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Effect of the seagrass *Zostera capricorni* on sediment microbial processes

Jens W. Hansen[2,*], James W. Udy[1], Christine J. Perry[1], William C. Dennison[1], Bente Aa. Lomstein[2]

[1]Department of Botany, University of Queensland, Brisbane, Queensland 4072, Australia
[2]Department of Microbial Ecology, Institute of Biological Sciences, University of Aarhus, Bldg. 540, Ny Munkegade, 8000 Aarhus C., Denmark

ABSTRACT: The effect of the seagrass *Zostera capricorni* on sediment microbial processes was studied in a tank experiment, where vegetated and unvegetated control sediments were incubated in 10 and 50% of incident light. Leaf and root-rhizome biomass, shoot density, and leaf productivity were significantly higher when plants were incubated in 50% than in 10% of incident light. Nitrogen fixation, sulphate reduction, and urea turnover in the *Z. capricorni* vegetated sediment were higher in the 50% than in the 10% light treatment and higher in the vegetated than in the unvegetated sediment. The stimulation of microbial processes in the *Z. capricorni* vegetated sediment took place in the rhizosphere, where nitrogen fixation and sulphate reduction in particular were stimulated. The sediment studies were supplemented by measurements of nitrogen fixation, sulphate reduction, and urea turnover by microorganisms associated with the roots and rhizomes of *Z. capricorni*. The rates of nitrogen fixation and sulphate reduction associated with root-rhizomes were up to 40- and 7-fold higher, respectively, than the highest respective sediment rates, whereas the root-rhizome associated urea turnover was lower than sediment rates. Nitrogen fixation and sulphate reduction associated with root-rhizomes could account for up to 39 and 4%, respectively, of the depth-integrated sediment rates. Nitrogen fixed by microorganisms associated with root-rhizomes could supply up to 65% of the nitrogen needed for plant growth. Further, it was estimated that 8 to 18% of the carbon fixed by *Z. capricorni* was released to the sediment by the roots and rhizomes. Urea turnover was suggested to be an important intermediate in the gross production of ammonium, and a low net production of ammonium indicated rapid internal nitrogen cycling within the sediment.

KEY WORDS: *Zostera capricorni* · Sediment · Roots · Rhizomes · Microbial processes · Light

INTRODUCTION

Several studies have demonstrated that seagrasses affect nutrient concentrations and microbial processes in the sediment (Short 1983, Caffrey & Kemp 1990, Short et al. 1993, Pedersen et al. 1997, Risgaard-Petersen et al. 1998). This is mainly due to the increased accumulation of nutrients and carbon in seagrass vegetated sediments caused by (1) plant biomass production within the seagrass meadow of which a part is mineralised within the seagrass bed, (2) increased sedimentation and decreased resuspension in seagrass beds, and (3) leakage of photosynthates from root-rhizomes.

The root-rhizome complex is an important component of the sediment in seagrass beds, as the total surface area and the total length of root-rhizomes often exceeds 1 m^2 and 100 m, respectively, per m^2 sediment (Smith et al. 1979). Also, the production of roots and rhizomes is often comparable to leaf production (Abal et al. 1994, Duarte et al. 1998). Leakage of photosynthates from root-rhizomes affects sediment microbial processes as demonstrated by the stimulation of nitrogen fixation, sulphate reduction, and bacterial growth in the rhizosphere and the diurnal variation in these processes (Moriarty & Pollard 1982, Pollard & Moriarty

*Present address: National Environmental Research Institute, Vejlsøvej 25, 8600 Silkeborg, Denmark.
E-mail: jwh@dmu.dk

1991, Blackburn et al. 1994, Welsh et al. 1996, Blaabjerg et al. 1998, McGlathery et al. 1998). Further, high rates of nitrogen fixation and sulphate reduction have been measured in incubations of roots and rhizomes of seagrasses (Capone & Budin 1982, Smith & Hayasaka 1982a,b, Blaabjerg & Finster 1998).

Light is the principal factor regulating growth, biomass, and distribution of seagrasses (Wium-Andersen & Borum 1984, Dennison 1987, Duarte 1991, Nelson & Waaland 1997). Accordingly, reduced light availability caused by an eutrophication-dependent increase in phytoplankton and epiphytes, and an increase in suspended inorganic particles, has been responsible for most of the decline in the distribution of seagrasses during the last few decades (Duarte 1995, Abal & Dennison 1996, Borum & Sand-Jensen 1996, Olesen 1996, Short & Wyllie-Echeverria 1996, Lee 1997). Due to the effect of seagrasses on sediment pools and processes, light-regulated changes in seagrass populations have an impact on microbial processes in vegetated sediments. However, the interaction between seagrasses and sediment microbial processes has not been thoroughly investigated.

The objective of this study was to evaluate the effect of *Zostera capricorni* on nitrogen fixation, sulphate reduction, urea turnover, and net ammonium production within the sediment. The experiment was carried out at 2 different light levels to examine the effect of reduced light availability on sediment microbial processes in areas vegetated by seagrasses. Nitrogen fixation, sulphate reduction, and urea turnover were also measured in incubations of root-rhizomes to estimate if the plant's effect on sediment rates were due to processes associated directly with root-rhizomes.

MATERIALS AND METHODS

Sampling and incubation. Seagrasses cover extensive areas in Moreton Bay, Queensland, Australia, and *Zostera capricorni* is the most dominant of the 6 species present (Hyland et al. 1989). Plants and sediment were collected in March 1996 from the intertidal part of a monotypic *Z. capricorni* meadow near Victoria Point on the western shore of Moreton Bay. The water temperature was 32°C and the salinity 34‰. Sediment cores with plants were taken with a corer (inner diameter, i.d., 15 cm) which removed a 15 cm deep plug. The sediment plugs were placed in plastic flower pots lined with a plastic bag, overlaid with ambient water and transported to seawater tanks.

The plants were incubated for 5 wk in 200 l (40 cm deep) outdoor Fiberglas tanks at the CSIRO Cleveland Marine Laboratory. Sand-filtered seawater from the western Moreton Bay was pumped through the tanks at a rate of ~140 l h^{-1} with a resultant water residence time in the tanks of ~1.4 h. Water temperature was 23 to 29°C during the incubation period with a daily variation of ~3°C, and salinity was ~34‰. The water was kept saturated with oxygen by bubbling with atmospheric air. The pots were distributed in 4 tanks with 8 pots in each tank. Leaves and most root-rhizomes were removed from 3 pots in each tank to establish unvegetated control sediment. The tanks were covered with shade screens, which reduced the light irradiance to ~50 or ~10% of incident light in each of 2 tanks. The growth of *Zostera capricorni* was light saturated in the 50% treatment (Abal et al. 1994), whereas the light irradiance at the 10% treatment was close to the light compensation point of seagrasses (Duarte 1991, Dennison et al. 1993). Once or twice a week the tanks were scrubbed clean of periphyton, and epiphytes were removed from the seagrass leaves by hand. Each pot was considered as 1 experimental unit.

