Influence of Sulfide Inhibition of Nitrification on Nitrogen Regeneration in Sediments

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Nitrification, a central process in the nitrogen cycle, converts ammonium to nitrite or nitrate. In experiments with estuarine sediment, addition of 60 and 100 μM hydrogen sulfide (HS^-) reduced nitrification by 50 and 100 percent, respectively. Aerobic incubation of ammonium-enriched sediment slurries showed that previous HS^- exposure reduced nitrification for at least 24 hours; nitrification rates recovered slowly after one-time HS^- exposure. Sulfide inhibition of nitrification could limit nitrogen loss through coupled nitrification-denitrification and may contribute to the previously observed difference in net nitrogen cycling between freshwater and marine sediments. This interaction could also exacerbate eutrophication in coastal environments.

Nitrifying bacteria connect the oxidized and reduced sides of the N cycle by nitrification, the conversion of ammonium to nitrogen oxides. Light, temperature, and substrate concentration affect nitrification, but other factors could also have an effect (*1*, *2*). By serving as a conduit between ammonium regeneration and denitrification, nitrification links N regeneration and N loss (*1*, *2*) (Fig. 1). The relative efficiency of this connection appears to differ between coastal marine or estuarine and freshwater sediments (*3*). Estuarine and marine sediments release similar amounts of ammonium and dinitrogen (N_2), whereas freshwater sediments release mainly N_2 by means of coupled nitrification-denitrification (*4*, *5*). The factor or factors that control this pattern are unclear.

The size of the exchangeable ammonium pool, biogeochemical zonation, and the dominant pathway of metabolism differ between freshwater and marine sediments and could influence the fate of regenerated N (*5*, *6*). In sediments, bacteria oxidize a significant fraction of organic matter using terminal electron acceptors other than oxygen (O_2). Two dominant anaerobic processes are dissimilatory sulfate reduction and methanogenesis. Generally, sulfate reduction (HS^- production) precedes methanogenesis (methane production) because sulfate-reducing bacteria outcompete methanogens for substrates (*6*). Freshwater has lower sulfate concentrations (10 to 200 μM) than does estuarine water (30 mM), and although sulfate reduction may occur in freshwater sediments (*7*), the process is usually less important than methanogenesis.

Because HS^- has been reported to inhibit nitrifying bacteria in biofilm reactors (*8*), we hypothesized that HS^- inhibition of nitrification might account for spatial and temporal differences in nitrification within estuarine environments as well as for the difference in N biogeochemistry between estuarine-marine and freshwater environments (*5*). We tested this hypothesis using sediment from Tomales Bay, California (38.5°N, 122.5°W) (*9*), a small estuary 40 km north of San Francisco. Estuaries and nearshore marine environments serve as centers of deposition for continentally derived organic materials. Most denitrification in marine sediments thus occurs in coastal environments rather than in deep-sea environments, where the sedimentation rate is low (*3*).

Nitrification was rapidly and substantially reduced when 60 or 100 μM HS^- was added to sediment slurries (Fig. 2) (*10*). We found that pulsing with HS^- significantly inhibited nitrification in slurries enriched for nitrifying bacteria, even when the slurries were allowed to recover for 24 hours before we assessed nitrification (Fig. 3) (*11*). The HS^- concentrations we used lie within the range common to estuarine sediments, 7 to 200 μM (*12*), and were much lower than those of organic-rich sediments (>1 mM) (*13*). The range of HS^- concentration in freshwater sediment pore water is much lower (0 to 30 μM) (*12*). In time-series experiments, slurries were amended with HS^- and HS^- plus synthetic goethite, an iron oxide that reacts with HS^-, to determine whether rapid HS^- removal eliminated or reduced the inhibitory effect. Sulfide inhibition persisted despite rapid (≤0.5 hour) removal of HS^- (Fig. 4) (*14*).

