

Putative functions and co-occurrence patterns of the microbial communities in natural and engineered ecosystems

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Abstract

Understanding functions and co-occurrence patterns of microbial communities in various ecosystems enriches the knowledge on ecosystem characteristics and microbial ecology. However, such analyses have rarely been reported. Herein, functions and inter-taxa correlations of microbial communities in a set of natural environments (farmland (SA), forest soil (SB) and Caspian Sea sediments (CSS)) and engineered ecosystems (wastewater treatment plants (FW, WA and WB) and anaerobic digesters (AD)) were studied based on FAPROTAX and network analyses, respectively, by a collection of 115 samples from seven published 16S rRNA gene datasets generated by high-throughput sequencing. The results show that chemoheterotrophy related populations were the most abundant in almost all the communities. Their relative abundances (RAs) in the AD systems were the highest ($43.7\% \pm 4.2\%$), followed by those of the soil environments ($40.2\% \pm 1.9\%$ in SA and $36.4\% \pm 2.0\%$ in SB). For each ecosystem, the indicative community and overall community showed differentiations in several function categories. For example, the SA and SB indicative communities showed higher RAs in aerobic chemoheterotrophy, the CSS indicative community showed higher RAs in sulfate respiration, the AD indicative community showed higher RAs in fermentation, and the WB indicative community included higher RAs of predatory/exoparasitic bacteria. Three molecular ecological networks of the communities from the AD, WB and SB datasets were constructed, respectively. The WB network showed the highest proportion of negative correlations (70.4%), possibly attributed to the environmental pressure which aggravated microbial competition. The positively correlated taxa showed lower phylogenetic distances than the negatively correlated taxa on average in each network.

Introduction

Microbial communities inhabit various natural environments like soil, lakes and sediments. They consist of diverse microorganisms that interact with each other. They play critical roles in energy flow and material cycling in these ecosystems primarily by functioning as decomposers [1, 2]. Also, microbial communities are applied in biotechnology such as wastewater treatment plants (WWTPs) and anaerobic digester, known as engineered ecosystems. For both natural and engineered ecosystems, the knowledge of microbial ecology is of great importance for understanding ecosystem characteristics and functioning.

The development of high-throughput techniques has advanced the studies on microbial components of ecosystems. The inventory diversity of the microbial communities both natural and engineered ecosystems have been intensively analyzed based on high-throughput sequencing in recently years [3-10]. These studies greatly facilitated comprehensive comparisons of the microbial diversity of different habitats. Engineered ecosystems were reported to harbor less diverse microbial communities than natural ecosystems, measured by diversity indices in our previous study [11] and other investigation [12]. Different habitats were also indicated to show differing microbial structures revealed by statistical analyses [11]. To further elucidate the relationship between single taxa and habitats, statistical methods has been suggested to define indicative communities of one or more groups of sites [13]. The composition of indicative taxa was suggested to be tightly correlated to habitat conditions [14].

As microbial ecologists have learned more about 'who's there', they have heightened interest in understanding 'what are they doing' in the ecosystems. In recent years, attempts have been made to unveil the functional profiles of microbial communities based on 16S rRNA genes with the development of bioinformatics softwares of FAPROTAX [15], Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) [16], Tax4Fun [17] and BugBase [18]. Among them, FAPROTAX is suggested to be a good tool for predicting microbial functions related to biogeochemical cycle processes such as carbon, nitrogen and sulfur cycling [15, 19]. The development of these bioinformatics tools provides convenience for understanding the functions of the microbial communities in different ecosystems, which have not been rarely systematically studied and compared.

