1   Effects of experimental warming and carbon addition on nitrate reduction and respiration in

2   coastal sediments

3

4   Lindsay D. Brin[1,2,3*], Anne E. Giblin[2], and Jeremy J. Rich[1]

5

6   [1]Department of Ecology and Evolutionary Biology, Brown University, 80 Waterman Street,

7   Providence, Rhode Island 02912

8   [2]Ecosystems Center, Marine Biological Laboratory, 7 MBL Street, Woods Hole, Massachusetts

9   02543

10   [3]Current address: Agriculture and Agri-Food Canada, Potato Research Centre, 850 Lincoln

11   Road, Fredericton, NB E3B 4Z7, Canada

12

13   *Corresponding author, email: lindsay.brin@gmail.com; phone: (506) 460-4520

14

17

18   Article type: General research

19

23   **Abstract**

24   Climate change may have differing effects on microbial processes that control coastal N

25   availability. We conducted a microcosm experiment to explore effects of warming and carbon

26   availability on nitrate reduction pathways in marine sediments. Sieved continental shelf

27   sediments were incubated for 12 weeks under aerated seawater amended with nitrate (~50 μM),

28   at winter (4°C) or summer (17°C) temperatures, with or without biweekly particulate organic C

29   additions. Treatments increased diffusive oxygen consumption as expected, with somewhat

30   higher effects of C addition compared to warming. Combined warming and C addition had the

31   strongest effect on nitrate flux across the sediment water interface, with a complete switch early

32   in the experiment from influx to sustained efflux. Supporting this result, vial incubations with

33   added ^{15}N-nitrate indicated that C addition stimulated potential rates of dissimilatory nitrate

34   reduction to ammonium (DNRA), but not denitrification. Overall capacity for both

35   denitrification and DNRA was reduced in warmed treatments, possibly reflecting C losses due to

36   increased respiration with warming. Anammox potential rates were much lower than DNRA or

37   denitrification, and were slightly negatively affected by warming or C addition. Overall, results

38   indicate that warming and C addition increased ammonium production through remineralization

39   and possibly DNRA. This stimulated nitrate production through nitrification, but without a

40   comparable increase in nitrate consumption through denitrification. The response to C of

41   potential DNRA rates over denitrification, along with a switch to nitrate efflux, raises the

42   possibility that DNRA is an important and previously overlooked source of internal N cycling in

43   shelf sediments.

Introduction

The amount of nitrogen (N) available in marine ecosystems is a determining factor for primary productivity at both local and global scales (Ryther and Dunstan 1971; Perry and Eppley 1981), and in excess causes eutrophication, a serious problem in coastal areas (Diaz and Rosenberg 2008). Available N is dependent not only on ecosystem inputs, but also on the balance of microbially mediated N cycling processes that lead to N removal or recycling. N is removed from coastal systems by denitrification and anammox, which reduce nitrate (NO_3^-) or nitrite (NO_2^-) to gaseous N_2 (Canfield 2005; Thamdrup 2012). Denitrification in coastal and marine sediments has been estimated to be one of the largest sinks of N in the marine environment, and may remove up to half of the total N inputs into the ocean (Christensen 1994; Codispoti et al. 2001). Anammox appears to be less important, especially in sediments where denitrification rates are high, but its importance is still being constrained (Trimmer and Engström 2011). In contrast to denitrification and anammox, dissimilatory NO_3^- reduction to ammonium (DNRA) retains N in the system as ammonium (NH_4^+) (Joye and Anderson 2008). While there is increasing evidence that DNRA can be as relevant as denitrification to net N availability and to NO_3^- reduction in shallow systems (Giblin et al. 2013), its importance in shelf sediments has not been well studied.

Changing temperatures in coastal ecosystems could alter the partitioning between these processes and thus the balance between N removal and recycling, but little is known about the specific effects of increased temperatures over relevant time scales. Studies that link seasonally changing temperatures to DNRA and denitrification rates suggest that warming may influence the balance of N cycling (Jørgensen 1989; Kelly-Gerreyn et al. 2001; Gruca-Rokosz et al. 2009). Higher temperatures may increase sediment O_2 consumption and thus be linked to lower

67   sediment redox potentials that favor DNRA (Giblin et al. 2013). Furthermore, DNRA has been

68   found to be dominant in warmer tropical ecosystems, and negligible in cold, deep sediments

69   (Dong et al. 2011; Crowe et al. 2012). If warming sediments have a similar effect as seasonally

70   or spatially changing temperatures, climate change could increase the relative importance of

71   DNRA to NO_3^- reduction.

72   Many of the impacts of temperature are likely to be mediated through its influence on C

73   availability, which may be the ultimate driver of the balance of the N cycle (Tiedje et al. 1982;

74   Christensen et al. 2000; Thamdrup and Dalsgaard 2002). Availability of organic C may influence

75   denitrification and DNRA more than anammox. Denitrification and DNRA can be directly

76   coupled to organic C respiration, while anammox is primarily autotrophic (Thamdrup 2012).

77   However, anammox can still be affected by variability in organic C through release of NH_4^+ and

78   NO_2^- during heterotrophic remineralization (Trimmer et al. 2003; Babbin et al. 2014).

79   Relationships between warming and sediment organic C availability could be manifested in

80   different ways, particularly through changes in phytoplankton delivery or sediment respiration.

81   Warming may change the timing and magnitude of spring phytoplankton blooms in coastal

82   ecosystems (Sommer and Lengfellner 2008; Nixon et al. 2009; Lewandowska and Sommer

83   2010), potentially altering C availability in benthic sediments. Warming may also interact with C

84   availability by increasing total benthic respiration, leading to a shortage of labile organic matter

85   in sediments (Alsterberg et al. 2012).

86   Changes in organic C availability could alter rates of denitrification, anammox, and

87   DNRA differently. Based on thermodynamic considerations, Tiedje et al. (1982) hypothesized

88   that DNRA should be favored over denitrification by a high ratio of organic matter to NO_3^-. This

89   hypothesis has been supported by culture experiments with a denitrifier and DNRA bacterium,

90   and by measurements in estuarine and reservoir sediments with varied organic matter loading

91   rates (Rehr and Klemme 1989; Christensen et al. 2000; Gruca-Rokosz et al. 2009; Gardner and

92   McCarthy 2009). These studies suggest that DNRA is favored over denitrification under very

93   high organic C loading rates, and therefore high respiration rates. However, for other types of

94   sediments typical of coastal systems, experimental evidence showing the relationship between C

95   availability and DNRA is more limited, as denitrification is also expected to increase with

96   organic C (Giblin et al. 2013). A recent modeling study showed that anammox was favored

97   under lowest organic C to NO_3^- ratios, denitrification at intermediate ratios, and DNRA at the

98   highest ratios (Algar and Vallino 2014). However, experimental evidence for this hypothesized

99   pattern is currently limited.

100   We conducted a microcosm experiment to investigate the effect of increased temperature

101   on sediment NO_3^- reducing processes in continental shelf sediments, and determine whether

102   effects are mediated by organic C availability. Building on previous studies that have used

103   microcosms or mesocosms to examine N cycling in sediments (Jensen et al. 1994; Fulweiler et

104   al. 2008; Neubacher et al. 2013), our microcosm experiment simultaneously tested the influence

105   of warming and C addition on all three processes currently known to respire NO_3^- or NO_2^-. We

106   hypothesized that warming alone would increase sediment respiration rates and eventually

107   decrease organic C availability. This would in turn lead to decreased denitrification potential

108   rates, and possibly decreased DNRA. Anammox might be favored under conditions with lower C

109   availability, but not if there was a direct negative effect of warming (Dalsgaard and Thamdrup

110   2002). As C was added at a modest rate comparable to *in situ* deposition rates, we hypothesized

111   that it would favor denitrification relative to DNRA, leading to increased net consumption of

112   inorganic N.

113

Methods

Sampling

116   On March 14, 2012, sediment was collected from a continental shelf site in Rhode Island

117 Sound (RIS2). This site was chosen as it was previously shown to exhibit appreciable potential

118 rates of anammox and denitrification (Brin et al. 2014). RIS2 has a water column depth of 38 m.

119 The water column is typically well mixed during winter, suggesting that O_2 was likely near air

120 saturation. At the time of collection, bottom water was 4°C and NO_3^- concentration was 0.3 μM.

121 During a two-year period when this experiment was conducted, bottom water temperature ranged

122 from 3 to 17°C (Brin et al. 2014), and NO_3^- concentration was 0.3-13 μM (Hardison, Giblin and

123 Rich, unpublished).

124   Sediments were collected using a box core, and the top 4 cm of sediment were collected

125 into coolers, covered with bottom water at in situ temperature, and brought back to the

126 laboratory. Sediment was held in the dark at 4°C, with aquarium bubblers used to keep the

127 overlying water oxic.

128

Microcosm experiment

130   Fifteen microcosms were set up four days later, with all processing done at 4°C. A

131 microcosm consisted of sieved sediment (1 mm) layered into a glass pan to a depth of ~4 cm

132 (20.9 x 11.1 cm). The pan was placed in an aquarium containing 6 L of 0.2 μm-filtered

133 Narragansett Bay seawater (salinity 32), which was kept air saturated with aquarium pumps, in

134 the dark. There were three replicate microcosms in each experimental treatment, as described

135 below. To decrease potential NO_3^- limitation in the experiment, we adjusted the NO_3^-

136   concentration in the aquarium water to 50 µM. We monitored NO_3^- concentration weekly in the

137   microcosms. In cases where there was net consumption, we adjusted the concentration back up to

138   50 µM. Every other week, half of the overlying water in each microcosm was refreshed with

139   initial seawater as a precaution against buildup of potential inhibitors or any other toxin.

