

Report for 2004AL24B: Nitrogen Cycling in Alabama Rivers: Effects of Nutrient Addition on the Composition of Functional Microbial Communities

There are no reported publications resulting from this project.

Report Follows

Synopsis of Research

Project Title: Nitrogen Cycling in Alabama Rivers: Effects of Nutrient Addition on the Composition of Functional Microbial Communities

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a. Statement of the Problem and Research Objectives

Many of Alabama's freshwater ecosystems have been or are continuously exposed to conditions that degrade water quality. These range from increased sedimentation to addition of excess nutrients, either from agricultural run-off or point and non-point source contamination. Wastewater treatment plants are responsible for point-source pollution that results in the degradation of Alabama's rivers and streams. While effluent standards have been created and enforced for designated pollutants, numerous other compounds and pollutants are unregulated. These include nitrate and phosphate introduced into the system via tertiary treated wastewater released directly into Alabama's rivers and streams. Since nitrate is a biologically-available form of nitrogen, its impact on the natural communities inhabiting these ecosystems must be examined.

This proposal examines two sites in the Upper Watershed of the Cahaba River, one that is relatively pristine and one that is heavily impacted by effluent from a nearby municipal wastewater treatment plant. While macroscopic changes in the vegetation can be readily seen between the sites, almost nothing is known about changes in the microbial communities that are responsible for cycling nitrogen. We propose to examine the overall microbial biodiversity at each site and the bacterial taxa richness of those microorganisms that are capable of carrying out the various processes of nitrogen transformation. If, as predicted, significant differences in the microbial communities are found, this suggests that water quality is not being maintained and that community shifts are occurring at a process (or functional) level. The methods outlined in this proposal can be utilized as an additional or alternate method to more effectively monitor water quality within Alabama's freshwater ecosystems.

The primary objective of this research is to determine the presence and genetic diversity of the overall microbial community and of organisms capable of each process within the N cycle in a riverine ecosystem.

b. Research Methodology

Sampling

Water and sediment samples were collected during June 2004 from 2 selected sites on the Cahaba River. The two sampling sites along the Cahaba River were selected based on specific, documented land use patterns. One site is located 200 m north of a wastewater treatment outflow (considered for our purposes the pristine environment) and the second is immediately (<20 m) south of the wastewater treatment plant outflow into the main channel of the Cahaba River (considered the impacted site). From previous water chemistry measurements, these sites have been shown to be critical locations within the river system for nutrient enrichment and

utilization. Multiple 10cc syringe corers were used to take sediment samples. Water samples were taken for water chemistry analyses.

Water Chemistry Measurements

Surface water samples were collected in acid-washed polyethylene bottles, placed in ice within coolers, and transported to the laboratory, where water was immediately filtered through 0.7 µm GF/F filters. A portion of the filtrate was acidified and analyzed for dissolved organic carbon (DOC) on a Shimadzu T5000 Total Organic Carbon Analyzer (Wetzel and Likens 2000). A additional portion of the filtrate was analyzed for ammonium (NH₄-N), nitrate (NO₃-N), nitrite (NO₂-N), and soluble reactive phosphorus (SRP) by flow injection analysis using a QuickChem Automated Ion Analyzer (Lachat Instruments). Dissolved organic nitrogen (DON) was determined by a modified persulfate autoclave method (Dafner et al. 1999; Schaefer 2001) in which the final end-product is nitrate; DON concentration is determined from the post-digestion nitrate concentration minus the sum of the initial ammonium, nitrate, and nitrite concentrations.

DNA Extraction

DNA was extracted from the sediment samples using the method of Zhou et al. (1996), involving grinding in liquid nitrogen, freeze-thawing, high salt, and sodium dodecyl sulfate-extended heating. The resulting solution was extracted with an equal volume of phenol:chloroform (1:1), precipitated in isopropanol, and resuspended in Tris-EDTA (TE) buffer (used effectively in Jackson et al. 2001). Samples showing signs of humic contamination, (usually designated by a yellow-brown color) were purified using Sepharose 4B columns (Jackson et al. 1997).

