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

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Research Article

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Abstract

Background

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.

Results

The bacterial diversity at the OTU level varied in accordance to organic substrates and geographic locations, but the functional groups are generally similar. The bacteria of *Marinifilaceae* were revealed as the key member; in addition, other key decomposers including *Spirochaetaceae*, *Psychromonadaceae*, *Vibrionaceae*, *Moritellaceae*, and *Fusobacteriaceae* were also recruited in most assemblages with varied abundance accordingly. Interestingly, nitrogen fixation inside the assemblages occurred in the process of polysaccharide decomposing. The microbial co-occurrence analysis revealed that nutrient availability and energy transfer determine relationships cooperation or competition. 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 proteinous substrates.

Conclusions

These in-situ observations revealed a unique bacterial assemblage in decomposing diverse newly input organic matters, which may constitute the microbial pumps involved in carbon, sulphur, and nitrogen cycles in oceanic interior.

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 remains, 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, lipids, 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, 4].

Sinking particulate organic matter (POM), which functions as a conduit for exporting organic materials from the euphotic zone to the deep ocean[5], critically contributes to the establishment of habitats for deep-sea benthic microbial communities[6]. 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[7, 8]. The sinking particulates that transit to the deep sea may contain energy-rich organic compounds or relatively energy-replete POM with lipids selectively preserved[5, 9]. High OM influx is usually accompanied by high sedimentation rates to the deep sea[10, 11]. The large and fast sinking particles may escape disaggregation, dissolution, solubilization, and even microbial decomposition en route to the deep sea[7], which determines the organic carbon and energy flux pattern of the deep sea and impacts the microbial communities in the deep sea[12, 13]. 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[14]. 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[5, 13, 15, 16]. Moreover, in the water column of pelagic oceans, the majority of prokaryotes remain uncultivated, such as SAR324, SAR406, and SAR202[16–19]. 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[5] 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 OM 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 materials rich in polysaccharides, proteins, or lipids using our newly developed deep-sea *in-situ* microbial incubator (DIMI), landed at different geographic locations (marginal sea and open sea). The OM input-spiked bacterial assemblages were subjected to bacterial diversity and meta-omics analyses to identify the key OM decomposers active *in situ* and their metabolic potentials. The results will provide knowledge about the microbial interactions with sinking POM and contribute to our understanding the roles of the microbial carbon pump in elemental biogeochemical cycles in global oceans.

Results

Deep-sea in situ incubation of OM-decomposing bacteria

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), the deep-sea basin beside the Southwest Indian Ridge in the Indian Ocean (IO, open sea), and a flat-topped seamount in the West Pacific Ocean (PO, open sea) at 3758 m, 4434 m, and 1622 m water depths for 375, 117, and 348 days (**Figs. S1-2**). Several kinds of natural materials rich in polysaccharides, proteins, or lipids were selected as fast-sinking POM (**Table S1**), loaded into 50 mL tubes and then placed into titanium alloy incubation chambers (designated TIC for the treatment incubation chamber). Meanwhile, two incubation chambers without OM supplementation (designated CIC for the control incubation chamber) were set up for collecting *in situ* seawater. Finally, all of the TICs and CICs were delivered to the deep sea floor with the deep-sea *in situ* microbial incubator (DIMI) device. 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**). After being recovered from the seabed and brought to the shipboard laboratory, all incubation tubes, even those with wood chips, exhibited obvious microbial growth indicated by turbidity and colour variation (**Fig. S3**); most samples smelled of hydrogen sulphide, and under a microscope, the field of view appeared with bacterial cells (**Table S1** and **Fig. S3**).

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

To profile the bacterial communities colonizing different types of OM, all 39 enrichments in TICs were subjected to community composition analysis via Illumina high-throughput sequencing and compared with the *in situ* seawater collected by CICs. In general, the α-diversity indexes (Shannon and Chao indexes) indicated that the bacterial diversity of the enrichments within the TICs was significantly lower than that of the *in situ* seawater in the CICs (**Fig. 1a and 1b**, $P < 0.01$). This indicated that bacterial communities were significantly enriched *in situ* by the OM supplements.

Principal coordinate analysis (PCoA) revealed that the structures of the enriched communities 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 based on the *β*-diversity index suggested that the enriched bacterial community structures were influenced by multiple factors, including geographic location (R^2 = 0.20, P = 0.001), the type of OM (R^2 = 0.13, P = 0.001), the enrichment time (R^2 = 0.094, P = 0.001), and other environmental factors, including temperature, water depth and salinity (for all factors, R^2 < 0.11, P < 0.0012) (**Table S2**). Among these factors, geographic location and OM type were the two most important determinants of the variation in microbial community structures.

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

Across the deep-sea *in-situ* enrichments, 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%) (**Fig. 2a**). 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_o_Clostridiales, *Fusobacteriaceae*, *Arcobacteraceae*, *Sulfurospirillaceae* and *Sulfurovaceae* (**Fig. 3** and **Fig. S4**). A total of 2,457 bacterial operational taxonomic units (OTUs) were retrieved from these 39 enrichments (**Table S4**). However, only 144 OTUs were shared among the three sites (**Fig. 2b**), indicating obvious variation among geographic locations at the OTU level.

Most of the dominant bacterial members listed above were anaerobes and constituted the main components of the microbial assemblages. In the *in situ* seawater collected by CICs, only 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[20] and benthic water[21–23], respectively. However, they were not detected in the enrichments with newly input OM (**Fig. 3**). Thus, special groups of heterotrophic bacteria were stimulated by the newly input OM, which were faster-growing and more competitive than the oligotrophs.

Keystone bacterial species involved in OM-decomposing in different in situ enrichments

To discriminate the keystone species enriched by different types of OM, we analysed the distribution of OTUs of each dominant family. The results showed that the bacteria within a family were divergent at the OTU level in response to different OMs (**Fig. 4**). Evidently, bacteria of the family *Marinifilaceae* were the most predominant, thriving in all OM enrichments and showing the highest abundance among all bacterial communities of 26.3% on average. Interestingly, six OTUs of this family exhibited a preference

for proteins, polysaccharides, and lipids. In detail, OTU6861 was mainly dominant in the consortia of substrates enriched with polysaccharides, such as wood debris, seaweed, and wheat bran, in the SCS and PO (**Fig. 4**). OTU4252 and OTU5909 both dominated the consortia of substrates enriched with proteins (**Fig. 4**), while OTU9995 occurred in all types of substrates at site PO, although it was predominant in proteinaceous enrichments. Moreover, OTU5914 not only occurred as the most predominant member in proteinaceous enrichments at the SCS site but also among all the enrichments of different OM types at the PO site.

Members of *Spirochaetaceae* are typical intestinal anaerobic bacteria that play an important role in the digestion of breakdown products from cellulose and hemicellulose in the termite gut[24]. In this study, *Spirochaetaceae* was the second predominant group in the enrichments, and a total of 28 OTUs were retrieved. However, most of the OTUs were obviously separated from those known to be from the animal intestine in the phylogenetic tree and formed several taxonomic branches with those from marine sediment, wood-fall ecosystems, and our coastal enrichments with OM (**Fig. S5**). Therefore, members of *Spirochaetaceae* displayed obvious ecotypic differentiation. Notably, one member (OTU5914) was noteworthy for its role in degrading polysaccharides and proteins in the deep sea *in situ*, which occurred in most polysaccharide- or protein-rich enrichments, 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 (**Fig. 4**).

In addition, the heterotrophic bacteria *Moritellaceae, Psychromonadaceae*[25], and *Vibrionaceae*[3, 26, 27] of Gammaproteobacteria also constituted the dominant members. The diversity within individual families varied with substrate and location at the OTU level (**Fig. 4**), which is described in detail in the text of the Supplementary Information and shown in **Figs. S6-S8**.

Members of *Desulfobulbaceae* are typical psychrophilic sulphate-reducing bacteria (SRBs) that can use the most common microbial fermentation products (e.g., acetate, propionate, butyrate, lactate, and hydrogen) in marine sediments as an energy source, coupled with sulphate, sulphite, and/or thiosulfate reduction[28]. Additionally, a recent report showed that the strain KaireiS1 belonging to the *Desulfobulbaceae* family could utilize hydrogen as an electron donor coupled with sulphate reduction[29]. Here, a total of 29 OTUs of *Desulfobulbaceae* were found in the *in situ* enrichments (**Fig. 4**). In the phylogenetic tree, they neighboured those from marine sediment, hydrothermal vents, and chemosynthetic wood-fall or whale-fall ecosystems (**Fig. S9**). They did not show a preference for certain OM substrates (**Fig. 4**). However, the dominant OTUs in this group varied with geographic location, and oddly, they were nearly absent at the IO site (**Fig. 4**).

Sulphur-oxidizing bacteria (SOBs) of *Arcobacteraceae* and *Sulfurovaceae*, which were enriched concomitantly with SRBs, are frequently detected in deep-sea hydrothermal chimneys, vent plumes[30], and vent animals as primary producers[31]. However, phylogenetic analysis showed that the dominant members of *Arcobacteraceae* (represented by OTU9977, OTU4225, OTU6340, and OTU1752) were obviously separated from those with hydrothermal origins but clustered onto a branch with those from the deep-sea wood-falls (**Fig. S11**). The same result was observed for the most abundant bacterium of

Sulfurovaceae, OTU4956 (**Fig. S12**). This indicates that SOBs in chemoorganoheterotrophic ecosystems display obvious ecotypic differentiation from those in chemolithoautotrophic ecosystems.

Function prediction of the deep-sea bacterial communities

To understand the relationships between bacterial communities and OM types, two different methods, PICRUSt and FAPROTAX, were applied to predict the functions of the enriched bacterial consortia. The PICRUSt analysis based on KEGG module clustering suggested that the properties of OM contributed more to microbial functional diversity (R^2 = 0.185, P = 0.01) than other factors, such as geographic location, enrichment time, temperature, depth and salinity (**Fig. 5** and **Table S3**). Protein and polysaccharide enrichments formed separate clusters, while lipid enrichments did not form any independent clusters (**Fig. 5**).

