

The influence of photoacclimation on the microscale oxygen environment and microbial niches in the coral *Galaxea fascicularis*

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Research Article

Keywords: photobiology, gastrovascular cavity, coral microbiome, *Endozoicomonas*

Posted Date: August 27th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10354255/v1>

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Additional Declarations: No competing interests reported.

Abstract

Light availability is a key environmental driver for symbiotic corals, as it regulates photosynthesis by endosymbiotic algae and hence strongly influences holobiont physiology. Light levels control symbiont photosynthetic oxygen production, which in turn shapes the internal and external physicochemical microenvironment of corals, potentially impacting the microbial communities associated with different anatomical compartments within coral polyps. Here, we experimentally investigated the effects of sustained exposure to contrasting light regimes on the oxygen microenvironment in the surface mucus layer (SML) and the gastrovascular cavity (GVC) of the coral *Galaxea fascicularis*. We found that sustained light-driven changes in photosynthetic activity can significantly alter internal oxygen conditions, but that responses varied across individual polyps, suggesting fine-scale physiological heterogeneity. Both SML and GVC samples were dominated by the genus *Endozoicomonas*, a key coral-associated taxon commonly found in intratissue aggregates. *Endozoicomonas* relative abundance declined in the GVC fluid under high light, but microbial community composition remained otherwise remarkably stable across light treatments. Our results underscore the need to further explore compartment-specific coral responses to environmental change.

Introduction

Tropical coral reefs are marine biodiversity hotspots supported by the symbiosis between corals and a multitude of microorganisms, which form the coral holobiont. Many reef-building corals live in obligate mutualism with photosynthetic dinoflagellates (family Symbiodiniaceae), which can contribute the majority of the coral's organic carbon demand via translocated photosynthates. The remainder of the coral's nutrition is obtained heterotrophically through food ingestion to gain essential nutrients. This photosymbiosis enables the growth of large biogenic reefs in nutrient poor tropical waters.

Beyond this algal symbiosis, corals harbor complex microbial communities (bacteria, archaea, fungi, viruses) in different anatomical compartments (Sweet et al. 2011; Bourne et al. 2016; Pollock et al. 2018; Liu et al. 2024). Microhabitats within the coral (e.g. ectodermal and endodermal tissue, the surface mucus layer (SML), skeleton, and gastrovascular cavity (GVC)) create distinct physicochemical niches, each hosting unique microbial assemblages (Sweet et al. 2011; Apprill et al. 2016; Bourne et al. 2016; Hughes et al. 2022). These compartment-specific microbiomes can vary in composition and responsiveness to environmental change (Lee et al. 2015; Pollock et al. 2018; Vellani et al. 2026), indicating that they may perform different functional roles. Understanding microenvironmental heterogeneity, compartment-level physiology and compartment-level holobiont composition is therefore an essential task towards understanding holobiont function, and ultimately to predict how corals respond to environmental stressors (Hughes et al. 2022). Much of the existing knowledge of coral physiological and microbial processes, however, comes from bulk (i.e. colony-scale) studies. Therefore, our knowledge of compartment-specific responses of corals to environmental change remains extremely limited.

One key microhabitat in this context is the coral gastrovascular cavity (GVC). Although corals lack a true differentiated gut, the GVC serves as a central compartment for processes such as prey digestion, nutrient cycling, reproduction, and symbiont recruitment (Agostini et al. 2012; Hughes et al. 2022; Bollati et al. 2024; Saper et al. 2025). The GVC's semi-enclosed structure leads to pronounced gradients of physicochemical variables (Agostini et al. 2012; Bollati et al. 2024; Zhang et al. 2026a,b). For example, anoxic zones have been detected at the bottom of the GVC of several coral species, even when strong illumination stimulates high photosynthetic oxygen production in the diffusive boundary layer and in the upper GVC (Agostini et al. 2012; Bollati et al. 2024). Such microenvironmental conditions, along with accumulated organic metabolites from feeding, suggest that the GVC can be a microaerobic, nutrient-rich niche ideal for specialized microbes (Agostini et al. 2012). Indeed, the coral GVC has been described as a rudimentary gut harboring a distinct "gut microbiome", which may play important functions for holobiont health (Bollati et al. 2024). Recent advances in microscale sampling methods, low-input DNA extraction and sequencing have enabled recovery of microbial DNA from single-polyp GVC samples (Bramucci et al. 2021; Hughes et al. 2022; Bollati et al. 2024). Coupling such microbiome sampling with microsensors measuring local physicochemical parameters now allows direct linkage of internal microenvironment conditions (e.g. O₂ levels) with the resident microbial community composition (Bollati et al. 2024; Dellisanti et al. 2026), an essential step towards a compartment-level understanding of holobiont functioning.

However, the field is still in its infancy (Hughes et al. 2022) and it is largely unknown how acclimation to external environmental factors translates to changes in the GVC microenvironment and microbiome. Importantly, studies have so far mainly characterized the effects of changes in external environmental conditions on the GVC microenvironment over temporal scales of minutes to hours (Agostini et al. 2012; Bove et al. 2020; Bollati et al. 2024; Zhang et al. 2026). These timescales are appropriate to capture the microenvironmental changes caused by e.g. the shifting balance between photosynthesis and respiration, or by active ventilation of the GVC through polyp contraction and expansion (Bove et al. 2020; Dellisanti et al. 2024; Zhang et al. 2026a). However, acclimatory physiological processes and microbial community composition shifts often occur over longer time scales (days to weeks). A recent study, for example, demonstrated that a three-week period of iron enrichment chronically reduced GVC oxygen availability and altered the GVC microbiome, highlighting the sensitivity of this compartment to sustained environmental changes (Dellisanti et al. 2026) .

Light availability is a particularly influential factor for coral acclimation to the reef environment, as it drives the photosynthetic activity of endosymbiotic Symbiodiniaceae thus altering physiological parameters and energy fluxes within the coral holobiont (Yu et al. 2023). Light availability on coral reefs is affected by depth gradients, seasonal changes in solar irradiance, water turbidity, and shading by neighboring organisms (Hoegh-Guldberg and Jones 1999; Warner et al. 2002). Corals acclimate to these changes over days to weeks by adjusting various physiological traits. For example, corals can regulate the density of endosymbiotic Symbiodinaceae, the concentration of photosynthetic pigments, as well as host photoprotective pigments produced by the host. These processes can optimize light harvesting

under low irradiance and reduce photodamage under high irradiances (Falkowski and Dubinsky 1981; Iglesias-Prieto and Trench 1997; Warner et al. 2002; Ferreira et al. 2023).

Oxygen measurements performed inside the coral GVC showed that short-term light-dark shifts directly affect oxygenation in the upper GVC, as measured for several tropical species (Agostini et al. 2012; Bollati et al. 2024; Zhang et al. 2026a), or even over the entire GVC, as measured for two Mediterranean corals (Dellisanti et al. 2024, 2026). These changes can be dramatic, ranging from anoxia in darkness to over 500% air saturation in bright light (Bollati et al. 2024). Alongside oxygen, short-term changes in external light levels cause pH shifts up to 1 unit, and affect the production of chemical species such as NO, N₂O, and H₂ (Zhang et al. 2026b).

Yet, we lack an understanding of how long-term differences in light (e.g. sustained high vs. low light regimes, as they occur across depth gradients or during photoacclimation) might reshape the GVC microenvironmental landscape or the composition of microbial communities.