140   Afterwards, the NO_3^- concentration was again adjusted back up to 50 µM. There was never a

141   smell of hydrogen sulfide, ammonia, or volatile fatty acids coming from the microcosms.

142   All microcosms received an initial pre-incubation at 4°C for 16 days, after which three

143   microcosms were sampled destructively, and potential rate experiments were conducted (t_0

144   experiments). Half of the remaining microcosms were kept at 4°C, and the other half were

145   warmed to 17°C, reflecting winter low and summer high temperatures at the collection site.

146   Sediment temperatures were monitored using iButtons (Dallas Semiconductor Corp) for the

147   duration of the experiment (12 weeks). Temperatures were maintained by placing microcosms in

148   a 4°C cold room or at 17°C in a large temperature-controlled water bath. Every two weeks, half

149   of the microcosms at each temperature received additions of C in the form of *Chlorella* algae,

150   resulting in four treatments (4°C, 4°C+C, 17°C, and 17°C+C) (Fig. 1). Before addition,

151   *Chlorella* was leached to remove soluble C as follows: for each microcosm, 240 mg of *Chlorella*

152   *vulgaris* powder (Jarrow Formulas, 100% pure Yaeyama Chlorella) was placed in a plastic

153   centrifuge tube with deionized water (45 ml), and tubes were shaken and allowed to sit

154   overnight. The next day, tubes were centrifuged (3,000 x g for 3 min) and the supernatant was

155   poured off, resulting in *Chlorella* pellets with a C:N ratio of 5.3. *Chlorella* pellets were

156   resuspended in filter sterilized seawater (15 ml), and suspensions were evenly added directly to

157   the top of the sediment in each microcosm after gently removing the overlying water. The algae

158   was mixed in by gently stirring the top 1 cm of sediment with a spatula, and then overlying

159　microcosm water was added back. C was added at a rate equivalent to 3.1 μmol C cm^{-2} d^{-1}. This

160　rate was chosen as it is somewhat higher than diffusive O_2 consumption measured at the site

161　(approximately 1.5 μmol cm^{-2} d^{-1}) (Brin et al. 2014), and was therefore expected to maintain

162　labile C stocks. Microcosms without C additions had the same treatment except using seawater

163　without *Chlorella*.

164

165　*Oxygen microprofiles, sediment C and N, and inorganic N measurements*

166　　　　To determine O_2 consumption rates, O_2 microprofiles were measured with a

167　microelectrode (OX100, Unisense) in 4 microcosms just prior to initiating treatments (t_0), in

168　17°C microcosms in week 1, and in all microcosms in weeks 5 and 11. Details about profiling

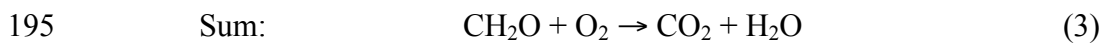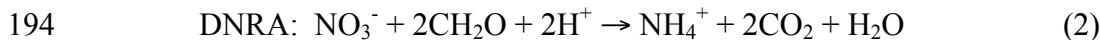169　methods and rate estimates were described previously (Brin et al. 2014).

170　　　　Total sediment C and N were measured on sediments harvested for rate measurements, at

171　(t_0) or after 12 weeks, using a CE Instruments NC2100 Elemental Analyzer (CE Elantech,

172　Lakewood, NJ).

173　　　　Overlying water samples were collected and frozen about three times a week to measure

174　concentrations of NO_3^-, NO_2^-, and NH_4^+. NO_3^- + NO_2^- were measured colorimetrically using

175　spongy cadmium (Jones 1984), and NO_2^- was measured without Cd reduction, either by hand or

176　on a Westco autoanalyzer (SmartChem 200, Westco Instruments, Brookfield, Connecticut). Pore

177　water NH_4^+ was measured using the phenol/hypochlorite method (Koroleff 1983), by hand or on

178　a Westco autoanalyzer. Net fluxes of DIN over the sediment-water interface were calculated

179　before t_0 and during weeks 1, 3, 5, 7, 9, and 11 as the change in aquarium water nutrient

180　concentration over a 3- or 4-day period during which no water column NO_3^- adjustments were

181　made.

182   To further assess nitrification and N losses during later stages of the experiment, we used

183   O_2 consumption rates from week 11 to calculate nitrification, assuming that (1) O_2 was used for

184   aerobic remineralization and nitrification of all NH_4^+ produced by remineralization, as there was

185   little to no NH_4^+ flux from the sediments throughout the experiment, (2) organic matter

186   composition followed Redfield stoichiometry, and (3) all heterotrophic respiration was

187   accounted for by O_2 consumption. In total, this would mean that 138 mol O_2 were consumed by

188   every 16 mol NO_3^- produced, i.e., 106 mol O_2 for remineralization of C and 32 mol O_2 for

189   nitrification of the 16 mol of NH_4^+ produced (Paulmier et al. 2009). The ratio of NO_3^- produced

190   to O_2 consumed is the same whether or not DNRA occurs, because coupled nitrification-DNRA

191   causes internal cycling of N, and the net reaction is simply aerobic respiration, rather than a

192   change in available NO_3^-:

193   Nitrification: $NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$ (1)

194   DNRA: $NO_3^- + 2CH_2O + 2H^+ \rightarrow NH_4^+ + 2CO_2 + H_2O$ (2)

195   Sum: $CH_2O + O_2 \rightarrow CO_2 + H_2O$ (3)

196   This is analogous to the internal recycling of oxidants and reductants involved in Fe, Mn and

197   SO_4^{2-} reduction (Canfield et al. 1993). Total N loss in the microcosms, assumed to be removal

198   mainly by denitrification, was calculated by adding NO_3^- influx to NO_3^- production by calculated

199   nitrification. Results were compared to similar calculations using week 5 fluxes.

200

201   *Potential rate measurements*

202   Potential rate measurements of denitrification, anammox, and DNRA were conducted at

203   the beginning of the experiment, just before the treatments were initiated (t_0), and then 12 weeks

204   later at the end of the experiment. Pans of sediment were removed from aquarium and the

205   overlying water was aspirated off. The sediment was homogenized into a beaker and then 1.5 mL

206   of sediment was dispensed into 5.9 mL Exetainer vials (LABCO, UK), which were flushed with

207   helium and held overnight at the same temperature treatment that they were incubated at during

208   the 12-week experiment (4°C or 17°C). This pre-incubation was conducted to remove any

209   residual NO_3^-, as described previously (Brin et al. 2014). After the overnight pre-incubation, half

210   of the vials continued to be incubated at the same temperature, while the other half of vials were

211   shifted to the opposite 12-week temperature treatment for 1 hour (i.e., 4°C to 17°C or 17°C to

212   4°C). This was enough time for all the sediment in the vial to reach the new temperature, at

213   which point potential rate measurements were initiated. Thus, potential rates were measured at

214   4°C and 17°C for both 12-week temperature treatments. This allowed us to distinguish whether

215   there was an effect due to factors such as physiological acclimation or microbial population

216   shifts during the 12-week experiment, rather than simply a direct kinetic effect on reaction rates

217   due to increasing or decreasing measurement temperatures.

218   To initiate potential rate measurements, $^{15}NO_3^- + ^{14}NH_4^+$ (100 nmol N mL^{-1} sediment) was

219   added to the vials, and production of $^{29}N_2$ and $^{30}N_2$ was measured after a 15-minute incubation, at

220   which point biological activity was halted with addition of 100 µL 7 M $ZnCl_2$. The produced

221   ^{15}N-N_2 was measured with an isotope ratio mass spectrophotometer (Isoprime Continuous Flow-

222   IRMS interfaced with Multiflow-Bio Unit). Calculation of denitrification and anammox rates

223   was conducted following Brin et al. (2014), using equations described by Thamdrup and

224   Dalsgaard (2002). DNRA rates were measured by determining $^{15}NH_4^+$ production over the same

225   time interval, correcting for significant background $^{15}NH_4^+$ production, which occurred in

226   samples that were killed immediately at the start of time course incubations. A small background

227   correction was also needed for anammox, but none was necessary for denitrification. DNRA was

228 measured following Holmes et al. (1998) by desorbing NH_4^+ in NaCl solution (1 M) and then

229 diffusing it onto acidified Teflon-wrapped GF-C filters. The $^{15}N\text{-}NH_4^+$ on the filters was

230 measured using a Europa ANCA-SL elemental analyzer–gas chromatograph preparation system

231 attached to a continuous-flow Europa 20-20 stable isotope ratio mass spectrometer, as described

232 in Koop-Jakobsen and Giblin (2010).

233 　　　　The presence of DNRA could lead to an overestimate of denitrification compared to

234 anammox in potential rate incubations due to conversion of $^{15}NO_3^-$ into $^{15}NH_4^+$ and its

235 subsequent conversion to $^{30}N_2$ by anammox (i.e., $^{15}NO_2^- + {}^{15}NH_4^+ = {}^{30}N_2$) (Kartal et al. 2007). To

236 address this, we calculated the potential magnitude of this effect, and determined that it would

237 have been negligible, due to the large background of added $^{14}NH_4^+$ and fairly low anammox

238 rates. In this calculation, we assumed an average atom% ^{15}N composition of $^{15}NH_4^+$ from linear

239 DNRA rates during the 15 min incubation. Given initial additions of 100 nmol $^{14}NH_4^+$ mL^{-1}

240 sediment, $^{15}NH_4^+$ from DNRA comprised 0 to 4.9% of total NH_4^+, causing a 0-5.1%

241 underestimate of potential anammox and a 0-0.7% overestimate of denitrification. These are

242 conservative estimates, as the actual pool of $^{14}NH_4^+$ was likely higher due to exchangeable NH_4^+,

243 decreasing the effect of $^{15}NH_4^+$. Because of the insignificance of this effect, we did not correct

244 for this in our reported rates.

