

## Influence of simulated bivalve biodeposition and microphytobenthos on sediment nitrogen dynamics: A laboratory study

Roger I. E. Newell, Jeffrey C. Cornwell, and Michael S. Owens

Horn Point Laboratory, University of Maryland Center for Environmental Science, P.O. Box 775, Cambridge, Maryland 21631

### Abstract

Suspension-feeding eastern oysters, *Crassostrea virginica*, were once abundant in Chesapeake Bay and may then have exerted top-down control on phytoplankton and also reduced turbidities, thereby increasing light available to benthic plants. Alternatively, oysters may have simply recycled inorganic nutrients rapidly back to the water column, with no long-lasting reduction in phytoplankton biomass resulting from oyster feeding activity. To help distinguish between these scenarios, we explored changes in nitrogen fluxes and denitrification in laboratory incubations of sediment cores held under oxic and anoxic conditions in response to loading by pelletized phytoplankton cells, an experimental analog for oyster feces and pseudofeces. When organics were regenerated under aerobic conditions, typical of those associated with oyster habitat, coupled nitrification–denitrification was promoted, resulting in denitrification of ~20% of the total added nitrogen. In contrast, under anoxic conditions, typical of current summertime conditions in main-stem Chesapeake Bay where phytoplankton is microbially degraded beneath the pycnocline, nitrogen was released solely as ammonium from the added organics. We postulate that denitrification of particulate nitrogen remaining in oyster feces and pseudofeces may enhance nitrogen removal from estuaries. In aerobic incubations with sufficient light ( $70 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), a benthic microalgal/cyanobacterial community grew that not only absorbed the inorganic nitrogen released from the added organics but also fixed  $\text{N}_2$ . This result suggests that an ecosystem dominated by benthic primary production may develop in shallow waters when reduced turbidity associated with bivalve feeding increases light penetration to a level that can sustain benthic microalgal production.

The Chesapeake Bay, in common with many estuaries worldwide, is subject to extreme anthropogenic nutrient enrichment that promotes high levels of phytoplankton production (Malone 1992). Phytoplankton ungrazed by meta-zoan consumers is advected to the bottom of the deep central channel in the middle and upper Chesapeake Bay (Kemp and Boynton 1992), where it is subject to microbial degradation. The resulting intense microbial respiration exceeds the rate at which surface oxygen is mixed to the bottom, so anaerobic microbial processes predominate in bottom waters in summer months (Boicourt 1992). Because bacterially mediated coupled nitrification–denitrification (Henriksen and Kemp 1988; Koike and Sorensen 1988) is inhibited under such anoxic conditions, nitrogen is regenerated solely as  $\text{NH}_4^+$ , which, when mixed into the euphotic zone, stimulates further phytoplankton production.

We hypothesize that abundant stocks of benthic suspension feeders will (1) consume phytoplankton in eutrophic coastal waters, thereby reducing the amount of material available to be regenerated through water-column microbial processes and (2) reduce turbidity by filtering phytoplankton and inorganic particles  $> \sim 3 \mu\text{m}$  from the water column and transferring in their feces and pseudofeces (collectively

called biodeposits) undigested material to the sediment surface. Conversely, if there is a reduction in the abundance of grazer organisms that normally exert top-down control, phytoplankton concentrations and turbidity may increase. Newell (1988) postulated that current high phytoplankton biomass in the Chesapeake Bay derives not only from the bottom-up effects of high nutrient inputs but also from a ~100-fold decline in stocks of the suspension-feeding eastern oyster, *Crassostrea virginica*, over the past 150 yr as a consequence of overharvesting and disease epizootics. These original stocks of eastern oysters were located in well-mixed and oxygenated waters in tributaries and along the shallow (<9 m) flanks of the main-stem bay (Newell 1988). It is important to measure remineralization of material from biodeposits under aerobic conditions when assessing the possible role of oyster populations in exerting top-down control on phytoplankton and in altering nitrogen regeneration processes. These regeneration processes can then be contrasted with degradation of the same amount of organic material under anoxic conditions more typical of current summer conditions beneath the pycnocline in the Chesapeake Bay (Boicourt 1992; Kemp and Boynton 1992).

The growth of benthic diatoms has declined in the Chesapeake Bay over the last two centuries. This decline has been attributed by Cooper and Brush (1993) to higher phytoplankton biomass and silt in the water column reducing the bottom area where the intensity of photosynthetically active radiation (PAR) is sufficient to support primary production. Microphytobenthos (MPB), including diatoms, are key components of the biota in shallow systems because they can stabilize sediments and limit nutrient fluxes from the sediment to the water column by assimilating inorganic nutrients, and they are an important food source for herbivores

<sup>1</sup> Corresponding author (newell@hpl.umces.edu).

### Acknowledgments

This research was funded by the Maryland Sea Grant Program (grant NA46RG0091) and the EPA STAR program as part of the Multiscale Experimental Ecosystem Research Center at HPL. Technical assistance was provided by Lora Pride and Shou-Chung Huang. We are especially grateful to our colleagues, W. Boynton, L. Codispoti, T. Fisher, M. Kemp, and V. Kennedy for reviewing an early draft of this manuscript.

(Rysgaard et al. 1995; Miller et al. 1996; Cerco and Seitzinger 1997). Therefore, we hypothesize that there is a complex interrelationship between suspension feeders, MPB, and nitrogen regeneration to the water column. Suspension feeders remove seston from the water column, thereby increasing PAR intensity at the sediment surface and transferring biodeposits containing nutrients to the sediments. In turn, MPB will flourish due to higher PAR levels and hence may absorb inorganic nutrients regenerated from the bivalves and their biodeposits. In the absence of MPB, some of these nutrients are released to the water column where they stimulate further phytoplankton production.

The overall objectives of our laboratory-based study were to measure rates of nitrogen regeneration from organic material deposited at the sediment surface under different oxygen conditions and in the presence of MPB. These data can be used to predict how changes in the rate and location of organic matter deposition to the sediment surface, as mediated by changes in the abundance of suspension-feeding bivalves, may influence patterns of nutrient cycling in a eutrophic estuary such as the Chesapeake Bay. We first determined whether the rate and form of inorganic nitrogen regenerated from organic material deposited on aerobic sediments differed from the situation in which the same mass of phytoplankton was degraded by anaerobic microbial processes. As a null hypothesis, we predicted that there would be no differences in the rates and amount of nutrient regenerated to the water column in the oxic and anoxic treatments. Our second objective was to determine whether the patterns of nutrient regeneration to the water column under aerobic conditions changed when there was sufficient light to permit an active MPB community to grow at the sediment surface. As a null hypothesis, we predicted that there would be no differences in the rates and amount of nutrient regenerated to the water column in the presence or absence of microphytobenthos.

## Methods

*Sediment collection and defaunation*—Sediment was collected from a small cove (38°35.33'N, 76°07.68'W) on the Choptank River, a mesohaline subestuary of the Chesapeake Bay, on 2 June (dark experiment) and 9 July (light experiment) 1997. The sediment was mixed by hand and poured to a depth of ~20 cm in 1-m<sup>2</sup> tanks. The sediment surface was covered with plastic food wrap to reduce oxygen diffusion and covered to ~2 cm with salt water (12 salinity) and allowed to become anoxic. Tanks were maintained at 22°C and covered with black plastic to ensure absolute darkness and hence preclude MPB growth. This protocol ensured that the sediment became anaerobic and thereby killed any infauna. By defaunating the sediments in this manner we followed a common approach (e.g., Sundbäck and Graneli 1988; Sloth et al. 1995) of simplifying our experimental system. It was important to ensure that bioturbation was uniform across all treatments because the burrowing and feeding processes of macroinfauna mix surficial sediments and alter rates of exchange of dissolved inorganic nitrogen (DIN) and oxygen between the sediments and water column (Kris-

Table 1. Description of the various POM and oxygen treatments used in the dark and illuminated sediment core incubations.

Addition (g C m <sup>-2</sup> d <sup>-1</sup> )	Classification of POM level	% O <sub>2</sub> saturation
Dark sediment core incubations		
0.25	Low	0
5.0	High	0
0.25	Low	100
5.0	High	100
Illuminated sediment core incubations		
0	Control	100
0.25	Low	100
2.5	Medium	100

tensen 1988; Pelegri et al. 1994). Yet it would have been impossible to contain sufficient numbers of macroinfauna (bivalves, polychaetes, etc.) in our relatively small cores (13.2 cm inner diameter [ID]) to ensure uniformity of response. We needed cores of this size in order to accommodate replicate cores for all treatments within our experimental holding tanks. After 4 d of anoxia, the plastic wrap was removed and the surficial ~0.5-cm sediment layer that might contain any dead benthic organisms was discarded. Each tank was filled to a depth of 50 cm with flowing unfiltered ambient salt water (9 to 14 salinity; 22°C) and held for 13 d. Such equilibration is necessary because nitrifying bacteria are killed by H<sub>2</sub>S (Henriksen and Kemp 1988; Sloth et al. 1995); consequently bacteria must recolonize the sediments from material carried in with the natural seawater. In parallel studies, a period of 13 d was shown to be sufficient to allow reestablishment of normal sediment biogeochemical processes, including nitrification (Porter 1999). Transparent acrylic plastic tubes (13.2 cm ID and 36 cm tall) were used to remove ~18-cm deep undisturbed sediment cores from these equilibration tanks for the experiments described below.

*Dark experiment*—Cores were randomly separated into four groups (Table 1) and placed in 120-liter plastic tanks held in the dark in a constant temperature room at 22°C. Cores were submerged to a depth of ~25 cm by filling each tank with ~90 liters of 15 salinity seawater. This water had been aged for >4 months in the dark while being aerated to reduce levels of organic matter; water from the same source was used for all water exchanges detailed below. Cores in two tanks (100% O<sub>2</sub> saturation) were left open at the top and a 2.5-cm (ID) PVC tube inserted such that it reached to within ~1 cm of the sediment surface. An air hose with air stone was placed into the tube, and active bubbling served as an air-lift pump that drew water from close to the sediment surface up the tube and into the tank. This system facilitated exchange of water overlying the sediment in each core tube and the larger volume of water in each tank. The water in each tank was also aerated vigorously, and 25% of the water was exchanged every day in each tank to maintain uniform water quality among tanks. Internal surfaces of the acrylic tubes holding aerobic cores were scraped with a piece

of flexible plastic to within 2 mm of the sediment surface every 2 d to remove any fouling material; scrapings were allowed to fall to the sediment surface. Cores in two other tanks (0% O<sub>2</sub> saturation) were sealed individually with gas-tight lids to allow the sediment and overlying water to become anoxic. Aerobic water was pumped at 30 ml h<sup>-1</sup> through the water overlying the sediment in each anaerobic core to facilitate nutrient exchange, and the outflow was discarded. This flow rate was low enough to maintain zero oxygen concentrations while exchanging ~30% of the water volume in the head space on a daily basis.

