Salinity Effects on Community Structure and Numbers of Ammonia-Oxidizing Bacteria and Nitrification Rates in Estuarine Sediment

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Abstract

We investigated the effects of salinity on nitrification and ammonia-oxidzing bacteria (AOB) community structure in estuarine sediments. Sites were chosen to represent a range of salinities (low, mid, and high). Potential nitrification rates and most probable numbers (MPNs) were measured at four salinities (0, 5, 10, and 30 ppt). Potential nitrification rates were higher in April than in August, but MPNs were higher in August. In April, rates and MPNs showed higher values near *in situ* salinities, but this was not the case in August. Community structure of AOBs was assessed by gene clone libraries of 16S rDNA and *amo*A subunit of the ammonia monooxygenase gene. Using primers specific for the 16S rDNA and *amo*A from AOB belonging to the beta and gamma Proteobacteria, we constructed clone libraries from low, mid, and high salinity sites. Phylogenetic analysis of the sequences revealed differences in the beta-AOB communities at these sites. Most notably, we did not recover any beta-AOB 16S rDNA or amoA clones from the low salinity site. Additionally, no 16S rDNA or amoA clones belonging to the gamma-AOB were recovered. These data indicate a seasonal cycle of nitrification, and a shift in AOB populations along the salinity gradient. By correlating changes in community structure with changes in process rates, we are beginning to resolve factors controlling nitrification in estuaries.

Objective

To characterize changes in the ammonia-oxidizing bacterial (AOB) communities along a salinity gradient and to correlate these changes in community structure with nitrification rates and abundance (MPN).

Hypotheses

Ammonia-oxidizing bacteria within the beta-Proteobacterial division will be most abundant at the freshwater end of the salinity gradient and will decrease with increasing salinity. Nitrification rates will also follow this pattern. These hypotheses are based on previous studies of AOB diversity in freshwater systems and nitrification rate data.

Methods

Duplicate sediment cores were collected from P22, P14, and R8 in April and August 2002. Cores were sectioned into 0.5 cm increments, homogenized, and stored at -80°C.

Potential nitrification rates were determined from sediment slurries at 4 different salinites with 300 µM ammonium and 60 µM phosphate additions. Incubations lasted 2-3 days, and nitrate was measured once a day.

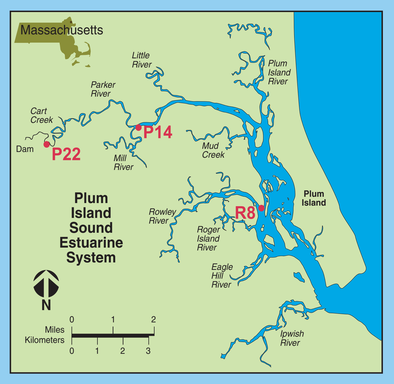
MPNs were determined using standard methods. Aliquots of sediment were incubated at 4 salinities under aerobic conditions.

DNA was extracted from 0.5 g aliquots using the Bio101 FastDNA kit for Soil.

16S rDNA genes were amplified using AMOf (1) and Nso1225r (2). AmoA genes were amplified using amoA-1F and amoA-2R (3), specific for the amoA subunit of the ammonia monooxygenase gene from the -AOBs.

Clone libraries were constructed using the TOPO TA cloning kit. Clones were randomly chosen for sequencing.

