

1 Response of the benthic food web to short- and long-term nutrient
2 enrichment in saltmarsh mudflats

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16 Pascal et al.: Nutrient influences on mudflat food web

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18 ABSTRACT: We examined the responses of biota at or near the base of the benthic food web
19 to nutrient enrichment in salt marsh mudflats in Plum Island estuary (Massachusetts, USA).
20 To simulate eutrophication, nitrate and phosphate loading rates were increased 10- to 15-fold
21 in creeks fertilized for 2 mo (i.e. short-term enrichment) or 6 yr (chronic enrichment). We
22 found that benthic invertebrate community structure was not altered by nutrient enrichment,

23 although the abundance of epifaunal, but not infaunal, grazers increased. Short-term
24 enrichment had no effect on the food web, but significant changes were detected with chronic
25 enrichment. Grazing experiments with ¹⁵N-enriched bacteria and ¹³C-enriched benthic algae
26 revealed higher per capita ingestion rates of benthic microalgae by nematodes, copepods and
27 hydrobiid snails in the creek with chronic nutrient enrichment where isotope composition also
28 indicated that algae increased in dietary importance. The fraction of bacterial biomass grazed
29 was not affected by nutrient enrichment; however, the fraction of benthic algal biomass
30 grazed increased by 235% with chronic enrichment. This higher grazing pressure was partly
31 the result of dietary changes (increases in per capita feeding rate or a change in selection) but
32 was mostly due to an increased abundance of the grazing consumer with the highest biomass,
33 the snail *Nassarius obsoletus*. This increased top-down control partially masked the bottom-
34 up effects of nutrient enrichment on algal biomass and helps explain the slow and inconsistent
35 response of microalgal biomass to chronic nutrient enrichment previously observed in this
36 estuary. Our research shows that eutrophication may subtly affect benthic food webs before
37 large, sustained increases in algal biomass are observed.

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INTRODUCTION

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Anthropogenic activities have contributed to a doubling of the rate of nitrogen entering the global biogeochemical cycle (Galloway et al. 2008, Gruber & Galloway 2008, Canfield et al. 2010). Through river, groundwater and atmospheric transport, a large fraction of this excess nutrient load passes through coastal ecosystems (Howarth et al. 1996, Nixon et al. 1996) causing harmful algal blooms, hypoxia and fisheries losses (Conley et al. 2009). Nutrient loading and subsequent eutrophication have been documented worldwide in coastal environments (Diaz & Rosenberg 2008), becoming major issues for both scientists and

47 managers. Among coastal environments, salt marshes appear particularly sensitive,
48 experiencing accelerated degradation and habitat loss (Silliman et al. 2010, Deegan et al.
49 2012).

50 The effects of eutrophication on salt marsh function are difficult to interpret and
51 predict. One reason is that benthic food webs are complex, and, in general, the nature and
52 strength of interactions are poorly understood. Chronic nutrient enrichment is expected to
53 increase the biomass and/or productivity of the 2 dominant primary producers in salt marshes:
54 cordgrass *Spartina* spp., primarily as aboveground production, and benthic algae (McComb
55 1995, Bertness et al. 2002, Deegan et al. 2007, Ferreira et al. 2011). This bottom-up
56 stimulation extends to benthic bacteria because bacteria utilize labile organic matter released
57 by benthic microalgae, which increases with nutrient enrichment (Bowen et al. 2009a).
58 Bottom-up stimulation may also extend to infaunal and epifaunal invertebrates that exploit
59 benthic bacteria and algae as food resources (Posey et al. 1999, Johnson & Fleeger 2009,
60 Wimp et al. 2010). An increased per capita consumption by benthic grazers associated with an
61 increased food supply may, over time, stimulate an increase in invertebrate biomass (Bertness
62 et al. 2008, Johnson 2011). Increases in per capita grazing rates and/or grazer biomass both
63 have the effect of increasing grazing pressure on bacteria and algae, possibly enhancing top-
64 down control which, in turn, may limit the increase in biomass of benthic algae caused by
65 eutrophication (Sardá et al. 1996, Posey et al. 2002). Because nutrient enrichment tends to
66 increase primary producer biomass and grazing tends to decrease it, primary producer
67 responses to nutrient enrichment depend on the relative strength of bottom-up and top-down
68 forces (Hillebrand et al. 2000, 2002, Haglund & Hillebrand 2005). Studies comparing the
69 relative importance of these forces as they affect organismal abundance are becoming
70 common (e.g. Posey et al. 2002, Hillebrand et al. 2007, Johnson et al. 2009). Our study
71 however differs from previous work because we also examined the rates of consumption of

72 benthic algae by both infaunal and epifaunal consumers to better elucidate the mechanisms by
73 which benthic food webs change with nutrient enrichment.

74 We took advantage of a whole-ecosystem manipulation (The USA 'TIDE' project;
75 Deegan et al. 2007, 2012) designed to investigate the effects of nutrient enrichment on salt
76 marshes in the Plum Island estuary, Massachusetts, USA. TIDE mimics the nutrient loadings
77 and the mechanism of nutrient delivery typically associated with anthropogenic
78 eutrophication in marsh creeks. Dissolved N and P have been added to creeks with the
79 flooding tide throughout whole growing seasons since 2004. Excess fertilizer and
80 phytoplankton production are removed on the falling tide due to the local 3 m tidal range and
81 nutrients diluted in the surrounding larger waterways (Drake et al. 2009). The large spatial
82 (60000 m^2) and temporal (>6 yr of seasonal nutrient enrichment) scales over which TIDE is
83 being conducted make this experimental perturbation more similar to human-induced
84 eutrophication than the typical application of dry fertilizer to small plots. These features
85 should contribute to a better understanding of the rate and mechanisms of change associated
86 with chronic nutrient enrichment.

87 We examined variation in the natural abundance of isotope concentrations and
88 conducted grazing experiments, with simultaneous dual labeling of bacteria and benthic algae
89 (Pascal et al. 2008a), to clarify our understanding of the effects of nutrient enrichment history
90 on mudflat food webs. The results allowed us to estimate per capita and, based on density
91 estimates, population-level grazing rates across the diversity of benthic grazers that occur
92 with a high biomass or a high abundance. Our labeling and isotope studies allowed an
93 evaluation of the effects of nutrient enrichment on the ability of each grazer to select food,
94 quantifying changes in diet. These methods also allowed an appraisal of the influence of
95 nutrient enrichment on top-down control by summing the quantity of bacteria and benthic
96 algae consumed by the total community of benthic grazers. The goal of the present study was

97 therefore to evaluate food web changes induced by nutrient enrichment in the low intertidal
98 marsh environment, the mudflat, in TIDE creeks.

99

100 MATERIALS AND METHODS

101 TIDE project

102 TIDE stands for ‘Trophic cascades and Interacting control processes in a Detritus-
103 based aquatic Ecosystem’ and is an ecosystem-level nutrient-enrichment experiment in the
104 salt marshes of Plum Island estuary (PIE), Massachusetts, (42°44'N, 70°52'W), USA (Fig. 1).
105 PIE is a 25 km long mesotidal estuary with a mean tidal range of 3 m. *Spartina alterniflora*
106 and *S. patens* are the dominant macrophytes on the creek edge and marsh platform in the
107 seawater-dominated intertidal zone where the salinity ranges from 8 to 28‰. Plant zonation in
108 PIE is considered typical of New England estuaries (Niering & Warren 1980), and PIE has
109 historically been unaffected by anthropogenic nutrient loading (background nutrients: <5 µM
110 NO₃⁻; 1 µM PO₄³⁻). Located in the lower intertidal area, mudflats consist of gently sloping
111 creek habitat of poorly consolidated sediments without macrophytes (Fig. 1).

112 Nutrient-enrichment experiments were conducted in 3 creeks (West, Clubhead,
113 Sweeney) having similar water chemistry, plant and infaunal communities (details in Deegan
114 et al. 2007, Johnson et al. 2007). Physical dimensions were also similar, with the watershed
115 area (including the marsh platform) ranging between 8.4 and 12.4 ha and the mean tidal
116 exchange volume ranging from 10560 to 21170 m³. Furthermore, all creeks drain completely
117 at low tide before being replenished with unenriched water from surrounding waterways on
118 the rising tide (Drake et al. 2009). Sweeney (hereafter referred to as the chronically fertilized
119 creek or CFC) was enriched for 6 field seasons (2004 to 2009), Clubhead (hereafter referred

120 to as the short-term fertilized creek or SFC) was enriched for about 2 mo (2009) and West
121 (hereafter referred to as the reference creek or RC) was not enriched. In order to maintain a
122 relatively constant enrichment through each tidal cycle, fertilizer was pumped into the creek
123 at flow rates based on a hydrologic model that estimates the volume of creek flooding water
124 throughout each tidal cycle. In CFC for approximately 150 d during the growing season,
125 nitrate and phosphate levels were increased on each rising tide to approximately $70 \mu\text{mol l}^{-1}$
126 NO_3^- and $4 \mu\text{mol l}^{-1} \text{PO}_4^{3-}$, resulting in a loading rate of $\sim 40 \text{ g N m}^{-2} \text{ yr}^{-1}$. For SFC, nutrient
127 enrichment was identical to CFC, but began about 2 mo before our experiments were
128 conducted. Nutrient concentrations were chosen because they designate an estuary to be
129 ‘moderately to severely eutrophied’ (US EPA 2002). Northeastern US estuaries have
130 experienced a large range in anthropogenically derived nutrient enrichment, mostly due to
131 variation in sewage input and differences in the regional frequency and types of agriculture
132 practiced. However, the total inorganic nitrogen concentrations achieved by fertilization in
133 CFC and SFC were similar to levels found in eutrophic Jamaica Bay, New York, USA,
134 although TIDE nitrogen loading rates were about an order of magnitude lower than most plot-
135 level nutrient enrichment studies that add dry fertilizer to salt marsh sediments (Deegan et al.
136 2012). Detailed background information on experimental design and biogeochemistry of the
137 tidal creeks can be found in Deegan et al. (2007, 2012) and Drake et al. (2009).

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139 **Natural abundance isotope analysis**

140 All biological samples (i.e. for isotope analysis, density estimates and grazing
141 experiments) were collected in the mudflats of each creek, at similar tidal elevation in areas
142 with notable physical similarities. Sample sites in nutrient-enriched creeks were located ~ 100

143 m upstream from the point at which fertilizer was added. An analogous location was selected
144 for collections in RC. All samples were collected during the second week of July, 2009.

145 For stable isotope analysis of the natural abundance of meiofauna, the upper 0.5 cm
146 layer of sediment was collected from 3 replicate plots of 900 cm² in each creek and frozen. In
147 the laboratory, samples were thawed, centrifuged with Ludox (de Jonge & Bouwman 1977),
148 and ≥700 nematode, ≥300 copepod and ≥10 ostracod specimens were haphazardly removed
149 and retained from each replicate. After centrifugation, foraminifers in the pellet fraction were
150 stained with Rose Bengal, and 300 individuals were picked from each replicate. Each
151 specimen was cleaned of debris and rinsed. Taxa containing high concentrations of calcium
152 carbonate (i.e. foraminifera and ostracods) were decalcified with 2.5% HCl. All specimens
153 from each taxon were pooled from a sample and then dried for isotope analysis (n = 3).

154 Snails were collected by hand near each of the plots used to sample meiofauna. Fifteen
155 specimens of hydrobiid snails were removed from the sediment from each sample, and snail
156 tissue was subsequently separated from their shells using forceps and pooled (mean longer-
157 shell height ± SD = 1.8 ± 0.4 mm). Three groups of 15 small (mean longer-shell height ± SD
158 = 6.7 ± 1.3 mm) and 10 large (mean longer-shell height ± SD = 16.3 ± 1.5 mm) specimens of
159 *Nassarius obsoletus* were also located and pooled by size. Snail tissues were homogenized
160 using a tissue grinder and freeze-dried before isotope analyses (n = 3).

161 Samples were analyzed at the Isotope Facility at the University of California, Davis,
162 using an elemental-analyzer isotope ratio mass spectrometer. Samples were reported relative
163 to the standards atmospheric N₂ and Vienna PeeDee Belemnite carbon. Stable isotope values
164 are reported in δ notation:

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$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$$

166 where R is respectively $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$.

