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Uptake of dissolved organic and inorganic nitrogen in microalgae-dominated sediment: comparing dark and light *in situ* and *ex situ* additions of ^{15}N

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ABSTRACT: Microbial communities within bare intertidal sediment have an active role in uptake of inorganic and organic nitrogen as it is transported through estuaries. ^{15}N-labeled dissolved inorganic nitrogen (DIN, NH_4^+; 250 µmol l^{-1}, 500 µmol l^{-1}) and dissolved organic nitrogen (DON, algal-derived; 125 µmol l^{-1}, 250 µmol l^{-1}) were applied to diatom-dominated sandy intertidal sediment under light and dark conditions to investigate short-term N uptake (24 h). Two experiments compared uptake in intact sediments (*in situ*) and homogenized slurries (*ex situ*). In both experiments, N uptake was similar in light and dark conditions, and benthic microalgae (BMA) dominated both biomass and DIN and DON uptake over heterotrophic bacteria. Substantially lower uptake of DON than DIN occurred for both experiments, likely because organic molecules require extracellular processing before uptake by BMA. Compared to intact sediments, sediment slurries had higher N uptake into sediment organic matter (3–36×), lower bacterial biomass (13.6 ± 3.5% versus 41.1 ± 7.6% intact) and low bacterial contribution to ^{15}N uptake (14 ± 0.8% versus 14 ± 3.0%). Differences are likely due to shifts within the microbial community and sediment environment caused by sediment homogenization or incubation effects. Consistently, uptake rates within slurries were greater than within intact sediments, and patterns of significant differences among treatments were different. Slurry incubations are therefore not reliable for quantification or comparison of *in situ* uptake rates across different N substrates, but biomarkers appear robust between the 2 methods, indicating low bacterial contribution to N uptake in BMA-dominated sediment.

KEY WORDS: Benthic microalgae · Bacteria · Microphytobenthos · DON · DIN · Slurry · Stable isotope

INTRODUCTION

Nitrogen (N) is a limiting nutrient within many coastal estuarine ecosystems that is predominantly present in 2 forms: dissolved inorganic nitrogen (DIN), the sum of NO_3^-, NH_4^+, and NO_2^-, and dissolved organic nitrogen (DON), a complex pool of labile and refractory molecules that is largely uncharacterized (80%) (Benner 2002, Sipler & Bronk 2015). Because DIN is the primary driver of organic matter production within estuaries, numerous studies have examined DIN uptake (e.g. Veuger et al. 2004, Hardison et al. 2011, Moneta et al. 2014). However, DON can also be a significant N input to estuaries (Eyre & Pont 2003, Sundbäck et al. 2006, Alkhatib et al. 2013) and contributes substantially to total sediment N flux (Eyre & Ferguson 2002, Ferguson et al. 2004, Ferguson & Eyre 2010). Despite considerable DON production by the sediment community, relatively low concentrations within more marine estuarine waters suggest that DON is either readily utilized (Bronk et al. 2007), or is refractory on the time scale of estuarine export and simply exported from the system (McCarthy et al. 1998, Ogawa et al. 2001, Ander-

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sson et al. 2006). It is clear that both DON and DIN are potentially important components of estuarine N cycling.

Much of the N processing within estuaries occurs within sediments (Hopkinson et al. 1999), largely mediated by heterotrophic bacteria (HB) and benthic microalgae (BMA) (Cook et al. 2007, Hardison et al. 2011, Anderson et al. 2014, Spivak & Ossolinski 2016). ^{15}N-labeling studies have highlighted the importance of the microbial community in the uptake of N (Cook et al. 2007, Evrard et al. 2008, Eyre et al. 2016). Although previous studies investigating the microbial uptake of individual DON compounds and DIN have provided valuable insights, these are limited by methodological constraints. First, all but 3 (Veuger et al. 2007a, Hardison et al. 2011, Eyre et al. 2016) previous labeling studies have been *ex situ*, using sediment slurries (e.g. Veuger & Middelburg 2007, Dähnke et al. 2012, Veuger et al. 2012), and there has been no comparison of *in situ* and *ex situ* N uptake. Second, the effect of light exposure on N uptake has been examined thoroughly within the water column (Middelburg & Nieuwenhuize 2000) and within sediment communities in the presence of macroalgae (Hardison et al. 2011), but there have been few comparisons of *in situ* dark and light uptake of DIN and DON into the sediment microbial community. Cook et al. (2007) and Evrard et al. (2008) observed uptake of N from NH_4^+ and NO_3^-, respectively, in light and dark incubations, but both studies lacked sufficient replication for statistical analysis. Finally, studies investigating DON uptake have focused on individual compounds that are relatively labile (e.g. urea or amino acids) (Veuger & Middelburg 2007, Veuger et al. 2007a) whereas naturally occurring DON is a complex mixture of compounds ranging from labile to refractory. Pelagic studies using more 'natural' algal-derived DON have shown uptake rates that are comparable to those of DIN (Bronk & Glibert 1993, Veuger et al. 2004, Moneta et al. 2014), but this substrate has not been applied to sediments.

In the present study, ^{15}N-labeled DIN (NH_4^+) and DON ('natural', algal-derived DON) were applied to intertidal sediments to compare apparent uptake after 24 h following application under different light conditions (light or dark). N uptake was also compared for *in situ* intact sediments and *ex situ* slurry incubations. We hypothesized that uptake of DON and DIN would be similar, with more rapid N uptake in light due to direct uptake of N by BMA, and that uptake rates would be higher within *ex situ* slurry incubations than for sediment incubated *in situ*.

MATERIALS AND METHODS

Site description

This study was conducted in October 2014 on a subtropical intertidal shoal in the lower Richmond River estuary in New South Wales, Australia (28° 52′ 30″ S, 153° 33′ 26″ E). The site has a semidiurnal tide range of ~2 m. Site water had a salinity of 34.6 and DIN and DON concentrations of 2.5 ± 0.04 and 14.8 ± 0.5 µmol N l^{-1}, respectively, measured via a 4-channel flow injection analyzer (Lachat Quickchem 8000) according to the method detailed in Eyre et al. (2011). Sediment porewater was not directly measured, but examination of N removed from sediment during KCl extraction of controls provided a maximum available ambient porewater N estimate of 1.8 ± 0.5 µmol N g^{-1} dry sediment (for calculations, see Supplement 1; all supplementary material in this article can be found at www.int-res.com/articles/suppl/m571p029_supp.pdf). Total N from these 2 sources represented 1.2% of the total N within control sediment. The river catchment is subject to infrequent episodic rainfall events and flooding (Eyre 1997, McKee et al. 2000), which has remodeled the shoal since it was studied by Oakes & Eyre (2014).

Sediment and BMA composition

Sediment within the site was sandy mud; details are provided in Supplement 1. The shoal was net autotrophic during the present study (production/respiration [P/R] = 1.19), but less so than reported by Oakes & Eyre (2014) (P/R = 2.23). Light microscopy (1000×) revealed a BMA community dominated by pennate diatoms, with only very occasional occurrences of filamentous cyanobacteria, and evidence of a minimal number of larger heterotrophs as has been observed previously at this site (Oakes & Eyre 2014). Reduced numbers of heterotrophs and macrofauna within site sediment is not atypical of systems at lower latitudes (Purwoko & Wolff 2008).

Chlorophyll *a* (chl *a*) was measured by colorimetry (Lorenzen 1967) for triplicate 1 cm^3 (0–1 cm depth) samples of wet sediment from each treatment plot. We did not measure bacterio-chlorophyll, but phospholipid fatty acid (PLFA) spectra did not include peaks for biomarkers associated with purple sulfur bacteria (19:0; Bühring et al. 2014), sulfate reducing bacteria (17:0, 17:1w8 or 17:1w5; Pagès et al. 2015) or cyanobacteria (18:2w6; Bellinger et al. 2009). PLFAs were extracted from freeze-dried, homogenized sed-

iment and prepared using a modified Bligh and Dyer technique as described by Oakes et al. (2012). BMA biomass was calculated assuming a carbon:chl *a* ratio of 40, comparable to that used previously for Australian subtropical estuaries (30–60; Ferguson et al. 2004, Oakes et al. 2012). Samples were transported on ice and stored frozen (–20°C) in the dark until analysis.

Preparation of ^{15}N-labeled substrates

^{15}N-labeled DON ($DO^{15}N$) was extracted and purified from an axenic culture of the diatom *Thalassiosira pseudonana* (CSIRO, ANACC culture CS-20) grown with 99% $^{15}NH_4Cl^+$ (Cambridge Isotope Laboratories NLM-467). The $DO^{15}N$ had a ^{15}N atom% value of 41.4 ± 0.2 and contained a small concentration of remaining DIN (0.6 µmol N l^{-1}), accounting for <0.02% of the ^{15}N present. Details of $DO^{15}N$ preparation are provided in Supplement 1.

