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Effect of organic loading on nitrification and denitrification in a marine sediment microcosm

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Abstract: The effects of organic additions on nitrification and denitrification were examined in sediment microcosms. The organic material, heat killed yeast, had a C/N ratio of 7.5 and was added to sieved, homogenized sediments. Four treatments were compared: no addition (control), 30 g dry weight (dw) m^{-2} mixed throughout the 10 cm sediment column (30M), 100 g dw m^{-2} mixed throughout sediments (100M), and 100 g dw m^{-2} mixed into top 1 cm (100S). After the microcosms had been established for 7–11 days, depth of O_2 penetration, sediment-water fluxes and nitrification rates were measured. Nitrification rates were measured using three different techniques: N-serve and acetylene inhibition in intact cores, and nitrification potentials in slurries. Increased organic additions decreased O_2 penetration from 2.7 to 0.2 mm while increasing both O_2 consumption, from 30 to 70 mmol O_2 m^{-2} d^{-1}, and NO_3^- flux into sediments. Nitrification rates in intact cores were similar for the two methods. Highest rates occurred in the 30M treatment, while the lowest rate was measured in the 100S treatment. Total denitrification rates (estimated from nitrification and nitrate fluxes) increased with increased organic addition, because of the high concentrations of NO_3^- (40 μM) in the overlying water. The ratio of nitrification: denitrification was used as an indication of the importance of nitrification as the NO_3^- supply for denitrification. This ratio decreased from 1.55 to 0.05 with increased organic addition.

Key words: Benthic fluxes; Nitrification; Organic loading; Microcosm; Denitrification

Introduction

Eutrophication, an increasing problem in many coastal ecosystems, has resulted in increased phytoplankton production [1,2] and led to increased organic inputs to the sediment from phytoplankton deposition [3]. The effects of organic loading on sediment O_2 consumption and NH_4^+ fluxes have been widely studied. Increased organic loading increases O_2 consumption, sediment NH_4^+ regeneration and NH_4^+ fluxes from the sediment [3–7]. In contrast, the effects of organic loading on nitrification and denitrification are not well understood. Nitrification, the oxidation of NH_4^+ to NO_2^- and NO_3^-, depends on both NH_4^+ and O_2 concentrations. In many marine sediments, nitrification is limited by O_2 concentrations [8,9], since O_2 penetration may be only several millimeters [10]. Denitrification, the reduction of NO_3^- to N_2, is controlled by organic carbon and

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NO_3^- concentrations where O_2 is absent. When NO_3^- concentrations in the overlying water are low, denitrification is dependent on NO_3^- supplied by nitrification in the sediments [11]. Increased denitrification associated with nutrient loading has been measured in estuarine mesocosms [12,13]. However, the direct effects of organic loading on nitrification and denitrification have not been studied.

Increased organic loading and associated decreased O_2 availability may have a significant impact where coupled nitrification–denitrification occurs. In Chesapeake Bay, nitrification and denitrification cease in the summer due to bottom water anoxia from the breakdown of the spring phytoplankton bloom [14]. Relationships between nitrification and denitrification under eutrophic conditions have been modelled and suggest that nitrification and denitrification may be reduced with high organic loads and low concentrations of NH_4^+ or NO_3^- in the water column [15].

The hypotheses for these experiments are as follows: (1) increased organic loading will increase NH_4^+ production (NH_4^+ supply to sediments) and increase O_2 consumption (decreasing O_2 concentrations and the depth of O_2 penetration). (2) Nitrification may initially be enhanced by increased NH_4^+ supply, but should decrease at 'high' loading rates because of reduced O_2 supply. (3) Denitrification may increase from increased organic carbon, but denitrification coupled to nitrification should decrease at 'high' loading rates when nitrification is inhibited. These hypotheses were tested using three different amounts of organic addition (which were determined based on a pilot experiment): no addition, low addition and high addition. We also believed that the way the organic material was added to sediments could be important, whether added to the surface or mixed throughout the sediment column. Organic matter mixed throughout the sediment core would simulate a sediment with high bioturbation, with animals present which could mix depositing organic matter through the sediment column, while the addition to the surface would simulate a situation with low bioturbation.

