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Response of a coastal tropical pelagic microbial community to changing salinity and temperature

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ABSTRACT: Studies on the responses of tropical microbial communities to changing hydrographic conditions are poorly represented. We present here the results from a mesocosm experiment conducted in coastal southwestern India to investigate how changes in temperature and salinity may affect a coastal tropical microbial community. The onset of algal and bacterial blooms, maximum production and biomass, and the interrelationship between phytoplankton and bacteria were studied in replicated mesocosms. The treatments were set up to feature ambient conditions (28°C, 35 PSU), hyposalinity (31 PSU), warming (31°C), and a double manipulation treatment with warming and hyposalinity (31°C, 31 PSU). The hyposaline treatment had the most considerable influence, manifested as significantly lower primary production, and the most dissimilar microphytoplankton species community. The increased temperature acted as a catalyst in the double manipulation treatment, and higher primary production was maintained. We investigated the dynamics of the microbial community with a structural equation model and found a significant interrelationship between phytoplankton biomass and bacterial abundance. Using this methodology, it became evident that temperature and salinity changes, individually and together, mediate direct and indirect effects that influence different compartments of the microbial loop. In the face of climate change, we suggest that in relatively nutrient-replete tropical coastal zones, salinity and temperature changes will affect nutrient assimilation, with subsequent significant effects on the quantity of microbial biomass and production.

KEY WORDS: Phytoplankton · Bacteria · Climate change · Temperature · Salinity · Structural equation modeling · Tropical microbial community

INTRODUCTION

Phytoplankton forms the base of the marine food chain and is intimately connected with higher and lower trophic levels. Additionally, nearly half of the global primary production stems from the phytoplankton of the world's oceans (Falkowski et al. 1998). Estimating the quantity and use of the carbon fixed during primary production and its potential variability under a changing external environment

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due to climate change is therefore fundamental for the understanding of the global carbon cycle and the energy transfer through the marine food chain. With respect to climate change scenarios, the future providence of oceanic global carbon fixation is a complex task. Uncertainties in generated predictive models are possibly due to the heterogeneity of the polyphyletic group of protists and bacteria responsible for photosynthetic carbon fixation and the great complexity of interlinkage in aquatic food webs (Boyce et al. 2010, Behrenfeld 2011).

Marine habitats are expected to face changing hydrographic conditions manifested as an increase in sea surface temperature (SST), higher freshwater runoff — resulting in decreasing sea surface salinity (SSS) —, stronger stratification, and ocean acidification (Meehl et al. 2005, Wootton et al. 2008). The microbial ecosystems of some polar habitats display rapid decline, whereas others are shifting towards different functional states with implications for the marine food web and biogeochemical cycling (e.g. Vincent 2010, Comeau et al. 2011). The tropics may also experience an early manifestation of climate change because of the relatively small natural variability and narrow climate bounds (Mora et al. 2013). The boundaries of species' niches are easily surpassed by relatively small hydrographic changes. Hence, changes in hydrographic conditions may induce considerable biological responses because species are adapted to a narrower climate window as opposed to organisms in temperate regions that have a broader physical tolerance (Deutsch et al. 2008, Thomas et al. 2012). Organisms from lower latitudes might not be able to escape the unfavorable condition, or survive through inherited plasticity and subsequently adapt to the new conditions (Mora et al. 2013).

Observations from the tropical Indian Ocean display a positive SST anomaly concurrent with extreme monsoon rain events (Anderson et al. 2002, Goswami et al. 2006). Increased precipitation over central India mediates the greater discharge of freshwater into the seas surrounding the peninsula, i.e. the Bay of Bengal and the Arabian Sea, and thereby creates a negative SSS anomaly (Gopalakrishna et al. 2005, Nyadjro et al. 2012). In the coastal southeastern Arabian Sea, the SST displays a long-term oscillation pattern in concurrence with the El Niño Southern Oscillation (ENSO) with an amplitude of >4°C, and the SSS displays a negative linear trend of approximately 0.1 yr^{-1} (Ramesh et al. 2010, Subrahmanyam et al. 2011, Godhe et al. 2015)

Marine bacteria and planktonic algae closely interact in the phycosphere, which is the immediate zone surrounding an algal cell (Amin et al. 2012). All active photosynthetic plankton release dissolved organic matter (DOM). The organic compounds are utilized by heterotrophic bacteria which may be free living near the surface of the algae or directly attached to it or occur as intracellular algal symbionts (Cole 1982). Extracellular DOM release promotes and enables close and intense algal–bacterial interactions on a cellular level, which mediate fast energy flow through the microenvironment (Passow et al. 2007, Gärdes et al. 2011). Recent studies expand on this emerging recognition that marine microbial communities are part of tightly connected networks by providing evidence that these interactions are mediated through the production and exchange of infochemicals (Amin et al. 2015). Several studies from temperate regions have explored temperature and salinity related traits and processes, such as optimal conditions for growth and tolerance, for marine phytoplankton and the marine microbial food web (Edwards & Richardson 2004, Hare et al. 2007, Levitan et al. 2010, Thomas et al. 2012). However, the interactions of tropical microbial communities and their responses to changing hydrographic conditions are presently not well investigated.

In the tropics, dissolved inorganic and organic nutrients are removed relatively fast from the sunlit ocean through assimilation by primary producers and heterotrophic bacteria (Dyhrman et al. 2009). Thus, the release of dissolved organic carbon (DOC) constitutes an important energy source for heterotrophic bacteria in tropical coastal waters with high turnover rates, and the dependence of heterotrophic bacteria on the phytoplankton standing stock mediates a strong interrelation between phytoplankton and bacterial biomass (Rehnstam-Holm et al. 2010). Field observations in the tropics have indicated that the density of algal biomass is more important for marine bacterial proliferation compared to other environmental parameters such as inorganic nutrients, temperature, and salinity (Asplund et al. 2011).

During this study, we examined the effect of warming, hyposalinity, and a combination of these on the tropical microbial community. Using a mesocosm setup, we aimed to investigate the following: (1) the effect of warming and hyposalinity on the maximum phytoplankton biomass, primary production, total bacterial abundance, and bacterial production; and (2) whether warming or hyposalinity, or a combination of the two, altered the timing of maximum phytoplankton biomass and bacterial abundance, and elicited an immediate response from the constituting microphytoplankton taxa. Our hypothesis was that

warming would stimulate phytoplankton growth and production. Hyposalinity, on the contrary, would affect the community negatively, with a decreased production and biomass. Further, we addressed (3) how the strong link between the phytoplankton and bacteria might differ under changed environmental conditions. We anticipated that the bacterial community would be affected by the phytoplankton biomass over consecutive days (Rehnstam-Holm et al. 2010, 2014); therefore, we used a structural equation model (SEM) which has the potential to capture the dynamics of the direct and indirect effects of the microbial community. It is well known that phytoplankton biomass stimulates the abundance of heterotrophic bacteria (Schut et al. 1997). Further, both phytoplankton and bacteria utilize inorganic nutrients to maintain growth (Kirchman 1994); consequently, the treatments will have indirect effects on inorganic nutrient concentrations. To achieve the overall objectives, a mesocosm experiment was set up using a microbial community growing under nutrient-replete conditions. We followed the bloom until biomass started to decline. The study was terminated when the system became limited to avoid negative feedback and competition between the compartments.

