Supplementary material:

Development and validation of an experimental life support system to study coral reef microbial communities

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Methods

Coral Fragmentation

Of the five model species, larger individuals were fragmented into 2-3 cm pieces and glued (acrylite) or stitched (*Sacrophyton glaucum*) to small carbonate sand stones (3 cm in diameter, 1 cm thickness). To recover from the fragmentation process, coral fragments were kept in coral reef aquaria (Rocha et al. 2015) for four months before transplantation to the ELSS.

Pore water sampling

Porewater samples were obtained using Rhizon flex samplers with a pore size of 0.6 µm (product number 19.60.25F, Rhizosphere Research Products, Wageningen, The Netherlands). To collect the sample, the sampler was connected to 50 ml disposable syringes. Nitrate NO^{3-}; nitrite NO^{2-}; ammonium NH^{4+}; and phosphate PO_4^{3-} were analysed on the day of sampling using photometric methods following the standard analytical protocol of the Supelco Spectroquant® test kits 1.14942, 1.14776, 1.14752 and 1.14848, respectively (Merck, Darmstadt, Germany). From the obtained absorption value, nutrient concentrations were calculated using standard curves, which were determined from standard solutions prior to sample analysis. Immediately after collection, aliquots of the pore water samples were sent to an external service provider (A3lab, Ilhavo, Portugal), for sulphate SO_4^{2-} and TOC analysis. SO_4^{2-} was measured by turbidimetry using discrete spectrophotometry following the standard analytical method: CZ_SOP_D06_02_016 (SM 4500-SO4).

TOC was measured using infra-red (IR) detection following the standard analytical method: CZ_SOP_D06_02_056 (SM 5310).

DNA extraction

Whole membrane filters (bacterioplankton communities) and ± 500 mg of sediment and host organisms were transferred to Lysing Matrix E tubes containing a mixture of ceramic and silica particles. Due to the small size of *Zoanthus* sp. (< 60 mg) and *Chondrilla* sp. (varied between 130 and 500 mg), whole organisms were used for extraction. For *M. digitata* and *M. capricornis*, fragments (tissue and skeleton) were first snap frozen in liquid nitrogen and subsequently ground using mortar and pestle. Blank negative controls, in which either no tissue or a sterile membrane filter was added to the Lysing Matrix E tubes, were also included. Microbial cell lysis was performed in the FastPrep Instrument (MP biomedicals) for 2 x 40 seconds 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 ºC until further use.

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-barcoding PCR library preparation approach. First 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 $pmol^{-1}µl^{-1}$. Reaction mixes were finalized 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 10 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.

Subsequently, 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 x 1 µl (concentration of 10pmol/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 resulted PCR products were measured on a fragment analyzer 5300 (Agilent) and subsequently, each 96-well plate was normalized 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 resulted pool of normalized 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 Id **PRJNA904682**.

References

Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glöckner FO (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res 41:1–11

