Microbial predictors of environmental perturbations in coral reef ecosystems

Bettina Glasl [a,b,c#], David G. Bourne [a,b,c], Pedro R. Frade [d], Torsten Thomas [e], Britta Schaffelke[a] and Nicole S. Webster [a,c,f]

[a] Australian Institute of Marine Science, Townsville, Qld, Australia

[b] College of Science and Engineering, James Cook University, Townsville, Qld, Australia

[c] AIMS@JCU, Townsville, Qld, Australia

[d] Centre of Marine Science, University of Algarve, Faro, Portugal

[e] Centre for Marine Bio-Innovation & School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, Australia

[f] Australian Centre for Ecogenomics, University of Queensland, Brisbane, Qld, Australia

Running Head: Coral reef microbial baseline

Address correspondence to Bettina Glasl (b.glasl@aims.gov.au).

Abstract

Incorporation of microbial community data into environmental monitoring programs could improve prediction and management of environmental pressures. Coral reefs have experienced dramatic declines due to cumulative impacts of local and global stressors. Here we assess the utility of free-living (i.e. seawater and sediment) and host-associated (i.e. corals, sponges and macroalgae) microbiomes for diagnosing environmental perturbation based on their habitat-specificity, environmental sensitivity and uniformity. We show that the seawater microbiome has the greatest diagnostic value, with environmental parameters explaining 56% of the observed compositional variation and temporal successions being dominated by uniform community assembly patterns. Host-associated microbiomes, in contrast, were five-times less affected by the environment and their community assembly patterns were generally less uniform. Further, seawater microbial community data provided an accurate prediction on the environmental state, highlighting the diagnostic value of microorganisms and illustrating how long-term coral reef monitoring initiatives could be enhanced by incorporating assessments of microbial communities in seawater.

Importance

The recent success in disease diagnostics based on the human microbiome has highlighted the utility of this approach for model systems. However, despite improved prediction and management of environmental pressures from the inclusion of microbial community data in monitoring programs, this approach has not previously been applied to coral reef ecosystems. Coral reefs are facing unprecedented pressure on a local and global scale, and sensitive and rapid markers for ecosystem

stress are urgently needed to underpin effective management and restoration strategies. In this study, we performed the first assessment of the diagnostic value of multiple free-living and host-associated reef microbiomes to infer the environmental state of coral reef ecosystems. Our results reveal that free-living microbial communities have a higher potential to infer environmental parameters than host-associated microbial communities due to their higher determinacy and environmental sensitivity. We therefore recommend timely integration of microbial sampling into current coral reef monitoring initiatives.

Introduction

Coral reef ecosystems are rapidly degrading due to local and global pressures (1). Overfishing, pollution, declining water quality, disease and outbreaks of coral predating crown-of-thorns starfish are responsible for localised reef degradation (2) while climate change is impacting reefs on a global scale, including remote reefs with little local anthropogenic pressure (3). For example, elevated sea surface temperatures caused back-to-back coral mass bleaching events in 2016 and 2017, resulting in a significant loss of shallow-water corals on the Great Barrier Reef (GBR) (4). Climate conditions predicted for the end of the century will result in even more frequent and severe coral mass bleaching events with dire projections for the future of coral reefs (5, 6). This global coral reef crisis is driving the development of new management, reef restoration and bioengineering tools to counteract reef loss and ensure the persistence of coral reefs (7, 8). Early prediction of ecosystem stress is critical for an effective implementation of local management and restoration strategies on threatened reef sites.

Microorganisms have considerable potential as a monitoring tool for coral reef ecosystem health (9-11). Microorganisms are fundamental drivers of biogeochemical cycling on coral reefs (12-14), they form intimate associations with the coral reef benthos (15-17), and they contribute significantly to host health and ecosystem homeostasis (18-20). The constant amendment of microbial communities to exploit available resources (21) can trigger differential abundances of specific microorganisms, hence shifts in community composition can provide an early indication of environmental change (22). For example, compositional and functional shifts of coral-associated microbial communities have been described along gradients of anthropogenic impact (23-25) and with changes in water quality (26). However, despite having many of the useful characteristics required of environmental indicators (9, 27), the diagnostic potential of microorganisms for coral reef monitoring is largely conceptual, with only a few studies elaborating on their potential value. For example, the 'microbialisation score' measures human impacts on coral reefs based on the ratio of microbial and fish metabolic rates (28). The main limitations to further develop and apply microbial-based monitoring approaches are the lack of temporal and spatial baselines for coral reef microbiomes (9, 29).

Coral reefs comprise a complex network of free-living and host-associated microbial communities with strong benthic-pelagic exchange (13, 30). Therefore, holistic assessments that combine different reef hosts and habitats are required to better understand microbial dynamics and sensitivities to environmental perturbations. The diagnostic value of microbial-based monitoring is likely to vary between distinct habitats of a coral reef ecosystem. For example, microbial communities occurring in seawater may be directly affected by the quality of the ambient reef water or climate conditions, however, the high heterogeneity of

seawater due to local hot-spots of available resources (31, 32) may diminish the specificity of these communities. In contrast, microbial communities that dwell in corals live in tight association with the most important frame-builders of reefs (29) and hence may provide crucial information not only on the environmental conditions but also on the effect of the environment on the coral host itself. Sponges, a highly abundant and diverse component of coral reefs (33), are renowned for their enormous filtration capacity (34) and form diverse and intimate associations with microbial communities (35). Hence, sponge microbiomes may provide suitable indicators to monitor water quality. Host-associated biofilms, such as those inhabiting the mucus layer of corals and the surface of macroalgae, provide another potential niche habitat informative for microbial indicators of environmental state. Coral mucus, for example, has been described as a suitable habitat to screen for enterobacteria from sewage contamination due to its ability to trap bacteria (36).

Given the complexity of microbial life on coral reefs we sought to identify the most suitable reef microbiomes for a microbial indicator program to pinpoint environmental state. To do this we quantified the 1) habitat-specificity, 2) determinacy of microbial community successions and 3) sensitivity towards environmental parameters of multiple free-living and host-associated microbiomes. Subsequently, we tested the microbiome's ability to infer environmental state using indicator value (37) and machine learning approaches (38).

Results

Samples were collected during a 16-month period (February 2016 - May 2017), at monthly (Magnetic Island - Geoffrey Bay) and seasonal (Orpheus Island – Pioneer Bay – Channel) intervals. The bacterial 16S rRNA genes of 381 samples including seawater, sediment, sponge tissue (*Coscinoderma matthewsi* and *Amphimedon queenslandica)*, coral tissue and mucus (*Acropora tenuis* and *Acropora millepora*), and macroalgal surfaces (*Sargassum sp.*) were sequenced (Figure 1). In total 231,316 zero-radius operational taxonomic units (zOTUs) were identified based on 100% sequence similarity (39).

Coral reef microbiomes are habitat-specific

Habitat-specificity of coral reef microbes was assessed by comparing the similarities of microbial communities associated with seawater (n=48), sediment (n=48), *A. queenslandica* (n=30), *C. matthewsi* (n=42), *A. tenuis* (tissue n=48, mucus n=46*), A. millepora* (tissue n=42, mucus n=42) and *Sargassum sp.* (n=35). Non-metric Multidimensional Scaling based on Bray-Curtis dissimilarities revealed a clear separation of the microbial communities from different reef habitats (Figure 1), and habitat-specificity was further confirmed with Permutational Multivariate Analysis of Variance (PERMANOVA, $p = 9.999 \times 10^{-5}$, Table Supplementary Table 1-2). Furthermore, alpha diversities (ANOVA, $F_{(8/372)} = 142$, $p < 2 \times 10^{-16}$) and zOTU richness (ANOVA, $F_{(8/372)} = 369$, $p < 2 \times 10^{-16}$) varied significantly between reef habitats (Supplementary Figure 1 and Supplementary Table 3-5). Sediment harboured by far the most diverse (Shannon Index 7.4 ± 0.2 SD) bacterial community, although microbial diversity was also high in coral surface mucus (Shannon Index 5.1 ± 0.9 SD), macroalgal biofilms (Shannon Index 4.5 ± 1.4 SD), seawater (Shannon Index 4.4 ± 0.2 SD) and in the tissue of the sponge *C. matthewsi*

(Shannon Index 4.4 ± 0.3 SD). Microbial diversity was lowest in coral tissue (Shannon Index 3.3 ± 0.8 SD) and in the sponge *A. queenslandica* (Shannon Index 2.7 ± 0.8 SD). These results suggest overall high habitat-specificity of free-living and host-associated microbial communities within coral reef ecosystems.

Uniform *vs* variable community assembly pattern

The uniformity *versus* variability of microbial community assembly patterns was explored through comparison of compositional similarity (Bray-Curtis index, 0 = dissimilar, 1 = identical) in samples collected monthly at Geoffrey Bay (Magnetic Island). The microbial communities of seawater (n = 30, Wilcoxon Rank-Sum test p = 3.1×10^{-7}) and sediment (n = 30; Wilcoxon Rank-Sum test p = 3×10^{-5}) had significantly higher similarities “within” than “among” sampling events (Figure 2a). This uniform response of the free-living microbial communities suggests that deterministic rather than stochastic processes drive their community assembly. For host-associated microbiomes, the overall response pattern varied between species. Microbial communities associated with the sponge *C. matthewsi* (n = 27; Wilcoxon Rank-Sum test, p = 0.0076), the coral *A. tenuis* (mucus n = 28, tissue n = 30; Wilcoxon Rank-Sum test, p = 0.0041 and p = 0.0096, respectively) and the macroalga *Sargassum sp.* (n = 30; Wilcoxon Rank-Sum test, p = 0.00013) followed the same trend as the free-living communities, with significantly higher similarities “within” than “among” sampling events (Figure 2a). In contrast, the microbiome of the sponge *A. queenslandica* (n = 30; Wilcoxon Rank-Sum test, p = 0.23) and the coral *A. millepora* (mucus n = 24, tissue n = 24; Wilcoxon Rank-Sum test, p = 0.15 and p = 0.11 respectively) showed no significant difference in similarities “within” and “among” time points (Figure 2a). Analysis of the compositional similarity of sample

replicates within each sampling time point indicated that the seawater microbial communities not only exhibit an overall higher similarity "within" replicates, but the high compositional similarity is conserved across all sampling events (Figure 2b). In contrast, host-associated microbial communities showed a generally lower compositional similarity and higher variation between sample replicates within each sampling time point (Figure 2b).

