Report as of FY2006 for 2006MN155B: "Ecological Stoichiometry and Microbial Biodiversity Effects on Water Quality in Minnesota Lakes"

Publications

Project 2006MN155B has resulted in no reported publications as of FY2006.

Report Follows

Ecological Stoichiometry and the Relevance of Prokaryotic Heterotroph Biodiversity

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Abstract

Prokaryotic heterotrophs are extremely abundant and have large impacts on global biogeochemistry and ecosystem processes such as nutrient regeneration and productivity (Cotner and Biddanda 2002). Ecological stoichiometry examines the balance of energy and chemical elements in living systems (Sterner and Elser 2002). In the work discussed here, the importance of microbial diversity and ecological stoichiometry to biogeochemical processes is being examined in aquatic systems.

Microbial stoichiometry and diversity interact to affect nutrient regeneration; stable interactions are promoted when decomposers are limited by organic carbon and the stoichiometry of decomposers is similar to that of autotrophs. Furthermore, biodiversity promotes redundancy and reliability in ecosystem function. However, the relationships among microbial stoichiometry, diversity, and ecosystem function have not been explored. This study is determining whether microbial diversity promotes stability in ecosystem function by providing increased stoichiometric diversity with subsequent effects on nutrient regeneration and productivity.

Hypotheses being tested are that (a) individual strains of bacteria are strongly homeostatic and (b) variable microbial community stoichiometry is achieved through variability in community composition. It is further hypothesized that (c) the efficiency at which nutrients are remineralized by the microbial community is directly dependent on the diversity present in a given lake/ecosystem.

Bacterial strains have been isolated from several lakes and characterized with respect to their stoichiometry under conditions of varying nutrients, resource ratios, and growth rates. Bacterial diversity is being ascertained using various PCR techniques and most of the previous year was spent developing those methods. We feel comfortable that we have some good measurements of microbial diversity now and the next year we will apply these methods using more field collected samples. Mathematical models are examining the importance of homeostasis at multiple levels, i.e., strains vs. communities.

Results to date:

Sampling

Last summer, ca. 10 lakes in Itasca State Park and surrounding areas were sampled twice (May and August) and 8 lakes in the Twin Cities Metro area were sampled multiple times (4-5). Water samples were collected from the mixed layer of the lakes. Samples were collected with a Van Dorn water sampler into acid-cleaned, sterilized containers.

Isolation and characterization of microbial strains

We have isolated over 50 strains of Bacteria from the lakes that we have sampled using the streak plate technique. Strains were isolated using several different types of media to increase the potential for greater metabolic and genetic diversity. Most of these isolates have been

screened initially by rep-PCR, which is a genomic fingerprinting technique that distinguishes bacteria to the sub-species or strain level (Rademaker and De Bruijn 1997) and are being sequenced in the Advanced Genetic Analysis Center at the University of Minnesota. Phylogenetic identification of nucleotide sequences will be determined by comparison with sequences available in the GenBank database (Benson et al. 1999) and the Ribosomal Database Project (RDP) database (Maidak et al. 1999).

In the coming year, we will manipulate substrate nutrient ratios and dilution/growth rates in culture to characterize strain stoichiometry and address some of the key components of our hypotheses. In mid-March 2007, we will add a post-doctoral fellow to our team (Thad Scott, from Baylor University) and he will be responsible for a large proportion of this culture work.

Community analysis and biodiversity quantification

To measure bacterial diversity in the lakes that we are studying, we have attempted the following techniques: (1) denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA gene fragments (PCR-DGGE), (2) terminal restriction fragment length polymorphism of nearly complete 16S rRNA genes (tRFLP), and (3) automated ribosomal intergenic spacer analysis (ARISA). We have determined that ARISA is best-suited to our purposes for the following reasons. PCR-DGGE is laborious, not well-liked by the scientific community, and it can detect only a limited number of taxa simultaneously (~15-20). tRFLP is less laborious and it has the potential to detect numerous taxa simultaneously. In contrast, ARISA is very simple to perform, which is of substantial importance because it would allow us to process many more samples (i.e., it can be very high throughput). It also targets a more variable region (the genetic material between the 16S rRNA gene and the 23S rRNA gene) than either PCR-DGGE or tRFLP. In theory, it is also biased against cyanobacteria, because they have a very large ITS region.

