

Nitrification and denitrification in Wadden Sea sediments (Königshafen, Island of Sylt, Germany) as measured by nitrogen isotope pairing and isotope dilution

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ABSTRACT: Seasonal variation of sediment denitrification and nitrification in darkness was measured by the nitrogen isotope pairing and the nitrate isotope dilution techniques in 3 different sediment types: coarse sand, fine sand and muddy sand (S, A and M, respectively) in intertidal Königshafen, Island of Sylt, Germany. The temporal pattern of denitrification based on nitrate from the overlying water could be explained by variations in the nitrate concentration and oxygen penetration depth in all 3 sediment types. Highest rates (8 to 48 $\mu\text{mol m}^{-2} \text{h}^{-1}$) were observed in April 1994 when nitrate concentration in the overlying water was 65 to 70 μM and the lowest (0.2 to 1.1 $\mu\text{mol m}^{-2} \text{h}^{-1}$) in summer and fall when nitrate was depleted from the water phase. The annual mean dark denitrification, of which 69 to 75% was due to overlying water nitrate, increased in the sequence A, S and M in accord with increasing sediment oxygen uptake. All 3 sediments types exhibited low nitrification in early winter. At S and M, maximum nitrification was measured in spring and late summer (8 to 17 and 27 to 28 $\mu\text{mol m}^{-2} \text{h}^{-1}$, respectively), whereas nitrification was repressed in mid summer. Maximum dark nitrification here generally occurred at the time of the highest activity of the benthic microalgae (measured as oxygen-based daily gross primary production). At Stn A, maximum nitrification was measured in mid summer (26 $\mu\text{mol m}^{-2} \text{h}^{-1}$), when daily gross primary production was low. The 2 different seasonal patterns of nitrification can be explained by differences in oxygen and ammonium availability. In all sediment types, a low degree of coupling between nitrification and denitrification (in relation to total nitrification) was evident, especially during summer or autumn. Annual mean dark nitrification, of which 11 to 27% was coupled to denitrification, was higher in the muddy sediment than in the 2 sandy sediments.

KEY WORDS: Intertidal · Sediment · Nitrification · Denitrification · Isotope pairing · Isotope dilution

INTRODUCTION

The increasing eutrophication of coastal areas has long been a matter of concern. However, it is not yet fully clear to what extent coastal marine sediments are capable of removing increased nitrogen loads. It is therefore necessary to obtain a better knowledge of key processes such as benthic nitrification and denitrification and especially the coupling between them, i.e. when the nitrate produced from nitrification is subsequently denitrified. A new tool, the isotope pairing technique, has recently been introduced for measuring

sediment denitrification (Nielsen 1992). By this technique, total denitrification is separated into denitrification of nitrate originating from the water phase and denitrification coupled to nitrification. The applicability of this technique has been shown in several investigations (e.g. Rysgaard et al. 1993, 1995, Risgaard-Petersen et al. 1994, Lohse et al. 1996, Nielsen & Glud 1996). The more traditional nitrate isotope dilution technique, on the other hand, provides a measure of the part of nitrification where nitrate is released to the overlying water (Koike & Hattori 1978, Chatarpaul et al. 1980, Nishio et al. 1983). This technique has recently been improved by applying new analytical methods (Risgaard-Petersen et al. 1993, Højberg et al.

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1994, Jensen et al. 1996). The 2 techniques have been successfully combined in studies of nitrification and denitrification under controlled conditions in manipulated sediment systems (Rysgaard et al. 1993, 1994, Risgaard-Petersen et al. 1994, Sloth et al. 1995, Pelegrí & Blackburn 1995).

The purpose of the present study was to determine seasonal variations of dark nitrification and denitrification in different sediment types of an intertidal area using the isotope pairing and isotope dilution techniques. The temporal and spatial variations in physical and chemical conditions required that the reliability of the techniques was thoroughly verified. The study is part of an integrated investigation of the biogeochemistry of sediments in the Königshafen bay (Island of Sylt, Germany) in the northern Wadden Sea

MATERIALS AND METHODS

Study site. The study was carried out in the shallow intertidal embayment Königshafen on the Island of Sylt, Germany. The bay is sheltered from winds and waves, but strongly influenced by semi-diurnal tides (amplitude 1.7 m; Reise 1985). Three stations in the intertidal zone, S, A and M (Fig. 1), were investigated on 8 occasions from April 1993 to July 1994. The sediment at S and A was composed of coarse and fine sand, respectively, whereas M had a higher content of fine-grained material (muddy sand). The mean porosity in the upper 10 cm at S and A varied between 0.32 and 0.38, whereas values between 0.44 and 0.58 were found at M; the highest values for all 3 stations were found in the upper 0 to 3 mm. Organic content, determined as loss on ignition (LoI) at 520°C according to Kristensen & Andersen (1987), decreased at S and A from 0.8% in the upper few millimeters to 0.2–0.4% at 10 cm depth. At Stn M, the organic content in the

upper 0 to 3 mm varied seasonally from 1% in winter to 6% in summer. At greater depths, the organic content at this station ranged from 0.5 to 4% in an irregular pattern. The macrofauna at Stn M was dominated by *Cerastoderma edule* (100 to 300 m⁻²) and *Macoma balthica* (40 to 300 m⁻²). At S, *Hydrobia ulvae* occurred in high numbers (4000 to >20000 m⁻²) for most of the year along with small specimens of *Arenicola marina* and various unidentified polychaetes (100 to 1000 m⁻²). At Stn A, large specimens of *A. marina* (30 to 70 m⁻²) dominated, whereas *H. ulvae* was absent. Stn Mx (Fig. 1), which was used only in preliminary studies, resembled Stn M in many respects. Salinity at all stations ranged between 23 and 33‰, lowest during winter and early spring.

Sediment collection and handling. Sediment cores were collected in daylight during water cover. Between 4 and 9 (usually 6) intact cores were taken to 9–11 cm depth with 8 cm (inner diameter) core tubes leaving 9 to 11 cm overlying water. The cores were brought to a nearby laboratory within 1 to 2 h and submersed uncapped into a darkened circular tank containing 40 l unfiltered aerated seawater from the location. The tank water was circulated by an Eheim pump and the water overlying the cores was stirred by spinning teflon-coated magnets (60 to 70 rpm) ca 6 cm above the sediment surface. During each study period (1 to 2 wk), the temperature of flood water at the beginning of the period was used as a standard incubation temperature (4.5 to 20°C).