Four treatments for this study were prepared using NaCl-amended Milli-Q (35 g l^{-1}), matching site salinity. $DO^{15}N$ was diluted to produce concentrations of 125 µmol N l^{-1} (treatment DON-125) and 250 µmol N l^{-1} (treatment DON-250). Inorganic nitrogen treatments were prepared through the addition of 99% $^{15}NH_4Cl$ to NaCl-amended Milli-Q, resulting in concentrations of 250 µmol N l^{-1} (DIN-250) and 500 µmol N l^{-1} (DIN-500). The treatment levels of DIN-250 and DON-250 were intended to allow comparison of the same amount of N added in 2 different forms. The lower concentrations used for DON treatments (125 and 250 µmol N l^{-1}) reflect the difficulty involved in selectively removing DIN from natural organic matter mixtures. Concentrations for treatment applications were based on pilot experiments examining uptake of ^{15}N into sediment organic matter (OM) for a range of concentrations for both $DI^{15}N$ and $DO^{15}N$, ensuring adequate uptake to allow for comparison between substrates. The use of 2 different concentrations of each substrate increased the chance of adequate label uptake, as well as providing the opportunity to explore the effect of increasing N availability on uptake of each substrate.

Labeled substrate application

Eight plots (1 light and 1 dark for each of 4 treatments) were established 2 m apart from one another at the same height within the intertidal zone in an area that was largely free of animal burrows. Plots were demarcated using rectangular frames divided into 12 sub-plots, each measuring 20 × 20 cm. Treatment applications occurred at low tide, soon after sediment was first exposed, allowing maximum time for potential label uptake (~4 h) before tidal inundation removed the bulk of any unincorporated label. Tidal flushing removed unincorporated ^{15}N-labeled DON and DIN applications from *in situ* sediment. After 24 h, $\delta^{15}N$ values for raw (non-KCl extracted) sediment OM for all treatments (175‰ DON-125, 157‰ DON-250, 210‰ DIN-250, and 259‰ DIN-500) were very low compared to those of the treatment applications, representing a loss of 99.6–99.9% of the ^{15}N that was applied to the sediments. Removal of label from the sediment likely occurred through replacement and turnover of porewater from flushing with relatively low nutrient seawater during tidal inundation. Four plots (one per treatment) were labeled in the light (13:30 h, 21 October 2014) and 4 in the dark (02:00 h, 22 October 2014), using motorized sprayers to apply 20 ml of relevant treatment onto each 400 cm^2 sub-plot. Labeling dosages for $^{15}NH_4Cl$ were 125 µmol N m^{-2} (DIN-250) and 250 µmol N m^{-2} (DIN-500) and for $DO^{15}N$ were 62.5 µmol N m^{-2} (DON-125) and 125 µmol N m^{-2} (DON-250). Higher concentrations were used than those that occur naturally in order to produce a pulse of ^{15}N large enough to be detectable within sediments after dilution through tidal flushing. Although the concentrations added are not representative of pristine waters, they are not unrealistic, as they fall within N concentrations reported for coastal environments receiving anthropogenic input (Cloern & Jassby 2012).

Label applications were spatially clustered by treatment to reduce the potential for cross-contamination across treatments. This would ordinarily reduce variance among samples (pseudoreplication). However, sediment microbial communities are extremely patchy at the millimetre to centimetre scale. Brown–Forsythe homogeneity of variance (HOV) tests ($\alpha = 0.05$) confirmed no significant decrease in variance for BMA biomass or porewater N removed with KCl extraction among sub-plots within each treatment when compared with that across treatments or controls (Supplement 1). The subplots were therefore considered independent of one another for the purpose of this study. Three control sediment cores for background isotope values were collected randomly within 1 m of sample plots prior to label application. Any unincorporated total hydrolyzable amino acids (THAAs) introduced from the algal-

derived application of DON remaining in the sediment would lead to an overestimate of ^{15}N incorporation by BMA. However, comparable D/L-alanine ratios between control plots (0.010 ± 0.001) and sediment treated with the DON application (0.013 ± 0.003) confirm that any contribution to THAAs from extracellular algal-derived DON were negligible and did not affect interpretation.

Sample collection

One core of sediment (9 cm diameter × 3 cm depth) was collected from each independently labeled subplot 24 h after label application using an acrylic core liner. Sediment was immediately extruded and sectioned into 1 cm depths to 3 cm. Sediment samples were stored frozen (–20°C) in plastic zip-lock bags. This yielded 6 samples per treatment (3 dark, 3 light × 4 treatments). Sediment (0–3 cm depth) was also collected from 4 cores between plots prior to labeling and transported at ambient temperature for use in the laboratory slurry experiment. Immediately after arrival, unlabeled sediment was homogenized in aerobic conditions and 20 ml aliquots were placed in 24 × 60 ml clear glass sample jars (2.5 cm diameter × 25 cm height) with opaque plastic lids containing rubber septa. Sediment was not sieved, as meiobenthos contained within site sediment are limited to foraminifera and have previously been found to have a minor contribution to total sediment biomass (up to 0.03%) (Oakes & Eyre 2014). When examined visually (1000×), sediment was largely devoid of biota ≥500 µm. Six sample jars were labeled with each treatment (either DIN-250, DIN-500, DON-125 or DON-250) through addition of 20 ml of each respective label application into the sample jars, with 3 incubated in the dark (foil wrapped) and 3 on a 12 h light:12 h dark cycle (unwrapped). Inclusion of the dark-only treatment was designed to maximize the potential for differences in uptake under altered light regimes to identify whether uptake of N was affected by application under light or dark conditions. All jars were then incubated for 24 h (as per previous slurry incubations [Cook et al. 2007, Veuger & Middelburg 2007, Evrard et al. 2008], allowing for direct comparison) in ambient sunlight (average of 629 ± 113 µE m^{-2} s^{-1} across 12 h) and temperature (21.3 ± 7.4°C). During recovery of sediment from sample jars, sediment was not sectioned for depth, as sediment was thoroughly mixed after treatment application and periodically during incubation. After incubation, sediment was stored frozen (–20°C).

Sample analysis

Sediments were freeze-dried, homogenized and extracted with 2 M KCl to remove adsorbed inorganic and organic N, as described in Supplement 1. Washed, KCl-extracted sediment was dried (60°C) and weighed into a tin capsule for analysis of $\delta^{15}N$ and %N using a Flash elemental analyzer coupled online to a Thermo Fisher Delta V Plus IRMS. Reproducibility of $\delta^{15}N$ values for samples with $\delta^{15}N$ enrichment <100‰ was ±0.2‰. Precision decreased with enrichment beyond 100‰.

A subsample of sediment from each sample and aliquots of the DON treatment (DON-125 and DON-250, to confirm algal culture was axenic) were analyzed for concentration and $\delta^{15}N$ of hydrolyzable amino acids (HAAs) according to the method of Veuger et al. (2005) (Supplement 1). Concentrations and $\delta^{15}N$ of the derivatized amino acids were determined by gas chromatography-combustion-isotope ratio mass spectrometry (GC-c-IRMS) on an HP 6890 GC interfaced via a Thermo Conflo III with a Thermo Delta V Plus IRMS. We compared uptake that occurred *in situ* at 0–1 cm depth within intact sediment to uptake that occurred in laboratory slurries of homogenized sediment taken from 0 to 3 cm depth. Within *in situ* samples, samples for biomarker analysis were only taken in the 0–1 cm depth range due to (1) bulk sediment analysis confirming that the majority of uptake (>85%) occurred at 0–1 cm depth (Fig. S1 in Supplement 5) and (2) limitations of time and cost associated with the laborious nature of biomarker analysis. Sediment slurries combined sediments from 0 to 3 cm depth, but were considered to not vary with depth after laboratory incubation due to thorough homogenization and repeated mixing that occurred during label addition.

Calculations

^{15}N data are presented as excess nmol ^{15}N per gram of dry sediment, as typically reported in other rare isotope tracer studies (e.g. Middelburg et al. 2000, Cook et al. 2007, Veuger et al. 2007a, Veuger et al. 2012, Oakes & Eyre 2014, Eyre et al. 2016), calculated as:

$$\text{Excess } ^{15}N = (\text{at\% } ^{15}N_{\text{sample}} - \text{at\% } ^{15}N_{\text{control}}) \times (\text{nmol N in sample}) / \text{g dry sediment} \quad (1)$$

where at% is the percentage of N atoms present as ^{15}N. Due to the difference in atom% of ^{15}N between DON and DIN treatments (41.4 and 99.0, respec-

tively), calculated excess ^{15}N values for DON treatments (sediment and amino acids) were multiplied by 2.4 to allow for direct comparison between substrates.

