Estuaries and Coasts Vol. 29, No. 6B, p. 1196–1211 December 2006

Nitrogen Dynamics in Nontidal Littoral Sediments: Role of Microphytobenthos and Denitrification

KRISTINA SUNDBÄCK*, ALISON MILES†, and FRANK LINARES

Department of Marine Ecology, Göteborg University, Post Office Box 461, SE-405 30 Göteborg, Sweden

ABSTRACT: Previous measurements from cool microtidal temperate areas suggest that microphytobenthic incorporation of nitrogen (N) exceeds N removal by denitrification in illuminated shallow-water sediments. The present study investigates if this is true also for fully nontidal sediments in the Baltic Sea. Sediment-water fluxes of inorganic (DIN) and organic nitrogen (DON) and oxygen, as well as denitrification, were measured in early autumn and spring, in light and dark, at four sites representing different sediment types. All sediments were autotrophic during the daytime both in the autumn and spring. On a 24-h time scale, they were autotrophic in the spring and heterotrophic in early autumn. Sediments functioned as sources of DIN and DON during the autumn and sinks during the spring, with DON fluxes dominating or being as important as DIN fluxes. Microphytobenthos (MPB) activity controlled fluxes of both DIN and DON. Significant differences between sites were found, although sediment type (sand or silt) had no consistent effect on the magnitude of MPB production or nutrient fluxes. The clearest effect related to sediment type was found for denitrification, although only in the autumn, with higher rates in silty sediments. Estimated N assimilation by MPB, based on both net primary production (0.7–6.5 mmol N m^{-2} d^{-1}) and on 80% of gross primary production (1.9–9.4 mmol N m^{-2} d^{-1}) far exceeded measured rates of denitrification (0.01–0.16 mmol N m^{-2} d^{-1}). A theoretical calculation showed that MPB may incorporate between 40% and 100% of the remineralized N, while denitrification removes <5%. MPB assimilation of N appears to be a far more important N consuming process than denitrification in these nontidal, shallow-water sediments.

Introduction

The worldwide problem of coastal eutrophication has focused our interest on the role of shallow-water coastal sediments as nitrogen (N) sinks. Illuminated sediments are productive land-margin ecosystems, with a high potential to influence N turnover (Borum 1996; Nielsen et al. 2004). N can be removed or retained mainly in 3 ways in these areas: bacterial production of N_2, burial into sediments, and incorporation by primary producers. Our study will consider two of these processes, denitrification and N assimilation into primary producers, in nontidal, shallow-water sediments.

Denitrification, along with the recently discovered anaerobic ammonium oxidation (Thamdrup and Dalsgaard 2002), is the main process by which N can be permanently removed from marine ecosystems. Communities of benthic primary producers, such as seagrasses, loose macroalgal mats, and microphytobenthos (MPB), can function as important temporary N sinks in shallow-water sediment areas (McGlathery et al. 2004 and references therein). MPB often accounts for more than 50% of the primary production in shallow soft-bottom estuaries and coastal embayments (Cahoon 1999; Underwood and Kromkamp 1999). The role of MPB communities in N retention is interesting in cool temperate areas, since they are the only primary producing component that is active throughout the year (Rysgaard et al. 1994; Sundbäck et al. 2000). In shallow lagoons and embayments, MPB N demand can be equal to, or even exceed, that of macroalgal mats (McGlathery et al. 2001; Sundbäck and Miles 2002; Sundbäck and McGlathery 2005). Although the retention time of N in MPB can be assumed to be short, microbial mats can reuse remineralized N rapidly at the sediment surface, keeping regenerated N away from the overlying water column (Kristensen 1993; Lomstein et al. 1998).

Sediments in tidal estuaries have been found to function as important sites for N removal by denitrification (e.g., Seitzinger 1988; Nedwell and Trimmer 1996; Dong et al. 2000 and references therein). Studies from coastal waters of Denmark and west Sweden suggest that denitrification may be a less important pathway for N removal in shallow microtidal sediments (Nielsen et al. 1995; Christensen 1998; Risgaard-Petersen et al. 1998; Sundbäck and Miles 2000; Nielsen et al. 2001). This apparent discrepancy can partly be explained by the different N status of coastal areas. It could also be related directly to the absence or presence of tides. In shallow microtidal and nontidal sediments, the absence of tide-induced turbidity and tide-synchronized vertical migration of MPB, allow primary production to occur during the entire daylight

---

* Corresponding author; tele: +46 31 7732703; fax +46 31 7732727; e-mail: kristina.sundback@marbot.gu.se

† Current address: National Marine Service, Environment Agency, Kingfisher House, Goldhay Way, Orton Goldhay, Peterborough, PE2 5ZR UK.

period, resulting in more autotrophic sediments on a diurnal time scale. This was actually hinted at by a previous European-scale study including areas with different tidal amplitudes and climatic zones (Dalsgaard et al. 1999a). The difference in the trophic status can in turn be expected to be reflected in the balance between the importance of algal N assimilation and denitrification (A:D ratio; Sundbäck and Miles 2000) in the sediment. This hypothesis is supported by the results of Risgaard-Petersen (2003), who showed, using data sets from 18 shallow coastal areas in Europe, that denitrification is lower in sediments rendered autotrophic by MPB activity. Generally, differences in the functional role of microorganisms between tidal and microtidal or nontidal areas have received little attention (Miles and Sundbäck 2000).

The nontidal Baltic Sea is one of the largest brackish water seas in the world and has been subjected to extensive research, including modeling of nutrient cycling (e.g., Elmgren 2001 and references therein). Hardly any quantitative data from the Baltic Sea exist on the role of shallow-water sediments for the retention and removal of N. A previous European-scale study on the importance of primary producers for N removal (Dalsgaard et al. 1999a) hinted that there could be some functional features that are typical of shallow, virtually nontidal sediment areas, particularly in cool waters, as reflected in the following hypotheses (Sundbäck and Miles 2000): sediments can be autotrophic throughout the majority of the year; MPB are a major controlling factor of nutrient dynamics; sediments, particularly those that are sandy, function as N sinks during the winter to early summer; N retention by microalgal assimilation is a more important pathway for removal of bioavailable N from the water column than denitrification; and while sediment type does not appear to determine the magnitude of MPB biomass, it influences the magnitude of oxygen and nutrient fluxes, and particularly denitrification (Sundbäck et al. 2000).

These tentative conclusions are based on an annual study at only a few shallow sites in the microtidal northeast Kattegat (tidal range 20–30 cm; salinity 18–34), at the entrance to the Baltic Sea (Sundbäck et al. 2000). Our aim was to test whether the above features also apply to sites in the Baltic Sea proper, which is nontidal with low and stable salinity (7–8). To our knowledge, denitrification has not been previously measured in the shallow sediments of the Baltic Sea, with the exception of a few measurements in the Riga Bay (Stockenberg et al. 1998). We also wanted to focus on the fluxes of dissolved organic nitrogen (DON), which often appear to dominate the N exchange between sediment and water in shallow areas (Lomstein et al. 1998; Tyler et al. 2003; Sundbäck et al. 2004).

Material and Methods

STUDY SITE AND SAMPLING

Our study was conducted in the Kalmar Sound, which is located between the Öland Island and the mainland on the southeast coast of Sweden in the Baltic Sea. This area is virtually nontidal, with a salinity range of 7–9. To cover variation on more than one spatial scale, we applied a hierarchical sampling design, including four different sites (ca. 10 km apart), with two sampling stations at each site, c. 50 m apart. Two sites, Kalmar North (KN; 56°40′N, 16°22′E) and Kalmar South (KS; 56°39′N, 16°22′E) were located on the mainland, while the other two, Öland North (ON; 56°39′N, 16°27′E) and Öland South (OS; 56°38′N, 16°27′E) were located on the west coast of Öland Island. The sediments at the mainland were composed of silt, although larger particles were included in the cores; the Öland sites were composed of sand (Table 1).

Based on the knowledge gained from a full seasonal study on the Swedish west coast (Sundbäck et al. 2000), two sampling seasons were chosen, early autumn, with normally warm water and low N concentration in the surface water, and early spring, with cold water and higher N concentrations. Sampling was made on three occasions: September (autumn) and March and April (spring).

For fluxes of oxygen and nutrients, as well as denitrification, sediment was collected with 24-cm long Plexiglas cores (i.d., 8 cm) at 0.5–1 m water depth. In September and March, 4 cores per station were collected, while 2 cores per station were sampled during April. At every sampling, 6 smaller (i.d., 6.7 cm) sediment cores were collected for the analysis of sediment properties. In situ measurements of salinity, temperature, and light were made prior to the collection of sediment cores. Samples for in situ nutrient concentrations were also taken.

FLUX MEASUREMENTS

Fluxes were measured in the laboratory under simulated in situ light and temperature conditions. The sediment cores were taken to the laboratory in cool boxes and randomly set into two incubation tanks, where nutrient and oxygen fluxes were measured in the light and dark. For a detailed description of the incubation system, see Sundbäck et al. (2000). The cores were left to settle overnight (with stirring, without lids) and on the following morning they were preincubated for 1 h in light (see below) after exchanging the water within the cores. Four cores per station (2 in April), i.e., 8 cores per site (4 in April), were incubated together

TABLE 1. Site and sediment characteristics during the sampling periods in September, March, and April. Site properties are for water overlying the sediment. As no significant difference in porosity, density, and sediment particle size was found between autumn and spring samples, only one mean is shown per site. Macrofaunal biomass includes shell weight. Mean values for each site are shown. TDN = total dissolved nitrogen, nv = no value measured, bd = below detection.

	Öland North			Öland South			Kalmar North			Kalmar South		
	Sep	Mar	Apr	Sep	Mar	Apr	Sep	Mar	Apr	Sep	Mar	Apr
Site properties												
Water depth, m (range)		0.6–0.9			0.6–0.9			0.5–0.9			0.5–0.9	
Temperature, °C	15	4.8	5.7	16.5	4.1	5.7	15	5	7	18.5	3.8	7.5
Salinity, psu	8	8	7	7.5	8	7	7.5	8	8	7.5	8.5	8.5
Nutrients, μmol l^{-1}												
$NO_3^- + NO_2^-$	0.16	2.46	0.25	bd	0.21	9.76	0.09	1.50	0.35	0.17	0.48	0.31
NH_4^+	0.15	0.14	0.63	bd	0.09	0.68	0.21	1.70	0.94	1.29	0.24	1.23
PO_4^{3-}	0.10	nv	nv	0.08	nv	nv	0.25	nv	nv	0.98	nv	nv
DON	22.4	22.2	32.8	19.27	22.7	23.3	36.7	23.2	33.6	22.8	22.5	33.4
Sediment properties												
Porosity	0.49			0.51			0.90			0.79		
Density, g ml^{-1}	1.96			1.75			1.04			1.01		
Size fractions, %												
>500 μm	1.1			1.2			39.0			45.8		
250–500 μm	6.3			3.0			18.3			19.9		
100–250 μm	92.1			96.2			24.7			26.1		
<63 μm	0.6			0.5			18.0			6.2		
TOC, %	0.217	0.063	nv	0.13	0.10	nv	7.36	3.90	nv	2.94	3.00	nv
TON, %	0.023	0.012	nv	0.01	0.013	nv	1.45	1.48	nv	0.60	0.84	nv
C:N ratio (molar)	10.8	6.2	nv	11.7	8.7	nv	5.9	3.1	nv	5.7	4.2	nv
Macrofauna, g ww m^{-2}	261	339	nv	238	97	nv	31	12	nv	71	81	nv

with one control core (in situ water only). The tank water was continuously circulated and the overlying water within the cores was stirred using magnets (60 rpm). Light-dark oxygen and nutrient flux incubations were run in the same cores, with dark incubation following the light incubation after a readjustment period (1–2 h). Denitrification (see below) was measured in separate cores, since the cores were slurred at the completion of the incubations.