MATERIALS AND METHODS

Mesocosm setup and sample collection

Mesocosms were set up for 4 different treatments to study the following phytoplankton and bacterial growth aspects: (1) ambient temperature and salinity (28°C, 35 PSU), (2) hyposalinity (28°C, 31 PSU), (3) warming (31°C, 35 PSU), and (4) warming and hyposalinity (31°C, 31 PSU). The treatments (1–4) were quadruplicated, resulting in 16 mesocosms. The experiment was conducted in December 2011 at the College of Fisheries in Mangalore, India. Seawater was collected 2 d before the plankton inoculum (see this section) 15 km south of Mangalore and was transported to the indoor mesocosm facility by trucks. The water was transferred to a common reservoir of 20 000 l. Before the release of water into the mesocosms, the tanks were thoroughly scrubbed and wiped with ethanol. Seawater was filtered through 0.5 µm sand filters to eliminate any coarse particles and was ozonized (Eltech ozone generator) to remove any non-marine contaminants brought along during transportation, while simultaneously being released into all the mesocosms. This treated water was checked for the presence of viable bacteria or protists. A pilot study revealed that the ozone treatment eliminated protists completely and that viable bacteria were reduced by 97 %. Each mesocosm unit contained a final volume of 1000 l water. Randomly, each mesocosm was assigned one of the 4 treatments. Light (Osram 36W Lumilux Daylight) intensity at the water surface was set to 50 µmol photons m^{-2} s^{-1} using tube lights at a 12 h light:12 h dark cycle. To minimize light scatter and prevent unwanted particles from falling in, reflective blankets were put up above each tank with slits to allow air circulation in the mesocosms. Before inoculating the natural microbial community, the ambient concentrations of dissolved inorganic nutrients, i.e. nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), silicate (SiO_3), and phosphate (PO_4^{3-}), were analyzed to confirm non-limiting nutrient conditions for the anticipated algal and bacterial growth.

The inoculum (phytoplankton assemblage with associated bacteria) was collected from the same source as the mesocosm water using plankton nets (10 µm mesh size). The method for concentrating the natural phytoplankton community was selected based on the dominance and functional importance of microphytoplankton in this and other coastal marine zones (e.g. McAllister et al. 1961). The phytoplankton species composition of the coastal southeastern Arabian Sea during the post-monsoon period is dominated by chain-forming phytoplankton species (Asplund et al. 2011). When chain-forming species, or species with spines or setae, occur in high abundances, the plankton community itself forms a fine network inside the net which retains smaller solitary cells (Tangen 1978). Hence, the inoculum also represented cells smaller than the pore size of the net. The sampled inoculum was thoroughly mixed in one large container and equally distributed among the 16 mesocosms. Chlorophyll concentration was estimated in 3 random mesocosms after inoculation to ensure that an equal amount was added to all units. This type of inoculum enabled a pre-bloom concentration (1.11 ± 0.15 µg chl *a* l^{-1}, average ± SD) of actively growing phytoplankton cells (McQuoid & Godhe 2004). Inoculation was followed by a 48 h period of acclimatization, where temperature was gradually increased (treatments 3 and 4) and/or salinity decreased (treatments 2 and 4). The temperature was increased by 1°C every 16th hour (from 28 to 31°C) using 4 heaters inside these tanks. The mesocosms for ambient temperature (treatments 1 and 2) also received a set of 3 heaters each to ensure a constant water temperature. Salinity was decrea-

sed from 35 PSU by exchanging 29 l of seawater with deionized fresh water every 12th hour to attain 31 PSU. After the acclimatization period was completed, the experiment started (experimental day 1). During the acclimatization and the experimental period, the mesocosms were continuously aerated with pressurized air through filter-plugged silicon tubes. Additionally, all mesocosms were stirred twice daily — once 30 min prior to sampling and once in the afternoon — with a long wooden stick. We used 1 individual stick for each mesocosm.

Temperature and salinity were measured daily by a thermometer and digital salinometer, respectively, to ensure stability in these parameters. Mesocosm sampling was performed at 09:00 h every alternate day for 14 d. In total, the mesocosms were sampled 7 times. The sampling was terminated when the chl *a* concentration started to decline, initiating an expected population crash due to (1) increased turbidity and, thus, decreased effective irradiance in the water column due to shading, and/or (2) nutrient depletion. Mesocosm samples were taken for different variables viz. inorganic nutrients, DOC, pH, chl *a*, primary production, total bacteria, bacterial production, copepod abundance and phytoplankton species identification, quantification and biovolume estimates.

The concentrations of inorganic PO_4^{3-} were measured spectrophotometrically (Shimadzu), following Strickland & Parsons (1972). NO_3^-, NO_2^-, and NH_4^+ concentrations were estimated spectrophotometrically using standard methods (Grasshoff et al. 1999). The analytical precision for NO_3^- and NH_4^+ concentrations was ±0.02 µM. SiO_3 concentrations were estimated with Merck kits for seawater (Merck KgaA 1.14794.0001, detection range 0.08 to 80 µM) using a spectrophotometer (Spectroquant® NOVA 60, Merck KGaA). For DOC, sample water was filtered through GF/F filters and immediately frozen. It was later analyzed in a TOC analyzer (model TOC-VCPH with total nitrogen unit TNM 1, Shimadzu).

Growth of phytoplankton biomass in the mesocosms was estimated using chl *a* concentrations. On each sampling occasion, 200 ml of water in duplicate from each mesocosm tank was vacuum filtered through GF/F filters (Whatman). Pigments were extracted overnight at 4°C in 3 ml of 90% acetone. The absorption of the extracted pigments was measured in a spectrophotometer (Shimadzu). Chl *a* concentrations (µg l^{-1}) in the water samples were calculated according to Jeffrey & Humphrey (1975).

Primary production was estimated by using carbon uptake rates. Carbon uptake rates were measured using the ^{13}C tracer technique in 4 of 16 mesocosms, each belonging to different conditions, on 4 different days (3rd, 6th, 8th, and 11th days). Water samples from each mesocosm were collected into 1 l acid-washed polycarbonate Nalgene bottles in duplicate, spiked with 99 atom% enriched ^{13}C-$NaHCO_3$ (<10% of ambient DIC), and incubated *in situ*, floating on the surface, for 4 h (approximately 2 h either side of local noon). Post-incubation samples were filtered onto Whatman GF/F filters (pre-combusted at 450°C) and oven dried at 50°C. Prior to the isotopic analysis, samples were acidified overnight using concentrated HCl to remove inorganic carbon content and further dried at 50°C. The mass spectrometric analyses of samples were performed using a continuous-flow isotope ratio mass spectrometer (Delta V Plus) connected to an elemental analyzer (Flash EA 2000), where ^{13}C atom% and particulate organic carbon (POC) content were measured. The uptake rates were calculated using the following equation adapted from Slawyk et al. (1977):

$$\text{Uptake rate (µmol C l}^{-1}\text{ h}^{-1}) = P \cdot \Delta I_p / [T \cdot (I_0 S_a + I_r S_t)/(S_a + S_t) - I_0]$$

where P is the POC in the post-incubation sample; ΔI_p is the increase in ^{13}C atom% in POC during incubation; S_a and S_t are ambient and added substrate concentrations, respectively; I_r and I_0 are ^{13}C atom% of the added tracer and natural ^{13}C atom%, respectively; and T is the incubation time.

