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Macroalgae degradation promotes microbial iron reduction via electron shuttling in coastal Antarctic sediments

David A. Aromokeye [a,b,1,*], Graciana Willis-Poratti [a,c,d,1], Lea C. Wunder [a,e], Xiuran Yin [a,b], Jenny Wendt [b], Tim Richter-Heitmann [a,b], Susann Henkel [b,f], Susana Vázquez [h], Marcus Elvert [b,g], Walter Mac Cormack [c,h], Michael W. Friedrich [a,b,*]

[a] Microbial Ecophysiology Group, Faculty of Biology/Chemistry, University of Bremen, Bremen, Germany
[b] MARUM – Center for Marine Environmental Sciences, University of Bremen, Bremen, Germany
[c] Instituto Antártico Argentino, San Martín, Buenos Aires, Argentina
[d] Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina
[e] Max Planck Institute for Marine Microbiology, Bremen, Germany
[f] Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany
[g] Faculty of Geosciences, University of Bremen, Bremen, Germany
[h] CONICET - Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Instituto de Nanobiotecnología (NANOBIOTEC), Buenos Aires, Argentina

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ABSTRACT

Colonization of newly ice-free areas by marine benthic organisms intensifies burial of macroalgae detritus in Potter Cove coastal surface sediments (Western Antarctic Peninsula). Thus, fresh and labile macroalgal detritus serves as primary organic matter (OM) source for microbial degradation. Here, we investigated the effects on post-depositional microbial iron reduction in Potter Cove using sediment incubations amended with pulverized macroalgal detritus as OM source, acetate as primary product of OM degradation and lepidocrocite as reactive iron oxide to mimic *in situ* conditions. Humic substances analogue anthraquinone-2,6-disulfonic acid (AQDS) was also added to some treatments to simulate potential for electron shuttling. Microbial iron reduction was promoted by macroalgae and further enhanced by up to 30-folds with AQDS. Notably, while acetate amendment alone did not stimulate iron reduction, adding macroalgae alone did. Acetate, formate, lactate, butyrate and propionate were detected as fermentation products from macroalgae degradation. By combining 16S rRNA gene sequencing and RNA stable isotope probing, we reconstructed the potential microbial food chain from macroalgae degraders to iron reducers. *Psychromonas*, *Marinifilum*, *Moritella*, and *Colwellia* were detected as potential fermenters of macroalgae and fermentation products such as lactate. Members of class deltaproteobacteria including Sva1033, *Desulfuromonas*, and *Desulfuromusa* together with *Arcobacter* (former phylum Epsilonbacteraeota, now Campylobacterota) acted as dissimilatory iron reducers. Our findings demonstrate that increasing burial of macroalgal detritus in an Antarctic fjord affected by glacier retreat intensifies early diagenetic processes such as iron reduction. Under scenarios of global warming, the active microbial populations identified above will expand their environmental function, facilitate OM remineralisation, and contribute to an increased release of iron and CO_2 from sediments. Such indirect consequences of glacial retreat are often overlooked but might, on a regional scale, be relevant for the assessment of future nutrient and carbon fluxes.

1. Introduction

Microbial iron reduction is an ancient form of metabolism on earth (Weber et al., 2006). In marine surface sediments, 17–50% of organic carbon mineralisation is estimated to be fuelled by dissimilatory iron reduction (DIR) (Canfield et al., 1993; Thamdrup, 2000).

* Corresponding authors.at: Microbial Ecophysiology Group, Faculty of Biology/Chemistry & MARUM, University of Bremen, Bremen, Germany.
E-mail addresses: david.aromokeye@uni-bremen.de (D.A. Aromokeye), willis.graciana@biotec.quimica.unlp.edu.ar (G. Willis-Poratti), le_wu@uni-bremen.de (L.C. Wunder), yin@uni-bremen.de (X. Yin), jwendt@marum.de (J. Wendt), trichter@uni-bremen.de (T. Richter-Heitmann), susann.henkel@awi.de (S. Henkel), svazquez@ffyb.uba.ar (S. Vázquez), melvert@marum.de (M. Elvert), wmac@ffyb.uba.ar (W. Mac Cormack), michael.friedrich@uni-bremen.de (M.W. Friedrich).
[1] David A. Aromokeye and Graciana Willis-Poratti contributed equally to this work.

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Microorganisms identified so far performing DIR predominantly include bacteria from the order Desulfuromonadales, such as those affiliated to the genera *Desulfuromonas*, *Desulfuromusa*, *Pelobacter*, *Geopsychrobacter*, *Geothermobacter* (Aromokeye et al., 2018; Holmes et al., 2004; Hori et al., 2015; Kashefi et al., 2003; Roden and Lovley, 1993; Vandieken et al., 2006a; Vandieken et al., 2006b; Vandieken and Thamdrup, 2013), and recently the clade Sva1033 (Ravenschlag et al., 1999; Wunder et al., 2021). In polar regions, global warming-induced glacial retreat results in increased reactive iron sedimentation to the surrounding marine sediments, through glacial associated erosion, weathering and melt water inputs (Death et al., 2014; Hawkings et al., 2014; Henkel et al., 2018; Monien et al., 2017; Raiswell et al., 2018; Raiswell et al., 2016; Wang et al., 2014). The increased supply of reactive iron phases can induce important changes in coastal sediments, such as broadening ferruginous zones, especially in sediments close to retreating glaciers (Henkel et al., 2018). Based on increased iron oxide availability, iron reducing microorganisms are likely to have a competitive advantage over sulfate reducers for common substrates (e.g. acetate) (Canfield et al., 1993; Vandieken et al., 2012).

Potter Cove is a small fjord on the southwest of King George Island/Isla 25 de Mayo, South Shetland Islands, located at the northern tip of the West Antarctic Peninsula (WAP). The cove is divided into an inner and outer section by a 30-m sill. The outer cove is characterized by predominantly hard substrate at its bottom and a complex macroalgal community at its coasts (Klöser et al., 1996; Quartino et al., 2005). In the inner cove, the accelerated retreat of the Fourcade Glacier (Rückamp et al., 2011) triggered macroalgal colonization in the newly ice-free areas close to the glacier front, being the large brown algae species of the order Desmarestiales and the red algae *Palmaria decipiens* the most abundant in biomass at these sites (Campana et al., 2018; Quartino et al., 2013). The increase in melt water fluxes into the inner cove, especially during summer seasons, prompts the accumulation of fine-grained material with high content of metal oxides (Henkel et al., 2018; Monien et al., 2017). The phytoplankton production is usually very low in Potter Cove (less than 3 mg/m^3 chlorophyll *a*) because of the combined effects of the high number of particles present in the water column, which greatly limits light penetration and wind-induced mixing (Schloss et al., 2012; Schloss and Ferreyra, 2002). Consequently, other organic matter (OM) substrates such as macroalgae and microphytobenthos have been proposed to represent the main pool available for benthic organisms and/or degrading microorganisms (Braeckman et al., 2019; Hoffmann et al., 2018; Quartino and Boraso de Zaixso, 2008).

As pore-water geochemistry in Potter Cove sediments indicates (Henkel et al., 2018; Monien et al., 2014), microbial iron reduction is the dominant terminal electron accepting process during OM degradation, especially in the newly ice-free areas and deeper troughs (Monien et al., 2014). Moreover, the intensified colonization of the sublittoral rocky substrates by macroalgae (Campana et al., 2018; Quartino et al., 2013) in Potter Cove and the consequent burial of macroalgal detritus in the surrounding sediments increases the amounts of labile OM accessible to microorganisms. Therefore, we hypothesize that a respiratory process such as microbial iron reduction, which is the dominant terminal electron accepting process in these sediments, might intensify as a result of the environmental change. To test this hypothesis, we investigated the potential impact of the above mentioned environmental change on microbial iron reduction in Potter Cove. Incubation experiments were set up using sediments from the inner and outer cove to achieve the following objectives: (I) to identify the influence of macroalgal detritus on iron reduction and microbial community composition; (II) to detect the potential for iron reduction in sediments by the addition of poorly crystalline iron oxide (lepidocrocite); (III) to test the influence of acetate as a typical substrate for iron reducers and the potential of the humic substances analogue anthraquinone-2,6-disulfonic acid (AQDS) to serve as electron shuttle and to stimulate potential terrigenous inputs from melt water streams into the cove; and (IV) to identify the key microorganisms involved in the food chain from macroalgae degradation to DIR via amplicon sequencing and RNA stable isotope probing (RNA-SIP).

2. Materials and methods

2.1. Sample collection

Sediment samples from Potter Cove were collected during austral summer 2018/2019, based on previous geochemical investigations (Henkel et al., 2018; Monien et al., 2014). Two sites were selected for this study: (I) close to the glacier front in the inner cove i.e. station 10 (STA10; 62° 13′ 30.7′’ S/ 58° 39′ 31.4′’ W), situated close to the rocky shore colonised by macroalgae and influenced by iron oxide inputs (Quartino et al., 2013); and (II) further from the glacier front in the outer cove i.e. station 14 (STA14; 62° 13′ 54.8′’ S/58° 40′ 6.6′’ W), which is proximal to the coast and not directly influenced by surficial melt water runoff (Henkel et al., 2018). Because of the shallow water depth at STA10 (13 m), sediments were retrieved with push cores by scuba divers from the Argentinian Diving Division. In contrast, sediment cores at STA14 (42 m) were collected using a small gravity corer (UWITEC, Austria). Immediately after retrieval, sediment cores (approximately 20–30 cm length) were carefully transported in vertical position to the field lab, sliced into 5-cm sections, and stored at 2 °C in 500 mL Schott-bottles sealed with rubber stoppers under N_2 headspace (99.999% purity, Linde, Germany). The sediments were kept at 2 °C in the dark to maintain near *in situ* conditions for 5 months until the start of incubation experiments in the home laboratory.

