

Role of microphytobenthos and denitrification for nutrient turnover in embayments with floating macroalgal mats: a spring situation

Kristina Sundbäck*, Alison Miles

Department of Marine Ecology, Marine Botany, Göteborg University, PO Box 461, 405 30 Göteborg, Sweden

ABSTRACT: The importance of the microphytobenthos (MPB) for nutrient turnover was studied in 2 shallow bays on the west coast of Sweden, during the period that floating green macroalgal mats generally become established in the area. Sediment-water nutrient fluxes (N, P and Si) and oxygen fluxes and denitrification were measured in light and dark laboratory incubations prior to and during the early growth of green-algal mats (April to June). The importance of microphytobenthic activity and hence, trophic status of the sediment system, magnitude of sediment fluxes and presence of macroalgal mats were shown to differ between the 2 bays. On a daily basis, the MPB decreased the efflux of inorganic N by 30 to 100%, P by 70 to 100% and Si by 10 to 95%. Thus, the MPB appears to efficiently control the availability of sediment-derived nutrients to ephemeral green algae during the period that is critical for the onset of the macroalgal growth. The MPB may even depress the development of green-algal mats, providing that these depend on the sediment nutrient efflux. Microphytobenthic N-assimilation was compared with other N-consuming processes, such as denitrification and green-macroalgal N-assimilation. In autotrophic sediments, the MPB was the dominating N-consuming component (1 to 5.3 mmol N m⁻² d⁻¹), denitrification (0.1 to 0.7 mmol N m⁻² d⁻¹) being a minor N-sink. In the net heterotrophic sediments, however (with no or little net MPB productivity, higher infaunal biomass and hence larger nutrient effluxes), green macroalgal growth was the main N-incorporating process (mean 2.3 mmol N m⁻² d⁻¹). This was followed by denitrification (0.3 to 1.4 mmol N m⁻² d⁻¹), which removed roughly 20% of the remineralised N. Thus, denitrification may be a significant N-sink in bays harbouring green-algal mats on the west coast of Sweden.

KEY WORDS: Sediment · Microphytobenthos · Nitrogen · Denitrification · Macroalgal mats · Sediment-water fluxes

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INTRODUCTION

Extensive coverage of shallow-water coastal areas by floating macroalgal mats is an increasing phenomenon worldwide, being generally interpreted as a symptom of coastal eutrophication (Valiela et al. 1997, Pihl et al. 1999 and references therein). So far, most studies dealing with the problem of fast-growing macroalgae have focused on the impact of established mats on

populations of other organisms or on the function of local food webs (e.g. Isaksson et al. 1994, Rafaelli 2000, Österling & Pihl 2001 and references therein). Established macroalgal mats have also been studied with regard to competition between these mats and other primary producers of shallow embayments. Mats of fast-growing macroalgae tend to outcompete benthic primary producers, as well as phytoplankton (e.g. Sundbäck et al. 1990, Fong et al. 1993, den Hartog 1994, Havens et al. 2001). Here, we have switched the angle of approach, focusing on the influence that other local primary producers might have on the onset and

*Email: kristina.sundback@marbot.gu.se

early growth of the macroalgal blooms rather than studying the effect of macroalgal mats themselves.

The sediment is known to function as an important source of regenerated nutrients for maintaining the growth of the macroalgal mats once they have become established (Thybo-Christesen et al. 1993, Jeffrey et al. 1995, Viaroli et al. 1996). However, the actual onset of the macroalgal growth may also be controlled by the local sediment nutrient efflux, rather than, for example, land run-off to the water column. This accords with the idea that embayments may have become, after several decades of nutrient loading, self-regenerating systems that favour the growth of opportunistic macroalgae (Hodgkin & Birch 1986, Pihl et al. 1999).

In this context, the role of microphytobenthos (MPB) as a competitor and controller of nutrient fluxes across the sediment-water interface (for references see Thornton et al. 1999, Sundbäck et al. 2000) is particularly interesting for several reasons. In contrast to seagrasses and ephemeral macroalgae, which start to grow in spring (Risgaard-Petersen et al. 1998, Pihl et al. 1999), the MPB is present and active throughout the year in temperate northern waters (Rysgaard et al. 1995, Sundbäck et al. 2000). Therefore, as the MPB is already well established at the time of initial macroalgal growth (spring), the MPB can be assumed to compete with the macroalgal mats for the sediment nutrient pool. By forming dense microbial mats at the sediment-water interface, the MPB could 'shut off' the sediment nutrient supply to the water column, thereby counteracting the development of extensive green-algal mats. This has been proposed in previous experiments (Sundbäck et al. 1996). The same study suggested that benthic diatom mats may continue to thrive under the 'shading roof' of macroalgal mats, thereby extending the controlling effect of MPB on the sediment-water nutrient flux further into the growth season of the ephemeral macroalgae.

The first aim of our study was to assess the role of the MPB in nutrient turnover in shallow embayments, prior to and during the onset of green-macroalgal blooms in the spring. Extended occurrence of filamentous macroalgae on the west coast of Sweden has occurred since the 1970s, paralleled with an increasing load of nutrients to the coastal zone (Pihl et al. 1996 and references therein). Macroalgal mats begin to develop in May and have been found to cover between 30 and 50% of the shallow soft-sediment habitats during summer (Pihl et al. 1996, 1999). The working hypothesis was that if the sediment nutrient efflux was critical for the initiation of the growth of the macroalgal mats, then the magnitude of microphytobenthic activity

could be an important factor controlling the early growth of the macroalgae. In other words, could the MPB reduce the sediment-nutrient efflux to such a degree that macroalgal growth would be prevented or delayed?

A second aim was to compare the quantitative importance of microphytobenthic nitrogen (N) assimilation and retention with other N-consuming processes, such as macroalgal N-assimilation and the final removal of N by denitrification. The approach taken was to measure sediment-water nutrient and oxygen fluxes as well as denitrification, prior to and during the early growth phase of the macroalgal mats (April to June) in 2 shallow embayments on the Swedish west coast where green-algal mats commonly occur.

