Received: 28 October 2022 | Revised: 23 August 2023 | Accepted: 30 August 2023

DOI: 10.1111/mec.17124

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Life-stage specificity and cross-generational climate effects on the microbiome of a tropical sea urchin (Echinodermata: Echinoidea)

Emma Marangon[1,2,3] | Sven Uthicke[2] | Frances Patel[2] | Ezequiel M. Marzinelli[4,5] | David G. Bourne[1,2,3] | Nicole S. Webster[2,6,7] | Patrick W. Laffy[2,3]

[1]College of Science and Engineering, James Cook University, Townsville, Queensland, Australia

[2]Australian Institute of Marine Science, Townsville, Queensland, Australia

[3]AIMS@JCU, Townsville, Queensland, Australia

[4]School of Life and Environmental Sciences, The University of Sydney, Sydney, New South Wales, Australia

[5]Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, Singapore

[6]Australian Centre for Ecogenomics, University of Queensland, Brisbane, Queensland, Australia

[7]Australian Antarctic Division, Kingston, Tasmania, Australia

Correspondence
Emma Marangon, College of Science and Engineering, James Cook University, Townsville, Qld, Australia.
Email: emma.marangon@my.jcu.edu.au

Funding information
Australian Institute of Marine Science

Handling Editor: Kayla King

Abstract

Microbes play a critical role in the development and health of marine invertebrates, though microbial dynamics across life stages and host generations remain poorly understood in most reef species, especially in the context of climate change. Here, we use a 4-year multigenerational experiment to explore microbe–host interactions under the Intergovernmental Panel on Climate Change (IPCC)-forecast climate scenarios in the rock-boring tropical urchin *Echinometra* sp. *A*. Adult urchins (F_0) were exposed for 18 months to increased temperature and pCO_2 levels predicted for years 2050 and 2100 under RCP 8.5, a period which encompassed spawning. After rearing F_1 offspring for a further 2 years, spawning was induced, and F_2 larvae were raised under current day and 2100 conditions. Cross-generational climate effects were also explored in the microbiome of F_1 offspring through a transplant experiment. Using 16S rRNA gene sequence analysis, we determined that each life stage and generation was associated with a distinct microbiome, with higher microbial diversity observed in juveniles compared to larval stages. Although life-stage specificity was conserved under climate conditions projected for 2050 and 2100, we observed changes in the urchin microbial community structure within life stages. Furthermore, we detected a climate-mediated parental effect when juveniles were transplanted among climate treatments, with the parental climate treatment influencing the offspring microbiome. Our findings reveal a potential for cross-generational impacts of climate change on the microbiome of a tropical invertebrate species.

KEYWORDS
climate change, coral reefs, development, microbe, sea urchin, transgenerational acclimatization

1 | INTRODUCTION

Microbial symbionts play a critical role in host health, contributing to host metabolism, immunity and development (Cavalcanti et al., 2020; McFall-Ngai, 2014; Robbins et al., 2021; Schuh et al., 2020; Weiland-Bräuer et al., 2020). In many animals, development and major life cycle transitions are influenced by the host-associated microbiome through interactions with regulatory networks at the base of these developmental processes (Russell & Castillo, 2020). In marine invertebrates with a biphasic life cycle, bacteria can provide the host with critical compounds necessary for regulating morphogenetic changes and completing the animal's life cycle (Cavalcanti et al., 2020; Song et al., 2021; Weiland-Bräuer et al., 2020). To maintain these crucial host–microbe relationships, key microbial taxa need to be faithfully acquired at each generation through vertical or horizontal transmission. In the sponge *Amphimedon queenslandica*, for example, vertical transmission from adult to embryos ensures the acquisition of three *Proteobacteria* taxa that are essential for settlement and metamorphosis (Fieth et al., 2016; Song et al., 2021). Instead, relationships like the squid-*Vibrio* symbiosis rely on the acquisition of mutualistic microbial symbionts from the surrounding seawater for the formation of functional organs, through complex host–symbiont interactions resulting in the monospecific colonization of *Vibrio fischeri* (Koropatnick et al., 2004; McFall-Ngai, 2014).

Following the establishment of host–microbe associations, restructuring of the microbiome may occur throughout ontogeny (Bernasconi et al., 2019; Carrier & Reitzel, 2019; Damjanovic et al., 2020; Fieth et al., 2016). These changes are species-specific; some coral larvae harbour less-diverse microbial communities than their early juvenile stages (Bernasconi et al., 2019), while some echinoid species are characterized by a decrease in microbial diversity from early development to juvenile stages (Carrier & Reitzel, 2019). Characterizing host–microbe relationships across life history stages is essential to better understand the role of microbes in host development and fitness, and identifying the impact of climate change on these functionally important microbial partnerships is critical.

In the marine environment, climate change not only compromises host fitness through physiological and behavioural impairments (Marangon et al., 2020; Pörtner & Farrell, 2008) but may also affect animal–microbe interactions. Ocean warming (OW) and acidification (OA) have been shown to drive compositional and functional shifts in the microbiome of marine invertebrates (Botté et al., 2019, 2020; Webster et al., 2016), with both positive and negative consequences for animal health depending on species, environmental stressors and environmental gradients (Egan & Gardiner, 2016; Marangon et al., 2021; Pita et al., 2018; Posadas et al., 2022; Ziegler et al., 2017). For example, a metagenomic study comparing the microbiome of the sponge *Coelocarteria singaporensis* between a CO_2 seep and an adjacent control reef found an enriched potential for energy-efficient archaeal carbon fixation at the seep, suggesting that microbes may contribute to host OA tolerance in this species (Botté et al., 2019). In contrast, detrimental changes in the microbial community associated with the reef sponge *Stylissa flabelliformis* were observed at both the compositional and functional levels under heat stress, including a reduced capacity for ammonia detoxification (Botté et al., 2023). Despite climate-induced changes in the microbiome structure being widely observed in marine organisms, other host-associated microbial communities are stable upon environmental disturbances (Grottoli et al., 2018; Luter et al., 2020; Webster et al., 2016). This stability may reduce the occurrence of diseases through pathogen exclusion, though may also preclude beneficial microbial changes from occurring (Voolstra & Ziegler, 2020). Given the importance of host-associated microbes for the stability of ecological community structure and broader ecological processes that underpin ecosystem function and services (Bourne et al., 2009; Pita et al., 2018), it is crucial to understand microbiome changes under OW-OA.

Host–microbe responses to altered environmental conditions may vary when the stressors are experienced over multiple generations (Donelson et al., 2012; Karelitz et al., 2020; Uthicke et al., 2021). Emerging evidence suggests that heritability of beneficial alterations to the microbiome may facilitate host acclimatization over generations (Baldassarre et al., 2022). For example, it was recently shown that sea anemone juveniles from temperature-acclimated F_0 females were characterized by high thermal tolerance and were associated with specific members from the parental acclimated microbiota (Baldassarre et al., 2022). Similarly, Luter et al. (2020) confirmed that transgenerational effects of climate treatments can alter the microbiome of the sponge *Carteriospongia foliascens*, with specific microbial taxa exclusively identified in recruits whose parents were pre-exposed to climate stressors. Hence, characterizing the contribution of microbes to transgenerational plasticity is required for generating realistic predictions of organisms' responses to future climate and informing reef conservation strategies.

To explore cross-generational effects of climate change on reef host–microbe dynamics, we employed a tropical sea urchin species as study system (*Echinometra* sp. *A*). Sea urchins are an emerging model for investigating animal–microbe symbiosis (Carrier et al., 2021), showing a re-shaping of the bacterial communities during major developmental transitions, from pelagic larvae to benthic juveniles and adults, with microbes inferred to contribute to larval immunity and nutrition in some species (Carrier et al., 2021; Carrier & Reitzel, 2019, 2020; Schuh et al., 2020). Urchins are susceptible to changing climate, in particular, during larval life stages (Byrne & Przeslawski, 2013), though little is known about the effects of OW and OA on microbial dynamics in adult urchins and early developmental stages (Brothers et al., 2018; Carrier & Reitzel, 2020; Ketchum et al., 2021; Webster et al., 2016). Along with an important role in reef resilience through its grazing activity, *Echinometra* sp. *A* is widely distributed in reef ecosystems (McClanahan & Muthiga, 2013) and characterized by relatively short-generation times, making it an ideal model for our multigenerational experiment. In this study, we employed 16S rRNA gene sequencing analyses to (1) characterize urchin ontogenetic microbial changes (aim 1);

(2) determine the combined effect of OW and OA on these host–microbe relationships (aim 2); and (3) identify transgenerational effects of climate treatments on the urchin microbiome through a transplant experiment (aim 3). Our 4-year multigenerational study demonstrated that urchin microbial communities differ across life stages, and although combined OW/OA can alter these associations, life-stage specificity was maintained under climate stress. Furthermore, transgenerational effects of climate treatments appeared to occur in *Echinometra* sp. *A* microbiome, with potential implications for host health.

2 | MATERIALS AND METHODS

2.1 | Urchin collection and experimental setup

Echinometra sp. *A* adults (F_0) were collected from the central Great Barrier Reef (GBR; Trunk Reef; 18.3188° S, 146.8662° E; Permit G12/35236.1) in February 2016 and transported to the National Sea Simulator (SeaSim) at the Australian Institute of Marine Science (AIMS). The experimental setup is described in detail by Uthicke et al. (2020). Briefly, urchins were randomly distributed across nine outdoor mesocosm tanks of 1260 L each ($n = 22–24$ per tank), at ambient conditions (28°C). Treatment conditions were gradually reached over a 4-week period, with target temperature and pCO_2 reflecting present-day conditions, predictions for year 2050 (+1°C; ~685 ppm) and year 2100 (+2°C; ~940 ppm) according to an RCP 8.5 scenario (Collins et al., 2013; Meinshausen et al., 2011), resulting in three mesocosms under each climate treatment (i.e. ambient, year 2050, year 2100). Ambient temperature mimicked natural fluctuations on the central GBR based on the daily average sea surface temperature at Davies Reef (temperature from 1991 to 2012; Australian Institute of Marine Science, 2020), and ambient pCO_2 reflected present-day conditions (~400 ppm; Uthicke, Furnas, et al., 2014). pCO_2 levels of all treatments simulated natural pCO_2 variations observed on the GBR (daily ±60 ppm fluctuations; Karelitz et al., 2020). Temperatures and pCO_2 levels were finely managed by three Programmable Logic Controllers (PLC), which were connected to temperature sensors and gas equilibration systems with Telaire CO_2 sensors. Urchins fed ad libitum on crustose coralline algae (CCA) and biofilms throughout the experimental period.

2.2 | F_0 urchin spawning and F_1 urchin larvae

F_0 adults were spawned after 18-month exposure to treatment conditions (15 November 2017) by injecting 0.5 mL of 0.5 M KCl (Uthicke et al., 2020). Sperm from multiple adults of the same mesocosm was pooled (1 µL of sperm per individual into 20 mL Filtered Seawater [FSW]) and 1 mL of diluted sperm was used to fertilize eggs from females of the respective mesocosm (spawned eggs pooled in 1 L FSW). Fertilization was accomplished in each mesocosm except for mesocosm #7 (2050 conditions) due to low abundance of individuals in this system. Fertilization success was high across treatments, as indicated by the presence of a fertilization envelope on 90%–100% of eggs (Uthicke et al., 2020). Cultures were reared in Schott bottles under parental treatment conditions (1 L of 0.5 µm FSW; $n = 1$ per treatment) at a density of ~5 embryos per mL. pCO_2 levels were stable over the incubation period (Karelitz et al., 2020), and target temperatures were maintained by placing Schott bottles on rollers in temperature-controlled water baths. Seawater of the larval cultures was changed every second day with FSW at the same treatment conditions, and feeding was carried out twice per day (*Chaetoceros muelleri*; 5000 cells/mL from 2 to 10 days, 8000 cells/mL thereafter). Larvae competent to settlement were transferred to 0.5 L aquaria under ambient conditions 19 and 26 days after fertilization, where settlement was induced by placing pre-conditioned aragonite plugs with CCA into the aquaria. Metamorphized juveniles were transferred to the mesocosm systems between 4 and 6 days post-settlement.

