Humic substances modulate bacterial communities and mitigate adverse effects of temperature stress in coral reef organisms

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Abstract

Aims: In the present study, we tested whether terrestrially derived humic substances (HS) could mitigate the adverse effects of elevated temperature and ultraviolet B (UVB) radiation on the bacterial communities of two hard corals (*Montipora digitata* and *M. capricornis*), one soft coral (*Sarcophyton glaucum*), sediment and water. We also examined the impact of temperature, UVB radiation, and HS supplementation on coral photosynthetic activity, a proxy for coral bleaching.

Methods and results: We performed a multifactorial experiment using a randomized-controlled microcosm setup. Coral photosynthetic efficiency was measured *in vivo* using a pulse amplitude modulation fluorometer. Bacterial communities were analyzed using 16S rRNA gene sequencing. Corals in HS-supplemented microcosms had significantly higher photosynthetic activities than those in microcosms subjected to elevated temperature and UVB radiation. Additionally, HS supplementation significantly influenced the composition of sediment, water, and host-associated bacterial communities. Reef organisms in HS supplemented microcosms contained distinct bacterial communities enriched with groups of potentially beneficial bacteria. In the hard coral *M. digitata*, we observed an interactive effect of HS supplementation, UVB radiation, and temperature.

Conclusion: Our findings indicate that HS significantly modulates coral reef bacterial communities and support the hypothesis that these substances contribute to improved reef resistance to the adverse effects of elevated temperature and UVB radiation.

Impact Statement

These findings have significant implications and highlight the potential importance of terrestrially derived humic substances in mitigating the adverse effects of severe heat stress and intense UVB exposure on corals.

Introduction

Tropical coral reefs are among the most diverse of marine ecosystems and provide numerous ecological and economic services (Moberg and Folke 1999, Fisher et al. 2015, Pellowe et al. 2023). Photosynthetic scleractinian corals are the foundational species of coral reefs. These organisms produce complex three-dimensional structures, which are colonized and influenced by other benthic species, including sponges, macro-benthic algae, microbial mats, and octocorals. Critical to the success of these benthic colonizers are their complex relationships with a diverse array of microorganisms (van Oppen and Blackall 2019, Burgsdorf et al. 2022, Wiedenmann et al. 2023). A well-known example is the symbiosis of corals with unicellular dinoflagellates also known as 'zooxanthellae'. Zooxanthellae supply corals with essential nutrients (nitrogen and phosphorus) and energy in the form of organic compounds (Wiedenmann et al. 2023). In return, the corals offer the zooxanthellae a protected environment and access to vital nutrients (Bourne et al. 2016). Zooxanthellae are of such high importance to their hosts, that the breakdown of this relationship, a phenomenon termed coral bleaching, can result in host death (Boilard et al. 2020, Helgoe et al. 2024). Large-scale coral bleaching events are currently threatening reefs worldwide and have been associated with elevated sea temperatures and increased solar radiation, particularly in relation to extreme El Niño Southern Oscillation (ENSO) events (Hughes et al. 2017, 2018, Van Woesik et al. 2022). As the frequency and severity of ENSO events is pre-

dicted to further increase in the coming decades (Ying et al. 2022), the future state of coral reef ecosystems will be determined by their resistance or resilience to these environmental perturbations.

In addition to their symbiosis with zooxanthellae, reef organisms also establish associations with a diverse array of other microbes, including viruses, archaea, and bacteria (Bourne and Webster 2013). While the roles of many of these microorganisms remain poorly understood, emerging research increasingly underscores their importance in supporting the health and functionality of their hosts. Bacteria, in particular, have been suggested to fulfill essential roles in host nutrient acquisition (Raina et al. 2009, Lema et al. 2012, Burgsdorf et al. 2022) and defense (Shnit-Orland and Kushmaro 2009, Alagely et al. 2011, Mohan et al. 2016). Coral-associated bacteria, for example, carry genes encoding enzymes involved in carbon fixation (Kimes et al. 2010) and are capable of utilizing simple and complex carbohydrates (Wegley et al. 2007). Additionally, their importance in nitrogen cycling has been inferred from the persistent detection of N_2-fixing bacteria (diazotrophs) in coral-associated bacterial communities (Benavides et al. 2017).

Given their importance for host survival, a growing body of research advocates the use of bacteria to promote coral resistance and resilience to severe environmental stressors and pathogens (Rosado et al. 2019, Doering et al. 2021). For example, microcosm experiments demonstrated that the bleaching response of the coral *Pocillopora damicornis* was partially mitigated when individuals were inoculated with potentially beneficial bacterial taxa, e.g. *Pseudoalteromonas* spp., *Halomonas taeanensis*, and *Colbetia marina*, prior to exposure to elevated temperature and pathogens (Rosado et al. 2019). However, the use of these microbiome modulation approaches based on the use of living microorganisms in coral reefs is challenging. Currently, there is a lack of established methodologies for the effective introduction of these microorganisms, and a lack of knowledge on the potential unintended effects they may have on other cohabiting organisms (Sweet et al. 2017). Chemical microbiome modulators, e.g. prebiotics, that promote the growth of naturally occurring beneficial microbes, can be a more natural and practical approach to stimulate the growth of beneficial bacterial taxa. Humic substances (HS), for example, have been previously used to improve soil properties, plant growth, and, importantly, soil bacterial diversity in agricultural systems (Nardi et al. 2021). They have, however, received limited attention as modulators of bacterial communities in aquatic systems (Louvado et al. 2021).

HS are complex organic compounds that are mainly formed through the decomposition of plant organic matter in terrestrial ecosystems (MacCarthy 2001) and enter the marine environment mainly via underwater cave systems or river runoff. HS concentrations are greatest near coastal ecosystems, and decrease with increasing distance to land (Esham et al. 2000, Riso et al. 2021). Moving from freshwater to marine ecosystems, HS are modified by biogeochemical processes, which alter their composition and concentration. A detailed study by Riso et al. (2021) found the HS pool in marine waters to be characterized by an increase in dissolved relative to particulate HS, a substantially lower percentage of electroactive HS, and a decrease in both molecular weight and % of aromatic carbon relative to river waters. These changes

can be explained by the individual and combined effects of multiple biological (Esham et al. 2000, Li et al. 2020, Yun et al. 2024) and physicochemical factors (Baalousha et al. 2006, Asmala et al. 2014). For example, exposure to sunlight, particularly UV radiation, can increase the bioavailability of HS by breaking down high-molecular weight compounds and aromatic rings into easier degradable compounds (Xu et al. 2024). Although susceptible to photodegradation, these aromatic rings are abundant chemical components of HS (Sharpless et al. 2014) and are thought to provide UV-protective properties to marine organisms due to their high absorbance of light in the UVB spectrum (280–320 nm, Ferrier-Pagès et al. 2007, Ayoub et al. 2012). While sunlight is essential for coral survival, high UV radiation can exacerbate the negative impacts of thermal stress on coral physiology (Downie et al. 2024).

