Heterotrophic Bacteria Respond Differently to Increasing Temperature and Dissolved Organic Carbon Sources in Two Tropical Coastal Systems

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Abstract  Temperature and substrate availability are important variables controlling marine heterotrophic bacterial activity. However, particularly in tropical regions it remains to be determined how these variables jointly affect bacterial activity. In this study we show how bacterial carbon cycling in two tropical coastal ecosystems (the Great Barrier Reef [GBR, Australia] and the Red Sea [Saudi Arabia]) are influenced by changing temperature (using a 6°C gradient) and the addition of dissolved organic carbon (DOC) from different sources (addition of mangroves and seagrass leafmates, plus natural seawater). Our study demonstrates that elevated temperatures in the GBR increased bacterial organic carbon processing, while in the Red Sea no clear effects were found. More of the added DOC was degraded in the Red Sea but this additional carbon did not increase the biomass production, due to low bacterial growth efficiencies in all treatments. In addition, increasing temperatures in the GBR resulted in lower bacterial growth efficiencies, while no clear impact were found in the Red Sea. In conclusion, this study suggests that site-specific ecosystem differences (e.g., different microbial and macrophyte community composition) may override general responses to temperature and substrate in tropical coastal waters.

Plain Language Summary  This is the first assessment of how bacterial carbon cycling in two tropical coastal ecosystems (the Great Barrier Reef [GBR] and the Red Sea) are influenced by changing temperature and organic carbon sources. Results from comparable experiments conducted in both ecosystems demonstrated that more of the added organic carbon was degraded in the Red Sea, but that this extra carbon was mainly respired. In addition, increasing temperature in the GBR enhanced the role of bacteria as a carbon dioxide source while no clear impact was found in the Red Sea. Overall, we conclude that substrate additions had a larger impact on bacterial carbon cycling than increasing temperature, but that these impacts varied between ecosystems.

1. Introduction

Around 10% of the world’s coastal water lies within the tropics (Jahnke, 2010). Tropical coastal waters naturally have high temperatures and sunlight levels, and are amongst the most active biogeochemical zones in the ocean (Brunskill, 2010; Jennerjahn, 2012; Lønborg et al., 2021). Despite this importance, comparatively few studies in tropical coastal waters have investigated the role of microbes in biogeochemical cycling and which environmental factors impact their physiology. In polar and temperate coastal waters, given its large variability, temperature emerges as a key control variable (Hute-Staufer et al., 2015; Pomeroy & Wiebe, 2001). On the contrary, in tropical coastal waters temperatures are more stable, but it is still uncertain whether temperature is a major controlling factor (Lønborg et al., 2021). Some studies suggest that in these waters microbial processes are already functioning close to their optima; therefore, increasing temperatures further should not have any large impact or a negative influence on biogeochemical process rates (Morán et al., 2017; Wiebe & Pomeroy, 1999). However, other studies suggest that tropical marine microbes actually increase their activity under warmer conditions (Lønborg et al., 2019; McKinnon et al., 2017).
Dissolved organic matter (DOM) is the most important carbon (dissolved organic carbon [DOC]) and nutrients (nitrogen and phosphorus among others) source for heterotrophic bacterial growth in coastal waters (Lønborg & Álvarez-Salgado, 2012). In coastal waters the main sources of DOM include phytoplankton, dominated in tropical regions by picoplankton (Buitenhuis et al., 2012; Furnas et al., 2005), and in nearshore areas coastal vegetation, which in some tropical regions are dominated by mangroves and seagrasses (Alongi, 2020; Davis et al., 2003). The uptake of DOM by heterotrophic bacteria depends on molecular size, with molecules in the size range 600–1,000 Da being directly transported across the cell membrane (Weiss et al., 1991), while those of larger size require enzymatic cleavage prior to uptake (Arnosti, 2011; Baltar, 2017; Hoppe, 1983). Following uptake the organic carbon can either be used for the production of bacterial biomass (bacterial production [BP]) or be respired (bacterial respiration [BR]), with the ratio between BP and the sum of BP and BR being termed the bacterial growth efficiency (BGE) (Giorgio & Cole, 1998). In coastal waters the BGE varies widely (approx. range from 0.1% to 63%) depending on factors such as substrate conditions (e.g., inorganic nutrient availability), DOM quality and environmental variables such as temperature and sunlight (Lønborg et al., 2011, 2016; Rivkin & Anderson, 1997; Rivkin & Legendre, 2001). Commonly, temperature, and substrate availability are believed to be interactive limiting factors for heterotrophic bacteria in parts of the ocean (Huete-Stauffer et al., 2015; López-Urrutia & Morán, 2007; Pomeroy & Wiebe, 2001), suggesting that studying only one limiting factor might be insufficient. Determining the impact of temperature on biogeochemical processes in tropical waters is especially important, as microbes in these waters have been suggested to already operate close to their temperature optima. Additionally, in oligotrophic conditions, as frequently found in many tropical coastal waters, it has been demonstrated that organic compounds contain the majority of the bioavailable carbon and nutrients (Lønborg et al., 2018). Therefore, while some studies have focused on bacterial organic carbon cycling in tropical coastal waters, no study has to our knowledge, made a cross-comparison of how changing temperature and DOM source influences these processes. Determining such potential links could help determine which factors regulate key biogeochemical processes in these underexplored low latitude regions.

In this study, we conducted similar short-term (4 days) laboratory incubation experiments in coastal waters of the Great Barrier Reef (GBR), Australia (19°S and 147°E) and the Red Sea, Saudi Arabia (22°N and 39°E), which present similar environmental conditions. The hypotheses to be tested in this study was that: (a) warming will increase the rates of microbial carbon processing in both the GBR and Red Sea and (b) substrate bioavailability will regulate the magnitude of the heterotrophic bacterioplankton activity response. To test these hypotheses we investigated how varying temperature and specific macrophyte DOM substrates from seagrasses and mangroves impact microbial organic carbon processing. We chose to use these macrophyte groups as they are important sources of organic matter sustaining the microbial activity in nearshore tropical coastal waters (Alongi & McKinnon, 2005). Our experimental approach consisted of incubating ambient surface heterotrophic bacterial assemblages with ambient and macrophyte DOM over 4 days at temperatures found in situ (26°C–27°C), plus at 3°C below and above this value. Changes in total heterotrophic bacterial biomass, physiological state (relative nucleic acid content, live and dead plus actively respiring cells), extracellular enzymatic activity (EEA), together with BGE were determined. Previous published studies have assessed in detail the results from the experiments conducted in the GBR (Baltar et al., 2017; Lønborg et al., 2019; Morán et al., 2020). This study complements those studies by including the experiments conducted in the Red Sea and compare for the first time how tropical planktonic bacteria from two different tropical systems are impacted by temperature and different macrophyte DOC sources. The GBR and Red Sea assessed here share virtually the same latitude (only 3° difference) and were at the time of sampling characterized by the same in situ temperature, but they differed in the mangrove and seagrass species present. Thus, while a number of mangrove and seagrass species are found in both ecosystems we chose in this study to use the species that were dominant at each site.

2. Material and Methods

2.1. Study Areas

The GBR is situated on the Australian North East coast covering an area of 224,000 km² (Hopley et al., 2007). About 7% and 20% of the shelf sediments are covered with coral reefs and seagrasses respectively, while mangrove forests alongside shore inhabit around 1.045 km² (Hopley et al., 2007). The system has a monsoonal climate, characterized by a wet summer (December–March) season and a dry winter season. In the GBR, there are large variations in surface water temperatures (ranging from around 20°C to 31°C) both temporally and spatially.
Both mangrove and seagrass ecosystems are large suppliers of carbon and nutrients in the coastal zone, thus both are important in influencing the biogeochemistry of the GBR (Alongi et al., 2013). The Red Sea is a narrow, semi-enclosed oceanic basin situated between the continents of Africa and Asia, and covers a sea surface area of 460,000 km² (Patzert, 1974). The Red Sea is surrounded by arid land masses with low precipitation, no riverine input and high evaporation rates (Sofianos & Johns, 2003), which makes it one of the warmest, most oligotrophic and saltiest marine basins in the world (Raitos et al., 2011). Beside its extreme environmental conditions, the Red Sea is home to extensive mangrove forests (Almahasheer et al., 2016) and the seagrass communities are highly diverse, with 12 species potentially dominating shallow environments (Kenworthy et al., 2006). Both the GBR and Red Sea are characterized by actively growing microbial communities which rapidly cycle carbon and nutrients (Carreira et al., 2021; Silva et al., 2019), with the DOM pool containing most of the carbon and nutrients used (Lønborg et al., 2018). In this study, we collected different initial microbial communities as well as used different seagrass and mangrove species at each site, so that the derived DOM likely had different chemical composition. Therefore some of the observed differences during our experiments could, to some degree, be due to differences in DOM composition and/or differences in the microbial populations.

