Metagenomic views of microbial communities in sand sediments associated with coral reefs

Xiyang Dong (dongxy23@mail.sysu.edu.cn)
Sun Yat-Sen University https://orcid.org/0000-0002-9224-5923
Haoyu Lan
Sun Yat-Sen University
Liangtian Huang
Sun Yat-Sen University
Haikun Zhang
Chinese Academy of Sciences
Xianbiao Lin
Sun Yat-Sen University
Shengze Weng
Sun Yat-Sen University
Yongyi Peng
Sun Yat-Sen University
Jia Lin
Sun Yat-Sen University
Jiang-hai Wang
Sun Yat-Sen University
Juan Peng
Sun Yat-Sen University
Ying Yang
Sun Yat-Sen University

Research Article

Keywords: marine sediment, coral reef, metagenomics, microbial ecology, antibiotic resistance genes

Posted Date: August 13th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-767957/v1

Version of Record: A version of this preprint was published at Microbial Ecology on February 3rd, 2022. See the published version at https://doi.org/10.1007/s00248-021-01957-8.

Abstract

Reef sediments, the home for microbes with high abundances, provide an important source of carbonates and nutrients for the growth and maintenance of coral reefs. However, there is a lack of systematic research on the composition of microbial community in sediments of different coral reef sites and the effect of microbial functional capabilities on the coral reef ecosystem. In combination of biogeochemical measurements and metagenomics, we assessed microbial community compositions and functional diversity, as well as profiles of antibiotic resistance genes in surface sediments of 16 coral reef sites from the Xisha islands in the South China Sea. Reef sediment microbiomes are diverse and novel at lower taxonomic ranks, dominated by Proteobacteria and Planctomycetota. Most reef sediment bacteria potentially participate in biogeochemical cycling via oxidizing various organic and inorganic compounds as energy sources. High abundances of Proteobacteria (mostly *Rhizobiales* and *Woeseiales*) are metabolically flexible and contain rhodopsin genes. Various classes of antibiotic resistance genes, hosted by diverse bacterial lineages, were identified to confer resistance to multidrug, aminoglycoside, and other antibiotics. Overall, by establishing a compressive microbial genome database, our findings expanded the understanding of reef sediment microbial ecology and provided insights for their link to the coral reef ecosystem health.

Introduction

Coral reefs, composed of coral reef communities and their surrounding marine environment, are one of the ecosystems with the highest level of biodiversity and community complexity in the ocean. However, corals and the reef ecosystems they support are facing enormous environmental pressures on local and global scales, such as coral bleaching caused by global warming and ocean acidification (Albright, et al. 2016, Hughes, et al. 2017). To tackle these threats and protect coral reef ecosystems effectively, it is important to comprehensively understand the fundamental contributions of each component in the system to the stability and ecological functions of the reef ecosystems.

Reef sediments, mainly composed of permeable calcium carbonate formed by biological breakdown processes, are one of the important components of coral reef ecosystems (Eyre, et al. 2018, Janßen, et al. 2017, Shadrack, et al. 2020). Reef sediments serve as an important source of carbonate for the growth and maintenance of coral reefs. Ocean acidification will increase the consumption of calcium carbonate in the sediments, affecting the carbonate input to coral reefs (Eyre, et al. 2018, Ning, et al. 2019). Additionally, reef sediments contain carbon, nitrogen, sulfur, phosphorus, iron and other chemical elements, most of which exist in the form of nutrient salts. These nutrients can help to maintain the high biomass and primary productivity of coral reefs in low-nutrient ocean environments (Duprey, et al. 2016, Ning, et al. 2019).

Microorganisms are the most abundant organisms in coral reef ecosystems. They are widely distributed in corals, sponges, coral mucus, surrounding water and sediments. By promoting primary production and remineralization of organic matter, microorganisms are critical to the biogeochemical cycling of various

elements in the coral reef ecosystem (Glasl, et al. 2019, Pernice, et al. 2020, Robbins, et al. 2019). To date, the research on the microbiome of coral reef ecosystems has focused on assessing microbial diversity and function related to corals, sponges or other invertebrate-related symbiotic bacteria and surrounding water (Glasl, et al. 2019, Glasl, et al. 2020, Matthews, et al. 2020, Pearman, et al. 2019), while less attention has been paid to the microbes in sediments. In fact, there are about 10,000 times more microbes in the surface sediments of coral reefs than in the seawater around them (Koren and Rosenberg 2006). Reef sediment microorganisms can affect the budget of sedimentary calcium carbonate by fixing and producing carbon dioxide (Kessler, et al. 2020). They are also directly involved in the recycling and utilization of nutrients such as carbon, nitrogen, phosphorus, and sulfur in the sediments (Glasl, et al. 2019). However, in general, there is a lack of systematic research on the composition of microbial species in the sediments of different coral reef sites and the effects of microbial functional characteristics on the coral reef ecosystem.

Antibiotic resistance genes (ARGs) have been detected in almost every environment studied (Cuadrat, et al. 2020, Van Goethem, et al. 2018, Zhao, et al. 2020). They are either endemic to natural environments or derived from human-dominated ecosystems. To date, however, it remains unclear about the diversity and hosts of ARGs in reef sediments. Highly abundant microorganisms mean that reef sediments might be potential reservoirs for ARGs. Additionally, reef ecosystems can become contaminated with antibiotics and ARGs from inputs from human and agricultural waste including urban surface run-off and effluent discharges (Ahasan, et al. 2017, Chen, et al. 2019). A case study in the coral reef regions in the South China Sea detected eighteen antibiotics and seven ARGs in the surface water with a potential link to anthropogenic activities (Liu, et al. 2020). Continuous discharge of antibiotics makes them pseudo persistent organic pollutants and their residues in aquatic systems will bind to particles and deposit to the sediment (Vizcaino, et al. 2010). These released antibiotics and ARGs may finally threaten the growth of coral and affect sedimentary microbial ecology. Thus, studying ARGs in reef sediments is an important part of assessing the coral reef ecosystem health.

The Xisha islands in the South China Sea cover an area of more than 500,000 km^2, with a total land area of about 10 km^2 and a coastline of 518 km (Fig. 1). Most islands are surrounded by large coral reefs rich in marine biological resources, with sediments being made of coral debris or sand grains. In this study, we selected 16 coral reef site surface sediment samples in the Xisha islands, based on their high coral species richness. The good quality and healthy coral reef made them ideal regions to provide baselines of the taxonomic, functional, and resistome diversity of reef sediment microbiomes. By combining biogeochemical characterization of reef sediments with an integrated metagenomic analysis of their microbial communities, we established a comprehensive genome database for reef sediment microbiomes and provided insights into the link between sediment microbiomes and coral reef ecosystem health.

Results And Discussion

Characteristics of coral reef sediments and overlying water

We collected 16 surface permeable sediment samples (0–5 cm) at water depths of 1.5–74 m from the reef slopes of different islands located in the Xisha Islands (Fig. 1). The physiochemical parameters of overlying seawater were quite different **(Table S1)**, including water temperature (20.6–32.3°C), salinity (30.30-30.98 ppt), pH values (7.81–8.15), dissolved oxygen (0.85–7.18 mg L^{-1}), and oxidation-reduction potential (176.2-242.5 mV). This is possibly related to various sampling depths. For example, the highest dissolved oxygen was observed at the shallowest sampling site (1.5 m, Zhaoshu Island) while lowest at the deepest (74 m, Yongle Blue Hole). Overall, these physicochemical parameters of water samples can sever as an imperfect proxy for the properties of surface sediment samples. For permeable carbonate sediments, water contents were relatively high (29–48%), with total inorganic carbon (13.2–14.5%) being the major component of the total carbon (**Table S2**). Sediment ammonium concentrations (1.8–8.8 μg N g^{-1}) were significantly higher than nitrate (0-1.13 μg N g^{-1}) or nitrite (0.01–0.02 μg N g^{-1}). These sediments were rich in sulfate and phosphate, corresponding to 0.8–2.5 mg S g^{-1} and 3.4–6.4 μg P g^{-1}, respectively.

Reef sediment microbiomes are highly diverse and dominated by Proteobacteria and Planctomycetota

To estimate coral reef sediment microbiome, we extracted genomic DNA from the 16 reef sediments and performed metagenomic sequencing. Alpha diversities of bacterial and archaeal communities in reef sediments were profiled based on 14 single-copy marker genes (Woodcroft, et al. 2018). Alpha diversity indicated diverse and rich microbial community members inhabited in these sediments, as evidenced by high values of Shannon (6.87 ± 0.06), Simpson (0.9988 ± 0.0001), and Chao1 (4478 ± 1012) across all the samples (**Figure S1**). Similar results were also reported in Great Barrier Reef sites where sediments showed the highest Shannon index (7.4 ± 0.2) among multiple coral reef microbiomes (i.e., seawater, sediment, corals, sponges and macroalgae) based on the 16S rRNA gene amplicon sequencing (Glasl, et al. 2019).