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Grazing experiments

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¹⁵N-enrichment of bacteria was carried out using the methods of Pascal et al. (2008b).

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Briefly, surficial sediment was sampled to a depth of 0.5 cm during the ebbing tide in RC.

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Bacteria were cultured in a liquid bacterial medium containing 1 g l⁻¹ of ¹⁵NH₄Cl (>99% ¹⁵N-

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enriched). Cultured bacteria were collected from their medium by centrifugation, frozen in

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liquid nitrogen and stored in a freezer (-20°C) until used in grazing experiments. Enriched

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bacteria abundance was 6.4 × 10⁹ bacteria ml⁻¹, and bacterium-produced nitrogen content was

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12.28% ¹⁵N.

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In each creek mudflat, 4 randomly placed plots of 900 cm² were staked. NaH¹³CO₃

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(>99% ¹³C-enriched) was dissolved in seawater and applied daily over 4 d with a garden

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sprayer directly to the sediment surface of each plot in each creek at a concentration of 1 g m⁻

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² (Middelburg et al. 2000). After spraying was completed, surficial sediment (0.5 cm) of each

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plot was collected, pooled from each creek and sieved, and the sediment fraction retained on a

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63 μm mesh containing meiofauna was removed. Three subsamples of sediment from each

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creek aggregate were freeze-dried, and phospholipid-derived fatty acids (PLFA) were

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extracted and their isotopic composition was determined (n = 3) using a gas-chromatograph

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combustion-interface isotope-ratio mass spectrometer (GC-c-IRMS) as in Boschker et al.

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(1999). Concentrations and δ¹³C PLFA specific to diatoms (C20:4ω6, C20:5ω3, C22:5ω3 and

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C22:6ω3), green algae and cyanobacteria (C18:2ω6c, C18:3ω3 and C18:4ω3) and bacteria

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(i14:0, i15:0, ai15:0, i16:0 and cy19:0) were used to estimate the relative contribution of these

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groups to the total PLFA pool and their weighted-average δ¹³C composition (Table 1).

189

¹³C-enriched sediment from each creek was mixed with ¹⁵N-enriched bacteria, and 157

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ml of this slurry was amended to each of 3 experimental and 3 control incubation microcosms

191 (314 cm²) established for each creek. Bacterial abundance was estimated (see below), and the
192 ratios of total bacteria to ¹⁵N-enriched bacteria in slurry sediments from RC, SFC and CFC
193 were, respectively, 1.86, 1.80 and 1.81. Superficial sediment (upper 0.5 cm) was collected in
194 each creek, and 157 ml of this sediment was sieved on a 63 μm mesh. This fraction of
195 sediment containing meiofauna was added to the experimental microcosms from each creek
196 along with 10 specimens of hydrobiid snails, 10 large and 15 small specimens (size
197 previously described) of *Nassarius obsoletus* collected from the same creek. Three controls
198 were also performed from each creek in which fauna were added to microcosms in the
199 quantities described above but after being frozen (−20°C) for 24 h to induce mortality. All
200 incubations were for 5 h and were conducted at the same time, in similar conditions of light
201 and temperature, close to natural ones. To ensure a near-instantaneous cessation of feeding,
202 incubations were ended by sieving sediment on 63 μm mesh and freezing the retained
203 sediment (−20°C). Samples were later thawed, and all isotope samples were prepared and
204 analyzed as previously described.

205 Isotopic analysis, including the measurement of carbon and nitrogen isotope ratios and
206 carbon and nitrogen content, was conducted on pooled specimens of each microcosm (n = 6).
207 Incorporation of ¹⁵N from bacteria into grazers is defined as excess above background ¹⁵N
208 (control experiment) and is expressed in terms of specific uptake (*I*). *I* was calculated as the
209 product of excess ¹⁵N (*E*) and biomass of N per taxon. *I* was converted to bacterial carbon
210 grazed using the C/N ratio of bacteria. *E* is the difference between the background ($F_{\text{background}}$)
211 and the sample (F_{sample}) ¹⁵N fraction: $E = F_{\text{sample}} - F_{\text{background}}$, with $F = \frac{^{15}\text{N}}{(^{15}\text{N} + ^{14}\text{N})} = \frac{R}{R}$
212 $+ 2)$ where *R* is the nitrogen isotope ratio. For $F_{\text{background}}$, we used values measured with the
213 control (frozen) taxa. *R* was derived from the measured δ¹⁵N values: $R = [(\delta^{15}\text{N} / 1000) + 1] \times$
214 R_{airN_2} . The uptake of bacteria was calculated as $[I \times (\%C_{\text{enriched bacteria}} / \%N_{\text{enriched bacteria}}) /$
215 $(F_{\text{enriched bacteria}} \times \text{incubation time})]$. This uptake was multiplied by the ratio between the

216 abundance of total and enriched bacteria. Incorporation of ^{13}C , and thus benthic microalgae
217 (BMA), into grazers was calculated analogously, with $F = ^{13}\text{C} / (^{13}\text{C} + ^{12}\text{C}) = R / (R + 1)$,
218 R_{airN_2} is replaced by R_{VPDB} and uptake = $I / (F_{\text{enriched BMA}} \times \text{incubation time})$. $F_{\text{enriched BMA}}$ was
219 determined using $\delta^{13}\text{C}$ of PLFA specific to diatoms.

220 Taxon-specific, individual weights were derived from stable isotope samples and were
221 used in grazing rate calculations. Weights averaged \pm SD were 344.1 ± 122.2 ng nematode $^{-1}$
222 (a total of **189000** specimens were examined isotopically from which weight was derived),
223 757.4 ± 276.8 ng copepod $^{-1}$ (**81000** specimens), 17.1 ± 3.4 μg foraminifera $^{-1}$ (**81000**
224 specimens, after acid treatment), 64.5 ± 13.1 μg ostracod $^{-1}$ (270 specimens, after acid
225 treatment), 92.7 ± 32.4 μg hydrobiid $^{-1}$ (405 specimens, after shell removal), 4.2 ± 2.6 mg
226 small *Nassarius obsoletus* $^{-1}$ (405 specimens, after shell removal) and 41.9 ± 10.4 mg large *N.*
227 *obsoletus* $^{-1}$ (270 specimens, after shell removal).

228

229 Population and community grazing rates

230 In order to estimate the biomass of bacteria and benthic algae on a surface area basis at
231 the time of grazing experiments, the upper 0.5 cm layer of sediment was collected from 3
232 plots of 900 cm^2 in each creek and homogenized. Three subsamples of this sediment (**each 20**
233 **ml**) were drawn from each homogenate for bacterial and algal analysis. Bacteria were
234 extracted from sediment particles (Carman 1993), stained using DAPI ($2500 \mu\text{g l}^{-1}$), filtered
235 onto $0.2 \mu\text{m}$ Nucleopore black filters, and then counted under an epifluorescence microscope
236 to estimate abundance ($n = 3$ from each creek). Bacterial carbon content was estimated from
237 bacterial abundances using conversion factors (Epstein 1997). Benthic microalgal biomass
238 was assessed using chlorophyll *a* (chl *a*) concentration as a proxy ($n = 3$ from each creek) and
239 measured after extraction with acetone using a Turner fluorometer (APHA 1992). Values

240 were compared to the studies of Bowen et al. (2009a) and Mitwally & Fleeger (H. M.
241 Mitwally & J. W. Fleeger unpubl. data) who examined the effects of nutrient enrichment on
242 benthic algae in CFC and RC. Algal biomass in units of carbon was estimated using a
243 carbon:chl *a* ratio of 40 (de Jonge 1980). Microalgal production was estimated using
244 empirically developed relationships between benthic chl *a* biomass and microalgal production
245 for estuarine mudflats (Pinckney 1994): production (mg C m⁻¹ yr⁻¹) = (3.2087 × biomass) –
246 0.0903, where biomass is in milligrams of chl *a* per square meter.

247 For meiofaunal abundance, 2 sediment cores (2.2 cm inner diameter) were sampled at
248 each site, fixed with 10% formalin and stained with Rose Bengal. Nematodes, copepods and
249 ostracods were extracted using Ludox centrifugation and enumerated (de Jonge & Bouwman
250 1977). Stained foraminifera from the pellet fraction were counted. Mature copepods were
251 identified to species. At least 80 nematode specimens per sample were categorized by trophic
252 group (Jensen 1987). In addition, our abundance estimates were compared to those generated
253 by a long-term study of nutrient-enrichment effects on meiofauna (H. M. Mitwally & J. W.
254 Fleeger unpubl. data) in which meiofaunal samples were collected in late summer in each
255 year of fertilization in CFC and RC from 2003 to 2009.

256 Hydrobiid snail abundances were estimated by enumerating specimens in the
257 previously described meiofaunal cores. Abundances of the 2 size-class groups of *Nassarius*
258 *obsoletus* (<14 and >14 mm) in each creek (n = 10 quadrats creek⁻¹) were derived from
259 extensive observations in a study of the effects of nutrient enrichment on snail density in 2009
260 in CFC and RC (Johnson & Short 2012).

261 Mean meiofaunal (n = 2 in each creek) and epifaunal (n = 10 in each creek) densities
262 were multiplied by mean grazing rates (n = 3 per taxon in each creek) to evaluate the total
263 amount of bacteria and algae grazed by each taxon. Grazing was also summed across all taxa
264 to estimate a community grazing rate. This total amount was compared to bacterial and algal

265 biomasses (n = 3 in each creek) to evaluate the amount of prey standing stock grazed per unit
266 of time.

267

268 **Data analyses**

269 To examine variation in nematode trophic guild composition and copepod community
270 structure among creeks, analysis of similarity (ANOSIM) was conducted with PRIMER 5.2.9
271 software (Clarke & Warwick 2001). One-way analysis of variance (ANOVA) was used to test
272 for differences in abundances, individual grazing rates and total amount of prey grazed in
273 each creek.

274

275 **RESULTS**

276 **Population responses and community structure**

277 Bacterial biomass at the time of the study was equivalent in all creeks (Table 2).
278 Microalgal biomass ranged from 15 to 45 mg chl *a* m⁻² and was less than similar estimates
279 derived from previous studies conducted in the same creeks, i.e. 73 to 88 mg chl *a* m⁻²
280 (Bowen et al. 2009a) and 51 to 101 mg chl *a* m⁻² (H. M. Mitwally & J. W. Fleeger unpubl.
281 data). Benthic algal biomass significantly differed among creeks at the time of the
282 experiment, with the highest concentration of chl *a* in CFC and the lowest in SFC.

283 Mean total abundances of infauna at the time of the grazing experiments were very
284 similar to the long-term averages found by Mitwally & Fleeger (H. M. Mitwally & J. W.
285 Fleeger unpubl. data), and ranges expressed as 10³ ind. m⁻² almost completely overlapped for
286 nematodes 287 to 1167 versus 172 to 1783 and ostracods 33 to 98 versus 2 to 112. However,

287 for copepods, means were higher at the time of the grazing experiments (267 to 562 versus 20
288 to 130). The density of *Nassarius obsoletus* was significantly (4-fold) higher in CFC than RC
289 (Johnson & Short 2012).

290 We used the composition of trophic groups as a surrogate for the functional diversity
291 of nematodes. Trophic groups did not differ among creeks based on ANOSIM (not
292 significant). Because this analysis did not reveal a creek effect, data from all creeks were
293 pooled, and the overall nematode community was composed of 0.5% deposit feeders, 1.1%
294 scavengers, 41.1% epistrate feeders and 57.3% predators. Similarly, species composition of
295 adult benthic copepods did not differ among creeks (ANOSIM, not significant), and data from
296 each creek were consequently pooled. The relative abundances of the 5 most abundant
297 copepod species in this community were *Stenhelia* sp. at 20%, *Pseudobradia* sp. at 17%,
298 *Enhydrosoma* sp. at 17%, *Scottolana canadensis* at 15% and *Microarthridon littorale* at 8%.

299

300

Natural abundance isotopic composition

301 The natural isotope abundance of large *Nassarius obsoletus* contained relatively more
302 ^{13}C than did other grazers in RC (Fig. 2). Small *N. obsoletus*, hydrobiid snails, foraminiferans
303 and ostracods in RC had a similar natural abundance of carbon isotope but copepods were
304 more depleted in ^{13}C compared to the other taxa. Ostracods were depleted in ^{15}N compared to
305 other taxa (Fig. 2).

