An Interplay of Phytoplankton Donor Species and Transformation of Released Compounds over Time Defines Bacterial Communities Following Phytoplankton DOMp Pulses

Running title: short-term determination of bacteria by phytoplankton

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ABSTRACT

Phytoplankton-bacteria interactions are stimulated by phytoplankton-released dissolved organic matter (DOMp). Two factors that shape the accompanying bacterial community are i) the “donor” phytoplankton species, defining the initial composition of released DOMp, and ii) the DOMp transformation over time. We

added phytoplankton DOMp from two globally abundant species - the diatom *Skeletonema marinoi* and the cyanobacterium *Prochlorococcus* MIT9312 - to natural bacterial communities in the Eastern Mediterranean and determined the bacterial responses over a time-course of 72 h in terms of cell numbers, bacterial production (BP), alkaline phosphatase activity (APA), and changes in active bacterial community compositions based on rRNA amplicon sequencing. Both DOMp types were demonstrated to serve the bacterial community as carbon and potentially phosphorus source. Diatom-derived DOM induced higher BP and lower APA compared to cyanobacterium DOM after 24 h, but not after 48 and 72 h of incubation, and also maintained higher Shannon diversities over the course of the experiment, indicating a better bacterial accessibility and broader disposability of diatom derived DOM. Bacterial communities significantly differed between DOMp types as well as different incubation times, pointing to a certain bacterial specificity for the DOMp donor as well as a successive utilization of phytoplankton DOM by different bacterial taxa. The highest differences in bacterial community composition with DOMp types occurred shortly after DOMp additions, suggesting a high specificity towards highly bioavailable DOMp compounds. We conclude that phytoplankton associated bacterial communities are strongly shaped by an interplay between phytoplankton donor and the transformation of its released DOMp over time.

IMPORTANCE

Phytoplankton-bacteria interactions maintain biogeochemical cycles of global importance. Phytoplankton photosynthetically fix carbon dioxide and subsequently release the synthesized compounds as dissolved organic matter (DOMp), which becomes processed and recycled by heterotrophic bacteria. Yet, the combined effect

of the phytoplankton donor species and time-dependent transformation of DOMp compounds on the accessibility to the bacterial community has not been explored until now. The diatom *Skeletonema marinoi* and the cyanobacterium *Prochlorococcus* MIT9312 are globally important phytoplankton species, and our study revealed that DOMp of both species was selectively incorporated by the bacterial community. The donor species had the highest impact shortly after DOMp appropriation, and its effect diminished over time. Our results significantly improve the understanding of biogeochemical cycles between phytoplankton and bacteria, and solve yet unresolved questions of phytoplankton-bacteria interactions.

KEYWORDS *Skeletonema*, *Prochlorococcus*, phytoplankton DOMp, phytoplankton-bacteria interactions, dissolved organic matter

INTRODUCTION

Phytoplankton is responsible for ~50% of the photosynthesis on Earth (1), but a high fraction of the afore photosynthetically fixed carbon is liberated shortly after the buildup (2) in the form of dissolved organic matter (DOMp). The released DOMp serves marine heterotrophic bacteria as energy and carbon (C) source (3), but also contains nutrients such as nitrogen (N) and phosphorus (P), which are likewise consumed and recycled by bacteria (4-7). Hence, interactions between phytoplankton and bacteria are crucial for the functioning of aquatic systems (8-13) and shape biogeochemical cycles at a global scale (2, 11, 14). Different phytoplankton groups and species have different biochemical compositions (15-17), and release different types of DOMp (8, 18-21). Diatoms, for instance, produce

especially polysaccharide-rich DOM (22), and diatoms and cyanobacteria own different ratios of specific amino acids in their released DOMp (23, 24). Such differing compositions engender different availabilities of carbon and nutrients, which, in turn may impact the accompanying bacterial community. Indeed, the phytoplankton host species may drive bacterial community dynamics (24-27) and shape the accompanying bacterial community (15, 28-30). The magnitude of interference of the phytoplankton host species on DOM utilization of the accompanying bacterial community, however, is still subject of debate. Earlier experiments with DOMp addition derived from cyanobacteria and diatoms revealed overall highly similar transcriptomic profiles of bacteria (31), and the two contrasting phytoplankton groups sometimes trigger the same bacterial communities in bloom events (28). On the other hand, only one out of many *Prochlorococcus* strains could meet the unique glycine requirements of SAR11 (32), and in the before mentioned studies, some bacteria were specialized in either cyanobacteria or diatom derived DOM (31), and responsive bacteria differed between other bloom events of diatoms or cyanobacteria (28). A second aspect that needs to be considered if analyzing bacterial utilization of DOMp is the temporal transformation of compounds and the concomitant succession of specific bacterial phyla after DOM pulses (28, 33-35). The DOMp composition changes with proceeding time from highly bioavailable, mostly low-molecular-weight (LMW) to less bioavailable, mostly high-molecular-weight (HMW) compounds, caused by the preferential utilization of labile LMW compounds by bacteria (36). Analogue to the phytoplankton source specificity of specific bacteria, different bacteria phyla are specialized in the utilization of different DOMp compounds. Typically, members of the Flavobacteria are the principle consumers of HMW compounds, whereas the genetic endowment of the Alphaproteobacterial Roseobacter clade reflect their preferences for LMW compounds (28, 31, 33, 34).

However, also the same bacterial phyla may adapt to the transformation of DOMp compounds by changing expression patterns of transporters (37), and various HMW compounds of the same molecular weight may reveal tremendous differences in their accessibility due to compositional and structural differences (38).

In a previous set of experiments, we showed that cyanobacteria DOM provide carbon, nitrogen and phosphorus to accompanying heterotrophic bacteria, and that the uptake of nitrogen and phosphorus bound to DOMp is preferred over inorganic forms (7). Here, we further explore how the interplay of DOM donor species and time after DOMp pulses impact the accompanying bacterial community dynamics. Therefore, we addressed the following questions: (i) do diatom and cyanobacterium DOM serve as P and C source in equal measure? (ii) to which magnitude does the phytoplankton donor species impact the accompanying bacterial community? (iii) how do bacterial communities change over time following DOMp pulses? (iv) which are the primary active bacteria in DOMp utilization from both phytoplankton donor species, and (v) does the specificity of heterotrophic bacteria to the phytoplankton donor species change with DOMp transformation over time?

In order to begin answering these questions, we performed DOMp addition experiments with two globally abundant phytoplankton species - the diatom *Skeletonema marinoi* and the cyanobacterium *Prochlorococcus* MIT9312 - over a time-course of 72 h. Diatoms and cyanobacteria are the two primary sources of marine organic carbon (39), where *Prochlorococcus* contributes up to 8.5% of oceanic photosynthesis and 50% of surface open-ocean chlorophyll (40), whereas diatoms execute up to 40% of primary production in coastal areas (41).

RESULTS

124 **Nutrient concentrations**

125 To test for possible nutrient limitations during the course of the experiment, we
126 measured PO_4, NO_x and NH_4 concentrations. PO_4 concentrations were ca. 0.1-0.2 µM
127 in the control and the diatom DOMDOM treatments, whereas ca. 1 µM PO_4 were
128 accidently added with the cyanobacterium DOMDOM. However, neither
129 cyanobacterium DOMDOM treatments with high PO_4 concentration, nor control or
130 diatom DOMDOM treatments showed pronounced changes in PO_4 concentrations
131 over the course of the experiment (Supplemental Fig. 1). NO_x concentrations at the
132 start of the experiment were ~ 2.5 µM and remained constant throughout the entire
133 experiment in control and cyanobacterium DOMDOM treatment, but decreased after
134 48 and 72 h in the diatom DOMDOM treatment (Supplemental Fig. 1), whereas NH_4
135 concentrations ranged between 1 and 15 µM but did not reveal obvious trends over
136 time. Nevertheless, control samples showed slightly higher NH_4 concentrations
137 compared to the DOMp (diatom and cyanobacterium) treatments, which were,
138 however, significantly higher only after 24 h of exposure (Supplemental Fig. 1).

139

140 **Bacterial cell numbers, Bacterial Production and Alkaline Phosphate Activity**

141 Initial bacterial cell numbers of winterly, coastal Mediterranean waters were ca. 2.25
142 million cells ml^{-1}, and elevated after 24 h of exposure in all treatments, but levelled off
143 to the original value after 48 and 72 h in both DOMp treatments, and decreased to
144 ca. 1.8 million cells ml^{-1} in the control treatment (Fig. 1A).

