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Substrate diversity affects carbon utilization rate and threshold concentration for uptake by natural bacterioplankton communities

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ABSTRACT: Persistence of dissolved organic matter (DOM) in aquatic environments may in part be explained by high diversity and low concentrations of carbon substrates. However, changes in dissolved substrate quality can modify aquatic bacterial community composition and rate of carbon uptake. The aim of this study was to test if the presence of multiple simple substrates affects the turnover of organic carbon. Natural bacterial communities were grown in continuous cultures supplied with either individual carbon substrates — salicylic acid (SA), tryptophan (Trp) or tyrosine (Tyr) — or a combination of the 3 substrates. Concentrations were tracked using fluorescence spectroscopy, and steady-state concentrations of a few nanomolar were reached. Bacterial growth efficiency was dependent on which carbon sources were present and reached an intermediate level in the combined treatment. The bacterial community maintained steady-state concentrations of Trp that were lower in the combined treatment than in the individual substrate treatment. In addition, steady-state concentrations were reached faster during growth on combined carbon substrates, although the maximum utilization rate of each individual compound was lower. However, the steady-state concentration of total carbon (sum of carbon content of SA, Trp and Tyr) was higher in the combined culture than in the individual substrate treatments, and seemed to be determined by the carbon substate for which the bacteria had the lowest affinity. The results from this study indicate that persistence of dissolved organic carbon can in part be explained by vast substrate diversity, which raises the threshold concentration for utilization by natural bacterial communities.

KEY WORDS: Carbon utilization · Chemical diversity · Continuous culture · Marine bacterial community · Excitation emission matrix spectroscopy · Steady-state concentration · Growth efficiency

1. INTRODUCTION

Dissolved organic carbon (DOC) represents a large reservoir of reduced carbon in aquatic environments (Cole et al. 2007, Hansell 2013) which is comparable in size to CO_2 in the atmosphere (860 Pg C) (Hansell 2013, Friedlingstein et al. 2019) and orders of magnitude higher than the carbon of marine biomass (Hansell 2013). The link between detrital organic matter and microorganisms represents one of the largest fluxes of carbon (Cole 1999) and in order to comprehend aquatic carbon cycling and sequestration it is important to understand which factors regulate microbial carbon cycling in those environments. Microbial utilization of DOC can be influenced by many factors, including environmental conditions, microbial community structure, molecular properties and the concentrations of dissolved molecules (Jiao et al. 2014, Carlsson & Hansell 2015, Dittmar 2015). In addition, it has been proposed that the molecular diversity of organic compounds, rather than the intrinsic properties of individual compounds, might

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control decomposition (Lehmann et al. 2020). Processing of organic matter requires extracellular enzymes for microbial uptake and metabolism, and producing enzymes is an energetically costly process (Vetter et al. 1998). A greater diversity of molecules can therefore lead to increased cost of metabolism and limit decomposition (Lehmann et al. 2020, Dittmar et al. 2021). However, molecular diversity has also been suggested to increase decomposition rates, with the utilization of one compound providing the resources required to decompose other compounds, a process often referred to as the priming effect (Lohnis 1926, Guenet et al. 2010, Bianchi 2011).

The utilization of mixtures of carbon substrates has been intensively studied under batch culture conditions at high initial concentrations (g l^{-1}). When microorganisms are grown under such conditions, they frequently exhibit diauxic growth, where initially only the substrate which supports the highest growth rate is utilized and the consumption of poorer substrates remains repressed (Harder & Dijkhuizen 1982). During diauxic growth there is a trade-off between the length of the lag phase and the long-term growth rate of the cell, which is related to the maintenance cost of the sensing apparatus for external nutrients. It is predicted that stable environments favor strains that grow fast, whereas in environments where the nutrient composition changes frequently, it will be more important to avoid delays due to long lag phases (Chu & Barnes 2016). However, in contrast to diauxic growth, a wide range of microorganisms are able to simultaneously utilize mixtures of substrates that serve the same physiological function (Harder & Dijkhuizen 1982, Wanner & Egli 1990). In batch cultures, simultaneous utilization of carbon substrates has been observed when initial substrate concentrations are low or when combinations of carbon substrates support only medium to low maximum specific growth rates (Egli et al. 1993).

The first experimental evidence for an influence of the simultaneous utilization of mixtures of carbon substrates on kinetics of growth was reported in a study where a *Corynebacterium* strain was grown in carbon-controlled chemostat cultures with various mixtures of glucose and amino acids at a constant growth rate. The steady-state concentration of glucose was lowered in the presence of amino acids (Law & Button 1977). Several subsequent studies confirmed that the steady-state concentration of a particular substrate is reduced during mixed substrate growth conditions (Egli et al. 1986, Babel et al. 1993), both for different isolates and on different combinations of carbon substrates (Egli 2010). The most extensively studied example of growth with mixed substrates is *Escherichia coli* grown in carbon-limited chemostat cultures with defined mixtures of up to 6 different sugars (Lendenmann et al. 1996). All of the sugars were utilized simultaneously and the results showed that steady-state concentrations were always lower during growth with sugar mixtures than during growth with a single sugar (Egli et al. 1993, Lendenmann et al. 1996). In addition, the reduction in steady-state concentrations was roughly proportional to their ratio in the medium (Egli et al. 1993, Lendenmann et al. 1996).

Although mixed substrate growth has been observed in many different microorganisms and combinations of carbon substrates, it has never been tested in natural complex bacterial communities (Egli et al. 1983, Lendenmann et al. 1996, Kovárová et al. 1996–1997, 1997). However, it has been suggested that the ability to simultaneously utilize many of the available carbon compounds is a crucial factor for the efficient and fast growth of microorganisms in natural systems. It has also been suggested that the reduction in threshold concentration (lowest concentration for utilization) of individual carbon substrates during growth on mixtures of carbon substrates is a general principle for heterotrophic microorganisms (Lendenmann et al. 1996). The natural environment differs greatly from cultivation under controlled conditions. In aquatic environments heterotrophic bacteria are exposed to a pool consisting of hundreds or thousands of organic compounds with individual concentrations that are extremely low, typically much less than nanomolar (Dittmar 2015). In addition, natural bacterial communities consist of a mixture of species and their composition is a direct reflection of the composition and availability of substrates for microbial growth (Brock 1987). Since bacterial species are adapted to growth on different carbon compounds and at different concentrations (Covert & Moran 2001), it could be expected that the bacterial community can always utilize each carbon substrate down to the same steady-state concentration.

In this study we used continuous cultures to investigate how carbon utilization was affected by diversity of carbon substrates. The hypothesis is that the persistence of dissolved organic matter (DOM) in aquatic environments may in part be explained by the high diversity and low concentrations of carbon substrates. Natural bacterial communities were grown on either single or mixtures of carbon substrates. Using fluorescence spectroscopy, we were able to easily follow the concentration of each of the carbon sources over time at nanomolar concentrations. The results show

that substrate diversity affects both utilization rate and threshold concentration for utilization.

2. MATERIALS AND METHODS

2.1. Experimental set-up

A chemostat experiment was set up to investigate the utilization of a selection of carbon substrates by natural bacterial communities. The experiment consisted of 12 chemostat cultures (4 treatments in triplicate) with a bacterial community sampled from the Skagerrak (a bulk water sample of several hundred m^3 used for plankton culturing at DTU Aqua). The medium consisted of a mix of aged seawater and artificial seawater which was supplemented with different combinations of naturally fluorescent carbon substrates. The chemostat cultures were run with a dilution rate of 0.04 h^{-1}. The concentrations of the carbon substrates were followed over time using excitation emission matrix (EEM) spectroscopy and bacterial abundances were determined by flow cytometry. The experiment was run for 14 d. Samples for bacterial abundance and fluorescence were taken from the cultures and medium every day (except Days 0, 11 and 13). Samples for bacterial community composition were taken every second day from the cultures.

