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

Eva Ortega-Retuerta, Quentin Devresse, Jocelyne Caparros, Barbara Marie, Olivier Crispi, Philippe Catala, Fabien Joux, Ingrid Obernosterer

▶ To cite this version:

HAL Id: hal-03007145

https://hal.archives-ouvertes.fr/hal-03007145

Submitted on 16 Nov 2020

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

Originality-Significance Statement

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

23

24   **Summary**

25   Marine heterotrophic prokaryotes (HP) play a key role in organic matter processing in

26   oceans; however, the view of HP as dissolved organic matter (DOM) sources remains

27   underexplored. In this study, we quantified and optically characterized the DOM

28   produced by two single marine bacterial strains. We then tested the availability of these

29   DOM sources to in situ Mediterranean Sea HP communities. Two bacterial strains were

30   used: *Photobacterium angustum* (a copiotrophic gammaproteobacterium) and

31   *Sphingopyxis alaskensis* (an oligotrophic alphaproteobacterium). When cultivated on

32   glucose as the sole carbon source, the two strains released from 7 to 23% of initial

33   glucose as bacterial derived DOM (B-DOM), the quality of which (as enrichment in

34   humic or protein-like substances) differed between strains. B-DOM promoted

35   significant growth and carbon consumption of natural HP communities, suggesting that

36   it was partly labile. However, B-DOM consistently promoted lower prokaryotic growth

37   efficiencies than in situ DOM. In addition, B-DOM changed HP exoenzymatic activities

38   enhancing aminopeptidase activity when degrading *P. angustum* DOM, and alkaline

39   phosphatase activity when using *S. alaskensis* DOM, and promoted differences in HP

40   diversity and composition. DOM produced by HP affects in situ prokaryotic metabolism

41   and diversity, thus changing the pathways for DOM cycling (e.g. respiration over

42   biomass production) in the ocean.

43

44   Keywords: microbial carbon pump, dissolved organic matter, bioavailability,

45   heterotrophic prokaryotes, prokaryotic diversity.

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 CO_2 (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 CO_2 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 $x10^6$ 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 $x10^6$ 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

128 nutrients, both strains used preferentially NH_4 over NO_3, but significant amounts of

129 NH_4, PO_4 and NO_3 remained in the cultures at the end of the incubations

130 (Supplementary Table 1), indicating that carbon was the limiting substrate for the

131 bacterial growth.

132 **Availability of bacterial-derived DOM for strain-derived natural heterotrophic**

133 **prokaryotic communities**

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

135 DOM originating from the bacterial strains promoted remarkable HP growth in all

136 biodegradation experiments (Fig. 2), with significant differences over time and between

137 treatments in all three experiments (repeated measures ANOVA, $p < 0.05$, Suppl. Table

138 2). In the open sea-March biodegradation (Table 1), DOM from *P. angustum* supported

139 a higher HP growth for surface and deep HP communities than their respective

140 treatments with in situ DOM (Fig. 2a), although only significant for surface

141 communities (Suppl. Table 3). In the coastal sea-April biodegradation experiment,

142 differences in the HP abundances among treatments were only significant at certain

143 timepoints (Fig. 2b, Suppl. Table 3). In the coastal sea-December biodegradation

144 experiment, as in the open sea-March one, the treatments with bacterial DOM promoted

145 significantly higher HP growth than *in situ* DOM (Fig. 2c, Suppl. Table 3). In the

146 coastal sea-December experiment we included a treatment with glucose (20 μmol C L$^-$

147 ^{1}), but although HP abundance was higher than that in the surface DOM treatment, it

148 was lower than HP abundances in the *S. alaskensis* DOM treatment (Suppl. Table 3).

149 We did not find significant differences in HP growth between treatments using *P.*

150 *angustum* vs. *S. alaskensis* DOM (Suppl. Table 3).

151 We performed controls in the degradation experiments, consisting of DOM from each

152 strain, with N and P additions similar to the treatments, but without inoculating with in

153 situ HP communities. The purpose of these controls was to check for potential growth

154 of the minimal amount of *P. angustum* and *S. alaskensis* cells that remained in the

155 filtrates. Little cell growth was observed in these controls (Fig. 2a-c).

156 At the onset of the three biodegradation experiments, the DOC concentration was

157 slightly different between treatments (Table 3). However, the amount of DOC

158 consumed per hour (during the total incubation) was consistently higher in the

159 treatments with strain derived DOM, except for coastal sea-April experiment

160 Biodegradation experiment and *S. alaskensis* DOM (Table 3). Average DOC

161 consumption in the incubations with strain DOM (both *P. angustum* and *S. alaskensis*

162 DOM) was 0.30 μmol L^{-1} h^{-1}, while in the treatments with in situ DOM, either deep or

163 surface, the average was 0.08 μmol L^{-1} h^{-1}. In the experiments with glucose additions

164 the DOC consumption was on average 0.18 μmol L^{-1} h^{-1}. When normalizing ΔDOC by

165 the initial DOC concentration in each treatment, these patterns were even more visible:

166 51% on average of the strain-derived DOC was degraded during the incubations,

167 compared to 13.6% of in situ DOC (Table 3).

168 We further wanted to elucidate whether differences in HP growth were due to different

169 initial amounts of DOC or to differences in its lability. For that purpose, we compared

170 HP biomass changes with initial DOC concentrations in every treatment (Table 3). In

171 contrast to total DOC utilization, we could not find consistent patterns in the relative

172 amount of initial DOC incorporated into HP biomass (Table 3). The %DOC invested

173 into prokaryotic biomass was lower (open sea-March), similar (coastal sea-April) or

174 higher (coastal sea-December) than in the in situ DOM (ndom) treatments. Comparing

175 the treatments with *P. angustum* (pdom) or *S. alaskensis* (sdom), the changes in HP

176 biomass compared to initial DOC were never significantly different. Interestingly,

177 prokaryotic growth efficiency (PGE) was consistently lower in treatments with strain-

178 derived DOM than in the treatments with in situ DOM in all biodegradation

179 experiments by a mean factor of 3.5 (range 1.3-7.0) (Fig. 3).

180 *Biodegradation experiments: Changes in FDOM and inorganic nutrients*

181 The different FDOM peaks did not decrease at the same level in every incubation

182 (Table 3): The peak T (surrogate of protein-like substances) tended to decrease up to a

183 70%, while the FDOM at peak C (surrogate of humic-like compounds) tended to

184 increase in almost every experiment and treatment (Table 3). Humic-like peaks A and

185 M were more variable among experiments and treatments.

186 The inorganic nutrient concentrations, particularly those of NO_3^-, were also higher in

187 the treatments with bacterial-derived DOM as compared to the in situ DOM, since *P.*

188 *angustum* and *S. alaskensis* did not consume all inorganic nutrients during the previous

189 strain incubations and equivalent amounts of all inorganic nutrients were added to each

190 treatment of the biodegradation experiments. At the end of the incubations, substantial

191 amounts of all inorganic nutrients remained in all treatments (Supplementary Table 1).