145

146 On the other hand, bulk measurements of bacterial production (BP) increased in all
147 but the control treatments over the course of the experiment, yet differed significantly

148 between the different treatments at all time-points (Fig. 1B). After 24 h, BP in the
149 diatom DOM treatment was higher than BP in the controls and the cyanobacterium
150 DOM treatments, whereas after 48 and 72 h both phytoplankton DOM treatments
151 yielded similar BP which were significantly higher compared to the control treatment
152 (Fig. 1B). To test for phosphorus starvation in the individual treatments, we measured
153 the production of alkaline phosphatase activity (APA). Bulk measurements of APA
154 yielded lower activity in the DOMp treatments compared to the controls after 48 and
155 72 h, without any statistical differences between both DOMp donors (Fig. 1C).
156 However, after 24 h, diatom DOM revealed ca. 4-times lower APA concentrations
157 compared to the cyanobacterium DOM (Fig. 1C).

158

159 **Bacterial community responses to DOMp additions**

160 In order to evaluate consequences of different DOMp donors and the succession of
161 DOMpDOMp on the richness of the active bacterial communities we calculated
162 Shannon diversities for the individual treatments and time-points. In all samples, the
163 diversity remained in a narrow margin with values ranging from ca. 5.4 to 6.2 (Fig. 2).
164 Nevertheless, after 24 and 48 h, samples in the diatom DOM treatments revealed
165 significantly higher diversities compared to cyanobacterium DOM and control
166 samples at this time point (Fig. 2). The increased diversity in diatom samples was
167 consistent also after 72 h of incubation, but not anymore in a significant way
168 ($p=0.055$, Supplemental table 2). Next, we asked for shared (generalist) and unique
169 (specialist) ASVs between treatments at different time-points using Venn analyses. If
170 shared ASVs between all treatments (control, diatom DOM, cyanobacterium DOM)
171 were compared with each other, no trend over time was obvious (13%, 22%, 20%
172 and 20% after 6, 24, 48, and 72 h of incubation, respectively), whereas ASVs that

173 were only shared between both DOMp treatments (i.e. DOMp generalists) showed a
174 slight increase over time with shares of 2.3%, 3.5%, 4.3% and 4.4% for 6 h, 24 h, 48
175 h, and 72 h of incubation, respectively (Supplemental Fig. 2). On the other hand, at
176 the different time points (6, 14, 48, 72 h) exclusive (i.e. specialist) ASVs in
177 cyanobacterium (22%, 20%, 20%, and 19%) and diatom (18%, 23%, 26%, and 25%,
178 respectively) DOMp revealed slightly decreasing (cyanobacterium) and variable
179 (diatom) trends over time (Supplemental Fig. 2).

180

181 We then explored the impact of the different treatments and progressing time on the
182 active bacterial communities with non-metric multidimensional scaling (NMDS) and
183 Bray-Curtis dissimilarities. Different treatments (t0, control, diatom DOMs,
184 cyanobacterium DOMs Fig. 3A) showed significantly different bacterial communities
185 (ANOSIM, R=0.3, p=0.001), and this difference remained if only bacterial
186 communities between both DOMp types were compared (ANOSIM, R=0.22, p=0.001,
187 Fig. 3A). However, if treatments were pooled together and samples grouped into
188 different incubation times (0 h, 6 h, 24 h, 48 h, and 72 h), significant differences in
189 communities between the different time-points could be detected (ANOSIM, R=0.4,
190 p=0.001, Fig. 3A).

191 In all treatments, time-dependent changes in the active bacterial community were
192 significantly higher in the first 24 h of exposure (Bray-Curtis dissimilarities between t0
193 and 6 h as well as between 6 h and 24 h), compared to dissimilarities between later
194 time-points (Figure 3B). Analogue to comparisons between different time-points in
195 the same treatments, community differences between different treatments at the
196 same time point were most pronounced already after 6 h, and the overall highest
197 difference was obtained between diatom and cyanobacterium DOM treatments (Fig.

3C). The differences between bacterial communities in diatom and cyanobacterium DOM treatments, however, diminished after 24 h, but successively increased again after 48 and 72 h (Fig. 3C).

General patterns revealing bacterial community differences between different treatments and incubation times were confirmed by abundance distributions of the overall 100 most abundant ASVs (defined as the sums of the subsampled reads for all samples, Fig. 4). A dendrogram of the ASVs yielded three major clusters, which were largely driven by the taxonomy, with *Sachharospirillaceae, Spongiispira*, SAR11 and KI89a ASVs in cluster I, *Ascidiaceihabitans, Glaciecola, and Aureimarina* ASVs dominating cluster II, and a diverse consortium of ASVs (including *Synechococcus*, Rhodobacteraceae, and others) in cluster III (Fig. 4). However, if the 100 most abundant ASVs were analysed for the dominant ASVs in the different treatments (t0, control, cyanobacterium DOMs, diatom DOMs), t0 samples were dominated by ASVs from SAR11 and *Synechococcus*, whereas 11 ASVs of *Saccharospirillaceae*, one *Spongiispira* ASV and one *Glaciecola* ASV dominated the control treatments (Fig. 4, SupplementalFig. 3). On the other hand, the cyanobacterium DOM treatments were exclusively dominated by ASVs of *Glaciecola*, whereas two Flavobacteriaceae, one *Ascidiaceihabitans* ASV, one SAR11 ASV, and four *Glaciecola* ASVs were dominating the diatom DOM samples (Fig. 4, Supplemental Fig. 3A). Additional tests for DOMp generalists, i.e. ASVs that dominate DOMp samples independent of the donor species (assigned treatments: all samples containing DOMp, t0, control), yielded 21 *Glaciecola* and two *Flavobacteriaceae* ASVs (Supplemental Fig. 3B). If different incubation times were analysed for their dominant ASVs, t0 samples were defined by a broad array of bacterial genera and ASVs, with the phototrophic

Synechococcus, and heterotrophic SAR11, OM43 clade, *Ascidiaceihabitans*, AEGEAN-169, NS2b, NS4, NS5, and NS9 marine group contributing several ASVs. Six-hour incubation samples were especially dominated by ASVs of the Rhodobacteraceae, 24 h incubations solely by *Glaciecola* ASVs, 48 h incubations by seven ASVs of *Glaciecola* and one ASV from *Pseudoalteromonas*, and the 72 h incubations by 11 ASVs of Saccharospirillaceae and one *Aureimarina* ASV (Fig. 4, Supplemental Fig. 3C).

DISCUSSION

In this study, we confirmed the bacterial utilization of DOMp derived from two phytoplankton primary sources of marine organic matter, i.e. diatoms and cyanobacteria (39) in coastal waters of the Eastern Mediterranean in winter. We found similar bacterial responses to diatom and cyanobacteria DOMp additions in terms of bacterial cell numbers, bacterial production (BP) and alkaline phosphatase activity (APA). Nevertheless, faster bacterial responses in BP and APA following the addition of diatom DOMs suggest better accessibility of carbon and phosphorus from these DOMp compared to cyanobacterium DOMs. Furthermore, via 16S rRNA sequencing we could demonstrate that different, DOMp-donor specific bacterial ASVs are active in DOMp utilization. However, the most pronounced differences between active bacterial communities of both DOMp types were obtained shortly after DOMp additions (6 h), indicating an important role of donor-specific DOMp compounds in the highly bioavailable DOMp fraction. We also observed bacterial community successions over time, which we hypothesize are due to a shift from ASVs effective in utilization of highly bioavailable, donor-specific DOMp compounds shortly after the DOMp pulse, towards ASVs specialized on less bioavailable

compounds at the later time points. In summary, we conclude that bacterial communities experiencing phytoplankton DOMp pulses are relevantly defined by an interplay between the original DOMp composition and temporal succession in substrate quality following the DOMp pulse.