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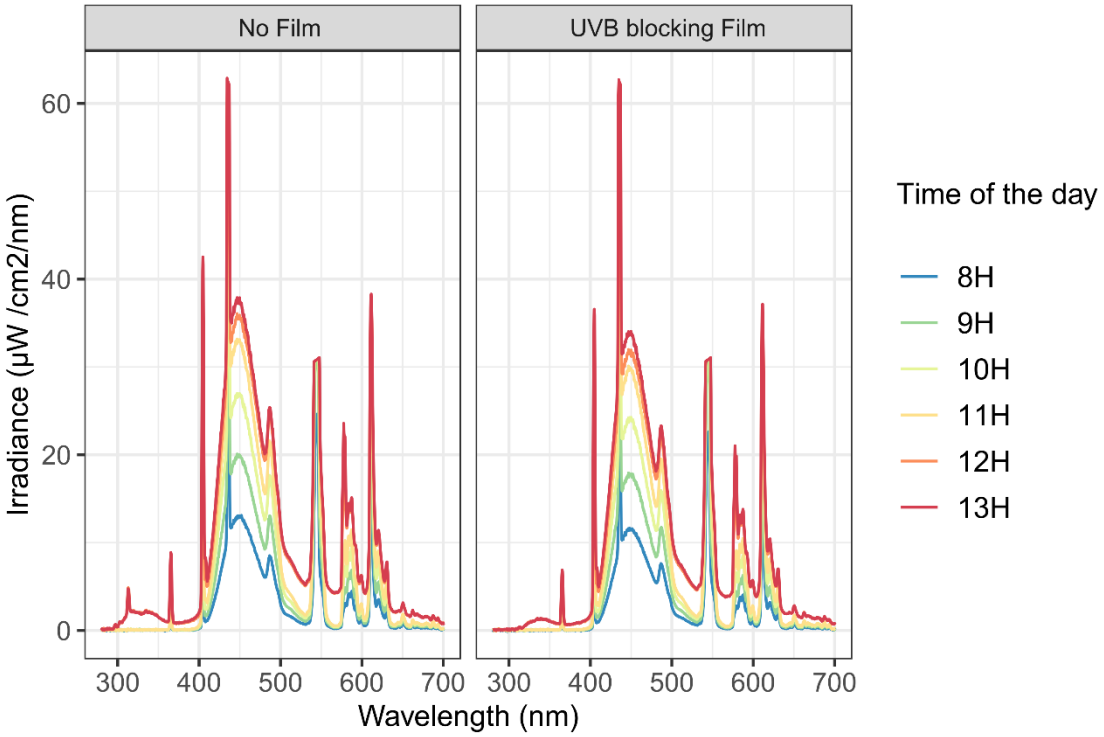
