Dissolved organic matter released by two marine heterotrophic bacterial strains and its bioavailability for natural prokaryotic communities

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1   **Dissolved organic matter released by two marine heterotrophic bacterial strains**
2   **and its bioavailability for natural prokaryotic communities**

3   Eva Ortega-Retuerta, Quentin Devresse^{*}, Jocelyne Caparros, Barbara Marie, Olivier

4   Crispi, Philippe Catala, Fabien Joux, Ingrid Obernosterer

5   CNRS/Sorbonne Université, UMR7621 Laboratoire d'Océanographie Microbienne, Banyuls sur Mer, France

6   ^{*} Present address: Geomar Helmholtz Centre for Ocean Research Kiel, Germany

7

8   Running title: Bacterial DOM release and bioavailability

10 **Originality-Significance Statement**

11 The role of heterotrophic prokaryotes as organic matter sources in the ocean is
12 increasingly receiving attention by the scientific community in view of its importance
13 for major biogeochemical fluxes, such as carbon sequestration via the “microbial carbon
14 pump”. Here we present novel data on dissolved organic matter (DOM) production by
15 bacterial strains and how this bacterial-derived DOM is degraded by in situ prokaryotic
16 communities and how bacterial DOM shapes microbial metabolism and diversity. We
17 demonstrate that DOM quality produced by bacteria depends on their taxonomy, which
18 translates into different degradation mechanisms and the growth of different microbial
19 communities. Even though a high amount of bacterial derived DOM can be degraded
20 within days, this DOM source would lower the prokaryotic growth efficiency of
21 degrading microbial communities compared to in situ DOM.

Summary

Marine heterotrophic prokaryotes (HP) play a key role in organic matter processing in oceans; however, the view of HP as dissolved organic matter (DOM) sources remains underexplored. In this study, we quantified and optically characterized the DOM produced by two single marine bacterial strains. We then tested the availability of these DOM sources to in situ Mediterranean Sea HP communities. Two bacterial strains were used: *Photobacterium angustum* (a copiotrophic gammaproteobacterium) and *Sphingopyxis alaskensis* (an oligotrophic alphaproteobacterium). When cultivated on glucose as the sole carbon source, the two strains released from 7 to 23% of initial glucose as bacterial derived DOM (B-DOM), the quality of which (as enrichment in humic or protein-like substances) differed between strains. B-DOM promoted significant growth and carbon consumption of natural HP communities, suggesting that it was partly labile. However, B-DOM consistently promoted lower prokaryotic growth efficiencies than in situ DOM. In addition, B-DOM changed HP exoenzymatic activities enhancing aminopeptidase activity when degrading *P. angustum* DOM, and alkaline phosphatase activity when using *S. alaskensis* DOM, and promoted differences in HP diversity and composition. DOM produced by HP affects in situ prokaryotic metabolism and diversity, thus changing the pathways for DOM cycling (e.g. respiration over biomass production) in the ocean.

Keywords: microbial carbon pump, dissolved organic matter, bioavailability, heterotrophic prokaryotes, prokaryotic diversity.

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47 **Introduction**

48 Heterotrophic prokaryotes (HP, bacteria and archaea) are key players in element cycling
49 in the ocean. Roughly 50% of carbon that is fixed by primary production in the ocean is
50 passing through HP (Azam et al., 1983), which can be respired into CO₂ (HP
51 respiration) or used to build up new biomass (HP production). The balance between
52 these two processes, expressed as prokaryotic growth efficiency, is a key parameter to
53 estimate carbon fluxes in the ocean and predict or model its changes (delGiorgio and
54 Cole, 1998). However, it has been demonstrated that HP also release carbon in the form
55 of dissolved organic matter (DOM). Pioneering studies showed that HP growing on
56 simple substrates (i.e. glucose) could produce a myriad of different DOM compounds
57 (Stoderegger and Herndl, 1998; Ogawa et al., 2001; Kawasaki and Benner, 2006). Since
58 then, numerous studies have attempted to characterize HP-derived DOM using
59 analytical techniques such as ultra-high-resolution Fourier transform-ion cyclotron
60 resonance-mass spectrometry (FT-ICR MS), high field nuclear magnetic resonance
61 (NMR) or 3D fluorescence spectroscopy, demonstrating that HP successively process
62 labile DOM and transform it into refractory DOM in an amount enough to sustain the
63 whole oceanic refractory DOM pool (Catalá et al., 2015; Lechtenfeld et al., 2015;
64 Osterholz et al., 2015). This process, termed the microbial carbon pump (MCP, (Jiao et
65 al., 2010) is an important carbon sequestration mechanism. It has been estimated that
66 carbon stored through the MCP accounts for 0.4% of primary production on a global
67 scale; this is in the same range as carbon stored through sinking particulate organic
68 matter (0.6 to 1.3% of primary production (Legendre et al., 2015)).
69 HP release DOM as metabolic by-products or as a consequence of the uncoupling of
70 anabolic and catabolic processes (Carlson et al., 2007). Since HP in the ocean exhibit a

wide range of metabolisms, DOM compounds released to the media are expected to differ among HP taxa, as demonstrated previously using experimental incubations (Shimotori et al., 2012; Noriega-Ortega et al., 2019). HP also actively release DOM compounds for different processes such as polymer enzymatic cleaving (Smith et al., 1992), nutrient acquisition (e.g. siderophores for iron uptake (Andrews et al., 2003), cell-cell communication (e.g. homoseryl lactones for quorum sensing (Gram et al., 2002)) or toxins (Christie-Oleza et al., 2012). Thus, environmental variables could shape the quantity and quality of the DOM released, affecting DOM fluxes. Finally, DOM degradation in the ocean, including HP-derived DOM, could depend on the taxonomic composition of the DOM processing community (Teeling et al., 2012). To better understand and predict changes in carbon sequestration through the microbial carbon pump, accurate information about DOM production by heterotrophic prokaryotes and its cycling in the ocean is a priority. How HP-derived DOM is degraded and cycled, and which fraction of it would be respired to CO₂ or would enter the oceanic refractory DOM pool, are questions that remain to be resolved.

In this study, we aimed to evaluate the availability of DOM released by two single bacterial strains as a substrate for Mediterranean Sea heterotrophic prokaryotic communities. Our working hypotheses were (1) DOM quality differs among bacterial strains and (2) these differences in quality promote the growth of different prokaryotic communities, translated into different DOM degradation mechanisms.

To test these hypotheses, two step experiments were conducted: First, two single bacterial strains with contrasting lifestyles and from different taxonomical classes (*Photobacterium angustum* and *Sphingopyxis alaskensis*) were axenically grown in minimum media with glucose as the only carbon source, and cultures were harvested at early stationary phase where DOM was extracted, quantified and characterized. Second,

96 DOM derived from the strains was used as substrate for biodegradation experiments,
97 where in situ prokaryotic communities from the Mediterranean Sea were grown either
98 on in situ DOM or on the DOM produced by the two strains (Table 1, see experimental
99 procedures). In these experiments, changes in heterotrophic prokaryotic abundance were
100 followed together with changes in carbon and nutrients, exoenzyme activities and
101 prokaryotic community composition.

102

Results

Bacterial strain growth: Glucose and nutrient consumption; BDOM production

The two single strains used in the present study to produce bacterial-derived DOM exhibited contrasting patterns both in their growth rates and carrying capacities (Table 2, Suppl. Fig. 1): *Photobacterium angustum* grew fast (growth rates 1.1-1.5 d⁻¹), this is, once the exponential growth started, the stationary phase was reached about 12h later, with a cell abundance of around 5 x10⁶ cell mL⁻¹ (Suppl. Fig. 1). Conversely, *Sphingopyxis alaskensis* grew slower (growth rates of 0.07-0.23 d⁻¹), with a longer lag-phase (3 to 5 days) but higher cell abundance (12 to 17 x10⁶ cell mL⁻¹, Suppl. Fig. 1) when the stationary phase was achieved.

When cells and DOM were harvested, 12 to 17 µM of carbon as glucose was remaining in the cultures (6-10% of initial glucose concentrations, Fig. 1). However, there was 42 to 73 µM of DOC remaining (Fig. 1). Comparing the decreases in glucose and in DOC at the beginning and at the end of the incubations (see Experimental Procedures), we estimated the amount of DOC that was produced by the bacterial strains. This DOC production was variable, ranging from 6.7% (*S. alaskensis*, March 2018) to 22.8% (*P. angustum*, March 2018) of the initial DOC. Bacteria passing through the filters represented less than 1% of cell abundances, and could only contribute to a tiny part of the DOC measured at the end of the cultures. No clear differences between strains could be observed in the %DOC produced (Table 2). However, the two strains produced DOM of different quality. DOM produced by *P. angustum* was relatively more enriched in protein-like compounds (FDOM Peak T after Coble (1996), 34.3 x10⁻³ R.U. on average) as compared to DOM from *S. alaskensis* (8.9 x10⁻³ R.U. on average). By contrast, all humic-like FDOM peaks were higher for *S. alaskensis*, in particular the M and A peaks that were twice as high as for *P. angustum* (Table 2). Regarding inorganic

nutrients, both strains used preferentially NH_4 over NO_3 , but significant amounts of NH_4 , PO_4 and NO_3 remained in the cultures at the end of the incubations (Supplementary Table 1), indicating that carbon was the limiting substrate for the bacterial growth.

