The effects of warming and ocean acidification on growth, photosynthesis and bacterial communities for the marine invasive macroalga *Caulerpa taxifolia*

Alexandra J. Roth-Schulze\(^1\), Torsten Thomas\(^1*\), Peter Steinberg\(^1,2\), Marty R. Deveney\(^3,5\), Jason E. Tanner\(^3,5\), Kathryn H. Wiltshire\(^3,5\), Stephanie Papantoniou\(^3,6\), John W. Runcie\(^7\), C. Frederico D. Gurgel\(^3,4,5\)

1. Centre for Marine Bio-Innovation, Sydney, NSW 2052, Australia
2. Sydney Institute of Marine Science, 2 Chowder Bay Rd., Mosman, NSW, Australia, 2088
3. The University of Adelaide, School of Earth and Environmental Sciences, DX 650-418, Adelaide, SA 5005 Australia.
4. South Australia State Herbarium, Department for Water, Environment and Natural Resources, PO Box 2732, Kent Town, SA 5071 Australia.
5. South Australian Research and Development Institute and Marine Innovation SA, West Beach, South Australia 5024 Australia.
6. Aquation Pty Ltd., PO Box 3146, Umina Beach, NSW 2257 Australia.
7. School of Biological Sciences, The University of Sydney, NSW 2006 Australia

*Corresponding author*: Torsten Thomas, Centre for Marine Bio-Innovation, The University of New South Wales, Sydney, NSW 2052, Australia
Telephone: (+612) 9385 3467, Fax: (+612) 9385 1483, E-mail: t.thomas@unsw.edu.au

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Abstract

*Caulerpa taxifolia* is a pantropical green benthic marine macroalga, and one of the best known marine invasive species in temperate coastal habitats. In Australia, this species has been introduced to seven estuaries along New South Wales and one in South Australia. How this alga will perform under future climate change scenarios is however not well defined. This study experimentally assessed the effects of ocean acidification and global warming on the growth, photosynthetic performance and the bacterial community on two populations of *C. taxifolia*, one native and one invasive. A range of complex significant interactive effects between pH, temperature, and initial plant size on the growth of *C. taxifolia* were observed, but no effect of population origin and PSII fluorescence quantum yield parameters were detected. No significant effects of the treatment combinations were observed on bacterial community richness or diversity. Only one bacterial species out of 1087 present on the algae showed significant changes between pH treatments at high temperature (24°C). This bacterium belonged to the genus *Planctomyces* and its relative abundance was more than 10 times higher in samples with low pH compared to the control. Higher plant growth was observed under all higher pCO$_2$ and lower pH conditions suggesting that *C. taxifolia* will benefit from climate change, posing a potential higher risk in invaded locations.
Introduction

Two of the main processes associated with anthropogenic climate change in the marine environment are ocean warming and ocean acidification (OA). A 2-4°C increase in average temperature (including sea surface temperatures), and an approximate 0.5 decrease in the ocean’s average pH, is expected by 2100, if greenhouse gas emissions, particularly CO₂, continue to grow at current rates (Royal Society 2005, IPCC 2014). The dimension and speed of these changes are unprecedented in the recent history of the planet. If unabated, continued increase in atmospheric CO₂ is expected to cause major changes in ocean chemistry, marine community structure and ecosystem services worldwide, many of which may be economically and environmentally negative for humans. Consequently, understanding how climate change will affect marine ecosystems is important.

The effects of ocean warming and acidification on a range of marine biota including bacteria, corals and other invertebrates, fish, macroalgae and seagrass have been assessed. An important conclusion from these studies is that marine organisms show different responses to the predicted changes in temperature, pH and CO₂ water chemistry, and these can be negative, neutral or positive (Hofmann et al. 2010; Kroeker et al. 2010; Munday et al. 2013). A species can, furthermore, have different ecophysiological behaviour under distinct combinations of global climate-related factors (e.g. temperature, pH, pCO₂), and regional or local factors, such as pollution (e.g. heavy metals), nutrients (N, P), light (photosynthetically active radiation, PAR, and UV) and biotic interactions (competition, herbivory, predation). These factors and responses make it hard to generalise how a species will behave under end-of-century climate scenarios. In the marine environment, meta-analyses of tank and whole community experiments from CO₂ volcano seeps, however, suggest that OA is highly detrimental to most tropical and temperate crustose coralline red macroalgae, and usually beneficial to temperate seagrasses (Harley et al. 2006; Porzio et al. 2011; Harley et al. 2012).
While most studies on the ecological consequences of climate change have focussed on responses of native fauna and flora, the impact on invasive species is less well understood (Stachowicz et al. 2002). Invasive marine species are important and often have a disproportionate influence on ecological processes (Molnar et al. 2008; Occhipinti-Ambrogi and Galil 2010). Invasive species often produce major shifts in species composition, diversity and abundance, leading to local extinction of native biota, and compromising regional industries (Mooney and Hobbs 2000; Clavero and García-Berthou 2005; Hejda et al. 2009). Understanding how climate change will affect invasive species is therefore important for understanding the overall response of marine ecosystems to climate change.

Marine macroalgae are major primary producers in coastal benthic ecosystems, contributing substantially to global primary productivity (Schiel and Foster 2006; Egan et al. 2013). Macroalgae also form biogenic habitats, for a range of other plants and animals. Moreover, macroalgae host a range of microbial symbionts (Friedrich 2012; Goecke et al. 2013). Several obligatory and facultative interactions have evolved between macroalgae and bacteria that include, for example, the provision of required vitamins (Croft et al. 2005; 2006), the production of secondary metabolites for the control of biofouling and pathogens (Wiese et al. 2009; Nasrolahi et al. 2012; Dobretsov et al. 2013), and cues of macroalgal morphogenetic developments (Provasoli and Pintner 1980; Matsuo 2005; Wichard 2015). Epiphytic microbial communities are also often assembled from bacteria in the surrounding seawater, which provides an opportunity to rapidly change community composition and function (Egan et al. 2013). Macrolalgae and their bacterial symbionts are increasingly seen as an interdependent, associational unit, called a holobiont (Rosenberg et al. 2007; Egan et al. 2013; Bordenstein and Theis 2015).

*Caulerpa* is one of the most common green macroalgal genera in tropical and temperate waters, with approximately 86 accepted species worldwide (Belton et al. 2014).
Caulerpa and other Bryopsidales have coenocytic thalli (Draisina et al. 2014) that host a wide range of bacteria (Dawes and Lohr 1978; Hollants et al. 2011; 2013a). The close association between Caulerpa and its bacterial communities has been described, including the characterization of bacterial diversity (Delbridge et al. 2004) and the stimulation of bacterial N₂ fixation in organic sediments (Chisholm and Moulin 2003). Successful attempts have also been made to determine the source of invasive Caulerpa populations using bacterial community profiling (Meusnier et al. 2001; Aires et al. 2013, Arnaud-Haond et al. 2017).