Sulfide removal from sediment pore water was likely facilitated by high concentrations of reactive metal oxides (for example, 100 μmol of reactive iron per gram of dry sediment). The availability of reactive iron varies in marine sediments (*15*) and may serve to modulate free HS^- concentration. Despite HS^- addition, the concentration of dissolved O_2 did not differ significantly between HS^--amended and control treatments (Fig. 4). Even though HS^- persisted for only a short time, samples amended with HS^- exhibited significantly lower nitrification rates than did controls (Fig. 4). Thus, only a brief exposure to HS^- was required for inhibition of nitrification.

In the above experiments, the sediment microbial community was exposed to low HS^- concentration in a uniform environment (serum bottles). In situ, nitrification and sulfate reduction are spatially and temporally variable. Both respond to environmental factors, such as bioturbation and burrow irrigation, inputs of organic matter, and benthic primary production, each of which affects the thickness of the aerobic oxidation

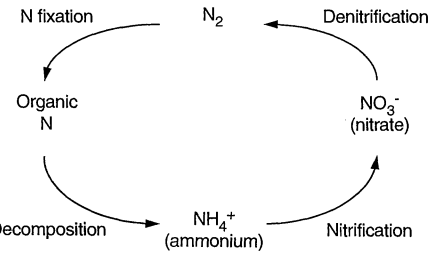

Fig. 1. The transformations of N between gaseous, reduced, and oxidized forms are mediated by bacteria. Gaseous N (N_2) is converted to organic N by N-fixing bacteria. Organic N undergoes decomposition to yield ammonium, which may be assimilated (by bacteria or phytoplankton) or may be nitrified. The processes of nitrification (NH_4^+ oxidation) and denitrification (NO_3^- reduction to N_2) combine to close the nitrogen cycle, returning inorganic, fixed N to the gaseous form. Blocking nitrification or denitrification serves to retain N in a biologically available form (NH_4^+ or NO_3^-).

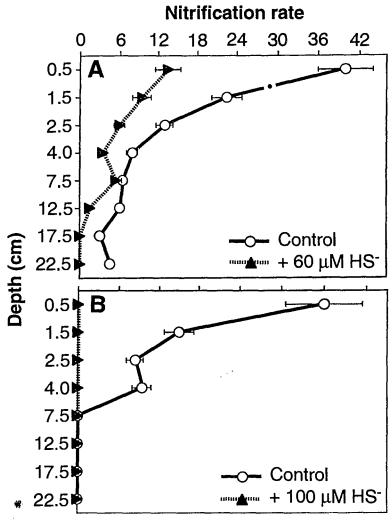

Fig. 2. Effect of HS^- addition [60 μM (**A**); 100 μM (**B**)] on nitrification in estuarine sediments (*10*). Symbols mark the mean of replicate samples (n = 3), and error bars indicate the standard deviation of the mean. Nitrification rate is expressed in nanomoles of NO_x (NO_3 + NO_2) per gram of wet sediment per hour.

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zone in the sediment (*1*, *12*). As a result, the boundary between oxic and anoxic conditions migrates on time scales of seconds to hours and on spatial scales of micrometers to centimeters. Nonmotile nitrifying bacteria are thus exposed to variable conditions that could include periods of HS^- exposure. These processes also vary on seasonal time scales (days to months) and larger spatial scales (meters to kilometers).

Several published studies on N cycling in coastal environments may reflect interactions between HS^- and nitrification. Kemp *et al.* (*16*) showed that during summer in the Chesapeake Bay, sediment nitrification and denitrification reached minima, whereas the benthic N flux reached a maximum. Nitrification increased with bottom water O_2 concentration, which implies that low O_2 concentration limits nitrification. However, this pattern could also reflect HS^- inhibition of nitrification. Under low O_2 conditions, HS^- production will be stimulated while HS^- oxidation is limited. The resulting increase in HS^- concentration could promote inhibition of nitrification by HS^-. Hansen *et al.* (*17*) also observed summer minima in nitrification in Danish coastal sediments. They suggested that HS^- might be an important factor influencing nitrification. Caffrey *et al.* (*18*) showed that nitrification increased with small increases in organic loading but decreased if loading was increased further. They hypothesized that the increase in N regeneration was due to O_2 limitation of nitrification; however, HS^- inhibition also may have been important.