Methods

Pilot experiment

To select the appropriate organic additions, a preliminary experiment was performed with six different additions: 20, 40, 60, 80, 100, and 120 g dry weight (dw) m^{-2} with two experimental treatments: mixed (M), where the organic material was mixed throughout the 8 cm sediment column, and surface (S), where organic material was mixed into the top 1 cm layer. Baker's yeast dried at 105°C was used as the organic substrate. The yeast, which were intact cells, had a C/N ratio of 7.5 which was similar to a phytoplankton C/N ratio of 6.6.

Sediment was collected from a sandy, intertidal area of the Limfjorden, Denmark in January and sieved through a 0.5 mm mesh to remove macrofauna. Core tubes (5.2 cm diameter) were filled with approximately 320 g of sieved wet sediment mixed with the appropriate organic additions. Because this was a pilot experiment, there was one core per addition per treatment, so a greater variety of additions could be made. Cores were placed in a reservoir with aerated, flowing seawater with water temperature between 15–18°C. The overlying water height was about 8 cm. Each tube was fitted with a magnetic stirrer to ensure mixing with the water in the reservoir.

Benthic fluxes of CO_2, O_2, NH_4^+ and NO_3^- were measured in the cores on day 5, 9, 13, 20 and 27 after the microcosm was set up. Water (60 ml) was removed without replacement for an initial measurement, then cores were capped with stoppers for the incubation and final samples were taken after 3–4 h. Regression analysis was done to determine the relationship between flux and organic addition [16].

Oxygen was analyzed by the Winkler method, CO_2 by the Gran titration [17], NH_4^+ was determined manually by the salicylate-hypochlorite technique [18], NO_2^- was also analyzed manually [17] and NO_3^- by the automated cadmium reduction method [17]. Oxygen profiles in sediments were made in control, 80 and 120 treatments at day 25 with O_2 microelectrodes. Electrodes were calibrated prior to measurements in air saturated water (100%) and anaerobic sediment (0%) [10].

Potential nitrification measurements of the 0–1 cm depth layer were made on day 27 following measurement of benthic fluxes. Sediment slurries (2 g wet sediment in 40 ml filtered Limfjorden water) were incubated for 8 h with 500 μM NH_4^+ at 25°C [19]. Slurries were incubated in centrifuge tubes with an aerobic headspace and agitated. NO_3^- concentrations in the water were about 40 μM, so chlorate was added to block NO_2^- oxidation to NO_3^-. A small volume (10 ml) was removed at 2 h intervals for NO_2^- analysis. Potential nitrification rates were calculated from NO_2^- production in the slurries.

Principal experiment

Four experimental treatments were chosen for the second experiment: control (no organic additions), 30 g dw m^{-2} mixed (30M), 100 g dw m^{-2} mixed (100M) and 100 g dw m^{-2} surface addition (100S). Sediment was collected in March, 1991 from the same area of the Limfjorden as in the pilot experiment and sieved through a 0.5 mm screen. The sediment was mixed and packed in acrylic boxes ($22 \times 30 \times 12$ cm) to a depth of approximately 10 cm. The yeast was dried at 105°C for at least 14 h and then rehydrated and mixed into the sediment. For the surface treatment, 200 ml of sediment and yeast were mixed and added to the surface, which represented an organic addition to the top 0.5 cm layer.

Boxes filled with sieved sediment were placed in a large reservoir of water about 4–5 cm from the water surface. Water in this reservoir was aerated and circulated to flow gently over the sediment surface. Temperature was controlled with a cooling system to 15 ± 2°C. Salinity was about 26 ‰. Based on the information collected from the pilot experiment, experiments were started when benthic fluxes were estimated to be high and relatively stable. The results from the pilot experiments suggested that the high organic addition treatments should be sampled between 7–14 days after the additions were made, while the low organic additions were stable over a 3 week period. One week after the boxes were set up, O_2 profiles were measured in situ. Then, boxes were subcored to measure benthic fluxes, nitrification, and potential nitrification. Boxes 100M and 100S were subcored at day 9, while control and 30M boxes were subcored at day 12. Cores were removed and incubated at 15°C.

Oxygen profiles were measured in each box with O_2 microelectrodes. Five profiles were taken at 5 cm intervals along the long axis of the box. Oxygen was measured at 0.05 mm depth intervals. An analysis of variance (ANOVA) was carried out to test for differences in oxygen consumption between microelectrode and cores measurements and for differences among the different treatments [16].

