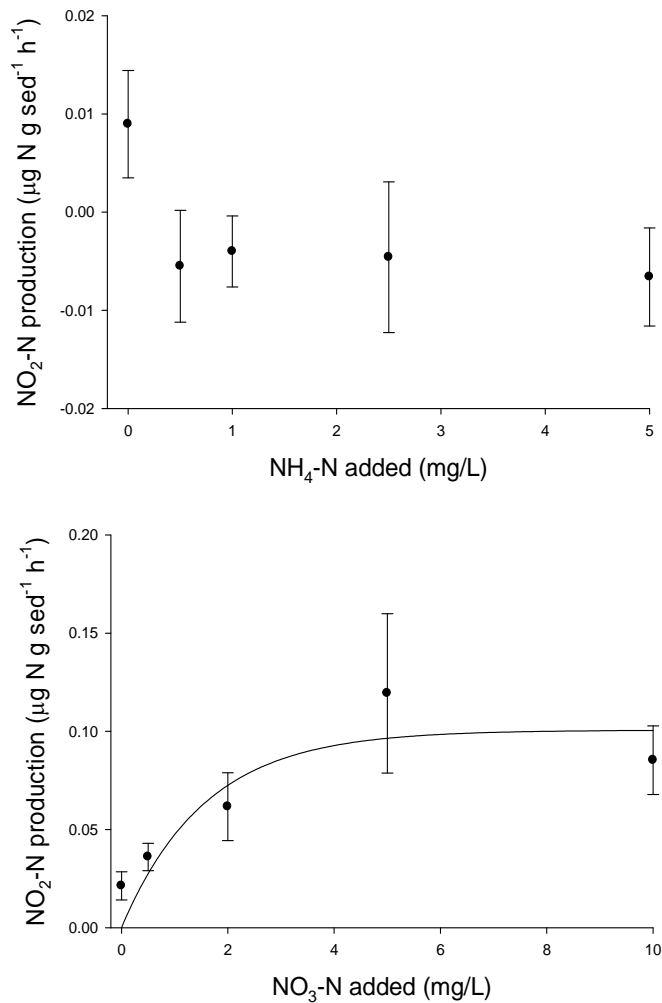
7

Fig. 5. Average (± 1 SE) NO_2-N production per g dry sediment as a function of treatment type and N concentration. Top panel corresponds to Hypothesis 1: NO_2-N generation via oxidation of NH_4-N under oxygenated conditions. Negative production denotes a net decrease in NO_2-N concentration relative to initial streamwater level. The bottom panel corresponds to Hypothesis 2: NO_2-N generation following addition of NO_3-N and elimination of dissolved oxygen. Line represents best fit curve ($p<0.05$).

determined definitively, and has strong implications for removal of groundwater-supplied NO_3^- discharged into EBP and other similar N-rich Wisconsin streams

CONCLUSIONS AND RECOMMENDATIONS

Referring back to the original alternative hypotheses (Fig. 1; H1: NO_2^- is generated via oxidation of NH_4^+ in soil and groundwater environments; H2: NO_2^- results from reduction of NO_3^- in near stream and stream bed environments), we found strong support for NO_2^- production resulting from reduction of NO_3^- in stream bed sediments, and conversely, no support for the alternative hypothesis. Thus, a key finding of this study is that, despite elevated NO_3^- concentrations, the groundwater environment does not appear to be the site of NO_2^- generation, which means that this ion is unlikely to pose an additional threat or stressor to drinking water derived from groundwater sources in most agricultural areas.

NO_2^- presence in agricultural streams is associated with elevated NO_3^- concentrations (Stanley and Maxted 2008), and its generation is favored under warm, low-oxygen, N-rich conditions in silty stream bed habitats. Discharge of NO_3^- rich groundwater into silty hyporheic sediments may be a common configuration favoring NO_2^- accumulation in surface water environments. Our interest in NO_2^- reflects the potential of this solute to pose human health threats if present in drinking water (and as noted above, we do not find evidence for this threat), as well as to sensitive aquatic biota such as amphibians and salmonids. NO_2^- generation that results from inputs of NO_3^- rich water to streams that also contain abundant stocks of sediments