Effects of DOMp additions on Bacterial Cell Numbers, Bacterial Production and Alkaline Phosphatase Activity

We confirmed bacterial utilization of phytoplankton DOMp as carbon source with measurements of bacterial cell numbers and production (BP) (Fig. 1), and found indications for phosphorus utilization due to lowered alkaline phosphatase activity (APA) in DOMp treatments (Fig. 1). Absolute cell-numbers appear to be surprisingly high for coastal Mediterranean Waters, with ~2.2 million cells ml^{-1} at t0 (Fig. 1A). Yet, these values correspond well to our previous study in winter (also ~2.2 million cells ml^{-1} at t0), F. Eigemann et al. (7)), as well as with a time series in the Eastern Mediterranean (~0.7 – 1.4 million cells ml^{-1} in January/February, O. Raveh et al. (42)), both sampled from the same station. Likewise, increased BP in both DOMp treatments corroborated bacterial utilization of phytoplankton derived DOC, with well corresponding values to O. Raveh et al. (42) (~0.8 – 1.6 µg C h^{-1} l^{-1} in January/February vs ~0.5 µg C h^{-1} l^{-1} at t0 in the present study), and our previous experiment (t0 ~0.13 fg C $cell^{-1}$ h^{-1}, F. Eigemann et al. (7) vs ~0.2 fg C $cell^{-1}$ h^{-1}, this study). Significantly lower APA in diatom DOM treatments compared to controls despite of identical inorganic P concentrations (Fig. 1C, Supplemental Fig. 1), suggests that diatom DOMs were used as an organic P source. This is in accordance with findings that bacterial communities in the Mediterranean Sea compensate for low inorganic nutrient levels with organic forms (43, 44), and phytoplankton DOMp

273 represent a substantial source of it(45). On the other hand, we added approximately
274 1 µM PO_4 with the cyanobacterium DOMs (Supplemental Fig 1), which makes a
275 definitive statement on P utilization derived from cyanobacterium DOMs difficult.
276 However, in our preceding study we showed a preferentially utilization of organic
277 nutrient forms provided from cyanobacterium DOMs compared to inorganic forms (7).
278 In the present study, we may have experienced a similar organic P utilization, which
279 is suggested by identical outcomes for t0 and control APA in both studies. The even
280 higher APA in the cyanobacterial DOMp treatments despite of our accidental PO_4
281 addition (Fig. 1) in parallel to constant inorganic nutrient levels with
282 contemporaneous decreasing APA over time (Fig. 1, Supplemental Fig. 1) supports
283 this notion.

284

285 **Faster accessibility of diatom DOMs and donor specific effects on bacterial**
286 **diversity**

287 DOMp differ in their composition between different phytoplankton groups (16, 21), as
288 well as between species in the same group (13), which has consequences on their
289 accessibility to the bacterial community (23, 24, 39). After 24 h of exposure, bacteria
290 exposed to diatom DOMs yielded approximately 2-times higher BP and 2.5-times
291 lower APA compared to bacteria exposed to the cyanobacterium DOMs (Fig. 1). This
292 pattern was consistent for bulk as well as per cell measurements, and thus not
293 attributed to the higher cell-numbers in cyanobacterium DOM treatments after 24 h of
294 exposure (Supplemental Fig. 4). Both results seem surprising at a glance,
295 considering that we added less carbon with the diatom than cyanobacterium DOMs
296 (14 vs 25 µM, see Experimental procedures), and also added inorganic PO_4 with the
297 cyanobacterium DOMs (see above). Indeed, our results suggest a faster and/or

298 higher accessibility of diatom DOMs for bacterial biomass synthesis and phosphorus
299 utilization, which is in accordance with the outcomes by B. Kieft et al. (39), who found
300 a higher ^{13}C enrichment in bacteria exposed to ^{13}C-labelled diatom than
301 cyanobacterium DOM after 15 h, and a mass spectrometric analysis of both DOMp
302 types, where diatom DOMs owned a higher fraction of P (23).

303 Higher Shannon diversities throughout the experiment and a higher diversity of
304 genera (and not ASVs) in the 50 most abundant ASVs in the diatom DOM samples
305 (Supplemental table 3), furthermore suggest that diatom DOMs can be used by a
306 larger fraction of the bacterial community compared to the cyanobacterium DOMs
307 (Fig. 2). This outcome may be attributed to the production of easy accessible DOM
308 such as the polysaccharide laminarin (46), and more evenly distributed individual
309 sugars and amino acids in diatom DOMs (which infer a higher diversity of polymers,
310 and thus can be used by a broader share of bacteria) compared to cyanobacteria
311 DOMp (23), which also contain lipo-polysaccharides (47), as well as fucose
312 containing polysaccharides (23). The latter requires sophisticated degradation
313 pathways. Nevertheless, cyanobacterium and diatom DOM treatments experienced
314 different DOC und P concentrations, which might have impacted bacterial responses.
315 Higher APA in higher PO_4, and lower BP in higher DOC environments, however,
316 indicate a DOM quality (i.e. source) and not quantity driven response. Further, higher
317 DOCp concentrations were, independent of the phytoplankton donor, utilized by a
318 larger fraction of bacteria (48), suggesting that the higher bacterial diversity in our
319 diatom vs. cyanobacterium DOM treatments indeed derives from DOM quality and
320 not quantity.

321

322 **DOMp donor specific ASVs**

We found multiple different active ASVs of Alphaproteobacteria, Gammaproteobacteria, Bacteroidetes, Verrucomicrobia, Firmicutes, Marinimicrobia, Latescibacteriota, and Actinobacteria in samples of both phytoplankton donors as abundant members (Supplemental table 3). The vast majority of abundant ASVs in DOMp enriched samples, however, were assigned to Gammaproteobacteria, the Bacteroidetes family Flavobacteriaceae, or the Rhodeobacterales clade of Alphaproteobacteria (Supplemental table 3). These three bacterial groups are primary utilizers of DOMp and are, independent from the phytoplankton donor species and time after DOMp pulses, well known as dominant members in associations with phytoplankton, and/or bacterial communities after phytoplankton DOMp additions (27, 28, 32-34, 39, 49-51).

At the ASV level, however, we determined 17 different *Glaciecola* ASVs in cyanobacteria and four different *Glaciecola* ASVs in diatom DOM samples dominating the respective treatments (Supplemental Fig. 3). The assignment of only *Glaciecola* (Gammaproteobacteria, Alteromonadaceae) ASVs to cyanobacterium DOM samples is very similar to the outcomes in our previous study (7), and also corroborated by studies of H. Sarmento and J. M. Gasol (51) and S. M. Kearney et al. (52), who found preferential uptake of Prochlorococcus DOMp by Alteromonadaceae, and specific associations of Alteromonadaceae to Piccocyanobacteria, respectively. However, *Glaciecola* ASVs were assigned to both DOMp types (Supplemental Fig. 3A, 3C), and obtained high relative abundances in conditions dominated by different phytoplankton species (53), and thus *Glaciecola* might be considered as a generalist phytoplankton associated bacterium (or DOMp generalist, Supplemental Fig. 3B). Besides of four *Glaciecola* ASVs, diatom DOM samples were dominated by two Flavobacteriaceae, and each one SAR11 and *Ascidiaceihabitans* ASVs (Supplemental Fig. 3A). These assignments are supported

349 by numerous studies that found general associations of Proteobacteria and
350 Bacteroidetes towards diatoms (reviewed by S. Amin (54)). However, despite of
351 several specific phytoplankton-bacteria associations that were found in our and other
352 studies, one should be careful to draw general conclusions. First, we indeed defined
353 several ASVs as phytoplankton donor specific, but one should keep in mind that also
354 other ASVs were abundant in either diatom or cyanobacteria samples, whose non-
355 consideration as donor specific might be a result of statistical cut-offs, which may not
356 reflect soft boarders in natural systems. Second, divergent outcomes were obtained
357 in similar studies. As examples, we predominantly assigned *Glaciecola* to
358 cyanobacteria DOMp, whereas M. Landa et al. (23) found it specific to diatom DOMs
359 (and did not find it in cyanobacteria DOMp treatments). Further, Rhodobacterales
360 were specifically associated with picocyanobacterial (39, 52), and Prochlorococcus
361 supported the growth of SAR11 in co-culture experiments (32), whereas we assigned
362 *Ascidiaceihabitans* (Rhodobacterales) and SAR11 only to diatom treatments. Thus,
363 general assignments of specific phytoplankton-bacteria associations are challenging,
364 which might be attributed to differences in abiotic parameters (which determine the
365 majority of bacterial associations with phytoplankton (55)) as well as bacterial source
366 communities (which greatly impact associations with phytoplankton (56)) between
367 studies.
368

369 **Diminishing impact of the DOMp donor on bacterial communities over time**

370 We observed the most pronounced bacterial community changes between 0 and 6,
371 as well as 6 and 24 h of exposure (Fig. 3B), suggesting a fast operation of treatment
372 specialized bacteria. Nevertheless, successions of the bacterial community occurred
373 throughout the complete incubation period (see Bray-Curtis dissimilarities between
374 48 and 72 h of exposure, Fig. 3B), and we were able to identify dominating ASVs for

