Unique deep-sea bacterial assemblages thriving on different natural organic matters delivered via *in situ* incubators

Jianyang Li
School of Environmental Science and Engineering, Tianjin University, Tianjin 300387, PR China; School of Environmental Science and Engineering, Tianjin University, Tianjin 300387, PR China.
https://orcid.org/0000-0002-2466-6881

Chunming Dong
Key Laboratory of Marine Genetic Resources, Third Institute of Oceanography, Ministry of Natural Resources of PR China; State Key Laboratory Breeding Base of Marine Genetic Resources; Key Laboratory of Marine Genetic Resources of Fujian Province, Xiamen 361005, PR China.

Qiliang Lai
Key Laboratory of Marine Genetic Resources, Third Institute of Oceanography, Ministry of Natural Resources of PR China; State Key Laboratory Breeding Base of Marine Genetic Resources; Key Laboratory of Marine Genetic Resources of Fujian Province, Xiamen 361005, PR China.

Zhaobin Huang
Key Laboratory of Marine Genetic Resources, Third Institute of Oceanography, Ministry of Natural Resources of PR China; State Key Laboratory Breeding Base of Marine Genetic Resources; Key Laboratory of Marine Genetic Resources of Fujian Province, Xiamen 361005, PR China.

Donghui Zhou
School of Mechanical Engineering, Hangzhou Dianzi University, Hangzhou 310018, PR China.

Linfeng Gong
Key Laboratory of Marine Genetic Resources, Third Institute of Oceanography, Ministry of Natural Resources of PR China; State Key Laboratory Breeding Base of Marine Genetic Resources; Key Laboratory of Marine Genetic Resources of Fujian Province, Xiamen 361005, PR China.

Guangyi Wang
School of Environmental Science and Engineering, Tianjin University, Tianjin 300387, PR China.

Zongze Shao (shaozz@163.com)
Key Laboratory of Marine Genetic Resources, Third Institute of Oceanography, Ministry of Natural Resources of PR China; State Key Laboratory Breeding Base of Marine Genetic Resources; Key Laboratory of Marine Genetic Resources of Fujian Province, Xiamen 361005, PR China; Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai), Zhuhai 519000, PR China

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Abstract

The transport of organic matters to the deep sea constantly occurs in global oceans and in accompany with simultaneous microbial remineralization. However, little is known about the impact of fast sinking organic complex on oceanic deep ecosystem and its interactions with microbes throughout the process of sinking and settling on the sea bottom. In this report, to observe the response of indigenous microorganisms to the newly input of organic matters, we developed a series of deep-sea *in situ* incubators loaded on the seabed and seamount with various organic substrates of plant and animal origins. Through high-throughput sequencing, we found the bacterial communities, *in situ* enriched for 4–12 months, were significant different from the control groups. The bacteria of *Marinifilaceae* were revealed as the key members; in addition, other key decomposers including *Spirochaetaceae*, *Psychromonadaceae*, *Vibrionaceae*, and *Moritellaceae* were recruited in most assemblages, with varied abundance accordingly and diversified at the operational taxonomic unit (OTU) level. Additionally, sulphate-reducing bacteria (*Desulfobulbaceae* and *Desulfobacteraceae*) and sulphur-oxidizing bacteria (*Arcobacteraceae* and *Sulfurovaceae*) were the dominant taxa. Interestingly, PICRUSt analysis demonstrated that nitrogen fixation inside the assemblages was enriched in the enrichments with plant detritus or fatty acids. Within the assemblages stimulated by substrates short of nitrogen sources, the microbial network was dominated by cooperative relationships, whereas competition relationships overwhelmed the communities thriving on protein-rich animal tissue. These unique bacterial assemblages driven by newly input organic matters, constitute the microbial carbon pump involved in carbon, sulphur, and nitrogen cycles in oceanic interior.

1 Introduction

Annually, a large amount of organic matter (OM) sinks to the deep sea bottom, among which 10-30 % is of terrestrial origin [1]. Both the terrestrially originated OM and those continuously generated from the oceanic euphotic zone will eventually sink to the deep sea. A single storm event can transport up to 1.8-4.0 teragrams of terrestrially derived OM into the ocean, as previously estimated by Bianchi et al. [2]. The zooplankton and phytoplankton remain, as well as a considerable part of the terrigenous OM originating from animal and plant tissues, are chemically characterized by high-weight proteins or polypeptides, fatty acids, polysaccharides (chitin, cellulose, and hemicellulose), and lignin content [2], respectively. The input of large amounts of OMs, such as plant fragments and animal carcasses, to the sea bottom contribute organic impulse to the deep-sea ecosystem, such as the well-known ecosystems of wood-fall and whale-fall [3-6].

Sinking particulate organic matter (POM), which functions as a conduit for exporting organic materials from the euphotic zone to the deep ocean [7], critically contributes to the establishment of habitats for deep-sea benthic microbial communities [8]. Through the water column, the microbial assemblages associated with sinking particulates in the upper ocean layer, such as photoautotrophs of *Cyanobacteria,* can be quickly delivered to the abyss, while deep-sea bacteria respond rapidly to the elevated nutrient delivery of sinking POM [9, 10]. The sinking particulates that transit to the deep sea may contain energy-

rich organic compounds or relatively energy-replete POM with fatty acids selectively preserved [7, 11]. High OM influx is usually accompanied by high sedimentation rates to the deep sea [12, 13]. The large and fast sinking particles may escape disaggregation, dissolution, solubilization, and even microbial decomposition en route to the deep sea [9], which determines the organic carbon and energy flux pattern of the deep sea and impacts the microbial communities in the deep sea [14, 15]. Considering the vast volume of the deep sea, processes of POM transformation and remineralization via microorganisms in benthic ecosystems are crucial to the global carbon cycle [16]. However, we only have scratches of pieces of information about the microbial processes on fast-sinking particles, let along the dynamic process in both microbial assemblage and organic transformation. Knowledge about the microbial decomposition process in the deep ocean is scarce, which hampers our ability to evaluate the fate and impacts of POM in pelagic waters.

With conductivity, temperature, and depth (CTD) sampling and high-throughput sequencing, quite diverse bacterial heterotrophs have been described in both deep-sea water and sediments; for example, in bathypelagic water, the Gammaproteobacteria *Alteromonas*, *Pseudoalteromomas*, *Alcanivorax*, *Halomonas*, and *Shewanella* are relatively abundant [7, 15, 17, 18]. Moreover, in the water column of pelagic oceans, the majority of prokaryotes remain uncultivated, such as SAR324, SAR406, and SAR202 [18-21]. We are unsure about their growth substrates *in situ*, even for those cultivated in the laboratory.

Moreover, the bacteria observed in the deep water column sampled with CTD cassettes, filter fractionation techniques, and sediment traps [7] may be different from the bacteria thriving on nutrients during the “hot time” of bulky POM sinking, such as the terrestrial debris transported via hurricanes and water currents or the dropping of marine animal corpses, because large pieces rich in OM do not constantly sink through the water column and settle to the seabed at every location. What we “see” via CTD may be just part of the story; a similar scenario is true for deep-sea sediments. Whether prevalent marine bacteria will be recruited to the community and the identity of the key taxon remineralizing the fresh POM in benthic environments are questions to answer as a prerequisite for discovering the fate of POM that sinks to the seabed. However, the big challenge is to catch up with the sporadic and instantaneous processes in the remote deep ocean. Measuring the responses of the benthic microbiome to the sudden input of OM requires *in situ* observations during the “hot time” of POM sinking.

We hypothesized that the key bacterial taxa that contribute to the decomposition of newly input POM in the deep sea would be different from the regular predominant bacteria mentioned above. To define the key microbial taxa potentially participating in the transformation and degradation of POM sunk to the deep sea *in situ*, in this study, we applied long-term *in situ* enrichments supplemented with natural plant and animal materials rich in polysaccharides, proteins, or fatty acids using our newly developed deep-sea *in situ* microbial incubator (DIMI), landed at different geographic locations (marginal sea and open sea). The POM input-spiked bacterial assemblages were subjected to bacterial diversity to identify the *in situ* keystone taxa during the OM remineralization. The results will provide knowledge about the microbial

interactions with sinking POM and contribute to our understanding of the roles of the microbial carbon pump in elemental biogeochemical cycles in global oceans.

2 Materials And Methods

2.1 Experimental design and *in situ* incubation experiments

From 2016 to 2018, the *in situ* incubation experiments were separately carried out on the seafloor in the South China Sea (SCS, the largest marginal sea of the West Pacific Ocean; 18.4925000° N, 116.2717833° E), the deep-sea basin beside the Southwest Indian Ridge in the Indian Ocean (IO, open sea; 33.2789333° S, 50.7510167° E), and a flat-topped seamount in the West Pacific Ocean (PO, open sea; 20.4059567° N, 160.7700883° E) at 3758 m, 4434 m, and 1622 m water depths, respectively (**Fig. S1** and **Fig. S2a**).

In this study, natural plant and animal origin materials were selected for *in situ* incubation (**Table S1**). The animal tissue substrates included fish muscle, fish scales, and shrimp muscle; the plant detritus included wood chips, wheat bran, wheat straw, and various seaweeds; in addition, fish oil (EPA and DHA) and vegetable oil as fatty acid were used here. Approximately 10 g of solid substrate was directly placed into a 50 ml tube. For the liquid fish oil and vegetable oil, 5 ml of oil was first absorbed into sintered silicate balls approximately two centimeters in diameter, and three balls were put into a 50 ml tube. All of the assembled tubes were wrapped with 75-μm nylon mesh to prevent potential ingestion by benthic fauna. Parallel tubes with the substrates were sterilized at 115 °C for 30 minutes and then placed into sterilized incubation chambers (ICs) that were made of titanium alloy. ICs filled with OM substrates were designated treatment incubation chambers (TICs). Meanwhile, two cleaned and sterilized empty ICs without any substrates (designated CICs, control incubation chambers) were reserved for collecting *in situ* seawater during each incubation. Finally, all the TICs and CICs were mounted on a DIMI device (**Fig. S2b**), which is a deep-sea microorganism *in situ* incubation system that can be landed on the seafloor at abyssal depths (6000 m) to conduct microbe incubation over the sediment for over 12 months. The details on the deployment and recovery of the DIMI is described in the Supplementary Materials. The temperature and salinity of the surrounding seawater and the depths were recorded using a SBE 37-SM microCAT CTD recorder (Sea-Bird Scientific, Bellevue, WA, USA) mounted on the DIMI during the *in situ* enrichment.

