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THE INFLUENCE OF COPPER, LEAD AND IRON ON STREAM SEDIMENT NITRIFICATION IN CENTRAL INDIANA STREAMS

Nicholas C. Reising and **Melody J. Bernot***: Department of Biology, Ball State University, Muncie, 47306

Klaus Neumann: Department of Geological Sciences, Ball State University, Muncie, IN 47306

ABSTRACT. Copper, lead, and iron have frequently been detected throughout Indiana freshwaters. Since microbial activity is a holistic measure of ecosystem function, changes in microbial activity in response to metal concentrations may indicate potential areas of concern. Metal concentrations in seven streams of the Upper White River watershed of central Indiana were measured during spring (May) and summer (August) in conjunction with measurement of sediment nitrification rates using the nitrapyrin-inhibition technique. Additionally, the influence of copper, lead, and iron on microbial nitrification was measured using *in vitro* mesocosms inoculated with stream sediment. Sediment metal concentrations ranged from 654–1,985 mg Fe/kg and 1.00–2.91 mg Cu/kg sediment. Dissolved metal concentrations ranged from below detection to 0.10 mg Fe /L and 0.01–0.02 mg Cu/L. Stream sediment nitrification rates were positively correlated to sediment copper concentrations. Metal concentrations of 127 mg/L may reduce stream sediment nitrification although stream physiochemical characteristics and history of metal exposure also influence microbial response. Further, stream sediment metal concentrations may affect nitrifying microbes more than dissolved metal concentrations.

Keywords: Copper, iron, lead, nitrification, metal concentrations, stream

INTRODUCTION

The rate of nitrification in the environment is dependent on multiple factors including the biological community, dissolved organic carbon (DOC), temperature, dissolved oxygen (DO), and pH (Kemp and Dodds 2001; Strauss et al. 2002; Earl et al. 2006). Nitrification is primarily limited by the concentration of ammonia and nitrite in an environment which can be influenced by stream organisms (Jones and Hood 1980; Villaverde et al. 1996; Lee et al. 1997; Ciudada et al. 2007) as well as surrounding land use (Galloway 1998). Nitrification is secondarily limited by physiochemical characteristics of the environment. Although much research has documented variation in nitrification rates, there is limited understanding of how anthropogenic contaminants may influence nitrification rates. Specifically, increased urbanization and industrial activities have raised concerns regarding metal pollution in freshwaters and their potential influence on microbial activity.

The effect of metal concentrations on nitrification rates is related to the type and concentration of metal and the species of nitrifying microbes present (Mertoglu et al. 2008). Metals are found naturally in the environment but can also enter an ecosystem via human activity including following fossil fuel combustion and leaching from disposed items including batteries and other metal products (Guinee et al.1999; Rimmer et al. 2006). Even as recycling efforts increase, the mining of many metals continues to grow, leading to an increase of metal concentrations in the environment (Guinee et al.1999). Landscape remediation and construction activities can cause metals trapped within soil to more enter aquatic ecosystems causing the sediment to act as a metal sink (Blake et al. 2007). Depending on water flow, sediment-bound metal may diffuse into the water column. The rate of diffusion is a function of water chemistry as well as stream discharge yielding variable metal concentrations over time. Freshwater metal concentrations and their potential

* *Corresponding author*: mjbernot@bsu.edu, Department of Biology, Ball State University, Cooper Life Science, Muncie, IN 47306, 765-285-8828, 765-285-8804 (fax).

effects on the ecosystem must be more comprehensively assessed to protect freshwater integrity.

Certain metal compounds are known to influence biological processes, including microbial activity. Microbial nitrification processes are typically not affected by low metal concentrations; however, as concentrations increase they can inhibit activity (Hu et al. 2004). Toxicity is related to free metal ion concentration, rather than total metal concentration, so metals that dissociate in water are generally more toxic to organisms (Semerci and Cecen 2007). Interestingly, nitrifying microbes exposed *in vitro* to low concentrations of a metal can develop a tolerance to the metal and even resist future exposures of high, normally inhibitory concentrations (Mertoglu et al. 2008). For example, in a stream exposed to mining runoff, long term exposure to copper and lead has been shown to cause changes in metal tolerance levels of the stream microbes and alters the dominant genera (microbial succession) of nitrifying bacteria present (Satchanska et al. 2005), indicating microbial adaptation following metal exposure (Mertoglu et al. 2008).

Copper is an essential trace element in nitrifying bacteria but becomes toxic as concentrations increase to levels that disrupt normal cellular function (Sato et al. 1988). The concentration at which copper becomes toxic depends on bacterial physiology. For example, copper concentrations between 1.27–12.7 mg/L are important for optimal ammonia monooxygenase (AMO) enzyme function (Ensign et al. 1993). However, in both *Nitrosomonas* and *Nitrobacter,* higher copper ion concentrations can decrease nitrification rates (Braam and Klapwijk 1981; Lee et al. 1997; Hu et al. 2004). At higher copper concentrations ($>$ 30 mg/L), ammonia oxidizing bacteria, such as the genus *Nitrosomonas,* show signs of growth delay greater than that of nitrite oxidizers, such as *Nitrobacter* (Lee et al. 1997). In addition, concentrations of only 0.5 mg/L copper can cause significant reduction (50%) in nitrification rates for the genus *Nitrosomonas* (Sato et al. 1988). Copper concentrations in sewer sludge have been reported at 0.10 mg/L (Sato et al. 1988).

Lead ions are highly toxic to nitrifying microbes and cause greater inhibition relative to copper (Mittal et al. 2004). Lead binds to soil particles and also to living and dead microbial cells (Stucznski et al. 2002; You et al. 2009). Lead also adheres to living cells, and may not enter bacterial cells limiting direct influence on bacterial enzymes (Stucznski et al. 2002; Sato et al. 1988). *In vitro* studies have found that lead has no significant influence on either step of the nitrification process when compared to cadmium and nickel (You et al. 2009). Lead concentrations in sewer sludge have been reported at 0.12 mg/L (Sato et al. 1988).

Similar to copper, iron is also an essential element for nitrifying bacteria. The optimal concentration for nitrification is 6 mg/L for *Nitrosomonas* and *Nitrobacter* (Meiklejohn 1957). The lowest concentration of iron needed for nitrification is 0.1 mg/L for *Nitrosomonas* and 0.3 mg/L for *Nitrobacter* (Meiklejohn 1957). Both *Nitrosomonas* and *Nitrobacter* can tolerate iron concentrations of 112 mg/L, although activity is reduced. Iron concentrations in sewer sludge have been reported at 3.0 mg/L (Sato et al. 1988).

