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Phytoplankton exudates provide full nutrition to a subset of accompanying heterotrophic bacteria via carbon, nitrogen and phosphorus allocation

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Summary

Marine bacteria rely on phytoplankton exudates as carbon sources (DOCp). Yet, it is unclear to what extent phytoplankton exudates also provide nutrients such as phytoplankton-derived N and P (DONp, DOPp). We address these questions by mesocosm exudate addition experiments with spent media from the ubiquitous pico-cyanobacterium *Prochlorococcus* to bacterial communities in contrasting ecosystems in the Eastern Mediterranean – a coastal and an open-ocean, oligotrophic station with and without on-top additions of inorganic nutrients. Inorganic nutrient addition did not lower the incorporation of exudate DONp, nor did it reduce alkaline phosphatase activity, suggesting that bacterial communities are able to exclusively cover their nitrogen and phosphorus demands with organic forms provided by phytoplankton exudates. Approximately half of the cells in each ecosystem took up detectable amounts of *Prochlorococcus*-derived C and N, yet based on 16S rRNA sequencing different bacterial genera were responsible for the observed exudate utilization patterns. In the coastal community, several phylotypes of *Aureimarina*, *Psychrosphaera* and *Glaciecola* responded positively to the addition of phytoplankton exudates, whereas phylotypes of *Pseudoalteromonas* increased and dominated the open-ocean communities. Together, our results strongly indicate that phytoplankton exudates provide coastal and open-ocean bacterial communities with organic carbon, nitrogen and phosphorus, and that phytoplankton exudate serve a full-fledged meal for the accompanying bacterial community in the nutrient-poor eastern Mediterranean.

Introduction

Approximately 50% of the global primary production is executed by phytoplankton (Field *et al*., 1998) with >100 Pg oceanically fixed carbon per year (Huang *et al*., 2021), and generally 50% of this photosynthetically fixed carbon is then consumed by oceanic heterotrophic bacteria (Azam and Malfatti, 2007). In addition to utilizing photosynthetically derived organic carbon, heterotrophic bacteria also recycle nutrients, such as nitrogen and phosphorus (N and P), and the recycling of these and other micro- and macro-nutrients may directly impact phytoplankton dynamics (Amin *et al*., 2012; Moore *et al*., 2013; Buchan *et al*., 2014; Amin *et al*., 2015). Thus, oceanic bacteria are key players in biogeochemical cycles with global importance (Azam and Malfatti, 2007; Amin *et al*., 2015; York, 2018), and interactions between phytoplankton and bacteria are crucial for carbon and nutrient fluxes through aquatic food webs (Cole, 1982; Amin *et al*., 2015; Christie-Oleza *et al*., 2017; Seymour *et al*., 2017; Mühlenbruch *et al*., 2018). Dissolved organic material (DOM) is released by phytoplankton (DOMp) via passive leakage, active release, as well as through lysis products of dead cells (Grossart, 1999; Thornton, 2014; Christie-Oleza *et al*., 2017), whereby the type of release may define its composition (Livanou *et al*., 2017). Besides dissolved organic carbon (DOCp), phytoplankton exudates contain organic nitrogen [DONp, e.g. amino acids, peptides and proteins (Beliaev *et al*., 2014; Roth-Rosenberg *et al*., 2021a)] and phosphorus [DOPp,

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e.g. DNA and RNA (Roth-Rosenberg *et al*., 2021a)]. These organic nutrient forms exuded by phytoplankton serve the accompanying bacterial community as N (Karlson *et al*., 2015) and P (Riemann *et al*., 2009) source, because inorganic forms of both nutrients are scarce in most oceanic environments (Moore *et al*., 2013; Saito *et al*., 2014; Liefer *et al*., 2019). However, the importance of such organic, phytoplankton-derived nutrients relative to inorganic nutrient sources for heterotrophic bacteria is still unclear. Also, the role of inorganic nutrients in the utilization of phytoplankton-derived dissolved organic carbon (DOCp) is not consistent, as inorganic nutrients may (Carlson *et al*., 2004; Thornton, 2014) or may not (Carlson and Ducklow, 1996) fuel the DOCp utilization by the bacterial community under nutrient limiting conditions, depending on so far unknown factors.

As suppliers of various carbon and nutrient sources, phytoplankton drive bacterial community dynamics (Rooney-Varga *et al*., 2005), and different phytoplankton release different types of DOMp (Mühlenbruch *et al*., 2018). This coupling between phytoplankton and heterotrophic bacteria, however, might be less pronounced in coastal environments compared to open-ocean sites, due to higher amounts of allochthonous material at coastal areas (Morán *et al*., 2002a), although DOCp is preferred over other carbon sources (Guillemette *et al*., 2016). Some bacteria are adapted to DOMp derived from specific phytoplankton species (Grossart *et al*., 2007; Sarmento and Gasol, 2012; Beier *et al*., 2015), phytoplankton source communities (Carlson *et al*., 2004) or growth-phases of the phytoplankton (Becker *et al*., 2019), and thus act as specialists. Others, however, apply generalist strategies and are more affected by DOM concentrations than by its composition (Sarmento *et al*., 2016; Becker *et al*., 2019). The questions of what percentage of the total bacteria are active in exudate utilization as well as which exudate compounds are used by them remained open in preceding studies.

In this study, we addressed the following questions: (i) Does phytoplankton-derived dissolved organic material (DOMp) serve as nitrogen and phosphorus source for the accompanying bacterial community? (ii) Do bacterial cells selectively incorporate exudate-derived organic carbon or nitrogen or do they incorporate both at a constant ratio? (iii) Which fraction of the total bacterial community is active in DOMp utilization? (iv) Which specific bacterial taxa utilize DOMp, (v) How is the bacterial DOCp utilization affected by inorganic nutrients? and (vi) Do bacterial communities from contrasting environments (coastal and open-ocean) show consistent patterns and magnitudes of reactions as responses to exudate and inorganic nutrient additions? We explore these questions in the Eastern Mediterranean at a coastal and an open ocean station. The pelagic Eastern Mediterranean is ultra-oligotrophic, comparable with major ocean gyres (Hazan *et al*., 2018; Reich *et al*., 2021), whereas conditions closer to the coast are somewhat richer in nutrients (Sisma-Ventura and Rahav, 2019). As a DOM source, we used spent media from *Prochlorococcus* strain MIT9312, labelled with ^{13}C and ^{15}N. The cyanobacterium *Prochlorococcus* is the most abundant phototrophic organism on Earth, with an annual global mean abundance of 2.9×10^{27} cells (Flombaum *et al*., 2013), and dominates phytoplankton biomass in many oligotrophic oceans despite its small size (Partensky *et al*., 1999). *Prochlorococcus* alone was suggested to exude as much as 75% of the daily photosynthetic organic carbon production in oligotrophic environments (Ribalet *et al*., 2015), resulting in feeding up to 40% of the total bacterial production (BP) (Bertilsson *et al*., 2005; Biller *et al*., 2015). In the Eastern Mediterranean, the community composition of the free-living heterotrophic bacteria is weakly but significantly correlated with the presence of divinyl Chlorophyll A, a diagnostic pigment of *Prochlorococcus*, further supporting a potential link through DOM production and uptake (Roth-Rosenberg *et al*., 2021b). The specific strain used, MIT9312, was selected because it is the most abundant ecotype globally (Johnson *et al*., 2006), although not in the Mediterranean (Mella-Flores *et al*., 2011), and has recently been shown to exude large amounts of DOC (Roth-Rosenberg *et al*., 2021a). To test for the above-raised questions, we amended coastal and open-ocean bacterial communities with MIT9312 exudates with and without additions of inorganic nutrients and analysed bacterial responses via 16S rRNA amplicon sequencing, cell numbers, BP, incorporation of DOCp and DONp, and alkaline phosphatase activity (APA).

