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Figure or Table # Please group Extended Data items by type, in sequential order. Total number of items (Figs. + Tables) must not exceed 10.	Figure/Table title One sentence only	Filename Whole original file name including extension. i.e.: Smith_ED_Fig1.jpg	Figure/Table Legend If you are citing a reference for the first time in these legends, please include all new references in the main text Methods References section, and carry on the numbering from the main References section of the paper. If your paper does not have a Methods section, include all new references at the end of the main Reference list.
Extended Data Fig. 1	**Chemotactic response of *Marinobacter adhaerens* HP15 to metabolites exuded by *Synechococcus***	Raina_ED_Fig1.eps	The chemotactic index, *Ic* denotes the concentration of cells within ISCA wells, normalized by the mean concentration of cells within wells containing no chemoattractants (filtered ESAW), after 30 min laboratory deployment. Wells containing *Synechococcus* exudates (1 mg ml^{-1}) and 10% Marine Broth (MB) contained significantly more bacteria than the ESAW control (ANOVA, n = 5 biologically independent samples, $p <$ 0.005; Table S5). Error bars represent standard error of the mean.
Extended Data Fig. 2	**Dissolved Organic Matter (DOM) exposure of model bacteria**	Raina_ED_Fig2.eps	Mean DOM exposure for three bacterial motility strategies across three different *Synechococcus* concentrations (leakage rate L = 0.052 pmol hr^{-1}). Chemotaxis conferred an enhancement in the DOM exposure by 2.1-, 1.3-, and 1.1-fold, for *Synechococcus* concentrations of 10^3, 10^4, and 10^5 cells ml^{-1} respectively, compared to non-chemotactic ($\Delta cheA$) or non-motile ($\Delta fliC$) mutants.
Extended Data Fig. 3	**Residence time of model bacteria**	Raina_ED_Fig3.eps	**(a)**. The bacterial residence time depends on the radius of the analysis zone and motility strategy. For $\Delta cheA$ mutants, the residence time grows linearly with radius. However, WT cells exhibit a steep increase for small radii, reflecting their capacity to detect the phytoplankton exudates. **(b)** The rate at which the residence time increases with radius reveals the zone in which chemotactic bacteria exhibit the strongest behavioral response to the DOM gradient. From this the encounter radius of 35 μm can be extracted. Other

			model parameters include $L = 0.052$ pmol hr^{-1}, $\rho = 10^3$ cells ml^{-1}.
Extended Data Fig. 4	**DOM profile does not depend strongly on bacterial consumption**	Raina_ED_Fig4.eps	In each plot, the steady state DOM profile emerges due to a balance between constant phytoplankton exudation and diffusion-limited uptake by bacteria. **(a)** DOM profile for four different bacterial concentrations. **(b)** Restricting bacteria to lie in the region $R < R_0$ has a minor influence on the resultant DOM profile.
Extended Data Fig. 5	**Growth of *Synechococcus* sp. CS-94 RRIMP N1 and *Marinobacter adhaerens* HP15**	Raina_ED_Fig5.eps	**(a)** Growth curves of *M. adhaerens* HP15 wild type (WT), non-chemotactic mutant ($\Delta cheA$), and non-motile mutant ($\Delta fliC$), each separately co-cultured with *Synechococcus* at an initial concentration of 10^3 cells ml^{-1} for both partners. **(b)** Simultaneous growth curve of *Synechococcus* for the same three co-culture experiments. Note: to clearly visualise differences in cell numbers during early timepoints, *Synechococcus* cell numbers are plotted on a logarithmic scale. Asterisks indicate timepoints at which treatments are significantly different (simple main effect test, p < 0.05, Table S9). Error bars represent standard error of the mean (n = 4 biologically independent samples). **(c)** Growth curves of *Marinobacter adhaerens* HP15 wild type (WT), non-chemotactic mutant ($\Delta cheA$), and non-motile mutant ($\Delta fliC$) in Marine Broth. Error bars represent standard error of the mean (n = 3 biologically independent samples). Asterisks indicate timepoints at which treatments are significantly different (simple main effect test, p < 0.05, Table S10).
Extended Data Fig. 6	**DOM concentration within a 2D cross-section of the full 3D profile**	Raina_ED_Fig6.eps	Results correspond to a *Synechococcus* concentration of $\rho = 10^3$ cells ml^{-1}. Other parameters as in Table S8. The white scale bar represents 1 mm.

Extended Data Fig. 7	**DOM exposure of model bacteria**	Raina_ED_Fig7.eps	The mean DOM concentration experienced by **(a)** non-chemotactic ($\Delta cheA$) mutants and **(b)** chemotactic (WT) bacteria, as a function of phytoplankton concentration (cells ml^{-1}) and DOM leakage rate L (pmol hr^{-1}).
Extended Data Fig. 8	**Phytoplankton exudation rate affects bacteria-phytoplankton distances and bacterial "trapping"**	Raina_ED_Fig8.eps	**(a)** Bacteria-phytoplankton distance is strongly affected by phytoplankton exudation rate. These data show the distance to the nearest hotspot, averaged over time (3 h co-incubation) and bacterial population (500 cells), as a function of DOM leakage rate L (pmol hr^{-1}). Results are shown for three different phytoplankton concentrations, 10^3 (dotted), 10^4 (dashed), 10^5 cells ml^{-1} (solid), and for three different bacterial mutants: chemotactic WT (blue), non-chemotactic $\Delta cheA$ (orange), non-motile $\Delta fliC$ (red). **(b)** Bacteria-phytoplankton trapping statistics. These data show the percentage of bacterial cells that are situated within 35 μm of a phytoplankton cell (phycosphere), as a function of DOM leakage rate L (pmol hr^{-1}). For each datapoint, results have been averaged over time (3 h co-incubation) and bacterial population (500 cells). Results are shown for three different phytoplankton concentrations, 10^3 (dotted), 10^4 (dashed), 10^5 cells ml^{-1} (solid), and for three different bacterial mutants: chemotactic WT (blue), non-chemotactic $\Delta cheA$ (orange), non-motile $\Delta fliC$ (red).
Extended Data Fig. 9	**Distribution of the single cell enrichment data reported in Figure 1 and 2**	Raina_ED_Fig9.eps	**(a)** ^{15}N uptake of *M. adhaerens* (10^3: n=166; 10^4: n=286; 10^5: n=172) and **(b)** ^{13}C uptake of *Synechococcus* (10^3: n=10; 10^4: n=17; 10^5: n=37).
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Item	Present?	Filename Whole original file name including extension. i.e.: Smith_SI.pdf. The extension must be .pdf	A brief, numerical description of file contents. *i.e.: Supplementary Figures 1-4, Supplementary Discussion, and Supplementary Tables 1-4.*
Supplementary Information	Yes	Raina_et_al_SI.pdf	Supplementary Figure 1, Supplementary Tables 1-10
Reporting Summary	Yes	nr-reporting-summary_jb_raina_Jan23.pdf	
Peer Review Information	No	*OFFICE USE ONLY*	

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Type	Number Each type of file (Table, Video, etc.) should be numbered from 1 onwards. Multiple files of the same type should be listed in sequence, i.e.: Supplementary Video 1, Supplementary Video 2, etc.	Filename Whole original file name including extension. i.e.: *Smith_ Supplementary_Video_1.mov*	Legend or Descriptive Caption Describe the contents of the file
Supplementary Table	Supplementary Tables 1-10	Raina_et_al_SupplementaryTables.xlsx	Full Supplementary Tables 1-10
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| Source Data Fig. 1 | Raina_et_al_SourceFig1.xlsx | Raw data for Figure 1 |
| Source Data Fig. 2 | Raina_et_al_SourceFig2.xlsx | Raw data for Figure 2 |

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41 **Chemotaxis increases metabolic exchanges between marine picophytoplankton and**
42 **heterotrophic bacteria**
43

44   Jean-Baptiste Raina[1][*][#], Marco Giardina[1][*], Douglas R. Brumley[2][*], Peta L. Clode[3,4,5],
45   Mathieu Pernice[1], Paul Guagliardo[3], Jeremy Bougoure[3], Himasha Mendis[6], Steven Smriga[7],
46   Eva C. Sonnenschein[8,9], Matthias S. Ullrich[10], Roman Stocker[11], Justin R. Seymour[1][#]
47
48

49 [1] University of Technology Sydney, Climate Change Cluster, Faculty of Science, Ultimo,
50 NSW, Australia
51 [2] School of Mathematics and Statistics, The University of Melbourne, Parkville, Victoria,
52 Australia,
53 [3] Centre for Microscopy Characterisation and Analysis, The University of Western Australia,
54 Perth, Australia
55 [4] UWA School of Biological Sciences, The University of Western Australia, Perth, Australia
56 [5] UWA Oceans Institute, The University of Western Australia, Perth, Australia
57 [6] Metabolomics Australia, Bio21 Institute, The University of Melbourne, Parkville, Australia
58 [7] Department of Earth Atmospheric and Planetary Sciences, Massachusetts Institute of
59 Technology, Cambridge, MA, USA
60 [8] Department of Biotechnology and Biomedicine, Technical University of Denmark, Kgs.
61 Lyngby, Denmark
62 [9] Department of Biosciences, Swansea University, Singleton Park, Swansea, SA2 8PP,
63 Wales, United Kingdom
64 [10] Department of Life Sciences & Chemistry, Jacobs University Bremen, Bremen, Germany
65 [11] Institute of Environmental Engineering, Department of Civil, Environmental and Geomatic
66 Engineering, ETH Zurich, Zurich, Switzerland
67
68 [*] These authors contributed equally to this work
69 [#] Corresponding authors
70 Jean-Baptiste.Raina@uts.edu.au
71 Justin.Seymour@uts.edu.au
72
73

Abstract

74

75 Behaviours such as chemotaxis can facilitate metabolic exchanges between phytoplankton and

76 heterotrophic bacteria, which ultimately regulate oceanic productivity and biogeochemistry.

77 However, numerically dominant picophytoplankton have been considered too small to be

78 detected by chemotactic bacteria, implying that cell-cell interactions might not be possible

79 between some of the most abundant organisms in the ocean. Here we examined how bacterial

80 behaviour influences metabolic exchanges at the single-cell level between the ubiquitous

81 picophytoplankton *Synechococcus* and the heterotrophic bacterium *Marinobacter adhaerens*,

82 using bacterial mutants deficient in motility or chemotaxis. Stable-isotope tracking revealed

83 that chemotaxis increased nitrogen and carbon uptake of both partners by up to 4.4-fold. A

84 mathematical model following thousands of cells confirmed that short periods of exposure to

85 small but nutrient-rich microenvironments surrounding *Synechococcus* cells provide a

86 considerable competitive advantage to chemotactic bacteria. These findings reveal that

87 transient interactions mediated by chemotaxis can underpin metabolic relationships among the

88 ocean's most abundant microorganisms.