To assess the influence of metal concentrations on sediment microbial nitrification, metal concentrations in central Indiana freshwaters were measured. Further, the influence of metal concentrations on sediment nitrification rates was experimentally quantified. The primary goal of this research was to comparatively quantify the influence of copper, lead, and iron on sediment nitrification rates in the streams of the Upper White River Watershed of central Indiana. It was hypothesized that microbial responses to metals are a function of the history of metal exposure and stream physiochemical characteristics. It was further hypothesized that sediment metal concentrations would affect nitrification rates more than dissolved metal concentrations.

METHODS

Site selection.—Seven sites were selected in the Upper White River Watershed of central Indiana to represent a range of agricultural and urban land use in the surrounding sub-watersheds (Fig. 1). All sites selected were 3rd order streams and topographic maps and aerial photographs obtained from the Indiana University GIS spatial data portal (topographic maps from USGS 1984; and aerial photography from Google Earth 2010) were used to determine stream order according to Cole (1994). The White River Watershed covers an area of 174,830 acres and is located in the

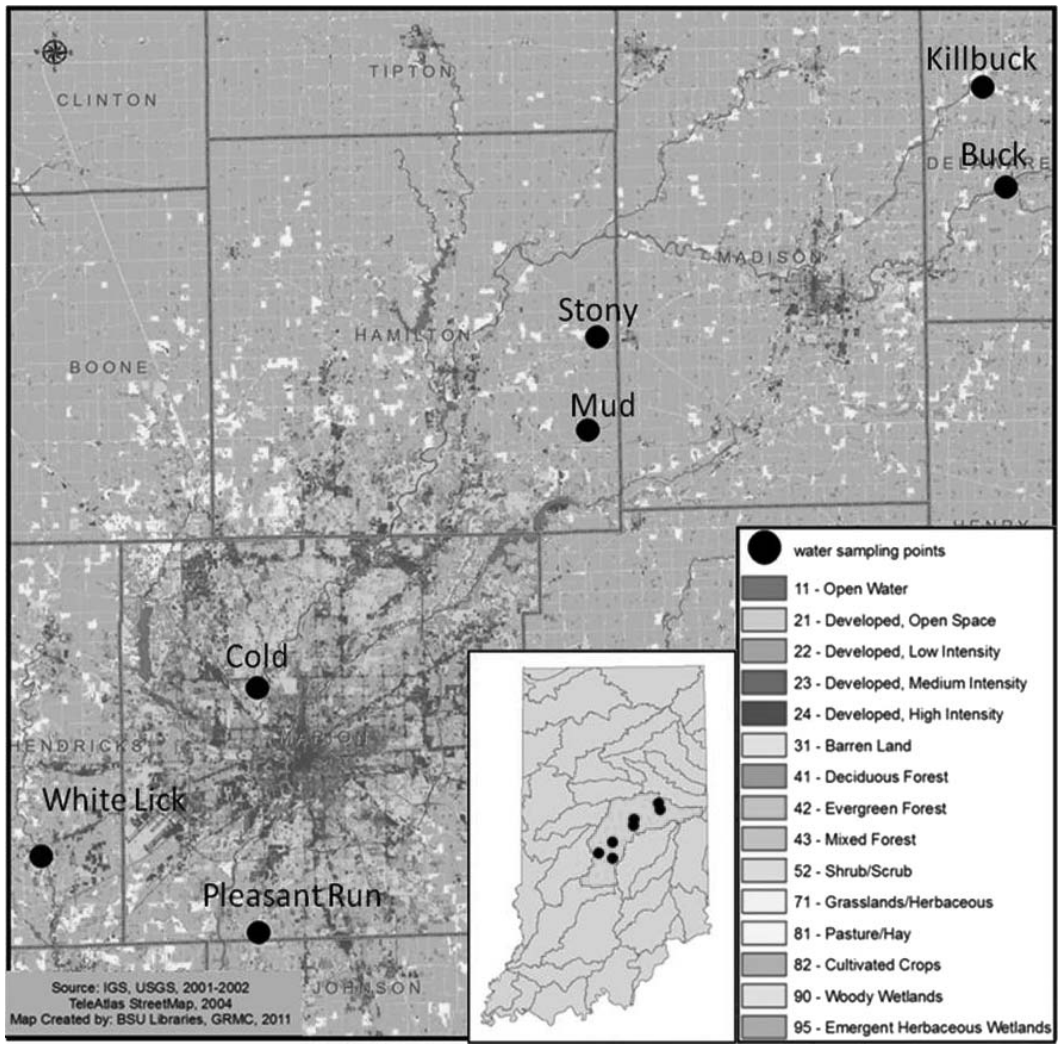

Figure 1.—Location of study sites in the Upper White River Watershed in central Indiana. Coloring denotes land use within the watershed and sites were selected to represent a gradient of land use from urban to agricultural.

Tipton Till plain of east-central Indiana. It contains mostly sand and gravel from glacial deposits. The predominant soil type has been classified as silt loam and highly erodible.

Streams were sampled in May and August 2010 to encompass stream flow at generally higher (May) and lower levels (August) corresponding to spring runoff and base flow. The May and August sampling times also facilitated incorporation of various seasonal changes in stream physiochemical properties such as changes in water temperature, stream biology, riparian characteristics, and land use.

Sediment and water collection.—At each site and sampling event, sediment and water were collected for laboratory nitrification assays. Specifically, a composite sediment sample was collected from the top 5–10 cm of the benthos at several points along the width of the stream channel. Sediment was placed into an acid-washed bucket with a lid. Additionally, ~2.5 L of stream water (unfiltered) was collected from a well-mixed portion of the stream into acid-washed 1 L Nalgene bottles. An additional 250 mL of stream water was collected and immediately filtered using a syringe fitted with

glass fiber filters (Whatman GF/F, 0.7 μm pore size) into a 250 mL Nalgene acid-washed bottle for subsequent analysis of dissolved nutrient and metal concentrations. At each site, stream physiochemical parameters were measured in the stream thalweg including pH, dissolved oxygen concentration, turbidity, total dissolved solids (TDS), and temperature using a Hydrolab minisonde equipped with an LDO oxygen sensor. After collection, samples were immediately placed on ice for transport to the laboratory. Filtered water samples were frozen within 12 h of collection for subsequent analyses of nutrient and metal concentrations. Sediment and unfiltered water were placed at 4°C (< 24 h) until the assay was begun. Filtered water samples were analyzed for anion and cation concentrations including nitrate (NO_3^--N), phosphate (PO_4-P), chloride (Cl^-), sulfate (SO_4^{2-}), bromide (Br^-), ammonium (NH_4^+-N), lithium (Li^+), potassium (K^+), magnesium (Mg^{2+}), and calcium (Ca^{2+}) using ion chromatography (DIONEX, ICS-3000 and 2000).