245

246 *Statistical analysis*

247 　　　　Statistical analyses were conducted using R version 2.15.0 (R Development Core Team).

248 Two-way or three-way ANOVAs were conducted to test for treatment differences for various

249 factors (O_2 consumption, potential rates, sediment C and N), depending on how often a particular

250 measurement was conducted during the 12-week experiment. We conducted two-way ANOVA

251   for treatment effects on total organic C and N and potential rate data, with the factors of 12-week

252   temperature treatment and C addition treatment, and an interaction between the two. In cases

253   where more time points were taken during the course of the experiment (i.e., O_2 consumption

254   rates and inorganic N fluxes), we conducted three-way ANOVA, with the factors of 12-week

255   temperature treatment, C addition treatment, week (as a continuous variable), and all interactions

256   as factors. In all cases, residuals were tested for normality and homogeneity of variance. In

257   general, the data met the assumptions of ANOVA. Linear regression was used to examine

258   relationships between potential rates and diffusive O_2 consumption, both measured at the 12-

259   week incubation temperature. Statistical tests were considered significant at $p<0.05$.

260

261   **Results**

262   *Diffusive O_2 consumption*

263   Complete O_2 consumption always occurred within the top 0.5 cm of the sediment,

264   indicating, as expected, that an oxic/anoxic interface was established in the microcosm sediments

265   at a similar depth to *in situ* (Brin et al. 2014). O_2 consumption increased significantly as a result

266   of C addition and warm temperature treatment over the 12-week experiment (Fig. 2). There was

267   no significant interactive effect between C addition and 12-week temperature treatment and no

268   effect of the week in which the measurements were conducted (Three-way ANOVA for weeks 5

269   and 11: 12-week temperature treatment, $F=14.3$, $p=0.002$; C treatment, $F=60.7$, $p<0.001$; Week,

270     $F=0.6$, $p=0.46$). C had a stronger effect than temperature based on the F statistic. Week was also

271   not a factor when only the 17°C data were analyzed in a two-way ANOVA, for weeks 1, 5, and

272   11, with C treatment and week as factors (Two-way ANOVA: C treatment, $F=75.0$, $p<0.001$;

273   Week, $F=4.3$, $p=0.06$). Thus, native pools of organic C were sufficient to maintain O_2

274   consumption rates in cores that lacked C addition during the course of the 12-week experiment.

275

276   *Sediment C and N*

277   At the end of the experiment, +C treatments had higher total organic C ($0.691 \pm 0.057\%$,

278   mean ± s.d.) than treatments without C added ($0.589 \pm 0.052\%$) ($F=8.82$, $p=0.02$) (Fig. 3). The

279   level of C buildup in sediments receiving C additions (~0.1%) was as expected based on the

280   cumulative amount of C that we added. Temperature did not influence total organic C, as

281   indicated by the lack of difference between microcosms subject to different temperature

282   treatments but similar C addition treatments. Sediment percent N was also higher in +C

283   treatments ($0.092 \pm 0.008\%$) compared to treatments without C added ($0.074 \pm 0.007\%$)

284   ($F=12.81$, $p=0.007$). There was no difference in total sediment C:N among treatments (Table 1)

285   (12-week temperature treatment, $F=0.039$, $p=0.74$; C treatment, $F=1.79$, $p=0.22$).

286

287   *Microcosm nutrient fluxes*

288   At the beginning of the experiment, all microcosm treatments consumed NO_3^- at similar

289   rates (Fig. 4). Microcosms held at 4°C continued to have the greatest net NO_3^- influx throughout

290   the rest of the experiment. In the other treatments, there was a shift in NO_3^- flux, ranging from

291   reduced influx in 4°C+C and 17°C to a complete reversal from influx to efflux in 17°C+C (Fig.

292   4). Treatment differences were evident by week 3 and remained consistent for the rest of the

293   experiment. These results were reflected in significant effects for all main factors in the three-

294   way ANOVA, as well as the C addition by week interaction (12-week temperature treatment,

295    $F=71.31$, $p<0.001$; C treatment, $F=45.7$, $p<0.001$; Week, $F=10.41$, $p=0.002$; C x Week

296   interaction, $F=16.05$, $p<0.001$). Treatment differences in NO_2^- or NH_4^+ were not as dramatic as

297   for NO_3^-, but differences were evident during a similar time frame to when NO_3^- flux shifted to

298   efflux for the 17°C+C treatment. In particular, there was production of NO_2^- and NH_4^+ efflux in

299   week 1 in the 17°C+C treatment that preceded the change in NO_3^-, and a similar efflux NH_4^+ in

300   week 3 in the 4°C+C treatment (NO_2^-: 12-week temperature treatment, $F=1.35$, $p=0.25$; C

301   treatment, $F=4.94$, $p=0.03$; Week, $F=5.71$, $p=0.02$; Temperature x Week interaction, $F=6.3$,

302     $p=0.01$; Temperature x C x Week interaction, $F=5.99$, $p=0.02$) (NH_4^+: no significant main effects

303   or interactions). After week 3, NO_2^- and NH_4^+ fluxes were usually around zero, with some

304   negligible treatment differences.

305   *In situ* nitrification, calculated from O_2 consumption rates from week 11, was comparable

306   to net NO_3^- influx in the 4°C treatments (Table 2). Nitrification was higher in the other

307   microcosm treatments, whereas NO_3^- flux into the sediments decreased. Calculated rates of

308   denitrification were comparable among treatments (Table 2). Conclusions were the same whether

309   the calculation was based on week 5 or week 11 fluxes.

310

311   *Denitrification, anammox and DNRA potential rates*

312   In t_0 sediments, and for all treatments at the end of the experiment, denitrification

313   potential rates were higher when measured at 17°C than when measured at 4°C for the same

314   treatment. However, regardless of whether denitrification potential rates were measured 4°C or at

315   17°C, rates were lower in sediments that had been subjected to the 17°C warming treatment than

316   in sediments held at 4°C (At the 4°C measurement temperature: $F=115.03$, $p<0.001$; at the 17°C

317   measurement temperature, $F=12.56$, $p=0.008$). C addition had no influence on this warming

318   effect (Fig. 5A).

319   Potential anammox rates were 7–46 % of denitrification rates and 7–31 % of total N loss

320   for all treatments at both measurement temperatures, with relatively higher importance when

321   measured at lower temperatures. After 12 weeks, potential anammox for all treatments measured

322   at 4°C was 26.3 ± 3.4 % of total N loss, while measurements at 17°C were 9.6 ± 2.2%. In 4C

323   microcosms, without warming or carbon addition, anammox was 23.1 ± 1.6% of total N loss

324   when measured at the treatment temperature of 4°C. Anammox rates showed slight but

325   statistically significant treatment effects depending on whether they were measured at low or

326   high temperature (Fig. 5B). When measured at the higher temperature, C additions decreased

327   anammox slightly (F=6.98, p=0.03), whereas warming decreased anammox slightly at the lower

328   measurement temperature (F=14.39, p=0.005).

329   Potential DNRA rates were of a similar order to denitrification. Similarly to

330   denitrification, DNRA rates within a treatment were higher when measured at 17°C than at 4°C.

331   However, DNRA rates were significantly lower in warmed treatments when measured at 17°C

332   (Fig. 5C) (F=20.35, p=0.002). At this same measurement temperature, DNRA rates were also

333   significantly higher in +C treatments (F=35.64, p<0.001). There were no interactive effects of C

334   addition and 12-week temperature treatment for any measured process at either high or low

335   measurement temperature. Potential DNRA rates correlated positively with O_2 consumption,

336   with measurements of both at temperatures corresponding to 12-week temperature treatments

337   (p=0.004, $adj\ r^2$=0.53), whereas denitrification and anammox rates did not (Fig. 6).

338   Warming for 12 weeks did not change the relative importance of DNRA or denitrification

339   to total NO_3^- reduction (i.e., DNRA or Denitrification/Total NO_3^- reduction). However, C

340   addition decreased relative denitrification rates and increased relative DNRA rates. This effect

341   was measured at the higher measurement temperature (17°C) (Relative denitrification, F=15.84,

342 *p*=0.004; relative DNRA, *F*=15.99, *p*=0.004), as well as at measurement temperatures

343 corresponding to the 12-week temperature treatments (Relative denitrification, *F*=6.89, *p*=0.03;

344 relative DNRA, *F*=5.85, *p*=0.04).