MATERIALS AND METHODS

Study sites and sampling protocol. Two shallow brackish-water bays on the microtidal (tidal amplitude ca. 20 cm) west coast of Sweden, Rågårdsvik (RÅ; 58° 12' N, 11° 27' E) and Bassholmen (BA; 58° 15' N, 11° 30' E), were studied. These 2 bays were chosen on the basis of a previous study (Pihl et al. 1996, 1999) as being representative of bays that regularly harbour extensive green-algal mats. Characteristics for the sites are given in Table 1. The study was conducted from 10 April to 28 June 2000. In each bay, 3 stations (20 m apart) were established and at each station, 2 replicate sediment cores were sampled with a 24 cm long Plexiglas core (8 cm in diameter) for incubation measurements. Fluxes of oxygen and inorganic nutri-

Table 1. Characteristics of the 2 bays during the study period (April to June 2000). Either range and/or mean is given. WW: wet weight; DW: dry weight

	Rågårdsvik	Bassholmen
Water column		
Water depth (m)	0.5	0.5
Salinity (PSU)	8–27 (22)	19–26 (22)
Water temperature (°C)	6–18	6–18
NH ₄ (µM)	0.6–4.4 (2.2)	0.8–3.9 (1.8)
NO ₃ + NO ₂ (µM)	0.2–21.2 (3.9)	0.2–3.5 (0.86)
PO ₄ (µM)	0.1–0.6 (0.4)	0.1–0.6 (0.4)
Si(OH) ₄ (µM)	2–71 (14.8)	2–10 (4.5)
Sediment		
Porosity	0.81	0.65
C-content (%)	2.04	1.45
N-content (%)	0.23	0.16
Infaunal biomass (g WW m ⁻²)	18–59 (35)	20–131 (73)
Green-algal mat		
Biomass (g DW m ⁻²)	0	40 ^a
^a Value for June		

ents (N, P and Si) were measured in light and dark under controlled laboratory conditions (see below). Chlorophyll *a* (chl *a*) and sediment properties were also measured (see Table 1 and 'Sediment properties'). Biomass of macroscopic infauna was measured in the top 5 cm (mesh size 0.5 mm). Incubations were run on 4 occasions, during calendar Weeks 15 (mid-April), 19 (mid-May), 24 (mid-June) and 26 (end of June). These sampling periods will be referred to as W15, W19, W24 and W26, respectively. Nutrient concentrations in the *in situ* overlying water were measured weekly from April to the end of June. Biomass, growth rate and N-content of green-algal mats were monitored during the study period (Sundbäck et al. in press). At BA, green-algal mats (dominated by *Enteromorpha* spp.) began to develop in May, reaching a mean biomass of 40 g DW m⁻² by the middle of June. No green-algal mats developed at RÅ during the study period.

Flux measurements. Sediment incubations for nutrient (NH₄⁺, NO₃⁻ + NO₂⁻, PO₄³⁻) and oxygen fluxes were carried out in the laboratory in light and dark. Two replicate and 1 blank core (only site water) for each station were incubated with magnetic stirring (60 rpm) at *in situ* temperature in specially designed tanks containing circulating site water (for details see Dalsgaard et al. 2000, Sundbäck et al. 2000). Light level during the incubation (individual 25 W halogen bulbs over each core) was adjusted to the daily mean (i.e. the mean for the time from sunrise to sunset) for that month. Incubation time was set so that changes in oxygen concentration within the cores did not exceed 20% and varied between 1.5 and 5 h, depending on temperature. Oxygen concentrations were measured using Winkler titration. Nutrient samples were immediately frozen (-80°C) and stored until analysis, which followed standard colorimetric methods using auto-analyser (TRAACS, Braun-Lubbe). As the sediment cores were exposed to a mean daily light level, oxygen and nutrient fluxes were recalculated to daily values using the number of light and dark hours obtained from a DELTA-T light sensor at the Kristineberg Marine Research Station.

Calculation of algal assimilation of nutrients. Microphytobenthic nutrient assimilation was calculated from daily net oxygen flux in light (net productivity, NP) and gross production (GP = the sum of NP and community respiration [CR] in the dark). For practical reasons, it was assumed that community respiration in the light equalled respiration in the dark. For conversion of oxygen to carbon produced, a photosynthetic quotient (PQ) of 1.2 was assumed (Wetzel & Likens 1991). N-assimilation by the MPB was calculated assuming a C:N molar ratio of 9 (Sundbäck et al. 2000 and references therein). The retention of N in the MPB

biomass was estimated from sediment chl *a* values, assuming a mean C:chl *a* ratio of 30 (de Jonge 1980, Sundbäck et al. 2000). Mean green macroalgal N-assimilation and retention were estimated from net macroalgal growth and the N and P content of the algae. Macroalgal growth (dry weight [DW] m⁻² d⁻¹) was calculated from the initial increase of algal biomass (5 replicate random samples of 350 cm²) between W19 and W24. As turnover or loss rates were not measured, the mean net growth represents a conservative estimate of mean biomass increase and nutrient accumulation in algal biomass.

Denitrification. Denitrification was measured in light and dark in the same laboratory incubation set-up that was used for flux measurements, using the isotope pairing technique (Nielsen 1992), which discriminates between denitrification supported by NO₃ in the water column (*D_w*) and nitrification-coupled denitrification (*D_n*). ¹⁵NO₃ (40 mM Na¹⁵NO₃, 99.6 at.%, Europa Scientific) was added to the headspace water in the cores to a final concentration corresponding to ca. 20% of the oxygen concentration (50 to 90 μM ¹⁵NO₃). The isotopic ¹⁵N:¹⁴N ratio of N₂ was analysed by mass spectrometry at the National Environmental Research Agency, Silkeborg, Denmark. Daily rates of total denitrification (*D_{tot}* = *D_w* + *D_n*) were calculated by multiplying the hourly rate in light by the number of daylight hours and the hourly rate in the dark by the number of dark hours. The total amount of N removed by denitrification during the 3 mo study period (April to June) was calculated by multiplying daily values by the number of days in the month. For June, the mean value of the 2 occasions (W24 and W26) was used.