Caulerpa species are prone to produce invasive species such as Caulerpa brachypus in Floripa (Lapointe et al. 2010), Caulerpa cylindraceae in the Mediterranean (Verlaque et al. 2003). However, Caulerpa taxifolia is by far the most famous invasive species in the genus. Caulerpa taxifolia is a pantropical species considered one of the world’s worst invasive species in temperate habitats (Lowe et al. 2000; Deudero et al. 2014). Since its discovery in Monaco in 1984, it quickly spread and by 1999 it occupied 97% of suitable benthic substrate between Toulon and Genes (i.e., France, Monaco and Italy; Bax et al. 2003). C. taxifolia is currently found in over seven Mediterranean countries (Stam et al. 2006) although recently local population retractions have been reported (Arnaud-Haond et al. 2017). Invasive populations of C. taxifolia in Australia were first reported in Port Hacking, Sydney, New South Wales in 2000 (Phillips and Price 2002). Between 2000 and 2007 invasive C. taxifolia populations were subsequently reported in five other water bodies in the greater Sydney area, eight other estuaries south of Sydney, and in one estuary north of Sydney (Glasby and Gibson 2007). In 2002, C. taxifolia was identified in West Lakes, Adelaide, South Australia (a manmade estuary), and subsequently in the Port River estuary (Cheshire et al. 2002). It was eradicated from West Lakes, but remains ineradicable in the Port River (Wiltshire 2010).

Caulerpa species in general are expected not to be negatively affected by OA (Hall-Spencer et al. 2008; Porzio et al. 2011), further increasing their competitive and potentially invasive
ability under future climate conditions. How global warming, or a combination of global warming and OA, specifically affect the ecology of invasive populations of *C. taxifolia* remains unknown.

We therefore assessed the combined effects of OA and global warming on the growth, photosynthetic performance and epiphytic bacterial communities of invasive and native Australian populations of *C. taxifolia*. We manipulated seawater temperature to mimic current and future summer averages for southern Australia (specifically Adelaide), and applied four pH treatments in an orthogonal, multi-factorial design. We also manipulated seawater pH with gaseous CO$_2$ and acid dosing to decouple the effects of lower pH from dissolved inorganic carbon enrichment to provide additional information about the mechanisms of carbon physiology on both algal host and its associate bacterial communities. Our null hypothesis states that under warmer and lower pH conditions (i.e. future climate scenarios) *C. taxifolia* populations will present no significant changes in mortality, growth, PAM fluorescence parameters, and bacterial community structure compared to control (i.e., present day) conditions.

**Material and methods**

**Experimental design**

We crossed four pH treatments with two temperature treatments. Each of the eight treatment combinations was replicated five times using a spatially randomized design, totalling 40 open-system glass tanks of 19 L each. Tanks were placed inside a controlled environment room at the South Australian Research and Development Institute (SARDI) Aquatic Sciences laboratories (Adelaide, Australia) and supplied with flow-through seawater from the Gulf St Vincent. The duration of the experiment was 21 days. Seawater nominal pH treatments corresponded to 8.05, 7.84, and 7.69. A further pH treatment equivalent to a nominal pH of
7.69 (= similar to the lowest pH or highest pCO₂), but manipulated with HCl dosing (acid solution treatment) was also included to distinguish the effects of low pH and to those of dissolved inorganic carbon enrichment. The range of pH tested are in agreement to an expected drop in ocean pH of 0.3 units by 2100 compared to present day levels (Gitay et al. 2002; Caldeira and Wickett 2003). The experimental set up also allowed for natural fluctuations in pH to occur within all tanks due to daily macroalge metabolic activity (= daily pH variation), at the same time that that average target pH levels were maintained constant (Figure S1). Temperatures corresponded to the average warmest seawater summer temperatures in Gulf St Vincent, South Australia (23°C), and an average one-degree higher future-warming scenario (24°C). A one-degree warming is a scenario that is highly likely to occur even under the most optimistic IPCC emission model (e.g. B1: IPCC 2007, RCP 2.4: IPCC 2014). Details of temperature, pH and CO₂ chemical properties of the seawater per treatment are described in Table 1.

**Experimental Set Up**

Natural seawater from the Gulf St Vincent was filtered (5 µm) and aerated with ambient-CO₂ air in a temperature-controlled sump tank. Water from the sump was supplied to each of the 40 experimental tanks at constant flow-through via drippers at a rate of 8 L.h⁻¹. Average light across all tanks was 109 µmol.m⁻².s⁻¹ (± 27.38 s.d.) supplied in a 14:10 light:dark cycle by an array of 12 x 150 W metal halide lights (Pierlite GMRS 150 MH). Light data collected with a Licor light logger positioned at four different points (front, middle, rear and back areas of each tank) and measured across all tanks, showed no significant differences in photosynthetically active radiation (PAR) among the eight treatment combinations (PERMANOVA, Pseudo-F₃,₁₂=0.4924, p=0.885). Individual submersible heaters (Aqua One 55W), water-circulation pumps (Aqua One, model PH101), temperature sensors (Aquatronica,
model ACQ001S) and pH electrodes (Aquatronica, model ACQ310N-PH) were set up in each tank. pH and temperature values were manipulated continuously and logged every three hours via Aquatronica controllers (model ACQ110) for each individual tank. pH was manipulated by CO$_2$ bubbling with Dupla solenoid control valves and atomizers (Dohse Aquaristik, Grafschaft-Gelsdorf, Germany) and in the case of the treatment, where pH was manipulated with acid addition, a 0.2 M HCl solution was delivered via peristaltic pumps (Aquatronica, model ACQ450). Pilot experiments showed photosynthesis would drive pH to >8.4 during light periods, therefore the low CO$_2$/pH treatments (= present day conditions) also included CO$_2$ addition to compensate for day-time photosynthesis and maintain a constant pH through the day.