2.2. Incubation experiments

Top sediments (1–10 cm) from STA10 and STA14 were used for slurry preparation. Sediment depths selection was based on a previous study, where the highest concentrations of dissolved iron in pore-water were observed at 1–10 cm at both sites (Henkel et al., 2018). Anoxic 30-mL slurries (1:6 w/v) were prepared in 50-mL serum bottles. The slurries were homogenized with anoxic, sterile and sulfate-free artificial sea water (ASW; composition [L^{-1}]: 26.4 g NaCl, 11.2 g $MgCl_2 \cdot 6\ H_2O$, 1.5 g $CaCl_2 \cdot 2\ H_2O$ and 0.7 g KCl), under N_2 headspace. Previously frozen macroalgal biomass (*Durvillaea* sp., obtained from Prof. Kai Bischof, Marine Botany, University of Bremen) was cut into small pieces and homogenized using a porcelain mortar under sterile conditions. Next, the homogenized macroalgal biomass was added to selected bottles in two different amounts (0.10 g or 0.25 g). This was followed by pre-incubation of all bottles at 2 °C for 3 days to equilibrate the slurries. After equilibration, a total of 10 different treatments were set up, each in biological triplicates, i.e. three separate bottles with the same treatments (Table S1). Briefly, for the various treatments, slurries were supplemented with lepidocrocite (insoluble iron oxide, equivalent to 10 mM final concentration, added in two portions of 5 mM), acetate (1 mM final concentration, added in two portions of 0.5 mM), macroalgae (0.10 g or 0.25 g), and AQDS (10 μM final concentration). Thus, while some treatments contained 0.10 g or 0.25 g macroalgae in combination with the aforementioned substrates, others were devoid of macroalgae amendment (Table S1). Although *Durvillaea* sp. is not typically found at Potter Cove, few specimens have been observed near the coast of King George Island/Isla 25 de Mayo (Fraser et al., 2018). *Durvillaea* spp. belong to the same class of brown algae (Phaeophyceae) as those present (Desmarestiales order, *Desmarestia* spp. and *Himantothallus* spp.) in Potter Cove (Quartino et al., 2013; Quartino et al., 2005; Velásquez et al., 2020). Those brown algae are known to stimulate *in situ* microbial degradation processes, while the red algae *Palmaria decipiens* is degraded faster by herbivores (Braeckman et al., 2019; Quartino et al., 2013). The algae species from the Phaeophyceae family only differ slightly in terms of carbon compounds between them. Specifically, their cell walls have more alginate than cellulose (Rioux and Turgeon, 2015; Velásquez et al., 2020), laminarin as the main storage polysaccharide and phlorotannins (Amsler et al., 2005; Velásquez et al., 2020). Thus,

the algal material used in our incubations is comparable to those present at Potter Cove.

Both lepidocrocite and acetate were added in two portions, at the beginning and at day-11 and day-12 for both STA10 and STA14, respectively, to simulate sporadic supply of electron donor acetate and fresh supply of reactive iron. As baseline control for this study, triplicate slurries amended only with 1 mM acetate ("acetate only") were used. All treatments were subsequently sampled for analytical measurements by collecting 1 mL of slurry under anoxic conditions prior (designated as day-0) to static incubation in the dark at 2 °C, followed by regular sampling at frequent intervals for 28 days.

Supplementary incubations were set up with STA10 sediments in biological triplicates for RNA stable isotope probing (RNA-SIP) similarly to the setups described above, with modifications to allow for successful labelling of target iron reducing populations (Table S2) (Hori et al., 2010). Firstly, 0.01 g macroalgae was added instead of 0.25 g to potentially reduce the extent of enrichment of primary macroalgae degraders. Secondly, after 7 days of pre-incubation with macroalgae, either unlabelled [^{12}C]acetate (i.e. with natural abundance of ^{13}C) or fully labelled [^{13}C]acetate (5 mM final concentration) was added to the incubations to provide excess acetate to reduce saturation effects caused by acetate potentially formed during macroalgae degradation. This ensures the possibility for the target microbial populations (ideally iron reducers) in the [^{13}C]acetate amended incubations to be labelled. Finally, 5 μM AQDS (final concentration) was added instead of the 10 μM provided to the initial sediment incubations. As physiological controls for SIP with [^{13}C]acetate, treatment sets with labelled dissolved inorganic carbon (DIC) [^{13}C]DIC and unlabelled [^{12}C]DIC (i.e. with natural abundance of ^{13}C) were set up. The expectation was that microorganisms targeted to incorporate [^{13}C]acetate will not be labelled with [^{13}C]DIC so as to allow for more robust conclusions from the RNA-SIP experiment. Lepidocrocite (insoluble iron oxide, equivalent to 5 mM final concentration) was directly added to the treatments as electron acceptor. For these supplementary incubations, a triplicate treatment set amended with only 0.01 g macroalgae as electron donor was used as baseline control. RNA-SIP targeted incubations were run statically in the dark at 2 °C for only 7 days to avoid over-enrichment of certain populations from macroalgae degradation and cross-feeding (Friedrich, 2011).

2.3. Analytical methods

Slurries (1 mL) retrieved from incubations were transferred under anoxic conditions to 1.5 mL reaction tubes pre-flushed with N_2. Supernatant from slurries was obtained by centrifugation (15,300 × *g*, 5 min at 4 °C) and used for dissolved iron (Fe^{2+}) and volatile fatty acids (VFAs) measurements. The formation of Fe^{2+} was monitored over time during incubations using the method by Viollier et al. (2000), with modifications: for all treatments (including acetate only controls) 200 μL of the supernatant were fixed with 50 μL of 0.5 M HCl. Next, 100 μL of the acidified mixture were directly transferred to the ferrozine-ammonium acetate buffer mixture in sealed cuvettes, which were subsequently incubated in the dark for 10 min prior to spectrophotometric measurements at 569 nm wavelength.

VFAs from the slurry water phase were measured using liquid chromatography coupled to isotope ratio mass spectrometry (LC-IRMS) using a Delta Plus XP interfaced to a LC Isolink (both Thermo Finnigan) (Heuer et al., 2009). VFAs were determined only in treatments amended with 0.25 g macroalgae, as these are expected to yield the highest concentrations of intermediates, and at specific time points (day-11/12 and day-28). The rest of the slurry was kept at −20 °C and later used for nucleic acid extractions, when required.

2.4. Nucleic acid extraction

Nucleic acids used for analysis of microbial communities in the initial incubation experiment were extracted from 1 mL of slurry from individual incubations at specific time points, from un-incubated slurries and from 0.25 g of original macroalgal biomass as a control to account for potential microbial populations introduced to the slurries via macroalgae addition. Nucleic acids were extracted following Lueders et al. (2004) with some modifications described in the supplementary material. To ensure sufficient RNA concentrations for SIP, triplicate incubations were pooled and RNA was extracted from a total of 30 mL slurry per treatment. For the RNA-SIP, extraction of RNA similarly followed the described protocol above, followed by DNase treatment.

2.5. RNA-SIP preparation, density centrifugation and fractionation

Density centrifugation and fractionation was performed as described in Yin et al. (2021). Extracted RNA was quantified with Quant-iT RiboGreen RNA assay kit (ThermoFisher Scientific) and 1 μg was used for density separation by ultracentrifugation. In total, 14 fractions were obtained, fraction 1 with the highest and fraction 14 with the lowest density. Separation was controlled and fractions were defined by an RNA concentration – density profile for each sample (Fig. S1). Two fractions were pooled as ultra-heavy = fraction 3 + 4 (1.818–1.837 g/mL), heavy = fraction 5 + 6 (1.806–1.822 g/mL), midpoint = fraction 7 + 8 (1.791–1.810 g/mL), light = fraction 9 + 10 (1.779–1.795 g/mL) and ultra-light = fraction 11 + 12 (1.764–1.783 g/mL) (see Table S3 for density of individual samples). cDNA synthesis was performed on pooled fractions.

2.6. 16S rRNA sequencing and analysis

A bacterial 16S rRNA amplicon library was prepared with the cDNA obtained from RNA-SIP and the DNA extracts from the initial enrichment incubations as described in Aromokeye et al. (2018). Paired-end sequencing was performed at Novogene Co. Ltd. (Cambridge, UK) on the Illumina Novaseq6000 platform (2x250 bp paired-end). Sequencing reads were analysed following Aromokeye et al. (2018) with modifications described in supplementary material.

2.7. Statistical analysis

All statistical analysis and figures were performed within the R environment version 3.6.1 (R Core Team, 2019). A Bray-Curtis distance matrix of the bacterial communities of macroalgae incubations was used for a non-metric multidimensional scaling (NMDS) ordination. To identify the variables influencing the clustering, the anova.cca function (vegan package, Oksanen et al., 2019) was used on a distance-based redundancy analysis (dbRDA) model with time point, station, and dummy coded substrate variables (acetate, lepidocrocite, macroalgae, AQDS). The dbRDA results were finally checked with PERMANOVA only with significant influencing variables (macroalgae, station and time point).

3. Results

3.1. Microbial iron reduction

Microbial iron reduction, indicated by increasing dissolved iron (Fe^{2+}) concentrations, was observed (Fig. 1) in most treatments for both the inner cove station (STA10) and the outer cove station (STA14). In treatments amended with only acetate ("acetate only"), iron reduction was not stimulated. However, iron reduction was fuelled in acetate and lepidocrocite amended treatments. More importantly, the combination of macroalgae and lepidocrocite amendment (with or without acetate) stimulated higher levels of iron reduction compared to treatments with acetate and lepidocrocite. Accordingly, treatments with 0.25 g macroalgae produced higher Fe^{2+} concentrations (2-fold increase at both sites) compared to the 0.10 g macroalgae treatment. The influence of adding 1