89

90 **Main text**

91 The substantial impact of microbial communities on the productivity and biogeochemistry of

92 the ocean is shaped by intricate networks of inter-organismal interactions[1,2]. Among pelagic

93 microbial relationships, the often-mutualistic metabolic associations between phytoplankton

94 and bacteria are some of the most important[3,4]. Reciprocal exchanges of metabolites, including

95 a diverse suite of organic and inorganic molecules, vitamins and minerals can support the

96 growth of both phytoplankton and bacterial partners[5-7]. However, within the vast expanses of

97 the ocean, the efficacy of these, often highly specific, chemical exchanges will be hindered by

98 large inter-cell distances (hundreds of micrometres on average) and the sharp diffusive decay

99 of metabolite concentrations with distance from exuding cells[8]. Phytoplankton-bacteria

100 partnerships may, nevertheless, overcome these constraints through the formation of close

101 spatial associations within the microenvironment immediately surrounding individual

102 phytoplankton cells, known as the phycosphere[9], which is characterized by elevated

103 concentrations of metabolites emanating from the phytoplankton cell. It has long been

104 theorized[9-11] that bacteria can locate and maintain position within the phycosphere using

105 chemotaxis – the capacity of motile cells to migrate up or down chemical gradients – resulting

106 in sustained spatial proximity of partners[12] and greatly enhanced metabolic exchanges[13].

107

108 There is substantial evidence for bacterial chemotaxis towards phytoplankton-derived
109 chemicals[9,12,14,15] and for the ability of bacteria to use chemotaxis to actively aggregate around
110 large phytoplankton cells, such as diatoms[16]. Yet, across vast areas of the ocean, phototrophic
111 biomass and primary production are dominated by picophytoplankton (< 3 µm), including
112 picoeukaryotes and the cyanobacteria *Prochlorococcus* and *Synechococcus*[17]. Like large
113 phytoplankton including diatoms and dinoflagellates, these picophytoplankton appear to have
114 important metabolic interdependencies with heterotrophic bacteria. Laboratory cultures of
115 *Prochlorococcus* and *Synechococcus* exhibit enhanced growth in the presence of specific
116 heterotrophic associates[18-20], and metabolic exchanges have been identified using
117 transcriptomic and proteomic approaches[18,21]. However, the nature of the ecological coupling
118 between picophytoplankton and heterotrophic bacteria in the environment is somewhat
119 paradoxical. While close proximity of partners is anticipated to strongly enhance and sustain
120 mutualistic phototroph-heterotroph interactions[12], physical constraints associated with the
121 small size of *Prochlorococcus* and *Synechococcus* cells are thought to prevent heterotrophic
122 bacteria from using chemotaxis to detect and retain position within their phycosphere[22]. Here
123 we show, however, that heterotrophic bacteria are indeed able to utilize chemotaxis to
124 substantially enhance their metabolic exchanges with picophytoplankton, demonstrating that
125 behavioural associations may shape the ecological relationships among some of the oceans'
126 most abundant microorganisms.
127

128 **RESULTS**
129 ***Synechococcus* and *Marinobacter* exchange N and C**
130 Using nano-scale secondary ion mass spectrometry (NanoSIMS), we directly quantified the
131 chemical exchanges between *Synechococcus* strain CS-94 RRIMP N1 and *Marinobacter*
132 *adhaerens* HP15, a heterotrophic Gammaproteobacterium associated with larger
133 phytoplankton (diatoms)[13,23]. Closely related *M. adhaerens* strains (more than 98% 16S rRNA
134 gene sequence identity) are also abundant in picophytoplankton cultures[20,24]. Given that
135 *Synechococcus* cells produce and release nitrogen-rich dissolved organic matter (DOM) that
136 can be utilised by heterotrophic bacteria[18], we tracked the transfer of nitrogen from
137 *Synechococcus* to *M. adhaerens*. *Synechococcus* cells were grown in f/2 medium with ^{15}N-
138 labelled $NaNO_3$ as the only source of nitrogen for one week prior to experiments, to ensure
139 high levels of ^{15}N enrichment in cells. To facilitate cell localization under NanoSIMS[25], *M.*
140 *adhaerens* cells were grown separately with ^{13}C-labelled amino acids. *Synechococcus* and *M.*
141 *adhaerens* cells were then thoroughly washed to remove isotopic labels from suspensions and

142   co-grown across a range of *Synechococcus* concentrations (10^3, 10^4 and 10^5 cells ml^{-1})

143   reflecting those occurring in different environments (from open ocean to coastal waters), with

144   a constant concentration of *M. adhaerens* (10^6 cells ml^{-1}). Following three hours of co-

145   incubation in stable light and temperature conditions[26], samples were collected for NanoSIMS

146   analysis.

147

148   After co-incubation, *M. adhaerens* cells were enriched in ^{15}N derived from *Synechococcus*,

149   with levels up to 2.7 times the natural abundance values of ^{15}N in unlabelled *M. adhaerens*

150   (^{15}N/^{14}N = 0.010 ± 0.00081, n = 172, compared to 0.0037 ± 0.000046, n = 300). Significant

151   uptake of *Synechococcus*-derived ^{15}N by *M. adhaerens* (the percentage of N incorporated into

152   the cells relative to the initial N content; see equation (1) in the Methods) occurred at all

153   *Synechococcus* concentrations (Kruskal-Wallis (KW), $p<0.05$; Table S1). ^{15}N uptake in *M.*

154   *adhaerens* increased strongly with the concentration of *Synechococcus* cells (KW, $p<0.05$;

155   Table S1), from 0.36% at 10^3 *Synechococcus* cells ml^{-1} to 0.98% at 10^5 *Synechococcus* cells

156   ml^{-1} (Figure 1a-b). These results deliver direct evidence that nitrogen-containing compounds

157   exuded from *Synechococcus* are taken up by heterotrophic bacteria, demonstrating that

158   chemical exchange takes place between these two organisms.

159

160   To identify which organic nitrogen compounds are exuded by *Synechococcus*, we used an

161   untargeted metabolomic approach. We identified 34 nitrogen-containing compounds exuded

162   by *Synechococcus*, using gas chromatography coupled with mass spectrometry (GC-MS). The

163   exuded compounds included amino acids (cysteine, phenylalanine, methionine, leucine),

164   amines (tyramine, ethanolamine), amides (urea), vitamins (nicotinamide, pantothenic acid),

165   and purines (xanthine; Table S2). Notably, an analysis of the *M. adhaerens* HP15 genome[27]

166   indicates that this bacterium has the capacity to catabolize at least 24 of these 34 compounds

167   (>70%; Table S2), implying the probable importance of these molecules in the metabolic

168   exchange between *Synechococcus* and heterotrophs[18].

169

170   Beyond the transfer of nitrogen from *Synechococcus* to *M. adhaerens*, our analysis also

171   revealed an unexpected exchange of carbon from *M. adhaerens* to *Synechococcus*, evidenced

172   by ^{13}C enrichment in *Synechococcus* cells that reached up to 2.9 times the natural abundance

173   values in unlabelled cells (^{13}C/^{12}C = 0.032 ± 0.0051, n = 10, compared to 0.011 ± 0.000027, n

174   = 102). ^{13}C uptake was not affected by the *Synechococcus* concentration (the percentage of C

175   incorporated into the cells relative to the initial C content; see equation (1) in Methods; KW,

176   *p*>0.05; Table S3, Figure 1c-d), likely because no competition for ^{13}C occurred at the

177   *Synechococcus* concentrations tested (see Methods). Using GC-MS, we identified 80 organic

178   compounds exuded by *M. adhaerens* that potentially contributed to the carbon enrichment in

179   *Synechococcus*. These compounds included sugars (galactose, mannose, sucrose), amino acids

180   (glycine, alanine, serine), organic acids (phosphoric acid, benzoic acid, pyroglutamic acid),

181   hormones (methoxytryptamine), and fatty acids (linoleic acid, palmitic acid, myristic acid;

182   Table S4). The identity of some of these compounds is consistent with previous reports of

183   photoheterotrophy in *Synechococcus* and their uptake of amino acids and urea[28,29]. Taken

184   together, these results provide the first direct evidence that *Synechococcus* cells can

185   simultaneously supply heterotrophic bacteria with nitrogen while acquiring carbon from them

186   (Figure 1e), pointing to a relationship that is akin to the reciprocal metabolic associations

187   observed between larger phytoplankton (*e.g.*, diatoms) and heterotrophic bacteria[6].

188

189   **Chemotaxis facilitates reciprocal metabolic exchanges**

190   *M. adhaerens* is motile[13] and we found, using a chemotaxis assay[30], that it is significantly

191   attracted towards dissolved organic matter exuded by *Synechococcus* (3.5 ± 0.3 times more

192   cells compared to controls, which consistent with levels of chemoattraction reported for other

193   marine Gammaproteobacteria the same experimental conditions[31]; ANOVA, *p*<0.05; Table S5,

194   Extended Data Figure 1). However, this observation does not provide confirmation that *M.*

195   *adhaerens* can use chemotaxis to home in on the phycosphere of individual *Synechococcus*

196   cells to gain a metabolic benefit. In fact, a previous mathematical model (parameterized with

197   the chemosensory capabilities of *Escherichia coli*), predicted that the chemical gradients in the

198   phycosphere of picophytoplankton are too small to be detectable by chemotactic bacteria[22].

199   This prediction has resulted in the paradigm that, whereas associations between heterotrophic

200   bacteria and large phytoplankton can be mediated by bacterial behaviour[11,22], those between

201   heterotrophic bacteria and picophytoplankton cannot[22]. To explicitly test the role of chemotaxis

202   in the exchange of resources between the two microorganisms, we compared the uptake of

203   *Synechococcus*-derived ^{15}N by *M. adhaerens* among three *M. adhaerens* phenotypes: the

204   motile and chemotactic wild-type (WT); a motile but non-chemotactic mutant (Δ*cheA*)[13]; and

205   a non-motile mutant (Δ*fliC*)[13] (Figure 2a).

206

207   These experiments revealed that the magnitude of the chemical exchange between

208   *Synechococcus* and *M. adhaerens* was substantially smaller when *M. adhaerens* cells were not

209   chemotactic. After three hours of co-incubation with ^{15}N-labelled *Synechococcus*, bacteria

210   from all three phenotypes were enriched in ^{15}N compared to unlabelled cells (Figure 2b), but

211   the level of enrichment was strongly determined by the bacteria's capacity for chemotaxis.