Availability of bacterial-derived DOM for strain-derived natural heterotrophic prokaryotic communities

Biodegradation experiments: Changes in HP abundance, DOC and growth efficiency

DOM originating from the bacterial strains promoted remarkable HP growth in all biodegradation experiments (Fig. 2), with significant differences over time and between treatments in all three experiments (repeated measures ANOVA, $p < 0.05$, Suppl. Table 2). In the open sea-March biodegradation (Table 1), DOM from *P. angustum* supported a higher HP growth for surface and deep HP communities than their respective treatments with *in situ* DOM (Fig. 2a), although only significant for surface communities (Suppl. Table 3). In the coastal sea-April biodegradation experiment, differences in the HP abundances among treatments were only significant at certain timepoints (Fig. 2b, Suppl. Table 3). In the coastal sea-December biodegradation experiment, as in the open sea-March one, the treatments with bacterial DOM promoted significantly higher HP growth than *in situ* DOM (Fig. 2c, Suppl. Table 3). In the coastal sea-December experiment we included a treatment with glucose ($20 \mu\text{mol C L}^{-1}$), but although HP abundance was higher than that in the surface DOM treatment, it was lower than HP abundances in the *S. alaskensis* DOM treatment (Suppl. Table 3). We did not find significant differences in HP growth between treatments using *P. angustum* vs. *S. alaskensis* DOM (Suppl. Table 3).

We performed controls in the degradation experiments, consisting of DOM from each strain, with N and P additions similar to the treatments, but without inoculating with in situ HP communities. The purpose of these controls was to check for potential growth of the minimal amount of *P. angustum* and *S. alaskensis* cells that remained in the filtrates. Little cell growth was observed in these controls (Fig. 2a-c).

At the onset of the three biodegradation experiments, the DOC concentration was slightly different between treatments (Table 3). However, the amount of DOC consumed per hour (during the total incubation) was consistently higher in the treatments with strain derived DOM, except for coastal sea-April experiment Biodegradation experiment and *S. alaskensis* DOM (Table 3). Average DOC consumption in the incubations with strain DOM (both *P. angustum* and *S. alaskensis* DOM) was $0.30 \mu\text{mol L}^{-1} \text{ h}^{-1}$, while in the treatments with in situ DOM, either deep or surface, the average was $0.08 \mu\text{mol L}^{-1} \text{ h}^{-1}$. In the experiments with glucose additions the DOC consumption was on average $0.18 \mu\text{mol L}^{-1} \text{ h}^{-1}$. When normalizing ΔDOC by the initial DOC concentration in each treatment, these patterns were even more visible: 51% on average of the strain-derived DOC was degraded during the incubations, compared to 13.6% of in situ DOC (Table 3).

We further wanted to elucidate whether differences in HP growth were due to different initial amounts of DOC or to differences in its lability. For that purpose, we compared HP biomass changes with initial DOC concentrations in every treatment (Table 3). In contrast to total DOC utilization, we could not find consistent patterns in the relative amount of initial DOC incorporated into HP biomass (Table 3). The %DOC invested into prokaryotic biomass was lower (open sea-March), similar (coastal sea-April) or higher (coastal sea-December) than in the in situ DOM (ndom) treatments. Comparing the treatments with *P. angustum* (pdom) or *S. alaskensis* (sdom), the changes in HP

biomass compared to initial DOC were never significantly different. Interestingly, prokaryotic growth efficiency (PGE) was consistently lower in treatments with strain-derived DOM than in the treatments with in situ DOM in all biodegradation experiments by a mean factor of 3.5 (range 1.3-7.0) (Fig. 3).

Biodegradation experiments: Changes in FDOM and inorganic nutrients

The different FDOM peaks did not decrease at the same level in every incubation (Table 3): The peak T (surrogate of protein-like substances) tended to decrease up to a 70%, while the FDOM at peak C (surrogate of humic-like compounds) tended to increase in almost every experiment and treatment (Table 3). Humic-like peaks A and M were more variable among experiments and treatments.

The inorganic nutrient concentrations, particularly those of NO_3^- , were also higher in the treatments with bacterial-derived DOM as compared to the in situ DOM, since *P. angustum* and *S. alaskensis* did not consume all inorganic nutrients during the previous strain incubations and equivalent amounts of all inorganic nutrients were added to each treatment of the biodegradation experiments. At the end of the incubations, substantial amounts of all inorganic nutrients remained in all treatments (Supplementary Table 1).

Coastal biodegradation experiments: Changes in exoenzymatic activity

Differences in the predominant exoenzymatic activities could be observed between treatments in Coastal sea-April and December experiments. In May, relatively high alpha- and beta- glucosidase production rates were observed in the treatment with surface DOM (Fig. 4 a,b) compared to the bacterial DOM treatments, but this result was not visible in December (Fig. 4 e,f). Remarkably, in both biodegradation experiments *P. angustum* DOM induced high rates of leucine aminopeptidase (Fig. 4 c and g), while the *S. alaskensis* DOM always induced high rates of alkaline phosphatase (Fig. 4 d and h).

Coastal-December Biodegradation experiment: Changes in prokaryotic community composition

Changes in HP diversity and community composition along the incubations were detected in all treatments and replicates of Coastal sea-December experiment, the only experiment where these samples were taken. A decrease in diversity between the onset and end of the incubations, expressed as richness, evenness or Shannon diversity, was detected in all treatments. Significant differences between treatments were observed at t (ANOVA, $p < 0.001$ for all richness, evenness and Shannon). HP communities growing on surface DOM had higher richness, evenness and Shannon than the communities growing on bacterial-derived DOM (Table 4).

At the onset of the experiments, the HP community was distributed among different orders, with Alphaproteobacteria (groups of SAR11, Rhodobacterales and Rhodospirillales), SAR86 (Gammaproteobacteria) and Flavobacteriales as the most abundant groups (Fig. 5). At the end of the incubations, differences between treatments could be detected (ANOSIM test, $p < 0.05$): The surface DOM treatment was dominated by Alteromonadales, Flavobacteriales and SAR86, the surface DOM +glucose was dominated by Vibrionales and Flavobacteriales (except for replicate 3 where Oceanospirillales were comparatively more abundant), the *P. angustum* DOM treatments were dominated by Alteromonadales, Rhodobacterales, Flavobacteriales (but replicate 2 showed a potential contamination by Campylobacter) and the *S. alaskensis* DOM treatment was dominated by Vibrionales, with more than half of the sequences corresponding to this order, but also Rhodospirillales. A MDS ordination based on Bray Curtis similarities showed separation among treatments with replicates grouping together, illustrating the significant differences (ANOSIM test, $p < 0.05$) in HP structure (Fig. 5).

Discussion

Comparison of DOC release rates among studies (strains vs in situ communities)

The bacterial DOC release rates reported here (7 to 23% of initial DOC, average 12%) are within those previously published for mixed/natural communities (3.5-22%) but higher than the four published studies using single bacterial strains (3.7% on average, Table 5). All previously published studies using single strains used 5 to 350 times higher initial DOC concentrations than in the present study, and some only quantified the DOM retained by solid-phase cartridges (Romano et al., 2014) or only the DOM released as extracellular polysaccharide (Zhang et al., 2015), making the values lower. The only study measuring DOC production by Archaea through chemoautotrophy also reported DOM release within our ranges when looking at DOM produced vs. biomass produced (Bayer et al., 2019). We could expect that the net DOM production by natural communities would be lower than the ones recorded using single strains, since DOM produced by some HP groups could be consumed by others. However, our DOM production values were within the range of previous studies using mixed communities (i.e., 3.5-22%, Table 5). The DOM production values obtained in our strain incubations could be conservative estimates. On one hand, the method used to quantify glucose (Myklestad et al. 1997) also determines other dissolved monosaccharides. Although it is very unlikely that the strains would have released these compounds without making use of them, their potential presence would lead to an underestimation of the DOM release rates in our experiments. On the other hand, HP release DOM not only as metabolic by-products, but also with functional roles such as cell-signaling (Gram et al., 2002) or particle degradation (Smith et al., 1992) among many others, processes that would not be happening in our simple glucose-amended monocultures, so DOM production in situ by HP would likely be higher than the calculated in our study. For instance, substrate

quality is likely crucial determining the quantity and quality of DOM produced: Ogawa et. al. (2001) and Wienhausen et. al. (2017) showed higher release of dissolved amino acids by heterotrophic bacterial strains when growing on glutamate than when growing on glucose, even though the amino acid composition was similar among treatments. If grown on complex substrates, their enzymatic degradation would likely enhance the release of DOM by HP (Aparicio et al., 2015). Further work is needed to explore which would be the main nutritional drivers, e.g. going beyond glucose amendments to testing the effect of the DOM source, of DOM production by HP in the sea.

Quantity and quality of bacterial DOM

Even though the quantity of DOM released by our two study strains was similar, we could evidence clear differences in its fluorescence signature, which suggests different chemical composition. The production of protein-like FDOM observed for *P. angustum* in the present study, has been previously reported for *Alteromonas macleodii* when grown on glucose (Goto et al., 2017) and for mixed bacterial communities grown on phytoplankton exudates (Romera-Castillo et al., 2011) or on lake water (Cammack et al., 2004). By contrast, the low protein-like FDOM signature in *S. alaskensis* DOM illustrates the importance of bacterial taxonomy for bacterial FDOM quality. *P. angustum* (our study) and *A. macleodii* (Goto et al., 2017) are both fast-growing copiotrophic bacteria, suggesting a link between aspects of bacterial metabolism and protein-like FDOM release. This is in line with the fact that copiotrophs have a higher number of secreted proteins than oligotrophs (Lauro et al., 2009), thus the quantity and composition of the secreted DOM would be likely dependent on the expression and production of these proteins. The production of humic-like DOM by bacteria has been shown using single strains (Shimotori et al., 2012) and experimental incubations with mixed communities (Rochelle-Newall and Fisher, 2002; Romera-Castillo et al., 2011).