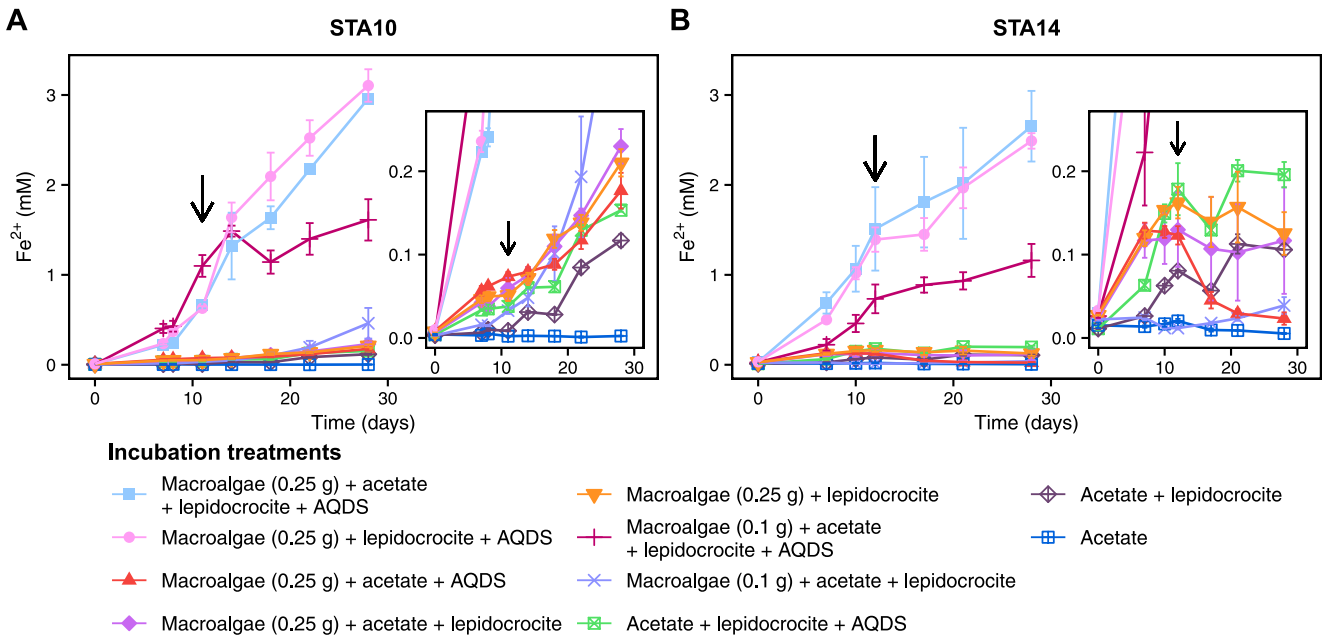

Fig. 1. Microbial iron reduction in sediments from Potter Cove promoted by macroalgal detritus via the electron shuttle AQDS in (a) the inner cove STA10 and (b) the outer cove STA14. SD of incubation triplicates is displayed as error bars. Black arrows indicate the second addition of 0.5 mM acetate and 5 mM lepidocrocite. The insets on the right side zoom in on the ferrous iron concentration in treatments with lower concentrations.

mM acetate to the macroalgae incubations showed no apparent effect to the overall rates of iron reduction, considering the concentrations in treatments without acetate. In incubations provided with macroalgae (0.25 g), lepidocrocite and AQDS, Fe^{2+} concentrations were similar to the same treatment amended with acetate. Due to the high rates of iron reduction measured in the first 7 – 10 days, additional 0.5 mM acetate and 5 mM lepidocrocite were added after 11/12 days to support continued iron reduction in all incubations. Addition of 10 μM of the electron shuttle AQDS to some treatments increased the rate of iron reduction in these sediment incubations. Direct comparison of the Fe^{2+} concentrations in the macroalgae (0.25 g) and lepidocrocite treatment from both stations with or without AQDS revealed a 15-fold increase in iron reduction rates just by the addition of electron shuttle. Likewise, treatments with acetate, lepidocrocite, AQDS (10 μM) and macroalgae (both 0.10 and 0.25 g) produced 3.5 – 30-fold more Fe^{2+} compared to same treatment without AQDS.

3.2. Fermentation products

The enhanced stimulation of iron reduction in incubations amended with macroalgae served as indirect indication for macroalgae degradation. Accordingly, we predicted the formation of fermentation products, which must have served as electron donors for iron reduction. Therefore, using LC-IRMS, VFAs as fermentation products from macroalgae degradation were tracked in all treatments amended with macroalgae (0.25 g) at both day-11/12 and day-28 of the incubations (Table 1). The treatments amended with 0.25 g of macroalgae were selected because the highest concentrations of fermentation products including acetate, formate, lactate, butyrate and propionate were expected for these treatments. Of these, both acetate (70 μM) and lactate (2 μM) were detected in the un-incubated slurry prior to macroalgae addition. Over the course of the incubation time, detected acetate concentrations reached up to 1400 μM in the various treatments sampled (Table 1).

Table 1
Volatile fatty acids concentrations (μM) as transient fermentation products detected during macroalgae degradation in selected incubations.

Treatment	Site	Time point	Formate	Acetate	Propionate	Butyrate	Lactate
Acetate + lepidocrocite + macroalgae	STA10	day-11	50 ± 25	1404 ± 356	322 ± 49	93 ± 35	ND
		day-28	6 ± 2	1381 ± 60	569 ± 49	91 ± 16	1.4 ± 0.3
	STA14	day-12	78 ± 24	1395 ± 74	100 ± 32	122 ± 25	21 ± 2
		day-28	N.D	1413 ± 57	186 ± 57	112 ± 34	14*
Acetate + lepidocrocite + macroalgae + AQDS	STA10	day-11	40 ± 1	1260 ± 50	244 ± 3	79 ± 8	79.9*
		day-28	1*	1271 ± 94	462 ± 60	60 ± 3	31.6*
	STA14	day-12	52 ± 29	1265 ± 130	71 ± 3	102 ± 28	16 ± 3
		day-28	ND	1578 ± 89	194 ± 51	95 ± 27	ND
Acetate + macroalgae + AQDS	STA10	day-11	39 ± 6	1244 ± 92	ND	70 ± 6	ND
		day-28	ND	1253 ± 123	503 ± 92	74 ± 12	ND
	STA14	day-12	45 ± 10	1154 ± 108	72 ± 4	75 ± 13	21 ± 3
		day-28	ND	1440 ± 55	216 ± 15	86 ± 4	ND
Macroalgae + lepidocrocite + AQDS	STA10	day-11	41 ± 26	1335 ± 438	325*	84 ± 42	15.4*
		day-28	ND	1109 ± 120	455 ± 57	58 ± 14	22 ± 5
	STA14	day-12	115 ± 72	1144 ± 53	76 ± 7	69 ± 5	ND
		day-28	ND	1393 ± 30	218 ± 79	64 ± 14	ND
Un-incubated slurry	STA10	–	ND	69.8	ND	ND	2.1
	STA14	–	ND	ND	ND	ND	2

* Numbers obtained from 1 replicate of the same treatment. The fermentation products were not detected from other replicates at the same time point. ND - not detected.

Next was propionate with concentrations up to 500 μM at day-28 compared to day-11/12 (up to 322 μM). At day-28, when the incubations were stopped, lactate and formate concentrations were either lower or undetectable (Table 1) while acetate and butyrate were detected in similar concentrations as on day-11/12.

3.3. Microbial communities in enrichments

Bacterial 16S rRNA genes were sequenced from biological triplicates from each initial enrichment incubation treatment to study the shift in microbial community compositions and identify the actively enriched communities over time. Similarly, technical triplicates of the freshly prepared slurry prior to substrate amendment (un-incubated), and the macroalgal material fed into the incubations were also sequenced to identify bacterial communities initially present in the sediment slurry and the associated bacterial community introduced via macroalgae addition. As expected, the shifts in bacterial communities were evident in the incubations amended with macroalgae compared to those without (Fig. 2, Fig. S2), as confirmed by PERMANOVA analysis ($F(2,117) = 266.98$, $P < .001$). In the macroalgae amended incubations, the genus *Psychromonas* was dominant (50 – 75% relative abundance) in all treatments regardless of the amount of macroalgae initially added (0.10 g or 0.25 g) and the incubation time (i.e. 11/12 or 28 days). Relative abundance of *Psychromonas* was less than 2% at the start of the incubation experiment (day-0, acetate, lepidocrocite, 0.25 g macroalgae, AQDS treatment). The other bacterial taxon enriched with macroalgae amendment in all treatments was the Bacteroidetes genus *Marinifilum*. With a relative abundance of 2.5 – 3% and ~ 0.5% at STA10 and STA14 respectively at day-0, *Marinifilum*'s relative abundance increased consistently over time to 7 – 8% and 3 – 5% at STA10 and STA14, respectively, after 28 days (Fig. 2). The relative abundance of the other bacteria present at day-0, in the un-incubated slurry and in the macroalgal material decreased over time during the incubation, except for the genus *Psychrilyobacter* (Fusobacteria). However, *Psychrilyobacter* was heavily associated with one of the two replicates of the macroalgal material (13%) initially fed into the incubation. The bacterial communities in the incubations without macroalgae clustered together with the un-incubated slurry and day-0 samples, as shown by both the NMDS (Fig. S3) and dbRDA ordination plots (Fig. S2). However, there were minor differences between them, especially in incubations from STA14. An example is the enrichment of the genus *Arcobacter* (up to 26% of bacterial 16S rRNA genes) in treatments amended with acetate and lepidocrocite with or without AQDS. Likewise, the clade Sva1033 (Desulfuromonadales) was abundant in the same treatments, with (7% – 13–14%) or without (4–5% – 8–9%) AQDS. Whereas clade Sva1033 was also present in incubations from STA10, a time course increase in relative abundance was not observed for this taxon unlike in STA14 (Fig. 2). In general, dbRDA and cluster analyses showed significant differences of the bacterial community between the stations ($F(1,120) = 57.61$, $P < .001$) and a minor difference between the time points ($F(2,120) = 34.59$, $P < .001$), mainly between un-incubated slurry and the more advanced time points. Differences in microbial community composition between day-11/12 and day-28 were only observed for samples containing macroalgae (Fig. S2).

3.4. Stable isotope probing experiment with unlabelled macroalgae and [^{13}C]acetate

Based on the 16S rRNA data obtained from initial enrichment incubations where *Psychromonas* dominated the microbial community assembly (Fig. 2), it was unclear which role other microorganisms played in the macroalgae amended treatments. Despite the strong indication for iron reduction (Fig. 1), known iron reducers were not enriched. Therefore, RNA-SIP incubations were set up with unlabelled macroalgae and [^{13}C]acetate (Table S2). The aim was to simulate iron reduction driven by macroalgae degradation with smaller amounts of macroalgae (0.01 g), thus ensuring active iron reducers and other players in the microbial food chain would be enriched. As negative biological control to test for the role of acetate as carbon source and electron donor for iron reducers, similar treatments were set up with labelled [^{13}C]DIC. To investigate the potential for macroalgae amendment alone to stimulate microbial iron reduction in the sediment, a control treatment amended with macroalgae, but excluding other substrates was set up. In this treatment, iron reduction could be observed (Fig. 3a) unlike with acetate only (Fig. 1). Iron reduction was notably stimulated in the [^{13}C]acetate amended treatments but not in the [^{13}C]DIC amended treatments either with or without macroalgae (Fig. 3a).

