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1 **Differential impacts of temperature increase on prokaryotes across**

2 **temperature regimes in subtropical coastal waters: Insights from field**

3 **experiments**

4

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23 **Abstract**

24 Prokaryotic communities play a dominant role in driving biogeochemical
25 cycling in marine ecosystems. How short-term temperature increase impacts
26 prokaryotes in subtropical coastal waters is still largely unknown. Here, 14
27 field experiments were conducted to investigate the response of prokaryotes
28 in subtropical coastal waters to temperature increases of 3˚C and 6˚C,
29 encompassing a range of ambient temperatures from 17˚C to 31˚C. We found
30 that responses of prokaryotic growth, grazing pressure, community and
31 transcriptomes to increased temperatures were largely affected by ambient
32 temperatures. Increased temperatures enhanced the growth rate and grazing
33 pressure of heterotrophic prokaryotes when ambient temperatures were
34 below 26-28˚C. The increased temperatures had greater negative effects on
35 the grazing rate compared to the growth rate, therefore the abundance of
36 heterotrophic prokaryotes generally increased after temperature increase
37 across all temperature regimes. Metatranscriptomics analysis showed that at
38 an ambient temperature of 30˚C, genes involved in the ATP synthase were
39 significantly down-regulated by the increased temperature. This could be a
40 major factor contributing to the decreased prokaryotic growth rate. In
41 comparison, autotrophic prokaryotes (*Synechococcus*) exhibited better
42 performance in response to elevated temperatures, thriving up to 35˚C,
43 beyond which their growth rate experienced a dramatic decline. When

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44 exposing to extremely high temperatures, genes involved in photosynthesis
45 significantly decreased. These findings highlight the differential ecological
46 impacts of temperature increase on prokaryotic communities, varying across
47 different ambient temperatures and taxa in subtropical coastal waters.

48

49 **Keywords:** Marine heatwaves, subtropical waters, prokaryotes, growth rate,
50 grazing pressure, metatranscriptomics.

51

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52 **Introduction**

53 Marine prokaryotes are the most abundant and diverse organisms in the
54 global ocean. *Synechococcus* is the major autotrophic prokaryote in coastal
55 waters, and contributes an estimated 16.7% of global net primary production
56 (Flombaum et al., 2013). Heterotrophic prokaryotes transform organic matter
57 to CO_2 and biomass. Furthermore, they are main drivers of the microbial
58 carbon pump (Jiao et al., 2010), a pathway for marine carbon sequestration
59 that transfers liable dissolved organic matter to refractory organic matter.
60 These prokaryotes are mainly grazed by protists, which in turn transfer energy
61 and nutrients across trophic levels, sustaining diverse ecosystems. A great
62 number of studies indicate that prokaryotic growth and community
63 composition are largely driven by temperature and organic carbon (Kirchman
64 et al., 2005; Morán et al., 2017; Lønborg et al., 2022). Global warming has
65 been one of the major environmental concerns nowadays, causing extinction
66 of organisms (Penn and Deutsch, 2022) and regime shift (Parmesan and
67 Yohe, 2003; Glibert et al., 2022). Compared with long-term warming, marine
68 heatwaves, which are on the rise (Oliver et al., 2018), are possibly more
69 problematic for marine microbes (Kling et al., 2020). While prokaryotes can
70 acclimate and adapt to slow, decades-long increases in temperature (Hall et
71 al., 2010; Kent et al., 2018), it could be difficult for them to survive sudden and

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72 severe warming events, especially in low-latitudes waters where marine
73 microbes often live near their optimal temperatures (Thomas et al., 2012;
74 Chen et al., 2014). However, how short-term heatwaves impact on
75 prokaryotes in low-latitude coastal waters is largely unknown (Carreira et al.,
76 2023).
77
78 Temperature can affect prokaryotes in two fundamentally different ways: (i) It
79 directly affects the metabolic rates including the respiration rate and
80 production rate (Paerl and Huisman, 2008; Chen et al., 2014; Lewandowska
81 et al., 2014; Smith et al., 2019); (ii) it indirectly affects the mortality of
82 prokaryotes via top-down control, which includes viral lysis and grazing by
83 protists (Pernthaler, 2005; Kirchman, 2015). The relationship between
84 temperature and the growth rate /grazing rate of prokaryotes generally fits a
85 unimodal model. Below the optimum temperature, rising temperature
86 increases the growth and grazing rates, while negative effects on these rates
87 could be observed once the temperature exceeds the optimum temperature
88 (Chen and Shakhnovich, 2010; Dell et al., 2011; Liu et al., 2018). Therefore,
89 the ambient temperature could be one of important factors controlling
90 ecological impacts of temperature increase on prokaryotic community. For
91 example, our previous study revealed that in subtropical coastal waters (Gu et
92 al., 2020), a 3˚C increase has positive impacts on heterotrophic prokaryotic

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93 growth in winter with an ambient temperature of 19.1˚C, while no significant
94 impacts in summer with an ambient temperature of 28.5˚C. This suggests that
95 the impacts of rising temperature on prokaryotes in subtropical waters varied
96 seasonally. However, we still do not know which ambient temperature is the
97 threshold where the impacts of temperature increase on prokaryotic
98 community changes from positive to negative in that area.
99
100 Thermal sensitivity differs considerably across diverse prokaryotic taxa (Smith
101 et al., 2019, 2021), suggesting a taxon-dependent effect of elevated
102 temperatures (Arandia-Gorostidi et al., 2020). For example, Smith et al.
103 (2021) showed varying optimal temperatures and thermal sensitivities for
104 carbon use efficiency and growth rates among 29 bacterial strains under lab
105 conditions. As autotrophs and heterotrophs play fundamentally different roles
106 in the carbon cycle, it is a topic of interest to understand how they differ in
107 sensitivity to temperature changes. Conventional analyses using an
108 exponential equation, like the metabolic theory of ecology, propose that the
109 metabolism of heterotrophs is more thermally sensitive than that of autotrophs
110 (Allen et al., 2005). However, this approach overlooks the fact that the
111 temperature performance curves of individual species exhibit a unimodal
112 shape (Chen, 2022) and is only applicable when temperatures are below the
113 optimum temperature. To our knowledge, there are no studies comparing the