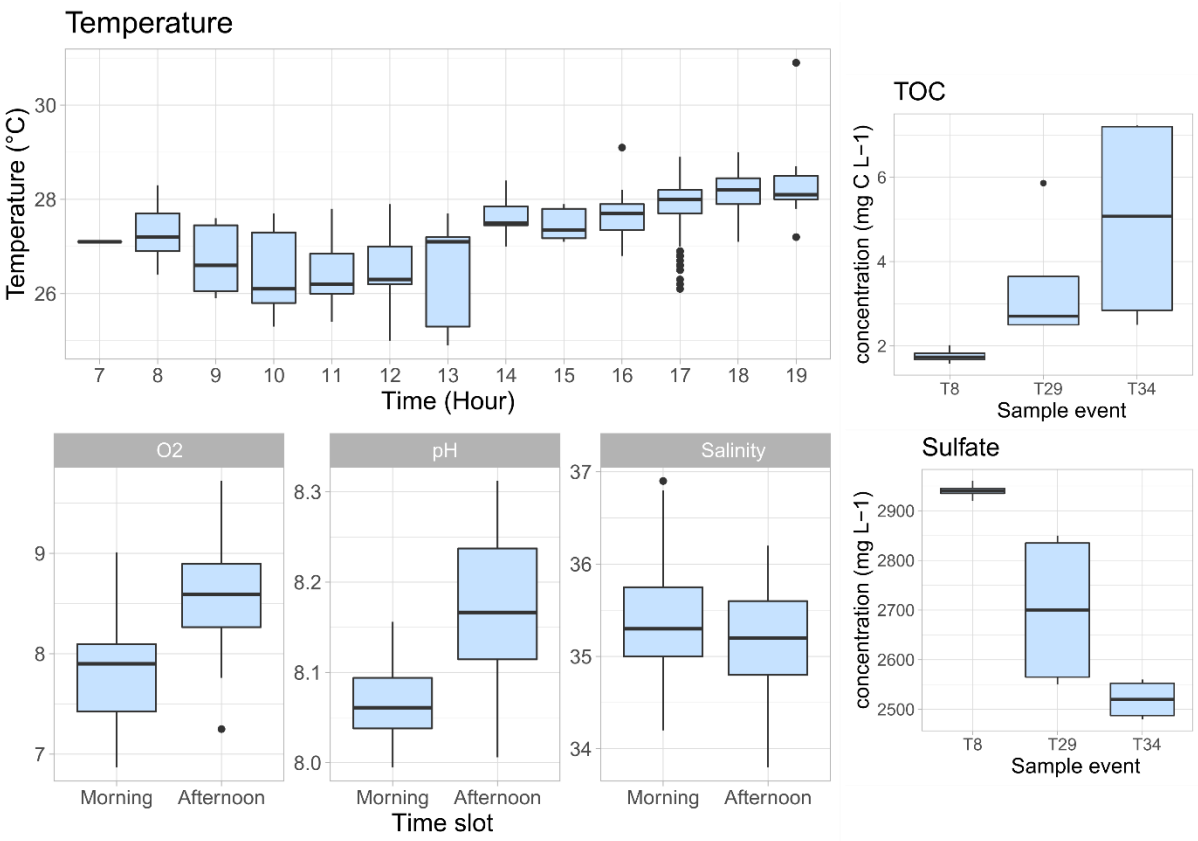
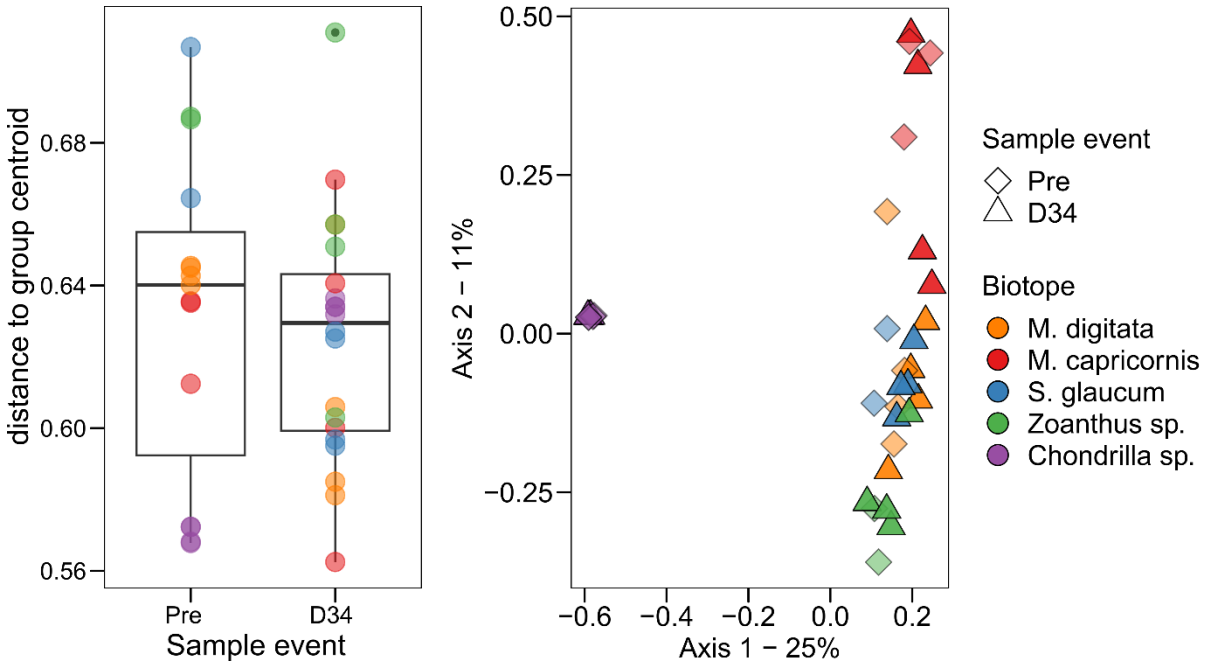