Bacterial community compositions obtained from the SIP experiments were clearly different between treatments (Fig. 3b). *Psychromonas* maintained a relative abundance of up to 44% in treatments with macroalgae and only 0.8% in treatments without. Relative abundance of *Psychromonas* however decreased in the heavy (less than 5%) compared to the light fractions (over 45%) of the [^{13}C]acetate and macroalgae amended treatments (Fig. 3b). In the DIC amended treatments with or without macroalgae, the enriched communities were similar both in the unlabelled SIP control and labelled amended treatments (Fig. 3b). There was a clear difference in the heavy fractions for [^{12}C]acetate and [^{13}C]acetate treatments. In the [^{13}C]acetate amended treatments, the order Desulfuromonadales was highly enriched, especially in the ultra-heavy fraction (Fig. 3b). Relative abundance of Desulfuromonadales in the relatively ultra-heavy fraction reached 50% with macroalgae and 30% without. Amongst this order, clade Sva1033 was the most enriched, up to 33% with macroalgae and 20% without. Other taxa enriched in the heavy fractions within the order Desulfuromonadales were *Desulfuromonas* (relative abundance 6% with macroalgae and 4% without), and *Desulfuromusa* (6% with macroalgae and 3% without). Besides Desulfuromonadales, some members of the class Gammaproteobacteria were also enriched in the heavy fractions with [^{13}C]acetate. Examples were *Moritella* and *Colwellia* in the heavy fractions with acetate and macroalgae (up to 13% and 3% respectively). *Arcobacter* (class Campylobacteria) was also enriched with acetate and macroalgae (up to 15% relative abundance), but more significantly enriched without macroalgae (up 38% relative abundance).

4. Discussion

The Fourcade Glacier has retreated at unprecedented rates over the last decades (mean rate of 20 m a^{-1} between 1956 and 2008; Rückamp et al., 2011). In Potter Cove, the glacier was marine terminating and became land based during the course of the last decades (Jerosch et al., 2018). This remarkable change, have further strengthened the colonization of the "newly ice-free areas" in the cove by a high biomass producing macroalgal community. *Desmarestia* spp., *Himantothallus* sp. and *Palmaria decipiens* were observed as the dominating species in the newly ice-free areas (Braeckman et al., 2019; Campana et al., 2018; Quartino et al., et al., 2013). Increased global warming ensures that macroalgal biomass accumulates at the seafloor in the area (Braeckman et al., 2019; Quartino et al., 2013), leading to intensified microbial degradation of accumulated macroalgal detritus (Braeckman et al., 2019; Lastra et al., 2018). Consequently, nutrient availability and microbial respiration activity rates increase in the surrounding sediments (Braeckman et al., 2019). This effect is, moreover, supported by easily reducible iron oxides that are delivered to the cove by glacial erosion and/or subglacial meltwater (Henkel et al., 2018, Monien et al., 2017). Therefore, macroalgal biomass, which is made labile by benthic organisms (Quartino and Boraso de Zaixso, 2008), potentially exerts a feedback mechanism to the overall OM degradation process in the cove as more labile OM is available, intensifying iron reduction and increasing fluxes of dissolved iron into the water column. This, in turn, will trigger growth of algae (phytoplankton and macroalgae), which will fuel even more intensified iron reduction in the sediments. The possible existence of the aforementioned scenario and the potential effects of this environmental-

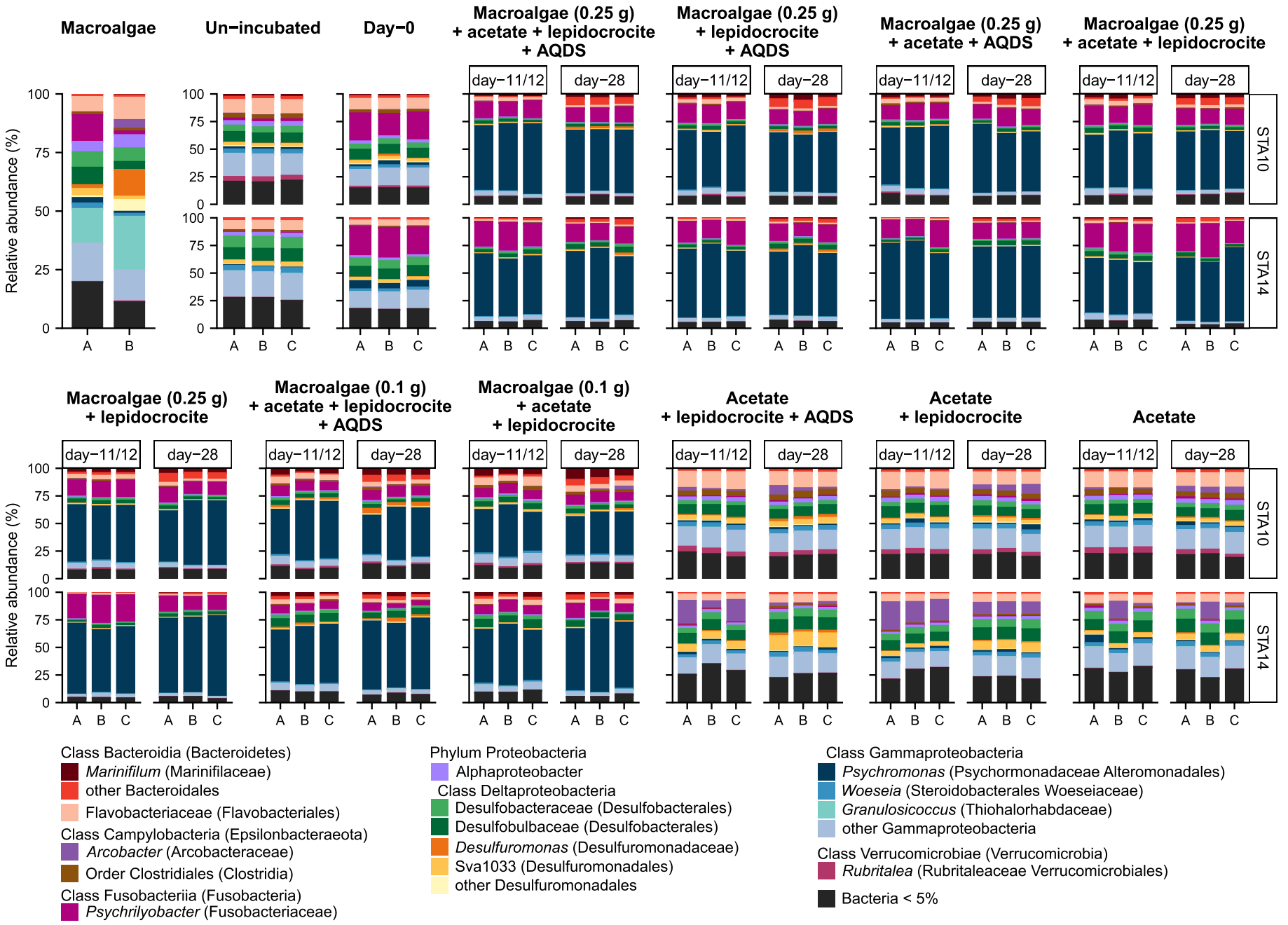

Fig. 2. Bacterial community composition in the sediment incubations identified by 16S rRNA gene sequencing. The day-0 sample is the 3 days pre-incubated slurry already amended with 0.25 g macroalgae. *Arcobacter* (former phylum Epsilonbacteraeota, now Campylobacterota).