Humic-like FDOM (peaks A, C and M) is released to the ambient seawater as a byproduct of organic matter remineralization by HP, and has been used as a tracer for refractory DOM in the ocean (Yamashita and Tanoue, 2008; Catalá et al., 2015). Therefore, we hypothesized that DOM produced by *S. alaskensis*, more rich in humic-like compounds, would be less available for HP degradation in our experiments. Few previous published studies have tested bacterial-DOM degradation by in situ HP communities. Both Kramer and Herndl (2004) and Zhang et al. (2015), these latter authors only using the exopolysaccharide fraction of bacterial DOM, concluded that DOM derived from mixed communities was mostly refractory, since no significant growth was detected during 2 to 14 days. However, Lønborg et al. (2009) followed DOC changes over longer time periods (30 d), and concluded that at least a fraction of HP-derived DOM can be utilized by other prokaryotic communities, indicating that HP-DOM is semilabile. This is in accordance with our results, showing that 51% of organic carbon produced by the strains was further used by in situ HP communities (Table 3).

Influence of HP community composition for the degradation of bacterial DOM

In contrast to our hypothesis, differences in the lability of *P. angustum* vs. *S. alaskensis* DOM could not be demonstrated by our biodegradation experiments, as inverse growth patterns on the two strain-derived DOM treatments were observed between coastal communities from April and December (Fig. 2). This suggests that not only the quality of the bacterial DOM, but also the in situ HP community composition would affect bacterial DOM degradation, as previously shown (Nelson and Wear, 2014). Our study strains released DOM that could not be used by themselves, since little bacterial growth of the residual cells was observed even when reinoculating with inorganic nutrients (control treatments in biodegradation experiments, Fig. 2), although this could be due to the possible damage of those cells after freezing. Whether the bacterial DOM can be

degraded by single strains, as shown for labile in situ DOM and *Alteromonas macleodii* (Pedler et al., 2014), or whether the complementary metabolic strategies of a diverse community are required, remains to be investigated. The remarkable growth of in situ HP communities was not only due to the glucose that remained in the pdom and sdom treatments, since HP abundance was in both cases higher than in the +glucose treatment. A possibility that we cannot exclude is that this remaining glucose boosted the utilization of other substrates. Differences in HP growth among experiments can be explained not only by differences in the in situ communities but also by in situ DOM variability: In late spring in the Mediterranean Sea, DOM is usually enriched in labile compounds after the spring phytoplankton bloom (Jones et. al., 2013, Ortega-Retuerta et. al., 2018), so the in situ DOM was likely more labile than bacterial derived DOM, in contrast to December, when DOM can have other signatures (i.e. terrestrial-like, Sanchez-Pérez et. al., 2020).

In fact, despite non-significant differences in HP growth, consistent patterns of exoenzyme activity were detected: *P. angustum* DOM always promoted high leucine-aminopeptidase activity rates (Fig. 4), in line with its relative enrichment in protein-like FDOM compounds (Table 2). By contrast, *S. alaskensis* DOM consistently promoted relatively high levels of alkaline phosphatase (Fig. 4). This last result was intriguing to us, since inorganic phosphorus was added at the beginning of each biodegradation experiments and thus HP communities were not P-limited. Although high alkaline phosphatase activities have been traditionally interpreted as P limitation (Cotner et al., 1997; Thingstad et al., 1998), HP can use these exoenzymes to release available dissolved organic carbon, as pointed out by previous studies (Benitez-Nelson and Buesseler, 1999; Van Wambeke et al., 2002). Our results suggest that *S. alaskensis* DOM could be rich in phosphorus-containing groups. We could see (only in the coastal

December experiment) that the *S. alaskensis* DOM treatment was the one with a higher proportion of Rhodospirillales. This order has been found in P-limited ocean areas linked to the presence of the carbon-phosphorus lyase pathway for phosphonate degradation (Sosa et al., 2019), in accordance with our hypothesis of P-rich DOM produced by *S. alaskensis*.

Bacterial DOM sustained a lower HP diversity as compared to the in situ DOM (Table 4). In line with this, Noriega-Ortega et al. (2019) compared the solid-phase extractable DOM produced by single bacterial strains with that of marine DOM from the North Pacific and observed higher diversity of compounds in marine DOM respect to bacterial-derived DOM. HP strains were shown to produce thousands of DOM molecules when using a single carbon substrate, however, the DOM chemical diversity produced by a single bacterium with respect to DOM from coastal seawater is likely to be lower. In any case, the significant differences in community composition between treatments suggest population-specific responses to the differences in quality of the DOM produced by *P. angustum* and *S. alaskensis*.

Bacterial DOM, PGE and carbon fluxes

The growth on strain-derived DOM induced significantly lower prokaryotic growth efficiency (PGE). To the best of our knowledge, our study provides the first measurements of PGE on bacterial-derived DOM, demonstrating that bacterial DOM is preferentially respired. HP do not only promote DOC accumulation in the ocean through the production of refractory DOM (the microbial carbon pump hypothesis shown in previous studies), but would also enhance carbon remineralization (therefore, acting as a CO₂ source). Noteworthy, we did not include DOM production by natural HP communities in our PGE calculations in the biodegradation experiments, and

including DOM production in our calculations would lead to even lower PGE values in all treatments.

Changing the paradigm of carbon fluxes through HP in the ocean

DOM release by HP has been repeatedly demonstrated, however, carbon (and other elements) release by HP as DOM is still overlooked when constraining carbon budgets or other elemental fluxes and models in the ocean. For instance, considering that HP produce, on average, 10% of DOC as DOM (average from values in Table 5), in situ HP production estimates (e.g. using isotopes incorporation such as ^3H -leucine, (Kirchman et al., 1985) would be underestimated. To better constrain this uncertainty, it is important to know whether DOM release is linearly related to HP biomass production during short incubation times. The release of DOM would also affect estimates of HP growth efficiency. Prokaryotic carbon demand can be calculated as the sum of HP biomass production and respiration or as differences in DOC concentrations in bottle incubations (the dilution approach (delGiorgio and Cole, 1998)), but in both cases PGE would be overestimated by roughly 10%, according to published results. The overestimation could even be higher if, as demonstrated in our study, this 10% of DOM produced by HP would enhance respiration over biomass production. More studies are needed to better understand the dynamics of DOM production by HP and their drivers, and therefore to constrain carbon fluxes through HP.

Conclusion and perspectives

In this study, we demonstrate that two marine heterotrophic bacterial strains growing on glucose release remarkable amounts of DOM (compared with biomass production), and that an important fraction of this bacterial derived DOM is available for Mediterranean Sea HP communities. Should this pattern be observed globally, the production of DOM

by prokaryotes in the sea would change carbon fluxes through HP towards a decrease in prokaryotic growth efficiency, as well as shaping HP community and exoenzyme activities. While in the present study DOM release is measured in single strains growing on glucose, the uptake of other organic compounds will likely affect the quantity and lability of the released DOM, hence affecting further metabolisms in the ocean. Further work is needed to better constrain the main mechanisms affecting HP DOM production and degradation in the ocean, thus improving our knowledge on carbon sequestration processes such as the microbial carbon pump.

Experimental procedures

Bacterial strains growth

In order to get dissolved organic matter (DOM) produced by marine bacteria, we grew axenically two strains with contrasting ecology and different taxonomical classes: The first one, *Photobacterium angustum* S14, is a Gammaproteobacterium (Vibrionales, Vibrionaceae) which was isolated from coastal waters (Humphrey et al., 1983). The second strain, *Sphingopyxis alaskensis* RB2256 (Sphingomonadales, Sphingomonadaceae), is an Alphaproteobacterium isolated from open waters in the North Pacific Ocean (Eguchi et al., 2001). These bacterial strains can be considered as models of copiotrophic (*P. angustum*) and oligotrophic (*S. alaskensis*) life styles (Lauro et al., 2009). The strains were selected based on their capacity to grow on glucose and the laboratory's experience in culturing them (Matallana-Surget et al., 2009; Koedooder et al., 2018). Both strains were preserved in glycerol at -80°C. The stocks were thawed and grown in minimum media, modified from Fegatella et al. (1998), composed of artificial seawater (salinity 35) with glucose as the sole carbon source ($\approx 200 \mu\text{M C}$), nitrogen (50:50 mixture of NH_4Cl and NO_3) and phosphorus (NaHPO_4) at a C:N:P ratio

of 45:9:1, equivalent to the internal stoichiometry of bacterial cells (Goldman et al., 1987), and trace amounts of metals and vitamins. In order to minimize the presence of glycerol in the media and get the cells adapted to the growth conditions, two acclimation steps were made, where cells were diluted on fresh media (initial concentration $\approx 10^4$ cell mL⁻¹) and grown to $1-2 \times 10^6$ cell mL⁻¹. Purity of the cultures was controlled at each step by plating samples on Marine Agar. Experimental incubations were set up in triplicate in 500 mL flasks, with two contamination controls, consisting of flasks containing culture media but without bacterial inoculation. Incubations were performed at 25°C with rotary agitation (90 rpm) until the stationary phase was reached (controlled several times per day by flow cytometry). No bacterial growth was detected in the contamination controls. Once the stationary phase was reached, the DOM was harvested by gentle filtration through pre-rinsed (Milli-Q water, 250 mL) 0.2 µm polycarbonate filters using an all-glass filtration system. All glass material used for the incubations and filtrations was previously combusted (450°C, 5h). Previous tests (dissolved organic carbon concentration and DOM fluorescence measurements on Milli-Q water filtered using the same device and filters) showed insignificant DOM leaching from the polycarbonate filters (data not shown). In the DOM filtrates, samples for glucose, dissolved organic carbon (DOC), inorganic nutrients, DOM fluorescence and flow cytometry (to check for the presence of cells passing through the filters) were taken, and the remaining DOM was kept at -20°C before being used as substrates for biodegradation experiments (see below). We chose to sample in the early stationary phase to ensure that minimal amounts of glucose were remaining in the DOM extracts for the biodegradation experiments, although at this stage some of the bacterial DOM could derive from cell lysis. Strain cultures p1 and s1 (Fig. 1) were used for the biodegradation experiments 1 and 2, while strain cultures p2

and s2 were used for biodegradation experiment 3. Being aware that freezing could alter some DOM properties, we chose this preservation method due to the inability to perform the biodegradation experiments shortly after the strain incubations were finished.

