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Effect of marine heatwaves and warming on kelp microbiota influence trophic interactions

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Abstract

The range-expansion of tropical herbivores due to ocean warming can profoundly alter temperate reef communities by overgrazing the seaweed forests that underpin them. Such ecological interactions may be mediated by changes to seaweed-associated microbiota in response to warming, but empirical evidence demonstrating this is rare. We experimentally simulated ocean warming and marine heatwaves (MHWs) to quantify effects on two dominant temperate seaweed species and their microbiota, as well as grazing by a tropical herbivore. The kelp *Ecklonia radiata*'s microbiota in sustained warming and MHW treatments was enriched with microorganisms associated with seaweed disease and tissue degradation. In contrast, the fucoid *Sargassum linearifolium*'s microbiota was unaffected by temperature. Consumption by the tropical sea-urchin *Tripneustes gratilla* was greater on *Ecklonia* where the microbiota had been altered by higher temperatures, while *Sargassum*'s consumption was unaffected. Elemental traits (carbon, nitrogen), chemical defences (phenolics) and tissue bleaching of both seaweeds were generally unaffected by temperature. Effects of warming and MHWs on seaweed holobionts (host plus its microbiota) are likely

species-specific. The effect of increased temperature on *Ecklonia*'s microbiota and subsequent increased consumption suggest that changes to kelp microbiota may underpin kelp-herbivore interactions, providing novel insights into potential mechanisms driving change in species' interactions in warming oceans.

KEYWORDS
climate change, *Ecklonia radiata*, macroalgae, microbiome, *Sargassum linearifolium*, tropicalisation

1 | INTRODUCTION

Ocean warming and marine heatwaves (MHWs) (Garrabou et al., 2009), that is discrete events where water temperatures exceed the 90th percentile of historical baseline for that area for five or more days (Hobday et al., 2016), are causing substantial changes to marine ecosystems. Warming and MHWs can potentially trigger widespread mortality of benthic invertebrates and kelp (Garrabou et al., 2009; Wernberg, Bennett, et al., 2016; Wernberg, de Bettignies, et al., 2016) and strong changes in genotypes within affected populations (Coleman et al., 2020; Coleman & Wernberg, 2020; Gurgel et al., 2020). Such effects can lead to physiological alterations, range shifts and regional extinctions of some species and concurrent population outbreaks of others (Smale et al., 2019). Impacts of warming and MHWs on foundation species such as corals or kelps are particularly important as they can cascade throughout the entire ecosystem (Hughes et al., 2017; Wernberg, Bennett, et al., 2016).

Warming and MHWs can create trophic disruptions such as overgrazing of habitat-forming organisms that lead to major cascading impacts on ecosystem (Scheffers et al., 2016). Often these complex interactions receive less attention than direct, physical or physiological effects of warming or MHWs on habitat-formers. For example, the poleward range-expansion of the herbivorous sea-urchin *Centrostephanus rodgersii* into Tasmania in southeastern Australia has caused significant declines of kelp forests, with major financial losses in associated fisheries (Ling et al., 2009). Range expansions of tropical herbivorous fishes are also leading to overgrazing and declines of seaweeds along their warm edge of distribution worldwide, a process coined "tropicalisation", and has been linked to shifts in dominant habitat-forming species, such as cool-water kelps becoming replaced by corals or low-biomass turf (Vergés et al., 2014; Wernberg, Bennett, et al., 2016). The frequency of such interactions and the consequences to temperate ecosystems are predicted to increase under future climate change scenarios (Castro et al., 2020; Provost et al., 2017).

Herbivory, particularly plant-insect interactions, is often mediated by microorganisms in terrestrial systems (Frago et al., 2012). Pathogenic microorganisms that are the direct cause of plant disease or stress may indirectly affect host plants by increasing their susceptibility to herbivore consumption (Stout et al., 2006). Conversely, wounds or damage from herbivores may provide infection sites for pathogens (Daleo et al., 2009). The effect of interactions between multiple natural enemies and marine diseases are predicted to increase as oceans warm (Lafferty et al., 2015). Warming can alter the microbiota of seaweeds, leading to tissue degradation or bleaching (Marzinelli et al., 2015; Qiu et al., 2019). Experimental simulations of ocean warming and acidification lead to strong changes in kelp-associated microbiota, followed by declines in photosynthetic efficiency and tissue degradation or bleaching (Qiu et al., 2019), which resembled changes observed in the field for the same kelp species (Marzinelli et al., 2015). Most studies to date, however, have focused on the direct effects of microbial changes on host seaweeds in the context of 'holobionts' (host plus the associated microbiota) (Dittami et al., 2021), and we know comparatively little about flow-on interactions with herbivores.

Changes to host-associated microbiota in response to environmental stressors can influence interactions between the host and other macro-organisms such as herbivores (Campbell et al., 2014). This may be mediated by changes to host chemical defences, nutritional quality or a combination of these factors. For example, temperature stress can lead to increased microbial disease susceptibility and reduced chemical defences for the red seaweed *Delisea pulchra*, which results in enhanced herbivory (Campbell et al., 2012). Microbes can also alter herbivory patterns by degrading the tissue of the host and enhancing palatability. Some terrestrial herbivores preferentially consume decaying plant tissue, potentially because these tissues harbour microbial communities that are distinct from healthy tissues and that reduces toughness and acts as cues for digestibility (Ihnen & Zimmer, 2008; Zimmer et al., 2003). Herbivores may also prefer decaying plant tissue because levels of stored nutrients and their release can differ from non-decaying tissue, particularly at high temperatures (Hanisak, 1993). As oceans become warmer and extreme MHWs more frequent (Frölicher et al., 2018; Oliver et al., 2019), understanding the mechanisms by which these stressors influence herbivory will enable a more holistic understanding of the response of seaweed forests to future environmental conditions.

