

Coupled nitrification–denitrification in autotrophic and heterotrophic estuarine sediments: On the influence of benthic microalgae

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Abstract

Field data obtained from 18 European estuaries using the isotope pairing technique were analyzed for trends in relationship between activity of benthic microalgae and coupled nitrification–denitrification. Kruskal–Wallis tests and analyses of covariance performed on the field dataset showed strong statistical evidence for the hypothesis that sediments colonized by microalgae whose activity exceeds community respiration display lower rates of coupled nitrification–denitrification than do heterotrophic sediments. In fully heterotrophic sediments, 90% of the measurements fell within the range 0–92 $\mu\text{mol N m}^{-2} \text{ h}^{-1}$ with a median of 20.3 $\mu\text{mol N m}^{-2} \text{ h}^{-1}$. In highly autotrophic sediments, 90% of the measurements fell within the range 0–34 $\mu\text{mol N m}^{-2} \text{ h}^{-1}$, and the median was 4.2 $\mu\text{mol N m}^{-2} \text{ h}^{-1}$. The hypothesis was tested experimentally using ^{15}N and microsensor (NO_3^-) techniques in prepared microcosms with and without algal activity. The results of the experimental studies were consistent with the hypothesis derived from the field data analysis. For the ^{15}N study, coupled nitrification–denitrification in alga-colonized sediments was between 4 and 51% of the activity in sediments without algae activity, depending on the N load. For the microsensor study, there was no indication of net NO_3^- production in alga-colonized sediments before addition of NH_4^+ . In contrast, NO_3^- accumulated in the oxic zone of a similar alga-free sediment. The experiments furthermore showed that compared to heterotrophic sediment, the presence of active microalgae might reduce the population of nitrifying bacteria capable of having an active metabolism. These bacterial populations could display diurnal variations in activity correlated with the diurnal variations in O_2 penetration depth, however. The results showed that induction of nitrogen limitation of the nitrifying bacteria population is a major controlling mechanism of coupled nitrification–denitrification in alga-colonized sediments.

Benthic microalgae might be important primary producers in shallow estuaries, and it has been estimated that these organisms can be responsible for more than 50% of estuarine primary production (Underwood and Kromkamp 1999). Benthic microalgae are concentrated at the sediment–water interface, and this allows them to significantly affect the chemical microenvironment in the upper few millimeters of the sediment. Via their assimilatory N uptake, they can reduce nutrient concentrations in the sediment porewater (Lorenzen et al. 1998) while, via photosynthesis and respiration, they can cause large diurnal fluctuations in both O_2 and dissolved inorganic carbon concentrations in the superficial sediment strata, affecting both the pH and the redox potential in this zone (Revsbech et al. 1988). In addition, the algae might excrete part of their photosynthetic products (Smith and Underwood 2000), thus providing the heterotrophic bacteria community with an additional carbon source (Middelburg et al. 2000). Nitrification and denitrification are bacterially mediated processes central to the N cycle. Nitrification is the aerobic oxidation of NH_4^+ to NO_2^- and NO_3^- , whereas denitrification represents the anoxic reduction of NO_3^- or

NO_2^- to gaseous end products such as N_2 or N_2O . In aquatic sediments, the two processes are located in the superficial sediment strata. Nitrification takes place in the oxic zone, and denitrification occurs just below the oxic/anoxic interface (Jensen et al. 1993, 1994).

Coupling between the two processes results from the transport of nitrification products ($\text{NO}_2^-/\text{NO}_3^-$) across the oxic/anoxic boundary (Jenkins and Kemp 1984), which are subsequently denitrified and thus lost from the system. The coupling between the two processes implies a loss of remineralized N from the system and is therefore important to overall system productivity because only a limited number of marine organisms are able to utilize N_2 as nitrogen source (Howarth et al. 1988).

The physical location of the nitrification and denitrification processes close to the sediment surface implies that presence of benthic microalgae could influence the two bacterial processes and the coupling between them by altering the chemical conditions in the niches they occupy.

In a review on nitrification, Henriksen and Kemp (1988) presented experimental data showing that the potential for nitrification in the superficial sediment strata was reduced in sediments exposed to light/dark (LD) cycles for 6 weeks compared to sediments incubated in the dark. It was suggested that light and a combination of factors linked to algal activity (i.e., high O_2 concentration, high pH, induction of NH_4^+ and CO_2 limitation, and excretion of bacteriostatic products) was responsible for the observed inhibition. As a consequence of their observation, the authors proposed that this reduced ability of nitrifying bacteria to survive in alga-colonized sediment would lead to a reduced loss of nitrogen via coupled nitrification–denitrification from the system, as compared to sediment without benthic microalgae.

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On quantification of nitrification and denitrification in sediments colonized by benthic microphytes, Risgaard-Petersen et al. (1994), Rysgaard et al. (1995), Lorenzen et al. (1998), and An and Joye (2001) observed higher rates of coupled nitrification–denitrification in light than in dark. This stimulation was attributed to a photosynthetically mediated increase in O_2 penetration depth, leading to an increased nitrification activity (Risgaard-Petersen et al. 1994). In the light of these results, Jeppesen et al. (1998) and Van Luijn et al. (1995) proposed that invasion of benthic microalgae in lakes after a shift to a clear-water state would promote the loss of nitrogen via coupled nitrification–denitrification and thus accelerate the process of recovery from eutrophication—a statement that apparently contradicts the hypothesis of Henriksen and Kemp (1988). However, several field studies have indicated that light-induced stimulation is not a general phenomenon (Rysgaard et al. 1995; Cabrita and Brotas 2000; Sundbäck and Miles 2000), and it has been suggested that stimulation of coupled nitrification–denitrification in light only occurs when competition between algae and nitrifiers and denitrifiers for dissolved inorganic nitrogen (DIN) is not limiting the bacterial processes (Rysgaard et al. 1995; Dong et al. 2000). Yet, the overall effect of benthic microalgae on coupled nitrification remains unclear.

In the present study, I focus on the effect of benthic microalgae on nitrification and denitrification with a special reference to the coupling between the two processes. The question I address is whether sediments with significant microphytobenthic activity display higher or lower rates of coupled nitrification–denitrification than heterotrophic sediments. The investigation is based on analysis of field data from 18 European estuaries collected over the past 10 yr. In this dataset, denitrification activity was measured with the isotope pairing technique (Nielsen 1992)—a technique that allows quantification of and distinction between the dependency of denitrification on bottom-water NO_3^- and on NO_3^- produced by sedimentary nitrification, respectively.

Interpretation of results from analysis of nonexperimental data can be complicated because of interference from both measured and unmeasured covariables. Therefore, controlled laboratory experiments in experimental microcosms with and without active benthic microalgae were performed in order to falsify the hypothesis derived from analysis of the field dataset. Two types of microcosm experiments were performed: a ^{15}N -isotope study and a NO_3^- microsensor experiment. In the ^{15}N study, coupled nitrification–denitrification rates measured in cores made from homogenized sediment and exposed to either LD cycles and different NO_3^- loads or to darkness were compared in order to evaluate the influence of benthic primary production and the N load on nitrification and denitrification. In this experiment, coupled nitrification–denitrification was measured using techniques equivalent to those used in the various field programs in consideration. In the NO_3^- microsensor experiment, microprofiles of NO_3^- were measured with a NO_3^- biosensor (Larsen et al. 1997) in alga-free sediment cores and in cores inoculated with benthic microalgae. The latter profiles were measured before and after pulse addition of NH_4^+ to analyze whether benthic microalgae introduced N limitation of nitrifying bacteria.

