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Vertical Migration in the Sediment-Dwelling Sulfur Bacteria *Thioploca* spp. in Overcoming Diffusion Limitations

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In order to investigate the environmental requirements of the filamentous sulfur bacteria *Thioploca* spp., we tested the chemotactic responses of these sedimentary microorganisms to changes in oxygen, nitrate, and sulfide concentrations. A sediment core with a *Thioploca* mat, retrieved from the oxygen-minimum zone on the Chilean shelf, was incubated in a recirculating flume. The addition of 25 μmol of nitrate per liter to the seawater flow induced the ascent of the *Thioploca* trichomes (length, up to 70 mm) in their mostly vertically oriented gelatinous sheaths. The upper ends of the filaments penetrated the sediment surface and protruded 1 to 3 mm into the flowing water before they bent downstream. By penetrating the diffusive boundary layer, *Thioploca* spp. facilitate efficient nitrate uptake in exposed trichome sections that are up to 30 mm long. The cumulative length of exposed filaments per square centimeter of sediment surface was up to 92 cm, with a total exposed trichome surface area of 1 cm^2. The positive reaction to nitrate overruled a negative response to oxygen, indicating that nitrate is the principal electron acceptor used by *Thioploca* spp. in the anoxic environment; 10-fold increases in nitrate fluxes after massive emergence of filaments strengthened this hypothesis. A positive chemotactic response to sulfide concentrations of less than 100 μmol $liter^{-1}$ counteracted the attraction to nitrate and, along with phobic reactions to oxygen and higher sulfide concentrations, controlled the vertical movement of the trichomes. We suggest that the success of *Thioploca* spp. on the Chilean shelf is based on the ability of these organisms to shuttle between the nitrate-rich boundary layer and the sulfidic sediment strata.

Thick microbial mats formed by giant filamentous sulfur bacteria of the genus *Thioploca* (Fig. 1) cover the shelf sediments at depths of 50 to 280 m off the Chilean and Peruvian coasts (5, 7–10, 15, 26). In this depth range the oxygen-poor and nutrient-enriched waters of the Peru-Chile subsurface countercurrent contact the seabed, creating a benthic environment with extended periods of suboxic and anoxic conditions. During spring and summer, upwelling events boost the primary production in the water column (up to values of 10 g of C m^{-2} day^{-1} [44]), and the *Thioploca* mats reach their maximum biomass of up to 1 kg (wet weight, including sheaths) m^{-2} (8, 10). Despite the vast area that they cover (from southern Peru to mid-Chile, an area which is 3,000 by 13 km [approximately 40,000 km^2] [10, 26, 29, 40]), the first report describing the mats by Gallardo (6) dates back only to 1963. The light brown, porous mats consist of an organic compound-rich sediment matrix containing up-to-15-cm-long interwoven bundles of *Thioploca* trichomes enclosed in gelatinous sheaths. One sheath may contain up to 100 of these filaments (consisting of one or even several *Thioploca* species [41]), which independently glide back and forth in their mucilaginous envelope.

At least three species, which are distinguishable by their 16S rRNA sequences (*Thioploca araucae* and *Thioploca chileae* [42]) or by their different trichome diameters (*T. araucae* trichomes are 30 to 40 μm in diameter, *T. chileae* trichomes are 12 to 20 μm in diameter, and *Thioploca* sp. trichomes are 2.5 to 5 μm in diameter [8, 41]) make up the mats. The uniseriate filaments, which are composed of several hundred to thousands of cells, reach lengths of 10 to 70 mm. The cylindrical cells are characterized by a large central vacuole and deposition of sulfur globules external to cytoplasmic invaginations (23, 28). Although bacteria belonging to the genus *Thioploca* were discovered by Lauterborn (24) in 1907 and several *Thioploca* species isolated from freshwater and marine environments have been described since that time (22, 25, 26, 47), little is known about the metabolism of these conspicuous microorganisms. One reason for this lack of information is that all attempts to grow *Thioploca* spp. in culture have failed so far.

The morphology of *Thioploca* spp., including the large vacuoles, is very similar to the morphology of some *Beggiatoa* species, which are filamentous sulfur bacteria assigned to the same family, the *Beggiatoaceae* (24, 30). 16S rRNA sequencing has shown that the genus *Beggiatoa* is phylogenetically the closest known relative of the genus *Thioploca* (42). *Beggiatoa* spp. obtain energy from the oxidation of sulfide with oxygen, and intermediate products of this oxidation are sulfur globules deposited in the cells (32, 46).

Because the water column above the Chilean *Thioploca* mats is oxygen depleted for extended periods of time (8, 26), utilization of oxygen by *Thioploca* spp. as its main electron acceptor, as found in *Beggiatoa* spp. (20), is unlikely. On the Chilean shelf, *Thioploca* mats occur only under the oxygen minimum zone, while *Beggiatoa* spp. cover the sulfidic sediments in shallow, oxygenated waters near the coast (4, 5, 41). So far, bacteria belonging to the genus *Thioploca* have been found only in environments where the oxygen concentration was <40 μmol $liter^{-1}$ and the sulfide concentration was <500 μmol $liter^{-1}$, indicating that the metabolism of *Thioploca* spp. is adapted to a different ecological niche than the metabolism of *Beggiatoa* spp. (23, 25, 26, 40). Autoradiographic experiments performed by Maier and Gallardo (27) with Chilean *Thioploca* species indicated that nutrition was mixotrophic with sulfide oxidation as the energy source.