192 *Coastal biodegradation experiments: Changes in exoenzymatic activity*

193 Differences in the predominant exoenzymatic activities could be observed between

194 treatments in Coastal sea-April and December experiments. In May, relatively high

195 alpha- and beta- glucosidase production rates were observed in the treatment with

196 surface DOM (Fig. 4 a,b) compared to the bacterial DOM treatments, but this result was

197 not visible in December (Fig. 4 e,f). Remarkably, in both biodegradation experiments *P.*

198 *angustum* DOM induced high rates of leucine aminopeptidase (Fig. 4 c and g), while the

199 *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 tf (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 Rhodospirilalles), 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 Oceanospirilalles 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 Rhodospirilalles. 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

250   quality is likely crucial determining the quantity and quality of DOM produced: Ogawa

251   et. al. (2001) and Wienhausen et. al. (2017) showed higher release of dissolved amino

252   acids by heterotrophic bacterial strains when growing on glutamate than when growing

253   on glucose, even though the amino acid composition was similar among treatments. If

254   grown on complex substrates, their enzymatic degradation would likely enhance the

255   release of DOM by HP (Aparicio et al., 2015). Further work is needed to explore which

256   would be the main nutritional drivers, e.g. going beyond glucose amendments to testing

257   the effect of the DOM source, of DOM production by HP in the sea.

258   **Quantity and quality of bacterial DOM**

259   Even though the quantity of DOM released by our two study strains was similar, we

260   could evidence clear differences in its fluorescence signature, which suggests different

261   chemical composition. The production of protein-like FDOM observed for *P. angustum*

262   in the present study, has been previously reported for *Alteromonas macleodii* when

263   grown on glucose (Goto et al., 2017) and for mixed bacterial communities grown on

264   phytoplankton exudates (Romera-Castillo et al., 2011) or on lake water (Cammack et

265   al., 2004). By contrast, the low protein-like FDOM signature in *S. alaskensis* DOM

266   illustrates the importance of bacterial taxonomy for bacterial FDOM quality. *P.*

267   *angustum* (our study) and *A. macleodii* (Goto et al., 2017) are both fast-growing

268   copiotrophic bacteria, suggesting a link between aspects of bacterial metabolism and

269   protein-like FDOM release. This is in line with the fact that copiotrophs have a higher

270   number of secreted proteins than oligotrophs (Lauro et al., 2009), thus the quantity and

271   composition of the secreted DOM would be likely dependent on the expression and

272   production of these proteins. The production of humic-like DOM by bacteria has been

273   shown using single strains (Shimotori et al., 2012) and experimental incubations with

274   mixed communities (Rochelle-Newall and Fisher, 2002; Romera-Castillo et al., 2011).

275 Humic-like FDOM (peaks A, C and M) is released to the ambient seawater as a

276 byproduct of organic matter remineralization by HP, and has been used as a tracer for

277 refractory DOM in the ocean (Yamashita and Tanoue, 2008; Catalá et al., 2015).

278 Therefore, we hypothesized that DOM produced by *S. alaskensis*, more rich in humic-

279 like compounds, would be less available for HP degradation in our experiments.

280 Few previous published studies have tested bacterial-DOM degradation by in situ HP

281 communities. Both Kramer and Herndl (2004) and Zhang et al. (2015), these latter

282 authors only using the exopolysaccharide fraction of bacterial DOM, concluded that

283 DOM derived from mixed communities was mostly refractory, since no significant

284 growth was detected during 2 to 14 days. However, Lønborg et al. (2009) followed

285 DOC changes over longer time periods (30 d), and concluded that at least a fraction of

286 HP-derived DOM can be utilized by other prokaryotic communities, indicating that HP-

287 DOM is semilabile. This is in accordance with our results, showing that 51% of organic

288 carbon produced by the strains was further used by in situ HP communities (Table 3).

289 **Influence of HP community composition for the degradation of bacterial DOM**

290 In contrast to our hypothesis, differences in the lability of *P. angustum* vs. *S. alaskensis*

291 DOM could not be demonstrated by our biodegradation experiments, as inverse growth

292 patterns on the two strain-derived DOM treatments were observed between coastal

293 communities from April and December (Fig. 2). This suggests that not only the quality

294 of the bacterial DOM, but also the in situ HP community composition would affect

295 bacterial DOM degradation, as previously shown (Nelson and Wear, 2014). Our study

296 strains released DOM that could not be used by themselves, since little bacterial growth

297 of the residual cells was observed even when reinoculating with inorganic nutrients

298 (control treatments in biodegradation experiments, Fig. 2), although this could be due to

299 the possible damage of those cells after freezing. Whether the bacterial DOM can be

300 degraded by single strains, as shown for labile in situ DOM and *Alteromonas macleodii*

301 (Pedler et al., 2014), or whether the complementary metabolic strategies of a diverse

302 community are required, remains to be investigated. The remarkable growth of in situ

303 HP communities was not only due to the glucose that remained in the pdom and sdom

304 treatments, since HP abundance was in both cases higher than in the +glucose

305 treatment. A possibility that we cannot exclude is that this remaining glucose boosted

306 the utilization of other substrates. Differences in HP growth among experiments can be

307 explained not only by differences in the in situ communities but also by in situ DOM

308 variability: In late spring in the Mediterranean Sea, DOM is usually enriched in labile

309 compounds after the spring phytoplankton bloom (Jones et. al., 2013, Ortega-Retuerta

310 et. al., 2018), so the in situ DOM was likely more labile than bacterial derived DOM, in

311 contrast to December, when DOM can have other signatures (i.e. terrestrial-like,

312 Sanchez-Pérez et. al., 2020).

313 In fact, despite non-significant differences in HP growth, consistent patterns of

314 exoenzyme activity were detected: *P. angustum* DOM always promoted high leucine-

315 aminopeptidase activity rates (Fig. 4), in line with its relative enrichment in protein-like

316 FDOM compounds (Table 2). By contrast, *S. alaskensis* DOM consistently promoted

317 relatively high levels of alkaline phosphatase (Fig. 4). This last result was intriguing to

318 us, since inorganic phosphorus was added at the beginning of each biodegradation

319 experiments and thus HP communities were not P-limited. Although high alkaline

320 phosphatase activities have been traditionally interpreted as P limitation (Cotner et al.,

321 1997; Thingstad et al., 1998), HP can use these exoenzymes to release available

322 dissolved organic carbon, as pointed out by previous studies (Benitez-Nelson and

323 Buesseler, 1999; Van Wambeke et al., 2002). Our results suggest that *S. alaskensis*

324 DOM could be rich in phosphorus-containing groups. We could see (only in the coastal

325 December experiment) that the *S. alaskensis* DOM treatment was the one with a higher

326 proportion of Rhodospirilalles. This order has been found in P-limited ocean areas

327 linked to the presence of the carbon-phosphorus lyase pathway for phosphonate

328 degradation (Sosa et al., 2019), in accordance with our hypothesis of P-rich DOM

329 produced by *S. alaskensis*.