**Abiotic data**

Seawater total alkalinity was measured weekly for all tanks for the duration of the experiment. Total alkalinity was analysed following ASTM D3875 using a Metrohm 719 S Titrino (Metrohm AG, Herisau, Switzerland). Electrodes were calibrated twice weekly using pH 7.0 and 10.0 National Institute of Standards and Technology (NIST) buffers. CO$_2$ speciation was calculated from logged pH, temperature, and average total alkalinity for each treatment using CO$_2$calc applying the constants of Lueker for carbonic acid and Dickson for sulphate (Robbins et al. 2010). Statistical analysis of abiotic data was performed with linear mixed effects models using the *lme* function of the package *nlme* (Pinheiro et al. 2014) in R (R Core Team 2013), with temperature and pH as fixed effects, and time and tank as random effects. Nine mesocosms were removed from the data set due to failures in either pH or temperature control during the experiment, resulting in an unbalanced design. All reported analyses only include the 31 remaining tanks (Table 1).
Macroalgal material

Invasive *C. taxifolia* specimens were collected from one site in the Port River, South Australia, and native *C. taxifolia* was collected from one site in Moreton Bay, Queensland. One specimen corresponded to a single independent thallus sometimes situated within a clump of thalli. Clumped thalli can represent clones of the same individual. To avoid pseudo-replication specimen were collected 1 to 2 m apart from each other and from non-continuous patches. Average *in situ* temperatures in South Australia and Brisbane during time of collection were 21°C and 23°C, respectively. Specimens from both populations were acclimated in the experimental system (tanks) at control temperature (23°C), pH and experimental lighting conditions for one week prior to the experiment. Three specimens per source population were randomly assigned to each tank, totalling six specimens per tank, and planted in small equidistant trays of clean kiln-dried sand. Initial plant size and number of fronds per specimen varied equally between invasive and native specimens. Fronds per specimen varied between one to three, and average initial plant size across all tanks, populations and replicates was 6.1 cm² ± 3.2 SD. Daily tank monitoring ensured absence of thallus overlapping or shading, and the presence of any herbivores inside tanks.

Macroalga growth response

Specimens were photographed immediately before and after the experiment over graph paper (8 mm² quadrats) with a Nikon D300 camera on a copy-stand at a fixed distance from the subject. Individual macroalgal planar areas were calculated using FIJI/ImageJ (Schindelin et al. 2012). Mortality was calculated as the number of macroalgal replicates that died per tank. Dead specimens were defined as completely translucent thalli (which often broke apart by touch) or specimens that completely disappeared from the tanks. The influence of temperature, pH and source population on mortality was investigated using logistic regression in SPSS.
v.21.0 (IBM Corp., Armonk, NY). Following this, growth of specimens that survived for the length of the experiment (83.9%) was examined using Analysis of Covariance (ANCOVA) in SPSS v. 21.0 using the Type III sums of squares (IBM Corp., Armonk, NY, USA). The response variable used was the natural logarithm of the difference between final and initial plant size, and the covariate was the logarithm of initial plant size. Preliminary analyses were also conducted with initial size untransformed, but a comparison of the two analyses using Akaike’s information criterion (AIC) showed the former to provide a better fit, and so only these results are presented. QQplots and Levene’s test were used to confirm normality and homogeneity of variances, respectively. In both analyses, the multi-factorial design comprised pH (4 levels), temperature (2 levels), and source population (2 levels) as independent variables, with tanks nested within the first two factors. Growth was calculated as the difference between initial and final macroalgal area divided by time in weeks. The experiment lasted 3 weeks.

**Chl-a fluorescence**

The photochemical parameters maximal quantum yield of PSII (Fv/Fm) measured 2 hours pre-dawn and the effective quantum yield (Φ_{PSII}) measured 4 hours after dawn were calculated based on fluorescence values measured with a Pulse Amplitude Modulated fluorometer (Aquation Pty Ltd, Australia). Fv/Fm and Φ_{PSII} values were calculated as described in Maxwell and Johnson (Maxwell and Johnson 2000). Fv/Fm and Φ_{PSII} were analysed using ANOVA (after establishing that macroalgal size did not play a significant role) after checking normality using QQ plots. Homogeneity of variances could not be tested, because only a single replicate of each species was measured per tank due to time constraints. As such, interactions with the tank effect could not be tested.
**Bacterial DNA extraction and sequencing**

At the end of the experiment subsamples of *C. taxifolia* thalli were frozen at -80°C. For the bacterial DNA extraction, one frond of each frozen thalli was swabbed with a cotton tip along the surface. Swabbed area comprised all the width (average width across all specimens = 9 ± 2 mm) and ~15 cm of the length of each frond on both sides, to sample surface-associated microorganisms. Microbial DNA was extracted from the swabs using the PowerSoil DNA isolation kit (MO BIO Laboratories Inc.). Samples from both origins and both temperatures, but only from control treatment = 8.05 (current atmospheric pCO$_2$, pH 7.69 equivalent to 1000 ppm CO$_2$ (enriched with dissolved inorganic carbon) and pH 7.69 equivalent to 1000 ppm CO$_2$, but manipulated with HCl dosing (acid solution addition), were analysed. The bacterial 16S rRNA gene was amplified from the DNA using the primers 28F: 5’-GAGTTTGATCNTGGCTCAGC3’ and 519R: 5’-GTNTTACNGCGGCKGCTGC-3’, which span the hypervariable regions V1 to V3 (Handl et al. 2011). Amplicons were sequenced on a Roche pyrosequencing platform FLX by the company Mr. DNA (Shallowater, Texas, USA).

**Analysis of 16S rRNA gene sequences**

Sequence data were processed with MOTHUR v1.29 (Schloss et al. 2009) using standard operating procedure (SOP) for Roche pyrosequencing data (Schloss et al. 2011)). Sequences were filtered using the flowgrams and clustered into operational taxonomic units (OTU) using a 97% cut-off. OTUs were taxonomically classified against the PDS database (i.e. RDP database plus 119 mitochondrial 16S rRNA gene sequences and four 18S rRNA gene sequences; see Supporting Information for more details). Due to a relatively high proportion of OTUs being unclassified with the PDS database, representative sequences for each OTU were further searched against the NCBI NT database using BLASTn algorithm (Myers et al. 1990). The BLASTn results were processed through the last common ancestor algorithm of...
MEGAN (Huson et al. 2011). Based on the resulting taxonomic assignment, sequences from mitochondria, chloroplasts or eukaryotes were removed. On average ~51% (s.d.= 17%) of the sequences were classified as mitochondria, chloroplast or eukaryota and removed from the analysis. The resulting OTU table (OTU per sample) was randomly subsampled to 160 sequences per sample (i.e. the number of sequences of the smallest sample) in order to perform the statistical analysis, resulting in 1087 different OTUs for all samples (Table S1 and S4).

