

1 **Fate of intertidal microphytobenthos nitrogen under enhanced nutrient availability:**

2 **Evidence for reduced nitrogen retention revealed through ^{15}N -labeling**

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18 **Running head:** Fate of microphytobenthos nitrogen

19 **Abstract**

20 Sediment microbial communities are an important sink for both organic and
21 inorganic nitrogen (N), with microphytobenthos (MPB) biomass having the largest
22 contribution to short-term N-assimilation and retention. Coastal waters are increasingly
23 subject to anthropogenic nutrient enrichment, but the effect of this nutrient enrichment on
24 microbial assimilation, processing, and fate of MPB-derived N (MPB-N) remains poorly
25 characterized. In this study, an MPB community was labeled in situ with a pulse of $^{15}\text{NH}_4^+$ -
26 N. Laboratory core incubations of this labeled sediment under different nutrient
27 concentrations (NH_4^+ and PO_4^{3-} : ambient, 2× ambient, 5× ambient, and 10× ambient) were
28 used to investigate changes in the processing and flux pathways of the ^{15}N -labeled MPB-N
29 across 10.5 d under nutrient enrichment. Initial production of MPB-N was stimulated by
30 nutrient addition, with higher ^{15}N incorporation into MPB in the nutrient amended treatments
31 (71-93%) than in the ambient treatment (38%). After 10.5 d, the nutrient amended treatments
32 had increased turnover of MPB-N out of MPB biomass into an uncharacterized pool of
33 sediment ON (45-75%). Increased turnover of MPB-N likely resulted from a decoupling
34 between EPS production and bacterial remineralization as inorganic nutrients were
35 preferentially used as an N source. This decoupling decreased the efflux of MPB-N via DON
36 in the amended (3.9-5.2%) versus the ambient treatment (10.9%). Exports of MPB-N to the
37 water column were relatively small, accounting for a maximum of 14% of ^{15}N exported from
38 the sediment, and were dominated by export of DON and N_2 (denitrification). Overall, there
39 was considerable retention of MPB-N over 10.5 d, but increased nutrient loading shifted N
40 from MPB biomass into other sediment ON.

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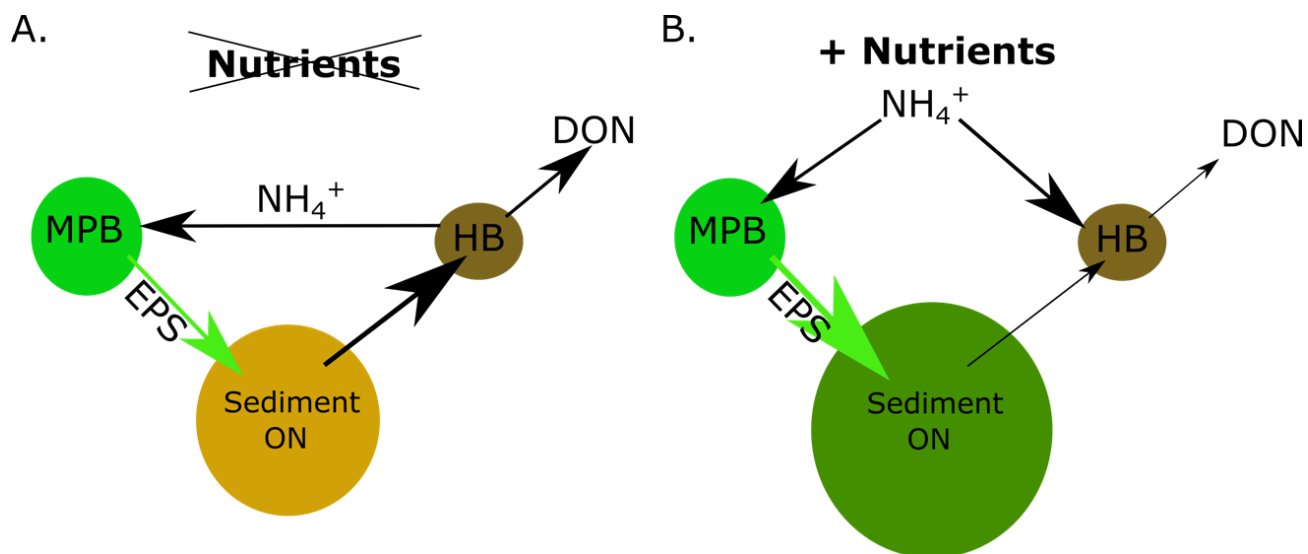
42 **1 Introduction**

43 Within shallow photic sediments, microphytobenthos (MPB) contribute significantly
44 to primary production and biomass by fixing carbon (C) and utilizing nitrogen (N) from the
45 water column and porewater (Dalsgaard, 2003; Ferguson et al., 2004; McGlathery et al.,
46 2007). Coupling between MPB production and bacterial remineralization within these
47 sediments can result in strong retention of N and recycling of C. Bacteria utilize the
48 extracellular polymeric substances (EPS) produced by MPB, while MPB rely on inorganic
49 sources or bacterially-excreted NH_4^+ from remineralization of organic matter (Cook et al.,
50 2007; Forehead et al., 2013). The fate and processing of MPB-derived C (MPB-C) within
51 sediments has been well described, with studies quantifying the incorporation of MPB-C into
52 sediment organic matter and its loss from the sediment via efflux of dissolved organic
53 carbon (DOC) and dissolved inorganic carbon (DIC) (Middelburg and Nieuwenhuize, 2001;
54 Oakes et al., 2012; Oakes and Eyre, 2014; Oakes et al., 2016). Multiple studies have
55 observed that respiration to DIC is the dominant pathway for MPB-C loss in coastal
56 sediments while little MPB-C was exported as DOC (Oakes et al., 2012; Oakes and Eyre,
57 2014; Oakes et al., 2016). In contrast to MPB-C, the processing and fate of MPB-derived N
58 (MPB-N) in coastal sediments is poorly understood, with only two comprehensive studies
59 having quantified MPB-N incorporation into sediment compartments and its loss via
60 sediment-water fluxes of dissolved inorganic nitrogen (DIN), dissolved organic nitrogen
61 (DON) and dinitrogen (N_2) (Eyre et al., 2016). These studies quantified processing and loss
62 of N via effluxes of DIN (37.3%, 1.9%, Eyre et al. 2016, Oakes et al., in press; respectively
63 throughout), DON (12.6%, 0.5%) and N_2 during tidal inundation (20.7%, 0.9%), with only
64 27% and 12.4% (lower due to physical loss) of the initially incorporated ^{15}N retained within

65 the sediment after ~30 d. MPB dominated initial uptake of ^{15}N (50% and 75% MPB vs. 2%
66 and 9% bacteria, respectively), but was only the dominant reservoir for MPB-N within
67 sediment organic nitrogen (sediment ON) by day 23 in the subtidal study (80.4 % MPB,
68 2.7% bacteria, and 15.9% uncharacterized; Eyre et al., 2016). In the intertidal study, MPB
69 and uncharacterized material contained comparable amounts of MPB-N by day 31 (50%
70 MPB, 1.6% bacteria, and 48.4%, Oakes et al., in press). Increased turnover into the
71 uncharacterized pool between these two studies may indicate quicker turnover of newly
72 assimilated MPB-N into sediment ON in intertidal settings.

73 Coastal waters are increasingly subject to anthropogenic nutrient enrichment
74 (Howarth and Marino, 2006) which alters biogeochemical pathways and can cause
75 eutrophication within otherwise healthy estuaries (Howarth and Marino, 2006; Rabalais et
76 al., 2009). Within intertidal sediments, increased availability of inorganic nutrients from
77 anthropogenic sources is expected to alter the processing of MPB-N as the microbial
78 community responds to an additional source of N that was previously in limited supply.
79 Efflux of N as DIN or N_2 occurs as the net result of mineralization and competition between
80 MPB, heterotrophic bacteria, and denitrifying bacteria for uptake of available NH_4^+ . In N-
81 limited settings, strong competition for nutrients arising from bacterial remineralization of
82 sediment ON results in strong retention of remineralized MPB-N within sediment organic
83 matter (OM) compartments (MPB, bacteria, and uncharacterized) (Fig.1A) and little N is
84 available for export via DIN or the coupled nitrification-denitrification pathways (Cook et
85 al., 2004; Cook et al., 2007; McGlathery et al., 2007; Sundback et al., 2000). With additional
86 nutrient inputs stimulation of MPB productivity has been observed to coincide with
87 decreasing efflux of DIN and N_2 to the water column (Dalsgaard, 2003; Ferguson and Eyre,

88 2013; Ferguson et al., 2004). Few studies have examined how increased N availability in the
89 water column affects the processing and fate of MPB-N within shallow photic coastal
90 settings. A laboratory study partitioned the algal and bacterial contribution to uptake of ^{15}N
91 within the sediment microbial community through the use a biomarker technique (D/L-
92 Alanine) and found that the addition of nutrients (NH_4^+ , $\text{Si}(\text{OH})_4$, HPO_4^-) increased MPB
93 incorporation of ^{15}N and decreased competition for inorganic nutrients between MPB and
94 bacteria (Cook et al. (2007); Fig. 1B). However, this study did not quantify water column
95 efflux of DIN, DON or N_2 .



96

97 **Figure 1: Conceptual diagram of the hypothesized mechanism and change in**
98 **relationship between microphytobenthos (MPB) and heterotrophic bacteria (HB)**
99 **under A) nutrient limiting and B) nutrient replete settings. A) When nutrients are**
100 **scarce there is a strong coupling between bacterial remineralization of MPB-N within**
101 **sediment ON and competition for nutrients between MPB and HB. Tight coupling**
102 **results in significant turnover of MPB-N between the three sediment compartments**
103 **and increased export of MPB-N as DON as EPS is remineralized. B) When inorganic**
104 **nutrients are available, bacterial remineralization decreases as inorganic nutrients are**
105 **preferentially used over sediment ON and results in decreased competition between**
106 **MPB and HB for nutrients. Decoupling between HB remineralization of MPB-derived**
107 **OM results in the buildup of MPB-N in sediment ON and decreased production of**
108 **DON as remineralization is decreased.**

109 In this ^{15}N pulse-chase study we aimed to quantify changes in the processing and fate
110 of MPB-N within subtropical intertidal sediments due to increased water column nutrient
111 availability. Pathways considered included transfer of MPB-N through sediment ON
112 compartments (MPB, bacteria and uncharacterized), and export via efflux of NH_4^+ , DON,
113 and N_2 to the overlying water column. We expected decreased remineralization, re-capture,
114 and recycling of processed MPB-N through the sediment compartments as introduced
115 inorganic nutrients were preferentially utilized by the microbial community. This could
116 result in either increased retention of MPB-N within the uncharacterized sediment organic
117 matter pool due to decreased utilization by both MPB and bacteria, or increased MPB-N
118 export as MPB-N is effluxed to the water column without being re-captured and recycled. As
119 a result of decreased recycling of MPB-N within the microbial community, we expect that
120 export of ^{15}N via efflux of NH_4^+ , DON, and N_2 would be reduced as a result of increased
121 sediment retention within N replete treatments (Fig. 1B).