330 Bacterial DOM sustained a lower HP diversity as compared to the in situ DOM (Table

331 4). In line with this, Noriega-Ortega et al. (2019) compared the solid- phase extractable

332 DOM produced by single bacterial strains with that of marine DOM from the North

333 Pacific and observed higher diversity of compounds in marine DOM respect to

334 bacterial-derived DOM. HP strains were shown to produce thousands of DOM

335 molecules when using a single carbon substrate, however, the DOM chemical diversity

336 produced by a single bacterium with respect to DOM from coastal seawater is likely to

337 be lower. In any case, the significant differences in community composition between

338 treatments suggest population-specific responses to the differences in quality of the

339 DOM produced by *P. angustum* and *S. alaskensis*.

340 **Bacterial DOM, PGE and carbon fluxes**

341 The growth on strain-derived DOM induced significantly lower prokaryotic growth

342 efficiency (PGE). To the best of our knowledge, our study provides the first

343 measurements of PGE on bacterial-derived DOM, demonstrating that bacterial DOM is

344 preferentially respired. HP do not only promote DOC accumulation in the ocean

345 through the production of refractory DOM (the microbial carbon pump hypothesis

346 shown in previous studies), but would also enhance carbon remineralization (therefore,

347 acting as a CO_2 source). Noteworthy, we did not include DOM production by natural

348 HP communities in our PGE calculations in the biodegradation experiments, and

349 including DOM production in our calculations would lead to even lower PGE values in

350 all treatments.

351 **Changing the paradigm of carbon fluxes through HP in the ocean**

352 DOM release by HP has been repeatedly demonstrated, however, carbon (and other

353 elements) release by HP as DOM is still overlooked when constraining carbon budgets

354 or other elemental fluxes and models in the ocean. For instance, considering that HP

355 produce, on average, 10% of DOC as DOM (average from values in Table 5), in situ HP

356 production estimates (e.g. using isotopes incorporation such as ^{3}H-leucine, (Kirchman et

357 al., 1985) would be underestimated. To better constrain this uncertainty, it is important

358 to know whether DOM release is linearly related to HP biomass production during short

359 incubation times. The release of DOM would also affect estimates of HP growth

360 efficiency. Prokaryotic carbon demand can be calculated as the sum of HP biomass

361 production and respiration or as differences in DOC concentrations in bottle incubations

362 (the dilution approach (delGiorgio and Cole, 1998)), but in both cases PGE would be

363 overestimated by roughly 10%, according to published results. The overestimation

364 could even be higher if, as demonstrated in our study, this 10% of DOM produced by

365 HP would enhance respiration over biomass production. More studies are needed to

366 better understand the dynamics of DOM production by HP and their drivers, and

367 therefore to constrain carbon fluxes through HP.

368

369 **Conclusion and perspectives**

370 In this study, we demonstrate that two marine heterotrophic bacterial strains growing on

371 glucose release remarkable amounts of DOM (compared with biomass production), and

372 that an important fraction of this bacterial derived DOM is available for Mediterranean

373 Sea HP communities. Should this pattern be observed globally, the production of DOM

374   by prokaryotes in the sea would change carbon fluxes through HP towards a decrease in

375   prokaryotic growth efficiency, as well as shaping HP community and exoenzyme

376   activities. While in the present study DOM release is measured in single strains growing

377   on glucose, the uptake of other organic compounds will likely affect the quantity and

378   lability of the released DOM, hence affecting further metabolisms in the ocean. Further

379   work is needed to better constrain the main mechanisms affecting HP DOM production

380   and degradation in the ocean, thus improving our knowledge on carbon sequestration

381   processes such as the microbial carbon pump.

382   **Experimental procedures**

383   **Bacterial strains growth**

384   In order to get dissolved organic matter (DOM) produced by marine bacteria, we grew

385   axenically two strains with contrasting ecology and different taxonomical classes: The

386   first one, *Photobacterium angustum S14*, is a Gammaproteobacterium (Vibrionales,

387   Vibrionaceae) which was isolated from coastal waters (Humphrey et al., 1983). The

388   second strain, *Sphingopyxis alaskensis* RB2256 (Sphingomonoadales,

389   Sphingomonadaceae), is an Alphaproteobacterium isolated from open waters in the

390   North Pacific Ocean (Eguchi et al., 2001). These bacterial strains can be considered as

391   models of copiotrophic (*P. angustum*) and oligotrophic (*S. alaskensis*) life styles (Lauro

392   et al., 2009). The strains were selected based on their capacity to grow on glucose and

393   the laboratory's experience in culturing them (Matallana-Surget et al., 2009; Koedooder

394   et al., 2018). Both strains were preserved in glycerol at -80ºC. The stocks were thawed

395   and grown in minimum media, modified from Fegatella et al. (1998), composed of

396   artificial seawater (salinity 35) with glucose as the sole carbon source ($\approx$200 μM C),

397   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 x10^6 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 25ºC 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 phased 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 (450ºC, 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 -20ºC 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

423 and s2 were used for biodegradation experiment 3. Being aware that freezing could alter

424 some DOM properties, we chose this preservation method due to the inability to

425 perform the biodegradation experiments shortly after the strain incubations were

426 finished.

427 **Biodegradation experiments**

428 To study how DOM derived from the bacterial strains is degraded by natural marine

429 heterotrophic prokaryotic (HP) communities, re-growth cultures were performed (Table

430 1). These consisted of 10% dilutions of natural HP communities from different NW

431 Mediterranean Sea locations (water prefiltered by 0.8 μm to remove most

432 phytoplankton and grazers) with 90% DOM (0.2 μm filtrates) from the following

433 sources: DOM sampled from the same waters as the HP communities (in situ surface or

434 deep DOM, ndom treatments) or DOM harvested from the strain cultures explained

435 above: *Photobacterium angustum* (*P. angustum* DOM treatments, pdom) or

436 *Sphingopyxis alaskensis* (*S. alaskensis* DOM treatments, sdom). DOM for ndom

437 treatments was prepared by filtering seawater from the same location as the prokaryotic

438 innocula through 0.2 μm filters as for the bacterial DOM. Inorganic nutrients (nitrogen

439 (50:50 mixture of NH_4Cl and NO_3) and phosphorus ($NaHPO_4$), final concentrations in

440 supplementary Table 1) were added to each treatment to force carbon limitation, and all

441 treatments were set up in triplicate in 250 mL flasks. Incubations were performed at

442 18ºC in the dark for 7-8 days.

