

The effect of eelgrass decomposition on sediment carbon and nitrogen cycling: A controlled laboratory experiment

Anna-Grethe U. Pedersen, Jørgen Berntsen,¹ and Bente Aa. Lomstein²

Department of Microbial Ecology, Institute of Biological Sciences, Aarhus University, Building 540, Ny Munkegade, DK-8000 Aarhus C, Denmark

Abstract

The initial benthic degradation of senescent *Zostera marina* leaves was studied in controlled flowthrough microcosm chambers for 33 d. Sediment chambers without added eelgrass leaves served as control chambers. The inflowing artificial seawater and outflowing seawater were analyzed for dissolved organic carbon and nitrogen (DOC and DON), total acid-hydrolyzable amino acids (THAA), dissolved free amino acids (DFAA), urea, NH_4^+ , NO_3^- , ΣCO_2 , and O_2 during the 33 d of incubation. Sediment profiles of particulate organic carbon and nitrogen, sediment acid-hydrolyzable amino acids, DON, DFAA, urea, NH_4^+ and the turnover rate of urea were measured at four different times during the 33 d of incubation. There was an immediate increase in carbon oxidation and the effluxes of DOC, DON, and NH_4^+ after the addition of eelgrass leaves to the sediment surface. During the course of incubation, 24.3% of the DON efflux was identified as acid-hydrolyzable amino acids, dissolved free amino acids, and urea in the chambers with eelgrass addition, whereas these compounds accounted for 33.8% of the DON efflux in the control chambers. There were indications of a stimulated bacterial growth on the eelgrass leaves during the first 7 d after leaf addition that was measured as an increase in acid-hydrolyzable amino acids. Further, there was a gradual increase in acid-hydrolyzable amino acids in the sediment throughout the incubation that could only be explained as bacterial growth (and/or protein synthesis). Most of the nitrogen for microbial growth was mobilized from the indigenous particulate organic nitrogen pool, whereas it could be inferred that the energy source for bacterial growth was mainly supplied from the added eelgrass leaves. Most of the nitrogen mineralized within the sediment was incorporated into the microbial biomass with a resultant low efflux of inorganic nitrogen from the sediment to the water column.

Shallow marine sediments are areas with an intense biogeochemical activity. The sources of organic matter to these sediments are benthic and pelagic primary producers and terrestrial organic matter. The quantity and quality of organic matter introduced into the sediment has been found to have an overall determining effect on benthic mineralization (e.g., Blackburn and Henriksen 1983; Lomstein et al. 1989; Therkildsen and Lomstein 1994; Sloth et al. 1995).

The eelgrass *Zostera marina* was one of the dominating primary producers in Danish coastal waters before the onset of eutrophication. *Z. marina* is characterized by a high molar C:N ratio (16.4–24.3; Enríquez et al. 1993). Further, the plant contains structural plant components (e.g., cellulose, hemicellulose, and phenolic constituents) that are not easily degraded by benthic microorganisms (Boulton and Boon 1991; Buchsbaum et al. 1991).

Fenchel et al. (1998) suggested that the large pool of dead organic matter in sediments may reflect the low rate at which

certain structural plant polymers can be hydrolyzed even under optimal conditions, thus explaining that decomposition lags behind production. Further, they suggested that the availability of nutrients limit the rate of mineralization in the sense that decomposing plant tissues have lower nutrient contents than the decomposer organisms. In support of this, Van Duyl et al. (1993) observed that the sediment–water fluxes of inorganic nutrients were inversely related to benthic bacterial production rates. This inverse relationship was explained by a temporary immobilization of nitrogen and phosphorus in bacterial biomass. Van Duyl et al. (1993) demonstrated that in response to a short-term sedimentation event, the increase in benthic bacterial biomass (over a 5-d period) may equal the decrease in sediment–water fluxes of dissolved inorganic nitrogen (DIN) and phosphorus. They suggested that heterotrophic benthic bacteria can act as sinks for nitrogen and phosphorus that only release nitrogen and phosphorus upon death.

The organic nitrogen that provides the nutrition in sediments has been parameterized in the literature primarily as total particulate organic nitrogen (PON) and as total acid-hydrolyzable amino acids (THAA). In the upper decimeter of surface sediments there is a relatively large pool of acid-hydrolyzable amino acids (THAA). THAA accounted for 24–38% of the PON pool in Buzzards Bay, USA (Henrichs and Farrington 1987), 37–44% in Saanich Inlet, Canada (Cowie et al. 1992), and 53% of the PON pool in the shallow Knebel Vig, Denmark (Lomstein et al. 1998). The sediment THAA pool has a high nitrogen content (molar C:N = 4.2:1.0), and more than 90% of this pool is composed of protein amino acids (i.e., Cowie et al. 1992; Lomstein et al. 1998).

¹ Present address: Research Center Foulum, Post box 50, DK-8830 Tjele, Denmark.

² Corresponding author.

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Dauwe and Middelburg (1998) found that although hexosamines are present in minor quantities compared to amino acids, they contribute considerably to sedimentary nitrogen in the more degraded North Sea sediment. Although there have been significant improvements in analytical techniques and considerable efforts to characterize sedimentary organic carbon, a large fraction of it remains biochemically uncharacterized (Hedges and Oades 1997).

The rate-limiting step in nitrogen and carbon mineralization is the extracellular hydrolysis of macromolecules (proteins, polysaccharides, lipids, and nucleic acids) into smaller molecules (amino acids, sugars, short-chained fatty acids, and nucleotides). Extracellular hydrolysis is an indispensable step in organic matter degradation as prokaryotes can only take up smaller molecules across their cell membrane. However, it was recently suggested by Burdige and Gardner (1998) that fermentative or perhaps respiratory processes may control carbon remineralization in estuarine sediments, whereas remineralization in continental margin sediments may be controlled by hydrolysis. In addition, it has been suggested that organic matter can be protected inside small pores (<10 nm in diameter [Mayer 1994]) and that adsorption to sediment surfaces can prevent hydrolysis of organic matter (Hedges and Keil 1995).

It is only within the past 10 years that attempts have been made to quantify the total dissolved organic matter (DOM) pool in marine sediments, the flux of DOM across the sediment-water interface, and the relative importance of the different components of DOM (e.g., Burdige and Zheng 1998; Lomstein et al. 1998). Burdige and Zheng (1998) showed that the C:N ratio in DOM escaping different estuarine sediment was lower (2–6) than the C:N ratio in porewater DOM (>10). Burdige and Zheng (1998) concluded that DOM accumulating in sediment porewaters was carbon-rich compared with the DOM that was either mineralized or escaped the sediment as a benthic flux. Lomstein et al. (1998) were able to identify 13% of the dissolved organic carbon (DOC) pool and 39% of the dissolved organic nitrogen (DON) pool as dissolved THAA, dissolved free amino acids, and urea. The turnover times of the identified DON pools were 7–52 h for THAA, 3–4 h for DFAA, and 1–3 h for urea.

During the last decades, there have been a number of studies on the degradation of marine primary producers, and this topic was recently reviewed by Enríquez et al. (1993). However, there are only a few studies where the aim has been to investigate the effect of plant decomposition on carbon oxidation and the fluxes of DOC and DON and DIN between the sediment and the water column. Among these studies are Andersen and Hargrave (1984) on the degradation of *Spartina alterniflora* leaves at depth in the sediment, Hansen and Blackburn (1991) on the degradation of the macroalgae *Chondrus crispus*, and Enoksson (1993) on the effect of a microalgae addition on nutrient cycling in coastal sediments.

The aim of the present study was to obtain detailed knowledge on the factors that control organic carbon and nitrogen mineralization during decomposition of senescent eelgrass leaves at the sediment surface. A continuous flowthrough system was established to measure carbon and nitrogen exchange rates between the sediment and the water column,

sediment and porewater carbon, and nitrogen pools and the turnover rate of urea. Senescent leaves were added to the sediment surface in one series of experiments, whereas the other series of sediment chambers without added leaves served as controls. The fluxes of dissolved organic carbon and nitrogen (DOC and DON), THAA, dissolved free amino acids (DFAA), urea, NH_4^+ , NO_3^- , ΣCO_2 , and O_2 were measured on a daily basis during the 33 d of incubation. Profiles of particulate organic carbon and nitrogen (POC and PON), sediment acid-hydrolyzable amino acids (THAA_s), DON, DFAA, urea, NH_4^+ , and the turnover rate of urea were measured at four different times during the incubation of eelgrass leaves and in the beginning and by the end of the control incubations. Conceptual models on carbon and nitrogen degradation in the two experimental series were constructed in which the area-integrated measured and estimated rates can be seen in relation to each other. There was specific focus on bacterial nitrogen incorporation, bacterial carbon incorporation efficiency, and the C:N ratio of the organic matter degraded.

Materials and methods

Study site and sampling—The sediment was collected in November 1994, at Sta. 6 in the Aarhus Bay, Denmark (56°09'10"N, 10°19'20"E). The bottom water temperature and the salinity were 12°C and 26.4‰, respectively, and the water depth was 16 m. Undisturbed sediment was collected with a box-corer (Jonasson and Olausson 1966).

The water overlying the sediment and the upper 10 mm of the sediment were carefully removed and discarded. Sediment from the 10-mm to 55-mm depth strata was sieved (0.5 mm mesh size) to remove macrofauna and transferred to 15 glass chambers. A water column was gently overlaid the sediment. The resultant volume of sediment in each chamber was 240 cm³, the sediment height was 4.5 cm, the sediment surface area was 55.4 cm², and the volume of the overlying water was 246 ml. The sediment was left overnight before the water flow was established. The water overlying the sediment (reservoir water) was artificial seawater (Kester et al. 1967) with the following modifications: the salinity was 26.4‰ and there was no addition of vitamins, nitrate (NO_3^-) or phosphate (PO_4^{3-}). The water overlying the sediment was stirred with a centrally placed magnet (45 rpm).

