Microphytobenthos activity and fluxes at the sediment-water interface: interactions and spatial variability

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Abstract

In this study oxygen and nutrient fluxes and denitrification rates across the sediment-water interface were measured via intact core incubations with a twofold aim: show whether microphytobenthos activity affects these processes and analyse the dispersion of replicate measurements. Eighteen intact sediment cores (i.d. 8 cm) were randomly sampled from a shallow microtidal brackish pond at Tjarno, on the west coast of Sweden, and were incubated in light and in darkness simulating *in situ* conditions. During incubation O_2, inorganic N and SiO_2 fluxes and denitrification rates (isotope pairing) were measured. Assuming mean values of 18 cores as best estimate of true average (BEA), the accuracy of O_2, NH_4^+, NO_3^- and SiO_2 fluxes calculated for an increasing number of subsamples was tested. At the investigated site, microalgae strongly influenced benthic O_2, inorganic N and SiO_2 fluxes and coupled (D_n) and uncoupled (D_w) denitrification through their photosynthetic activity. In the shift between dark and light conditions NH_4^+ and SiO_2 effluxes (~60 and ~110 μmol m^{-2} h^{-1}) and D_n (~5 μmol m^{-2} h^{-1}) dropped to zero, NO_3^- uptake (~70 μmol m^{-2} h^{-1}) showed a 30% increase, while D_w (~20 μmol m^{-2} h^{-1}) showed an 80% decrease. For O_2 and NO_3^- dark fluxes, 4 core replicates were sufficient to obtain averages within 5–10% of the best estimated mean, while 10–20% accuracy was obtained with 4–12 replicates for SiO_2 and > 10 replicates for NH_4^+ dark fluxes. Mean accuracy was considerably lower for all light incubations, probably due to the patchy distribution of the benthic microalgal community.

Introduction

Benthic microalgae form more or less heterogeneous thin layers at the sediment surface; their occurrence is generally regulated by water column light penetration, while their distribution quite unpredictably depends on factors like grazing pressure, physical disturbance or the redox state of the surficial sediment (MacIntyre et al. 1996). The uptake of nutrients by benthic microalgae can attenuate the net regeneration to the water column and may result in a temporary trap for inorganic N, PO_4^{3-} and SiO_2 (Sundbäck and Graneli 1988; Rizzo 1990; Sundback et al. 1991; Sundback et al. 2000). Together with this direct ef-

fect, microalgae can improve indirectly, via reoxidation of Fe(II), the retention of PO_4^{3-} (Sundbäck and Graneli 1988). Furthermore the production of O_2 at the interface can expand the oxic sediment layer and stimulate the N-removal via coupled nitrification-denitrification. Simultaneously, the uptake of NH_4^+ and the subtraction of porewater CO_2 can result in a competition with nitrifiers (Wiltshire 1992; Risgaard-Petersen et al. 1994; Rysgaard et al. 1995; Lorenzen et al. 1998). Additionally the activity of microalgae at the interface can rise the porewater pH to values above 9, and nitrifiers performance is generally low at very alkaline pH values probably due to the occurrence of free NH_3, which is toxic to the bacteria (Fo-

cht and Verstraete 1977). Fluxes at the sediment-water interface and microphytobenthos activity have been measured in a range of environments with more or less sophisticated methods. In the literature, rates have been obtained with *in situ* incubations of benthic chambers (Klump and Martens 1981; Hall 1984; Boynton and Kemp 1985; Reay et al. 1995), models for the interpretation of porewater profiles (Klump and Martens 1981; Lerat et al. 1990; Glud et al. 1992, Lorenzen et al. 1998) and boat or laboratory incubations of sediment cores (Sundbäck and Graneli 1988; Nielsen et al. 1990; Sundbäck et al. 1991; Rysgaard et al. 1995).