An increased understanding of the potential protective effects of HS on coral reefs is crucial, not only for assessing coral responses to thermal and UV-induced stress but also for understanding the broader impacts of land-use changes on marine ecosystems. Coastal and riparian forests, mangroves, and seagrass meadows have experienced extensive degradation and habitat loss due to large-scale urban development, industrial expansion, and the intensification of agriculture and aquaculture (Crain et al. 2009). This development has resulted in increased transfer of nutrients and pollutants to coastal marine environments (Petersen et al. 2017), adversely affecting corals that would normally thrive under oligotrophic conditions (Ferrier-Pagès et al. 2000, Hall et al. 2018). This development has, furthermore, resulted in qualitative and quantitative shifts in HS that are transferred from inland coastal watersheds to adjacent marine environments (dos Santos et al. 2019). For example, soils of deforested agricultural fields showed a 38% to 53% reduction in HS relative to soils of natural forests (dos Santos et al. 2019).

Although the impact of nutrient enrichment on tropical coral reefs has been studied extensively (Ferrier-Pagès et al. 2000, Hall et al. 2018), the specific effects related to the loss and modification of HS in these ecosystems are relatively unknown. Interestingly, some observational studies (Ayoub et al. 2012, Nelson and Siegel 2013, Cleary et al. 2016) found a positive correlation between coral ecosystem health and chromophoric dissolved organic matter, often used as a proxy for HS, originating from river plumes of intact forest-dominated coastlines (Cleary et al. 2016). Few studies have explicitly investigated the potential protective effects of HS on coral reef organisms exposed to elevated temperatures and UV radiation. In our recent study (Stuij et al. 2024a), we showed that HS significantly influenced the structural diversity of bacterial communities of the marine sponge *Chondrilla* sp., including an increase in the relative abundance of bacterial populations potentially involved in the degradation of recalcitrant organic matter. However, UVB alone did not have a significant independent effect, nor were there any significant interactive effects (HS, heat, and UVB) on sponge-associated bacterial communities. In the present study, we extended the scope of our previous study to investigate the effects of HS, elevated temperature, and UVB radiation, independently and interactively, on bacterial communities associated with two hard corals (*Montipora digitata* and *M. capricornis*), one soft coral (*Sarcophyton glaucum*), sediment and water, while also examining how these treatments influenced

the photosynthetic activity of the corals as a proxy for coral bleaching.

Methods

Experimental design

The experimental life support system (ELSS) developed 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. (2024b) and was previously employed to investigate the effects of HS, elevated temperatures, and UVB radiation on the sponge *Chondrilla* sp. (Stuij et al. 2024a). In the present study, we expanded on this work by analysing additional organisms, two hard corals (*M. digitata* and *M. capricornis*) and one soft coral (*S. glaucum*), along with sediment and water from the same microcosm experiment. These corals represent key taxa commonly found in Indo-Pacific coral reef environments. Briefly, the ELSS included 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 (Stuij et al. 2024b). No water circulation took place between individual microcosm-reservoir units. Using the ELSS, a multi-factorial experiment was designed for the testing of independent and interactive effects of HS supplementation (technical grade humic acid, Sigma–Aldrich, batch number: BCCD6371, see also the certificate of origin in the supplements), temperature and UVB radiation. Each factor had two levels, namely, HS supplementation (with *versus* without), temperature (28°C versus 32°C), and UVB radiation (with *versus* without) for a total of eight treatments with four replicates each. The full experiment, thus, consisted of 32 microcosms. The temperature treatment was randomized in groups of four microcosms, whereby HS supplementation, UVB radiation, and the combination of both were randomly assigned within each group of microcosms with equal temperature. During the experiment, each microcosm consisted of a sediment layer, artificial seawater, and five reef organisms: the sponge *Chondrilla* sp., the hard corals *M. digitata* and *M. capricornis*, the soft coral *S. glaucum*, and the zoanthid *Zoanthus* sp. A graphical summary of the ELSS and different phases of the experiment can be found in Fig. 1. Note that the present study does not include data from the Zoanthid *Zoanthus* sp., as low sequence quality for this organism resulted in a significant loss of replicates. Additional details about the ELSS setup, including biogeochemical analyses, can be found in Table S1 and Stuij et al. (2024a).

In vivo chlorophyll fluorescence analysis

Before addition to the microcosms and at the end of the experiment, the chlorophyll fluorescence of the corals was measured *in vivo* using a pulse amplitude modulation (PAM) fluorometer (Walz™). Fluorescence was measured in dark-adapted samples (for 20 min), with Junior PAM and WinControl3 software (Walz™). Saturating light pulses (450 nm) were performed perpendicularly to the sample surface, with a 1.5 mm fiber optic. The maximum quantum yield (Fv/Fm) of photosystem II was calculated as $Fv/Fm = \frac{Fm-F0}{Fm}$.

Bacterial community analysis

Sampling and DNA extraction

Samples of sediment, water, and organisms were collected 5 days after the beginning of the experiment. From each microcosm, a composite sediment sample was collected haphazardly by taking four sub-samples (1 cm surface sediment cores with a diameter of approximately 2 cm). One sample from the sterilized commercial sediment was obtained as control for ELSS contamination with environmental DNA (Torti et al. 2015) and sample collection (Hornung et al. 2019). Bacterioplankton communities were sampled by filtering 250 ml of water through a 0.22 μm-pore-size polycarbonate membrane using an EZ-Fit™ vacuum filtration system (Millipore). One specimen of each reef organism was sampled from each microcosm. After sampling, the organisms were rinsed with sterilized artificial seawater (filtered through 0.22 μm pores) and removed from their carbonate stones. All sediment samples, whole membrane filters and reef organisms were frozen at −80°C until DNA extraction. An overview of the sample metadata can be found in Table S2. Polymerase chain reaction (PCR)-ready genomic DNA was isolated from all samples using the FastDNA® SPIN soil Kit (MP biomedicals) following the manufacturer's instructions. Briefly, the whole membrane filter (bacterioplankton communities) and sediment (~500 mg) and host organisms were transferred to Lysing Matrix E tubes containing a mixture of ceramic and silica particles. *Montipora digitata* and *M. capricornis*, fragments (tissue and skeleton) (~500 mg) were first snap frozen in liquid nitrogen and subsequently ground in a heat-sterilized mortar and pestle. Blank negative controls (without samples) were also included to evaluate sample contamination during DNA extraction. Extracted DNA was eluted in 50 μl of DNase/pyrogen-free water and stored at −20°C until further use.

Bacterial community analysis

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. The current study followed the same PCR amplification and sequencing protocol as described in Stuij et al. (2024a); further details are available in that publication.