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 polysaccharide enrichment, such as glycolysis (KEGG modules: M00001 and M00002), pentose phosphate pathway (M00007), Entner-Doudoroff pathway (M00008), pectin degradation (M00081), D-galacturonate degradation (M00631), and galactose degradation (M00632) (**Table S5**). Intriguingly, the module for nitrogen fixation was selectively enriched in the nitrogen-deficient enrichments of polysaccharides and lipids. The relative abundance of the nitrogen fixation module (M00175) in polysaccharide enrichments was 2.7 times higher than that in protein enrichments (**Table S5**). In lipid enrichments, the modules thought to be responsible for fatty acid metabolism 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 OM 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 tightly coupled with the cycles of nitrogen, sulphur, and hydrogen (**Fig. S16**).

Metagenomic and metatranscriptomic analyses of the enriched bacterial communities

To confirm the response of the bacterial assemblages to different OM inputs, metagenomic and metatranscriptomic investigations were further conducted to retrieve the abundance and expression of the functional genes potentially involved in carbon, nitrogen, and sulphur metabolism. To this end, five enrichments of P-MX (wood chips), P-FP (wheat bran), P-YR (fish muscle), P-YL (fish scales), and P-YY (fish oil) were selected for metagenomic and metatranscriptomic sequencing based on the analysis results using MicroPITA software[32] (**Table S6**). A large number of genes responsible for hydrolysing

polysaccharides and proteins and fatty acid oxidization were found in these metagenomes (**Fig. 6** and **Table S7**). Approximately 1,407 to 3,034 genes that matched hits in the Carbohydrate-Active enZYmes (CAZy) database were detected in these metagenomes. The CAZy number was the highest in polysaccharide incubations but was the lowest in protein incubations.

In contrast, the genes involved in protein metabolism ranked highest (5406 genes) in the enrichment with collagen-rich fish scales (P-YL). These results indicated that the corresponding genes involved in the degradation of polysaccharides and proteins were selectively enriched in different OM substrates. In these metagenomes, genes encoding peptidases, oligopeptides, amino acid transporters, aminotransferases, and amino acid dehydrogenases for protein hydrolysis and their further metabolism were obviously enriched (**Fig. 6** and **Table S7**).

Moreover, the genes involved in fermentation (e.g., production of formate (*pflD*), ethanol (*aldH* and *adhE*), acetate (*acdAB* and *ackA*), propionate (*mmdAC* and *pccB*), lactate (*lldF* and *dlD*), and hydrogen (*hydABC*, *hydS*, and *hydM*)) were enriched in all OM enrichments (**Fig. 6** and **Table S9**). These simple compounds are the major end products of OM fermentation, which subsequently drive sulphate reduction by SRBs.

Similar to those involved in carbon metabolism, the genes involved in sulphur metabolism were also selectively enriched. The following processes of sulphur metabolism were detected in all five metagenomes: oxidation genes of hydrogen sulphide (*sqr*), thiosulfate (Sox complex system), sulphite (*soeABC* and *suoX*), and various dissimilatory reductions of sulphite, tetrathionate, and thiosulfate, in addition to the respiration of dimethyl sulfoxide (**Fig. 6** and **Table S9**).

With respect to nitrogen metabolism, we detected the enrichment of genes involved in both nitrogen fixation (*nifDKH*) and nitrogen reduction, such as dissimilatory nitrate reduction (*napAB*), nitrite reduction (*NirA* and *narB*), nitric oxide reduction (*norBC*), and nitrous oxide reduction (*nosZ*), with variation in abundance in these enrichments (**Fig. 6** and **Table S9**). More intriguingly, the metagenome-assembled genomes (MAGs), possessing nitrogen fixation-related genes, were mostly binned from polysaccharide and lipid metagenomes. These MAGs were mainly affiliated with *Marinifilaceae*, *Spirochaetaceae*, *Psychromonadaceae*, *Desulfobacteraceae*, and *Desulfobulbaceae* (data not shown).

To confirm that these metabolic pathways were active *in situ*, metatranscriptomic sequencing analyses were also performed with the five aforementioned enrichments. Approximately 87.0–97.0% of filtered paired reads were mapped to their own metagenomic scaffold dataset, indicating a high utilization rate of metatranscriptomic reads (**Table S6**). Metatranscriptomic data showed that in the polysaccharide enrichments, such as that with wood chips, the total transcript per million reads (TPM) values of genes encoding glycoside hydrolase (GH) were at least 4 times than those in other enrichments (**Fig. 6** and **Table S7-S8**). In contrast, the total TPM values of peptidase-encoding genes were obviously higher in both fish scale and fish muscle enrichments. The difference in the transcriptional pattern of genes for polymer hydrolysis between polysaccharide and protein enrichments reconfirmed the active response to corresponding organic substrates *in situ* (**Fig. S17**). For example, glycoside hydrolase (families GH13, GH3, GH32, GH57, and GH43) was enriched and expressed in the wood chip and wheat bran enrichments

(**Table S8**), while protein metabolism-related genes, such as those encoding peptidase and aminotransferase, were enriched and expressed in the fish scale and fish muscle enrichments.

In addition, the genes associated with nitrogen and sulphur metabolism, as well as organic fermentation, were also actively transcribed *in situ*. In particular, the genes involved in dissimilatory sulphite reduction in protein (P-YR) and lipid (P-YY) enrichments had higher transcriptional levels than those in other enrichments. The genes encoding Sqr, NapA, NorB, and NosZ also showed high transcriptional activity in enrichments with wood chips (P-MX) and fish scales (P-YL). Ethanol and acetate production genes were more actively transcribed in enrichments with wheat bran (P-FP) and fish scales (P-YL). Furthermore, genes involved in hydrogen production via fermentation were actively transcribed in wood chip (P-MX), wheat bran (P-FP), and fish scale (P-YL) enrichments (**Fig. 6** and **Table S9**).

Microbial co-occurrence networks within the bacterial assemblages of different OMs

Since the functional differences of enriched microbial communities were mainly affected by the properties of the organic substrates (**Fig. 5** and **Table S3**), to further understand the co-occurrence relationships among bacteria during the process of OM degradation, we constructed three co-occurrence networks for polysaccharide-, protein-, and lipid-enriched communities, respectively (**Fig. 7**). The complexity of the three network diagrams was high, and there were two modules in each network diagram (**Fig. 7**). Each network diagram consisted of 58 to 75 OTUs, mainly belonging to the dominant families mentioned above (**Fig. 7** and **Table S10**). The network results reconfirmed that these dominant microorganisms were the key members and played a pivotal role in the establishment of OM-degrading consortia in the deep sea.

The co-occurrence relationships of the bacterial OTUs were closely related to the enrichment substrates. The relationships among bacteria within the communities of proteinaceous substrates were negative in 90.7% of cases (the relationship lines in red) (**Fig. 7b** and **Table S11**). However, cooperative relationships among bacteria dominated in the communities in the polysaccharide and lipid incubations (the cross lines are mainly green) (**Fig. 7a, Fig. 7c**, and **Table S11**). This may be because nutrients in proteinaceous substrates are replete and full of not only nitrogen, carbon, and phosphate sources but also other nutrients, such as vitamins; in contrast, many nutrients are quite limited in cellulose, lignin, and lipids. Therefore, microorganisms in proteinaceous enrichments showed competitive relationships and were more independent from each other. In contrast, the other two kinds of consortia displayed cooperative relationships to maintain nutrient balance and sustain the whole community. For example, in the polysaccharide substrates, the potential OM degraders *Marinifilaceae*, *Spirochaetaceae*, *Vibrionaceae*, and *Moritellaceae* were generally positively correlated with SRBs, SOBs, and *Fusobacteriaceae.* At the OTU level, the co-occurrence of the keystone species is described in detail in the Supplementary Materials.

Discussion

Organic matter decomposition and mineralization in global oceans is a key process in global carbon cycling[14]. 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[5, 16]. Filtration methods can be biased by the volume of water filtered[33] 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). The organic pulse with different POMs enriched unique bacterial communities different from those in deep-sea water or sediment[34]. The degradation and further mineralization of POM in fast-sinking particles were mainly carried out by anaerobic bacteria via extracellular depolymerization, hydrolyte fermentation, and final mineralization coupled with dissimilatory sulphate reduction (**Fig. 6, Fig. S16** and **Table S9**). This is congruent with the opinion that high-molecular-weight OM, usually in the form of insoluble particles, tends to go through anaerobic processes even in oxic seawater[35].

The features of bacterial assemblages in enriched communities

The bacterial communities of the *in situ* enrichments varied according to the OM substrates and geographical locations (**Fig. 1c** and **Table S2**). Most microbial assemblages were composed of bacteria belonging to Bacteroidia, Gammaproteobacteria, Deltaproteobacteria, Spirochaetia, Campylobacteria, Clostridia, and Fusobacteriia, including families of *Marinifilaceae*, *Spirochaetaceae*, *Psychromonadaceae*, *Vibrionaceae*, *Moritellaceae*, *Desulfobulbaceae*, *Desulfobacteraceae*, Family_XII_o_Clostridiales, *Fusobacteriaceae*, *Arcobacteraceae*, *Sulfurospirillaceae*, and *Sulfurovaceae.* However, as a minority, only a few families were detected in the seawater of empty chambers, such as *Flavobacteriaceae* and the genera *Colwellia* and *Shewanella* (**Fig. 3** and **Table S4**). 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 and Sogin[6, 36].

The above bacteria propagating on newly input POM on the sea floor are different from bathypelagic bacteria in the global ocean water column and deep-sea sediments[16, 34]. For example, in the oceans, Gammaproteobacteria are the most prevalent among deep-sea pelagic prokaryotes, including the genera *Alteromonas*, *Halomonas*, *Pseudoalteromonas*, *Psychrobacter*, and so on[5, 13, 15, 16], while in deep-sea sediments, the phyla Chloroflexi, Planctomycetes, and Actinobacteria are usually predominant[16, 34, 37]. However, they are not the components thriving on the newly input POM, suggesting the remoulding of bacterial assemblages. Although uncultivated SAR324, SAR406, and SAR202 are prevalent in deep-sea pelagic oceans[16], they are probably not involved in the remineralization of the newly input OM, 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 bottom water, were also missing in all enrichments (**Fig. 3** and **Table S4**).

