

Microbial response to nitrogen availability:

Preferential and adaptive community uptake

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Project Abstract

This project was designed to assess the ability of natural sediment microbial communities and single species microbial populations to preferentially utilize inorganic forms of nitrogen (ammonium, $\text{NH}_4\text{-N}$, and nitrate, $\text{NO}_3\text{-N}$, specifically). The first chapter addressed two primary questions: 1) Do sediment microbial communities preferentially assimilate $\text{NH}_4\text{-N}$ or $\text{NO}_3\text{-N}$?; and, 2) Does preferential uptake of nitrogen change with increased $\text{NH}_4\text{-N}$ or $\text{NO}_3\text{-N}$ availability? The second chapter furthered these analyses by assessing shifts in microbial nitrogen assimilation in response to sustained nitrogen enrichments. Primary questions addressed were: 1) Are microbial communities able to adapt to nitrogen enrichment and preferentially utilize a more available source?; and, 2) Are initial microbial responses to nitrogen enrichment different from sustained responses? Questions were addressed with *in vitro* laboratory experiments quantifying microbial activity. Overall, microbial community activity changed in response to the form of nitrogen available, enrichment type, and duration of exposure. Data demonstrate sediment microbial communities in the Midwestern US may prefer $\text{NO}_3\text{-N}$ over other forms of nitrogen. However, microbial communities became saturated with $\text{NO}_3\text{-N}$ with increases in concentrations >0.75 mg $\text{NO}_3\text{-N/L}$. Microbial communities were able to adapt to higher nitrogen concentration and increase rates of assimilation for both $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$. Thus, microbial communities are robust in response to nitrogen increases in an ecosystem, even in high nitrogen environments like the Midwestern US.

Project Introduction

Nitrogen Cycle

Nitrogen is an essential nutrient for all living organisms and is required for basic structural molecules including amino acids, nucleic acids, enzymes, and many lipids and carbohydrates. Thus, the relative abundance of nitrogen in the environment can limit growth and reproduction of organisms at all trophic levels (Vitousek and Howarth, 1991). In the natural environment, nitrogen exists in several forms including organic nitrogen and inorganic nitrogen, the latter of which is composed of ammonia (NH_3), ammonium ($\text{NH}_4\text{-N}$), nitrite (NO_2), nitrate ($\text{NO}_3\text{-N}$), nitrous oxide (N_2O) and dinitrogen gas (N_2) (Figure 1). Organic nitrogen is manifested as hundreds of compounds including basic amino acids, enzymes, complex proteins and carbohydrates. The most abundant form of nitrogen is N_2 , which comprises 78% of the atmosphere and is the least biologically reactive form of nitrogen due to its triple bond that is energetically difficult for organisms to access (Schlesinger, 1997). A few specialized bacteria with the enzyme nitrogenase can utilize N_2 via nitrogen fixation. Other bacteria can oxidize $\text{NH}_4\text{-N}$ into NO_2 or $\text{NO}_3\text{-N}$ via nitrification. This NO_2 and NO_3 can also be utilized by microbes and reduced to N_2 or N_2O via denitrification. Many organisms can also directly incorporate organic nitrogen, $\text{NH}_4\text{-N}$, NO_2 , and $\text{NO}_3\text{-N}$ into cells by both heterotrophic and autotrophic assimilatory uptake.

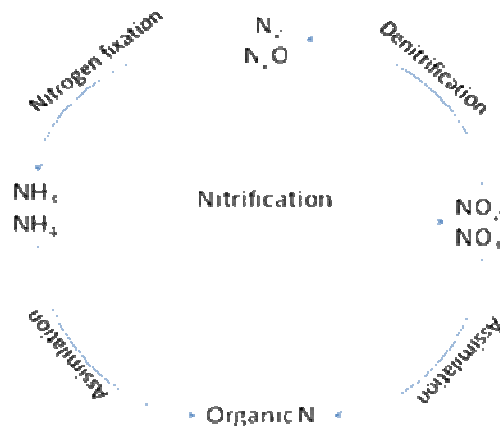


Figure 1. Potential nitrogen pathways for inorganic nitrogen forms into organic nitrogen.

Microbial Uptake of Nitrogen

In any ecosystem, microbes are ubiquitous and essential to nitrogen processing. The importance of microbes to nitrogen cycling has been advanced within the context of the microbial loop. The microbial loop is the return of energy lost from dissolved organic matter and returned to the food chain via microorganisms including bacteria, fungus, and protozoa (Azam et al., 1983; Figure 2). Approximately 60% of nutrients in a given ecosystem are assimilated through the microbial community whereas only 10-20% of nutrients are assimilated through higher trophic levels (Payne, 1970). For example, bacteria in oceans assimilate 10-50% of photosynthetically fixed carbon (Azam et al., 1983); sediment microbes in lakes provide half of the carbon requirements for organisms at higher trophic levels (Hart et al., 2000); and, 86% of total autotrophic production in oligotrophic Mediterranean freshwaters is used by protozoa and heterotrophic bacteria for respiration (Hagstrom et al, 1988). A similar phenomenon is observed in terrestrial ecosystems where microbial decomposers are more influential to ecosystem nitrogen cycling than plants with respect to overall rates of nitrogen transformation (Knops et al.,

2002; Chapman et al., 2006). Microbial organisms also account for a majority of inorganic nitrogen uptake in streams (Dodds et al., 2000; Peterson et al., 2001). Thus, mitigation of nitrogen pollution associated with human activities is likely to be most effective when the activity of the microbial community is fully understood.

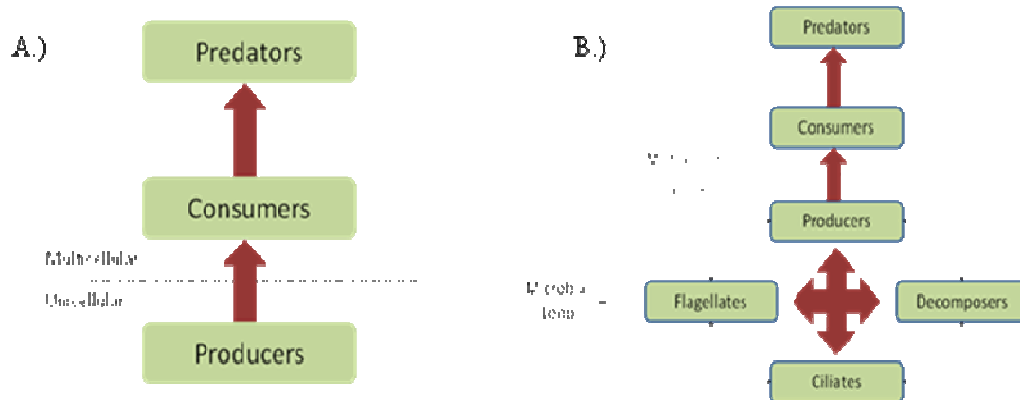


Figure 2: A.) Traditional diagram showing the flow of energy and nutrients beginning with consumption and release by primary producers which then flows to consumers and predators. B.) Actual flow of nutrients (i.e., nitrogen) and energy. Nutrients and energy are cycled, not just released by producers, and involve more complex interactions between other microorganisms (decomposers, ciliates, and flagellates).

Chapter 1: Preferential uptake of available nitrogen forms

Abstract

All organisms require nitrogen and the relative abundance of different forms of nitrogen can influence microbial growth and reproduction. We measured preferential uptake of inorganic forms of nitrogen (ammonium, $\text{NH}_4\text{-N}$, and nitrate, $\text{NO}_3\text{-N}$) in both cultured *Pseudomonas fluorescens* populations and mixed species microbial communities in natural stream sediment. Uptake kinetics of nitrogen assimilation by mixed-microbial communities was also quantified. Microbial communities were placed into mesocosms with filtered stream water. Microbes were then exposed to high concentrations of both $\text{NO}_3\text{-N}$ ($>1\text{mg N/L}$) and $\text{NH}_4\text{-N}$ ($> 30 \mu\text{g N/L}$), to determine if preferential uptake varied with either concentration or form of available nitrogen. After 3 d incubation, filtered water extracts were collected for measurement of ammonium and nitrate concentrations using standard colorimetric ($\text{NH}_4\text{-N}$) and ion chromatography procedures ($\text{NO}_3\text{-N}$). Increased availability of $\text{NO}_3\text{-N}$ in natural mixed-microbial communities decreased $\text{NH}_4\text{-N}$ uptake, likely due to preferential uptake of the more abundant $\text{NO}_3\text{-N}$. In contrast, increasing $\text{NH}_4\text{-N}$ concentrations yielded higher $\text{NH}_4\text{-N}$ uptake. Increasing $\text{NO}_3\text{-N}$ yielded microbial saturation of activity when concentrations were $>0.75 \text{ mg N/L}$. These data indicate both single-species microbial cultures and natural mixed-microbial communities can preferentially utilize $\text{NH}_4\text{-N}$ or $\text{NO}_3\text{-N}$ in response to changing availability.

Introduction

Nitrogen is an essential nutrient that is commonly limiting to organism production in many different ecosystems (Webster et al., 2003). However, concentrations and composition of available nitrogen in ecosystems is changing due to human activities including fossil fuel combustion, wastewater discharge, and fertilizer runoff (Howarth, 1996; Vitousek et al., 1997; Middelburg and Nieuwenhuize, 2000). These human alterations to the environment have approximately doubled the nitrogen availability in ecosystems worldwide (Vitousek et al., 1997). For example, nitrate ($\text{NO}_3\text{-N}$) concentrations in the Mississippi River have doubled since 1965 (Turner and Rabalias, 1991). Similarly, a study on the Ave River in northwestern Portugal noted a 2-fold increase in $\text{NO}_3\text{-N}$ and ammonium ($\text{NH}_4\text{-N}$) concentrations at agriculturally-influenced sites relative to reference sites (Pascoal et al., 2005). In streams, agricultural activity can increase $\text{NO}_3\text{-N}$ an order of magnitude and $\text{NH}_4\text{-N}$ approximately 4-fold relative to unimpacted sites (Kemp and Dodds, 2001; Mulholland et al., 2008).

Given the increase in nitrogen concentrations and the importance of microbial communities to nitrogen cycling, a more comprehensive understanding of microbial response to nitrogen availability is needed. Specifically, we do not yet understand if microbial communities can preferentially utilize one form of nitrogen over another based on availability. Bacteria can use both organic and inorganic nitrogen (Brown, 1980; Kemp and Dodds, 2001; Kemp and Dodds, 2002), but there is a paucity of information regarding the relative rates of assimilation in natural ecosystems. Insight on how microbes utilize these different forms of nitrogen may help in mitigating environmental problems associated with increased nitrogen availability. Problems associated with

increased nitrogen include eutrophication, decreased biodiversity, fish kills, and human health concerns associated with drinking water including links to non-Hodgkins lymphoma and methylhemoglobinemia (US EPA, 1990; Carpenter et al., 1998).

Preferential uptake is defined here as selection of one form of nitrogen over another (e.g., selectively utilizing $\text{NH}_4\text{-N}$ and not $\text{NO}_3\text{-N}$). Microbes may preferentially utilize one form of nitrogen to meet growth requirements when nitrogen is limiting or to avoid competitive constraints associated with acquiring needed nitrogen. The phenomenon of preferential nutrient uptake is most pronounced with limiting nutrients (Thomas, 1999) and in many ecosystems nitrogen is the primary element limiting growth. Such preferential uptake of nitrogen likely provides species with effective coexistent properties under nitrogen limiting conditions (McKane, 2002). Nitrogen limitation was demonstrated in Sycamore Creek, Arizona, when microbial activity increased after nitrogen additions but did not increase after phosphorus additions (Grimm and Fisher, 1986). Nitrogen limitation has also been observed in terrestrial ecosystem net primary production (Gutschik 1981, 1987; Lee et al., 1983; Binkley, 1986; Kimmins, 1987; Tilman, 1988). Specific terrestrial ecosystems that have shown nitrogen limitation with additions of nitrogen increasing production are arctic and alpine tundra (Bliss, 1963; Shaver & Chapin, 1980), boreal forests (Bonan, 1990), and temperate forests (Hunt et al., 1988). Several lake environments also exhibit nitrogen limitation on net primary production including both tropical (Hecky and Kilhan, 1988) and temperate-zone (Goldman, 1988) lake ecosystems. Howarth (1988) also found that net primary production was limited by nitrogen availability in coastal marine ecosystems. Thus, despite increasing nitrogen concentrations world-wide, diverse ecosystems still exhibit

conditions of nitrogen limitation. Preferential uptake in response to nitrogen limitation has been observed in aquatic communities including lakes (McCarthy et al., 1982), streams (Mullholland, 2004), and rivers (Naudin et al., 2001) as well as terrestrial ecosystems but the mechanisms remain unknown.

Individual organisms can periodically exhibit preferential uptake of nutrients. For example, plant (Watson, 1986; Padgett and Leonard, 1996; Nordin et al., 2001; Weigelt et al., 2005), animal (Wilkerson and Muscatine, 1984), and microbial (McCarthy et al., 1982; Wheeler and Kirchman, 1986) organisms have all been shown to exhibit preferential uptake for specific forms of nitrogen under certain conditions. Specifically, low soil temperatures (Watson, 1986) and forest type (Nordin et al., 2001) yield different nitrogen preferences in plants. Furthermore, light availability can be a factor controlling preferential uptake in freshwaters (McCarthy et al., 1982). Presumably, nutrient availability is a foundational property controlling preferential uptake though relationships between nitrogen concentrations and preferential uptake are inconsistent (McCarthy et al., 1982), likely due to interacting physiochemical controls.