Laboratory mesocosms.—Laboratory mesocosms were prepared by sieving collected sediment, separately for each site, using a nylon (2.54 mm × 1.25 mm) screen to homogenize the sediment and remove debris. After sediment homogenization, sediment from each site was separately sub-divided for preparation of laboratory mesocosms by filling a graduated cylinder with 40 cm^3 of sediment and placing into 250 mL glass containers. After sediment addition, 56 mL of site-appropriate stream water was added to each mesocosm. Five paired replicate mesocosms (N = 10) were prepared for each site and treatment. Prepared laboratory mesocosms from each site were randomly assigned one of 4 treatments including a control (no metal addition), copper addition (127 mg/L), lead addition (127 mg/L), and iron addition (127 mg/L). Metal additions were made using 14 mL of prepared TraceCERT standards for ICP (1000 mg/L Cu, Pb, Fe dissolved in a 2% nitric acid solution) metal stock (Sigma Aldrich) which is 7.62 mg metal per 60 mL total flask content volume. The control mesocosms received 14 mL of deionized water to bring to equal volume relative to treatments. Each treatment had 5 paired replicates prepared for each stream sampled (N = 280 total mesocosms).

Nitrification activity.—Nitrification activity was measured using nitrapyrin-inhibition assays on paired replicate mesocosms. Specifically, five replicate mesocosms were treated with nitrapyrin dissolved in dimethly sulfoxide (DMSO) to reach a mesocosm concentration of 10 mg/L nitrapyrin (Kemp and Dodds 2001). The remaining 5 paired replicates were treated with an equal volume of DMSO only. After nitrapyrin and DMSO were added to mesocosms, the mesocosms were gently bubbled with air for ~10 s and covered with a tarp to block light. Mesocosms were incubated for 5 d with all mesocosms briefly uncovered, bubbled with air for ~10 s, and immediately re-covered every 24 h to ensure mesocosms remained oxic.

After incubation, ammonium was extracted from sediment by adding 10 mL of 1 N potassium chloride (KCl), mixing the flasks, then incubating for 10 min, followed by a 30 min sediment settling period. Overlying water was then filtered with glass fiber filters (Whatman GF/F, 0.7 μm pore size) into an acid washed 15 mL Falcon tube and immediately refrigerated (< 24 h) for subsequent analyses of ammonium concentrations using the phenol-hypochlorite technique (Weatherburn 1967). Remaining water was decanted and mesocosms were placed in a 75°C drying oven overnight, followed by measurement of sediment dry mass in each individual mesocosm. Nitrification rates were calculated for each paired replicate mesocosm (N = 5 for each treatment) by subtracting the measured ammonium concentration in the nitrapyrin-treated paired replicate from the DMSO-only paired replicate, and then dividing by mesocosm sediment dry mass and total incubation time for expression of the nitrification rate as μg NH_4-N/gdm/d.

Bioavailable sediment metal concentrations.—Biologically available metal concentrations in sediments were quantified according to McKeague (1978). Using the collected homogenized and dried stream sediment from each site, 0.5 g of sediment was placed into a 15 mL acid-washed Falcon tube. For each stream site, two replicates were prepared for a total of two tubes per site, in addition to three water and acid oxalate blanks. Acid oxalate (10 mL) was added to each tube and the tubes were capped. All tubes were then shaken horizontally in the dark for 4 h. After shaking, tubes were centrifuged at 2000 × g for 13 min, and the supernatant decanted and saved for metal analysis on the ICP-OES, Perkin Elmer Optima

2100 DV. Remaining sediment was discarded. Due to high iron concentrations in the sediment, a 1/10 dilution was used to keep the samples within the standard range.

Total sediment metal concentrations.—To determine the total metal concentration in stream sediment, a multi-acid digestion was used, modified from Briggs and Meyer (2002). To perform the assay, 0.2 g of dry homogenized stream sediment was placed into a Teflon vessel with subsequent addition of 3 mL (30% w/v) H_2O_2. After 24 h, 2 mL of concentrated nitric acid (65% w/v) was added to each vessel, followed by 1 mL of concentrated (40% w/v) hydrofluoric acid. The vessels were then capped and heated (~100 °C) overnight, then uncapped and heated until dry. The nitric and hydrofluoric acid steps were repeated 3 times due to undissolved materials. Three mL of H_2O_2 was then added to each vessel and heated until dry to remove remaining organics. The H_2O_2 addition was also repeated 3 times. Due to high iron concentrations in the sediment, iron samples were run with an additional 1/10 dilution.

To determine metal concentrations in the water column, 15 mL acid washed Falcon tubes were used. To each tube, 10 mL of acidified stream water (5 ml of 65% w/v nitric acid per 100 mL of stream water) was added. Three replicates were made for each site for a total of 21 tubes. The samples were then refrigerated until analyzed on the ICP-OES for metal concentration. All transference of liquid was performed using acid washed bottles and pipette tips.

Calculations and statistical analyses.—Differences in control nitrification rates and metal concentrations among streams were compared using one way analysis of variance (ANOVA). Two Sample t-tests were used to compare differences in nitrification rates, sediment and water metal concentrations between the sampling events (May, August). Bonferroni corrected Pearson correlations were used to identify relationships between stream physicochemical parameters, nitrification rates, and metal concentrations (Pearson correlation coefficients, r, and probability, p, reported). ANOVA and t-tests were performed using MiniTab 16 Software, and correlation statistics were performed using SAS Statistical software using p-*values* ≤ 0.05 to determine significance.

RESULTS

Sediment bioavailable metal concentrations.—Overall, bioavailable copper, lead, and iron concentrations in stream sediment varied among sites (Fig. 2). Sediment concentrations ranged from 654–1,985 mg Fe/kg sediment and from1.00–2.91 mg Cu/kg sediment (Fig. 2). Lead concentrations were below detection limits at all sites except in May at one site (Pleasant Run Creek, 0.47 mg Pb/kg sediment) and in August at one site (Mud Creek, 0.38 mg Pb/kg sediment). Across sites, significant differences in bioavailable metal concentrations were identified between sampling events (May *vs.* August; $p < 0.05$). Specifically, bioavailable iron in Buck Creek was ~25% higher in May (876 mg Fe/kg) relative to August (654 mg Fe/kg; $p = 0.030$, Fig. 2). Similarly, bioavailable iron in Killbuck Creek was ~41% higher in May (1,985 mg Fe/kg) relative to August (1161 mg Fe/kg; $p = 0.01$; Fig. 2). Bioavailable copper concentrations in Killbuck Creek were ~51% higher in May (2.63 mg Cu/kg) relative to August (1.29 mg Cu/kg; $p = 0.004$).