Plant and sediment characteristics. Leaf growth rate was measured by the leaf marking technique (Sand-Jensen 1975). Leaves in 1 pot from each tank were punched with a needle at the end of the 5 wk incubation period and harvested 10 d later. The number of shoots in each of these pots was counted at harvest, and the plant material was washed and dried at 60°C to constant weight. Leaf production was estimated from the dry weight of the new growth by 10 shoots pot^{-1}. The maximum canopy height was noted as the length of the longest leaf. Leaf productivity, maximum canopy height, and plant biomass were measured on plants from the same pots. The turnover time of leaves was estimated as the total leaf biomass divided by the leaf production. The vertical distribution of the root-rhizome biomass was determined in 1 sediment core (3.2 cm i.d.) from each tank.

Total organic carbon (TOC) and total organic nitrogen (TON) contents of leaves, root-rhizomes, and sediment (with root-rhizomes removed) were determined on dried, H_2SO_3 treated, and homogenised plant material or sediment using a Carlo Erba NA 1500 HCN analyser. The concentrations of acid hydrolysable amino acids were measured in the sediment ($THAA_{sed}$) and in the root-rhizomes ($THAA_{rt+rh}$). $THAA_{sed}$ and $THAA_{rt+rh}$ were determined from 1 cm^3 sediment and 0.16 g fresh weight root-rhizomes, respectively, to which 1 ml 12 N HCl was added. The samples were hydrolysed at 105°C for 24 h. After hydrolysis, 100 µl of the hydrolysate was evaporated in a desiccator under vacuum and subsequently redissolved in 100 µl Milli-Q water. This 100 µl was evaporated again and finally dissolved in 1 ml Milli-Q water. The THAA was determined as the concentration of dissolved free amino acids measured by high performance liquid chromatography (HPLC, Waters Chromatographic System)

on *o*-phthaldialdehyde derivatized products (Lindroth & Mopper 1979).

To determine sediment porosity, 1 sediment core (3.2 cm i.d.) was sampled from both a vegetated and an unvegetated sediment pot from each tank, which resulted in 2 parallel cores from each treatment. The sediment was sectioned into the following depth intervals: 0–2, 2–5, and 5–10 cm. Sediment from parallel cores was pooled for each depth interval. Sediment density was measured as the fresh weight of a known volume of sediment from each depth interval, and the porewater content was determined as the weight loss of sediment dried at 60°C to constant weight. Sediment porosity was calculated as the density multiplied by the porewater content.

Porewater pools. Porewater concentrations of ammonium (NH_4^+), urea, dissolved free amino acids (DFAA), and acid hydrolysable amino acids ($THAA_{pw}$) were measured in sediment sampled as described above, with the exception that samples from the 0–2 cm depth interval were pooled from 4 cores to gain enough porewater. The root-rhizomes were removed from both the vegetated and the unvegetated control sediment before porewater extraction to avoid leakage of NH_4^+ and dissolved organic nitrogen from root-rhizomes (Hansen & Lomstein 1999). The sediment was homogenised, and porewater was obtained by centrifugation at 1000 × *g* for 6 min. The supernatant was 0.2 µm filtered (Sartorius) and frozen for later analysis. Porewater for sulphate analysis was obtained from separate cores, sectioned with root-rhizomes, and frozen for later analysis.

Concentrations of NH_4^+ and urea were determined by the methods described in Bower & Holm-Hansen (1980) and Price & Harrison (1987), respectively. The concentration of SO_4^- was analysed on a Dionex 2010 ion chromatograph. The concentration of $THAA_{pw}$ was determined on 1 ml porewater samples as described in a previous section for $THAA_{sed}$ and $THAA_{rt+rh}$. The concentration of $THAA_{pw}$ was obtained after correction for DFAA.

Sediment microbial processes. Rates of sulphate reduction, urea turnover, nitrogen fixation, and net ammonium production were measured in 3 depth intervals (0–2, 2–5, and 5–10 cm) in the vegetated and the unvegetated sediment. Urea turnover and sulphate reduction were measured in intact cores (3.2 cm i.d.), whereas nitrogen fixation and net ammonium production were measured in homogenised sediment. All incubations were performed in the dark at 24°C.

Sulphate reduction was measured in duplicate in the vegetated and the unvegetated sediment from each tank by the radiotracer method described in Jørgensen (1978) and Fossing & Jørgensen (1989). Ten µl carrier-free $^{35}SO_4^-$ (19 kBq µl^{-1}, ICN Pharmaceuticals) was injected through silicone-filled side ports in the cores in a horizontal line through the sediment at 1 cm depth intervals with a resultant activity of 23 kBq cm^{-3}. The sediment cores were incubated for 5 h. The sediment was transferred to 20% (w/v) ZnAc in a 2:1 v/v ratio to stop biological activity and fix the produced ^{35}S-sulphide. The sediment-ZnAc slurry was mixed and frozen for later analysis of ^{35}S activity. The slurries were distilled as described in Fossing & Jørgensen (1989). The distillates were mixed with scintillation liquid (Beckman, Ready Safe) and counted in a Packard 1600 TR liquid scintillation analyser.

Urea turnover was determined once in the vegetated and the unvegetated sediment from each tank by the radiotracer method described in Lund & Blackburn (1989). In each sediment core 10 µl of ^{14}C-urea tracer (56 Bq µl^{-1}, ICN Pharmaceuticals) was injected in a horizontal line at 1 cm depth interval with a resultant activity of 70 Bq cm^{-3}. The urea enrichment due to ^{14}C-urea was always <3%. Cores were incubated as a time course incubation (0, 40, and 80 min). The sediment was transferred to 2.5% (w/v) NaOH in a 2:1 v/v ratio, to stop biological activity and fix the produced $^{14}CO_2$. The sediment-NaOH slurry was mixed and frozen for later analysis of ^{14}C activity. The scintillation liquid and the counter were as described for $^{35}SO_4^-$. Changes in the porewater concentration of urea were determined in a parallel time course incubation of 1 cm^3 sediment with root-rhizomes removed. The urea turnover rate was calculated by the non-steady state Model I described in Lund & Blackburn (1989).

Nitrogen fixation was measured in triplicate under both oxic and anoxic conditions in the vegetated and the unvegetated sediment from each tank by the acetylene reduction technique (Capone 1993). Sediment slurries were prepared from 10 cm^3 sediment including root-rhizomes and 5 ml 0.45 µm filtered (Whatman) seawater in a 125 ml Erlenmeyer flask. At the start of the incubation 10% of head space was exchanged with acetylene, and the slurries were incubated as a time course incubation (3, 6, 9, 12, and 24 h). The concentration of ethylene in samples from head space was measured on a Shimadzu Mini-2 Gas Chromatograph. The ethylene production rate was calculated from the initial linear increase in the concentration of ethylene with time. A ratio of moles ethylene produced to nitrogen fixed of 3.0:1.9 was used to calculate nitrogen fixation from ethylene production. This ratio was obtained in *Zostera capricorni* sediment from Moreton Bay by O'Donohue et al. (1991).

