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The impact of heat stress, UVB radiation and humic substances on the diversity and structure of prokaryotic communities inhabiting host and environmental coral reef biotopes

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ABSTRACT

Coral cover has declined across the globe over the past decades. A key contributor to this decline has been the increased prevalence of bleaching events due to heat stress. A number of field observational studies have suggested that the impact of heat stress and intense ultraviolet B (UVB) radiation may be mitigated by the presence of humic substances (HS) in the form of Chromophoric Dissolved Organic Matter (CDOM) in the water column. Here, we examined the independent and interactive effects of heat stress, UVB exposure, and humic substance (HS) modulation on prokaryotic diversity and community structure, namely, their alignment with neutral model predictions, across two coral species (*Montipora digitata* and *Sarcophyton glaucum*), a sponge (*Chondrilla* sp.), and sediment under controlled microcosm conditions. Prokaryotic evenness was significantly lower in *M. digitata* under UVB exposure, but significantly higher under HS modulation. Evenness was also significantly higher in sediment under HS modulation. Rarefied richness, in turn, was significantly higher in *M. digitata* and sediment under HS modulation. There were also significant two- and three-way interactions with respect to rarefied richness among heat stress, UVB radiation and HS modulation on the prokaryotic communities of *Chondrilla* sp. and *M. digitata*. For example, in *M. digitata*, HS modulation appeared to mitigate the adverse effects of heat stress and UVB exposure on rarefied richness. Prokaryotic communities from each biotope with and without HS modulation were fitted to a neutral model. Fits to the neutral model varied substantially and were lowest for the soft coral *Sarcophyton glaucum* ($R^2 = 0.547$ without HS and $R^2 = 0.596$ with HS) and highest for the sponge *Chondrilla* sp. ($R^2 = 0.922$ without HS and $R^2 = 0.949$ with HS). In all biotopes the fit to the neutral model was higher under HS modulation. This difference was most pronounced for *M. digitata* where there was a relatively poor fit without HS modulation ($R^2 = 0.303$), but a much better fit with HS modulation ($R^2 = 0.636$). Taken together, these results suggest that HS have biotope-specific effects on prokaryotic diversity and appear to promote neutral community assembly of prokaryotic communities, particularly in *M. digitata*. HS may, furthermore, help to increase resilience against heat stress and UVB radiation by stimulating the growth of richer and more even prokaryotic assemblages.

1. Introduction

Coral reefs are among the most biologically diverse ecosystems on Earth and provide essential ecological services, which benefit marine life, coastal communities, and the global environment (Moberg and Folke, 1999). For example, coral reefs protect coastal regions by

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acting as barriers against storm surges, thereby reducing erosion and safeguarding communities from extreme weather events (Ferrario et al., 2014). Many marine species depend on coral reefs for shelter, food, and breeding grounds, and the decline in reef health can disrupt these relationships. Loss of corals and coral reef structure can cascade through the food web, affecting both predators and herbivores, reducing overall ecosystem stability and eventually leading to the loss of key services (Rogers et al., 2018). Coral reefs are also of considerable economic importance. For example, they support fishing and tourism industries, estimated to be worth billions of dollars worldwide, and are essential to the livelihoods of millions of people (Barbier et al., 2011).

Despite their importance, coral reefs face significant threats, including heat-induced bleaching (Hughes et al., 2017a,b), pollution (Dubinsky and Stambler, 1996), and overfishing (Shantz et al., 2020). Bleaching occurs when corals, under stress from elevated sea temperatures, in addition to other factors, expel the symbiotic zooxanthellae that provide them with nutrients and colour. Without these dinoflagellates, corals lose both their pigmentation and their primary energy source, leaving them vulnerable to disease, reduced growth rates, and increased mortality (Hughes et al., 2017a,b, 2018; Hoegh-Guldberg et al., 2019). Bleaching events resulting from heat stress have been linked to structural degradation, reduced biodiversity, weakened ecosystem services and declines in reef coral and fish populations (Magel et al., 2019; Sato et al., 2020). Bleaching also impacts several species across a range of taxa that rely on coral reefs for habitat complexity and food availability. Studies estimate that coral bleaching and associated reef degradation could result in significant economic losses, especially in regions heavily dependent on reef-related activities (Spalding et al., 2017). In addition to elevated sea temperatures, a number of other factors can influence coral bleaching including bacterial infections (Kushmaro et al., 1996), algal contact (Rasher and Hay, 2010), and elevated light levels/UV radiation (Brown et al., 1994; Fitt et al., 2001). Coastal degradation, particularly the loss of coastal forests and mangroves may also play an important role in the susceptibility to bleaching due to reduced export of humic substances (HS) into the marine environment (Roche et al., 2014).

In agriculture, HS, which include complex organic compounds derived from the decomposition of terrestrial plants and microbial material, are known to enhance soil fertility by improving soil structure, water retention, and nutrient availability (Yang et al., 2021). They act as electron acceptors, influencing organismal oxidative stress and aiding survival in variable environmental conditions (Steinberg et al., 2006). Their negative charge attracts and holds essential cations (e.g., potassium, calcium, and magnesium) and trace elements, preventing them from leaching and making them more accessible to plants (Canellas and Olivares, 2014). Additionally, HS promote root development and nutrient absorption, increasing plant growth and resilience under stress (Nardi et al., 2002). The binding affinity for heavy metals and organic pollutants makes HS valuable for soil and water remediation. For example, HS have been shown to increase the uptake of cadmium by plants, accelerating phytoremediation of contaminated soils (Evangelou et al., 2004). HS also reduced cadmium bioavailability and uptake by filter feeders, as demonstrated by Voets et al. (2004) with the zebra mussel, *Dreissena polymorpha*.

HS are transported from rivers and soils into the ocean, where they interact with marine microbes by providing carbon and other nutrients, affecting bacterial abundance, composition, and metabolic processes (Amon and Benner, 1996). They offer numerous environmental benefits due to their chemical properties and biological activities and can, for example, impact coral health by altering water quality, oxidant stress, light penetration, and nutrient availability, ultimately influencing coral growth, metabolism, and stress response (Aeschbacher et al., 2012; Ayoub et al., 2012). HS have shown promise in reducing inflammation in human and animal cells, with applications in medicine and animal feed (Vucskits et al., 2010). HS, furthermore, indirectly support microbial metabolic activities, by modulating the availability of trace metals, which are crucial for microbial enzymatic processes (Hertkorn et al., 2006).

One of the primary effects of HS in the marine environment is related to light attenuation. HS absorbs light, particularly in the blue and UV spectrum, which can limit the light available for photosynthesis to symbiotic dinoflagellates (e.g., *Symbiodinium* spp.) that corals rely on for energy (Ayoub et al., 2012; Hongo and Yamano, 2013). Reduced light penetration may, however, increase photosynthetic efficiency (Rocha et al., 2013), and impact coral growth and energy reserves in a complex manner (Baker et al., 2018). HS may also protect corals from intense UVB radiation and help to reduce bleaching (Ayoub et al., 2012). Additionally, HS serve as carbon sources and energy for marine bacteria, enhancing microbial productivity and supporting food webs in coastal and oceanic waters (Ogawa et al., 2001). Furthermore, they influence bacterial community structure by promoting the growth of specific taxa capable of degrading these complex molecules, thereby diversifying bacterial functional roles within the ecosystem (Kellerman et al., 2015).

Although HS have been shown to have a profound effect on bacterioplankton communities (Moran and Hodson, 1994a,b; Carlsson et al., 1995; Cooper et al., 2005), little is known about their effect on host-associated bacterial communities. Importantly, these communities play an essential role in host organismal health, and contribute to functions such as nutrient cycling, pathogen defense, and stress response. They are also highly diverse and vary considerably among coral species, environments, and even among individual coral colonies (Bourne et al., 2016). The prokaryotic community, part of the host microbiome, includes symbiotic bacteria that support resilience against environmental stressors, including bleaching, disease, and temperature fluctuations (Rosenberg et al., 2007). Certain prokaryotic species within the coral microbiome are, furthermore, thought to assist in nitrogen fixation, an essential process in nutrient-poor tropical waters (Lema et al., 2012). This microbial nitrogen cycling is especially important as it supports coral growth by supplementing nutrients to the symbiotic dinoflagellates, which provide energy through photosynthesis (Lesser et al., 2004).

To evaluate the relative importance of environmental stressors and HS modulation, this study compared four benthic biotopes: a scleractinian (hard) coral, soft coral, marine sponge and sediment. These habitats span a broad range of structural complexities and surface morphologies, which shape local hydrodynamics, diffusive boundary-layer regimes and microbial immigration and colonisation rates (Jørgensen and Revsbech, 1985; Shashar et al., 1993; Battin et al., 2003; Wahl et al., 2012). They also differ in the degree of host control over microbial recruitment although both sponges and corals tend to harbour host-specific microbiomes (Rohwer et al., 2002; van de Water et al., 2018; Cleary and de Voogd, 2024; Cleary et al., 2020, 2021, 2022, 2023, 2024, 2025). Under neutral and metacommunity frameworks, these differences in host architecture and substrate dynamics are expected to alter dispersal limitation and mass effects, that is, the extent to which local communities are shaped by immigration from the surrounding pool rather than by local selection (Hubbell, 2001; Sloan et al., 2006; Leibold et al., 2004; Nemergut et al., 2013; Burns et al., 2016). Comparing these biotopes therefore provides a useful contrast for testing how host identity and substrate type shape microbial assembly and diversity, and whether HS modulation acts consistently across them.

The finger coral, *Montipora digitata* (Family: Acroporidae), is a widely distributed, branching scleractinian coral species in the Indo-Pacific region (Jia et al., 2023). It is commonly found in shallow, turbulent waters, from reef flats to back reefs, where it forms colonies that contribute to reef structural complexity. The species has shown potential for reef rehabilitation, with successful transplantation experiments conducted in the Philippines (Gomez et al., 2011). It is also known for its rapid growth rates, making it a popular choice in reef restoration projects where fast recovery is desired (Rinkevich, 2014). It exhibits various growth forms and pigmentation, often influenced by water flow, light availability, and nutrient levels (Alessi et al., 2024). Like other corals,

M. digitata relies on a symbiotic relationship with dinoflagellates for photosynthetic energy, but it can also feed heterotrophically, capturing plankton from surrounding waters (Sangmanee et al., 2020). Despite its potential for reef rehabilitation, It has been shown to be sensitive to environmental perturbations, including heat stress, pollution, and acidification, which contribute to bleaching and tissue loss (Kavousi et al., 2016; Hughes et al., 2017).

The genus *Chondrilla* comprises a group of demosponges found in marine environments worldwide. *Chondrilla* sponges are typically found in shallow coastal waters, including coral reefs, seagrass beds, and rocky shores. They are effective filter feeders and play a role in nutrient cycling (Diaz and Ward, 1997). Some species can form symbiotic relationships with photosynthetic cyanobacteria, which live within the sponge's tissues and provide it with a source of energy (Arillo et al., 1993). This symbiotic relationship can also be responsible for the sponges' colour, which may vary depending on the amount and type of symbiont present. *Sarcophyton glaucum*, commonly known as the toadstool leather coral or mushroom leather coral, is a species of soft coral widely found in the Indo-Pacific region across a variety of marine environments from shallow reef crests to deeper lagoon habitats. They are a rich source of bioactive compounds (Carroll et al., 2024).

In a previous study (Stuij et al., 2025), we showed that HS modulation was a significant predictor of variation in the composition of all biotopes. We (Stuij et al., 2025) also measured minimum and maximum fluorescence of *M. digitata* in order to calculate the Fv/Fm ratio, which has been linked to damage to the photosynthetic machinery in hard corals under thermal stress (Bhagooli and Yakovleva, 2004; Manullang et al., 2020). The F_V/F_M ratio reflects the PSII maximum potential quantum efficiency of coral zooxanthellae and serves as an indicator of coral photo-oxidative stress (Downs et al., 2002; Warner et al., 1999; Wiedenmann et al., 2013). According to oxidative stress theory, coral bleaching results from PSII dysfunction in zooxanthellae chloroplasts under elevated heat and UVB stress, leading to reactive oxygen species (ROS) buildup and the expulsion of zooxanthellae from coral tissue (Downs et al., 2002). We (Stuij et al., 2025) showed that F_V/F_M ratios were significantly lower in corals kept in heat-stressed microcosms than control microcosms, but only in the absence of HS modulation. With HS modulation, there was no significant difference. The adverse impact of heat stress on Fv/Fm ratios was, thus, mitigated by HS modulation.

In the present study, we expanded on our previous work (Stuij et al., 2025) to investigate the independent and interactive effects of heat stress, UV exposure and HS modulation on diversity and structure in relation to neutral model predictions (Hubbell, 2001). We tested the following hypotheses 1. HS modulation will increase prokaryotic evenness and rarefied richness, and 2. HS modulation will promote prokaryotic community structure in line with neutral model predictions.