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114 temperature sensitivity between autotrophic and heterotrophic prokaryotes
115 when temperatures exceed optimum growth temperatures- something which
116 typically occurs in tropical and subtropical summers (Chen et al., 2014; Gu et
117 al., 2020). If autotrophic and heterotrophic prokaryotes exhibit different
118 temperature sensitivities, warming could shift carbon fluxes within prokaryotic
119 communities, potentially driving feedback loops that contribute to climate
120 change in subtropical marine ecosystems.
121
122 In microbial ecology, field incubation stands as a potent tool routinely
123 employed to understand responses to changes in a variety of ecosystems.
124 This method has widespread application in studying the effects of temperature
125 increase on marine prokaryotic community in diverse marine environments
126 (von Scheilbner et al., 2014; Bergen et al., 2016; von Scheilbner et al., 2018;
127 Gu et al., 2020; Liu et al., 2021; Lønborg et al., 2022). However, few studies
128 have integrated physiological processes and omics to offer a comprehensive
129 understanding of the effects of rising temperatures on prokaryotic community.
130 Here, by integrating field incubations, flow cytometry, 16S rRNA sequencing
131 and metatranscriptomics, we conducted 14 temperature-manipulated
132 experiments (with an ambient temperature ranging from 17˚C to 31˚C) to
133 estimate the effects of short-term warming on growth rate, grazing pressure
134 and community functions of heterotrophic and autotrophic prokaryotes in

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135 Daya Bay and the Pearl River Estuary. These areas are the typical subtropical
136 coastal environments in the northern South China Sea which have
137 experienced a drastic increase in the frequency and severity of marine
138 heatwaves in recent years (Tan et al., 2022). Subtropical and tropical seas are
139 experiencing marine heatwaves with an annual mean intensity of 1-3˚C and
140 duration of 10-15 days (Oliver et al., 2018). Extreme heatwaves have also
141 been recorded, such as during the summer of 2010-2011, when coastal
142 waters in Australia saw temperature anomalies exceeding 5˚C (Pearce and
143 Feng, 2013). We used 3˚C and 6˚C above the ambient temperature as the
144 warming treatments to represent moderately and extremely hot events,
145 respectively. We hypothesized that varying temperature increases could have
146 differential effects on prokaryotes. Moderate temperature increases may
147 remain within the optimal range for prokaryotic growth, potentially enhancing
148 growth rates. In contrast, more significant temperature increases could
149 exceed the optimal range, leading to negative impacts on prokaryotes.
150

151 **Materials and Methods**

152 **Sampling.** Samples were collected during the day in six field sampling trips
153 and three cruises experiments between July 2020 and July 2021. The
154 estuarine sampling sites A1, A3, E3, S10, S15, S19 and S20 were located in

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155 the Pearl River Estuary and the coastal sampling site DYB was located in the
156 Daya Bay **(Fig. S1a)**. Part of the estuarine samples (A1, A3 and E3) were
157 collected during a cruise in June-July 2021, and surface seawater samples
158 were collected using a conductivity-temperature-depth (CTD, Sea-Bird)
159 rosette fitted with 12-L Niskin bottles. The remaining estuarine samples (S10,
160 S15, S19, S20) were collected during cruises in January and April 2021. For
161 these estuarine samples, as well as the coastal samples from station DYB,
162 surface seawater samples were collected using several 25-L acids-washed
163 polycarbonate (PC) bottles. In each case, the ambient temperature of the
164 seawater was measured immediately with a mercury thermometer. At each
165 sampling site, 32-72 L seawater was pre-filtered through a 200-μm mesh and
166 then transported to a land-based or onboard laboratory within ~20 min for the
167 incubation experiments.
168
169 **Short-term warming manipulation experiments**. A total of 14 incubation
170 experiments were performed **(Table S1)** and schematic of the experimental
171 design is shown in **Fig. S1b**. One fraction of the seawater samples was not
172 filtered and thus contained grazers of size < 200 μm (hereafter called with
173 grazers group). The other fraction was filtered through 1.2-μm pore size PC
174 membranes (Millipore) and thus contained nearly no grazers (hereafter called
175 without grazers group). Each group was then separated into three 1-L PC

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176 transparent bottles (Nalgene, ThermoFisher) for incubation at the ambient
177 temperature, 3˚C and 6˚C over the ambient temperature. All the incubations
178 were carried out in near-transparent 280-L tanks temperature-controlled by
179 pump- equipped macro chillers (CW-0500, RESUM) located on the deck and
180 outdoors on the shore for 24 h. To ensure the accuracy of temperature
181 control, we measured the temperature of the tank every hour during the
182 incubation period.
183
184 **Sample collection.** To determine the prokaryotic abundance, 2 mL seawater
185 was fixed with glutaraldehyde (1% final concentration; Sangon Biotech) before
186 and after incubation. They were then flash frozen in liquid nitrogen and stored
187 at -80˚C until further analysis. To determine ambient nutrients and DOC
188 before incubation, 100 mL seawater was filtered through a GF/F membrane
189 (47 mm; Whatman) and stored at -20˚C until further analysis. Filters for DNA
190 extraction of three size fractions were collected via sequential filtration of
191 approximately 100 ml of seawater from in-situ (before incubation) waters and
192 from each bottle post-incubation using (after incubation). Each sample was
193 sequentially filtered 5-μm, 1.2-μm, and 0.2-μm PC membranes (47 mm;
194 Millipore) which stored at -80˚C until required. Two experiments (Jan19 and
195 Jul15 from DYB) were also collected for RNA extraction of the with grazers
196 group. In brief, approximately 700 mL seawater from each bottle post-

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197 incubation was filtered through a 0.2-μm PC membrane (47 mm; Millipore)
198 and subsequently transferred to 2-mL tubes containing 0.2 mL RNA hold
199 (TransGen Biotech), flash frozen with liquid nitrogen and stored at -80˚C.
200
201 **Measurements of prokaryotic abundance, and concentrations of**
202 **nutrients and DOC.** To measure prokaryotic abundance, glutaraldehyde-fixed
203 samples were analyzed with a flow cytometer (Cytoflex S, Beckman). The
204 samples were stained 1x SYBR Green I (10,000x, Invitrogen) according to the
205 published protocol (Marie et al., 1997) for assessing the abundance of
206 heterotrophic prokaryotes, whereas the abundance of *Synechococcus* was
207 determined by pigment fluorescence. The concentrations of inorganic
208 nutrients (i.e., ammonium, nitrate, nitrite, phosphate, and silicate) were
209 determined with a SEAL AutoAnalyzer 3 (Bran-Luebbe) according to the
210 manufacturer's instructions. The concentrations of DOC were measured with
211 a TOC analyzer (TOC-VCPH, Shimadzu).
212
213 **Growth rate of and grazing rate on prokaryotes.** We assumed an
214 exponential growth rate and nearly no grazing in the without grazers group
215 since most of the grazers, such as nanoflagellates and ciliates were lager
216 than 1.2 μm in nearby seas (Gu et al., 2021). We used the following formula
217 to calculate the intrinsic growth rate of prokaryotes including both

