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Salinity Effects on Nitrogen Dynamics in Estuarine Sediment Investigated by a Plug-flux Method

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The efficiency of nitrification and coupled denitrification of regenerated N is greater in fresh water than in the marine and estuarine environments (1). Salinity or factors related to salinity thus play a major role in determining the fate of regenerated N. Increasing salinity decreases the amount of exchangeable ammonium, which is thought to diminish the substrate availability for nitrifiers (1). Sulfide, which is associated with sulfate reduction in saline environments, inhibits nitrification and denitrification (2). Lastly, nitrifiers and denitrifiers presumably suffer direct physiological salinity stress (3).

The relative significance and interaction of these relations are unclear. The purpose of this study was to investigate the effect of salinity on estuarine sediment in the absence of potential sulfide effects. We employed a plug-flux method (4) in which thin layers of sediment are incubated with a small volume of overlying water after porewater concentrations have achieved steady state in a large volume of overlying water. The advantage of the method is that steady state can be attained relatively fast, and that linear fluxes during incubation can be interpreted as production rates (4).

Surface sediment (0–2 cm) was collected in April 1999 from a station with seasonally varying salinity in the Parker River Estuary, Massachusetts. Salinity was ~0‰ at collection. Sediment was sieved (1 mm), kept dark at 2°C, and stirred daily for 2 weeks prior to incubations. Sediment was incubated at different concentrations of artificial seawater lacking sulfate (0, 3, 10, and 30‰ under oxic conditions, and 0 and 30‰ under anoxic conditions). At each treatment, 14 plugs (0.8 cm deep, 4.8 cm diameter) filled with 14.5 cm^3 of sediment were each placed in open 125-ml cups, and afterwards placed in a tank containing 25 l of treatment water. The experiment was kept at 20°C. After 50 h, steady state was assumed and the cups were sealed. Exchangeable and porewater ammonium concentrations were hereafter measured to be constant in each treatment between 0 and 72 h, thus indicating that steady state had been achieved. Microelectrode measurements of oxygen concentrations indicated that only the top 2 mm of sediment in the plugs was oxygenated. Rates of ammonification, nitrification, denitrification, and oxygen consumption were calculated from differences in final and initial concentrations in the overlying water in the sealed cups after 0, 18, 40, 48, 72, and 161 h. At every timepoint, two cups from each treatment were removed for analysis. For sediments removed after 161 h, potential nitrification was measured as nitrate production in oxic conditions. The samples were shaken with treatment water enriched with 500 μM NH_4^+ and 200 μM PO_4^{3-} (5).

Ammonium concentrations were measured fluorometrically (6). Nitrate (+nitrite) concentrations were measured on a Lachat autoanalyzer. Oxygen consumption and denitrification were measured by membrane inlet mass spectrometry (MIMS) (7). Differences ($P < 0.05$) in flux rates among treatments were tested using a one-way ANOVA after determining whether data were homogeneous and normally distributed. If the differences were significant, treatment means were compared using a Tukey test. Nitrate efflux data were not homogeneous and were therefore analyzed with a Kruskal-Wallace nonparametric ANOVA.

The ammonification rates (Fig. 1A), expressed on an areal basis were ~0.6–0.9 mmol $m^{-2}d^{-1}$, which is typical for estuarine sediment. The ammonification rate was significantly higher at 3‰ than at all other salinities. There was no significant difference between the anoxic and oxic treatments at either 0 or 30‰. Measured nitrification rates (Fig. 1B) were very low (0–2% of ammonification rates), suggesting either low nitrification or efficient coupling to denitrification. Nitrate decreased at all treatments, suggesting net denitrification. Potential nitrification (Fig. 1C) was significantly higher at 3‰ than at 30‰. Oxygen consumption (Fig. 1D) was similar for all four oxic salinity treatments, suggesting that the difference in ammonification at 3‰ might reflect a difference in anaerobic metabolism. If Redfield ratio (C:N = 7:1) is assumed for the decomposed organic matter, then an oxygen consumption of 500 nmol would produce ~70 nmol of ammonification. Thus oxygen consumption alone is not sufficient to explain the ammonification rate at 3‰. The measured rates of denitrification (Fig. 1E) were highly variable and not significantly different from 0 in any treatment. The measurements ranged from 0 to 100 nmol N g^{-1} ww d^{-1}. However, because ammonification did not differ between anoxic and oxic treatments, because there was no nitrate in overlying water at the beginning of the experiment, and because there was very low measured nitrification, we believe that denitrification was very low.

