

Nitrogen incorporation and retention by bacteria, algae, and fauna in a subtropical intertidal sediment: An in situ ^{15}N -labeling study

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Abstract

We performed a ^{15}N -labeling study to investigate nitrogen incorporation and retention by the benthic microbial community (bacteria and benthic microalgae) and fauna in the intertidal sediment of the subtropical Australian Brunswick Estuary. The main experiment involved an in situ ^{15}N pulse-chase experiment. After injection of $^{15}\text{NH}_4^+$ into the sediment, ^{15}N was traced into bulk sediment, total hydrolyzable amino acids (THAAs, representing bulk proteinaceous biomass), the bacterial biomarker D-alanine, and fauna over a 30-d period. Additional experiments included short-term (24 h) incubations of sediment cores injected with different ^{15}N -labeled substrates (NH_4^+ , NO_3^- , urea, and an amino acid mixture) and sediment core incubations for analysis of benthic fluxes of O_2 , dissolved inorganic carbon, NH_4^+ , NO_x^- , dissolved organic nitrogen, and N_2 . ^{15}N was rapidly incorporated and strongly retained in microbial biomass (THAAs) during the 30-d period in situ, indicating efficient recycling of ^{15}N by the benthic microbial community. Analysis of ^{15}N in D-alanine revealed a major bacterial contribution (50–100%) to total microbial ^{15}N incorporation and retention. ^{15}N was also incorporated into fauna via grazing on ^{15}N -labeled microbial biomass, but this was a negligible fraction (<1%) of total ^{15}N in the sediment. Altogether, results show that efficient recycling of nitrogen by the benthic microbial community can be an important mechanism for nitrogen retention in the sediment and an important pathway supporting benthic microbial production.

Nitrogen is often a limiting element in coastal sediments, which results in strong competition between different groups of microorganisms (bacteria and benthic microalgae [BMA]) that need dissolved nitrogenous substrates to support their growth as well as for catabolic processes such as nitrification and denitrification (e.g., Rysgaard et al. 1995, Risgaard-Petersen 2003, Sundbäck et al. 2004). Moreover, one would expect the benthic microbial community to be very economical with available nitrogen. This is reflected by results from studies on benthic nitrogen

fluxes in different subtropical Australian estuaries (Ferguson et al. 2004, Eyre and Ferguson 2005) as well as other coastal systems (e.g., Lomstein et al. 1998, Anderson et al. 2003, Cook et al. 2004) that suggest a relatively strong retention of nitrogen in the sediment. This retention is thought to be due to incorporation of remineralized nitrogen by BMA or bacteria or both (e.g., Lomstein et al. 1998, Anderson et al. 2003, Ferguson et al. 2004) and possibly by transfer of nitrogen to higher trophic levels (Eyre and Ferguson 2005). However, “traditional” measurements of sediment–water nitrogen fluxes only allow analysis of the net result of all nitrogen cycling processes in the sediment (i.e., the sediment remains a “black box”), meaning that elucidation of the numerous nitrogen transformation processes and nitrogen flows through (micro-)organisms within the sediment requires an alternative approach.

A useful approach is stable isotope labeling with the nitrogen isotope ^{15}N . ^{15}N -labeling has previously been used to measure uptake of nitrogenous substrates by the microbial community in the water column (e.g., Dugdale and Goering 1967, McCarthy and Eppley 1972). However, applications in studies on sediments have so far been limited to studies on nitrogen transformation processes such as nitrification and denitrification (e.g., Rysgaard et al. 1993; Ottosen et al. 2001) or analysis of ^{15}N uptake by

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the whole sediment (e.g., Tobias et al. 2003; Gribsholt et al. 2005). Analysis of ^{15}N incorporation by the total benthic microbial community and by specific groups within the microbial community has long been hampered by methodological problems (see Veuger et al. 2005). Only recently, ^{15}N -labeling has been combined with analysis of ^{15}N incorporation into hydrolyzable amino acids (HAAs). ^{15}N incorporation into total hydrolyzable amino acids (THAAs) provides a measure for ^{15}N incorporation in total microbial biomass, whereas analysis of ^{15}N incorporation into bacteria-specific amino acids makes it possible to trace ^{15}N into bacteria (Tobias et al. 2003, Veuger et al. 2005). In the present study, ^{15}N -labeling was used to elucidate nitrogen flows through bacteria, BMA, and fauna in a subtropical intertidal sediment. Analysis of ^{15}N in HAAs, including the bacterial biomarker D-alanine (D-Ala) (Veuger et al. 2005), was used to investigate ^{15}N incorporation and retention by the total benthic microbial community and the role of bacteria therein.

Materials and methods

Location—The present study was undertaken in the Brunswick Estuary, a small, shallow, subtropical estuary in northern New South Wales, Australia. Together with the smaller Simpsons River, the Brunswick River drains a catchment area of $\sim 220\text{ km}^2$ dominated by woodland and low-intensity agriculture. The estuaries in this area can experience heavy rainfalls and flooding during the summer season (Eyre and Ferguson 2005). A small flood event occurred in the week before the sampling period with a peak of $56\text{ m}^3\text{ d}^{-1}$ ($16\text{ m}^3\text{ d}^{-1}$ gauged). River discharge was low ($8 \pm 4\text{ m}^3\text{ d}^{-1}$) and gradually decreased during the sampling period. The site for the present study was a small ($\leq 5\text{ m}$ wide) intertidal mud bank in the upper estuary, $\sim 50\text{ m}$ downstream from site RW sampled by Ferguson et al. (2003, 2004) and Eyre and Ferguson (2005). The mud bank was bordered by mangrove trees and shrubs, resulting in a substantial amount of higher plant material in the sediment. Average water column salinity at the study site was 8 ± 4 and water temperature during the field work period was $\sim 29^\circ\text{C}$. More details on the Brunswick Estuary and the study site can be found in Ferguson et al. (2003, 2004) and Eyre and Ferguson (2005).

Experimental setup—The whole study consisted of three components. The main component was a $^{15}\text{NH}_4^+$ pulse-chase experiment in two in situ sediment plots in which ^{15}N was traced into various pools up to 30 d after labeling. The second component involved short-term (24 h) sediment core incubations with various ^{15}N -labeled substrates to investigate the use of these substrates as nitrogen sources by the benthic microbial community. The third component involved three series of core incubations for analysis of benthic fluxes. These benthic flux incubations were performed to obtain an overview of total nitrogen cycling in the sediment, which provides a link with previous benthic flux studies in the Brunswick Estuary and thereby places the ^{15}N results in a broader context. The three different components are presented in detail below.

Benthic flux incubations—Three series of benthic flux incubations were performed in three consecutive weeks during the sampling period for the ^{15}N plots (corresponding to sampling days 1, 9, and 16, see below). Each week, three sediment cores (internal diameter 10 cm, $\sim 20\text{ cm}$ deep) were collected from the sediment surrounding the ^{15}N plots. Cores were transported to the laboratory within a few hours and placed in an incubator containing site water at in situ temperature. Each core was stirred at a rate just below the threshold for sediment resuspension by a 20-mm magnet placed 10 cm above the sediment surface driven by a rotating external magnet. After a 24-h pre-equilibrium period, cores were incubated at the daily average in situ light ($400\text{ }\mu\text{mol photons m}^{-2}\text{ s}^{-1}$) and temperature ($29^\circ\text{C} \pm 2^\circ\text{C}$) over a 24-h dark–light cycle (10 h dark and 14 h light). Dissolved oxygen (DO) concentrations and pH were measured electrochemically and samples for analysis of alkalinity and concentrations of NH_4^+ , NO_x^- , total dissolved N (TDN), and N_2 were taken three to four times during the light and dark periods. Alkalinity, NH_4^+ , NO_x^- , and TDN samples were withdrawn with a plastic syringe and withdrawn water was replaced with site water from a gravity-feed reservoir. Samples were filtered ($0.45\text{ }\mu\text{m}$) and immediately frozen (-20°C), except alkalinity samples, which were kept cold (10°C). Concentrations of NH_4^+ , NO_x^- , and TDN were measured colorimetrically using LachatTM flow injection analysis. Total dissolved inorganic nitrogen (DIN) was calculated as $\text{NH}_4^+ + \text{NO}_x^-$ and dissolved organic nitrogen (DON) as $\text{TDN} - \text{DIN}$. N_2 samples were collected in triplicate by allowing water to flow, driven by the reservoir head, directly into 7-mL gastight glass vials with glass stoppers. Vials were allowed to overflow to minimize the introduction of bubbles. N_2 samples were poisoned with $20\text{ }\mu\text{L}$ of 5% HgCl_2 and stored submerged at about $1-2^\circ\text{C}$ below ambient temperature. N_2 concentrations were measured using a modified membrane inlet mass spectrometer with O_2 removal (Eyre et al. 2002). Changes in alkalinity and pH were used to calculate fluxes of dissolved inorganic carbon (DIC). See Eyre and Ferguson (2005) for details on analytical procedures. Fluxes across the sediment–water interface were calculated by linear regression of the concentration data, corrected for the addition of replacement water, as a function of incubation time, core water volume, and surface area. Net flux rates represent the net result of 10 h of dark fluxes and 14 h of light fluxes.