In this study, we used the reef coral *Galaxea fascicularis* as a model to examine how sustained light regimes modulate internal microhabitats. We chose *G. fascicularis* due to its relatively large polyps, which have been successfully used in prior GVC studies (Agostini et al. 2012; Bollati et al. 2024), and to contribute to the development of this taxon as an emerging coral model organism (Puntin et al. 2023).

We used O₂ microsensors to measure fine-scale oxygen concentration profiles both inside the GVC and on the external surface of corals maintained under different light intensities for 20 weeks, and combined such measurements with a characterization of the microbial communities residing in the GVC fluid and the surface mucus layer (SML) via 16S rRNA gene metabarcoding. We hypothesized that corals acclimated to higher irradiance would exhibit chronically elevated internal oxygenation and harbor distinct microbial assemblages enriched in aerobic taxa in the GVC and SML as compared to low-light acclimated corals.

Methods and Materials

Aquarium set-up

One colony of *G. fascicularis* was originally sourced from the European ornamental trade (De Jong Marinelife, the Netherlands) and maintained in the Marine Biology Section tropical aquarium facilities at the University of Copenhagen (recirculating artificial seawater) for at least 6 months prior to the start of the experiment. Individual polyps (n = 12) were then fragmented from the colony, placed on ceramic tiles, and transferred to a 60 L aquarium. Temperature was kept constant at 25°C through a 25 W aquarium heater placed directly in the tank. The polyps were fed with live *Artemia* sp. nauplii once and with Reefpearls (D. van Houten Import-Export B.V., Groningen, the Netherlands) twice per week. Once per week, ten liters of seawater were exchanged with freshly prepared artificial seawater (salinity 35‰, prepared with coral salt (Hoss, Roskilde, Denmark). Salinity was kept constant by topping up with distilled water to counteract evaporation using an automatic top-up system (D&D Aquarium Solutions,

Ilford, UK). Lighting was provided by two LED tiles (Fluval Nano Reef, Montreal, Canada) set to a 12:12 hours dark:light cycle with a photon irradiance of photosynthetically active radiation (PAR; 400–700 nm) of $\sim 60 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (low light treatment; LLT) and $\sim 200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (high light treatment; HLT), respectively. HLT and LLT corals were kept in the same aquarium and placed underneath the respective lamp for the assigned light treatment. The photon scalar irradiance in the aquarium was measured just above the coral polyps with a Universal Light Meter (ULM-500) connected to a spherical micro quantum sensor (US-SQS/L, Heinz Walz, Effeltrich, Germany).

Variable chlorophyll fluorometry

Photosynthetic performance was measured via variable chlorophyll fluorescence using an imaging PAM fluorometer (IMAG-MINI/R, Walz, Effeltrich, Germany) (Ralph et al. 2005). The maximum quantum yield of PSII (F_v/F_m) was assessed after dark adaptation of coral fragments for 15 min prior to measurement. Then, corals were exposed to a sequence of increasing photon irradiance levels (17 steps from 3 to 2268 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and the effective quantum yield was measured at each irradiance level, with 2-min intervals between measurement steps, to generate rapid light curves. Rapid light curves can be used to assess photosynthetic activity following photoacclimation in plants and corals, as their key parameters (e.g. initial slope and asymptote) are strongly influenced by the long-term light exposure history of the organism (Ralph and Gademann 2005). The photon irradiance levels were calibrated using the ULM light meter mounted with a photon irradiance sensor (LiCor; LI-250).

Oxygen Microsensor Measurements

After 20 weeks of exposure to the different light levels, three polyps per group ($n = 6$) were randomly chosen to perform oxygen microsensor measurements in the SML (on the external surface of the oral disk) and inside the GVC in darkness, under low ($60 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and high photon irradiance ($200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$). Measurements were performed with a Clark-type O₂ microsensor (OX50, 50 μm tip diameter; Unisense, Aarhus, Denmark) in oxygenated artificial seawater (25°C, 35‰ salinity) in a custom-made flow chamber (0.5 cm s^{-1} , laminar water flow), as previously described (Bollati et al. 2024; Dellisanti et al. 2024). Light was provided by an LED tile identical to those used for the experimental aquarium, set to a photon irradiance of $60 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (low light) or $200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (high light), measured as described for the aquarium set up.

The oxygen microsensors were calibrated linearly from sensor readings in artificial seawater (25°C, 35‰ salinity) at 100% air-saturation and 0% saturation, after adding a Na_2SO_3 solution to deplete oxygen. The microsensors were mounted on a motorized micromanipulator (Unisense) and connected to a multichannel microsensor meter (Unisense). Data were recorded with the software SensorSuite Profiler v3.2 (Unisense). A stereo microscope allowed for precise tracking of the microsensor's position.

Prior to measurement, polyps were incubated for 20 min at the target light level to allow for the establishment of a steady state. Then, SML point measurements were taken in 3–6 different locations on the oral disk for each polyp. For the GVC, we measured vertical profiles in 2–5 technical replicates per polyp. The deepest point of the GVC was determined as the first position of the microsensor that caused a small polyp contraction, presumably due to the microsensor tip touching the bottom of the GVC (Zhang et al. 2026a). In addition to vertical profiles, we recorded temporal oxygen fluctuations in the GVC by exposing the polyps to darkness, low light, and high light, for 90 min each, while recording data every second with the microsensor tip positioned near the GVC bottom (50 μm above the point where the microsensor caused a small contraction to avoid piercing the tissue during the measurement).

Microsampling

Alongside oxygen and photosynthesis measurements, at the end of the 20-week experiment microvolume fluid samples were taken from the GVC and the SML of all polyps ($n = 12$, including the 6 that were used for the oxygen measurements) and used for DNA extraction and 16S rRNA gene metabarcoding of the microbial communities. Prior to sampling, to ensure the sterility of the equipment, all centrifuge tubes and nylon swabs were UV-sterilized in a UV-crosslinker for 1 h. Polyps were then placed in an autoclaved glass dish (15 cm diameter, 6 cm height) with filter-sterilized (mesh size 0.2 μm) artificial seawater. After each sampling, the artificial seawater was exchanged, and the glass dish was sterilized again with 70% ethanol, to reduce cross-sample contamination. The polyp mouth was placed as closely as possible to the water surface to minimize the distance travelled by the sampling instruments.

Samples were then taken from the GVC with a sterile low-dead volume needle (The Invisible Needle, TSK Laboratory, Japan, 34G, 9 mm long) connected to a 1 mL sterile syringe, and with a precision nylon swab (TX 730, Texwipe, Kernersville, USA), as previously described (see Bollati et al., 2024). Needles and swabs were inserted into the gastric cavity through the coral mouth, using a manual micromanipulator, allowing for high precision movements. The swab was moved three times back and forth inside the cavity to collect material from the inner surface of the gastric cavity as well as the gastric fluid. Then, the micromanipulator was retracted and the sample transferred into a sterile 1.5 mL centrifuge tube. For the swab, this was done by cutting the tip with a pair of sterilized scissors directly into the tube.

For mucus sampling, the polyp was taken out of the water for one minute to excite mucus production. Subsequently, $\sim 10 \mu\text{L}$ of the mucus were collected using a P10 pipette with a sterile tip.

Additionally, water samples (100 μL) were taken from the glass beaker after GVC sampling of three randomly chosen polyps using a P1000 pipette ($n = 3$). Sampling negative controls for the needle were taken by aspirating 10 μL sterile (autoclaved and UV-crosslinked for 1h) milliQ water through the needle and for the swabs by collecting an unused swab tip into a sterile centrifuge tube ($n = 2$ needle and 2 swab samples). Finally, 100 μL samples of aquarium seawater from the experimental tank were sampled with a P1000 pipette with a sterile tip ($n = 3$). The samples were stored at -80°C until further processing.