### 2.2. Experimental Setup

To jointly test the impact of temperature and source-specific DOM on the pelagic heterotrophic bacterial communities, we added DOM from three different sources: marine surface water as control, DOM leached from the seagrasses *Halodule uninervis* (GBR) and *Cymodocea serrulata* (Red Sea), and from leaves of the mangroves *Rhizophora stylosa* (GBR) and *Avicennia marina* (Red Sea), respectively. These species were used, as they are abundant seagrass and mangrove species in each respective ecosystem. Seawater samples were collected during the spring period (18 October 2016 in the GBR and 9 March 2017 in the Red Sea) at sites (Figure S1 in Supporting Information) distant from direct anthropogenic impact. At both sites water was collected at 5 m depth using acid-cleaned Niskin bottles and thereafter pooled into 50 L acid-washed carboys. After collection, the seawater was stored in the dark until used approximately 2 hr later. The water temperature was measured directly after collection; while salinity levels were determined, using a laboratory based Portasal Model 8410 A (GBR) or a conductivity-temperature-depth (CTD) (Red Sea) sensor. Water samples for chlorophyll *a* (chl *a*) analysis were at both sites collected by filtering seawater (200 mL) through a GF/F filter and analyzed after 90% acetone extraction with a Turner Designs 10000R fluorometer.

Seawater filtrations was initiated at both sites within 1 hr after sample collection. To establish a microbial culture one part of the collected seawater was filtered through pre-combusted (450°C, 4 hr) GF/C filters (~1.2 μm pore size) with the filtrate being kept in the dark at in situ temperature (~27°C in the GBR and ~26°C in the Red Sea) until used approximately 2 hr later. The remaining seawater was gravity filtered through a dual-stage (0.8/0.2 μm) filter cartridge ( Pall-Acropak Supor membrane), which had been pre-washed with ultraclean water (>10 L). The 0.2 μm filtered seawater was subsequently used as the control experiment and to dilute the DOM derived from the seagrass and mangrove leaves.

In the GBR fresh seagrass and mangrove leaves were collected in Cleveland Bay (Figure S1 in Supporting Information), Australia, while in the Red Sea they were collected in Al Qadimah lagoon (Figure S1 in Supporting Information), Saudi Arabia. Both the seagrass and mangrove leaves were collected at similar nearshore stations with a water depth of around 1 m (Figure S1 in Supporting Information). Additionally in both case the time of sampling was at same period of the seasonal cycle (i.e., late spring).

In the laboratory, the seagrass and mangrove leaves were rinsed carefully with 0.2 μm prefiltered surface seawater. The seagrass and mangrove-derived DOM was extracted by adding approximately 25 g of dried leaves (60°C) to a glass bottle containing 1–2 L of 0.2 μm-filtered seawater. After extraction (24 hr) in the dark the water was filtered firstly through a pre-combusted GF/C filter and, then, through a dual-stage filter cartridge (0.8/0.2 μm Pall-Acropak Supor membrane) to isolate the DOM fraction. The DOC and inorganic nutrient concentrations in all these solutions were then measured. The seagrass and mangrove derived DOM were added to different 20–40 L carboys to reach a DOC enrichment of approximately 40 μmol L⁻¹ (which resembled the seasonal build-up of DOM in both systems; (Calleja et al., 2019; Lønborg et al., 2017)). The GF/C filtered microbial culture was added as 1 part to 9 parts of water (Control, seagrass, and mangrove). The water was in both experiments distributed into 135 acid cleaned glass bottles (500 mL) and incubated in the dark at three temperatures (in situ, −3°C, and +3°C). Three replicates of each treatment were collected for analysis at Days 0, 1, 2, 3, and 4. The
experiments were performed in Australia at the National Sea Simulator (SeaSim) of the Australian Institute of Marine Science and in Saudi Arabia at the Coastal and Marine Resources Core Lab of King Abdullah University of Science at Technology, which can keep the temperature constant with an accuracy of ±0.1°C.

Changes in total bacterial abundance (BA), cell size as well as the groups of low and high nucleic acid content (LNA and HNA, respectively), live and dead cells (membrane-intact and membrane-damaged, respectively) and actively respiring cells (those able to reduce CTC) were monitored daily in unfiltered water samples (Giorgio & Gasol, 2008). Total EEA was also measured with unfiltered water. Samples for the dissolved phase were collected by filtration through prewashed (250 mL Milli-Q water) 0.2 μm filters (Pall, Supor membrane Disc Filter) to track changes in ammonium (NH$_4^+$, GBR only), nitrate/nitrite (NO$_3^−$/NO$_2^−$), dissolved inorganic phosphate (DIP: HPO$_4^{2−}$), DOC, total dissolved nitrogen (TDN), and total dissolved phosphorus (TDP, GBR only). The water samples for inorganic nutrient and TDP analysis were stored in 20 mL acid washed polyethylene bottles and kept frozen (−20°C) until analysis. Sub-samples (10 mL) for DOC and TDN analysis were collected in pre-combusted (450°C, 12 hr) glass containers and preserved by adding 50 μL of 25% H$_2$PO$_4$.

### 2.3. Sample Measurements

The inorganic nutrient concentrations (NH$_4^+$, NO$_3^−$/NO$_2^−$, and DIP) were determined using segmented flow analysis (Hansen & Koroleff, 1999). Please note that NH$_4^+$ concentrations could only be analyzed in the GBR, due to problems with the segmented flow analysis during the Red Sea experiment.

The concentrations of DOC and total dissolved organic nitrogen (TDN) were measured using a Shimadzu TOC-L carbon analyzer coupled in series with a nitric oxide chemiluminescence detector. Concentrations were calculated by subtracting a Milli-Q blank and dividing by the slope of a daily standard curve. During the measurement runs we regularly analyzed deep ocean reference samples, with average DOC and TDN concentration (43 ± 1 μmol C L$^{-1}$ and 32.2 ± 2.0 μmol N L$^{-1}$—GBR; 41 ± 1 and 32.3 ± 2.4 μmol L$^{-1}$—Red Sea) being comparable with those obtained by the reference laboratory (Prof. Hansell Lab; 41–44 μmol C L$^{-1}$ and 32.2–33.7 μmol N L$^{-1}$). The difference between the initial and minimum DOC concentration over the experimental period was in this study defined as the bioavailable DOC (BDOC). Analysis of TDP was conducted by oxidation (121°C, 70 min) under alkaline conditions by persulphate digestion of the water samples (Valderrama, 1981), which were then analyzed for inorganic nutrients. Dissolved organic nitrogen (DON) concentrations were calculated as the difference between TDN and dissolved inorganic nitrogen (DIN, NH$_4^+$+ NO$_3^−$/NO$_2^−$) (DON = TDN − DIN) and dissolved organic phosphorus (DOP) as the difference between TDP and DIP (DOP = TDP − DIP).

Initial fluorescence of colored DOM was in the GBR recorded with a Jasco FP-8500 luminescence spectrofluorometer, while in the Red Sea an HORIBA Jobin Yvon AquaLog spectrofluorometer was used. Both instruments used a 1 cm path length quartz cuvette and Excitation (Ex) and emission (Em) slit widths were 10 nm. Concentrations could only be analyzed in the GBR, due to problems with the segmented flow analysis during the Red Sea experiment. Single-point measurements were performed at peak-T (aromatic amino acids, average Ex/Em: 280/350 nm), peak-A (general humic compounds, average Ex/Em: 250/435 nm), peak-C (terrestrial humic substances, average Ex/Em: 320/410 nm; which were obtained by the reference laboratory (Prof. Hansell Lab; 41–44 μmol C L$^{-1}$ and 32.2–33.7 μmol N L$^{-1}$). A Milli-Q water blank was also recorded in order to subtract these from the sample values and thereby eliminate the water Raman scatter. Subsequently, fluorescent DOM (FDOM) peak values were normalized to the Milli-Q water Raman peaks to account for potential instrument-dependent intensity factors and results are reported in Raman units (R.U.).

The samples for BA (3 mL) were initially fixed with glutaraldehyde (0.5% final concentration) for 30 min at 4°C, and thereafter flash frozen in liquid nitrogen and stored at −80°C until analyzed, typically within less than 4 days. After thawing, samples were stained with SYBR Green I (Invitrogen-Molecular Probes) for 15 min in the dark and analyzed using a flow cytometer (FACSCaliber—GBR and FACSCanto II—Red Sea), with abundances calculated using daily flow rates as described previously (Gasol & Morán, 2015). Bacterial side scatter (SSC) signals were standardized using 1 μm fluorescent latex beads (Molecular Probes, ref. F-13081) in order to estimate the mean biovolume for which we used an empirical calibration between relative SSC and cell diameter (Calvo-Díaz & Morán, 2006), therefore assuming a spherical cell shape. Finally, biovolume was converted into cellular carbon content using a previously established relationship (Norland, 1993).