Individual 50-W halogen bulbs placed directly above each core provided light. Layers of mesh were used to adjust for in situ irradiance. The light intensity used corresponded to the daily mean for that month, which was 320–330 μmol photons m^{-2} s^{-1} for September and 270–300 μmol photons m^{-2} s^{-1} for March and April. The light incubations were run during the natural daylight period. The incubation time was set so that the change in oxygen concentration (production or consumption) would not exceed 20% (Dalsgaard et al. 2000). The flux times varied between 3 and 5 h for the silty Kalmar sites and 5 and 10 h for the sandy Öland sites.

Samples were taken through holes in the transparent core-lids, using glass (oxygen) or disposable plastic (nutrients) syringes. Oxygen was analyzed by Winkler titration (Strickland and Parsons 1972). Inorganic nutrients (NO_3^- + NO_2^-, NH_4^+, PO_4^{3-}) were analyzed using an autoanalyzer (TRAACS, Bran and Lubbe, Norderstedt, Germany; NO_3^- + NO_2^- will hereafter be referred to as NO_3^-). Total dissolved nitrogen (TDN) was measured using the persulfate oxidation method described by Valderrama (1981) and modified by Bronk et al. (2000). DON concentrations were calculated as the difference between TDN and dissolved inorganic nitrogen (DIN). Hourly flux rates of nutrients were recalculated to daily values using the number of light and dark hours for the time period in question.

MICROPHYTOBENTHIC PRIMARY PRODUCTIVITY AND TROPHIC STATE

Oxygen flux in the light represented net primary production (NPP) of MPB, while gross primary production (GPP) was calculated as NPP minus community respiration (CR, a negative flux) in the dark. For practical reasons, it was assumed that CR in the light equaled CR in the dark. Net oxygen flux over a 24-h period was calculated as hourly light values times the number of daylight hours plus hourly dark values times the number of dark hours. Oxygen production was converted to carbon (C) produced by using a photosynthetic quotient (PQ) of 1.2 (e.g., Glud et al. 2002). Since the irradiance used during the incubations represented a daily mean for the light hours of each month, daily values of primary production were calculated by multiplying hourly values by the number of daylight hours. Trophic state (balance between autotrophy and heterotrophy) of the sediment was assessed using the Trophic Oxygen State Index (TOSI; Viaroli and Christian 2003), derived from the Benthic Trophic Status Index BTSI (Rizzo et al. 1996). In practice, TOSI is assessed by plotting hourly NPP values of individual cores against CR values (not shown).

CALCULATION OF ALGAL NITROGEN ASSIMILATION

N assimilation by MPB was calculated from daily primary production values (NPP and GPP), assuming a C:N molar ratio of 9 (for justification of this ratio, see Sundbäck and Miles 2000; Hillebrand and Kahlert 2002). Calculations based on NPP (Ass_{NPP}) probably underestimate actual MPB assimilation rates, since they are affected by the respiration of the total sediment community; so Ass_{NPP} is a conservative measure of net N assimilation into MPB. Assimilation rates were also calculated for 80% of GPP (Ass_{GPP80}), assuming a MPB respiration of 20% of GPP (Brotas and Catarino 1995).

DENITRIFICATION

Denitrification was measured in light and dark using the isotope pairing technique (Nielsen 1992). This method discriminates between denitrification supported by water column NO_3^- (D_w) and nitrification-coupled D in the sediment (D_n). For the purpose of this paper, only total denitrification ($D_{tot} = D_w + D_n$) will be presented. $^{15}NO_3^-$ (100 mM $Na^{15}NO_3^-$; 99.6 atom %; Europa Scientific Ltd., Cheshire, U.K.) was added to the overlying water of each core, with the final concentration corresponding (60–90 μM $^{15}NO_3^-$) to ca. 20% of the oxygen concentration (Dalsgaard et al. 2000). The depth of the oxic zone in the sediment was measured using microelectrodes (Revsbech 1989) in order to calculate optimal diffusion time (Dalsgaard et al. 2000). Adding 2 ml of $ZnCl_2$ (1 g ml^{-1}) to the water phase, and quickly mixing the sediment with the water stopped denitrification. N_2 samples were collected from the slurry into 12.5 ml gas-tight vials, to which 250 μl of $ZnCl_2$ were added. The isotopic ratios in the N_2 samples were analyzed by mass spectrometry at the National Environmental Research Agency in Silkeborg, Denmark. Daily rates of D_{tot} were calculated by using the number of light and dark hours for the time period in question.

CHLOROPHYLL AND MICROPHYTOBENTHOS

Samples (top 5 mm) for the analysis of chlorophyll *a* (chl *a*) content were collected from sediment cores on completion of the nutrient and oxygen fluxes, using a cutoff 20-ml disposable syringe. Chl *a* was extracted using 90% acetone, and the extract was analyzed using High Performance Liquid Chromatography according to Wright et al. (1991), with the modification of the solvent protocol according to Kraay et al. (1992); for details see Wulff et al. (2000). The composition of the MPB was assessed semiquantitatively using epifluorescence microscopy.

SEDIMENT PROPERTIES

Density and water content (porosity) for the top 5 mm of sediment was measured by standard gravimetric methods (e.g., Dalsgaard et al. 2000). Grain size distribution was measured by dry sieving the sediment. The content of particulate organic carbon (TOC) and nitrogen (TON) of the sediment was determined from dried samples with a Carlo Erba CHN elemental analyzer (Carlo-Erba, Milano, Italy). Before analysis, samples for TOC content were treated with HCl to remove carbonates.

MACROFAUNA

After flux measurements and sampling for chlorophyll, the entire core was wet sieved (500-μm mesh size). The collected fauna was identified and their wet weight (ww; including shell weight) for each taxon (genus or species level) was assessed.

STATISTICAL ANALYSES

Spatial variability for each sampling occasion was tested using nested analysis of variance (ANOVA) with site and station(site) as factors. Since no significant difference was found between stations within a site except for chl *a*, further testing was done at the site level. The results were analyzed by two-way ANOVA with time and site, time and light-dark treatment, and time and sediment type (sand or silt) as fixed factors. Cochran's test for homogenous variances was applied, and pairwise testing of means was made with Student-Newman-Keul's test (SNK) if the interaction was significant. Differences were accepted as being significant when $p < 0.05$. Any time the word significant appears in connection with ANOVA or SNK, a probability level <0.05 applies. The Pearson correlation analysis was used to look for correlations between variables.

Results

SITE CHARACTERISTICS

Water depth, salinity, and mean temperature across the sampling sites are listed in Table 1. In both autumn and spring, DON (19–36 μM) dominated the pool of TDN in the water overlying the sediment, making up >90% of the TDN pool. In September, DIN ($NO_3^- + NH_4^+$) concentrations were generally <1 μM. In spring, DIN concentrations were higher (1–10 μM), with one exception (OS in March). NO_3^- concentrations mostly exceeded those of NH_4^+ in March, while the opposite applied for April, except for the highest NO_3^-

concentration (10 μM) that was found in April at OS. PO_4^{3-} concentrations varied between 0.1 and 1.0 $\mu mol\ l^{-1}$ in September (no values for spring).

The sites differed in sediment characteristics, with fine sand at the Öland sites and silty sediment at the Kalmar sites (Table 1). The sites also differed significantly as to the organic C and N content of the sediment, with TOC contents <1% (weight) at Öland, and 2.9% and 7.4% at the Kalmar sites. TON contents showed a similar pattern. TOC contents were higher in autumn than in spring (except at KS), while TON content was similar in both seasons. C:N molar ratios were significantly higher at the Öland sites (6.2–11.7) than at the Kalmar sites (3.1–5.9), and generally lower in spring for all sites.

MPB species composition varied between sites, although diatoms dominated at all stations. Epipsammic (attached to sand grains) communities characterized the sandy Öland sites, with small-sized (<30 μm) diatoms from the genera *Achnanthes*, *Navicula*, *Amphora*, and *Opephora*. Benthic photoautotrophic dinoflagellates (e.g., *Amphidinium* sp. and *Gymnodinium* cf. *variabile* Herdman) were also common both in autumn and spring, together with some cyanobacteria. In September, a visible brown diatom mat was found at ON, which was reflected in high chl *a* values (cf., Fig. 1). The Kalmar sites, with soft sediments, had a higher proportion of large epipelic diatoms (e.g., *Gyrosigma*, *Pleurosigma* spp.) and colony-forming diatoms (*Melosira*, *Fragilaria* spp.), which were also found in the flocculent material. The most well-developed cohesive MPB mats were found at KN in September, consisting of a mixture of motile diatoms and cyanobacteria (*Oscillatoria* sp.).

Macrofauna biomass was significantly higher at the sandy Öland sites (97–339 g ww m^{-2}) than at the softer Kalmar sites (12–81 g ww m^{-2}; Table 1). The fact that gastropods and bivalves (shell weight was included in the wet weight) dominated at the Öland sites (*Hydrobia* spp., *Bithynia tentaculata* L., *Macoma baltica* L., *Cerastoderma edule* L.) may partly contribute to this difference, although the number of macrofauna individuals was higher for the Öland sites. In particular mud snails (*Hydrobia*) were abundant in September at the ON site (c. 10,000 individuals m^{-2}). The polychaete *Nereis* sp. was the most common taxon among the burrowing infauna.

CHLOROPHYLL *A*

Chl *a* content of the sediment ranged between 11 and 144 mg m^{-2} (Fig. 1). During September, there was no significant difference between the two Öland sites (Kalmar values are missing). There was a significant difference (nested ANOVA; station (site); $p < 0.05$) between the two ON stations, but not between the two OS stations. In March, there were significant differences between sites, as well as between stations within the sites (nested ANOVA; station(site); $p < 0.05$). The mean value for ON (122 mg m^{-2}) was significantly higher than all other sites. In April, the spatial variation in chl *a* content was lower.

OXYGEN FLUX

During daytime, the sediment was autotrophic ($NPP > 0$) at all sites in both autumn and spring, but on a 24-h time scale, sediments were heterotrophic in the autumn (Fig. 1). The TOSI approach classified all sites as highly autotrophic (BTSI category 3) in the spring. In September, all sites were mostly net heterotrophic (BTSI category 2), with ON being the most autotrophic site.

