# Benthic Carbon fixation and cycling in diffuse hydrothermal and background sediments in the Bransfield Strait, Antarctica

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**Abstract**

Sedimented hydrothermal vents are likely to be widespread compared to hard substrate hot vents. They host chemosynthetic microbial communities which fix inorganic C at the seafloor, as well as a wide range of macroinfauna, including vent-obligate and background non-vent taxa. There are no previous direct observations of Carbon cycling at a sedimented hydrothermal vent. We conducted $^{13}$C isotope tracing experiments at 3 sedimented sites in the Bransfield Strait, Antarctica, which showed different degrees of hydrothermalism. Two experimental treatments were applied, with $^{13}$C added as either algal detritus (photosynthetic C), or as bicarbonate (substrate for benthic C fixation). Algal $^{13}$C was taken up by both bacteria and metazoan macrofaunal, but its dominant fate was respiration, as observed at deeper and more food limited sites elsewhere. Rates of $^{13}$C uptake and respiration suggested that the diffuse hydrothermal site was not the hotspot of benthic C-cycling that we hypothesised it would be. Fixation of inorganic C into bacterial biomass was observed at all sites, and was measurable at 2 out of 3 sites. At all sites, newly fixed C was transferred to metazoan macrofauna. Fixation rates were relatively low compared to similar experiments elsewhere, thus C fixed at the seafloor was a minor C source for the benthic ecosystem. However, as the greatest amount of benthic C fixation occurred at the

26    off vent (non-hydrothermal) site ($0.077 \pm 0.034$ mg C m$^{-2}$ fixed during 60 h), we suggest that benthic fixation of

27    inorganic C is more widespread than previously thought, and warrants further study.

## 1. Introduction

Sedimented hydrothermal vent (SHV) sites are those where hydrothermal fluid diffuses through soft sediment cover on its way to mixing with oceanic bottom water. This creates hot (up to ~100°C) sediments with porewaters rich in dissolved sulphide and methane. This supports microbes that conduct chemosynthetic C fixation through a range of pathways (Bernardino et al., 2012). These hydrothermally influenced sediments are likely to be more spatially extensive than hard substrate vents, although their diffusive nature makes their extent hard to quantify. Sedimented hydrothermal vents have been shown to influence biological community composition and nutrition at adjacent sites which were otherwise characterised as 'inactive' or 'off-vent' (Levin et al., 2009; Bell et al., 2016a; Bell et al. 2016b; Bell et al., 2017a). However, the ecology of sedimented hydrothermal sites has received relatively little study. There is only one modelling study that has focused on the interaction between benthic ecosystems and C-cycling at SHVs (Bell et al., 2017b), and there are no direct observations of SHV C-cycling by components of the benthic ecosystem.

So far, a limited number of studies have used natural stable isotopic analysis to determine carbon sources and their fixation pathways utilised by infauna at SHVs (Levin et al., 2009; Soto, 2009; Sweetman et al., 2013; Bell et al. 2016b; Portail et al. 2016). Evidence has shown that C fixed during anaerobic oxidation of methane, oxic methanotrophy, sulphide oxidation, as well photosynthetic organic matter (OM) sinking from the surface, are all utilised by macrofauna to varying extents at SHVs (Levin et al., 2009; Bernardino et al., 2012). It is challenging to quantify the relative contributions of different C sources to macrofaunal diets, both because the natural isotopic ranges of some C sources overlap, and because often the isotopic compositions of those end members could not be measured (Levin et al., 2009; Bell et al., 2016b). Unknown variability in trophic discrimination factors also currently preclude quantitative estimates of the relative contribution of different C sources.

Stable isotope tracing experiments offer a way to overcome some of these issues. The experimental addition of labelled C sources, either photosynthetic OM or dissolved inorganic C (bicarbonate) to SHV sediment allows production of chemosynthetic OM, and the transfer of different OM types into the macrobenthos and other C pools in the short term to be directly observed. Such experiments (using only photosynthetic OM) have been conducted at a wide range of (ostensibly) non-chemosynthetic benthic sites, and have shown a wide variation in the relative importance of different biological C processing pathways (Woulds et al., 2009; 2016). At food limited sites in the deep-sea, respiration tends to be the dominant fate of added OM (van Oevelen et al. 2011; 2012). Shallower, more food rich settings such as coastal fjords and estuaries, with greater sedimentary organic C concentrations and higher macrofaunal biomass, show a pattern of biological C processing in which uptake by

59    fauna is a more important process, and at unusual and particularly food rich sites, such as the lower margin of

60    the Arabian Sea oxygen minimum zone (~1000 m depth), macrofaunal C uptake can even be the dominant

61    process (Woulds et al., 2009; 2016).

62    The occurrence of chemosynthesis in a benthic habitat represents an additional source of fresh, labile OM in an

63    environment that would otherwise be more severely food limited. For this reason, it has been suggested that

64    hydrothermally influenced sites can be biomass hotspots, where biogeochemical cycling is rapid (Bernardino et

65    al., 2012). However, due to the environmental toxicity created by hydrothermal fluid, and the fact that the

66    majority of taxa inhabiting SHVs are background rather than vent-endemic, the difference in faunal biomass

67    between SHVs and adjacent non-vent sites is highly variable (Levin et al., 2009; Bernardino et al., 2012; Bell et

68    al., 2016). It therefore seems possible that biological C processing at SHVs will show a distinct complement of

69    biological C processing patterns unlike those observed elsewhere in the deep sea. The food rich, high biomass

70    characteristics of some SHVs may lead to biological C processing that is and more similar to shallower, food

71    rich environments. On the contrary, spatially variable biomass patterns, as well as the metabolic costs associated

72    with potentially high temperatures and porewater toxicity could counteract the effect of enhanced food

73    availability. As direct measurements of biological C processing rates and pathways have not previously been

74    made at SHVs or in the Southern Ocean, there remains a gap in our understanding of sedimentary C and N-

75    cycling.

76    In this study we conducted stable isotope tracing experiments at three sites of variable hydrothermal activity in

77    the Bransfield Strait, Antarctica. To the best of our knowledge this is the first isotope tracing experiment in this

78    type of system. The following hypotheses were tested:

79    • Hydrothermally influenced sites exhibiting chemosynthesis will show elevated rates of biological C

80      processing.

81    • At hydrothermally influenced sites bicarbonate will be fixed by chemoautotrophs and transferred to the

82      macrofauna.

83    • Preference for feeding on photosynthetic versus chemosynthetic OM will be taxon dependent.

## 2. Methods

### 2.1 2.1 Study sites

In this study we focus on a SHV in the Bransfield Strait, close to the tip of the Antarctic peninsula. The discovery of hydrothermal venting in the Bransfield Strait was reported by Klinkhammer et al. (2001), who detected hydrothermal plumes in the water column, and recovered hot 'soupy' sediment from Hook Ridge. In addition, a species of *Sclerolinum* (Sahling et al., 2005; Georgieva et al., 2015) there has been described, and porewater geochemistry and hydrothermal flux rates have been published (Sahling et al., 2005; Aquilina et al., 2013).

Experiments were conducted at three sites in the Bransfield Strait, Antarctica (Fig. 1). Two of the sites lay on raised edifices, known as Hook Ridge and Middle Sister, along the axis of the basin, and were selected as being likely to exhibit diffuse hydrothermal venting, and the former was the location where diffuse venting had been identified. A third site, at a similar depth but along the north side of the basin, was chosen as an off-vent control (hereafter known as 'Off-Vent').

Porewater geochemistry at Middle Sister and Off-Vent were consistent with microbial processes without influence of hydrothermal activity. Porewater $NO_3^-$ and $NH_4^+$ profiles were indicative of nitrate reduction, but downcore declines in $SO_4^{2-}$ and $Cl^-$ were lacking over the ~40 cm depth sampled. In contrast, at Hook Ridge $SO_4^{2-}$ was depleted by up to 11% compared to seawater, and $Cl^-$ by up to 7%, allowing calculation of hydrothermal advection of 9-33 cm $y^{-1}$ (Aquilina et al., 2013).

Sediment organic carbon (Corg) concentrations were lower at Hook Ridge (0.97 wt% Corg) than at the Off-Vent and Middle Sister sites, which showed similar values (1.35 and 1.4 wt% Corg respectively, Table 1). The sites differed in biomass of different groups, with Hook Ridge and Middle Sister showing higher bacterial biomass and lower macrofaunal biomass than the Off-Vent site (Table 1). Hook Ridge was the only site classified as hydrothermally active by Aquilina et al. (2013). Porewaters were enriched in sulphide, methane and dissolved metals and depleted in chloride, and the calculated hydrothermal advection rate was 9-33 cm $y^{-1}$. Macrofauna tended to be representative of the background taxa of the region. Polychaetes were numerically dominant (41-56%), except at Hook Ridge, which was dominated by peracarids. Oligochaetes were the next most dominant at all sites. Vent endemic fauna were represented by two species of siboglinid polychaete; S. *contortum* at Hook Ridge, and *Siboglimun sp*. elsewhere (Bell et al., 2016a). Each site also supported one species of siboglinid

112    polychaete. In the case of Hook Ridge this was *S. contortum*, and at Middle Sister and the Off-Vent site it was

113    *Siboglinum sp.*, and they were always a minority constituent of the community (Bell et al., 2016 a).

114    **2.2 Isotope tracing experiments**

115    Sediment cores (10 cm i.d.) were recovered using a multiple corer, and kept in the dark at seafloor temperatures

116    (Table 1) using cooled incubators. Experiments were initiated by addition of isotopically enriched substrates.

117    Cores were then sealed and incubated for ~60 h, during which core-top water was continuously stirred.

118    Duplicate cores were subjected to each of two treatments. In the 'algae' treatment, lyophilized algal cells

119    (*Chlorella*, Cambridge Isotope Laboratories, CNLM-455-1) enriched in $^{13}$C and $^{15}$N (both ~100 at %) were

120    allowed to settle on the sediment surface, giving a final dose of $436\pm30$ mg C m$^{-2}$. This was equivalent to ~1.6%

121    of total OC in the surface 1 cm of sediment, or ~9% of annual OC input (Bell et al., 2017b). It is recognised that

122    such organic detritus is less degraded than the sinking photosynthetic material which normally reaches the

123    depths of our study sites. This is a limitation of the method common to all such experiments in the literature, and

124    means that rates for processing of added C in 'algae' experiments should be considered maximal. Further,

125    diatom detritus would have been more representative of local photosynthetic material, but was unfortunately not

126    available.