In situ ^{15}N plots—Two $50 \times 50 \times 4\text{-cm}$ aluminum frames were placed side by side on a flat section of the mud bank parallel to the river. Plots were labeled with $^{15}\text{NH}_4^+$ at low tide on 05 February 2005 by injecting a $^{15}\text{NH}_4^+$ solution in the upper 8 cm of the sediment using a 1-mL syringe with stainless steel needle ($0.9 \times 80\text{ mm}$). The syringe was emptied gradually while retracting the needle from the sediment to achieve a uniform depth distribution of the label. Per plot, 361 injections with 0.5 mL of $^{15}\text{NH}_4^+$ solution were made (one injection per 6.3 cm^2). The $^{15}\text{NH}_4^+$ solution contained 0.6 g of $(^{15}\text{NH}_4)_2\text{SO}_4$ ($\geq 98\%$ ^{15}N) dissolved in 400 mL of Milli-Q water and 100 mL of seawater from the mouth of the estuary (yielding a salinity

similar to that of the river water at the study site). The $^{15}\text{NH}_4^+$ concentration in the label solution was 18 mmol L^{-1} , which resulted in a pore-water $^{15}\text{NH}_4^+$ concentration of 320 $\mu\text{mol L}^{-1}$. During label injection and sampling, wooden walking boards were used to reduce disturbance of the surrounding sediment. Plots were sampled at low tide at 1, 2, 4, 9, 16, and 30 d after label injection by collecting 2–3 sediment cores (Ø 5 cm, \sim 10 cm deep) per plot. Resulting holes in the plots were filled with dummy cores containing sediment from outside the plots to prevent physical disturbance of the remaining sediment. Unlabeled control samples were taken from the sediment surrounding the plots. Cores were transported to the laboratory within 2 h after sampling and processed directly or frozen (-20°C) and processed later. Processing involved homogenization of sediment from the 0–8-cm layer of the cores, after which subsamples were taken for analysis of ^{15}N in the bulk sediment and HAAs. Sediment samples were stored frozen (-20°C) until oven drying (48 h at 60°C). At sampling days 2, 9, and 30, remaining sediment was used for extraction of fauna, which were also analyzed for ^{15}N enrichment (see below).

^{15}N core incubations—Small sediment cores (Ø 2.5 cm, 8–12 cm deep) were collected from the sediment surrounding the ^{15}N plots at sampling day 9. Cores were transported to the laboratory within 2 h after sampling and placed in a water bath under the same temperature and light regime as used for the benthic flux incubations. Cores were left to settle overnight (in the dark, keeping the sediment surface submerged). The following day, cores were injected with $^{15}\text{NH}_4^+$, $^{15}\text{NO}_3^-$, ^{15}N -urea, or a ^{15}N -amino acid mixture (Cambridge Isotope Laboratories, NLM-2161, hereafter called “AA-mix”) dissolved in 0.45- μm -filtered site water. All ^{15}N substrates were $\geq 98\%$ ^{15}N . For each substrate, four cores were labeled by three injections of 0.4 mL of substrate solution per core (6 cm deep, using a 1-mL syringe with a 0.9×80 mm stainless steel needle). Injection resulted in addition of $\sim 0.6 \mu\text{mol}$ of ^{15}N in the upper 6 cm of the cores ($\sim 20 \text{ nmol mL}^{-1}$ ^{15}N in wet sediment) for $^{15}\text{NH}_4^+$, $^{15}\text{NO}_3^-$, and the ^{15}N -AA-mix and $\sim 1.2 \mu\text{mol}$ of ^{15}N for ^{15}N -urea. After injection, labeled cores together with a set of unlabeled control cores were incubated in a water bath under the same temperature and light regime as used for the benthic flux incubations. Cores were kept saturated with water by maintaining a small volume of water over the sediment. Incubations were terminated after 6 h and 24 h (two cores per incubation time per substrate) by freezing the 0–6-cm layer (-20°C). Later, samples were defrosted, homogenized, and oven-dried (48 h at 60°C), after which subsamples were analyzed for ^{15}N enrichment of bulk sediment and HAAs (both in duplicate).

^{15}N analyses— ^{15}N in the bulk sediment was measured using a Fisons CN elemental analyzer coupled on-line to a Finnigan Delta S isotope ratio mass spectrometer (EA-IRMS) with a typical reproducibility of $\delta^{15}\text{N}$ within 0.4‰.

^{15}N in HAAs, including D-Ala, was analyzed following the protocol described in Veuger et al. (2005). Briefly, samples (~ 1 g) of freeze-dried sediment were washed with

HCl (2 mol L^{-1}) and Milli-Q water (removing dissolvable and sediment-bound amino acids) and subsequently hydrolyzed in HCl (6 mol L^{-1}) at 110°C for 20 h. After purification by cation exchange chromatography, amino acids were derivatized with isopropanol and pentafluoropropionic anhydride and samples were further purified by solvent extraction. Concentrations and relative abundance of ^{15}N for the derivatized D- and L-amino acids were analyzed by gas chromatography–combustion–isotope ratio mass spectrometry (GC-c-IRMS) on a HP 6890 GC with a Thermo type III combustion interface and Thermo Delta Plus IRMS.

For analysis of ^{15}N in fauna, sediment from the upper 8 cm of two to three cores was sieved over a 500- μm sieve to collect particles $> 500 \mu\text{m}$ (including larger animals). The organic fraction of the $< 500\text{-}\mu\text{m}$ material was separated from inorganic particles by elutriation using water collected at the study site and concentrated on a 63- μm sieve. Animals from the $> 500 \mu\text{m}$ and 63–500- μm fractions were hand picked using a microscope and rinsed with Milli-Q water. Larger animals were transferred to plastic vials (one to five animals per vial), whereas smaller animals (“meio-fauna”) were pooled in tin boats (50–200 individuals for nematodes and 1–10 individuals for other groups). All fauna samples were oven-dried (18 h at 50°C). Before ^{15}N analysis, subsamples from larger animals were transferred to tin boats. ^{15}N in the fauna samples was measured by EA-IRMS as for bulk sediment. The amount of faunal biomass-N used for EA-IRMS analysis was 2–20 μg for small animals and 5–150 μg for larger animals.

Additional analyses—Total organic carbon and nitrogen content of the sediment was measured with the same elemental analyzer as used for bulk ^{15}N analysis after acidification of the samples for removal of carbonates. Separate sediment cores were collected and freeze-dried for analysis of photosynthetic pigments by high-performance liquid chromatography (HPLC) (Barranguet et al. 1997) and phospholipid-derived fatty acids (PLFAs; see Middelburg et al. [2000] and Boschker [2004]).

Data treatment— ^{15}N data are presented as excess nmol of ^{15}N per gram of dry sediment or as $\Delta\delta^{15}\text{N}$ values that were calculated as follows:

$$\begin{aligned} \text{excess } ^{15}\text{N} \text{ (nmol)} &= \\ & \left[(\text{at}\%^{15}\text{N}_{\text{sample}} - \text{at}\%^{15}\text{N}_{\text{control}}) / 100 \right] \times \\ & \left[\text{nmol of N in sample} \right] \end{aligned} \quad (1)$$

$$\begin{aligned} \text{at}\%^{15}\text{N} &= \left[100 \times R_{\text{standard}} \times (\delta^{15}\text{N}_{\text{sample}} / 1000) + 1 \right] / \\ & \left[1 + R_{\text{standard}} \times (\delta^{15}\text{N}_{\text{sample}} / 1000) + 1 \right] \end{aligned} \quad (2)$$

$$\delta^{15}\text{N} (\text{‰}) = \left[(R_{\text{sample}} / R_{\text{standard}}) - 1 \right] 1000 \quad (3)$$

$$\Delta\delta^{15}\text{N}_{\text{sample}} = \left[\delta^{15}\text{N}_{\text{sample}} \right] - \left[\delta^{15}\text{N}_{\text{control(unlabeled)}} \right] \quad (4)$$

where $R = ^{15}\text{N}/^{14}\text{N}$. D-Ala data were corrected for the

formation of D-Ala from L-Ala by hydrolysis-induced racemization as follows:

$$\begin{aligned} \text{excess } ^{15}\text{N in D-Ala} = \\ \left[\text{measured excess } ^{15}\text{N in D-Ala} \right] - \\ \left[0.017 \times \text{excess } ^{15}\text{N in L-Ala} \right], \end{aligned} \quad (5)$$

where 0.017 represents the average fraction of L-Ala that is converted to D-Ala during hydrolysis of microbial biomass under the used hydrolysis conditions (see update of Veuger et al. 2005). The same correction was used for D-Ala concentrations. Excess ^{15}N in D-Ala is also expressed relative to that in L-Ala as excess ^{15}N D/L-Ala ratios that were calculated as:

$$\begin{aligned} \text{excess } ^{15}\text{N D/L-Ala} = \\ \left(\text{excess } ^{15}\text{N in D-Ala} \right) / \left(\text{excess } ^{15}\text{N in L-Ala} \right), \end{aligned} \quad (6)$$

using the measured excess ^{15}N in D-Ala (i.e., not corrected for racemization). Instead of correcting for racemization, the racemization background (D/L-Ala ratio of ~ 0.017) will be indicated graphically. Excess ^{15}N in total microbial biomass was estimated from excess ^{15}N in THAAs using the following equation:

$$\begin{aligned} \text{excess } ^{15}\text{N in total microbial biomass} = \\ \left(\text{excess } ^{15}\text{N in THAAs} \right) X, \end{aligned} \quad (7)$$

where $X = 2$ yields a maximum estimate (corresponding to a THAA content of 50%) and $X = 1.25$ gives a minimum estimate (corresponding to 80%). These values are based on a THAA content of about 50–60% for bacterial biomass (Simon and Azam 1989; Cowie and Hedges 1992) and 60–80% for algal biomass (Cowie and Hedges 1992). The bacterial contribution to total ^{15}N incorporation was estimated from excess ^{15}N D/L-Ala ratios as follows:

$$\begin{aligned} \text{Bacterial contribution (\%)} = \\ \left[\left(\text{excess } ^{15}\text{N D/L-Ala} - 0.017 \right) / \right. \\ \left. \left(\text{bacterial D/L-Ala} - 0.017 \right) \right] \times 100, \end{aligned} \quad (8)$$

where 0.017 represents the racemization background and the bacterial D/L-Ala ratio ranges between 0.05 and ~ 0.1 , with 0.05 giving a maximum estimate and 0.1 giving a minimum estimate (see update Veuger et al. 2005). Interpretation of these results will be clarified graphically.

Results

Sediment properties—Sediment at the study site consisted of fine mud with a high plant litter content. The average nitrogen content of the bulk sediment was $0.24\% \pm 0.02\%$ (weight %) and the organic carbon (OC) content was $3.7\% \pm 0.5\%$. The corresponding mole-based C:N ratio was 19 ± 1.4 . The average concentration of THAAs (summed concentrations of individual HAAs) was $47 \pm 5 \mu\text{mol g}^{-1}$ (5.7 mg g^{-1}). THAA-C was 7.6% of bulk OC

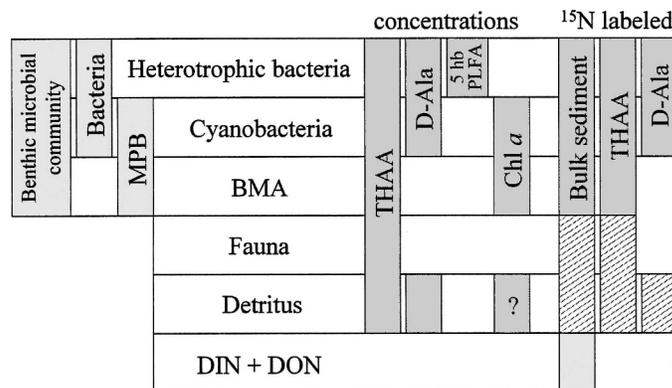


Fig. 1. Schematic overview of benthic nitrogen pools, microbial community composition, and corresponding biogeochemical proxies for the present study. MPB, microphytobenthos; BMA, benthic microalgae; THAAs, total hydrolyzable amino acids; D-Ala, D-alanine, 5 hb PLFAs, five phospholipid-derived fatty acids specific for heterotrophic bacteria; Chl *a*, chlorophyll *a*. Hatched boxes indicate negligible contributions. ? indicates uncertainty about Chl *a* in detritus (see text for details).

and THAA-N was 28% of bulk N. The average D-Ala concentration (corrected for hydrolysis-induced racemization) was $0.40 \pm 0.04 \mu\text{mol g}^{-1}$. The average D/L-Ala ratio (not corrected for racemization) was 0.071 ± 0.005 .

Microbial community—An overview of the different microbial groups in the sediment and their biogeochemical proxies is presented in Fig. 1. HPLC analysis of photosynthetic pigments in the sediment showed high abundance of degradation products such as phaeophytin and phaeophorbide, causing poor peak separation that prohibited precise quantification of most pigments (data not shown). The average chlorophyll *a* (Chl *a*) concentration in the upper 8 cm was $3 \mu\text{g g}^{-1}$, which corresponds to $\sim 250 \text{ mg m}^{-2}$. When assuming a C:Chl *a* ratio of 50, these values represent an estimated photosynthetic biomass of $14 \pm 8 \mu\text{mol g}^{-1} \text{ C}$. The sediment also contained detectable but not quantifiable amounts of other pigments, including fucoxanthin (specific for diatoms) and zeaxanthin (specific for cyanobacteria). Note that estimates of total photosynthetic biomass above include both algal and cyanobacterial biomass (see Fig. 1). Heterotrophic bacterial biomass was estimated from concentrations of the heterotrophic bacteria-specific PLFAs i14:0, i15:0, a15:0, i16:0, and 18:1 ω 7c assuming that these together make up $\sim 28\%$ of total bacterial PLFAs and that PLFA-C makes up $\sim 6\%$ of total bacterial C (see Middelburg et al. 2000 and references therein), which yielded a biomass estimate of $21 \pm 11 \mu\text{mol C g}^{-1} \text{ C}$. Note that this estimate does not include cyanobacteria (see Fig. 1).

Benthic fluxes—Benthic fluxes of DO and especially DIC showed considerable variation between the three series performed in three consecutive weeks (Fig. 2). DO showed a clear influx in the dark and efflux in the light, resulting in a very small average net efflux of DO from the sediment. DIC fluxes in the dark were always directed out of the sediment, with highest rates for week 1. DIC fluxes in the

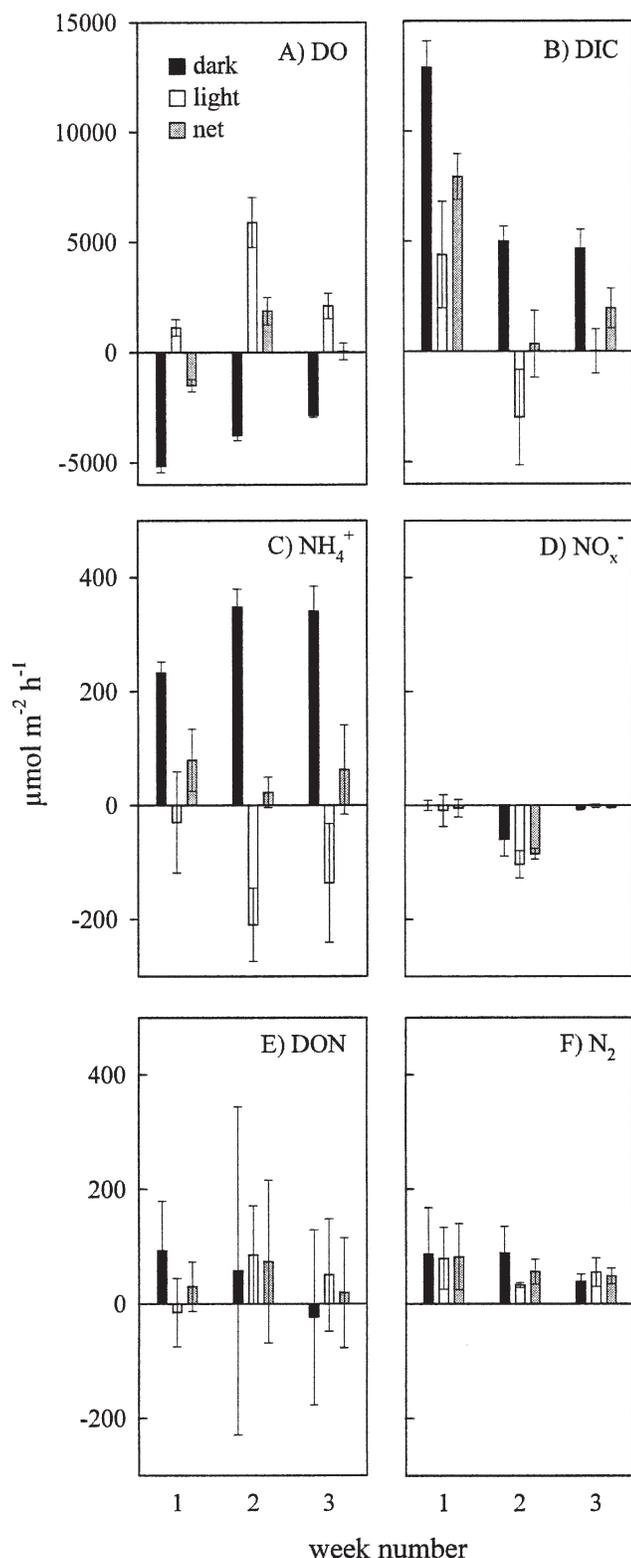


Fig. 2. Benthic flux rates of (A) DO, (B) DIC, (C) NH_4^+ , (D) NO_x^- , (E) DON, and (F) N_2 in the dark, light, and net (on the basis of 10 h of darkness and 14 h of light) for three series performed in three consecutive weeks. Positive values represent effluxes, negative values are influxes. Error bars indicate standard deviation for three replicate cores.

light were very different for all 3 weeks, with a clear efflux in week 1, a clear influx in week 2, and a very small efflux in week 3. For the net DIC fluxes, this resulted in a clear efflux in week 1 and much lower effluxes for weeks 2 and 3.