Dissolved bioavailable metal concentrations.—Copper and iron concentrations in stream water varied among sites and ranged from below detection to 0.10 mg Fe /L, and from 0.01–0.02 mg Cu/L (Fig. 3). Lead concentrations were below detection limits at all sites and sampling events. Overall, bioavailable iron concentrations (mean = 0.026 mg/L) in stream water were greater than copper concentrations (mean = 0.013 mg/L; $p = 0.01$). Across sites, significant differences in bioavailable iron concentrations in the water column were identified between sampling events (May, August; $p < 0.05$) in all streams, except White Lick ($p = 0.904$), with higher dissolved iron concentrations in August relative to May (Fig. 3). Killbuck Creek was the only site with significant differences in dissolved copper concentrations between May and August (0.02 mg/L vs. 0.01 mg/L; $p = 0.037$; Fig. 3).

Control nitrification rates.—Overall, control nitrification rates were ~76% greater in May (mean = 4.31 μg NH_4-N/gdm/d) than August (mean = 1.05 μg NH_4-N/gdm/d; $p = 0.001$; Fig. 4). Across sites, significant differences in control nitrification rates were identified between sampling events (May, August; $p < 0.05$) only in Killbuck Creek ($p = 0.001$). No other

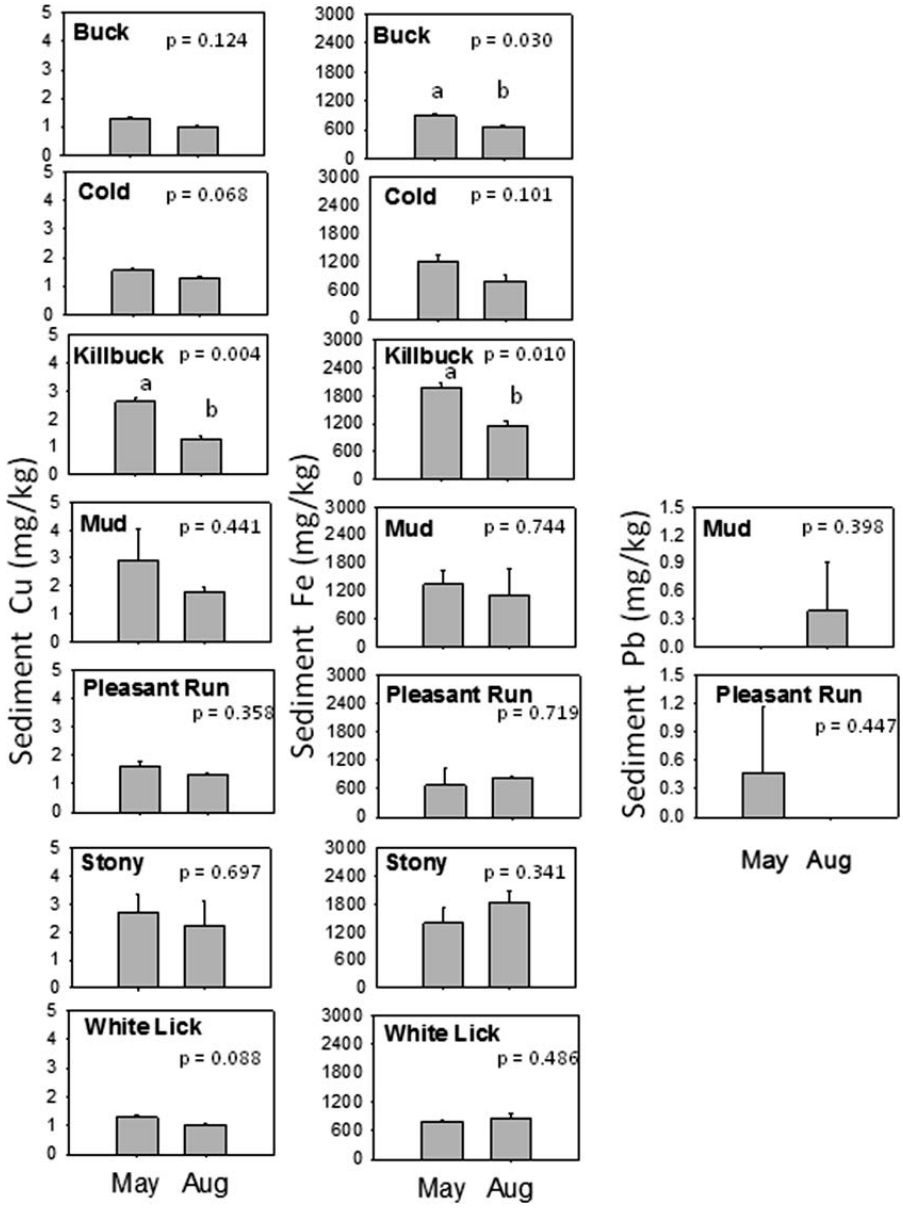

Figure 2.—Mean (N = 5) sediment copper, iron, and lead concentrations in sampled streams during May and August ± 1 standard error (SE). Sediment lead concentrations were below detection except for two sites during one sampling event. Different letters denote significant difference in concentrations between May and August for a given stream. See Figure 1 for stream locations.

stream showed significant differences in nitrification rates with sampling time (Fig. 4).

Nitrification response to metals.—Overall, there was not a consistent nitrification response to metal enrichment across sites (Fig. 5). Nitrification response to metal enrichment did not differ between May and August ($p > 0.05$; *data not shown*). Iron enrichments did reduce nitrification rates compared to the control during August at some sites ($p < 0.05$: Buck Creek - 0.00 vs. 1.72 µg NH_4-N/gdm/d; Mud Creek - 0.00 vs. 8.89 µg NH_4-N/gdm/d; Pleasant Run Creek − 0.02 vs. 1.29 µg NH_4-N/gdm/d; White Lick Creek - 0.03 vs. 0.715 µg