122 **2 Methods**

123 2.1 *Study site*

124 The study site was a subtropical intertidal shoal ~2 km upstream of the mouth of the
125 Richmond River estuary in New South Wales, Australia ($28^\circ 52'30''\text{S}$, $153^\circ 33'26''\text{E}$) that
126 was simultaneously used for a ^{13}C pulse-chase study (Riekenberg et al. 2018). The 6900 km^2
127 Richmond River catchment has a mean annual rainfall of 1300 mm (McKee et al., 2000) and
128 an average flow rate of 2200 ML d^{-1} (daily gauged flow adjusted for catchment area,
129 averaged over years for which data was available; 1970–2013). Although the Richmond
130 River estuary has highly variable flushing, salinity, and nutrient concentrations associated

131 with frequent episodic rainfall events and flooding (Eyre, 1997; McKee et al., 2000), this
132 study was undertaken during a period of average rainfall (~200 mm in the month prior). The
133 site experiences semidiurnal tides with a range of ~2 m. Samples were collected in January
134 2015 (summer) with average site water temperature of $25.6 \pm 2.3^{\circ}\text{C}$. Sediment at depths of 0-
135 2 cm, 2-5 cm and 5-10 cm was dominated by fine sand (66%-73%) and sediment across 0-10
136 cm had an organic N content of $1.2 \pm 0.1 \text{ mol N m}^{-2}$. Sediment molar C:N was lowest at 2-5
137 cm, but comparable across other depths (0-2 cm 17.2 ± 1.7 , 2-5 cm 10.9 ± 0.5 , 5-10 cm 16.2
138 ± 2.2). The MPB assemblage was dominated by pennate diatoms with few larger
139 heterotrophs ($>500 \mu\text{M}$) and no cyanobacteria observed under light microscopy ($1000\times$) as
140 has been described previously for this site (Oakes and Eyre, 2014; Riekenberg et al., 2017;
141 Riekenberg et al., 2018). Foraminifera were the dominant heterotrophs observed within site
142 sediments, but were not considered in the current study, as they have previously been found
143 to make only a minimal contribution to uptake of MPB-N (~1%) in sediments in the adjacent
144 Brunswick Estuary (Eyre et al., 2016).

145 *2.2 Experimental overview*

146 An in situ application of ^{15}N (99% NH_4^+) was used to introduce a pulse of ^{15}N -
147 labeled MPB-N into sediment. Unincorporated ^{15}N was flushed from the sediment during the
148 next tidal inundation of the site. Sediment cores were then collected and incubated in the
149 laboratory under four nutrient enrichment scenarios (ambient, minimal, moderate, and
150 elevated) using pulsed nutrient additions. Laboratory incubation of cores allowed for explicit
151 control of nutrient additions and was used to examine the fate and processing of the pulse of
152 MPB-N over 10.5 d. Sediments remained inundated during incubation to minimize loss of
153 MPB-N through physical processes as we were primarily interested in biological sediment

154 processing and previous studies have shown that physical loss processes can have an
155 important seasonal role in the processing of MPB-N (Nielsen et al., 2017).

156 2.3 ¹⁵N labeling

157 An experimental plot (2 m²) of bare sediment free of large animal burrows was
158 labeled with ¹⁵N (99% ¹⁵NH₄⁺) when sediments were initially exposed during the ebbing tide
159 in the middle of the day. To ensure even application, the plot was divided into 400 cm²
160 subplots and a 20 ml aliquot of 40 μmol ¹⁵NH₄⁺ solution was sprayed onto each subplot using
161 motorized sprayers. This gave a label application rate of 2 mmol ¹⁵NH₄⁺ m⁻². The labeled
162 solution was prepared with NaCl amended Milli-Q to match site salinity (34.6). Assimilation
163 of ¹⁵N by the sediment community occurred during the 4 h prior to tidal inundation. The tide
164 then removed unincorporated ¹⁵N; as confirmed by measurement of removal of ¹⁵N from the
165 initial application (90.3%) in 0-10 cm of sediment during initial sampling.

166 2.4 Sample collection

167 Prior to application of ¹⁵N, 3 cores (9 cm diameter, 20 cm depth) were collected
168 immediately adjacent to the treatment plot and were immediately extruded and sectioned (0-
169 2 cm, 2-5 cm, and 5-10 cm) to provide unlabeled control samples for sediment ON δ¹⁵N. At
170 the next low tide (11 h after label application), 43 sediment cores were collected from the
171 labeled plot using clear acrylic core liners (9 cm diameter, 47 cm height). Immediately, three
172 cores were extruded and sectioned, as described above. The sediment samples were placed
173 into plastic bags, transported to the laboratory on ice, stored frozen in the dark (-20 °C), and
174 were later used to determine initial ¹⁵N uptake, grain size distribution across sediment
175 depths, and chlorophyll-*α* (Chl-*α*) concentration within 0-1 cm sediments. The 40 remaining

176 core liners were sealed with acrylic bottom plates and transported to the laboratory for
177 incubation within 2 h of sampling. Site water (400 L) was collected and transported to the
178 laboratory for use during incubations.

179 *2.5 Nutrient Amendment*

180 Sediment cores were incubated in the laboratory with a range of nutrient
181 concentrations in the overlying water that were below the sediment capacity for uptake.
182 Incubation tanks (85 L volume), each containing ten cores, were established with nutrients at
183 ambient concentration (site water: DIN of $2.5 \pm 0.04 \mu\text{M N L}^{-1}$, TP $0.9 \pm 0.09 \mu\text{M P L}^{-1}$
184 measured on incoming tide), and with N (NH_4^+) and P (H_3PO_4) amendments to site water at
185 $2\times$ (minimal treatment), $5\times$ (moderate treatment), and $10\times$ (elevated treatment) average
186 water column concentrations near the study site ($4 \mu\text{M L}^{-1} \text{NH}_4^+$ and $5 \mu\text{M L}^{-1} \text{PO}_4^{3-}$, Eyre
187 (2000)). Nutrient amendments were added to the incubation tanks and to bags of
188 replacement water an hour prior to cores being transferred into treatment tanks for
189 incubation. Two additional identical amendments of NH_4^+ were added to maintain the
190 respective treatment concentrations to the incubation tanks after sampling at 1.5 d and 3.5 d
191 of incubation to ensure that nutrient limitation did not develop after uptake of the initial
192 treatment addition (Appendix Fig. 1). There was no significant accumulation of NH_4^+ within
193 treatment tank waters, as nutrients were readily processed and removed from the water
194 column within 24 h of additions. An addition of sodium metasilicate (Na_2SiO_3 , $17 \mu\text{mol Si}$
195 L^{-1}) was also added to all incubation tanks at the end of the 2.5 d of incubation to prevent
196 secondary limitation of Si.

197 *2.6 Benthic flux incubations*

198 In the laboratory, cores were fitted with magnetic stir bars positioned 10 cm above
199 the sediment surface, filled with ~2 L of site water, and randomly allocated to one of the four
200 treatment tanks (ambient, minimal, moderate, elevated; ten cores per treatment). Water
201 within treatment tanks and cores was continuously recirculated, held at in situ temperature
202 ($25 \pm 1^\circ\text{C}$) using a temperature controller, and aerated. Cores were stirred at a rate below the
203 threshold for sediment resuspension (Ferguson et al., 2003) via a rotating magnet at the
204 center of each treatment tank, which interacted with the magnetic stir bars. Three sodium
205 halide lamps suspended above the treatment tanks approximated the average light level
206 measured at the sediment surface during inundation ($941.4 \pm 139 \mu\text{E m}^{-2} \text{ s}^{-1}$) by providing
207 $824 \pm 40 \mu\text{E m}^{-2} \text{ s}^{-1}$ to the sediment/water interface within the cores on a 12 h light/12 h dark
208 cycle. Cores were allowed to acclimate in the treatment tanks for 6 h prior to the start of
209 incubation, allowing sediment microhabitats to re-establish. Cores remained open to the tank
210 water until 30 min before sampling when clear Plexiglas lids were fitted to each core liner to
211 seal in overlying water within the core for the duration of the incubation (~16 h). On each
212 sampling occasion, 8 cores (2 per treatment at 1.5, 2.5, 3.5 and 10.5 d) were sampled for
213 dissolved oxygen ($\pm 0.01 \text{ mg/L}$) and temperature ($\pm 0.01^\circ\text{C}$) using a Hach HQ40d multi-
214 parameter meter via a sampling port in the core lid. For each sampling period, measurements
215 were made at three time points (initial, dark end/light start, and light end) to allow dark,
216 light, and net flux calculations. Initial samples were taken 30 min after closure of the lids,
217 dark end/light start samples were taken after ~12 hours incubation with no light, and light
218 end samples were taken 3 hours after illumination at the end of the dark sampling. At each
219 initial, dark end/light start, and light end sampling duplicate samples for $\text{N}_2:\text{Ar}$ analysis were
220 collected by allowing water from suspended replacement water bags to gently displace

221 sample water out of the core via tubing into 7 ml gastight glass-stoppered glass vials. These
222 vials were allowed to overflow by 2-3 volumes, killed with 20 μl of saturated HgCl_2 and
223 stored submerged at ambient temperature. To determine NH_4^+ , NO_3^- , and DON
224 concentrations and $\delta^{15}\text{N}$ values, sample water was syringe-filtered (0.45 μm cellulose
225 acetate) into 10 ml and 50 ml polyethylene vials, leaving a headspace, and stored frozen (-
226 20°C). To determine $\delta^{15}\text{N}$ values for N_2 , sample water was filtered (0.45 μm cellulose
227 acetate) into a 20 ml glass vial containing 500 μl of 2M NaOH. These samples were sealed
228 without headspace using a lid containing a teflon-coated septum and refrigerated for storage.
229 After completion of the dark/light flux incubation, cores were sacrificed and sediment was
230 extruded and sectioned (top scrape, 0.2-2 cm, 2-5 cm, and 5-10 cm depths). A subsample (1
231 cm^3) of sediment was taken for Chl- α analysis from the 0-1 cm depth prior to sectioning of
232 the 0-2 cm layer using a spatula and placed in a 10 ml centrifuge vial containing 5 ml of 90%
233 acetone. Sediment samples were placed in ziplock bags and stored in the dark at -20°C until
234 analysis.