Cores were allowed to stabilize for 4 d in the aerobic and anaerobic tanks. We determined baseline sediment–water exchange rates by sealing three randomly selected cores from each aerobic tank and measuring solute (NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>) and gas concentrations (O<sub>2</sub>, N<sub>2</sub>) at four time points over a period of 5 h. Fluxes were calculated from the change of the solute or gas concentration in the known volume of water (~2.3 liters) overlying each core plotted over time and expressed relative to core surface area. Water from the holding tank was incubated in triplicate and used as a control to assess water-column activity. Rates calculated for these water-column blanks were then subtracted from rates measured for the sediment cores. Water overlying the sediment was stirred by magnetic stirrers held centrally in the water column and driven by an external magnet on a turntable. The stirring rate was adjusted to just below the level that caused sediment resuspension. The small amounts of water withdrawn for each sampling were replaced by water taken from the holding tank. This replacement water was introduced by gravity through tubing connected to a reservoir into the water overlying the core, thereby ensuring that no air bubbles became trapped in the system. Nutrient analyses followed the procedures of Parsons et al. (1984). Concentrations of O<sub>2</sub> and N<sub>2</sub> were measured with a mass spectrometer (Kana et al. 1998); concentrations were corrected for the formation of NO and N<sub>2</sub>O in the spectrometer. Denitrification rates were calculated from measured changes in N<sub>2</sub> concentration.

We added two different amounts of particulate organic matter (POM) made from centrifuged unialgal cultures of *Thalassosira pseudonana* that were prepared beforehand and stored at 4°C. This material served as an experimental analog of phytoplankton that settles naturally to the sediment surface and the biodeposits of eastern oysters. The two POM loadings were designed to simulate (1) the current levels of organic deposition with reduced benthic–pelagic coupling and (2) historic conditions when high abundances of oysters may have delivered large amounts of biodeposits to sediments surrounding oyster bars. Kemp and Boynton (1992) determined that POM sedimentation rates in the mesohaline portion of the Chesapeake Bay ranged between ~0.5 and 1.2 g C m<sup>-2</sup> d<sup>-1</sup> in 1986 and 1987. Based on these data, we added 0.25 g C m<sup>-2</sup> d<sup>-1</sup> carbon to 50% of the cores loading for the low-POM treatment and 5.0 g C m<sup>-2</sup> d<sup>-1</sup> to the remaining cores for the high-POM treatment (Table 1). Daily additions of POM were made for 14 d to allow sufficient material to be added to the cores to obtain measurable flux rates, although the biochemical processes within the cores were unlikely to reach a stable equilibrium within 14 d. The airlift circulating system in all aerobic cores was turned off

for 1 h during POM additions to allow material to be deposited uniformly from a 5-ml syringe to the sediment surface. In the anaerobic treatment tanks, half the cores were supplied with low POM and half with high POM by allowing an algal suspension to be sucked in with irrigating water.

Sixteen days after POM was first added, three aerobic cores from the high- and low-POM treatment were randomly selected and incubated for nutrient fluxes and gas samples (O<sub>2</sub>, N<sub>2</sub>) as described above. Samples of water entering and leaving three sealed anaerobic cores for the high- and low-POM treatments were also collected and analyzed for solutes and gases. Fluxes were calculated based on differences in concentrations between inflow and outflow water and the flow rate of water pumped through each core and expressed relative to core surface area. The remaining three low- and high-POM addition aerobic cores were supplied with the appropriate daily addition of POM and then we added NaNO<sub>3</sub> to the overlying water column to assess changes in sediment fluxes in response to increases in NO<sub>3</sub><sup>-</sup> concentrations from ambient (12 μmol) to about 80 μmol. Microbial processes were allowed to adjust to the change in water-column NO<sub>3</sub><sup>-</sup> for 36 h before nutrient and gas fluxes (O<sub>2</sub> and N<sub>2</sub>) were measured as described above. In previous work we have shown that a stable response to added NO<sub>3</sub><sup>-</sup> is induced within <24 h of NO<sub>3</sub><sup>-</sup> addition (Kana et al. 1998). The NO<sub>3</sub><sup>-</sup> levels were then further increased to about 160 μmol in both aerobic tanks by adding additional NaNO<sub>3</sub>, and 36 h later the fluxes in the same three cores were again measured.

The percent water in the 0- to 6-mm sediment surface layer of each core was determined gravimetrically. The remaining sediment in each core was poured through a 500-μm sieve to ascertain whether any invertebrates survived the initial anoxic treatment.

*Light : dark experiment*—Cores were randomly submerged in three separate 120-liter plastic tanks filled with 90 liters of 15 salinity aged seawater from the same source used in the dark experiments. The tanks were held in a constant temperature room at 22°C and with a uniform illumination of 70 to 80 μmol m<sup>-2</sup> s<sup>-1</sup> at the sediment surface from high output fluorescent lamps operated on a 12 : 12 h light : dark cycle. The water in each tank was vigorously aerated and 25% of water was exchanged every day in each tank to maintain uniform water quality; aeration and water exchange for the unsealed cores were maintained with an airlift tube as described for the dark experiments. To each core we added a single 300-ml dose of a unialgal log growth phase culture of the diatom *Navicula incerta* that was allowed to settle for 12 h before we restarted the airlift system. Internal surfaces of the core tubes were cleaned as in the dark experiment.

The *Navicula incerta*, together with algae and cyanobacteria present naturally, developed an active MPB community within 9 d after initial inoculation. Cores were then randomly allocated to three levels of daily additions of *Thalassosira pseudonana* (Table 1). The no-POM addition control and low-POM addition (0.25 g C m<sup>-2</sup> d<sup>-1</sup>) were the same as we used in the dark experiment. The medium addition of 2.5 g C m<sup>-2</sup> d<sup>-1</sup> was only 50% of the high-POM loading we used in the dark experiment in order to avoid completely over-

whelming the MPB community in a flocculent organic layer. The cores were maintained for 17 d under these conditions to ensure that sufficient POM had been added to obtain measurable flux rates. Cores (four from each of the two POM levels and two no-POM addition) were incubated by sealing the cores 5 h before the end of the dark cycle and continuing the flux measurements until 5 h into the succeeding light cycle. Solute and gas concentrations (including concentrations of  $\Sigma\text{CO}_2$  analyzed coulometrically following Johnson et al. 1993) were measured in the overlying water as described for the dark experiments at four time points in the dark and three times in the light commencing 1 h after the light came on at the usual time in the diel cycle.

We determined how the fluxes changed in response to changes in overlying water-column  $\text{NO}_3^-$  concentrations. Two cores from each of the three POM treatments were placed together in one tank in which the  $\text{NO}_3^-$  concentration was increased from ambient ( $12 \mu\text{mol}$ ) to about  $80 \mu\text{mol}$ . An additional two cores from the low- and medium-POM treatments were placed together in a separate tank in which  $\text{NO}_3^-$  concentrations were increased to about  $160 \mu\text{mol}$ . The light, temperature, and daily POM addition regimes remained unchanged. After 36 h at the enhanced  $\text{NO}_3^-$  levels, nutrient fluxes were measured.

The percent water in the 0- to 6-mm sediment surface layer of each core was determined gravimetrically and Chl *a* content in triplicate-weighed aliquots of this surface layer was measured by HPLC (Van Heukelem et al. 1994). The remaining sediment in each core was poured through a  $500\text{-}\mu\text{m}$  sieve to ascertain whether any invertebrates survived the initial anoxic treatment.

Statistical relationships between parameters were tested using linear regression and correlation analysis (Sokal and Rohlf 1981).

## Results

**Dark experiment**—The results of our experiments reflect microbially mediated responses, as the anoxic pretreatment of the sediments was effective at removing all benthos. The solute and gas flux time series data for all incubations were linear over the 5-h duration of the incubations. The changes in  $\text{N}_2$  concentration were resolved by precise mass-spectrometric measurement of the  $\text{N}_2:\text{Ar}$  ratio. The baseline gas fluxes (mean  $\pm$  SD), measured before POM additions, were  $-1696 \pm 90 \mu\text{mol O}_2 \text{ m}^{-2} \text{ h}^{-1}$  ( $n = 6$ ) and  $125 \pm 17 \mu\text{mol N}_2\text{-N m}^{-2} \text{ h}^{-1}$  ( $n = 5$ ). (Note that negative fluxes indicate movement from the water column into the sediments.)