Trends in the temporal community assembly pattern of free-living, host tissue- and biofilm-associated microbial communities were analysed using Analysis of Similarity (ANOSIM) as a proxy to describe similarity patterns (R = 0 indicates equal similarity "within" and "among" time point replicates and R = 1 indicates higher "within" than "among" sampling time point similarities; Figure 2b and Supplementary Figure 2). Overall, free-living microbiomes had R values closer to 1 (seawater R = 0.9919 and sediment R = 0.7322), whereas host tissue-associated microbiomes had R values closer to 0 (*A. queenslandica* R = 0.2927, *C. matthewsi* R = 0.3449, *A. tenuis* tissue R = 0.4547 and *A. millepora* tissue R = 0.2151). Host biofilm-associated microbiomes showed R values of approximately 0.5 (*A. tenuis* mucus R = 0.4613 *A. millepora* mucus R = 0.3090 and *Sargassum sp.* biofilm R = 0.4440). These results suggest that free-living microbiomes (seawater and sediment) exhibit a uniform compositional succession, whereas host-associated microbiomes (coral, sponge and macroalgae) are more stochastic in their temporal community succession. Interestingly, host biofilm-associated microbiomes exhibited a higher uniformity (higher ANOSIM R values) in temporal community succession than tissue-associated microbiomes, most likely reflecting greater environmental influence. The uniform temporal response of free-living microbiomes suggests a high diagnostic

value of these microbial communities; hence seawater and sediment microbiomes should provide an accurate prediction of environmental variables.

Microbiomes in seawater (n=48) and sediment (n=48) were further tested for their compositional similarity between all three sampling sites (Geoffrey Bay, Pioneer Bay and Channel). The microbial community composition of sediment samples varied significantly between all three sampling sites (PERMANOVA, $p = 9.999 \times 10^{-5}$, 10,000 permutations; Supplementary Figure 3a). The seawater microbiome, in contrast, showed high temporal variability (ANOSIM R = 0.9934, p = 0.001) and low spatial variability (ANOSIM R = 0.2343, p = 0.002; Supplementary Figure 3b). The high spatial variability of sediment microbiomes indicates that habitat characteristics rather than environmental fluctuations are the main drivers structuring community composition.

Environmental sensitivity

Environmental sensitivity of the different microbiomes was assessed by comparing how much of the compositional variation was explained by sea surface temperature, light and water quality parameters (Supplementary Figures 4 and 5). The compositional variability of the seawater microbiome (n=30) was significantly explained by sampling date, season (summer versus winter) and water quality parameters, such as average seawater temperature, average hours of daylight, total suspended solids (TSS), particulate organic carbon (POC), Chlorophyll *a* (Chl *a)*, and non-purgeable organic carbon (NPOC) concentration (PERMANOVA for Bray Curtis distance based Redundancy Analysis (dbRDA); Figure 3a and Supplementary Table 6a-b). In total, these parameters explained 66% of the observed compositional variation in seawater, with 56% being significantly explained by environmental

variables (Variation Partitioning Analysis, Figure 3b). Season (summer *versus* winter) and sampling date explained 6% and 4%, respectively (Variation Partitioning Analysis, Figure 3b). In comparison, sampling site significantly explained 24% of the variation in sediment microbial communities (n=48), which overlapped by 12% with the variation explained by sediment characteristics, such as particle size and total organic carbon (TOC) content (PERMANOVA for dbRDA and Variation Partitioning Analysis; Supplementary Table 6b and 7). Water quality parameters and sea surface temperature explained only 3% of the observed variability in the sediment microbiome (Variation Partitioning Analysis).

Host-associated microbiomes varied substantially in their response to environmental parameters (PERMANOVA for dbRDA and Variation Partitioning Analysis, Figure 3b-c, Supplementary Table 6c-i and 7). On average, 11% of the observed community variations in host-associated microbiomes were explained by the environment, which is five-times less than what we found for the seawater associated microbial community (Supplementary Table 7). This suggests that compositional variations of the seawater microbiome are more likely to reflect environmental changes. Host-associated microbiomes, are comparatively stable to changes in environmental factors.

Predictability of environmental metadata

Due to the seawater microbiomes uniform temporal pattern and high sensitivity to changing environmental parameters, the ability to infer environmental state based on microbial community data was tested using an Indicator Value analysis (37) and a Random Forest machine learning approach. In total, 110 zOTUs were identified as significant indicators for temperature (Indicator Value $p < 0.01$). Microbial zOTU

assemblages that were indicative of high, low and average seawater temperatures (classification based on their variation around observed annual averages) were present throughout the sampling period. However, higher relative abundances and lower variation (as calculated by coefficient of variation) were evident at certain time points (Figure 4a). Furthermore, we were able to identify microbial indicator taxa for high and low Chl *a*, TSS and POC levels (Supplementary Material Figure 6). Indicators for low and high seawater temperatures were identified in the bacterial phyla Proteobacteria, Bacteroidetes, Cyanobacteria, Actinobacteria and Planctomycetes (Figure 4b). High temperatures were indicated by an increase of zOTUs belonging to the bacterial family *Rhodobacteraceae* and the presence of *Cryomorphaceae*, *Synechococcaeae*, *Vibrio* and *Flavobacterium* (Figure 4b). In contrast, the occurrence of zOTUS belonging to the family *Pelagibacteriaceae* and the genus *Prochlorococcus* were indicative for low seawater temperatures. The phyla Proteobacteria, Bacteroidetes and Cyanobacteria had the greatest number of indicator zOTUs for temperature and other water quality parameters (Supplementary Figure 6). *Flavobacteriaceae*-affiliated zOTUs were significant indicators for temperature, Chl *a*, TSS and POC. *Halomonadaceae* significantly associated with high Chl *a* and TSS and zOTUs belonging to the phylum Verrucomicrobia were significant indicators for high TSS levels.

The diagnostic value of the seawater microbiome (n=48) was further evaluated by applying a Random Forest machine learning classification and regression analysis with 1,213 zOTUs preselected based on a non-zero abundance threshold in at least 10% of the samples (n=48). The seawater microbiome enabled the prediction of seawater temperature classes (low, average, high) with 92% accuracy (Kappa = 88%, Figure 5a-b and Supplementary Figure 7). Highest

accuracy (lowest Out of Bag (OOB) estimated error rate) was achieved with m_{try} = 100 zOTUS. Random Forest regression of the seawater microbiome predicted temperature values (R^2 = 0.67, RMSE = 0.5) (Figure 5c-d and Supplementary Figure 8) with the highest accuracy (lowest OOB estimated error rate) when m_{try} = 400 zOTUs. The effectiveness of zOTUs in reducing uncertainty and variance (also referred to as 'feature importance') within the machine learning algorithm was measured by the decrease in mean accuracy for classification and mean-squared error (%incMSE) for regression. The most important zOTUs belong to the bacterial taxa *Flavobacteriaceae*, *Pelagibacteraceae*, Cyanobacteria, *Rhodobacteraceae*, *Synechococcaceae* and *Pirrelulacae*. These results demonstrate that the microbial community associated with coral reef seawater allows for the accurate prediction of fluctuations in sea surface temperature and water quality parameters.

Discussion

Sensitive and rapidly responding markers of coral ecosystem stress are needed to underpin effective management and restoration strategies. In this study, we used a range of statistical tests and machine learning approaches across multiple free-living and host-associated reef microbiomes to assess their diagnostic value as sensitive indicators of environmental state. Our results show that the microbial community in reef seawater has the highest diagnostic value when compared to other free-living (e.g. sediment) and host-associated microbiomes (e.g. coral, sponge and macroalgae). Our conclusion is based on the microbiome's 1) habitat-specificity, 2) uniformity of its community assembly, 3) sensitivity towards environmental fluctuations and 4) accuracy to predict environmental parameters. This assessment

290 of the diagnostic capacity of various free-living and host-associated coral reef
291 microbiomes to extrapolate environmental variations provides crucial information for
292 ecosystem management initiatives aimed at incorporating microbial monitoring.

293 In general, high habitat-specificity was observed across free-living and host-
294 associated microbiomes, confirming previous reports on the compositional variability
295 of microbial communities between coral reef habitats (40), host species (15, 41-43)
296 and even between host compartments (44). High compositional divergence of
297 microbial communities across different reef habitats can be due to the variation of
298 available resources and/or biotic interactions (21). High habitat-specificity contributes
299 to the overall high diversity and complexity across different microbial communities on
300 coral reefs, highlighting the importance of holistic studies that focus on microbial
301 interactions across the benthic-pelagic realm.

302 Bacterial community structure associated with water and sediment is thought
303 to be primarily governed by deterministic processes (45). Our results are consistent
304 with this, showing uniform community assembly patterns within time point replicates.
305 In contrast, host-associated microbiomes displayed little compositional similarity
306 within a sampling time point, suggesting a non-uniform temporal response. Host-
307 associated microbiomes were also only marginally affected by environmental
308 parameters, indicating that their community assembly pattern are variable between
309 conspecific individuals (45). A higher variability in community assembly can lead to
310 increased community heterogeneity, also referred to as dispersion, which has been
311 described as a common characteristic of host-associated microbiomes (18, 46-48).
312 Furthermore, lower microbial compositional similarities amongst replicates may be
313 driven by increased niche space (e.g. host compartments) (44) and host genotype
314 effects (e.g. host genetics) (42). Collectively, our results show that free-living

315 microbial communities have a higher potential to infer environmental parameters
316 (such as standard measures in environmental monitoring programs) than host-
317 associated microbial communities due to their higher uniformity and environmental
318 sensitivity. Importantly however, previous metaproteomic research on reef sponges
319 has shown that while microbial community composition can appear stable when
320 seawater temperatures increase, disruption to nutritional interdependence and
321 molecular interactions (such as reduced expression of transporters involved in the
322 uptake of sugars, peptides and other substrates) actually occurs prior to detectable
323 changes in community structure (49). Hence, considering the importance of
324 microbes to reef invertebrate health, more sensitive transcriptomic / proteomic
325 approaches may still be warranted for sensitive detection of microbial responses to
326 environmental perturbations.

327 The diagnostic potential of microbial communities, especially in combination
328 with machine learning approaches, has gained momentum across multiple research
329 fields, including disease identification by characterisation of the human gut-
330 microbiome (50), evaluation of the environment and host genetics on the human
331 microbiome (51), prediction of hydrological functions in riverine ecosystems (52) and
332 assessment of macroecological patterns in soil samples (53). This development of
333 microbial-based diagnostics is largely due to availability of high-throughput
334 sequencing of the 16S rRNA gene and streamlined analytical pipelines that facilitate
335 rapid assessment of microbial community composition (54, 55). In addition to its
336 utility for inferring environmental fluctuations, the seawater microbiome possesses
337 numerous characteristics desirable for environmental monitoring programs: i) non-
338 destructive collection and simple processing methods facilitate large-scale
339 collections alongside existing programs that sample water quality measurements, ii)

high fractional contribution of abundant microbes minimises the impacts of sequencing biases (Supplementary Figure 9) and iii) sampling is conducive to future automated, high throughput analyses such as in-line flow cytometry on vessels and real-time DNA/RNA sequencing for community characterisation.