To assess the overall microbial community structure in these sediments (Fig. 2a), we retrieved and classified shotgun metagenomic reads of the universal single-copy ribosomal protein gene *rplB* (Boyd, et al. 2018, Ortiz, et al. 2020). The dominant community members from bacterial phyla were Proteobacteria (classes of *Gammaproteobacteria* and *Alphaproteobacteria*, on average 24.5% of the whole community) and Planctomycetota (18.7%), followed by Desulfobacterota (6.0%). Different from previous reports using 16S rRNA gene amplicon sequencing focusing on bacterial community in carbonate sands (Hernandez-Zulueta, et al. 2016, Kegler, et al. 2017, Polonia, et al. 2015, Schottner, et al. 2011), we also observed high numbers of archaeal members (6.9%) in this ecosystem, comprising Thermoproteota (5.3%, mainly the order of *Nitrososphaerales*) and Nanoarchaeota (1.1%). Members from the order *Nitrososphaerales* were predicted to be responsible for aerobic ammonia oxidization in the carbonate sediment of a coral reef in Kaneohe Bay, Hawaii (Rusch and Gaidos 2013). Beta diversity analysis based on the *rplB* OTU table using Bray-Curtis dissimilarity confirmed taxonomic compositions of these microbial communities were largely

similar among different sampled depths or island sites within the Xisha Islands (p = 0.499, **Figure S2**). However, physicochemical parameters did explain the minor differences at the phylum level in microbial communities, such as depths, nitrate, ammonium, and temperatures, with depth being the strongest correlates (Fig. 2b).

Assembly and binning of metagenomes resulted in 273 metagenome-assembled genomes (MAGs, with > 50% completeness and < 10% contamination) dereplicated at species level, that represented 9–16% of the whole community (**Table S3**) based on the genus-level recovery estimates (Singleton, et al. 2021). The high strain heterogeneity level (average, 40% based on checkM) possibly explained the relatively low recovery of MAGs (Bowers, et al. 2017). These MAGs included 270 bacterial and three archaeal MAGs, assigned to 21 phyla and at least 65 orders (Fig. 3a). The degree of taxonomic novelty in this MAG dataset increased towards lower taxonomic ranks, with 59% of MAGs having an unassigned genus and only one MAG being classified at the species level (Fig. 3b). In the Bacteria domain, members of Proteobacteria (n = 114, mainly belonging to classes of *Gammaproteobacteria* and *Alphaproteobacteria*), Actinobacteriota (n = 33, mainly *Acidimicrobiia*), Desulfobacterota (n = 27, mainly *Desulfobacteria*), and Planctomycetota (n = 27, mainly *Planctomycetes*) were highly represented. As for the Archaea domain, *Nitrososphaeria* (n = 2) and *Bathyarchaeia* (n = 1) were reconstructed. The relative abundance of each species MAG was generally at low level, mostly below 0.5% of the community (**Table S3 and Figure S3**). The highest abundances were observed for members from *Gammaproteobacteria* XS9-1 and XS8-1 (0.46% and 0.23 % of the community on average, respectively), and *Desulfobacterota* XS12-4 and XS16-14 (0.27% and 0.25%, respectively). Taxonomic comparisons of these sediment MAGs with bacterial and archaeal MAGs recovered from microbial symbionts or seawater microbiome of coral or sponge species showed that these communities were distinct and possibly experienced different biogeochemical cycling patterns (Engelberts, et al. 2020, Glasl, et al. 2020, Robbins, et al. 2019, Robbins, et al. 2021).

Reef sediment bacteria harbor the flexibility to oxidize various organic and inorganic compounds as energy sources

The marine sediments of the first five centimeters are often stratified and structured, with a mix of various microbial processes driven by the nature of chemical gradients of these sediments (Chen, et al. 2017). To explore the main representative biogeochemical cycling in reef sediments, we screened key metabolic genes in the microbial reference gene catalog (**Table S4; see Methods**) for metabolic functions involved in aerobic respiration, carbon fixation, nitrogen cycling, phototrophy, sulfur cycling, and urea utilization. Marker gene abundances in each metagenome were divided by the averaged abundances of 14 universal single-copy ribosomal genes in this catalog as a proxy for the percentage of microbial cells encoding each function (**Table S5 and** Fig. 4a) (Acinas, et al. 2021, Bay, et al. 2021). Presence of these metabolic marker genes were also predicted for reconstructed MAGs to infer their microbial hosts (**Table S6 and** Fig. 4b).

In accordance with dissolved oxygen levels (**Table S1**), the community appears to be dominated by lineages capable of aerobic respiration (**Table S5 and** Fig. 4a), with the aid of three different types of

respiratory oxygen reductases including *cbb*$_3$-type cytochrome c oxidase (43.9% of the microbial community cells; on average), *bd*-type cytochrome (quinone) oxidase (32.5%), and *caa3*-type cytochrome c oxidase (18.9%). Both *cbb3*-type cytochrome c oxidase and *bd*-type cytochrome (quinone) oxidase can be functional under low-oxygen conditions. This is beneficial to those microorganisms, as reef sediments often shift between oxic and anoxic habitats over short distances and timescales due to porewater advection and physical disruptors as induced by e.g. waves and currents (Huettel, et al. 2014, Rusch and Gaidos 2013). Additionally, other terminal electron acceptors were predicted that potentially allowed the growth of microbial cell under anoxic conditions, including inorganic compounds detected in the sediments (**Table S2**) like nitrate (*napA*, 15.1%; *narG*, 6.1%), nitrite (*nirK*, 1.4%; *nirS*, 15%; *octR*, 5.8%) and sulfate (reductive *dsrA*, 11%), and possibly gaseous compounds such as nitric oxide (*norB*, 11.2%) and nitrous oxide (*nosZ*, 4.8%). Functional annotations of reconstructed MAGs suggest that many bacterial members encoded partial enzymes for several steps in a dissimilatory denitrification pathway, but none were found to carry out all the steps for the whole sequential redox transformations (**Table S6 and** Fig. 4b), as also observed in other ecosystems, e.g. groundwater (Anantharaman, et al. 2016). Genes encoding dissimilatory sulfite reductases (reductive *dsrA* genes) were mainly found in genomes of typical sulfate-reducing taxa Desulfobacterota as well as Planctomycetota that awaits experimental proof (Anantharaman, et al. 2018). Genes encoding methyl-coenzyme reductase (*mcr*) were absent in the gene catalog, suggesting the potential lack of methanogenic groups.

The electron donors for aerobic respiration can be organic compounds but can also be inorganic compounds such as CO (*coxL*, 11.4%), sulfide *(sqr*, 2.4%; oxidative *dsrA*, 7.3%; *sor*, 0.4%), nitrite (*nxrA*, 7.4%), thiosulfate (*soxB*, 0.9%), and ammonium (*amoA*, 0.1%). These relatively high proportions of encoded genes point to the oxidation of organic substrates as an important energy supplement for heterotrophs in the reef sediments (Kessler, et al. 2019). Aerobic CO oxidation is proposed to be of major importance in changeable environments such as grassland and rainforest soils, coastal and mesopelagic seawater, and salt marshes (Cordero, et al. 2019). In benthic zone like reef sediments, CO may be produced through photochemical organic matter degradation or by benthic algae (King 2007). In line with previous literature (Cordero, et al. 2019), the oxidation of carbon monoxide was most likely carried out by members from Acidobacteriota, Actinobacteria, *Alphaproteobacteria* and *Gammaproteobacteria* based on the occurrence of CO dehydrogenase in their genomes (Fig. 4b). The detection of multiple pathways for sulfide and thiosulfate oxidations at high abundances suggests that reduced sulfur is another important energy source that potentially sustains multiple ecological niches in reef sediments. Reflecting this, abundant taxa *Gammaproteobacteria* (*sqr* and oxidative *dsrA*) and *Alphaproteobacteria* (*soxB*) potentially performed sulfide and thiosulfate oxidation, respectively (**Table S6 and** Fig. 4b). The genes encoding nitrate reductase/nitrite oxidoreductase related to nitrite oxidation were not found in the genome of typical nitrite oxidizing bacteria Nitrospirota (Park, et al. 2020) possibly due to genome incompleteness, but in Planctomycetes, Actinobacteriota, Verrucomicrobiota, Methylomirabilota and *Gammaproteobacteria*. The functional annotations of MAGs also highlighted that members from Proteobacteria (mostly orders of *Rhizobiales* and *Woeseiales*) tended to be the most highly metabolically flexible (Fig. 4), explaining their high abundance and accommodating environmental fluctuations in

electron acceptor availability, in agreement with other studies on sand sediments (Buongiorno, et al. 2020, Dyksma, et al. 2016).