In situ incubations are generally very expensive, as they require specific and sophisticated instrumentation, the same is true for sediment microprofiling; for routine measurements of fluxes the incubation of sediment cores is conversely very practical and inexpensive. Even if sediment core incubation is a relatively easy experimental approach, scientists tend to minimise the number of replicates, and reported fluxes are often based on the average of 3 to 5 cores. Among the investigated environments are sediments from the open sea, which are generally considered to be homogeneous, and sediments from coastal shallow areas, which on the contrary are always heterogeneous. This heterogeneity is a consequence of the benthic primary producers patchiness (Admiraal 1984), the distribution of bioturbating fauna (Fleeger and Decho 1987; Kristensen 1988), the hydrodynamism of the water column, the different rate of particle sedimentation and the salinity gradients (Wilken et al. 1990).

In this paper we present results from measurements of light and dark benthic fluxes of O_2, inorganic N and Si and rates of nitrification-coupled (D_n) and uncoupled (D_w) denitrification in a shallow brackish pond colonised by a mat of benthic diatoms. In order to evaluate the net effect of microphytobenthos activity on sediment-water exchanges, 18 cores (for a total investigated surface of approximately 900 cm^2) were incubated in light and darkness for O_2 and inorganic nutrient measurements, while 9 cores were incubated for light and dark denitrification measurements. The dispersion of the measurements was then analysed with respect to the two incubation conditions and the target compounds. This was performed assuming mean values of 18 cores to provide the best estimate of true average (BEA) and evaluating the accuracy of mean O_2, NH_4^+, SiO_2 and NO_3^- fluxes calculated for an increasing number of subsamples.

Study area

Sediment cores were collected from a very shallow microtidal brackish pond at Tjarno (58°52' N, 11°09' E) on the west coast of Sweden. The sampling site had a muddy, reduced black sediment covered by a mat of benthic microalgae; the height of the water column was 20–25 cm and the salinity was between 22 and 35‰. The mat was mostly composed by diatoms, mainly the large epipelic diatom *Gyrosigma balticum*, but also *Pleurosigma angulatum* and some *Amphora* species and by cyanobacteria (*Oscillatoria sp.*) (Sundbäck, personal communication). Sediment samples were collected along a 15 cm horizon via 8 cm i.d. plexiglass cores and sieved with a 500 μm mesh net for a qualitative recognisement of benthic fauna whilst surface samples (~0.5 cm) were collected for chlorophyll *a* determination. The macrofauna community was dominated by the crustacean *Corophium insidiosum* and by the larvae of *Chironomus salinarius,* which were quite homogeneously distributed at densities of respectively 350 and 500 ind m^{-2}. No large size animals were found in the surficial sediment. Mean microalgal biomass, expressed as chl *a* concentration, was 76.3 ± 12.5 mg m^{-2} (Miles, personal communication).

Materials and methods

Benthic fluxes and denitrification rates

Surface sediments were collected by hand from an area of approximately 2 m^2 using transparent Plexiglas cores on 09/12/98. Five cores (i.d. 5 cm, height 30 cm) were sampled for pore water analysis: 5 sediment slices (0–0.5; 0.5–1, 2–3, 4–5, 9–10 cm) were squeezed under nitrogen and interstitial water collected into preevacuated gas-tight vials. Eighteen cores (i.d. 8 cm, height 30 cm) were randomly taken for flux measurements; after sampling the height of the sediment in each core was levelled to 20 cm. The cores were then submerged and pre-incubated in an incubation system in which running *in situ* water was vigorously bubbled with air; the stirring system of the water inside the cores (a teflon-coated 5 cm long magnet driven by an external motor at 60 rpm) was on during the whole preincubation period. The incubations were started the day after the sampling; light incubations were performed at midday while dark incubations were performed in the night. The top lids,

made of transparent plexiglass, were screwed to the core body at the beginning of each incubation. The same set of cores was incubated for light and dark denitrification measurements on 09/14/98 (n = 9). For both experiments the incubation temperature was 16 °C and the water salinity was 25 ‰; the incubation apparatus was provided with a series of lamps each positioned on top of a core with a homogeneous light intensity (~500 μE m^{-2} s^{-1} in the 400 to 700 nm range) close to the *in situ* average conditions.