127    In the 'Bicarbonate' treatment a solution of 100 % $^{13}$C labelled sodium bicarbonate and 100 % $^{15}$N labelled

128    ammonium chloride was injected in the surface 5 cm of sediment porewater, to give a dose of 306 mg C m$^{-2}$ and

129    2.52 mg N m$^{-2}$, and an estimated porewater bicarbonate concentration of 1 mM.

130    At intervals (T0 and every ~12 h thereafter) during the incubation, core top water samples were withdrawn from

131    Algae treatment cores, and stored in crimp-cap vials poisoned with HgCl$_2$ for dissolved inorganic carbon (DIC)

132    analysis. At the end of the experiment cores were extruded and sectioned at intervals of 0-1, 1-2, 2-3, 3-5 and 5-

133    10 cm. Half of each section was frozen at -20°C, and the other half was preserved in buffered 10% formalin.

134    **2.3 Sample processing and analysis**

135    Overlying water samples were analysed for concentration and isotopic composition of DIC in triplicate on a

136    Thermalox TOC analyser coupled to a Thermo Delta V Advantage IRMS via a Conflo IV interface, using a

137    Thermo TriPlus autosampler. The reaction column was filled with H$_3$PO$_4$-coated beads.

138    Frozen sediment samples were freeze dried, and surface 0-1 cm horizons were analysed for phospholipid fatty

139    acids (PLFAs) following Main et al. (2015). Briefly, samples were extracted in a modified Bligh and Dyer

140      extraction solution of chloroform:methanol:citrate buffer, 1:2:0.8. The polar fraction was obtained by loading

141      samples onto ISOLUTE SPE columns, washing with chloroform and acetone, and eluting with methanol. After

142      addition of nonadecanoic acid (C19:0) as an internal standard, extracts were derivatised in the presence of KOH

143      in methanol. Derivatisation was quenched with water and acetic acid, and the organic fraction was extracted by

144      washing with 4:1 isohexane:chloroform. Samples were dried and then taken up in isohexane for analysis on a

145      Trace Ultra GC, connected via a GC Combustion III to a Delta V Advantage IRMS (Thermo Finnigan,

146      Bremen). The isotopic signature of each PLFA was measured against a $CO_2$ reference gas which is traceable to

147      IAEA reference material NBS 19 TS-Limestone, with a precision of $\pm$ 0.31 ‰, and corrected for the C atom

148      added during derivatization.

149      Sediment horizons between 0 and 10 cm preserved in formalin were sieved over a 300$\mu$m mesh. Macrofauna

150      were extracted under a binocular microscope, identified to broad taxonomic level, air dried in pre-weighed tin

151      capsules, and weighed. In some cases multiple individuals were pooled to create samples large enough for

152      analysis. Fauna were de-carbonated by dropwise addition of 0.1M HCl, followed by air drying at 50°C.

153      Calcareous foraminifera and bivalves which were too small for manual removal of shells were de-carbonated

154      with 6N HCl. Fauna were analysed for their C contents and isotopic signature using a Flash EA 1112 Series

155      Elemental Analyser connected via a Conflo III to a Delta$^{Plus}$ XP isotope ratio mass spectrometer (all Thermo

156      Finnigan, Bremen). Carbon contents was quantified using the area under the mass spectrometer response curve,

157      against National Institute of Standards and Technology reference material 1547 peach leaves (repeat analysis

158      gave precision $\pm$ 0.35 %). Isotopic data were traceable to IAEA reference materials USGS40 and USGS41 (both

159      L-glutamic acid), with a precision $\pm$ 0.13 ‰.

160      **2.4 Data treatment**

161      Respiration of added algal C was calculated for cores subjected to the algae treatment. The amount of excess

162      DI$^{13}$C in each sample was calculated by first subtracting the natural abundance of $^{13}$C in DIC. This was scaled

163      up to give the total amount of DIC from the added algae at each sample timepoint, and corrected for water

164      removed and added during sampling. Respiration rate was calculated for each core by placing a line of best fit

165      through the amount of added $^{13}$C over time, and normalised to surface area.

166      Bacterial incorporation of $^{13}$C was calculated by first subtracting the natural abundance of $^{13}$C from the isotopic

167      signature of each PLFA (data published in Bell et al., 2017), where the difference exceeded the precision of the

168      analytical technique, to give the amount of added C in each compound. Bacterial incorporation was then

169     calculated using the 4 bacteria-specific PLFAs isoC14:0, isoC15:0, antisoC15:0, and isoC16:0, following

170     Boschker and Middelburg (2002). Uptake of $^{13}$C into these bacteria-specific PLFAs was summed, and scaled up

171     on the basis that they together account for 14% of total bacterial PLFA, and that PLFAs account for 5.6% of

172     total bacterial biomass. For samples in the bicarbonate treatment further scaling up was applied, to account for

173     the fact that the addition of $^{13}$C bicarbonate was calculated to result in a porewater DIC pool that was 22 atom %

174     $^{13}$C.

175     Faunal uptake of added $^{13}$C was calculated by subtracting $^{13}$C attributable to its natural abundance in the

176     appropriate taxon (data published in Bell et al., 2017 a) from faunal isotopic signatures, where the difference

177     exceeded the precision of the analytical technique, and multiplying by the quantity of organic C in each

178     specimen. Specimens were summed for each core, and the value multiplied by 2, to account for only half of

179     each horizon being used for faunal extraction.

180     **3.   Results**

181     Data files can be accessed at DOIxxxx.

182     **3.1  3.1 Respiration**

183     Respiration rates measured in algae addition experiments varied from 0.03 mg C m$^{-2}$ h$^{-1}$ at the off vent site to

184     0.15 mg C m$^{-2}$ h$^{-1}$ at Middle Sister (Fig. 2).

185     **3.2  3.2 Bacterial uptake and PLFA suite**

186     In the algae addition experiments, total bacterial uptake of C throughout the experiment was maximal at Middle

187     Sister and Hook Ridge (1.30-1.91 and 1.25 mg C m$^{-2}$, respectively), and minimal at the off vent site (0.25-0.77

188     mg C m$^{-2}$, Fig. 3). In bicarbonate addition experiments, in which incorporation of $^{13}$C into bacterial PLFAs

189     represents chemosynthesis, bacterial incorporation of bicarbonate was maximal at the off vent site (0.05-0.10

190     mg C m$^{-2}$), and was also detectable in one of the replicates at Middle Sister (0.003 mg C m$^{-2}$, close to detection

191     limits, so this value is treated with caution), however it was not detectable at Hook Ridge.

192     The PLFA suites at all sites were qualitatively similar. They were dominated by C16:0, C16:1ω7c, and

193     C18:1ω7, which together constituted 42 ± 2% of total PLFAs (Fig. 4). This is at the high end of contributions

194     from these compounds elsewhere, such as 34-45% in the Arabian Sea, and 41% on the Galicia Bank (Kunihiro

195     et al., 2014). The relatively high proportions of C16:1ω7 and C18:1ω7 are indicative of the presence of

196     chemosynthetic and specifically sulphide oxidising bacteria (Colaco et al., 2007). In addition C18:1ω9, which is

197      linked to endosymbionts in vent mussels, and C18:1ω13, which is associated with methylotrophic bacteria were

198      also present (Colaco et al., 2007).

199      In both algae and bicarbonate addition experiments, $^{13}$C incorporation into PLFAs was dominated by C16:0,

200      followed by C18:1ω9 and the sulphide oxidiser indicators C16:1ω7 and C18:1ω7 (Fig 4).

201      ### 3.3  3.3 Faunal uptake

202      Faunal uptake of added C differed between A and B replicate cores in all experiments except the algae addition

203      at the off vent site, and bicarbonate addition at Middle Sister (Fig. 5).

204      In algae addition experiments faunal uptake was similar between the off vent site and one of the Hook Ridge

205      cores (~0.03 mg C m$^{-2}$), while the other Hook Ridge core showed very low faunal C uptake. Considerably

206      greater faunal uptake (0.12 mg C m$^{-2}$) was observed in one of the replicate cores from Middle Sister (Fig. 5).

207      In bicarbonate addition experiments, measurable uptake of $^{13}$C by fauna was observed at all sites. It was

208      maximal at Hook Ridge (0.02 mg C m$^{-2}$ in one replicate), and the off vent and Middle Sister sites showed

209      similar values (Table 2, Fig. 5).

210      Small size of individuals meant that organisms had to be pooled for isotopic analysis, limiting the taxonomic

211      resolution of the faunal uptake data. Although limited in this way, the data show that faunal uptake of $^{13}$C in

212      both algae and bicarbonate addition experiments was mostly carried out by either polychaetes, or 'mixed

213      macrofauna' (Fig. 6). This latter category contained variously bivalves, crustaceans, echinoderms, nematodes

214      and foraminifera, in cases where those groups were not present in sufficient numbers for separate reporting of

215      their C uptake. When a group was present in sufficient quantity it was analysed separately. As with total

216      macrofaunal $^{13}$C uptake, there was considerable variability between replicate cores in the most abundant

217      taxonomic groups. In addition, meiofaunal organisms took up $^{13}$C at Middle Sister, and the bicarbonate $^{13}$C that

218      was transferred to macrofauna at Hook Ridge was mostly observed in amphipod crustaceans.

219      ## 4.  Discussion

220      ### 4.1 Occurrence of inorganic C fixation

221      The results of bicarbonate addition experiments show evidence for occurrence of benthic C-fixation at all sites,

222      and transfer of that C to the macrofauna, in the form of isotopic enrichment of bacterial PLFAs at the off-vent

223      and Middle Sister sites (Fig. 3), and of macrofauna at the Hook Ridge and Middle Sister sites (Fig. 5). The

224    quantities of bicarbonate ^{13}C detected in bacterial and faunal biomass were low, and tended to be 1 to 2 orders of

225    magnitude smaller than equivalent values for algae addition experiments (Table 2). We have confidence that the

226    values reported are above detection limits, in that data were only used where the enrichment of organisms or

227    PLFAs above their natural background signatures was greater than the analytical precision of the method. The

228    greatest quantities of bacterial uptake were measured at the off-vent site (Fig. 3), and the greatest quantity

229    transferred to the fauna was measured at Hook Ridge (Fig. 5), however, due to the low values measured and the

230    evident patchiness of faunal communities we do not feel these differences are suitable for further discussion.

231    The most striking result of the bicarbonate addition experiments was that evidence for benthic C fixation was

232    found at all sites, not only at the hydrothermally influenced Hook Ridge. Further, the site showing the largest

233    amount of incorporation of bicarbonate ^{13}C into bacterial PLFAs was the off-vent 'control' site (Table 2, Fig. 3).