Seasonal ¹⁵N-incubations. Denitrification was measured by the isotope pairing method of Nielsen (1992). This technique provides rates of denitrification due to nitrate from the overlying water as well as denitrification coupled to nitrification. Furthermore, by monitoring the isotope dilution of added ¹⁵NO₃⁻ the rate of nitrification not coupled to denitrification can be measured simultaneously (Koike & Hattori 1978, Nishio et al. 1983).

After 2 h preincubation in darkness, the overlying water of the sediment cores was enriched with ¹⁵NO₃⁻ (30 to 60 μM) by addition of a ¹⁵NO₃⁻ stock solution to the tank water followed by vigorous stirring for about 15 min. Initial water samples for analysis of dissolved O₂, dissolved inorganic nitrogen (DIN: NH₄⁺, NO₃⁻, NO₂⁻) and ¹⁵N distribution in dissolved N₂ and NO₃⁻ (+NO₂⁻) were taken before capping the cores (within 0.5 h after addition of ¹⁵NO₃⁻). Incubation was done in darkness with stirred water phases for 0.6 to 16 h, depending on season and station. During most incubations, sampling was performed in a time-series to test for linearity of the measured rates. The O₂ concentration rarely decreased below 75% of air saturation at the end of incubation to prevent an influence from diminished O₂ penetration on nitrification and denitri-

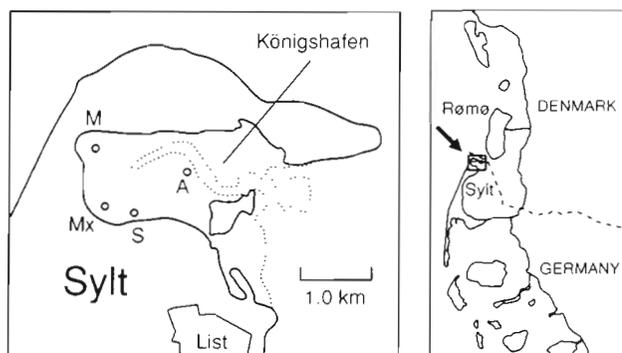


Fig. 1 Study area: Königshafen, Island of Sylt, Germany. Sampling stations situated in the intertidal zone are indicated. Dotted line: mean low water level

fication. The incubation was terminated by uncapping the cores and quickly repeating the water sampling before carefully mixing the sediment and the remaining overlying water into a homogenous slurry. Samples for mass spectrometric determination of ^{15}N in N_2 and O_2 content in the slurry were taken after a few minutes of sedimentation; the O_2 content was measured in order to check for proper inhibition of denitrification in the slurry prior to sampling and preservation. Finally, the sediment slurry was sieved (1 mm mesh) for quantification of larger macrofauna.

Water samples for dissolved O_2 were taken in 25 ml Winkler bottles. Water samples for determination of ^{15}N in N_2 were stored in 8.5 (or 12.5) ml gas-tight containers (Exetainers, Labco, High Wycombe, UK) with screwcap lids (excluding any air bubbles) and preserved with 100 μl 80% w/w ZnCl_2 or 10 μl 0.06 M HgCl_2 . Samples for parallel determination of ^{15}N in N_2 and for O_2 content were taken in 8.5 ml Exetainers and preserved with 20 μl 10 M NaOH. The DIN water samples and the samples for ^{15}N content in NO_3^- were GF/C-filtered and stored frozen in 20 ml scintillation vials.

Test incubations. Influence of $^{15}\text{NO}_3^-$ addition: To test for interference of added nitrate on denitrification of ambient nitrate and to assure that an even distribution of nitrate isotopes existed in the denitrification zone (Nielsen 1992), a series of incubations using different additions of ^{15}N -nitrate was performed. In April 1994, when ambient nitrate concentration was high (65 to 71 μM), 9 cores from S, A and M were enriched with 30, 60 and 120 μM $^{15}\text{NO}_3^-$ in triplicate. In July 1994, when ambient nitrate concentration was low (<0.1 μM), 7 concentrations between 5 and 120 μM $^{15}\text{NO}_3^-$ were used on 1 core each from A and M. The incubation temperatures in April and July were approximately 6.5 and 20°C, respectively.

Microalgal interference: To test for a possible impact of microphytobenthic dark uptake of dissolved inorganic nitrogen on the nitrification/denitrification assay, 500 μM NH_4^+ was added to 3 out of 6 cores incubated with 60 μM ^{15}N -nitrate, assuming that the microphytobenthos would preferentially assimilate NH_4^+ (e.g. Nielsen et al. 1990b). The incubations were done on sediment cores which had just previously been used for the measurement of unamended fluxes in a light-dark cycle (E. Kristensen, M. J. Jensen & K. M. Jensen unpubl.). The cores were preincubated in light for 8 h and then received the standard 2 h preincubation in darkness. These ammonium addition (AA) incubations were performed on S, A and M in April 1994. In another series, cores were preincubated for approximately 48 h in darkness to eliminate (or at least greatly reduce) the microalgal assimilation of inorganic nitrogen before 30 μM ^{15}N -nitrate was added. These con-

stant-dark (CD) incubations were conducted on S and Mx in June 1993. Both the AA and the CD incubations were otherwise performed as the standard seasonal ^{15}N -incubations.

Chemical analysis. O_2 was analyzed by the standard Winkler technique. DIN samples were analyzed for NO_3^- , NO_2^- and NH_4^+ on an autoanalyzer by standard methods (Armstrong et al. 1967, Solorzano 1969) or for NH_4^+ by a manual salicylate hypochlorite method (Bower & Holm-Hansen 1980). Since NO_2^- was always low in concentration, it is included in NO_3^- . The ^{15}N labelling of dissolved N_2 in the ZnCl_2 (or HgCl_2) preserved samples were measured by GC-MS (Sira Series II, VG-Isotech, Middelwich, UK) as described by Rysgaard et al. (1994). The NaOH preserved samples were analyzed for ^{15}N labelling and O_2 content by membrane inlet-MS as described by Jensen et al. (1996). The samples for ^{15}N in NO_3^- were measured by membrane inlet-MS after bacterial reduction of NO_3^- to N_2 (Jensen et al. 1996).