2. Material and methods

2.1. Experimental life support system (ELSS) design

The ELSS used in this study was based on a microcosm system previously designed to assess the effects of global climate change and environmental contamination on sediment communities (Coelho et al., 2013). This system was modified and validated for coral reef microcosm experiments under laboratory-controlled conditions as described in detail in Stuij et al. (2024). Briefly, the ELSS consisted of 32 glass aquaria (referred to as microcosms, 23 cm in height, 16 cm length and 12 cm width), which were individually connected to another aquarium (referred to as reservoir, 30 cm in height, 12 cm length and 12 cm width). Each reservoir-microcosm unit contained a functional water volume of ~5 L. Water was circulated within microcosm-reservoir units using small hydraulic pumps, resulting in a constant flow rate of 8.64 ml/s (s = 0.66 ml/s). Water temperature was regulated using water bath tanks each surrounding four microcosm-reservoir units, equipped with water heaters with an internal thermostat (V2Therm 100 Digital heater). Constant aeration within microcosms was maintained using diffusing air stones connected to an air pump (530L/h, Resun) via small hoses. Lighting was controlled by four programmable luminaire systems (Reef - SET, Rees, Germany), each holding eight fluorescent lamps (Coelho et al., 2013). During the current experiment, four UV fluorescent tubes (SolarRaptor, T5/54W, Rees, Germany) and four full spectra fluorescent tubes (ATI AquaBlue Special, T5/54W) were connected alternately and programmed to a 12 h diurnal light cycle, simulating photoperiod conditions of tropical latitudes. To block radiation in the UVB spectrum (290 – 320 nm), microcosms were covered independently from each other by a transparent polyester film (Folanorm SF-AS, Folex coating, Köln, Germany). The film has been previously used in multiple studies to block UVB radiation (Müller et al., 2009; Rautenberger et al., 2013). In our experimental set-up, the film absorbed 90% of the UVB, 31% of UVA and 9% of PAR irradiance.

Using the ELSS, a multi-factorial experiment was designed to test the independent and interactive effects of HS modulation, heat stress and UVB radiation on prokaryotic diversity and structure. Each treatment had two levels, namely, HS modulation (with *versus* without), heat stress (28 °C *versus* 32 °C) and UVB radiation (with *versus* without) for a total of eight groups with four replicates each. The full experiment, thus, consisted of 32 microcosms. The heat stress treatment was randomised in groups of four microcosms, whereby HS modulation, UVB radiation and the combination of both were randomly assigned within each group of microcosms with equal temperature. A graphical summary of the ELSS and different phases of the experiment can be found in Stuij et al. (2025).

Each microcosm received a coral reef sediment layer of ~3 cm, consisting of a mixture of commercially available (Reef Pink dry aragonite sand, Red Sea) and natural coral reef sediment. The commercial sediment was washed and sterilised (3 times autoclavation at 121 °C for 20 min (Otte et al., 2018) before use. Natural sediment was collected from a coral reef south of Fongguei, Penghu, Taiwan (22°19′50.5″N 120°22′19.8″E). Synthetic seawater used in the experiment was prepared by mixing coral reef salt (CORAL PRO SALT, Red Sea) with deionized water (V2Pure 360), with salinity adjusted to 35 ppt. A concentrated HS stock solution (10 gL-1) was first prepared by dissolving commercially available HS (technical grade humic acid, Sigma-Aldrich) in 0.2M NaOH water solution. Subsequently, the solution was neutralised to a pH of 8.0 – 8.2 by adding concentrated HCL. This concentrated stock solution was then used to enrich the synthetic seawater to a final HS concentration of 7.5 mg L^{-1}. This concentration falls within the range of dissolved organic carbon (DOC) concentrations measured in coastal coral reef ecosystems, which were shown to vary from 0.05 to up to 17 mg L^{-1} (Kegler et al., 2017; Teichberg et al., 2018). Synthetic seawater with and without HS was poured into microcosms containing sediment, and the temperature was set to 28 °C. Partial seawater renewal was carried out daily by replacing 1 L of seawater with newly prepared synthetic seawater, with or without HS, in each microcosm. HS concentration within the water column of the microcosms was monitored using UV spectrometry (Eaton, 1995). The absorbance at a wavelength of 300 nm was relatively stable over the course of the experiment and averaged 0.017 ± 0.003 AU among microcosms (referenced against non-HS modulated microcosm water).

After eight days of initial stabilisation of the system, specimens of *M. digitata* (Dana, 1846) previously grown on carbonate stones (3 cm in diameter, 1 cm thickness) were added to each individual microcosm. All animals used in this study were obtained from the collection of marine invertebrates cultivated at ECOMARE (University of Aveiro, Portugal). ECOMARE holds validated coral reef culture systems (Rocha et al., 2015), which have previously been used to study environmental effects on coral reef species under experimentally controlled conditions (Rocha et al., 2020).

After adding the corals, the microcosms were kept in the conditions mentioned above for an additional 21 days. After this period, heat stress and UVB treatments were applied to the microcosms for 5 days as follows. In the heat-treated microcosms, temperature was gradually

increased over the course of three days from 28.0 ± 0.6 °C on day 1 to 31.0 ± 0.6 °C on day 3 and averaged 31.4 ± 0.5 °C and 31.2 ± 0.7 °C on days 4 and 5. These temperatures were chosen to match temperatures previously observed in Pacific coral reefs, which ranged between 26 °C and 32 °C. In the UVB treated microcosms, the UVB absorbing film was removed, which resulted in daily doses of 2.43 * 10 -2 J cm^{-2} UVB radiation.

2.2. Sampling and DNA extraction

One hard coral specimen from each microcosm was sampled. After sampling, the coral specimens were rinsed with sterilised artificial seawater (filtered through 0.22-μm pores), removed from their carbonate stones, and frozen at −80 ^{0}C until DNA extraction. A composite sediment sample was collected from each microcosm by haphazardly taking four sub-samples (1 cm surface sediment cores with a diameter of approximately 2 cm). An overview of the sample metadata can be found in Supplementary data 1.

PCR-ready genomic DNA was isolated from all coral samples using the FastDNA® SPIN soil Kit (MP biomedicals) following the manufacturer's instructions. Coral fragments (tissue and skeleton) (~500 mg) were first snap frozen in liquid nitrogen and subsequently ground with a heat-sterilised mortar and pestle. Soft coral and sponge specimens were cut into small pieces with sterilised scissors. Approximately 500 mg of the ground coral, sediment, sponge and soft coral pieces were transferred to Lysing Matrix E tubes containing a mixture of ceramic and silica particles. Blank negative controls (without samples) were also included to evaluate sample contamination during DNA extraction. Microbial cell lysis was performed in the FastPrep Instrument (MP biomedicals) for 2 × 40 s at a speed setting of 6.0 ms^{-1}. Extracted DNA was eluted in 50 μl of DNase/pyrogen-free water and stored at −20 ^{0}C until further use.

2.3. 16S rRNA gene library preparation and sequencing

The V3/V4 variable region of the 16S rRNA gene was amplified using primers 341F 5′CCTACGGGNGGCWGCAG'3 and 785R 5′GACTACHVGGGTATCTAATCC'3 (Klindworth et al., 2013) with Illumina Nextera XT overhang adapters for a dual-PCR library preparation approach. PCRs were performed using 1 to 3 μl of DNA template, 10 μl of HS ReadyMix (KAPA HiFi Roche), and 0.6 μl of the forward and reverse primers in a concentration of 10 pmol/μl. Reaction mixes were finalised by the addition of mQ water (Ultrapure) to a final volume of 20 μl. The PCR conditions consisted of initial denaturing at 95 °C for 3 min, followed by 30 cycles of 98 °C for 20 s, 57 °C for 30 s, and 72 °C for 30 s, after which a final elongation step at 72 °C for 1 min was performed. We checked for the success of amplification, relative intensity of the bands, and contamination using 2% Invitrogen E-gels with 3 μl of PCR product.

PCR products were cleaned with magnetic beads at a ratio of 0.9:1 using a magnetic extractor stamp, after which a second PCR was performed. The 25 μl reaction mix consisted of 4 μl of the first PCR product, 12.5 μl HS ReadyMix (KAPA HiFi Roche), 2 × 1 μl (concentration of 10 pmol/μl) MiSeq Nextera XT adapters (dual indexed, Illumina), and 6.5 μl of mQ water (Ultrapure). PCR conditions consisted of initial denaturing at 95 °C for 3 min, followed by 8 cycles of 98 °C for 20 s, 55 °C for 30 s, and 72 °C for 30 s, after which a final elongation step at 72 °C for 5 min was performed. DNA molarity and fragment sizes of the PCR products were measured on a fragment analyser 5300 (Agilent) and subsequently, each 96-well plate was normalised and pooled together in subpools using the Qiagen QIAgility. The DNA molarity of the subpools was measured on the Agilent 4150 TapeStation to combine the subpools into a final pool. The pool of normalised DNA was cleaned one last time using magnetic beads at a ratio of 0.65:1 and thereafter sequenced at a commercial company (Baseclear, Leiden, the Netherlands) on the Illumina MiSeq platform using 2 × 300 bp paired-end sequencing (Illumina MiSeq PE300). Three negative control samples were included to detect possible contamination during library preparation and sequencing. Sequences from each end were paired following Q25 quality trimming and removal of short reads (<150 bp). The DNA sequences generated in this study can be downloaded from NCBI BioProject Ids: PRJNA904682.

2.4. Prokaryotic community analysis

Demultiplexed FASTQ files, which contained paired forward and reverse reads for each sample, were imported and visualised using QIIME2 (Bolyen et al., 2019). Subsequently, forward and reverse sequences were trimmed to a length of 245 and 200 nt, respectively, using the DADA2 plugin (Callahan et al., 2016). The DADA2 analysis produced a quality filtered table of all operational taxonomic units (OTUs), a fasta file of representative sequences, and a table summarising the denoising statistics. Using the described method, the OTUs generated are equal to exact amplicon sequence variants (ASVs). Following this, the QIIME2 feature-classifier plugin with the extract-reads option was used to extract reads from the Silva database with the silva-138-99-seqs.qza file as input and the forward and reverse PCR primers as parameters. This produced a file of reference sequence reads, which was used as input for the feature-classifier plugin with the fit-classifier-naive-bayes option. The feature-classifier plugin was then used with the classify-sklearn method and the i-reads argument set to the representative sequences file generated by the DADA2 analysis, which produced a table with taxonomic classifications for all OTUs. Mitochondria, chloroplasts, and Eukaryota were filtered out from the obtained OTU table using the QIIME2 taxa plugin with the filter-table method. OTUs with total abundances > 2 observed in the blank controls were also removed prior to subsequent analyses. The DNA sequences generated in this study can be downloaded from NCBI BioProject Ids: PRJNA904682.

2.5. Analyses

Tables containing the OTU counts of all *M. digitata*, *S. glaucum*, and *Chondrilla* sp., and sediment samples were imported into R. The tables consisted of 32 *S. glaucum*, and *Chondrilla* sp. samples, 31 *M. digitata* samples, and 29 sediment samples. Samples with less than 5000 sequences were removed resulting in the lower number of *M. digitata* and sediment samples. Diversity indices were obtained using the rarefy and diversity functions from the vegan package in R (R Core Team, 2022). We tested for significant differences in evenness and rarefied richness with an analysis of deviance with heat stress, UV radiation and HS modulation as treatments using the glm function in R. The optimal generalised linear models for species richness and evenness were determined through a comprehensive evaluation of data support, residual dispersion ratios, and diagnostic visualisations. For rarefied richness, a continuous, positive metric, the Tweedie model was selected using the tweedie function in R with var.power = 1.5 and link.power = 0 (a compound Poisson–gamma distribution) because it achieved a balanced dispersion ratio of alongside clean Q-Q and scale-location plots. In contrast, for species evenness, a bounded proportion on the interval [0, 1], the Quasibinomial model was chosen.

2.6. Neutral model

The neutral model of biodiversity, first formalised as the Unified Neutral Theory of Biodiversity and Biogeography (Hubbell, 2001), proposes that biodiversity patterns can be explained by random processes, rather than by niche differentiation or competition among species. According to this model, individuals within a community are considered functionally equivalent, meaning that species are assumed to have similar birth, death, and dispersal rates (Hubbell, 2001). Here, we used a script provided by Burns et al. (2016) to estimate the migration rate m in addition to providing a goodness of fit measure for Sloan's neutral model. Using a beta distribution, Sloan's model predicts the association between a taxon's mean relative abundance in the

metacommunity and the probability of observing it in a local community. The immigration rate m, which represents the rate at which new microbes enter the community, can be estimated with a nonlinear fit (Sieber et al., 2019). The fit to the neutral model was also tested against an alternative binomial model with the Akaike information criterion (AIC) method (Akaike, 1973). AIC estimates are provided in addition to 95% confidence intervals based on bootstrapping with 1000 repetitions (Burns et al., 2016). Note that a binomial distribution represents the scenario where local communities are simply random selections from the entire metacommunity with no influence from drift or dispersal limitation.

Following Burns et al. (2016), OTUs were categorised into three partitions based on their frequency in hosts compared to neutral model predictions. The "Above" partition consisted of OTUs, which were more frequently observed than expected based on their abundance in the 'metacommunity', which entails that they may be actively selected. The "below" partition consisted of OTUs, which appeared less frequently than expected, possibly indicative of active suppression by the host or pronounced dispersal limitation, and the "neutral" partition consisted of OTUs, which were consistent with model predictions.