Microorganisms do not exist in isolation but form complex ecological networks through microbial interactions [20]. The possible combinations of two interaction participants can bring win (positive), loss (negative) and neutral outcomes [21]. Interspecies interactions are important drivers of microbial community assembly [22-24] and affect ecosystem functioning [25]. Microbial co-occurrence patterns are becoming one of the emerging fronts for microbiome research to predict taxa interactions in recent years. Through microbial co-occurrence network analyses, taxa interactions in the microbial communities of several habitats such as soil [26, 27], lakes [14], wastewater treatment systems [28] and anaerobic digesters [29] were deciphered, respectively. The understanding of microbial co-occurrence patterns of various ecosystems enriches knowledge on ecosystem characteristics and microbial ecology. Very recently, a study analyzed the co-occurrence patterns of microbial communities in 14 environments including animal corpus, animal gut, plant corpus, plant surface, rhizosphere, sediments, surface, soil and water and so on, suggesting distinct network characteristics of each environment [30]. An earlier publication on the co-occurrence patterns of microbes across different environments indicated More frequent co-occurrences of phylogenetically close taxa than by chance based on an analysis of 298,591 16S rRNA sequences [31]. However, co-occurrence patterns of microbes inhabiting engineered ecosystems have been rarely compared with those of other environments.

In this study, putative functions and co-occurrence patterns of microbial populations of farmlands contaminated by heavy metals [10], forests [3], Caspian Sea sediments [4], municipal WWTPs [7, 8], a food WWTP [6] and anaerobic digesters (ADs) [5] were studied by a collection of 115 samples from seven published 16S rRNA gene datasets, following our previous study [11]. The scientific questions we address are: 1) what the functional compositions of the microbial communities in the investigated ecosystems are and how they differ; 2) how the indicative taxa of each ecosystem differ and whether they can reflect ecosystem characteristics; 3) how microbial co-occurrence patterns of these ecosystems differ.

Materials And Methods

Dataset information and data pretreatment

The sequencing data were retrieved from seven public datasets on the National Center for Biotechnology Information (NCBI) database (<https://www.ncbi.nlm.nih.gov/>) and the European Nucleotide Archive (ENA) database (<http://www.ebi.ac.uk/ena/>), generated by Illumina sequencing using the 515F and 806R primer set. The samples were collected from heavy metal contaminated farmland (SA), forest (SB), Caspian Sea sediment (CSS), WWTPs

(WA, WB, and FW) and anaerobic digesters (AD). After pretreatment, 20,195 sequences remained in each sample. Detailed information about the dataset and data pretreatment methods was displayed in our previous study [11].

Function prediction of microbial communities by using FAPROTAX

The software FAPROTAX [15] was used to unveil the functions of the microbial communities. This tool extrapolates taxonomic profiles into putative functional profiles of microbial communities based on a database of cultured microorganisms. To evaluate the differences between the functional compositions of any two groups of the communities, analysis of Dunn's Kruskal-Wallis multiple comparisons was conducted. As the FW dataset only included one sample, this dataset was excluded for this analysis.

Identification of indicative operational taxonomic units (OTUs)

The relationship between single species and their sites was studied by analyzing the indicative operational taxonomic units (OTUs) of each ecosystem type. The indicative OTUs of each ecosystem type were identified using the `multipatt` function in the 'indicpecies' package [13] under the R environment. `Multipatt` function calculates the `IndVal` index for every OTU and each dataset. The statistical significance of the relationship between OTUs and datasets is tested using a permutation test. The OTU showing a significant association with a dataset ($p < 0.05$) was identified as its indicative taxa. To evaluate the differences of the RAs of a certain functional group or phylum between the indicative community and overall community for each dataset, a paired t test was performed. As the FW and WA dataset only included one sample and four samples, respectively, they were excluded for this analysis. To evaluate the correlations between indicative community composition and environmental parameters, a mantel test was performed.