Materials and methods

Field studies—The field data source: The dataset analyzed is a compilation of measurements from 18 European estuaries. Data originate from monitoring programs conducted by the National Environmental Research Institute (NERI), Denmark, and from the EU-funded Nitrogen Cycling In Estuaries (NICE) project (Table 1). The database consists of data on denitrification, DIN and O_2 fluxes, nutrient concentrations, temperature, and salinity, all measured with a standardized methodology as described by Dalsgaard et al. (2000b). In short, 6–10 intact sediment cores were collected in Plexiglas core tubes in the field during each sampling event. In the laboratory, the core tubes were immersed in a large tank with magnetic Teflon-coated bars, stirring the water column inside the tubes. Fluxes of nutrients, O_2 , and rates of denitrification were measured in light and in the dark at in situ temperature. In the case of the NERI dataset a standard irradiance ($100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) was used for all incubations (see Rysgaard et al. 1995). For this subset of data, there is unfortunately no information about in situ irradiance; therefore, it is not possible to evaluate how well the applied irradiance matched the field situation and thus how well, for instance, photosynthesis estimates reflect in situ activity. In the NICE dataset, irradiance was adjusted to in situ levels (see Sundbäck and Miles 2000). For this set of data, the reported irradiance ranged between 2 and 1,300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

Denitrification was measured by the ^{15}N isotope pairing technique (Nielsen 1992; Risgaard-Petersen and Rysgaard 1995) and separated into coupled nitrification–denitrification (Dn) and denitrification of bottom-water NO_3^- (DW) as described by Nielsen (1992). For a detailed description of the sampling and incubation procedures applied, see the primary sources listed in Table 1.

Statistical analysis of the field data: Statistical analysis of the data was performed on a subsection of the entire reported dataset. Data were selected according to the following criteria. Data from seagrass beds and macroalga-dominated areas were omitted from the analysis because the study focuses on interactions between microalgae and nitrification–denitrification. Only data where measurements were performed both in light and dark, where coupled nitrification–denitrification was ≥ 0 , and where rates measured in darkness were $< 50\%$ of the O_2 consumption rate were considered. Values outside these boundaries are not biologically possible and should therefore be considered as artifacts. When these criteria are applied, the dataset analyzed in the present study comprises 392 sampling events and 2,892 individual cores. A copy of the dataset is available on request.

In order to establish whether the relationship between the activity of benthic microalgae and coupled nitrification–denitrification was significant and at the same time facilitate an analysis of covariance, data were grouped according to the classification scheme for shallow sediments proposed by Rizzo et al. (1996). This scheme—the benthic trophic state index (BTSI)—operates with four different sediment types: (0) fully heterotrophic, (1) net heterotrophic, (2) net autotrophic, and (3) highly autotrophic. In the given context, BTSI

Table 1. Sampling site, number of stations, number of sampling events, year of investigation, and references for the entire field dataset. Note that the analysis is performed on a subset of this dataset (*see text*). DK: Denmark, I: Italy, P: Portugal, S: Sweden, UK: United Kingdom.

Site	Stations	Sampling events sta ⁻¹	Year	Program	Reference
Colne (UK)	5	12	1997–98	NICE	Dalsgaard et al. 2000a
Giralda (I)	1	12	1997–98	NICE	Bartoli et al. 2001; Viaroli et al. unpubl. data
Gorino (I)	1	12	1997–98	NICE	Bartoli et al. 2001; Viaroli et al. unpubl. data
Guldborg Sund (DK)	1	9	1994–95	NERI monitoring program	Dalsgaard unpubl. data
Horsens Fjord (DK)	4	6	1996	NERI monitoring program	Christensen et al. 2000
Kertinge Nor (DK)	4	9	1992	NERI monitoring program	Rysgaard et al. 1995
Kertinge Nor (DK)	2	8	1995	NERI monitoring program	Dalsgaard et al. 1999; Rysgaard unpubl. data
Odense Fjord (DK)	4	10	1995	NERI monitoring program	Risgaard-Petersen and Rysgaard unpubl. data
Ouse (UK)	4	12	1997–98	NICE	Dong et al. 2000
Pancas (P)	2	12	1997–98	NICE	Cabrita and Brotas 2000
Randers Fjord (DK)	7	10	1994–95	NERI monitoring program	Nielsen et al. 2001
Rosario (P)	2	12	1997–98	NICE	Cabrita and Brotas 2000
Rörtången (S)	2	12	1997–98	NICE	Sundbäck and Miles 2000
Skive (DK)	1	19	1993–94	NERI monitoring program	Dalsgaard et al. 1999
Smarlacca (I)	1	12	1997–98	NICE	Bartoli et al. 2001; Viaroli et al. unpubl. data
Ulbjerg (DK)	1	12	1997–98	NICE	Dalsgaard pers. comm.
Vadehavet (DK)	2	10	1997–98	NERI monitoring program	Christensen and Pedersen unpubl. data
Vallda (S)	2	12	1997–98	NICE	Sundbäck and Miles 2000
Virksund (DK)	1	12	1997–98	NICE	Dalsgaard per. comm.

reflects a gradient of increasing algal activity compared to overall sediment metabolism. The classification is based on sedimentary O₂ metabolism (*see Table 2*).

The Kruskal–Wallis chi-square test (Stokes et al. 2000) was used to test the hypothesis that coupled nitrification–denitrification was similar for the different levels of BTSI. This nonparametric test was used because the assumptions of the analysis of variance (normal distribution and homogeneity of variances) were violated even after log transformation of the data. This analysis was performed on the raw data level ($n = 2,892$). Wilcoxon’s signed ranks test (Stokes et al. 2000) was used to test the hypothesis that there was no general significant effect of light on coupled nitrification–denitrification in the dataset. The Student’s *t*-test was used to analyze each individual sampling event for an effect of light.

Analysis of covariance (ANCOVA, Sokal and Rohlf 1995) was used to test the hypothesis that apparent differences in coupled nitrification–denitrification for the different levels of BTSI could be explained by a covarying factor. This analysis was performed on the mean values for each

sampling event ($n = 392$) since, for instance, O₂ fluxes and denitrification were not consistently measured in identical cores. To satisfy the assumptions of the classical ANCOVA (e.g., normality of error terms, equality of error variance, and equality of slopes), it was necessary to log-transform the response variable (coupled nitrification–denitrification activity, i.e., Dn) and, in order to include rates reported as zero values, the following response variable was used: $\text{resp} = \ln(1 + \text{Dn})$. Covariates considered in this analysis were selected using the following criteria. (1) A significant correlation between Dn and the controlling factor considered was required. Spearman’s rank correlation analysis (Sokal and Rohlf 1995) was applied in this step. (2) There had to be a significant difference in the factor considered as covariate for the different levels of BTSI. Kruskal–Wallis chi-squared test was used in this step. For the analysis of covariance, a simple linear relationship between the response variate and the individual covariate was assumed. All analyses were performed using the SAS system for Windows, release 8.02 (Statistical Analysis Systems Institute).

Table 2. The benthic trophic state index (BTSI) as defined by Rizzo et al. (1996) and distribution of samples within the different categories. The criteria are based on mean O₂ fluxes in light and dark for each sampling event. For sediment defined as “fully heterotrophic” the equality term was defined as the absence of a statistically significant difference between O₂ flux in the light and O₂ flux in the dark (Student’s *t*-test, $P < 0.05$) and a numerical difference $\leq 25\%$.

BTSI	Description	Criteria	Sampling events	Cores
0	Fully heterotrophic	O ₂ flux light \leq O ₂ flux dark < 0	82	550
1	Net heterotrophic	O ₂ flux light $>$ O ₂ flux dark < 0	114	898
2	Net autotrophic	$0 <$ O ₂ flux light $<$ O ₂ flux dark	88	645
3	Highly autotrophic	$0 <$ O ₂ flux dark $<$ O ₂ flux light	108	792

Experimental studies—Two types of microcosm experiments were performed to test the hypothesis derived from the field data: a ^{15}N study and a NO_3^- microsensor experiment. Because of difficulties in constructing the NO_3^- sensor, it was not possible to run these experiments simultaneously in the same microcosms. The protocol for preparation of the microcosms for these experiments was also slightly different (see below). Therefore, rates are not directly comparable.