2.2. Chemostat cultures

Ten liters of medium was prepared for each chemostat. The medium was a mixture of Atlantic seawater and artificial seawater. The Atlantic seawater had been stored at room temperature for several years and therefore the labile DOM had already been utilized, resulting in a low background DOC (~60 µM). The seawater was filtered through a 0.22 µm filter using a peristaltic pump. Artificial seawater with a salinity of 35‰ was prepared and mixed 1:1 with Atlantic seawater. The medium was autoclaved and stored at room temperature until the start of the experiment. The carbon substrates as well as nitrogen (as NH_4Cl) and phosphorus (as KH_2PO_4) were added to the autoclaved medium at the start of the experiment. A total concentration of 1 µM carbon substrate was added and nitrogen and phosphorus were added at 5 times excess of that required for marine heterotrophic bacteria, according to the cell C/N/P ratios (Jover et al. 2014).

Four treatments were run in triplicate: (1) seawater supplemented with 1 µM salicylic acid (SA); (2) seawater with 1 µM tryptophan (Trp); (3) seawater with 1 µM tyrosine (Tyr); and (4) a combination of seawater with 0.33 µM SA, 0.33 µM Trp and 0.33 µM Tyr. The 3 carbon compounds were selected because they fulfilled 3 criteria: they all have a high molar fluorescence, their fluorescence signals can easily be separated using parallel factor (PARAFAC) analysis (Wünsch et al. 2015) and they are all natural carbon substrates in marine systems. Labile compounds, such as amino acids and glucose, are known to account for up to 10–30% of the daily organic carbon uptake by bacteria (Kirchman 2003). In addition, UVA fluorescence is often detected in oceanic samples and has been shown to be correlated with the presence of Tyr and Trp (Yamashita et al. 2015). SA is an important signaling molecule in plants (Wildermuth et al. 2001, Rivas-San Vicente & Plasencia 2011) and is excreted by diatoms, such as the ubiquitous diatom *Asterionellopsis glacialis* (Shibl et al. 2020). Microbial communities have been widely shown to utilize and benefit from diatom excretions that accumulate within the microenvironment surrounding phytoplankton cells (Shibl et al. 2020).

The inoculum originated from the bulk seawater sample taken from the Skagerrak and stored for general plankton cultivation at the Technical University of Denmark. The water had a salinity of approximately 28 and was filtered through pre-combusted 0.6 µm filters. Each chemostat was started with 400 ml of inoculum, which was diluted to 500 ml by the incoming medium. The medium was fed in drops into the culture through a glass tube to prevent back growth (Hagström et al. 1984). Air was passed through a 0.2 µm pore size PTFE Acrodisc CR filter (Pall) and used to force the inflow into the cultures to prevent a water column from building up in the glass tube. A gentle flow of air was maintained to allow bubbles to be continuously produced and to stir the culture (Hagström et al. 1984, Zweifel et al. 1996).

2.3. Fluorescence measurements

Samples for fluorescence measurements were filtered through 0.2 µm filters and collected in acid washed and pre-combusted 40 ml glass vials with Teflon-lined caps. Fluorescence EEMs were measured in a 1 cm quartz cuvette using a HORIBA Jobin Yvon Aqualog fluorometer. Fluorescence emission wavelengths were 240 to 600 nm (increment 2 nm) and excitation wavelengths were 240 to 600 nm (increment 5 nm). Absorption spectra were collected simultaneously at wavelengths equal to the excita-

tion. EEMs were corrected for inner filter effects and for Raman and Rayleigh scattering. All data processing was carried out using the drEEM toolbox (version 0.6.3, Murphy et al. 2013) in Matlab (MathWorks). The EEM correction process consisted of the application of excitation and emission correction factors (supplied by the manufacturer), a blank EEM subtraction, scatter excision, correction for inner filter effects, and normalization to Raman Units (RU). The resulting corrected dataset was then subjected to PARAFAC analysis with the drEEM toolbox (n = 347).

2.4. Bacterial abundance and bacterial growth efficiency

Samples for bacterial abundance (BA) were fixed with glutaraldehyde (1% final conc.) and stored at −80°C. Bacteria were stained with Sybr Green 1 (Invitrogen) and counted on a FASCanto II flow cytometer (Becton Dickinson) (Troussellier et al. 1999, Gasol & del Giorgio 2000) using fluorescent beads (Trucounts, Becton Dickinson) to calibrate the flow rate.

Eq. (1) describes the dynamics of substrate concentrations in a chemostat.

$$\frac{\mathrm{d}c}{\mathrm{d}t} = D \times (C_\mathrm{m} - C_\mathrm{c}) - S\mu \times \frac{W}{V} \quad (1)$$

where C_m is the substrate concentration in the medium, C_c is the concentration in the culture vessel; $C_\mathrm{m} - C_\mathrm{c}$ therefore represent substrate uptake. D is the dilution rate (flow divided by volume of culture). W/V is the bacterial biomass concentration in the culture vessel, μ is the relative growth rate and the factor S describes the stoichiometry of the conversion of substrate into biomass. S (substrate uptake, nmol per cell) was calculated for each treatment and converted into bacterial growth efficiency (BGE) based on a constant carbon content per cell of 20 fg cell^{-1} (1.6×10^{-15} moles carbon cell^{-1}) (Lee & Fuhrman 1987). The use of a constant carbon content per cell might introduce uncertainty in the data since estimates of carbon content per cell vary between locations and depths (Buitenhuis et al. 2012). However, we believe that this has minor effect in our experiment since the bacteria in all treatments originate from the same inoculum (location).

2.5. Bacterial community composition

Water (200 ml) was filtered through 0.2 μm pore size Supor filters (25 mm; Pall) to collect bacterial biomass for DNA extraction. The filters were stored at −20°C until extraction. DNA was extracted using a Qiagen Power Soil kit.

DNA was amplified using the 16S V3 (341F) forward and V4 (805R) reverse (Herlemann et al. 2011) primer pairs with added Illumina adapter overhang nucleotide sequences (Zheng et al. 2015). Amplicon synthesis was performed using thermocycling with 2 μl of genomic DNA, 2 μl of amplicon PCR forward primer (0.8 μM), 2 μl of amplicon PCR reverse primer (0.8 μM), 1 μl BSA (0.8mg ml^{-1}), 5 μl 5× buffer, 0.5 μl dNTP (0.2 mM), 0.5 μl Phusion (0.04 units μl^{-1}) and 12 μl PCR-grade water at 98°C initial denaturation for 45 s, followed by 29 cycles of 98°C for 10 s, 55°C for 20 s, and 72°C for 30 s, and a final extension at 72°C for 8 min.

Attachment of dual indices and Illumina sequencing adapters was performed using 2 μl of amplicon PCR product DNA, 3 μl of Illumina Nextera XT Index Primer 1 (N7xx), 3 μl of Nextera XT Index Primer 2 (S5xx), 0.25 μl of BSA (0.25 mg ml^{-1}), 5 μl 5× buffer, 0.5 μl dNTP (0.25 mM) and 0.25 μl Phusion (0.025 units μl^{-1}) and 6 μl of PCR-grade water with thermocycling at 98°C for 45 s, followed by 8 cycles of 98°C for 10 s, 55°C for 30 s, and 72°C for 60 s, and a final extension at 72°C for 5 min.

PCR products were purified with Agencourt AMPure kit (Agencourt Bioscence) to remove residual salts, primers and primer dimers. The concentration of the purified PCR products was measured with the PicoGreen ds DNA Quantification Kit (Molecular Probes) on a FLUOstar OPTIMA (BMG LABTECH). PCR products were mixed in equimolar amounts and sequenced on an Illumina MiSeq system (DNA sequencing Facility, Lund University).

2.6. Bioinformatics analysis and statistics

Sequences were analyzed using the dada2 pipeline version 1.14.0 (Callahan et al. 2016). Processing was done in 3 stages: filtering, sample inference and removing chimeras/assigning taxonomy. Taxonomy was assigned using the Silva reference database.

After quality control, our data consisted of 80 757 ± 24 573 (mean ± SD) reads per sample (26 056 to 110 840) and the amplicon sequence variant (ASV) table resulted in 768 ASVs. ASVs occurring less than 10 times in all samples were removed and the final ASV table then included 413 ASVs. DNA sequences have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive under accession number PRJNA682042.