All of these examples of shifts in N regeneration are from estuarine environments. Oxygen concentration also fluctuates in freshwater sediments, but without concomitant production of HS^-. Although O_2 availability might be expected to limit nitrification during summer when benthic metabolism is high, annual maxima in nitrification often occur at this time (*19*). This pattern suggests that O_2 concentration is not the primary variable regulating nitrification in freshwater systems, at least during summer.

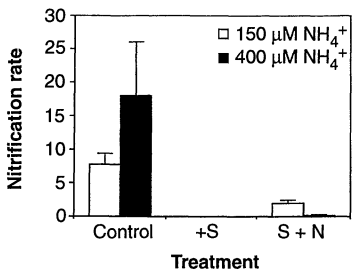

Fig. 3. Effect of HS^- pulses on nitrification in slurries enriched for nitrifying bacteria. No HS^- was present during nitrification assays (*11*). Bars represent the mean nitrification rate (as expressed in Fig. 2) (n = 3), and error bars indicate the standard deviation of the mean. Clear and black bars denote 150 and 400 μM NH_4^+ addition, respectively, during the nitrification assay. Rates in HS^--amended treatments were significantly lower than control rates ($P \leq 0.05$, Fisher's exact test).

Sulfide inhibition of nitrification thus appears to be important in regulating the N cycle of estuarine and marine sediments. It may also explain the pattern of increased N regeneration and less efficient, coupled nitrification-denitrification in estuarine and marine sediments compared with that in freshwater sediments (*5*). Cultural eutrophication or bloom events that increase organic matter sedimentation and stimulate HS^- production in sediments (*20*) could uncouple N regeneration from denitrification by blocking the requisite intermediate step, nitrification. Increased N regeneration could act as a positive feedback loop, enhancing primary production in the water column and accentuating the cycle of cultural eutrophication. For example, increased primary production would stimulate organic matter delivery to sediments, which would stimulate organic matter oxidation and sulfate reduction, leading to higher HS^- concentrations and HS^- inhibition of nitrification. Sulfide inhibition of nitrification would increase benthic N regeneration; higher ammonium fluxes to the water column could further stimulate primary production. Denitrification is also inhibited by HS^- (*21*); thus, the effect of HS^- on N cycling in marine sediments includes both portions of the N-sink couple. Other reduced S compounds, such as polysulfides, which may have a longer half-life than does HS^-, may also inhibit nitrification.

The seasonality of sulfate reduction, HS^- production, and HS^- inhibition of nitrification and denitrification should lead to enhanced ammonium regeneration during summer, when sulfate reduction rates are high compared with those in winter (*12*). Such seasonal or episodic enhancement of ammonium regeneration could result in shifts in the nutrient (N, P, or Si) limiting primary production (*22*). Internal control of the sedimentary N cycle by HS^- appears to be an important factor regulating the fate of sediment N and demonstrates a link between the global biogeochemical cycles of N and S.