The separation of live (membrane-intact) from dead (membrane-damaged) cells, was done after double staining with SYBR Green I (Molecular Probes) and addition of propidium iodine (PI, Sigma) (Grégori et al., 2001). Dead cells were excluded from the analysis due to problems with the segmented flow analysis during the Red Sea experiment.
cells were distinguished from live ones by a higher red fluorescence signal due to PI. The abundances of LNA and HNA bacteria were determined on samples that had been fixed with glutaraldehyde (0.5% final concentration), flash frozen in liquid nitrogen and stored at −80°C until analysis within 1 week of collection. After staining with SYBR Green I LNA and HNA cells could be clearly separated in green fluorescence versus SSC plots. At both sites Prochlorococcus cyanobacteria were present in the inoculum but after GF/C pre-filtering, their abundance in the experiments were around 10 times lower (4.5 × 10^3—GBR and 4.2 × 10^3 cells ml^-1—Red Sea) and they could be easily separated from the HNA cells. The heterotrophic bacteria with an active electron transport chain (CTC+; i.e., those capable of reducing 5-cyano-2,3-dinitol tetrazolium chloride) were identified in red fluorescence and green fluorescence versus SSC cytograms of fresh samples incubated with 5 mmol L^-1 CTC for 1.5 hr.

The BGE over the 4 days experiment was calculated as bacterial growth (BG: net increase in bacterial biomass between day 0 and the maximum, calculated from abundance and volume) divided by the bioavailable DOC (BDOC, BGE = BG/BDOC).

The potential activity rates of leucine aminopeptidase (LAPase), β-glucosidase (BGase), and alkaline phosphatase (APase) was determined using the hydrolysis of the fluorogenic substrate analogs L-leucine-7-amido-4-methylcoumarin, 4-methylumbelliferyl (MUF)-β-D-glucoside and MUF-phosphate, respectively (Hoppe, 1983). Here LAPase is a proteolytic enzyme, BGase is a glycolytic enzyme, and APase is an esterase involved in the acquisition of P from DOM. The procedure used followed approaches previously described (Baltar et al., 2017; Lønborg et al., 2019). Briefly, EEA was determined after substrate addition and incubation using a spectrofluorometer fitted with a microwell plate reader (Biotek Cytation 3—GBR and Tecan—Red Sea) at excitation and emission wavelengths of 365 and 445 nm, respectively. Samples (300 μL) were incubated in the dark at the separate temperatures (in situ, −3°C, and +3°C) for 1.5–3 hr depending on activity. The increase in fluorescence over time was thereafter converted into activity using a standard curve consisting of the fluorochromes 4-methylumbelliferyl and 7-amido-4-methylcoumarin added to 0.2 μm filtered sample water. A final substrate concentration of 250 μmol L^-1 was used in both experiments, which was determined as saturating substrate concentrations for these experiments.

2.4. Data and Statistical Analysis

In this manuscript, we for some key variables reported trapezoidal integrated values (the area under the curve) over the experimental period in order to provide a collective response of these variables to the different treatments. In order to test the significance of the effects of site (GBR, Red Sea), DOM source (control, mangrove, seagrass), and temperature treatment (using in situ, −3°C, and +3°C as categories) we used three-way analysis of variance (ANOVA) for the variables where real replicates and not only average values can be reported (i.e., BDOC and BGE). Regression analyses (Figure S2 in Supporting Information S1) were conducted using the best-fit between the two variables X and Y (Sokal & Rohlf, 1995).

3. Results

3.1. Environmental Conditions

Generally, the environmental conditions during seawater sample collection were similar in the two systems and comparable to the typical spring conditions found in the GBR and the Red Sea (Al-Otaibi et al., 2020; Carreira et al., 2020; Lønborg et al., 2017). The salinities (35.9—GBR and 39.1—Red Sea) were similar to open ocean conditions found in both regions. Temperatures were, characteristically elevated for tropical waters and comparable to the typical spring conditions found in the GBR and the Red Sea (Al-Otaibi et al., 2017). The salinities (35.9—GBR and 39.1—Red Sea) were in both systems close to the detection limit, while the DOC concentrations (83 ± 1—GBR and 80 ± 1 μmol C L^-1—Red Sea) were within the range found in coastal waters globally (Lønborg & Álvarez-Salgado, 2012). The initial TDN varied between treatments with highest concentrations in the GBR mangrove treatment (28.5 ± 1.8 μmol N L^-1), followed by the same treatment in the Red Sea (20.9 ± 1.1 μmol N L^-1, Table 1). The DOC:TDN ratio consistently showed at both sites the lowest values in the mangrove and the highest in the seagrass treatments (Table 1). In the GBR both the TDP and DOP concentrations had the highest levels in the mangrove treatment (1.15 ± 0.10 and 0.28 ± 0.11 μmol P L^-1), followed by the seagrass (0.58 ± 0.01 and 0.27 ± 0.03 μmol P L^-1) and control

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The FDOM signals showed clear differences in both total and the C-specific signals between treatments and sites suggesting differences in the CDOM chemical composition (Table S1 in Supporting Information S1). Initial levels in the seawater experiments had generally the lowest FDOM signals, while the mangrove samples exhibited

(0.33 ± 0.03 and 0.25 ± 0.04 µmol P L⁻¹; Table 1). The initial C:N:P stoichiometry for the DOM pool was in the GBR 336(±63): 24(±5): 1 in the control, 474(±56): 24(±4): 1 in the seagrass and 447(±183): 102(±47): 1 in the mangrove treatment (Table 1).

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### Table 1

**Initial Concentrations of Dissolved Inorganic Phosphate and Nitrate/Nitrite (NO₃⁻/NO₂⁻) Are Shown**

|                | Control | Seagrass | Mangrove |
|----------------|---------|----------|----------|
|                | −3°C    | In situ  | +3°C     | −3°C     | In situ  | +3°C     |
| DIP (µmol P L⁻¹) | 0.08 ± 0.02 | 0.30 ± 0.02 | 0.87 ± 0.01 |
| NO₃⁻/NO₂⁻ (µmol N L⁻¹) | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.02 ± 0.01 |
| DOC₀ (µmol C L⁻¹) | 83 ± 1 | 128 ± 1 | 126 ± 1 |
| DOC₆₀₀ (µmol C L⁻¹) | 72 ± 1 | 66 ± 1 | 68 ± 1 |
| BDOC (µmol C L⁻¹) | 11 ± 1 | 14 ± 1 | 15 ± 2 |
| BDOC (%) | 13 ± 2 | 17 ± 2 | 18 ± 3 |
| TDN | 6.2 ± 0.1 | 6.7 ± 0.2 | 28.5 ± 1.8 |
| DON | 5.7 ± 0.1 | 6.4 ± 0.3 | 28.2 ± 1.9 |
| TDP | 0.33 ± 0.03 | 0.58 ± 0.01 | 1.15 ± 0.10 |
| DOP | 0.25 ± 0.04 | 0.27 ± 0.03 | 0.28 ± 0.11 |
| DOC:TDN | 13(±1): 1 | 19(±1): 1 | 4(±1): 1 |
| DOC:DON | 14(±1): 1 | 20(±1): 1 | 4(±1): 1 |
| DOC:DOP | 24(±5): 1 | 23(±4): 1 | 102(±47): 1 |
| DOC:DON:DOP | 336(±63): 24(±5): 1 | 474(±56): 24(±4): 1 | 447(±183): 102(±47): 1 |

#### Red Sea

|                | Control | Seagrass | Mangrove |
|----------------|---------|----------|----------|
|                | −3°C    | In situ  | +3°C     | −3°C     | In situ  | +3°C     |
| DIP (µmol P L⁻¹) | 0.05 ± 0.01 | 0.59 ± 0.02 | 0.20 ± 0.02 |
| NO₃⁻/NO₂⁻ (µmol N L⁻¹) | 0.03 ± 0.04 | 0.07 ± 0.05 | 0.13 ± 0.15 |
| DOC₀ (µmol C L⁻¹) | 80 ± 1 | 125 ± 1 | 125 ± 2 |
| DOC₆₀₀ (µmol C L⁻¹) | 78 ± 1 | 72 ± 2 | 77 ± 1 |
| BDOC (µmol C L⁻¹) | 6 ± 2 | 2 ± 1 | 3 ± 1 |
| BDOC (%) | 8 ± 4 | 2 ± 2 | 4 ± 1 |
| TDN | 8.4 ± 0.6 | 11.3 ± 0.3 | 20.9 ± 1.1 |
| DON | N.D | N.D | N.D |
| TDP | N.D | N.D | N.D |
| DOP | N.D | N.D | N.D |
| DOC:TDN | 9(±1): 1 | 11(±1): 1 | 6 (±1): 1 |
| DOC:DON | N.D | N.D | N.D |
| DOC:DOP | N.D | N.D | N.D |
| DOC:DON:DOP | N.D | N.D | N.D |