A statistical method applied for differential gene expressions was used to rank ASVs consistently present in replicates as representative for each treatment (Robinson & Smyth 2008). In brief, a tagwise dispersion function (edgeR package; Robinson et al. 2010) was used to rank ASVs according to their consistency among replicates and analyze which ASVs differed significantly between treatments. Using a generalized linear model, we tested for differential representation of ASVs between treatments using the toptag function, an analysis similar to an ANOVA. For alpha diversity measures we subsampled to 26 000 reads per sample. Species richness (observed number of species) and evenness (Pielou's evenness index) were calculated using R (version 3.6.1) and RStudio (version 1.1.456) and the package vegan (Oksanen et al. 2010). To test for differences between treatments, an ANOVA was performed. When criteria of normality and heterogeneity were not met, a Kruskal-Wallis test was applied.

3. RESULTS

3.1. Fluorescence measurements: concentrations in medium and steady-state concentrations in cultures

Using fluorescence spectroscopy, we were able to follow the concentration of each of the carbon substrates over time at nanomolar concentrations, in both the medium and the cultures (Figs. 1 & 2). The fluorescence signature could be best characterized by a 5-component PARAFAC model, which explained 99.91% of the data with an 89% core consistency (see Figs. S1 & S2 in the Supplement at www.int-res.com/articles/suppl/a088p095_supp.pdf). Three of the 5 components were matched to the pure spectra of SA, Trp, and Tyr with spectral congruence coefficients larger than 0.99 in excitation and emission spectra (Fig. S3). Fluorescence intensities were converted to concentrations using standard curves for the different carbon substrates used in the medium. The fourth component was detected in treatments containing SA and found to have similar emission spectrum to SA but shifted to lower wavelengths (~400 nm), and excitation maxim shifted to longer wavelengths. No direct match with pure compounds or spectra in the OpenFluor database were found (Murphy et al. 2014). As the identity of this component is unknown, conversion from fluorescence intensities to concentrations was not possible. The appearance of this component in SA treatments coincided with an apparent 40%

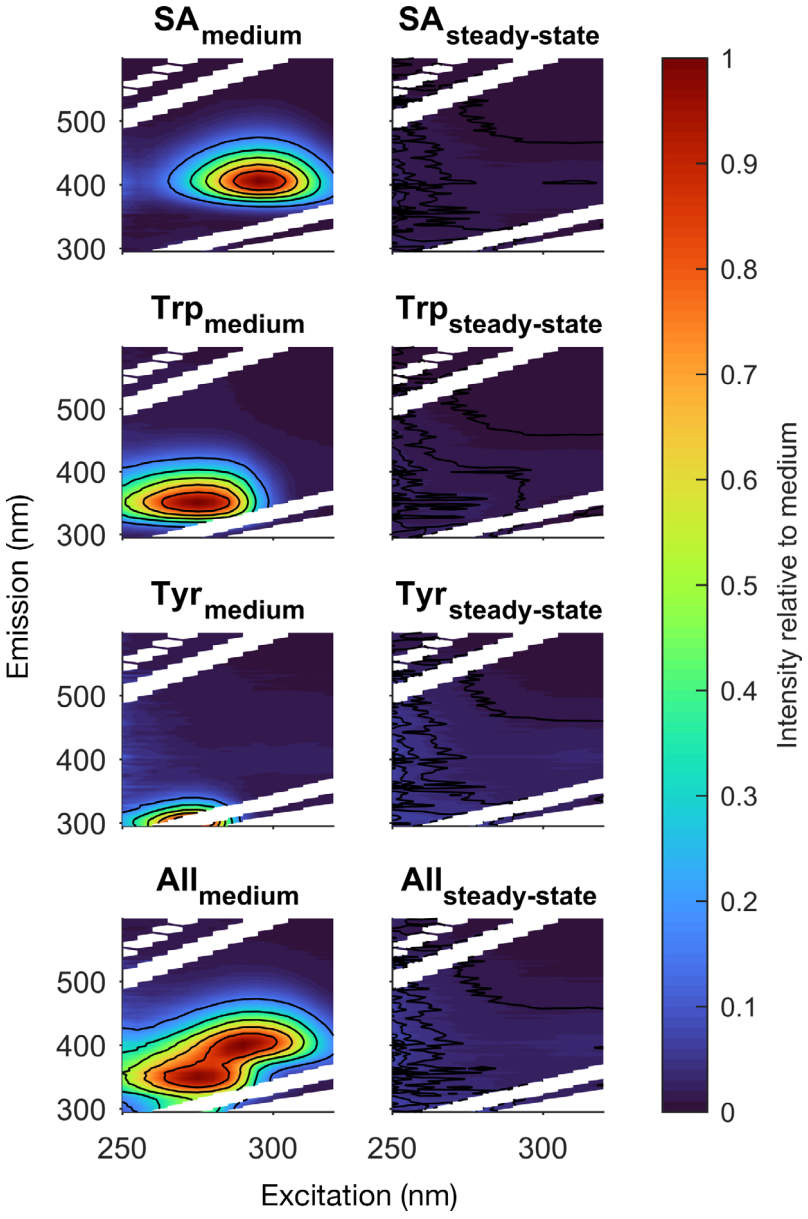

Fig. 1. Examples of excitation emission matrices (EEM) comparing the fluorescence signal from carbon substrates between the medium (left column, Day 1) and the culture at steady state (right column, Day 12). SA: salicylic acid; Trp: tryptophan; Tyr: tyrosine; All: Combined (SA, Trp and Tyr)

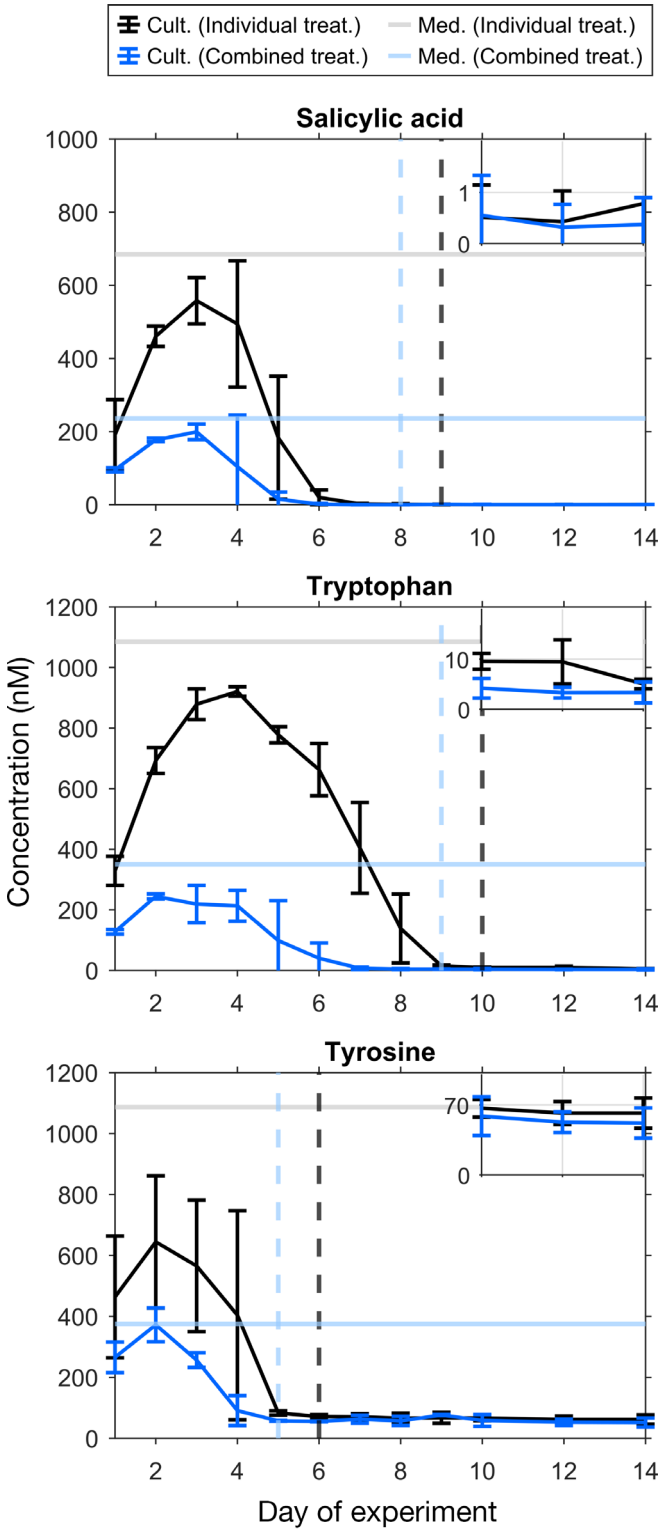

Fig. 2. Average concentration of carbon substrates in cultures grown on individual and combined carbon substrates. Error bars indicate ±SD. Dashed vertical lines indicate the time to reach steady state (light blue: combined treatment; black: individual treatment). Boxes in the upper right corner of each panel show concentrations for the 3 last sampling points (Days 10, 12 and 14)