All fluxes were measured in four replicate cores (5.2 cm diameter) from each treatment. Cores were stirred with a magnetic stirring system during flux incubations. The first samples for the flux measurements were taken 1 h after the cores were sealed and stirring began. A supply of water from the reservoir was used to replace the 60 ml of water removed from the flux cores. The replacement water was thoroughly mixed with the water in the cores by gently filling and emptying the syringe several times while collecting the overflow from the sealed core in a 60 ml syringe during the mixing. Samples were also taken of the replacement water and concentrations in the cores were corrected for dilution. Four samples were taken at 2 h intervals for 100M and 100S treatments and 3 h intervals for control and 30M. Analyses of O_2, and NO_3^- were performed as described above.

Nitrification was measured in intact cores by two methods: N-serve inhibition [19] and acetylene inhibition [20]. For the N-serve method, 10 cores (2.5 cm i.d.) from each treatment were incubated for 2 days. Half of the cores had 15 and 8 μl of N-serve (10 mg/ml dissolved in acetone) injected to the overlying water (30 ml volume) and the top 2 cm of sediment, respectively, to give a final N-serve concentration of 5 ppm. The overlying water was stirred with gentle aeration, which also kept O_2 concentrations close to saturation. Overlying water was replaced each day and fresh N-serve was added to the water. Ammonium concentrations were measured in overlying water and in the 0–3 cm sediment layer which was extracted in 25 ml of 2 M NaCl. Nitrification was calculated based on the differ-

ence between NH_4^+ production in control and N-serve treated cores. For the acetylene inhibition method, 8 cores (5.2 cm i.d.) were incubated for 8 h. To balance O_2 consumption by the sediment in the different treatments, a headspace enriched with oxygen was established, with 26% O_2 in control and 30M, and 41% in 100M and 100S cores. Samples for NH_4^+ were taken every hour and frozen for later analysis. Samples for O_2 were taken at the beginning and end of the incubation to monitor oxygen concentrations in the water overlying the sediment, and analyzed by Winkler titration. Acetylene was added to 5 cores after 4 h to inhibit nitrification. Three cores were left uninhibited to check the stability of the NH_4^+ flux. Flux rates were calculated by linear regression of the NH_4^+ accumulation in the overlying water in individual cores before and after inhibition. Nitrification rates were calculated by subtraction of NH_4^+ fluxes before inhibition from rates after inhibition. Results from the two methods were compared with ANOVA [16]. Potential nitrification was measured in the 0–0.5 cm layer as described above [19].

Denitrification rates were calculated by subtracting the NO_3^- fluxes from nitrification rates. Assumptions associated with these calculations are: 1) that no NO_3^- was reduced to NH_4^+; 2) that NO_3^- pools in the sediments were not changing over the course of the flux incubation.

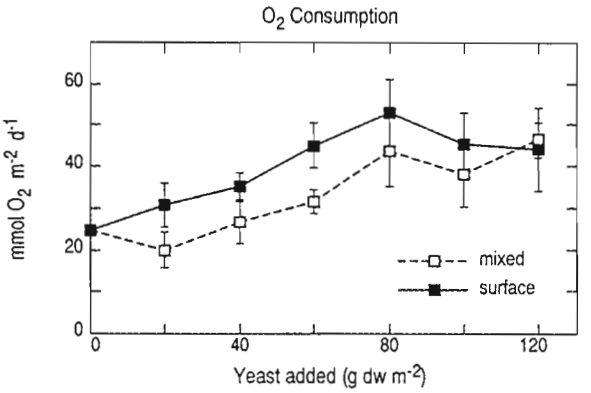

Fig. 1. Oxygen consumption (mmol O_2 m^{-2} d^{-1}) in the pilot experiment at different organic additions (g dw m^{-2}) with organic material added to surface or mixed throughout sediment. Results presented as mean and standard error of five flux measurements.

Results and Discussion

Pilot experiment

O_2 consumption increased with increased organic addition in surface (S; $y = 26.5 + 0.22\ x$,

Table 1

Depth of O_2 penetration and O_2 consumption from the pilot experiment measured at the days indicated following setup of microcosm

Treatment	O_2 penetration Depth (mm)		O_2 consumption (mmol O_2 m^{-2} d^{-1})		
Days following setup:	5	24	20 [a]	24 [b]	27 [a]
Control	0.90	0.94	41	48 (9)	26
40M	n.d.	0.81	45	32 (5)	22
80M	n.d.	0.37	42	63 (5)	48
120M	n.d.	0.30	58	76 (12)	40
40S	n.d.	0.60	35	49 (20)	n.d.
80S	0.33	1.10	39	36 (9)	42
120S	n.d.	0.36	n.d.	63 (17)	11

[a] measured by single core incubation.
[b] calculated from O_2 profile, mean of four profiles (±S.D.).
n.d. no data.