212   Specifically, in the treatments with the low and intermediate *Synechococcus* concentrations

213   (10^3 and 10^4 cells ml^{-1}), the ^{15}N uptake of the WT *M. adhaerens* was more than double that of

214   the Δ*fliC* (2.6 and 2.4-fold increase, for 10^3 and 10^4 cells ml^{-1}, respectively) and Δ*cheA* (2.0

215   and 1.8-fold increase, for 10^3 and 10^4 cells ml^{-1}, respectively) mutants (KW, *p*<0.05; Table S6,

216   Figure 2b). At high *Synechococcus* concentrations (10^5 cells ml^{-1}) this difference vanished,

217   possibly because a high background concentration of substrates renders chemotaxis less

218   advantageous. These results overturn the paradigm that chemotaxis of heterotrophic bacteria

219   towards picophytoplankton is not possible, showing instead that it can deliver bacteria with a

220   substantial advantage in metabolic uptake.

221

222   Chemotaxis of *M. adhaerens* also influenced the uptake of *M. adhaerens*-derived organic

223   carbon by *Synechococcus*. At concentrations of 10^4 and 10^5 cells ml^{-1}, *Synechococcus* co-

224   incubated with WT *M. adhaerens* were up to four times more enriched in ^{13}C than cells co-

225   incubated with Δ*fliC* (1.2 and 1.5-fold increase, for 10^4 and 10^5 cells ml^{-1}, respectively) and

226   Δ*cheA* (1.8 and 4.4-fold increase, for 10^4 and 10^5 cells ml^{-1}, respectively) mutants (KW,

227   *p*<0.001; Table S7, Figure 2c). Therefore, chemotaxis by heterotrophic bacteria not only

228   enhances the uptake of picophytoplankton-derived metabolites by the bacteria, but also

229   increases picophytoplankton uptake of bacteria-derived metabolites, identifying chemotaxis as

230   an important behaviour in the establishment of reciprocal resource exchange between two of

231   the most numerous groups of microorganisms in the ocean.

232

Phycosphere interactions are short-lived yet repeated

234   The role of chemotaxis in enhancing bacterial uptake of picophytoplankton metabolites was

235   supported by a mathematical model that simulates the motion of chemotactic bacteria in a

236   three-dimensional dissolved organic matter (DOM) landscape representative of experimental

237   conditions. A suspension of *Synechococcus* cells was modelled as a collection of randomly

238   positioned spherical 'hotspots', each exuding DOM at a steady rate $L = 0.052$ pmol h^{-1}, which

239   was determined by calibrating the model with experimental parameters (see Methods, Table

240   S8) and is consistent with predictions based on previous estimates of phytoplankton exudation

241   rates[12]. Based on the high proportion of amino acids we measured in the *Synechococcus*

242   exudates by metabolomics (Table S2), the DOM was represented in the model as a single

243   chemoattractant molecule of amino-acid size (diffusivity: 608 μm^2 s^{-1})[32]. The model predicted

244   how the DOM concentration changes in space and time as a result of exudation, diffusion and

245   bacterial uptake, and used this information to compute the three-dimensional trajectories of

246   bacteria executing run-reverse-flick locomotion representative of monotrichous marine

247   bacteria[22,32]. The amount of DOM taken up by 500 individual *M. adhaerens* cells (at 10^6 cells

248   ml^{-1}) was calculated over three hours (see Methods), for the same three bacterial phenotypes

249   (WT, $\Delta cheA$, $\Delta fliC$) used in the experiments. Results revealed an increased uptake of DOM by

250   WT cells when compared to both $\Delta cheA$ and $\Delta fliC$ mutants (Extended Data Figure 2), in

251   agreement with experimental results (Figure 2b). Specifically, chemotactic cells (WT)

252   exhibited a 2.1-, 1.3-, and 1.1-fold increase in DOM uptake over the $\Delta cheA$ mutants, for

253   *Synechococcus* concentrations of 10^3, 10^4, and 10^5 cells ml^{-1}, respectively.

254

255   Investigation of individual bacterial trajectories from the mathematical model revealed the

256   fleeting nature of the interactions between bacteria and picophytoplankton. We defined the

257   phycosphere radius, R_p = 35 µm, as the distance from individual *Synechococcus* cells at which

258   the bacterial chemotactic response was strongest (see Methods; Extended Data Figure 3). This

259   allowed us to quantify from the model the residence time τ associated with each encounter of

260   a bacterium with a *Synechococcus* phycosphere (Figure 3), defined as the time between entry

261   to and departure from the phycosphere (Figure 3a). Computing the residence time for all

262   bacteria-phycosphere encounters revealed that WT cells spend on average three times longer

263   in *Synechococcus* phycospheres (τ=3.2±11.5 s, *n*=82,242 encounters) than $\Delta cheA$ mutants

264   (τ=1.0±1.4 s, *n*=2,626 encounters; Figure 3b-c). The difference was even more pronounced for

265   longer encounters, with residence times greater than 10 s being 16 times more likely for WT

266   cells (7.3% of encounters) than for $\Delta cheA$ mutants (0.46% of encounters).

267

268   Beyond the duration of individual encounters, our trajectory analysis revealed that chemotaxis

269   strongly affects the number of unique phycosphere encounters per day. At a *Synechococcus*

270   concentration of 10^3 cells ml^{-1}, chemotaxis more than tripled the mean encounter rate of

271   bacteria with phycospheres (WT: 12.6 day^{-1}; $\Delta cheA$: 4.1 day^{-1}; Figure 3d). The distribution of

272   encounters revealed that 29% of WT cells but only 0.7% of $\Delta cheA$ cells encountered more than

273   15 unique phycospheres per day. Moreover, the top 5% of WT cells encountered on average

274   36 unique phycospheres per day, compared to only 13 for the top 5% of $\Delta cheA$ cells (Figure

275   3d).

276

277   The phycosphere afforded WT bacteria a substantial fraction of their uptake, even though time
278   spent in the phycosphere was short. Our model revealed that WT bacteria derived 30% of their
279   total DOM uptake from the phycosphere, despite spending merely 1.7% of time in it. In stark
280   contrast, for $\Delta cheA$ bacteria, the proportion of DOM uptake originating from the phycospheres
281   was ~100-fold smaller – they derived just 0.38% of their DOM uptake from the phycosphere,
282   where they spent only 0.02% of time. We conclude that the differences in residence time,
283   coupled with the sharp spatial decay of the DOM concentration in the phycosphere (Extended
284   Data Figure 4), is the cause of the significant enhancement in DOM uptake conferred by
285   chemotaxis.
286
287   **Chemotaxis provides growth advantages**
288   To further explore how chemotactic behaviour may affect the fitness of heterotrophic bacteria,
289   we conducted a multi-day co-culture experiment between *Synechococcus* and each of the three
290   *M. adhaerens* phenotypes (at a starting concentration of 10^3 cells ml^{-1} for both partners). Our
291   results demonstrate that WT *M. adhaerens* grew significantly faster in co-culture with
292   *Synechococcus* than the non-motile mutant and the non-chemotactic mutant (repeated measure
293   ANOVA, $p < 0.05$; Table S9, Extended Data Figure 5). This effect was sustained over four
294   days while *Synechococcus* concentrations remained low, but disappeared as *Synechococcus*
295   increased in abundance beyond 10^5 cell ml^{-1} (Extended Data Figure 5). Importantly, this growth
296   enhancement occurred despite both $\Delta cheA$ and $\Delta fliC$ mutants growing significantly faster than
297   the WT under nutrient-replete conditions (repeated measure ANOVA, $p < 0.05$; Table S10,
298   Extended Data Figure 5). These results reveal that the greater nutrient uptake achieved by
299   chemotactic cells over short timescales (minutes to hours) ultimately increases cell fitness over
300   longer timescales (days).
301
302   Our numerical simulations allowed us to extend the analysis to phytoplankton cells of different
303   sizes, and explore the relative advantage provided by chemotaxis across a range of realistic
304   nutrient sources (Figure 4). Indeed, our simulations revealed that the advantage of chemotaxis
305   is most pronounced at low phytoplankton concentrations, regardless of the phytoplankton cell
306   sizes (or equivalently nutrient leakage rate). However, the relative advantage provided by
307   chemotaxis increased with phytoplankton size, with a >50-fold enhancement in DOM exposure
308   over $\Delta cheA$ mutants when interacting with large, but widely separated phytoplankton (Figure
309   4). In addition, larger phytoplankton sizes dramatically increase the mean residence time of
310   chemotactic bacteria (Figure 3c), ultimately suppressing the transient interactions reported for

Synechococcus and limiting bacterial dispersal (Figure 3e-f). These data indicate qualitatively distinct bacterial interactions with small and large phytoplankton, respectively, and can serve as a blueprint for studying ecological interactions in different regimes.

Our experiments were carried out in laboratory conditions whereby cells were suspended in a nitrogen and phosphorous-rich medium, which is not directly reflective of the nutrient-limiting conditions prevailing in the oligotrophic ocean, and this could potentially influence rates of exudation by *Synechococcus* cells. The factors governing the exudation rates of organic substrates from phytoplankton cells are still largely unresolved[12], and ambient nutrient levels may potentially influence exudations rates. While some evidence suggests that exudation rates are enhanced in oligotrophic conditions[33], it is also possible that under nutrient-limited conditions *Synechococcus* cells may reduce the amount of nitrogen they exude. This uncertainty notwithstanding, our numerical simulations indicate that even if the leakage rate of *Synechococcus* cells were substantially smaller than the one observed in our experiments (i.e. $L=0.01$ pmol h^{-1}), the relative enhancement in nutrient exposure due to chemotaxis would still be sizeable (e.g. 11% enhancement if the exudation rate was five times smaller; Figure 4).

DISCUSSION

The principal goal of our study was to determine whether chemotaxis enhances heterotrophic bacterial exposure to, and assimilation of, substrates released into the phycosphere by picophytoplankton. Motility comes at an energetic cost for cells[34], which we have here not explicitly considered. This energetic cost would partly offset the nutrient uptake advantage and could result in there being a *Synechococcus* cell concentration below which motility no longer provides an advantage. However, in our experiments, no significant difference in ^{15}N uptake between non-chemotactic and non-motile cells was recorded over short co-incubations (Figure 2b), suggesting that the energetic cost of swimming was not detectable over that timeframe (3 hours). However, during longer co-incubations (12 days), the non-motile cells grew on average 24.5% faster than the non-chemotactic ones (Extended Data Figure 5), potentially due to the cost of building and operating the flagellar apparatus.

Taken together, our experimental and modelling results both (i) provide the first quantitative demonstration that chemotaxis enhances the uptake of phytoplankton-derived metabolites by motile heterotrophic marine bacteria and (ii) overturns the paradigm (previously based on the chemotactic parameters from *E. coli*[22]) that marine bacteria will not be able to use chemotaxis

345 towards individual picophytoplankton. The latter points to a greater chemotactic sensitivity of
346 marine bacteria compared to *E. coli*, which is in line with prior observations on the chemotaxis
347 of marine bacteria[32,35]. Picophytoplankton collectively amount to a biomass similar to diatoms
348 at the global scale (12.7 and 16.5 µg C L^{-1}, respectively)[36], despite their diameter being 10-
349 100-fold smaller[36], and are the dominant phototrophic organisms in many parts of the
350 oligotrophic ocean[17]. Our findings therefore expand the potential for chemotaxis to govern the
351 ecological and metabolic interactions between heterotrophic bacteria and phytoplankton to a
352 major fraction of phototrophic biomass in the ocean.