Nitrogen fluxes showed more consistent results for the three consecutive weeks. NH_4^+ showed high efflux rates in the dark and high influx rates in the light, resulting in a relatively small net efflux. NO_3^- showed a net influx in both light and dark with very low rates for weeks 1 and 3. Fluxes of DON were highly variable with average values showing a net efflux of DON from the sediment. N_2 fluxes showed a consistent release of N_2 from the sediment in the dark as well as in the light.

^{15}N core incubations—Sediment cores showed moderate ^{15}N enrichment of the bulk sediment and HAAs following injection of the ^{15}N -labeled substrates with $\Delta\delta^{15}\text{N}$ values up to $\sim 50\%$. Excess ^{15}N values for the bulk sediment were highest for the cores injected with ^{15}N -urea and lowest for those injected with $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ (Fig. 3A). Although these differences roughly reflect differences in added amounts of ^{15}N , values were relatively low for $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ cores. Bulk excess ^{15}N showed little to no difference between 6 h and 24 h of incubation.

Excess ^{15}N in THAAs (summed excess ^{15}N in individual HAAs, Fig. 3B) showed roughly the same relative differences between the four ^{15}N -substrates as bulk excess ^{15}N , except for relatively low values for ^{15}N -urea cores. Unlike bulk ^{15}N , excess ^{15}N in THAAs did show a difference between 6 h and 24 h, with up to ~ 1.5 times higher values after 24 h. Excess ^{15}N in THAAs was 14–27% of bulk excess ^{15}N after 6 h and 19–35% after 24 h, with lowest values for cores injected with ^{15}N -urea (14–19%) and highest values for those injected with $^{15}\text{NH}_4^+$ (25–29%) and the ^{15}N -AA-mix (27–35%). Application of Eq. 7 to the ^{15}N core results after 24 h yielded estimates of excess ^{15}N in total microbial biomass that were 36–58% of bulk excess ^{15}N for NH_4^+ cores, 38–60% for NO_3^- cores, 24–30% for urea cores, and 44–70% for the AA-mix cores (with minimum values corresponding to $X = 1.25$ and maximum values to $X = 2$).

Trends in excess ^{15}N in D-Ala (Fig. 3C) for the four substrates after 6 h roughly matched those for excess ^{15}N in THAAs (including L-Ala) with a relatively high excess ^{15}N in the cores injected with $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$. However, unlike ^{15}N in THAAs, ^{15}N in D-Ala did not increase between 6 h and 24 h but actually decreased slightly for cores injected with $^{15}\text{NO}_3^-$, ^{15}N -urea, and the ^{15}N -AA-mix. This resulted in a decrease in excess ^{15}N D/L-Ala ratios between 6 h and 24 h (Fig. 3D). After 6 h, average excess ^{15}N D/L-Ala ratios were very similar (0.09–0.11) for all four substrates, whereas ratios were lower after 24 h, with highest values for $^{15}\text{NH}_4^+$ cores (with considerable variation) and lowest values for the ^{15}N -AA-mix cores. Estimated bacterial contributions to total microbial ^{15}N incorporation after 24 h on the basis of the excess ^{15}N D/L-Ala ratios (Eq. 8 and Fig. 3D) were 80–100% for $^{15}\text{NH}_4^+$ cores, 55–100% for cores injected with $^{15}\text{NO}_3^-$, and ^{15}N -urea, and 35–100% for the ^{15}N -AA-mix cores (minimal

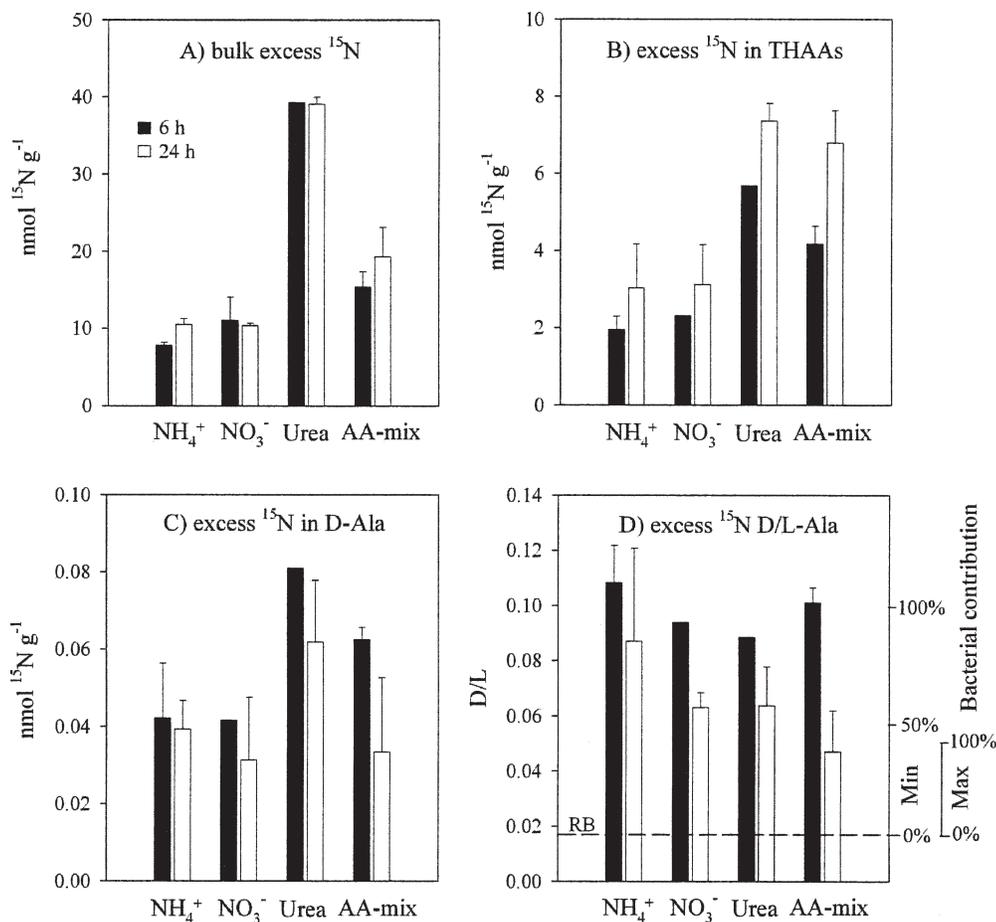


Fig. 3. Excess ^{15}N in (A) bulk sediment, (B) total hydrolyzable amino acids (THAAs), (C) D-Ala (corrected for hydrolysis-induced racemization), and (D) excess ^{15}N D/L-Ala ratio (not corrected for racemization) for 0–6-cm layer from sediment cores incubated with $^{15}\text{NH}_4^+$, $^{15}\text{NO}_3^-$, ^{15}N -urea, or a ^{15}N -AA-mix for 6 h and 24 h. Dashed line in panel D indicates racemization background (RB). Values on second y-axis in panel D indicate minimum and maximum estimate of bacterial contribution to total microbial ^{15}N incorporation (see text for details). Error bars represent standard deviation for two replicate cores. When no error bars are shown, only one sample was analyzed.

and maximal contributions calculated with a bacterial D/L-Ala ratio of 0.1 and 0.05 respectively).

In situ ^{15}N plots: ^{15}N in bulk sediment—Injection of $^{15}\text{NH}_4^+$ into the sediment plots resulted in ^{15}N enrichment of the bulk sediment well above natural abundance, with $\Delta\delta^{15}\text{N}$ values up to 150‰. Bulk excess ^{15}N was highest 1 d after injection ($\sim 86 \text{ nmol g}^{-1} \text{ }^{15}\text{N}$) (Fig. 4A), which corresponds to $\sim 50\%$ of the total amount of injected ^{15}N . Bulk excess ^{15}N decreased over the 30-d sampling period to $\sim 40 \text{ nmol g}^{-1}$ with a relatively rapid decrease during the first 4 d.