^{15}N studies: The sediment used for the microcosms was collected in Norsminde Fjord, Denmark, and filtered through a 1-mm mesh screen in order to remove larger animals and shell fragments. Approximately 85 ml of filtered sediment was then transferred to 44 Plexiglas core tubes (50 mm i.d., height 300 mm). Black plastic was wrapped around the core tubes from the bottom of the tube to the sediment surface in order to prevent microalgae from colonizing the sides. Thirty-two of these cores were placed in a transparent aquarium containing filtered (1- μm , Millipore) seawater. The NO_3^- concentration was $3 \mu\text{mol L}^{-1}$, and NH_4^+ was $<1 \mu\text{mol L}^{-1}$. The aquarium was subdivided into four 30-liter chambers, and an additional supply of NO_3^- ($200 \mu\text{mol L}^{-1}$) was added to two of these chambers. In the following, cores incubated in the nitrate-enriched chambers will be designated “alga + N sediments,” whereas cores incubated in chambers without the supplementary NO_3^- addition will be designated “alga sediments.” The cores were exposed to a 12:12 h LD cycle (irradiance: $200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$). Light was provided by 400-W greenhouse lamps (HPI-T + mercury, Philips). Twelve additional cores were immersed in a darkened reservoir covered by an opaque lid and containing 20 liters of filtered seawater. In the following, these cores will be designated “heterotrophic sediments.”

The water inside all cores was stirred by Teflon-coated magnetic stir bars, positioned approximately 6 cm above the sediment surface and driven by external rotating magnets (~ 50 rpm). The setups were placed in a thermostatically controlled room, and the reservoirs were aerated to ensure a constant water temperature (19°C). The cores were preincubated for 3 weeks, during which water in the reservoirs was renewed every 3–4 d. During the preincubation period, O_2 fluxes were monitored regularly. Because there was no significant difference in O_2 fluxes in cores incubated in different chambers but exposed to the same LD conditions and N load, it was safe to treat the cores as replicates.

Measurements of denitrification and fluxes: After the preincubation period, denitrification and exchange of $\text{NO}_3^-/\text{NO}_2^-$, NH_4^+ , and O_2 between the sediment and the water column were measured. In the LD preincubated sediments, fluxes and denitrification were measured both in light and dark. Fluxes were measured in the same cores in light and in dark ($n = 8$ for each treatment), whereas denitrification was measured on one set of cores in light and another set of cores in dark ($n = 7$ for each treatment). In the heterotrophic sediment, fluxes and denitrification were measured only in the dark, and the same cores were used for both denitrification and flux measurements ($n = 8$). Incubations were performed in two sessions: fluxes were measured first, and after an equilibrium period of 20 h, the denitrification

measurements were performed. All measurements were initiated 4 h after a change in light regime.

Flux measurements were initiated by closing the Plexiglas tubes with transparent lids. The incubation time was 2–6 h depending on the preincubation conditions. Water samples for nutrient and O_2 analyses were collected from the water column within the Plexiglas tubes using a glass syringe immediately prior to sealing of the tubes and immediately after completion of the incubations. Samples for nutrient analysis were frozen in polyethylene vials. Oxygen samples were analyzed within 12 h by the Winkler titration technique (Grasshoff et al. 1983). Nitrate plus NO_2^- was determined using the vanadium chloride reduction method (Braman and Hendrix 1989) on a NO_x analyzer (model 42c, Thermo Environmental Instruments). Ammonium was determined using the salicylate-hypochlorite method (Bower and Holm-Hansen 1980) and analyzed automatically on a robotic sample processor (Tecan RSP-5051, Tecan AG) in line with a spectrophotometer (Camspec M330, Camspec). Fluxes of the measured components were calculated from the differences in concentrations in the initial and the final samples.

Denitrification measurements: Denitrification was measured using the ^{15}N isotope pairing technique (Nielsen 1992): ^{15}N -labeled NO_3^- was added to the water in the reservoirs (up to $100 \mu\text{mol L}^{-1} \text{ }^{15}\text{NO}_3^-$), after which water in the Plexiglas tubes was replaced with reservoir water. Following an equilibrium period of $\sim 4:1$ h LD (Dalsgaard et al. 2000b), the Plexiglas tubes were sealed and incubation was initiated. Incubation of individual cores was stopped at fixed intervals within 2–6 h depending on the pretreatment. On finishing incubation of a core, samples of the water column and sediment porewater were collected for determination of the $^{15}\text{N}_2$ ($^{29}\text{N}_2$, $^{30}\text{N}_2$) concentration and ^{15}N enrichment of the NO_3^- pool. The water column was sampled immediately on removal of the lids, and $250 \mu\text{l}$ of a 7 M ZnCl_2 solution was then added to the sediment surface to inhibit microbial activity. Water column and sediment was mixed carefully with a Plexiglas rod, and a sample of the resultant sediment slurry was collected with a syringe. Water or slurry samples for gas analysis were transferred to 12-ml glass vials (Exetainer[®], Labco) and preserved with an additional $250 \mu\text{l}$ of 7 M ZnCl_2 , whereas samples for $^{15}\text{NO}_3^-$ analysis were frozen in 10-ml polyethylene vials. Abundance of $^{29}\text{N}_2$ and $^{30}\text{N}_2$ in water samples and sediment–water suspensions was determined by gas chromatography/mass spectrometry (Robo-Prep-G+ in line with Tracermass, Europa Scientific) as described by Risgaard-Petersen and Rysgaard (1995). The ^{15}N atom percentage of NO_3^- was estimated by mass spectrometry after biological reduction to N_2 (Risgaard-Petersen et al. 1993). The production rate of $^{29}\text{N}_2$ and $^{30}\text{N}_2$ was calculated from the slope of the regression line obtained from plots of ^{15}N -labeled gases versus time for each treatment. Coupled nitrification–denitrification was then estimated from the production rates of $^{29}\text{N}_2$ and $^{30}\text{N}_2$ and the ^{15}N atom percentage of NO_3^- in the water column as described by Nielsen (1992).

Potential nitrification and chlorophyll *a* measurements: Chlorophyll *a* (Chl *a*) was determined at the beginning and at the end of the experiment on sediment subsamples. At the

beginning, samples were collected from the homogenized sediment pool used for preparing the microcosms. At the end of the experiment, sediment collected from the upper 0.5 cm of three cores from each treatment was used for these measurements. Chl *a* was measured by acetone extraction followed by photometry (Lorenzen 1967). The subsamples collected at the end of the experiment were also used for measurements of potential nitrification. Potential nitrification was estimated from the production of NO_3^- in NH_4^+ -enriched ($\sim 600 \mu\text{mol L}^{-1}$) slurries as described by Henriksen (1980). Concentrations of NO_3^- and NH_4^+ in these samples were determined as described above.

Microsensor study—The sediment used for preparing the microcosms in this experiment was collected at Møllerup, Randers Fjord, Denmark, where nitrification rates are high (Nielsen et al. 2001). The sediment was filtered through a 1-mm mesh screen and preincubated in a darkened plastic box for 4 weeks in order to assure sufficiently high populations of active nitrifying bacteria in the oxic zone. Undisturbed sediment cores were then collected in 200-mm coring tubes (55 mm i.d.), and one core was inoculated with 0.5 ml of a suspension of benthic diatoms (corresponding to $30 \mu\text{g}$ Chl *a*). The inoculum was prepared from a dense mat of benthic microalgae collected in the field: subsamples of this mat were transferred to 50-ml falcon tubes containing 30 ml of seawater and vortexed. Sediment particles were allowed to settle for a few minutes, after which the supernatant was transferred to falcon tubes and centrifuged at 2,000 rpm for 5 min. The pellets were then suspended in seawater.