306 Confidence intervals for the natural abundance of isotope composition among creeks
307 generally overlapped (Fig. 3). However, 2 trends were observed in all creeks: all grazers from
308 RC were more enriched in ^{15}N and, except for small *N. obsoletus*, all taxa from CFC were
309 more depleted in ^{13}C . The most common method of fertilizer production is industrial fixation
310 of atmospheric nitrogen, resulting in fertilizer products with $\delta^{15}\text{N}$ values close to zero.

311 Nutrient enrichment in CFC likely led to decreased algal $\delta^{15}\text{N}$ ratios, and grazers consuming
312 those algae should be similarly depleted.

313

314 **Individual grazing rates**

315 Mean per capita grazing rates on bacteria measured in each creek ranged between 12
316 and 20 $\text{pg C ind.}^{-1} \text{h}^{-1}$ for nematodes, 78 and 118 $\text{pg C ind.}^{-1} \text{h}^{-1}$ for copepods, 15 and 26 pg C
317 $\text{ind.}^{-1} \text{h}^{-1}$ for foraminiferans, 336 and 397 $\text{pg C ind.}^{-1} \text{h}^{-1}$ for ostracods, 31 and 50 ng C ind.^{-1}
318 h^{-1} for hydrobiid snails, 1.1 and 1.4 $\mu\text{g C ind.}^{-1} \text{h}^{-1}$ for small *Nassarius obsoletus* and 3.3 and
319 3.4 $\mu\text{g C ind.}^{-1} \text{h}^{-1}$ for large *N. obsoletus* (Fig. 4). Nematodes from CFC grazed bacteria at a
320 significantly lower per capita rate than nematodes from the other creeks (ANOVA, $p < 0.01$).
321 All other taxa ingested bacteria at a similar rate, regardless of nutrient enrichment history
322 (ANOVA, not significant).

323 Mean per capita grazing rates of benthic algae measured in each creek ranged between
324 41 and 395 $\text{pg C ind.}^{-1} \text{h}^{-1}$ for nematodes, 1012 and 2369 $\text{pg C ind.}^{-1} \text{h}^{-1}$ for copepods, 31 and
325 77 $\text{pg C ind.}^{-1} \text{h}^{-1}$ for foraminifera, 344 and 6046 $\text{ng C ind.}^{-1} \text{h}^{-1}$ for ostracods, 47 and 167 ng
326 $\text{C ind.}^{-1} \text{h}^{-1}$ for hydrobiid snails, 0.5 and 1.0 $\mu\text{g C ind.}^{-1} \text{h}^{-1}$ for small *Nassarius obsoletus* and
327 1.1 and 2.3 $\mu\text{g C ind.}^{-1} \text{h}^{-1}$ for large *N. obsoletus* (Fig. 5). Ingestion of algae was significantly
328 higher in CFC than in the other 2 creeks for copepods (ANOVA, $p < 0.05$) and hydrobiid
329 snails (ANOVA, $p < 0.01$). Only nematodes and ostracods differed, with lower per capita
330 ingestion at SFC (ANOVA, $p < 0.05$ and $p < 0.01$, respectively). Foraminiferans and *N.*
331 *obsoletus* (small and large) ingested benthic microalgae at similar rates in all creeks
332 (ANOVA, not significant).

333 Specific fatty acids of diatoms and bacteria allow evaluation of their respective
334 biomasses. The ratio of diatoms:bacteria in RC sediment used for grazing experiments was

335 1.9 ± 1.2 (Table 1). Ingestion rates of bacteria and BMA were measured simultaneously
336 during the grazing experiment. When compared to the ratio of food sources available, the
337 ratio of BMA:bacteria grazed indicates food selection (Pascal et al. 2008a). In RC, this ratio
338 was 10.5 ± 1.9 for nematodes, 17.1 ± 3.1 for copepods, 6.4 ± 4.5 for foraminifera, 16.9 ± 2.4
339 for ostracods, 2.4 ± 0.6 for hydrobiids, 0.7 ± 0.1 for small *Nassarius obsoletus* and 0.6 ± 0.2
340 for large *N. obsoletus*. In RC, this ratio was not significantly different between small and
341 large *N. obsoletus* (ANOVA, not significant). This ratio was significantly higher for
342 nematodes and hydrobiids from CFC than from the other 2 creeks (ANOVA, $p < 0.01$).

343

344 **Population and community grazing rates**

345 Population-level grazing rates (m^{-2}) on bacteria were calculated as the product of the
346 per capita grazing rate and density for each taxon (Table 3). Nematodes and large *Nassarius*
347 *obsoletus* from CFC and copepods from SFC ingested significantly more bacteria than in the
348 other creeks (ANOVA, $p < 0.05$ and $p < 0.01$, respectively). Hydrobiid snails and small *N.*
349 *obsoletus* from RC ingested significantly fewer bacteria than in the other creeks (ANOVA, p
350 < 0.01 and $p < 0.05$, respectively). The amount of bacteria grazed by total meiofauna, total
351 epifauna and total fauna did not differ significantly in the 3 treatment creeks (ANOVA, not
352 significant). The entire community of benthic invertebrates ingested, respectively, 4.2, 9.3 and
353 14.5% of bacterial biomass daily in RC, SFC and CFC.

354 Population-level grazing rates (m^{-2}) on benthic algae were calculated as the product of
355 the per capita grazing rate and density for each taxon (Table 4). Nematodes, hydrobiid snails
356 and large *Nassarius obsoletus* ingested significantly more algae in CFC than in the other
357 creeks (ANOVA, $p < 0.001$, $p < 0.01$ and $p < 0.01$, respectively). Population-level grazing
358 was summed across all taxa, and was significantly higher in CFC than in other creeks

359 (ANOVA, $p < 0.01$). Together, the community of benthic invertebrates ingested daily 235%
360 more benthic algae in CFC than in RC. Compared to the algal biomass of each creek, the
361 community of grazers ingested daily 1.9, 5.1 and 4.7% of algal biomass, respectively, in RF,
362 SFC and CFC. The grazing community ingested 9.9, 26.3 and 23.9% of benthic algal
363 production, respectively, in RF, SFC and CFC. Overall, hydrobiid snails consumed more
364 benthic algae than any other taxon. However, the fraction ingested by *N. obsoletus* showed
365 the largest increase in CFC, and *N. obsoletus* accounted for about 40% of all benthic
366 microalgae grazed under the conditions of chronic nutrient enrichment.

367

368

DISCUSSION

369 We studied the effects of nutrient enrichment on benthic food webs in mudflats, with
370 emphasis on the potential for top-down control over benthic microalgae and bacteria. Nutrient
371 enrichment may alter the food web in 3 ways that could act singly or in combination: (1)
372 benthic grazers may change their dietary preference by becoming more selective in their diet,
373 (2) per capita grazing rates of individual taxa on bacteria or algae may vary and (3) changes in
374 the population density of grazing species may affect the total consumption of algae or
375 bacteria. We found that the food web was altered in the creek with a long history (6 yr) of
376 nutrient enrichment through all 3 mechanisms. Some grazers became more selective in their
377 diet, increasing their preference for algae over bacteria, and most grazers increased per capita
378 consumption rates of algae with chronic nutrient enrichment. In addition, the epifaunal snail
379 *Nassarius obsoletus* responded to nutrient enrichment with increased abundance (Johnson &
380 Short 2012), and contributed most to the increased grazing pressure in nutrient-enriched
381 creeks. As a result, total community grazing on benthic algae increased with chronic nutrient
382 enrichment.

383

384

Diet composition

385 The diet of most benthic grazers differed in the chronically nutrient-enriched creek.
386 Isotope composition integrates diet over long-time intervals, and benthic invertebrates in PIE
387 have relatively depleted natural abundance $\delta^{13}\text{C}$ values compared to salt marsh macrophyte
388 detritus (Galván et al. 2008, 2011, present study), indicating an importance of benthic algae to
389 their diet. However, 5 of the 6 taxa we examined had lower $\delta^{13}\text{C}$ values in CFC, consistent
390 with increased long-term consumption of benthic algae (Fig. 3). We also calculated the ratio
391 of ingested algae and bacteria from our simultaneous, dual-label experiment. This ratio is a
392 function of the ability of the grazing species to select diatoms from the sediment–alga–
393 bacterium aggregate. In the reference creek, ratios observed for nematodes (10.5), copepods
394 (17.1) and ostracods (16.9) indicate that benthic diatoms were the primary carbon source,
395 while ratios observed for hydrobiid snails (2.4) and for *Nassarius obsoletus* (0.6) were
396 smaller, indicating a limited capacity to select algae. Previous research has also found that
397 many meiofauna selectively ingest food (Buffan-Dubau et al. 1996, Moens et al. 1999,
398 Buffan-Dubau & Carman 2000, Wyckmans et al. 2007), often preferring diatoms, while
399 estuarine snails have limited selectivity (Brown 1969, Lopez & Kofoed 1980, Levinton 1987,
400 Haubois et al. 2005). However, nematodes and hydrobiid snails became significantly more
401 selective in their diet in the chronically nutrient-enriched creek, increasing their preference for
402 benthic microalgae over bacteria. Finally, short-term grazing rate experiments revealed that
403 nematodes, copepods and hydrobiid snails ingested more microalgae on a per capita basis
404 under conditions of chronic eutrophication. The per capita grazing rate on bacteria was altered
405 in only 1 taxon (such that nematode grazing rates were significantly lower in CFC).

406 Previous research has shown that opportunistic species may benefit from nutrient
407 enrichment, leading to communities dominated by generalist feeders (Heip 1995, Keats et al.
408 2004). However, we found no evidence for a change in benthic community structure in our
409 study creeks, as the macroinfaunal community (Johnson & Fleeger 2009), the relative
410 abundance of nematode trophic groups and the species composition of meiobenthic copepods
411 were all unaffected by nutrient enrichment. Moreover *Nassarius obsoletus* was the most
412 abundant species of epifauna regardless of nutrient enrichment history (Johnson & Short
413 2012). The dietary changes we observed at the community scale were therefore due to
414 changes in feeding behavior or population density at the species level rather than a change in
415 community composition. Previous studies in estuaries also indicate considerable diet
416 plasticity in benthic grazers. Diet become more varied (Armitage & Fourqurean 2009) and
417 omnivores switched to herbivory (Keats et al. 2004, Fox et al. 2009) when the abundance of
418 the basal resource was altered by nutrient enrichment. In a previous study of nutrient
419 enrichment in PIE, 2 surface-feeding polychaete species switched to a microalgal resource
420 after 10 wk of enrichment (Galván 2008). Estuarine grazers appear to readily adapt to
421 changing food resource availability in their environment under conditions of nutrient
422 enrichment.

423

424 **Total consumption of bacteria and algae**

425 We calculated population-level grazing rates by multiplying per capita grazing rates
426 with population density. We then employed an estimate of grazing by the benthic community
427 as a whole by summing grazing across the populations of benthic grazers. At the population
428 level, the non-selective epifaunal snails, hydrobiids and *Nassarius obsoletus* were responsible
429 for the greatest grazing pressure on bacteria and microalgae (averaging from 64 to 98% of all

430 grazing) (Figs. 4, 5 & 6). The potential to exert top-down control over benthic algae and
431 bacteria is therefore much greater for epifauna than for meiofauna, as has been found in other
432 mudflats (van Oevelen et al. 2006, Pascal et al. 2009).

433 Previous research at our study site has shown that nutrient enrichment indirectly
434 benefits the microbial community because of increased algal exudation, although bacterial
435 productivity and community structure are unaffected by nutrient enrichment (Bowen et al.
436 2009a,b, 2011). A similar indirect nutrient effect was observed on bacteria associated with
437 periphyton (Hepinstall & Fuller 1994, Hillebrand et al. 2002). Our research shows that
438 consumption of bacteria by the grazing community was not affected by nutrient enrichment,
439 even though the most important grazer, *Nassarius obsoletus*, increased in density and
440 population-level consumption of bacteria in CFC. We estimate that the total community of
441 grazers ingested between 3 and 14% of the bacterial standing stock each day. These
442 observations suggest grazing by benthic invertebrates did not mask a bottom-up stimulation
443 of bacterial production and that increased grazing had little effect on the biomass of bacteria.
444 Mudflat bacterial communities appear to be strongly resistant to change from exogenous
445 perturbation to the food web (Bowen et al. 2009b, 2011), including from grazing
446 invertebrates.