**Note.** The dissolved organic carbon (DOC) concentration at day 0 (DOC₀) and the minimum concentration (DOC₆₀₀) measured during the experiments are also shown, together with the bioavailable fraction (BDOC). Initial total dissolved nitrogen (TDN) and phosphorus (TDP), and dissolved organic nitrogen (DON) and phosphorus (DOP) concentrations are where available shown. The DOC:TDN, DOC:DON, DOC:DOP as well as the DOC:DON:DOP stoichiometry ratios are also shown where available. Values are averages of the three replicate bottles ± standard deviation. N.D.—not determined.
the highest in the GBR and the seagrass showed highest values in the Red Sea (Table S1 in Supporting Information S1). For peak-T, the mangrove in the GBR had the highest C-specific fluorescence, while the seagrass treatment in the Red Sea had a generally higher humic (Peak-A, Peak-C, and Peak-M) contribution (Table S1 in Supporting Information S1). The ratio of humic-like to protein-like fluorescence (peak C/peak T) had highest levels in seagrass experiment in the Red Sea (Table S1 in Supporting Information S1).

3.2. Dissolved Organic Carbon Degradation With Changing Source and Temperature

Over the 4 days incubation, different bioavailable DOC (BDOC) levels were found between experiments (Figure 1, Table 1). The highest BDOC values were found in the Red Sea mangrove treatment (between 43% ± 3% and 51% ± 2% of initial DOC), followed by the same treatment in the GBR (between 19% ± 1% and 27% ± 1% of initial DOC (Table 1). The TDN concentrations were correlated with BDOC ($r = 0.85$, $p = 0.0033$, $n = 9$) and the slope (3.0) was indicating a preferred N uptake compared with C. The addition of seagrass DOC also had different impacts, with higher BDOC in the Red Sea compared with the GBR (Figure 1, Table 1). In the GBR higher temperature consistently resulted in higher DOC degradation (BDOC), while no systematic effect of temperature was found in the Red Sea (Figure 1, Table 1). It is noteworthy that in the GBR the addition of seagrass extract reduced the BDOC even below the level of the control. Previous studies have suggested that some seagrass extracts appear to have “antibacterial” compounds which could depress growth (Kannan et al., 2010). However, as the BB and BGE in the seagrass were on the contrary similar or higher than in the control experiments we are

![Figure 1. The measured (a and c) bioavailable dissolved organic carbon (BDOC) and (b and d) bacterial growth efficiency (BGE) integrated over 4 days in the (a and b) Great Barrier Reef (GBR) and (c and d) Red Sea in the Control, Seagrass, and Mangrove treatments performed at −3°C (+), in situ (○), and +3°C (●). Error bars represent standard deviations. The integrated BGE was calculated as the bacterial growth divided by the BDOC. Please note that a bracket is used in the y-axis for the BGE in the Red Sea due to the large difference compared to the GBR.](https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2022JG006890)
not 100% certain for the precise explanation for these results. Despite that DOP was only measured in the GBR experiments, no significant increase in the initial levels was observed in the seagrass or mangrove leachates with respect to the control (Table 1). However, the DIP measurements, available for all sites and DOM substrates, showed that phosphorus tended to be consumed in most cases (72% of the 18 DOM and temperature experiments), suggesting strong P limitation of the DOM available for bacterial processing, especially in the macrophyte treatments (Figure S2 in Supporting Information S1). On average, DIP was produced at a slow rate in both control experiments (0.003 ± 0.006 and 0.007 ± 0.002 μmol L\(^{-1}\) d\(^{-1}\) at the GBR and Red Sea, respectively), which showed also very low initial NO\(_3^-\)/NO\(_2^-\) concentrations (Table 1). The higher the DIP initial concentration, the higher its consumption rate (see Figure S2 in Supporting Information S1).

### 3.3. Bacterial Response to Different Substrates and Temperatures

The number of initial cells (around 1 × 10\(^5\) Cells mL\(^{-1}\)) were similar in all experiments independent of treatment or site (Figure S3 in Supporting Information S1, Table 2). The BAs were highest in the GBR mangrove treatment (between 32.04 ± 2.96 and 30.05 ± 1.86 × 10\(^5\) Cells mL\(^{-1}\)), followed by the seagrass treatment in the Red Sea (between 6.75 ± 0.74 and 8.01 ± 1.41 × 10\(^5\) Cells mL\(^{-1}\)) (Table 2). As expected, bacterial biomass (BB) increased in all experiments following the consumption of DOC (Figure 2, Table 2). Although clear differences in cell size were observed, BB was largely driven by changes in BA. Higher biomass increases were found in the GBR than in the Red Sea following DOC addition, with the exception of the seagrass treatment, where higher growth was found in the Red Sea (Figure 2, Table 2). The highest BB (10 μmol C L\(^{-1}\)) was reached in the mangrove treatment (day 3) in the GBR experiment (Figure 2, Table 2). Both the study site and DOM source yielded significant differences in the maximum BAs attained but not temperature (Table S2 in Supporting Information S1).

In the Red Sea all treatments had low integrated BGE values (range 2%–8%), which were comparable to those found in the control and seagrass treatments in the GBR (Figure 1). The mangrove treatment in the GBR had notably higher BGE values (between 27% and 43%). In the GBR, increasing temperature decreased the BGE within treatments, while no impacts were found in the mangrove and seagrass treatments in the Red Sea. In the Red Sea, the control and seagrass treatments showed marginally higher BGE at in situ than at the other temperatures (Figure 1).

In both sites the abundance of HNA cells increased notably from initially low contributions (~40%; GBR; ~20%; Red Sea) and became the dominant group (Figure S4 in Supporting Information S1). In the GBR average %HNA values ranged from 59% (Control) to almost 100% (Mangrove), with seagrass reaching 70%, while in the Red Sea %HNA values were less variable, with averages between 56% (Control) and close to 70% (Mangrove and seagrass) (Figure S4 in Supporting Information S1). Temperature did not have any clear impact on the relative contribution of HNA cells in the GBR, although the %HNA increased consistently with temperature in the Red Sea experiments (1.5%–1.9°C\(^{-1}\)). Maximum %HNA values reached during the experiments were however significantly different for each individual factor assessed in the three-way ANOVA, as well as the interactions between site and DOM source and between site, DOM source and temperature (Table S2 in Supporting Information S1).

Live cells made up the bulk of the community in all treatments except in the seagrass treatment in the GBR, where dead cells (i.e., cells with compromised membranes) occasionally became dominant (Figure S5 in Supporting Information S1). The contribution of live cells (%live) therefore tended to be higher in the Red Sea (Figure S5 in Supporting Information S1). Since one of the most conspicuous findings was the decrease in %live over time in some treatments, we also conducted a three-way ANOVA with the minimum value reached during the incubations. As well as between the study areas, significant differences were observed with DOM source and temperature levels (Table S2 in Supporting Information S1).

The CTC+ cells generally increased over the course of the experiment from initial values of 3% (GBR) and 74% (Red Sea), reaching generally higher mean values in the Red Sea than in the GBR (Figure S6 in Supporting Information S1), which was statistically significant when maximum %CTC+ values attained were considered (Table S2 in Supporting Information S1). A common feature at both sites is the significant negative impact of increasing temperature on the %CTC+, with values ranging from −0.1% to −6.5°C\(^{-1}\). This decrease was more marked in the Red Sea experiments (Figure S6 and Table S2 in Supporting Information S1).
Table 2

|                   | Control | Seagrass | Mangrove |
|-------------------|---------|----------|----------|
|                   | -3°C    | In situ  | +3°C     | -3°C     | In situ  | +3°C     | -3°C     | In situ  | +3°C     |
| BA₀ (Cells × 10⁶ ml⁻¹) | 1.24 ± 0.09 | 1.43 ± 0.10 | 1.41 ± 0.08 |
| BA_max (Cells × 10⁶ ml⁻¹) | 2.75 ± 0.17 | 3.02 ± 0.25 | 3.37 ± 0.33 |
| BB₀ (μmol C L⁻¹) | 0.09 ± 0.01 | 0.10 ± 0.01 | 0.09 ± 0.01 |
| BB_max (μmol C L⁻¹) | 0.36 ± 0.02 | 0.36 ± 0.01 | 0.63 ± 0.04 |
| BG (μmol C L⁻¹) | 0.27 ± 0.02 | 0.27 ± 0.03 | 0.53 ± 0.08 |
| %HNA₀ | 39 ± 3 | 38 ± 3 | 35 ± 3 |
| %HNA_max | 67 ± 5 | 75 ± 4 | 99 ± 1 |
| %Live₀ | 90 ± 9 | 75 ± 3 | 76 ± 6 |
| %Live_max | 91 ± 2 | 67 ± 5 | 96 ± 1 |
| %CTC₀ | 3 ± 1 | 3 ± 1 | 3 ± 1 |
| %CTC_max | 15 ± 2 | 24 ± 1 | 140 ± 80 |
| APase_int (nmol L⁻¹) | 12 ± 1 | 26 ± 3 | 273 ± 22 |
| BGase_int (nmol L⁻¹) | 0.6 ± 0.3 | 6.8 ± 1.3 | 102 ± 16 |
| LAPase_int (nmol L⁻¹) | 43 ± 3 | 201 ± 26 | 241 ± 43 |

Note. The integrated enzymatic activities over the 4 days experimental period for alkaline phosphatase (APase_int), β-glucosidase (BGase_int), and leucine aminopeptidase (LAPase_int) are also shown. Values are averages of the three replicate bottles ± standard deviation.

