

Spatio-temporal dynamics of the microbiome of *Acropora millepora* corals and their symbiotic fish *Dascyllus emano* along an environmental gradient in Ahe atoll, French Polynesia

Tony Gardon

PSL Université Paris: EPHE-UPVD-CNRS, UAR 3278 CRIOBE

Camille Clerissi

PSL Université Paris: EPHE-UPVD-CNRS, Université de Perpignan

Camille Gache

PSL Université Paris: EPHE-UPVD-CNRS, UAR 3278 CRIOBE

Orphé Bichet

OECHO

Corinne Belliard

Ifremer, ILM, IRD, Univ French Polynesia, UMR 241 SECOPOL

Jérémy Le Luyer

`jeremy.le.luyer@ifremer.fr`

Ifremer

David Lecchini

PSL Université Paris: EPHE-UPVD-CNRS, UAR 3278 CRIOBE

Research Article

Keywords: Coral, fish, microbiome, symbiosis, dysbiosis, Endozoicomonas, Vibrio

Posted Date: October 9th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11035827/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

We investigated the spatio-temporal variations of bacterial communities associated with *Acropora millepora* corals and their symbiotic *Dascyllus emano* fish in the Ahe Lagoon (French Polynesia). Sites differing in bathymetry, oceanic connectivity, anthropogenic pressure, and pollutant loads (i.e microplastics), were sampled during both the cold and warm seasons.

Significant spatial and seasonal variations in microbial community structure, diversity, and core composition were observed. Localized environmental conditions strongly influenced both coral and fish microbiota during the cold season, which coincided with a marked microbial shift, consistent with dysbiosis. Coral microbiota exhibited a sharp decline in beneficial symbionts such as *Endozoicomonas* together with an enrichment of opportunistic taxa. Concurrently, the microbiota of associated *Dascyllus emano* showed a pronounced loss of diversity and richness and became overwhelmingly dominated by *Vibrio*.

The parallel restructuring of microbial communities in both symbiotic partners suggests a coordinated response to local environmental disturbance and highlights the potential of host-associated microbiomes as sensitive indicators of reef condition. These findings highlight the value of integrated, multi-host microbiome analyses for detecting localized environmental degradation in aquaculture-influenced reefs, but emphasizes the need for broader temporal validation before microbial indicators can be reliably incorporated into long-term reef monitoring and lagoon management.

Background

Hosting nearly a quarter of the world's marine life in less than 1% of the ocean's area (~ 250,000 km²), coral reefs provide key ecosystem services such as food supply, shoreline protection, erosion regulation, and habitat support (Woodhead et al., 2019). These services are closely related to the ecosystem functioning supported by diverse taxa mediating ecological processes depending on their composition and structure (Brandl et al., 2019). Within coral reefs, hard coral species are crucial for reef-building, maintaining the structure that supports marine biodiversity (Perry and Alvarez-Filip, 2019). However, these ecosystems are under threat from anthropogenic pressures such as climate change, pollution, and overfishing, which jeopardize their resilience and survival (Ellis et al., 2019).

In French Polynesia (FP), tourism and pearl farming are key economic resources but also exert significant pressures on coral reef ecosystems. Pearl farming, in particular, generates plastic waste due to the lack of waste management measures on remote islands and atolls where this activity has been practiced for over 40 years (Crusot et al., 2023). This waste, along with operational farming structures, contributes to the release of microplastics and chemicals (Gardon et al., 2020, 2021). These stressors amplify the impacts on vulnerable coral reef ecosystems (Hughes et al., 2017; Gardon et al., 2025), which are already under pressure from fluctuating environmental conditions, including temperature variations, nutrient loading, and water quality changes, particularly in semi-closed lagoons. For instance, corals exposed to nitrogen pollution exhibited higher rates of bleaching and mortality compared to those in low-

nitrogen conditions in Mo'orea lagoon, FP (Burkepile et al., 2020). Excess nitrogen exposure may lower the temperature threshold at which corals bleach, triggering bleaching events even when typical thermal stress thresholds have not been exceeded (Burkepile et al., 2020). Thus, fluctuations in environmental conditions, influenced by both human activities and lagoon geomorphology (Bongaerts et al., 2017), contribute to the complex exposome faced by coral species (Brandl et al., 2019).

Recent research has highlighted the importance of microbiomes associated with corals and fish in modulating their response to environmental stress (Webster and Reusch, 2017; Santoro et al., 2025). Corals are meta-organisms, hosting microbial communities (e.g., bacteria, archaea, fungi, protists, and viruses) that play crucial roles in nutrient acquisition, waste processing, and pathogen resistance (Bourne et al., 2016; Peixoto et al., 2017). Fish species that engage in symbiotic relationships with corals also contribute to ecosystem functions and resilience through interactions with their coral hosts and microbial communities (Bellwood et al., 2004). Moreover, studies showed that anthropogenic and environmental disturbances significantly affect bacterial community structures and nitrogen cycling along physical gradients (Highton et al., 2016; Zaneveld et al., 2016; Stevick et al., 2021). For example, overfishing, nutrient pollution, and temperature impacted reefs at microbial scales, killing corals by sensitizing them to predation and bacterial opportunism (Zaneveld et al., 2016). Understanding microbial community distribution in key reef species is critical for elucidating coral reef ecosystem dynamics and their vulnerability to environmental changes (Nemergut et al., 2013).

In this study, we employed 16S DNA gene amplicon sequencing to analyze bacterial communities in seawater and the microbiomes of damselfish *Dascyllus emano* and its symbiotic coral host *Acropora millepora*. While both microbiomes are expected to show a species-specific signature, they may however show differences associated with seasonal temperature variations and anthropogenic disturbances, such as nutrient loading or pollution from aquaculture. Accordingly, the survey was conducted at three sites along a pearl farming gradient in the Ahe lagoon (FP) across both cold and warm seasons. The North-South gradient in Ahe is characterized by intensive pearl farming, human settlement, and relatively restricted water renewal. Combined with the strong seasonal variability typical of Tuamotu lagoons, these geomorphological and hydrodynamic features are expected to generate pronounced temporal changes in environmental conditions (Pagano et al. 2017). This research provides a snapshot of microbiomes in reef species in a lagoon ecosystem affected by aquaculture. It also examines how these host-associated microbiomes were influenced by their local environment, considering the different ecological roles and mobility of corals and fishes. We aimed to identify spatial and temporal disparities in microbial composition, highlighting coral reef ecosystem vulnerability and providing insights into microbiome dynamics in response to disturbances, potentially identifying microbial indicators of reef health.

Results

Sampled sites showed variability in environmental conditions

Three sampling locations were selected along an anthropogenic gradient of pearl farming activity in the lagoon of Ahe atoll with site 1 (S1) located in the North with the lower activity, site 2 (S2) located next to the pass corresponding to a moderate pressure, and site 3 (S3) located in the South with the highest anthropogenic pressure (**Fig. 1**). In addition to varying levels of pearl farming inputs, these sites were characterized by specific environmental conditions (*i.e.*, temperature, dissolved oxygen, pH, salinity and nutrients) according to season (cold vs. warm) (**Table S1**). The principal component analysis of the recorded environmental conditions exhibited eigenvalues of dimensions one and two representing 74.7% of the environmental variation between sites (**Fig. 2A**). Each site was characterized by a subset of environmental factors over the cold and the warm seasons. The most contributing factors to both dimensions (>15%) were the organic silica (Si(OH)_4) concentration, the pH, the salinity and the temperature (**Fig. S1**); mostly explaining the divergence we observed between study sites in cold and warm seasons. Globally, study sites were similar depending on the season except site S3 which was particularly divergent regarding Si(OH)_4 and nitrite (NO_2) concentrations during the cold season (**Fig. 2A**). In this way, a North-South lagoon gradient was detected regarding NO_2 with a significant increase in concentration from S1 to S3 (Pearson's correlation, $R = -0.83$, $p = 0.041$; **Fig. 2B**).

Metabarcoding dataset

A final dataset of 150 samples, including 4 quality-control blanks, were sequenced using Illumina MiSeq technology. In the bacteria-only dataset, mean sequencing depth was $75.4\text{k} \pm 20.3\text{k}$ reads per library (mean $\pm$ SD; all samples), and $76.6\text{k} \pm 19.1\text{k}$ reads per library after excluding blanks. A total of 11,182,106 quality-controlled bacterial 16S reads were retained in non-blank samples, including 664,155 reads from seawater ($n = 12$), 4,046,456 from coral ($n = 53$), and 6,471,495 from fish ($n = 81$), across the three study sites.

After bacteria-only filtering and blank decontamination, the full dataset (including blanks) contained 5,324 ASVs, and 5,201 ASVs were retained in non-blank samples used for downstream analyses. In non-blank samples, 1,012 ASVs were detected in seawater, 1,717 in coral, and 3,202 in fish, with 108 ASVs shared across all three sample types.

Diverging microbial community structures according to sample type

ASV analysis identified 229 bacterial orders across 45 phyla in all samples. The most dominant phyla in seawater samples, averaged across sites and seasons (mean $\pm$ SD), were Cyanobacteriota (60.6 $\pm$ 12.1%) and Proteobacteria/Pseudomonadota (23.4 $\pm$ 7.0%). In coral samples, the dominant phyla were Proteobacteria/Pseudomonadota (93.8 $\pm$ 15.0%) and Cyanobacteriota (1.5 $\pm$ 4.2%), whereas fish samples were dominated by Proteobacteria/Pseudomonadota (80.4 $\pm$ 19.6%), Firmicutes/Bacillota (11.3 $\pm$ 15.0%), and Cyanobacteriota (4.2 $\pm$ 9.2%). A total of 108 ASVs were shared among seawater, coral, and fish samples; Proteobacteria/Pseudomonadota was the most represented shared phylum ($n = 52$), including among others Flavobacteriaceae ($n = 7$), Vibrionaceae ($n = 7$), and Endozoicomonadaceae ($n =$

4) (**Fig. 3A**. Significant differences in bacterial community structure among sample types were observed for richness (Kruskal-Wallis, $P < 0.001$), Shannon diversity ($P < 0.001$), and evenness ($P < 0.001$) (**Fig. 3B**). Bray-Curtis β -diversity also indicated marked dissimilarities in community composition among sample types (ANOSIM, $R = 0.91$, $P = 0.001$; **Fig. 3C**).

Effects of sites and seasons on alpha diversity of bacterial community

Seawater-associated communities showed moderate overlap across all three sites in both seasons, with 19.3% of ASVs shared among sites in the warm season (S1; 99/513) and 19.5% in the cold season (S2; 110/565) (**Fig. 4A**). In the cold season, site S3 contained 69.2% of total seawater ASVs (391/565), including 41.1% that were unique to S3 (232/565). Coral-associated communities also showed stronger site structuring in the cold season (**Fig. 4B**). The total number of coral ASVs increased from 681 in the warm season to 1,090 in the cold season (1.60-fold). Of these, 40.0% were detected at S3 (436/1,090), and 33.9% were S3-specific (370/1,090). In fish samples, overlap among sites remained low in both seasons (shared ASVs: 5.12% in warm and 5.16% in cold), with strong site-specific components (**Fig. 4C**). During the cold season, S1 had the highest unique fraction (40.5%; 706/1,745). Fish ASV totals in the cold season were highest at S1 (1,003), then S2 (611), and S3 (544).