Fluxes were defined as the difference between final and initial concentrations in the water column after 1.5 hours of incubation as calculated by Equation (1); incubation time was chosen in order to keep oxygen variation within 20% of the initial value.

$$F_x = \frac{(C_f - C_i) \times V}{A \times t}$$

Fx = flux of the x species (μmol m^{-2} h^{-1})
Cf = final concentration of x (μM)
Ci = initial concentration of x (μM)
V = volume of water (l)
A = surface area (m^2)
t = incubation time (h)

The isotope-pairing technique (Nielsen 1992) was used to measure sedimentary denitrification. This method makes it possible to split total denitrification into denitrification of nitrate diffusing to the anoxic sediment from the water column (D_w) and denitrification of nitrate produced within the sediment due to nitrification (D_n). At the beginning of the experiment, $^{15}NO_3^-$ was added to the water column to produce a final concentration of ~20 μM. The NO_3^- concentration was measured prior to the addition of $^{15}NO_3^-$ and at the time the cores were closed (within 5 minutes from the addition of $^{15}NO_3^-$) in order to calculate the $^{14}N/^{15}N$ ratio in the NO_3^- pool. At the end of incubation, 10 ml of ZnCl$_2$ (7 M) were added to the water phase and then sediment and water were mixed. Part of the slurry was then transferred in 12.5 ml gas-tight vials; $^{14}N^{15}N$ and $^{15}N^{15}N$ abundance in N$_2$ was analysed by mass spectrometry at the National Environmental Research Agency, Silkeborg, Denmark. The rates of denitrification were calculated by using the equations and principles developed by Nielsen (1992):

D_{15} = p($^{15}N^{14}N$)+2p($^{15}N^{15}N$) and D_{14} = p($^{15}N^{14}N$) + 2p($^{14}N^{14}N$), where D_{15} and D_{14} = rates of denitrification based on $^{15}NO_3^-$ and $^{14}NO_3^-$, respec-

tively; and p($^{14}N^{14}N$), p($^{15}N^{14}N$) and p($^{15}N^{15}N$) = rates of production of labelled and unlabelled N$_2$ species. Because the p($^{14}N^{14}N$) cannot be readily measured, estimation of D_{14} was obtained from: D_{14} = $D_{15} \times$ p($^{15}N^{14}N$)/2p($^{15}N^{15}N$). The proportion of D_{14} supported by unlabelled NO_3^- from the water column (D_w) was calculated from: $D_w = D_{15} \times f/(1-f)$, where f = mole fraction of $^{14}NO_3^-$ in the water column. The coupled nitrification-denitrification (D_n) difference was calculated as: $D_n = D_{14} - D_w$.

O$_2$ concentration was measured by Winkler titration (Strickland and Parsons, 1972); NO_3^- was determined after reduction to NO_2^- in the presence of cadmium, NO_2^- was determined spectrophotometrically using sulphanilamide and N-(1-naphtyl)ethylendiamine (Golterman et al. 1978). NH_4^+ was determined spectrophotometrically using salicylate and hypochlorite in the presence of sodium nitroprussiate (Bower and Holm-Hansen 1980). SiO$_2$ was determined after reaction with sodium molybdate and H$_2$SO$_4$ as discussed by Golterman et al. (1978); PO_4^{3-} was determined spectrophotometrically after reaction with ammonium molybdate and potassium antimonyl tartrate and reduction by ascorbic acid (Valderrama 1977).

Differences between light and dark fluxes and denitrification rates were tested with a one way ANOVA.

Distribution of the measurements

1000 sets of n subsamples with n comprised between 4 and 18 were randomly resampled from each group of 18 light and dark O$_2$, NH_4^+, NO_3^- and SiO$_2$ fluxes using a bootstrap technique (Resampling Stats package, Version 4.1 for Windows 95). Each bootstrap set is a random sample of n values selected with replacement from the original group of fluxes. Because a bootstrap sample is taken and replaced, some of the original observations could be chosen more than once and others might not be chosen at all (Efron and Tibshirani 1986). The bootstrap technique was thus used to generate all possible subsets of size n from the original finite population of 18 fluxes.