Our study investigated the effects of sustained ocean warming and MHWs on (1) seaweed-associated microbiota, (2) seaweed susceptibility to herbivory by a tropical sea-urchin and (3) the relationship between these factors. We focused on two forest-forming species of seaweeds distributed throughout Australia's Great Southern Reef: the dominant kelp *Ecklonia radiata* (Wernberg et al., 2019) and the fucoid *Sargassum linearifolium* (Coleman &

Wernberg, 2017), hereafter *Ecklonia* and *Sargassum*, respectively. Following exposure to sustained warming and MHWs, we examined herbivory by the tropical herbivore *Tripneustes gratilla* (hereafter *Tripneustes*), which currently inhabits tropical and subtropical Indo-Pacific regions. *Tripneustes* outbreaks have caused overgrazing and phase-shifts in many regions around the world (Eklöf et al., 2008; Valentine & Edgar, 2010). In Australia, *Tripneustes* has already been identified as a key consumer of *Ecklonia* in warming tropical-temperate transition zones where kelp is declining (Vergés et al., 2016). A recent study predicts novel regions of co-occurrence between *Tripneustes* and *Ecklonia* in response to continued ocean warming (Castro et al., 2020). These novel interactions between tropical consumers and temperate prey due to tropicalisation is a global phenomenon that can cause profound shifts in ecological communities (Vergés et al., 2014; Wernberg, Bennett, et al., 2016).

We hypothesised that sustained warming and MHWs would alter the seaweed's microbiota (alpha- and/or beta-diversity) which would, in turn, influence herbivory. Differences in susceptibility to consumption by urchins were further related to changes in macroalgal nutrient (carbon and nitrogen), total phenolic (chemical defences) content and levels of tissue bleaching to understand other potential mechanisms that can influence feeding. We compared the microbial data from the kelp *Ecklonia* under different temperature treatments with a published microbial dataset from a field study on the same species (Marzinelli et al., 2015) to assess the ecological relevance of any observed changes in microbial composition.

2 | MATERIALS AND METHODS

2.1 | Seaweed collection

Juvenile *Ecklonia radiata* (length ~ 15 cm; $N = 140$) and *Sargassum linearifolium* (length ~ 10 cm; $N = 140$) were collected haphazardly (>2 m apart) at Cronulla rocky reef, Sydney, Australia (34.0706° S, 151.1566° E; representative of south-eastern Australian reefs) on 1 March 2017 at 3–5 m depth by SCUBA. Seaweeds were placed in moist, cool containers and transported by plane to the National Marine Science Centre (NMSC) in Coffs Harbour (30.3022° S, 153.1189° E). Seaweeds were placed in mesocosms within 4 h of collection.

2.2 | Temperature treatments

We exposed seaweeds to one of four temperature profiles over 7 weeks (1 March to 18 April 2018): (1) Ambient/control ('C') at 22°C (mean 21.96, SD 0.35) throughout the experiment (0 degree-days), a typical summer water temperature at the collection site (Schaeffer & Roughan, 2017); (2) sustained warming ('W') at 24°C (mean 24.13, SD 0.58) throughout the experiment (96 degree-days) reflecting a 2°C increase under RCP 4.5 (emission stabilisation) scenario (IPCC, 2013); (3) marine heatwave ('MHW') at 22°C until day 22, when it was increased to 27°C (mean 27.69, SD 0.50) for 18 days and then brought back to 22°C (85 degree-days); (4) marine heatwave plus variability ('MHW + V') similar to the MHW profile (mean 27.65, SD 0.53) plus three 24°C heat spikes (2-day-long each) starting on day seven (97 degree-days; temperature was decreased to 22°C for 1 day between spikes, Figure S1). MHW profiles mimicked a recent heatwave in Western Australia, with the 5°C increase in temperature to 27°C reflecting the upper range of temperature anomalies experienced and the smaller heat spikes representing the temperature fluctuations that occurred during the event (Hobday et al., 2016). MHWs are also becoming more frequent and intense along the east coast of Australia (Oliver et al., 2017). W and MHW + V treatments had similar degree-day values (i.e. cumulative temperature over the experiment calculated using Method 1 in (McMaster & Wilhelm, 1997)) to decouple cumulative versus profile-specific effects (Figure S1).

Experiments were conducted in shaded, outdoor mesocosms, with flow-through seawater (Qiu et al., 2019) pumped directly from the ocean and filtered (50 μm) into three large header tanks, where the water temperature was regulated at either 22, 24 or 27°C by heater-chiller units (Aquahort Ltd., Auckland, NZ). Header tanks fed into 230 L mesocosms (80 cm diameter × 45 cm height) randomly allocated to each temperature treatment ($n = 5$). Mesocosms only received water from one header tank at any one time (constant flow rate of 2.5 L min^{-1}), but MHW and MHW + V mesocosms were plumbed to either two or three header tanks. The tank source changed during the experiment depending on the temperature treatment to maintain flow rates among mesocosms. Mesocosm's water temperature, pH and conductivity were measured daily using a Hach HQ40d multi-probe calibrated with NIST buffers (Qiu et al., 2019). Each mesocosm contained seven haphazardly selected *Ecklonia* and *Sargassum* individuals fixed to mesh bags containing small rocks/gravel. Air was constantly pumped into the mesocosms via weighted air stones.