Sediment properties. Samples for chl *a* and pheopigment content of the top 5 mm sediment were taken from the sediment cores, on completion of the nutrient flux measurements, with a 20 ml cut-off disposable syringe. The samples were immediately frozen (-80°C). Pigment concentration was measured spectrophotometrically (Lorenzen 1967) after extraction with 90% acetone. The composition of the MPB was assessed semi-quantitatively using epifluorescence microscopy. Porosity of the top 5 mm of sediment was measured by gravimetric standard methods (e.g. Dalsgaard et al. 2000). The content of particulate organic carbon (POC) and nitrogen (PON) of the sediment was determined on dried samples using a Carlo Erba CHN elemental analyser. Before analysis, samples for POC content were treated with HCl fumes to remove metal carbonates.

Statistical analyses. Spatial variation was tested using 3-way nested ANOVA with time, bay and station (nested within bays) as factors. As no significant differences between stations within a bay were found, further testing of treatments was based on 6 replicates.

Differences between fluxes in light and dark were tested using a 2-way ANOVA, with time and treatment as factors. Homogeneity of variances was checked using Cochran's test and when found to be heterogeneous, the data were transformed according to Underwood (1997). In cases of a significant interaction term, pair wise comparisons of the means were made using Student-Newman-Keul's test (SNK). Differences were accepted as significant at $p < 0.05$.

RESULTS

Microphytobenthic biomass and composition

Mean chl *a* content of the top 5 mm sediment varied only slightly during the 3 mo study period (range 61 to 137 mg m⁻²), being occasionally (W15 and W24) different between the 2 bays (week × bay interaction, $p = 0.0001$) (Fig. 1a). Pheopigment concentrations (range 40 to 92 mg m⁻²) were significantly higher at BA than at RÅ (Fig. 1a). Epifluorescence microscopy revealed a well-developed community consisting of both motile and epipsammic (attached) diatom species. Dominat-

ing species in both bays during the study period were the centric diatom *Paralia sulcata* (Ehrenberg) Cleve and sigmoid motile species, such as *Gyrosigma fasciola* (Ehrenberg) Griffith & Henfrey, and small *Fragilaria*-like epipsammic cells. In April, *Cylindrotheca closterium* (Ehrenberg) Reimann & Lewin, and sedimented chains of the planktonic species *Skeletonema costatum* (Greville) Cleve, were also common. In June, the number of colonies of the cyanobacterium *Microcrocis* sp. had increased at both sites.

Oxygen flux

There was a significant effect of light on the hourly oxygen flux, reflecting MPB primary productivity. Daily GP varied between 30 and 115 mmol O₂ m⁻² d⁻¹ (equivalent to 300–1150 mg C m⁻² d⁻¹), while NP varied between 0 and 58 mmol O₂ m⁻² d⁻¹ (0 to 580 mg C m⁻² d⁻¹). From May onwards, both GP and NP were significantly higher at RÅ than at BA (2-way ANOVA, week × bay interaction) (Fig. 1b). Net oxygen fluxes showed that RÅ was an autotrophic system during daytime, while BA was heterotrophic, with the exception of late June. CR was within the same range in both bays (30 to 86 mmol O₂ m⁻² d⁻¹) (Fig. 1b).

Nutrient fluxes

Water-column concentrations of DIN during the study period were generally below 5 μM, with the exception of a NO₃ peak of 21 μM at RÅ in April (Table 1). PO₄ concentrations were low, generally close to the detection limit (Table 1). Si content (range 2 to 71 μM) was higher at RÅ than BA.

Three general patterns emerged from the hourly nutrient fluxes: (1) light had a significant effect on the fluxes (Table 2); (2) effluxes were significantly higher at BA than at RÅ, where frequent net uptake occurred; and (3) fluxes increased significantly between April and May, coinciding with a temperature increase of 10°C (Fig. 2).

Light had a significant effect on NH₄ and NO₃ fluxes in both bays (Table 2). At RÅ, NH₄ was taken up by the sediment in the light and released in the dark, with the exception of May (Fig. 2a). At BA, NH₄ was released, although the efflux decreased by 40 to 60% in the light (Fig. 2a). Nitrate fluxes were low (<10 μmol m⁻² h⁻¹) at RÅ, but increased to greater than 100 μmol at BA

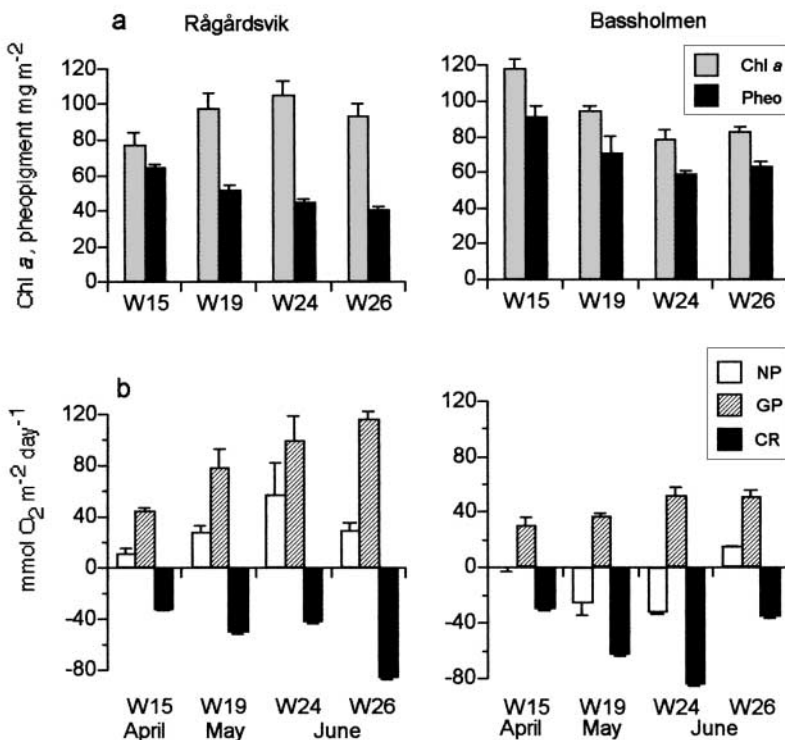


Fig. 1. (a) Chlorophyll *a* and pheopigment content of the top 5 mm sediment and (b) daily net (NP) and gross (GP) primary productivity and community respiration (CR) on 4 occasions during April to June 2000 at Rågårdsvik (RÅ) and Bassholmen (BA). Bars show mean + SE, $n = 6$. Numbers on x-axes denote calendar week (W) numbers