**Statistical analysis of bacterial communities**

The Shannon diversity index, Chao richness estimator and Good's index were obtained from the OTU table (Details in Supporting Information and Table S1 and S2) and the alpha diversity values were compared by ANOVA (Analysis of Variance) using the R package “stats” with function anova. The OTU abundances were square root or presence/absence transformed (for structural and compositional analyses, respectively) and beta-diversity was calculated using the Bray-Curtis coefficient. Bray-Curtis similarities were visualised with two-dimensional, non-metric multidimensional scaling (nMDS) diagrams. Canonical Analysis of Principal coordinates (CAP) analysis with pH as a discriminating factor and a distance-based test for homogeneity of multivariate dispersions (PERMDISP) were performed with Primer v6 (Clarke and Gorley 2006). For testing the effect of pH and temperature treatments, only one thallus from each tank was randomly selected (i.e. ignoring the collection site of the thallus; Table S1) due to the probable non-independence between microbial communities from thalli sharing a tank (i.e. microbial communities on different thalli from the same tank are likely to be exposed to more similar microbial communities than thalli from different tanks). The statistical design included two fixed factors, temperature (2 levels) and pH (3 levels). Mvabund (Wang et al. 2012) was used to test if the bacterial communities or specific
OTUs were significantly different between treatments. Mvabund tests were performed for abundance and presence/absence data with a generalised linear model using negative binomial and binomial distributions, respectively. In order to control for family-wise error rates across variables (i.e. OTUs) the p.uni argument was set to “adjusted”. nBoot = 10'000 ("resampling iterations") was used.

Results

Abiotic data

pH and temperature treatments were consistent throughout the course of the experiment. The pH differed as intended between pH treatments (LMM: F₃,₂₂=75.8, p<0.0001). Post-hoc tests showed that all pH treatments differed (p<0.0006), except between the 1000 ppm CO₂ and the acid treatments (F₁,₁₀=4.4, p=0.062), for which targeted pH levels were the same. Temperature varied between temperature treatments (F₁,₂₂=53, p<0.0001).

The fugacity of CO₂ for air (fCO₂air), seawater (fCO₂SW), carbonate (CO₃²⁻) and bicarbonate (HCO₃⁻) differed amongst pH treatments (LMM: F₃,₂₂=32.6, 32.6, 87.2 and 82.3 respectively, p<0.0001 for all), but not with temperature (F₁,₂₂=0.18, 0.20, 0.67 and 0.75, p=0.68, 0.66, 0.42 and 0.40, respectively) or the interactions (F₃,₂₂=1.47, 1.47, 1.90 and 1.92, p=0.25, 0.25, 0.16, and 0.16, respectively). Post-hoc tests indicated that these variables differed between all pH treatments, except between 1000 ppm CO₂ and the acid treatment (F₁,₁₀=0.31, p=0.59) for CO₃²⁻. Alkalinity varied with pH (LMM: F₃,₂₂=49.3, p<0.0001), but not temperature (F₁,₂₂=0.83, p=0.37) or the interaction (F₃,₂₂=0.28, p=0.84). These system parameters met the guidelines from Dickson et al. (2007) and Riebesell et al. (Dickson et al. 2007; Riebesell et al. 2010)

Macroalgal growth response

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Mortality was not influenced by any of the factors investigated (GLM: p>0.222 for all terms). Only 13% of all 201 plant replicates in the experiment died. This mortality was evenly spread across all treatments (i.e., from a minimum value of 8% at pH 8.05 + 23°C to a maximum value of 18% at pH 7.69 HCl + 23°C). A QQplot confirmed normality with natural log transformation, but Levene’s test showed heterogeneous variances (F_{61,131}=1.56, p=0.019). As analysis of variance is known to be robust to departures of this assumption, it was decided to proceed despite this.

Final specimen size was determined by a complex three-way interaction between initial plant size, pH, and temperature (Figure 1, ANCOVA, F_{3,61}=1.06, p=0.028, Figure S1). Population origin (i.e. whether macroalgae came from invasive SA or native QLD populations) did not have an effect on final size (F_{1,13.6} = 1.5, p=0.24) and did not interact with any of the factors in the experimental design (p>0.051 for all interaction terms). At pH 8.05 macroalgal size remained static at 23°C, whereas at 24°C small thalli grew and large thalli decreased in size. At pH 7.84 and 23°C small thalli did not grow and large thalli tended to decrease in size slightly, whereas at 24°C larger thalli grew. At pH 7.69 using CO_{2} there was no influence of temperature, with smaller thalli remaining static and larger thalli growing. There was also no effect of temperature in the acid treatment (pH 7.69) with small thalli growing and larger thalli decreasing in size (see Figure S1 for a detailed breakdown of change in plant size results). Small and large initial plant sizes for the SA population corresponded to 3.0 ± 0.72 cm^2 and 6.3 ± 1.82 cm^2, respectively. Small and large initial plant size for QLD population were in average 4.8 ± 1.49 cm^2 and 9.7 ± 2.35 cm^2, respectively.

*Chl-a fluorescence*

For Φ_{PSII} there were no significant effects across all factors and their interactions (p>0.12 for all terms). For Fv/Fm there were no significant effects of pH, temperature or tank (for
temperature: $F_{1,11.7}=4.21$, $p=0.063$; for all other terms, $p>0.64$), but differences between populations were significant ($F_{1,11.2}=6.36$, $p=0.028$). Native *Caulerpa* specimens from Moreton Bay, QLD, had an average Fv/Fm of 0.64 ($\pm 0.02$ SE), while invasive specimens from the Port River, SA, had an 11% greater Fv/Fm value ($= 0.71 \pm 0.02$ SE).

**Analysis of bacterial communities**

Coverage of the bacterial community by 16S rRNA gene sequencing ranged from 58% to 90% per sample, Shannon diversity indices varies from 1.44 to 4.31 and richness estimates were between 74 and 259 OTUs (see Table S2). There are no significant differences in the estimated richness and diversity of samples from different treatments (Table S3). An interaction between temperature and pH was found with both presence/absence and abundance data (Table 2).

Therefore, mvabund test comparisons were done within each level of pH and temperature. No significant differences were detected between bacterial communities of samples from different temperatures at any of the pH levels (Table 2). An overall significant difference between communities of samples from different pH was detected only at 24°C (only abundance data; Mvabund Dev$_{6,2} = 1159$, $p = 0.031$; Table 2). Additionally, nMDS plots and CAP analysis showed that samples from different pH treatments clearly separate at 24 °C (Figure 2 and 3). This was not due to differences in dispersion of samples from different pH treatments (PERMDISP: F: 1.66 P(perm): 0.78).

