Metagenomic Views of Microbial Communities in Sand Sediments Associated with Coral Reefs

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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 geographic sites and their potential effect on nutrient recycling and health of the coral reef ecosystem. In combination of biogeochemical measurements with gene- and genome-centric 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 at different depths 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, our findings expanded the understanding of reef sediment microbial ecology and provided insights for their link to the coral reef ecosystem health.

Keywords Marine sediment · Coral reef · Metagenomics · Microbial ecology · Antibiotic resistance genes

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 reef ecosystems are facing enormous local and global environmental changes, such as global warming and ocean acidification [1, 2]. To protect coral reef ecosystems effectively, it is important to comprehensively understand the contributions of each component of the reef ecosystem to the whole system’s stability and ecological function.

Reef sediments, mainly composed of permeable calcium carbonate formed by biological breakdown processes, are one of the important components of coral reef [3–5]. 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 [3, 6]. 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 maintain the high biomass and primary productivity of coral reefs in low-nutrient ocean environments [6, 7].

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 [8–10]. 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 [8, 11–13], 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 [14]. Reef sediment microorganisms can affect the budget of sedimentary calcium carbonate by fixing and producing carbon dioxide [15]. They are also directly involved in the recycling and utilization of nutrients such as carbon, nitrogen, phosphorus, and sulfur in the [8]. 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 [16–18]. 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. If these microorganisms resistant to various antibiotics are coral pathogens (e.g., *Vibrio coralliilyticus*), this might make coral disease outbreaks even worse [8, 19]. 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 [20, 21]. 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 [22]. 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 [23]. 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 km², with a total land area of about 10 km² 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, where relatively high coral species richness was observed based on visual

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**Fig. 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 total land area of each island: Lingyang Reef (13 km²), Langhua Reef (67.3 km²), Huaguang Reef (170 km²), Jinqing Island (0.21 km²), East Island (1.6 km²), North Reef (41 km²), Panshi Island (0.4 km²), Ganquan Island (0.31 km²), Zhaoshu Island (0.22 km²), and Zhongjian Island (1.2 km²). The map was generated using the ArcGIS v10.8.1.
inspection. 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 gene- and genome-centric 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 physicochemical parameters of overlying seawater were quite different (Table S1 and Figure 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 the lowest at the deepest (74 m, Yongle Blue Hole). These physicochemical parameters of water samples can serve as an imperfect proxy for the properties of surface sediment samples, due to distributions of waves and currents. 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 and Figure S1). 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 [24]. 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 S2a). Spearman correlations between alpha diversity and water depths suggest that the microbial diversity increased with water depths from 1.5 to 74 m (Figure S2b).

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* [25, 26]. 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 [27–29], 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 oxidation in the carbonate sediment of a coral reef in Kaneohe Bay, Hawaii [30]. 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 S3). However, physico-chemical 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 high- or medium-quality [31, 32] 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 [33]. The high strain heterogeneity level (average, 40% based on checkM) possibly explained the relatively low recovery of MAGs [31]. 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 *Acidimicrobia*), Desulfobacterota (*n* = 27, mainly Desulfobacteria), and Planctomycetota (*n* = 27, mainly *Planctomycetes*) were highly represented. As for the Archaea domain, *Nitrososphaera* (*n* = 2) and Bathyarchaeota (*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 S4). 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).

### Reef Sediment Bacteria Harbor the Flexibility to Oxidize Various Organic and Inorganic Compounds as Energy Sources

The marine sediments of the first 5 cm are often stratified and structured, with a mix of various microbial processes driven by the nature of chemical gradients of these sediments [34]. 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, 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) [35, 36]. The 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 in the bottom water (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*$_F$-type cytochrome c oxidase (43.9% of the microbial community cells, on average), *bd*-type cytochrome (quinone) oxidase (32.5%), and *caa*$_3$-type cytochrome c oxidase (18.9%). Both *cbb*$_F$-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 [30, 37]. 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 with some being abundant encoded partial enzymes for several steps in a dissimilatory denitrification pathway, but none was 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 [38]. 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 [39]. 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 (Table S5 and Fig. 4a) 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 [40]. 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 [41]. In benthic zone like reef sediments, CO may be produced through photochemical organic matter degradation or by benthic algae [42]. In line with previous literature [41], 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 [43] possibly due to genome incompleteness (Figure S5) 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 [44, 45].