The average, the standard deviation and the variation coefficient were calculated for each of these sets. Results for all combinations of experimental condition and target compound (i.e., dark nitrate fluxes) were populations of 1000 averages, 1000 standard deviations and 1000 variation coefficients, each in

turn characterised by a mean and a standard deviation.

The mean light and dark fluxes of O_2, NH_4^+, NO_3^- and SiO_2, experimentally calculated on 18 core replicates, were assumed to be the best estimate of the true average fluxes (BEA). The accuracy of mean fluxes obtained from the bootstrap-generated sets of n replicates was tested calculating their "distance" from the BEA according to Equation (2); 1000 d values were thus calculated for each value of n.

$$d = \frac{BEA - An}{BEA}$$

Where:

BEA = Best Estimate of the true Average flux (assumed as the average of 18 replicates)

An = Average flux of n bootstrap–generated subsamples with $4 \leq n \leq 18$

The "distance" d expresses how "far" a mean calculated on a certain number of subsamples is situated from the true one. For example, if $d = 0.05$ it means that the considered average is within the interval "true average $\pm 5\%$" (Sfriso et al. 1991).

Results

Benthic fluxes and denitrification rates

Nutrient concentrations in the water sampled from the pond on 9/12/98 were below 1 μM for NO_2^- and PO_4^{3-} and respectively 6.3, 8.0 and 9.8 μM for NH_4^+, NO_3^- and SiO_2. Porewater profiles of PO_4^{3-}, NH_4^+ and SiO_2 showed increasing concentrations with depth for all ions with values comprised respectively between 1 and 5, 18 and 270 and 9 and 54 μM (Figure 1). NO_2^- and NO_3^- concentrations were below the detection limit of our methods (1 μM) in the upper sediment layer.

NO_2^- and PO_4^{3-} concentrations at the beginning and at the end of the core incubations were close to the detection limit of our methods, thus PO_4^{3-} and NO_2^- fluxes were not considered to be reliable. Net O_2 light fluxes were always positive (from the sediment-water interface to the water column) and ranged between 1.83 and 7.95 mmol m^{-2} h^{-1}; dark fluxes ranged between –3.11 and –6.87 mmol m^{-2} h^{-1}. O_2 fluxes were significantly different in light and darkness (ANOVA, $P < 0.01$, n = 18) being respectively 4.31 $\pm$ 0.39 and
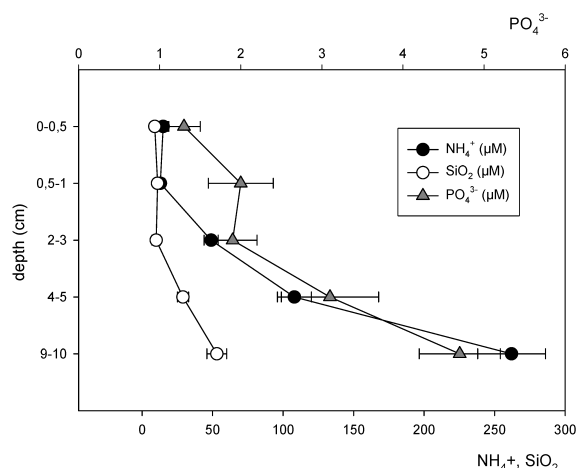

Figure 1. Porewater profiles of PO_4^{3-}, NH_4^+ and SiO_2.