353

354 Our results show that chemotactic bacteria benefit from phytoplankton not just by migrating
355 into and retaining position within their phycosphere, as occurs for larger phytoplankton[16], but
356 through transient spatial associations with the phycospheres. These brief encounters still
357 provide conspicuous advantages, because spending even a small fraction of time in the highly
358 DOM-enriched vicinity of phytoplankton cells translates into large increases in DOM uptake[16].
359 Because of the abundance of picophytoplankton in the global ocean[17], these fleeting
360 interactions will be numerous, providing a viable strategy for nutrient exchanges in the water
361 column. Rather than stably associating with *Synechococcus*, chemotactic heterotrophs
362 therefore derive a competitive advantage over their non-chemotactic counterparts because they
363 can considerably extend the frequency and duration of their spatial association with
364 picophytoplankton, even if each encounter is highly transient.

365

366 Our experiments reveal that these transient interactions increase not only the uptake of
367 *Synechococcus*-derived dissolved organic nitrogen by heterotrophs, but also the uptake of
368 heterotroph-derived carbon by *Synechococcus* cells. This indicates that chemotactic behaviour
369 can foster reciprocal metabolic exchange between marine microorganisms and thereby
370 potentially enhance primary and secondary production levels and rates of nutrient recycling,
371 even in the large regions of ocean dominated by small phytoplankton cells. Chemotaxis is
372 recognized as a pervasively important behaviour enabling the onset and maintenance of
373 symbiotic interactions across different hosts and environments[14], however, symbiosis
374 commonly refers to spatially close and temporally extended interactions between organisms[37].
375 Although very different from this traditional view, the short-lived yet repeated encounters
376 described here benefit both partners and may contribute to their survival in the resource-poor
377 open ocean. These partnerships might therefore represent "transient" symbioses, at the opposite
378 end of obligate intracellular associations on the symbiotic continuum. Together, these

379 observations suggest that even across the large areas of the ocean where phototrophic biomass

380 is dominated by very small cells, sophisticated metabolic interactions among the plankton,

381 facilitated by microbial foraging behaviours, can influence oceanic productivity and

382 biogeochemical cycling. Furthermore, our quantification of the benefits of chemotactic

383 interactions between very small cells highlights that chemotaxis may play an unexpected role

384 in the metabolic exchanges between individual bacterial cells across all environments.

385

386 **METHODS**

387 *Cultures*

388 *Synechococcus* sp. CS-94 RRIMP N1[38] was grown in Enriched Seawater Artificial Water

389 (ESAW)[39] complemented with f/2 nutrients[40]. The cells were maintained at 23°C on a 12:12 h

390 dark:light cycle at ~ 180 µmol photons m^{-2} s^{-1}. In addition, we used the wild type (WT) marine

391 bacterium *Marinobacter adhaerens* HP15[41] (both motile and chemotactic), Δ*cheA* a motile but

392 non-chemotactic mutant[13], and Δ*fliC* a non-motile mutant[42]. In Δ*cheA* or Δ*fliC* mutants, the

393 genes *cheA* or *fliC*, respectively, were replaced by a chloramphenicol resistance cassette using

394 homologous recombination on the up- and downstream regions of the genes, as described

395 previously[13,42]. Both mutants were complemented with medium-copy number plasmids

396 (pBBR1MCs-based) containing *cheA* or *fliC*, downstream of the *lac* promoter of the vector.

397 This was needed since neither of these two genes carry their own promoter. These

398 complemented mutants were tested in the respective assays with 10-fold diluted MB 0.3% soft

399 agar (for *cheA* mutant transformant) and the MB 0.3% soft agar assay (*fliC* mutant

400 transformant), showing restoration of wild type levels of motility.

401

402 To determine the growth dynamics of the three *M. adhaerens* HP15 phenotypes (WT, Δ*cheA*

403 and Δ*fliC*), single colonies were picked from Marine Agar plates (Difco Laboratories,

404 Michigan) and resuspended in Marine Broth (Difco Laboratories, Michigan). Cell

405 concentrations were quantified using flow cytometry (CytoFLEX S, using CytExpert Version

406 2.4, Beckman Coulter, California), using filtered MilliQ water as the sheath fluid and a flow

407 rate of 25 µl min^{-1}. Cells were fixed with glutaraldehyde (final conc. 2%) and then stained with

408 SYBR Green (final conc. 1:10,000) for 15 minutes in the dark[43]. For each sample, forward

409 scatter (FSC), side scatter (SSC), green (488 nm, SYBR) and red (650 nm) fluorescence were

410 recorded. *Marinobacter* cells were characterized according to SSC and SYBR Green

411 fluorescence[44]. Flow cytometric counts were used to normalize the starting concentration of

412 *Marinobacter* cells (WT, $\Delta cheA$ and $\Delta fliC$, n=3 for each treatment) to 10,000 cells ml^{-1} in

413 Marine Broth (Difco Laboratories, Michigan). Cells were incubated at 23°C with shaking (180

414 rpm) and 100 µl were sampled every two hours from each culture. Cells were then immediately

415 fixed with glutaraldehyde and enumerated as outlined above. To enumerate cell concentrations

416 in each treatment over a 24-hour period, triplicate starting cultures for each treatment were set

417 up twice 12 hours apart. The first set of cultures was enumerated for the first 10 hours, and 12

418 hours later, the second set of cultures was enumerated between 10 and 24 hours.

419

420 ***Isotope labelling***

421 To quantify the reciprocal exchanges of nutrients between *Synechococcus* and *M. adhaerens*,

422 the cells were pre-labeled with the stable isotopes ^{15}N and ^{13}C, respectively. *Synechococcus*

423 cells were inoculated into ESAW complemented with f/2 with ^{15}N-labeled sodium nitrate

424 (NaNO$_3$, ^{15}N, 98 %+, Cambridge Isotopes Laboratories, Massachusetts) as sole source of

425 nitrogen (0.882 mM; same concentration as f/2). The cells were grown in 50 ml for one week,

426 under the same conditions as above, to ensure high level of ^{15}N enrichment in the cells. Two

427 days before the experiment, glycerol stocks of the three *M. adhaerens* phenotypes were

428 streaked onto respective Difco 2216 Marine Agar plates (Difco Laboratories, Michigan) and

429 incubated at 30°C. The day before the experiment, single colonies of each of the *Marinobacter*

430 phenotypes were suspended into ESAW medium enriched with ^{13}C-labeled amino-acids (1 g l^{-}

431 ^{1} Celtone Base Powder; 98%+ ^{13}C, Cambridge Isotope Laboratories, Massachusetts) and grown

432 for 12 h at 30°C with shaking (180 rpm), to ensure that the three *Marinobacter* phenotypes

433 were in the same growth phase prior to the start of the experiment. Note: *M. adhaerens* HP15

434 lacks all genes required for dissimilatory or assimilatory nitrate reduction based on its genome

435 annotation (https://www.genome.jp/entry/gn:T01922).

436

437 ***Experimental conditions***

438 On the day of the experiment, both *Synechococcus* and *M. adhaerens* cells were rinsed three

439 times to remove all residual stable isotopes from their respective media. Specifically,

440 *Synechococcus* cells were centrifuged at 1,500 g for 15 minutes, the supernatant was discarded

441 and replaced with fresh f/2 medium containing natural abundances of ^{15}N. These washing steps

442 were performed three times before resuspending the cells in 50 ml of f/2. These repeated media

443 exchanges (from ^{15}N enriched f/2 to natural abundance) were carried out to ensure that no

444 enriched level of ^{15}NaNO$_3$ was present in the growth medium when the co-incubation started.

445   The same washing procedure was applied to the overnight *Marinobacter* cultures to remove

446   ^{13}C from the medium before inoculation.

447

448   The cell concentrations of both *Synechococcus* and the three *Marinobacter* phenotypes were

449   then determined by flow cytometry (CytoFLEX S, Beckman Coulter, California), using filtered

450   MilliQ water as the sheath fluid and a flow rate of 25 µl min^{-1}. Cells were fixed with

451   glutaraldehyde (final conc. 2%) for 15 minutes. Prior to analysis, the *Marinobacter* samples

452   were stained with SYBR Green (final conc. 1:10,000) for 15 minutes in the dark[43]. For each

453   sample, forward scatter (FSC), side scatter (SSC), green (488 nm, SYBR) and red (650 nm)

454   fluorescence were recorded (Figure S1). *Marinobacter* cells were characterized according to

455   SSC and SYBR Green fluorescence[44], while *Synechococcus* were discriminated according to

456   SSC and red fluorescence (through the autofluorescence of photosynthetic pigments). Cell

457   counts were used to adjust the *Synechococcus* densities to three discrete concentrations: 1,000;

458   10,000; and 100,000 cells ml^{-1}. Each of the three strains of *M. adhaerens* were inoculated

459   separately at a final concentration of 10^6 cells ml^{-1} in each *Synechococcus* cell density.