DNA extraction, amplification, and sequencing

DNA was extracted from the microsamples following a low-input extraction protocol (Bramucci et al. 2021) under a UV-clean hood, using 10 μL (mucus and GVC needle samples) or 100 μL (seawater and GVC swab samples) of input volume. GVC needle, mucus, and seawater samples were taken directly as input. GVC swab samples were first sonicated on ice for 5 minutes in 30 s on/off bursts to release bacterial cells, then 100 μL PCR-grade water were added into the tube before performing the extraction, taking care that the swab tip was always submerged during the procedure. The swabs were then removed with a P1000 pipette immediately before bead-cleanup. Each extraction batch included an extraction negative control.

The V3-V4 hypervariable region of the 16S rRNA gene was amplified with the primer pair 341F (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTAYGGGRBGCASCAG) and 805R (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACNNGGTATCTAAT) including Illumina adapters in a reaction volume of 25 μL (5 μL DNA template, 0.5 μL of each 10 μM primer, 1 μL bovine serum albumin (2 mg mL^{-1}), 12.5 μL of KAPA HiFi HotStart Ready Mix (Roche, Basel, Switzerland), and PCR grade water to obtain the total reaction volume of 25 μL). The PCR program was: 98°C for 2 min (initial denaturation and activation of polymerase), 35 cycles of 98°C for 30 s (denaturation), 55°C for 30 s (primer annealing), 72°C for 30 s (elongation), followed by 72°C for 10 min (final elongation). A PCR negative control was included for each batch. Amplicons were visualized on an agarose gel after electrophoresis to verify successful amplification and assess contamination. The amplicons ($n = 63$) were submitted to the Australian Genome Resource Facility (Melbourne, Australia) for barcoding and sequencing on an Illumina MiSeq platform (300 bp paired-end), in a run including samples from a separate project (total $n = 146$). Reads were demultiplexed by the sequencing facility.

Bioinformatics

The sequencing data processing and analysis was performed in R v4.3.2. The primers and Illumina adaptors were trimmed using cutadapt v3.5 (Martin 2011). The trimmed reads were further processed using the dada2 pipeline (v.1.30.0) to create an Amplicon Sequence Variant (ASV) table (Callahan et al. 2016).

The reads were truncated at 280 bp (forward) and 260 bp (reverse). The maximum number of expected errors was set to 2. Taxonomy was assigned using the default dada2 settings based on the SILVA database v138.1 (Quast et al. 2012). All ASVs assigned as eukaryote, chloroplast, or mitochondria were removed from the dataset, as well as ASVs that were identified as bacteria but were unassigned at phylum level. As low input extraction of bacterial DNA is highly susceptible to contamination, the dataset underwent a thorough decontamination procedure. First, all ASVs detected in any negative controls (needle and swab sampling negatives, extraction negatives, and PCR negatives) were removed. Additionally, contaminants from the sampling containers were identified using the prevalence method in the decontam package v1.22.0 with the threshold set to 0.5 (Davis et al. 2018). All identified

contaminants were removed from all samples. Rarefaction curves (Fig. S1) were created using the vegan package (v.2.7.5) (Oksanen et al. 2026) and inspected for saturation. Samples with fewer than 1000 reads remaining after decontamination were removed from the dataset.

Data Analysis and statistics

Rapid light curves were analyzed by fitting the hyperbolic tangent model to the relative electron transport rate (rETR) calculated from effective quantum yield measurements (Jassby and Platt 1976) in nlme v3.1-168 (Pinheiro and Bates 2023), with light treatment as a fixed effect and polyp replicate as a random effect. As this model does not include a photoinhibition term, the dataset was cropped to photon irradiance levels below 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The effect of light treatment on the curves was assessed by comparing the full model to a reduced model without fixed effects, using a likelihood ratio test.

Normality of maximum quantum yield (F_v/F_m) data was assessed using Shapiro–Wilk tests. As normality assumptions were not met, differences between high-light (HLT) and low-light (LLT) acclimated corals were analyzed using a two-sided Wilcoxon rank-sum test.

For time series oxygen measurements, pairwise root mean square errors (RMSE) were calculated from 90-minute oxygen time series for all biological replicate pairs within each treatment and light level. RMSE was used as a descriptive measure of the similarity between oxygen time series, with higher values indicating greater differences in oxygen dynamics between polyps.

As a first approach to understanding the relationship between bacterial 16S samples, we calculated pairwise community dissimilarities among samples using the Bray-Curtis index on relative abundance data (after removing zero-abundance ASVs). The resulting Bray-Curtis dissimilarity matrix (Fig. S2) was visualized as a heatmap using pheatmap (v 1.0.13) (Kolde 2025) in R, with hierarchical clustering by complete linkage applied directly to the Bray-Curtis dissimilarity matrix. Coral samples that clustered with environmental samples (aquarium seawater and the sampling container) were flagged as potentially contaminated during sampling (see Results) and excluded from subsequent analyses.

Shannon's alpha diversity index was calculated using phyloseq v 1.56.0 (McMurdie and Holmes 2013). Alpha diversity was compared between HLT and LLT corals within each sampling method using Wilcoxon rank-sum tests, with Bonferroni correction applied to account for multiple comparisons. Beta diversity was analyzed using non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities calculated from relative abundance data in phyloseq. Ordinations were performed with a fixed random seed to ensure reproducibility. Homogeneity of multivariate dispersion among groups was tested with the betadisper function from the vegan package (v. 2.7.5) (Oksanen et al. 2026). Comparisons between HLT and LLT corals were performed for each sample type separately, using two-sided Wilcoxon rank-sum tests. Differences in community composition were tested using ANOSIM with 1000 permutations.

Pairwise ANOSIM comparisons were performed between HLT and LLT samples within each sample type, and p-values were adjusted using the Benjamini–Hochberg method.

Pairwise comparisons of *Endozoicomonas* relative abundance between HLT and LLT were performed separately for each sampling method using Wilcoxon rank-sum tests. Because mucus and gastric swab samples were overwhelmingly dominated by *Endozoicomonas* in both treatments, statistical tests for other taxa in these compartments were deemed not informative and were therefore omitted.

Results

Photoacclimation and oxygen microenvironment

To assess photoacclimation to the long-term experimental light conditions, we used variable chlorophyll fluorescence measurements and performed rapid light curves on individual polyps (Fig. 1). After 20 weeks of light acclimation, LLT polyps appeared darker than HLT polyps (Fig. 1a). In addition, LLT polyps had a significantly higher maximum quantum yield of PSII (Fv/Fm; median = 0.537) compared to HLT polyps (median = 0.449; two-sided Wilcoxon rank-sum test, $W = 46$, $p = 0.004$; Fig. 1b, Table S1). The light treatment had a significant influence on the relationship between rETR and light intensity (Likelihood ratio test, $p < 0.01$, Fig. 1c, Table S2); specifically, the maximum rETR was significantly higher in HLT corals (estimate = 78.92) than LLT corals (estimate = 54.64; $p < 0.001$, Fig. 1c, Table S2), while the initial slope of the curve was not significantly different between the two groups ($p = 0.87$, Fig. 1c, Table S2).