Study site

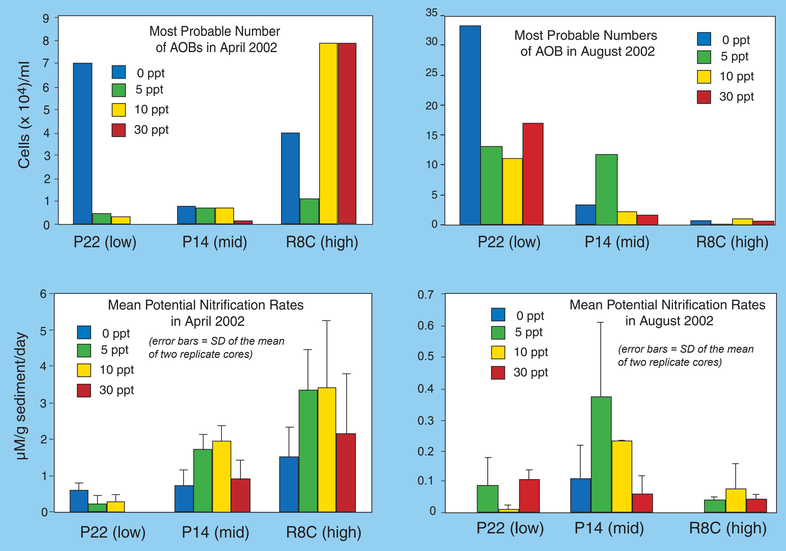


Plum Island Sound is a Long-Term Ecological Research site on the northeast coast of Massachusetts, U.S. Sites were selected to represent low salinity (P22), mid-salinity (P14), and high salinity (R8) conditions.

Plum Island Sound is a productive, riverine, tidal marsh system, with vegetation typical of a Northeastern salt marsh, including *Spartina alterniflora* and *S. patens*.

Site	April 2002 Salinity (ppt)	August 2002 Salinity (ppt)
P22	0	10
P14	2	25
R8	20	30

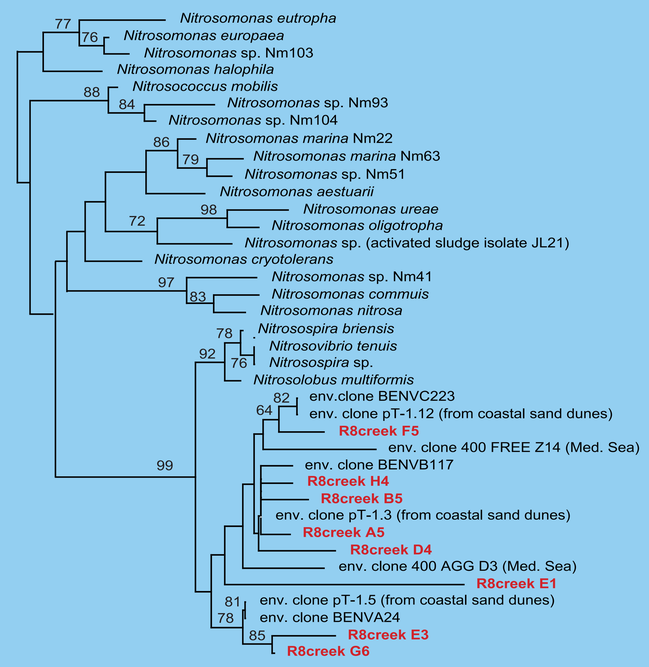
AOB Abundance and Nitrification Rates



- **At the high salinity site (R8), MPNs and potential rates were higher in April than in August. However, there was no correlation between MPNs and rates at the low and mid salinity sites.**
- **Potential nitrification rates were an order of magnitude higher in April than in August, but MPNs were higher in August.**

16S rDNA Phylogeny

(Tree was inferred by the neighbor-joining algorithm using the Kimura 2-parameter correction. This tree is based on 283 nucleotide positions.)



- No beta-AOBs were found in the clone library from the low salinity site (P22).
- Similarly, we have been unsuccessful at cloning *amo*A genes from the low salinity site (P22).
- Results from *amo*A phylogeny (not shown) were congruent with the 16S rDNA phylogeny

Conclusions

1) Based on findings from 16S rDNA and *amo*A clone libraries, beta-AOBs are more abundant at the high salinity site than the low salinity site. This is contrary to our initial hypothesis.

2) AOBs at the high salinity site are most closely related to the *Nitrosospiras*.

3) The conflicting results from nitrification rates and MPNs suggest significant culture biases, particularly at the low and mid salinity sites.

4) Despite potential biases, MPNs and nitrification rates at the low salinity site suggest the presence of novel nitrifying bacteria at this site.

References

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