For total bacterial abundance, 50 ml water samples were preserved in formaldehyde (1% final concentration) and stored in the dark at room temperature. These samples were stained with SYBR Green nucleic acid gel stain (0.5% final concentration, Invitrogen), and total bacterial abundance (cells ml^{-1}) was estimated by flow cytometer (FACSCalibur, BD Biosciences). A minimum of 10 000 events min^{-1} was recorded. Bacterial production was measured by the analysis of amino acid uptake after incubation with radioactively labeled leucine (Smith & Azam 1992). To survey the potential effects of grazing on phytoplankton, water samples were screened for mesozooplankton twice during the experiment, on experimental days 1 and 7, and 500 ml of water sample from each mesocosm was poured through a 100 µm stainless sieve mesh. Copepods and nauplii larvae retained were fixed with 1% Lugol's solution (Throndsen et al. 2003) and quantified in a stereomicroscope. Phytoplankton species identification, abundance, and sizes were microscopically determined for 1 replicate of each treatment on experimental day 1 and from all 4 replicates of each treat-

ment on experimental days 3, 7, and 11. Then, 50 ml water samples were fixed with 1% Lugol's and settled in sedimentation chambers (Utermöhl 1958). Phytoplankton cells were identified to the lowest taxonomic level possible and counted under 200 to 400× magnification. Small (diameter 3 to 5 µm) cells with lost flagella or without a flagellum were categorized as unicells. Species abundances (cells l^{-1}) were of limited interest because of the large size differences between the species. For better comparison, the total cell numbers were converted to total cell volume per taxon (μm^3 l^{-1}) based on the procedure stated in Olenina et al. (2006).

Data analysis

The net effects of warming, hyposalinity, and their interaction on maximum phytoplankton biomass, primary production, bacterial abundance, and bacterial production were tested using type I ANOVA. Temperature (high vs. ambient) and salinity (low vs. ambient) were used as fixed factors. Significance levels were set at $p < 0.05$. ANOVA analysis was also used to test for the differences in phytoplankton biomass and bacterial abundance induced by the treatments separately on each sampling occasion. Before running the analysis, all response variables were checked for homogeneity of variances and normality. All analyses were performed using SPSS v. 21 (IBM Statistics).

To investigate the dynamics between the phytoplankton and bacteria, as well as their respective influence on the nutrient pool (represented by dissolved inorganic nitrogen, DIN) an SEM was used. SEM is a multi-statistical tool with a multiple regression base (Grace 2006) which can be used to partition net effects of experimental treatments into direct and indirect effects (Wootton 1994, Alsterberg et al. 2013). Data from 4 sampling occasions (experimental days 5, 7, 9, and 11) were combined, rendering 64 (16 × 4) data points. The measurement of bacterial abundance was missing for one of the replicates on experimental day 9. Since SEM requires complete datasets (Allison 2003), all data from this replicate were omitted from the analysis. Thus, 63 data points were analyzed. The 2 initial samplings (experimental days 1 and 3) as well as the last sampling occasion (day 13) were excluded to allow the experiment to reach a stable state and to exclude the deteriorating community of the last day. The treatments (hyposalinity, warming, and their interaction, i.e. warming and hyposalinity) were modeled as binary numbers (Grace 2006, Whalen et al. 2013). To obtain normality, the continuous response variables, phytoplankton biomass (µg chl *a* l^{-1}) and bacterial abundance (cells ml^{-1}), were $\log_{10}$ transformed, while DIN concentrations were square root transformed. The effect of the treatments on phytoplankton biomass and bacterial abundance could then be evaluated simultaneously when the influence of all the 5 variables on the DIN concentrations was estimated. To evaluate the model's fit to the data, the observed covariance matrix was compared to that predicted by the model, and the residuals were used to derive a chi-square (χ^2) value. Contrary to traditional hypothesis testing, a lower p-value here indicates large residuals and a poor model fit, and generally models with $p \geq 0.3$ are accepted as probable (Arhonditsis et al. 2007, Blake & Duffy 2012). Standardized path coefficients were created through normalization against the standard deviation, and this parameter thus represents the approximate percentage to percentage change that the predicting variable exerts on the dependent variable. These coefficients were used to compare the relative strength of different paths in the model. Significant levels for individual paths within the model were accepted at $p < 0.05$, and SEM was run in AMOS v. 21 (IBM SPSS).

Patterns in the assemblage structure of the phytoplankton taxa as a function of treatment were derived from the proportional biovolumes of each taxon and examined by non-parametric multivariate analysis in the PRIMER software (Clarke 1993). A 2-way nested ANOSIM (experimental day [3, 7, and 11] nested within treatment) was used to test the null hypothesis of no difference between the treatments by 999 permutations; 33 taxa that contributed ≥2% of the total biovolume in any replicated treatment at any sampling time were included. The input data were $\log_{10}$ transformed. No data were weighted. SIMPER was used to determine the degree of difference (% dissimilarity) between the treatments and examine which phytoplankton taxa contributed most to the observed differences. SIMPER analysis was also used to investigate the change of species composition over time within each treatment. The analysis was performed on transformed biovolume proportions, arranged in a Bray-Curtis similarity matrix. The diversity of the phytoplankton community in the different treatments was estimated using the Shannon-Wiener index (H') and calculated based on abundance data of the different taxa. Changes in phytoplankton species diversity over time were analyzed by linear regression using SPSS v. 21 (IBM Statistics).

RESULTS

The recorded temperatures in the ambient and warming treatments were 28.6 ± 0.4 and 31.5 ± 0.4°C, respectively. The salinity was 35.1 ± 0.3 in the ambient treatments and 30.9 ± 0.4 in the hyposaline treatments. The range, average, and standard deviation of all measured variables per treatment are stated in Table S1 in the Supplement at www.int-res.com/articles/suppl/a077p037_supp.pdf.