After inoculation, the sediment surface of the sediment core was positioned in line with the upper rim of the core tube by pushing a rubber stopper from below. The microcosm was then carefully immersed in a tank containing filtered seawater ($[\text{NO}_3^-] \approx 1 \mu\text{mol L}^{-1}$) and exposed to an alternating 12:12 LD cycle (irradiance: $200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$). Hereafter, this core will be designated “the alga core.” Another core—the “alga-free core”—was incubated in darkness in filtered seawater at 20°C . After a preincubation period of 2 weeks, depth profiles of O_2 and NO_3^- concentrations were measured. Oxygen concentration profiles were measured with a Clark-type microsensor (Revsbech 1989) and NO_3^- concentration profiles with a NO_3^- biosensor (Larsen et al. 1997) equipped with an electrophoretic sensitivity control to optimize the sensitivity of the sensor (Kjær et al. 1999). The microsensors were mounted on a PC-controlled, motor-driven micromanipulator (Oriel) and analog signals from the sensors were digitized and fed into a PC as described by Revsbech and Jørgensen (1986). Prior to profile measurements, the cores were placed in filtered seawater in a temperature-controlled (20°C) container. The water was aerated to ensure stirring and a constant O_2 concentration during the measurements. In the LD-incubated sediment, five depth profiles of NO_3^- and O_2 were measured in both light and darkness. In the dark-incubated sediment five depth profiles of each species were measured only in the dark. After measurements, NH_4^+ was added (final concentration $\approx 1 \text{ mmol L}^{-1}$) to the LD-incubated sediment, which was then left for 16 h in light before additional NO_3^- profiles were measured in the light and in the dark. For all measurements

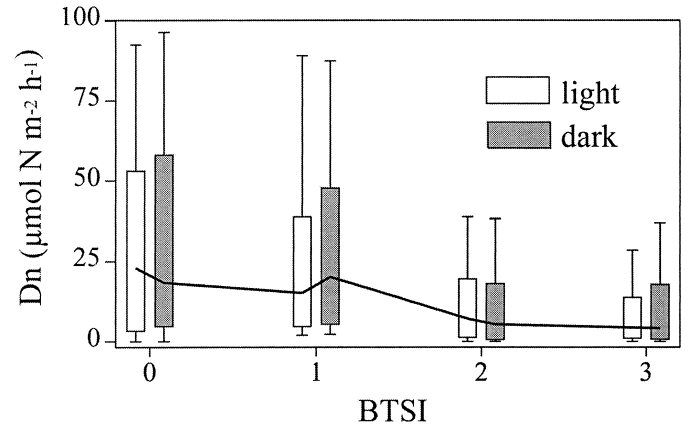


Fig. 1. Box whiskers plot of coupled nitrification–denitrification in light and in dark for the different levels of BTSI. Median (line), 25 and 75% quantiles (boxes), and 10 and 90% quantiles (bars) are shown.

involving changes in light regime, the sediment was preincubated for 4 h at the appropriate irradiance before measurements were performed. Production profiles were obtained using the numerical method described by Berg et al. (1998). The bulk sediment diffusion coefficient used in these calculations was estimated from the free solution diffusion coefficient for NO_3^- (Li and Gregory 1974) and sediment porosity.

Results

Field data analysis—Coupled nitrification–denitrification and BTSI: Coupled nitrification–denitrification rates were significantly different for the different levels of BTSI (Kruskal–Wallis, $p < 0.0001$, Fig. 1). Highest rates were observed in sediments classified as fully and net heterotrophic (BTSI 0 and BTSI 1). Rates for sediments belonging to these groups were not significantly different (Wilcoxon, $p = 0.7226$). Lowest activity was found in sediments classified as highly autotrophic (BTSI 3). In fully heterotrophic sediments, 90% of the measurements fell within the range $0\text{--}92 \mu\text{mol N m}^{-2} \text{ h}^{-1}$, with a median of $20.3 \mu\text{mol N m}^{-2} \text{ h}^{-1}$. In highly autotrophic sediments, 90% of the measurements fell within the range $0\text{--}34 \mu\text{mol N m}^{-2} \text{ h}^{-1}$, and the median was $4.2 \mu\text{mol N m}^{-2} \text{ h}^{-1}$.

For the 310 sampling events in the categories indicating benthic primary production ($\text{BTSI} \geq 1$), there was no overall significant difference between rates of coupled nitrification in light and rates of coupled nitrification in the dark (Wilcoxon, $p = 0.36$). No deviation from this observation was found when only data from net autotrophic and highly autotrophic sediments were considered. A Student's *t*-test performed on the individual sampling events showed no significant difference between rates obtained in the dark and rates obtained in light in 72% of sampling events (significance level: 0.05). Significant ($p < 0.05$) stimulation of Dn in the light was observed in 11% of the sample events, while significant ($p < 0.05$) reduction of Dn in the light was observed in 17% of the sampling events.

Table 3. Mean, standard error of the mean (SE); 10 and 90% quantiles of sedimentary O₂ consumption rates (SOD); bottom water NO₃⁻, NH₄⁺, and O₂ concentrations; salinity; and temperature for the different levels of BTSI.

BTSI	N	Parameter	Mean	SE	10% quantile	90% quantile
0	82	SOD (mmol m ⁻² h ⁻¹)	2.61	0.23	0.51	5.72
		NO ₃ ⁻ (μmol L ⁻¹)	154.11	28.47	0.36	606.51
		NH ₄ ⁺ (μmol L ⁻¹)	21.14	6.07	0.74	44.67
		O ₂ (μmol L ⁻¹)	303.23	9.68	215.85	405.00
		Salinity	15.48	1.16	2.50	30.00
		Temperature (°C)	12.42	0.74	3.7	19.5
1	114	SOD (mmol m ⁻² h ⁻¹)	2.91	0.27	0.58	6.01
		NO ₃ ⁻ (μmol L ⁻¹)	59.16	12.08	0.1	147.96
		NH ₄ ⁺ (μmol L ⁻¹)	29.36	9.95	0.25	44.74
		O ₂ (μmol L ⁻¹)	301.94	7.35	208.82	387.9
		Salinity	17.83	0.805	4.7	29
		Temperature (°C)	12.75	0.648	3.85	20.75
2	87	SOD (mmol m ⁻² h ⁻¹)	1.97	0.20	0.36	5.02
		NO ₃ ⁻ (μmol L ⁻¹)	80.74	15.46	0.43	219.58
		NH ₄ ⁺ (μmol L ⁻¹)	26.56	10.47	0.68	44.64
		O ₂ (μmol L ⁻¹)	320.71	6.15	255.06	394.00
		Salinity	19.40	0.80	10.00	29.00
		Temperature (°C)	12.60	0.91	3.70	26.00
3	108	SOD (mmol m ⁻² h ⁻¹)	1.49	0.12	0.21	3.07
		NO ₃ ⁻ (μmol L ⁻¹)	83.65	18.63	0.35	246.66
		NH ₄ ⁺ (μmol L ⁻¹)	56.01	16.01	0.82	105.46
		O ₂ (μmol L ⁻¹)	319.85	5.43	250.00	392.00
		Salinity	25.02	0.83	14.00	34.00
		Temperature (°C)	10.57	0.62	3.00	20.50

Correlation analysis and distribution of potential covariates: Significant differences in sediment O₂ demand (measured as O₂ uptake in the dark), bottom-water salinity, bottom-water NO₃⁻ and NH₄⁺ concentrations, and temperature were found among the different levels of BTSI (Table 3). Sediment O₂ demand was highest in the fully and the net heterotrophic sediments (Kruskal–Wallis, $p < 0.0001$). Bottom-water salinity was lowest in sediments classified as fully heterotrophic and highest in sediments classified as highly autotrophic (Kruskal–Wallis, $p < 0.0001$). Bottom-water NO₃⁻ was highest in sediments classified as fully heterotrophic, whereas no significant difference in NO₃⁻ concentration was found between sediments in the three remaining groups (Kruskal–Wallis, $p = 0.1494$). Bottom-water NH₄⁺ concentrations were highest in sediments classified as highly autotrophic (Kruskal–Wallis, $p = 0.0160$), whereas no significant differences were found in sediments belonging to the other groups (Kruskal–Wallis, $p = 0.2013$). Bottom-water temperatures were lowest for sediments classified as highly autotrophic, (Kruskal–Wallis $p = 0.0379$), whereas bottom-water temperatures were similar for the other groups. (Kruskal–Wallis, $p = 0.7964$). There was no statistical evidence for differences in the seasonal distribution of the different categories (Kruskal–Wallis, $p = 0.6134$), and no significant differences in bottom-water O₂ concentrations (Kruskal–Wallis, $p = 0.1478$).