3. Results

Prokaryotic evenness was significantly lower ($\Delta_{Deviance} = 0.30$, $F_{1,29} = 10.86$, $p < 0.001$) in *M. digitata* under UVB exposure (Fig. 1), but significantly higher in *M. digitata* ($\Delta_{Deviance} = 0.25$, $F_{1,28} = 9.18$, $p = 0.002$) and sediment ($\Delta_{Deviance} = 0.01$, $F_{1,27} = 20.85$, $p < 0.001$) under HS modulation. There was a marginally significant effect of heat stress on evenness in *S. glaucum* ($\Delta_{Deviance} = 0.24$, $F_{1,30} = 4.10$, $p = 0.043$) with evenness higher under heat stress. There was no significant ($p > 0.05$) effect of heat stress, HS modulation or UVB radiation on the evenness of *Chondrilla* sp. (Supplementary data 2).

Rarefied richness was significantly higher in *M. digitata* ($\Delta_{Deviance} = 22.39$, $F_{1,28} = 12.46$, $p < 0.001$) and sediment ($\Delta_{Deviance} = 18.15$, $F_{1,26} = 7.15$, $p = 0.012$) under HS modulation (Fig. 2 and Supplementary data 3). Rarefied richness was marginally significantly lower in *Chondrilla* sp. under UVB radiation ($\Delta_{Deviance} = 0.63$, $F_{1,29} = 4.60$, $p = 0.030$). There were also significant three-way interactions among heat stress, UVB radiation and HS modulation on the rarefied richness of *Montipora digitata* ($\Delta_{Deviance} = 22.02$, $F_{1,28} = 12.25$, $p < 0.001$), and *Chondrilla* sp. ($\Delta_{Deviance} = 0.82$, $F_{1,24} = 5.99$, $p = 0.014$), and significant two-way interactions between heat stress and HS modulation ($\Delta_{Deviance} = 1.00$, $F_{1,26} = 7.34$, $p = 0.007$), and UVB exposure and HS modulation ($\Delta_{Deviance} = 0.74$, $F_{1,25} = 5.99$, $p = 0.014$) on the rarefied richness of *Chondrilla* sp.

In *M. digitata*, rarefied richness was higher under heat stress and UVB exposure with HS modulation, but lower in its absence. HS modulation, thus, appeared to mitigate the adverse effects of heat stress and UVB radiation on rarefied richness. In *Chondrilla* sp. in the absence of UVB exposure, rarefied richness was higher under heat stress in the absence of HS modulation, but lower in its presence. There was no significant ($p > 0.05$) effect of heat stress, HS modulation or UVB radiation on the rarefied richness of *S. glaucum*.

Cumulative richness was higher under HS supplementation in *Chondrilla* sp., *Montipora digitata*, and sediment, but lower in *S. glaucum* (Fig. 3). The net difference in the number of OTUs per phylum in *M. digitata* with and without HS supplementation is presented in Fig. 4 with positive values indicating a net difference under HS supplementation and negative values a net difference without HS supplementation. There were far greater numbers of OTUs classified to most of the 40 most

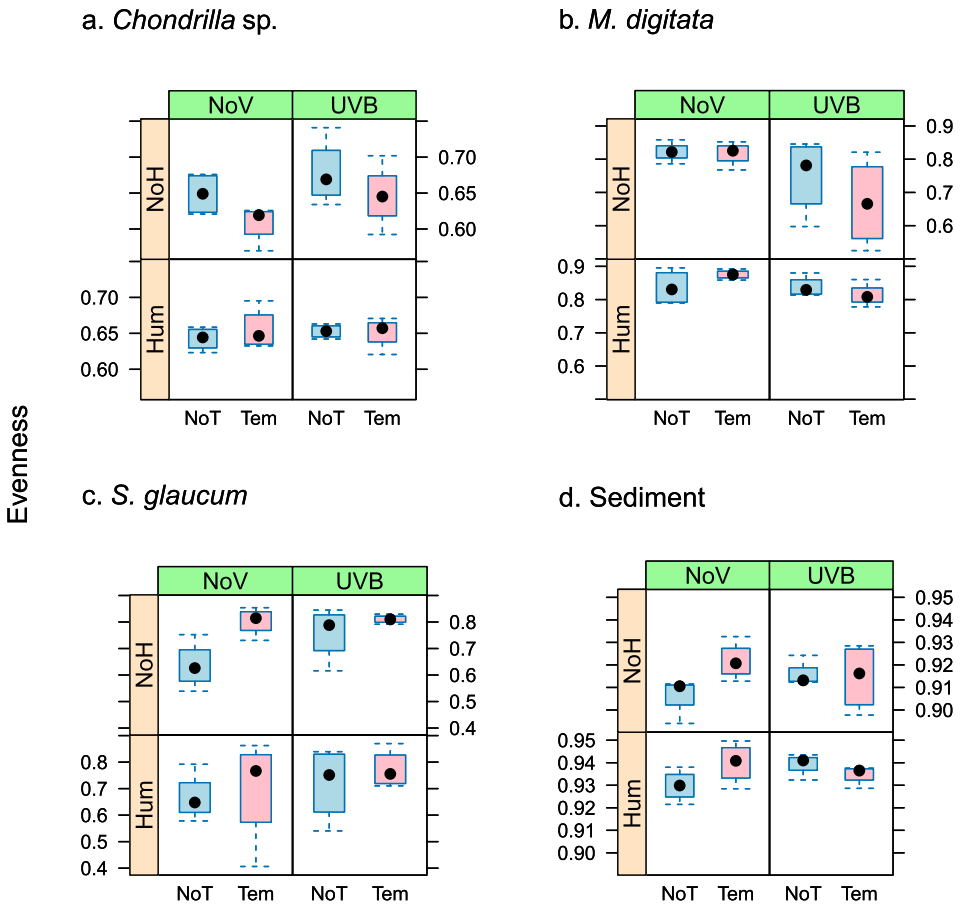


Fig. 1. Boxplots of evenness for a. *Chondrilla* sp., b. *M. digitata*, c. *S. glaucum*, and d. sediment for prokaryotic communities with (Hum) and without (NoH) HS modulation, with (UVB) and without (NoV) UVB exposure, and with (Tem) and without (NoT) heat stress.

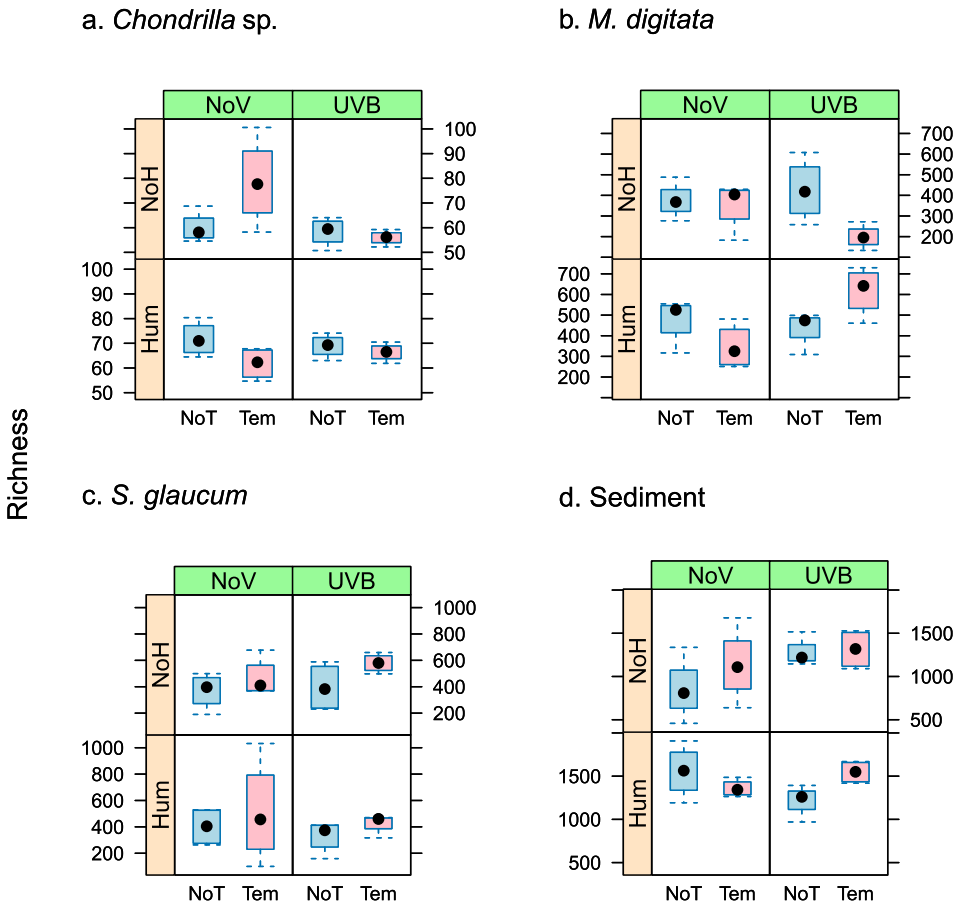


Fig. 2. Boxplots of rarefied richness for a. *Chondrilla* sp., b. *M. digitata*, c. *S. glaucum*, and d. sediment for prokaryotic communities with (Hum) and without (NoH) HS modulation, with (UVB) and without (NoV) UVB exposure, and with (Tem) and without (NoT) heat stress.

abundant orders under HS modulation. The greatest differences in terms of numbers of OTUs in the HS modulated *versus* non-modulated microcosms were in the orders Pirellulales, Verrucomicrobiales, Microtrichales, Chitinophagales, OM190, Planctomycetales, Cellvibrionales, Cytophagales, Rhodobacterales, and NB1−j.

Fits to the neutral model were $R^2 = 0.547$ (without HS) and $R^2 = 0.596$ (with HS) for *S. glaucum*, $R^2 = 0.303$ (without HS) and $R^2 = 0.636$ (with HS) for *M. digitata*, $R^2 = 0.806$ (without HS) and $R^2 = 0.840$ for sediment, and $R^2 = 0.922$ (without HS) and $R^2 = 0.948$ (with HS) for *Chondrilla* sp.(Supplementary data 4). When comparing the neutral and binomial models, the neutral model provided substantially better fits for all biotopes indicated by lower AIC values. The estimated immigration rates "m" were higher in all biotopes in the presence of HS and varied from 0.014 ± 0.001 (without HS) and 0.025 ± 0.001 (with HS) for *M. digitata*, 0.030 ± 0.002 (without HS) and 0.035 ± 0.002 (with HS) for *S. glaucum*, 0.145 ± 0.004 (without HS) and 0.227 ± 0.006 (with HS) for sediment, and 0.146 ± 0.031 (without HS) and 0.375 ± 0.077 (with HS) for *Chondrilla* sp. (Supplementary data 4).

Fig. 5 illustrates these fit differences across conditions, depicting three partitions of operational taxonomic units (OTUs) according to their alignment with neutral model predictions. These partitions include: (1) the "neutral" partition (green symbols), where OTUs fall within a 95% confidence interval around the model prediction (black line), (2) the "above" partition (blue symbols) for OTUs more frequently observed than predicted by their abundances, and (3) the "below" partition (red symbols) for OTUs less frequently observed than expected. OTUs within the neutral partition conformed to neutral model predictions, while those in the above and below partitions indicated significant deviations from these predictions. The presence of HS appeared to enhance the predictability of prokaryotic distributions by the neutral model.

4. Discussion

The presence of HS significantly influenced the rarefied richness of *M. digitata*-associated and sediment prokaryotic communities in addition to having interactive effects on the rarefied richness of the *Chondrilla* sp.-associated prokaryotic community, aligning with our hypotheses. There was, however, no significant effect on the rarefied richness of *S. glaucum*. HS modulation was also associated with a better alignment with neutral model predictions in all biotopes although this was most pronounced with *M. digitata*. Our previous study (Stuij et al., 2025), furthermore, showed that HS buffered the effects of heat and UVB radiation on coral photo-oxidative stress, crucial given the roles of heat stress and UVB radiation in coral bleaching and symbiont diversity shifts (Lesser, 1996). Prokaryotic communities play vital roles in coral health and resilience, with environmental disturbances potentially reshaping microbiomes in ways that influence coral acclimatisation and survival (Tran, 2022).

Previous studies have demonstrated that coral bleaching can affect microbial communities associated with corals, although the extent and nature of these changes can vary (Pootakham et al., 2019; Zou et al., 2024; Sun et al., 2022). These microbiome shifts can affect coral health and environmental adaptability, potentially increasing disease risk (Sun et al., 2022). In the absence of disturbance, Dunphy et al. (2019) showed that various coral species possessed signature microbiomes that remained stable, with host identity playing a dominant role in structuring the microbial community (Dunphy et al., 2019). Coral microbiomes, however, did vary across space.