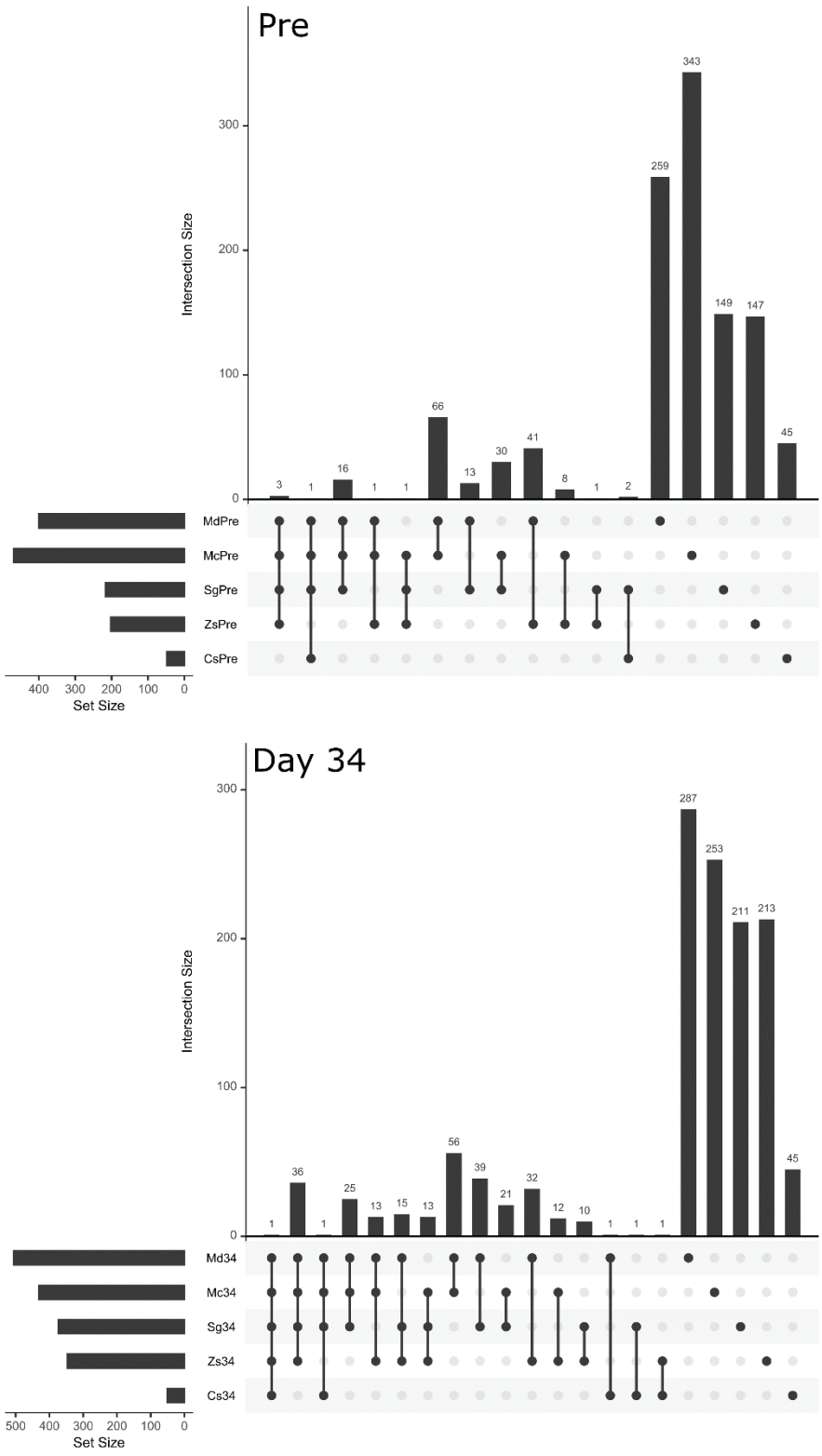
Supplementary Fig. 1 Irradiance spectrum of the fluorescent lamps measured at the point where the light would hit the water surface with and without the transparent polyester film (Folanorm SF-AS, Folex coating, Köln, Germany). Light intensity increased from 8.00 in the morning to 13.00 in the afternoon.