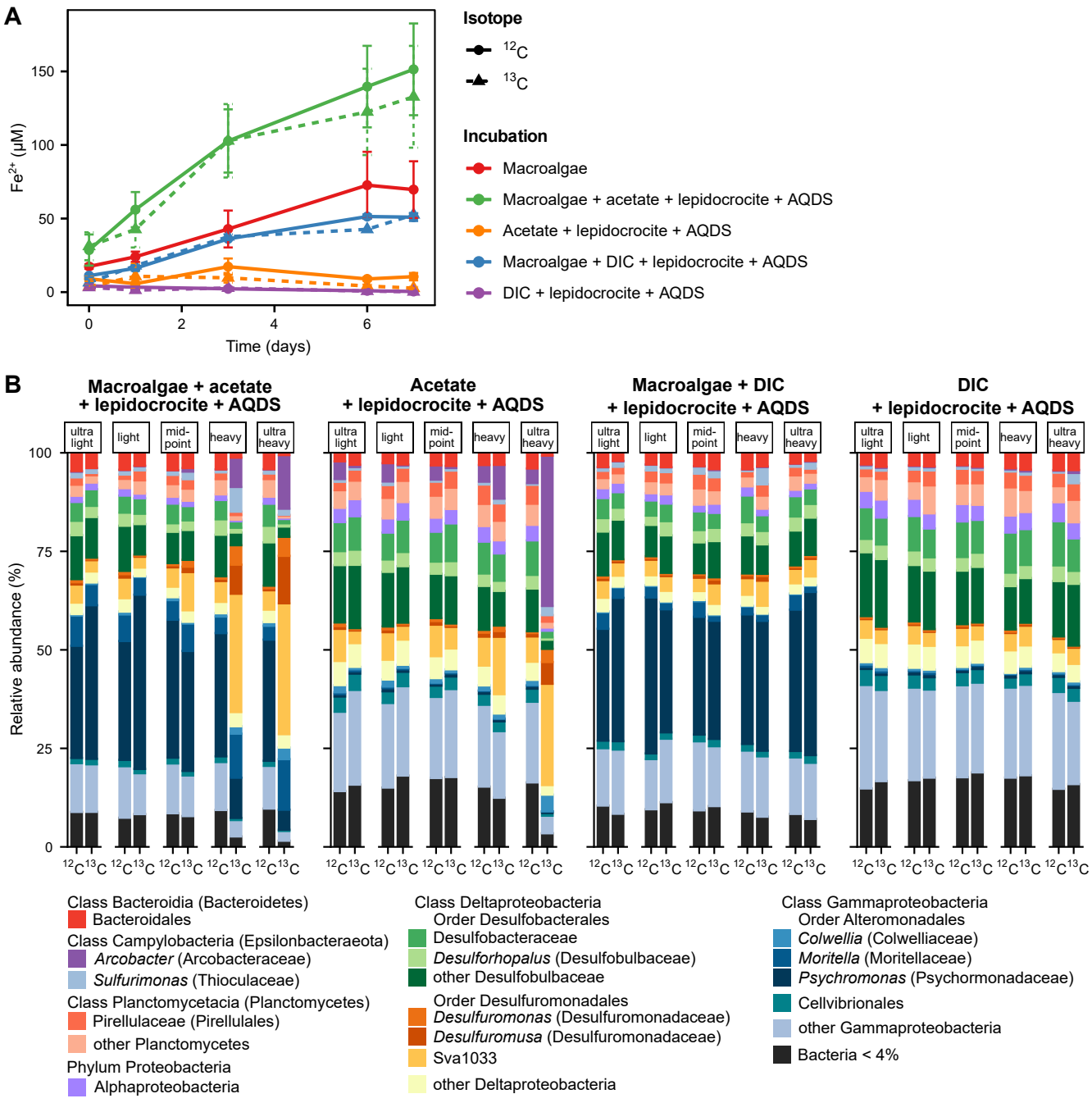

Fig. 3. RNA-SIP to identify other key bacterial players other than *Psychromonas* performing microbial iron reduction during macroalgae degradation using [^{13}C] acetate. Incubations with [^{13}C]DIC were set up as control for the acetate amended treatments. **a** Mean dissolved Fe^{2+} concentrations of individual treatments (n = 3) with SD as error bars. **b** bacterial community composition in the RNA-SIP fractions from the different treatments. *Arcobacter* (former phylum Epsilonbacteraeota, now Campylobacterota).

change-induced bacterial degradation of macroalgae on microbial iron reduction were investigated in this study and key findings are discussed below.

4.1. *Macroalgae degradation directly contributes to iron reduction in Potter Cove sediments*

Previous studies have shown that dissolved Fe^{2+} in Potter Cove sediments emanates from DIR (Henkel et al., 2018; Monien et al., 2014). Therefore competition, e.g. by iron and sulfate reducers for acetate produced from degradation of OM, could be a major constraint for *in situ* iron reduction. As established in previous studies from Potter Cove, macroalgae are the dominant primary producers (Quartino and Boraso de Zaixso, 2008) and thus, macroalgae detritus represents a major OM source for microbial degradation (Braeckman et al., 2019; Lastra et al., 2018). A direct impact of the degradation of the macroalgal biomass added to our anoxic sediment incubations performed at near *in situ* temperature of 2 °C was the rapid stimulation of high rates of microbial iron reduction in a short time frame (Fig. 1). The role of macroalgae degradation as an important source of electrons for iron reduction was revealed by the differences in Fe^{2+} produced in macroalgae, lepidocrocite and AQDS amended treatments when compared to the similar treatments with acetate as sole electron donor. While macroalgal biomass might presumably not have served directly as electron donor for

iron reduction, it was degraded into simpler carbohydrates, proteins and VFAs (lactate, butyrate, and propionate; Table 1) that must have undergone secondary fermentation into acetate and formate (Table 1). For example, lactate was no longer detectable at day-28 of the incubation experiment reflecting its role as a transient intermediate of macroalgae degradation either directly oxidized or further fermented most likely into propionate and acetate (Oude Elferink et al., 2001; Soubes et al., 1989). At day-11 and day-12 of the incubation experiments in the inner cove (STA10) and outer cove (STA14), intermediate acetate concentrations reached 1335 ± 438 and 1578 ± 89 μM, respectively (Table 1). This newly produced acetate must have triggered the increased levels of iron reduction observed in the incubations amended with macroalgae compared to acetate only treatments (Fig. 1), thus demonstrating how degradation of macroalgae fuels microbial iron reduction in Potter Cove. Beside acetate, all fermentation products detected from macroalgae degradation in our study, which could undergo secondary fermentation, i.e. lactate, butyrate and propionate, were previously shown as electron donors for iron reduction in Arctic sediments from Svalbard with similar environmental conditions (Finke et al., 2007). Therefore, these fermentation products may have directly served as electron donors for iron reduction as well. While addition of 1 mM acetate alone during the course of the incubation experiment could not stimulate microbial iron reduction (Fig. 1), as little as 0.01 g macroalgae alone was sufficient to stimulate iron reduction with endogenous iron oxides in the sediments (Fig. 3a). One explanation for this observation might be the formation of excess intermediate acetate concentrations, which provided sufficient electrons to overcome the strong kinetic limitation posed by the low solubility of endogenous iron oxides in the sediments (Kappler et al., 2004). Another explanation could be the adaptation to intensified iron reduction by the active players of the microbial food chain from macroalgae degradation to iron reduction. The latter is likely the case as revealed in the treatment amended with 25 times less macroalgae compared to the initial incubation set. Iron reduction increased over 7 days in this treatment showing affinity of the active microorganisms for a microbial food chain, which was primed by macroalgae degradation and terminates at iron reduction (Fig. 4).

Total organic carbon (TOC) contents found in Potter Cove sediments vary between 0.5 wt% at the inner cove to 1.3 wt% at the outer cove (Monien et al., 2014) and an intense macroalgal colonization has been observed at the inner cove within the newly ice-free areas during the last decade (Quartino et al., 2013). This suggests that direct burial of macroalgal detritus increases OM amounts in Potter Cove. The addition of similar amounts of macroalgal biomass and lepidocrocite to the incubations from the inner (STA10) and outer cove (STA14), resulted in similar rates of iron reduction (Fig. 1). These results predict that, in a future scenario of augmented colonization of the cove by macroalgae and supply of easily reducible iron oxides, microbial iron reduction might increase in relative importance, extending iron reduction zones beyond the average 10 – 15 cm sediment depth currently observed in the outer cove (Henkel et al., 2018).

Humic substances and extracellular quinones are known to act as electron shuttles facilitating electron transfer to Fe(III) in soils, aquifers, and freshwater sediments (Kappler et al. 2004; Lipson et al., 2010; Nevin and Lovley, 2000) but their role in microbial respiration has not been duly explored in marine sediments. Humic-like substances with aromatic moieties are typically transported to marine sediments during delivery of terrestrial OM (Chen et al., 2016; Schubert and Calvert, 2001) and may have similar stimulatory effects on iron reduction in marine sediments as in other environments. Besides, humic substances serve alternatively as electron shuttles, not just for iron reducers but also for a number of other microbial populations such as sulfate reducers and methanogens (Kappler et al., 2004). Addition of 10 μM of the humic substance analogue AQDS significantly increased iron reduction rates in these marine sediments incubations (3.5 – 30-fold increase, Fig. 1), facilitating further electron transfer on amended lepidocrocite and other endogenous Fe(III) complexes in our Antarctic sediment incubations. A 2 – 4-fold enhancement of iron reduction by humic substances was previously shown in coastal sediments from the North Sea receiving high amounts of terrigenous OM (Sztejrenszus, 2016). Our results now show that a similar stimulation of microbial iron reduction by humic substances could occur in Antarctic sediments. Thus, terrigenous inputs from nearby melt water streams in Potter Cove might enhance *in situ* iron reduction rates.

4.2. *Active players in microbial food chain from macroalgae degradation to iron reduction*

A significant shift in the bacterial community as response to macroalgae amendment was observed via the strong enrichment of the psychrophilic bacterial genus *Psychromonas* (up to 75%; Fig. 2, S2 and S3) after 11/12 days of incubation with macroalgae and lepidocrocite. This strong response of *Psychromonas* was not visible after the 3 days of pre-incubation of the sediment with macroalgal biomass alone, as their relative abundance was below 2% at day-0 in the acetate, lepidocrocite, 0.25 g macroalgae, and AQDS treatment. Therefore, the enrichment of *Psychromonas* was not only due to macroalgae degradation alone but also partly linked to the onset of iron reduction. Similarly, in the RNA-SIP experiment, *Psychromonas* dominated the lighter fractions (up to

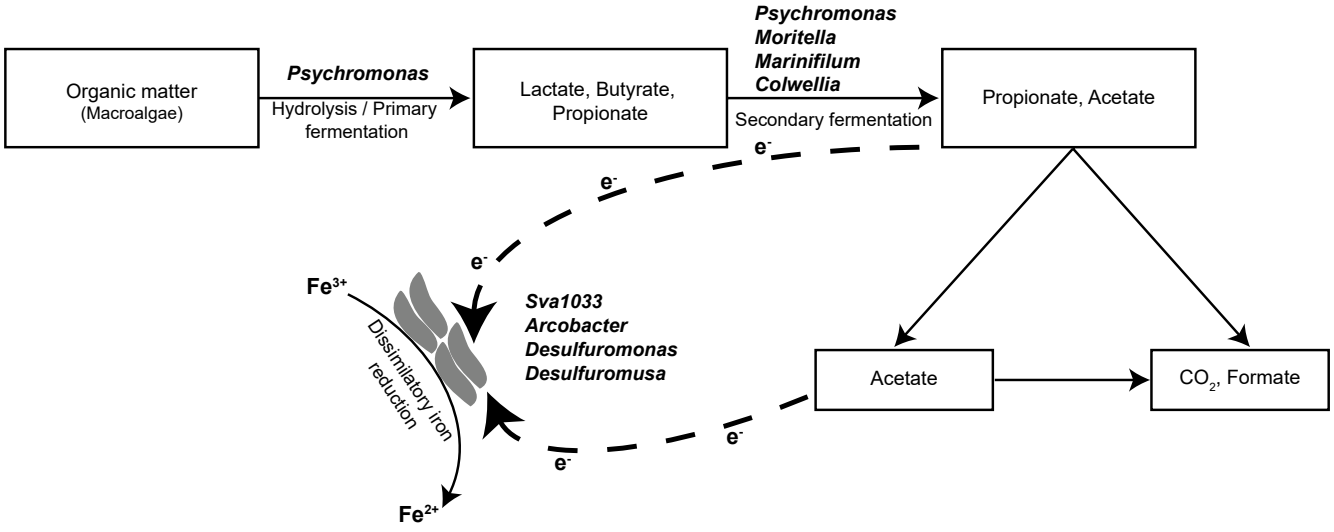

Fig. 4. Proposed microbial food chain from macroalgae degradation to microbial iron reduction in the investigated sediment incubations.