460

461   *Synechococcus* and *M. adhaerens* strains were co-incubated in triplicates for three hours (based

462   on pilot studies), during the light cycle, under the same light and temperature conditions used

463   for maintaining *Synechococcus*. At the end of the experiment, samples were fixed with

464   glutaraldehyde (final conc. 2%) for 30 minutes. A *Synechococcus* culture maintained in natural

465   abundance of ^{15}N, and a *M. adhaerens* culture maintained in natural abundance of ^{13}C were

466   used as unlabeled controls, these cells were treated identically to all other experimental

467   cultures. To remove any residual glutaraldehyde, the samples were washed with ESAW after

468   pelleting the cells by centrifugation (1,500 g for 15 minutes). Finally, the cells were

469   resuspended in 50 µl of sterile filtered MilliQ water (to remove ESAW salts) and the full

470   volume was immediately placed onto silicon wafers (7.07 mm × 7.07 mm, Type P / <111>,

471   ProSciTech), dried at 45°C and stored inside a desiccator, protected from light until NanoSIMS

472   analysis. Finally, the samples were coated with 5 nm of gold before being loaded in the

473   NanoSIMS.

474

475   *NanoSIMS analysis*

476   We used the NanoSIMS 50 (Cameca, Gennevilliers, France) at the Centre for Microscopy,

477   Characterisation and Analysis (CMCA) at The University of Western Australia. This

478   instrument allows for simultaneous collection of up to five isotopic species (here: $^{12}C_2^-$, $^{12}C^{13}C^-$

479 , $^{12}C^{14}N^-$, $^{12}C^{15}N^-$, ^{32}S). Enrichment of the rare isotopes ^{15}N and ^{13}C was confirmed by an

480 increase in the $^{15}N/^{14}N$ or $^{13}C/^{12}C$ ratio above the natural abundance value recorded in the

481 control (equal to 0.003716 ± 0.00005 for nitrogen in *Marinobacter* cells and $0.011167 \pm$

482 0.000027 for carbon in *Synechococcus* cells). The NanoSIMS was performed using a chain

483 analysis: samples were pre-sputtered for 3.5 minutes at 500 pA Cs$^+$ beam (D1=1) on 30 µm²

484 areas (256 × 256 pixel), followed by automatic horizontal and vertical secondary ion beam

485 centering. The analysis was then performed by rastering a 2 pA beam (D1=2) over 25 µm²

486 areas (256 × 256 pixels); three planes were recorded per area with a dwell time of 3 ms per

487 pixel. The instrument was operated with a high mass resolving power (in the range of 9,000),

488 allowing the separation of isobaric interferences, and was calibrated daily using yeast cells

489 harboring natural abundance of C, N and S. Images were analyzed using the Fiji software

490 package (version 1.53c) (http://fiji.sc/Fiji)[45] combined with the OpenMIMS plug-in

491 (http://nrims.harvard.edu/software). All images were dead-time corrected[46], the individual

492 planes were then summed prior to extracting counts from the images. Isotopic quantification

493 data were extracted from the mass images by manually drawing regions of interest around each

494 bacterial cell using the $^{12}C^{14}N^-$ image as mask. No cell attachment was observed between the

495 two bacterial species in any of the experiments.

496

497 *Calculation of net fixation (uptake)*

498 We converted our NanoSIMS data into percentage of C or N incorporated into the

499 microorganism relative to the initial C or N content, respectively. This net fixation (Fx_{net})[47] is

500 equal to:

501

$$Fx_{net} = \frac{R_f \left(1 - \frac{R_i}{R_i + 1}\right) - \frac{R_i}{R_i + 1}}{\frac{R_s}{R_s + 1} - R_f \left(\frac{1}{R_s + 1}\right)} \times 100 \qquad (1)$$

502

503 where R_i is the initial isotopic ratios of the organism prior, R_f the final isotopic ratio of the

504 sampled organism, and R_s the isotopic ratios in the pre-enriched partner organism.

505

506 *Characterization of Synechococcus and M. adhaerens HP15 WT metabolites*

507 A 2-liter *Synechococcus* culture was grown for one week in ESAW supplemented with f/2

508 nutrients as described above. A 2-liter *M. adhaerens* culture (WT) was grown overnight as

509   described above (10% Celtone Base Powder in ESAW; Cambridge Isotope Laboratories,

510   Massachusetts). To characterize the cell exudates, cells were pelleted at 1,500 g for 15 minutes

511   and resuspended individually in fresh ESAW supplemented with f/2 nutrients for three hours.

512   Following this incubation, cells were pelleted at 1,500 g for 15 minutes and the supernatant

513   was filtered through a 0.45 µm filter, and then through a 0.2 µm filter to ensure the removal of

514   all cells. The filtrate was acidified to pH 2 using 10% HCl (made with HPLC-water from HCl

515   puriss. 32%, Fluka, Sigma), spiked with internal standards (0.5% final concentration; $^{13}C_6$-

516   Sorbitol; ^{13}C-^{15}N -Valine, penta-fluorobenzoic acid and 2-aminoanthracene) and subjected to

517   a solid phase extraction (SPE; 12 cc, 500 mg sorbent; HLB cartridges, Oasis). After the

518   complete elution of the filtrate, the SPE cartridges were washed twice with 6 ml of 0.01 N HCl

519   to remove residual salts and dried for 20 minutes under vacuum. Finally, the metabolites were

520   eluted with 4 ml of HPLC-grade methanol (Sigma-Aldrich, USA) into glass vials and stored at

521   -20ºC until needed.

522

Metabolomics: sample derivatization

524   Dried samples for targeted analysis were prepared by adding 25 µl of Methoxyamine

525   Hydrochloride (30 mg/ml in Pyridine) followed by shaking at 37°C for 2 h. Samples were then

526   derivatized with 25 µl of *N*,*O-bis* (Trimethylsilyl)trifluoroacetamide with

527   Trimethylchlorosilane (BSTFA with 1% TMCS, Thermo Scientific) for 1h at 37°C. Samples

528   were left for 1 h before 1 µl was injected onto the gas chromatography column using a hot

529   needle technique. Split (1:10) injections were done for each sample.

530

Metabolomics: analytical instrumentation

532   The gas chromatography–mass spectrometry (GC-MS) system used comprised of an AOC6000

533   autosampler, a 2030 Shimadzu gas chromatograph and a TQ8050 quadrupole mass

534   spectrometer (Shimadzu, Japan). The mass spectrometer was tuned according to the

535   manufacturer's recommendations using tris-(perfluorobutyl)-amine (CF43). GC-MS was

536   performed on a 30 m Agilent DB-5 column with 1 µm film thickness and 0.25 mm internal

537   diameter column. The injection temperature (Inlet) was set at 280°C, the MS transfer line at

538   280°C and the ion source adjusted to 200°C. Helium was used as the carrier gas at a flow rate

539   of 1 ml min^{-1} and Argon gas was used as the collision cell gas to generate the MRM product

540   ion. Sample analysis was performed under the following temperature program; start at injection

541   100°C, a hold for 4 minutes, followed by a 10°C min^{-1} oven temperature ramp to 320°C

542   following final hold off for 11 minutes. Approximately 520 quantifying MRM targets were

543   collected using Shimadzu Smart Database along with qualifier for each target which covers

544   about 350 endogenous metabolites and multiple ^{13}C-labeled internal standards. Both

545   chromatograms and MRMs were evaluated using the Shimadzu GC-MS browser and

546   LabSolutions Insight software. This approach is classified as Level 1-2[48] or level C according

547   to the proposed reporting standards by the Metabolite Identification Task Group of the

548   Metabolomics   Society   (http://metabolomicssociety.org/board/scientific-task-

549   groups/metabolite-identification-task-group). Resulting area responses were normalized to the

550   internal standard ^{13}C$_6$ Sorbitol area response.

551

552   ***Chemotaxis Assay***

553   To test the chemotactic response of *M. adhaerens* HP15 (WT) towards *Synechococcus*

554   metabolites, we performed a chemotaxis assay using the ISCA (*In Situ* Chemotaxis Assay)[30].

555   The ISCA is a microfluidic device composed of an array of microwells which can be filled

556   with different chemoattractants. Here, we used: *i*) ESAW as negative control, *ii*)

557   *Synechococcus* exudates (generated for metabolite analysis above; 1 mg ml^{-1}) and *iii*) 10%

558   Marine Broth (BD Difco) as positive control. Each chemical was resuspended in ESAW and

559   filtered (0.2 µm).

560

561   *Marinobacter adhaerens* HP15 (WT) cells were grown on plate for 3 days. Colonies were then

562   transferred into 0.22-µm-filtered ESAW (room temperature). *M. adhaerens* cells numbers were

563   then adjusted to 10^6 cells ml^{-1} with 0.22-µm-filtered ESAW. Each ISCA (n =5) was deployed

564   in the *M. adhaerens* suspension (80 ml) for 30 minutes[49]. At the end of the incubation, ISCA

565   well contents were collected and fixed with glutaraldehyde (2% final concentration) for 15

566   minutes. Cell abundance in each ISCA treatment (*n*=5) was quantified by running a

567   standardized volume of sample (50 µl) by flow cytometry as described above. To quantify the

568   strength of chemotaxis, the chemotactic index *Ic* was calculated by dividing the number of cells

569   present in each treatment by the number of cells present in the filtered seawater control[30].

570

571   ***Co-growth experiments***

572   Co-cultures were established between each of the three *Marinobacter* phenotypes (WT, Δ*cheA*

573   and Δ*fliC*) and *Synechococcus*. *Marinobacter* were grown on Marine Agar plates (Difco

574   Laboratories, Michigan) for 3 days, single colonies were transferred into Marine Broth and

575   grown overnight (12 h). Cells were washed with sterile f/2 media and diluted 1:100 in f/2. A

576   200 µL aliquot was fixed with glutaraldehyde and stained with SYBR Green for enumeration

577   via flow cytometry as described above. *Synechococcus* from a 7-day culture were also diluted

578   1:100 in f/2 and enumerated using flow cytometry as described above. Co-cultures were then

579   set up by inoculating both *Synechococcus* and *Marinobacter* into fresh f/2 medium at a

580   standardized cell density of ~1,000 cells ml^{-1} (n=4). Co-cultures were incubated at 23°C on a

581   12:12 h dark:light cycle at ~ 180 μmol photons m^{-2} s^{-1} and shaken every 4 hours (180 rpm for

582   15 min) to keep cells in suspension. Cell densities were then enumerated every day for 12 days

583   using flow cytometry as described above.

584

585   ***Statistical analysis***

586   Growth data were analyzed using repeated-measure ANOVA after assessing the normality and

587   sphericity of the data, simple main effect tests were then used to assess if treatments were

588   significantly different at each timepoint (*p*-values were corrected using Bonferroni). The

589   chemotaxis responses of *Marinobacter* were then analyzed using a one-way analysis of

590   variance (ANOVA).   As the NanoSIMS data were not normally distributed and/or not

591   homogeneous, Kruskal-Wallis test, followed with pairwise Wilcoxon tests were carried out to

592   test the ^{15}N and ^{13}C of the target cells. All *p* values were corrected using the Benjamini–

593   Hochberg procedure for multiple comparisons. All statistical analyses were carried out in R

594   v4.1.1[50] and analysis scripts are available on GitHub (https://github.com/JB-Raina-

595   codes/Synechococcus-paper).