We then characterized the oxygen microenvironment in the GVC and the SML using microsensors (Fig. 2). We performed the measurements under darkness, low photon irradiance ($60 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), and high photon irradiance ($200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) for all corals, allowing us to estimate the chronic daytime and oxygen conditions experienced by the polyps under the two light treatments, as well as the responsiveness of each compartment to short-term changes in illumination.

In darkness, the SML oxygen concentration was zero or near zero in both groups (pairwise Wilcoxon rank-sum test, $W = 5$, $p = 1.0$. Figure 2a, Table S3). Under low photon irradiance, median oxygen concentrations were lower than air saturation, but fell in the normoxic range for both groups; the LLT polyps (median = $115.1 \mu\text{mol O}_2 \text{L}^{-1}$) appeared to have higher oxygen concentrations than HLT polyps (median = $68.7 \mu\text{mol O}_2 \text{L}^{-1}$) at the same light level, yet the difference was not significant, possibly due to the low sample size ($W = 2$, $p = 0.4$, Fig. 2a, Table S3).

Under high photon irradiance, hyperoxic conditions were detected in both HLT (median = $319.1 \mu\text{mol L}^{-1}$) and LLT (median = $322.7 \mu\text{mol L}^{-1}$) polyps, with no significant difference between the experimental groups ($W = 3$, $p = 0.7$. Figure 2a, Table S3). Surface oxygen concentrations under the respective acclimation irradiances were consistently higher in HLT corals than LLT corals (median 319 vs. $115 \mu\text{mol L}^{-1}$). Although all HLT values exceeded all LLT values, this difference did not reach statistical significance

(two-sided Wilcoxon rank-sum test, $W = 9$, $p = 0.10$, Table S3), likely due to the small number of biological replicates ($n = 3$ per treatment).

Inside the GVC, in darkness we measured a steep oxycline from the mouth towards the bottom of the GVC, with anoxia detected below the depth of 1 mm in all polyps from both groups (Fig. 2b). Under low photon irradiance, the oxygen conditions within the GVC resembled those measured in darkness for both groups, with most GVCs being anoxic or hypoxic below a depth of 1 mm from the mouth. This resemblance was particularly marked in LLT corals, for which the low light oxygen profiles appeared almost indistinguishable from those measured in darkness (Fig. 2b). When exposed to high light, all polyps reached oxygen supersaturation in at least some parts of the GVC. For most polyps (except for one HLT polyp), an oxygen maximum between 350 and 400 $\mu\text{mol O}_2 \text{ L}^{-1}$ was observed either near the mouth or in the upper region of the GVC, followed by a decrease in oxygen concentration towards the deeper region. Hypoxia was only detected in one HLT polyp under high light, while LLT polyps had less intragroup variability than HLT polyps (Fig. 2b).

Time series measurements of oxygen concentrations near the bottom of the GVC revealed remarkable temporal variability of the oxygen conditions within the coral GVC, even under constant light intensity over a period of 90 minutes (Fig. 2c). In darkness, anoxia persisted for the majority of the measurement duration across most polyps (median oxygen concentrations $\sim 0 \mu\text{mol O}_2 \text{ L}^{-1}$). However, occasional fluctuations of up to $\sim 8.5\text{--}108 \mu\text{mol O}_2 \text{ L}^{-1}$ across individual polyps were recorded (Fig. 2c, Fig. S3).

Under low photon irradiance, HLT polyps exhibited temporal profiles that resembled those measured in darkness with median concentrations at or near $0 \mu\text{mol O}_2 \text{ L}^{-1}$ for all three polyps (Fig. 2c, Table S4). LLT polyps showed greater inter-polyp variability, with medians ranging from -1.2 to $52.1 \mu\text{mol O}_2 \text{ L}^{-1}$ and peak values ranging from 7.7 to $115 \mu\text{mol O}_2 \text{ L}^{-1}$ (Fig. 2c, Table S4).

Under high photon irradiance, oxygen dynamics differed substantially among HLT polyps, whereas LLT polyps exhibited more similar profiles (Fig. 2c), consistent with the pattern observed for the spatial profiles (Fig. 2b). Individual HLT measurements ranged from ~ 0 to $704 \mu\text{mol O}_2 \text{ L}^{-1}$, with medians for each polyp being ~ 74.6 , 209.8 , and $444.3 \mu\text{mol O}_2 \text{ L}^{-1}$ (Fig. 2c). In comparison, LLT polyp measurements under high light ranged from 159 to $374 \mu\text{mol O}_2 \text{ L}^{-1}$, with medians more similar to each other ($20\text{--}327 \mu\text{mol O}_2 \text{ L}^{-1}$; Table S4).

Comparing the temporal data for HLT polyps measured under high photon irradiance to LLT polyps measured under low photon irradiance (i.e., under the respective experimental long-term light conditions) showed that median GVC oxygen concentrations ranged from 74.6 to $444.3 \mu\text{mol O}_2 \text{ L}^{-1}$ among HLT polyps under high photon irradiance, compared with -1.2 to $52.1 \mu\text{mol O}_2 \text{ L}^{-1}$ (Fig. 2c, Table S4) among LLT polyps under low photon irradiance, indicating larger inter-polyp variability under the HLT coral's acclimation condition than under the LLT acclimation condition.

Slightly negative median and minimum values observed in some polyps reflect sensor noise near the anoxic detection limit rather than “true” sub-zero values.

Microbial community

At the end of the 20-week experimental light treatment, we used 16S rRNA gene metabarcoding to characterize the microbial community present in the GVC fluid (sampled with a microneedle), the GVC tissue interface (sampled with a microswab), and the SML (sampled with a pipette) of *G. fascicularis* polyps (Fig. 3). Sequencing generated 4,998,808 raw read pairs for the samples included in the final dataset (excluding negative extraction and PCR controls and low-read samples) (Table S5, S6). After quality filtering, 3,907,845 read pairs remained, of which 3,776,270 were successfully merged. Following contaminant removal and exclusion of samples with fewer than 1000 reads, the final dataset comprised 41 samples, 3,347,114 reads, and 9,827 ASVs. The median sequencing depth was 55,709 reads per sample (IQR: 35,305 – 73,902; range: 3,434–429,658 reads; Table S6). Saturation of the rarefaction curves indicated sufficient sequencing depth to properly represent the bacterial diversity in the analyzed samples (Fig. S1). Heatmap clustering of relative ASV abundances using Bray-Curtis dissimilarities revealed two clearly distinct groups of samples. One cluster contained only coral samples, whereas the other included all environmental samples (aquarium seawater and seawater from the sampling container) together with five GVC needle samples (Fig. S2). Within the environmental cluster, seawater samples grouped closely together and remained well separated from all coral samples. The five GVC samples that clustered with the seawater collected from the sampling container were flagged as potential failed sampling attempts or contaminated samples, and excluded from subsequent analyses.

Median alpha diversity (measured as Shannon’s H index) ranged between 1.76 (in HLT GVC swab samples) and 2.57 (in HLT GVC needle samples), while tank seawater samples had median H = 2.56 (Fig. 3a). The light treatments did not have an apparent effect on alpha diversity in any of the examined compartments, since no significant difference was found between HLT and LLT samples of the same type (GVC needle/swab, and mucus; Wilcoxon rank-sum test: GVC needle $W = 12$, $P = 0.343$; GVC swab $W = 9$, $P = 0.548$; mucus $W = 17$, $P = 0.937$; Fig. 3a, Table S7).