Results

Contrasting conditions at the coastal versus open-ocean sites

We performed two experiments – one at a coastal site and one at an open-ocean station in the Eastern Mediterranean Sea. At both sites, 25 μM C *Prochlorococcus* MIT9312 exudates were added to the natural bacterial community with and without on top additions of inorganic nutrients (for details see Experimental procedures and Table 2). The two sampling sites exhibited distinctively different chemical characteristics. The inorganic nutrient concentrations at the coastal site under influence of a storm event were 0.12 ± 0.01 μM PO_4, 3.00 ± 0.63 μM NO_{2+3} and 0.50 ± 0.11 μM NH_4. In contrast, the open

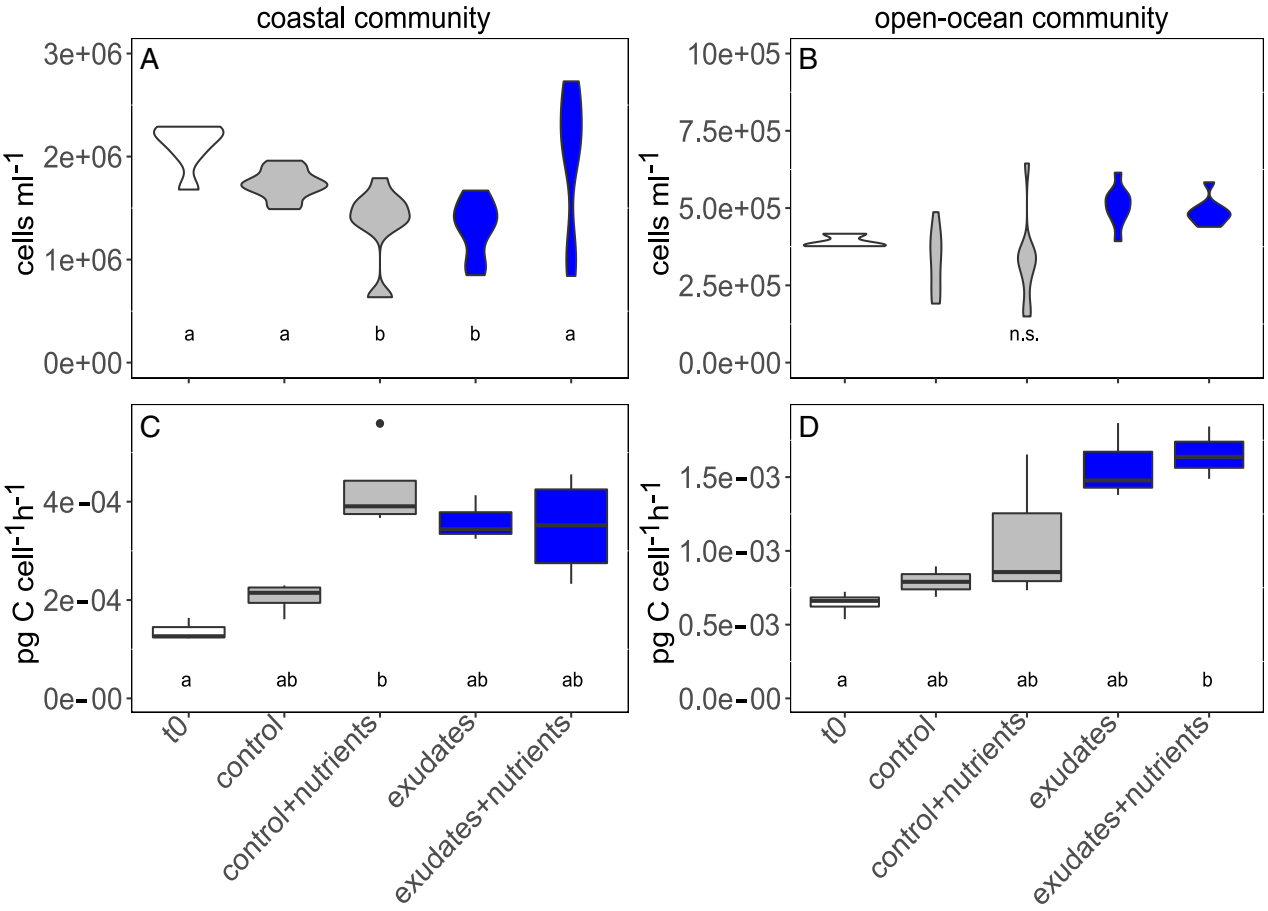

Fig. 1. Cell numbers (violin plots, top row) and bacterial production (box plots, bottom row) in the different treatment microcosm bottles. The letters in the panels represent the outcomes of Tukey *post hoc* tests. Please note the different *y*-scales for the coastal and open-ocean communities. T0 samples are displayed in white, control samples in grey and exudate samples in blue.

ocean site was highly oligotrophic even though the samples were taken during winter when the water column was relatively well mixed; 0.003 μM PO_4, 0.22 μM NO_3 and 0.0025 μM NH_4.

Cell numbers and cell-specific bacterial production

In the coastal community, after 24 h, cell numbers significantly decreased in the control + nutrients and exudate without nutrients treatments (Fig. 1A), whereas in the open-ocean community, both exudate treatments (with and without nutrients) showed elevated cell numbers (~1.5-fold), which were, however, not significant (Fig. 1B). Thus, neither the inorganic nutrient nor the exudate additions resulted in a clear impact on cell numbers after 24 h of incubation.

Although the cell-specific BP increased in all treatments after 24 h, it differed significantly from the control only in the nutrient amendment for the coastal community (Fig. 1C). In the open-ocean community the exudate + nutrient treatment revealed increased cell-specific BP (Fig. 1D).

Incorporation of organic carbon and nitrogen

In order to verify that bacteria incorporate carbon and nitrogen from phytoplankton-derived dissolved organic material (DOMp), we investigated their uptake by means of NanoSIMS measurements. Both coastal and open-ocean bacterial communities showed incorporation of labelled organic carbon and nitrogen derived from the phytoplankton exudates, revealed by significantly increased $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios (Fig. 2). As expected, the coastal community at time 0 and all control treatments revealed values around the naturally occurring ratios of 0.011 ($^{13}C/^{12}C$) and 0.00367 ($^{15}N/^{14}N$) (Fig. 2, no $t = 0$ samples were available for the open-ocean location). Nutrient additions had neither an effect on the DOCp (Fig. 2A and B) nor on the DONp incorporation (Fig. 2C and D). We further tested the percentage of active cells in DOCp and DONp utilization