Experimental set-up—The experimental flowthrough system (Fig. 1) was essentially as described by Binnerup et al. (1992). The reservoir water was pumped through the sediment chambers with a Watson-Marlow peristaltic pump with a flow rate of $0.40 \pm 0.01 \text{ ml min}^{-1}$. The reservoir water was bubbled with gas from a Brooks mass flow controller (5850 TR series) where the composition of CO_2 , O_2 , and N_2 could be controlled. The O_2 concentration in the reservoir water was adjusted twice during the experiment: in the beginning of the experiment where the O_2 concentration was 440 μM and after the eelgrass addition, where the reservoir O_2 concentration was increased to 928 μM . The increased O_2 concentration in the inflowing water to the eelgrass chambers ensured that the O_2 concentration in the water overlay-

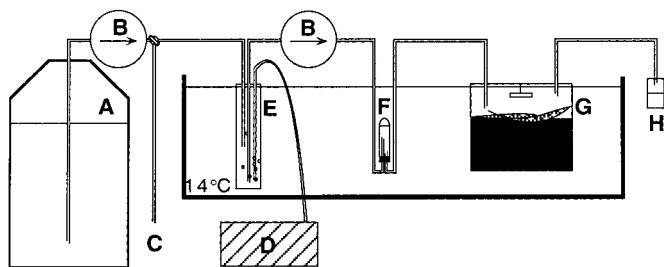


Fig. 1. The continuous flowthrough system was composed of a reservoir with artificial seawater (A), a Watson–Marlow peristaltic pump (B), sampling for inflowing water (C), a Brooks gas mass flow controller (D), a gas bubbling chamber (E), a debubbler (F), sediment chambers (G), and a sampling flask for outflowing water (H).

ing the sediment remained close to air saturation in all sediment chambers.

All tubes from the water reservoir to the chambers were glass and all connections were gas-tight Viton VA 70 (Verneret). The outflow tubes from the chambers to the sampling station were Teflon tubes coated with Tygon tubes. The Teflon tubes were used to avoid binding of dissolved solutes to tube walls and the Tygon coating was used to prevent gas exchange. All reservoirs, tubes, and chambers were submerged into a 14°C water bath and kept in the dark. On days 4 and 22 after the eelgrass addition the tube system was disconnected from the sediment chambers and tested for O_2 consumption. The tube system was rinsed with 1 N HCl followed by a thorough rinse with artificial seawater before it again was connected to the sediment chambers.

Sediment–water fluxes—The sediment–water fluxes were high and variable during the first 11 d of incubation, and this period was considered as a preincubation period (data not shown). Thus, day 0 in the following refers to day 11 in the preincubation period. Intact senescent eelgrass leaves (leaves 4 and 5 according to Pedersen and Borum [1993]) were added to the sediment surface of nine sediment chambers at day 0 (E+S treatment), and the remaining six sediment chambers served as controls (S treatment). The wet weight of the eelgrass added to each sediment chamber was 6.75 ± 0.03 g and the dry weight was 1.00 ± 0.01 g. The POC and the PON contents in the added eelgrass leaves were $6,487 \text{ mmol C m}^{-2}$ and $292 \text{ mmol N m}^{-2}$ sediment surface, respectively. The addition of eelgrass leaves corresponded to 18 d of eelgrass leaf loss during the fall in a Danish eelgrass bed (Pedersen and Borum 1993).

Inflow and outflow water from the S and E+S chambers were collected daily during the 33 d of incubation and were analyzed for O_2 , ΣCO_2 , NO_3^- , NH_4^+ , urea, DOC and DON, THAA, and DFAA. The sediment–water fluxes were calculated by use of the Nishio et al. (1982) formula: $F = DC \cdot V / A$, where DC is the concentration change between outflow and inflow water, V is the flow rate, and A is the sediment surface area in the chamber.

O_2 was analyzed as described in Revsbech (1989) with an O_2 microelectrode in inflow and outflow water. ΣCO_2 was analyzed immediately after sample collection in gas-tight

Labco exetainers on a flow-injection system as described in Hall and Aller (1992).

Samples for DON, DOC, and DFAA were filtered through a 0.2- μm Sartorius filter, whereas the samples for THAA, urea-N, NO_3^- , and NH_4^+ analysis were unfiltered. All samples were frozen for later analysis. NO_3^- , NH_4^+ , and urea were measured on an α ALFA-LAVAL Bran+Luebbe autoanalyzer by the following methods: NO_3^- (Grasshoff et al. 1983), NH_4^+ (Bower and Holm-Hansen 1980), and urea (Price and Harrison 1987), respectively.

The concentration of DFAA was determined by high-performance liquid chromatography (HPLC; Waters Chromatographic System) on *o*-phthaldialdehyde-derivatized products (Lindroth and Mopper 1979). THAA was determined on 1-ml unfiltered water samples to which there was added 1 ml of 12 N HCl. Hydrolysis of the samples was carried out at 110°C for 24 h. After hydrolysis, 100 μl of the sample was dried in a vacuum desiccator for 4 h. The sample residue was dissolved in 5 ml Milli-Q water and filtered through a 0.2- μm Sartorius filter. The THAA was measured as DFAA as described above.

Samples for total dissolved nitrogen (TDN) and DOC were analyzed on a modified Antek 7000 system as described in Lomstein et al. (1998). An IR detector (Licor LI-6252) for CO_2 analysis was placed in series before the chemiluminescence detector for NO. Data were acquired on a Macintosh LC-III with a Lab View (National Instruments) program. The volume of sample analyzed was 50 μl . The area of samples in both the DOC and TDN analyses were within the same order of magnitude as the blank areas, when there was no or very little efflux of DOC or TDN from the sediment to the water column. At maximal efflux rates the area of DOC and TDN samples were 37 and 10 times larger than the area of blanks, respectively. The concentrations of DOC and TDN were calculated from a five-point calibration curve made on Tris buffer. The concentration of DON was determined as TDN minus the concentration of $NH_4^+ + NO_2^- + NO_3^-$.

Sediment and eelgrass characteristics—Before eelgrass addition, three S chambers were removed from the flowsystem, and the overlaying water was gently removed. The sediment was sectioned into 1.6-mm slices in the upper 6.4 mm of the sediment, into 4.8-mm slices in the 6.4–16.0-mm zone, and into 8-mm slices in the 16.0–32.0-mm zone. Sediment segments from the same depth strata were thoroughly mixed. Eelgrass was gently removed from three E+S chambers on days 7, 19, and 33 after eelgrass addition, and the sediment was sectioned as described above. On day 33 the last three S chambers were sectioned as described above.

Sediment-specific density was determined gravimetrically on duplicate 1-cm³ sediment samples, and the porewater content was determined as the weight loss of fresh sediment dried at 105°C for 24 h. The POC and PON contents were determined on duplicate dried, H_2SO_3 -treated sediment in a Carlo Erba NA-1500 CHN analyzer.

THAA_s were determined on 0.5-cm³ fresh sediment to which there was added 9.5 ml of 6 N HCl (pro analysis). Hydrolysis was carried out at 110°C for 24 h. Further handling of THAA_s samples was as described for the determi-

nation of THAA in water samples. THAA_s samples from day 0 and 7 in the E+S treatment were analyzed twice in order to obtain information about the analytical precision.

Eelgrass water content and the contents of POC, PON, and eelgrass total hydrolyzable amino acids (THAA_{eel}) were determined in duplicates on day 0, 7, 19, and 33 when the sediment chambers were sacrificed. Eelgrass water content was measured as the weight loss of leaves dried at 105°C for 24 h. The POC, PON, and THAA_{eel} contents of dried eelgrass were determined as described above for sediment analysis with the exception that THAA_{eel} was determined on 22.5 mg dry weight eelgrass leaves to which there was added 5 ml of 6 N HCl.

Porewater—Porewater was obtained by centrifugation at $2,000 \times g$ for 10 min. Samples were analyzed for NH_4^+ , urea, DFAA, and DON. Samples for DON and DFAA were immediately filtered ($0.2 \mu\text{m}$, Sartorius) and frozen for later analysis. NH_4^+ , urea, DFAA, and DON were analyzed as described above. O_2 profiles were measured 25 d after eelgrass addition with an O_2 microelectrode (Revsbech 1989) in the S and E+S treatments. The depth resolution of the O_2 profiles was $100 \mu\text{m}$.

Urea turnover—Urea turnover was measured as described in Lund and Blackburn (1989) with the following modifications: $2 \mu\text{l}$ ^{14}C -urea ($1.8 \text{ nCi } \mu\text{l}^{-1}$, 55 nCi nmol^{-1} , Amersham) was injected into 0.5 cm^3 sediment and the urea turnover activity was stopped by the addition of 1 ml of 2.5% w/v NaOH after 0 and 1 h of incubation. The incubations were performed in the dark, at 14°C in 6.5 ml N_2 -flushed Labco exetainers. The turnover rate of urea was calculated by use of the Lund and Blackburn (1989) steady-state model II, where the pool size of urea is assumed to remain constant during incubation and the ^{14}C -urea pool to decrease exponentially with time.