44% relative abundance; Fig. 3b). Although the labelling approach of the RNA-SIP experiment was not designed to target the primary degraders of macroalgae given the labelled substrate (acetate) provided, the dominance of *Psychromonas* in the lighter RNA fractions reflected their activity and role as the dominant microbial taxa in both set of incubation experiments (Figs. 2 and 3). The substantial enrichment of *Psychromonas* in this study strongly reflects their role as active degraders of fresh and labile OM such as the macroalgal biomass in Potter Cove, Antarctica. A previous study showed *Psychromonas* excrete alginate lyase enzymes which catalyse the degradation of alginate, a major component of brown algae cell wall (Dong et al., 2012). Metabolic flexibility of *Psychromonas* in permanently cold sediments is, however, not limited to degradation of seaweeds. *Psychromonas* were recently shown to be prominent degraders of lipids and proteins, components of detrital OM, in Svalbard sediments (Pelikan et al., 2020). Besides, *Psychromonas* metabolic flexibility and affinity for fresh OM was demonstrated in sulfidic sediments as well, where *Spirulina* (Cyanobacteria) necromass served as suitable degradation substrate (Müller et al., 2018). Strong correlations were also observed between phytodetritus fluxes and *Psychromonas* abundance in the Arctic Laptev Sea (Bienhold et al., 2012). While there is no known direct link to fermentative iron reduction metabolism in *Psychromonas*, they were previously found to be dominant in psychrophilic enrichments performing microbial iron reduction with glucose as substrate in incubations with deep sediment of the North Sea (Aromokeye et al., 2018).

Since we obtained no distinct evidence for *Psychromonas* performing iron reduction in the initial enrichment incubations, we hypothesize that dissimilatory iron reducers might remain important. However, relative abundance of dissimilatory iron reducers might have been overshadowed by the strong enrichment of *Psychromonas*. To test this hypothesis, the RNA-SIP experiment with acetate was set up. Based on the knowledge that detected VFAs such as lactate, propionate and butyrate undergo secondary fermentation to form acetate of up to 1.5 mM (Table 1), we provided the RNA-SIP treatments with fewer amounts of macroalgae (0.01 g) and 5 mM [^{13}C]acetate. The goal was to stimulate a positive response of dissimilatory iron reducers to the excess [^{13}C]acetate provided. The RNA-SIP experiment enabled the observation that Sva1033, with 33% relative abundance in the heavy fractions, indeed performed iron reduction during macroalgae degradation by *Psychromonas* (Fig. 3b). Although enriched in lower relative abundance in the heavy fractions compared to Sva1033, other known iron reducers such as *Desulfuromonas* and *Desulfuromusa* (Roden and Lovley, 1993; Vandieken et al., 2006b) were also involved in iron reduction (Fig. 3b).

The other bacterial taxon enriched in the initial enrichments without macroalgae was the genus *Arcobacter*, which was previously identified as manganese reducer in various other marine sediments (Thamdrup et al., 2000; Vandieken et al., 2012). Their enrichment with acetate and lepidocrocite suggests they are capable of growth via DIR which was previously reported for *Arcobacter* (Roalkvam et al., 2015). The possible role of *Arcobacter* as iron reducer in the acetate-amended treatments from the initial incubations (Fig. 2) was supported by the RNA-SIP experiment with macroalgae and [^{13}C]acetate (Fig. 3b) where *Arcobacter* reached a relative abundance of up to 15%. The absence of label incorporation by bacteria in the biological control [^{13}C]DIC treatments (Fig. 3b) confirms that the microorganisms enriched with [^{13}C]acetate are involved in the food chain of OM degradation in Potter Cove sediments. We therefore conclude that dissimilatory iron reducers (Sva1033, *Desulfuromonas, Desulfuromusa* and *Arcobacter*) were active members of the microbial food chain participating in the terminal step of acetate oxidation coupled to iron reduction.

Besides *Psychromonas*, members of the iron reducing Desulfuromonadales order, and *Arcobacter*, other bacterial taxa were stimulated either in the initial enrichment incubations or found in the heavy fractions of the RNA-SIP incubations. An example was the Bacteroidetes genus *Marinifilum* (up to 13% relative abundance at day-28) of the initial incubation experiment (Fig. 2). Likewise, genera *Moritella* and *Colwellia* (Class Gammaproteobacteria) were enriched in the heavy fractions in the SIP experiment with [^{13}C]acetate. Due to the unique position of genus *Marinifilum* (STA10 2.5 – 10%, STA14 0.5 – 5% relative abundance, Fig. 2) as the second largest taxa enriched after *Psychromonas*, we propose they participate in the fermentation of simple fermentable substrates such as lactate (Table 1).

Several psychrophilic *Moritella* strains have been previously isolated from marine sediments especially in the Arctic (Kim et al., 2008; Xu et al., 2003). Examples are *M. abyssi* and *M. profunda* with maximum growth rates at 2 °C, which was the temperature of incubation of the Antarctic sediments in our study. Some *Moritella* strains are capable of both fermentative (e.g. glucose) and respiratory (e.g. acetate, propionate) metabolism (Xu et al., 2003). Given their enrichment in heavy fractions, specifically with [^{13}C]acetate and macroalgae (13% relative abundance, Fig. 3b), it is clear that they incorporate acetate to form biomass. However, *Moritella* was not enriched in the heavy fractions of the [^{13}C]acetate treatment without macroalgae (Fig. 3b). Thus, while macroalgae degradation by *Moritella* was not previously shown, macroalgae addition facilitated the enrichment of *Moritella* in our incubations. We propose that *Moritella* may act as fermenters of both macroalgae and the VFAs formed in the incubations, such as propionate (Xu et al., 2003). The genus *Colwellia* was previously identified by RNA-SIP to perform acetate-dependent manganese reduction (Vandieken et al., 2012). Here, *Colwellia* was more abundant in the [^{13}C]acetate treatment without macroalgae, suggesting their role in acetate oxidation which may have been coupled to iron reduction.

5. Conclusion

The West Antarctic experiences significant ice mass loss with effects on coastal and shelf environment that are not fully assessed so far. We investigated the interdependence of OM supply, sedimentary iron reduction and nutrient recycling in a small fjord of King George Island/Isla 25 de Mayo that experienced significant glacier retreat over the past 60 years. Bacterial degradation of macroalgal detritus triggered high rates of microbial iron reduction via electron shuttling with the humic substance analogue AQDS within a short-time frame. The stimulation of microbial iron reduction from macroalgae degradation at the near *in situ* temperature of 2 °C presents one of the possible microbial responses that could be currently ongoing due to environmental change in Potter Cove sediments. The implication of our findings is that future glacier retreat and enhanced macroalgal colonization of newly ice-free areas in Antarctica will likely intensify microbial activity, increasing CO_2 fluxes from these environments. A potential network of key bacterial populations involved in the anaerobic microbial food chain was constructed from the observations in our incubation experiments (Fig. 4). We speculate that a future scenario of global-warming induced environmental change will favour the activity of metabolically versatile chemoorganotrophs such as *Psychromonas*, which are involved in the hydrolysis/primary fermentation of fresh and labile detrital OM. Other bacteria such as *Moritella*, *Colwellia* and *Marinifilum*, which may be involved in macroalgae primary fermentation or secondary fermentation of VFA, could also benefit from this environmental change. The bacterial populations identified to be involved in microbial iron reduction (Sva1033, *Desulfuromonas, Desulfuromusa* and *Arcobacter*) may perform more important ecological roles in Antarctic sediments in the future, as they account for the terminal steps of the carbon remineralisation in the environment.

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7. Data availability

The raw sequence data of this study was submitted to GenBank Short Reads Archive (SRA) under the accession number PRJNA668691.

CRediT authorship contribution statement

David A. Aromokeye: Writing - original draft, Writing - review & editing, Investigation, Conceptualization, Methodology, Project administration. **Graciana Willis-Poratti:** Writing - original draft, Writing - review & editing, Investigation, Methodology, Conceptualization, Data curation. **Lea C. Wunder:** Writing - original draft, Writing - review & editing, Investigation, Data curation, Formal analysis. **Xiuran Yin:** Methodology, Validation, Writing - review & editing. **Jenny Wendt:** Investigation, Data curation, Resources. **Tim Richter-Heitmann:** Formal analysis, Writing - review & editing. **Susann Henkel:** Writing - review & editing. **Susana Vázquez:** Writing - review & editing. **Marcus Elvert:** Resources, Writing - review & editing. **Walter Cormack:** Writing - review & editing, Supervision. **Michael W. Friedrich:** Writing - review & editing, Conceptualization, Funding acquisition, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.106602.

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Macroalgae degradation promotes microbial iron reduction via electron shuttling in coastal Antarctic sediments

David A. Aromokeye,[a,b,1*] Graciana Willis-Poratti,[a,c,d,1] Lea C. Wunder,[a,e] Xiuran Yin,[a,b] Jenny Wendt,[b] Tim Richter-Heitmann,[a,b] Susann Henkel,[b,f] Susana Vázquez,[h] Marcus Elvert,[b,g] Walter Mac Cormack,[c,h] and Michael W. Friedrich,[a,b]*

[a]Microbial Ecophysiology Group, Faculty of Biology/Chemistry, University of Bremen, Bremen, Germany
[b]MARUM – Center for Marine Environmental Sciences, University of Bremen, Bremen, Germany
[c]Instituto Antártico Argentino, San Martín, Buenos Aires, Argentina
[d]Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina
[e]Max Planck Institute for Marine Microbiology, Bremen, Germany
[f]Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany
[g]Faculty of Geosciences, University of Bremen, Bremen, Germany
[h]CONICET - Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Instituto de Nanobiotecnología (NANOBIOTEC), Buenos Aires, Argentina

* Address correspondence to David A. Aromokeye and Michael W. Friedrich at: Microbial Ecophysiology Group, Faculty of Biology/Chemistry, University of Bremen, Bremen, Germany.

[1] David A. Aromokeye and Graciana Willis-Poratti contributed equally to this work.