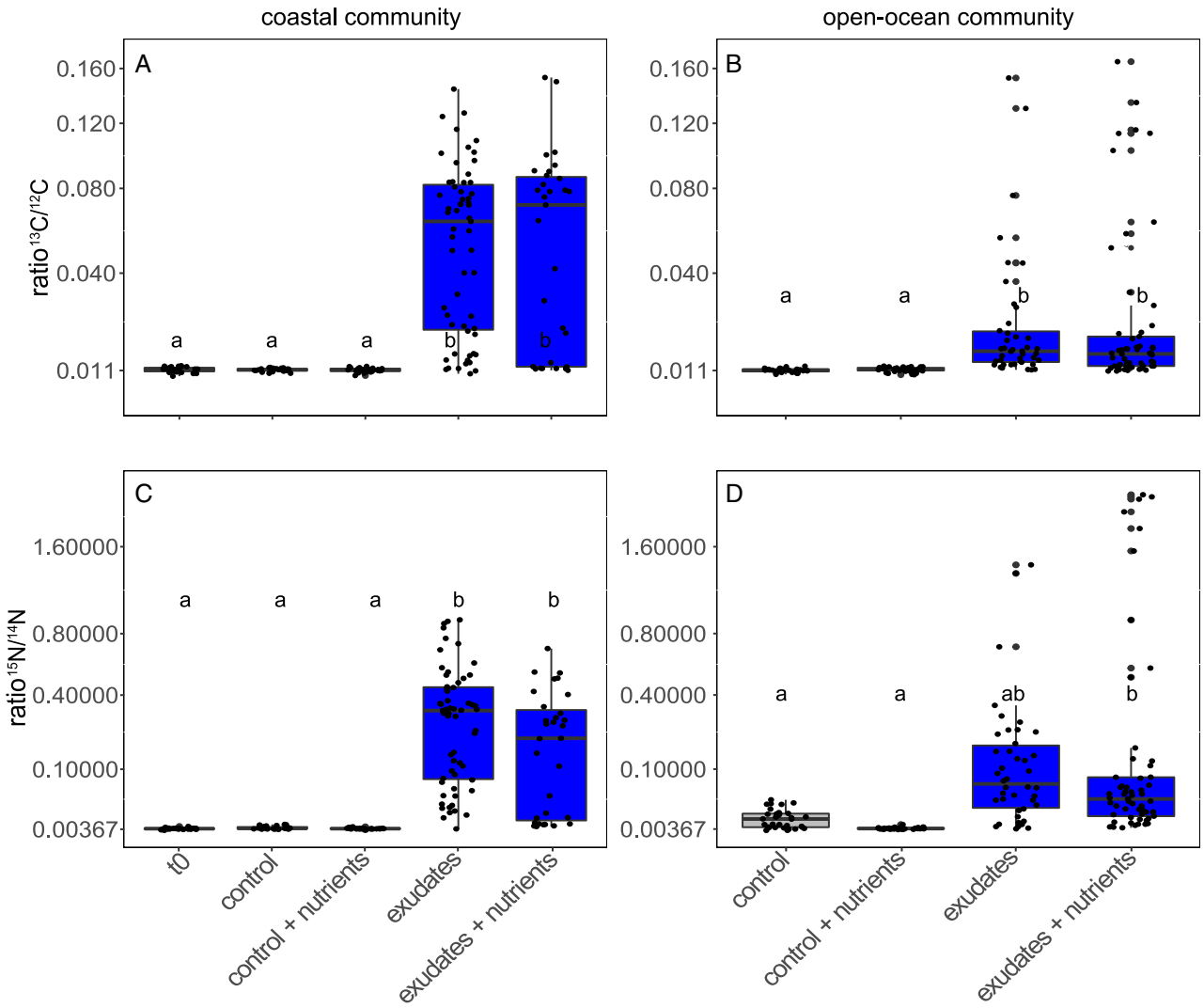

Fig. 2. $^{13}C/^{12}C$ ratios (A, B) and $^{15}N/^{14}N$ ratios (C, D) of the coastal (A, C) and the open-ocean (B, D) bacterial community following 24 h incubations and in t0. T0 samples are displayed in white, control samples in grey and exudate samples in blue.

(active cells defined as cells with ratios above the 95% percentile of pooled t0, control and control + nutrient measurements, Supplement 1). After 24 h of incubation, in the coastal bacterial community, 54% of the cells in the pooled exudate and exudate + nutrients treatments revealed increased ^{15}N values and 47% increased ^{13}C values. In the open-ocean community, 39% and 51% cells (pooled exudate and exudate + nutrients treatment) with increased ^{15}N and ^{13}C values were found. In the coastal as well as in the open-ocean bacterial community ^{13}C and ^{15}N uptake were highly correlated (Table 1). If the single treatments were considered separately, for both, the coastal and open-ocean communities both exudate treatments revealed significant correlations, but as expected, none of the other treatments (Table 1). The slopes (^{13}C = *y*-axis, ^{15}N = *x*-axis) of the correlations in all exudate treatments were steeper for the coastal community compared to the open-ocean one (Table 1).

Cell-specific alkaline phosphatase activity

To test whether bacterial communities satisfy their phosphorus demand via DOMp, we analysed the activity of the alkaline phosphatase enzyme (APA) in the different treatments and environments. In both environments, additions of phytoplankton exudates lowered the APA, without any effect of on top additions of inorganic nutrients. Nutrient additions in the controls also lowered the APA, but not as strong as the exudate additions (Fig. 3). In the coastal bacterial community, the control treatments without nutrient additions revealed a significantly higher cell-specific APA compared to all other treatments, and in the open-ocean community the exudate treatments had

Table 1. Correlations between ^{13}C and ^{15}N incorporation derived from NanoSIMS analyses.

Experiment	Treatment	Linear function	r^2	p
Exp. 1, coastal	All treatments	$y = 0.14x$	0.74	<0.0001
	t0	$y = -0.13x$	−0.03	0.63
	Control	$y = -0.07x$	0.01	0.39
	Control + nutrients	$y = 0.48x$	0.1	0.05
	Exudates	$y = 0.12x$	0.72	<0.0001
	Exudates + nutrients	$y = 0.15x$	0.48	<0.0001
Exp. 2, open-ocean	All treatments	$y = 0.06$	0.91	<0.0001
	Control	$y = 0.002x$	−0.03	0.77
	Control + nutrients	$y = -0.04x$	−0.02	0.78
	Exudates	$y = 0.1x$	0.94	<0.0001
	Exudates + nutrients	$y = 0.06x$	0.95	<0.0001

The y-axis was defined as = $^{13}C/^{12}C$ ratio, the x-axis as = $^{15}N/^{14}N$ ratio.

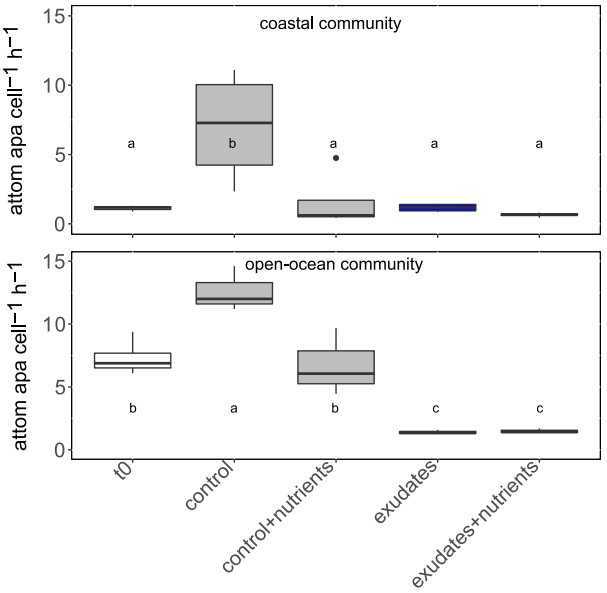

Fig. 3. Cell-specific alkaline phosphatase activity for the coastal (top panel) and the open-ocean (bottom panel) bacterial community. The letters in the panels represent the outcomes of Tukey *post hoc* tests. T0 samples are displayed in white, control samples in grey and exudate samples in blue.

not only lower APA compared to the control treatment, but also lower APA compared to the t0 and control + nutrient treatments (Fig. 3).

Dynamics in bacterial community composition

To answer how the active bacterial communities develop in response to exudate additions, we analysed the diversity and composition in the 16S rRNA derived dataset. Since the amplicons were derived from RNA molecules, these represent primarily the active members of the bacterial community. Shannon diversities did not show significant differences between the treatments in the coastal communities (ANOVA, $p = 0.81$), but in the open-ocean community exudate treatments yielded lower Shannon diversities (ANOVA, $p = 0.00$, Fig. 4). If only t0 bacterial communities were compared, both environments revealed slightly higher Bray–Curtis dissimilarities compared to comparisons of t0 samples in the same environment (average between the environments 0.76 ± 0.06, between coastal t0 samples 0.63 ± 0.13, and between open-ocean t0 samples 0.67 ± 0.01, Supplement 2).

NMDS analyses of the bacterial communities showed differences between experiments [analysis of similarities (ANOSIM), $p = 0.001$, $R = 0.44$, Fig. 5], and in the coastal community additionally between the free and attached fractions (ANOSIM, $p = 0.001$, $R = 0.45$, Fig. 5). However, the different treatments also clustered significantly differently (ANOSIM, $p = 0.02$, $R = 0.11$), where strong differences occurred between samples with added exudates and those without. The addition of nutrients did not show any clear effects in either experiment (Fig. 5). If only the 40 most abundant ASVs in both experiments were considered and illustrated in heatmaps, likewise strong community shifts were observed between the treatments (Fig. 6A and B). LEfSe (linear discriminant analysis effect size) analyses suggested *Fluviicola*, *Pseudofulvibacter*, *Synechococcus* and NS4 marine group being predominant in t0 samples, *Spongiispira* predominant in the controls, whereas the exudate treatments were dominated by *Glaciecola* in the coastal environment [Supplement 3, Fig. 6A, see also the 20 most abundant ASVs for every single treatment (Supplement 4)]. In the open-ocean community, *Pseudoalteromonas* was strikingly abundant in the exudate treatments, whereas SAR11, SAR202 and KI89a clade bacteria were predominant at t0 and in the control samples (Fig. 6B, Supplements 4 and 5).

Discussion

In this study, we wanted to identify which fraction (and which bacterial taxa) of the total bacterial community are

active in phytoplankton-derived dissolved organic material (DOMp) utilization, whether DOMp serves as a substantial nitrogen and phosphorus source for the accompanying bacterial community, and if so, whether bacterial cells selectively incorporate carbon or nitrogen. To test for general patterns as well as for the impact of allochthonous material on utilization of DOMp, we addressed these questions in two contrasting systems in the Eastern Mediterranean: a coastal station after a storm event with lots of suspended material and an oligotrophic open-ocean station. Our experimental results strongly indicate that DOMp serves as a substantial N and P source for a subset of approximately 50% of the bacterial community at the times and places sampled, and that the active community members can fully satisfy their demands with the phytoplankton-derived organic nutrient sources. Thus, the Mediterranean Sea bacterial communities may compensate for inorganic nutrient limitations (Krom *et al*., 2010; Krom *et al*., 2014) with organic forms derived from phytoplankton exudates. Another raised question was which factors limit heterotrophic production: Are marine bacteria predominantly carbon (Christie-Oleza *et al*., 2017) or nutrient (Carlson *et al*., 2004; Fouilland *et al*., 2014) limited? To address this point, N and P derived from the provided exudates seem to be more important for the open-ocean community if compared to the coastal community despite similar overall reactions to DOMp additions in both environments.