2.3 | F_1 urchin growth

In the mesocosms, juveniles were raised in 50 mL polypropylene centrifuge tubes covered with 150 µm mesh (40 juveniles per tube, $n = 2$ tubes per mesocosm) and fed on CCA-encrusted aragonite plugs. In addition to rearing juveniles in the mesocosms under the respective parent treatments, juveniles from adults held at ambient conditions were also reared under 2050 and 2100 conditions, and juveniles from adults at 2050 conditions were also raised under 2100 conditions generating a transplant experiment (Figure 1). For logistic reasons, 6-month juveniles (F_1) were transferred to indoor 50 L flow-through aquaria under the respective treatment conditions ($n = 3$ tanks under ambient conditions; $n = 2$ tanks for the other climate treatments), where they fed ad libitum on CCA and reared until maturity (~25 months). In these aquaria, temperature and pCO_2 levels were finely controlled by a SeaSim computer system, and temperature was further stabilized by temperature-controlled water baths. Light simulated the natural 12 h light/dark cycle (6:00 AM–6:00 PM) with 4-h ramping at sunrise and sunset (AquaIllumination SOL LED lights; light 50 µE). At the end of October 2018 (~10.5-month juveniles), a pathogen-induced disease outbreak affected urchin survival across all treatments, with ambient–ambient juveniles showing a significantly higher survival rate than the other treatment groups (see Uthicke et al., 2021 for detailed information on the outbreak).

2.4 | F_1 urchin spawning and F_2 urchin growth

F_1 urchins reached maturity after >2 years of exposure to future climate scenarios. Urchins under ambient–ambient and 2100–2100 were spawned on 15 January 2020, following the same methodology as described above for F_0 adults. F_2 larvae were maintained

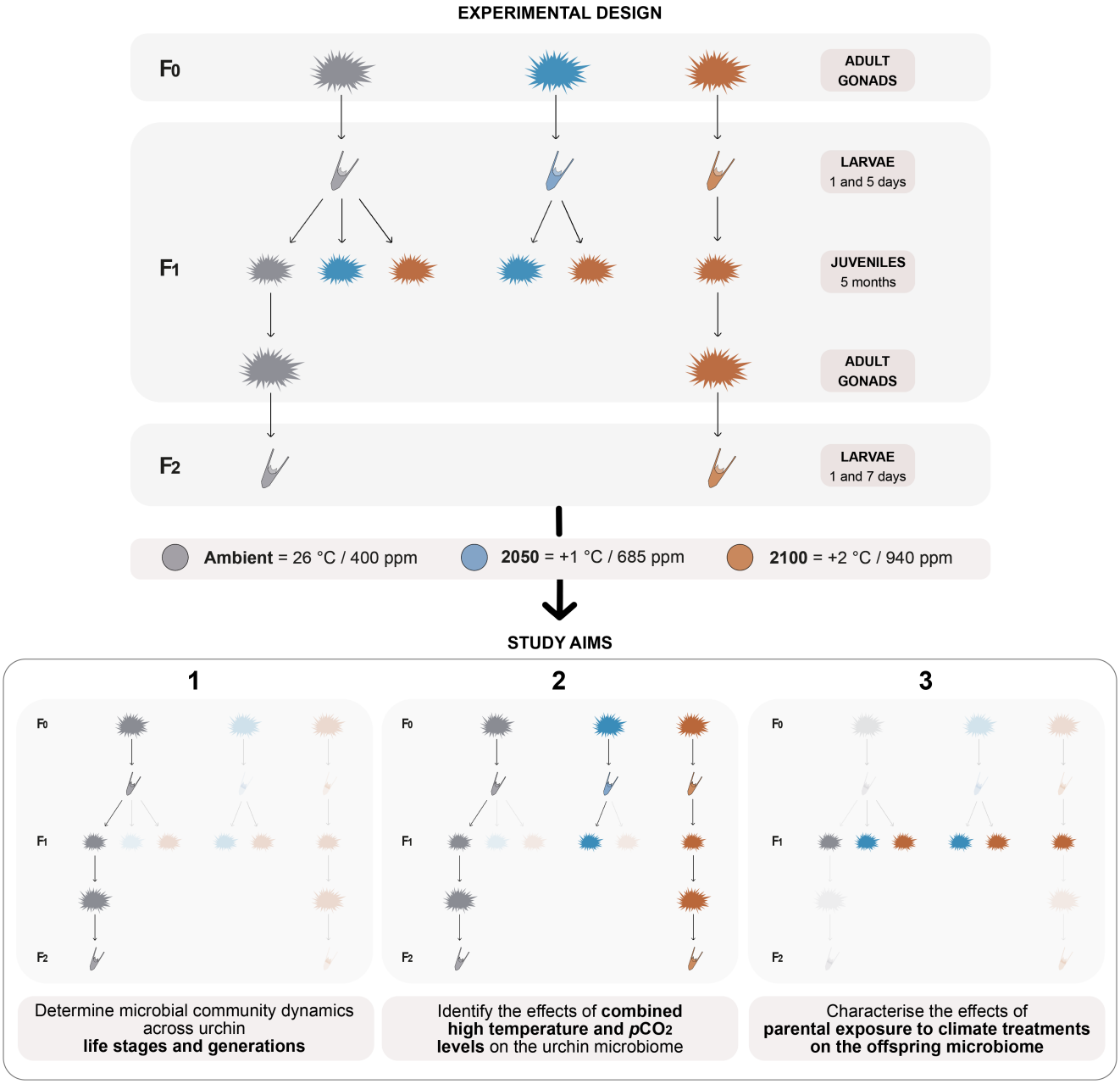

FIGURE 1 Experimental design and research aims of the transgenerational urchin experiment. Ambient temperature mimicked natural fluctuations of the central GBR, with an average of 26°C. Samples used for addressing each study aim are highlighted in the illustration. Sample replication: F_0 adult gonads $n \geq 5$; F_1 larvae $n = 3$; F_1 juveniles $n \geq 8$; F_1 adult gonads $n \geq 3$; F_2 larvae $n = 2$. Details in Table S1.

under parental conditions in 1 L Schott bottles ($n = 2$ per treatment) on rollers in temperature-controlled water baths.

2.5 | Urchin sampling

To assess microbial community dynamics across urchin life stages, generations and climate treatments, samples were collected from F_0 adult gonad tissue, most major life stages in F_1 (1-day larvae, 5-day larvae, 5-month juveniles, adult gonad) and F_2 larvae (1- and 7-day). Following F_0 spawning, six female adults were dissected per treatment (ambient, 2050, 2100) and gonads were sampled using sterile tweezers and scissors. Samples were rinsed with 0.2 μm filtered-sterilized seawater, snap frozen in liquid nitrogen and stored at −80°C until DNA extraction. F_1 larvae were sampled 1 day and 5 days after fertilization under ambient, 2050 and 2100 conditions. First, 500 mL of larval culture per each treatment was filtered through 50 μm membrane and rinsed in 0.2 μm filtered-sterilized seawater. Larvae within each treatment were then distributed into three aliquots, concentrated into a pellet by centrifugation to remove excess seawater, snap frozen and stored at −80°C until DNA extractions. Five-month juveniles (F_1)

were sampled under all treatment conditions ($n = 6$ treatments, Table S1), snap frozen in liquid nitrogen and stored at −80°C until processing. For F_1 adults, gonads were sampled as described per F_0 gonads. F_2 larvae were sampled 1 and 7 days after fertilization under ambient and 2100 conditions following the same sampling procedure as per F_1 larvae. Seawater samples were collected as environmental control in parallel with sampling of F_0 gonad adults, F_1 larvae, F_1 juveniles and F_1 gonad adults. For each seawater sampling time, 1 L seawater was collected from each tank or larval culture and filtered through 0.2 µm sterivex filters (Millipore) and stored at −80°C until DNA extraction.

2.6 | DNA extraction and sequencing

Genomic DNA was extracted from major life stages across the two generations and seawater samples (see Figure 1; $n = 136$, details in Table S1). DNA extractions were undertaken using the DNeasy PowerSoil Pro kit (QIAGEN) following the Manufacturer's protocol, with samples homogenized using the FastPrep-24 5G instrument (MP Biomedicals). DNA purity was examined using the Nanodrop 2000 spectrophotometer (Thermo Scientific), and DNA concentration was quantified using the Qubit 3.0 Fluorometer (Life Technologies) and then standardized within each sample type. The V4 variable region of the 16S rRNA gene was amplified with the primers 515F (Parada et al., 2016) and 806rB (Apprill et al., 2015), for targeting bacteria and archaea. PCRs were performed using the AmpliTaq Gold 360 Master Mix (Thermo Fisher Scientific), 0.2 µM primers (Sigma-Aldrich) and 3.1 µM $MgCl_2$ (QIAGEN). PCR conditions were the following: initial denaturation at 95°C for 10 min, 30 cycles at 95°C for 30 s, 55°C for 1 min, 72°C for 30 s, and final extension at 72°C for 7 min. PCR amplification products were analysed by agarose gel electrophoresis and then stored at −20°C before submission to the Ramaciotti Centre for Genomics (UNSW, Australia) for completion of the 16S library preparation and sequencing on the Illumina MiSeq platform (2 × 250 bp paired-end reads). In addition to urchin and seawater samples, three blank DNA extractions were sequenced to detect any potential reagent contamination.

2.7 | 16S rRNA gene data processing and statistical analyses

Demultiplexed paired-end reads were analysed in QIIME 2 (v 2020.8; Bolyen et al., 2019). First, poor quality reads and chimeras were removed using DADA2 (Callahan et al., 2016). High-quality sequences were then grouped into amplicon sequence variants (ASVs) based on 100% sequence similarity in DADA2 (Callahan et al., 2016). Taxonomic assignment was performed using a naïve Bayes classifier trained with the feature-classifier plugin using the primer set 515F/806rB on the SILVA 132 database (Quast et al., 2012). FastTree method was used to build phylogenetic relationships. ASVs table, taxonomic table, phylogenetic tree and metadata were imported into R (v 4.0.3; R Core Team, 2020) for data analyses. Contaminants were identified and removed using the R package decontam (Davis et al., 2018) using stringent thresholds (p threshold = .5; $n = 32$ contaminants identified, Table S2). Reads assigned to Chloroplasts, Eukaryotes and Mitochondria, as well as singletons and low-quality samples (<8400 reads; $n = 2$, F_1 juvenile from 2050 treatment and F_0 gonads from ambient conditions) were removed prior to the analyses.