Results

Sediment O_2 uptake and the efflux of ΣCO_2 and DOC

In the S treatment, the average O_2 uptake and the average ΣCO_2 efflux were $16.2 \pm 0.5 \text{ mmol m}^{-2} \text{ d}^{-1}$ and $12.5 \pm 1.8 \text{ mmol m}^{-2} \text{ d}^{-1}$, respectively (Fig. 2A,B). The O_2 uptake in the E+S treatment increased during the first 12 d after eelgrass addition from the control level to an average O_2 uptake of $68.3 \pm 1.5 \text{ mmol m}^{-2} \text{ d}^{-1}$ (days 12–33; Fig. 2A). The ΣCO_2 efflux increased during the first 12 d after eelgrass addition from the control level to an average of $79.9 \pm 2.4 \text{ mmol m}^{-2} \text{ d}^{-1}$ (Fig. 2B). There was no O_2 consumption in the tube system when tested on days 4 and 22.

The DOC efflux in the S treatment remained $<4 \text{ mmol m}^{-2} \text{ d}^{-1}$ throughout the incubation, whereas the DOC efflux increased from the control level to $145 \text{ mmol m}^{-2} \text{ d}^{-1}$ at day 5 after the addition of eelgrass leaves (Fig. 2C). After day 7, the DOC efflux approximated the control level with the exception of day 10 where the DOC efflux was increased to $43 \text{ mmol m}^{-2} \text{ d}^{-1}$.

Efflux of DON and DIN—There was a decrease in the DON efflux from $3.4 \text{ mmol m}^{-2} \text{ d}^{-1}$ at the first time of DON

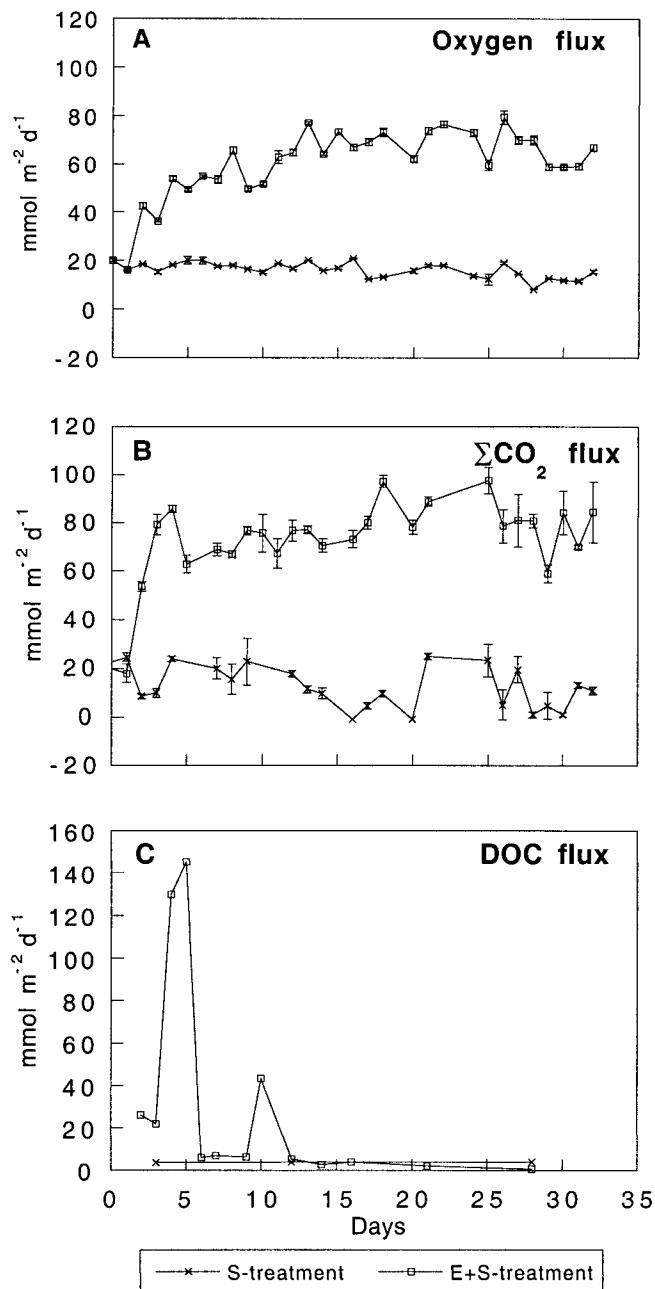


Fig. 2. Fluxes of O_2 (A), ΣCO_2 (B), and DOC (C) in the E+S treatment and in the S treatment. Eelgrass leaves were added to the E+S treatment on day 0.

sampling after eelgrass addition (day 2), to the control level ($0.25 \text{ mmol m}^{-2} \text{ d}^{-1}$) at day 9 (Fig. 3A). From day 16 to the end of the experiment there was a gradual increase in the DON efflux from the control level to $1.2 \text{ mmol m}^{-2} \text{ d}^{-1}$ at day 33 (Fig. 3A).

The THAA efflux was stimulated during the first 12 d after eelgrass addition (up to $0.61 \text{ mmol m}^{-2} \text{ d}^{-1}$), whereupon it approximated the THAA-N efflux in the S treatment of $0.05 \text{ mmol m}^{-2} \text{ d}^{-1}$ (Fig. 3B). The effluxes of DFAA-N from the S and E+S treatments were highly variable and $<0.1 \text{ mmol m}^{-2} \text{ d}^{-1}$ (Fig. 3C). The efflux of urea-N was

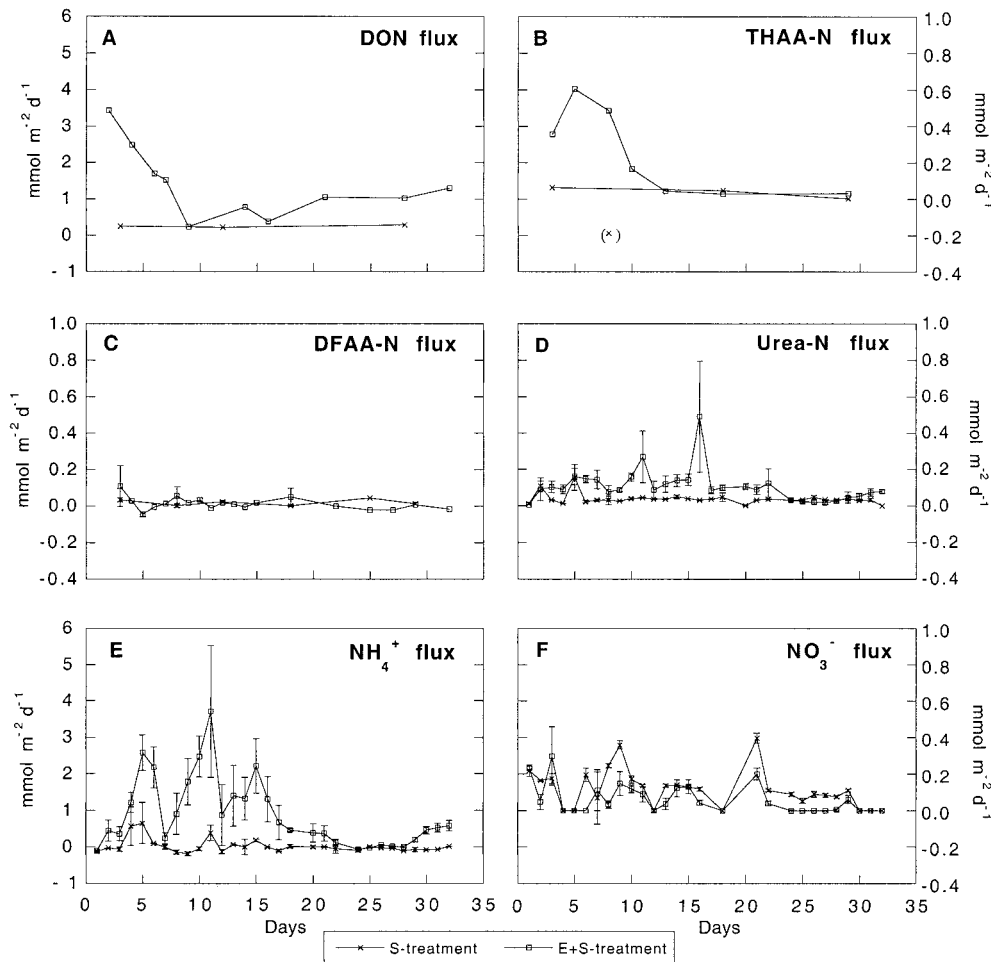


Fig. 3. Fluxes of DON (A), THAA-N (B), DFAA-N (C), urea-N (D), NH_4^+ (E), and NO_3^- (F) in the E+S treatment and in the S treatment. Eelgrass leaves were added to the E+S treatment on day 0.

stimulated during the first 22 d after eelgrass addition and was on average $0.13 \pm 0.02 \text{ mmol m}^{-2} \text{ d}^{-1}$ (Fig. 3D). During the remaining part of the experiment, the urea-N efflux remained at the control level ($0.039 \pm 0.005 \text{ mmol m}^{-2} \text{ d}^{-1}$; Fig. 3D).

The efflux of inorganic nitrogen (NH_4^+ and NO_3^-) was dominated by NH_4^+ in the E+S treatment. The NH_4^+ efflux was on average $1.38 \pm 0.24 \text{ mmol m}^{-2} \text{ d}^{-1}$ during the first 17 d after eelgrass addition, whereupon it decreased to $0.24 \pm 0.06 \text{ mmol m}^{-2} \text{ d}^{-1}$ during the remaining part of the in-

cubation (Fig. 3E). The average NH_4^+ efflux in the S treatment was $0.02 \pm 0.04 \text{ mmol m}^{-2} \text{ d}^{-1}$ (Fig. 3E). The average NO_3^- efflux was slightly higher in the S treatment $0.12 \pm 0.02 \text{ mmol m}^{-2} \text{ d}^{-1}$ than the NO_3^- efflux in the E+S treatment $0.06 \pm 0.02 \text{ mmol m}^{-2} \text{ d}^{-1}$ (Fig. 3F).