The 16 d of high and low rates of POM loading stimulated different levels of sediment metabolism and nitrogen cycling rates (Fig. 1). The major form of N release from these sediments was via  $\text{NH}_4^+$  efflux. Denitrification of N in the added POM, stemming from coupled nitrification–denitrification, averaged 17% of the total N remineralization in the high-POM loaded cores and 24% in the low-POM loaded cores. These denitrification rates were within those found in incubation of natural sediment cores from the Choptank River (Cornwell, unpubl. data). We measured negligible  $\text{NO}_3^-$  fluxes across the sediment–water interface during our experi-

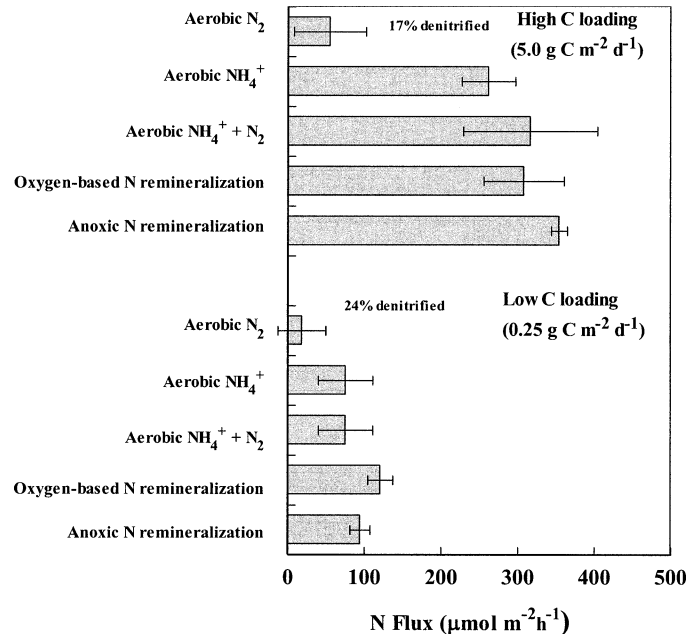


Fig. 1. Dark experiment. Mean ( $\pm$ SD;  $n = 3$ ) fluxes of nitrogen species 16 d after the start of high and low-POM additions to cores maintained either aerobically or anaerobically. Oxygen-based remineralization rates were converted to N remineralization numbers by assuming an  $\text{O}_2:\text{N}$  ratio of 6.625. Denitrification rates averaged 17% of total N remineralization in the high and 24% in the low-POM cores. Positive fluxes indicate a flux from sediment to the overlying water.

ments, indicating that nitrification in the aerobic sediment was the source of  $\text{NO}_3^-$  for denitrification. When we experimentally increased  $\text{NO}_3^-$  concentrations above ambient levels in the overlying water, the rates of denitrification in low and high-POM loading treatments increased substantially (Fig. 2).

The sediments were not at steady state balance between loading and remineralization. In the high-POM treatment, daily N inputs from algal paste loading were equivalent to  $\sim 1,400 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ ; the low-POM treatment cores had  $\sim 70 \mu\text{mol N m}^{-2} \text{ h}^{-1}$  of N loading. For the high-POM treatment this amount of N was not released, and therefore a considerable amount of particulate organic nitrogen (PON) was probably accumulating in the sediments. Although the organic matter loading rates were 20 times higher in the high POM relative to the low-POM treatment, we found that the rates of oxygen consumption of the sediment differed only by a factor of  $\sim 2.5$ . This indicates that in the high treatment a large amount of organic matter was not being microbially metabolized and was accumulating in the sediment. Nitrogen remineralization rates were estimated by assuming a 106:16  $\text{O}_2:\text{N}$  stoichiometry (a value consistent with low proportional rates of nitrification). These predicted N production rates from oxygen consumption are similar to our experimental measurements based on (1) anoxic  $\text{NH}_4^+$  fluxes and (2) the sum of aerobic  $\text{NH}_4^+$  and  $\text{N}_2$  fluxes ( $\text{NO}_3^-$  concentrations and fluxes were low). The oxygen-based N remineralization rates had a coefficient of variation of 13% for the low and 17% for the high-POM treatment cores.

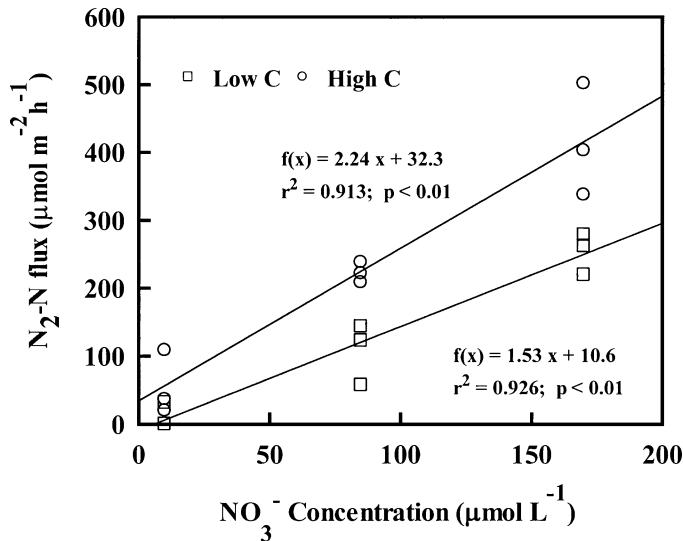


Fig. 2. Dark experiment. The effect of experimental additions of nitrate to the overlying water column on sediment–water exchange of  $N_2-N$  for high and low-POM addition cores maintained aerobically. Positive values indicate a flux of  $N_2$  out of the sediment. The lines are linear regressions fitted to the nine data points shown.

*Light: dark experiment*—To capture changes in fluxes associated with the light: dark cycle, we measured fluxes from cores incubated for the last 5 h of a dark period and for the first 5 h of the succeeding light period. In general, we observed linear changes in  $O_2$ ,  $N_2$ ,  $NO_3^-$ , and  $NH_4^+$  during the incubations (Fig. 3). Dissolved oxygen concentrations decreased under dark conditions and increased with illumination. The greatest temporal changes in oxygen concentration occurred in the medium-POM treatment. In general, the fluxes of  $\Sigma CO_2$  were the inverse of the oxygen fluxes, with increased  $\Sigma CO_2$  under dark conditions and a leveling off or a decrease under light conditions. Illumination resulted in net autotrophic conditions ( $O_2$  production >  $CO_2$  production), particularly in the cores loaded with POM at a medium rate.

Sediment oxygen fluxes varied by treatment, with the highest fluxes ( $\sim 3,000 \mu mol O_2 m^{-2} h^{-1}$ ) under illuminated conditions occurring in the medium-POM treatment (Fig. 4). Surprisingly, rates of dark oxygen uptake were similar in the two POM treatments and the control. Under both light and dark conditions sediment  $\Sigma CO_2$  fluxes were weakly correlated ( $p < 0.05$ ) with sediment oxygen fluxes (Fig. 5A,B), with the greatest deviation from a 1:1 relationship occurring with the medium-POM treatment. Simplistically assuming that net primary production under illuminated conditions for these systems may be calculated as the difference between dark and light fluxes, the net primary production may be estimated using both  $O_2$  and  $\Sigma CO_2$  flux rates (Fig. 5C). Despite weak correlations between  $O_2$  and  $\Sigma CO_2$  fluxes for the light and dark incubations, net primary production by both techniques was highly correlated ( $p < 0.01$ ) and close to a 1:1 relationship.

Chlorophyll *a* concentrations showed variability between cores within each treatment, especially at the medium-POM treatment (Fig. 6A). Primary production estimated from  $O_2$  production was only weakly correlated ( $p < 0.05$ ) to the

mean chlorophyll *a* concentration of each core (Fig. 6A). This result suggests that only some of the chlorophyll *a* was associated with actively photosynthesizing microphytobenthos, while some was derived from the added *Thalassosira pseudonna* paste in which the cells were either dormant or dead.

Relative to fluxes measured in the dark,  $NH_4^+$  fluxes in the light decreased for the two levels of POM addition, and  $NO_3^-$  uptake in the light increased for the medium-POM treatment (Fig. 4). In general, the  $NO_3^-$  and  $NH_4^+$  fluxes into the sediment from the water indicate a switch to dissolved inorganic nitrogen uptake during photosynthesis. The  $N_2-N$  fluxes showed high variability in denitrification between the triplicate cores in the low-POM treatment, negligible denitrification in the control cores, and  $N_2$  uptake in the cores with medium-POM additions. This  $N_2$  uptake results from N fixation in the cores. To distinguish between gross N fixation and the net  $N_2$  measurements made in this study, we use the term net N fixation. The  $N_2-N$  uptake in these experiments does not preclude the presence of denitrification; it may be occurring, but apparently at an overall rate lower than that of N fixation.

The N requirements to sustain the rates of algal production in these cores may be estimated from the oxygen produced by applying an  $O_2:N$  stoichiometric ratio of 6.625. If gross photosynthesis is the sum of oxygen production in the light plus dark respiration, there is a photosynthetic requirement of  $703 \pm 209$  ( $n = 4$ )  $\mu mol N m^{-2} h^{-1}$  for medium-POM treatment,  $312 \pm 179$  ( $n = 4$ )  $\mu mol N m^{-2} h^{-1}$  for the low-POM treatment, and  $384 \pm 86$  ( $n = 2$ )  $\mu mol N m^{-2} h^{-1}$  for the control cores. Remineralization can supply 32 to 56% of the N requirements for all of these treatments, indicating a need for a substantial amount of N fixation to support photosynthesis in the medium-POM treatment. The high  $N_2$  and  $NO_3^-$  uptake rates in the medium-POM treatment are consistent with the N nutritional requirements for the microalgae. Uptake of  $N_2$  in the dark was observed in the medium and low-POM treatments, but not in the control treatment. The uptake of  $N_2$  in the light and the estimated primary production were significantly correlated (Fig. 6B,  $p < 0.01$ ).

In contrast to the dark experiment (Fig. 2), experimental additions of  $NO_3^-$  to the overlying water column of cores with MPB did not increase net denitrification (Fig. 7). Added  $NO_3^-$  in the medium-POM treatment either decreased the rate of N fixation or increased the rate of denitrification; at lower rates of loading, there was no effect. Nitrate additions under dark conditions resulted in lower  $N_2-N$  fluxes into the sediment, consistent with a lowered N demand from nitrogen fixing autotrophic organisms.