To test for differences in microbial community structure between host life stages, generations and climate treatments (see below), we calculated beta-diversity on non-rarefied data, with Bray–Curtis dissimilarities applied on square-root transformed data normalized using proportions (McKnight et al., 2019). For beta-diversity analyses, rare ASVs with an overall relative abundance <0.001% were removed (34,412 ASVs). Analyses include Non-metric Multidimensional Scaling (NMDS, 'phyloseq package'; McMurdie & Holmes, 2013), Permutation Multivariate Analysis of Variance using 10,000 permutations (Adonis, 'vegan package'; Oksanen et al., 2020), Multivariate Homogeneity of Group Dispersions ('vegan package'; Oksanen et al., 2020) and pairwise comparisons using Benjamini–Hochberg correction ('RVAideMemoire package'; Hervé, 2021). Differential abundance analyses ('DESeq2 package'; Love et al., 2014) were run on filtered data using $p = .01$, and significant ASVs that were present across at least 50% of the samples in one of the compared groups were identified as differentially abundant between groups. Furthermore, a 1% mean relative abundance cut-off was included when determining differentially abundant ASVs across life stages (ambient conditions). Principal component analysis (PCA) and multilevel sparse Partial Least Squares–Discriminant Analysis (multilevel sPLS-DA) were performed in the mixOmics package (Rohart et al., 2017) on the same pre-processed data as above but applying a centred log-ratio transformation (offset by +1; Chua et al., 2017). To determine which ASVs were shared between life stages/generations/climate conditions, ASVs present in at least 50% of samples within each group were analysed using the function 'ps_venn' in 'MicEco' (https://github.com/Russel88/MicEco) on rarefied data (8400 sequences).

Analyses of alpha diversity were based on Shannon diversity index calculated on a dataset rarefied to 8400 sequences ('phyloseq package'; McMurdie & Holmes, 2013), with the effect of host life stages, generations and climate treatments tested through linear mixed effect models ('glmmTMB package'; Brooks et al., 2017) and pairwise comparisons with Benjamini–Hochberg correction ('emmeans package'; Lenth, 2022). The assumption of normality and homogeneity of variances was tested both visually and through DHARMa residual diagnostics ('DHARMa package'; Hartig, 2021). Additional alpha diversity measures (i.e. Pielou's evenness, Simpson's diversity, richness, Faith's phylogenetic diversity) were calculated using the phyloseq (McMurdie & Holmes, 2013) and btools (https://github.com/twbattaglia/btools/) packages. Data manipulation was performed using the dpylr,

forcats and tidyr packages (Wickham et al., 2019), graphs were generated using ggplot2 and RColorBrewer (https://github.com/cran/RColorBrewer) packages, and illustrations were further stylized in Affinity Designer. All analyses were performed in R (v 4.0.3; R Core Team, 2020).

2.7.1 | Aim 1: Microbial community across urchin life stages and generations under ambient conditions

To examine microbial community dynamics across life stages and generations, we first investigated urchins under ambient conditions (see Figure 1, aim 1). The interaction between life stage (fixed factor with four levels: 'adult gonad', 'larvae [1-day and older larvae were pooled]', ' juvenile', 'seawater') and generation (fixed factor with three levels: 'F_0', 'F_1,', 'F_2') was explored using Permutation Multivariate Analysis of Variance (Adonis, 'vegan package'; Oksanen et al., 2020) including tank as fixed factor fitted first in the model to account for tank-to-tank variation. The effect of age on the larval microbial community was assessed across generations using Permutation Multivariate Analysis of Variance (Adonis, 'vegan package'; Oksanen et al., 2020), with larval age (two levels: '1 day', '>1 day') and tank as fixed factors. To test for differences in Shannon diversity among life stages, linear mixed effect models ('glmmTMB package'; Brooks et al., 2017) were performed with life stage and generation tested as fixed effect (seven levels: 'F_0 adult gonad', 'F_1 larvae', 'F_1 juvenile', 'F_1 adult gonad', 'F_2 larvae', 'F_0 seawater', 'F_1 seawater') and tank as random, nested effect.

2.7.2 | Aim 2: Effects of combined OW and OA on the urchin microbiome

Effects of future climate scenarios predicted for years 2050 and 2100 on urchin microbial community structure (see Figure 1, aim 2) were investigated using Permutation Multivariate Analysis of Variance (Adonis, 'vegan package'; Oksanen et al., 2020), including climate scenario (three levels: 'ambient', '2050', '2100'), life stage (three levels: 'F_0 adult gonad', 'F_1 larvae [1- and 5- day larvae were pooled]', 'F_1 juvenile') and tank as fixed factors, as well as climate scenario × life stage. As F_1 adults were not reared under 2050 conditions, the effect of climate scenario (two levels: 'ambient', '2100'; fixed factor) on the microbiome associated with adult gonads (F_1) was tested separately. Climate effects on each F_1 larval stage (1- and 5-day) were also explored, with climate scenario (three levels: 'ambient', '2050', '2100') included as fixed factor. Finally, the effect of climate treatments on the seawater microbiome was tested, including climate scenario (three levels: 'ambient', '2050', '2100'), time point ('F_0 adult gonad', 'F_1 larvae', 'F_1 juvenile', 'F_1 adult gonad') and tank as fixed factors, as well as climate scenario × time point. Climate effects on F_2 larvae were not statistically investigated due to insufficient replication. Liner mixed effect models ('glmmTMB package'; Brooks et al., 2017) were used to test any climate treatment, life stage and generation effects on the Shannon diversity index.

2.7.3 | Aim 3: Effects of parental exposure to climate treatments on the offspring microbiome

Finally, transgenerational effects of climate change on the urchin microbiome were tested through a transplant experiment (see Figure 1, aim 3). Permutation Multivariate Analysis of Variance (Adonis, 'vegan package'; Oksanen et al., 2020) was performed to test any effect of the interaction between parental (F_0) climate treatment (three levels: 'ambient', '2050', '2100') and offspring climate treatment (three levels: 'ambient', '2050', '2100') on the 5-month juvenile microbiome, with tank included as fixed effect. Differences in alpha diversity (Shannon index) among juveniles exposed to the six reciprocal climate treatments were tested using linear mixed effect models ('glmmTMB package'; Brooks et al., 2017) with climate treatment as fixed factor and tank as random, nested effect. Multilevel sPLS-DA was run following the example Case Study Multilevel sPLS-DA: Vac18 available on the mixOmics website (http://mixomics.org/case-studies/multilevel-vac18-case-study/). For this analysis, ambient–ambient juveniles were excluded. Multilevel sPLS-DA is a supervised approach for data classification of repeated measures ('tank' in our study). Briefly, a sPLS-DA was run with 10 components and then tuned using the 'perf' and 'tune.splsda' ($n=3$ folds, $n=50$ repeat, Maximum Distance, Balanced Error Rate measure) functions to select the optimal number of components and variables.

3 | RESULTS

We performed a 4-year multigenerational experiment to characterize the combined effect of high temperature and pCO_2 levels on the microbial community dynamics in the tropical sea urchin *Echinometra* sp. *A* across major life stages (experimental design illustrated in Figure 1; replicates per life stage/treatment conditions listed in Table S1). A total of 4,767,687 high-quality 16S rRNA amplicon reads was obtained, with an average of 35,580 reads per sample ranging between 8495 and 104,419 reads. Following quality trimming, chimera removal and data filtration, a total of 40,599 ASVs were identified. Rarefaction curves based on sample diversity reached an asymptote, confirming sequencing depth for this study was adequate (Figure S1).

3.1 | Microbial dynamics across urchin life stages and generations under ambient conditions (aim 1)

The microbial community across life history stages and generations held under ambient conditions, including adult gonads (F_0 and F_1), larvae (F_1: 1- and 5-day; F_2: 1- and 7-day) and 5-month juveniles (F_1)

were characterized (Figure 1, aim 1). Although there were significant differences in dispersion (dispersion in adult gonads was lower than larvae and juvenile), NMDS and permutational multivariate analysis of variance revealed that microbial communities shifted significantly during developmental stages and between generations, with changes in the presence/absence of taxa as well as in their relative abundance (PERMANOVA, life stage×generation: pseudo-$F=4.1$, $p<.001$; Figures 2 and 3, Table S3). Each life stage (i.e. adult [gonad], larvae and juvenile) was significantly different from one another ($p<.001$), as well as from the seawater-associated microbial community ($p<.001$; Figure 2, Table S3). Age also affected the microbial community associated with urchin larvae, with 1-day larvae significantly different from older larvae (PERMANOVA, pseudo-$F=8.1$, $p<.001$; Figure 2, Table S3b). In addition to these microbial changes during host development, microbial community structure shifted across generations within adult gonads (PERMANOVA, $p=.002$; Figure 2, Table S3) and larvae (larval ages were pooled; PERMANOVA, $p=.03$; Figure 2, Table S3).

Overall, 23 microbial classes represented at least 1% mean relative abundance across life stages and generations, of which *Alphaproteobacteria* (*Proteobacteria*), *Deltaproteobacteria* (*Proteobacteria*), *Gammaproteobacteria* (*Proteobacteria*) and *Bacteroidia* (*Bacteroidetes*) were the only classes with >1% abundance in all life stages (Figure 3a). Some microbial classes were characteristic of specific life stages, for example, *Fusobacteriia*-affiliated sequences were highly abundant only in adult gonads (F_0: 14.3%, F_1: 13.5%), *Nitrososphaeria* in F_1 1d-larvae (8.2%) and *Oxyphotobacteria* in juveniles (7.5%). To better characterize the potential drivers of the differences among life history stages, we explored microbial dynamics at the family level (Figure 3b and Figure S2). The microbiome of adult gonads (F_0 and F_1) was primarily composed of Vibrionaceae (*Gammaproteobacteria*), Desulfobulbaceae (*Deltaproteobacteria*), Fusobacteriaceae (*Fusobacteriia*), Cryomorphaceae (*Bacteroidia*) and Prolixibacteraceae (*Bacteroidia*); and F_1 gonads showed a high relative abundance also in Kiritimatiellaceae (*Kiritimatiellae*)-affiliated sequences (Figure S2). In contrast, larval stages were dominated by Rhodobacteraceae (*Alphaproteobacteria*)- and Alteromonadaceae (*Gammaproteobacteria*)-affiliated sequences, constituting up to 27% and 32%, respectively, in mean relative abundances (Figure 3b). Other bacterial families were abundant at this life stage, such as Halomonadaceae (*Gammaproteobacteria*) in F_1 larvae, and Oleiphilaceae (*Gammaproteobacteria*) and Cellvibrionaceae (*Gammaproteobacteria*) in F_2 larvae (Figure S2). In juveniles, Rhodobacteraceae relative abundance was similar to the larvae life stage (11.9%), while Alteromonadaceae and Vibrionaceae represented 1.5% and 16.3% of the community, respectively (Figure 3b). The most abundant families in the urchin-associated tissue across life stages were a minor component of the seawater samples, indicating that urchin microbial communities are host-specific (Figure 3b and Figure S2), which was also confirmed by the small proportion of ASVs shared between life stages and seawater (Figure S3).