Daily mean NPP varied between 5 and 80 mmol $O_2\ m^{-2}\ d^{-1}$ (Fig. 1), which is equivalent to 50–800 mg C $m^{-2}\ d^{-1}$. NPP was significantly lower in autumn than in spring due to higher CR (see below). NPP varied significantly between sites depending on month (time × site interaction; $p < 0.001$), but not between stations within a site (nested ANOVA; station(site); $p = 0.91$). There was no consistent difference in NPP between the sandy (Öland) and silty (Kalmar) sites, but on each sampling occasion, NPP was significantly higher at one of the Kalmar sites when compared with the other sites. GPP (range 24–137 mmol $O_2\ m^{-2}\ d^{-1}$) was roughly twice as high in September as in spring. In contrast to NPP, significantly different GPP rates were found for the two sediment types, with higher rates for the silty sites in spring.

Daily CR was significantly higher in September (32–100 mmol $O_2\ m^{-2}\ d^{-1}$) than in spring (2–38 mmol $O_2\ m^{-2}\ d^{-1}$; Fig. 1). As for GPP, the daily CR was related to sediment type, with significantly higher rates in silty sediments in spring, but not in September.

FLUX OF INORGANIC NITROGEN

DIN fluxes did not vary significantly between stations within a site (nested ANOVA; station(site); $p > 0.05$), so fluxes are shown for sites only (Figs. 2 and 3). Some general patterns were observed: DIN was released in autumn and taken up in spring, DIN fluxes were significantly affected by light, and although fluxes varied significantly between the sites, fluxes were significantly higher in silty sediment only in spring.

Light and Dark Fluxes of Inorganic Nitrogen

NO_3^- fluxes were undetectable in September, except for KS (efflux of 5–10 $\mu mol\ m^{-2}\ h^{-1}$; Fig. 2). In spring, NO_3^- was mainly taken up at all sites (range 4–26 $\mu mol\ m^{-2}\ h^{-1}$), with significantly

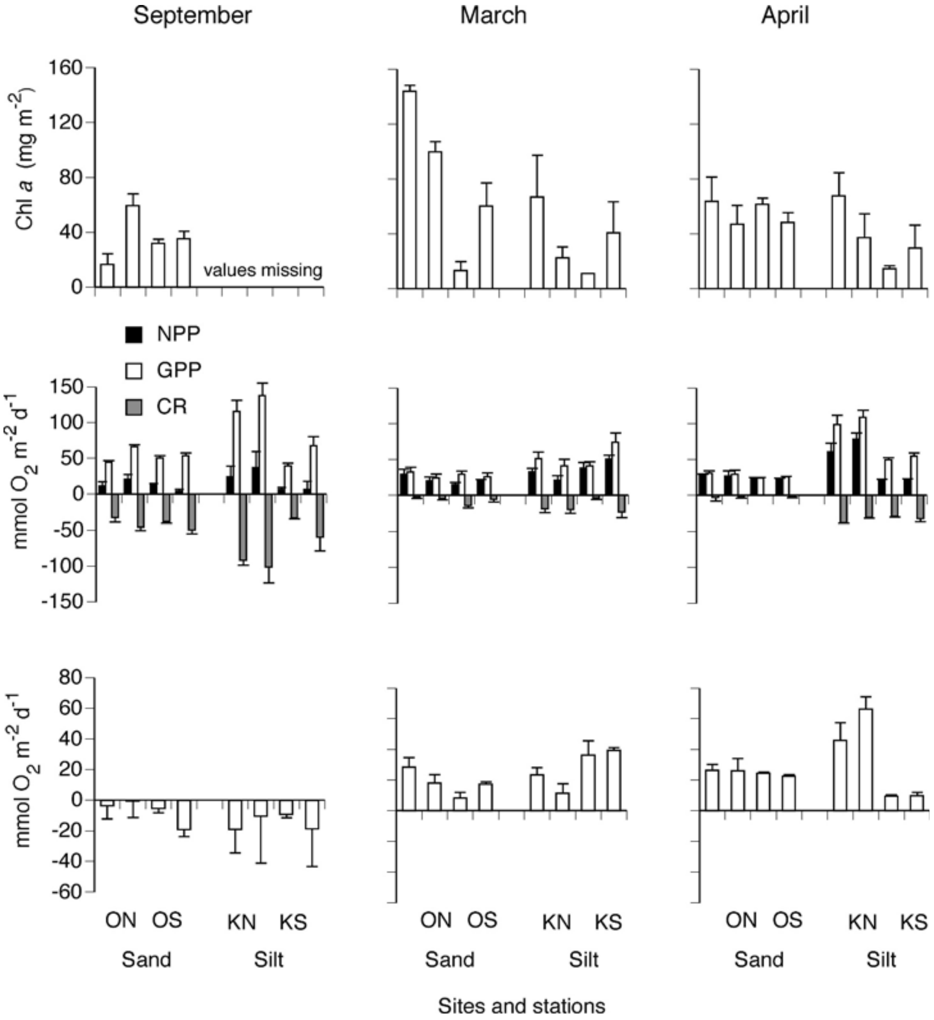

Fig. 1. Chlorophyll *a* content (mean values + SE, n = 3 for each station) of the top 5 mm sediment, net primary production (NPP), gross primary production (GPP), community respiration (CR), and 24-h net oxygen flux (mean + SE, n = 4 September, n = 2 April for each station) between sediment and water at four sites (ON = Öland North, OS = Öland South, KN = Kalmar North, KS = Kalmar South) in the Kalmar Sound in September, March, and April.

higher uptake in light. In March, light fluxes were significantly higher at KN, while in April, fluxes were highest at the Öland sites.

In September, NH_4^+ dominated DIN flux and was mainly released from the sediment (mean flux rates −10 to 112 µmol m^{-2} h^{-1}; Fig. 2). In spring, NH_4^+ was mostly taken up in the light, with mean fluxes varying between +5 and −23 µmol m^{-2} h^{-1}. Significant differences between sites were found; the highest fluxes were found at one or both of the Kalmar sites. There was a trend of higher uptake or lower efflux in light, although it was significant only once.

Daily DIN Flux

While comparing light and dark fluxes tells us whether N flux is regulated by MPB, comparing daily values tells us whether sediments function as sources or sinks of DIN. In September, sediments functioned as N sources (efflux 0.18–2.8 mmol m^{-2} d^{-1}), with significantly higher efflux at KS (SNK, $p < 0.05$; Fig. 3). In spring, sediments were sinks for DIN (influx 0.18–0.9 mmol m^{-2} d^{-1}), with KN showing significantly higher influx in March.

DON Flux

DON generally dominated TDN flux and showed the same pattern as DIN, with efflux in autumn and uptake in spring (Figs. 2 and 3). Significant light effects were observed.

Light and Dark Fluxes of DON

The net release of DON out of the sediment in September was 15–100 µmol m^{-2} h^{-1} (exception

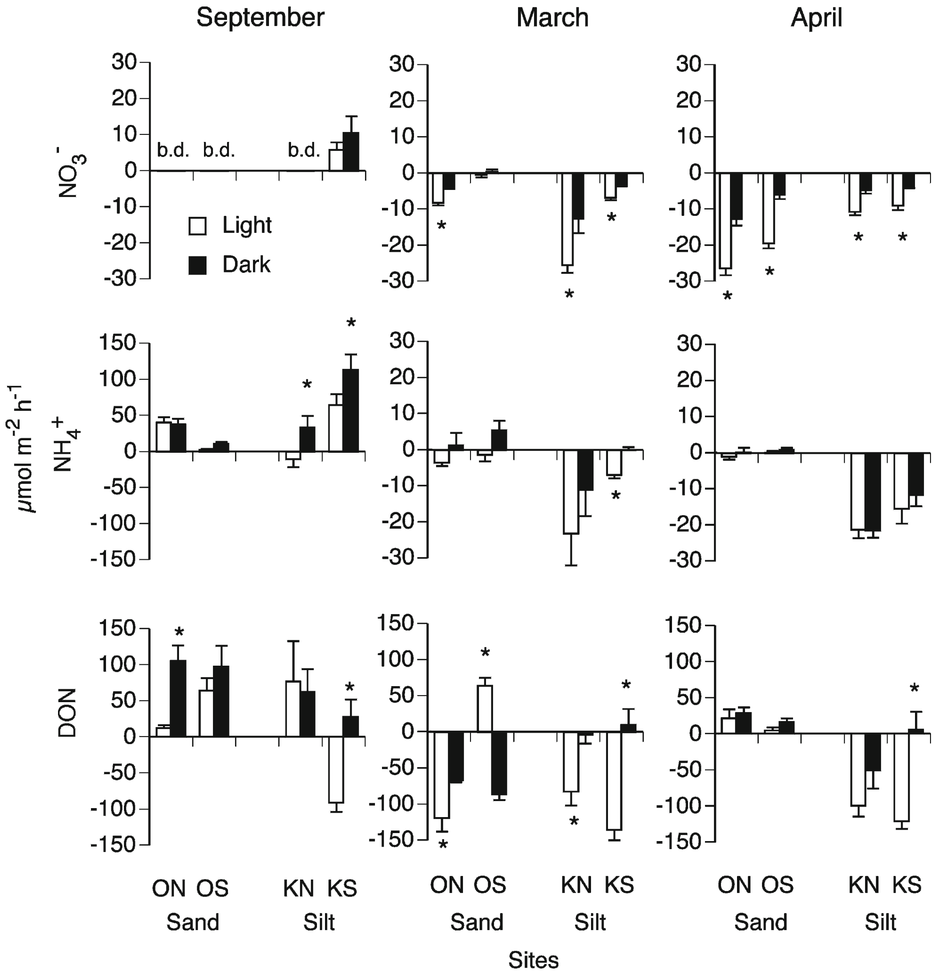

Fig. 2. Hourly nutrient fluxes in light and dark at four sites in the Kalmar Sound in September, March, and April. Rates shown are means for sites (n = 8 in September and March, n = 4 in April). Nitrate values below detection for 3 sites in September. Note the difference in scales. * denotes significant light-dark effect. ON = Öland North, OS = Öland South, KN = Kalmar North, KS = Kalmar South.

KS) with no significant difference between sites (Fig. 2). In March, DON fluxes were mostly into the sediment (-4 to -140 μmol m^{-2} h^{-1}) in both light and dark, with dark fluxes being significantly different between Öland and Kalmar sites. In April, there was a DON influx only at the Kalmar sites (50–140 μmol m^{-2} h^{-1}), while DON was released (10–30 μmol m^{-2} h^{-1}) at the Öland sites. Significant light effects were observed, where uptake was higher or efflux lower in light (with one exception, OS in March).

Daily DON Flux

In September, sediments functioned as sources of DON (1 and 2.3 mmol m^{-2} d^{-1}), with the exception of KS (uptake ≈ 1 mmol m^{-2} d^{-1}; Fig. 3). In March and April the sediments were DON sinks (influx 0.3–2.2 mmol m^{-2} d^{-1}) with the exception of the Öland sites in April. A significant effect of sediment type was seen only in April.