345

346 **Discussion**

347   Climate change may alter benthic N cycling in coastal ecosystems, either through direct

348 temperature effects or through indirect effects mediated by changes in C availability. These

349 indirect effects may be due to changes in sediment remineralization rates or to shifts in

350 phytoplankton blooms and organic matter delivery to the benthos (Sommer and Lengfellner

351 2008; Nixon et al. 2009; Lewandowska and Sommer 2010), which may maintain C availability at

352 times when it would otherwise be depleted by respiration, such as between spring and fall

353 phytoplankton blooms. Impending and already occurring changes have prompted microcosm

354 studies that explore the effects of multiple stressors on benthic N cycling, but few have examined

355 the effects of temperature in conjunction with other factors in coastal systems (Fitch and Crowe

356 2011; Alsterberg et al. 2012). Most of the knowledge of the effect of temperature and organic C

357 on NO_3^- reduction comes from seasonal studies and comparisons of rates with changing

358 environmental factors measured in the field (Dalsgaard et al. 2005; Trimmer and Engström 2011;

359 Giblin et al. 2013). The present study extends knowledge of these responses by examining both

360 warming and organic C addition in a mechanistic laboratory microcosm experiment (Jensen et al.

361 1993; Fulweiler et al. 2008; Neubacher et al. 2013). Although our temperature manipulation does

362 not simulate gradual temperature increases expected due to future climate change, it

363 demonstrates that multiple factors can elucidate potential changes in N cycling that may not be

364 observed in response to varying a single factor, and that indirect effects of warming may have

365   stronger effects than direct effects. In particular, the combination of both warming and C

366   addition was required to reverse the net flux of NO_3^- over the sediment-water interface (Fig. 4).

367   However, contrary to expectations, warming did not influence the relative importance of DNRA

368   as a NO_3^- reduction pathway (Ogilvie et al. 1997; Kelly-Gerreyn et al. 2001). Instead, DNRA

369   increased in response to C addition, supporting the hypothesis that warming effects observed in

370   previous studies of benthic sediments may have been mediated by changes in availability of

371   organic C (King and Nedwell 1984; Brin et al. 2014).

372

373   *O_2 consumption and microcosm sediment C*

374   Oxygen consumption reflects overall sediment reactivity as driven by organic C

375   availability. Our biweekly C additions were at the low end of the range in values reported for

376   primary production in the Middle Atlantic Bight (de Haas et al. 2002). O_2 consumption rates in

377   microcosms with and without C additions agreed well with rates measured over two years in

378   intact sediment cores from the study site, which are typical of near shore continental shelf

379   sediments in general (Glud 2008; Brin et al. 2014). We acknowledge that the timing and nature

380   of our C additions did not mimic *in situ* variability. However, O_2 consumption rates suggested

381   that our level of C addition was within the range encountered *in situ*.

382   Sediment O_2 consumption was significantly greater in microcosms with added C than in

383   sediments that did not receive C additions, demonstrating that treatments were effective in

384   increasing sediment organic C availability (Fig. 2). Increased O_2 consumption due to C additions

385   at either temperature indicated that respiration rates were stimulated by organic C inputs, even at

386   winter temperatures. Differences in O_2 consumption rates between sediments with and without C

387   additions (i.e., ~1 μmol cm^{-2} d^{-1}) were about one-third the rate of C addition, suggesting an

388   equivalent fraction of the added C was rapidly mineralized. This enabled a detectable difference

389   in sediment organic C in sediments that received C additions compared to those without added C

390   (Fig. 3).

391   Warming also increased O_2 consumption, after accounting for differences due to C

392   addition (Fig. 2). This suggests that eventually, given enough time, O_2 consumption rates would

393   begin to decline in the 17°C microcosms, although we were not able to detect a statistically

394   significant decline after 11 weeks of treatment. This lack of decline in warmed treatments

395   without C additions indicates a relatively constant supply of native organic C fueling respiration.

396   Regardless of the lack of a rate decline over time, greater O_2 consumption in 17°C treatments

397   than in 4°C treatments indicate that more organic C was being consumed at the warmer

398   temperature. This may have led to decreased labile C that was available for heterotrophic NO_3^-

399   reduction during potential rate incubations at the end of the experiment, as has been observed

400   previously in microcosm sediments in response to experimental warming (Alsterberg et al.

401   2011).

402

403   *Nutrient fluxes in microcosms*

404   Measuring net fluxes of inorganic N across the sediment-water interface allowed us to

405   examine treatment effects on sediment N cycling throughout the experiment. It is noteworthy

406   that there was a strong influx of NO_3^- into sediments at 4°C without C additions for the duration

407   of the experiment (Fig. 4). This influx of NO_3^- with little to no associated NH_4^+ or NO_2^- efflux

408   indicated a net loss of N from the system. Potential rate measurements of anammox and

409   denitrification suggested that this N loss could be largely attributed to denitrification, with

410   anammox responsible for approximately 23.1%. This partitioning is slightly lower than that

411   predicted assuming remineralization of organic C by denitrification with a C/N ratio of Redfield

412   (6.6), producing NH_4^+ and equivalent NO_2^- that was consumed by anammox (28%) (Trimmer

413   and Engström 2011; Babbin et al. 2014). This comparison neither indicates nor refutes the

414   presence of DNRA; although DNRA could affect the relative proportion of NO_2^- and NH_4^+ from

415   remineralization available for denitrification and anammox, this effect may be countered by

416   subsequent nitrification. Consistent NO_3^- consumption in sediments at 4°C without C addition

417   throughout the experiment suggests capacity for significant N loss during winter months, even in

418   the absence of any new organic C inputs. This agrees with comparable N_2 fluxes during winter

419   and other seasons at the site (Heiss et al. 2012).

420   Warming and C addition individually decreased the net influx of NO_3^- over the sediment-

421   water interface. However, the most striking result occurred under both warming and C addition,

422   as there was complete reversal from net NO_3^- consumption to production early on in the

423   experiment, which was preceded by a transient pulse of NH_4^+ production (Fig. 4). The delayed

424   pulse of NH_4^+ production in the 4°C+C treatment as compared to the 17°C+C treatment could

425   indicate a temperature effect on the rate of heterotrophic response to organic C additions.

426   Furthermore, no significant NH_4^+ effluxes occurred after these pulses, indicating that from that

427   point forward, NH_4^+ produced in sediments was completely consumed by nitrification and

428   possibly heterotrophic uptake.

429   The result of reduced NO_3^- consumption was at first puzzling, as we expected C addition

430   at this level to stimulate denitrification and increase NO_3^- consumption. However, the switch

431   could have been due to increased remineralization of added organic C, releasing NH_4^+, and

432   subsequent nitrification of this NH_4^+ to NO_3^-, without any change in N loss. Based on Redfield

433   conversion of O_2 consumption rates, nitrification in 4°C treatments was comparable to net NO_3^-

434   influx, thereby making the estimate of N loss about double that of measured NO_3^- influx (Table

435   2). In contrast, in the 17°C+C treatments, in which increased O_2 consumption indicated increased

436   nitrification, nitrification supplied the majority of the NO_3^- used to support sediment NO_3^-

437   reduction processes (Table 2). These calculations suggest that *in situ* denitrification rates did not

438   change across experimental treatments, despite increases in nitrification by both warming and C

439   addition, with the net effect of positive N efflux from 17°C+C microcosm sediments. Although

440   this mass balance can be explained solely in terms of nitrification and denitrification, it is still

441   counter-intuitive that denitrification was not stimulated by C addition. A possible further

442   explanation is provided by invoking results from potential rate experiments, which indicated that

443   DNRA rates were increased with warming and C addition, but denitrification rates were not. This

444   may have led to a disproportionate increase in internal N recycling over N removal in sediments

445   that received C additions, i.e., DNRA was stimulated when denitrification was not.

446   Our stiochiometric calculations should be viewed with caution in light of our simplifying

447   assumptions, i.e. (1) that the remineralized organic C had a constant Redfield C:N ratio, an

448   assumption supported by past research (e.g., Burdige 1991), (2) that all of the NH_4^+ released

449   through heterotrophic respiration was completely nitrified, an assumption supported by the very

450   little overall NH_4^+ release from the sediments, and (3) that all heterotrophic respiration was

451   accounted for by O_2 consumption. However, heterotrophic denitrification is not balanced by re-

452   oxidation of end products with O_2 (Canfield et al. 1993), and so NH_4^+ released through this

453   pathway would not be included in the calculation, causing nitrification to be underestimated.

454   Additionally, if there were a NH_4^+ sink other than nitrification, such as microbial assimilation,

455   the ratio of O_2 consumption to NO_3^- production would be higher than the assumed 138:16, and

456   our calculated nitrification and denitrification rates would be overestimates.

457   Relatively few studies have examined the effects of experimental warming and organic C

458   addition on DIN fluxes from coastal sediments, and those that do exist have found different

459   results from our study. Warming or organic C addition increased remineralization but usually

460   with a stimulation of denitrification as well (Caffrey et al. 1993; Fitch and Crowe 2011;

461   Alsterberg et al. 2012), which we did not observe. A potential explanation for the difference is

462   the presence of DNRA and cycling of N between DNRA and nitrification in our experimental

463   sediments (Burgin and Hamilton 2007). This link has been quantified in a moist tropical forest

464   soil, in which DNRA accounted for about 35% of NO_3^- production by nitrification (Templer et

465   al. 2008), and a positive relationship between DNRA and nitrification was predicted in a

466   modeling study of a coastal freshwater sediment (Canavan et al. 2007). Several other studies of

467   estuarine or marine sediments have noted links between DNRA and nitrification, in terms of

468   DNRA outcompeting denitrification as a sink for NO_3^- produced by nitrification (Tobias et al.

469   2001), *in situ* nitrification providing $^{14}NO_3^-$ for DNRA in $^{15}NO_3^-$ addition experiments (Gardner

470   and McCarthy 2009), and nitrification preventing NH_4^+ accumulation when DNRA rates were

471   high (Jäntti and Hietanen 2012). To our knowledge, however, this link has not been carefully

472   studied in aquatic sediments.