Net ammonium production was measured in duplicate in the vegetated and the unvegetated sediment from an anoxic time course incubation (0, 9, 21, 28, and 42 h) of sediment with root-rhizomes. At the end of each incubation, 1 ml 2 M KCl was added to the 1 cm^3

sediment sample, and the mixture was vortexed and extracted for 30 min at 5°C, before it was centrifuged at 1000 × *g* for 6 min. The supernatant was frozen for later analysis. The KCl extractable NH_4^+ ($NH_4^+{}_{KCl}$) was analysed as described for porewater NH_4^+. The net ammonium production rate was calculated from the change in concentration with time.

Processes associated with roots and rhizomes. Rates of urea turnover, sulphate reduction, and nitrogen fixation associated with root-rhizomes were in principle measured as described for the respective processes in the sediment. Root-rhizomes (~0.1 g fresh weight) were incubated in 5 ml 0.2 µm filtered (Sartorius) porewater. Glucose and acetate were added as substrates to the porewater to a final concentration of 0.8 mM together with 1 mg Poly (A) per 5 ml. Poly (A) is a polymer of the nucleotide adenosine, which stimulate the turnover of urea (Therkildsen et al. 1996). Root-rhizomes from the 10 and 50% tanks were incubated under both oxic and anoxic conditions. Samples were incubated in the dark on a shaking table at 24°C.

Rates of urea turnover and sulphate reduction were measured in triplicate by the methods described for the sediment incubations except that 5 µl of tracer were added with a resultant activity of 56 Bq ml^{-1} (^{14}C-urea) and 19 kBq ml^{-1} ($^{35}SO_4^-$). Urea turnover was measured in a time course incubation (0, 30, and 60 min). Samples without tracer were incubated in parallel to follow the change in the concentration of urea with time. Sulphate reduction was measured in an end point incubation (4 h). The incubations were stopped by the addition of 2.5 ml 2.5% (w/v) NaOH (urea turnover) and 20% (w/v) ZnAc (sulphate reduction). Nitrogen fixation was measured in 5 parallel incubations by the methods described for the sediment incubations.

Statistical analysis. Plant characteristics (except carbon and nitrogen contents), sediment processes, and root-rhizome processes are presented as mean values ± standard error (SE) of the mean. A 2-tailed Student's *t*-test was used for comparison of data.

RESULTS

Plant characteristics

After 5 wk of incubation the root-rhizome biomass of *Zostera capricorni* was 87 g dry wt m^{-2} in the 10% light treatment and 239 g dry wt m^{-2} in the 50% light treatment (Table 1). However, the vertical distribution of root-rhizome biomass within the upper 10 cm of the sediment was similar in the 2 treatments: ~70% in the

Table 1. Characteristics of *Zostera capricorni* after 5 wk incubation at light intensities corresponding to 10 and 50% of the incident light. p-values are from a comparison of 10 and 50% data. Values in parentheses are standard errors of mean (n = 2). *Parameters that are significantly different ($p < 0.05$) in the 10 and 50% treatment

		Treatment		p
		10%	50%	
Morphology				
Maximum canopy height (cm)		18.5 (0.5)	12.0 (0.3)	<0.01*
Shoot density (shoots m^{-2})		1413 (88)	3850 (450)	0.03*
Root-rhizome vol. ($vol_{rt\text{-}rh}/vol_{sed} \times 100\%$)[a]		0.35 (0.02)	0.95 (0.06)	0.01*
Biomass				
Leaves	(g dw m^{-2})	13.2 (0.9)	30.5 (3.4)	0.03*
	(mg dw $shoot^{-1}$)	9.3 (0.1)	8.1 (1.8)	0.57
Roots-rhizomes	(g dw m^{-2})	87 (4)	239 (16)	0.01*
	(mg dw $shoot^{-1}$)	62 (1)	63 (11)	0.90
Total	(g dw m^{-2})	100 (5)	269 (19)	0.01*
	(mg dw $shoot^{-1}$)	71 (1)	71 (13)	0.90
Growth and turnover				
Leaf production	(g dw m^{-2} d^{-1})	0.69 (0.11)	1.70 (0.11)	0.02*
	(mg dw $shoot^{-1}$ d^{-1})	0.48 (0.05)	0.45 (0.08)	0.78
Leaf turnover	(d)	20 (2)	18 (1)	0.48
Carbon and nitrogen content				
Leaves	(% C of dw)	36.3	33.0	
	(% N of dw)	2.85	2.17	
	(molar C:N ratio)	14.8	17.7	
Roots-rhizomes	(% C of dw)	25.0	23.1	
	(% N of dw)	0.84	0.55	
	(molar C:N ratio)	34.8	48.7	

[a]The density of roots-rhizomes was assumed to be 1.0 in the calculation of the volume of roots-rhizomes in the sediment

0–2 cm stratum, ~20% in the 2–5 cm stratum, and ~10% in the 5–10 cm stratum. Also, leaf biomass, shoot density, and leaf production of *Z. capricorni* were ~2.5-fold higher when plants were exposed to 50% rather than 10% of the incident light (Table 1). The maximum canopy height was 12.0 cm in the 50% light treatment, which was significantly lower than the 18.5 cm measured in the 10% light treatment. The shoot-specific leaf and root-rhizome biomass and leaf production, and the leaf turnover rate did not differ significantly with light exposure (Table 1). The organic carbon and nitrogen contents were lower in the 50% than in the 10% treatment for both leaves and root-rhizomes, and the molar C:N ratio was higher in the 50% than in the 10% treatment for both leaves and root-rhizomes (Table 1).

Sediment pools

Sediment porewater concentrations of NH_4^+, urea, DFAA, and $THAA_{pw}$ in the unvegetated control sediment are presented as a mean of the results obtained in the 50 and 10% light treatments, as no consistent differences were noted. The concentration of NH_4^+ was higher in the unvegetated than in the vegetated sediment (Fig. 1a). The concentration of NH_4^+ decreased with depth in the vegetated sediments from ~25 µM in the upper 2 cm to ~15 µM in the 5–10 cm depth stratum. In the unvegetated sediment, the NH_4^+ concentration increased from 23 µM in the upper 2 cm to 69 µM in the 5–10 cm depth stratum. In all treatments there was a maximum in the concentration of urea in the 2–5 cm depth interval of ~9 µM in the vegetated sediment and 17 µM in the unvegetated sediment. There was an almost linear decrease in the concentration of DFAA with depth in both the vegetated and unvegetated sediments. The highest DFAA concentration (76 µM) was obtained in the 0–2 cm depth interval in the 50% treatment of the vegetated sediment (Fig. 1c). The concentration of $THAA_{pw}$ was lowest in the 0–2 cm depth interval in both the vegetated and unvegetated sediment. The highest $THAA_{pw}$ concentration (34 µM) was obtained in the 2–5 cm depth interval in the 50% treatment of the vegetated sediment (Fig. 1d). The depth-integrated pool of $THAA_{pw}$ was ~1 mmol m^{-2} in both the vegetated and unvegetated sediment, and constituted <0.1% of $THAA_{sed}$.