In agreement with the total inorganic carbon concentrations (Table S2) and the presence of autotrophs, pathways for inorganic carbon fixation (Table S5 and Fig. 4a) were predicted to be primarily through the Wood–Ljungdahl

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**Fig. 4** Metabolic profile of reef sediment microbial communities. 

**a** 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. **b** 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.
highlighting another potential source of sulfur provided
49 MAGs mostly assigned to the phylum of Proteobacteria,
were identified in 17% of microbial cells, corresponding to
seawater, e.g., the hydrolysis of urea into carbon dioxide
on the TARA Oceans [51, 52]. Additional nitrogen may
were also found in surface waters of the open ocean based
nitrogen fixation capabilities for these microorganisms
Desulfobulbales, Polyangiales, and Chromatiales. Putative
fixation marker gene
cells on average in reef sediments. Searches for the nitrogen
microorganisms to thrive in nutrient-poor environments.
Bacteroidota, and Chloroflexota in these sediments.
spread genes in the photic zone worldwide [48]. They are
rhodopsins have been shown to be among the most wide-
trophs in coastal sediments [45]. Putative energy-converting
This is consistent with the expectation that carbon fixation is
primarily driven by chemoautotrophs rather than photoauto-
trophs in coastal sediments [45]. Putative energy-converting
rhopdopsins have been shown to be among the most wide-
spread genes in the photic zone worldwide [48]. They are
very diverse and are distributed throughout most taxa, pre-
present in several of the most dominant orders of Desulfo-
bacterota, Planctomycetota, Actinobacteriota, Proteobacteria,
Bacteroidota, and Chloroflexota in these sediments.
Efficient nitrogen and sulfur acquisition is important for
microorganisms to thrive in nutrient-poor environments.
Diazotrophic bacteria can convert gaseous dinitrogen to
ammonia [49, 50], 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 [51, 52]. Additional nitrogen may
come from organic compounds present in the surrounding
seawater, e.g., the hydrolysis of urea into carbon dioxide
and ammonia [10]. Genes encoding urease α, β, and
γ subunits (ureABC) were identified in 14.2–20.4% of
microorganisms and in MAGs mostly from high abundances
of 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 [53],
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 [54]. Accordingly, genes for anaerobic
dimethylsulfoxide reductase (dmsA) that converts DMSO to
dimethyl sulfide DMS were also identified in Proteobacteria,
Desulfo bacterota, Planctomycetota, and Bacteroidota
genomes, accounting for 1.9% of total microbial cells and
providing an alternative energy source under anoxic
conditions [55].

Diverse Bacterial Lineages Potentially Harbor
Antibiotic Resistance Genes

We further screened for the abundance and diversity of
ARGs in the microbial gene cataloge. 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 [56, 57], suggesting
that high biodiversity might also lead to enrichment of
ARGs. Additionally, these findings also indicate that coral
reef sediments are an overlooked but potentially important
reservoir of ARGs. Main ARG classes detected in river and
mangrove sediments were genes resistant to multidrug and
macrolide-lincosamide-streptogramin (MLS) antibiotics
[56, 57]. In contrast, among our samples, the top four most
abundant ARG classes (excluding unclassified ARGs) 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%). Based
on Spearman's correlation coefficient, environmental factors
were related to various classes of ARGs at different degrees
(Figure S6). For example, ARG genes resistance to multidrug
were observed to be positively related to total organic
carbon, while a significant negative correlation was found
for salinity and ARG genes resistance to aminoglycoside.
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 [58]. 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;
and public policy making on coral reef protection. However, genome reference database for environmental assessments genes, emphasizing their potential influence in the coral reef that bacterial lineages harbor various antibiotic resistance sulfur cycling. Another important finding of this study is bic respiration and carbon fixation, as well as nitrogen and nutrient recycling of coral reef ecosystems, including aero- ury of microorganisms. They have potential roles in overall and Planctomycetota, along with vast yet uncultured major- 