473

474   *Denitrification, anammox and DNRA potential rates*

475   Potential rate measurements in homogenized sediments are often criticized for generally

476   overstimulating rates compared to *in situ*, although this is not always the case (Laverman et al.

477   2006; Behrendt et al. 2013). To assess this, we converted O_2 consumption- and NO_3 flux-based

478   denitrification rates in Table 2 to volumetric rates assuming a depth interval of NO_3^- reduction of

479   0.2 cm, which was similar to the depth interval of O_2 penetration. The depth interval of NO_3^-

480   reduction could theoretically vary with both temperature and C addition, but for simplicity, we

481   used a constant 0.2 cm depth interval for all treatments. Converted rates of N loss from Table 2

482   ranged from 33 to 68 nmol N h^{-1} ml^{-1} sediment, which were similar to or somewhat higher than

483   measured potential rates of denitrification (Fig. 5).

484   Perhaps more concerning is the potential for slurry incubations to overstimulate specific

485   NO_3^- reduction processes compared to others, with denitrification being favored over anammox,

486   or DNRA over denitrification (Revsbech et al. 2006; Trimmer et al. 2006; Behrendt et al. 2013).

487   Explanations for this are not currently certain, but could relate to more denitrifiers or DNRA

488   bacteria being exposed to favorable conditions in slurries. Overstimulation of denitrification

489   relative to anammox is generally observed in shallower sediments (Trimmer and Engström

490   2011). Although we cannot completely rule out overstimulation of denitrification over anammox

491   in our potential rate incubations, our anammox rates as a percent of denitrification were typical

492   for shelf sediments measured by slurries or intact cores (Trimmer and Engstrom 2011). Slurry

493   incubations have also been found to overstimulate DNRA relative to denitrification by disrupting

494   natural gradients and increasing NO_3^- availability for DNRA bacteria (Behrendt et al. 2013).

495   DNRA bacteria may reside deeper than denitrifiers in intact sediments, and so have less access to

496   NO_3^- in sediments in which NO_3^- is typically supplied by diffusion rather than advection. It is not

497   known whether this overstimulation occurred in our experiment. However, taken together with

498   DIN fluxes, potential rate measurements support the hypothesis that DNRA became a more

499   important process in the microcosms due to warming and C additions.

500   DNRA is typically associated with sediments with high organic matter availability and O_2

501   consumption rates (MacFarlane and Herbert 1984; Tomaszek and Gruca-Rokosz 2007; Gardner

502   and McCarthy 2009; Nizzoli et al. 2010). We also found a relationship between potential DNRA

503   rates and O_2 consumption in our experiment (Fig. 6). Furthermore, C addition increased the

504   relative importance of DNRA versus denitrification in NO_3^- reduction. This agrees with

505   theoretical considerations and chemostat experiments that indicate that DNRA is favored over

506   denitrification in sediments with a greater relative supply of organic C compared to NO_3^- (Tiedje

507   1988; Kraft et al. 2014; Algar and Vallino 2014).

508   This link between C and DNRA could also occur via sulfur cycling. Addition of C could

509   stimulate sulfate reduction and flux of hydrogen sulfide into the NO_3^- reducing layer. Sulfide has

510   been shown to inhibit the last step in denitrification, while not adversely influencing DNRA, and

511   the presence of DNRA has been positively correlated with the presence of sulfide in marine

512   sediments (Brunet and Garcia-Gil 1996; An and Gardner 2002). However, there is also evidence

513   that sulfide may favor denitrification over DNRA, depending on NO_3^- availability, or have no

514   strong influence on the competition between the two processes (Dong et al. 2011; Kraft et al.

515   2014). Furthermore, nitrification is also inhibited by sulfide (Joye and Hollibaugh 1995), but our

516   organic C addition increased, not decreased, nitrification rates based on stoichiometric

517   calculations. This suggests that if sulfide production was stimulated, it was too low to have any

518   negative influence on nitrification.

519   Stimulation of DNRA over denitrification due solely to the level of C added in our

520   experiment would be surprising, as DNRA is generally favored in sediments with substantially

521   higher O_2 consumption rates than we measured in our study (Christensen et al. 2000; Revsbech

522   et al. 2006). Perhaps differences in the chemical composition of added compared to native C was

523   a factor in the tradeoff between reduction of NO_3^- to NH_4^+ or N_2 (Akunna et al. 1993; Bonin et

524   al. 1999; Gardner and McCarthy 2009). Gardner and McCarthy (2009) found that DNRA was

525   higher than expected based on O_2 consumption alone during a cyanobacterial depositional event

526   in shallow tropical sediments, suggesting that composition of organic C may have been a factor.

527   In our experiment, the effects of warming on potential rates and O_2 consumption may similarly

528   suggest the influence of chemical composition of organic C. Both denitrification and DNRA

529   potentials decreased in 12-week warmed treatments relative to cool treatments measured at the

530   same temperature (Fig. 5), with a more consistent result for denitrification than DNRA. We

531   attribute this decrease to increased respiration and thus C depletion in warmed compared to

532   winter 12-week temperature treatments, as reflected by O_2 consumption rates. C additions

533   increased potential rates of DNRA, potentially by mitigating C limitation, but had no effect on

534   denitrification. This may suggest that denitrifying bacteria in our experiment were better adapted

535   to utilize native organic C compared to added organic C. In contrast, DNRA bacteria appeared to

536   show a response to either type of C.

537

538   **Conclusions**

539   Effects of increasing coastal water temperatures on benthic N cycling processes may be

540   mediated primarily by changes in sediment organic C availability. These effects are generally

541   examined by correlation of rates with environmental factors that change *in situ* (Dalsgaard et al.

542   2005; Trimmer and Engström 2011), and few microcosm studies have examined the effects of

543   warming in conjunction with other factors (Fitch and Crowe 2011; Alsterberg et al. 2012). By

544   examining both warming and organic C addition in a mechanistic laboratory microcosm

545   experiment, this study provided new insights about potential controls on relationships between

546   NO_3^- reduction processes and shifts from N removal to recycling. We demonstrated that

547   temperature and changes in organic C, alone or in concert, could affect NO_3^- reduction processes

548   and the net balance of benthic N cycling. In particular, nutrient and O_2 fluxes indicated that

549   warming and C addition increased the relative importance of N recycling over removal. This

550   change was driven by increased NH_4^+ production by remineralization, which increased

551   nitrification but not N loss, shifting net NO_3^- fluxes towards efflux over the sediment-water

552   interface. Potential rate measurements of microcosm sediments indicated significant capacity for

553   DNRA, which increased due to C addition and at the higher measurement temperature. Although

554   results from potential rate measurements must be viewed with caution, stimulation of DNRA but

555   not denitrification in microcosm sediments is consistent with a switch towards internal N

556   recycling and NO_3^- efflux. This suggests stimulation of N cycling between nitrification and

557   DNRA, a coupling that has not been well studied in aquatic sediments.

558   **Figure legends**

559   *Note: All figures were created with R and outputted directly to PDF files.*

560   **Fig 1** Timeline of experimental treatments, and temperatures for each treatment. Dashed lines

561   indicate 4°C and 17°C. Dark lines indicate means of iButton measurements for each

562   temperature treatment, and light lines indicate minimum and maximum iButton

563   measurements, both of which deviated slightly from 4°C or 17°C. Arrows indicate dates of

564   *Chlorella* addition

565   **Fig 2** O_2 consumption rates, calculated from sediment microprofiles measured during the

566   experiment. Means ± s.d. are plotted for each treatment (*n*=3). Temperature and C addition

567   are significant, but week is not, in a 2-way ANOVA analysis including weeks 5 and 11

568   **Fig 3** Fluxes of (a) NO_3^-, (b) NO_2^- and (c) NH_4^+ across the sediment water interface in

569   microcosms exposed to warming and organic C addition. Means ± s.d. are plotted for each

570   treatment (*n*=3). Negative fluxes indicate influx

571   **Fig 4** Sediment organic C in microcosm sediments exposed to warming and organic C additions.

572   Means ± s.d. are plotted for each treatment (*n*=3). Asterisks indicate significant differences as

573   assessed by 2-way ANOVA with temperature and C addition as factors (*$p<0.05$, **$p<0.01$,

574   ***$p<0.001$)

575   **Fig 5** Potential rates of (a) denitrification, (b) anammox and (c) DNRA in sediments from

576   microcosms harvested before initiation of treatments (t_0) and from all experimental

577   treatments at the end of the experiment. For each treatment, potential rates were measured at

578   4°C and at 17°C. Means ± s.d. are plotted for each treatment (*n*=3). Asterisks indicate

579   significant differences as assessed by 2-way ANOVA analysis conducted for assays at each

580 measurement temperature, with 12-week temperature treatment and C addition treatment as

581 factors (*p<0.05, **p<0.01, ***p<0.001)

582 **Fig 6** Relationship between sediment O_2 consumption measured in week 11 and potential rates of

583 denitrification, anammox, and DNRA, measured at the same temperature as O_2 consumption,

584 i.e., 12-week temperature treatment. Each symbol represents an individual microcosm.

585 Symbol shape indicates treatment: 4°C, diamonds; 4°C+C, circles; 17°C, squares; 17°C+C,

586 triangles. Symbol color indicates process: denitrification, black; anammox, gray; DNRA,

587 open symbols. Lines show linear regressions (denitrification, solid black line: r^2=-0.01,

588 p=0.381; anammox, gray line: r^2=0.04, p=0.25; DNRA, dotted black line: r^2=0.53, p=0.004).