443 Using this approach, we performed three different experiments: Open sea-March

444 experiment: We selected waters from a station located on the edge of the continental

445 shelf (hereafter termed as "open sea") in the North Western Mediterranean Sea

446 (42°27'205 N – 03°32'565 E, 600m depth) at two different depths: 5m (surface

447 communities) and 500m (deep communities), in March 2018. These natural HP

448 communities were grown on in situ DOM (surface or deep DOM) or on *P. angustum*

449 DOM, with the purpose to evaluate the differences in HP growth depending on the

450 initial community (surface vs. deep marine communities). For this experiment, samples

451 for DOC, FDOM and nutrients were taken at the onset and end of the incubations, while

452 HP abundance was monitored daily by flow cytometry. For the Coastal sea-April

453 experiment, we collected surface waters from a coastal station (42º29'3N, 3º8'7E, 20m

454 bottom depth) in April 2018 and grew HP communities on in situ DOM, DOM

455 produced by *P. angustum,* and DOM produced by *S. alaskensis,* to evaluate growth of

456 the same HP community on different DOM sources. In this experiment, we monitored

457 the same variables as in Open sea-April experiment and, in addition, we measured the

458 exoenzyme activity at the onset, day 3, and end of the experiment. For the Coastal sea-

459 December experiment, we collected surface waters from the same coastal station as for

460 biodegradation Experiment 2 (42º29'3N, 3º8'7E, 20m depth), but in December 2018.

461 The treatments were the same as in May 2018 and thus allowed us to investigate

462 seasonal differences. We included an additional "+Gluc" treatment to evaluate the effect

463 of residual glucose amounts in the bacterial DOM sources. In this last experiment, we

464 monitored the same variables as in biodegradation experiment 2 (DOM, nutrients, HP

465 abundance and exoenzyme activities) and, additionally, we measured the HP

466 community structure (16s rRNA Illumina sequencing) at the onset and end of the

467 experiment.

468 **Chemical and biological analyses**

469 *Dissolved organic carbon (DOC)*

470 Samples (10 mL) for DOC were transferred into precombusted glass tubes, acidified

471 with 85% H_3PO_4 (final pH 2), closed with Teflon lined screw caps and stored in the

472   dark at room temperature until analysis (less than 2 months after sampling). Calibration

473   curves were made using an acetanilide solution (C_8H_9NO; M= 135.17 g mol^{-1}). DOC

474   was analysed using the high temperature catalytic oxidation (HTCO) technique (Benner

475   and Strom, 1993) with a Shimadzu TOC-V-CSH analyser. Standards of 44–45 μmol C

476   L^{-1}, provided by D.A. Hansell and Wenhao Chen (Univ. of Miami), were used to assess

477   the accuracy of the measurements.

478   *Glucose*

479   Glucose was quantified using a modification of the colorimetric protocol described by

480   Myklestad et al. (1997). Samples (200 μL) were stored in pre-combusted glass vials at -

481   20°C until analysis. All reagents were downscaled to 200 μL samples, and incubations

482   after reagent A additions were performed during 20 min in a 100ºC water bath. The

483   reagents were calibrated daily using a standard curve made of D-glucose in the same

484   batch of artificial seawater used for the strain incubations. Milli-Q water and artificial

485   sea water blanks were run daily in triplicate. Glucose concentrations in μmol were

486   converted in carbon equivalent (C-Glucose).

487   *Fluorescent dissolved organic matter (FDOM)*

488   FDOM samples were freshly (within 1-3 h) analyzed with a Perkin Elmer luminescence

489   spectrometer LS 55 equipped with a xenon discharge lamp, equivalent to 20 kW. Slit

490   widths were 10.0 nm for the wavelengths of excitation and emission. We characterized

491   4 groups of fluorophores using the following excitation/emission (ex/em) pairs (Coble,

492   1996): 280 nm/350 nm (peak T, surrogate of protein-like substances); 250 nm/435 nm

493   (peak A); 340 nm/440 nm (peak C) and 320 nm/410 nm (peak M) the later three due to

494   humic substances of different origins (Coble, 1996). Fluorescence intensities of the

495   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.

Dissolved inorganic nutrients

498   Samples for inorganic nutrient analyses were kept frozen at -20°C. NO_3+NO_2, and PO_4

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 NO_3+NO_2, and 0.03 μM for PO_4. NH_4

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).

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).

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 ß-D-glucopyranoside (for ß-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 μmol 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 °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 °C after adding 41 μL of freshly prepared

538 lysozyme solution (36 mg mL^{-1}), and a second incubation at 55 °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.

543   The V4-V5 region of the 16S rRNA gene from both fractions was amplified with the

544   primer sets 515F-Y (5'-GTGYCAGCMGCCGCGGTAA) and 926-R (5'-

545   CCGYCAATTYMTTTRAGTTT) as described in Parada et al. (2016) with a

546   modification to the PCR amplification step. Triplicate 20μL reaction mixtures contained

547   2 μg DNA, 5 μl KAPA2G Fast HotStart ReadyMix, 0.2 μM forward primer and 0.2 μM

548   reverse primer. Cycling reaction started with a 3 min heating step at 95°C followed by

549   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

550   for 5 min. The presence of amplification products was confirmed by 1% agarose

551   electrophoresis and triplicate reactions were pooled. The pooled PCR amplicons were

552   purified using Sephadex G-50 Superfine resin (GE Healthcare Bio-Sciences, New

553   Jersey, USA) following the protocol. The purification step aims to desalt the samples

554   and eliminate unincorporated nucleotides and excess PCR primers. 16S rRNA gene

555   amplicons were sequenced with Illumina MiSeq 2 × 250 bp chemistry on one flow-cell

556   at GeT-PlaGe platform (Toulouse, France). A mock community DNA (LGC standards,

557   UK) was used as a standard for subsequent analyses and considered as a DNA sample

558   for all treatments.

559   All samples from the sequencing run were demultiplexed by GeT-PlaGe and barcodes

560   were trimmed off. Processing of sequences was performed using the DADA2 pipeline

561   (version 1.10) (Callahan et al., 2016) in R (version 3.4.2) with following parameters:

562   trimLeft=c(19, 20), truncLen=c(240, 200), maxN=0, maxEE=c(2, 2), truncQ=2. Briefly,

563   the pipeline combines the following steps: filtering and trimming, dereplication, sample

564   inference, chimera identification, and merging of paired-end reads. It provides exact

565   amplicon sequence variants (ASVs) from sequencing data with one nucleotide

566   difference instead of building operational taxonomic units (OTUs) based on sequence

567   similarity. ASVs were assigned against SILVA release 132 database (Quast et al.,

568 2013). Singletons and sequences assigned to chloroplast and mitochondria were

569 removed prior to subsequent analyses. Sequences were submitted to NCBI under the

570 accession number PRJNA630555

571

572 **Data analyses**

573 In the strain incubations, we calculated the % of initial DOC transformed to bacterial-

574 derived DOM (%DOC) as the difference between final carbon as glucose (C-Glucose)

575 and dissolved organic carbon (DOC) along the incubations as follows:

576
$$\%DOC = \frac{DOC_{tf}(\mu mol\ L^{-1})-C-Glucose_{tf}(\mu mol\ L^{-1})}{DOC_{t0}(\mu mol\ L^{-1})} \qquad (1)$$

577 This equation would be applicable only if at t0, DOC and glucose values were equal.

578 However, there were slight differences between these two pools at t0 in the *S.*

579 *alaskensis* cultures s1 and s2 (Fig. 1). Because glucose was the sole organic carbon

580 source added to the cultures (final concentration of 200 μM C-glucose), the difference is

581 likely due to the introduction of DOC contamination while manipulating the cultures, or

582 due to analytical differences between the glucose and DOC analyses.