Temperature was an important factor in setting the phytoplankton growth pace of the experiment. The warming (31°C) treatments displayed significantly faster initial growth of the phytoplankton community. Irrespective of salinity, higher phytoplankton biomass, based on chl *a* concentrations (µg l^{-1}), was recorded on experimental day 5 ($p = 0.007$, $F = 10.61$, Fig. 1A). The warming treatments attained maximum phytoplankton biomass on experimental day 7 and declined thereafter, whereas the treatment exposed to ambient conditions maintained high phytoplankton biomass until experimental day 11 (Fig. 1A). On experimental day 13, all replicated treatments had declined in phytoplankton biomass, and the experiment was subsequently terminated. The interaction between warming and hyposalinity had significant effects on bacterial abundance in the initial phase of the experiment (experimental days 1, 3, and 5, Fig. 1B). Pairwise comparisons displayed a significant difference between the 2 temperature treatments (28 and 31°C) at low salinity ($p < 0.05$). A significant difference between the 2 temperature treatments irrespective of salinity was observed on experimental day 7 ($p = 0.02$, $F = 7.46$, Fig. 1B).

We recorded a trend towards lower maximum phytoplankton biomass in the hyposaline treatment, but variation among replicates for each treatment was high; therefore, the net effect was not significant (Fig. 2A). Maximum primary production was significantly affected by the treatments ($p = 0.001$, $F = 70.45$, Fig. 2B, Fig. S1A in the Supplement), where salinity ($p < 0.001$) and the interaction of temperature and salinity ($p = 0.001$) showed significant effects. The hyposaline treatment (28°C, 31 PSU) displayed the lowest maximum primary production (average 98.6 ± 14.8 µg C l^{-1} d^{-1}), and the ambient treatment exhibited the highest maximum primary production (average 580.3 ± 54.3 µg C l^{-1} d^{-1}). On pairwise comparison, we observed a significant difference between the 2 salinity treatments at ambient temperature (28°C) ($p < 0.001$). Also, the 2 temperature treatments (28 and 31°C) were significantly different at low salinity of 31 ($p = 0.008$) and at ambient salinity

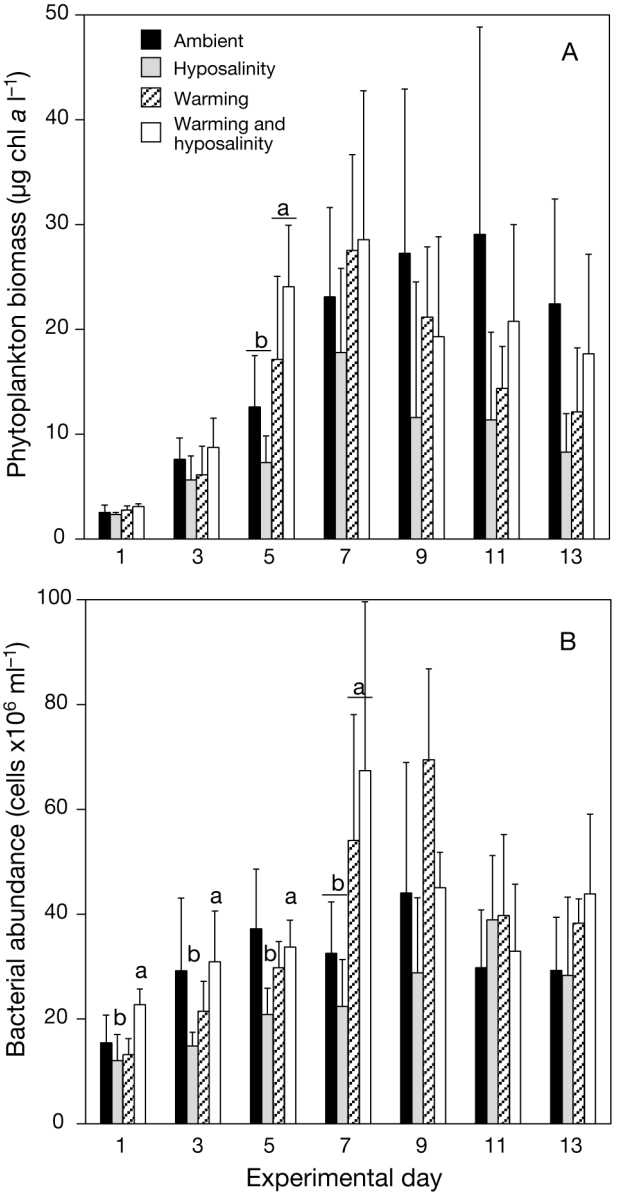


Fig. 1. (A) Phytoplankton and (B) bacterial growth in the 4 treatments during the course of the experiment, i.e. from experimental days 1 to 13. n = 4, error bars depict standard error of mean. The effect of the treatments on phytoplankton biomass and bacterial abundance was tested by ANOVA on each experimental day. Different letters on the same experimental day denote significant difference ($p < 0.05$) between treatments. Phytoplankton biomass (estimated as µg chl *a* l^{-1}) (A) was significantly larger in the warming treatments on experimental day 5, irrespective of salinity; bacterial abundance (B) was estimated as 10^6 cells ml^{-1}. On experimental days 1, 3, and 5, the interaction between warming and hyposalinity was significant. Pairwise comparisons displayed a significant difference between the 2 temperature treatments (28 and 31°C) in the hyposaline treatments. On experimental day 7, the bacterial abundance was significantly larger in the warming treatments irrespective of salinity

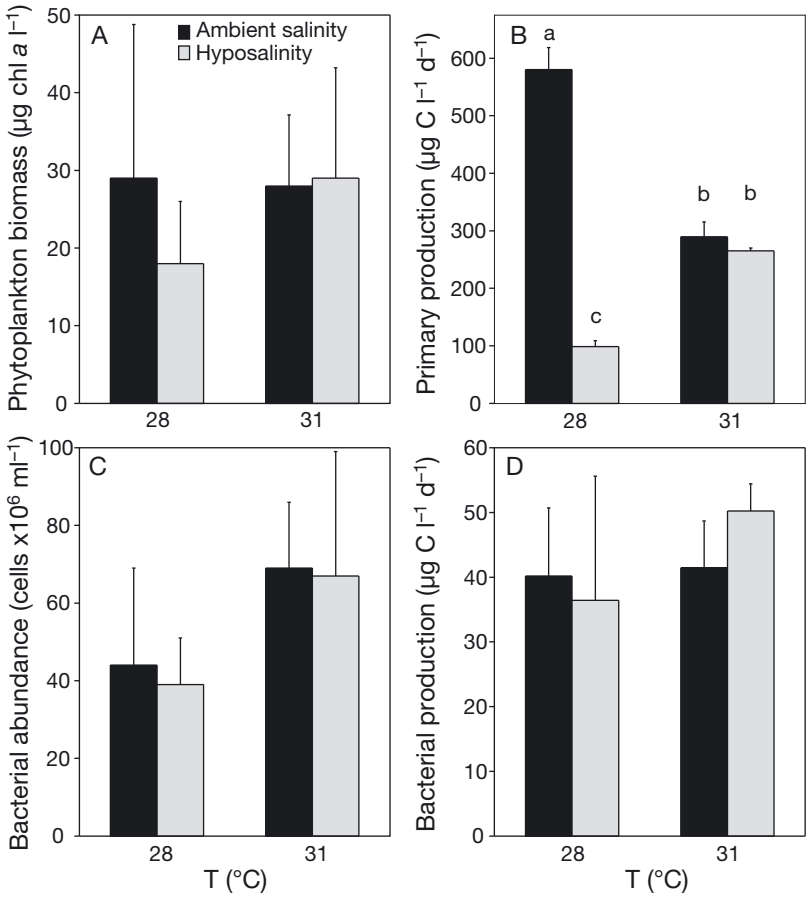


Fig. 2. (A) Maximum phytoplankton biomass, (B) primary production, (C) bacterial abundance and (D) bacterial production recorded in the different treatments. n = 4, except in (B), where n = 2. Error bars represent standard error of mean. The effect of the treatments on phytoplankton biomass and bacterial abundance and on primary and bacterial production was tested by ANOVA. The effect was significant for primary production (B). Different letters denote significant difference ($p < 0.001$) between treatments. T: temperature