Coupled nitrification was positively correlated to sediment O₂ consumption and to bottom-water NO₃⁻ and NH₄⁺ concentrations and negatively correlated with salinity (Table 4). No significant correlation was found at the 0.05 significance level between Dn and temperature and Dn and bottom-water O₂ concentrations.

From a statistical point of view, the variation in Dn among the different levels of BTSI could thus be the result of variations in sediment O₂ consumption, salinity, and bottom-water NO₃⁻ concentrations, and these parameters were therefore selected for an analysis of covariance. Because of the observation that bottom-water NH₄⁺ concentrations were highest in the group with the lowest Dn (BTSI 3) and that Dn was generally positively correlated to the NH₄⁺ concentration, variations in NH₄⁺ concentrations were not considered as an explaining factor.

Analysis of covariance: Only O₂ consumption and salinity were included in the ANCOVA performed on the entire dataset. Including bottom-water NO₃⁻ concentrations violated the fundamental assumption of constant slopes ($p = 0.0051$ for the BTSI \times NO₃⁻ interaction term). An ANCOVA based on O₂ consumption and salinity alone showed strong statistical evidence for a difference among mean values of Dn for the different levels of BTSI (Table 5). Tukey comparisons suggest two distinct sets of means. Sediments classified as heterotrophic exhibited the same level of activity, as did sediments classified as autotrophic. Lowest coupled nitrification–denitrification rates were found in the autotrophic sediments (BTSI 2 and 3, Fig 2A).

It was possible to perform the ANCOVA using O₂ consumption, salinity, and bottom-water NO₃⁻ concentration as covariates for data belonging to groups 0, 2, and 3. The results of the analysis were consistent with the results from the analysis described above: there was strong statistical evidence for a difference among mean values of Dn for the different levels of BTSI (Table 6). Tukey comparisons suggest two distinct sets of means, with lowest coupled nitrifi-

Table 4. Spearman's rank correlation (r), $\text{Prog} > |r|$ under $H_0: r = 0$ (p), and number of observations (n) for relationships between coupled nitrification–denitrification rates (Dn, mean of light/dark rates); sediment oxygen demand (SOD); bottom water NO_3^- , NH_4^+ , and O_2 concentrations; temperature; and salinity.

Variable	Parameter	Dn	SOD	NO_3^-	NH_4^+	O_2	Temperature	Salinity
Dn	r	1	0.30005	0.39163	0.11549	-0.0565	0.0966	-0.4001
	p		<0.0001	<0.0001	0.0301	0.3029	0.0744	<0.0001
	n	392	392	352	353	335	342	337

cation–denitrification rates in sediments classified as net or fully autotrophic (BTSI 2 and 3, Fig 2B). This analysis furthermore suggested that the effect of bottom-water NO_3^- concentrations on coupled nitrification–denitrification was not significant (Table 6).

Experimental data: ^{15}N -isotope studies—Coupled nitrification–denitrification: Rates of coupled nitrification–denitrification in light and in dark are shown in Fig 3. There was a significant effect of the treatment on the denitrification activity (ANOVA, $p = 0.0017$). Rates of coupled nitrification–denitrification were highest in heterotrophic sediments and lowest in alga sediments. Integrated over a full diurnal cycle (12:12 LD), coupled nitrification–denitrification was $1.49 \pm 0.17 \text{ mmol N m}^{-2} \text{ d}^{-1}$ in heterotrophic sediment, $0.76 \pm 0.2 \text{ mmol N m}^{-2} \text{ d}^{-1}$ in alga + N sediment, and $0.08 \pm 0.17 \text{ mmol N m}^{-2} \text{ d}^{-1}$ in alga sediment. Thus, Dn in alga-colonized sediments was between 4 and 51% of the activity in heterotrophic sediments. In alga + N sediments, total denitrification (Dn + Dw) was $6.3 \pm 1.2 \text{ mmol N m}^{-2} \text{ d}^{-1}$.

In both types of alga-colonized sediments, coupled nitrification–denitrification rates were highest in light. In alga + N sediments, rates in light were not significantly different from rates obtained in heterotrophic sediments (ANOVA, $p = 0.23$).

Potential nitrification: Rates of potential nitrification activity are shown in Fig. 4. According to the ANOVA, the preincubation conditions had a significant effect on the activity (ANOVA, $p = 0.0017$). Potential nitrification in alga sediments and alga + N sediments was 29 and 52%, respectively, of the activity measured in heterotrophic sediments. Ammonium concentrations were $657 \pm 31 \mu\text{mol L}^{-1}$ at the end of incubation, with no significant difference between the different treatments (ANOVA, $p = 0.5638$). Thus, the observed differences in potential nitrification activity cannot be explained by depletion of the NH_4^+ pool in samples with alga-colonized sediment during incubation.

Table 5. Analysis of variance. Type III sum of squares for the model including sediment O_2 consumption (SOD) and salinity as covariates and the BTSI as a class variable for the entire dataset.

Source	df	F	p
Salinity	1	43.25	<0.0001
SOD	1	21.38	<0.0001
BTSI	3	7.906	<0.0001
SOD \times BTSI	3	1.31	0.27080
Salinity \times BTSI	3	1.521	0.20890

O_2 fluxes: Rates of O_2 exchange between the sediment and the water column are shown in Fig. 5. There was a significant effect of treatment on sediment O_2 uptake rates in the dark (ANOVA, $p = 0.0420$). Highest uptake rates were observed in alga + N sediment, whereas O_2 uptake rates in heterotrophic sediments and alga sediment were similar (Student's t -test, $p = 0.5760$). Oxygen efflux rates in light were highest in alga + N sediments (Student's t -test, $p = 0.0004$). Integrated over a full diurnal cycle, net O_2 production was

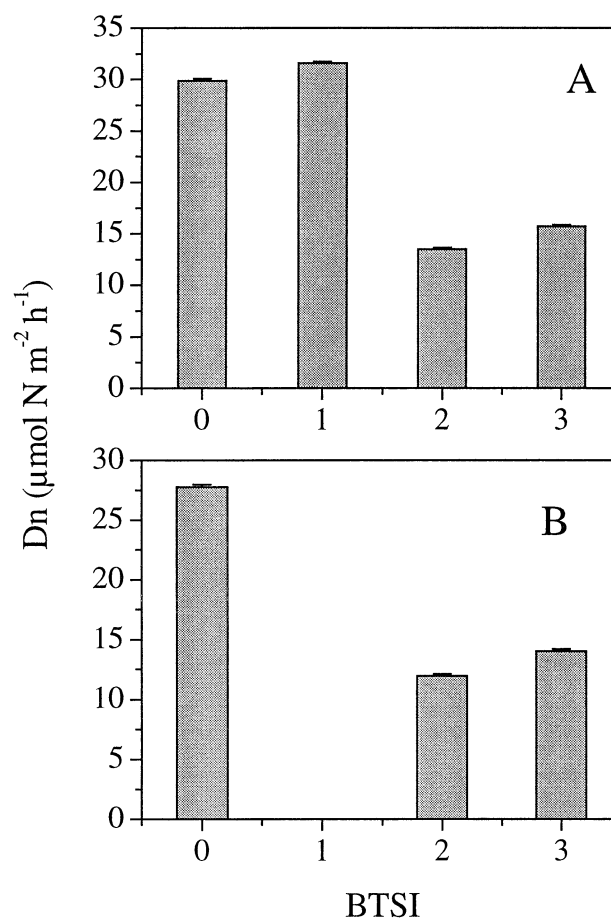


Fig. 2. Predicted least squares means for coupled nitrification–denitrification for the different levels of BTSI when accounting for the effect of O_2 consumption and salinity for the entire dataset (A), and when accounting for the effect of O_2 consumption, salinity, and bottom-water NO_3^- concentrations for data classified as either BTSI 0, BTSI 2, or BTSI 3 (B). Error bars are the standard error of the mean.