583 Thus, we decided to consider this initial DOC excess (DOC(t0)-C-glucose(t0)) as a non-

584 reactive pool and subtract it from the concentrations at the beginning and end of each

585 incubation. This is the most conservative way to calculate the %DOC released as DOM

586 by the strains, since, alternatively, the strains could have taken up some of this extra

587 DOC and then released a fraction of it back as bacterial DOM.

588 Prokaryotic growth efficiency (PGE) in the biodegradation experiments was calculated

589 as the increase in cell biomass between t0 and tf divided by the decrease in DOC

590 between t0 and tf in the incubations using this formula:

591
$$PGE\ (\%) = \frac{\Delta Biomass\ (\mu mol\ L^{-1})}{\Delta DOC\ (\mu mol\ L^{-1})} \times 100 \qquad (2)$$

592 using a general cell to biomass conversion factor of 20 fg cell^{-1} (Watson et al., 1977).

593 Differences in HP abundance between treatments in the biodegradation experiments

594 were tested using repeated measures ANOVA and post-hoc pairwise t-tests (package

595 "rstatix" in R). Differences in prokaryotic community structure were tested by

596 ANOSIM tests (package "vegan" in R).

607 **References**

608 Andrews, S.C., Robinson, A.K., and Rodríguez-Quiñones, F. (2003) Bacterial iron

609 homeostasis. *FEMS Microbiology Reviews* **27**: 215-237.

610 Aparicio, F.L., Nieto-Cid, M., Borrull, E., Romero, E., Stedmon, C.A., Sala, M.M. et al.

611 (2015) Microbially-mediated fluorescent organic matter transformations in the deep

612 ocean. Do the chemical precursors matter? *Frontiers in Marine Science* **2**.

613 Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyerreil, L.A., and Thingstad, F. (1983)

614 The ecological role of water-column microbes in the sea. *Marine Ecology-Progress*

615 *Series* **10**: 257-263.

616 Bayer, B., Hansman, R.L., Bittner, M.J., Noriega-Ortega, B.E., Niggemann, J., Dittmar,

617 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 heterotrophicmetabolism. *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 Sarcrasso 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., Kérouel, 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) Signiticance 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., Guéneuguès, A., Van Geersdaële, R., Vergé, 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 angsutum explained by an

705   unexpected role of photolyase. *Photochem Photobiol Sci* **8**: 1313-1320.

706   Myklestad, 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., Sarmento, 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

783　**Table 1. Overview of the biodegradation experiments**: Incubation date, treatment
784　abbreviations, dissolved organic matter (DOM) and heterotrophic prokaryote (HP)
785　innocula sources. deep.n: Deep DOM-Deep HP. deep.p: *P. angustum* DOM -Deep HP
786　surf.n: Surface DOM-Surface HP. surf.p: *P. angustum* DOM -Surface HP. ndom:
787　Surface DOM-Surface HP. +gluc: Surface DOM+ glucose-Surface HP. pdom : *P.*
788　*angustum* DOM -Surface HP. sdom: *S. alaskensis* DOM-Surface HP
789

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	*P. angustum*	"			
		surf.n	5m	5m			
		surf.p	*P. angustum*	"			
Coastal Sea-April	10 Apr 2018				√	√	
		ndom	5m	5m			
		pdom	*P. angustum*	"			
		sdom	*S. alaskensis*	"			
Coastal Sea-December	12 Dec 2018				√	√	√
		ndom	5m	5m			
		+gluc	*5m (+glucose)*	"			
		pdom	*P. angustum*	"			
		sdom	*S. alaskensis*	"			

790

791 **Table 2:** Characteristics of bacterial-derived DOM (mean ± standard deviation):
792 percentage of initial DOC released as bacterial dissolved organic matter (%DOC), final-
793 initial (Δ) values of fluorescent DOM peaks C, M, A (all surrogates for humic-like
794 DOM, x10^{-3} Raman Units) and T (surrogate for protein-like DOM, x10^{-3} Raman Units)
795 and the ratio between Peaks C and T (C/T, indicative of the prevalence of protein-like
796 over humic-like DOM). p: *Photobacterium angustum* s: *Sphingopyxis alaskensis*. p 1
797 and p 2, and s 1 and s 2 refer to independent experiments (See Experimental Procedures
798 for details).
799
800

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

801

802   Table 3. See horizontal Tables document

803

804

805　　Table 4. Diversity indexes: Richness, Evenness and Shannon for every treatment of the
806　　Biodegradation experiment 3
807
808

	Richness		Evenness		Shannon	
Treatment	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- *P. angustum* DOM	81	10	0.59	0.03	2.60	0.16
Surf HP- *S. alaskensis* DOM	40	4	0.46	0.11	1.69	0.39