Original concentrations of D- and L-alanine were determined from measured concentrations by correcting for racemization using the equations of Kaiser & Benner (2005) (Supplement 2). Biomass of HB, BMA, and total microbial were calculated as described by Veuger et al. (2005, 2007b) (see Supplement 2). ^{15}N uptake into HB, BMA, and total microbial biomass was calculated using the procedure described above using excess ^{15}N in place of total N concentrations (see Supplement 2). Uncertainties associated with the variables used within the D/L-alanine biomarker technique are thoroughly discussed in Veuger et al. (2005, 2007b).

To determine hourly uptake rates for intact sediment and slurry incubations, the excess ^{15}N within total microbial biomass was divided by 4 and 24 h, respectively, based on the estimated time available for ^{15}N incorporation. For intact sediments, this equated to the 4 h before tidal inundation and flushing occurred and assumed no loss from the sediment over the 20 h between inundation and sampling. Finally, microbial N production (mmol ^{15}N uptake mol^{-1} N biomass) was calculated as:

$$\text{Microbial nitrogen production} = \text{Excess } ^{15}\text{N total microbial biomass} / \text{Total microbial biomass} \quad (2)$$

Microbial N production is useful as a measure of uptake of ^{15}N per unit of biomass to examine uptake of ^{15}N independently from changes in microbial biomass, and should not be confused with uptake into microbial biomass.

The full statistical analysis is provided in Supplement 3.

RESULTS

Sediment characteristics

Control samples had higher chl *a* biomass (30.2 ± 4.8 mmol C m^{-2}) than either light or dark treatment plots (18.1 ± 8 and 22.8 ± 8.3 mmol C m^{-2}, respectively). There were no significant effects of treatment on chl *a* biomass in the light or dark (2-way ANOVAs, $F_{1,23}$ = 0.8, p = 0.4 and $F_{3,23}$ = 0.1, p = 1.0, respectively).

Total N content and microbial biomass varied significantly among control, intact and slurried sediments. After 24 h, total N of 0–1 cm sediments was similar for control and ^{15}N-labeled *in situ* intact sediment (14.8 ± 2.0 and 13.2 ± 1.1 µmol N g^{-1}, respectively). Conversely, after 24 h, total N within homogenized *ex situ* sediments was 1.85× higher (42.4 ± 3.4 µmol N g^{-1}) than within intact sediments from the same depth (0–3 cm, 22.9 ± 1.4 µmol N g^{-1}), with control sediment falling between both experiments (0–3 cm, 31.8 ± 1.4 µmol N g^{-1}). Within *ex situ* slurry incubations, bacterial biomass was 0.5× lower and algal biomass ~2× higher (0.7 ± 0.2 and 4.4 ± 0.8 µmol ^{15}N g^{-1}, respectively) than within intact sediments (1.2 ± 0.4 and 1.9 ± 0.4 µmol ^{15}N g^{-1}, respectively). Total microbial biomass after 24 h was higher in both intact sediment and slurry incubations than in control sediments (intact: 3.2 ± 0.6 µmol ^{15}N g^{-1}; slurry: 5.1 ± 0.8 µmol ^{15}N g^{-1}; control: 2.2 ± 0.6 µmol ^{15}N g^{-1}).

Intact sediments

Light exposure

There was little effect of light exposure on ^{15}N uptake into intact sediments, although HB contributed more to uptake in the light (15.4 ± 3.4%) than in the dark (12.6 ± 5.4%) (p = 0.012; Table S1 in Supplement 5). For intact sediments, there was no significant effect of light exposure on total or hourly ^{15}N uptake into sediment OM, hourly uptake into HAAs, microbial biomass or HB contribution to biomass. The average natural light exposure over 12 h following treatment application was 748 ± 136 µE m^{-2} s^{-1} (max. 2039 µE m^{-2} s^{-1}) at the surface of intact sediments and 629 ± 113 µE m^{-2} s^{-1} (max 2035 µE m^{-2} s^{-1}) for the slurry incubation.

^{15}N uptake into sediment OM

Across the 4 treatments, 59.3 ± 10.6% of the added ^{15}N was assimilated into OM within 0–3 cm intact sediments. Of this, 87.6 ± 2.0% was within 0–1 cm intact sediments (Supplement 4, Fig. S1 in Supplement 5); this uppermost sediment layer was therefore the primary focus of the present study. Details of ^{15}N uptake in deeper sediments are detailed in Figs. S1 & S2 in Supplement 5.

Total ^{15}N uptake into sediment OM within intact sediments (0–3 cm) 24 h after application varied significantly among treatments (Fig. 1A, Table S1 in Supplement 5). The DIN-500 treatment had signifi-

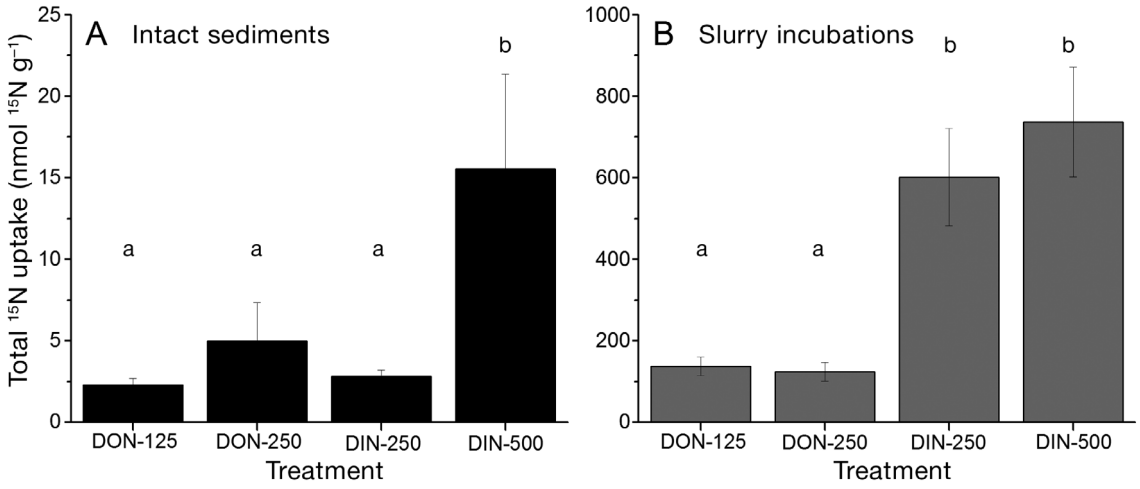

Fig. 1. Total uptake of ^{15}N into sediment organic matter for (A) intact sediments and (B) slurry incubations (means ± SE) for 0–3 cm depth. Different letters indicate significant differences between treatments at $\alpha = 0.05$ (Tukey's test). Note the large difference between the *y*-axes scales for intact sediment and slurry incubations

cantly greater uptake (15.6 ± 5.8 nmol ^{15}N g^{-1}) than all other treatments (2.3 ± 0.4 nmol ^{15}N g^{-1} for DON-125, 5.0 ± 2.4 nmol ^{15}N g^{-1} for DON-250, and 2.8 ± 0.4 nmol ^{15}N g^{-1} for DIN-250; Fig. 1A).

Hourly ^{15}N uptake into sediment OM (0–1 cm) varied significantly among treatments, with 3–6× higher uptake for DIN-500 than for all other treatments (Fig. 2A). Hourly ^{15}N uptake into HAAs and microbial biomass was significantly higher for DIN-500 than for all other treatments (HAAs: 8–39×, microbial biomass: 8–58×) (Fig. 2B,C). Doubling of DON concentrations in treatment applications had no effect on uptake rates in sediment OM, HAAs or microbial biomass for intact sediments.

Contribution of heterotrophic bacteria to biomass and uptake

Total biomass within intact sediment treatments was dominated by BMA, with HB representing 41.1 ± 7.6% (range of 1–80% within individual samples) of this biomass (Fig. 3A) at 0–1 cm. Although there were no significant differences in HB contribution to biomass among treatments, HB contribution to ^{15}N uptake was significantly higher (2–4×) for DON-125 (30.1 ± 2.2%) than for all other treatments (14.5 ± 5.3% DON-250, 7.5 ± 2.5% DIN-250, and 6.9 ± 1.3% DIN-500) (Fig. 4A, Table S1 in Supplement 5). Microbial N production (mmol uptake of ^{15}N mol^{-1} N biomass) was significantly higher for DIN-500 (14–60×) than for all other treatments (Fig. 5A), i.e. per unit biomass, there was greater ^{15}N uptake.

