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Interactive climate change and runoff effects alter O_2 fluxes and bacterial community composition of coastal biofilms from the Great Barrier Reef

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ABSTRACT: Global (i.e. ocean warming) and local (i.e. land runoff) anthropogenic disturbances affect coastal coral reefs worldwide. Terrestrial runoff, leading to reduced light and increased nutrient availability, may have interactive effects with ocean warming in promoting shifts in benthic communities. Because microbial biofilms rapidly reflect environmental changes, we investigated the quantitative (C, N and chlorophyll *a* contents) and qualitative (microbial community composition) parameters and metabolic responses (O_2 fluxes) of biofilms established on glass slides to combinations of manipulated water temperatures (26, 29 and 31°C), nitrate (0.5, 1.0 and 1.4 µM), and light availabilities (40 and 200 µmol photons m^{-2} s^{-1}) in a 28 d flow-through aquarium experiment. The findings revealed that, independent of light availability, a temperature of 31°C significantly decreased the 24 h net O_2 production and all of the quantitative parameters. Under high light, additive effects of 31°C and 1.4 µM nitrate reduced the 24 h net O_2 production. Terminal restriction fragment length polymorphisms (T-RFLP) of 16S rRNA genes showed light-independent temperature-induced microbial community shifts driven by increases in the relative abundance of *Oceanospirillum* (*Gammaproteobacteria*) and decreases of *Cyanobacteria*. The relative abundances of diatom plastids increased in response to elevated nitrate only in high-light-exposed biofilms. Overall, high temperatures altered microbial biofilm community composition, biomass and productivity. Under predicted near-future inner reef scenarios (low light and high nitrate availability), biofilms become light-limited through sedimentation, while outer (high light and low nitrate availability) inshore reef biofilms remain nitrate-limited. Understanding the interactive effects of environmental changes on microbial biofilm communities may contribute to bioindicator development and improved coastal management strategies for coral reefs.

KEY WORDS: Light · Nitrate · Temperature · Water quality · Terrestrial runoff · Coral reef

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INTRODUCTION

Several natural and anthropogenic impacts, such as rising sea surface temperatures (SST) and increased inorganic nutrient imports from land, may affect the functioning of coastal coral reef ecosystems worldwide. Climate change has already increased annual average SSTs on the Great Barrier Reef (GBR) by 0.7°C within the last century (Lough 2001, Lough et al. 2006). Furthermore, forecasts by the Intergovernmental Panel on Climate Change (IPCC 2007) predict that GBR waters may warm by a further 1 to 3°C by 2100. In addition to global warming, local terrestrial runoff from coastal agriculture (e.g. sugar cane) may contribute to the deterioration of water quality along coastal regions of the GBR. Terrestrial runoff is the largest source of new nitrate to the inshore GBR (Furnas 2003) and

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drastically increases during the wet season (Devlin & Schaffelke 2009), and these nutrient inputs have increased 10-fold over the pre-agriculture baseline (Wooldridge et al. 2006). Runoff leads to reduced light and increased nutrient availability for coral reef communities at inner shelf reefs in comparison to outer nearshore reefs, where nutrient availability is low and light availability high. Such inner nearshore conditions shift the benthic community composition, resulting in dominance by macroalgae that spatially compete with coral recruits, and consequently may promote decline in coral cover (Szmant 2002, Fabricius 2005, Schaffelke et al. 2005). Local chronic eutrophication continues to affect coral reefs and may have an additive interaction with global stressors, such as rising SSTs, therefore posing a threat to coral reefs of the GBR (Uthicke & Altenrath 2010, Schmidt et al. 2011, Uthicke et al. 2012b). Coastal water quality management of local disturbances may help preserve coral reefs from the projected effects of climate change (Wooldridge 2009, Negri et al. 2011, Schmidt et al. 2011).

Coral reefs harbour abundant microorganisms that predominantly exist as surface-attached communities (Costerton et al. 1995, Crump et al. 1998, Teske & Wooldridge 2001). Surface-colonising biofilms are complex communities comprising macromolecules and microorganisms embedded in an extracellular polymeric matrix (Mihm et al. 1981). These highly dynamic microbial biofilm communities respond rapidly to and are integrative of changing ambient environmental conditions (Paerl & Pinckney 1996, Snyder et al. 2005). Microbial biofilm communities also contribute significantly to biogeochemical nutrient cycling in aquatic ecosystems, such as coral reefs (Battin et al. 2003), and influence the settlement and metamorphosis of important reef building organisms, such as corals, therefore affecting the establishment, recovery and resilience of coral reefs (Wieczorek & Todd 1998, Webster et al. 2004). The immense surface area available for biofilm colonisation and development highlights the important contribution of biofilms to coral ecosystem functioning.

Coral bleaching is promoted by increased SST (Hoegh-Guldberg 1999), and these effects may be exacerbated by elevated nitrate availability (Zhu et al. 2004, Wooldridge 2009, Wooldridge & Done 2009), leading to phase shifts from coral- to algae-dominated communities with concomitant reef decline (Fabricius 2005, Schaffelke, et al. 2005). Further, chronically disturbed inshore reefs of the GBR exhibit increased disease occurrence in sponges and corals (Webster et al. 2008, Haapkylä et al. 2011). Thus, shifts within biofilm communities may substantially alter bacterial community composition and function, subsequently affecting coral reef productivity (O_2 fluxes) and biomass.

Despite the importance of biofilm communities, the possible impacts of future environmental change (e.g. eutrophication, increased SST and ocean acidification) on these communities have rarely been investigated. Several studies have demonstrated shifts of bacterial community composition in biofilms in response to local anthropogenic nutrient impacts (Meyer-Reil & Koster 2000, Lawrence et al. 2004, Chénier et al. 2006, Nocker et al. 2007, Chiu et al. 2008) and in response to increased SST (Boivin et al. 2005, Lau et al. 2005, Chiu et al. 2006). However, these investigations have either tested individual factors alone or one of these factors in combination with e.g. salinity, pollutants and their consequent effect on invertebrate larval settlement. Testing environmental parameters individually prevents the identification of additive or synergistic effects, making future predictions about the potential effects of climate change difficult. The environmental parameters of temperature, light and nutrients (in particular, nitrate from fertiliser input) are critical parameters that may affect biological entities in coral reef environments. Although the effects of seasonal environmental inshore conditions on benthic organisms have been investigated *in situ* along a water quality gradient in the GBR (Uthicke & Altenrath 2010, Uthicke et al. 2010, Kriwy & Uthicke 2011, Witt et al. 2011b), interactions between these 3 specific parameters on aquatic bacterial biofilms remain poorly understood.

To disentangle interactive effects and determine disturbance tolerance levels, the present study investigates metabolic (O_2 fluxes), quantitative (C, N and chlorophyll *a* [chl *a*] contents) and qualitative (bacterial community structure) changes in coastal marine biofilms in response to controlled combinations of elevated temperature and increased nitrate concentrations, under 2 different light availabilities, in a 28 d aquarium study. We directly measured metabolic activity (O_2 fluxes) and simultaneously investigated changes in community composition using molecular tools on marine biofilms in response to environmental factors (continued from a previous study on the effects of pCO_2) (Witt et al. 2011a). Such investigations of the biofilm communities associated with coral reefs may elucidate the interactive effects of the environmental stressors potentially leading to deleterious environmental change. These results can support future coastal management.