Incorporation of seawater microbial community data into coral reef monitoring approaches should enhance our ability to describe environmental conditions and changes more holistically. For example, temperature fluctuations drive structural variations in seawater microbial communities (56, 57), and elevated seawater temperatures on coral reefs are highly correlated with coral bleaching (1, 58). The inclusion of microbial community data alongside water quality parameters could therefore improve our ability to predict the likelihood of ecosystem stress. For instance, our sample sites, located in the central sector of the GBR, were not affected by the 2016 bleaching that primarily affected the northern sector (59), however they were impacted by the 2017 bleaching event (60). In the months prior to bleaching (late December 2016 till March 2017) we observed two to four times higher relative abundances of high temperature indicator assemblages than when compared to the equivalent period at the beginning of 2016 (Figure 4a), where no bleaching was observed. Interestingly, high temperature indicator assemblages included putative coral pathogens (e.g. *Vibrio*) and opportunistic bacteria (e.g. Rhodobacteraceae, *Verrucomicrobia* and *Flavobacterium*). Coral pathogens, such as *Vibrio coralliilyticus* increase their efficiency and motility behaviours with rising seawater temperatures (61-63), and the higher abundance of these microbes may explain the increased prevalence of coral disease post bleaching (64). Hence, microbial monitoring could help inform managers about impending disease outbreaks.

While microbial inventories for reef biofilms and seawater have been established within the Red Sea (57) and Florida coastal areas (65), our study provides the first holistic microbial baseline spanning multiple free-living and host-associated microbiomes for selected GBR sites. Results suggest that there is realistic scope to enhance long-term reef monitoring initiatives by incorporating seawater microbiome observations for assessments of environmental change over space and time, especially for rapid and sensitive identification of early signs of declining ecosystem health. The establishment of microbial observatories (66) and DNA biobanks for long-term biomonitoring (67) will be paramount to successfully inferring ecosystem state and / or perturbations from microbial communities. We therefore recommend timely integration of microbial sampling into current coral reef monitoring initiatives. Further refinement of the sampling and data analysis techniques should focus on selection and validation of additional indicator taxa as well as assessment of ecologically important microbial functions. A further consideration is to explore which monitoring objectives would benefit most from assessments of microbial communities. For example, it is likely that the rapid response time of microbial indicators makes them better suited to early-warning, impact or compliance monitoring programs than to monitoring of slower, long-term changes.

Materials and Methods

Sample collection

Samples for microbial community characterization were collected monthly (Magnetic Island) and seasonally (Orpheus Island) from seawater, sediment and multiple host

organisms (i.e. corals, sponges and macroalgae), along with environmental metadata, between February 2016 and May 2017 at three Great Barrier Reef sites (Figure 1). Samples were collected under the permit G16/38348.1 issued by the Great Barrier Reef Marine Park Authority.

Samples (n= 3/ sample type/ sampling event) for molecular analysis and additional environmental metadata were collected following the standard operational procedures of the Australian Marine Microbial Biodiversity Initiative (AMMBI; https://data.bioplatforms.com/organization/pages/australian-microbiome/methods). In brief, seawater for molecular analysis was collected with collapsible sterile bags close to the reef substrate at 2 m depth and pre-filtered (50 µm) to remove large particles and subsequently filtered (2 L) onto 0.2 µm Sterivex-filters (Millepore). The sediment surface layer was sampled with sterile 50 mL tubes at 2 m depth and subsampled immediately into 2 mL cryogenic vials. The sponges *Coscinoderma matthewsi* and *Amphimedon queenslandica* were removed from the substrate (at 7 m and 3 m respectively) with sterile scalpel blades, rinsed with 0.2 µm filter-sterilised seawater and subsampled into 2 mL cryogenic vials. The surface mucus layer of the two acroporid coral species, *Acropora tenuis* and *Acropora millepora*, was sampled with sterile cotton swabs (18). Additionally, coral fragments of each sampled coral were collected at 3 m depth. Coral fragments were rinsed with 0.2 µm filtered-sterilised seawater and placed into 5 mL cryogenic vials. The thallus (including stem, floats and blades) of the macroalgae *Sargassum sp.* was sampled with sterile scalpels at 3 m depth, rinsed with 0.2 µm filtered-sterilised seawater and placed into 2 mL cryogenic vials. All samples were immediately flash frozen in liquid nitrogen after processing and stored at -80°C until DNA extraction.

Additional seawater samples were collected with a diver-operated Niskin bottle close to the reef substrate at 2 m depth at each sampling occasion. Water was subsampled in duplicate for analyses of salinity and concentrations of dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), particulate organic carbon (POC), dissolved inorganic nutrients (DIN), total suspended solids (TSS) and chlorophyll a (Chl a) concentration. Samples were further analysed according to the standard procedures of the Australian Institute of Marine Science (AIMS, Townsville, Australia)(68). Sediment samples were collected with 100 ml glass jars at 2 m depth and characteristics, such as grain size distribution and total organic carbon (TOC) and nitrogen (TON) content, were assessed for each sampling event. Seawater temperatures were obtained from AIMS long-term monitoring temperature records (http://eatlas.org.au/).

DNA extraction

Prior to extraction, the macroalgal biofilm was separated from the algal tissue by overnight incubation at 200 rpm in 10 mL 1 x PBS at 37°C. Coral fragments were defrosted on ice and the tissue was stripped from the skeleton with an airgun into 1 x PBS solution, homogenised for 1 min at 12.5 rpm with a tissue homogeniser, pelleted (10 min at 16,000 rcf) and snap frozen in liquid nitrogen prior to DNA extraction. DNA from seawater, sediment, sponge and macroalgal biofilms was extracted with the DNeasy PowerSoil kit (Qiagen) and DNA of coral tissue and mucus samples was extracted using the DNeasy PowerBiofilm kit (Qiagen) following the Manufacturer's instructions. DNA extracts were stored at -80°C until being sent for sequencing.

438 **16S rRNA gene sequencing**

439 DNA extracts were sent on dry ice to the Ramaciotti Centre for Genomics (Sydney,
440 Australia) for sequencing. The bacterial 16S rRNA genes were sequenced using the
441 27F (69) and 519R (70) primer pairs on the Illumina MiSeq platform utilising a duel
442 indexed 2 x 300 bp paired end approach. Further documentation outlining the
443 standard operating procedures for generating and sequencing amplicons is available
444 at https://data.bioplatforms.com/organization/pages/bpa-marine-microbes/methods.

445

446 **Sequence analysis**

447 Sequencing data were analysed as single nucleotide variants in a standardized
448 platform alongside other Australian microbial biodiversity initiative samples (39, 71).
449 In brief, forward and reverse reads were merged using FLASH (72). FASTA
450 formatted sequences were extracted from FASTQ files and those < 400 bp in length
451 or containing N's or homopolymer runs of > 8 bp were removed using MOTHUR
452 (v1.34.1) (73). USEARCH (64 bit v10.0.240) (74) package was used to de-replicate
453 sequences and to order them by abundance. Sequences with < 4 representatives
454 and Chimeras were removed. Quality-filtered sequences were mapped to chimera-
455 free zero-radius operational taxonomic units (zOTUs) and a sample by read
456 abundance table created. zOTUs were taxonomically classified with SILVA v132 (75)
457 database using MOTHUR's implementation of the Wang classifier (76) and a 60%
458 Bayesian probability cut-off.

459 Chloroplast and mitochondria derived reads as well as singletons were
460 removed from the dataset. Remaining data were rarefied to 3,600 reads per sample
461 and transformed to relative abundances using the phyloseq package (77) in R (78).

462

463 **Habitat and host-specificity**

464 Habitat and host-specificity of a microbiome was assessed by calculating the
465 compositional similarities of all 381 samples with the Bray-Curtis Index and
466 illustrating them in a Non-Metric Multidimensional Scaling (NMDS) plot using the
467 phyloseq package (77). To confirm habitat and host-specificity, Permutational
468 Multivariate Analysis of Variance (PERMANOVA) was applied using the adonis()
469 function of the vegan package (79) with 10,000 permutations.

470

471 **Uniform response pattern**

472 The microbiome similarity of replicates for sampling time points *versus* the
473 microbiome similarity among sampling time points was compared by obtaining the
474 Bray-Curtis Similarity for each habitat individually. The variation between the overall
475 within and among time point replicates was tested with a Wilcoxon Rank-sum test in
476 R (78). The dispersion of the Bray-Cutis similarities within a sampling time point was
477 calculated as the coefficient of variation. Analysis of Similarity (ANOSIM; anosim()
478 function of the vegan package (79)) based on Bray-Curtis similarities was used to
479 further evaluate within and among time point similarities in the microbial
480 communities.

481

482 **Environmental sensitivities**

483 Environmental metadata were z-score standardized (80) and checked for collinearity
484 using the Pearson correlation coefficient. Collinearity was assumed if correlation was
485 > 0.7 or < -0.7 (81). Collinear variables were considered redundant and removed
486 from the analysis.

zOTU relative abundance, environmental metadata (e.g. average seawater temperature, average hours of daylight, Chl a, POC, NPOC and TSS concentration), season (summer *versus* winter) and sampling date were used for Bray-Curtis distance-based redundancy analysis (db-RDA) using the phyloseq package (77). The significance of each response variable was confirmed with an Analysis of Variance (ANOVA) for the db-RDA (anova.cca() function in the vegan package (79)). Only significant (p-value < 0.05) response variables were kept in the model. The explanatory value (in %) of significant response variables (e.g. environmental parameters, season and sampling date) was assessed with a Variation Partitioning Analysis of the vegan package (79).

Indicator value analysis

Indicator taxa were identified with the indicator value analysis (indicspecies package (37)) using the following thresholds: 1,000 permutations, minimum specificity (At) and minimum sensitivity (Bt) set to 70% and p-value ≤ 0.01.

Random forest machine learning

Random forest machine learning was performed with the caret (82) and random forest package (83) in R (78). zOTUs with non-zero abundance values in at least 10% of the samples (n=48) were preselected and z-score standardised prior to model training. Random Forest (with n_{tress} = 10,000) prediction error was measured with out-of-bag (OOB) error. Highest accuracy (lowest OOB estimated error rate) for classification was achieved with m_{try} = 100 zOTUS and for regression with m_{try} = 400 zOTUs. Importance of zOTUs was measured using the decrease in mean accuracy for classification and mean-squared error (%incMSE) for regression.

512

513 **Data availability**

514 Sequencing data, metadata and protocols are available at the Bioplatforms Australia
515 data portal under the Australian Microbiome project (www.data.bioplatforms.com).
516 Full usage requires free registration. To search for the sequencing data, navigate to
517 “Processed data”, select “Amplicon is 27f519r_bacteria” and “Environment is
518 Marine”. To search for the Great Barrier Reef sampling sites, add an additional
519 contextual filter, select “Sampling Site” from the dropdown menu and search for
520 “Geoffrey Bay”, “Pionner Bay” and “Channel”.

521

522 ## Acknowledgements

523 We thank Michele Skuza, Neale Johnston and the AIMS water quality team for their
524 help with analysing the water quality samples. We also thank Heidi Luter, Katarina
525 Damjanovic and Joe Gioffre for their assistance in the field and Sara Bell for her
526 expertise in the laboratory. We would like to acknowledge the contribution of the
527 Marine Microbes (MM) and Biomes of Australian Soil Environments (BASE) projects,
528 through the Australian Microbiome Initiative in the generation of data used in this
529 publication. The Australian Microbiome Initiative is supported by funding from
530 Bioplatforms Australia through the Australian Government National Collaborative
531 Research Infrastructure Strategy (NCRIS). The study was further funded by the
532 Advance Queensland PhD Scholarship, the Great Barrier Reef Marine Park Authority
533 Management Award and a National Environmental Science Program (NESP) grant
534 awarded to B.G.