2.2 Sampling and DNA extraction

After recovery from the deep sea, the enriched biomass in the liquid phase was immediately filtered with a 0.22-μm pore size polycarbonate membrane (Millipore, USA) and stored at -80 °C. The remaining solid substrates were resampled in a sterile plastic bag and stored at -80 °C until subsequent processing. For the controls, two 0.2-μm-pore size polycarbonate membranes were used to harvest the *in situ* microbial cells from the CICs with no substrates. The solid substrate, as well as the silicate ball, was ground to

powder in liquid nitrogen immersion for DNA extraction. Total DNA was extracted and purified with a DNeasy PowerWater Kit (Qiagen, Germany) according to the manufacturer's protocol. DNA concentrations were measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA).

2.3 Illumina high-throughput sequencing and analysis

The V3-V4 hypervariable region of the bacterial 16S rRNA gene (~454 bp) was amplified from the enrichment and control samples using the primer pair 338F (5'- ACTCCTACGGGAGGCAGCAG-3') and 806R (5'- GGACTACHVGGGTWTCTAAT-3'). The 20 μl reaction solution comprised 4 μl of 5× PCR buffer, 2 μl of each 2.5 mM dNTP, 0.8 μl of each 5 μM primer, 2.5 U of DNA polymerase (rTaq; TaKaRa), and 10 ng of total DNA. Preliminary amplification of some randomly selected samples was conducted to determine the optimal PCR conditions, and the results were as follows: 3 min at 95 °C; 29 cycles of 30 s at 95 °C, 30 s at 53 °C and 45 s at 72 °C; 10 min at 72 °C; and infinite hold at 4 °C. No amplification products were observed for the negative PCR controls. All barcoded amplicons were pooled in equimolar amounts and subjected to paired-end (PE) sequencing using a PE300 strategy on an Illumina MiSeq platform at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). The raw dataset is available in NODE (https://www.biosino.org/node/) at the accession number OEX011322.

Further analysis for raw data was conducted with the QIIME standard pipeline (version 1.9.1) [22]. In brief, PE reads were merged, yielding ~454 bp fragments, using FLASH software version 1.2.11 [23]. The dataset was then quality filtered to remove ambiguous reads and chimeric sequences using UCHIME version 4.2 [24]. Optimized sequences were clustered into operational taxonomic units (OTUs) using UPARSE version 7 with a 97 % sequence similarity threshold [25]. Taxonomic classification of each representative OTU was performed using the Ribosomal Database Project (RDP) classifier against the SILVA 132 database [26]. Random subsampling of sequences was conducted with an equal depth of 25,904 sequences for the subsequent comparative analysis.

The Chao community richness estimator, as well as the Shannon diversity index, was computed using the QIIME pipeline. Bray–Curtis distance- and UniFrac distance-based β-diversity at the OTU level was calculated using the R 'vegan' package and QIIME, and principal coordinate analysis (PCoA) plots were drawn by the 'ggplot2' package in R. Adonis analysis was carried out to show the important determinants for the variation in microbial community structure based on the Bray–Curtis distance algorithm for 999 permutations through the 'vegan' package. A heatmap was constructed to show the community composition at the family level using R version 3.3.2 with the 'vegan' package. The relationships between abundant genera and samples were displayed using CIRCOS software version 0.67-7 [27].

2.4 Phylogenetic analysis

Multiple sequence alignment of 16S rRNA genes was performed using the MAFFT program with default parameters, and a phylogenetic tree was reconstructed with the neighbor-joining method [28]. The reference sequences were retrieved from NCBI.

2.5 Function prediction of each enrichment

Prokaryotic function prediction of each enrichment was performed using PICRUSt based on the KEGG database [29] and using FAPROTAX based on the metabolic functional group database of marine microorganisms [30] with the rarefied OTU tables as inputs. Adonis analysis was carried out to show the contribution of each factor to microbial functional diversity based on the Bray–Curtis distance algorithm for 999 permutations through the 'vegan' package.

2.6 Network analysis

Network analyses were carried out using the molecular ecological network analyses (MENA) pipeline at the OTU level [31]. Network complexity was assessed by calculating the number of edges, average degree, and average clustering coefficient. Networks were constructed and visualized in Cytoscape version 3.7.1 [32].

3 Results

3.1 Deep-sea *in situ* incubation of OM-decomposing bacteria

After incubation at SCS, IO, and PO for 375, 117, and 348 days, respectively, all incubation tubes, even those with wood chips, exhibited obvious microbial growth indicated by turbidity and color variation (**Fig. S3**); many organic substrates remained in each centrifuge tube, even for fish oil and vegetable oil we could see oily residue on the surface of sintered silicate balls; most samples smelled of hydrogen sulphide, and under a microscope, the field of view appeared with bacterial cells (**Table S1** and **Fig. S3**). The *in situ* temperature and salinity of seawater at those sites were approximately 2.39-2.40 °C and 34.62-34.65 ‰, respectively (**Table S1**).

3.2 Bacterial diversity and its environmental determinants of the OM-decomposing microbial assemblages

The *α*-diversity (Shannon and Chao indexes) analysis showed that the bacterial diversity of the enrichments was significantly lower than that of the *in situ* seawater in the CICs (**Fig. 1a** and **1b**, $P < 0.01$), which indicated that bacterial communities were significantly enriched *in situ* by the POM supplements.

The *β*-diversity analysis revealed that the enriched bacterial community structures were significantly different from those of the seawater in CICs and were quite diverse at the three sites, separated by both organic substrate and geographic location (**Fig. 1c**). Additionally, adonis analyses suggested that the enriched bacterial community structures at OTU level might be influenced by multiple factors, including geographic location, the type of OM, the enrichment time, temperature, water depth, and salinity, among which geographic location ($R^2 = 0.20$, $P = 0.001$), the type of OM ($R^2 = 0.13$, $P = 0.001$), were the two most important determinants of the variation in microbial community structures (**Table S2**).

3.3 Bacterial composition of deep-sea *in situ* enrichments with natural organic materials

A total of 2457 OTUs were retrieved from the incubation samples (**Table S3**) and 1461, 961, and 651 OTUs were obtained from IO, SCS, and PO enrichment sites, respectively (**Fig. 2a**), however, only 144 OTUs were shared among the three sites (**Fig. 2b**). The dominant bacteria were affiliated with the classes Bacteroidia (average abundance of 28.4 %), Gammaproteobacteria (20.4 %), Deltaproteobacteria (13.5 %), Spirochaetia (12.0 %), Campylobacteria (8.4 %), Clostridia (7.2 %) and Fusobacteriia (6.1 %) across the deep-sea *in situ* enrichments (**Fig. 2c**). Although Gammaproteobacteria (25.4 %) and Deltaproteobacteria (8.8 %) also thrived in the communities of the *in situ* seawater in the CICs, however, unlike the enriched communities, Alphaprotebacteria (14.1 %), Dehalococcoidia (11.1 %), Actinobacteria (3.7 %), Gemmatimonadetes (4.1 %), and Phycisphaerae (3.1 %) were additionally detected in large numbers. These results indicated that the newly input POMs had a significant impact on the indigenous microorganisms in the deep sea.

At the family level, the dominant bacterial groups were shared across these deep-sea enrichments even though the sites were far away on the oceanic scale (**Fig. 3** and **Fig. S4**). The bacteria were affiliated with the families *Marinifilaceae*, *Spirochaetaceae*, *Psychromonadaceae*, *Vibrionaceae*, *Moritellaceae*, *Desulfobulbaceae*, *Desulfobacteraceae*, Family_XII_Clostridiales, *Fusobacteriaceae*, *Arcobacteraceae*, *Sulfurospirillaceae*, and *Sulfurovaceae* (**Fig. 3** and **Fig. S4**). Most of these bacterial members were anaerobes or facultative anaerobes, like *Marinifilaceae* [33], *Spirochaetaceae* [34], *Psychromonadaceae* [35], *Fusobacteriaceae* [36], sulphur-oxidizing bacteria (SOBs) of *Arcobacteraceae* and *Sulfurovaceae* [37-39], sulphur-reducing bacteria of *Sulfurospirillaceae* [40], and

sulphate-reducing bacteria (SRBs) of *Desulfobulbaceae* and *Desulfobacteraceae* [41, 42]. In the *in situ* seawater collected by CICs, the putative anaerobic bacteria *Woeseiaceae*/JTB255 and SAR202 were recovered, which have been recognized as cosmopolitan and abundant core members of deep-sea surface sediments [43] and benthic water [44-46], respectively. However, they were not detected in the enrichments with newly input OM (**Fig. 3**). Thus, unique deep-sea bacterial assemblages were stimulated by the newly input POM, which were fast-growing and more competitive in OM-rich habitats than in oligotrophic ones.

3.4 Keystone bacteria showing a preference for organic substrates and geographic locations at the OTU level

To discriminate the keystone species enriched by different types of POM, we analyzed the distribution of OTUs of each dominant family. Bacteria of the family *Marinifilaceae* were the most predominant, thriving in all POM enrichments and showing the highest abundance among all bacterial communities (**Fig. S4**). Interestingly, OTU6861 was mainly dominant in the consortia enriched with plant detritus, such as wood detritus, seaweeds, and wheat bran in the SCS and PO sites, while both OTU4252 and OTU5909 dominated the consortia of substrates-rich in animal tissue (**Fig. 4**). Moreover, OTU5914 not only occurred as the most predominant member in animal tissue enrichments at the SCS site but also among all the enrichments of different POM types at the PO site (**Fig. 4**). Therefore, these OTUs exhibited discrepant preferences for plant detritus or animal tissue.

Spirochaetaceae was the second predominant group in the enrichments (**Fig. S4**). OTU5914 occurred in most plant detritus and animal tissue enrichments at both PO and SCS sites (**Fig. 4**), particularly with relative abundances of 90.0 % and 52.0 % in the wheat bran enrichment at the PO site and shrimp muscle enrichment at the SCS site, respectively. However, little was detected in the enrichments at the IO site. These results indicated that *Spirochaetaceae* perhaps showed a preference for geographic locations.

In addition, the heterotrophic bacteria *Moritellaceae, Psychromonadaceae* [35], and *Vibrionaceae* [3, 47, 48] of Gammaproteobacteria are also the predominant member and varied with substrate and location at the OTU level (**Fig. 4**), which are described in detail in the text of the Supplementary Information.

Collectively, the keystone species for each predominant group were divergent at the OTU level in response to different POMs and geographic locations, which were also confirmed by adonis analyses (**Table S2**). Meanwhile, this result implied that these predominant groups were characterized by functional diversity, that is, some species may mainly degrade polysaccharides and/or lignin, and some may mainly degrade proteins and/or fatty acids, or both.