235 *2.7 Sample analysis-*

236 A portion of each sediment sample was freeze-dried and KCl-extracted (2M KCl) for
237 analysis of concentration and $\delta^{15}\text{N}$ of sediment ON. Both washed (KCl-extracted) and raw
238 (non KCl-extracted) sediment samples were dried (60°C) and weighed into tin capsules for
239 analysis of $\delta^{15}\text{N}$ and %N using a Flash elemental analyzer coupled on-line to a Thermo
240 Fisher Delta V Plus isotope ratio mass spectrometer (IRMS). Reproducibility of $\delta^{15}\text{N}$ values
241 for samples with $\delta^{15}\text{N}$ enrichment $<100\text{‰}$ was $\pm 0.2\text{‰}$. Precision decreased with enrichment
242 beyond 100%. Additional freeze-dried sediment was analyzed for D- and L- alanine

243 concentration and $\delta^{15}\text{N}$ to determine the relative contribution to uptake of ^{15}N by both MPB
244 and bacteria, as described below.

245 N_2 concentrations were analyzed using $\text{N}_2:\text{Ar}$ measured with a membrane inlet mass
246 spectrometer with O_2 removal (Eyre et al., 2002). Concentrations for DON , NH_4^+ , and NO_3^-
247 were determined through colorimetric analysis on a four channel Flow Injection Analyzer
248 (FIA, Lachat QuickChem 8000) (Eyre et al., 2011). DON was quantified via measurement of
249 total nitrogen (TN) through flow injection analysis using persulfate digestion (Valderrama,
250 1981) and subsequent subtraction of DIN ($\text{NH}_4^+ + \text{NO}_3^-$) concentrations (Lachat, 1994).
251 Prior to analysis, vials for N_2 analysis had 4 ml of water displaced with a helium headspace
252 and were held at ambient temperature. $\delta^{15}\text{N}_2$ concentration of the He headspace in N_2
253 samples was determined via gas chromatography isotope ratio mass spectrometry using a
254 Thermo Trace Ultra Gas Chromatograph coupled to a Delta V Plus IRMS via a Thermo
255 Conflo III interface.

256 $\delta^{15}\text{N}$ for NH_4^+ was measured for samples with a concentration of $>0.5 \mu\text{mol N L}^{-1}$ (as
257 determined by Flow Injection Analysis) via chemical conversion of NH_4^+ to NO_2^- followed
258 by azide conversion of NO_2^- to N_2O (Zhang et al., 2007). Samples with a concentration <0.5
259 $\mu\text{mol N L}^{-1}$ were below the detection limit for this method and were assumed to contribute a
260 negligible amount of $\delta^{15}\text{N}$ for calculations of MPB-N efflux. Concentrations of NO_3^- were
261 consistently below the detection limit ($0.5 \mu\text{mol N L}^{-1}$) for the denitrifier method (Sigman et
262 al., 2001) for the analysis of $^{15}\text{NO}_3^-$; NO_3^- was assumed to have a minimal contribution to the
263 efflux of MPB-N. The isotopic composition of DON was determined after persulfate
264 oxidation of TN to NO_3^- , followed by conversion to N_2O using the denitrifier method. The
265 $\delta^{15}\text{N}$ -DON was calculated using a two source mixing model based on $\delta^{15}\text{N}$ of TN and

266 $^{15}\text{NH}_4^+$ for samples with sufficient concentrations (Erler et al., 2014). Where NH_4^+
267 concentrations were $<0.5 \mu\text{mol N L}^{-1}$, no correction was applied and $\delta^{15}\text{N-TN}$ was used as
268 $\delta^{15}\text{N-DON}$. NO_3^- was not considered in this calculation because it was consistently below
269 $0.5 \mu\text{mol N L}^{-1}$ while DON concentrations were $\sim 18 \mu\text{mol N L}^{-1}$ ($\sim 36 \times$ greater).

270 The relative contribution of MPB and bacteria to ^{15}N uptake and transfer was
271 determined through compound-specific analysis of D- and L- alanine following acid
272 hydrolysis and extraction of total hydrolysable amino acids from 7 g of freeze-dried
273 sediment. Due to the laborious extraction process, only single replicates from the 0.2-2 cm,
274 2-5 cm and 5-10 cm depths of each core at each time were analyzed. Extraction and analysis
275 was performed as described by Veuger et al. (2005) and Veuger et al. (2007a), with minor
276 modifications. Briefly, freeze-dried sediment (7 g) was rinsed with 2 M HCl, centrifuged (5
277 min, 900 g), and the supernatant discarded. This was repeated with milli-Q water $3\times$, and the
278 sediment pellet was then hydrolyzed with 6 M HCl (110°C , 20 h). After hydrolysis, a
279 standard spike of L-norleucine (2.5 mg ml^{-1}) was added and the sample shaken and
280 centrifuged again. The supernatant was removed and retained. The pellet was then
281 resuspended in 10 ml milli-Q water, centrifuged $2\times$, and the additional supernatant also
282 retained. The combined supernatant (25 ml) was purified through cation exchange
283 chromatography (Dowex 50WX8-100), and the amino acids were derivatized with
284 isopropanol and penta-fluoropropionic anhydride, and further purified via solvent extraction
285 with chloroform. Concentrations and $\delta^{15}\text{N}$ of the derivatized amino acids were determined
286 via gas chromatography-combustion-isotope ratio mass spectrometry (GC-c-IRMS) on a HP
287 6890 GC interfaced via a Thermo Conflo III interfaced with a Thermo Delta V Plus IRMS
288 using the column and ramp schedule described in Eyre et al. (2016).

289 Chl- α was extracted by adding a 9/1 acetone/water mix followed with sonication (15
290 min), centrifugation (5 min, 9 g), and removal of the supernatant for analysis by colorimetry
291 (Lorenzen, 1967). ON within bulk sediment was analyzed for $\delta^{15}\text{N}$ after KCl-extraction to
292 remove any N adsorbed to sediment particles. Briefly, 2 g of homogenized freeze-dried
293 sediment were combined with 5 ml of 2M KCl in a centrifuge tube, shaken for 5 min, and
294 centrifuged (15 min, 9 g). The supernatant was discarded, and three times thereafter 5 ml of
295 milli-Q was added to the pellet prior to shaking (5 min), centrifugation (15 min, 9 g), and
296 removal of the supernatant. The KCl-extracted pellet was dried at 60 °C to constant weight
297 and weighed into a tin capsule for analysis of $\delta^{15}\text{N}$ and %N using a Flash elemental analyzer
298 coupled on-line to a Thermo Fisher Delta V Plus IRMS. Reproducibility of $\delta^{15}\text{N}$ for samples
299 with enrichment < 100 ‰ was ± 0.2 ‰. Precision decreased with enrichment beyond 100
300 ‰.

301 2.8 Calculations

302 The total ON mass (biomass) of sediment was calculated as the product of %N and
303 dry mass of sediment per unit area. Incorporation of ^{15}N into sediment ON, bacteria and
304 MPB ($\text{mmol } ^{15}\text{N m}^{-2}$) was calculated as the product of excess ^{15}N (fraction ^{15}N in sample –
305 fraction ^{15}N in control) and the mass of ON within each pool.

306 Biomass and excess ^{15}N contained in bacteria and MPB was estimated based on the
307 excess ^{15}N contained within the amino acids D- and L- alanine (Ala). The assumptions and
308 uncertainty associated with this method are discussed in detail by Veuger et al. (2005) and
309 Veuger et al. (2007b) and estimates of error propagation for individual variables are
310 provided in Riekenberg et al. (2017). During acid hydrolysis, some racemization of L- Ala to

311 D- Ala occurs, resulting in a D- : L- Ala ratio of 0.015-0.02 for algal cultures (Veuger et al.,
312 2007b) and 0.006 for dissolved free amino acids (Kaiser and Benner, 2005). In the current
313 study, uncorrected D/L-Ala ratios as low as 0.009 were observed, and therefore a
314 racemization rate of 0.006 was applied to correct for L-Ala racemization. This value
315 corresponded well to measured values for racemization of L-Ala standards during hydrolysis
316 under laboratory conditions. Measured D- and L- Ala concentrations were corrected for
317 racemization using the equations of Kaiser and Benner (2005):

318 1. $L^*_0 = (L^* - D^* (0.6)) / 100 - 0.6$

319 2. $D^*_0 = D^* + L^* - L^*_0$

320 where L^*_0 and D^*_0 and L^* and D^* are the concentrations of L- and D-Ala occurring originally
321 and after hydrolysis, respectively. Original concentrations of D- and L-Ala represent
322 concentrations of both forms of alanine derived from bacterial and algal biomass prior to
323 hydrolysis induced racemization.