Biodegradation experiments

To study how DOM derived from the bacterial strains is degraded by natural marine heterotrophic prokaryotic (HP) communities, re-growth cultures were performed (Table 1). These consisted of 10% dilutions of natural HP communities from different NW Mediterranean Sea locations (water prefiltered by 0.8 μm to remove most phytoplankton and grazers) with 90% DOM (0.2 μm filtrates) from the following sources: DOM sampled from the same waters as the HP communities (in situ surface or deep DOM, ndom treatments) or DOM harvested from the strain cultures explained above: *Photobacterium angustum* (*P. angustum* DOM treatments, pdom) or *Sphingopyxis alaskensis* (*S. alaskensis* DOM treatments, sdom). DOM for ndom treatments was prepared by filtering seawater from the same location as the prokaryotic inocula through 0.2 μm filters as for the bacterial DOM. Inorganic nutrients (nitrogen (50:50 mixture of NH_4Cl and NO_3) and phosphorus (NaHPO_4), final concentrations in supplementary Table 1) were added to each treatment to force carbon limitation, and all treatments were set up in triplicate in 250 mL flasks. Incubations were performed at 18°C in the dark for 7-8 days.

Using this approach, we performed three different experiments: Open sea-March experiment: We selected waters from a station located on the edge of the continental shelf (hereafter termed as “open sea”) in the North Western Mediterranean Sea (42°27'205 N – 03°32'565 E, 600m depth) at two different depths: 5m (surface communities) and 500m (deep communities), in March 2018. These natural HP

communities were grown on in situ DOM (surface or deep DOM) or on *P. angustum* DOM, with the purpose to evaluate the differences in HP growth depending on the initial community (surface vs. deep marine communities). For this experiment, samples for DOC, FDOM and nutrients were taken at the onset and end of the incubations, while HP abundance was monitored daily by flow cytometry. For the Coastal sea-April experiment, we collected surface waters from a coastal station (42°29'3N, 3°8'7E, 20m bottom depth) in April 2018 and grew HP communities on in situ DOM, DOM produced by *P. angustum*, and DOM produced by *S. alaskensis*, to evaluate growth of the same HP community on different DOM sources. In this experiment, we monitored the same variables as in Open sea-April experiment and, in addition, we measured the exoenzyme activity at the onset, day 3, and end of the experiment. For the Coastal sea-December experiment, we collected surface waters from the same coastal station as for biodegradation Experiment 2 (42°29'3N, 3°8'7E, 20m depth), but in December 2018. The treatments were the same as in May 2018 and thus allowed us to investigate seasonal differences. We included an additional “+Gluc” treatment to evaluate the effect of residual glucose amounts in the bacterial DOM sources. In this last experiment, we monitored the same variables as in biodegradation experiment 2 (DOM, nutrients, HP abundance and exoenzyme activities) and, additionally, we measured the HP community structure (16s rRNA Illumina sequencing) at the onset and end of the experiment.

Chemical and biological analyses

Dissolved organic carbon (DOC)

Samples (10 mL) for DOC were transferred into precombusted glass tubes, acidified with 85% H₃PO₄ (final pH 2), closed with Teflon lined screw caps and stored in the

dark at room temperature until analysis (less than 2 months after sampling). Calibration curves were made using an acetanilide solution ($\text{C}_8\text{H}_9\text{NO}$; $M = 135.17 \text{ g mol}^{-1}$). DOC was analysed using the high temperature catalytic oxidation (HTCO) technique (Benner and Strom, 1993) with a Shimadzu TOC-V-CSH analyser. Standards of $44\text{--}45 \mu\text{mol C L}^{-1}$, provided by D.A. Hansell and Wenhao Chen (Univ. of Miami), were used to assess the accuracy of the measurements.

Glucose

Glucose was quantified using a modification of the colorimetric protocol described by Myklestad et al. (1997). Samples ($200 \mu\text{L}$) were stored in pre-combusted glass vials at 20°C until analysis. All reagents were downscaled to $200 \mu\text{L}$ samples, and incubations after reagent A additions were performed during 20 min in a 100°C water bath. The reagents were calibrated daily using a standard curve made of D-glucose in the same batch of artificial seawater used for the strain incubations. Milli-Q water and artificial sea water blanks were run daily in triplicate. Glucose concentrations in μmol were converted in carbon equivalent (C-Glucose).

Fluorescent dissolved organic matter (FDOM)

FDOM samples were freshly (within 1-3 h) analyzed with a Perkin Elmer luminescence spectrometer LS 55 equipped with a xenon discharge lamp, equivalent to 20 kW. Slit widths were 10.0 nm for the wavelengths of excitation and emission. We characterized 4 groups of fluorophores using the following excitation/emission (ex/em) pairs (Coble, 1996): 280 nm/350 nm (peak T, surrogate of protein-like substances); 250 nm/435 nm (peak A); 340 nm/440 nm (peak C) and 320 nm/410 nm (peak M) the later three due to humic substances of different origins (Coble, 1996). Fluorescence intensities of the peaks were reported in Raman units (R.U.) obtained by dividing the fluorescence units

496 by the Milli-Q blank peak area (Raman scatter) excited at 350 nm.

497 *Dissolved inorganic nutrients*

498 Samples for inorganic nutrient analyses were kept frozen at -20°C. NO₃+NO₂, and PO₄
499 were quantified with a segmented flow analyser (Bran Luebbe) with colorimetric
500 detection using methods described in Holmes (1999). The accuracy of the methods was
501 assessed using reference material (Certipur, Merck). The precisions were in the range of
502 1–4%, and the detection limits were 0.02 µM for NO₃+NO₂, and 0.03 µM for PO₄. NH₄
503 samples were collected into pre-rinsed 20 mL HDPE bottles and stored frozen at -20°C
504 before being analysed by fluorometric detection (Holmes, 1999).

505 *Bacterial and heterotrophic prokaryotic abundance*

506 Cell abundances in all experiments were monitored by flow cytometry. Subsamples
507 were fixed with glutaraldehyde (0.5% final concentration) and preserved at -80°C prior
508 to analysis (within days). Cell abundance was measured by using Beckman CytoFLEX
509 and BD FACSCanto flow cytometers equipped with lasers exciting at 488 nm. Samples
510 were thawed and stained with SYBR Green (0.025% (v/v) final concentration).
511 Fluorescent beads (1 µm) were added as internal standards. Samples were run at low
512 speed (10 to 16 µL min⁻¹) for 60 to 90 seconds. Prokaryote communities were identified
513 in plots of side scattered light vs. green fluorescence (del Giorgio et al., 1996).

514 *Extracellular enzyme activities*

515 Extracellular enzyme activities were quantified with the use of fluorogenic substrates
516 (Hoppe, 1983) with the modifications for plate readers described in Sala et al. (2016).
517 Each sample was pipetted in triplicate into 96-well black plates with the following
518 substrates: 4-methylumbelliferyl β-D-glucopyranoside (for β-glucosidase), 4-

519 methylumbelliferyl α -D-glucopyranoside (for α -glucosidase), 4-methylumbelliferyl
520 phosphate (for alkaline phosphatase), and L-leucine-7-amido-4-methyl coumarin (for
521 leu-aminopeptidase) added (final concentration 120 $\mu\text{mol L}^{-1}$). Fluorescence was
522 measured immediately after addition of the substrate and after incubations (up to 5h
523 with one middle timepoint) in the dark at the same temperature as for the experimental
524 incubations. Fluorescence readings were done with a Victor3 Perkin Elmer
525 spectrofluorometer at 355/450 nm ex/em wavelengths. The increase of fluorescence
526 units during the period of incubation was converted into enzymatic activity with a
527 standard curve prepared with the end products of the reactions, 7-amido-4-
528 methylcoumarin (MCA) for leu-aminopeptidase and 4-methylumbelliferone (MUF) for
529 the rest of enzymes.

530 *DNA extraction and sequencing*

531 Samples (110 mL) for bacterial diversity were filtered onto 0.2 μm pore size
532 polycarbonate filters. The 0.2 μm filters were stored at $-80\text{ }^{\circ}\text{C}$ until analysis. For
533 analysis, frozen filters were cut with sterilized scissors into small strips and vortexed
534 briefly in 750 μL of lysis buffer (50 mmol L^{-1} Tris hydrochloride pH 8.3, 40 mmol L^{-1}
535 EDTA and 0.75 mol L^{-1} sucrose) and then subjected to three freeze-thaw cycles
536 (immersed successively into liquid nitrogen and hot water). Cell lysis was accomplished
537 by an initial incubation for 45 min at $37\text{ }^{\circ}\text{C}$ after adding 41 μL of freshly prepared
538 lysozyme solution (36 mg mL^{-1}), and a second incubation at $55\text{ }^{\circ}\text{C}$ for 1 h after adding
539 80 μL of 10% sodium dodecyl sulfate and 8 μL of proteinase K (20 mg mL^{-1}). After
540 denaturation and degradation of proteins, DNA was purified using a Quick-DNA^{TM}
541 fungal/bacteria miniprep kit (Zymo Research, Catalog No. D6005) according to the
542 manufacturer's instructions.