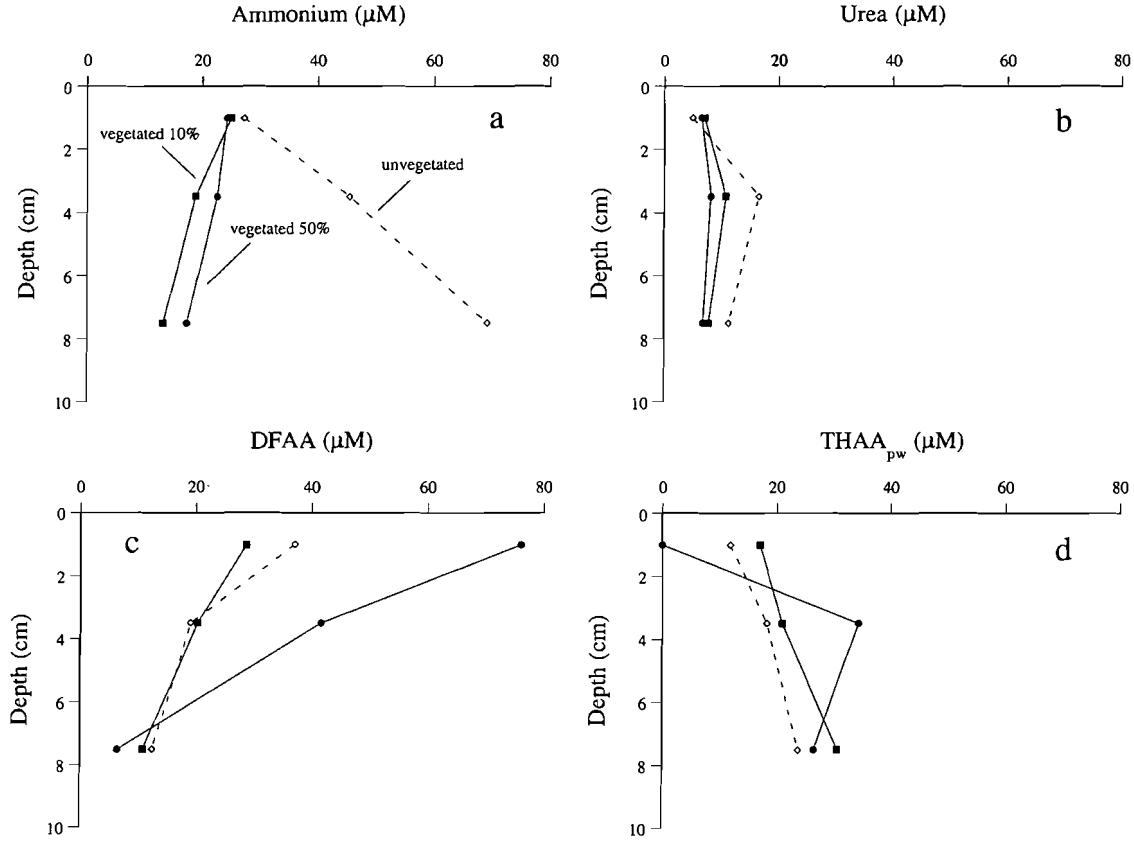

Fig. 1. Profiles of porewater concentrations of (a) NH_4^+, (b) urea, (c) DFAA, and (d) $THAA_{pw}$ in vegetated sediment exposed to 50% (●) and 10% (■) of incident light and in unvegetated sediment (◇)

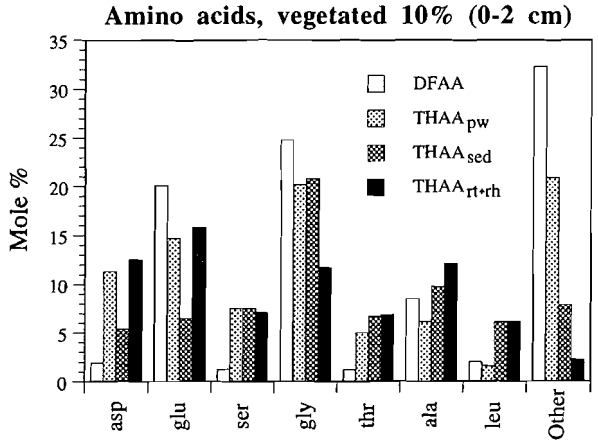

Fig. 2. Molar composition of amino acids in DFAA, $THAA_{pw}$, $THAA_{sed}$, and $THAA_{rt+rh}$ in the upper 2 cm of the vegetated sediment adapted to 10% of the incident light. Other: unidentified amino acids

The depth-integrated pools of $THAA_{sed}$ and $THAA_{rt+rh}$ in the upper 10 cm of the vegetated sediment were 1282 and 42 mmol m^{-2}, respectively, in the 50% treatment and 1271 and 14 mmol m^{-2}, respectively, in the 10% treatment. The $THAA_{rt+rh}$ concentrations were high, with a maximum of 62 mM.

The molar composition of DFAA, $THAA_{pw}$, $THAA_{sed}$, and $THAA_{rt+rh}$ in the upper 2 cm of the vegetated sediment exposed to 10% of the incident light is presented in Fig. 2. Glutamic acid and glycine were the dominant protein amino acids. Histidine, arginine, tyrosine, methionine, valine, phenylalanine, iso-leucine, and lysine each accounted for less than 6% of the amino acid pools (data not shown). Unidentified amino acids accounted for 32, 21, 8, and 2% of DFAA, $THAA_{pw}$, $THAA_{sed}$, and $THAA_{rt+rh}$, respectively. A similar molar composition of amino acids was obtained in the other depth intervals for both the vegetated and the unvegetated sediment at both light levels (data not shown).

The molar C:N ratio of total sediment organic matter did not show any significant difference between the vegetated and the unvegetated sediment or between the 2 light treatments, and there was no marked change with depth (data not shown). The average molar C:N ratio in the total sediment organic matter was 14.1 ± 0.4 mol mol^{-1} (n = 12). The average sediment TOC and TON contents were 70.0 ± 4.6 mol C m^{-2} (n = 12) and 4.8 ± 0.4 mol N m^{-2} (n = 12), respectively.

Sediment microbial processes

Sediment processes in the unvegetated sediment are presented as a mean of the results obtained in the 50 and 10% light treatment, as there was no significant difference between results obtained in the 2 treatments.

The rate of nitrogen fixation decreased with depth from 94, 45, and 15 nmol N cm^{-3} d^{-1} in the upper 2 cm strata of the vegetated (50%), the vegetated (10%), and the unvegetated sediment, respectively, to 16, 12, and 4 nmol N cm^{-3} d^{-1} in the 5–10 cm depth stratum (Fig. 3a). Nitrogen fixation rates were significantly higher in the vegetated than in the unvegetated sediment at all depths ($p < 0.01$). Further, the rate of nitrogen fixation in the main root zone (rhizosphere: 0–5 cm) was significantly higher in the 50% than in the 10% light treatment ($p < 0.01$). The depth-integrated rates were 5.4, 2.6, and 0.7 mmol N m^{-2} d^{-1} in the vegetated (50%), the vegetated (10%), and the unvegetated sediment, respectively (Fig. 4). The nitrogen fixation rates presented are mean values of rates obtained under oxic and anoxic conditions, as these rates were not significantly different ($p > 0.19$).