535

536 **Author contributions**

537 Samples were collected by B.G., D.G.B., P.R.F. and N.S.W. Samples were
538 processed in the laboratory by B.G. and P.R.F. B.G. analysed and prepared the
539 manuscript. All authors reviewed and edited the manuscript.

540

541 # References

542 1. Hughes TP, Barnes ML, Bellwood DR, Cinner JE, Cumming GS, Jackson
543 JBC, Kleypas J, van de Leemput IA, Lough JM, Morrison TH, Palumbi SR,
544 van Nes EH, Scheffer M. 2017. Coral reefs in the Anthropocene. Nature
545 546:82-90.
546 2. De'ath G, Fabricius KE, Sweatman H, Puotinen M. 2012. The 27-year decline
547 of coral cover on the Great Barrier Reef and its causes. Proceedings of the
548 National Academy of Sciences of the United States of America 109:17995-
549 17999.
550 3. Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P,
551 Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin
552 CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatziolos ME. 2007.
553 Coral Reefs Under Rapid Climate Change and Ocean Acidification. Science
554 318:1737-1742.
555 4. Hughes TP, Kerry JT, Baird AH, Connolly SR, Dietzel A, Eakin CM, Heron SF,
556 Hoey AS, Hoogenboom MO, Liu G, McWilliam MJ, Pears RJ, Pratchett MS,
557 Skirving WJ, Stella JS, Torda G. 2018. Global warming transforms coral reef
558 assemblages. Nature 556:492-496.

559 5. van Hooidonk R, Maynard J, Tamelander J, Gove J, Ahmadia G, Raymundo
560 L, Williams G, Heron SF, Planes S. 2016. Local-scale projections of coral reef
561 futures and implications of the Paris Agreement. Sci Rep 6:39666.
562 6. Hughes TP, Anderson KD, Connolly SR, Heron SF, Kerry JT, Lough JM,
563 Baird AH, Baum JK, Berumen ML, Bridge TC, Claar DC, Eakin CM, Gilmour
564 JP, Graham NAJ, Harrison H, Hobbs JA, Hoey AS, Hoogenboom M, Lowe
565 RJ, McCulloch MT, Pandolfi JM, Pratchett M, Schoepf V, Torda G, Wilson SK.
566 2018. Spatial and temporal patterns of mass bleaching of corals in the
567 Anthropocene. Science 359:80-83.
568 7. Anthony K, Bay LK, Costanza R, Firn J, Gunn J, Harrison P, Heyward A,
569 Lundgren P, Mead D, Moore T, Mumby PJ, van Oppen MJH, Robertson J,
570 Runge MC, Suggett DJ, Schaffelke B, Wachenfeld D, Walshe T. 2017. New
571 interventions are needed to save coral reefs. Nat Ecol Evol 1:1420-1422.
572 8. Damjanovic K, Blackall LL, Webster NS, van Oppen MJH. 2017. The
573 contribution of microbial biotechnology to mitigating coral reef degradation.
574 Microb Biotechnol 10:1236-1243.
575 9. Glasl B, Webster NS, Bourne DG. 2017. Microbial indicators as a diagnostic
576 tool for assessing water quality and climate stress in coral reef ecosystems.
577 Marine Biology 164.
578 10. Glasl B, Bourne DG, Frade PR, Webster NS. 2018. Establishing microbial
579 baselines to identify indicators of coral reef health. Microbiology Australia
580 39:42-46.
581 11. Roitman S, Joseph Pollock F, Medina M. Coral Microbiomes as Bioindicators
582 of Reef Health, p 1-19 doi:10.1007/13836_2018_29. Springer International
583 Publishing, Cham.

584 12. Gast GJ, Wiegman S, Wieringa E, Duyl FCv, Bak RPM. 1998. Bacteria in
585 coral reef water types: removal of cells, stimulation of growth and
586 mineralization. Mar Ecol Prog Ser 167:37-45.
587 13. Bourne D, Webster N. 2013. Coral Reef Bacterial Communities, p 163-187. *In*
588 Rosenberg E, DeLong E, Lory S, Stackebrandt E, Thompson F (ed), The
589 Prokaryotes doi:10.1007/978-3-642-30123-0_48. Springer Berlin Heidelberg.
590 14. Sorokin YI. 1973. Trophical role of bacteria in ecosystem of coral reef. Nature
591 242:415-417.
592 15. Rohwer F, Seguritan V, Azam F, Knowlton N. 2002. Diversity and distribution
593 of coral-associated bacteria. Mar Ecol Prog Ser 243:1-10.
594 16. Webster NS, Luter HM, Soo RM, Botte ES, Simister RL, Abdo D, Whalan S.
595 2012. Same, same but different: symbiotic bacterial associations in GBR
596 sponges. Front Microbiol 3:444.
597 17. Egan S, Harder T, Burke C, Steinberg P, Kjelleberg S, Thomas T. 2013. The
598 seaweed holobiont: understanding seaweed–bacteria interactions. FEMS
599 Microbiol Rev 37:462-476.
600 18. Glasl B, Herndl GJ, Frade PR. 2016. The microbiome of coral surface mucus
601 has a key role in mediating holobiont health and survival upon disturbance.
602 ISME J doi:10.1038/ismej.2016.9.
603 19. Hentschel U, Schmid M, Wagner M, Fieseler L, Gernert C, Hacker J. 2001.
604 Isolation and phylogenetic analysis of bacteria with antimicrobial activities
605 from the Mediterranean sponges Aplysina aerophoba and Aplysina
606 cavernicola. FEMS Microbiol Ecol 35:305-312.
607 20. Webster NS, Reusch TBH. 2017. Microbial contributions to the persistence of
608 coral reefs. Isme Journal 11:2167-2174.

609 21. Martiny JB, Jones SE, Lennon JT, Martiny AC. 2015. Microbiomes in light of
610 traits: A phylogenetic perspective. Science 350:aac9323.
611 22. Garza DR, van Verk MC, Huynen MA, Dutilh BE. 2018. Towards predicting
612 the environmental metabolome from metagenomics with a mechanistic model.
613 Nat Microbiol 3:456-460.
614 23. Ziegler M, Roik A, Porter A, Zubier K, Mudarris MS, Ormond R, Voolstra CR.
615 2016. Coral microbial community dynamics in response to anthropogenic
616 impacts near a major city in the central Red Sea. Mar Pollut Bull
617 doi:http://dx.doi.org/10.1016/j.marpolbul.2015.12.045.
618 24. Kelly LW, Williams GJ, Barott KL, Carlson CA, Dinsdale EA, Edwards RA,
619 Haas AF, Haynes M, Lim YW, McDole T, Nelson CE, Sala E, Sandin SA,
620 Smith JE, Vermeij MJA, Youle M, Rohwer F. 2014. Local genomic adaptation
621 of coral reef-associated microbiomes to gradients of natural variability and
622 anthropogenic stressors. Proceedings of the National Academy of Sciences
623 111:10227-10232.
624 25. Dinsdale EA, Pantos O, Smriga S, Edwards RA, Angly F, Wegley L, Hatay M,
625 Hall D, Brown E, Haynes M, Krause L, Sala E, Sandin SA, Thurber RV, Willis
626 BL, Azam F, Knowlton N, Rohwer F. 2008. Microbial ecology of four coral
627 atolls in the northern Line Islands. PLoS ONE 3:e 1584.
628 26. Angly FE, Heath C, Morgan TC, Tonin H, Rich V, Schaffelke B, Bourne DG,
629 Tyson GW. 2016. Marine microbial communities of the Great Barrier Reef
630 lagoon are influenced by riverine floodwaters and seasonal weather events.
631 PeerJ 4:e1511.

632 27. Cooper TF, Gilmour JP, Fabricius KE. 2009. Bioindicators of changes in water
633 quality on coral reefs: review and recommendations for monitoring
634 programmes. Coral Reefs 28:589-606.
635 28. McDole T, Nulton J, Barott KL, Felts B, Hand C, Hatay M, Lee H, Nadon MO,
636 Nosrat B, Salamon P, Bailey B, Sandin SA, Vargas-Angel B, Youle M,
637 Zgliczynski BJ, Brainard RE, Rohwer F. 2012. Assessing coral reefs on a
638 pacific-wide scale using the microbialization score. Plos One 7.
639 29. Bourne DG, Morrow KM, Webster NS. 2016. Coral Holobionts: Insights into
640 the coral microbiome: Underpinning the health and resilience of reef
641 ecosystems. Annual Reviews of Microbiology 70:317-340.
642 30. Lesser MP. 2006. Benthic-pelagic coupling on coral reefs: Feeding and
643 growth of Caribbean sponges. Journal of Experimental Marine Biology and
644 Ecology 328:277-288.
645 31. Azam F. 1998. Microbial control of oceanic carbon flux: the plot thickens.
646 Science 280:694-696.
647 32. Stocker R. 2012. Marine microbes see a sea of gradients. Science 338:628-
648 633.
649 33. Diaz MC, Rützler K. 2001. Sponges: an essential component of Caribbean
650 coral reefs. Bull Mar Sci 69:535-546.
651 34. Reiswig HM. 1971. *In situ* pumping activities of tropical Demospongiae.
652 Marine Biology 9:38-50.
653 35. Taylor MW, Radax R, Steger D, Wagner M. 2007. Sponge-associated
654 microorganisms: evolution, ecology, and biotechnological potential.
655 Microbiology and Molecular Biology Reviews 71:295-347.

656 36. Lipp EK, Griffin DW. 2004. Analysis of coral mucus as an improved medium
657 for detection of enteric microbes and for determining patterns of sewage
658 contamination in reef environments. EcoHealth 1:317-323.
659 37. De Cáceres M, Legendre P. 2009. Associations between species and groups
660 of sites: indices and statistical inference. Ecology 90:3566-3574.
661 38. Knights D, Costello EK, Knight R. 2011. Supervised classification of human
662 microbiota. FEMS Microbiol Rev 35:343-59.
663 39. Brown MV, Kamp Jvd, Ostrowski M, Seymour JR, Ingleton T, Messer LF,
664 Jeffries T, Siboni N, Laverock B, Bibiloni-Isaksson J, Nelson TM, Coman F,
665 Davies CH, Frampton D, Rayner M, Goossen K, Robert S, Holmes B, Abell
666 GCJ, Craw P, Kahlke T, Sow SLS, McAllister K, Windsor J, Skuza M,
667 Crossing R, Patten N, Malthouse P, Ruth PDv, Paulsen I, Fuhrman JA,
668 Richardson A, Koval J, Bissett A, Fitzgerald A, Moltmann T, Bodrossy L. in
669 press. Systematic, continental scale temporal monitoring of marine pelagic
670 microbiota by the Australian Marine Microbial Biodiversity Initiative. Scientific
671 Data SDATA-18-00035B.
672 40. Tout J, Jeffries TC, Webster NS, Stocker R, Ralph PJ, Seymour JR. 2014.
673 Variability in Microbial Community Composition and Function Between
674 Different Niches Within a Coral Reef. Microb Ecol 67:540-552.
675 41. Carlos C, Torres TT, Ottoboni LMM. 2013. Bacterial communities and
676 species-specific associations with the mucus of Brazilian coral species. Sci
677 Rep 3.
678 42. Glasl B, Smith CE, Bourne DG, Webster NS. 2018. Exploring the diversity-
679 stability paradigm using sponge microbial communities. Sci Rep 8:8425.
680 43. Webster NS, Thomas T. 2016. The Sponge Hologenome. MBio 7.