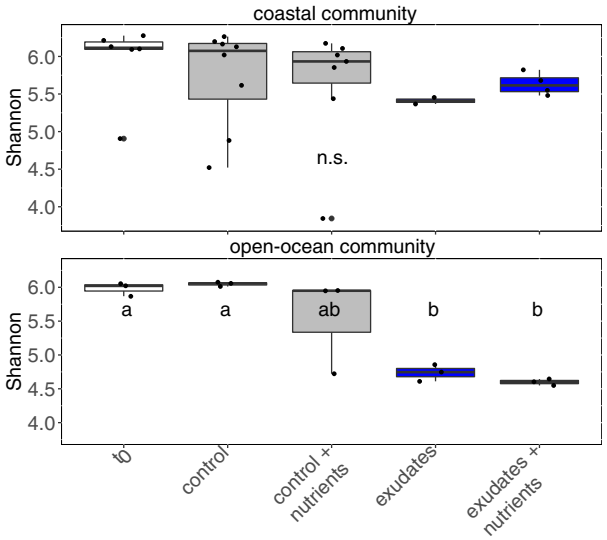

Fig. 4. Shannon diversity for the coastal (top panel) and the open-ocean (bottom panel) bacterial community. The letters in the panels represent the outcomes of Tukey *post hoc* tests. Please note that in coastal communities size-fractionated (5 and 0.2 μm pore width) samples were analysed, whereas in the open-ocean communities no size-fractionation was performed (only 0.2 μm filter pore width). T0 samples are displayed in white, control samples in grey and exudate samples in blue.

Bacterial production and carbon incorporation following phytoplankton exudate addition

Increased BP in both environments following exudate additions (Fig. 1) suggests that exudate DOCp provide carbon for both bacterial communities, which was confirmed by NanoSIMS measurements (Fig. 2). However, as stated above, DOCp incorporation might be limited by inorganic nutrients (Carlson *et al*., 2004; Fouilland *et al*., 2014). For example, the conversion of carbon from polysaccharides into bacterial biomass was enhanced by inorganic nitrogen for *de novo* synthesis of cellular proteins (Grossart *et al*., 2007; Piontek *et al*., 2011), and additions of inorganic nitrogen and high nitrate concentrations increased glucose assimilation by bacterioplankton (Bianchi *et al*., 1998; Skoog *et al*., 2002). However, also organic nutrients (e.g. dissolved free amino acids) were already shown to increase BP rates in marine environments (Carlson and Ducklow, 1996). Our results suggest that the nutrient demand for DOCp utilization in both

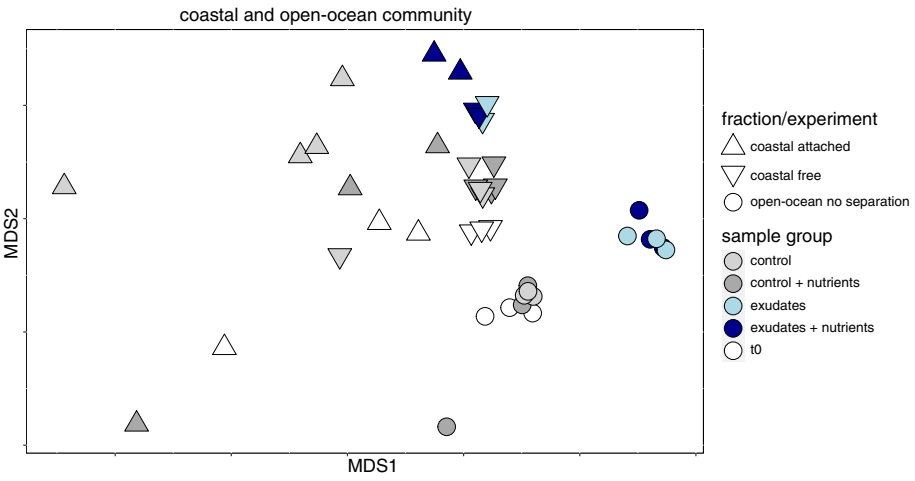

Fig. 5. NMDS plot of the bacterial communities (stress = 0.15). The symbol and colour key is given on the right-hand side. T0 samples are displayed in white, control samples in grey and exudate samples in blue.

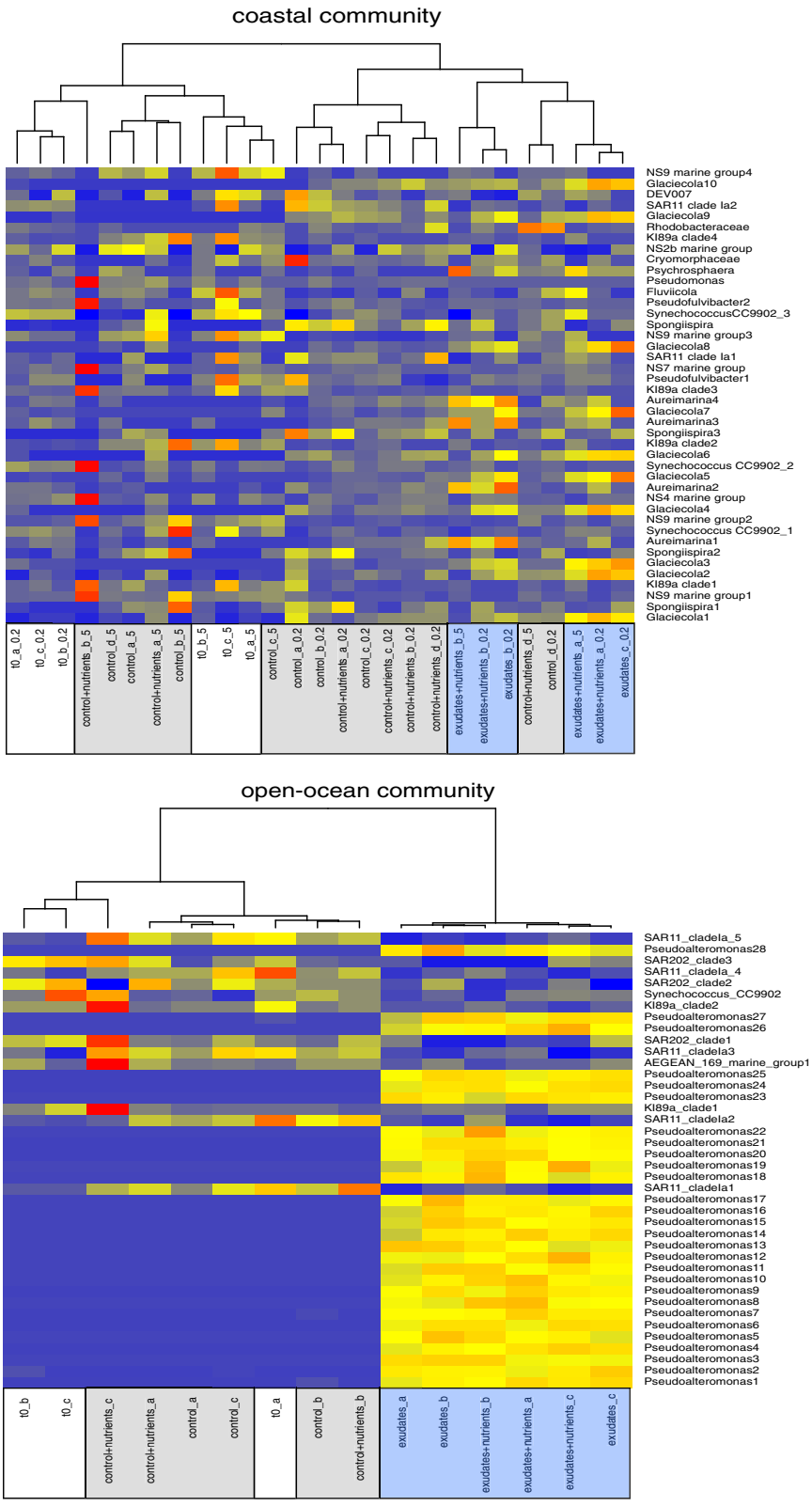

Fig. 6. Heat maps of the 40 most abundant ASVs (normalized absolute read counts) in the coastal (top) and open-ocean (bottom) environment. Samples can be seen below the heatmap, ASVs on the right side. If several ASVs with the same taxonomy were present, the taxonomy was numbered. Blue colour indicates low read numbers and red colour indicates high read numbers. The letter behind the treatment names refer to the replicate, the number behind this letter indicates the filter pore width and thus the fraction (only coastal experiment, 0.2 μm = free fraction, 5 μm = attached fraction). Dendrograms on top are calculated based on Euclidian distances, the ordering of ASVs refers to read counts (top ASV: lowest read counts, bottom ASV: highest read counts). T0 samples are displayed in white, control samples in grey and exudate samples in blue.