Eelgrass and sediment characteristics—The POC and PON content in the added eelgrass leaves decreased throughout the experimental period. The POC content in eelgrass leaves decreased from 6,487 to 4,296 mmol m^{-2} sediment surface from days 0 to 33, and the PON content decreased from 292 to 236 mmol m^{-2} sediment surface during the same period (Table 1). There was a slight increase in the $\text{THAA}_{\text{eel-N}}$ content during the first 7 d of incubation from 140 to 146 mmol m^{-2} . During the remaining part of the experimental period, there was a decrease in $\text{THAA}_{\text{eel-N}}$ from 146 to 119 mmol m^{-2} (Table 1). The $\text{THAA}_{\text{eel-N}}$ accounted for 48–54% of the eelgrass PON content.

In both treatments and at the different dates of sampling there was always an almost linear increase in the POC and PON content with depth from the 0.0–1.6-mm to the 4.8–6.4-mm depth strata (Fig. 4A,C). Below this depth there was

Table 1. The contents of POC, PON, and THAA-N and the relative importance of THAA for the PON pool in eelgrass leaves after 0, 7, 19, and 33 d of incubation. The amount of eelgrass/ m^2 sediment surface was 180.5 g dry weight.

Day	POC (mmol m^{-2})	PON (mmol m^{-2})	THAA-N (mmol m^{-2})	THAA-N/ PON, %
0	6,487	292	140	48
7	6,054	270	146	54
19	5,226	249	128	51
33	4,296	236	119	50

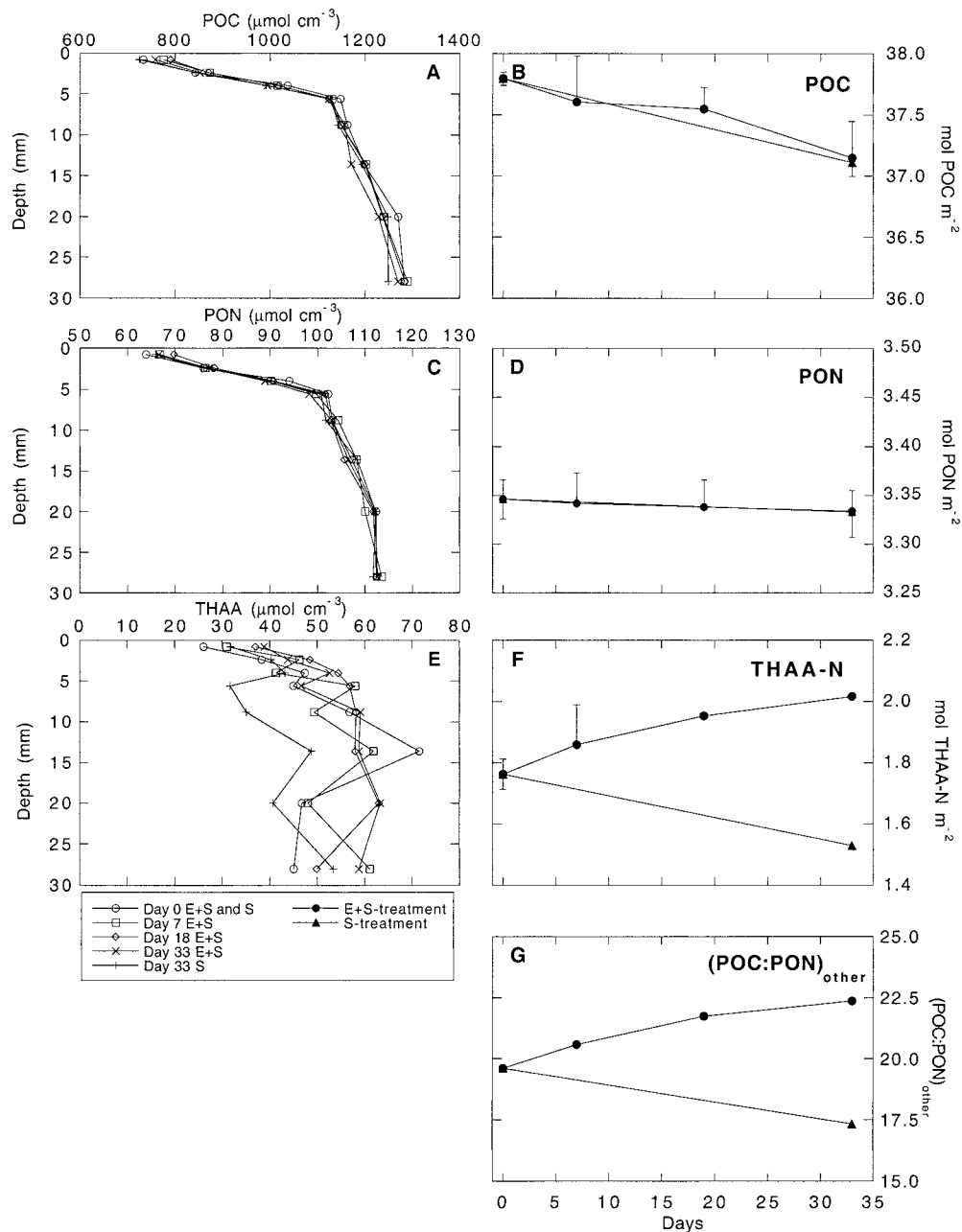


Fig. 4. Depth profiles of POC (A), PON (C), and THAA_s-N (E) on days 0, 7, 18, and 33 in the E+S treatment and days 0 and 33 in the S treatment. Sediment area integrated pools ($\Sigma 0-32$ mm) of POC (B), PON (D), THAA_s-N (F), and the C:N ratio of unidentified POC and PON (G) in the E+S treatment and the S treatment.

only a slight increase in the POC and PON contents (Fig. 4A,C). The area-integrated POC and PON contents decreased to the same level in the two treatments during the 33-d incubation period from 37.8 to 37.1 mol m⁻² and from 3.35 to 3.33 mol m⁻², respectively (Fig. 4B,D).

The THAA_s-N content was somewhat variable with sediment depth (26–72 μmol cm⁻³) in the two treatments and at the different dates of sampling (Fig. 4E). However, there was an increase in the THAA_s-N content with time after eelgrass addition at all depths except in the 11.2–16.0-mm-

depth strata (Fig. 4E). The area-integrated THAA_s-N increased from 1.76 to 2.02 mol m⁻² in the E+S treatment during the 33 d of incubation, whereas the THAA_s-N content in the S treatment decreased from 1.76 to 1.53 mol m⁻² during the same period (Fig. 4F). The proportion of THAA_s-N in the PON pool increased from 51 to 60% during the 33 d of incubation in the E+S treatment and decreased from 51 to 46% in the S treatment during the same period.

The area-integrated POC:PON ratio decreased slightly from 11.3 to 11.1 during the 33 d of incubation in both

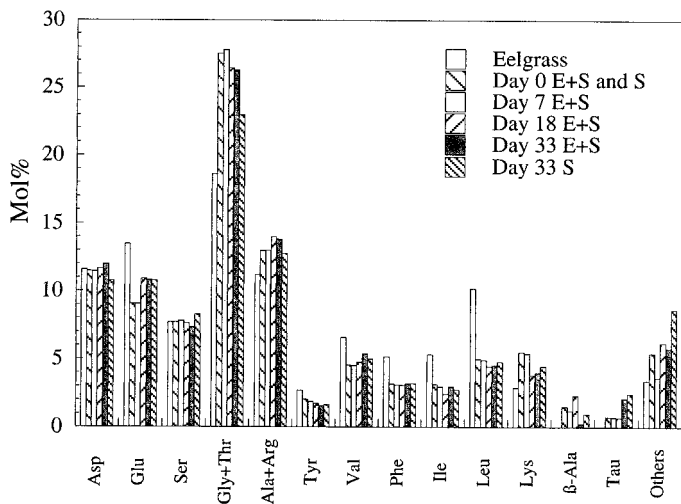


Fig. 5. Mol percent contributions of the individual protein and nonprotein amino acids to the THAA pool in the sediment and in the added eelgrass leaves.

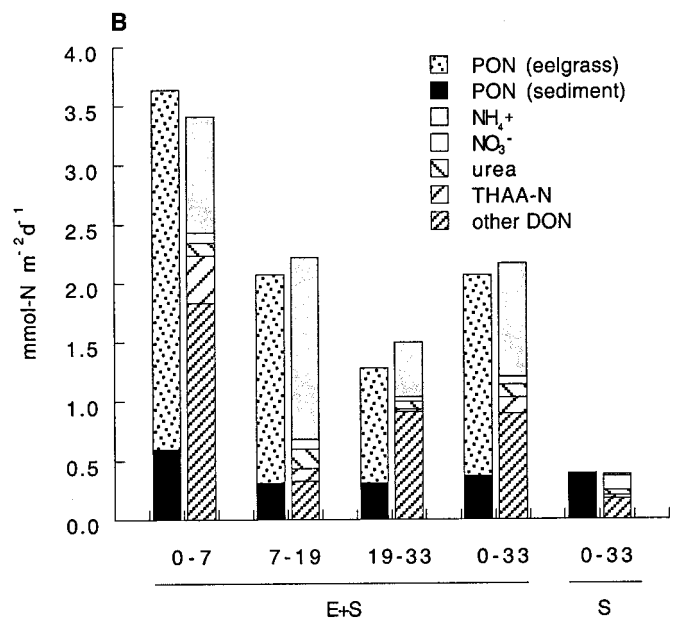
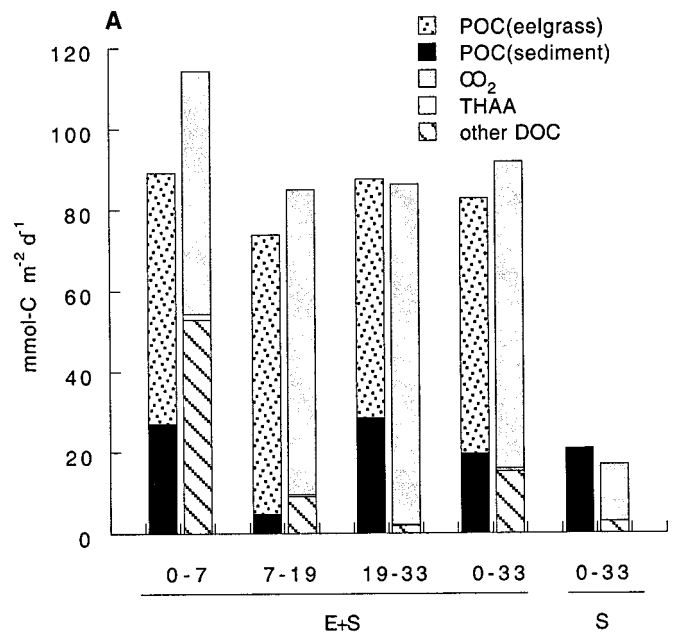