loss of SA when comparing expected concentrations in the chemostat media with those quantified using fluorescence. This additional fluorophore was not observed in standard curves, indicating that it does not represent an impurity. The media remained sterile during the experiment, suggesting that microbial metabolism was not the source of this component, which was more likely due to abiotic auto-oxidation in the media. The fifth component did not represent properties of typical fluorophores but rather represented background interference that improved the estimation in part due to the removal of instrument noise and artefacts (Fig. S2).

Carbon substrates were added to the medium to obtain a concentration of 1 µM of substrate. In the media with single carbon substrates, the measured concentrations were stable throughout the experiment (Days 1 to 14). Based on the fluorescence results, concentrations were 672 ± 28 nM, 1081 ± 44 nM and 1096 ±97 nM, respectively, for SA, Trp and Tyr (Table 1, Fig. 2). The concentrations of substrates decreased over time in one of the combined substrate media, indicating that the medium was no longer sterile. This treatment was therefore excluded from the study. In the 2 other combined media, the measured concentrations were 235 ± 8 nM SA, 348 ± 20 nM Trp and 375 ± 69 nM Tyr (Table 1, Fig. 2).

Table 1. Substrate concentration (nM) and carbon concentration (nM C) at start of incubation and at steady state for each carbon substrate in the individual and combined treatments. Start values represent mean (±SD) in the medium (Days 1 to 14) and steady-state values represent mean (±SD) in culture (based on the 4 d directly after steady state is reached). Removal represents % carbon removed

	Start		Steady state		Removal
	nM	nM C	nM	nM C	
Individual treatment					
Salicylic acid	672.4 (±28.5)	4706.9 (±199.5)	0.53 (±0.50)	3.69 (±3.50)	99.9
Tryptophan	1081.4 (±43.7)	11 895.7 (±481.1)	8.21 (±3.10)	90.3 (±34.1)	99.2
Tyrosine	1096.2 (±97.4)	9866.0 (±877.0)	63.4 (±10.8)	570.4 (±96.8)	94.2
Combined treatment					
Salicylic acid	235.0 (±7.7)	1649.5 (±54.9)	0.41 (±0.48)	2.90 (±3.36)	99.8
Tryptophan	347.6 (±19.5)	3826.2 (±209.9)	3.61 (±1.43)	39.7 (±15.8)	98.9
Tyrosine	375.0 (±69.2)	3328.7 (±572.4)	54.4 (±12.4)	489.9 (±111.5)	85.2
Sum		8804.4 (±733.8)		532.5 (±130.4)	94.0

Based on the average concentrations during the 3 last sampling points (Days 10, 12 and 14), the bacterial communities reduced the steady-state concentration of each carbon substrate to lower levels in the combined treatment than in the individual treatments (Table 1, Fig. 2). However, only the steady-state concentration of Trp was significantly lower for growth in the combined treatment relative to the single substrate (Kruskal-Wallis, $p = 0.004678$). In addition, the steady-state concentration for Tyr was (based on mol basis) more than 5 times higher than the steady-state concentrations for SA and Trp and the difference was significant in both the individual and combined treatments (Kruskal-Wallis, $p < 0.01$).

Although lower steady-state concentrations were reached in the combined treatments, a smaller fraction of each of the carbon substrates was utilized (Table 1). This was most evident for Tyr, where the removal was 94.2% in the individual treatment and 85.2% in the combined treatment. In addition, the average total carbon utilization was lower in the combined treatment than in any of the individual treatments and the steady-state concentration of total carbon was significantly higher in the combined treatment than in the SA treatment and the Trp treatment (Table 1, Kruskal-Wallis, $p < 0.05$). However, the concentration of total carbon was similar between the Tyr and combined treatments (Table 1, Kruskal-Wallis, $p = 0.69$).

3.2. Rate of utilization and time for reaching steady state

Maximum utilization rate of each carbon substrate was compared in individual and combined treatments. Maximum utilization rates were estimated by fitting polynomials to the data describing the decrease in substrate concentration from maximum substrate concentration to steady-state concentration. Combined substrate treatments resulted in slower utilization rates. For cultures on individual substrates, the average maximum utilization rate varied between 254 and 357 nM d^{-1}, whereas the utilization rate in the cultures with combined substrates varied between 117 and 261 nM d^{-1} (Table 2).

Steady-state concentrations were reached between Days 5 and 10. The time for reaching steady state was defined as the time point when the concentration was below steady-state concentration + 1 SD. Based on the average concentrations within each treatment, steady state was reached 1 d earlier in the combined treatments than in the single treatments (Table 2, Fig. 2).

Table 2. Maximum uptake rates (± SD) and time to reach steady-state concentration for each carbon substrate in the individual and combined treatments. Time to steady state is based on the average concentrations within each treatment

Carbon substrate	Individual treatment		Combined treatment	
	Uptake rate (nM d^{-1})	Steady state (d)	Uptake rate (nM d^{-1})	Steady state (d)
Salicylic acid	357.3 (±140.8)	9	242.7 (±43.8)	8
Tryptophan	253.6 (±19.2)	10	117.1 (±12.2)	9
Tyrosine	337.9 (±14.7)	6	260.5 (±35.6)	5

3.3. Bacterial abundances and bacterial growth efficiency

BA varied over time in all treatments and increased in response to the addition of carbon substrates. The highest abundances were reached around Day 5 (Fig. S4). Based on the 3 last sampling days (Days 10, 12 and 14), there were no significant differences in BA between treatments (ANOVA, $p > 0.05$). The average BA (± SD) was $1.24 \pm 0.68 \times 10^6$ cell ml^{-1} in the SA treatment, $1.09 \pm 0.46 \times 10^6$ cell ml^{-1} in the Trp treatment and $1.39 \pm 0.79 \times 10^6$ cell ml^{-1} in the Tyr treatment (Table 3). In the combined culture, the bacterial abundance reached $1.63 \pm 0.66 \times 10^6$ cell ml^{-1} (Table 3).

Table 3. Total DOC uptake, bacterial abundance (BA) and bacterial growth efficiency (BGE) in the different treatments during steady state. DOC uptake is calculated as the difference between the average carbon concentration at the start of the experiment (in the medium) and at steady state. BA and BGE are presented as mean (±SD) based on the 4 d directly after steady state was reached

Treatment	DOC uptake (nM C)	BA (cells ml^{-1})	BGE
Salicylic acid	4703	1.24×10^6 ($\pm0.68\times10^6$)	0.49 (±0.25)
Tryptophan	11805	1.09×10^6 ($\pm0.46\times10^6$)	0.14 (±0.09)
Tyrosine	9296	1.39×10^6 ($\pm0.79\times10^6$)	0.19 (±0.16)
Combined	8281	1.63×10^6 (0.66×10^6)	0.41 (±0.22)

DOC assimilation, based on nM carbon removed, was highest in the Trp treatment and lowest in the SA treatment (Table 3). Despite having the highest carbon assimilation, the Trp treatment had the lowest BA. These 2 factors resulted in the lowest BGE in the Trp treatment. The average BGE (± SD, based on the same days as BA) was 0.14 ± 0.09 in the Trp treatment, 0.19 ± 0.16 in the Tyr treatment, 0.49 ± 0.25 in the SA treatment and 0.41 ± 0.22 in the combined treatment (Table 3). BGE was significantly higher in the SA treatment than in the Trp and Tyr treatments (Kruskal-Wallis, $p < 0.05$).