bacterial communities was satisfied with organic sources derived from *Prochlorococcus* exudates because nutrient additions on top of the exudates did not result in higher cell-specific bacterial productivity nor in higher incorporation rates of ^{13}C-labelled carbon (Figs 1 and 2, also see the next paragraph). Despite similar bulk measurements

in both environments (Supplement 6), we observed lower cell-specific BP and higher $^{13}C/^{12}C$ ratios in the coastal community if compared to the open-ocean community (Figs 1 and 2). The lower cell-specific BP in the coastal community might partly be explained with the storm event that introduced sediment and soil bacteria to the water column. The re-suspension might have contributed significantly to our cell counts [at the start of the experiment (t0), the coastal community revealed approximately five times higher cell numbers compared to the open-ocean community (Fig. 1A and B)], but not to the cell-specific activity, lowering only the cell-specific BP. This assumption is supported by Raveh *et al*. (2015), where much lower bacterial counts ($\sim 5 \times 10^5$ cells ml^{-1} in January) but comparable bacterial bulk productions were determined for a coastal area in the Eastern Mediterranean Sea. The higher $^{13}C/^{12}C$ ratios in the coastal communities, on the other hand, appear surprising taking into account the storm event with possible input of carbon and contradict previous studies, where direct carbon coupling between phytoplankton and bacteria was weak in coastal waters due to substantial allochthonous carbon sources (Morán *et al*., 2002b). Yet, our findings suggest a preferential utilization of DOMp compared to other carbon sources, which fits with observations from freshwater bacterial communities that preferentially utilized phytoplankton-derived carbon over allochthonous, terrestrial one (Guillemette *et al*., 2016) and of coastal bacterial communities in the Mediterranean satisfying more than half of their carbon demand from phytoplankton exudates (Fouilland *et al*., 2014). This can be explained by the fact that pico-cyanobacteria (i.e. *Prochlorococcus* and *Synechococcus*) exudates contain high fractions of low-molecular-weight DOC, which is highly labile and can be rapidly utilized by heterotrophic bacteria (Bertilsson *et al*., 2005; Sharma *et al*., 2014), e.g. organic acids, organo-halogens and isoprene (Shaw *et al*., 2003; Bertilsson *et al*., 2005) as well as lipids, proteins and fragments of DNA and RNA (Biller *et al*., 2015). The strain *Prochlorococcus* MIT9312 used in our experiments releases ~90% of the fixed carbon as DOCp (Roth-Rosenberg *et al*., 2021a). Together, we did not find indications that allochthonous carbon and nutrients at the coastal site impacted the utilization of DOMp.

Incorporation of DONp and DOPp

Our NanoSIMS measurements, as well as APA analyses, suggest that the coastal and open-ocean bacterial communities cover their nitrogen and phosphorus demands via DOMp (Figs 2 and 3). This notion is in accordance with previous studies, showing that pico-cyanobacteria produce nitrogen and phosphorus-rich DOMp (Beliaev *et al*., 2014; Christie-Oleza *et al*., 2017) that serves as energy and also nutrient source for bacteria (Livanou *et al*., 2017). For example, bacteria exposed to phytoplankton exudates upregulated transcription of nitrogen and phosphorus utilization genes (McCarren *et al*., 2010), and co-cultures of picocyanobacteria with several different heterotrophic bacteria revealed an increased expression of genes for transporters of amino acids and peptides (Beliaev *et al*., 2014). It has been previously shown that *Prochlorococcus* MIT9312 exudes considerable amounts of organic nitrogen under laboratory conditions (Roth-Rosenberg *et al*., 2021a), possibly in the form of proteins, amino acids, DNA, RNA and nucleotides. This could provide labile DONp to the bacterial community (Sharma *et al*., 2014). In general, phytoplankton exudates provide manifold organic nitrogen species to heterotrophic bacteria, including urea, dissolved and free amino acids, proteins, nucleic acids, amino sugars (Meon and Kirchman, 2001; Berman and Bronk, 2003) and methylated amines (Lidbury *et al*., 2015), and so on. In co-culture and exudate addition experiments, especially dissolved free amino acids and urea were used by bacteria (Grossart and Simon, 2007; Bradley *et al*., 2010; Sarmento *et al*., 2013; Beliaev *et al*., 2014). Similar to nitrogen, phytoplankton exudates may offer substantial amounts of organic phosphorus in various forms (Livanou *et al*., 2017), and our results indicate that both bacterial communities appease their phosphorus demand with the provided phytoplankton exudates (Fig. 3).

A previous modelling study has suggested that, under conditions of N starvation, *Prochlorococcus* releases P-containing molecules such as nucleobases and nucleosides (Ofaim *et al*., 2021), although the magnitude of exudation of DOP is not well constrained (Roth-Rosenberg *et al*., 2021a). Increased APA in the control incubations of both environments indicates phosphorus depletion in these treatments, which is consistent with the low initial phosphorus concentrations and our 'batch-like' bottle incubations. Indeed, we could show in the open-ocean community a significantly lowered APA after exudate additions compared to the control + nutrient treatments, indicating not only a complete phosphorus supply by the exudates but a preferential utilization (Fig. 3).

However, organic N and P forms provided with the phytoplankton exudates seem to have a higher importance for the open-ocean community than for the coastal one: At time 0, cell-specific APA was approximately seven times higher in the open-ocean community compared to the coastal community (Fig. 3) [whereas bulk analyses showed comparable outcomes in both environments (Supplement 7)], suggesting a severe P limitation in the first, which was completely diminished with the addition of exudates. Likewise, correlations of C and N uptake revealed steeper slopes (i.e. relatively higher C

compared to N incorporation) in the coastal community, indicating preferential uptake of N by the open-ocean community (Table 1). The higher R^2 values of C to N correlations furthermore suggest a more uniform behaviour of the open-ocean community (Table 1). The ratios of ^{13}C to ^{15}N incorporations derived from NanoSIMS analyses in our experiments were in the same range as for phytoplankton associated bacteria in the Baltic Sea (Eigemann *et al*., 2019), and different ^{13}C to ^{15}N ratios in NanoSIMS measurements of different treatments suggest selective uptakes of either carbon or nitrogen.

In summary, our results strongly suggest that phytoplankton-derived organic nitrogen as well as phosphorus is incorporated into bacterial biomass and provide the primary nitrogen and phosphorus source for the coastal as well as open-ocean bacterial communities. Phytoplankton exudates seem to be a more important N and P source for the open-ocean communities compared to the coastal ones, where the latter may satisfy their N and P demand partly with allochthonous sources. Our experimental conditions, however, do not fully mimic natural conditions, for example, the added organic matter is at relatively high concentration, and comes from a single course (a single phytoplankton strain). Further research is required in order to determine whether these patterns also occur when considering naturally derived phytoplankton exudates in natural environments.