324 Biomass of heterotrophic bacteria (HB), MPB, and total microbial biomass were
325 calculated as described by Veuger et al. (2005) and Veuger et al. (2007b), as follows:

326 3. $HB\ L\text{-Ala} = D^*_0\text{-Ala} \times 20$

327 4. $HB\ \text{contribution to microbial biomass (\%)} = (HB\ L\text{-Ala} / L^*_0\text{-Ala}) \times 100$

328 5. $MPB\ \text{contribution to microbial biomass (\%)} = 1 - HB\ \text{contribution to microbial}$
329 biomass

330 6. $HB\ \text{biomass} = D^*_0\text{-Ala} \times 400$

331 where 20 is the conversion for excess ^{15}N in D-Ala (5%) that represents excess ^{15}N present
332 in bacterial L-Ala (20 \times). This conversion factor is based on culture analysis (Veuger et al.,

333 2005; Veuger et al., 2007b), is further discussed in Eyre et al. (2016), and assumes a
334 negligible contribution of Gram positive bacteria or cyanobacteria to the benthic community.
335 The lack of cyanobacteria in the current study was confirmed through both microscope work
336 and PLFA analysis (Riekenberg et al. 2018). The conversion factor of 400 (Veuger et al.,
337 2005) accounts for the % N in dry bacterial biomass (12%; (Madigan et al., 2000), and yields
338 a D-Ala content of 0.25. ^{15}N uptake into HB, MPB, and total microbial biomass were
339 calculated using equations 3-8, substituting excess ^{15}N values for L^*_{0-} and D^*_{0-} Ala in place
340 of L^*_{0-} and D^*_{0-} Ala contents.

341 Total excess ^{15}N within water column NH_4^+ , DON and N_2 were calculated for the
342 beginning and end of dark incubation period and the end of the light incubation period as the
343 product of excess ^{15}N in NH_4^+ , DON and N_2 (excess $^{15}\text{N}_{\text{sample}} - \text{excess } ^{15}\text{N}_{\text{control}}$), core volume,
344 and concentration of NH_4^+ , DON, and N_2 . Total excess flux of ^{15}N in NH_4^+ , DON, and N_2 was
345 calculated as:

346 7.
$$\text{Excess } ^{15}\text{N flux} = (\text{Excess } ^{15}\text{N}_{\text{start}} - \text{Excess } ^{15}\text{N}_{\text{end}}) / \text{SA} / t$$

347 where excess $^{15}\text{N}_{\text{start}}$ and excess $^{15}\text{N}_{\text{end}}$ represent excess ^{15}N in NH_4^+ , DON, and N_2 at the start
348 and end of the dark and light incubation period, SA is sediment surface area, and t is the
349 incubation period (h) in the light or the dark. Net flux of excess ^{15}N (excess $^{15}\text{N m}^{-2} \text{ h}^{-1}$) for
350 NH_4^+ , DON, and N_2 was calculated as:

351 8.
$$\text{Net flux} = (\text{dark flux} * \text{dark hours}) + (\text{light flux} * \text{light hours}) / 24 \text{ h}$$

352 export of ^{15}N to the water column via $^{15}\text{NH}_4^+$, DO^{15}N , and $^{15}\text{N}_2$ was estimated through
353 interpolation between efflux measurements for each treatment by calculation of the area
354 beneath the curve during each sampling period. Incubations for dark periods were ~12 h
355 while light periods were limited to ~4 h as required to prevent supersaturation and bubble

356 development samples taken to measure N_2/Ar for N_2 fluxes. Fluxes of excess $^{15}NO_3^-$ were
357 assumed to be negligible and were therefore not included in excess flux measurements for
358 DIN because core water column concentrations were consistently low and below the
359 detection limit required for analysis of $\delta^{15}N$ values via the denitrifier method ($0.5 \mu M N L^{-1}$;
360 Sigman et al. (2001).

361 *2.9 Data Analysis*

362 In the initial sampling period, there was incorporation of MPB-N below 2 cm (2-10
363 cm) within all of the treatments. A one-way analysis of variance was used (ANOVA) to
364 determine whether there was a significant difference amongst treatments ($\alpha = 0.05$). A one-
365 way ANOVA was also used to determine if there was a significant difference among
366 treatments in ^{15}N uptake into MPB during the initial sampling period. Levene's tests
367 indicated that variances were homogenous in all cases. When significant differences were
368 indicated, post-hoc Tukey tests investigated the differences between treatments ($\alpha = 0.05$).

369 Two-way ANOVAs were separately applied to Chl- α and ^{15}N incorporation with
370 pooled depths of 0-2 cm and 2-10 cm across sampling times to determine whether significant
371 differences occurred between treatments or depths within each time period. Additional two-
372 way ANOVAs were used to investigate whether significant differences occurred between
373 sediment compartments (uncharacterized, MPB, and bacteria) within ambient or nutrient
374 amended treatments. Levene's tests indicated that variances were homogenous in all cases,
375 and there were no significant interactions indicated during these analyses. Where ANOVAs
376 indicated a significant difference ($\alpha = 0.05$), post-hoc Tukey tests were applied to investigate
377 significant differences between variables ($\alpha = 0.05$).

378 **3 Results**

379 *3.1 Sediment characteristics*

380 Sediment Chl- α at 0.2-2 cm depth averaged $48.3 \pm 2.9 \text{ mg m}^{-2}$ (mean \pm SE) across all
381 cores collected in this study and was not affected by time or treatment (two-way ANOVA:
382 $F_{3,20}=0.8, p=0.5$). Sediment organic matter across the 0-10 cm depth had a molar C:N ratio of
383 18.1 ± 1.1 and contained $1.2 \pm 0.1 \text{ mol N m}^{-2}$. The %N of sediment was low and even across
384 all sediment layers (0.02%). Within initial cores taken after labeling, but prior to incubation,
385 MPB biomass had the greatest contribution to sediment ON (20%) in the 0-2 cm depth, with
386 bacteria representing only 4% (Table 1). Due to uptake of ^{15}N being largely confined to ON
387 in the uppermost sediment layer (0-2 cm) in the initial cores, deeper depths (2-5 cm and 5-10
388 cm) were not examined for uptake into MPB, bacteria, and uncharacterized sediment
389 compartments. Within the ambient treatment across samplings, MPB had the greatest
390 contribution to ON in the uppermost layer (0-2 cm, 28.4%) and contributed less in deeper
391 depths (2-5 cm, 10.7%; 5-10 cm, 6.4 %, Table 1). Bacterial contribution to ON was less than
392 MPB in the uppermost layer (0-2 cm, 17.4%) and decreased with depth (2-5 cm, 7.1%; 5-10
393 cm, 6.0%). Uncharacterized ON made the largest contribution to both initial (75%) and
394 ambient (54-88%, Table 1) sediments within this study.

395

	0-2 cm			2-5 cm			5-10 cm		
	mmol N m ⁻²	SE	%N	mmol N m ⁻²	SE	%N	mmol N m ⁻²	SE	%N
Initial									
Sediment	268.3	19.1	21.2	465.7	29.7	36.9	529.7	44.7	41.9
MPB	53.8	3.1	20.1						
Bacteria	11.1	0.0	4.1						
Uncharacterized	201.6	36.1	75.1						
Ambient									
Sediment	231.1	18.8	19.8	421.4	93.3	36.1	515.7	73.3	44.1
MPB	65.6	8.1	28.4	45.2	10.5	10.7	33.2	13.2	6.4
Bacteria	40.3	10.5	17.4	29.8	4.6	7.1	31.1	7.3	6.0
Uncharacterized	125.2	23.0	54.2	346.4	94.0	82.2	451.3	74.9	87.5

396

397 **Table 1: Mean biomass for sediment compartments for 0-2 cm for initially sampled**
 398 **cores after tidal flushing and for 0-2 cm, 2-5 cm, and 5-10 cm for all cores sampled in**
 399 **the ambient treatment (mean ± SE). %N is the percentage N within each sediment**
 400 **depth for individual sediment compartments (MPB, Bacteria, and Uncharacterized)**
 401 **except for sediment N, where it represents the portion of N relative to the total N in 0-**
 402 **10 cm of sediment.**

403 *3.2 Uptake of ¹⁵N*

404 The pulse of ¹⁵N was rapidly incorporated into sediment ON, with 193 ± 44.8 μmol
 405 ¹⁵N m⁻² detected in 0-2 cm of sediment 11 h after label application, when the first ¹⁵N-
 406 labeled cores were collected after tidal flushing. At 0-2 cm, ¹⁵N uptake into the microbial
 407 community was dominated by MPB (MPB 53.8 mmol N m⁻², 83%; bacteria 11.1 mmol N m⁻
 408 ², 17%, Table 1). At this time ¹⁵N was largely confined to the top scrape (upper 2 mm of
 409 sediment) and 0-2 cm depths (84.8 mmol N m⁻², 43.8% and 85.4 mmol N m⁻²; 44.1% of total
 410 incorporated ¹⁵N, 193.4 mmol N m⁻², respectively), but some downward transport of ¹⁵N was
 411 evident in the 2-5 cm and 5-10 cm depths (10.2 mmol N m⁻², 5.3%; 13.1 mmol N m⁻², 6.8%,
 412 respectively). Due to limited labeling uptake within the lower depths during the initial
 413 sampling, we did not run D/L-Ala analysis for the microbial community in the 2-5 cm and 5-
 414 10 cm depths.

415 ^{15}N was incorporated into deeper sediments (2-5 cm and 5-10 cm) within all
416 treatments by the initial sampling time (Fig. 2). By 0.5 d, comparable amounts of the
417 assimilated MPB-N (8-16%) was in sediment below 2 cm (One-way ANOVA: $F_{3,7}=5.3$,
418 $p=0.07$, ambient 12%; minimal 16%; moderate 8%; and elevated 10%). After 10.5 d there
419 was significantly more ^{15}N contained in the 0-2 cm layer (TS + 0.2-2 cm) than the 2-5 cm or
420 5-10 cm layers across all treatments (two-way ANOVA: $F_{2, 108}=1070$, $p<0.001$; Fig. 2;
421 ambient 83.4%, minimal 81.9%, moderate 79.1%, and elevated 78.6%). Tukey tests
422 indicated that incorporation into the 2-5 and 5-10 cm depths were similarly low for all
423 treatments across sampling times.