METHODS

Aquarium set-up and sampling

An indoor flow-through experiment for manipulation of nitrate concentrations, temperature and light conditions was conducted over 28 d in October 2009. The experimental design consisted of 3 water temperatures (26, 29 and 31°C) and 3 nitrate concentrations (0.5, 1.0 and 1.4 µM) each replicated in 3 separate tanks, resulting in a total of 27 aquaria (volume: 16 l).

The aquaria for each temperature were heated by 2 and 3 kW titanium heating bars and connected to a computer control system (CR1000 Measurement and Control Datalogger, Campbell Scientific). Automatic temperature sensors (Campbell Scientific) were placed randomly into each temperature treatment to log the temperature over the course of the experiment, and additional manual measurements revealed constant temperatures of 26.4 ± 0.3, 29.2 ± 0.3 and 31.0 ± 0.3°C (means ± standard deviation [SD]) in the different treatments.

Prior to entering the aquarium system, inshore seawater directly pumped from the sea was stored in a settlement tank and then filtered through a series of filters (pore width: 25 µm, 10 µm and 5 µm). Seawater flow rates of 500 ml min^{-1} ensured a steady supply of fresh seawater to each aquarium. Peristaltic pumps (Masterflex L/X multichannel Extech Equipment) delivered nitrate into each aquarium at near constant flow rates of 0.8 to 1.0 ml min^{-1} to enhance the baseline nitrate concentration (average: 0.5 ± 0.3 µM NO_3^-, n = 9), which was very similar to that of typical local inshore seawater (~0.5 µM) (Cooper et al. 2007). Nitrate concentrations were chosen based on naturally occurring nutrient concentrations during flood-plume events in the GBR (Devlin & Schaffelke 2009), and baseline nitrate concentrations were therefore enhanced to 1.0 (average: 1.0 ± 0.4) and 1.4 (average: 1.4 ± 0.8) µM NO_3^- using potassium nitrate stock solutions (1 M KNO_3). Duplicate nutrient samples for dissolved inorganic nitrogen (DIN) were taken from each aquarium twice weekly and were analysed by the Australian Institute Laboratory Services using the methods of Ryle et al. (1981). Salinity, determined daily using a handheld refractometer, remained constant (between 35 and 36 PSU) throughout the experiment.

All of the aquaria were maintained under a diel light:dark cycle of 12:12 h. As an additional factor to the main setup, each aquarium was further divided into 'high' light (200 µmol photons m^{-2} s^{-1}) and 'low' light (40 µmol photons m^{-2} s^{-1}) conditions by covering half of the tank with a 70% shade cloth.

Glass microscope slides (75 × 25 mm) were used as settlement substrata for biofilm development because they allow growth of biofilms with bacterial communities that are not significantly different from those grown on natural substrata, such as coral skeletons or reef sediments (Witt et al. 2011b). Bacteria initially colonising specific artificial and natural substrates may be different depending on the surface properties of the substrate (Sweet et al. 2011); however, biofilms undergo distinct temporal shifts where the effect of substrate type diminishes, and biofilms tend to form more similar community structures over time (Huggett et al. 2009, Chung et al. 2010). The slides were fixed onto PVC holders, and biofilms were allowed to develop for 14 d in outdoor flow-through aquaria from the AIMS facility directly located at Cape Cleveland Bay with untreated water pumped directly from the sea. At the start of the experiment, randomly selected glass slides (n = 6) with attached biofilms were sampled to represent initial communities. Two pre-conditioned biofilm slides were then immersed into each aquarium, one per light condition (total n = 108). At the end of the experiment (after 28 d), a total of 54 biofilm slides (1 per treatment combination) was sampled for subsequent microbial community analyses by carefully scraping off the biofilm material from the glass substrate into cryovials using sterile no. 11 scalpel blades (the yield was usually >2 g). Samples were snap-frozen in liquid nitrogen and stored at −80°C until further processing.

Determination of O_2 production and O_2 consumption rates

The remaining 54 biofilm slides were used for measurements of net O_2 production as described by Witt et al. (2011a). Briefly, individual glass slides from each aquarium per treatment were transferred into airtight 50 ml glass vials filled with seawater from the corresponding aquaria. Dissolved O_2 (DO) concentrations were measured in the individual vials (using a hand-held luminescent HACH optode HQ10-HQ20) prior to carefully enclosing the biofilm slides and after incubation for 30 min at light conditions of 200 µmol photons m^{-2} s^{-1} or 40 µmol photons m^{-2} s^{-1}. Subsequently, the same procedure was repeated for a 60 min dark incubation. The resulting hourly net O_2 production and consumption were calculated as the 24 h net O_2 production and expressed as µmol O_2 cm^{-2} biofilm area d^{-1}. Production/respiration (P/R) ratios were calculated as follows: P/R ratio = (hourly

net production + hourly respiration) × 12/(hourly respiration × 24). Subsequent to the O_2 measurements, half of the biofilm material from each slide (8.1 cm^2) was scraped into vials using scalpel blades, snap-frozen in liquid nitrogen and stored at −80°C for chl *a* analysis, while the other half was scraped onto pre-combusted glass fiber filters (GF/F, 25 mm in diameter, Whatman) for organic C and N analyses.

Spectrometric determination of biofilm chl *a* content

The analysis of the total chl *a* content was adapted for biofilms from protocols for freshwater phytoplankton and marine sediments (Sartory & Grobbelaar 1984, Uthicke 2006). Samples were kept in low light conditions and on ice during analysis. The material collected from each slide (corresponding area: 8.1 cm^2) was extracted in hot (78°C) ethanol (95%) for 5 min and incubated for 24 h at room temperature in the dark while shaken occasionally. After 24 h, the biofilm extractions were centrifuged at 5200 rpm for 5 min. Subsequently, 320 µl of each extract was measured using a Synergy plate reader (Bio-Tek) at wavelengths of 665 nm and 750 nm. To correct for phaeophytin content, 18 µl of 0.1 N HCl was added to each sample after the first reading, and the samples were re-measured at both wavelengths. Chl *a* concentrations were calculated as described by Schmidt et al. (2011).

Determination of carbon and nitrogen contents in biofilms

The GF/F filters with biofilm material were rinsed with freshwater to remove salts, then dried at 60°C and homogenised using a mortar and pestle. The total organic carbon (TOC) and total organic nitrogen (TON) contents were determined on a parallel sample for each slide on a Shimadzu elemental analyser (TOC5000A) using standard reference material (MESS-1 and Round 40).