E-mail addresses: david.aromokeye@uni-bremen.de (David A. Aromokeye), willis.graciana@biotec.quimica.unlp.edu.ar (Graciana Willis-Poratti), le_wu@uni-bremen.de (Lea C. Wunder), yin@uni-bremen.de (Xiuran Yin), jwendt@marum.de (Jenny Wendt), trichter@uni-bremen.de (Tim Richter-Heitmann), susann.henkel@awi.de (Susann Henkel), svazquez@ffyb.uba.ar (Susana Vázquez), melvert@marum.de (Marcus Elvert), wmac@ffyb.uba.ar (Walter Mac Cormack), Michael.friedrich@uni-bremen.de (Michael W. Friedrich).

Supplementary figures and tables

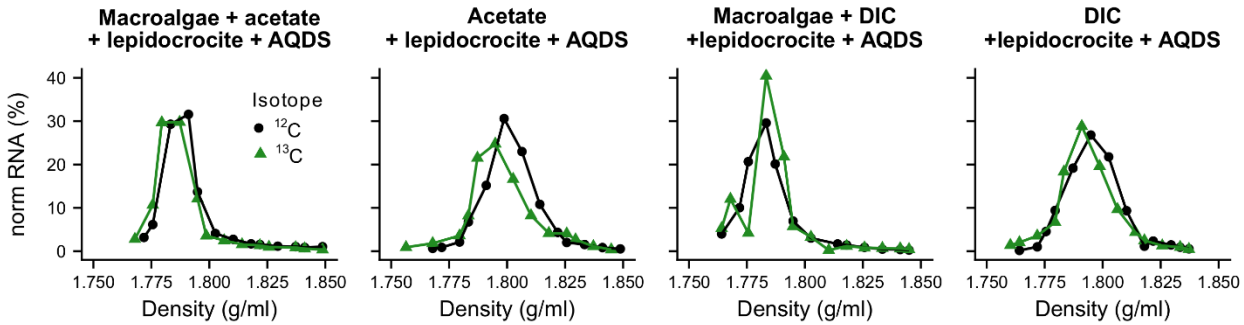


Figure S.1: Fractionation of RNA stable isotope probing incubations. RNA content was normalized by dividing ng RNA per fraction by total ng RNA recovered per sample.

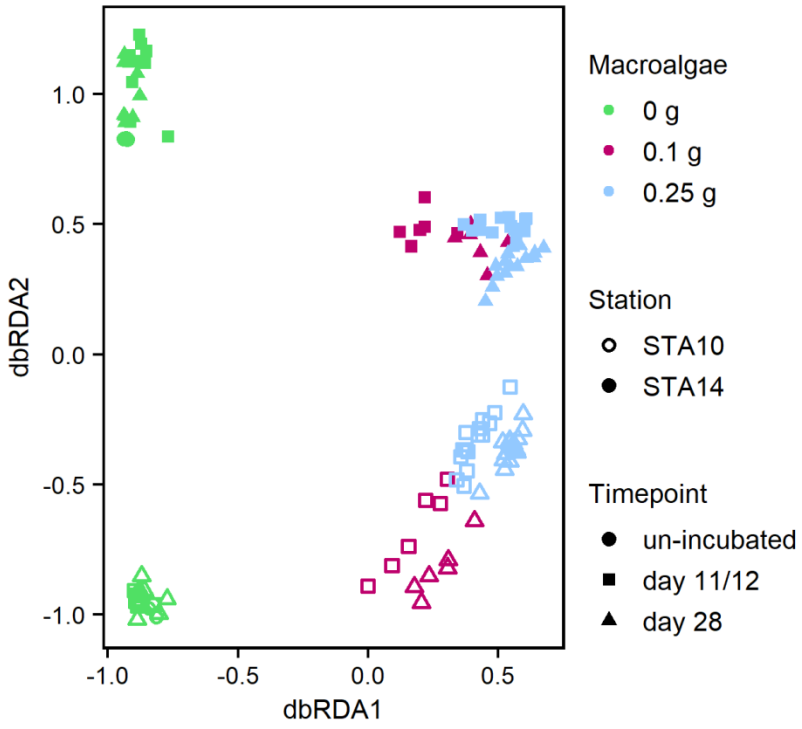


Figure S.2: Ordination plot of distance-based redundancy analysis of macroalgae incubations with significant variables macroalgae, station and time point.

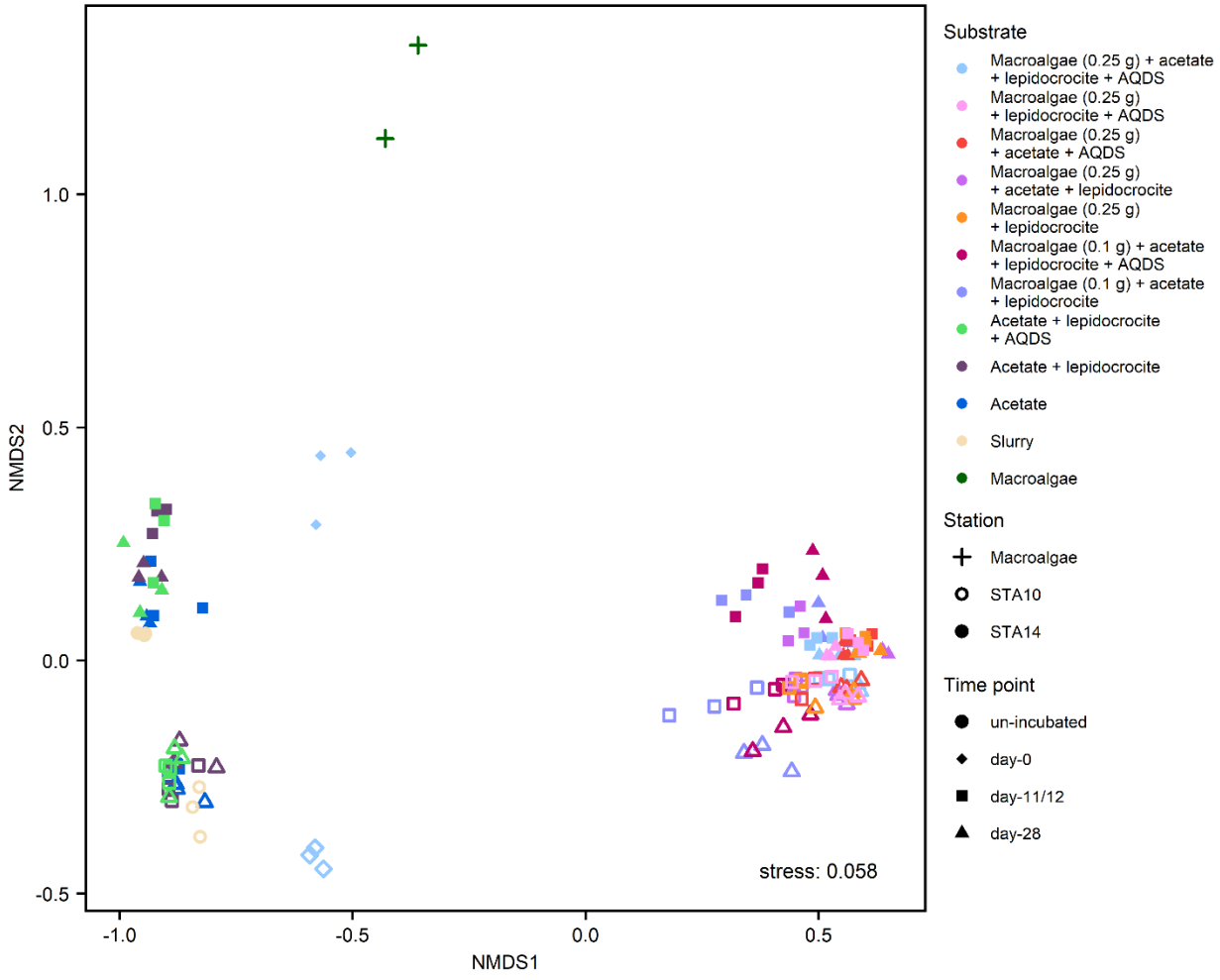


Figure S.3: Non-metric multidimensional scaling (NMDS) of macroalgae incubations based on Bray-Curtis distance matrix of bacterial community.

Table S.1: Initial incubation experiment set up with sediments from both STA10 and STA14.

Treatment	Macroalgae (0.25 g)	Macroalgae (0.1 g)	Acetate (0.5 mM)[a]	Lepidocrocite (5 mM)[a]	AQDS[b] (10 µM)
Macroalgae + acetate + lepidocrocite + AQDS	+	-	+	+	+
Macroalgae + lepidocrocite + AQDS	+	-	-	+	+
Macroalgae + acetate + AQDS	+	-	+	-	+
Macroalgae + acetate + lepidocrocite	+	-	+	+	-
Macroalgae + lepidocrocite	+	-	-	+	-
Macroalgae + acetate + lepidocrocite + AQDS	-	+	+	+	+
Macroalgae + acetate + lepidocrocite	-	+	+	+	-
Acetate + lepidocrocite + AQDS	-	-	+	+	+
Acetate + lepidocrocite	-	-	+	+	-
Acetate	-	-	+	-	-

[a] Final concentration of substrates. [b] 2,6-anthracenedisulfonic acid

Table S.2: RNA stable isotope probing incubations with sediments from STA10. Treatments containing acetate or dissolved inorganic carbon (DIC) were set up in two sets with either labelled [^{13}C] substrate or natural isotope [^{12}C] substrate.