of 35 ($p = 0.001$). All treatments recorded maximum primary production on experimental day 8, except the warming and hyposalinity treatment that recorded maximum primary production on experimental day 6 (Fig. S1A in the Supplement). There was no significant net effect of treatments either on the maximum bacterial abundance, estimated as average maximum total bacterial count ($p = 0.429$, Fig. 2C), or on the net maximum bacterial production ($p = 0.524$, Fig. 2D, Fig. S1B in the Supplement).

The SEM did not deviate significantly from the observed data ($\chi^2 = 0.016$, 1 df, $p = 0.899$), which justified further analysis of the model output data. The model revealed that hyposalinity had a significant direct negative influence on the dynamics of the phytoplankton biomass, whereas warming showed no significant influence. The interaction between warming and hyposalinity had a direct positive influence on the phytoplankton biomass (Fig. 3, Table 1). Additionally, the phytoplankton biomass displayed a strong positive influence on the bacterial abundance (Fig. 3, Table 1). Warming alone positively affected the dynamics of the bacterial abundance, but hyposalinity and the interaction (warming and hyposalinity) had no significant effects on bacterial abundance. As the phyto-

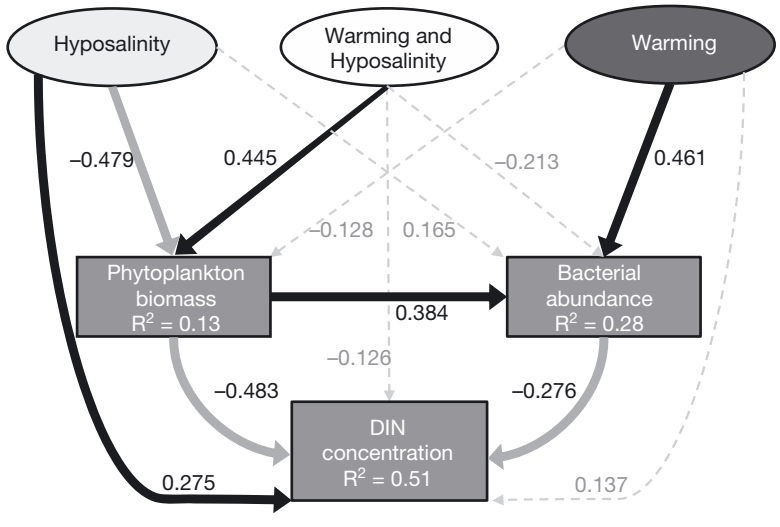


Fig. 3. The structural equation model (SEM) was formulated based on the estimates of phytoplankton biomass (measured as µg chl *a* l^{-1}) and bacterial abundance (measured as cells ml^{-1}) from experimental day 5 until 11. Ovals represent treatments, and rectangles represent measured variables. Black and grey (thick arrow) paths indicate significant ($p < 0.05$) positive and negative influences, respectively. Dashed-line paths indicate non-significant ($p > 0.05$) influences. Values next to the paths are standardized coefficients interpreted as follows: If, for example, the interaction (warming and hyposalinity) goes up by 1 SD, the chlorophyll value goes up by 0.445 SD. R^2 value for endogenous variables indicates the degree of variance explained by the model

Table 1. Results from the structural equation model (SEM) examining the experimental effects of warming, hyposalinity, and their interaction (warming and hyposalinity). Each row indicates 1 path in the model (Fig. 3), with the arrow showing the direction of the effect. Path coefficients represent the unit per unit change of the 2 connected variables as predicted by the SEM, while the standardized path coefficients allow for strength comparison (the standard deviation per standard deviation effect, i.e. at perfect normalized distribution, the percent change). SE: standard error; CR: critical ratio; DIN: dissolved inorganic nitrogen. For individual paths, $p < 0.05$ is considered significant. Whole-model chi-square (χ^2) = 0.016, df = 1, p = 0.899, sample size = 63