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218 *Synechococcus* and heterotrophic prokaryotes: $\mu = (\ln_{Af} - \ln_{Ai}) / (t_f - t_i)$, where
219 A_f and A_i are the abundance of prokaryotes at the final (t_f) and initial (t_i) time
220 point of the incubation, respectively. Growth rates estimated by this method
221 are conservative because viruses that pass through 1.2-μm membranes
222 would cause prokaryotic mortality during the incubations (Yokokawa and
223 Nagata, 2005). The grazing rate (day^{-1}) of grazers on prokaryotes
224 (*Synechococcus* and heterotrophic prokaryotes) was calculated by the
225 following formula: $g = (\ln_{\text{abundance of without grazers group}} - \ln_{\text{abundance of with grazers group}}) /$
226 $(t_f - t_i)$, where the abundances of without and with grazers groups were
227 measured at the final time point of incubation.
228
229 **Estimating effects of short-term warming on growth and grazing rate**.
230 Generalized linear models are flexible in handling dependent variables (e.g.
231 growth and grazing rate) and independent variables (e.g. temperature),
232 allowing for precise estimation of treatment effects and providing robust
233 statistical inference (Nelder and Wedderburn, 1972). We fitted generalized
234 linear models with Gaussian distributions with the *lme4* v1.1-27.1 package
235 (Bates et al., 2015) in R v4.1.0 (R Core Team, 2018) to calculate the effect
236 size (parameter estimates from models; see below) of temperature increase
237 on growth rate and grazing rate in each experiment, and then assessed
238 statistical significance using a chi-squared test (Schulhof et al., 2019).

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239 Parameter estimates and standard error values from the generalized linear
240 models were used to represent effect sizes of the warming treatments on
241 response variables (Schulhof et al., 2019). Then, a linear regression model
242 was employed to check if there was a linear relationship between growth rate
243 and grazing rate for each experiment with seawater ambient temperatures.
244 Before running models, growth rate and grazing rate were standardized to a
245 mean of zero and a standard deviation of 1 in R with the function of *scale* to
246 ensure that they followed a normal distribution.
247
248 **DNA extraction, PCR, pyrosequencing, amplicon processing and**
249 **prokaryotic community analysis.** To evaluate the effect of warming on the
250 prokaryotic communities, we extracted genomic DNA from four experiments
251 (i.e., summer: Jul15, Aug14, and Sep07; winter: Jan19). Genomic DNA stored
252 on 0.2-μm membranes (described above) was extracted using a modified
253 enzyme/phenol-chloroform extraction protocol (Xia et al., 2020; Gu et al.,
254 2022). In brief, each membrane was cut into small pieces and then transferred
255 into a 2-mL tube with 0.5 mL solution I (50 mM EDTA, 50 mM Tris-HCL and 50
256 mM sucrose; pH 8.0). These were then subjected to three freeze-and-thaw
257 cycles using liquid nitrogen and a 60˚C dry bath, after which 5 mg mL^{-1}
258 lysozyme (final concentration; Sangon Biotech) was added to each tube and
259 incubated for 1 h at 37˚C. 2 mg mL^{-1} proteinase K (final concentration; Sangon

260 Biotech) and sodium dodecyl sulfate (0.5%, w/v; Sangon Biotech) were then
261 added and the samples were incubated for 1 h at 60˚C. DNA was then
262 extracted from each sample by the addition of an equal volume of phenol-
263 chloroform-isoamyl alcohol (Sigma-Aldrich) at a ratio of 25:24:1. The samples
264 were centrifuged for 10 min at 12,000 g, after which the upper aqueous layer
265 was transferred to a new microcentrifuge tube. These samples were then
266 extracted twice with an equal volume of chloroform-isoamyl alcohol (Sigma-
267 Aldrich) at a ratio of 24:1. After the final extraction step, the supernatant was
268 transferred to a new microcentrifuge tube and isopropyl alcohol (Sangon
269 Biotech) at 70% volume of the supernatant was added. The samples were
270 incubated overnight at -20˚C, after which the DNA pellets were washed twice
271 using 0.2 mL 70% ethanol (Sangon Biotech) and then resuspended in 35 μL
272 TE buffer (1 mM EDTA, 10 mM Tric-Hcl; pH 8.0; Sangon Biotech). The V4-V5
273 regions of the 16S rRNA gene were amplified with the following primers: 515F
274 (5'-GTGCCAGCMGCCGCGGTAA-3') and 907R (5'-
275 CCGTCAATTCCTTTGAGTTT-3') for pyrosequencing (Turber et al., 1999).
276 The PCR reaction was carried out in a 25-μL master mix, including 1 μL DNA,
277 0.5 μM of each primer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1× PCR buffer,
278 and 1.0 unit Platinum® Taq DNA polymerase (Invitrogen). Sterile water was
279 used as the negative control. The PCR for each sample was carried out in
280 triplicate with the following thermal cycles: 5 min initial denaturation at 95˚C,

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281 followed by 30 cycles of 95˚C for 30 s, 55˚C for 30 s, and 72˚C for 60 s,
282 followed by a final extension step at 72˚C for 7 min before holding at 4˚C.
283 Paired-end amplicon sequencing was then conducted by Novogene Company
284 using the Illumina Hiseq 2500 platform. Read quality control, trimming, and
285 inference of amplicon sequence variants (ASVs) were analyzed by *DADA2*
286 v1.20.0 (Callahan et al., 2016) in R v4.1.0. The truncation lengths of the
287 forward and reverse reads and primer trimming (e.g., trim-left or trim-right)
288 were defined based on the read-quality profiles. The maximum number of
289 expected errors (MaxEE) was set to 2 for the forward and reverse reads.
290 Default DADA2 parameters (e.g., maxN) were used for other flags. Taxonomy
291 was assigned using the Silva database v138 (Quast et al., 2013). Bray-Curtis
292 dissimilarity between warming treatments and control (ambient temperature
293 treatments) was analyzed by permutational multivariate ANOVA using the
294 *Adonis* function in the *vegan* package v2.5-7 (Oksanen et al., 2020) in R
295 v4.1.0.
296
297 **RNA extraction, metatranscriptome sequencing and metatranscriptomic**
298 **analysis.** For metatranscriptomics, the total RNA of the microbial community
299 was extracted using TRIzol reagent (ThermoFisher). RNA samples were
300 collected and stored on PC membranes in RNA hold, as described above.
301 The RNA hold was removed from the PC membranes by centrifugal, and