Treatment (n = 3)	**Macroalgae (0.01 g)**	**Acetate (5 mM)**	**DIC (10 mM)**	**Lepidocrocite (5 mM)**	**AQDS[a] (5 µM)**
Macroalgae	+	-	-	-	-
Macroalgae + acetate + lepidocrocite + AQDS	+	[^{12}C]/[^{13}C]	-	+	+
Acetate + lepidocrocite + AQDS	-	[^{12}C]/[^{13}C]	-	+	+
Macroalgae + DIC + lepidocrocite + AQDS	+	-	[^{12}C]/[^{13}C]	+	+
DIC + lepidocrocite + AQDS	-	-	[^{12}C]/[^{13}C]	+	+

[a] 2,6-anthracenedisulfonic acid

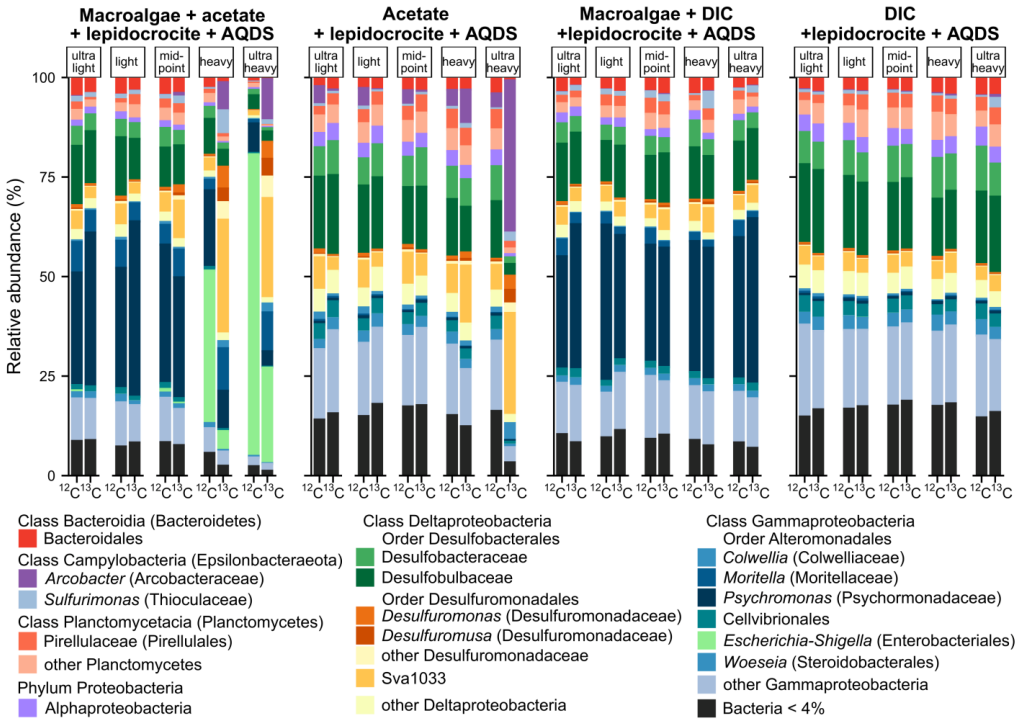
Table S.3: Density of individual fractions from RNA stable isotope probing incubations.

Treatment	Isotope	Fraction	Density (g/mL)
Macroalgae + acetate + lepidocrocite + AQDS	[^{12}C]	ultra-light	1.776-1.783
		light	1.791-1.795
		midpoint	1.803-1.810
		heavy	1.818-1.822
		ultra-heavy	1.830-1.837
	[^{13}C]	ultra-light	1.776-1.779
		light	1.787-1.795
		midpoint	1.799-1.806
		heavy	1.814-1.822
		ultra-heavy	1.826-1.837
Acetate + lepidocrocite + AQDS	[^{12}C]	ultra-light	1.772-1.779
		light	1.783-1.791
		midpoint	1.799-1.806
		heavy	1.814-1.822
		ultra-heavy	1.826-1.833
	[^{13}C]	ultra-light	1.768-1.779
		light	1.783-1.787
		midpoint	1.795-1.803
		heavy	1.810-1.818
		ultra-heavy	1.826-1.830
Macroalgae + DIC + lepidocrocite + AQDS	[^{12}C]	ultra-light	1.772-1.776
		light	1.783-1.787
		midpoint	1.795-1.803
		heavy	1.814-1.818
		ultra-heavy	1.826-1.833
	[^{13}C]	ultra-light	1.768-1.776
		light	1.783-1.791
		midpoint	1.795-1.803
		heavy	1.810-1.818
		ultra-heavy	1.826-1.833
DIC + lepidocrocite + AQDS	[^{12}C]	ultra-light	1.772-1.776
		light	1.779-1.787
		midpoint	1.795-1.803
		heavy	1.810-1.818
		ultra-heavy	1.822-1.830
	[^{13}C]	ultra-light	1.764-1.772
		light	1.779-1.783
		midpoint	1.791-1.799
		heavy	1.806-1.814
		ultra-heavy	1.818-1.826

Removal of unexpected taxa *Escherichia-Shigella* in RNA stable isotope probing incubations

During the analysis of sequence data from the SIP incubations, sequences belonging to the taxa *Escherichia-Shigella* were observed in two SIP fractions (heavy and ultra-heavy) from the macroalgae + acetate + lepidocrocite + AQDS treatment. The most abundant OTUs from these treatments were blasted on NCBI website blastn tool (not shown) to obtain the exact closest taxonomic relative. However, the sequences were both affiliated to *Escherichia* and *Shigella* (99.59% similarity, 100% coverage) without any clear distinctions, a known phenomenon for these taxa on 16S rRNA level (Devanga Ragupathi et al. 2017). To investigate the merits of the enrichment of these sequences as actual biological observations representing activity of *Escherichia-Shigella* in these anaerobic incubations, we compared these treatments with the other treatment containing the labelled substrate [^{13}C]Acetate), however they were not present (see Figure S4 below). Next, comparing the macroalgae + acetate + lepidocrocite + AQDS treatment with similar macroalgae + acetate + lepidocrocite + AQDS treatments from the initial sediment incubation also revealed absence of these sequences (Figure 2, main text). Thus, it is like that these sequences were contaminations introduced to the heavy and ultra-heavy fractions of this RNA-SIP treatment during the molecular work for SIP and they are not present as a result of biological activity. To investigate this, we sequenced samples from the various steps after extracting the original nucleic acid from both the original slurry used to set up the treatment and the treatment slurry itself after 7 days of incubation for RNA-SIP. The un-incubated slurry contained nearly no sequences (< 0.02% relative abundance, considered absent), belonging to *Escherichia-Shigella*. Thus the source of contamination is limited to the ‘after 7-days slurry incubation’. Introduction of contamination by added substrates could be out-ruled because the same substrates (macroalgae, acetate, [^{13}C]Acetate, lepidocrocite, AQDS) were used to amend

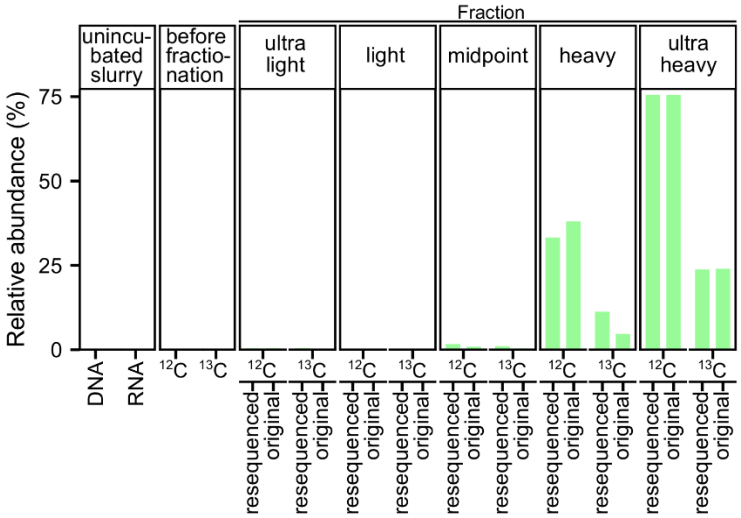
70 the other RNA-SIP treatments, which did not contain any *Escherichia-Shigella* (Figure S4). Prior
71 to density centrifugation and fractionation, sequences affiliated to *Escherichia-Shigella* were
72 very low (0.2% [^{12}C], 0.1% [^{13}C] with low sequence depth of sample, Figure S5) in the RNA and
73 therefore considered absent. Therefore, we conclude that the material used for density
74 centrifugation contained no *Escherichia-Shigella* affiliated sequences. Although these sequences
75 appeared in high relative abundance in the heavy and ultra-heavy RNA-SIP fractions after
76 density centrifugation, it is clear that the contaminants were most likely introduced during
77 handling of the nucleic acids after fractionation. Therefore, these sequences were removed for
78 further analysis of the overall RNA-SIP results. The microbial community composition expected
79 due to macroalgae addition, similarly to the other RNA-SIP treatments (Figure 3, main text) was
80 obtained after removal of the *Escherichia-Shigella* sequences unlike the community composition
81 including the *Escherichia-Shigella* sequences (Figure S4). This therefore validates the decision
82 to remove these sequences for the analysis and interpretation of the RNA-SIP results in the
83 macroalgae + acetate + lepidocrocite + AQDS treatment.



84

85 **Figure S.4:** Bacterial 16S rRNA community of RNA stable isotope probing incubations

86 including taxa *Escherichia-Shigella*.



87

88 **Figure S.5:** Relative abundance of taxa *Escherichia-Shigella* in re-sequenced and original

89 samples at different stages of the working procedure in Potter Cove SIP incubation Macroalgae +

90 acetate + lepidocrocite + AQDS.

Supplementary Materials and Methods

Nucleic Acid Extraction

Modifications from (Lueders et al. 2004) during nucleic acid extraction procedure: briefly, precipitation of nucleic acids from solution was done with two volumes of polyethylene glycol (PEG-6000) followed by centrifugation for 90 min at 15,300 x *g* and 4 ºC. Pellets were washed twice with 500 µL of 70% ethanol (15,300 x g, 5 min at 4 ºC) and eluted in 50 µL of diethylpyrocarbonate (DEPC) treated water (Carl Roth, Germany). Nucleic acid concentrations were measured with a NanoDrop 1000 spectrophotometer (Peqlab Biotechnologie, Erlangen, Germany).

16S rRNA Sequencing and Analysis

The following modifications were made to analyse sequencing reads: before de-replication, forward and reverse reads were joined with minimum 10 bp overlap and de-multiplexed. Quality filtering was performed with a minimum sequence length of 242 bp and an expected error of < 0.5 using QIIME 1.9.0 (Caporaso et al. 2010) and USEARCH 11.0 (Edgar 2010). The 16S rRNA database SILVA release 132 (Quast et al. 2012) was used for taxonomic assignment.
Reads classified as unassigned, archaea, chloroplast and mitochondria, and taxa considered as contamination (see above removal of unexpected taxa Escherichia-Shigella in RNA stable isotope probing incubations) were removed from the OTU table. Different sequencing depth was normalized by scaling OTU abundance to observation totals in each sample ("relative data").

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