234    This is consistent with the occurrence of siboglinids at all sites. These host chemosynthetic endosymbionts most

235    of which conduct sulphide oxidation (Thornhill et al., 2008; Georgieva et al., 2015). It should be noted that the

236    evidence for inorganic C fixation comes from PLFAs in the bulk sediment, while isotopic signatures of

237    siboglinids did not show enrichment above background values. Therefore the occurrence of benthic C fixation is

238    not only associated with siboglinids.

239    Experiments were designed to replicate natural conditions as far as practically possible, while being limited to

240    shipboard rather than in situ methods. One result of this is that the sediment contained in cores was detached

241    from the upward flux of hydrothermal fluid, and the electron donors it supplied. This could have limited

242    inorganic C fixation, which would have impacted the rates measured at Hook Ridge. We suggest however that

243    this is not a serious limitation, as Hook Ridge was rather mildly hydrothermal. Vent endemic fauna were almost

244    absent (Bell et al., 2016), there was no increase in faunal biomass close to venting, downcore profiles of

245    alkalinity, nitrate and ammonium were consistent with normal microbial processes, and hydrothermal advection

246    rates were 9-33 cm y$^{-1}$ (Aquilina et al., 2013). At these low advection rates we suggest that there would not have

247    been sufficient time during our ~60 h experiments for a noticeable depletion in availability of electron donors

248    supplied by hydrothermal fluid.

249    The evidence suggests that while the amount of benthic C-fixation was always low, it was not restricted to

250    environments typically thought of as chemosynthetic (sedimented or hard substrate hydrothermal vents, methane

251    seeps, or organic falls (Bernardino et al., 2012)). Thus, benthic C-fixation appears to play a role in benthic C-

252    cycling at a much wider range of sites and over a much larger area of the seafloor than previously thought. This

253    is supported by linear inverse modelling of C-cycling at the sites in this study, which led Bell et al. (2017b) to

254  suggest that chemosynthetic support for ecosystems may have a far greater spatial extent than previously

255  thought, extending beyond those which are directly hydrothermally influenced. Similar results have also been

256  reported in non-hydrothermal, but methane rich sediments on the South Georgia margin, where assimilation of

257  $^{13}$C labelled bicarbonate into bacterial biomass, and transfer into macrofauna was also observed (Would et al.,

258  2019). In addition, in situ observations of benthic C fixation have also been made at mesotrophic, abyssal sites

259  in the eastern equatorial Pacific, which were not associated with hydrothermal or methane seep activity

260  (Sweetman et al. 2018). In that study incorporation of $^{13}$C labelled bicarbonate into bacterial PLFAs was

261  observed at 2 sites separated by 100's of kilometres, at rates similar to bacterial assimilation of phytodetritus C

262  at the same sites. Together with global scale modelling completed by Middelburg (2011), these studies suggest

263  that chemoautotrophic C fixation may be considerably more widespread than previously thought. It is therefore

264  deserving of further study so that it can be quantitatively incorporated into our understanding of the marine C-

265  cycle.

266  In their study using linear inverse modelling of the benthic food web and C cycle, based on natural isotopic and

267  biomass data, Bell et al. (2017b) modelled a rate for chemosynthesis of 5.76-8.4 mg C $m^{-2}$ $d^{-1}$ at Hook Ridge,

268  and <0.006 mg C $m^{-2}$ $d^{-1}$ at the off-vent site. These modelled rates at Hook Ridge are considerably higher than

269  Hook Ridge benthic C-fixation measured in this study, for which there was evidence (labelled PLFAs), but a

270  rate could not be calculated. The higher modelled rates by Bell et al. (2017 b) may be explained by the fact that

271  a temperature of 50°C was used for the Hook Ridge site, based on previously published conditions of the site

272  (Klinkhammer et al., 2001). Unfortunately, equipment was not available while at sea for measurement of

273  sediment temperature at the study sites, therefore all experiments, including that at Hook Ridge, were conducted

274  at measured bottom water temperatures of 0-1°C. It is likely that the rates measured here for chemosynthetic

275  incorporation of labelled bicarbonate are lower than those that would have been measured in situ. It is also

276  probable that measurable rates could have been detected at Hook Ridge had more samples been available for

277  replicate analyses.

278  The maximal rate of benthic C-fixation measured in this study was 0.050 mg C $m^{-2}$ $d^{-1}$ , which occurred in one

279  core at the off-vent site. This remains considerably lower than the 0.24-1.02 mg C $m^{-2}$ $d^{-1}$ measured by Molari et

280  al. (2013, rates calculated in Sweetman et al., 2018) at depths ranging between 1207-4381 m on the Iberian

281  margin and in the Mediterranean, and the 1.29 mg C $m^{-2}$ $d^{-1}$ measured by Sweetman et al. (2018) at ~4100 m

282  depth in the Clarion Clipperton Zone. The Bransfield Strait sites in this study were shallower, had higher

283  concentrations of sedimentary organic C, and slightly lower bottom water temperatures than either of the

284 previous studies cited. The very low temperatures at which experiments were conducted (1°C at Hook Ridge

285 and 0°C at the off vent site) is likely to have contributed to the slow measured rates of benthic C-fixation.

286 Another factor which may influence benthic C-fixation is the annual flux of photosynthetic C from the surface

287 (Molari et al., 2013; Bell et al., 2017a). The annual flux of POC to the sediments in the Bransfield Strait is

288 greater than in the Clarion Clipperton Zone, and probably than in the Mediterranean as well (Masque et al.,

289 2002; Sweetman et al., 2017), and this may be an additional driver behind the low benthic C-fixation rates

290 observed. Archaeal abundance has been shown to correlate with dark C-fixation, and addition of labile organic

291 material has been shown to increase inorganic C fixation rates, perhaps through a combination of heterotrophy

292 and mixotrophy (Molari et al., 2013). Overall, the factors governing benthic C-fixation rates require

293 investigation. In addition, the pathways (i.e. autotrophic C fixation versus anapleurotic C fixation by

294 heterotrophs, Wegener et al., 2012), energy sources (e.g. sulphide, methane) and organisms responsible for

295 benthic inorganic C fixation have not been identified, and warrant further study.

296 4.2 Carbon uptake by macrofauna

297 Uptake of added C by fauna in isotope tracer experiments usually shows a degree of spatial patchiness (e.g.

298 Woulds et al., 2007), but this seems to have been particularly marked in the Bransfield Strait, mainly at those

299 sites with hydrothermal influence. This is consistent with the patchiness of *Sclerolinum contortum* in replicate

300 cores at Hook Ridge (Bell et al. 2016a). At both Hook Ridge and Middle Sister there was a very marked

301 difference in faunal uptake of algal C between the A and B replicate cores in algae addition experiments (Fig.

302 5), and this was considerably greater than that observed, for example, in experiments on the Pakistan margin

303 Woulds et al. (2007). This is likely to be due to difference in the biomass of fauna present in each core, and such

304 marked small scale patchiness in faunal communities has been noted previously as a particular feature of SHVs

305 (Levin et al., 2009; Bernardino et al., 2012). Fine scale distribution of fauna is related to variations in

306 concentrations of substrates such as sulphide and methane (Levin et al., 2003), therefore the patchiness observed

307 especially at Hook Ridge is likely related to spatial and temporal fluctuation in hydrothermal advection.

308 Faunal uptake of added C appeared to be greatest at Middle Sister in algae addition experiments, and at Hook

309 Ridge in bicarbonate addition experiments, however the variation between replicate cores limits conclusions that

310 can be drawn. Previous isotope tracing experiments have noted correlations between biomass of taxa and the

311 amount of C they take up (e.g. Woulds et al., 2007). Further, there was no systematic variation in biomass-

312 specific C uptake (0.026-0.13 ug C uptake / mg C biomass) between sites, therefore the patterns observed here

313 in faunal C uptake are likely to result from variation in biomass present in each experimental core.

314 Similarly, the identities of fauna responsible for ^{13}C uptake was variable between replicate cores (Fig. 6), and

315 this is also likely to have been driven by variation in the macrofaunal community present in each core. The

316 prevalence and variable importance of the 'mixed macrofauna' category indicates that in some cases a fairly

317 diverse assemblage was engaged in C uptake and processing.

318 Previous studies have suggested that SHVs tend to exhibit relatively high biomass macrofaunal communities,

319 sustained by the additional food source provided by chemosynthesis (Bernadino et al., 2012), and this leads to

320 an expectation that the macrofauna may be particularly active in processing of organic C in the sediment, in line

321 with other food rich environments such as estuaries and fjords (Moodley et al., 2000; 2005; Witte et al., 2003a).

322 This was not the case in the algae addition experiments, with faunal uptake accounting for only 0.05-2.2 % of

323 total biological ^{13}C processing (Fig. 7). This is similar to the role of faunal C uptake in overall C processing seen

324 at deep, organic carbon poor sites such as at 2170 m depth off NW Spain (2.2 %, Moodley et al., 2002), or at

325 1552 m depth in the Eastern Mediterranean (0.2 %, Moodley et al., 2005), and is lower than that at 4800 m

326 depth on the Porcupine Abyssal Plain (1.5-26 %, Witte et al., 2003b). Such sites tend to have lower OC

327 concentrations and lower macrofaunal biomass (Woulds et al., 2016) than was observed in the Bransfield Strait,

328 therefore the unusually small role of macrofaunal in C uptake in the Bransfield Strait may be due to low

329 temperatures. Both low temperature and food scarcity have previously been observed to limit metabolic rates in

330 polar environments (Brockington and Peck, 2001; Sommer and Portner, 2002). Another possible explanation for

331 the rather small amount of macrofaunal C uptake at the Hook Ridge site may be that the macrofaunal

332 community, which was composed almost entirely of non vent-obligate, ambient Southern Ocean taxa (Bell et

333 al., 2016a), had reduced levels of function due to the stress imposed by living at a site influenced by

334 hydrothermal fluid. The toxicity and relatively high temperature of their environment (compared to non-

335 hydrothermal Southern Ocean benthic settings) may have resulted in reduced C uptake activity. Therefore,

336 macrofaunal biomass and C processing activity were limited by a hydrothermal flux that was sufficient to limit

337 functioning and preclude occurrence of some some locally common taxa, but insufficient to sustain a high

338 biomass, vent endemic macrofaunal community as seen in other SHVs (Bell et al., 2016 a).