The V4-V5 region of the 16S rRNA gene from both fractions was amplified with the primer sets 515F-Y (5'-GTGYCAGCMGCCGCGGTAA) and 926-R (5'-CCGYCAATTYMTTTRAGTTT) as described in Parada et al. (2016) with a modification to the PCR amplification step. Triplicate 20 µL reaction mixtures contained 2 µg DNA, 5 µl KAPA2G Fast HotStart ReadyMix, 0.2 µM forward primer and 0.2 µM reverse primer. Cycling reaction started with a 3 min heating step at 95°C followed by 30 cycles of 95°C for 45 s, 50°C for 45 s, 68°C for 90 s, and a final extension of 68°C for 5 min. The presence of amplification products was confirmed by 1% agarose electrophoresis and triplicate reactions were pooled. The pooled PCR amplicons were purified using Sephadex G-50 Superfine resin (GE Healthcare Bio-Sciences, New Jersey, USA) following the protocol. The purification step aims to desalt the samples and eliminate unincorporated nucleotides and excess PCR primers. 16S rRNA gene amplicons were sequenced with Illumina MiSeq 2 × 250 bp chemistry on one flow-cell at GeT-PlaGe platform (Toulouse, France). A mock community DNA (LGC standards, UK) was used as a standard for subsequent analyses and considered as a DNA sample for all treatments.

All samples from the sequencing run were demultiplexed by GeT-PlaGe and barcodes were trimmed off. Processing of sequences was performed using the DADA2 pipeline (version 1.10) (Callahan et al., 2016) in R (version 3.4.2) with following parameters: trimLeft=c(19, 20), truncLen=c(240, 200), maxN=0, maxEE=c(2, 2), truncQ=2. Briefly, the pipeline combines the following steps: filtering and trimming, dereplication, sample inference, chimera identification, and merging of paired-end reads. It provides exact amplicon sequence variants (ASVs) from sequencing data with one nucleotide difference instead of building operational taxonomic units (OTUs) based on sequence similarity. ASVs were assigned against SILVA release 132 database (Quast et al.,

2013). Singletons and sequences assigned to chloroplast and mitochondria were removed prior to subsequent analyses. Sequences were submitted to NCBI under the accession number PRJNA630555

Data analyses

In the strain incubations, we calculated the % of initial DOC transformed to bacterial-derived DOM (%DOC) as the difference between final carbon as glucose (C-Glucose) and dissolved organic carbon (DOC) along the incubations as follows:

$$\%DOC = \frac{DOC_{tf}(\mu\text{mol L}^{-1}) - C\text{-Glucose}_{tf}(\mu\text{mol L}^{-1})}{DOC_{t0}(\mu\text{mol L}^{-1})} \quad (1)$$

This equation would be applicable only if at t_0 , DOC and glucose values were equal. However, there were slight differences between these two pools at t_0 in the *S. alaskensis* cultures s1 and s2 (Fig. 1). Because glucose was the sole organic carbon source added to the cultures (final concentration of 200 μM C-glucose), the difference is likely due to the introduction of DOC contamination while manipulating the cultures, or due to analytical differences between the glucose and DOC analyses.

Thus, we decided to consider this initial DOC excess ($DOC(t_0) - C\text{-glucose}(t_0)$) as a non-reactive pool and subtract it from the concentrations at the beginning and end of each incubation. This is the most conservative way to calculate the %DOC released as DOM by the strains, since, alternatively, the strains could have taken up some of this extra DOC and then released a fraction of it back as bacterial DOM.

Prokaryotic growth efficiency (PGE) in the biodegradation experiments was calculated as the increase in cell biomass between t_0 and t_f divided by the decrease in DOC between t_0 and t_f in the incubations using this formula:

$$PGE (\%) = \frac{\Delta\text{Biomass} (\mu\text{mol L}^{-1})}{\Delta\text{DOC} (\mu\text{mol L}^{-1})} \times 100 \quad (2)$$

using a general cell to biomass conversion factor of 20 fg cell⁻¹ (Watson et al., 1977).

Differences in HP abundance between treatments in the biodegradation experiments were tested using repeated measures ANOVA and post-hoc pairwise t-tests (package “rstatix” in R). Differences in prokaryotic community structure were tested by ANOSIM tests (package “vegan” in R).

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References

- Andrews, S.C., Robinson, A.K., and Rodríguez-Quñones, F. (2003) Bacterial iron homeostasis. *FEMS Microbiology Reviews* **27**: 215-237.
- Aparicio, F.L., Nieto-Cid, M., Borrull, E., Romero, E., Stedmon, C.A., Sala, M.M. et al. (2015) Microbially-mediated fluorescent organic matter transformations in the deep ocean. Do the chemical precursors matter? *Frontiers in Marine Science* **2**.
- Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyerreil, L.A., and Thingstad, F. (1983) The ecological role of water-column microbes in the sea. *Marine Ecology-Progress Series* **10**: 257-263.
- Bayer, B., Hansman, R.L., Bittner, M.J., Noriega-Ortega, B.E., Niggemann, J., Dittmar, T., and Herndl, G.J. (2019) Ammonia-oxidizing archaea release a suite of organic

618 compounds potentially fueling prokaryotic heterotrophy in the ocean. *Environ*
619 *Microbiol* **21**: 4062-4075.

620 Benitez-Nelson, C., and Buesseler, K.O. (1999) Variability of inorganic and organic
621 phosphorus turnover rates in the coastal ocean. *Nature* **398**: 502-505.

622 Benner, R., and Strom, M. (1993) A critical evaluation of the analytical blank
623 associated with DOC measurements by high-temperature catalytic oxidation. *Marine*
624 *Chemistry* **41**: 153-160.

625 Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J., and Holmes,
626 S.P. (2016) DADA2: High-resolution sample inference from Illumina amplicon data.
627 *Nat Methods* **13**: 581-583.

628 Cammack, W.K.L., Kalff, J., Prairie, Y., and Smith, E.M. (2004) Fluorescent dissolved
629 organic matter in lakes: Relationships with heterotrophic metabolism. *Limnol Oceanogr*
630 **49**: 2034-2045.

631 Carlson, C.A., Giorgio, P.A.d., and Herndl, G.J. (2007) Microbes and the Dissipation of
632 Energy and Respiration: From Cells to Ecosystems. *Oceanography* **20**: 89-100.

633 Catalá, T.S., Reche, I., Fuentes-Lema, A., Romera-Castillo, C., Nieto-Cid, M., Ortega-
634 Retuerta, E. et al. (2015) Turnover time of fluorescent dissolved organic matter in the
635 dark global ocean. *Nat Commun* **6**: 5986.

636 Christie-Oleza, J.A., Pina-Villalonga, J.M., Bosch, R., Nogales, B., and Armengaud, J.
637 (2012) Comparative proteogenomics of twelve *Roseobacter* exoproteomes reveals
638 different adaptive strategies among these marine bacteria. *Mol Cell Proteomics* **11**:
639 M111 013110.

640 Coble, P.G. (1996) Characterization of marine and terrestrial DOM in seawater using
641 excitation-emission matrix spectroscopy. *Marine Chemistry* **51**: 325-346.

642 Cotner, J.B., Ammerman, J.W., Peele, E.R., and Bentzen, E. (1997) Phosphorus-limited
643 bacterioplankton growth in the Sargasso Sea. *Aquat Microb Ecol* **13**: 141-149.

644 del Giorgio, P.A., Bird, D.F., Prairie, Y.T., and Planas, D. (1996) Flow Cytometric
645 Determination of Bacterial Abundance in Lake Plankton with the Green Nucleic Acid
646 Stain SYTO 13. *Limnology and Oceanography* **41**: 783-789.

647 delGiorgio, P., and Cole, J.J. (1998) Bacterial growth efficiency in natural aquatic
648 ecosystems. *Annu Rev Ecol Syst* **29**: 503-541.

649 Eguchi, M., Ostrowski, M., Fegatella, F., Bowman, J., Nichols, D., Nishino, T., and
650 Cavicchioli, R. (2001) *Sphingomonas alaskensis*, Strain AF01: an abundant oligotrophic
651 ultramicrobacterium from the North Pacific. *Appl Environ Microbiol* **67**.

652 Fegatella, F., Lim, J., Kjelleberg, S., and Cavicchioli, R. (1998) Implications of rRNA
653 Operon Copy Number and Ribosome Content in the Marine Oligotrophic
654 Ultramicrobacterium *Sphingomonas* sp. Strain RB2256. *Appl Environ Microbiol* **64**:
655 4433-4438.

656 Goldman, J.C., Caron, D.A., and Dennett, M.R. (1987) Regulation of gross growth
657 efficiency and ammonium regeneration in bacteria by substrate c-n ratio. *Limnology and*
658 *Oceanography* **32**: 1239-1252.

659 Goto, S., Tada, Y., Suzuki, K., and Yamashita, Y. (2017) Production and Reutilization
660 of Fluorescent Dissolved Organic Matter by a Marine Bacterial Strain, *Alteromonas*
661 *macleodii*. *Front Microbiol* **8**: 507.

662 Gram, L., Grossart, H.P., Schlingloff, A., and Kiorboe, T. (2002) Possible quorum
663 sensing in marine snow bacteria: production of acylated homoserine lactones by
664 *Roseobacter* strains isolated from marine snow. *Appl Environ Microbiol* **68**: 4111-4116.