The Mvabund univariate analysis identified only a significant difference in the abundance of one OTU (OTU 13; Mvabund Dev$_{6,2} =17.7$, $p = 0.048$) in samples from different pH within temperature treatment of 24 °C. OTU 13, which was assigned to the genus *Planctomyces* and was not found in the control pH samples, while in samples treated with high CO$_2$ (pH 7.69) had an average relative abundance of 5%. In acid-dosed low pH
treatment (pH 7.69) this OTU’s average relative abundance was 2%.

Discussion.
We showed that, in the absence of any other driving factors, combinations of ocean acidification, global warming and plant size drive higher growth rates in Australian *C. taxifolia* populations, regardless of whether those populations are native (Qld) or invasive (SA). All four scenarios at which higher growth rates were observed occurred under higher temperature, higher pCO$_2$ or lower pH scenarios. However, growth response was strongly influenced by initial thallus size. Our results concur with published field observations from volcanic CO$_2$ seeps showing that *Caulerpa* species are not negatively affected by projected end-of-the-century pCO$_2$ levels, similar to those we tested (Hall-Spencer et al. 2008; Porzio et al. 2011). Our results also agree with studies showing variable intra-specific algal responses to different combinations of pH, pCO$_2$ and temperature (Langer et al. 2009; Olischlager and Wiencke 2013). The only non-positive growth observed under the lowest pH/highest pCO$_2$ tested was 0.7 times smaller than the observed at the control pH 8.05 (Figure S1). The observed significant effect of higher temperature under lower pCO$_2$ also agrees with published laboratory data showing that a few degrees of warming markedly influences *C. taxifolia* growth rates in temperate habitats (Komatsu 1997). Published field survey data have also shown a positive response of *C. taxifolia* to temperature under current atmospheric pCO$_2$, with invasive *C. taxifolia* populations tending to expand in the summer/autumn when temperatures (and light intensity) are higher and shrink during winter (Thibaut et al. 2004). This summer vs. winter population expansion and contraction behaviour is also observed in South Australian populations (Tanner 2011). Komatsu *et al.* (Komatsu 1997) observed that *C. taxifolia* growth seems to increase linearly with temperature until a limit point around 30°C is reached, after which survivorship declines sharply. The optimal lower and upper temperatures
for thallus growth were 20˚C and 30˚C, respectively, which encompasses both temperatures tested in this study (Komatsu 1997).

The pronounced effect of initial plant size on *C. taxifolia* growth rates has also been observed before. Stolon growth from larger fragments, consisting of four fronds or more, is nearly four times greater than those of smaller fragments consisting of only one frond (Komatsu 1997). Similarly, larger clumps of *C. taxifolia* experience greater survivorship after desiccation and transport than smaller clumps (West et al. 2007). Published laboratory results and field observations therefore imply that larger *C. taxifolia* fragments have greater potential to successfully invade new habitats due to higher growth rates and survivorship than smaller fragments. This study however was the first time that macroalgal thallus size was tested in a climate change experimental scenario. The presence of a plant size interactive effect must therefore be considered in future experimental designs and forecasts.

The significant changes in *Caulerpa* growth observed in this study occurred in the absence of any significant change in chlorophyll fluorescence. While PAM fluorescence analysis has become one of the most widely used tools to detect physiological stress in autotrophs (Baker 2008), its ability to detect effects related to processes associated with climate change vary. This is because seaweeds display differing response to warming and ocean acidification and, in many cases, responses are species-specific (Roleda and Hurd 2012; Koch et al. 2013). Even the same species can present differing results. For example, recent ocean acidification experiments on the green benthic macroalga *Ulva lactuca* showed no effects of higher CO$_2$ concentrations/low pH on PAM fluorescence parameters (Rautenberger et al. 2015). However when temperature levels, which were 10˚C apart, were crossed with CO$_2$-enrichment treatments, *U. lactuca* displayed significant effects on chlorophyll-a fluorescence parameters (Liu and Zou 2015).
Different lines of evidence suggest that *C. taxifolia*, like most marine macroalgae, has a carbon concentrating mechanism (CCM) allowing it to take up and use both CO₂ and HCO₃⁻ as a source of inorganic carbon, the latter via an energy-costly active transport (Roleda and Hurd 2012). CCMs involve diverse processes such as the presence of carbonic anhydrases and pyrenoids in the chloroplasts (Roleda and Hurd 2012). CCMs allow algae to regulate and optimize their intracellular pCO₂ demand, diminishing their sensitivity to external variations in environmental pCO₂. Therefore, algae with a CCM, such as *Caulerpa*, are expected to show no response to increases in pCO₂ compared to species lacking CCM. However, it is also expected that CCM-containing macroalgae are capable of adjusting their CCM, down-regulating its expression under higher pCO₂ conditions and saving energy in the process. This energy can be used for other biological processes, such as growth. As such, OA can have substantial effects even on species with CCM (Roleda and Hurd 2012). Furthermore, because CCM can be up or down regulated, a range of physiological responses to increased pCO₂ can be observed in the same species. Such a wide range of responses was observed in our results. Results suggested that under the two lowest pH scenarios (pH 7.69) plant size and CO₂ enrichment drove the outcome that varied from negative, to neutral to positive differences in final plant size.

We further showed that an increase in temperature did not change the overall bacterial community structure or composition, nor the abundances or presence of individual community members associated with *C. taxifolia*. However, changes in pH had some effect on the overall bacterial community structure, but only at elevated temperature. This was driven by significant changes in the abundance of one OTU and many small, but non-significant, changes of other community members. In agreement with our analysis, changes in pH (from pH 8.15-8.26 to pH 7.82 and 7.67) have previously been shown to cause shifts in bacterial communities of seawater under controlled laboratory conditions, where many
members of the taxa Gammaproteobacteria, Flavobacteriaceae, Rhodobacteriaceae and Campylobacteraceae were found to change in abundance (Krause et al. 2012). Similarly, recent studies have reported that a decrease in pH alters the microbial communities associated with marine hosts, such as corals (Meron et al. 2012; Webster et al. 2013a) and the sea anemone Anemonia viridis (Meron et al. 2013). The tropical crustose coralline macroalga Hydrolithon (Porolithon) onkodes is highly sensitive to OA and ocean warming (Anthony et al. 2008) and its bacterial associates also change significantly when the pH was decreased from 8.1 to 7.9 (Webster et al. 2013b). Changes in the bacterial community continued as the pH was decreased to 7.7 and 7.5 to a point where certain bacteria were no longer present and new taxa appeared. Our analysis revealed a substantial change in the abundance of a bacterial epiphyte belonging to the genus Planctomyces (low at pH 8.05 and high at pH 7.69). A Planctomycetes bacterium on the coral Porites cylindrical has also recently been seen to increase its relative abundance of 3.7% at a control sites (pH: 7.91–8.09) to 5.4% at a CO$_2$ seep (pH:7.21–8.01) (Morrow et al. 2015).