3.5 Function prediction of the deep-sea bacterial communities

Adonis analyses based on the PICRUSt function prediction suggested that the type of POM contributed more to microbial functional diversity (R^2 = 0.185, *P* = 0.01) than other factors (**Table S4**). Moreover, enrichments with plant detritus or animal tissue tended to cluster together, while fatty acid enrichments were scattered among all the enrichments (**Fig. S5**). Furthermore, the enrichment patterns of key metabolic pathways of the bacterial communities varied among OM substrates. For example, several key modules for carbohydrate metabolism were obviously enriched in plant detritus enrichment, such as glycolysis (KEGG modules: M00001 and M00002), pentose phosphate pathway (M00007), pectin degradation (M00081), D-galacturonate degradation (M00631), and galactose degradation (M00632) (**Table S5**). Intriguingly, the module for nitrogen fixation was particularly enriched in the nitrogen-deficient enrichments of plant detritus and fatty acid enrichments. The relative abundance of the nitrogen fixation module (M00175) in plant detritus enrichments was 2.7 times higher than that in protein enrichments (**Table S5**). In fatty acid enrichments, the modules thought to be responsible for fatty acid oxidation were significantly enriched (**Table S5**), such as beta-oxidation (M00087), acyl-CoA synthesis (M00086), the ethylmalonyl-CoA pathway (M00373 and M00774), and acylglycerol degradation (M00098).

In addition, the FAPROTAX predictions revealed that the top ten functional groups were chemoheterotrophy, fermentation, respiration of sulphur compounds, sulphate respiration, hydrogen oxidation, sulphur respiration, nitrate reduction, thiosulfate respiration, oxidation of sulphur compounds, and iron respiration. These results indicated that the bacterial decomposition of newly input POM during the “hot time” in the deep sea was an anaerobic process achieved via fermentation and diverse forms of anaerobic respiration, and these processes were perhaps tightly coupled with the cycles of nitrogen, sulphur, and hydrogen (**Fig. S6**).

3.6 Microbial co-occurrence networks within the bacterial assemblages of different POMs

To further understand the co-occurrence relationships among bacteria during the process of OM degradation, we constructed three co-occurrence networks for plant detritus-, animal tissue-, and fatty acid-enriched communities, respectively (**Fig. 5**). Each network diagram consisted of 58 to 75 OTUs, mainly belonging to the dominant families mentioned above (**Fig. 5** and **Table S6**).

The co-occurrence relationships of the bacterial OTUs were closely related to the enrichment substrates. The relationships among bacteria within the communities of animal tissue were negative in 90.7 % of cases (**Fig. 5a** and **Table S7**). However, cooperative relationships among different bacteria taxa were

observed in the plant detritus and fatty acid incubations (**Fig. 5b**, **Fig. 5c**, and **Table S7**). These two kinds of distinct relationships could be explained as follows: nutrients in animal tissue are replete and full of not only nitrogen, carbon, and phosphate sources but also other nutrients, such as vitamins. In contrast, many kinds of nutrients are quite limited in fatty acids and plant detritus. Therefore, bacteria in animal detritus enrichments showed competitive relationships and were more independent from each other. However, the fatty acid- and plant detritus-enriched consortia displayed more cooperative relationships to maintain nutrient balance and sustain the whole community. The details of the co-occurrence of the keystone species are described in the Supplementary Materials.

4 Discussion

Organic matter decomposition and mineralization in global oceans is a key process in global carbon cycling [16]. Characterizing the microorganisms involved in these processes in the deep sea is important for understanding how element cycling works in this vast ecosystem. Previous studies have used serial filtration and sediment trapping techniques to characterize suspended and sinking particle-associated microbes [7, 18]. Filtration methods can be biased by the volume of water filtered [49] and undersample fast-sinking particles. In this report, *in situ* incubations were conducted to observe bacteria involved in the transformation and mineralization of OM within the sinking “compact” biomasses in the deep sea of open areas (the Pacific Ocean and the Indian Ocean) and a marginal sea (the South China Sea). Our results showed that the organic pulse with different POMs enriched unique bacterial communities different from those in deep-sea water, or deep-sea sediment [50]. The *in situ* degradation and further mineralization of POM were mainly carried out by anaerobic processes via a special bacterial assemblage, though our incubation chambers were open to deep seawater. High-molecular-weight OM, usually in the form of insoluble particles, tends to go through anaerobic processes even in oxic seawater, as reported previously [51].

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4.1 The bacterial assemblages in enriched communities are unique

Most enriched microbial assemblages were composed of bacteria belonging to the classes of Bacteroidia, Gammaproteobacteria, Deltaproteobacteria, Spirochaetia, Campylobacteria, Clostridia, and Fusobacteriia, including families of *Marinifilaceae*, *Spirochaetaceae*, *Psychromonadaceae*, *Vibrionaceae*, *Moritellaceae*, *Desulfobulbaceae*, *Desulfobacteraceae*, Family_XII_Clostridiales, *Fusobacteriaceae*, *Arcobacteraceae*, *Sulfurospirillaceae*, and *Sulfurovaceae* (**Fig. 1c** and **Table S2**). However, they were only present as a minority in the surrounding seawater of empty chambers (**Fig. 3**). Routinely, these bacteria should exist as a rare species of famine waiting for the feast of sinking bulky POM on the cold barren seabed, as suggested previously by Jorgensen [8].

The above bacteria propagating on newly input POM are different from bathypelagic bacteria in the global ocean water column and deep-sea sediments [18, 50]. In the oceans, Gammaproteobacteria are the most prevalent among deep-sea pelagic prokaryotes, including the genera *Alteromonas*, *Halomonas*, *Pseudoalteromonas*, *Psychrobacter*, and so on [7, 15, 17, 18], and in deep-sea sediments, the phyla Chloroflexi, Planctomycetes, and Actinobacteria are usually predominant [50, 52]. However, they are not the components thriving on the newly input POM, suggesting the remolding of bacterial assemblages. Although uncultivated SAR324, SAR406, and SAR202 are prevalent in deep-sea pelagic oceans [18], they are probably not involved in the remineralization of the macromolecular OM, e.g., polysaccharides and proteins, as only SAR202 occurred in a few enrichments with very low abundance. Similarly, *Woeseiaceae*/ JTB255, the prevalent uncultivated bacteria in surface sediment as well as those present in the bottom water, were also missing in all enrichments (**Fig. 3** and **Table S3**).

Intriguingly, in contrast to the bacterial diversity sampled via the CTD water sampler mentioned above [18], our observation was partially consistent with those associated with sinking particles sampled by sediment traps in the oceanic interior described below. In abyssal sinking POM, DeLong and colleagues found that *Arcobacteraceae* (Campylobacterales) was the most predominant bacterial group [7], which also was the predominant member in all our enrichments (**Fig. 3**); additionally, the genera *Colwellia*, *Moritella*, and *Shewanella* of Gammaproteobacteria constituted the second predominant group in their report [7], which are frequently reported as psychrophilic and piezophilic deep-sea bacteria. Congruently, those Gammaproteobacteria taxa were present among the top 50 predominant members in almost all our enrichments. In particular, *Moritella* was even among the top 5 ones in some enrichments (**Table S3**).

Even in eutrophic coastal sediment, our parallel *in situ* incubation also revealed some predominant taxa shared with deep-sea enriched assemblages, e.g., *Desulfobulbaceae*, *Desulfobacteraceae*, *Arcobacteraceae*, *Vibrionaceae*, *Spirochaetaceae*, and *Fusobacteriaeae* [53]. However, several special groups, including *Marinilifaceae*, *Moritellacea*, and *Psychromonadaceae,* that dominated the deep-sea enriched consortia were barely detected in our coastal enrichments [53]. Moreover, in deep-sea chemoorganotrophic ecosystems of wood and whale falls, the taxa of *Spirochaetaceae*, *Marinilifaceae*, *Vibrionaceae*, and *Fusobacteriaeae* also occur in bacterial assemblages with high abundance [3]. In contrast, the bacteria *Desulfobulbaceae*, *Psychromonadaceae*, *Moritellaceae*, *Arcobacteraceae*, *Sulfurospirillaceae*, and *Sulfurovaceae* were rarely enriched in the wood-falls sunk in the Mediterranean Sea compared to our deep-sea incubations [47].

4.2 Bacterial assemblages may form a unique ecological niche

Some of the keystone taxa enriched *in situ* in the deep sea were quite diverse in phylogeny from those in other conventional habitats but were closely related to those from wood- and/or whale-fall ecosystems. For example, *Spirochaetaceae* are typical intestinal anaerobic bacteria [34], most OTUs of which in this study were obviously separated from those known to be from the animal intestine in the phylogenetic tree (**Fig. S7**). However, they formed several taxonomic branches with those from wood-fall ecosystems, and our coastal enrichments (**Fig. S7**). Therefore, members of *Spirochaetaceae* displayed obvious ecotypic differentiation.

A total of 29 OTUs of *Desulfobulbaceae* were found in the *in situ* enrichments. In the phylogenetic tree, they neighboured those from the chemosynthetic wood-fall or whale-fall ecosystems but separated from those from hydrothermal vents (**Fig. S8**). *Arcobacteraceae* have been identified as emerging pathogens potentially cause health risks to humans and animals [54] and also occur in hydrothermal vents like *Sulfurovaceae* [37, 55]. However, phylogenetic analysis showed that the dominant species of *Arcobacteraceae* (represented by OTU9977, OTU4225, OTU6340, and OTU1752) were obviously separated from those associated with humans and animals or with hydrothermal origins, but clustered onto a branch with those from the deep-sea wood-falls (**Fig. S9**). The same phenomenon was observed for the most abundant bacterium of *Sulfurovaceae,* OTU4956 (**Fig. S10**). This indicates that SOBs in chemoorganoheterotrophic ecosystems display obvious ecotypic differentiation from those in chemolithoautotrophic ecosystems. Thus, bacterial assemblages via the deep-sea *in situ* enrichment may form a unique ecological niche, like whale-fall and wood-fall ecosystems, described as one of the four chemosynthetic deep-sea ecosystems [48].

4.3 The key bacterial taxa play important and different roles in POM mineralization

Functional prediction results showed that the hydrolysis of proteins and polysaccharides, oxidation of fatty acids, fermentation, sulphate reduction, and sulphur oxidation were distinctly enriched in the enrichment assemblages (**Table S5** and **Fig. S6**), suggesting that multiple functional groups were involved in the *in situ* mineralization of POMs in the deep sea.