Calculations. The rate of denitrification was calculated according to the procedures and assumptions of Nielsen (1992). The concentration of dissolved N_2 in air-saturated water was used as an internal standard to obtain the areal production rate of single and double labelled N_2 ($p^{14}\text{N}^{15}\text{N}$ and $p^{15}\text{N}^{15}\text{N}$) from the measured isotope distribution in N_2 from the overlying water and the slurry. The denitrification rates were then calculated from the areal production rates according to:

$$D_{15} = p^{14}\text{N}^{15}\text{N} + 2 \times p^{15}\text{N}^{15}\text{N} \quad (1)$$

$$D_{14} = D_{15} \times \frac{p^{14}\text{N}^{15}\text{N}}{2 \times p^{15}\text{N}^{15}\text{N}} \quad (2)$$

$$D^{\text{tot}} = D_{15} + D_{14} \quad (3)$$

where D_{15} and D_{14} are the denitrification rates of $^{15}\text{NO}_3^-$ and $^{14}\text{NO}_3^-$, respectively, and D^{tot} is the total rate of denitrification in the $^{15}\text{NO}_3^-$ amended core system.

The rate of denitrification based on added plus ambient nitrate from the overlying water (D_w^{tot}), the rate of denitrification coupled to nitrification (D_n) and the denitrification of ambient nitrate from the water (D_w) were then calculated as:

$$D_w^{\text{tot}} = D_{15}/f \quad (4)$$

$$D_n = D^{\text{tot}} - D_w^{\text{tot}} \quad (5)$$

$$D_w = \frac{D_w^{\text{tot}}}{[\text{NO}_3^-]_i} \times [\text{NO}_3^-]_a \quad (6)$$

where f is the fraction of ^{15}N labelled NO_3^- in the overlying water, $[\text{NO}_3^-]_i$ is the nitrate concentration in the water phase after $^{15}\text{NO}_3^-$ addition prior to capping the cores and $[\text{NO}_3^-]_a$ is the ambient NO_3^- concentration

(measured in the tank water before preincubation). Total unamended denitrification is the sum of D_w and D_n .

The rate of nitrification not coupled to denitrification, but released as NO_3^- to the overlying water (N_w), was measured from the fractional changes in ^{15}N labelling of NO_3^- in the overlying water during incubation, Δf (adapted from Nishio et al. 1983):

$$N_w = \frac{[\text{NO}_3^-] \times \Delta f}{0.00366 - f} \times \frac{h}{\Delta t} \quad (7)$$

where $[\text{NO}_3^-]$ is the mean concentration during incubation, h is the water phase height and Δt the incubation time. Total nitrification is the sum of D_n and N_w .

RESULTS

Seasonal study

The ambient NO_3^- concentration increased from near zero in summer and fall 1993 to 65–70 μM in late winter/early spring 1994. Ambient NH_4^+ concentrations varied from near zero found in summer to about 10 μM in winter (Fig. 2A). The dark O_2 uptake in sum-

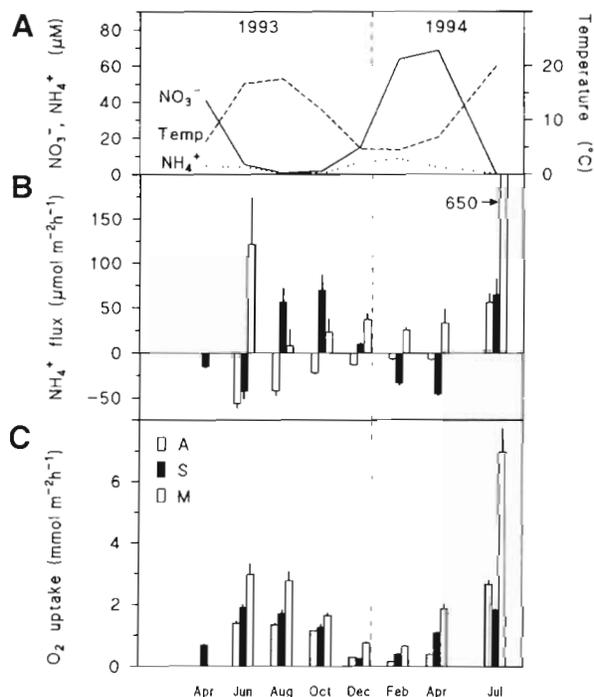


Fig. 2. Seasonal variation in (A) ambient NO_3^- (+ NO_2^-) and NH_4^+ concentration in the water phase and incubation temperature, (B) dark NH_4^+ flux (positive values indicate release) and (C) dark O_2 uptake. Standard error of the mean is indicated. Only Stn S was included in April 1993

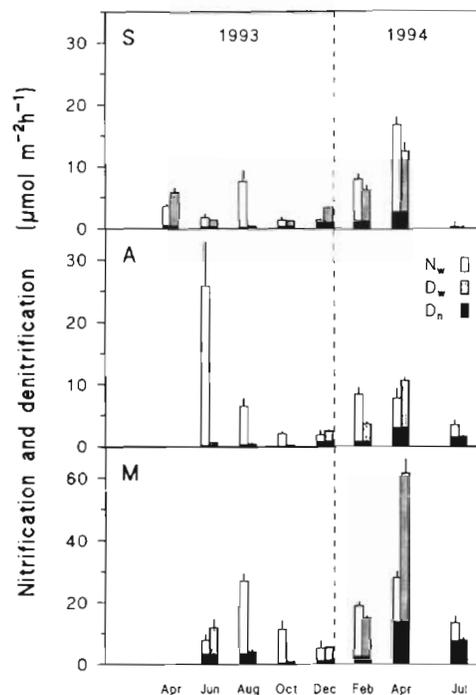


Fig. 3. Seasonal variation of dark nitrification and denitrification at the 3 stations. Nitrification is the sum of D_n and N_w , whereas denitrification is the sum of D_n and D_w . Standard error of the mean is indicated. Only S was included in April 1993. Note the different scales on the vertical axes