Slurry incubations

Light exposure

Light exposure increased ^{15}N uptake into HAAs (0.8 ± 0.2 and 0.4 ± 0.1 nmol ^{15}N g^{-1} h^{-1} in light and dark treatments, respectively) and microbial biomass (3.6 ± 1.2 and 1.6 ± 0.6 nmol ^{15}N g^{-1} h^{-1}). However, there was no significant effect of light exposure on total and hourly ^{15}N uptake into sediment OM, HB contribution to biomass and ^{15}N uptake, or microbial N production (Table S1 in Supplement 5).

^{15}N uptake into sediment OM

Across the 4 treatments, 99.9 ± 14.8% of the added ^{15}N was assimilated into OM within the slurry incubations. Total ^{15}N uptake into sediment OM was 4–5× higher for DIN-250 (601 ± 119 nmol ^{15}N g^{-1}) and DIN-500 (737 ± 135 nmol ^{15}N g^{-1}) than for DON-125 (137.5 ± 22.6 nmol ^{15}N g^{-1}) or DON-250 (124.1 ± 22.8 nmol ^{15}N g^{-1}). Increasing DON application resulted in no additional uptake. Although increasing the concentration of the DIN application increased ^{15}N uptake, this was not statistically significant (Fig. 1B, Table S1 in Supplement 5).

Hourly rates of ^{15}N uptake into sediment OM were significantly higher for both DIN treatments than for either DON treatment (Fig. 2A), whereas hourly ^{15}N uptake rates for HAAs and microbial biomass in slurry incubations were significantly higher for DIN-500 than for all other treatments (HAAs: 1.9–8.7×, microbial biomass: 2.4–14.6×) (Fig. 2B,C, Table S1).

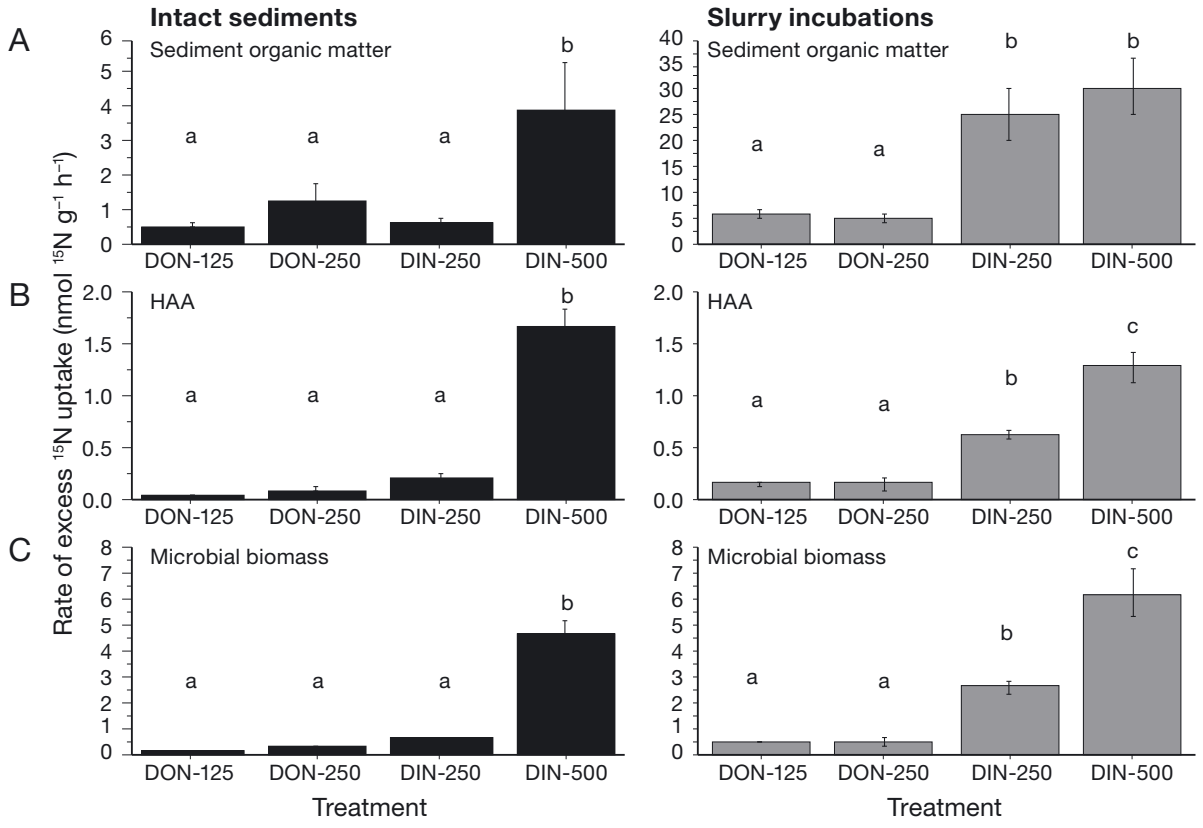

Fig. 2. Hourly rates of excess ^{15}N uptake into (A) sediment organic matter, (B) hydrolyzable amino acids (HAA) and (C) microbial biomass (means ± SE) from 0 to 1 cm depth for intact sediment and homogenized sediment from 0 to 3 cm for slurry incubations. Different letters indicate significant differences between treatments at $\alpha = 0.05$ (Tukey's test). Some error bars are too small to be seen. Note the large difference between the *y*-axes scales for intact sediment and slurry incubations for sediment organic matter (A)

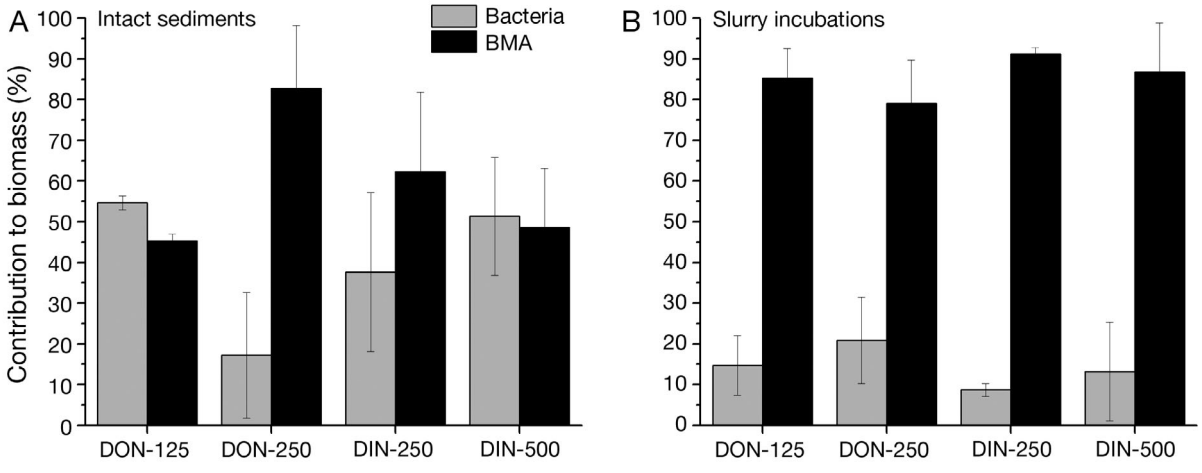

Fig. 3. Percentage of bacterial and benthic microalgae (BMA) contribution to biomass calculated from D- and L-alanine content, corrected for racemization (means ± SE). No significant differences were found between treatments at $\alpha = 0.05$, but intact sediments (A) had significantly higher bacterial contribution than slurry incubations (B)

However, uptake rates for DIN-250 for HAAs and microbial biomass were still significantly higher than those of both DON treatments for HAAs. Increasing the concentration of DIN significantly increased ^{15}N uptake for HAAs (Fig. 2B) and microbial biomass (Fig. 2C), but not for sediment OM (Fig. 2A, Table S1). Doubling the DON concentration resulted in no additional ^{15}N uptake.