589 Note that only the regression line for DNRA is statistically significant, and that one data

590 point for 17°C+C DNRA (open triangle) is mostly obscured by a 17°C+C denitrification

591 point (black triangle)

592   **Tables**

593   **Table 1** Total C:N ratio for all treatments, from measurements of total sediment C and N.

Treatment	C:N
t_0	7.3 ± 1.1
4°C	8.0 ± 0.3
4°C + C	7.5 ± 0.3
17°C	7.9 ± 0.9
17°C + C	7.6 ± 0.2

594   **Table 2** Nitrification and denitrification in sediment microcosms, calculated from measured NO_3^- flux and stoichiometric conversion

595   of organic C remineralization based on measured O_2 consumption. Nitrification was calculated from O_2 consumption assuming

596   that for every 138 mol of O_2 consumed, 16 mol of NO_3^- was produced. Denitrification was calculated as nitrification minus NO_3^-

597   flux (negative values denote influx). Means ± s.d. are shown for each treatment (n=3).

Treatment	NO_3^- flux (μmol N cm^{-2} d^{-1})	Diffusive O_2 flux (μmol O_2 cm^{-2} d^{-1})	Calculated remineralization (μmol C cm^{-2} d^{-1})	Calculated nitrification (μmol N cm^{-2} d^{-1})	Calculated N loss (denitrification) (μmol N cm^{-2} d^{-1})
4°	-0.129 ± 0.059	1.016 ± 0.183	0.780 ± 0.141	0.118 ± 0.021	0.246 ± 0.079
4° + C	0.001 ± 0.015	1.734 ± 0.226	1.332 ± 0.173	0.201 ± 0.026	0.200 ± 0.012
17°	-0.032 ± 0.007	1.229 ± 0.156	0.944 ± 0.120	0.142 ± 0.018	0.175 ± 0.024
17° + C	0.063 ± 0.016	2.198 ± 0.071	1.689 ± 0.055	0.255 ± 0.008	0.192 ± 0.009

598

607   **Conflict of Interest:** The authors declare that they have no conflict of interest.

608 **References**

609 Akunna JC, Bizeau C, Moletta R (1993) Nitrate and nitrite reductions with anaerobic sludge
610   using various carbon sources: Glucose, glycerol, acetic acid, lactic acid and methanol.
611   Water Res 27:1303–1312.

612 Algar CK, Vallino JJ (2014) Predicting microbial nitrate reduction pathways in coastal
613   sediments. Aquat Microb Ecol 71:223–238. doi: 10.3354/ame01678

614 Alsterberg C, Hulth S, Sundbäck K (2011) Response of a shallow-water sediment system to
615   warming. Limnol Oceanogr 56:2147–2160. doi: 10.4319/lo.2011.56.6.2147

616 Alsterberg C, Sundbäck K, Hulth S (2012) Functioning of a shallow-water sediment system
617   during experimental warming and nutrient enrichment. PLoS One 7:e51503. doi:
618   10.1371/journal.pone.0051503

619 An S, Gardner WS (2002) Dissimilatory nitrate reduction to ammonium (DNRA) as a nitrogen
620   link, versus denitrification as a sink in a shallow estuary (Laguna Madre/Baffin Bay,
621   Texas). Mar Ecol Prog Ser 237:41–50.

622 Babbin AR, Keil RG, Devol AH, Ward BB (2014) Organic matter stoichiometry, flux, and
623   oxygen control nitrogen loss in the ocean. Science (80-) 344:406–8. doi:
624   10.1126/science.1248364

625 Behrendt A, de Beer D, Stief P (2013) Vertical activity distribution of dissimilatory nitrate
626   reduction in coastal marine sediments. Biogeosciences Discuss 10:8065–8101. doi:
627   10.5194/bgd-10-8065-2013

628 Bonin PC, Omnes P, Chalamet A (1999) The influence of nitrate and carbon inputs on the end
629   products of bacterial nitrate dissimilation in marine sediment. Toxicol Environ Chem
630   73:67–79.

631 Brin LD, Giblin AE, Rich JJ (2014) Environmental controls of anammox and denitrification in
632   southern New England estuarine and shelf sediments. Limnol Oceanogr 59:851–860. doi:
633   10.4319/lo.2014.59.3.0851

634 Brunet RC, Garcia-Gil LJ (1996) Sulfide-induced dissimilatory nitrate reduction to ammonia in
635   anaerobic freshwater sediments. FEMS Microbiol Ecol 21:131–138. doi: 10.1016/0168-
636   6496(96)00051-7

637 Burdige DJ (1991) The kinetics of organic matter mineralization in anoxic marine sediments. J
638   Mar Res 49:727–761. doi: 10.1357/002224091784995710

639 Burgin AJ, Hamilton SK (2007) Have we overemphasized the role of denitrification in aquatic
640   ecosystems? A review of nitrate removal pathways. Front Ecol Environ 5:89–96. doi:
641   10.1890/1540-9295(2007)5[89:HWOTRO]2.0.CO;2

642 Caffrey JM, Sloth NP, Kaspar HF, Blackburn TH (1993) Effect of organic loading on
643   nitrification and denitrification in a marine sediment microcosm. FEMS Microbiol Ecol
644   12:159–167.

645 Canavan RW, Laverman AM, Slomp CP (2007) Modeling nitrogen cycling in a coastal fresh
646   water sediment. Hydrobiologia 584:27–36. doi: 10.1007/s10750-007-0583-z

647 Canfield DE (2005) The Nitrogen Cycle. In: Canfield DE, Thamdrup B, Kristensen E (eds)

648   Aquatic Geomicrobiology: Advances in Marine Biology. Elsevier Academic Press, San
649   Diego, CA; London, pp 205–267

650 Canfield DE, Jørgensen BB, Fossing H, et al (1993) Pathways of organic carbon oxidation in
651   three continental margin sediments. Mar Geol 113:27–40.

652 Christensen J (1994) Carbon export from continental shelves, denitrification and atmospheric
653   carbon dioxide. Cont Shelf Res 14:547–576.

654 Christensen PB, Rysgaard S, Sloth NP, et al (2000) Sediment mineralization, nutrient fluxes,
655   denitrification and dissimilatory nitrate reduction to ammonium in an estuarine fjord with
656   sea cage trout farms. Aquat Microb Ecol 21:73–84.

657 Codispoti LA, Brandes JA, Christensen JP, et al (2001) The oceanic fixed nitrogen and nitrous
658   oxide budgets: Moving targets as we enter the anthropocene? Sci Mar 65:85–105.

659 Crowe SA, Canfield DE, Mucci A, et al (2012) Anammox, denitrification and fixed-nitrogen
660   removal in sediments from the Lower St. Lawrence Estuary. Biogeosciences 9:4309–4321.
661   doi: 10.5194/bg-9-4309-2012

662 Dalsgaard T, Thamdrup B (2002) Factors controlling anaerobic ammonium oxidation with nitrite
663   in marine sediments. Appl Environ Microbiol 68:3802–3808. doi: 10.1128/AEM.68.8.3802

664 Dalsgaard T, Thamdrup B, Canfield DE (2005) Anaerobic ammonium oxidation (anammox) in
665   the marine environment. Res Microbiol 156:457–464. doi: 10.1016/j.resmic.2005.01.011

666 de Haas H, van Weering TCE, de Stigter H (2002) Organic carbon in shelf seas: Sinks or
667   sources, processes and products. Cont Shelf Res 22:691–717. doi: 10.1016/S0278-
668   4343(01)00093-0

669 Diaz RJ, Rosenberg R (2008) Spreading dead zones and consequences for marine ecosystems.
670   Science (80-) 321:926–929. doi: 10.1126/science.1156401

671 Dong LF, Sobey MN, Smith CJ, et al (2011) Dissimilatory reduction of nitrate to ammonium,
672   not denitrification or anammox, dominates benthic nitrate reduction in tropical estuaries.
673   Limnol Oceanogr 56:279–291. doi: 10.4319/lo.2011.56.1.0279

674 Fitch JE, Crowe TP (2011) Combined effects of temperature, inorganic nutrients and organic
675   matter on ecosystem processes in intertidal sediments. J Exp Mar Bio Ecol 400:257–263.
676   doi: 10.1016/j.jembe.2011.02.005

677 Fulweiler RW, Nixon SW, Buckley B a., Granger SL (2008) Net sediment N2 fluxes in a coastal
678   marine system - Experimental manipulations and a conceptual model. Ecosystems 11:1168–
679   1180. doi: 10.1007/s10021-008-9187-3

680 Gardner WS, McCarthy MJ (2009) Nitrogen dynamics at the sediment–water interface in
681   shallow, sub-tropical Florida Bay: Why denitrification efficiency may decrease with
682   increased eutrophication. Biogeochemistry 95:185–198. doi: 10.1007/s10533-009-9329-5

683 Giblin AE, Tobias CR, Song B, et al (2013) The importance of dissimilatory nitrate reduction to
684   ammonium (DNRA) in the nitrogen cycle of coastal ecosystems. Oceanography 26:94–101.

685 Glud RN (2008) Oxygen dynamics of marine sediments. Mar Biol Res 4:243–289. doi:
686   10.1080/17451000801888726

687 Gruca-Rokosz R, Tomaszek JA, Koszelnik P (2009) Competitiveness of dissimilatory nitrate
688   reduction processes in bottom sediment of Rzeszów reservoir. Environ Prot Eng 35:5–13.

