Diversity of Ammonia Oxidizing Bacteria Along an Estuarine Gradient

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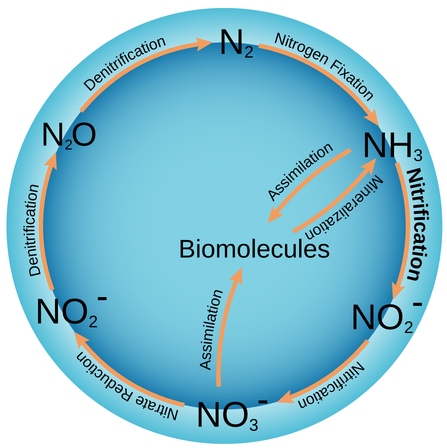
Abstract

Diversity of Ammonia Oxidizing Bacteria (AOB) Along an Estuarine Gradient

Nitrification, the oxidation of ammonia to nitrite and nitrate, is an important part of the global nitrogen cycle and is greatly mediated by bacteria. Although many species of bacteria involved in this process have been identified, their ecology is not well described. We analyzed differences in the community structure of ammonia oxidizing bacteria (AOB) relative salinity, depth, and season along the Plum Island Estuary in Massachusetts. Our approach uses the diversity of the ammonia monooxygenase (amo) gene to estimate community diversity. The diversity of each site is determined by "fingerprinting" each sample through terminal restriction fragment length polymorphism (TRFLP) of the A subunit of the ammonia monooxygenase gene (amoA). Each terminal restriction fragment (TRF) represents a specific microbial population, and the number of different TRFs within a sample can be used to estimate community diversity. Preliminary analyses of these data through multivariate ordination methods show clear differences in AOB community composition between the sample sites. We are currently building amoA gene libraries for each site to link the predominant gene fragment types in the TRFLP analyses to a particular gene clone. Clones matching the amoA gene fragment types will be sequenced and identified by comparison to sequences in the GenBank DNA sequence database. These data can then be used to link particular species and their prevalence to site-specific conditions such as salinity and oxygen concentration.

Introduction

The Nitrogen Cycle

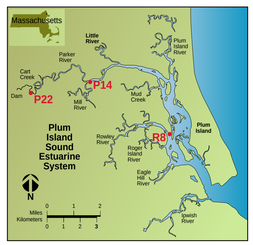


Electron Mictograph of an Ammonia Oxidizing Bacterium

Ammonia Oxidizing Bacteria are the predominant group of organisms that carry out the transformation of ammonia to nitrite. This process is known as **nitrification**. It is an important part of the global nitrogen cycle, affecting sewage treatment, agriculture, and ecosystem health.

Plum Island Sound

Plum Island Estuary has been identified for Long-Term Ecological Research. Sites represent low (P22), mid (P14), and high (R8c, R8m) salinity.



Methods

- Sediment cores were collected at each site and sectioned in 0.5 cm increments.
- DNA was extracted from each sediment slice.
- The amoA gene was amplified via PCR using primers specific to ammonia monooxygenase.

Study Goals & Hypotheses

Purpose

Estimate the diversity of Ammonia Oxidizing Bacteria (AOB) in estuarine sediment.

Hypotheses

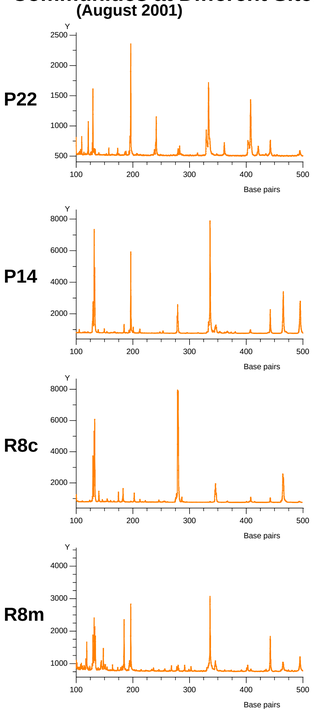
We expect to see changes in community structure based on:

1) **Sample depth**. Differences in oxygen availability may affect the AOB species composition, since different species have different oxygen needs.
2) **Site salinity**. Previous research has shown that certain AOB's predominate in freshwater, while other groups predominate in marine ecosystems.
3) **Seasonal variation**. Differences in nutrient availability, temperature, and other environmental conditions may be reflected in

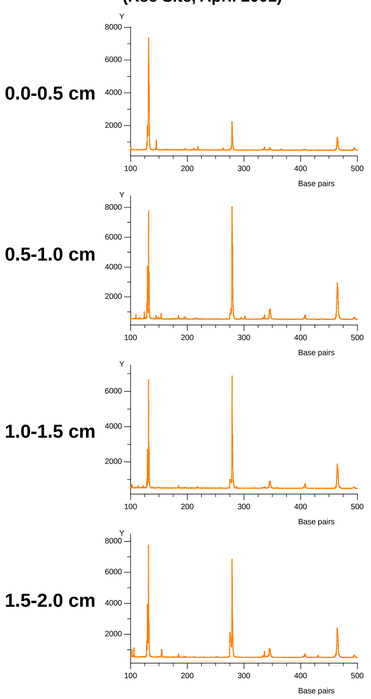
TRFLP Fragment Analysis

TRFLP: Each gene fragment length-type represents a specific subset of amoA genes that fragment in a similar way, based on their DNA

Communities at Different Sites
(August 2001)

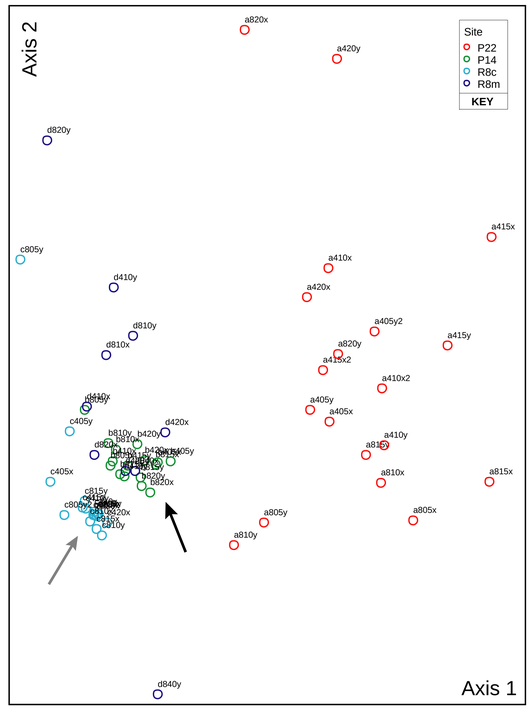


Communities at Different Depths
(R8c Site, April 2001)



- TRFLP Peaks show clear differences at different sites along the estuary, indicating a shift in the AOB community.
- The populations are stable within sites regardless of sample depth (oxygen concentration).
- No seasonal changes were visible.

Ordination



Ordination: A process of data reduction where each community is represented as a point. Distance between points is proportional to dissimilarity.

- Ordination of the TRFLP samples shows both a clear grouping of the AOB communities from sites P14 (black arrow) and R8c (gray arrow).
- Communities from sites P22 and R8m do not show a clear grouping and appear scattered in the ordination above.

Conclusions

The AOB Communities in our study:
Did not vary with sample depth
Shifted with site salinity
Did not reflect seasonal changes

Our data suggest that site salinity, rather than oxygen concentration or seasonal changes, drives the shift in comunity composition of AOBs.

Our future work will be to identify the particular species shown by representative TRFLP peaks.