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302 these were then incubated with 1 mL TRIzol in 2-mL tubes for 5 min at room
303 temperature, after which 0.2 mL chloroform was added and the samples were
304 incubated for 3 min, again at room temperature. The samples were
305 centrifuged for 15 min at 12,000 g and a temperature of 4˚C, after which the
306 upper aqueous layer was transferred to a fresh microcentrifuge tube, and
307 incubated with 0.5 mL isopropyl alcohol for 10 min at 4˚C. The samples were
308 centrifuged again (for 10 min at 12,000 g and 4˚C) to pellet the RNA. The
309 RNA pellets were then washed twice using 1 mL 70% ethanol and
310 resuspended in 35 μL nuclease-free water (Invitrogen). Whole mRNAseq
311 libraries were generated by Magigene Biotechnology Company using the NEB
312 Next® UltraTM Nondirectional RNA Library Prep kit for Illumina® (New
313 England Biolabs), following the manufacturer’s recommendations. The library
314 was sequenced on an Illumina Novaseq6000 platform and paired-end reads
315 were generated. Metatranscriptomic analysis was conducted using the
316 *SqueezeMeta* pipeline v1.6.2 (Tamames et al., 2019). Metatranscriptomic
317 reads were quality checked and trimmed for low-quality regions using
318 *Trimmomatic* v0.38 (Bolger et al., 2014), after which the sequences were
319 assembled using *Megahit* with default settings (Bankevich et al., 2012). The
320 open reading frames (ORFs) of the assembled contigs (> 200 bp), which were
321 identified with the *Prodigal* software (Hyatt et al., 2012), were further
322 annotated using *DIAMOND* v2.0.15.153 (Buchfink et al., 2015) against both

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323 the NR and KEGG databases, with an e-value cutoff of 1×10^{-3}. Raw reads
324 were mapped to the contigs using *Bowtie* v1.1.2 (Langmead et al., 2009) to
325 calculate the abundance of each ORF. The abundance of each ORF was
326 calculated as: the transcripts per million (TPM) = $rg \times rl \times 10^6 / cl \times T$, where rg
327 indicates the reads mapped to gene g, rl is read length, cl is the coding
328 sequence (CDS) length, and T is the sum of rg × rl / cl for all genes (Wagner
329 et al., 2012). To distinguish between the cyanobacterial genes and other
330 heterotrophic prokaryotic genes, we subdivided the genes annotated as
331 Cyanobacteria at the phylum level as being cyanobacterial genes, and those
332 annotated as other phyla as heterotrophic prokaryotic genes. The significant
333 differential abundance of functional genes between groups of samples was
334 identified using the *DESeq2* v1.32.0 package with an adjusted p-value (p_{adj}) <
335 0.05 (Love et al., 2014).
336

337 **Data availability.** The sequence data have been deposited in the National
338 Center for Biotechnology Information sequence read archive (BioProject
339 accession no. PRJNA822857; 16S rRNA amplicon Biosample accession nos.
340 SAMN27280266- SAMN27280409, metatranscriptomics-seq BioSample
341 accession nos. SAMN27280410- SAMN27280424).
342

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343 **Results**

344 **Environmental parameters and initial abundance of prokaryotes.** Ambient
345 temperatures in the experimental stations varied from 17 to 31˚C. At the
346 various coastal experimental stations, the surface seawater was characterized
347 by high concentrations of dissolved organic carbon (DOC) with mean ±
348 standard deviations (SD) ranging from 92.94 ± 14.58 to 233.75 ± 49.12 μmol
349 L. Concentrations of phosphate and nitrite in these stations were below 1.5
350 μM and 1.1 μM, respectively, while ammonium and nitrate were at a wide
351 range of concentrations, i.e., 0.53-6.18 μM and 0.10-17.18 μM, respectively
352 **(Table S1)**.
353
354 For heterotrophic prokaryotes, the initial abundances before 1.2-μm filtration
355 ranged from 0.47 ± 0.01 (Jul01) to 40.71 ± 2.70 × 10^5 cells/mL (Aug14).
356 These values were comparable to those after 1.2-μm filtration, which ranged
357 from 0.42 ± 0.01 (Jul01) to 38.25 ± 2.37 × 10^5 cells/mL (Aug14) (Table S2).
358 For *Synechococcus*, the initial abundances of non-summer samples (Jan12,
359 Jan13, Jan19, Jan21, Apr15, Apr16, and Apr18) were quite low, falling below
360 the detection limit. The abundance of the summer sample Jul03 was also
361 below the detection limit, likely because it was collected at offshore station A3
362 (Fig. S1a), which could be dominated by *Prochlorococcus*. The initial

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363 *Synechococcus* abundances for other samples ranged from 1.53 ± 0.06
364 (Jul08) to 79.24 ± 3.39 × 10^4 cells/mL (Aug14) (Table S2).
365
366 **Effects of temperature increase on the abundance, growth rate and**
367 **grazing loss of heterotrophic prokaryotic communities across**
368 **temperature regimes**. In the ambient temperature incubations (24 h), the
369 abundance of heterotrophic prokaryotes varied from 0.98 ± 0.08 to 58.43 ±
370 2.98 × 10^5 cells/mL, with an average of 24.19 ± 18.29 × 10^5 cells/mL in the
371 1.2-μm filtered group (Fig. 1a), while they were generally lower in the
372 unfiltered group that the abundance varied from 0.58 ± 0.04 to 16.57 ± 4.84 ×
373 10^5 cells/mL, with an average of 13.21 ± 8.57 × 10^5 cells/mL (Fig. 1b). After
374 exposure to 3˚C and 6˚C increases, abundances of heterotrophic prokaryotic
375 communities were enhanced only when the ambient temperatures were
376 ≤25˚C in the filtered group (Fig. 1a), while they were enhanced in all
377 temperature regimes in the unfiltered group (Fig. 1b).
378
379 In the ambient temperature incubations, the growth rate of heterotrophic
380 prokaryotes was at its lowest on Jan 12, with a value of 0.08 ± 0.04 d^{-1}. It then
381 increased with rising ambient temperatures, peaking on Jul 01 (1.58 ± 0.09 d^{-}
382 ^{1}), and subsequently decreased in these experiments with ambient
383 temperatures exceeded 29˚C **(Fig. S2a)**. We found that the responses of