375 each individual time-point (Supplemental Fig. 3C). Bacterial community successions
376 after DOMp pulses follow the exploitation of different DOMp compounds over time,
377 from highly bioavailable compounds shortly after the pulse towards less bioavailable
378 compounds at later time-points (33). The broad consortium of different bacteria
379 dominating t0, changed to four Rhodobacter (Alphaproteobacteria) ASVs and each
380 one *Glaciecola* (Gammaproteobacteria) and NS2b marine group (Flavobacteriaceae)
381 ASVs dominating the 6 h exposure (Supplemental Fig. 3C). The high share of
382 Alphaproteobacteria at this time-point underlines their task as first responders after
383 DOMp pulses, possibly as a result of their high expression of ABC and TRAP
384 transporters that enable the uptake of various LMW compounds (33, 39). After 24
385 and 48 h, we only assigned Gammaproteobacteria (and only one none *Glaciecola*
386 ASV), and after 72 h eleven Gammaproteobacteria (all Saccharospirillaceae), and
387 only one Flavobacterium as time-point dominating (Supplemental Fig. 3C).
388 Interestingly, these outcomes exactly reflect the patterns from H. Teeling et al. (33),
389 who also found a succession of peaking Gammaproteobacterial clades, after the
390 primary response of Alphaproteobacteria to a diatom bloom. The secondary peak of
391 Gammaproteobacteria and to a lesser extent Flavobacteria is a recurrent
392 phenomenon after DOMp pulses (34), and can be explained by their genetic
393 endowment that enable the utilization of numerous HMW compounds (33, 57).
394 The donor specific responses as well as bacterial community differences between
395 both DOMp types were most pronounced shortly after DOMp additions (Figs. 1, 3),
396 suggesting a strong impact of the donor type shortly after DOMp addition. We
397 hypothesize that this is caused by high shares of donor-specific compounds in the
398 highly bioavailable DOMp fractions, whereas less bioavailable compounds may have
399 a more general composition. This assumption is supported by increases in shared
400 ASVs between both DOMp types over time (when highly bioavailable compounds

401 decrease, Supplemental Fig. 2), the decrease of Bray-Curtis dissimilarities from 6 to
402 24 h of exposure (Fig. 3C), and the different outcomes for BP, APA and cell numbers
403 after 24, but not after 48 and 72 h (Fig. 1). Contradictory, M. Landa et al. (23)
404 suggested that labile compounds have a minor effect on the bacterial community and
405 that pronounced effects of the donor species occur if enzymatic machineries are
406 required. In their study, however, a continuous supply of cyanobacterium as well as
407 diatom DOMs was applied, which may have masked a succession from highly to less
408 bioavailable compounds. Furthermore, lower recurrences of phytoplankton species in
409 blooms, compared to heterotrophic bacterial OTUs days to weeks after the blooms
410 between years (34), suggest high similarities in the less bioavailable fraction of
411 phytoplankton DOMp derived from different species, and supports our conclusions on
412 DOMp generalists (Supplemental Fig. 3B). Finally, a mechanistic interference study
413 on the same dataset revealed ~3-times higher recurrences of DOM species
414 compared to phytoplankton species between years in spring blooms (58), which,
415 taking into account that highly bioavailable compounds may be rapidly consumed,
416 further supports our hypothesis.
417
418 **Limitations and synthesis**
419 Despite of reconstructing the occurring environmental conditions in our mesocosm
420 experiments, we experienced pronounced bottle effects, illustrated by increased
421 bacterial production in the controls (Fig. 1B), similar temporal developments of
422 bacterial communities in all treatments (including the controls, Fig.3A), and an
423 increase in shared ASVs between all treatments with increasing time (Supplemental
424 Fig. 2). However, first, it is almost impossible to completely omit bottle effects in
425 these types of experiments (e.g., S. Elovaara et al. (59) or C. M. Luria et al. (60)).
426 Second, despite of obvious changes in control communities from t0 to 6 h of

427 incubation, the highest community differences for these time steps were achieved
428 between both DOMp treatments (Fig. 3C). Furthermore, we found pronounced
429 differences between the phytoplankton donors in APA, BP, and cell numbers after 24
430 h of exposure (Fig. 1), and were able to define phytoplankton donor-specific ASVs,
431 as well as DOMp generalist ASVs (Supplemental Fig. 3B). Thus, the observed bottle
432 effects did not prohibit the gain of valuable information.
433
434 As synthesis, our results and that of similar studies suggest that bacterial diversity,
435 Alkaline Phosphatase activity (APA), Bacterial Production (BP), and cell numbers of
436 phytoplankton accompanying bacterial communities are determined by the
437 phytoplankton donor species (Fig. 5), which may be caused by different DOMp
438 compositions of cyanobacteria and diatoms (20). Additionally, the bacterial
439 community participates in the succession of DOMp from highly bioavailable to less
440 bioavailable (33, 61), with time-point defining ASVs (Supplemental Fig. 3C).
441 However, decreasing differences with increasing time of bacterial communities in the
442 same treatment (Fig. 3B), as well as high community differences between both
443 DOMp treatments after 6 h incubation (Fig. 3C), suggest a particularly high impact of
444 the host shortly after DOMp additions (Fig. 5). After exploitation of species-specific
445 highly bioavailable compounds, the remaining DOMp of different phytoplankton may
446 become more similar, and bacterial communities might be especially impacted by the
447 DOM accessibility (Fig. 5), i.e. the state of DOMp succession from highly bioavailable
448 to less bioavailable compounds.
449
450 MATERIALS AND METHODS
451 **Experimental set-up**

The experiment was performed in winter from 14. January 2019 until 17. January 2019 at the Israel Oceanographic and Limnological Research center in Haifa, Israel. Shaded 20 l Nalgene bottles were filled with 18 l of 50 µm pre-filtered sea-water (intake pipe at 5 m depth, ca. 50 m off the shore), amended with phytoplankton DOM, and placed in 1 m³ natural seawater flow-through tanks to maintain temperature. We added 25 µM cyanobacterium (*Prochlorococcus* MIT9312) DOC, in order to produce comparable outcomes with similar studies (see F. Eigemann et al. (7)), and 14 µM diatom (*Skeletonema marinoi*) DOC (due to technical issues, several DOM containing bottles broke at an air-transfer). Each of the three treatments (control, diatom DOM, cyanobacterium DOM) contained four biological replicates, and samples were taken at t0 (nutrients, RNA, APA, BP, cell-numbers), after 1 (only nutrients), 6 (nutrients and RNA), 24, 48 and 72 h (APA, BP, RNA, cell-numbers, nutrients). A graphical overview of the experimental set-up is given as Fig. 6.

Culture conditions and harvesting of DOMp

Prochlorococcus MIT9312 was grown under constant light (20 µ E m^{-2} sec^{-1}) at 22°C in Pro99 media where the NH_4 concentration was reduced from 800 µM to 100 µM, resulting in the cells entering stationary stage due to N starvation (62). *Skeletonema marinoi* was grown in F/2 + Si medium (63) at 45 µ E m^{-2} sec^{-1} at 17°C in a conditioning-cabinet. To obtain cell-free DOMp, batch cultures were harvested at the early decline phase by centrifugation followed by filtration through a 0.22 µm polycarbonate filter. Phytoplankton derived dissolved organic matter (DOMp) from the early decline phase may reflect natural DOM compositions better than from exponentially growing cultures (12), and includes DOM from phytoplankton exudation

476 as well as cell lysis. The different DOMp from both phytoplankton were kept at -20°C
477 until utilization.

478

479 **Nutrient measurements**

480 Nutrients were measured colorimetrical according to H. P. Hansen and F. Koroleff
481 (64) by means of a Seal Analytical QuAAtro constant flow analyzer. Concentrations
482 of PO_4, NO_x (NO_2 + NO_3), and NH_4 in the different treatments over the course of the
483 experiment are given in Supplemental Fig. 1.

484

485 **Cell numbers, Bacterial production, alkaline phosphatase activity, RNA**
486 **extraction, DNA digestion, cDNA synthesis and sequencing**

487 All analyzes were performed as described in F. Eigemann et al. (7). Brief descriptions
488 of the used techniques and protocols are given below.

489

490 **Cell numbers**

491 Duplicate samples were fixed with cytometry-grade glutaraldehyde (0.125% final
492 concentration), flash-frozen with liquid nitrogen and stored at -80 °C until analyses.
493 For measurements, samples were thawed in the dark at room temperature, stained
494 with SYBR Green I (Molecular Probes/ ThermoFisher) for 10 min at room
495 temperature, vortexed, and run on a BD FACSCanto™ II Flow Cytometry Analyzer
496 Systems (BD 146 Biosciences).