In conclusion, there was a relative stimulation of ammonification and potential nitrification at 3‰. Neither sulfide inhibition nor the relative amount of exchangeable ammonium could explain this result, pointing to a direct physiological response of the bacterial community to salinity. This response could be due either to a larger number of active bacteria or to an increase in bacterial activity. Potential and actual nitrification was lowest at 30‰, which was expected due to severe salinity stress. Decrease in substrate availability could also acount for the low actual nitrification, but not for the low potential nitrification. However, the experimental setup was not optimal for investigating actual nitrification and denitrification. First, changes in oxygen conditions influenced the conditions for nitrification and denitrification, so that fluxes were nonlinear at several treatments. To investigate the effects on actual rates of nitrification and denitrification more thoroughly, it would be advisable to look at the two processes separately. This could have been done by investigating nitrification at thinner sediment plugs without an anoxic interface, and by investigating denitrification in plugs with an oxic anoxic interface as described above, but with a substantial addition of nitrate to the treatment water.

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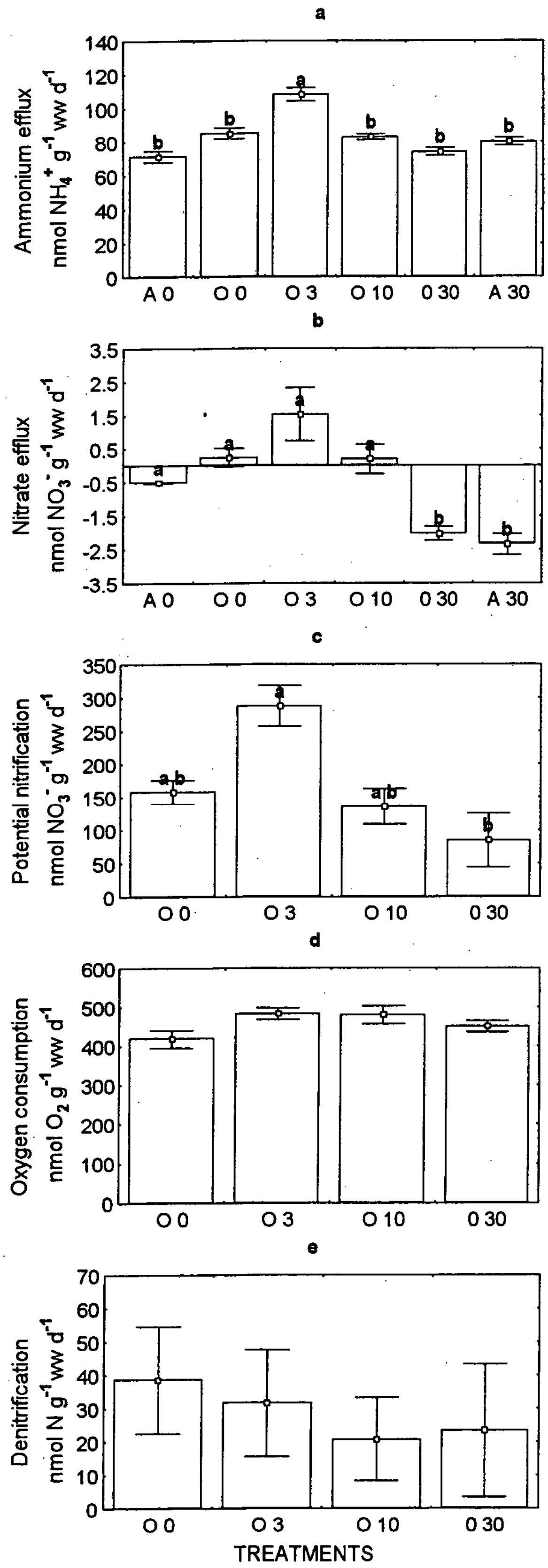

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Figure 1. *Average flux rates ± SE for each treatment (A 0 and A 30 = anoxic 0 and 30‰, O 0, O 3, O 10, and O 30 = oxic 0, 3, 10, and 30‰): (A) ammonium efflux, (B) nitrate efflux, (C) potential nitrification, (D) oxygen consumption, and (E) denitrification. Treatments shown to be significantly different (*$P < 0.05$*) by ANOVA following multiple comparison are indicated with different letters (A and B).*