Table 6. Analysis of variance. Type III sum of squares for the model including sediment O₂ consumption (SOD) salinity and bottom water NO₃⁻ concentrations as covariates and the BTSI as a class variable for data classified as either BTSI 0, BTSI 2, or BTSI 3.

Source	df	F	p
Salinity	1	22.25	<0.0001
BTSI	2	5.771	0.0036
SOD	1	7.062	0.0084
NO ₃ ⁻	1	3.383	0.0672
SOD × BTSI	2	1.976	0.1412
Salinity × BTSI	2	0.594	0.5529
NO ₃ ⁻ × BTSI	2	0.52	0.5952

40 ± 3.6 mmol m⁻² d⁻¹ in alga sediment and 82 ± 10 mmol m⁻² d⁻¹ in alga + N sediment.

Nutrient fluxes: Both NH₄⁺ and NO₃⁻ were released from the heterotrophic sediment (Fig. 6). The diurnal integrated DIN flux was 4.9 ± 0.2 mmol m⁻² d⁻¹. In alga sediments, there was a small net release of NO₃⁻ to the water column and a net uptake of NH₄⁺ in light. In darkness, the pattern was reversed: NO₃⁻ was taken up and NH₄⁺ was released to the water column.

The release of NO₃⁻ or NH₄⁺ from the sediment to the water column was less than 12% of the release from heterotrophic sediments, and integrated over a full diurnal cycle, there was a net uptake of DIN from the water column amounting to 0.10 ± 0.09 mmol m⁻² d⁻¹.

In alga + N sediment, NO₃⁻ was taken up from the water column throughout the diurnal cycle. The NO₃⁻ uptake was highest in light. A small net uptake of NH₄⁺ was observed in light, whereas a small net release in the dark was observed. The diurnal net uptake of DIN was 16.4 ± 1.4 mmol m⁻² d⁻¹.

The amount of nitrogen available for assimilation can be estimated as the sum of ammonification and DIN uptake minus the loss of nitrogen via denitrification. Because the same sediment was used for all treatments, ammonification

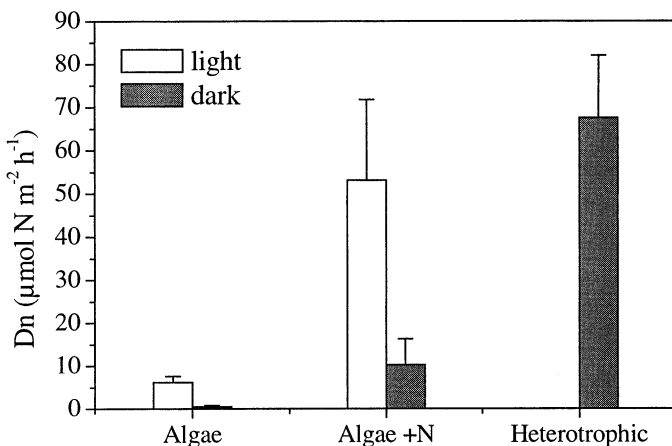


Fig. 3. Coupled nitrification-denitrification rates in “alga sediment,” “alga + N sediment,” and “heterotrophic sediment.” Columns represent mean values and error bars represent the standard error of the mean.

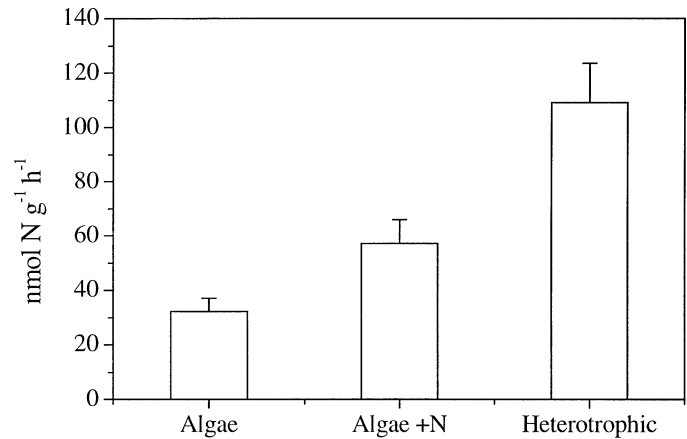


Fig. 4. Rates of potential nitrification in “alga sediment,” “alga + N sediment,” and “heterotrophic sediment.” Columns represent mean values and error bars represent the standard error of the mean.

levels can be assumed to be similar. Ammonification can be estimated from the denitrification rates and the DIN flux measured in heterotrophic sediment (Risgaard et al. 1993). Ammonification was 6.4 ± 0.4 mmol m⁻² d⁻¹. Thus, in alga sediment, assimilation was 6.4 + 0.1 - 0.08 (ammonification + net DIN uptake - denitrification) = 6.4 mmol N m⁻² d⁻¹. The ratio between net diurnal O₂ production and N assimilation was approximately 6. In alga + N sediment assimilation was 6.4 + 16.4 - 6.3 = 16.5 mmol N m⁻² d⁻¹, and the ratio between net diurnal O₂ production and nitrogen assimilation was approximately 5.

Chl *a*: In both types of LD-incubated sediments, Chl *a* increased significantly during the course of the experiment. Chl *a* was 105 ± 17 mg m⁻² at the beginning of the experiment, and at the end of the experiment, Chl *a* was 220 ± 3 mg m⁻² in the alga sediment, 317 ± 16 mg m⁻² in the NO₃⁻-enriched alga sediment, and 77 ± 14 mg m⁻² in the heterotrophic sediment.

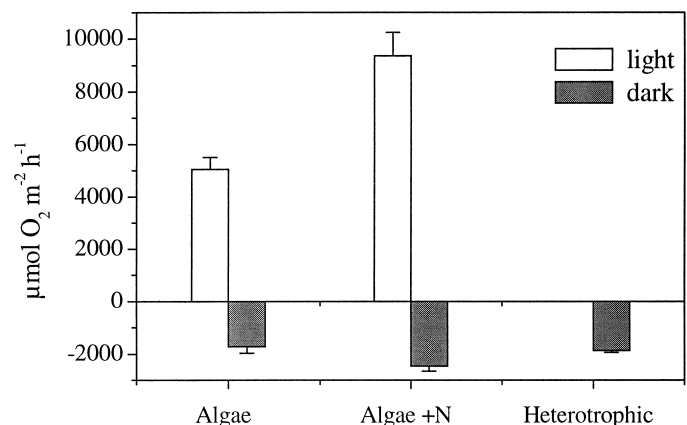


Fig. 5. Oxygen exchange between the water column and the sediment in “alga sediment,” “alga + N sediment,” and “heterotrophic sediment.” Columns represent mean values and error bars represent the standard error of the mean.