Table 2. Results of 2-way ANOVA testing the significance of light on hourly sediment nutrient fluxes and denitrification rates. Factors used were week (W) and light/dark treatment (L/D). Probability (p) values for either an overall single-factor effect of L/D treatment or a $W \times L/D$ interaction are shown. Week number denotes the number of the calendar week when significant effects were found based on Student-Newman-Keul's (SNK) tests, ns = no significant effects; n = 6. DIN = dissolved inorganic nitrogen, D_w = denitrification based on water column NO_3 , D_n = nitrification-coupled denitrification

Variable	Rågårdsvik (RÅ)			Bassholmen (BA)		
	Factor	p	Week	Factor	p	Week
NH_4	$W \times L/D$	0.0001	19, 24	L/D	0.0135	
$\text{NO}_3 + \text{NO}_2$	$W \times L/D$	0.0001	15, 19, 26	$W \times L/D$	0.0014	24, 26
DIN	$W \times L/D$	0.0001	19, 24	L/D	0.0001	
PO_4	$W \times L/D$	0.0001	15, 19, 24	$W \times L/D$	0.0001	19, 24, 26
$\text{Si}(\text{OH})_4$	L/D	0.0257		ns		
D_w	L/D	0.0005		ns		
D_n	$W \times L/D$	0.0173	19	ns		

(Fig. 2b). When calculated over the day, total fluxes of DIN ($\text{NO}_3 + \text{NO}_2 + \text{NH}_4$) were significantly higher at BA than at RÅ from May onwards (2-way ANOVA, week \times bay interaction, $p = 0.0002$). Thus, at RÅ, sediments functioned mainly as a sink of DIN, while at BA sediments functioned as a source of DIN.

Fluxes of PO_4 were low and the light effect, although significant (Table 2), did not always follow the usual pattern of less efflux in the light (Fig. 2c). Si showed a significant light/dark effect at RÅ, with frequent uptake in light, while no such light effect was seen for BA, the flux being mainly out of the sediment (Table 2, Fig. 2d).

Denitrification

D_{tot} varied between ca. 5 and 60 $\mu\text{mol N m}^{-2} \text{ h}^{-1}$ (equivalent to 100–1400 $\mu\text{mol m}^{-2} \text{ d}^{-1}$) and was dominated by D_n , which was an order of magnitude higher than D_w (Figs. 3 & 4). Light had a significant effect on denitrification rates at RÅ (Table 2), with an overall negative effect on D_w , while D_n was only affected in May. No significant light effect was seen at BA (Table 2, Fig. 3). Daily total denitrification was significantly higher at BA (Fig. 4).

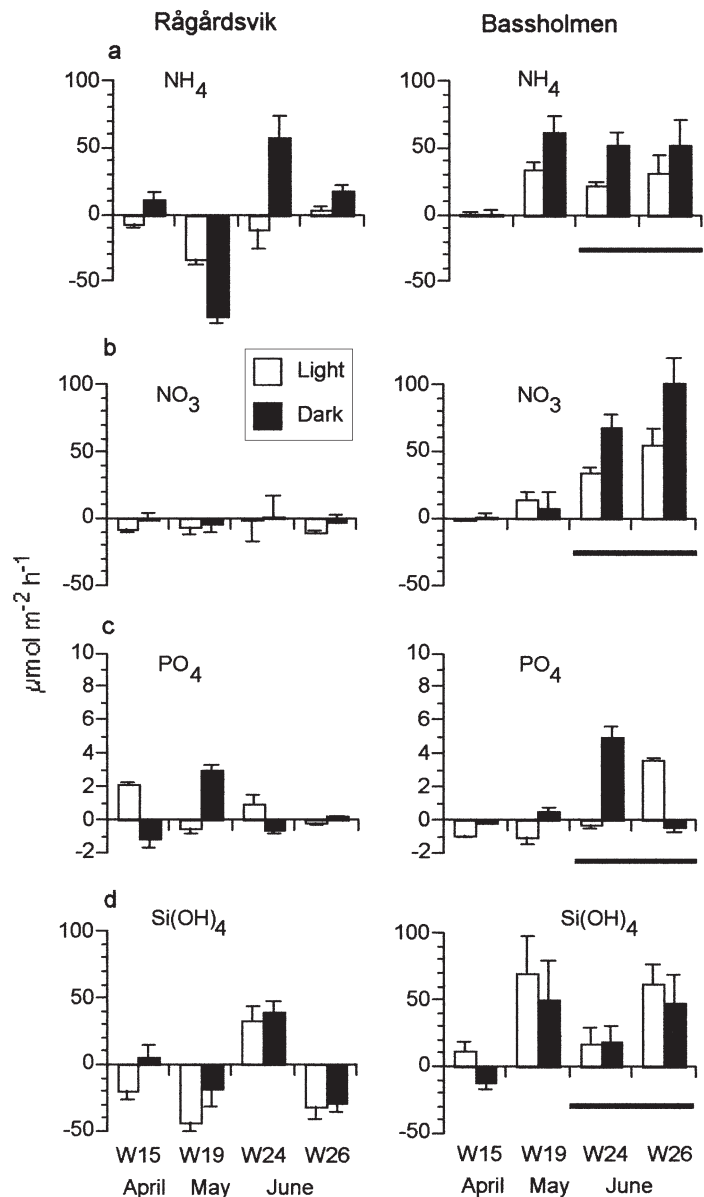


Fig. 2. Sediment-water fluxes of inorganic nutrients (NH_4 , $\text{NO}_3 + \text{NO}_2$, PO_4 , $\text{Si}(\text{OH})_4$) in light and dark conditions on 4 occasions during April to June 2000 at Rågårdsvik (RÅ) and Bassholmen (BA). Bars show mean \pm SE, n = 6. Numbers on x-axes denote calendar week (W) numbers. The horizontal line indicates the period when macroalgae were present at BA