In addition to a significant independent effect of HS on the rarefied

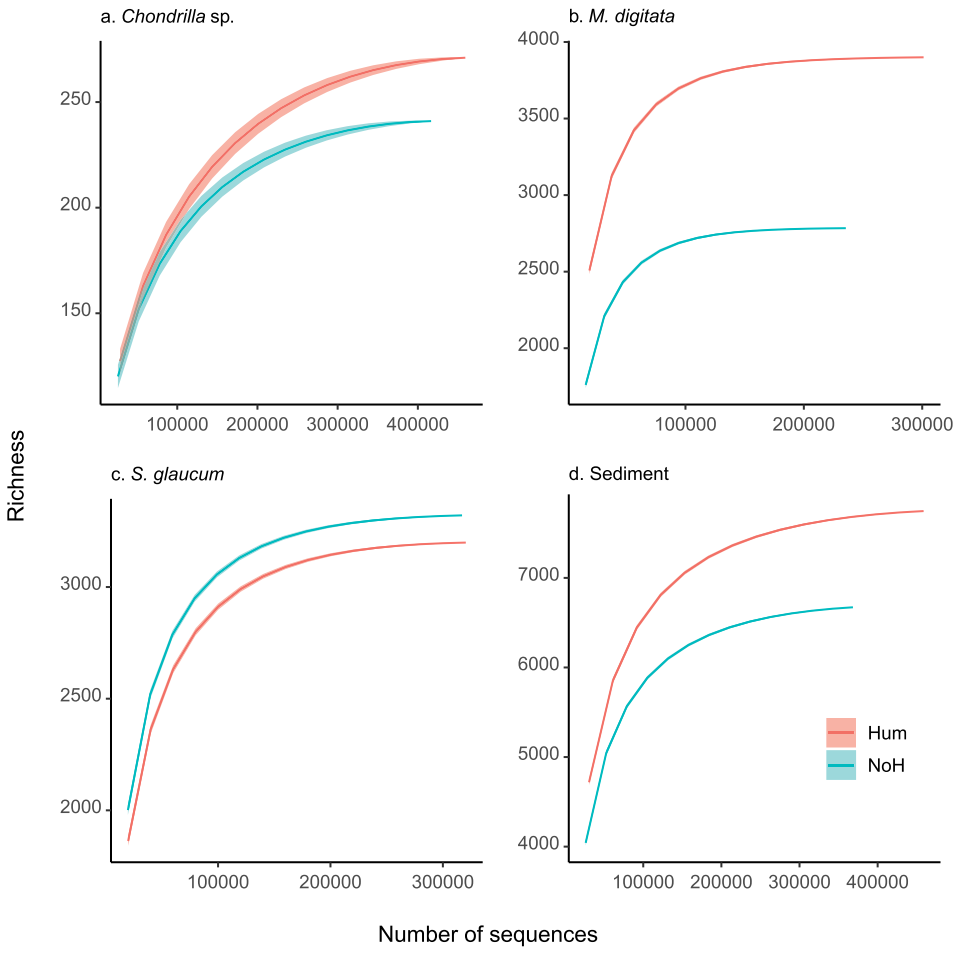


Fig. 3. Cumulative\ richness curves showing the number of OTUs as a function of the number of sequences for microcosms with (Hum) and without (NoH) HS modulation for a. *Chondrilla* sp., b. *M. digitata*, c. *S. glaucum*, and d. sediment.

richness of *M. digitata*-associated prokaryotic communities, we also identified a significant interactive effect of heat stress, UVB exposure and HS modulation. In particular, UVB exposure was associated with increased rarefied richness in the presence of HS, but lower richness in its absence.

In line with *M. digitata*, prokaryotic communities in sediment were also more diverse in HS modulated microcosms. Cumulative richness was also much higher for *M. digitata*, *Chondrilla* sp. and sediment in HS-modulated microcosms. The question arises as to the mechanism of impact of HS on the diversity of host-associated and sediment prokaryotic communities. HS in the water column may, for example, directly stimulate bacterial growth and inoculate the coral mucus surface. Alternatively, HS may stimulate bacterial growth in sediment and indirectly influence *M. digitata*-associated prokaryotic communities through resuspension of sediment and colonisation of the coral surface. Previous studies have shown corals to share microorganisms with sediment. Carlos et al. (2013), for example, observed that coral mucus shared more OTUs with sediment than with water and suggested that sediment may act as a microbial seed bank. Likewise, Hamamoto et al. (2024) highlighted the potential importance of sediment as a reservoir of microbial taxa for coral reef ecosystems. Cleary et al. (2019) also showed that corals and sediment were more similar and shared more OTUs than corals and seawater.

Sediment acts as a seed bank housing a vast diversity of microbial taxa, many of which are extremely rare or undetected in the overlying water column (the rare biosphere; Lynch and Neufeld, 2015). This reservoir of genetic and functional diversity, contributes to the stability and adaptability of marine ecosystems (Jørgensen and Boetius, 2007). They also contribute to critical processes such as carbon storage, nutrient cycling, and organic matter decomposition (Gaidos et al., 2011; LaRowe et al., 2020). Members of the rare biosphere may also be important drivers of microbiome function and play roles in biogeochemical cycles out of proportion to their abundance (Jousset et al., 2017). Sediments also contain a mix of active, dormant, and dead microbes. Dormant cells can persist for extended periods, ready to activate when conditions such as nutrient availability, oxygen levels, or temperature change, ensuring continuity of ecosystem processes (Lomstein et al., 2012; Orsi, 2018). Resuspension can, furthermore, mobilise microbes from sediments back into the water column, spreading microbial diversity across regions and supporting ecosystem connectivity. The higher cumulative richness across *M. digitata* and sediment in HS-modulated microcosms suggests that HS exerts ecosystem-wide effects, likely promoting the development of less dominant microbial members within these interconnected biotopes.

Of the 40 most abundant orders, most were more speciose under HS modulation. This included the orders Pirellulales, Verrucomicrobiales, Microtrichales, Chitinophagales, OM190, Planctomycetales, Cellvibrionales, Cytophagales, Rhodobacterales, and NB1−j. The order Pirellulales (Phylum: Planctomycetes) consists of Gram-negative, budding, and chemoheterotrophic members (Dedysh, 2025). They are reported to be ubiquitous in aquatic environments and are often found in anoxic or micro-oxic environments (Dedysh et al., 2020). The order Pirellulales had the greatest difference in OTU number between HS-modulated and non-modulated microcosms. Other Planctomycetes orders enriched in terms of OTU number under HS modulation included OM190 and Planctomycetales. Members of the OM190 order have been shown to be

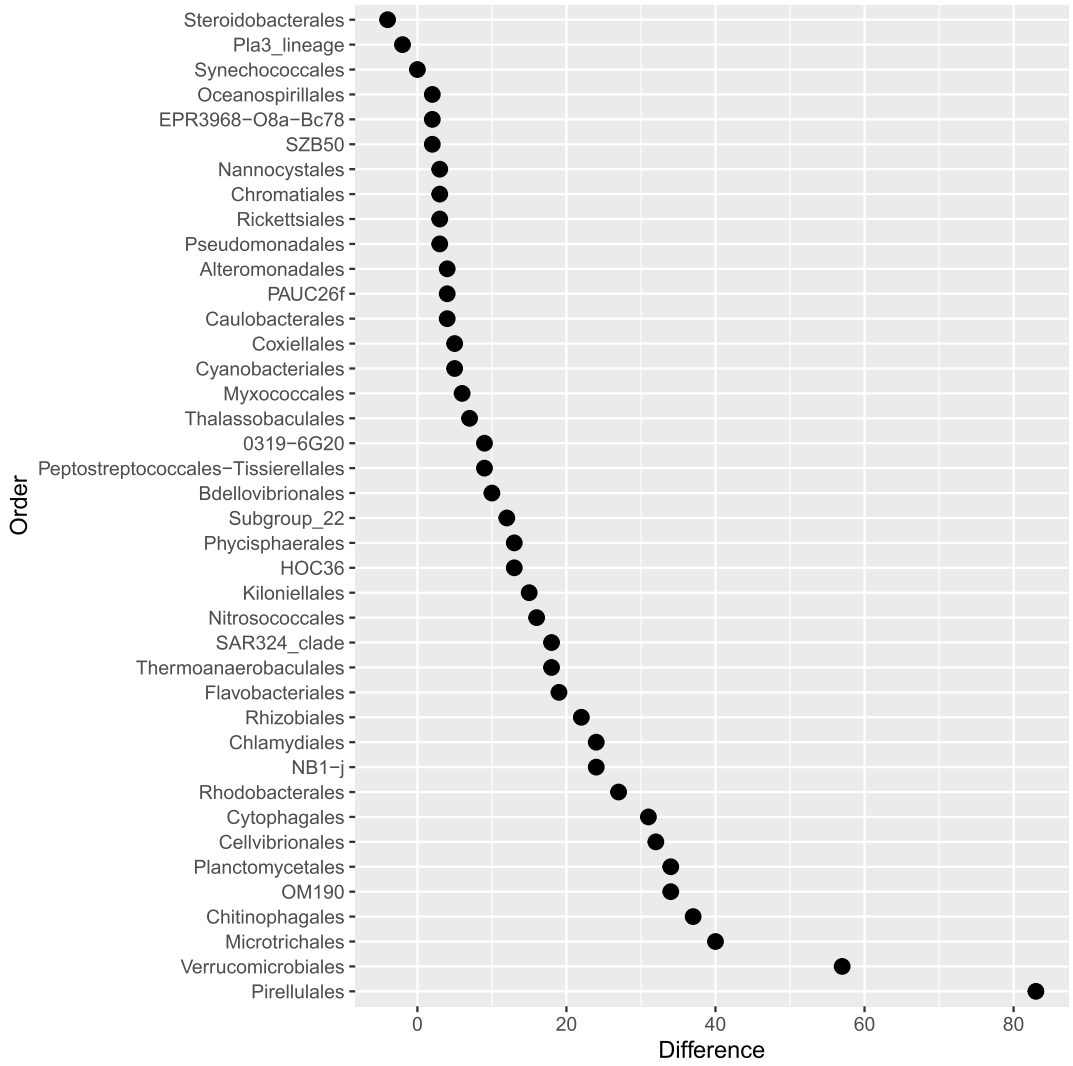


Fig. 4. Differences in the total number of OTUs per order sampled from *M. digitata* in microcosms with (Hum) and without (NoH) HS supplementation. Positive numbers indicate a difference in favour of HS-modulated microcosms whereas negative values indicate a difference in favour of no HS supplementation.

dominant components on the surfaces of macroalgae (Bondoso et al., 2017) while Planctomycetales have been reported as key players in global carbon and nitrogen cycles (Wiegand et al., 2018). The Bacteroidetes orders Chitinophagales and Cytophagales include members capable of degrading complex biopolymers such as chitin (Kirchman, 2002; Rangel et al., 2022) while the proteobacterial Rhodobacterales includes several potentially beneficial bacteria, e.g., members of the *Roseobacter* clade (Brinkhoff et al., 2008; Porsby et al., 2008; Sonnenschein et al., 2021). The marked enrichment in the numbers of species of these orders under HS modulation suggests a substantial enrichment of diverse functional roles. By providing substrate for a diverse range of functionally distinct bacterial lineages, HS potentially contribute to microbiome homeostasis and resilience to environmental stress as exemplified by the mitigation of heat stress on coral-associated photosynthetic activity (Stuij et al., 2025).

The biotopes tested in the present study differed in their alignment to neutral model predictions with the best fits observed in sponge and sediment communities and the worst fits in both coral communities. There was, however, a marked difference in *M. digitata*-associated prokaryotic communities dependent on HS modulation. *Montipora digitata*-associated prokaryotic communities largely aligned with neutral model predictions in the presence of HS despite being subjected to heat stress and UVB radiation. In contrast, in the absence of HS modulation, these communities deviated from neutral model predictions.

Estimated migration rates were higher for sediment than for host-associated prokaryotic communities. The estimated migration rates were more than twice as high for *M. digitata*-associated and sediment-associated prokaryotic communities under HS modulation. In Hubbell's neutral theory (2001), the migration rate 'm' represents the probability of a species migrating into a local community from a larger metacommunity. It reflects the degree of connectivity between the local community and the surrounding metacommunity. A higher migration rate indicates that the local community is more open to immigration, leading to higher species turnover and increased similarity with the metacommunity. Conversely, a lower rate implies limited immigration, making the local community more dependent on local processes such as speciation and extinction. The migration rate balances local ecological drift and regional dispersal. When high, local species richness and composition are heavily influenced by the metacommunity. When the migration rate is low, local dynamics dominate, and the community becomes more isolated. Immigration helps maintain species diversity in local communities by counteracting the effects of ecological drift, which tends to reduce diversity over time in isolated systems.