3.4. Extracellular Enzymatic Activity (EEA) With Changing Substrate and Temperature

The EEA were lower in the Red Sea than in the GBR, with significant main effects (site, DOM source and temperature) differences and interactions for each enzyme (Table S2 in Supporting Information S1). In both experiments, the 4 days integrated leucine aminopeptidase (LAPase) activity (up to 394—GBR and 391 nmol L⁻¹ h⁻¹—Red Sea) was the most active extracellular enzyme followed by alkaline phosphatase (APase) (up to 341—GBR and 42 nmol L⁻¹ h⁻¹—Red Sea) and β-glucosidase (BGase) activity (up to 149—GBR and 2.6 nmol L⁻¹ h⁻¹—Red Sea) (Figures 3–5). All of the three EEEs showed a general increase over the initial experimental period (from day 1 to 3) at both sites, particularly in the mangrove and seagrass treatments (Figures 3–5). However, strong
differences were found in EEAs between the mangrove and seagrass treatments in the GBR. The LAPase was at the end of the experiments higher in the seagrass (5 times—GBR and 16 times—Red Sea) and mangrove (35 times—GBR and 20 times—Red Sea) relative to the control (Figure 3). The APase activity in the GBR increased only in the mangrove compared with the control (by >20 times, Figure 4); moreover APase in the Red Sea increased in both the mangrove (by 5 times) and seagrass (by 7 times) treatments relative to the control. The BGase activity in both the GBR and Red Sea was at the end of the experiment higher in the seagrass (4 times—GBR and 6 times—Red Sea) and mangrove (90 times—GBR and 29 times—Red Sea), relative to the control (Figure 5). In the GBR, maximum APase and BGase activities increased linear with higher temperature in all treatments (Table S3 in Supporting Information S1; except for BGase in the seagrass treatment) and LAPase activity increased in response to increasing temperature in both the seagrass and mangrove treatments while there was no clear impact in the control (Figures 3–5, Table S3 in Supporting Information S1). In the Red Sea, the response in maximum enzymatic activity to increasing temperature was only clear in the control and for BGase in the seagrass treatment (Figures 3–5, Table S3 in Supporting Information S1).

4. Discussion

Marine bacterial communities are fundamental to the ocean as they are key drivers of all major elemental cycles (Moran, 2015). Although some studies have determined bacterial cycling of DOM in coastal tropical waters (Antai et al., 2013; Carreira et al., 2021; Guenther et al., 2008, 2017; Lee et al., 2009; Lønborg et al., 2019; Ram
et al., 2003, 2007; Silva et al., 2019), none has to our knowledge compared how this might vary between tropical ecosystems. Determining such potential difference may contribute toward understanding which factors are regulating bacterial DOM cycling in these understudied environments. Our experiments were therefore designed to fill this knowledge gap and assess how combined changes in organic matter source and temperature influence bacterial processing of DOC, in two ecosystems located at approximately the same latitude in the southern and northern hemispheres, the GBR and the Red Sea, respectively. When using short-term manipulation experiments, such as in this study, the conclusions drawn have clear limitations and our results can therefore not be directly used to make predictions at the ecosystem scale as many physical, chemical and biological processes and interactions that occur in complex natural systems cannot all be included in our experiments (Carpenter, 1996; Fraser & Keddy, 1997). Nevertheless, simplistic manipulation experiments, like in our study, are a suitable approach to investigate cause and effect relationships in response to specific manipulations resembling possible natural changes.

4.1. Initial Environmental Conditions and Substrate Additions

In this study, we determined differences in the composition of the initial DOM substrate using stoichiometry and FDOM. Thus, we are not able to quantify whether there were differences in the contribution of specific molecules in the two experiments. However, with this in mind, it should be remembered that many important properties of the DOM pool (e.g., bioavailability) is not only intrinsically linked to specific molecules, but also depends on for example, environmental conditions (Baltar et al., 2021).
Comparing the DOC:TDN ratios between sites, they were slightly lower in the Red Sea, showing that the added substrate here was marginally N-richer than in the GBR. Further in the GBR comparing the ratios with the average bacterial C:N:P biomass stoichiometry of 50:10:1 (Fagerbakke et al., 1996), it indicates that bacteria at this site could have been limited by nutrients for biomass build up. However, in all our GBR experiments inorganic N accumulated over time, suggesting that the bacteria were not N limited. Although DOP was only measured in the GBR experiments, no significant increase was observed in the seagrass or mangrove leachates with respect to the control (Table 1). However, some conclusions might be drawn from the DIP dynamics, available for all sites and DOM substrates. The DIP tended to be consumed in most cases (72% of all treatments), suggesting strong P limitation of the DOM available for bacterial processing, especially that of macrophyte origin. On average, DIP was produced at a slow rate in both control treatments, which showed also very low initial DIN concentrations (Table 1). The highest DIP initial concentration and mean consumption rates corresponded to the GBR mangrove and Red Sea seagrass treatments, which were also those yielding highest bacterial biomasses, further supporting the view that P was limiting DOM degradation at both sites in the macrophyte treatments. The FDOM technique traces a sub-fraction of the CDOM pool, which fluoresces when irradiated with UV-light. Using this technique, numerous studies have demonstrated how differences in the signals can be used to trace changes in the DOM chemical composition (Catala et al., 2015; Lønborg & Álvarez-Salgado, 2014). At both sites, the FDOM levels were elevated in the mangrove and seagrass treatments compared with the control, with relative highest levels in the mangrove (GBR) and seagrass (Red Sea). Comparing the protein-like FDOM (peak-T) which is associated with bioavailable compounds and the humic-like component peak-C, it suggests clearly differences in the chemical composition of the added DOM. The C-specific contribution of peak-T in the GBR mangrove treatment suggests

**Figure 4.** Time course changes in the estimated potential enzyme activity rates of alkaline phosphatase in the 4 days experiment in the (a and d) Control, (b and e) Seagrass, and (c and f) Mangrove treatments performed at −3°C (○), in situ (●), and +3°C (●) in the (a–c) Great Barrier Reef and (d–f) Red Sea. Error bars represent standard deviations of three replicates. Please note that brackets are used in some y-axis.
a relative larger contribution of more available compounds in this treatment. Coupled with this the C-specific humic-like substances and the ratio of fluorescence of humic-like to more bioavailable protein-like compounds (peak-C/peak-T ratio), all point towards a more labile nature of the added DOM in the GBR mangrove, which also fits well with the higher BDOC levels found in this treatment.

4.2. Bacterial Response to Dissolved Organic Carbon Sources and Temperature

The physiological structure of the heterotrophic bacteria showed similarities and discrepancies in their response in the GBR and Red Sea. The different physiological structure was evidenced in the different initial values of the three indices used here, the percent contribution of HNA (%HNA), membrane-intact or live (%live) and actively respiring or CTC+ (%CTC+) cells. The contribution of HNA cells informed of changes in the contribution of the most active taxa and demonstrated a prevalence of typically copiotrophic groups as the amount of resources available per cell increased, either by dilution (Control) or by the addition of extra DOM (Mangrove and seagrass) (Vila-Costa et al., 2012). Temperature increased %HNA consistently in the Red Sea but only occasionally in the GBR (Figure S4 in Supporting Information S1), showing that at both sites the bacterial physiological structure changed during our experiments (Morán et al., 2018). However, the most obvious effect of increasing temperature, the decrease in the contribution of live and CTC+ cells, was shared at both sites. Our study shows that although warming may momentarily result in slightly higher heterotrophic bacterioplankton stocks, which we only found in the Red Sea (Figure 2) it can also consistently impact their “healthiness” (i.e., membrane-integrity and electron transport chain activity; Figures S5 and S6 in Supporting Information S1). This finding is well in line with...
with large-scale studies suggesting that low latitude plankton may be negatively affected by temperature, contrary to temperate and Polar Regions (Kirchman et al., 2009; Morán et al., 2018).