## Discussion

*Aerobic and anaerobic nutrient regeneration*—Our investigation was designed to explore how the location of organic material deposition in estuaries, associated perhaps with changes in the magnitude of biodeposition from benthic suspension-feeding bivalves, may alter sediment nitrogen regeneration processes. Such possible influences of bivalve biodeposition on rates and processes of sediment nitrogen

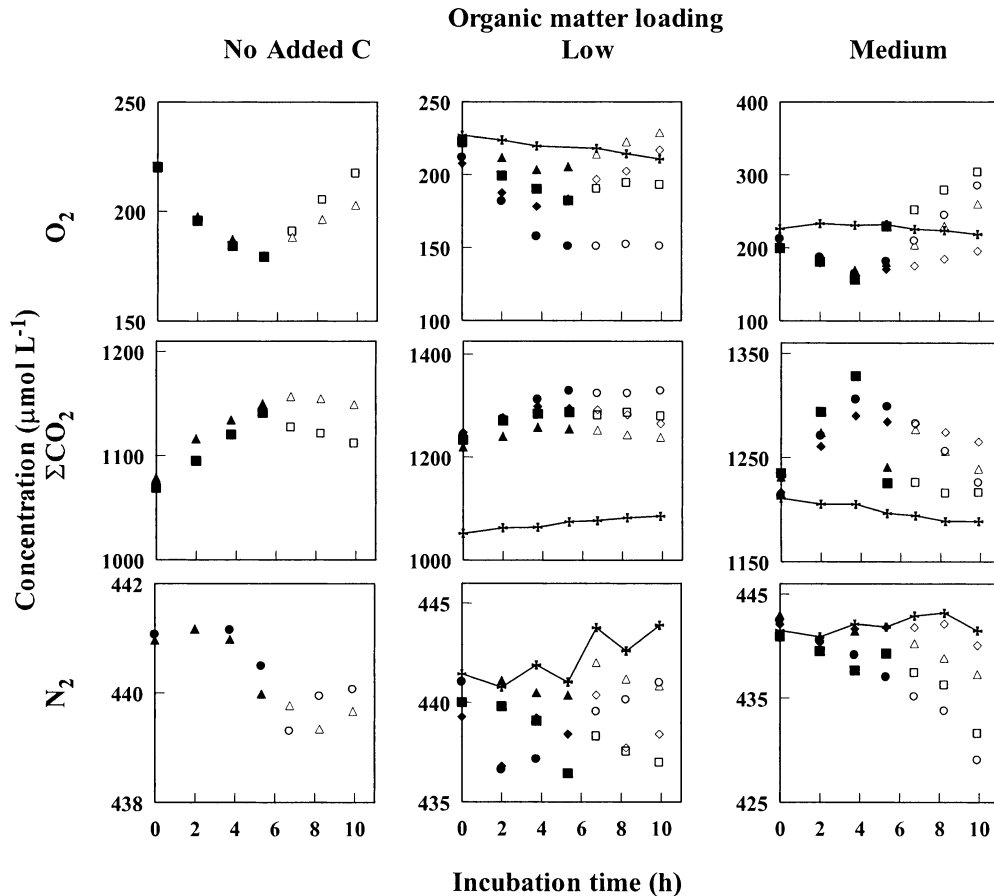


Fig. 3. Light:dark experiment. Time course of O<sub>2</sub>, ΣCO<sub>2</sub>, and N<sub>2</sub> fluxes for the individual control no-POM-addition cores ( $n = 2$ ) and low and medium-POM treatment cores ( $n = 4$ ) incubated first in darkness (filled symbols) and then in light (open symbols). Results for the two water blanks (core tubes incubated without sediment) are illustrated by the solid lines in the low and medium-POM panels. Note scale differences between panels.

dynamics have not been fully characterized, mainly due to difficulty of performing controlled studies under natural field conditions. We developed a laboratory-based experimental system to constrain the variation in our study of nitrogen regeneration under different scenarios typical of eutrophic estuaries. We used algal paste as an experimental analog of oyster biodeposits and of phytoplankton that sinks to the sediment surface. This was similar to the method used by Sloth et al. (1995) of adding organic material in the form of yeast in their study of nutrient regeneration from sediments. Our method enabled us to control precisely the daily POM loaded to sediment cores in the various experimental treatments. Such reproducibility would have been impossible to achieve if we had attempted to direct biodeposits from actively feeding oysters onto the surface of defaunated and homogenous natural sediments held in tubular cores. We believe that this is a realistic approach because bivalves only digest and absorb ~50% of the filtered particulate nitrogen, and hence their voided biodeposits contain a large proportion of residual particulate organic nitrogen (Newell and Jordan 1983). Of the nitrogen absorbed, most is used for tissue growth and some is excreted, predominately as ammonium (Bayne and Hawkins 1992), which returns to the DIN pool

in the water column and is available for both MPB and phytoplankton production (Kaspar et al. 1985; Doering et al. 1986; Asmus and Asmus 1991; Swanberg 1991).

We observed that N was released primarily as NH<sub>4</sub><sup>+</sup> from organic material added to the anaerobic cores incubated in the dark and that nitrification in these cores was precluded. The situation in the dark-incubated aerobic cores was more complex, with about 76 to 83% N being regenerated as NH<sub>4</sub><sup>+</sup> and a moderate amount (17 to 24%) being denitrified. Interestingly, the low NO<sub>3</sub><sup>-</sup> effluxes we found in all cores are typical of conditions in Chesapeake Bay sediments (Cowan and Boynton 1996) and suggest a close proximity of nitrifying and denitrifying bacteria. The relatively high coefficient of variation (13 to 17%) of flux rates after POM amendment suggests either losses of organic matter from the core due to our aeration system or uneven distribution of organic matter within the cores. Our observed differences in N release from POM added to laboratory-incubated cores maintained at different oxygen levels are consistent with remineralization processes observed previously in natural sediments from the Chesapeake Bay (Kemp et al. 1990) and in other systems (Rysgaard et al. 1994). We observed that total N remineralization rates were similar for both the high and

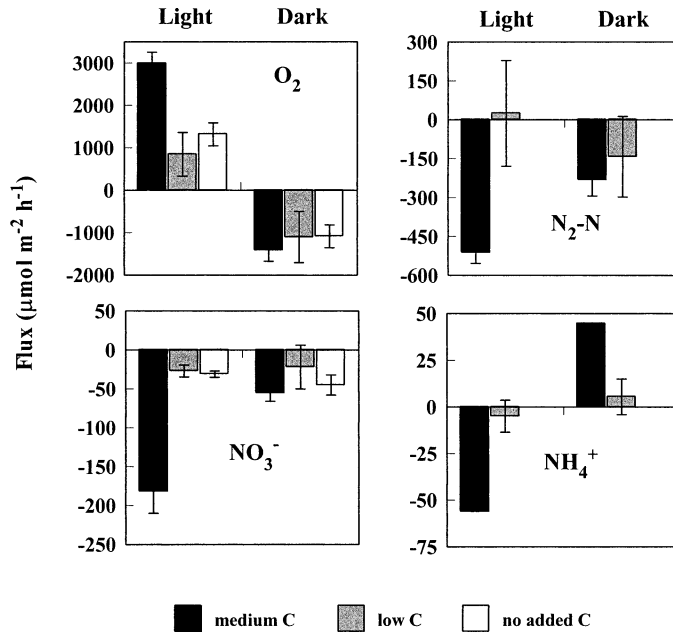


Fig. 4. Light:dark experiment. Fluxes of  $O_2$ ,  $N_2$ ,  $NO_3^-$ , and  $NH_4^+$  17 d after the start of medium, low, and control additions of POM. Values are means ( $\pm$ SD;  $n = 4$ ) for the medium and low additions and mean (with range bars) for the two control cores. Positive values indicate a flux out of the sediment to the overlying water (for  $N_2$  = net denitrification); negative values indicate flux into the sediment (for  $N_2$  = net nitrogen fixation). Control cores exhibited minor  $N_2$  and  $NH_4^+$  fluxes that do not show at the scale of these figures.

low-POM loadings under aerobic and anaerobic conditions, even though the organic matter loading rates were 20 times higher in the high POM relative to the low-POM treatment. The proportionally lower N regeneration from the high-POM treatment indicates that a considerable amount of PON was buried in the sediments. Estimates of remineralization based on the sum of DIN fluxes,  $O_2$ -based stoichiometry, and anaerobic  $NH_4^+$  fluxes were similar for both the high and low-POM loading treatments, and the N mass balances were as predicted based on geochemical ratios. We have observed similar agreement between direct and stoichiometric approaches for the estimation of N remineralization in cores taken from the Choptank River near where we collected the sediments used in this study (Cornwell and Owens, unpubl. data).

We found that when  $NO_3^-$  concentrations were increased above ambient levels in the overlying water column for cores without MPB, the denitrification rates in both low and high-POM loading treatments also increased (Fig. 2). The increase in denitrification shows that under low to moderate  $NO_3^-$  concentrations in the overlying water, denitrification rates may be limited by diffusion of nitrate to the anaerobic zone. Previous work on intact cores taken from the Choptank River near our sediment collection site showed a similar linear relationship (Kana et al. 1998), with a slope intermediate between the high and low-POM treatments in our experiment. While additions of  $NO_3^-$  increased concentrations to levels considerably above summer ambient levels in the