For beta diversity, the ordination did not reveal a clear grouping into the individual experimental groups (Fig. 3b). However, beta dispersion was significantly different between groups overall (ANOVA, $F = 2.9$, $p = 0.03$; Fig. 3c, Table S8). Comparisons between HLT and LLT within each sample type using two-sided Wilcoxon rank-sum tests revealed a trend towards greater beta dispersion in GVC needle samples from HLT corals compared to LLT corals ($W = 15$, $p = 0.061$), whereas no significant differences were detected for GVC swab ($W = 7$, $p = 0.296$) or mucus samples ($W = 17$, $p = 0.936$; Table S8).

ANOSIM showed a small, but significant degree of dissimilarity between groups ($p = 0.016$, $R = 0.15$). Pairwise ANOSIM for comparing HLT to LLT polyps for each sampling method showed a significant difference for the GVC swab samples only ($p = 0.03$, $R = 0.34$; Table S8).

Interestingly, almost all coral samples were overwhelmingly dominated by the genus *Endozoicomonas*, which accounted for ~ 90% of the detected community in mucus and GVC swab samples (Fig. 3d). No significant differences in *Endozoicomonas* relative abundance were detected between HLT and LLT polyps in either mucus (median = 93.4% vs. 92.2%; Wilcoxon rank-sum test, $W = 17$, $p = 0.9$) or GVC swab samples (median = 96.3% vs. 99.9%; $W = 5$, $p = 0.15$, Table S9). In GVC needle samples, *Endozoicomonas* remained the dominant genus but its relative abundance was significantly lower in HLT polyps (38.3%) than in LLT polyps (85.4%; $W = 0$, $p = 0.03$; Fig. 3d, Table S9).

While Endozoicomonadaceae remained the dominant family in all coral-associated microbial communities, the reduced dominance of Endozoicomonadaceae in HLT GVC needle samples was accompanied by greater contributions of Saccharospirillaceae, Alteromonadaceae, Pseudoalteromonadaceae, Flavobacteriaceae, and Rhodobacteraceae (Table S10, Fig. S5).

Seawater samples were compositionally distinct from coral-associated communities and were primarily dominated by Rhodobacteraceae, Clade 1, PS1 clade, and Flavobacteriaceae, which together accounted for the majority of the bacterial community (Fig. S5, Table S11).

Discussion

Light acclimation and oxygen microenvironments

In this study, we explored the effects of long-term acclimation to different light conditions on photosynthetic performance, oxygen microenvironments and microbial communities associated with surface tissue and gastrovascular cavity compartments of *G. fascicularis*.

After 20 weeks exposure to high ($200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) or low ($60 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) photon irradiance, low-light acclimated corals appeared darker and exhibited a higher maximum quantum yield of PSII (F_v/F_m) as well as a lower maximum rETR than high-light acclimated corals. Additionally, corals acclimated to low light showed higher oxygen concentration at the tissue surface compared to high-light acclimated ones when measured under $60 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, indicating either increased photosynthetic production or reduced consumption at this light intensity compared to high-light acclimated corals. Together, these measures are likely indicative of an enhanced capacity of the photosymbionts to exploit light at reduced intensities, consistent with previous studies on photoacclimatory processes in corals (Cohen and Dubinsky 2015; Toniolo et al. 2026).

However, for all corals photosynthetic production was higher under $200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ than at $60 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, as indicated by the higher rETR and surface oxygen concentration measured under $200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. This indicates that *G. fascicularis* polyps maintained under high and low light experienced radically different microenvironmental conditions. Specifically, over our 20-week experiment low-light acclimated corals experienced chronically reduced levels of oxygen in the GVC and SML as well as reduced diel fluctuations compared to high-light acclimated ones.

Oxygen levels in the GVC and the SML are driven by the combination of production and consumption by different holobiont members, active ventilation through tissue contraction/expansion, ciliary action, and boundary layer flow dynamics (Kühl et al. 1995; Pachерres et al. 2022, 2026; Dellisanti et al. 2024; Zhang et al. 2026a). Differences in the resulting oxygen environment under high and low light are likely to affect microbial processes associated with these compartments. For example, a hypoxic or anoxic GVC environment has been proposed to support specialized microbial communities enriched in facultative anaerobes (Bollati et al. 2024), while catalase positive (i.e. oxygen tolerant) taxa may decrease in GVCs that do not experience daily hyperoxia (Zhang et al. 2026b). The oxygen environment directly affects aerobic and anaerobic processes, as evidenced for example by measurements of hydrogen or nitric oxide production in anoxic coral GVCs (Zhang et al. 2026b). Alongside oxygen and other dissolved species, the higher photosynthetic rates detected in high-light corals may also affect the concentration of organic carbon as well as organic and inorganic nutrients within the GVC. Unfortunately, measurements of these parameters in the GVC are currently lacking. Enhanced mucus production and lipid release has been observed under high light (Crossland 1987), while no effect of light intensity on dissolved organic carbon release was detected in one study using bulk measurements (Mueller et al. 2014). However, considering the complex nutrient cycles and high recycling rates encountered in reef-building corals (Rädecker et al. 2015), microscale dynamics are likely to be very different from what is detected in the bulk. Future studies quantifying organic matter, carbon and nutrients in the GVC fluid would help resolve these microscale dynamics and provide a more complete picture of how the external light environment affects the availability of these substrates to GVC microbial communities.

Besides having higher daytime oxygen concentrations, the GVCs of high-light acclimated corals harbored an overall more variable oxygen environment compared to those found under low light. This was true not only in terms of diel range or short-term fluctuations, but also in terms of variability between polyps. While higher temporal variability within a single polyp may select for microbial taxa with broader environmental tolerance, intra-colony variability would create a wider range of microhabitats, potentially enriching the overall diversity of taxa and functions. Intracolony variability in the GVC microenvironment in intact colonies (as compared to individually fragmented polyps) and its role in microbiome diversity and holobiont functioning remains to be investigated.

Dominance of Endozoicomonas in the G. fascicularis GVC

After 20 weeks of acclimation to high- and low-light conditions, *Endozoicomonas* was the dominant taxon in all *G. fascicularis* polyps, especially in mucus and GVC swab samples, which exhibited relative abundances close to 100%. On the other hand, this genus was virtually absent from seawater samples. *Endozoicomonas* are ubiquitous bacteria in coral tissue, where they often form coral-associated microbial aggregates (CAMAs) (Neave et al. 2017; Wada et al. 2022; Maire et al. 2023). The coral-*Endozoicomonas* relationship has been proposed to range over the entire spectrum of symbiosis, from mutualism, to commensalism, to parasitism (Pogoreutz and Ziegler 2024). Previous work on *G. fascicularis*, however, reported that while *Endozoicomonas* was highly abundant in the tissue, it was absent from the GVC (Liu et al. 2024). A low relative abundance of *Endozoicomonas* was also reported

from the GVC of other coral species on the Great Barrier Reef (Bollati et al. 2024). Thus, the finding of a high relative abundance of this taxon in the *G. fascicularis* GVC is in contrast with previous reports. We suggest that the *Endozoicomonas* detected here may originate from CAMAs located in the gastrodermis (Bayer et al. 2013; Neave et al. 2016; Gotze et al. 2025), the tissue layer lining the GVC and in direct contact with the GVC fluid. These aggregates host high bacterial numbers compared to the GVC fluid (cf. 2,800 cells per small endodermal CAMA, Wada et al., 2019, against ~ 1,000 total cells per one 10 uL GVC fluid sample, Bollati et al. 2024), thus could have contributed considerable amounts of DNA in our samples, for example through shedding of DNA or dead cells. Gastrodermal CAMAs are also consistent with our finding that swabs taken at the GVC tissue-fluid interface had higher *Endozoicomonas* dominance compared to needle samples.