In the absence of HS, disturbances such as thermal stress in combination with intense UVB radiation may significantly impact coral microbiomes (Downs et al., 2002) and may also lead to deviations from neutral model predictions of community structure by introducing selective pressures that contrast with the assumptions of neutrality. In

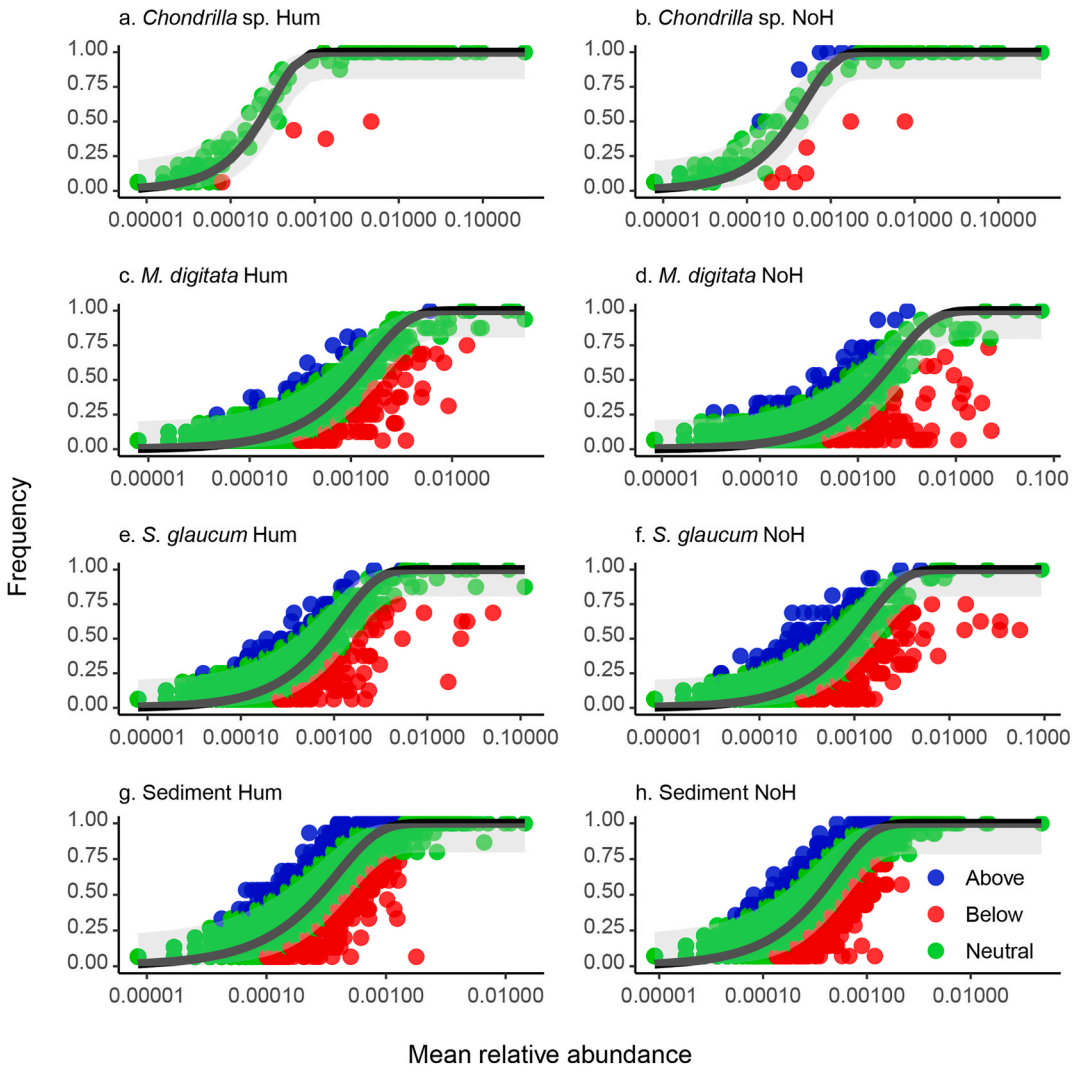


Fig. 5. Neutral model fit for prokaryotic communities of a. *Chondrilla* sp. in the presence of HS, b. *Chondrilla* sp. in the absence of HS, c. *M. digitata* in the presence of HS, d. *M. digitata* in the absence of HS, e. *S. glaucum* in the presence of HS, f. *S. glaucum* in the absence of HS, g. sediment in the presence of HS, and h. sediment in the absence of HS. The black line represents the model fit. Symbols in green represent OTUs within the 95% confidence interval of the neutral model fit (neutral partition). Symbols in blue represent OTUs above the 95% confidence interval (above partition) and symbols in red represent OTUs below the 95% confidence interval (below partition).

neutral models, species are assumed to have similar fitness and equal probability of reproduction, death, and dispersal, which leads to community structure being shaped primarily by stochastic processes such as ecological drift and dispersal limitation (Hubbell, 2001). However, disturbances such as habitat loss, heat stress, pollution, or species invasions often disrupt these processes in several ways. Disturbances, for example, can introduce or amplify selective pressures, causing certain species to outcompete others due to their unique traits or environmental tolerances (Tilman, 2004). For example, after a disturbance, only species with specific resistance or resilience traits may survive, leading to community shifts that neutral models cannot fully predict. This selective pressure results in community assemblages that reflect environmental filtering rather than neutral processes. Neutral models rely on immigration from a metacommunity to maintain diversity (Sloan et al., 2006). Disturbances, however, can fragment habitats or create physical barriers that reduce dispersal, thereby isolating communities and altering species composition in ways that differ from neutral predictions (Leibold et al., 2004). Disturbances often intensify ecological drift by increasing local extinctions, particularly of rare species, leading to reduced biodiversity. This impacts community dynamics by decreasing overall community resilience and shifting the community structure to reflect the dominance of a few species rather than a neutral balance (Chase, 2007). Disturbances can alter resource distribution and availability, which can lead to niche differentiation among species and reduce the randomness predicted by neutral models. Species may start to diverge in their ecological roles and functions, reducing the homogeneity in fitness that neutral models assume (Chesson, 2000). Disturbances, therefore, often shift community structure toward a more deterministic outcome based on environmental filtering and competitive interactions rather than the randomness neutral models suggest. The altered conditions post-disturbance can lead to more predictable patterns based on species traits and environmental compatibility, diverging from the outcomes expected under neutral assumptions.

The question arises as to why HS appear to foster a community consistent with neutral model predictions in *M. digitata* and increase the estimated migration rate suggesting enhanced connectivity and what are the implications within a microcosm setting. Neutral theory posits that community composition is influenced mainly by stochastic events such as random dispersal and ecological drift rather than by strong selective forces. HS can contribute to a neutral community structure by providing a stable yet diffuse source of organic matter that is less selective in its promotion of specific microbial growth. HS are highly complex,

recalcitrant, that is resistant to immediate microbial degradation, mixtures, containing a vast array of molecular structures including aromatic rings, aliphatic chains, and nitrogen-containing compounds (Piccolo, 2001; Calvo et al., 2014; Lipczynska-Kochany, 2018). Although certain bacterial taxa cannot directly degrade many of these compounds, some specialised bacterial taxa have evolved metabolic pathways to utilise them, particularly under low-energy conditions (Arrieta et al., 2015). Specific bacteria capable of degrading recalcitrant compounds, such as members of the Actinobacteria, Chloroflexi and Proteobacteria, can become abundant in ecosystems rich in these compounds (Kujawinski, 2011; Saw et al., 2020). Certain bacteria also produce extracellular enzymes such as peroxidases and laccases to break down recalcitrant compounds, making smaller, more accessible molecules available for uptake and metabolism. This enzymatic activity is often coupled to other biogeochemical processes (Azam and Malfatti, 2007). Because of their structural complexity, HS can support diverse microbes with potentially overlapping metabolic capabilities, creating a level of redundancy where multiple species are capable of using available resources (Kellerman et al., 2014). This redundancy may reduce strongly selective pressures as individual species do not outcompete others due to specific resource advantages. As a result, microbial populations may persist and fluctuate more by random birth-death processes (ecological drift) rather than by competition-driven exclusion, which is characteristic of communities fitting neutral model predictions.

An important nuance in interpreting neutral community dynamics is the causal relationship between environmental stress alleviation and assembly processes. While our findings indicate that HS modulation shifts community assembly toward greater neutrality, it is critical to distinguish whether HS actively engineers neutral conditions or whether it functions by alleviating environmental stressors (e.g., acute heat stress and UVB radiation). In stressed ecosystems, strong deterministic filtering typically drives compositional homogenisation by selecting for specialised stress-tolerant taxa. By buffering these environmental extremes, HS reduces harsh selective gradients, allowing the system to revert towards a state where stochastic immigration, birth-death dynamics, and neutral drift predominate. Consequently, increased neutrality in HS-modulated systems can be interpreted as a downstream symptom of environmental stability and stress release, reflecting the recovery of a flexible, resilient baseline rather than a direct deterministic driver of community structure.

Taken together, our findings suggest that HS play a potentially important role in structuring both environmental and host-associated prokaryotic communities. Specifically, HS appear to enhance community richness and evenness. Our results are consistent with previous experimental and observational studies indicating a protective effect of HS or CDOM against coral bleaching (Otis et al., 2004; Zepp et al., 2008; Shank et al., 2010; Ayoub et al., 2012; Oxenford and Vallès, 2016; Ranith et al., 2016; Stuij et al., 2025). These earlier studies, however, primarily focused on the photo-protective properties of CDOM against UVB radiation. Here, we highlight an additional potential mechanism, namely, the enhancement of richness and neutral community dynamics in host-associated and environmental prokaryotic communities.

Such enrichment may support host health by promoting microbiome homeostasis, resilience to environmental stress, and resistance to pathogen invasion. Greater prokaryotic richness provides a broader pool of functional traits and ecological roles, buffering the system against environmental fluctuations (Tilman et al., 1997; Allison, 2004; Downing and Leibold, 2010; De Roy et al., 2013). This diversity fosters functional redundancy, ensuring that essential ecosystem functions persist even when specific taxa are lost (Loreau et al., 2001). Moreover, a diverse prokaryotic community creates competitive interactions that limit pathogen establishment while enhancing recovery and stabilisation following disturbances (Elton, 1958; Walker et al., 1999; Levine et al., 2004). Finally, the maintenance of neutral dynamics, diversity sustained by stochastic rather than niche-based processes, supports long-term ecosystem stability and homeostasis. Collectively, these factors highlight the potential of HS to promote both microbial diversity and host ecosystem health over time.

While our microcosm and amplicon sequencing approach provide robust insights into multi-taxon host-microbiome interactions, several limitations of our study should be noted. First, 16S rRNA gene amplicon sequencing captures only a fraction of the total bacterial community under highly controlled conditions and during a limited time interval. Second, while marker-gene profiling effectively characterises broader taxonomic shifts, it inherently lacks the strain-level resolution and functional metabolic tracking offered by shotgun metagenomics. Finally, although controlled experimental conditions isolate the interactive effects of targeted stressors/modulators, natural marine habitats involve complex, fluctuating gradients of temperature, salinity, and biological interactions that can alter the magnitude of both biomarker responses and microbiome restructuring.

5. Conclusion

The use of microcosms enables the controlled manipulation of environmental conditions, allowing researchers to isolate specific variables and test hypotheses that would be impossible to examine within the complexity of natural reef ecosystems. Aligned with our hypotheses, we have shown that the presence of HS had a significant impact on microbial community evenness, rarefied richness, and alignment with neutral model predictions. *Montipora digitata*-associated rarefied richness and evenness were both higher in the presence of HS. We, furthermore, identified a highly significant interaction between heat stress, UV radiation and HS modulation on coral-associated prokaryotic rarefied richness. The presence of UVB radiation and HS together was associated with increased rarefied richness under heat stress conditions, while the reverse held in the absence of HS. The neutral model also fit *M. digitata*-associated prokaryotic communities more effectively under HS-modulation and with higher immigration rates, suggesting that HS facilitates microbial distributions aligning with neutral model predictions. Together with our previous study (Stuij et al., 2025), our findings demonstrate that HS can significantly shape certain host-associated prokaryotic communities and may play an essential role in mitigating the effects of heat stress on corals. Future work should test to what extent this holds for other coral reef species and to develop methods based on HS modulation that can be applied to increase coral resistance and resilience to heat stress. The susceptibility of many coral reefs to bleaching may be a result of the loss of HS inputs into the marine environment due to the loss of mangroves, seagrass beds, and coastal, riparian forests.

CRediT authorship contribution statement

Daniel F.R. Cleary: Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Tamara M. Stuij:** Writing – review & editing, Investigation. **Davide A. Machado e Silva:** Writing – review & editing, Investigation, Conceptualization. **Antonio Louvado:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Vanessa Oliveira:** Writing – review & editing, Investigation. **Rui J.M. Rocha:** Writing – review & editing, Methodology, Conceptualization. **Yusheng M. Huang:** Writing – review & editing, Resources. **Newton C.M. Gomes:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Conceptualization. **Nicole J. de Voogd:** Writing – review & editing, Supervision, Resources, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2026.110345.

Data availability

Sequences generated in this study can be downloaded from the NCBI Sequence Read Archive under the BioProject accession number PRJNA904682.

References

Aeschbacher, M., Graf, C., Schwarzenbach, R.P., Sander, M., 2012. Antioxidant properties of humic substances. Environ. Sci. Technol. 46, 4916–4925. https://doi.org/10.1021/es300039h.

Alessi, C., Randall, C.J., Dumas, M., Lemonnier, H., Rodolfo-Metalpa, R., 2024. Spawning window and fecundity in three Acroporid corals from the environmentally variable semi-enclosed lagoon of Bouraké. Coral Reefs 43, 1469–1482. https://doi.org/10.1007/s00338-024-02551-3.