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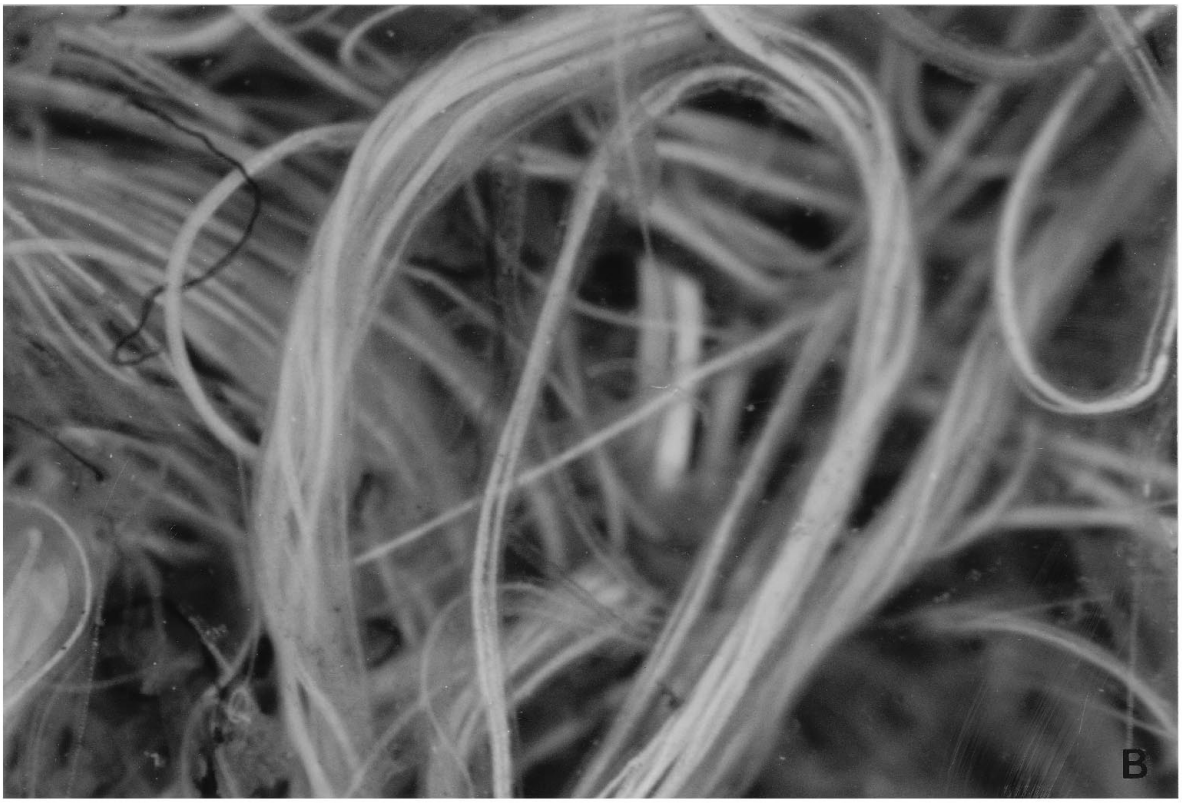
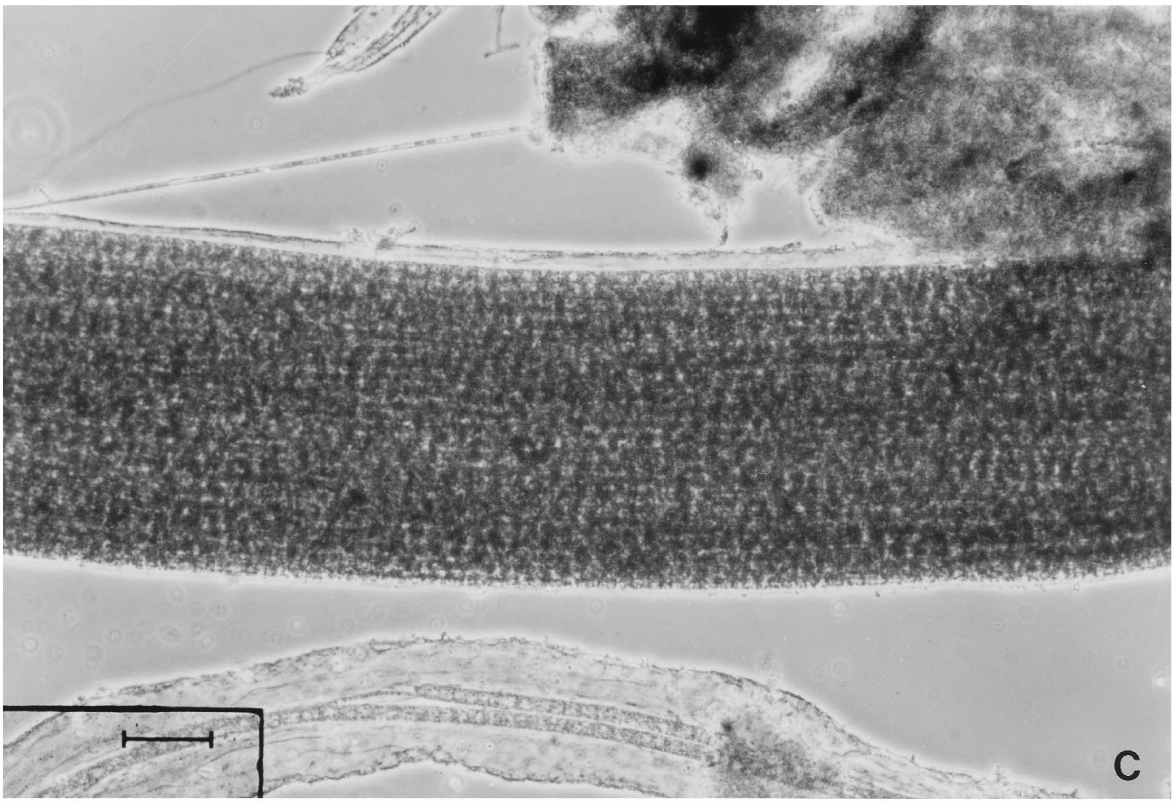
1 mm



0.5 mm

FIG. 1. (A) *Thioploca* trichomes protruding from the sediment surface. The exposed filaments reached 1 to 3 mm vertically into the water column. (B) *Thioploca* filaments within their sheaths. The "spaghetti-like" filament bundles were washed out from the sediment through a 125-μm-pore-size sieve. (C) *Thioploca* bundle containing approximately 100 trichomes. A sheath containing only two filaments is at the bottom. (D) Three *Thioploca* trichomes within their common gelatinous sheath. The small globules in the cells are elemental sulfur.



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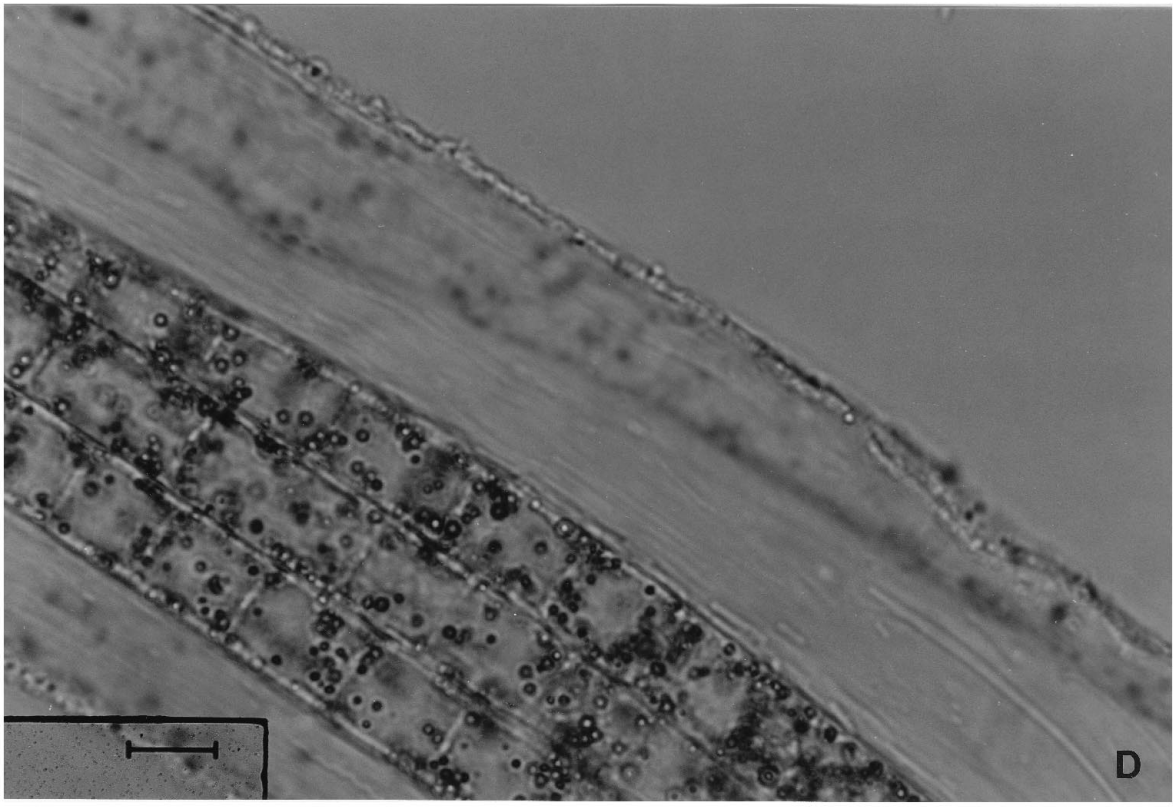