Briefly, samples were 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 (dataset reference: Stuij et al. access date: 23-11-2022). In QIIME2 (Bolyen et al. 2019), raw sequences were quality trimmed using the DADA2 plugin (Callahan et al. 2016) and subsequently classified using the QIIME2 feature-classifier plugin with the SILVA 138 reference database. The produced amplicon sequence variants (ASVs) along with their abun-
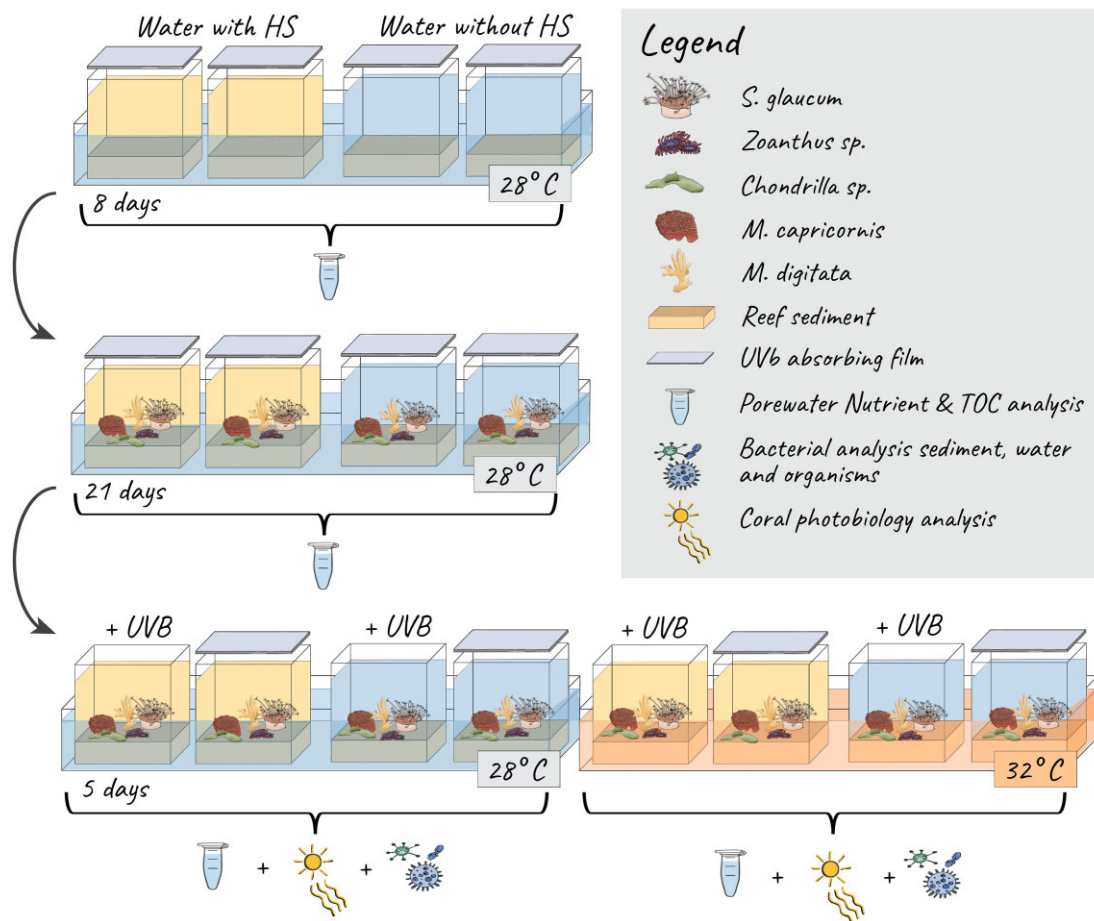
Figure 1. Graphical representation of the experimental design.

dance per sample and taxonomic assignment are listed in Table S3.

Subsequently, Archaea, ASVs unassigned at the phylum level and ASVs that occurred in the triple-autoclaved commercial sediment (control for sampling and eDNA contamination) and negative controls were removed. All ASVs removed following detection in the control samples are listed in Table S4. Overall, the removed ASVs were assigned to known contaminants, for example, the genera *Ralstonia*, *Burkholderia–Caballeronia–Paraburkholderia*, *Reyranella*, *Bacillus*, and *Bradyrhizobium* (Weyrich et al. 2019). After these filtering steps, we removed four samples with low (<4000 reads) read counts. Among these were two sediment samples, one water sample, and one sample *of M. capricornis*. The 10 most abundant ASVs of each biotope were referenced against the NCBI nucleotide database using NCBI Basic Local Alignment Search Tool (Zhang et al. 2000).

Statistical analyses

All statistical tests were performed within R, using functions included in the packages 'stats' (R Core Team 2022), 'vegan' (Oksanen et al. latest access date: 30-11-2024), and 'Boruta' (Kursa et al. 2010). Figures were created using the R package 'ggplot2' (Wickham 2016).

To evaluate the effect of transplantation to the microcosms, we compared the Fv/Fm ratios of the corals before addi-

tion to the microcosms to those of corals kept in the microcosms for 34 days. The results of this analysis are discussed in Stuij et al. (2024b). To analyse the effect of our treatments, we checked for deviations from normality in Fv/Fm ratios with the shapiro.test() function and tested for homogeneity of variance with the bartlett.test() function of the R package 'stats'(http://www.r-project.org/; Accessed June 2022). We tested for significant differences among treatments in normally distributed Fv/Fm ratios (*M. capricornis, S. glaucum*) with a three-way analysis of variance (ANOVA) using the aov() function of the R package 'stats' and tested for significant differences in non-normally distributed Fv/Fm ratios (*M. digitata*) with a permutational analysis of variance (PERMANOVA, 999 permutations) using the adonis2() function of the R package 'vegan'. In the aov() function, the Fv/Fm ratio was the response variable and HS, temperature and UVB were the independent variables. In the adonis2() analysis, the Euclidean distance matrix of the Fv/Fm ratio was the response variable with HS, temperature and UVB as independent variables.

A table containing the ASV counts was imported into R. Differences in higher taxon abundances were assessed using the glm() function of the R package 'stats'. A number of these variables included an excess of zero counts in the samples, therefore, we set the family argument to 'tweedie' using the tweedie function in R with var.power = 1.5 and link.power = 0 (a compound Poisson–Gamma distribution). Using the glm model, we tested for significant variation us-

ing the anova() function with the F-test of the R package 'stats'.

Variation in bacterial composition among treatments was visualized with principal coordinates analysis (PCO). For the PCO analysis, the ASV table was rarefied per biotope to the minimum sample size using the rrarefy() function of the R package 'vegan'. The rarefied ASV table was log $(x + 1)$ transformed and a distance matrix constructed using the Bray–Curtis index with the vegdist() function of the R package 'vegan'. We tested for significant differences in ASV composition among treatments within each biotope with a permutational analysis of variance (PERMANOVA) using the adonis2() function and for homogeneity of multivariate dispersion using the betadisper() and permutest() functions of the R package 'vegan' (999 permutations).

Finally, we used the Boruta() function of the R package 'Boruta' to identify specific ASVs, which were positively or negatively associated with HS supplementation. Boruta is a random forest wrapper, which is used to evaluate feature importance (Kursa et al. 2010).

Results

Photobiology of the host organisms

HS supplementation proved to be a significant independent predictor of variation in the Fv/Fm ratios of *M. digitata*, *M. capricornis*, and *S. glaucum* (Fig. 2, (PERM)ANOVA, $P = 0.007$, $P < 0.001$, and $P = 0.005$, respectively, Table S5). In all three biotopes, the Fv/Fm ratios were higher on average under HS supplementation. Temperature was, furthermore, a significant independent predictor of the photosynthetic efficiencies of *M. digitata* and *M. capricornis* [(PERM)ANOVA, $P = 0.031$ and $P < 0.001$, respectively, Table S5]. In both biotopes the Fv/Fm ratios were lower on average in heat-treated microcosms. In addition to this, there was a significant interactive effect of temperature and HS supplementation on the photosynthetic efficiency of *M. digitata* (PERMANOVA, $P = 0.008$, Table S5). Fv/Fm ratios were lower in heat-treated microcosms without HS supplementation, whereas there was no such effect with HS supplementation.