Energetically, organisms likely prefer $\text{NH}_4\text{-N}$ over $\text{NO}_3\text{-N}$ due to the reduced state which is more energetically favorable for incorporating into organic nitrogen (e.g., Watson, 1986; Wheeler and Kirchman, 1986; Vermeer et al., 2003). To fix 1 g of nitrogen requires 8-12 g of glucose by a symbiotic fixer; $\text{NO}_3\text{-N}$ requires a similar amount of energy to be reduced to organic nitrogen to necessary organic forms (Gutschick, 1981). Microbial decomposers in oat straw prefer $\text{NH}_4\text{-N}$ over $\text{NO}_3\text{-N}$ when availability is equal (Jansson et al., 1955). However, some organisms preferentially utilize $\text{NO}_3\text{-N}$ or organic nitrogen, despite energetic constraints. Specifically, some

grassland plants utilize $\text{NO}_3\text{-N}$ preferentially relative to reduced forms of nitrogen regardless of availability (Miller and Bohman, 2003) to relieve competitive constraints for limited nitrogen. Jorgensen et al. (1999) documented microbial communities preferentially utilize organic nitrogen (as amino acids) over inorganic nitrogen ($\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$) in both the Gulf of Mexico (open-water) and Santa Rosa Sound (estuary). Thus, preferential uptake is common across ecosystems and organisms though clear predictive mechanisms are lacking. If microbes can respond to variation of availability of different form of nitrogen by altering preferential use of different nitrogen compounds, mitigation of nitrogen pollution may be better managed to maximize preferential uptake of specific forms of nitrogen.

To assess preferential nitrogen uptake by microbial communities, sediment microbial community response to nitrogen availability as $\text{NH}_4\text{-N}$ assimilation and $\text{NO}_3\text{-N}$ assimilation was quantified. Preferential nitrogen uptake was compared between a cultured single-species microbial population and a natural sediment microbial community to assess potential differences noted in laboratory studies relative to previous field studies. Two specific research questions were addressed: 1) Do sediment microbial communities preferentially assimilate $\text{NH}_4\text{-N}$ or $\text{NO}_3\text{-N}$?; and, 2) Does preferential uptake of nitrogen change with increased $\text{NH}_4\text{-N}$ or $\text{NO}_3\text{-N}$ availability? I hypothesized that both microbial communities would prefer $\text{NH}_4\text{-N}$ relative to $\text{NO}_3\text{-N}$, regardless of concentration, due to the more energetically favorable assimilation process.

Materials and Methods

Microbial Inoculum

Two microbial inocula were used in parallel experiments comparing cultured single-species microbial uptake to natural stream sediment microbial community uptake. *Pseudomonas fluorescens* cultures were obtained from dehydrated populations for single-species assessments. The *P. fluorescens* culture was isolated on Pseudomonas agar F and tryptone-glucose-yeast (TGY) after incubation at 25°C. Isolated colonies were then inoculated onto Trypto-Soy agar (TSA) and sub-cultured on TSA slants once every two weeks for the duration of the experiment. Sub-cultures were stored at 2°C. Natural microbial community inoculum, as stream sediment, was collected from a stream in central Indiana by randomly gathering the top 5 cm of sediment from several locations and homogenizing the sample through a USGS no. 5 sieve to remove debris and macroinvertebrates. The stream site was characteristic of streams throughout central Indiana (Bernot et al. 2006). Sediment was collected <24 h prior to experiment start and preliminary experiments indicated microbial activity did not change after holding periods <72 h.

Laboratory Mesocosms

Laboratory mesocosms (N=80) for two experiments (preferential uptake and uptake kinetics) were prepared to quantify nitrogen uptake in response to varying nitrogen forms and concentrations. All mesocosms were constructed of 120 mL HDPE specimen cups. For the natural microbial community, 30 mL homogenized stream sediment was placed into each mesocosm along with 60 mL filtered stream water. For *P.*

fluorescens, 10 mL suspended cells were placed into 80 mL of filtered stream water. All experiments were conducted at room temperature.

Preferential Nitrogen Uptake Experiment

Laboratory mesocosms were used to quantify preferential nitrogen uptake by cultured single-species and natural sediment microbial communities. Mesocosms, prepared as described above, were randomly assigned four nitrogen treatments including a negative control, NO₃-N addition at 1 mg NO₃-N/L, NH₄-N addition at 30 µg NH₄-N/L, and combined NH₄-N and NO₃-N at 30 µg/L NH₄-N and 1 mg/L NO₃-N. Four replicates for each treatment were prepared and analyzed. Treatment concentrations were selected to approximate mean nitrogen concentrations observed in streams throughout the Midwestern United States (Bernot et al., 2006) and were additive to nitrogen available in stream water. Mesocosms were incubated for 3 d followed by filtration of water extract. Extracts were frozen for subsequent chemical analyses detailed below.

Nitrogen Uptake Kinetics Experiment

To further assess potential microbial response to nitrogen, an experiment quantifying uptake kinetics of nitrogen associated with natural sediment microbial communities was conducted. Prepared laboratory mesocosms, as described above, were randomly assigned one of 6 ammonium (NH₄-N) or 6 nitrate (NO₃-N) treatments to quantify microbial uptake of nitrogen with increasing concentration. NH₄-N treatments included a control (no nitrogen addition) and 10, 20, 30, 40, or 50 µg NH₄-N/L additions to mesocosms. NO₃-N treatments included a control (no nitrogen addition) and 0.25, 0.5, 0.75, 1, and 1.25 mg NO₃-N/L addition to mesocosms. Treatment concentrations of ammonium and nitrate were selected to represent the range of nitrogen concentrations

observed in stream ecosystems of the Midwestern United States (Bernot et al., 2006). All $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ treatments had 3 replicate mesocosms for a total of 48 mesocosms. After addition of nitrogen treatments, mesocosms were incubated at room temperature for 3 d. Following incubation, filtered extracts were collected using 25 mm Whatman GF/F glass fiber filters (0.7 μm pore size). Filtered extracts were frozen for subsequent chemical analyses detailed below.

Chemical Analyses

All chemical analyses were conducted on filtered water extracts thawed <24 h prior to analysis. $\text{NO}_3\text{-N}$ concentration was measured by ion chromatography on a Dionex 3000 Ion Chromatograph comparing unknown samples to known standards and incorporating analytical replicates for sample concentrations. $\text{NH}_4\text{-N}$ concentration was measured using the colorimetric phenol-hypochlorite technique (APHA, 1995; Aminot et al., 1997) followed by quantifying absorbance for samples and known standards on a Shimadzu dual-beam spectrophotometer.

Statistical Analyses

Preferential uptake of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ was analyzed by one-way analysis of variance (ANOVA), with nitrogen treatment as the main effect, followed by Tukey's pairwise comparisons for significant differences among means using Minitab software. Net ammonium and nitrate uptake rates were calculated as the decline in $\text{NH}_4\text{-N}$ or $\text{NO}_3\text{-N}$ over time per mass of substrate (*sensu* Kemp and Dodds, 2002). Uptake kinetics were determined by fitting a polynomial curve to data using Sigma Plot statistical software.

Results

Preferential Nitrogen Assimilation

In the natural sediment microbial cultures, uptake of NO₃-N increased with nitrogen enrichment as either NH₄-N or NO₃-N (P<0.001; Figure 1B). NO₃-N uptake by the singles-species culture was not significantly affected by nitrogen enrichment in either form (P>0.1; Figure 1A). No synergistic or antagonistic effects of increased NH₄-N in combination with increased NO₃-N were observed for NO₃-N uptake associated with either the cultured single-species or natural sediment microbial community.

Remineralization rates exceeded uptake rates of NH₄-N in the *P. fluorescens* cultures, yielding a net increase in NH₄-N (Figure 1C). Despite a net increase in NH₄-N over the incubation period in the *P. fluorescens* populations, significant treatment effects were observed (P=0.004; Figure 1C). Specifically, the combined NH₄-N and NO₃-N treatment yielded less remineralization relative to assimilation when compared to control treatments. In contrast, the natural sediment microbial community did not exhibit significant differences in NH₄-N uptake with different nitrogen treatments (P>0.1; Figure 1D).

The mean net uptake rate for NH₄-N and NO₃-N across all treatments in *P. fluorescens* was <0.001 mg N/L/d and 13.11 mg N/L/d, respectively. Average net uptake of NH₄-N and NO₃-N for the natural stream microbial communities was <0.001 mg N/L/d and 58.35 mg N/L/d, respectively. Further, nitrogen uptake was greater in the natural microbial community relative to the *P. fluorescens* population (p<0.001 from F-test).

Nitrogen Uptake Kinetics

The natural sediment microbial communities exhibited changes in nitrogen assimilation with changes in concentration of specific nitrogen forms (Figure 2). Overall, rates of $\text{NO}_3\text{-N}$ uptake were >2x greater than $\text{NH}_4\text{-N}$ uptake (mean = 0.0068 mg $\text{NO}_3\text{-N/L/d}$, 0.0029 mg $\text{NH}_4\text{-N/L/d}$; $p < 0.001$ from F-test) in uptake kinetics experiments, consistent with preferential uptake experiments. Exponential decay of $\text{NO}_3\text{-N}$ uptake rates occurred at concentrations > 0.019 mg $\text{NO}_3\text{-N/L}$ with $\text{NO}_3\text{-N}$ uptake below detection at concentrations > 0.75 mg $\text{NO}_3\text{-N/L}$ (Figure 2C). $\text{NH}_4\text{-N}$ uptake increased with $\text{NH}_4\text{-N}$ concentration and followed a quadratic growth trend (Figure 2B). $\text{NO}_3\text{-N}$ uptake rates did not change with increasing ammonium concentrations; $\text{NH}_4\text{-N}$ uptake rates did not change with increasing nitrate concentrations.

Discussion

Do microbial communities preferentially assimilate NH₄-N or NO₃-N?

Contrary to the hypothesis and published literature, these data demonstrate that some microbial communities may preferentially utilize NO₃-N over NH₄-N, as evidenced by higher uptake rates in both the natural microbial community and cultured *P. fluorescens*.

This is in contrast to other studies that found heterotrophic bacteria often prefer NH₄-N over other forms of nitrogen (Wheeler and Kirchman, 1986; Middleburg and Nieuwenhuize, 2000). Two potential hypotheses may explain this inconsistency: 1) Microbial inoculum used in this study may have adapted to higher NO₃-N concentrations found in the Midwestern US; or, 2) Differing incubation periods or treatment concentrations may affect conclusions. Although NO₃-N uptake rates increased with higher NO₃-N availability in preferential uptake experiments (Figure 1), the treatment concentration used in this experiment (1 mg NO₃-N/L) was near saturation based on uptake kinetic experiments (Figure 2). Increases in NO₃-N led to decreases in NH₄-N uptake across the entire range of treatments from 0-1.2 mg NO₃-N/L (Figure 2). When concentrations of NO₃-N were greater than 0.75 mg/L NO₃-N, increased availability decreased NO₃-N assimilation rates (Figure 2).

Different microbial processes take varying amounts of time to reach steady state equilibria (Koch, 1997). Thus, NO₃-N assimilation may reach steady state at a different time than NH₄-N assimilation. Incubation periods for both experiments were ~3 d, consistent with time necessary to achieve steady state for nitrogen assimilation identified in previous studies (Kemp and Dodds, 2001; Inwood et al., 2007). Although we assume

the incubation period was sufficient to achieve steady-state, differences in microbial communities assessed may have resulted in variable responses. Further, mesocosm environments (e.g., oxygen, pH, temperature) may have changed during the incubation period inhibiting steady-state conditions.

Despite the abundance of literature suggesting preferential uptake of $\text{NH}_4\text{-N}$, several studies have also noted preferential uptake of $\text{NO}_3\text{-N}$, particularly after prolonged exposure to high $\text{NO}_3\text{-N}$ concentrations, consistent with these data (Middleburg and Nieuwenhuize, 2000). Middleburg and Nieuwenhuize (2000) determined that $\text{NO}_3\text{-N}$ was the main substrate for heterotrophic bacteria in an inner estuary, with $\text{NH}_4\text{-N}$ assimilation accounting for only 40 percent of total nitrogen uptake. Further, estuarine $\text{NO}_3\text{-N}$ assimilation was $\sim 3.4\times$ greater than $\text{NH}_4\text{-N}$ assimilation; ranging from 0.05-7.58 and 0.06-89.29 $\mu\text{g N/L/h}$ of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$, respectively (Middleburg and Nieuwenhuize, 2000). Similarly, phytoplankton preference of $\text{NO}_3\text{-N}$, not $\text{NH}_4\text{-N}$, during the day has been observed (Cochlan et al., 1991), suggesting temporal variation in preferential uptake. In microbial communities the assimilation of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ can vary depending on physiological, genetic, and biochemical restrictions (Zehr and Ward, 2002). Adaptations by microbial communities in Midwestern US streams, which are characterized by high $\text{NO}_3\text{-N}$ concentrations, may have led to the preference of $\text{NO}_3\text{-N}$ in our study.

Variation in $\text{NH}_4\text{-N}$ was primarily associated with relatively high rates of remineralization that exceeded $\text{NH}_4\text{-N}$ assimilation yielding a net increase in $\text{NH}_4\text{-N}$ concentrations over the incubation period (Figure 1). However, $\text{NH}_4\text{-N}$ assimilation exceeded remineralization rates in uptake kinetics experiments.

Results from this study can be explained from previous literature. Uptake of specific inorganic forms of nitrogen can be affected not only by nitrogen availability but also by availability of other nutrients and physiochemical parameters. For instance, the addition of glucose in subarctic Pacific tundra stimulated $\text{NH}_4\text{-N}$ uptake (Kirchman et al., 1990). Further, in arctic streams with long-term phosphorus additions, $\text{NH}_4\text{-N}$ availability and assimilation rates decreased over time in conjunction with changes in the microbial community (Wollheim et al., 2001). Temperature and precipitation can also influence biological uptake, $\text{NO}_3\text{-N}$ transport, and nitrification rates within sediment profiles (Fenn et al., 1998) yielding fluctuations in nitrogen assimilation. Nitrogen assimilation is directly affected by physiochemical conditions in the salt marsh grass species *Spartina alterniflora* (Bradley and Morris, 1990). In salt marsh ecosystems, low concentrations of sulfide inhibit $\text{NH}_4\text{-N}$ uptake and lower saturation concentrations under hypoxic conditions. Confounding factors, such as carbon, phosphorus and oxygen, make it difficult to compare and predict assimilation response to nitrogen availability across studies (Cirmo and McDonnell, 1997) making it essential that nitrogen assimilation dynamics in individual ecosystems are quantified.