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FIG. 1—*Continued.*

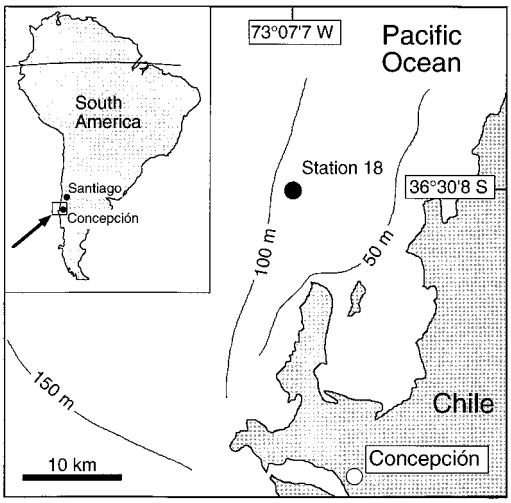

FIG. 2. Location of station 18 off the coast of Chile, where the sediment core with *Thioploca* mat was obtained.

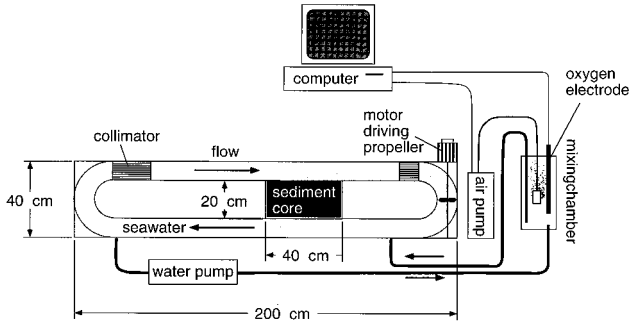

FIG. 3. Laboratory flume system. Water was circulated at a velocity of 6 cm s^{-1} over the sediment core. The oxygen content in the closed system was regulated with a computer which read the oxygen sensor and switched the aeration pump on and off.

The anoxic conditions on the Chilean shelf, combined with the nutrient-enriched upwelling waters, favor metabolic pathways in which nitrate or sulfate is used as the electron acceptor. In this paper we describe experiments that were performed in a closed-flume system designed to assess the chemotactic responses of *Thioploca* spp. to different oxygen, nitrate, and sulfide concentrations in the water flowing over a mat. We describe the unique emergence and shuttling behavior of this bacterium and discuss its advantages and consequences. Our results show that mat structure, filament mobility, and bundle formation are important and integral parts of the ecological niche of *Thioploca* spp.

MATERIALS AND METHODS

Sampling site. In March 1994 workers from the University of Concepción and the Max Planck Institute for Marine Microbiology in Bremen, Germany, went on a research cruise to investigate the ecology of *Thioploca* spp. (5). The sediment core with a *Thioploca* mat used for the flume experiments originated from station 18 located on the Chilean shelf off the Bay of Concepción (73°07′7″W, 36°30′8″S) (Fig. 2). At the sampling site the water depth was 87 m, the bottom water temperature was 11.0°C, and the salinity was 34‰. From a depth of 60 m down to the bottom, the water column was anoxic (oxygen concentration, <1 μmol $liter^{-1}$) (5, 11). Nonetheless, sulfide could not be detected (sulfide concentration, <0.5 μmol $liter^{-1}$) in the bottom water (11).

The uppermost 2 cm of the muddy sediment had an elastic, spongy consistency and contained abundant sheaths of *Thioploca* spp. and the U-shaped burrows of the polychaete *Paraprionospio pinnata* ($35 \times 10^4 \pm 27 \times 10^4$ individuals m^{-2}; $n = 29$). The small polychaetes (length, 5 to 8 mm), all of which belonged to the same recently settled age group, revealed that bottom currents containing oxygen and polychaete larvae had passed the sampling area.

The upper, porous sediment layer (depth, 0 to 2 cm) was separated by an abrupt boundary from the lower part of the sediment, which was homogeneous and without visible stratification. At the study site, nitrate was present in the porewater down to a sediment depth of 2 cm (5, 43). Despite the fact that high sulfate reduction rates were measured (4, 5, 43), interstitial sulfide concentrations were undetectable (<0.5 μmol $liter^{-1}$).

Experimental setup. Immediately after retrieval, onboard the ship, the sediment core with a thin film of overlying water was transferred from the box corer which was used into an acrylic container (length, 40 cm; width, 20 cm; depth, 20 cm), which was sealed with a lid to avoid contact with air. The core then was transported to the Field Station of the University of Concepción at Dichato, Chile, where it was incubated in a gas-tight flume (Fig. 3). The container with the sediment core fit into a drop box in the flow channel (length, 200 cm; width, 30 cm; depth, 12 cm) of the recirculating system. The flume was filled with 160 liters of water that originated from the sampling site and was sealed with an acrylic lid. All air bubbles were then removed. An axial pump was adjusted to produce a flow velocity of 6 cm s^{-1} at a height of 6 cm above the sediment surface. A cooling coil connected to a cooling unit with an external model PT 100 temperature sensor kept the water temperature at 11.0 ± 0.1°C. All experiments were performed with this core within 2 weeks, and the *Thioploca* mat could be maintained in good condition throughout this period.

To assess the chemotactic responses of *Thioploca* spp., we manipulated the concentrations of oxygen, nitrate, and sulfide in the recirculating seawater. The oxygen concentration was regulated via a computer which switched an aeration pump on and off according to the readings of an oxygen sensor (WTW instruments) located in an aeration chamber connected to the flume (Fig. 3). When removal of oxygen was necessary, the water was flushed with nitrogen gas. Winkler titrations of water samples taken at intervals ranging from 0.5 to 8 h were used to calibrate the oxygen sensor.

To manipulate the nitrate and sulfide concentrations, we added preweighed amounts of sodium nitrate (initial concentration after addition, 27 μmol $liter^{-1}$) and sodium sulfide (initial concentrations, 50 and 150 μmol $liter^{-1}$) to the water. The concentrations of these compounds in the flowing seawater were analyzed by the method of Grasshoff et al. (13).