681 44. Sweet MJ, Croquer A, Bythell JC. 2011. Bacterial assemblages differ between
682 compartments within the coral holobiont. Coral Reefs 30:39-52.
683 45. Wang J, Shen J, Wu Y, Tu C, Soininen J, Stegen JC, He J, Liu X, Zhang L,
684 Zhang E. 2013. Phylogenetic beta diversity in bacterial assemblages across
685 ecosystems: deterministic versus stochastic processes. ISME J 7:1310-21.
686 46. Casey JM, Connolly SR, Ainsworth TD. 2015. Coral transplantation triggers
687 shift in microbiome and promotion of coral disease associated potential
688 pathogens. Sci Rep 5:11903.
689 47. Zaneveld JR, McMinds R, Thurber RV. 2017. Stress and stability: applying the
690 Anna Karenina principle to animal microbiomes. Nature Microbiology 2.
691 48. Zaneveld JR, Burkepile DE, Shantz AA, Pritchard CE, McMinds R, Payet JP,
692 RoryWelsh, Correa AMS, Lemoine NP, Rosales S, Fuchs C, Maynard JA,
693 Thurber RV. 2016. Overfishing and nutrient pollution interact with temperature
694 to disrupt coral reefs down to microbial scales. Nature Communications
695 7:11833.
696 49. Fan L, Liu M, Simister R, Webster NS, Thomas T. 2013. Marine microbial
697 symbiosis heats up: the phylogenetic and functional response of a sponge
698 holobiont to thermal stress. ISME J 7:991-1002.
699 50. Duvallet C, Gibbons SM, Gurry T, Irizarry RA, Alm EJ. 2017. Meta-analysis of
700 gut microbiome studies identifies disease-specific and shared responses. Nat
701 Commun 8:1784.
702 51. Rothschild D, Weissbrod O, Barkan E, Kurilshikov A, Korem T, Zeevi D,
703 Costea PI, Godneva A, Kalka IN, Bar N, Shilo S, Lador D, Vila AV, Zmora N,
704 Pevsner-Fischer M, Israeli D, Kosower N, Malka G, Wolf BC, Avnit-Sagi T,
705 Lotan-Pompan M, Weinberger A, Halpern Z, Carmi S, Fu J, Wijmenga C,

706 Zhernakova A, Elinav E, Segal E. 2018. Environment dominates over host
707 genetics in shaping human gut microbiota. Nature 555:210-215.
708 52. Good SP, URycki DR, Crump BC. 2018. Predicting hydrologic function with
709 aquatic gene fragments. Water Resources Research 54:2424-2435.
710 53. Ramirez KS, Knight CG, de Hollander M, Brearley FQ, Constantinides B,
711 Cotton A, Creer S, Crowther TW, Davison J, Delgado-Baquerizo M, Dorrepaal
712 E, Elliott DR, Fox G, Griffiths RI, Hale C, Hartman K, Houlden A, Jones DL,
713 Krab EJ, Maestre FT, McGuire KL, Monteux S, Orr CH, van der Putten WH,
714 Roberts IS, Robinson DA, Rocca JD, Rowntree J, Schlaeppi K, Shepherd M,
715 Singh BK, Straathof AL, Bhatnagar JM, Thion C, van der Heijden MGA, de
716 Vries FT. 2018. Detecting macroecological patterns in bacterial communities
717 across independent studies of global soils. Nat Microbiol 3:189-196.
718 54. Schuster SC. 2008. Next-generation sequencing transforms today's biology.
719 Nat Methods 5:16-8.
720 55. Waldor MK, Tyson G, Borenstein E, Ochman H, Moeller A, Finlay BB, Kong
721 HH, Gordon JI, Nelson KE, Dabbagh K, Smith H. 2015. Where next for
722 microbiome research? PLoS Biol 13:e1002050.
723 56. Sunagawa S, Coelho LP, Chaffron S, Kultima JR, Labadie K, Salazar G,
724 Djahanschiri B, Zeller G, Mende DR, Alberti A, Cornejo-Castillo FM, Costea
725 PI, Cruaud C, d'Ovidio F, Engelen S, Ferrera I, Gasol JM, Guidi L, Hildebrand
726 F, Kokoszka F, Lepoivre C, Lima-Mendez G, Poulain J, Poulos BT, Royo-
727 Llonch M, Sarmento H, Vieira-Silva S, Dimier C, Picheral M, Searson S,
728 Kandels-Lewis S, Tara Oceans c, Bowler C, de Vargas C, Gorsky G, Grimsley
729 N, Hingamp P, Iudicone D, Jaillon O, Not F, Ogata H, Pesant S, Speich S,
730 Stemmann L, Sullivan MB, Weissenbach J, Wincker P, Karsenti E, Raes J,

731 Acinas SG, et al. 2015. Ocean plankton. Structure and function of the global
732 ocean microbiome. Science 348:1261359.
733 57. Roik A, Rothig T, Roder C, Ziegler M, Kremb SG, Voolstra CR. 2016. Year-
734 Long Monitoring of Physico-Chemical and Biological Variables Provide a
735 Comparative Baseline of Coral Reef Functioning in the Central Red Sea.
736 PLoS One 11:e0163939.
737 58. Brown EB. 1997. Coral bleaching: causes and consequences. Coral Reefs
738 16:S129-S138.
739 59. Hughes TP, Kerry JT, Alvarez-Noriega M, Alvarez-Romero JG, Anderson KD,
740 Baird AH, Babcock RC, Beger M, Bellwood DR, Berkelmans R, Bridge TC,
741 Butler IR, Byrne M, Cantin NE, Comeau S, Connolly SR, Cumming GS,
742 Dalton SJ, Diaz-Pulido G, Eakin CM, Figueira WF, Gilmour JP, Harrison HB,
743 Heron SF, Hoey AS, Hobbs JA, Hoogenboom MO, Kennedy EV, Kuo CY,
744 Lough JM, Lowe RJ, Liu G, McCulloch MT, Malcolm HA, McWilliam MJ,
745 Pandolfi JM, Pears RJ, Pratchett MS, Schoepf V, Simpson T, Skirving WJ,
746 Sommer B, Torda G, Wachenfeld DR, Willis BL, Wilson SK. 2017. Global
747 warming and recurrent mass bleaching of corals. Nature 543:373-377.
748 60. ARC_Centre_of_Excellence. 2017. Two-thirds of Great Barrier Reef hit by
749 back-to-back mass coral bleaching. Media Release
750 https://www.coralcoe.org.au/media-releases/two-thirds-of-great-barrier-reef-
751 hit-by-back-to-back-mass-coral-bleaching.
752 61. Garren M, Son K, Raina J-B, Rusconi R, Menolascina F, Shapiro OH, Tout J,
753 Bourne DG, Seymour JR, Stocker R. 2014. A bacterial pathogen uses
754 dimethylsulfoniopropionate as a cue to target heat-stressed corals. The ISME
755 journal 8:999-1007.

62. Garren M, Son K, Tout J, Seymour JR, Stocker R. 2016. Temperature-induced behavioral switches in a bacterial coral pathogen. ISME J 10:1363-72.

63. Tout J, Jeffries TC, Petrou K, Tyson GW, Webster NS, Garren M, Stocker R, Ralph PJ, Seymour JR. 2015. Chemotaxis by natural populations of coral reef bacteria. ISME J 9:1764-1777.

64. Muller EM, Rogers CS, Spitzack AS, van Woesik R. 2008. Bleaching increases likelihood of disease on Acropora palmata (Lamarck) in Hawksnest Bay, St John, US Virgin Islands. Coral Reefs 27:191-195.

65. Campbell AM, Fleisher J, Sinigalliano C, White JR, Lopez JV. 2015. Dynamics of marine bacterial community diversity of the coastal waters of the reefs, inlets, and wastewater outfalls of southeast Florida. Microbiologyopen 4:390-408.

66. Buttigieg PL, Fadeev E, Bienhold C, Hehemann L, Offre P, Boetius A. 2018. Marine microbes in 4D-using time series observation to assess the dynamics of the ocean microbiome and its links to ocean health. Curr Opin Microbiol 43:169-185.

67. Jarman SN, Berry O, Bunce M. 2018. The value of environmental DNA biobanking for long-term biomonitoring. Nat Ecol Evol 2:1192-1193.

68. Devlin MJ, Lourey MJ. 2000. Water quality - field and analytical procedures. Reef L-tMotGB, Australian Institute of Marine Science Townsville.

69. Lane DJ. 1991. 16S/23S rRNA sequencing, p 115–175. *In* Stackebrandt E, Goodfellow M (ed), Nucleic acid techniques in bacterial systematics. John Wiley and Sons, New York.

780 70. Turner S, Pryer KM, Miao VP, Palmer JD. 1999. Investigating deep
781 phylogenetic relationships among cyanobacteria and plastids by small subunit
782 rRNA sequence analysis. J Eukaryot Microbiol 46:327-38.
783 71. Bissett A, Fitzgerald A, Meintjes T, Mele PM, Reith F, Dennis PG, Breed MF,
784 Brown B, Brown MV, Brugger J, Byrne M, Caddy-Retalic S, Carmody B,
785 Coates DJ, Correa C, Ferrari BC, Gupta VVSR, Hamonts K, Haslem A,
786 Hugenholtz P, Karan M, Koval J, Lowe AJ, Macdonald S, McGrath L, Martin
787 D, Morgan M, North KI, Paungfoo-Lonhienne C, Pendall E, Phillips L, Pirzl R,
788 Powell JR, Ragan MA, Schmidt S, Seymour N, Snape I, Stephen JR, Stevens
789 M, Tinning M, Williams K, Yeoh YK, Zammit CM, Young A. 2016. Introducing
790 BASE: the Biomes of Australian Soil Environments soil microbial diversity
791 database. Gigascience 5.
792 72. Magoc T, Salzberg SL. 2011. FLASH: fast length adjustment of short reads to
793 improve genome assemblies. Bioinformatics 27:2957-63.
794 73. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB,
795 Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B,
796 Thallinger GG, Van Horn DJ, Weber CF. 2009. Introducing mothur: open-
797 source, platform-independent, community-supported software for describing
798 and comparing microbial communities. Appl Environ Microbiol 75:7537-41.
799 74. Edgar RC. 2010. Search and clustering orders of magnitude faster than
800 BLAST. Bioinformatics 26:2460-1.
801 75. Yilmaz P, Parfrey LW, Yarza P, Gerken J, Pruesse E, Quast C, Schweer T,
802 Peplies J, Ludwig W, Glockner FO. 2014. The SILVA and "All-species Living
803 Tree Project (LTP)" taxonomic frameworks. Nucleic Acids Res 42:D643-8.