Network construction

Network construction was performed to unveil possible taxa-taxa interactions of the investigated natural and engineered ecosystems based on random matrix theory (RMT) using a comprehensive Molecular Ecological Network Analysis Pipeline (MENAP) (<http://ieg4.rccc.ou.edu/MENA/main.cgi>) [32]. Molecular ecological networks of the microbial communities in eight WWTPs (from the WB dataset), 13 ADs (from the AD dataset) and ten sites of a natural broadleaved forest were constructed (from the SB dataset), respectively. To remove poorly represented OTUs and reduce network complexity [33], only the OTUs present in over 50% samples were used for each network construction. The nodes were separated into modules by the fast greedy modularity optimization. According to within-module connectivity (z_i) and among module connectivity (P_i), the topological roles of the nodes were divided into (i) peripheral nodes; (ii) connectors; (iii) module hubs; and (iv) network hubs [34]. Module hubs and connectors may be ecologically considered as generalists [32]. Cytoscape_3.8.2 was used for network visualization of the nodes and their associations.

Results And Discussion

Putative functions of overall microbial communities

The putative functions of the microbial communities in the ecosystems were analyzed by using FAPROTAX. In each community, 8.9% ~ 60.0% sequences were assigned to a certain or several functional processes. A total of 64 functional groups were detected, with 29 to 57 groups present in each community. The average RA distribution of the known functional taxa in each dataset is shown in Fig. 1. The functional populations with an average RA lower than 1% were classified into the group of 'Others'. RAs of several functional categories of the communities in every two datasets showed significant differences, revealed by Dunn's Kruskal-Wallis multiple comparisons (Table 1).

In the carbon cycling process, chemoheterotrophy related taxa were the most abundant in each dataset except FW. Their RAs in the AD systems were the highest ($43.7\% \pm 4.2\%$), followed by those of the soil systems ($40.2\% \pm 1.9\%$ in SA and $36.4\% \pm 2.0\%$ in SB). On the one hand, aerobic chemoheterotrophy related taxa were with the lowest RAs in the AD systems ($5.0\% \pm 2.8\%$) and with the highest RAs in the soil systems ($34.2\% \pm 3.7\%$ in SA and $34.8\% \pm 2.0\%$ in SA). On the other hand, fermentation associated populations were with the highest RAs in AD ($36.6\% \pm 6.6\%$), and took up $4.6\% \pm 2.9\%$ and $1.2\% \pm 0.4\%$ in SA and SB, respectively. These results indicate that chemoheterotrophic bacteria were relatively abundant in the AD systems and soil environments, with aerobic chemoheterotrophs abundant in the soil ecosystems and anaerobic chemoheterotrophs abundant in AD, respectively. This can be possibly explained by the characteristics of the habitats. AD systems are designed to convert organic wastes into a methane-rich gas by microorganisms in the absence of oxygen. They thus harbor higher abundances of chemoheterotrophs and fermenters. SA and SB contained certain organic matters [3, 10] and can support a variety of chemoheterotrophs, especially aerobic chemoheterotrophs.

In the nitrogen cycling process, nitrate reduction populations were the most abundant in the WWTPs, accounting for 17.2%, $10.9\% \pm 1.5\%$ and $7.1\% \pm 1.2\%$ in FW, WA and WB, respectively. Aeration in WWTPs supports the process of nitrification, with nitrate as the final form of oxidized nitrogen. This can give an explanation to the high RAs of nitrate respiration populations in WWTP systems in this study. Also, the nitrogen/nitrate respiration populations were the most abundant in WWTPs, accounting for 17.0%, $9.9\% \pm 1.6\%$ and $6.5\% \pm 1.2\%$ in the FW, WA and WB, respectively. This may result from the high loading rate of nitrogen in WWTPs compared with other ecosystems. The ureolysis populations were the most abundant in soil systems, accounting for $2.9\% \pm 1.5\%$ and $2.9\% \pm 1.3\%$ in SA and SB, respectively, higher than those of other ecosystems ($p < 0.05$). Urea is usually added to soils as a fertilizer, which can be a possible reason for the high proportions of ureolysis populations in SA and SB.

In the sulfur cycling, the taxa participating in sulfur compound respiration/sulfate respiration were with the highest RAs ($16.9\% \pm 13.0\%$) in CSS. Sulfate is a typical electron acceptor in marine sediment as it is with a high concentration in seawater (~ 28 mM) [35]. The biogeochemical sulfur cycle of marine sediments is intensively discussed and reviewed in previous studies [35, 36].