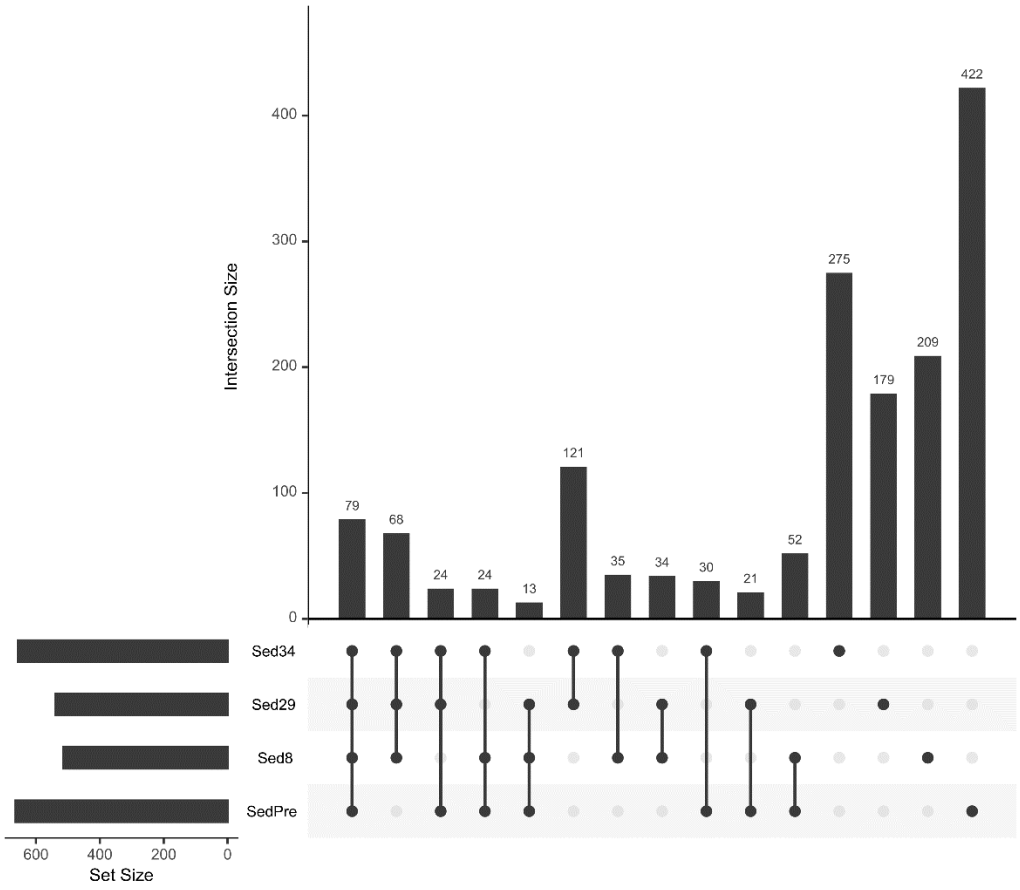
Supplementary Fig. 2 Physical and chemical parameters measured at different timepoints during the experiment. Temperature (hourly), oxygen, pH and salinity (two times per day) were measured in the water column. TOC and Sulphate were measured in the sediment porewater at day 8, 29, and 34.