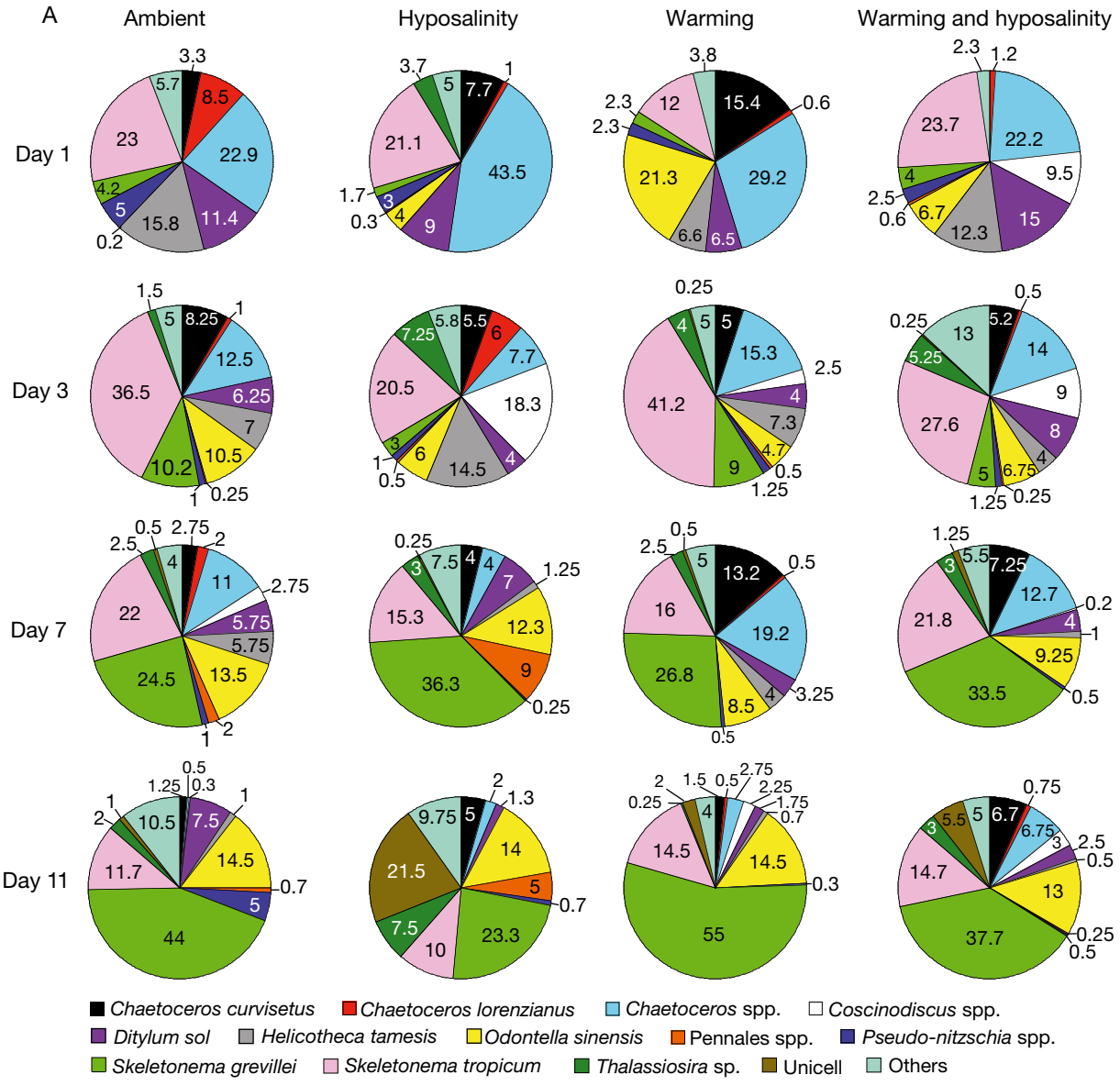
Path	Path coefficient	Standardized path coefficient	SE	CR	p-value
Warming → Phytoplankton biomass	−0.085	−0.128	0.110	−0.77	0.441
Interaction → Phytoplankton biomass	0.340	0.445	0.157	2.17	0.030
Hyposalinity → Phytoplankton biomass	−0.317	−0.479	0.112	−2.84	0.005
Warming → Bacterial abundance	0.247	0.461	0.082	3.00	0.003
Interaction → Bacterial abundance	−0.131	−0.213	0.120	−1.08	0.275
Hyposalinity → Bacterial abundance	0.088	0.165	0.088	0.99	0.317
Phytoplankton biomass → Bacterial abundance	0.311	0.384	0.094	3.30	<0.001
Phytoplankton biomass → DIN concentration	−0.950	−0.483	0.204	−4.65	<0.001
Bacterial abundance → DIN concentration	−0.670	−0.276	0.257	−2.60	0.009
Warming → DIN concentration	0.177	0.137	0.175	1.01	0.311
Interaction → DIN concentration	−0.189	−0.126	0.243	−0.80	0.436
Hyposalinity → DIN concentration	0.358	0.275	0.117	2.02	0.044

plankton biomass had a significant direct positive influence on the bacterial abundance, the bacterial abundance was not only directly affected by the treatment, i.e. salinity and temperature changes, but also indirectly affected through the phytoplankton biomass and its response to the treatment (Fig. 3, Table 2, Table S2 in the Supplement). For instance, the direct influence of hyposalinity on the bacterial abundance was eliminated by the indirect negative influence mediated by the phytoplankton biomass. SEM indicates that the phytoplankton community contributed most to the usage of the nutrient pool and had a significant direct negative influence on DIN ($NH_4^+ + NO_3^-$) concentrations (Fig. 3, Table 1). Additionally, the bacterial community utilized the inorganic nitrogen pool and displayed a direct negative influence. Our model indicated that in the hyposaline treatment, less DIN was consumed and thus provided a more nitrogen-replete mesocosm environment. On the contrary, the interaction had an indirect negative effect on the nitrogen pool, which was mainly mediated by the phytoplankton (Table 2).

Diatoms, irrespective of the treatment, dominated the phytoplankton community in the mesocosms. Towards the later phase of the experiment, small (<5 µm diameter) flagellated cells (unicells, Fig. 4A) and the microzooplankton *Protoperidinium* spp. (max 9% of the total biovolume in 1 of 4 replicates from experimental day 11, Table S3 in the Supplement) increased proportionally in the hyposaline treatment. The 2-way nested ANOSIM displayed a significant separation in phytoplankton community structure between the treatments and sampling days ($R = 0.32$, $p < 0.001$). The SIMPER analysis revealed that the hyposaline treatment displayed the most dissimilar species community based on proportional biovolumes (Fig. 4B). Based on the estimated biovolumes per individual taxa from experimental days 3, 7, and 11, we found that the phytoplankton community in the ambient and hyposaline treatments was the most different (average 55% dissimilarity). The warming and interaction (warming and hyposalinity) treatments were the least different (average 36% dissimilarity). The proportional variation of the biovolumes of the 2 *Skeletonema* species,

Table 2. Standardized total effects from the structural equation model (SEM). Values are the sum of the direct and indirect effects that the variables in the columns have on the variable in the rows, as predicted by the SEM (Fig. 3). An indirect effect equals the product of the 2 (or several) paths that create it (e.g. the indirect effect of Warming on Bacterial abundance is the product of the path between Warming → Phytoplankton biomass and Phytoplankton biomass → Bacterial abundance). DIN: dissolved inorganic nitrogen

	Warming	Interaction	Hyposalinity	Phytoplankton biomass	Bacterial abundance
Phytoplankton biomass	−0.128	0.445	−0.479	0.000	0.000
Bacterial abundance	0.412	−0.042	−0.020	0.384	0.000
DIN concentration	0.085	−0.330	0.513	−0.589	−0.276



B Average dissimilarity between treatments (%)

	Ambient	Hyposalinity	Warming	Warming and hyposalinity
Ambient	–			
Hyposalinity	55	–		
Warming	42	52	–	
Warming and hyposalinity	46	49	36	–

Fig. 4. Patterns in the assemblage structure of the phytoplankton taxa per treatment assessed by relative biovolumes of each taxon. (A) Taxa contributing an average (n = 4; for variability among replicates, see Table S3A–D in the Supplement at www.int-res.com/articles/suppl/a077p037_supp.pdf) of ≥5% of the total biovolume in the 4 treatments at any sampling time were included (14 species). Proportion of each taxon is indicated in the circle diagrams. The numbers feature the percentage (%) of each taxon of the total biovolume. For day 1, percentage is based on 1 replicate per treatment; for days 3, 7, and 11, the percentage of each taxon is based on the average of 4 replicates (for variability among replicates, see Table S3A–D in the Supplement). Coloration of taxa as indicated. (B) Pairwise degree of difference (% dissimilarity) between the treatments in respect of species composition. ANOSIM analysis yielded significant difference ($p < 0.001$) in respect of phytoplankton community composition among the 4 treatments. Subsequently, SIMPER analysis was used to quantify the difference. Taxa contributing ≥2% of the total biovolume in any replicated treatment at any sampling time were included (33 taxa, Table S3A–D in the Supplement) and examined with a SIMPER analysis