Table 1. Average O_2 and nutrient fluxes and denitrification rates ($\pm$ standard deviation) determined during light and dark incubations. O_2, NH_4^+, NO_3^- and SiO_2 fluxes are mean values for 18 replicates, while denitrification rates are mean values for 9 replicates. D_w stands for denitrification of nitrate from the water column; D_n stands for denitrification of nitrate produced within the sediment due to nitrification

Flux	Light	Dark
O_2 (mmol m^{-2} h^{-1})	4.31 ± 0.39	-5.46 ± 0.26
NH_4^+ (μmol m^{-2} h^{-1})	1.9 ± 4.7	60.9 ± 12.0
NO_3^- (μmol m^{-2} h^{-1})	-88.1 ± 7.8	-64.1 ± 3.7
SiO_2 (μmol m^{-2} h^{-1})	-1.0 ± 6.4	107.1 ± 15.2
Dw (μmol m^{-2} h^{-1})	3.5 ± 1.2	16.8 ± 1.8
Dn (μmol m^{-2} h^{-1})	0.3 ± 0.2	5.2 ± 1.2

–5.46 $\pm$ 0.26 mmol m^{-2} h^{-1} (average $\pm$ standard deviation) (Table 1). Light and dark NH_4^+ fluxes were significantly different (ANOVA, $P < 0.01$, n = 18) being respectively 1.9 $\pm$ 4.7 and 60.9 $\pm$ 12.0 μmol m^{-2} h^{-1}. Dark NH_4^+ fluxes were all directed from the sediment to the water column and ranged between 14.2 and 208.5 μmol m^{-2} h^{-1}; light fluxes on the contrary were both positive and negative (and comprised between -33.3 and 40.9 μmol m^{-2} h^{-1}). Light and dark NO_3^- fluxes were all directed from the water column to the sediment; there was a significant difference between the two incubations (ANOVA, $P < 0.05$, n = 18). In light incubations, the average NO_3^- flux was -88.1 ± 7.8 μmol m^{-2} h^{-1} having values ranging between -6.4 and –132.0 μmol m^{-2} h^{-1}; in the dark, the average NO_3^- flux was –64.1 $\pm$ 3.7 μmol m^{-2} h^{-1} with values ranging between –44.3 and –104.2 μmol m^{-2} h^{-1}.

Light and dark SiO_2 fluxes (-1.0 ± 6.5 and 107.1 ± 15.2 μmol m^{-2} h^{-1}) were significantly different (ANOVA, P < 0.01, n = 18).

Denitrification rates of NO_3^- diffusing from the water column (D_w) and of NO_3^- produced within the sediment (D_n) were low (less than 20 μmol N m^{-2} h^{-1}) but significantly different in the light and in the dark (ANOVA, n = 18 P < 0.01 for both measurements). Dark D_w was in fact 5 times higher than light D_w (16.8 $\pm$ 1.8 and 3.5 $\pm$ 1.2 μmol m^{-2} h^{-1} respectively). Dn was also higher in the dark than in the light incubation (5.2 $\pm$ 1.2 and 0.3 $\pm$ 0.1 μmol m^{-2} h^{-1}).

Distribution of the measurements

After the resampling procedure, the bootstrap-generated mean fluxes and standard deviations were equal to those determined experimentally, confirming that the technique did not affect the characteristics of the original populations. An example of the results for light and dark O_2 fluxes is reported in Figure 2. All the means plotted in Figure 2 are fluctuating around the same value (be it the "true" average, standard deviation or variation coefficient), while their standard deviations, as expected, decrease as the number of extracted replicates (*n*) increases. Mean standard deviations (Figure 2, graph b) were ~1 and ~1.5 respectively for dark and light O_2 measurements, while mean variation coefficients (Figure 2, graph c) were approximately 35% for light and 18% for dark measurements. Similar results, with higher standard deviations and variation coefficients in the light incubations, were obtained for NH_4^+, NO_3^- and SiO_2 (data not shown).