The sulphate reduction rate in the vegetated sediments decreased with depth from 408 and 273 nmol cm^{-3} d^{-1} in the upper 2 cm of the 50 and 10% light treatment, respectively, to 157 and 135 nmol cm^{-3} d^{-1} in the 5–10 cm stratum (Fig. 3b). In the unvegetated sediment there was a maximum of 254 nmol cm^{-3} d^{-1} in the 2–5 cm depth interval of the unvegetated sediment. The sulphate reduction rate was significantly higher in the vegetated than in the unvegetated sediment in the upper 2 cm ($p < 0.03$). In the vegetated sediment the sulphate reduction rate was higher, although not significantly, in the 50% than in the 10% light treatment at all depths ($p > 0.18$). Depth-integrated rates were 26.6, 19.2, and 18.1 mmol m^{-2} d^{-1} in the vegetated (50%), the vegetated (10%), and the unvegetated sediment, respectively (Fig. 4).

The urea turnover rate was highest in the upper 2 cm of the sediment in all treatments (Fig. 3c). The urea turnover rate was higher, although not significantly, in the vegetated than in the unvegetated sediment in the upper 2 cm ($p > 0.09$). The depth-integrated rates were 18.5, 17.2, and 15.2 mmol N m^{-2} d^{-1} in the vegetated (50%), the vegetated (10%), and the unvegetated sediment, respectively (Fig. 4).

The net ammonium production was not significantly different from zero at any depths in any treatments ($p > 0.07$, data not shown).

Processes associated with roots and rhizomes

The rates of nitrogen fixation, sulphate reduction, and urea turnover associated with root-rhizomes were up to 8669, 8157, and 462 nmol g^{-1} dry wt root-rhizome d^{-1}, respectively, and could potentially account for 39,

4, and 1% of the respective depth-integrated rates in the sediment (Table 2). The rates obtained with root-rhizomes from the 50% light treatment were not significantly different from the rates obtained with root-rhizomes from the 10% light treatment ($p > 0.05$). The nitrogen fixation rates associated with root-rhizomes were significantly higher under oxic than under anoxic conditions ($p < 0.01$). However, the rates of sulphate reduction and urea turnover were not significantly different under oxic than under anoxic conditions ($p > 0.28$, data not shown), and are, therefore, presented as the mean of the results from the 2 treatments.

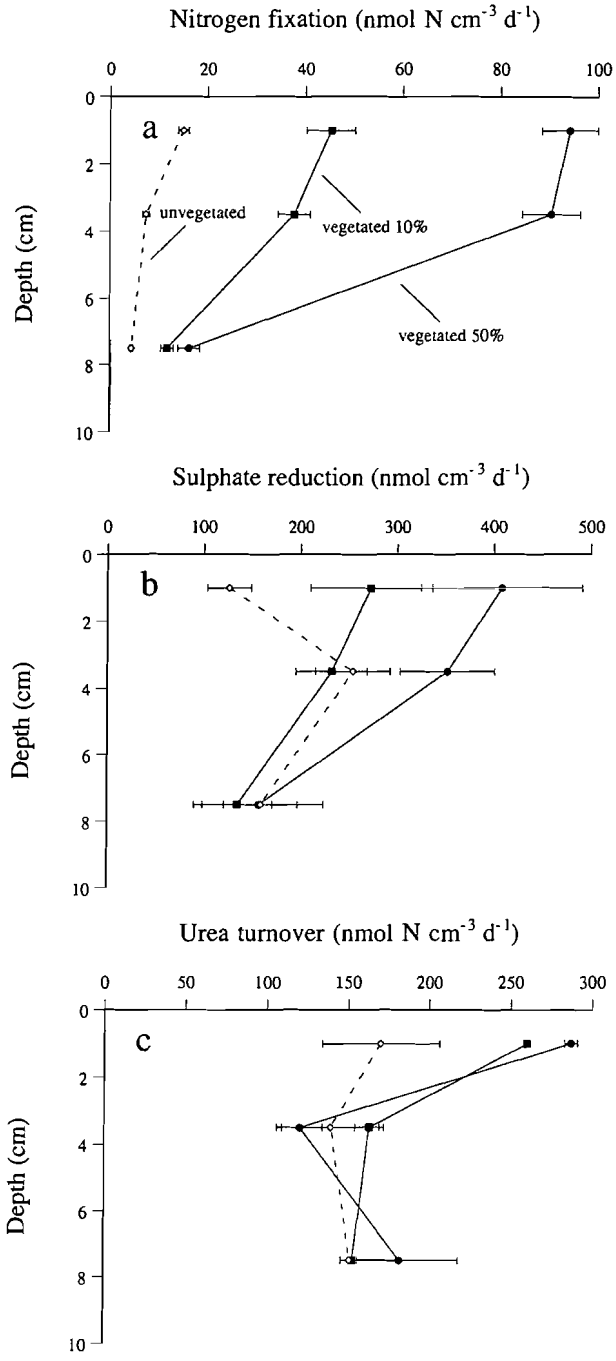

Fig. 3. Sediment profiles for rates of (a) nitrogen fixation, (b) sulphate reduction, and (c) urea turnover in vegetated sediment exposed to 50% (●) and 10% (■) of incident light and in unvegetated sediment (◇). Error bars represent standard errors of means (n = 6 for nitrogen fixation, n = 4 for sulfate reduction, and n = 2 for urea turnover; n was 12, 8, 4, respectively, in the unvegetated sediment)

DISCUSSION

Plant response to different light irradiances

The estimated leaf turnover time of 18 to 20 d indicates that *Zostera capricorni* was able to adapt to the 2 light irradiances, of 50 and 10% of incident light, within the 5 wk tank incubation (Table 1). Adaptation to the low-light environment was also indicated from the plant characteristics; as leaf and root-rhizome biomass, shoot density, and leaf productivity were significantly lower and the maximum canopy height significantly higher in the 10% than in the 50% light treatment ($p < 0.05$). These changes in plant characteristics seem to be a general adaptation to low light in seagrasses (Backman & Barilotti 1976, Dennison & Alberte 1985, Olesen & Sand-Jensen 1993, Abal et al. 1994, Czerny & Dunton 1995, Philippart 1995, Short et al. 1995). There was no significant difference in the shoot-specific biomass or the shoot-specific leaf production in the 50 and 10% light treatment ($p > 0.55$). Hence, *Z. capricorni* adapted to the 2 light irradiances through changes in the shoot density and morphology rather than through changes of the shoot-specific biomass or productivity. However, we can not exclude that a longer incubation period could have lowered shoot-specific growth in the 10% light treatment, as irradiance in this treatment was close to the minimum light requirement of seagrasses (Duarte 1991, Dennison et al. 1993). In shorter periods with severe light limitation, seagrasses can survive by mobilisation of carbon reserves stored in root-rhizomes (Alcoverro et al. 1999).