4.3. Total Extracellular Enzymatic Activity (EEA) With Changing Substrate and Temperature

Hydrolitic extracellular enzymes are primarily produced by bacteria and they represent the initial step in organic matter degradation, with their activity therefore often used to indicate microbial organic matter processing (Arnosti et al., 2014). The response of the extracellular enzymatic activities showed similarities and differences between sites, revealing potential changes in microbial organic matter processing as a response to different DOC sources and temperatures. In both the GBR and Red Sea the same order in the responses of the relative enzymatic activity to the altered conditions were found: LAPase > APase > BGase, which is consistent with patterns typically found in marine environments (Baltar et al., 2009, 2017; Lønborg et al., 2019; Nichols & Martín, 2021; Shi et al., 2019). Such relatively higher LAPase activity is commonly linked with the microbial use of organic matter for protein synthesis and increasing supply of leucine containing compounds (Kellogg & Deming, 2014; Piontek et al., 2014; Shi et al., 2019). The maximum EEA rates were generally lower in the Red Sea than in the GBR, suggesting that the response of EEA to different DOC sources will depend on the ambient microbial communities but also on the composition of the main DOC source (e.g., mangrove and seagrass species). The pronounced response of APase activity relative to the control in the GBR mangrove, and in the mangrove and seagrass treatments in the Red Sea experiment relative to the control, might indicate, as also described above, a potential P limitation of the microbial community during the experiments. Alternatively, these increased APase activities could likely be associated to the response of the organic carbon substrates, since strong increases in APase activity have been reported for microbes supplemented with labile organic carbon compounds in the presence of sufficient inorganic phosphorus (Baltar et al., 2016). In addition, the EEA response to increasing temperature was different between experiments, indicating the difficulties associated with identifying a common response of the organic matter hydrolysis capabilities of microbes to increasing temperature (Figure 6). The enzymatic activity depends on both the chemical structure of the substrates and the natural capabilities of the microbial communities (Arnosti et al., 2014), but contrary changes in enzymatic activity induced by substrate availability or other environmental factors can also impact the bacterial community composition (Teeling et al., 2012).

4.4. Bacterial Organic Matter Cycling and Response to Sources and Temperature

The bacterial degradation of DOC and the conversion of these compounds into new biomass (particulate organic carbon) is a key process describing whether they act as links (biomass) or sinks (CO₂ source). The efficiency of this transformation is known as the BGE. Previous studies have shown that substrate availability (such as nitrogen and phosphorus), quality and stoichiometry, are important regulators of BGE (Farjalla et al., 2006; Hall & Cotner, 2007; Lønborg et al., 2011; Sarmento & Gasol, 2012). However, factors such as distance of the study site from shore (La Ferla et al., 2005), season (Reinthaler & Herndl, 2005), depth (Eichinger et al., 2006),
earlier sunlight exposure (Lønborg et al., 2016) together with temperature are also important in explaining differences in BGE (Apple et al., 2006; Lønborg et al., 2019; Rivkin & Legendre, 2001). However, these factors can also be interconnected (López-Urrutia & Morán, 2007). As an example, the temperature sensitivity of bacterial processes can be strongly affected by substrate availability (Arandia-Gorostidi et al., 2017; Degerman et al., 2013; López-Urrutia & Morán, 2007; Maske et al., 2017). The studies focusing on bacterial DOC cycling in tropical coastal waters have shown a high variability in BGE (spanning from 2% to 64%; (Antai et al., 2013; Carreira et al., 2021; Guenther et al., 2008, 2017; Lee et al., 2009; Lønborg et al., 2019; Ram et al., 2003, 2007; Silva et al., 2019). In our control treatments the average BGE value (2%—GBR and 5%—Red Sea) are within this wide range but they were at the lower end of global averages reported for coastal waters (27%, (Giorgio & Cole, 1998) and 19%, (Robinson, 2008)), yet comparable to previous estimates in the GBR (2%, (Carreira et al., 2021)) and Red Sea (7%, (Silva et al., 2019)) as well as values reported for, for example, the subtropical southern East China Sea (6%, (Chen et al., 2020)). These BGE values suggest that in our control experiment around 95%–98% of the DOC degraded was respired.

The GBR and Red Sea both are commonly nutrient depleted ecosystems and it is assumed that the BGE is lower in oligotrophic than eutrophic environments (Giorgio & Cole, 1998; Reinthaler et al., 2006), which may be linked to system productivity through changes in the quantity and quality of organic carbon substrates available (Biddanda et al., 1994). Furthermore it has been suggested that at least for inland aquatic ecosystems tropical systems have lower BGE than temperate ecosystems (Amado et al., 2013). Our relatively low BGE suggest that low BGEs are also found in tropical coastal waters but whether this is a general phenomenon is not known as both high and low values have been previously reported (Carreira et al., 2021; Silva et al., 2021). In our GBR experiment, it was clear that additional bioavailable substrate increased the BGE, while contrary in the Red Sea the additions did not have any marked impact. In fact, more of the added seagrass and mangrove DOC was degraded in the Red Sea than in the GBR but this did not translate into a higher BGE (Figure 6). The comparatively higher apparent rates of BR in the Red Sea could also explain why we found much higher contributions of CTC+ cells at this site. In our experiments, there were indications that P was limiting and might partially explain the low BGE found in the experiments. However, we cannot exclude the possibility that other factors such as micronutrients or vitamins, not measured in this study, could have been limiting and precluded an efficient transformation into microbial biomass. A previous study in a tropical lagoon in Brazil has demonstrated that, similarly to our GBR experiment, the addition of different organic substrates increased BGE compared with the control, which in that study was suggested to be linked either to changes in bacterial community composition, potentially promoting a more efficient use of DOC, or to “priming” of the organic compounds present in the mixture that would allow a more efficient conversion (Fonte et al., 2013). Consistently, in our experiments the generalized and sustained increases in the contribution of HNA cells suggested changes in community composition that could have influenced the utilization efficiency. In addition, the chemical nature of the added DOC was likely different, which could have resulted in very different BGE values (del Giorgio et al., 2011). It should be noted that the seagrass and mangrove species from which the DOC was derived were different at the two sites. As there surely were species-specific differences in their biochemical composition, this also could have impacted the differences observed in BDOC and BGE between sites (Atkinson & Smith, 1983; Nordhaus et al., 2017). Additionally, the environmental conditions (light, salinity, etc.) under which the seagrass and mangrove species grew were also different to some extent, which could have impacted their biochemical composition (Mbaga & Mvungi, 2019; Twilley et al., 1997). However, as we do not have data to determine such difference we cannot conclude whether or how such changes could have affected our results. Previous studies have indicated that marine bacteria are unable to efficiently grow when large amounts of substrate are added (Eichinger et al., 2010), as such additions sustain the growth of bacteria with a lower BGE than when substrate is added at lower concentration (Landa et al., 2013). As the DOC concentration added in our experiments were relatively low and within the natural ranges found at both sites, we do not expect DOC concentration to be a major regulator of the BGE in our experiments. Rather, we suggest that the chemical composition and quality of the organic matter was likely more important (Eiler et al., 2003). Other studies have indicated that bacterial energy demand varies between systems and depends strongly on the substrate available, and therefore with the same BDOC value different bacteria may have different BGE (Giorgio & Gasol, 2008). In our experiments the production of extracellular enzymes were generally lower in the Red Sea (Figure 6), which might be connected with a higher energy demand associated with enzyme production (Farjalla et al., 2002) and therefore a lower BGE. Another factor that could have impacted our BGE results is potential differences in the impact of protistan grazers and/or viruses (i.e., top-down control), but this impact is likely minor, as was previously demonstrated for our GBR experiment (Morán et al., 2020).
Numerous studies have shown how higher temperatures can increase BP, respiration and therefore carbon demand (Amado et al., 2013; López-Urrutia & Morán, 2007; Lønborg et al., 2019), while impacts on BGE are more variable (Alonso-Sáez et al., 2008; Lemée et al., 2002; Silva et al., 2019). While some studies have proposed that BGE declines with higher temperature (Rivkin & Legendre, 2001), others have found the reverse (Jiménez-Mercado et al., 2007; Silva et al., 2019). This study demonstrates that, while exoenzymatic activity in both ecosystems was higher at increased temperatures, in the GBR elevated temperatures lowered BGE while in the Red Sea no impact was found (Figure 6). The cause of this difference could be due to variances in the species-specific responses to temperature (Bidle et al., 2002; Fuhrman et al., 2006), but also changes in the energy demand needed to uphold their metabolism and substrate affinity could explain this result (Harder, 1997; Nedwell, 1999; Piontek et al., 2015).