Bacterial community analysis

Community composition

HS supplementation proved to be a significant predictor of variation in ASV composition of all biotopes with the exception of *M. capricornis* (PERMANOVA: $P < 0.05$, Table S6). In sediment samples, HS explained 9.1% of the variation in the community data but slight heterogeneity in group dispersions was detected (PERMDISP: $P = 0.043$). No significant heterogeneity in dispersion was detected in the other biotopes (PERMDISP: $P > 0.05$, Table S6). The variation explained by HS varied from 4.8% in *M. digitata* to 15.6% in water (see Table S6 for all R^2 values). Temperature also proved a significant predictor of variation in ASV composition in water and *S. glaucum* (PERMDISP: $P > 0.05$, PERMANOVA: $P = 0.043$ and $R^2 = 0.044$; $P = 0.028$ and $R^2 = 0.05$, respectively, Table S6). Finally, there was a significant interactive effect of temperature, UVB radiation, and HS supplementation in *M. digitata* (PERMANOVA: $P = 0.039$ and $R^2 = 0.041$). PCO ordinations presented in Fig. 3 show that samples subjected to HS supplementation clearly separated along the first two axes. For *M. digitata*, the ordination, furthermore, showed a
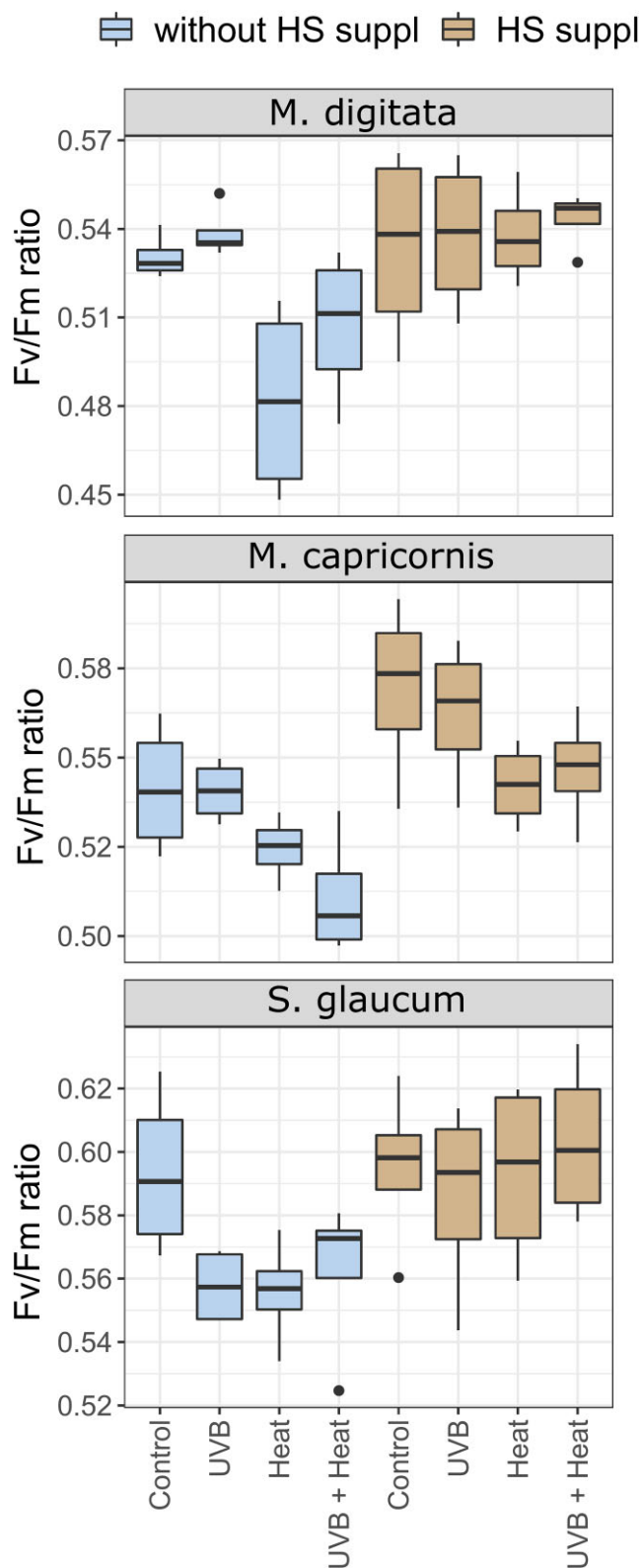
Figure 2. Boxplots of the maximum quantum yield of photosystem II (Fv/Fm ratio) of *M. digitata, M. capricornis*, and *S. glaucum*. Fv/Fm ratios are grouped per treatment within each biotope.
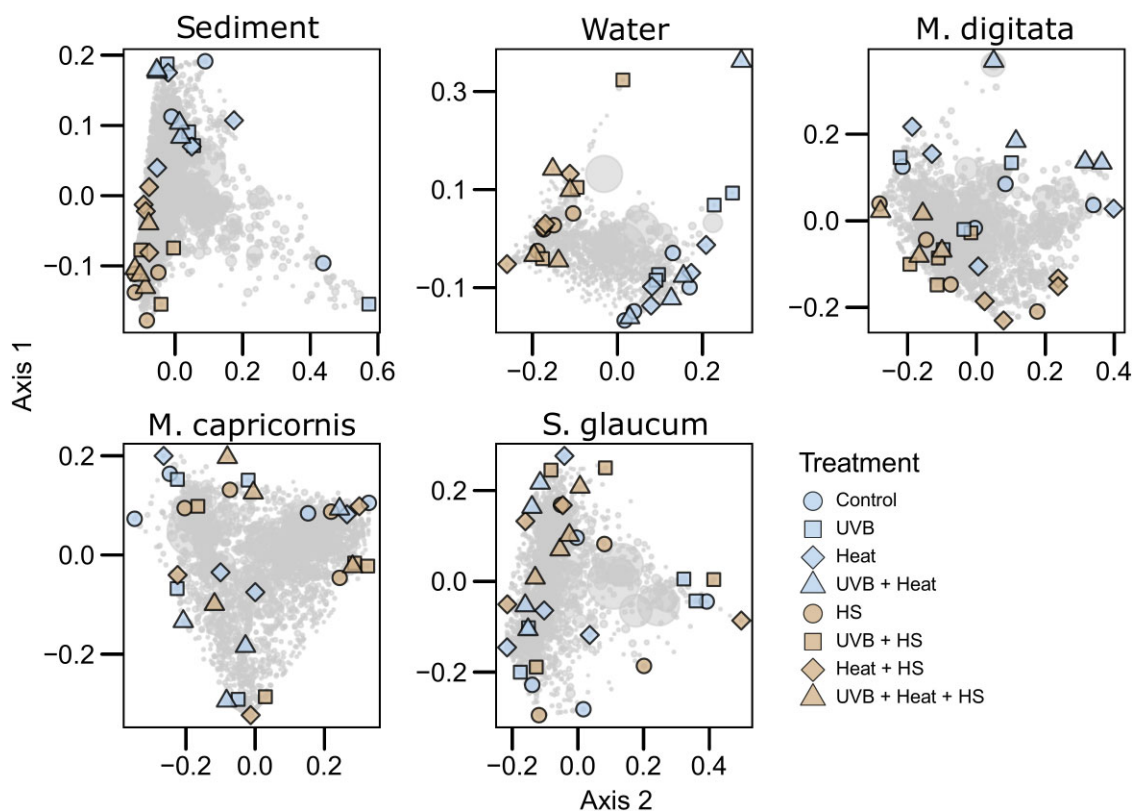
Figure 3. Ordinations showing the first two axes of the PCO of prokaryotic ASV composition of the studied biotopes. The PCO was generated using the cmdscale() function in the R base package and wascores() function in vegan. Prior to the PCO, the raw data were log(x + 1) transformed and used to produce a distance matrix based on the Bray–Curtis distance with the vegdist() function in vegan (Oksanen et al. latest access date: 30-11-2024). Light grey symbols represent operational taxonomic unit (ASV) scores with the symbol size proportional to their abundance (number of sequence reads). The percentage of variation explained by the first two axes was 21.37% in sediment, 27.64% in water, 18.38% in *M. digitata*, 28.16% in *M. capricornis*, and 24.7% in *S. glaucum*.