497

498 **Bacterial production and alkaline phosphatase activity (APA)**

499 Bacterial production was measured using the [4,5-^{3}H]-leucine incorporation method
500 (65). Samples were amended with 100 nmol of leucine L^{-1} (Perkin Elmer, specific
501 activity 156 Ci $mmol^{-1}$) and incubated for 4 h in the dark under ambient surface sea
502 water temperature (~19 °C). Incubations were stopped by the addition of 100 µL of
503 ice-cold 100% trichloroacetic acid (TCA). APA was determined by the 4-
504 methylumbeliferyl phosphate (MUF-P: Sigma M8168) method according to (66).
505 Substrate was added to a final concentration of 50 µM and incubated in the dark at
506 ambient temperature for 4 h (same as BP).

507

508 **RNA extraction, DNA digestion, cDNA synthesis**

509 In order to analyze active bacteria of the bacterial community, rRNA was extracted
510 and transcribed into cDNA. Approximately 1.5 l of each incubation bottle was filtered
511 directly onto 0.2 µm filters, and extracted with TRI reagent. Remaining DNA was
512 digested using the Turbo DNA free kit (Invitrogen) using the manufacturer's
513 instructions, and successful digestions were tested by PCRs. RNA was transcribed
514 into cDNA using MultiScribe reverse transcriptase following the manufacturer's
515 instructions (Invitrogen).

516

517 **Sequencing and Sequence analyses**

518 Sequencing was performed using primers 515F and 926R (67) and an Illumina
519 MiniSeq mid-output flow cell. Forward and backward sequence reads were deposited
520 at the European Nucleotide Archive under the accession number PRJEB44710. All
521 sequences were analyzed using the Dada2 (68) pipeline and the software packages
522 R (70, 71) and RStudio (72). Briefly, forward and backward primers were trimmed,
523 forward and backward reads truncated after quality inspections to 280 and 210

524 bases, respectively, and after merging of forward and backward sequences, a
525 consensus length only between 404 and 417 bases was accepted. For taxonomic
526 assignment, Silva database version 138 (73) was used. All chloroplasts,
527 mitochondria, archaea, eukaryotes and Amplicon sequence variants (ASVs) without
528 any taxonomic affiliation were discarded from downstream analyses. The complete
529 ASV table with absolute, subsampled read counts, and meta data for all samples is
530 accessible as Supplemental Table 1. After inspections of rarefaction curves, samples
531 with less than 10,154 reads were discarded from further analyses (t0, replicate four
532 (41 reads), and cyanobacterium DOM, replicate four, 6 h incubation (1,955 reads)),
533 and all remaining samples subsampled to 10,154 reads. One additional sample was
534 discarded from downstream analyses, because of a sandy RNA filter appearance,
535 and NMDS clustering far away from all other samples (control, replicate four, 6 h
536 incubation). Thus, from originally 52 samples, 49 were used for elaborated analyses.
537

538 **Statistics**

539 We tested for differences between the treatments at individual timepoints with
540 Shannon indices, cell numbers, Alkaline Phosphatase Activity (APA), and bacterial
541 production. Variance homogeneities between the different treatments for each
542 individual time-point were tested with Levene tests with the R package car (74). If
543 homogeneities were given, ANOVAs with subsequent Tukey post-hoc tests were
544 calculated. If no homogeneities of variances were given, Kruskal-Wallis tests with
545 subsequent Tukey-Nemenyi post-hoc tests were executed with the R package
546 PMCMR (75). To test for unique and shared ASVs between treatments, we merged
547 biological replicates as follows: All subsampled, absolute read counts >0 were set to
548 1, and subsequently the mean abundance of the biological replicates was calculated

549 for each treatment. An ASV was set as present if the calculated mean for the
550 treatment was ≥ 0.5. With these matrices, Venn counts were calculated and Venn
551 diagrams generated with the limma package (76). Changes in bacterial communities
552 were examined with Shannon diversities, non-metric-multidimensional-scaling
553 (NMDS), Bray-Curtis dissimilarities between treatments and time-points, and
554 heatmaps of the overall 100 most abundant ASVs. Differences of Bray-Curtis
555 dissimilarities between samples were tested with ANOVAs (homogeneities of
556 variances given) or Kruskal-Wallis tests (homogeneities of variances not given), with
557 subsequent Tukey post-hoc or Tukey-Nemenyi post-hoc tests, respectively (see
558 above). NMDS was executed with Bray-Curtis distances in the vegan package
559 (Oksanen et al. 2019) with subsampled absolute ASV reads. To test for differences
560 between bacterial communities, analyses of similarities (ANOSIM) as well as envfit
561 (environmental fits on ordinations) analyses were conducted using the vegan
562 package. Heatmaps for the overall 100 most abundant, subsampled ASVs were
563 executed using the heatmap 3 package (70, 77). To test if specific ASVs of these 100
564 most abundant ASVs are associated with specific time-points or treatments (control,
565 diatom DOMs, cyanobacterium DOMs), LDA effect size (LEfSe) analyses (78) were
566 executed with the online tool https://huttenhower.sph.harvard.edu/galaxy. Time-
567 points and treatments were assigned as class, and samples as subjects. Then, a
568 "one-against-all" strategy was applied with normalization and the LDA effect size
569 threshold set to 2.5. All analyses, except denoted otherwise, were executed with R
570 (71) and RStudio (72). Statistical outcomes are summarized in Supplemental Table
571 2. All graphics, except denoted otherwise, were executed with the ggplot2 package
572 (79), and refined with the freeware Inkscape (https://inkscape.org).

573

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Fig. 1 (A): Bacterial cell numbers, (B) Bacterial Production, and (C) Alkaline Phosphatase Activity (APA) for different time-points and treatments. The letters in the panels represent the outcomes of Tukey post-hoc tests between the treatments for each individual time-point (see Supplemental statistics for further information).

Figure 2: Shannon diversities for the different time-points and treatments. The letters in the panels represent the outcomes of Tukey post-hoc tests between the treatments for each individual time-point (see Supplemental table 2 for further information).

Figure 3: Effects of different treatments and proceeding time on bacterial communities. (A) Non-metric multidimensional scaling (NMDS) for the different treatments and time-points combined with EnvFit analyses. The red squares indicate

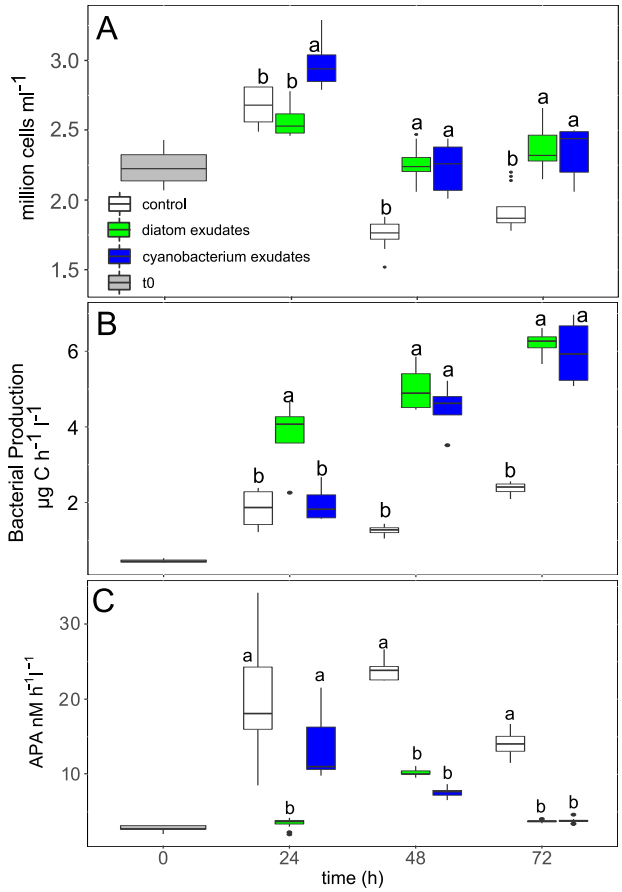
805 the calculated centres of the respective treatments, the arrow indicates the direction
806 of proceeding time, (B) Bray-Curtis dissimilarities of bacterial communities between
807 different time-points in the same treatment, and (C) Bray-Curtis dissimilarities of
808 bacterial communities between different treatments at the same time-point. (B, C)
809 Letters on top of the box-plots refer to the outcomes of Tukey post-hoc tests for
810 differences over time in the same treatment (B) and for differences of time-dependent
811 differences between different treatments (C). Further information and additional tests
812 (different treatments, same time-point comparisons (B), and different treatment
813 comparisons at the same time-point (C)) are provided in Supplemental Table 2.