Sediment microbial processes

Nitrogen fixation

The sediment nitrogen fixation rates obtained in the present study were high, but within the range of rates encountered in other tropical and subtropical seagrass meadows (Patriquin & Knowles 1972, Capone & Taylor 1980, O'Donohue et al. 1991, Moriarty & O'Donohue 1993), which are often much higher than rates from

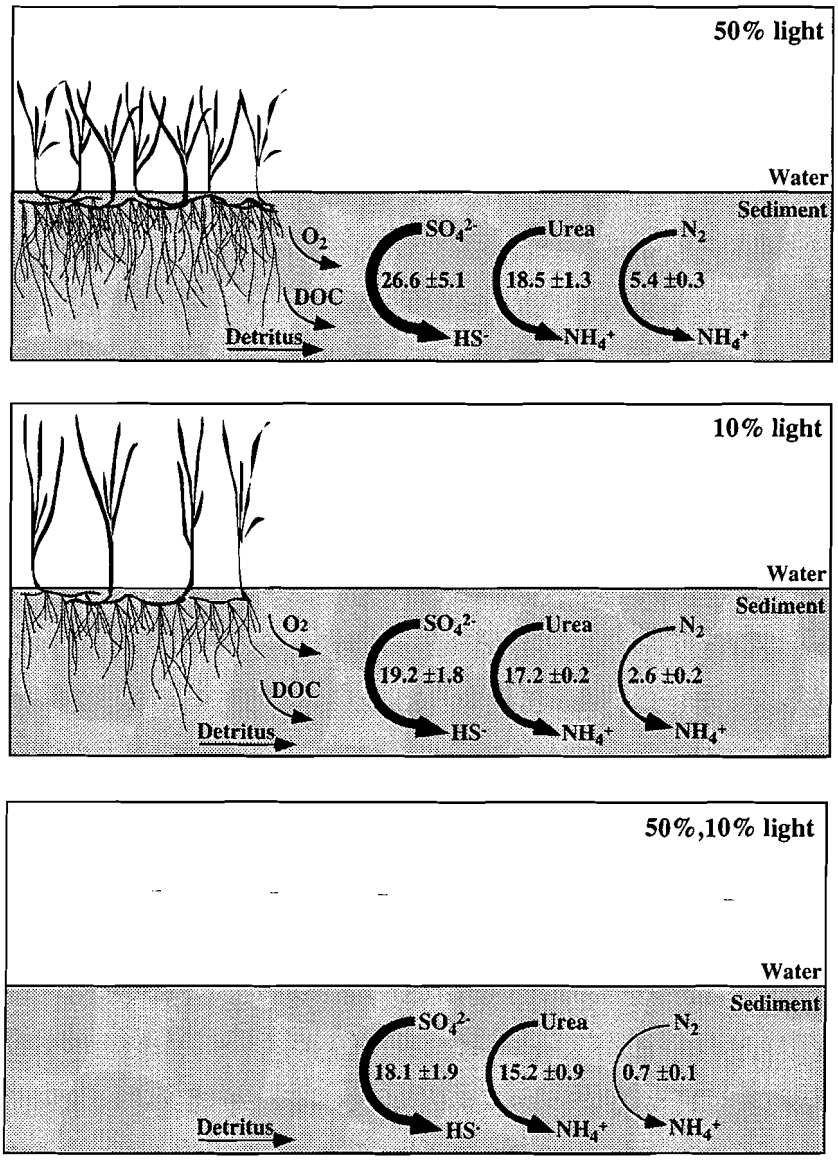

Fig. 4. Depth-integrated, 0 to 10 cm, sediment rates of nitrogen fixation, sulphate reduction, and urea turnover given in mmol m^{-2} d^{-1}. Upper and middle box present rates from the vegetated sediments that received 50 and 10% of the incident light, respectively, and the lower box present values from the unvegetated sediment. The ± values represents standard errors of means (n = 6 for nitrogen fixation, n = 4 for sulphate reduction, and n = 2 for urea turnover; n was 12, 8, 4, respectively, in the unvegetated sediment)

temperate seagrass meadows (Capone & Budin 1982, Welsh et al. 1996, McGlathery et al. 1998). The nitrogen fixation rates obtained in this study were higher in the vegetated than in the unvegetated sediment due to a stimulation of the nitrogen fixation in the rhizosphere, 0–5 cm (Figs. 3a & 4). A rapid transfer of fixed nitrogen from the rhizosphere to the plant has been demonstrated for *Zostera capricorni* and *Zostera marina* (Capone 1988, O'Donohue et al. 1991). O'Donohue et al. (1991) estimated that 50% of the nitrogen fixed in the rhizosphere of a *Z. capricorni* sediment was transferred to the leaves within 6 h. In the present study, nitrogen fixation in the rhizosphere could potentially supply ~90% of the nitrogen requirement for growth, if 50% of the fixed nitrogen was transferred to the plant. The nitrogen requirement for growth was estimated from leaf and root-rhizome growth and the average nitrogen content in the leaves and the root-rhizomes. The root-rhizome growth was assumed to be 89 and 21% of the leaf growth in the 50 and 10% light

Table 2. Root-rhizome associated process rates and their contribution to the area sediment rates in the *Zostera capricorni* bed. Rt-rh: roots-rhizomes. Values in parentheses are standard errors of mean: n = 6 for sulphate reduction and urea turnover, n = 5 for nitrogen fixation

	10%	50%
Nitrogen fixation, aerobic		
Rates per weight rt-rh (nmol g^{-1} dw d^{-1})	7300 (683)	8669 (979)
Rates per volume rt-rh (nmol cm^{-3} d^{-1})	1825 (171)	2173 (245)
Rt-rh contribution to areal sediment rates (%)[a]	27.8 (2.6)	39.0 (4.4)
Nitrogen fixation, anaerobic		
Rates per weight rt-rh (nmol g^{-1} dw d^{-1})	3106 (214)	3779 (337)
Rates per volume rt-rh (nmol cm^{-3} d^{-1})	777 (53)	945 (84)
Rt-rh contribution to areal sediment rates (%)[a]	11.8 (0.8)	17.0 (1.5)
Sulphate reduction		
Rates per weight rt-rh (nmol g^{-1} dw d^{-1})	8157 (2165)	4641 (1681)
Rates per volume rt-rh (nmol cm^{-3} d^{-1})	2039 (541)	1160 (420)
Rt-rh contribution to areal sediment rates (%)[a]	4.2 (1.1)	4.2 (1.5)
Urea turnover		
Rates per weight rt-rh (nmol g^{-1} dw d^{-1})	92 (93)	462 (105)
Rates per volume rt-rh (nmol cm^{-3} d^{-1})	23 (23)	115 (26)
Rt-rh contribution to areal sediment rates (%)[a]	0.1 (0.1)	0.6 (0.1)

[a]The ratio $Vol_{rt\text{-}rh}/Vol_{sed}$ (Table 1) was used to calculate the contribution of the root-rhizome associated rates to the sediment rates. Rates associated with roots-rhizomes were assumed to be the same in the root-rhizome incubations and in the sediment incubations

treatment, respectively, as found for *Z. capricorni* adapted to 50 and 15% of incident light (Abal et al. 1994). If the difference between the depth-integrated nitrogen fixation rates in the vegetated and the unvegetated sediment is assumed to represent a potential nitrogen uptake by the plants, this could supply ~140% of the nitrogen needed for growth of *Z. capricorni*. However, part of this estimated potential nitrogen uptake may be incorporated in bacteria instead of plants, since bacterial activity was higher in the vegetated than the unvegetated sediment. Based on the estimates above, we suggest that nitrogen fixed in the rhizosphere was an important nitrogen source for *Z. capricorni*.