separate cluster of Heat and UVB-treated samples in microcosms not supplemented with HS.

Higher taxon abundance

Relative abundances of the four most abundant classes and orders were significantly impacted by HS supplementation (in sediment and *M. digitata*), temperature (in water and *S. glaucum*) and interactive effects of HS, temperature and UVB exposure (water, *M. digitata*) (GLMs, $P < 0.05$, Fig. 4, Fig. S1 and Table S6). In sediment, the classes Gammaproteobacteria and Bacteroidia had significantly higher, and the orders Rhodobacterales, Pirellulales, and Microtrichales significantly lower relative abundances with HS supplementation. In *M. digitata*, the relative abundance of the order Pirellulales was significantly higher with HS supplementation. The relative abundance of the order Cytophagales was significantly lower with heat and UVB exposure, but this effect was not observed with heat, UVB and HS supplementation. In *S. glaucum*, the relative abundances of the classes Firmicutes and Bacilli and order Entoplasmatales were lower, and the order Rhodobacterales higher with heat. In water, the ABY1 order was significantly more abundant with HS supplementation, the order Rhodobacterales with heat and the order Caulobacterales with heat and HS supplementation, but this effect was not apparent under UVB exposure. No significant differences were observed for the four most abundant classes and orders in *M. capricornis*.

Abundant ASVs

The 10 most abundant ASVs in sediment, water, *M. digitata*, and *S. glaucum* are shown in Fig. 5. In sediment, we observed that ASV-11, assigned to family Rhodobacteraceae and closely related to an organism detected in the coral *Porites cylindrica* (100% sequence similarity), was particularly abundant in micrococsms without HS supplementation (Table S7). In contrast, ASVs 17 and 66, assigned to the genera *Filomicrobium* and *Halieaceae*, respectively, were particularly abundant in microcosms with HS supplementation.

In water, we found that ASV-23, assigned to the genus *Maricaulis* (family Hyphomonadaceae), was particularly abundant in microcosms without HS supplementation. ASV-62, in turn, also assigned to the Hyphomonadaceae family, was particularly abundant with HS supplementation. Additionally, ASV-9, assigned to the NS3a marine group, was markedly more abundant under the individual and combined treatment of heat and HS supplementation. ASV-29, assigned to the family Hyphomonadaceae, was particularly abundant under the combined treatment of heat and HS supplementation. These previously mentioned ASVs were all closely related to organisms previously detected or isolated from seawater samples (100% sequence similarities, Table S7).

In *M. digitata*, ASV-66, assigned to the genus *Halieaceae*, occurred in all treatments with HS supplementation, but was absent from heat, and heat + UVB treated micro-
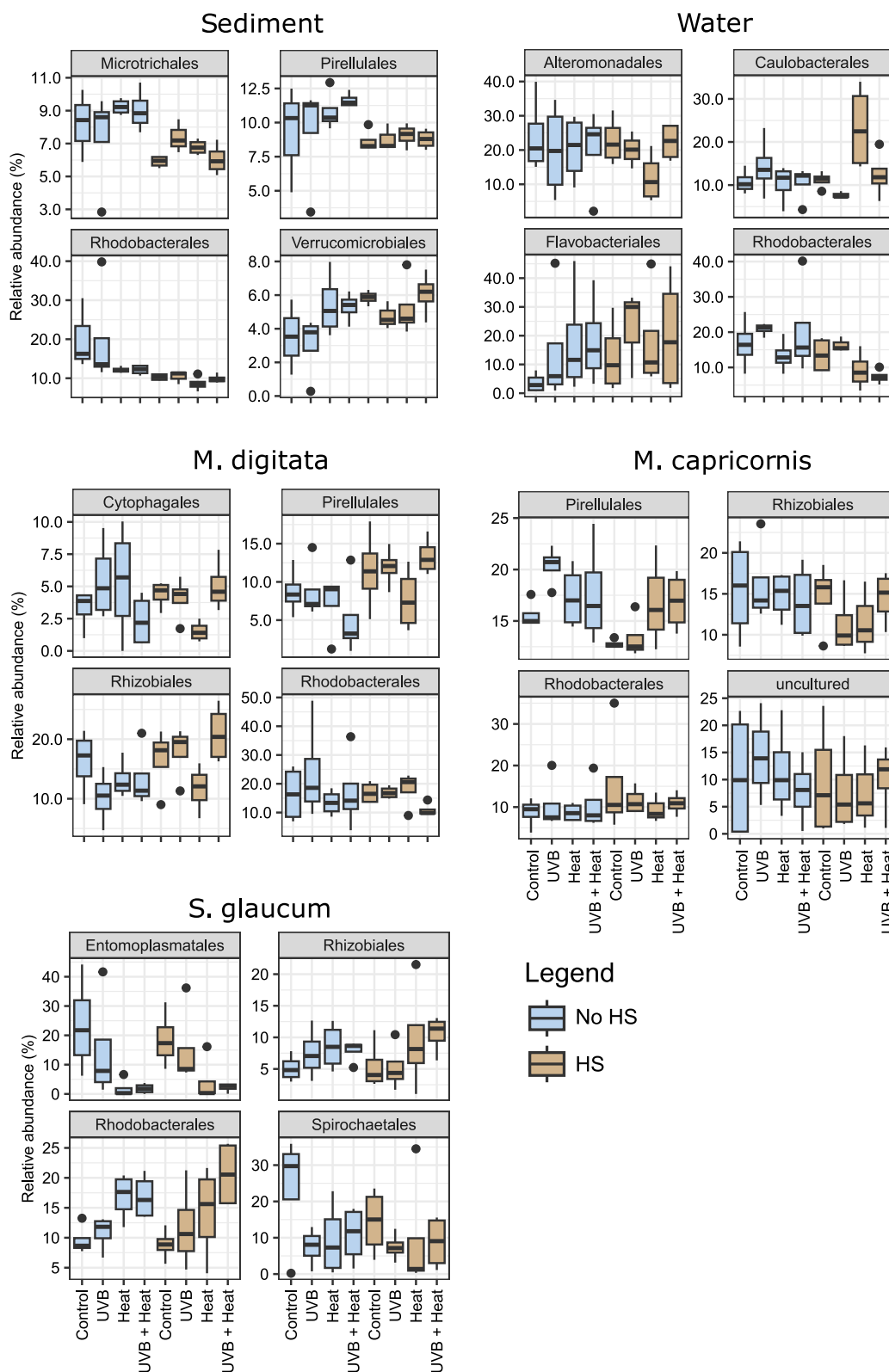
Figure 4. Boxplots of the relative abundance of the four most abundant orders in sediment, water, *M. digitata*, *M. capricornis*, and *S. glaucum* under the independent and combined effects of UVB, heat, and HS supplementation. Relative abundances are grouped per treatment within each biotope.