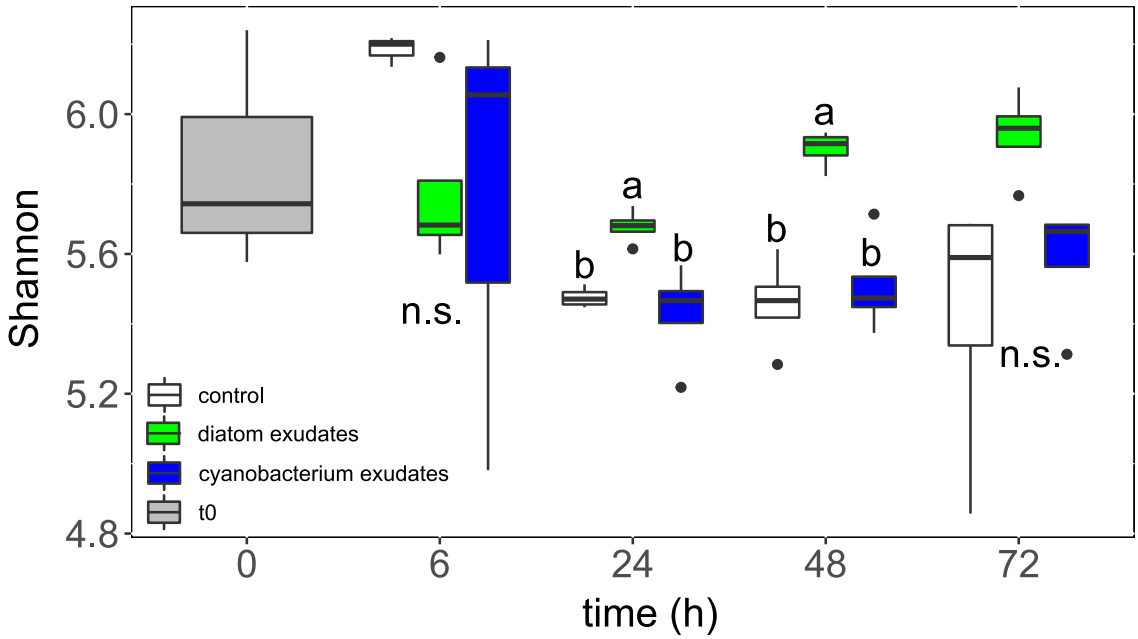
814 Figure 4: Heatmap of the overall 100 most abundant ASVs for the different
815 treatments and time-points. The dendrogram on the left side of the heatmap is based
816 on hierarchical clustering of ASVs. The table on the right side of the heatmap
817 provides ASV taxonomy (first column with colour code), and dominant ASVs for the
818 different treatments (t0, diatom DOMs, cyanobacterium DOMs, control, backed with
819 light-grey) as well as different time-points (0 h, 6 h, 24 h, 48 h, 72 h, different
820 treatments pooled, backed with brown-grey, Supplemental Fig. 3A, C). Dominant
821 ASVs of the treatments/time-points are indicated by filled boxes in the colour of the
822 respective taxonomy. ASVs of the same genus are defined by numbers in the
823 taxonomic colour code.

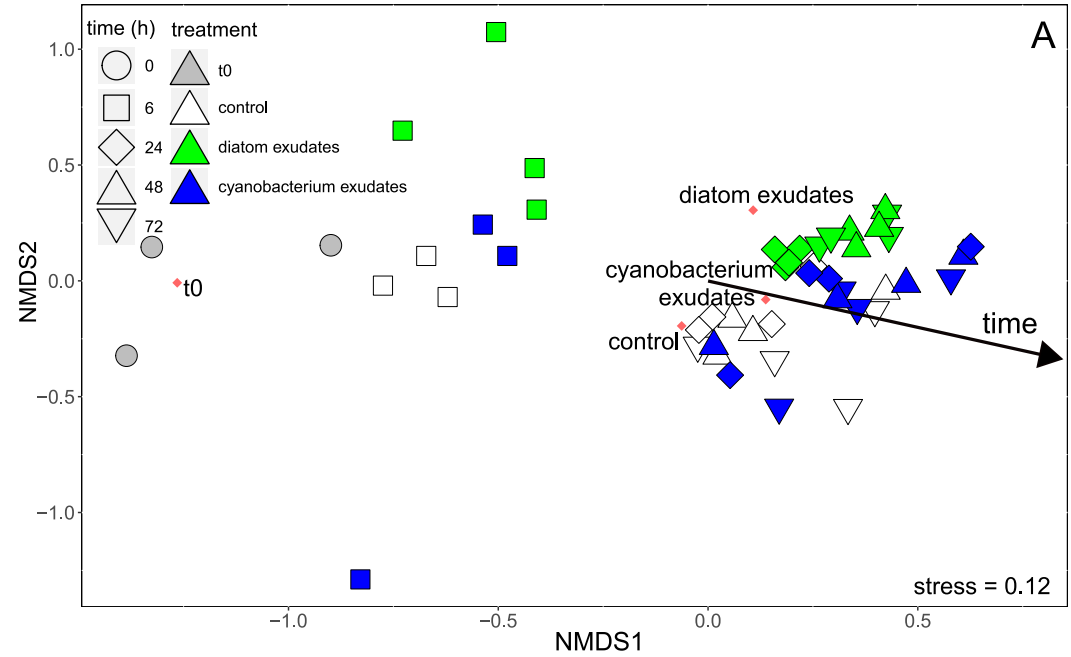
824 Fig. 5: Summary figure of the obtained results (indicated by *), and outcomes of
825 similar studies on bacterial utilization of cyanobacteria and diatom derived DOM (a =
826 B. Kieft et al. (39), b = H. Sarmento and J. M. Gasol (51), c = M. Landa et al. (23), d =
827 F. Eigemann et al. (7)). If different outcomes at different time-points were obtained, a
828 time-specification is given after the reference. Right side: Different colored bacteria
829 indicate taxonomically different bacteria, with higher similarities between colors

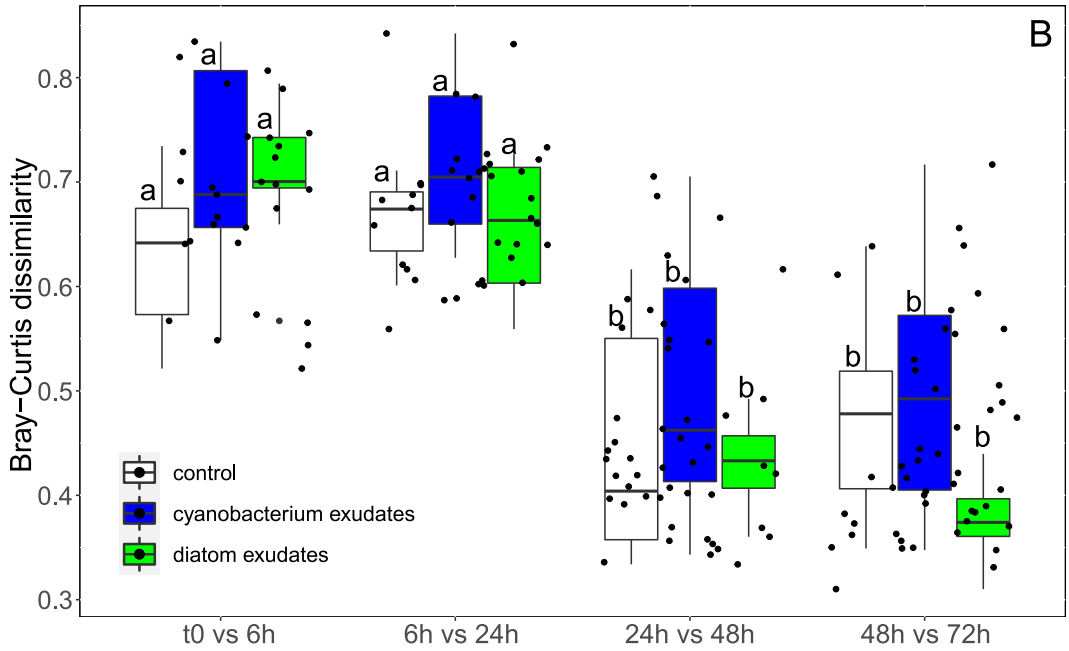
indicating higher taxonomical similarities. The approximating colors of diatom and cyanobacteria DOM with progressing time indicate the succession of DOM compounds from highly to less bioavailable, which might become more similar.

