Environmental Microbiome

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Composition and rhythmic variations in the microbiome of Southwestern Atlantic corals

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Abstract

Background Diel and tidal rhythms can regulate the metabolism, physiology, behavior, and gene expression patterns of different organisms, with evidence of an integration on the circadian behavior of host species and their microbial community. Corals host a diverse and dynamic microbial community, with variable diversity and abundance across geographic and temporal scales. Within scleractinian corals, those that host endosymbiotic algae (i.e., zooxanthellate) display a diel variation in the oxygen levels, an oscillation in their internal environment that has the potential to influence its microbiome abundance and/or composition. Here we investigate in situ daily fluctuations on the microbial community of two zooxanthellate (*Madracis decactis* and *Mussismilia hispida*) and two azooxanthellate coral species (*Tubastraea coccinea* and *T. tagusensis*) along a 72-hour period.

Results Day and night alpha diversity values were similar for all species, with *Ma. decactis* hosting a significantly more diverse community. Similarly, there was no fluctuation in the microbiome composition at the Amplicon Sequence Variants (ASV) level between day and night within species, but all species were significantly different from each other. Interestingly, *Mu. hispida*, an endemic species to the Southwestern Atlantic, had a high proportion of unidentified microbial taxa at genus level, suggesting a species-specific microbiome community composed by unidentified taxa. Significant rhythmicity in the abundance of individual ASVs was observed for one ASV (genus *Pseudoalteromonas*) in *T. tagusensis* and one (genus *Woeseia*) in *Ma. decactis*, with 24 and 12-hour fluctuations, respectively. In addition, DESeq2 recovered 13 ASVs (four in *Ma. decactis*, two in *Mu. hispida*, six in *T. coccinea*, and one in *T. tagusensis*) with different abundances between day and night.

Conclusions Results show divergent microbial communities when comparing zooxanthellate and azooxanthellate species, with few significant changes within a 24-hour period. Future studies should focus on metabolic pathways to better understand how the microbiome community can adjust to environmental changes within the coral host in short time scales.

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Background

Biological processes following environmental rhythms are present in nearly all living forms, but the most ubiquitously observed cycle affecting behavior, physiological responses, and even gene expression is the day/night cycle. Bacteria are not an exception. In the Hawaiian bobtail squid, the bioluminescent bacteria *Aliivibrio fischerii* present in a specific organ display bioluminescence during the night, when the population is large, and are expelled at dawn, to then increase again in abundance during the day [1, 2]. The reproductive cycle of *Epulopiscium* spp., a bacteria present in the gut of a surgeonfish (*Naso lituratus*), also follows a diel pattern, with mature endospores only being found at night [3]. In mice, a few strains of bacteria from their gut microbiome vary in abundance between day and night [4]. These works show that animal microbiome can display its own circadian clock, integrating with the host circadian rhythm. It has been shown in humans that the host circadian clock can regulate the gut microbiome, such as the daily change in mobility in the gastrointestinal system induced by the presence of melatonin [5]. At the same time, the microbiome can also influence the host, as observed for plants, when the period of host diel cycles was modified in disrupted rhizosphere microbial community [6].

For corals, the microbiome relevance is remarkable, once it has been related to coral diseases (e.g., white-plague disease [7, 8]) as well as disease-avoidance mechanisms (e.g., some bacteria can inhibit the growth of other bacteria [9]). As observed in other life forms, the coral microbiome can metabolize and provide nutrients to the host [10–12]. Part of the coral microbiome is dynamic, as it can change along a species distribution, over the life cycle, or as a response to environmental factors [13]. Over a diel cycle, it is still unknown how microbiome changes affect coral's circadian rhythms, but the coral host may be able to influence its microbial community composition/balance. External cyclic signals, like sunlight, have been associated with different processes in corals, such as larval settlement [14], tentacle expansion [15], calcification rates (higher during the day than at night in zooxanthellate coral species, i.e., species that host photosynthetic dinoflagellates of family Symbiodiniaceae) [16–18], and timing of reproduction in corals [19]. In addition, the photosynthetic oxygen production in zooxanthellate corals displays a diel variation [20], which, in turn, can provide an environment with a marked diel oscillation to its microbiome. To date, studies reported that the microbial community diversity did not change following a diel rhythm [21–23], but changes in abundance have been observed for a few bacterial strains in the anemone *Nematostella vectensis* [22] as well as in the Pacific species *Porites lutea*, *P. cylindrica*, and *Pocillopora damicornis* [23]. The activity of endosymbiotic cyanobacteria has also been shown to change in a daily basis (possibly peaking at dusk and dawn) in the coral *Montastraea cavernosa* [24]. Nevertheless, all available studies that focused on endosymbiotic bacteria composition and/or abundance targeted Pacific zooxanthellate species, where coral reefs are remarkably different from those in the Southwestern Atlantic, considering water temperature and turbidity [25–29]. Azooxanthellate species, on the other hand, lack the potential diel variation caused by Symbiodiniaceae photosynthetic activity, thus corresponding to an interesting model to better understand the host and microbiome circadian patterns in a somewhat constant internal environment.

In addition to a nearly 24-hour oscillation, as a response to the day/night cycles, biological rhythms with 12-hour oscillation have been recently observed in some anthozoans, with circatidal patterns of gene expression in the facultative photosymbiotic coral *Euphyllia paradivisa* (i.e., the symbiotic relationship with Symbiodiniaceae is facultative) and in the aposymbiotic anemone *N. vectensis* [30, 31]. For the facultative anemone *Exaiptasia diaphana*, aposymbiotic morphs had the majority of genes being expressed with a tidal rhythm while, in symbiotic morphs, the majority of genes were expressed in a diel rhythm [32].

Rhythms allow organisms to cope/prepare to cyclic changes in their environment, such as food availability and the likelihood of predation [33], and a disruption in this pattern may threaten species' survival. For example, corals that spawn at night have been shown to be affected by artificial light at night (ALAN) [34], resulting in reduced synchrony and increased predation on coral larvae [34–36]. Consequently, ALAN is currently considered a major anthropogenic impact affecting organisms globally [37]. To date, diel and tidal rhythms in coral microbial communities have only been investigated in a few species (i.e., *Mussismilia braziliensis*' mucus [21]; *Nematostella vectensis* [22]; *Porites lutea*, *P. cylindrica*, and *Pocillopora damicornis* [23]). In this study, we investigate the diel and tidal changes in the composition and abundance of the microbial community in four sympatric scleractinian corals, including two zooxanthellate (*Madracis decactis* and *Mussismilia hispida*) and two azooxanthellate species (*Tubastraea coccinea* and *T. tagusensis*), along a 72-hour period within a marine protected area in the Southwestern Atlantic, correlating the findings of microbial strain abundance with abiotic variables. Understanding how corals' different rhythms behave in less impacted areas will allow us to differentiate natural variations from those that may be caused by stressors such as pollution and climate change.