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 [19]. 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 eco- systems. Here, our investigation of the microbiome com- position and functionality in reef sediments from the Xisha islands suggests that reef sand microbiomes are highly diverse with dominance of members from Proteobacteria and Planctomycetota, along with vast yet uncultured major- ity of microorganisms. They have potential roles in overall nutrient recycling of coral reef ecosystems, including aerobic respiration and carbon fixation, as well as nitrogen and sulfur cycling. Another important finding of this study is that bacterial lineages harbor various antibiotic resistance genes, emphasizing 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 (e.g., at different seasons) is 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 by scuba divers and immediately stored at −80 °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; https://www.ysi.com/ prodss), 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 [59]. Sediment exchangeable phosphate was extracted from fresh sediments with 1 M HCl and measured colorimetrically by the ascorbic acid-molybdate blue method [60]. Sediment exchangeable ammonium, nitrite, and nitrate were extracted by 2 M potassium chloride (purged with N2) according to a previous protocol [61] and measured by a continuous flow nutrient analyzer (Futura, Alliance, France). Sulfate concentrations were determined colorimetrically with barium chloro- ride [62]. 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 [63].

DNA Extraction and Metagenome Sequencing

Genomic DNA for metagenomic sequencing was extracted from the untreated sediments (5–6 g) using the cetyltrimethylammonium bromide (CTAB) method [64]. 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/wwwood/singlem), followed by summarizing OTU tables via rarefying and clustering (command, singlem summarise). 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 [24]. 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 [65]. The 16 quality-controlled metagenomes were individually assembled using MEGAHIT v1.1.3 with default parameters [66], which might not work well for samples with ultra-complex metagenomics data. Each of the 16 assemblies (for detail, see Table S10) 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 dereplicated using dRep v2.5.4 at 95% average nucleotide identities [67], for species level [68, 69]. SingleM was used to determine genome recovery efforts at genus-level (singleton 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) [32]. Completeness, contamination, and heterogeneity of each bin were estimated using CheckM. Numbers of rRNAs and tRNAs of each MAG were predicted based on DRAM [70]. Relative abundance of MAGs depreciated at species level was calculated with CoverM v0.4.0 (https://github.com/wwwood/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 [71]. 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 [35, 72]. 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 [73]. DIAMOND blastp algorithm was used against reference datasets [26] with a minimum percentage identity of 50% for identification of genes for photosystem I reaction center protein (PsA), photosystem II reaction center protein (PsB), and microbial rhodopsin (energy-converting type, RHO). The screening for ARGs was performed with DeepARG-LS using gene models [74]. GraftM v0.13.1 was used to identify 14 universal single-copy ribosomal genes [25]. For the phylogenetic analysis of dissimilatory sulfite reductase subunit A (DsrA), amino acid sequences were aligned using the MUSCLE algorithm [75] included in MEGA X [76]. 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 [77] 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.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00248-021-01957-8.

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**Author Contribution** 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.

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**Data Availability** DNA raw reads have been deposited in NCBI Bio-Project databases with accession number PRJNA724996. Individual assembly for metagenome-assembled genomes, SingleM OTU tables for 14 universal single-copy genes, the microbial reference gene catalog and their function assignments, and the 17 metagenomic assemblies can be found at figshare: https://figshare.com/s/cd5bf6b6e8cd6be6b60. 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.

**Declarations**

**Conflict of Interest** The authors declare no competing interests.

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