Particularly, *Marinifilaceae*, *Spirochaetaceae*, *Psychromonadaceae,* and *Vibrionaceae* may be the direct decomposers and fermenters of macromolecular OM. *Marinifilaceae* family contains some psychrotolerant and facultatively anaerobe bacteria that are capable of degrading a large variety of macromolecules polysaccharides, e.g., starch, cellulose, and alginate, and protein, e.g., casein and gelatin, and generate various small molecular organic acids via fermentation [33, 56-58]. In addition, through omics data, we found that *Marinifilaceae* OTU6861, which mainly occurred in plant detritus enrichments, possessed the metabolic potential in hydrolyzing cellulose and xylan, while OTU4252 and OTU5909 within this family have no corresponding potential, but hydrolyze proteins (unpublished data in another

separate paper attached for reference). Previous studies showed that some members of *Spirochaetaceae* played an important role in the digestion of breakdown products from cellulose and hemicellulose in the termite gut [34]. Moreover, *Pleomorphochaeta multiformis* MO-SPC2 [59] and *Spirochaeta perfilievii* [60] within *Spirochaetaceae*, could utilize mono-, di- and poly-saccharides (xylan, trehalose, and pectin) and protein, and can generate formate, acetate, ethanol, pyruvate, and hydrogen via fermentation. Therefore, bacteria OTU5914 and OTU4953, sharing high similarity with *P. multiformis* MO-SPC2 (99 %) and *S. perfilievii* (97 %), respectively, may play key roles in the degradation of polysaccharide and/or protein in plant detritus and animal tissue enrichments in this study. Additionally, previous studies also found that some members within *Vibrionaceae* and *Psychromonadaceae*, could degrade various macromolecular substrates, e.g., starch, xylan, alginate, mannan, gelatin, casein, chitin, and lecithin [35, 61], suggesting that those enriched taxa may play an important role in the mineralization of plant detritus and animal tissues in the deep sea *in situ* as well.

Although the above taxa have not been found or proved to have fatty acid oxidation capacity in previous reports, the functional prediction results showed that fatty acid oxidation-related modules were significantly enriched in fatty acid enrichment (**Table S5**), suggesting that bacteria with potential in fatty acid oxidation occurred in the fatty acid enrichments.

Common SRBs and SOBs were found in most of the enrichments (**Fig. 3**). As well, respirations of sulphur compounds, e.g., sulphate, sulphur, thiosulfate, and oxidation of sulphur compounds were detected by function prediction (**Fig. S6**). Both *Arcobacteraceae* and *Sulfurovaceae* are typical chemoautotrophic marine SOBs [37-39]. In our enrichments, *Arcobacteraceae* was more abundant, prevalent, and diverse than *Sulfurovaceae* (**Fig. 3** and **Fig. 4**); in contrast, the bacterial *Sulfurovaceae* is the predominant SOB in the hydrothermal plume close to a vent [62]. Evidently, they are not directly involved in macromolecular OM decomposition but oxidizing sulphides generated by SRBs, such as *Desulfobulbaceae* and *Desulfobacteraceae*, which might use the most common microbial fermentation products (e.g., acetate, propionate, butyrate, lactate, and hydrogen) as an energy source, coupled with sulphate, sulphite, and/or thiosulfate reduction, like cultured strain KaireiS1 and members of *Desulfofrigus*, *Desulfofaba*, and *Desulfotalea* [41, 42]. Moreover, the chemoautotrophic lifestyle of *Arcobacteraceae* and *Sulfurovaceae* in the enrichments will suppress the accumulation of hydrogen sulphide; otherwise, sulphide may have detrimental effects on other microorganisms. These results indicated POM anaerobic degradation coupled with sulphate reduction inside the organic particles. The inorganic chemical energy retained in sulphide was conserved by SOBs via sulphur oxidation and preserved in the form of new organic carbon to avoid energy escape from the particle ecosystem to the extremely oligotrophic surroundings.

At the community level based on functional prediction, the metabolic modules for nitrogen fixation were selectively enriched in the enrichments of plant detritus and fatty acids (**Table S5**). This result indicated nitrogen fixation was accompanied by POM remineralization in nitrogen-nutrient-depleted enrichments. A recent study showed that some members within Acidobacteria, Firmicutes, Nitrospirae,

Gammaproteobacteria, and Deltaproteobacteria are active diazotrophs in the deep-sea sediments, among which Desulfobacterales and Desulfuromonadales of Deltaproteobacteria are the most abundant diazotrophs [63]. In this study, the dominant *Desulfobacteraceae* belonging to Desulfobacterales, might play the role of nitrogen fixation, in addition to sulphur metabolism. Moreover, the *Marinifilaceae* OTU6861 also possesses the ability of nitrogen fixation (unpublished data, in another separate paper attached for reference). Same as our observation in the enrichments, more and more deep-sea nitrogen fixation has been found to occur at regional sites of enhanced productivity, such as methane seeps, mud volcanoes, and hydrothermal vents [64, 65]. Cross the ocean, nitrogen fixation accompanies chemoheterotrophic processes, as we observed, which are stimulated by OMs inside organic aggregates short of nitrogen sources in the deep water column. Nitrogen deficiency may be one of the key factors restricting the degradation and transformation of nonnitrogenous OM; therefore, diazotrophs play an important role in the biogeochemical process of POMs in the deep sea, which agrees with one of the predictions on nitrogen fixation made by Chakraborty: “nitrogen fixation can occur on large particles with high concentrations of polysaccharides and polypeptides in fully oxygenated marine waters” [66].

Interestingly, cooperative relationships dominate the bacterial relationships within the assemblages enriched with polysaccharides or fatty acids. In contrast, the relationships of the dominant taxa in protein enrichments were almost competitive (**Fig. 5**). Presumably, the co-occurrence of the key taxa within each assemblage is determined by the availability of nutrients and the supply of energy, both influenced by the OM provided. We hypothesized that the relative scarcity of nitrogen sources may be one of the main factors leading to the cooperative relationship among microorganisms, in addition to the type of organic substrates themselves. Therefore, in communities supported by polysaccharides or fatty acids, different members may cooperate to deal with the shortage of nitrogen sources.

5 Summary And Conclusion

Based on the results of this study, we aimed to delineate the roles of microbial key taxa in the degradation of POM and the relationships among them. As shown in **Figure 6**, the key taxa belonging to *Marinifilaceae*, *Spirochaetaceae*, *Psychromonadaceae*, and *Vibrionaceae* were inferred to be the direct depolymerizers and degraders of polysaccharides and/or protein polymers in the plant detritus or animal tissue. They depolymerize the natural polymers into monomers and simultaneously produce low-molecular weight compounds by fermentation (**Fig. 6**). These fermentation products can be supplied to SRBs of *Desulfobulbaceae* and *Desulfobacteraceae* or sulphur-reducing bacteria of *Sulfurospirillaceae* to generate reduced hydrogen sulphide. The reduced sulphur compounds further provide energy for chemoautotrophic bacteria of SOBs belonging to *Arcobacteraceae* and *Sulfurovaceae*, these primary producers that may feed the community in return by newly synthesized carbon sources and reduce the potential negative effects of high concentrations of sulphide. Therefore, bacterial assemblages developed over the new organic inputs and formed a unique ecological niche in the deep sea.

In conclusion, this report revealed unique deep-sea benthic bacterial communities responsive to diverse natural POM input, by which macromolecules in the natural plant detritus and animal tissue sinking to the seabed is decomposed and remineralized *in situ* in the deep sea. This suggested these communities which are different from those dominating the water column and sediment, play an unneglectable role in carbon biogeochemical cycling in global oceans.

Declarations

Conflict of interest

The authors declare that they have no conflict of interest.

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Author contributions

Zongze Shao conceived the idea, applied the financial supports, organized and performed *in situ* incubation and processed samples on board during the cruise of COMRA DY40 in the Indian Ocean. Chunming Dong organized and conducted in situ incubation and processed samples on board during the cruise of COMRA DY45 in the Pacific Ocean and the South China Sea. Zongze Shao, Jianyang Li, and Zhaobin Huang participated in equipment deployment during the cruise of COMRA DY45 in the South China Sea and participated in the next year's cruise for equipment recovery with Qiliang Lai. Jianyang Li and Chunming Dong conducted further sample processing. Jianyang Li analyzed the 16S rRNA sequence data. Zongze Shao, Jianyang Li, and Chunming Dong interpreted the results and wrote the manuscript. Donghui Zhou and Zongze Shao were involved in equipment development and maintenance. Linfeng Gong was responsible for building the analysis platform. Guangyi Wang reviewed the paper.

References

1. Burdige DJ: **Burial of terrestrial organic matter in marine sediments: A re-assessment**. *Global Biogeochemical Cycles* 2005, **19**(4):GB4011.

2. Bianchi TS: **The role of terrestrially derived organic carbon in the coastal ocean: A changing paradigm and the priming effect**. *Proceedings of the National Academy of Sciences* 2011, **108**(49):19473-19481.

3. Kalenitchenko D, Le Bris N, Dadaglio L, Peru E, Besserer A, Galand PE: **Bacteria alone establish the chemical basis of the wood-fall chemosynthetic ecosystem in the deep-sea**. *The ISME journal* 2017.

4. Soltwedel T, Guilini K, Sauter E, Schewe I, Hasemann C: **Local effects of large food-falls on nematode diversity at an arctic deep-sea site: Results from an in situ experiment at the deep-sea observatory HAUSGARTEN**. *J Exp Mar Biol Ecol* 2017.

5. Goffredi SK, Orphan VJ: **Bacterial community shifts in taxa and diversity in response to localized organic loading in the deep sea**. *Environ Microbiol* 2010, **12**(2):344-363.

6. Kalenitchenko D, Fagervold SK, Pruski AM, Vetion G, Yucel M, Bris NL, Galand PE: **Temporal and spatial constraints on community assembly during microbial colonization of wood in seawater**. *The ISME journal* 2015, **9**(12):2657-2670.

7. Boeuf D, Edwards BR, Eppley JM, Hu SK, Poff KE, Romano AE, Caron DA, Karl DM, DeLong EF: **Biological composition and microbial dynamics of sinking particulate organic matter at abyssal depths in the oligotrophic open ocean**. *Proceedings of the National Academy of Sciences* 2019:201903080.

8. Jorgensen BB, Boetius A: **Feast and famine–microbial life in the deep-sea bed**. *Nat Rev Microbiol* 2007, **5**(10):770-781.

9. Grabowski E, Letelier RM, Laws EA, Karl DM: **Coupling carbon and energy fluxes in the North Pacific Subtropical Gyre**. *Nat Commun* 2019, **10**(1):1895.

10. Poff KE, Leu AO, Eppley JM, Karl DM, DeLong EF: **Microbial dynamics of elevated carbon flux in the open ocean's abyss**. *Proc Natl Acad Sci U S A* 2021, **118**(4).