339 Siboglinid polychaetes, known to host chemosynthetic endosymbionts, were present at all study sites (Bell et al.,

340 2016 a), but were not found to make a substantial contribution to uptake of added [13]C. This is to be expected in

341 the algae addition experiments, as siboglinids would have direct access to algal C (except possibly via DOC).

342 Most specimens recovered from biocarbonate addition experiments also showed $\delta^{13}C$ values indistinguishable

from their natural signature, with one exception at the Middle Sister site which was enriched compared to the

natural signature by 3.2 ‰. The fact that sioglinids did not have a major role in C fixation and cycling in our

experiments may have been partly due to their low abundances in experiment cores compared to patches where

they were maximally abundant (Bell et al., 2016a), or because experiments were not long enough for uptake by

endosymbionts. Nonetheless, our findings show a much reduced role for siboglinids compared to suggestions

made in previous publications. Aquilina et al. (2014) suggested that *Siboglinum sp.* at Hook Ridge may be

sufficiently abundant to be conduits for a quantitatively meaningful flux of dissolved iron out of the sediment,

and Bell et al. (2017 b) found that they may be a key taxon facilitating input of chemosynthetic C into the food

web. In agreement with the point made by Bell et al. (2016a), the spatial distribution of siboglinids is extremely

patchy, and thus their role in benthic biogeochemical processes is spatially heterogeneous (Bell et al., 2017a, b).

4.3 Carbon processing and SHVs as biogeochemical hotspots

Respiration rates measured in the algae addition experiments were maximal at Middle Sister, and minimal at the

off-vent site (Fig. 2). Temperature is often recognised as a dominant control on benthic respiration rates (e.g.

Moodley et al., 2005; Woulds et al., 2009), however these experiments were all conducted within 1°C of each

other, so temperature is unlikely to have driven differences in respiration rates. Instead, the differences between

sites may have been driven by differences in bacterial biomass (Table 1), which was maximal at Middle Sister

and minimal at the off-vent site. The bacteria are often found to account for a large majority of benthic

community biomass, and are thus usually assumed to be responsible for the majority of benthic community

respiration (e.g. Heip et al., 2001). The measured respiration rates were similar to those measured at 2170 m on

the NW margin of Spain (Moodley et al., 2002), and on the Porcupine Abyssal Plain (Witte et al., 2003b), both

of which were considerably deeper, and had lower sediment organic C concentrations, but higher bacteria

biomass (Woulds et al., 2016). They were also lower than respiration rates measured at similar depths in the

Eastern Mediterranean (Moodley et al., 2005), and Arabian Sea (Woulds et al., 2009). These sites showed

similar bacteria biomass to the Bransfield Strait, but were all considerably warmer (7-14°C, Woulds et al.,

2016), therefore the low ambient temperatures of the Southern Ocean appeared to reduce respiration rates.

It has been suggested that reducing benthic environments are often hotspots of faunal biomass and

biogeochemical cycling due to the increased availability of labile food sources supplied by chemosynthesis

(Bernardino et al., 2012). In this study, the hydrothermally active site Hook Ridge showed rates of respiration

and bacterial uptake of algal C that were intermediate between the two non-hydrothermally active sites (Figs. 2,

3). Whilst comparison between sites is limited by very marked faunal patchiness, the amount of faunal uptake of

algal $^{13}$C at Hook Ridge was similar to that at the off-vent control site, while that at Middle Sister was, in one

replicate, considerably greater (Fig. 5). This suggests that SHVs are not necessarily biogeochemical cycling

hotspots, as in algae addition experiments the overall amount of added C processed by the benthic community

was not greater than that observed at non-hydrothermal sites (Fig. 8). In line with this, biological processing of

added C in the algae addition experiments did not show a major role for faunal C uptake as we hypothesised, but

was instead dominated by respiration, as is typically observed at relatively deep, cold sites (Woulds et al., 2009).

The Middle Sister site showed the greatest amount of biological processing of added algal C, which was

probably attributable to it having the greatest bacterial biomass and organic carbon concentrations, and the fact

that the macrofaunal community, composed mostly of ambient Southern Ocean taxa, will have been functioning

without the stress imposed by hydrothermal fluid.

## 5. Conclusions

The main fate of photosynthetic C was respiration in common with other deeper and more food limited sites.

The rates of respiration and C uptake by both macrofaunal and bacteria that we measured were comparatively

low, and this is attributable to the low temperature of the experiments, and the toxicity and thermal stress caused

by hydrothermal fluid. The hydrothermal site (Hook Ridge) in this study did not show more rapid C-cycling

than other similar experiments, as we hypothesised it would.

Benthic fixation of inorganic was observed at all sites, and quantified at 2 out of 3 sites. While the rates were

low compared to other similar experiments, the fact that the greatest amount of benthic C fixation occurred at

the off vent site suggests that benthic C fixation may not be restricted to hydrothermal and other reducing

settings. We suggest that it could be an important aspect of the marine C-cycle, and warrants further study.

**Data Availability**

Data sets can be found at DOIxxxx

**Author Contributions**

Experiments were conducted by C. Woulds and A. Glover. All authors contributed to analysis of samples, and

commented on the manuscript.

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**References**

Aquilina, A., Connelly, D. P., Copley, J. T., Green, D. R. H., Hawkes, J. A., Hepburn, L. E., Huvenne, V. A. I., Marsh, L., Mills, R. A., and Tyler, P. A.: Geochemical and Visual Indicators of Hydrothermal Fluid Flow through a Sediment-Hosted Volcanic Ridge in the Central Bransfield Basin (Antarctica), Plos One, 8, 2013.

Aquilina, A., Homoky, W. B., Hawkes, J. A., Lyons, T. W., and Mills, R. A.: Hydrothermal sediments are a source of water column Fe and Mn in the Bransfield Strait, Antarctica, Geochimica Et Cosmochimica Acta, 137, 64-80, 2014.

Bell, J. B., Aquilina, A., Woulds, C., Glover, A. G., Little, C. T. S., Reid, W. D. K., Hepburn, L. E., Newton, J., and Mills, R. A.: Geochemistry, faunal composition and trophic structure in reducing sediments on the southwest South Georgia margin, Royal Society Open Science, 3, 2016b.

Bell, J. B., Reid, W. D. K., Pearce, D. A., Glover, A. G., Sweeting, C. J., Newton, J., and Woulds, C.: Hydrothermal activity lowers trophic diversity in Antarctic hydrothermal sediments, Biogeosciences, 14, 5705-5725, 2017a.

Bell, J. B., Woulds, C., Brown, L. E., Sweeting, C. J., Reid, W. D. K., Little, C. T. S., and Glover, A. G.: Macrofaunal ecology of sedimented hydrothermal vents in the Bransfield Strait, Antarctica, Frontiers in Marine Science, 3, 2016a.

Bell, J. B., Woulds, C., and Oevelen, D. v.: Hydrothermal activity, functional diversity and chemoautotrophy are major drivers of seafloor carbon cycling, Scientific Reports, 7, 12025, 2017b.

Bernardino, A. F., Levin, L. A., Thurber, A. R., and Smith, C. R.: Comparative Composition, Diversity and Trophic Ecology of Sediment Macrofauna at Vents, Seeps and Organic Falls, Plos One, 7, 2012.

Boschker, H. T. S. and Middelburg, J. J.: Stable isotopes and biomarkers in microbial ecology, FEMS Microbiology Ecology, 40, 85-95, 2002.

424 Brockington, S.,  Peck, L. S.: Seasonality of respiration and ammonium excretion in the Antarctic   echinoid

425 Sterechinus neumayeri, Marine Ecology Progress Series, 219, 159-168, 2001.

426 Colaco, A., Desbruyeres, D., and Guezennec, J.: Polar lipid fatty acids as indicators of trophic associations in a

427 deep-sea vent system community, Marine Ecology-an Evolutionary Perspective, 28, 15-24, 2007.

428 Georgieva, M. N., Wicklund, H., Bell, J. B., Eilertsen, M. H., Mills, R. A., Little, C. T. S., Glover, A. G.: A

429 chemosynthetic weed: the tubeworm *Sclerolinum contortum* is a bipolar, cosmopolitan species, BMC

430 Evolutionary Biology, 15, article 280, 2015.

431 Heip, C. H. R., Duineveld, G., Flach, E., Graf, G., Helder, W., Herman, P. M. J., Lavaleye, M., Middelburg, J.

432 J., Pfannkuche, O., Soetaert, K., Soltwedel, T., de Stigter, H., Thomsen, L., Vanaverbeke, J., and de Wilde, P.:

433 The role of the benthic biota in sedimentary metabolism and sediment-water exchange processes in the Goban

434 Spur area (NE Atlantic), Deep Sea Research Part II, 48, 3223-3243, 2001.

435 Klinkhammer, G. P., Chin, C. S., Keller, R. A., Dahlmann, A., Sahling, H., Sarthou, G., Petersen, S., and Smith,

436 F.: Discovery of new hydrothermal vent sites in Bransfield Strait, Antarctica, Earth and Planetary Science

437 Letters, 193, 395-407, 2001.

438 Kunihiro, T., Veuger, B., Vasquez-Cardenas, D., Pozzato, L., Le Guitton, M., Moriya, K., Kuwae, M., Omori,

439 K., Boschker, H. T. S., and van Oevelen, D.: Phospholipid-Derived Fatty Acids and Quinones as Markers for

440 Bacterial Biomass and Community Structure in Marine Sediments, Plos One, 9, 2014.

441 Levin, L. A., Mendoza, G. F., Konotchick, T., and Lee, R.: Macrobenthos community structure and trophic

442 relationships within active and inactive Pacific hydrothermal sediments, Deep-Sea Research Part Ii-Topical

443 Studies in Oceanography, 56, 1632-1648, 2009.

444 Levin, L. A., Ziebis, W., Mendoza, G. F., Growney, V. A., Tryon, M. D., Brown, K. M., Mahn, C., Gieskes, J.

445 M., Rathburn, A. E.: Spatial heterogeneity of macrofauna at northern California methane seeps: influence of

446 sulphide concentration and fluid flow, Marine Ecology Progress Series, 265, 123-139, 2003.