DENITRIFICATION

Denitrification rates are shown only for sites, as rates did not vary between stations within a site (except once: KS in September). Denitrification rates were generally low, hourly light and dark rates varying between 0.02 and 6.6 μmol N m^{-2} h^{-1} (Fig. 4), equivalent to daily rates of 0.01 and 0.16 mmol m^{-2} d^{-1} (Fig. 4). In September, a significant effect of sediment type was found, with significantly higher rates at the silty sites, the highest rate being found at KS (Fig. 4). In April, hourly D_{tot} rates were more similar between sites (range 0.8–3.3 μmol m^{-2} h^{-1}), although dark denitrification was significantly higher at OS, when the only significant light effect was observed (Fig. 4).

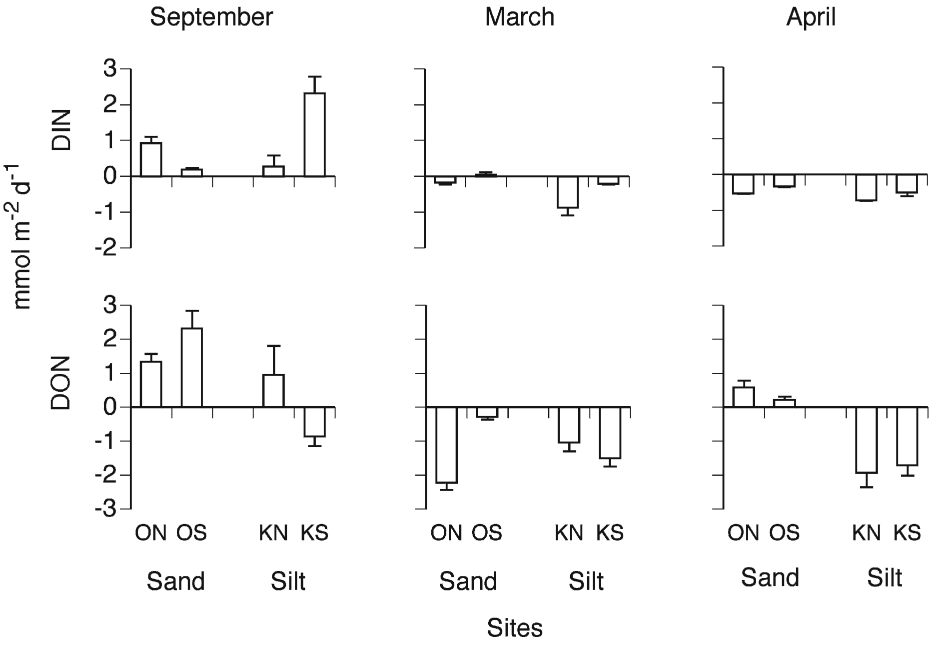

Fig. 3. Daily fluxes of dissolved inorganic (DIN) and organic nitrogen (DON; mean + SE, n = 8 in September and March, n = 4 in April) at four sites in the Kalmar Sound in September, March, and April. ON = Öland North, OS = Öland South, KN = Kalmar North, KS = Kalmar South.

BALANCE BETWEEN ASSIMILATION AND DENITRIFICATION

Calculated rates for microphytobenthic N assimilation based on NPP (Ass_{NPP}) varied between 0.7 and 6.5 mmol N m^{-2} d^{-1}, with rates being roughly 2–3 times higher in spring (Fig. 5). In autumn, Ass_{GPP80} rates were 3–6 times higher than Ass_{NPP}, but equaled Ass_{NPP} in spring due to low CR rates.

The ratio between MPB N assimilation and denitrification, expressed as the A:D ratio (Sundbäck and Miles 2000), varied between 4 and 154 for Ass_{NPP} and 24 to 428 for Ass_{GP80} (Fig. 5). MPB assimilation appeared a far more important N consuming process than denitrification. The highest and lowest ratios were found in September, for OS and KS, respectively. In April the difference between sites was smaller.

THE FATE OF REMINERALIZED NITROGEN

The rate of N mineralization can be roughly calculated from daily oxygen consumption and C:N ratios of the sediment (cf., Trimmer et al. 2000) assuming a mean respiratory quotient (RQ) of 1.25 (Glud et al. 2002). Calculated mean N mineralization rates varied between 0.12 and 13 mmol N m^{-2} d^{-1}. The mean rate for the sandy Öland sites was 3.1 mmol m^{-2} d^{-1} in September and 0.46 mmol m^{-2} d^{-1} in spring. For the Kalmar sites, the equivalent values were more similar for the two seasons (9.6 and 5.8 mmol N m^{-2} d^{-1}, respectively). Comparing these rates with the calculated algal N assimilation (adopting the conservative measure Ass_{NPP}) suggests that in September MPB consumed between 10% and 50% (Öland 25–50%, Kalmar sites 10–20%) of the remineralized N. In spring, theoretical MPB N demand far exceeded N mineralization (up to 200%; Table 2) at the sandy Öland sites (with extremely low CR). Also at the Kalmar site, MPB consumed more (35–100%) of the remineralized N in spring. Denitrification, on the other hand, only appeared to remove 0.3–7%, with one exception, 44% in April at OS (for an overview of ranges, see also Table 2).

Discussion

Our aim was to test whether previous findings of MPB-sediment interactions in the microtidal northeast Kattegat (Sundbäck et al. 2000) would also apply to shallow sites in the nontidal Baltic Sea. One criticism against our study could be that we included only two seasons and one study area (Kalmar Sound). The rationale behind this cost effective sampling was to cover two opposite seasons (but with similar light conditions), one with warm water and low DIN concentrations in the overlying water (early autumn) and one with cold water and higher DIN concentrations (spring). The results from a full seasonal study from sandy and silty sites in the Kattegat area (Sundbäck et al. 2000) suggested that substantial MPB primary productivity and denitrifi-

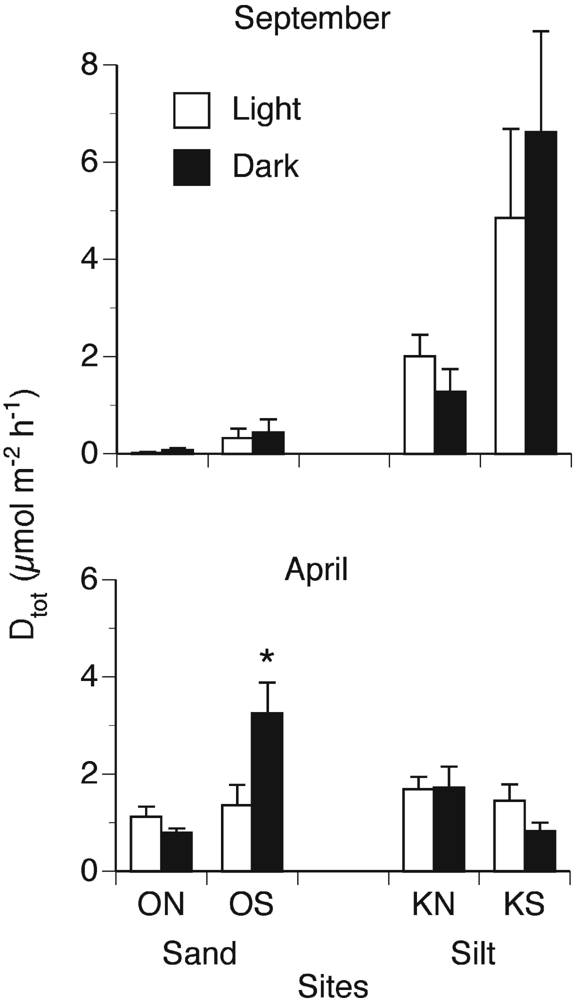

Fig. 4. Hourly total denitrification (D_{tot}) in light and dark at four sites in the Kalmar Sound in September and April (mean + SE, n = 4). ON = Öland North, OS = Öland South, KN = Kalmar North, KS = Kalmar South.

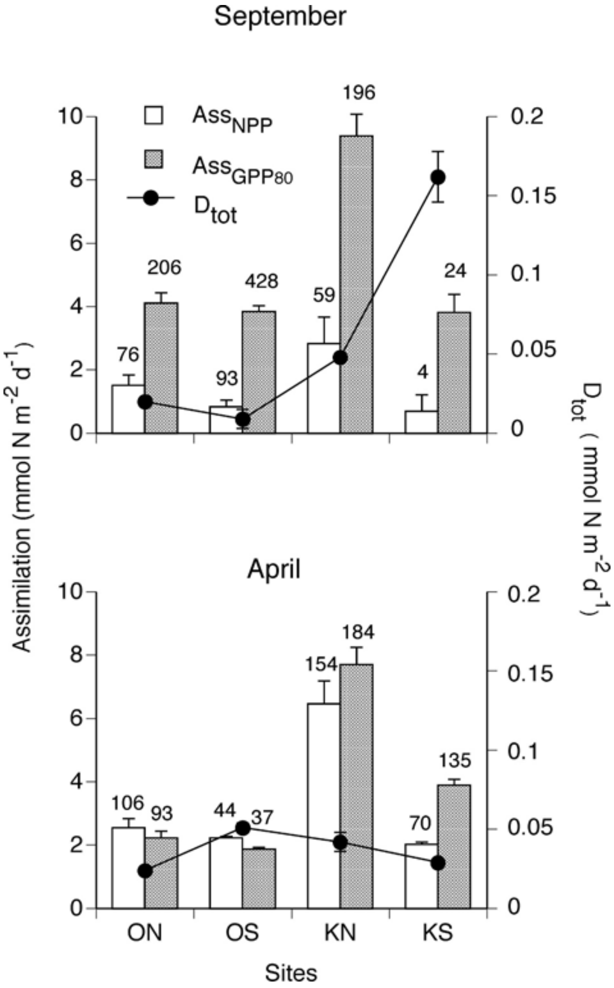

Fig. 5. Balance between daily N assimilation by microphytobenthos calculated from net primary production (Ass_{NPP}) and 80% gross primary production (Ass_{GPP80}) (bars; mean + SE, n = 8), and measured daily denitrification (line; mean + SE, n = 3) at four sites in the Kalmar Sound in September and April. Numbers above bars represent the Ass_{NPP}:D_{tot} and Ass_{GPP80}:D_{tot} ratios. ON = Öland North, OS = Öland South, KN = Kalmar North, KS = Kalmar South.

cation could be expected to occur during these two periods. Other studies from Scandinavian microtidal waters have observed two denitrification peaks, one in spring, and one in autumn (e.g., Jørgensen 1989; Dalsgaard et al. 1999b). Although our study is certainly limited in time and space, it should give us a hint about the ranges of the quantitative role of benthic primary producers and denitrifiers in the N dynamics in these shallow nontidal sediments.