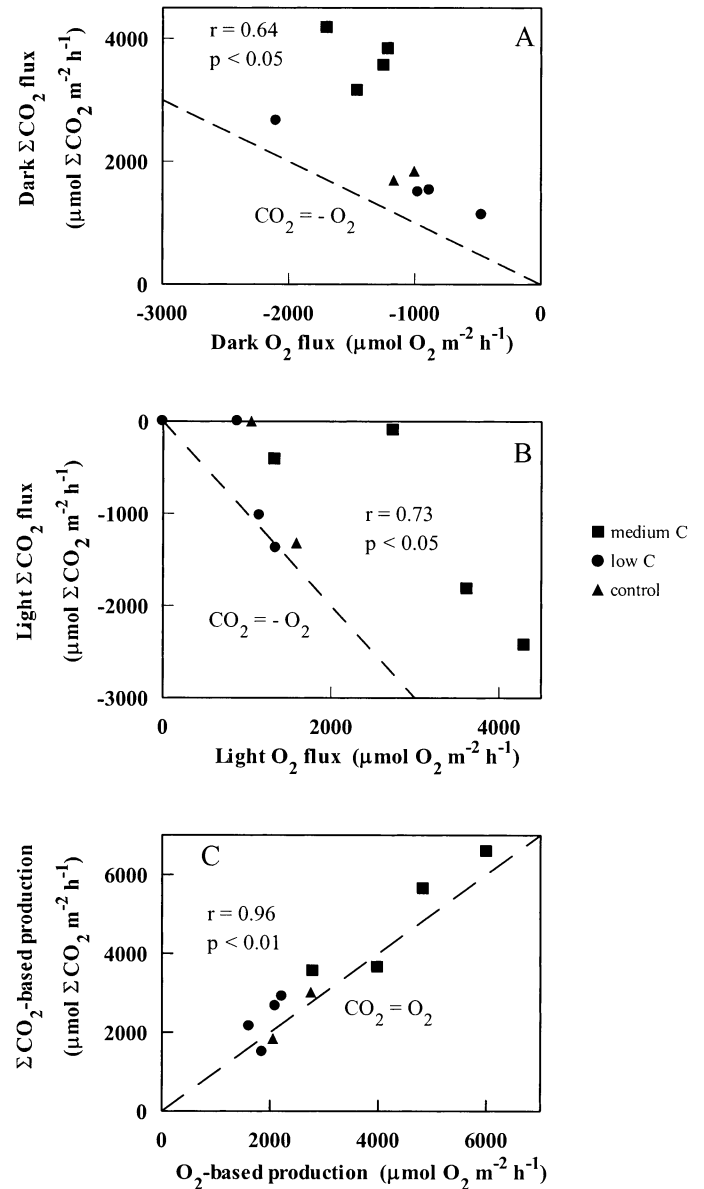


Fig. 5. Light:dark experiment.  $\Sigma CO_2$  and  $O_2$  relationships measured concurrently in the same cores for medium ( $n = 4$ ), low ( $n = 4$ ), and control ( $n = 2$ ) levels of POM additions incubated in the dark and the light. The 1:1 relationships between the parameters are shown as dashed lines. (A) Correlation (significant at  $p < 0.05$ ) between dark  $\Sigma CO_2$  flux and dark  $O_2$  flux. (B) Correlation (significant at  $p < 0.05$ ) between light  $\Sigma CO_2$  flux and light  $O_2$  flux. (C) Correlation (significant at  $p < 0.01$ ) between  $O_2$ -based and  $\Sigma CO_2$ -based primary production, which are two independent measures of production for cores from all POM treatments.

Choptank River, the additions illustrated the close relationship between denitrification and  $[NO_3^-]$ . Our data show that at higher  $[NO_3^-]$ , whether from nitrification or from overlying waters, there is an enhanced N loss via denitrification. In contrast,  $NO_3^-$  additions to illuminated cores with an active microphytobenthic community did not increase net denitrification rates (Fig. 7). Added  $NO_3^-$  in the medium-POM loading case either decreased the rate of N fixation or in-

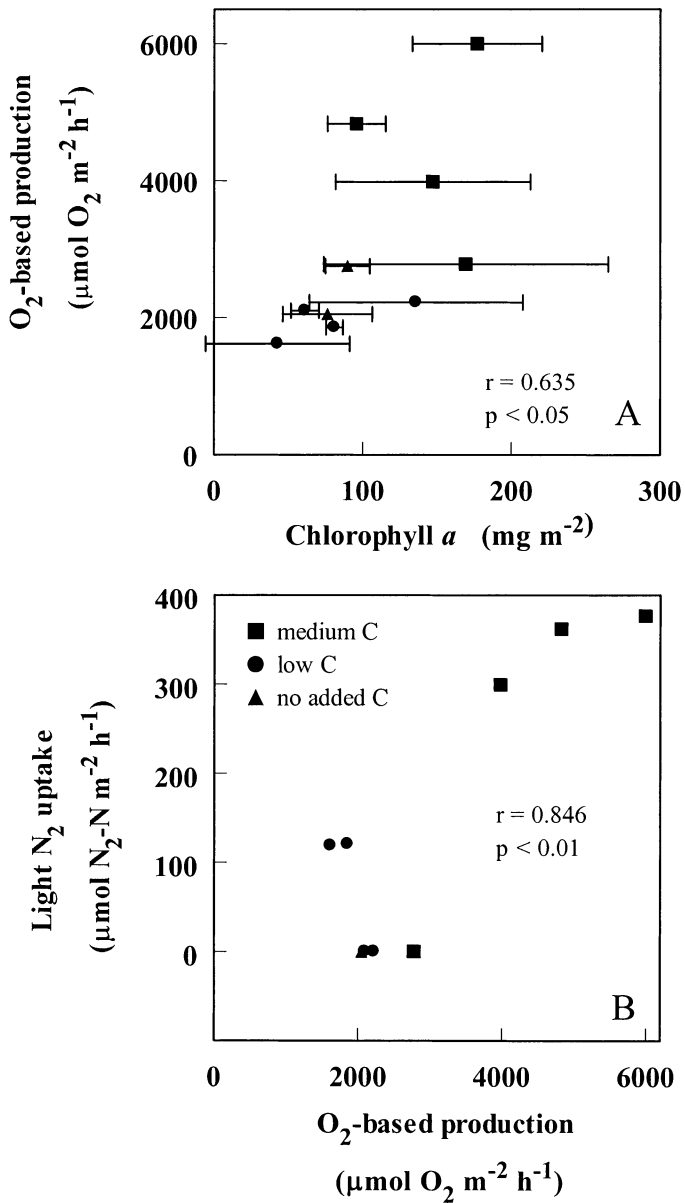


Fig. 6. Light:dark experiment. (A) Calculated oxygen-based production correlated with mean ( $\pm$ SE;  $n = 3$ ) areal chlorophyll *a* concentration (correlation significant at  $p < 0.05$ ). (B) Light N<sub>2</sub> uptake correlated with O<sub>2</sub>-based primary production (correlation significant at  $p < 0.01$ ). ( $n = 2$  for control and  $n = 4$  for low and medium-POM addition cores.)

creased the rate of denitrification; at the lower rates of POM loading, there was no effect. In darkness, these same cores with MPB exhibited lower N<sub>2</sub>-N fluxes into the sediment, consistent with a lowered N demand from nitrogen fixing autotrophic organisms.

The weak correlation between  $\Sigma$ CO<sub>2</sub>-based and O<sub>2</sub>-based light and dark fluxes (Fig. 5A, B) did not appear to stem from analytical or experimental artifacts because  $\Sigma$ CO<sub>2</sub>-based and O<sub>2</sub>-based photosynthesis measurements were proportional (Fig. 5C). Under dark conditions, net  $\Sigma$ CO<sub>2</sub> flux exceeded that expected by O<sub>2</sub> uptake, particularly under me-

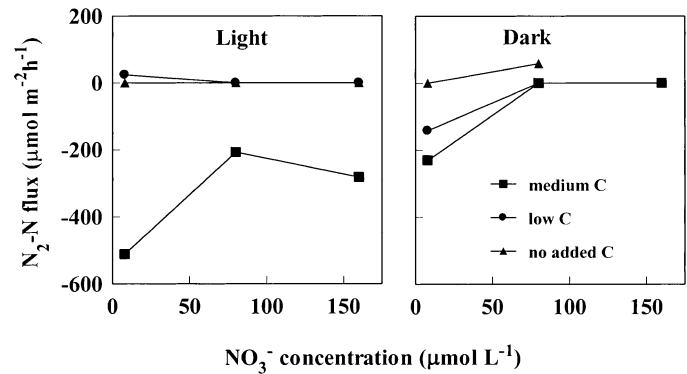


Fig. 7. Light:dark experiment. The effect of experimental additions of nitrate to the overlying water column on light and dark sediment-water exchange of N<sub>2</sub>-N for control-, medium-, and low-POM addition cores maintained aerobically. Positive values indicate a flux of N<sub>2</sub> out of the sediment; negative values indicate net nitrogen fixation. Each point is the mean of data from two cores.

dium-POM loading conditions. Under light conditions, there was an excess of O<sub>2</sub> relative to  $\Sigma$ CO<sub>2</sub>. Both gases are affected by photosynthesis and respiration. The different behavior of O<sub>2</sub> relative to  $\Sigma$ CO<sub>2</sub> may relate to the role of oxygen in the degradation of oxidizable organic substrates (Epping et al. 1999) and the reoxidation of reduced inorganic compounds such as hydrogen sulfide, reduced iron and manganese, and iron sulfide minerals in the sediments.

The light-dark change in net N<sub>2</sub> fluxes indicates the important role of light in the N cycle. Our calculation of net N<sub>2</sub> uptake could be an overestimate, however, because the method we employed to measure N<sub>2</sub> concentrations (Kana et al. 1998) relies on measuring N<sub>2</sub>:Ar ratios, which can be altered by oxygen bubbles generated by algae that strip N<sub>2</sub> relative to Ar. This could potentially lead to an apparent N<sub>2</sub> flux into the sediment when there was none. This experimental artifact does not seem to be a factor in our study because N<sub>2</sub> uptake was observed during the dark incubations when the dissolved O<sub>2</sub> concentrations in the overlying water were depleted below saturation. Furthermore, we did not observe oxygen bubble formation during these experiments.

Our study demonstrates the importance of making direct N<sub>2</sub> flux measurements. While stoichiometric estimates of N<sub>2</sub> flux may work well in systems in which denitrification is the major DIN flux pathway (Cornwell et al. 1999), that approach is inadequate when N<sub>2</sub> fluxes are a small proportion of the DIN flux. The complex pathways of nitrogen uptake and recycling in microphytobenthic communities, including the induction of N<sub>2</sub> fixation, preclude simple stoichiometric models. Even expected relationships between  $\Sigma$ CO<sub>2</sub> and O<sub>2</sub> are complicated by small-scale temporal and spatial complexity.

Our anaerobic core incubations indicate that if organic material settles on sediments with restricted oxygen supply, most nitrogen will be released in the form of NH<sub>4</sub><sup>+</sup> and denitrification will be inhibited. This observation is similar to phytoplankton degradation in the Chesapeake Bay today, where substantial amounts of nitrogen are regenerated in anoxic waters beneath the pycnocline, which has limited O<sub>2</sub> concentrations due to minimal mixing of oxygenated surface



waters (Kemp et al. 1990; Kemp and Boynton 1992). This lack of oxygen limits nitrification during summer months, a time when the spring supply of nitrate from the watershed is depleted by algal uptake. In our experiments, when POM was regenerated aerobically in the dark incubations, 17 to 24% of the total PON added to the sediments was released as  $N_2$  gas and was unavailable to support further phytoplankton production. The rest, regenerated as  $NH_4^+$ , would enter the DIN pool in the water column.

Our assessment that about 20% of N from added POM was denitrified is at the low end of the range (20 to 70% of total DIN flux) calculated from literature data by Seitzinger (1988). The denitrification rates reviewed by Seitzinger (1988) were measured by techniques with methodological flaws (Seitzinger et al. 1993; Cornwell et al. 1999), making direct comparison with modern studies problematic. The  $^{15}N$  ion pairing approach (Nielsen 1992) and the membrane inlet mass-spectrometry technique (Kana et al. 1998) that we used in our study appear to measure coupled denitrification–nitrification more accurately.