Magnitude of the microphytobenthic effect on nutrient turnover

Microphytobenthos exerted a significant effect on both nutrient fluxes and denitrification although to a different degree in the 2 bays. The magnitude of microphytobenthic influence on daily nutrient effluxes can be roughly estimated by comparing calculated daily fluxes with a hypothetical 24 h dark flux. Then, MPB could decrease daily DIN efflux by 40 to >100% at RÅ, but only by 30 to 40% at BA. Si efflux was decreased by 10 to 95% at RÅ, whereas no significant decrease was observed at BA. The light effect on PO_4 was not consistent at RÅ. However, at BA microphytobenthos decreased daily PO_4 efflux by 70 to >100% during the first 3 sampling weeks.

Fig. 4 summarises the magnitude of calculated daily microalgal assimilation of DIN (based on NP and GP, MPB_{NP} and MPB_{GP} , respectively), and D_{tot} as well as daily fluxes of DIN. At RÅ, where no green macroalgal growth occurred during the study period, MPB assimilation from NP (MPB_{NP} : 1 to $5.3 \text{ mmol N m}^{-2} \text{ d}^{-1}$) was the dominating N-consuming process on a daily basis, denitrification (0.1 to $0.7 \text{ mmol N m}^{-2} \text{ d}^{-1}$) having a minor role (Fig. 4a). At BA, on the other hand, macroalgal assimilation was the most important N-consuming process from May onwards (mean $2.3 \text{ mmol N m}^{-2} \text{ d}^{-1}$), followed by denitrification (D_{tot} 0.3 to $1.4 \text{ mmol N m}^{-2} \text{ d}^{-1}$), at least when adopting the more conservative measure of MPB_{NP} . At the end of June, however, MPB_{NP} ($1.4 \text{ mmol N m}^{-2} \text{ d}^{-1}$) equalled denitrification (Fig. 4a).

Two types of daily DIN fluxes are shown in Fig. 4. Measured fluxes, calculated as daily rates (Fig. 4b), suggest that the sediments at RÅ act mainly as a N-sink, while those at BA act mainly as a N-source. Calculated daily light-related uptake (Fig. 4c), which is the difference between a hypothetical 24 h dark flux and the daily flux, roughly mirrors the uptake related to microphytobenthic activity and was within the same range for both bays (0 to $1150 \text{ } \mu\text{mol N m}^{-2} \text{ d}^{-1}$). Light-related uptake at RÅ corresponded roughly to 20 to 50% of the calculated microphytobenthic DIN demand (cf. Fig. 4a), while at BA, light-related uptake roughly equalled MPB_{NP} .

Balance between N-consuming processes

Table 3 summarises the magnitude of N-consuming components and processes calculated over the 3 mo study period. The 3 pro-

cesses include calculated microphytobenthic and macroalgal N-assimilation, and measured total denitrification. For an explanation and calculations of these variables, see Table 3. The $A:D$ ratio is the balance between algal N-assimilation and N-removal by denitrification (Sundbäck & Miles 2000). The estimated amounts of N consumed by the 3 processes were either within the same order of magnitude or they differed, at most, by 1 order of magnitude (Table 3). When considering the balance between these processes, we have adopted the more conservative measure of microphytobenthic N-assimilation, i.e. that based on NP (MPB_{NP}).

At RÅ, where no green-macroalgal growth occurred, microphytobenthic assimilation consumed most N (235 mmol m^{-2}), while total denitrification removed only 44 mmol N m^{-2} ($\approx 20\%$ of MPB_{NP}) (Table 3). It is interesting to note that MPB_{NP} at RÅ exceeded, by a factor of 2, the green-macroalgal consumption calculated for BA. At BA, macroalgal assimilation ($118 \text{ mmol N m}^{-2}$) was the dominating N-consuming process, followed closely by denitrification (87 mmol m^{-2}), which in turn exceeded microphytobenthic net

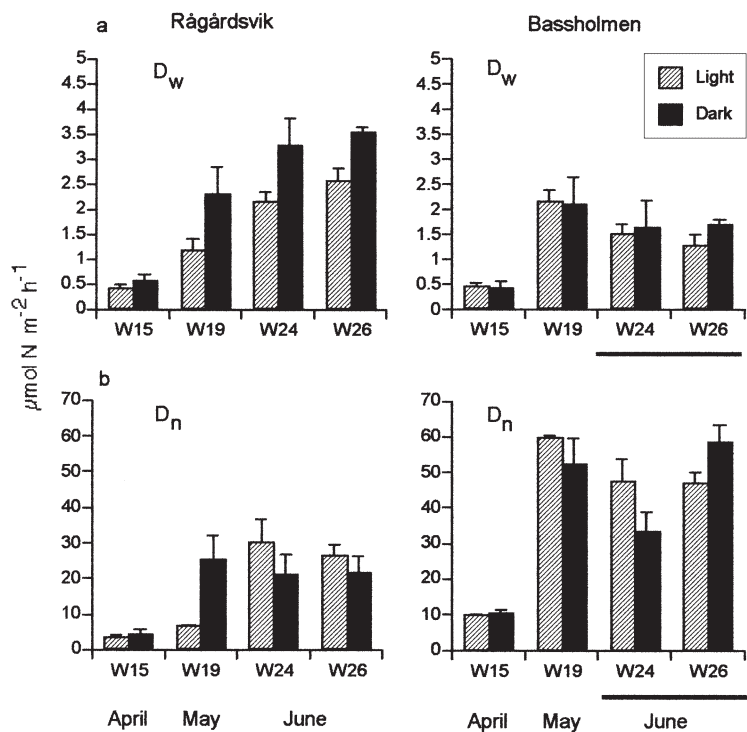


Fig. 3. Denitrification based on water column NO_3 (D_w) and nitrification-coupled denitrification (D_n) in light and dark conditions on 4 occasions during April to June 2000 at Rågårdsvik (RÅ) and Bassholmen (BA). Bars show mean + SE, $n = 4$. Numbers on x-axes denote calendar week (W) numbers. The horizontal line indicates the period when macroalgae were present at BA.