804 76. Wang Q, Garrity GM, Tiedje JM, Cole JR. 2007. Naïve bayesian classifier for
805 rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl
806 Environ Microbiol 73:5261-5267.
807 77. McMurdie PJ, Holmes S. 2013. phyloseq: an R package for reproducible
808 interactive analysis and graphics of microbiome census data. PLoS One
809 8:e61217.
810 78. R Development Core Team. 2008. R: A language and environment for
811 statistical computing. R Foundation for Statistical Computing.
812 79. Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB,
813 Simpson GL, Solymos P, Stevens MHH, Wagner H. 2013. vegan: Community
814 Ecology Package. R package version 20-9.
815 80. Clark- Carter D (ed). 2014. z Scores. Wiley StatsRef,
816 81. Dormann CF, Elith J, Bacher S, Buchmann C, Carl G, Carre G, Marquez JRG,
817 Gruber B, Lafourcade B, Leitao PJ, Munkemuller T, McClean C, Osborne PE,
818 Reineking B, Schroder B, Skidmore AK, Zurell D, Lautenbach S. 2013.
819 Collinearity: a review of methods to deal with it and a simulation study
820 evaluating their performance. Ecography 36:27-46.
821 82. Kuhn M. 2008. Caret package. Journal of Statistical Software 28.
822 83. Liaw A, Wiener M. 2002. Classification and Regression by randomForest. R
823 News 2:18-22.
824

825

826

827

828

829 **Figure 1. Habitat-specificity of coral reef microbiomes**. Seawater, sediment,
830 coral (*Acropora tenuis* and *Acropora millepora*), sponge (*Amphimedon*
831 *queenslandica* and *Coscinoderma matthewsi*) and macroalgae (*Sargassum sp.)*
832 samples were collected for 16S rRNA gene sequencing at fringing reefs surrounding
833 Magnetic Island (Geoffrey Bay) and Orpheus Island (Pioneer Bay and Channel;
834 Queensland, Australia). Non-metric multidimensional scaling (NMDS) based on
835 Bray-Curtis dissimilarities revealed high habitat-specificity of coral reef microbiomes.

836

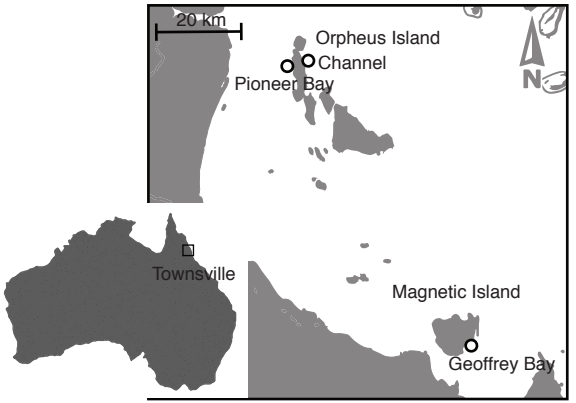
837 **Figure 2. Compositional similarity of coral reef microbiomes over time** a)
838 Variations in the compositional similarity among and within sampling time points of
839 various coral reef microbiomes collected at Geoffrey Bay (Magnetic Island). A higher
840 similarity within time point replicates than among time point replicates suggests a
841 uniform response of the microbial community to temporal variations. Similarities were
842 calculated with Bray-Curtis Similarity Index (0=no similarity, 1=high similarity) and
843 significances tested with Wilcoxon rank-sum test. b) The within sampling time point
844 similarities of replicates (n=3) is indicated in colour and the coefficient of variation
845 (dispersion) is displayed as size. Analysis of Similarity (ANOSIM) was further used
846 as a proxy for the within and among time point variation. R-values of 1 indicate high
847 similarity within sampling time points and high variability among sampling time
848 points, whereas 0 indicates equal similarity within and among sampling time points.

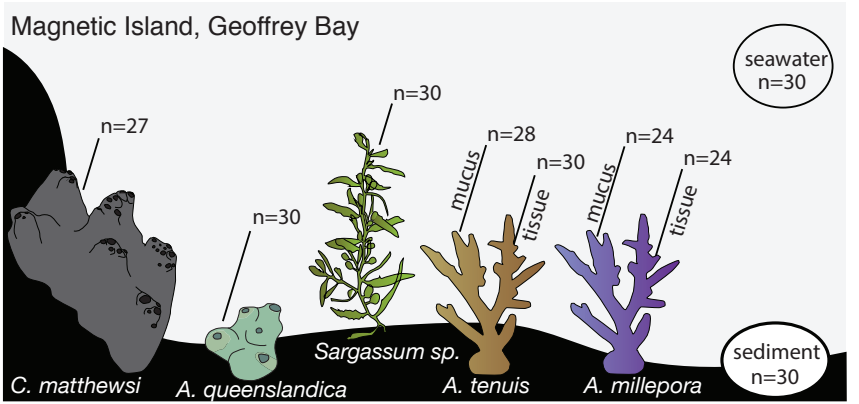
849

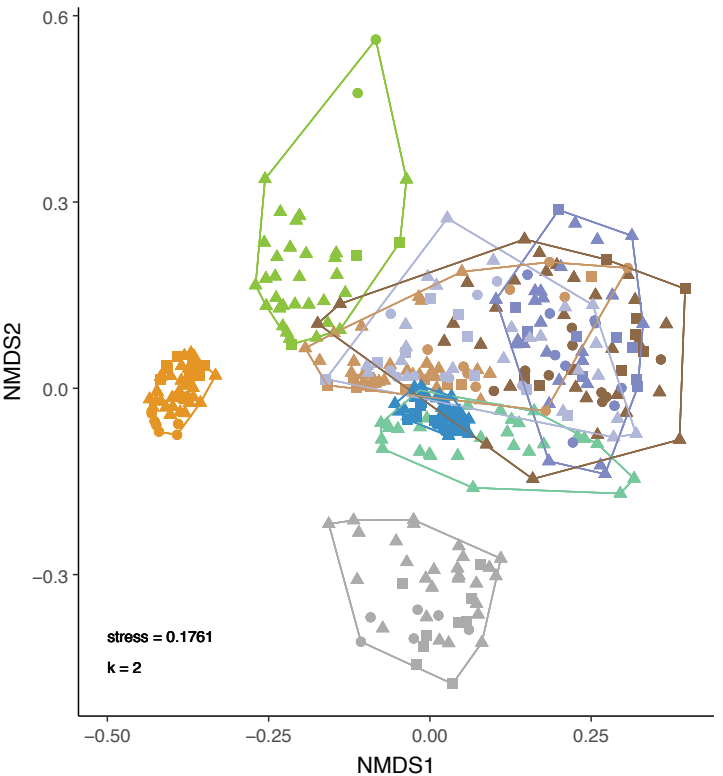
Figure 3. Coral reef microbiome sensitivity to environmental parameters. Bray-Curtis distance-based RDA (dbRDA) was used to evaluate the effect of environmental fluctuations on the microbial community composition of various coral reef habitats/hosts. a) Environmental factors significantly explained 56% of the observed compositional variation in the seawater associated microbial community. b) Variation partitioning shows that environmental parameters rather than season and sampling date explain observed community composition structures in the seawater microbiome. c) Coral mucus and algae biofilm as well as d) coral and sponge tissue microbial communities were significantly influenced by environmental factors; however, environmental parameters only explain on average 11% of the observed community variation.

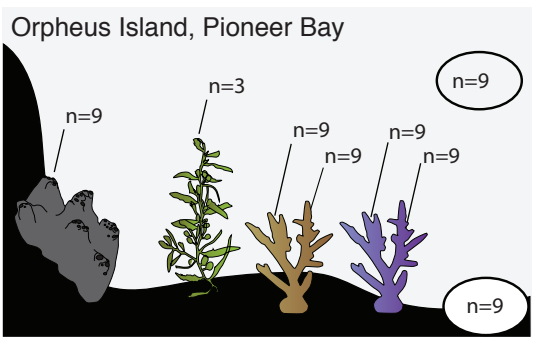
Figure 4. Microbial indicator taxa for seawater temperature fluctuations. Seawater temperatures were z-score standardised and, based on their variation around their mean, classified into low (< -0.5), average (-0.5 – 0.5) and high (> 0.5) temperature groups. Indicator zOTUs were identified with the Indicator Value analysis (IndVal). a) The average relative abundance of the sum of low, average and high temperature indicators is represented for each sampling time point. Significant indicator zOTUs assemblages ($p<0.01$) for the respective temperature group are indicated in black and size represents the coefficient of variation. Colour gradient further represents the seawater temperature at the given sampling timepoints. b) Relative abundances and taxonomic affiliation of zOTUs identified to be significant ($p<0.01$) indicators for high and low seawater temperatures. Each dot represents a unique zOTU.

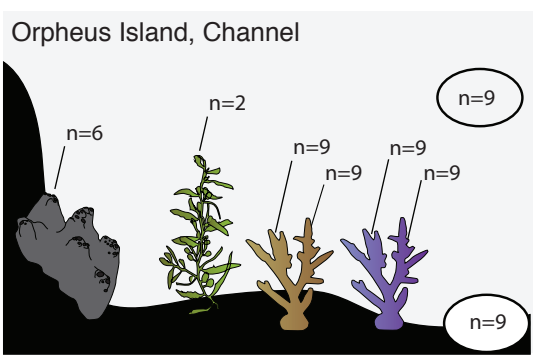
875 **Figure 5. Random Forest machine learning** a) The 30 most important zOTUs
876 reducing the uncertainty in the prediction of seawater temperature classes (low,
877 average, high) based on their mean decrease in accuracy and b) their enrichment in
878 the temperature classes. c) The 30 most important zOTUs reducing the variance
879 (mean squared error (% Inc MSE)) in regression based prediction of seawater
880 temperatures. d) Predicted seawater temperature values *versus* actual seawater
881 temperature values based on Random Forest regression.
882