689   Heiss EM, Fields L, Fulweiler RW (2012) Directly measured net denitrification rates in offshore
690   New England sediments. Cont Shelf Res 45:78–86. doi: 10.1016/j.csr.2012.06.002

691   Holmes RM, McClelland JW, Sigman DM, et al (1998) Measuring 15N-NH4+ in marine,
692   estuarine and fresh waters: An adaptation of the ammonia diffusion method for samples
693   with low ammonium concentrations. Mar Chem 60:235–243.

694   Jäntti H, Hietanen S (2012) The effects of hypoxia on sediment nitrogen cycling in the Baltic
695   Sea. Ambio 41:161–169. doi: 10.1007/s13280-011-0233-6

696   Jensen K, Revsbech NP, Nielsen LP (1993) Microscale distribution of nitrification activity in
697   sediment determined with a shielded microsensor for nitrate. Appl Environ Microbiol
698   59:3287–3296.

699   Jensen K, Sloth NP, Risgaard-Petersen N, Rysgaard S (1994) Estimation of nitrification and
700   denitrification from microprofiles of oxygen and nitrate in model sediment systems. Appl
701   Envioronmental Microbiol 60:2094–2100.

702   Jones MN (1984) Nitrate reduction by shaking with cadmium: Alternative to cadmium columns.
703   Water Res 18:643–646.

704   Jørgensen KS (1989) Annual pattern of denitrification and nitrate ammonification in estuarine
705   sediment. Appl Environ Microbiol 55:1841–1847.

706   Joye SB, Anderson IC (2008) Nitrogen cycling in coastal sediments. In: Capone DG, Bronk D,
707   Mulholland D, Carpenter EJ (eds) Nitrogen in the Marine Environment. pp 867–915

708   Joye SB, Hollibaugh JT (1995) Influence of sulfide inhibition on nitrogen regeneration in
709   sediments. Science (80-) 270:623–625.

710   Kartal B, Kuypers MMM, Lavik G, et al (2007) Anammox bacteria disguised as denitrifiers:
711   Nitrate reduction to dinitrogen gas via nitrite and ammonium. Environ Microbiol 9:635–42.
712   doi: 10.1111/j.1462-2920.2006.01183.x

713   Kelly-Gerreyn BA, Trimmer M, Hydes D. J (2001) A diagenetic model discriminating
714   denitrification and dissimilatory nitrate reduction to ammonium in a temperate estuarine
715   sediment. Mar Ecol Prog Ser 220:33–46.

716   King D, Nedwell DB (1984) Changes in the nitrate-reducing community of an anaerobic
717   saltmarsh sediment in response to seasonal selection by temperature. J Gen Microbiol
718   130:2935–2941.

719   Koop-Jakobsen K, Giblin AE (2010) The effect of increased nitrate loading on nitrate reduction
720   via denitrification and DNRA in salt marsh sediments. Limnol Oceanogr 55:789–802. doi:
721   10.4319/lo.2009.55.2.0789

722   Koroleff F (1983) Determination of nutrients. In: Grasshoff K (ed) Methods of seawater analysis.
723   Verlag Chemie, pp 125–187

724   Kraft B, Tegetmeyer HE, Sharma R, et al (2014) The environmental controls that govern the end
725   product of bacterial nitrate respiration. Science (80-) 345:676–679. doi:
726   10.1126/science.1254070

727   Laverman AM, Van Cappellen P, van Rotterdam-Los D, et al (2006) Potential rates and
728   pathways of microbial nitrate reduction in coastal sediments. FEMS Microbiol Ecol
729   58:179–92. doi: 10.1111/j.1574-6941.2006.00155.x

730 Lewandowska A, Sommer U (2010) Climate change and the spring bloom: A mesocosm study
731   on the influence of light and temperature on phytoplankton and mesozooplankton. Mar Ecol
732   Prog Ser 12:101–111. doi: 10.3354/meps08520

733 MacFarlane GT, Herbert RA (1984) Dissimilatory nitrate reduction and nitrification in estuarine
734   sediments. J Gen Microbiol 130:2301–2308.

735 Neubacher EC, Parker RE, Trimmer M (2013) The potential effect of sustained hypoxia on
736   nitrogen cycling in sediment from the southern North Sea: a mesocosm experiment.
737   Biogeochemistry 113:69–84. doi: 10.1007/s10533-012-9749-5

738 Nixon SW, Fulweiler RW, Buckley BA, et al (2009) The impact of changing climate on
739   phenology, productivity, and benthic-pelagic coupling in Narragansett Bay. Estuar Coast
740   Shelf Sci 82:1–18. doi: 10.1016/j.ecss.2008.12.016

741 Nizzoli D, Carraro E, Nigro V, Viaroli P (2010) Effect of organic enrichment and thermal regime
742   on denitrification and dissimilatory nitrate reduction to ammonium (DNRA) in
743   hypolimnetic sediments of two lowland lakes. Water Res 44:2715–24. doi:
744   10.1016/j.watres.2010.02.002

745 Ogilvie BG, Rutter M, Nedwell DB (1997) Selection by temperature of nitrate-reducing bacteria
746   from estuarine sediments: species composition and competition for nitrate. FEMS Microbiol
747   Ecol 23:11–22.

748 Paulmier A, Kriest I, Oschlies A (2009) Stoichiometries of remineralisation and denitrification in
749   global biogeochemical ocean models. Biogeosciences 6:923–935. doi: 10.5194/bg-6-923-
750   2009

751 Perry MJ, Eppley RW (1981) Phosphate uptake by phytoplankton in the central North Pacific
752   Ocean. Deep Sea Res Part A Oceanogr Res Pap 28:39–49.

753 R Development Core Team R: A language and environment for statistical computing.
754   http://www.r-project.org. http://www.r-project.org/.

755 Rehr B, Klemme J-H (1989) Competition for nitrate between denitrifying Pseudomonas stutzeri
756   and nitrate ammonifying enterobacteria. FEMS Microbiol Lett 62:51–57.

757 Revsbech NP, Risgaard-Petersen N, Schramm A, Nielsen LP (2006) Nitrogen transformations in
758   stratified aquatic microbial ecosystems. Antonie Van Leeuwenhoek 90:361–375. doi:
759   10.1007/s10482-006-9087-5

760 Ryther JH, Dunstan WM (1971) Nitrogen, phosphorus, and eutrophication in the coastal marine
761   environment. Science (80-) 171:1008–1013. doi: 10.1126/science.171.3975.1008

762 Sommer U, Lengfellner K (2008) Climate change and the timing, magnitude, and composition of
763   the phytoplankton spring bloom. Glob Chang Biol 14:1199–1208. doi: 10.1111/j.1365-
764   2486.2008.01571.x

765 Templer PH, Silver WL, Pett-Ridge J, et al (2008) Plant and microbial controls on nitrogen
766   retention and loss in a humid tropical forest. Ecology 89:3030–3040.

767 Thamdrup B (2012) New pathways and processes in the global nitrogen cycle. Annu Rev Ecol
768   Evol Syst 43:407–428. doi: 10.1146/annurev-ecolsys-102710-145048

769 Thamdrup B, Dalsgaard T (2002) Production of N2 through anaerobic ammonium oxidation
770   coupled to nitrate reduction in marine sediments. Appl Environ Microbiol 68:1312–1318.

771   doi: 10.1128/AEM.68.3.1312

772 Tiedje JM (1988) Ecology of denitrification and dissimilatory nitrate reduction to ammonium.
773   In: Zehnder AJB (ed) Biology of Anaerobic Microorganisms. John Wiley and Sons, New
774   York, NY, pp 179–244

775 Tiedje JM, Sexstone AJ, Myrold DD, Robinson JA (1982) Denitrification: Ecological niches,
776   competition and survival. Antonie Van Leeuwenhoek 48:569–83.

777 Tobias CR, Anderson IC, Canuel EA, Macko SA (2001) Nitrogen cycling through a fringing
778   marsh-aquifer ecotone. Mar Ecol Prog Ser 210:25–39. doi: 10.3354/meps210025

779 Tomaszek JA, Gruca-Rokosz R (2007) Rates of dissimilatory nitrate reduction to ammonium in
780   two Polish reservoirs: impacts of temperature, organic matter content, and nitrate
781   concentration. Environ Technol 28:771–778.