When exploring microbial community structure at the ASV level, DESeq analyses identified ASVs that were differentially abundant between life stages. In F_1 larvae, seven ASVs were present exclusively at 1- or 5-day, and sequences affiliated to the archaea *Nitrosopumilus* were significantly reduced from 1-day to older larvae (F_1; from 7.3% to 2.2%) (Figure 3d). Some ASVs were also differentially abundant between F_1 larvae and juveniles (Figure 3e). For example, one ASV assigned to *Ruegeria* constituted on average 2.1% of the microbial community in juveniles, though was absent in F_1 larvae; and *Alteromonas*-affiliated sequences showed about a 10-fold reduction

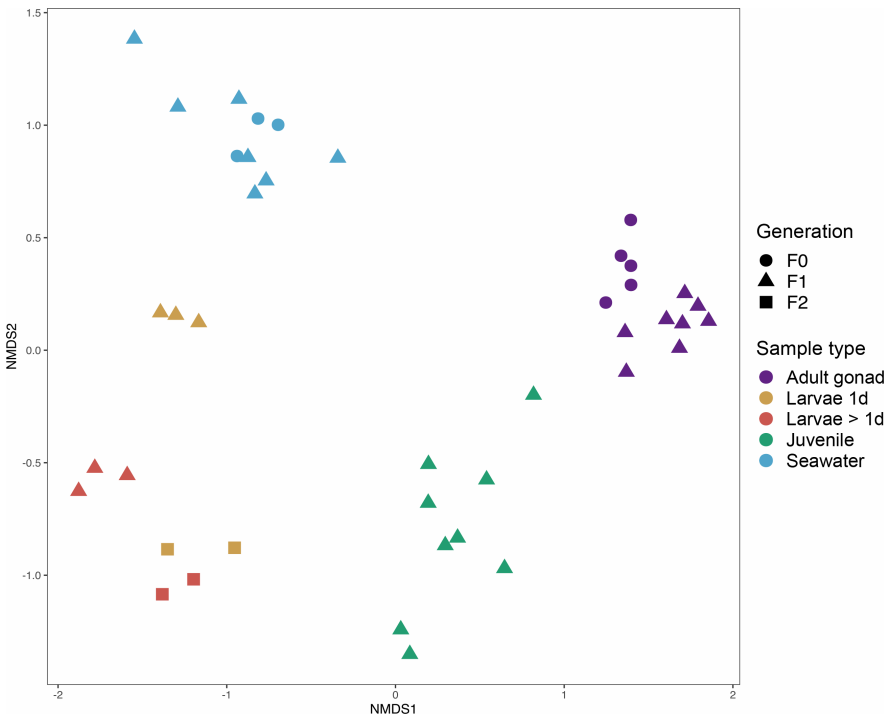

FIGURE 2 Non-metric Multidimensional Scaling (NMDS; stress = 0.13) based on Bray–Curtis dissimilarities on the microbial communities (ASVs level) associated with urchin adult gonad, juvenile, larvae (1 day; >1 day) and seawater across generations (F_0, F_1, F_2) under ambient conditions. Relative abundances were square-root transformed.

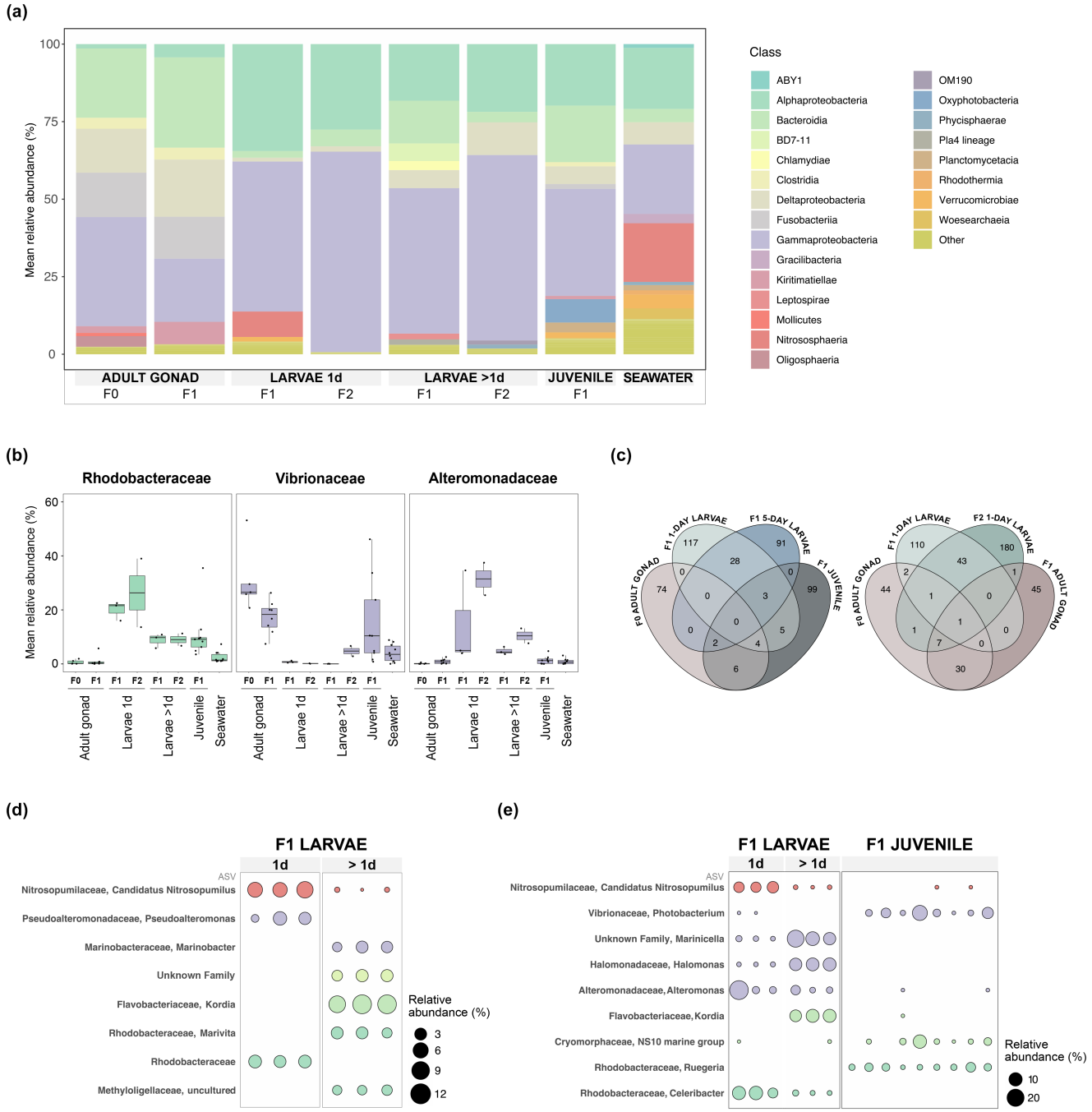

FIGURE 3 Urchin microbial community structure under ambient conditions. (a) Mean relative abundance of prevalent microbial classes (>1% relative abundance; relative abundance mean in larvae F_2 is based on two replicates/larval age) and (b) boxplot of dominant three families across sample types and generations (colours based on taxonomic classes, see a). Box = inter-quartile range (IQR), line in box = median, whiskers = minimum and maximum values not outliers (i.e. −/+1.5 × IQR). Seawater represents an environmental control, and we here show the seawater-associated microbial community pooled across time and generations. (c) Venn diagrams illustrating the number of shared ASVs between life stages and generations. Only ASVs present in at least 50% of the samples within each life stage/generation were considered; data were rarefied. (d) ASVs with significantly different relative abundances between 1- and 5-day F_1 larvae, and (e) larvae F_1 (pooled 1- and 5-day) and juvenile F_1 identified using DESeq analyses (1% mean relative abundance cut-off). Taxonomy is shown as Family and Genus, and colours are based on taxonomic classes, see (a).

in juveniles compared to F_1 larvae. This clear distinction between life history stages was also reflected in the low number of ASVs observed across all life stages, with few ASVs present in at least 50% of the samples and shared between developmental stages (Figure 3c; Table S4). Only four microbial taxa were shared between F_0 adult gonads and F_1 1-day larvae, which were not retained in older larvae (Figure 3c; Table S4). While a high number of ASVs ($n = 31$) were retained from 1-day to older larvae, only three of them remained

in association with the juveniles (Figure 3c; Table S4). These three ASVs were also present in F_2 larvae at both sampling times and were assigned to *Alcanivorax*, *Hyphomonas* and *Oleiphilus* strains. When comparing the microbial composition between generations, 44% of ASVs associated with F_0 adult gonads was also present in F_1 gonads, and 29% of the microbiome in F_1 1-day larvae was retained in the next generation (Figure 3c). Some ASVs associated with the urchin tissue were also present in the seawater, with the proportion of shared microbial taxa between urchin and seawater varying among life stages (Figure S3).

Alpha diversity based on Shannon index varied significantly across life stages but not generations under ambient conditions (Figure 4a, Table S5). Specifically, post-hoc comparisons revealed that juveniles (4.97 ± 0.77 SE) had significantly higher alpha diversity than F_1 larvae (3.83 ± 0.40 SE; $p = .002$), F_2 larvae (3.47 ± 0.32 SE; $p = .0005$), F_0 adult gonads (3.42 ± 0.38 SE; $p = .0002$) and F_1 adult gonads (3.30 ± 0.50 SE; $p < .0001$; Figure 4a, Table S5). Similar patterns were observed for Pielou's evenness, Simpson's diversity, richness (total number of ASVs) and Faith's phylogenetic diversity (Figure S4).

3.2 | Effects of combined OA and OW predicted for years 2050 and 2100 on the urchin microbiome (aim 2)

Urchins were reared under future RCP 8.5 climate scenarios predicted for years 2050 and 2100 (see Figure 1, aim 2; experimental design described in Uthicke et al., 2021) and the microbial community changes to OW/OA across host generations were assessed. NMDS visualization showed that the greatest difference in microbial communities was driven by life stage rather than combined temperature and pCO_2 levels (Figure 5a). However, PCA revealed that climate treatment had an effect on the microbial structure within life stages (Figure 5b) and permutational multivariate analysis of variance confirmed this climate effect on F_0 adult gonads and F_1 juveniles (PERMANOVA, life stage*climate scenario: pseudo-$F = 1.86$, $p = .01$), 1-day F_1 larvae (PERMANOVA, climate scenario: pseudo-$F = 3.38$, $p = .004$), 5-day F_1 larvae (PERMANOVA, climate scenario: pseudo-$F = 6.69$, $p = .003$) and F_1 adult gonads (PERMANOVA, climate scenario: pseudo-$F = 1.84$, $p = .028$, Table S6). Microbial community dispersion was significantly different across life stages. Post-hoc comparisons showed that the microbial community of adult gonads (F_0) reared under ambient conditions was significantly different from adult gonads under the 2050 treatment ($p = .01$; Figure 5b, Table S6), and juveniles (F_1) under ambient were different from juveniles under 2050 ($p < .001$) and 2100 ($p = .04$, Table S6) treatments.