809
810
811
812

813   Table 5: see horizontal Tables document
814

Figure Captions

816

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

821

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

830

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

837

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

841

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

Table 3. Initial and final values of dissolved organic carbon (DOC) concentration (μmol L^{-1}), ΔDOC per time (μmol L^{-1} h^{-1}), ΔDOC/DOCt0 (%), ΔBiomass/DOC (%) and DOM fluorescence (protein-like peak T, humic-like peaks A, C and M, Raman Units) in all biodegradation experiments; tf: mean ± 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**
Open Sea-March													
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- *P. angustum* 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- *P. angustum* 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
Coastal Sea- April													
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- *P. angustum* 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- *S. .alaskensis* 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
Coastal Sea-December													
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- *P. angustum* 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- *S. alaskensis* 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 (μmol L^{-1})	Time (d)	DOC final (μmol L^{-1})	Biomass final (μmol 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 (μmol L^{-1})	Time (d)	DOC final (μmol L^{-1})	Biomass final (μmol L^{-1})	%DOC Released as DOM	%DOC into biomass
(Romano et al., 2014)	Glucose, P limited	*Pseudovibrio sp. FO-BEG1*	60000		na	2000	0.9[*]	3.3
"	Glucose, P replete	"					0.2[*]	
(Zhang et al., 2015)	Glucose	*Alteromonas sp. JL2810*	3333	5			8[**]	
(Goto et al., 2017)	Glucose	*Alteromonas macleodii*	995	1	58	6.1	5.8	0.6
(Noriega-Ortega et al., 2019)	Acetate	*Phaeobacter inhibens*	70050	na	2400	na	3.4	na
Present Study	Glucose	*Photobacterium angustum*	198		55	15.4	16.3	4.4
		Sphingopyxis alaskensis	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, µmol L^{-1}, dark grey columns) and glucose-C (µmol 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.
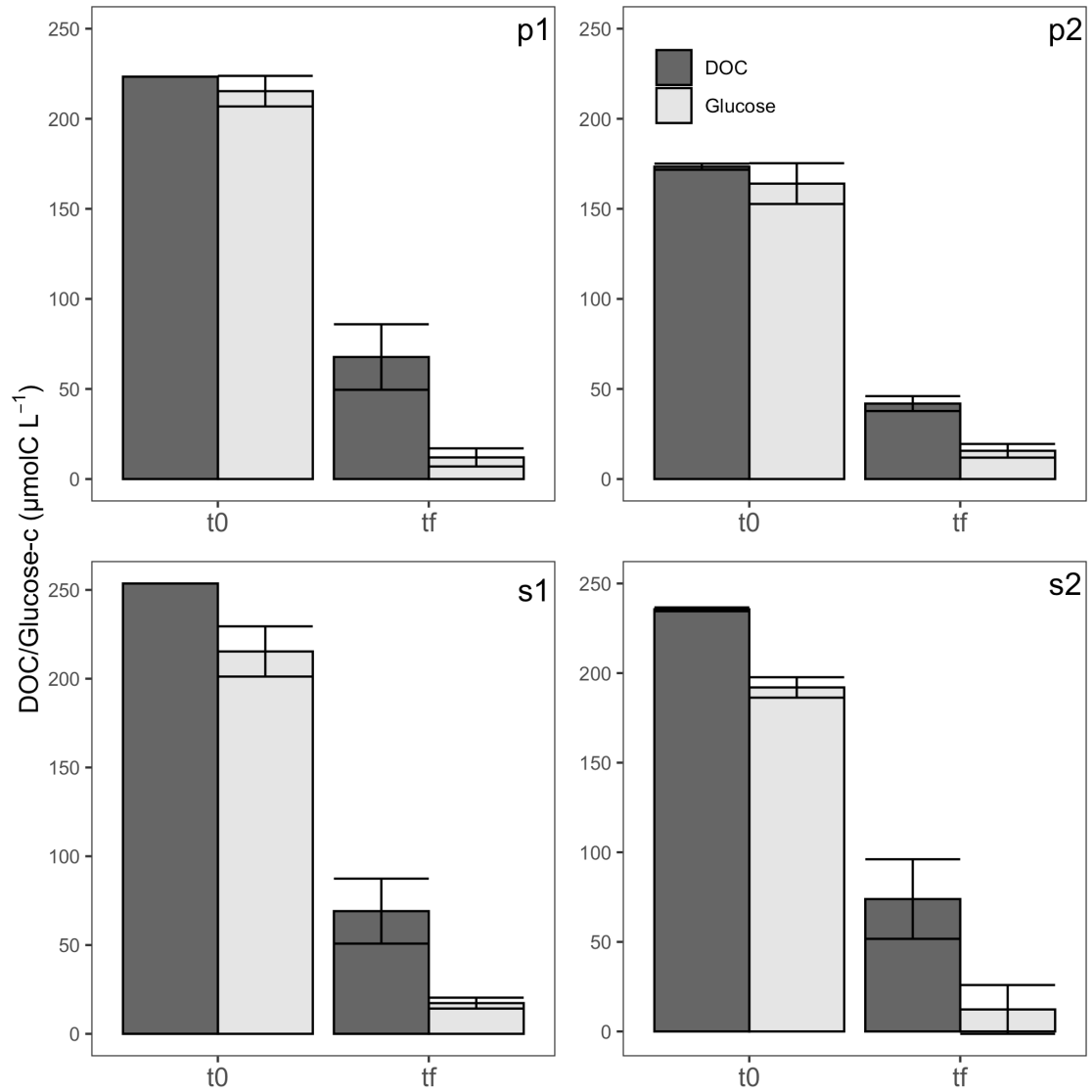
Figure 2. Changes in prokaryotic heterotrophic abundance (PHA, cells mL^{-1}) over incubation time in the three biodegradation experiments. Open sea-March (A): deep.n: deep water HP and DOM, deep.p: deep water HP, *P. angustum* DOM. surf.n: surface water HP and DOM, surface.p: surface water HP, *P. angustum* DOM. Coastal sea-April (B) and coastal sea-December (C): ndom: surface water HP and DOM; +gluc: surface water HP and DOM, +20 µm 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
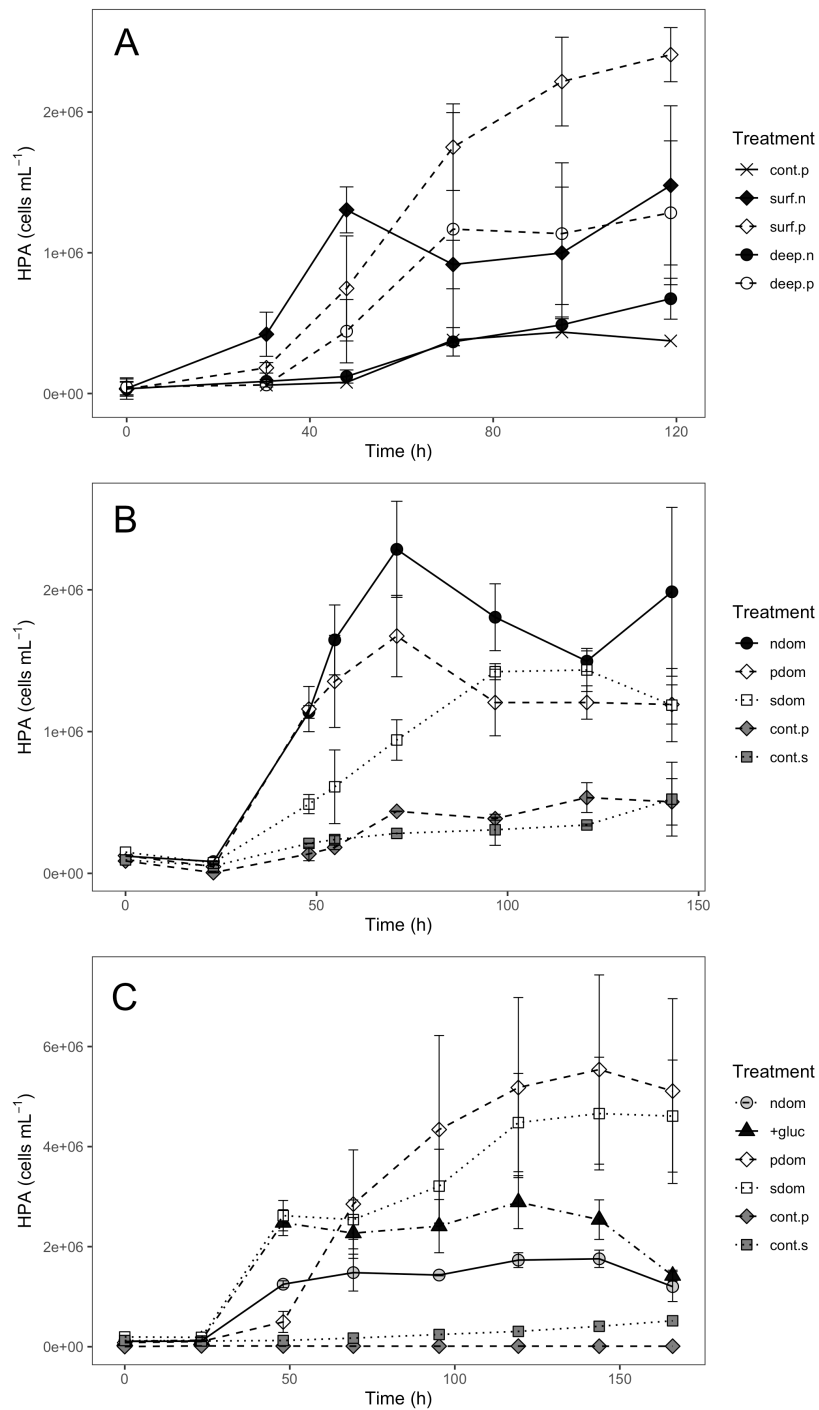
Figure 3. Prokaryotic Growth efficiency (PGE, %) in the biodegradation experiments (Biodeg). Open Sea-March: deep.n: deep water HP and in situ DOM, deep.p: deep water HP, *P angustum* DOM. surf.n: surface water HP and in situ DOM, deep.p: surface water HP, *P angustum* DOM. Coastal sea experiments: ndom: surface water HP and in situ DOM; +gluc: surface water HP and DOM, +20 μm L^{-1} glucose-C added. pdom: surface water HP, *P. angustum* DOM. sdom: surface water HP, *S. alaskensis* DOM
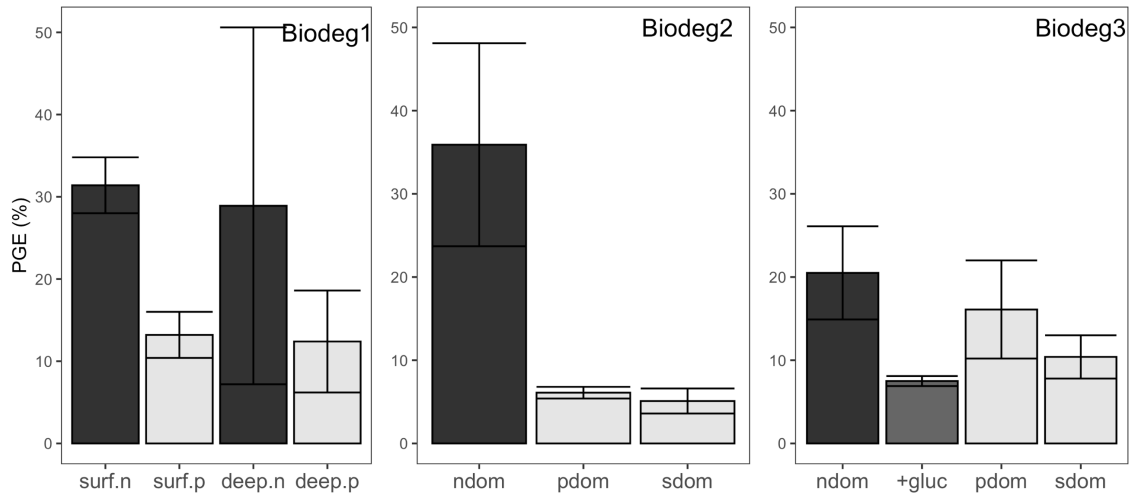
Figure 4. Changes in exoenzymatic activity over time in coastal biodegradation experiments (April: a, b, c, d. December: e, f, g, h). Columns: different study enzymes. Rows: Different experiments