In situ ^{15}N plots: ^{15}N in HAAs—HAAs were also highly enriched in ^{15}N ($\Delta\delta^{15}\text{N}$ 50–200‰). Excess ^{15}N in THAAs was $17 \pm 2 \text{ nmol g}^{-1}$ and, contrary to bulk excess ^{15}N , did not decrease during the 30-d sampling period (i.e., no net loss of ^{15}N from THAAs) (Fig. 4A). At day 1, excess ^{15}N in THAAs was only $17\% \pm 1\%$ of bulk excess ^{15}N , but this

fraction increased to $43\% \pm 1\%$ at day 30. Corresponding estimates of excess ^{15}N in total microbial biomass were calculated with Eq. 7 using $X = 2$ since ^{15}N incorporation was dominated by bacteria (see discussion) with a THAA content of $\sim 50\%$. These estimates are indicated by the dotted line in Fig. 4A.

Trends for excess ^{15}N in D-Ala over time (Fig. 4B) were very similar to those for the THAAs (including L-Ala). Corresponding excess ^{15}N D/L-Ala ratios (Fig. 4C) were relatively high at day 1 (~ 0.08 , similar to that measured in the cores incubated with $^{15}\text{NH}_4^+$ after 24 h [Fig. 3D]) and decreased during the following week to level off at 0.055–0.060 after day 9. The estimated bacterial contribution to total microbial ^{15}N incorporation (Eq. 8 and Fig. 4C) was 50–100% (corresponding to a D/L-Ala ratio for the active bacterial community of 0.1 and 0.05 respectively).

In situ ^{15}N plots: ^{15}N in fauna—Animals from various faunal groups were extracted from the sediment but

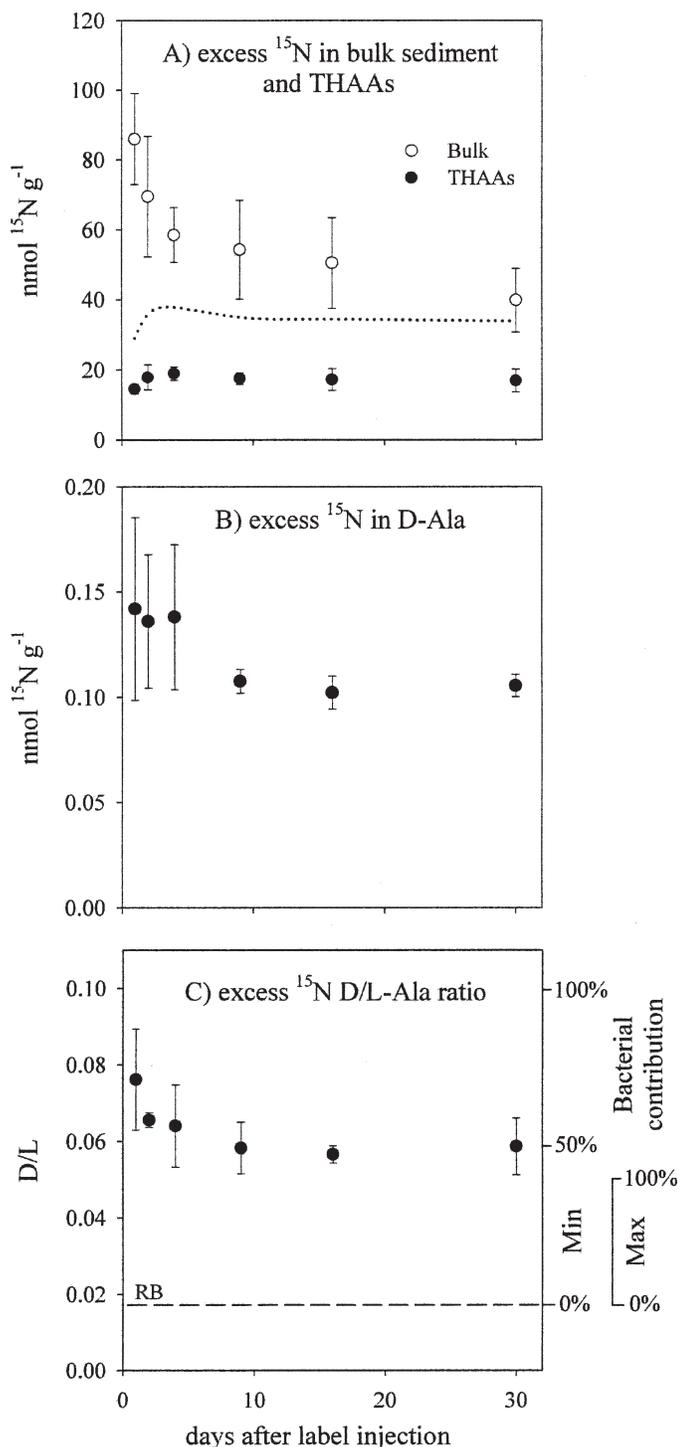


Fig. 4. (A) Excess ^{15}N in bulk sediment and THAAs. (B) Excess ^{15}N in D-Ala (corrected for hydrolysis-induced racemization) and (C) excess ^{15}N D/L-Ala ratios (not corrected for racemization). Dotted line in panel A indicates estimated excess ^{15}N in total microbial biomass (excess ^{15}N in THAAs \times 2). Dashed line in panel C indicates racemization background (RB). Values on second y-axis in panel C indicate minimum and maximum estimate of bacterial contribution to total microbial ^{15}N incorporation. Error bars represent standard deviation for replicate plots.

densities of most groups were low. As a result, part of the fauna samples contained insufficient biomass for ^{15}N analysis while other samples (from different subgroups or sampling days) had to be pooled for proper ^{15}N analysis. The only groups that were abundantly present at all sampling days were nematodes and polychaetes. Nematodes dominated the 63–500- μm fraction with densities of 100–300 individuals per core and showed clear ^{15}N enrichment with highest $\Delta\delta^{15}\text{N}$ values after 9 d and $\Delta\delta^{15}\text{N}$ remaining high up to day 30 (Fig. 5A). The polychaetes *Notomastus estuarius* and *Simplisetia aequisetis* were extracted from the >500- μm fraction at densities of 2–5 individuals per core (~ 30 g wet biomass m^{-2}). *N. estuarius* showed lower $\Delta\delta^{15}\text{N}$ values than those for the nematodes but a similar trend over time (highest ^{15}N -enrichment after 9 d.) (Fig. 5B). $\Delta\delta^{15}\text{N}$ values for *S. aequisetis* (Fig. 5B) were very similar to those for the nematodes. However, the lack of data for day 9 and the substantial variation between samples from day 30 precludes assessment of a clear trend over time. Results for other faunal groups are summarized in Table 1. Although these results are too limited to reveal consistent trends over time, it does show that ^{15}N -enrichment of these animals was similar to that of the nematodes and polychaetes and that they remained well labeled up to 30 d after injection.

To clarify the importance of the ^{15}N transfer to fauna as part of the total ^{15}N budget, a “back of the envelope” calculation of total excess ^{15}N incorporated into faunal biomass was made using measured densities of nematodes and polychaetes and nitrogen contents of 50 ng per individual for nematodes and 100 μg per individual for polychaetes (determined directly from EA-IRMS analyses). Resulting estimates were 0.2–0.5 μmol of ^{15}N per plot in nematodes and ~ 2 μmol of ^{15}N per plot in the polychaetes. Together, these estimates are $\sim 0.2\%$ of total excess ^{15}N in the plots.

To determine the contribution of ^{15}N -labeled microbial biomass to the total nitrogenous diet of the animals from Fig. 5, a simple isotope model (Hamilton et al. 2004) was fitted to reproduce the dynamics of $\Delta\delta^{15}\text{N}$ values for microbial and faunal biomass under the non-steady-state situation in the present experiment. This approach has been described in detail for an in situ ^{13}C -labeling study similar to the present study (van Oevelen et al. 2006b). $\Delta\delta^{15}\text{N}$ values for living microbial biomass were estimated from those for the HAAs by calculating the fraction of THAAs in the whole sediment present in living microbial biomass (derived from biomass estimates of phototrophs [Chl *a*] + heterotrophic bacteria [PLFAs]). This indicated that only $\sim 7\%$ of THAAs were present in living microbial biomass, meaning that $\Delta\delta^{15}\text{N}$ values for HAAs in living biomass were ~ 14 times higher than those for the HAAs in the whole sediment (i.e., $\Delta\delta^{15}\text{N}$ for “living” HAAs was $\sim 1,000$ – $1,800\%$). Comparison of microbial and faunal $\Delta\delta^{15}\text{N}$ values, taking into account their dynamics over time, yielded an estimated dependence on ^{15}N -labeled microbial biomass (likely dominated by bacteria) of $42\% \pm 12\%$ for nematodes, $12\% \pm 4\%$ for *N. estuarius*, and $37\% \pm 12\%$ for *S. aequisetis*.