11. Fontanez KM, Eppley JM, Samo TJ, Karl DM, DeLong EF: **Microbial community structure and function on sinking particles in the North Pacific Subtropical Gyre**. *Front Microbiol* 2015, **6**:469.

12. van Waveren I, Visscher H: **Analysis of the composition and selective preservation of organic matter in surficial deep-sea sediments from a high-productivity area (Banda Sea, Indonesia)**. *Palaeogeography, Palaeoclimatology, Palaeoecology* 1994, **112**(1):85-111.

13. Orsi WD: **Ecology and evolution of seafloor and subseafloor microbial communities**. *Nature Reviews Microbiology* 2018.

14. Arnosti C: **Microbial Extracellular Enzymes and the Marine Carbon Cycle**. *Annual Review of Marine Science* 2011, **3**(1):401-425.

15. Liu R, Wang L, Liu Q, Wang Z, Li Z, Fang J, Zhang L, Luo M: **Depth-Resolved Distribution of Particle-Attached and Free-Living Bacterial Communities in the Water Column of the New Britain Trench**. *Front Microbiol* 2018, **9**.

16. Karl DM: **Microbial oceanography: paradigms, processes and promise**. *Nat Rev Microbiol* 2007, **5**(10):759-769.

17. Buchan A, LeCleir GR, Gulvik CA, González JM: **Master recyclers: features and functions of bacteria associated with phytoplankton blooms**. *Nature Reviews Microbiology* 2014, **12**:686.

18. Salazar G, Cornejocastillo FM, Benitezbarrios VM, Frailenuez E, Alvarezsalgado XA, Duarte CM, Gasol JM, Acinas SG: **Global diversity and biogeography of deep-sea pelagic prokaryotes**. *The ISME journal* 2016, **10**(3):596-608.

19. Bergauer K, Fernandez-Guerra A, Garcia JAL, Sprenger RR, Stepanauskas R, Pachiadaki MG, Jensen ON, Herndl GJ: **Organic matter processing by microbial communities throughout the Atlantic water column as revealed by metaproteomics**. *Proceedings of the National Academy of Sciences of the United States of America* 2018, **115**(3):E400-E408.

20. Pham VD, Konstantinidis KT, Palden T, DeLong EF: **Phylogenetic analyses of ribosomal DNA-containing bacterioplankton genome fragments from a 4000 m vertical profile in the North Pacific Subtropical Gyre**. *Environ Microbiol* 2008, **10**(9):2313-2330.

21. Giovannoni SJ, Thrash JC, Temperton B: **Implications of streamlining theory for microbial ecology**. *The ISME journal* 2014, **8**(8):1553-1565.

22. Kuczynski J, Stombaugh J, Walters WA, González A, Caporaso JG, Knight R: **Using QIIME to analyze 16S rRNA gene sequences from microbial communities**. *Curr Protoc Bioinformatics* 2011, **Chapter 10**:Unit10.17-10.17.

23. Magoč T, Salzberg SL: **FLASH: fast length adjustment of short reads to improve genome assemblies**. *Bioinformatics* 2011, **27**(21):2957-2963.

24. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R: **UCHIME improves sensitivity and speed of chimera detection**. *Bioinformatics (Oxford, England)* 2011, **27**(16):2194-2200.

25. Edgar RC: **UPARSE: highly accurate OTU sequences from microbial amplicon reads**. *Nat Methods* 2013, **10**(10):996-998.

26. Lan Y, Wang Q, Cole JR, Rosen GL: **Using the RDP Classifier to Predict Taxonomic Novelty and Reduce the Search Space for Finding Novel Organisms**. *PLOS ONE* 2012, **7**(3):e32491.

27. Krzywinski MI, Schein JE, Birol I, Connors J, Gascoyne R, Horsman D, Jones SJ, Marra MA: **Circos: An information aesthetic for comparative genomics**. *Genome Res* 2009.

28. Katoh K, Rozewicki J, Yamada KD: **MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization**. *Briefings in Bioinformatics* 2017, **20**(4):1160-1166.

29. Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Vega Thurber RL, Knight R *et al*: **Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences**. *Nat Biotechnol* 2013, **31**(9):814-821.

30. Louca S, Parfrey LW, Doebeli M: **Decoupling function and taxonomy in the global ocean microbiome**. *Science* 2016, **353**(6305):1272-1277.

31. Deng Y, Jiang Y-H, Yang Y, He Z, Luo F, Zhou J: **Molecular ecological network analyses**. *BMC Bioinformatics* 2012, **13**(1):113.

32. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T: **Cytoscape: a software environment for integrated models of biomolecular interaction networks**. *Genome Res* 2003, **13**(11):2498-2504.

33. Wu W-J, Zhao J-X, Chen G-J, Du Z-J: **Description of *Ancylomarina subtilis* gen. nov., sp. nov., isolated from coastal sediment, proposal of Marinilabiliales ord. nov. and transfer of *Marinilabiliaceae*, *Prolixibacteraceae* and *Marinifilaceae* to the order Marinilabiliales**. *Int J Syst Evol Microbiol* 2016, **66**(10):4243-4249.

34. Abt B, Han C, Scheuner C, Lu M, Lapidus A, Nolan M, Lucas S, Hammon N, Deshpande S, Cheng J-F *et al*: **Complete genome sequence of the termite hindgut bacterium Spirochaeta coccoides type strain (SPN1T), reclassification in the genus Sphaerochaeta as Sphaerochaeta coccoides comb. nov. and emendations of the family Spirochaetaceae and the genus Sphaerochaeta**. *Standards in Genomic Sciences* 2012, **6**(2):194-209.

35. Kawasaki K, Nogi Y, Hishinuma M, Nodasaka Y, Matsuyama H, Yumoto I: ***Psychromonas marina* sp. nov., a novel halophilic, facultatively psychrophilic bacterium isolated from the coast of the Okhotsk Sea**. *Int J Syst Evol Microbiol* 2002, **52**(5):1455-1459.

36. Staley JT, Whitman WB: ***Fusobacteriaceae* fam. nov**. In: *Bergey's Manual of Systematics of Archaea and Bacteria.* 1-1.

37. Wang Y, Bi HY, Chen HG, Zheng PF, Zhou YL, Li JT: **Metagenomics Reveals Dominant Unusual Sulfur Oxidizers Inhabiting Active Hydrothermal Chimneys From the Southwest Indian Ridge**. *Front Microbiol* 2022, **13**:861795.

38. Callbeck CM, Pelzer C, Lavik G, Ferdelman TG, Graf JS, Vekeman B, Schunck H, Littmann S, Fuchs BM, Hach PF *et al*: ***Arcobacter peruensis* sp. nov., a Chemolithoheterotroph Isolated from Sulfide- and**

Organic-Rich Coastal Waters off Peru. *Appl Environ Microbiol* 2019, **85**(24):e01344-01319.

39. Wirsen CO, Sievert SM, Cavanaugh CM, Molyneaux SJ, Ahmad A, Taylor LT, DeLong EF, Taylor CD: **Characterization of an autotrophic sulfide-oxidizing marine *Arcobacter* sp. that produces filamentous sulfur**. *Appl Environ Microbiol* 2002, **68**(1):316-325.

40. Kodama Y, Ha LT, Watanabe K: ***Sulfurospirillum cavolei* sp. nov., a facultatively anaerobic sulfur-reducing bacterium isolated from an underground crude oil storage cavity**. *Int J Syst Evol Microbiol* 2007, **57**(4):827-831.

41. Adam N, Han Y, Laufer-Meiser K, Bährle R, Schwarz-Schampera U, Schippers A, Perner M: **Deltaproteobacterium Strain KaireiS1, a Mesophilic, Hydrogen-Oxidizing and Sulfate-Reducing Bacterium From an Inactive Deep-Sea Hydrothermal Chimney**. *Front Microbiol* 2021, **12**.

42. Knoblauch C, Sahm K, Jørgensen BB: **Psychrophilic sulfate-reducing bacteria isolated from permanently cold Arctic marine sediments: description of *Desulfofrigus oceanense* gen. nov., sp. nov., *Desulfofrigus fragile* sp. nov., *Desulfofaba gelida* gen. nov., sp. nov., *Desulfotalea psychrophila* gen. nov., sp. nov. and *Desulfotalea arctica* sp. nov**. *Int J Syst Evol Microbiol* 1999, **49**(4):1631-1643.

43. Mussmann M, Pjevac P, Kruger K, Dyksma S: **Genomic repertoire of the Woeseiaceae/JTB255, cosmopolitan and abundant core members of microbial communities in marine sediments**. *ISME J* 2017, **11**(5):1276-1281.

44. Mehrshad M, Rodriguez-Valera F, Amoozegar MA, Lopez-Garcia P, Ghai R: **The enigmatic SAR202 cluster up close: shedding light on a globally distributed dark ocean lineage involved in sulfur cycling**. *ISME J* 2018, **12**(3):655-668.

45. Varela MM, van Aken HM, Herndl GJ: **Abundance and activity of Chloroflexi-type SAR202 bacterioplankton in the meso- and bathypelagic waters of the (sub)tropical Atlantic**. *Environ Microbiol* 2008, **10**(7):1903-1911.

46. Landry Z, Swan BK, Herndl GJ, Stepanauskas R, Giovannoni SJ: **SAR202 Genomes from the Dark Ocean Predict Pathways for the Oxidation of Recalcitrant Dissolved Organic Matter**. *MBio* 2017, **8**(2).

47. Kalenitchenko D, Dupraz M, Le Bris N, Petetin C, Rose C, West NJ, Galand PE: **Ecological succession leads to chemosynthesis in mats colonizing wood in sea water**. *The ISME journal* 2016, **10**(9):2246-2258.

48. Bienhold C, Ristova PP, Wenzhofer F, Dittmar T, Boetius A: **How deep-sea wood falls sustain chemosynthetic life**. *PLOS ONE* 2013, **8**(1).

49. Padilla CC, Ganesh S, Gantt S, Huhman A, Parris DJ, Sarode N, Stewart FJ: **Standard filtration practices may significantly distort planktonic microbial diversity estimates**. *Front Microbiol* 2015, **6**:547.

50. Hoshino T, Doi H, Uramoto G-I, Wörmer L, Adhikari RR, Xiao N, Morono Y, D'Hondt S, Hinrichs K-U, Inagaki F: **Global diversity of microbial communities in marine sediment**. *Proceedings of the National Academy of Sciences* 2020, **117**(44):27587-27597.