Our denitrification values may be low because we removed all burrowing and deposit-feeding benthic organisms (bioturbators) in our controlled experiments. Bioturbation serves to increase the surface-area-to-volume ratio of the oxic–anoxic sediment interface and accelerate the delivery of oxidants and reductants to the sediment microbial communities (Kristensen 1988). In this spatially complex interface between aerobic and anaerobic zones, denitrification rates are enhanced compared to sediments without bioturbators (Kaspar et al. 1985; Henriksen and Kemp 1988; Kristensen 1988; Pelegri et al. 1994). A further complicating factor is that we used a fine mud sediment of low porosity that, without the presence of active bioturbators, also limited oxygen penetration into the sediment, hence reducing nitrification. Low rates of denitrification were also observed by Sloth et al. (1995) when they added organics to sandy sediment as a single dose at high concentrations (30 to 100 g  $dw^{-2}$ ), with most N being released as  $NO_3^-$  and  $NH_4^+$ . They concluded that the high nitrification rates were because  $O_2$  could penetrate deep into the coarse sediment. Denitrification was low because the sediment remained predominately aerobic, which is exactly the opposite of the situation in our study using fine grain sediments. This result confirms the need to have aerobic conditions that promote high levels of nitrification in close proximity to anaerobic conditions where denitrification can take place.

The complex interactions between sediment porosity and bioturbation is illustrated in a study by Sundbäck et al. (2000) using intact natural sediments of two different grain sizes. They reported that coupled nitrification–denitrification rates were about an order of magnitude higher annually in finer grain sediments with active bioturbators than in sediments of higher porosity and with a slightly lower biomass of bioturbators. Because the sediments used by Sundbäck et al. (2000) were natural and did not have POM added experimentally, the higher denitrification rates measured in their finer grain sediment can also be explained by the background amounts of POM that are higher in fine than in coarse grain sediments.

Our data showing that growing MPB can directly assim-

ilate all inorganic nitrogen released by bacterial decomposition of added POM support previous studies showing how MPB limit nutrient release to the water column (e.g., Sundbäck and Graneli 1988; Rysgaard et al. 1995; Cerco and Seitzinger 1997; Sundbäck et al. 2000; Souchu et al. 2001). The magnitude of these fluxes can vary with nutrient concentrations in the overlying water, MPB biomass and species composition, and PAR conditions. In addition, our observation of a strong  $N_2$  flux into the sediment when an active MPB community was growing agrees with results of Cerco and Seitzinger (1997) and numerous observations of nitrogen fixation in estuarine and coastal shallow water sediments (Nedwell and Aziz 1980; Joye and Paerl 1994). The high N requirement of MPB photosynthesis in our treatments required an N source other than regeneration and the rates of  $N_2$  uptake in our experiments were consistent with that demand.

In addition to the role of MPB in intercepting DIN being regenerated from the sediments, the oxygen produced from their photosynthesis can alter sediment microbial processes, including rates of denitrification. Where denitrification is driven by  $NO_3^-$  diffusing into the sediment from the water column, increased sediment oxygenation results in a longer diffusion path for  $NO_3^-$  to encounter the reducing conditions necessary for denitrification (Christensen et al. 1990; Risgaard-Petersen et al. 1994) and reduces denitrification rates. Conversely, in systems with coupled nitrification–denitrification, oxygen can be used by bacteria in the microzone at the sediment–water interface to maintain nitrification (Sundbäck et al. 1991; Risgaard-Petersen et al. 1994; Rysgaard et al. 1995; An and Joye 2001). This pattern changes when MPB growth is high because the demand by the microalgae for inorganic nutrients outcompetes the nitrifying bacteria for  $NH_4^+$ , resulting in a decline or cessation of denitrification (Risgaard-Petersen et al. 1994; Rysgaard et al. 1995; An and Joye 2001). Our observation of “net N fixation” in the illuminated cores suggests that the MPB outcompeted the nitrifying bacteria and hence inhibited denitrification. Even when we added high concentrations of  $NO_3^-$  to the water column (Fig. 7), net denitrification was absent in the dark, which suggests that the MPB were still taking up  $NO_3^-$ .

Our results suggest that when organic matter, such as biodeposits from suspension-feeding bivalves, are transferred to well-oxygenated sediments, coupled nitrification–denitrification can produce  $N_2$  gas. Absent N fixation, this gaseous  $N_2$  is in a form unavailable to plankton and so it passes to the atmosphere without stimulating further primary production. In addition, MPB, perhaps growing over a larger bottom area due to increased water clarity, further reduces the amount of N regenerated to the water column.

*Influence of bivalves on nutrient regeneration*—Based on our results and literature information, we have developed a conceptual model (Fig. 8) illustrating some of the complex interrelationships among benthic and pelagic processes that may be influenced by bivalve suspension feeders. Bivalves actively filter particles  $> \sim 3 \mu m$  from the water column (Bayne and Hawkins 1992). In shallow systems such filtration can reduce turbidities to the point where PAR levels at the sediment surface are sufficient to support MPB produc-

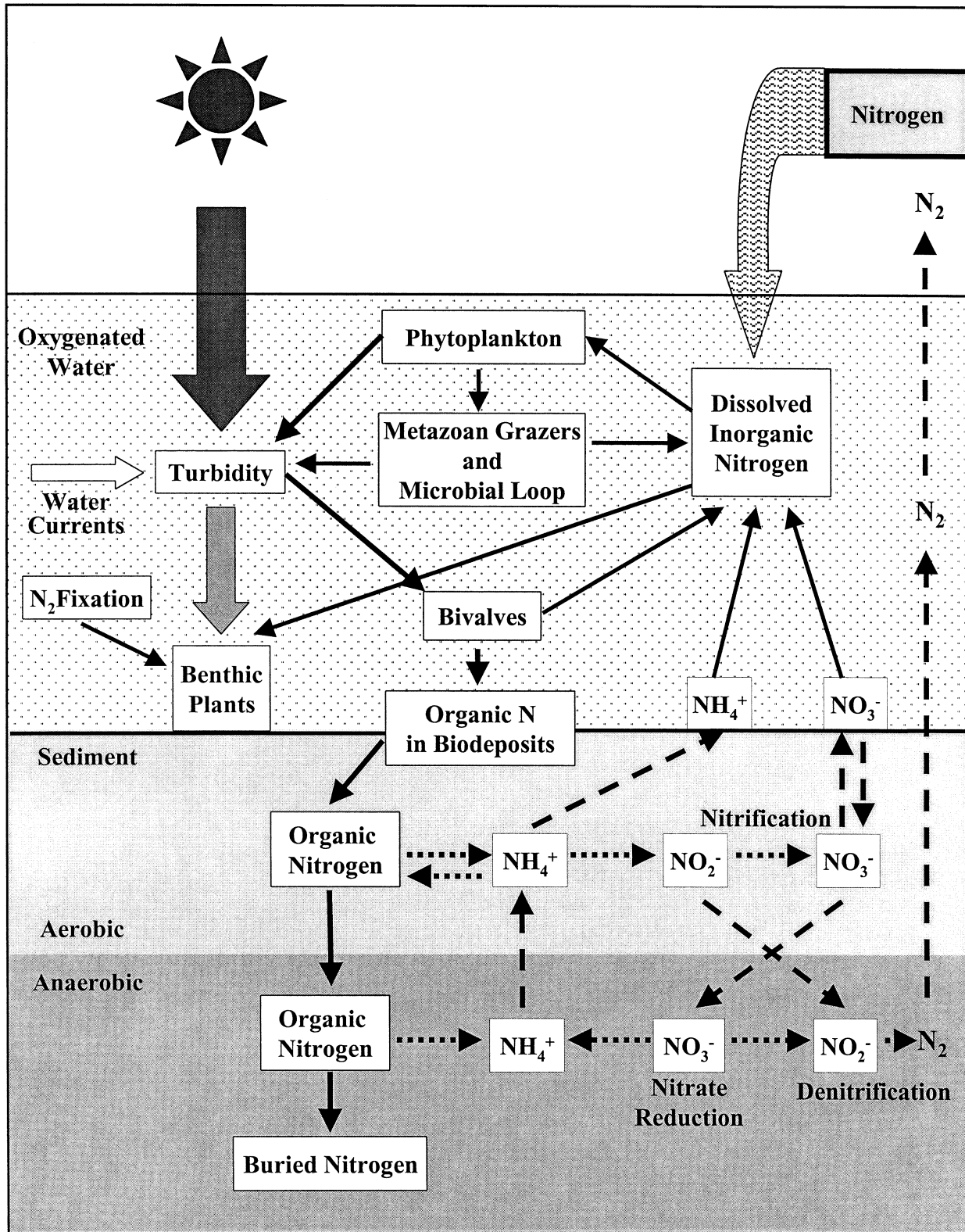


Fig. 8. Conceptual diagram of how suspension-feeding bivalves serve to reduce turbidities in the water column and transfer undigested POM to the sediment surface. Within the aerobic sediment layers, the microbially mediated process of nitrification occurs, which, when linked to denitrification within the underlying anaerobic sediment, leads to the release of N<sub>2</sub> gas. Solid lines indicate movement of materials; dashed lines indicate diffusion of materials; dotted lines indicate microbially mediated reactions. (*Sediment nitrogen transformation schematic modified from Henriksen and Kemp 1988*).

tion (Souchu et al. 2001). Filtered particles are sorted on pallial organs and less nutritious and excess particles are rejected as pseudofeces before ingestion (Newell and Jordan 1983). In some circumstances, up to 90% of the total volume of filtered particulate material can be rejected as pseudofeces (Bayne and Hawkins 1992). Because feces and pseudofeces are voided from bivalves as mucus-bound aggregates, they have a higher sinking velocity than nonaggregated particles and settle out at rates up to 40 times that of nonaggregated particles (Kautsky and Evans 1987; Widdows et al. 1998). If bottom currents are below the critical erosional velocity, the biodeposits undergo a dewatering process and gradually become incorporated into the sediments (Haven and Morales-Alamo 1966, 1968; Dame 1987; Jaramillo et al. 1992) leading to an increase in sediment N content (Kaspar et al. 1985; Kautsky and Evans 1987; Deslous-Paoli et al. 1992; Hatcher et al. 1994). High abundances of bivalves can overenrich sediments with biodeposits, thereby generating high microbial respiration leading to sediment anoxia that both inhibits nitrification and kills bioturbating benthic infauna (Tenore et al. 1982; Rodhouse and Roden 1987). Such local adverse effects can be ameliorated by moderate water currents or wave action that allows biodeposits to be spread across a larger bottom area (Haven and Morales-Alamo 1968; Dame 1987; Dame et al. 1991).