In agreement with the total inorganic carbon concentrations (**Table S2**) and the presence of autotrophs, pathways for inorganic carbon fixation were predicted to be primarily through the Wood-Ljungdahl pathway (*cdhD*, *cdhE* and *cooS*; 9.4% on average), and the Calvin-Benson-Bassham (CBB) cycle (*rbcL*, 6.9%; mostly Form I type), followed by the reductive tricarboxylic acid (rTCA) cycle (*aclB*, 0.7%). The genes for the Wood-Ljungdahl pathway were mostly found in the genomes of Desulfobacterota, with encoded enzymes possibly also operating in reverse process under anoxic conditions (Ragsdale and Pierce 2008). The complete reductive tricarboxylic acid cycle was only identified in a Nitrospirota MAG. We identified 15 MAGs encoded the ribulose bisphosphate carboxykinase: one Actinobacteriota, one Cyanobacteria, one Methylomirabilota, 12 *Gammaproteobacteria* MAGs. As expected (Sanchez-Baracaldo and Cardona 2020), the one MAG from Cyanobacteria was also found to encode two photosystems (PS I and PS II) based on the photosynthesis marker genes, *psaA* and *psbA*. These two genes are also very abundant in certain sites possibly due to the extracellular or relic DNA. PS II sequences were also detected in seven alphaproteobacterial (e.g. *Rhodobacterales*, *Rhizobiales*, and *Sphingomonadales*) MAGs with PS I sequences found in one *Coxiellales* MAG, potentially serving as anoxygenic phototrophs. This is consistent with the expectation that carbon fixation is primarily driven by chemoautotrophs rather than photoautotrophs in coastal sediments (Dyksma, et al. 2016). Putative energy-converting rhodopsins have been shown to be among the most widespread genes in the photic zone worldwide (Haro-Moreno, et al. 2018). They are very diverse and are distributed throughout most taxa, present in several of the most dominant orders of Desulfobacterota, Planctomycetota, Actinobacteriota, Proteobacteria, Bacteroidota and Chloroflexota in these sediments.

Efficient nitrogen and sulfur acquisition are important for microorganisms to thrive in nutrient-poor environments. Diazotrophic bacteria can convert gaseous dinitrogen to ammonia (Bednarz, et al. 2015, Devol 2015), which comprised 3.2% of microbial cells on average in reef sediments. Searches for the nitrogen fixation marker gene *nifH* identified four MAGs that fell into non-cyanobacterial clades from orders of UBA8473, *Desulfobulbales*, *Polyangiales*, and *Chromatiales*. Putative nitrogen fixation capabilities for these microorganisms were also found in surface waters of the open ocean based on the TARA Oceans metagenomes (Delmont, et al. 2018, Salazar, et al. 2019). Additional nitrogen may come from organic compounds present in the surrounding seawater, e.g. the hydrolysis of urea into carbon dioxide and ammonia (Robbins, et al. 2019). Genes encoding urease *α*, *β* and *γ* subunits (*ureABC*) were identified in 14.2–20.4% of microorganisms and in MAGs mostly from *Alphaproteobacteria* and *Gammaproteobacteria*, e.g. *Rhizobiales* and UBA4575. Dissolved sulfate constitutes the main source of sulfur for the sediment microbiome (**Table S2**). Taurine dioxygenases used for sulfite production from organic sulfur molecules e.g. animal tissues (Schuller-Levis and Park 2003) were identified in 17% of microbial cells, corresponding to 49 MAGs mostly assigned to the phylum of Proteobacteria, highlighting another potential source of sulfur provided to the sedimentary microbial community. Dimethylated sulfur compounds e.g. dimethyl sulfoxide (DMSO) are particularly abundant in coral reef and permeable coral reef carbonate sediments (Deschaseaux, et al. 2019). Accordingly, genes for anaerobic dimethylsulfoxide

reductase (*dmsA*) that converts DMSO to dimethyl sulfide DMS were also identified in Proteobacteria, Desulfobacterota, Planctomycetota, and Bacteroidota genomes, accounting for 1.9% of total microbial cells and providing an alternative energy source under anoxic conditions (Vigneron, et al. 2021).

Diverse bacterial linages potentially harbor antibiotic resistance genes

We further screened for the abundance and diversity of ARGs in the microbial gene catalogue. A total of 819 hits were annotated as ARG-like sequences in the 16 samples **(Table S7)**. The 819 ARG-like genes were assigned to 51 ARG subtypes belonging to 14 ARG classes, with high proportions being unclassified (Fig. 5a **and Table S8)**. Among the classified types, the total ARG abundance of different types ranged from 0.01 to 10.3% of total microbial cells. The overall numbers of ARG classes and subtypes are surprisingly larger than those identified in sediments related to river and mangrove, which were heavily influenced by anthropogenic activities (Imchen and Kumavath 2021, Li, et al. 2021). Main ARG classes detected in river and mangrove sediments were genes resistant to multidrug and macrolide-lincosamide-streptogramin (MLS) antibiotics (Imchen and Kumavath 2021, Li, et al. 2021). In contrast, among our samples, the top four most abundant ARG classes were beyond 1% as compared to total microbial cells, including genes conferring resistance to multidrug (resistance to at least three classes of antibiotics; 10.3%), aminoglycoside (5.3%), tetracycline (3.2%), and fosfomycin (1.6%). The ARG composition in reef sediments is also different from that in pristine marine sediments e.g., from deep ocean with lower diversity of ARGs dominated by genes related to polypeptide resistance (Chen, et al. 2013). The highest abundant identified ARG was predicted to be resistant to cAMP receptor protein (unclassified; 14.2%), followed by AAC(3)-I (aminoglycoside; 2.9%), truncated putative response regulator ArlR (unclassified; 2.7%) and OmpR (multidrug; 2.1%). These findings suggest that reef sediments in the Xisha islands are possibly contaminated by anthropogenic ARGs to a certain extent.

Among the 273 recovered MAGs, a total of 110 MAGs were identified to carry ARGs **(**Fig. 5b **and Table S9)**. These MAGs were assigned to 11 phyla and the hosts of ARGs mostly belonged to the phylum Proteobacteria (69 MAGs), which contained two classes *Gammaproteobacteria* (50 MAGs) and *Alphaproteobacteria* (19 MAGs). Moreover, 44 MAGs were found to carry at least two ARGs. For example, Cobin198, a MAG assigned to *Sphingomonas paucimobilis* harbored 11 ARGs conferring resistance to multidrug, aminoglycoside, peptide, and fosmidomycin. *Sphingomonas* species have been linked to the death of coral reefs off the coast of Florida (Richardson, et al. 1998). However, this MAG had a low relative abundance (average 0.12%) compared to other MAGs. *Methyloceanibacter* Cobin10 and *Tardiphaga* Cobin107 encoded five and eight ARGs from both multidrug and fosmidomycin, respectively. The diverse hosts of ARGs with potential multiple antibiotic resistance suggested that these bacteria are reservoirs of ARGs and may play a critical role in the acquisition and spread of antibiotic resistance in reef sediments.

Conclusions

Previously studies largely ignored the role of sediment microbiome in maintaining the stability of coral reef ecosystems. Here, our investigation of the microbiome composition and functionality in reef sediments from the Xisha islands suggest that reef sand microbiomes are highly diverse with dominance of members from Proteobacteria and Planctomycetota, along with vast yet uncultured majority of microorganisms. They have potential roles in overall nutrient recycling of coral reef ecosystems, including aerobic respiration, carbon fixation, as well as nitrogen and sulfur cycling. Another important finding of this study is that bacterial lineages harbor various antibiotic resistance genes, emphasising their potential influence in the coral reef ecosystem health. Overall, this study provides a valuable genome reference database for environmental assessments and public policy making on coral reef protection. However, further continuous sampling of these as well as additional sites at the different depths and conditions are still needed to track the interactions between reef sediment microbiome, reef ecosystems and humans.

Materials And Methods

Sampling and characterization of seawater quality

Surface sediments (0–5 cm) were taken in April 2019 from 16 coral reefs sites in the Xisha islands (Fig. 1): Lingyang Reef (water depth of 16 m), Langhua Reef (7.5 m, 12 m, 25 m, 41 m), Huaguang Reef Cliff (25 m), Huaguang Reef (29 m), Jinqing Island (13.4 m), East Island (47.8 m), North Reef (16 m, 27 m), Panshi Island (73 m), Ganquan Island (26 m), Zhaoshu Island (1.5 m), Zhongjian Island (24 m), and Yongle Blue Hole (74 m). All samples were collected using Falcon 50 mL conical centrifuge tubes and immediately stored at -80 ^{o}C. A variety of parameters of the overlying seawater were measured *in situ* with ProDSS multiparameter water quality meter (YSI, Yellow Springs Instruments Inc., USA), including temperature, salinity, pH, dissolved oxygen, and oxidation-reduction potential.