447 Algal biomass in mudflats was subtly (if at all), rather than dramatically, altered in
448 nutrient-enriched creeks after 6 yr of fertilization in PIE (H. M. Mitwally & J. W. Fleeger
449 unpubl. data). This absence of a persistent and easily perceivable change in algal biomass in
450 the chronically nutrient-enriched creek mudflat is of interest because algal biomass is often
451 used as an indicator of eutrophication (Juanes et al. 2008, Giordani et al. 2009, Ferreira et al.
452 2011). Our research allowed us to determine what role, if any, benthic grazing had in
453 maintaining the levels of algal biomass in mudflats experiencing nutrient enrichment.

454 Daily grazing by the community of meiofauna and small macroinfauna has been
455 shown to exceed the benthic algal standing stock (Montagna et al. 1995, Carman et al. 1997).
456 Daily consumption by epifauna has also been shown to exceed benthic algal biomass (Connor
457 et al. 1982). Although grazing pressure by benthic invertebrates at our study site did not reach
458 the levels observed in the studies cited above, it was substantial. Meiofauna and epifauna
459 together ingested 2 to 5% of algal stock daily, and the proportion of the daily algal primary
460 production consumed increased from 10% in the reference creek to 24% in the creek with
461 chronic nutrient enrichment. Total grazing pressure on benthic algae in the creek with chronic
462 nutrient enrichment was increased by 235% compared to the reference creek. The difference
463 in algal production grazed between RC and CFC (about 20% more of primary production was
464 grazed per day at CFC) suggests that algal biomass at CFC would increase in the absence of
465 benthic grazers and that algae would reach a higher maximum biomass (Blanchard et al.
466 2001). This difference is relatively small in the absolute amount of benthic algal biomass, but
467 the increased grazing pressure on benthic algae lessened potential increases in algal biomass
468 associated with chronic eutrophication. Similar results were observed in a sandy environment
469 (Hillebrand et al. 2000). Our results indicate that at least part of the slow and inconsistent
470 response of benthic algae to nutrient enrichment in PIE mudflats was due to top-down control
471 because increased grazing restrained changes in algal biomass.

472 Our results also show that nutrient-enrichment effects on food webs may take longer
473 than a single growing season to occur. Findings in the creek with short-term nutrient
474 enrichment and the reference creek were very similar and contrasted sharply with those from
475 the chronically fertilized creek. For example, natural abundance isotopes and per capita
476 grazing rates in SFC were not increased compared to RC for any taxon. Similarly, benthic
477 algal biomass did not increase during the first year (2004) of nutrient enrichment in CFC (data
478 in Deegan et al. 2007), but increased by 60% in the second year of enrichment in treatments

479 in which a nektonic grazer was reduced in abundance. Previous research has identified effects
480 of nutrient enrichment on abundance or on consumer diet that occurred within weeks to
481 months after nutrient enrichment was begun (Posey et al. 2006, McFarlin et al. 2008), while
482 other studies have found that food web changes or abundance changes take longer to develop.
483 For example, in a tundra river, fertilization experiment effects were also first noted in the
484 second year of nutrient enrichment (Peterson et al. 1993), and Sardá et al. (1996) found that
485 an infauna community was affected by nutrient enrichment, but over decadal time scales.
486 Although some infaunal species at our study site responded quickly to nutrient enrichment by
487 changes in dietary selectivity (Galván et al. 2008), large non-selective epifauna were the most
488 important grazers at our mudflat site. These large species are slow growing, and changes in
489 abundance occur over long time scales, perhaps explaining the lack of a rapid change in
490 grazing pressure on benthic algae in the nutrient-enriched creeks at our study site.

491

492 **Other considerations**

493 Salt marsh nekton can consume benthic bacteria and algae but were not considered
494 here. Mummichog *Fundulus heteroclitus* is a key omnivorous fish able to ingest algae, bulk
495 detritus and the microbial community, as well as animal prey (D'Avanzo & Valiela 1990).
496 However, adults likely graze filamentous algae not found on mudflats (Lockfield 2011), and
497 juveniles can graze on mudflat microalgae only for a short time as the tide rises over the
498 mudflat before epipellic diatoms vertically migrate. Grass shrimp *Palaemonetes pugio* are not
499 effective grazers on the epipellic microalgal community (Fleeger et al. 1999). Macroinfauna
500 also consume benthic microalgae (Galván et al. 2008), although the biomass of epifaunal
501 snails was >2 orders of magnitude higher than the combined biomasses of all macroinfauna
502 (Johnson & Fleeger 2009, Johnson & Short 2012). Our estimation of total community

503 grazing, therefore, focuses on the most abundant and highest biomass members of the
504 infaunal (the meiofauna) and epifaunal (snail) communities.

505 Estimates of biomass are needed to evaluate the trophic fate of benthic microalgae and
506 bacteria. Our algal and bacterial biomass estimates were based on 3 large (each 900 cm²)
507 sediment samples from each creek that were subsequently homogenized and subsampled. This
508 procedure should reduce small-scale variation and increase confidence in the means we
509 generated (Moreno & Niell 2004). To allow comparisons with algal grazing rates, algal
510 abundance must be converted from units of chl *a* to carbon using a conversion factor known
511 to vary (de Jonge 1980, Frost et al. 2005). Bacterial biomass measurements are also
512 potentially biased because a conversion factor from volume to carbon content (Bratbak 1985)
513 is required, and a physical separation of attached bacteria from sediment particles is necessary
514 for accurate counts (Carman 1993). Like all methods previously developed and applied to
515 measure benthic microalgal and bacterial biomasses, the methods used in the present study
516 present potential methodological shortcomings that must be kept in mind when interpreting
517 results.

518 Our calculation of algal ingestion is based on the assumption that enrichment of grazer
519 tissue is due only to the ingestion of ¹³C-enriched diatoms. However, other potential food
520 resources may also have become enriched in ¹³C. Fatty acid isotopic analysis revealed that
521 green algae and cyanobacteria were, on average, 20% more enriched than diatoms. However,
522 their contribution to grazer enrichment was limited because their biomasses were, on average,
523 1 order of magnitude lower than those of diatoms. Dominance by diatoms is common (Vander
524 Grinten et al. 2004), particularly in muddy sediments (Watermann et al. 1999). PLFA-specific
525 markers for bacteria were also enriched in ¹³C, but bacterial dietary contributions may be
526 limited because they were, on average, 5-fold less enriched than diatoms. Those potential
527 biases could lead to a small overestimation of diatom importance in the diet of grazers.

528 However, grazing rates measured using the ^{15}N pre-labeled bacteria method were comparable
529 with those reported for a French intertidal mudflat (Pascal et al. 2008b, 2009).

530 Another uncertainty in our calculations of the impact of the grazing community on
531 microalgae and bacteria is associated with faunal density estimates. *Nassarius obsoletus* was
532 the most important contributor to changes in the food web in CFC, and its density was
533 surveyed during the time that the present study was conducted (Johnson & Short 2012).
534 However, meiofauna were sampled with minimal replication at the time of our study because
535 density was estimated simultaneously in the same creeks as part of a long-term assessment of
536 nutrient-enrichment effects (H. M. Mitwally & J. W. Fleeger unpubl. data). This long-term
537 assessment could not detect consistent effects on meiofauna associated with nutrient
538 enrichment in the mudflat habitat, and mean values were very similar to those used in our
539 calculations. To determine whether the conclusions of our estimates of grazing would be
540 altered by using the long-term density estimates of meiofauna, we recalculated grazing rates
541 using the meiofauna densities of Mitwally & Fleeger (H. M. Mitwally & J. W. Fleeger
542 unpubl. data). The amount of algae grazed hourly by meiofauna increased by 11 and 10%,
543 whereas the amount of bacteria grazed increased by 24 and 4%, respectively, in RC and CFC.
544 These reduced fluctuations suggest that bias due to low replication of density measurements is
545 limited.

546

547

CONCLUSIONS

548 Increased algal biomass is often used as an indicator of eutrophication (Juanes et al.
549 2008, Giordani et al. 2009, Ferreira et al. 2011). Similar to the conclusion of Schramm
550 (1999), our research shows that top-down control by an extant benthic invertebrate
551 community may mask the effects of eutrophication when measured by algal biomass.

552 Although changes in diet preference and increases in species-specific per capita grazing rates
553 both contributed, grazing pressure on benthic algae increased largely because the abundance
554 of the non-selective feeding snail *Nassarius obsoletus* increased with chronic nutrient
555 enrichment. This increased abundance accounted for about 40% of the increase in community
556 grazing rates. Our results also indicate that nutrient-induced changes in the benthic food web
557 were observed sometime after 1 field season and before 6 yr of fertilization, and that large
558 changes in benthic algal biomass were not observed during this time. Thus, we detected
559 changes in food web structure that occurred prior to any large, sustained increases in algal
560 biomass, suggesting that effects of nutrient enrichment on food webs may take place before
561 eutrophication is apparent.

562

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572

573

LITERATURE CITED

574 <bok>APHA (American Public Health Association) (1992) Standard methods for the
575 examination of water and wastewater, 18th edn. American Waterworks Association and
576 Water Pollution Control Federation, Washington, DC</bok>

577 <jrn>Armitage AR, Fourqurean JW (2009) Stable isotopes reveal complex changes in trophic
578 relationships following nutrient addition in a coastal marine ecosystem. *Estuaries Coasts*
579 32:1152–1164 [doi:10.1007/s12237-009-9219-z](https://doi.org/10.1007/s12237-009-9219-z)</jrn>

580 <jrn>Bertness MD, Ewanchug PJ, Silliman BR (2002) Anthropogenic modification of New
581 England salt marsh landscapes. *Proc Natl Acad Sci USA* 99:1395–1398 [PubMed](#)
582 [doi:10.1073/pnas.022447299](https://doi.org/10.1073/pnas.022447299)</jrn>

583 <jrn>Bertness MD, Crain C, Holdrege C, Sala N (2008) Eutrophication and consumer control
584 of New England salt marsh primary productivity. *Conserv Biol* 22:131–139 [PubMed](#)
585 [doi:10.1111/j.1523-1739.2007.00801.x](https://doi.org/10.1111/j.1523-1739.2007.00801.x)</jrn>

586 <jrn>Blanchard GF, Guarini JM, Orvain F, Sauriau PG (2001) Dynamic behaviour of benthic
587 microalgal biomass in intertidal mudflats. *J Exp Mar Biol Ecol* 264:85–100
588 [doi:10.1016/S0022-0981\(01\)00312-4](https://doi.org/10.1016/S0022-0981(01)00312-4)</jrn>

589 <jrn>Boschker HTS, Brouwer JFC, Cappenberg TE (1999) The contribution of macrophyte-
590 derived organic matter to microbial biomass in salt-marsh sediments: stable carbon
591 isotope analysis of microbial biomarkers. *Limnol Oceanogr* 44:309–319
592 [doi:10.4319/lo.1999.44.2.0309](https://doi.org/10.4319/lo.1999.44.2.0309)</jrn>

593 <jrn>Bowen JL, Crump BC, Deegan LA, Hobbie JE (2009a) Increased supply of ambient
594 nitrogen has minimal effect on salt marsh bacterial production. *Limnol Oceanogr* 54:713–
595 722 [doi:10.4319/lo.2009.54.3.0713](https://doi.org/10.4319/lo.2009.54.3.0713)</jrn>

596 <jrn>Bowen JL, Crump BC, Deegan LA, Hobbie JE (2009b) Salt marsh sediment bacteria:
597 their distribution and response to external nutrient inputs. *ISME J* 3:924–934 [PubMed](#)
598 [doi:10.1038/ismej.2009.44](#)</jrn>

599 <jrn>Bowen JL, Ward BB, Morrison HG, Hobbie JE, Valiela I, Deegan LA, Sogin ML
600 (2011) Microbial community composition in sediments resists perturbation by nutrient
601 enrichment. *ISME J* 5:1540–1548 [PubMed](#) [doi:10.1038/ismej.2011.22](#)</jrn>