mer was 2 to 2.5 and 3 to 6.5 $\text{mmol m}^{-2} \text{h}^{-1}$ on the sandy and muddy sediments, respectively, and decreased to below 1 $\text{mmol m}^{-2} \text{h}^{-1}$ in winter (Fig. 2C). At Stn A, the NH_4^+ flux (Fig. 2B) was always directed into the sediment (from -6 to $-56 \mu\text{mol m}^{-2} \text{h}^{-1}$) with lowest uptake in winter and spring, except for July 1994 when NH_4^+ was released significantly ($56 \mu\text{mol m}^{-2} \text{h}^{-1}$). At M, NH_4^+ was always released from the sediment. Highest rates were observed in summer (120 and 650 $\mu\text{mol m}^{-2} \text{h}^{-1}$), whereas the range was 8 to 37 $\mu\text{mol m}^{-2} \text{h}^{-1}$ the rest of the year. At S, NH_4^+ was released from late summer to early winter (10 to 65 $\mu\text{mol m}^{-2} \text{h}^{-1}$), but taken up during the rest of the year (from -16 to $-46 \mu\text{mol m}^{-2} \text{h}^{-1}$). For all study periods and stations the addition of 30 μM $^{15}\text{NO}_3^-$ (or higher) in the denitrification incubations did not influence the NH_4^+ flux as judged by comparison to parallel unamended incubations (E. Kristensen et al. unpubl.).

The overall pattern of denitrification generally showed low rates from summer to early winter for all stations (0 to 8 $\mu\text{mol m}^{-2} \text{h}^{-1}$) when NO_3^- concentration in the overlying water was below 2 μM (Fig. 3). The increasing rates in late winter and spring (maximum around 11 $\mu\text{mol m}^{-2} \text{h}^{-1}$ in sandy sediment and up to 62 $\mu\text{mol m}^{-2} \text{h}^{-1}$ at M in April) corresponded to the

increasing NO_3^- concentration in the water phase (maximum of 65 to 70 μM in April). Denitrification of NO_3^- from the overlying water (D_w) accounted for 60 to 90% of the total denitrification during most of the year, but during summer, when NO_3^- was depleted, the contribution of D_w was only a few percent. The annual mean dark denitrification rate at S, A and M (from June 1993 to April 1994) was 4.2, 3.0 and 17 $\mu\text{mol m}^{-2} \text{h}^{-1}$, respectively, of which D_w accounted for 75, 69 and 74%.

Nitrification rates ranged from 1 to 17 $\mu\text{mol m}^{-2} \text{h}^{-1}$ at S. The rates at A were in the same range except for 1 high value of 26 $\mu\text{mol m}^{-2} \text{h}^{-1}$ in June 1993. At the muddy Stn M nitrification rates were somewhat higher, ranging between 5 and 28 $\mu\text{mol m}^{-2} \text{h}^{-1}$. The nitrification rates were generally lowest in early winter at all 3 stations. At S and M, nitrification showed 2 maxima, one in spring and one in late summer. At A, however, a maximum in nitrification was evident in June. The highest rates of coupled nitrification-denitrification were found in spring (April 1994). The fraction of total nitrification which was coupled to denitrification [$D_n/(N_w + D_n)$] was with few exceptions below 50%. The lowest values ranging from 1 to 5% were found at A in June, S in August and M in October. The annual mean rate of dark nitrification at S, A and M (June 1993 to April 1994) was 6.1, 8.7 and 16 $\mu\text{mol m}^{-2} \text{h}^{-1}$, respectively, of which 17, 11 and 27% was coupled to denitrification.

Test incubations

Influence of $^{15}\text{NO}_3^-$ additions

The rates of O_2 uptake, DIN exchange and denitrification in cores with variable $^{15}\text{NO}_3^-$ additions showed consistent results irrespective of station and season (Fig. 4). The presence of the cockle *Cerastoderma edule* at Stn M influenced fluxes and process rates considerably and forced us to separate cores in this specific experiment into those with and those without this actively filtrating animal. The O_2 uptake was independent of the NO_3^- concentration whereas the NO_3^- uptake (measured net NO_3^- uptake plus N_w) increased linearly with NO_3^- concentration within the range measured. The NH_4^+ release was generally independent of the NO_3^- concentration, but positively correlated with the O_2 uptake in the M sediment ($r^2 > 0.64$; not shown). An NH_4^+ uptake was generally observed in April except in muddy cores containing *C. edule*.

Proportionality between the water phase NO_3^- concentration and the corresponding denitrification of NO_3^- from the water phase (D_w^{tot}) is indicated by regression lines in Fig. 4. Higher D_w^{tot} than expected