Characterization of coral reef sediments

Sediment water contents were calculated gravimetrically from fresh sediment dried at 60°C to a constant value (Wang, et al. 2016). Sediment exchangeable phosphate was extracted from fresh sediments with 1 M HCl and measured colorimetrically by the ascorbic acid-molybdate blue method (Hou, et al. 2008). Sediment exchangeable ammonium, nitrite, and nitrate were extracted by 2 M potassium chloride (purged with N_2) according to a previous protocol (Hou, et al. 2013), and measured by a continuous flow nutrient analyzer (Futura, Alliance, France). Sulfate concentrations were determined colorimetrically with barium chloride (Roy, et al. 2011). The sediment total carbon (TC) and total organic carbon (TOC) were analyzed using an elemental analyzer (ELTRA CS 800, German), and the freeze-dried sediments for TOC measurements were treated with 3 M hydrochloric acid for 48 h to remove inorganic carbon (Xu, et al. 2018).

DNA extraction and metagenome sequencing

Genomic DNA for metagenomic sequencing was extracted from the untreated sediments using the cetyltrimethylammonium bromide (CTAB) method (Zhou, et al. 1996). DNA concentrations were

quantified through Qubit Fluorometer and agarose gel electrophoresis was used to examine DNA quality. Metagenomic shotgun libraries were prepared following the manufacturer's instructions (Illumina Inc.) and subject to paired-end sequencing (2×150 bp) on an Illumina Novaseq 6000 platform at Berry Genomics Co.Ltd., Beijing. Each sample generated ~ 20 Gb of raw data.

Microbial community profiling

For each of 14 universal single copy genes, operational taxonomic units (OTUs) were extracted from raw metagenomic reads using SingleM v0.13.2 (https://github.com/wwood/singlem). Alpha diversity (Chao1, Simpson and Shannon) of microbial communities was calculated using vegan package v2.5 based on the SingleM OTU table across each of the 14 single copy marker genes (Woodcroft, et al. 2018). Community composition profiles in sequenced metagenomes were generated based on metagenomic reads of the single-copy marker gene *rplB* with reference to GTDB R05-RS95. For the beta diversity analysis using *rplB* OTU table, Bray-Curtis dissimilarity was calculated and visualized using a non-metric multidimensional scaling ordination (NMDS) plot.

Metagenomic assembly and binning

Raw reads were quality-controlled by clipping off primers and adapters, and filtering out artifacts and low-quality reads using Read_QC module within the metaWRAP pipeline v1.2.2 (Uritskiy, et al. 2018). The 16 quality-controlled metagenomes were individually assembled using MEGAHIT v1.1.3 with default parameters (Li, et al. 2016). Each of the 16 assemblies was binned using the binning module (-metabat2 -maxbin2 -metabat1) and consolidated using the Bin_refinement module (-c 50 -x 10) within the metaWRAP pipeline. Additionally, the 16 metagenomes were co-assembled using MEGAHIT (-k-min 27 -kmin-1pass). The co-assembly was binned using the binning module (-metabat2 -metabat1) and consolidated using the Bin_refinement module (-c 50 -x 10) within the metaWRAP pipeline. The produced 17 bin sets were aggregated and de-replicated using dRep v2.5.4 at 95% average nucleotide identities (Olm, et al. 2017), for species level (Jain, et al. 2018, Olm, et al. 2020). SingleM was used to determine genome recovery efforts at genus level (singlem appraise -imperfect -sequence_identity 0.89).

After dereplication, the taxonomy of each MAG was assigned using GTDB-Tk v1.3.0 with the Genome Taxonomy Database (GTDB) (Release 05-RS95) (Chaumeil, et al. 2019). Completeness, contamination, and heterogeneity of each bin were estimated using CheckM. Numbers of rRNAs and tRNAs of each MAG were predicted based on DRAM (Shaffer, et al. 2020). Relative abundance of MAGs depreciated at species level was calculated with CoverM v0.4.0 (https://github.com/wwood/CoverM), with parameters specified as follows: --min-read-percent-identity 0.95 --min-read-aligned-percent 0.75 --trim-min 0.10 --trim-max 0.90.

Functional annotations

To generate the reference gene catalog for microbial communities in sand sediments associated with coral reefs, metagenomic contigs from 16 single-sample assemblies and one co-assembly were annotated using MetaErg v1.2.1 (Dong and Strous 2019). All the predicted genes were pooled (n = 10,732,682) and clustered at 95% of sequence similarity and 90% alignment coverage of the shorter

sequence using *cd-hit-est* option in CD-HIT v 4.8.1 (Acinas, et al. 2021, Fu, et al. 2012). The parameters are as follows: -c 0.95 -T 0 -M 0 -G 0 -aS 0.9 -g 1 -r 1 -d 0. This produced 7,925,822 nonredundant gene clusters, with the longest sequence of each cluster being selected for downstream analysis. Contigs in MAGs were also annotated using MetaErg.

Predicted genes from microbial reference gene catalog and MAGs were assigned with metabolic potential functions for the main biogeochemical cycles based on METABOLIC v4.0 (Zhou, et al. 2020). DIAMOND *blastp* algorithm was used against reference datasets (Ortiz, et al. 2020) with a minimum percentage identity of 50% for identification of genes for photosystem I reaction centre protein (PsaA), photosystem II reaction centre protein (PsbA), and microbial rhodopsin (energy-converting type, RHO). The screening for ARGs was performed with DeepARG-LS using gene models (Arango-Argoty, et al. 2018). GraftM v0.13.1 was used to identify 14 universal single-copy ribosomal genes (Boyd, et al. 2018). For the phylogenetic analysis of dissimilatory sulfite reductase subunit A (DsrA), amino acid sequences were aligned using the MUSCLE algorithm (Edgar 2004) included in MEGA X (Kumar, et al. 2018). All positions with less than 95% site coverage were eliminated. The bootstrapped maximum-likelihood phylogenetic tree was constructed in MEGA X based on the JTT matrix-based model with 50 replicates.

To quantify gene abundance from the reference gene catalog in different metagenomes, Salmon v1.4.0 (Patro, et al. 2017) was used in mapping-based mode (salmon quant, –validateMappings –meta). The TPM of each metabolic gene was divided by averaged TPM across the 14 single-copy ribosomal genes, to find the estimated percentage of the community with the gene, assuming one copy per genome.

Declarations

Data availability

DNA raw reads have been deposited in NCBI BioProject databases with accession number PRJNA724996. Individual assembly for metagenome-assembled genomes can be found at figshare: https://figshare.com/s/cd5bfb6be8cdadb6eb60. The authors declare that all other data supporting the findings of this study are available within the article and its supplementary information files, or from the corresponding authors upon request.

Acknowledgements

The work was supported by the National Natural Science Foundation of China (No. 41906076), the Fundamental Research Funds for the Central Universities (No. 19lgpy90), the Science and Technology Projects in Guangzhou (No. 202102020970), and the Regional Marine Geological Survey Projects of China Geological Survey (DD20191002). We gratefully acknowledge the sampling work by cruise members of Xisha Comprehensive Scientific Expedition in 2019, as part of the South China Sea Scientific

Expedition Program led by Sun Yat-sen University. We thank Guihao Li for help with biogeochemical measurements.

Author contributions

XD designed this study. XD, HL, LH, SW and YP analyzed metagenomic data and prepared figures. HL, XL, JL and JHW performed biogeochemical measurements. XD, YY and HZ contributed to ARG analyses. JP performed sampling. XD, YY and HZ wrote the paper that was read, edited and approved by all authors.

Conflicts of interest

The authors declare that they have no conflict of interest.