Does preferential uptake of nitrogen change with increased $\text{NH}_4\text{-N}$ or $\text{NO}_3\text{-N}$ availability?

The results of the uptake kinetic experiment indicate increased $\text{NH}_4\text{-N}$ concentrations may yield preferential assimilation of $\text{NH}_4\text{-N}$ but increased $\text{NO}_3\text{-N}$ concentrations will inhibit $\text{NO}_3\text{-N}$ assimilation, also yielding preferential assimilation of $\text{NH}_4\text{-N}$. $\text{NO}_3\text{-N}$ assimilation is thought to be significant in environments with high C:N

ratios, high microbial turnovers, and high $\text{NO}_3\text{-N}$ concentrations (Middleburg & Niewenhuize, 2000; Royer et al., 2004; Inwood et al., 2007). These characteristics are becoming more predominant in freshwater ecosystems with increased agricultural activity. However, increased agricultural activity may yield $\text{NO}_3\text{-N}$ concentrations above the threshold identified in this study (~ 0.75 mg $\text{NO}_3\text{-N/L}$), yielding inhibition of $\text{NO}_3\text{-N}$ assimilation. Kemp & Dodds (2001) directly linked higher $\text{NO}_3\text{-N}$ assimilation to higher $\text{NO}_3\text{-N}$ concentrations associated with an agriculturally-influenced stream. However, this relationship was identified in Kansas streams with relatively low $\text{NO}_3\text{-N}$ (~ 0.5 mg $\text{NO}_3\text{-N/L}$). It is likely that in the Midwestern US, where $\text{NO}_3\text{-N}$ concentrations in freshwater often exceed 10 mg $\text{NO}_3\text{-N/L}$, continued nitrogen loading associated with agricultural activity may saturate $\text{NO}_3\text{-N}$ assimilation resulting in reduced rates of assimilation and decreased mitigation by microbes.

In many ecosystems, lower concentrations of $\text{NH}_4\text{-N}$, < 30 $\mu\text{g/L}$, may stimulate nitrification coupled with de-nitrification (Kemp and Dodds, 2001). If nitrification and denitrification are tightly coupled, $\text{NH}_4\text{-N}$ assimilation may be higher than estimates made in this study. Because $\text{NH}_4\text{-N}$ assimilation was measured as net change in $\text{NH}_4\text{-N}$ concentration, any tight coupling of nitrification and denitrification would quickly remove water-column $\text{NH}_4\text{-N}$ and convert to nitrogen gases. Tight coupling of nitrification and denitrification is predominantly found in oligotrophic ecosystems with low nitrogen concentrations. Thus, tight coupling of these processes was likely minimal in these experiments which utilized water and sediment from ecosystems with relatively high nitrogen concentrations.

Overall, $\text{NO}_3\text{-N}$ assimilation decreased with increasing $\text{NO}_3\text{-N}$ concentration but not with increasing $\text{NH}_4\text{-N}$ concentration from uptake kinetic experiments (Figure 2). Thus, if $\text{NO}_3\text{-N}$ concentrations increase relative to $\text{NH}_4\text{-N}$, we would predict preferential use of $\text{NH}_4\text{-N}$ by microbial communities. Further, $\text{NH}_4\text{-N}$ assimilation increased with increasing $\text{NH}_4\text{-N}$ concentrations, but not $\text{NO}_3\text{-N}$ assimilation, also supporting predictions of increased preferential use of $\text{NH}_4\text{-N}$ by microbial communities. As human activities are increasing $\text{NO}_3\text{-N}$ concentrations in freshwater at a greater rate than $\text{NH}_4\text{-N}$ concentrations (Caraco and Cole, 1999; Schlesinger, 2009), mitigation of this nitrogen pollution may not be achieved through shifts in the microbial community. Microbial communities may be able to mitigate $\text{NH}_4\text{-N}$ loading (e.g., that associated with wastewater or animal feeding operations) to some degree, through increased assimilation and preferential uptake, although microbial communities will likely become inhibited, exacerbating the problem, in the context of increased $\text{NO}_3\text{-N}$ loading (e.g., nitrogen associated with fertilizer runoff).

Literature Cited

- Aminot, A., Kirkwood, D.S., and Kerouel, R. 1997. Determination of ammonium in seawater by the indophenol-blue method: Evaluation of the ICES NUTS I/C 5 questionnaire. *Mar. Chem.* 56: 59-75.
- APHA. 1995. Standard Methods for the Examination of Water and Wastewater. 19th Ed. American Public Health Association, Washington, D.C.
- Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyer-Reil, and Thingstad, F. 1983. The Ecological Role of Water-Column Microbes in the Sea. *Marine Ecology*. 10: 257-263.
- Bernot, M.J., Tank, J.L., Royer, T.V., and David, M.B. 2006. Nutrient uptake in streams draining agricultural catchments of the Midwestern United States. *Freshwaer Biol.* 51: 499-509.
- Binkley, D. 1986. Forest Nutrition Management: John Wiley and Sons, New York. 299 pp
- Bliss, L.C. 1963. Alpine plant communities of the Presidential Range, New Hampshire. *Ecology* 44: 678-697
- Bradley, P.M., and Morris, J.T. 1990. Influence of oxygen and sulfide concentration on nitrogen kinetics in *Spartina alterniflora*. *Ecology*. 71: 282-287.
- Brown, C.M. 1980. Ammonia assimilation and utilization in bacteria and fungi. Microorganisms and nitrogen sources. Wiley. pp 511-535.
- Bonan, G.B. 1990. Carbon and nitrogen cycling in North American boreal forests. II. Biogeographic patterns. *Can. J. Forest Res.* 20: 1077-1088
- Carpenter, S.R., Caraco, N.F., Correll, D.L., Howarth, R.W., Sharpley, A.N., and Smith, V.H. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecol. Apps.* 8: 559-568.
- Caraco, N.F., and Cole, J.J. 1999. Human impact on nitrate export: An analysis using major world rivers. *Ambio* 28: 167-170.
- Cirno, C.P., and McDonnell, J.J. 1997. Linking the hydrologic and biogeochemical controls of nitrogen transport in near-stream zones of temperate-forested catchments: a review. *J. of Hydrol.* 199: 88-120.

- Chapman, S.K., Langley, J.A., Hart, S.C., and Koch G.W. 2006. Plants actively control nitrogen cycling: uncorking the microbial bottleneck. *New Phytologist*. 169: 27-34.
- Cochlan, W.P., Harrison, P.J., and Denman, K.L. 1991. Diel periodicity of nitrogen uptake by marine phytoplankton nitrate-rich environments. *Limnol. Oceanogr.* 36: 1689-1700.
- Dodds, W.K., Evans-White, M.A., Gerlanc, N.M., Gray, L., Gudder, D.A., Kemp, M.J., Lopez, A.L., Stagliano, D., Strauss, E.A., Tank, J.L., Whiles, M.R., and Wollheim, W.M. 2000. Quantification of the nitrogen cycle in a prairie stream. *Ecosys.* 3: 574-589.
- Fenn, M.E., Poth, M.A., Aber, J.D., Baron, J.S., Bormann, B.T., Johnson, D.W., Lemly, A.D., McNully, S.G., Ryan, D.F., and Stottlemeyer R. 1998. Nitrogen excess in North American Ecosystems: Predisposing factors, ecosystem responses, and management strategies. *Ecol. Apps.* 8: 706-733.
- Grimm, N.B. and Fisher, S.G. 1986. Nitrogen limitation in a Sonoran Desert stream. *J. N. Am. Benthol. Soc.* 5: 2-15.
- Goldman, C.R. 1988. Primary productivity, nutrients, and transparency during the early onset of eutrophication in ultra-oligotrophic Lake Tahoe. *Limnol. Oceanogr.* 33: 1321-1333
- Gutschick, V.P. 1981. Evolved strategies in nitrogen acquisition by plants. *Am. Nat.* 118: 607-637.
- Gutschick, V.P. 1987. A Functional Biology of Crop Plants. Timber Press, Portland. 230 pp
- Hart, D.R., Stone, L., and Berman, T. 2000. Seasonal dynamics of the Lake Kinneret food web: The importance of the microbial loop. *Limnol. Oceanogr.* 45: 350-361.
- Hagstrom, A., Azam, F., Andersson, A., Wikner, J., and Rassoulzadegan, F. 1988. Microbial loop in an oligotrophic pelagic marine system: possible roles of cyanobacteria and nanoflagellates in the organic fluxes. *Marine Ecology.* 49: 171-178.
- Hecky, R.E., and Kilham, P. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. *Limnol. Oceanogr.* 33: 796-822

- Howarth, R.W. 1988. Nutrient limitation of net primary production in marine ecosystems. *Ann. Rev. Ecol. & Syst.* 19: 89-110
- Howarth, R.W., Billen, G., Swaney, D., Townsend, A., Jaworski, N., Lajtha, K., Downing, J.A., Elmgren, R., Caraco, N., Jordan, T., Berendse, F., Freney, J., Kudeyarov, V., Murdoch, P., and Zhao-Liang, Z. 1996. Regional nitrogen budgets and riverine N & P fluxes for the drainages to the North Atlantic Ocean: Natural and human influences. *Biogeochem.* 35: 75-139.
- Hunt, H.W., Ingham, E.R., Coleman, D.C., Elliott, E.T., and Reid, C.P.P. 1988. Nitrogen limitation of production and decomposition in prairie, mountain meadow, and pine forest. *Ecology* 69: 1009-1016.
- Inwood, S.E., Tank, J.L., and Bernot, M.J. Factors controlling sediment denitrification in Midwestern streams of varying land use. *Microbial Ecol.* 2: 247-258.
- Jansson, S.L., Hallam, H.J., and Bartholeomew, W.V. 1955. Preferential utilization of ammonium over nitrate by micro-organisms in the decomposition of oat straw. *Plant and Soil.* 6: 382-390.
- Jorgensen, N.O.G., Kroer, N., Coffin, R.B., and Hoch, M.P. 1999. Relations between bacterial nitrogen metabolism and growth efficiency in an estuarine and an open-water ecosystem. *Aq. Microb. Ecol.* 18: 247-261.
- Kemp, M.J., and Dodds, W.K. 2001. Spatial and temporal patterns of nitrogen concentrations in pristine and agriculturally influenced prairie streams. *Biogeochem.* 53: 125-141.
- Kemp MJ, Dodds WK. 2002. The influence of ammonium, nitrate, and dissolved oxygen concentrations on uptake, nitrification, and denitrification rates associated prairie stream substrata. *Limnol. & Oceanogr.* 47: 1380-1393.
- Kimmins J.P. 1987. Forest Ecology. Macmillian Publishing, New York. 531 pp
- Kirchman D.L., Keil R.G., and Wheeler P.A. 1990. Carbon limitation of ammonium uptake by heterotrophic bacteria in the subarctic Pacific. *Limnol Oceanogr.* 35:1258-1266
- Koch, A.L. 1997. Microbial physiology and ecology of slow growth. *Microbiol. & Molecular Biol. Rev.* 61: 305-318.
- Knops, J.M.H., Bradley, K.L., and Wedin, D.A. 2002. Mechanisms of plant species impacts on ecosystem nitrogen cycle. *Eco. Letters.* 5: 454-466.

- Lee J.A., Harmer R. and Ignaciuk R. 1983. Nitrogen as a limiting factor in plant communities. In: Lee JA, McNeill S Rorison IH (Eds) Nitrogen as an Ecological Factor (pp 95-112). Blackwell Scientific, Oxford
- McCarthy, J.J., Wynne, D., and Berman, T. 1982. The uptake of dissolved nitrogenous nutrients by Lake Kinneret (Israel) microplankton. *Limnol. & Oceanogr.* 27: 673-680.
- McKane, R.B., Johnson, L.C., Shaver, G.R., Nadelhoffer, K.J., Rastetter, E.B., Fry, B., Giblin, A.E., Kielland, K., Kwiatkowski, B.L., Laundre, J.A., and Murray, G. 2002. Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. *Nature.* 415: 68-71.
- Middleburg, J.J, and Nieuwenhuize, J. 2000. Nitrogen uptake by heterotrophic bacteria and phytoplankton in the nitrate-rich Thames estuary. *Mar. Ecol. Prog. Ser.* 192: 79-88.
- Miller, A.E., and Bowman, W.D. 2003. Alpine plants show species-level differences in the uptake of organic and inorganic nitrogen. *Plant and Soil.* 250: 283-292.
- Mullholland, P.J. 2004. The importance of in-stream uptake for regarding stream concentrations and outputs of N and P from a forested watershed; evidence from long-term chemistry records for Walker Branch Watershed. *Biogeochem.* 70: 403- 426.
- Mulholland, P.J., Helton, A.M., Poole, G.C., Hall Jr., R.O., Hamilton, S.K., Peterson, B.J., Tank, J.L., Ashkenas, L.R., Cooper, L.W., Dahm, C.N., Dodds, W.K., Findlay, S.E., Gregory, S.V., Grimm, N.B., Johnson, S.L., McDowell, W.H., Meyer, J.L., Valett, H.M., Webster, J.R., Arango, C.P., Beaulieu, J.J., Bernot, M.J., Potter, J.D., Sheibley, R.W., Sobota, D.J., and Thomas, S.M. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. *Nature.* 452: 202-206.
- Naudin, J.-J., Cauwet, G., Fajon, C., Oriol, L., Terzic, S., Devenon, J.-L., and Broche, P. 2001. Effect of mixing on microbial communities in the Rhone River plume. *J. of Mar. Syst.* 28: 203-227.
- Nordin, A., Hogberg, P., and Nasholm, T. 2001. Soil nitrogen form and plant nitrogen uptake along a boreal forest productivity gradient. *Oecologia.* 129: 125-132.
- Padgett, P.E., and Leonard, R.T. 1996. Free amino acid levels and the regulation of nitrate uptake in maize cell suspension cultures. *Journ. Experiment. Bot.* 47: 871-883