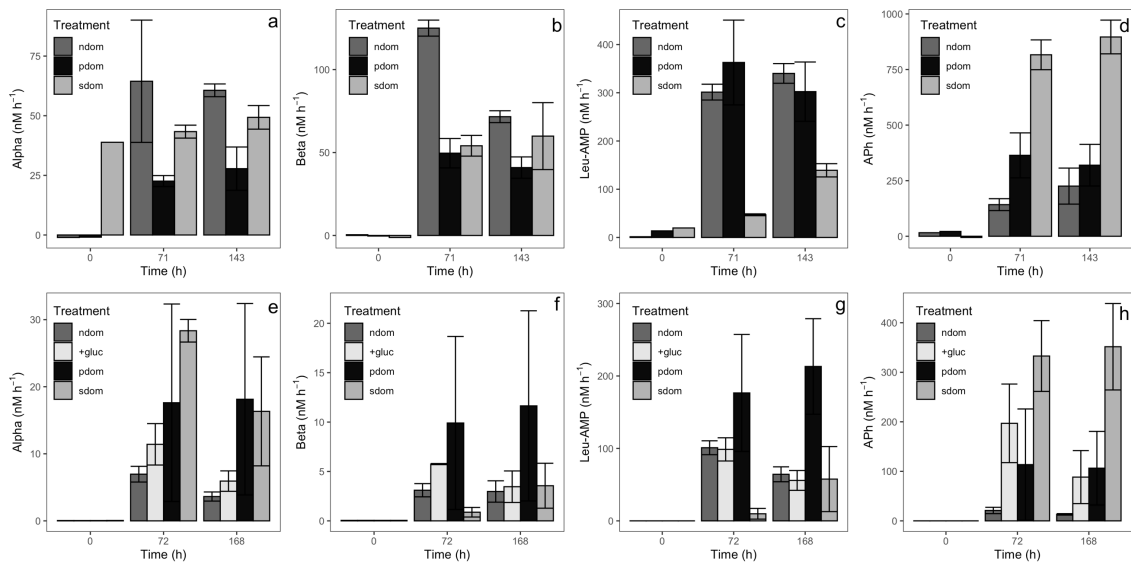**Figure 5.** Left panel: MDS plot of all treatments and replicates based on bray Curtis dissimilarity. Differences in prokaryotic community structure among treatments are significantly (ANOSIM test, p < 0.05). Right panel: Changes in prokaryotic community composition based in 16s rRNA Illumina sequencing in the coastal-December biodegradation experiment. Only orders having ASV's with a relative abundance higher than 2% are plotted.
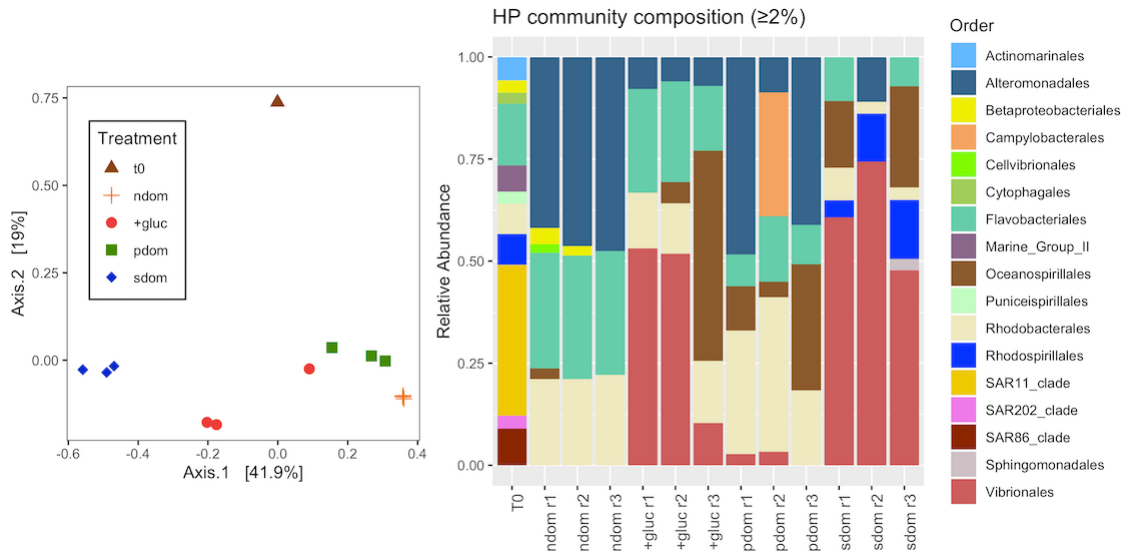
1   Supplementary Table 1. Inorganic nutrients (phosphate, nitrate+nitrite, ammonium
2   µmol L^{-1}) at the onset (t0) and end (tf) of all strain incubations and biodegradation
3   experiments.
4