Alpha-diversity comparisons across sample types were performed using either ANOVA or non-parametric pANOVA to assess the effects of season, site, and their interaction. Due to the limited number of seawater replicates per site and season, seawater samples were grouped either by season (pooling all sites within season) or by site (pooling both seasons within site). In seawater, no significant effect was detected for any metric (Observed: season $P = 0.070$, site $P = 0.879$; Shannon: season $P = 0.184$, site $P = 0.845$; Evenness: season $P = 0.216$, site $P = 0.831$; **Fig. 5A**). In corals, all three metrics (Observed, Shannon, Evenness) showed significant effects of season, site, and season $\times$ site interaction (Observed: $P = 0.0087$; Shannon: $P < 0.001$). In fish, Shannon and Evenness (ANOVA) were significantly affected by season, site, and interaction (Shannon: $P < 0.001$; Evenness: $P < 0.001$). Fish Observed richness was significantly affected by season $\times$ site interaction (pANOVA, $P = 0.0009$). Post-hoc comparisons showed site-specific seasonal reversals: at fish site S3, warm season values were higher than cold for Observed, Shannon, and Evenness (all adjusted $P < 0.001$), whereas at fish site S1, cold season values were higher for Shannon and Evenness (both adjusted $P < 0.001$) (**Fig. 5A**).

Effects of sites and season on beta diversity of bacterial community

By performing non-metric multidimensional scaling (NMDS) on Bray-Curtis dissimilarities, β -diversity analyses confirmed significant compositional shifts across seasons and/or sites in seawater, coral, and fish microbiota (**Fig. 5B**). For seawater samples (sites pooled by season to avoid groups with $n = 1$), PERMANOVA showed a significant seasonal effect ($F = 3.98$, $P = 0.025$). The homogeneity of multivariate dispersion test was not significant (PERMDISP: $F = 0.92$, $P = 0.38$), indicating that this

separation was not explained by unequal dispersion. In corals, ANOSIM detected significant effects of both season ($R = 0.505$, $P = 0.001$) and site ($R = 0.366$, $P = 0.001$). Dispersion differed among site $\times$ season groups (PERMDISP: $F = 26.42$, $P = 0.001$), with the highest distances to centroid at site S3 in the cold season, significantly higher than all other coral groups (pairwise adjusted $P = 0.007$). In fish, PERMANOVA revealed significant effects of season $\times$ site interaction ($F = 13.38$, $P = 0.001$). Fish dispersion also differed among site $\times$ season groups (PERMDISP: $F = 12.10$, $P = 0.001$), with notably lower distances to centroid at site S3 during the cold season than in all other groups (pairwise adjusted $P = 0.004$).

Spatio-temporal variation in core microbiome composition and abundance

The core microbiome (i.e taxa with relative abundance $\geq 1\%$ in at least 80% of samples within that group) comprised 18 ASVs in seawater (10 genera, including one unidentified genus), 5 ASVs in corals (4 genera), and 13 ASVs in fish (7 genera). In seawater, *Synechococcus* CC9902 dominated the core across groups (54.7–87.0% of core composition), while other genera showed lower and group-dependent contributions (e.g., *Incertae Sedis*: 3.7–15.5%). In corals, *Endozoicomonas* (2 ASVs) dominated most groups (42.7–92.1%), but was absent at site S3 in the warm season (S1), where *Parendoicomonas* represented 100% of the core. At site S3 in the cold season (S2), *Endozoicomonas* remained dominant ($81.5 \pm 22.4\%$; mean $\pm$ 95% CI), with *Synechococcus* CC9902 ($14.3 \pm 19.8\%$) and MD3-55 ($4.2 \pm 3.0\%$) also present. In fish, *Vibrio* (4 ASVs) was generally dominant (33.7–100%), but was absent from the warm season at site S2, where *Synechococcus* CC9902 ($79.4 \pm 4.1\%$) and other uncharacterized ($20.6 \pm 4.1\%$) defined the fish core microbiome (Fig. 6A).

Total core abundance was then tested across groups. For seawater, Kruskal-Wallis tests detected no significant effect of season or site (Season: $P = 0.462$; Site: $P = 0.936$; Fig. 6B). In corals, two-way ANOVA showed significant effects of season ($F = 15.59$, $P < 0.001$), site ($F = 38.75$, $P < 0.001$), and interaction ($F = 29.17$, $P < 0.001$). Post-hoc Tukey tests confirmed that in the cold season (S2), site S3 had significantly lower core abundance ($47.8 \pm 25.0\%$) than site S1 ($93.7 \pm 3.1\%$, $P = 0.001$) and site S2 ($98.9 \pm 0.4\%$, $P < 0.001$). In fish, ANOVA also showed significant effects of season ($F = 10.94$, $P = 0.002$), site ($F = 24.46$, $P < 0.001$), and interaction ($F = 4.24$, $P = 0.018$). During the cold season (S2), core abundance was highest at site S3 ($88.5 \pm 14.9\%$), significantly above site S1 ($59.4 \pm 18.7\%$, $P < 0.001$) and site S2 ($44.5 \pm 29.7\%$, $P < 0.001$). During the warm season (S1), site S2 showed significantly lower core abundance ($26.6 \pm 13.0\%$) than site S1 ($63.5 \pm 27.6\%$, $P = 0.003$) and site S3 ($60.6 \pm 15.7\%$, $P = 0.007$).

Discussion

Our study reveals pronounced spatial and seasonal variation in bacterial community structure, diversity, and core composition across lagoon ecosystems exposed to differing environmental conditions and anthropogenic pressures in the Ahe atoll. By comparing the microbiome of seawater, corals, and fish along a north–south gradient of pearl farming intensity and physico-chemical conditions, our findings indicate that localized environmental factors, particularly at site S3, strongly shape microbial

assemblages. These effects are evident not only in the taxonomic composition and diversity indices, but also in the stability and structure of the core microbiomes associated with the two symbiotic coral reef species. In particular, several *Endozoicomonas* ASV were less abundant on corals at site S3 during both cold and warm season. In contrast, several taxa had different abundances in fish microbiota at site S3 but only during the cold season, highlighting a temporal variability.

Environmental drivers of microbial divergence

In the context of the Ahe atoll, local variations in nutrient concentrations, hydrodynamic isolation, and contaminant exposure appear to play a foundational role in structuring host-associated microbiomes.

Environmental parameters varied substantially between sites and seasons, with strong negative correlation between nitrite levels and geographic positioning (north–south gradient) suggesting that S3 may act as a nutrient accumulation zone. This pattern is likely driven by the cumulative effects of intensive pearl farming operations, which have been previously associated with nutrient loading, organic matter enrichment, and reduced water circulation in the Ahe lagoon (Thomas et al., 2016; Rodier et al., 2021). A recent study also reported that the southwestern part of Ahe lagoon (corresponding to site S3) accumulates the highest concentrations of microplastics (Gardon et al., 2021), reinforcing the idea that this site acts as a convergence zone for multiple stressors. This pattern is likely driven by the prevailing east to northeast trade winds and the lagoon’s orientation, which, combined with the northern location of the main pass, promote the accumulation of particles and contaminants in the southwestern sector. Such accumulation may exacerbate local environmental stressors, including microplastics and nutrient enrichment, which can destabilize microbial communities by enriching for opportunistic taxa (Wright et al., 2020), altering local biogeochemical processes (Seeley et al., 2020), and reducing microbial evenness and functional redundancy (Tang et al., 2021; Bian et al., 2024). The co-localization of nutrient and contaminant accumulation raises concerns about the cumulative and potentially synergistic effects of multiple anthropogenic stressors on the taxonomic composition and functional dynamics of microbial communities in seawater, coral, and fish samples at this site.

Host-specific patterns and ecological niches

Microbial communities differed significantly across sample types, with coral and fish hosts maintaining distinct microbiota rather than showing convergence such as previously observed (Émie et al., 2021), suggesting strong host-specific filtering. Coral microbiota were dominated by *Endozoicomonas*, a known symbiont involved in host nutrient cycling and immune modulation (Neave et al., 2016), whereas fish-associated communities were more variable and often enriched in *Vibrio* and *Photobacterium*, which are common genera in fish gut microbiota (Egerton et al., 2018), whose composition is typically influenced by diet and trophic behaviour (Huang et al., 2020). *D. emano*, a planktivorous species, predominantly harbours these genera, which contribute to nutrient assimilation but may also include opportunistic taxa whose proliferation can be favoured under environmental stress or host imbalance (Medina-Félix et al., 2023). The absence of convergence between coral and fish microbiota likely also reflects fundamental differences in microbial niches, since the coral microbiome represents an epithelial consortium directly

interacting with the surrounding seawater (Lima et al., 2023), whereas the fish microbiome analysed here corresponds to gut-associated communities that are more physiologically regulated and less exposed to environmental exchange (Sylvain et al., 2020). In parallel, seawater samples were dominated by *Cyanobacteria*, particularly *Synechococcus*, a picoplanktonic genus typical of oligotrophic marine systems (Partensky et al., 1999), and this pattern was also linked to the pre-filtration of seawater applied prior to DNA extraction in the present study. While these patterns reflect baseline host-specificity, more nuanced insights emerge when examining how these communities shift across environmental gradients and seasons, particularly in the damselfish *D. emano* and its symbiotic host coral *A. millepora*, where marked changes in diversity and core composition were observed.

Differences in coral microbiota structure under site-specific environmental stress

A marked influx of unique ASVs in corals was observed at site S3 during the cold season, contributing to an increase in α -diversity. While microbial evenness is often considered indicative of ecosystem resilience, in this case it more likely reflects ecological destabilization. The rise in α -diversity does not seem to result from functional diversification, but rather from environmental filtering, where environmental perturbations such as elevated nitrite and silica concentrations may promote the proliferation of opportunistic taxa (Zhu et al., 2023), or from physiological stress disrupting host-microbe specificity (Ziegler et al., 2017). This increase in taxonomic richness coincided with substantial shifts in the composition and structure of the core microbiome, suggesting a deeper functional disruption of the coral holobiont. Notably, the symbiont *Endozoicomonas* showed a marked lower relative abundance and represent a reduced proportion of core microbiome of individuals from site S3 during this season. A more moderate yet significant lower abundance was also observed at site S3 during the warm season, underscoring the persistent environmental stress affecting this location across both sampling periods. This pattern is consistent with the known sensitivity of *Endozoicomonas* to environmental stressors, particularly nitrogen enrichment (Maher et al., 2019; Rice et al., 2019).