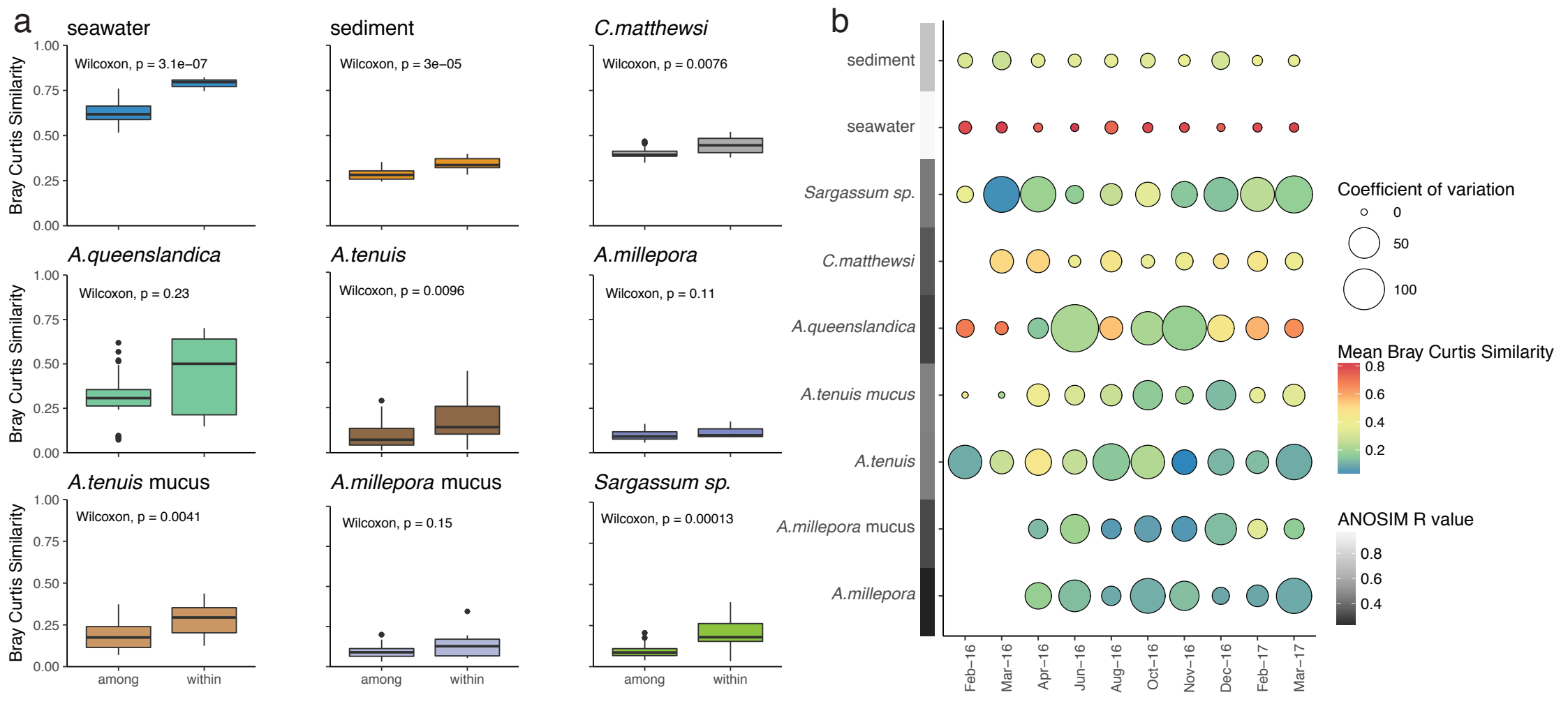

20 km
Orpheus Island
Channel
Pioneer Bay
N
Townsville
Magnetic Island
Geoffrey Bay

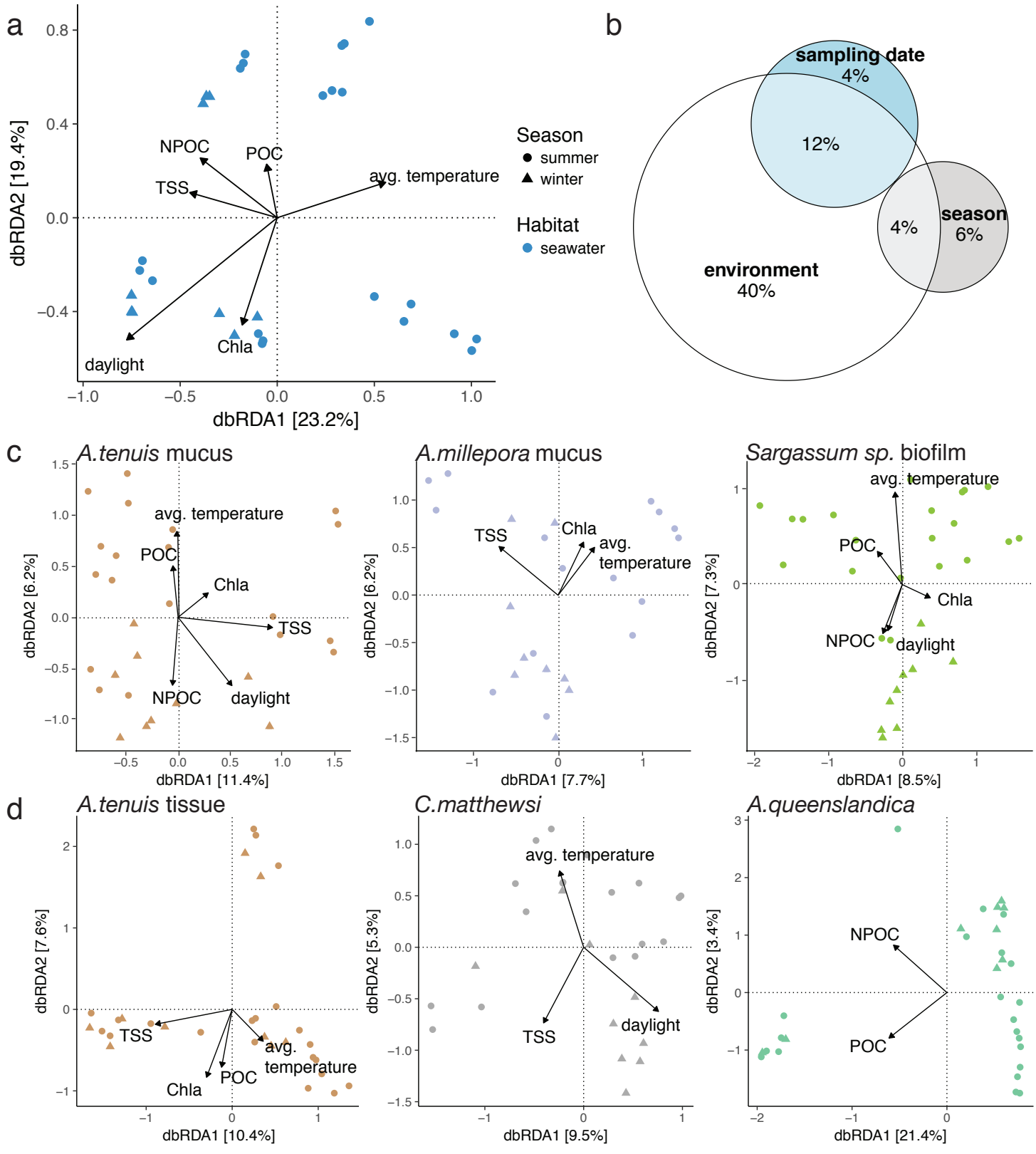

Magnetic Island, Geoffrey Bay
seawater
n=30
n=27
n=30
n=30
n=28
mucus
tissue
n=30
n=24
mucus
tissue
n=24
Sargassum sp.
sediment
n=30
C. matthewsi
A. queenslandica
A. tenuis
A. millepora

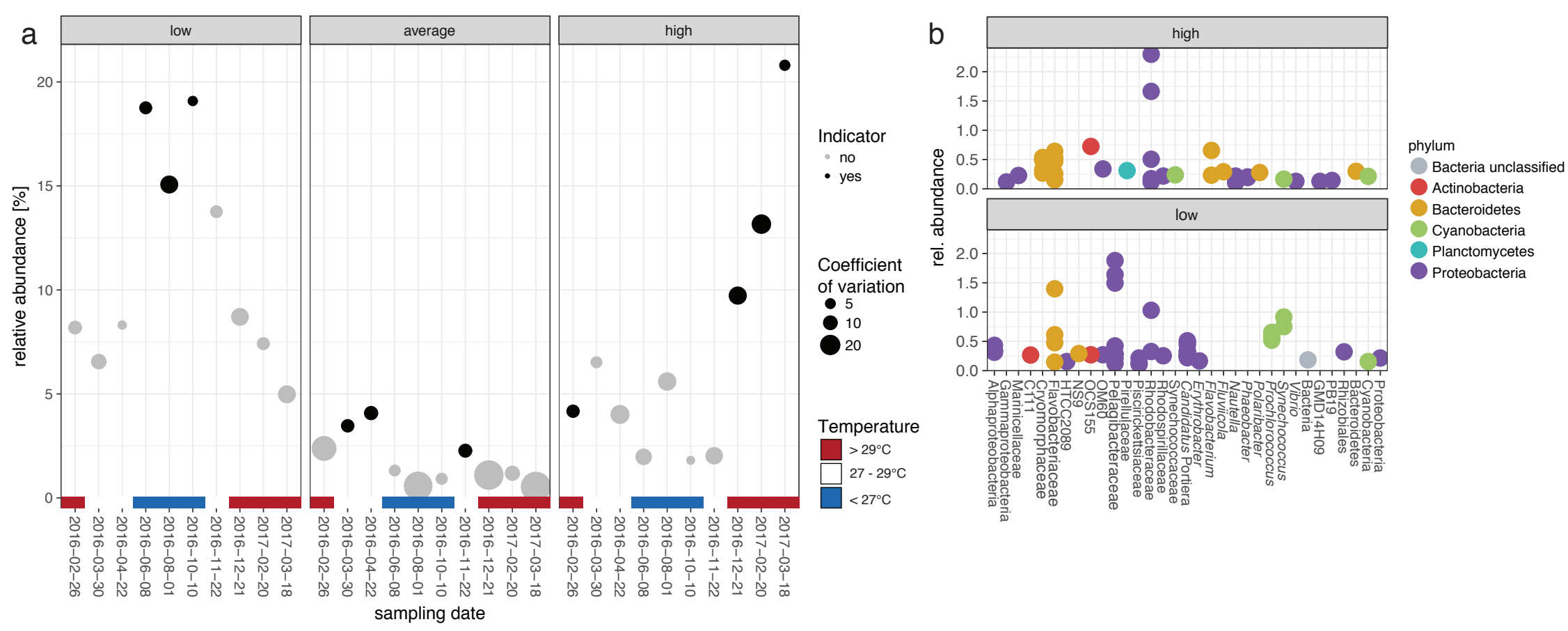

0.6
0.3
0.0
−0.3
NMDS2
−0.50
−0.25
0.00
0.25
NMDS1
stress = 0.1761
k = 2
Site
Channel
Geoffrey Bay
Pioneer Bay
Host/Habitat
sediment
Sargassum sp.
A.queenslandica
C.matthewsi
A.millepora
A.millepora mucus
A.tenuis
A.tenuis mucus
seawater