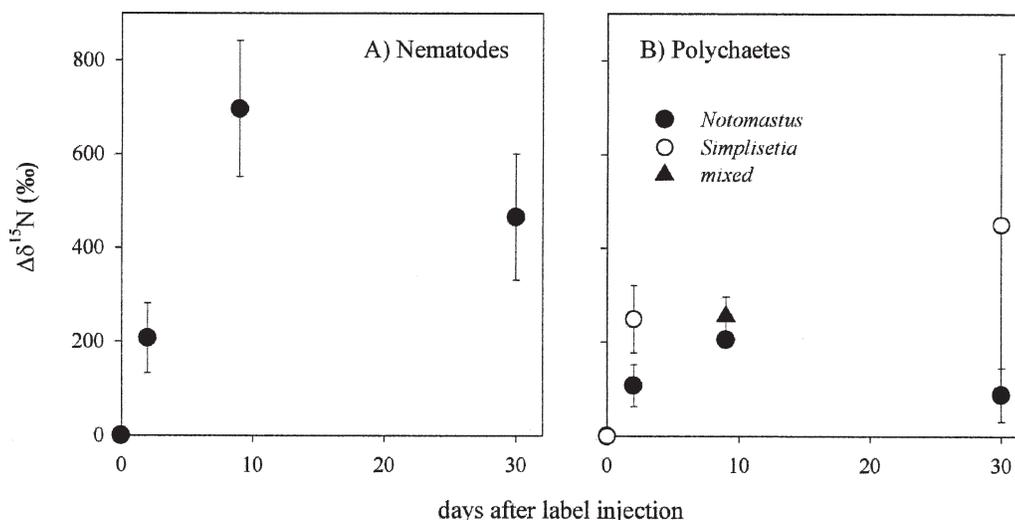


Fig. 5. ^{15}N -enrichment ($\Delta\delta^{15}\text{N}$) of (A) nematodes and (B) polychaetes (*Notomastus estuarius*, *Simplisetia aequisetis*, and a mix of these two). Error bars indicate standard deviation; $n = 6\text{--}8$ for nematodes and $1\text{--}3$ for polychaetes.

Discussion

Benthic fluxes—Benthic fluxes in the Brunswick Estuary and other subtropical Australian estuaries have already been studied and discussed extensively (e.g., Ferguson et al. 2004, Eyre and Ferguson 2005, 2006). Therefore, the present discussion of the benthic fluxes is focused on the general nitrogen cycling picture for comparison with previous studies in the Brunswick Estuary and other systems to place our ^{15}N results in a broader context.

The benthic fluxes of DO and DIC provide information on the overall metabolism of the sediment. The net efflux of DIC from the sediment indicates that the sediment was net heterotrophic (i.e., DIC release from organic matter degradation exceeded DIC consumption for primary production). In addition, the fact that DO fluxes were quite well balanced while DIC fluxes were not suggests that organic matter was partly degraded via anaerobic pathways. These results are consistent with those from more

extensive studies on the benthic metabolism of the Brunswick Estuary (Ferguson et al. 2003, 2004, Eyre and Ferguson 2005). In general, measured nitrogen fluxes (Fig. 2) were comparable with those previously measured for the upper Brunswick Estuary (Eyre and Ferguson 2005). The strong efflux of NH_4^+ in the dark most likely consisted of NH_4^+ produced during organic matter degradation. In the light, this production likely continued, but simultaneous with NH_4^+ uptake, which resulted in a net influx of NH_4^+ in the light. The light dependence of the NH_4^+ uptake indicates that this influx was due to uptake by phototrophs (BMA or cyanobacteria). The influx of NO_3^- in the light as well as the dark indicates steady consumption in the sediment. This may have included uptake of NO_3^- as a nitrogen source for microbial growth (both algae and bacteria) as well as the use of NO_3^- for denitrification ($\text{NO}_3^- \rightarrow \text{N}_2$). The latter is consistent with the continuous efflux of N_2 . Average efflux rates for $\text{N}_2\text{-N}$ were about four times higher than influx rates of $\text{NO}_3^- \text{-N}$,

Table 1. $\delta^{15}\text{N}$ values for fauna not included in Fig. 5 (natural abundance $\delta^{15}\text{N} = 5\text{--}20\text{‰}$). For comparison, $\delta^{15}\text{N}$ values of bulk sediment and THAAs were $60\text{--}160\text{‰}$ and estimated $\delta^{15}\text{N}$ values for living microbial biomass were $\sim 1,000\text{--}1,800\text{‰}$.

Phylum	Class: Order	Family	Genus + species	$\delta^{15}\text{N}$ (‰)			
				Day 2	Day 9	Day 30	Days 2 + 9 + 30 (pooled)
63–500 μm fraction							
Arthropoda	Crustacea: Copepoda	Harpacticoida			110	21–53	
Arthropoda	Crustacea: Ostracoda						194
Annelida	Polychaeta	Nereididae + Spionidae		134			
Annelida	Oligochaeta				178		
Kinorhyncha							36
>500- μm fraction							
Mollusca	Bivalvia	Galleomatidae	<i>Arthritica helmsi</i> (mussel)	991		174	
Annelida	Polychaeta	Eunicidae		38–64			
Arthropoda	Crustacea: Decapoda	Ocypodidae	<i>Macrophthalmus sp.</i> (crab)			261	
Annelida	Polychaeta (mixed)					462	

indicating that denitrification not only used NO_3^- from the water column, but also NO_3^- produced within the sediment by nitrification of NH_4^+ produced during organic matter degradation. The average estimated rate for this coupled nitrification–denitrification on the basis of the discrepancy between fluxes of NO_x^- and N_2 is $94 \mu\text{mol m}^{-2} \text{h}^{-1}$. The net efflux of DON probably involved loss of DON produced during organic matter degradation and possibly grazing of BMA. The strong variation in DON fluxes may partly have been due to the indirect way in which DON fluxes were calculated ($\text{DON} = \text{TDN} - [\text{NH}_4^+ + \text{NO}_3^-]$).

Comparison of effluxes of remineralized nitrogen (net efflux of $\text{DIN} + \text{N}_2$) versus carbon (net DIC efflux) yielded remineralized C:N ratios of 33, 7, and 12 for weeks 1, 2, and 3 respectively. These values are similar to the C:N ratio of 19 for the total organic matter in the bulk sediment (consisting of bacteria [C:N ~5], BMA [C:N ~7–20], bacterial and BMA remnants [C:N higher than that of living organisms], and material from higher plants [C:N 25–500; Cowie and Hedges 1992]), indicating that effluxes of remineralized N and C (resulting from organic matter degradation) were more or less balanced during the sampling period. Combined with the sediment being a net source of dissolved nitrogen to the water column, these balanced fluxes of N and C suggest that the sediment was not as nitrogen limited as it can be in other parts of the estuary and during other times of the year (Ferguson et al. 2004; Eyre and Ferguson 2005). This apparent absence of strong nitrogen limitation may have been due to an increased nitrogen input in the form of phytodetritus from a phytoplankton bloom following the small flood event in the week before the start of the sampling period.

¹⁵N core incubations: total ¹⁵N incorporation—Core incubations with the different ¹⁵N-labeled substrates were performed to investigate the potential importance of DIN (NH_4^+ and NO_3^-) and labile DON (urea and amino acids) as nitrogen sources for the microbial community, focusing on the relative contributions by bacteria versus BMA to total microbial incorporation of the nitrogen from these substrates. It was assumed that ¹⁵N incorporation in these short-term core incubations primarily involved direct incorporation of ¹⁵N from the added ¹⁵N-substrates by the microbial community (bacteria or BMA or both). ¹⁵N incorporation into faunal biomass was considered negligible given that this mainly occurs indirectly via grazing of ¹⁵N-labeled microbial biomass, which causes a delay of more than 24 h (see ¹⁵N plots).

Excess ¹⁵N in the bulk sediment (Fig. 3A) may have included added ¹⁵N-substrates (dissolved in pore water or bound to the sediment) and ¹⁵N incorporated in microbial biomass as well as ¹⁵N-compounds (detritus, DON, and DIN) formed by degradation of ¹⁵N-labeled biomass (see Fig. 1). However, the latter was probably not very relevant for the short-term core incubations. The differences in bulk excess ¹⁵N between cores injected with different ¹⁵N-substrates roughly reflect differences in added amounts of ¹⁵N since two times more ¹⁵N was added to the urea cores than to the other cores. The relatively low bulk excess ¹⁵N

for NO_3^- and NH_4^+ cores (in comparison with values for the AA-mix cores and 50% of those for the urea cores) may have been due to loss of some ¹⁵N₂ from the sediment by nitrification–denitrification. Conversion of the estimated rate of coupled nitrification–denitrification per square meter (see Discussion, benthic fluxes) to a rate per gram of dry sediment for direct comparison with ¹⁵N fluxes yielded a rate of $0.6 \text{ nmol g}^{-1} \text{ h}^{-1}$. This indicates that the loss of ¹⁵N in the cores incubated with NH_4^+ and NO_3^- may indeed have been due to nitrification–denitrification.