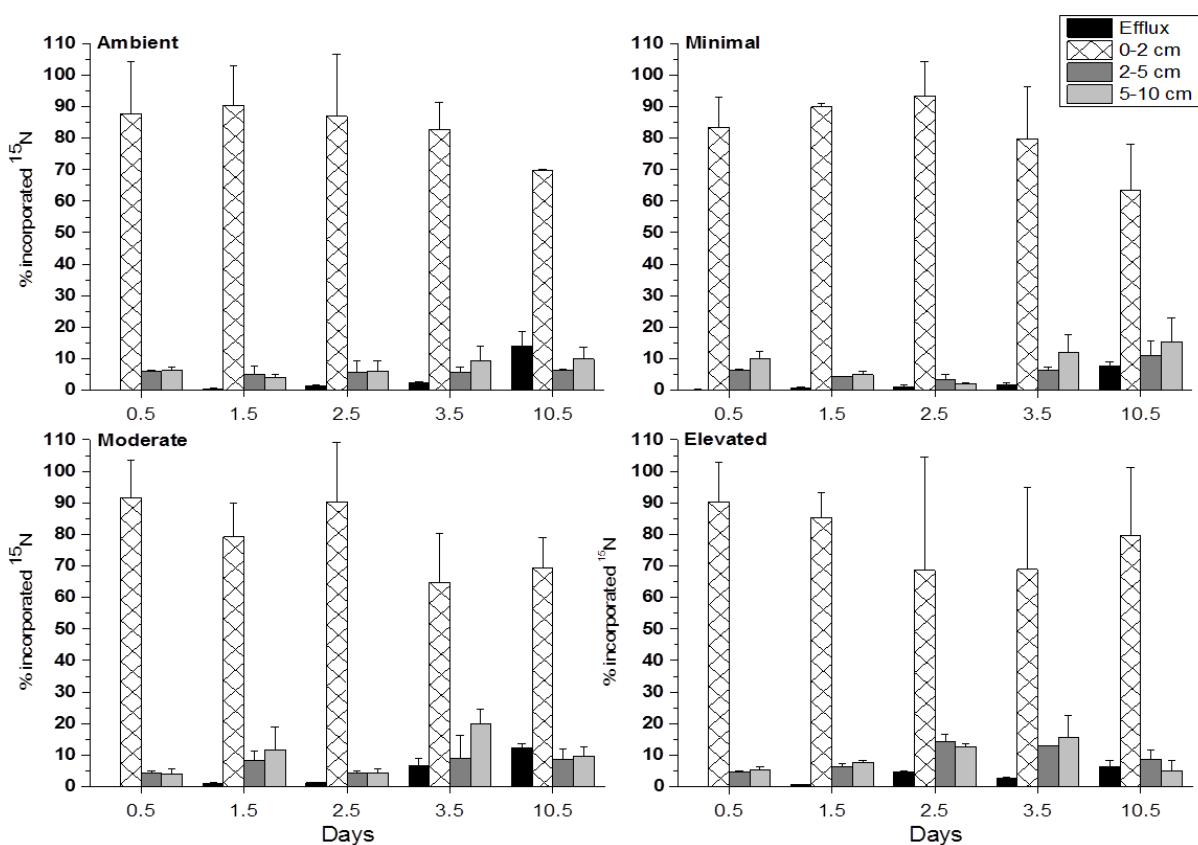
424 Uptake of ^{15}N into microbial biomass (MPB and bacteria) accounted for 22-95% of
425 the total ^{15}N incorporated into sediment ON across all treatments and sampling times (Fig.
426 3). Initial ^{15}N contained (0.5 d) in MPB during the incubation was higher in the elevated
427 treatment (one-way ANOVA: $F_{3,7}=6.9$, $p=0.046$, minimal, $71\pm 21\%$; moderate, $75\pm 12\%$;
428 and elevated, $93\pm 1\%$, mean \pm SE) than in the ambient treatment ($38\pm 4\%$) with Tukey tests
429 indicating a significant difference between elevated and ambient treatments ($p=0.04$) with
430 values for minimal and moderate treatments falling intermediate between them. This
431 indicates that initial production of MPB-N was stimulated by increased nutrient availability
432 and remained in MPB at 0.5 d. Within the microbial community after 0.5 d, distribution of
433 ^{15}N continued to be dominated by MPB for all treatments (ambient $83\pm 3\%$; minimal $79\pm 4\%$;
434 moderate $74\pm 2\%$; elevated $75\pm 5\%$, Fig. 4). Bacterial contribution to ^{15}N uptake was
435 comparable or higher during the later sampling times (1.5 to 10.5 d) in the nutrient amended
436 treatments (minimal, 15-23%; moderate, 2-49%; elevated, 2-40%, Fig.15) compared to the
437 ambient treatment (10-23%).

438 In the ambient treatment, distribution of ^{15}N between microbial biomass and
439 uncharacterized sediment ON remained comparable across the 10.5 d incubation (one-way
440 ANOVA: $F_{4,9}=0.5$, $p=0.8$; Fig.14). In contrast, in the nutrient amended treatments ^{15}N
441 incorporation was initially dominated by microbial biomass, primarily MPB, but shifted
442 towards increased incorporation into the uncharacterized pool as the incubations progressed
443 (two-way ANOVAs: minimal, $F_{1,10}=10$, $p=0.01$; moderate, $F_{1,10}=10$, $p<0.001$; elevated, $F_{1,10}$
444 $=10$, $p<0.001$; Fig. 3). In the nutrient amended treatments, the ^{15}N contributing to the
445 increased uncharacterized pool appeared to be largely sourced from MPB, resulting in
446 reduced MPB contributions by 10.5 d (minimal 38%; moderate 12%; elevated 16%; Fig. 3).
447 Bacterial contributions to ^{15}N remained comparable across the incubation period in nutrient
448 amended treatments (two-way ANOVA: $F_{2,15}=0.1$, $p=0.9$).

449 3.3 Loss of ^{15}N from sediments

450 There was relatively little efflux of ^{15}N from the sediment to the water column in this
451 study, with loss pathways accounting for a maximum of 14% of the initially incorporated
452 ^{15}N across treatments (Fig. 2). Across all treatments, most of this loss to the water column
453 occurred in the form of DON and N_2 fluxes (Fig. 5), with only a minor contribution from
454 DIN (NH_4^+ , maximum of 0.3%). Within the ambient treatment, DON was the largest export
455 pathway accounting for loss over 10.5 d of 10.5% of the initially incorporated ^{15}N (Fig. 5 &
456 6). Loss of ^{15}N from the sediments via DON effluxes was lower within the nutrient amended
457 treatments (minimal, 4.8%; moderate, 5.2%; elevated, 5.2%) but was not statistically
458 significant. Export of ^{15}N via N_2 was comparably low between ambient, minimal and
459 elevated treatments (3.3%, 2.8%, and 2.3%, respectively), but was higher in the moderate
460 treatment (7.1%, Fig. 4 & 6). Overall export via combined efflux pathways was low across

461 all treatments, with the bulk of MPB-N remaining within the sediment by 10.5 d (ambient 86
462 $\pm 4\%$, minimal $90 \pm 2\%$, moderate $88 \pm 2\%$, and elevated $94 \pm 3\%$, Fig. 6). The majority of
463 ^{15}N remaining in the sediment was found in the 0-2 cm depth for all treatments (ambient
464 70%; minimal 63%; moderate 69%; and elevated 80%, Fig.2 while ^{15}N incorporation
465 remained lower for depths below 2 cm (ambient 16%; minimal 27%; moderate 18%; and
466 elevated 14%).



467

468 **Figure 2: Nitrogen budget for excess ^{15}N within sediment ON at 0 to 2 cm, 2 to 5 cm,**
469 **and 5-10 cm, and cumulative excess ^{15}N exported to the water column combined via**
470 **effluxes of NH_4^+ , DON, and N_2 for each treatment at each sampling time. All values are**
471 **as a percentage of the ^{15}N initially incorporated into sediment ON (0-10 cm). Some**
472 **error bars are too small to be seen (mean + SE).**

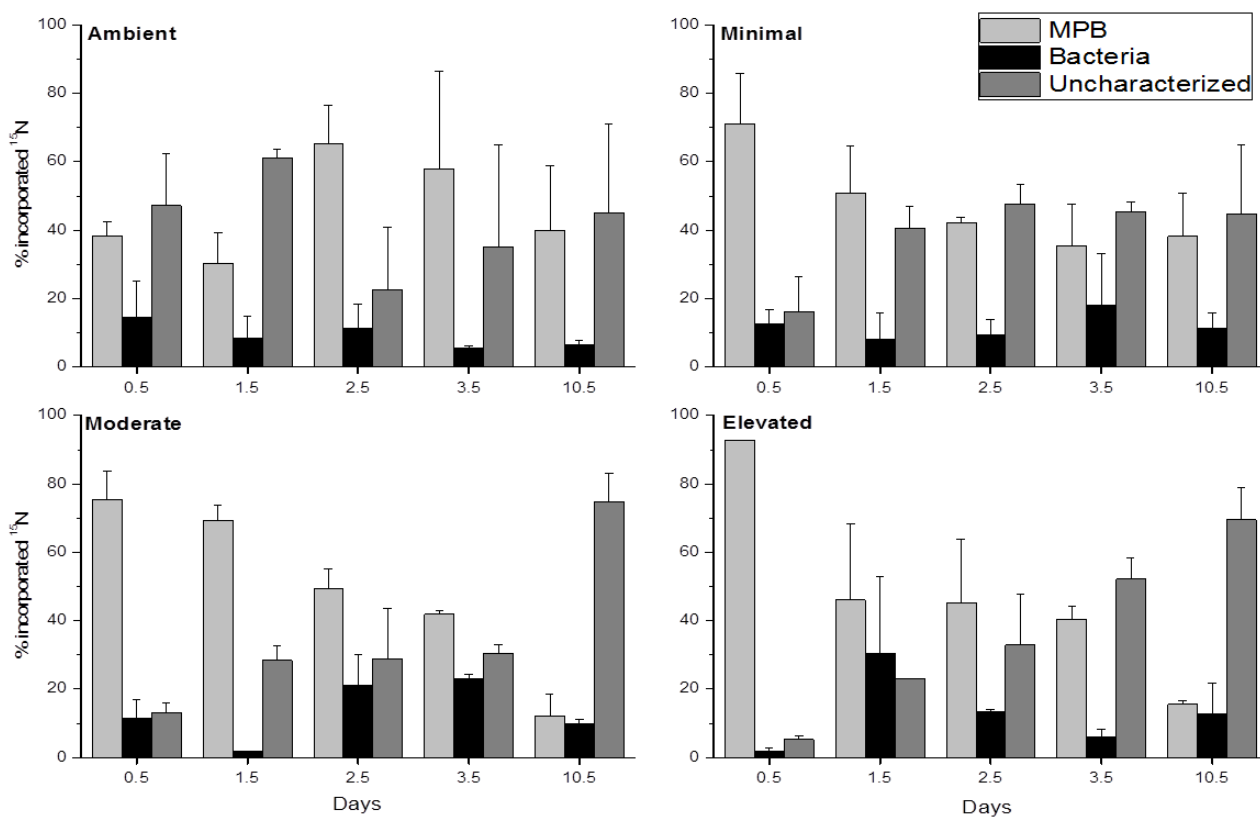
473 **4 Discussion**

474 By combining a pulse-chase application of the rare isotope ^{15}N with the D/L-Ala
475 biomarker technique, we identified that increased nutrient availability as a pulsed addition of
476 5 to 50 $\mu\text{M L}^{-1} \text{NH}_4^+$ across 3 treatments: 1) stimulated initial production of MPB-N 2)
477 increased microbial turnover of MPB-N into the uncharacterized sediment ON compartment,
478 and 3) decreased the amount of MPB-N lost via DON effluxes. By the end of the incubation
479 (10.5 d) 86-94% of the ^{15}N incorporated remained in the sediment, with 3.9 - 10.9% effluxed
480 as DON, 2.3 - 7.1% effluxed as N_2 , and less than 1% effluxed as NH_4^+ (Fig. 6). Of the ^{15}N
481 contained in the sediment 12-40% was in MPB, 6-15% was in bacteria and the remaining
482 45-75% was uncharacterized (Fig. 6). This study is the first to track the fate and processing
483 of a pulse of MPB-N produced in situ under an increasing gradient of water column nutrient
484 availability.