Identification of indicative OTUs of the habitats

A total of 683, 228, 208, 715, 776, 505 and 2551 OTUs were detected to be indicative to the AD, WA, WB, FW, SA, SB and CSS datasets, respectively. The indicative OTUs accounted for 18.0%, 10.8%, 4.2%, 60.5%, 9.6%, 7.2% and 41.8% of the total OTUs detected in each of the above dataset, respectively. The cumulative abundances of the indicative populations for the above ecosystems were 41.5%, 20.0%, 14.1%, 50.5%, 31.1%, 42.6% and 78.4% on average, respectively. This indicates the indicative OTUs were relatively abundant compared with other OTUs in most of the ecosystems. The high proportion of indicative community in CSS can be partly explained by its unique geographic location in the investigated sampling sites. The indicative communities were significantly correlated to pH ($r = 0.1476$, $p < 0.05$), temperature ($r = 0.3471$, $p = 0.001$), latitude ($r = 0.0975$, $p < 0.05$) and longitude ($r = 0.4295$, $p = 0.001$), respectively, as revealed by mantel test. In our previous study, however, overall communities did not show significant correlations to pH values or longitude. These together indicate that indicative communities can reflect the abiotic state environment well, consistent with the results of previous studies [14].

Comparisons of phylum composition of indicative and overall communities

Comparisons of the phylogenetic composition of the indicative community and the overall community of each dataset were made at phylum level. In the SA dataset, *Crenarchaeota*, *Gemmatimonadetes* and *Verrucomicrobia* were more abundant in the indicative communities (accounting for $4.50\% \pm 3.27\%$, $11.24\% \pm 4.49\%$ and $4.34\% \pm 1.13\%$, respectively) than in the overall communities (accounting for $1.56\% \pm 1.19\%$, $4.77\% \pm 2.12\%$ and $2.65\% \pm 0.59\%$, respectively) ($p < 0.05$, $p < 0.001$ and $p < 0.001$, respectively). In the SB dataset, *Verrucomicrobia* was greatly more abundant in the indicative communities ($22.51\% \pm 8.18\%$) than in the overall communities ($10.62\% \pm 3.05\%$) ($p < 0.001$). As discussed in our previous study [11], the phyla *Gemmatimonadetes* [37] and *Verrucomicrobia* [37, 38] are known typically as *K*-strategists capable of efficient utilization of scarce resources in the environment. *Crenarchaeota* has been reported to be present in heavy metal contaminated soil [27, 39]. This phylum was reported to show significant positive correlations with Cd, As and Zn concentrations in SA [27]. The higher RAs of *Crenarchaeota* in the indicative community reflect SA's environmental characteristics that the investigated farmlands were with heavy metal contamination.

In the CSS dataset, *Chloroflexi* was also slightly more abundant in the indicative community than in the overall community, accounting for $8.64\% \pm 3.68\%$ and $7.66\% \pm 3.27\%$, respectively ($p < 0.001$). In the AD dataset, this phylum was also with higher RAs in the indicative communities ($14.53\% \pm 15.37\%$) than in the overall communities ($7.58\% \pm 7.80\%$) ($p < 0.01$). The higher RAs of *Chloroflexi* in the indicative communities of ADs and sea sediments suggest the importance of this phylum in these ecosystems. *Chloroflexi* is reported to be present in a wide range of habitats including microbial mats, the deep oceans, fresh water aquifers and lakes, as well as wastewater treatment systems [40]. *Chloroflexi* in ADs was suggested to have the functions of carbohydrate degradation and cellular matter degradation [41]. Other isolates of this phylum from subseafloor sediment also show metabolic pathways of carbohydrate degradation [42].