- Pascoal, C., Cassio, F., Marcotecui, A., Sanz, B., and Gomes, P. 2005. Role of fungi, bacteria, and invertebrates in leaf litter breakdown in a polluted river. *J. N. Am. Benthol. Soc.* 24:784-797.
- Payne, W.J. 1970. Energy yields and growth of heterotrophs. *Annu. Rev. Microbiol.* 24: 17-52.
- Peterson, B.J., Wollheim, W.M., Mullholland, P.J., Webster, J.R., Meyer, J.L., Tank, J.L., Marti, E., Bowden, W.B., Valett, M., Hershey, A.E., McDowell, W.H., Dodd, W.K., Hamilton, S.K., Gregory, S., Morrall, D.D. 2001. Control of Nitrogen Export from Watersheds by Headwater Streams. *Science.* 292:86-89.
- Royer, T.V., Tank, J.L., and David, M.B. 2004. Transport and fate of nitrate in headwater agricultural streams in Illinois. *J. Environ. Qual.* 33: 1296-1304.
- Schlesinger, W.H. 1997. Biogeochemistry: An analysis of global change. Academic Press. (2nd ed.) San Diego, California.
- Schlesinger, W.H. 2009. On the fate of anthropogenic nitrogen. *PNAS.* 106: 203-208.
- Shaver, G.R., and Chapin, F.S. III 1980. Response to fertilization by various plant growth forms in an Alaskan tundra: nutrient accumulation and growth. *Ecology* 61: 662-675
- Tilman, D. 1988. Plant Strategies and the Dynamics and Structure of Plant Communities. Princeton University Press, Princeton. 360 pp
- Thomas, H., Ittekkot, V., Osterroht, C., and Schneider, B. 1999. Preferential Recycling of Nutrients-The Ocean's Way to Increase New Production and to Pass Nutrient Limitation? *Limnol. Oceanogr.* 44: 1999-2004.
- Turner, R. E. and Rabalais, N. N. 1991. Changes in the Mississippi River water quality this century - implications for coastal food webs. *BioScience* 41: 140-147.
- U.S. EPA (U.S. Environmental Protection Agency). 1990. National water quality inventory in the United States. EPA 841-R-96-002. USEPA. Office of Water (4503F). U.S. Government Printing Office. Washington, D.C. USA.
- Vermeer, C.P., Escher, M., Portielje, R., Klein, J.J.M. 2003. Nitrogen uptake and translocation by *Chara*. *Aquat. Bot.* 76: 245-258.
- Vitousek, P. M., Aber, J.D., Howarth, R.W., Liens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., and Tilman, D.G. 1997. Human alteration of the global nitrogen cycle sources and consequences. *Ecol. App.* 7: 737-750.

- Watson, C.J. 1986. Preferential uptake of ammonium nitrogen from soil by ryegrass under simulated spring conditions. *J. agric. Sci., Camb.* 107: 171-177.
- Webster, J. R., et al. 2003. Factors affecting ammonium uptake in streams—an inter-biome perspective. *Freshwater Biology* 48:1329–1352.
- Weigelt, A., Bol, R., and Bardgett, R.D. 2005. Preferential uptake of soil nitrogen forms by grassland plant species. *Oecologia*. 142: 627-635.
- Wheeler, P.A, and Kirchman, D.L. 1986. Utilization of inorganic and organic nitrogen by bacteria in marine systems. *Limnol. Oceanogr.* 31: 998-1009.
- Wilkerson, F.P., and Muscatine, L. 1984. Uptake and assimilation of dissolved inorganic nitrogen by a symbiotic sea anemone. *Proc. R. Soc. Lond. B* 221: 71-86.
- Wollheim, W.M., Peterson, B.J., Deegan, L.A., Hobbie, J.E., Hooker, B., Bowden, W.B., Edwardson, K.J., Arscott, D.B., Hershey, A.E., and Finlay. 2001. Influence of stream size on ammonium and suspended particulate nitrogen processing. *Limnol. Oceanogr.* 46: 1-13.
- Zehr, J.P., and Ward, B.B. 2002. Nitrogen cycling in the ocean: New perspectives on processes and paradigms. *Appl. & Environ. Microbiol.* 68: 1015-1024.

Figure Legends

Figure 1: Mean+SE $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ uptake by *Pseudomonas fluorescences* (A,C) and natural sediment microbial communities (B,D) in response to nitrogen treatments.

Treatments are a control (no N addition), nitrate addition (1 mg $\text{NO}_3\text{-N/L}$), ammonium addition (30 $\mu\text{g NH}_4\text{-N/L}$), and a combined $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ addition. N=4 replicates for each bar. Significance determined with Analysis of Variance and denoted by p-values. Different letters indicate significant pairwise comparisons. Raw data are provided in Appendix I.

Figure 2: $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ uptake rates associated with natural sediment microbial communities in response to increasing nitrogen concentrations. Significant polynomial fit where appropriate is presented and equations and p-values noted. Red lines indicate preferential uptake experiment concentrations (Figure 1). Raw data are provided in Appendix II.

Figure 1

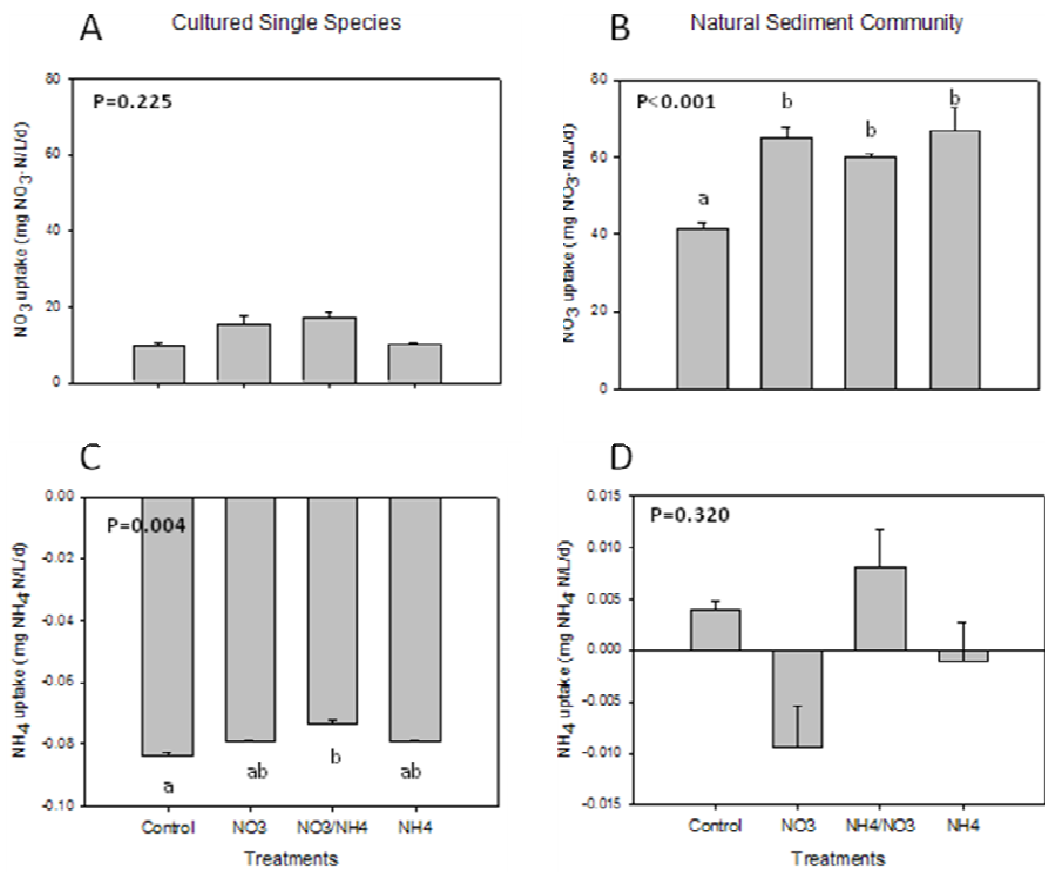
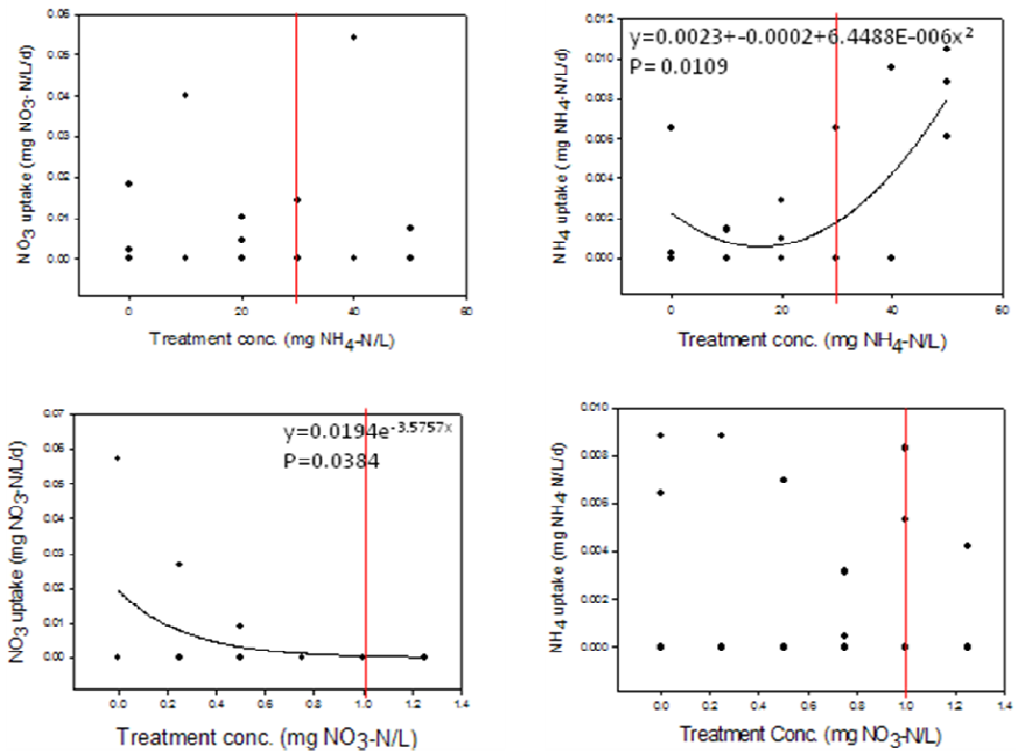


Figure 2



Chapter 2: Adaptive Uptake in Microbial Communities

Abstract

Anthropogenic activities have increased nitrogen concentration in many ecosystems. Because microbes have higher metabolic rates relative to larger organisms, microbial activity may influence nitrogen movement and degradation significantly in ecosystems. Thus, the ability for microorganisms to adapt to increasing nitrogen concentrations is essential to ecosystem sustainability. We measured sediment microbial community nitrogen assimilation after sustained nitrogen enrichments using *in vitro* and isotopic techniques. Mixed-microbial communities were exposed to high concentrations of NO₃-N (1 mg/L) and NH₄-N (30 μg/L) for four weeks. Each week, filtered water samples were collected from each mesocosm and sediment was removed to quantify rates of nitrogen assimilation by the sediment microbial community. During the fourth week, isotopic tracers ¹⁵NO₃ and ¹⁵NH₄ were added to mesocosms to directly measure nitrogen incorporation into microbial cells. Initial microbial responses to nitrogen enrichment were distinctly different than the sustained microbial community responses. NH₄-N uptake was initially stimulated with NH₄-N enrichments but increased uptake rates were not sustained over time. Sustained responses to changing nitrogen availability equilibrated within 1-3 weeks (depending on nitrogen form) indicating that even though microbial communities can respond to increased availability, potential for increased assimilation is limited.

Introduction

Myriad changes have occurred in ecosystems worldwide due to anthropogenic activities such as urban and agricultural development (Howarth et al., 1996; Vitousek et al., 1997; Fenn et al., 1998; Galloway, 1998). However, it remains unknown as to whether, or more precisely how and when, microbes are responding to these changes. Notably, dissolved inorganic nitrogen concentrations in freshwater has significantly increased throughout North America due to human activity (Middleburg and Nieuwenhuize 2000; Schlessinger 2000). Response to this increased nitrogen may include either 1) inhibition of activity due toxicity; or, 2) increased or status quo activity associated with adaptation. Both laboratory and field studies have documented varied microbial response to these increasing nitrogen concentrations (Kirchman et al., 1990; Kirchman, 1994; Hoch and Kirchman, 1995; Dodds et al. 2002; Hall and Tank, 2003) with both toxicity and stimulation of nitrogen assimilation identified with increasing nitrogen concentration. Inconsistencies in results are likely due in part to historical nitrogen conditions for a given ecosystem that may allow for adaptation or tolerance to nitrogen loading in freshwater. To effectively mitigate and manage nitrogen loading to freshwater, a better understanding of adaptive uptake by microbial communities is needed.