51. Zhang Y, Xiao W, Jiao N: **Linking biochemical properties of particles to particle-attached and free-living bacterial community structure along the particle density gradient from freshwater to open ocean**. *Journal of Geophysical Research: Biogeosciences* 2016, **121**(8):2261-2274.

52. Zhang Y, Yao P, Sun C, Li S, Shi X, Zhang X-H, Liu J: **Vertical diversity and association pattern of total, abundant and rare microbial communities in deep-sea sediments**. *Mol Ecol* 2021, **30**(12):2800-2816.

53. Qi M, Jianyang L, Xianhua L, Zongze S: ***In situ* enrichment and diversity analysis of natural macromolecular polymer degrading bacteria in offshore sediments and water bodies**. *Journal of Applied Oceanography* 2020, **39**(04):566-573.

54. Khan IUH, Becker A, Cloutier M, Plötz M, Lapen DR, Wilkes G, Topp E, Abdulmawjood A: **Loop-mediated isothermal amplification: Development, validation and application of simple and rapid assays for quantitative detection of species of Arcobacteraceae family- and species-specific Aliarcobacter faecis and Aliarcobacter lanthieri**. *J Appl Microbiol* 2021, **131**(1):288-299.

55. Dick GJ, Anantharaman K, Baker BJ, Li M, Reed DC, Sheik CS: **The microbiology of deep-sea hydrothermal vent plumes: ecological and biogeographic linkages to seafloor and water column habitats**. *Front Microbiol* 2013, **4**:124.

56. Na H, Kim S, Moon EY, Chun J: ***Marinifilum fragile* gen. nov., sp. nov., isolated from tidal flat sediment**. *Int J Syst Evol Microbiol* 2009, **59**(9):2241-2246.

57. Yadav S, Villanueva L, Bale N, Koenen M, Hopmans EC, Damsté JSS: **Physiological, chemotaxonomic and genomic characterization of two novel piezotolerant bacteria of the family Marinifilaceae isolated from sulfidic waters of the Black Sea**. *Syst Appl Microbiol* 2020, **43**(5):126122.

58. Ruvira MA, Lucena T, Pujalte MJ, Arahal DR, Macián MC: **Marinifilum flexuosum sp. nov., a new Bacteroidetes isolated from coastal Mediterranean Sea water and emended description of the genus Marinifilum Na et al., 2009**. *Syst Appl Microbiol* 2013, **36**(3):155-159.

59. Miyazaki M, Sakai S, Ritalahti KM, Saito Y, Yamanaka Y, Saito Y, Tame A, Uematsu K, Löffler FE, Takai K *et al*: ***Sphaerochaeta multiformis* sp. nov., an anaerobic, psychrophilic bacterium isolated from subseafloor sediment, and emended description of the genus *Sphaerochaeta***. *Int J Syst Evol Microbiol* 2014, **64**(Pt 12):4147-4154.

60. Dubinina G, Grabovich M, Leshcheva N, Rainey FA, Gavrish E: **Spirochaeta perfilievii sp. nov., an oxygen-tolerant, sulfide-oxidizing, sulfur- and thiosulfate-reducing spirochaete isolated from a saline**

spring. *Int J Syst Evol Microbiol* 2011, **61**(Pt 1):110-117.

61. Li B, Liu J, Zhou S, Fu L, Yao P, Chen L, Yang Z, Wang X, Zhang X-H: **Vertical variation in *Vibrio* community composition in Sansha Yongle Blue Hole and its ability to degrade macromolecules**. *Marine Life Science & Technology* 2020, **2**(1):60-72.

62. Meier DV, Pjevac P, Bach W, Hourdez S, Girguis PR, Vidoudez C, Amann R, Meyerdierks A: **Niche partitioning of diverse sulfur-oxidizing bacteria at hydrothermal vents**. *The ISME journal* 2017, **11**(7):1545-1558.

63. Kapili BJ, Barnett SE, Buckley DH, Dekas AE: **Evidence for phylogenetically and catabolically diverse active diazotrophs in deep-sea sediment**. *The ISME journal* 2020, **14**(4):971-983.

64. Dekas AE, Fike DA, Chadwick GL, Green-Saxena A, Fortney J, Connon SA, Dawson KS, Orphan VJ: **Widespread nitrogen fixation in sediments from diverse deep-sea sites of elevated carbon loading**. *Environ Microbiol* 2018, **20**(12):4281-4296.

65. Dekas AE, Chadwick GL, Bowles MW, Joye SB, Orphan VJ: **Spatial distribution of nitrogen fixation in methane seep sediment and the role of the ANME archaea**. *Environ Microbiol* 2014, **16**(10):3012-3029.

66. Chakraborty S, Andersen KH, Visser AW, Inomura K, Follows MJ, Riemann L: **Quantifying nitrogen fixation by heterotrophic bacteria in sinking marine particles**. *Nature communications* 2021, **12**(1):4085.

Figures

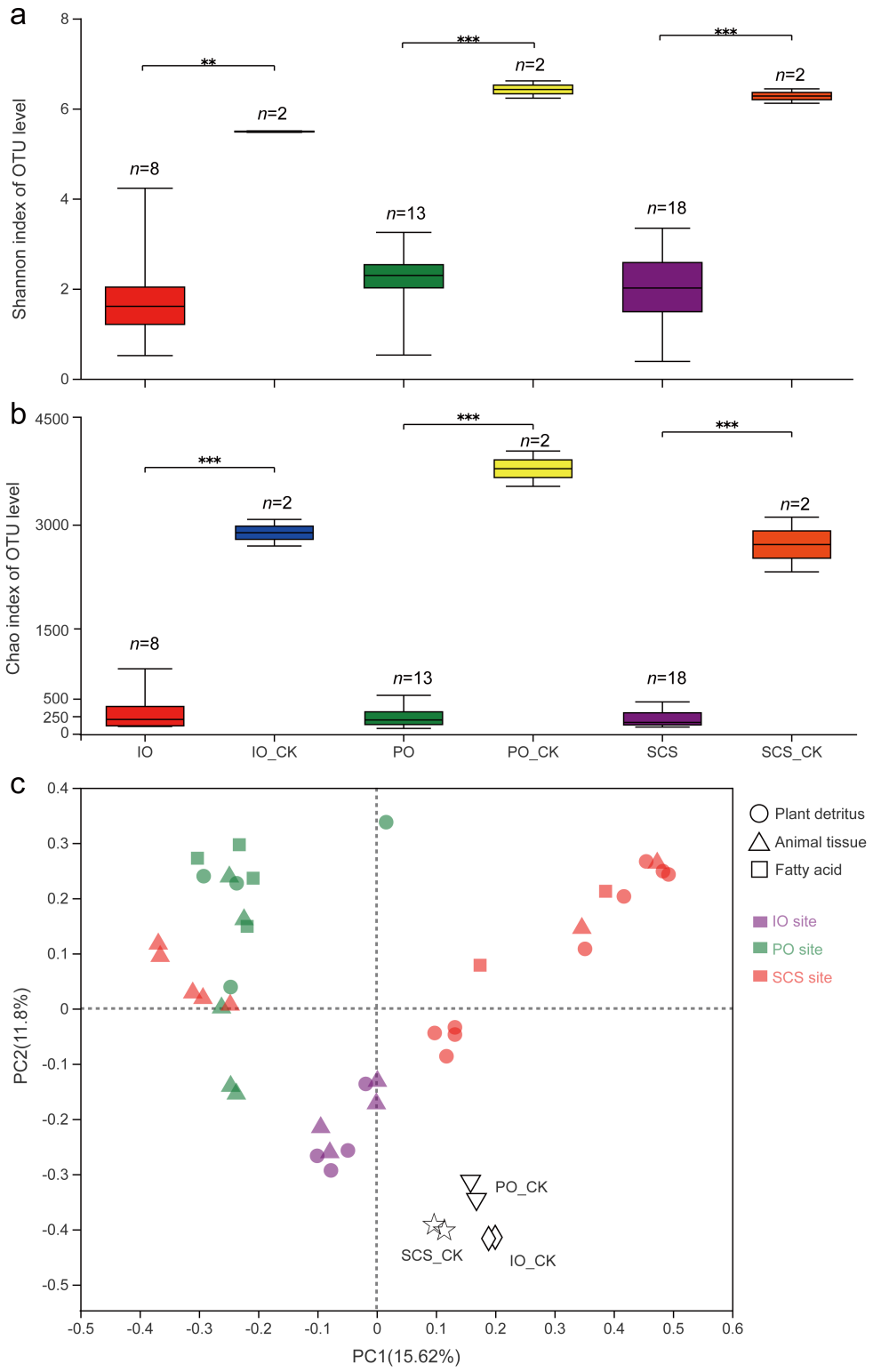

Figure 1

Comparison of bacterial diversity and compositions between enriched microbial assemblages and control samples. These deep-sea microbial assemblages were enriched respectively with natural POMs *in situ* in different oceans. Control samples were the surrounding seawater without any POM. (**a**) Shannon index, (**b**) Chao index, and (**c**) principal coordinate analysis (PCoA) of beta diversity (Bray–Curtis distances) at the OTU level. Welch's t-test was used to estimate the significance of differences among the

samples. 0.001 < $P \leq 0.01$ (**), $P \leq 0.001$ (***). IO, Indian Ocean; SCS, South China Sea; PO, Pacific Ocean. CK, control sample without POM supplements.