In WB dataset, *Proteobacteria* was more abundant in the indicative communities than in the overall communities, accounting for $69.95\% \pm 11.55\%$ and $56.12\% \pm 9.38\%$, respectively ($p < 0.001$). *Proteobacteria* (with an average rrn number of 4.9) [43, 44] are generally classified as *r*-strategists. Taxa from this phylum have been reported to degrade diverse carbohydrates [45] and participate in denitrification [46, 47]. The higher RAs of *Proteobacteria* in the WB indicative community may be attributed to the high pollutant concentrations of WWTPs.

At genus level, two genera *Mycobacterium* and *Herbaspirillum* were associated with six datasets (CSS, FW, SA, SB, WA and WB), indicating their strong ability of habitat adaptation. In FAPROTAX database, *Mycobacterium* is shown to play a role in the processes of aerobic chemoheterotrophy and chemoheterotrophy. *Herbaspirillum* is suggested to be linked with the processes of ureolysis and nitrogen fixation.

Comparisons of functional composition of indicative and overall communities

Functional compositions of the indicative community and the overall community of each dataset were compared, with significant differentiations detected (Table 2). For each dataset, only the functional categories with average RAs over 5% in both overall and indicative communities and increased significantly by over 5% in the indicative community were listed. In the SA and SB datasets, aerobic chemoheterotrophy related microbes were more abundant in the indicative communities. The CSS indicative community showed higher RAs in sulfate respiration, respiration of sulfur compounds and fermentation. The AD indicative community showed higher RAs in fermentation and chemoheterotrophy. This can be explained by the environmental characteristics of each kind of habitat discussed in Part 3.1. The WB indicative community showed higher RAs in aerobic chemoheterotrophy, chemoheterotrophy and predatory/exoparasitic related bacteria. Noticeably, predatory/exoparasitic populations were greatly more abundant ($40.06\% \pm 8.92\%$) than that of the overall community ($5.60\% \pm 2.19\%$) ($p < 0.001$). Predatory/exoparasitic related population in the investigated communities were from the families of *Burkholderiaceae*, *Bacteriovoraceae*, *Bdellovibrionaceae*, *Haliangiaceae* and *Phaselicytidaceae*. *Bacteriovoraceae* and *Bdellovibrionaceae* are classified as *Bdellovibrio*-and-like organisms (BALOs) [48]. BALOs are obligate predators of a variety of gram-negative bacteria including human pathogens [49]. *Bdellovibrio bacteriovorus* is a typical BALO and serves as a model organism for bacterial predation [49]. *B. bacteriovorus* was reported to be present in activated sludge [50]. The RAs of the genus *Bdellovibrio* were $1.28 \pm 2.41\%$ in the WB indicative communities and can be up to 11.19% in some sample, while its RAs were $0.47 \pm 0.52\%$ in the WB overall community. Predatory bacteria are suggested to be a key factor in limiting waste activated sludge production [51], which can be an explanation of the abundant predatory/exoparasitic population in the WB overall community.

Microbial co-occurrence patterns of the ecosystems

Potential microbial interactions were shown by analyzing the co-occurrence patterns of the microbial communities from WB, SB and AD datasets through network construction based on a RMT algorithm. A network of 1,065 nodes and 2,086 links was obtained for the activated sludge (AS) microbial communities from the WB dataset, with 29.6% correlations positive and 70.4% negative. A network of 219 nodes and 349 links was obtained for the AD microbial communities from the AD dataset, with 53.3% correlations positive and 46.7% negative. A network of 849 nodes and 1758 links was obtained for the microbial communities in the broadleaved forest from the SB dataset, with 63.2% correlations positive and 36.8% negative. The networks of AD, SB and WB are displayed in Figure 2. The topological characteristics of the three networks are shown in Table 3. The constructed networks exhibited general topological features including scale free, small world and modularity (Supplementary Text S1 online).