3.4. Bacterial community composition, richness, evenness and diversity

Bacterial community composition was determined after each culture reached steady state and differed significantly between treatments (Fig. 3, perm-ANOVA, $p = 0.003996$). Based on average Bray-Curtis similarity values, similarity was higher within treatments than between treatments for SA and Trp (Table S1). However, variation was high across replicates in the Tyr and combined treatments, and the average similarity was comparable within these treatments and between the Tyr and combined treatments.

The bacterial community composition was dominated by *Bacteroidetes*, *Alphaproteobacteria* and *Gammaproteobacteria*. Within *Gammaproteobacteria*, *Alteromonadales* was the dominant order in the SA treatment, whereas the orders *Alteromonadales* and *Oceanospirillales* were both dominant in the Trp and Tyr treatments. Within *Alphaproteobacteria*, *Rhodospirillales* was the dominant order in all treatments and within *Bacteroidetes*, *Flavobacteriales* was the dominant order. However, based on tagwise dispersion, it was not possible to identify any indicator species linked to specific treatments. In addition, there were no significant differences in evenness and richness between treatments (Table S2).

4. DISCUSSION

It has been suggested that the ability to simultaneously utilize several available carbon compounds is a crucial factor for the efficient and fast growth of microorganisms in natural systems (Lendenmann et al. 1996). Furthermore, reduction in threshold concentration of individual carbon substrates during growth on mixtures of carbon substrates has been proposed as a general kinetic principle for heterotrophic microorganisms (Lendenmann et al. 1996). However, these theories have previously not been tested for diverse natural bacterial communities. Our results show that substrate combination affects utilization rate, threshold concentration and the fraction of carbon utilized. BA and BGE, however, show large variation depending on carbon substrate supplied.

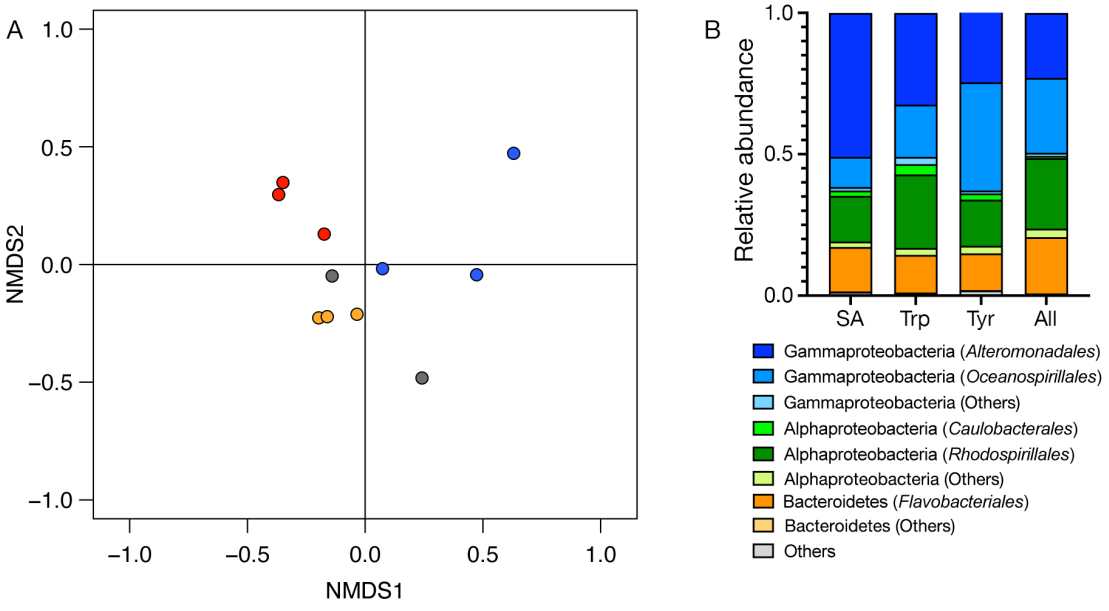

Fig. 3. (A) NMDS ordination plot based on 16S rRNA gene amplicon sequencing showing differences in bacterial community composition between treatments. Analysis is based on data from Illumina sequencing and Bray–Curtis dissimilarities. Stress = 0.09. Yellow = salicylic acid (SA), Red = tryptophan (Trp), Blue = tyrosine (Tyr), Grey = All (SA, Trp and Tyr combined). (B) Bacterial community composition in each treatment at steady state

4.1. Effect of individual and combinations of carbon substrate

In agreement with previous studies performed with isolates, we showed that growth on combined substrates can result in lower steady-state concentrations and thresholds for utilization even for diverse natural bacterial communities. However, there was only a significant difference in steady-state concentration for 1 of 3 tested substrates. In addition, we could not verify that the steady-state concentration of a particular carbon substrate is proportional to its contribution to the combined carbon pool (Egli et al. 1993). This proportionality pattern has however been suggested to only apply to substrates for which both the affinity constants and the growth yields are in the same range (Egli et al. 1983, Lendenmann et al. 1996). Based on differences in steady-state concentrations for the carbon substrates used in our experiment, we conclude that affinities differed and that the affinity was significantly lower for Tyr than the other 2 substrates. Moreover, BGE also differed significantly for the 3 substrates.

It has frequently been pointed out that for growth of a microbial strain in continuous cultures, the steady-state concentration of the growth-controlling substrate is related to the content of the enzymes involved in transport and catabolism of this substrate (Button 1985, Rutgers et al. 1991). One explanation for the lower steady state concentration of Trp in the combined cultures might therefore be related to the widely reported phenomenon known as the priming effect (Kuzyakov 2010). The process has also been observed in a few aquatic studies (Guenet et al. 2010, Bianchi 2011) and explains that generally labile carbon sources trigger uptake of previously unreactive organic matter. The main mechanism is thought to be the use of labile carbon for sustenance and subsequent investment into extracellular enzymes. These in turn then degrade less available organic matter (Kuzyakov 2010). The presence of SA and Tyr in the combined treatment might support the production of enzymes with a higher affinity for Trp, leading to lower steady-state concentrations of Trp. In our experiment, Trp might be the carbon source that is hardest for the bacteria to utilize. Growth experiments with the heterotrophic marine bacterium *Pseudoalteromonas haloplanktis* showed that the order of nutrient uptake is partially determined by the biomass yield that can be achieved when the same compounds are provided as single carbon substrates. Trp was one of the last amino acids to be consumed and was slowly metabolized (Perrin et al. 2020). This is also in agreement with our results showing the lowest utilization rates and BGE for Trp.

During amino acid degradation, the α-amino acid group is removed and carbon is transformed into major metabolic intermediates which can be converted into glucose or oxidized by the citric acid cycle (Berg et al. 2002). The pathway for degradation of Trp is longer and includes more enzymatic steps than the pathway for Tyr (Berg et al. 2002). Another explanation for lower steady-state concentrations of Trp when grown on combined substrates might be that some enzymatic steps are shared with the SA and/or Tyr degradation pathways. Some microbes can, via an alternative pathway (the Kynurenine pathway), degrade Trp to catechol (Parthasarathy et al. 2018) which is the same intermediate as that formed in the degradation of SA. SA (salicylate) is first converted to catechol by salicylate hydroxylase as part of the naphthalene pathway. Catechol is then converted to pyruvate and acetaldehyde in the nitrobenzene pathway (Peng et al. 2008). For natural bacterial communities, the combination of carbon compounds included in the carbon pool might therefore be important for the effect on steady-state concentration.

Steady state for each substrate was achieved 1 d earlier in the combined cultures than in the cultures with single carbon substrates (Table 2). This might indicate a faster adaption to utilization of carbon sources in a mixture and is in agreement with experiments showing that simultaneous utilization of DOC together with pollutants influences the rate and extent of pollutant degradation (Kovárová-Kovar & Egli 1998). The rate of degradation of pollutants was enhanced and the acclimation phase was shortened when the growth of the population was supported by supplementary carbon sources (Kovárová-Kovar & Egli 1998). Although steady-state concentrations were reached faster in the combined cultures, maximum utilization rates were lower. This could be an effect of a lower concentration of each individual substrate in the mixed cultures but might also be because consumption of carbon at a specific rate supports a particular cellular specific growth rate. In the chemostats the cultures are forced to the same growth rate, which can be achieved by either consuming one substrate at a high rate or consuming several substrates at a reduced rate (Egli 1995).