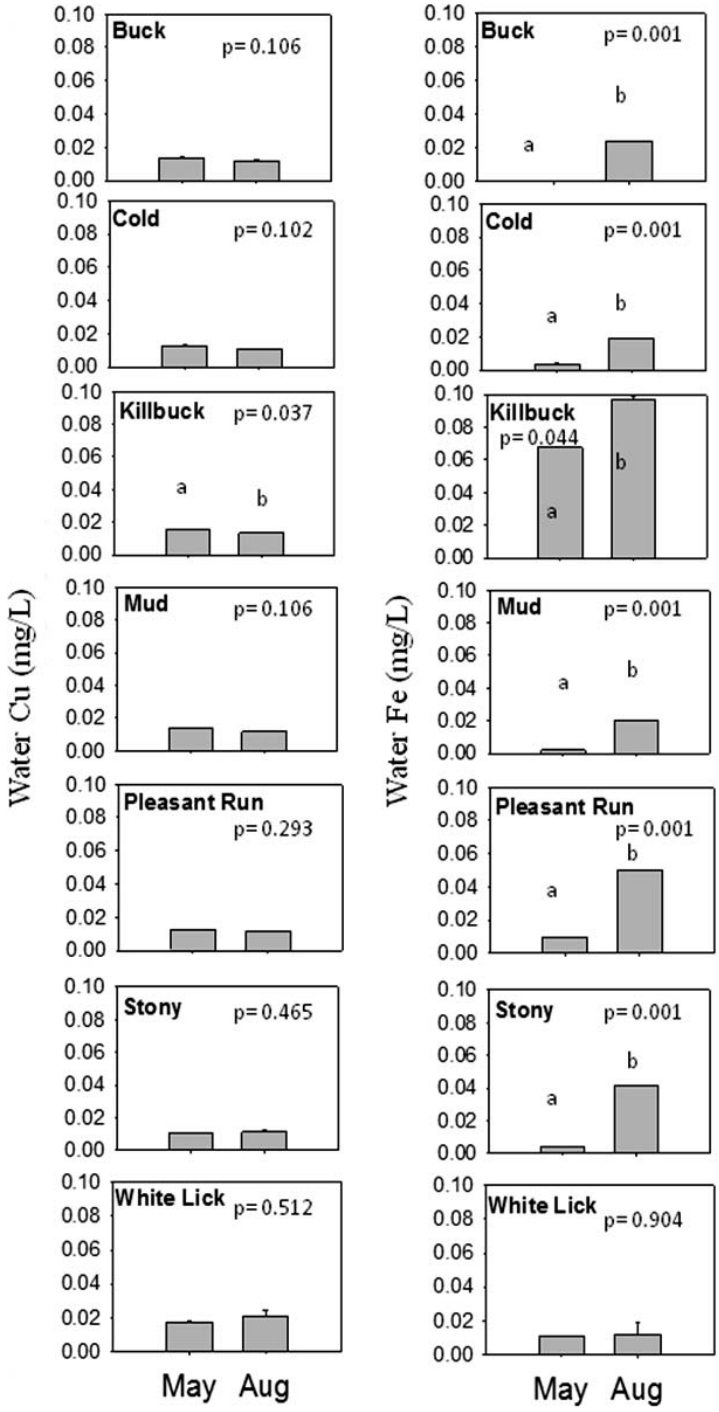

Figure 3.—Mean (N = 5) water column (i.e., dissolved) copper and iron concentrations in sampled streams during May and August ± 1 standard error (SE). Water column lead concentrations were below detection in all samples. Different letters denote significant difference in concentrations between May and August for a given stream. See Figure 1 for stream locations.

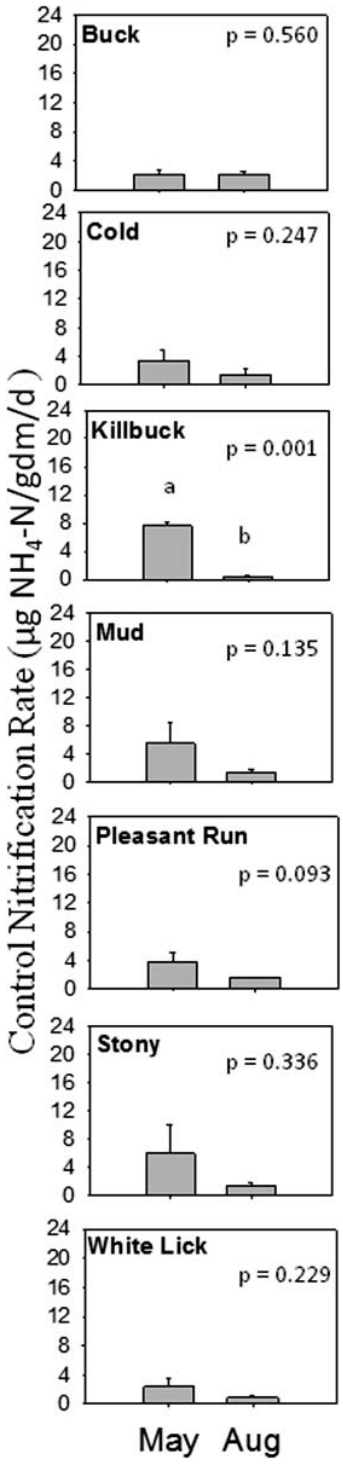

Figure 4.—Mean (N = 5) control sediment nitrification rates in sampled streams during May and August ± 1 standard error (SE). Different letters denote significant difference in nitrification rates between May and August for a given stream. See Figure 1 for stream locations.

NH_4-N/gdm/d; Fig. 5). Copper enrichments decreased nitrification rates in May compared to the control in Killbuck Creek (1.34 vs. 7.58 µg NH_4-N/gdm/d; $p = 0.020$) and Pleasant Run Creek (0.00 vs. 3.86 µg NH_4-N/gdm/d; $p = 0.001$; Fig. 5). Copper enrichments also increased nitrification rates compared to the control during August in Killbuck Creek (1.01 vs. 0.279 µg NH_4-N/gdm/d; $p = 0.024$). Significant decreases in August nitrification rates with copper enrichment compared to the control were found in Buck Creek (0.17 vs. 1.72 µg NH_4-N/gdm/d; $p = 0.032$), Pleasant Run Creek (0.09 vs. 1.29 µg NH_4-N/gdm/d; $p = 0.021$), and White Lick Creek (0.05 vs. 0.715 µg NH_4-N/gdm/d; $p = 0.045$). Lead enrichments decreased nitrification rates in May compared to the control in Killbuck Creek (0.99 vs. 7.58 µg NH_4-N/gdm/d; $p = 0.001$). Lead enrichments also significantly decreased August nitrification rates compared to the control in Buck Creek (0.13 vs. 1.72 µg NH_4-N/gdm/d; $p = 0.029$), Cold Creek (0.00 vs. 1.14 µg NH_4-N/gdm/d; $p = 0.001$), Pleasant Run Creek (0.00 vs. 1.29 µg NH_4-N/gdm/d; $p = 0.001$), Stony Creek (0.01 vs. 1.13 µg NH_4-N/gdm/d; $p = 0.037$), and White Lick Creek (0.00 vs. 0.715 µg NH_4-N/gdm/d; $p = 0.001$).