The best indication of total ¹⁵N incorporation into benthic microbial biomass is provided by ¹⁵N incorporation into THAAs, as these represent proteinaceous biomass, a substantial fraction of total biomass of all organisms. Although THAAs are also present in detritus, this does not bias short-term ¹⁵N incorporation results since these only deal with the THAA pool in which ¹⁵N was incorporated (i.e., THAAs present in active biomass). The estimates of excess ¹⁵N in total microbial biomass after 24 h (24–70% of bulk excess ¹⁵N) indicate that a substantial fraction of total ¹⁵N had been incorporated into microbial biomass after 24 h. The relatively high values for the AA-mix cores (44–70%) and the relatively low values for the urea cores (24–30%) may suggest a relatively high preference for amino acids and a relatively low preference for urea. However, because of various reasons (such as binding of ¹⁵N-labeled substrates to the sediment) these results do not allow any conclusion concerning microbial preferences for the different substrates. What these results do show is that nitrogen from all four substrates was rapidly incorporated into microbial biomass. This suggests that nitrogen was a limiting element for the benthic microbial community even though benthic fluxes of nitrogen and carbon were more or less balanced (see Discussion, benthic fluxes).

¹⁵N core incubations: ¹⁵N in bacteria—Studies on benthic nitrogen cycling in shallow coastal systems tend to focus on BMA as the main microbial group involved. Since they are concentrated at the sediment surface, BMA can indeed be the main group responsible for uptake of dissolved nitrogen from the overlying water. However, in most marine sediment, including those in the upper Brunswick Estuary, most nitrogen enters the sediment in the form of detritus rather than as dissolved nitrogen. As this detritus is degraded and remineralized within the sediment by bacteria, these bacteria play an important role in benthic nitrogen cycling. In systems like the Brunswick Estuary, nitrogen flows through bacteria may be even more important than in temperate systems since bacterial abundance and activity are relatively high in these (sub)tropical sediments (Alongi 1994).

The importance of ¹⁵N incorporation by bacteria in the present study was investigated by analysis of ¹⁵N incorporation into the bacterial biomarker D-Ala (see Fig. 1). The best indication of the bacterial contribution to total microbial ¹⁵N incorporation is provided by the ratio between excess ¹⁵N in D-Ala versus that in L-Ala, a common protein amino acid present in all organisms (Eq. 6). Excess ¹⁵N D/L-Ala ratios for the ¹⁵N core incubations (Fig. 3D)

were well above the racemization background (i.e., ^{15}N in D-Ala represented ^{15}N incorporated in bacteria) and within the range of D/L-Ala ratios for natural bacterial communities (0.05 to \sim 0.1, *see* update of Veuger et al. 2005). Highest excess ^{15}N D/L-Ala ratios (0.09–0.12) occurred after 6 h and are among the highest values measured in ^{15}N - and ^{13}C -labeling studies so far (Veuger 2006). These high excess ^{15}N D/L-Ala ratios indicate a substantial contribution by Gram-positive (G+) bacteria or cyanobacteria (or both) to total microbial ^{15}N incorporation during the first 6 h of incubation, since both have a relatively high D/L-Ala ratio (related to their cell wall structure). Although estuarine and marine bacterial communities are generally considered to be dominated by Gram-negative (G-) bacteria, G+ bacteria have been shown to contribute up to 30% of the total bacterial community in deeper, anaerobic sediment from a subtropical Australian coastal embayment (Moriarty and Hayward 1982), indicating that they may have been equally abundant in the sampled 0–6-cm layer of our ^{15}N -cores. The presence of cyanobacteria in the studied sediment is also likely given the high temperatures and nitrogen-limited conditions and was confirmed by the presence of the cyanobacterial pigment zeaxanthin. Unfortunately, the quantitative contribution of cyanobacteria to total photosynthetic biomass (and hence to bacterial ^{15}N incorporation) remains unclear since zeaxanthin concentrations could not be quantified properly. The subsequent rapid decrease in excess ^{15}N D/L-Ala ratios between 6 h and 24 h was considerable (Fig. 3D), especially for the cores injected with the ^{15}N -AA-mix, and was the combined effect of a loss of ^{15}N from D-Ala (Fig. 3C) and a continued increase of excess ^{15}N in THAAs, including L-Ala (Fig. 3B). The net loss of ^{15}N from D-Ala between 6 h and 24 h indicates rapid turnover of the bacteria that dominated ^{15}N incorporation during the first 6 h. Such rapid turnover of bacterial biomass is consistent with bacterial turnover times of <1 d reported for (sub)tropical intertidal sediments (Alongi 1994). The continued increase of excess ^{15}N in THAAs between 6 h and 24 h indicates net ^{15}N incorporation into total microbial biomass up to 24 h. Given the net loss of ^{15}N from bacteria with a high D/L-Ala ratio, this implies that this continued ^{15}N incorporation was accounted for by bacteria with a lower D/L-Ala ratio (likely G-; *see* update of Veuger et al. 2005) or BMA (corresponding to a D/L-Ala ratio around racemization background). Moreover, it seems that these groups were characterized by slower ^{15}N incorporation or biomass turnover.

Precise quantification of the bacterial contribution to total microbial ^{15}N incorporation was prohibited by the uncertainty about the different bacterial groups involved (G+, G-, and cyanobacteria) and the associated variation in D/L-Ala ratios. However, the minimum and maximum estimates (calculated from a bacterial D/L-Ala ratio of 0.1 and 0.05 respectively, *see* Fig. 3D) of 35–100% indicate that bacteria played a major role in total microbial ^{15}N incorporation from all four substrates.

In situ ^{15}N plots: Methodological aspects—Labeling of the in situ sediment plots with $^{15}\text{NH}_4^+$ was undertaken to investigate incorporation and retention of nitrogen within

the sediment under in situ conditions, including a potential transfer to fauna. $^{15}\text{NH}_4^+$ was selected as ^{15}N -substrate since NH_4^+ is an abundant form of dissolved nitrogen in the sediment (e.g., Ferguson et al. 2004) and because of its central role in benthic nitrogen cycling and microbial nitrogen uptake (i.e., almost all forms of bioavailable N in the sediment must be converted to NH_4^+ before incorporation into microbial biomass). The $^{15}\text{NH}_4^+$ solution was injected into the sediment (i.e., not sprayed on top of the sediment or added to overlying water) to best mimic the release of dissolved nitrogen during degradation of organic matter within the sediment.

One day after injection of the $^{15}\text{NH}_4^+$, \sim 50% of total added ^{15}N was recovered in the bulk sediment, indicating that the other \sim 50% had been lost from the sediment during the first 24 h. Most of this loss probably involved loss of $^{15}\text{NH}_4^+$ to the overlying water since the plots were submerged within 1 h after injection. Additional losses may have included loss of $^{15}\text{NH}_4^+$ to sediment outside the plots or to sediment below the 8-cm sampling depth as well as loss of $^{15}\text{NO}_x^-$ or $^{15}\text{N}_2$ resulting from nitrification and denitrification (*see* rate estimate in Discussion, ^{15}N cores). However, these initial losses did not interfere with the aim of the study since it only deals with the \sim 50% of total added ^{15}N that was retained in the upper 8 cm of the plots.

Although ambient concentrations of NH_4^+ in the pore water were not measured for the present study, the added $^{15}\text{NH}_4^+$ concentration of \sim 300 $\mu\text{mol L}^{-1}$ was within the range of concentrations of free NH_4^+ (100–500 $\mu\text{mol L}^{-1}$) and freeze-lysable NH_4^+ (100–1000 $\mu\text{mol L}^{-1}$) reported for Brunswick Estuary sediments (Ferguson et al. 2004), whereas total ambient NH_4^+ concentrations (including NH_4^+ bound to the sediment) may have been even higher. Moreover, the sediment also contained other forms of bioavailable nitrogen, including NO_x^- and DON as well as nitrogen in microbial biomass (*see* discussion below), where concentrations of the latter were an order of magnitude higher than the added $^{15}\text{NH}_4^+$ concentration. Therefore, addition of $^{15}\text{NH}_4^+$ did not cause a major increase in total bioavailable nitrogen in the sediment, meaning that measured ^{15}N flows can be considered as representative for ambient nitrogen flows.

In situ ^{15}N plots: Total ^{15}N incorporation—Similar to the ^{15}N -core incubations, ^{15}N in the in situ plots was rapidly incorporated into biomass (represented by THAAs, Fig. 4A). Comparison of the estimated excess ^{15}N in total microbial biomass (dotted line in Fig. 4A) with bulk excess ^{15}N indicates that a large fraction of the ^{15}N in the sediment was present in microbial biomass, especially after day 4, with this fraction increasing over time. The relatively large fraction of bulk excess ^{15}N not present in biomass during the first few days likely comprised added $^{15}\text{NH}_4^+$ dissolved in the pore water or bound to the sediment. Later in the experiment, this fraction may also have included some other ^{15}N -labeled material (detritus, DIN, DON) resulting from degradation of ^{15}N -labeled microbial biomass (*see* Fig. 1 and discussion below).