References

Acinas SG, Sanchez P, Salazar G et al (2021) Deep ocean metagenomes provide insight into the metabolic architecture of bathypelagic microbial communities. Commun Biol 4:604

Ahasan MS, Picard J, Elliott L et al (2017) Evidence of antibiotic resistance in Enterobacteriales isolated from green sea turtles, Chelonia mydas on the Great Barrier Reef. Mar Pollut Bull 120:18–27

Albright R, Caldeira L, Hosfelt J et al (2016) Reversal of ocean acidification enhances net coral reef calcification. Nature 531:362–365

Anantharaman K, Brown CT, Hug LA et al (2016) Thousands of microbial genomes shed light on interconnected biogeochemical processes in an aquifer system. Nat Commun 7:13219

Anantharaman K, Hausmann B, Jungbluth SP et al (2018) Expanded diversity of microbial groups that shape the dissimilatory sulfur cycle. ISME J 12:1715–1728

Arango-Argoty G, Garner E, Pruden A et al (2018) DeepARG: a deep learning approach for predicting antibiotic resistance genes from metagenomic data. Microbiome 6:23

Bay SK, Dong X, Bradley JA et al (2021) Trace gas oxidizers are widespread and active members of soil microbial communities. Nat Microbiol 6:246–256

Bednarz VN, van Hoytema N, Cardini U et al (2015) Dinitrogen fixation and primary productivity by carbonate and silicate reef sand communities of the Northern Red Sea. Mar Ecol Prog Ser 527:47–57

Bowers RM, Kyrpides NC, Stepanauskas R et al (2017) Minimum information about a single amplified genome (MISAG) and a metagenome-assembled genome (MIMAG) of bacteria and archaea. Nat Biotechnol 35:725–731

Boyd JA, Woodcroft BJ, Tyson GW (2018) GraftM: a tool for scalable, phylogenetically informed classification of genes within metagenomes. Nucleic Acids Res 46:e59

Buongiorno J, Sipes K, Wasmund K et al (2020) Woeseiales transcriptional response to shallow burial in Arctic fjord surface sediment. PLoS One 15:e0234839

Chaumeil PA, Mussig AJ, Hugenholtz P et al (2019) GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. Bioinformatics 36:1925–1927

Chen B, Yang Y, Liang X et al (2013) Metagenomic profiles of antibiotic resistance genes (ARGs) between human impacted estuary and deep ocean sediments. Environ Sci Technol 47:12753–12760

Chen J, Hanke A, Tegetmeyer HE et al (2017) Impacts of chemical gradients on microbial community structure. ISME J 11:920–931

Chen J, McIlroy SE, Archana A et al (2019) A pollution gradient contributes to the taxonomic, functional, and resistome diversity of microbial communities in marine sediments. Microbiome 7:104

Cordero PRF, Bayly K, Man Leung P et al (2019) Atmospheric carbon monoxide oxidation is a widespread mechanism supporting microbial survival. ISME J 13:2868–2881

Cuadrat RRC, Sorokina M, Andrade BG et al. Global ocean resistome revealed: Exploring antibiotic resistance gene abundance and distribution in TARA Oceans samples. *GigaScience* 2020;9

Delmont TO, Quince C, Shaiber A et al (2018) Nitrogen-fixing populations of Planctomycetes and Proteobacteria are abundant in surface ocean metagenomes. Nat Microbiol 3:804–813

Deschaseaux E, Stoltenberg L, Hrebien V et al (2019) Dimethylsulfide (DMS) fluxes from permeable coral reef carbonate sediments. Mar Chem 208:1–10

Devol AH (2015) Denitrification, anammox, and N_2 production in marine sediments. Ann Rev Mar Sci 7:403–423

Dong X, Strous M (2019) An Integrated Pipeline for Annotation and Visualization of Metagenomic Contigs. Front Genet 10:999

Duprey NN, Yasuhara M, Baker DM (2016) Reefs of tomorrow: eutrophication reduces coral biodiversity in an urbanized seascape. Glob Chang Biol 22:3550–3565

Dyksma S, Bischof K, Fuchs BM et al (2016) Ubiquitous Gammaproteobacteria dominate dark carbon fixation in coastal sediments. ISME J 10:1939–1953

Edgar RC (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5:113

Engelberts JP, Robbins SJ, de Goeij JM et al (2020) Characterization of a sponge microbiome using an integrative genome-centric approach. ISME J 14:1100–1110

Eyre BD, Cyronak T, Drupp P et al (2018) Coral reefs will transition to net dissolving before end of century. Science 359:908–911

Fu L, Niu B, Zhu Z et al (2012) CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinformatics 28:3150–3152

Glasl B, Bourne DG, Frade PR et al (2019) Microbial indicators of environmental perturbations in coral reef ecosystems. Microbiome 7:94

Glasl B, Robbins S, Frade PR et al (2020) Comparative genome-centric analysis reveals seasonal variation in the function of coral reef microbiomes. ISME J 14:1435–1450

Haro-Moreno JM, Lopez-Perez M, de la Torre JR et al (2018) Fine metagenomic profile of the Mediterranean stratified and mixed water columns revealed by assembly and recruitment. Microbiome 6:128

Hernandez-Zulueta J, Araya R, Vargas-Ponce O et al. First deep screening of bacterial assemblages associated with corals of the Tropical Eastern Pacific. *FEMS Microbiol Ecol* 2016;92

Hou L, Zheng Y, Liu M et al (2013) Anaerobic ammonium oxidation (anammox) bacterial diversity, abundance, and activity in marsh sediments of the Yangtze Estuary. J Geophys Res-Biogeo 118:1237–1246

Hou LJ, Liu M, Ou DN et al. Influences of the macrophyte (Scirpus mariqueter) on phosphorous geochemical properties in the intertidal marsh of the Yangtze Estuary. *J Geophys Res-Biogeo* 2008;113

Huettel M, Berg P, Kostka JE (2014) Benthic exchange and biogeochemical cycling in permeable sediments. Ann Rev Mar Sci 6:23–51

Hughes TP, Kerry JT, Alvarez-Noriega M et al (2017) Global warming and recurrent mass bleaching of corals. Nature 543:373–377

Imchen M, Kumavath R (2021) Metagenomic insights into the antibiotic resistome of mangrove sediments and their association to socioeconomic status. Environ Pollut 268:115795

Jain C, Rodriguez RL, Phillippy AM et al (2018) High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. Nat Commun 9:5114

Janßen A, Wizemann A, Klicpera A et al. Sediment Composition and Facies of Coral Reef Islands in the Spermonde Archipelago, Indonesia. *Front Mar Sci* 2017;4

Kegler HF, Lukman M, Teichberg M et al (2017) Bacterial Community Composition and Potential Driving Factors in Different Reef Habitats of the Spermonde Archipelago, Indonesia. Front Microbiol 8:662

Kessler AJ, Chen YJ, Waite DW et al (2019) Bacterial fermentation and respiration processes are uncoupled in anoxic permeable sediments. Nat Microbiol 4:1014–1023

Kessler AJ, Rogers A, Cyronak T et al (2020) Pore water conditions driving calcium carbonate dissolution in reef sands. Geochim Cosmochim Ac 279:16–28

King GM (2007) Microbial carbon monoxide consumption in salt marsh sediments. FEMS Microbiol Ecol 59:2–9

Koren O, Rosenberg E (2006) Bacteria associated with mucus and tissues of the coral Oculina patagonica in summer and winter. Appl Environ Microbiol 72:5254–5259

Kumar S, Stecher G, Li M et al (2018) MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol Biol Evol 35:1547–1549

Li D, Luo R, Liu CM et al (2016) MEGAHIT v1.0: A fast and scalable metagenome assembler driven by advanced methodologies and community practices. Methods 102:3–11

Li Y, Chen H, Song L et al (2021) Effects on microbiomes and resistomes and the source-specific ecological risks of heavy metals in the sediments of an urban river. J Hazard Mater 409:124472

Liu S, Su H, Pan YF et al (2020) Spatial and seasonal variations of antibiotics and antibiotic resistance genes and ecological risks in the coral reef regions adjacent to two typical islands in South China Sea. Mar Pollut Bull 158:111424

Matthews JL, Raina JB, Kahlke T et al (2020) Symbiodiniaceae-bacteria interactions: rethinking metabolite exchange in reef-building corals as multi-partner metabolic networks. Environ Microbiol 22:1675–1687

Ning Z, Yu K, Wang Y et al (2019) Carbon and nutrient dynamics of permeable carbonate and silicate sands adjacent to coral reefs around Weizhou Island in the northern South China Sea. Estuar Coast Shelf S 225:106229

Olm MR, Brown CT, Brooks B et al (2017) dRep: a tool for fast and accurate genomic comparisons that enables improved genome recovery from metagenomes through de-replication. ISME J 11:2864–2868

Olm MR, Crits-Christoph A, Diamond S et al (2020) Consistent Metagenome-Derived Metrics Verify and Delineate Bacterial Species Boundaries. mSystems 5:e00731–e00719

Ortiz M, Leung PM, Shelley G et al. A genome compendium reveals diverse metabolic adaptations of Antarctic soil microorganisms. *bioRxiv* 2020, DOI 10.1101/2020.08.06.239558

Park SJ, Andrei AS, Bulzu PA et al (2020) Expanded Diversity and Metabolic Versatility of Marine Nitrite-Oxidizing Bacteria Revealed by Cultivation- and Genomics-Based Approaches. Appl Environ Microbiol 86:e01667–e01620