Community responses to exudate additions

In the open-ocean communities, as a reaction to exudate additions a decrease in Shannon diversity was obvious (Fig. 4), which was caused by the dominance of a single genus, namely, *Pseudoalteromona*s (Fig. 6, Supplement 6), whereas diversity in the coastal community remained constant, but with different abundant genera in the different treatments (Figs 4 and 6). In general, exudate additions boosted copiotrophic members of the heterotrophic bacterial community to the costs of oligotrophic genera (Fig. 5, Supplement 4). However, one should keep in mind that our analyses are based on rRNA, and thus only the active part of the bacterial community is appropriately reflected. Together, after exudate additions, *Pseudoalteromonas* in the open-ocean communities, and *Glaciecola*, *Psychrosphaera* and *Aureimarina* in the coastal communities revealed strong positive responses, whereas SAR11 showed negative responses to exudate additions, especially in the open-ocean community (Fig. 6, Supplements 3, 4, 5). *Pseudoalteromonas* and *Glaciecola* belong to the order Alteromonadales which significantly contribute to carbon cycling in the surface ocean (Pedler *et al*., 2014), possesses effective degradation systems for a wide array of polysaccharides (Gobet *et al*., 2018), and showed positive responses to phytoplankton exudates (Seymour *et al*., 2009; Taylor and Cunliffe, 2017). The responsive bacteria in our experiments possess a variety of carbohydrate-active enzymes (CAZymes). For example, *Glaciecola* sp. 4H-3-7YE-5 possesses the genomic possibility for the degradation of several polysaccharides (Klippel *et al*., 2011), which constitute major components of phytoplankton exudates (Meon and Kirchman, 2001; Mühlenbruch *et al*., 2018). Furthermore, cyanobacteria are known to produce glycogen as a storage polysaccharide (Bertocchi *et al*., 1990; Bhatnagar and Bhatnagar, 2019), and *Pseudoalteromonas*, *Psychrosphaera* as well as *Glaciecola* possess effective utilization systems for glycogen (Lombard *et al*., 2014), and other polysaccharides common in phytoplankton (Klippel *et al*., 2011; Pheng *et al*., 2017; Gobet *et al*., 2018). The negative responses of SAR11 related ASVs to exudate additions might be partly attributed to the point in time when *Prochlorococcus* spent medium was harvested, i.e. the early stationary phase (see Experimental procedures why this point in time was chosen). In co-culture experiments between several *Prochlorococcus* strains and SAR11 HTCC7211, stable long-term coexistence was maintained if *Prochlorococcus* was kept in the log-phase, but strong detrimental effects on SAR11 occurred when *Prochlorococcus* entered the stationary phase (Becker *et al*., 2019). These results were ascribed to growth phase–dependent releases of metabolites, where log-phase growing *Prochlorococcus* exudates fulfilled the central carbon requirement of SAR11, but *Prochlorococcus* metabolites from the stationary phase caused a rapid decline in SAR11 cell numbers. Simultaneously, however, sympatric copiotroph bacteria in co-cultures were boosted in cell numbers when *Prochlorococcus* entered the stationary phase, highlighting the higher functional and regulatory facilities of copiotrophs compared to oligotrophs such as SAR11 (Becker *et al*., 2019). Despite clear responses of RNA-based amplicon sequencing of specific genera to *Prochlorococcus* exudates in our experiments, we cannot relate these responses to specific demands of N, P, or C, because the used methods (BP, APA and isotope labelling) focused on bulk reactions. Nevertheless, we could demonstrate the relative increase/decrease of specific genera, implying niche partitioning within the bulk community.

We observed higher discrepancies and variability between the control and the control + nutrient samples in the attached fraction of the coastal community compared to the free fraction and the open-ocean samples (Fig. 5). The higher discrepancies between the control and control + nutrient samples in the attached fraction might be an indirect effect of the storm event with bacteria colonizing introduced particles, whose utilization is fuelled by

the addition of inorganic nutrients, whereas in the open-ocean the necessary carbon for a boost effect of inorganic nutrients is just lacking. The higher variability in the attached fraction might also be an indirect effect of the storm event and may represent more chaotic communities attached to re-suspended and newly introduced particles. However, we can only speculate on this because we did not perform analyses confirming the above-raised hypothesis. Despite considerable overlap in the bacterial communities at t0 in coastal and open-ocean environments (Supplement 2), different responders to exudate additions appeared at the contrasting sites (Fig. 5, Supplements 3, 4, 5). Below, we list three possible explanations for the different development after exudate additions: First, the source communities differed although several abundant members overlapped. Indeed, *Pseudoalteromonas*, the main responder in the open-ocean treatments, showed even higher relative read abundances at t0 in the coastal community (mean subsampled read-sums of *Pseudoalteromonas* ASVs in t0 samples: five for open-ocean and 19 for coastal communities, Supplement 8), but the most responsive genera of the coastal community were completely lacking in the open-ocean community (*Psychrosphaera*, *Aureimarina*, Supplement 8) or present at low abundances (*Glaciecola*, Supplement 8). This outcome suggests a high importance of the bacterial source communities on the effectiveness in DOC utilization as well as the ability to use different DOC sources (Carlson *et al*., 2004; Grossart *et al*., 2007). Second, environmental conditions favour certain bacterial genera over others (Nemergut *et al*., 2013; Wu *et al*., 2019), which may reflect the ability to effectively utilize DOMp. Thus, under open-ocean conditions paired with the additions of DOMp, *Pseudoalteromonas* was the most successful genus that outcompeted other genera, whereas under coastal conditions it was outcompeted, despite its higher relative abundances at t0. Our data suggest that other members in the open-ocean community were not able to show such a strong response in the 24 h of incubation, and Alteromonadales are known as opportunists (Eilers *et al*., 2000) and fast growers (Pedler *et al*., 2014). We did not assess the effect of bacterivorous protists on either of the two communities which may affect bacterial abundances and communities after nutrient additions (Lebaron *et al*., 2001). However, the overall effect of grazers on bacterial community composition in Mediterranean mesocosm experiments was not significant (Baltar *et al*., 2016), and it is unlikely that different grazing pressures affected the differential outcomes of our experiments (Baltar *et al*., 2016). Third, the dominant response of the copiotrophic generalist *Pseudoalteromonas* (Gammaproteobacteria) in the open-ocean environments may be explained with drastic changes in relative DOM concentrations, whereas in the coastal environment additions of DOM induced more specialists responses (Sarmento *et al*., 2016). Absolute DOC concentrations are an important factor for the uptake ability of heterotrophic bacteria, as only a few specialists were able to incorporate DOCp at low concentrations, but a broader range of bacteria could use the same source of DOCp at high concentrations (Sarmento *et al*., 2016). This might be also true in our experiments, where the background DOC concentrations probably have been much higher in the coastal (especially after the storm event) compared to the open-ocean environment.

Besides specific outcomes at the genus level, some general patterns occur under phytoplankton bloom conditions, with Flavobacteria, Alphaproteobacteria and Gammaproteobacteria being dominant classes (Buchan *et al*., 2014). This is partly reflected in our experiments (Fig. 6), with especially Gammaproteobacteria (*Pseudoalteromonas*, *Psychrosphaera*, *Glaciecola*) and Flavobacteria (*Aureimarina*) showing strong positive responses to exudate additions, whereas we did not observe positive responses but rather relative declines of Alphaproteobacteria (Supplement 4). This relative decline of Alphaproteobacteria as response to phytoplankton exudates partly contradicts previous studies, where especially the Roseobacter clade (class Alphaproteobacteria) reveals numerous positive interactions with phytoplankton (Romera-Castillo *et al*., 2011; Lidbury *et al*., 2015). However, despite being capable of using a wide array of substrates (Lidbury *et al*., 2015), Roseobacter did not take up *Prochlorococcus* exudates in a similar experiment (Sarmento and Gasol, 2012), emphasizing the selective uptake of DOMp by different bacteria (Sarmento *et al*., 2013).

A point not addressed in our experimental set-up is the successive degradation from labile to recalcitrant DOM. DOMp utilization by heterotrophic bacteria undergoes a succession with different responsive organisms at different times after DOMp pulses (Teeling *et al*., 2012; Buchan *et al*., 2014; Teeling *et al*., 2016). Oligotrophic organisms like SAR11 utilize highly labile low-molecular-weight compounds, whereas copiotrophic bacteria such as Alteromonadales utilize high-molecular-weight compounds such as polysaccharides (Sharma *et al*., 2014). With our experiments, we only reflect the community response at a single time-point, i.e. 24 h after DOMp addition, which may resemble daily changes in DOMp concentrations accompanied with photosynthesis during phytoplankton blooms. Furthermore, as mentioned above, *Prochlorococcus* exudes high amounts of labile DOMp, which can be rapidly utilized by heterotrophic bacteria (Roth-Rosenberg *et al*., 2021a). Thus, the concentration of the added DOC as well as the incubation time allow for a maximal comprehensive picture (for a

single pulse), and thus should reflect responses of oligotrophic as well as copiotrophic community members at environmental relevant concentrations (McCarren *et al*., 2010; Seymour *et al*., 2010; Sarmento and Gasol, 2012; Sharma *et al*., 2014; Beier *et al*., 2015; Sarmento *et al*., 2016). However, to fully comprehend the dynamics of DOCp utilization and the community succession additional time-course studies are needed.