Adaptive uptake is defined here as a change in selectivity (i.e., preferential uptake) for nitrogen forms in response to changes in availability over a sustained period of time. Organisms, particularly fast-growing microbial species, may be capable of adapting to different conditions and changing utilization rates over time. Microbes are associated with genetic and edaphic processes, such as genetic alteration, to allow

adaptation to changing conditions (Kearney and Kellogg, 1985). Several studies have identified adaptive nutrient uptake by organisms in natural ecosystems. For example, nutrient enrichment in aquatic ecosystems can change algal community composition (Pedersen and Borum, 1996), likely due to differential affinities for changing nutrient availabilities or species responses that alter competitive interactions. Further, studies have identified inhibition of nitrate (NO_3) uptake by microbes in response to sustained increases in organic nitrogen concentrations (Padgett and Leonard, 1996). Alpine herbaceous species can adapt nitrogen preference in response to sustained changes in availability, likely because these ecosystems are nitrogen limited (Miller and Bowman, 2003). Microbes can also adapt to xenobiotic compounds resulting in more efficient degradation after sustained exposure (Swindoll et al., 1988). Adaptive uptake is essential if organisms are able to cope with the ever changing environment. Despite this recognition, understanding of adaptive uptake is limited as most research is conducted at shorter time scales (days) than necessary to quantify sustained shifts in activity. Further, research conducted over longer time periods (months-years) are often field-based and adaptive uptake is difficult to isolate within an ever-changing environment (Bernot et al., 2005).

Using long-term *in vitro* experiments with the addition of stable isotopes, we quantified nitrogen assimilation rates of natural sediment microbial communities exposed to increased ammonium ($\text{NH}_4\text{-N}$) and nitrate ($\text{NO}_3\text{-N}$) to assess potential for adaptive uptake. I hypothesized that the microbial community would alter rates of nitrogen assimilation over time to preferentially utilize the most abundant form of nitrogen. I

further hypothesized that initial responses to nitrogen conditions would not be characteristic of the sustained response.

Materials and Methods

Culture Preparation

One natural sediment microbial community inoculum was used for a mesocosm experiment. This natural community was collected in spring 2009 from a stream in central Indiana by randomly gathering the top 5 cm of sediment from several locations and homogenizing the sample through a USGS no. 5 sieve to remove debris and macroinvertebrates. The stream site was characteristic of streams throughout central Indiana with $\text{NH}_4\text{-N}$ concentrations ranging from 0.03-0.05 mg N/L, $\text{NO}_3\text{-N}$ concentrations ranging from 0.8- 4.7 mg N/L, and DOC concentrations reaching 6.2 mg/L (Bernot et al. 2006). Sediment was collected <24 h prior to experiment start and preliminary experiments indicated microbial activity did not change after holding periods <72 h.

Experimental Mesocosms

Laboratory mesocosms were constructed and treated with variable nitrogen concentrations as either $\text{NH}_4\text{-N}$ or $\text{NO}_3\text{-N}$. Mesocosms consisted of 2 L homogenized sediment and 3 L stream water in 6 L HDPE plastic containers. Nitrogen treatments were addition of 30 $\mu\text{g/L}$ of $\text{NH}_4\text{-N}$, 1 mg/L of $\text{NO}_3\text{-N}$, or control (no nitrogen addition). Each nitrogen treatment was replicated in four separate mesocosms resulting in 12 laboratory mesocosms. Mesocosms were incubated at room temperature, under constant fluorescent light for 4 weeks. Mesocosms were carefully monitored throughout the incubation to maintain water volume (appropriately N-enriched stream water was added as necessary).

Each week, initial filtered water samples were collected from each mesocosm and sediment was removed (carefully to ensure minimal disruption) to quantify rates of nitrogen assimilation by the sediment microbial community. Filtered water samples were also collected each week to quantify available nitrogen concentrations. Sediment removed from each mesocosm was placed into 50 mL falcon tubes (10 mL sediment into each tube, N=5 replicates per mesocosm) with an additional 30 mL stream water from same mesocosm. Falcon tubes with collected sediment and water were incubated 3 d followed by nitrogen extraction and collection of filtered water extracts (post samples). Filtered extracts were frozen for subsequent analysis of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations detailed below. Ammonium and $\text{NO}_3\text{-N}$ uptake rates were calculated each week as the difference between initial and post-incubation water sample concentrations divided by the 3 d incubation time (i.e., mg N/d). Week 0 uptake measurements were taken immediately after N enrichment.

Chemical Analyses

All filtered water extracts were analyzed for nitrogen concentrations <24 h post-thaw. Ammonium concentration was measured using the colorimetric phenol-hypochlorite technique (Aminot et al., 1997, APHA, 1995) followed by quantifying absorbance for samples and known standards on a Shimadzu dual-beam spectrophotometer. Nitrate concentration was measured by ion chromatography on a Dionex 3000 Ion Chromatograph also comparing samples to known standards.

Isotopic Analysis

To ensure *in vitro* laboratory experiments were robust, direct measurements of nitrogen uptake via ^{15}N incorporation were made in conjunction with sediment assays for nitrogen assimilation. Isotopic tracers ($^{15}\text{NH}_4$ and $^{15}\text{NO}_3$ as $>99\%$ $^{15}\text{NH}_4\text{Cl}$ and K^{15}NO_3 , respectively) were added to the adaptive uptake mesocosms at the end of the 4 week incubation. Isotopes were added to label $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ pools $\sim 1,000\%$ and mesocosms were incubated with isotope for one week prior to sample analysis. Enrichment of $\delta^{15}\text{N}$ to $\sim 1,000\%$ increased available nitrogen <0.01 mg N/L in all treatments. Background enrichment of $\delta^{15}\text{N}$ in sediment was $\sim 10\%$. All isotope samples were prepared in the laboratory by drying mesocosm sediment for 3 d at 65°C after rinsing with deionized water. Sediment was then homogenized by grinding with a mortar and pestel. Samples were analyzed at the Stable Isotope Mass Spectrometry Laboratory at the Marine Biological Laboratory (MBL) for measurement of ^{15}N and percent nitrogen. At MBL, sediment samples were assayed for ^{15}N and % nitrogen by mass spectrometry using an automated sample combustion system and a Finnigan Delta S isotope ratio mass spectrometer.

Isotopic enrichment was calculated by dividing control mesocosms (no nitrogen enrichment) from nitrogen enriched mesocosms with appropriate isotope addition (i.e., $\delta^{15}\text{N}$ $\text{NH}_4\text{-N}$ enriched mesocosm labeled with $^{15}\text{NH}_4$ / $\delta^{15}\text{N}$ control mesocosm labeled with $^{15}\text{NH}_4$) yielding a nitrogen assimilation rate ($\text{g}^{15}\text{N}/\text{g}^{15}\text{N}/\text{d}$).

Statistical Analyses

Ammonium and NO₃-N uptake rates were calculated as the decline in NH₄-N or NO₃-N over time per mass of substrate (*sensu* Kemp and Dodds, 2002). One-way ANOVA with treatment as main effect was conducted on weekly estimates of N uptake and available mesocosm concentrations to determine significant differences among treatments within a given week (0-4). Differences in isotopic enrichment were also determined using one-way ANOVA followed by Tukey's pairwise comparisons with treatment as the main effect.

Results

Ammonium uptake rates were significantly different among mesocosm treatments initially with NH₄-N enriched mesocosms having higher rates than control mesocosms and NO₃-N enriched mesocosms having lower rates than control mesocosms (P<0.01; Figure 1A, Table 1). Control mesocosms had consistent NH₄-N uptake rates throughout the incubation. Initially, net NH₄-N uptake was negative (net remineralization) in response to NO₃-N enrichment. Over the incubation period, differences in NH₄-N uptake rates among treatments declined though NH₄-N enriched mesocosms had higher NH₄-N uptake rates throughout the incubation. Concentrations of NH₄-N in the water column declined throughout the incubation period in all mesocosm treatments though NH₄-N concentrations in NH₄-N enriched mesocosms were always higher than control and NO₃-N enriched mesocosms (Figure 1B). After 2 weeks, NH₄-N uptake and NH₄ water-column concentration became near constant for the duration of the incubation across all treatments.

There were no significant differences in $\text{NO}_3\text{-N}$ uptake rates among treatments at weeks 0 and 2 ($P>0.1$; Figure 1). Week 1, 3, and 4, $\text{NO}_3\text{-N}$ enriched mesocosms had significantly higher $\text{NO}_3\text{-N}$ uptake than $\text{NH}_4\text{-N}$ enriched and control mesocosms ($P<0.01$; Figure 1, Table 1). Mesocosm $\text{NO}_3\text{-N}$ concentration declined throughout the incubation although $\text{NO}_3\text{-N}$ concentrations in the $\text{NO}_3\text{-N}$ enriched treatment were always significantly higher than $\text{NO}_3\text{-N}$ concentrations in control or $\text{NH}_4\text{-N}$ enriched mesocosms (Figure 2B).

Isotopic data quantifying direct assimilation of $^{15}\text{NH}_4\text{-N}$ and $^{15}\text{NO}_3\text{-N}$ by the sediment microbial community at the end of the 4 wk incubation period identified significant differences in nitrogen assimilation for both $\text{NH}_4\text{-N}$ enriched and $\text{NO}_3\text{-N}$ enriched treatments (Figure 3). Specifically, both $\text{NH}_4\text{-N}$ enriched and $\text{NO}_3\text{-N}$ enriched treatments had higher $^{15}\text{NO}_3\text{-N}$ incorporation relative to controls ($P<0.01$). No significant differences in $^{15}\text{NH}_4\text{-N}$ assimilation were observed relative to controls with either nitrogen enrichment ($P>0.10$).

Discussion

These data supported our hypothesis that microbial communities can adapt to changes in nutrient availability. If sediment microbial communities were not adapting to nutrient availability, no differences in nitrogen uptake among treatments would be expected. Sediment microbial communities adapted to higher concentrations of $\text{NH}_4\text{-N}$, exhibiting higher rates of $\text{NH}_4\text{-N}$ uptake, relative to control and $\text{NO}_3\text{-N}$ enriched treatments, throughout the incubation (Figure 1A). Although $\text{NO}_3\text{-N}$ enriched sediment microbial communities did exhibit higher rates of $\text{NO}_3\text{-N}$ uptake at times, this pattern was inconsistent through the 4 wk incubation (Figure 2A). Variability in $\text{NO}_3\text{-N}$ uptake rates among treatments was observed early in the incubation period, becoming more consistent within and across treatments over time. Thus, microbial community adaptations observed were more pronounced with $\text{NH}_4\text{-N}$ relative to $\text{NO}_3\text{-N}$. Inconsistency in net uptake of $\text{NO}_3\text{-N}$ up to week 2 may have resulted from a delayed response by microbial communities to nitrogen enrichment. Ammonium-N is generally a preferred source of N for microbial communities (Jorgensen et al., 1999; Reay et al., 1999; Middleburg and Nieuwenhuize, 2000) and competition for available N may dictate stronger adaptive characteristics relative to $\text{NO}_3\text{-N}$.

As hypothesized, initial microbial responses were not indicative of sustained microbial response over the incubation period. At the onset of nitrogen enrichment, $\text{NH}_4\text{-N}$ uptake in $\text{NH}_4\text{-N}$ enriched mesocosms was ~10x higher in weeks 0 and 1 relative to the uptake after sustained exposure (>1 wk). This suggests an initial stimulatory response consistent with previous studies (Mulholland et al., 2000). Previous assessments of microbial response to $\text{NH}_4\text{-N}$ enrichment may over-estimate capacity for $\text{NH}_4\text{-N}$

assimilation if only initial responses are assessed. In contrast, $\text{NO}_3\text{-N}$ enrichment did not yield an initial stimulatory effect (Figure 2A). Rather, increased $\text{NO}_3\text{-N}$ uptake was observed after prolonged exposure to $\text{NO}_3\text{-N}$ (>1 wk). Increased assimilation of $\text{NO}_3\text{-N}$ with sustained $\text{NO}_3\text{-N}$ enrichment is further verified with isotopic measurements indicating greater incorporation of $^{15}\text{NO}_3\text{-N}$ in $\text{NO}_3\text{-N}$ enriched treatments relative to $\text{NH}_4\text{-N}$ enriched treatments (Figure 3).

Patterns of $\text{NH}_4\text{-N}$ uptake with $\text{NO}_3\text{-N}$ enrichment suggest $\text{NO}_3\text{-N}$ may inhibit $\text{NH}_4\text{-N}$ assimilation with initial exposure to higher concentrations (Figure 1A). Nitrification, the microbial oxidation of $\text{NH}_4\text{-N}$ to $\text{NO}_3\text{-N}$, is inhibited by $\text{NO}_3\text{-N}$ (Burford and Longmore, 2001), consistent with these data. Interestingly, $\text{NH}_4\text{-N}$ enrichment did not yield higher $^{15}\text{NH}_4\text{-N}$ incorporation, despite higher rates of assimilation (Figure 1A). This suggests $\text{NH}_4\text{-N}$ used by the microbial community was either quickly remineralized or not assimilated into organic nitrogen in cells. Previous research has noted microbial communities in some ecosystems have higher remineralization rates relative to assimilation (Harrison, 1978; Wheeler and Kirchman, 1986). This is particularly true when organisms are exposed to non-limiting nitrogen concentrations (Bronk et al. 1998) as is characteristics of Midwestern freshwaters (Bernot et al. 2006) and the conditions of our laboratory mesocosms. Alternatively, if $\text{NH}_4\text{-N}$ uptake was associated with alternative pathways, such as nitrification, $\text{NH}_4\text{-N}$ would be quickly converted to $\text{NO}_3\text{-N}$. Isotopic data suggests nitrification was likely a predominant form of $\text{NH}_4\text{-N}$ uptake in these laboratory mesocosm as $\text{NH}_4\text{-N}$ enriched mesocosms had higher $^{15}\text{NO}_3\text{-N}$ incorporation relative to controls (Figure 3). Further, the higher $^{15}\text{NO}_3\text{-N}$ incorporation in $\text{NH}_4\text{-N}$ enrichments suggests tight coupling between

nitrification and denitrification (microbial reduction of $\text{NO}_3\text{-N}$ to dinitrogen gas, N_2) in these sediment microbial communities.