485 *4.1 Initial incorporation and downward transfer of ^{15}N*

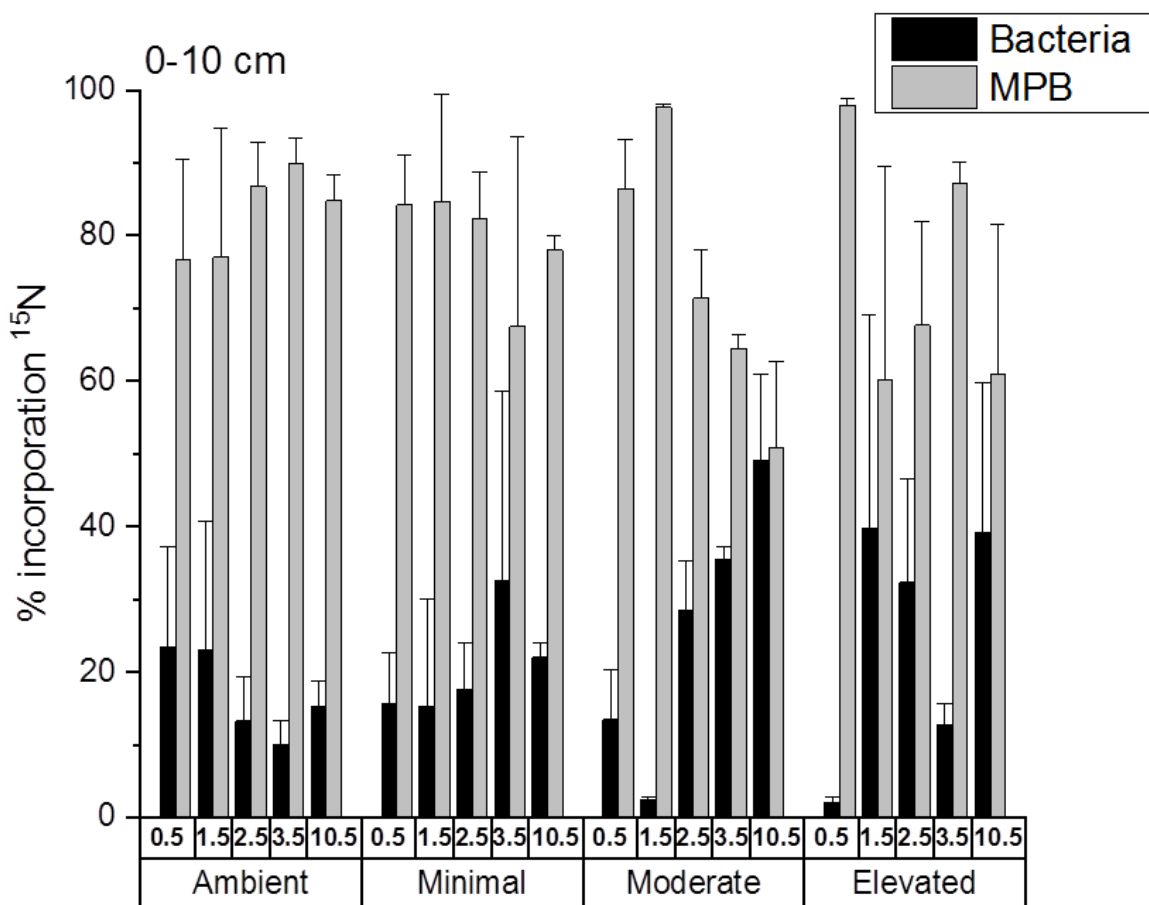
486 Initial uptake of ^{15}N into microbial biomass was dominated by MPB across all
487 treatments (0.5 d, Fig. 4) and increased nutrient amendment stimulated initial retention of
488 MPB-N within MPB (ambient 53% versus 84-95% nutrient amended treatments, 0.5 d, Fig.
489 3). Retention of MPB-N may have resulted from the decoupling of bacterial processing from
490 MPB-N production, with inorganic nutrients preferentially utilized in lieu of MPB-N in the
491 nutrient amended treatments. Decoupling is further supported by a decrease in MPB-N
492 turnover into uncharacterized ON; there was the considerable initial turnover of MPB-N into
493 the uncharacterized ON in the ambient treatment (47.3%, 0.5 d), but turnover within the
494 nutrient amended treatments (5-16%, Fig. 3) was considerably lower ($t= 3.8, p= 0.009$). The

495 uncharacterized pool represents ^{15}N within bulk sediment OM that was not accounted for by
496 ^{15}N contained within microbial biomass. This pool is composed of ^{15}N -containing
497 compounds such as EPS, enzymes, and OM derived from the remineralization of MPB-N.
498 Increased breakdown and initial transfer of MPB-N into the uncharacterized pool is
499 consistent with increased bacterial utilization of MPB-N during remineralization that
500 resulted in increased initial turnover in low nutrient settings (Fig. 3). Increased coupling
501 between MPB and bacteria in low nutrient settings (Cook et al., 2007; Oakes et al., 2012)
502 has previously been attributed to increased reliance on the limited nutrients arising from
503 bacterial remineralization.



505 **Figure 3: Incorporation of ^{15}N into bacteria, MPB, and uncharacterized sediment**
506 **compartments (mean + SE). The uncharacterized compartment is derived by**
507 **subtracting ^{15}N contained in microbial biomass from bulk sediment organic ^{15}N .**

508



509

510 **Figure 4: Excess ^{15}N incorporation into MPB and bacterial biomass within 0-10 cm**
511 **depth as a percentage of the total ^{15}N incorporated into the microbial community for**
512 **each treatment at each time period (mean + SE).**

513 Ratios of $^{13}\text{C}:$ ^{15}N uptake into microbial biomass were estimated based on the excess
514 ^{13}C incorporation determined in a complementary study (Riekenberg et al., 2018). Due to
515 phospholipid fatty acid analysis only accounting for the ^{13}C contained in living biomass and
516 D/L-Ala accounting for ^{15}N in both living and dead biomass, this $^{13}\text{C}:$ ^{15}N ratio potentially
517 over-estimates the relative uptake of ^{15}N into the microbial community, particularly as

518 incubations progress. Therefore, we only considered the $^{13}\text{C}:^{15}\text{N}$ ratios for microbial biomass
519 during the initial sampling (0.5 d) and not for subsequent samplings. The $^{13}\text{C}:^{15}\text{N}$ ratio in the
520 ambient treatment (16.7 ± 2.8) was considerably higher than in the nutrient amended
521 treatments (minimal 8.3 ± 3.8 ; moderate 5.2 ± 3.4 , and elevated 7.5 ± 2 ; One-way ANOVA,
522 $F_{3,7}=5.4$, $p=0.068$), and aligns well with previous estimates where preferential excretion of
523 fixed C as EPS would occur due to N limitation within algal cells (e.g., ~20, Van den
524 Meersche et al. (2004). The lower $^{13}\text{C}:^{15}\text{N}$ ratios within nutrient amended treatments indicate
525 uptake occurring at a ratio closer to that of the Redfield ratio expected for algal uptake (6.7)
526 as a result of increased N availability (Cook et al., 2007). The current study suggests that
527 under N replete conditions, bacteria will utilize inorganic nutrients that are present to support
528 production of low C:N biomass regardless of the C:N ratio of the substrate being processed
529 to produce that biomass, as has been previously proposed (Goldman and Dennett, 2000).

530 Over 10.5 d, the majority (81%) of the ^{15}N incorporated into the sediment was
531 recovered from the 0-2 cm depth, but incorporation of ^{15}N into deeper sediment layers
532 occurred rapidly (within 0.5 d, Fig. 2), equating to a transport rate of ^{15}N to sediment below
533 2 cm of $2.7 - 4.4 \mu\text{mol } ^{15}\text{N m}^{-2} \text{ h}^{-1}$. This rate of downward transport was substantially lower
534 than previously observed at this site (e.g., $19.6 \mu\text{mol } ^{15}\text{N m}^{-2} \text{ h}^{-1}$ over 1.5 d, Oakes et al., in
535 press) which may have been driven by the draining and re-filling of cores to simulate
536 intertidal conditions in that study resulting in additional transport. The rates of downward
537 transport for ^{15}N in this study are similar to that observed for MPB-N in subtropical subtidal
538 sediments (e.g., $\sim 2.7 \mu\text{mol } ^{15}\text{N m}^{-2} \text{ h}^{-1}$ over 3 d with ambient DIN of $\sim 8 \mu\text{mol L}^{-1}$, Eyre et al.
539 (2016), but represent a smaller portion of the total incorporated ^{15}N (8.3 – 16.4% compared
540 to ~29%). The similar rates of downward transport for MPB-N between the two studies

541 likely reflect comparable downward migration by MPB within subtropical sands (Saburova
542 and Polikarpov, 2003). Incorporation of ^{15}N into the 2-10 cm depths increased relatively
543 slowly thereafter but resulted in higher downward transport in the nutrient amended
544 treatments as the incubations progressed (average over 3.5-10.5 d; ambient $15.6 \pm 2.0\%$;
545 minimal $22.6 \pm 2.8\%$; moderate $23.6 \pm 2.7\%$; and elevated $21.2 \pm 2.0\%$, Fig. 2). Downward
546 migration of MPB can be enhanced by light stress (Underwood 2002), but given that light
547 intensity was consistent across treatments, the increased downward transfer of MPB-N
548 within the nutrient amended treatments more likely reflects increased downward migration
549 for nutrients and mitosis as water column nutrients quickly became limiting later in the
550 incubations (Saburova and Polikarpov, 2003).