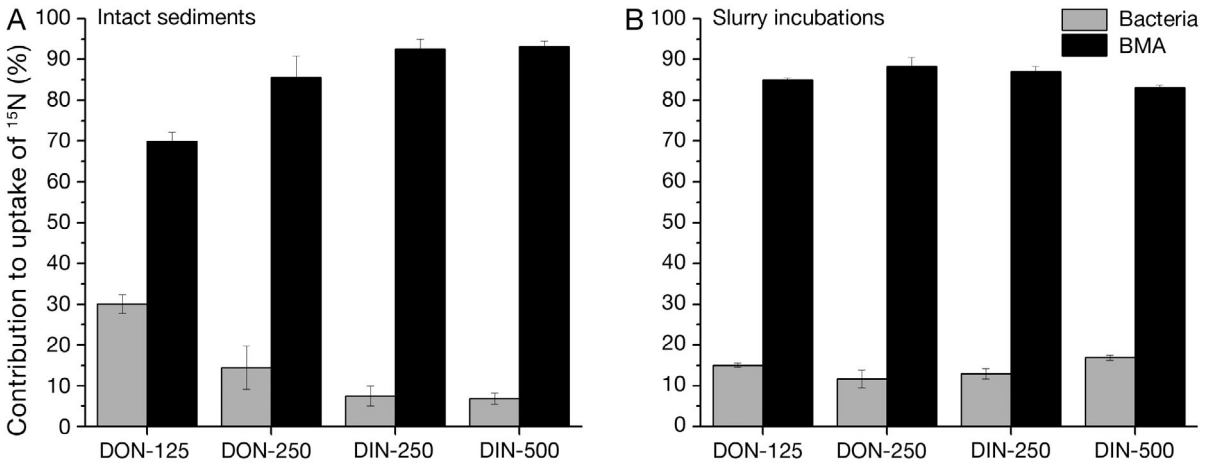

Fig. 4. Percentage of bacterial and benthic microalgae (BMA) contribution to uptake of excess ^{15}N into microbial biomass 24 h after treatment application (means ± SE) in both (A) intact sediments and (B) slurry incubations

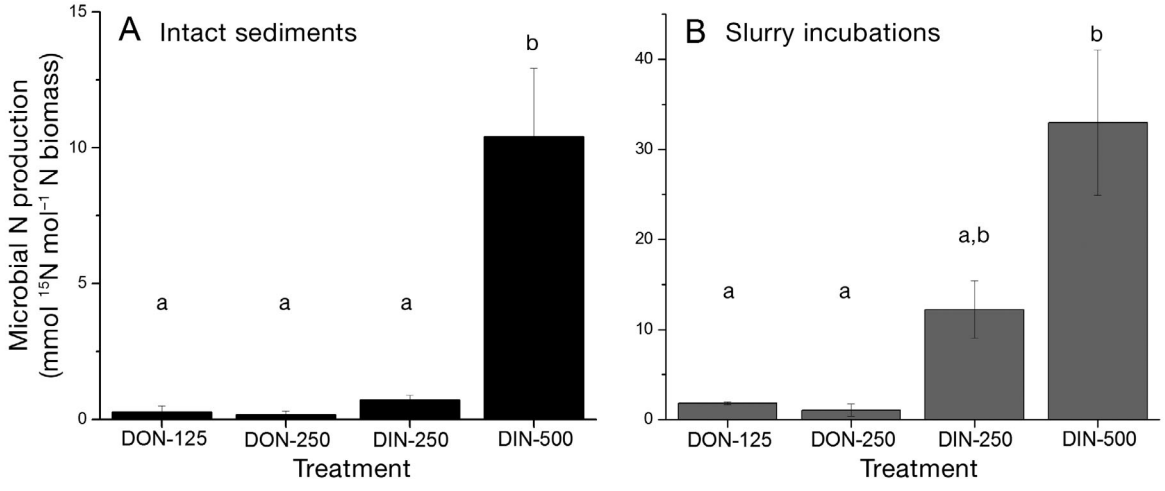

Fig. 5. Microbial N production (mmol ^{15}N mol^{-1} N biomass) for all treatments (means ± SE) in both (A) intact sediments and (B) slurry incubations. Different letters indicate significant differences between treatments at $\alpha = 0.05$ (Tukey's test). Note the large difference between the *y*-axes scales for intact sediment and slurry incubations

Contribution of heterotrophic bacteria to biomass and uptake

Microbial biomass in the slurry incubations was dominated by BMA, with HB contributing only 13.6 ± 3.5% to the total microbial biomass within sediments across treatments (Fig. 3B). HB contribution to biomass was statistically similar across treatments (Table S1). HB contribution to ^{15}N was also statistically similar across treatments (average of 14.0 ± 0.8%; Fig. 4B). Microbial N production was significantly higher in the DIN-500 treatment (33.0 ± 8.1 mmol ^{15}N mol^{-1} N biomass) than in either DON treatment (1.8 ± 0.2 mmol ^{15}N mol^{-1} N biomass DON-125, 1.1 ± 0.7 mmol ^{15}N mol^{-1} N biomass DON-250; Fig. 5B, Table S1). Microbial N production in the DIN-250 treatment (12.2 ± 3.2 mmol ^{15}N mol^{-1} N biomass) was intermediate to that in the DON treatments and the DIN-500 treatment.

Comparing slurry incubations and intact sediments

Total ^{15}N uptake into sediment OM was significantly higher in slurry incubations than in intact sediments (60× for DON-125, 25× for DON-250, 214× for DIN-250, and 47× for DIN-500; Fig. 1, Table S2 in Supplement 5), and hourly rates of ^{15}N uptake into sediment OM (Fig. 2A) and microbial biomass (Fig. 2C) were also significantly higher for slurries than for intact sediments.

HB contribution to biomass was significantly lower in slurry incubations (13.6 ± 3.5%) than in intact sediments (41.1 ± 7.6%; Fig. 3A,B, Table S2), and in both cases was higher after 24 h than within control sediments (3.6 ± 1.4%). No significant differences were observed between intact sediments and slurry incubations for hourly ^{15}N uptake into HAAs (Fig. 2B), HB contribution to ^{15}N uptake (Fig. 4), and microbial N production (Fig. 5).

Patterns of uptake across treatments (Tukey test results) were also different for intact sediments and slurry samples, specifically for total and hourly ^{15}N uptake into sediment OM (Figs. 1A & 2A), hourly ^{15}N uptake into HAAs (Fig. 2B) and microbial biomass (Fig. 2C), HB contribution to ^{15}N uptake (Fig. 4), and microbial N production (Fig. 5, Table S2). Only HB contribution to biomass (Table S1) displayed the same pattern of significant differences among treatments (no differences) for both slurry samples and intact sediments.

DISCUSSION

Patterns in the uptake of DI^{15}N and DO^{15}N in the present study were compared for *in situ* sediments and incubations of sediment slurries in a laboratory setting. Slurry incubations are commonly used in combination with stable isotope tracer pulses to examine uptake dynamics within the benthic microbial community, but there are few comparisons between uptake in slurry incubations and *in situ* settings. This study confirmed that uptake of both DIN and DON was largely dominated by MPB in *in situ* sediments and slurries, but *in situ* uptake rates for sediment OM, THAAs and microbial biomass were substantially lower than those found in sediment slurries. Throughout the study, light had little effect on the assimilation of ^{15}N from DON or DIN for either *in situ* sediments or slurries. Uptake of algal-derived DO^{15}N was substantially lower in both experiments than uptake of DIN, although comparable uptake was observed *in situ* for the DIN- and DON-250 treatments.

Effect of light exposure

There was little effect of light exposure on ^{15}N uptake in intact sediments, with only HB contribution to ^{15}N uptake affected (increased). BMA dominated ^{15}N uptake in the light and the dark. Although NH_4^+ uptake by BMA can be reduced in the dark (Longphuirt et al. 2009) and flux differences have been attributed to the effects of light on BMA (e.g. Eyre & Ferguson 2005 and references therein, Anderson et al. 2014), BMA are capable of dark DIN (NO_3^- and NH_4^+) assimilation (Rysgaard et al. 1993, Evrard et al. 2008), and some studies have reported no differences in light and dark uptake of $^{15}NH_4^+$ by algae (Middelburg & Nieuwenhuize 2000, Cook et al. 2007). It is likely that N uptake in the dark becomes more similar to that in light when N demand is greater, for example, when carbon is in excess (Raimbault & Mingazzini 1987, Clark et al. 2002) or N is limiting (Antia et al. 1991).

Previous studies have attributed DON assimilation by the microbial community to HB due to the lack of diurnal changes in uptake rates that are usually indicative of BMA activity (Eyre & Ferguson 2005, Eyre et al. 2011), and strong inhibition of uptake when the microbial community is treated with antibiotics (Middelburg & Nieuwenhuize 2000). However, the low contribution of HB within intact sediments to ^{15}N uptake from DON (30% for DON-125 and 14.5% for DON-250), suggests that N assimilation from DON in the present study was largely mediated by BMA under both light and dark conditions. This agrees with previous findings of BMA dominating DON uptake (Veuger & Middelburg 2007, Sundbäck et al. 2011).