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384 prokaryote growth rates to short-term temperature rising depended on
385 ambient temperatures. After exposure to 3˚C and 6˚C increases, growth rates
386 of heterotrophic prokaryotic communities were enhanced only when the
387 ambient temperatures were ≤26˚C **(Fig. S2a)**. Once ambient temperatures
388 exceeded 26˚C, negative impacts of warming on the growth rate were
389 observed. These observations were also supported by the generalized linear
390 model, which was applied to quantify the effects of temperature increase on
391 the growth rate of heterotrophic prokaryotes. The positive effects of
392 temperature increase on the growth rate of heterotrophic prokaryotes were
393 observed at an ambient temperature ≤26˚C, while the growth rate of
394 heterotrophic prokaryotes of all eight experiments with ambient temperatures
395 ≥28˚C showed negative responses to short-term warming **(Fig. 1c)**,
396 suggesting 26-28˚C is a tipping temperature for the effects of warming on
397 heterotrophic prokaryotic growth. The effects of a 6˚C temperature increase
398 were greater than those of a 3˚C temperature increase **(Fig. 1e)**, except for
399 the Apr16 and Apr18 experiments, which exhibited an ambient temperature of
400 24-25˚C. In addition, the slope of effect/ambient temperatures was larger in
401 the 6˚C warming treatment than the 3˚C warming treatment **(Fig. 1f and 1g)**,
402 suggesting the impact of warming was largely determined by the degree of
403 temperature change.
404

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405 The grazing rates on heterotrophic prokaryotic community varied from 0.06 ±
406 0.03 d^{-1} (Jan13) to 1.20 ± 0.16 d^{-1} (Jul29) in the ambient temperature
407 incubations of fourteen experiments. Regarding the responses of grazing rate
408 to temperature increase, a similar trend to growth rate was observed **(Fig.**
409 **S2b)**. When the ambient temperature exceeded 26˚C, the grazing on
410 heterotrophic prokaryotic community was strongly suppressed by the warming
411 of 6˚C. In addition, grazing rates on heterotrophic prokaryotes decreased
412 more than growth rate of heterotrophic prokaryotes in response to warming
413 **(Fig. 1e)**. While the decrease was not significant (t-test, $p > 0.05$) with a 3˚C
414 increase, it was significant (t-test, $p < 0.05$) with a 6˚C increase **(Fig. 1e)**. This
415 suggested that grazers may be more sensitive to warming than heterotrophic
416 prokaryotes. This may explain why the abundance of heterotrophic
417 prokaryotes increased in summer in the warming treatments **(Fig. 1b)**, even
418 though the growth rate was decreased by warming.
419
420 **Effects of temperature increase on the growth rate and grazing loss of**
421 **autotrophic prokaryotic communities.** Autotrophic prokaryotic
422 *Synechococcus* is one of the major groups among the prokaryotic community
423 in summer (Fig. 2b). Therefore, we further compared the effects of
424 temperature increase between the heterotrophic prokaryotes and
425 *Synechococcus* community in summer experiments which conducted on

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426 Jul01, Jul08, Jul15, Jul29, Aug14 and Sep07. In the ambient temperature
427 incubations, the abundance of *Synechococcus* varied from 3.38 ± 0.14 to
428 97.93 ± 3.34 × 10^4 cells/mL, with an average of 31.81 ± 32.66 × 10^4 cells/mL
429 in the 1.2-μm filtered group (**Fig. 2a**), while they were generally lower in the
430 unfiltered group that the abundance varied from 1.77 ± 0.03 × 10^4 to 85.00 ±
431 4.11 × 10^4 cells/mL, with an average of 21.91 ± 29.90 × 10^4 cells/mL (**Fig. 2b**).
432 After exposure to 3˚C increases, abundances of *Synechococcus* were
433 generally stable compared to ambient temperature incubations in both filtered
434 and unfiltered groups (**Fig. 2a and b**). When exposed to 6˚C increases,
435 abundances of *Synechococcus* in both filtered and unfiltered groups
436 significantly decreased in most of the experiments (**Fig. 2a and b**).
437
438 The 3˚C increases showed little effects on the growth rate of *Synechococcus*
439 **(Fig. 2c and S3a)**, and the average warming effects (n=5; -0.06 ± 0.16) were
440 smaller than that on the growth of heterotrophic prokaryotes (n=5; -0.15 ±
441 0.15) **(Fig. 3a)**. However, the 6˚C increases demonstrated predominantly
442 negative impacts in most of the incubation experiments **(Fig. 2c and S3a)**,
443 and the average warming effects (n=5; -1.04 ± 0.33) were significantly larger
444 than that on the growth of heterotrophic prokaryotes (n=5; -0.31 ± 0.16) **(Fig.
445 3a)**. This indicated the different responses between heterotrophic and
446 autotrophic prokaryotes to temperature increase. Similar to heterotrophic

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447 prokaryotes, the grazing pressure on *Synechococcus* decreased after
448 temperature increase **(Fig. 2d)**. The negative effects of temperature increase
449 on *Synechococcus* growth were higher than that on grazing pressure **(Fig. 2c**
450 **and 2d)**. The different responses of heterotrophic prokaryotes and
451 *Synechococcus* to warming might lead to a shift in their abundance ratio in the
452 subtropical oceans of the future, which potentially results in a change in the
453 local carbon budget. We investigated the relationships between the ratio of
454 heterotrophic prokaryotes and *Synechococcus* abundance **(Fig. 3b)**. The ratio
455 reached a minimum at 34˚C and increased at 36˚C **(Fig. 3b)**, indicating that
456 extremely warming in summer would decrease the proportion of
457 *Synechococcus* among the pico-prokaryotes.
458
459 **Responses of prokaryotic communities to temperature increase.** To
460 investigate the impact of elevated temperature on prokaryotic community
461 composition, we sequenced the 16S rRNA gene of samples from four
462 experiments (Jan19, Jul15, Aug14, and Sep07) which were conducted in the
463 DYB. Our findings revealed that during the summer months (Jul15, Aug14,
464 and Sep07), the prokaryotic communities in the sampled areas were primarily
465 comprised of Cyanobiaceae, Rhodobacteraceae, and Flavobacteriaceae.
466 Conversely, in winter (Jan19), the dominant groups shifted to SAR11 clade I,
467 Rhodobacteraceae, and Flavobacteriaceae **(Fig. 4)**. The Bray-Curtis analysis

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468 showed that warming had stronger effects on prokaryotic community
469 composition in summer than in winter. In addition, in summer, an increase of
470 6˚C affected greater changes in prokaryotic community composition than an
471 increase of 3˚C **(Fig. S4)**. This indicates that the response of the prokaryotic
472 community to increased temperatures was largely determined by ambient
473 temperatures— a conclusion which is further supported by the ternary plot
474 illustrating different bacterial families positioned near the middle of the plot in
475 winter, while different bacterial families were positioned closer to the ends of
476 the axes representing the different temperature treatments **(Fig. 4)**. For
477 example, Cyanobacteria were generally close to the ambient temperature and
478 ambient temperature +3˚C treatments, which is consistent with the response
479 of *Synechococcus* abundance to temperature increase **(Fig. 2b)**. In contrast,
480 Bacteroidia (NS9 marine group and Flavobacteriaceae) were generally
481 enriched in the ambient temperature +6˚C treatment **(Fig. 4)**.
482
483 **Responses of the prokaryotic metatranscriptome to warming.** To explore
484 the response of metatranscriptome to warming at different ambient
485 temperatures, we selected samples from the experiments conducted at the
486 ambient temperatures of 17˚C (Jan19) and 30˚C (Jul15) for further analysis.
487 Principal coordinate analysis showed that warming had little effects on the
488 transcriptome profiles of prokaryotic community in winter **(Fig. S5)**. In summer