602 <jrn>Bratbak G (1985) Bacterial biovolume and biomass estimations. *Appl Environ*
603 *Microbiol* 49:1488–1493 [PubMed](#)</jrn>

604 <jrn>Brown SC (1969) The structure and function of the digestive system of the mud snail
605 *Nassarius obsoletus* (Say). *Malacologia* 9:447–500</jrn>

606 <jrn>Buffan-Dubau E, Carman KR (2000) Diel feeding behavior of meiofauna and their
607 relationships with microalgal resources. *Limnol Oceanogr* 45:381–395
608 [doi:10.4319/lo.2000.45.2.0381](#)</jrn>

609 <jrn>Buffan-Dubau E, de Wit R, Castel J (1996) Feeding selectivity of the harpacticoid
610 copepod *Canuella perplexa* in benthic muddy environments demonstrated by HPLC
611 analyses of chlorin and carotenoid pigments. *Mar Ecol Prog Ser* 137:71–82
612 [doi:10.3354/meps137071](#)</jrn>

613 <jrn>Canfield DE, Glazer AN, Falkowski PG (2010) The evolution of Earth's nitrogen cycle.
614 *Science* 330:192–196 [PubMed](#) [doi:10.1126/science.1186120](#)</jrn>

615 <edb>Carman KR (1993) Microautoradiographic detection of microbial activity. In: Kemp
616 PF, Sherr BF, Sherr EB, Cole JJ (eds) *Handbook of methods in aquatic microbial ecology*.
617 Lewis Publishers, Boca Raton, FL</edb>

618 <jrn>Carman KR, Fleeger JW, Pomarico SM (1997) Response of a benthic food web to
619 hydrocarbon contamination. *Limnol Oceanogr* 42:561–571
620 [doi:10.4319/lo.1997.42.3.0561](https://doi.org/10.4319/lo.1997.42.3.0561)</jrn>

621 <bok>Clarke KR, Warwick RM (2001) Change in marine communities: an approach to
622 statistical analysis and interpretation. PRIMER-E, Plymouth</bok>

623 <jrn>Conley DJ, Paerl HW, Howarth RW, Boesch DF and others (2009) Controlling
624 eutrophication: nitrogen and phosphorus. *Science* 323:1014–1015 [PubMed](https://pubmed.ncbi.nlm.nih.gov/19311111/)
625 [doi:10.1126/science.1167755](https://doi.org/10.1126/science.1167755)</jrn>

626 <jrn>Connor MS, Teal JM, Valiela I (1982) The effect of feeding by mud snails, *Ilyanassa*
627 *obsoleta* (Say), on the structure and metabolism of a laboratory benthic algal community.
628 *J Exp Mar Biol Ecol* 65:29–45 [doi:10.1016/0022-0981\(82\)90174-5](https://doi.org/10.1016/0022-0981(82)90174-5)</jrn>

629 <jrn>D'Avanzo C, Valiela I (1990) Use of detrital foods and assimilation of nitrogen by
630 coastal detritivores. *Estuaries* 13:20–24 [doi:10.2307/1351428](https://doi.org/10.2307/1351428)</jrn>

631 <jrn>de Jonge VN (1980) Fluctuations in the organic carbon to chlorophyll *a* ratios for
632 estuarine benthic diatom populations. *Mar Ecol Prog Ser* 2:345–353
633 [doi:10.3354/meps002345](https://doi.org/10.3354/meps002345)</jrn>

634

635 <jrn>de Jonge VN, Bouwman LA (1977) A simple density separation technique for
636 quantitative isolation of meiobenthos using the colloidal silica Ludox-TM. *Mar Biol*
637 42:143–148 [doi:10.1007/BF00391564](https://doi.org/10.1007/BF00391564)</jrn>

638 <jrn>Deegan LA, Bowen JL, Drake D, Fleeger JW and others (2007) Susceptibility of salt-
639 marshes to nutrient enrichment and predator removal. *Ecol Appl* 17:S42–S63
640 [doi:10.1890/06-0452.1](https://doi.org/10.1890/06-0452.1)</jrn>

641 <jrn>Deegan LA, Johnson DS, Warren RS, Peterson B, Fleeger JW, Fagherazzi S, Wollheim
642 WM (2012) Coastal eutrophication as a driver of salt marsh loss. *Nature* 490:388–392
643 [PubMed](#)</jrn>

644 <jrn>Diaz RJ, Rosenberg R (2008) Spreading dead zones and consequences for marine
645 ecosystems. *Science* 321:926–929 [PubMed](#) [doi:10.1126/science.1156401](https://doi.org/10.1126/science.1156401)</jrn>

646 <jrn>Drake DC, Peterson BJ, Galván KA, Deegan LA and others (2009) Salt marsh
647 ecosystem biogeochemical responses to nutrient enrichment: a paired ¹⁵N tracer study.
648 *Ecology* 90:2535–2546 [PubMed](#) [doi:10.1890/08-1051.1](https://doi.org/10.1890/08-1051.1)</jrn>

649

650 <jrn>Epstein SS (1997) Microbial food webs in marine sediments. I. Trophic interactions and
651 grazing rates in two tidal flat communities. *Microb Ecol* 34:188–198 [PubMed](#)
652 [doi:10.1007/s002489900048](https://doi.org/10.1007/s002489900048)</jrn>

653 <jrn>Ferreira JG, Andersen JH, Borja A, Bricker SB and others (2011) Overview of
654 eutrophication indicators to assess environmental status within the European Marine
655 Framework Strategy Framework Directive. *Estuar Coast Shelf Sci* 93:117–131
656 [doi:10.1016/j.ecss.2011.03.014](https://doi.org/10.1016/j.ecss.2011.03.014)</jrn>

657 <jrn>Fleeger JW, Carman KR, Webb S, Hilbun N, Pace ML (1999) Consumption of
658 microalgae by the grass shrimp *Palaemonetes pugio*. *J Crustac Biol* 19:324–336
659 [doi:10.2307/1549239](https://doi.org/10.2307/1549239)</jrn>

660 <jrn>Fox SE, Teichberg M, Olsen YS, Heffner L, Valiela I (2009) Restructuring of benthic
661 communities in eutrophic estuaries: lower abundance of prey leads to trophic shifts from
662 omnivory to grazing. *Mar Ecol Prog Ser* 380:43–57 [doi:10.3354/meps07917](https://doi.org/10.3354/meps07917)</jrn>

663 <jrn>Frost PC, Cross WF, Benstead JP (2005) Ecological stoichiometry in freshwater benthic
664 ecosystems: an introduction. *Freshw Biol* 50:1781–1785 [doi:10.1111/j.1365-](https://doi.org/10.1111/j.1365-2427.2005.01457.x)
665 [2427.2005.01457.x](https://doi.org/10.1111/j.1365-2427.2005.01457.x)</jrn>

666 <jrn>Galloway JN, Townsend AR, Erisman JW, Bekunda M and others (2008)
667 Transformation of the nitrogen cycle: recent trends, questions and potential solutions.
668 *Science* 320:889–892 [PubMed doi:10.1126/science.1136674](https://pubmed.ncbi.nlm.nih.gov/1136674/)</jrn>

669 <bok>Galván K (2008) The diet of saltmarsh consumers. Louisiana State University, Baton
670 Rouge, LA</bok>

671 <jrn>Galván K, Fleeger JW, Fry B (2008) Stable isotope addition reveals dietary importance
672 of phytoplankton and microphytobenthos to saltmarsh infauna. *Mar Ecol Prog Ser*
673 359:37–49 [doi:10.3354/meps07321](https://doi.org/10.3354/meps07321)</jrn>

674 <jrn>Galván K, Fleeger JW, Peterson B, Drake D, Deegan LA, Johnson DS (2011) Natural
675 abundance stable isotopes and dual isotope tracer additions help to resolve resources
676 supporting a saltmarsh food web. *J Exp Mar Biol Ecol* 410:1–11
677 [doi:10.1016/j.jembe.2011.08.007](https://doi.org/10.1016/j.jembe.2011.08.007)</jrn>

678 <jrn>Giordani G, Zaldivar JM, Viaroli P (2009) Simple tools for assessing water quality and
679 trophic status in transitional water ecosystems. *Ecol Indic* 9:982–991
680 [doi:10.1016/j.ecolind.2008.11.007](https://doi.org/10.1016/j.ecolind.2008.11.007)</jrn>

681 <jrn>Gruber N, Galloway JN (2008) An earth-system perspective of the global nitrogen
682 cycle. *Nature* 451:293–296 [PubMed doi:10.1038/nature06592](https://pubmed.ncbi.nlm.nih.gov/101038/)</jrn>

683 <jrn>Haglund AL, Hillebrand H (2005) The effect of grazing and nutrient supply on
684 periphyton associated bacteria. *FEMS Microbiol Ecol* 52:31–41 [PubMed](https://pubmed.ncbi.nlm.nih.gov/101016/)
685 [doi:10.1016/j.femsec.2004.10.003](https://doi.org/10.1016/j.femsec.2004.10.003)</jrn>

686 <jrn>Haubois AG, Guarini JM, Richard P, Fichet D, Radenac G, Blanchard GF (2005)
687 Ingestion rate of the deposit-feeder *Hydrobia ulvae* (Gastropoda) on epipelagic diatoms:
688 effect of cell size and algal biomass. *J Exp Mar Biol Ecol* 317:1–12
689 [doi:10.1016/j.jembe.2004.11.009](https://doi.org/10.1016/j.jembe.2004.11.009)</jrn>

690 <jrn>Heip CHR (1995) Eutrophication and zoobenthos dynamics. *Ophelia* 41:113–136</jrn>

691 <jrn>Hepinstall JA, Fuller RL (1994) Periphyton reactions to different light and nutrient
692 levels and the response of bacteria to these manipulations. *Arch Hydrobiol* 131:161–
693 173</jrn>

694 <jrn>Hillebrand H, Worm B, Lotze HK (2000) Marine microbenthic community structure
695 regulated by nitrogen loading and grazing pressure. *Mar Ecol Prog Ser* 204:27–38
696 [doi:10.3354/meps204027](https://doi.org/10.3354/meps204027)</jrn>

697 <jrn>Hillebrand H, Kahlert M, Haglund AL, Berninger UG, Nagel S, Wickham S (2002)
698 Control of microbenthic communities by grazing and nutrient supply. *Ecology* 83:2205–
699 2219 [doi:10.1890/0012-9658\(2002\)083\[2205:COMCBG\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[2205:COMCBG]2.0.CO;2)</jrn>

700 <jrn>Hillebrand H, Gruner DS, Borer ET, Bracken MES and others (2007) Consumer versus
701 resource control of producer diversity depends on ecosystem type and producer
702 community structure. *Proc Natl Acad Sci USA* 104:10904–10909 [PubMed](https://pubmed.ncbi.nlm.nih.gov/)
703 [doi:10.1073/pnas.0701918104](https://doi.org/10.1073/pnas.0701918104)</jrn>

704 <jrn>Howarth RW, Billen G, Swaney D, Townsend A and others (1996) Regional nitrogen
705 budgets and riverine N and P fluxes for the drainage to the North Atlantic Ocean: natural
706 and human influences. *Biogeochemistry* 35:75–139 [doi:10.1007/BF02179825](https://doi.org/10.1007/BF02179825)</jrn>

707 <jrn>Jensen P (1987) Feeding ecology of free-living aquatic nematodes. *Mar Ecol Prog Ser*
708 35:187–196 [doi:10.3354/meps035187](https://doi.org/10.3354/meps035187)</jrn>

709 <jrn>Johnson DS (2011) High-marsh invertebrates are susceptible to eutrophication. Mar
710 Ecol Prog Ser 438:143–152 [doi:10.3354/meps09306](https://doi.org/10.3354/meps09306)</jrn>

711 <jrn>Johnson DS, Fleeger JW (2009) Weak response of saltmarsh infauna to ecosystem-wide
712 nutrient enrichment and fish predator reduction: a four-year study. J Exp Mar Biol Ecol
713 373:35–44 [doi:10.1016/j.jembe.2009.03.003](https://doi.org/10.1016/j.jembe.2009.03.003)</jrn>

714 <bok>Johnson DS, Short M (2012) Chronic nutrient enrichment increases the density and
715 biomass of the mudsnail, *Nassarius obsoletus*. Estuaries Coasts ■:■–■</bok>