Interestingly, bacteria from the phylum Planctomycetes are able to colonise diverse ecosystems, including extreme environments, such as hot springs and acidophilic habitats (Ivanova and Dedysh 2012; Bohorquez et al. 2012; Lucheta et al. 2013). In fact, the marine bacterium Rhodopirellula baltica SH1$^T$ (phylum Planctomycetes) has been experimentally shown to be highly responsive and adaptable to environmental stressors (temperature and salinity; (Wecker et al. 2009). Planctomycetes also live generally attached to surfaces and members of the genera Blastopirellula, Rhodopirellula and Planctomyces are commonly found on marine macroalgae (Bengtsson et al. 2010; Hollants et al. 2013b; Lage and Bondoso 2014), including C. taxifolia (Meusnier et al. 2001). Given the general physiological adaptability of Planctomycetes and its frequent association to macroalgae, it is possible that the Planctomyces OTU identified in the current study has a role in the adaptation of the algal
holobiont to OA.

In conclusion, this study detected and described the magnitude of ecophysiological responses of *C. taxifolia*’s and its associated bacterial community to changes in a range of pH, pCO$_2$ and temperature forecasted in the IPCC models, mostly the business-as-usual carbon emission scenarios. Other important drivers of algal and microbial community biology, such as nutrients, light and biotic interactions (e.g. herbivory) are necessarily excluded in experiments like these. Our observations though indicate that climate change will promote *C. taxifolia* growth (albeit dependent on initial fragment size), but might not cause major changes in its associated bacterial communities. Indeed, only one bacterial taxa (OTU) out of 1087 was presumably involved in the response to OA. This OTU is likely to be part of the holobiont, a unified functional entity of host and associated microorganisms that interacts in order to maintain its overall health and stability, overcoming environmental changes. If native temperate benthic species are negatively affected by ocean warming and OA, invasive populations of *C. taxifolia* might be granted competitive advantage over them, leading to a more invasive behaviour of this often nefarious weed.

**Acknowledgements**

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**Data Accessibility Required for DNA sequence:**

Sequencing data are available through the Sequence Read Archive (SRA) database in the
National Centre of Biotechnology Information (accession number SRP073482).
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Table 1. Physicochemical parameter for *Caulerpa taxifolia* climate change experiment. Parameters of the carbonate system in each of eight mesocosm treatments. Three pH levels manipulated with CO₂ bubbling: pH 8.05 = present day, pH 7.84 ~ 700 ppm CO₂, pH 7.69 ~ 1000 ppm CO₂; and one level with pH 7.69 manipulated with HCl dripping. Nominal temperature in °C. N = number of tank replicates per treatment; maximum = 5 tank replicates per treatment.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Nominal pH</th>
<th>Nominal Temperature</th>
<th>Temperature (°C)</th>
<th>pH (NBS scale)</th>
<th>TA (mmol kg⁻¹)</th>
<th>pCO₂ (ppm)</th>
<th>aqCO₂ (µmol kg⁻¹)</th>
<th>CO₃²⁻ (µmol kg⁻¹)</th>
<th>HCO₃⁻ (µmol kg⁻¹)</th>
<th>TIC (µmol kg⁻¹)</th>
<th>Ωcalc</th>
<th>Ωarag</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (5)</td>
<td>8.05</td>
<td>23</td>
<td>23.1 ± 1.6</td>
<td>8.09 ± 0.15</td>
<td>2.336 ± 0.596</td>
<td>558.49</td>
<td>15.77</td>
<td>193.77</td>
<td>1849.78</td>
<td>2059.33</td>
<td>4.46</td>
<td>2.94</td>
</tr>
<tr>
<td>2 (3)</td>
<td>8.05</td>
<td>24</td>
<td>24.3 ± 3.0</td>
<td>8.01 ± 0.12</td>
<td>2.342 ± 1.292</td>
<td>687.96</td>
<td>18.67</td>
<td>172.48</td>
<td>1904.64</td>
<td>2095.80</td>
<td>3.97</td>
<td>2.63</td>
</tr>
<tr>
<td>3 (5)</td>
<td>7.84</td>
<td>23</td>
<td>23.6 ± 1.4</td>
<td>7.84 ± 0.13</td>
<td>2.358 ± 0.686</td>
<td>1052.97</td>
<td>29.19</td>
<td>122.55</td>
<td>2029.05</td>
<td>2180.79</td>
<td>2.82</td>
<td>1.86</td>
</tr>
<tr>
<td>4 (3)</td>
<td>7.84</td>
<td>24</td>
<td>24.2 ± 1.1</td>
<td>7.84 ± 0.10</td>
<td>2.351 ± 0.614</td>
<td>1025.90</td>
<td>27.93</td>
<td>125.54</td>
<td>2022.18</td>
<td>2175.65</td>
<td>2.89</td>
<td>1.91</td>
</tr>
<tr>
<td>5 (4)</td>
<td>7.69</td>
<td>23</td>
<td>23.6 ± 1.3</td>
<td>7.67 ± 0.27</td>
<td>2.352 ± 0.560</td>
<td>1952.36</td>
<td>54.08</td>
<td>96.56</td>
<td>2094.09</td>
<td>2244.72</td>
<td>2.22</td>
<td>1.47</td>
</tr>
<tr>
<td>6 (3)</td>
<td>7.69</td>
<td>24</td>
<td>24.1 ± 1.2</td>
<td>7.71 ± 0.08</td>
<td>2.347 ± 0.717</td>
<td>1417.54</td>
<td>38.92</td>
<td>94.78</td>
<td>2098.86</td>
<td>2232.56</td>
<td>2.18</td>
<td>1.44</td>
</tr>
<tr>
<td>7 (4)</td>
<td>7.69</td>
<td>23</td>
<td>23.5 ± 1.6</td>
<td>7.78 ± 0.07</td>
<td>2.104 ± 1.777</td>
<td>1092.05</td>
<td>30.52</td>
<td>98.24</td>
<td>1910.67</td>
<td>2039.43</td>
<td>2.26</td>
<td>1.49</td>
</tr>
<tr>
<td>8 (4)</td>
<td>7.69</td>
<td>24</td>
<td>24.2 ± 1.2</td>
<td>7.76 ± 0.09</td>
<td>2.046 ± 1.853</td>
<td>1138.03</td>
<td>30.93</td>
<td>97.32</td>
<td>1912.51</td>
<td>2040.76</td>
<td>2.24</td>
<td>1.48</td>
</tr>
</tbody>
</table>

The values reported are means ± SD of data collected from 23rd November to 18th of December 2012. pH and temperature data were collected every 3 hours. Alkalinity was measured weekly. Carbon speciation parameters were calculated using CO2Calc software. TA, total alkalinity; pCO₂, atmospheric CO₂ partial pressure; aqCO₂, aqueous CO₂; TIC, total dissolved inorganic carbon; Ωcalc and Ωarag refer to the saturation state of calcite and aragonite, respectively.
Table 2. Mvabund test results (Deviance and p-values) for the comparison of presence/absence (composition) and abundance (structure) of OTUs in microbial communities from *Caulerpa taxifolia* for different treatments. Test done using nBoot = 10'000. Res.df: the residual degrees of freedom. Statistically significant p-values (<0.05) shown in bold.