Patro R, Duggal G, Love MI et al (2017) Salmon provides fast and bias-aware quantification of transcript expression. Nat Methods 14:417–419

Pearman JK, Aylagas E, Voolstra CR et al (2019) Disentangling the complex microbial community of coral reefs using standardized Autonomous Reef Monitoring Structures (ARMS). Mol Ecol 28:3496–3507

Pernice M, Raina JB, Radecker N et al (2020) Down to the bone: the role of overlooked endolithic microbiomes in reef coral health. ISME J 14:325–334

Polonia AR, Cleary DF, Freitas R et al (2015) The putative functional ecology and distribution of archaeal communities in sponges, sediment and seawater in a coral reef environment. Mol Ecol 24:409–423

Ragsdale SW, Pierce E (2008) Acetogenesis and the Wood-Ljungdahl pathway of CO_2 fixation. Biochim Biophys Acta 1784:1873–1898

Richardson LL, Goldberg WM, Kuta KG et al (1998) Florida's mystery coral-killer identified. Nature 392:557–558

Robbins SJ, Singleton CM, Chan CX et al (2019) A genomic view of the reef-building coral Porites lutea and its microbial symbionts. Nat Microbiol 4:2090–2100

Robbins SJ, Song W, Engelberts JP et al (2021) A genomic view of the microbiome of coral reef demosponges. ISME J 15:1641–1654

Roy A, Das BK, Bhattacharya J (2011) Development and Validation of a Spectrophotometric Method to Measure Sulfate Concentrations in Mine Water without Interference. Mine Water Environ 30:169–174

Rusch A, Gaidos E (2013) Nitrogen-cycling bacteria and archaea in the carbonate sediment of a coral reef. Geobiology 11:472–484

Salazar G, Paoli L, Alberti A et al (2019) Gene Expression Changes and Community Turnover Differentially Shape the Global Ocean Metatranscriptome. Cell 179:1068–1083 e21

Sanchez-Baracaldo P, Cardona T (2020) On the origin of oxygenic photosynthesis and Cyanobacteria. New Phytol 225:1440–1446

Schottner S, Pfitzner B, Grunke S et al (2011) Drivers of bacterial diversity dynamics in permeable carbonate and silicate coral reef sands from the Red Sea. Environ Microbiol 13:1815–1826

Schuller-Levis GB, Park E (2003) Taurine: new implications for an old amino acid. FEMS Microbiol Lett 226:195–202

Shadrack RS, Pohler S, Dutra LXC et al (2020) Carbonate sediments from Maui bay (coral coast, Fiji) reflect importance of coral reef conservation. Ocean Coast Manag 198:105381

Shaffer M, Borton MA, McGivern BB et al (2020) DRAM for distilling microbial metabolism to automate the curation of microbiome function. Nucleic Acids Res 48:8883–8900

Singleton CM, Petriglieri F, Kristensen JM et al. Connecting structure to function with the recovery of over 1000 high-quality metagenome-assembled genomes from activated sludge using long-read sequencing. *Nat Commun* 2021;**12**: 2009

Uritskiy GV, DiRuggiero J, Taylor J (2018) MetaWRAP-a flexible pipeline for genome-resolved metagenomic data analysis. Microbiome 6:158

Van Goethem MW, Pierneef R, Bezuidt OKI et al (2018) A reservoir of 'historical' antibiotic resistance genes in remote pristine Antarctic soils. Microbiome 6:40

Vigneron A, Cruaud P, Culley AI et al (2021) Genomic evidence for sulfur intermediates as new biogeochemical hubs in a model aquatic microbial ecosystem. Microbiome 9:46

Vizcaino MI, Johnson WR, Kimes NE et al (2010) Antimicrobial resistance of the coral pathogen Vibrio coralliilyticus and Caribbean sister phylotypes isolated from a diseased octocoral. Microb Ecol 59:646–657

Wang JL, Du JZ, Baskaran M et al (2016) Mobile mud dynamics in the East China Sea elucidated using 210Pb, 137Cs, 7Be, and 234Th as tracers. J Geophys Res-Oceans 121:224–239

Woodcroft BJ, Singleton CM, Boyd JA et al (2018) Genome-centric view of carbon processing in thawing permafrost. Nature 560:49–54

Xu X, Zhu Q, Zhou Q et al (2018) An improved method for quantitatively measuring the sequences of total organic carbon and black carbon in marine sediment cores. J Oceanol Limnol 36:105–113

Zhao R, Yu K, Zhang J et al (2020) Deciphering the mobility and bacterial hosts of antibiotic resistance genes under antibiotic selection pressure by metagenomic assembly and binning approaches. Water Res 186:116318

Zhou J, Bruns MA, Tiedje JM (1996) DNA recovery from soils of diverse composition. Appl Environ Microbiol 62:316–322

Zhou Z, Tran PQ, Breister AM et al. METABOLIC: a scalable high-throughput metabolic and biogeochemical functional trait profiler based on microbial genomes. *bioRxiv* 2020, DOI 10.1101/761643

Figures

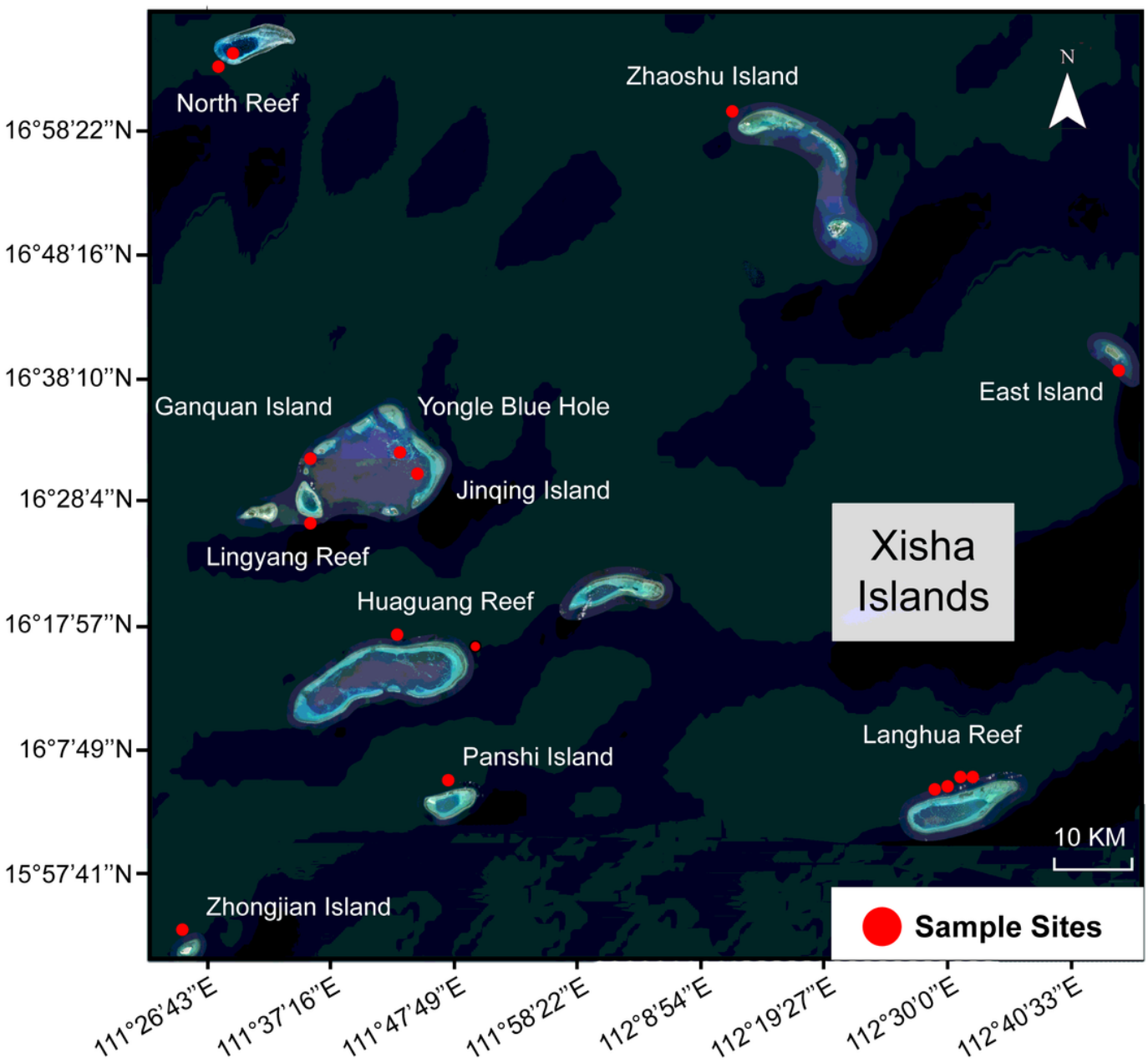

Figure 1

Geographic distribution of the 16 sediment sampling locations. Surface permeable sediment samples (0-5 cm) were taken from the reef slopes of different islands located in the Xisha Islands. The map was generated using the ArcGIS v10.8.1 .