The dominance of *Endozoicomonas* in our investigated corals also contrasts common reports that *Endozoicomonas* diminish in long-term captivity (Barreto et al. 2021; Puntin et al. 2024). The corals used in this study were reared in captivity for years prior to the experiment, so it was unexpected to detect such high *Endozoicomonas* abundance. It has been suggested that the putatively beneficial roles of *Endozoicomonas* (e.g. in nutrient cycling or B vitamin production) might become redundant in an aquarium setting with regular feeding (Pogoreutz and Ziegler 2024). However, at least for cold water corals, feeding with specific diets has been linked to the retention of *Endozoicomonas* in captivity (Galand et al. 2020). We therefore hypothesise that the combined feeding of live *Artemia* with zooplankton pellets used in our study may have contributed to this result.

Effects of light acclimation on the GVC microbiome

Given the chronic differences in the GVC oxygen microenvironment under our two light conditions, we hypothesized that 20 weeks of light acclimation would induce a major shift in the GVC microbiome composition of *G. fascicularis*. The most notable difference we detected was in the relative abundance of *Endozoicomonas*, with high-light acclimated corals showing a lower relative abundance of this bacterial genus in the GVC fluid than low-light acclimated ones. *Endozoicomonas* have been reported to decrease when corals are exposed to highly variable environments (Pantos et al. 2015; Camp et al. 2020; Pogoreutz and Ziegler 2024), which could explain why their relative abundance was lower in the highly variable GVC of high-light corals. *Endozoicomonas* have been shown to correlate positively with phosphates (Ruiz-Toquica et al. 2025), which may become more rapidly depleted under high light due to faster uptake by the algal symbiont (Godinot et al. 2009), and negatively with total suspended solids, including organic matter (Ruiz-Toquica et al. 2025), which may accumulate in the GVC as a result of higher photosynthetic production. Alternatively, photon irradiance may affect the rate at which *Endozoicomonas* cells or DNA are shed from the coral tissue into the GVC.

Another possibility is that the observed decrease in *Endozoicomonas* relative abundance under high light was caused by an increase in the abundance of other bacterial taxa. While alpha diversity was not significantly different between treatments, this may have been due to low statistical power caused by low replication, and we note that the highest individual alpha diversity values in our dataset were detected in the GVC fluid of high-light corals. A previous study on a Mediterranean coral species reported

an increase in alpha diversity in the GVC in response to chronic iron enrichment (Dellisanti et al. 2026), a condition which similarly to high-light acclimation may stimulate photosynthetic production and increase the magnitude of diel GVC oxygen fluctuations (Dellisanti et al. 2024, 2026). Similarly, changes in the availability of carbon and nutrients in the GVC under high light may have promoted colonization by other taxa, resulting in a lower relative abundance of *Endozoicomonas*. Due to the small sample size and the heavy *Endozoicomonas* dominance, we did not attempt formal differential abundance analysis on the rest of the community. However, we note that several families and genera made up a larger proportion of the microbial community in high-light GVCs compared to low-light. The most enriched families included *Saccharospirillaceae*, *Alteromonadaceae*, *Pseudoalteromonadaceae*, *Flavobacteriaceae*, and *Rhodobacteraceae*, while the most enriched genera were *Alteromonas*, *Bermanella*, *Pseudoalteromonas*, *Tenacibaculum*, and *Ruegeria*.

Alongside the difference in *Endozoicomonas* relative abundance, we detected a trend for higher beta dispersion in the gastric fluid of high-light acclimated corals, indicating that the high-light GVC communities might be more different from each other, as compared to low-light ones. This observation would be consistent with the higher inter-polyp variability we detected in the GVC oxygen microenvironment and should be further investigated in colony-level studies to confirm whether this variability is retained when polyps are connected by tissue. If confirmed, this would indicate that corals exposed to higher light intensities indeed harbor a wider range of microenvironmental niches.

Despite the difference in beta dispersion, we did not detect a clear shift in beta diversity in response to photoacclimation. This contradicts our hypothesis that a radically different GVC oxygen environment would be associated with distinct microbial communities. While it is possible that the *G. fascicularis* GVC community is relatively stable and more resilient to environmental changes than hypothesized, the high abundance of *Endozoicomonas* in our dataset makes it challenging to interpret this result with confidence, as it may have masked trends in the rest of the community. Comparison with non *Endozoicomonas*-dominated samples will help clarify this in future studies.

Methodological challenges and future developments

Besides the novel insights into the microbiome and physicochemical conditions inside the GVC in response to differing light levels, our study also highlights a few limitations of the applied methods, which should be considered when working with microbial ecology inside the coral GVC.

First, a subset of samples collected from the *G. fascicularis* GVC using either the needle or swab method hosted microbial communities closely resembling those detected in environmental samples (aquarium seawater and the sampling container). Environment-like samples could represent “failed” sampling attempts, in which more ambient seawater than actual GVC fluid was sampled (Bollati et al. 2024). Alternatively, these datapoints could indicate that some GVCs predominantly contain taxa from the surrounding seawater, rather than specialized communities (Bollati et al. 2024). The interpretability of these “environment-like” GVC microbiome samples is limited, as it is difficult to distinguish between a real biological signal and sampling artifacts or contaminations during the sampling process (Bollati et al.

2024; Dellisanti et al. 2026). In this study, we attempted to minimize contamination by lowering the water level in the sampling environment as much as possible to reduce the distance travelled by the sampling instrument through the water column to a minimum (Bollati et al. 2024), and we sampled the GVC fluid for each polyp separately in a sterilized glass dish containing filtered artificial seawater. To avoid including failed or contaminated samples in our final dataset, we excluded the environment-like GVC samples from any downstream analysis. However, understanding whether some GVCs are truly more similar to seawater in terms of microbial community composition is highly relevant to studies of intra-colony heterogeneity. Future studies should attempt to resolve this by comparing, for example, GVC samples taken in different sampling environments (sterile vs raw seawater, or seawater spiked with a mock community) to differentiate contamination from biological variability.

Second, while controlled laboratory experiments are invaluable for disentangling specific drivers of microbial and physicochemical dynamics, they inevitably simplify the complexity of natural reef environments. *In situ* conditions are shaped by fluctuating light regimes, diel and seasonal photoperiods, complex flow dynamics, and variable nutrient and microbial inputs from the surrounding seawater. These factors can interact in non-linear ways, influencing oxygen gradients, microbial community assembly, and host physiology at scales that are difficult to replicate *ex situ* (Pendleton et al. 2016; Linsmayer et al. 2020). Future research should therefore include field-based sampling of GVC fluid under ecologically relevant conditions, ideally integrating microsensor profiling with molecular approaches to capture both physicochemical heterogeneity and microbial dynamics *in situ*. Such efforts will be critical to validate laboratory findings, assess intra-colony variability, and refine our understanding of how environmental variability shapes coral internal microhabitats and their associated microbiomes.

Conclusion

Our study has shown that changes in environmental conditions, such as light levels, can have a profound effect on the chemical environment found in the GVC of coral polyps. While further studies are needed to understand to what extent these microenvironmental changes affect the microbial community, our results suggest that GVC microbiome heterogeneity and *Endozoicomonas* abundance are responsive to external light levels. These findings underscore the importance of studying physiological processes and microbiome dynamics at relevant spatial scales, and to consider compartment-specific responses when investigating how corals acclimate and adapt to environmental change. Continued improvements in compartment sampling methods will enable more studies to include this approach in the future. Ultimately, such efforts will yield mechanistic insight that can help predict coral resilience and vulnerability under future scenarios.