cosms without HS supplementation. This is in line with the significant interaction of HS supplementation, heat and UVB exposure on the bacterial composition of *M. digitata*. ASVs 36 and 37, assigned to the genera *Methy-*

loceanibacter and *Filomicrobium*, in turn, had markedly lower relative abundances in heat + UVB treated microcosms, but HS supplementation appeared to mitigate this effect.
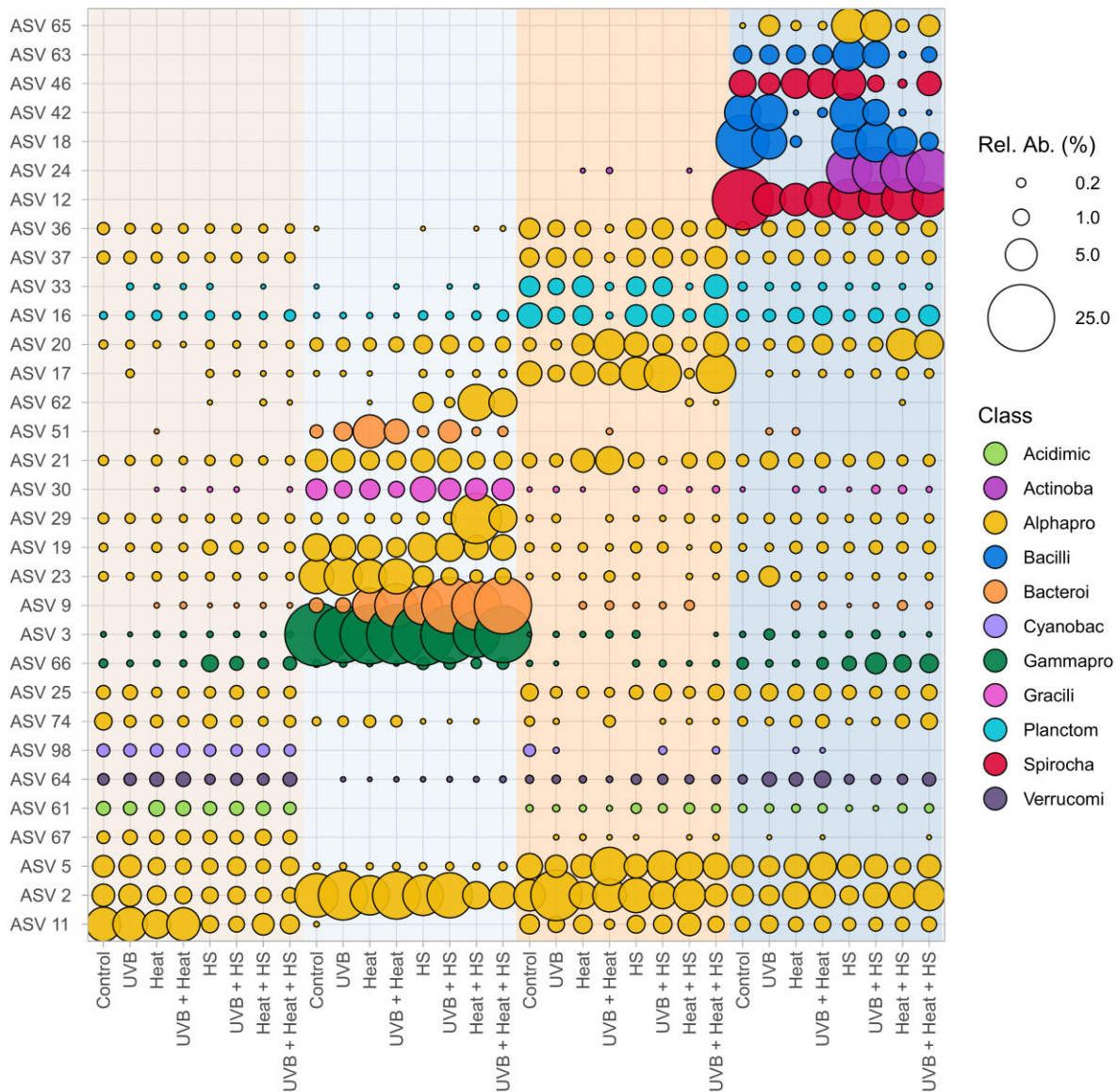
Figure 5. Mean relative abundances of the 10 most abundant ASVs in sediment, water, *M. digitata*, and *S. glaucum*. Relative abundances are grouped per treatment within each biotope. Symbols are proportional to the relative abundance of the respective ASV and colour-coded following their class-level taxonomic assignment.

In consonance with the significant effects of temperature and HS supplementation on the bacterial composition of *S. glaucum*, ASV 24, assigned to the genus *Rhodococcus*, was highly abundant in microcosms with and absent from microcosms without HS supplementation. Interestingly, this ASV was closely related to a humus utilizing *Rhodococcus* sp. *strain* isolated from marine sediment (100% sequence similarity; GenBank MT626344.1). ASVs 18 and 42, both assigned to the genus candidatus *Hepatoplasma*, were highly abundant under ambient temperatures, but markedly less so in heat-treated microcosms. In contrast, ASVs 2 and 20, assigned to the alphaproteobacterial families Rhodobacteraceae and Methyloligellaceae, were markedly more abundant in heat-treated microcosms. ASV-2 was closely related to a *Tritonibacter litoralis* isolate, and ASV-20 to an organism obtained from the sponge *Terpios hoshinota* (both 100% sequence similarity; GenBank MN544912.1 and KX177464.1, respectively).

Significant predictor ASVs

Significant predictor ASVs based on the Boruta analysis which had an importance level >5.00% are presented in Fig. 6 for sediment, water, *M. digitata*, and *S. glaucum*. Several ASVs exhibited consistent responses to HS supplementation across biotopes (sediment, water, *M. digitata*, and *S. Glaucum*). ASVs 39, 43, 81, and 310, for example, were all more abundant in microcosms without HS supplementation, whereas ASVs 50, 66, and 256 were all more abundant in microcosms with HS supplementation (see Table S8 for statistical results).