Changes in relative abundances of Fusobacteriaceae, Prolixibacteraceae and Desulfobulbaceae were observed in gonads of adults exposed to 2050 treatments (Figure S5), and for example, an ASV assigned to Desulfobulbaceae was significantly enriched in both 2050 and 2100 treatments compared to ambient conditions (Figure S6). In juveniles (F_1), *Haliangium* strains (Haliangiaceae) were differentially abundant among ambient and 2100 juveniles (F_1), while two ASVs assigned to Flavobacteriaceae were differentially abundant between ambient and 2050 F_1 juveniles (Figure 5c). Climate treatment appeared to have an effect on the larval microbial structure at each age/generation (Figure 5), but low replicate numbers precluded statistical confirmation of this pattern in F_2 larvae. An increase in Pseudomonadaceae and decrease in Rhodobacteraceae-affiliated sequences were observed in 1-day F_1 larvae reared under climate treatments, while a decrease in Flavobacteriaceae and increase in Alteromonadaceae and Vibrionaceae occurred in 5-day F_1 larvae under 2100 conditions (Figure S5). DESeq analyses revealed that distinct ASVs

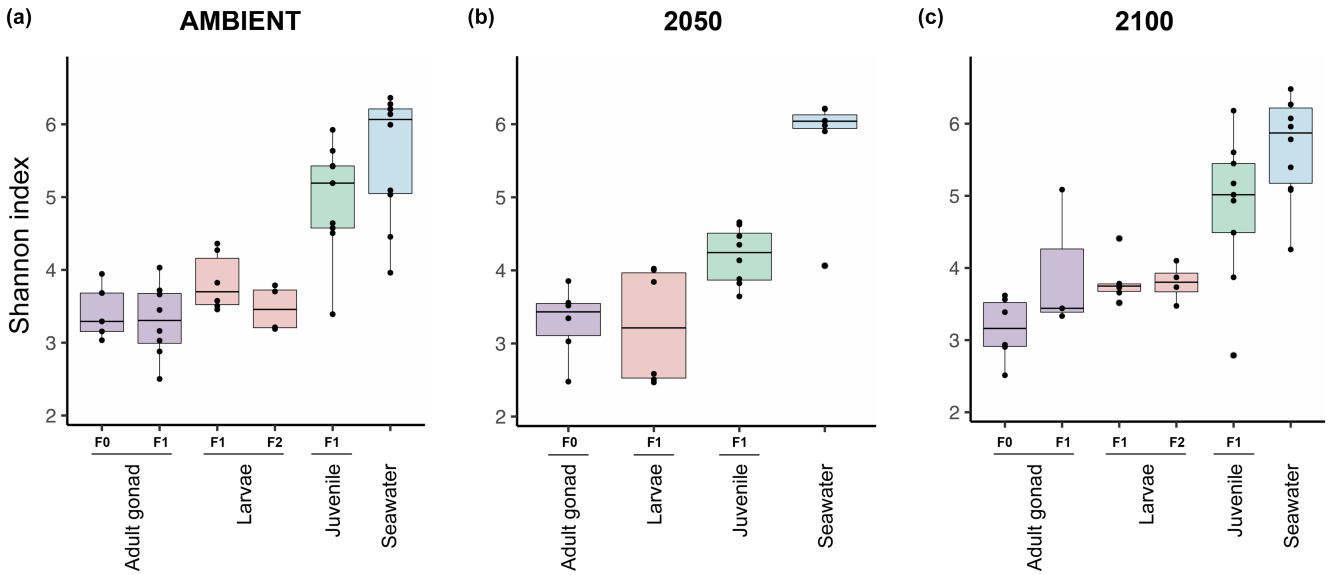

FIGURE 4 Boxplots of Shannon diversity index representing alpha diversity of the microbiome in adult gonads, larvae (1d and >1d larvae were pooled), juveniles and seawater under (a) ambient, (b) 2050 and (c) 2100 treatments.

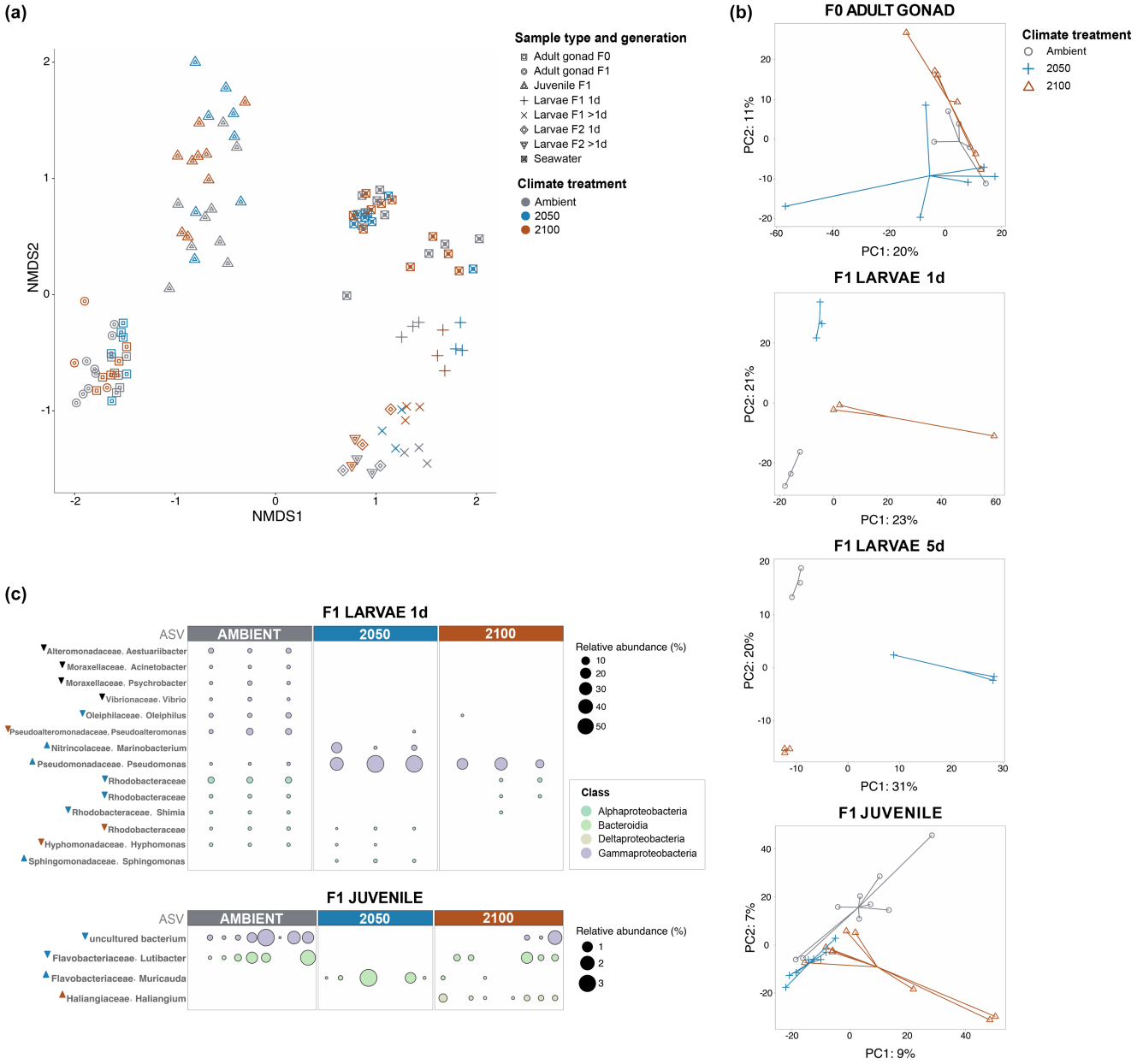

FIGURE 5 Urchin microbial responses to combined OW and OA predicted for years 2050 and 2100 (RCP 8.5). (a) Non-metric Multidimensional Scaling (NMDS, square-rooted data; stress = 0.14) based on Bray–Curtis dissimilarities calculated on relative abundance of ASVs present in adult gonad, juvenile, larvae and seawater across generations (F_0, F_1, F_2) and climate treatments (ambient, 2050, 2100). (b) Principal Component Analysis (PCA, centred log-ratio transformation) on relative abundance of ASVs in F_0 adult gonads, F_1 larvae at 1 and 5 days, and F_1 juveniles. (c) ASVs with significantly different relative abundances between climate treatments within 1-day F_1 larvae and F_1 juveniles identified using DESeq analyses. Only ASVs present across at least 50% of the samples in one of the compared groups are illustrated. Taxonomy is shown as Family and Genus, and a significant increase (▲) or decrease (▼) in relative abundance between ambient and 2050 treatment (blue), ambient and 2100 (red), and ambient and both climate treatments (black) is illustrated for each ASV.

were differentially abundant among the ambient and two climate treatments (Figure 5c and Figure S6). Importantly, 48% of the microbial community in 1d-larvae (F_1) under 2050 conditions comprised one ASV identified as a *Pseudomonas* strain, and ASVs identified as *Psychrobacter*, *Acinetobacter*, *Aestuariibacter* and *Vibrio* were lost in 1-day larvae reared under 2050 and 2100 climate scenarios (Figure 5c). Despite the microbial variation observed in urchins exposed to future climate scenarios, linear mixed effects models revealed that Shannon diversity was not affected by climate treatment, with life stage being the major driver of differences in microbial diversity among samples (Figure 4, Table S7, Figure S7).

We also explored the number of ASVs retained across life stages and generations under climate treatments. No ASVs were shared between adult gonads (F_0) and 1-day larvae (F_1) under 2050 conditions, while one ASV was shared under 2100 conditions (Figure S8). In contrast, 31% and 38% of ASVs associated with 1-day larvae were also

present in 5-day larvae (F_1) under 2050 and 2100 conditions, respectively. These findings indicate that a higher proportion of microbial taxa was retained during larval development under these treatments compared to ambient conditions (20%, Figure 3c). Finally, the proportion of ASVs retained from 5-day larvae to 5-month juveniles was minor (2% under 2050; 4% under 2100; Figure S8). The three ASVs that were consistently associated with larvae and juveniles under ambient conditions were not present at each life stage under climate treatments (Tables S8 and S9). Under 2050 and 2100 conditions, the ASV affiliated with *Alcanivorax* was absent in juveniles; and 1-day larvae (F_1) were not associated with *Oleiphilus* under 2050 conditions, and *Hyphomonas* under 2100 conditions (Tables S8 and S9). When exploring differences in microbial composition between generations of urchins reared under 2100 treatment, we found that 42% of ASVs associated with F_0 adult gonads was also present in F_1 gonads, and 35% of ASVs in F_1 1-day larvae was present in F_2 1-day larvae (Figure S8), similar to the pattern observed under ambient conditions (Figure 3c).

3.3 | Effects of parental exposure to climate treatments on the offspring microbiome (aim 3)

Parental effect on the urchin microbiome was investigated through a transplant experiment in which F_1 juveniles from ambient parents were reared under ambient, 2050 and 2100 conditions, and juveniles from 2050 parents were reared under 2050 and 2100 conditions (Figure 1, aim 3). Parental climate treatment, as well as offspring climate treatment, was sufficient for discrimination of the F_1 juvenile samples by multilevel sparse Partial Least Squares–Discriminant Analysis (sPLS-DA, six optimal components; Figure 6a). Permutational multivariate analysis of variance confirmed this pattern (PERMANOVA, parental climate treatment*offspring climate treatment: pseudo-$F = 1.78$ $p < .001$; Table S10), with the microbiome of juveniles whose parents were exposed to 2050 or 2100 conditions different to juveniles from parents under ambient conditions (Figure 6a). However, the dominant microbial families (>2%) appeared to have similar relative abundances across parental climate treatments (Figure S9). At the ASV level, DESeq analyses identified a wide range of ASVs differently abundant across parental and offspring treatments (Figure 6b). However, parental treatment did not have a significant effect on the Shannon diversity index of F_1 juveniles, although highest diversity was observed in parents exposed to ambient conditions and lowest under 2050 treatment (Figure S10, Table S11).

4 | DISCUSSION

Marine organisms are frequently associated with microbial communities that underpin their health and survival (McFall-Ngai, 2014; Robbins et al., 2021). Characterizing microbial dynamics throughout the development of marine invertebrate species and determining the effect of climate stressors on microbe–host relationships is critical for making predictions on ecological community structure in the context of environmental change. Using 16S rRNA gene sequencing, we determined that within the tropical sea urchin *Echinometra* sp. *A*, microbial life-stage specificity was maintained across generational exposure to future OW/OA conditions. However, results also indicated that shifts in microbial community composition occurred for specific urchin life stages under climate treatments, with a transplant experiment demonstrating that exposure of parents to climate treatments influenced the microbiota in subsequent urchin generations, thereby indicating potential for transgenerational effects of climate change on the microbiome.