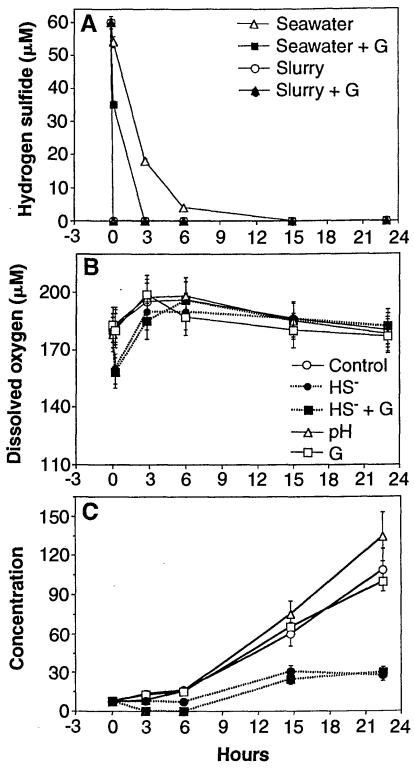

Fig. 4. Concentration of HS^- (**A**), dissolved O_2 (**B**), and NO_x accumulation (**C**) in sediment slurries (*12*). For (A), data from seawater only (seawater) and seawater plus sediment (slurry) treatments, with or without goethite, are shown. Symbols represent the mean (n = 3); error bars denote the standard deviation of the mean. Nitrification rates were significantly lower (analysis of variance, $P <$ 0.05) in HS^--amended compared with control treatments. Concentration in (C) is a function of nanomoles of NO_x per gram of wet sediment. Symbols in (C) are the same as those in (B).

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10. Two sediment cores (30 cm) were collected (January 1994) and sectioned into discrete depth intervals; the salinity of the bay was 30 practical salinity units. Samples from each depth were slurried [1.5 g of wet sediment + 50 ml of filtered (glass fiber filler, 0.7 μm nominal pore size) seawater] and placed in 70-ml serum bottles. Bottles were amended with ammonium (NH_4^+, 300 μM) or NH_4^+ plus 60 or 100 μM HS^- and sealed. Samples were incubated in the dark with constant shaking (100 rpm). Replicates were collected and analyzed at intervals between 0 to 24 hours to ensure linearity in nitrification rates [linear regressions of (NO_3 + NO_2) versus time had r^2 values of ≥0.98]. Samples did not become anoxic during the incubation (*see* Fig. 4). After incubation, a 15-ml aliquot was filtered (GF/F) and immediately frozen for subsequent NO_x and NH_4^+ analyses. [NO_x] was determined with the spongy cadmium reduction method (*23*), and nitrification rates were calculated from the change in NO_x concentration over time.
11. Surface (top 5 cm) sediment was sieved (1-mm

mesh) and slurried with filtered bottom water. Three sterile flasks each received 500 ml of sediment slurry. The three flasks were as follows: control, amended with 400 μM NH_4^+; +S, pulsed with 100 μM HS^- twice daily; or S + N, amended with 400 μM NH_4^+ and pulsed with 100 μM HS^- twice daily. During enrichment, hydrated air flowed continuously through the cultures. Approximately 24 hours after the last HS^- pulse was administered, nitrification rates were assessed in subsamples amended with either 150 or 400 μM NH_4^+.

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14. Surface sediments (0 to 3 cm) were sieved, homogenized, and randomly distributed [1 gws (gram of wet sediment)] into 40-ml serum bottles. Thirty milliliters of filtered seawater, amended with 300 μM NH_4^+, was added to each bottle; 18 replicates were prepared for each treatment group. Treatment groups were as follows: control, NH_4^+ only; pH, pH adjusted with NaOH to match that in HS^--amended samples; goethite (G), amended with 700 μM goethite; HS^-, amended with 60 μM HS^-; and HS^- + G, amended with 60 μM HS^-, then with 700 μM goethite. Triplicate samples from each group were collected and analyzed at approximately 0.5, 3, 6, 12, and 23 hours. Sulfide was determined as in (*24*). We quantified oxygen concentration by inserting microelectrodes (Diamond General, Ann Arbor, MI) into bottles before collecting nutrient samples. Oxygen was not limiting during these experiments. NO_x concentration was determined as described previously (*10*).
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Cross-Arc Geochemical Variations in the Kurile Arc as a Function of Slab Depth

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Lavas from transects across the Kurile Islands arc showed geochemical variations related to changes in the compositions of fluids derived from the subducting slab. Enrichment factors for boron, cesium, arsenic, and antimony, all elements with strong affinities for water, decreased across the arc. This decrease is presumably related to losses of water-rich fluids during the dehydration of the subducting plate. Enrichments of potassium, barium, beryllium-10, and the light rare earth elements remained constant; these species may move in silica-rich fluids liberated from the slab at greater depths.