596

597   ***Mathematical model for bacteria-phytoplankton interactions***

598   <u>Modelling chemical microenvironments of *Synechococcus*</u>

599   In order to model the dissolved organic matter (DOM) landscape, individual *Synechococcus*

600   cells were considered as point-wise particles, exuding DOM at a rate *L* (molecules per unit

601   time). We begin by considering the DOM concentration around a single *Synechococcus* cell in

602   an unbounded, quiescent fluid. The exuded molecules diffuse radially and are consumed by

603   bacteria distributed throughout the domain. Owing to the spherical symmetry of the problem,

604   both the DOM concentration, *C(r, t)*, and the bacterial concentration, *B(r, t)*, may be written as

605   functions of distance, *r*, from the *Synechococcus* cell, and time, *t*. The DOM profile varies in

606   space and time according to the diffusion equation[16]:

$$\frac{\partial C}{\partial t} = D\nabla^2 C - [4\pi a D B(r,t)]C. \tag{2}$$

23

607 The first and second terms on the right-hand side of Eq. (2) represent the molecular diffusion
608 of DOM and the diffusion-limited uptake by bacteria, respectively. Amino acids constitute a
609 significant fraction of the *Synechococcus* exudates (see Table S2), and given their similar
610 respective molecular weights, we represent the exudate as a single molecular species.
611 Glutamate was chosen (diffusivity $D = 608 \ \mu m^2 s^{-1}$), since its capacity to elicit chemotaxis is
612 well-studied[32]. The second term on the right-hand side of Eq. (2) represents diffusion-limited
613 consumption of the DOM source by bacteria. The parameter a is the cell radius of
614 *Marinobacter adhaerens*. The distribution of bacteria, $B(r, t)$, will in general not be uniform,
615 and will depend on $C(r, t)$. However, if we assume that bacteria are approximately uniformly
616 distributed with concentration B_0, Eq. (2) may be rewritten as:

$$\frac{\partial C}{\partial t} = D\nabla^2 C - kC, \tag{3}$$

617 where the diffusion-limited consumption rate is given by $k = 4\pi a D B_0$. The steady state
618 solution to Eq. (3) in spherical coordinates, which is finite at $r \to \infty$, is given by

$$C(r) = \frac{A}{r} \exp\left(-\sqrt{\frac{k}{D}}r\right) = \frac{A}{r}\exp\left(-\sqrt{4\pi a B_0}\ r\right), \tag{4}$$

619 where $A > 0$ is an arbitrary constant. The radial flux of DOM through a spherical surface at
620 $r = \epsilon \ll 1$ must match the leakage rate from the *Synechococcus* cell. That is,

$$\lim_{r \to 0}\left(-D\frac{dC}{dr}4\pi r^2\right) = 4\pi A D = L. \tag{5}$$

621 It follows that

$$C(r) = \frac{L}{4\pi Dr}\exp\left(-\sqrt{4\pi a B_0}\ r\right). \tag{6}$$

622 Note that the above expression diverges at $r \to 0$. However, for any bacterium in the vicinity
623 of the DOM source, the maximum concentration of DOM it may experience occurs at the
624 surface of *Synechococcus* (with radius $r_0 = 1 \ \mu m$). The DOM profile is therefore capped by
625 this value, so that

$$C(r) = \begin{cases} \dfrac{L}{4\pi Dr_0}\exp\left(-\sqrt{4\pi a B_0}r_0\right), & r \leq r_0 \\[2mm] \dfrac{L}{4\pi Dr}\exp\left(-\sqrt{4\pi a B_0}r\right), & r > r_0 \end{cases} \tag{7}$$

626   We note that the total amount of DOM present in the domain, $\int C(r)dV$, is finite, as the

627   phytoplankton leakage is balanced by bacterial consumption. It is possible to recover the DOM

628   profile in the absence of bacterial consumption by setting $B_0 = 0$. The resulting functional

629   form, $C(r) = L/4\pi Dr$, is used elsewhere[12] in the case of single hotspots. However, for a

630   suspension of *Synechococcus* cells, the long-range nature of this function results in a divergent

631   DOM concentration. It is therefore necessary to utilize the more realistic profile shown in Eq.

632   (7), which encompasses the effect of bacterial consumption. For the purposes of calculating

633   the DOM profile in Eq. (7), the experimental value of $B_0 = 10^6$ cells ml^{-1} is used.

634

635   The DOM profile presented in Eq. (6) assumes an infinite bacterial suspension surrounding an

636   individual *Synechococcus* cell. We explicitly examine the role that diffusion-limited uptake

637   has in shaping the DOM profile (Extended Data Figure 4a). For four different bacterial

638   concentrations, B_0 (cells ml^{-1}), the DOM profile is shown. Dilutions by factor 2 and 5 from the

639   experimental concentration of $B_0 = 10^6$ cells ml^{-1} only slightly affect the resultant DOM

640   profile.

641

642   We also test the effect of truncating bacterial density beyond a critical radius, so that $B = B_0$

643   for $r < R_0$ and $B = 0$ beyond this radius. This is essential for assessing the role of interacting

644   phycospheres, where the background concentration of bacteria would not necessarily exhibit

645   the same diffusion-limited uptake for multiple patches simultaneously. Extended Data Figure

646   4b shows the exact solution to Eq. (2) with a step change in bacterial concentration outlined

647   above. Both the dark green curve (bacteria everywhere: $R_0 = \infty$) and black curve (no bacteria:

648    $R_0 = 0$) are identical to those presented in panel A. Truncating the bacterial concentration to

649   lie only in $R_0 < 1000$ μm and $R_0 < 500$ μm barely modifies the DOM concentration profile.

650   In other words, the uptake of DOM by bacteria in the far field is not significant for regulating

651   the DOM profile in the vicinity of the phytoplankton cell. As a result, we are able to directly

652   apply Eq. (6) for a 3D suspension of multiple *Synechococcus* cells.

653

654   <u>Model for multiple resources</u>

655   To mimic the experimental system, we considered a rectangular box with dimensions

656    $l_x = l_y = l_z = l$ in the x, y, z directions respectively. This box is seeded with N identical DOM

657   sources at random positions in space $\{x_i = (x_i, y_i, z_i) | i = 1,2, \dots, N\}$, so that the total

658   concentration of *Synechococcus* cells is $\rho = N/l^3$. In all simulations conducted, we set $N =$

659   250, and vary ρ by changing the dimensions of the box, l. Linearity of the diffusion equation

660   enables the superposition of multiple solutions from Eq. (7). It follows that the total DOM

661   concentration at position $\boldsymbol{x}$ is given by

$$C(\boldsymbol{x}) = \sum_{i=1}^{N} C_i(\boldsymbol{d_i}), \tag{8}$$

662   where C_i is the expression in Eq. (7) and d_i is the distance between points $\boldsymbol{x}$ and $\boldsymbol{x_i}$. We utilise

663   periodic boundary conditions to evaluate d_i, so that the concentration resulting from each

664   *Synechococcus* cell is evaluated by taking the shortest distance to it within the periodic domain.

665   From Eqs. (7) and (8), it is also possible to directly evaluate the spatial gradient of the DOM

666   field, given by $\nabla C(\boldsymbol{x})$. A single 2D slice of the DOM profile through the box domain with

667   *Synechococcus* concentration $\rho = 10^3$ cells ml^{-1} is shown in Extended Data Figure 6.

668

669   <u>Model for bacterial chemotaxis</u>

670   We introduce bacteria into the three-dimensional DOM field defined by Eq. (8) and investigate

671   their collective dynamics. The relative performance (DOM exposure) of wild type bacteria

672   compared to their non-chemotactic or non-motile counterparts is examined. To begin with, we

673   outline the agent-based model for bacterial chemotaxis. This model incorporates the essential

674   features of bacterial navigation, and accurately captures the chemotaxis of another marine

675   bacterium, *Vibrio ordalii*[32] responding to dissolved glutamate sources (less than 1% fitting

676   error). Where possible, we have updated specific model parameters for the case of *M.*

677   *adhaerens* (see Table S8 for all model parameters).

678

679   In the laboratory frame, the DOM concentration is given by the smooth function $C(\boldsymbol{x})$. In each

680   simulation time-step, $\Delta t = 0.10$ s, a bacterium with velocity $\boldsymbol{v}$ and position $\boldsymbol{x}$ performs a noisy

681   measurement of the concentration change in its reference frame, $\partial C_N / \partial t = N(\mu, \sigma^2)$. This

682   stochastic measurement is normally distributed with mean $\mu = \boldsymbol{v} \cdot \nabla C$ and standard deviation

683   $\sigma = \Pi[3C(\boldsymbol{x},t)/\pi a D T^3]^{1/2}$, and therefore directly incorporates the fundamental precision

684   with which a cell can measure the gradient. Here Π is the chemotactic precision factor and T is

685   the timescale over which the bacteria measure the gradient (see[32]). For each bacterium, we

686   model an internal state variable, $S(t)$, which evolves according to

$$\frac{dS}{dt} = -\frac{S}{t_M} + \kappa \, N(\mu, \sigma^2), \tag{9}$$

26

687   where $t_M = 1.3$ s is the adaptation timescale[51] and κ is the effective receptor gain – the receptor

688   gain rescaled by the half-saturation constant (see[32]). The cell's mean run time is modified

689   according to the following equation:

$$\tau(S) = \frac{2\tau_0}{1 + \exp(-\Gamma S)} \tag{10}$$

690   where τ_0 is the mean run time of bacteria in their fully adapted state and Γ is the (constant)

691   dimensionless flagellar motor gain. During each time-step, the probability of reorientation is

692   given by $\Delta t / \tau$. Run-reverse-flick reorientation dynamics were included explicitly using known

693   parameters derived for *Vibrio alginolyticus*[52], and rotational diffusion with $D_r =$

694   0.0349 rad^2 s^{-1} perturbed the swimming direction at each time-step. Cell motility occurs in

695   three dimensions, as in experiments, with swimming bacteria subject to periodic boundary

696   conditions. The sensory integration timescale is given as $T = 0.1$ s, the cell radius is taken to

697   be $a = 0.5\,\mu m$, the swimming speed $v = |\boldsymbol{v}| = 45\,\mu m\,s^{-1}$, and we use the diffusivity for

698   glutamate, $D = 608\,\mu m^2\,s^{-1}$. For the WT cells, we utilize the recently measured parameter

699   for *Vibrio ordalii*, $\Pi_{\text{sim}} = 6.6$. Initially seeded randomly within the domain, we simulated the

700   3D motion of 500 bacteria as they respond to the DOM landscape. Within the context of this

701   model, it is straightforward to simulate non-chemotactic ($\Delta cheA$) or non-motile ($\Delta fliC$) mutants

702   by fixing $\tau = \tau_0$ or $v = 0$ respectively. We considered the same concentrations of

703   phytoplankton cells (10^3, 10^4 and 10^5 cells ml^{-1}) as used in the experiments.

704

705   <u>DOM uptake by model bacteria</u>

706   The bacterial trajectories from the numerical simulations were cross-referenced against the 3D

707   DOM landscape to reveal the time-series of DOM exposure for all model bacteria. Diffusion-

708   limited uptake is proportional to the DOM concentration, and so acts as an effective proxy for

709   actual uptake. We investigate the DOM exposure, averaged over time and across the

710   population, as a function of DOM leakage rate and concentration of phytoplankton. The results

711   in Extended Data Figures 7a and 7b illustrate the results for non-chemotactic ($\Delta cheA$) and

712   chemotactic (WT) cells, respectively. Cells in the $\Delta cheA$ strain swim randomly, and therefore

713   sample all areas of their environment with equal probability. The average DOM exposure is

714   therefore proportional to the product $L \times \rho$ for low hotspot concentrations, a feature reflected

715   in the straight-line level contours of Extended Data Figure 7a. These contours are distorted in

716   the case of WT cells (Extended Data Figure 7b) which are able to respond to chemical gradients

717   and attain a relatively high DOM exposure, particularly at low hotspot concentrations. Figure

718   4 in the main text is calculated by taking the ratio of Extended Data Figures 7b and 7a, directly

719   quantifying the advantage due to chemotaxis.