551 *4.2 Transfer of MPB-N within sediments*

552 The transfer and processing of MPB-N within the sediment compartments varied
553 between the ambient and nutrient amended treatments, potentially as a result of tight
554 coupling between bacterial remineralization and EPS production by MPB providing efficient
555 recycling and transfer of MPB-N between all sediment compartments within sediment ON
556 (Fig. 5). In the ambient treatment, as the incubation progressed, the contribution of MPB to
557 ^{15}N within microbial biomass increased (77-90%), bacterial contributions declined (10-23%,
558 Fig. 3), and the uncharacterized pool of ^{15}N contributed considerably to the excess ^{15}N in
559 sediment ON (23-61%, Fig. 3). These combined factors suggest that MPB-N is efficiently
560 recycled when nutrients are relatively scarce. Bacteria in the ambient treatment likely re-
561 mineralized MPB-N within the uncharacterized pool (Fig. 1A), providing inorganic nutrients
562 that were increasingly used by MPB they competed for available nutrients under nutrient
563 limitation (Cook et al., 2007).

564 In contrast, as incubations progressed in the nutrient amended treatments, decoupling
565 between MPB and recycled nutrients from bacterial remineralization likely resulted in
566 accumulation of ^{15}N within sediment ON as MPB-N was not utilized by bacteria and quickly
567 recycled. With increased nutrient loading, MPB contributions to ^{15}N within microbial
568 biomass declined, bacterial contributions increased (Fig. 3), and coincided with an increased
569 contribution of ^{15}N to the uncharacterized pool (minimal, 41-47%; moderate 29-75%;
570 elevated 23-70%; Fig. 3). The accumulation of ^{15}N in the uncharacterized sediment
571 compartment reflects decreased recycling of MPB-N contained in EPS and detritus (derived
572 from MPB and bacterial biomass) as bacteria preferentially utilize inorganic nutrients instead
573 of MPB-N within the uncharacterized pool (Fig. 1B). The increased contribution of bacteria
574 to the microbial community largely reflects increased turnover of ^{15}N out of MPB as
575 increased production shunts MPB-N into the uncharacterized pool.

576 4.3 *Effluxes of MPB-N*

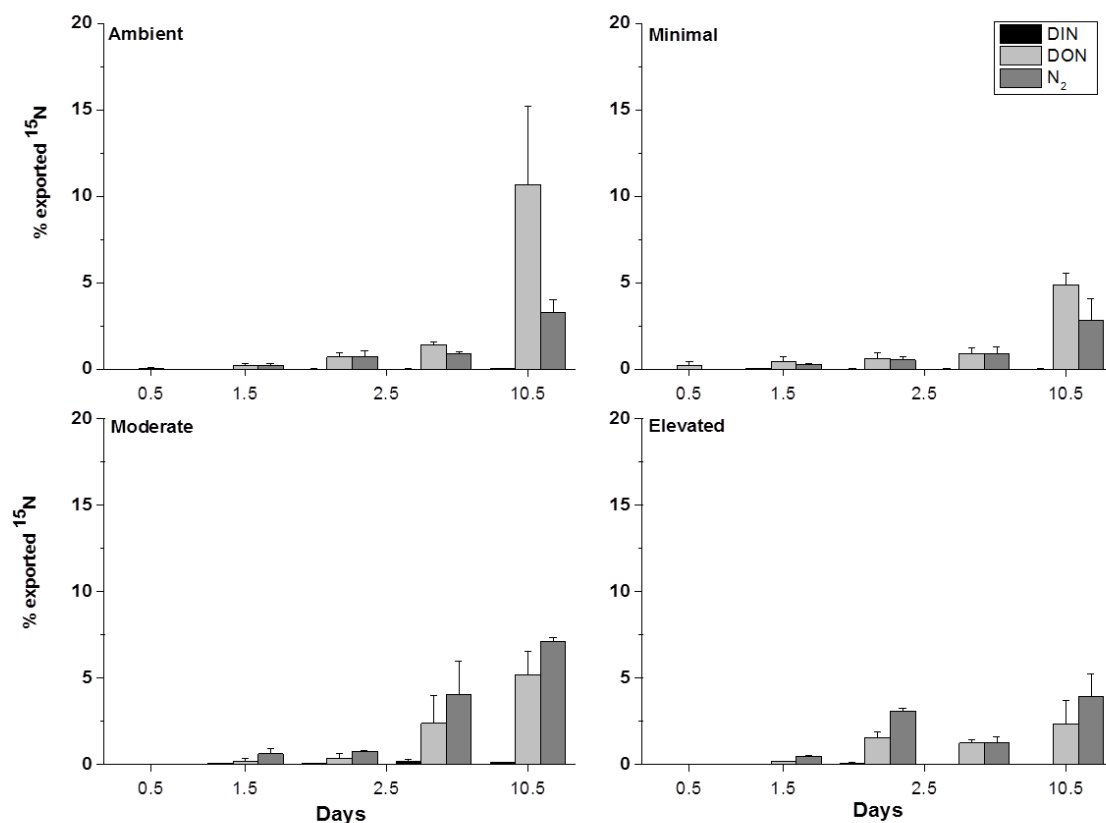
577 Export of ^{15}N from the sediments to the water column primarily occurred via fluxes
578 of DON, N_2 , and NH_4^+ (Fig. 2 & 16). Fluxes of NH_4^+ resulting from remineralization (0-
579 0.3%) contributed little to the export of ^{15}N across all treatments, indicating strong retention
580 of nutrients arising from MPB-N remineralization. Strong retention of this N is not
581 unexpected, as competition between MPB and heterotrophic bacteria for N has been
582 previously observed under nutrient limiting settings (Agogue et al., 2014). Limited export of
583 ^{15}N as NH_4^+ across all treatments in this study suggests that the microbial community
584 maintained capacity for further N uptake throughout the incubations. The cumulative flux of
585 NH_4^+ observed in this study was considerably smaller than that observed in subtropical
586 subtidal sediments over 33 d (20.8%, Eyre et al. 2016) but was comparable to that found

587 previously for this site across 31 d (0.2%, Oakes et al. in press). These comparable fluxes are
588 likely due to the decreased bacterial remineralization and increased retention of MPB-N that
589 was observed within this study.

590 Low fluxes of N_2 may also relate to the competition for inorganic nutrients between
591 MPB and bacteria. The intense competition for recycled inorganic nutrients between MPB
592 and bacteria would have starved denitrifying bacteria of NO_3^- (McGlathery et al., 2007;
593 Sundbäck and Miles, 2002) and greatly reduced the amount of N_2 efflux from denitrification
594 (2.3-7.1%, 10.5 d; Fig. 6). This finding is comparable with previously reported
595 denitrification rates reported for this site (2.6% by 31 d; Oakes et al. in press), and suggests
596 that competition from MPB for NH_4^+ was similarly limiting during that period as ambient
597 NH_4^+ was higher in Oakes et al. in press ($2.1 \pm 1.8 \mu\text{molL}^{-1}$) than observed in this study
598 ($0.9 \pm 0.1 \mu\text{molL}^{-1}$). Limited availability of NO_3^- required for denitrification due to intense
599 competition for the substrate for nitrification would help to explain the low N_2 efflux
600 observed within this study compared to that found previously for this site as well as in
601 subtropical subtidal sediments (20.7%, Eyre et al. (2016). The enhanced efflux of MPB-N as
602 both N_2 and NH_4^+ that was previously observed in a subtidal subtropical setting (20.7% N_2
603 and 20.8% NH_4^+ , Eyre et al. 2016a versus 2.3-7.1% N_2 and 0.02-0.09% NH_4^+ , this study;
604 Figs. 3 & 6) suggests that remineralization produced inorganic nutrients in excess of what
605 was required by the microbial community in that system and resulted in increased export of
606 ^{15}N via DIN and denitrification.

607 Within the current study, increased inorganic nutrient availability appeared to
608 decouple remineralization of MPB-N by bacteria as inorganic nutrients were preferentially

609 utilized instead of sediment ON (Fig. 1B). In the ambient treatment, efflux of MPB-N via
610 DON accounted for 10% of the ^{15}N assimilated within the system and is evidence of
611 considerable remineralization, hydrolysis of freshly deposited material (Ferguson et al.,
612 2004), and export of MPB-N from the uncharacterized pool within sediment ON. With
613 nutrient amendment, export of MPB-N via DON decreased to 3.9-5.2% (Fig. 6) and occurred
614 despite enhanced MPB-N production and the equal or increased presence of ^{15}N within the
615 uncharacterized pool within the nutrient amended treatments. This indicates that bacteria
616 preferentially utilized inorganic nutrients instead of MPB-N derived from ^{15}N -labeled EPS
617 contained within the uncharacterized sediment pool, likely as a result of preferential
618 remineralization of other non-labeled EPS or organic matter that is present in the sediments.