In parallel, the decline of *Endozoicomonas* was accompanied by the emergence of other dominant taxa, such as *Synechococcus* and MD3-55, all considered opportunistic or potentially pathogenic. The increased abundance of MD3-55 (sensu *Candidatus Aquarickettsia*) is particularly concerning, as this taxon has been associated with parasitic lifestyles and elevated nitrogen environments (Klinges et al., 2020). It is hypothesized that *Ca. Aquarickettsia* species impair coral health by depleting host nutrients and energy, weakening and killing host cells, which may increase disease susceptibility through two mechanisms: (i) inducing host nutritional deficiencies that disrupt the symbiotic state, and (ii) creating ecological space for secondary pathogens under stress conditions (Klinges et al., 2020). This hypothesis is particularly relevant given the concurrent presence of *Synechococcus* within the coral core microbiome at S3 during the cold season. The unexpected prevalence of *Synechococcus*, a taxon typically abundant in seawater, may reflect increased tissue permeability or a breakdown of host filtering mechanisms, potentially enabling the invasion of free-living cyanobacteria into coral-associated niches. Coral mucus is known to trap picocyanobacteria such as *Synechococcus*, concentrating them up to 40-fold (Naumann et al., 2010), especially under stress-induced changes in epithelial integrity. In addition,

non-heterocystous cyanobacteria, including *Synechococcus*-related lineages, have been detected in coral-associated diazotrophic communities and may contribute to nitrogen cycling within the holobiont (Moynihan et al., 2022). However, their proliferation under disturbed environmental conditions may also signal a breakdown in microbial homeostasis and impaired host regulation (Ziegler et al., 2017). Notably, this enrichment coincided with significantly elevated nitrite concentrations at site S3, suggesting that altered nitrogen cycling and nutrient enrichment may favour the proliferation of cyanobacteria or reflect broader disruptions of host–microbe interactions. The co-occurrence of *Vibrio* species further supports this interpretation, as these bacteria are well-known opportunistic coral pathogens that can become virulent under stress conditions, such as thermal anomalies and nutrient enrichment, by exploiting compromised coral hosts (Munn, 2015; McDevitt-Irwin et al., 2017).

Reduced diversity and *Vibrio*-associated dysbiosis in fish microbiota

Fish-associated communities exhibited an opposite seasonal trend compared to corals when considering the total number of ASVs identified at each site, particularly at site S3. During the cold season, site S1 hosted the highest number of ASVs ($n = 1,003$), followed by a substantial reduction at S2 ($n = 611$) and S3 ($n = 544$). This sharp decrease in richness at S3 contrasts with the simultaneous increase in coral microbial diversity observed at the same site and time, suggesting that environmental stress can affect host-associated microbiomes differently depending on the host. A comparable host-specific response was reported by Clerissi et al., (2025), who found that a marine heatwave altered the microbiota of sea anemones but had little effect on their associated clownfish microbiota. Together with the lower number of ASVs, a drastic reduction in taxonomic, and most likely functional, diversity with the microbiome being almost entirely monopolized by a single *Vibrio* species (ASV944) accounting for over 90% of the microbial community. Conversely, other genera typically constituting a more balanced core microbiota in fish, such as *Synechococcus*, *Turicibacter*, *Shewanella*, and *Photobacterium*, showed markedly reduced contributions at S3 fish samples at this time, with *Synechococcus* being entirely absent. A comparable pattern of reduced diversity and *Vibrio* dominance was observed in the stomach microbiota of the damselfish *Abudefduf vaigiensis*, along a 2000-km latitudinal gradient in a global warming hotspot in eastern Australia. The study revealed that at the fish's temperate cold-range edge, its stomach microbiome showed a 59% decrease in bacterial diversity and a 164% increase in *Vibrio* abundance compared to its tropical core range (Hayes et al., 2025). This observation aligns with our results and underscores that the observed differences in the microbiome composition of *D. emano* suggest dysbiosis to the environmental conditions at site S3 during the cold season.

While the cold season exhibited a clear north–south gradient in fish microbiome diversity and composition, this spatial structure largely disappeared during the warm season, giving way to more irregular and site-specific patterns. At site S1, the fish core microbiome was composed exclusively of *Vibrio* species, similar to the dominance observed at S3 during the cold season, although multiple *Vibrio* ASVs co-occurred with low-abundance non-core taxa. In contrast, site S2 exhibited a marked and significant decrease in core microbiome abundance, likely reflecting a dilution effect driven by the presence of a broader range of transient taxa.

While some *Vibrio* species can exist as commensals in healthy fish microbiomes, many are opportunistic pathogens that thrive under stress, either environmental or host-related (Egerton et al., 2018; Manchanayake et al., 2023). Their proliferation can disrupt intestinal function, suppress immune responses, and increase disease occurrence and mortality (Manchanayake et al., 2023). Environmental pressures at site S3, including nutrient loading, reduced water exchange, and cumulative aquaculture impact, correlate to the altered state of the fish microbiome observed at this site. This likely led to a filtering process favoring stress-tolerant or opportunistic bacteria like *Vibrio*, resulting in community homogenization. This simplification and skewing of the microbiota exemplify a dysbiotic shift, similar to patterns observed in other aquatic systems under chronic stress (Zaneveld et al., 2017).

Ecological implications and microbial indicators of reef degradation

Together, these observations highlight the impact of environmental disturbances on both coral and fish microbiota, leading to dysbiosis in both symbiotic organisms at site 3 during the cold season. In corals, the difference in microbial composition was marked by significant reduction of beneficial symbionts like *Endozoicomonas* (this observation was also done during the warm season) and the rise of opportunistic taxa such as *Vibrio*, which also became prevalent in the microbiome of associated symbiotic fish. This reflects a consistent response between the two species, indicating a destabilized microbiome that could undermine the health and resilience of these coral reef organisms (Glasl et al., 2019; MacKnight et al., 2021). These changes suggest site-specific ecological degradation, likely driven by cumulative anthropogenic pressures and disrupted hydrodynamic conditions, making it a focal point of microbiome destabilization within the Ahe lagoon at this time.

These findings also highlight the potential of microbial community analyses as sensitive indicators of reef health. Shifts in both taxonomic composition and core microbiota structure can provide early warning signals of environmental disturbance before visible impacts on macroorganisms occur. Notably, taxa such as *Endozoicomonas*, *Vibrio*, and MD3-55 may serve as microbial indicators of ecosystem status, offering valuable tools for reef biomonitoring and restoration. In particular, several *Endozoicomonas* ASV were less abundant in coral microbiota at site S3 for both cold and warm seasons. Furthermore, *Candidatus Aquarickettsia* MD3-55 has been previously proposed as a marker of disease resistance, with promising applications for non-invasive reef health assessments through surface or mucus sampling (Klinges et al., 2020; Aguirre et al., 2021). The consistent association between elevated nitrogen concentrations, altered microbiome structure, and host dysbiosis underscores the urgency of mitigating land-based inputs and aquaculture-derived nutrient enrichment in coral reef habitats. These results emphasize the importance of microbial surveillance, particularly in remote atolls such as Ahe, where conventional environmental monitoring remains limited. Integrating microbiome-based diagnostics with traditional ecological approaches could substantially improve early detection and management of emerging stressors in reef ecosystems.

Conclusions

This study highlights the complexity of microbial community dynamics in lagoon ecosystems exposed to both natural seasonal fluctuations and anthropogenic pressures, particularly those associated with pearl farming. We demonstrated that microbial diversity and composition are highly responsive to localized environmental conditions, especially at sites experiencing high nutrient enrichment and reduced water quality. Microbiomes of coral reef species exhibit contrasting and season-dependent responses to environmental stress. Coral-associated microbiomes showed structural shifts and changes in core composition, whereas fish-associated microbiomes displayed reduced diversity and *Vibrio* dominance under site-specific and seasonal stress conditions. In particular, coral-associated *Endozoicomonas* were less abundant at site S3 during both cold and warm seasons, whereas differences between sites for fish microbiota were mostly identified during the cold season. These findings reveal the potential of developing biomarkers based on *Endozoicomonas* abundances, but also that host-associated microbiomes do not respond uniformly to environmental gradients and that their responses vary substantially between species and seasons, highlighting the need for temporal replication before considering their potential use in environmental monitoring.

Methods

Study area and sample collection

The present study focused on the pearl farming lagoon of Ahe (14° 30' S, 146° 20' W; Fig. 1A) located in the Tuamotu archipelago (18° 47' S, 141° 35' W, French Polynesia), at the North-East of Tahiti Island (17° 40' S, 149° 28' W, French Polynesia). Ahe is a semi-enclosed lagoon environment with 12 km² of emerged land and 491 inhabitants. The lagoon (145 km²) is reached by a unique pass, which plays an important role in the water renewal, with about 34 days of residence time (Pagès et al., 2001). This study area was chosen because of its pearl farming history and activity, and marked by a low tourist activity, estimated at 4,500 people in transit per year. Specific areas are devoted to pearl farming activities (i.e. maritime concessions for pearl oyster rearing) with additional collecting stations authorized by the government. Collecting stations are composed of spat collectors placed in specific zones (mostly in the South) of the atoll corresponding to optimal locations for spat settlement, notably based on hydrodynamic conditions (Thomas et al., 2016). Authorized surface areas for pearl farming marine concessions in Ahe are 831 ha representing 5.6% of the total lagoon area with additional 1,536 collecting stations authorized (DRM, 2017).

Three sites were selected along the lagoon of Ahe (S1, S2 and S3; Fig. 1A) according to a pearl farming activity gradient ranging from low (S1) to high (S3). Sample collections were completed at each site in warm (May 15-17, 2019) and cold (September 14-16, 2019) season and consisted of seawater, fish (*Dascyllus emano*), and its symbiotic host coral (*Acropora millepora*) samples at each of the 3 sites in accordance with the collection regulations established by the DIREN of French Polynesia in 2019 (<https://www.service-public.pf/diren/>). A total of 12, 58, and 81 samples of seawater, coral, and fish,

respectively, were collected within an approximately 50 m² area at each of the three sampling sites. Coral and seawater samples were randomly collected within each area. Fish were captured in close proximity to their associated coral colony hosts, and seawater samples were collected adjacent to these colonies, ensuring spatial correspondence among sample types. Fish were weighed, measured for length, and had entire gut tissue dissected. All samples, including intact coral nubbins, were preserved in DNA/RNA Shield™ for subsequent DNA extraction. Preserved tissue samples were stored at -80 °C until DNA extractions. The depth at which the fish and corals were collected was recorded, and up to 5 L of surface seawater was collected in triplicate from each location. Seawater samples were filtered using a peristaltic pump onto a 0.22 µm pore-size filters (Isopore), filled with 2mL of DNA/RNA Shield™, and then stored at -80 °C until DNA extraction. Additional unfiltered samples of seawater were collected in light-proof 500 ml containers for nutrient analyses and stored at -20 °C until analysis of inorganic nutrients, including nitrite (NO₂⁻), nitrate (NO₃⁻), phosphate (PO₄²⁻), ammonium (NH₄⁺), and silicate (Si(OH)₄), using a Technicon AutoAnalyzer system (Southampton, United Kingdom). Environmental data for temperature, pH, DO, and salinity were measured *in situ* using a multi-parameter probe (WTW®) at each site during sampling. A summary table of the study site characteristics is given in the Supplementary Information file (**Table S1**).