The potential ecosystem effects of bivalve grazing (Fig. 8) support previous literature reports that populations of suspension-feeding bivalves can exert top-down control on phytoplankton production in estuarine and coastal waters (e.g., blue mussels, Riemann et al. 1988; Prins et al. 1995; Pacific oysters, Souchu et al. 2001; and nonnative bivalves in San Francisco Bay, Cloern 1982; Officer et al. 1982). In freshwater systems, Asiatic clams in the Potomac River, Maryland, reduced phytoplankton stocks (Cohen et al. 1984), and nonnative zebra mussels have greatly reduced phytoplankton biomass in the eutrophic Great Lakes (Holland 1993) and the Hudson River, New York (Strayer et al. 1999).

Conversely, some investigators contend that bivalves may not reduce phytoplankton levels appreciably. This is based on their observations of high rates of N excretion by bivalves, N regeneration to the water column from bivalve biodeposits, and either estimates or direct measures of higher primary production and phytoplankton biomass associated with bivalve grazing (e.g., Doering et al. 1986; Prins and Smaal 1990; Asmus and Asmus 1991; Dame and Libes 1993; Yamamuro and Koike 1993). Rates of  $\text{NH}_4^+$  flux from natural bivalve communities (direct excretion plus regeneration from the sediments) can be substantial, ranging from  $\sim 1$  to  $10 \text{ mmol N m}^{-2} \text{ h}^{-1}$  (Dame et al. 1991, 1992; Asmus and Asmus 1991) with rates being greater in summer than in winter months (Dame et al. 1992). The nitrogen released directly by the bivalves and regenerated from their biodeposits comes not only from ingested phytoplankton but also from nonphytoplankton material, such as N-rich bacteria and flagellates (Asmus and Asmus 1991), that are readily captured and digested by bivalves (Bayne and Hawkins 1992). The regenerated DIN will stimulate phytoplankton production, hence explaining the enhanced primary production observed in the vicinity of the bivalves. However, the only way to assess at the ecosystem level the influence of bivalve graz-

ing on phytoplankton is to measure the fluxes of DIN and PON (derived from both microautotrophs and microheterotrophs) moving into and out from the bivalve community. Such mass balance calculations have not been made. We suggest that  $\Sigma \text{DIN} + \text{PON}$  in the water moving into the bivalve community is greater than that leaving, with the difference being due to the loss of N by denitrification. It is this systematic removal of N from the ecosystem via denitrification that has not been considered in these earlier investigations that focused on the local enhancement of phytoplankton production by the N regenerated by bivalves. In one of the few studies of the influence of bivalves on ecosystem-level processes in which denitrification was evaluated, Kaspar et al. (1985) found appreciably higher denitrification in sediments underlying rope-cultured mussels than in reference sites without mussels. (Note that Kaspar et al. measured denitrification using the acetylene block technique that provides information on denitrification potential rather than absolute rates; Seitzinger et al. 1993; Cornwell et al. 1999.)

We propose that increased top-down control on phytoplankton associated with bivalve grazing in eutrophied estuaries may lead to permanent removal of nitrogen through enhanced rates of denitrification. In the Chesapeake Bay, for example, the once abundant eastern oyster stocks were located in shallow ( $< 9 \text{ m}$ ), well-mixed, and fully oxygenated waters (Newell 1988). When biodeposits from those oysters became incorporated in the sediments, they likely served to enhance denitrification and reduce the advection of POM to deeper water underlying the pycnocline. Today, much of the phytoplankton in the Chesapeake Bay remains unconsumed by metazoans and instead accumulates beneath the pycnocline in the deep central channel. The resulting intense microbial respiration in summer generates anoxic bottom waters, leading to N being regenerated via anaerobic pathways predominately as  $\text{NH}_4^+$  (Kemp and Boynton 1992). Conservation and enhancement of stocks of eastern oysters for their ability to promote denitrification may provide a useful supplement to current management activities designed to curb point and non-point sources of N inputs into the Chesapeake Bay. If the water where the oysters are located is shallow, the reduction in turbidity associated with bivalve feeding could be sufficient to increase light penetration to a level that can sustain benthic microalgal production. Such microalgae absorb inorganic nutrients released from bivalve biodeposits at the sediment-water interface and provide an important food resource for benthic grazers.

## References

- AN, S., AND S. B. JOYE. 2001. Enhancement of coupled nitrification-denitrification by benthic photosynthesis in shallow estuarine sediments. *Limnol. Oceanogr.* **46**: 62–74.
- ASMUS, R. M., AND H. ASMUS. 1991. Mussel beds: Limiting or promoting phytoplankton? *J. Exp. Mar. Biol. Ecol.* **148**: 215–232.
- BAYNE, B. L., AND A. J. S. HAWKINS. 1992. Ecological and physiological aspects of herbivory in benthic suspension-feeding molluscs, p. 265–288. *In* D. M. John, S. J. Hawkins, and J. H. Price [eds.], *Plant-animal interactions in the marine benthos*. Systematics Association Special Volume No. 46. Clarendon.

- BOICOURT, W. C. 1992. Influences of circulation processes on dissolved oxygen in the Chesapeake Bay, p. 7–59. *In* D. E. Smith, M. Leffler, and G. Mackiernan [eds.], Oxygen dynamics in the Chesapeake Bay. Maryland Sea Grant Publication.
- CERCO, C. F., AND S. P. SEITZINGER. 1997. Measured and modeled effects of benthic algae on eutrophication in Indian River-Rohoboth Bay, Delaware. *Estuaries* **20**: 231–248.
- CHRISTENSEN, P. B., L. P. NIELSEN, J. SORENSEN, AND N. P. REVSBECH. 1990. Denitrification in nitrate-rich streams: Diurnal and seasonal variation related to benthic oxygen metabolism. *Limnol. Oceanogr.* **35**: 640–651.
- CLOERN, J. E. 1982. Does the benthos control phytoplankton biomass in South San Francisco Bay? *Mar. Ecol. Prog. Ser.* **9**: 191–202.
- COHEN, R. R. H., P. V. DRESLER, E. J. P. PHILIPS, AND R. L. CORY. 1984. The effect of the asiatic clam, *Corbicula fluminea*, on phytoplankton of the Potomac River, Maryland. *Limnol. Oceanogr.* **29**: 170–180.
- COOPER, S. R., AND G. R. BRUSH. 1993. A 2,500-year history of anoxia and eutrophication in Chesapeake Bay. *Estuaries* **16**: 617–626.
- CORNWELL, J. C., W. M. KEMP, AND T. M. KANA. 1999. Denitrification in coastal ecosystems: Methods, environmental controls, and ecosystem level controls, a review. *Aquat. Ecol.* **33**: 41–54.
- COWAN, J. L. W., AND W. R. BOYNTON. 1996. Sediment-water oxygen and nutrient exchanges along the longitudinal axis of Chesapeake Bay: Seasonal patterns, controlling factors and ecological significance. *Estuaries* **19**: 562–580.
- DAME, R. F. 1987. The net flux of inorganic sediments by an intertidal oyster reef. *Cont. Shelf. Sci.* **7**: 1421–1424.
- , N. DANKERS, T. C. PRINS, H. JONGSMA, AND A. C. SMAAL. 1991. The influence of mussel beds on nutrients in the western Wadden Sea and eastern Scheldt estuaries. *Estuaries* **14**: 130–138.
- , AND S. LIBES. 1993. Oyster reefs and nutrient retention in tidal creeks. *J. Exp. Mar. Biol. Ecol.* **171**: 251–258.
- , J. D. SPURRIER, AND R. G. ZINGMARK. 1992. In situ metabolism of an oyster reef. *J. Exp. Mar. Biol. Ecol.* **164**: 147–159.
- DESLOUS-PAOLI, J.-M., A.-M. LANNOU, P. GEAIRON, S. BOUGRIER, O. RAILLARD, AND M. HERAL. 1992. Effects of the feeding behaviour of *Crassostrea gigas* (bivalve molluscs) on biodegradation of natural particulate matter. *Hydrobiologia* **231**: 85–91.
- DOERING, P. H., C. A. OVIATT, AND J. R. KELLY. 1986. The effects of the filter-feeding clam *Mercenaria mercenaria* on carbon cycling in experimental marine mesocosms. *J. Mar. Res.* **44**: 839–861.
- EPPING, E. H. G., A. KHALILI, AND R. THAR. 1999. Photosynthesis and the dynamics of oxygen consumption in a microbial mat as calculated from transient oxygen microprofiles. *Limnol. Oceanogr.* **44**: 1936–1948.
- HATCHER, A., J. GRANT, AND B. SCHOFIELD. 1994. Effects of suspended mussel culture (*Mytilus* spp.) on sedimentation, benthic respiration and sediment nutrient dynamics in a coastal bay. *Mar. Ecol. Prog. Ser.* **115**: 219–235.
- HAVEN, D. S., AND R. MORALES-ALAMO. 1966. Aspects of biodeposition by oysters and other invertebrate filter feeders. *Limnol. Oceanogr.* **11**: 487–498.
- , AND ———. 1968. Occurrence and transport of faecal pellets in suspension in a tidal estuary. *Sediment. Geol.* **2**: 141–151.
- HENRIKSEN, K., AND W. M. KEMP. 1988. Nitrification in estuarine and coastal marine sediments, p. 205–249. *In* T. H. Blackburn and J. Sorensen [eds.], Nitrogen cycling in coastal marine environments. Wiley.
- HOLLAND, R. E. 1993. Changes in planktonic diatoms and water transparency in Hatchery Bay, Bass Island area, western Lake Erie since the establishment of the zebra mussel. *J. Gt. Lakes Res.* **19**: 717–724.
- JARAMILLO, E., C. BERTRAN, AND A. BRAVO. 1992. Mussel biodeposition in an estuary in southern Chile. *Mar. Ecol. Prog. Ser.* **82**: 85–94.
- JOHNSON, K. M., K. D. WILLS, D. BUTLER, W. K. JOHNSON, AND C. S. WONG. 1993. Coulometric total carbon dioxide analysis for marine studies: Maximizing the performance of an automated gas extraction system and coulometric detector. *Mar. Chem.* **44**: 167–188.
- JOYE, S. B., AND H. W. PAERL. 1994. Nitrogen cycling in microbial mats: Rates and patterns of denitrification and nitrogen fixation. *Mar. Biol.* **119**: 285–295.
- KANA, T. M., M. B. SULLIVAN, J. C. CORNWELL, AND K. GROSZKOWSKI. 1998. Denitrification in estuarine sediments determined by membrane inlet mass spectrometry. *Limnol. Oceanogr.* **43**: 334–339.
- KASPAR, H. F., P. A. GILLESPIE, I. C. BOYER, AND A. L. MACKENZIE. 1985. Effects of mussel aquaculture on the nitrogen cycle and benthic communities in Kenepuru Sound, Marlborough Sounds, New Zealand. *Mar. Biol.* **85**: 127–136.
- KAUTSKY, N., AND S. EVANS. 1987. Role of biodeposition by *Mytilus edulis* in the circulation of matter and nutrients in a Baltic coastal ecosystem. *Mar. Ecol. Prog. Ser.* **38**: 201–212.
- KEMP, W. M., AND W. R. BOYNTON. 1992. Benthic-pelagic interactions: Nutrient and oxygen dynamics, p. 149–221. *In* D. E. Smith, M. Leffler, and G. Mackiernan [eds.], Oxygen dynamics in the Chesapeake Bay. Maryland Sea Grant Publication.
- , P. A. SAMPOU, J. M. CAFFREY, M. S. MAYER, K. HENRIKSEN, AND W. R. BOYNTON. 1990. Ammonium recycling versus denitrification in Chesapeake Bay sediments. *Limnol. Oceanogr.* **35**: 1545–1563.
- KOIKE, I., AND J. SORENSEN. 1988. Nitrate reduction and denitrification in marine sediments, p. 251–273. *In* T. H. Blackburn and J. Sorensen [eds.], Nitrogen cycling in coastal marine environments. Wiley.
- KRISTENSEN, E. 1988. Benthic fauna and biogeochemical processes in marine sediments: Microbial activities and fluxes, p. 275–299. *In* T. H. Blackburn and J. Sorensen [eds.], Nitrogen cycling in coastal marine environments. Wiley.
- MALONE, T. C. 1992. Effects of water column processes on dissolved oxygen, nutrients, phytoplankton and zooplankton, p. 61–112. *In* D. E. Smith, M. Leffler, and G. Mackiernan [eds.], Oxygen dynamics in the Chesapeake Bay. Maryland Sea Grant Publication.
- MILLER, D. C., R. J. GEIDER, AND H. L. MACINTYRE. 1996. Microphytobenthos: The ecological role of the “secret garden” of unvegetated, shallow-water marine habitats. II. Role in sediment stability and shallow-water food webs. *Estuaries* **19**: 202–212.
- NEDWELL, D. B., AND S. AZIZ. 1980. Heterotrophic nitrogen fixation in an intertidal salt marsh sediment. *Estuar. Coast. Mar. Sci.* **10**: 699–702.
- NEWELL, R. I. E. 1988. Ecological changes in Chesapeake Bay: Are they the result of overharvesting the eastern oyster (*Crassostrea virginica*)?, p. 536–546. *In* M. P. Lynch and E. C. Krome [eds.], Understanding the estuary: Advances in Chesapeake Bay research. Chesapeake Research Consortium Publication 129.
- , AND S. J. JORDAN. 1983. Preferential ingestion of organic material by the American Oyster *Crassostrea virginica*. *Mar. Ecol. Prog. Ser.* **13**: 47–53.