Fig. 6. (A) Estimated daily loss of POC from leaves and sediment and average daily carbon efflux during three periods in the E+S incubation: days 0–7, 7–19, and 19–33 and during the 33 d of incubation the S treatment. (B) Estimated daily loss of PON from leaves and sediment and average daily nitrogen efflux rates in the same periods as described above.

sediment during the 33 d of incubation in the S treatment (Fig. 6B).

Porewater profiles and depth-integrated pools of DON, DFAA-N, urea-N, and NH₄⁺—Figure 7A–H gives the porewater profiles and the depth-integrated pools of DON, DFAA-N, urea-N, and NH₄⁺ at days 0, 7, 19, and 33 in the E+S treatment and at days 0 and 33 in the S treatment. The

treatments. On the contrary, the C:N ratio of the unknown pool of POC and PON increased from 19.6 to 22.4 in the E+S treatment, whereas it decreased from 19.6 to 17.3 in the S treatment (Fig. 4G). THAA_s was the only identified component of the POC and PON pools and the C:N ratio in THAA_s remained at 4.2.

Figure 5 gives the change in the mol% of individual amino acids in the added eelgrass leaves and in the area-integrated THAA_s pool with time of incubation. Glycine + threonine were the dominating protein amino acids in the added eelgrass leaves and in the sediment. Compared to the sediment, eelgrass leaves were relatively enriched in glutamate, valine, phenylalanine, isoleucine, and leucine, whereas they were depleted in glycine + threonine, alanine + arginine, lysine, and unidentified others. There was an increase in the mol% of alanine + arginine and in glutamate in both the E+S treatment and in the S treatment with time of incubation (Fig. 5). The mol% of unidentified others increased in the S treatments, whereas it was variable with time in the E+S treatment. The mol% of taurine increased in both the E+S treatment and in the S treatment. The mol% of glycine + threonine decreased with time of incubation in both the E+S treatment and in the S treatment. However, the decrease in the mol% of glycine + threonine in the S treatment from 27.5% at day 0 to 23.0% at day 33 greatly exceeded the decrease in the E+S treatment (from 27.5% to 26.3%).

The average accumulated efflux of $\Sigma\text{CO}_2 + \text{DOC}$ (given in $\text{mmol m}^{-2} \text{d}^{-1}$) could explain 128, 115, and 99% of the carbon loss from the leaves and the sediment in the following periods after eelgrass addition: days 0–7, 7–19, and 19–33, respectively, and 81% of the carbon loss from the sediment in the S treatment (Fig. 6A). In parallel, the average accumulated efflux of $\text{NH}_4^+ + \text{NO}_3^- + \text{urea-N} + \text{THAA-N} + \text{other DON}$ compounds (given in $\text{mmol m}^{-2} \text{d}^{-1}$) could explain 94, 107, and 117% of the nitrogen loss from the leaves and the sediment in the three periods after eelgrass addition, respectively, and 98% of the nitrogen loss from the

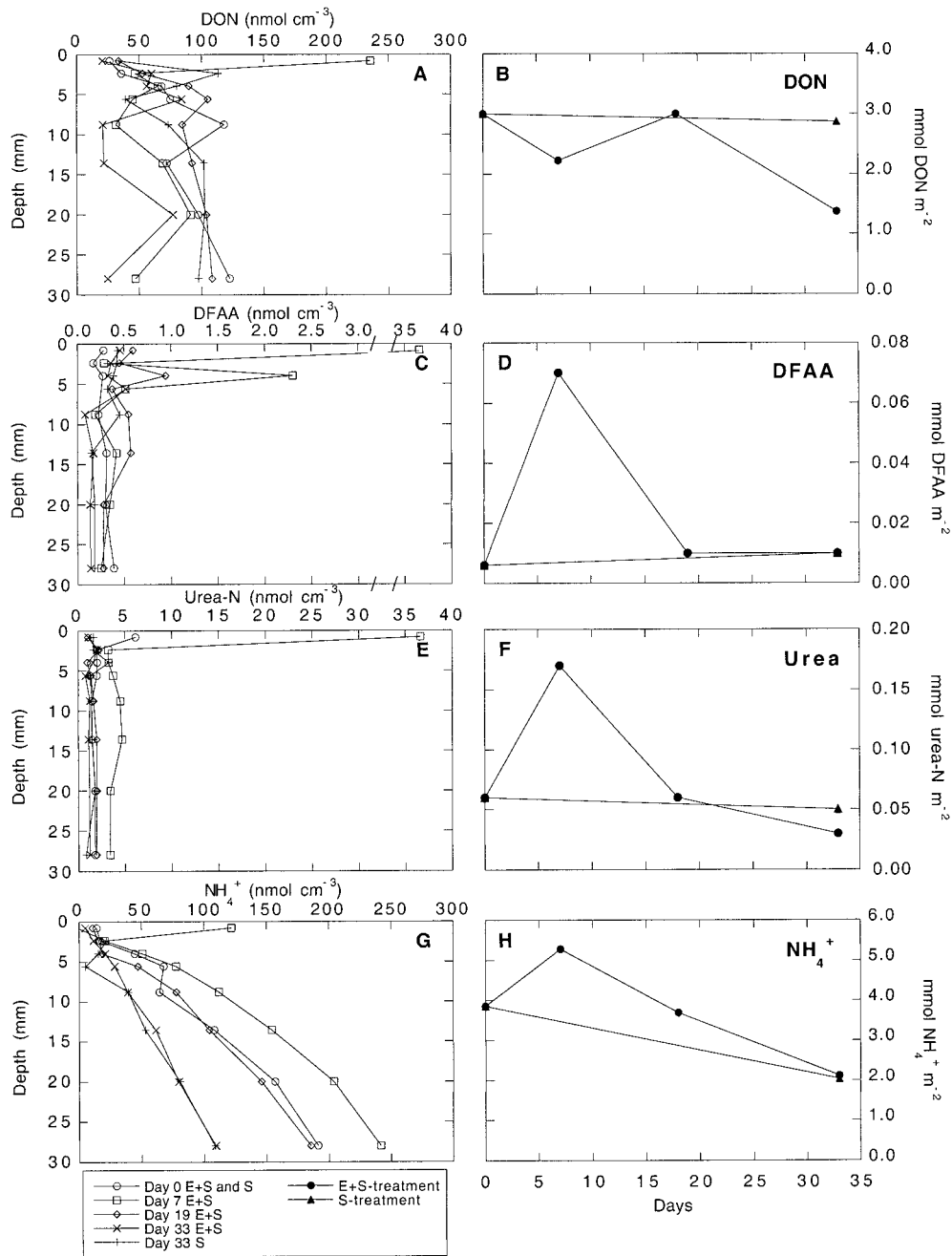


Fig. 7. Depth profiles of DON (A), DFAA-N (C), urea-N (E), and NH_4^+ (G) on days 0, 7, 18, and 33 in the E+S treatment and days 0 and 33 in the S treatment. Sediment area integrated pools ($\Sigma 0\text{--}32$ mm) of DON (B), DFAA-N (D), urea-N (F), and NH_4^+ (H) in the E+S treatment and the S treatment.

concentration of DON varied with depth within the range from 21 to 235 nmol cm^{-3} in the E+S treatment and between 26 and 122 nmol cm^{-3} in the S treatment (Fig. 7A). The area-integrated DON pool was somewhat variable with time in the E+S treatment, but the DON pool was lower by the end of the experiment (1.4 mmol m^{-2}) compared to the beginning (3.0 mmol m^{-2} ; Fig. 7B). In the S treatment there was a slight decrease in the area-integrated DON pool from 3.0 at day 0 to 2.9 mmol m^{-2} at day 33 (Fig. 7B).

