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Carbon processing by the benthic ecosystem and benthic C fixation in methane rich sediments on the South Georgia margin

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Abstract

As bottom water warms, destabilisation of gas hydrates may increase the extent of methane rich sediments. We present an assessment of organic carbon processing by the benthic community in methane rich sediments, including one of the first investigations of inorganic C fixation in a non-hydrothermal vent setting. This topic was previously poorly studied, and there is much need to fill the gaps in our knowledge of such ecosystems. We hypothesised that benthic C fixation would occur, and that a high biomass macrofaunal community would play a substantial role in organic C cycling.

Experiments were conducted at a 257 m deep site off South Georgia. Sediment cores were amended with ^{13}C and ^{15}N labelled algal detritus, or ^{13}C labelled bicarbonate solution.

In the bicarbonate experiment, labelling of bacteria-specific phospholipid fatty acids provided direct evidence of benthic C fixation, with transfer of fixed-C to macrofauna and DOC. In the algae experiment, macrofauna played an active role in organic carbon cycling. Compared to similar experiments, low temperature supressed the rates of community respiration, and macrofaunal C uptake. While benthic C fixation occurred, the biological processing of organic carbon was dominantly controlled by low temperature and high photic zone productivity.

Keywords: Carbon-cycle, isotope tracer, ^{13}C, macrofauna, South Georgia, seep, chemosynthesis.

Introduction

Understanding the immediate fate of organic carbon (OC) deposited on the seafloor is crucial to our knowledge of both benthic ecosystem function, and sedimentary C sequestration. Carbon cycling in methane rich seafloor environments is of considerable interest, owing to the possibility of increased destabilisation of sub-seafloor gas hydrate deposits under warming climate conditions. This issue is particularly pertinent in shallow, high latitude sediments, where gas hydrate deposits are larger, pressure is minimal, and where sediment warming is most likely to occur on century timescales (Ciais et al., 2013).At the type of methane-rich sites which may result, methane flux can be low enough to be entirely consumed by sedimentary communities, preventing loss to the water column, but still sufficient to influence the composition and function of the resident biota (Bell et al., 2016).

Increasingly, dark fixation of inorganic C is being observed in non-hydrothermal vent or methane seep settings, and may be an important and overlooked aspect of benthic C-cycling (Molari et al., 2013; Boschker et al., 2014; Pruski et al., 2017; Sweetman et al. in press). Methane can support a range of metabolic strategies within the sediment, including methanotrophy, aerobic and anaerobic oxidation of methane (e.g. Treude et al., 2003), and associated sulphide oxidation (Bernardino et al., 2012). Methane derived carbon and has been shown to sustain chemoautotrophy, in some cases resulting in unusually high infaunal density and biomass (Levin and Michener, 2002; Levin et al., 2005; Bernardino et al., 2012). Thus, the occurrence of benthic C fixation and its role in seafloor C-cycling at methane rich and other sites requires considerable investigation.

Isotope tracing experiments (ITEs) have been used in a wide range of benthic environments to investigate carbon processing by benthic ecosystems, and thus the fate of organic Carbon (OC) deposited in the sediment (e.g. Moodley et al., 2002; Witte et al., 2003; Woulds et al., 2009). A comparison of previous experiments (Woulds et al., 2009) shows that in the generally OC poor and low biomass deep-sea, C tends to be dominantly routed towards total community respiration, thus the majority of OC processed by the benthic community in such locations is returned to the water

column as dissolved CO_2. In shallower settings, such as estuaries and fjords, increased OC supply sustains a larger and more active faunal community, and thus a greater proportion of biologically processed OC is routed through the faunal biomass (Woulds et al., 2009). Methane rich sites potentially exhibit benthic C fixation, which may support high biomass faunal communities, which would play a key role is short-term C-cycling. However, while the sources and turnover of OC has been investigated at some methane rich sites (Pozzato et al., 2017; Pruski et al., 2017; Rabouille et al., 2017), they have not previously been the focus of isotope tracing experiments. Here we present the first assessment, using stable isotope tracing, of C-cycling by the benthic ecosystem in methane rich sediments.

The aim of this study was to characterise C-cycling by the benthic ecosystem and quantify process rates in methane rich reducing sediments on the southwest South Georgia shelf. We hypothesised that benthic C fixation would occur, and that a high biomass macrofaunal community related to benthic production would play a substantial role in the immediate fate of deposited organic C (Woulds et al., 2009; Bell et al., 2016).

Methods

Field site

The continental shelf surrounding South Georgia has recently been found to be methane rich, with gas flares identified in fjords on the northern margin (Romer et al., 2014), and sites of weaker methane seepage in the southwest (Bell et al., 2016). Benthic faunal communities from the southwest shelf are reported to be similar to those typically found in reducing sediments, and seep endemic fauna are absent (Bell et al., 2016).

This study was conducted at a site 257 m deep on the southwest margin of South Georgia, at 54.1575° S, 37.9761° W (station JC55 111 in Bell et al., 2016). Surface sediments (0-1 cm) were 1.6% (dry weight) organic C. Oxygen penetrated no deeper than 2 cm into the sediment. Porewater CH_4

concentrations increased continually downcore, but particularly from ~15 cm downwards, at which point S^{2-} also increased. Maximal CH_4 concentrations of ~25 μM were observed at the maximum sampling depth (~27 cm, Bell et al., 2016). Macrofaunal density and biomass were 25,402 ind m^{-2} and 2606 mg C m^{-2} respectively (Bell et al., 2016; Bell, pers. comm.), and the community was dominated by polychaetes (particularly cirratulids), tubificid oligochaetes, and thyasirid bivalves (Bell et al., 2016).