The reactions of *Thioploca* spp. to the overlying water chemistry were recorded with a video camera and by time-lapse photography through the transparent acrylic flume walls. The white *Thioploca* filaments could be seen clearly on the monitor and photographs. To count the filaments protruding from the sediment and to measure their lengths, we analyzed projected color slides taken vertically through the transparent lid of the flume. These photographs permitted measurement of only the horizontal length component of the exposed filaments. From side views of protruding filaments, we derived a function (by polynomial regression) which allowed us to calculate the total length of the exposed bacterial filaments from the horizontal component (Fig. 4B).

The boundary layer conditions above the *Thioploca* mat in the flume were analyzed by releasing a nonpoisonous red dye (carotene) in 1- to 30-s pulses at defined heights (1, 3, 5, 7, 10, 20, 27, and 35 mm) above the sediment surface. The lateral transport of the tracer was recorded with the video camera through the sidewalls of the flume. From the video recordings the velocity profile above the core was deduced. Small stoppered holes in the flume lid permitted the measurements of oxygen profiles in the sediment with microelectrodes (38, 39).

At the end of the flume experiment (after 14 days), 32 sediment subcores were removed with acrylic cylinders. Subcores 1 to 9 (diameter, 26 mm) were used to assess the abundance and depth penetration of the *Thioploca* sheaths. These cores were cut into three sections (0 to 1, 1 to 5, and 5 to 10 cm), which were carefully resuspended and passed through a 125-μm-pore-size sieve to extract the *Thioploca* sheaths. The sheath fragments were counted and weighed prior to drying and combustion (550°C, 8 h) to measure their organic content. Subcores 10 to 15 (diameter, 26 mm) were analyzed with a light microscope to determine the *Thioploca* filament orientation, sediment composition, and grain size distribution. Porewater was extracted from subcores 16 to 21 (diameter, 18 mm) to measure the concentrations of nitrate, nitrite, ammonium, and sulfide (13). The hydraulic conductivity of the upper porous layer (depth, 0 to 2 cm) and the underlying sediment (depth, 2 to 6 cm) was measured in subcores 22 to 29 (diameter, 18 mm) by using a permeameter as described by Holme and McIntyre (16). Finally, subcores 30 to 32 (diameter, 4 cm) were cut at 1-cm intervals and combusted at 550°C for 8 h to provide the data used to determine the depth distribution of organic matter.

RESULTS

Characteristics of the *Thioploca* mat. In the incubated sediment core, the concentration of *Thioploca* sheaths was greatest (15.2 ± 1.6 sheaths cm^{-2}; $n = 5$) on the downstream side of three small sediment ripples (height, 0.8 cm) that were aligned perpendicular to the flume flow. The concentration in

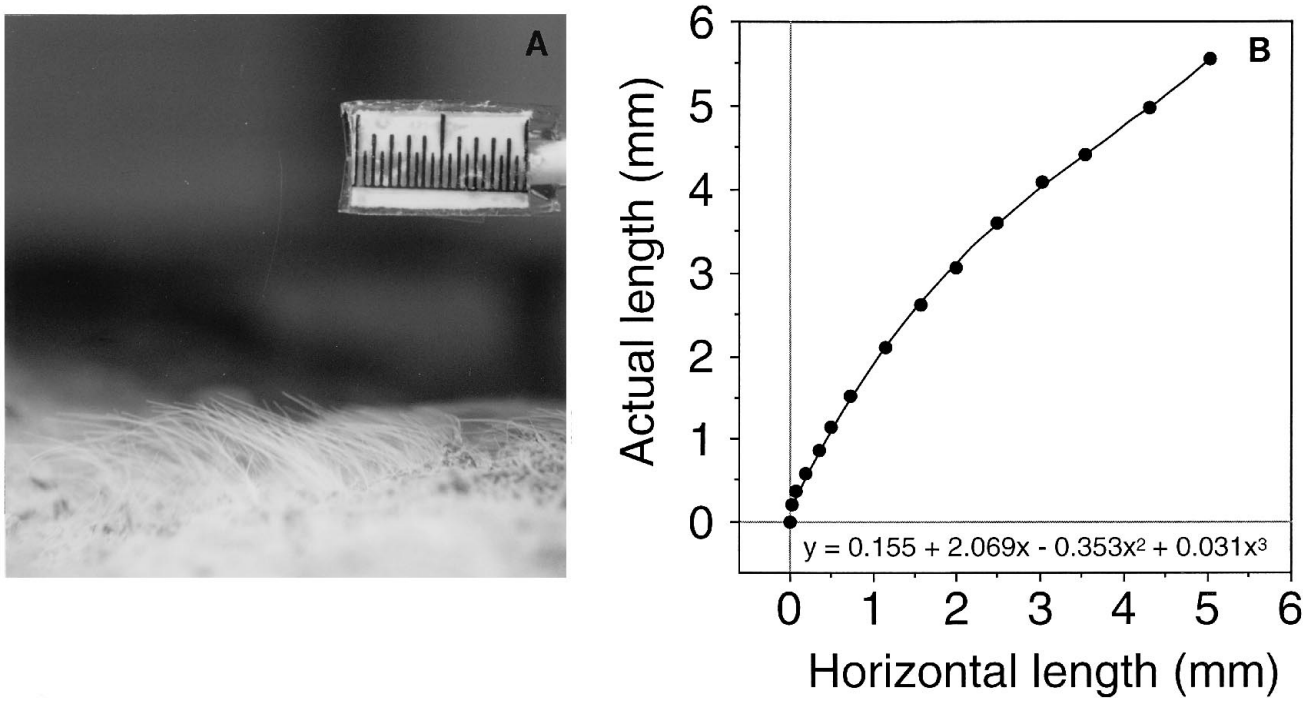

FIG. 4. (A) Bending of protruding *Thioploca* filaments in the boundary layer flow. Bar = 1 cm. (B) Function derived to deduce the actual lengths of protruding filaments from pictures taken through the transparent lid of the flume. The function describes the length of the protruding filament as seen from above (horizontal component) in relation to the actual length as measured in side views.

the level area of the core was 8.2 ± 1.8 sheaths cm^{-2} (n = 4). Below the surface the sheaths penetrated, bending irregularly, downward into deeper sediment layers (a more detailed description of the orientation of the sheaths is given in the accompanying paper [41]). The biomass of *Thioploca* spp. reached maximum values at sediment depths of 1 to 5 cm (Fig. 5A). Only a few sheaths were found deeper than 13 cm, and the maximum recorded penetration depth was 18 cm. A mean wet weight of *Thioploca* spp. with sheaths of 356.4 ± 329.3 g m^{-2} (n = 9), which was equivalent to 32.1 ± 22.6 g (dry weight) m^{-2} or 11.1 ± 2.3 g (ash-free dry weight) m^{-2}, was measured for the upper 10 cm of the incubated sediment core.