Degree of Autotrophy

The trophic status can be used to describe general properties of the benthic system, such as system stability (stable autotrophy increases the buffering capacity; de Wit et al. 2001), the importance of sediment for nutrient cycling (autotrophic sediments are mainly sinks of nutrients; Eyre and Ferguson 2002), and N removal by denitrification (denitrification decreases with increasing autotrophy; Risgaard-Petersen 2003). The TOSI-BTSI indices categorized all sites in our study as either highly autotrophic (spring) or net autotrophic (autumn). This finding supports our first tentative hypothesis that littoral sediments in cool microtidal areas can be autotrophic throughout the majority of the year (Sundbäck et al. 2000). TOSI, which is based on hourly fluxes in light and dark, does not describe the trophic state on a diurnal (24-h) time scale. Calculated diurnal net oxygen fluxes showed that the benthic system was actually net heterotrophic in autumn, but autotrophic in spring. This

TABLE 2. Average percentage and range of remineralized N assimilated by microphytobenthos and denitrified at sites on the west coast of Sweden and in the Baltic Sea. A_{NPP} = assimilation based on net primary production, $A_{80\%GPP}$ = assimilation based on 80% of the gross primary production (assuming a C:N ratio of 9 and a PQ of 1.2), and D = denitrification. Remineralization is calculated from oxygen consumption and C:N ratio of sediment. All denitrification measurements are by isotope-pairing technique (Nielsen 1992).

Site		A_{NPP} %	$A_{80\%GPP}$ %	D %	Source
Rågårdsvik, Skagerrak[a]	<1 m	62 (33–130)	100 (72–160)	10 (3–17)	Sundbäck and Miles 2002
Bassholmen, Skagerrak[a]	<1 m	10 (0–42)	47 (28–91)	20 (9–36)	Sundbäck and Miles 2002
Gullmarfjord, Skagerrak[b]	1 m	94 (88–300)	>100 (130–300)	12 (2–22)	Sundbäck et al. 2004
	5 m	0	38 (28–48)	15 (5–24)	Sundbäck et al. 2004
	10 m	0	7 (0–14)	30 (4–56)	Sundbäck et al. 2004
	15 m	0	17 (14–19)	14 (7–21)	Sundbäck et al. 2004
Kalmar Sound, Baltic Sea[b]	silt, <1 m	42 (11–112)	76 (62–116)	1 (0.4–2.6)	This study
	sand, <1 m	89 (52–183)	>100 (114–227)	2[c] (0.3–44)	This study

[a] April to June.
[b] April and September.
[c] When including one value that is 44%.

difference in the trophic state was reflected in the N fluxes, so that the sediment functioned as a source of TDN in autumn and a sink in spring.

We suggested earlier that the general dominating autotrophy of microtidal and nontidal sediments during daytime (Sundbäck and Miles 2000) could partly be related to the absence of tide-induced turbidity and MPB downward migration, which on muddy tidal flats restrict benthic photosynthesis to daytime emersion periods. In a previous study (Dalsgaard et al. 1999a), the MPB primary productivity of 16 European shallow-water sites with different tidal ranges and climates was measured using standardized methods. That study showed that when daily primary production values for macrotidal sites were calculated taking into account the number of light hours of emersion, daily GPP and NPP (expressed as m^{-2}) were significantly higher for northern microtidal sites when compared with both macrotidal sites and warm microtidal sites (Sundbäck et al. unpublished data); a larger data set is needed to verify this difference.

INFLUENCE OF MPB ON NITROGEN DYNAMICS

MPB appeared to regulate both DIN and DON fluxes across the sediment-water interface, supporting our second hypothesis, which stated that MPB is a major controlling factor of sediment–water nutrient fluxes. This was apparent from significant light-dark differences. Light increased DIN uptake, particularly in spring, by between 30% and 200%, and DON uptake increased even more. When fluxes were directed out of the sediment (September), light decreased DIN release by 40–90% and DON release by 25–90%. The MPB effect on DON flux will be discussed later. The influence of MPB on DIN fluxes was also corroborated by a significant negative correlation between mean daily NPP and DIN flux ($r = -0.513$, $p = 0.05$; Fig. 6). DON fluxes correlated better with GPP, but only in spring ($r = -0.672$, $p = 0.01$). While the regulating role of MPB for sediment–water fluxes of DIN is today well documented for both intertidal and subtidal sediments in varying climate conditions (e.g., Henriksen et al. 1980; Sundbäck et al. 1991; Reay et al. 1995; Eyre and Ferguson 2002; Tyler et al. 2003 and references therein), less is known about MPB regulation of DON.

We did not find any consistent effect from light exposure on denitrification rates in this study, although light effects have previously been observed for other Swedish coastal sites (Sundbäck et al. 2000, 2004; Sundbäck and Miles 2002). This effect has been mostly seen as a suppression of D_{tot}, and appears to be typical of sediments rendered autotrophic by MPB particularly in N-poor areas where MPB compete for NH_4^+ with nitrifying bacteria, decreasing D_n (Rysgaard et al. 1994; Risgaard-Petersen 2003). At higher DIN concentrations, MPB photosynthesis can stimulate D_n by enhancing nitrification (Risgaard-Petersen et al. 1994; Dong et al. 2000; An and Joye 2001). The reason for a lack of a significant suppression of denitrification by light (except once; Fig. 4) in our study could be that denitrification rates were already very low, making interpretation difficult.

DON FLUXES

While DON has been recognized as being important in the N turnover of pelagic ecosystems for at least two decades (Ward and Bronk 2001), only recently has DON started to gain more interest in shallow-water ecosystems where benthic primary producers are present (Tyler et al. 2001, 2003; Eyre and Ferguson 2002). As found in other shallow sites (e.g., Tyler et al. 2003; Eyre and Ferguson 2002), DON dominated the TDN pool of the overlying water. The fact that DON dominated the efflux of N in September in our study agrees with previous results from other shallow-water, subtidal sediments

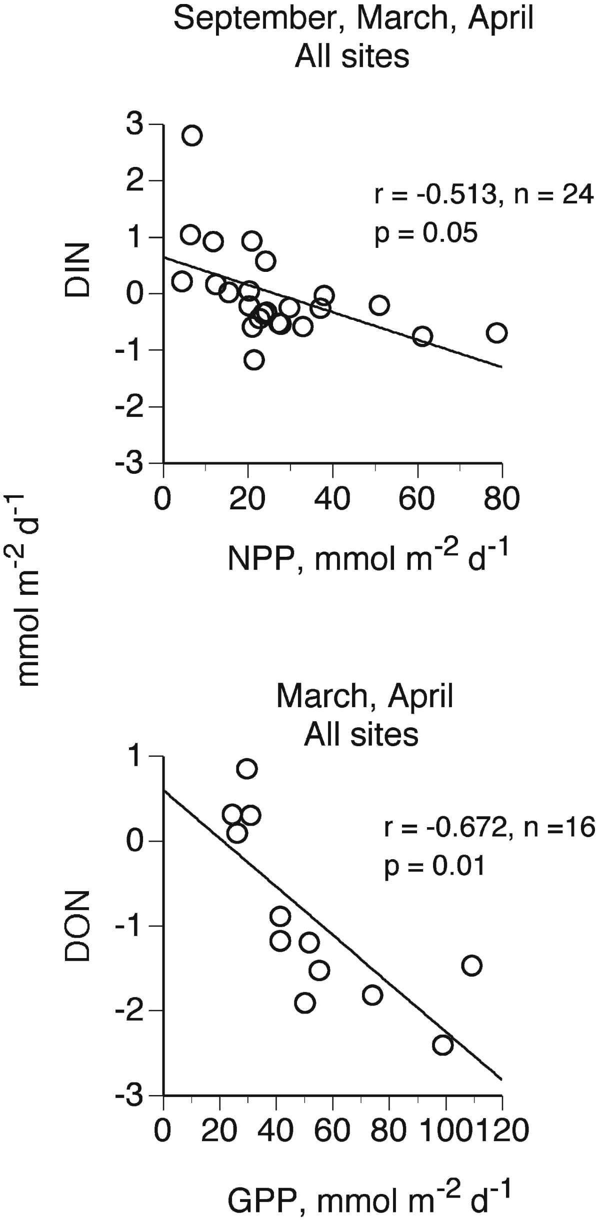

Fig. 6. Daily fluxes of dissolved inorganic nitrogen (DIN) versus daily net primary production (NPP), and dissolved organic nitrogen (DON) versus daily gross primary production (GPP) for the sampling period at all sites.

in the warm season (Hansen and Blackburn 1991; Sloth et al. 1995; Lomstein et al. 1998). A large DON efflux relative to NH_4^+ flux (see Fig. 2) is considered to reflect a situation where fresh (and autochthonous) organic matter is being hydrolyzed at the sediment surface (Blackburn and Blackburn 1993; Burdige and Zheng 1998; Eyre and Ferguson 2002). This could well have been the case in September, when well-developed microbial mats were present. A higher grazing pressure on MPB in autumn may also have contributed to the DON efflux (Eyre and Fergusson 2002; Cook et al. 2004).

In spring, DON was taken up by the sediment at a rate of 0.3–2.2 mmol m^{-2} d^{-1}, i.e., at a rate similar to DIN uptake. DON fluxes are expected to be directed out of the sediment, but uptake of DON, particularly in the light, has also been found in other shallow estuaries (Dollar et al. 1991; Cowan and Boynton 1996; Cook 2002) and temperate lagoons (Tyler et al. 2001, 2003; Eyre and Ferguson 2002). Light effects and significant negative correlations with primary productivity suggest that some of this DON uptake may be by MPB, although bacterial uptake by DON is probably also substantial (e.g., Guldberg et al. 2002). Sediments that are rendered autotrophic by MPB activity have been found to take up DON (Cook et al. 2004) and such sediments can function as DON sinks on an annual scale (Tyler et al. 2003). A high DON uptake ($\approx$100 µmol m^{-2} h^{-1}) in early spring has been found to coincide with high MPB productivity (Cook et al. 2004).

The actual quantitative role of DON as a N source for MPB communities still remains unclear. It has been suggested that DON may be an important alternative N source for sustaining MPB growth when DIN concentrations are low (Linares 2006; Linares and Sundbäck 2006); e.g., substantial DON uptake by cyanobacteria mats has been observed (Rondell et al. 2000). The fact that our calculated N demand of MPB in March–April (2–2.5 mmol N m^{-2} d^{-1}) far exceeded the influx of DIN (0.3–0.7 mmol N m^{-2} d^{-1}) suggests that DON could have been a complementing N source for the growing populations of both benthic microalgae and bacteria in the spring. Low sediment C:N ratios, particularly at the Kalmar sites (<6), suggest the presence of fresh N-rich material, such as bacteria and microalgae (cf., Kristensen 1993). In September, high N mineralization (cf., high CR) probably provided a sufficient N source for MPB, which incorporated $\leq$50% of the mineralized N, as opposed to >100% in spring.