Factors influencing metal concentrations and nitrification rates.—Stream pH, temperature, total dissolved solids (TDS), and dissolved oxygen concentrations (DO) were not significantly correlated with sediment iron concentrations ($p > 0.05$; *data not shown*). Dissolved iron concentrations were negatively correlated with stream DO ($r = -0.75$, $p = 0.003$; Fig. 6). Stream pH, temperature and TDS were not significantly correlated with dissolved iron concentrations ($p > 0.05$; Fig. 6). Sediment copper concentrations were negatively correlated with stream temperature ($r = -0.66$, $p = 0.010$; *data not shown*). Stream pH, TDS, and DO were not significantly correlated with sediment copper concentrations ($p > 0.05$; *data not shown*). Stream pH, temperature, TDS, and DO were not significantly correlated with dissolved copper concentrations ($p > 0.05$; *data not shown*).

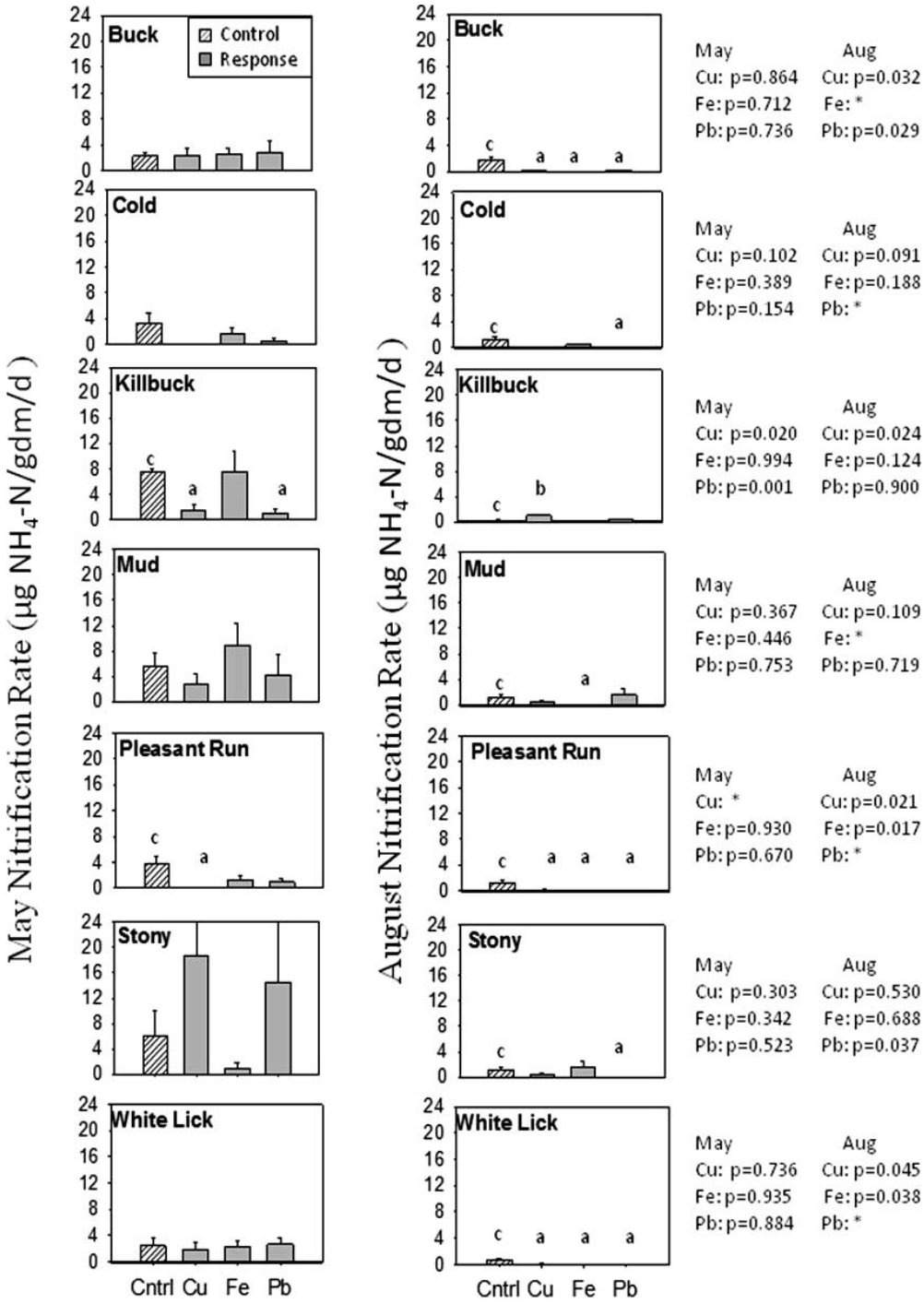

Figure 5.—Mean (N = 5) nitrification rates in metal enriched mesocosms in May and August ± 1 standard error (SE). "a" indicates value is significantly greater than the control. "b" indicates value is significantly less than the control and "c" denotes the control value. P-values are indicated for the comparison of control rates to metal enrichments treatments (a,b,c) (*) indicates no detected nitrification.

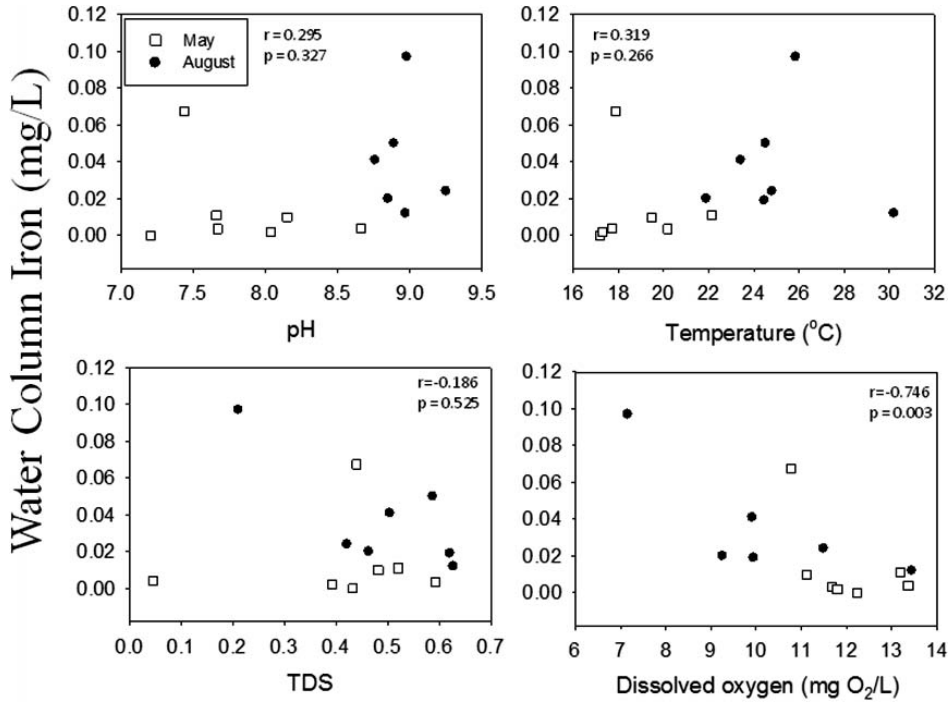

Figure 6.—Correlations between water column (i.e., dissolved) iron concentrations and stream physiochemical parameters: water column pH, total dissolved solids (TDS), dissolved oxygen, and temperature. N = 14.