The upper layer of the mat (depth, 0 to 2 cm) had a high hydraulic conductivity (2.4×10^{-3} to 1.4×10^{-2} cm s^{-1}; n = 6) (Fig. 5B) compared with the muddy sediment underneath (1.0×10^{-5} to 1.1×10^{-4} cm s^{-1}). The flow velocity profile indicated that there was a 7- to 9-mm-thick boundary layer overlying the core and that there was some turbulence in the water flow above this layer (Fig. 6A). With 27.4 μmol of O_2 per liter in the flume water at the beginning of incubation, the microelectrodes revealed that the diffusive boundary layer was 250 to 300 μm thick and traced oxygen down to a sediment depth of 1.5 mm despite the relatively high organic matter content (17%) in the uppermost 1 cm of the sediment (Fig. 5A and 6B). The oxygen fluxes calculated from these profiles were lower (3.31 ± 0.37 mmol m^{-2} day^{-1}; n = 6) than the fluxes deduced from the oxygen consumption in the flume at the same time (37.28 ± 4.19 mmol m^{-2} day^{-1}; n = 4).

The porewater nitrate and nitrite concentrations measured at the end of the experiment, 2 days after the last addition of nitrate to the recirculating water, showed that these substances were depleted below sediment depths of 3.5 and 5 cm, respectively (Fig. 7A). The concentrations of ammonium and sulfide increased with depth, reaching values of 297.0 ± 26.6 and 55.4 ± 15.0 μmol $liter^{-1}$ (n = 6), respectively, at a sediment depth of 10 cm (Fig. 7B).

Protrusion of filaments. The *Thioploca* filaments adjusted their vertical positions in the sediment according to changes in the overlying water chemistry. The initial conditions in the flume water were as follows: salinity, 34‰; water temperature, 11°C; oxygen concentration, 166 μmol $liter^{-1}$ (60% of air saturation); NO_3^- concentration, 8.5 μmol $liter^{-1}$; NO_2^- concentration, 0.8 μmol $liter^{-1}$; NH_4^+ concentration, 5.2 μmol $liter^{-1}$; and pH 7.8. Reduction of the oxygen content in the recirculating water to 10% of air saturation (27.7 μmol $liter^{-1}$) triggered an upward movement of the *Thioploca* trichomes in their sheaths. Individual filaments emerged vertically from the

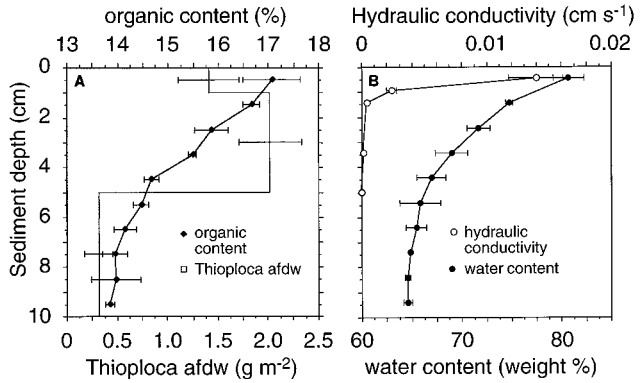

FIG. 5. (A) *Thioploca* spp. ash-free dry weight (afdw [shaded]) in the incubated core and organic content (expressed as a percentage of dry weight) of the sediment. (B) Water content and hydraulic conductivity of the sediment as a function of the sediment depth.

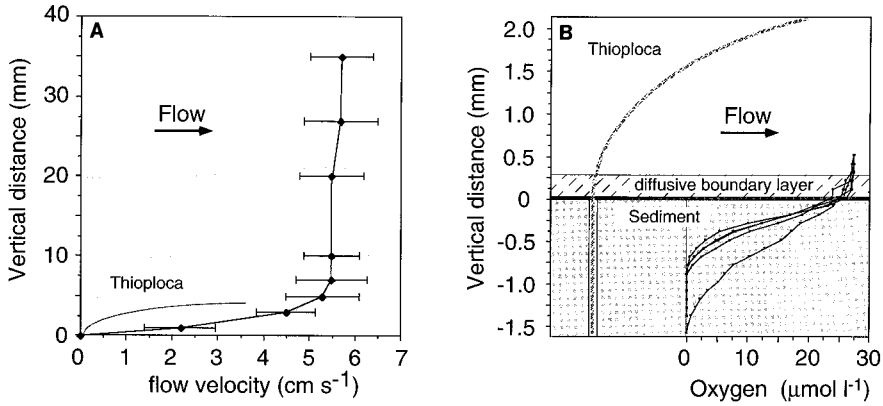

FIG. 6. (A) Boundary layer flow velocities above the incubated core. The thick shaded lines indicate the levels of turbulence observed after dye injection. The thin curved line represents a protruding *Thioploca* filament. (B) Oxygen profiles measured with microelectrodes and the diffusive boundary layer (laminar lower section of the boundary layer) (cross-hatched area) as indicated by these profiles. The shaded line represents a *Thioploca* trichome.

sediment surface (Fig. 1). Because of their relative stiffness and the reduced flow velocity near the sediment-water interface, the trichomes reached 1 to 3 mm into the water column before, forced by the boundary flow, they bent downstream (Fig. 4A). Under reduced flow conditions (<1 cm s^{-1}) the trichomes could project up to 6 mm into the water column.

The trichomes moved individually and independently from the sheaths, which remained stationary and hidden in the sediment. During emergence the average number of filaments protruding per sheath, as well as the average lengths of the exposed filament sections, increased constantly (0.23 filament h^{-1} [r^2 = 0.76], 0.30 mm h^{-1} [r^2 = 0.94]) (Fig. 8). After approximately 8 h, the average number of exposed trichomes (7.5 ± 0.2 trichomes; n = 768) and average length of exposed trichomes (4.8 ± 0.1 mm; n = 768) reached a steady state.

Responses to oxygen concentration increase. An increase in the oxygen concentration in the flume water caused the retreat of the protruding *Thioploca* trichomes into the sediment. This reaction started promptly after aeration of the recirculating water began. Again, filaments moved independently. The average velocity of the retreating trichomes (0.37 mm h^{-1} [r^2 = 0.95]; n = 768) was similar to the average velocity of the trichomes when they moved upward. Repeated cycles of aeration and oxygen depletion (Fig. 8) revealed that the chemotactic responses of *Thioploca* spp. to oxygen changes were consistent as long as the nitrate concentrations in the water were low (<5 µmol $liter^{-1}$).

Responses to nitrate concentration increase. After the experiment began, the nitrate concentration in the recirculating water decreased at a fairly constant rate, 1.1 µmol $liter^{-1}$ day^{-1}, which was equivalent to a flux of 2.24 ± 0.12 mmol m^{-2} day^{-1} into the sediment. After 9 days the nitrate was depleted. With no nitrate present in the water, the *Thioploca* spp. did not emerge from the sediment when the oxygen concentration in the flume was reduced to 10% of air saturation or even 0% of air saturation. In contrast, subsequent addition of nitrate (25 µmol $liter^{-1}$) to the water resulted in immediate emergence of the filaments from the sediment (Fig. 9). In the nitrate-enriched anoxic water, trichomes protruded up to 30 mm from the sediment, but they never completely emerged from the core.