The DFAA and urea-N pools ranged from 0.1 to 2.3 nmol cm^{-3} and from 0.8 to 36.6 nmol cm^{-3} , respectively, in the E+S treatment (Fig. 7C,E). In the S treatment the DFAA-N and the urea-N pools varied between 0.1 and 0.5 nmol cm^{-3} and between 0.8 and 6.2 nmol cm^{-3} , respectively (Fig. 7C,E). Generally, the DFAA-N and the urea-N pools were highest within the upper 4.8 mm of the sediment in both treatments (Fig. 7C,E). There was an increase in the area-integrated DFAA-N and urea-N pools during the first 7 d

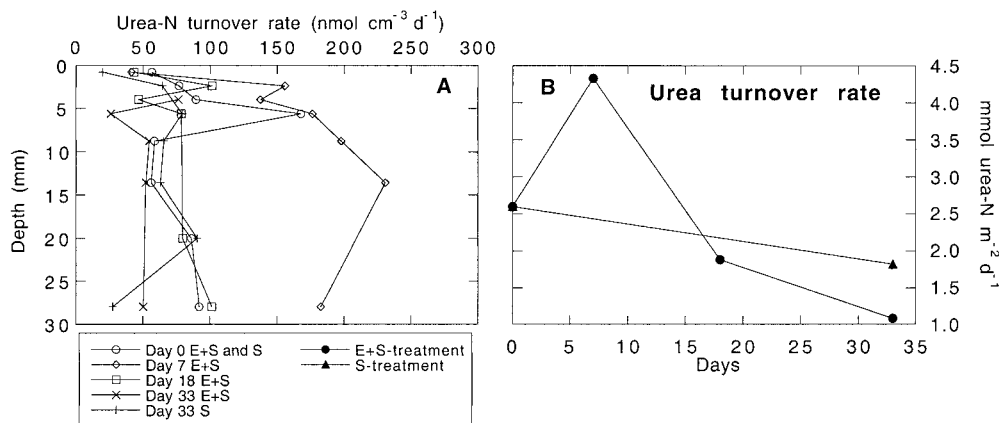


Fig. 8. Depth profiles of urea-N turnover rates (A) on days 0, 7, 18, and 33 in the E+S treatment and days 0 and 33 in the S treatment. Sediment area integrated pools ($\Sigma 0$ –32 mm) of urea-N turnover (B) in the E+S treatment and the S treatment.

after eelgrass addition from 6 to 70 $\mu\text{mol m}^{-2}$ and from 60 to 170 $\mu\text{mol m}^{-2}$, respectively (Fig. 7D,F). After day 7 the integrated DFAA-N and urea-N pools decreased in the E+S treatment to approximate the integrated DFAA-N and urea-N pools in the S treatment at day 33 (Fig. 7D,F).

There was a gradual increase in the NH_4^+ pool with depth in the sediment in both treatments with the exception of day 7 in the E+S treatment (Fig. 7G). At day 7 after eelgrass addition, the concentration of NH_4^+ was increased to 122 nmol cm^{-3} within the upper 1.6 mm of the sediment compared to 14 nmol cm^{-3} at day 0. The NH_4^+ pool was also elevated at all other depths compared to day 0 (Fig. 7G). The area-integrated NH_4^+ pool increased from 3.9 to 5.4 mmol m^{-2} during the first 7 d after the eelgrass addition, after which it decreased to 2.1 mmol m^{-2} at day 33 (Fig. 7H). The area-integrated NH_4^+ pool in the S treatment decreased from 3.9 to 2.0 mmol m^{-2} during 33 d of incubation (Fig. 7H).

Sediment urea-N turnover—The turnover rate of urea-N was stimulated at all sediment depths on day 7 after eelgrass addition (Fig. 8A). On this day there was a gradual increase in the urea-N turnover rate from 41 $\text{nmol urea-N cm}^{-3} \text{d}^{-1}$ within the upper 1.6 mm of the sediment to 231 $\text{nmol urea-N cm}^{-3} \text{d}^{-1}$ in the 11.2–16.0-mm depth strata. At all other dates, the urea turnover rate varied with depth within the range from 20 to 168 $\text{nmol urea-N cm}^{-3} \text{d}^{-1}$ (Fig. 8A). The area-integrated urea turnover rate increased after eelgrass addition from 2.6 $\text{mmol urea-N m}^{-2} \text{d}^{-1}$ at day 0 to 4.4 $\text{mmol urea-N m}^{-2} \text{d}^{-1}$ 7 d after eelgrass addition. After day 7 there was a decrease in the urea-N turnover rate to 1.1 $\text{mmol m}^{-2} \text{d}^{-1}$ on day 33 (Fig. 8B). The area-integrated urea turnover rate decreased from 2.6 to 1.8 $\text{mmol m}^{-2} \text{d}^{-1}$ during the 33 d of incubation in the S treatment (Fig. 8B).

Discussion

Composition and the C:N ratio in efflux solutes from the sediment—There was a rapid response in the efflux of DOC and DON after the addition of eelgrass leaves to the sedi-

ment surface. The stimulated efflux of DON during the first few days after the addition of organic matter to the sediment surface is in accordance with the findings of Enoksson (1993) and Sloth et al. (1995) after the addition of diatom cells and yeast cells to the sediment surface, respectively. Enoksson (1993) suggested that the DON released might have consisted partially of autolysis products.

During the course of incubation 24.3% of the DON efflux was identified as THAA-N, DFAA-N, and urea in the E+S treatment, whereas 33.8% of the DON efflux was identified as these compounds in the control chambers. THAA-N and urea-N were the most important components of the identified DON efflux and accounted for 14.4 and 9.9% of the total DON efflux, respectively, in the E+S treatment. THAA, DFAA, and urea only comprised a minor fraction of the DOC efflux in the S treatment (<5%). Although THAA was measured on unfiltered in- and outflow water from the flow system, there was strong evidence that THAA was a component of the DON pool. DON was measured on selected unfiltered samples collected during the first 7 d after eelgrass addition, where the DON efflux was highest. However, there was no difference between the DON concentrations in filtered and unfiltered samples.

The unidentified DOM efflux was likely to have been composed of N-free DOM molecules together with nitrogen-containing organic molecules. Among the N-free DOM molecules that may have been of importance were polysaccharides, sugars, lipids, and fatty acids. The nitrogen-containing organic molecules that were not identified was likely to have been DNA, RNA, aliphatic amines, and amino sugars as these compounds have been identified in sediment porewater and seawater (Antia et al. 1991). Further, the unidentified nitrogen-containing organic molecules could have been products of hydrolysis and intermediates in nitrogen mineralization.

The average molar DOC: DON ratio in the DOM efflux from the sediment decreased from 23.1 during the first 7 d of incubation to 2.1 during the last 2 weeks of incubation in the E+S treatment (Table 2). The average DOC: DON ratio was 11.6 in the control chambers. The elevated DOC: DON

Table 2. Ratios between carbon and nitrogen efflux components in the E+S treatments during three periods after eelgrass addition and in the S treatment during the 33 d of incubation. The DIN efflux was the efflux of $\text{NH}_4^+ + \text{NO}_3^- + \text{N}_2$. It was assumed that the N_2 efflux balanced the NO_3^- efflux.

Treatment	Period (d)	DOC:DON	ΣCO_2 :DIN	(DOC + ΣCO_2):(DON + DIN)
E+S	0–7	23.1	52.1	33.5
E+S	7–19	15.9	44.4	38.3
E+S	19–33	2.1	155.8	57.7
S	0–33	11.6	54.5	44.2

efflux ratio during the first week after eelgrass addition may have been due to leaching of plant storage compounds after autolysis of the cells. As leaching can be assumed to be a temporary phenomenon, it is hypothesized that hydrolysis and mineralization products gradually became more important components of the DOM efflux with time. The low DOC:DON efflux ratio by the end of the E+S incubation supports this hypothesis. However, a DOC:DON efflux ratio of 2 demands that the unidentified components of the DOM efflux were composed of DON compounds with a low C:N ratio such as RNA and amines. A low DOC:DON efflux could be due to accumulation of carbon-rich DOM in the sediment as proposed by Burdige and Zheng (1998).

There was a gradual increase in carbon oxidation and the NH_4^+ efflux during the first 4–5 d after eelgrass addition that was suggestive of an increased bacterial mineralization activity. The net increase in eelgrass THAA-N (6 mmol-N m^{-2}) during the first week of incubation may indicate bacterial colonization. The resultant net increase in bacterial cell number caused by the increase in leaf THAA-N was estimated from the nitrogen content in bacterial cells. Fagerbakke et al. (1996) reports that the nitrogen content in marine bacteria fall in the range from 1.6 to 5.0 fg cell^{-1} and we assume that protein (THAA) is the most important organic nitrogen compound in bacteria cells. The increase in leaf THAA_{eel} was equivalent to an increase in bacterial cell number of 9.3×10^{10} – $2.9 \times 10^{11} \text{ cells g dry weight}^{-1}$ eelgrass leaf. In comparison Blum and Mills (1991) found an increase of $1.5 \times 10^{10} \text{ cells g dry weight}^{-1}$ leaf material during the first week of *Z. marina* leaf decomposition.

Opposed to the stimulated NH_4^+ efflux after eelgrass addition there was a decrease in the efflux of NO_3^- that could be explained by a reduction in O_2 penetration from 2 mm in the S treatment to <1 mm in the E+S treatment. Denitrification was possibly also reduced after eelgrass addition as the major source of NO_3^- for denitrification was from sediment nitrification. There was no NO_3^- in the inflow water to the continuous flowthrough system. Thus, denitrification was based solely on NO_3^- from sediment nitrification, and it could be anticipated that the upward flux (efflux) of NO_3^- was equal to the downward flux of NO_3^- (denitrification). This has previously been shown in a seasonal study from the same location (Jørgensen 1996). Further, an enhanced bacterial production in the sediment can lower the efflux of

inorganic nitrogen compounds after a sedimentation event (Van Duyl et al. 1993).