$P < 0.01$) and mixed (M; $y = 17.9 + 0.24\ x$, $P < 0.001$) treatments (Fig. 1); rates in the surface treatments were often higher than mixed treatments. The control core had a flux of 25 mmol O_2 m^{-2} d^{-1}, while the 80S had the highest O_2 consumption, 52 mmol O_2 m^{-2} d^{-1}. O_2 consumption in the high surface additions (80S, 100S, 120S) peaked 9–13 days after setup and declined rapidly, while mixed treatments generally began to decline after 20 days (data not shown). O_2 penetration in sediments was greatest in the control, intermediate in the 40M and 40S treatments, and least in the high treatments (Table 1). In the 80S treatment, O_2 penetration increased from 0.33 mm 13 days after setup to 1.1 mm 24 days after set up (Table 1). This pattern is consistent with decreases in O_2 consumption in surface treatments over this period.

Nitrate fluxes were usually into the sediment. The response of the surface addition treatments was similar to that found for O_2 consumption; NO_3^- uptake by the sediment was higher at higher organic additions ($y = -1.62 - 0.05\ x$; $P < 0.001$; Fig. 2). This was not true for the mixed treatments ($y = -1.57 - 0.02\ x$; $P < 0.13$), where uptake only increased at the 100 and 120 additions (Fig. 2). Nitrification potential ranged from 40 to 100 nmol cm^{-3} h^{-1} (Fig. 3a). In contrast to the O_2 and NO_3^- fluxes, nitrification potential increased at intermediate organic additions (20–80 for mixed, 40–60 for surface) and then decreased at the higher additions. Potential nitrification rates in surface treatments were lower than mixed treatments except at 60 and 120 g dw m^{-2} additions (Fig. 3a).

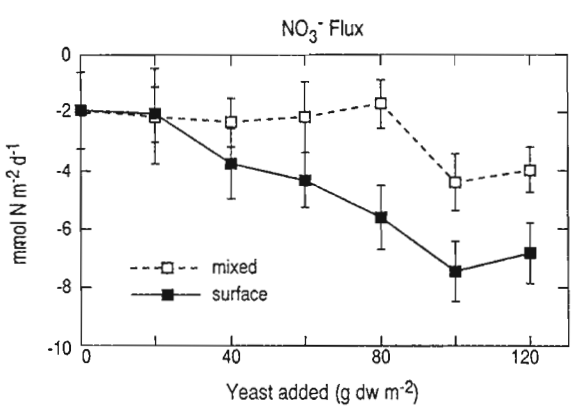

Fig. 2. Nitrate flux (mmol N m^{-2} d^{-1}) in the pilot experiment at different organic additions (g dw m^{-2}) for surface and mixed treatments (positive fluxes are out of sediment, negative fluxes are into sediment). Results presented as mean and standard error of five flux measurements.