447 Main, C. E., Ruhl, H. A., Jones, D. O. B., Yool, A., Thornton, B., and Mayor, D. J.: Hydrocarbon contamination

448 affects deep-sea benthic oxygen uptake and microbial community composition, Deep-Sea Research Part I-

449 Oceanographic Research Papers, 100, 79-87, 2015.

450 Masque, P., Isla, E., Sanchez-Cabeza, J. A., Palanques, A., Bruach, J. M., Puig, P., and Guillen, J.: Sediment

451 accumulation rates and carbon fluxes to bottom sediments at the Western Bransfield Strait (Antarctica), Deep-

452 Sea Research Part Ii-Topical Studies in Oceanography, 49, 921-933, 2002.

453 Middelburg, J. J.: Chemoautotrophy in the ocean, Geophysical Research Letters, 38, 2011.

454 Molari, M., Manini, E., and Dell'Anno, A.: Dark inorganic carbon fixation sustains the functioning of benthic

455 deep-sea ecosystems, Global Biogeochemical Cycles, 27, 212-221, 2013.

456 Moodley, L., Boschker, H. T. S., Middelburg, J. J., Pel, R., Herman, P. M. J., de Deckere, E., and Heip, C. H.

457 R.: Ecological significance of benthic foraminifera: $^{13}$C labelling experiments, Marine Ecology Progress Series,

458 202, 289-295, 2000.

459 Moodley, L., Middelburg, J. J., Boschker, H. T. S., Duineveld, G. C. A., Pel, R., Herman, P. M., and Heip, C. H.

460 R.: Bacteria and foraminifera: Key players in a short-term deep-sea benthic response to phytodetritus, Marine

461 Ecology Progress Series, 236, 23-29, 2002.

462 Moodley, L., Middelburg, J. J., Soetaert, K., Boschker, H. T. S., Herman, P. M., and Heip, C. H. R.: Similar

463 rapid response to phytodetritus deposition on shallow and deep-sea sediments, Journal of Marine Research, 63,

464 457-469, 2005.

465 Portail, M., Olu, K., Dubois, S. F., Escobar-Briones, E., Gelinas, Y., Menot, L., and Sarrazin, J.: Food-Web

466 Complexity in Guaymas Basin Hydrothermal Vents and Cold Seeps, Plos One, 11, 2016.

467 Sahling, H., Wallmann, K., Dahlmann, A., Schmaljohann, R., and Petersen, S.: The physicochemical habitat of

468 Sclerolinum sp at Hook Ridge hydrothernial vent, Bransfield Strait, Antarctica, Limnology and Oceanography,

469 50, 598-606, 2005.

470 Sommer, A. M., Portner, H. O.: Metabolic cold adaptation in the lugworm Arenicola marina: comparison of a

471 North Sea and a White Sea population, Marine Ecology Progress Series, 240, 171-182, 2002.

472 Soto, L. A.: Stable carbon and nitrogen isotopic signatures of fauna associated with the deep-sea hydrothermal

473 vent system of Guaymas Basin, Gulf of California, Deep-Sea Research Part Ii-Topical Studies in Oceanography,

474 56, 1675-1682, 2009.

475 Sweetman, A. K., Levin, L. A., Rapp, H. T., and Schander, C.: Faunal trophic structure at hydrothermal vents on

476 the southern Mohn's Ridge, Arctic Ocean, Marine Ecology Progress Series, 473, 115-+, 2013.

477 Sweetman, A. K., Smith, C. R., Shulse, C. N., Maillot, B., Lindh, M., Church, M. J., Meyer, K., Oevelen, D. v.,

478 Stratmann, T., and Gooday, A. J.: Key role of bacteria in the short-term cycling of carbon at the abyssal

479 seafloor, Limnology and Oceanography, 9999, 1-20, 2018.

480 Sweetman, A. K., Thurber, A. R., Smith, C. R., Levin, L. A., Mora, C., Wei, C. L., Gooday, A. J., Jones, D. O.

481 B., Rex, M. A., Yasuhara, M., Ingels, J., Ruhl, H. A., Frieder, C. A., Danovaro, R., Wurzberg, L., Baco, A. R.,

482 Grupe, B. M., Pasulka, A., Meyer, K. S., Dunlop, K. M., Henry, L.-A., and Roberts, M.: Major impacts of

483 climate change on deep-sea benthic ecosystems, Elementa: Science of the Anthropocene, 5, 2017.

484 Thornhill, D. J., Wiley, A. A., Campbell, A. L., Bartol, F. F., Teske, A., Halanych, K. M.: Endosymbionts of

485 *Siboglinum fjordicum* and the phylogeny of bacterial endosymbionts in siboglinidae (Annelida), Biological

486 Bulletin, 214, 135-144, 2008.

487 van Oevelen, D., Bergmann, M., Soetaert, K., Bauerfeind, E., Hasemann, C., Klages, M., Schewe, I., Soltwedel,

488 T., and Budaeva, N. E.: Carbon flows in the benthic food web at the deep-sea observatory HAUSGARTEN

489 (Fram Strait), Deep-Sea Research Part I-Oceanographic Research Papers, 58, 1069-1083, 2011.

490 van Oevelen, D., Soetaert, K., and Heip, C.: Carbon flows in the benthic food web of the Porcupine Abyssal

491 Plain: The (un)importance of labile detritus in supporting microbial and faunal carbon demands, Limnology and

492 Oceanography, 57, 645-664, 2012.

493 Wegener, G., Bausch, M., Holler, T., Thang, N. M., Mollar, X. P., Kellermann, M. Y., Hinrichs, K. U., and

494 Boetius, A.: Assessing sub-seafloor microbial activity by combined stable isotope probing with deuterated water

495 and 13C-bicarbonate, Environmental Microbiology, 14, 1517-1527, 2012.

496 Witte, U., Aberle, N., Sand, M., and Wenzhofer, F.: Rapid response of a deep-sea benthic community to POM

497 enrichment: an *in situ* experimental study, Marine Ecology Progress Series, 251, 27-36, 2003 a.

498 Witte, U., Wenzhofer, F., Sommer, S., Boetius, A., Heinz, P., Aberle, N., Sand, M., Cremer, A., Abraham, W.-

499 R., Jorgensen, B. B., and Pfannkuche, O.: In situ experimental evidence of the fate of a phytodetritus pulse at the

500 abyssal sea floor, Nature, 424, 763-766, 2003 b.

501 Woulds, C., Andersson, J. H., Cowie, G. L., Middelburg, J. J., and Levin, L. A.: The short-term fate of organic

502 carbon in marine sediments: Comparing the Pakistan margin to other regions, Deep Sea Research Part II:

503 Topical Studies in Oceanography, 56, 393-402, 2009.

504    Woulds, C., Bouillon, S., Cowie, G., Drake, E., Middelburg, J. J., and Witte, U.: Patterns of carbon processing

505    at the seafloor: the role of faunal and microbial communities in moderating carbon flows, Biogeosciences, 13, 1-

506    15, 2016.

507    Woulds, C., Cowie, G. L., Levin, L. A., Andersson, J. H., Middelburg, J. J., Vandewiele, S., Lamont, P. A.,

508    Larkin, K. E., Gooday, A. J., Schumacher, S., Whitcraft, C., Jeffreys, R. M., and Schwartz, M. C.: Oxygen as a

509    control on seafloor biological communities and their roles in sedimentary carbon cycling, Limnology and

510    Oceanography, 52, 1698-1709, 2007.

511

| Site | Lat. | Long. | Depth (m) | Temperature | Sediment wt%Corg in 0-1 cm horizon | Macrofaunal Biomass (mg C m$^{-2}$) | Bacterial Biomass (mg C m$^{-2}$) |
|------|------|-------|-----------|-------------|-----------------------------------|-------------------------------------|-----------------------------------|
| Off-Vent | 62.3842 S | 57.2440 W | 1150 | 0 | 1.35 | 1091 | 314±145 |
| Hook Ridge | 62.1924 S | 57.2783 W | 1054 | 1 | 0.97 | 318 | 451±21 |
| Middle Sister | 62.6552 S | 59.0502 W | 1311 | 0 | 1.40 | 374 | 575±394 |

512     **Table 1. Site characteristics, all except bacterial biomass are from Bell et al. (2016).**

513

| Site | Treatment and Replicate | Amount Respired (mg C m$^{-2}$) | Respiration Rate (mg C m$^{-2}$ h$^{-1}$) | Bacterial Uptake (mg C m$^{-2}$) | Macrofaunal Uptake (mg C m$^{-2}$) |
|---|---|---|---|---|---|
| Off-Vent | Algae A | 1.23 | 0.025 | 0.25 | 0.027 |
| Off-Vent | Algae B | 0.75 | 0.015 | 0.77 | 0.034 |
| Off-Vent | Bicarbonate A | N/A | N/A | 0.053 | 0.0009 |
| Off-Vent | Bicarbonate B | N/A | N/A | 0.102 | low |
| Hook Ridge | Algae A | 4.97 | 0.087 | n.d. | 0.033 |
| Hook Ridge | Algae B | 4.06 | 0.071 | 1.25 | 0.003 |
| Hook Ridge | Bicarbonate A | N/A | N/A | n.d. | 0.021 |
| Hook Ridge | Bicarbonate B | N/A | N/A | low | low |
| Middle Sister | Algae A | 7.16 | 0.13 | 1.91 | 0.004 |
| Middle Sister | Algae B | 8.37 | 0.15 | 1.30 | 0.12 |
| Middle Sister | Bicarbonate A | N/A | N/A | 0.00 | 0.003 |
| Middle Sister | Bicarbonate B | N/A | N/A | 0.003* | 0.003 |

514 **Table 2. Amount of C in pools at experiment end, and respiration rates (algae addition experiments only). N/A indicates**

515 **that it was not appropriate to measure respiration in bicarbonate addition experiments, n.d. indicates no data due to**

516 **missing sample, and 'low' indicates unmeasurably low value. The value marked * indicates detectable bacterial $^{13}$C**

517 **uptake, but very close to detection limits, so value to be treated with caution.**