Isotope tracing experiments

The isotope tracing experiments were conducted using replicate 10 cm diameter megacores collected from a single deployment, and treated as follows. Cores subjected to the 'algae' treatment were amended with 100% ^{13}C and ^{15}N labelled *Chlorella* cells (Cambridge Isotope Laboratories) at a dose of 392 mg C m^{-2}, which were allowed to settle onto the sediment surface. The algal dose was chosen to represent a minimal addition to natural present organic C (~0.5% of that present in the surface 1 cm), and is similar to other published experiments (Woulds et al., 2009). Cores subjected to the 'bicarbonate' treatment had a 10 ml solution of 100% ^{13}C labelled $NaHCO_3$ and 100% ^{15}N labelled NH_4Cl injected into the porewater of the surface 5 cm of sediment, to give a final dose of 306 mg C m^{-2}, and 2.52 mg N m^{-2}.

Two cores were used for each treatment. This limited replication was necessary due to the time and cost intensive nature of sample processing and analysis, and is standard for such experiments. Thus, while there is limited scope for statistical analyses, and care must be taken with data interpretation, the replicates do indicate the extent of spatial variability, and in many cases allow firm conclusions to be drawn.

After labelling, cores were closed with caps that allowed stirring of the core top water throughout the experiment such that re-suspension of surface sediment did not occur, and incubated at local bottom temperature 1.2°C for ~60 h. Core top dissolved oxygen concentrations did not decline below ~60 % saturation during incubations. At intervals during the incubation, samples of core-top

water were withdrawn through sampling ports, and the volume was replaced with filtered bottom water. Water samples were preserved for dissolved inorganic carbon (DIC) analysis in 10 ml crimp cap vials, and poisoned with $HgCl_2$.

At the end of the experiment, cores were sectioned at 1 cm intervals to 10 cm depth. Half of each section was frozen, and the other half was preserved in buffered formalin (10% in filtered seawater).

Analysis

Preserved sediment was sieved over a 300 μm mesh, and macrofaunal organisms were extracted under a binocular microscope. They were identified to broad taxonomic groups (family level for some polychaetes), and air dried at 50°C in pre-weighed silver boats. Re-weighed fauna were decarbonated using 0.1 N HCl and air dried again at 50°C. A stronger treatment with 6N HCl was used for organisms with carbonate shells/tests (e.g. bivalves and foraminifera). Fauna were analysed for isotopic signatures using a Flash EA 1112 Series Elemental Analyser connected via a Conflo III to a Delta^{Plus} XP isotope ratio mass spectrometer (all Thermo Finnigan, Bremen). Carbon contents were quantified using the area under the mass spectrometer response curve, standardised using National Institute of Standards and Technology reference material 1547 peach leaves (repeat analysis gave precision ± 0.35 %). Isotopic data were traceable to IAEA reference materials USGS40 and USGS41 (both L-glutamic acid), with a precision ± 0.13 ‰.

Time series core top water samples were analysed for ^{13}C-enrichment of DIC and DIC concentrations, by injecting samples in triplicate on a Thermalox TOC analyser coupled to a Thermo Delta V Advantage IRMS via a Conflo IV interface, using a Thermo TriPlus autosampler. The reaction column was filled with H_3PO_4-coated beads. They were also analysed for ^{13}C in DOC using a method similar to the setup used by De Troyer et al. (2010) whereby a Thermalox TOC analyser was coupled to a Thermo Delta V Advantage isotope ratio mass spectrometer via a custom cryofocusing unit.

Frozen sediment samples were freeze dried and analysed for ^{13}C in phospholipid fatty acids (PLFAs) following a modified Bligh-Dyer extraction (Main et al., 2015). Lipids were extracted in a 1:2:0.8 chloroform:methanol:citrate buffer solution, and separated on ISOLUTE SI SPE columns. Columns were washed with chloroform and acetone, and polar lipids were collected by elution with methanol. These were dried and then derivitised using KOH in methanol, following addition of the C19 internal standard nonadecanoic acid. The reaction was quenched with water and acetic acid, and the organic fraction was extracted in 4:1 isohexane: chloroform. Samples were dried, and taken up in isohexane prior to quantification using gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS). This was performed on a Trace Ultra GC, connected via a GC Combustion III to a Delta V Advantage IRMS (Thermo Finnigan, Bremen). The $\delta^{13}C_{VPDB}$ values of each fatty acid methyl ester were measured with respect to a reference CO_2 gas traceable to IAEA reference material NBS 19 TS-Limestone, with a precision of ± 0.31 ‰, and corrected for the C atom added during derivatization.

Data processing

Respiration rates were calculated using the volume of core tope water, DIC concentration, and isotopic signature to calculate the total amount of ^{13}C DIC in the chamber at each sampling time point. These values were corrected for removal and addition of ^{13}C DIC during the sampling process. Linear regression was then applied to corrected ^{13}C DIC quantities against time (R^2 >0.9, see associated data set for graphs), and the slope of the line gave the respiration rate for each of the algae treatment cores. Respiration rates were not calculated for bicarbonate treatment cores, as their treatment involved the addition of highly ^{13}C labelled DIC to the surface porewater. DOC consumption rates were calculated in the same way, following subtraction of naturally present ^{13}C DOC using data from the unlabelled replacement water.

Transfer of the added ^{13}C and ^{15}N label into fauna was calculated by first correcting for naturally occurring ^{13}C and ^{15}N based on natural isotopic signatures from Bell et al. (2016). Corrected isotopic signatures were then multiplied by the C or N contents of each sample.

Bacterial uptake of ^{13}C was calculated using the concentrations and isotopic signatures (after subtraction of naturally occurring ^{13}C based on results from un-labelled sediment) of four bacteria-specific PLFAs (iso C14:0, iso C15:0, antiso C15:0 and iso C16:0), which are estimated to represent 14% of total bacterial PLFA, which in turn were assumed to constitute 5.6% of total bacterial biomass (Boschker and Middelburg, 2002). In the case of bicarbonate addition, uptake into bacterial biomass was scaled up to account for the fact that the quantity of $NaHCO_3$ added would have resulted in a DIC pool that was 22% ^{13}C labelled.