Another task of importance in the following year will be to further develop this method and to make comparisons among lakes and seasons to see if we can get an idea of how diversity varies. We anticipate that use of ARISA for making diversity measurements among lakes is sufficiently novel that it will merit publication (possibly Applied and Environmental Microbiology).

Chemostats and nutrient measurements

As mentioned above, we have sampled ca. 18 different lakes and all of the lakes have been sampled multiple times. We have collected samples for measurements of both seston and bacterial elemental composition. Currently, we have processed nearly 500 samples for particulate P content and should be obtaining results for the carbon and nitrogen content this winter from analyses performed elsewhere. In the coming year, chemostat experiments will be performed on individual strains that have been isolated. We will manipulate the elemental composition of their media and observe the effects on strain homeostasis and elemental composition.

We will also assemble 10 microbial communities by randomly selecting 2-5 strains and running them in chemostats at constant dilution rates with varying C:P ratios. However, we will focus primarily on high C:P ratios as this is the region where we expect diversity to matter most. We will measure biomass stoichiometry, dissolved nutrient content, DOC, dissolve inorganic C (DIC) to examine growth and re-generation efficiencies.

Mathematical modeling of biodiversity, homeostasis, and ecological stoichiometry

Both presence and absence of homeostasis of individual strains can result in bacterial communities that are not homeostatic. Whether or not bacterial strains are homeostatic can only be determined empirically. We can, however, use models to predict under which environmental conditions homeostasis at the strain level is favored. Building on standard chemostat models (Monod model and Droop model, as described in (Thingstad and Pengerud 1985) for bacterial

growth, investigated the growth of mixed bacterial communities under various nutrient-limited conditions (C or P; Neuhauseer, submitted). The Monod model assumes fixed cell quota, which imposes the constraint that individual strains cannot modulate the ratio at which they consume other nutrients (e.g., nitrogen or phosphorus). The Droop model assumes flexible cell quota and can be extended to allow the maximum nutrient uptake rate to vary with cell quota ((Morel et al. 1987)). Under constant nutrient supply the number of coexisting bacterial strains cannot exceed the number of resources according to resource competition theory (Tilman 1981). However, it has been shown under the fixed cell quota assumption that the number of strains coexisting at variable supply stoichiometry may be higher. Few theoretical studies have been conducted to investigate the effects of variable supply stoichiometry on diverse communities under the variable cell quota assumption (Grover 1991). The two proposed models will allow us to compare the ecological consequences of fixed and variable cell quota in the two models, and to determine which environmental conditions (amplitude and frequency of nutrient supply) favor one mode versus the other. Both analytical tools and numerical simulations (Matlab) will be used to investigate the proposed models.

Publications, Presentations, or Published Abstracts:

Presentation

Cotner, J.B.; Tim LaPara; Andre Amado; Meghan Funke, and Audrey Wiley. Bacterial diversity and its effects on nutrient and carbon cycling in lakes. American Museum of Natural History Conference on Microbial Conservation, To be held April 26-27, NY, NY.

Student(s) supported by this project:

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Awards
None to date

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Table 1. Diversity measures in Itasca Lakes, May and Aug 2006.

Sample Description	Number of Populations	Total Peak Area	Shannon Index	Simpson Index
Arco (Aug)	156	462303	1.6	16
Boot (Aug)	143	366615	1.46	8.66
Josephine ISP May 20	142	304144	1.6	11.18
Itasca (aug 1)	141	463052	1.5	14.2
Itasca (May)	123	389754	1.43	11.92
Long (august)	113	336276	1.46	13.13
Arco (May)	108	311849	1.16	3.87
Mary (Aug)	101	243999	1.52	17
Elk (Aug)	100	226923	1.59	21.3
Deming (May)	94	250894	1.31	8.21
Mary (May)	90	145444	1.56	14.1
Josephine ISP Aug	67	144963	1.39	12.61
Elk (May)	48	74872	1.25	6.5
Long May 20	41	130172	1.15	6.52
East Twin May 20	38	60001	1.42	16.1
Deming (Aug)	25	188680	1.02	6.97
Ozawindib (Aug)	19	49178	1.05	7.35
West Twin May 20	14	25858	1.05	9.93

Figure 1. ARISA results from Itasca State Park lakes, Aug 2006.