Addition of nitrate caused filament protrusion, even when the oxygen concentration in the water was as high as 166 µmol $liter^{-1}$ (60% of air saturation). A subsequent reduction in the oxygen concentration in the recirculating flow to 0% of air saturation led to an increase in the average protrusion length

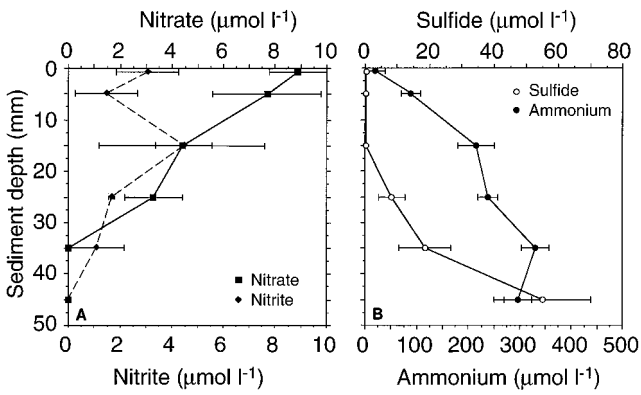

FIG. 7. (A) Porewater nitrate (shaded) and nitrite concentration profiles measured at the end of the experiment 2 days after nitrate (25 µmol $liter^{-1}$) had been added to the flume water. (B) Ammonium and sulfide profiles in the incubated core measured at the end of the experiment.

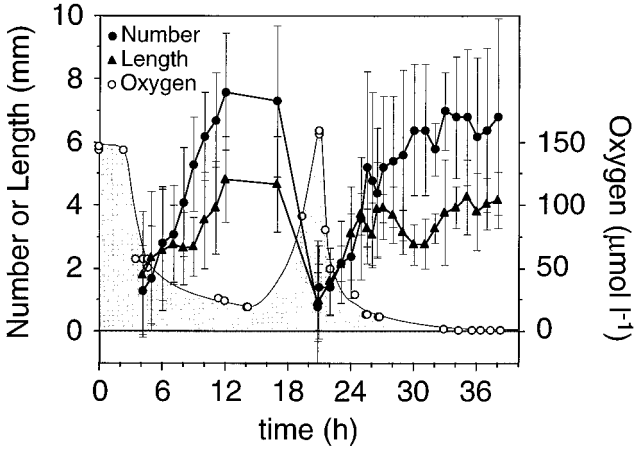

FIG. 8. Chemotactic responses of *Thioploca* spp. to changes in oxygen concentrations (shaded) in the water overlying the sediment. The changes in the average numbers of trichomes protruding per sheath and the average protrusion lengths are shown (n = 768). During the time studied, nitrate was present in the recirculating seawater at low concentrations (<5 µmol $liter^{-1}$).

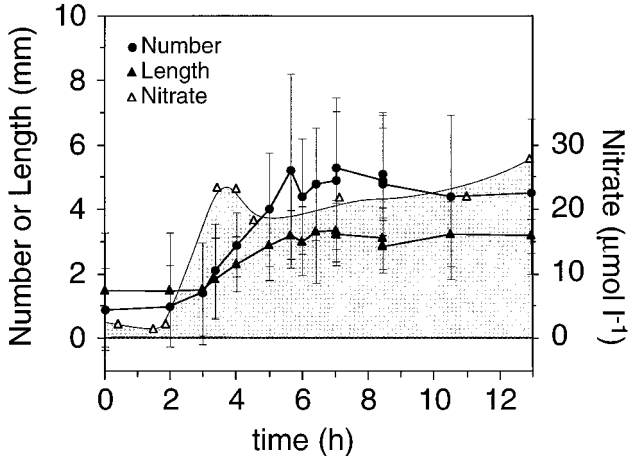

FIG. 9. Chemotactic responses of *Thioploca* spp. to addition of nitrate (shaded) to the flume water. The attraction to the increased nitrate concentrations overruled the negative response to oxygen, which was present during the entire experiment at concentrations of >10 μmol liter^{-1}. The average numbers of trichomes protruding per sheath and the average protrusion lengths are shown (n = 641).

from 3.59 ± 0.11 to 6.29 ± 0.24 mm (n = 641), a 175% increase (Fig. 10). The average number of emerging filaments, however, increased less (7.00 ± 0.16 versus 8.90 ± 0.35 filaments [n = 641], a 127% increase, demonstrating that oxygen affected the protrusion length but not the general upward movement of the trichomes when nitrate was present in the water.

Following the first nitrate addition, the rate of nitrate consumption in the flume increased significantly to 19.77 ± 0.83 mmol m^{-2} day^{-1} (n = 3). A second addition of nitrate (after the concentration had dropped to 1.5 μmol liter^{-1}) resulted in a similarly strong response of *Thioploca* spp. and a similar high rate of nitrate consumption (22.48 ± 1.40 mmol m^{-2} day^{-1}). An increase in the nitrite concentration in the flume flow from 1.1 to 10.8 μmol liter^{-1} 1 day after the first nitrate addition and an increase in the ammonium concentration after 2 days (from 20 to 23 μmol liter^{-1}) indicated that enhanced denitrification and ammonification occurred in the recirculating flume system.

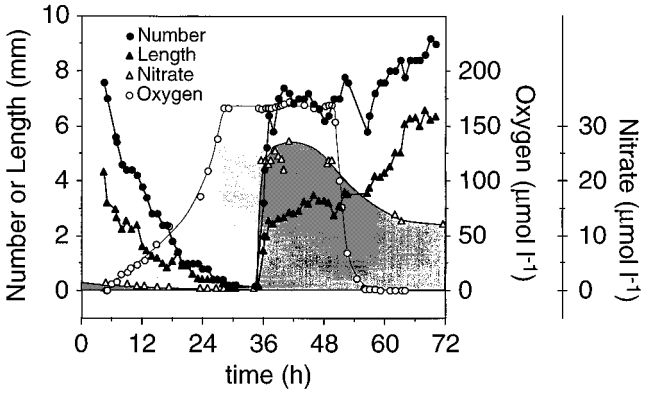

FIG. 10. Response of *Thioploca* spp. to changes in oxygen (light shading) and nitrate (dark shading) concentrations in the water. At low initial nitrate concentrations the negative response to oxygen caused the filaments to retreat into the sediment. After nitrate was added, the trichomes protruded from the sediment surface. Removing the oxygen resulted in a further increase in the average number of protruding trichomes and the average protrusion length (n = 650).

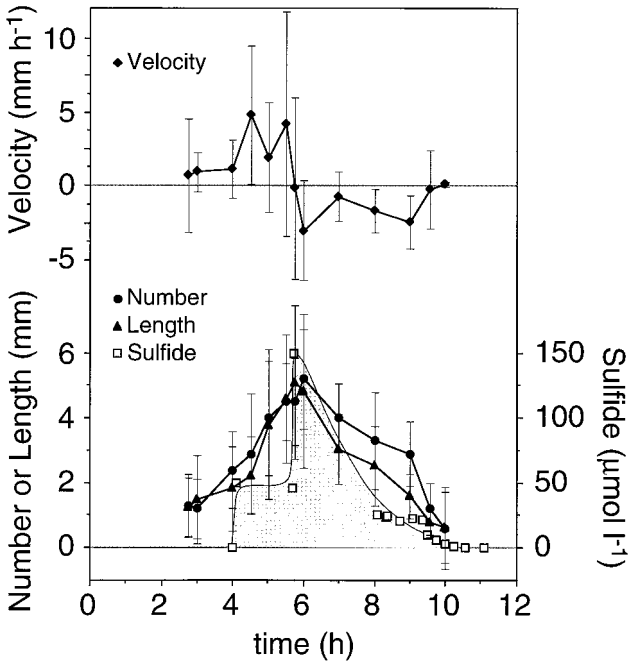

FIG. 11. (Upper lines) Average velocities of individual *Thioploca* trichomes moving upward (positive values) and downward (negative values) during stepwise increases in the sulfide concentration in the flume water. (Lower lines) Increases and decreases in the sulfide concentration in the water (shaded area) and the chemotactic reactions of the *Thioploca* trichomes to the concentration changes. The graph shows the average numbers of trichomes protruding per sheath and the average protrusion lengths (n = 561).