2.3 | Microbiota

After seven weeks of exposure to temperature treatments, a subset of individuals from each species/temperature treatment (*Ecklonia*: $n = 4–6$; *Sargassum*: $n = 3$) were randomly selected. Sterile cotton swabs were used to sample microbiota on algal surfaces, with the same area (20 cm^2) and swabbing time (30 s) sampled for all individuals (Marzinelli et al., 2015, 2018). *Ecklonia* were swabbed mid-thallus, that is blade area above the meristem, while *Sargassum*'s total area of a randomly chosen single branch (including blades) was swabbed due to their different morphology. Swabs were immediately stored in liquid nitrogen and transported to the University of New South Wales (UNSW, Sydney) and kept at −80°C until DNA extraction. Swabbed seaweeds were not used in feeding assays to avoid confounding due to potential effects of the swabbing process on herbivory. Given the random selection of these individuals, their microbiota was deemed representative of seaweeds used in feeding

assays. We also quantified levels of tissue bleaching, that is visible as a whitening of algal tissue, often associated with loss of surface integrity and known to be associated with microbial disease in other macroalgae (defined in Marzinelli et al., 2015) on an independent, randomly selected subset of seaweeds of each species/temperature treatment using categories: 1 = 0%–5% tissue bleaching; 2 = 6%–25%; 3 = 25%–49%; 4 = 50%–74%; 5 = 75%–100%.

2.4 | Herbivory

After 7 weeks, we performed feeding assays with *Tripneustes gratilla*, an abundant herbivorous sea-urchin in tropical and subtropical Indo-Pacific reefs (Lawrence & Agatsuma, 2013) (not to be confused with the similar, recently described *T. kermadecences* that is sympatric with *T. gratilla* at Coffs Harbour and extends into temperate waters [Bronstein et al., 2019]). Urchins used were the offspring of brood stock collected in Cairns (QLD, Australia) ensuring they were *T. gratilla*, and were maintained in a 3000 L outdoor aquaria at NMSC where they had been cultured at ambient temperature and fed a diet of mixed macroalgae. Urchins were haphazardly collected ($n = 71$) and placed in individual plastic containers (~0.7 L) with flow-through seawater at ambient temperature (~23.8°C). Urchins were starved for 15 h before the start of herbivory assays for acclimation and to enhance feeding rates (Dworjanyn et al., 2007). The random allocation of urchins to treatments resulted in similar sizes in each treatment (i.e. no significant differences in urchin wet-weight among temperature/species treatments; Table S4b; Figure S7). Nevertheless, larger urchins generally consumed more algae throughout all treatments for both *Ecklonia* and *Sargassum* (Table S4a; Figure S6), so this relationship was explicitly accounted for in analyses by fitting initial urchin wet-weight as a continuous covariate in models (see below).

Ecklonia ($n = 6$–10) and *Sargassum* ($n = 9$–10) exposed to temperature treatments were collected from the mesocosms, patted dry with paper towels (different towel per individual), weighed and offered to the urchins (one urchin/seaweed per 0.7 L container) in separate, no-choice feeding assays at ambient temperature following established methodology (Poore & Steinberg, 1999). The level of replication (urchin/tank) per treatment was: C = control/ambient ($n = 10$ for both algal spp.); MHW = marine heatwave (*Ecklonia* $n = 10$, *Sargassum* $n = 9$); MHW + V = marine heatwave plus variability (*Ecklonia* $n = 6$, *Sargassum* $n = 10$); W = sustained warming (*Ecklonia* $n = 7$, *Sargassum* $n = 9$). Initial *Ecklonia* weight ranged between 1.0–23.4 g (mean 5.6 ± SE 0.5 g), whereas *Sargassum* ranged between 0.2–5.1 g (mean 1.5 ± SE 0.2 g). Controls with seaweeds in containers arranged randomly without urchins ($n = 5$ per species/temperature treatment) were included to measure autogenic changes in mass. Assays ended when approximately half of the available seaweed was consumed in one of the treatments (*Sargassum*: after 8 h due to smaller initial size of individuals; *Ecklonia*: 52 h). Remaining seaweeds were carefully removed from the tanks, patted dry and reweighed, then freeze-dried for tissue chemistry analyses.

2.5 | Seaweed chemistry

Freeze-dried seaweeds ($n = 5$ per species/temperature treatment) were finely ground using a ball mill (Retsch MM 200) and carbon (C) and nitrogen (N) analysis performed using a LECO TruSpec at Mark Wainwright Analytical Centre (UNSW). *Ecklonia* total phenolic content was measured using the Folin–Ciocalteu method (van Alstyne, 1995). Each sample of 4–4.5 mg ground tissue was extracted in 1 mL Methanol:H_2O [1:1], sonicated for 15 min. and dark-incubated at 4°C for 24 h. 50 mg/L solution of gallic acid was used to determine the standard curve (Kubanek et al., 2004). Absorbance was read at 765 nm (van Alstyne, 1995). *Sargassum* phenolics were not analysed as there was not enough tissue sample remaining. Some samples yielded values below detection levels and were not analysed.

2.6 | DNA extractions and bioinformatics

DNA was extracted from swabs using the DNeasy PowerSoil Kit (Qiagen) and amplified using Polymerase Chain Reaction (PCR) primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 785R (5′-GACTACHVGGGTATCTAATCC-3′), targeting the 16S rRNA gene V3-V4 regions (bacteria and archaea) (Herlemann et al., 2011; Klindworth et al., 2012). Primers incorporated standard Illumina overhangs. These overhangs were then targeted in a second round PCR to incorporate samples-specific barcodes assigned to one primer. Each PCR reaction contained 25 μL of EconoTaq DNA Polymerase plus green Master Mix (Lucigen Corporation), 2.5 μL of primer 341f (10 pmol/μL), 2.5 μL of primer 785r (10 pmol/μL), 15 μL of Molecular Biology Grade Water and 4 μL of DNA extract. Initial denaturation occurred at 94°C for 2 min, followed by 35 cycles of: (1) denaturation at 94°C for 30 s, (2) annealing at 55°C for 30 s, and (3) extension at 72°C for 45 s. Lastly the final extension was performed at 72°C for 7 min. PCR included negative controls, that is swabs that were not used to sample algal surfaces but that were otherwise handled in the same was as those used to sample algal surfaces. PCR on these controls showed no amplification visible on the gels. Zymo DNA-5 Clean Concentrator was used to purify amplicons; their quality and quantity were assessed via NanoDrop 1000 and Qubit 2.0 Fluorometer (Thermo Fisher Scientific) and were sequenced with a 2 × 250 bp MiSeq reagent kit v2 on the Illumina MiSeq2000 Platform at Ramaciotti Centre for Genomics.