prone to anoxia introduces a solute that is known to have chronic effects on sensitive aquatic biota at low concentrations. Highest concentrations during warm summer months may add to the stress of warmer temperatures on organisms such as some cool water fish species. Restoration activities at EBP were intended, in part, to eliminate the thick layer of anoxic sediments that are pervasive in Driftless Area streams (and in fact, in many agricultural areas; Wood and Armitage 1997), and thus, may help to reduce NO_2^- production. However, while NO_2^- build-up is not ideal, our experimental results demonstrated that its presence indicates active nitrogen cycling in stream sediments. If denitrification is the primary process responsible for NO_2^- generation, then removal of a stream's silt layer may reduce ecosystem capacity to remove at least some of the NO_3^- that is, unfortunately, prevalent in these agricultural systems. Denitrification is a process that many natural resource managers are now targeting in management and restoration activities (Craig et al. 2008), so if the silt layer is an active site of denitrification, and if restoration activities favor its removal, then additional actions (e.g., establishment of effective lateral riparian wetlands)

Fig. 6. Average (± 1 SE) change in NO_2-N, NO_3-N, and NH_4-N (bottom) concentrations in water mixed with silt, gravel, or mixed composition stream bed sediments. Control represents unfiltered stream water only.

should to maintain some denitrification capacity. However, while results of one of our experiments pointed strongly to denitrification as the process responsible for both NO_2^- generation and NO_3^- loss, a later experiment was more ambiguous and suggested that excess NO_3^- may simply be converted to a different form of N (NH_4^+ via DNRA) rather than being removed from the aquatic environment, as is the case with denitrification. Thus, the logical next scientific step is to determine if denitrification or DNRA prevails, if these processes vary in space and time, and if so, what drives the shift from the N-removing to the N-transforming process in streams receiving steady high doses of groundwater NO_3^-, such as the East Branch Pecatonica.
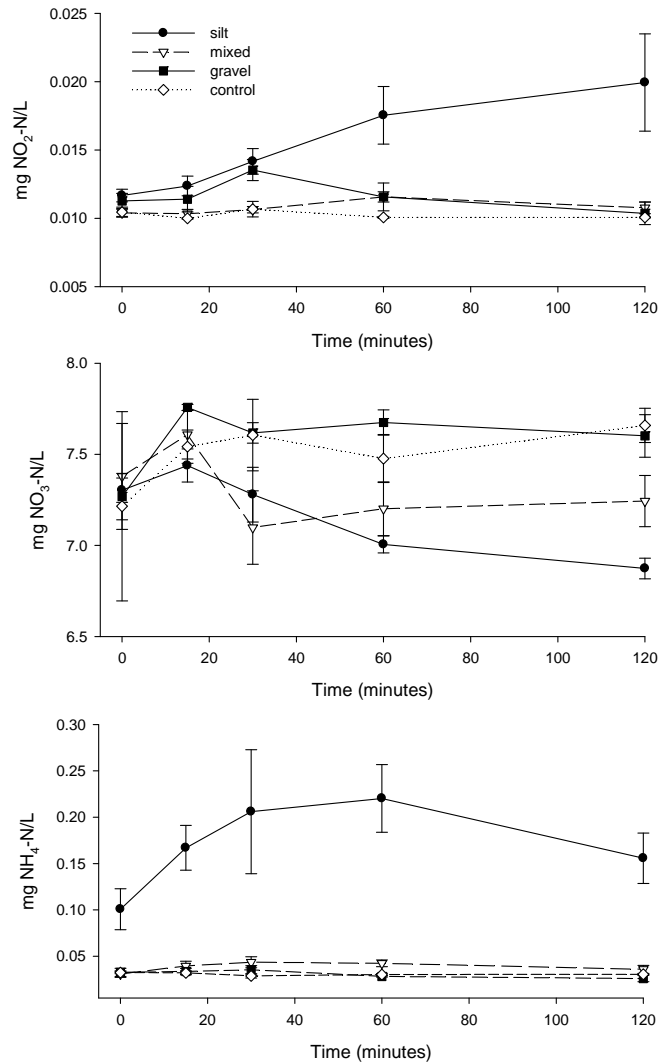
REFERENCES

APHA [American Public Health Association]. 1998. Standard methods for the examination of water and wastewater. Twentieth edition. American Public Health Association, Washington, D.C., USA.