The nitrogen fixation rates obtained in the incubation of the rinsed *Zostera capricorni* root-rhizomes were comparable to rates of nitrogen fixation obtained from incubations of root-rhizomes of other seagrasses (Capone & Taylor 1980, Capone & Budin 1982, Smith & Hayasaka 1982a,b). The nitrogen fixation rate associated with the *Z. capricorni* root-rhizomes were up to 70-fold higher than the average nitrogen fixation rate in the vegetated sediment and accounted for up to 39% of the nitrogen fixation in the vegetated sediment (Table 2). Other studies have demonstrated that nitrogen fixation associated with root-rhizomes can account for a significant proportion of the total nitrogen fixation in *Z. capricorni* and *Syringodium isotifolium* sediments (O'Donohue et al. 1991, Moriarty & O'Donohue 1993). In the present study nitrogen fixation associated with root-rhizomes could potentially supply a substantial part (21 to 65%) of the nitrogen required for growth in *Z. capricorn* as also demonstrated by O'Donohue et al. (1991).

The nitrogen fixation rates associated with root-rhizomes were significantly higher under oxic than under anoxic conditions ($p < 0.01$, Table 2) a result also found for other seagrasses (Smith & Hayasaka 1982a,b). This seems to be an adaptation to oxic conditions at the surface of root-rhizomes caused by leakage of oxygen from root-rhizomes (Sand-Jensen et al. 1982, Smith et al. 1984, Thursby 1984, Pedersen et al. 1998, 1999, Connell et al. 1999). Root-rhizomes are an important site for nutrient uptake, and leakage of oxygen could be a secondary effect of the nutrient uptake. However, leakage of oxygen in an anoxic environment could also be a protection of root-rhizomes against reduced phytotoxins like sulphide (Penhale & Wetzel 1983, Goodman et al. 1995, Pedersen et al. 1998).

In the present study, the nitrogen fixation rates associated with root-rhizomes were potential rates, as the root-rhizomes were incubated in substrate enriched porewater. However, the root-rhizomes of seagrasses release dissolved organic carbon (Wetzel & Penhale 1979, Moriarty et al. 1986), and the microorganisms associated with the root-rhizome complex are therefore exposed to higher substrate concentrations than microorganisms in unvegetated sediments. Leakage of photosynthates from root-rhizomes has been suggested to be an important carbon source for nitrogen

fixing bacteria, as light has been shown to stimulate nitrogen fixation in the rhizosphere of seagrasses (Capone 1988, O'Donohue et al. 1991, Welsh et al. 1996, 1997, McGlathery et al. 1998). In addition, nitrogen fixing bacteria have been demonstrated in high numbers both on the surface and intercellularly in roots and rhizomes of seagrasses, and the seasonal variation in the no. of bacteria attached to root-rhizomes followed the productivity of the seagrasses (Smith & Hayasaka 1982b, Schmidt & Hayasaka 1985, Shieh et al. 1989, Glazebrook et al. 1996). These studies indicated a close nutritional association between bacteria and seagrasses, which may be of mutual advantad especially in oligotrophic systems.

Sulphate reduction

Sulphate reduction rates in the *Zostera capricorni* sediment were comparable to rates measured in other seagrass sediments (Pollard & Moriarty 1991, Blackburn et al. 1994, Isaksen & Finster 1996, Welsh et al. 1996, Holmer & Nielsen 1997, Blaabjerg et al. 1998). The sulphate reduction rates obtained here were higher in the vegetated than in the unvegetated sediment due to elevated rates in the rhizosphere (Figs. 3b & 4), a result also found in other seagrass sediments (Pollard & Moriarty 1991, Isaksen & Finster 1996, Holmer & Nielsen 1997). Sulphate reduction in seagrass sediments is stimulated by light, which suggests that sulphate reduction in seagrass sediments, like nitrogen fixation, is stimulated by leakage of photosynthates from root-rhizomes (Blaabjerg et al. 1998). We estimated, from the difference between the depth-integrated sulphate reduction rate in the vegetated and the unvegetated sediments, the leakage of organic carbon from the root-rhizomes to be 8 to 18% of the carbon fixed by *Z. capricorni*. The total amount of fixed carbon was estimated as the carbon needed for plant growth (based on carbon content and growth data from this study and Abal et al. 1994) plus the estimated leakage from root-rhizomes. Direct measurements of carbon loss due to leakage from root-rhizomes of *Zostera marina*, *Halodule wrightii*, and *Thalassia testudinum* accounted for ~1 to 17% of the carbon fixed (Penhale & Smith 1977, Wetzel & Penhale 1979, Moriarty et al. 1986, Blaabjerg et al. 1998).

The sulphate reduction rates associated with the rinsed *Zostera capricorni* root-rhizomes were up to 11-fold higher than the average sulphate reduction rate in the vegetated sediment and accounted for 4% of the total sulphate reduction in the vegetated sediment (Table 2). High sulphate reduction rates associated with root-rhizomes were also demonstrated in a study of *Zostera marina* (Blaabjerg & Finster 1998). In addition, Blaabjerg & Finster (1998) demonstrated a high tolerance of root-rhizome associated sulphate reducing bacteria to oxygen as an adaptation to oxygen leakage from roots-rhizomes. The sulphate reducers associated with the rootrhizomes in the present study must also have been tolerant to oxygen, as sulphate reduction rates were not significantly different under oxic and anoxic conditions ($p > 0.28$).

Urea turnover and net ammonium production

The urea turnover rate was ~1.5-fold higher in the vegetated sediment when compared to the unvegetated sediment within the upper 2 cm of the sediment, which indicates that the turnover of urea was stimulated by the presence of root-rhizomes (Fig. 3c). However, the urea turnover rate associated with root-rhizomes was lower than the urea turnover rate in the sediment (Table 2). This indicates that the bacteria responsible for the turnover of urea were only loosely associated to the root-rhizomes, and therefore poorly represented in the incubation of rinsed root-rhizomes. The urea turnover rate and the gross ammonium production rate in *Zostera capricorni* sediment in Moreton Bay obtained in the present study and by Boon et al. (1986), respectively, indicate that more than half of the gross ammonium production could be due to the turnover of urea. We therefore suggest that urea is an important intermediate in the nitrogen cycle in this area.

There was no net production of ammonium in the *Zostera capricorni* sediment, contrary to what has been found in other seagrass sediments (Iizumi et al. 1982, Caffrey & Kemp 1990, Blackburn et al. 1994). Boon et al. (1986) also found low rates of net ammonium production in *Z. capricorni* sediments of Moreton Bay, despite the fact that rates of gross ammonium production were high. Consequently, the low net ammonium production indicated an efficient uptake and a rapid turnover of NH_4^+ as expected for an oligotrophic and presumably nitrogen limited system.

Amino acids in the sediment

The acid hydrolysable amino acids accounted for ~30% of TON in the *Zostera capricorni* sediment, which is comparable to the proportions (53%) found in an unvegetated sediment (Lomstein et al. 1998). The depth-integrated pools of acid hydrolysable amino acids in the sediment ($THAA_{sed}$) and in the root-rhizomes ($THAA_{rt+rh}$) were 3 and 1 order of magnitude higher, respectively, than the depth-integrated pool of acid hydrolysable amino acids in the porewater ($THAA_{pw}$).