- NIELSEN, L. P. 1992. Denitrification in sediment determined from nitrogen isotope pairing. *FEMS Microbiol. Ecol.* **86**: 357–362.
- OFFICER, C. B., T. J. SMAYDA, AND R. MANN. 1982. Benthic filter feeding: A natural eutrophication control. *Mar. Ecol. Prog. Ser.* **9**: 203–210.
- PARSONS, T. R., Y. MAITA, AND C. M. LALLI. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon.
- PELEGRI, S. P., L. P. NIELSEN, AND T. H. BLACKBURN. 1994. Denitrification in estuarine sediment stimulated by the irrigation activity of the amphipod *Corophium volutator*. *Mar. Ecol. Prog. Ser.* **105**: 285–290.
- PORTER, E. T. 1999. Physical and biological scaling of benthic-pelagic coupling in experimental ecosystem studies. Doctoral dissertation. University of Maryland, College Park.
- PRINS, T. C., V. ESCARAVAGE, A. C. SMAAL, AND J. C. H. PEETERS. 1995. Nutrient cycling and phytoplankton dynamics in relation to mussel grazing in a mesocosm experiment. *Ophelia* **41**: 289–315.
- , AND A. C. SMAAL. 1990. Benthic-pelagic coupling: The release of inorganic nutrients by an intertidal bed of *Mytilus edulis*, p. 89–103. In M. Barnes and R. N. Gibson [eds.], *Trophic relationships in the marine environment*. Aberdeen Univ. Press.
- RIEMANN, B., T. G. NIELSEN, S. J. HORSTED, P. K. BJØRNSSEN, AND J. POCK-STEEN. 1988. Regulation of phytoplankton biomass in estuarine enclosures. *Mar. Ecol. Prog. Ser.* **48**: 205–215.
- RISGAARD-PETERSEN, N., S. RYSGAARD, L. P. NIELSEN, AND N. P. REVSBECH. 1994. Diurnal variation of denitrification and nitrification in sediments colonized by benthic microphytes. *Limnol. Oceanogr.* **39**: 573–579.
- RODHOUSE, P. G., AND C. M. RODEN. 1987. Carbon budget for a coastal inlet in relation to intensive cultivation of suspension-feeding bivalve molluscs. *Mar. Ecol. Prog. Ser.* **36**: 225–236.
- RYSGAARD, S., P. B. CHRISTENSEN, AND L. P. NIELSEN. 1995. Seasonal variation in nitrification and denitrification in estuarine sediment colonized by benthic microalgae and bioturbating infauna. *Mar. Ecol. Prog. Ser.* **126**: 111–121.
- , N. RISGAARD-PETERSEN, N. P. SLOTH, K. JENSEN, AND L. P. NIELSEN. 1994. Oxygen regulation of nitrification and denitrification in sediments. *Limnol. Oceanogr.* **39**: 1643–1652.
- SEITZINGER, S. P. 1988. Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. *Limnol. Oceanogr.* **33**: 702–724.
- , L. P. NIELSEN, J. CAFFREY, AND P. B. CHRISTENSEN. 1993. Denitrification measurements in aquatic sediments: A comparison of three methods. *Biogeochemistry* **35**: 235–260.
- SLOTH, N. P., H. BLACKBURN, L. S. HANSEN, N. RISGAARD-PETERSEN, AND B. A. LOMSTEIN. 1995. Nitrogen cycling in sediments with different organic loadings. *Mar. Ecol. Prog. Ser.* **116**: 163–170.
- SOKAL, R. R., AND F. J. ROHLF. 1981. *Biometry*, 2nd ed. Freeman.
- SOUCHU, P., A. VAQUER, Y. COLLOS, S. LANDREIN, J.-M. DESLOUS-PAOLI, AND B. BIBENT. 2001. Influence of shellfish farming activities on the biogeochemical composition of the water column in Thau lagoon. *Mar. Ecol. Prog. Ser.* **218**: 141–152.
- STRAYER, D. L., N. F. CARACO, J. J. COLE, S. FINDLAY, AND M. L. PACE. 1999. Transformation of freshwater ecosystems by bivalves: A case study of zebra mussels in the Hudson River. *Bioscience* **49**: 19–27.
- SUNDBÄCK, K., V. ENOKSSON, W. GRANIELI, AND K. PETTERSSON. 1991. Influence of sublittoral microphytobenthos on the oxygen and nutrient flux between sediment and water: A laboratory continuous-flow study. *Mar. Ecol. Prog. Ser.* **74**: 263–279.
- , AND W. GRANIELI. 1988. Influence of microphytobenthos on the nutrient flux between sediment and water: A laboratory study. *Mar. Ecol. Prog. Ser.* **43**: 63–69.
- , A. MILES, AND E. GORANSSON. 2000. Nitrogen fluxes, denitrification and the role of microphytobenthos in microtidal shallow-water sediments: An annual study. *Mar. Ecol. Prog. Ser.* **200**: 59–76.
- SWANBERG, I. L. 1991. The influence of the filter-feeding bivalve *Cerastoderma edule* L. on microphytobenthos: A laboratory study. *J. Exp. Mar. Biol. Ecol.* **151**: 93–111.
- TENORE, K. R., AND OTHERS. 1982. Coastal upwelling in the Rias Bajas, NW Spain. Contrasting the benthic regimes of the Rias de Arosa and de Muros. *J. Mar. Res.* **40**: 701–768.
- VAN HEUKELEM, L., A. J. LEWITUS, T. M. KANA, AND N. E. CRAFT. 1994. Improved separations of phytoplankton pigments using temperature-controlled high performance liquid chromatography. *Mar. Ecol. Prog. Ser.* **114**: 303–313.
- WIDDOWS, J., M. D. BRINSLEY, P. N. SALKELD, AND M. ELLIOTT. 1998. Use of annular flumes to determine the influence of current velocity and bivalves on material flux at the sediment-water interface. *Estuaries* **21**: 552–559.
- YAMAMURO, M., AND I. KOIKE. 1993. Nitrogen metabolism of the filter-feeding bivalve *Corbicula japonica* and its significance in primary production of a brackish lake in Japan. *Limnol. Oceanogr.* **38**: 997–1007.

Received: 21 August 2001

Accepted: 16 April 2002

Amended: 12 June 2002