Böhlke, J.K., R.L. Smith, and J.E. Hannon. 2007. Isotopic analysis of N and O in nitrite and nitrate by sequential selective bacterial reduction to N_2O. Analytical Chemistry 79:5888-5895.

Booth, E.G. and S.P. Loheide. 2010. Effects of evapotranspiration partitioning, plant water stress response and topsoil removal of a floodplain wetland: implications for restoration. on the soil moisture regime. Hydrological Processes 24:2934-2946.

Booth EG, Loheide SP, Hansis RD II. 2009. Postsettlement alluvium removal: a novel floodplain restoration technique (Wisconsin). Ecological Restoration 27:136–139.

Burgin, A.J. and S.K. Hamilton. 2007. Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. Frontiers in Ecology and the Environment 5:89-96.

Chen, G., X. Cao, C. Song, and Y. Zhou. 2010. Adverse effects of ammonia on nitrification process: the case of Chinese shallow freshwater lakes. Water, Air, and Soil Pollution 210:297-306.

Craig L.S., M.A. Palmer, D.C. Richardson, S. Filoso, E.S. Bernhardt, B.P. Bledsoe, M.W. Doyle, P.M Groffman, B.A. Hassett, S.S. Kaushel, P.M. Meyer, S.M. Smith, and P.R. Wilcock. 2008. Stream restoration strategies for reducing river nitrogen loads. Frontiers in Ecology and the Environment 6, doi:10.1890/070080.

Kelso, B.H., R.V. Smith, R.J. Laughlin, and S.D. Lennox. 1997. Dissimilatory nitrate reduction in anaerobic sediments leading to river nitrite accumulation. Applied and Environmental Microbiology 63:4679-4685.

Smith, R.L., L.K. Baumgartner, D.N. Miller, D.A. Repert, and J.K. Böhlke. 2006. Assessment of nitrification potential in ground water using short-term, single well injection experiments. Microbial Ecology 51:22-35.

Stanley, E.H. and J.T. Maxted. 2008. Changes in the dissolved nitrogen pool across land cover gradients in Wisconsin streams. Ecological Applications 18:1579-1590.

Stief, P., D. De Beer, and D. Neumann. 2002. Small-scale distribution of interstitital nitrite in freshwater sediment microcosms: the role of nitrate and oxygen availability, and sediment permeability. Microbial Ecology 43:367-378.

Triska, F.J., A.P. Jackman, J.H. Duff, and R.J. Avanzino. 1994. Ammonium sorption to channel and riparian sediments: A transient storage pool for dissolved inorganic nitrogen. Biogeochemistry 26:67-83.

Wood, P. J., and P. D. Armitage. 1997. Biological effects of fine sediment in the lotic environment. Environmental Management 21:203-217.

APPENDIX A

Presentations and Seminars

Stanley, E.H. 2008. Potential sources of nitrite in southern Wisconsin agricultural streams. American Society of Limnology and Oceanography annual meeting, St. John's, Newfoundland.

Stanley, E.H. 2008. On the receiving end: nitrogen in Wisconsin streams. Invited seminar, Program in Ecology, Duke University, Durham, N.C.

Powers, S.M. and E.H. Stanley. 2009. Terrestrial and aquatic controls of inter-annual variability in phosphorus yields. North American Benthological Society annual meeting, Grand Rapids, MI.

Stanley, E.H. 2009. On the receiving end: nitrogen in Wisconsin streams. Department of Zoology, University of Wisconsin, Madison, WI.

Powers S.M. and E.H. Stanley. 2010. Stream and wetland nitrate uptake across extremes in channel form. Joint meeting of American Society of Limnology and Oceanography and North American Benthological Society. Santa Fe, NM.

Stanley E.H. 2010. Nitrogen dynamics in Wisconsin streams. St. Olaf College, Northfield, MN.

Stanley, E.H. 2010. Occurrence and generation of nitrite in ground and surface waters in an agricultural watershed. Wisconsin Groundwater Coordinating Council, Madison, WI.

Publications

Powers, S.M., R.A. Johnson, and E.H. Stanley. Nutrient retention and the problem of hydrologic disconnection in streams and wetlands. In review.