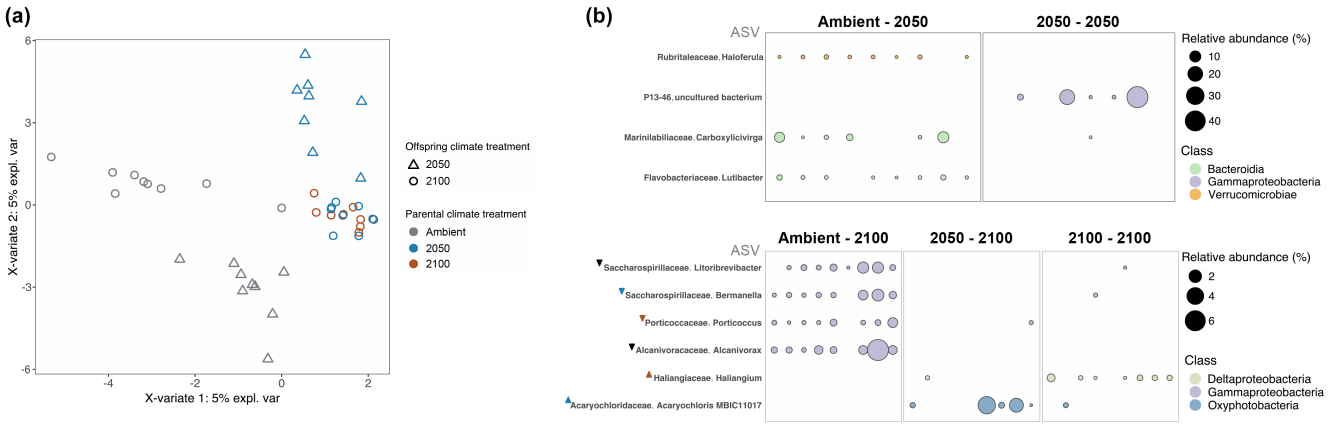

FIGURE 6 F_1 5-month juvenile urchin microbiome in the transplant experiment. (a) Multilevel sPLS-DA on the relative abundance of ASVs in F_1 juveniles following centred log-ratio transformation (visualization of the first two components). (b) Top ASVs with significantly different relative abundances across parental climate treatments (DESeq analysis). Only ASVs present across at least 50% of the samples in one of the compared groups are illustrated. Treatments are reported as 'parental (F0) climate treatment – juvenile (F1) climate treatment', and taxonomy is shown as Family and Genus. A significant increase (▲) or decrease (▼) in relative abundance between ambient-2100 and 2050–2100 (blue), ambient-2100 and 2100–2100 (red), and ambient-2100 and both parental climate treatments (black) is illustrated for each ASV.

4.1 | Major life history stages harbour a distinct microbiome under ambient conditions (aim 1)

We first investigated microbial community dynamics throughout the urchin life cycle (Figure 1, aim 1) and found that adult gonads, larvae and juveniles harboured a distinct microbiota under ambient conditions (Figures 2 and 3). Microbiome tissue specificity has been widely reported for many reef marine invertebrates, including other urchin species (Bernasconi et al., 2019; Carrier & Reitzel, 2019). In urchins, Carrier and Reitzel (2019) suggested that the establishment of a microbial community commences prior to fertilization since bacteria were detected in unfertilized eggs of three confamilial echinoids using 16S rRNA gene sequencing. However, ASVs retrieved from adult female gonads were not similar to larvae in our experiment; while only four ASVs were shared between F_0 gonads and 1-day F_1 larvae (Figure 3c), a higher number of ASVs were shared between 1-day F_1 larvae and seawater (Figure S3), indicating that microbial vertical transmission from parents to offspring may not be the primary mode of acquisition of these communities in *Echinometra* sp. *A*. Importantly, gonad sampling was performed following spawning; hence, the sampling methodology could also have precluded the detection of some microbial taxa associated with the pre-spawning gonads. In regard to microbial diversity, an increase was observed from larvae to juveniles (Figure 4a), suggesting that more bacterial niches become available for colonization throughout development. Although these results contrast with previous findings in other urchin species (Carrier & Reitzel, 2019), they are consistent with studies on other reef species with biphasic life cycles, where microbial alpha diversity increases during development, such as in the coral *Acropora digitifera* (Bernasconi et al., 2019). Although urchin microbial diversity was conserved within each life stage between generations (Figure 4a), transgenerational changes in microbial community composition were observed. For example, a higher abundance of Alteromonadaceae-, Oleiphilaceae- and Cellvibrionaceae-affiliated taxa was present in F_2 larvae compared to F_1 larvae (Figure S2). And in the adult gonads, less than 50% of ASVs were shared between generations (Figure 3c). Given that *Echinometra* sp. *A* appears to acquire microbes primarily from the surrounding environment, we hypothesize that microbial composition may be influenced by the available environmental microbial pool at each generation. We do not exclude that these microbial changes may be driven by stochastic mechanisms, as previously observed in the microbiota associated with echinoids eggs over multiple years (Carrier et al., 2020).

The microbiome of urchin larvae was dominated by Alteromonadaceae and Rhodobacteraceae families at 1-day in both F_1 and F_2 generations (Figure 3b). These bacterial taxa have been observed at high relative abundance in larvae of other reef species, including the corals *A. digitifera* and *Acropora tenuis* (Bernasconi et al., 2019; Damjanovic et al., 2020). The larval microbial community structure was shaped by age (Figure 3d), with the observed microbial changes likely linked to the emergence of new niches available for colonization during development. However, these changes may also occur due to the commencement of feeding in >1-day larvae, as food has been shown to be the main source of bacterial uptake within larvae across multiple urchin species (Carrier et al., 2021; Schuh et al., 2020).

In urchin juveniles, members of the class *Oxyphotobacteria* were at higher abundances compared to all other life stages (Figure 3a), and ASVs affiliated to Rhodobacteraceae and Vibrionaceae were also dominant at this stage (Figure 3b and S2), in line with previous studies on cnidarian species (Bernasconi et al., 2019; Mortzfeld et al., 2016; Quigley et al., 2020). In contrast, Alteromonadaceae-affiliated sequences were present in low relative abundances (Figure 3b), mirroring the observed reduction of Alteromonadaceae reported in the coral *A. digitifera* during development into juveniles (Bernasconi et al., 2019). While members of the Rhodobacteraceae family were abundant in both juveniles and larvae, ASV level abundance within this family was widely variable across these life stages (Figure 3e).

Urchin adult gonads (female) were dominated by *Gammaproteobacteria*, *Bacteroidia*, *Deltaproteobacteria* and *Fusobacteriia* classes (Figure 3a), as well as several families, including Vibrionaceae, Desulfobulbaceae, Cryomorphaceae and Fusobacteriaceae (Figure S2). In contrast to juvenile and larvae, adult gonads were characterized by low abundance in Rhodobacteraceae (Figure 3b). However, it is worth noting that our sampling design was unable to separate tissue-type effects (gonad) from life stage effects in adults. Hence, the observed differences between adult gonads and larvae/juveniles may be due to gonad microbiome specificity. Although the echinoderm gonad microbiome remains considerably underexplored, the microbiome associated with female gonads in the crown-of-thorns sea star *Acanthaster* cf. *solaris* appears to be highly variable (Høj et al., 2018), and a recent study reports high relative abundance of *Tenericutes* and *Spirochaetae* in multiple sea star taxa (Jackson et al., 2018), which has not been observed in the *Echinometra* gonads collected within our study.

4.2 | Microbial changes under exposure to 2050 and 2100 climate scenarios (aim 2)

To determine the multigenerational effects of OW and OA on the urchin microbiome, we assessed microbial community composition in urchin adult gonads, larvae and juveniles reared under future climate scenarios across three generations (Figure 1, aim 2). Despite microbial life-stage specificity being maintained under OW/OA (Figure 5a), treatment-attributed microbial changes were observed within each life stage (Figure 5b). In adult gonads (F_0), microbial shifts were observed in urchins raised under 2050 and 2100 for ~2 years, with an ASV belonging to Desulfobulbaceae being present only at both high OW/OA conditions (Figure S6). Although these results contrast with previous experimental studies on adult urchins (spines, gut) showing that the urchin microbial community is stable under exposure to OW/OA (Brothers et al., 2018; Webster et al., 2016), the discrepancy in microbial responses may be explained by the different tissue types as well as the extended exposure periods investigated within

this study. Indeed, Ketchum et al. (2021) recently confirmed that temperature-driven microbial shifts occur in the gut of the urchin *Echinometra* sp. *EZ* in natural conditions, suggesting that temperature can be a predictor of community variation.

Similar to adult gonads, the microbial community associated with larvae and juveniles was also altered under climate treatments. In 1-day larvae (F_1), for example, an ASV identified as *Pseudomonas* significantly increased in larvae exposed to 2050 conditions, representing the dominant ASV under this climate treatment (Figure 5c). In juveniles, *Muricauda* strains, which have been suggested to mitigate light and temperature stress through the provision of the antioxidant carotenoid zeaxanthin in cultured Symbiodiniaceae (Motone et al., 2020), were absent under ambient conditions but present at both climate treatments (Figure 5c). Furthermore, the three ASVs consistently associated with each developmental stage (1-day larvae, older larvae and juvenile) under ambient conditions, were not present at each life stage under 2050 and 2100 conditions, indicating potential disruption of critical host–microbe interactions under climate treatments.

In parallel to characterizing the urchin microbial community, host health was also assessed under exposure to future climate conditions (see Uthicke et al., 2020, 2021). In contrast to previous short-term experiments (Uthicke, Liddy, et al., 2014), F_0 adult health (growth, respiration rates and gonad development) was not impacted by climate conditions in our long-term experiment (analysed in Uthicke et al., 2020), suggesting that adult urchins may have acclimated to the treatment conditions. Despite the maintenance of standard physiological performances in F_0 adults, a negative carryover effect was observed between F_0 and F_1 generations (Karelitz et al., 2020; Uthicke et al., 2021). Indeed, Karelitz et al. (2020) assessed F_1 larval survival rates under ambient and 2100 conditions in the same experiment and found that larvae from 2100-acclimated parents were characterized by a reduced survival. Furthermore, juveniles from 2050 and 2100 parents showed a decline in physiological performances at 9 months of age, until a pathogenic mortality event occurred in 10-month juveniles, which may have selected for phenotypes adapted to climate conditions (Uthicke et al., 2021).

Host physiological responses may be associated with the restructuring of the microbial community, as observed in other marine invertebrates (Botté et al., 2019). While changes in the gonad microbiome could be related to positive acclimatory mechanisms in our urchin species, the microbial alteration observed in larvae reared under climate treatments is likely a sign of dysbiosis occurring in parallel to a decrease in survival. The dominance of specific ASVs under climate conditions, which were absent or a minor component under ambient, further supports the hypothesis that larval microbial changes were detrimental. Similarly, microbial shifts in 5-month juveniles may represent an early sign of decline in urchin health due to negative parental carryover effects, although juvenile's size was not impacted by climate treatments at this sampling time (Uthicke et al., 2020). Finally, microbial alterations observed in F_2 adult gonads may be linked to the host phenotypic adaptation under treatment conditions following the mortality event. Stochastic processes, however, may also underlie the observed microbial changes (Sieber et al., 2019). It should also be noted that the urchin genetic diversity was reduced over generations, with potential implications for host acclimatory responses. To conclude, although microbial restructuring occurred under climate treatments, the influence of the microbiome in mitigating host stress and maintaining host physiology cannot be determined and additional studies investigating the link between microbial metabolic activities and host performance under environmental stress would be required.