Conclusions

Short-term responses of coastal and open-ocean bacterial communities to phytoplankton exudates addition with and without inorganic nutrients revealed similar overall bacterial response patterns, but different responders in the coastal versus the open ocean communities as well as a higher importance of N and P provided with the exudates for the open-ocean community. The different responders suggest environmental factors, such as the ambient DOM concentrations or the initial bacterial communities to determine DOMp utilization effectiveness of the respective bacterial communities to some extent. Our results strongly indicate that the allocated phytoplankton exudates provide a major fraction of the bacterial community (~50%) with organic carbon, nitrogen and phosphorus as combined additions of exudates and inorganic nutrients neither enhance cell-specific BP nor lowered incorporation of DONp and cell-specific APA. Utilization of DOCp seems not to be limited by inorganic nutrients, because addition of inorganic nutrients did not elevate the incorporation of DOCp into bacterial cells. Consequently, phytoplankton exudates may function as a full-fledged meal for the accompanying bacterial communities, and can be used as energy and nutrient sources by bacteria independent of the surrounding inorganic nutrient concentrations. *Prochlorococcus* is a major component of the global phytoplankton (Flombaum *et al*., 2013), and its exudates likely substantially contribute to BP in oligotrophic environments (Biller *et al*., 2015; Ribalet *et al*., 2015). Hence, together with the naturally occurring amount of added DOM (in bloom occasions) (Sharma *et al*., 2014; Beier *et al*., 2015), our results may reflect important patterns in marine environments. Together, our study emphasizes the dependency of heterotrophic bacteria on phytoplankton exudates and illustrates that abiotic factors may resign beyond biotic interactions for marine heterotrophic bacteria. Therewith, our outcomes further strengthen the importance of phytoplankton–bacteria interactions in carbon, nutrient and mineral cycling, and thus in functioning of marine ecosystems.

Experimental procedures

Experimental set-up

Prochlorococcus MIT9312 was grown in 2 L bottles under constant light (20 μE m^{-2} s^{-1}) at 22°C in Pro99 media where the NH_4 concentration was reduced from 800 to 100 μM, resulting in the cells entering stationary stage due to N starvation (Grossowicz *et al*., 2017). For several generations before harvest, 98% labelled ^{15}N-NH_4 was used as the sole N source, and the media was amended with 1 mM of 98% labelled ^{13}C-HCO_3 as a C source, resulting in a fully labelled culture. To obtain cell-free exudates, batch cultures were harvested at the early decline phase by centrifugation followed by filtration through a 0.22 μm polycarbonate filter. Early stationary phase was chosen to minimize the carryover of ^{15}N-NH_4 (which should be depleted from the media, see below), to increase the amount of released DOC (Roth-Rosenberg *et al*., 2021a), and because spent media from this stage may reflect natural DOMp composition better than DOMp from exponentially growing cultures (Christie-Oleza *et al*., 2017). We note that the DOM in the media is likely a result of both exudation and cell mortality. The spent media was maintained at −20°C until use.

Using the ^{15}N and ^{13}C labelled spent media we performed two incubation experiments in order to test the bacterial response to DOMp in coastal (Exp 1) compared to open-ocean (Exp 2) systems. Each of these experiments included the spent media with and without inorganic nutrient additions of 20 μM NH_4, and 2 μM PO_4, to eliminate nutrient limitation in the 24 h of exposure in the nutrient spiked treatments (Table 2). Exp 1 was carried out in the dark on January 9, 2019, in 1 m^3 natural seawater flow-through tanks to maintain ambient temperature at the Israel Oceanographic and Limnological Research centre in Haifa, Israel. Each treatment consisted of four biological replicates. The coastal site was a 5 m intake pipe during a winter storm with high waves

Table 2. Summary of the exudate and nutrient additions to surface Eastern Mediterranean Seawater in January 2019.

Ingredient/treatment	Control	Control + nutrients	Exudates	Exudates + nutrients
Prochlorococcus MIT9312 exudates	–	–	25 μM C	25 μM C
Nutrients	–	20 μM NH_4, 2 μM PO_4	–	20 μM NH_4, 2 μM PO_4
^{15}N NH_4	5 nM	5 nM	–	–

Values shown are the final concentration.

and significant turbulence. Additionally, heavy rainfalls caused considerable land-to-sea run-offs. As a result, the water was brownish in colour, which may have added allochthonous material, as well as soil and sediment bacteria. Exp 2 was carried out on board of the R/V Mediterranean Explorer on January 23, 2019, at station THEMO-2 (Reich *et al*., 2021), in an on-board flow-through system, and each treatment consisted of three biological replicates. For both experiments, 4.5 L Nalgene bottles were filled with 3 L of 50 μm pre-filtered seawater with the respective DOCp and nutrient amendments (Table 2). In order to receive a clear response of the bacterial community in the range of naturally occurring concentrations of DOC, we chose the addition of 25 μM DOCp (Seymour *et al*., 2010; Beier *et al*., 2015; Sarmento *et al*., 2016). Likewise, the incubation time was set to 24 h, in order to see a meaningful response of both, fast and slow responsive bacteria to phytoplankton exudates at the above-mentioned DOC concentration (McCarren *et al*., 2010; Sarmento and Gasol, 2012; Sharma *et al*., 2014; Beier *et al*., 2015). The spent *Prochlorococcus* medium contained 0.9 μM PO_4 and 0.16 μM NH_4, resulting in a final concentration of 5 nM $^{15}NH_4$ in the exudate treatments (32× diluted). We accounted for these labelled, inorganic leftovers in the control and control + nutrient treatments in order to differentiate between uptake of DOMp and inorganic nutrients by the bacterial communities in our NanoSIMS measurements (Table 2).

Nutrient measurements

For measurements of inorganic nutrients, samples were pre-filtered through 0.2 μm pore width filters and collected in acid-clean plastic vials. Dissolved nutrients in the cultures and for Exp. 1 and 2 were then determined using a three-channel segmented flow auto-analyser system (AA-3 Seal Analytical).

Cell numbers

Bacterial cell numbers were determined by flow cytometry. Briefly, duplicate samples were fixed with cytometry-grade glutaraldehyde (0.125% final concentration), flash-frozen with liquid nitrogen and stored at −80°C until analyses. For measurements, samples were thawed in the dark at room temperature, stained with SYBR Green I (Molecular Probes/Thermo Fisher) for 10 min at room temperature, vortexed, and run on a BD FACSCanto™ II Flow Cytometry Analyser Systems (BD 146 Biosciences) with 2 μm diameter fluorescent beads (Polysciences, Warminster, PA, USA) as a size and fluorescence standard. Bacterial cells were detected at Ex494nm/Em520nm (FITC channel) and by the size of cell (forward scatter). Phytoplankton cells were identified based on their cell chlorophyll (Ex482nm/Em676nm, PerCP channel) and by the size of cell (forward scatter). Flow rates were determined several times during each running session by weighing tubes with double-distilled water. Finally, the data were analysed with the free software 'Flowing Software' (https://bioscience.fi/).

Bacterial production

The activity of heterotrophic production was measured using the [4,5-^{3}H]-leucine incorporation method (Simon *et al*., 1990). To this end, triplicate 1.7 ml of seawater samples were collected from each microcosm bottle and incubated with a 7:1 mixture of 'cold' leucine and 'hot' ^{3}H-leucine respectively, at a final concentration of 100 nmol leucine L^{-1} (Perkin Elmer, specific activity 156 Ci $mmol^{-1}$) for 4 h in the dark under ambient surface seawater temperature (∼19°C). Incubations were stopped by the addition of 100 μl ice-cold 100% trichloroacetic acid. Next, the samples were briefly spun with a desk-centrifuge and 1 ml of scintillation cocktail (Ultima-Gold) was added to each vial. Disintegrations per minute were measured using a TRI-CARB 2100 TR (Packard) liquid counter. A conversion factor of 1.5 kg C mol^{-1} per mol leucine was used with an isotope dilution factor of 2 (Simon and Azam, 1989).

Alkaline phosphatase activity

APA was determined by the 4-methylumbelliferyl phosphate (MUF-P: Sigma M8168) method according to Thingstad and Mantoura (2005). Substrate was added to triplicate 1 ml water samples (final concentration of 50 μM) and incubated in the dark at ambient temperature for 4 h (same as BP). The increase in fluorescence by the cleaved 4-methylumbelliferone (MUF) was measured at 365 nm excitation, 455 nm emissions (GloMax®-Multi Detection System E9032) and calibrated against a MUF standard (Sigma M1508).