Control nitrification rates were positively correlated with sediment copper concentrations ($r = 0.78$, $p = 0.001$; Fig. 7). There was no significant correlation between control nitrification rates and sediment iron concentrations, water iron concentrations, water copper concentrations, stream pH, or TDS ($p > 0.05$; Fig. 7). Nitrification response to metal enrichment was positively correlated to total iron concentrations ($r = 0.61$, $p = 0.02$; Fig. 8) and total copper concentrations ($r = 0.742$, $p = 0.002$; Fig. 8).

DISCUSSION

Metal concentrations in central Indiana streams.—These data suggest iron is the most abundant metal in the selected study sites relative to copper, and lead. Copper was the second most abundant and lead was undetectable in all but two samples (N = 14 total). These findings are consistent with previous reports from the Indiana Department of Environmental Management (IDEM) (Holdeman et al. 1999). The higher presence of iron compared to copper and lead may be attributed to less federal and state monitoring of these contaminants. Iron concentrations are not regulated due to minimal adverse effects at environmentally-relevant concentrations (Holdeman et al. 1999). In contrast, copper is toxic to humans and can function as a biocide to aquatic organisms at environmentally-relevant concentrations (Nirel and Pasquini 2010; Moore and Ramamoorthy 1984). Similarly, lead is toxic to both humans and aquatic organisms at environmentally-relevant concentrations (Moore and Ramamoorthy 1984). Higher concentrations of iron in central Indiana streams may also be due to greater natural occurrences of these elements.

Factors controlling metal concentrations in central Indiana streams.—Geochemical processes and sediment dynamics (i.e., sorption) may influence dissolved metal concentrations in streams, with some evidence suggesting metal concentrations may follow diel cycles (Nimick et al. 2003). Urban et al. (1990) found soluble iron concentrations in lakes were positively correlated with dissolved organic carbon (DOC) concentrations and negatively correlated with

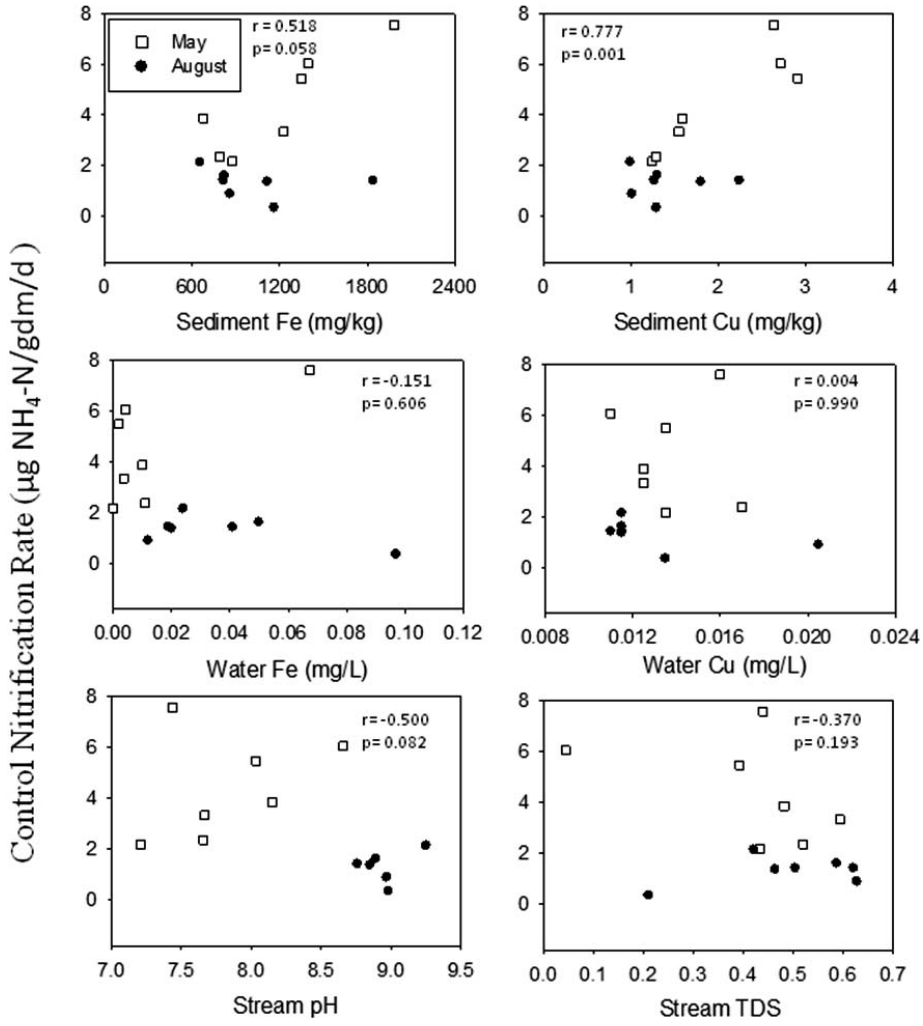

Figure 7.—Correlation between control nitrification rates and stream sediment and water column iron and copper concentrations as well as stream physicochemical parameters (pH and total dissolved solids, TDS). N = 14.

stream pH. Dissolved organic carbon enhances iron mineral phase solubility above pH 5 and buffers dissolved iron content below pH 5 through binding and flocculation processes (Urban et al. 1990). Wen et al. (1998) found pH was positively correlated with copper adsorption rates. Aquatic plants have also been shown to absorb metal from the environment, reducing metal concentrations (Miretzky et al. 2004). The rate at which aquatic plants can remove metals depends on plant species and water conditions such as dissolved oxygen and pH (Miretzky et al. 2004). The relationship between dissolved oxygen and plant metal uptake may be the cause of decreasing dissolved iron concentrations as steam dissolved oxygen increases. Observed relationships between metal concentrations and physiochemical parameters may also be due to biogeochemical processes and factors not measured in this study, such as dissolved organic carbon concentrations and macrophyte abundance.