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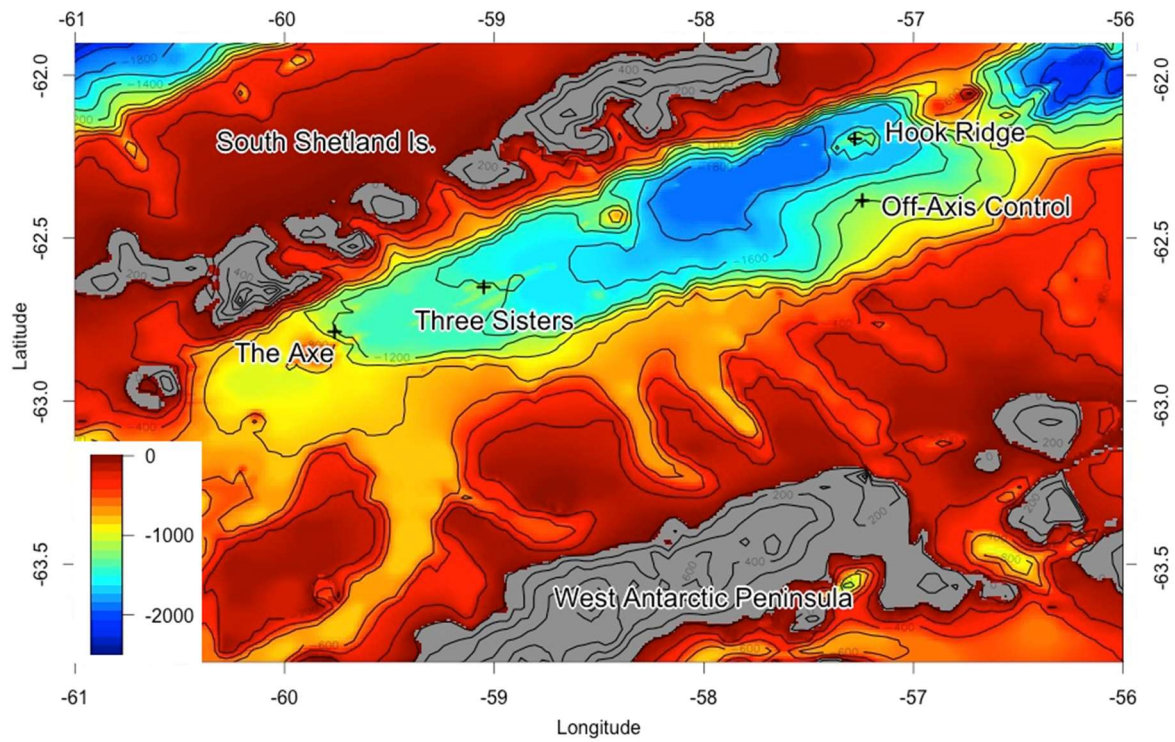
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520 **Figure 1. Map of study sites, adapted from Bell et al. 2016 a. The Off Vent site is marked 'Off-Axis Control', and the**

521 **Middle Sister site is located where 'Three Sisters' is marked. Depths in m.**

522

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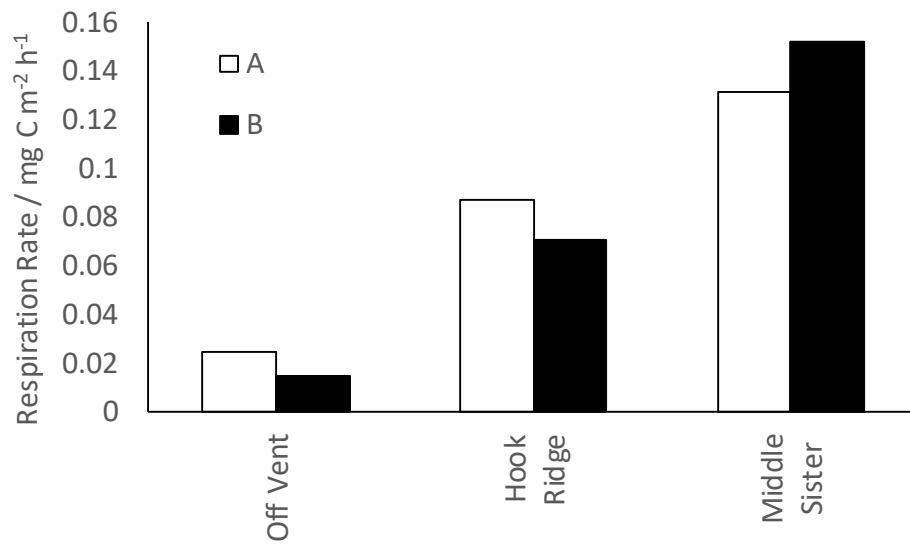
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526 **Figure 2. Respiration rates measured in algae addition experiments. A and B refer to the two replicate cores in each**

527 **experiment.**

528

529

530    A

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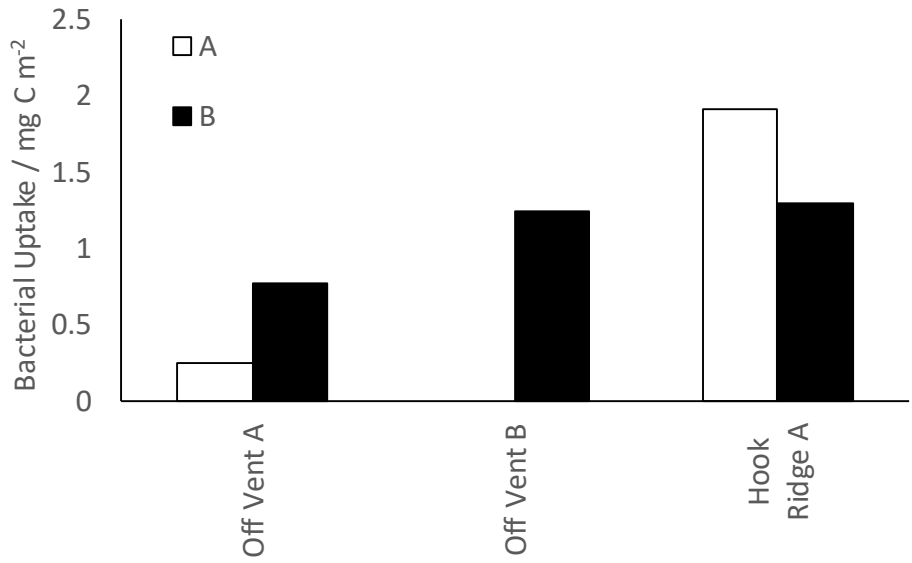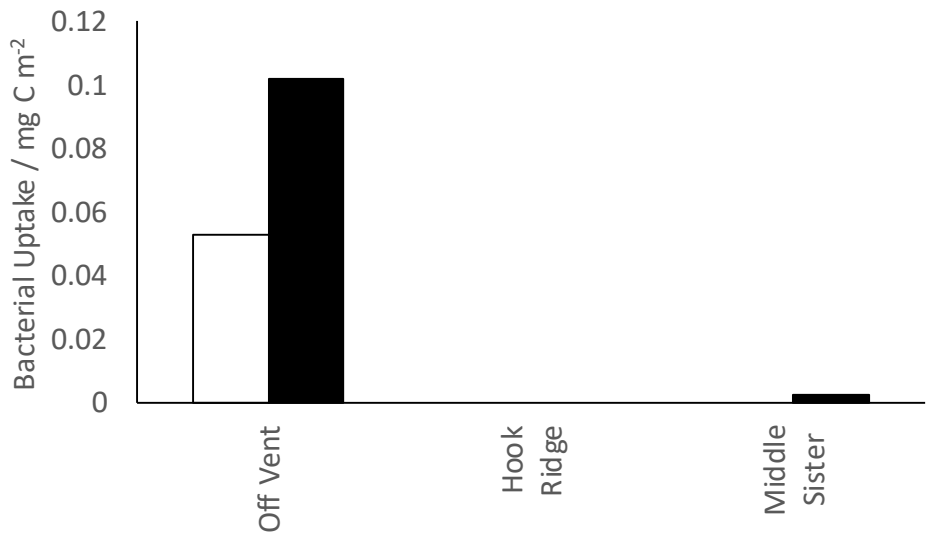
532    B