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489 samples, a 3˚C temperature rise resulted in 4.1% of Cyanobacteria genes
490 (i.e., 96/2322) being significantly changed (i.e., 2.8% up-regulated and 1.3%
491 down-regulated), and 2.2% heterotrophic prokaryotes genes (i.e., 200/8997)
492 being changed (i.e., 0.8% up-regulated and 1.4% down-regulated; DESeq2,
493 p_{adj} <0.05). Short-term warming by 6˚C resulted in 13.4% Cyanobacteria
494 genes (314/2342; 5.7% up-regulated and 7.7% down-regulated), and 7.5%
495 heterotrophic prokaryotes genes (689/9146; 2.7% up-regulated and 4.8%
496 down-regulated), being significantly changed (DESeq2, p_{adj} < 0.05). These
497 results strongly suggest that the effects of seawater temperatures increase on
498 the prokaryotic community are dependent on the ambient temperatures.
499
500 We further examined the effects of temperature increase on the
501 metatranscriptome profile of the prokaryotic communities during summer. The
502 50 pathways with the most differentially expressed genes were mostly
503 associated with amino acid metabolism, carbohydrate metabolism, energy
504 metabolism, environmental adaption (thermogenesis), lipid metabolism, and
505 genetic information processing, as well as signaling and cellular processes
506 **(Fig. 5a)**. For heterotrophic prokaryotes, it is particularly interesting that most
507 genes relating to oxidative phosphorylation related genes (i.e., *ATPF0C*,
508 *ATP1A*, *cox1*, *2*, *3*, *15*, *coxACD*, *nD1*, *2*, *4L*, *6*, *ndufs123*, and *nuoAM*) were
509 significantly (DESeq2, p_{adj} < 0.05) decreased under heat stress in summer,

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510 both as a result of a 3˚C and 6˚C temperature rise **(Fig. 5b)**. For
511 cyanobacteria, some of the photosynthesis related genes (i.e., *psaADEIJK*,
512 *psbE*, *cpcABC*, and *cpeAB*) were significantly down-regulated (DESeq2, p_{adj} <
513 0.05) with a 6˚C temperature increase, but most of these were not significantly
514 affected (DESeq2, p_{adj} > 0.05) by a 3˚C temperature increase **(Fig. 5c)**.
515

516 Discussion

517 Our primary goal was to analyze the impact of temperature increase on the
518 prokaryotes in subtropical waters and to predict how prokaryotes respond to
519 short-term warming events, such as marine heatwaves, which have increased
520 in frequency and length over the past century (Oliver et al., 2018). We
521 conducted fourteen field experiments (outdoors or ship-deck), closely
522 mimicking natural conditions. This approach minimized the influence of
523 missing sunlight on experimental results (Teira et al., 2019), a factor that can
524 be significant in the laboratory-based studies. Additionally, short-term (24-
525 hour) incubations were employed to minimize potential changes in prokaryotic
526 composition due to the bottle effect. In subtropical coastal waters, bottle
527 incubations lasting 24 h are longer than local prokaryotic division times (Gu et
528 al., 2020; Tsai et al., 2015). In this study, the average intrinsic growth rates
529 under ambient temperature were 0.59 ± 0.40 for heterotrophic prokaryotes
530 and 0.86 ± 0.17 for *Synechococcus* **(Fig. S2a and Fig. S3a)**. This enables us

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531 to evaluate the effects of short-term temperature increase on prokaryotes. We
532 believe that our approach is a valid method to examine the effects of
533 temperature increase on current prokaryotic communities, and it can generate
534 plausible hypotheses about changes of prokaryotic communities in response
535 to short-term warming events.
536
537 **Effects of temperature increase on prokaryotes were highly dependent**
538 **on ambient temperatures.** The effects of experimental warming on
539 heterotrophic prokaryotes growth rate have been evaluated by many studies
540 (e.g., Bergen et al., 2016; Lindh et al., 2013; von Scheilbner et al., 2014;
541 2018). However, many of these studies were limited to a narrow temperature
542 range or only one temperature point, resulting in different conclusions about
543 the effects of temperature increase on heterotrophic prokaryotes growth. In
544 our prior study, we found distinct seasonal effects of temperature increase on
545 heterotrophic prokaryotes growth and community dynamics in subtropical
546 coastal waters (Gu et al., 2020). Specifically, we observed a significant
547 enhancement in heterotrophic prokaryotes growth rate during winter (19˚C),
548 whereas the effect was minimal during summer (28.5˚C), consistent with the
549 results presented here **(Fig. 1c)**. In the present study, we extended the
550 temperature range to 17-31˚C and identified a critical temperature range (26-
551 28˚C) where the effects of moderate warming (+3˚C) transitioned from positive

552 to negative on heterotrophic prokaryotes growth. This shift strongly indicates
553 that the typical unimodal model of the temperature-growth rate relationship of
554 single strains in the laboratory (Chen and Shakhnovich, 2010) is also suitable
555 for the prokaryotic community in the environment. Below the optimum
556 temperature, growth rates of the community generally increase with
557 temperature, while adverse effects become apparent once the temperature
558 surpasses the optimum temperature. Although the optimum temperature of
559 the prokaryotic community may be site-dependent (Lønborg et al., 2022)
560 because of the different substrates and communities, in-situ prokaryotes may
561 all follow the unimodal model when they are responding to temperature
562 increase.
563
564 Although the growth rate of heterotrophic prokaryotes decreased in warming
565 treatments when ambient temperatures exceeded 28˚C, we observed an
566 increased abundance of heterotrophic prokaryotes in response to warming
567 across all temperature regimes we tested (17-31˚C) **(Fig. 1b)**. It is well-known
568 that the abundance of heterotrophic prokaryotes is determined by both top-
569 down (e.g. grazing and viral lysis) and bottom-up control (e.g. substrates and
570 temperature). Below 28˚C, the higher abundance after warming is primarily
571 caused by the higher growth rates of heterotrophic prokaryotes after
572 temperature increase **(Fig. 1c)**. However, once temperatures exceeded 28˚C,