665 Holmes, R.M., Aminot, A., Krouel, R., Hooker, B. A., and Peterson, B. J. (1999) A
 666 simple and precise method for measuring ammo- nium in marine and freshwater
 667 ecosystems. *Can J Fish Aquat Sci* **56**: 1801–1808.
 668 Hoppe, H.G. (1983) Significance of exoenzymatic activities in the ecology of brackish
 669 water: measurements by means of methylumbelliferyl substrates. *Mar Ecol Progr Ser*
 670 **11**: 299-308.
 671 Humphrey, B., Kjelleberg, S., and Marshall, K.C. (1983) Responses of marine bacteria
 672 under starvation conditions at a solid-water interface. *Appl Environ Microbiol* **45**:**43–**
 673 **47**.
 674 Jiao, N., Herndl, G.J., Hansell, D.A., Benner, R., Kattner, G., Wilhelm, S.W. et al.
 675 (2010) Microbial production of recalcitrant dissolved organic matter: long-term carbon
 676 storage in the global ocean. *Nature Reviews Microbiology* **8**: 593-599.
 677 Kawasaki, N., and Benner, R. (2006) Bacterial release of dissolved organic matter
 678 during cell growth and decline: Molecular origin and composition. *Limnol Oceanogr*
 679 **51**: 2170-2180.
 680 Kirchman, D., Knees, E., and Hodson, R. (1985) Leucine incorporation and its potential
 681 as a measure of protein-synthesis by bacteria in natural aquatic systems. *Applied and*
 682 *Environmental Microbiology* **49**: 599-607.
 683 Koedooder, C., Guneugus, A., Van Geersdale, R., Verg, V., Bouget, F.-Y.,
 684 Labreuche, Y. et al. (2018) The Role of the Glyoxylate Shunt in the Acclimation to Iron
 685 Limitation in Marine Heterotrophic Bacteria. *Frontiers in Marine Science* **5**.
 686 Kramer, G.D., and Herndl, G.J. (2004) Photo- and bioreactivity of chromophoric
 687 dissolved organic matter produced by marine bacterioplankton. *Aquat Microb Ecol* **36**:
 688 239-246.

689 Jones, V., Meador, T.B., Gogou, A., Migon, C., Penkman, K.E.H., Collins, M.J., and
 690 Repeta, D.J. (2013) Characterisation and dynamics of dissolved organic matter in the
 691 Northwestern Mediterranean Sea. *Progress in Oceanography* **119**: 78-89.
 692 Lauro, F.M., McDougald, D., Thomas, T., Williams, T.J., Egan, S., Rice, S. et al.
 693 (2009) The genomic basis of trophic strategy in marine bacteria. *Proc Natl Acad Sci*
 694 *USA* **106**.
 695 Lechtenfeld, O.J., Hertkorn, N., Shen, Y., Witt, M., and Benner, R. (2015) Marine
 696 sequestration of carbon in bacterial metabolites. *Nat Commun* **6**.
 697 Legendre, L., Rivkin, R.B., Weinbauer, M.G., Guidi, L., and Uitz, J. (2015) The
 698 microbial carbon pump concept: Potential biogeochemical significance in the globally
 699 changing ocean. *Progress in Oceanography* **134**: 432-450.
 700 Lønborg, C., Álvarez-Salgado, X.A., Davidson, K., and Miller, A.E.J. (2009)
 701 Production of bioavailable and refractory dissolved organic matter by coastal
 702 heterotrophic microbial populations. *Estuarine, Coastal and Shelf Science* **82**: 682-688.
 703 Matallana-Surget, S., Douki, T., Cavicchioli, R., and Joux, F. (2009) Remarkable
 704 resistance to UVB of the marine bacterium *Photobacterium angustum* explained by an
 705 unexpected role of photolyase. *Photochem Photobiol Sci* **8**: 1313-1320.
 706 Mykkestad, S.M., Skanoy, E., and Hestmann, S. (1997) A sensitive and rapid method for
 707 analysis of dissolved mono- and polysaccharides in seawater. *Marine Chemistry* **56**:
 708 279-286.
 709 Nelson, C.E., and Wear, E.K. (2014) Microbial diversity and the lability of dissolved
 710 organic carbon. *Proc Natl Acad Sci U S A* **111**: 7166-7167.
 711 Noriega-Ortega, B.E., Wienhausen, G., Mentges, A., Dittmar, T., Simon, M., and
 712 Niggemann, J. (2019) Does the Chemodiversity of Bacterial Exometabolomes Sustain
 713 the Chemodiversity of Marine Dissolved Organic Matter? *Front Microbiol* **10**: 215.

714 Ogawa, H., Amagai, Y., Koike, I., Kaiser, K., and Benner, R. (2001) Production of
 715 Refractory Dissolved Organic Matter by Bacteria. *Science* **292**: 917-920.
 716 Ortega-Retuerta, E., Marrasé, C., Muñoz-Fernández, A., Sala, M.M., Simó, R., and
 717 Gasol, J.M. (2018) Seasonal dynamics of transparent exopolymer particles (TEP) and
 718 their drivers in the coastal NW Mediterranean Sea. *Science of the Total Environment*
 719 **631**: 180-190.
 720 Osterholz, H., Niggemann, J., Giebel, H.A., Simon, M., and Dittmar, T. (2015)
 721 Inefficient microbial production of refractory dissolved organic matter in the ocean. *Nat*
 722 *Commun* **6**: 7422.
 723 Parada, A.E., Needham, D.M., and Fuhrman, J.A. (2016) Every base matters: assessing
 724 small subunit rRNA primers for marine microbiomes with mock communities, time
 725 series and global field samples. *Environ Microbiol* **18**: 1403-1414.
 726 Pedler, B.E., Aluwihare, L.I., and Azam, F. (2014) Single bacterial strain capable of
 727 significant contribution to carbon cycling in the surface ocean. *Proc Natl Acad Sci U S*
 728 *A* **111**: 7202-7207.
 729 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P. et al. (2013) The
 730 SILVA ribosomal RNA gene database project: improved data processing and web-
 731 based tools. *Nucleic Acids Res* **41**: D590-596.
 732 Rochelle-Newall, E., and Fisher, T.R. (2002) Production of chromophoric dissolved
 733 organic matter fluorescence in marine and estuarine environments: an investigation into
 734 the role of phytoplankton. *Marine Chemistry* **77**: 7-21.
 735 Romano, S., Dittmar, T., Bondarev, V., Weber, R.J., Viant, M.R., and Schulz-Vogt,
 736 H.N. (2014) Exo-metabolome of *Pseudovibrio* sp. FO-BEG1 analyzed by ultra-high
 737 resolution mass spectrometry and the effect of phosphate limitation. *PLoS One* **9**:
 738 e96038.

739 Romera-Castillo, C., Sarmiento, H., Alvarez-Salgado, X.A., Gasol, J.M., and Marrase,
 740 C. (2011) Net production and consumption of fluorescent colored dissolved organic
 741 matter by natural bacterial assemblages growing on marine phytoplankton exudates.
 742 *Appl Environ Microbiol* **77**: 7490-7498.

743 Sala, M.M., Aparicio, F.L., Balagué, V., Boras, J.A., Borrull, E., Cardelús, C. et al.
 744 (2016) Contrasting effects of ocean acidification on the microbial food web under
 745 different trophic conditions. *ICES Journal of Marine Science: Journal du Conseil* **73**:
 746 670-679.

747 Sánchez-Pérez, E.D., Pujo-Pay, M., Ortega-Retuerta, E., Conan, P., Peters, F., and
 748 Marrasé, C. (2020) Mismatched dynamics of dissolved organic carbon and
 749 chromophoric dissolved organic matter in the coastal NW Mediterranean Sea. *Science*
 750 *of The Total Environment* **746**: 141190.

751 Shimotori, K., Watanabe, K., and Hama, T. (2012) Fluorescence characteristics of
 752 humic-like fluorescent dissolved organic matter produced by various taxa of marine
 753 bacteria. *Aquatic Microbial Ecology* **65**: 249-260.

754 Smith, D.C., Simon, M., Alldredge, A.L., and Azam, F. (1992) Intense hydrolytic
 755 enzyme-activity on marine aggregates and implications for rapid particle dissolution
 756 *Nature* **359**: 139-142.

757 Sosa, O.A., Repeta, D.J., DeLong, E.F., Ashkezari, M.D., and Karl, D.M. (2019)
 758 Phosphate-limited ocean regions select for bacterial populations enriched in the carbon-
 759 phosphorus lyase pathway for phosphonate degradation. *Environ Microbiol* **21**: 2402-
 760 2414.

761 Stoderegger, K., and Herndl, G.J. (1998) Production and release of bacterial capsular
 762 material and its subsequent utilization by marine bacterioplankton. *Limnol Oceanogr*
 763 **43**: 877-884.