d values calculated for light/dark O_2, NH_4^+, NO_3^- and SiO_2 are shown in Figure 3. Differences are evident between incubation conditions and target compounds: for all 4 species, *d* values were lower in the dark incubations than in the light ones, indicating that a lower number of cores were needed for dark measurements to obtain the same level of accuracy (same distance from BEA). For example, *d* values < 0.1 (mean of *n* subsamples within the 10% of the BEA) were determined when n = 4 cores in the dark O_2 incubation, while 8 cores were necessary in the light one to obtain the same level of precision. For a greater accuracy (*d* < 0.05) 8 cores were necessary for the dark incubations, while 18 cores were not enough for the measurements in the light.
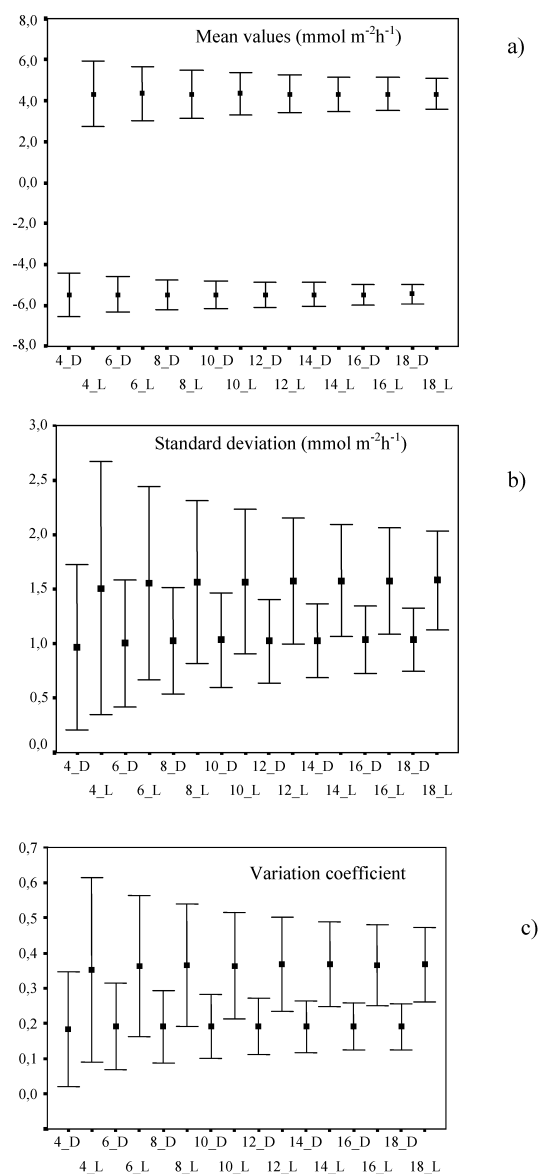
Figure 2. Mean average (a), standard deviation (b) and variation coefficient (c) $\pm$ standard deviation for 8 populations of dark and light O_2 fluxes. Each population was generated with a bootstrap extracting 1000 sets of *n* replicates (with *n* comprised between 4 and 18) from original populations of 18 light and dark measured fluxes.