<table>
<thead>
<tr>
<th>Source</th>
<th>Presence/absence</th>
<th>Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Res.df</td>
<td>Df.diff</td>
</tr>
<tr>
<td>Temperature x pH</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>For levels of factor temperature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control pH</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Acid addition pH</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Carbon dioxide pH</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>For levels of factor pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23°C</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>24°C</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>
Figure Legends

**Figure 1.** Diagram illustrating the complexity of ANCOVA result obtained for plant growth data of *Caulerpa taxifolia* exposed to eight treatment combinations of temperature (23°C and 24°C) and pH manipulated with CO₂ (390, 700 and 1000 ppm) and HCl (~1000 ppm) during 3 weeks. Outcome is relative to the present day (reference) growth rate of +0.84 cm²/week (±0.13 SE). No effect = no significative changes in growth rate relative to reference; positive = significant increase in average growth rate; negative = significant decrease in average growth rate. Statistical significance level set at 0.05.

**Figure 2.** Multidimensional scaling plot based on the Bray Curtis similarity of the relative abundance of OTUs of the bacterial community associated to *Caulerpa taxifolia* under different pH and temperature treatments. Open figures: 23°C, filled figures: 24°C; circles: control pH, squares: enriched with dissolved inorganic carbon pH and triangle: acid solution addition pH treatments.

**Figure 3:** CAP analysis of the relative abundance of OTUs of the bacterial community associated to *Caulerpa taxifolia* under different pH treatments at 24°C. Circles: control pH, squares: enriched with dissolved inorganic carbon pH and triangle: acid solution addition pH treatments.

**Figure 4:** Bar diagram of OTUs and their taxonomic classifications with an average relative abundance of greater 1%.
<table>
<thead>
<tr>
<th>pH Treatment (CO₂ or HCl)</th>
<th>Temperature</th>
<th>Initial Plant Size</th>
<th>Outcome</th>
<th>Average degree of change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>23°C</td>
<td>small = large</td>
<td>no effect</td>
<td>reference</td>
</tr>
<tr>
<td>390 ppm</td>
<td>24°C</td>
<td>small</td>
<td>positive</td>
<td>+ 9.4 x</td>
</tr>
<tr>
<td></td>
<td>23°C</td>
<td>small</td>
<td>negative</td>
<td>- 2.0 x</td>
</tr>
<tr>
<td>700 ppm</td>
<td>24°C</td>
<td>large</td>
<td>negative</td>
<td>- 1.1 x</td>
</tr>
<tr>
<td></td>
<td>23°C</td>
<td>small</td>
<td>no effect</td>
<td></td>
</tr>
<tr>
<td>1000 ppm</td>
<td>24°C</td>
<td>large</td>
<td>positive</td>
<td>+ 2.8 x</td>
</tr>
<tr>
<td></td>
<td>23°C = 24°C</td>
<td>small</td>
<td>no effect</td>
<td></td>
</tr>
<tr>
<td>HCl</td>
<td>23°C = 24°C</td>
<td>large</td>
<td>positive</td>
<td>+ 9.6 x</td>
</tr>
<tr>
<td></td>
<td></td>
<td>small</td>
<td>positive</td>
<td>+ 4.4 x</td>
</tr>
<tr>
<td></td>
<td></td>
<td>large</td>
<td>negative</td>
<td>- 0.7 x</td>
</tr>
</tbody>
</table>
Figure 3: CAP analysis of the relative abundance of OTUs of the bacterial community associated to Caulerpa taxifolia under different pH treatments at 24°C. Circles: control pH, squares: enriched with dissolved inorganic carbon pH and triangle: acid solution addition pH treatments.
Rest <1%

- Otu0033: Genus Litoreibacter
- Otu0024: Unclassified bacteria
- Otu0021: Genus Porticoccus
- Otu0023: Class Gammaproteobacteria
- Otu0018: Family Alteromonadaceae
- Otu0017: Unclassified bacteria
- Otu0012: Class Gammaproteobacteria
- Otu0011: Unclassified bacteria
- Otu0009: Unclassified bacteria
- Otu0006: Family Rhodobacteraceae
- Otu0004: Unclassified bacteria

- MB118
- MB114
- MB04
- MB15
- MB58
- MB104
- MB132
- MB104
- MB129
- MB27
- MB31
- MB94
- MB68

**Temperature Conditions:***
- **AA**: 23°C to 24°C
- **Control**: 23°C to 24°C
- **HCD**: 23°C to 24°C
Supporting information: The effects of warming and ocean acidification on host growth, photosynthesis and bacterial communities for the marine invasive macroalga *Caulerpa taxifolia*

Supporting Methods:

**Details on the analysis of 16S rRNA gene sequences with MOTHUR’s 454 standard operating procedure (SOP)**

Sequences were quality filtered using flowgrams. Improved sequences were aligned to the SILVA bacterial reference alignment making sure that all sequences overlap in the alignment space using the "screen.seqs" command and optimising the start and end positions. Contaminant taxa (mitochondria, chloroplast, eukaryote) and unknown sequences were removed with the command "remove.lineage" and parameter cutoff=80 after the classification of the sequences (using the PDS database). The error analysis step was not applied and no groups were removed from the analysis with mothur. Option 1 of the SOP was used for the generation of a distance matrix and the cluster of sequences into OTUs. OTUs were classified with the "classify.otu" command with parameters cutoff=80 and basis=sequence.