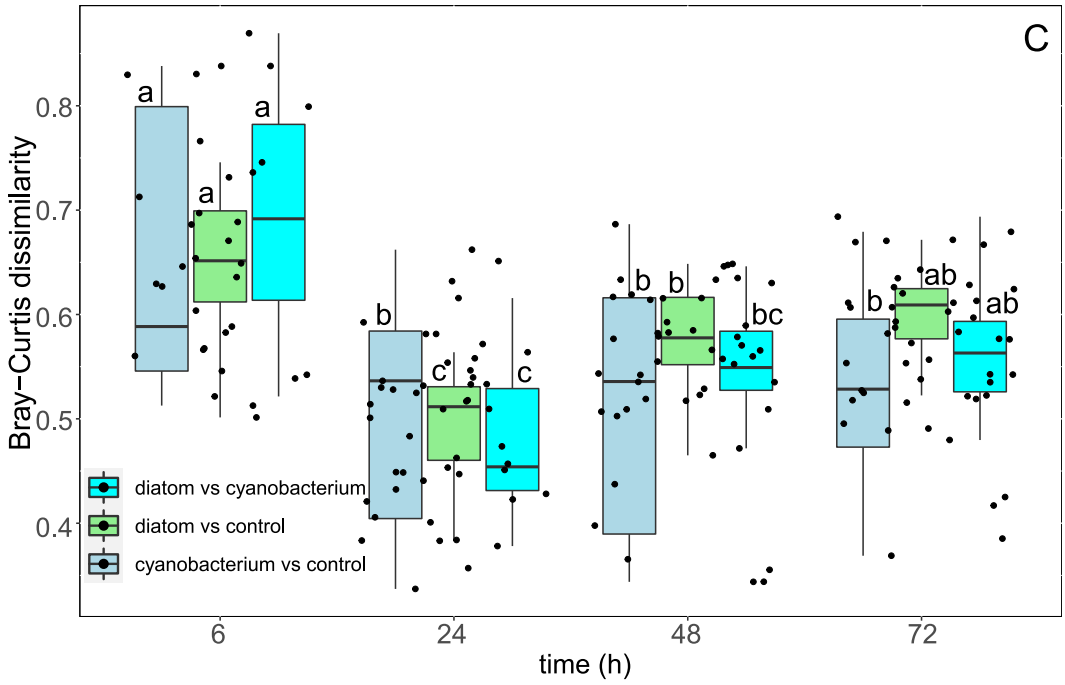
Figure 6: Schematic overview of the experimental set-up. *Prochlorococcus* MIT9312 (cyanobacterium) and *Skeletonema marinoi* (diatom) were grown till the early decline phase, cell-free DOMp harvested, and mixed with pre-filtered natural water. Incubation bottles were placed in darkened flow-through tanks.

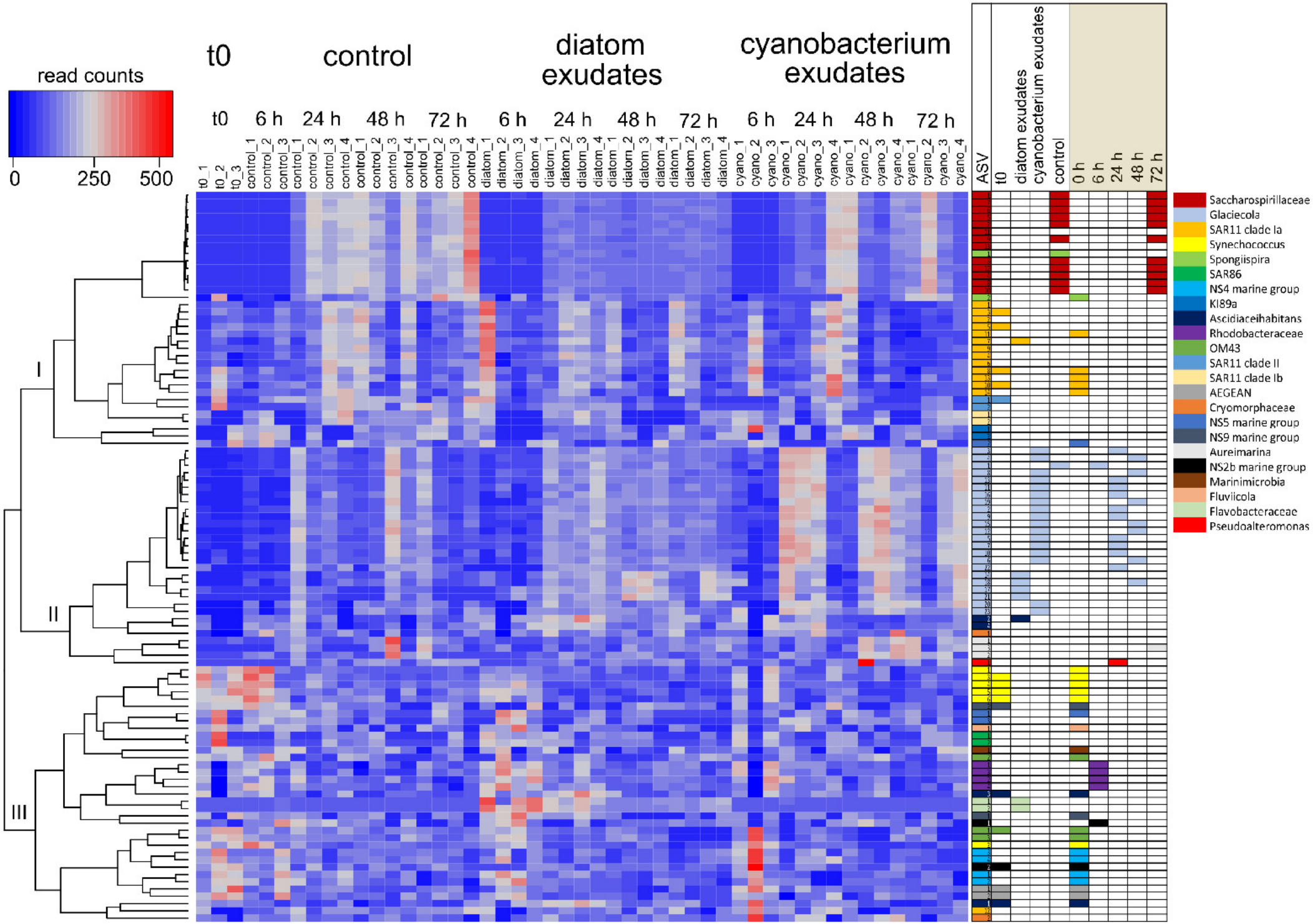

A
million cells ml^{-1}
3.0
2.5
2.0
1.5
control
diatom exudates
cyanobacterium exudates
t0
B
Bacterial Production
µg C h^{-1} l^{-1}
6
4
2
C
APA nM h^{-1}l^{-1}
30
20
10
0
24
48
72
time (h)