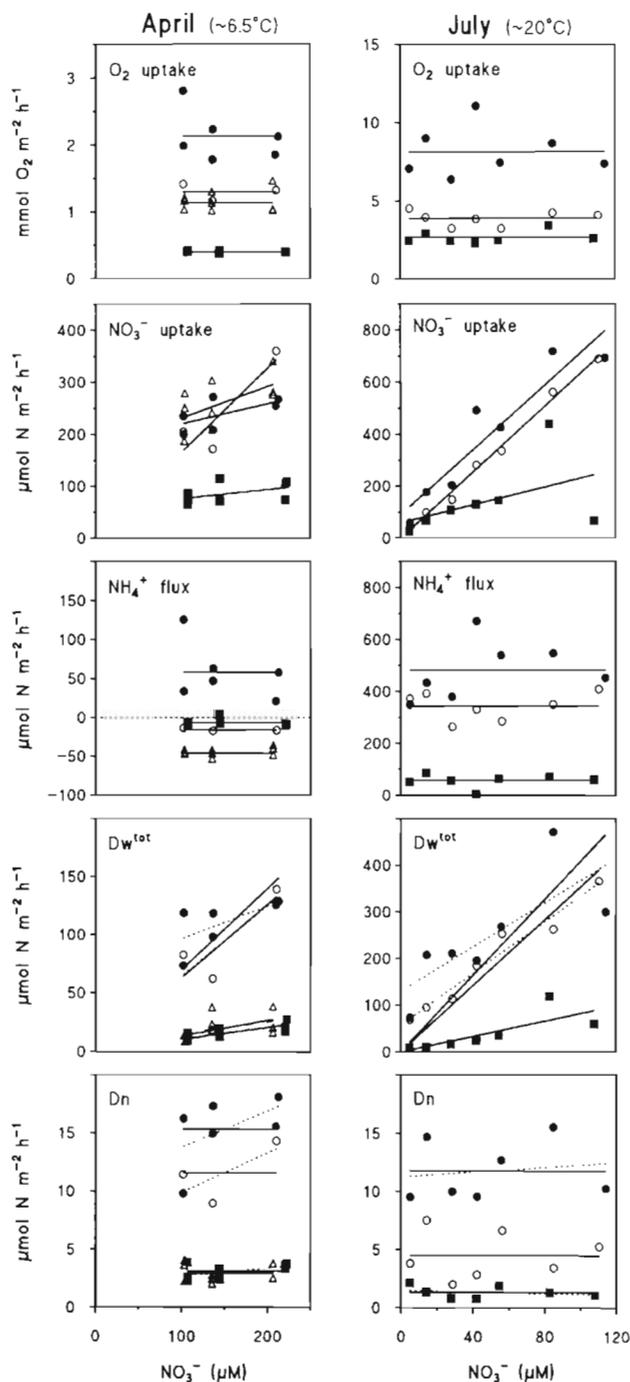


Fig. 4. Effect of variable ^{15}N -nitrate additions on measured dark fluxes of O_2 and NH_4^+ , NO_3^- (+ NO_2^-) uptake and estimated D_w^{tot} and D_n . The test was carried out in a situation of low ($< 0.1 \mu\text{M}$, July 1994) and high ambient nitrate (65 to 71 μM , April 1994). (●) M⁺; (○) M⁻; (Δ) S; (■) A; + and - indicate cores with and without *Cerastoderma edule*. For O_2 and NH_4^+ fluxes the mean values are indicated as horizontal lines and for NO_3^- uptake the free linear regression lines are shown. For D_w^{tot} the lines for linear regression forced through zero as well as the free linear regression lines (dotted) are given. For D_n the mean values and the free linear regression lines (dotted) are given. Positive NH_4^+ fluxes indicate release

were found for the lowest NO_3^- additions in the M sediment. Using free linear regression, significant deviation of the intercept from zero (t -test, $\alpha = 0.05$; Zar 1984) was only found in the M sediment without *Cerastoderma edule* in July. Omitting the 2 lowest concentrations from the regression, the intercept was not significantly different from zero. The rate of coupled nitrification-denitrification (D_n) was not significantly affected by the added $^{15}\text{NO}_3^-$ regardless of ambient NO_3^- , i.e. significant deviation of the slope from zero was not detected.

Microalgal interference

The addition of NO_3^- in the AA incubations (Table 1; Incubation 2) did not affect NH_4^+ and O_2 fluxes compared to the initial unamended incubation (1). In the $^{15}\text{NO}_3^-$ incubations (2 & 3) the NO_3^- concentration was approximately doubled compared to the unamended situation (69 to 76 μM) causing almost a doubling of NO_3^- uptake in the cores with no NH_4^+ added. Addition of NH_4^+ on the other hand reduced this increased NO_3^- uptake by 20 to 43%. In the $^{15}\text{NO}_3^-$ incubation without NH_4^+ approximately 14% of the NO_3^- uptake

was denitrified in S and A sediment compared to 35% for M sediment. When NH_4^+ was added, denitrification of NO_3^- from the water phase was increased by 41 to 44% in the S and A sediment and reduced by 13% in the M sediment. About one third of the NO_3^- taken up was then denitrified in all sediment types. At the 2 sandy sediments, NH_4^+ additions increased the coupled nitrification-denitrification significantly, whereas at M the effect was insignificant (t -test, $\alpha = 0.05$). For all 3 sediments, the effect of NH_4^+ addition on N_w (the part of nitrification not coupled to denitrification but released as NO_3^- to the overlying water) was smaller than (S, A) or similar to (M) the effect on coupled nitrification, D_n .

The constant-dark (CD) treatment (Table 2; Incubation 3) showed no significant impact on O_2 uptake compared to the standard 2 h dark preincubated cores with NO_3^- (Incubation 2), but NH_4^+ release was increased and at Stn S, NO_3^- uptake was decreased. In S and Mx sediments, 8 and 22%, respectively, of the NO_3^- uptake was denitrified in the standard $^{15}\text{NO}_3^-$ incubation, compared to 27 and 57% when the dark preincubation time was prolonged to 48 h. Both parts of the nitrification (D_n and N_w) were increased several-fold after the 48 h dark preincubation period.

Table 1. Effect of adding surplus (500 μM) NH_4^+ on O_2 and DIN (dissolved inorganic nitrogen) uptake, nitrification and denitrification in April 94 (AA incubations). Results for the first incubation (1) are taken from Kristensen et al. (unpubl.), and the unamended flux cores were used in the other incubations (2 and 3) where half of the cores received 500 μM NH_4^+ . NO_3^- uptake was determined by adding N_w to the measured net NO_3^- uptake (N_w from Incubation 2 was used to correct the measured net NO_3^- uptake in Incubation 1). Stimulation of D_w^{tot} is normalized to the same nitrate concentration. Negative sign for NH_4^+ flux denotes uptake. Standard error of the mean is indicated