Table 3. Calculated N-consumption and retention by microphytobenthos (MPB) and green macroalgal mats (MA) and measured denitrification (D) for the period April to June 2000: assimilation by MPB is calculated from gross production (MPB_{GP}) and net production (MPB_{NP}). N retained in MPB biomass is based on mean chlorophyll a (MPB_{bio}), assuming a C:chl a ratio of 30. Assimilation by green algae is based on net growth (MA_{NP}), N retained in green-macroalgal biomass (MA_{bio}) is based on mean biomass dry weight (DW) m^{-2} and measured N content. Values for green macroalgae are from mid-May to June. Ratios between algal N-assimilation and denitrification ($A:D$) based on both GP and NP are shown. Consumption rates are expressed as $mmol N m^{-2} 3 mo^{-1}$ and retention in biomass as $mmol N m^{-2}$

Bay	MPB_{GP}	MPB_{NP}	MPB_{bio}	MA_{NP}	MA_{bio}	D	$A:D$ ratios		
							$MPB_{GP}:D$	$MPB_{NP}:D$	$MA_{NP}:D$
Rågårdsvik (RÅ)	517	235	26			44	11.8	5.3	
Bassholmen (BA)	204	21	26	118	58	87	2.3	0.24	1.4

assimilation ($21 mmol m^{-2}$) by a factor of ~ 4 . However, if microphytobenthic assimilation was based on GP ($MPB_{GP}:204 mmol m^{-2}$), it would be double that of denitrification. The $A_{NP}:D$ ratio for the MPB was ca. 5 for RÅ, but below 1 for BA, emphasising the varying importance of the MPB in the 2 bays that were studied. The $A:D$ ratio for green macroalgae (1.4) suggested that macroalgal assimilation and denitrification were nearly balanced at BA in terms of DIN consumption.

If N-retention is estimated as the amount of N that at any time is retained in the mean amount of algal standing stock (MPB_{bio} , MA_{bio} in Table 3), we find that approximately double the amount of N is retained in macroalgae as in the MPB. However, mean standing stock is an underestimation of N-retention, as no attention is paid to the fact that N is both moved farther in the food web and buried in the sediment.

DISCUSSION

Although our study is limited, covering only 1 spring period and 2 bays, it provides some indicators regarding the role of the MPB in embayments subjected to eutrophication. The MPB appears to compete successfully for the local sediment pool of regenerated nutrients during the period that is critical for the onset of the green macroalgal growth. At the same time, the role of seemingly similar microphytobenthic communities appears to vary between apparently similar sediment systems and bays.

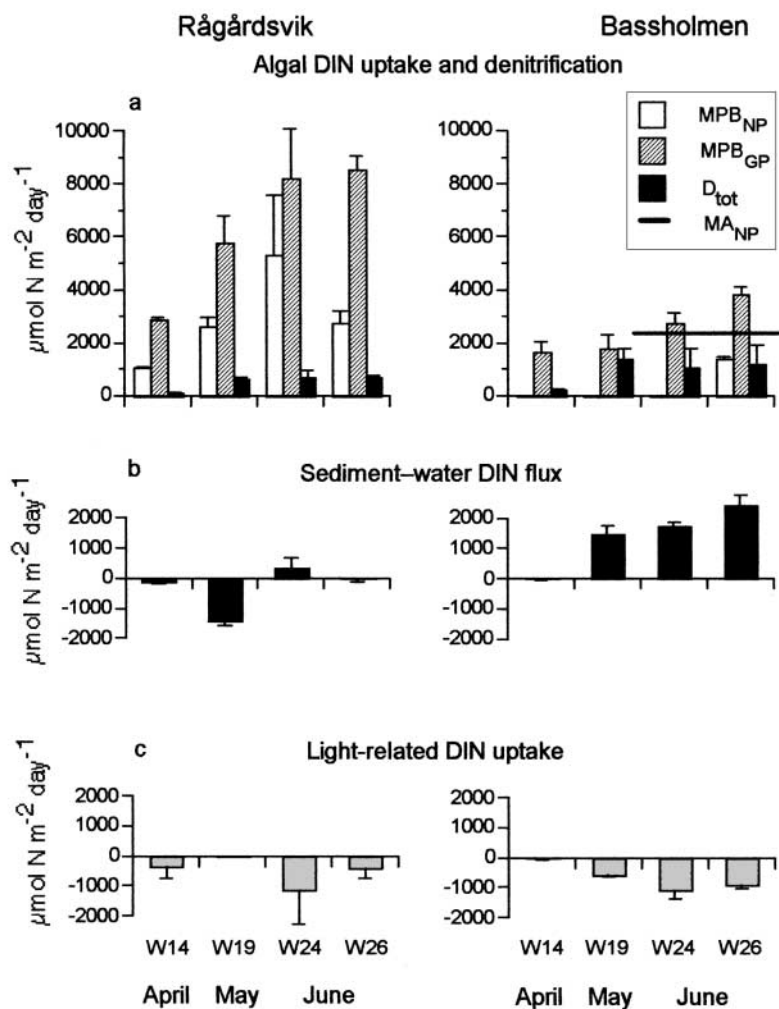


Fig. 4. Importance of microphytobenthic (MPB) N-assimilation, calculated from net productivity (MPB_{NP}) and gross productivity (MPB_{GP}), and measured total denitrification (D_{tot}) on 4 occasions during April to June 2000 at Rågårdsvik (RÅ) and Bassholmen (BA). The mean rate of green-macroalgal assimilation at BA, calculated from net growth (MA_{NP}), is indicated by a horizontal line. Bars show mean + SE, $n = 6$ for MPB_{NP} and MPB_{GP} , $n = 5$ for MA_{NP} and $n = 4$ for denitrification. Numbers on x-axes denote calendar week number