16S rRNA gene sequences were quality-filtered using Trimmomatic v0.36 (Bolger et al., 2014) with a 4:15 base-pairs (bp) sliding window trim to remove adaptors and low-quality sequences with <36 bp. Paired-end reads were merged (length: 400–500 bp) using USEARCH v9.2.64 (Edgar, 2010). UNOISE was then used to remove chimeras, producing 5777 amplicon sequence variants (ASV; 2,911,853 sequences) (Edgar, 2016). Quality-filtered reads were mapped back to ASV with USEARCH. ASV sequences were searched with BlastN against the SILVA SSU-RefNR99 taxonomic classification database (Quast et al., 2012). ASV assigned to chloroplasts

(3% of total) were removed. Sequencing coverage, assessed by Michaelis–Menten kinetics, was 75%–88% per sample, indicating sampling of the majority of the microbial community. ASV table was normalised using the "DESeq2" R-package, with size-factor dispersions calculated for each treatment combination, which allows accounting for differences in sequencing depth without loosing sequences, as with other common methods, for example rarefaction (McMurdie & Holmes, 2014). ASV with a relative abundance <0.01% were removed.

To examine whether ASV that differed significantly among treatments in mesocosms (1) occur in the field and (2) differ between healthy and bleached/tissue-degraded kelp, we searched them by BLASTN (strict identity) against a dataset that comprises the microbiota of healthy and bleached/tissue-degraded *Ecklonia* from nine sites ($n = 5–6$ healthy and bleached/site) spanning ~500 km along the NSW coast, sampled as in this study (details in [Marzinelli et al., 2015]). To make sequencing data comparable, we ran field sequences through the same bioinformatic pipeline described above. Abundances of this subset of "field" ASV were compared between healthy and bleached kelp (details below).

2.7 | Statistical analyses

Microbial community data were analysed using PERMANOVA in PRIMER-6 with the PERMANOVA add-on (PRIMER-E, UK), which examines differences among centroids (a location parameter for multivariate data, analogous to the mean in univariate data). Data were square-root transformed, similarity matrices were calculated using Bray-Curtis dissimilarities. Seaweed species and temperature treatment were fixed factors, with mesocosm a random factor nested within treatment. The model was run with 9999 permutations of residuals. Differences in dispersion (analogous to variance for univariate data) between groups were tested using PERMDISP. Where significant differences in dispersion were detected, we used a more conservative critical value of alpha (0.01) to determine centroid differences for those factors. Due to large differences in the dispersion of microbial communities found between the two species ($F_{1,30} = 99.672$, $p = .001$; Table S1), the same PERMANOVA analysis was run separately for *Ecklonia* and *Sargassum* to better resolve the effect of temperature treatments for each species.

Species richness and Simpson's diversity indices were calculated using "vegan" R-package. Linear mixed models (LMM) were fitted using "lme4" R-package (also used for all univariate analyses below), with seaweed species, temperature treatment and their interaction as fixed, and mesocosm as random. LMM significance was assessed using *F*-statistic with denominator degrees-of-freedom estimated using Kenward–Roger's method ("lmerTest" R-package).

To determine which microbial taxa differed between treatments for each macroalgal species, a negative-binomial generalised linear model (GLM) for each ASV and accounting for multiple testing was fitted using DESeq2 (Love et al., 2014). Wald pairwise tests were used to compare relative abundances of taxa among treatments. Dozens of taxa differed among treatments; we focused on taxa with an absolute effect size three times greater than the control standard error, as this method has been shown to reveal important ASVs linked to changes in host condition (Qiu et al., 2019). GLMs were also used to compare abundances of ASV from field samples (that matched those found to differ among treatments in the mesocosm experiment) between healthy and bleached/tissue-degraded *Ecklonia*.

A LMM was fitted to test effects of seaweed species, temperature treatments and their interaction on relative consumption, calculated as $([\text{weight}_{\text{start}} - \text{weight}_{\text{end}}]/\text{weight}_{\text{start}}) \times 100$. Urchin biomass was accounted for by adding urchin weight as a continuous covariate first in the model. Mesocosm was a random effect. To test for autogenic changes unrelated to herbivory, a similar model was fitted for controls without urchins.

Mantel tests (9999 permutations, mantel. test function in "ape" R-package) were used to test for relationships between dissimilarities in microbial community structure and absolute differences in % algal tissue consumed by sea-urchins among each pair of temperature treatments for *Ecklonia* and *Sargassum*. Spearman's rank correlation (ρ) were calculated to determine the strength of the association between these variables for the two algal species.

To test for temperature effects on algal chemistry and bleaching, LMM were fitted for %C, %N, C:N and bleaching. Algal species, temperature treatments and their interaction were fixed effects, and mesocosm was random. For phenolics, temperature was the sole fixed factor (*Ecklonia* only).

For all univariate analyses, normality and variance homogeneity assumptions were examined with residual quantile plots and fitted vs residual plots. Post-hoc Tukey HSD ("emmeans" R-package) were used to resolve differences between treatment levels.

3 | RESULTS

3.1 | Seaweed microbiota

Temperature effects on microbiota were species-specific (Figure 1; pseudo-$F_{1,3} = 1.934$, $p < .02$, Table S1). *Ecklonia*'s microbiota under heatwave (MHW, MHW + V) and sustained warming (W) treatments differed significantly from controls (pseudo-$F_{3,12} = 2.16$, $p < .01$, Table S2) and those in MHW + V differed significantly from sustained warming (W), which clustered between heatwave treatments and controls (Figure 1). In contrast, *Sargassum*'s microbiota was unaffected by temperature (Figure 1; Table S2).