Declarations

Acknowledgements

This study was supported by the Gordon and Betty Moore Foundation (grant no. GBMF9206, <https://doi.org/10.37807/GBMF9206>; M.K.) and a PhD scholarship from the China Scholarship Council (grant no. 202207940005; Q.Z.). We thank Sofie Jakobsen (University of Copenhagen) and Jean Baptiste Raina (Max Plank Institute for Marine Microbiology) for their invaluable assistance with coral husbandry and 16S sequencing.

Data availability

Raw sequencing data are available from SRA (PRJNA1417609). All remaining datasets are available from [10.5281/zenodo.21293875](https://doi.org/10.5281/zenodo.21293875).

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Figures

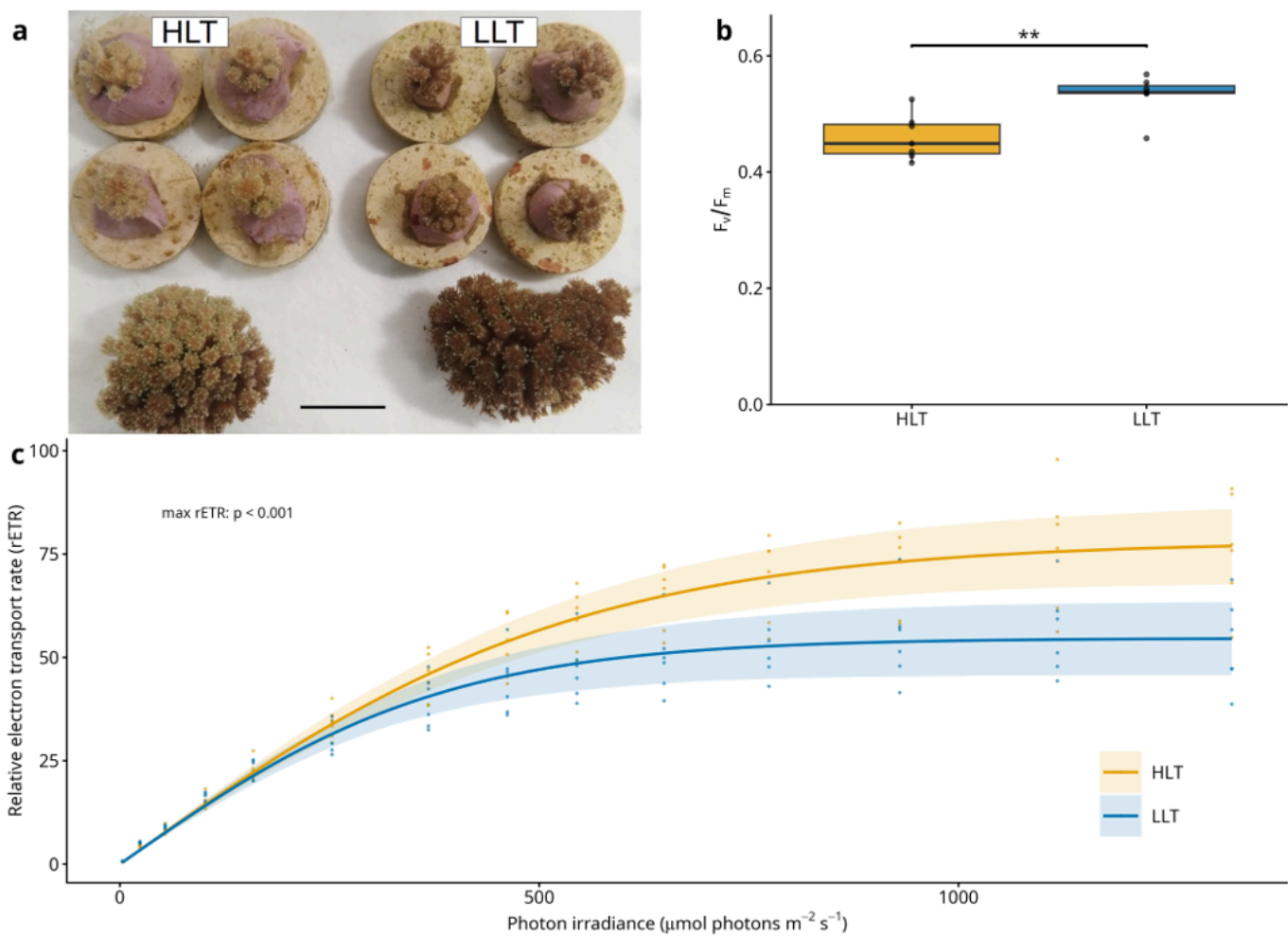


Figure 1

Photoacclimation of *G. fascicularis* polyps. Variable chlorophyll fluorescence measurements taken on the oral disc after 20 weeks of acclimation to high-light (HLT; 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) or low-light (LLT; 60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). **(a)** *G. fascicularis* fragments after 20 weeks of acclimation. The bar indicates a length of 2cm **(b)** Maximum quantum yield of photosystem II (F_v/F_m) ($n = 7$ polyps per treatment). Asterisks represent $p < 0.05$ in Wilcoxon rank-sum test. **(c)** Rapid light curves showing the relative electron transport rate (rETR) as a function of photon irradiance. Spheres represent individual measurements, solid lines represent model fits, and shaded areas indicate 95% confidence intervals.