Dark NH_4^+ fluxes were more variable compared to the O_2 ones with *d* values of ~0.3 calculated on sets of 4 subsamples and < 0.2 calculated when *n* > 10; *d* values for light incubations were one order of magnitude higher probably because, at Tjarno, average light NH_4^+ fluxes were very low and close to zero. A
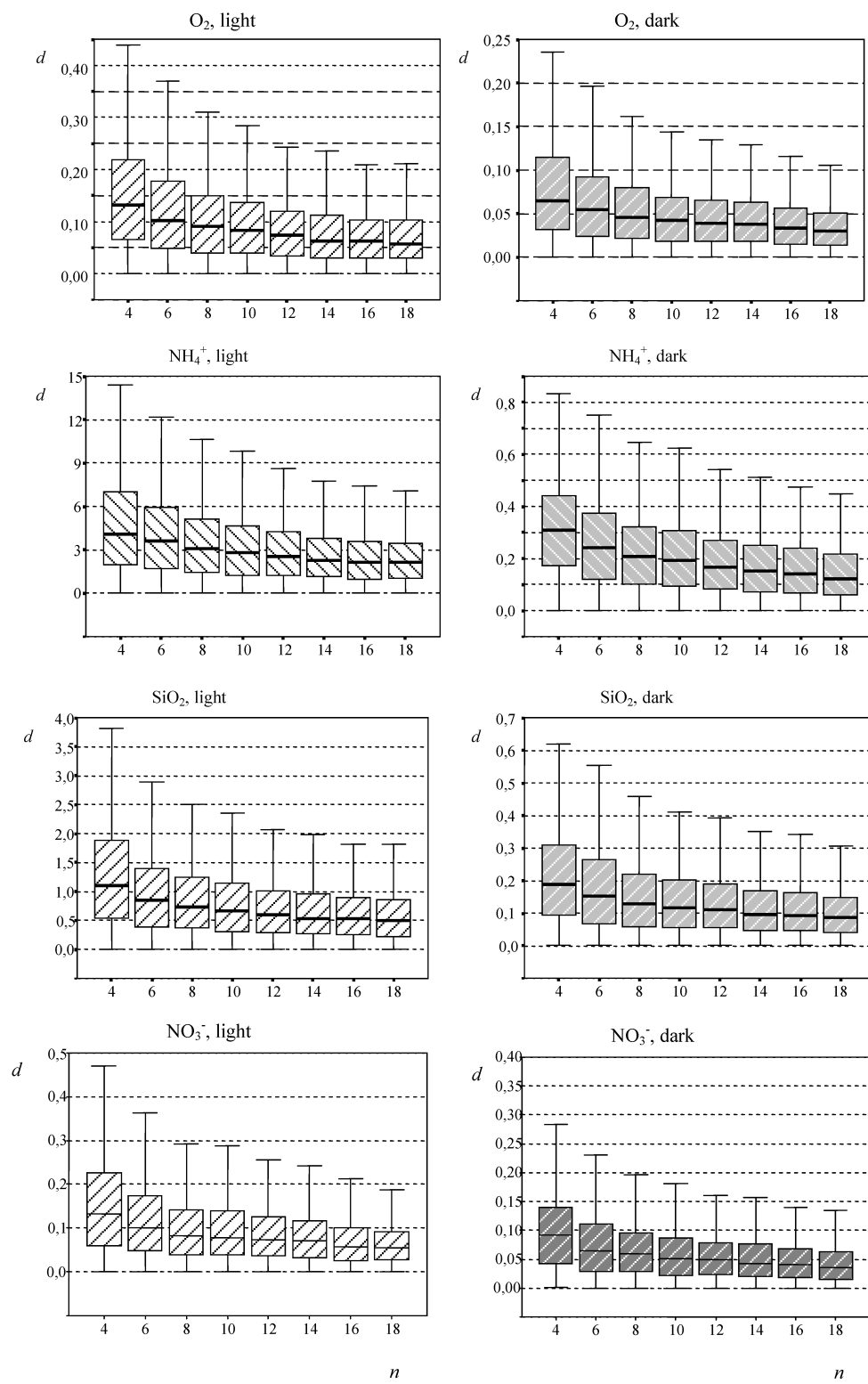
Figure 3. Deviation from BEA (Best Estimate of the true Average) in the light (left) and dark incubations for O_2, NH_4^+, NO_3^- and SiO_2 fluxes. (See text for further details).

similar result, with a great difference between light and dark d values, was obtained for SiO_2 fluxes with $0.1 < d < 0.2$ for n comprised between 4 and 12 in the dark and $0.5 < d < 1$ for $n > 4$ in the light. Finally, d ranges for NO_3^- fluxes were similar to the O_2 ones with lower values for the dark incubations $(0.03 < d < 0.1)$ than in those determined in the light $(0.07 < d < 0.13)$.

Discussion and conclusions

At Tjarno, the photosynthetic activity of microphytobenthos resulted in a net O_2 efflux to the water column, efficient trapping of dissolved inorganic N and SiO_2 at the water-sediment interface and significant reduction of N_2 loss via nitrification-coupled and uncoupled denitrification.