Sargassum had greater microbial richness (~700–800 ASV, $F_{1,18} = 61.8$, $p < .01$, Table S3; Figure S2) and Simpsons index (~0.95, $F_{1,20} = 14.5$, $p < .01$, Table S3; Figure S2) across all treatments than *Ecklonia* (~300–600 ASV, Simpson's: ~0.90), but these diversity metrics were unaffected by temperature (Table S3; Figure S2).

Differences in *Ecklonia*'s microbiota among temperature treatments were primarily influenced by 31 ASV, which accounted for 0.95% of all *Ecklonia*-associated ASV (Figures 2 and S3). Most

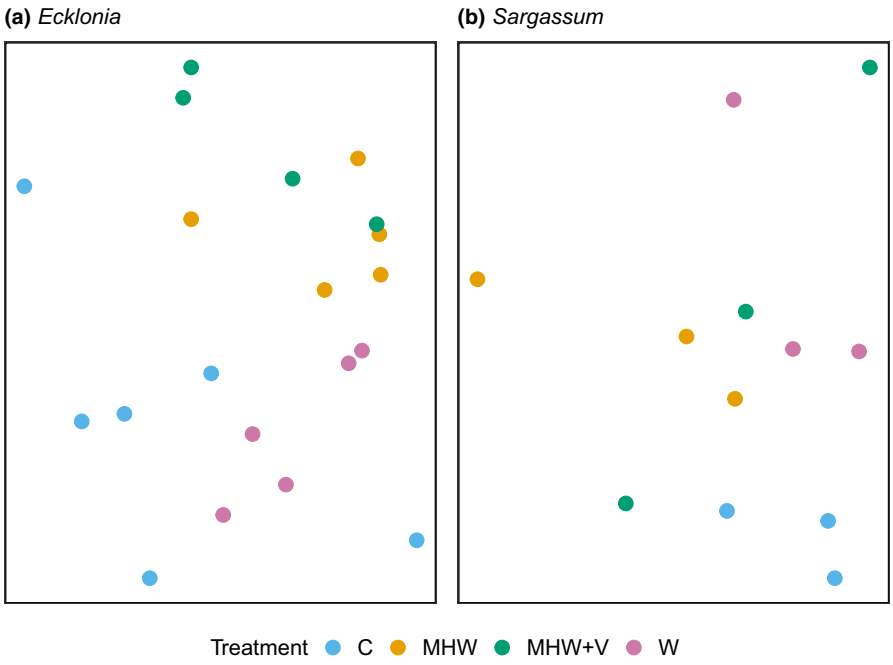

FIGURE 1 nMDS ordinations of *Ecklonia* (a; $n=4–6$, stress = 0.11) or *Sargassum* (b; $n=3$, stress = 0.15) microbiota after 7 weeks of exposure to temperature treatments: C, control/ambient; MHW, marine heatwave; MHW + V, marine heatwave plus variability; W, sustained warming.

ASV had higher relative abundances in warmer temperature treatments (W, MHW, MHW + V) than in controls; other ASVs did not differ between warming and control treatments, except for one (Gammaproteobacteria) which had higher relative abundances in the control (Figure S3). For example, an ASV assigned to the OM190 class (phylum Planctomycetes) was significantly more abundant in MHW + V than all other treatments, while an ASV assigned to *Roseibacillus* dominated in the W treatment and the *Flavobacteriaceae* family was only found in W. ASVs assigned to the families *Erythrobacteraceae*, *Rhodobacteraceae* and *Verrucomicrobiaceae* (*Haloferula* genus) were significantly more abundant in W, MHW and MHW + V treatments than in controls (Figure 2). Relative abundances of some ASV, however, were not consistent among warming treatments. For instance, *Roseibacillus* had higher abundances in W than C, but the lowest abundances were in the MHW treatments (Figure 2).

All 31 ASV that differed significantly among our experimental temperatures in mesocosms were found associated with *Ecklonia* in the field samples from (Marzinelli et al., 2015). Of these, 14 ASV had significantly higher relative abundances on bleached field *Ecklonia* (Figure S4) and in mesocosms they were enriched under warming treatments (Figures 2 and S3). Only one ASV had higher relative abundances on healthy field kelp (Figure S4) and in mesocosms it was more abundant in controls (Figure S3).

3.2 | Herbivory

Temperature effects on algal consumption by urchins differed between species (Figure 3a; $F_{3,55}=4.9$, $p<.01$, Table S4a). Consumption of *Ecklonia* was ~40% higher in MHW + V than controls, and there was a non-significant trend for higher consumption in MHW and W than C (~20% increase; Figure 3a; Table S4a). In contrast, consumption of *Sargassum* was unaffected by temperature, despite a trend for ~30% higher consumption in controls (Figure 3a; Table S4a). There were no differences in consumption between species in C and W, however *Ecklonia*'s consumption was ~20%–40% higher than *Sargassum* in MHW and MHW + V (Figure 3a; Table S4a). Algal weight changes in procedural controls without urchins were unaffected by temperature/species (Figure S5; Table S4c).

3.3 | Microbiota-herbivory relationships

Ecklonia's microbial community dissimilarity was positively related with differences in consumption between temperature treatments (Figure 3b; $\rho=0.9$, Mantel test: $z=6786$, $p<.04$). The highest dissimilarities occurred between control and heatwave treatments for *Ecklonia*, which coincided with the largest differences in consumption (Figure S8; Table S2). Dissimilarities in *Sargassum*'s microbial community structure did not differ between temperature treatments (Figure S8; Table S2) and were not related with consumption (Figure 3c; $\rho=0.4$, $z=2127$, $p=.13$).