**Figure 3. Bacterial uptake measured in A) algae addition experiments; B) bicarbonate addition experiments. Uptake**

**was not quantifiable at Hook Ridge B and Middle Sister A, and sample was not available from Hook Ridge A. A and B**

**refer to the two replicate cores in each experiment.**

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537

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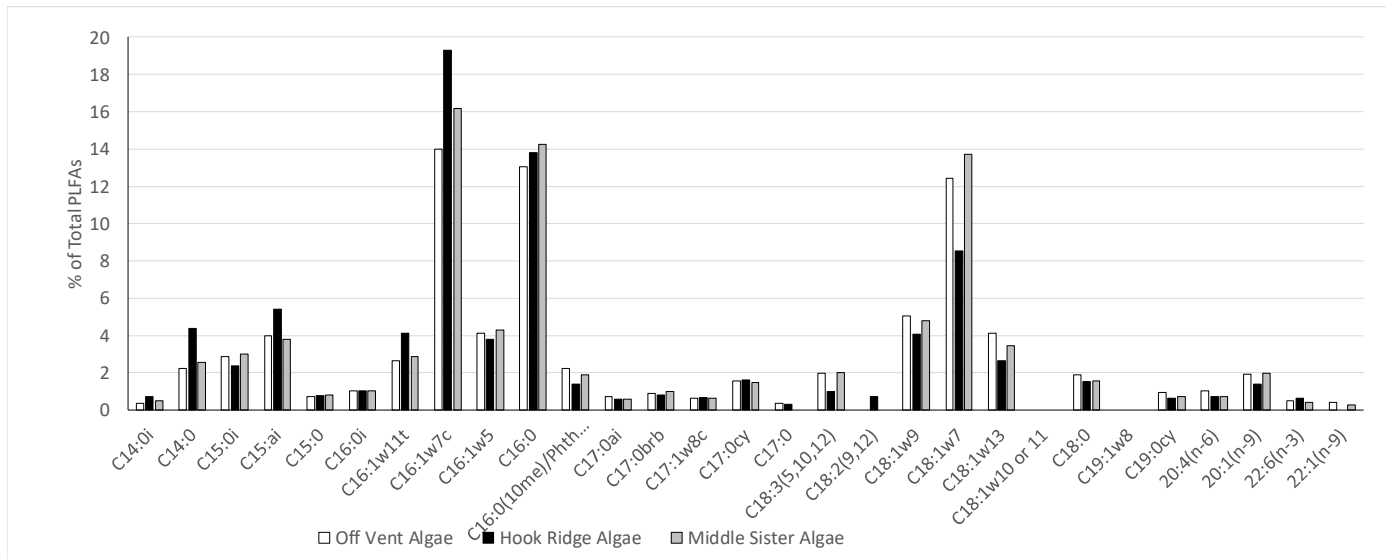
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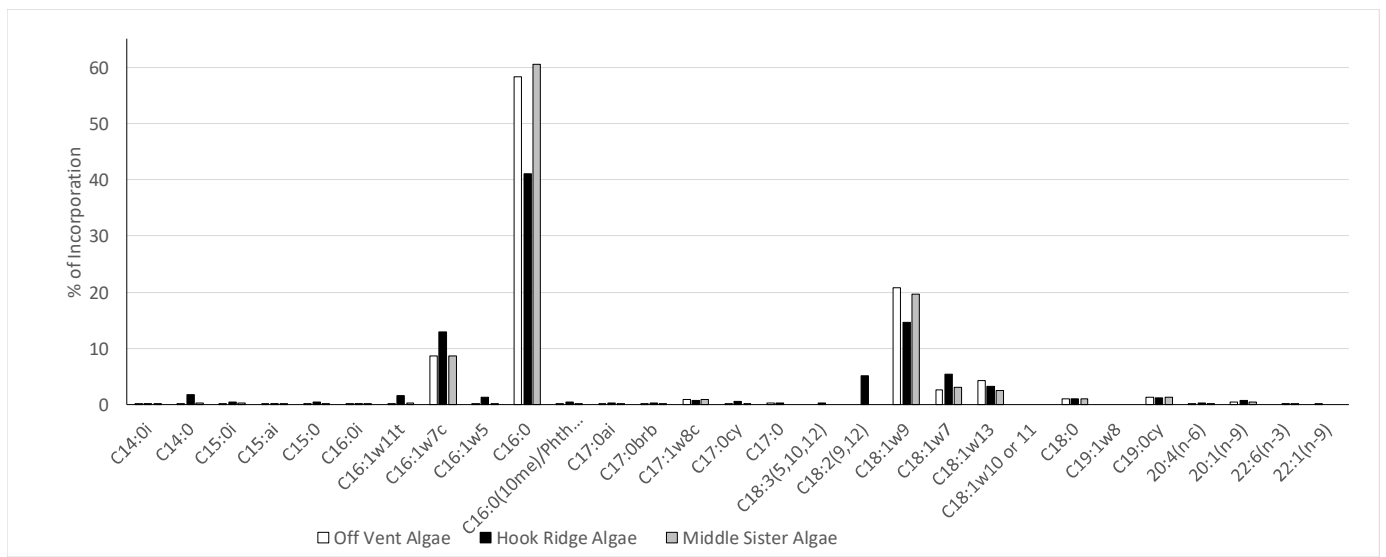
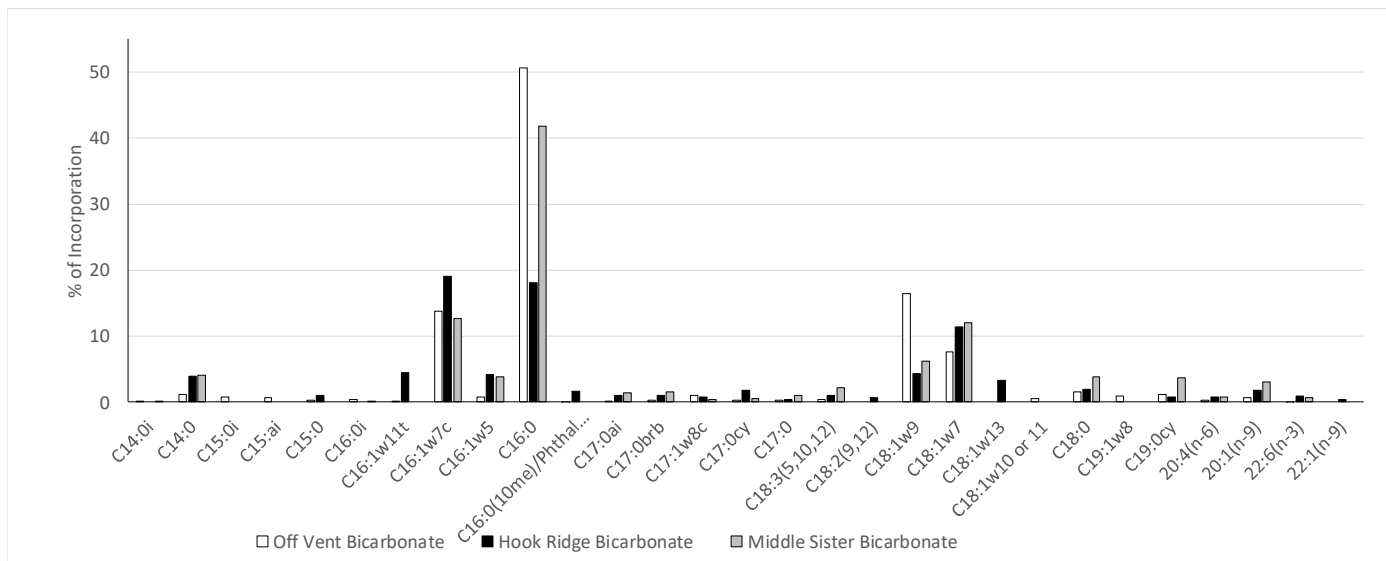

543    B

546 C

547 **Figure 4. Example PLFA suites – each data series is from one sample, as opposed to being an average across two**

548 **replicates. A) PLFA suite as % of total PLFAs in algae addition experiments (figure for bicarbonate addition**

549 **experiments very similar and not shown), B) Composition of $^{13}$C uptake into PLFAs in algae addition experiments, and**

550 **C) Composition of $^{13}$C uptake into PLFAs in bicarbonate addition experiments.**

551

552

553   A

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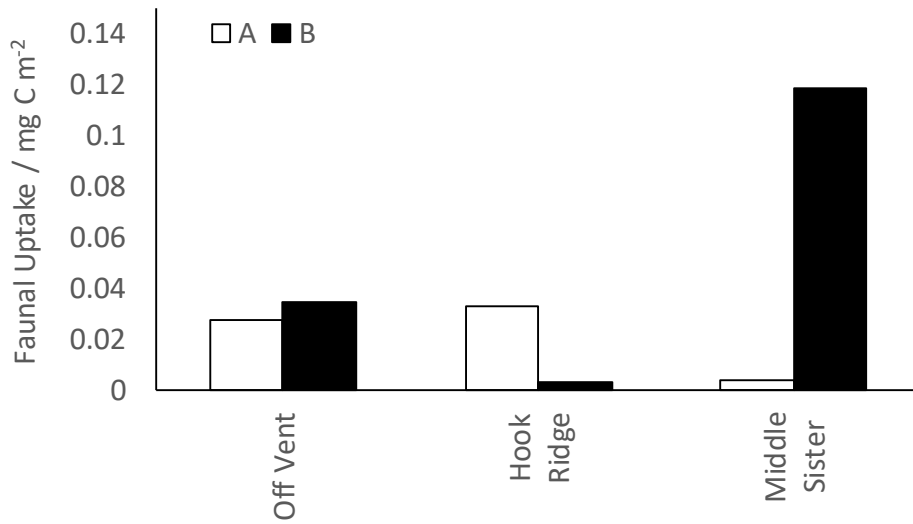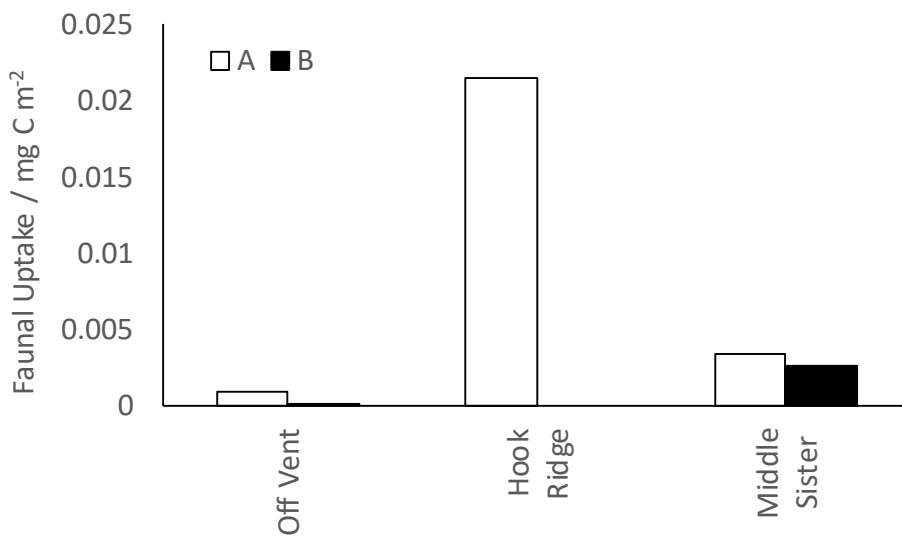
555   B

**Figure 5. Faunal uptake in A) algae addition experiments, and B) bicarbonate addition experiments. A and B refer to the two replicate cores in each experiment.**