The AD network showed the highest proportion of positive correlations. This can be explained by the microbiology of anaerobic transformation of organic wastes. This process involves several different bacterial species, such as hydrolytic, acid forming, acetogenic, and methanogenic bacteria with CO₂ and CH₄ as the main products [52, 53]. Acetogenic bacteria and methanogenic bacteria cannot directly metabolize polymers, without the help of hydrolytic bacteria who digest polymer into simpler soluble monomers. From this perspective, microbial cooperation widely exists in ADs. Thus, a high proportion of positive correlations can be detected between the taxa. The proportion of positive correlations of AD microbial communities detected in this investigation was comparable to the values of other networks built for AD communities [29].

The WB network showed the highest proportion of negative correlations. Negative correlations may possibly indicate competition between the taxa [54]. WWTPs are designed to remove pollutants from anthropogenic activities. The microbial inhabitants are confronted with environmental pressure such as influent fluctuations and exposure to toxic substances [55], which may aggravate the competition between the taxa. Similar results were reported in previous studies which indicated that salinity and lower food to microorganism ratio (F/M) may possibly lead to increased negative links of microbial components in lakes [14] and wastewater treatment systems [28], respectively.

The nodes of AD, SB and WB networks showed an average PD of 0.51, 0.50 and 0.59, respectively, lower than the average PD of all the detected OTUs. Noticeably, the positively linked OTUs showed a lower PD value than the negatively linked OTUs in each network. In the AD network, the average PD of negatively correlated OTUs was 0.59, and that of positively correlated OTUs was 0.44. In the WB network, the average PD of negatively correlated OTUs was 0.60, and that of positively correlated OTUs was 0.56. In the SB network, the average PD of negatively correlated OTUs was 0.54, and that of positively correlated OTUs was 0.48. This is consistent with the results of other studies. These studies together suggest that positively correlated taxa are more phylogenetically close in general. This is usually attributed to niche overlap of phylogenetically similar taxa [56].

Two OTUs were present in all the networks constructed, both assigned to the order *Actinomycetales*. This indicates that *Actinomycetales* may play important roles in a wide range of habitats. One of them was from the genus *Janibacter*, the other with unknown affiliation at genus level. *Janibacter* is reported to show both antibiotic resistance/pathogenicity and the ability to degrade pollutants [57].

The nodes were classified into four subcategories by within-module connectivity (z_i) and among module connectivity (P_i). In each network, most OTUs were peripheral nodes, accounting for 97.3%, 97.1% and 99.0% in the AD, WB and SB networks, respectively. This reveals a general topological feature of the microbial association networks that they are scale-free. This feature generally implies the presence of many taxa with only a few links and a few highly connected (hub) taxa.

In the AD network, a connector and five module hubs were detected. The connector was affiliated to the genus *Aminomonas*. *Aminomonas* was reported to be a dominant genus and characterized by anaerobic fermentation of amino acids in an anaerobic membrane bioreactor (AnMBR) treating urban wastewater and food waste [58]. Three module hubs (60%) were from *Firmicutes*, suggesting the importance of this phylum in the network. Two of them were from the genus *Clostridium sensu stricto*. *Clostridium sensu stricto* was shown to be abundant in anaerobic reactors in previous studies [59, 60]. Also, this genus was shown to be abundant exoelectrogens in anaerobic digestion with direct interspecies electron transfer stimulation through nickel supplementation [61], which may indicate intensive connections between *Clostridium sensu stricto* and other microorganisms.

In the SB network, seven connectors and 18 module hubs were detected. Among the module hubs, seven were from *Proteobacteria* (38.9%) and five were from *Acidobacteria* (27.8%). Among the connectors, three were from *Proteobacteria* (42.9%) and three were from *Acidobacteria* (42.9%). These results together suggest the importance of *Proteobacteria* and *Acidobacteria* in the SB network. These *Acidobacteria* OTUs were assigned to the genera Gp2, Gp5 and Gp6. Their high abundance in the soil samples was also reported in our previous investigation [11]. Similarly, *Acidobacteria* Gp2 was identified as hubs in most of subnetworks of the microbiomes in various environments in a previous publication [30].