Determination of biofilm community composition using terminal restriction fragment length polymorphism (T-RFLP) analysis

The total DNA was extracted from 0.5 g of biofilm (wet weight) sample using the MoBio UltraClean Soil kit (MoBio Laboratories) according to the manufacturer's protocol with the following modifications. Bead-beating (Mini-Bead-Beater, Biospec Products) (2 × 30 s) cycles were performed, and the DNA was eluted with 2 × 50 µl of 1× TE buffer. The DNA extracts were examined using standard 1% agarose gel electrophoresis and quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific). Bacterial 16S rRNA genes were amplified by PCR using the 5 -Cy5-labelled 63F (5′-CAG GCC TAA CAC ATG CAA GTC-3′) and 1389R primers (5′-ACG GGC GGT GTG TAC AAG-3′) (Sigma-Proligo) (Marchesi et al. 1998). Each biofilm sample was amplified in triplicate 25 µl reactions containing 2.5 µM non-acetylated bovine serum albumin (New England Biolabs), 2 µM (2 mM each) dNTPs (Astral Scientific), 2.5 µM forward primer 63F, 1.25 µM reverse primer 1389R, 1 µM MgCl (Qiagen), 1.25U HotStar *Taq* (Qiagen), 2.5 µl HotStar Buffer (Qiagen) and ~2 ng of template DNA. Amplification was performed with an initial incubation at 95°C for 15 min, followed by 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1.5 min, and a final extension at 72°C for 10 min. After PCR, triplicate amplicons were pooled to avoid PCR bias and were subsequently purified using the MinElute PCR purification kit (Qiagen). The PCR products were quantified using the NanoDrop spectrophotometer (Thermo Fisher Scientific), and 150 ng of each purified product was digested with the restriction enzyme MspI (New England Biolabs) according to the manufacturer's instructions. The digested fragments were desalted using the DyeEx 2.0 Spin kit (Qiagen) and vacuum dried for 40 min at low temperature in the dark. Terminal restriction fragments (T-RFs) were resolved and visualised using the CEQ 8800 Genetic Analysis System (Beckman-Coulter) with a 600 bp size standard (Beckman-Coulter). Replicate samples were compared using the software T-align (Smith et al. 2005) with a range of 0.5 bp peak area to identify the consensus peaks between duplicates. The relative fluorescence intensities of the peak area of T-RFs were used as a relative abundance measure for T-RFs in further statistical analyses detailed below. To identify T-RFs, the T-RFLP data were examined in relation to an extensive database of 16S rRNA genes identified from previous Great Barrier Reef biofilm studies (Witt et al. 2011a,b).

Statistical analyses

To assist with the interpretation and to evaluate the effects of the 2 key stressors (nitrate and temperature) in this experiment, the data obtained under the

2 different light levels were analysed separately. A 2-way analysis of variance (ANOVA) was applied to the 24 h net O_2 production data (mean ± SD) and the C, N and chl *a* contents at different temperatures and nitrate concentrations for each light condition. The homogeneity of variance was tested using Levene's test, and the Tukey-Kramer test was used as a post-hoc test. Linear regression analyses were used to identify relationships between the 24 h net O_2 production data and the C, N and chl *a* contents. The linear regressions and ANOVA analyses were performed using the Number Cruncher Statistical System (NCSS) 2007 statistical software (Hintze 2007).

Prior to the statistical community analyses, the T-RF peak area values were fourth-root transformed and standardised to 100%. Principle component analysis (PCA) was used to determine whether the microbial assemblages in samples grouped by temperature and/or nitrate level. The significance of the overall assemblage dissimilarities between temperature and nitrate treatments was tested by applying 2-way permutational multivariate analysis of variance (PERMANOVA) based on permutation procedures (9999 permutations) using the Bray-Curtis distance measure and p-values derived from Monte-Carlo simulations. Pairwise *t*-tests were used as post-hoc tests. The contributions of each taxon to the total dissimilarities of treatments were analysed using the similarity percentage (SIMPER) routine and represented by vectors in the PCA. The PCA and PERMANOVA (Clarke 1993) were performed using the Primer 6.0 statistical software (Clarke & Gorley 2006). To further identify significant differences between relative abundances (peak area) for contributing T-RFs, a 2-way ANOVA analysis with the respective post-hoc test was applied as stated above.

RESULTS

Net oxygen production was measured for biofilms grown under both high and low light conditions for each combination of nitrate and temperature treatments. Under high light conditions (200 µmol photons m^{-2} s^{-1}), the biofilms were net autotrophic (Fig. 1A), with P/R ratios ranging from 3.33 ± 0.91 (SD) to 7.19 ± 1.48 (see Table S1 in the supplement at www.int-res.com/articles/suppl/a066p117_supp.pdf). The 24 h net O_2 production in high-light-exposed biofilms after 28 d was significantly affected by both temperature and nitrate levels (2-way ANOVA, t_0 = 8.91 ± 0.72 µmol O_2 cm^{-2} biofilm area d^{-1}) (Table 1, Fig. 1A). The average 24 h net O_2 production of biofilms exposed to 31°C was significantly (Tukey-Kramer post-hoc test, $p < 0.05$) (Table 1) lower (8.6 µmol O_2 cm^{-1} biofilm area d^{-1}) compared to biofilms exposed to 26°C (16.6 µmol O_2 cm^{-1} biofilm area d^{-1}) and 29°C (13.7 µmol O_2 cm^{-1} biofilm area d^{-1}), a decrease by 48% and 17%, respectively. Biofilms at 1.0 µM NO_3^- produced significantly ($p < 0.05$) more O_2 than biofilms in 0.5 µM NO_3^- treatments, with an increase of

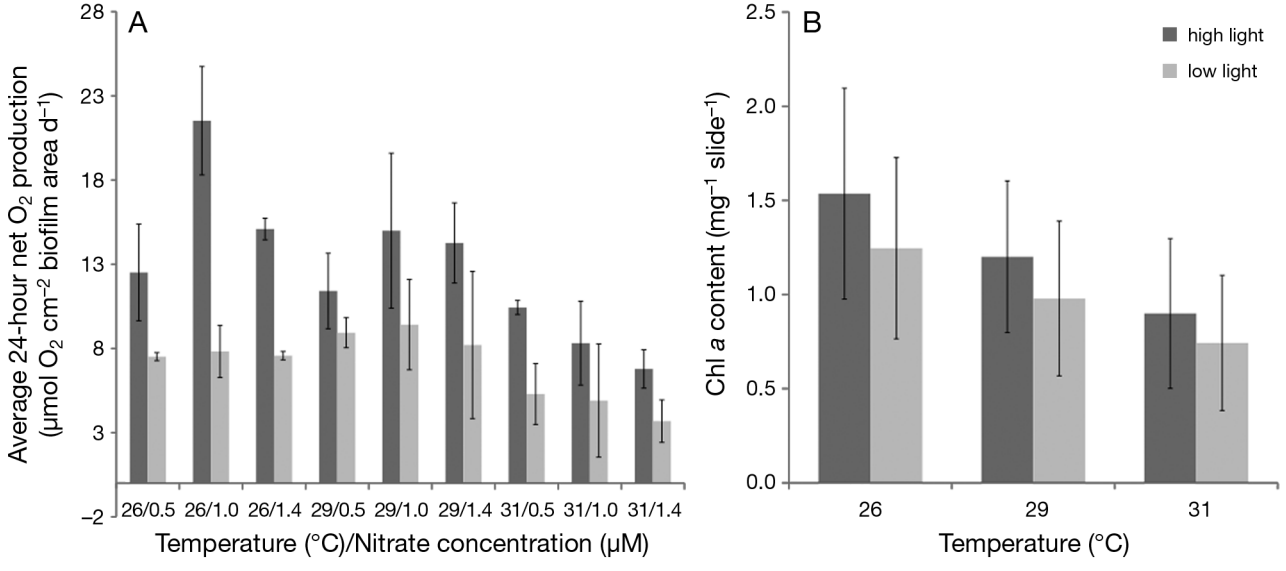

Fig. 1. (A) Average (± SD) 24 h net O_2 production and (B) average chlorophyll *a* content of biofilm communities grown in different (A,B) temperature and (A) nitrate treatments under high light (200 µmol photons m^{-2} s^{-1}) and low light (40 µmol photons m^{-2} s^{-1}) availability