Coral and fish gut DNA extraction

Due to different matrices sampled, different DNA extraction protocols were used. For fish and corals, total nucleic acids were extracted using TRIZOL Reagent (Life Technologies, USA) at a ratio of 1 ml per 100 mg of tissue. The samples were subjected to bead grinding using a Mixer Mill MM200 (Retsch™) for 5 min at 300 rpm and a frequency of 30 Hz. The supernatant was then transferred to a new microcentrifuge tube and the protocol continued according to the manufacturer's recommendations, using a high salt precipitation procedure (0.8 M sodium citrate and 1.2 M NaCl per 1 mL of TRIZOL reagent used for the homogenisation) to reduce proteoglycan and polysaccharide contamination. For seawater samples, filters were transferred to bead-beating tubes, and DNA was extracted using a Zymo Research environmental DNA extraction kit following the manufacturer's protocol, with mechanical lysis by bead beating. Excess DNA/RNA Shield was removed before lysis, while a small residual volume was retained with the filter to maximize DNA recovery.

Quantity, integrity, and purity of total DNA were validated by both NanoDrop readings (NanoDrop Technologies Inc., USA) and a 2100 Bioanalyzer system (Agilent Technologies, USA). DNA contaminants were removed using a DNase/RNase-free kit (Ambion™). DNA samples were dried in DNA-stable solution (Thermo Fisher Scientific, USA) following manufacturer's recommendations.

Libraries preparation and sequencing

In order to obtain a comprehensive representation of the free-living bacteria in seawater and the microbial communities associated with coral and fish guts, a broad-range amplification of the 16S DNA

gene was performed using PCR. Amplicons of the 16S DNA gene were prepared using the variable V3V4 loops (341F: 5'CCTACGGGNGGCWGCAG-3'; 805R: 5'-GACTACHVGGGTATCTAATCC-3'; Klindworth et al., 2013). The PCR mixtures (25 µl final volume) contained 10 ng of total DNA template or 10 µl of water, with 0.4 µM final concentration of each primer, 3% DMSO and 1X Phusion Master Mix (Thermo Fisher Scientific, Waltham, MA, USA). PCR amplifications (98°C for 2 min; 30 cycles of 30 s at 98°C, 30 s at 55°C, 1 min at 72°C; and 72°C for 10 min) of all samples were carried out in triplicate in order to smooth out intra-sample variance. Triplicates of PCR products were pooled and purified by 1x AMPure XP beads (Beckmann Coulter Genomics) clean-up. Amplicon lengths were measured on an Agilent Bioanalyzer using the DNA High Sensitivity LabChip kit, then quantified with a Qubit Fluorometer. Amplicon libraries were multiplexed and sequenced on a single lane of a MiSeq 250bp PE Illumina machine at the Genome Québec McGill platform (Montreal, Canada).

Sequence assembly, quality control and taxonomic assignment

Bioinformatic processing was conducted with the **SAMBA v4.0.0** workflow (<https://gitlab.ifremer.fr/bioinfo/workflows/samba>). Raw data integrity was first verified, and primers were trimmed with **QIIME 2 v2022.11.1** and **Cutadapt v4.2** (error rate = 0.1); reads without primers were discarded. **DADA2 v1.26.0** was then used to infer ASVs (trunQ = 2, FmaxEE = 2, RmaxEE = 2, n_read_learn = 1,000,000), applying independent denoising, merging without primer removal, and consensus-based chimera detection. Optimal DADA2 parametrization was performed with Figaro v1.1.2 (Weinstein et al., 2019). To minimize false positives, ASVs were clustered with **dbOTU3 v1.5.3** (genetic criterion = 0.1, abundance = 10, P = 0.0005). Taxonomy was assigned via a Naïve Bayesian classifier trained on **SILVA v138.2** (Quast et al., 2013).

Contaminant filtering was then applied using negative controls ($n = 4$, including PCR blanks) following a prevalence/enrichment strategy adapted from Davis et al. (2018). After restricting the dataset to bacterial ASVs, taxa were removed when both criteria were met: (i) prevalence in blanks $\geq 30\%$ and (ii) mean relative abundance in blanks at least twofold higher than in non-blank samples. This procedure retained taxa unlikely to represent reagent/background contamination while preserving biologically informative ASVs.

Analysis of shared and unique ASVs

To assess ASV overlap among sample types (seawater, coral, and fish) and, within each sample type, among sites (S1, S2, S3) across seasons (cold and warm), Venn diagrams were generated in R using phyloseq and ggVennDiagram. ASV tables were transformed to relative abundance, and ASV detection was defined using a minimum threshold of 0.001 (0.1% relative abundance per sample). For each group (sample type, or site $\times$ season within sample type), ASV sets were built from taxa detected at or above this threshold, and overlaps were visualized as counts and percentages of shared versus unique ASVs.

Diversity analysis of microbial communities

We assessed α - and β -diversity using the phyloseq and vegan R packages (McMurdie and Holmes, 2013; Oksanen et al., 2024). For α -diversity, we calculated observed richness (number of ASVs), Shannon diversity, and Pielou's evenness ($J = H'/\log(S)$, with H' the Shannon diversity index and S the observed richness in number of ASVs). Observed richness and presence/absence summaries were computed on rarefied counts to ensure comparable sampling depth across samples: we rarefied to the minimum sequencing depth observed across non-blank samples (35,120 reads per sample). Shannon diversity and Pielou's evenness were computed on non-rarefied data using sample-wise normalized abundances (relative abundances) to avoid information loss from rarefaction (Fig. S2). Differences were tested either (i) among sample types (seawater, coral, fish) or (ii) within sample type as a function of site, season, and site $\times$ season interaction. Model choice was based on residual normality: ANOVA was used when assumptions were met, and permutation-based ANOVA (pANOVA) was used otherwise. For seawater, because of limited replication per site $\times$ season cell, effects were tested separately by season (sites pooled) and by site (seasons pooled). Post-hoc comparisons used Tukey's HSD for parametric models and Wilcoxon pairwise tests with BH correction for non-parametric cases. Significance threshold was set at $P < 0.05$. For β -diversity, Bray-Curtis dissimilarities were computed from relative-abundance ASV tables, and ordinations were visualized using NMDS (metaMDS, vegan package). Homogeneity of multivariate dispersion was evaluated with betadisper followed by permutation testing (permutest). When dispersion homogeneity was acceptable, community composition effects were tested with PERMANOVA (adonis2) with marginal tests (`by="margin"`). When dispersion heterogeneity was significant, ANOSIM was additionally used to test factor-specific group separation. Pairwise dispersion differences were evaluated from distances to centroid using post-hoc pairwise tests (Wilcoxon with BH correction). All permutation-based analyses were run with 999 permutations, and results were considered significant at $P < 0.05$.

Core bacterial community analysis

Core microbiome analysis was performed in R using a custom prevalence/detection workflow on relative-abundance ASV tables. For each sample type (seawater, coral, fish) and each site $\times$ season group, core ASVs were defined as taxa with relative abundance $\geq 1\%$ in at least 80% of samples within that group. Core ASVs were then mapped to genus-level taxonomy to summarize core composition across groups.

For each sample, total core abundance was calculated as the sum of relative abundances of core ASVs defined for its corresponding site $\times$ season group, and group-level summaries were reported as mean $\pm$ 95% CI. Differences in core abundance were tested using two-way ANOVA (Season, Site, and Season $\times$ Site) for coral and fish. For seawater, because of limited replication per site $\times$ season cell, effects were tested with Kruskal–Wallis tests after pooling by season or by site. Pairwise post-hoc comparisons for coral and fish were performed using Tukey's HSD. Statistical significance was set at $P < 0.05$.

Environmental statistical analysis

Environmental statistical analyses were carried out in R (v.4.1.2) to characterize and relate site-specific physico-chemical parameters. After data import and cleaning, a principal component analysis (PCA) was performed on the scaled variables using *'prcomp()'* from the *factoextra* package (Kassambara and Mundt, 2020). Variable coordinates and contributions were extracted with *'get_pca_var()'* and percentage of variance explained per component (dimension) was visualized via *'fviz_eig()'*. A correlation circle of variable loadings was generated using *'corrplot()'* and the contributions of the eight variables to the first two dimensions were plotted with *'fviz_contrib()'*. The PCA was then plotted using *'autoplot()'* enabled by *ggfortify* package (Tang et al., 2016). Host–environment relationships were explored by first assessing the normality of site latitude and each environmental parameter with Shapiro–Wilk tests. Pearson correlation coefficients between latitude and all variables were subsequently calculated, revealing a significant negative correlation with nitrite concentration (NO₂). Finally, the latitude–NO₂ association was illustrated with scatterplots and linear regression fits using *'ggscatter()'* from *ggpubr* package (Kassambara, 2025).

Declarations

Availability of data and material

The sequencing data relevant to this publication is available at ENA under BioProject ID PRJEB125596.

Competing interests

Authors declare no competing interests.

Funding

This work was supported by MITI CNRS PLASTICOR (collaboration CNRS/IFREMER/EPHE).

Authors' contributions

DL, CC and JLL conceived the study. CC, CG, JLL, OB and TG conducted the sampling. CB, CG process the DNA and water samples, respectively. CC, JLL and TG analysed the data and drafted the paper. All authors contribute to data acquisition and manuscript revision.

Acknowledgements

We first warmly thank pension Raita for providing accommodation and boat transport logistics during sampling.