Responses to changes in sulfide concentrations in the water. Addition of 50 μmol of sulfide per liter to the flume water (O_2 concentration, <1 μmol liter^{-1}; NO_3^- concentration, 6 μmol liter^{-1}) triggered a positive chemotactic response in *Thioploca* spp., and the trichomes emerged from the sediment (Fig. 11). In individual filaments the average velocity of the upward movement was 4.8 ± 4.6 mm h^{-1} (n = 10) and the maximum velocity observed was 14.8 mm h^{-1} (the velocity of individual filaments was approximately 10 times greater than the average velocity of the entire bundle because of uneven movement of trichomes). A further increase in the sulfide concentration to 150 μmol liter^{-1} stopped and reversed the movement of the filaments (single-filament velocity, 3.0 ± 3.3 mm h^{-1}). The retreat into the sediment continued even when the sulfide concentration dropped below 45 μmol liter^{-1}.

DISCUSSION

The thick microbial mats that constitute up to 80% of the benthic biomass (29) reflect the successful adaptation of *Thioploca* spp. to the nutrient-rich but dysaerobic conditions in the coastal upwelling regimes of Peru and Chile. The dominance of *Thioploca* spp. is related to the unique physiology and behavior of these organisms, which may be viewed as the result of parallel evolution with the ecological adaptations found in the closely related organisms *Beggiatoa* spp. Below, we describe our findings in relation to similarities to and differences from *Beggiatoa* spp. which may be useful in understanding the ecological niche of *Thioploca* spp.

Chemotaxis and filament orientation. The sedimentary sulfur bacteria *Beggiatoa* spp. live in aquatic environments at the oxygen-sulfide transition (19, 20). A phobic response to oxygen

concentrations of >50 μmol liter^{-1}, which has been interpreted as a mechanism to compensate for a lack of catalase (3, 20, 34), and avoidance of high sulfide concentrations (33) keep these bacteria in the zone of optimal growth. In our experiments we found similar phobic responses in *Thioploca* spp. Oxygen concentrations of >15 μmol liter^{-1} and sulfide concentrations of ≥150 μmol liter^{-1} in the flume water caused the *Thioploca* trichomes to retreat into the sediment. These results support the observations of Maier and Gallardo (26), who found that even low oxygen concentrations (10% of air saturation) or sulfide concentrations of >500 μmol liter^{-1} were harmful to *Thioploca* spp. As in *Beggiatoa* spp., these negative chemotactic reactions keep the *Thioploca* filaments in a sediment layer where the oxygen and sulfide concentrations are low; one difference between the two groups of organisms is that *Thioploca* spp. have lower tolerances toward both gases (21, 25, 26, 40).

The orientation of the *Thioploca* sheaths within the growth zone is not random but is more or less vertical (41). Our flume experiments revealed that in addition to the phobic reactions, two positive chemotactic responses may be responsible for the vertical orientation of the sheaths. Nitrate (concentration, <50 μmol liter^{-1}) and low concentrations of sulfide (<150 μmol liter^{-1}) attracted *Thioploca* spp. Attraction toward sulfide has been detected previously by Koppe (22). These positive chemotactic responses may promote the vertical alignment of the trichomes between the nitrate-rich overlying water and the deeper sulfidic sediment strata after sulfide consumption by *Thioploca* spp. and advective porewater flushing (see below) have removed the free sulfide from the uppermost sediment layers.

Filament protrusion, nitrate reduction, and oxidation of sulfide. The positive reaction to nitrate and the vertical orientation of the sheaths within the sediment are fundamental to the emergence of *Thioploca* trichomes from the sediment. Shortly after the oxygen concentration in the flume water was reduced, the filaments penetrated the interface and appeared like growing white hair swaying in the boundary flows. This behavior explains the enigmatic observations of "white lawns" (40) and "slimy grass" (9) reported in sediments in upwelling regions. Such white lawns have also been observed and videotaped during dives of the research submersible *ALVIN* on the Peruvian shelf (2).

Although anoxic conditions in the flume water enhanced trichome emergence (Fig. 10), the *Thioploca* filaments reached even into oxygenated flume flows when nitrate was added to the water after 3 days of deprivation (i.e., there was no nitrate in the flume). In contrast, when no nitrate was present in the flume water, *Thioploca* spp. did not emerge even when the oxygen concentration was lowered to 0% of air saturation. The strong attraction to nitrate and the dominance of this positive chemotactic response over the phobic reaction toward oxygen suggest that nitrate is the principal electron acceptor in *Thioploca* spp. in the anoxic environment. This hypothesis is supported by the 10-fold increase in nitrate consumption in the flume water (2 versus 20 mmol m^{-2} day^{-1}) after nitrate was added and the subsequent massive emergence of *Thioploca* filaments. Such an increase could be explained by the findings of Nielsen (5, 35), who found that *Thioploca* spp. accumulate nitrate. Since oxygen was not present, *Thioploca* spp. presumably also use nitrate to oxidize sulfide. Sulfide oxidation linked to the reduction of nitrate to nitrite has been found in *Thiobacillus denitrificans* (1).

Penetration of the diffusive boundary layer. With their ability to protrude 1 to 6 mm into the boundary layer, the *Thioploca* trichomes may outcompete sedimentary nitrate-consuming processes. The relative rigidity of the filaments, which may be related to the vacuoles that function as a hydroskeleton (similar to turgor in plant cells), allows them to penetrate the diffusive boundary layer (thickness, 300 μm at a flow velocity of 6 cm s^{-1} [Fig. 6B]) which coats the sediment surface and limits interfacial solute fluxes (14). The protruding filaments gain access to the higher nitrate concentrations in the water column and reach into the flow (Fig. 6A), thereby minimizing the thickness of the diffusive layer enclosing the bare trichomes.

As determined from the average maximum protrusion length (6.6 mm) and the presence of 9.2 trichomes per sheath and 15.2 sheaths cm^{-2}, the total length of exposed filament sections per square centimeter of sediment was almost 1 m (92 cm). The total surface area of the exposed filaments was as large as the sediment area from which they emerged (92 cm × 0.011 cm [the maximum average circumference] ≈ 1 cm^2 of exposed trichome surface). This indicates a high potential of nitrate uptake by *Thioploca* spp.