Intriguingly, in contrast to the bacterial diversity sampled via the CTD water sampler mentioned above[16], 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[5, 16], which was the predominant member in all our enrichments; additionally, the genera *Colwellia*, *Moritella*, and *Shewanella* of Gammaproteobacteria constituted the second predominant group[5, 16], which are frequently reported as psychrophilic and piezophilic deep-sea bacteria. Congruently, they were present among the top 50 predominant members in almost all our enrichments (**Table S4**). In particular, *Moritella* was even among the top 5 in some enrichments (**Fig. 3**).

Even in eutrophic coastal sediment, our parallel in-situ incubation also revealed some predominant taxa shared with deep sea assemblages, such as SRBs of *Desulfobulbacteae* and *Desulfobacteraceae* and SOBs of *Arcobacteraceae*, in addition to the depolymerizing bacteria of *Vibrionaceae*, *Spirochaetaceae*, and *Fusobacteriaeae*[38]; moreover, they neighboured the deep-sea bacteria of this report in the phylogenetic trees (Supplementary Materials and figures: **Figs. S5**, **S6**, **S9**, **S10**, **S11**, and **S14**). In contrast, special groups, including *Marinilifaceae*, *Moritellacea*, and *Psychromonadaceae*, that dominated the deep-sea enriched consortia were barely detected in our coastal enrichments[38]. On the other hand, in deep-sea chemoorganotrophic ecosystems of wood and whale falls, the decomposer and fermenters of *Spirochaetaceae*, *Marinilifaceae*, *Vibrionaceae*, and *Fusobacteriaeae* also occur in bacterial assemblages with high abundance[3, 39]. In contrast, the bacteria *Desulfobulbaceae*, *Psychromonadaceae*, *Moritellaceae*, *Arcobacteraceae*, *Sulfurospirillaceae*, and *Sulfurovaceae* were rarely enriched in the wood-falls sunk in the Mediterranean Sea[26] compared to our deep-sea incubations. These results indicate the variations and common features of OM-decomposing bacterial assemblages among different marine environments.

The key bacterial taxa responsible for OM decomposition and fermentation

Deep-sea *in situ* incubation results revealed that the bacterial taxa thriving on newly input bulky POM play a key role in the hydrolysis of proteins/polysaccharides and further organic fermentation. At the level of the whole community, the metabolism modules of carbohydrates, amino acids, and fatty acids of the resulting bacterial assemblages were selectively enriched in different OM types (**Table S5**). These functions were reconfirmed by metagenomic and metatranscriptomic results (**Fig. 6, Fig. S17**, and **Tables S7-S9**).

Marinifilaceae, *Spirochaetaceae*, *Psychromonadaceae* and *Vibrionaceae* may be the OM decomposer. *Marinifilaceae* family contains some psychrotorelant and facultatively anaerobe bacteria that are capable of degrading a large variety of macromolecules and micromolecules and fermenting to generate various small molecular organic acids[40–42]. Some strains of *Labilibaculum* within *Marinifilaceae* generate formate, acetate, succinate, and minor amounts of propionate and malate via glucose fermentation[43].

Bacterium OTU6861, close to *Labilibaculum antarcticum* SPP2, may be able to use polysaccharide such as xylan[42].

Some cultured strains within *Spirochaetaceae*, such as *Pleomorphochaeta multiformis* MO-SPC2[44] and *Spirochaeta perfilievii*[45], are anaerobic, psychrophilic bacteria that can utilize mono-, di- and poly-saccharides (xylan, trehalose, and pectin) and protein, and also generate formate, acetate, ethanol, pyruvate, and hydrogen via fermentation. Therefore, bacteria OTU5914 and OTU4953, sharing high similarity with *P. multiformis* MO-SPC2 and *S. perfilievii*, respectively, both may play key roles in the degradation of polysaccharide and/or protein. The MAG corresponding to OTU4953 encoded genes for starch and xylan metabolism, but no genes for cellulose hydrolysis were found (data not shown).

Psychromonadaceae and *Vibrionaceae* were frequently found in the enrichments, even as dominant members in SCS enrichments (**Fig. 3**). Consistently, previous studies also found that the abundance of *Vibrionaceae* was significantly enhanced with phytoplankton blooms in Delaware's inland bays, indicating that it plays an important role in marine carbon and other element circulation[46]. In another report, poisoned trap microbial assemblages were enriched in *Vibrio*, but the authors supposed they were associated with eukaryotic surfaces and intestinal tracts as symbionts, pathogens, or saprophytes but not POM degraders in the deep sea[9]. In the case of *Psychromonadaceae*, two dominant OTUs showed the highest similarity (450 bp; over 99.0% similarity) with cultured *Psychromonas marina* 4-22^{T}, which is a facultative anaerobic psychrophilic bacterium that can degrade starch and alginic acid[25].

Sulphur cycling driven by the new input POM and energy conservation coupled with carbon fixation

Both *Arcobacteraceae* and *Sulfurovaceae* are typical chemoautotrophic marine sulphur-oxidizing bacteria[47]. 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[48, 49]. Evidently, they are not directly involved in OM decomposition or transformation but oxidizing sulphides generated by SRBs, such as *Desulfobulbacteae* and *Desulfobacteraceae*, which occurred as key taxa in our *in situ* enrichments. The chemoautotrophic lifestyle of *Arcobacteraceae* and *Sulfurovaceae* is supported by the oxidation of thiosulfate through the Sox complex and the oxidation of hydrogen sulphide through sulphide quinone oxidoreductase (Sqr). Moreover, their presence in the enrichments will suppress the accumulation of hydrogen sulphide; otherwise, sulphide may have detrimental effects on other microorganisms. A large number of sulphur oxidation-related genes were detected and actively transcribed in the enrichments, according to metagenomic and metatranscriptomic data (**Fig. 6** and **Table S9**). These results indicated OM 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.

The new organic carbon produced by chemolithotrophs in the dark ocean has drawn accumulative attention and is probably high relative to the organic carbon supplied by sinking particles[50]. In this study, the predominance of SOBs within the community thriving on newly input POM highlights the significance of chemolithotrophs for energy conservation (producing new organic carbon) in such an ecosystem. Recently, in addition to SOBs, chemoautotrophic nitrifiers were found to produce a series of new organic compounds to maintain community metabolism through POM degradation, particularly with nitrogenous compounds, indicating that nitrifiers may play an important role in the processes of POM transformation and remineralization in the aphotic ocean layer[51]. However, in this study, we found that nitrifiers were scarcely detected in all enrichments, as only a few copies of nitrification-related genes (such as *amoA*) in the omics data, even in the proteinaceous-rich OMs.

Nitrogen fixation coupled with OM mineralization in nitrogen-nutrient depleted environments

The contribution of nitrogen fixation in the dark ocean remains enigmatic. There has been a lack of consensus on whether the nitrogen losses caused by microbial removal pathways are balanced by biological nitrogen fixation and other inputs, such as atmospheric nitrogen deposition and terrestrial runoff[52]. At the community level based on functional prediction and omics analyses, the metabolic module, encoding genes and corresponding transcripts for nitrogen fixation were selectively enriched in the enrichment of polysaccharides and lipids (**Fig. 6, Table S5**, and **Table S9**). These results collectively support nitrogen fixation accompanied by remineralization of these organics.

A previous study showed that nitrogen fixation, which was mostly from the cyanobacterium *Trichodesmium* spp., accounts for up to half of the nitrogen required to maintain total annual particulate nitrogen export in the oligotrophic North Pacific Ocean[53]. Much evidence for deep-sea nitrogen fixation to date has been collected at regional sites of enhanced productivity, such as methane seeps, mud volcanoes, and hydrothermal vents[54, 55].

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. Similarly, a previous report showed that nitrogen fixation rates were higher within methane-laden seep sediments than within nearby background sediments, suggesting that the input of nonnitrogenous carbon source (methane) led to nitrogen limitation in the seep community and thus diazotrophy[56]. More recently, heterotrophic diazotrophs have been found to be associated with more organic-rich detrital POM[57], where the physical structure of the particle can limit diffusion, allowing O_2 concentrations to be reduced and even anoxic and nitrogen fixation to occur[52]. Consistently, in this study, the input of nonnitrogenous carbon sources obviously stimulated nitrogen fixation (**Fig. 6**). We suppose that anaerobes of bacterial classes of Spirochaetia, Bacteriodia, and Gammaproteobacteria, such as *Spirochaetaceae*, *Marinilifaceae*, and *Psychromonadaceae*, function as organic substrate decomposers and diazotrophs in the global ocean. 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”[58].

Nutrient availability and energy transfer within microbial assemblages determine the occurrence relationships

Cooperative relationships dominate the bacterial relationships within the assemblages enriched with polysaccharides or lipids. In contrast, the relationships of the dominant taxa in protein enrichments were almost competitive (**Fig. 7**). 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. For example, in communities supported by polysaccharides or lipids, different members may cooperate to deal with the shortage of nitrogen sources. From metagenomic and functional predictions, a large number of nitrogen fixation-related genes were found in the two nitrogen-deficient OMs (**Fig. 6** and **Table S9**). Moreover, the MAGs with nitrogen fixation-related genes were mostly retrieved from polysaccharide and lipid metagenomes (data not shown). However, most of the dominant taxa in the protein-enriched consortia competed for limited kinds of sources or inhabiting niches. Generally, the nutrients in proteinaceous substrates are replete and thus reduce the dependence of each other and even compete for the restricted niche space, as previously reported. Microbial aggregation promotes competition when particles are labile and turnover rates are high[59]. On the other hand, when the OM is recalcitrant, such as lignin in plant detritus, cooperation relationships will promote the microbial community to access or make up limited resources to maintain the population size and grow[59]. Thus, the lability of POM determines the relationship among bacteria within a microbial assemblage to a certain degree.

Aerobic or anaerobic respiration dominates POM mineralization in oxic deep water?