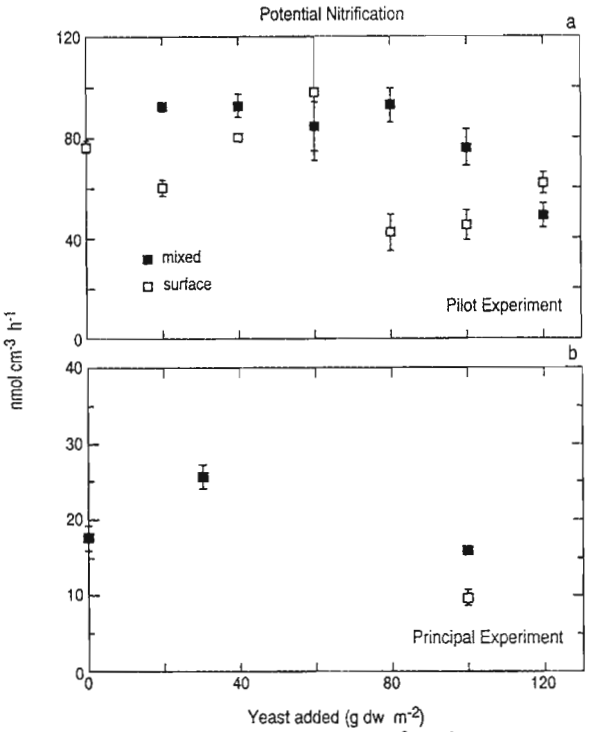

Fig. 3. Nitrification potential (nmol cm^{-3} h^{-1}) at different organic additions (g dw m^{-2}) in surface and mixed treatments a) pilot experiment ($n = 2$) b) principal experiment ($n = 4$). Results are presented as mean and standard error.

Principal experiment

Oxygen consumption and nitrate fluxes

The control and 30M boxes both had a light brown color (indicating the presence of iron oxyhydroxides) extending to depth of 10 mm in the sediments. The high treatments were much more reduced, with the light brown color extending to 5 mm in the 100M and only about 0.5 mm in the 100S. These color changes were mirrored in the depths of O_2 penetration in the different treatments. Depth of O_2 penetration decreased an order of magnitude at the high organic addition rates from 2.7 mm in control and 30M to 0.39 mm

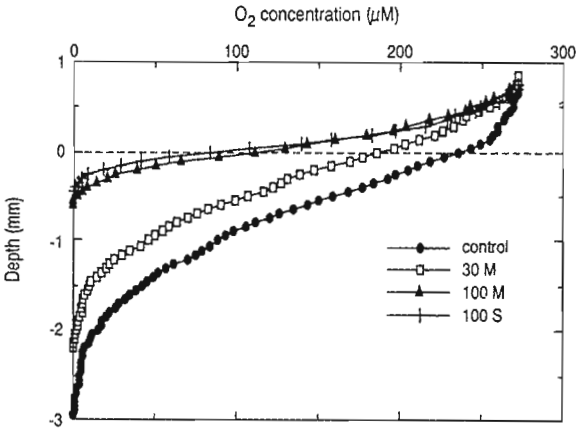

Fig. 4. Selected O_2 profiles measured with O_2 microelectrodes at 4 different treatments: no addition (control), 30 g dw m^{-2} mixed (30M), 100 mixed (100M), and 100 surface (100S).

and 0.19 mm in 100M and 100S treatments, respectively (Fig. 4).

Oxygen consumption was similar in the control and 30M treatments, about 30 mmol O_2 m^{-2} d^{-1}. Rates were much higher in the 100M and 100S treatments, about 70 mmol O_2 m^{-2} d^{-1} (Table 2). Oxygen fluxes were also calculated from the O_2 profiles and were not significantly different from the fluxes measured in core incubations ($P < 0.31$), although the different treatments were significantly different ($P < 0.001$). NO_3^- flux was near zero in the control treatments, while NO_3^- flux into the sediments increased with increased organic addition (Table 2). NO_3^- uptake by sediments was the greatest in the 100S treatment.

Increased O_2 consumption and NO_3^- flux into sediments at the higher organic additions in the principal experiment were consistent with the results observed in the pilot experiment. There was good agreement between the measurement of O_2 consumption by benthic fluxes and that calculated from O_2 profiles in the principal experiment, while there were some discrepancies in the pilot experiment. Differences between measurements in cores and from profiles were greatest at the higher additions. The differences between the two techniques in the pilot experiment may have been due to uneven distribution of yeast within the sediments and accumulation of material in the center of the cores below the stirring magnet. In the principal experiment, organic matter was evenly distributed throughout the boxes and water flow over the sediment surface did not affect this distribution.

These organic additions are similar to those used in other experiments [4,7,21], but are much higher than 'typical', estuarine sedimentation events. Sedimentation rates range from 0.1 to 1.6 g C m^{-2} d^{-1} in estuaries [22,23]. Organic additions in the principal experiment (30M and 100M) represent between 10 and 30 days worth of deposition to sediments, at the high sedimentation rates. Oxygen consumption, and the increase in O_2 consumption with addition, was similar to organic loading with macroalgae [7, 21], but higher than those reported for Narragansett Bay sediments supplemented with particulate organic material [4]. The response of the microbial community in the sediments with these yeast additions