The C:N ratio in the mineralization products (ΣCO_2 :DIN) that effluxed the sediment (i.e., $\Sigma\text{CO}_2/[\text{NH}_4^+ + 2 \text{NO}_3^-]$) remained high ($>44 \text{ mol mol}^{-1}$) and almost unchanged during the first 19 d after leaf addition (Table 2). The high ΣCO_2 :DIN ratios fall in the range of in situ measurements reported by Blackburn et al. (1996) from Arctic sediments (40–135) and from a Danish coastal sediment (64; Lomstein et al. 1998). As stated by Fenchel et al. (1998), the C:N ratio in the organic matter degraded is often inferred from the ΣCO_2 :DIN ratio in efflux mineralization products. This assumption is only valid if it can be assumed that the bacterial biomass remains in steady state. However, we hypothesize that the addition of eelgrass leaves stimulated bacterial growth, as indicated by the increase in THAA_{eel} during the first 7 d of incubation. Bacterial growth demands nitrogen incorporation into essential cell components with a resultant relatively high ΣCO_2 :DIN efflux. Consequently, the mineralization was through a closed cycle of alternate organic nitrogen degradation and resynthesis, driven by carbon oxidation as suggested by Lomstein et al. (1989, 1998).

Indications on stimulated bacterial growth in the sediment after eelgrass addition—There was a continuous net increase in THAA_s throughout the sediment after eelgrass addition (Fig. 4F), whereas there was a slight decrease in the POC and PON pools with time (Fig. 4B,D). The net increase in THAA_s (237 mmol m^{-2}) could not be explained by the loss of THAA_{eel} (21 mmol m^{-2}) during the 33 d of incubation. Thus, the net increase in THAA_s could only be explained by a net increase in bacterial biomass and/or microbial protein production. A net synthesis of THAA_s required that non- THAA_s -PON was mineralized to NH_4^+ and incorporated into THAA_s . We are fully aware that DFAA was a potential source for THAA_s production, but DFAA incorporation into THAA_s would not have led to any net increase in THAA_s , as DFAA was part of the measured THAA_s pool.

Further discussion will be related to Fig. 9A–D in which the average area integrated measured and estimated carbon and nitrogen transformation rates (in $\text{mmol m}^{-2} \text{ d}^{-1}$) can be seen in relation to each other in the entire 33-d incubation period in the E+S treatment and the S treatment. The average integrated urea turnover rate always exceeded the efflux of DIN, which further indicated that there was another sink for NH_4^+ than the measured efflux of DIN. The only possible sink for NH_4^+ was bacterial incorporation, as the change in the NH_4^+ pool could not balance the difference between urea turnover and the DIN efflux. It is not likely that urea was the only source for NH_4^+ production in the sediment, as it has been shown that the turnover of urea and DFAA are independent processes that both lead to NH_4^+ production (Therkildsen et al. 1996; Lomstein et al. 1998). In addition, it can be anticipated that the direct deamination of nucleic acids, especially RNA, may contribute to NH_4^+ production as suggested by Lomstein et al. (1998). Thus, the net increase in THAA_s with time was used as a minimum estimate of bacterial NH_4^+ incorporation (*i*, Table 3) in the E+S treatment, as this allowed other sources than urea for NH_4^+ production.

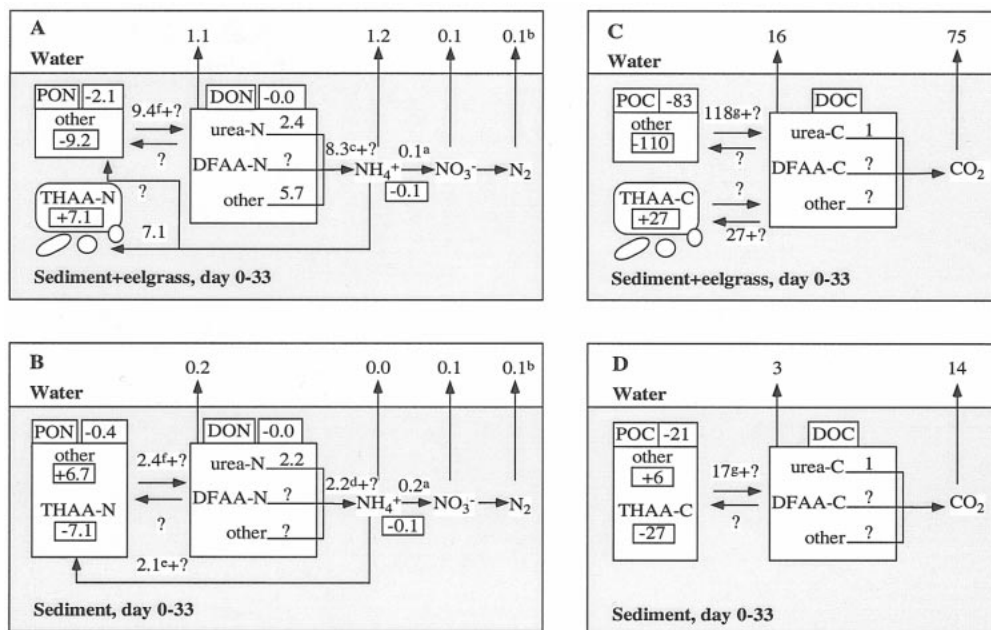


Fig. 9. Conceptual model of the average nitrogen (A,B) and carbon (C,D) transformation rates in the E+S treatment and S treatment, respectively, during the 33 d of incubation. All rates are in $\text{mmol m}^{-2} \text{d}^{-1}$. The efflux of DOC, DON, NH_4^+ , NO_3^- , N_2 , and ΣCO_2 are shown as arrows directed from the sediment to the water column. We assumed that the nitrification rate (a) was twice the net efflux of nitrate, as the probability for an upward and a downward diffusion of NO_3^- was considered equal (b). The numbers in the small bordered boxes are the measured pool changes in eelgrass leaves + the sediment ($\Sigma 0-32$ mm). The numbers in the open boxes are process rates between the sediment pools. The NH_4^+ -mineralization (c) in the E+S treatment was calculated as minimum NH_4^+ incorporation + NH_4^+ efflux + net change in the NH_4^+ pool + nitrification, where the minimum NH_4^+ incorporation was the increase in THAA_s. The NH_4^+ mineralization rate (d) in the S treatment was calculated as the urea turnover. The NH_4^+ incorporation (e) in the S treatment was calculated as NH_4^+ mineralization - (net change of NH_4^+ pool + DIN efflux). The minimum hydrolysis of PON (f) was calculated as NH_4^+ mineralization + DON efflux. The minimum POC hydrolysis (g) was calculated as DOC efflux + ΣCO_2 efflux + carbon incorporation.

Our use of the THAA_s increase with time as a measure of net bacterial growth implies that there was a stimulation of bacterial growth or protein synthesis down to a depth of 3.2 cm in the sediment. In agreement with this, Graf (1987) showed that benthic biomass and metabolism were stimulated down to at least 7 cm after the addition of diatoms to the sediment surface. Benthic biomass was measured as ATP and metabolism as heat production. In addition, diffusion estimates show that diffusion can easily account for high activities at a depth of 3–4 cm within 1 week. This estimate was based on the Carslaw and Jaeger (1959) formulation and a diffusion coefficient of $5 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ (Blackburn and Blackburn 1993). Meiofauna may also have enhanced the transport rates, because they could pass through the 0.5-mm sieve.

The increase in bacterial cell number during the 33-d period after eelgrass addition could be estimated from the THAA_s-N increase and the nitrogen content in natural aquatic bacterial cells that varies from 1.6 to 5.0 fg N cell⁻¹ (Fagerbakke et al. 1996). We assumed that protein was the dominant nitrogen-containing organic molecule in bacterial cells. Thus, the net increase in the cell number fell in the range from 2.1×10^{10} to 6.4×10^{10} cells cm^{-3} during the

33 d of incubation. The average daily increase in bacterial cell number within the three periods investigated ranged from 3.4×10^8 to 4.0×10^9 cell cm^{-3} . In comparison, the daily bacterial production in the upper layer of the sediment in an open bay at the island Groote Eylandt, Australia, was within the range from 1.0×10^9 to 3.4×10^{10} cell cm^{-3} (Pollard and Moriarty 1991). The Australian bacterial production rates were converted from bacterial carbon productivity by the mean carbon content in marine bacterial cells given by Fagerbakke et al. (1996).

The total volume of bacterial cells by the end of the E+S incubation was estimated from the mean cell volumes of natural bacteria that are in the range from 0.11 to 0.41 μm^3 (Fagerbakke et al. 1996). The increase in bacterial cells occupied 0.3–3.1% of the pore volume. Further, we assume that the initial cell number did not exceed 6.41×10^{10} cells cm^{-3} , which allows us to conclude that the increase in cell number in the E+S treatment was realistic compared to the available pore volume. Further, as bacterial NH_4^+ incorporation decreased with time of incubation, this indicated that bacterial growth would have ceased if the experimental period had been extended.

The change in the molar composition of individual amino

Table 3. Summary of carbon oxidation (C_o), gross NH_4^+ mineralization (d), NH_4^+ incorporation (i), carbon incorporation efficiency (E) and the C:N ratio in the substrate degraded (C:N_{sub}) during three periods of the E+S treatment and during the 33 d of incubation in the E+S and S treatment. The numbers in brackets are the estimates based on an assumed NH_4^+ -mineralization that was twice the urea-N turnover in the S treatment (see text for further explanation).