Glutamic acid and glycine were the most abundant of the identified amino acids in DFAA, $THAA_{pw}$, $THAA_{sed}$, and $THAA_{rt+rh}$ (Fig. 2), as also found in 2 other studies of DFAA in seagrass sediments (Jørgensen et al. 1981, Boon 1986). The contribution (mole percent, mol%) of unidentified amino acids in the upper 10 cm of the *Zostera capricorni* sediment increased from 3% in $THAA_{rt+rh}$ through 8% in $THAA_{sed}$ and 14% in $THAA_{pw}$ to 40% in DFAA. Similarly, Lomstein et al. (1998) found that the mol% of unidentified amino acids increased from $THAA_{sed}$ (~6%) and $THAA_{pw}$ (~13%) to DFAA (~26%) in an unvegetated sediment. In addition, Burdige & Martens (1990) found that the mol% of unidentified amino acids was higher in DFAA than in $THAA_{sed}$, and they suggested that the unidentified amino acids were transient intermediates in the mineralisation of protein amino acids. This was supported by Macko et al. (1994), who found that the content of protein amino acids in *Halodule wrightii* decreased by ~50% during a decomposition study.

Sources and sinks of ammonium

Nitrogen is supplied to the sediment as particulate organic nitrogen (PON) and through nitrogen fixation (Fig. 5). In addition, dissolved inorganic nitrogen (DIN) is taken up from the water column by seagrass leaves and by benthic microalgae.

The average urea turnover rate in the vegetated sediment was 18 mmol N m^{-2} d^{-1} and the nitrogen fixation was 4 mmol N m^{-2} d^{-1}, leading to a gross ammonium production of at least 22 mmol m^{-2} d^{-1} (Fig. 5). Denitrification and nitrification were not measured, but recent ^{15}N-tracer studies in sediments vegetated by *Zostera marina* and *Zostera noltii* indicate low rates (Rysgaard et al. 1996, Risgaard-Petersen et al. 1998, Ottosen et al. 1999). The net requirement of nitrogen for growth of *Zostera capricorni* was ~3 mmol m^{-2} d^{-1}. Uptake of nitrogen by seagrasses is assumed to occur both from the water column and the sediment with a maximum of 2/3 of the demand supplied from one of the sources (Zimmerman et al. 1987, Pedersen & Borum 1992). The plant uptake of nitrogen in the present study was relatively low compared to the gross ammonium production, which suggests, as there was no net ammonium production, a high rate of nitrogen incorporation into the bacteria. According to Blackburn (1983) the nitrogen incorporation into anaerobic bacteria can be estimated as $C_o \cdot N_c \cdot E/(1-E)$, where C_o is the carbon oxidation rate, N_c is the N/C ratio of bacterial cells, and E is the efficiency of C incorporation. Assuming that sulphate reduction was the only anaerobic process of importance for carbon oxidation, the anaerobic carbon oxidation should account for ~46 mmol m^{-2} d^{-1}, resulting in an estimated nitrogen incorporation into anaerobic bacteria of 4 mmol m^{-2} d^{-1} (E = 0.3 and N_c = 0.2; Blackburn 1983). Similarly, the nitrogen incorporation into aerobic bacteria can be calculated if the respiration by aerobic bacteria is known. Nitrogen incorporation into aerobic bacteria in the present study was 7 to 21 mmol m^{-2} d^{-1}, assuming that the aerobic respiration was responsible for 25 to 50% of the carbon oxidation (E = 0.7 and N_c = 0.2). In addition, nitrogen was presumably incorporated into benthic microalgae. Together with the observation that there was no net ammonium production, these estimates on nitrogen incorporation into the microbial biomass demonstrate high rates of internal nitrogen cycling in the sediment. We propose that mineralisation within the sediment, was through an almost closed cycle of alternate organic nitrogen degradation and resynthe-

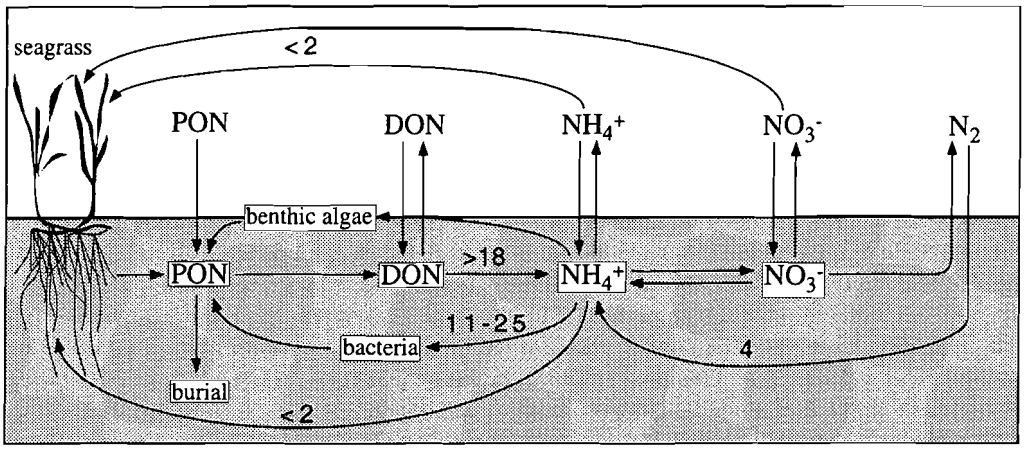

Fig. 5. Nitrogen cycle in the *Zostera capricorni* bed. Rates are presented as the mean of the rates obtained in the 10 and 50% light treatment and are given as mmol N m^{-2} d^{-1}. See text for further discussion

sis, driven by carbon oxidation as also suggested by Lomstein et al. (1989, 1998). *Z. capricorni* functioned as a temporary storage of nitrogen and supplied, partly as leakage from root-rhizomes, some of the carbon that fuelled the recycling of nitrogen. The rapid recycling of nitrogen in an almost closed cycle within the sediment implies that porewater concentrations of nitrogen was kept low and that most of the nitrogen, at least temporarily, was unavailable to phytoplankton. Thereby, high microbial activity in the sediment and relatively high growth rates of seagrasses was sustained, despite the fact that Moreton Bay is characterised as a low nutrient environment (Boon 1986, Udy & Dennison 1997).

In summary, the presence of *Zostera capricorni* stimulated the rates of nitrogen fixation, sulphate reduction, and to a lesser extent urea turnover in the sediment. The processes were enhanced in the rhizosphere, and there were high rates of nitrogen fixation and sulphate reduction associated directly with the root-rhizomes. This indicated a close nutritional association between the plant and the microorganisms attached to the root-rhizomes.

The stimulation of *Zostera capricorni* on sediment microbial processes was less pronounced at reduced light irradiance. Accordingly, reduced light availability in the water column will have an immediate effect on the microbial activity in vegetated sediments, and presumably the microbial activity will be displaced towards the water column as also found for plant growth.

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