DIN versus DON and effect of concentration

For the same level of N application (DIN-250 and DON-250) in the slurry incubations, there was greater ^{15}N uptake from DIN than from DON into sediment OM, HAAs and microbial biomass, after correcting for treatment-specific differences in ^{15}N atom% (Figs. 1 & 2). This concurs with previous studies (Veuger et al. 2005, 2007a, 2012, Cook et al. 2007). However, given that these studies mostly also used slurry incubations, DIN uptake is not necessarily greater than DON uptake in the natural environment. Reflecting this, for intact sediments in the present study, there was equivalent uptake of organic and inorganic N (DON-250 and DIN-250) into sediment OM (Figs. 1A & 2A), HAAs (Fig. 2B) and microbial biomass (Fig. 2C). Although this may indicate low demand for new N due to the availability of porewater N, this seems unlikely. Porewater samples were not collected, but comparison of raw and KCl-extracted control sediments yielded an estimate of 1.8 ± 0.5 µmol N g^{-1} dry sediment with a $\delta^{15}N$ of 3.6 ± 1‰ for ambient total N. This estimate is likely to overestimate the porewater concentration of NH_4^+ as

exchangeable NH_4^+ has previously been found to be significantly higher than porewater concentrations within intertidal sediment (Garcia-Robledo et al. 2016). The KCl-extracted material is a combination of porewater N that remained during freeze-drying and material displaced from sediment interaction sites by strong salts. This pool of N was largely ^{15}N-free, with a $\delta^{15}N$ value lower than the control $\delta^{15}N$ value used for excess calculations (7.2‰) and likely of limited bioavailability as it was tightly bound to the sediment. Additionally, measured rates of $^{15}NH_4^+$ uptake over 24 h were generally high for benthic microbial communities (Veuger et al. 2005, 2007a, 2012, Cook et al. 2007) and N uptake increased with greater DIN application (DIN-500), suggesting that sediments were not N replete.

Previous studies reporting little difference in DIN and DON uptake have used relatively labile DON compounds, such as amino acids and urea (Veuger & Middelburg 2007, Veuger et al. 2007a), which comprise only 6–12% of total oceanic DON (Benner 2002, Sundbäck et al. 2011, Sipler & Bronk 2015), potentially overestimating utilization of the DON pool. In contrast, the algal-derived DON used in the present study contains a complex mix of molecules, many of which are likely relatively refractory compared to DIN or single amino acids. Although full characterization of this algal-derived DON is beyond the scope of this study, uptake rates within the present study should be more representative of 'natural' estuarine DON uptake. Bioavailability of DON largely decreases with increasing complexity of organic molecules. Therefore, the magnitude of difference between uptake of ^{15}N derived from DON and DIN should increase with an elevated contribution of refractory molecules to the pool of DON. Apparent rates of DON utilization may also depend on the composition of the microbial community; whereas BMA can utilize labile DON (Veuger & Middelburg 2007, Sundbäck et al. 2011), their capacity to utilize more refractory compounds is unknown, and may be low compared to that of some HB (See et al. 2006). If N is limiting, increased HB biomass would be expected to increase DON utilization and the availability of N for BMA.

Uptake of ^{15}N from DON showed no increase with increased concentration for either intact sediment or slurry incubations, indicating limited utilization of DON as a N source. Lower ^{15}N uptake from DON, compared to DIN, most likely reflects the requirement for extracellular or bacterial processing of DON molecules prior to uptake (See et al. 2006, Bronk et al. 2007, Arnosti 2011). This may limit uptake of DON-derived N, particularly where HB biomass is low, as was observed in the present study (Fig. 3). N can be a limiting substrate for both BMA and HB within sediments (Cook et al. 2007). Under limiting conditions, addition of N should increase N uptake, but if the need for N has already been met or the N is not accessible, then no additional uptake will occur and N will be exported from the system via tidal flushing. It is possible that only a subset of molecules within the DON application were labile over the time scale of the present study (4 to 24 h), but it seems unlikely that marine diatom-derived DON is not readily bioavailable, given that multiple studies have observed ready uptake of algal-derived DON (Veuger et al. 2004, Moneta et al. 2014) based on assumptions of comparable bioavailability of algal-derived DON to amino acids or natural dissolved OM pools. Another possibility is that uptake enzymes were saturated and unable to process additional available DON, resulting in the comparable uptake observed between both DON applications in the sediment slurries. The non-significant increase in ^{15}N uptake between DON-125 and DON-250 for intact sediment indicates that saturation of uptake enzymes was unlikely to have occurred under *in situ* conditions. It may be useful in future studies to perform spike and recovery applications to assess the portion of DON application that adsorbs to sediment OM and to assess recovery rates for KCl extraction. Additionally, investigation of the uptake and fate of ^{15}N across time periods longer than 4–24 h would also improve the applicability of these findings to *in situ* processes.

In contrast to DON, DIN was more readily utilized at higher concentrations (DIN-500), with ^{15}N uptake rates in excess of those for DON within both intact and slurry incubations (Figs. 1A,B & 2). DIN availability apparently limits N uptake within the study site, as doubling DIN concentrations more than doubled ^{15}N uptake rates within intact sediment (Fig. 2). Decreased ^{15}N uptake observed in the DIN-250 treatment within intact sediment is unlikely to be a result of substrate limitation, as the uptake observed represented utilization of only 30% of ^{15}N within the treatment application. Increased utilization of ^{15}N with substrate availability was reflected in increased microbial N production for DIN-500 compared to DIN-250 for both slurries and intact sediments (Fig. 5A,B). This suggests that there is an efficient method for DIN uptake within the microbial community that is not present for DON, such as a secondary uptake pathway or increased efficiency of transport across the membrane (diffusion) at higher concentrations (Longphuirt et al. 2009). Due to the dominance

of biomass by BMA (Fig. 3) and the limited contribution of HB to ^{15}N uptake (Fig. 4), increased DIN uptake efficiency at higher concentrations is likely to be associated with a physiological pathway of BMA. Previous work has identified a similar pathway for increased uptake of NO_3^- within diatoms at concentrations greater than 60 µmol l^{-1} (Lomas & Glibert 2000), but uptake efficiency at higher concentrations is rarely investigated due to the paucity of such conditions occurring within the environment (Longphuirt et al. 2009). Presence of such pathways would allow for opportunistic uptake of nutrients during short-lived conditions and may function as an adaptation to rapidly changing conditions in intertidal sediments.

Uptake of DIN and DON into microbial sediment compartments

Dominance of pennate diatoms within site sediment and the BMA community was an important characteristic that influenced both DIN and DON uptake (Figs. 3 & 4). Diatom dominance within the intertidal microbial community can occur due to continual disturbance through tidal flushing and exposure at low tide disrupting the growth of bacterial populations (Underwood et al. 2005, Coelho et al. 2011). Bacterial populations recover more slowly from disturbance, with long-lasting effects on population structure and biomass (Langezaal et al. 2003). In contrast, diatoms are well adapted to disturbance, migrating through the sediment to optimize light and nutrient availability (Saburova & Polikarpov 2003) and using extracellular polymeric substances to mediate harmful effects of light intensity and inundation (Decho 2000).

BMA dominated uptake in the present study (Fig. 4), and HB experienced intense competition for newly available N. This is reflected in the relatively low contribution of HB to ^{15}N uptake from DIN and DON across both experiments. The contributions of HB to ^{15}N uptake from DIN in both experiments (14.4 ± 1.1% intact and 7.2 ± 1.9% slurries; Fig. 4) were comparable to, or lower than, values previously reported for sediments in laboratory studies (20–30%, Cook et al. 2007; 60%, Veuger et al. 2012), in field studies (0–30%, Eyre et al. 2016; 50–100%, Veuger et al. 2007a) and in the water column (15–60%, Moneta et al. 2014). HB contributions to the ^{15}N uptake from DON within both experiments (13.4 ± 1.3% intact and 22.3 ± 4.3% slurries; Fig. 4) were comparable to, or lower than, those found within sediments using labile DON compounds (6–15% urea, 19–48% amino acid mix; Veuger & Middelburg 2007). A pelagic study using a similar algal-derived DON reported a comparable contribution of HB to DON uptake within the water column (0–30%; Moneta et al. 2014).

Although the contribution of HB to ^{15}N uptake was generally low in the present study, there was a significantly greater contribution of HB to ^{15}N uptake in intact sediments for the DON-125 treatment (Fig. 4A). This demonstrates that HB are able to compete with BMA when available N is lower (Cook et al. 2007) and sediments have not undergone homogenization. Within slurry incubations, no significant differences for HB contribution to uptake of ^{15}N (Fig. 4B) were observed between treatments (Table S1 in Supplement 5) and rates were comparable to those found within intact sediments, indicating successful competition by BMA for available substrates.