Microbial communities in this study were able to respond to increased nitrogen availability, by significantly increasing net uptake regardless of magnitude, indicating potential to mitigate nitrogen pollution as either $\text{NH}_4\text{-N}$ or $\text{NO}_3\text{-N}$. Notably, both $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ pollution to freshwater can occur as either an acute or chronic increase in concentrations. These data suggest two primary limits to microbial mitigation of nitrogen pollution. First, initial responses are distinctly different than sustained microbial community responses. Thus, potential for microbial mitigation of nitrogen will vary depending on acute relative to chronic exposure. Second, sustained responses to changing nitrogen availability appear to equilibrate within 1-3 weeks indicating that even though microbial communities can adapt to changing availability, this potential is limited. With sustained exposure, nitrogen uptake by the sediment microbial community likely becomes saturated with maximum uptake rates being achieved. It is likely that secondary enrichments of nitrogen would not further stimulate rates of assimilation. Future studies are needed to assess even longer time scales and document microbial community thresholds for nitrogen assimilation.

Literature Cited

- Aminot, A., Kirkwood, D.S., and Kerouel, R. 1997. Determination of ammonium in seawater by the indophenol-blue method: Evaluation of the ICES NUTS I/C 5 questionnaire. *Mar. Chem.* 56: 59-75.
- APHA. 1995. Standard Methods for the Examination of Water and Wastewater. 19th Ed. American Public Health Association, Washington, D.C.
- Bernot, M.J., and Dodds, W.K. 2005. Nitrogen retention, removal, and saturation in lotic ecosystems. *Ecosystems*. 8: 442-453.
- Bernot, M.J., Tank, J.L., Royer, T.V., and David, M.B. 2006. Nutrient uptake in streams draining agricultural catchments of the Midwestern United States. *Freshwaer Biol.* 51: 499-509.
- Bronk, D.A., Glibert, P.M., Malone, T.C., Banahan, S., and Sahlsten, E. 1998. Inorganic and organic nitrogen cycling in Chesapeake Bay: autotrophic versus heterotrophic processes and relationships to carbon flux. *Aquat. Microb. Ecol.* 15: 177-189.
- Burford, M.A., and Lonmore, A.R. 2001. High ammonium production from sediments in hypereutrophic shrimp ponds. *Mat. Ecol. Prog. Ser.* 224: 187-195.
- Dodds, W.K., Lo´pez, A.J., Bowden, W.B., Gregory, S., Grimm, N.B., Hamilton, S.K., Hershey, A.E., Marti´, E., McDowell, W.B., Meyer, J.L., Morrall, D., Mulholland, P.J., Peterson, B.J., Tank, J.L., Vallet, H.M., Webster, J.R., Wollheim, W. 2002. N uptake as a function of concentration in streams. *J. N. Am. Benthol. Soc.* 21:206–220.
- Fenn, M.E., Poth, M.A., Aber, J.D., Baron, J.S., Bormann, B.T., Johnson, D.W., Lemly, A.D., McNully, S.G., Ryan, D.F., and Stottlemyer R. 1998. Nitrogen excess in North American Ecosystems: Predisposing factors, ecosystem responses, and management strategies. *Ecol. Apps.* 8: 706-733.
- Galloway, J.N. 1998. The global nitrogen cycle: changes and consequences. *Environ. Poll.* 102: 15-24.
- Hall, R.O. Jr., and Tank J.L. 2003. Ecosystem metabolism controls nitrogen uptake in streams in Gran Teton National Park, Wyoming. *Limnol. Oceanogr.* 48: 1120-1128.
- Harrison, W.G. 1978. Experimental measurements of nitrogen remineralization in coastal waters. *Limnol. Ocaenogr.* 23: 684-694.

- Hoch, M.P. and Kirchman, D.L. 1995. Ammonium uptake by heterotrophic bacteria in the Delaware estuary and adjacent coastal waters. *Limnol. Oceanogr.* 40: 886-897.
- Howarth, R.W., Billen, G., Swaney, D., Townsend, A., Jaworski, N., Lajtha, K., Downing, J.A., Elmgren, R., Caraco, N., Jordan, T., Berendse, F., Freney, J., Kudeyarov, V., Murdoch, P., and Zhao-Liang, Z. 1996. Regional nitrogen budgets and riverine N & P fluxes for the drainages to the North Atlantic Ocean: Natural and human influences. *Biogeochemistry.* 35: 75-139.
- Jorgensen, N.O.G., Kroer, N., Coffin, R.B., and Hoch, M.P. 1999. Relations between bacterial nitrogen metabolism and growth efficiency in an estuarine and an open-water ecosystem. *Aquat. Microb. Ecol.* 18: 247-261.
- Kemp, M.J. and Dodds, W.K. 2001. Spatial and temporal patterns of nitrogen concentrations in pristine and agriculturally influenced prairie streams. *Biogeochem.* 53: 125-141.
- Kemp M.J. and Dodds W.K. 2002. The influence of ammonium, nitrate, and dissolved oxygen concentrations on uptake, nitrification, and denitrification rates associated prairie stream substrata. *Limnol. and Oceanogr.* 47: 1380-1393.
- Kearney, P.C., and Kellogg, S.T. 1985. Microbial adaptation to pesticides. *Pure & Appl. Chem.* 57: 389-403.
- Kirchman, D.L. 1990. Limitation of bacterial growth by dissolved organic matter in the subarctic Pacific. *Marine Ecol. Prog. Ser.* 62: 47-54.
- Kirchman, D.L. 1994. The uptake of inorganic nutrients by heterotrophic bacteria. *Microb. Ecol.* 28: 255-271.
- Middleburg, J.J., and Nieuwenhuize, J. 2000. Uptake of dissolved inorganic nitrogen in turbid, tidal estuaries. *Mar. Ecol. Prog. Ser.* 192: 79-88.
- Miller, A.E., and Bowman, W.D. 2003. Alpine plants show species-level differences in the uptake of organic and inorganic nitrogen. *Plant and Soil.* 250: 283-292.
- Mulholland, P.J., Tank, J.L., Sanzone, D.M., Wollheim, W.M., Peterson, B.J., Webster, J.R., and Meyer, J.L. 2000. Nitrogen cycling in a forest stream determined by a ¹⁵N tracer addition. *Ecolog. Monogr.* 70: 471-493.

- Padgett, P.E., and Leonard, R.T. 1996. Free amino acid levels and the regulation of nitrate uptake in maize cell suspension cultures. *Journ. Experiment. Bot.* 47: 871-883.
- Pedersen, M.F., and Borum J. 1996. Nutrient control of algal growth in estuarine waters. Nutrient limitation and the importance of nitrogen requirements and nitrogen storage among phytoplankton and species of macroalgae. *Mar. Ecol. Prog. Ser.* 142: 261-272.
- Reay, D.S., Nedwell, D.B., Priddle, J., and Ellis-Evans, J.C. 1999. Temperature dependence of inorganic nitrogen uptake: Reduced affinity for nitrate at suboptimal temperatures in both algae and bacteria. *Appl. & Environ. Microbiol.* 65: 2577-2584.
- Schlesinger, W.H. 1997. Biogeochemistry: An analysis of global change. Academic Press. (2nd ed.) San Diego, California.
- Swindoll, C.M., Aelion, C.M., and Pfaender, F.K. 1988. Influence of inorganic and organic nutrients on aerobic biodegradation and on the adaptation response of subsurface microbial communities. *App. & Environ. Microbiol.* 54: 212-217.
- Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., and Tilman, D.G. 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecol. App.* 7: 737-750.
- Wheeler, P.A., and Kirchman, D.L. 1986. Utilization of inorganic and organic nitrogen by bacteria in marine systems. *Limnol. Oceanogr.* 31: 998-1009.

Figure Legends

Figure 1: Mean A) $\text{NH}_4\text{-N}$ uptake rates; and, B) mesocosm $\text{NH}_4\text{-N}$ concentrations over the incubation period. Each symbol is the mean of 4 replicates +SE. Asterisk notes significant treatment differences within each week (ANOVA, $p < 0.05$). Raw data are provided in appendix III & IV.

Figure 2: Mean A) $\text{NO}_3\text{-N}$ uptake rates; and, B) mesocosm $\text{NO}_3\text{-N}$ concentrations over the incubation period. Each symbol is the mean of 4 replicates +SE. Asterisk notes significant treatment differences within each week (ANOVA, $p < 0.05$). Raw data are provided in appendix III & IV.

Figure 3: Mean $^{15}\text{NO}_3\text{-N}$ and $^{15}\text{NH}_4\text{-N}$ incorporation rates by sediment microbial communities at the end of the incubation period in $\text{NH}_4\text{-N}$ enriched and $\text{NO}_3\text{-N}$ enriched treatments. Each bar is the mean of 4 replicates +SE. Letters indicate significant Tukey's post hoc comparisons. Raw data in appendix V.

Table 1. Analysis of variance (ANOVA) statistics comparing NH₄-N and NO₃-N uptake rates among nitrogen enrichment treatments each week.

	NH ₄ -N Uptake		NO ₃ -N Uptake	
	F	<i>p</i>	F	<i>p</i>
Week 0	6.26	0.004	0.18	>0.50
Week 1	3.90	0.027	17.60	<0.001
Week 2	14.75	<0.001	0.32	>0.50
Week 3	20.59	<0.001	87.98	<0.001
Week 4	32.98	<0.001	44.96	<0.001

Table 2. Analysis of variance (ANOVA) statistics comparing NH₄-N and NO₃-N concentrations among nitrogen enrichment treatments each week.

	NH ₄ -N Concentration		NO ₃ -N Concentration	
	F	<i>p</i>	F	<i>p</i>
Week 0	170	<0.001	2250	<0.001
Week 1	45	<0.001	68	<0.001
Week 2	103	<0.001	317	<0.001
Week 3	46	<0.001	83	<0.001
Week 4	61	<0.001	27	<0.001

Figure 1:

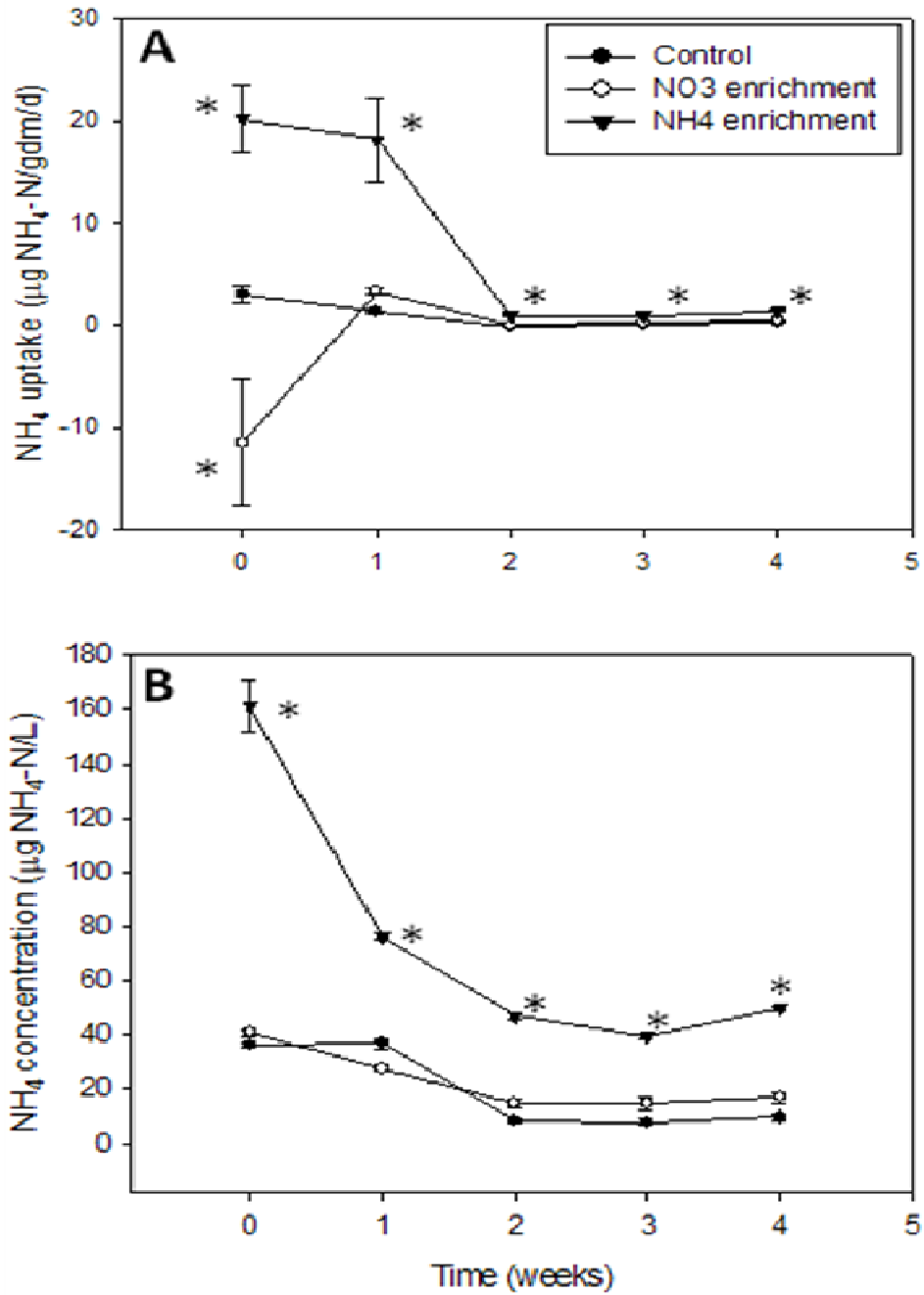


Figure 2:

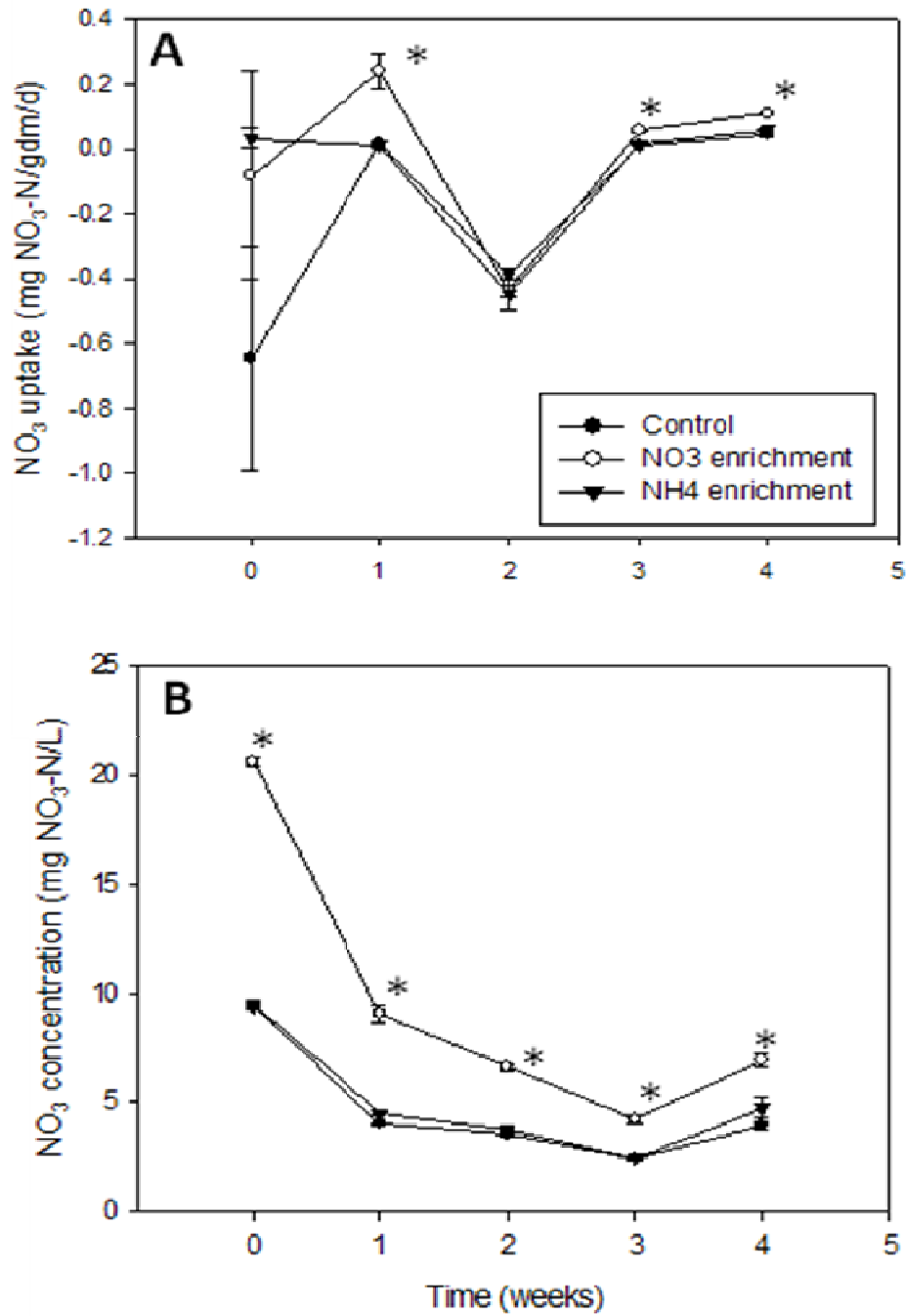
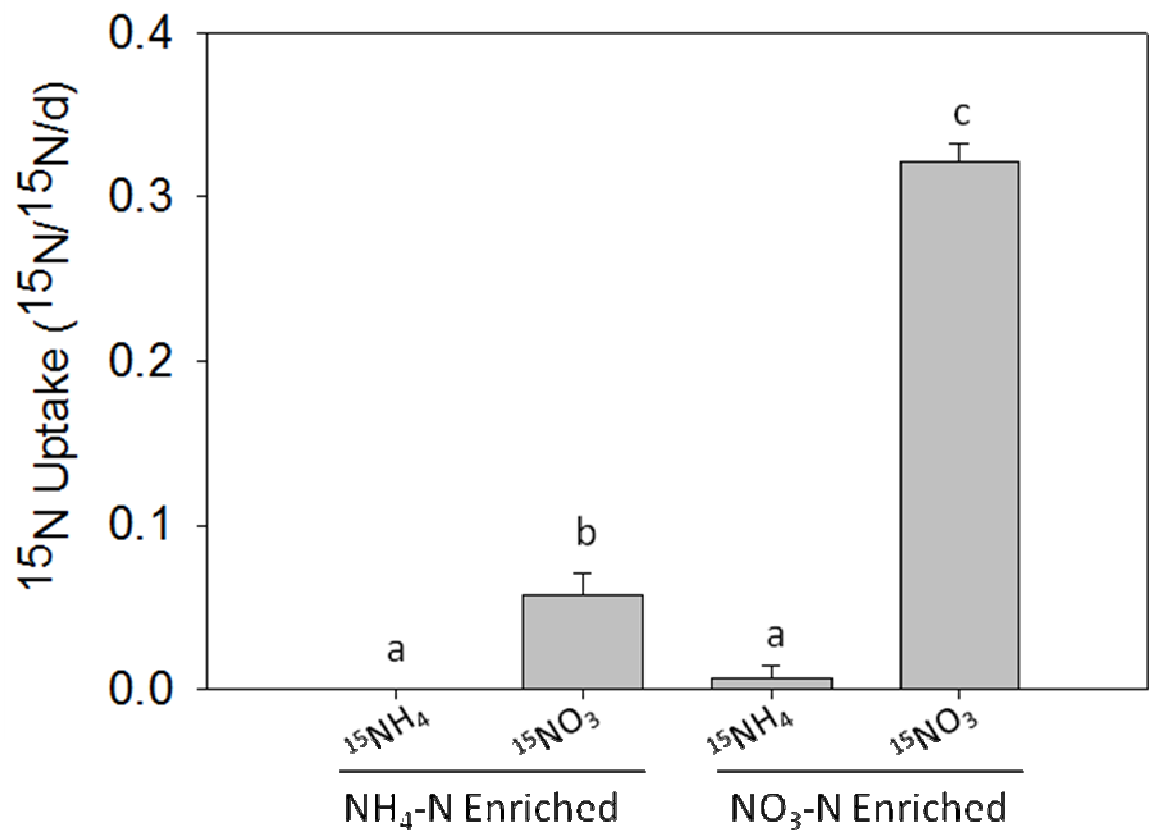


Figure 3:



Project Conclusion

The results of this study showed that $\text{NO}_3\text{-N}$ was the preferred source of inorganic nitrogen by mixed microbial communities in our *in vitro* studies of Midwestern US stream sediment. This was also true for *Pseudomonas fluorescens* cultured microbial populations. Uptake kinetics experiments illustrated saturation of $\text{NO}_3\text{-N}$ at concentration $>0.75\text{mg/L}$. Ammonium-N assimilation did not saturation at concentrations tested (up to $0.05\text{ mg NH}_4\text{-N/L}$).

This study also found microbial communities preferentially utilized the more available form of inorganic nitrogen over a four week incubation period (adaptation), and this response was more pronounced with $\text{NH}_4\text{-N}$ additions relative to $\text{NO}_3\text{-N}$ additions. Initial response to higher concentrations of nitrogen was different from the sustained response in mixed microbial communities. Higher $\text{NH}_4\text{-N}$ uptake was observed initially (up to week 2) with $\text{NH}_4\text{-N}$ enrichment. However, with sustained $\text{NH}_4\text{-N}$ enrichment, differences in uptake rates relative to controls diminished. Similarly, $\text{NO}_3\text{-N}$ uptake was variable up to week 3, and then became more consistent across replicates and treatments. Isotopic analysis illustrated higher incorporation of $^{15}\text{NO}_3\text{-N}$ in both enrichments ($\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$).

These *in vitro* assessments of microbial nitrogen uptake are limited in several respects. For example, nitrification in our experiments could have been a source of $\text{NH}_4\text{-N}$ uptake that was not measured. Additional studies are needed to assess nitrification response. Mesocosms also did not allow for exact replication of a natural stream environment and conditions may have not been comparable to *in situ* responses by

microbial communities. Parallel laboratory and field experiments should be conducted for more direct comparisons. Replication of experiments in several different streams could also be conducted to determine potential variations in response both throughout Midwestern streams and across different biomes.

With ever changing environments from anthropogenic activities, more experiments are needed to comprehensively assess microbial response to nitrogen loading. Longer time scales are needed to assess nitrogen thresholds and response of microbial community structure and activity. Furthermore, additional nutrients should be assessed in conjunction with $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ to see if there are any synergistic or antagonistic effects on uptake that would affect microbial communities' ability to preferentially utilize or adapt to altered nutrient availability. More research would allow for a better understanding of how excess nitrogen is influencing microbial activity and stream ecosystems.

Appendix I: Preferential Uptake. Uptake and remineralization rates in response to nitrogen enrichments (Chapter 1: Figure 1).

Treatment	Mixed Microbial Community		<i>Pseudomonas fluorescens</i>	
	Ammonium Uptake mg NH ₄ -N/L/d	Nitrate Uptake mg NO ₃ -N/L/d	Ammonium Uptake mg NH ₄ -N/L/d	Nitrate Uptake mg NO ₃ -N/L/d
NH4	-0.005	69.853	-0.080	11.719
NH4	-0.019	67.223	-0.077	8.932
NH4	0.002	72.056	-0.082	7.942
NH4	0.017	58.710	-0.078	11.896
NO3	0.011	50.216	-0.078	7.044
NO3	-0.023	72.521	-0.081	7.562
NO3	-0.006	70.955	-0.077	27.050
NO3	-0.019	67.260	-0.081	19.117
NH4/NO3	-0.006	61.615	-0.070	14.345
NH4/NO3	0.026	53.870	-0.073	15.746
NH4/NO3	-0.001	58.624	-0.078	24.697
NH4/NO3	0.013	65.341	-0.073	14.870
Control	0.003	43.720	-0.080	4.214
Control	0.004	43.556	-0.087	9.609
Control	0.000	44.993	-0.080	10.854
Control	0.009	33.170	-0.088	14.113

Appendix II: Uptake Kinetics. Uptake rates in response to increasing of nitrogen concentrations (Chapter 1: Figure 2).

Stream Sediment Microbial Community		
	Ammonium Uptake	Nitrate Uptake
Treatments	mg NH ₄ -N/L	mg NO ₃ -N/L
NO3 0	0.000	0.057
NO3 0	6.404	0.000
NO3 0	8.840	0.000
NO3 .25	0.000	0.000
NO3 .25	0.000	0.027
NO3 .25	8.840	0.000
NO3 .5	0.000	0.000
NO3 .5	0.000	0.000
NO3 .5	6.945	0.009
NO3 .75	0.000	0.000
NO3 .75	3.157	0.000
NO3 .75	0.451	0.000
NO3 1	0.000	0.000
NO3 1	5.322	0.000
NO3 1	8.298	0.000
NO3 1.25	0.000	0.000
NO3 1.25	4.239	0.000
NO3 1.25	0.000	0.000
NH4 0	0.271	0.002
NH4 0	6.494	0.000
NH4 0	0.000	0.018
NH4 10	1.439	0.000
NH4 10	0.000	0.000
NH4 10	0.000	0.040
NH4 20	0.000	0.010
NH4 20	0.984	0.004
NH4 20	2.878	0.000
NH4 30	6.482	0.000
NH4 30	0.000	0.000
NH4 30	0.000	0.014
NH4 40	9.545	0.000
NH4 40	0.000	0.054
NH4 40	0.000	0.000
NH4 50	6.113	0.000
NH4 50	10.443	0.000
NH4 50	8.819	0.007

Appendix III: Adaptive Uptake. Uptake and remineralization rates in response to chronic nitrogen enrichment (Chapter 2: Figures 1 and 2).

Ammonium Uptake ($\mu\text{g NH}_4\text{-N/gdm/d}$)					
Treatments	Week 0	Week 1	Week 2	Week 3	Week 4
C 1-1	1.133	1.794	-0.901	-0.048	0.000
C 1-2	-0.244	1.413	-0.109	-0.494	-0.435
C 1-3	1.165	1.421	-0.119	-0.079	0.059
C 1-4	1.501	3.069	-0.353	0.196	0.123
C 2-1	-0.638	0.937	-0.230	-0.443	0.380
C 2-2	-10.699	2.515	-0.540	1.084	0.829
C 2-3	-0.289	1.214	-0.329	-0.192	0.012
C 2-4	12.163	0.997	-0.104	0.024	0.065
C 3-1	-1.734	1.085	0.017	0.518	0.000
C 3-2	-3.001	1.283	-0.177	0.192	0.696
C 3-3	0.429	0.360	0.048	0.127	0.617
C 3-4	17.894	0.536	-0.426	0.314	0.057
C 4-1	-0.814	1.423	0.122	0.420	0.329
C 4-2	15.931	0.541	0.078	-0.145	0.111
C 4-3	4.358	0.937	0.029	-0.416	0.877
C 4-4	10.403	0.986	0.350	0.049	-0.256
NO3 1-1	-30.057	1.952	-0.283	-0.257	0.452
NO3 1-2	-149.776	12.934	-1.695	-0.079	0.412
NO3 1-3	23.204	0.671	-1.020	0.175	0.430
NO3 1-4	-36.554	2.137	-0.614	0.000	0.648
NO3 2-1	-3.502	0.704	-0.818	0.229	0.837
NO3 2-2	-15.508	0.471	0.329	0.292	0.484
NO3 2-3	-3.692	14.240	-0.244	0.182	0.476
NO3 2-4	1.882	1.198	-0.016	0.115	0.696
NO3 3-1	-0.499	0.506	0.470	0.655	0.610
NO3 3-2	-1.375	0.453	0.401	0.579	0.651
NO3 3-3	13.844	4.658	0.326	0.386	1.186
NO3 3-4	1.363	2.891	0.622	0.585	0.037
NO3 4-1	0.615	1.291	0.466	0.692	-0.209
NO3 4-2	11.996	1.309	0.298	0.469	0.288
NO3 4-3	3.376	6.129	0.781	-0.597	-0.170
NO3 4-4	0.442	1.369	0.374	0.992	0.022
NH4 1-1	10.429	5.991	1.224	0.893	1.661
NH4 1-2	23.247	130.198	0.463	1.087	0.289
NH4 1-3	30.788	16.946	1.461	1.330	1.731
NH4 1-4	23.806	11.916	0.759	1.493	1.557
NH4 2-1	9.831	1.552	0.583	1.115	1.146
NH4 2-2	5.022	7.565	0.582	1.391	1.338
NH4 2-3	4.940	42.288	0.913	0.975	1.764
NH4 2-4	7.611	8.979	0.604	-0.054	1.259
NH4 3-1	8.616	2.208	0.494	1.706	1.786
NH4 3-2	22.061	2.130	0.390	1.191	0.469
NH4 3-3	28.705	1.828	0.906	1.005	1.001
NH4 3-4	-2.219	3.196	0.758	1.007	1.214
NH4 4-1	11.946	1.200	1.166	0.786	2.165
NH4 4-2	24.716	11.017	0.443	-0.103	2.274
NH4 4-3	57.297	34.195	1.062	0.998	1.210
NH4 4-4	56.611	9.864	3.974	1.077	1.269