We did not measure individual DON compounds, but labile compounds, such as dissolved free amino acids (DFAA), make up a substantial portion of sediment DON (Guldberg et al. 2002; McCarthy 2003). DFAA have been found to stimulate the growth of benthic diatom cultures (Admiraal and Peletier 1979; Admiraal et al. 1984), as well as natural MPB communities (Linares 2006). DON was estimated to provide c. 10% of the MPB N demand in littoral waters and substantially more (>50%) in deeper waters (Linares and Sundbäck 2006). Rapid recycling of DON within the microbial mats may be an important mechanism that can sustain high primary production in generally N-poor sandy sediments (e.g., Lomstein et al. 1998). In a temper-

TABLE 3. Mean daily total denitrification ($D_{tot} = D_w + D_n$) measured in some microtidal and nontidal shallow-water sediments (< 2 m) in Nordic coastal waters. Rates are given as μmol N m^{-2} d^{-1}. All measurements are by isotope-pairing technique (Nielsen et al. 1992).

Site	Sediment	Depth (m)	D_{tot}	Source
Norsminde Fjord, Kattegat[a]	sand-mud	0.6 (mean)	567	Nielsen et al. 1995
Randersfjord, Kattegat	Silt	1	3200	Nielsen et al. 2001
Zostera marina bed, Limfjord		0.6–1.2	400	Risgaard–Petersen 1998
Riga Bay (southeast Baltic Sea)[b]	Sand	0.2–0.1	58	Stockenberg et al. 1998
Vallda Bay, Kattegat[b]	Sand	0.5	33	Sundbäck et al. 2000
Rörtången Bay, Kattegat[b]	Silt	0.5	353	Sundbäck et al. 2000
Bassholmen Bay, Skagerrak[c]	Silty sand	0.5	1000	Sundbäck and Miles 2002
Rågårdsvik Bay, Skagerrak[c]	Silty sand	0.5	535	Sundbäck and Miles 2002
9 Danish fjords (average)[a]	Varying	Varying	665	Dalsgaard et al. 1999b
Average for inner Danish coastal waters[a]	Varying	Varying	400	Christensen 1998
Gullmar fjord, Skagerrak	Silty sand	1	115	Sundbäck et al. 2004
Öland, Kalmar Sound (Baltic Sea)[d]	Sand	0.5	22	This study
Kalmar, Kalmar Sound (Baltic Sea)[d]	Silt	0.5	70	This study

[a] Recalculated from annual value.
[b] Recalculated from hourly values.
[c] April to June.
[d] April, September.

ate lagoon, the highest turnover rate of the TDN pool was found in sediments with the lowest C content (Anderson et al. 2003), which hints that these types of sediments exhibit an efficient N turnover. This conclusion also agrees with Kristensen's (1993) observations for low-organic, shallow-water sediments. The general importance of DON as a N source for primary producers in low nutrient areas has been highlighted recently (van Breeman (2002).

SEDIMENTS AS NITROGEN SINKS

Sediments functioned as sinks for N in spring. This finding supports our third hypothesis (Sundbäck et al. 2000), stating that particularly sandy autotrophic sediments tend to be sinks of N (cf., Eyre and Ferguson 2002; Sundbäck et al. 2003; Tyler et al. 2003). The role of shallow-water, illuminated sediments as N sinks has been emphasized in a number of studies from different types of shallow-water ecosystems in temperate areas (e.g., Simon 1988; Rizzo et al. 1992; Cerco and Seitzinger 1997; Kristensen et al. 1997; Thornton et al. 2002), as well as for subtropical and tropical areas (Miyajima et al. 2001; Suzumura et al. 2002). The fact that the sediments functioned as N sources in early autumn, despite daytime net autotrophy, was due to high N mineralization rates, as suggested by a much higher CR compared with the spring situation. In this context, it must be kept in mind that bacteria also contribute to the immobilization of remineralized N in the sediment (e.g., Guldberg et al. 2002; Anderson et al. 2003). The fact that shallow-water sediments, due to MPB activity, may often function as sinks rather than sources of nutrients, may have consequences for other autotrophs within the system, such as phytoplankton and floating macroalgal mats, by controlling the availability of nutrients remineralized in the sediment (Fong et al. 1993; Havens et al. 2001; Sundbäck et al. 2003; Sundbäck and McGlathery 2005).

RELATIVE IMPORTANCE OF MICROPHYTOBENTHIC N ASSIMILATION AND DENITRIFICATION

Denitrification rates (mean < 0.1 mmol N m^{-2} d^{-1}) were some of the lowest ever reported for shallow, northern microtidal sites (Table 2). Since we measured denitrification only twice, we may have underestimated the degree of annual variation. Similar low values have also been found in sandy sediments in the Kattegat and Riga Bay (Baltic Sea), as well as in seagrass beds in Denmark (see Table 3). As discussed earlier, low denitrification rates are typical of autotrophic sediments in areas with generally low DIN concentrations, partly because benthic microalgae tend to suppress denitrification (Rysgaard et al. 1995; Risgaard-Petersen 2003). This fact is also supported by a comparison of 18 European estuaries, where the lowest D_n rates (median value 4.2 μmol N m^{-2} h^{-1}) were found in highly autotrophic sediments (Risgaard-Petersen 2003). Denitrification, particularly D_w, could have been expected to be higher in April, when water column NO_3^- concentrations are normally higher. This was true for the sandy sites, with the highest denitrification rate coinciding with the highest NO_3^- concentration (dark D at OS; Fig. 4). At the silty sites, denitrification was actually lower in the spring. High photosynthetic oxygen production, in combination with low mineralization rates (when compared with autumn), probably kept denitrification rates at a low level.

The high ratios between MPB assimilation and denitrification (A:D; 4–428; Fig. 5) observed in our study support our fourth hypothesis, stating that N retention by MPB is more important than denitrification in shallow-water (autotrophic) sediments of cool microtidal areas. It should be noted that we apply here $\mathrm{Ass}_{\mathrm{NPP}}$, which is a conservative measure of MPB incorporation of N (see Material and Methods and Sundbäck and McGlathery 2005). The lower A:D ratios observed at KS in September, as compared with those observed at the other sites, suggest that denitrification was more important in removing N at this site. The A:D ratios were generally up to an order of magnitude higher than those previously reported for the northeast Kattegat (Sundbäck and Miles 2000; Sundbäck and Miles 2002), the Belt Sea (Rysgaard et al. 1995), and the Skive Fjord in Denmark (Dalsgaard et al. 1999b; Table 3 in Sundbäck and Miles 2000). This result could imply that microalgal assimilation may even be more important for N retention in the nontidal sediments studied here, than in previously studied microtidal sediments; more data are needed. In tidal areas, N retention by MPB can also be important, but denitrification has often been found to be relatively more important as a N sink (e.g., Cabrita and Brotas 2000; Dong et al. 2000).

The hypothetically large role of MPB in regulating N turnover in our nontidal sediments was also highlighted by the fact that they appear to incorporate up to 100% (or more) of the estimated amount of remineralized N (Table 2). The calculated N demand of MPB often far exceeded estimated N mineralization rates, as also found in other studies in Swedish coastal waters. There might be both methodological and biogeochemical explanations for this finding. We did not measure actual N mineralization rates (cf., Anderson et al. 2003), neither did we consider possible anoxic mineralization nor the N pool of the overlying water. Others have also found that MPB N demand can exceed N mineralization rates in shallow-water sediments (Anderson et al. 2003). Clearly, even if we have done only rough estimations, it seems that MPB incorporation of N in the nontidal system studied here is far more important than removal by denitrification. MPB retention of N is only temporary, and we can assume short turnover times. We can also assume a rapid reutilization of old N (both as DIN and DON), leading to an efficient retention of N in the sediment community.

SEDIMENT TYPE AND PROCESS RATES

The fifth tentative hypothesis stated that we could expect higher rates of nutrient fluxes and denitrification in silty sediments. No consistent influence of sediment type (sand and silt) was found, and when differences were found they depended on the season. The finding that NPP was not dependent on sediment type agrees with previous results from the Kattegat area (Sundbäck et al. 2000). The largest effect related to sediment type was seen for denitrification, but only in the autumn, when rates were significantly higher in silty sediment. The site that had the highest TOC and TON content (KN) differed most often from other sites with regard to oxygen fluxes, whereas nutrient fluxes and denitrification were not consistently higher.

While sediment type determines the type of indigenous microalgal community (e.g., Paterson and Hagerthey 2001 and references therein), sediment type per se does not appear to necessarily determine the MPB biomass (Barranguet et al. 1997; Miles and Sundbäck 2000) or magnitude of process rates, at least not when extremes are excluded (Sundbäck et al. 2000). All sites irrespective of sediment type, functioned as sources of dissolved N in the autumn and as sinks during the spring. Higher N remineralization rates, and higher N efflux, could have been expected at the Kalmar sites because of their higher TOC and TON content and CR, but this was not the case.

ROLE OF BENTHIC FAUNA

A detailed study of the macrofauna was outside the scope of our study. As the activity of benthic fauna increases mineralization, nutrient efflux, and denitrification (Christensen et al. 2000; Dong et al. 2000; Emmerson and Raffaelli 2000; Hulth et al. 2002) we will briefly consider macrofauna as well. In terms of wet weight (including shell weight), faunal biomass was higher at the sandy sites than at the silty sites, but fluxes were lower. The reason for this could be that epifauna, such as gastropods and bivalves, dominated the sandy sites, while burrowing infauna, known to enhance fluxes of $\mathrm{NH_4^+}$ (Emmerson et al. 2001), dominated at the silty sites. In September and April, KN had the highest primary production and the most well-developed (visible) diatom mats, but the lowest faunal biomass. This finding probably reflected low bioturbation and grazing pressure. Low animal activity is known to favor the development of distinct cohesive microbial mats at the sediment surface (Paterson 1989; Paterson and Hagerthey 2001).

Conclusions

Our study on nontidal sediments confirmed previous tentative hypotheses about the functioning of illuminated microtidal sediments in cool, low N areas. Due to an active community of benthic microalgae, sediments can be autotrophic during daytime in both spring and autumn. Both sandy and silty sediments can function as sinks of DIN and DON in the spring, but as sources in the warm season. Not only does MPB control sediment–water fluxes of DIN, but that of DON as well. Influx of DON in spring, along with observed higher uptake in the light, suggest that DON may be an important N source for growing MPB, supporting primary production when DIN levels are low. The importance of sediment type (sand and silt) for rates of oxygen and nutrient fluxes is not consistent and depends on the season. Denitrification rates can be expected to be higher in soft sediments, but mainly in the warm season, when mineralization rates are high and oxygen penetration depth smaller. Retention of N by MPB in these nontidal sediments appears to be a far more important process than N removal by denitrification, even to higher degree than previously found for cool microtidal sediments. Although denitrification generally increases with increasing N loading, denitrification cannot be expected to balance additional anthropogenic N inputs in the shallow-water areas of the Baltic Sea proper.

ACKNOWLEDGMENTS

Funding was received from the Foundation for Strategic Environmental Research (MISTRA), the Royal Society of Arts and Sciences in Göteborg, the Foundation in Memory of Birgit and Birger Wåhlström, and the Captain Stenholm Fund. We want to thank Professor Edna Granéli and the staff at the Department of Marine Sciences at Kalmar University for giving us access to their excellent facilities during the field campaigns. We are grateful to Dr. Lars-Erik Persson for guiding us to suitable sampling sites. We also thank Jenny Toth for assisting in the sample analyses and Lars Håkansson for assisting in field sampling.