S. grevillei and *S. tropicum*, contributed most to the within-treatment (among replicates and experimental days) differences, i.e. 51 to 55% (Fig. 4A). The variation of these 2 taxa contributed less in respect of between-treatment variation (30 to 37%). In the pairwise comparisons with the 2 hyposaline treatments, the variation of unicells and *Coscinodiscus* spp. biovolumes contributed more to the observed differences (up to 7.2 and 6.9%, respectively). In the pairwise comparisons with the 2 warming treatments, the variation of proportional biovolumes of *Chaetoceros* spp. contributed more to the observed differences (up to 8.7%). The microphytoplankton species diversity displayed a declining trend in all the treatments (Fig. 5A–D) during the course of the experiment, with a significant decline in the warming treatment ($p < 0.01$, $F = 11.05$, Fig. 5C). The phytoplankton community displayed a tendency of higher taxon diversity in the ambient temperature treatments, but we recorded large variation among replicates and no significant difference among the treatments ($p = 0.54$).

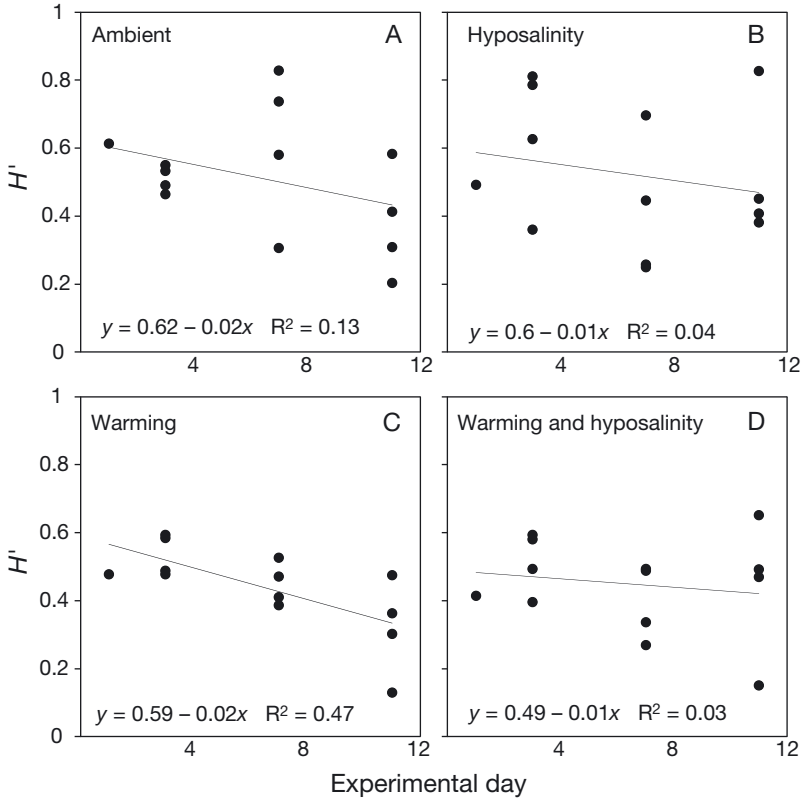


Fig. 5. Phytoplankton taxon diversity displayed as Shannon diversity index (H') during the course of the experiment, i.e. on experimental days 1, 3, 7, and 11. (A) Ambient conditions; (B) hyposalinity; (C) warming; (D) warming and hyposalinity. All the treatments displayed a declining trend during the course of the experiment in respect of diversity. Only the warming treatment (31°C, 35 PSU) displayed a significant decline ($F = 11,045$, $p < 0.01$). Species data from experimental day 1 were present from 1 replicate per treatment and from all 4 replicates on experimental days 3, 7, and 11

DISCUSSION

The results obtained from this mesocosm study highlight that hydrographic change, manifested by hyposalinity and warming, will have a significant impact on the tropical coastal pelagic microbial community. Warming triggered phytoplankton growth and caused a faster bloom development of both phytoplankton biomass and bacterial abundance. It also contributed to a change in microphytoplankton species composition. The lower salinity had even more pronounced effects as displayed by significantly lower primary production and the most dissimilar microphytoplankton species community. While hyposalinity alone caused a decrease in primary production, hyposalinity coupled with warming dampened its negative effect. Also, our results highlight the dependency of bacterial abundance on algal biomass. A strong positive effect of phytoplankton biomass on bacterial abundance means that any impact of climate change will have a consequential effect on the associated bacteria.

The hyposaline treatment had the most considerable influence on the estimated rate of processes, biomasses, and taxonomic structure of the microphytoplankton community. The reduced primary production recorded in the hyposaline treatment was probably due to fitness reduction in sub-optimal conditions, resulting in an overall slower growth. The lower salinity could potentially have resulted in decreased cell abundance by increased lysis. If cell lysis was the causative driver of lower phytoplankton biomass and reduced primary production, the DOC concentration is expected to be higher in the low-salinity treatment (Passow et al. 2007). In contrast, the estimated DOC concentration was low and uniform among the treatments during the course of the experiment, and there was no significant difference between the treat-

ments ($p = 0.28$). At ambient salinity, primary production was higher at the control temperature. However, at low salinity, increased temperature enhanced the primary production. Thus, interestingly, the increased temperature acted as a catalyst only in combination with low salinity, while at ambient salinity, higher temperature reduced the maximum primary production. Additionally, the warming treatments displayed faster growth of the phytoplankton as well as the bacterial community, irrespective of the salinity treatments. Our results suggest that increased temperature will promote faster growth and development of microbial blooms.

In coastal communities, microphytoplankton, especially diatoms, are the most important primary producers and the dominant contributors to carbon cycling (McAllister et al. 1961, Yool & Tyrell 2003). In our study, the phytoplankton species community was almost exclusively made up of diatoms, as anticipated, granted the time and the geographical site for the collection of the inoculum. In the coastal southeastern Arabian Sea, diatoms may constitute up to 99% of the phytoplankton community during the post-monsoon period, i.e. from September to December (Madhupratap et al. 1996, Härnström et al. 2007, D'Costa & Anil 2010, Asplund et al. 2011). It is a common observation that autotrophic, mixotrophic, or heterotrophic flagellates dominate mesocosms at the very end of an experiment due to silicate deficiency (Jacobsen et al. 1995). In our study, the silica concentration remained high throughout the experimental period (Table S1 in the Supplement at www.int-res.com/articles/suppl/a077p037_supp.pdf), which probably favoured diatom growth. There are also other limitations to mesocosm experiments, such as the wall effects of enclosed systems, which can affect the species composition and therefore not exactly mimic scenarios in the coastal environment (Chen & Kemp 2004).