Methods

Experiment setting and collection

This study was conducted in Alcatrazes Archipelago, São Paulo, Brazil, starting with the collection and preparation of zooxanthellate species in December 2018 and May 2019. To avoid potential biases from the recovery process, one colony of each zooxanthellate species (~5 m distant from each other) was collected and fragmented into 30 pieces with ~5 cm^2 (*Ma. decactis*) and 19 pieces with ~15 to 30 cm^2 (*Mu. hispida*). All fragments were transplanted near the colony's original sampling sites (1 m apart) following the same orientation (vertical, or 90º, for *Ma. decactis* and horizontal, or 180º, for *Mu. hispida*). For *Tubastraea tagusensis* and *T. coccinea* (both azooxanthellate), whole colonies were sampled from a 1 m^2 area from a negatively oriented surface. *Tubastraea* was introduced to the Brazilian coast in the late 1980's [25] and invasive populations within the sampling area were shown to be highly clonal [38] with a gregarious settlement pattern, ensuring that colonies in close proximity are genetically identical. By the time of sampling (late October/2020), all fragments of *Mu. hispida* and *Ma. decactis* were fully recovered, with no signs of injuries, and growing. A fragment/colony of each species (one genotype per species) was collected every 4 h for 72 h (from 12 pm October 26th to 8 am October 29th; $N = 18$/species) and preserved in ethanol 100% (to avoid contamination, one set of collection tools was designated for each species and they were cleaned and sterilized with 70% ethanol and sodium hydroxide between samples). Two approaches and instruments measured oceanographic variables during sampling. Vertical profiles were performed using a CTD (Conductivity (C), Temperature (T), and Depth (D)) AAQ-RINKO from JFE Advantech Co. Ltd., equipped with sensors for pH, Photosynthetically Active Radiation (PAR), chlorophyll-a, turbidity, and dissolved oxygen, sampling at 5 Hz acquisition rates. Profiles were made every 2 h, starting at 12 pm on 26th Oct and ending at 10 pm on 29th Oct 2020, and all data was binned at 0.5 m vertical resolution. An additional CTD (RBR®) was moored at 11.5 m deep, about 50 m from the experimental settlement, equipped with optical sensors for detecting fluorescence by Colored Dissolved Organic Matter (CDOM), chlorophyll-a, and phycoerythrin (cyclops Turner Designs®), and Photosynthetic Available Irradiance (PAR) sensor from SeaBird®. The RBR CTD acquired data every 10 min, with 1 Hz frequency, from 6 pm on 26th Oct to 12 pm on 29th Oct 2020. On the last day of the experiment, both CTDs (RBR and AAQ-RINKO) were deployed together in a vertical cast for data comparison. The pressure sensor in the moored CTD strongly indicated periodic changes in sea level due to tides. As there are no tidal gauges present in Alcatrazes, we included in the analyses the sea level changes predicted by the forecast model recently developed for Alcatrazes by Carvalho et al. [39]. Comparison of predicted sea level and the pressure values registered by the CTD were in agreement (r2 above 0.8).

DNA extraction, PCR, and sequencing

Total DNA was extracted using the DNeasy PowerSoil Pro Kit (Qiagen) following the manufacturer's instructions. The quality and concentration of extracted DNA were verified by electrophoresis in agarose gel and spectrophotometer (NanoDrop 2000). Regions V3 and V4 of the 16S rRNA gene were then partly amplified with the universal primers Bakt_341F and Bakt_805R [40], following the same protocol used by Zanotti et al. [8] but with 28 cycles and using three PCR reactions per sample. DNA concentration of all samples was standardized to 5 ng/ul prior to PCR. The three PCR replicates per sample were pooled, purified using magnetic beads (Agencourt AMPure XP), and prepared for sequencing following Illumina's protocol for the 16S Metagenomic Sequencing Library Preparation [41] with those adaptations from Zanotti et al. [8]. The final concentration of each library was measured with Qubit® dsDNA HS Assay Kit and their average size was estimated using agarose gel electrophoresis. One sample (Mhi54) was removed from sequencing due to low concentration after library preparation. Libraries were pooled and sequenced on an Illumina MiSeq platform (two paired-end runs of 600 cycles). DNA sequencing was performed at the Genome Investigation and Analysis Laboratory (Genial– CEFAP/USP). Raw sequences are available in the National Center for Biotechnology Information Sequence Read Archive (BioProject ID: PRJNA1227542).

Data analysis

Read quality control, identification of Amplicon Sequence Variants (ASVs), and taxonomy inference were performed using the DADA2 pipeline [42], SILVA version 138.1 as database, and parameters maxEE 2,5 and truncLen (270,240) but mergepairs of 8 bp. Two samples were excluded from further analysis: Mde45 did not reach a *plateau* in the rarefaction curve (Additional file 01: Supplementary Figure S1) and Mde33 had substantially fewer reads (11,388) than all other samples (at least 20,371 reads).

The following analyses were based on multiple R packages (readxl, ggplot2, vegan, RColorBrewer, reshape2, scales, data.table, microbiome, dplyr, phyloseq, DT, microbiomeutilities, mirlyn, tibble, MetaCycle, GUniFrac, pairwiseAdonis, DESeq2, pals, Polychrome [43–62]; R script used for analyses and plots and *input* data files are included in Additional Files 02–35). After removing sequences of chloroplast, mitochondria, and *Archaea*, ASVs with less than five reads were excluded and the

dataset was repeatedly rarefied using mirlyn software [51] with parameters libsize = 20,371, rep = 100, set.seed = 120, replace = FALSE. The average ASV abundance found in all 100 replicates was used, for each species, in alpha diversity analyses using the Shannon index by period (day and night; ASV taxonomic level). This approach was chosen to prevent any biases on the recovered diversity due to the loss of rare taxa during rarefaction. Alpha diversity indexes were tested for normality with the Shapiro-Wilk test, and the non-parametric Kruskal-Wallis test was used to investigate significant differences among species and between day and night within species. When significant differences were detected, the Wilcoxon rank sum test was applied for pairwise comparisons, with the p-value adjusted by the Bonferroni correction. For the following analyses, the dataset was rarefied for the smallest number of ASVs (20,371) using GUniFrac [53]. The relative abundance of the 20 most abundant taxa was assessed at four taxonomic levels (phylum, order, family, and genus). Beta diversity (using absolute abundance at ASV taxonomic level) was evaluated using PERMANOVA and PERMDISP based on Bray-Curtis distance and plotted in a DCA to investigate differences in the microbial community between coral species, time of sampling, and presence of light (day vs. night). When PERMDISP was significant, ANOSIM was run instead of PERMANOVA. A post hoc test (using the pairwise.adonis function in the pairwiseAdonis library [48]) was used to verify significant differences. To investigate further patterns, beta diversity was also calculated between and within zooxanthellate and azooxanthellate groups and separately for each species (grouped by time and presence of light).

For rhythmicity analyses each species was rarefied separately, also using GUniFrac [53] to the smallest number of ASVs (*Ma. decactis*: 20,371; *Mu. hispida*: 39,403; *T. tagusensis*: 30,977 and *T. coccinea*: 65,675). The R package 'MetaCycle' [50] was used to investigate potential rhythmicity on the relative abundance of ASVs from all four species using the JTK_CYCLE algorithm [63] (JTK). To test for circadian and tidal rhythmicity, both 12-hour (minper = 12, maxper = 12) and 24-hour (minper = 24, maxper = 24) were tested for each coral species using Bonferroni correction in all cases. ASVs with ADJ.$P < 0.01$ in the JTK result table were considered significant (p-value cutoff obtained from [64]). DESeq2 was performed to test for significant differences in the abundance of ASVs between day and night in each coral species, based on ASV absolute abundance tables. Herein, only the ASVs showing a p-value < 0.05 and present in more than one sampling time period at night or daytime were considered significant.