4.2. Effect of bacterial community composition

For this experiment it is also important to keep in mind that the bacterial community composition was

significantly different between treatments. This concurs with previous studies showing that bacterial communities are tightly coupled to the concentration and composition of DOM (Cottrell & Kirchman 2000, Kirchman et al. 2004, Judd et al. 2006, Alonso-Sáez & Gasol 2007, Amaral et al. 2016, Broman et al. 2019, Bruhn et al. 2021).

At steady state, based on average Bray-Curtis similarity values, the similarity was higher within treatments than between treatments for SA and Trp (Table S1). However, variation was high among replicates in the Tyr and combined treatments. Since the utilization was similar within these treatments anyway, this indicates that the bacterial communities were functionally redundant, meaning that contrasting bacterial species could perform the same functions and utilize the same carbon sources (Wohl et al. 2004, Sjöstedt et al. 2012, Andersson 2017, Louca et al. 2018). The composition of the bacterial community that establishes can be related to priority effects and depends on which species colonize the chemostat first (Fukami 2015, Vass & Langenheder 2017, Svoboda et al. 2018).

When the community composition in the mixed culture was compared to each culture with single carbon sources, it was not possible to identify any indicator species linked to specific treatments. Differences in community composition between the treatments therefore seem to be an effect of differences in proportion rather than species identity. In addition to co-metabolism, another explanation for reduced steady-state concentrations of individual substrates in mixed substrate growth might therefore be that the abundant bacterial species, which differed between the treatments, had different affinities for the substrates. The growth parameters, μ_{max} and K_s, are not constants but variables that depend on the composition of the bacterial community and physiological state of the species responsible for growth and substrate consumption (Peil & Gaudy 1971, Ghosh & Pohland 1972). However, since the same pattern of reduced steady-state concentration has been reported for several isolates, this does not seem to be an effect of different bacterial community composition but instead a general pattern for carbon utilization. The mechanistic explanation is still unclear but might be related to the priming effect or that fact that specific bacteria can exhibit different kinetic properties under different growth conditions (Senn et al. 1994).

Higher diversity of carbon substrates could either select for species specialized in utilizing 1 of the 3 different carbon substrates, resulting in a higher species diversity, or generalists utilizing all 3 substrates simultaneously. In our experiment, there were no significant differences in richness or evenness across treatments, which might indicate that the dominant species were generalists. Previous enrichment experiments have shown that the same phylotypes can become abundant in response to addition of several different carbon compounds (Mou et al. 2008, Gómez-Consarnau et al. 2012). In our experiment the dominant orders within *Gammaproteobacteria* were *Altermondales* and *Oceanospirillales*. *Alteromondales* are known to utilize labile DOC and their genome contains genes for utilizing a diverse set of organic matter compounds (McCarren et al. 2010, Pedler et al. 2014, Koch et al. 2020). Bacteria within this order contribute significantly both to abundance and activity in natural bacterioplankton communities (Teeling et al. 2012) and outcompete other bacteria under high nutrient conditions (McCarren et al. 2010, Pedler et al. 2014). In addition, enrichment experiments have shown that bacteria within the order *Altermondales* are stimulated and become dominant in response to addition of several different carbon compounds (Gómez-Consarnau et al. 2012, Bryson et al. 2017, Pontiller et al. 2020). Similarly to *Alteromondales*, phylotypes within the order *Oceanospirillales* have been shown to become dominant in enrichment studies (Pontiller et al. 2020), and genomic analyses of uncultured *Oceanospirillales* representatives found genes for transporters for nutrients such as amino acids, fatty acids and carboxylic acids (Mason et al. 2012, Delmont et al. 2015). *Flavobacteriales*, which was the dominant order within *Bacteroidetes*, has also been shown to increase in abundance in response to addition of several carbon substrates (Bryson et al. 2017). Although different bacterial taxa, even those of the same order or genus, can have different ecological roles in the transformation of the marine DOM pool (Konstantinidis et al. 2009, Newton et al. 2010), our results combined with results from previous studies indicate a rapid response to incubation conditions (Pedler et al. 2014) and a selection of fast-growing generalist bacteria (Gómez-Consarnau et al. 2012).

Producing enzymes for utilizing more than one carbon substrate might lead to increased cost of metabolism (Lehmann et al. 2020) and it has been shown that there is a negative correlation between the maximum growth rate and the total activity of carbon, nitrogen, and phosphorus degrading extracellular enzymes (Ramin & Allison 2019). However, in the chemostats the bacterial communities are forced to grow at the same speed and this trade-off is instead seen as lower utilization rates and

intermediate growth efficiencies in the combined treatments. Although priming can help lower the concentrations, there is a threshold concentration needed to support energy-efficient bacterial growth (Traving et al. 2015). Individual carbon concentrations cannot be reduced below this level and the total carbon concentration might therefore increase with higher molecular diversity. In our experiment, the steady-state concentration of total carbon was higher in the combined treatment than in the SA and Trp treatments and similar to the concentration in the Tyr treatment. This is in agreement with studies where *Escherichia coli* were grown on 2 substrates for which the affinity constants were very different and where results show that the total steady-state carbon concentration in the culture was essentially determined by the steady-state concentration of the substrate for which *E. coli* exhibited lowest affinity (higher K_s) (Kovárová et al. 1996–1997, 1997). Our study therefore lends support to the dilution theory, which states that the apparent persistence of DOC can be explained by a multitude of compounds at low concentrations rather than low intrinsic chemical reactivity (Jannasch 1967, Arrieta et al. 2015).

4.3. Conclusion and implications

Recently there has been much discussion about what controls the utilization of DOC in aquatic environments and subsequently the size of the refractory pool. A recent perspective paper concludes that the persistence of refractory DOC is dependent on both intrinsic (chemical composition and structure, e.g. molecular properties), and extrinsic properties (amount or external factors, e.g. molecular concentrations, ecosystem properties) (Baltar et al. 2021). Our study indicates that the diversity of organic compounds is likely to also be an important factor. Although individual steady-state concentrations were lower at higher substrate diversity, maximum utilization rates were reduced and the fraction of total carbon utilized decreased. In a highly complex mixture with low substrate concentrations this would result in apparent slow degradation. However, utilization started earlier and steady-state concentrations were reached faster when bacterial communities were grown on combined compared to single carbon sources, which might, in systems with a short residence time, determine whether organic matter is mineralized *in situ* or transported to the ocean (Steen et al. 2016).

This experiment was run at natural concentrations of DOC and using continuous cultures which has been suggested to be the method that most closely resembles the growth conditions bacteria encounter in natural systems (Kovárová-Kovar & Egli 1998). The use of simple substrates supplied at higher concentrations shows that even labile substrates can persist, having an apparent recalcitrance. It is also clear that the affinity towards specific substrates depends not only on the match between chemical composition and an appropriate enzyme repertoire, but can also be enhanced by metabolism of auxiliary substrates. This blurs the importance of individual substrate affinities in complex mixtures such as those found in natural environments. These findings warrant further study with increasingly complex combinations of substrates. However, future studies need to take into account that microorganisms in nature may not be controlled by only a single nutrient but by 2 or more nutrients simultaneously.