Compared to the POM breakdown in whale-falls, wood-falls, and seaweed-falls, our enrichment *in situ* is simple with a small size. Generally, the interface of particles and seawater is supposed to change from aerobic to microaerobic, while the inner segment is anoxic. Although we did not analyse the degradation process of POMs in temporal and special sequences, the existence of certain microaerophilic respiration was confirmed according to both metagenome and metatranscriptome data, in addition to anaerobic respiration (**Fig. 6** and **Table S9**). This result suggests that aerobic degradation processes also occurred *in situ* around the substrates, and a higher aerobic proportion would be expected in open seawater flash. Previous studies have also found that both anaerobic and aerobic respiration coexist in wood-fall[3, 60] and that a large number of anaerobic or facultative anaerobic microorganisms were found in both whale-falls and wood-falls[3, 39]. In this study, the transcription levels of genes related to microaerophilic respiration were lower than those related to anaerobic respiration. Therefore, anaerobic respiration might be dominant during the degradation of OMs in fast sinking particles even resting on the seafloor, which resembling might be sustained over years or even decades, like whale falls[61].

Synthesis of the story

Based on the results of this study and those of previous reports, we aimed to delineate the roles of microbial key taxa in the degradation of OM and the relationships among them. As shown in **Fig. 8**, the key taxa belonging to *Marinifilaceae*[43, 62, 63], *Spirochaetaceae*[44], *Psychromonadaceae*[25], and *Vibrionaceae*[64] were inferred to be the direct depolymerizers and degraders of polysaccharides and/or protein polymers. They depolymerize the natural polymers into monomers by polysaccharide hydrolase (or lyase) and protein hydrolase and simultaneously produce low-molecular weight compounds by fermentation (**Fig. 8**). These fermentation products can be supplied to SRBs of *Desulfobulbaceae* and *Desulfobacteraceae*[28] or sulphur-reducing bacteria of *Sulfurospirillaceae*[65, 66] to generate reduced H_2S. The reduced sulphur compounds further provide energy for chemoautotrophic bacteria of SOBs belonging to *Arcobacteraceae* and *Sulfurovaceae*[47], 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.

Summary And Conclusion

This report revealed unique deep-sea benthic bacterial communities responsive to OM input. These communities play an important role in the mineralization of diverse macromolecules in compact POM sinking to the seabed. Regardless of the type of POM, the bacterial assemblages included similar functional groups. However, the bacteria within each family of key taxa were diverse and differentially enriched in accordance with organic substrate and location. Among them, bacteria of *Marinifilaceae* were most prevalent and predominant in all communities, probably playing an important role in OM decomposition *in situ*. Therefore, POM is decomposed and remineralized through unique bacterial assemblages in deep sea, which are different with those dominating the water column, but play an unneglectable role in carbon biogeochemical cycling in global oceans.

Methods

Experimental design and in situ incubation experiments

From 2016 to 2018, *in situ* incubations of OM-degrading bacteria were performed in three different marine environments: a marginal sea (South China Sea, SCS), an abyssal ocean (Indian Ocean, IO), and a seamount (Pacific Ocean, PO) (**Fig. S1**). Site SCS was located in a deep-sea basin with a water depth of 3758 m in the northern SCS. Site IO was located in the deep-sea basin near the Southwest Indian Ridge at a water depth of 4434 m in the Indian Ocean. Site PO was located at the top of a flat-topped seamount with a water depth of 1622 m in the West Pacific Ocean.

In this study, three categories of natural OM substrates were selected for *in situ* incubation, including proteins, lipids, and polysaccharides (**Table S1**). The substrates of proteins included fish muscle, fish scales, and shrimp muscle; the substrates of lipids included fish oil (EPA and DHA) and vegetable oil; and the substrates of polysaccharides included wood chips, wheat bran, and seaweed. 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 centimetres 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. In this study, 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, 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 and the subsample of the incubated samples are 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.

Sampling, DNA and RNA 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), preserved in 1 ml of RNAlater stabilization solution (Thermo Scientific, 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). Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. DNA and RNA were transported to the MAGIGENE Company (Guangzhou, China) on dry ice for subsequent procedures to obtain metagenomic and metatranscriptomic datasets, respectively.

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')[67]. 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).

High-quality sequences were extracted from the raw Illumina reads and sorted into individual samples according to the unique barcodes. All the primers and barcodes were removed to obtain a FASTQ format file. Further analysis was conducted with the QIIME standard pipeline (version 1.9.1)[68]. In brief, PE reads were merged, yielding ~ 454 bp fragments, using FLASH software version 1.2.11[69]. The dataset was then quality filtered to remove ambiguous reads and chimeric sequences using UCHIME version 4.2[70]. Optimized sequences were clustered into operational taxonomic units (OTUs) using UPARSE version 7 with a 97% sequence similarity threshold[71]. Taxonomic classification of each representative OTU was performed using the Ribosomal Database Project (RDP) classifier[72] against the SILVA 132 database. 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 (http://circos.ca/).

Multiple sequence alignment of 16S rRNA genes was performed using the MAFFT program with default parameters, and a phylogenetic tree was reconstructed with the neighbour-joining method (https://mafft.cbrc.jp/alignment/server/).

Function prediction of each enrichment and network analysis

Prokaryotic function prediction of each enrichment was performed using PICRUSt based on the KEGG database[73] and using FAPROTAX based on the metabolic functional group database of marine microorganisms[74] with the rarefied OTU tables as inputs (**Table S4**). 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. Network analyses were carried out using the molecular ecological network analyses (MENA) pipeline at the OTU level[75]. 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[76].

Metagenomic and metatranscriptomic analyses

MicroPITA[32] was used to select representative enrichments (n = 5; 2 of polysaccharides, 2 of proteins, and 1 of lipid) for meta-omics analysis based on the Bray–Curtis distance algorithm by using the Shannon and Chao diversity indexes. Metagenomic and metatranscriptomic sequencing and analysis of the *in situ* enrichments were performed according to a previous report[77]. In detail, the sequencing

platform used here was the Illumina NovaSeq 6000 platform (PE 150-bp mode) by the MAGIGENE Company (Guangzhou, China). Raw data were QC-processed by using fastp v0.19.3 with default parameters[78]. For metagenomics data, cleaned reads were assembled de novo by MateSPAdes v3.13.0 with the settings “-k 21,33,55”[79]. Each sample metagenome was dividedly assembled. To calculate the abundance of each gene, all high-quality reads from metagenomics datasets were mapped to the assembled scaffolds using Bowtie2 with default parameters[80]. Then, fragments (PE reads) assigned to each gene were counted using the FeatureCounts program with the parameters “-p -F GTF -g ID -t CDS[81]. For metatranscriptomic data, clean PE reads without rRNA were mapped to the metagenomic scaffold dataset using HISAT v2.1.0[82] with the settings “–rna-strandness RF”. HISAT is a fast and sensitive alignment program for transcriptome reads[82]. Afterwards, fragments (PE reads) assigned to each gene were counted using the FeatureCounts program with the parameters “-p -F GTF -g ID -t CDS -s 1 -M –fraction”[81]. The transcriptional expression of each gene was normalized with the transcripts per million (TPM) method in each metatranscriptomic dataset[83], as well as the gene abundance from metagenomic datasets.

Gene functional annotations for metagenomes

Protein-coding genes were predicted using Prodigal v2.6.3[84]. Protein sequences were functionally annotated against the KEGG, eggNOG, Pfam, and CAZy databases. The online software KAAS v2.1[85] (https://www.genome.jp/kegg/kaas/) with the GHOSTZ program was applied for homology searching of protein sequences against the KEGG database. Annotation using the eggNOG database was performed by eggnog-mapper v1.0.3 software with the Diamond Blastp (v0.8.36.98) method[86]. To identify carbohydrate degradation-related enzymes, we used the online dbCAN2 meta server (http://bcb.unl.edu/dbCAN2/) to annotate protein sequences by HMMER against the CAZy database, including glycoside hydrolases (GHs), carbohydrate esterases (CEs), glycosyl transferases (GTs), carbohydrate-binding modules (CBMs), polysaccharide lyases (PLs), and auxiliary activities (AAs)[87]. Additionally, eggNOG annotations were used as auxiliary results for GH identification. Pfam 31.0[88] was used as the reference database for the annotation of peptidases, aminotransferases, and transporters of oligopeptides and amino acids by HMMER v3.1b2 (cut-off e value: 1e-10, best hits reserved). In addition, nonredundant peptidase unit sequences included in MEROPS (Release 12.1)[89] were used for peptidase annotation by Diamond Blastp v0.8.36.98 (cut-offs e value: 1e-10, best hits reserved)[90]. The prediction of signal peptides was carried out by using the online SignalP-5.0 Server (http://www.cbs.dtu.dk/services/SignalP/). When a protein sequence was annotated to the same peptidase family with the above two databases, its annotation result was accepted and then used for subsequent analyses.

Declarations

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Authors' contributions

Z.Z.S. 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. C.M.D. 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. Z.Z.S., J.Y.L. and Z.B.H. participated DIMI deployment during the cruise of COMRA DY45 in the South China Sea, and participated the next-year's cruise for DIMI recovery with Q.L.L. J.Y.L. and C.M.D. conducted further sample processing. J.Y.L. analysed the 16S rRNA sequence, metagenomic and metatranscriptomic data. Z.Z.S., J.Y.L. and C.M.D. interpreted the results and wrote the manuscript. D.H.Z. and Z.Z.S. were involved in DIMI development and maintenance. L.F.G. was responsible for building the omics analysis platform. G.Y.W. reviewed the paper.

Availability of data and materials

The dataset supporting the conclusions of this article is available in NODE (https://www.biosino.org/node/) at the accession number OEP001506.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have read and approved the submission of the manuscript and provide consent for publication.

Competing interests

All authors declare that they have no competing interests.

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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. 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.
6. Jorgensen BB, Boetius A: **Feast and famine–microbial life in the deep-sea bed**. Nat Rev Microbiol 2007, **5**(10):770–781.
7. 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.
8. 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).
9. 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.