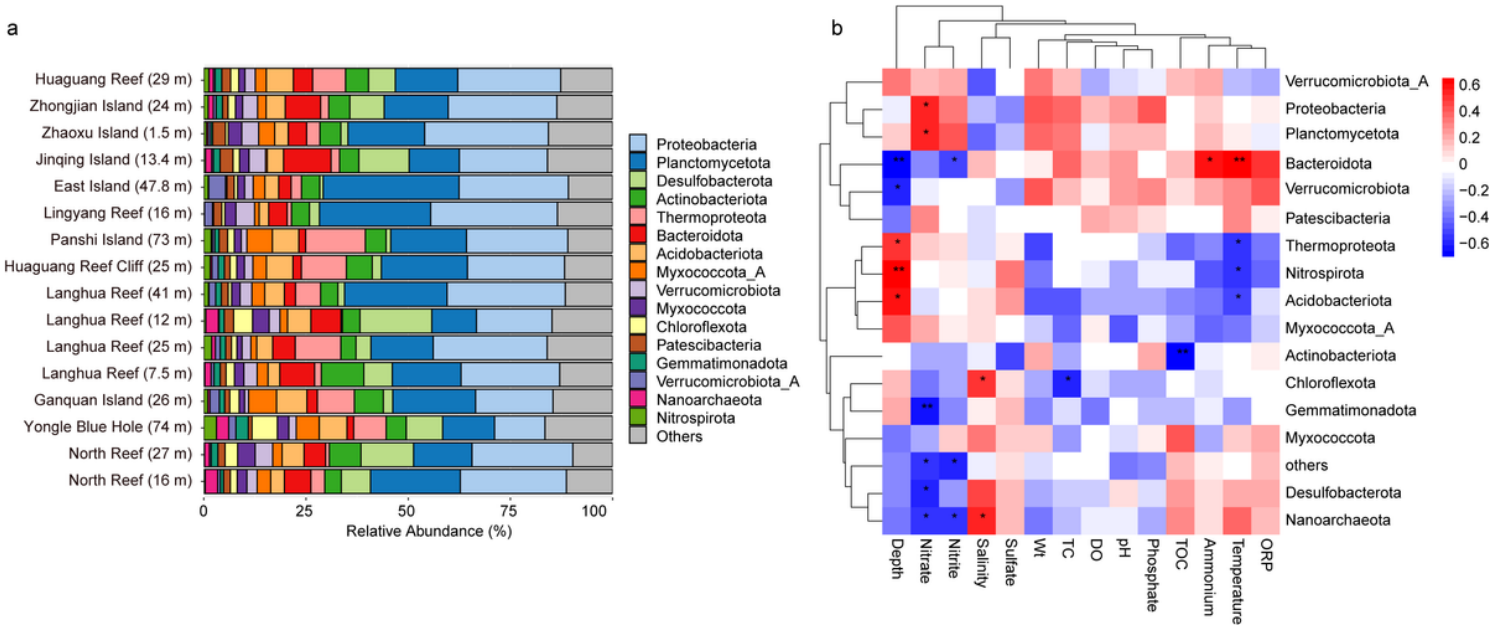

Figure 2

Composition and diversity of coral reef sediment microbiome in the Xisha Islands. (a) Relative abundances of phylum-level bacterial and archaeal taxa based on metagenomic reads of the single-copy marker gene rplB. (b) Correlation between phylum-level microbial abundance and physicochemical parameters. Bacterial and archaeal taxonomy is based on Genome taxonomy database (GTDB) release 05-RS95. The stars symbolize p values of correlation. *** means $p < 0.001$; ** means $p < 0.01$; *means $p < 0.05$.

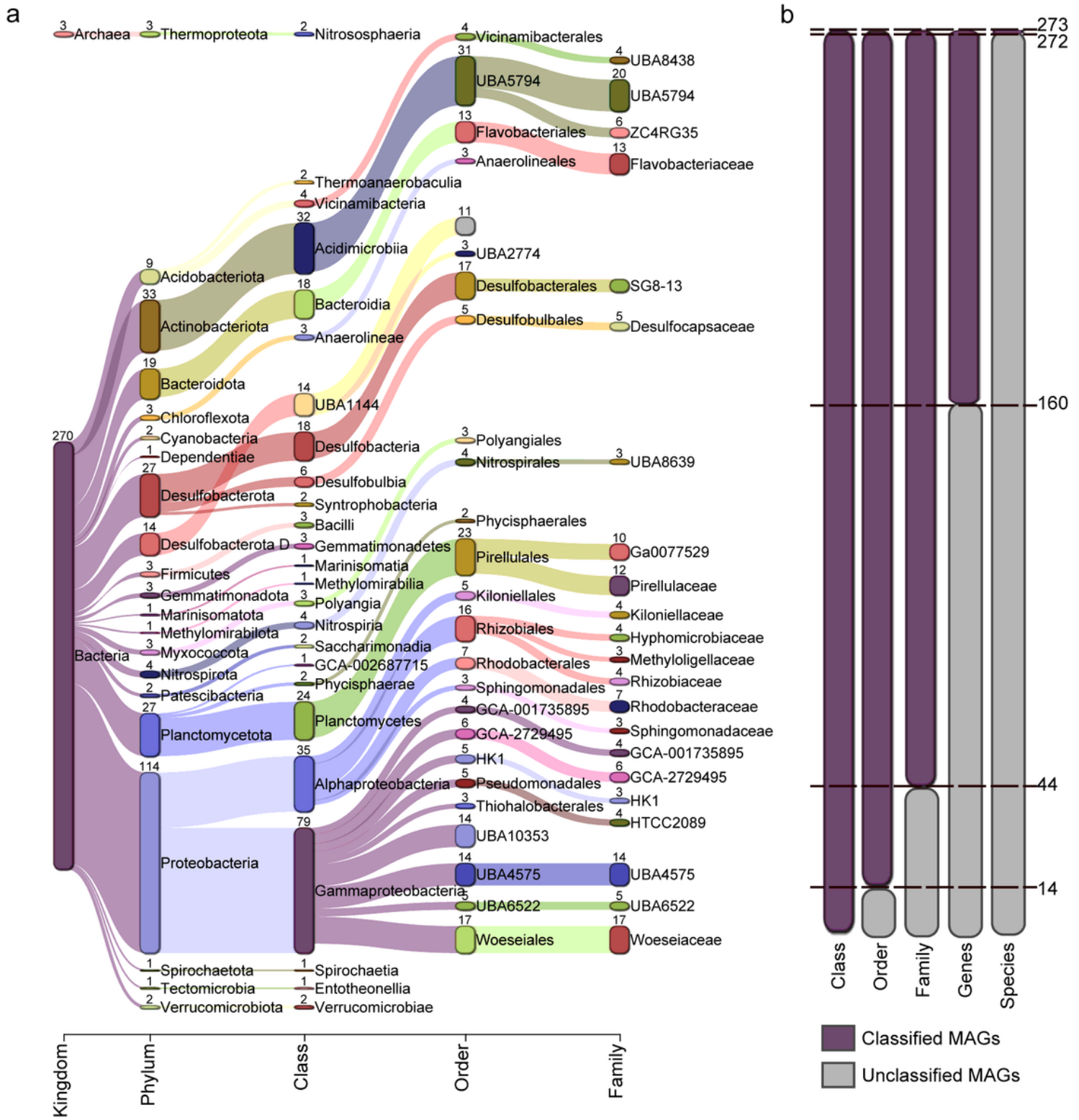

Figure 3

Recovered MAG information of coral reef sediment microbiome across taxonomic levels. (a) Sankey based on assigned taxonomy showing populations at different phylogenetic levels based on GTDB-Tk classification. Numbers indicate the number of MAGs recovered for the lineage. (b) Total MAGs unclassified by GTDB-Tk at each taxonomic level. MAGs were dereplicated at 95% average nucleotide

identity for species level. Pavian was used to create the Sankey figure. Detailed statistics for recovered MAGs can be found in Table S3.