Table 1. Two-way ANOVA of 24 h net O_2 production of biofilms after 28 d in different temperature (26, 29 or 31°C) and nitrate (0.5, 1.0 or 1.4 µM) treatments. High light: 200 µmol photons m^{-2} s^{-1}; low light: 40 µmol photons m^{-2} s^{-1}. Degrees of freedom (df), mean square (MS), Tukey-Kramer (MC) post-hoc test, nd: no data available. *p < 0.05 (bold)

Source of variation	df	MS	*F*	p	Conclusion: MC post-hoc test
High light					
Temperature	2	148.66	16.80	**<0.0001***	26, 29 > 31
Nitrate	2	46.02	5.20	**0.0165***	0.5 < 1.0
Temperature × Nitrate	4	18.04	2.04	0.1318	nd
Residual	18	8.85			
Low light					
Temperature	2	34.12	4.56	**0.0251***	26, 29 > 31
Nitrate	2	4.34	0.58	0.5703	nd
Temperature × Nitrate	4	5.08	0.68	0.6156	nd
Residual	18	7.49			

28 % (Table 1). The 24 h net O_2 production relative to the control treatment (26°C and 0.5 µM NO_3^-) showed a 72 % increase at 26°C and 1.0 µM NO_3^- but only a 20.6 % increase at 26°C and 1.4 µM NO_3^-. The net O_2 production showed an 8.8 to 19 % increase at 29°C + 0.5 µM NO_3^- and 17 to 46 % decrease at 31°C + 0.5 µM NO_3^- compared to the control treatment (26°C and 0.5 µM NO_3^-). Nitrate concentrations averaged by treatment were 0.53 (SD = 0.26) for the baseline, 1.00 (SD = 0.44) for the intermediate and 1.42 (SD = 0.76) µM NO_3^- for the highest nitrate concentration treatment. Although there was some variation, nutrient addition clearly resulted in the targeted nitrate concentrations, and these concentrations differed among treatments.

Similar to high-light-exposed biofilms, low-light-exposed (40 µmol photons m^{-2} s^{-1}) biofilms were net autotrophic (with P/R ratios ranging from 3.31 ± 1.50 to 9.25 ± 1.40) (see Table S1 in the supplement), but overall production was decreased by 56 % compared to high-light-exposed biofilms (Fig. 1A). There was a significant effect of temperature on 24 h net O_2 production rates in low-light-exposed biofilms after 28 d; however, there was no effect of nitrate (2-way ANOVA) (Table 1). The 24 h net O_2 production decreased significantly at 31°C compared to 26°C and 29°C by ~40 % and ~48 %, respectively. Thus, for both light intensities, net O_2 production significantly decreased at 31°C. However, in high-light-exposed biofilms, conditions of 31°C combined with 1.4 µM NO_3^- significantly lowered 24 h net O_2 production with an additive effect.

Chlorophyll *a* contents of biofilm communities

Under both light conditions, water temperature had a significant effect (Table 2) on the chl *a* content of the biofilms, while nitrate concentrations showed no significant effect. Under high light, the average chl *a* concentration at 31°C (0.8 ± 0.4 mg^{-1} cm^{-2}) was significantly lower than at 26°C (1.5 ± 1.0 mg^{-1} cm^{-2}). Similarly, under low light, the average chl *a* concentration at 31°C (0.7 ± 0.4 mg^{-1} cm^{-2}) was significantly lower than at 26°C (1.2 ± 0.8 mg^{-1} cm^{-2}) (Fig. 1B, Table 2). On average, the chl *a* concentration in low light was ~20 % lower than in high-light-exposed biofilms.

Carbon and nitrogen contents of biofilm communities

The total organic carbon (TOC) and total nitrogen (TN) contents of high-light-exposed biofilms were both significantly affected by temperature but not by nitrate (Table 3, Table S2 in the supplement at www.int-res.com/articles/suppl/a066p117_supp.pdf). TOC was significantly different ($p < 0.05$) (Table 3) among all temperatures, with TOC highest at 26°C and lowest at 31°C. TN was significantly higher at 26°C than at 31°C (Table 3).

In low-light-exposed biofilm communities, TOC was affected by temperature and nitrate (Table 3). TOC was significantly lower at 31°C than at 26 and 29°C (by 59 and 68 %, respectively; $p < 0.05$) and significantly lower at 1.4 µM NO_3^- than at 1.0 µM NO_3^-

Table 2. Two-way ANOVA of chlorophyll *a* contents in biofilms after 28 d in different temperature (26, 29 or 31°C) and nitrate (0.5, 1.0 or 1.4 µM) treatments. High light: 200 µmol photons m^{-2} s^{-1}; low light: 40 µmol photons m^{-2} s^{-1}. Degrees of freedom (df), mean square (MS), Tukey-Kramer (MC) post-hoc test, nd: no data available. *p < 0.05 (bold)

Source of variation	df	MS	*F*	p	Conclusion: MC post-hoc test
High light					
Temperature	2	1.060	6.49	**0.0075***	26 > 31
Nitrate	2	0.385	2.36	0.1228	nd
Temperature × Nitrate	4	0.384	2.35	0.0927	nd
Residual	18	0.163			
Low light					
Temperature	2	0.601	5.29	**0.0156***	26 > 31
Nitrate	2	0.126	1.07	0.3620	nd
Temperature × Nitrate	4	0.230	2.02	0.1340	nd
Residual	18	0.114			

Table 3. Two-way ANOVA of carbon/nitrogen contents of biofilms at final measurements in different temperature (26, 29 or 31°C), nitrate (0.5, 1.0 or 1.4 µM) and light treatments. High light: 200 µmol photons m^{-2} s^{-1}; low light: 40 µmol photons m^{-2} s^{-1}. Total organic carbon (TOC), total organic nitrogen (TN), degrees of freedom (df), mean square (MS), Tukey-Kramer (MC) post-hoc test, nd: no data available. *p < 0.05 (bold)

Source of variation	df	MS	*F*	p	Conclusion: MC post-hoc test
High light					
TOC					
Temperature	2	0.907	24.24	**<0.0001***	26 > 29 >31
Nitrate	2	8.095×10^{-2}	0.81	0.4617	nd
Temperature × Nitrate	4	7.416×10^{-2}	0.74	0.5773	nd
Residual	18	0.1			
TN					
Temperature	2	8.849×10^{-3}	3.87	**0.0399***	26 > 31
Nitrate	2	4.017×10^{-3}	1.43	0.2642	nd
Temperature × Nitrate	4	3.124×10^{-3}	1.11	0.3802	nd
Residual	18	2.802×10^{-3}			
Low light					
TOC					
Temperature	2	6.726×10^{-2}	18.15	**<0.0001***	26, 29 > 31
Nitrate	2	1.723×10^{-2}	4.66	**0.0233***	1.0 > 1.4
Temperature × Nitrate	4	8.182×10^{-3}	2.21	0.109	nd
Residual	18	3.706×10^{-3}			
TN					
Temperature	2	5.810×10^{-3}	11.28	**0.0007***	26, 29 > 31
Nitrate	2	8.155×10^{-4}	1.58	0.2325	nd
Temperature × Nitrate	4	7.149×10^{-4}	1.38	0.2816	nd
Residual	18	5.149×10^{-4}			

(by 71%; p < 0.05) (see Table S2 in the supplement). TN was solely affected by temperature ($F_{2,18}$ = 5.30, p = 0.0016), and TN was lower in biofilms grown at 31°C than at 26 and 29°C (by 70%; p < 0.05), while nitrate addition had no effect (p > 0.05) (Table 3). TOC was on average 66% lower and TN up to 80% lower in low light than in high light. A regression analysis showed significant relationships between 24 h net O_2 production, chl *a*, TOC and TN (Table S4 in the supplement).