3.4 | Algal chemistry and bleaching

Temperature treatments had no effect on algal chemistry (Figure S9a–d; Table S5a–d). Carbon content and carbon-nitrogen ratio were higher for *Sargassum* than *Ecklonia* (Figure S9a,c; $F_{1,26}=10.4$ and $F_{1,19}=8.5$, respectively, $p<.01$, Table S5). *Ecklonia* phenolic content was unaffected by temperature (Figure S9d; Table S5). Tissue bleaching levels were generally low and did not differ among temperature treatments or algal species, with the

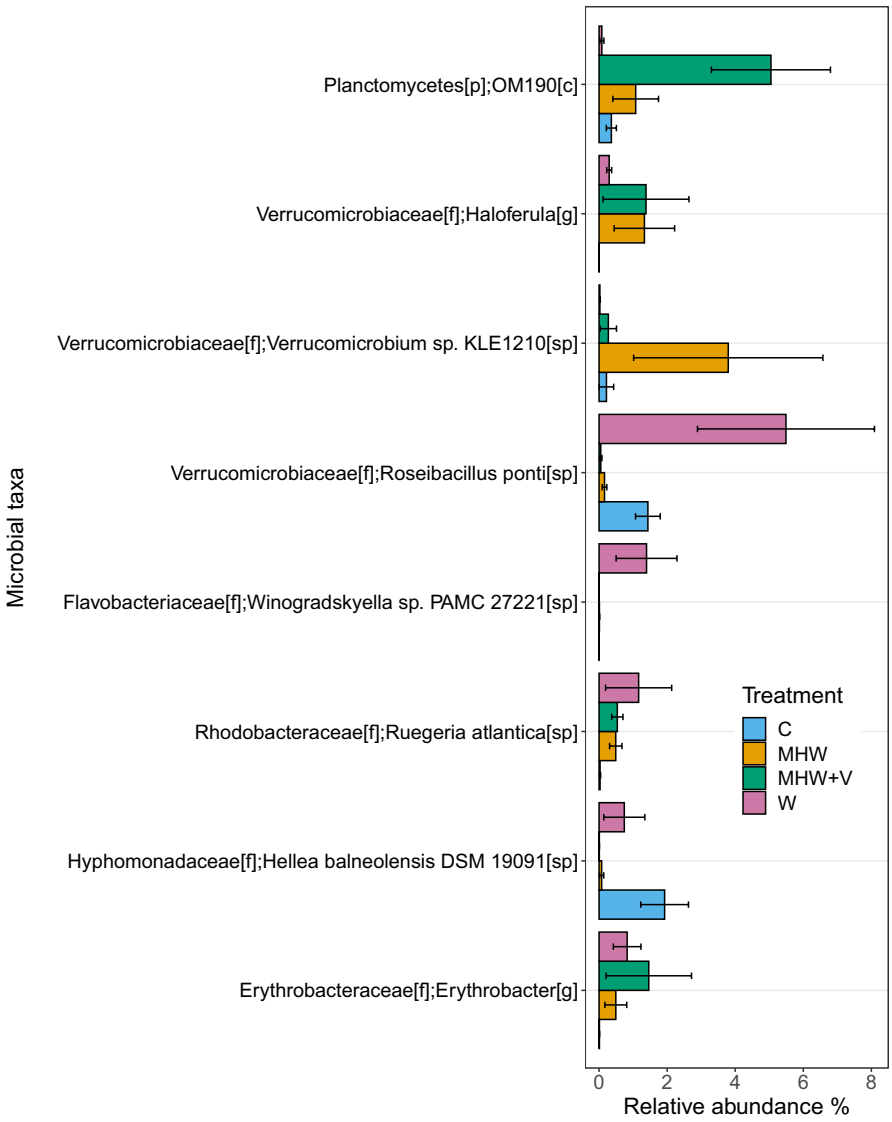

FIGURE 2 Relative abundances (%; mean ± SE) of abundant (>1%) microbial taxa associated with *Ecklonia* that differed among temperature treatments after 7 weeks. C, control/ambient ($n = 6$); [c], Class; [f], Family; [g], Genus; MHW, marine heatwave ($n = 5$); MHW + V, marine heatwave plus variability ($n = 4$); [p], Phylum; [sp], species; W, sustained warming ($n = 5$).

exception of *Sargassum* individuals in controls, with higher bleaching than all other treatments (Figure S9e; Table S5e).

4 | DISCUSSION

Sustained warming and MHWs can have strong effects on marine habitat-formers by altering interactions between species, which can lead to cascading effects on entire ecosystems (Hughes et al., 2017; Vergés et al., 2016; Wernberg, Bennett, et al., 2016). We showed that sustained warming and in particular MHWs affected microbiota associated with the kelp *Ecklonia radiata* and this was linked to enhanced consumption by the sea-urchin *Tripneustes gratilla* in the MHW treatment. In contrast, neither sustained warming nor MHWs influenced consumption of the fucoid *Sargassum linearifolium* or affected its microbiota. Given that microbial changes on *Ecklonia* occurred before the consumption trials, changes to host-associated microbiota in response to environmental changes may have been a mechanism underpinning the observed differences in herbivory among the different temperature treatments. Our results indicate that changes to complex, kelp-microbiome-herbivore interactions induced by sustained warming and, in particular, extreme variable MHWs may underpin the decline of *Ecklonia*, the dominant kelp along Australia's Great Southern Reef. The species-specificity of these changes suggests that such interactions may lead to transitions to more resistant, but ephemeral habitats such as *Sargassum* forests.