10. 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.
11. Orsi WD: **Ecology and evolution of seafloor and subseafloor microbial communities**. Nature Reviews Microbiology 2018.
12. Arnosti C: **Microbial Extracellular Enzymes and the Marine Carbon Cycle**. Annual Review of Marine Science 2011, **3**(1):401–425.
13. 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**. Frontiers in Microbiology 2018, **9**.
14. Karl DM: **Microbial oceanography: paradigms, processes and promise**. Nat Rev Microbiol 2007, **5**(10):759–769.
15. 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.
16. 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.
17. 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.
18. 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.
19. Giovannoni SJ, Thrash JC, Temperton B: **Implications of streamlining theory for microbial ecology**. Isme Journal 2014, **8**(8):1553–1565.
20. 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.
21. 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.
22. 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.
23. 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).

24. 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.
25. 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.
26. 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.
27. Bienhold C, Ristova PP, Wenzhofer F, Dittmar T, Boetius A: **How deep-sea wood falls sustain chemosynthetic life**. PLOS ONE 2013, **8**(1).
28. 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.
29. 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**. Frontiers in Microbiology 2021, **12**.
30. 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.
31. Petersen JM, Ramette A, Lott C, Cambon-Bonavita M-A, Zbinden M, Dubilier N: **Dual symbiosis of the vent shrimp Rimicaris exoculata with filamentous gamma- and epsilonproteobacteria at four Mid-Atlantic Ridge hydrothermal vent fields**. Environ Microbiol 2010, **12**(8):2204–2218.
32. Tickle TL, Segata N, Waldron L, Weingart U, Huttenhower C: **Two-stage microbial community experimental design**. The ISME journal 2013, **7**(12):2330–2339.
33. 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.
34. 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.
35. 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.

36. Sogin ML, Morrison HG, Huber JA, Mark Welch D, Huse SM, Neal PR, Arrieta JM, Herndl GJ: **Microbial diversity in the deep sea and the underexplored "rare biosphere"**. Proc Natl Acad Sci U S A 2006, **103**(32):12115–12120.
37. 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.
38. 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.
39. Voight JR: **Xylotrophic bivalves: aspects of their biology and the impacts of humans**. J Molluscan Stud 2015, **81**(2):175–186.
40. 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.
41. Xu Z-X, Mu X, Zhang H-X, Chen G-J, Du Z-J: **Marinifilum albidiflavum sp. nov., isolated from coastal sediment**. Int J Syst Evol Microbiol 2016, **66**(11):4589–4593.
42. Han Z, Shang-Guan F, Yang J: **Molecular and Biochemical Characterization of a Bimodular Xylanase From Marinifilaceae Bacterium Strain SPP2**. Front Microbiol 2019, **10**:1507.
43. Vandieken V, Marshall IPG, Niemann H, Engelen B, Cypionka H: *Labilibaculum manganireducens* **gen. nov., sp. nov. and** *Labilibaculum filiforme* **sp. nov., Novel** *Bacteroidetes* **Isolated from Subsurface Sediments of the Baltic Sea**. Frontiers in Microbiology 2018, **8**(2614).
44. 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.
45. 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.
46. Main CR, Salvitti LR, Whereat EB, Coyne KJ: **Community-Level and Species-Specific Associations between Phytoplankton and Particle-Associated** *Vibrio* **Species in Delaware's Inland Bays**. Appl Environ Microbiol 2015, **81**(17):5703–5713.
47. Ghosh W, Dam B: **Biochemistry and molecular biology of lithotrophic sulfur oxidation by taxonomically and ecologically diverse bacteria and archaea**. FEMS Microbiol Rev 2009, **33**(6):999–1043.
48. 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.

49. Li Z, Pan D, Wei G, Pi W, Zhang C, Wang J-H, Peng Y, Zhang L, Wang Y, Hubert CRJ *et al*: **Deep sea sediments associated with cold seeps are a subsurface reservoir of viral diversity**. Isme Journal 2021.
50. Kirchman DL: **Microbial proteins for organic material degradation in the deep ocean**. *Proceedings of the National Academy of Sciences* 2018, **115**(3):445–447.
51. Zhang L, Chen M, Chen X, Wang J, Zhang Y, Xiao X, Hu C, Liu J, Zhang R, Xu D *et al*: **Nitrifiers drive successions of particulate organic matter and microbial community composition in a starved macrocosm**. Environ Int 2021, **157**:106776.
52. Zehr JP, Capone DG: **Changing perspectives in marine nitrogen fixation**. Science 2020, **368**(6492):eaay9514.
53. Karl D, Letelier R, Tupas L, Dore J, Christian J, Hebel D: **The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean**. Nature 1997, **388**(6642):533–538.
54. 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.
55. 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.
56. 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.
57. Farnelid H, Turk-Kubo K, Ploug H, Ossolinski JE, Collins JR, Van Mooy BAS, Zehr JP: **Diverse diazotrophs are present on sinking particles in the North Pacific Subtropical Gyre**. The ISME journal 2019, **13**(1):170–182.
58. 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.
59. Ebrahimi A, Schwartzman J, Cordero OX: **Cooperation and spatial self-organization determine rate and efficiency of particulate organic matter degradation in marine bacteria**. *Proceedings of the National Academy of Sciences* 2019, **116**(46):23309–23316.
60. 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.
61. 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.
62. Watanabe M, Kojima H, Fukui M: *Labilibaculum antarcticum* **sp. nov., a novel facultative anaerobic, psychrotorelant bacterium isolated from marine sediment of Antarctica**. Antonie Van Leeuwenhoek 2020, **113**(3):349–355.

63. 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.
64. Zhang X, Lin H, Wang X, Austin B: **Significance of Vibrio species in the marine organic carbon cycle—A review**. Science China Earth Sciences 2018, **61**(10):1357–1368.
65. Finster K, Liesack W, Tindall BJ: **Sulfurospirillum arcachonense sp. nov., a New Microaerophilic Sulfur-Reducing Bacterium**. Int J Syst Evol Microbiol 1997, **47**(4):1212–1217.
66. 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.
67. Dennis KL, Wang Y, Blatner NR, Wang S, Saadalla A, Trudeau E, Roers A, Weaver CT, Lee JJ, Gilbert JA *et al*: **Adenomatous polyps are drivenbymicrobe-instigated focal inflammation and are controlled by IL-10-producing T cells**. Cancer Research 2013, **73**(19):5905–5913.
68. 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, Chap. 1**0**:Unit10.17-10.17.
69. Magoč T, Salzberg SL: **FLASH: fast length adjustment of short reads to improve genome assemblies**. Bioinformatics 2011, **27**(21):2957–2963.
70. 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.
71. Edgar RC: **UPARSE: highly accurate OTU sequences from microbial amplicon reads**. Nat Methods 2013, **10**(10):996–998.
72. Wang Q, Garrity GM, Tiedje JM, Cole JR: **Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy**. Appl Environ Microbiol 2007, **73**(16):5261–5267.
73. 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.
74. Louca S, Parfrey LW, Doebeli M: **Decoupling function and taxonomy in the global ocean microbiome**. Science 2016, **353**(6305):1272–1277.
75. Deng Y, Jiang Y-H, Yang Y, He Z, Luo F, Zhou J: **Molecular ecological network analyses**. BMC Bioinformatics 2012, **13**(1):113.
76. 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.
77. Tan S, Liu J, Fang Y, Hedlund BP, Lian Z-H, Huang L-Y, Li J-T, Huang L-N, Li W-J, Jiang H-C *et al*: **Insights into ecological role of a new deltaproteobacterial order Candidatus Acidulodesulfobacterales by metagenomics and metatranscriptomics**. The ISME journal 2019, **13**(8):2044–2057.

78. Chen S, Zhou Y, Chen Y, Gu J: **fastp: an ultra-fast all-in-one FASTQ preprocessor**. Bioinformatics 2018, **34**(17):i884-i890.
79. Nurk S, Meleshko D, Korobeynikov A, Pevzner PA: **metaSPAdes: a new versatile metagenomic assembler**. Genome Res 2017, **27**(5):824–834.
80. Langmead B, Salzberg SL: **Fast gapped-read alignment with Bowtie 2**. Nat Methods 2012, **9**(4):357–359.
81. Liao Y, Smyth GK, Shi W: **featureCounts: an efficient general purpose program for assigning sequence reads to genomic features**. Bioinformatics 2013, **30**(7):923–930.
82. Kim D, Langmead B, Salzberg SL: **HISAT: a fast spliced aligner with low memory requirements**. Nat Methods 2015, **12**(4):357–360.
83. Wagner GP, Kin K, Lynch VJ: **Measurement of mRNA abundance using RNA-seq data: RPKM measure is inconsistent among samples**. Theory in biosciences = Theorie in den Biowissenschaften 2012, **131**(4):281–285.
84. Hyatt D, Chen G-L, LoCascio PF, Land ML, Larimer FW, Hauser LJ: **Prodigal: prokaryotic gene recognition and translation initiation site identification**. BMC Bioinformatics 2010, **11**(1):119.
85. Moriya Y, Itoh M, Okuda S, Yoshizawa AC, Kanehisa M: **KAAS: an automatic genome annotation and pathway reconstruction server**. Nucleic Acids Res 2007, **35**(Web Server issue):W182-185.
86. Huerta-Cepas J, Forslund K, Coelho LP, Szklarczyk D, Jensen LJ, von Mering C, Bork P: **Fast Genome-Wide Functional Annotation through Orthology Assignment by eggNOG-Mapper**. Mol Biol Evol 2017, **34**(8):2115–2122.
87. Zhang H, Yohe T, Huang L, Entwistle S, Wu P, Yang Z, Busk PK, Xu Y, Yin Y: **dbCAN2: a meta server for automated carbohydrate-active enzyme annotation**. Nucleic Acids Res 2018, **46**(W1):W95-W101.
88. El-Gebali S, Mistry J, Bateman A, Eddy SR, Luciani A, Potter SC, Qureshi M, Richardson LJ, Salazar GA, Smart A *et al*: **The Pfam protein families database in 2019**. Nucleic Acids Res 2018, **47**(D1):D427-D432.
89. Rawlings ND, Barrett AJ, Thomas PD, Huang X, Bateman A, Finn RD: **The MEROPS database of proteolytic enzymes, their substrates and inhibitors in 2017 and a comparison with peptidases in the PANTHER database**. Nucleic Acids Res 2017, **46**(D1):D624-D632.
90. Buchfink B, Xie C, Huson DH: **Fast and sensitive protein alignment using DIAMOND**. Nat Methods 2014, **12**:59.