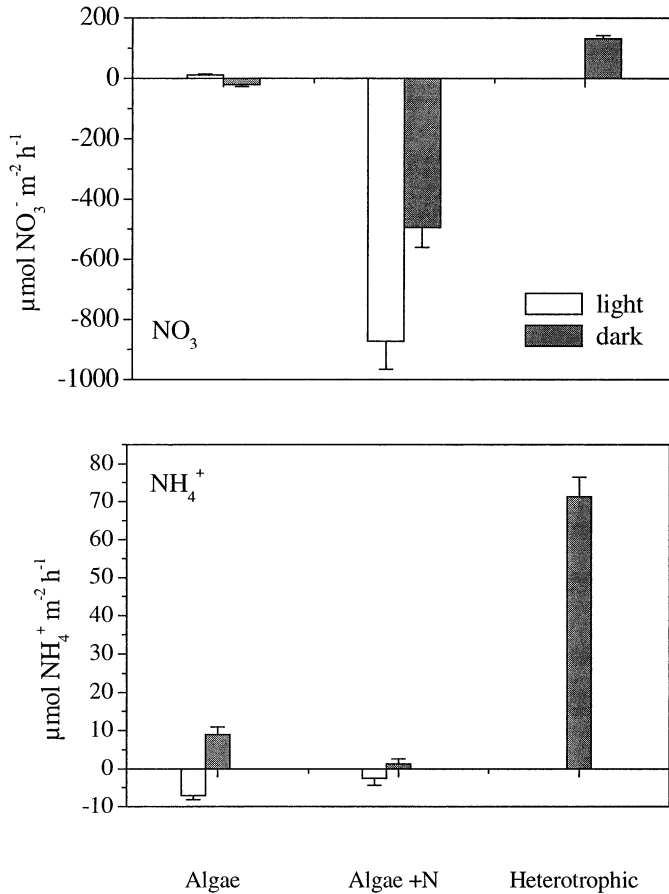


Fig. 6. Exchange of NO_3^- and NH_4^+ between the water column and the sediment in “alga sediment,” “alga + N sediment,” and “heterotrophic sediment.” Columns represent mean values and error bars represent the standard error of the mean.

Microsensor studies—There were no indications of net NO_3^- production in the “alga core” (Figs. 7, 8). In both light and darkness, the NO_3^- profiles indicated only consumption (Fig. 8). In light, NO_3^- was consumed in the sediment strata that coincided with maximum O_2 concentrations, suggesting that assimilation was the major sink. In the dark, the calculated consumption profile suggested consumption both in the oxic and in the anoxic sediment strata. In contrast, the “alga-free core” displayed significant NO_3^- production in the entire oxic zone, and a NO_3^- peak of $27 \mu\text{mol L}^{-1}$ was observed at the oxic/anoxic interface (Fig. 7). In the alga core, the O_2 penetration depth was 2.3 mm in light and 0.85 mm in dark. In the dark-incubated core, the O_2 penetration depth was 1.6 mm. Addition of NH_4^+ had a profound effect on the NO_3^- concentration in the “alga-core.” After 16 h of NH_4^+ exposure, a NO_3^- peak ($12 \mu\text{mol L}^{-1}$) was observed in the illuminated sediment at a depth of 1.2 mm. Darkening of this sediment resulted in disappearance of NO_3^- , suggesting no capacity for nitrification in the superficial sediment strata. The NO_3^- profile measured in light probably did not reflect a steady state situation; therefore, no attempt was made to calculate a production profile.

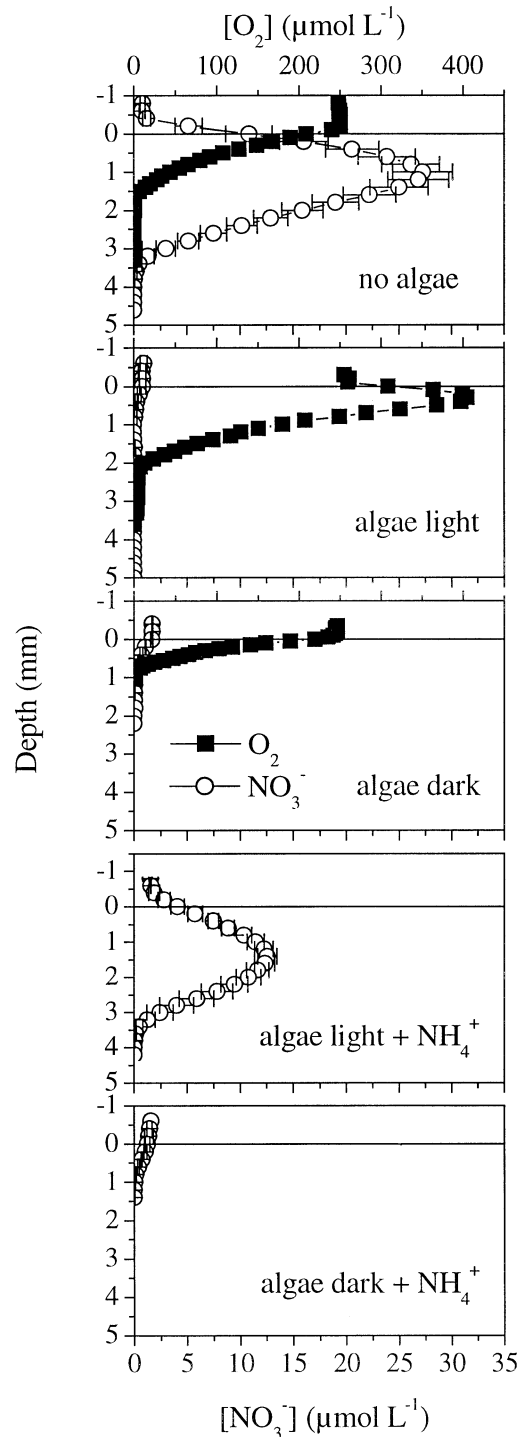


Fig. 7. Microprofiles of O_2 and NO_3^- in alga-free sediment and in alga-inoculated sediment before and after addition of NH_4^+ to a final water column concentration of 1 mmol L^{-1} .

Discussion

Reduced coupled nitrification–denitrification in alga-colonized sediments—In the present study, field data obtained from 18 European estuaries using the isotope pairing technique were analyzed for trends in relationship between ac-

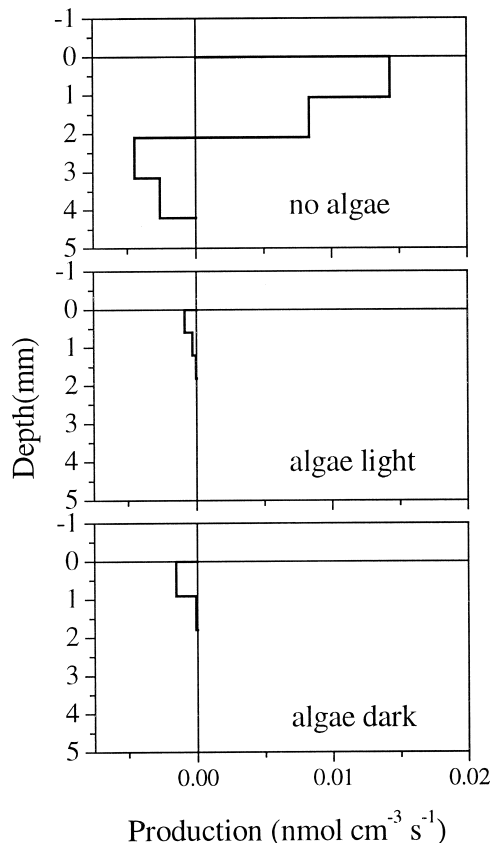


Fig. 8. Production profile of NO_3^- in the alga-free sediment and in the alga-inoculated sediment.

tivity of benthic microalgae and coupled nitrification–denitrification. When applying the BTSI as an index for activity of microalgae compared to overall sediment metabolism, the dataset showed a clear and significant inverse relationship between the trophic state of the sediment and the coupled nitrification–denitrification activity. This trend was still conserved after an analysis of covariance with O_2 consumption and salinity as covariates on the entire dataset and with bottom-water NO_3^- concentration, O_2 consumption, and salinity on a subset of the dataset. Therefore, there is strong evidence for the hypothesis that sediments with microalgae displaying an activity that exceeds community respiration (i.e., net and fully autotrophic sediments) exhibit lower rates of coupled nitrification–denitrification than do heterotrophic sediments.