716 <jrn>Johnson DS, Fleeger JW, Galván KA, Moser EB (2007) Worm holes and their space-
717 time continuum: spatial and temporal variability of macroinfaunal annelids in a northern
718 New England salt marsh. Estuaries Coasts 30:226–237</jrn>

719 <jrn>Johnson DS, Fleeger JW, Deegan LA (2009) Large-scale manipulations reveal that top-
720 down and bottom-up controls interact to alter habitat utilization by saltmarsh fauna. Mar
721 Ecol Prog Ser 377:33–41 [doi:10.3354/meps07849](https://doi.org/10.3354/meps07849)</jrn>

722 <jrn>Juanes JA, Guinda X, Puente A, Revilla JA (2008) Macroalgae, a suitable indicator of
723 the ecological status of coastal rocky communities in the NE Atlantic. Ecol Indic 8:351–
724 359 [doi:10.1016/j.ecolind.2007.04.005](https://doi.org/10.1016/j.ecolind.2007.04.005)</jrn>

725 <jrn>Keats RA, Osher LJ, Neckles HA (2004) The effect of nitrogen loading on a brackish
726 estuarine faunal community: a stable isotope approach. Estuaries 27:460–471
727 [doi:10.1007/BF02803538](https://doi.org/10.1007/BF02803538)</jrn>

728 <jrn>Levinton JS (1987) The body size–prey size hypothesis and *Hydrobia*. Ecology 68:229–
729 231 [doi:10.2307/1938827](https://doi.org/10.2307/1938827)</jrn>

730 <bok>Lockfield K (2011) Chronic nutrient enrichment effects on mummichog, *Fundulus*
731 *heteroclitus* in a northeastern Massachusetts salt marsh. Masters thesis, Louisiana State
732 University, Baton Rouge, LA</bok>

733 <jrn>Lopez GR, Kofoed LH (1980) Epipsammic browsing and deposit-feeding in mud snails
734 (Hydrobiidae). J Mar Res 38:585–599</jrn>

735 <bok>McComb AJ (1995) Eutrophic shallow estuaries and lagoons. CRC Press, Boca Raton,
736 FL</bok>

737 <jrn>McFarlin CR, Brewer JS, Buck TL, Pennings SC (2008) Impact of fertilization on a salt
738 marsh food web in Georgia. Estuaries Coasts 31:313–325 [doi:10.1007/s12237-008-9036-](https://doi.org/10.1007/s12237-008-9036-9)
739 [9](https://doi.org/10.1007/s12237-008-9036-9)</jrn>

740 <jrn>Middelburg JJ, Barranguet C, Boschker HTS, Herman PMJ, Moens T, Heip CHR
741 (2000) The fate of intertidal microphytobenthos carbon. An *in situ* ¹³C labelling study.
742 Limnol Oceanogr 45:1224–1234 [doi:10.4319/lo.2000.45.6.1224](https://doi.org/10.4319/lo.2000.45.6.1224)</jrn>

743 <jrn>Moens T, Verbeeck L, de Maeyer A, Swings J, Vincx M (1999) Selective attraction of
744 marine bacterivorous nematodes to their bacterial food. Mar Ecol Prog Ser 176:165–178
745 [doi:10.3354/meps176165](https://doi.org/10.3354/meps176165)</jrn>

746 <jrn>Montagna PA, Blanchard GF, Dinet A (1995) Effect of production and biomass of
747 intertidal microphytobenthos on meiofaunal grazing rates. J Exp Mar Biol Ecol 185:149–
748 165 [doi:10.1016/0022-0981\(94\)00138-4](https://doi.org/10.1016/0022-0981(94)00138-4)</jrn>

749 <jrn>Moreno S, Niell XF (2004) Scales of variability in the sediment chlorophyll content in
750 the shallow Palmones River estuary, Spain. Estuar Coast Shelf Sci 60:49–57
751 [doi:10.1016/j.ecss.2003.06.006](https://doi.org/10.1016/j.ecss.2003.06.006)</jrn>

752 <jrn>Niering WA, Warren RS (1980) Vegetation patterns and processes in New England salt
753 marshes. BioScience 30:301–307 [doi:10.2307/1307853](https://doi.org/10.2307/1307853)</jrn>

754 <jrn>Nixon SW, Ammerman JW, Atkinson LP, Berounsky VM and others (1996) The fate of
755 nitrogen and phosphorus at the land–sea margin of the North Atlantic Ocean.
756 Biogeochemistry 35:141–180 [doi:10.1007/BF02179826](https://doi.org/10.1007/BF02179826)</jrn>

757 <jrn>Pascal PY, Dupuy C, Haubois AG, Richard P, Niquil N (2008a) Influence of
758 environmental factors on bacterial ingestion rate of the deposit-feeder *Hydrobia ulvae* and
759 comparison with meiofauna. *J Sea Res* 60:151–156
760 [doi:10.1016/j.seares.2008.05.003](https://doi.org/10.1016/j.seares.2008.05.003)</jrn>

761 <jrn>Pascal PY, Dupuy C, Mallet C, Richard P, Niquil N (2008b) Bacterivory by benthic
762 organisms in sediment: quantification using ¹⁵N-enriched bacteria. *J Exp Mar Biol Ecol*
763 355:18–26 [doi:10.1016/j.jembe.2007.11.012](https://doi.org/10.1016/j.jembe.2007.11.012)</jrn>

764 <jrn>Pascal PY, Dupuy C, Richard P, Mallet C, Armynot du Chatelet E, Niquil N (2009)
765 Seasonal variation in consumption of benthic bacteria by meio- and macrofauna in an
766 intertidal mudflat. *Limnol Oceanogr* 54:1048–1059 [doi:10.4319/lo.2009.54.4.1048](https://doi.org/10.4319/lo.2009.54.4.1048)</jrn>

767 <jrn>Peterson BJ, Deegan LA, Helfrich J, Hobbie JE and others (1993) Biological response
768 of a tundra river to fertilization. *Ecology* 74:653–672 [doi:10.2307/1940794](https://doi.org/10.2307/1940794)</jrn>

769 <edb>Pinckney JL (1994) Development of an irradiance-based ecophysiological model for
770 intertidal benthic microalgal production. In: Krumbein WE, Paterson DM, Stal LJ (eds)
771 Biostabilization of sediments. ■, Oldenberg</edb>

772 <jrn>Posey MH, Alphin TD, Cahoon L, Lindquist D, Becker ME (1999) Interactive effects of
773 nutrient additions and predation on infaunal communities. *Estuaries* 22:785–792
774 [doi:10.2307/1353111](https://doi.org/10.2307/1353111)</jrn>

775 <jrn>Posey MH, Alphin TD, Cahoon L, Lindquist D, Malin MA, Nevers MB (2002) Top-
776 down versus bottom-up limitation in benthic infaunal communities: direct and indirect
777 effects. *Estuaries* 25:999–1014 [doi:10.1007/BF02691347](https://doi.org/10.1007/BF02691347)</jrn>

778 <jrn>Posey MH, Alphin TD, Cahoon L (2006) Benthic community responses to nutrient
779 enrichment and predator exclusion: influence of background nutrient concentrations and

780 interactive effects. *J Exp Mar Biol Ecol* 330:105–118
781 [doi:10.1016/j.jembe.2005.12.020](https://doi.org/10.1016/j.jembe.2005.12.020)</jrn>

782 <jrn>Sardá R, Valiela I, Foreman K (1996) Decadal shifts in a salt marsh macroinfaunal
783 community in response to sustained long-term experimental nutrient enrichment. *J Exp*
784 *Mar Biol Ecol* 205:63–81 [doi:10.1016/S0022-0981\(96\)02600-7](https://doi.org/10.1016/S0022-0981(96)02600-7)</jrn>

785 <jrn>Schramm W (1999) Factors influencing seaweed responses to eutrophication: some
786 results from EU-project EUMAC. *J Appl Phycol* 11:69–78
787 [doi:10.1023/A:1008076026792](https://doi.org/10.1023/A:1008076026792)</jrn>

788 <bok>Silliman BR, Grosholz ED, Bertness MD (2010) Human impacts on salt marshes: a
789 global perspective. University of California Press, Berkeley, CA</bok>

790 <edb>US EPA (US Environmental Protection Agency) (2002) ■. EPA/620/R-02/003, US
791 EPA, Office of Research and Development, National Health and Research Laboratory,
792 Atlantic Ecology Division, Narragansett, RI</edb>

793 <jrn>van Oevelen D, Middelburg JJ, Soetaert K, Moodley L (2006) The fate of bacterial
794 carbon in sediments: modeling an *in situ* isotope tracer experiment. *Limnol Oceanogr*
795 51:1302–1314 [doi:10.4319/lo.2006.51.3.1302](https://doi.org/10.4319/lo.2006.51.3.1302)</jrn>

796 <jrn>Vander Grinten E, Sismis SGH, Barranguet C, Admiraal W (2004) Dominance of
797 diatoms over cyanobacterial species in nitrogen-limited biofilms. *Arch Hydrobiol* 161:98–
798 111 [doi:10.1127/0003-9136/2004/0161-0099](https://doi.org/10.1127/0003-9136/2004/0161-0099)</jrn>

799 <jrn>Watermann F, Hillebrand H, Gerdes G, Krumbein WE, Sommer U (1999) Competition
800 between benthic cyanobacteria and diatoms as influenced by different grain sizes and
801 temperatures. *Mar Ecol Prog Ser* 187:77–87 [doi:10.3354/meps187077](https://doi.org/10.3354/meps187077)</jrn>

802 <jrn>Wimp GM, Murphy SM, Finke DL, Huberthy AF, Denno RF (2010) Increased primary
803 production shifts the structure and composition of a terrestrial arthropod community.
804 Ecology 91:3303–3311 PubMed doi:10.1890/09-1291.1</jrn>

805 <jrn>Wyckmans M, Chepurnov VA, Vanreusel A, De Troch M (2007) Effect of food
806 diversity on diatom selection by harpacticoid copepods. J Exp Mar Biol Ecol 345:119–128
807 doi:10.1016/j.jembe.2007.02.002</jrn>

808 Table 1. Contribution (%) of fatty acids (FA) specific to diatoms, green algae/cyanobacteria and bacteria to total fatty acid methyl esters (FAME)
 809 and weighted $\delta^{13}\text{C}$ of each compound in the 3 creeks studied (means \pm SD; n = 3). RC: reference creek; SFC: short-term fertilized creek; CFC:
 810 chronically fertilized creek

	FAME (%)			Weighted $\delta^{13}\text{C}$ (%)		
	RC	SFC	CFC	RC	SFC	CFC
Diatom FA	15.9 \pm 1.6	11.3 \pm 0.7	17.0 \pm 0.9	2973 \pm 123	2697 \pm 73	2180 \pm 38
Green algal/cyanobacterial FA	1.8 \pm 0.3	1.8 \pm 0.1	1.6 \pm 0.0	3164 \pm 105	2633 \pm 194	3654 \pm 217
Bacterial FA	8.3 \pm 0.1	10.8 \pm 0.2	6.6 \pm 0.1	472 \pm 7	323 \pm 6	808 \pm 8

811 Table 2. Biomasses of bacteria and benthic microalgae (BMA) (in mg C m⁻², n = 3) of taxa in
812 the 3 creeks studied. An asterisk indicates significant (p < 0.01) differences with the 2 other
813 creeks (ANOVA; Tukey test) for meiofauna (nematodes, copepods, foraminiferans and
814 ostracods, in 10³ ind. m⁻² and mg C m⁻², n = 2) and epifauna (hydrobiid snails and *Nassarius*
815 *obsoletus*, in ind. m⁻² and g C m⁻², n = 10). RC: reference creek; SFC: short-term fertilized
816 creek; CFC: chronically fertilized creek