In addition to these analyses, microbiome data were correlated with abiotic data with a Redundancy Analysis (RDA), using the vegan R package [56], to investigate its influence on the microbial community patterns. The abiotic data used in the RDA analysis corresponded to the RBR® multi-channel logger data observed at 120, 60, and 30 min before the sampling time. To further confirm the results, another RDA was performed with the AAQ-RINKO data taken at 11 and 12 m depth bins (the same range level as the experiment). As stated in the methods, sea level changes were obtained by the forecast model developed by Carvalho et al. [39].

Results

Sequencing generated on average 4.6 million reads per species, which corresponded to an average of 166,909 reads per sample assigned to 44,743 ASVs. Removal of ASVs with less than five reads and sequences from mitochondria, chloroplast, and *Archaea* led to a dataset with 31,904 ASVs. The taxonomy and ASV tables with non-rarefied data and rarefied data (with mirlyn and GUniFrac) are available as Additional files 03–07.

Alpha diversity was based on an average of 100 replicates generated by mirlyn (Fig. 1). Non-parametric analyses were used to compare diversity within and between coral species, since the dataset did not present a normal distribution (Shapiro-Wilk test; W = 0.95949, $p = 0.025$ and W = 0.95984, p-value = 0.02613 for log-transformed data). *Madracis decactis* showed higher alpha diversity values when compared with all other species (Kruskal-Wallis chi-squared = 26.055, df = 3, p-value = 9.286e-06; Additional file 01: Supplementary Table S1). Within species comparison between day and night microbial communities revealed no significant differences.

Results from the beta diversity indicated an associated microbial community significantly different between the four host species ([PERMDISP] F-value: 14.227; p-value: 0.001; [ANOSIM] R: 0.7184; significance: 0.001) and between zooxanthellate and azooxanthellate corals (two large groups represented by zooxanthellate and azooxanthellate corals in Fig. 2 as well as [PERMDISP] F-value: 57.739; p-value: 0.001; [ANOSIM] R: 0.7878; p-value: 0.001). It is interesting to note that *Ma. decactis* had the lowest dispersion (distance to centroid) among the microbial communities (Additional file 01: Supplementary Figure S6). Time of sampling and presence of light (day vs. night) did not correspond to clearly grouped samples and beta diversity within species was not significantly different between these groups ([PERMANOVA] Mde by Time $p = 0.623$; Mde Day vs. Night $p = 0.839$; Mhi by Time $p = 0.776$; Mde Day vs. Night $p = 0.754$; Tta by Time $p = 0.47$; Tta Day vs. Night $p = 0.575$; Tco by Time $p = 0.358$; Tco Day vs. Night $p = 0.106$). The post hoc test indicated that the microbiomes from all species were significantly different from each other (Additional file 01: Supplementary Table S2).

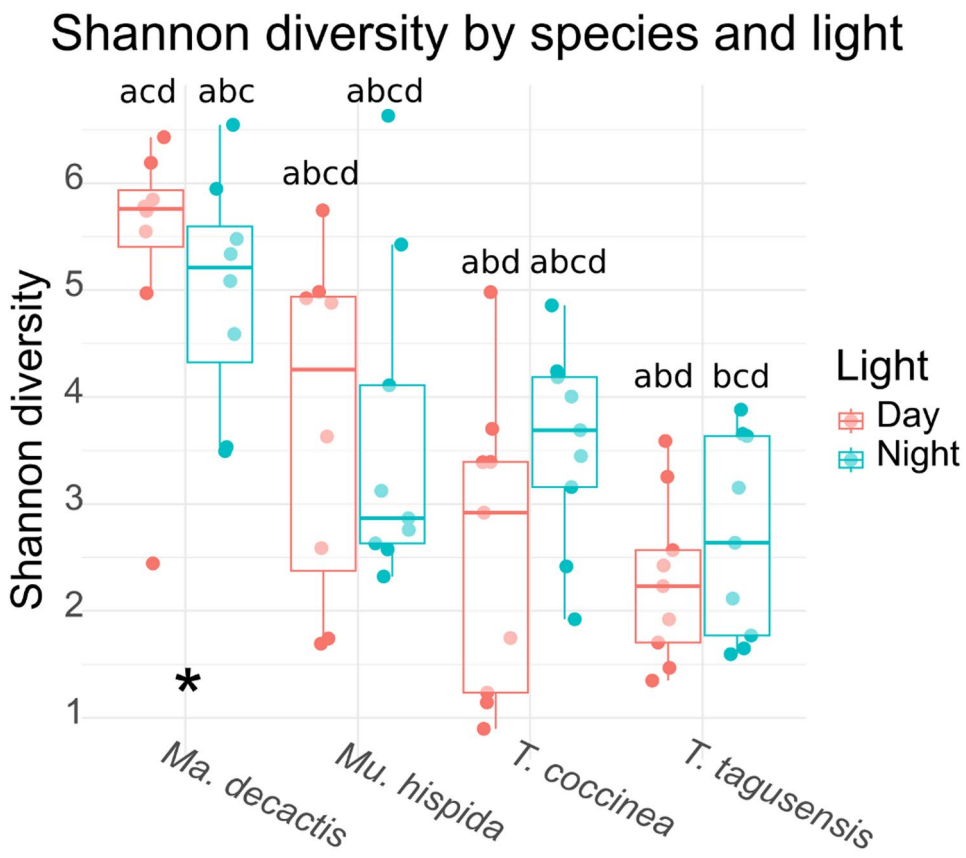

Fig. 1 Box plots showing ASVs alpha diversity based on the Shannon index for each species. The asterisk (*) indicates that *Madracis decactis* is significantly different in terms of diversity when compared to the other species and letters on each box plot indicate which species and light condition were significantly different from each other

Regarding the microbial community composition, there were no remarkable patterns over a 24-hour cycle (Additional file 01: Supplementary Figures S2-S5). Overall, *Proteobacteria* was, by far, the most abundant Phylum in all four scleractinians (Fig. 3). For family and order levels, *Ma. decactis* microbiome had a more equitable distribution of the 20 most abundant taxa, while the other species had one dominant taxon (family *Stappiaceae* and order *Rhizobiales* for *Mu. hispida*, and family *Endozoicomonadaceae* and order *Pseudomonadales* for *T. coccinea* and *T. tagusensis*; Fig. 3). At the genus level, *Mu. hispida* had a large proportion of unclassified taxa. *Ma. decactis* also had some unclassified genera, but with a more even abundance of the classified ones (*Endozoicomonas*, *Marine Methylotrophic Group 3*, *MBIC10086*, *Pelagibius*, *Vibrio*, and *Woeseia*) in comparison to the other species. For *T. coccinea* and *T. tagusensis*, two bacterial genera were notably more abundant (*Endozoicomonas* and *Marine Methylotrophic Group 3*; Fig. 3). Redundancy analysis (RDA) using abiotic data obtained with the RBR® multi-channel logger (temperature, depth, salinity, chlorophyll-a, PAR, phycoerythrin) and the AAQ-RINKO profiler (i.e. temperature, salinity, chlorophyll-a, PAR, dissolved oxygen, pH, turbidity) showed no significant correlations with the microbial community.