5. Conclusion
Overall, this study shows that in nutrient-poor tropical coastal waters such as found in the GBR and the Red Sea, the bacterial response to changing resource availability and temperature are very complex. Our results suggest that when it comes to tropical regions, it is not all the same; while substrate additions had larger importance than changing temperatures, the impacts of additional DOM may be different between ecosystems.

Data Availability Statement
All raw data is now available at Figshare: https://figshare.com/articles/dataset/Result_overview_Figshare_-_L_nborg_et_al_Xlsx/20028095.

References
Almahasheer, H., Aljowair, D., Duarte, C. M., & Igo andien, X. (2016). Decadal stability of Red Sea mangroves. Estuarine, Coastal and Shelf Science, 169, 164–172. https://doi.org/10.1016/j.ecss.2015.11.027
Alongi, D. M. (2020). Carbon balance in salt marsh and mangrove ecosystems: A global synthesis. Journal of Marine Science and Engineering, 8(10), 787. https://doi.org/10.3390/jmsea1007076
Alongi, D. M., Bouillon, S., Duarte, C. M., Ramanathan, A., & Robertson, A. (2013). Carbon and nutrient fluxes across tropical river-coastal boundaries in the Anthropocene. In Biogeochemical dynamics at major river-coastal interfaces: Linkages with global change, (pp. 373–396). Cambridge University Press.
Alongi, D. M., & McKinney, A. D. (2005). The cycling and fate of terrestrially-derived sediments and nutrients in the coastal zone of the Great Barrier Reef shelf. Marine Pollution Bulletin, 51(1–4), 239–252. https://doi.org/10.1016/j.marpolbul.2004.10.033
Alonso-Sáez, I., Vazquez-Dominguez, E., Cardelus, C., Pinhasi, J., Sala, M. M., Lekumberry, I., et al. (2008). Factors controlling the year-round variability in carbon flux through bacteria in a coastal marine system. Ecosystems, 11(3), 397–409. https://doi.org/10.1007/s10021-008-9129-0
Al-Otaibi, N., Huete-Stauffer, T. M., Calleja, M. L., Igo andien, X., & Moran, X. A. G. (2020). Seasonal variability and vertical distribution of autotrophic and heterotrophic picoplankton in the Central Red Sea. PeerJ, 8, e8612. https://doi.org/10.7717/peerj.8612
Amado, A., Meirelles-Pereira, F., Vidal, L., Sarmento, H., Suhett, A., Farjalla, V., et al. (2013). Tropical freshwater ecosystems have lower bacterial growth efficiency than temperate ones. Frontiers in Microbiology, 4, 167. https://doi.org/10.3389/fmicb.2013.00167
Antai, E. E., Asiotel, A. D., & Paul, A. (2013). Bacterial growth efficiency, respiration and secondary production and in the cross river estuary, Nigeria and the near coast. International Journal of Science and Research, 4(10), 1791–1798.
Apple, J. K., Giorgio, P. A. D., & Kemp, W. M. (2006). Temperature regulation of bacterial production, respiration, and growth efficiency in a temperate salt-marsh estuary. Aquatic Microbial Ecology, 41, 243–254. https://doi.org/10.3354/ame03243
Arandia-Gorostidi, N., Huete-Stauffer, T. M., Alonso-Sáez, L., & Morán, X. A. G. (2017). Testing the metabolic theory of ecology with marine bacteria: Different temperature sensitivity of major phylogenetic groups during the spring phytoplankton bloom. Environmental Microbiology, 19(11), 4493–4505. https://doi.org/10.1111/1462-2920.13898
Arnott, C. (2011). Microbial extracellular enzymes and the marine carbon cycle. Annual Review of Marine Science, 3(1), 401–425. https://doi.org/10.1146/annurev-marine-120709-142731
Arnott, C., Bell, C., Moorhead, D. L., Sinsabaugh, R. L., Steen, A. D., Stromberg, M., et al. (2014). Extracellular enzymes in terrestrial, freshwater, and marine environments: Perspectives on system variability and common research needs. Biogeochemistry, 117(1), 5–21. https://doi.org/10.1007/s10533-013-9906-5
Atkinson, M., & Smith, S. (1983). C: N: P ratios of benthic marine plants 1. Limnology and Oceanography, 28(3), 568–574. https://doi.org/10.4319/lo.1983.28.3.0568
Baltr, F. (2017). Watch out for the “living dead”: Cell-free enzymes and their fate. Frontiers in Microbiology, 8, 2438. https://doi.org/10.3389/fmicb.2017.02438
Baltr, F., Alvarez-Salgado, X. A., Aristeu, J., Benner, R., Hassell, D. A., Hernd, G. J., & Lønborg, C. (2021). What is refractory organic matter in the ocean? Frontiers in Marine Science, 8, 642637. https://doi.org/10.3389/fmars.2021.642637
Baltr, F., Aristeu, J., Sinteu, B., Van Aken, H. M., Gasol, J. M., & Hernd, G. J. (2009). Prokaryotic extracellular enzymatic activity in relation to biomass production and respiration in the meso- and bathypelagic waters of the (sub)tropical Atlantic. Environmental Microbiology, 11(8), 1998–2014. https://doi.org/10.1111/j.1462-2920.2009.01922.x
Baltr, F., Lundin, D., Palovaara, J., Lekumberry, I., Reinthaler, T., Hernd, G. J., & Pinhasi, J. (2016). Prokaryotic responses to ammonium and organic carbon reveal alternative CDX fixation pathways and importance of alkaline phosphatase in the mesopelagic North Atlantic. Frontiers in Microbiology, 7, 1670. https://doi.org/10.3389/fmicb.2016.01670
Baltar, F., Morán, X. A. G., & Lønborg, C. (2017). Warming and organic matter sources impact the proportion of dissolved to total activities in marine extracellular enzymatic rates. *Biogeochemistry, 133*(3), 307–316. https://doi.org/10.1007/s10533-017-0334-9

Biddanda, B., Opsahl, S., & Benner, R. (1994). Plankton respiration and carbon flux through bacterioplankton on the Louisiana shelf. *Limnology and Oceanography, 39*(6), 1259–1275. https://doi.org/10.4319/lo.1994.39.6.1259

Bidle, K. D., Manganello, M., & Azam, F. (2002). Regulation of oceanic silicon and carbon preservation by temperature control on bacteria. *Science, 296*(5560), 1980–1984. https://doi.org/10.1126/science.1076076

Brunskill, G. J. (2010). An overview of tropical margins. In K.-K. Liu, L. Atkinson, R. Quinones, & L. Talaue-McManus (Eds.), *Carbon and nutrient fluxes in continental margins: A global synthesis* (pp. 423–426). Springer.

Buitenhuis, E., Li, W. K. W., Vaulot, D., Lomas, M. W., Landry, M. R., Partensky, F., & et al. (2012). Picophytoplankton biomass distribution in the global ocean. *Earth System Science Data, 4*(1), 37–46. https://doi.org/10.5194/essd-4-37-2012

Callegari, M. L., Al-Otaibi, N., & Morán, X. A. G. (2019). Dissolved organic carbon contribution to oxygen respiration in the Central Red Sea. *Science Reports, 9*(1), 4690. https://doi.org/10.1038/s41598-019-40753-w

Calvo-Díaz, A., & Morán, X. A. G. (2006). Seasonal dynamics of picophytoplankton in shelf waters of the southern Bay of Biscay. *Aquatic Microbial Ecology, 42*, 159–174. https://doi.org/10.3354/ame042159

Carpenter, S. R. (1996). Microcosm experiments have limited relevance for community and ecosystem ecology. *Ecology, 77*(3), 677–680. https://doi.org/10.2307/2265490

Carreira, C., Carvalho, J. P. S., Talbot, S., Pereira, I., & Lønborg, C. (2020). Small-scale distribution of microbes and biochemistry in the Great Barrier Reef. *PeerJ*, 8, e10049. https://doi.org/10.7717/peerj.10049

Carreira, C., Talbot, S., & Lønborg, C. (2021). Bacterial consumption of total and dissolved organic carbon in the Great Barrier Reef. *Biogeochemistry, 154*(3), 489–508. https://doi.org/10.1007/s10533-021-00802-x

Catala, T. S., Reche, I., Fuentes-Lema, A., Romero-Castillo, C., Nieto-Cid, M., Ortega-Retuerta, E., & et al. (2015). Turnover time of fluorescent dissolved organic matter in the dark global ocean. *Nature Communications, 6*(1), 5986. https://doi.org/10.1038/ncomms6986

Chen, T.-Y., Lai, C.-C., Shiah, F.-K., & Gong, G.-C. (2020). Dissolved and particulate primary production and subsequent bacterial C consumption in different natural organic carbon concentrations. *Archiv für Hydrobiologie, 156*(1), 97–119. https://doi.org/10.1127/0003-9136/2020/0156-0097