The most striking point from Fig. 4A is the strong retention of ^{15}N in THAAs (representing total microbial

biomass). Theoretically, this strong retention may have been due to retention in the organisms that were responsible for the initial incorporation of ^{15}N from $^{15}\text{NH}_4^+$. However, given the rapid turnover (hours to days) of bacteria and BMA in (sub)tropical sediments like those in the Brunswick Estuary (Alongi 1994; Ferguson et al. 2004), which likely resulted in rapid loss of ^{15}N from the original ^{15}N -labeled microbial biomass, this was probably not the main cause for the strong retention of ^{15}N in microbial biomass. Another theoretical explanation for the strong retention of ^{15}N in THAAs is accumulation of ^{15}N -labeled detritus (microbial biomass components that escaped or resisted degradation) (see Fig. 1). However, accumulation of a substantial pool of ^{15}N -labeled detritus is unlikely for the present study given the high temperatures and microbial activity that likely resulted in rapid degradation of the majority of dead microbial biomass. This was confirmed by the lack of compositional changes of the ^{15}N -labeled THAA pool during the whole 30-d sampling period (data not shown). A change in the composition of this pool would be expected if accumulation of a substantial pool of labeled microbial detritus occurred (see Veuger et al. 2006). Therefore, the strong retention of ^{15}N in THAAs must primarily have been due to retention of ^{15}N in living microbial biomass by continuous recycling of the ^{15}N (i.e., ^{15}N released during degradation of ^{15}N -labeled biomass [as ^{15}N -DON or ^{15}N -DIN] was rapidly reincorporated into new microbial biomass). The net ^{15}N retention in THAAs may have included continued incorporation of ^{15}N from injected $^{15}\text{NH}_4^+$ bound to the sediment, which compensated for some loss of ^{15}N from THAAs (i.e., excess ^{15}N in THAAs may have decreased after day 30 when all $^{15}\text{NH}_4^+$ had been incorporated). Despite this potential bias, results still show efficient microbial recycling of the ^{15}N and thereby indicate that efficient recycling of nitrogen by the microbial community within the sediment can serve as a nitrogen sink over a period of at least several weeks (without net accumulation of nitrogen) and that recycling of nitrogen within the sediment can be an important pathway supporting benthic microbial production. Although benthic fluxes indicated that there was no net accumulation of nitrogen in the sediment in the present study, our ^{15}N results clearly show the potential of the microbial community as a (temporary) net nitrogen sink in situations of net nitrogen accumulation that regularly occur in the Brunswick Estuary (Ferguson et al. 2004, Eyre and Ferguson 2005) and other coastal sediments (e.g., Lomstein et al. 1998, Anderson et al. 2003, Cook et al. 2004). In addition to microbial nitrogen recycling, the retention of nitrogen in sediments over longer periods of time (months to years) is also governed by loss processes such as resuspension by bioturbation or during flood events (Eyre and Ferguson 2006).

In situ ^{15}N plots: ^{15}N in bacteria—Like in the ^{15}N cores, different microbial groups may have been involved in the incorporation and recycling of ^{15}N in the *in situ* sediment plots, including heterotrophic bacteria, cyanobacteria, and BMA (Fig. 1). Although ^{15}N was also incorporated into benthic fauna (Fig. 5 and Table 1), this was only a

negligible fraction of ^{15}N in total benthic biomass, meaning that ^{15}N incorporation and recycling were primarily microbial processes. A direct indication of the bacterial contribution to total microbial ^{15}N incorporation and recycling is provided by the excess ^{15}N D/L-Ala ratios (Fig. 4C). The high excess ^{15}N D/L-Ala ratio of ~ 0.08 1 d after labeling indicates that total microbial ^{15}N incorporation during the first day was dominated by bacteria, including G+ bacteria and cyanobacteria (see Discussion, ^{15}N cores). The decrease in excess ^{15}N D/L-Ala ratio up to day 9 while excess ^{15}N in total biomass (THAAs) remained stable (Fig. 4A) suggests a net transfer of ^{15}N to bacteria with a lower D/L-Ala ratio or BMA during recycling of the ^{15}N .

The estimated bacterial contribution to total microbial ^{15}N incorporation and recycling of 50–100% (Fig. 4C) leaves a theoretical contribution of 0–50% by BMA. Although these data alone do not allow more specific quantification, the actual bacterial contribution was most likely near 100%, whereas the role of BMA was negligible. A substantial contribution by BMA seems counterintuitive because active BMA are typically concentrated in the upper few millimeters of the sediment while bacteria are abundantly present throughout the whole 0–8-cm layer, meaning that bacteria predominated in most of the 0–8-cm layer. This may seem to conflict with our biomass estimates that yielded roughly similar biomasses for phototrophs (on the basis of Chl *a* concentrations) and heterotrophic bacteria (on the basis of bacteria-specific PLFA concentrations). However, these estimates may not reflect the biomass of BMA versus bacteria because of the presence of cyanobacteria that are included in phototrophic biomass and not in bacterial biomass (see Fig. 1). Additional bias may have resulted from the presence of Chl *a* in phytodetritus from the water column or higher plant detritus next to Chl *a* in active BMA. Another point that supports bacterial domination of nitrogen recycling within the sediment is that bacteria are generally considered to mediate organic matter degradation and to be better at utilizing nutrients at low concentrations (i.e., when gradually released during organic matter degradation) (Kirchman 1994; Goldman and Dennett 2001). Altogether, our ^{15}N D-Ala results clearly show that bacteria played a major role in recycling of the ^{15}N and therefore confirm the potential importance of bacteria in recycling and retention of nitrogen in sediments.

In situ ^{15}N plots: ^{15}N in fauna— ^{15}N was traced into fauna to investigate the importance of faunal biomass as a temporary sink for bacterial- or algal-derived nitrogen (via grazing) and its role as a first step in a potential nitrogen flow to higher trophic levels. Extraction and ^{15}N -analysis of fauna had an experimental character since little was known about faunal densities in the sediment and the required numbers of individuals for ^{15}N analysis of small fauna such as nematodes. Recovered faunal densities may have been somewhat lower than actual densities in the sediment because of the relative coarse extraction method that was used. However, recovered polychaete densities are within the range of densities reported for subtidal sediment

in the upper Brunswick Estuary (Eyre and Ferguson 2005) and recovered nematode densities are comparable with densities reported for other intertidal sediments (e.g., Steyaert et al. 2001), suggesting that recovered densities did not severely underestimate actual densities.

The clear ^{15}N enrichment of animals from different faunal groups with $\Delta\delta^{15}\text{N}$ values in the same range as those for bulk sediment and HAAs (Fig. 5 and Table 1) indicates substantial incorporation of ^{15}N into fauna. Moreover, the delay in ^{15}N enrichment of faunal biomass compared with that of the bulk sediment and THAAs confirms that ^{15}N incorporation into fauna did not occur via direct incorporation of ^{15}N from $^{15}\text{NH}_4^+$ but indirectly via grazing on ^{15}N -labeled microbial biomass.

The estimated dependence on ^{15}N -labeled microbial biomass (likely dominated by bacteria) of $12\% \pm 4\%$ for *N. estuarius* as derived from the model simulation of the dynamics of $\Delta\delta^{15}\text{N}$ values of microbial and faunal biomass is comparable with previously reported low (<20%) dependence on bacterial carbon for various faunal groups (van Oevelen et al. 2006b). Conversely, the $37\% \pm 12\%$ for *S. aequisetis* and the $42\% \pm 12\%$ for nematodes indicate that ^{15}N -labeled microbial biomass was an important nitrogen source for these faunal groups, contributing up to 50% to their total nitrogen incorporation. These values are relatively high in comparison with the faunal dependence on bacterial carbon for a temperate intertidal mudflat (van Oevelen et al. 2006b), which may be due to preferential incorporation of microbial nitrogen over carbon or due to the relatively high bacterial biomass in (sub)tropical sediments like in the present study (Alongi 1994). However, results are too limited for further discussion.

Despite this importance of ^{15}N -labeled microbial biomass as a nitrogen source for the benthic fauna, it appears that the total amount of ^{15}N incorporated into faunal biomass was only a very small fraction of total ^{15}N in benthic biomass ($\sim 0.2\%$). Even when taking into account the ^{15}N in other faunal groups (Table 1) and the possible underestimate of faunal densities, it seems that only a negligible fraction (<1%) of total microbial ^{15}N was transferred to faunal biomass via grazing. This is consistent with results from other studies in which only a small fraction (typically no more than 20–25%) of bacterial production is consumed by fauna (Kemp 1990; van Oevelen et al. 2006a). The very low values for the present study may be related to the high temperatures and high organic matter content of the sediment, which results in very high bacterial biomass and productivity relative to faunal biomass and grazing rates (Alongi 1994). Altogether, the ^{15}N -fauna results show that, despite the importance of microbial biomass as a nitrogen source for the benthic fauna, the role of fauna in total benthic nitrogen cycling was negligible.

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