Allison, G., 2004. The influence of species diversity and stress intensity on community resistance and resilience. Ecol. Monogr. 74, 117–134. https://doi.org/10.1890/02-0681.

Amon, R.M., Benner, R., 1996. Bacterial utilization of different size classes of dissolved organic matter. Limnol. Oceanogr. 41, 41–51. https://doi.org/10.4319/lo.1996.41.1.0041.

Arillo, A., Bavestrello, G., Burlando, B., Sarà, M., 1993. Metabolic integration between symbiotic Cyanobacteria and sponges: a possible mechanism. Mar Biol 117, 159–162. https://doi.org/10.1007/BF00346438.

Arrieta, J.M., Mayol, E., Hansman, R.L., Herndl, G.J., Dittmar, T., Duarte, C.M., 2015. Ocean chemistry. Dilution limits dissolved organic carbon utilization in the deep ocean. Science 348, 331–333. https://doi.org/10.1126/science.1258955.

Ayoub, L.M., Hallock, P., Coble, P.G., Bell, S.S., 2012. MAA-like absorbing substances in Florida Keys phytoplankton vary with distance from shore and CDOM: implications for coral reefs. J. Exp. Mar. Biol. Ecol. 420, 91–98. https://doi.org/10.1016/j.jembe.2012.03.026.

Azam, F., Malfatti, F., 2007 Oct. Microbial structuring of marine ecosystems. Nat. Rev. Microbiol. 5 (10), 782–791. https://doi.org/10.1038/nrmicro1747. Erratum in: Nat Rev Microbiol. 2007 Dec;5(12):966.

Baker, D.M., Freeman, C.J., Wong, J.C.Y., Fogel, M.L., Knowlton, N., 2018. Climate change promotes parasitism in a coral symbiosis. ISME J. 12, 921–930. https://doi.org/10.1038/s41396-018-0046-8.

Barbier, E.B., Hacker, S.D., Kennedy, C., Koch, E.W., Stier, A.C., Silliman, B.R., 2011. The value of estuarine and coastal ecosystem services. Ecol. Monogr. 81, 169–193. https://doi.org/10.1890/10-1510.1.

Battin, T.J., Kaplan, L.A., Newbold, J.D., Cheng, X., Hansen, C., 2003. Effects of current velocity on the nascent architecture of stream microbial biofilms. Appl. Environ. Microbiol. 69, 5443–5452. https://doi.org/10.1128/AEM.69.9.5443-5452.2003.

Bhagooli, R., Yakovleva, I., 2004. Differential bleaching susceptibility and mortality patterns among four corals in response to thermal stress. Symbiosis 37, 121–136.

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. Nat. Biotechnol. 37, 852–857. https://doi.org/10.1038/s41587-019-0209-9.

Bondoso, J., Godoy-Vitorino, F., Balagué, V., Gasol, J.M., Harder, J., Lage, O.M., 2017. Epiphytic Planctomycetes communities associated with three main groups of macroalgae. FEMS Microbiol. Ecol. 93, fiw255. https://doi.org/10.1093/femsec/fiw255.

Bourne, D.G., Morrow, K.M., Webster, N.S., 2016. Insights into the coral microbiome: underpinning the health and resilience of reef ecosystems. Annu. Rev. Microbiol. 70, 317–340. https://doi.org/10.1146/annurev-micro-102215-095440.

Brinkhoff, T., Giebel, H.A., Simon, M., 2008. Diversity, ecology, and genomics of the *Roseobacter* clade: a short overview. Arch. Microbiol. 189, 531–539. https://doi.org/10.1007/s00203-008-0353-y.

Brown, B.E., Dunne, R.P., Scoffin, T.P., Le Tissier, M.D.A., 1994. Solar damage in intertidal corals. Mar. Ecol. Prog. Ser. 105, 219–230. https://doi.org/10.3354/meps105219.

Burns, A.R., Stephens, W.Z., Stagaman, K., Wong, S., Rawls, J.F., Guillemin, K., Bohannan, B.J.M., 2016. Contribution of neutral processes to the assembly of gut microbial communities in the zebrafish over host development. ISME J. 10, 655–664. https://doi.org/10.1038/ismej.2015.142.

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. Nat. Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869.

Calvo, P., Nelson, L., Kloepper, J.W., 2014. Agricultural uses of plant biostimulants. Plant Soil 383, 3–41. https://doi.org/10.1007/s11104-014-2131-8.

Canellas, L.P., Olivares, F.L., 2014. Physiological responses to humic substances as plant growth promoter. Chem. Biol. Technol. Agric. 1, 3. https://doi.org/10.1186/2196-5641-1-3.

Carlos, C., Torres, T.T., Ottoboni, L.M., 2013. Bacterial communities and species-specific associations with the mucus of Brazilian coral species. Sci. Rep. 3, 1624. https://doi.org/10.1038/srep01624.

Carlsson, P., Granéli, E., Tester, P., Boni, L., 1995. Influences of riverine humic substances on bacteria, protozoa, phytoplankton, and copepods in a coastal plankton community. Mar. Ecol. Prog. Ser. 127, 213–221. https://doi.org/10.3354/meps127213.

Carroll, A.R., Copp, B.R., Grkovic, T., Keyzers, R.A., Prinsep, M.R., 2024. Marine natural products. Nat. Prod. Rep. 41, 162–207. https://doi.org/10.1039/d3np00061c.

Chase, J.M., 2007. Drought mediates the importance of stochastic community assembly. Proc. Natl. Acad. Sci. U. S. A. 104, 17430–17434. https://doi.org/10.1073/pnas.0704350104.

Chesson, P., 2000. Mechanisms of maintenance of species diversity. Annu. Rev. Ecol. Evol. Syst 31, 343–366. https://doi.org/10.1146/annurev.ecolsys.31.1.343.

Cleary, D.F.R., de Voogd, N.J., 2024. The impact of space, host dissimilitude, and environment on prokaryotic communities of golf ball sponges. Mar. Ecol. 45, e12815. https://doi.org/10.1111/maec.12815.

Cleary, D.F.R., de Voogd, N.J., Stuij, T., Swierts, T., Oliveira, V., Polónia, A.R.M., Louvado, A., Gomes, N.C.M., Coelho, F.J.R.C., 2023. A study of sponge symbionts from different light habitats. Microb. Ecol. 86, 2819–2837. https://doi.org/10.1007/s00248-023-02267-x.

Cleary, D.F.R., Gomes, N.C.M., Ambo-Rappe, R., de Voogd, N.J., 2025. The impact of coral reef habitat and microbial abundance status on sponge-associated prokaryotic communities. Coral Reefs 44, 15–30. https://doi.org/10.1007/s00338-024-02568-8.

Cleary, D.F.R., Polónia, A.R.M., de Voogd, N.J., 2021. Composition and diversity of prokaryotic communities sampled from sponges and soft corals in Maldivian waters. Mar. Ecol. 42, e12638. https://doi.org/10.1111/maec.12638.

Cleary, D.F.R., Polónia, A.R.M., Reijnen, B.T., Berumen, M.L., de Voogd, N.J., 2020. Prokaryote communities inhabiting endemic and newly discovered sponges and octocorals from the Red Sea. Microb. Ecol. 80, 103–119. https://doi.org/10.1007/s00248-019-01465-w.

Cleary, D.F.R., Polónia, A.R.M., Swierts, T., Coelho, F.J.R.C., de Voogd, N.J., Gomes, N.C.M., 2022. Spatial and environmental variables structure sponge symbiont communities. Mol. Ecol. 31, 4932–4948. https://doi.org/10.1111/mec.16631.

Cleary, D.F.R., Swierts, T., Coelho, F.J.R.C., Polónia, A.R.M., Huang, Y.M., Ferreira, M.R.S., Putchakarn, S., Carvalheiro, L., van der Ent, E., Ueng, J.P., Gomes, N.C.M., de Voogd, N.J., 2019. The sponge microbiome within the greater coral reef microbial metacommunity. Nat. Commun. 10, 1644. https://doi.org/10.1038/s41467-019-09537-8.

Cleary, D.F.R., van Bendegom, D.J., Gomes, N.C.M., de Voogd, N.J., 2024. Abundance and composition of sponge-associated microbes in the Twilight zone of Curaçao. Symbiosis 93, 81–97. https://doi.org/10.1007/s13199-024-00992-6.

Coelho, F.J.R.C., Rocha, R.J.M., Pires, A.C.C., Ladeiro, B., Castanheira, J.M., Costa, R., Almeida, A., Cunha, Â., Lillebø, A.I., Ribeiro, R., Pereira, R., Lopes, I., Marques, C., Moreira-Santos, M., Calado, R., Cleary, D.F.R., Gomes, N.C.M., 2013. Development and validation of an experimental life support system for accessing the effects of global climate change on marine benthic communities. Glob. Change Biol. 19, 2584–2595. https://doi.org/10.1111/gcb.12227.

Cooper, L.W., Benner, R., McClelland, J.W., Peterson, B.J., Holmes, R.M., et al., 2005. Linkages among runoff, dissolved organic carbon, and the stable oxygen isotope composition of seawater and other water mass indicators in the Arctic Ocean. J. Geophys. Res. 110, G02013. https://doi.org/10.1029/2005JG000031.

De Roy, K., Marzorati, M., Negroni, A., Thas, O., Balloi, A., Fava, F., Verstraete, W., Daffonchio, D., Boon, N., 2013. Environmental conditions and community evenness determine the outcome of biological invasion. Nat. Commun. 4, 1383. https://doi.org/10.1038/ncomms2392.

Dedysh, S.N., 2025. Pirellulales. In: Trujillo, M.E., Dedysh, S., DeVos, P., Hedlund, B., Kämpfer, P., Rainey, F.A., Whitman, W.B. (Eds.), Bergey's Manual of Systematics of Archaea and Bacteria. https://doi.org/10.1002/9781118960608.obm00179.

Dedysh, S.N., Kulichevskaya, I.S., Beletsky, A.V., Ivanova, A.A., Rijpstra, W.I.C., Damsté, J.S.S., Mardanov, A.V., Ravin, N.V., 2020. Lacipirellula parvula gen. nov., sp. Nov., representing a lineage of planctomycetes widespread in low-oxygen habitats, description of the family Lacipirellulaceae fam. nov. and proposal of the orders Pirellulales ord. nov., Gemmatales ord. nov. and Isosphaerales ord. nov. Syst. Appl. Microbiol. 43, 126050. https://doi.org/10.1016/j.syapm.2019.126050.

Diaz, M.C., Ward, B.B., 1997. Sponge-mediated nitrification in tropical benthic communities. Mar. Ecol. Prog. Ser. 156, 97–107. https://doi.org/10.3354/meps156097.

Downing, A.L., Leibold, M.A., 2010. Species richness facilitates ecosystem resilience in aquatic food webs. Freshw. Biol. 55, 2123–2137. https://doi.org/10.1111/j.1365-2427.2010.02472.x.

Downs, C.A., Fauth, J.E., Halas, J.C., Dustan, P., Bemiss, J., Woodley, C.M., 2002. Oxidative stress and seasonal coral bleaching. Free Radic. Biol. Med. 33, 533–543. https://doi.org/10.1016/s0891-5849(02)00907-3.

Dubinsky, Z., Stambler, N., 1996. Marine pollution and coral reefs. Glob. Change Biol. 2, 511–526. https://doi.org/10.1111/j.1365-2486.1996.tb00064.x.

Dunphy, C.M., Gouhier, T.C., Chu, N.D., Vollmer, S.V., 2019. Structure and stability of the coral microbiome in space and time. Sci. Rep. 9, 6785. https://doi.org/10.1038/s41598-019-43268-6.

Eaton, A., 1995. Measuring UV-absorbing organics: a standard method. J. Am. Water Works Assoc. 87 (2), 86–90.

Elton, C.S., 1958. The Ecology of Invasions by Animals and Plants. Springer, Dordrecht, p. 181. https://doi.org/10.1007/978-94-009-5851-7.

Evangelou, M.W.H., Daghan, H., Schaeffer, A., 2004. The influence of humic acids on the phytoextraction of cadmium from soil. Chemosphere 57, 207–213. https://doi.org/10.1016/j.chemosphere.2004.06.017.

Ferrario, F., Beck, M.W., Storlazzi, C.D., Micheli, F., Shepard, C.C., Airoldi, L., 2014. The effectiveness of coral reefs for coastal hazard risk reduction and adaptation. Nat. Commun. 5, 3794. https://doi.org/10.1038/ncomms4794.

Fitt, W., Brown, B., Warner, M., Dunne, R., 2001. Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. Coral Reefs 20, 51–65. https://doi.org/10.1007/s003380100146.

Gaidos, E., Rusch, A., Ilardo, M., 2011. Ribosomal tag pyrosequencing of DNA and RNA from benthic coral reef microbiota: community spatial structure, rare members and nitrogen-cycling guilds. Environ. Microbiol. 13, 1138–1152. https://doi.org/10.1111/j.1462-2920.2010.02422.x.

Gomez, E.D., Yap, H.T., Cabaitan, P.C., Dizon, R.M., 2011. Successful transplantation of a fragmenting coral, Montipora digitata, for reef rehabilitation. Coast. Manag. 39, 556–574. https://doi.org/10.1080/08920753.2011.600240.