Ecklonia's consumption by *Tripneustes* was highest in the MHW + V treatment, which characterises MHW with fluctuating temperature anomalies over a prolonged period (Hobday et al., 2016), particularly in coastal areas (Schaeffer & Roughan, 2017). It is unlikely that differences in herbivory were solely caused by the impacts of higher cumulative temperature of the MHW + V treatment compared with the MHW treatment with no prior temperature fluctuations (97 vs. 85 degree-days), as the sustained warming treatment (W; 96 degree-days) experienced similar degree-days warming to the MHW + V treatment yet did not result in similar herbivory levels. We speculate that the

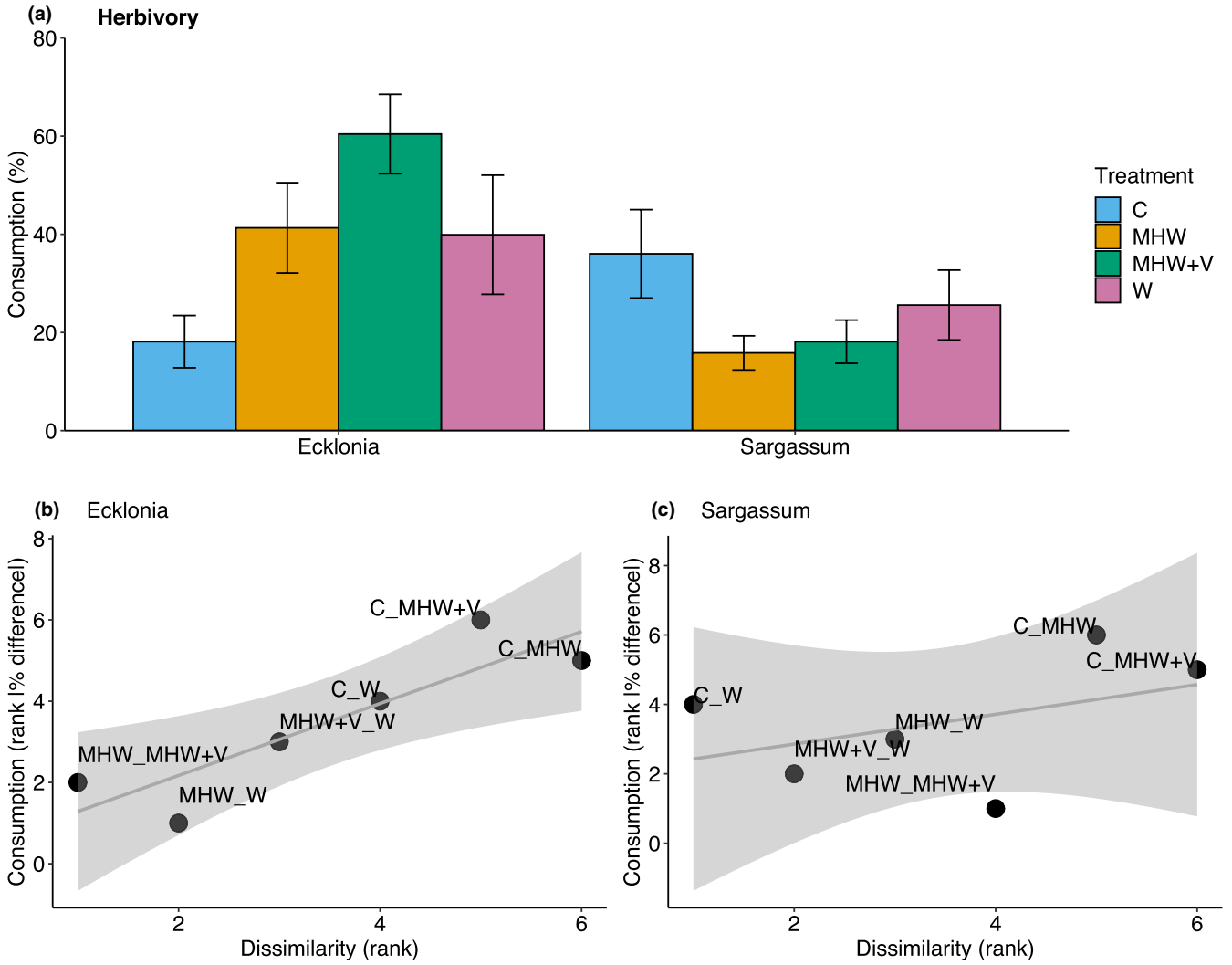

FIGURE 3 (a) Sea-urchin consumption of *Ecklonia* or *Sargassum* ([(weight$_{start}$ − weight$_{end}$)/weight$_{start}$] × 100; means ± SE) after exposure of algae to temperature treatments for 7 weeks. (b), (c) Relationships between ranked microbial community Bray-Curtis dissimilarities and absolute differences in % algal tissue consumed among each temperature treatment pair (paired treatments separated by "_") for *Ecklonia* (b) or *Sargassum* (c). Line = predicted values from linear model; grey area = 95% CI. C, control/ambient ($n = 10$); MHW, marine heatwave (*Ecklonia* $n = 10$, *Sargassum* $n = 9$); MHW + V, marine heatwave plus variability (*Ecklonia* $n = 6$, *Sargassum* $n = 10$); W, sustained warming (*Ecklonia* $n = 7$, *Sargassum* $n = 9$).

initial heat-spikes may have stressed *Ecklonia* before reaching the heatwave maximum, making it less resistant. Although sustained warming and MHWs are both important drivers of change in marine ecosystems (Smale et al., 2019; Vergés et al., 2016), the differences in kelp consumption between these temperature stressors provide further evidence that their effects are likely to differ. Our results suggest that short-term experiments focused on predicted, sustained ocean conditions are likely to underestimate effects in kelp-dominated systems.