T-RFLP of bacterial communities in response to temperature, nitrate and light

Across all treatments, molecular fingerprinting of bacterial communities with T-RFLP analysis revealed 37 terminal restriction fragments (T-RFs). Most of these fragments (36) were found in the low-light communities, whereas only 65% (24 T-RFs) were found in the high-light communities (GenBank accession no. JF261709, JF261753, JF261762, JF261782, JF261784, JF261789, JF261796, JF261798, JF261814, JF261820, JF261830, JF261837, JF261845, JF261854, JF261881, JF261891, JF261915, JF261929, JF261950, HQ601725, HQ601742, HQ601790, HQ601837 and unidentified) (see Table S3 in the supplement at www.int-res.com/articles/suppl/a066p117_supp.pdf). Therefore, 12 T-RFs (mainly *Gammaproteobacteria*; accession no. HQ601620, HQ601722, HQ601725, HQ601730, HQ601823, JF261867, JF261871, JF261872, JF261919, JF261935, JF261950 and unidentified) were specific to the low-light communities, but only one (accession no. JF261929 *Flavobacteriaceae*, *Bacteroidetes*) was specific for the high-light-exposed communities.

For the high-light data set, significant differences in community structures were detected between temperature and nitrate treatments, with a significant interaction term (2-way PERMANOVA) (Table 4A). Post-hoc tests of that interaction (presented as nitrate within temperature) (Table 4B) revealed that communities with no nitrate addition were not different among temperature treatments (p [MC] < 0.05). In contrast, under enhanced nitrate levels (both 1.0 and 1.4 µM NO_3^- addition), the incubated biofilm communities were significantly different at 26 and 29°C from those at 31°C.

Bacterial communities in high-light-exposed treatments formed distinct community assemblages in response to different temperatures, as demonstrated in a PCA analysis (Fig. 2). Within the temperature treatments, each nitrate concentration resulted in different community structures, as illustrated by each nitrate concentration forming distinct assemblages at 29°C and 31°C compared to assemblages at 26°C (Fig. 2). Vectors in the PCA and SIMPER analyses illustrated that community dissimilarities for the high-light treatment at different temperatures were mainly driven by the increasing relative abundance of *Oceanospirillum* (*Gammaproteobacteria*) and diatom plastids and decreasing relative abundance of *Cyanobacteria* (Table 5). Further contributing groups were the *Roseobacter* clade and *Flavobacteriaceae* (Table 5).

Under high light, differences in the relative abundance of the species identified as important contributors to group differences based on SIMPER were also investigated using separate 2-way ANOVA. *Oceanospirillum* was influenced only by temperature (2-way ANOVA Temperature: $F_{2,27}$ = 19.37, p < 0.0001; Nitrate: $F_{2,27}$ = 1.97, p = 0.1687), with its

Table 4. (A,C) Results of PERMANOVA analysis of temperature (26, 29 and 31°C) and nitrate (0.5, 1.0 and 1.4 µM) groups in (A) high light (200 µmol photons m^{-2} s^{-1}) and (C) low light (40 µmol photons m^{-2} s^{-1}) biofilms. (B) Post-hoc tests in the significant interaction term (in A) presented as nitrate within temperature. Tests are based on 9999 permutations. p (MC): p-value based on Monte Carlo random draws. *p < 0.05 (bold)

(A) Source	df	SS	MS	Pseudo-*F*	p (MC)
Temperature	2	8101.6	4050.8	5.4017	**0.0001***
Nitrate	2	6456.8	3228.4	4.3051	**0.0008***
Temperature × Nitrate	4	5697.9	1424.5	1.8995	**0.0357***
Residual	19	13 498	749.91		
Total	27	33 755			

(B)	0.5		1.0		1.4	
Groups	T	p (MC)	T	p (MC)	T	p (MC)
26, 29	1.1688	0.2947	1.0688	0.3626	1.6667	0.0841
26, 31	1.4175	0.1611	3.2553	**0.0086***	2.5664	**0.0232***
29, 31	1.1407	0.309	2.0202	**0.0382***	2.2712	**0.0255***

(C) Source	df	SS	MS	Pseudo-*F*	p (MC)
Temperature	2	9066.7	4533.3	3.3335	**0.0125***
Nitrate	2	5418.3	2709.2	1.9921	0.084
Temperature × Nitrate	4	6963.3	1740.8	1.2801	0.2516
Residual	19	23 119	1359.9		
Total	27	44 600			

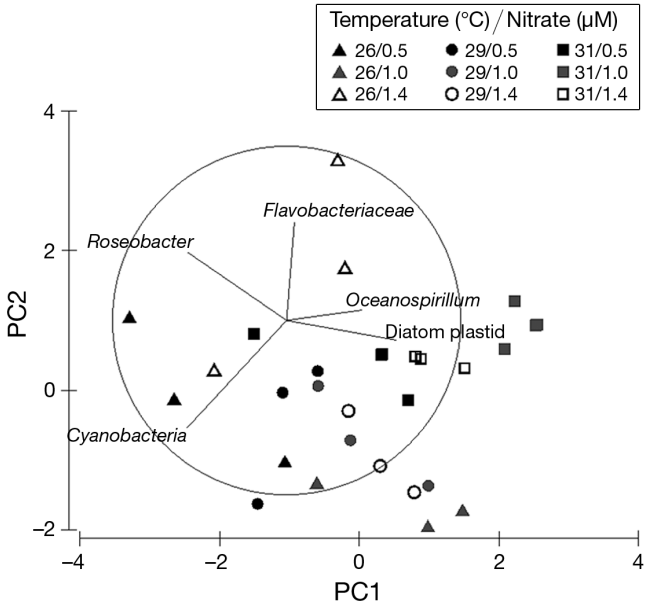

Fig. 2. Principal component analysis (PCA) incorporating relative abundances of terminal restriction fragments (T-RFs; based on the relative fluorescence peak intensity matrix) showing bacterial assemblages at different temperatures in high light (200 µmol photons m^{-2} s^{-1}) availability (PC1: 57.2%, PC2: 16.5%). Vectors of the most important T-RFs based on contribution percentages are shown in the biplot and labelled with the corresponding taxon

abundance significantly lower at 26°C than at 29 and 31°C (Fig. 3). Similarly, T-RFs representing *Cyanobacteria* and *Synechococcus* were affected by temperature and were significantly less abundant at 31°C than at 26 and 29°C (ANOVA $F_{2,27}$ = 6.20, p = 0.0049; $F_{2,27}$ = 0.05, p = 0.9468; and $F_{2,27}$ = 8.08, p = 0.0021, respectively) (Fig. 3). High temperature also affected *Flavobacteria*, which increased significantly in abundance at 29°C compared to 31°C (ANOVA $F_{2,27}$ = 4.46, p = 0.0226). The relative abundance of *Roseobacter* was affected by temperature and nitrate (2-way ANOVA $F_{2,27}$ = 5.51, p = 0.0036; $F_{2,27}$ = 6.22, p = 0.0210): this clade decreased significantly (p < 0.05) in the high-temperature treatments (29 and 31°C) in comparison to the 26°C treatments and were more abundant at 1.4 µM NO_3^- than at 1.0 µM NO_3^- (Fig. 3).