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LITERATURE CITED

Alonso-Sáez L, Gasol JM (2007) Seasonal variations in the contributions of different bacterial groups to the uptake of low-molecular-weight compounds in northwestern Mediterranean coastal waters. Appl Environ Microbiol 73:3528–3535

Amaral V, Graeber D, Calliari D, Alonso C (2016) Strong linkages between DOM optical properties and main clades of aquatic bacteria. Limnol Oceanogr 61:906–918

Andersson M (2017) Extent and limitations of functional redundancy among bacterial communities towards dissolved organic matter. PhD dissertation, Uppsala University

Arrieta JM, Mayol E, Hansman RL, Herndl GJ, Dittmar T, Duarte CM (2015) Dilution limits dissolved organic carbon utilization in the deep ocean. Science 348:331–333

Babel W, Brinkmann U, Muller RH (1993) The auxiliary substrate concept — an approach for overcoming limits of microbial performances. Acta Biotechnol 13:211–242

Baltar F, Alvarez-Salgado XA, Arístegui J, Benner R, Hansell DA, Herndl GJ, Lønborg C (2021) What is refractory organic matter in the ocean? Front Mar Sci 8:642637

Berg JM, Stryer L, Tymoczko J, Gatto G (2002) Protein turnover and amino acid catabolism. In: Moran S (ed) Bio-

chemistry, 5th edn. WH Freeman and Company, New York, NY, p 633–663
Bianchi TS (2011) The role of terrestrially derived organic carbon in the coastal ocean: a changing paradigm and the priming effect. Proc Natl Acad Sci USA 108: 19473–19481
Brock TD (1987) The study of microorganisms in situ: progress and problems. In: Fletcher M, Gray TRG, Jones JG (eds) Ecology of microbial communities. Cambridge University Press, Cambridge, p 1–18
Broman E, Asmala E, Carstensen J, Pinhassi J, Dopson M (2019) Distinct coastal microbiome populations associated with autochthonous- and allochthonous-like dissolved organic matter. Front Microbiol 10:2579
Bruhn AD, Stedmon CA, Comte JM, Matsuoka A and others (2021) Terrestrial dissolved organic matter mobilized from eroding permafrost controls microbial community composition and growth in Arctic coastal zones. Front Earth Sci 9:640580
Bryson S, Li Z, Chavez F, Weber PK and others (2017) Phylogenetically conserved resource partitioning in the coastal microbial loop. ISME J 11:2781–2792
Buitenhuis ET, Li WKW, Lomas MW, Karl DM, Landry MR, Jacquet S (2012) Picoheterotroph (Bacteria and Archaea) biomass distribution in the global ocean. Earth Syst Sci Data 4:101–106
Button DK (1985) Kinetics of nutrient-limited transport and microbial growth. Microbiol Rev 49:270–297
Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 13:581
Carlsson CA, Hansell DA (2015) DOM sources, sinks, reactivity, and budgets. In: Hansell DA, Carlson CA (eds) Biogeochemistry of marine dissolved organic matter, 2nd edn. Academic Press, San Diego, CA, p 65–126
Chu D, Barnes DJ (2016) The lag-phase during diauxic growth is a trade-off between fast adaptation and high growth rate. Sci Rep 6:25191
Cole JJ (1999) Aquatic microbiology for ecosystem scientists: new and recycled paradigms in ecological microbiology. Ecosystems 2:215–225
Cole JJ, Prairie YT, Caraco NF, McDowell WH and others (2007) Plumbing the global carbon cycle: integrating inland waters into the terrestrial carbon budget. Ecosystems 10:172–184
Cottrell MT, Kirchman DL (2000) Natural assemblages of marine Proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. Appl Environ Microbiol 66:1692–1697
Covert JS, Moran MA (2001) Molecular characterization of estuarine bacterial communities that use high- and low-molecular weight fractions of dissolved organic carbon. Aquat Microb Ecol 25:127–139
Delmont TO, Eren AM, Vineis JH, Post AF (2015) Genome reconstructions indicate the partitioning of ecological functions inside a phytoplankton bloom in the Amundsen Sea, Antarctica. Front Microbiol 6:1090
Dittmar T (2015) Reasons behind the long-term stability of dissolved organic matter. In: Hansell DA, Carlson CA (eds) Biogeochemistry of marine dissolved organic matter, 2nd edn. Academic Press, San Diego, CA, p 369–388
Dittmar T, Lennartz ST, Buck-Wiese H, Hansel DA and others (2021) Enigmatic persistence of dissolved organic matter in the ocean. Nat Rev Earth Environ 2:570–583
Egli T (1995) The ecological and physiological significance of growth of heterotrophic microorganisms with mixtures of substrates. In: Jones JG (ed) Advances in microbial ecology, Book 14. Plenum Press, New York, NY, p 305–386
Egli T (2010) How to live at very low substrate concentration. Water Res 44:4826–4837
Egli T, Lindley ND, Quayle JR (1983) Regulation of enzyme-synthesis and variation of residual methanol concentration during carbon-limited growth of kloeckera sp-2201 on mixtures of methanol and glucose. J Gen Microbiol 129:1269–1281
Egli T, Bosshard C, Hamer G (1986) Simultaneous utilization of methanol glucose mixtures by hansenula-polymorpha in chemostat — influence of dilution rate and mixture composition on utilization pattern. Biotechnol Bioeng 28: 1735–1741
Egli T, Lendenmann U, Snozzi M (1993) Kinetics of microbial growth with mixtures of carbon sources. Ant Van Leeuwenhoek 63:289–298
Friedlingstein P, Jones MW, O'Sullivan M, Andrew RM and others (2019) Global carbon budget 2019. Earth Syst Sci Data 11:1783–1838
Fukami T (2015) Historical contingency in community assembly: integrating niches, species pools, and priority effects. Annu Rev Ecol Evol Syst 46:1–23
Gasol JM, Del Giorgio PA (2000) Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. Sci Mar 64:197–224
Ghosh S, Pohland FG (1972) Kinetics of assimilation of multiple substrates in dispersed growth systems. Water Res 6:99
Gómez-Consarnau L, Lindh MV, Gasol JM, Pinhassi J (2012) Structuring of bacterioplankton communities by specific dissolved organic carbon compounds. Environ Microbiol 14:2361–2378
Guenet B, Danger M, Abbadie L, Lacroix G (2010) Priming effect: bridging the gap between terrestrial and aquatic ecology. Ecology 91:2850–2861
Hagström Å, Ammerman JW, Henrichs S, Azam F (1984) Bacterioplankton growth in seawater: II. Organic matter utilization during steady-state growth in seawater cultures. Mar Ecol Prog Ser 18:41–48
Hansell DA (2013) Recalcitrant dissolved organic carbon fractions. Annu Rev Mar Sci 5:421–445
Harder W, Dijkhuizen L (1982) Strategies of mixed substrate utilization in microorganims. Philos Trans R Soc B 297: 459–480
Herlemann DP, Labrenz M, Jurgens K, Bertilsson S, Waniek JJ, Andersson AF (2011) Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME J 5:1571–1579
Jannasch HW (1967) Growth of marine bacteria at limiting concentrations of organic carbon in seawater. Limnol Oceanogr 12:264–271
Jiao N, Robinson C, Azam F, Thomas H and others (2014) Mechanisms of microbial carbon sequestration in the ocean — future research directions. Biogeosciences 11: 5285–5306
Jover LF, Effler TC, Buchan A, Wilhelm SW, Weitz JS (2014) The elemental composition of virus particles: implications for marine biogeochemical cycles. Nat Rev Microbiol 12:519–528
Judd KE, Crump BC, Kling GW (2006) Variation in dissolved

organic matter controls bacterial production and community composition. Ecology 87:2068–2079

Kirchman DL (2003) The contribution of monomers and other low-molecular weight compounds to the flux of dissolved organic matter in aquatic ecosystems. In: Findlay SEG, Sinsabaugh RL (eds) Aquatic ecosystems: interactivity of dissolved organic matter. Elsevier Science, New York, NY, p 217–241

Kirchman DL, Dittel AI, Findlay SEG, Fischer D (2004) Changes in bacterial activity and community structure in response to dissolved organic matter in the Hudson River, New York. Aquat Microb Ecol 35:243–257

Koch H, Germscheid N, Freese HM, Noriega-Ortega B and others (2020) Genomic, metabolic and phenotypic variability shapes ecological differentiation and intraspecies interactions of *Alteromonas macleodii*. Sci Rep 10:809

Konstantinidis KT, Serres MH, Romine MF, Rodrigues JLM and others (2009) Comparative systems biology across an evolutionary gradient within the *Shewanella* genus. Proc Natl Acad Sci USA 106:15909–15914