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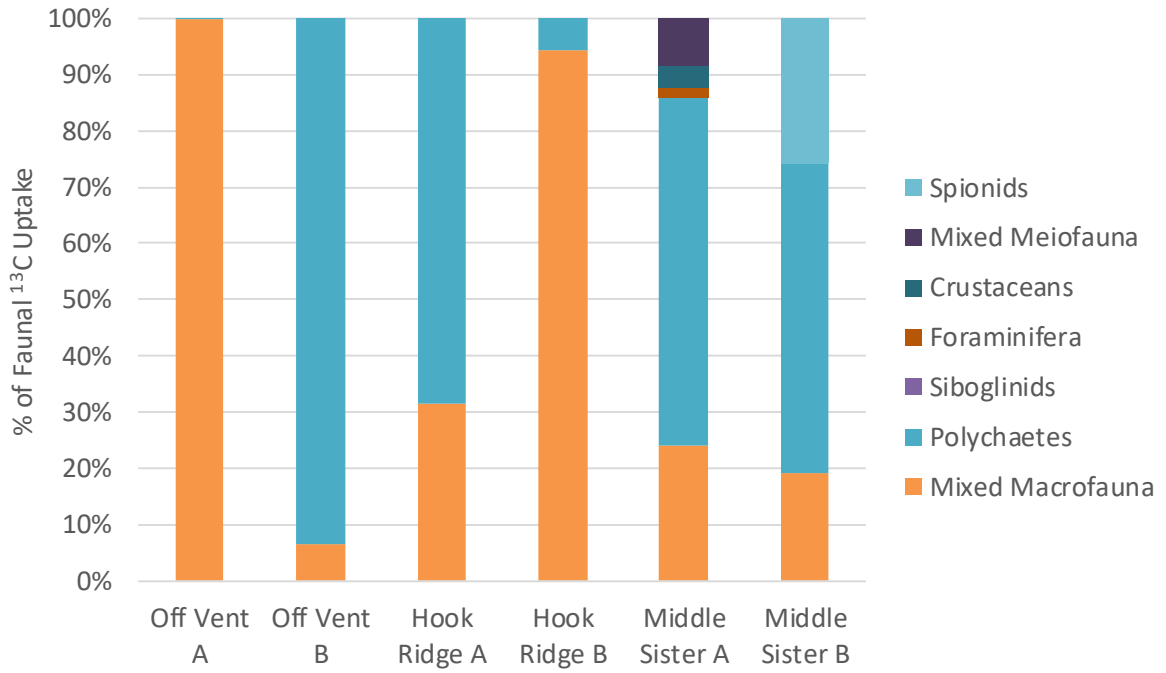
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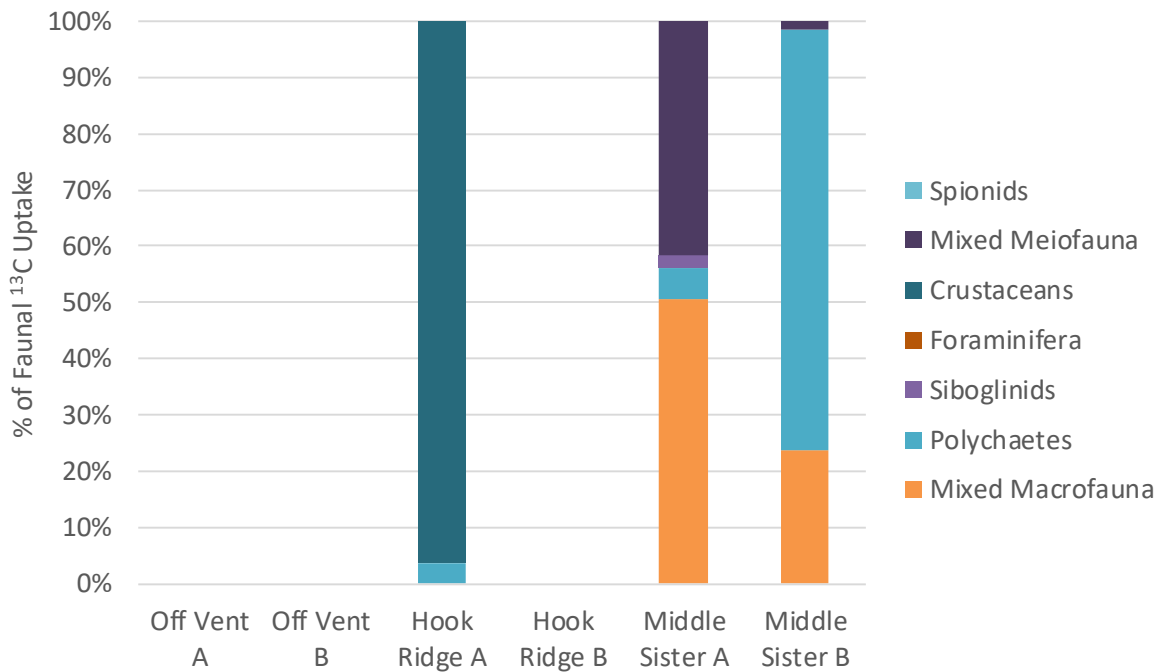
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562    B

563    **Figure 6. Distribution of C uptake amongst taxonomic groups in A) algae addition experiments, and B) bicarbonate**

564    **addition experiments.**

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566

567    A

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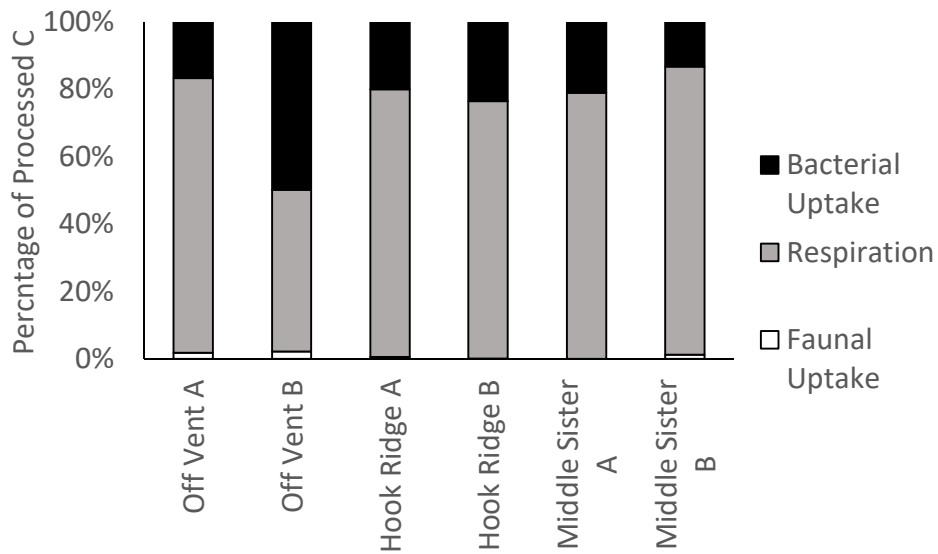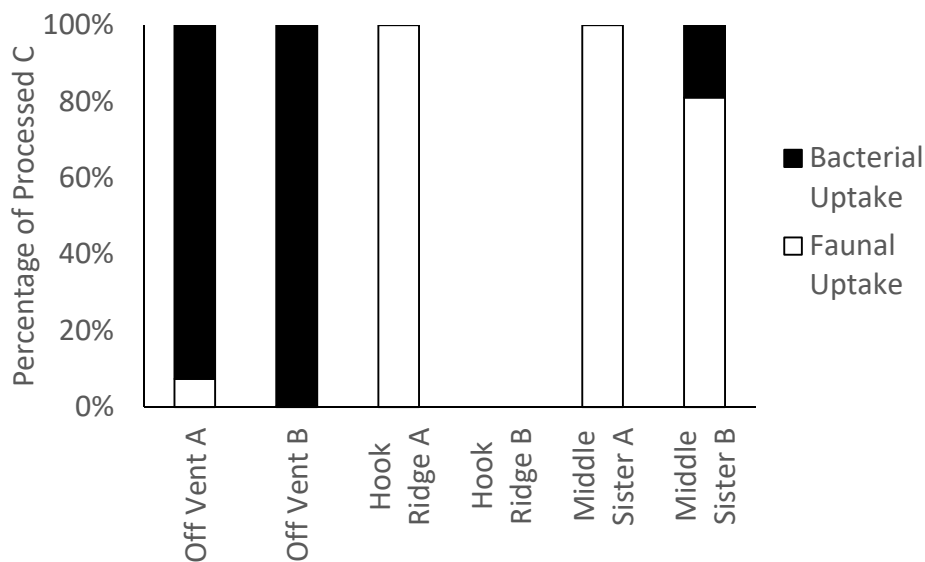
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**Figure 7. Distribution of biologically processed C between processes for A) algae addition experiments, and B)**

**bicarbonate addition experiments. Where data are absent this is a combination of lack of sample, and undetectable**

**faunal $^{13}$C uptake.**

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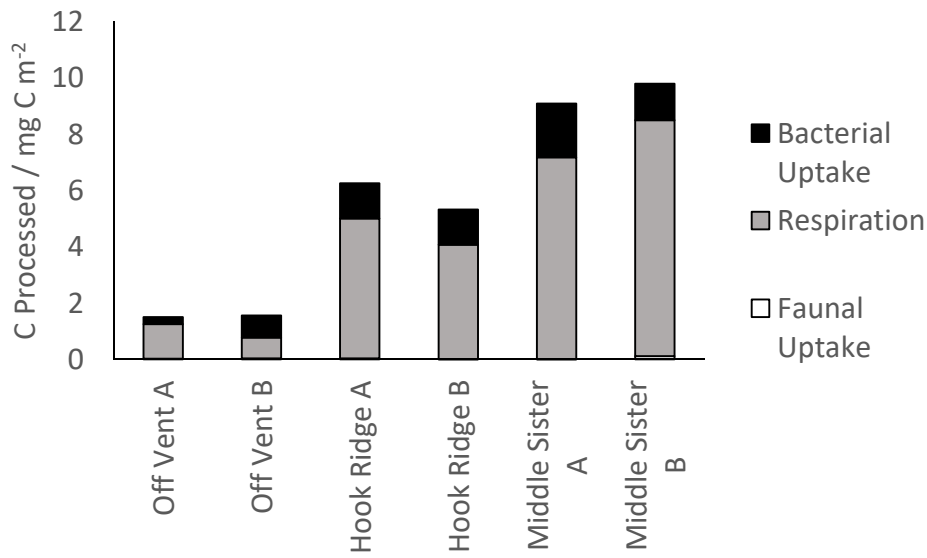
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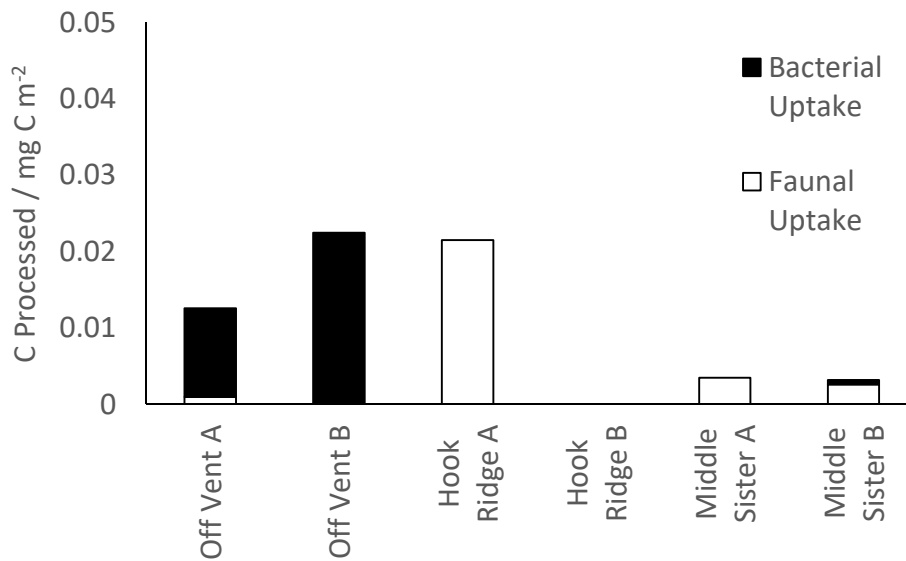
576    A

577

578    B

579    **Figure 8. Total biological C processing during A) algae addition experiments, B) bicarbonate addition experiments.**

580    **Where data are absent this is a combination of lack of sample, and undetectable faunal $^{13}$C uptake.**

581