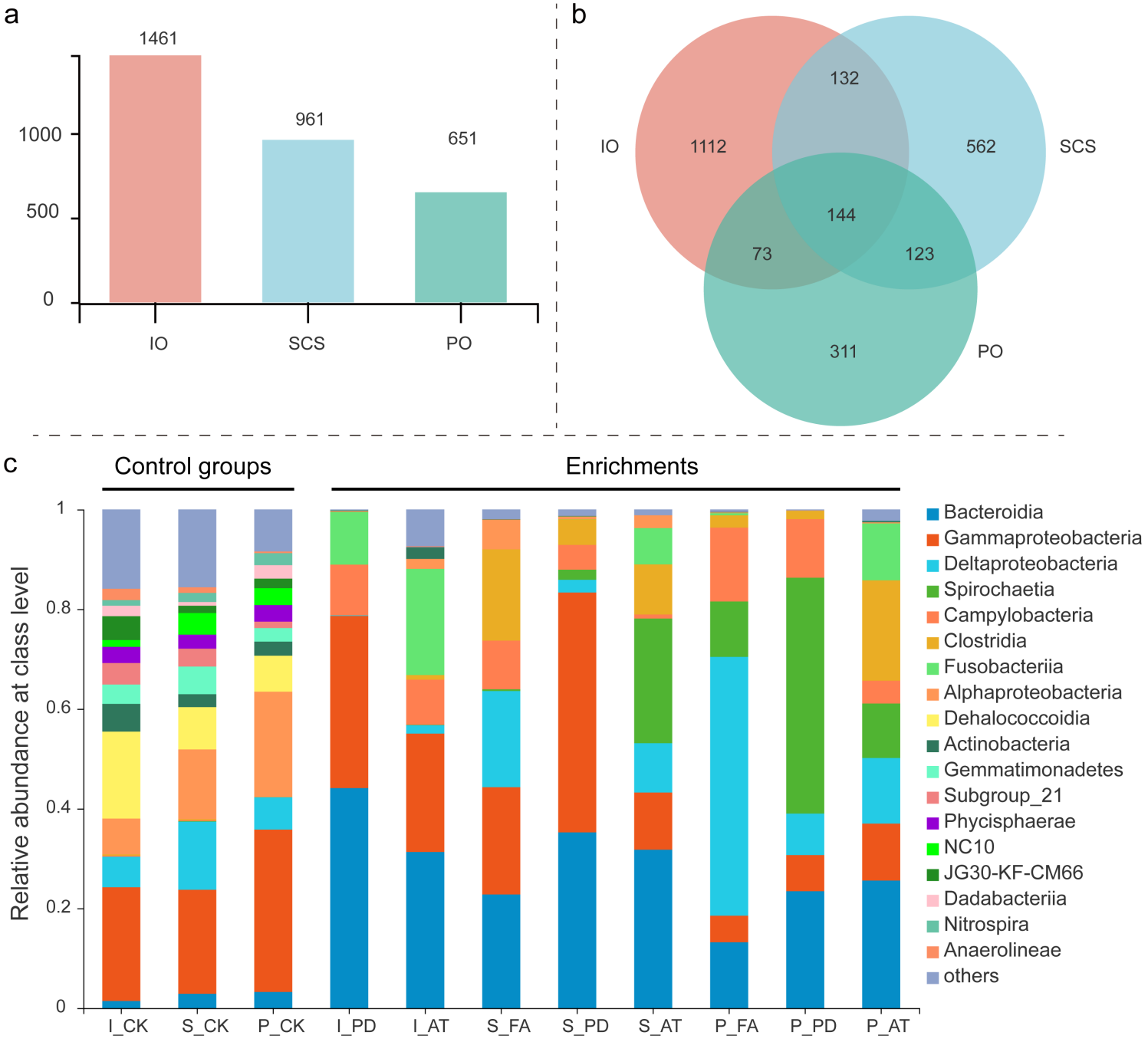

Figure 2

Bacterial community compositions and shared OTUs among the deep-sea microbial assemblages. (**a**) Bar chart showing the total number of OTUs at each incubation site, (**b**) Venn diagram showing the shared and specific OTUs among the POM enrichments at the three deep-sea sites, and (**c**) Average

relative abundance of bacteria at the class level. IO, Indian Ocean; SCS, South China Sea; PO, Pacific Ocean. PD, plant detritus; AT, animal tissue; FA, fatty acid.

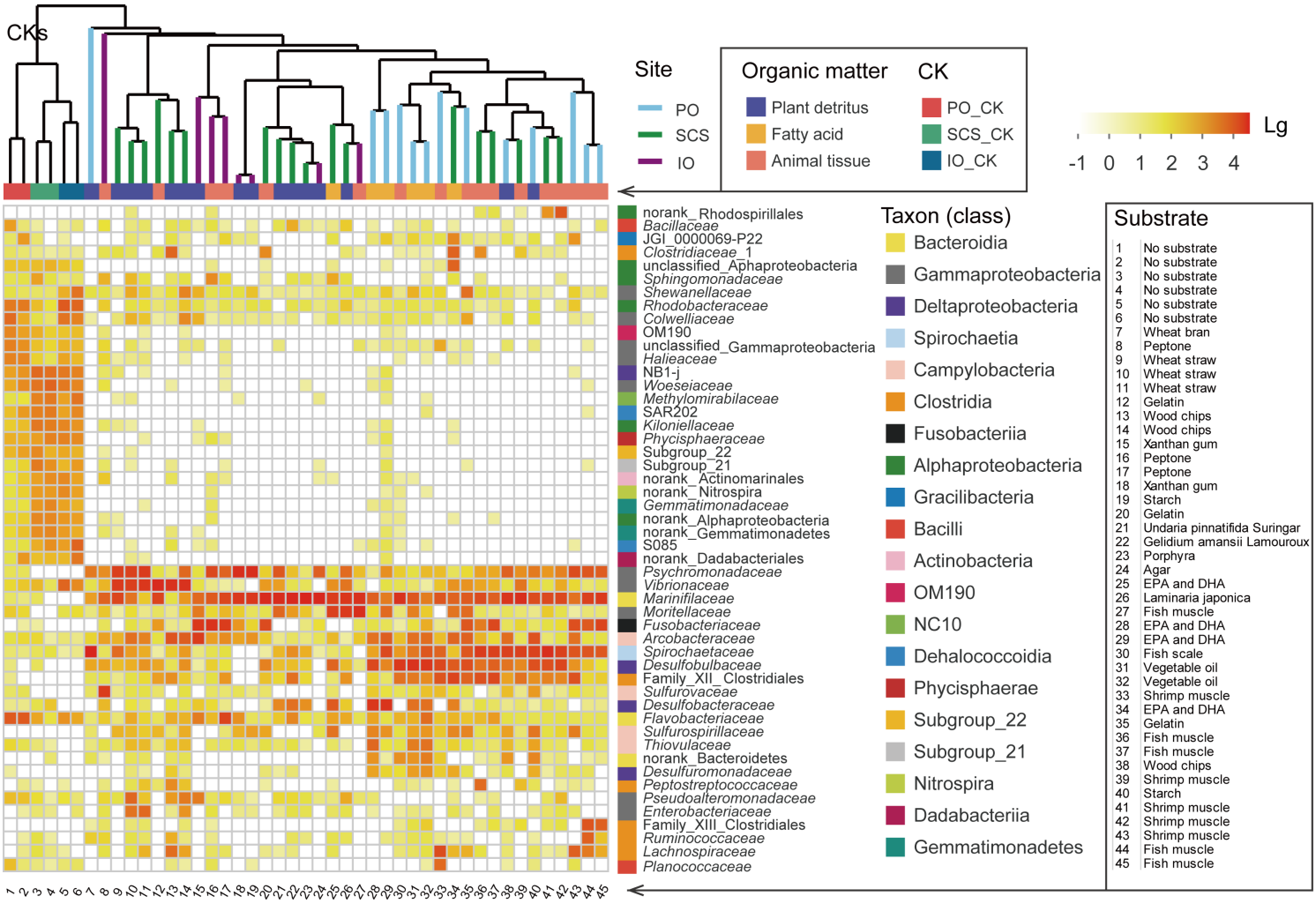

Figure 3

Heatmap showing the relative abundances of the 50 most abundant family-level taxa. Columns 1-6 are the control samples and columns 7-45 are the 39 incubations at three sites. Samples were clustered using the UPGMA technique based on the Bray–Curtis similarity index (top). Enrichment substrates for each sample are listed on the right. IO, Indian Ocean; SCS, South China Sea; PO, Pacific Ocean. CK, control sample without POM supplements.

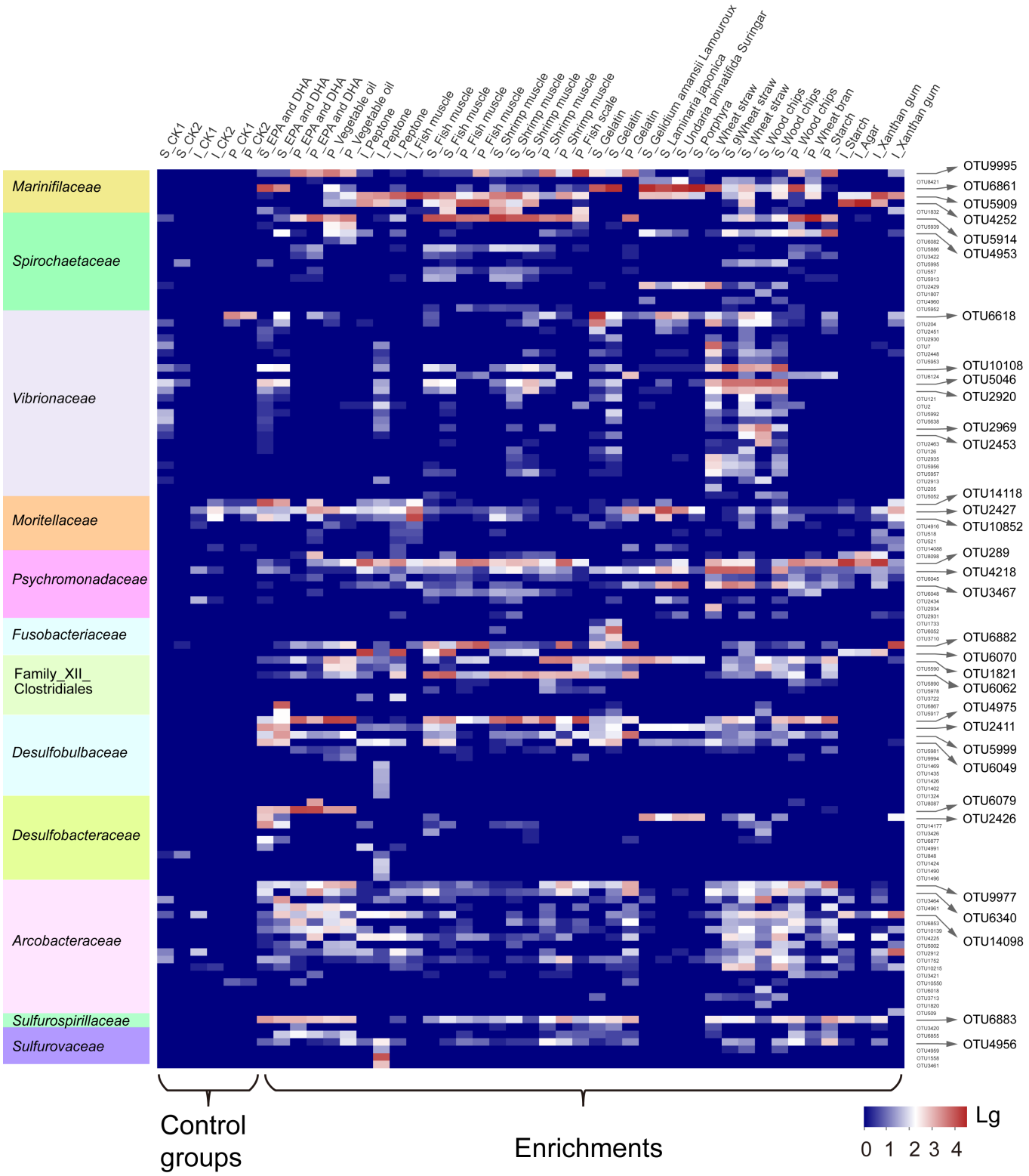

Figure 4

Heatmap showing the relative abundances of OTUs within each dominant family. This diagram shows the high diversity of these families, especially *Spirochaetaceae*, *Vibrionaceae*, *Desulfobacteraceae*, and *Arcobacteraceae*, among the 39 incubations at the three incubation sites. These families were the dominant groups, while members of each family varied at the OTU level according to substrate type and incubation location.