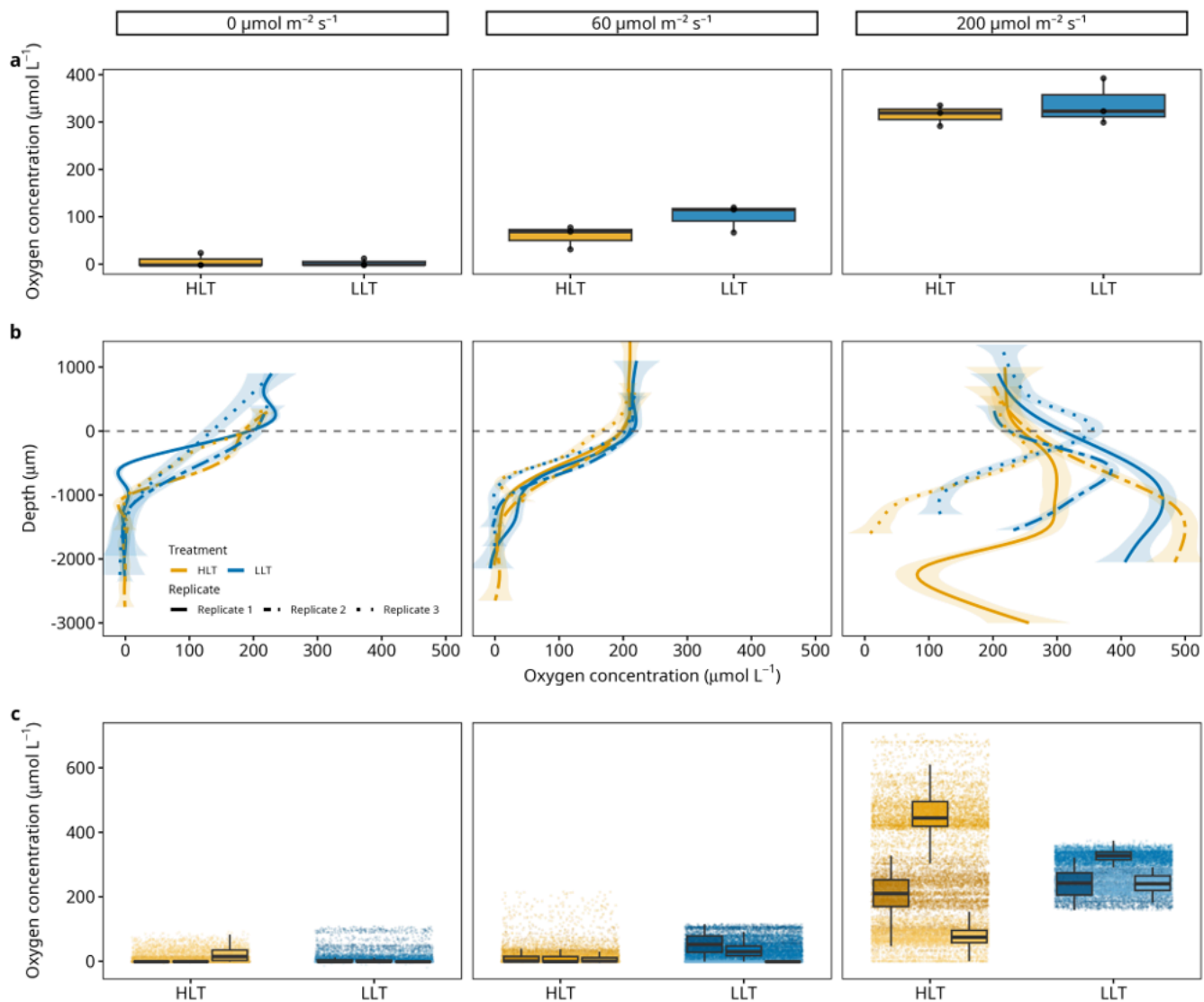


Figure 2

Oxygen microenvironment of *G. fascicularis* polyps acclimated to different light levels. Measurements performed under three different incident light intensities (0, 60, and 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) in the surface mucus layer (SML; **a**) and gastrovascular cavity (GVC; **b,c**) of high-light (HLT; 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and low-light (LLT; 60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) acclimated polyps ($n = 3$ per condition). In (**a**), each data point represents the mean of 3–6 technical replicates measurements for a single biological replicate (polyp). In (**b**) spatial oxygen profiles were recorded inside the GVC from the deepest point to 0 μm , defined as the position of the polyp mouth (dashed grey line). Solid lines represent the mean of 2–5 technical replicate profiles for each polyp, line type distinguishes individual polyps, and shaded areas indicate 95% confidence intervals. In (**c**), individual data points represent oxygen concentrations measured at the bottom of the GVC every second over a 90 min incubation. Different shades of the treatment colors distinguish biological replicates (polyps).

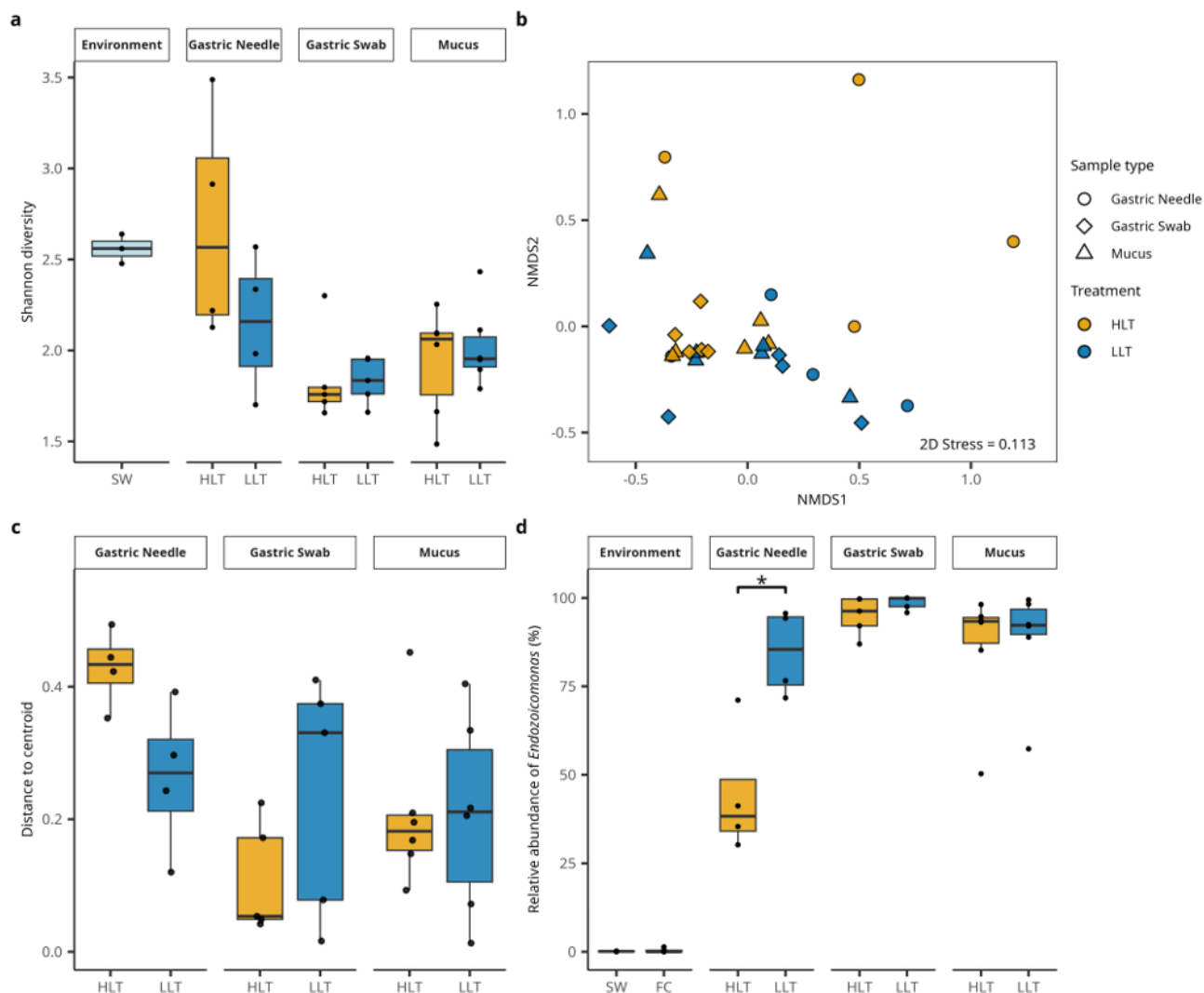


Figure 3

Microbial diversity in the gastrovascular cavity and the surface mucus layer of *G. fascicularis*. (a) Alpha diversity measured as the Shannon-Wiener H index after 20 weeks at high (HLT; 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) or low (LLT; 60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) photon irradiance. SW = seawater. (b) Beta diversity represented as NMDS ordination based on Bray-Curtis dissimilarities. Seawater samples are excluded from the ordination plot to facilitate visualization; see Fig. S4 for the complete plot. (c) Multivariate dispersion (distance to group centroid) based on Bray-Curtis dissimilarities for each sample type. The p-value indicates the result of a two-sided Wilcoxon rank-sum test comparing HLT and LLT within gastric needle samples. (d) Relative abundance of *Endozoicomonas* across treatments and sample types. The asterisk indicates $p = 0.03$ (two sided Wilcoxon rank-sum test).

Supplementary Files

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