Table 2

Oxygen consumption and NO_3^- fluxes from core incubations, O_2 consumption calculated from O_2 profiles, and depth of O_2 penetration in sediment microcosms with different organic additions. Results presented as means and standard errors (n = 4 for cores, n = 5 for profiles)

Treatment	O_2 consumption		NO_3^- fluxes	O_2 penetration (mm)
	Cores (mmol m^{-2} d^{-1})	Profiles (mmol m^{-2} d^{-1})	Cores (mmol m^{-2} d^{-1})	
Control	35 (5)	24 (7)	0.9 (5.3)	2.70
30M	29 (10)	25 (4)	−1.9 (2.4)	1.90
100M	73 (25)	47 (19)	−7.1 (3.9)	0.39
100S	69 (10)	69 (24)	−8.2 (7.2)	0.19

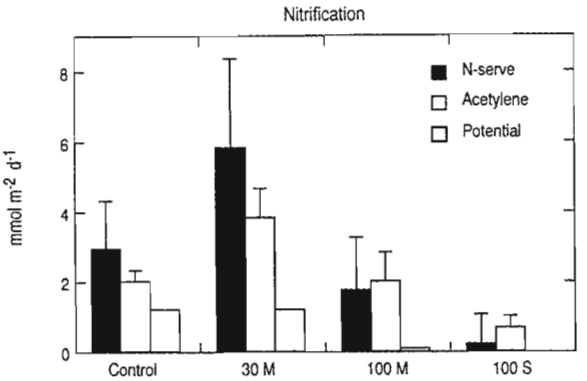

Fig. 5. Nitrification rates (mmol N m^{-2} d^{-1}) at 4 different treatments: no addition, 30 g dw m^{-2} mixed, 100 mixed, and 100 surface, measured using N-serve and acetylene inhibition techniques in intact cores and nitrification potentials in sediment slurries, mean and standard error ($n = 5$).

was very similar to experiments performed with other types of organic material: rapid increase in O_2 consumption or NH_4^+ efflux, with a peak around 1 week after addition and then a slow decline in rate [6,7,21]. The C/N ratio for yeast (7.5) is similar to that of phytoplankton (6.6); as was the POM (6.8–10) [4,5,23] and macroalgae (5.6–9.7) [7,21], which have been used for other organic loading experiments.

Nitrification rates

Nitrification was enhanced in the 30M treatment over the control, but decreased at the higher treatments. Rates of nitrification measured with N-serve inhibition ranged from a low of 0.23 mmol N m^{-2} d^{-1} in 100S treatment to 5.81 mmol N m^{-2} d^{-1} in the 30M treatment (Fig. 5) and were significantly different ($P < 0.005$) between the different treatments. The acetylene method gave slightly lower nitrification rates than the N-serve method, although rates measured by the two methods were not significantly different ($P < 0.48$). Oxygen concentrations in the acetylene treated cores were within 10% of the start concentration at the end of the experiment: +3% in control, −6% in 30M, +8% in 100M and −1% in 100S cores. There was no significant change in the NH_4^+ fluxes in the control cores during the incubation.

The response in the potential nitrification slurries was similar to nitrification rates measured in intact cores (Fig. 3b). Potential nitrification was highest in the 30M treatment and decreased with increased organic addition. The surface treatment (100S) potential nitrification was less than the mixed treatment (100M), which was similar to the results observed in the pilot experiment. Although these patterns were the same in the pilot and principal experiments, the absolute rates in the principal experiment were about 1/4 those of the pilot. Perhaps this was because the 4 weeks duration of the experiment allowed nitrifying bacterial populations time to grow.

The enhancement of nitrification at intermediate amount of organic addition (30M treatment) supports the hypothesis that low organic addition may stimulate nitrification due to increased NH_4^+ supply. Porewater NH_4^+ concentrations, as well as NH_4^+ flux out of sediments increased with increasing additions (pilot experiment, not shown). Depression of nitrification at high additions, particularly the surface treatment (100S) even with high porewater NH_4^+ concentrations, suggests that in this situation O_2 supply is controlling the process. Nitrification rates in intact cores from the 100S treatment were about 20% the rate of 100M treatment, while O_2 penetration only decreased by 50% between these two treatments. Nitrifying bacteria are inhibited by sulfide [9], and sulfide concentrations were probably higher closer to the sediment-water interface in the surface treatments. The presence of patches of a white, filmy layer on the surface of 100S cores suggests that *Beggiatoa*, which oxidizes sulfide, was present.