4.3 | Parental cross-generational effects observed on the offspring microbiome (aim 3)

Cross-generational effects on the microbiome have been observed in some marine species, but few studies have confirmed this mechanism in coral reef invertebrates. In our transplant experiment, parental (F_0) exposure to climate treatment affected the offspring microbiome (F_1 5-month juvenile; Figure 1 aim 3, and Figure 6), with potential implications for host health. For example, some ASVs belonging to *Gammaproteobacteria* were present in juveniles raised under 2100 conditions whose parents were under ambient treatment, but not in juveniles whose parents were exposed to 2050 or 2100 conditions (Figure 6b). Given the likelihood that most *Echinometra* sp. *A* ASVs are acquired from the environment, experimental treatment conditions combined with host physiology and niche colonization likely drive these microbiome changes, rather than cross-generational transmission. Despite these differences at the ASV level, major microbial changes were not detectable at the family level (Figure S9). However, larvae were kept at the same treatment conditions as their parents, and thus, our experimental design may have impacted our ability to fully identify cross-generational effects. Given older juveniles (9 months old) whose parents were reared under climate treatments showed signs of carryover effects on their physiology and behavioural responses (Uthicke et al., 2021), we hypothesize the microbial shifts observed in 5-month juveniles may be linked to early signs of decline in host health. Functional studies are needed to validate this hypothesis and characterize any metabolic change associated with the observed microbiome restructuring.

5 | CONCLUSIONS

Understanding how microbe–host interactions vary across host generations in a warming and acidifying ocean is critical for predicting realistic responses of reef species to future climate. Our 4-year experiment provides the first insights into microbial dynamics across life stages in a tropical urchin species exposed to OW/OA over multiple generations. Microbial communities were distinct across life stages, and this life-stage specificity was maintained under temperature and pCO_2 levels predicted for years 2050 and 2100 (RCP 8.5).

However, microbial dynamics were affected by exposure to OW/OA within life stages, and the offspring microbiome was also influenced by parental exposure to climate treatments. As *Echinometra* sp. *A* appears to acquire microbes primarily from the surrounding environment, we exclude that inheritance of shuffled microbes is the basis of the observed parental effect but postulate that urchin hosts may modify microbiome recruitment in response to parental exposure to OW/OA.

AUTHOR CONTRIBUTIONS

Nicole S. Webster, Sven Uthicke and Emma Marangon designed the research. Sven Uthicke and Frances Patel performed the research. Emma Marangon collected the samples, performed laboratory work and analysed the data. Ezequiel M. Marzinelli assisted with the statistical analyses. Emma Marangon wrote the manuscript and generated the figures. Patrick W. Laffy, David G. Bourne and Nicole S. Webster revised the manuscript and made substantial contributions to its intellectual content.

ACKNOWLEDGEMENTS

We are very grateful to Dr. Sam Karelitz for his assistance in maintaining the larval cultures, Dr. Sara Bell and Dr. Katarina Damjanovic for their guidance in processing the samples, and Dr. Murray Logan and Hillary Smith for their advices on statistical analyses. We thank the National Sea Simulator (SeaSim) staff for assisting during this long-term experiment and maintaining the experimental aquaria. We also would like to acknowledge the Traditional Owners of the land and sea country where this study took place, the Wulgurukaba and Bindal People. The research was funded through the AIMS Evolution21 project, and Emma Marangon was supported by an AIMS@JCU PhD scholarship. JCU supported open access publication of this study. Open access publishing facilitated by James Cook University, as part of the Wiley - James Cook University agreement via the Council of Australian University Librarians

CONFLICT OF INTEREST STATEMENT

We declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

Sequence data are deposited in NCBI (BioProject PRJNA1013433). Metadata are also accessible through the AIMS Research Data Platform (Australian Institute of Marine Science, 2023). R scripts for microbial analyses are available at https://github.com/emarangon/Cross-generational-climate-effects-on-the-urchin-microbiome.

ORCID

Emma Marangon https://orcid.org/0000-0002-9546-4822
Sven Uthicke https://orcid.org/0000-0002-3476-6595
Ezequiel M. Marzinelli https://orcid.org/0000-0002-3762-3389
Nicole S. Webster https://orcid.org/0000-0002-4753-5278
Patrick W. Laffy https://orcid.org/0000-0002-6754-1691

REFERENCES

[dataset] Australian Institute of Marine Science (AIMS). (2023). Microbiome dynamics across life stages, generations and climate treatments in a tropical sea urchin. http://apps.aims.gov.au/metadata/view/b5cbcbcd-0b19-4344-9aa8-7bbdd9201eb7

Apprill, A., McNally, S., Parsons, R., & Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquatic Microbial Ecology*, *75*(2), 129–137. https://doi.org/10.3354/ame01753

Australian Institute of Marine Science. (2020). Northern australia automated marine weather and oceanographic stations, sites: [Davies Reef], Parameters: [water temperature]. https://doi.org/10.25845/5c09bf93f315d

Baldassarre, L., Ying, H., Reitzel, A. M., Franzenburg, S., & Fraune, S. (2022). Microbiota mediated plasticity promotes thermal adaptation in the sea anemone *Nematostella vectensis*. *Nature Communications*, *13*(1), 3804. https://doi.org/10.1038/s41467-022-31350-z

Bernasconi, R., Stat, M., Koenders, A., Paparini, A., Bunce, M., & Huggett, M. J. (2019). Establishment of coral-bacteria symbioses reveal changes in the core bacterial community with host ontogeny. *Frontiers in Microbiology*, *10*, 1529. https://doi.org/10.3389/fmicb.2019.01529

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., … Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8), 852–857. https://doi.org/10.1038/s41587-019-0209-9

Botté, E. S., Bennett, H., Engelberts, J. P., Thomas, T., Bell, J. J., Webster, N. S., & Luter, H. M. (2023). Future ocean conditions induce necrosis, microbial dysbiosis and nutrient cycling imbalance in the reef sponge *Stylissa flabelliformis*. *ISME Communications*, *3*(1), 53. https://doi.org/10.1038/s43705-023-00247-3

Botté, E. S., Luter, H. M., Marangon, E., Patel, F., Uthicke, S., & Webster, N. S. (2020). Simulated future conditions of ocean warming and acidification disrupt the microbiome of the calcifying foraminifera *Marginopora vertebralis* across life stages. *Environmental Microbiology Reports*, *12*(6), 693–701. https://doi.org/10.1111/1758-2229.12900

Botté, E. S., Nielsen, S., Abdul Wahab, M. A., Webster, J., Robbins, S., Thomas, T., & Webster, N. S. (2019). Changes in the metabolic potential of the sponge microbiome under ocean acidification. *Nature Communications*, *10*(1), 4134. https://doi.org/10.1038/s41467-019-12156-y

Bourne, D. G., Garren, M., Work, T. M., Rosenberg, E., Smith, G. W., & Harvell, C. D. (2009). Microbial disease and the coral holobiont. *Trends in Microbiology*, *17*(12), 554–562. https://doi.org/10.1016/j.tim.2009.09.004

Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Mächler, M., & Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, *9*(2), 378. https://doi.org/10.32614/RJ-2017-066

Brothers, C. J., Van Der Pol, W. J., Morrow, C. D., Hakim, J. A., Koo, H., & McClintock, J. B. (2018). Ocean warming alters predicted microbiome functionality in a common sea urchin. *Proceedings of the Royal Society B: Biological Sciences*, *285*, 20180340. https://doi.org/10.1098/rspb.2018.0340

Byrne, M., & Przeslawski, R. (2013). Multistressor impacts of warming and acidification of the ocean on marine invertebrates' life histories. *Integrative and Comparative Biology*, *53*(4), 582–596. https://doi.org/10.1093/icb/ict049

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Carrier, T. J., Leigh, B. A., Deaker, D. J., Devens, H. R., Wray, G. A., Bordenstein, S. R., Byrne, M., & Reitzel, A. M. (2021). Microbiome reduction and endosymbiont gain from a switch in sea urchin life history. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(16), 1–7. https://doi.org/10.1073/pnas.2022023118

Carrier, T. J., Lessios, H., & Reitzel, A. (2020). Eggs of echinoids separated by the Isthmus of Panama harbor divergent microbiota. *Marine Ecology Progress Series*, *648*, 169–177. https://doi.org/10.3354/meps13424

Carrier, T. J., & Reitzel, A. (2019). Bacterial community dynamics during embryonic and larval development of three confamilial echinoids. *Marine Ecology Progress Series*, *611*, 179–188. https://doi.org/10.3354/meps12872

Carrier, T. J., & Reitzel, A. M. (2020). Symbiotic life of echinoderm larvae. *Frontiers in Ecology and Evolution*, *7*(January), 1–7. https://doi.org/10.3389/fevo.2019.00509

Cavalcanti, G. S., Alker, A. T., Delherbe, N., Malter, K. E., & Shikuma, N. J. (2020). The influence of bacteria on animal metamorphosis. *Annual Review of Microbiology*, *74*(1), 137–158. https://doi.org/10.1146/annurev-micro-011320-012753

Chua, L. L., Rajasuriar, R., Azanan, M. S., Abdullah, N. K., Tang, M. S., Lee, S. C., Woo, Y. L., Lim, Y. A. L., Ariffin, H., & Loke, P. (2017). Reduced microbial diversity in adult survivors of childhood acute lymphoblastic leukemia and microbial associations with increased immune activation. *Microbiome*, *5*(1), 35. https://doi.org/10.1186/s40168-017-0250-1

Collins, M., Knutti, R., Arblaster, J., Dufresne, J.-L., Fichefet, T., Friedlingstein, P., Gao, X., Gutowski, W., Johns, T., Krinner, G., Shongwe, M., Tebaldi, C., Weaver, A., & Wehner, M. (2013). Long-term climate change: Projections, commitments and irreversibility. In T. F. Stocker, D. Qin, G. K. Plattner, M. Tignor, et al. (Eds.), *Climate change 2013: The physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 1029–1136). Cambridge University Press.

Damjanovic, K., Menéndez, P., Blackall, L. L., & van Oppen, M. J. H. (2020). Early life stages of a common broadcast spawning coral associate with specific bacterial communities despite lack of internalized bacteria. *Microbial Ecology*, *79*(3), 706–719. https://doi.org/10.1007/s00248-019-01428-1

Davis, N. M., Proctor, D. M., Holmes, S. P., Relman, D. A., & Callahan, B. J. (2018). Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome*, *6*(1), 226. https://doi.org/10.1186/s40168-018-0605-2

Donelson, J. M., Munday, P. L., McCormick, M. I., & Pitcher, C. R. (2012). Rapid transgenerational acclimation of a tropical reef fish to climate change. *Nature Climate Change*, *2*(1), 30–32. https://doi.org/10.1038/nclimate1323

Egan, S., & Gardiner, M. (2016). Microbial dysbiosis: Rethinking disease in marine ecosystems. *Frontiers in Microbiology*, *7*, 991. https://doi.org/10.3389/fmicb.2016.00991

Fieth, R. A., Gauthier, M.-E. A., Bayes, J., Green, K. M., & Degnan, S. M. (2016). Ontogenetic changes in the bacterial symbiont community of the tropical demosponge *Amphimedon queenslandica*: Metamorphosis is a new beginning. *Frontiers in Marine Science*, *3*, 228. https://doi.org/10.3389/fmars.2016.00228

Grottoli, A. G., Dalcin Martins, P., Wilkins, M. J., Johnston, M. D., Warner, M. E., Cai, W.-J., Melman, T. F., Hoadley, K. D., Pettay, D. T., Levas, S., & Schoepf, V. (2018). Coral physiology and microbiome dynamics under combined warming and ocean acidification. *PLoS One*, *13*(1), e0191156. https://doi.org/10.1371/journal.pone.0191156

Hartig, F. (2021). DHARMa: Residual diagnostics for hierarchical (multi-level/mixed) regression models (0.4.4).