720

721   We calibrated the leakage rate in the mathematical model using the following method. For each

722   phytoplankton concentration (10^3, 10^4 and 10^5 cells ml^{-1}) and across a fine mesh of L values,

723   we calculated the ratio of the mean DOM uptake for the WT cells compared to the $\Delta cheA$

724   mutants. The value $L = 0.052\ \mathrm{pmol\ hr^{-1}}$ resulted in the closest agreement between the

725   numerical simulations (Extended Data Figure 2) and experimental measurements (Figure 2b).

726   This value of L was applied in simulations designed to mimic experimental conditions.

727

728   The mathematical model does not simulate the release of ^{13}C-enriched compounds from *M.*

729   *adhaerens*, or subsequent uptake by *Synechococcus* cells. Nevertheless, the dynamic results of

730   the numerical simulations provide insight into the experimental findings of Figure 2c. For

731    $\rho = 10^3$ cells ml^{-1}, the ^{13}C uptake is significantly higher for WT cells than for $\Delta cheA$ and $\Delta fliC$

732   strains, demonstrating that bacteria-phytoplankton spatial associations influence the ^{13}C

733   transfer. Interestingly, however, the ^{13}C uptake in *Synechococcus* does not vary significantly

734   with concentration of suspended *Synechococcus* cells, even though the concentration of *M.*

735   *adhaerens* is constant across all treatments (10^6 cells ml^{-1}). If the ^{13}C exchange was dominated

736   by bulk background concentration of ^{13}C-enriched *Marinobacter* exudates, then the measured

737   enrichment would decrease with *Synechococcus* concentration, as more cells compete for fixed

738   supply of DOM. Similarly, if *Synechococcus* derived chemical gradients were strong enough

739   to trap bacteria for sustained periods of time, then the ^{13}C enrichment would also be expected

740   to drop with increasing *Synechococcus* concentration, ρ. The insensitivity of the results to ρ is

741   consistent with the physical model of fleeting bacteria-phytoplankton interactions. Since

742   chemotaxis prolongs the bacteria-phytoplankton interactions by only a few seconds on average,

743   the number of bacteria entering and departing a given analysis zone per unit time – and

744   therefore the ^{13}C uptake in *Synechococcus* – is only weakly affected by the *Synechococcus*

745   concentration itself.

746

747   For any individual *Synechococcus* cell, the associated DOM concentration profile converges

748   to zero in the limit as $r \to \infty$ (see Eq. (7)). However, because the simulation volume contains

749   multiple *Synechococcus* cells, the minimum nutrient concentration in the domain – which is

750   found approximately midway between cells – is non-zero. We examined our simulation data

751 to identify the lowest ('background') concentration in each treatment and found that the range

752 of concentrations is commensurate with average free amino-acid concentrations in the ocean

753 (~10-20 nM average, with concentrations up to 100s or 1000 nM in bloom conditions[53,54]).

754

755 Specifically, the lowest local nutrient concentration occurs in the treatments with 10^3

756 *Synechococcus* cells ml^{-1}, since the phycospheres are most widely separated. For non-

757 chemotactic cells in this case – which explore the landscape uniformly – simulated bacterial

758 trajectories reveal the average nutrient concentration experienced by bacteria to be 3.7 nM

759 (averaged over the simulation time). Conversely, the maximum value of the background

760 concentration occurs in the treatments with 10^5 Synechococcus cells ml^{-1}. We examined the

761 simulation data corresponding to these treatments and found nutrient concentrations of

762 approximately 200 nM, a value that is commensurate with free amino acid concentrations

763 occurring in bloom conditions.

764

765 <u>Bacteria-phytoplankton dynamic interactions</u>

766 The full trajectories of all model bacteria are recorded in the simulations. This facilitates

767 exploration of the dynamic interactions between DOM sources and model bacteria. The region

768 immediately surrounding a phytoplankton cell rich in phytoplankton exudates is known as the

769 phycosphere[9]. The exact definition of the phycosphere remains challenging, since the

770 phycosphere is composed of a wide range of chemicals, with different concentrations and

771 diffusivities, which can be used by bacteria as either growth substrates or signals. One way to

772 operationally define the phycosphere is through a threshold DOM concentration compared to

773 the background value[12]. Because our work focuses on chemotaxis, we instead define the

774 phycosphere based on the chemotactic properties of WT bacteria, and the behavioral

775 associations with individual phytoplankton. We determine the effective phycosphere radius of

776 the *Synechococcus* cell by examining the behavioral properties of model bacteria as a function

777 of distance from the *Synechococcus* cell. We calculate the residence time, τ, of bacteria within

778 a distance d (μm) of a *Synechococcus* cell. For WT bacteria, the rate at which this residence

779 time increases with d is greatest for $d \leq 35$μm (Extended Data Figure 3), demonstrating that

780 within this zone, chemotaxis allows bacteria to prolong their spatial association with

781 *Synechococcus*. Conversely, non-chemotactic cells ($\Delta cheA$) exhibit a residence time which

782 grows linearly with d at all distances, with no behavioral biases. We utilize the value $d =$

783 35μm for the phycosphere radius throughout the manuscript. Crucially, the specific choice of

784   phycosphere radius does not influence the total DOM exposure by model bacteria (and does
785   not enter the actual simulations), only the statistics of encounters with phycospheres and time
786   spent within them.

787

788   At every instant in time and for each bacterium, we calculate the distance to the nearest hotspot.
789   The results in Extended Data Figure 8a show the time- and population-averaged minimum
790   distance, as a function of DOM leakage rate, L. The leakage rate may be approximated as
791   scaling with cell radius according to $L \propto r^{2.28}$ [55,56]. By matching the fitted leakage rate for
792   *Synechococcus* ($L = 0.052 \, \text{pmol hr}^{-1}$) with its known cell radius (Table S8), leakage rate
793   (horizontal axis of Extended Data Figure 8a) can instead be recast in terms of cell radius. As
794   the leakage rate L is increased, WT bacteria are increasingly able to detect and respond to the
795   chemical gradients, resulting in closer physical association with the phytoplankton cells. The
796   grey vertical line in Extended Data Figure 8a corresponds to the fitted value $L = 0.052 \, \text{pmol}$
797   hr^{-1}.

798

799   Extended Data Figure 8b displays the percentage of the bacterial population within a distance
800   of 35 μm from a hotspot. The role of chemotaxis is clear, with the fraction of cells co-localized
801   with DOM sources increasing dramatically with DOM leakage rate. At the highest leakage rate
802   studied, more than 85% of chemotactic bacteria are within 35 μm of a phytoplankton cell. This
803   percentage plateaus at a value less than 100% since the stochastic nature of bacterial run-
804   reverse-flick motion (mean run distance $\sim 21 \, \mu$m) precludes a cell from residing indefinitely
805   within the analysis zone.

806

807   **Data availability**
808   All chemotaxis, growth, metabolomics and NanoSIMS data are available in Zenodo
809   (https://zenodo.org/record/7509161#.Y7fUcRVBw2w); DOI: 10.5281/zenodo.7509161

810

811   **Code availability**
812   All analysis scripts are available on GitHub (https://github.com/JB-Raina-
813   codes/Synechococcus-paper)

814

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828

Author contributions

830 J.B.R, D.R.B, S.S., R.S and J.R.S. designed the experiments. M.G. and J.B.R. conducted the

831 experimental work. M.G., P.L.C., P.G., J.B. conducted the NanoSIMS work. J.B.R. and H.M.

832 conducted the metabolomics. D.R.B. conducted the agent-based simulations. E.C.S. and

833 M.S.U. provided the bacterial strains and mutants. J.B.R., D.R.B, R.S and J.R.S. wrote the

834 manuscript and all authors edited subsequent versions.

835

Competing interests

837 The authors declare no competing interests.

838

Figures captions

840

Figure 1: Reciprocal exchanges between *Synechococcus* and *Marinobacter adhaerens* HP15. **(a)** Representative $^{15}N/^{14}N$ ratio image using NanoSIMS (highlighting the highly enriched *Synechococcus* cell, pink; at least 20 images were acquired per treatment; scale bar: 1 μm), **(b)** ^{15}N uptake of *M. adhaerens* HP15 wild type (WT) originating from *Synechococcus* (10^3: n=166; 10^4: n=286; 10^5: n=172). **(c)** Representative $^{13}C/^{12}C$ ratio image using NanoSIMS (identifying highly enriched *Marinobacter adhaerens* cells, pink; at least 20 images were acquired per treatment; scale bar: 1 μm), and **(d)** ^{13}C uptake in *Synechococcus* cells originating from *M. adhaerens* (10^3: n=10; 10^4: n=17; 10^5: n=37), at different *Synechococcus* concentrations. **(e)** Reciprocal exchange of chemical currencies through diffusion in the bulk. Stable isotope uptake from **(b)** and **(d)** were calculated according to[47] (see equation (1) in the Methods). Error bars in **(b)** and **(d)** represent standard error of the mean. Significant differences (Kruskal-Wallis) are indicated by using different letters, see also Tables S1 and S3. The full distribution of the data is presented in Extended Data Figure 9.

Figure 2: Reciprocal exchanges between *Synechococcus* and *Marinobacter adhaerens* HP15 are enhanced by chemotaxis. (a) The chemotactic (WT), non-chemotactic ($\Delta cheA$) and non-motile ($\Delta fliC$) strains of *Marinobacter* interact with *Synechococcus* phycospheres in qualitatively different ways, resulting in strong differences in the **(b)** ^{15}N uptake of *M. adhaerens* (10^3: n=166; 10^4: n=286; 10^5: n=172) and **(c)** ^{13}C uptake in *Synechococcus* (10^3: n=10; 10^4: n=17; 10^5: n=37). Error bars in **(b)** and **(c)** represent standard error of the mean. Significant differences (Kruskal-Wallis) are indicated using different letters (see also Tables S6-7). Stable isotope uptake from **(c)** and **(d)** were calculated according to[47] (see equation (1) in the Methods). The full distribution of the data is presented in Extended Data Figure 9.