NanoSIMS analyses

Before analyses, filter pieces were covered with approximately 30 nm gold in a sputter coater (Cressington108 auto-sputter coater). SIMS imaging was performed as described in Eigemann *et al*. (2019) using a NanoSIMS 50 L instrument (Cameca, France). The scanning parameters were 512 × 512 px for areas of 20–30 μm, with a dwell time of 250 μs per pixel and a primary beam of 1 pA. All NanoSIMS measurements were analysed with the Matlab based program look@nanosims (Polerecky *et al*., 2012). Briefly, the 60 measured planes were checked for inconsistencies, all usable planes accumulated, regions of interest (i.e. bacterial cells) defined based on $^{12}C^{14}N$ mass pictures and $^{13}C/^{12}C$ as well as $^{15}N/^{14}N$ ratios calculated from the

ion signals for each region of interest. For analyses of each measurement, first the means of background measurements were determined (i.e. regions on the filter without bacterial cells), and this mean was factorized for theoretical background values (0.11 for $^{13}C/^{12}C$ and 0.00367 for $^{15}N/^{14}N$). These factors were applied to all non-background regions of interest in the same measurement. For each treatment, measurements of different spots on the same filter as well as replicate filters (two replicates for each treatment) were pooled.

RNA extraction, DNA digestion, cDNA synthesis

For RNA extraction, approximately 1.5 L of each incubation bottle was filtered successively onto 5 and 0.2 μm pore width polycarbonate filters (Exp 1) or directly onto 0.2 μm filters (Exp 2). Filters were stored in 1 ml lysis buffer (40 mM EDTA, 50 mM Tris pH 8.3, 0.75 M sucrose), flash-frozen in liquid nitrogen and stored at −80°C upon extraction, approximately 3 months after the experiments. For extraction, filters were cut into half with scissors and placed in Eppendorf tubes. Then, 1 ml TRI Reagent was added (to one half of a filter), filters were vortexed and incubated for 10 min at room temperature on an orbital shaker with 55 rpm. Then, 200 μl of chloroform was added, and filters were vortexed again and incubated for 15 min at room temperature. Following, the tubes were centrifuged at 12 000*g* for 12 min at 4°C, the supernatant was transferred to fresh tubes, centrifuged again as described above, and the aqueous phase was transferred to fresh tubes. Next, 1 ml ice-cold 100% ethanol was added, vortexed and incubated for 1 h on ice, centrifuged at 12 000*g* for 10 min, the supernatant was discarded, 1 ml ice-cold 75% ethanol was added, tubes were vortexed, and incubated for 10 min at −20°C. Last, tubes were centrifuged for 5 min at 7500*g*, the ethanol removed, and the RNA pellet air-dried in a hood. The resulting pellet was resolved in autoclaved DEPC-treated water and quality controlled with a Nanodrop device. Remaining DNA was digested using the Turbo DNA free kit (Invitrogen) using the manufacturer's instructions, and successful digestions were tested by PCRs using primers com1f and com2rph (Schwieger and Tebbe, 1998), with initial denaturation at 94°C for 3 min, 30 cycles of denaturation at 94°C for 1 min, annealing for 1 min at 50°C and elongation for 90 s at 72°C, finalized by elongation at 72°C for 4 min. RNA was transcribed into cDNA using MultiScribe reverse transcriptase following the manufacturer's instructions (Invitrogen).

Sequencing

Complementary DNA was PCR amplified with primers 515F and 926R (Walters *et al*., 2016) targeting the V4 and V5 variable regions of the microbial small subunit ribosomal RNA gene using a two-stage 'targeted amplicon sequencing' protocol (Naqib *et al*., 2018). Primers were modified to include linker sequences at the 5′ ends (i.e. so-called 'common sequences' or CS1 and CS2 on forward and reverse primers respectively). First stage PCR amplifications were performed in 10 μl reactions in 96-well plates, using the MyTaq HS 2× mastermix (BioLine, Taunton, MA, USA). PCR conditions were 95°C for 5 min, followed by 28 cycles of 95°C for 30″, 50°C for 60″ and 72°C for 90″. Subsequently, a second PCR amplification was performed in 10 μl reactions in 96-well plates. A mastermix for the entire plate was made using the MyTaq HS 2× mastermix. Each well received a separate primer pair with a unique 10-base barcode, obtained from the Access Array Barcode Library for Illumina (Fluidigm, South San Francisco, CA, USA; Item# 100-4876). These Access Array primers contained the CS1 and CS2 linkers at the 3′ ends of the oligonucleotides. Cycling conditions were as follows: 95°C for 5 min, followed by 8 cycles of 95°C for 30″, 60°C for 30″ and 72°C for 30″. A final, 7-min elongation step was performed at 72°C. Samples were pooled in equal volume using an EpMotion5075 liquid handling robot (Eppendorf, Hamburg, Germany). The pooled library was purified using an AMPure XP cleanup protocol (0.6×, vol./vol.; Agencourt, Beckman-Coulter) to remove fragments smaller than 300 bp. The pooled libraries, with a 20% phiX spike-in, were loaded onto an Illumina MiniSeq mid-output flow cell (2 × 150 paired-end reads). Based on the distribution of reads per barcode, the amplicons (before purification) were re-pooled to generate a more balanced distribution of reads. The re-pooled library was purified using AMPure XP cleanup, as described above. Next, the re-pooled libraries, with a 15% phiX spike-in, were loaded onto a MiSeq v3 flow cell and sequenced using an Illumina MiSeq sequencer. Fluidigm sequencing primers, targeting the CS1 and CS2 linker regions, were used to initiate sequencing. Library preparation, pooling and MiniSeq sequencing were performed at the University of Illinois at Chicago Sequencing Core (UICSQC), Research Resources Center (RRC), University of Illinois at Chicago (UIC). All forward and backward sequence reads were deposited at the European Nucleotide Archive under the accession number PRJEB44710.

Sequence analyses

All sequences were analysed using the Dada2 (Callahan *et al*., 2016) pipeline and the software packages R (R Development Team, 2020) and RStudio (RStudio Team, 2020). Briefly, forward and backward primers were trimmed, forward and backward reads truncated after quality inspections to 280 and 210 bases respectively,

and after merging of forward and backward sequences, a consensus length only between 404 and 417 bases was accepted. For taxonomic assignment, Silva database version 138 (Quast *et al*., 2013) was used. All chloroplasts, mitochondria, archaea, eukaryotes and amplicon sequence variants (ASVs) without any taxonomic affiliation were discarded from downstream analyses. The complete ASV table with absolute read counts, sequences, sequence lengths as well as metadata for all samples is accessible as Supplement 9. After inspections of rarefaction curves, all samples with <2509 reads were discarded from further analyses, and all remaining samples subsampled to 2509 reads.

Statistical analyses

Bacterial communities were analysed with Shannon diversities, non-metric-multidimensional-scaling (NMDS) and heatmaps of the most abundant ASVs. NMDS was performed using the 'metaMDS' command and the Bray distance in the 'vegan' package (Oksanen *et al*., 2013) in order to analyse differences between the communities based on the subsampled absolute ASV tables. An ANOSIM, which tests for significant differences between communities, was conducted using the 'vegan' package. Heatmaps were calculated separately for both experiments using the 40 most abundant ASVs (i.e. the ASVs with the highest sum of subsampled reads for all samples in the same experiment) and the heatmap 3 package (Zhao *et al*., 2014). Homogeneities of variances were tested by Bartlett or Levene's (Shannon diversity) tests. Differences between the different treatments in terms of Shannon diversity, incorporation of ^{13}C and ^{15}N, cell numbers and BP were tested by ANOVAs with subsequent Tukey *post hoc* tests if homogeneity of variances was given. If homogeneity was not given, Kruskal–Wallis tests were calculated, with subsequent Tukey–Nemenyi *post hoc* tests. For all analyses, significance was assumed for p values <0.05. To test for specific ASVs associated with treatments, LDA effect size (LEfSe) analyses (Segata *et al*., 2011) were executed with the online tool https://huttenhower.sph.harvard.edu/galaxy. For the open-ocean community, treatments were assigned as class without subclass, whereas for the open-ocean community, treatments were assigned as class, and the fraction (free and attached) assigned as subclass. For this multi-class analysis, a 'one-against-all' strategy was applied. All analyses, except LEfSe were performed with R (R Development Team, 2020) and RStudio (RStudio Team, 2020), and all graphics were executed with the ggplot2 package (Ginestet, 2011), and refined with the freeware Inkscape (https://inkscape.org).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1: Supporting Information.