Intact sediments versus slurry

Slurry experiments are widely acknowledged as having considerable deficiencies in their ability to replicate *in situ* conditions and, at best, represent potential rates of uptake (Veuger et al. 2005). However, many investigations continue to rely on slurry incubations as a lower-cost, lower-effort method for investigating biogeochemical N cycling. This method has provided valuable initial insight into these processes, but the comparison in the present study emphasizes the caution required when interpreting results from slurry experiments and extrapolating to the natural environment. OM processing pathways within slurries are likely to be affected by destruction of anoxic boundary layers and sediment fine structure due to physical disturbance (Langezaal et al. 2003, Glud 2008, McKew et al. 2013) and shifts within bacterial communities (Böer et al. 2009). These effects could be minimized through the use of mini- or micro-cores, small incubation devices that allow for the maintenance of fine sediment structure, redox gradients and the microbial community, while remaining small enough to be convenient in the laboratory despite requirements for replication. Homogenization is likely to affect competition between BMA and HB for N, with more negative effects on HB than BMA (Langezaal et al. 2003). This is evident in the significantly lower contribution of HB to microbial biomass within sediment slurries (13.6 ± 3.5%) compared to intact sediments (41.1 ± 7.6%, $p = 0.005$; Table S2, Fig. 3B). BMA had a proportionally greater

role in ^{15}N uptake and processing within slurry incubations, largely due to effects of homogenization on HB biomass. With few exceptions, rates and patterns of significant differences for uptake within slurries were not comparable to those observed within intact sediments (Table S2). Although uptake rates were comparable to ranges reported for $^{15}NH_4^+$ in the water column (Veuger et al. 2004, Moneta et al. 2014), hourly ^{15}N uptake rates into sediment OM within slurry incubations were higher than for intact sediment for both DIN (8–36×) and DON (4–10×) treatments (Fig. 2A). Elevated uptake within sediment slurries is likely due to a lack of tidal flushing and limited diffusion through sediment structure present within *in situ* applications. Uptake rates for HAAs and microbial biomass were more similar between experiments, but slurry incubations had different patterns of significant differences between treatments, and hourly rates of ^{15}N uptake into microbial biomass were significantly higher ($p = 0.002$; Table S2). Microbial N production was significantly elevated within slurry incubations for DON-125, DIN-250 and DIN-500, and displayed different patterns of significant differences between treatments (Table S2). Elevated microbial N production indicates a substantial incubation effect within the slurry experiments. This was likely caused by a combination of lack of tidal flushing and increased mixing of label throughout the sediment slurries, in addition to the effects of homogenization.

For *in situ* sediments, tidal flushing removed ^{15}N and label did not diffuse much beyond surface sediments, preventing microbial uptake and processing within deeper sediments and limiting total N uptake (Fig. S1). Within slurries, elevated ^{15}N uptake within sediment OM (Fig. 2A) did not correspond with elevated uptake into HAAs or biomarkers (Fig. 2B,C). There are 2 possible explanations: (1) inefficient KCl extraction of ^{15}N, resulting in elevated apparent uptake rates within OM due to potential laboratory error during extraction, or (2) extensive uptake and processing of N within slurry samples that resulted in considerably more metabolized ^{15}N byproducts that are no longer contained within living microbial biomass. These metabolic products likely compose a large portion of the 'uncharacterized' ^{15}N, that is, ^{15}N contained in sediment OM that is not held within microbial biomass after KCl extraction (Veuger et al. 2012). We repeated KCl extraction on sediment within DIN-250 and -500 in the slurry experiment and observed a negligible additional loss of ^{15}N, confirming that the elevated uptake observed for OM in the slurry incubation was not a result of ineffective KCl extraction. We did not observe discrepancies in patterns of significant differences between extractions for bulk sediment OM and THAA for treatments other than DIN-250 in the slurries. This indicates that additional removal of OM from sediment due to HCl extraction during acid hydrolysis was similar across treatments and that the differences observed between the methods were real, and not methodological artefacts. Elevated ^{15}N incorporated into sediment OM in excess of incorporation into microbial biomass indicated the presence of a large pool of uncharacterized material (a mixture of metabolic byproducts, ^{15}N containing compounds produced from living biomass) in sediment slurries (Fig. 2A,C). This large pool of uncharacterized material further indicates a substantial incubation effect as uncharacterized material within intact sediments formed a considerably smaller pool of ^{15}N. Increased ^{15}N uptake within slurry incubations suggests that the microbial community had greater access to, and/or greater incorporation of, the added ^{15}N-labeled substrates.

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LITERATURE CITED

Alkhatib M, del Giorgio PA, Gelinas Y, Lehmann MF (2013) Benthic fluxes of dissolved organic nitrogen in the lower St. Lawrence estuary and implications for selective organic matter degradation. Biogeosciences 10:7609–7622

Anderson IC, Brush MJ, Piehler MF, Currin CA and others (2014) Impacts of climate-related drivers on the benthic nutrient filter in a shallow photic estuary. Estuaries Coasts 37:46–62

Andersson MGI, van Rijswijk P, Middelburg JJ (2006) Uptake of dissolved inorganic nitrogen, urea and amino acids in the Scheldt estuary: comparison of organic carbon and nitrogen uptake. Aquat Microb Ecol 44:303–315

Antia NJ, Harrison PJ, Oliveira L (1991) The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology and ecology. Phycologia 30:1–89

Arnosti C (2011) Microbial extracellular enzymes and the marine carbon cycle. Ann Rev Mar Sci 3:401–425

Bellinger BJ, Underwood GJC, Ziegler SE, Gretz MR (2009) Significance of diatom-derived polymers in carbon flow dynamics within estuarine biofilms determined through isotopic enrichment. Aquat Microb Ecol 55:169–187

Benner R (2002) Chemical composition and reactivity. In:

Hansell DA, Carlson CA (eds) Biogeochemistry of marine dissolved organic matter. Academic Press, San Diego, CA, p 59–90

Böer SI, Hedtkamp SC, van Beusekom JE, Fuhrman JA, Boetius A, Ramette A (2009) Time- and sediment depth-related variations in bacterial diversity and community structure in subtidal sands. ISME J 3:780–791

Bronk DA, Glibert PM (1993) Application of a ^{15}N tracer method to the study of dissolved organic nitrogen uptake during spring and summer in Chesapeake Bay. Mar Biol 115:501–508

Bronk DA, See JH, Bradley P, Killberg L (2007) DON as a source of bioavailable nitrogen for phytoplankton. Biogeosciences 4:283–296

Bühring SI, Kamp A, Wörmer L, Ho S, Hinrichs K (2014) Functional structure of laminated microbial sediments from a supratidal sandy beach of the German Wadden Sea (St. Peter-Ording). J Sea Res 85:463–473

Clark DR, Flynn KJ, Owens NJP (2002) The large capacity for dark nitrate-assimilation in diatoms may overcome nitrate limitation of growth. New Phytol 155:101–108

Cloern JE, Jassby AD (2012) Drivers of change in estuarine-coastal ecosystems: discoveries from decades of study in San Francisco Bay. Rev Geophys 50:4001–4034

Coelho H, Vieira S, Serôdio J (2011) Endogenous versus environmental control of vertical migration by intertidal benthic microalgae. Eur J Phycol 46:271–281

Cook PLM, Veuger B, Böer S, Middelburg JJ (2007) Effect of nutrient availability on carbon and nitrogen incorporation and flows through benthic algae and bacteria in near-shore sandy sediment. Aquat Microb Ecol 49:165–180

Dähnke K, Moneta A, Veuger B, Soetaert K, Middelburg JJ (2012) Balance of assimilative and dissimilative nitrogen processes in a diatom-rich tidal flat sediment. Biogeosciences 9:4059–4070

Decho AW (2000) Microbial biofilms in intertidal systems: an overview. Cont Shelf Res 20:1257–1273

Evrard V, Cook PLM, Veuger B, Huettel M, Middelburg JJ (2008) Tracing carbon and nitrogen incorporation and pathways in the microbial community of a photic subtidal sand. Aquat Microb Ecol 53:257–269

Eyre B (1997) Water quality changes in an episodically flushed sub-tropical Australian estuary: a 50 year perspective. Mar Chem 59:177–187

Eyre BD, Ferguson AJP (2002) Comparison of carbon production and decomposition, benthic nutrient fluxes and denitrification in seagrass, phytoplankton, benthic microalgae- and macroalgae-dominated warm-temperate Australian lagoons. Mar Ecol Prog Ser 229:43–59

Eyre BD, Ferguson AJP (2005) Benthic metabolism and nitrogen cycling in a subtropical East Australian estuary (Brunswick): temporal variability and controlling factors. Limnol Oceanogr 50:81–96