Comparing the microbial core among species, the proportion of ASVs, genera, and families present in all cases was lower than 5%. The only genus common among all species was *Endozoicomonas* and the few families present in all species are *Flavobacteriaceae*, *Cyanobiaceae*, *Rhodobacteraceae*, *Vibrionaceae*, and *Endozoicomonadaceae*. Within species, one to four ASVs were found in all samples: ASV12 (*Marine Methylotrophic Group 3*) for *Ma. decactis*; ASV4 (*Synechococcus CC9902*) and ASV6 (family *Stappiaceae*) for *Mu. hispida;* ASV1 (*Endozoicomonas*), ASV2 (*Marine Methylotrophic Group 3*), and ASV4 (*Synechococcus CC9902*) for *T. tagusensis*; and ASV1 (*Endozoicomonas*), ASV2 (*Marine Methylotrophic Group 3*), ASV4 (*Synechococcus CC9902*), and ASV7 (*Endozoicomonas*) for *T. coccinea*. When comparing day *versus* night samples within each species, two to eight ASVs were always present (Table 1).

Significant rhythmicity was observed only for the *T. tagusensis*' genus *Pseudoalteromonas* (oscillation period = 24 h) and for the *Woeseia* genus in *Ma. decactis* (oscillation period = 12 h) (Table 2; Fig. 4). For the

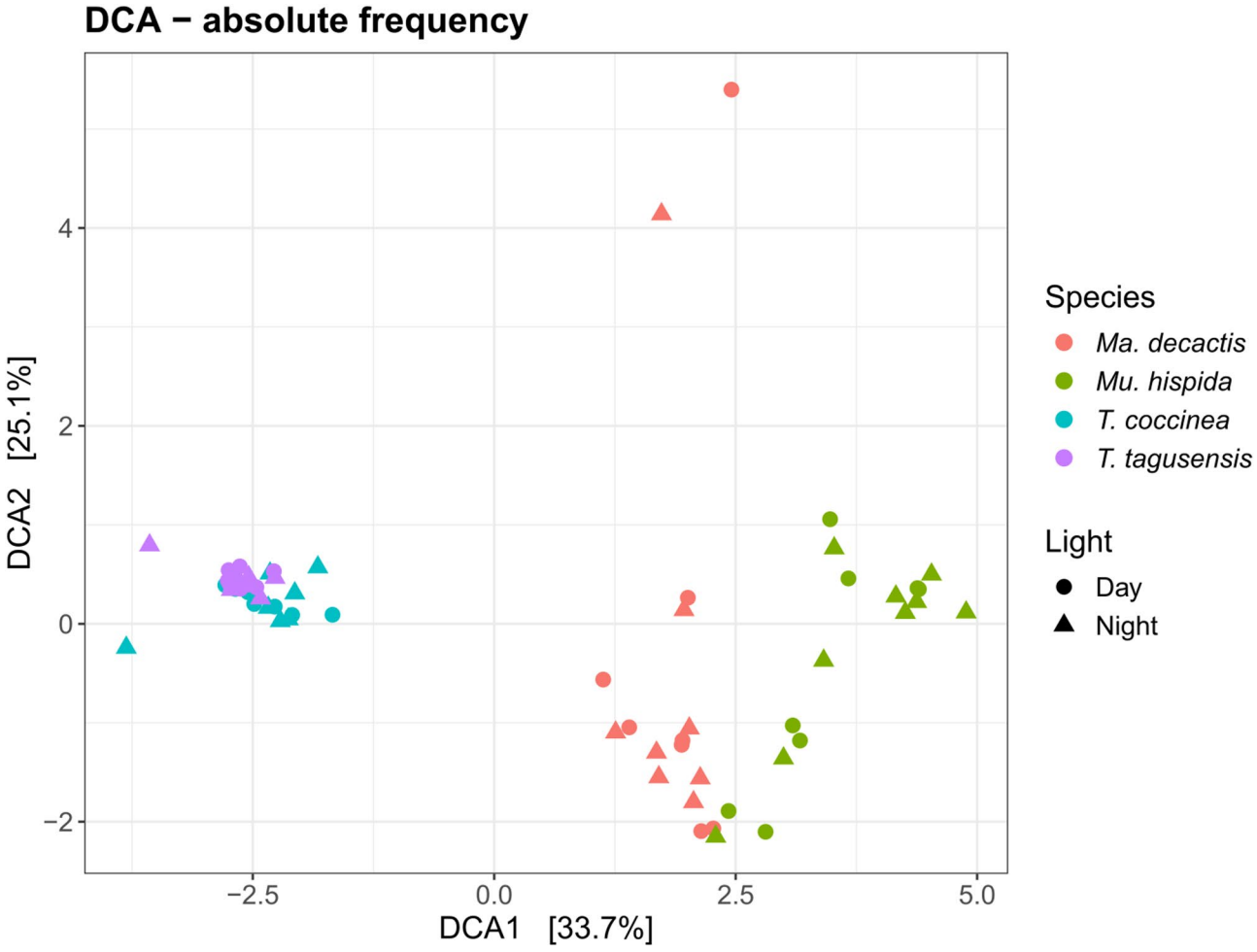

Fig. 2 Beta diversity analysis considering species and light (samples collected at day vs. night time). Detrended correspondence analysis (DCA) was performed at the ASV level

DESeq2, even though results identified 58 ASVs with different absolute abundances between day and night (two for *Mu. hispida*, one for *T. tagusensis*, six for *T. coccinea*, and 49 for *Ma. decactis*; Additional file 36), only 13 of them had more than one time period during day or night in which the ASV was present (Table 2; Fig. 4). While results from JTK and DESeq2 were distinct, with no overlap of significant results, both ASVs considered significant for *T. tagusensis* (ASV_148, significant in JTK, and ASV_377, significant in DESeq2) belong to the genus *Pseudoalteromonas* and seem to be more abundant at 4 am (Fig. 4).

Discussion

Here we investigated the microbiome composition and diel changes in their abundance for sympatric zooxanthellate and azooxanthellate coral species from the Southwestern Atlantic. Results show that each species hosts a significantly different community, with the highest diversity observed for *Ma. decactis*. Only a few ASVs showed a pattern of rhythmicity or differential abundance between day and night, suggesting that although likely changing the behavior, gene expression pattern, and physiological responses (as previously observed for other species; [14–19; 30]), the light oscillation during a day/night cycle did not have a strong effect on the abundance and composition of the microbial communities associated with the studied shallow-water corals. Although previous studies have shown that rhythmicity analyses can tolerate uneven sampling and missing data [65], it is important to consider that missing data for *Ma. decactis* and *Mu. hispida* could lead to uncertainties in the rhythmicity estimates.