764 Teeling, H., Fuchs, B.M., Becher, D., Klockow, C., Gardebrecht, A., Bennke, C.M. et
 765 al. (2012) Substrate-Controlled Succession of Marine Bacterioplankton Populations
 766 Induced by a Phytoplankton Bloom. *Science* **336**: 608-611.
 767 Thingstad, T.F., Zweifel, U.L., and Rassoulzadegan, F. (1998) P limitation of
 768 heterotrophic bacteria and phytoplankton in the northwest Mediterranean. *Limnol*
 769 *Oceanogr* **43**: 88-94.
 770 Van Wambeke, F., Christaki, U., Giannakourou, A., Moutin, T., and Souvemerzoglou,
 771 K. (2002) Longitudinal and vertical trends of bacterial limitation by phosphorus and
 772 carbon in the Mediterranean Sea. *Microbial Ecology* **43**: 119-133.
 773 Watson, S.W., Novitsky, T.J., Quinby, H.L., and Valois, F.W. (1977) Determination of
 774 bacterial number and biomass in the marine environment. *Appl Envir Microb* **33**: 940-
 775 946.
 776 Yamashita, Y., and Tanoue, E. (2008) Production of bio-refractory fluorescent
 777 dissolved organic matter in the ocean interior. *Nature Geoscience* **1**: 579-582.
 778 Zhang, Z., Chen, Y., Wang, R., Cai, R., Fu, Y., and Jiao, N. (2015) The Fate of Marine
 779 Bacterial Exopolysaccharide in Natural Marine Microbial Communities. *PLoS One* **10**:
 780 e0142690.
 781
 782

Table 1. Overview of the biodegradation experiments: Incubation date, treatment abbreviations, dissolved organic matter (DOM) and heterotrophic prokaryote (HP) inocula sources. deep.n: Deep DOM-Deep HP. deep.p: *P. angustum* DOM -Deep HP surf.n: Surface DOM-Surface HP. surf.p: *P. angustum* DOM -Surface HP. ndom: Surface DOM-Surface HP. +gluc: Surface DOM+ glucose-Surface HP. pdom : *P. angustum* DOM -Surface HP. sdom: *S. alaskensis* DOM-Surface HP

Experiment	Date	Treatment	DOM Source (90% vol)	HP Innocula	Abundance DOC Nuts. FDOM	Enzymes	16s
Open Sea- March	29 Mar 2018				✓		
		deep.n	500m	500m			
		deep.p	<i>P. angustum</i>	“			
		surf.n	5m	5m			
		surf.p	<i>P. angustum</i>	“			
Coastal Sea- April	10 Apr 2018				✓	✓	
		ndom	5m	5m			
		pdom	<i>P. angustum</i>	“			
		sdom	<i>S. alaskensis</i>	“			
Coastal Sea- December	12 Dec 2018				✓	✓	✓
		ndom	5m	5m			
		+gluc	5m	“			
			(+glucose)				
		pdom	<i>P. angustum</i>	“			
		sdom	<i>S. alaskensis</i>	“			

Table 2: Characteristics of bacterial-derived DOM (mean $\pm$ standard deviation): percentage of initial DOC released as bacterial dissolved organic matter (%DOC), final-initial (Δ) values of fluorescent DOM peaks C, M, A (all surrogates for humic-like DOM, $\times 10^{-3}$ Raman Units) and T (surrogate for protein-like DOM, $\times 10^{-3}$ Raman Units) and the ratio between Peaks C and T (C/T, indicative of the prevalence of protein-like over humic-like DOM). p: *Photobacterium angustum* s: *Sphingopyxis alaskensis*. p 1 and p 2, and s 1 and s 2 refer to independent experiments (See Experimental Procedures for details).

Experiment	p 1	p 2	s 1	s 2
Date	03/2018	07/2018	03/2018	11/2018
%DOC	22.2 $\pm$ 10.6	10.3 $\pm$ 2.1	6.3 $\pm$ 7.1	10.3 $\pm$ 4.6
Δ FDOM.C	3.21 $\pm$ 0.14	2.47 $\pm$ 0.12	2.64 $\pm$ 1.68	5.72 $\pm$ 0.19
Δ FDOM.M	4.84 $\pm$ 0.44	2.79 $\pm$ 0.18	9.03 $\pm$ 3.38	12.88 $\pm$ 0.02
Δ FDOM.A	4.29 $\pm$ 0.55	5.43 $\pm$ 0.41	13.74 $\pm$ 6.63	16.36 $\pm$ 0.52
Δ FDOM.T	35.37 $\pm$ 1.60	33.29 $\pm$ 1.4	6.38 $\pm$ 0.54	11.37 $\pm$ 0.70
C/T	0.09 $\pm$ 0.008	0.07 $\pm$ 0.006	0.42 $\pm$ 0.30	0.50 $\pm$ 0.06

802 Table 3. See horizontal Tables document
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Table 4. Diversity indexes: Richness, Evenness and Shannon for every treatment of the Biodegradation experiment 3

Treatment	Richness		Evenness		Shannon	
	mean	SD	mean	SD	mean	SD
Initial	308		0.86		4.93	
Surf HP- Surf DOM	112	6	0.50	0.02	2.36	0.10
Surf HP- Surf DOM+Gluc	87	9	0.44	0.04	1.98	0.16
Surf HP- <i>P. angustum</i> DOM	81	10	0.59	0.03	2.60	0.16
Surf HP- <i>S. alaskensis</i> DOM	40	4	0.46	0.11	1.69	0.39

813 Table 5: see horizontal Tables document
814

Figure Captions

Figure 1. Dissolved organic carbon (DOC, $\mu\text{mol L}^{-1}$, dark grey columns) and glucose-C ($\mu\text{mol C L}^{-1}$, light grey columns) at the beginning (t0) and end (tf) of the growth experiments of each strain. Whiskers: Standard deviation; p1, p2: *P. angustum* s1, s2: *S. alaskensis* as described on Table 2.

Figure 2. Changes in prokaryotic heterotrophic abundance (PHA, cells mL^{-1}) over incubation time in the three biodegradation experiments. Open sea-March (A): deep.n: deep water HP and DOM, deep.p: deep water HP, *P. angustum* DOM. surf.n: surface water HP and DOM, surface.p: surface water HP, *P. angustum* DOM. Coastal sea-April (B) and coastal sea-December (C): ndom: surface water HP and DOM; +gluc: surface water HP and DOM, +20 $\mu\text{m L}^{-1}$ glucose-C added. pdom: surface water HP, *P. angustum* DOM. sdom: surface water HP, *S. alaskensis* DOM. cont.p and cont.s: *P. angustum* and *S. alaskensis* DOM, no HP inoculum added

Figure 3. Prokaryotic Growth efficiency (PGE, %) in the biodegradation experiments (Biodeg). Open Sea-March: deep.n: deep water HP and in situ DOM, deep.p: deep water HP, *P. angustum* DOM. surf.n: surface water HP and in situ DOM, deep.p: surface water HP, *P. angustum* DOM. Coastal sea experiments: ndom: surface water HP and in situ DOM; +gluc: surface water HP and DOM, +20 $\mu\text{m L}^{-1}$ glucose-C added. pdom: surface water HP, *P. angustum* DOM. sdom: surface water HP, *S. alaskensis* DOM

Figure 4. Changes in exoenzymatic activity over time in coastal biodegradation experiments (April: a, b, c, d. December: e, f, g, h). Columns: different study enzymes. Rows: Different experiments

Figure 5. Left panel: MDS plot of all treatments and replicates based on bray Curtis dissimilarity. Differences in prokaryotic community structure among treatments are significantly (ANOSIM test, $p < 0.05$). Right panel: Changes in prokaryotic community composition based in 16s rRNA Illumina sequencing in the coastal-December biodegradation experiment. Only orders having ASV's with a relative abundance higher than 2% are plotted.

Table 3. Initial and final values of dissolved organic carbon (DOC) concentration ($\mu\text{mol L}^{-1}$), ΔDOC per time ($\mu\text{mol L}^{-1} \text{h}^{-1}$), $\Delta\text{DOC}/\text{DOC}_{t0}$ (%), $\Delta\text{Biomass}/\text{DOC}$ (%) and DOM fluorescence (protein-like peak T, humic-like peaks A, C and M, Raman Units) in all biodegradation experiments; tf: mean $\pm$ SD of biological triplicates

	DOC		ΔDOC/t	ΔBiomass/ DOC (%)	ΔDOC/ DOCt0 (%)	FDOM.C		FDOM.M		FDOM.A		FDOM.T	
Experiment-Treatment	t0	tf				t0	tf	t0	tf	t0	tf	t0	tf
<i>Open Sea-March</i>													
Deep HP- Deep DOM	60.8	56.2 ± 2.1	0.04	8.4 ± 0.5	7.8	1.8	5.0	7.7	6.1	18.9	14.0	10.9	7.2
Deep HP- <i>P. angustum</i> DOM	52.7	30.5 ± 4.5	0.18	4.9 ± 1.6	41.1	1.6	5.4	8.1	5.6	14.4	12.7	38.7	28.1
Surf HP- Surf DOM	70.9	61.8 ± 2.8	0.08	4.0 ± 1.3	12.6	12.9	7.2	15.8	7.5	83.3	19.8	18.8	8.6
Surf HP- <i>P. angustum</i> DOM	72.7	32.8 ± 8.1	0.33	1.4 ± 0.3	54.6	2.2	5.7	7.9	5.5	15.3	12.8	34.1	22.0
<i>Coastal Sea- April</i>													
Surf HP- Surf DOM	99.2	75.5 ± 12.8	0.16	3.3 ± 1.1	23.4	10.3	16.2	11.6	12.1	27.4	29.1	11.3	13.2
Surf HP- <i>P. angustum</i> DOM	98.1	34.1 ± 15.5	0.45	2.0 ± 0.2	65.3	9.2	11.8	16.5	6.8	25.1	15.2	53.7	14.0
Surf HP- <i>S. alaskensis</i> DOM	76.0	57.0 ± 4.1	0.13	2.5 ± 0.7	25.0	16.5	21.9	32.3	17.1	41.2	16.9	16.5	14.4
<i>Coastal Sea-December</i>													
Surf HP- Surf DOM	83.0	74.1 ± 2.3	0.05	2.0 ± 0.6	10.8	11.2	11.2	12.1	12.5	28.4	29.2	14.3	11.2
Surf HP- Surf DOM+Gluc	107.3	78.3 ± 2.4	0.18	1.9 ± 0.2	27.3	11.5	10.7	11.9	11.3	29.1	27.2	13.2	12.2
Surf HP- <i>P. angustum</i> DOM	90.1	38.0 ± 2.4	0.31	9.2 ± 3.4	57.8	6.5	6.3	7.6	8.4	15.5	19.7	35.3	37.0
Surf HP- <i>S. alaskensis</i> DOM	110.0	39.1 ± 4.0	0.43	6.4 ± 1.7	64.5	8.4	9.0	15.2	13.5	23.4	22.8	13.3	13.5