An attempt to measure bacterial N uptake by tracing ^{15}N in to D-Alanine (Veuger et al. 2005) was made, but was below detection limits, thus the method is not given in full, and data are not presented.

Results

Algae treatment

Algal C was respired to DIC at a rate of 0.11±0.04 mg C m^{-2} h^{-1}, meaning that 6.76±2.34 mg C m^{-2} was respired during the experiment (Table 1, Fig. 1). Total bacterial uptake of added C was 2.51±0.72 mg C m^{-2} (Table 1).

Faunal C uptake was particularly variable between the two replicate cores, ranging over an order of magnitude from 0.45 to 4.44 mg C m^{-2} (Fig. 1). Polychaetes were the most dominant taxonomic group, accounting for between 55% and 97 % of total faunal C uptake (Fig. 2). Bivalves also played a role in the A core (9 % of faunal C uptake), as did nematodes (5 % of faunal C uptake), and in the B core crustaceans (amphipods and isopods) accounted for nearly 2 % of faunal C uptake. To achieve sufficient sample masses, it was often necessary to pool multiple individual specimens, resulting in

the category 'mixed metazoans', also accounting for significant proportions of total faunal C uptake (30 % and 1.5 % in the A and B cores respectively). It should be noted that some of the mixed metazoan C uptake will have been by individuals belonging to the other categories, but which had to be preserved and analysed along with other taxonomic groups.

Faunal N uptake was similarly spatially variable, ranging from 0.06 mg N m^{-2} in the B core to 1.29 mg n m^{-2} in the A core (Fig. 3). The distribution of N uptake amongst taxonomic groups was similar as for C uptake, although in both cores the polychaetes accounted for less N uptake than they had for C uptake (by ~4 %), and this was instead mostly accounted for by the mixed macrofauna (Fig. 3).

The mean molar C/N ratio of uptake into fauna was 4.7±2.7, and showed a relatively wide range between 0.3 and 9.9. These values showed some patterning with taxon, with bivalves tending to have a C/N ratio of ~4-5, and polychaetes tending to show ratios of either 1-3 or 7-8.

The amount of ^{13}C DOC derived from the added algae present in overlying water tended to decrease through the experiment, due to initial release of DOC from the freeze-dried algal cells and subsequent uptake by the microbial community (Fig. 4). The mean DOC consumption rate was 0.14±0.02 mg C m^{-2} h^{-1} (Table 1). Despite overall DOC consumption however, the isotopic signature of DOC in the overlying water increased throughout the experiment up to a maximum of 986 ‰ in core B.

Respiration was the dominant fate of biologically processed added C, accounting for 53-68 %. The fate of the remainder varied between the two replicates, with macrofaunal uptake being particularly important in one (28 %), and bacterial uptake being the second most important fate (27 %) in the other (Fig. 1B).

Bicarbonate treatment

Measurable isotopic enrichment of all 4 bacterial specific PLFAs was only present in the B core, where a value of 0.03 mg C m^{-2} bacterial C uptake was measured (Table 1, Fig. 5). The lower value

measured for the A core in figure 5 should be treated with caution, as one of the PLFAs was isotopically lighter than in the natural background sediment, and two were sufficiently close to the natural background value to be within the margin of error.

Almost all faunal specimens from the bicarbonate treatment had isotopic signatures that were indistinguishable from natural background values (Bell et al., 2016). Three faunal samples (two of syllid polychaetes, and one of mixed polychaetes) were exceptions to this trend and showed $\delta^{13}C$ values that were 1-3 ‰ higher than natural background values (data not shown). Thus although the rate of transfer of bacterial C into the macrofauna could not be confidently quantified, the data nonetheless showed that in some cases it had occurred.

Isotopic signatures of DOC were similarly close to natural background values as for other C pools. The baseline value was -26.6 ‰, and a $\delta^{13}C$ DOC value as high as -20.6 ‰ was measured at the end of the incubation in the B core. Therefore there is evidence for cycling of chemosynthetically produced OC into the DOC pool, but this remains a tentative result.

Discussion

Benthic OC production and trophic support

In the bicarbonate experiment we directly observed incorporation of bicarbonate ^{13}C into bacterial biomass, and subsequent transfer into faunal biomass and the DOC pool. In one of the two replicate cores that received bicarbonate addition, uptake of inorganic C into the bacterial biomass was detected (Table 1, Fig. 5). Although rates were close to detection limits, this is direct evidence of benthic C fixation occurring in methane rich sediments on the South Georgia margin. The most likely pathway for the observed benthic generation of OC is sulphide oxidation, and this conclusion is supported to some extent by the PLFA suite found in unlabelled sediment at the site (Bell et al., 2016, supplementary data). These data showed that C16:1ω7 and C18:1ω7, which have been associated with sulphur oxidising chemoautotrophs (Guezennec and Fiala-Medioni, 1996; Pond et

al., 1998; Yamanaka and Sakata, 2004; Boschker et al., 2014), together constituted a reasonably high 28 % of total PLFAs. Further, although the observed rates of bicarbonate assimilation were very low, this may be due to the suite of compounds used in the calculation. The four generic bacterial PLFAs used (i-C14:0, i-C15:0, ai-C15:0 and i-C16:0) each accounted for a maximum of ~0.5 % of total ^{13}C incorporation into PLFAs. In contrast, the two PLFAs which are often associated with sulphur oxidation (C16:1ω7 and C18:1ω7; Guezennec and Fiala-Medioni, 1996; Pond et al., 1998; Yamanaka and Sakata, 2004; Boschker et al., 2014) together accounted for 13.8 % and 46.7 % of total incorporation of ^{13}C into PLFAs in the A and B replicate cores respectively (Table 3). Thus the apparently low C fixation rates may to some extent be an artefact of the established transfer function not being well suited to a chemosynthetic setting. They could also indicate that incorporation of ^{13}C from DIC into organic compounds was via anaplerotic carboxylation. However, there is potential for this in all heterotrophs, therefore we suggest that it would not produce the observed pattern of labelling amongst the PLFAs, for which ^{13}C incorporation was focussed on compounds associated with suphide oxidisers.