Hamamoto, K., Mizuyama, M., Nishijima, M., Maeda, A., Gibu, K., Poliseno, A., Iguchi, A., Reimer, J.D., 2024. Diversity, composition and potential roles of sedimentary microbial communities in different coastal substrates around subtropical Okinawa Island, Japan. Environ Microbiome 19, 54. https://doi.org/10.1186/s40793-024-00594-1.

Hertkorn, N., Benner, R., Frommberger, M., Witt, M., Kaiser, K., Kettrup, A., Hedges, J.I., 2006. Characterization of a major refractory component of marine dissolved organic matter. Geochem. Cosmochim. Acta 70, 2990–3010. https://doi.org/10.1016/j.gca.2006.03.021.

Hoegh-Guldberg, O., Jacob, D., Taylor, M., Guillén Bolaños, T., Bindi, M., Brown, S., Camilloni, I.A., Diedhiou, A., Djalante, R., Ebi, K., Engelbrecht, F., Guiot, J., Hijioka, Y., Mehrotra, S., Hope, C.W., Payne, A.J., Pörtner, H.O., Seneviratne, S.I., Thomas, A., Warren, R., Zhou, G., 2019. The human imperative of stabilizing global climate change at 1.5°C. Science 365, eaaw6974. https://doi.org/10.1126/science.aaw6974.

Hongo, C., Yamano, H., 2013. Species-specific responses of corals to bleaching events on anthropogenically turbid reefs on Okinawa Island, Japan, over a 15-year period (1995-2009). PLoS One 8, e60952. https://doi.org/10.1371/journal.pone.0060952.

Hubbell, S.P., 2001. The Unified Neutral Theory of Biodiversity and Biogeography. Princeton University Press, Princeton, p. 392. https://doi.org/10.1515/9781400837526.

Hughes, T.P., Barnes, M.L., Bellwood, D.R., Cinner, J.E., Cumming, G.S., Jackson, J.B.C., Kleypas, J., van de Leemput, I.A., Lough, J.M., Morrison, T.H., Palumbi, S.R., van Nes, E.H., Scheffer, M., 2017a. Coral reefs in the Anthropocene. Nature 546, 82–90. https://doi.org/10.1038/nature22901.

Hughes, T.P., Kerry, J.T., Álvarez-Noriega, M., Álvarez-Romero, J.G., Anderson, K.D., Baird, A.H., Babcock, R.C., Beger, M., Bellwood, D.R., Berkelmans, R., Bridge, T.C., Butler, I.R., Byrne, M., Cantin, N.E., Comeau, S., Connolly, S.R., Cumming, G.S., Dalton, S.J., Diaz-Pulido, G., Eakin, C.M., Figueira, W.F., Gilmour, J.P., Harrison, H. B., Heron, S.F., Hoey, A.S., Hobbs, J.A., Hoogenboom, M.O., Kennedy, E.V., Kuo, C. Y., Lough, J.M., Lowe, R.J., Liu, G., McCulloch, M.T., Malcolm, H.A., McWilliam, M. J., Pandolfi, J.M., Pears, R.J., Pratchett, M.S., Schoepf, V., Simpson, T., Skirving, W. J., Sommer, B., Torda, G., Wachenfeld, D.R., Willis, B.L., Wilson, S.K., 2017b. Global warming and recurrent mass bleaching of corals. Nature 543, 373–377. https://doi.org/10.1038/nature21707.

Jia, S., Li, Y., Chen, S., Cai, Z., Shen, J., Wang, Y., Wu, Z., Wang, D., 2023. Microsatellite markers for Montipora digitata designed using restriction-site associated DNA sequencing. Front. Mar. Sci. 10, 1019419. https://doi.org/10.3389/fmars.2023.1019419.

Jørgensen, B.B., Boetius, A., 2007. Feast and famine–microbial life in the deep-sea bed. Nat. Rev. Microbiol. 5, 770–781. https://doi.org/10.1038/nrmicro1745.

Jørgensen, B.B., Revsbech, N.P., 1985. Diffusive boundary layers and the oxygen uptake of sediments and detritus. Limnol. Oceanogr. 30, 111–122. https://doi.org/10.4319/lo.1985.30.1.0111.

Jousset, A., Bienhold, C., Chatzinotas, A., Gallien, L., Gobet, A., Kurm, V., Küsel, K., Rillig, M.C., Rivett, D.W., Salles, J.F., van der Heijden, M.G., Youssef, N.H., Zhang, X., Wei, Z., Hol, W.H., 2017. Where less may be more: how the rare biosphere pulls ecosystems strings. ISME J. 11, 853–862. https://doi.org/10.1038/ismej.2016.174.

Kavousi, J., Tanaka, Y., Nishida, K., Suzuki, A., Nojiri, Y., Nakamura, T., 2016. Colony-specific calcification and mortality under ocean acidification in the branching coral *Montipora digitata*. Mar. Environ. Res. 119, 161–165. https://doi.org/10.1016/j.marenvres.2016.05.025.

Kegler, H.F., Lukman, M., Teichberg, M., Plass-Johnson, J., Hassenrück, C., Wild, C., Gärdes, A., 2017. Bacterial Community Composition and Potential Driving Factors in Different Reef Habitats of the Spermonde Archipelago, Indonesia. Front. Microbiol. 8, 662. https://doi.org/10.3389/fmicb.2017.00662.

Kellerman, A., Kothawala, D., Dittmar, T., Tranvik, L.J., 2015. Persistence of dissolved organic matter in lakes related to its molecular characteristics. Nat. Geosci. 8, 454–457. https://doi.org/10.1038/ngeo2440.

Kellerman, A.M., Dittmar, T., Kothawala, D.N., Tranvik, L.J., 2014. Chemodiversity of dissolved organic matter in lakes driven by climate and hydrology. Nat. Commun. 5, 3804. https://doi.org/10.1038/ncomms4804.

Kirchman, D.L., 2002. The ecology of Cytophaga-Flavobacteria in aquatic environments. FEMS Microbiol. Ecol. 39, 91–100. https://doi.org/10.1111/j.1574-6941.2002.tb00910.x.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 41, e1. https://doi.org/10.1093/nar/gks808.

Kujawinski, E.B., 2011. The impact of microbial metabolism on marine dissolved organic matter. Ann. Rev. Mar. Sci 3, 567–599. https://doi.org/10.1146/annurev-marine-120308-081003.

Kushmaro, A., Loya, Y., Fine, M., Rosenberg, M., 1996. Bacterial infection and coral bleaching. Nature 380, 396. https://doi.org/10.1038/380396a0.

LaRowe, D.E., Arndt, S., Bradley, J.A., Estes, E.R., Hoarfrost, A., Lang, S.Q., Zhao, R., 2020. The fate of organic carbon in marine sediments-new insights from recent data and analysis. Earth Sci. Rev. 204, 103146. https://doi.org/10.1016/j.earscirev.2020.103146.

Leibold, M.A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J.M., Hoopes, M.F., Holt, R.D., Shurin, J.B., Law, R., Tilman, D., Loreau, M., Gonzalez, A., 2004. The metacommunity concept: a framework for multi-scale community ecology. Ecol. Lett. 7, 601–613. https://doi.org/10.1111/j.1461-0248.2004.00608.x.

Lema, K.A., Willis, B.L., Bourne, D.G., 2012. Corals form characteristic associations with symbiotic nitrogen-fixing bacteria. Appl. Environ. Microbiol. 78, 3136–3144. https://doi.org/10.1128/AEM.07800-11.

Lesser, M.P., 1996. Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in symbiotic dinoflagellates. Limnol. Oceanogr. 41, 271–283. https://doi.org/10.4319/lo.1996.41.2.0271.

Lesser, M.P., Mazel, C.H., Gorbunov, M.Y., Falkowski, P.G., 2004. Discovery of symbiotic nitrogen-fixing Cyanobacteria in corals. Science 305, 997–1000. https://doi.org/10.1126/science.1099128.

Levine, J.M., Adler, P.B., Yelenik, S.G., 2004. A meta-analysis of biotic resistance to exotic plant invasions. Ecol. Lett. 7, 975–989. https://doi.org/10.1111/j.1461-0248.2004.00657.x.

Lipczynska-Kochany, E., 2018. Humic substances, their microbial interactions and effects on biological transformations of organic pollutants in water and soil: a review. Chemosphere 202, 420–437. https://doi.org/10.1016/j.chemosphere.2018.03.093.

Lomstein, B.A., Langerhuus, A.T., D'Hondt, S., Jørgensen, B.B., Spivack, A.J., 2012. Endospore abundance, microbial growth and necromass turnover in deep sub-seafloor sediment. Nature 484, 101–104. https://doi.org/10.1038/nature10905.

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A., Hooper, D.U., Huston, M.A., Raffaelli, D., Schmid, B., Tilman, D., Wardle, D.A., 2001. Biodiversity and ecosystem functioning: current knowledge and future challenges. Science 294, 804–808. https://doi.org/10.1126/science.1064088.

Lynch, M.D., Neufeld, J.D., 2015. Ecology and exploration of the rare biosphere. Nat. Rev. Microbiol. 13, 217–229. https://doi.org/10.1038/nrmicro3400.

Magel, J.M.T., Burns, J.H.R., Gates, R.D., Baum, J.K., 2019. Effects of bleaching-associated mass coral mortality on reef structural complexity across a gradient of local disturbance. Sci. Rep. 9, 2512. https://doi.org/10.1038/s41598-018-37713-1.

Manullang, C., Millyaningrum, I.H., Iguchi, A., Miyagi, A., Tanaka, Y., Nojiri, Y., Sakai, K., 2020. Responses of branching reef corals Acropora digitifera and Montipora digitata to elevated temperature and pCO2. PeerJ 8, e10562. https://doi.org/10.7717/peerj.10562.

Moberg, F., Folke, C., 1999. Ecological goods and services of coral reef ecosystems. Ecol. Econ. 29, 215–233. https://doi.org/10.1016/S0921-8009(99)00009-9.

Moran, M.A., Hodson, R.E., 1994a. Dissolved humic substances of vascular plant origin in a coastal marine environment. Limnol. Oceanogr. 39, 762–771. https://doi.org/10.4319/lo.1994.39.4.0762.

Moran, M.A., Hodson, R.E., 1994b. Support of bacterioplankton production by dissolved humic substances from three marine environments. Mar. Ecol. Prog. Ser. 110, 241–247.

Müller, R., Wiencke, C., Bischof, K., Krock, B., 2009 Jul-Aug. Zoospores of three Arctic laminariales under different UV radiation and temperature conditions: exceptional