Chen, T.-Y., Lai, C.-C., Shiah, F.-K., & Gong, G.-C. (2021). Dissolved and particulate primary production and subsequent bacterial C consumption in different natural organic carbon concentrations. *Archiv für Hydrobiologie, 157*(1), 97–119. https://doi.org/10.1127/0003-9136/2020/0156-0097

Coble, P. G. (1996). Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. *Marine Chemistry, 51*(4), 325–346. https://doi.org/10.1016/0149-0430(95)00622-3

Davis, S. E., Childers, D. L., Day, J. W., Rudnick, D. T., & Sklar, F. H. (2003). Factors affecting the concentration and flux of materials in two southern Everglades mangrove wetlands. *Ecology Progress Series, 235*, 85–96. https://doi.org/10.1146/ans10533-021-00802-x

Eichinger, M., Poggiale, J. C., Wambbeke, F. V., Lefèvre, D., & Sempère, R. (2006). Modelling DOC assimilation and bacterial growth efficiency in biodegradation experiments: A case study in the Northeast Atlantic Ocean. *Aquatic Microbial Ecology, 43*, 139–151. https://doi.org/10.3354/ame043139

Eichinger, M., Sempère, R., Grégoir, G., Charrière, B., Poggiale, J. C., & Lefèvre, D. (2010). Increased bacterial growth efficiency with environmental variability: Results from DOC degradation by bacteria in pure culture experiments. *Biogeosciences, 7*(6), 1861–1876. https://doi.org/10.5194/bg-7-1861-2010

Eiler, A., Langenheder, S., Bertilsson, S., & Tranvik, L. J. (2003). Heterotrophic bacterial growth efficiency and community structure at different natural organic carbon concentrations. *Applied and Environmental Microbiology, 69*(7), 3701–3709. https://doi.org/10.1128/AEM.69.7.3701-3709.2003

Fagerbakke, K. M., Heldal, M., & Norland, S. (1996). Content of carbon, nitrogen, oxygen, sulphur and phosphorus in native aquatic and cultured bacteria. *Aquatic Microbial Ecology, 10*, 15–27. https://doi.org/10.3354/ame010015

Farjalla, V. F., Arevedo, D. A., Esteves, F. A., Bozelli, R. L., Roland, F., & Enrich-Prast, A. (2006). Influence of hydrological pulse on bacterial growth and DOC uptake in a clear-water Amazonian lake. *Microbial Ecology, 52*(2), 334–344. https://doi.org/10.1007/s00248-006-0921-4

Farjalla, V. F., Faria, B. M., & Esteves, F. A. (2002). Bacterial growth efficiency at different natural organic carbon concentrations. *Biogeosciences, 9*(1), 97–119. https://doi.org/10.5194/bg-9-97-2012

Fonte, E. S., Amado, A. M., Morellés-Pereira, F., Esteves, F. A., Rosado, A. S., & Farjalla, V. F. (2013). The combination of different carbon sources enhances bacterial growth efficiency in aquatic ecosystems. *Microbial Ecology, 66*(4), 871–878. https://doi.org/10.1007/s00248-013-0277-1

Furnas, M., Mitchell, A., Skuja, M., & Brodie, J. (2005). In the other 90%: Phytoplankton responses to enhanced nutrient availability in the Great Barrier Reef Lagoon. *Marine Pollution Bulletin, 51*(1–4), 253–265. https://doi.org/10.1016/j.marpolbul.2004.11.010

Gasol, J. M., & Morán, X. A. G. (2015). Flow cytometric determination of microbial abundances and its use to obtain indices of community structure and relative activity. In T. J. McGinnity, K. N. Timmis, & B. Nogales (Eds.), *Hydrocarbon and lipid microbiology protocols* (pp. 159–187). Springer.

Giorgio, P. A. D., & Cole, J. J. (1998). Bacterial growth efficiency in natural aquatic systems. *Annual Reviews Ecological Systems, 29*(1), 503–541. https://doi.org/10.1146/annurev.ecolsys.29.1.503

Giorgio, P. A. D., & Gasol, J. M. (2008). Physiological structure and single-cell activity in marine bacterioplankton. *Microbial Ecology of the Oceans, 2*, 243–298.

Grégoir, G., Citterio, S., Ghiani, A., Labra, M., Sgopbi, S., Brown, S., & Denis, M. (2001). Resolution of viable and membrane-compromised bacteria in freshwater and marine waters based on analytical flow cytometry and nucleic acid double staining. *Applied and Environmental Microbiology, 67*(10), 4662–4670. https://doi.org/10.1128/aeam.67.10.4662-4670.2001

Guenthner, M., Gonzalez-Rodriguez, E., Flores-Montes, M., Araújo, M., & Neumann-Leitão, S. (2017). High bacterial carbon demand and low growth efficiency at a tropical hypereutrophic estuary. Importance of dissolved organic matter remineralization. *Brazilian Journal of Oceanography, 65*(3), 382–391. https://doi.org/10.1590/1679-87592017ame01154

Guenthner, M., Paranhos, R., Rezende, C. E., Gonzalez-Rodriguez, E., & Valentim, J. L. (2008). Dynamics of bacterial carbon metabolism at the entrance of a tropical eutrophic bay influenced by tidal oscillation. *Aquatic Microbial Ecology, 50*, 123–133. https://doi.org/10.3354/ame01154
Moran, X. A. G., Calvo-Díaz, A., Arandia-Gorostidi, N., & Huete-Stauffer, T. M. (2018). Temperature sensitivities of microbial plankton net growth rates are seasonally coherent and linked to nutrient availability. *Environmental Microbiology*, 20(10), 3798–3810. https://doi.org/10.1111/1462-2920.14393

Moran, X. A. G., Gasol, J. M., Percine, M. C., Mangot, J. F., Massana, R., Lara, E., et al. (2017). Temperature regulation of marine heterotrophic prokaryotes increases latitudinally as a breach between bottom-up and top-down controls. *Global Change Biology*, 23(9), 3956–3964. https://doi.org/10.1111/gcb.13730

Nedwell, D. B. (1999). Effect of low temperature on microbial growth: Lowered affinity for substrates limits growth at low temperature. *FEMS Microbiology Ecology*, 30(2), 101–111. https://doi.org/10.1111/j.1574-6941.1999.tb00639.x

Nichols, R. S., & Martin, P. (2021). Low Biodegradability of dissolved organic matter from southeast Asian peat-draining rivers. *Journal of Geophysical Research: Biogeosciences*, 126(6), e2020JG005682. https://doi.org/10.1029/2020JG005682

Northaus, I., Salewski, T., & Jennerjahn, T. C. (2017). Interspecific variations in mangrove leaf litter decomposition are related to labile nitrogenous compounds. *Estuarine, Coastal and Shelf Science*, 192, 137–148. https://doi.org/10.1016/j.ecss.2017.04.029

Norland, S. (1993). The relationship between biomass and volume of bacteria. In P. Kemp, B. Sherr, E. Sherr, & J. Cole (Eds.), *Handbook of methods in aquatic microbial ecology*, (pp. 303–307). Lewis Publishers.

Patzert, W. C. (1974). Wind-induced reversal in Red Sea circulation. *Deep-Sea Research and Oceanographic Abstracts*, 21(2), 109–121. https://doi.org/10.1016/0011-7471(74)90068-0

Piontek, J., Sperling, M., Nothig, E. M., & Engel, A. (2014). Regulation of bacterioplankton activity in Fram Strait (Arctic Ocean) during early summer: The role of organic matter supply and temperature. *Journal of Plankton Research*, 35(2), 83–94. https://doi.org/10.1093/plankt/bft069

Piontek, J., Sperling, M., Nothig, E. M., & Engel, A. (2015). Multiple environment changes induce interactive effects on bacterial degradation activity in the Arctic Ocean. *Limbology and Oceanography*, 60(4), 1392–1410. https://doi.org/10.1002/lno.11112

Pomeroy, L. R., & Wiebe, W. J. (1999). The temperature-substrate controversy resolved? In C. R. Bell, M. Brylinsky, & P. Johnson-Green (Eds.), *Weiss, M. S., Abele, U., Weckesser, J., Welte, W., Schulz, G. E. (1991). Molecular architecture and electrostatic properties of a bacterial porin. *Science.*, 254(5038), 1627–1630. https://doi.org/10.1126/science.1721142

Pomeroy, L. R., & Wiebe, W. J. (1999). The temperature-substrate controversy resolved? In C. R. Bell, M. Brylinsky, & P. Johnson-Green (Eds.), *Microbial biosystems: New frontiers proceedings of the 6th international symposium on microbial ecology*. https://doi.org/10.1029/2022JG006890