References

1. Aguirre, E., Million, W., Bartels, E., Krediet, C., Kenkel, C., 2021. Host-specific epibiomes of distinct *Acropora cervicornis* genotypes persist after field transplantation. <https://doi.org/10.1101/2021.06.25.449961>
2. Bellwood, D.R., Hughes, T.P., Folke, C., Nyström, M., 2004. Confronting the coral reef crisis. *Nature* 429, 827–833. <https://doi.org/10.1038/nature02691>
3. Bian, W., Zeng, Y., Li, Y., Na, G., Mu, J., Lv, S., Liu, M., 2024. Microplastic pollution in tropical coral reef ecosystems from the coastal South China Sea and their impacts on corals *in situ*. *J. Hazard. Mater.* 480, 135898. <https://doi.org/10.1016/j.jhazmat.2024.135898>
4. Bongaerts, P., Riginos, C., Brunner, R., Englebert, N., Smith, S.R., Hoegh-Guldberg, O., 2017. Deep reefs are not universal refuges: Reseeding potential varies among coral species. *Sci. Adv.* 3, e1602373. <https://doi.org/10.1126/sciadv.1602373>
5. Bourne, D.G., Morrow, K.M., Webster, N.S., 2016. Insights into the Coral Microbiome: Underpinning the Health and Resilience of Reef Ecosystems. *Annu. Rev. Microbiol.* 70, 317–340. <https://doi.org/10.1146/annurev-micro-102215-095440>
6. Brandl, S.J., Rasher, D.B., Côté, I.M., Casey, J.M., Darling, E.S., Lefcheck, J.S., Duffy, J.E., 2019. Coral reef ecosystem functioning: eight core processes and the role of biodiversity. *Front. Ecol. Environ.* 17, 445–454. <https://doi.org/10.1002/fee.2088>
7. Burkepile, D.E., Shantz, A.A., Adam, T.C., Munsterman, K.S., Speare, K.E., Ladd, M.C., Rice, M.M., Ezzat, L., McIlroy, S., Wong, J.C.Y., Baker, D.M., Brooks, A.J., Schmitt, R.J., Holbrook, S.J., 2020. Nitrogen Identity Drives Differential Impacts of Nutrients on Coral Bleaching and Mortality. *Ecosystems* 23, 798–811. <https://doi.org/10.1007/s10021-019-00433-2>
8. Clerissi, C., Beldade, R., Mejait, A., Haguenauer, A., Jobet, E., Titus, B.M., Mills, S.C., 2026. Microbiota of host sea anemones and their associated anemonefish show contrasting responses to a bleaching event. *Coral Reefs* 45, 817–830. <https://doi.org/10.1007/s00338-025-02799-3>
9. Crusot, M., Gaertner, J.-C., Rodriguez, T., Lo, C., Gaertner-Mazouni, N., 2023. Assessment of plastic waste generated by the aquaculture industry: The case study of pearl farming in French Polynesia. *J. Clean. Prod.* 425, 138902. <https://doi.org/10.1016/j.jclepro.2023.138902>
10. Davis, N.M., Proctor, D.M., Holmes, S.P., Relman, D.A., Callahan, B.J., 2018. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome* 6, 226. <https://doi.org/10.1186/s40168-018-0605-2>
11. Egerton, S., Culloty, S., Whooley, J., Stanton, C., Ross, R.P., 2018. The Gut Microbiota of Marine Fish. *Front. Microbiol.* 9. <https://doi.org/10.3389/fmicb.2018.00873>
12. Ellis, J.I., Jamil, T., Anlauf, H., Coker, D.J., Curdia, J., Hewitt, J., Jones, B.H., Krokos, G., Kürten, B., Hariprasad, D., Roth, F., Carvalho, S., Hoteit, I., 2019. Multiple stressor effects on coral reef ecosystems. *Glob. Change Biol.* 25, 4131–4146. <https://doi.org/10.1111/gcb.14819>

13. Émie, A.-G., François-Étienne, S., Sidki, B., Nicolas, D., 2021. Microbiomes of clownfish and their symbiotic host anemone converge before their first physical contact. *Microbiome* 9, 109. <https://doi.org/10.1186/s40168-021-01058-1>
14. Gardon, T., Dromard, C.R., Gaertner, J.C., Gaertner-Mazouni, N., 2025. Vulnerability of Coral Reefs to Contaminants of Emerging Concern, in: Planes, S. (Ed.), *The Future of Coral Reefs: Evidence from Research*. Springer Nature Switzerland, Cham, pp. 103–122. https://doi.org/10.1007/978-3-031-98584-3_6
15. Gardon, T., El Rakwe, M., Paul-Pont, I., Le Luyer, J., Thomas, L., Prado, E., Boukerma, K., Cassone, A.-L., Quillien, V., Soye, C., Costes, L., Crusot, M., Dreanno, C., Le Moullac, G., Huvet, A., 2021. Microplastics contamination in pearl-farming lagoons of French Polynesia. *J. Hazard. Mater.* 419, 126396. <https://doi.org/10.1016/j.jhazmat.2021.126396>
16. Gardon, T., Huvet, A., Paul-Pont, I., Cassone, A.-L., Sham Koua, M., Soye, C., Jezequel, R., Receveur, J., Le Moullac, G., 2020. Toxic effects of leachates from plastic pearl-farming gear on embryo-larval development in the pearl oyster *Pinctada margaritifera*. *Water Res.* 179, 115890. <https://doi.org/10.1016/j.watres.2020.115890>
17. Glasl, B., Bourne, D.G., Frade, P.R., Thomas, T., Schaffelke, B., Webster, N.S., 2019. Microbial indicators of environmental perturbations in coral reef ecosystems. *Microbiome* 7, 94. <https://doi.org/10.1186/s40168-019-0705-7>
18. Hayes, C., Mitchell, A., Huerlimann, R., Jolly, J., Li, C., Booth, D.J., Ravasi, T., Nagelkerken, I., 2025. Stomach Microbiome Simplification of a Coral Reef Fish at Its Novel Cold-Range Edge Under Climate Change. *Mol. Ecol.* 34, e17704. <https://doi.org/10.1111/mec.17704>
19. Highton, M.P., Roosa, S., Crawshaw, J., Schallenberg, M., Morales, S.E., 2016. Physical Factors Correlate to Microbial Community Structure and Nitrogen Cycling Gene Abundance in a Nitrate Fed Eutrophic Lagoon. *Front. Microbiol.* 7. <https://doi.org/10.3389/fmicb.2016.01691>
20. Huang, Q., Sham, R.C., Deng, Y., Mao, Y., Wang, C., Zhang, T., Leung, K.M.Y., 2020. Diversity of gut microbiomes in marine fishes is shaped by host-related factors. *Mol. Ecol.* 29, 5019–5034. <https://doi.org/10.1111/mec.15699>
21. Hughes, T.P., Barnes, M.L., Bellwood, D.R., Cinner, J.E., Cumming, G.S., Jackson, J.B.C., Kleypas, J., van de Leemput, I.A., Lough, J.M., Morrison, T.H., Palumbi, S.R., van Nes, E.H., Scheffer, M., 2017. Coral reefs in the Anthropocene. *Nature* 546, 82–90. <https://doi.org/10.1038/nature22901>
22. Kassambara, A., 2025. ggpubr: “ggplot2” Based Publication Ready Plots.
23. Kassambara, A., Mundt, F., 2020. Factoextra: Extract and Visualize the Results of Multivariate Data Analyses. R Package Version 1.0.7 [WWW Document]. URL <https://CRAN.R-project.org/package=factoextra> (accessed 7.18.25).
24. Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* 41, e1. <https://doi.org/10.1093/nar/gks808>

25. Klinges, G., Maher, R.L., Vega Thurber, R.L., Muller, E.M., 2020. Parasitic 'Candidatus Aquarickettsia rohweri' is a marker of disease susceptibility in *Acropora cervicornis* but is lost during thermal stress. *Environ. Microbiol.* 22, 5341–5355. <https://doi.org/10.1111/1462-2920.15245>
26. Lima, L.F.O., Alker, A.T., Papudeshi, B., Morris, M.M., Edwards, R.A., de Putron, S.J., Dinsdale, E.A., 2023. Coral and Seawater Metagenomes Reveal Key Microbial Functions to Coral Health and Ecosystem Functioning Shaped at Reef Scale. *Microb. Ecol.* 86, 392–407. <https://doi.org/10.1007/s00248-022-02094-6>
27. MacKnight, N.J., Cobleigh, K., Lasseigne, D., Chaves-Fonnegra, A., Gutting, A., Dimos, B., Antoine, J., Fuess, L., Ricci, C., Butler, C., Muller, E.M., Mydlarz, L.D., Brandt, M., 2021. Microbial dysbiosis reflects disease resistance in diverse coral species. *Commun. Biol.* 4, 679. <https://doi.org/10.1038/s42003-021-02163-5>
28. Maher, R.L., Rice, M.M., McMinds, R., Burkepile, D.E., Vega Thurber, R., 2019. Multiple stressors interact primarily through antagonism to drive changes in the coral microbiome. *Sci. Rep.* 9, 6834. <https://doi.org/10.1038/s41598-019-43274-8>
29. Manchanayake, T., Salleh, A., Amal, M.N.A., Yasin, I.S.M., Zamri-Saad, M., 2023. Pathology and pathogenesis of *Vibrio* infection in fish: A review. *Aquac. Rep.* 28, 101459. <https://doi.org/10.1016/j.aqrep.2022.101459>
30. McDevitt-Irwin, J.M., Baum, J.K., Garren, M., Vega Thurber, R.L., 2017. Responses of Coral-Associated Bacterial Communities to Local and Global Stressors. *Front. Mar. Sci.* 4. <https://doi.org/10.3389/fmars.2017.00262>
31. McMurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PloS One* 8, e61217. <https://doi.org/10.1371/journal.pone.0061217>
32. Medina-Félix, D., Garibay-Valdez, E., Vargas-Albores, F., Martínez-Porchas, M., 2023. Fish disease and intestinal microbiota: A close and indivisible relationship. *Rev. Aquac.* 15, 820–839. <https://doi.org/10.1111/raq.12762>
33. Moynihan, M.A., Goodkin, N.F., Morgan, K.M., Kho, P.Y.Y., Lopes dos Santos, A., Lauro, F.M., Baker, D.M., Martin, P., 2022. Coral-associated nitrogen fixation rates and diazotrophic diversity on a nutrient-replete equatorial reef. *ISME J.* 16, 233–246. <https://doi.org/10.1038/s41396-021-01054-1>
34. Munn, C.B., 2015. The Role of Vibrios in Diseases of Corals. *Microbiol. Spectr.* 3. <https://doi.org/10.1128/microbiolspec.VE-0006-2014>
35. Naumann, M.S., Haas, A., Struck, U., Mayr, C., el-Zibdah, M., Wild, C., 2010. Organic matter release by dominant hermatypic corals of the Northern Red Sea. *Coral Reefs* 29, 649–659. <https://doi.org/10.1007/s00338-010-0612-7>
36. Neave, M.J., Apprill, A., Ferrier-Pagès, C., Voolstra, C.R., 2016. Diversity and function of prevalent symbiotic marine bacteria in the genus *Endozoicomonas*. *Appl. Microbiol. Biotechnol.* 100, 8315–8324. <https://doi.org/10.1007/s00253-016-7777-0>