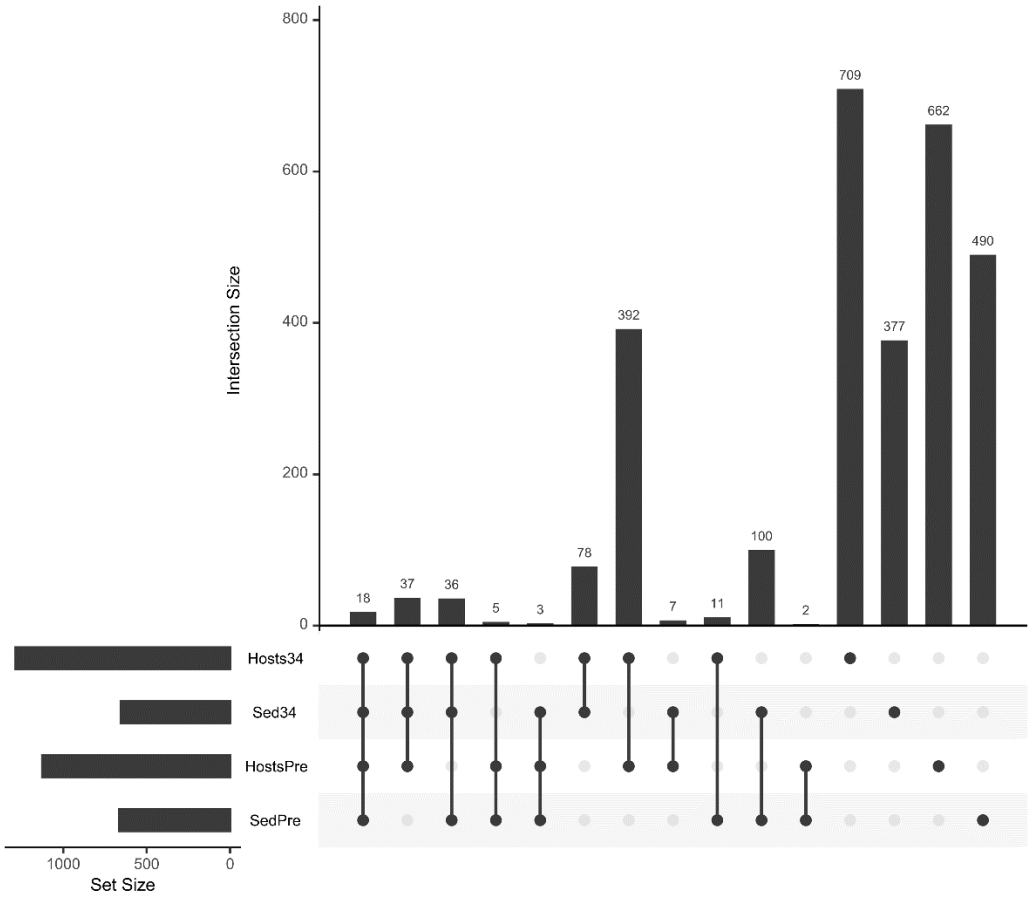
Supplementary Fig. 3 Distance to the group centroid (left) and ordination (right) showing the first two axes of the principal coordinates analysis (PCO) of prokaryote ASV composition for host organisms based on Bray-Curtis transformed distances. Within group variance is not significantly different between Pre and D34 samples of host organisms (left graph, ANOVA, $F_{1,33} = 0.26$, $p = 0.61$). Biotope explained 48% of the variation in the dataset (PERMANOVA, $R^2 = 0.48$, $F_{4,30} = 6.93$, $p = 0.001$).



Supplementary Fig. 4 Shared ASV analysis among sample groups. Set size: Total amount of ASVs observed within Pre and day 34 host organisms with ≥ 0.1% abundance in at least one sample. Intersection size: Total amount of unique and shared ASVs among groups. Md: *Montipora digitata*, Mc: *Montipora capricornis*, Sg: *Sarcophyton glaucum*, Zs: *Zoanthus* sp., Cs: *Chondrilla* sp..



Supplementary Fig. 5 Shared ASV analysis among sample groups. Set size: Total amount of ASVs observed among sediments at different sample events with ≥ 0.1% abundance in at least one sample. Intersection size: Total amount of unique and shared ASVs among groups.



Supplementary Fig. 6 Shared ASV analysis among sample groups. Set size: Total amount of ASVs observed among Pre and day 34 host organisms and sediment with ≥ 0.1% abundance in at least one sample. Intersection size: Total amount of unique and shared ASVs among groups.