Light/dark effects on nutrient fluxes and denitrification

Light significantly decreased sediment effluxes of all nutrients that were measured, although to a different degree in the 2 bays studied. The effect of the MPB on sediment-water nutrient fluxes, through both uptake and oxygenation of the sediment surface, is today well documented for both intertidal and subtidal sediments (e.g. Henriksen et al. 1980, Rizzo 1990, Sundbäck et al. 1991, Reay et al. 1995, Thornton et al. 1999, Cabrita & Brotas 2000). However, the actual ecological implication of this effect on sediment-water nutrient fluxes has rarely been discussed, although it could be of particular interest in the type of coastal sheltered embayments considered here. Assuming that the MPB was responsible for the main reduction of nutrient efflux from the sediment in light, our results suggest that the activity of MPB could, on occasion, reduce the daily efflux of dissolved inorganic N, P and Si by up to 100%. This would imply that there is no net efflux of regenerated nutrients from the sediment to the water column, a situation that makes green macroalgal growth dependant on external nutrient supply to the water column.

An effect of light exposure was also seen on denitrification, but only in one of the bays (RÅ). Light effects on denitrification are difficult to interpret, as D_w and D_n may be affected in opposite ways by microphytobenthic activity (Risgaard-Petersen et al. 1994, Dong et al. 2000, An & Joye 2001). The general effect of light (and MPB), however, appears to be a reduction in denitrification rates, particularly when NO_3 values are low (Risgaard et al. 1995, Sundbäck et al. 2000). A significant effect at RÅ, but not at BA, suggests that the competition for NO_3 between denitrifiers and MPB was larger at RÅ, which agrees with the higher microphytobenthic activity at RÅ when compared with BA. D_w decreased by up to 50% in light, but as coupled denitrification dominated, the effect on D_{tot} was marginal, with one exception; in May D_n decreased by 60% in light, coinciding with DIN uptake both in light and dark. At BA, denitrification equaled DIN efflux and did not respond significantly to light, which suggests that N was less limiting at BA than RÅ.

Spatial variation of light effect on flux rates

Although the 2 bays presented seemingly similar systems (cf. Table 1), the MPB were more productive, exerting a stronger control on the sediment system at RÅ than BA. At RÅ, the magnitude of the light effect was greater (cf. Fig. 2) and more variables were significantly influenced by light (Table 2), which agrees with

the higher microphytobenthic GP and NP found at RÅ. However, the difference between the bays does not apparently result from a greater microphytobenthic biomass at BA (chl *a* concentrations were similar between bays), nor higher community respiration at BA, which could have balanced the oxygen production. The benthic diatom community appeared to be more active at RÅ, being also reflected by a significant light effect on Si uptake at RÅ, but not at BA. One possible explanation for the difference is that other sediment processes, such as faunal activity, exert a stronger control than the MPB on nutrient fluxes at BA. Although the chl *a* content in the top 5 mm was similar in the 2 bays, there may have been differences in the vertical distribution of chl *a* within this interval. A higher density of burrowing infauna at BA (Sundbäck et al. in press) could redistribute algal cells away from the shallow photic zone (generally <3 mm, Kühl et al. 1994), thereby decreasing microphytobenthic productivity. Thus, animal activity may, to some extent, have overridden the effect of the MPB at BA. Higher effluxes, due to more burrowing fauna at BA (cf. Emmerson et al. 2001) and fewer significant light effects on fluxes and denitrification, also support this explanation.

Can microphytobenthos control the onset of macroalgal growth in spring?

In our study, we targeted the season most critical for the onset of the growth of the green macroalgal mats. The prerequisite for a microphytobenthic control of the establishment of these mats is that the green algae actually depend on the flux of regenerated nutrients from the sediment rather than on external import of nutrients to the water column of the bay. Such a scenario could prevail when land run-off is low due to dry weather conditions, or when nutrient loads have been decreased as a measure to counteract coastal eutrophication. Our data, although limited, suggest that MPB can under such circumstances delay, or even prevent, the establishment of green-macroalgal mats (cf. Sundbäck et al. 1996).

A microphytobenthic control could apply at RÅ, where MPB activity was high and sediment efflux low, frequently directed into the sediment. Although this bay usually harbours green-macroalgal mats, no such mats developed during the study period. Macroalgal mats, however, did form at BA. Comparing the N-demand of the mats with the measured fluxes out of the sediment, showed that the DIN efflux at RÅ accounted for up to 35% of the green-algal N-demand, but up to 100% at BA (Sundbäck et al. in press). Assuming that the main reduction of nutrient efflux in

light was due to microphytobenthic activity, then this activity apparently turned the sediment from a N-source to a N-sink at RÅ. Shallow, particularly sandy, sediments have been found to frequently function as N-sinks in spring and summer (e.g. Nilsson et al. 1991, Rizzo et al. 1992, Sundbäck et al. 2000). However, it must also be kept in mind that other factors such as water movement and wind conditions are important factors regulating the occurrence of green-macroalgal mats, as shown in a modelling approach by Stigebrandt & Eilola (1999).