spectral absorbance properties and lack of phlorotannin induction. Photochem. Photobiol. 85 (4), 970–977. https://doi.org/10.1111/j.1751-1097.2008.00515.x.
Nardi, S., Pizzeghello, D., Muscolo, A., Vianello, A., 2002. Physiological effects of humic substances on higher plants. Soil Biol. Biochem. 34, 1527–1536. https://doi.org/10.1016/S0038-0717(02)00174-8.
Nemergut, D.R., Schmidt, S.K., Fukami, T., O'Neill, S.P., Bilinski, T.M., Stanish, L.F., Knelman, J.E., Darcy, J.L., Lynch, R.C., Wickey, P., Ferrenberg, S., 2013. Patterns and processes of microbial community assembly. Microbiol. Mol. Biol. Rev. 77, 342–356. https://doi.org/10.1128/MMBR.00051-12.
Ogawa, H., Amagai, Y., Koike, I., Kaiser, K., Benner, R., 2001. Production of refractory dissolved organic matter by bacteria. Science 292, 917–920. https://doi.org/10.1126/science.1057627.
Orsi, W.D., 2018. Ecology and evolution of seafloor and subseafloor microbial communities. Nat. Rev. Microbiol. 16, 671–683. https://doi.org/10.1038/s41579-018-0046-8.
Otis, D.B., Carder, K.L., English, D.C., Ivey, J.E., 2004. CDOM transport from The Bahamas Banks. Coral Reefs 23, 152–160. https://doi.org/10.1007/s00338-003-0356-8.
Otte, J.M., Blackwell, N., Soos, V., Rughöft, S., Maisch, M., Kappler, A., Kleindienst, S., Schmidt, C., 2018 Dec 1. Sterilization impacts on marine sediment---Are we able to inactivate microorganisms in environmental samples? FEMS Microbiol. Ecol. 94 (12). https://doi.org/10.1093/femsec/fiy189.
Oxenford, H.A., Vallès, H., 2016. Transient turbid water mass reduces temperature-induced coral bleaching and mortality in Barbados. PeerJ 4, e2118. https://doi.org/10.7717/peerj.2118.
Piccolo, A., 2001. The supramolecular structure of humic substances. Soil Sci. 166, 810–833. https://doi.org/10.1097/00010694-200111000-00007.
Pootakham, W., Mhuantong, W., Yoocha, T., Putchim, L., Jomchai, N., Sonthirod, C., Naktang, C., Kongkachana, W., Tangphatsornruang, S., 2019. Heat-induced shift in coral microbiome reveals several members of the Rhodobacteraceae family as indicator species for thermal stress in Porites lutea. Microbiologyopen 8, e935. https://doi.org/10.1002/mbo3.935.
Porsby, C.H., Nielsen, K.F., Gram, L., 2008. Phaeobacter and Ruegeria species of the Roseobacter clade colonize separate niches in a Danish Turbot (*Scophthalmus maximus*)-rearing farm and antagonize *Vibrio anguillarum* under different growth conditions. Appl. Environ. Microbiol. 74, 7356–7364. https://doi.org/10.1128/AEM.01738-08.
R Core Team, 2022. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. Available from: https://www.R-project.org/.
Rangel, F., Santos, R.A., Monteiro, M., Lavrador, A.S., Gasco, L., Gai, F., Oliva-Teles, A., Enes, P., Serra, C.R., 2022. Isolation of chitinolytic bacteria from European Sea Bass gut microbiota fed diets with distinct insect meals. Biology 11, 964. https://doi.org/10.3390/biology11070964.
Ranith, R., Senthilnathan, L., Machendiranathan, M., Thangaradjou, T., Kumar, A.S., Sasamal, S.K., Choudhury, S.B., 2016. Assessment of chromophoric dissolved organic matter (CDOM) distribution in tropical coastal waters using in situ and satellite observations. Curr. Sci. 110, 1832–1838.
Rasher, D.B., Hay, M.E., 2010. Chemically rich seaweeds poison corals when not controlled by herbivores. Proc. Natl. Acad. Sci. U. S. A. 107, 9683–9688. https://doi.org/10.1073/pnas.0912095107.
Rautenberger, R., Wiencke, C., Bischof, K., 2013. Acclimation to UV radiation and antioxidative defence in the endemic Antarctic brown macroalga Desmarestia anceps along a depth gradient. Polar Biol. 36, 1779–1789. https://doi.org/10.1007/s00300-013-1397-2.
Rinkevich, B., 2014. Rebuilding coral reefs: does active reef restoration lead to sustainable reefs? Curr. Opin. Environ. Sustain. 7, 28–36. https://doi.org/10.1016/j.cosust.2013.11.018.
Rocha, R.J.M., Bontas, B., Cartaxana, P., Leal, M.C., Ferreira, J.M., Rosa, R., Serôdio, J., Calado, R., 2015. Development of a Standardized Modular System for Experimental Coral Culture. J World Aquacult Soc 46, 235–251. https://doi.org/10.1111/jwas.12186.
Rocha, R.J.M., Calado, R., Cartaxana, P., Furtado, J., Serôdio, J., 2013. Photobiology and growth of leather coral *Sarcophyton* cf. *glaucum* fragments stocked under low light in a recirculated system. Aquaculture 235-242, 24–29. https://doi.org/10.1016/j.aquaculture.2013.08.018.
Rocha, R.J.M., Rodrigues, A.C.M., Campos, D., Cícero, L.H., Costa, A.P.L., Silva, D.A.M., Oliveira, M., Soares, A.M.V.M., Patrício Silva, A.L., 2020 Apr 15. Do microplastics affect the zoanthid Zoanthus sociatus? Sci. Total Environ. 713, 136659. https://doi.org/10.1016/j.scitotenv.2020.136659.
Roche, R.C., Perry, C.T., Smithers, S.G., Leng, M.J., Grove, C.A., Sloane, H.J., Unsworth, C.E., 2014. Mid-Holocene sea surface conditions and riverine influence on the inshore Great Barrier Reef. Holocene 24, 885–897. https://doi.org/10.1177/095968361453473.
Rogers, A., Blanchard, J.L., Mumby, P.J., Arlinghaus, R., 2018. Fisheries productivity under progressive coral reef degradation. J. Appl. Ecol. 55, 1041–1049. https://doi.org/10.1111/1365-2664.13051.
Rohwer, F., Seguritan, V., Azam, F., Knowlton, N., 2002. Diversity and distribution of coral-associated bacteria. Mar. Ecol. Prog. Ser. 243, 1–10. https://doi.org/10.3354/meps243001.
Rosenberg, E., Kellogg, C.A., Rohwer, F., 2007. Coral microbiology. Oceanography (Wash. D. C.) 20, 146–154. https://doi.org/10.5670/oceanog.2007.60.
Sangmanee, K., Casareto, B.E., Nguyen, T.D., Sangsawang, L., Toyoda, K., Suzuki, T., Suzuki, Y., 2020. Influence of thermal stress and bleaching on heterotrophic feeding of two scleractinian corals on pico-nanoplankton. Mar. Pollut. Bull. 158, 111405. https://doi.org/10.1016/j.marpolbul.2020.111405.
Sato, M., Nanami, A., Bayne, C.J., Makino, M., Hori, M., 2020. Changes in the potential stocks of coral reef ecosystem services following coral bleaching in Sekisei Lagoon, southern Japan: implications for the future under global warming. Sustain. Sci. 15, 863–883. https://doi.org/10.1007/s11625-019-00778-6.
Saw, J.H.W., Nunoura, T., Hirai, M., Takaki, Y., Parsons, R., Michelsen, M., Longnecker, K., Kujawinski, E.B., Stepanauskas, R., Landry, Z., Carlson, C.A., Giovannoni, S.J., 2020. Pangenomics analysis reveals diversification of enzyme families and niche specialization in globally abundant SAR202 bacteria. mBio 11. https://doi.org/10.1128/mBio.02975-19.
Shank, G.C., Zepp, R.G., Vähätalo, A., Lee, R., Bartels, E., 2010. Photobleaching kinetics of chromophoric dissolved organic matter derived from mangrove leaf litter and floating Sargassum colonies. Mar. Chem. 119, 162–171. https://doi.org/10.1016/j.marchem.2010.01.003.
Shantz, A.A., Ladd, M.C., Burkepile, D.E., 2020. Overfishing and the ecological impacts of extirpating large parrotfish from Caribbean coral reefs. Ecol. Monogr. 90, e01403. https://doi.org/10.1002/ecm.1403.
Shashar, N., Cohen, Y., Loya, Y., 1993. Extreme diel fluctuations of oxygen in diffusive boundary layers surrounding stony corals. Biol Bull 185, 455–461. https://doi.org/10.2307/1542485.
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 Jun 19. Neutrality in the metaorganism. PLoS Biol. 17 (6), e3000298. https://doi.org/10.1371/journal.pbio.3000298.
Sloan, W.T., Lunn, M., Woodcock, S., Head, I.M., Nee, S., Curtis, T.P., 2006. Quantifying the roles of immigration and chance in shaping prokaryote community structure. Environ. Microbiol. 8, 732–740. https://doi.org/10.1111/j.1462-2920.2005.00956.x.
Sonnenschein, E.C., Jimenez, G., Castex, M., Gram, L., 2021. The Roseobacter-group bacterium Phaeobacter as a safe probiotic solution for aquaculture. Appl. Environ. Microbiol. 87. https://doi.org/10.1128/AEM.02581-20.
Spalding, M., Burke, L., Wood, S.A., Ashpole, J., Hutchison, J., zu Ermgassen, P., 2017. Mapping the global value and distribution of coral reef tourism. Mar Policy 82, 104–113. https://doi.org/10.1016/j.marpol.2017.05.014.
Steinberg, C.E.W., Kamara, S., Prokhotskaya, V.Y., Manusadžianas, L., Karasyova, T.A., Timofeyev, M.A., Jie, Z., Paul, A., Meinelt, T., Farjalla, V.F., Matsuo, A.Y.O., Kent Burnison, B., Menzel, R., 2006. Dissolved humic substances – ecological driving forces from the individual to the ecosystem level? Freshw. Biol. 51, 1189–1210. https://doi.org/10.1111/j.1365-2427.2006.01571.x.
Stuij, T.M., Cleary, D.F.R., de Voogd, N.J., Rocha, R.J.M., Polónia, A.R.M., Silva, D.A.M., Frommlet, J., Louvado, A., Huang, Y.M., Gomes, N.C.M., 2025. Humic substances modulate bacterial communities and mitigate adverse effects of temperature stress in coral reef organisms. J. Appl. Microbiol. 136, lxaf012. https://doi.org/10.1093/jambio/lxaf012.
Stuij, T.M., Cleary, D.F.R., Rocha, R.J.M., Polonia, A.R.M., Machado, E., Silva, D.A., Frommlet, J.C., Louvado, A., Huang, Y.M., De Voogd, N.J., Gomes, N.C.M., 2024. Development and validation of an experimental life support system to study coral reef microbial communities. Sci. Rep. 14, 21260. https://doi.org/10.1038/s41598-024-69514-0.
Sun, F., Yang, H., Zhang, X., Tan, F., Shi, Q., 2022. Response characteristics of bacterial communities in multiple coral genera at the early stages of coral bleaching during El Niño. Ecol. Indic. 144, 109569. https://doi.org/10.1016/j.ecolind.2022.109569.
Teichberg, M., Wild, C., Bednarz, V.N., Kegler, H.F., Lukman, M., Gärdes, A.A., Heiden, J.P., Weiand, L., Abu, N., Nasir, A., Miñarro, S., Ferse, S.C.A., Reuter, H., Plass-Johnson, J.G., 2018. Spatio-temporal patterns in coral reef communities of the Spermonde Archipelago, 2012–2014, I: comprehensive reef monitoring of water and benthic indicators reflect changes in reef health. Front. Mar. Sci. 5, 33. https://doi.org/10.3389/fmars.2018.00033.
Tilman, D., 2004. Niche tradeoffs, neutrality, and community structure: a stochastic theory of resource competition, invasion, and community assembly. Proc. Natl. Acad. Sci. U. S. A. 101, 10854–10861. https://doi.org/10.1073/pnas.0403458101.
Tilman, D., Lehman, C.L., Thomson, K.T., 1997. Plant diversity and ecosystem productivity: theoretical considerations. Proc. Natl. Acad. Sci. U. S. A. 94, 1857–1861. https://doi.org/10.1073/pnas.94.5.1857.
Tran, C., 2022. Coral-microbe interactions: their importance to reef function and survival. Emerg. Top. Life Sci. 6, 33–44. https://doi.org/10.1042/ETLS20210229.
van de Water, J.A.J.M., Allemand, D., Ferrier-Pagès, C., 2018. Host-microbe interactions in octocoral holobionts - recent advances and perspectives. Microbiome 6, 64. https://doi.org/10.1186/s40168-018-0431-6.
Voets, J., Bervoets, L., Blust, R., 2004. Environ. Sci. Technol. 38, 1003–1008. https://doi.org/10.1021/es034742e.
Vucskits, A.V., Hullár, I., Bersényi, A., Andrásofszky, E., Kulcsár, M., Szabó, J., 2010. Effect of fulvic and humic acids on performance, immune response and thyroid function in rats. J. Anim. Physiol. Anim. Nutr. 94, 721–728. https://doi.org/10.1111/j.1439-0396.2010.01023.x.
Wahl, M., Goecke, F., Labes, A., Dobretsov, S., Weinberger, F., 2012. The second skin: ecological role of epibiotic biofilms on marine organisms. Front. Microbiol. 3, 292. https://doi.org/10.3389/fmicb.2012.00292.
Walker, B., Kinzig, A., Langridge, J., 1999. Plant attribute diversity, resilience, and ecosystem function: the nature and significance of dominant and minor species. Ecosystems 2, 95–113. https://doi.org/10.1007/s100219900062.
Warner, M.E., Fitt, W.K., Schmidt, G.W., 1999. Damage to photosystem II in symbiotic dinoflagellates: a determinant of coral bleaching. Proc. Natl. Acad. Sci. U. S. A. 96, 8007–8012. https://doi.org/10.1073/pnas.96.14.8007.
Wiedenmann, J., D'Angelo, C., Smith, E.G., Hunt, A.N., Legiret, F.E., Postle, A.D., Achterberg, E.P., 2013. Nutrient enrichment can increase the susceptibility of reef

corals to bleaching. Nat. Clim. Change 3, 160–164. https://doi.org/10.1038/nclimate1661.
Wiegand, S., Jogler, M., Jogler, C., 2018. On the maverick Planctomycetes. FEMS Microbiol. Rev. 42, 739–760. https://doi.org/10.1093/femsre/fuy029.
Yang, F., Tang, C., Antonietti, M., 2021. Natural and artificial humic substances to manage minerals, ions, water, and soil microorganisms. Chem. Soc. Rev. 50, 6221–6239. https://doi.org/10.1039/d0cs01531n.
Zepp, R.G., Shank, G.C., Stabenau, E., Patterson, K.W., Cyterski, M., Fisher, W., Bartels, E., Anderson, S.L., 2008. Spatial and temporal variability of solar ultraviolet exposure of coral assemblages in the Florida Keys: importance of colored dissolved organic matter. Limnol. Oceanogr. 53, 1909–1922. https://doi.org/10.4319/lo.2008.53.5.1909.
Zou, Y., Ip, J.C., Xie, J.Y., Yeung, Y.H., Wei, L., Guo, Z., Zhang, Y., Qiu, J.W., 2024. Dynamic changes in bacterial communities in three species of corals during the 2017 bleaching event in subtropical Hong Kong waters. Mar. Pollut. Bull. 199, 116002. https://doi.org/10.1016/j.marpolbul.2023.116002.