	RC	SFC	CFC
Bacteria (mg C m ⁻²)	432 ± 26	408 ± 23	410 ± 53
BMA (mg C m ⁻²)	1625 ± 58*	799 ± 72*	2256 ± 52*
Nematodes (10 ³ ind. m ⁻²)	317 ± 67	287 ± 9	1167 ± 8
Copepods (10 ³ ind. m ⁻²)	213 ± 74	367 ± 104	199 ± 41
Foraminiferans (10 ³ ind. m ⁻²)	157 ± 2	213 ± 41	40 ± 33
Ostracods (10 ³ ind. m ⁻²)	33 ± 22	98 ± 8	58 ± 28
Total meiofauna (mg C m ⁻²)	272 ± 81	546 ± 50	392 ± 105
Hydrobiids (10 ³ ind. m ⁻²)	9 ± 7	18 ± 5	6 ± 8
<i>N. obsoletus</i> small (ind. m ⁻²)	22 ± 13	146 ± 87	237 ± 107
<i>N. obsoletus</i> large (ind. m ⁻²)	120 ± 74	158 ± 95	515 ± 232
Total epifauna (g C m ⁻²)	74 ± 48	135 ± 85	404 ± 164

817

818

819 Table 3. Amount of bacterial carbon grazed daily ($\mu\text{g C m}^{-2} \text{d}^{-1}$) by meiofauna (nematodes,
820 copepods, foraminiferans and ostracods), epifauna (hydrobiid snails, small and large
821 *Nassarius obsoletus*) and total fauna in surficial sediments (0.5 cm) from the 3 creeks studied
822 (means \pm SD; n = 3). Percentage values correspond to the fractions of total bacteria ingested
823 by each grazer. An asterisk indicates significant ($p < 0.05$) differences with the 2 other creeks
824 (ANOVA; Tukey test). RC: reference creek; SFC: short-term fertilized creek; CFC:
825 chronically fertilized creek

	RC	SFC	CFC
Nematodes	6.4 \pm 1.0	5.8 \pm 0.3	15.2 \pm 1.3*
Copepods	16.7 \pm 3.1	55.4 \pm 16.7*	23.5 \pm 6.4
Foraminiferans	2.4 \pm 1.2	5.5 \pm 1.7	0.6 \pm 0.5
Ostracods	12.1 \pm 5.7	33.0 \pm 21.0	22.2 \pm 3.5
Total meiofauna	37.7 \pm 6.5	99.6 \pm 3.7	61.5 \pm 9.2
Percent total meiofauna	5	6	2
Hydrobiid snails	285.3 \pm 23.7*	838.4 \pm 125.7	587.1 \pm 137.0
Percent hydrobiid snails	38	53	23
<i>N. obsoletus</i> (small)	28.7 \pm 10.9*	193.9 \pm 16.7	263.0 \pm 77.4
<i>N. obsoletus</i> (large)	399.4 \pm 183.6	544.2 \pm 106.3	1692.6 \pm 616.2*
Percent <i>N. obsoletus</i>	57	44	75
Total epifauna	713.4 \pm 214.9	1576.5 \pm 241.0	2542.7 \pm 771.3
Total grazers	751.0 \pm 214.3	1676.1 \pm 223.6	2604.3 \pm 762.1

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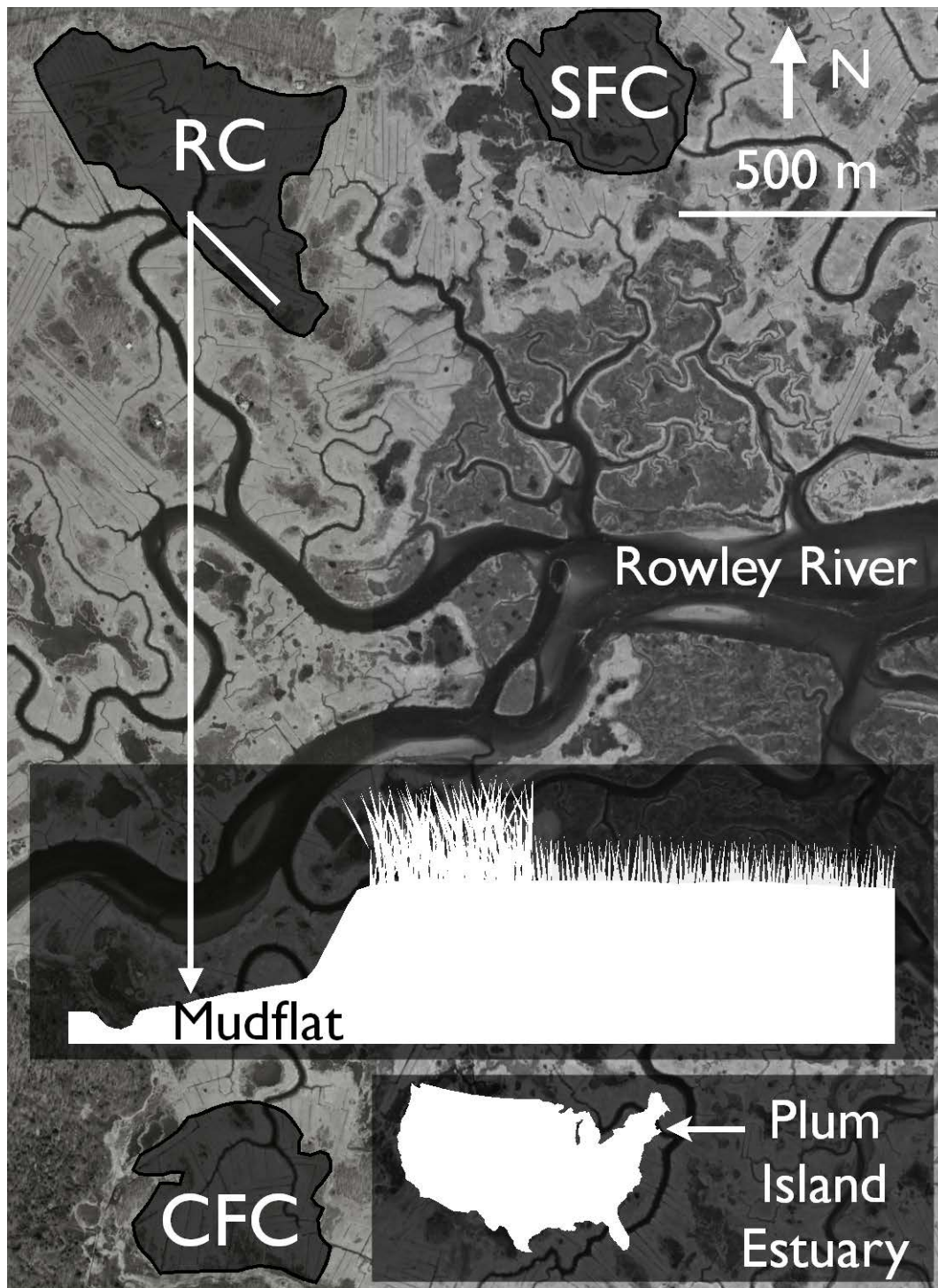
827

828 Table 4. Amount of algal carbon grazed daily ($\mu\text{g C m}^{-2} \text{d}^{-1}$) and fraction of algal standing
829 stock grazed daily (%) by meiofauna (nematodes, copepods, foraminiferans, and ostracods)
830 and epifauna (hydrobiid snails, small and large *Nassarius obsoletus*) in surficial sediment (0.5
831 cm) of the 3 creeks studied (means \pm SD; n = 3). Percentage values correspond to the
832 fractions of **total algae** ingested by each grazer. An asterisk indicates significant differences (p
833 < 0.05) with the 2 other creeks (ANOVA; Tukey test). RC: reference creek; SFC: short-term
834 fertilized creek; CFC: chronically fertilized creek

	RC	SFC	CFC
Nematodes	66.8 \pm 17.2	11.8 \pm 2.6	493.6 \pm 128.3*
Copepods	267.5 \pm 63.5	562.2 \pm 287.6	470.8 \pm 89.2
Foraminiferans	12.1 \pm 1.0	6.6 \pm 5.1	2.9 \pm 1.4
Ostracods	4.0 \pm 2.0	33.7 \pm 28.1	105.9 \pm 15.1*
Total meiofauna	349.3 \pm 46.0	614.3 \pm 287.4	1073.2 \pm 136.9
Percent total meiofauna	27	36	24
Hydrobiid snails	674.5 \pm 210.7	842.2 \pm 84.9	1977.8 \pm 559.0*
Percent hydrobiid snails	51	49	45
<i>N. obsoletus</i> (small)	21.0 \pm 12.4	78.2 \pm 2.9	222.1 \pm 106.8
<i>N. obsoletus</i> (large)	267.3 \pm 218.3	170.7 \pm 67.5	1112.3 \pm 459.1*
Percent <i>N. obsoletus</i>	22	15	30
Total epifauna	962.7 \pm 434.2	1091.2 \pm 153.9	3312.2 \pm 1061.7*
Total grazers	1312.0 \pm 388.2	1705.5 \pm 150.1	4385.4 \pm 954.5*

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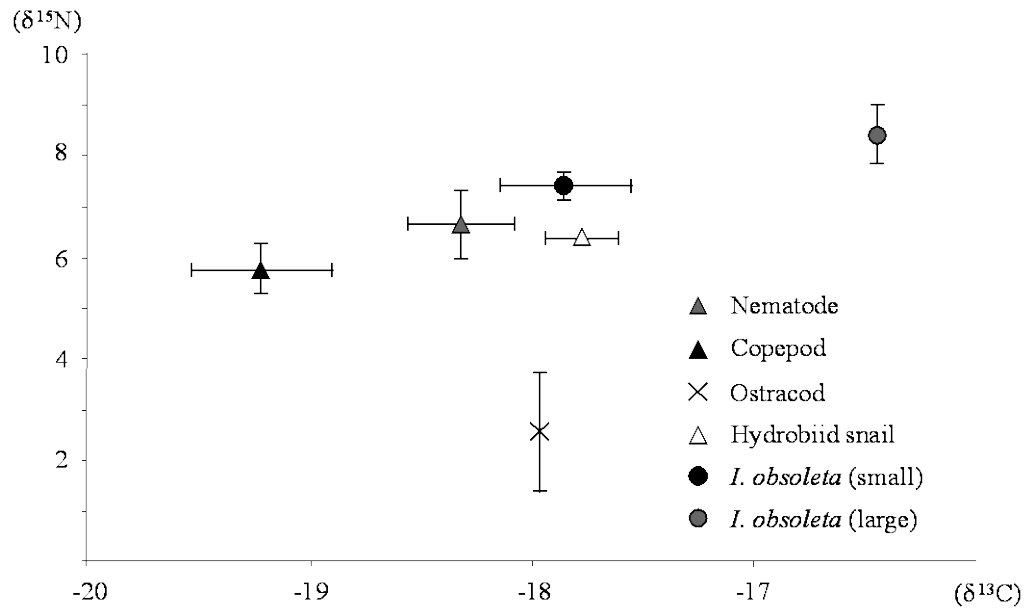
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838 Fig. 1. Rowley River region salt marshes of the Plum Island estuary, Massachusetts, USA,
 839 and a profile of salt marsh habitat with the locations of mudflats studied. RC: reference creek;
 840 SFC: short-term fertilized creek; CFC: chronically fertilized creek (not drawn to scale)