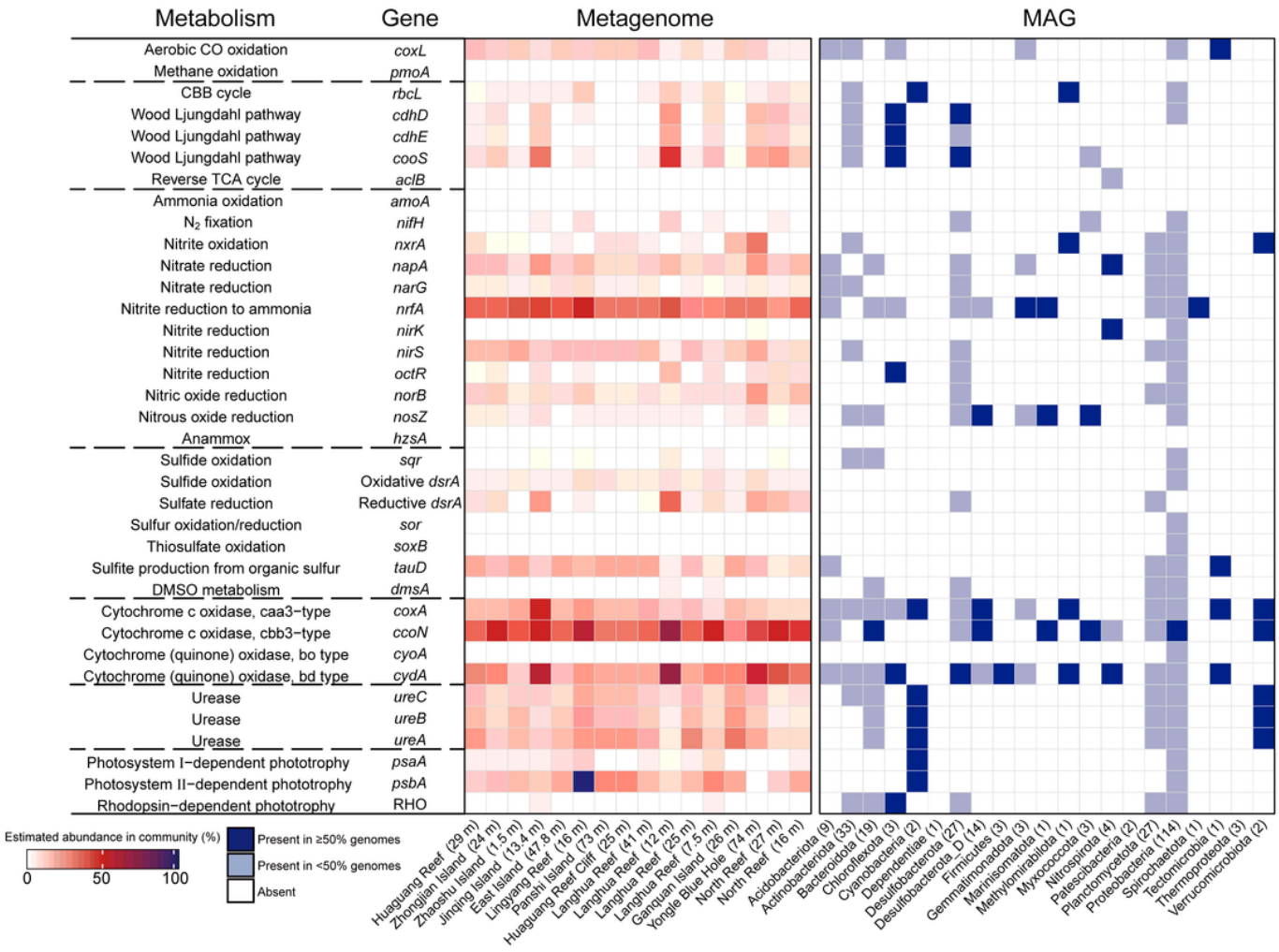

Figure 4

Metabolic profile of reef sediment microbial communities. Left panel, Heatmap of selected metabolic gene abundances in the microbial gene catalog. Relative abundances were computed using by dividing the RPKM (Reads per kilo base per million mapped reads) value of a gene sequence by the mean RPKM value estimated from 14 single-copy marker genes. Right panel, the percentage of MAGs that containing each gene at phylum level. The numbers next to the taxonomic groups represent the numbers of MAGs. Detailed annotation data for reads and predicted genes of MAGs can be found in Tables S4-S6.

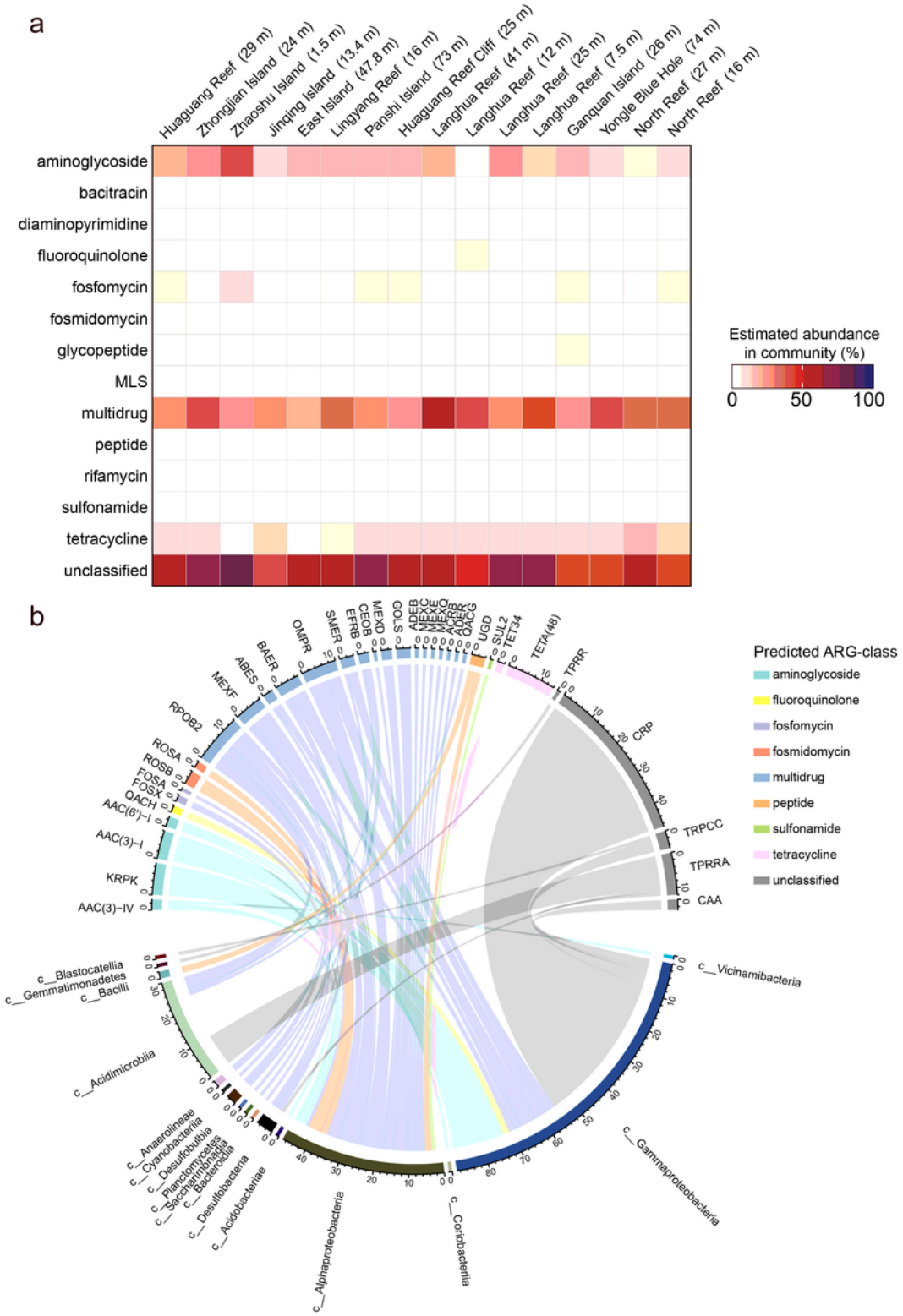

Figure 5

Abundances and hosts of antibiotic resistance genes for reef sediment microbial communities. (a) Relative abundance of antibiotic resistance genes at class level in 16 sediment sampling locations. Relative abundances were computed using by dividing the RPKM (Reads per kilo base per million mapped reads) value of each ARG by the mean RPKM value estimated from 14 single-copy marker genes. MLS, macrolide-lincosamide-streptogramin. (b) Mapping relationships between ARGs and ARG-

carrying MAGs grouped at class level. The width of the bands on the inner ring represented the total number of ARGs carried by the MAGs or the number of MAGs that carrying this ARG. Detailed ARG annotation data can be found in Tables S7-S9.

Supplementary Files

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

- Supplementarymaterial.docx
- TableS3.xlsx
- TableS4.xlsx
- TableS5.xlsx
- TableS6.xlsx
- TableS7.xlsx
- TableS8.xlsx
- TableS9.xlsx