Bell et al. (2016) suggested a limited amount of methane derived C (MDC) was incorporated into the food web, based on low faunal $\delta^{13}C$ values, which may be indicative of some dietary contribution from MDC, and low faunal $\delta^{34}S$ values, which are indicative of sulphate reduction associated with AOM. Further, they observed dolomite and aragonite structures consistently precipitated onto shells of the thyasirid bivalve *Axinulus antarcticus*, which were likely authigenic, and thus indicative of AOM. However, in both the previous study and this one, PLFAs normally associated with methanotrophs (C16:1ω6, C16:1ω8, C18:1ω6 and C18:1ω8; Guezennec and Fiala-Medioni, 1996; Pond et al., 1998; Zhang et al., 2005) were not detected (Table 3; Bell et al., 2016, supplementary data). In the future, these findings could be expanded upon using experimental addition of ^{13}C labelled methane. We suggest that for best results these would need to be run for 1-2 weeks and include observations down to 10-20 cm depth, where most porewater methane was apparently consumed (Bell et al., 2016).

Interestingly, the taxa which showed natural isotopic signatures indicative of some MDC contribution to their diets included syllids (Bell et al., 2016), which also accounted for 2 out of the 3 faunal specimens from the bicarbonate treatment which were measurably ^{13}C enriched compared to the natural background value. Thus, the two studies are consistent in suggesting that there may have been inclusion of chemosynthetic C in the diets of syllids.

Both this study and that by Bell et al. (2016) indicate the occurrence of benthic C fixation, through two different pathways, and in both cases it was a relatively minor process. This is consistent with the porewater geochemistry and natural faunal ^{13}C isotopic signatures published by Bell et al. (2016), which showed that the dominant C source to the benthic food web was from surface derived photosynthetic material. However, other studies have shown that benthic C fixation, including use of methane derived C, can be more important processes under different conditions. On the Congo fan a high input of terrestrial OC in turbidites sustains a high biomass macrofaunal community, sediment community oxygen uptake and dissolved inorganic C fluxes (Rabouille et al., 2017). That C is re-mineralised, fixed once more into biomass through anaerobic oxidation of methane, and transferred to the macrofauna (Pruski et al., 2017). In this case the methane flux is also sufficient to sustain Vesicomyid bivalves and microbial mats, which did not occur at our study site (Pruski et al., 2017; Rabouille et al., 2017). Further, Sweetman et al. (in press) observed bacterial assimilation of dissolved inorganic C in the eastern equatorial Pacific at rates similar to that of bacterial assimilation of C from organic detritus. Therefore we suggest that benthic C fixation is a relatively widespread and important process, and can play a greater role in the benthic C cycle than we observed.

Macrofaunal OC Uptake

In the algae experiment, metazoan macrofaunal uptake was responsible for up to ~30% of the total biological processing of the added C, and this substantial role was in line with our hypothesis. Other sites which have shown this biological C processing pattern include estuarine and shelf sea sites and sites of a similar depth in the Arabian Sea oxygen minimum zone (Woulds et al., 2009 and references

therein), all of which had relatively OC rich sediments compared to continental margin and deep-sea sites. Thus the active role of macrofaunal in ^{13}C processing off South Georgia is likely to be attributable to the relatively high concentration of organic C in the surface sediments (1.6 wt %), supporting a high biomass macrofaunal community (Table 2). However, the high concentration of OC in surface sediments is attributable to productivity in the photic zone, not benthic primary production, as hypothesised (Bell et al., 2016).

It is worth noting that the macrofaunal community biomass was markedly higher than any previously studied sites which have shown similar macrofaunal C uptake rates (Table 2). For example, in the Sognefjord, where macrofaunal biomass was an order of magnitude lower than on the South Georgia margin, Witte et al. (2003a) observed a mean macrofaunal uptake rate of 0.158 mg C m^{-2} h^{-1}, more than twice the maximum of only 0.073 mg C m^{-2} h^{-1} off South Georgia (Table 2). In contrast, sites that showed similar macrofaunal uptake rates to our study, included the NE Atlantic (2170m, Moodley et al., 2002) and the Porcupine Abyssal Plain (~4800m, Witte et al., 2003b). Both of these sites had macrofaunal biomasses that were an order of magnitude lower than off South Georgia (Table 2). Since faunal uptake is usually strongly related to biomass (e.g. Woulds et al., 2007), the faunal biomass at South Georgia might have led to an expectation of much greater uptake of added C by the macrofauna. The low temperature of the South Georgia site (1.2°C compared to 7°C in the Sognefjord) may have contributed to this observation of comparatively low faunal activity.

The molar C/N ratio of the added algae was ~7.1, therefore the majority of fauna (mean molar C/N 4.7±2.7) showed a de-coupling of C and N. The lower C/N ratios of labelled material in the faunal pool indicates preferential retention of algal N versus preferential metabolism and loss of algal C. In a few instances this pattern was reversed, with single samples of maldanid, ampharetid and cirratulid polychaetes, as well as 2 samples of mixed polychaetes (largely unidentified) showing C/N ratios higher (~8-9) than the added algae. In general, the C/N ratio of material taken up from the added algae was similar to the C/N ratio of natural faunal tissues (mean 4.8±0.4), with no systematic

differences between these two parameters. Therefore the relative retention of algal C and N may be driven by organism needs, as indicated by their bulk composition (Hunter et al., 2012). The selective retention of algal N over C is consistent with observations of meiofauna (although not macrofauna) in sandy subtidal sediments, and in this case it was attributed to different feeding strategies (targeted grazing upon algae by meiofauna as opposed to bulk deposit feeding by macrofauna). Further, in the Whittard Canyon, Hunter et al. (2013) observed strong demand for N over C, even compared to the biomass composition of the organisms concerned, and strong preferential uptake by macrofauna of macroalgal N compared to macroalgal C was observed in sandy intertidal sediments (Rossi, 2007). The preferential retention of algal N seen here contrasts with C and N decoupling seen in Arabian Sea oxygen minimum zone macrofauna, which showed uptake of ^{13}C and ^{15}N with a ratio of between 10 and 60, from algae with a C/N ratio of 4.04 (Hunter et al., 2012). In that case, the retention of algal ^{13}C was hypothesised to be due to a build up in tissues of lactate from respiration in low oxygen conditions. Indeed, biomass-specific $^{13}C/^{15}N$ ratios in the same study also showed the preference for retention of algal N observed here.