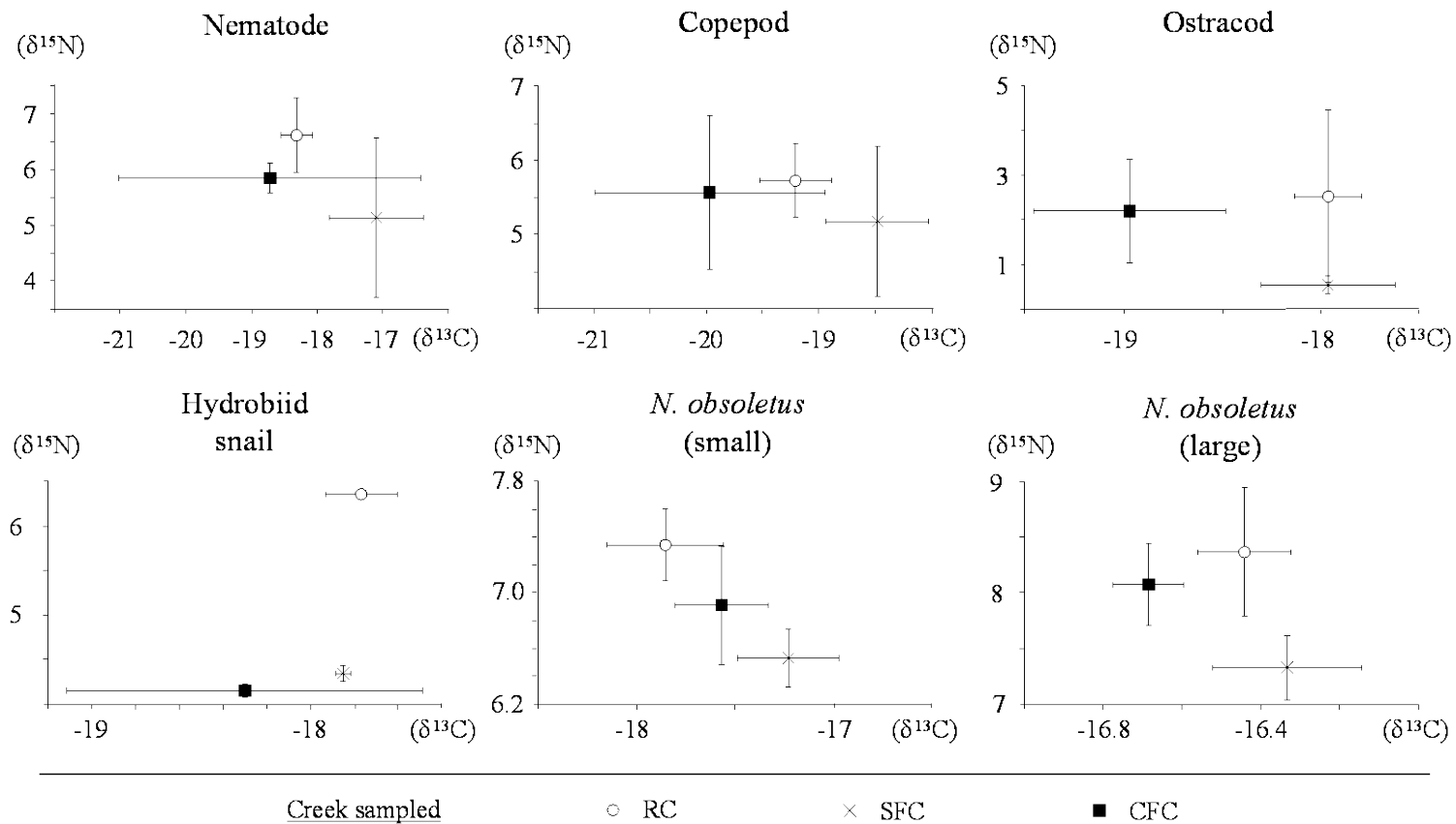
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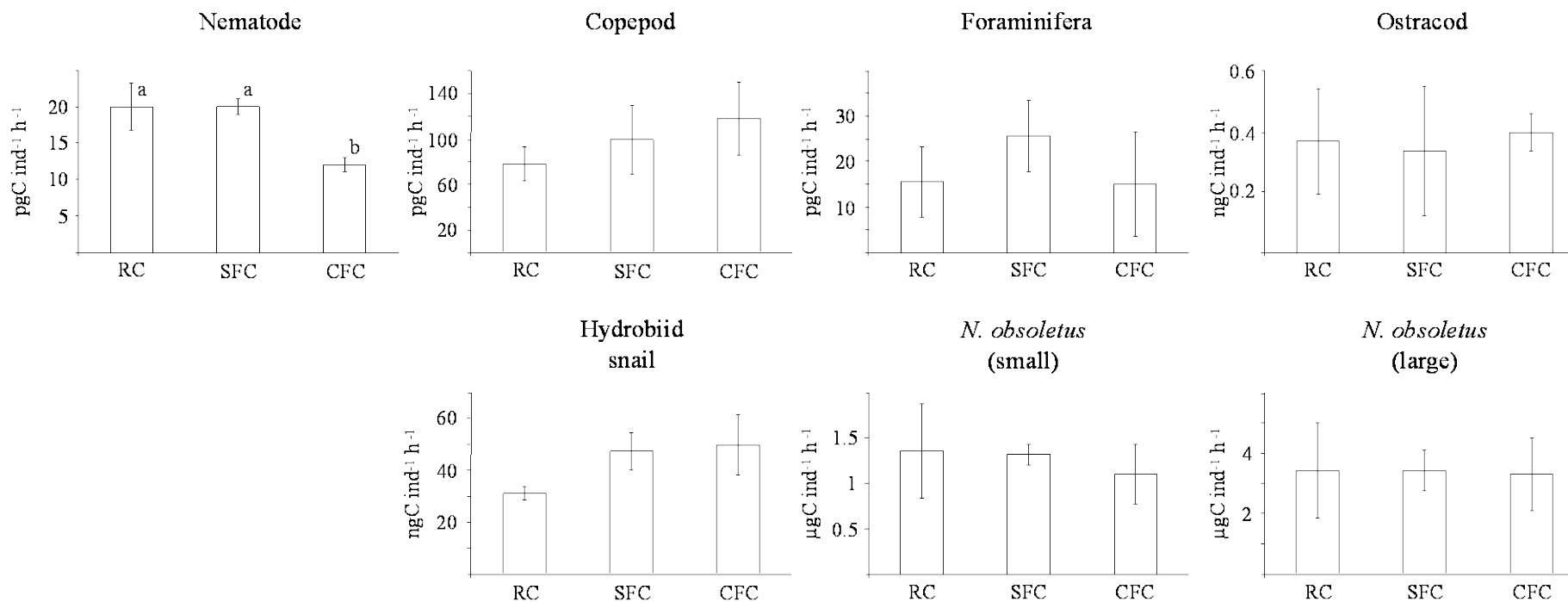
843 Fig. 2. Natural isotopic composition ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) of meiofauna (nematodes, copepods and
 844 ostracods) and epifauna (hydrobiid snails and small and large *Nassarius obsoletus*) in the
 845 reference creek (means \pm SD; n = 3)

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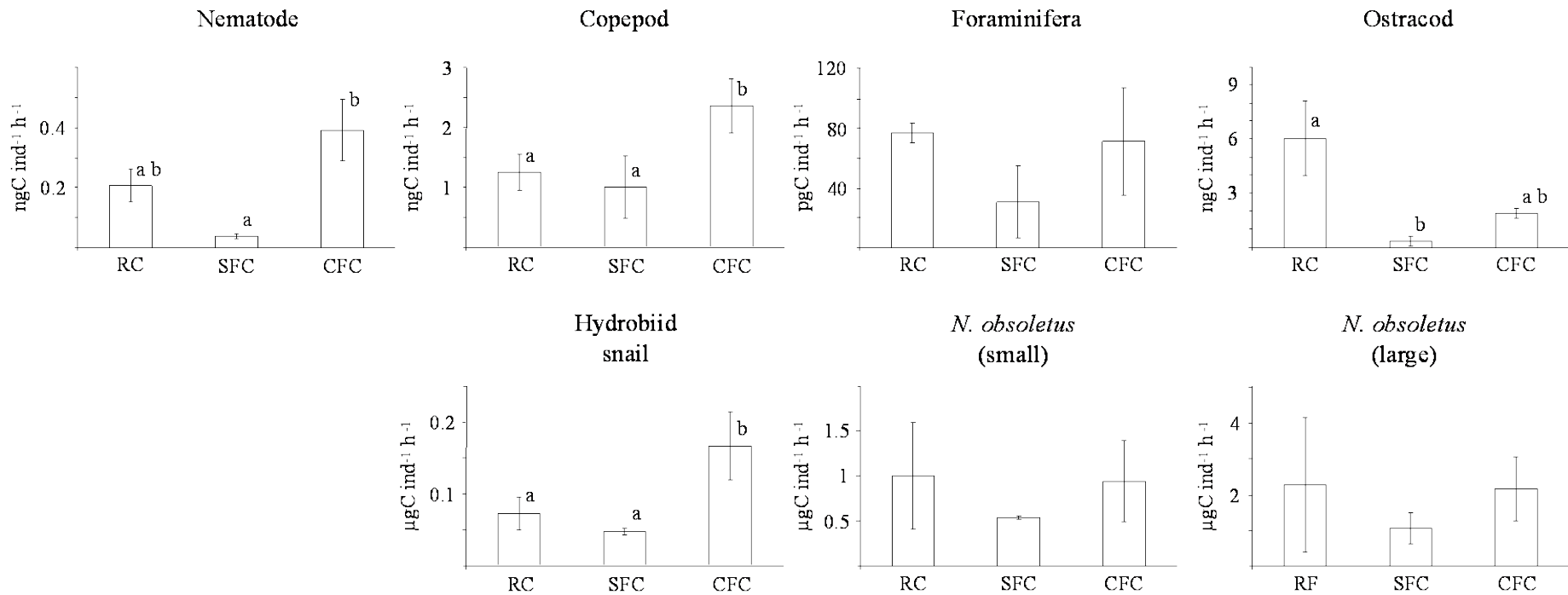
847

848 Fig. 3. Natural isotopic composition ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) of meiofauna (nematodes, copepods and ostracods) and epifauna (hydrobiid snails and small
 849 and large *Nassarius obsoletus*) in the 3 creeks studied (means \pm SD; n = 3). RC: reference creek; SFC: short-term fertilized creek; CFC:
 850 chronically fertilized creek



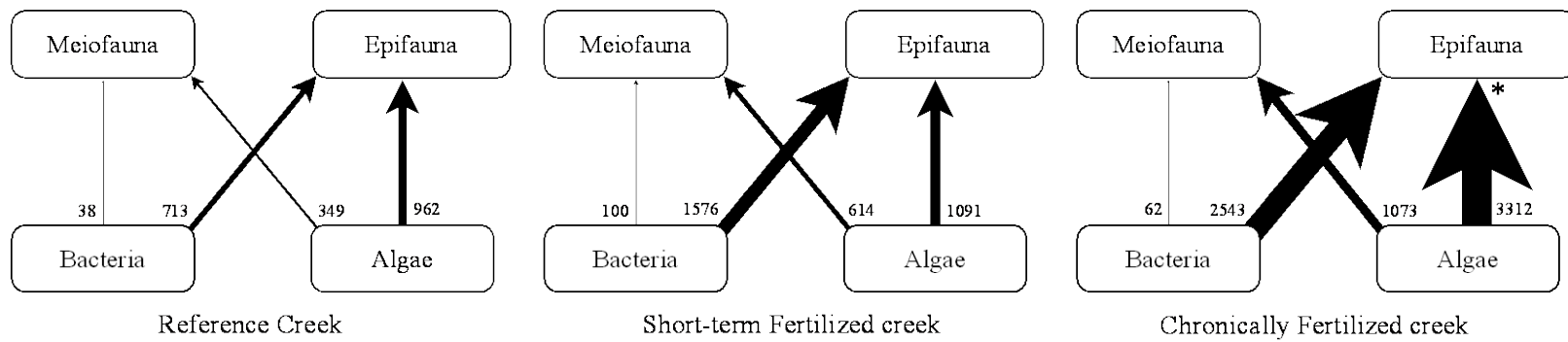
851

852 Fig. 4. Grazing rates of bacterial carbon, per individual grazer per hour, of meiofauna (nematodes, copepods, foraminiferans and ostracods) and
 853 epifauna (hydrobiid snails and small and large *Nassarius obsoletus*) in the 3 creeks studied (means ± SD; n = 3). Different letters above bars
 854 indicate significant differences (p < 0.05) between grazing rates (ANOVA; Tukey test). RC: reference creek; SFC: short-term fertilized creek;
 855 CFC: chronically fertilized creek



857

858 Fig. 5. Grazing rates of algal carbon, per individual grazer per hour, of meiofauna (nematodes, copepods, foraminiferans and ostracods) and
 859 epifauna (hydrobiid snails and small and large *Nassarius obsoletus*) in the 3 creeks studied (means \pm SD; n = 3). Different letters above bars
 860 indicate significant differences ($p < 0.05$) between grazing rates (ANOVA; Tukey test). RC: reference creek; SFC: short-term fertilized creek;
 861 CFC: chronically fertilized creek



862

863 Fig. 6. Fluxes between benthic bacteria and algae and their meiofaunal and epifaunal grazers ($g\ C\ m^{-2}\ d^{-1}$) in the 3 creeks studied. An asterisk

864 indicates significant ($p < 0.01$) differences in flux (ANOVA; Tukey test)

865