Hervé, M. (2021). RVAideMemoire: testing and plotting procedures for biostatistics (0.9-80).

Høj, L., Levy, N., Baillie, B. K., Clode, P. L., Strohmaier, R. C., Siboni, N., Webster, N. S., Uthicke, S., & Bourne, D. G. (2018). Crown-of-Thorns Sea star *Acanthaster* cf. *solaris* has tissue-characteristic microbiomes with potential roles in health and reproduction. *Applied and Environmental Microbiology*, *84*(13), e00181-18. https://doi.org/10.1128/AEM.00181-18

Jackson, E. W., Pepe-Ranney, C., Debenport, S. J., Buckley, D. H., & Hewson, I. (2018). The microbial landscape of sea stars and the anatomical and interspecies variability of their microbiome. *Frontiers in Microbiology*, *9*, 1829. https://doi.org/10.3389/fmicb.2018.01829

Karelitz, S., Lamare, M., Patel, F., Gemmell, N., & Uthicke, S. (2020). Parental acclimation to future ocean conditions increases development rates but decreases survival in sea urchin larvae. *Marine Biology*, *167*(1), 2. https://doi.org/10.1007/s00227-019-3610-5

Ketchum, R. N., Smith, E. G., Vaughan, G. O., McParland, D., Al-Mansoori, N., Burt, J. A., & Reitzel, A. M. (2021). Unraveling the predictive role of temperature in the gut microbiota of the sea urchin *Echinometra* sp. *EZ* across spatial and temporal gradients. *Molecular Ecology*, *30*(15), 3869–3881. https://doi.org/10.1111/mec.15990

Koropatnick, T. A., Engle, J. T., Apicella, M. A., Stabb, E. V., Goldman, W. E., & McFall-Ngai, M. J. (2004). Microbial factor-mediated development in a host-bacterial mutualism. *Science*, *306*(5699), 1186–1188. https://doi.org/10.1126/science.1102218

Lenth, R. V. (2022). emmeans: Estimated Marginal Means, aka Least-Squares Means (1.7.4-1). https://cran.r-project.org/package=emmeans

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12), 550. https://doi.org/10.1186/s13059-014-0550-8

Luter, H. M., Andersen, M., Versteegen, E., Laffy, P., Uthicke, S., Bell, J. J., & Webster, N. S. (2020). Cross-generational effects of climate change on the microbiome of a photosynthetic sponge. *Environmental Microbiology*, *22*(11), 4732–4744. https://doi.org/10.1111/1462-2920.15222

Marangon, E., Goldenberg, S. U., & Nagelkerken, I. (2020). Ocean warming increases availability of crustacean prey via riskier behavior. *Behavioral Ecology*, *31*(2), 287–291. https://doi.org/10.1093/beheco/arz196

Marangon, E., Laffy, P. W., Bourne, D. G., & Webster, N. S. (2021). Microbiome-mediated mechanisms contributing to the environmental tolerance of reef invertebrate species. *Marine Biology*, *168*(6), 89. https://doi.org/10.1007/s00227-021-03893-0

McClanahan, T. R., & Muthiga, N. A. (2013). Echinometra. In J. M. Lawrence (Ed.), *Developments in aquaculture and fisheries science* (Vol. *38*, pp. 337–353). Elsevier. https://doi.org/10.1016/B978-0-12-396491-5.00023-X

McFall-Ngai, M. J. (2014). The importance of microbes in animal development: Lessons from the squid-vibrio symbiosis. *Annual Review of Microbiology*, *68*(1), 177–194. https://doi.org/10.1146/annurev-micro-091313-103654

McKnight, D. T., Huerlimann, R., Bower, D. S., Schwarzkopf, L., Alford, R. A., & Zenger, K. R. (2019). Methods for normalizing microbiome data: An ecological perspective. *Methods in Ecology and Evolution*, *10*(3), 389–400. https://doi.org/10.1111/2041-210X.13115

McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*, *8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217

Meinshausen, M., Smith, S. J., Calvin, K., Daniel, J. S., Kainuma, M. L. T., Lamarque, J.-F., Matsumoto, K., Montzka, S. A., Raper, S. C.

B., Riahi, K., Thomson, A., Velders, G. J. M., & van Vuuren, D. P. P. P. (2011). The RCP greenhouse gas concentrations and their extensions from 1765 to 2300. *Climatic Change*, *109*(1–2), 213–241. https://doi.org/10.1007/s10584-011-0156-z

Mortzfeld, B. M., Urbanski, S., Reitzel, A. M., Künzel, S., Technau, U., & Fraune, S. (2016). Response of bacterial colonization in *Nematostella vectensis* to development, environment and biogeography. *Environmental Microbiology*, *18*(6), 1764–1781. https://doi.org/10.1111/1462-2920.12926

Motone, K., Takagi, T., Aburaya, S., Miura, N., Aoki, W., & Ueda, M. (2020). A zeaxanthin-producing bacterium isolated from the algal phycosphere protects coral endosymbionts from environmental stress. *mBio*, *11*(1), 1–13. https://doi.org/10.1128/mBio.01019-19

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., Dan, M., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2020). Vegan: Community ecology package (2.5-7). https://cran.r-project.org/package=vegan

Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2016). Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environmental Microbiology*, *18*(5), 1403–1414. https://doi.org/10.1111/1462-2920.13023

Pita, L., Rix, L., Slaby, B. M., Franke, A., & Hentschel, U. (2018). The sponge holobiont in a changing ocean: From microbes to ecosystems. *Microbiome*, *6*(1), 46. https://doi.org/10.1186/s40168-018-0428-1

Pörtner, H. O., & Farrell, A. P. (2008). Physiology and climate change. *Science*, *322*(5902), 690–692. https://doi.org/10.1126/science.1163156

Posadas, N., Baquiran, J. I. P., Nada, M. A. L., Kelly, M., & Conaco, C. (2022). Microbiome diversity and host immune functions influence survivorship of sponge holobionts under future ocean conditions. *The ISME Journal*, *16*(1), 58–67. https://doi.org/10.1038/s41396-021-01050-5

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, *41*(D1), D590–D596. https://doi.org/10.1093/nar/gks1219

Quigley, K. M., Alvarez Roa, C., Torda, G., Bourne, D. G., & Willis, B. L. (2020). Co-dynamics of Symbiodiniaceae and bacterial populations during the first year of symbiosis with *Acropora tenuis* juveniles. *Microbiology Open*, *9*(2), e959. https://doi.org/10.1002/mbo3.959

R Core Team. (2020). R: A language and environment for statistical computing. https://www.r-project.org/

Robbins, S. J., Song, W., Engelberts, J. P., Glasl, B., Slaby, B. M., Boyd, J., Marangon, E., Botté, E. S., Laffy, P., Thomas, T., & Webster, N. S. (2021). A genomic view of the microbiome of coral reef demosponges. *The ISME Journal*, *15*(6), 1641–1654. https://doi.org/10.1038/s41396-020-00876-9

Rohart, F., Gautier, B., Singh, A., & Lê Cao, K.-A. (2017). mixOmics: An R package for omics feature selection and multiple data integration. *PLoS Computational Biology*, *13*(11), e1005752. https://doi.org/10.1371/journal.pcbi.1005752

Russell, S. L., & Castillo, J. R. (2020). Trends in symbiont-induced host cellular differentiation. In M. Kloc (Ed.), *Symbiosis: Cellular, molecular, medical and evolutionary aspects* (Vol. *69*, pp. 137–176). Springer. https://doi.org/10.1007/978-3-030-51849-3_5

Schuh, N. W., Carrier, T. J., Schrankel, C. S., Reitzel, A. M., Heyland, A., & Rast, J. P. (2020). Bacterial exposure mediates developmental plasticity and resistance to lethal *Vibrio lentus* infection in Purple Sea urchin (*Strongylocentrotus purpuratus*) Larvae. *Frontiers in Immunology*, *10*(January), 1–17. https://doi.org/10.3389/fimmu.2019.03014

Sieber, M., Pita, L., Weiland-Bräuer, N., Dirksen, P., Wang, J., Mortzfeld, B., Franzenburg, S., Schmitz, R. A., Baines, J. F., Fraune, S., Hentschel, U., Schulenburg, H., Bosch, T. C. G., & Traulsen, A. (2019). Neutrality in the metaorganism. *PLOS Biology*, *17*(6), e3000298. https://doi.org/10.1371/journal.pbio.3000298

Song, H., Hewitt, O. H., & Degnan, S. M. (2021). Arginine biosynthesis by a bacterial symbiont enables nitric oxide production and facilitates larval settlement in the marine-sponge host. *Current Biology*, *31*(2), 433–437.e3. https://doi.org/10.1016/j.cub.2020.10.051

Uthicke, S., Furnas, M., & Lønborg, C. (2014). Coral reefs on the edge? Carbon chemistry on inshore reefs of the great barrier reef. *PLoS One*, *9*(10), e109092. https://doi.org/10.1371/journal.pone.0109092

Uthicke, S., Liddy, M., Nguyen, H. D., & Byrne, M. (2014). Interactive effects of near-future temperature increase and ocean acidification on physiology and gonad development in adult Pacific sea urchin, *Echinometra* sp. *A*. *Coral Reefs*, *33*(3), 831–845. https://doi.org/10.1007/s00338-014-1165-y

Uthicke, S., Patel, F., Karelitz, S., Luter, H., Webster, N., & Lamare, M. (2020). Key biological responses over two generations of the sea urchin *Echinometra* sp. *A* under future ocean conditions. *Marine Ecology Progress Series*, *637*, 87–101. https://doi.org/10.3354/meps13236

Uthicke, S., Patel, F., Petrik, C., Watson, S., Karelitz, S. E., & Lamare, M. D. (2021). Cross-generational response of a tropical sea urchin to global change and a selection event in a 43-month mesocosm study. *Global Change Biology*, *27*(15), 3448–3462. https://doi.org/10.1111/gcb.15657

Voolstra, C. R., & Ziegler, M. (2020). Adapting with microbial help: Microbiome flexibility facilitates rapid responses to environmental change. *BioEssays*, *42*(7), 2000004. https://doi.org/10.1002/bies.202000004

Webster, N. S., Negri, A. P., Botté, E. S., Laffy, P. W., Flores, F., Noonan, S., Schmidt, C., & Uthicke, S. (2016). Host-associated coral reef microbes respond to the cumulative pressures of ocean warming and ocean acidification. *Scientific Reports*, *6*(1), 19324. https://doi.org/10.1038/srep19324

Weiland-Bräuer, N., Pinnow, N., Langfeldt, D., Roik, A., Güllert, S., Chibani, C. M., Reusch, T. B. H., & Schmitz, R. A. (2020). The native microbiome is crucial for offspring generation and fitness of *Aurelia aurita*. *mBio*, *11*(6), 1–20. https://doi.org/10.1128/mBio.02336-20

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., … Yutani, H. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, *4*(43), 1686. https://doi.org/10.21105/joss.01686

Ziegler, M., Seneca, F. O., Yum, L. K., Palumbi, S. R., & Voolstra, C. R. (2017). Bacterial community dynamics are linked to patterns of coral heat tolerance. *Nature Communications*, *8*, 14213. https://doi.org/10.1038/ncomms14213

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Marangon, E., Uthicke, S., Patel, F., Marzinelli, E. M., Bourne, D. G., Webster, N. S., & Laffy, P. W. (2023). Life-stage specificity and cross-generational climate effects on the microbiome of a tropical sea urchin (Echinodermata: Echinoidea). *Molecular Ecology*, *32*, 5645–5660. https://doi.org/10.1111/mec.17124