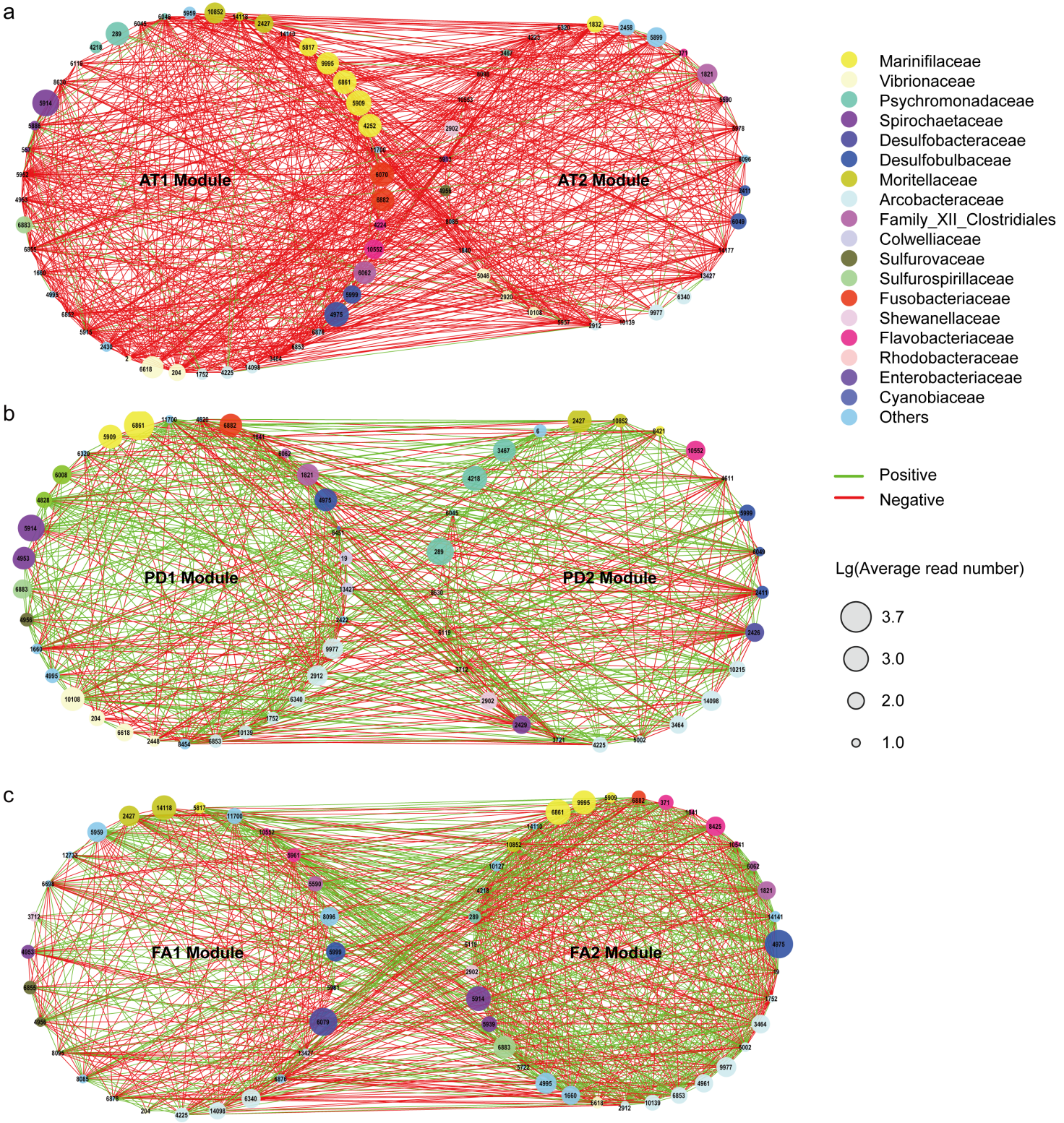

Figure 5

Networks showing bacterial co-occurrence within the microbial assemblages. The co-occurrence relationships of the bacterial OTUs were closely related to the enrichment substrates. Competitive relationships overwhelmingly dominated the protein-based communities (**a**) with more than 90 % negative edges, while cooperative relationships slightly dominated the bacterial communities grown with polysaccharides (**b**) or fatty acids (**c**) with more than 62 % and 54 % positive edges, respectively. Each

node represents an OTU, with colour indicating family-level taxonomy. OTU IDs are overlaid on each node, and edge relationships are indicated with green and red lines for positive and negative connections, respectively. The size of the node represents the average relative abundance of the OTU. PD, plant detritus; AT, animal tissue; FA, fatty acid.

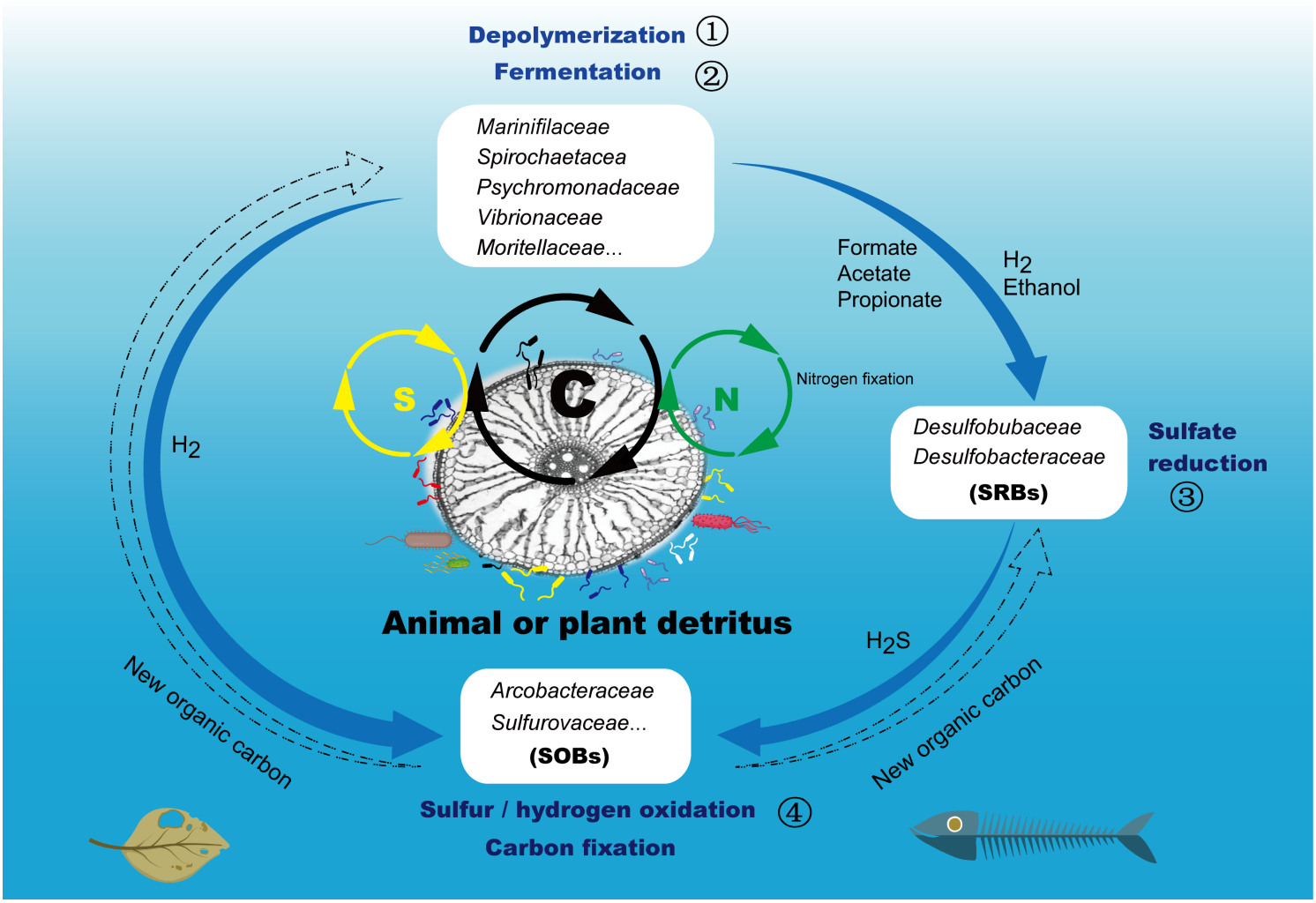

Figure 6

Schematic model of microbial degradation and transformation of animal or plant detritus in the deep sea. Within the bacterial assemblages thriving on newly input POM *in situ*, biopolymers are decomposed to drain energy driving the carbon cycle coupled with the nitrogen and sulphur cycles at the microscale. Four functional groups (□-□) were found to be key players involved in the mineralization process, initiated by heterotrophic bacteria, including *Marinifilaceae*, *Spirochaetaceae*, *Psychromonadaceae*, *Vibrionaceae*, and *Moritellaceae*, which play key roles in the depolymerization of polysaccharides and polypeptides of animal or plant detritus and ferment monomers to low-molecular-weight compounds, including formate, ethanol, acetate, propionate, and hydrogen. Subsequently, with the depletion of oxygen, SRBs of *Desulfobulbaceae* and *Desulfobacteraceae* grow up by utilizing these fermentation products, coupled with sulphate reduction to generate reduced H_2S, which further stimulates the thriving of chemoautotrophic SOBs of *Arcobacteraceae* and *Sulfurovaceae* and in return produces new organic

carbon feeding back to the heterotrophs within the microbial assemblages. However, protein and polysaccharides result in different bacterial communities at low taxonomic levels; details can be found in the main text of this report and specific reports on specific families in the future.

Supplementary Files

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

- 0713Additionalfile1.pdf
- 0713Additionalfile2.xlsx