The thick *Thioploca* sheaths, which would lower the diffusional gradients around the exposed filaments and thereby the uptake rate, remain stationary within the sediment and act as holdfasts for the protruding trichomes. The extreme length of the bacterial filaments is the functional counterpart of this anchoring mechanism; a relatively long section of the trichome can emerge while the section remaining in the sediment prevents dislodging of the filament by the water current. In contrast to the *Beggiatoa* mats, which usually are only a few hundred micrometers thick (31, 33), the *Thioploca* mats can reach thicknesses of several centimeters (sheaths may reach down to a depth of 23 cm [15]), suggesting that these organisms live longer without contact with their electron acceptor. This may be possible because nitrate can be stored in the cells of *Thioploca* spp. (5, 35).

Nitrate as transportable electron acceptor. *Thioploca* spp. accumulate nitrate to high concentrations (up to 500 mmol liter^{-1}) in the large vacuole in each cell (5, 35). This adaptation enables *Thioploca* spp. to carry the electron acceptor with it and to reach sulfidic sediment layers that are remote from the nitrate-supplying bottom flow. With vertical transport of nitrate *Thioploca* spp. not only may gain access to deep sediment layers but also may push the upper boundary of the sulfidic zone deeper. Investigations have shown that *Beggiatoa* spp. can quantitatively remove sulfide from the porewater and that sulfide oxidation in the *Beggiatoa* mats is 10,000- to 100,000-fold faster than the autocatalytic sulfide oxidation that occurs in oxygenated water (20, 33, 36). Thus, the depletion of sulfide in the upper sediment layers on the Chilean shelf may result in part from sulfide consumption by *Thioploca* spp. (a considerable fraction of the sulfide in the upper sediment layers is probably consumed by chemical reactions [4, 12, 43] or flushed out of the bed [see below]).

Because sulfide uptake moves the sulfidic zone away from the sediment surface, *Thioploca* spp. may inhibit the growth of competing sulfide oxidizers which depend on concurrently supplied nitrate (or oxygen) and reduced sulfur. However, the observation that only a fraction of the long filaments emerge from the seabed and the fact that thick sheaths with living trichomes were found down to a sediment depth of 18 cm raise the questions of how the metabolic requirements of the deeper sections of the trichomes are satisfied and how the vertical positioning is controlled.

Permeable mat structure. Our *Thioploca* mat had a highly porous surface and elastic properties which gave it a spongelike appearance. Reimers (37) found that the fabric of the uppermost 5 cm of the *Thioploca* sediment off the Peruvian coast was characterized by a maze of extremely large pores

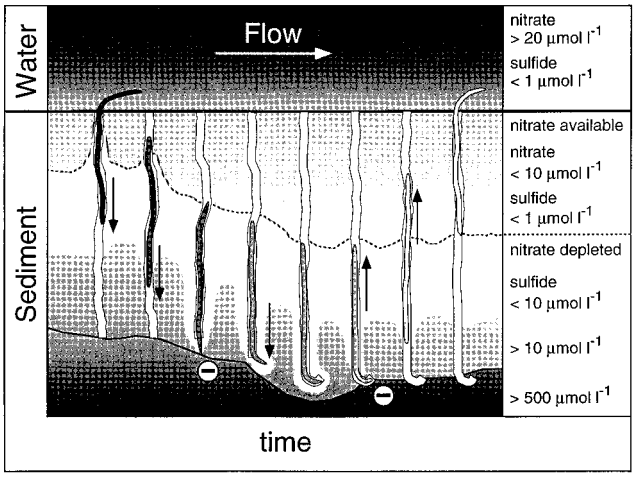

FIG. 12. Diagram showing how chemotactic responses and the concentration of an internal trigger may control vertical shuttling in *Thioploca* spp. The shading of the trichomes reflects the concentrations of the trigger (e.g., nitrate) in the filaments. The arrows indicate the chemotactic attraction of and movement toward sulfide and nitrate, respectively. The minus signs indicate phobic responses to high oxygen concentrations or high sulfide concentrations. For further explanation see the text.

(13% of the volume), a high water content (90%), and exceptional plasticity. The last is a result of bacterial mucus that coats the loosely deposited fecal pellets which account for most of the sediment (45). Our measurements revealed a hydraulic conductivity in the upper layer of our *Thioploca* sediment (depth, 0 to 2 cm) on the order of 10^{-3} to 10^{-2} cm s^{-1} (Fig. 5B). Thus, despite the small grain size of the sediment (95% of the grains had diameters of less than 63 μm), the matrix with cavities and small channels resulted in a permeability more typical of coarse, well-sorted sands (17).

Even slow boundary currents (velocity, 2 to 10 cm s^{-1}) can drive significant interfacial fluid flows in sediments that have such high hydraulic conductivities (18, 48). The relatively deep penetration depths of oxygen (1.5 mm at 10% air saturation in the water [Fig. 6B]) and nitrate (35 mm 2 days after nitrate was added to the recirculating water [Fig. 7A]) indicate that advective porewater flows enhanced the exchange process between the *Thioploca* mat and the overlying water. Thus, besides exposing the trichomes to the boundary layer flow, the upward migration also moves the lower cells of the filaments into the nitrate-rich permeable sediment layer. With nitrate penetrating 35 mm into the seabed and our maximum filament protrusion length of 30 mm, most cells in the longest trichome that we found (70 mm) could have access to nitrate. We suggest that the permeability of the mat structure is an important factor for the nitrate supply of the trichomes in the upper sediment layer.

Vertical positioning of the trichomes. The protrusion lengths of the *Thioploca* trichomes and their positions within the sediment may be controlled by chemotactic responses and the concentration of an internal trigger (e.g., nitrate) in the filament (Fig. 12). After a protruding trichome has absorbed nitrate from the boundary layer flow and the porewater of the uppermost sediment layer, the attraction toward sulfide may outweigh the affinity toward nitrate and result in downward movement. The contact of most cells with sulfide or sulfide concentrations that are too high then may stop the descent. In the sediment the cells take up sulfide until the internal trigger (e.g., a decrease in the internal nitrate pool concentration to a certain value) initiates movement. Possibly after some random reversals until the nitrate gradient is reached, the attraction to nitrate then could cause the ascent. The upward movement may stop when most cells contact nitrate, and the cycle starts anew.

Maier and Gallardo (26) reported that changes in oxygen or sulfide concentrations resulted in relocation of buried trichomes in a new suitable sediment horizon. A sulfide concentration of 100 μmol $liter^{-1}$ was most beneficial for *Thioploca* spp. Likewise, when the bottom flows are depleted of oxygen as well as nitrate and sulfide builds up to high concentrations in the porewater, the *Thioploca* filaments can completely emerge from the sediment and may be entrained by the bottom flows. Such an emergence was observed in sediment samples incubated in aquaria with stagnant water (10) and has also been reported for *Thioploca ingrica* (25). This behavior can be regarded as a mechanism for retreat of *Thioploca* spp. from adverse environments and may be beneficial for the dispersion of the species.

We concluded that the success of *Thioploca* spp. in the upwelling regimes of the Chilean and Peruvian shelf is based on the ability of these sulfur bacteria to gain access to the high nitrate concentrations in the boundary flow and their ability to exploit the sulfide resources in a layer of sediment that is up to 18 cm thick.

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