37. Nemergut, D.R., Schmidt, S.K., Fukami, T., O'Neill, S.P., Bilinski, T.M., Stanish, L.F., Knelman, J.E., Darcy, J.L., Lynch, R.C., Wickey, P., Ferrenberg, S., 2013. Patterns and Processes of Microbial Community Assembly. *Microbiol. Mol. Biol. Rev. MMBR* 77, 342–356.
<https://doi.org/10.1128/MMBR.00051-12>
38. Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Caceres, M.D., Durand, S., Evangelista, H.B.A., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M.O., Lahti, L., McGlinn, D., Ouellette, M.-H., Cunha, E.R., Smith, T., Stier, A., Braak, C.J.F.T., Weedon, J., 2024. *vegan: Community Ecology Package*.
39. Pagès, J., Andrefouët, S., Delesalle, B., Prasil, V., 2001. Hydrology and trophic state in Takapoto Atoll lagoon: comparison with other Tuamotu lagoons. *Aquat. Living Resour.*, Pearl oysters in French Polynesia 14, 183–193. [https://doi.org/10.1016/S0990-7440\(01\)01113-5](https://doi.org/10.1016/S0990-7440(01)01113-5)
40. Partensky, F., Hess, W.R., Vaulot, D., 1999. *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. *Microbiol. Mol. Biol. Rev. MMBR* 63, 106–127.
<https://doi.org/10.1128/MMBR.63.1.106-127.1999>
41. Peixoto, R.S., Rosado, P.M., Leite, D.C. de A., Rosado, A.S., Bourne, D.G., 2017. Beneficial Microorganisms for Corals (BMC): Proposed Mechanisms for Coral Health and Resilience. *Front. Microbiol.* 8. <https://doi.org/10.3389/fmicb.2017.00341>
42. Perry, C.T., Alvarez-Filip, L., 2019. Changing geo-ecological functions of coral reefs in the Anthropocene. *Funct. Ecol.* 33, 976–988. <https://doi.org/10.1111/1365-2435.13247>
43. Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41, D590-596. <https://doi.org/10.1093/nar/gks1219>
44. Rice, M.M., Maher, R.L., Thurber, R.V., Burkepile, D.E., 2019. Different nitrogen sources speed recovery from corallivory and uniquely alter the microbiome of a reef-building coral. *PeerJ* 7, e8056.
<https://doi.org/10.7717/peerj.8056>
45. Rodier, M., Pinazo, C., Seceh, C., Varillon, D., 2021. Pelagic stocks and carbon and nitrogen uptake in a pearl farming atoll (Ahe, French Polynesia). *Mar. Pollut. Bull.* 167, 112352.
<https://doi.org/10.1016/j.marpolbul.2021.112352>
46. Santoro, E.P., Cárdenas, A., Villela, H.D.M., Vilela, C.L.S., Ghizelini, A.M., Duarte, G.A.S., Perna, G., Saraiva, J.P., Thomas, T., Voolstra, C.R., Peixoto, R.S., 2025. Inherent differential microbial assemblages and functions associated with corals exhibiting different thermal phenotypes. *Sci. Adv.* 11, eadq2583. <https://doi.org/10.1126/sciadv.adq2583>
47. Seeley, M.E., Song, B., Passie, R., Hale, R.C., 2020. Microplastics affect sedimentary microbial communities and nitrogen cycling. *Nat. Commun.* 11, 2372. <https://doi.org/10.1038/s41467-020-16235-3>
48. Stevick, R.J., Post, A.F., Gómez-Chiarri, M., 2021. Functional plasticity in oyster gut microbiomes along a eutrophication gradient in an urbanized estuary. *Anim. Microbiome* 3, 5.

<https://doi.org/10.1186/s42523-020-00066-0>

49. Sylvain, F.-É., Holland, A., Bouslama, S., Audet-Gilbert, É., Lavoie, C., Val, A.L., Derome, N., 2020. Fish Skin and Gut Microbiomes Show Contrasting Signatures of Host Species and Habitat. *Appl. Environ. Microbiol.* 86, e00789-20. <https://doi.org/10.1128/AEM.00789-20>
50. Tang, J., Wu, Z., Wan, L., Cai, W., Chen, S., Wang, X., Luo, J., Zhou, Z., Zhao, J., Lin, S., 2021. Differential enrichment and physiological impacts of ingested microplastics in scleractinian corals in situ. *J. Hazard. Mater.* 404, 124205. <https://doi.org/10.1016/j.jhazmat.2020.124205>
51. Tang, Y., Horikoshi, M., Li, W., 2016. ggfortify: Unified Interface to Visualize Statistical Results of Popular R Packages. *R J.* 8, 474–485.
52. Thomas, Y., Dumas, F., Andréfouët, S., 2016. Larval connectivity of pearl oyster through biophysical modelling; evidence of food limitation and broodstock effect. *Estuar. Coast. Shelf Sci., St:Sustainable pearl-culture* 182, 283–293. <https://doi.org/10.1016/j.ecss.2016.03.010>
53. Webster, N.S., Reusch, T.B.H., 2017. Microbial contributions to the persistence of coral reefs. *ISME J.* 11, 2167–2174. <https://doi.org/10.1038/ismej.2017.66>
54. Weinstein, M.M., Prem, A., Jin, M., Tang, S., Bhasin, J.M., 2019. FIGARO: An efficient and objective tool for optimizing microbiome rRNA gene trimming parameters. <https://doi.org/10.1101/610394>
55. Woodhead, A.J., Hicks, C.C., Norström, A.V., Williams, G.J., Graham, N.A.J., 2019. Coral reef ecosystem services in the Anthropocene. *Funct. Ecol.* 33, 1023–1034. <https://doi.org/10.1111/1365-2435.13331>
56. Wright, R.J., Erni-Cassola, G., Zadjelovic, V., Latva, M., Christie-Oleza, J.A., 2020. Marine Plastic Debris: A New Surface for Microbial Colonization. *Environ. Sci. Technol.* 54, 11657–11672. <https://doi.org/10.1021/acs.est.0c02305>
57. Zaneveld, J.R., Burkepile, D.E., Shantz, A.A., Pritchard, C.E., McMinds, R., Payet, J.P., Welsh, R., Correa, A.M.S., Lemoine, N.P., Rosales, S., Fuchs, C., Maynard, J.A., Thurber, R.V., 2016. Overfishing and nutrient pollution interact with temperature to disrupt coral reefs down to microbial scales. *Nat. Commun.* 7, 11833. <https://doi.org/10.1038/ncomms11833>
58. Zaneveld, J.R., McMinds, R., Vega Thurber, R., 2017. Stress and stability: applying the Anna Karenina principle to animal microbiomes. *Nat. Microbiol.* 2, 17121. <https://doi.org/10.1038/nmicrobiol.2017.121>
59. Zhu, W., Liu, X., Zhang, J., Zhao, H., Li, Z., Wang, H., Chen, R., Wang, A., Li, X., 2023. Response of coral bacterial composition and function to water quality variations under anthropogenic influence. *Sci. Total Environ.* 884, 163837. <https://doi.org/10.1016/j.scitotenv.2023.163837>
60. Ziegler, M., Seneca, F.O., Yum, L.K., Palumbi, S.R., Voolstra, C.R., 2017. Bacterial community dynamics are linked to patterns of coral heat tolerance. *Nat. Commun.* 8, 14213. <https://doi.org/10.1038/ncomms14213>

Figures

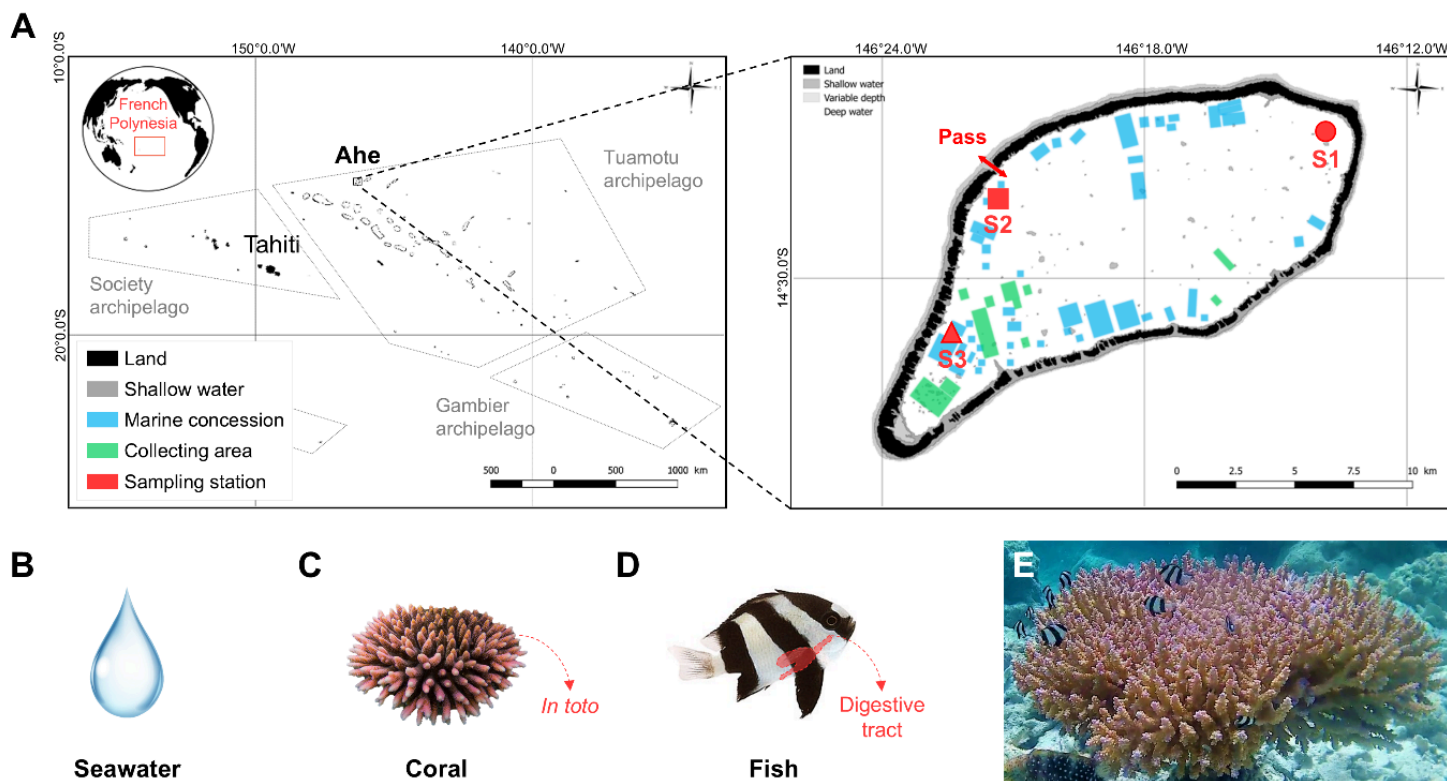


Figure 1

Biological samples collected in the pearl farming lagoon of Ahe, French Polynesia. (A) Map of the study area showing pearl farming concessions and the three sampling locations (S1, S2, and S3) within the Ahe atoll lagoon, located in the Tuamotu archipelago of French Polynesia. A previous study of microplastic (MP) contamination in Ahe lagoon reported an average concentration of $82.6 \pm 67.6 \text{ MP m}^{-3}$ (mean $\pm$ standard deviation) in the water column, with site S3 reaching the highest levels ($\sim 150 \text{ MP m}^{-3}$) compared to sites S1 and S2 ($\sim 20\text{--}40 \text{ MP m}^{-3}$) (Gardon et al., 2021). Colours indicate land (black), shallow water (grey), pearl farming marine concessions (blue; leased zones equipped with rafts and longlines for rearing pearl oysters), spat-collecting areas (green; submerged collectors deployed to capture settling oyster larvae), and sampling locations (red). Schematics of the three sample types collected at each site are shown: (B) seawater, (C) the coral *Acropora millepora* (whole colony), (D) the fish *Dascyllus emano* (digestive tract), and (E) a photograph illustrating the symbiotic relationship between both marine species.