Station Incubation	NO_3^- (μM)	O_2 uptake ($\text{mmol m}^{-2} \text{h}^{-1}$)	NH_4^+ flux	NO_3^- uptake	D_w^{tot} ($\mu\text{mol m}^{-2} \text{h}^{-1}$)	D_n	N_w
S							
(1) Unamended	71	1.06 ± 0.08	-27 ± 2	176 ± 25			
(2) $^{15}\text{NO}_3^-$	132	1.21 ± 0.04	-34 ± 2	265 ± 20	37 ± 6	3.2 ± 0.4	10 ± 1
(3) $^{15}\text{NO}_3^- + \text{NH}_4^+$	134	1.27 ± 0.16	-958	152 ± 43	52 ± 6	6.1 ± 0.7	11 ± 2
Stimulation:				-43% ^c	+41% ^a	+91% ^b	+3%
A							
(1) Unamended	76	0.55 ± 0.04	-18 ± 5	66 ± 7			
(2) $^{15}\text{NO}_3^-$	128	0.66 ± 0.09	-20 ± 1	107 ± 20	15 ± 2	1.7 ± 0.1	2.9 ± 0.7
(3) $^{15}\text{NO}_3^- + \text{NH}_4^+$	127	0.64 ± 0.03	-458	63 ± 9	21 ± 0	3.7 ± 0.2	4.9 ± 0.4
Stimulation:				-41% ^c	+44% ^b	+120% ^b	+67% ^c
M							
(1) Unamended	69	2.2 ± 0.2	36 ± 20	197 ± 44			
(2) $^{15}\text{NO}_3^-$	134	2.4 ± 0.4	49 ± 23	294 ± 44	102 ± 19	13 ± 2	20 ± 10
(3) $^{15}\text{NO}_3^- + \text{NH}_4^+$	132	2.1 ± 0.3	-1815	236 ± 13	88 ± 2	16 ± 2	27 ± 2
Stimulation:				-20%	-13%	+25%	+32%

^a 187% if compared to the seasonal study

^b Significant difference (t -test, $\alpha = 0.05$)

^c $\alpha = 0.10$

Table 2. Effect of preincubation time in darkness on O₂ and DIN uptake, nitrification and denitrification in June 1993 (CD incubations). Results for the first incubation (1) are taken from Kristensen et al. (unpubl.). A standard 2 h dark preincubation was used in the second incubation, whereas in the third incubation the cores were preincubated for 48 h in darkness before ¹⁵NO₃⁻ addition. Corrected NO₃⁻ uptake, stimulation of D_w^{10t}, NH₄⁺ flux sign and standard error as in Table 1

Station Incubation	NO ₃ ⁻ (μM)	O ₂ uptake (mmol m ⁻² h ⁻¹)	NH ₄ ⁺ flux	NO ₃ ⁻ uptake	D _w ^{10t} (μmol m ⁻² h ⁻¹)	D _n	N _w
S							
(1) Unamended	6.3	2.5 ± 0.3	33 ± 11	40 ± 4			
(2) 2 h ¹⁵ NO ₃ ⁻	41	1.9 ± 0.1	-43 ± 9	147 ± 14	12 ± 2	0.34 ± 0.06	1.4 ± 0.7
(2) 48 h ¹⁵ NO ₃ ⁻	39	1.6 ± 0.2	152 ± 29	70 ± 8	19 ± 2	0.85 ± 0.19	5.6 ± 0.5
Stimulation:				-52% ^a	+66% ^b	+150% ^a	+307% ^a
Mx							
(1) Unamended	5.3	2.3 ± 0.1	261 ± 15	14 ± 7			
(2) 2 h ¹⁵ NO ₃ ⁻	43	2.3 ± 0.1	250 ± 22	192 ± 11	42 ± 6	2.0 ± 0.2	10 ± 2
(2) 48 h ¹⁵ NO ₃ ⁻	46	2.4 ± 0.1	440 ± 16	178 ± 3	101 ± 13	11 ± 3	59 ± 26
Stimulation:				-7%	+125% ^a	+453% ^b	+492% ^b
^a Significant difference (<i>t</i> -test, α = 0.05)							
^b α = 0.10							

DISCUSSION

Reliability of the methods

Three basic assumptions have to be satisfied when the ¹⁵N pairing technique is used for ambient denitrification measurements (Nielsen 1992): (1) the added nitrate should not interfere with the denitrification of ambient water phase nitrate (*D_w*), implying that denitrification of nitrate from the water phase should be proportional to the nitrate concentration; (2) the rate of coupled nitrification-denitrification (*D_n*) should be unaffected by the ¹⁵N-nitrate concentration indicating an even distribution of the nitrate isotopes in the denitrification zone; and (3) the measured rates should be unaffected by the incubation time.

In the sandy S and A sediments the first and second assumptions were fulfilled at ¹⁵N-nitrate concentrations from 5 to 120 μM (Fig. 4). In the muddy M sediment the second assumption was satisfied but the lowest ¹⁵N-nitrate additions tended to give higher *D_w* rates. This could be caused by the pronounced heterogeneity of this muddy sediment. Other studies using the same technique on sandy or silty sediments found no such effect on *D_w* (Pelegri et al. 1994, Rysgaard et al. 1995, Nielsen & Glud 1996). Since a minimum of 30 μM ¹⁵N-nitrate additions were used in our seasonal incubations we therefore assumed a proportionality between *D_w*^{10t} and the nitrate concentration.

The third assumption is critical as the present isotope pairing technique requires a relatively long time for the ¹⁵N-nitrate gradient to stabilize within the sediment. The same stabilization time (15 min) was used

during all study periods and this time was probably somewhat too long in summer and too short in winter. However, much longer incubation times were used in order to make the effect of the stabilization time small. In most of the seasonal incubations presented here, different durations of incubation were used and the effect of time proved insignificant (*t*-test, α = 0.05).