Because we were interested in effects of warming on algal susceptibility to herbivory, rather than on the herbivores and their feeding rates or capacity per se, urchins were not exposed to the same temperature treatments as the seaweeds. Warmer temperatures can, however, increase the metabolism of herbivores and, in turn, enhance consumption rates (Burnell et al., 2013; Provost et al., 2017). It is therefore possible that seaweed consumption by urchins under warming or MHW treatments would have been even higher than observed here.

Host-associated microbiota play important roles in the condition and performance of seaweeds, and it has been argued that both components (host plus its microbiota) need to be studied together as holobionts to improve our ecological understanding and management of these systems (Wernberg et al., 2019; Wood et al., 2019). Disruptions of seaweed-associated microbiota in response to environmental stressors can result in host tissue degradation (Qiu et al., 2019) and enhanced herbivory (Campbell et al., 2014). *Ecklonia*'s surface-associated microbiota was affected by all warming treatments, although the greatest effect was caused by MHWs. Kelp microbiota in the warming and MHW treatments had higher relative abundances of taxa previously associated with bleached/

tissue-degraded kelp in field surveys (Marzinelli et al., 2015, 2018). However, bleaching levels in this experiment were low (<25%) and generally did not differ among temperature treatments or species. This suggests that some of these microbial taxa may affect tissue integrity in other ways. For example, taxa within the phylum Bacteriodetes, the class Gammaproteobacteria, the families *Rhodobacteraceae* and *Flavobacteriaceae* and the species *Vibrio* are known to produce enzymes associated with cell-wall degradation (Goecke et al., 2010) and can degrade polysaccharides, and members of the phylum Planctomycetes are considered important in the ocean carbon-cycle because they can degrade sulfated polysaccharides on algal cell walls (Bengtsson & Øvreås, 2010). Some of these taxa and members of the family *Saprospiraceae* have been associated with tissue degradation and warming in *Ecklonia* in mesocosms and in the field (Marzinelli et al., 2015; Qiu et al., 2019). The increase in the relative abundances of these microorganisms associated with the likely potential of cell-wall and polymer degradation could therefore partially explain why *Ecklonia* was consumed more in all warming treatments. We note, however, the limitations in accurately assigning taxonomy using amplicon sequencing, particularly at the genus/species level. Other sequencing methods and/or isolation of these taxa may help with identification to finer taxonomic levels.

Warming and other stressors can alter algal nutritional quality and reduce chemical defences, which could have led to differences in consumption irrespective of microbial changes (Steinberg, 1985, 1988). Differences in nitrogen availability—an essential, often limiting macronutrient for herbivores (Mattson, 1980)—could lead to differences in consumption. While some Australian herbivores are insensitive to variation in phenolic levels in brown algae (Steinberg & van Altena, 1992), higher phenolic concentrations can reduce kelp grazing, and warming could cause algae to shift resource allocation away from the production of these secondary metabolites, increasing vulnerability to herbivores (Steinberg, 1985, 1988). Carbon-nitrogen ratios and phenolic concentrations in our experiment were unaffected by temperature, reinforcing the idea that kelp consumption may have been mediated by microbial changes, not by changes in the chemical composition of the kelp itself. Further investigations assessing tissue integrity and composition (e.g. levels of iodine and polysaccharide compounds) coupled with experimental manipulations of microbiota are necessary to better understand the relationship between microbial changes and host consumption observed here.

In contrast to *Ecklonia*, there were no effects of warming and MHWs on *Sargassum*'s microbiota and consumption, suggesting higher resistance to temperature/herbivory. To our knowledge, *Sargassum linearifolium*'s thermal threshold is unknown. However, other temperate *Sargassum* species have a higher thermal threshold than *Ecklonia* (Wernberg, de Bettignies, et al., 2016), which encompass the temperatures experienced in this experiment. *Sargassum* maintained higher microbial richness and diversity than *Ecklonia*, potentially influencing its resistance to warming and/or its palatability. Independent of the mechanism, lower susceptibility to grazing and a life history that allows persistence through stress and rapid regeneration (Loffler & Hoey, 2018) and widespread dispersal could potentially lead to increases in abundances of *Sargassum* on temperate reefs as dominant kelp species decline. This may help preserve some of the ecosystem functions provided by forest-forming seaweeds in temperate reefs (Vergés et al., 2019), although other stressors such as acidification may impact *Sargassum* in the future (Poore et al., 2013).

Our study adds to the growing body of evidence showing that extreme events such as MHWs may become primary drivers of rapid ecological (Oliver et al., 2019; Smale et al., 2019) and evolutionary (Coleman et al., 2020; Coleman & Wernberg, 2020; Gurgel et al., 2020) change in future oceans. Indirect impacts of extreme events on temperate reefs are likely to result in continued kelp loss and transitions to new ecosystem states. Understudied seaweed-microbiota-herbivore interactions are a potential mechanism underpinning these changes that can manifest in species- and context-specific ways that may redefine temperate ecosystems under future climates. Understanding the interactive role of microorganisms (Wood et al., 2019) and exploring transformative methods to harness microbial interactions (Coleman & Goold, 2019; Trevathan-Tackett et al., 2019) will be vital for ensuring persistence of kelp forests into the future.

AUTHOR CONTRIBUTIONS

LCC, AV, SCS, MAC, TW, AHC, PS, PCH, MR and EMM conceived the ideas. LCC, AV, SCS, MAC, TW and EMM designed the experiment. LC, AV, SCS, MAC and EMM ran the experiment with assistance of SD and collected the data. LCC, TT and EMM analysed the data. LCC, AV and EMM led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data available through the Dryad repository: https://doi.org/10.5061/dryad.vhhmgqns7. This is the Reviewer sharing link while data is curated/becomes fully available: https://datadryad.org/stash/share/v19zzgb4OUuwIMP29nt3AMGbztTLHF9LSpQfuvzKZDY.

BENEFIT-SHARING STATEMENT

Benefits from this research accrue from sharing of research and development results.

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SUPPORTING INFORMATION

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