**Details on the calculation of Shannon diversity index, Chao richness estimator and Good's coverage index using MOTHUR**

The OTU table was used to obtain the coverage (Good's coverage), Chao estimated richness and Shannon diversity index with the command "summary.single" using parameter subsample=160. Representative sequences per OTU were obtained with the command "get.oturep" and after this step the OTU table was subsampled to 160 sequences per sample for statistical analysis.
Supporting tables and figures:

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Number of raw sequences</th>
<th>Number of filtered sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB118</td>
<td>2220</td>
<td>651</td>
</tr>
<tr>
<td>PR78</td>
<td>3712</td>
<td>2048</td>
</tr>
<tr>
<td>PR19</td>
<td>2118</td>
<td>721</td>
</tr>
<tr>
<td>MB114</td>
<td>3960</td>
<td>874</td>
</tr>
<tr>
<td>PR145</td>
<td>2061</td>
<td>305</td>
</tr>
<tr>
<td>MB04</td>
<td>3140</td>
<td>1531</td>
</tr>
<tr>
<td>PR15</td>
<td>3462</td>
<td>1155</td>
</tr>
<tr>
<td>MB58</td>
<td>3140</td>
<td>904</td>
</tr>
<tr>
<td>PR132</td>
<td>2963</td>
<td>1333</td>
</tr>
<tr>
<td>MB104</td>
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<td>265</td>
</tr>
<tr>
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<td>1859</td>
<td>691</td>
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<tr>
<td>PR77</td>
<td>2336</td>
<td>792</td>
</tr>
<tr>
<td>MB130</td>
<td>2063</td>
<td>802</td>
</tr>
<tr>
<td>PR129</td>
<td>694</td>
<td>160</td>
</tr>
<tr>
<td>MB27</td>
<td>3454</td>
<td>1005</td>
</tr>
<tr>
<td>PR17</td>
<td>4216</td>
<td>1699</td>
</tr>
<tr>
<td>MB31</td>
<td>2406</td>
<td>953</td>
</tr>
<tr>
<td>PR94</td>
<td>2207</td>
<td>1559</td>
</tr>
<tr>
<td>MB68</td>
<td>932</td>
<td>543</td>
</tr>
</tbody>
</table>

Table S1: Number of raw and filtered sequences for the epiphytic bacterial community of *Caulerpa taxifolia*.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Temperature treatment</th>
<th>pH Treatment</th>
<th>Coverage</th>
<th>Shannon</th>
<th>Chao</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB118</td>
<td>AA</td>
<td>T1</td>
<td>0.85</td>
<td>2.08</td>
<td>114.41</td>
</tr>
<tr>
<td>PR78</td>
<td>AA</td>
<td>T1</td>
<td>0.74</td>
<td>3.39</td>
<td>187.95</td>
</tr>
<tr>
<td>PR19</td>
<td>AA</td>
<td>T1</td>
<td>0.89</td>
<td>1.81</td>
<td>77.55</td>
</tr>
<tr>
<td>MB114</td>
<td>AA</td>
<td>T2</td>
<td>0.8</td>
<td>2.88</td>
<td>149.24</td>
</tr>
<tr>
<td>PR145</td>
<td>AA</td>
<td>T2</td>
<td>0.75</td>
<td>2.86</td>
<td>143.15</td>
</tr>
<tr>
<td>MB04</td>
<td>AA</td>
<td>T2</td>
<td>0.82</td>
<td>2.41</td>
<td>115.04</td>
</tr>
<tr>
<td>PR15</td>
<td>AA</td>
<td>T2</td>
<td>0.74</td>
<td>3.33</td>
<td>191.1</td>
</tr>
<tr>
<td>MB58</td>
<td>Control</td>
<td>T1</td>
<td>0.74</td>
<td>3.35</td>
<td>164.79</td>
</tr>
<tr>
<td>PR132</td>
<td>Control</td>
<td>T1</td>
<td>0.65</td>
<td>3.79</td>
<td>246.5</td>
</tr>
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<td>MB104</td>
<td>Control</td>
<td>T1</td>
<td>0.88</td>
<td>2.18</td>
<td>78.39</td>
</tr>
<tr>
<td>PR104</td>
<td>Control</td>
<td>T1</td>
<td>0.62</td>
<td>3.79</td>
<td>240.75</td>
</tr>
<tr>
<td>PR77</td>
<td>Control</td>
<td>T2</td>
<td>0.63</td>
<td>4.09</td>
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<td>258.83</td>
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<tr>
<td>PR129</td>
<td>Control</td>
<td>T2</td>
<td>0.58</td>
<td>4.31</td>
<td>242.94</td>
</tr>
<tr>
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<td>HCD</td>
<td>T1</td>
<td>0.9</td>
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<td>73.9</td>
</tr>
<tr>
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<td>HCD</td>
<td>T1</td>
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<td>2.61</td>
<td>168.23</td>
</tr>
<tr>
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<td>HCD</td>
<td>T1</td>
<td>0.76</td>
<td>2.69</td>
<td>170.94</td>
</tr>
<tr>
<td>PR94</td>
<td>HCD</td>
<td>T2</td>
<td>0.76</td>
<td>3.57</td>
<td>170.64</td>
</tr>
<tr>
<td>MB68</td>
<td>HCD</td>
<td>T2</td>
<td>0.65</td>
<td>3.94</td>
<td>234.83</td>
</tr>
</tbody>
</table>

Table S2. Good's coverage, Shannon diversity index and Chao estimated richness statistics of bacterial communities associated with *Caulerpa taxifolia*. Information about the tank in which each thallus was held during the experiment are also shown.
<table>
<thead>
<tr>
<th>Source</th>
<th>Alpha diversity measure</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>P-value</th>
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<td>pH</td>
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<td>3.2</td>
<td>0.07</td>
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<tr>
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Table S3. ANOVA test results (F values and P-values) for the Chao estimated richness and the Shannon diversity index of *Caulerpa taxifolia* samples. Num.df: degrees of freedom for the numerator. Den.df: Degrees of freedom for the denominator.
Figure S1. Average and standard deviation of the average for daily pH fluctuation for each pH treatment in an experiment with Caulerpa taxifolia samples. Seawater nominal pH treatments corresponded to 8.05, 7.84, and 7.69. At nominal pH 7.69, one treatment was manipulated with CO$_2$ dosing, the other with HCl dosing.
Figure S2. Growth of *Caulerpa taxifolia* (thallus area in cm$^2$) as a function of initial thallus size (= covariate) under the present (23°C – top panel) and future (24°C – bottom panel) temperature conditions. pH1 (control) = 8.06 (current atmospheric pCO$_2$), pH2 = 7.84, pH3 = 7.69, pH4 = 7.69 (pH equivalent to 1000 ppm CO$_2$, but manipulated with HCl dosing). Lines indicate exponential growth fits to each treatment combination. Experiment duration = 21 days.
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Author/s:
Roth-Schulze, AJ; Thomas, T; Steinberg, P; Deveney, MR; Tanner, JE; Wiltshire, KH; Papantoniou, S; Runcie, JW; Gurgel, CFD

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