A second indication of the competitive role of the MBP is that its N-demand can be of the same magnitude as that of the green macroalgae. The calculated mean microphytobenthic demand based on NP at RÅ ($3.2 \text{ mmol N m}^{-2} \text{ d}^{-1}$) was in fact higher (by a factor of 1.4) than that of growing green-algal mats at BA ($2.3 \text{ mmol N m}^{-2} \text{ d}^{-1}$). A similar dominance of microphytobenthic N-uptake over macroalgal N-assimilation was found for a lagoon on the coast of Virginia, although the observed microalgal assimilation rates were even higher than we observed (6 to $10.6 \text{ mmol N m}^{-2} \text{ d}^{-1}$ for MBP vs 3.1 to $9.5 \text{ mmol N m}^{-2} \text{ d}^{-1}$ for macroalgae, McGlathery et al. 2001). At BA, where the sediment system was mainly heterotrophic, the N-demand of the MPB was at most only 50% of the green macroalgal demand (cf. Fig. 4a). This suggests that the MPB exerted a strong competition for the DIN pool of the sediment at RÅ. It could be argued that the retention time of N in the MPB is much shorter than in the macroalgal mats. However, previous studies suggest that remineralised N is rapidly recycled back to the microbiota at the very sediment surface (Lomstein et al. 1998), keeping DIN concentrations in the water column low. In this context, it could also be argued that our calculated macroalgal nutrient demand could be underestimated, as the growth rate is based on only few measuring occasions and the loss rate is not known. However, the growth rates of the *Enteromorpha* mats in our study are well within the range of growth rates found in field cage experiments on *Enteromorpha* species from the same region (Pihl et al. 1996, for further discussion see Sundbäck et al. in press).

Balance between the N-retaining and removing processes

Although our study considered only 1 season and 3 N-removal processes (2 temporary and 1 final), it should still give an idea of the main paths of N-turnover in spring. The role of rooted perennial benthic primary producers (seagrasses) was not included in our study. However, the only occasion when sea-

grasses (*Ruppia* sp.) could have affected the processes to any degree was in late June at RÅ. Because of a shallow water column, the influence of phytoplankton was negligible, as indicated by the oxygen and nutrient fluxes in the blank cores.

Our results suggest that when the MPB is the only benthic primary producer (RÅ), it is responsible for the main assimilation of sediment-derived DIN, denitrifiers being of minor importance. This is in accordance with a previous hypothetical conclusion that, in cool shallow microtidal waters where the MPB dominates primary productivity, denitrification plays only a minor role for the removal of N from the ecosystem, *A:D* ratios being generally >5 (Table 3 in Sundbäck & Miles 2000 and references therein). Low denitrification rates also apply for areas dominated by seagrass productivity in Scandinavian waters (Risgaard-Petersen et al. 1998, Ottosen et al. 1999). Such high *A:D* ratios do not apply to cool temperate macrotidal areas in general, such as estuaries in the UK, where the *A:D* ratio may be <1 (Dong et al. 2000), or warmer macrotidal estuaries, such as the Tagus estuary in Portugal (Cabrita & Brotas 2000). However, this regional difference also reflects a difference in the N-status of these areas. Studied Swedish sites had maximum winter values of $30 \mu\text{mol NO}_3$ (Sundbäck et al. 2000, K. Sundbäck, F. Linares & A. Miles unpubl.), while maximum water column values of NO_3 may be an order of magnitude higher in other European estuaries (e.g. Dong et al. 2000).

Once macroalgal mats were established (BA), they appeared to dominate the N-turnover. This has also been found for the Danish microtidal Limfjorden (Dalsgaard 1999) and shallow coastal lagoons of Virginia (McGlathery et al. 2001). However, the share of denitrification in N-consumption was also higher at BA, equivalent to 75% of the green-macroalgal assimilation, suggesting that denitrification was a more important pathway of N-removal than if the MPB was the only primary producer (RÅ). The difference in the *A:D* balance between the 2 bays also agrees with the hypothesis by An & Joye (2001) that benthic microalgal production dominates the benthic N cycle when N is limiting, denitrification becoming more important when N is in excess.

The measured hourly rates of total denitrification (4 to $60 \mu\text{mol m}^{-2} \text{ h}^{-1}$) are within the lower range reported for other shallow-water coastal sediments (Cabrita & Brotas 2000 and references therein). However, rates were higher than those previously found for shallow sediments on the west coast of Sweden (<1 to $40 \mu\text{mol m}^{-2} \text{ h}^{-1}$; Sundbäck et al. 2000) and the Baltic Proper (<1 to $12 \mu\text{mol m}^{-2} \text{ h}^{-1}$; Sundbäck et al. unpubl.). Assuming that oxygen uptake provides a reliable measure of mineralisation rates within the

sediment, we can roughly estimate the share of mineralised N that was denitrified from values for community respiration and the C:N ratio of the surface sediment (cf. Trimmer et al. 2000). Assuming an $O_2:CO_2$ mean ratio of 1 (see Hulth et al. 1997), then denitrification accounted for, on average, 10% (range 4 to 18%) at RÅ and 20% (range 9 to 37) at BA of the N-mineralisation. These figures are higher than those found by Trimmer et al. (2000) for some harbours in southern England (<2% of N-mineralisation), but similar to those found in the Great Ouse estuary (8 to 26%, Nedwell & Trimmer 1996), which were considered rather high. Thus, in contrast to what has been previously suggested for Scandinavian microtidal shallow-water sediments (Sundbäck & Miles 2000), denitrification may be a significant N-sink in bays harbouring green-macroalgal mats on the west coast of Sweden.

CONCLUSIONS

Microphytobenthos can significantly reduce inorganic nutrient efflux from the sediment during the period that is critical for the onset of the growth of green-macroalgal mats. Thus, microphytobenthos appear to efficiently control the availability of sediment-derived nutrients to the green macroalgae that are in their early growth phase, and hypothetically delay, or even prevent, the onset of the growth of floating macroalgal mats. The prerequisite for this scenario is, however, that green-macroalgal growth depends mainly on sediment efflux of regenerated nutrients, and that other benthic processes, such as high infaunal activity, do not override the microphytobenthic control. Estimates of N-consuming processes show that microphytobenthic N-demand can exceed green-macroalgal N-demand and that denitrification plays only a minor role as an N-sink in autotrophic sediment systems dominated by microphytobenthic primary producers. However, in a net heterotrophic shallow-water system, with no or little net microphytobenthic productivity, a more active fauna, and hence, larger nutrient effluxes, green macroalgal growth is the main N-consuming process, followed closely by denitrification. Thus, denitrification may be a significant N-sink in eutrophicated bays harbouring green-macroalgal mats on the west coast of Sweden.

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