Table 5. Review of previous estimates of dissolved organic matter (DOM) vs. biomass production by heterotrophic prokaryotes as percentage of the initial dissolved organic carbon (DOC) concentration

Innocula from mixed bacterial communities								
Study	Substrate used	Procedence	DOC Initial ($\mu\text{mol L}^{-1}$)	Time (d)	DOC final ($\mu\text{mol L}^{-1}$)	Biomass final ($\mu\text{mol L}^{-1}$)	%DOC Released as DOM	%DOC into biomass
(Ogawa et al., 2001)	Glucose	Coastal (Gulf of Mexico)	208	2	30	na	15.0	7.0
“	Glutamate	Coastal (Sagami Bay)	132	2	18	na	13.0	22.0
	Glucose	Coastal (North Sea)	220	5	24	13.3	10.9	6.0
(Kawasaki and Benner, 2006)	Glucose	Lake (Murray)	529	1	37	8	7.0	1.5
“	Glucose	Estuary (Winyah Bay)	513	2	115	47	22.4	9.2
“	Glucose	Coastal (Folly Beach)	310	2	25	0.83	8.1	0.3
(Lønborg et al., 2009)	Glucose	Fjord (Loch Creran)	na	30	na	na	11	na
(Koch et al., 2014)	Glucose	Antarctic	326	21	45	30	13.8	9.2
(Lechtenfeld et al., 2015)	Glucose+Glutamic Acid	Coastal seawater	283	1.5	10	6.7	3.5	2.4
(Arai et al., 2017)	Glucose	Coastal (Shizoka, Japan)	833	5	60.4	272	7.3	32.4
Innocula from single bacterial strains								
Study	Substrate used	Strain	DOC initial ($\mu\text{mol L}^{-1}$)	Time (d)	DOC final ($\mu\text{mol L}^{-1}$)	Biomass final ($\mu\text{mol L}^{-1}$)	%DOC Released as DOM	%DOC into biomass
(Romano et al., 2014)	Glucose, P limited	<i>Pseudovibrio sp. FO-BEG1</i>	60000		na	2000	0.9*	3.3
	“ Glucose, P replete	“					0.2*	
(Zhang et al., 2015)	Glucose	<i>Alteromonas sp. JL2810</i>	3333	5			8**	
(Goto et al., 2017)	Glucose	<i>Alteromonas macleodii</i>	995	1	58	6.1	5.8	0.6
(Noriega-Ortega et al., 2019)	Acetate	<i>Phaeobacter inhibens</i>	70050	na	2400	na	3.4	na
Present Study	Glucose	<i>Photobacterium angustum</i>	198		55	15.4	16.3	4.4
		<i>Sphingopyxis alaskensis</i>	245	7	72	8.4	8.5	7.6

bdl: below detection limit; na: not available (not measured or not reported in the paper)

* Only SPE extractable fraction quantified ** Only exopolysaccharide quantified

Figure 1. Dissolved organic carbon (DOC, $\mu\text{mol L}^{-1}$, dark grey columns) and glucose-C ($\mu\text{mol C L}^{-1}$, light grey columns) at the beginning (t0) and end (tf) of the growth experiments of each strain. Whiskers: Standard deviation; p1, p2: *P. angustum* s1, s2: *S. alaskensis* as described on Table 2.

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Supplementary Table 1. Inorganic nutrients (phosphate, nitrate+nitrite, ammonium $\mu\text{mol L}^{-1}$) at the onset (t0) and end (tf) of all strain incubations and biodegradation experiments.

Strain	Date	PO ₄ t0	PO ₄ tf	NO ₃ t0	NO ₃ tf	NH ₄ t0	NH ₄ tf
<i>S. alaskensis</i>	mar-18	2.5	1.4	26	23.4	25	7.5
"	nov-18	3.4	2.5	28	26.8	18.9	10.5
<i>P. angustum</i>	mar-18	2.8	1.4	19.9	20	20.3	7.1
"	jul-18	2.8	1.4	23.8	23.3	nd	nd
Experiment	Treat	PO ₄ t0	PO ₄ tf	NO ₃ t0	NO ₃ tf	NH ₄ t0	NH ₄ tf
Open /March	deep.n	1.3	1.23 $\pm$ 0.1	14.3	13.42 $\pm$ 0.8	nd	8.94 $\pm$ 0.5
"	deep.p	2.0	1.87 $\pm$ 0.3	27.6	24.8 $\pm$ 2.3	10.6	8.47 $\pm$ 2.3
"	surf.n	1.3	1.12 $\pm$ 0.1	13.5	11.60 $\pm$ 0.2	9.2	8.94 $\pm$ 0.5
"	surf.p	2.5	1.77 $\pm$ 0.4	28.2	27.91 $\pm$ 4.2	11.9	9.57 $\pm$ 0.6
Coastal/ April	ndom	1.2	0.90 $\pm$ 0.1	12.1	11.33 $\pm$ 0.2	nd	nd
"	pdom	2.6	1.63 $\pm$ 0.1	26.7	25.13 $\pm$ 0.5	nd	nd
"	sdom	2.0	1.66 $\pm$ 1.9	31.7	29.79 $\pm$ 0.4	nd	nd
Coastal / Dec	ndom	1.6	1.55 $\pm$ 0.1	9.3	8.62 $\pm$ 0.1	7.3	6.86 $\pm$ 0.3
"	+gluc	1.4	1.21 $\pm$ 0.0	9.9	9.43 $\pm$ 0.2	8.2	6.45 $\pm$ 0.1
"	pdom	2.5	2.03 $\pm$ 0.1	31.2	29.64 $\pm$ 0.2	14.6	12.57 $\pm$ 0.4
"	sdom	3.3	2.54 $\pm$ 0.1	32.6	30.53 $\pm$ 0.5	16.2	11.12 $\pm$ 0.3

Supplementary Figure 1. Changes in cell abundance in the strain growth experiments. Symbols and whiskers are, respectively, averages and standard deviations of three biological triplicates. Samples for DOM characterization were taken at the t_0 and t_{final} points in each growth curve. Note the log-10 scale in the y axis

Supplementary Table 2. Results of the repeated measures ANOVA tests to compare the changes in cell abundance over the different biodegradation experiments and treatments. DF_n: Degrees of freedom in the numerator. DF_d: Degrees of freedom in the denominator. F: F-value p: level of significance. ges: Generalized Eta-Squared measure of effect size.

Open Sea/March					
Effect	DF _n	DF _d	F	p	ges
treatment	3	6	25.973	0.000778	0.806
time	4	8	58.986	5.63E-06	0.806
treat:time	12	24	9.901	1.35E-06	0.697
Coastal Sea/April					
Effect	DF _n	DF _d	F	p	ges
treatment	2	4	12.73	0.018	0.649
time	6	12	75.287	8.04E-09	0.877
treat:time	12	24	7.134	2.44E-05	0.603
Coastal Sea/December					
Effect	DF _n	DF _d	F	p	ges
treatment	3	6	7.242	0.02	0.602
time	6	12	67.137	1.56E-08	0.729
treat:time	18	36	10.226	2.87E-09	0.561

Supplementary Table 3. Results of pairwise t-tests between each treatment and time in the three biodegradation experiments. Different letters denote significantly different treatments at $p < 0.05$

Open Sea/March				
Time/Treatment	deep.n	deep.p	surf.n	surf.p
1	a	a	b	c
2	a	a	ab	b
3	a	ac	b	c
4	a	a	a	b
5	a	ab	ab	b
Coastal Sea/April				
Time/Treatment	ndom	pdom	sdom	
1	a	b	ab	
2	a	a	b	
3	a	ab	b	
4	a	ab	b	
5	a	a	a	
6	a	b	ab	
7	a	a	a	
Coastal Sea/December				
Time/Treatment	ndom	+gluc	pdom	sdom
1	a	b	c	d
2	a	b	c	b
3	a	b	ab	ab
4	a	ab	ab	b
5	a	b	abc	c
6	a	a	ab	b
7	a	ab	bc	c

26 Supplementary Table 4. Composition of the minimum media used for strain growth
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Sea Salts	Final Concentration (g L ⁻¹)
NaCl	24
Na ₂ SO ₄	4
KCl	0.68
KBr	0.1
H ₃ BO ₃	0.025
NaF	0.002
MgCl ₂ .6H ₂ O	10.8
CaCl ₂ .2H ₂ O	1.5
SrCl ₂ .6H ₂ O	0.024
NaHCO ₃	0.2
Macronutrients	Final Concentration (μM)
Glucose	33 μM (=200 μM C)
NaNO ₃	22 μM N
NH ₄ Cl	22 μM N
NaHPO ₄	4.4 μM P
Trace Metals	Final Concentration (μg L ⁻¹)
CuCl ₂ .2H ₂ O	0.015
NiCl ₂ .H ₂ O	0.025
Na ₂ MOO ₄ .2H ₂ O	0.025
ZnSO ₄ .7H ₂ O	0.07
MnCl ₂ .4H ₂ O	0.1
CoCl ₂ .6H ₂ O	0.12
FeCl ₃ .6H ₂ O	4
EDTA	2 (0.068 μM C)
Vitamins	Final Concentration (μg L ⁻¹ / μM)
P-aminobenzoic acid	0.05 / 2.6
D-biotin	0.02 / 0.8
Folic Acid	0.02 / 0.9
Niacinamide	0.02 / 0.9
D-pantothenic acid	0.05 / 1.8
Pyridoxal	0.02 / 0.8
Pyridoxamine	0.10 / 3.3
Pyridoxine	0.10 / 3.9
Riboflavin	0.05 / 2.3
Thiamine	0.02 / 0.9
D L-6,8-thioctic acid	0.02 / 0.7
Vitamin B12	0.05 / 2.3
Nicotinamide	0.05 / 2.5

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