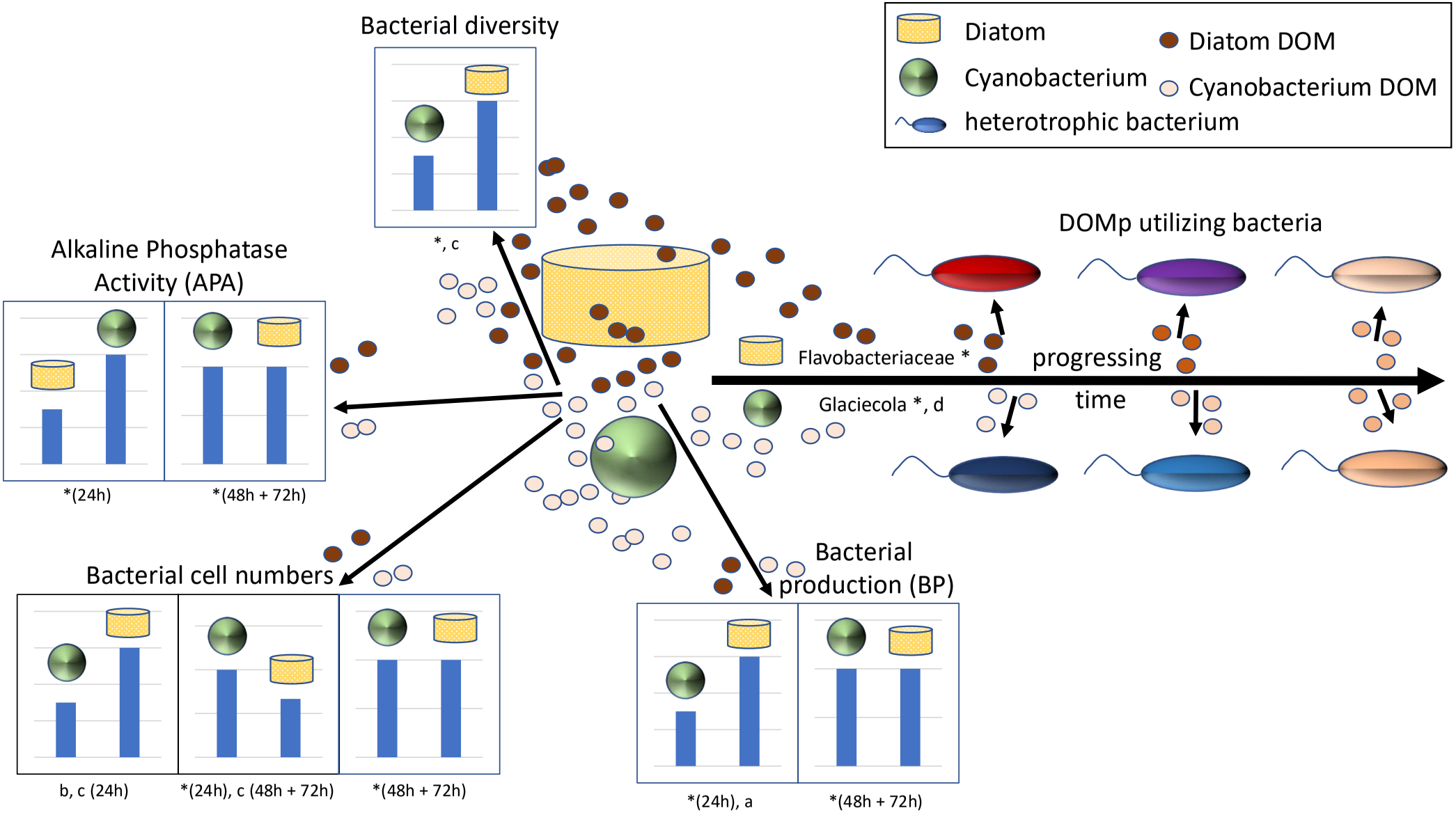

Shannon
6.0
5.6
5.2
4.8
0
6
24
48
72
time (h)
n.s.
b
a
b
b
a
b
n.s.
control
diatom exudates
cyanobacterium exudates
t0

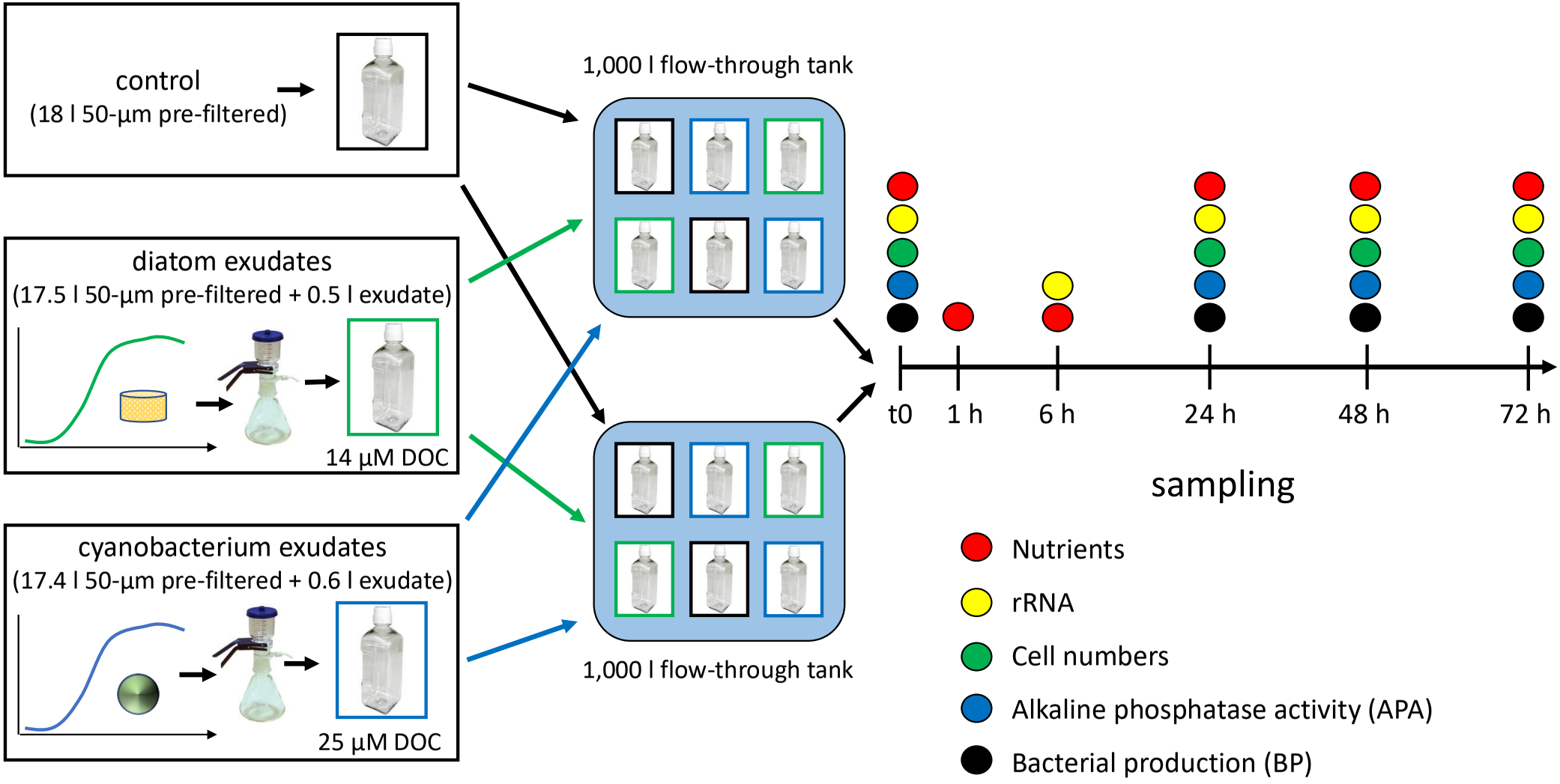

A
time (h)
treatment
0
6
24
48
72
t0
control
diatom exudates
cyanobacterium exudates
diatom exudates
cyanobacterium
exudates
control
time
t0
stress = 0.12
NMDS2
NMDS1
1.0
0.5
0.0
−0.5
−1.0
−1.0
−0.5
0.0
0.5


B
Bray−Curtis dissimilarity
0.8
0.7
0.6
0.5
0.4
0.3
control
cyanobacterium exudates
diatom exudates
t0 vs 6h
6h vs 24h
24h vs 48h
48h vs 72h


C
Bray−Curtis dissimilarity
0.8
0.7
0.6
0.5
0.4
diatom vs cyanobacterium
diatom vs control
cyanobacterium vs control
6
24
48
72
time (h)


read counts
0
250
500
t0
control
diatom exudates
cyanobacterium exudates
t0
6 h
24 h
48 h
72 h
I
II
III
ASV
t0
diatom exudates
cyanobacterium exudates
control
0 h
6 h
24 h
48 h
72 h
Saccharospirillaceae
Glaciecola
SAR11 clade Ia
Synechococcus
Spongiispira
SAR86
NS4 marine group
KI89a
Ascidiaceihabitans
Rhodobacteraceae
OM43
SAR11 clade II
SAR11 clade Ib
AEGEAN
Cryomorphaceae
NS5 marine group
NS9 marine group
Aureimarina
NS2b marine group
Marinimicrobia
Fluviicola
Flavobacteraceae
Pseudoalteromonas


Diatom
Cyanobacterium
heterotrophic bacterium
Diatom DOM
Cyanobacterium DOM
Bacterial diversity
*, c
Alkaline Phosphatase Activity (APA)
*(24h)
*(48h + 72h)
DOMp utilizing bacteria
Flavobacteriaceae *
Glaciecola *, d
progressing
time
Bacterial cell numbers
b, c (24h)
*(24h), c (48h + 72h)
*(48h + 72h)
Bacterial production (BP)
*(24h), a
*(48h + 72h)


control
(18 l 50-µm pre-filtered)
diatom exudates
(17.5 l 50-µm pre-filtered + 0.5 l exudate)
14 µM DOC
cyanobacterium exudates
(17.4 l 50-µm pre-filtered + 0.6 l exudate)
25 µM DOC
1,000 l flow-through tank
1,000 l flow-through tank
t0
1 h
6 h
24 h
48 h
72 h
sampling
Nutrients
rRNA
Cell numbers
Alkaline phosphatase activity (APA)
Bacterial production (BP)