Figure 3: Numerical simulations reveal the dynamic interactions between *Marinobacter* and *Synechococcus*. (a) The duration of every bacterium-phytoplankton interaction (residence time τ) was recorded throughout the simulations, **(b)** revealing that WT *M. adhaerens* cells spend, on average, more than 3 times longer within each phycosphere ($R_p = 35 \ \mu m$) than chemotaxis-deficient mutants (WT: 3.2 ± 11.5 s; $\Delta cheA$: 1.0 ± 1.4 s). For WT cells, 0.58% of encounters were for more than 60 s, while the longest residence time for $\Delta cheA$ cells was 23 s (n= 2626 encounters). **(c)** Mean residence time as a function of leakage rate L (or equivalently phytoplankton radius) for three *Synechococcus* concentrations (10^3 dotted, 10^4 dashed, 10^5 solid). **(d)** The number of unique phytoplankton encounter per day depended strongly on the bacterial motility strategy (mean WT: 12.6 day^{-1}; $\Delta cheA$: 4.1 day^{-1}). **(e)** The mean rate of unique phytoplankton encounters as a function of phytoplankton leakage rate and cell concentration for the WT simulations. **(f)** Bacterial trajectories for three different phytoplankton radii, (i) $R = 0.36 \ \mu m$ ($L = 0.1L_0$), (ii) $R = 1 \ \mu m$ ($L = L_0$), (iii) $R = 2.7 \ \mu m$ ($L = 10L_0$), where L_0 is the value for *Synechococcus*. Bacterial trajectories are colour-coded based on the instantaneous distance to the nearest phytoplankton cell.

Figure 4: DOM enhancement due to chemotaxis depends strongly on the size and concentration of phytoplankton cells. (a) The relative DOM exposure (ratio) for chemotactic bacteria (WT) compared to non-chemotactic mutants ($\Delta cheA$) as a function of phytoplankton size (controlled through the leakage rate, L), and phytoplankton concentration. Grey circles correspond to the experimental treatments in Figures 1 and 2. Roman numeral placement in the parameter space correspond to image panels. **(b)** Schematic representation of phytoplankton sizes and densities depicted by the Roman numerals in (a).

Extended Data Figures

Extended Data Figure 1: Chemotactic response of *Marinobacter adhaerens* HP15 to metabolites exuded by *Synechococcus*. The chemotactic index, Ic denotes the concentration of cells within ISCA wells, normalized by the mean concentration of cells within wells containing no chemoattractants (filtered ESAW), after 30 min laboratory deployment. Wells containing *Synechococcus* exudates (1 mg ml^{-1}) and 10% Marine Broth (MB) contained significantly more bacteria than the ESAW control (ANOVA, $n = 5$ biologically independent samples, $p < 0.005$; Table S5). Error bars represent standard error of the mean.

Extended Data Figure 2: Dissolved Organic Matter (DOM) exposure of model bacteria. Mean DOM exposure for three bacterial motility strategies across three different *Synechococcus* concentrations (leakage rate $L = 0.052$ pmol hr^{-1}). Chemotaxis conferred an enhancement in the DOM exposure by 2.1-, 1.3-, and 1.1-fold, for *Synechococcus*

904 concentrations of 10^3, 10^4, and 10^5 cells ml^{-1} respectively, compared to non-chemotactic
905 ($\Delta cheA$) or non-motile ($\Delta fliC$) mutants.
906
907 **Extended Data Figure 3: Residence time of model bacteria. (a)**. The bacterial residence
908 time depends on the radius of the analysis zone and motility strategy. For $\Delta cheA$ mutants, the
909 residence time grows linearly with radius. However, WT cells exhibit a steep increase for small
910 radii, reflecting their capacity to detect the phytoplankton exudates. **(b)** The rate at which the
911 residence time increases with radius reveals the zone in which chemotactic bacteria exhibit the
912 strongest behavioral response to the DOM gradient. From this the encounter radius of 35 μm
913 can be extracted. Other model parameters include $L = 0.052$ pmol hr^{-1}, $\rho = 10^3$ cells ml^{-1}.
914
915 **Extended Data Figure 4: DOM profile does not depend strongly on bacterial**
916 **consumption.** In each plot, the steady state DOM profile emerges due to a balance between
917 constant phytoplankton exudation and diffusion-limited uptake by bacteria. **(a)** DOM profile
918 for four different bacterial concentrations. **(b)** Restricting bacteria to lie in the region $R < R_0$
919 has a minor influence on the resultant DOM profile.
920
921 **Extended Data Figure 5: Growth of *Synechococcus* sp. CS-94 RRIMP N1 and**
922 ***Marinobacter adhaerens* HP15. (a)** Growth curves of *M. adhaerens* HP15 wild type (WT),
923 non-chemotactic mutant ($\Delta cheA$), and non-motile mutant ($\Delta fliC$), each separately co-cultured
924 with *Synechococcus* at an initial concentration of 10^3 cells ml^{-1} for both partners. **(b)**
925 Simultaneous growth curve of *Synechococcus* for the same three co-culture experiments. Note:
926 to clearly visualise differences in cell numbers during early timepoints, *Synechococcus* cell
927 numbers are plotted on a logarithmic scale. Asterisks indicate timepoints at which treatments
928 are significantly different (simple main effect test, p < 0.05, Table S9). Error bars represent
929 standard error of the mean (n = 4 biologically independent samples). **(c)** Growth curves of
930 *Marinobacter adhaerens* HP15 wild type (WT), non-chemotactic mutant ($\Delta cheA$), and non-
931 motile mutant ($\Delta fliC$) in Marine Broth. Error bars represent standard error of the mean (n = 3
932 biologically independent samples). Asterisks indicate timepoints at which treatments are
933 significantly different (simple main effect test, p < 0.05, Table S10).
934
935 **Extended Data Figure 6: DOM concentration within a 2D cross-section of the full 3D**
936 **profile.** Results correspond to a *Synechococcus* concentration of $\rho = 10^3$ cells ml^{-1}. Other
937 parameters as in Table S8. The white scale bar represents 1 mm.
938
939 **Extended Data Figure 7: DOM exposure of model bacteria.** The mean DOM concentration
940 experienced by **(a)** non-chemotactic ($\Delta cheA$) mutants and **(b)** chemotactic (WT) bacteria, as a
941 function of phytoplankton concentration (cells ml^{-1}) and DOM leakage rate L (pmol hr^{-1}).
942
943 **Extended Data Figure 8: Phytoplankton exudation rate affects bacteria-phytoplankton**
944 **distances and bacterial "trapping". (a)** Bacteria-phytoplankton distance is strongly affected
945 by phytoplankton exudation rate. These data show the distance to the nearest hotspot, averaged
946 over time (3 h co-incubation) and bacterial population (500 cells), as a function of DOM
947 leakage rate L (pmol hr^{-1}). Results are shown for three different phytoplankton concentrations,
948 10^3 (dotted), 10^4 (dashed), 10^5 cells ml^{-1} (solid), and for three different bacterial mutants:
949 chemotactic WT (blue), non-chemotactic $\Delta cheA$ (orange), non-motile $\Delta fliC$ (red). **(b)** Bacteria-
950 phytoplankton trapping statistics. These data show the percentage of bacterial cells that are
951 situated within 35 μm of a phytoplankton cell (phycosphere), as a function of DOM leakage
952 rate L (pmol hr^{-1}). For each datapoint, results have been averaged over time (3 h co-incubation)
953 and bacterial population (500 cells). Results are shown for three different phytoplankton

954   concentrations, 10^3 (dotted), 10^4 (dashed), 10^5 cells ml^{-1} (solid), and for three different bacterial
955   mutants: chemotactic WT (blue), non-chemotactic $\Delta cheA$ (orange), non-motile $\Delta fliC$ (red).
956
957   **Extended Data Figure 9: Distribution of the single cell uptake data reported in Figure 1**
958   **and 2. (a)** ^{15}N uptake of *M. adhaerens* (10^3: n=166; 10^4: n=286; 10^5: n=172) and **(b)** ^{13}C
959   uptake of *Synechococcus* (10^3: n=10; 10^4: n=17; 10^5: n=37).
960
961
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1126

a

b *Marinobacter*

¹⁵N uptake (%)

0.0037   0.02

c

0.011   0.05

d *Synechococcus*

¹³C uptake (%)

Synechococcus conc. (cells ml⁻¹)

e

Marinobacter adhaerens
¹³C enriched

¹⁵N   ¹³C

Synechococcus
¹⁵N enriched

a

WT

Synechococcus

Δ*cheA*

Δ*fliC*

b

□ WT
□ Δ*cheA*
□ Δ*fliC*

^{15}N uptake (%)

1

0.75

0.50

0.25

0

a   b b   c   d d

c

^{13}C uptake (%)

15

10

5

0

e   f e   g   h i

10^3 10^4 10^5

Synechococcus concentration (cells ml^{-1})

a residence time τ

phycosphere radius

b Residence time distribution

$L = 0.052$ pmol hr^{-1}
$\rho = 10^3$ cells ml^{-1}

1.0s 3.2s

WT

$\Delta cheA$

Probability

Time (sec)

c Mean residence time

Time (sec)

WT

$\Delta cheA$

Leakage rate (pmol hr^{-1})

d Encounter rate distribution

$L = 0.052$ pmol hr^{-1}
$\rho = 10^3$ cells ml^{-1}

$\Delta cheA$
4.1 day^{-1}

WT
12.6 day^{-1}

Probability

Unique phytoplankton
encounters (day^{-1})

e Unique phytoplankton encounters (day^{-1})

1000

300

300

300

100

100

100

30

30

30

10

3

(i) (ii) (iii)

Phytoplankton (cells ml^{-1})

Leakage rate (pmol hr^{-1})

Phytoplankton radius (µm)

0.5 1 2 5 10

f Bacterial tracks

(i)

500 µm

(ii)

(iii)

Distance to nearest
hotspot (µm)

0 500 1000

a Chemotactic advantage (WT/Δ*cheA*)

Phytoplankton concentration (cells ml^{-1})

Leakage rate (pmol hr^{-1})

Phytoplankton radius (μm)

b

I   II   III   IV

a Residence time

b Residence time gradient

a DOM profile

b DOM profile

Concentration (a.u.)

Distance from *Synechococcus* (μm)

Legend (a):
- $B_0 = 10^6$ cells ml^{-1}
- $B = B_0/2$
- $B = B_0/5$
- $B = 0$

Legend (b):
- $R_0 = \infty$
- $R_0 = 1000$ μm
- $R_0 = 500$ μm
- $R_0 = 0$

a DOM exposure (Δ*cheA*)   **b** DOM exposure (WT)

μM

1000
10
0.1
0.001

Synechococcus (cells ml⁻¹)

Leakage rate (pmol hr⁻¹)

a Mean distance to nearest hotspot

b Percentage of bacteria within a phycosphere

a

WT
$\Delta cheA$
$\Delta fliC$

^{15}N uptake (%)

b

^{13}C uptake (%)

$Synechococcus$ concentration (cells ml^{-1})