Figures

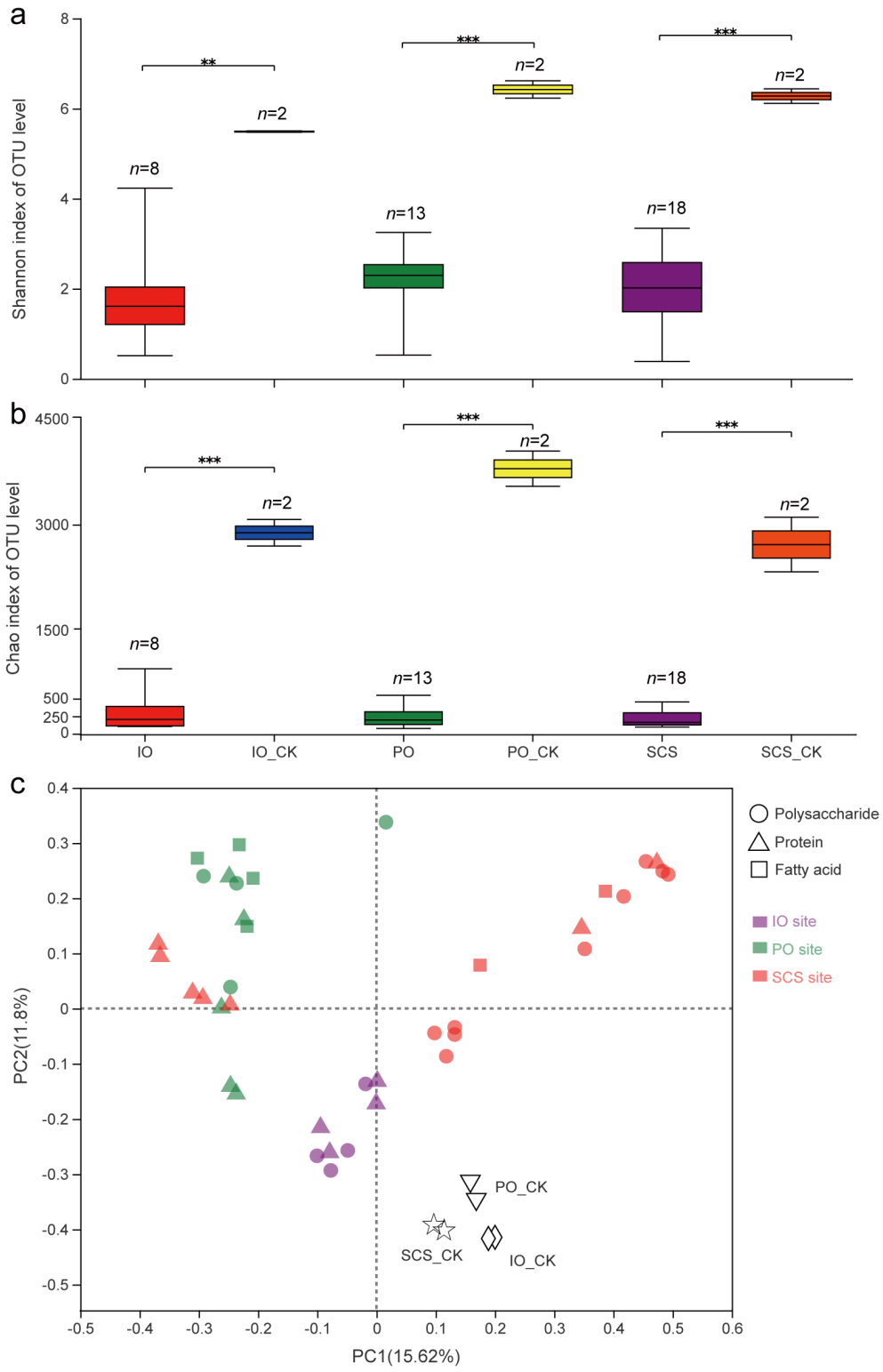

Figure 1

Comparison of bacterial diversity and compositions between enriched microbial assemblages and control samples. These deep-sea microbial assemblages were enriched respectively with POMs rich in three kinds of biological macromolecules *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.

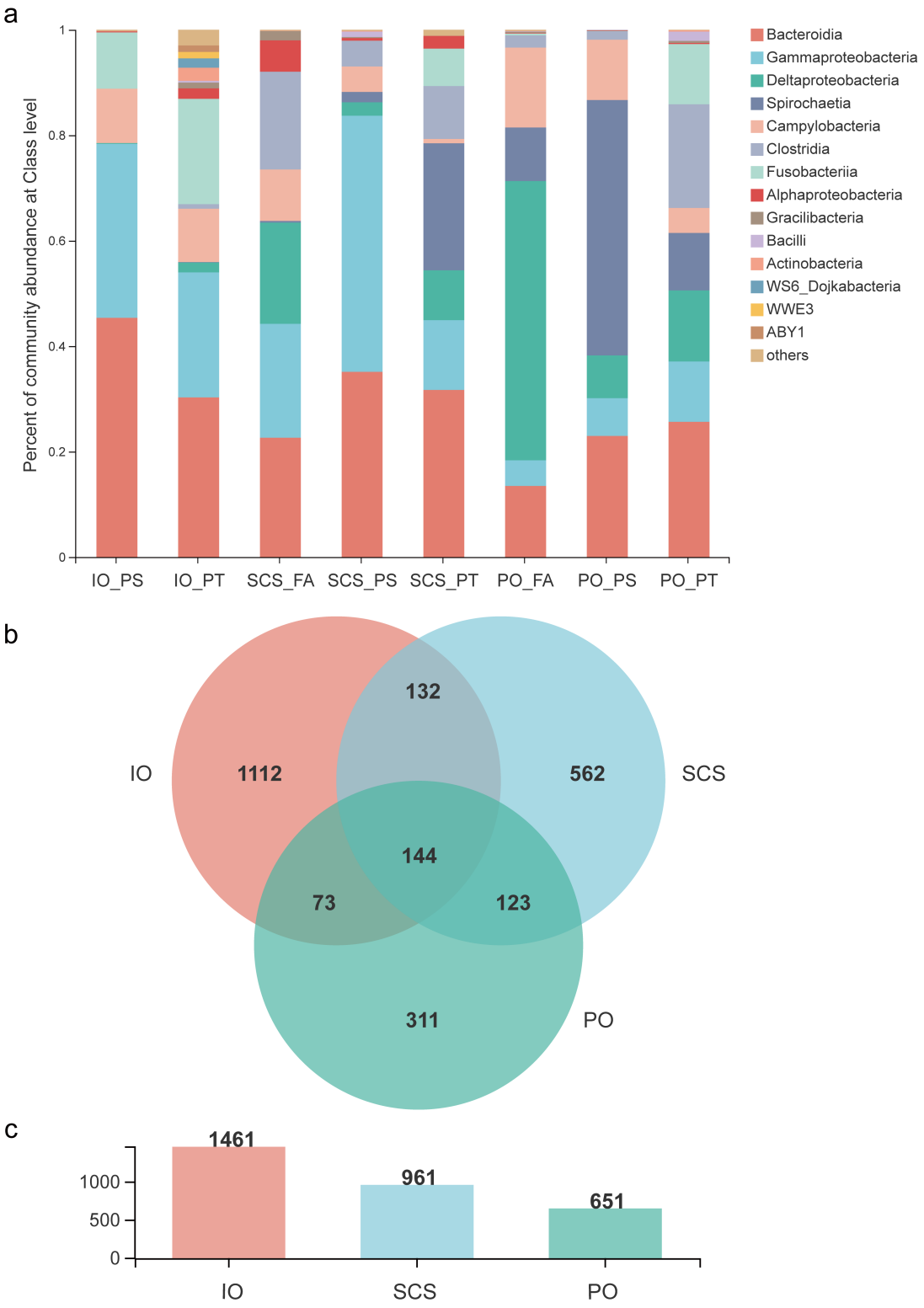

Figure 2

Bacterial community compositions and shared OTUs among the deep-sea microbial assemblages. (**a**) Relative abundance of bacteria at the class level, (**b**) Venn diagram showing the shared and specific

OTUs among the three kinds of POM enrichments at the three deep sea sites, and (**c**) Bar chart showing the total number of OTUs at each incubation site. IO, Indian Ocean; SCS, South China Sea; PO, Pacific Ocean. PS, polysaccharide; PT, protein; 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.