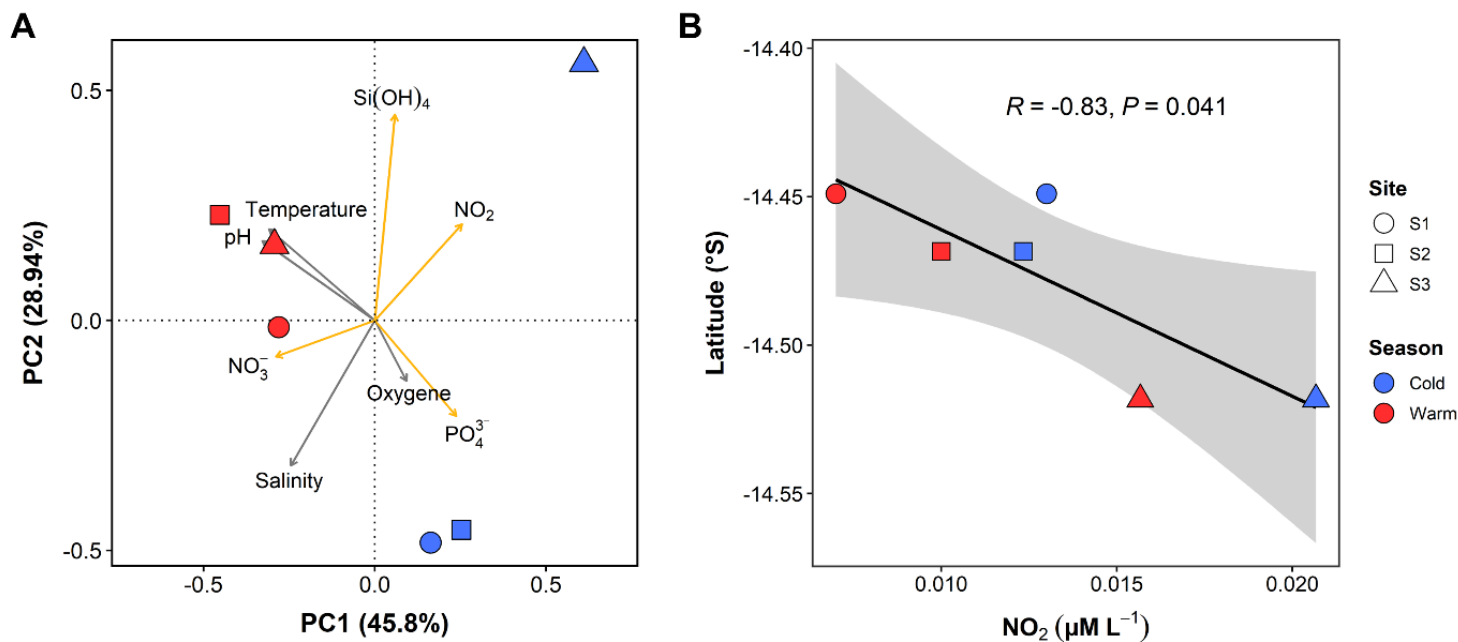


Figure 2

Environmental conditions characterizing each study site across seasons. (A) Principal component analysis of environmental variables. Each site is represented by a coloured symbol, and each environmental variable by an arrow. Grey arrows indicate *in situ* measurements taken during sampling, while yellow arrows represent average nutrient concentrations from seawater samples ($n = 3$). (B) Scatter plot of study sites across seasons, showing a significant Pearson correlation ($R = -0.83$, $P = 0.041$) between latitude (from north to south) and measured nitrite (NO_2) concentrations.

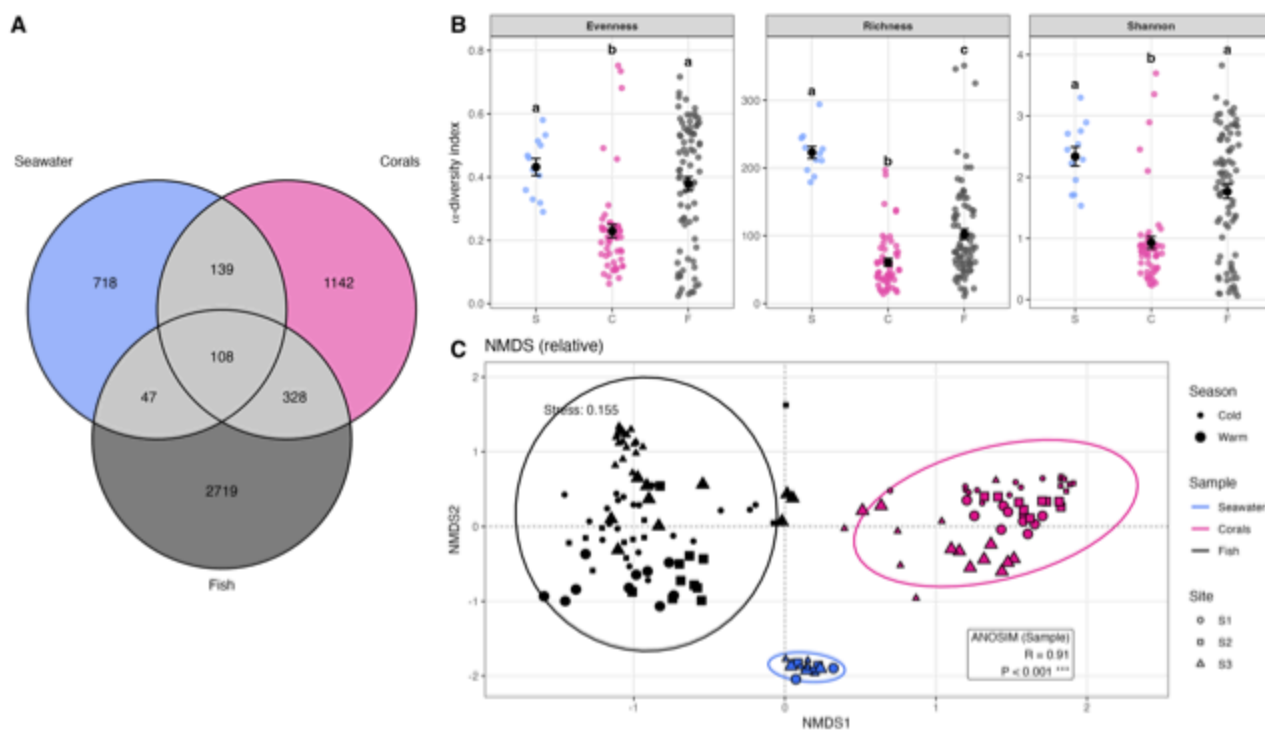


Figure 3

Effect of sample type on diversity indices in bacterial community structure. (A) Venn diagram showing the number of shared ASVs between sample types (detection threshold of $\geq 0.1\%$ per sample). (B) Dot plots of α -diversity indices (observed richness, Shannon index, and evenness) across all samples by type ($12 \leq n \leq 81$), calculated at the ASV level. Each point represents an individual sample, with horizontal bars indicating the mean $\pm$ 95% confidence interval (CI). Letters indicate significant differences between groups based on post-hoc permutation test. S: seawater, C: coral, F: fish. (C) Non-metric multidimensional scaling (NMDS) plot based on Bray-Curtis β -diversity ($k=2$) at the ASV level, grouped by sample type across sites ($1 \leq n \leq 20$) and seasons ($4 \leq n \leq 49$). The ellipse lines show the 95% CI for each sample type. The R and P -values displayed inside the black box indicate the significance of group (sample types) differences, as assessed by the one-way non-parametric analysis of similarity (ANOSIM) test.