N-serve and acetylene techniques gave very similar results; and nitrification rates were not significantly different ($P < 0.48$). There was lower variability with the acetylene method because rates of nitrification were calculated for individual cores and the variable NH_4^+ sediment pools were not included in the calculations. Another advantage of the acetylene technique is that the short incubation time (8 h) does not allow much NH_4^+ accumulation in the water phase, which could stimulate nitrification. Although acetylene and N-serve are effective at inhibiting nitrifica-

tion, they also inhibit other processes as well, particularly acetylene which can inhibit denitrification, methanogenesis, methane oxidation, and dissimilatory NO_3^- reduction to NH_4^+ [24]. Acetone, used to dissolve the N-serve, is a carbon source to sediments and inhibits nitrification [24]. Inhibition of dissimilatory NO_3^- reduction to NH_4^+ by acetylene may explain why the nitrification rates from the acetylene method were slightly lower than the N-serve method. However, these differences were not significant, suggesting that rates of dissimilatory NO_3^- reduction to NH_4^+ were low. Nitrification rates were also calculated from nitrification potentials and O_2 penetration [19]. Rates ranged from 0.1 and 0.0 mmol N m^{-2} d^{-1} in 100M and 100S treatments to 0.8 and 1.2 mmol N m^{-2} d^{-1} in control and 30M treatments, respectively (Fig. 5). The rates measured in intact sediments were 3–5 times greater. One possible explanation for this difference is that the potential measurement from the 0–5 mm layer may underestimate the nitrification activity if the bacteria are concentrated in the top 2 mm oxic layer.

Dentrification

Denitrification rates were high in these experiments, particularly at the high addition treatments (Table 3). High NO_3^- concentrations in the overlying water (40 μM) combined with the organic carbon inputs were able to support high denitrification rates in the sediments. Nitrification as a source of NO_3^- for denitrifying bacteria became much less important at the high additions because nitrification was restricted by lack of O_2. The ratio of nitrification to denitrification provides an indication of the importance of different NO_3^- sources for denitrification. Ratios greater than or equal to 1 suggest that nitrification is the source of NO_3^- for denitrification, while some external supply of NO_3^- is required when the ratio is less than 1. The ratio of nitrification: denitrification declined from 1.55 in the control to about 0.05 in the 100S (Table 3). We assumed that reduction of NO_3^- to NH_4^+ was insignificant, although this may not be correct, especially for the high organic addition. If this process were significant in these sediment, our denitrification rates would be overestimated. Dissimilatory NO_3^- reduction to NH_4^+ is thought to be highest at low NO_3^- and high organic carbon concentrations [25]. Since NO_3^- concentrations were high (40 μM) in the overlying water for these experiments, dissimilatory NO_3^- reduction to NH_4^+ may not have been very significant in these sediments.

A similar relationship between oxygen, nitrification and denitrification has been observed in Chesapeake Bay [14]. Nitrification and denitrification declined in the spring and summer as bottom O_2 concentrations decreased. The responses by nitrifiers and denitrifiers were very different in mesocosms with NH_4^+ loading to the overlying water [12]. Both nitrification and denitrification increased with increased NH_4^+ loading. Denitrification: nitrification was greater than 1, except at the highest loading rate where it was 0.68. Apparently, increased organic production and sedimentation in these systems did not affect depth of O_2 penetration in the sediment as it did in our system.

Differences between surface and mixed additions of organic material were greatest for nitrification, and least for O_2 or NO_3^- fluxes, or depth of O_2 penetration. This model system has demonstrated that organic loading has a signifi-

Table 3

Denitrification rates (mmol N m^{-2} d^{-1}) and ratio of nitrification:denitrification (N/D) at different organic additions

Treatment	Total denitrification [a] (mmol N m^{-2} d^{-1})	N/D ratio
Control	1.6	1.55
30M	6.8	0.71
100M	8.9	0.21
100S	8.6	0.05

[a] calculated from nitrification rates (mean of N-serve and acetylene rates) and nitrate fluxes.

cant impact on both nitrification and denitrification. In this high NO_3^- system, denitrification was enhanced at the higher additions. With low NO_3^- concentrations in the overlying water, denitrification would probably have been reduced at high organic additions.

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