In our study, the warming treatment represented exposure to the local temperature amplitude of 1 ENSO cycle, and the hyposaline treatments are in accordance with the ongoing sea surface freshening of southeastern Arabian Sea coastal waters on a decadal scale (Ramesh et al. 2010, Subrahmanyam et al. 2011, Godhe et al. 2015). If the environment and the selection pressure change, populations of selected species will have to adapt to the new conditions or move to a more favorable location which provides an environment for optimal growth, or the population will not survive and will go extinct (Orr & Unckless 2008). Obviously, much longer experimental periods are needed to expose populations or species communities to natural selection and to examine adaptation potential at the population or community level as a consequence of imposed environmental change (Collins & Bell 2004, Lohbeck et al. 2012, Schlüter et al. 2014). In our study, the species community present in any of the manipulation treatments will reflect the plasticity and tolerance of the phytoplankton species community to these relatively sudden changes. For example, the success and the dominance of *Skeletonema grevillei* in all treatments are probably due to inherent plasticity. But, we also expected candidate species to take advantage and proliferate during the course of the experiment. Estuarine species that normally recede in the coastal environment at relatively low densities under suboptimal conditions could potentially prosper in any of the manipulated conditions when the success of other competing species diminishes. For instance, the species *Asterionellopsis glacialis*, which may be classified as an estuarine species (Hustedt 1959, O'Boyle & Silke 2010), was proportionally more abundant in the low-salinity treatments (Table S3 in the Supplement).

The phytoplankton species diversity declined with increased phytoplankton density in all mesocosms irrespective of treatment. The inverse relationship between richness and abundance is commonly observed in enclosed experiments (McQuoid & Godhe 2004, Murray et al. 2007). The warming treatment displayed a significant decline of diversity index over time, which most likely was caused by the proliferation of the fast-growing and opportunistic species *S. grevillei*. Hyposalinity, on the contrary, appeared to counteract the fast decay in species diversity, probably by allowing estuarine species to prosper to a greater extent. The hyposaline treatment also displayed the most dissimilar species community. Initially, the large diatoms *Coscinodiscus* sp. and *Helicotheca tamesis* contributed proportionally more in the reduced-salinity treatment. However, these large diatoms sink fast, and probably they were outcompeted due to light limitation by shading from fast-growing smaller suspended cells. Subsequently, *Thalassiosira* spp., small pennate diatoms, and nanoflagellates (Pennales and unicells, Fig. 4A) became dominant in the hyposaline treatment. We have not conducted any single-species salinity tolerance experiments, and thereby it is not possible to conclude with certainty that these latter taxa are specifically tolerant to low salinity. However, our findings indicate that due to freshening of the coastal southeastern Arabian Sea in the future, the phytoplankton species composition is likely to change, possibly with a more prominent contribution of estuarine taxa.

In terms of temporal succession, we observed a change of species over the experimental period. This change was most pronounced in the hyposaline treatment and the double manipulation treatment. The temporal changes in species community composition were the least in the warming treatment. This was probably due to major changes, such as the rapid decay of diversity and elimination of selected species already during the acclimatization period, which rendered fewer tolerant species left to prosper during the actual experiment.

In our study, the use of SEM assisted in presenting a distinct relationship between algae and its associated bacteria and the ability to perceive the indirect treatment effects on the bacterial abundance. The results suggest that the close coupling between the 2 trophic levels, which had previously been observed in the same coastal region under ambient conditions in the field and in mesocosms (Rehnstam-Holm et al. 2010, 2014, Asplund et al. 2011), also persists during changed environmental conditions. The interrelationship was strictly positive between these 2 compartments irrespective of treatment (Spearman's Rho test 0.592, $p < 0.001$), which indicates that bacterial cells benefited from the organic material released by live algae. However, as dead algal cells also sustain heterotrophic bacteria (Rehnstam et al. 1993, Mouriño-Pérez et al. 2003), this was probably an additional carbon source for the bacteria in the mesocosms. Our data featured the dependency of bacterial abundance on algal biomass and explains the subsequent increase observed in total bacterial counts with increasing phytoplankton biomass. We have no detailed information on the grazing intensity of the heterotrophic bacteria or the microphytoplankton community. Ciliates and heterotrophic nanoflagellates are important grazers of heterotrophic marine bacteria (Azam et al. 1983). Whereas ciliates were absent in the mesocosms, heterotrophic nanoflagellates were grouped under the category unicells, which are defined as small cells with no visible flagella. However, it is difficult to differentiate between the small heterotrophic and autotrophic flagellates in Lugol's-fixed samples. The pool of heterotrophic microflagellates was made up of dinoflagellates (90% small, <10 000 μm^3, *Protoperidinium* species; 9.6% larger, >10 000 μm^3, *Protoperidinium* species; 0.4% Gymnodiales; Tables S1 & S3 in the Supplement). These are important microzooplankton which feed on diatoms and other dinoflagellates (Menden-Deuer et al. 2005), but they also have a considerable grazing impact on populations of marine bacteria (Jeong et al. 2008). The abundances of heterotrophic microflagellates and nanoflagellates (unicells) increased over time, although at similar rates ($r = 0.7$). Therefore, we believe that the grazing pressure on the phytoplankton and bacteria was comparable; hence, the bacterial abundance was more strongly coupled to the DOC source, i.e. the phytoplankton biomass.

Complex trophic interrelationships, such as the compartments of the microbial loop, interacting with a combination of environmental variables may not be completely assessed by the examination of direct effects only (Alsterberg et al. 2013). An interesting observation from the application of the SEM model is that the mode and the pathway for the buildup of high bacterial abundance in the coastal Arabian Sea will be modified depending on the set of environmental changes. In this respect, warming would directly benefit the heterotrophic bacterial community, as reported from other geographic locations (Hsieh et al. 2008, Martinez-Urtaza et al. 2008). Contrary to this, freshening of surface water may not directly affect the bacterial community, but indirectly through the phytoplankton community, hyposalinity will mediate a negative effect on bacterial biomass. The increased temperature scenario coupled to sea surface water freshening will additionally account for higher bacterial biomass, but indirectly through the accumulation of phytoplankton biomass. Total bacterial abundance was estimated without taxonomic resolution, but we observed a changed phytoplankton species community as a function of the changed hydrographic conditions. It is expected that the functional and taxonomic units of the bacterial community will be modified due to environmental changes but also according to the altered phytoplankton species community as phytoplankton species co-occur with specific bacterial taxa (Amin et al. 2012).

The present study indicates the importance of salinity and temperature to biogeochemical cycling in, and the biology of, a tropical coastal region. Lower primary production during decreased salinity conditions and the nutrient-replete environment of the present study suggest that potential change in salinity due to extreme rain events in the Indian subcontinent may lead to a decrease in carbon fixation in coastal regions, which can have an important consequence for the regional carbon budget and the marine trophic food chain. Coastal regions, although generally replete with nutrients, may also undergo a change in the nutrient pool due to the shift in phytoplankton and bacterial community structure and their ability to assimilate nitrogen and carbon, as evidenced during the present study.

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