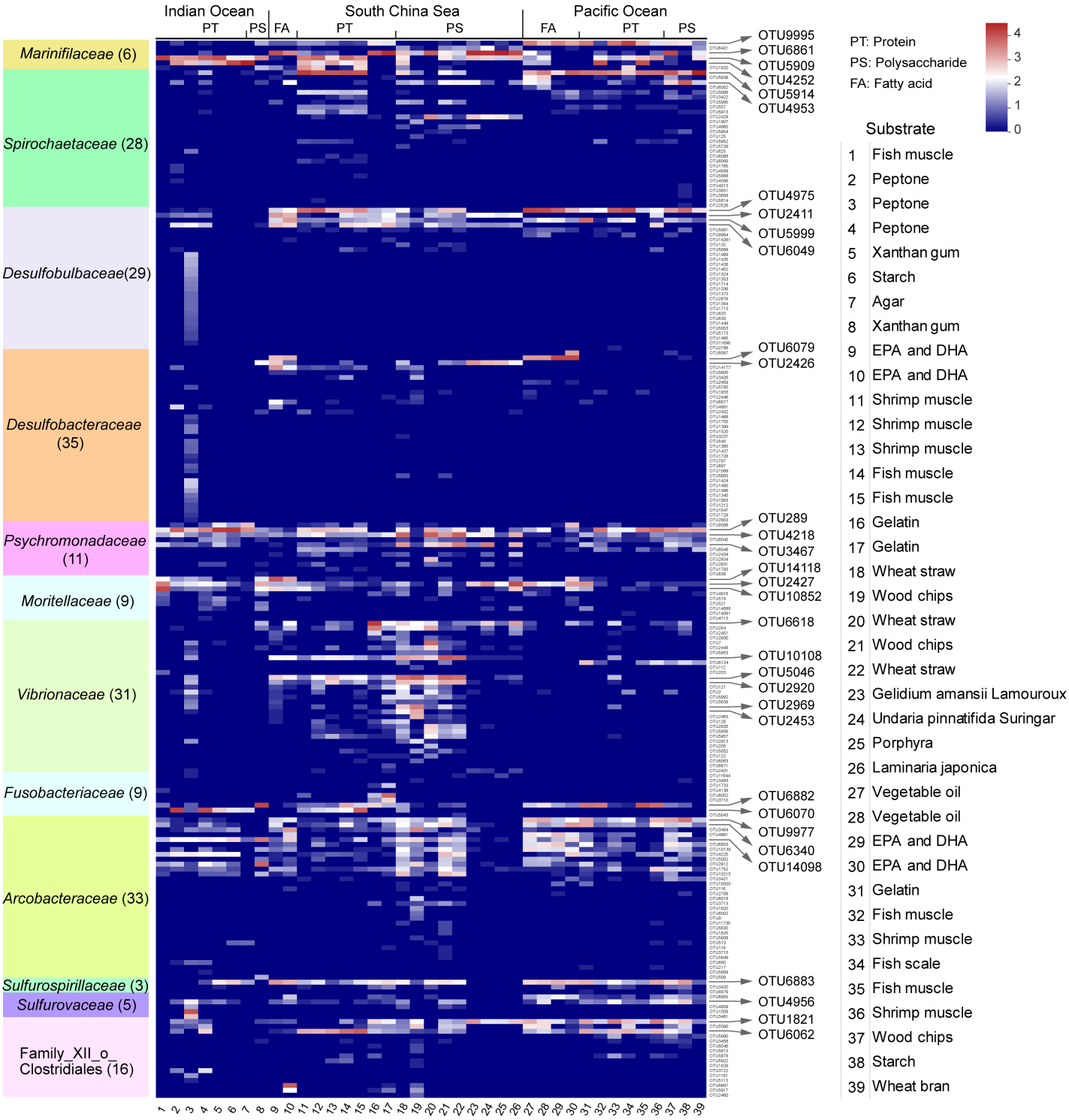

Figure 4

Heatmap showing the relative abundances of all OTUs within each dominant families. 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 based on the results from Figure 2 and Figure S5, while members of each family varied at the OTU level according to substrate type and incubation location. The numbers in brackets after each

family show the number of OTUs in each family. Enrichment substrates for each sample are listed on the right.

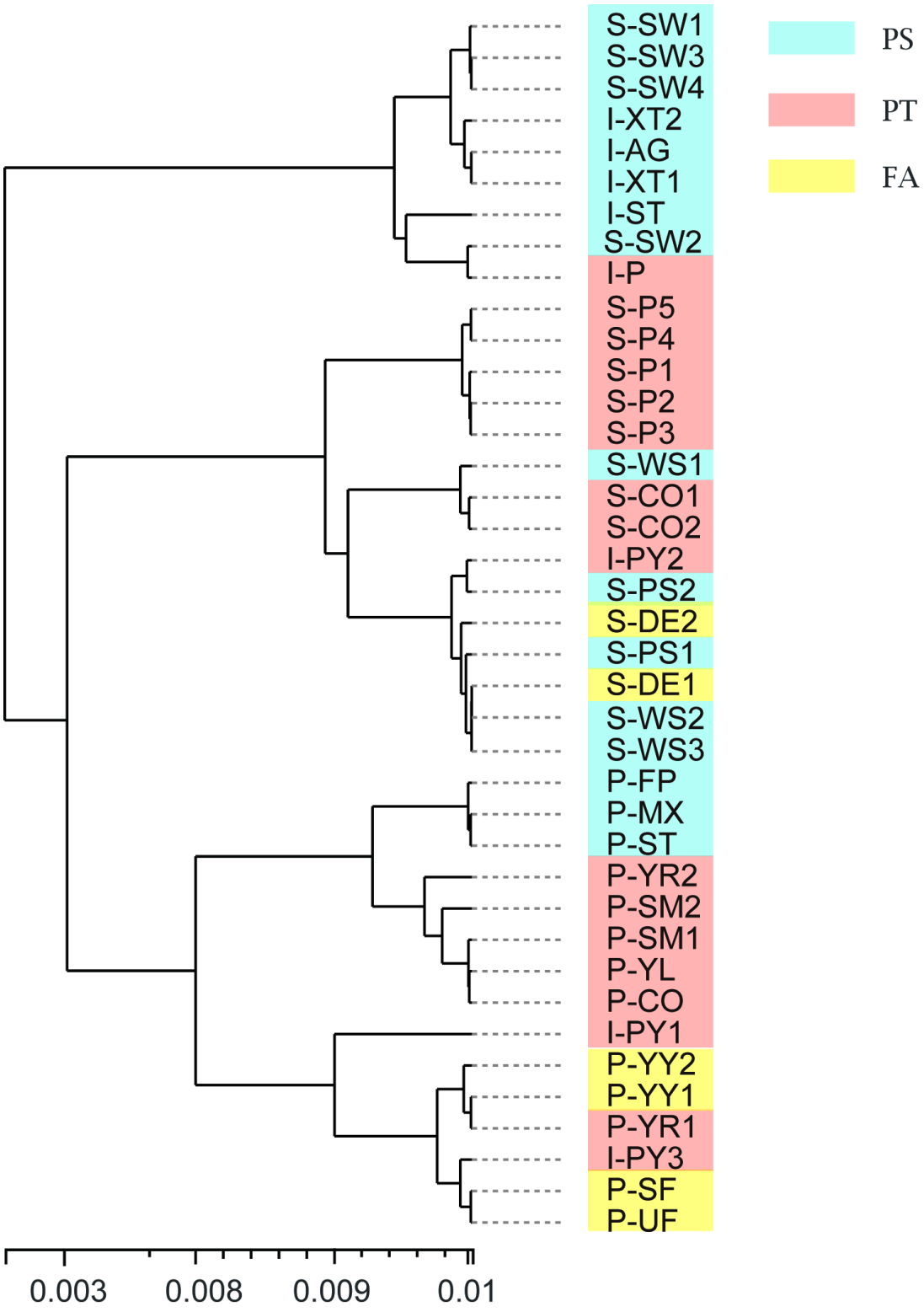

Figure 5

Functional cluster profile of enrichments based on PICRUSt prediction. This profile showed differences in microbial function under different organic substrates, especially between polysaccharide and protein

enrichment. Overall, protein and polysaccharide enrichments formed separate clusters, while lipid enrichments did not form any independent clusters. Hierarchical clustering was performed using the abundance Jaccard distance algorithm. PS, polysaccharide; PT, protein; FA, fatty acid. The names of each natural substrate used are listed in Supplementary Table 1. Details on the functional prediction results are provided in Supplementary Table 5.