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573 the increased abundance of heterotrophic prokaryotes after warming may
574 have resulted from the disproportionate response of growth rate and grazing
575 pressure to temperature increases, with the grazing activities showing higher
576 sensitivity to temperature increases compared to the growth of heterotrophic
577 prokaryotes **(Fig. 1e-g)**. Only few studies explored the effects of temperature
578 increase on protist grazing rates on heterotrophic prokaryotes in the field,
579 particularly in subtropical waters. Tsai et al. (2015) reported increased grazing
580 rates on prokaryotes under experimental warming conditions in the coastal
581 waters of the western subtropical Pacific Ocean, mirroring our findings of
582 positive effects at temperatures below 28˚C **(Fig. 1c)**. This study expands the
583 temperature regimes and observed negative effects on grazing activities
584 exceeding 28˚C. The higher temperature sensitivity of grazing activity when
585 compared with heterotrophic prokaryotes growth might be explained by that
586 the growth efficiency of protists decreases faster than that of bacteria with
587 increasing temperature (Rivkin and Legendre, 2001). Taken together,
588 although the growth rate of heterotrophic prokaryotes decreased after short-
589 term warming when ambient temperatures reached 28˚C **(Fig. 1c)**, the higher
590 temperature sensitivity of predators resulted in fewer prokaryotes being
591 subjected to grazing. As seawater temperature increased, this led to a net
592 increase in prokaryotic cell abundance.
593

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594 **Different responses to temperature increase between *Synechococcus***
595 **and heterotrophic prokaryotes in subtropical coastal waters in summer.**
596 The impact of temperature on *Synechococcus* abundance has been explored
597 globally (Flombaum et al., 2013) and specifically in subtropical seas as well
598 (Liu et al., 2021). These two studies predicted an increase in the growth rate
599 and abundance of *Synechococcus* with warming in tropical and subtropical
600 waters, but the highest temperatures they tested were both ≤30˚C. Our study
601 expands the knowledge about the effects of temperature increase on
602 *Synechococcus* over 30˚C. In this study, *Synechococcus* growth showed little
603 response to +3˚C warming treatments, while they were significantly
604 suppressed in the +6˚C warming treatments in summer **(Fig. 2c and S3a)**.
605 This pattern was further supported by the ternary plot analysis of 16S rRNA
606 gene data, showing that Cyanobacteria were primarily associated with
607 treatments at ambient temperatures (30-31˚C) or ambient temperature +3˚C
608 treatments (33-34˚C), but were distant from ambient temperature +6˚C
609 treatments (36-37˚C) **(Fig. 4)**. These indicate that *Synechococcus* are not
610 able to adapt to temperatures exceeding 35˚C in the natural environment, and
611 the same applies under laboratory conditions with nutrient-rich culture
612 medium (Labban et al., 2021; Pittera et al., 2014). In addition, our
613 experimental results were consistent with a field investigation (Ren et al.,
614 2019) in regions influenced by thermal effluents for 30 years from a nuclear

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615 power plant in the DYB. In that study, a significant decline in the relative
616 abundance of *Synechococcus* within the prokaryotic community (inferred from
617 16S rRNA gene amplicon data) was observed when ambient temperatures
618 rose from 31˚C to approximately 39˚C (Ren et al., 2019). Collectively, these
619 observations indicate that *Synechococcus* can adapt to moderate warming
620 conditions below 35°C. However, intense warming during summer with
621 temperatures over 35°C, will have a profound negative impact on
622 *Synechococcus* growth in subtropical waters.
623
624 The metabolic theory of ecology suggests that the metabolism of heterotrophs
625 is more thermally sensitive than that of autotrophs (Allen et al., 2005).
626 However, this approach is only applicable when temperatures are below the
627 optimum temperature. In our study, the +6˚C treatments can be considered as
628 temperatures exceeding the optimum temperature for both heterotrophic
629 prokaryotes and *Synechococcus* **(Fig. 1c and 2c)**. Our results demonstrated
630 that the growth of autotrophs (*Synechococcus*) were more negatively affected
631 by experimental warming beyond the optimum temperature compared to
632 heterotrophs **(Fig. 3a)**. However, in the +3˚C treatments, *Synechococcus*
633 outperformed heterotrophic prokaryotes, which may be attributed to the higher
634 optimum temperature of *Synechococcus* relative to heterotrophic prokaryotes.
635 Due to the differential response to temperature increase between

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636 heterotrophic prokaryotes and *Synechococcus*, our model predicted that the
637 prokaryotic community would become more autotrophic under moderate
638 warming conditions and more heterotrophic under extreme warming
639 conditions in subtropical summer **(Fig. 3b and 4)**.
640
641 **Potential mechanisms of decreased prokaryotic growth under thermal**
642 **stress in summer.** Several covariates are associated with warming, including
643 community structure, thermal acclimation, and nutrient and light levels
644 resulting from stratification. Here we only discuss the potential mechanisms
645 caused by temperature increase. Microorganisms often respond to
646 environmental stresses by diverting cellular resources from biomass synthesis
647 to the restoration of homeostasis (Lopez-Maury et al., 2008). For example,
648 increased lipid metabolism under thermal stress was reported in both
649 prokaryotes (Koga et al., 2012; Hassan et al., 2020) and phytoplankton (Leles
650 and Levine, 2023; Zhang et al., 2022) as a mechanism to mitigate oxidative
651 stress. This is also observed in this study indicated by the significant down-
652 regulation of genes involved in carbohydrate metabolism and concurrent up-
653 regulation of genes involved in lipid metabolism in the +6˚C treatments **(Fig.**
654 **5a)**. This transition of resources investment could be one reason for the lower
655 growth rate of heterotrophic prokaryotes under thermal stress.
656

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657 Furthermore, genes related to oxidative phosphorylation were mostly down-
658 regulated in this study **(Fig. 5a)**. *ATPF0C*, *ATP1A*, *cox1*, *cox2*, *cox3*, *cox15*
659 significantly decreased under heat stress in summer, both as a result of a 3˚C
660 and 6˚C temperature rise **(Fig. 5b)**. Oxidative phosphorylation is involved in
661 the synthesis of adenosine triphosphate (ATP) (Senior, 1988), which supports
662 the growth and survival of heterotrophic bacteria (Cook et al., 2017). Thus, the
663 decline in oxidative phosphorylation-associated transcript levels that was
664 observed with short-term warming in the summer likely led to reduced ATP-
665 synthesis and a subsequent decrease in the growth rate of heterotrophic
666 prokaryotes **(Fig. 1c)**. The down-regulated ATP synthase of *Escherichia coli*
667 upon heat stress was also observed *in vitro* (Kim et al., 2020). Additionally,
668 ATP-synthesis can be blocked by the lack of inorganic phosphate (Nicholls,
669 1982) and Kim et al. (2020) showed that the phosphate required for ATP
670 synthesis decreased upon the temperature increase by metabolome analysis.
671 The ambient concentrations of phosphate were extremely low (mostly < 0.2
672 μM) in our summer experiments **(Table S1)**. Considering the general pattern
673 of low phosphate concentrations in the subtropical gyres (Martiny et al.,
674 2019), the decrease of heterotrophic prokaryotes growth in response to
675 temperature increase could be common in these areas.
676
677 In contrast, photosynthesis is generally less sensitive to temperature than