The use of the isotope dilution technique in shallow areas requires that the measurement of *N_w*, the part of nitrification where the produced nitrate is released to the water phase, is independent of microphytobenthic activity. As the calculation of *N_w* is based on the dilution of the water phase ¹⁵N-nitrate during incubation, algal assimilation of the ¹⁴N-nitrate produced by nitrification before entering the water phase could create a serious underestimation of *N_w*. A decrease in algal DIN assimilation is therefore expected to stimulate the measured *N_w* more than *D_n*. The ammonium addition (AA) and the constant-dark (CD) experiments showed that microphytobenthic nitrate uptake only interfered to a limited extent with the measurement of *N_w*, despite an effect of the microalgae on the overall nitrification rate. We only observed an effect in the sandy S sediment in June which may have been coincidental. The low interference from the microalgae on the measurement of *N_w* was somewhat surprising, since more than two thirds of the nitrate uptake in the dark denitrification incubation was assimilated by the algae (Tables 1 & 2). The AA and CD treatments might have altered the depth distribution of nitrification in the oxic zone as has been shown in experiments with ammonium addition to dark incubated homogenized sediment (Jensen et al. 1993), but this should actually

increase the ratio $N_w/(N_w + D_n)$. A plausible explanation could be that the microphytobenthic activity was restricted to a very narrow surface zone, where the concentration of nitrate diffusing from the water column was much higher than the concentration of nitrate diffusing from a nitrification zone located deeper in the oxic zone (Jensen et al. 1993, 1994).

Previous ^{15}N -nitrate isotope dilution studies using continuous flow systems with estuarine diatom-inhabited sediments in day-night cycles have provided contradictory results. Thus, Risgaard-Petersen et al. (1993) observed a stabilization of the isotope dilution within few hours after light was turned off indicating a subsequent stable N_w measurement; whereas Rysgaard et al. (1993) in a similar sediment found that algal assimilation of nitrate continued for about 25 h after light was turned off and more than 10 h was required for N_w to stabilize. Since we have used the same preincubation procedure in all our investigations and found no significant changes of N_w in our time course incubations, we believe that the present measurement of dark nitrification not coupled to denitrification is not seriously affected by microphytobenthic activity.

Seasonal variations of denitrification

The dominating source of nitrate for dark denitrification was from the water phase at all stations and for most of the year. D_w followed largely the water phase nitrate concentration (Figs. 2 & 3) with highest rates in April 1994. The coupled rate (D_n) dominated only during summer when the overlying water was almost nitrate depleted. The highest rates of coupled denitrification were, nevertheless, found in spring. Similar results have previously been found in other coastal sediments (Nielsen et al. 1994, Rysgaard et al. 1995). Spring maxima of sediment denitrification at relatively low, but increasing, temperatures in near-coastal areas (Jørgensen & Sørensen 1985, Jensen et al. 1988) have been explained by high water phase nitrate concentrations combined with progressively lower oxygen penetration depth (i.e. small diffusion distance for nitrate) due to increasing O_2 consumption (Jensen et al. 1990).

Assuming that nitrate is denitrified in a narrow zone just underneath the oxic zone and that microphytobenthic assimilation of nitrate or other nitrate-consuming processes do not interfere with the flux of nitrate to the denitrification zone, Fick's first law can be used to calculate the denitrification of nitrate from the water phase:

$$D_w = D_{\text{NO}_3} \times \phi^m \times [\text{NO}_3^-]/Z_{\text{O}_2} \quad (8)$$

where D_{NO_3} is the diffusion coefficient of nitrate in seawater (30‰) corrected for temperature (T): $D_{\text{NO}_3} =$

$(9.36 + 0.33 \times T) \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$; ϕ is the mean porosity in the oxygen penetration zone Z_{O_2} and $m = 2$ or 3 depending on the porosity being below or above 0.7 (Li & Gregory 1974, Ullman & Aller 1982, Christensen et al. 1990). The oxygen penetration depths in the dark varied seasonally from 5 to 20 mm at S, and from 0.4 to 4 mm at M (Bruns et al. 1995); oxygen penetration depths were not measured at Stn A. This diffusion-based approach has been intensively used and verified in previous denitrification studies (Christensen et al. 1990, Nielsen et al. 1990b, Jensen et al. 1994, Risgaard-Petersen et al. 1994, Rysgaard et al. 1994). It should be noted, however, that the oxygen penetration depths used here were not measured during the same incubations or in the same cores as the denitrification rates and differences in stirring conditions and general core variability could be of importance. Another problem in the present study is the low depth resolution of the porosity measurement in the upper sediment layer (3 mm), since diffusivity may change considerably in the upper few millimeters of the sediment (Jensen et al. 1994). Despite these considerations, the calculated values were generally close to the corresponding measured values of D_w (Fig. 5). Large deviations were only found at S in April 1994, and at M in June 1993 probably due to microalgal interference (see Tables 1 & 2). There were, however, no indications that assimilation by the benthic microalgae severely affected the transport of nitrate to the denitrification zone at other times, even though D_w only accounted for 13% (range 1 to 66%) of the total NO_3^- uptake (i.e. minus measured NO_3^- flux from Fig. 6 plus N_w from Fig. 3) with highest

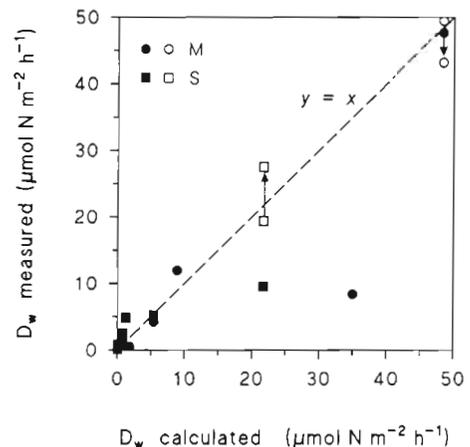


Fig. 5. Measured versus calculated D_w . Calculated D_w is based on Fick's first law using measured water phase NO_3^- concentration and dark O_2 penetration depths (see text). Open symbols: data from the AA experiment (Table 1; Incubations 2 and 3), where arrows indicate the effect of the ammonium addition (the results are normalized to the ambient nitrate concentration)