Microbiome community composition

The microbiome community plays a crucial role in coral health [66], and its composition and abundance can respond to changes in environmental conditions [67, 68]. Microbiome composition differed significantly among species, with a marked distinction between

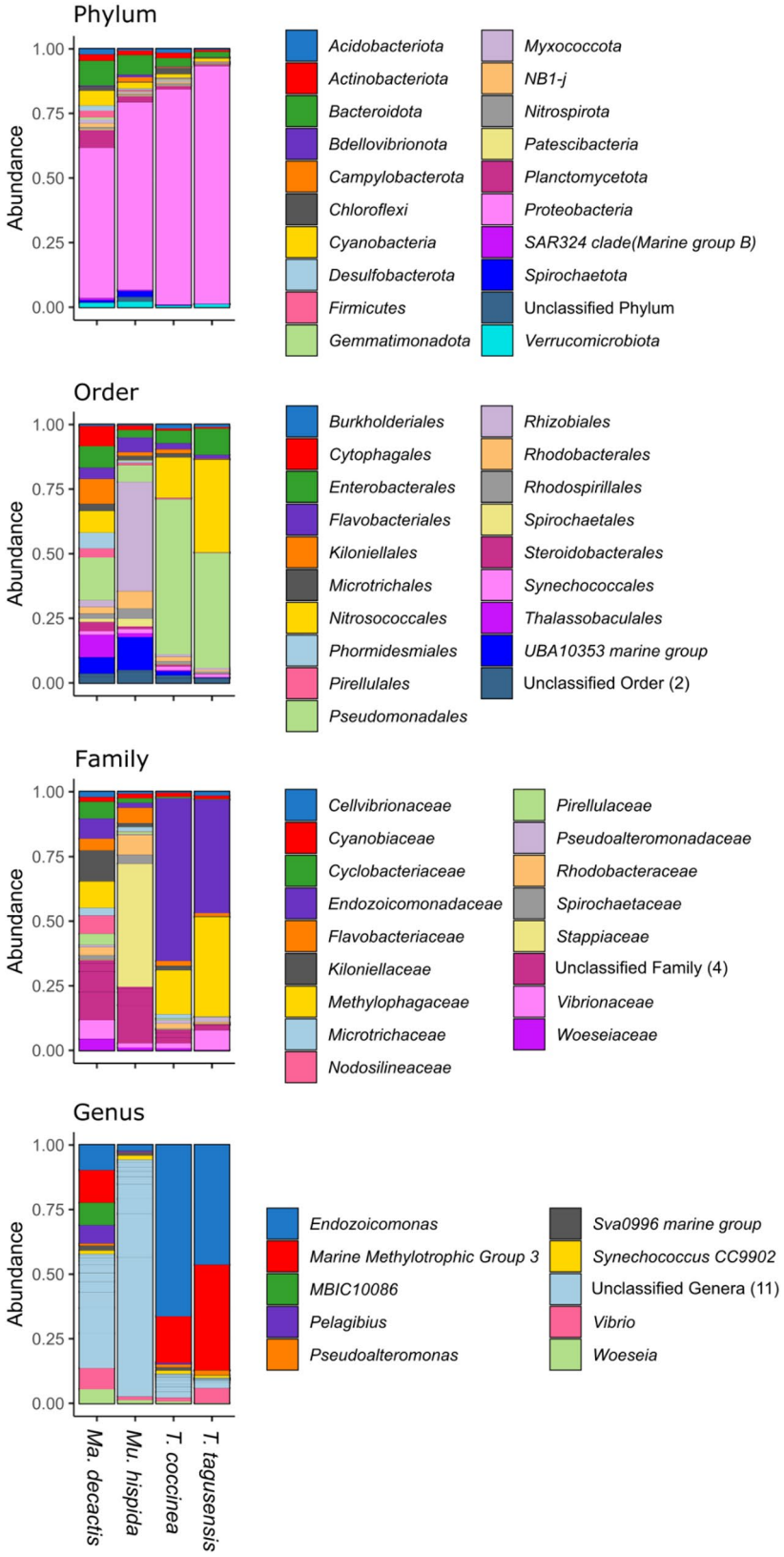

Fig. 3 Relative abundance of the 20 most abundant (**A**) Phyla, (**B**) Orders, (**C**) Families, and (**D**) Genera associated with the four species investigated, *Madracis decactis*, *Mussismilia hispida*, *Tubastraea coccinea*, and *T. tagusensis*. Unidentified taxa are grouped as Unclassified

Table 1 List of ASVs found in all day or night samples for each species. Taxa without an identification were assigned 'NA'

Species	Sampling time	ASV	Phylum	Class/Order/Family	Genus
Ma. decactis	Day	ASV4	*Cyanobacteria*	*Cyanobacteriia/Synechococcales/Cyanobiaceae*	*Synechococcus CC9902*
	Day	ASV12	*Proteobacteria*	*Gammaproteobacteria/Nitrosococcales/Methylophagaceae*	*Marine Methylotrophic Group 3*
	Night	ASV12	*Proteobacteria*	*Gammaproteobacteria/Nitrosococcales/Methylophagaceae*	*Marine Methylotrophic Group 3*
	Night	ASV22	*Proteobacteria*	*Alphaproteobacteria/Thalassobaculales/NA*	NA
Mu. hispida	Day	ASV4	*Cyanobacteria*	*Cyanobacteriia/Synechococcales/Cyanobiaceae*	*Synechococcus CC9902*
	Day	ASV6	*Proteobacteria*	*Alphaproteobacteria/Rhizobiales/Stappiaceae*	NA
	Night	ASV4	*Cyanobacteria*	*Cyanobacteriia/Synechococcales/Cyanobiaceae*	*Synechococcus CC9902*
	Night	ASV6	*Proteobacteria*	*Alphaproteobacteria/Rhizobiales/Stappiaceae*	NA
	Night	ASV8	*Proteobacteria*	*Alphaproteobacteria/Rhizobiales/Stappiaceae*	NA
	Night	ASV91	*Proteobacteria*	*Alphaproteobacteria/Rhodospirillales/Terasakiellaceae*	NA
	Night	ASV119	*Bdellovibrionota*	*Bdellovibrionia/Bacteriovoracales/Bacteriovoracaceae*	*Peredibacter*
	Night	ASV413	*Proteobacteria*	*Gammaproteobacteria/Pseudomonadales/Endozoicomonadaceae*	*Endozoicomonas*
	Night	ASV444	*Actinobacteriota*	*Acidimicrobiia/Microtrichales/Microtrichaceae*	*Sva0996 marine group*
T. tagusensis	Day	ASV1	*Proteobacteria*	*Gammaproteobacteria/Pseudomonadales/Endozoicomonadaceae*	*Endozoicomonas*
	Day	ASV2	*Proteobacteria*	*Gammaproteobacteria/Nitrosococcales/Methylophagaceae*	*Marine Methylotrophic Group 3*
	Day	ASV4	*Cyanobacteria*	*Cyanobacteriia/Synechococcales/Cyanobiaceae*	*Synechococcus CC9902*
	Day	ASV7	*Proteobacteria*	*Gammaproteobacteria/Pseudomonadales/Endozoicomonadaceae*	*Endozoicomonas*
	Day	ASV11	*Actinobacteriota*	*Acidimicrobiia/Actinomarinales/Actinomarinaceae*	*Candidatus Actinomarina*
	Day	ASV60	*Proteobacteria*	*Alphaproteobacteria/Rhodospirillales/Terasakiellaceae*	NA
	Day	ASV98	*Proteobacteria*	*Gammaproteobacteria/Burkholderiales/Burkholderiaceae*	*Ralstonia*
	Day	ASV254	*Proteobacteria*	*Gammaproteobacteria/Nitrosococcales/Methylophagaceae*	*Marine Methylotrophic Group 3*
	Night	ASV1	*Proteobacteria*	*Gammaproteobacteria/Pseudomonadales/Endozoicomonadaceae*	*Endozoicomonas*
	Night	ASV2	*Proteobacteria*	*Gammaproteobacteria/Nitrosococcales/Methylophagaceae*	*Marine Methylotrophic Group 3*
	Night	ASV4	*Cyanobacteria*	*Cyanobacteriia/Synechococcales/Cyanobiaceae*	*Synechococcus CC9902*
	Night	ASV101	*Proteobacteria*	*Gammaproteobacteria/Xanthomonadales/Xanthomonadaceae*	*Stenotrophomonas*
T. coccinea	Day	ASV1	*Proteobacteria*	*Gammaproteobacteria/Pseudomonadales/Endozoicomonadaceae*	*Endozoicomonas*
	Day	ASV2	*Proteobacteria*	*Gammaproteobacteria/Nitrosococcales/Methylophagaceae*	*Marine Methylotrophic Group 3*
	Day	ASV4	*Cyanobacteria*	*Cyanobacteriia/Synechococcales/Cyanobiaceae*	*Synechococcus CC9902*
	Day	ASV7	*Proteobacteria*	*Gammaproteobacteria/Pseudomonadales/Endozoicomonadaceae*	*Endozoicomonas*
	Day	ASV112	*Proteobacteria*	*Gammaproteobacteria/Burkholderiales/Burkholderiaceae*	*Cupriavidus*
	Day	ASV176	*Proteobacteria*	*Gammaproteobacteria/Pseudomonadales/Endozoicomonadaceae*	*Endozoicomonas*
	Day	ASV219	*Campylobacterota*	*Campylobacteria/Campylobacterales/Helicobacteraceae*	NA
	Day	ASV228	*Actinobacteriota*	*Actinobacteria/Micrococcales/Microbacteriaceae*	*Microbacterium*
	Night	ASV1	*Proteobacteria*	*Gammaproteobacteria/Pseudomonadales/Endozoicomonadaceae*	*Endozoicomonas*
	Night	ASV2	*Proteobacteria*	*Gammaproteobacteria/Nitrosococcales/Methylophagaceae*	*Marine Methylotrophic Group 3*
	Night	ASV4	*Cyanobacteria*	*Cyanobacteriia/Synechococcales/Cyanobiaceae*	*Synechococcus CC9902*
	Night	ASV7	*Proteobacteria*	*Gammaproteobacteria/Pseudomonadales/Endozoicomonadaceae*	*Endozoicomonas*