Discussion

Our results indicate that HS modulate bacterial communities and mitigate the detrimental effects of UVB radiation and temperature stress on corals.
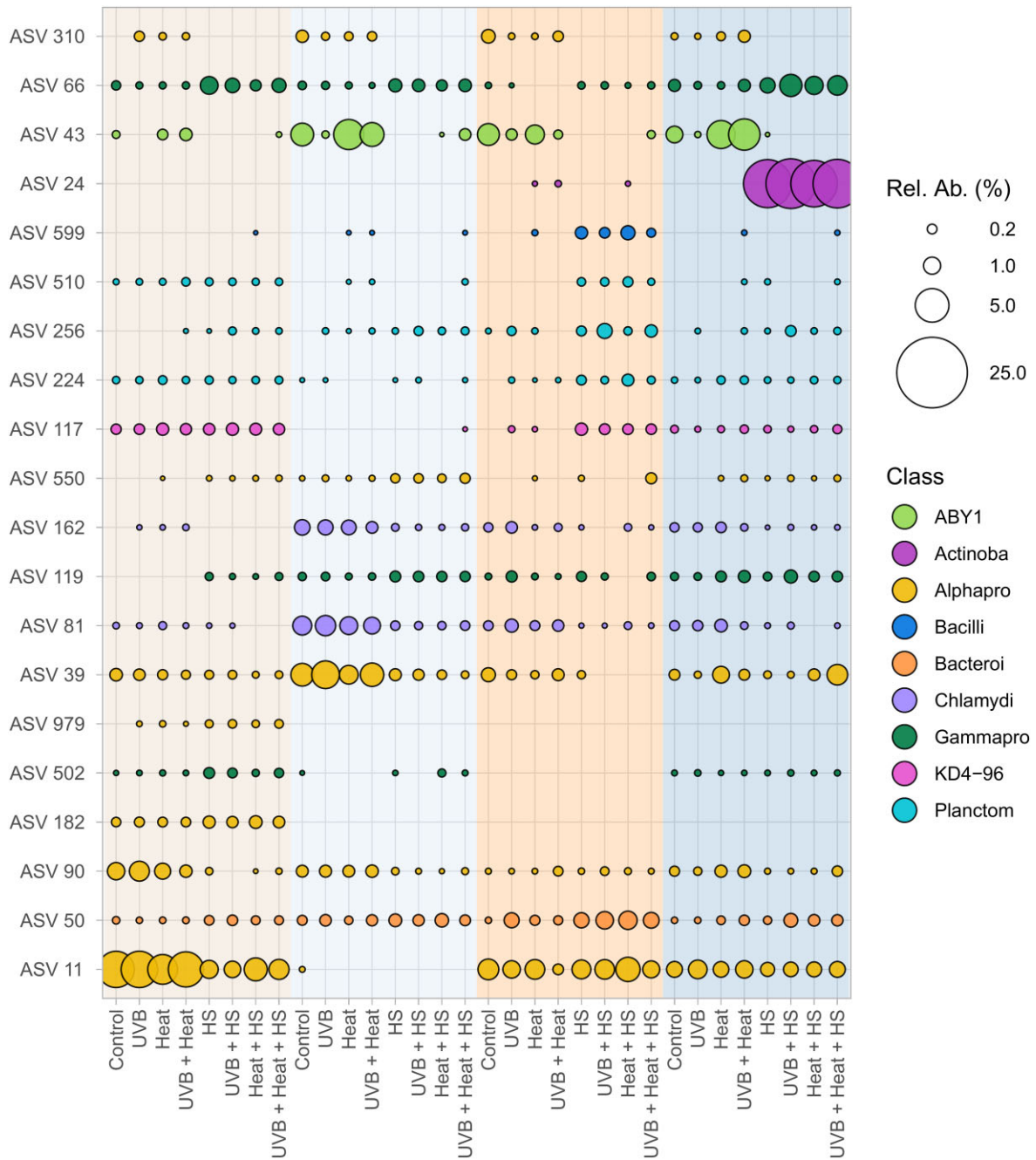
Figure 6. Mean relative abundances of the ASVs with more than 5.00% importance in the Boruta analysis in sediment, water, *M. digitata*, and *S. glaucum*. Relative abundances are grouped per treatment within each biotope. Symbols are proportional to the relative abundance of the respective ASV and colour-coded following their class-level taxonomic assignment.

Effects of HS, temperature, and UVB on photosynthetic activity

The photo-system type II (PSII) maximum potential quantum efficiency of the coral-associated zooxanthellae is widely used as a quantitative indicator of coral photo-oxidative stress (Downs et al. 2002, Travesso et al. 2023). According to the 'oxidative stress theory', temperature and UVB-induced coral bleaching result from a PSII-dysfunction in the zooxanthellae chloroplasts leading to reduced photosynthetic efficiency, excessive production of reactive oxygen species (ROS) and de-activation of various ROS-neutralizing pathways. ROS can subsequently accumulate and diffuse into the coral-host tissue causing oxidative stress and result in zooxanthellae expulsion (Downs et al. 2002). In the present study, we found that elevated temperatures were associated with significantly lower Fv/Fm ratios in the hard corals *M. digitata* and *M. capricornis*, but not in the soft coral *S. glaucum*. The results for both hard coral species confirm previous laboratory studies and *in situ* observations (Higuchi et al. 2013, Manullang et al. 2020) and suggest that the photosynthetic machinery of the coral symbionts were damaged by the high-temperature treatment (Downs et al. 2002). In contrast to our results, an-

other study found a significant reduction in Fv/Fm ratios in *S. glaucum*, following exposure to a laboratory-induced heat-wave (Travesso et al. 2023). Travesso et al. (2023), however, exposed their specimens for a period of 10 days to an elevated temperature in contrast to the 5 days of our study. The lack of a significant response of *S. glaucum* in our study might, therefore, be related to the shorter duration of our experiment. Although non-significant, Fv/Fm ratios of *S. glaucum* in heat-treated microcosms without HS supplementation were lower than the controls and longer exposure might have resulted in additional reductions in photosynthetic efficiency.

In contrast to heat, HS supplementation alone had a significantly positive effect on Fv/Fm ratios, and, most importantly, HS supplementation appeared to mitigate the adverse effect of elevated temperature in all three tested species. While rising seawater temperatures can trigger coral bleaching, prior research indicates that corals are particularly susceptible to bleaching when subjected to higher levels of UV radiation (Ayoub et al. 2012). Therefore, the effects of HS supplementation on coral photosynthetic efficiency may be explained by their optical properties, which are characterized by a particularly high absorbance of wavelengths in the UV spectrum (Del Vecchio and Blough 2004). It is also plausible that in microcosms supplemented with HS, corals exhibited increased photosynthetic efficiency through a process known as photoacclimation (Roth 2014), responding to altered light conditions. HS are furthermore known to have various beneficial effects on animals, including improved nutrient utilization, antioxidant properties, immunomodulation, anti-inflammatory effects, and enrichment of beneficial bacteria (De Lourdes et al. 2022). Hence, the observed enhancement in coral photosynthetic efficiency due to HS supplementation likely results from complex interactions that warrant further investigation.