Diatom plastids were affected by nitrate only and showed a significant interaction among temperature and nitrate (2-way ANOVA $F_{2,27}$ = 2.14, p = 0.1468; $F_{2,27}$ = 3.90, p = 0.0391; $F_{4,27}$ = 5.25, p = 0.0056 in the three nitrate treatments). Although post-hoc tests were negative, the relative abundance of diatom plastids increased with increasing nitrate addition, and in interaction with rising temperature, the relative abundance of diatom plastids decreased at 1.0 µM NO_3^-. *Synechococcus* was only present at 0.5 µM NO_3^- (average relative abundance 5.28%) and was not detected at either of the enhanced nitrate concentrations (1.0 and 1.4 µM NO_3^-).

For the low-light data set, significant differences in community structures were detected among temperatures (2-way PERMANOVA Pseudo-$F_{2,27}$ = 3.33, p [MC] = 0.0125) (Table 4C), while nitrate availability and the temperature-nitrate interaction showed no significant effect (p > 0.05) (Table 4C). Post-hoc tests showed that low-light biofilm communities were significantly different at 29°C than at 26 and 31°C.

A PCA analysis of low-light-exposed bacterial biofilm assemblages revealed pronounced overlap between 26 and 31°C, while the 29°C treatment separated from these 2 groups (Fig. 4), confirming patterns detected by PERMANOVA. SIMPER analysis revealed that community dissimilarities at different temperatures were mainly driven by the increas-

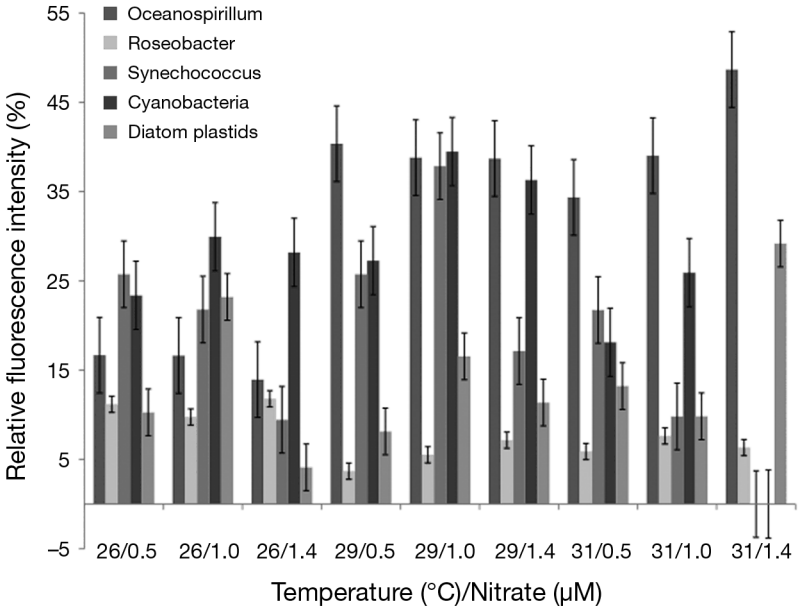

Fig. 3. Relative abundances based on fluorescence intensity of the dominant terminal restriction fragments in the different combinations of temperature and nitrate treatments under high light (200 µmol photons m^{-2} s^{-1}). Error bars are SE

ing relative abundances of *Gammaproteobacteria*, *Oceanospirillum* and *Cyanobacteria*, which were highest at 29°C (Fig. 3).

Because the PERMANOVA showed no nutrient effects, the abundances of individual taxa were only tested using 1-way ANOVA with temperature as a factor. The relative abundance of *Cyanobacteria* was significantly higher at 29°C than at 31°C and 26°C (ANOVA $F_{2,26}$ = 6.66, p = 0.0052, Tukey's Honestly Significant Difference post-hoc 26, 31°C < 29°C). The relative abundance of both *Oceanospirillum* and an unclassified gammaproteobacterium increased with rising temperature (ANOVA $F_{2,26}$ = 4.15, p = 0.0289; $F_{2,26}$ = 6.73, p = 0.0050) (Fig. 5). Finally, when comparing high contributing T-RFs occurring in both high- and low-light treatments, *Oceanospirillum* significantly decreased in low light conditions ($F_{1,52}$ = 29.2, p < 0.0001), while *Synechococcus* remained unaffected by light ($F_{1,52}$ = 3.9, p = 0.0601).

In summary, changes in the relative abundance of *Oceanospirillum*, *Cyanobacteria* and *Flavobacteriaceae* illustrated temperature-induced community shifts in high-light-exposed biofilms, while microbial communities were affected by a temperature-nitrate interaction driven by diatom plastids and *Roseobacter*. In low-light biofilms, temperature-driven microbial community shifts mainly resulted from changes in the relative abundance of *Gammaproteobacteria* and *Cyanobacteria*.

Table 5. Similarity percentage (SIMPER) analysis with overall average dissimilarity values of temperature treatment data in different light availability (high light: 200 µmol photons m^{-2} s^{-1}, low light: 40 µmol photons m^{-2} s^{-1}) and contribution (%) of bacterial taxa (with T-RF length in basepairs [bp]) contributing most to the community dissimilarities. Zero values may indicate either absence from the T-RF or that the contribution is 1% or lower

Temperature treatment	High light	Low light
Overall average dissimilarity (%)	56.6	64.4
Taxon (T-RF, bp)	Taxon contribution (%)	
Oceanospirillum (95)	13.3	6
Cyanobacteria (457)	10.7	10.7
Diatom plastid (461)	6.1	0
Synechococcus (456)	4.1	0
Roseobacter (401)	2.8	0
Vibrionaceae (469)	0	5.4
Flavobacteriaceae (55)	2.5	3.2
Gammaproteobacteria (451)	0	4.2

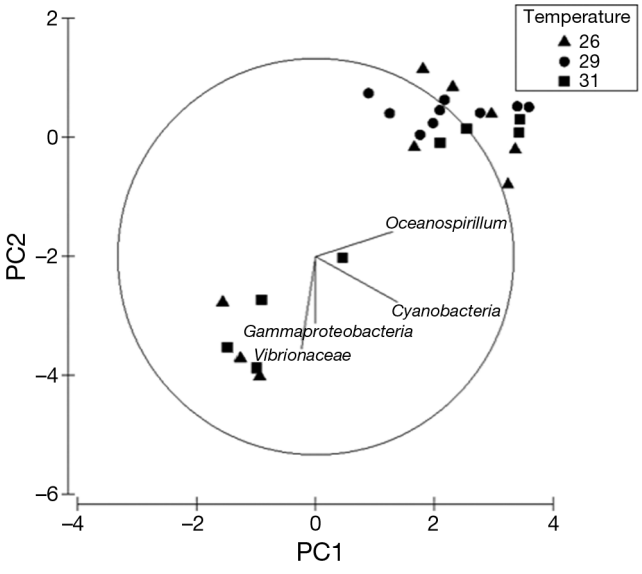

Fig. 4. Principal component analysis (PCA) incorporating relative abundances of terminal restriction fragments (T-RFs; based on the relative fluorescence peak intensity matrix) showing bacterial assemblages at different temperatures (°C) in low light (40 µmol photons m^{-2} s^{-1}) availability (PC1: 57.2%, PC2: 16.5%) with 40% similarity level. Vectors of the most importantly contributing T-RFs are shown in the biplot and labelled with the corresponding taxon