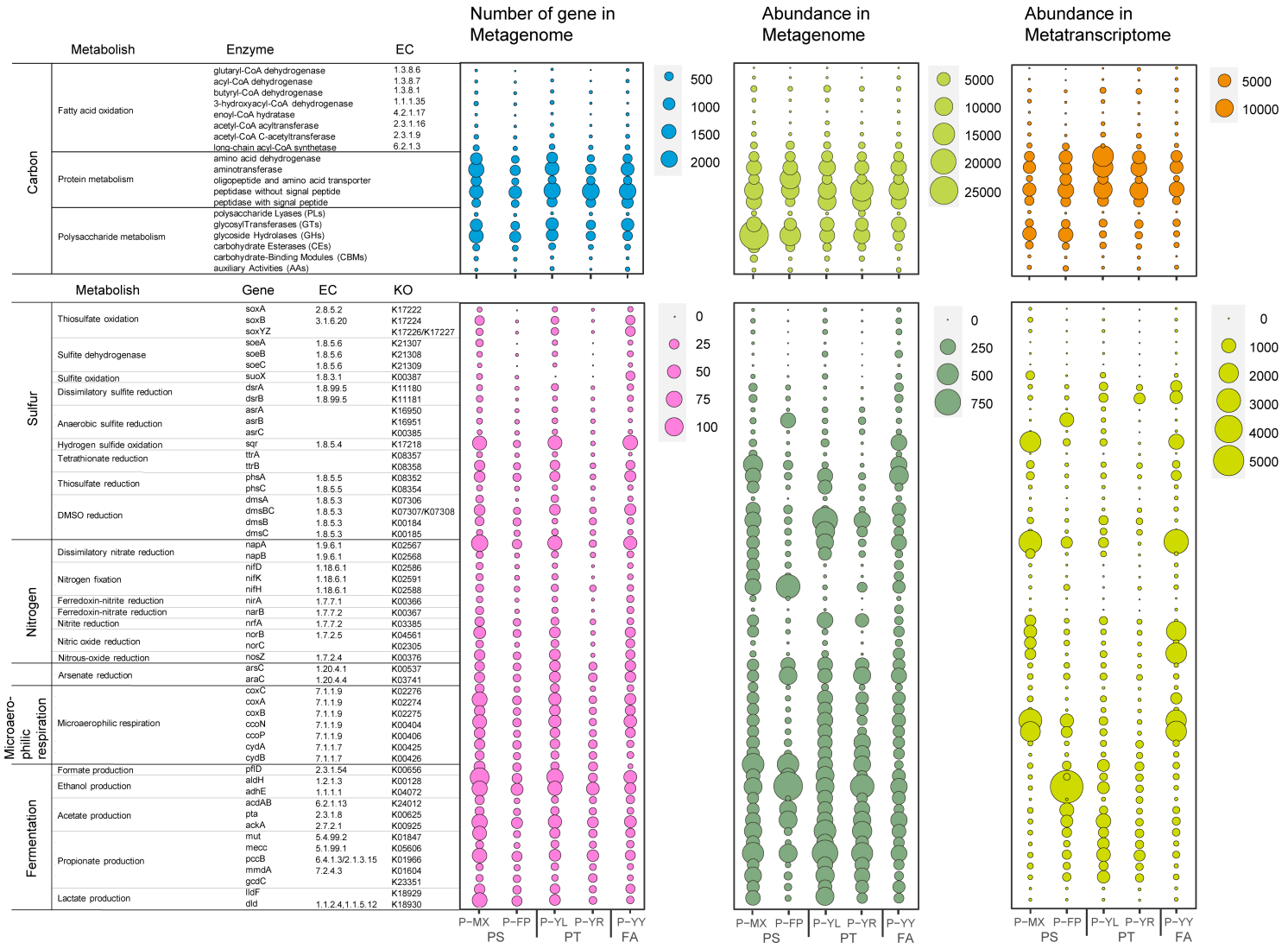

Figure 6

Abundance profiles of functional genes or gene sets based on metagenome and metatranscriptome. Metabolic pathways were reconstructed in each metagenome for polysaccharide and protein degradation, fatty acid beta-oxidation, and sulphur and nitrogen metabolism, as well as various fermentations and microaerophilic respirations. The total number of genes or gene sets from the metagenomic data (**Left**) and the relative abundance (normalization, TPM value) of the genes or gene sets in metagenomic data (**Middle**) and in metatranscriptomic data (**Right**). Most genes and enzymes were identified by their KEGG Orthologue (KO) number, gene name, and Enzyme Commission (EC) number (when applicable). PS, polysaccharide; PT, protein; FA, fatty acid. Detailed abundance information of these genes or gene sets is presented in Supplementary Table 7 and Table 9.

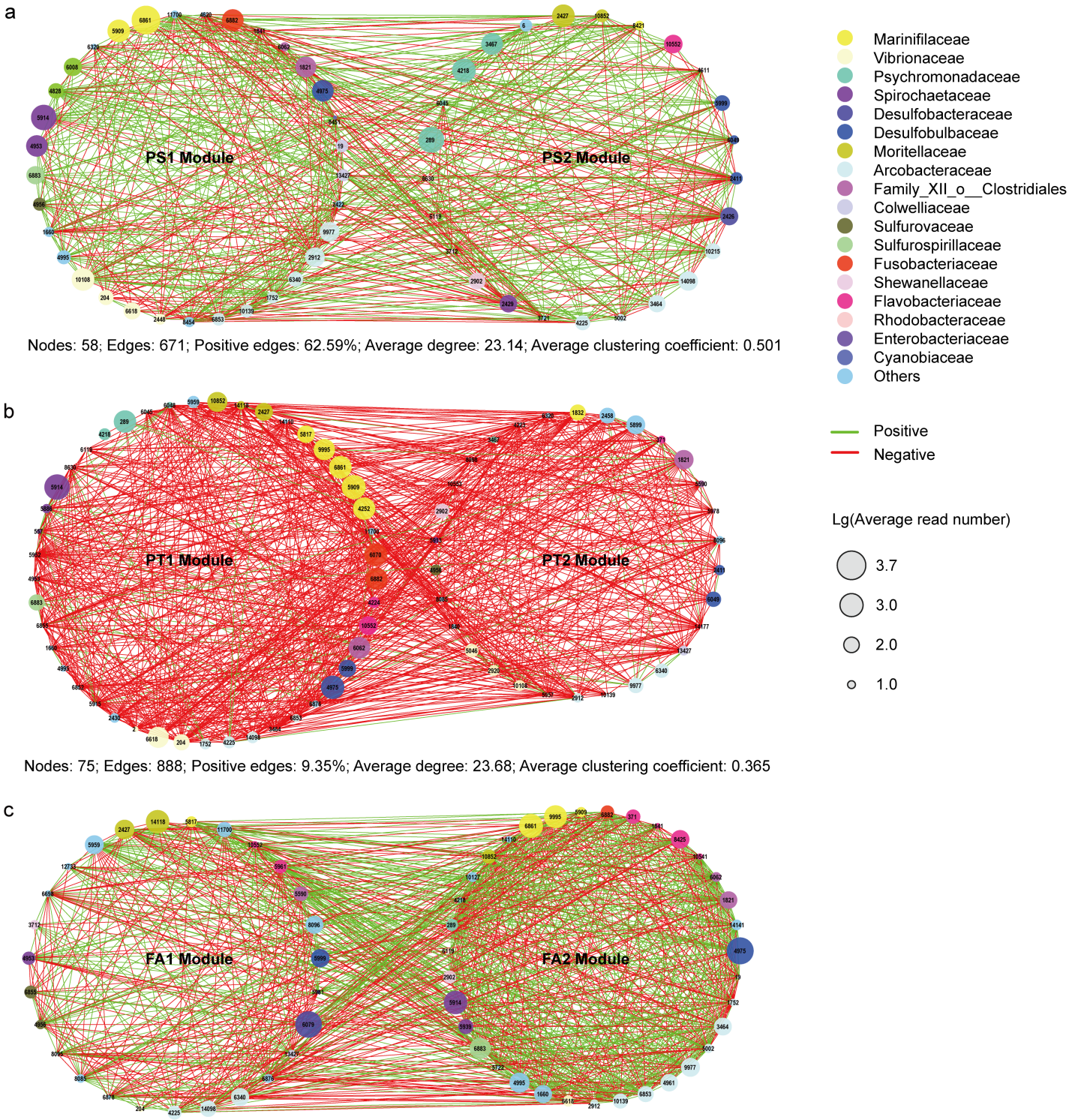

Figure 7

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

node represents an OTU, with colour indicating family-level taxonomy. Network analyses were carried out using the molecular ecological network analyses (MENA) pipeline at the OTU level[75]. Network complexity was assessed by calculating the number of edges, average degree, and average clustering coefficient. 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.

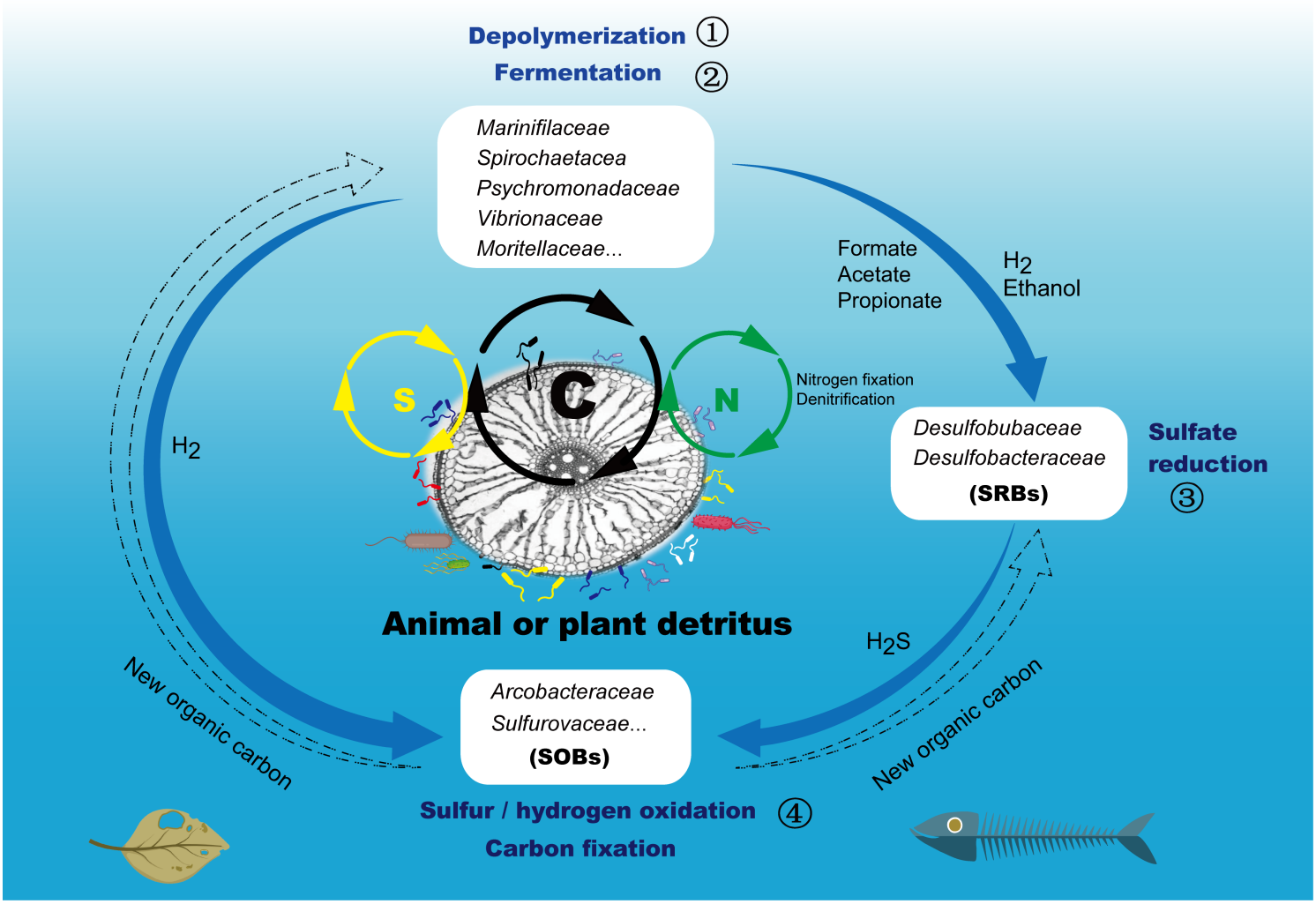

Figure 8

Schematic model of microbial degradation and transformation of animal or plant detritus in deep sea. Within the bacterial assemblages thriving on new 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 (j-m) 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 to

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.

- Additionalfile1.pdf
- Additionalfile2.xlsx