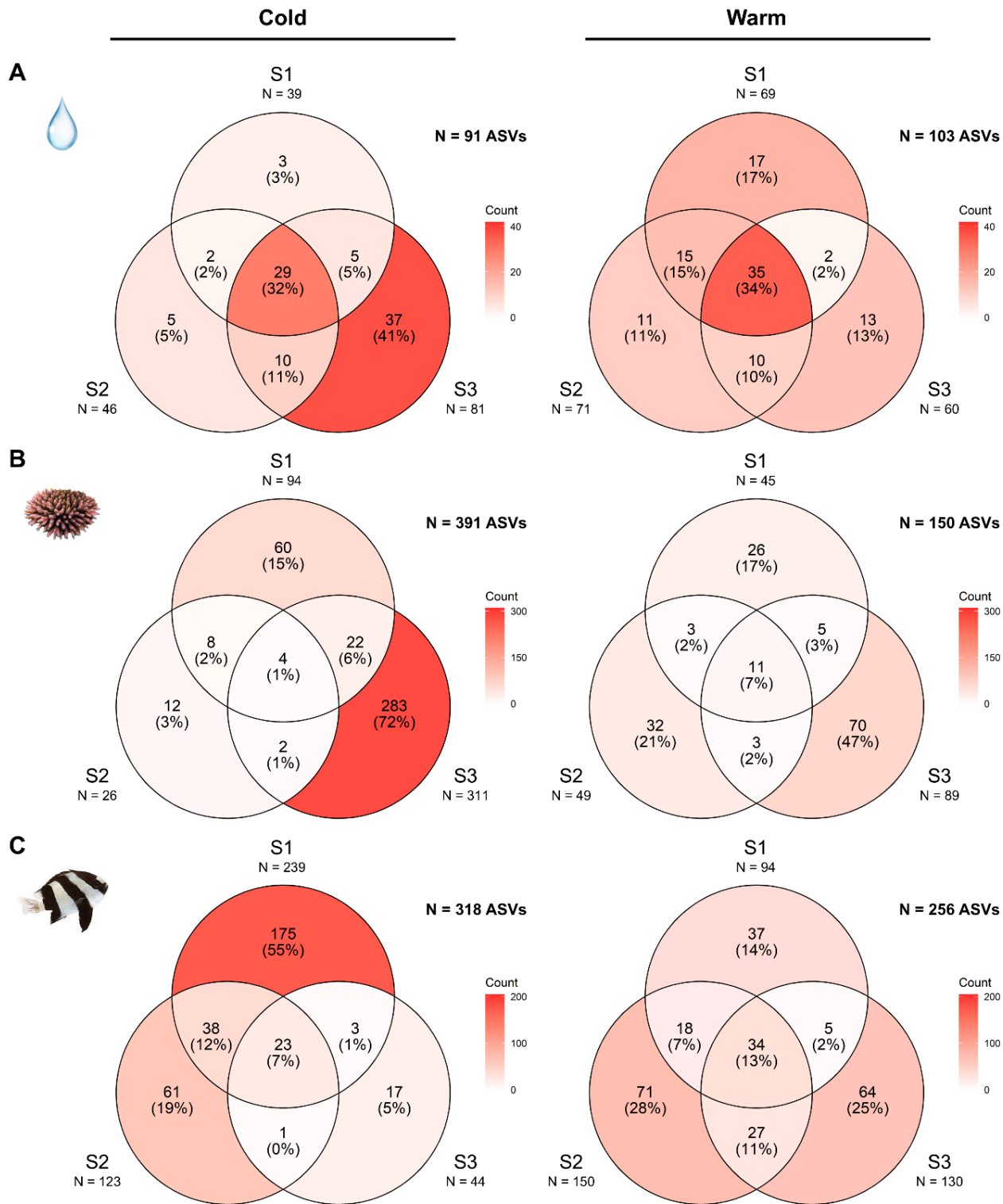


Figure 4

Shared ASVs between study sites across seasons. Venn diagrams showing the number and relative abundance of ASVs (detection threshold of $\geq 0.1\%$ per sample) that are unique to or shared between study sites during the cold (left) and warm (right) seasons in **(A)** seawater, **(B)** coral, and **(C)** fish samples.

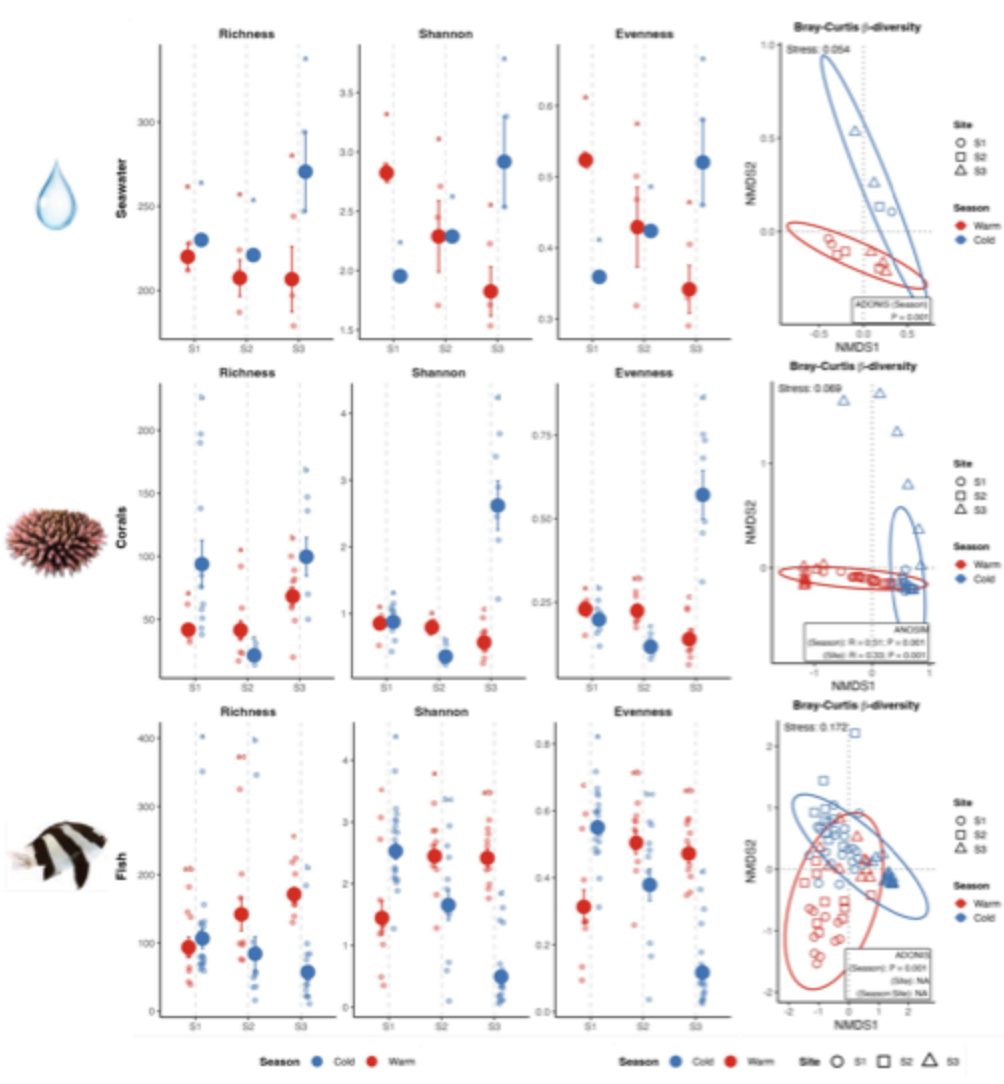


Figure 5

Effect of season and site on diversity indices in bacterial community structures across sample types. Dot plots of α -diversity indices (observed richness, Shannon index, and evenness) in seawater ($n = 12$), coral ($n = 52$), and fish ($n = 81$) samples, shown by season (warm, S1; cold, S2) within sites (S1, S2, S3). Small points are individual samples; large points are group means with error bars (mean $\pm$ SE). Letters indicate significant pairwise differences among site $\times$ season groups based on post-hoc Tukey HSD or Wilcoxon tests (BH-adjusted), depending on model assumptions. (B) NMDS ordination of Bray-Curtis β -diversity at ASV level ($k = 2$), colored by season and shaped by site; ellipses show 95% confidence regions for seasons. Insets report group-difference tests: PERMANOVA (adonis2) for seawater (season effect) and fish (season, site, and season $\times$ site), and ANOSIM for corals (season and site), used when dispersion assumptions were not met.

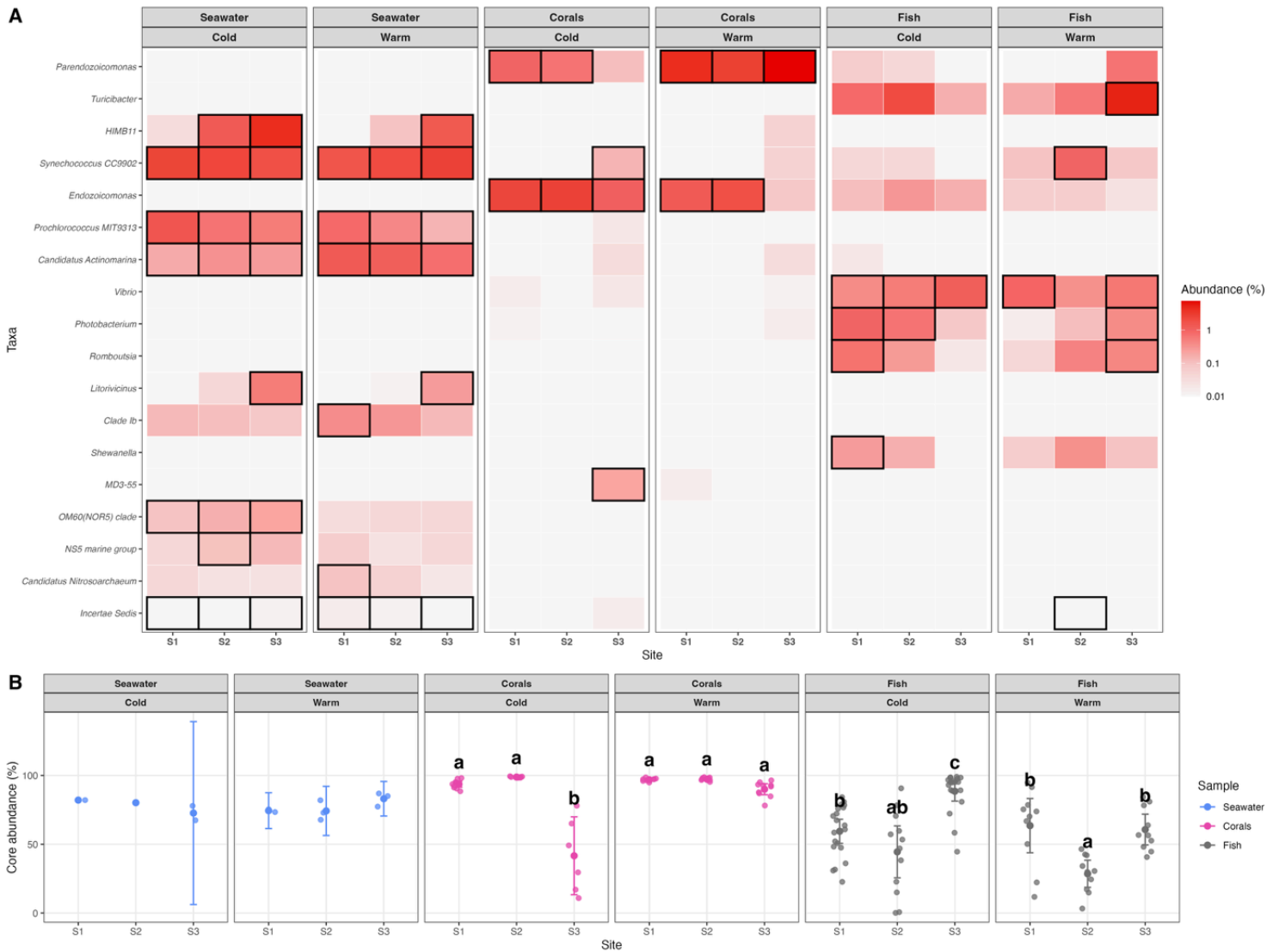


Figure 6

Core microbiome composition and relative abundance among sample groups within each sample type.

(A) Heatmap of the core bacterial community agglomerated at the genus level, based on 33 ASVs identified as core members ($\geq 1\%$ relative abundance in at least 80% of samples) within site $\times$ season groups for each sample type (seawater, coral, and fish). Black frames delineate the composition of the core microbiome for each group. **(B)** Dot plots showing the relative abundance of the core microbiome across sample groups within each sample type. Horizontal bars represent the mean $\pm$ 95% CI. Different letters indicate significant differences between groups based on post hoc pairwise comparisons using Tukey's HSD test.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SIGardonetal.Cor.Reefs.docx](#)

- [image1.png](#)