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678 respiration (Padfield et al., 2016). In this study, a 3˚C increase in temperature
679 had minimal influence on the expression levels of *Synechococcus*
680 photosynthesis-related genes. However, during summer, when temperatures
681 exceeded 36°C, certain photosynthesis-related genes, including *psaADEIJK*,
682 *psbE*, *cpcABC*, and *cpeAB*, were significantly down-regulated (DESeq2, $p_{adj} <$
683 0.05). Previous research has indicated that the PS-II light-harvesting antenna
684 of *Synechococcus* is thermally sensitive (Pittera et al., 2017). Our findings
685 further reveal that among all antenna protein genes, *cpcB* was the most
686 strongly downregulated gene **(Fig. S6)**. Taken together, we attribute the
687 apparent reduction in the growth of heterotrophic prokaryotes and
688 *Synechococcus* under short-term warming conditions in the summer to a
689 down regulation of oxidative phosphorylation and photosynthesis,
690 respectively.
691

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713

714 **Author Contributions**

715 **Bowei Gu:** Investigation; field experiments; formal analysis; methodology;
716 visualization; writing – original draft. **Xiao Ma:** Investigation; field experiments;
717 writing – review and editing. **Bingzhang Chen:** Writing – review and editing.

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718 **Hongbin Liu:** Writing – review and editing. **Yang Zhang:** Writing – review and
719 editing. **Xiaomin Xia:** Conceptualization; methodology; supervision;
720 validation; writing – review and editing.
721

722 **Conflict of Interest Statement**

723 The authors declare no conflicts of interest.
724

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953 **FIGURE LEGEND**

954 **Fig. 1. Effects of temperature increase on heterotrophic prokaryotes**
955 **were highly dependent on ambient temperatures**. Thermal responses of
956 heterotrophic prokaryotic abundance at the final point (24 h) of the filtered
957 incubations (**a**), abundance at the final point (24 h) of the unfiltered
958 incubations (**b**), intrinsic growth rate (**c**), and grazing pressure (**d**).
959 Experimental warming effects on heterotrophic prokaryotes growth rate (μ_{HP})
960 and grazing pressure (g_{HP}) were quantified by generalized linear model (see
961 methods). The letters in the first and second rows of the figure represent the
962 experimental date and the ambient temperatures (T_{amb}), respectively. The
963 asterisks in panels a-c indicate significance values (*$p < 0.05$, **$p < 0.01$; chi-
964 squared test), and the control temperatures (the same as the ambient
965 temperatures) of each experiment are shown in panels. Comparisons
966 between the negative effects of warming on growth and grazing (**e**). Note that
967 only the negative effects were included. *Represent the significant difference
968 with $p < 0.05$ (t-test). The linear relationships between the control
969 temperatures and the effects of warming on the growth rate (**f**) and grazing
970 pressure (**g**). These effects were scaled before running the model.
971

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972 **Fig. 2. Autotrophic prokaryotes *Synechococcus* hardly survived under
973 6˚C experimental warming in summer**. Thermal responses of
974 *Synechococcus* abundance at the final point of the filtered incubations (**a**),
975 abundance at the final point of the unfiltered incubations (**b**), intrinsic growth
976 rate (**c**), and grazing pressure (**d**) in summer. Experimental warming effects
977 on *Synechococcus* growth rate (μ_{Syn}) and grazing pressure (g_{Syn}) were
978 quantified by generalized linear model (see methods). The asterisks indicate
979 significance values (*p < 0.05, **p < 0.01; t-test), and the control temperatures
980 (the same as the ambient temperatures, T_{amb}) of each experiment are shown
981 in panels.

982

983 **Fig. 3. Different responses to temperature increase between
984 heterotrophic prokaryotes and *Synechococcus***. (**a**) The comparison of the
985 warming effects on growth rates (μ) between heterotrophic prokaryotes (HPs)
986 and *Synechococcus* (*Syn*) when ambient temperatures (T_{amb}) were ≥30˚C.
987 The asterisks indicate significance values (**p < 0.01; t-test). (**b**) The
988 standardized ratio of heterotrophic prokaryotes and *Synechococcus*
989 abundance over ambient temperatures.

990

991 **Fig. 4. Thermal preference of different prokaryotes**. Ternary plots showed
992 responses of prokaryotic taxa to temperature increase (at family level colored

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993 by class) in the experiments of Jan19 (**a**), Jul15 (**b**), Aug14 (**c**) and Sep07 (**d**).
994 The experimental date and ambient temperatures (T_{amb}) of each experiment
995 were shown in each panel. The dot size represented the relative abundance
996 of each family based on 16S rRNA data. The top 10 abundant families were
997 labeled in panels b, c, d, except a, because all the families gathered together
998 in that experiment. Arrows highlight the position of Cyanobacteria in the
999 panels. Abbreviations: Actin., Actinomarinaceae; Alter., Alteromonadaceae;
1000 Bacte., Bacteriovoracaceae; Balne., Balneolaceae; Bdell., Bdellovibrionaceae;
1001 Croci., Crocinitomicaceae; Cryom., Cryomorphaceae; Cyano., Cyanobiaceae;
1002 Flavo., Flavobacteriaceae; Halie., Halieaceae; Micro., Microbacteriaceae;
1003 Rhodo., Rhodothermaceae; Rhodob., Rhodobacteraceae; Sapro.,
1004 Saprospiraceae.
1005
1006 **Fig. 5. Potential mechanisms indicated by metatranscriptomics for the**
1007 **reduced prokaryotic growth under thermal stress.** (**a**) Overview of the
1008 pathways with metatranscriptional expression genes that significantly respond
1009 to experimental warming. Blue and red bars indicate the number of
1010 significantly down-regulated and up-regulated genes, respectively. The
1011 significantly changed genes related to oxidative phosphorylation of
1012 heterotrophic prokaryotes (**b**) and photosynthesis of cyanobacteria (**c**) in
1013 response to extreme temperature increase in summer. Significant (DESeq2,

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1014 $p_{adj} < 0.05$) and non-significant (DESeq2, $p_{adj} > 0.05$) effects are marked with

1015 solid and hollow dots, respectively.

1016