zooxanthellate and azooxanthellate species. *Ma. decactis* had a higher biodiversity and a microbiome community with low variability among samples. For the genus *Tubastraea*, while the two species were significantly different in beta diversity, they had a more similar composition, as observed in the DCA and in the fact that the two most abundant groups were the same even at the genus level. Host species have been indicated as an important factor of microbiome composition differences in Anthozoa [69–71]. This difference can be related to many different aspects, including the different morphologies [69] and evolutionary histories (observed for a subset of

Table 2 JTK and DESeq2 results table including only ASVs with $p < 0.01$ (for JTK) and significantly different ASVs between day and night (for DESeq2). Taxa without an identification were assigned 'NA'

Species	ASV	padj	Phylum	Class/Order/Family	Genus
JTK-12 h					
Mde	ASV_477	0.00857	*Proteobacteria*	*Gammaproteobacteria/ Steroidobacterales/Woeseiaceae*	*Woeseia*
JTK-24 h					
Ttag	ASV_148	0.006383	*Proteobacteria*	*Gammaproteobacteria/ Enterobacterales/Pseudoalteromonadaceae*	*Pseudoalteromonas*
DESeq2 day x night					
Mde	ASV_236	3.69E-11	*Bacteroidota*	*Bacteroidia/ Flavobacteriales/Flavobacteriaceae*	*Aquimarina*
Mde	ASV_247	2.32E-12	*Proteobacteria*	*Gammaproteobacteria/ Pseudomonadales/SAR86 clade*	NA
Mde	ASV_304	1.07E-12	*Proteobacteria*	*Alphaproteobacteria/ Rhodospirillales/Magnetospiraceae*	NA
Mde	ASV_2368	1.39E-12	*Bdellovibrionota*	*Bdellovibrionia/ Bacteriovoracales/Bacteriovoracaceae*	NA
Mhi	ASV_137	2.04E-08	*Proteobacteria*	*Gammaproteobacteria/UBA10353 marine group/ NA*	NA
Mhi	ASV_356	2.56E-12	*Proteobacteria*	*Alphaproteobacteria/ Rhodobacterales/Rhodobacteraceae*	NA
Tco	ASV_104	2.81E-13	*Cyanobacteria*	*Cyanobacteriia/ Synechococcales/Cyanobiaceae*	*Synechococcus CC9902*
Tco	ASV_388	2.69E-10	*Proteobacteria*	*Gammaproteobacteria/ Burkholderiales/ EC94*	*NA*
Tco	ASV_836	7.66E-11	*Proteobacteria*	*Alphaproteobacteria/ Kordiimonadales/Kordiimonadaceae*	*Kordiimonas*
Tco	ASV_1060	8.03E-12	*Proteobacteria*	*Gammaproteobacteria/ Chromatiales/Sedimenticolaceae*	*Sedimenticola*
Tco	ASV_1072	6.86E-11	*Proteobacteria*	*Gammaproteobacteria/ Enterobacterales/Vibrionaceae*	*Vibrio*
Tco	ASV_1931	1.22E-09	*Proteobacteria*	*Gammaproteobacteria/ HOC36/ NA*	*NA*
Ttag	ASV_377	7.59E-11	*Proteobacteria*	*Gammaproteobacteria/ Enterobacterales/Pseudoalteromonadaceae*	*Pseudoalteromonas*