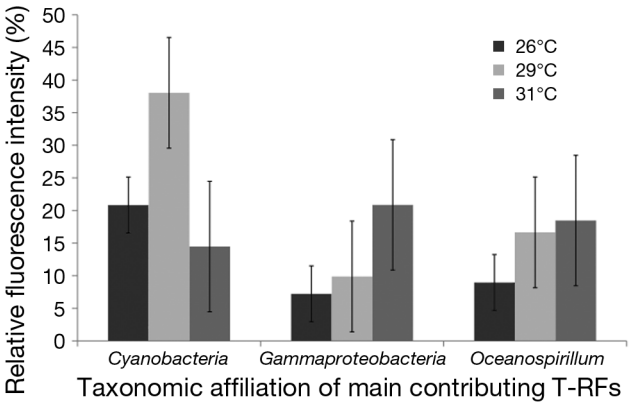

Fig. 5. Relative abundances based on fluorescence intensity of the dominant terminal restriction fragments, i.e. bacterial assemblages, at different temperatures under low light (40 µmol photons m^{-2} s^{-1}). Error bars are SE

DISCUSSION

We tested for interactive temperature and nitrate effects in a 28 d flow-through aquaria experiment mimicking inner (low light and high nutrient availability) and outer (high light and low nutrient availability) GBR inshore reef conditions (Uthicke 2006, Cooper et al. 2007). Irrespective of nitrate and light availability, high (elevated by 2 to 3°C over current summer maxima) water temperatures of 31°C significantly reduced biofilm community 24 h net O_2 production and biomass (organic C, N and chl *a* contents). Experimental results suggested that under high light availability, the effects of high temperature and nitrate were additive. In addition, high-light-exposed biofilms were nitrate-limited, illustrated by the 24 h net O_2 production increase at 1.0 µM NO_3^-. However, high temperatures had the most distinct effect on bacterial community composition, as elevated nitrate concentrations were only important in conjunction with high temperature (31°C). In contrast, biofilms under low light availability were presumably light-limited; neither community composition nor productivity changed under increased nitrate. Our findings suggest that an increase in water temperatures of ~1 to 2°C above the current average summer temperatures within the study area (28 to 29°C), which is predicted to occur by 2100 (Lough 2001, Lough et al. 2006), would have a detrimental effect on photosynthesis by local biofilms.

Balance between autotrophy and heterotrophy

In addition to global warming, coastal water quality is influenced by periodical terrestrial runoff that causes increased nitrate and reduced light availability, which have become chronic disturbances to inshore coral reefs (Devlin & Schaffelke 2009). In the present study, both high- and low-light-exposed biofilms were net autotrophic, as illustrated by 24 h net O_2 productions and high P/R ratios (>1). Over the 28 d period, communities did not shift from autotrophy towards predominant heterotrophy under the experimental conditions; however, under longer exposure to low light, biofilms may turn heterotrophic. Biofilms exhibited significantly lower 24 h net O_2 production and chl *a* contents at 31°C, but the effects of temperature were more pronounced under low than high light (i.e. 31°C reduced 24 h net O_2 production by ~40% from 26°C). Nevertheless, low-light-exposed biofilms were very productive, with the 24 h net O_2 production being half of that of high-light-exposed biofilms even with only 20% of the light availability. A regression analysis showed a significant relationship between 24 h net O_2 production, chl a, TOC and TN, suggesting that at least part of the production decrease was coupled to a reduction in biomass (50% reduction of TOC, TN and chl *a*).

Light and temperature alter productivity and nutrient uptake

In agreement with other studies, lowered productivity and chl *a* contents at 30°C were also detected in marine coastal (Chiu et al. 2006) and estuarine biofilms (Nayar et al. 2005). Similarly, endosymbiotic diatoms in benthic low-light-adapted foraminifera showed reduced photosynthetic rates and chl *a* at 31°C (Schmidt et al. 2011, Uthicke et al. 2012b), resulting from photo-inactivation caused by thermal and oxidative damage of the photosynthetic apparatus (Warner et al. 1996). In the present study, at higher temperatures, 24 h net O_2 production decreased proportionally with chl *a*. Additionally, the 50% reduced TOC content, indicating less photosynthetically fixed C, indicated lower photosynthetic activity. Therefore, temperature-induced changes detected in biofilms may be a consequence of a malfunctioning photosynthetic apparatus, potentially caused by thermal damage distorting enzymes in the dark reaction (e.g. ribulose-1,5-biphosphate-carboxylase/-oxygenase [RuBisCO]) responsible for photosynthetic carbon fixation. As demonstrated in higher plants, RuBisCO can become inhibited by temperatures >30°C (Feller et al. 1998), which likely may be the case in our study in the highly productive diatoms and *Cyanobacteria* (Underwood & Kromkamp 1999).

Lower TN contents at high temperature may be explained by increased membrane permeability in microalgae; hence, less fixed N is incorporated into cells. Additionally, N-uptake mechanisms are largely light activated (Pennock 1987, Boyer et al. 1994, Tuchman et al. 2006). Therefore, we propose that fast-growing microalgae in biofilms may suffer N-limitation and outcompete slow-growing microalgae at inner nearshore locations that are chronically affected by flood plumes. In contrast, slow-growing microalgae usually remain unaffected by increased N availability; however, their growth is restricted by shading instead (Underwood & Kromkamp 1999).

Enhanced nitrate influences biofilm productivity

In addition to high temperature, high-light-exposed biofilms were influenced by enhanced nitrate concentrations. Increased 24 h net O_2 production (photosynthetic production) in response to intermediate nitrate concentrations (1.0 µM NO_3^-), as used in the present study, has previously been observed for corals (Marubini & Thake 1999, Zhu et al. 2004). Compared to the increased 24 h net O_2 production at 26 and 29°C and 1.4 µM NO_3^- and decreased 24 h net O_2 production at 31°C and 1.4 µM NO_3^- in high-light biofilms observed in the present study, in coral symbionts photosynthesis at 2 µM NO_3^- remained unaltered (Ferrier-Pagès et al. 2001). Our findings indicate that the temperature and nitrate tolerance limits of the investigated microbial biofilm communities range between 29 and 30°C and ~1 µM NO_3^-. Beyond these levels, their photosynthetic functioning (O_2 fluxes) is impacted or inhibited. Given the additive effect under high light, elevated temperatures become more relevant under high nitrate regimes. When calculating percentage changes of each factor from the control treatment (26°C and 0.5 µM NO_3^-), a high temperature of 31°C reduced 24 h net O_2 production by 17%, and additive effects with 1.4 µM NO_3^- reduced that production by an additional 28% (thus, a total reduction of 45%). Similarly, warming effects became more pronounced under elevated nutrient conditions in river biofilms, suggesting that high temperatures may promote faster biofilm re-colonisation after disturbances (Diaz Villanueva et al. 2011). As also demonstrated in the present study, higher nutrient availability did not result in higher biomass. This was due to increased presence of ciliates at high temperature and hence increased grazing on biofilm bacteria. Therefore, grazing effects should be investigated in future studies.

Biofilm communities at inner nearshore sites

Community shifts induced by increasing temperature and nitrate concentrations in high-light-exposed biofilms were driven by changes in the relative abundance of *Cyanobacteria*, *Gammaproteobacteria* and diatom plastids. *Alphaproteobacteria* and *Cytophaga-Flavobacteria-Bacteroides* were also contributing phylogenetic bacterial groups and have been previously identified as major contributors to community shifts in biofilms in temperate estuaries (Jones et al. 2007), at mid-shelf reefs (Webster et al. 2004) and in response to nutrient and light stress at inshore GBR sites (Kriwy & Uthicke 2011).