Factors controlling stream nitrification rates.—Stream sediment nitrification rates measured in this study (1–7 µg NH_4-N/gdm/d) were comparable to rates previously measured in lake sediments (0.4–2.3 µg NH_4-N/gdm/d; Strauss and Dodds 1997). Differences in stream physiochemical factors may have influenced

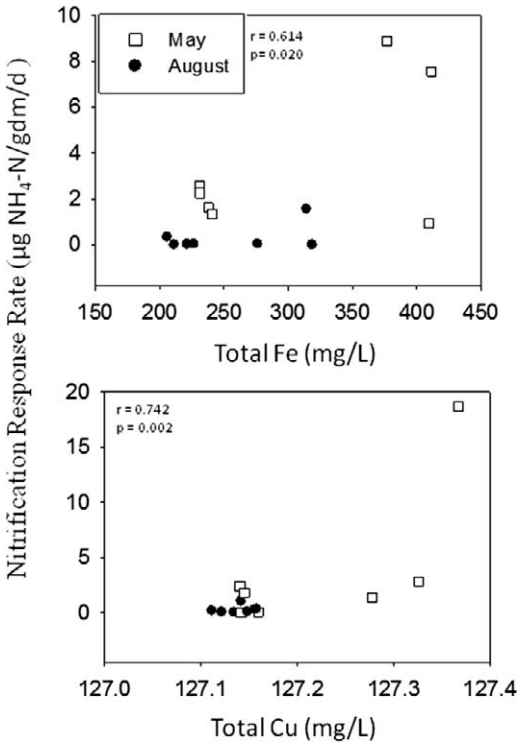

Figure 8.—Correlation between nitrification response and total metal concentration (sum of sediment, dissolved and experimental addition) for iron and copper. N = 14.

nitrification rates measured across sampling events and explain variation between the May and August sampling events. Overall, nitrification rates were greater in the May relative to August. In May, nitrification rate increased with pH around 7.0 to just above 8.5; in contrast, August samples were closely clustered between pH 8.5 and 9. Previous studies (Strauss et al. 2002) show nitrification rate tends to increase with stream pH and optimal conditions of pH 7.5. This is consistent with this study for May samples, but August samples had higher pH (above the optimum of pH 7.5) and lower nitrification rates, potentially due to confounding factors. This also supports the conclusion noted by Strauss et al. (2002) that additional factors other than pH may influence nitrification responses including levels of organic carbon and available ammonium as well as available light. Light can inhibit the growth of nitrifying bacteria (Hagopian and Riley 1998) and even under ideal growth conditions nitrifying bacteria have a relatively slow mean generation time (up to 60 h). Ward et al. (1982) suggested that light levels may strongly influence the location and depth at which nitrifying bacteria are found, with higher nitrification activity occurring below the photic zone of coastal waters. The greater depth of streams in May relative to August (*data not shown*) may have shielded the stream sediment from light, thus allowing for increased activity in May relative to August.

The influence of metals on sediment nitrification rates.—Since nitrifying microbes grow best when bound to a surface and shielded from light (Hagopian and Riley 1998), the stream sediment is the ideal habitat for nitrifying bacteria. Thus, sediment metal concentrations should be more influential than water column metal concentrations. The correlation between nitrification rates and sediment iron and copper concentration suggest that nitrification may be facilitated with increasing concentrations under some conditions. Observations by Dollhopf et al. (2005) indicated an increase in nitrification rate as sediment iron concentrations increased in salt marshes. This increase was attributed to possible protection of ammonium monoxygenase by iron from sulfide. While sulfide was not measured in this study, iron could be performing a similar type of protection which would explain increased nitrification rates as iron concentrations increased. Copper has also been shown to protect nitrifying bacteria against some nitrification inhibition compounds (Campbell and Aleem 1965) which also supports the relationship between increased copper concentrations and increased nitrification rates.

Copper enrichment of Killbuck Creek sediment collected in May decreased nitrification rates relative to controls but increased rates in sediment collected in August when sediment copper concentrations were lower in the ecosystem. Thus, an increase in nitrification rate was observed only when additional copper was added during a period of lower *in situ* copper concentrations in Killbuck Creek. This may have been due to microbial adaption to higher metal concentrations (e.g., Mertoglu et al 2008) or to other factors such as changes in temperature.

The lack of a significant nitrification rate response with iron enrichments in May suggest iron additions of 127 mg/L may not influence microbial nitrification during the May condi-

tions. Sediment iron concentrations were similar in May compared to August with only Killbuck Creek and Buck Creek having lower concentrations in August. Killbuck Creek had the highest dissolved iron concentration and the only evidence of inhibition of nitrification rates with enrichment in August. The lack of a nitrification rate response to iron enrichment may be due to the higher concentrations of iron naturally found in the sampled streams compared to copper. Significant differences were more prevalent for copper treatments including an increase in Killbuck Creek. Nitrifying microbes sampled were from an environment with higher iron concentrations compared to copper. Thus 127 mg/L iron addition did not consistently influence stream nitrification rates. Rather, the total metal concentrations (sum of *in situ* concentration and experimental addition) dictated nitrification response.

Because most samples had lead concentrations below detection and inconsistent responses to lead enrichment, it is difficult to determine with certainty, the influence of lead on microbial nitrification. Observations by You et al. (2009) found that 40 mg/L of lead did not affect nitrification rates in sludge. In this study, the addition of 127 mg/L of lead also did not have a direct influence on nitrification in stream sediment.

Conclusions.—Previous research has indicated that certain metal compounds can influence the physiology of nitrifying microbes although the influence of metals at environmentally-relevant concentrations on stream sediment nitrification rates is not well understood. We found that stream sediment metal concentrations may have a greater influence on nitrification rates relative to dissolved metal concentrations. Nitrification rates in central Indiana streams were comparable to previous nitrification estimates in aquatic ecosystems. Similarly, sediment and dissolved metal concentrations were within previously reported ranges. The nitrification response to metal enrichment in stream ecosystems is likely a function of both physiochemical characteristics of the stream ecosystem and the history of metal exposure. Overall, nitrification rates were lower during August compared to May sampling events regardless of metal enrichment.

The May sampling event was characterized by lower water temperature and pH, but greater dissolved oxygen concentration relative to the August sampling event. Relationships between these physiochemical characteristics and stream sediment nitrification rates suggest that stream physicochemical properties are more influential on microbial nitrification than the 127 mg/L enrichment of copper, lead, and iron. Although a 127 mg/L metal concentration enrichment may reduce stream sediment nitrification rates; stream physiochemical characteristics such as oxygen, pH and light likely dictate the majority of observed microbial responses by affecting biotic activity (i.e., metabolic rates).

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