Dissolved Organic Carbon

This study is one of only a few to have quantified the role of DOC in a seafloor isotope tracing experiment, and to our knowledge the first to present direct measurements. This showed both a net consumption of the initial pulse of added DOC from the source algae (Fig.4 a, b), and also continual generation of labelled DOC throughout the experiment (Fig.4 c, d). This reflects substantial cycling of ^{13}C between the bacterial and DOC pools, which has previously been identified using linear inverse modelling of isotope tracer experiments and natural isotopic data (Van Oevelen et al., 2006; Gontikkaki et al., 2011; Bell et al., 2017). Notably, the rate of labelled DOC consumption was of a similar magnitude to respiration measured as labelled DIC production (Table 1). This is in line with results from another cold adapted site, at 1080 m depth in the Faero-Shetland channel, where Gontikakki et al. (2011) found that the DOC flux out of the sediment was equivalent to 39% of total

sediment community oxygen consumption (a measure of respiration). They noted this was higher than the typical value for continental margins (10%, Burdige et al., 1999), but that similarly high percentages had also been observed in the NE Atlantic and Arabian Sea. In contrast, an experiment using intertidal sandy sediment found that very little ^{13}C fixed by microphytobenthos was lost to the water column as DOC, and that the DOC was presumably being bound to the sediment (Cook et al., 2007). Therefore, DOC production and consumption is undoubtedly an important aspect of sedimentary short-term C-cycling, however it appears to be particularly variable between sites. Further study is warranted to determine the factors responsible for this variability.

Respiration and Bacterial Uptake

Sediment community respiration rates are strongly controlled by temperature (e.g. Moodley et al., 2005; Woulds et al., 2016). Therefore, it is unsurprising that the respiration rates observed off South Georgia were most similar to those reported from previous experiments in relatively cold settings (Table 2), such as at 2170 m depth in the NE Atlantic (Moodley et al., 2002), and at 4800m depth on the Porcupine Abyssal Plain (Witte et al., 2003b). The respiration rates reported here are some of the lowest seen in this kind of ITE, since they are from the coldest experiment reported so far. Other experiments showing similar temperatures and respiration were from much deeper sites, whereas the low temperature off South Georgia was due to its high latitude. These low respiration rates may contribute to enhanced C storage in South Georgia margin sediments, and an abundant food supply for the benthic community.

Bacterial uptake rates were comparable with those at a 1540 m deep site in the Cretan Sea (Buhring et al., 2006), and a 2170 m deep NE Atlantic site (Moodley et al., 2002). In the case of the Cretan Sea this corresponds to similar bacterial biomass (Table 2), however the NE Atlantic site showed similar bacterial C uptake rate despite showing bacterial biomass that was lower by two orders of magnitude. Similarly, the Porcupine Abyssal Plain showed much greater bacterial biomass, but much lower bacterial C uptake (Table 2). These differences are characteristic of the fact that bacterial

biomass and C processing are poorly coupled, since large fractions of bacterial biomass are often inactive, and bacterial growth efficiency (the balance between biomass production and respiration) is highly variable in space and time (Del Giorgio and Cole, 1998).

Conclusions

In summary, the fate of freshly deposited OC on the South Georgia margin is controlled by low bottom water temperature combined with high photic zone productivity. While benthic C fixation does occur, the extent of methane enrichment observed off South Georgia did not appear to have a major bearing on nutrition of the benthos or C-cycling.

The following specific conclusions were reached:

- Direct evidence was found for benthic C-fixation, and transfer of fixed C to the macrofauna and the DOC pool, although rates were very low.
- Macrofauna played an active role in benthic ecosystem carbon processing, which is attributable to the high biomass macrofaunal community supported by OC rich sediments. This is likely to be derived principally from high productivity in the photic zone, rather than to benthic primary production.
- Low bottom water temperatures on the South Georgia margin appeared to supress the rates of respiration and macrofaunal uptake of added ^{13}C compared to similar ITEs conducted elsewhere.
- In common with other sites, macrofaunal uptake and retention of C and N were decoupled, with a tendency for macrofaunal to preferentially retain N.
- Both uptake and production of DOC were notable processes, deserving further study.

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Author Contributions

CW designed and conducted the experiments with assistance from AG, and wrote the manuscript. Sample processing and analyses were conducted by CW, JB, SB and LB, and all co-authors commented on the manuscript.

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Treatment	Core	Respiration Rate (mg C m^{-2} h^{-1})	Total respired C (mg C m^{-2})	Bacterial Uptake (mg C m^{-2})	Faunal Uptake (mg C m^{-2})	DOC Consumption Rate (mg C m^{-2})
Algae	A	0.14	8.41	3.02	4.44	0.12
Algae	B	0.09	5.11	2.00	0.45	0.15
Bicarbonate	A	n/a	n/a	0.0033*	0.0009	n/a
Bicarbonate	B	n/a	n/a	0.030	0.0029	n/a

Table 1. Quantities of added C processed to carbon pools, and respiration rates. * indicates data to be treated with caution, as value was close to detection limit.