619

620 **Figure 5: Cumulative excess ¹⁵N lost via efflux of DIN, DON, and N₂ at each sampling**
621 **time. All values are as a percentage of the ¹⁵N incorporated into sediment ON (0-10**
622 **cm). Some bars and error bars are too small to be seen (mean + SE).**

623 *4.4 Retention of MPB-N*

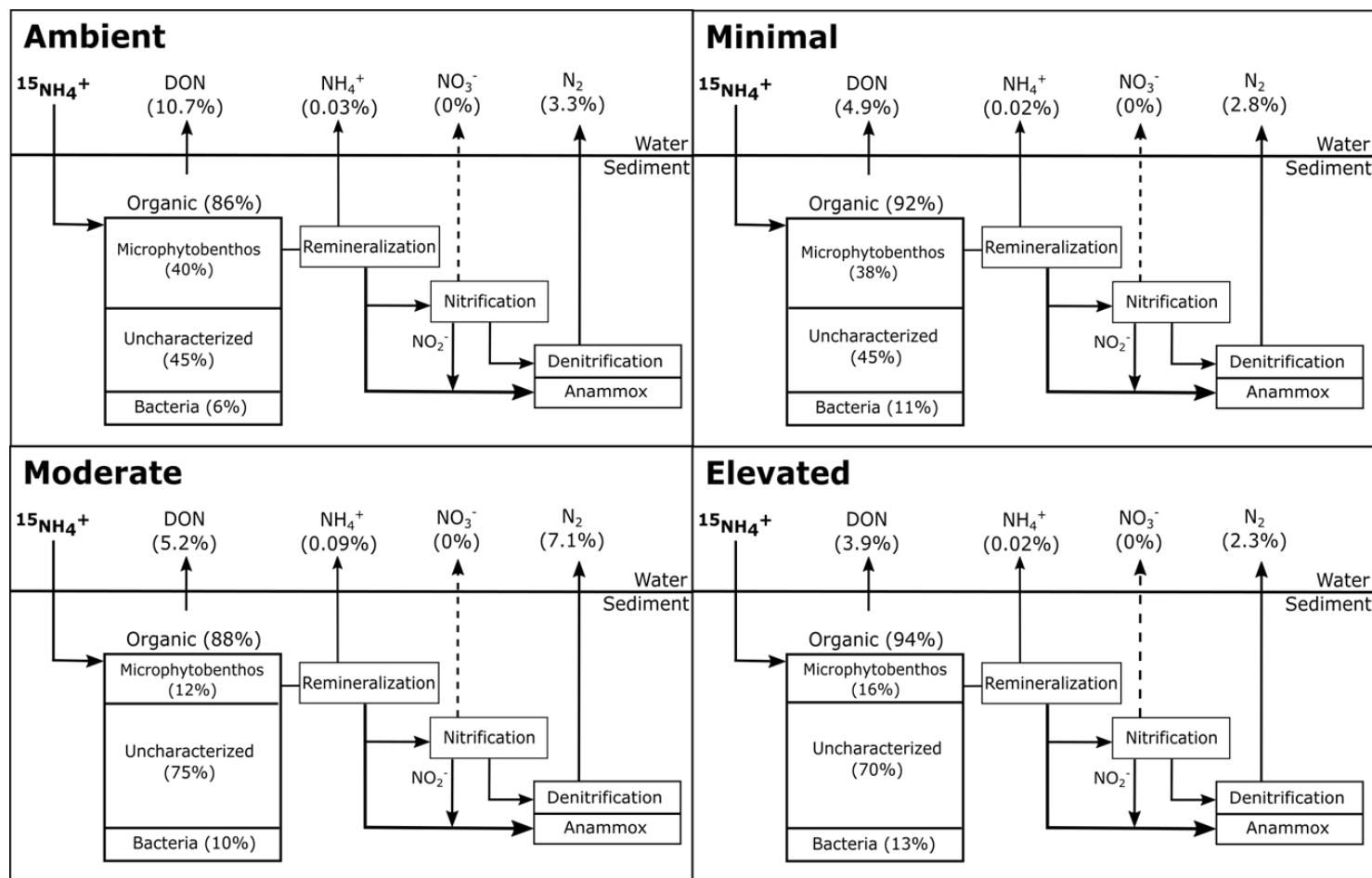
624 MPB have a considerable role in regulating the processing of nutrients within
625 shallow unvegetated benthic settings through primary production and N uptake (Ferguson
626 and Eyre, 2013; Ferguson et al., 2004; McGlathery et al., 2007; Nielsen et al., 2017;
627 Sundbäck and Miles, 2002). Newly assimilated N has been typically considered to be
628 retained within MPB biomass, with increased MPB biomass resulting in reduced N efflux
629 from sediments (Sundbäck et al., 2006). Excluding Eyre et al. (2016) and Oakes et al. (in

630 press), previous studies have not used biomarkers to partition sediment ON into
631 compartments representing the contributions from microbial groups (MPB, bacteria) as well
632 as processed MPB-N remaining in the sediment (uncharacterized). Partitioning out the
633 uncharacterized pool within sediment ON reveals that much of the initially assimilated
634 MPB-N is transferred into the uncharacterized pool, presumably via excretion of EPS by
635 MPB and turnover of microbial cellular material. Under nutrient-limiting conditions, this
636 EPS is quickly remineralized by bacteria, releasing $^{15}\text{NH}_4^+$ that is then recycled as MPB and
637 bacteria compete for available nutrients (Fig. 1A). Under ambient conditions, this coupling
638 resulted in efficient recycling of MPB-N and caused considerable turnover of ^{15}N between
639 the uncharacterized, MPB and bacteria sediment compartments, although sediment retention
640 was somewhat reduced by increased efflux of DON through increased hydrolysis and
641 bacterial remineralization of freshly deposited MPB-N. When nutrient amendments were
642 added, hydrolysis and bacterial remineralization of MPB-N appeared to slow, resulting in
643 reduced processing and accumulation of MPB-N within sediment ON and decreased loss of
644 ^{15}N via DON efflux. The net result of these changes is increased retention of MPB-N under
645 nutrient amendment, as processing and export of assimilated N is reduced. Where MPB-N
646 was retained it was largely contained outside of the microbial biomass, largely within the
647 uncharacterized sediment ON pool (i.e., it was retained as EPS and other N-containing
648 molecules). This is in stark contrast to previous findings that MPB-N is predominately
649 strongly retained in MPB-biomass (Eyre et al., 2016; Nielsen et al., 2017) and likely reflects
650 the considerable turnover of MPB-N within this system.

651 **5 Conclusion**

652 The microbial community is an important sink for both organic and inorganic N
653 within benthic coastal settings (Ferguson et al., 2004; McGlathery et al., 2007; Thornton et
654 al., 2002). Uptake of N is typically viewed as being MPB dominated, with N predominately
655 retained in MPB biomass (Evrard et al., 2008; Hardison et al., 2011; Sundbäck et al., 2006;
656 Veuger et al., 2007a) unless grazing leads to trophic transfer of MPB-N. The addition of
657 inorganic nutrients appears to initially stimulate incorporation of N into MPB, but then leads
658 to decoupling between bacterial remineralization and production of MPB-N as inorganic
659 nutrients are utilized to drive breakdown of other non-labeled organic matter. This leads to
660 decreased MPB-N retention within MPB biomass, greater accumulation of MPB-N within
661 sediment ON as uncharacterized material and corresponds with decreased efflux of MPB-N
662 as DON. This material is composed of a mixture of EPS, enzymes and other N-bearing OM
663 that is either associated with the biofilm formed by MPB and bacteria and fairly resistant to
664 export or has accumulated due to the low physical transport processes associated with this
665 study. In either case, turnover of MPB-N between the sediment compartments has decreased,
666 with MPB-N being less efficiently recycled, and no longer strongly solely retained within
667 MPB biomass. The net effect of these changes appears to be increased presence of relatively
668 labile MPB-N in the shallow surface sediments which may lead to longer term support of
669 increased bacterial respiration. If MPB production slows, there is potential to shift the
670 benthic metabolism from net autotrophy towards net heterotrophy.

671



672

673 **Figure 6: Conceptual model comparing the processing and fate of microphytobenthos nitrogen after 10.5 d of incubation**
 674 **amongst treatments. Sediment organic nitrogen is partitioned into sediment compartments (microphytobenthos, bacteria, and**
 675 **uncharacterized) as a percentage of ^{15}N contained in 0-10 cm depth using biomarker analyses for 0-2 cm, 2-5 cm, and 5-10 cm**
 676 **depths. The dashed line for efflux of NO_3^- indicates that concentrations were consistently below the detection limit for $^{15}\text{NO}_3^-$**
 677 **analysis (figure modified from Eyre et al. 2016)**

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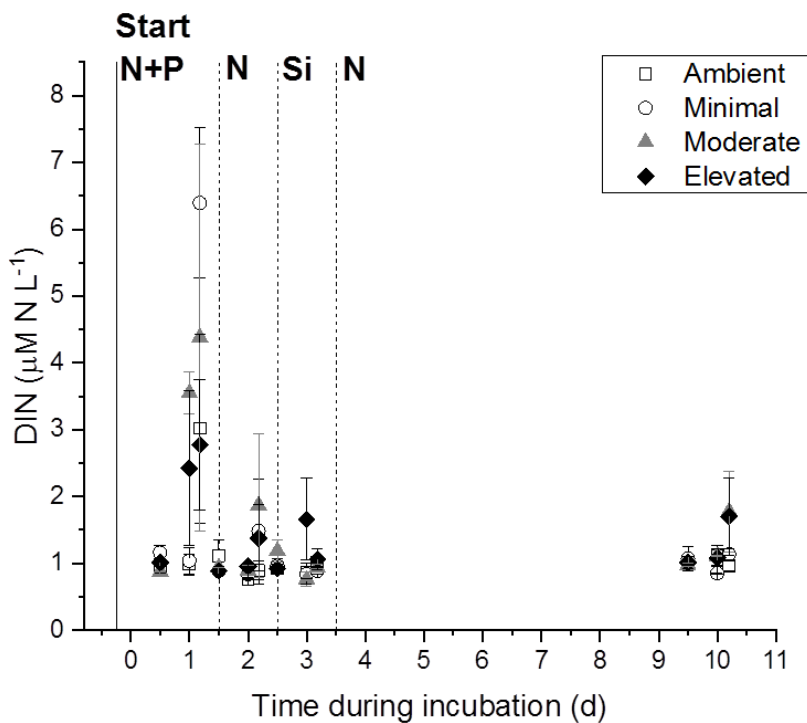
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807 Appendix



808

809 **Appendix Figure 1:** Dissolved inorganic nitrogen in the overlying core waters during sampling
810 for light and dark incubations. The y-axis intercept represents the initial application of $^{15}\text{NH}_4^+$ to
811 the sediment. The solid line is when cores were placed into treatment tanks prior to the start of
812 incubation. Dashed lines represent additional treatment pulses (N= NH_4^+ , P= PO_4^{3-} , Si= SiO_3) that
813 occurred during incubation.