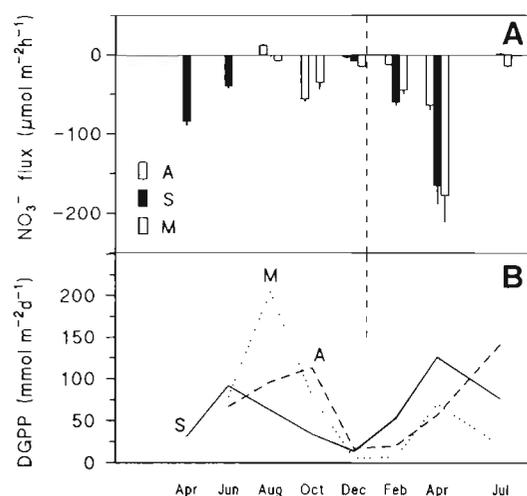


Fig. 6. Seasonal variation in (A) dark unamended NO_3^- ($+\text{NO}_2^-$) flux (negative values denotes uptake) and (B) daily gross primary production (DGPP is O_2 production in light + O_2 uptake in dark corrected for length of day). Results are obtained from Kristensen et al. (unpubl.); these incubations were conducted in parallel with the ^{15}N incubations. In April and June 1993, NO_3^- flux was only measured at Stn S; NO_3^- flux was not measured at S in October 1993 and at A in July 1994

values in winter and in the middle of summer. Micro-sensor investigations and model calculations have previously shown that competition between NO_3^- assimilation in a narrow surface zone and denitrification in deeper anoxic zones could be negligible (Nielsen et al. 1990b).

The annual mean dark denitrification rate of water phase NO_3^- (D_w) increased in the sequence A, S, M. This was probably due to smaller diffusional distances for water phase NO_3^- to the denitrification zone with increasing oxygen uptake (Fig. 2). Similar results were obtained from biofilms (Nielsen et al. 1990a) and marine sediments (Jensen et al. 1988, Caffrey et al. 1993, Sloth et al. 1995) with different organic loadings. The difference in mean dark denitrification at the 3 stations is probably a result of organic matter content and reactivity and thus differences in mineralization rate.

Seasonal variations of nitrification

Dark nitrification was low in the early winter in all sediments probably due to low temperature and low mineralization (i.e. low NH_4^+ production). Low nitrification during winter has frequently been observed in seasonal investigations of coastal sediments (MacFarlane & Herbert 1984, Seitzinger et al. 1984), but the opposite pattern has also been reported (Hansen et al.

1981). In all 3 sediments the nitrification increased in February, probably as a response to increasing NH_4^+ availability, although the NH_4^+ concentration in the water column only increased slightly (Fig. 2A).

A spring maximum in nitrification rate was detected in April at M and S whereas nitrification was repressed in the middle of summer. Low nitrification rates during warm summer months have been explained by a combination of low oxygen availability, H_2S inhibition and competition for NH_4^+ (Hansen et al. 1981, Jenkins & Kemp 1984, Seitzinger et al. 1984, Henriksen & Kemp 1988, Kemp et al. 1990, Joye & Hollibaugh 1995). In the present study, conditions of low oxygen availability and presence of H_2S could prevail and inhibit nitrification since intense sulfate reduction was observed throughout the summer (maximum $2.3 \text{ mmol m}^{-2} \text{ h}^{-1}$ at Stn M; Kristensen et al. 1996) with peak rates close to the sediment surface. The sulfate reduction was still high in August when a late summer maximum in nitrification rate was evident at M and S, but it was observed that maxima in nitrification at these 2 stations generally occurred at the time of maxima in daily gross primary production, DGPP (Fig. 6). Although no correlation was evident between dark nitrification and dark oxygen penetration, the microalgal oxygen production during daylight hours may have had a prolonged oxidizing effect during the night. The correspondence between DGPP and nitrification indicates that competition for NH_4^+ was of minor importance especially on M, where ammonium was always released from the sediment (Fig. 2). The NH_4^+ addition experiment (AA) also indicated that microalgal assimilation of NH_4^+ could be less inhibitory to nitrification in the muddy M sediment than in the sandy S sediment. However, an inhibition of nitrification by the microalgae was indicated in the CD incubation in the sandy S sediments during early summer (June 1993, July 1994) when the correspondence between DGPP and nitrification was absent.

A different pattern of seasonal control of nitrification may be present at Stn A. Thus, in this sediment no relationship was evident between nitrification and DGPP (Fig. 6). Instead the highest nitrification was found in summer (June 1993) when DGPP was relatively low. Ammonium was nearly always taken up by this sediment (Fig. 2) and the effect of NH_4^+ addition (AA experiment) indicated that the inorganic nitrogen availability was more limited at A than at S, which diminished the role of O_2 availability. Nitrification at Stn A was probably controlled by competition for ammonium with the benthic microalgae combined with a positive influence of temperature on both mineralizing and nitrifying bacteria. Similar seasonal trends have been found by Nixon et al. (1976), Seitzinger et al. (1984) and MacFarlane & Herbert (1984). It should be noted that the rates of nitrification presented

here may be underestimated, especially at Stn A, due to the absence of deep burrowing *Arenicola marina* in our cores. Previous results from Königshafen (Hüttel 1990) have indicated that bioturbation by these animals has a stimulating effect on nitrification deep in the sediment.

Coupling of nitrification and denitrification [$D_n/(N_w + D_n)$] was generally low, especially during late summer or autumn in all sediment types (Fig. 3). A low coupling in autumn has been observed from investigations in deeper North Sea sediments (Lohse et al. 1993). Nitrification during this period may have occurred at the very top of the oxic zone, fed by NH_4^+ from mineralization and animal excretion in the aerobic zone.

The 2 different patterns of seasonal control of nitrification in the 3 sediment types was probably caused by increasing mineralization in the sequence A, S, M (Fig. 2). This is in accordance with the higher organic contents of the muddy sediment compared to the 2 sandy sediments, although a significant difference in bulk organic content between the 2 sandy sediments was not evident. Similar conclusions were drawn by Lohse et al. (1993) from studies of nitrification in sediments of the southeastern North Sea. The annual mean dark nitrification rate was higher in the muddy M than in the sandy sediments. Increased nitrification under moderate organic loading have been predicted from models (Blackburn 1990, Blackburn & Blackburn 1993) and experimentally verified in marine sediment systems (Caffrey et al. 1993, Sloth et al. 1995). Another controlling factor could be the dependence of nitrifying bacteria on surface area (Henriksen & Kemp 1988). It seems plausible that the higher mean dark nitrification rate at the muddy sediment compared to the 2 sandy sediments was an effect of a higher content of organic rich fine particles combined with higher mineralization rates.

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