Planktonic diatoms frequently dominate nearshore waters (Revelante & Gilmartin 1982) with high nutrient input and low light availability. This has also been demonstrated for benthic diatoms (Gottschalk et al. 2007, Uthicke & McGuire 2007) and biofilms from the GBR (Kriwy & Uthicke 2011). However, diatoms have also previously been identified as a key driver of community composition in high-light-exposed biofilms in response to elevated pCO_2 (Witt et al. 2011a). This finding is possibly due to phototrophic diatoms using light to produce exudates (extracellular polymeric substances), fuelling other microbes (reviewed by Thornton 2002).

The high contribution of *Gammaproteobacteria*, in particular *Oceanospirillum*, in biofilms from simulated inner inshore conditions could be confirmed in field-grown biofilms at inner inshore locations (5 km distance from the coast) of reduced water quality on the GBR (Kriwy & Uthicke 2011, Witt et al. 2011b) and the Atlantic coast (Dang et al. 2008). In estuarine biofilms, increasing relative abundance of *Gammaproteobacteria*, in particular sulphate-reducing species, was correlated with decreasing dissolved O_2 availability in the water column, while enhanced nutrient availability had a secondary effect (Nocker et al. 2007). The increasing relative abundance of *Oceanospirillum* with rising temperature in high- and low-light conditions may also be correlated with high temperatures that result in reduced dissolved O_2 availability and heat-stressed or decaying microalgae cells, providing more detritus to heterotrophic bacteria. The lower relative abundance of *Oceanospirillum* (by 50%) at low compared to high light availability indicated higher competition of bacteria under low light availability. *Gammaproteobacteria* species also contributed the most to microbial community shifts in field-grown biofilms at shallow water sites (high light availability) along a water depth gradient on the GBR (Webster et al. 2004).

Biofilm communities at outer nearshore sites

Cyanobacteria prefer warm water temperatures and high-light environments (Paerl 1985, Robarts & Zohary 1987), and although *Cyanobacteria* are dominant in outer inshore locations, light has little effect. *Cyanobacteria* are able to adapt their growth in altered light quality and quantities through gene regulation of their chromatic phycobilisome structure (Grossman 1990, Grossman et al. 1993) and control of their photosystem (Kulkarni et al. 1992, Kulkarni & Golden 1994, Bhaya et al. 2000). Increasing relative abundance of *Cyanobacteria*, in particular *Synechococcus*, at 29°C and decrease at 31°C with a concomitant decrease in net O_2 production and chl *a* content has also been observed in *Synechococcus*-dominated tropical estuarine biofilm communities grown on glass substrata (Nayar et al. 2005). These findings imply that 30 to 31°C may also exceed the temperature optimum of *Cyanobacteria* species found in our study. Temperature-induced differences in the relative abundances of *Synechococcus* were only found within the 0.5 µM NO_3^- treatments. Further, the absence of *Synechococcus* at higher nutrient concentrations may be explained by their N-fixing ability, which decreases at >30°C (Breitbarth et al. 2006). Therefore, a consequent selection towards N-fixers at low N regimes leads to the predominant occurrence of N-fixers in tropical oligotrophic offshore waters (Crosbie & Furnas 2001a,b, Moisan et al. 2010, Nelson et al. 2011), such as the outer inshore GBR (>30 km from the coast) (Kriwy & Uthicke 2011).

Several bioindicator studies have confirmed *Roseobacter* as a dominant group in microbial biofilms in estuarine (Jones et al. 2007), polar (Webster & Negri 2006), temperate (Dang et al. 2008) and coral reef environments (Kriwy & Uthicke 2011). *Roseobacter* clade members were more abundant at outer than inner nearshore sites in the GBR (Kriwy & Uthicke 2011, Witt et al. 2011b), possibly because of a competitive advantage due to rapid absorbance of nutrients dissolved in coastal waters (Alonso-Sáez & Gasol 2007) and anoxygenic phototrophy (Allgaier et al. 2003). A decrease in *Alphaproteobacteria* was also detected in biofilms in response to elevated temperature (Webster et al. 2010) and elevated pCO_2 (Witt et al. 2011a) with a concomitant increase in *Bacteroidetes*, in particular *Flavobacteria*. These findings were reconfirmed in this study, indicating that *Alphaproteobacteria*, in particular *Roseobacter*, and *Bacteroidetes* respond to environmental stress and may serve as indicator species for future biofilm bioindicator applications.

Finally, unconsidered factors that have previously been demonstrated to influence bacterial community composition, such as the impact of grazers on bacteria (Pernthaler 2005, Pernthaler & Amann 2005, Weinbauer et al. 2010) and viral infection (Hewson et al. 2003, Weinbauer et al. 2010), are worth exploring in further studies.

Overall, biofilms at both inner (5 km from coast) and outer nearshore (>30 km from the coast) locations in the study area are primarily affected by warming SSTs, as illustrated by the changes in 24 h net O_2 production and bacterial community compositions. Inner reefs, adapted to high-nitrate regimes, are additionally affected by light reduction resulting from eutrophication and sedimentation. Hence, inner reef biofilms harbour more heterotrophic bacterial species (e.g. *Gammaproteobacteria*) limited by light, while outer reef biofilms are mainly composed of nitrate-limited autotrophic species (e.g. diatoms and *Cyanobacteria*).

Inner nearshore reefs are suffering from chronic low light availability. From a historical point of view, these reefs may either have always experienced higher sediment loads or have been altered through increased sediment loading since European settlement (mid-18th century) and intense agricultural development of the Queensland coastline. Historical data on coral communities or biofilms is difficult to obtain. However, sediment cores have demonstrated that benthic foraminifera communities on some outer nearshore reefs have remained the same over nearly 2 millennia, while inner reef communities remained stable until recently, having changed since European settlement (Uthicke et al. 2012a).

CONCLUSIONS

Global warming and eutrophication simultaneously affect microbial biofilms established in coral reef habitats. Our findings add to the growing body of evidence that runoff alters community composition of coral reefs. Because inner reefs are already experiencing light reduction due to increased sediment input, it appears that the associated biofilms are less vulnerable to nitrate enhancement but clearly vulnerable to temperature increases. Hence, our findings are consistent with a runoff and temperature interaction for inshore reefs. Unexpectedly, the runoff interaction was not an interaction with nitrate but with sedimentation (leading to light reduction). Rising SSTs are difficult to influence because long-lasting reductions in greenhouse gas emissions can

only be reached by a concerted global management effort. In contrast, local nitrate and sediment input through farming can be controlled by sustainable regulations and coastal management. Therefore, a reduction of sediment input (increasing light availability) requires a simultaneous reduction of nitrate load to prevent temperature-vulnerable outer reef degradation scenarios. Although biofilms mimicking outer (presumed high light) reef conditions were affected by additive effects of temperature and nitrate, current flood plumes are unlikely to reach these reefs, except under cyclonic conditions (Cooper et al. 2007). This finding suggests higher resilience to temperature at outer than inner reefs, assuming that runoff does not worsen in the near future.

Therefore, the effect suggests that stringent control over land-based pollution to ameliorate water quality may be equivalent to reducing SSTs by 1 to 2°C. Extending the knowledge of qualitative and metabolic responses (i.e. O_2 fluxes) of biofilms is important for the future development of coastal management and bioindicator systems for coral reef health.

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