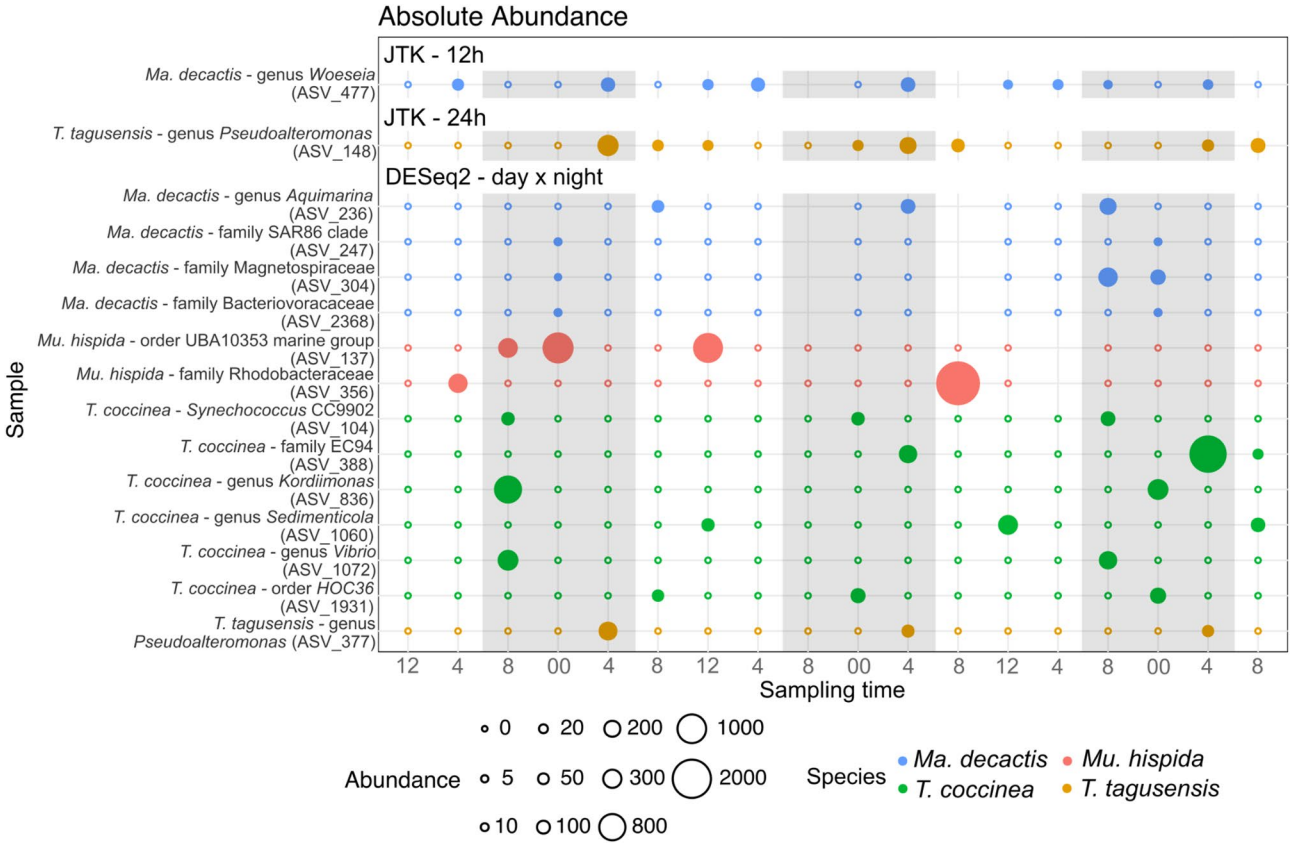

Fig. 4 ASVs displaying significant diel (24 h) or tidal (12 h) variation (measured by JTK and based on relative abundance), and with significant differences between day and night (measured by DESeq2 based on absolute abundance) for *Madracis decactis* (blue), *Mussismilia hispida* (red), *Tubastraea coccinea* (green) and *T. tagusensis* (orange). Gray shaded areas approximately represent night time

the microbiome in Pollock et al. [71]). Within the studied species, *Ma. decactis* is encrusting and knobby, *Mu. hispida* is massive and *Tubastraea* spp. can be massive and branching. Studies on the evolutionary history of microbiomes and their hosts have shown that microbial community composition can be correlated with the host phylogeny (known as phylosymbiosis) [71, 72]. There are exceptions to this phylosymbiotic pattern, such as what was observed in a microbial diel rhythm study, in which *Porites cylindrica* had a more similar microbiome, in terms of taxa abundance, to *Pocillopora damicornis* than to *Porites lutea* [23]. Indeed, environmental factors might be more important than taxonomy, as observed for three species of *Acropora* sampled at two distinct sites at the Great Barrier Reef [73] (see also Hernandez-Agreda et al. [66]). Although the general composition of the microbiome has been shown to be similar to corals' phylogeny, the long-term evolution of only a few microbial lineages coincides with that of corals, indicating that several other factors are also relevant in determining corals' microbiome composition [71]. It remains to be determined how much the coral host favors certain microbiome members.

Our results also recovered the core microbiome of the four studied species from the Alcatrazes Archipelago. When comparing the core microbiome of *T. tagusensis* — the only one with a previously described core (specimens from Búzios Island, nearly 60 km north of Alcatrazes [74]) — we found that only the cyanobacteria genus *Synechococcus* CC9902 was common to both locations. This pattern is similar to the findings of Galand et al. [75], who did not identify a specific core microbiome from three Pacific coral species from different regions. Nevertheless, it is important to note that, for each studied species, we were working with one single genotype. Host genotype might be another key "force" modulating the microbiome that deserves to be further studied in corals as it has been done in other groups, such as humans [76], wheat [77], phytoplankton [78], to cite a few. These results highlight the significant influence of environmental factors on the microbiome composition, indicating that they reflect the historical context, environmental conditions (including day/night), and host genotype.

At the genus level, the microbiome from *Mu. hispida* was composed mainly of Unclassified genera. This result can be related to the fact that *Mu. hispida* is an endemic genus in an understudied geographical region. A similar issue was previously reported for functional analyses in *Mu. hispida*'s endemic congener *Mu. braziliensis* [79]. Potential coevolving microbial strains have already been identified in corals. For instance, the comparison of *Endozoicomonas* strains in different coral species revealed that no OTU was found in more than one species [80]. In Australian corals, four microbial strains (i.e., *Clostridiaceae*, *Endozoicomonas*-like bacteria, unclassified *Kiloniellales*, and unclassified *Myxococcales*) have long standing relationships with their coral host lineages [71]. Future studies with *Mu. hispida* and its microbiome may include investigations on the coevolution, as well as, attempts to isolate and identify unknown bacterial strains in this species.

Proteobacteria was the dominant phylum in all four studied species, similar to what has been previously observed for Southwestern Atlantic corals (i.e., *Siderastrea stellata* and *Mu. hispida* [81],; *Mu. hispida*, *Ma. decactis*, *T. coccinea* and *Palythoa caribaeorum* [82],; *T. tagusensis* [74]),. Considering the most abundant orders in all species, eight of them were among the ten most common orders associated with Pacific corals (i.e., *Cytophagales*, *Flavobacteriales*, *Kiloniellales*, *Phormidesmiales*, *Pirellulales*, *Rhizobiales*, *Rhodobacterales*, *Spirochaetales*; [75]). At the family level, *Stappiaceae*, the most abundant taxa in *Mu. hispida*, was also previously found to be abundant in both diseased and healthy parts of colonies of *Mu. hispida* from the same area [8]. Genera *Marine Methylotrophic Group 3* and *Endozoicomonas* were remarkably more abundant in *Tubastraea* but were also found within the top 10 bacterial genera of the two zooxanthellate species. *Marine Methylotrophic Group 3* has been commonly related to methane-releasing cold seeps (e.g. Ruff et al. [83]) and has been found in calcareous coralline algae, in which they were positively correlated to coral larval settlement [84]. The genus is also a recurrent component of *T. tagusensis* microbiome [74]. *Endozoicomonas*, the only genus found on all samples of all species, was pointed as one of the most abundant genera in the coral microbiome [85]. The genus has been remarkably associated with healthy corals [69, 86], and seems to contribute with B vitamins to both coral host and Symbiodiniaceae [12]. Even though it may also take part in non-beneficial associations, these occur with non-cnidarian hosts, such as clams and fish (discussed in Pogoreutz and Ziegler [87]). *Pseudoalteromonas* was also one of the most abundant groups and, as previously observed, some of its strains have antibacterial activity [9]. *Vibrio* is interestingly among the ten most abundant genera in all four species (although it is not present in all samples). This genus has been related to diseases in corals (e.g., Garcia et al. [79]), but has also been found in healthy organisms, including *Ma. decactis* from St. Peter and St. Paul Archipelago [88] and *Mu. hispida* and *Mu. braziliensis* from the Abrolhos Bank [89]. In general, taxa found in our samples are compatible with other coral studies. There were no significant correlations between the abiotic variables and the microbial community, suggesting that the measured abiotic variables did not affect the microbial community's abundance in a short time scale. However, it is possible that these abiotic variables

have some influence in the activity of some strains, which could be assessed by analyzing bacterial RNA.