Strain	Date	PO$_4$ t0	PO$_4$ tf	NO$_3$ t0	NO$_3$ tf	NH$_4$ t0	NH$_4$ tf
S. alaskensis	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
P. angustum	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 ± 0.1	14.3	13.42 ± 0.8	nd	8.94 ± 0.5
"	deep.p	2.0	1.87 ± 0.3	27.6	24.8 ± 2.3	10.6	8.47 ± 2.3
"	surf.n	1.3	1.12 ± 0.1	13.5	11.60 ± 0.2	9.2	8.94 ± 0.5
"	surf.p	2.5	1.77 ± 0.4	28.2	27.91 ± 4.2	11.9	9.57 ± 0.6
Coastal/ April	ndom	1.2	0.90 ± 0.1	12.1	11.33 ± 0.2	nd	nd
"	pdom	2.6	1.63 ± 0.1	26.7	25.13 ± 0.5	nd	nd
"	sdom	2.0	1.66 ± 1.9	31.7	29.79 ± 0.4	nd	nd
Coastal / Dec	ndom	1.6	1.55 ± 0.1	9.3	8.62 ± 0.1	7.3	6.86 ± 0.3
"	+gluc	1.4	1.21 ± 0.0	9.9	9.43 ± 0.2	8.2	6.45 ± 0.1
"	pdom	2.5	2.03 ± 0.1	31.2	29.64 ± 0.2	14.6	12.57 ± 0.4
"	sdom	3.3	2.54 ± 0.1	32.6	30.53 ± 0.5	16.2	11.12 ± 0.3

5

6   Supplementary Figure 1. Changes in cell abundance in the strain growth experiments.
7   Symbols and whiskers are, respectively, averages and standard deviations of three
8   biological triplicates. Samples for DOM characterization were taken at the t0 and t final
9   points in each growth curve. Note the log-10 scale in the y axis
10
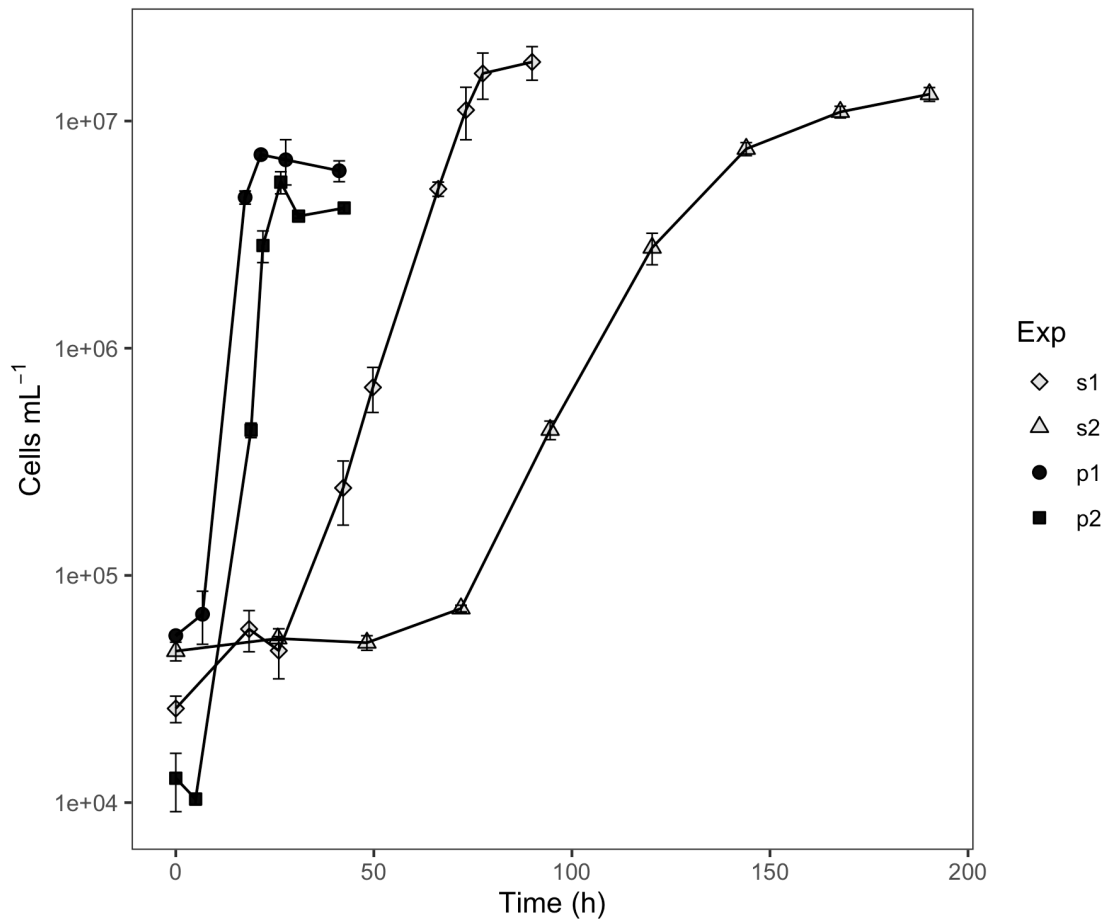
11
12

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

Open Sea/March					
Effect	DFn	DFd	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	DFn	DFd	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	DFn	DFd	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

18
19

20 Supplementary Table 3. Results of pairwise t-tests between each treatment and time in
21 the three biodegradation experiments. Different letters denote significantly different
22 treatments at $p < 0.05$
23

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

24
25

26   Supplementary Table 4. Composition of the minimum media used for strain growth
27

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

28