Treatment	Period (d)	C_o (mmol C $\text{m}^{-2} \text{d}^{-1}$)		d (mmol N $\text{m}^{-2} \text{d}^{-1}$)	i (mmol N $\text{m}^{-2} \text{d}^{-1}$)	E^*	$\text{C:N}_{\text{sub}}^\dagger$ (mol mol^{-1})
		C	d				
E+S	0–7	60	16.3	14.7	0.55	8.2	
E+S	7–19	75	8.0	6.4	0.30	13.4	
E+S	19–33	84	4.3	3.9	0.19	24.1	
E+S	0–33	75	8.3	7.1	0.32	13.4	
S	0–33	14	2.2	2.0	0.41	10.8	
(S)	(0–33)	(14)	(4.2)	(4.4)	(0.60)	(7.9)	

* Estimated according to Blackburn (1986) from the formula $E = i / [(C_o N_c) + i]$, where C_o was the measured rate of carbon oxidation, i was NH_4^+ incorporation and N_c was the N:C ratio in the bacterial cells according to Fagerbakke et al. (1996).

† Estimated according to Blackburn (1980) as $1/N_s$ from the formula $N_s = EN_c d / i$, where N_s was the N:C ratio in the substrate degraded and E , N_c , d , and i were as described above.

acids in THAA_s with sediment depth, and, hence, longer time scales, has been attributed to differences in reactivity of individual amino acids (Cowie and Hedges 1992; Cowie et al. 1992; Dauwe and Middelburg 1998). It is generally found that amino acids such as glycine, threonine, serine, and arginine become relatively enriched, whereas isoleucine, leucine, and glutamate become depleted when the organic matter becomes more degraded (e.g., Dauwe and Middelburg 1998). During the relatively short time scale over which degradation was followed in the present study, we did not observe these trends in individual amino acids of the THAA_s pool. On the contrary, there was a marked decrease in the mol% of glycine + threonine with time of incubation in both the E+S and the S treatment, whereas there was an increase in glutamate with time. The observed trend in the E+S treatment may have been due to the generation of microbial biomass. There was an increase in the mol% of taurine and unidentified others with time of incubation in the S treatment. It is likely that the unidentified others were nonprotein amino acids produced as transient intermediates in the mineralization of certain protein amino acids and other biologically produced nitrogen compounds (Henrichs and Farrington 1987; Burdige and Martens 1990). The change in the mol% of individual amino acids in the uppermost few millimeters of the sediment in the E+S treatment did not reflect the mol% of individual amino acids in the added eelgrass leaves (data not shown). In agreement with this, Cowie and Hedges (1992) were not able to provide any source-specific information from the amino acid composition in sediment from Dabob Bay, Washington, USA.

Bacterial carbon incorporation efficiencies and the C:N ratios in the organic matter degraded—The carbon incor-

poration efficiency (E) was estimated from the Blackburn (1986) nonsteady-state formulation on the relationship between carbon oxidation (C_o) and NH_4^+ incorporation into bacterial cells (i):

$$E = i / [(C_o N_c) + i] \quad (1)$$

where N_c was the N:C ratio in bacterial cells (mean of 0.2 mol-mol⁻¹; Fagerbakke et al. 1996). As described above, there were several independent indications on nonsteady-state conditions in the E+S treatment. The S treatment was also assumed to be in nonsteady state as ecosystems are not usually in a steady state and net synthesis or breakdown of microbial cells can take place (Fenchel et al. 1998). The estimated E -values are given in Table 3. E decreased from a mean of 0.55 during the first 7 d after eelgrass addition to 0.19 in the period from days 19 to 33, whereas E on average was 0.41 in the S treatment. The relatively high E -values during the first 7 d after eelgrass addition compared to days 7–19 and days 19–33 may reflect that a greater proportion of carbon degradation was by aerobic microorganism than later in the experiment. Oxygen penetrated deeper in the S treatment (2 mm) than in the E+S treatment (<1 mm 25 d after eelgrass addition). Thus, it is likely that aerobic carbon degradation was more important during the first 7 d of the E+S treatment and in the S treatment than during the later part of the E+S incubation. The estimated E -values were surprisingly high compared to E -values obtained from pure cultures of anaerobic bacteria. Dependent on the organism and the substrates used, E can vary between 0.05 and 0.27 in anaerobic pure cultures (Stouthamer 1979). However, our present knowledge on the physiology of bacteria is based on isolates that represent <1% of the natural population of bacteria and that may not functionally be the most important ones in situ. Aerobic bacteria have much higher E -values than anaerobic bacteria. For instance, Goldman and Dennett (1991) found E -values of natural assemblages of marine aerobic bacteria to vary between 0.36 and 0.65 dependent on the substrates degraded. One should keep in mind that the E -values estimated in the present study are area integrated and thus include both aerobic and anaerobic bacteria. It was possible to estimate E -values from bacterial production rates and oxygen consumption in a mesocosm study mimicking seasonal development of microbial variables in North Sea sediments (Van Duyl et al. 1993). In this study the E -value increased from 0.33 before the addition of *Phaeocystis pouchetii* to a silty sediment (similar to the sediment in the present study) to 0.46 after the addition. Further, our E -values are within the range of previously obtained data on carbon incorporation efficiencies (0.06–0.67) obtained within the upper 4 cm of the sediment in a study on sediment nitrogen cycling in different types of sediments from Danish waters (Blackburn and Henriksen 1983).

The C:N ratio in the substrate degraded ($1/N_s$) was estimated from the Blackburn (1980) formulation based on the carbon incorporation efficiency (E), NH_4^+ incorporation into the microbial biomass (i), gross NH_4^+ mineralization (d), and the N:C ratio in bacterial cells (N_c):

$$N_s = EN_c d / i \quad (2)$$

A minimum estimate of d was the sum of the increase in THAA_s with time, the DIN efflux and the net change in the NH₄⁺ pool during the three periods encountered in the E+S treatment. The minimum d in the S treatment was the urea-N turnover rate. The C:N ratio in the substrate degraded (C:N_{sub}) increased from a mean of 8.2 during the first 7 d after eelgrass addition to 24.1 in the period from days 19 to 33 (Table 3). The C:N_{sub} was 10.8 in the S treatment. The increase in the substrate C:N ratio with time after eelgrass addition is in agreement with the general expectation of a preferential nitrogen mineralization of organic matter, where the most labile and nutrient-rich components are mineralized first. However, there are several indications of the source of energy and nitrogen for bacterial growth in the present data set. The energy source was most likely DOC produced from the added eelgrass leaves, whereas most of the NH₄⁺ incorporated into the bacterial biomass was mobilized from the unidentified sediment PON pool as discussed above. Independent measurements of the POC loss from eelgrass leaves and carbon oxidation + carbon incorporation showed that the POC loss from eelgrass leaves potentially could have supplied 63% of the carbon degraded in the sediment during the 33 d of incubation. Contrary to this, it was only 20% of the gross NH₄⁺ mineralization in the sediment that could have been supplied from the added eelgrass leaves. The stimulated mineralization of sediment organic nitrogen due to the addition of eelgrass leaves is reflected in the C:N ratio of the unidentified POM that increased from 19.4 to 21.5 during the 33 d of incubation, which indicated a preferential N-mineralization. In accordance with the present study, Boetius and Lochte (1996) found that the addition of glucose to Arctic deep-sea sediment increased bacterial growth, and it was suggested that the bacteria had sequestered organic or inorganic sedimentary nitrogen sources to synthesize amino acids. The decrease in the C:N ratio of the unidentified POM pool from 19.4 to 17.2 in the S treatment was due to a net conversion of THAA_s into more nitrogen-rich unidentified PON components than the ones that were present in the beginning of the experiment. A possible explanation for this phenomenon could be that there was a net breakdown of bacterial THAA into other nitrogen-containing organic molecules.

The estimates of the carbon incorporation efficiency (E) and the substrate C:N ratio in the S treatment was based on the assumption that the gross NH₄⁺ production (d) was equal to the measured urea turnover rate. Alternative estimates of nitrogen incorporation into bacterial cells (i), carbon incorporation efficiency (E), and the C:N_{sub} were made based on an assumed NH₄⁺ production from other DON components than urea that balanced urea turnover. Besides urea, DFAAs are another major source for NH₄⁺ production in marine sediments. However, the relative importance of urea for the total urea + DFAA turnover is variable. Lomstein et al. (1998) found that urea turnover accounted for 27% of the total urea + DFAA turnover in the shallow Knebel Vig sediment, Denmark, and Lomstein et al. (unpubl. data) found that urea turnover accounted for 60% and 99% of the urea + DFAA turnover in a *Z. marina* vegetated sediment in April and August, respectively. The alternative estimates of E and C:N_{sub} in the S treatment are presented

in Table 3 together with the data discussed above. If gross NH₄⁺ mineralization was underestimated by a factor of 2, this would result in an increase in E from 0.41 to 0.60 and a decrease in the substrate C:N ratio from 10.8 to 7.9. However, a doubling of the gross NH₄⁺ mineralization did not alter the overall conclusion in the present study. The mineralization was through a closed cycle of alternate organic nitrogen degradation and resynthesis, driven by carbon oxidation.

In conclusion, there were indications of different mineralization scenarios in sediment enriched with eelgrass leaves compared to unamended sediment. Most of the carbon oxidation in the eelgrass-amended sediment was based on carbon supplied from the added leaves, and this carbon oxidation caused mineralization of indigenous sediment nitrogen. Thus, the supply of energy sources stimulated bacterial growth with a resultant temporary retention of nitrogen within the microbial biomass. Organic carbon and nitrogen mineralized in the unamended sediment were from the indigenous POC and PON pools. The decrease in THAA_s with time and the relatively low C:N ratio in the substrate degraded indicated that part of the organic matter degraded was bacterial cells. The mobilization of indigenous sediment nitrogen after the addition of eelgrass leaves (energy source) can give some explanation to why PON accumulates in marine sediments: the mineralization of indigenous organic nitrogen is partly limited by the availability of energy sources. However, this hypothesis does not exclude other recent suggestions of factors that control carbon preservation such as adsorption of organic matter to mineral grain surfaces (Keil et al. 1994; Mayer 1994).

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