LITERATURE CITED

ADMIRAAL, W., R. W. P. M. LAANE, AND H. PELETIER. 1984. Participation of diatoms in the amino acid cycle of coastal waters; uptake and excretion in cultures. *Marine Ecology Progress Series* 15:303–306.

ADMIRAAL, W. AND H. PELETIER. 1979. Influence of organic compounds and light limitation on the growth rate of estuarine benthic diatoms. *British Phycological Journal* 14:197–206.

AN, S. AND S. B. JOYE. 2001. Enhancement of coupled nitrification-denitrification by benthic photosynthesis in shallow estuarine sediments. *Limnology and Oceanography* 46:62–74.

ANDERSON, I. C., K. J. MCGLATHERY, AND A. C. TYLER. 2003. Microbial mediation of 'reactive' nitrogen transformations in a temperate lagoon. *Marine Ecology Progress Series* 246:73–84.

BARRANGUET, C., P. M. J. HERMAN, AND J. J. SINKE. 1997. Microphytobenthos biomass and community composition studied by pigment biomarkers: Importance and fate in the carbon cycle of the tidal flat. *Journal of Sea Research* 38:59–70.

BLACKBURN, T. H. AND N. D. BLACKBURN. 1993. Rates of microbial processes in sediments. *Philosophical Transactions of the Royal Society of London* 344:49–58.

BORUM, J. 1996. Shallow waters and land/sea boundaries, p. 179–203. *In* B. Jørgensen and K. Richardson (eds.), Coastal Estuarine Studies: Eutrophication in Coastal Marine Ecosystems, Volume 52. American Geophysical Union, Washington, D.C.

BRONK, D. A., M. W. LOMAS, P. M. GLIBERT, K. J. SCHUKERT, AND M. P. SANDERSSON. 2000. Total dissolved nitrogen analysis: Comparisons between the persulfate, UV and high temperature oxidation methods. *Marine Chemistry* 69:163–178.

BROTAS, V. AND F. CATARINO. 1995. Microphytobenthos primary production of Tagus estuary intertidal flats (Portugal). *Netherlands Journal of Aquatic Ecology* 29:333–339.

BURDIGE, D. J. AND S. ZHENG. 1998. The biogeochemical cycling of dissolved organic nitrogen in estuarine sediments. *Limnology and Oceanography* 43:1796–1813.

CABRITA, M. T. AND V. BROTAS. 2000. Seasonal variation in denitrification and dissolved nitrogen fluxes in intertidal sediments of the Tagus estuary. *Marine Ecology Progress Series* 202:51–65.

CAHOON, L. B. 1999. The role of benthic microalgae in neritic ecosystems. *Oceanography and Marine Biology: An Annual Review* 37:47–86.

CERCO, C. F. AND S. P. SEITZINGER. 1997. Measured and modeled effects of benthic algae on eutrophication in Indian River-Rehoboth Bay, Delaware. *Estuaries* 20:231–248.

CHRISTENSEN, P. B. 1998. The Danish marine environment: Has action improved its state? Danish Environmental Protection Agency Report 62, Copenhagen, Denmark.

CHRISTENSEN, P. B., S. RYSGAARD, N. P. SLOTH, T. DALSGAARD, AND S. SCHWAERTER. 2000. Sediment mineralization, nutrient fluxes, denitrification and dissimilatory nitrate reduction to ammonium in an estuarine fjord with sea cage trout farms. *Aquatic Microbial Biology* 21:73–84.

COOK, P. L. M. 2002. Carbon and nitrogen cycling on intertidal mudflats in a temperate Australian estuary. Ph.D. Dissertation, University of Tasmania, Launceston, Tasmania, Australia.

COOK, P. L. M., A. T. REVILL, E. C. V. BUTLER, AND B. D. EYRE. 2004. Carbon and nitrogen cycling on intertidal mudflats of a temperate Australian estuary. II. Nitrogen cycling. *Marine Ecology Progress Series* 280:39–54.

COWAN, J. L. AND W. R. BOYNTON. 1996. Sediment-water oxygen and nutrient exchanges along the longitudinal axis of Chesapeake Bay: Seasonal patterns, controlling factors and ecological significance. *Estuaries* 19:562–580.

DALSGAARD, T. (ED.). 1999a. Nitrogen Cycling in Estuaries. Final Scientific Report for NICE, a project under the EU research programme: Marine Science and Technology (MAST III). National Environmental Research Institute, Silkeborg, Denmark.

DALSGAARD, T., P. B. CHRISTENSEN, S. RYSGAARD, AND N. RISGAARD-PETERSEN. 1999b. Nitrogen removal in Danish waters: Importance and regulation (In Danish). Havmiljøet ved årtusindskiftet (The Marine Environment at the Turn of the Millenium). L. B. A. Fredensborg, Olsen and Olsen: 101–118.

DALSGAARD, T., L. P. NIELSEN, V. BROTAS, P. VIAROLI, G. UNDERWOOD, D. B. NEDWELL, K. SUNDBÄCK, S. RYSGAARD, A. MILES, M. BARTOLI, L. DONG, D. C. O. THORNTON, L. D. M. OTTOSEN, G. CASTALDELLI, AND N. RISGAARD-PETERSEN. 2000. Protocol handbook for NICE - Nitrogen Cycling in Estuaries: A project under the EU research programme: Marine Science and Technology (MAST III). National Environmental Research Institute, Silkeborg, Denmark.

DE WIT, R., L. J. STAL, B. A. LOMSTEIN, R. A. HERBERT, H. VAN GEMERDEN, P. VIAROLI, V. U. CECHERELLI, F. RODRIGUEZ-VALERA, M. BARTOLI, G. GIORDANI, R. AZZONI, B. SCHAUB, D. T. WELSH, A. DONELLI, A. CIFUENTES, J. ANTON, K. FINSTER, L. B. NIELSEN, A. G. U. PEDERSEN, A. T. NEUBAER, M. A. COLANGELO, AND S. K. HEJS. 2001. ROBUST: The ROle of BUffering capacities in STabilising coastal lagoon ecosystems. *Continental Shelf Research* 21:2021–2041.

DOLLAR, S. J., S. V. SMITH, S. M. VINK, S. OBRESKI, AND J. T. HOLLIBAUGH. 1991. Annual cycle of benthic nutrient fluxes in Tomales Bay, California, and contribution of the benthos to total ecosystem metabolism. *Marine Ecology Progress Series* 79:115–125.

Dong, L. F., D. C. O. Thornton, D. B. Nedwell, and G. J. C. Underwood. 2000. Denitrification in sediments of the River Colne estuary, England. *Marine Ecology Progress Series* 203:109–122.

Elmgren, R. 2001. Understanding human impact on the Baltic ecosystem: Changing views in recent decades. *Ambio* 30:222–231.

Emmerson, M. C. and D. G. Raffaelli. 2000. Detecting the effects of diversity on measures of ecosystem function: Experimental design, null models and empirical observations. *OIKOS* 91:195–203.

Emmerson, M. C., M. Solan, C. Ames, D. M. Paterson, and D. Raffaelli. 2001. Consistent patterns and the idiosyncratic effects of biodiversity in marine ecosystems. *Nature* 411:73–77.

Eyre, B. D. and A. J. Ferguson. 2002. Comparison of carbon production and decomposition, benthic nutrient fluxes and denitrification in seagrass, phytoplankton, benthic microalgal and macroalgal dominated warm temperate Australian lagoons. *Marine Ecology Progress Series* 229:43–59.

Fong, P., R. M. Donohoe, and J. B. Zedler. 1993. Competition with macroalgae and benthic cyanobacterial mats limits phytoplankton abundance in experimental microcosms. *Marine Ecology Progress Series* 100:97–102.

Glud, R. N., M. Kühl, F. Wenzhöfer, and S. Rysgaard. 2002. Benthic diatoms of a high artic fjord (Young sound, NE Greenland): Importance for ecosystem primary production. *Marine Ecology Progress Series* 238:15–29.

Guldberg, L. B., K. Finster, N. O. G. Jørgensen, M. Middelboe, and B. Lomstein. 2002. Utilization of marine sedimentary dissolved organic nitrogen by native anaerobic bacteria. *Limnology and Oceanography* 47:1712–1722.

Hansen, L. S. and T. H. Blackburn. 1991. Aerobic and anaerobic mineralization of organic material in marine sediment microcosms. *Marine Ecology Progress Series* 75:283–291.

Havens, K. E., J. Hauxwell, A. C. Tyler, S. Thomas, K. J. McGlathery, J. Cebrian, I. Valiela, A. D. Steinman, and S.-J. Hwang. 2001. Complex interactions between autotrophs in shallow marine and freshwater ecosystems: Implications for community responses to nutrient stress. *Environmental Pollution* 113:95–107.

Henriksen, K., J. Hansen, and T. H. Blackburn. 1980. The influence of benthic infauna on exchange rates of inorganic nitrogen between sediment and water. *Ophelia* 1:249–256.

Hillebrand, H. and M. Kahlert. 2002. Effect of grazing and water column nutrient supply on biomass and nutrient content of sediment microalgae. *Aquatic Botany* 72:143–159.

Hulth, S., P. Engström, E. Selander, and R. C. Aller. 2002. A pH plate fluorosensor for early diagenetic studies of marine sediments. *Limnology and Oceanography* 47:212–220.

Jonge, V. N. 1980. Fluctuations in the organic carbon to chlorophyll *a* ratios for estuarine benthic diatom populations. *Marine Ecology Progress Series* 2:345–353.

Jørgensen, K. S. 1989. Annual pattern of denitrification and nitrate ammonification in estuarine sediment. *Applied Environmental Microbiology* 55:1841–1847.

Kraay, G. W., M. Zapata, and M. J. W. Veldhuis. 1992. Separation of chlorophylls c^1, c^2, and c^3 of marine phytoplankton by reversed-phase-C-18 high performance liquid chromatography. *Journal of Phycology* 28:708–712.

Kristensen, E. 1993. Seasonal variations in benthic community metabolism and nitrogen dynamics in a shallow, organic-poor Danish lagoon. *Estuarine Coastal and Shelf Science* 36:565–586.

Kristensen, E., M. H. Jensen, and K. M. Jensen. 1997. Temporal variations in microbenthic metabolism and inorganic nitrogen fluxes in sandy and muddy sediments of a tidally dominated bay in the northern Wadden Sea. *Helgoländer Meeresunters* 51:295–320.

Linares, F. 2006. Effect of dissolved free amino acids (DFAA) on the biomass and production of microphytobenthic communities. *Journal of Experimental Marine Biology and Ecology* 330:469–481.

Linares, F. and K. Sundbäck. 2006. Uptake of dissolved free amino acids (DFAA) by microphytobenthic communities. *Aquatic Microbial Ecology* 42:175–186.