The experimental data obtained in the present study support the hypothesis that coupled nitrification–denitrification activity is reduced in alga-colonized sediments. In the ^{15}N studies, diurnal integrated coupled nitrification–denitrification rates in alga-colonized sediments were between 4 and 51% of the activity measured in heterotrophic sediment (Fig. 3). In the microsensor experiment, the NO_3^- profile measured before NH_4^+ addition suggested much lower nitrification and denitrification activity in alga-colonized sediment than in sediment without algae (Figs. 7, 8).

The experimental dataset furthermore showed that a general suppression of coupled nitrification–denitrification in alga-colonized sediments is fully compatible with the ability

of these sediments to promote coupled nitrification–denitrification via oxygenic photosynthesis, which has been questioned in previous studies (Risgaard-Petersen et al. 1994; Rysgaard et al. 1995). Thus, the results of the experimental study unite two apparently opposing views on the interaction between microalgae and nitrifying or denitrifying bacteria. Presence of active microalgae might allow only a reduced population of nitrifying/denitrifying bacteria to have an active metabolism, as compared to a heterotrophic sediment (Henriksen and Kemp 1988), and these bacterial populations can display diurnal variations in activity correlated with diurnal variations in O_2 penetration depth (Risgaard-Petersen et al. 1994; Rysgaard et al. 1995; Lorenzen et al. 1998; An and Joye 2001).

In the field dataset, stimulation of coupled nitrification–denitrification via oxygenic photosynthesis was not a general measurable trend, however. For most observations (72%) there was no significant difference between rates measured in light and in dark. A power analysis of the t -test applied on this set of observations showed that the average probability of rejecting a false null hypothesis (i.e., H_0 : No difference between light and dark rates) was only 31%. This may indicate that failure to measure significant differences between light and dark rates to some extent was due to the experimental design, i.e., the replicates were too few: 3–5 for each LD treatment to account for the spatial heterogeneity.

On the factors selected as covariates in the analysis of covariance—The factors used as covariates in the analysis of covariance were selected on the basis of simple statistical considerations (i.e., their distribution among the different sediment categories and the correlation with Dn). The observed correlation between Dn and the chosen factors could reflect both dependency and interdependency.

The positive correlation between sedimentary O_2 consumption and coupled nitrification–denitrification found in the present study and incorporated in the analysis of covariance is consistent with data reported by Seitzinger and Giblin (1996) and Trimmer et al. (2000). Sedimentary O_2 consumption is linked to mineralization of organic bound nitrogen and hence to NH_4^+ generation. Therefore, low O_2 consumption rates reflect low NH_4^+ generation and limitation of nitrification by the availability of electron donors, whereas higher O_2 consumption rates reflect better NH_4^+ availability for the nitrifiers (Sloth et al. 1995).

The negative correlation between salinity and Dn (Table 4) found in the present study is consistent with previous biogeochemical studies. Slurry experiments and experiments with intact estuarine sediments have shown that both the potential for nitrification and rates of coupled nitrification–denitrification decrease with increasing salinity (Rysgaard et al. 1999). However, because several marine strains of nitrifiers are known, the salinity effect is probably not just a simple inhibition effect. Gardner et al. (1991) suggested that ion pair formation of NH_4^+ and seawater anions and blockage of cation exchange sites with seawater cations might allow an increasing fraction of NH_4^+ to diffuse out of the sediment before it is nitrified as salinity is increased.

The positive correlation between bottom-water NO_3^- and

Dn found in the dataset and incorporated into the ANCOVA is apparently a conceptual mismatch because Dn is regarded solely as a dependency between sedimentary nitrification and denitrification (Nielsen 1992). In full agreement with this concept, Dong et al. (2000) showed experimentally that Dn was independent of the concentration of NO_3^- in the water column. Most likely, the correlation between Dn and NO_3^- found in the present dataset as a whole reflects interdependency rather than dependency (e.g., a covariance with bottom-water salinity). The lack of significant correlation between NO_3^- and Dn in the analysis of covariance (Table 6) supports this idea.

Factors responsible for reduced Dn in alga-colonized sediments—The results of the present study suggest that a major factor responsible for the reduction in coupled nitrification–denitrification in alga-colonized sediments is the generation of unfavorable growth conditions or conditions that promote higher death rates for the nitrifying bacteria population in these systems. This is reflected in a lower potential for nitrification in both types of alga-colonized sediments as compared to heterotrophic sediments (Fig. 4). In a review on nitrification, Henriksen and Kemp (1988) presented experimental data in agreement with this finding. Without further experimental documentation, the authors proposed that reduction of the potential nitrification activity in alga-colonized sediments was caused by a combination of several alga-induced factors, such as NH_4^+ limitation, high pH, high O_2 concentration, CO_2 limitation, and organic excretion products. The results of the present study do not exclude any of these factors. However in the present study, reduction of both coupled nitrification–denitrification and nitrification potential was most marked in the alga-colonized sediments that were least photosynthetically active (i.e., the alga sediments). This suggests that factors such as O_2 toxicity, CO_2 limitation, and inhibition of nitrification by high pH were of minor importance. The data point toward the idea that induction of nitrogen limitation of the nitrifying bacteria population was a major controlling mechanism. In the alga sediment, the sediment was the main source of N for assimilation, and the estimates of assimilation in this type of sediment almost balanced sedimentary ammonification after the 21 d of incubation, suggesting a strong competitive pressure on the nitrifying population. For the alga + N sediment, the water column represented an additional N source for assimilation, and this could imply a less intense competition for nitrogen.

The idea that nitrogen limitation of nitrification is induced in alga-colonized sediments is further supported by the results from the microsensor studies, where it was shown that net NO_3^- production could be partly restored in light after the addition of NH_4^+ to the “alga core” (Fig. 7).

Nitrogen limitation of nitrification in alga-colonized sediments might be induced both directly by microalgae and indirectly via alga-induced stimulation of growth of heterotrophic bacteria. Results from the microsensor study (Figs. 7, 8) showed that the effect of alga colonization on nitrification was not restricted to the sediment surface but could propagate to the entire oxic zone. This might suggest that aerobic heterotrophic bacteria were responsible for the ob-

served suppression of net NO_3^- production below the photic zone. Photosynthesizing microalgae can contribute substantially to the carbon flow in sediments (Underwood and Kromkamp 1999; Middelburg et al. 2000; Perkins et al. 2001) via the release of photoassimilates in the form of carbohydrate (Smith and Underwood 2000) to the sediment environment. The released carbon can be incorporated rapidly into the heterotrophic bacterial biomass (Middelburg et al. 2000) and stimulate the growth of these bacteria and could thus induce N limitation of nitrification. Strauss and Lamberti (2000) demonstrated that addition of easily accessible carbon to stream sediments resulted in a decrease in nitrification activity but an overall increase in total microbial activity. The authors suggested that this shift in bacterial population structure was the result of competition between heterotrophs and nitrifiers for nitrogen in favor of the fast growing and generally more abundant heterotrophic bacteria. Butturini et al. (2000) observed a similar mechanism in stream biofilms, and it is likely that a mechanism such as this acts in microalga-colonized sediments.

In the present study, analysis of both field data and data from controlled laboratory experiments showed that presence of active benthic microphytes led to reduced loss of N via coupled nitrification–denitrification as compared to sediments without benthic photosynthesis.

The suppression of coupled nitrification–denitrification in these sediments makes the coupling between sedimentary N regeneration and N assimilation more efficient and thus will promote internal recycling and temporal storage of nitrogen. The observation that suppression of coupled nitrification–denitrification is likely to be linked to induction of N limitation of nitrifying bacteria points toward the existence of a mechanism by which nitrogen-limited systems limit N loss and conserve internal resources.

Borum and Sand-Jensen (1996) have suggested that changes in nutrient load to the estuarine environment are accompanied by qualitative changes in plant community composition and primary production. At high N loads, the plant community is dominated by pelagic primary producers, whereas reduced N loading will favor benthic primary production. The results of the present study suggest that such changes in plant community composition will significantly alter the benthic N cycle.

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