Source	Site	Depth (m)	Temperature (°C)	Macrofaunal Biomass (g C m^{-2})	Bacterial Biomass (g C m^{-2})	Respiration Rate (mg c m^{-2} h^{-1})	Bacterial Uptake Rate (mg c m^{-2} h^{-1})	Macrofaunal Uptake Rate (mg c m^{-2} h^{-1})
This Study	South Georgia A	257	1.2	2.6	0.60	0.14	0.050	0.073
This Study	South Georgia B	257	1.2	2.6	0.51	0.09	0.035	0.008
Buhrin et al., 2006	Cretan Sea	1540	13	0.06	0.4	0.042	0.044	n/a
Witte et al., 2003b	Porcupine Abyssal Plain	4800	n/a	0.12	2.5	0.167	0.00	0.058
Moodley et al., 2002	NE Atlantic	2170	3.6	0.04	0.002	0.083	0.039	0.006

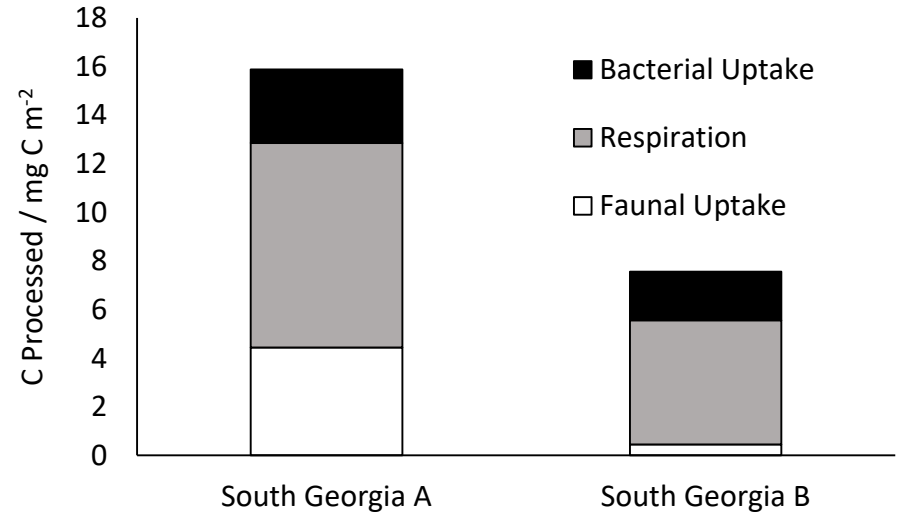
Witte et al., 2003a	Sognefjord	1265	7	0.25	8.5	0.539	0.083	0.158

Table 2. Biological C processing rates in algae addition experiments from this study, and other studies showing comparable rates.

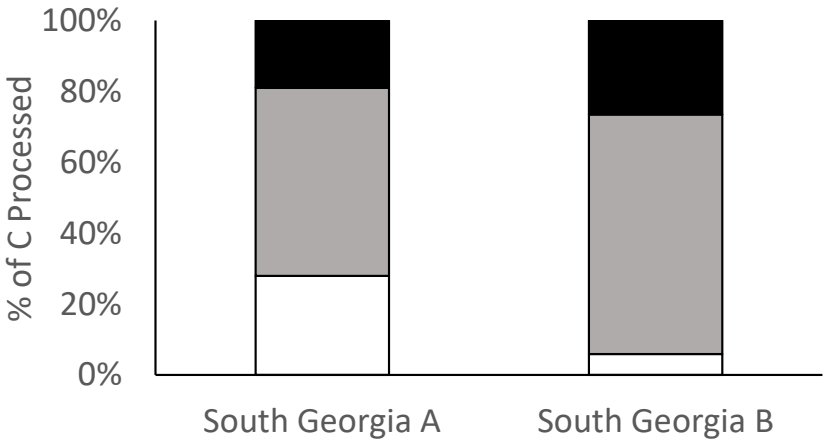
	Algae A		**Algae B**		**Bicarb A**		**Bicarb B**	
Compound	**% Conc.**	**% Incorp.**	**% Conc.**	**% Incorp.**	**% Conc.**	**% Incorp.**	**% Conc.**	**% Incorp.**
C14:0i	0.32	0.01	0.37	0.01	0.30	0.02	0.37	0.00
C14:0	4.29	0.40	3.28	0.31	3.36	0.45	4.86	3.53
C15:0i	2.43	0.57	2.41	0.35	2.17	0.07	2.81	0.50
C15:ai	4.46	0.22	4.34	0.14	4.21	0.00	4.53	0.51
C15:0	1.36	0.17	1.09	0.14	1.16	0.00	1.11	0.34
C16:1i	0.00	0.00	0.10	0.03	0.00	0.00	0.00	0.00
C16:1w11c	0.63	0.00	0.68	0.00	0.75	0.00	0.66	0.23
C16:0i	0.90	0.10	0.90	0.09	0.89	0.00	0.89	0.09
C16:1w11t	2.55	0.27	0.13	0.00	2.14	0.00	3.06	1.43
C16:1w7c	17.12	7.90	16.36	7.57	15.52	9.03	16.56	31.20
C16:1w5	3.48	0.01	3.01	0.03	3.05	0.82	3.22	3.85
C16:0	19.79	61.58	17.81	58.71	16.60	12.83	16.74	28.38
C16:0(10me)/ Phthalate	1.08	0.05	1.22	0.00	1.48	0.23	1.30	2.68
C17:0i	0.00	0.00	0.40	0.00	0.20	0.05	0.00	0.00
C17:0ai	0.77	0.10	0.55	0.06	0.81	0.36	0.58	0.00
C17:0brb	0.73	0.03	0.54	0.00	0.87	0.80	0.74	0.34
C17:1w8c	0.80	0.76	0.97	0.72	0.75	0.00	0.54	0.00
C17:0cy	1.43	0.07	1.05	0.00	1.28	0.48	0.76	0.00
C17:0	0.88	0.38	0.63	0.30	0.98	0.98	0.74	1.26
C18:3(5,10,12)	0.76	0.00	1.32	0.06	0.65	0.84	0.79	1.02
C18:2(9,12)	0.84	3.57	0.74	3.96	0.87	0.75	0.69	0.00
C18:1w9	5.54	18.29	5.92	19.36	4.78	3.63	4.83	3.84