Gross O_2 production was estimated at > 9 mmol m^{-2} h^{-1}, resulting probably in an expansion of the oxic sediment layer during daylight hours (Revsbech et al. 1981). Assuming for microphytobenthos an O_2:CO_2 ratio of 1 and a C:N molar ratio of 10 (Whitaker and Richardson 1980; Brzezinski 1985), gross O_2 production can be converted into a N demand of approximately 900 μmol m^{-2} h^{-1}. Dissolved nutrient concentrations in the water column were low and their requirements by microphytobenthos were probably satisfied by sedimentary pools; this could explain also low PO_4^{3-}, NH_4^+ and SiO_2 concentrations in the porewater of the upper sediment layers (Figure 1). Dark NH_4^+ efflux (close to 60 μmol m^{-2} h^{-1}) was almost reduced to zero in the light while dark NO_3^- influx was enhanced in the light of about 30 μmol m^{-2} h^{-1}. The resulting DIN difference of 90 μmol m^{-2} h^{-1} between light and dark conditions is probably due to inorganic N incorporated in microalgal biomass; substantial rates of NO_3^- assimilation in the light were also evidenced by Lorenzen et al. (1998). Inorganic N flux represents, in any event, only 10% of the theoretical microalgal requirements, which suggests that about 90% come from porewater or are retained and translocated within the mat. As for NH_4^+, SiO_2 dark efflux (110 μmol m^{-2} h^{-1}) dropped to zero in the light incubation due probably to incorporation in diatom exoskeleton. The role of microphytobenthos as a temporary sink for regenerated nutrients, already evidenced by different authors, is thus confirmed (Sundbäck and Graneli 1988; Sundbäck et al. 1991).

Both D_w and D_n were low at Tjarno (respectively less than 20 and 10 μmol m^{-2} h^{-1}) due to low NO_2^- and NO_3^- concentration in the water column and low nitrification activity (data not shown); the two processes were significantly affected by the activity of microphytobenthos. D_w was inhibited in the light due to increased diffusion pathlength to reach the anoxic sediment horizon and probably due to competition between primary producers and denitrifiers for NO_3^-; similar results were obtained by Dong et al. (2000). Contrarily to what was evidenced by Lorenzen et al. (1998) and Dong et al. (2000), D_n was inhibited by microphytobenthos photosynthetic activity despite higher O_2 availability in sediments due probably to competition between primary producers and nitrifiers for NH_4^+.

The analysis of the distribution of measurements revealed that fluxes detected in the light incubation were affected by a great variability than those detect in the dark; this result is probably explained by the patchy distribution of microalgae. At Tjarno, a low number of replicates (4) was enough to estimate with a good accuracy $(d < 0.1)$ dark O_2 and NO_3^- fluxes whose intensity and direction, in this low-bioturbated site, were probably determined by microbial processes. In the light, O_2 and NO_3^- fluxes calculated on 4 replicates were 2 times less accurate but still within 15% of the true means best estimate. Dark NH_4^+ and SiO_2 fluxes were respectively within 30 and 20% of their BEA for $n = 4$; this increased variability is probably due to the occurrence of some dark microalgal assimilation. In the light incubation, NH_4^+ and SiO_2 fluxes were extremely variable (for NH_4^+ in particular $2 < d < 5$!), a finding suggesting that true fluxes could have been underestimated or overestimated by a factor 5. This is probably explained by the rates which were low and close to zero. Previous papers report average microalgal patch sizes of 79 cm^2 (Blanchard 1990) and ranges between 30 and 191 cm^2 (Sandulli and Pinckney 1999) determined respectively in muddy and sandy sediments. Results presented in this paper, despite the rather large cores used for flux measurements (50 cm^2), suggest that different numbers of replicates are necessary to obtain the same accuracy in light and dark conditions, as well as when analysing different target compounds. The net effect of microphytobenthos on O_2, NH_4^+, NO_3^-, SiO_2 fluxes and on D_w and D_n, here confirmed, can in fact be masked by the distribution of the measurements.

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Acknowledgements

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