Effects of HS, temperature, and UVB on environmental and host-associated bacterial communities

Our results showed that HS supplementation significantly influenced the bacterial composition of all studied biotopes, with the exception of *M. capricornis*. This aligns with previous studies on the effects of HS on marine bacterioplankton and bacterial communities associated with fish and sponges (Lindh et al. 2015, Louvado et al. 2021, Stuij et al. 2024a). It further strengthens the evidence for the substantial modulatory role these substances play across a wide range of marine biotopes. Several ASVs were positively or negatively associated with HS supplementation across multiple biotopes. ASVs associated with HS supplementation were assigned to a range of taxa including the gammaproteobacterial family *Halieaceae*. Interestingly, Shore et al. (2021) previously observed an enrichment of *Halieaceae* members in bacterial communities of the sponges *Xestospongia muta* and *Agelas clathrodes* following increased terrestrial run-off. In *M. digitata*, ASVs assigned to the families *Gimesiaceae* (previously *Planctomycetaceae*) and *Pirellulaceae,* were also associated with HS supplementation. Members of both families have previously been detected in association with coral and sponge hosts (Mohamed et al. 2010, Ostria-Hernández et al. 2022, Sun et al. 2022). *Gimesiaceae* are part of the order Planctomycetales. Coral-associated Planctomycetales displayed genomic potential for the anaerobic oxidation of ammonium, suggesting their importance in the nitrogen cycle within coral-hosts (Zhang et

al. 2015). Moreover, in a study which evaluated the bacterial communities of 26 coral genera, ASVs assigned to the order Pirellulales were mostly associated with healthy coral microbiomes, and their abundances were lower in bleached individuals (Sun et al. 2022). In *S. glaucum,* an abundant ASV assigned to the genus *Rhodococcus*, was a significant predictor of HS supplementation, and was completely absent in microcosms without HS supplementation. *Rhodococcus* members are widely distributed in the environment and have also been observed in association with coral hosts (Hackbusch et al. 2020, Ramaprasad et al. 2018); they are known for their ability to degrade a wide range of complex organic compounds (Hackbusch et al. 2020). Several *Rhodococcus* strains obtained from tundra soils were identified as putative HS degraders (Park et al. 2021). ASV-24, furthermore, had 100% similarity with a strain classified as *Rhodococcus* and described as a 'humus-utilizing bacteria' isolated from sediment in the Pacific Ocean (accession number MT626344.1). In addition, they may also act as antagonists of bacterial pathogens. Several *Rhodococcus* strains from the coral species *Coscinaraea columna*, *Platygyra daedalea*, and *Po. harrisoni* exhibited antibiotic activity against potential pathogenic bacteria (Mahmoud and Kalendar 2016), suggesting a role in host defense.

In *M. digitata*, we observed a significant interactive effect of elevated temperature, UVB radiation, and HS supplementation on bacterial community composition and higher taxon abundance. Several dominant ASVs were less abundant in heat and UVB-treated microcosms without HS, but this effect was not apparent under HS supplementation. This included ASVs assigned to the genera *Methyloceanibacter* and *Filomicrobium*, which have been previously identified as core members of several coral species of the genus *Montipora* (Cai et al. 2018). In contrast, an ASV assigned to the genus *Pyruvatibacter* was more abundant in heat and UVB treated microcosms without HS supplementation. Recently, genomic analysis of a *Pyruvatibacter* strain isolated from a marine microalga revealed that it could utilize various antioxidants to deal with oxidative stress (Rong et al. 2021). Therefore, the enrichment of this bacteria in *M. digitata* exposed to elevated temperatures and UVB radiation may be associated with host oxidative stress; HS supplementation, however, appeared to mitigate this.

In contrast to the interactive effect in *M. digitata*, we observed a significant, independent effect of heat on the bacterial community composition of the soft coral *S. glaucum*. Previously, a similar response to elevated temperature was observed in the coral *Acropora tenuis* (Littman et al. 2010), but not in the soft coral *Lobophytum pauciflorum* (Wessels et al. 2017).

Conclusion

Our results showed that elevated temperature and UVB affected the photosynthetic activities of all corals studied. Importantly, the adverse effects of these abiotic factors were mitigated by HS supplementation. With respect to bacterial composition, our results showed a pronounced effect of HS across all biotopes with the exception of *M. capricornis*. Temperature, in contrast, only had a significant independent effect on *S. glaucum* and a significant interactive effect on *M. digitata*. Reef organisms in HS-supplemented microcosms exhibited distinct bacterial community profiles enriched with known groups of potentially beneficial bacteria. Particularly

in the hard coral *M. digitata*, we observed an interactive effect of HS, UVB, and temperature. For example, potentially beneficial symbiotic bacteria were relatively more abundant in HS-supplemented microcosms subjected to heat stress and UVB exposure. Our findings indicate that HS significantly modulates coral reef bacterial communities and further support the hypothesis that these substances contribute to improved resistance to the adverse effects of elevated temperature and UVB radiation.

Periodic bleaching during severe ENSO-induced warming events has induced large-scale destruction of coral reef habitat (Hughes et al. 2017). Coral reef resilience and resistance has been suggested to play key roles in bleaching susceptibility (Hughes et al. 2010). Importantly, both resistance and resilience are largely determined by site-specific conditions, for example, inputs of nutrients, pollutants, turbidity, sedimentation rates, and grazing activity (Babcock and Davies 1991, Mumby et al. 2006, MacNeil et al. 2019, Zweifler et al. 2021). An improved understanding of the interplay between these different environmental factors is crucial to successfully restore and protect coral reefs. The reported effects of HS suggest that natural forests and wetlands, the predominant sources of HS inputs into coastal ecosystems, may have an important, but understudied, role in promoting coral reef resistance and resilience. As such, large-scale clearance of coastal and riparian forests may have significantly contributed to the increased vulnerability of certain coral reefs to climate-induced bleaching. Management efforts, with a focus on restoring natural forests on previously cleared lands will not only restore habitat for terrestrial organisms, but may also lead to a cascade of beneficial effects for adjacent marine ecosystems.

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Author contributions

Tamara M. Stuij (Formal analysis, Investigation, Writing – original draft, Writing – review & editing), Daniel.F.R. Cleary (Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing), Nicole J. de Voogd (Conceptualization, Resources, Supervision, Writing – review & editing), Rui.J.M. Rocha (Conceptualization, Resources), Ana Rita M. Polónia (Investigation, Writing – review & editing), Davide A.M. Silva (Conceptualization, Investigation, Methodology, Writing – review & editing), Jörg C. Frommlet (Resources, Writing – review & editing), Antonio Louvado (Conceptualization, Investigation, Methodology, Writing – review & editing), Yusheng M. Huang (Resources, Writing – review & editing), and Newton.C.M. Gomes (Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing)

Supplementary data

Supplementary data is available at *JAMBIO Journal* online.

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Data availability

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

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