Lomstein, B. A., A.-G. U. Jensen, J. W. Hansen, J. B. Andreasen, L. S. Hansen, J. Berntsen, and H. Kunzendorf. 1998. Budgets of sediment nitrogen and carbon cycling in the shallow water of Knebel Vig, Denmark. *Aquatic Microbial Ecology* 14:69–80.

McCarthy, M. D. 2003. What is Dissolved Organic Nitrogen. Aquatic Sciences Meeting, American Society of Limnology and Oceanography, Salt Lake City, Utah.

McGlathery, K. J., I. C. Anderson, and A. C. Tyler. 2001. Magnitude and variability of benthic and pelagic metabolism in a temperate coastal lagoon. *Marine Ecology Progress Series* 216:1–15.

McGlathery, K., K. Sundbäck, and I. C. Anderson. 2004. The importance of primary producers for benthic nitrogen and phosphorus cycling, p. 231–261. *In* S. L. Nielsen, B. T. Banta, and M. F. Pedersen (eds.), The Influence of Primary Producers on Estuarine Nutrient Cycling. Kluwer, Dordrecht, The Netherlands.

Miles, A. and K. Sundbäck. 2000. Diel variation in microphytobenthic productivity in areas of different tidal amplitude. *Marine Ecology Progress Series* 205:11–22.

Miyajima, T., M. Suzumura, Y. Umezawa, and I. Koike. 2001. Microbiological nitrogen transformation in carbonate sediments of coral-reef lagoon and associated seagrass beds. *Marine Ecology Progress Series* 217:273–286.

Nedwell, D. B. and M. Trimmer. 1996. Nitrogen fluxes through the upper estuary of the Great Ouse, England: The role of the bottom sediments. *Marine Ecology Progress Series* 142:273–286.

Nielsen, K., L. P. Nielsen, and P. Rasmussen. 1995. Estuarine nitrogen retention independently estimated by the denitrification rate and mass balance methods: A study of Norsminde Fjord, Denmark. *Marine Ecology Progress Series* 119:275–283.

Nielsen, K., N. Risgaard-Petersen, B. Sømod, S. Rysgaard, and T. Bergø. 2001. Nitrogen and phosphorus retention estimated independently by flux measurements and dynamic modeling in the estuary, Randers Fjord, Denmark. *Marine Ecology Progress Series* 219:25–40.

Nielsen, L. P. 1992. Denitrification in sediment determined from nitrogen isotope pairing. *FEMS Microbiology Ecology* 86:357–362.

Nielsen, S. L., G. T. Banta, and M. F. Pedersen (eds.). 2004. Estuarine nutrient cycling: The influence of primary producers. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Paterson, D. M. 1989. Short-term changes in the erodibility of intertidal cohesive sediments related to the migratory behavior of epipelic diatoms. *Limnology and Oceanography* 34:223–234.

Paterson, D. M. and S. E. Hagerthey. 2001. Microphytobenthos in Contrasting Coastal Ecosystems: Biology and Dynamics. Ecological Comparisons of Sedimentary Shores, edition 151. Springer-Verlag, Berlin, Germany.

Reay, W. G., D. L. Gallagher, and G. M. J. Simmons. 1995. Sediment-water column oxygen and nutrient fluxes in nearshore environments of the lower Delmarva Peninsula, USA. *Marine Ecology Progress Series* 118:215–227.

Revsbech, N. P. 1989. An oxygen microelectrode with a guard cathode. *Limnology and Oceanography* 34:474–478.

Risgaard-Petersen, N. 2003. Coupled nitrification-denitrification in autotrophic and heterotrophic estuarine sediments: On the influence of benthic microalgae. *Limnology and Oceanography* 48:93–105.

Risgaard-Petersen, N., T. Dalsgaard, S. Rysgaard, P. B. Christensen, J. Borum, K. McGlathery, and L. P. Nielsen. 1998. Nitrogen balance of a temperate eelgrass *Zostera marina* bed. *Marine Ecology Progress Series* 174:281–291.

Risgaard-Petersen, N., S. Rysgaard, L. P. Nielsen, and N. P. Revsbech. 1994. Diurnal variation of denitrification and nitrification in sediments colonized by benthic microphytes. *Limnology and Oceanography* 39:573–579.

Rizzo, W. M. 1990. Nutrient exchanges between the water column and a subtidal benthic microalgal community. *Estuaries* 13:219–226.

Rizzo, W. M., S. K. Dailey, G. J. Lackey, R. R. Christian, B. E. Berry, and R. L. Wetzel. 1996. A metabolism-based trophic index for comparing the ecological values of shallow-water sediment habitats. *Estuaries* 19:247–256.

Rizzo, W. M., G. J. Lackey, and R. R. Christian. 1992. Significance of euphotic, subtidal sediments to oxygen and nutrient cycling in a temperate estuary. *Marine Ecology Progress Series* 86:51–61.

Rondell, J. B., K. W. Finster, and B. A. Lomstein. 2000. Urea and DON uptake by a *Lyngbya gracilis* dominated microbial mat: A controlled laboratory experiment. *Aquatic Microbial Ecology* 21: 169–175.

Rysgaard, S., P. B. Christensen, and L. P. Nielsen. 1995. Seasonal variation in nitrification and denitrification in estuarine sediment colonized by benthic microalgae and bioturbating infauna. *Marine Ecology Progress Series* 126:111–121.

Rysgaard, S., N. Risgaard-Petersen, N. P. Sloth, K. Jensen, and L. P. Nielsen. 1994. Oxygen regulation of nitrification and denitrification in sediments. *Limnology and Oceanography* 39: 1643–1652.

Seitzinger, S. P. 1988. Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. *Limnology and Oceanography* 33:702–724.

Simon, N. S. 1988. Nitrogen cycling between sediment and the shallow-water column in the transition zone of the Potomac River and estuary. I. Nitrate and ammonium fluxes. *Estuarine Coastal and Shelf Science* 26:483–497.

Sloth, N. P., H. Blackburn, L. S. Hansen, N. Risgaard-Peteresen, and B. A. Lomstein. 1995. Nitrogen cycling in sediments with different organic loading. *Marine Ecology Progress Series* 116:163–170.

Stockenberg, A., K. Sundbäck, and C. Nilsson. 1998. Denitrification and microphytobenthos - sinks of nitrogen in the Gulf of Riga. The role of sediments in nitrogen cycling in the larger Baltic Sea. A. Stockenberg. Stockholm University, Stockholm, Sweden.

Strickland, J. D. H. and T. R. Parsons. 1972. A Practical Handbook of Sewater Analysis, 2nd edition. Bulletin of Fishery Research Board of Canada, Ottawa, Canada.

Sundbäck, K., V. Enoksson, W. Granéli, and K. Pettersson. 1991. Influence of sublittoral microphytobenthos on the oxygen and nutrient flux between sediment and water: A laboratory continuous-flow study. *Marine Ecology Progress Series* 74:263–279.

Sundbäck, K., F. Linares, F. Larson, A. Wulff, and A. Engelsen. 2004. Benthic nitrogen fluxes along a depth gradient in a microtidal fjord: The role of denitrification and microphytobenthos. *Limnology and Oceanography* 49:1095–1107.

Sundbäck, K. and K. McGlathery. 2005. Interactions between benthic macroalgal and microalgal mats, p. 7–29. *In* E. Kristensen, R. R. Haese, and J. E. Kostka (eds.), Interactions Between Macro- and Microorganisms in Marine Sediments, Volume 60. American Geophysical Union, Washington, D.C.

Sundbäck, K. and A. Miles. 2000. Balance between denitrification and microalgal incorporation of nitrogen in microbial sediments, NE Kattegat. *Aquatic Microbial Ecology* 22:291–300.

Sundbäck, K. and A. Miles. 2002. Role of microphytobenthos and denitrification for nutrient turnover in embayments with floating microalgal mats: A spring situation. *Aquatic Microbial Ecology* 30:91–101.

Sundbäck, K., A. Miles, and E. Göransson. 2000. Nitrogen fluxes, denitrification and the role of microphytobenthos in microtidal shallow-water sediments: an annual study. *Marine Ecology Progress Series* 200:59–76.

Sundbäck, K., A. Miles, S. Hulth, L. Pihl, P. Engström, E. Selander, and A. Svenson. 2003. Importance of benthic nutrient regeneration during initiation of macroalgal blooms in shallow bays. *Marine Ecology Progress Series* 246:115–126.

Suzumura, M., T. Miyajima, H. Hata, Y. Umezawa, H. Kayanne, and I. Koike. 2002. Cycling of phosphorus maintains the production of microphytobenthic communities in carbonate sediments of a coral reef. *Limnology and Oceanography* 47:771–781.

Thamdrup, B. and T. Dalsgaard. 2002. Production of N_2 through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. *Applied Environmental Microbiology* 68:1312–1318.

Thornton, D. C. O., L. F. Dong, G. J. C. Underwood, and D. B. Nedwell. 2002. Factors affecting microphytobenthic biomass, species composition and production in the Colne Estuary (UK). *Aquatic Microbial Ecology* 27:285–300.

Trimmer, M., D. B. Nedwell, D. B. Sivyer, and S. J. Malcolm. 2000. Seasonal organic mineralization and denitrification in intertidal sediments and their relationship to the abundance of *Enteromorpha* sp. and *Ulva* sp. *Marine Ecology Progress Series* 203: 67–80.

Tyler, A. C., K. J. McGlathery, and I. C. Anderson. 2001. Macroalgae mediation of dissolved organic nitrogen fluxes in a temperate coastal lagoon. *Estuarine Coastal and Shelf Science* 53: 155–168.

Tyler, A. C., K. J. McGlathery, and I. C. Anderson. 2003. Benthic algae control sediment-water column fluxes and inorganic nitrogen compounds in a temperate lagoon. *Limnology and Oceanography* 48:2125–2137.

Underwood, G. J. C. and J. Kromkamp. 1999. Primary production by phytoplankton and microphytobenthos in estuaries. *Advances in Ecological Research* 29:93–153.

Valderrama, J. C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. *Marine Chemistry* 10:109–122.

van Breemen, N. 2002. Natural organic tendency. *Nature* 415:381–382.

Viaroli, P. and R. R. Christian. 2003. Description of trophic status, hyperautotrophy and dystrophy of a coastal lagoon through a potential oxygen production and consumption index—TOSI: Trophic Oxygen Status Index. *Ecological Indicators* 3:237–250.

Ward, B. B. and D. A. Bronk. 2001. Net nitrogen uptake and DON release in surface waters: Importance of trophic interactions implied from size fractionation experiments. *Marine Ecology Progress Series* 219:11–24.

Wright, S. W., S. W. Jeffrey, R. F. C. Mantoura, C. A. Llewellyn, T. Bjørnland, D. Repeta, and N. Welschmeyer. 1991. Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. *Marine Ecology Progress Series* 77: 183–196.

Wulff, A., S. Å. Wängberg, K. Sundbäck, C. Nilsson, and G. Underwood. 2000. Effects of UVB radiation on a marine microphytobenthic community growing on a sand-substratum under different nutrient conditions. *Limnology and Oceanography* 45:1144–1152.

Received, December 14, 2005
Accepted, June 14, 2006