Nitrate Uptake (mg NO₃-N/gdm/d)

Treatments	Week 0	Week 1	Week 2	Week 3	Week 4
C 1-1	0.027	0.010	-0.410	0.008	0.025
C 1-2	-0.045	0.016	-0.368	-0.004	0.047
C 1-3	0.209	0.011	-0.370	-0.005	0.039
C 1-4	-0.006	-0.032	-0.466	0.002	0.029
C 2-1	-0.022	0.013	-0.304	0.007	0.034
C 2-2	0.503	0.018	-0.307	0.003	0.028
C 2-3	-0.828	0.029	-0.371	0.012	0.023
C 2-4	0.922	0.026	-0.395	0.014	0.027
C 3-1	-0.371	0.019	-0.374	0.015	0.064
C 3-2	-1.209	0.025	-0.392	0.007	0.066
C 3-3	-2.423	0.028	-0.371	0.007	0.055
C 3-4	-4.779	0.011	-0.487	0.011	0.052
C 4-1	-0.577	0.027	-0.297	0.019	0.044
C 4-2	-0.044	0.019	-0.323	0.012	0.057
C 4-3	0.053	-0.024	-0.336	0.017	0.046
C 4-4	-1.765	0.038	-0.607	0.009	0.038
NO3 1-1	-0.005	-0.216	-0.362	0.051	0.090
NO3 1-2	2.581	0.540	-0.423	0.053	0.104
NO3 1-3	-1.612	0.236	-0.342	0.039	0.091
NO3 1-4	1.170	0.189	-0.438	0.054	0.086
NO3 2-1	0.420	0.124	-0.426	0.038	0.144
NO3 2-2	-3.312	0.152	-0.406	0.042	0.140
NO3 2-3	-1.410	0.655	-0.428	0.047	0.085
NO3 2-4	-0.326	0.207	-0.477	0.041	0.077
NO3 3-1	0.464	0.126	-0.458	0.073	0.125
NO3 3-2	0.111	0.140	-0.402	0.073	0.116
NO3 3-3	-0.363	0.434	-0.422	0.059	0.174
NO3 3-4	0.188	0.209	-0.620	0.101	0.085
NO3 4-1	-0.114	0.119	-0.530	0.052	0.094
NO3 4-2	0.278	0.233	-0.402	0.052	0.102
NO3 4-3	0.387	0.533	-0.436	0.053	0.110
NO3 4-4	0.238	0.141	-0.456	0.053	0.101
NH4 1-1	0.044	0.027	-0.396	-0.001	0.054
NH4 1-2	0.068	-0.050	-0.344	0.013	0.049
NH4 1-3	0.053	0.016	-0.414	0.008	0.045
NH4 1-4	-0.130	-0.066	-0.363	0.005	0.034
NH4 2-1	0.146	0.028	-0.452	0.012	0.071
NH4 2-2	0.156	0.069	-0.392	0.020	0.090
NH4 2-3	0.154	0.016	-0.412	0.017	0.074
NH4 2-4	0.144	0.016	-0.447	0.011	0.070
NH4 3-1	0.119	0.033	-0.360	0.019	0.039
NH4 3-2	0.025	0.029	-0.353	0.016	0.041
NH4 3-3	-0.086	0.030	-0.394	0.008	0.026
NH4 3-4	0.037	0.041	-0.400	0.015	0.035
NH4 4-1	0.143	0.029	-0.389	0.027	0.084
NH4 4-2	-0.019	0.024	-0.346	0.026	0.075
NH4 4-3	-0.135	-0.077	-0.584	0.016	0.051
NH4 4-4	-0.197	0.010	-1.139	0.017	0.070

Appendix IV: Adaptive Uptake- Initial Concentrations. Mesocosm nitrogen concentrations (Chapter 2: Figures 1 and 2).

Treatments	Initial Ammonium concentration ($\mu\text{g NH}_4\text{-N/L}$)				
	Week 0	Week 1	Week 2	Week 3	Week 4
C 1-1	8.512	3.856	3.507	2.121	3.678
C 1-2	9.230	3.928	3.954	2.077	3.624
C 1-3	9.235	3.610	3.965	2.082	3.649
C 1-4	9.442	3.287	3.953	2.148	3.696
C 2-1	9.137	3.989	3.666	2.571	3.182
C 2-2	9.532	3.985	3.803	2.516	3.242
C 2-3	9.472	4.053	3.737	2.588	3.245
C 2-4	9.532	4.027	3.691	2.590	3.292
C 3-1	9.444	4.162	3.306	2.623	4.721
C 3-2	9.505	4.124	3.332	2.613	4.834
C 3-3	9.498	4.195	3.330	2.537	4.816
C 3-4	9.473	4.207	3.401	2.528	4.726
C 4-1	9.457	3.965	3.063	2.475	4.136
C 4-2	9.329	3.955	3.130	2.461	4.144
C 4-3	9.312	4.304	3.076	2.467	4.097
C 4-4	9.497	4.281	3.122	2.460	4.010
NO3 1-1	18.914	1.000	6.575	3.682	6.312
NO3 1-2	19.688	9.009	6.656	3.692	6.393
NO3 1-3	19.749	8.969	6.665	3.639	6.383
NO3 1-4	20.047	9.014	6.672	3.693	6.404
NO3 2-1	19.983	10.123	7.346	3.767	6.421
NO3 2-2	19.994	10.251	7.307	3.680	6.508
NO3 2-3	20.013	10.234	7.249	3.787	6.436
NO3 2-4	20.251	10.290	7.275	3.697	6.365
NO3 3-1	21.718	10.226	6.731	5.359	8.620
NO3 3-2	21.730	10.509	6.809	5.388	8.798
NO3 3-3	20.932	10.561	6.725	5.433	8.916
NO3 3-4	20.862	10.298	6.782	5.384	8.731
NO3 4-1	21.470	8.325	5.882	4.008	6.395
NO3 4-2	21.343	8.463	5.771	4.029	6.369
NO3 4-3	21.674	8.448	5.865	4.012	6.443
NO3 4-4	21.576	8.736	5.771	4.008	6.376
NH4 1-1	9.999	4.149	3.809	2.260	4.477
NH4 1-2	9.433	4.179	3.747	2.261	4.483
NH4 1-3	9.428	4.190	3.747	2.253	4.449
NH4 1-4	9.412	3.586	3.638	2.182	4.475
NH4 2-1	9.228	4.602	3.951	2.559	6.813
NH4 2-2	9.384	4.635	4.033	2.507	7.069
NH4 2-3	9.460	4.666	3.701	2.532	7.070
NH4 2-4	9.367	4.655	3.936	2.544	7.111
NH4 3-1	9.485	4.531	3.379	2.411	2.447
NH4 3-2	9.411	4.576	3.375	2.409	2.420
NH4 3-3	9.513	4.558	3.392	2.351	2.404
NH4 3-4	9.465	4.827	3.436	2.342	2.425
NH4 4-1	9.220	4.756	3.965	2.723	5.172
NH4 4-2	9.378	4.737	3.975	2.716	5.034
NH4 4-3	9.492	4.764	3.833	2.699	5.161
NH4 4-4	9.323	4.760	3.947	2.721	5.181

Initial Nitrate concentration (mg NO₃-N/L)

Treatments	Week 0	Week 1	Week 2	Week 3	Week 4
C 1-1	39.172	51.773	9.815	0.446	0.000
C 1-2	37.577	49.695	7.749	0.000	0.000
C 1-3	31.197	22.673	9.815	1.990	1.762
C 1-4	33.589	43.459	5.166	5.078	8.612
C 2-1	47.945	35.145	4.649	1.990	17.518
C 2-2	33.589	80.874	4.133	39.049	26.425
C 2-3	20.828	37.223	7.749	8.167	0.391
C 2-4	59.110	27.870	7.232	2.762	8.612
C 3-1	47.945	36.184	5.682	15.115	0.000
C 3-2	32.792	42.420	5.682	8.167	22.999
C 3-3	21.626	14.359	11.881	4.306	20.259
C 3-4	32.792	16.437	9.815	9.711	1.762
C 4-1	20.031	44.498	10.331	15.115	10.668
C 4-2	53.528	24.752	10.848	2.762	5.872
C 4-3	30.399	30.987	6.715	0.000	28.480
C 4-4	37.577	29.948	17.563	8.167	0.000
NO3 1-1	46.350	43.459	4.133	8.167	18.204
NO3 1-2	43.957	43.459	6.199	8.939	16.833
NO3 1-3	43.160	7.084	14.464	10.483	16.833
NO3 1-4	34.387	34.105	3.099	0.000	20.259
NO3 2-1	51.933	23.712	19.113	10.483	26.425
NO3 2-2	34.387	13.319	17.563	8.939	14.778
NO3 2-3	23.221	39.302	5.682	8.167	19.574
NO3 2-4	36.779	21.634	8.265	3.534	36.701
NO3 3-1	26.411	16.437	18.080	26.696	21.629
NO3 3-2	42.362	15.398	16.530	24.380	31.905
NO3 3-3	42.362	26.830	12.398	20.519	27.110
NO3 3-4	34.387	41.380	23.246	11.255	3.817
NO3 4-1	44.755	43.459	25.312	22.063	1.077
NO3 4-2	41.565	15.398	14.981	25.152	10.668
NO3 4-3	39.969	20.594	28.928	1.990	0.000
NO3 4-4	64.693	40.341	17.563	45.997	3.817
NH4 1-1	192.791	57.870	60.994	43.571	56.425
NH4 1-2	202.361	85.931	35.166	35.850	32.447
NH4 1-3	183.220	59.948	50.663	51.291	60.535
NH4 1-4	196.778	90.088	38.265	49.747	48.204
NH4 2-1	188.005	62.027	38.782	35.850	47.518
NH4 2-2	203.956	77.616	51.179	46.659	44.778
NH4 2-3	171.257	78.656	40.331	31.990	65.331
NH4 2-4	180.828	78.656	41.881	40.483	48.204
NH4 3-1	119.417	70.341	34.649	51.291	66.016
NH4 3-2	113.834	67.223	39.815	35.078	30.391
NH4 3-3	105.061	60.987	51.696	38.939	38.612
NH4 3-4	110.644	93.206	62.544	29.674	46.148
NH4 4-1	158.496	44.359	55.312	46.659	60.535
NH4 4-2	163.282	93.206	35.682	31.218	68.071
NH4 4-3	140.951	104.638	42.914	31.218	41.353
NH4 4-4	145.736	95.284	72.875	32.762	42.723

Appendix V: Isotopic Analysis. Isotopic abundance in response to ¹⁵N tracer additions after chronic nitrogen enrichments (Chapter 2: Figure 3).

Treatment	¹⁵N addition	δ¹⁵N o/oo vs AIR
Control 1	No addition	6.9
Control 2	No addition	6.1
Control 3	No addition	6.5
Control 4	No addition	6.4
NH ₄ -N 1	No addition	6.5
NH ₄ -N 2	No addition	6.8
NH ₄ -N 3	No addition	6.7
NH ₄ -N 4	No addition	6.5
NO ₃ -N 1	No addition	6.1
NO ₃ -N 2	No addition	5.5
NO ₃ -N 3	No addition	6.4
NO ₃ -N 4	No addition	5.8
Control 1	¹⁵ NO ₃	169.6
Control 2	¹⁵ NO ₃	108.3
Control 3	¹⁵ NO ₃	109.6
Control 4	¹⁵ NO ₃	156.7
NH ₄ -N 1	¹⁵ NO ₃	213.6
NH ₄ -N 2	¹⁵ NO ₃	122.7
NH ₄ -N 3	¹⁵ NO ₃	134.0
NH ₄ -N 4	¹⁵ NO ₃	127.2
NO ₃ -N 1	¹⁵ NO ₃	134.9
NO ₃ -N 2	¹⁵ NO ₃	163.2
NO ₃ -N 3	¹⁵ NO ₃	123.8
NO ₃ -N 4	¹⁵ NO ₃	139.7
Control 1	¹⁵ NH ₄	563.7
Control 2	¹⁵ NH ₄	483.6
Control 3	¹⁵ NH ₄	404.0
Control 4	¹⁵ NH ₄	512.0
NH ₄ -N 1	¹⁵ NH ₄	548.5
NH ₄ -N 2	¹⁵ NH ₄	506.4
NH ₄ -N 3	¹⁵ NH ₄	474.1
NH ₄ -N 4	¹⁵ NH ₄	431.4
NO ₃ -N 1	¹⁵ NH ₄	425.6
NO ₃ -N 2	¹⁵ NH ₄	482.4
NO ₃ -N 3	¹⁵ NH ₄	408.6
NO ₃ -N 4	¹⁵ NH ₄	461.8