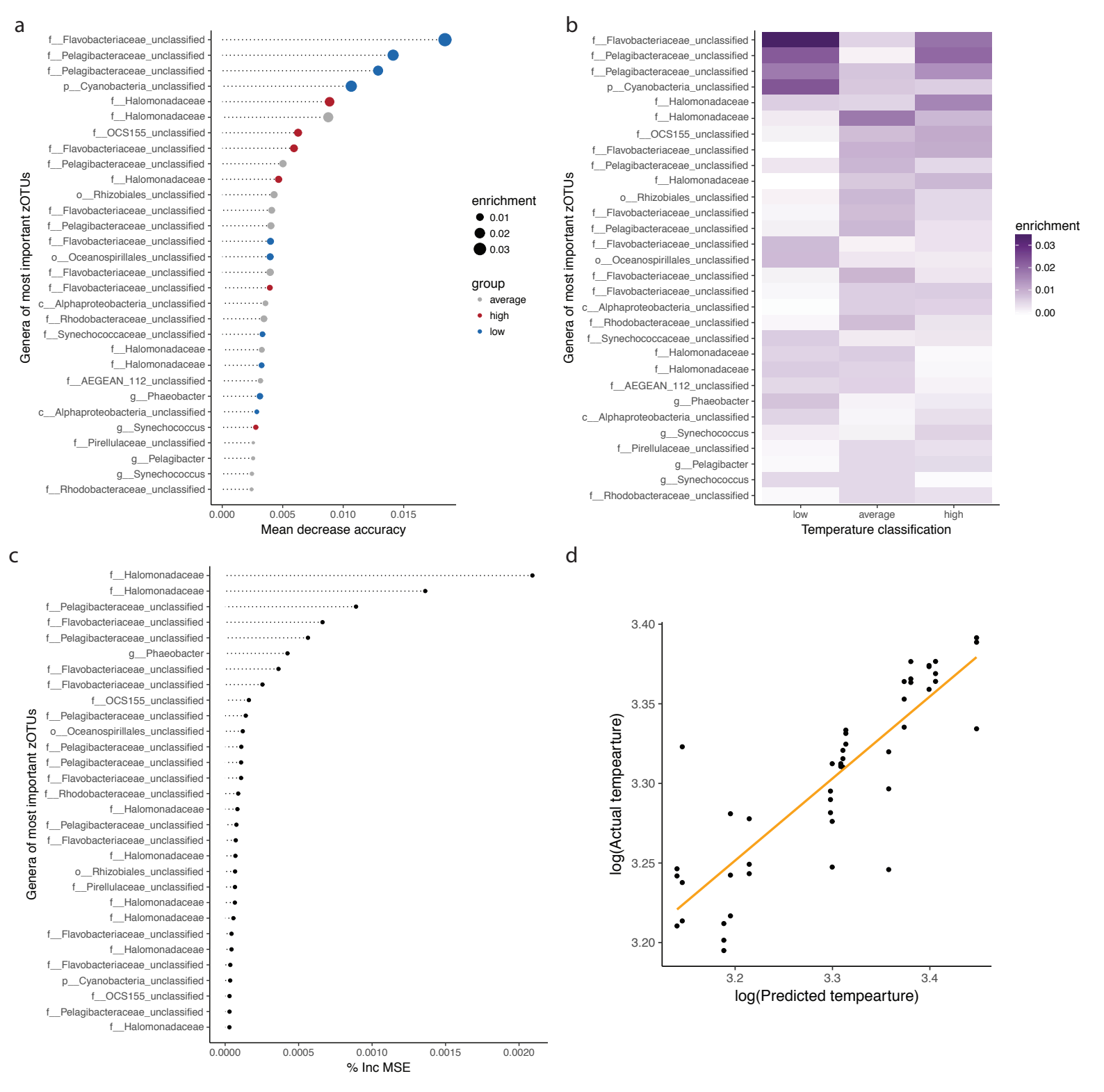

Orpheus Island, Pioneer Bay
n=9
n=9
n=3
n=9
n=9
n=9
n=9
n=9


Orpheus Island, Channel
n=9
n=6
n=2
n=9
n=9
n=9
n=9
n=9


a
seawater
Wilcoxon, p = 3.1e−07
sediment
Wilcoxon, p = 3e−05
C.matthewsi
Wilcoxon, p = 0.0076
A.queenslandica
Wilcoxon, p = 0.23
A.tenuis
Wilcoxon, p = 0.0096
A.millepora
Wilcoxon, p = 0.11
A.tenuis mucus
Wilcoxon, p = 0.0041
A.millepora mucus
Wilcoxon, p = 0.15
Sargassum sp.
Wilcoxon, p = 0.00013
Bray Curtis Similarity
1.00
0.75
0.50
0.25
0.00
among
within
b
sediment
seawater
Sargassum sp.
C.matthewsi
A.queenslandica
A.tenuis mucus
A.tenuis
A.millepora mucus
A.millepora
Feb−16
Mar−16
Apr−16
Jun−16
Aug−16
Oct−16
Nov−16
Dec−16
Feb−17
Mar−17
Coefficient of variation
0
50
100
Mean Bray Curtis Similarity
0.8
0.6
0.4
0.2
ANOSIM R value
0.8
0.6
0.4


a
NPOC
POC
TSS
avg. temperature
Chla
daylight
dbRDA2 [19.4%]
dbRDA1 [23.2%]
Season
summer
winter
Habitat
seawater
b
sampling date
4%
12%
season
6%
4%
environment
40%
c
A.tenuis mucus
avg. temperature
POC
Chla
TSS
NPOC
daylight
dbRDA2 [6.2%]
dbRDA1 [11.4%]
A.millepora mucus
TSS
Chla
avg. temperature
dbRDA2 [6.2%]
dbRDA1 [7.7%]
Sargassum sp. biofilm
avg. temperature
POC
Chla
NPOC
daylight
dbRDA2 [7.3%]
dbRDA1 [8.5%]
d
A.tenuis tissue
TSS
avg. temperature
Chla
POC
dbRDA2 [7.6%]
dbRDA1 [10.4%]
C.matthewsi
avg. temperature
TSS
daylight
dbRDA2 [5.3%]
dbRDA1 [9.5%]
A.queenslandica
NPOC
POC
dbRDA2 [3.4%]
dbRDA1 [21.4%]


a
low
average
high
relative abundance [%]
0
5
10
15
20
2016–02–26
2016–03–30
2016–04–22
2016–06–08
2016–08–01
2016–10–10
2016–11–22
2016–12–21
2017–02–20
2017–03–18
sampling date
Indicator
no
yes
Coefficient of variation
5
10
20
Temperature
> 29°C
27 - 29°C
< 27°C
b
high
low
rel. abundance
0.0
0.5
1.0
1.5
2.0
Alphaproteobacteria
Gammaproteobacteria
Marinicellaceae
C111
Cryomorphaceae
Flavobacteriaceae
HTCC2089
NS9
OCS155
OM60
Pelagibacteraceae
Pirellulaceae
Piscirickettsiaceae
Rhodobacteraceae
Rhodospirillaceae
Synechococcaceae
Candidatus Portiera
Erythrobacter
Flavobacterium
Fluviicola
Nautella
Phaeobacter
Polaribacter
Prochlorococcus
Synechococcus
Vibrio
Bacteria
GMD14H09
PB19
Rhizobiales
Bacteroidetes
Cyanobacteria
Proteobacteria
phylum
Bacteria unclassified
Actinobacteria
Bacteroidetes
Cyanobacteria
Planctomycetes
Proteobacteria


a
f__Flavobacteriaceae_unclassified
f__Pelagibacteraceae_unclassified
f__Pelagibacteraceae_unclassified
p__Cyanobacteria_unclassified
f__Halomonadaceae
f__Halomonadaceae
f__OCS155_unclassified
f__Flavobacteriaceae_unclassified
f__Pelagibacteraceae_unclassified
f__Halomonadaceae
o__Rhizobiales_unclassified
f__Flavobacteriaceae_unclassified
f__Pelagibacteraceae_unclassified
f__Flavobacteriaceae_unclassified
o__Oceanospirillales_unclassified
f__Flavobacteriaceae_unclassified
f__Flavobacteriaceae_unclassified
c__Alphaproteobacteria_unclassified
f__Rhodobacteraceae_unclassified
f__Synechococcaceae_unclassified
f__Halomonadaceae
f__Halomonadaceae
f__AEGEAN_112_unclassified
g__Phaeobacter
c__Alphaproteobacteria_unclassified
g__Synechococcus
f__Pirellulaceae_unclassified
g__Pelagibacter
g__Synechococcus
f__Rhodobacteraceae_unclassified
Genera of most important zOTUs
0.000
0.005
0.010
0.015
Mean decrease accuracy
enrichment
0.01
0.02
0.03
group
average
high
low
b
f__Flavobacteriaceae_unclassified
f__Pelagibacteraceae_unclassified
f__Pelagibacteraceae_unclassified
p__Cyanobacteria_unclassified
f__Halomonadaceae
f__Halomonadaceae
f__OCS155_unclassified
f__Flavobacteriaceae_unclassified
f__Pelagibacteraceae_unclassified
f__Halomonadaceae
o__Rhizobiales_unclassified
f__Flavobacteriaceae_unclassified
f__Pelagibacteraceae_unclassified
f__Flavobacteriaceae_unclassified
o__Oceanospirillales_unclassified
f__Flavobacteriaceae_unclassified
f__Flavobacteriaceae_unclassified
c__Alphaproteobacteria_unclassified
f__Rhodobacteraceae_unclassified
f__Synechococcaceae_unclassified
f__Halomonadaceae
f__Halomonadaceae
f__AEGEAN_112_unclassified
g__Phaeobacter
c__Alphaproteobacteria_unclassified
g__Synechococcus
f__Pirellulaceae_unclassified
g__Pelagibacter
g__Synechococcus
f__Rhodobacteraceae_unclassified
Genera of most important zOTUs
low
average
high
Temperature classification
enrichment
0.03
0.02
0.01
0.00
c
f__Halomonadaceae
f__Halomonadaceae
f__Pelagibacteraceae_unclassified
f__Flavobacteriaceae_unclassified
f__Pelagibacteraceae_unclassified
g__Phaeobacter
f__Flavobacteriaceae_unclassified
f__Flavobacteriaceae_unclassified
f__OCS155_unclassified
f__Pelagibacteraceae_unclassified
o__Oceanospirillales_unclassified
f__Pelagibacteraceae_unclassified
f__Pelagibacteraceae_unclassified
f__Flavobacteriaceae_unclassified
f__Rhodobacteraceae_unclassified
f__Halomonadaceae
f__Pelagibacteraceae_unclassified
f__Flavobacteriaceae_unclassified
f__Halomonadaceae
o__Rhizobiales_unclassified
f__Pirellulaceae_unclassified
f__Halomonadaceae
f__Halomonadaceae
f__Flavobacteriaceae_unclassified
f__Halomonadaceae
f__Flavobacteriaceae_unclassified
p__Cyanobacteria_unclassified
f__OCS155_unclassified
f__Pelagibacteraceae_unclassified
f__Halomonadaceae
Genera of most important zOTUs
0.0000
0.0005
0.0010
0.0015
0.0020
% Inc MSE
d
3.40
3.35
3.30
3.25
3.20
log(Actual tempearture)
3.2
3.3
3.4
log(Predicted tempearture)