Variation in diel and tidal rhythms

Only two ASVs showed a pattern of rhythmicity, with an abundance peak every 12 h (ASV_477, *Woeseia* in *Ma. decactis*) and 24 h (ASV_148, *Pseudoalteromonas* in *T. tagusensis)*. ASV_477 was more abundant during the transition from high to low tide. Tidal rhythms are complex, but they have been found to control physiological and behavioral rhythms in corals [90]. Circatidal cycles of 12 h have been observed for the gene expression in aposymbiotic *Exaiptasia diaphana*, contrasting with the most common circadian pattern observed on symbiotic morphs [32]. Another example of a tidal pattern is that of *Montastraea cavernosa* containing cyanobacteria, in which nitrification rates were higher at 6 to 8 am and 6 to 8 pm and it was hypothesized to occur because those time periods would have intermediate levels of oxygen, promoting respiration in the cyanobacteria without inactivating the enzyme nitrogenase [24]. The genus *Woeseia* (ASV_477) has been previously identified in *Porites lutea* [91] and it has genes related to different stages of the nitrogen cycle, as shown for lineages found in mangrove sediment [92]. It is possible that 4 am and 4 pm peaks in abundance are also related to intermediate conditions in the coral, or because the bacteria could be anticipating optimal conditions for performing nitrogen cycle reactions, similar to the mechanism observed for cyanobacteria in *Mo. cavernosa* [24]. For *Pseudoalteromonas*, two strains displayed a diel rhythm with their abundance peak at approximately 4 am (one based on JTK and the other based on DESeq2) in *T. tagusensis*.

Among the 13 ASVs with a significant change in abundance between day and night, six belong to *Gammaproteobacteria* and three to *Alphaproteobacteria*, which is somewhat similar to what has been observed for *Nematostella vectensis* [22] (although the only similar taxon at lower levels was the family *Rhodobacteraceae*). At the genus level, our results showed a significant difference in the abundance of *Vibrio* between day and night in *T. coccinea*. Similarly, *Vibrio* was one of the genera displaying higher abundance during the day than at night in *Porites lutea* [23]. The genus also had significant differences in abundance in *N. vectensis*, kept under constant darkness, when compared to day/night natural cycle [22]. Interestingly, three significantly cycling ASVs are phylogenetically close to a few bacteria that are known to have endogenous circadian clocks. ASV_104 (*Synechococcus* CC9902) belongs to the same genus as the well-studied cyanobacteria *Synechococcus* sp. RF-1 and *Synechococcus elongatus* [93–95], and *Pseudoalteromonas* (ASV_148 and ASV_377) belongs to order *Enterobacterales* together with the human gut bacteria *Enterobacter aerogenes* [5]. In addition, ASV_304 (*Magnetospiraceae*) is a member of the order *Rhodospirillales*, as well as *Rhodopseudomonas palustris*, which presents a timekeeping mechanism that is not self-sustained in constant conditions and, thus, does not correspond to a true circadian clock [96]. These could be candidate microbial lineages for investigating the presence of circadian rhythms while circatidal rhythms could be searched for in ASV_477 (*Woeseia*).

Apart from a few ASVs, there was no remarkable variation in the microbiome composition between day and night for the four investigated coral species. This pattern is similar to that observed by previous studies [21–23], indicating that day-night oscillation is not a significant factor guiding coral microbial communities. As hypothesized by Zanotti et al. [97] based on Sharp and Foster [98], the coral host may somehow control microbial community abundances, which could explain the little variation observed at a daily time scale and the lack of significant results with other abiotic parameters. Furthermore, abundance may not necessarily be related to relevance, once rare taxa may be highly active, contribute to metabolic pathways, and induce other taxa to produce specific compounds (discussed in Jousset et al. [99]). The activity of strains (assessed with cDNA) and their abundance (assessed with microbial DNA) may vary in different ways, as observed when both microbial DNA and cDNA were assessed [23]. Thus, although only a few ASVs oscillate in a diel or tidal pattern, most of the microbiome may function differently within the diel rhythm. Future studies might focus on the bacterial metabolic pathways within diel and/or tidal rhythmicity.

Conclusions

Our results show divergent microbial communities when comparing zooxanthellate and azooxanthellate species, following a similar pattern to the phylogeny of the host species. Interestingly, a large proportion of the microbial community at the genus level found for the zooxanthellate endemic species *Mussismilia hispida* are unknown lineages, highlighting the paucity of data on the microbiome associated with Southwestern Atlantic corals. Abiotic variables do not seem to affect microbiome abundance in a short time scale, but there were a few significant changes on ASV abundance within 24-hour and 12-hour periods. It is important to note that the inclusion of biological replicates could improve the accuracy of the results. Among the few ASVs which varied in abundance, some are phylogenetically related to bacteria with endogenous clock mechanisms previously described and could be candidates for investigating the presence of circadian or circatidal rhythms. Future studies should focus on investigating metabolic pathways to better understand how the microbiome community can adjust to environmental changes within the coral host in short time scales.

Supplementary Information

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Supplementary Material 1: Additional file 01: Supplementary figures S1-S6, Tables S1-S2. Additional file 02: R script used to perform statistical analyses (R). Additional file 03: ASV table used as input for the R script (xlsx). Additional file 04: Taxonomy table used as input for the R script (xlsx). Additional file 05: Metadata table used as input for the R script (xlsx). Additional file 06: Rarefied data table used for alpha diversity analyses (csv). Additional file 07: Rarefied data table used for beta diversity and diel variation analyses (csv). Additional files 08–11: Alpha diversity values separated by species, used as input to assess whether alpha diversity varies in 24-hour rhythms (csv). Additional files 12–15: Rarefied ASV tables separated by species, used as input for diel and tidal variation analyses (csv). Additional files 16–19: rarefied ASV tables separated by species with relative abundance values, used as input for diel and tidal variation analyses (csv). Additional files 20–35: abiotic data used in the Redundancy Analysis, separated by species and amount of time before collection time. "RBR" refers to data obtained with the RBR CTD and "RINKO" corresponds to data obtained with AAQ-RINKO CTD (csv). Additional file 36: All ASVs with significantly different abundance between day and night according to DESeq2 (xlsx).

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Author contributions

K.C.C.C., and M.V.K. conceptualized the study; I.G.L.S., K.C.C.C., R.R.O., C.Z., C.A.M.M.C., C.L.B.F., and M.V.K. contributed to the experiment design and sample collection; I.G.L.S., K.C.C.C., R.R.O., A.A.Z., and A.M.C. performed the data analysis; I.G.L.S. and K.C.C.C. wrote the first draft; all authors reviewed the manuscript and approved the final version.

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Data availability

The datasets supporting the conclusions of this article are included within the article (and its additional files). Raw sequencing data are available on the Sequence Read Archive database under accession number PRJNA1227542.

Declarations

Ethics approval and consent to participate

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Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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