Kovárová K, Käch A, Chaloupka V, Egli T (1996–1997) Cultivation of *Escherichia coli* with mixtures of 3-phenylpropionic acid and glucose: dynamics of growth and substrate consumption. Biodegradation 7:445–453

Kovárová K, Käch A, Zehnder AJB, Egli T (1997) Cultivation of *Escherichia coli* with mixtures of 3-phenylpropionic acid and glucose: steady-state growth kinetics. Appl Environ Microbiol 63:2619–2624

Kovárová-Kovar K, Egli T (1998) Growth kinetics of suspended microbial cells: from single-substrate-controlled growth to mixed-substrate kinetics. Microbiol Mol Biol Rev 62:646–666

Kuzyakov Y (2010) Priming effects: interactions between living and dead organic matter. Soil Biol Biochem 42: 1363–1371

Law AT, Button DK (1977) Multiple-carbon-source-limited growth kinetics of a marine coryneform bacterium. J Bacteriol 129:115–123

Lee S, Fuhrman JA (1987) Relationships between biovolume and biomass of naturally derived marine bacterioplankton. Appl Environ Microbiol 53:1298–1303

Lehmann J, Hansel CM, Kaiser C, Kleber M and others (2020) Persistence of soil organic carbon caused by functional complexity. Nat Geosci 13:529–534

Lendenmann U, Snozzi M, Egli T (1996) Kinetics of the simultaneous utilization of sugar mixtures by *Escherichia coli* in continuous culture. Appl Environ Microbiol 62: 1493–1499

Lohnis F (1926) Nitrogen availability of green manures. Soil Sci 22:253–290

Louca S, Polz MF, Mazel F, Albright MBN and others (2018) Function and functional redundancy in microbial systems. Nat Ecol Evol 2:936–943

Mason OU, Hazen TC, Borglin S, Chain PSG and others (2012) Metagenome, metatranscriptome and single-cell sequencing reveal microbial response to Deepwater Horizon oil spill. ISME J 6:1715–1727

McCarren J, Becker JW, Repeta DJ, Shi YM and others (2010) Microbial community transcriptomes reveal microbes and metabolic pathways associated with dissolved organic matter turnover in the sea. Proc Natl Acad Sci USA 107:16420–16427

Mou X, Sun S, Edwards RA, Hodson RE, Moran MA (2008) Bacterial carbon processing by generalist species in the coastal ocean. Nature 451:708–711

Murphy KR, Stedmon CA, Graeber D, Bro R (2013) Fluorescence spectroscopy and multi-way techniques. PARAFAC. Anal Methods 5:6557–6566

Murphy KR, Stedmon CA, Wenig P, Bro R (2014) OpenFluor- an online spectral library of auto-fluorescence by organic compounds in the environment. Anal Methods 6: 658–661

Newton RJ, Griffin LE, Bowles KM, Meile C and others (2010) Genome characteristics of a generalist marine bacterial lineage. ISME J 4:784–798

Oksanen JF, Blanchet G, Kindt R, Legrende P and others (2010) vegan: community ecology package. R package version 1.17-5. https://CRAN.R-project.org/package=vegan

Parthasarathy A, Cross PJ, Dobson RCJ, Adams LE, Savka MA, Hudson AO (2018) A three-ring circus: metabolism of the three proteogenic aromatic amino acids and their role in the health of plants and animals. Front Mol Biosci 5:29

Pedler BE, Aluwihare LI, Azam F (2014) Single bacterial strain capable of significant contribution to carbon cycling in the surface ocean. Proc Natl Acad Sci USA 111:7202–7207

Peil KM, Gaudy AF (1971) Kinetic constants for aerobic growth of microbial populations selected with various single compounds and with municipal wastes as substrates. Appl Microbiol 21:253

Peng RH, Xiong AS, Xue Y, Fu XY and others (2008) Microbial biodegradation of polyaromatic hydrocarbons. FEMS Microbiol Rev 32:927–955

Perrin E, Ghini V, Giovannini M, Di Patti F and others (2020) Diauxie and co-utilization of carbon sources can coexist during bacterial growth in nutritionally complex environments. Nat Commun 11:3135

Pontiller B, Martinez-Garcia S, Lundin D, Pinhassi J (2020) Labile dissolved organic matter compound characteristics select for divergence in marine bacterial activity and transcription. Front Microbiol 11:588778

Ramin KI, Allison SD (2019) Bacterial tradeoffs in growth rate and extracellular enzymes. Front Microbiol 10:2956

Rivas-San Vicente M, Plasencia J (2011) Salicylic acid beyond defence: its role in plant growth and development. J Exp Bot 62:3321–3338

Robinson MD, Smyth GK (2008) Small-sample estimation of negative binomial dispersion, with applications to SAGE data. Biostatistics 9:321–332

Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26:139–140

Rutgers M, Vandam K, Westerhoff HV (1991) Control and thermodynamics of microbial growth: rational tools for bioengineering. Crit Rev Biotechnol 11:367–395

Senn H, Lendenmann U, Snozzi M, Hamer G, Egli T (1994) The growth of *Escherichia coli* in glucose-limited chemostat cultures: a re-examination of the kinetics. Biochim Biophys Acta 1201:424–436

Shibl AA, Isaac A, Ochsenkuhn MA, Cardenas A and others (2020) Diatom modulation of select bacteria through use of two unique secondary metabolites. Proc Natl Acad Sci USA 117:27445–27455

Sjöstedt J, Koch-Schmidt P, Pontarp M, Canbäck B and others (2012) Recruitment of members from the rare biosphere of marine bacterioplankton communities after environmental disturbance. Appl Environ Microbiol 78: 1361–1369

Steen AD, Quigley LNM, Buchan A (2016) Evidence for the priming effect in a planktonic estuarine microbial community. Front Mar Sci 3:6

Svoboda P, Lindstrom ES, Osman OA, Langenheder S (2018) Dispersal timing determines the importance of priority effects in bacterial communities. ISME J 12:644–646

Teeling H, Fuchs BM, Becher D, Klockow C and others (2012) Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. Science 336:608–611

Traving SJ, Thygesen UH, Riemann L, Stedmon CA (2015) A model of extracellular enzymes in free-living microbes: which strategy pays off? Appl Environ Microbiol 81: 7385–7393

Troussellier M, Courties C, Lebaron P, Servais P (1999) Flow cytometric discrimination of bacterial populations in seawater based on SYTO 13 staining of nucleic acids. FEMS Microbiol Ecol 29:319–330

Vass M, Langenheder S (2017) The legacy of the past: effects of historical processes on microbial metacommunities. Aquat Microb Ecol 79:13–19

Vetter YA, Deming JW, Jumars PA, Krieger-Brockett BB (1998) A predictive model of bacterial foraging by means of freely released extracellular enzymes. Microb Ecol 36: 75–92

Wanner U, Egli T (1990) Dynamics of microbial growth and cell composition in batch culture. FEMS Microbiol Rev 75:19–44

Wildermuth MC, Dewdney J, Wu G, Ausubel FM (2001) Isochorismate synthase is required to synthesize salicylic acid for plant defence. Nature 414:562–565

Wohl DL, Arora S, Gladstone JR (2004) Functional redundancy supports biodiversity and ecosystem function in a closed and constant environment. Ecology 85:1534–1540

Wünsch U, Murphy KR, Stedmon CA (2015) Fluorescence quantum yields of natural organic matter and organic compounds: implications for the fluorescence-based interpretation of organic matter composition. Front Mar Sci 2:98

Yamashita Y, Fichot CG, Shen Y, Jaffe R, Benner R (2015) Linkages among fluorescent dissolved organic matter, dissolved amino acids and lignin-derived phenols in a river-influenced ocean margin. Front Mar Sci 2:92

Zheng W, Tsompana M, Ruscitto A, Sharma A, Genco R, Sun YJ, Buck MJ (2015) An accurate and efficient experimental approach for characterization of the complex oral microbiota. Microbiome 3:48

Zweifel UL, Blackburn N, Hagström Å (1996) Cycling of marine dissolved organic matter. I. An experimental system. Aquat Microb Ecol 11:65–77

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