C18:1w7	12.09	3.42	14.48	4.46	13.54	4.74	13.66	15.31
C18:1w13	0.38	0.00	0.00	0.00	0.69	61.32	0.00	0.00
C18:1w10 or 11	1.93	0.59	3.24	0.57	3.07	0.00	3.23	0.00
C18:0	3.06	1.13	3.23	1.13	4.20	0.58	3.47	1.70
C19:1w8	0.00	0.00	1.02	0.09	0.45	0.62	1.76	0.93
C19:0cy	0.00	0.00	0.26	1.37	0.00	0.00	0.16	0.98
C19:0	9.54	0.03	10.25	0.00	10.26	0.20	8.21	0.00
20:5(n-3)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20:1(n-9)	2.02	0.33	2.60	0.46	2.73	0.60	2.93	1.35
22:6(n-3)	0.34	0.00	0.37	0.00	0.50	0.49	0.13	0.46
22:1(n-9)	0.49	0.03	0.55	0.11	1.56	0.00	0.21	0.00
24:1(n-9)	0.00	0.00	0.19	0.00	0.15	0.11	0.11	0.07

Table 3. The percentage contribution of each PLFA to total PLFA concentration, and total incorporation of ^{13}C into PLFAs in each experimental core.



A



B

Figure 1. Amount of C processed by the benthic community in the algae experiment with indication of the C pools from which it was recovered A) absolute quantities, and B) as percentages of total biological C processing.

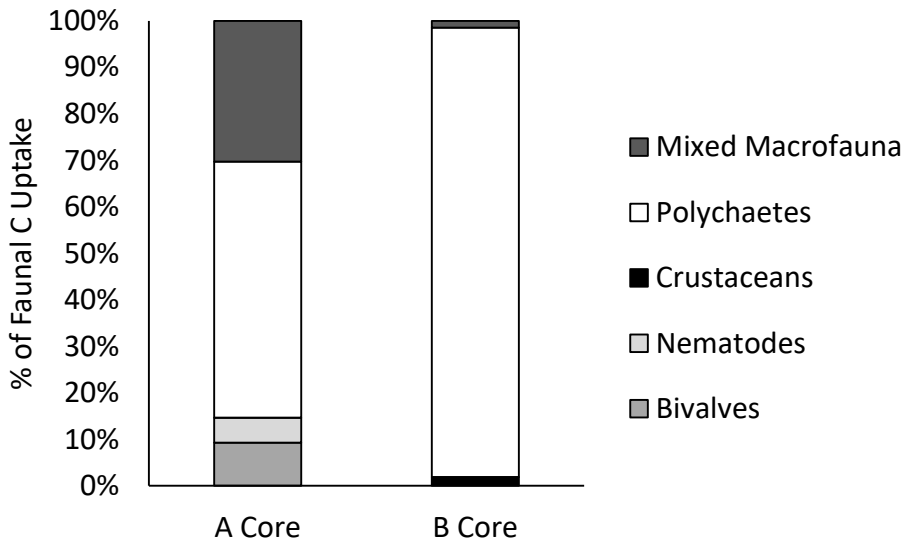
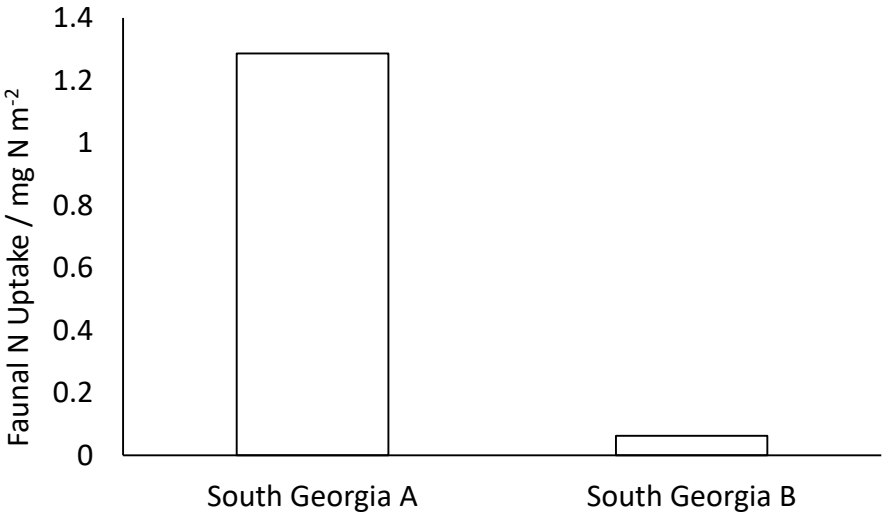


Figure 2. Distribution of faunal C uptake between taxonomic groups.