Eyre B, Pont D (2003) Intra- and inter-annual variability in the different forms of diffuse nitrogen and phosphorous delivered to seven sub-tropical east Australian estuaries. Estuar Coast Shelf Sci 57:137–148

Eyre B, Ferguson AP, Webb A, Maher D, Oakes J (2011) Denitrification, N-fixation and nitrogen and phosphorus fluxes in different benthic habitats and their contribution to the nitrogen and phosphorus budgets of a shallow oligotrophic sub-tropical coastal system (southern Moreton Bay, Australia). Biogeochemistry 102:111–133

Eyre B, Oakes JM, Middelburg JJ (2016) Fate of microphytobenthos nitrogen in subtropical sediments: a ^{15}N pulse-chase study. Limnol Oceanogr 61:2108–2121

Ferguson AJP, Eyre BD (2010) Carbon and nitrogen cycling in a shallow productive sub-tropical coastal embayment (western Moreton Bay, Australia): the importance of pelagic-benthic coupling. Ecosystems 13:1127–1144

Ferguson AJP, Eyre BD, Gay JM (2004) Benthic nutrient fluxes in euphotic sediments along shallow sub-tropical estuaries, northern New South Wales, Australia. Aquat Microb Ecol 37:219–235

Garcia-Robledo E, Bohorquez J, Corzo A, Jimenez-Arias JL, Papaspyrou S (2016) Dynamics of inorganic nutrients in intertidal sediments: porewater, exchangable, and intracellular pools. Front Microbiol 7:761–775

Glud RN (2008) Oxygen dynamics of marine sediments. Mar Biol Res 4:243–289

Hardison A, Anderson I, Canuel E, Tobias C, Veuger B (2011) Carbon and nitrogen dynamics in shallow photic systems: interactions between macroalgae, microalgae, and bacteria. Limnol Oceanogr 56:1489–1503

Hopkinson CS, Giblin AE, Tucker J, Garritt RH (1999) Benthic metabolism and nutrient cycling along an estuarine salinity gradient. Estuaries 22:863–881

Kaiser K, Benner R (2005) Hydrolysis-induced racemization of amino acids. Limnol Oceanogr Methods 3:318–325

Langezaal AM, Ernst SR, Haese RR, van Bergen PF, van der Zwaan GJ (2003) Disturbance of intertidal sediments: the response of bacteria and foraminifera. Estuar Coast Shelf Sci 58:249–264

Lomas MW, Glibert PM (2000) Comparisons of nitrate uptake, storage, and reduction in marine diatoms and flagellates. J Phycol 36:903–913

Longphuirt SN, Lim JH, Leynaert A, Claquin P, Choy EJ, Kang CK, An S (2009) Dissolved inorganic nitrogen uptake by intertidal microphytobenthos: nutrient concentrations, light availability and migration. Mar Ecol Prog Ser 379:33–44

Lorenzen C (1967) Determinations of chlorophyll and pheopigments: spectrophotometric equations. Limnol Oceanogr 12:343–346

McCarthy MD, Hedges JI, Bennar R (1998) Major bacterial contribution to marine dissolved organic nitrogen. Science 281:231–234

McKee L, Eyre B, Hossain S (2000) Intra- and interannual export of nitrogen and phosphorus in the subtropical Richmond River catchment, Australia. Hydrol Processes 14:1787–1809

McKew BA, Dumbrell AJ, Taylor JD, McGenity TJ, Underwood GJC (2013) Differences between aerobic and anaerobic degradation of microphytobenthic biofilm-derived organic matter within intertidal sediments. FEMS Microbiol Ecol 84:495–509

Middelburg JJ, Nieuwenhuize J (2000) Nitrogen uptake by heterotrophic bacteria and phytoplankton in the nitrate-rich Thames estuary. Mar Ecol Prog Ser 203:13–21

Middelburg JJ, Barranguet C, Boschker HTS, Herman MJ, Moens T, Heip C (2000) The fate of intertidal microphytobenthos carbon: an *in situ* ^{13}C-labeling study. Limnol Oceanogr 45:1224–1225

Moneta A, Veuger B, van Rijswijk P, Meysman F, Soetaert K, Middelburg JJ (2014) Dissolved inorganic and organic nitrogen uptake in the coastal North Sea: a seasonal study. Estuar Coast Shelf Sci 147:78–86

Oakes JM, Eyre BD (2014) Transformation and fate of microphytobenthos carbon in subtropical, intertidal sediments: potential for long-term carbon retention revealed by ^{13}C-

labeling. Biogeosciences 11:1927–1940
Oakes JM, Eyre BD, Middelburg JJ (2012) Transformation and fate of microphytobentos carbon in subtropical shallow subtidal sands: a ^{13}C-labeling study. Limnol Oceanogr 57:1846–1856
Ogawa H, Amagai Y, Koike I, Kaiser K, Benner R (2001) Production of refractory dissolved organic matter by bacteria. Science 292:917–920
Pagès A, Grice K, Welsh DT, Teasdale PT, Van Kranendonk MJ, Greenwood P (2015) Lipid biomarker and isotopic study of community distribution and biomarker preservation in a laminated microbial mat from Shark Bay, Western Australia. Microb Ecol 70:459–472
Purwoko A, Wolff WJ (2008) Low biomass of macrobenthic fauna at a tropical mudflat: An effect of latitude? Estuar Coast Shelf Sci 76:869–875
Raimbault P, Mingazzini M (1987) Diurnal variations of intracellular nitrate storage by marine diatoms: effects of nutritional state. J Exp Mar Biol Ecol 112:217–232
Rysgaard S, Risgaard-Petersen N, Nielsen LP, Revsbech NP (1993) Nitrification and denitrification in lake and estuarine sediments measured by the ^{15}N dilution technique and isotope pairing. Appl Environ Microbiol 59:2093–2098
Saburova MA, Polikarpov IG (2003) Diatom activity within soft sediments: behavioural and physiological processes. Mar Ecol Prog Ser 251:115–126
See JH, Bronk DA, Lewitus AJ (2006) Uptake of *Spartina*-derived humic nitrogen by estuarine phytoplankton in nonaxenic and axenic culture. Limnol Oceanogr 51: 2290–2299
Sipler RE, Bronk DA (2015) Dynamics of dissolved organic nitrogen. In: Hansell DA, Carlson CA (eds) Biogeochemistry of marine dissolved organic matter. Elsevier, San Diego, CA, p 128–213
Spivak AC, Ossolinski J (2016) Limited effects of nutrient enrichment on bacterial carbon sources in salt marsh tidal creek sediments. Mar Ecol Prog Ser 544:107–130
Sundbäck K, Miles A, Linares F (2006) Nitrogen dynamics in nontidal littoral sediments: role of microphytobenthos and denitrification. Estuaries Coasts 29:1196–1211
Sundbäck K, Lindehoff E, Granéli E (2011) Dissolved organic nitrogen: an important source of nitrogen for the microphytobenthos in sandy sediment. Aquat Microb Ecol 63:89–100
Underwood GJC, Perkins RG, Consalvey MC, Hanlon ARM, Oxborough K, Baker NR, Paterson DM (2005) Patterns in microphytobenthic primary productivity: species-specific variation in migratory rhythms and photosynthetic efficiency in mixed-species biofilms. Limnol Oceanogr 50:755–767
Veuger B, Middelburg JJ (2007) Incorporation of nitrogen from amino acids and urea by benthic microbes: role of bacteria versus algae and coupled incorporation of carbon. Aquat Microb Ecol 48:35–46
Veuger B, Middelburg JJ, Boschker HTS, Nieuwenhuize J, van Rijswijk P, Rochelle-Newall EJ, Navarro N (2004) Microbial uptake of dissolved organic and inorganic nitrogen in Randers Fjord. Estuar Coast Shelf Sci 61:507–515
Veuger B, Middelburg JJ, Boschker HTS, Houtekamer M (2005) Analysis of ^{15}N incorporation into D-alanine: a new method for tracing nitrogen uptake by bacteria. Limnol Oceanogr Methods 3:230–240
Veuger B, Eyre BD, Maher D, Middelburg JJ (2007a) Nitrogen incorporation and retention by bacteria, algae, and fauna in a subtropical intertidal sediment: an *in situ* ^{15}N-labeling study. Limnol Oceanogr 52:1930–1942
Veuger B, Middelburg JJ, Boschker HTS, Houtekamer M (2007b) Update of 'Analysis of ^{15}N incorporation into D-alanine: a new method for tracing nitrogen uptake by bacteria'. Limnol Oceanogr Methods 5:192–194
Veuger B, van Oevelen D, Middelburg JJ (2012) Fate of microbial nitrogen, carbon, hydrolysable amino acids, monosaccharides, and fatty acids in sediment. Geochim Cosmochim Acta 83:217–233

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