PCR Amplification

Samples of sediment DNA were used to amplify 16S rRNA genes and genes specific for organisms with the capacity for nitrogen fixation, nitrification, and denitrification. Multiple amplification reactions (3 per sample) were run to ensure that DNA representative of the entire microbial community was used. Functional gene primer sets used included: nitrification-ammonia oxidizer partial 16S rRNA gene, *Nitrobacter* partial 16S rRNA gene; nitrogen fixation-*nifH* gene; denitrification- nitrite reductase *nirS* gene, nitrous oxide reductase *nosZ* gene.

Clone Library Formation

Amplified PCR products were ligated into pGEM (Promega Corporation) vectors and transformed into chemically competent *E. coli* cells. Resulting colonies were screened for successful insertion of the PCR product. PCR was used to amplify the insert and cloning was conducted by Macrogen (Korea).

Phylogenetic Analyses

Sequencher was used to align the resulting sequences. PAUP* will be used to perform phylogenetic analyses to determine if different communities are present at the two sites.

c. Principal Findings and Significance

Water Chemistry Data from June 21, 2004

Site	Total N (µgN/L)	NH₃-N (µg/L)	NO₃-N (µg/L)	NO₂-N (µg/L)	DON (µgN/L)	O-PO₄ (µg/L)	DOC (mgCL ⁻¹)
Upstream	361.48	11.60	226.83	2.19	120.86	8.78	6.07

Downstream	1512.00	26.02	1151.54	1.90	332.54	212.41	5.32
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Significance of Water Chemistry Data

These data indicate that the addition of the treated wastewater effluent into the main branch of the Cahaba River introduces a significant amount of nutrients, primarily as N and P. Total N increases by an order of magnitude, while PO₄ concentrations increase by two orders of magnitude. It has not yet been determined if these nutrient additions are sufficient to alter the structure of the functional microbial communities residing at the two sampling sites.

Molecular Data

Various genes (described in the research methodology section) were used to examine the potential functional microbial communities at the two sites. The following table indicates the number of sequences that have currently been obtained for each of the genes selected. Sequencing and data analysis are on-going. A phylogenetic tree generated using parsimony in PAUP* for recovered ammonia oxidizer 16S rRNA genes is shown below (Figure 1).

Functional gene	Upstream (pristine)	Downstream (impacted)	Total
<i>nifH</i>	0	6	6*
Ammonia oxidizer 16S rRNA gene	15	17	32
<i>Nitrobacter</i> 16S rRNA gene	13	14	27
<i>nirS</i>	7	11	18
<i>nosZ</i>	NA	NA	74^

* = additional clones are currently being sequenced

^ = these sequences have not yet been aligned

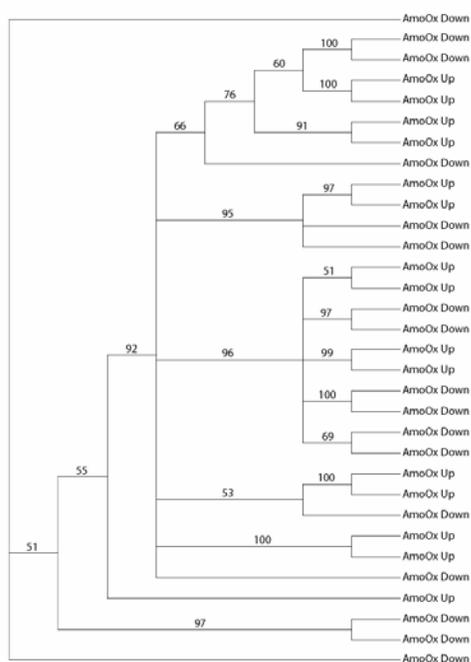


Figure 1. Strict consensus tree prepared in PAUP* using ammonia oxidizer partial 16S rRNA gene sequences. Bootstrap values from 1000 iterations that are greater than 50% are shown.

Significance of Molecular Data

This information will allow us to determine if the functional microbial communities involved in N-cycling are altered by the addition of nutrients from the wastewater effluent. Changes in these communities could serve as an early indicator of watershed water quality degradation.