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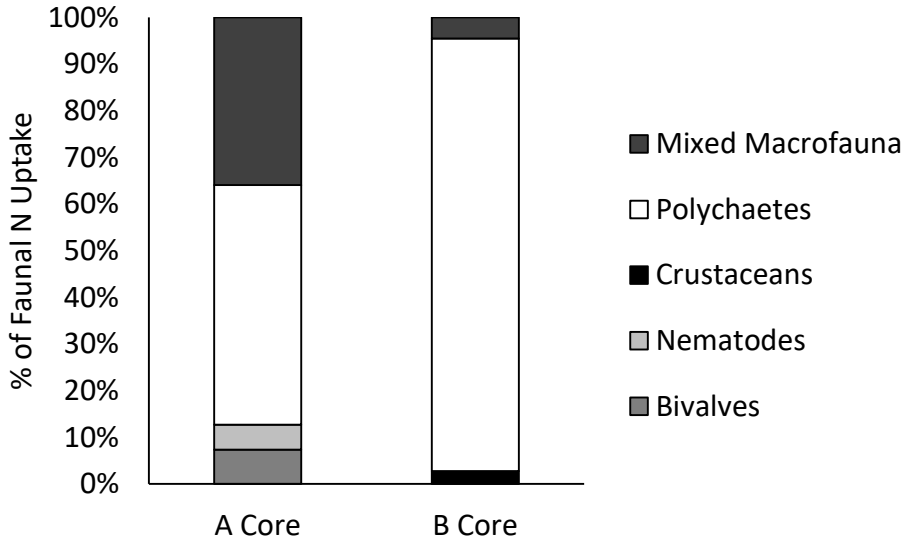
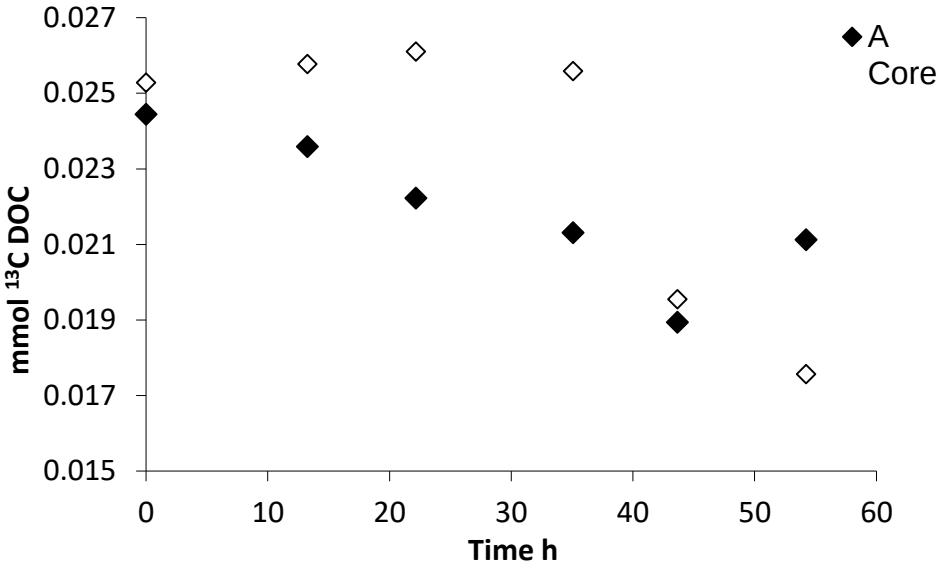


B

Figure 3. A) Faunal N uptake, and B) its distribution amongst taxonomic groups.



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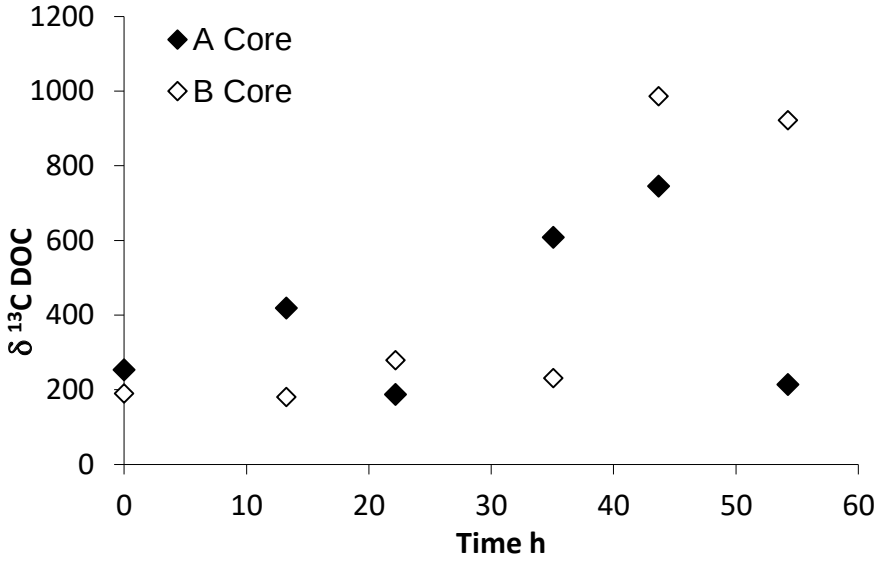

B

Figure 4. ^{13}C DOC quantities in overlying water (A), and isotopic labelling of DOC (B) in the algae experiment.

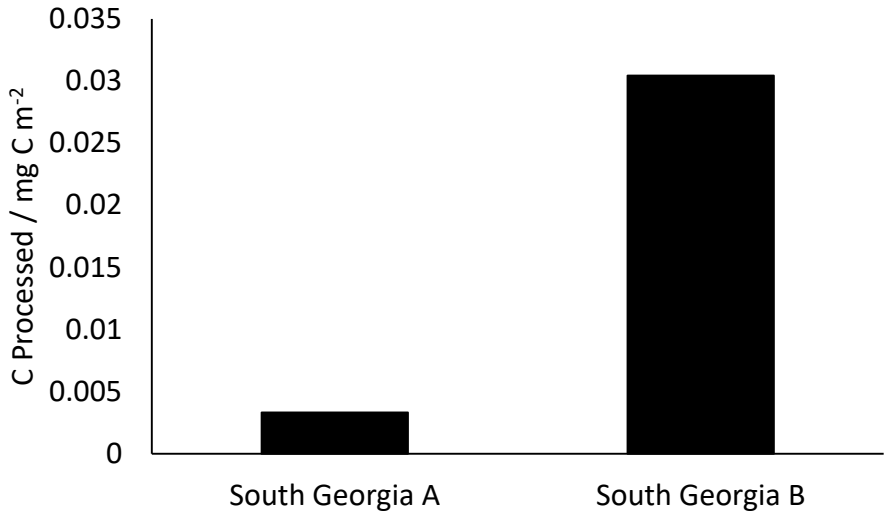

Figure 5. Bacterial C uptake in the bicarbonate addition treatment. Note that values for core A should be regarded with caution as they are very close to the detection limit.