782 Trimmer M, Engström P (2011) Distribution, activity, and ecology of anammox bacteria in
783   aquatic environments. In: Ward BB, Arp DJ, Klotz MG (eds) Nitrification, 1st edn. ASM
784   Press, Washington, DC, pp 201–236

785 Trimmer M, Nicholls JC, Deflandre B (2003) Anaerobic ammonium oxidation measured in
786   sediments along the Thames Estuary, United Kingdom. Appl Environ Microbiol 69:6447–
787   6454. doi: 10.1128/AEM.69.11.6447

788 Trimmer M, Risgaard-Petersen N, Nicholls JC, Engström P (2006) Direct measurement of
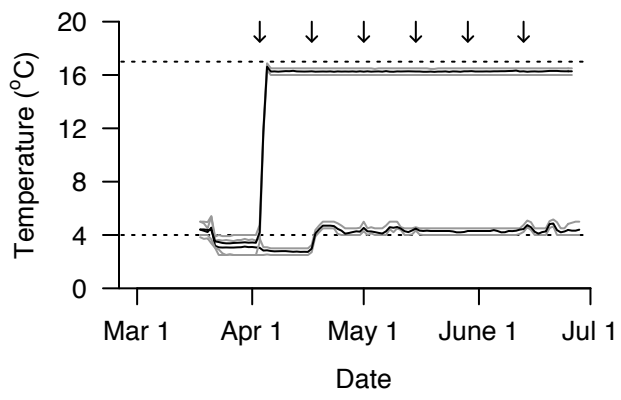789   anaerobic ammonium oxidation (anammox) and denitrification in intact sediment cores.
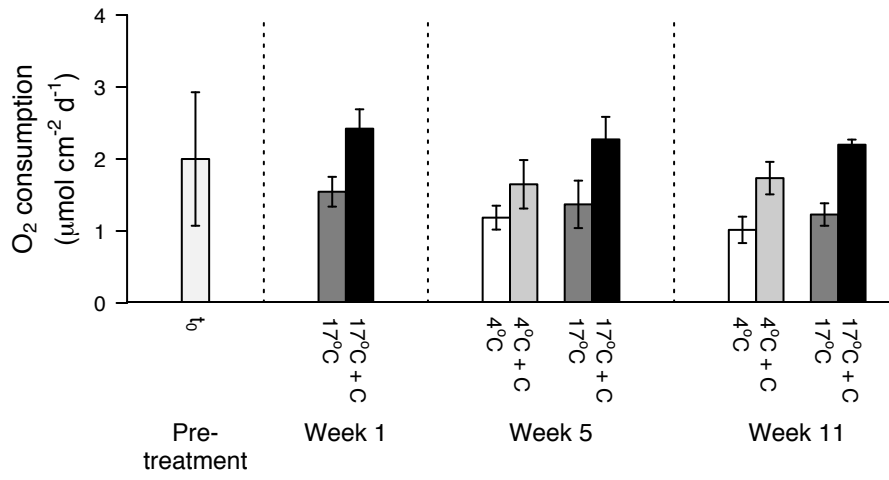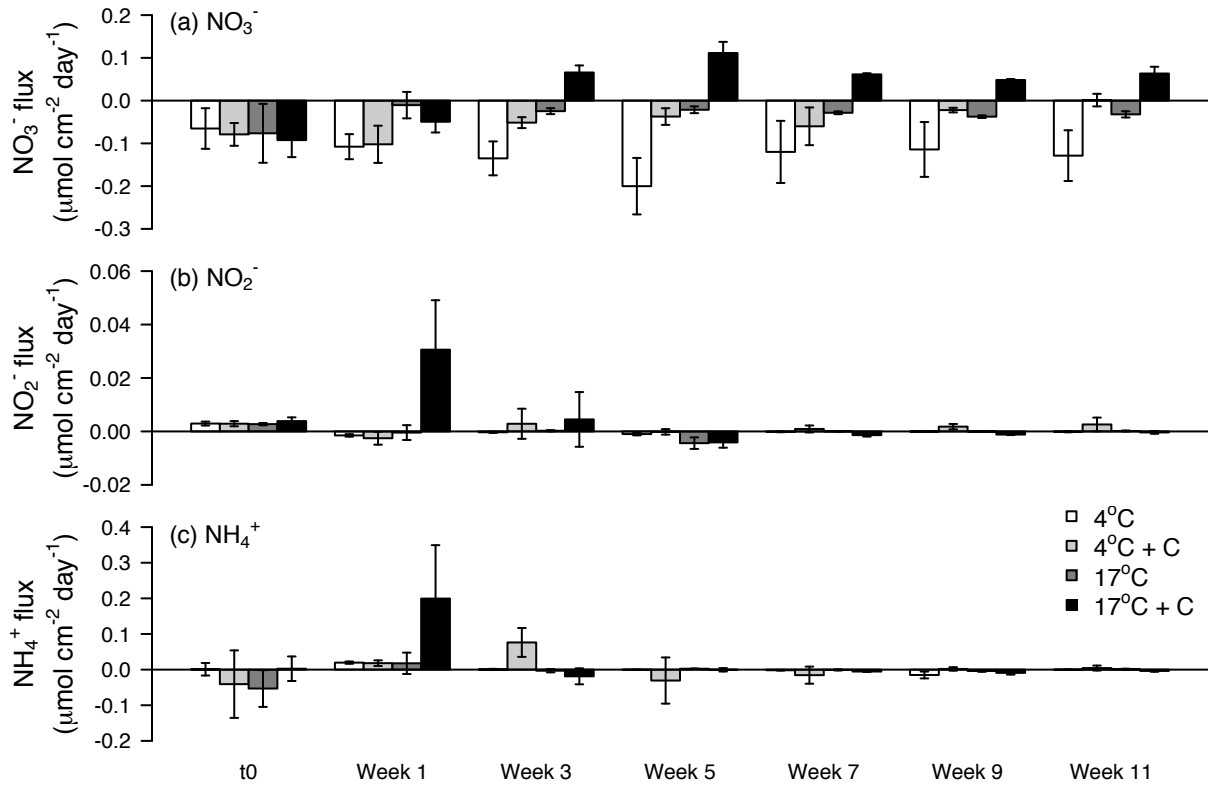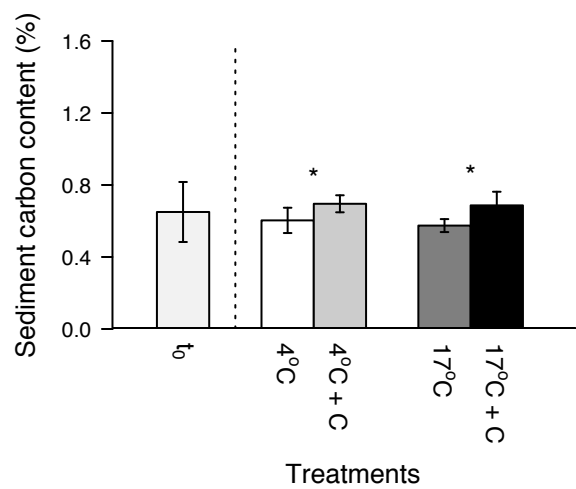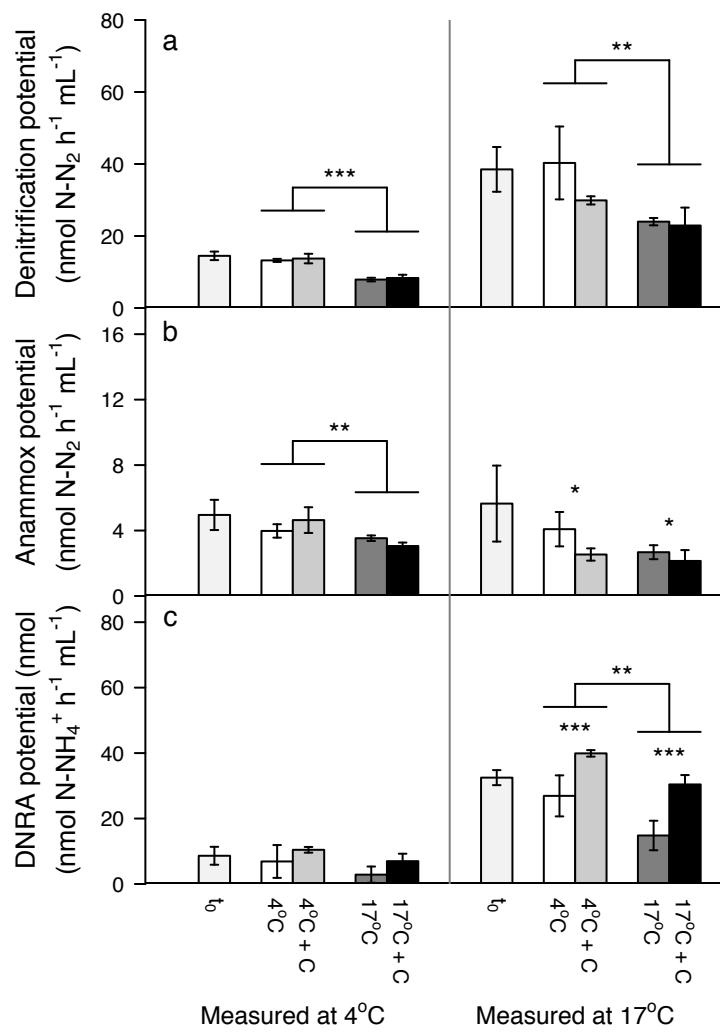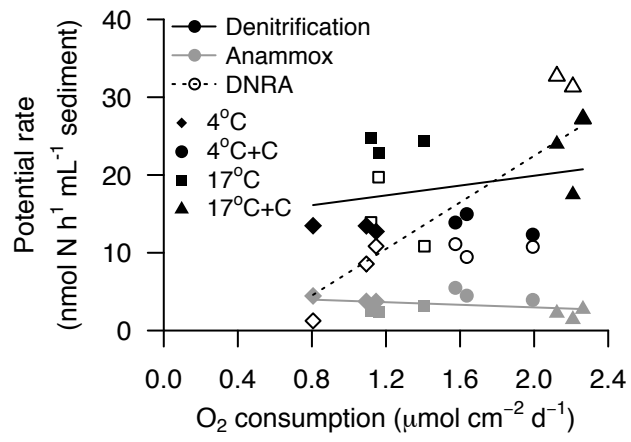790   Mar Ecol Prog Ser 326:37–47.

791

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6