The involvement of slab-derived melts (*1*, *2*) and the importance of nonmagmatic (that is, fluid) slab components (*3*–*6*) in arc magmatism are uncertain and much debated. Only recently have clear indicators for slab contributions been identified: High concentrations of B and ^{10}Be in arc lavas require material inputs to arc source regions from subducted oceanic sediments and altered oceanic crust (*4*, *7*). To better understand magma generation at arcs and the significance of subduction in crustal recycling, we need to know what processes control material fluxes from the subducting plate and how these fluxes interact with the overlying mantle.

We examined trace element systematics in suites of lavas from a series of "cross-arc transects" across the arc of the Kurile Islands. The volcanoes sampled lie 120 to 250 km above the subducting plate and may thus reflect slab-mantle interactions occurring over a range of pressure and temperature conditions. The Kurile Islands are a stereotypic island arc, erupting medium K calc-alkaline lavas across an unusually wide volcanic zone (Table 1) (*8*). Most Kurile lavas that have been analyzed have ^{10}Be contents greater than 1.0×10^6 atoms/g, which indicates subducted sediment (slab) involvement during magma genesis. Limited isotopic variation ($^{87/86}Sr \approx 0.703$ to 0.7034 and $^{143/144}Nd \approx 0.5130$ to 0.5131) (*9*) suggests that neither enriched mantle sources nor crustal assimilation strongly affects lava chemistry.

Data for six Kurile Islands cross-arc transects are presented in Table 1 and Fig. 1 (*10*). Incompatible trace elements show a spectrum of across-arc variation patterns. B and Sb (*11*) reflect one extreme (Fig. 1A)—high concentrations in volcanic front (VF) lavas, with abundances declining across the arc. Cs and Rb (Fig. 1C) show no clear pattern of concentration variation, whereas K, Ba, and most other elements (Fig. 1, E and G) increase in concentration across the arc. These element abundance patterns result from slab inputs, which may vary with increasing slab depth, and from varying extents of partial melting and crystal fractionation. We disentangled these effects by determining the ratios of the elements of interest to other elements with similar solid-melt distribution coefficients (*D*s) but much lower apparent solid "slab fluid" *D*s. Use of these element ratios minimized the effects of processes other than subduction modification of the mantle. K_2O, La, and B all have solid or melt *D*s similar to that of Be; the solid or melt *D* for Cs is more similar to that of Th. Thus, in

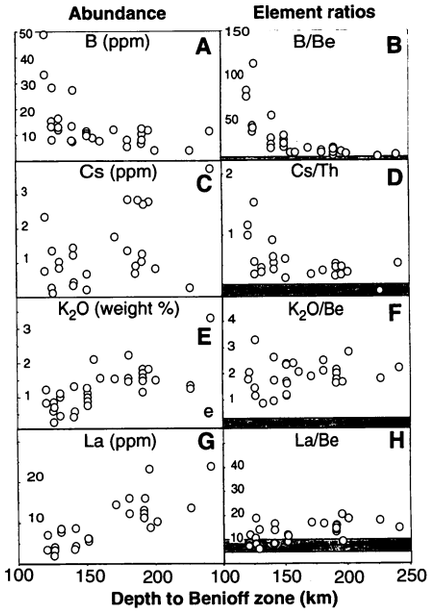

Fig. 1. Plots of element abundance and ratios for cross-arc transects in the Kurile Islands. Depths to Benioff zone were determined with the use of data from (*32*) and (*33*); absolute uncertainties in depths are ±10 km, but volcano-to-volcano uncertainties are much smaller. Gray fields on ratio diagrams represent ranges for mid-ocean ridge basalt and Ocean island basalt sources based on (*5*) and (*35*) and references therein. (**A** and **B**) B and B/Be versus depth. (**C** and **D**) Cs and Cs/Th versus depth. (**E** and **F**) K_2O and K_2O/Be versus depth. (**G** and **H**) La and La/Be versus depth.

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