In the WB network, four connectors and seven module hubs were detected. Three module hubs (42.9%) and two connectors (50%) were from *Proteobacteria*, indicating the importance of this phylum in the WB network. Two connectors in WB were assigned to the genera of *Thauera* and *Acidobacteria* Gp4, respectively. *Thauera* has been reported to be frequently detected in WWTPs [62, 63] and play important roles in denitrification [46, 47] and carbon degradation [63, 64]. The wide presence of *Acidobacteria* Gp4 in WWTPs has been shown by several investigations [62, 65]. Gp4 was reported to be one of the characteristic genera for protein hydrolysis and short chain fatty acids (SCFAs) accumulation in waste activated sludge fermentation [66]. The high abundance and versatile functions of *Thauera* and *Acidobacteria* Gp4 may explain their important topologic roles in the WB network. One module hub was from *Byssovorax*, which was reported to be able to degrade cellulose [67].

Conclusion

Understanding the functions and co-occurrence patterns of microbial communities in different ecosystems is important to enrich microbial ecology knowledge. This study analyzed putative functions and co-occurrence patterns of the microbial populations in both natural and engineered ecosystems based on FAPROTAX and network analyses, respectively. The chemoheterotrophy related populations accounted for the largest proportions in almost all the communities. Their relative abundances (RAs) in the AD systems were the highest (43.7%±4.2%), followed by those of the soil systems (40.2%±1.9% in SA and 36.4%±2.0% in SB). The indicative communities showed higher RAs of aerobic chemoheterotrophy, sulfate respiration, fermentation and predatory/exoparasitic related bacteria than the overall communities for the SA and SB, AD, CSS and WB datasets, respectively. The above-mentioned differences in putative function distributions can be attributed to the characteristics of environmental conditions of the systems. The AD, WB and SB networks exhibited general topological features of scale free, small world and modularity. The WB network showed the highest proportion of negative correlations (70.4%), which may result from the environmental pressure in WWTPs. In each network, the taxa with positive correlations showed lower phylogenetic distances than the negatively correlated taxa on average.

Declarations

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Availability of data and material Not applicable

Code availability Not applicable

Ethics approval Not applicable

Consent to participate Not applicable

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Tables

Table 1. Significant differences between the overall community and the indicative community in each dataset (FW and WA excluded).

Dataset	Functional category	RAs in indicative community	RAs in overall community	Adjusted <i>p</i> value
SA	Aerobic chemoheterotrophy	37.78%±3.53%	34.16±3.75%	7.71 × 10 ⁻⁷ ***
SB	Aerobic chemoheterotrophy	42.69% ± 4.09%	34.79% ± 2.01%	4.25 × 10 ⁻⁹ ***
	Chemoheterotrophy	43.91% ± 3.87%	36.44 ± 2.00%	1.27 × 10 ⁻⁸ ***
CSS	Sulfate respiration	19.72%±12.67%	16.52%±12.93%	2.34 × 10 ⁻⁴ ***
	Respiration of sulfur compounds	19.88%±12.61%	16.88±12.99%	1.29 × 10 ⁻³ **
	Fermentation	6.65%±4.04%	5.54%±2.85%	0.014 *
AD	Fermentation	40.07%±6.62%	36.58%±6.63%	0.038 *
	Chemoheterotrophy	47.81±2.25%	43.69±4.15%	8.00 × 10 ⁻³ **
WB	Aerobic chemoheterotrophy	25.29%±4.88%	19.07%±2.14%	5.93 × 10 ⁻⁷ ***
	Chemoheterotrophy	25.29%±4.88%	22.70%±1.99%	9.00 × 10 ⁻³ **
	Predatory/exoparasitic	40.06%±8.92%	5.60%±2.19%	3.41 × 10 ⁻²⁰ ***

Table 2. Major topological properties of the empirical association networks of the microbial communities from WB, SB and AD datasets and their associated random networks.

Empirical networks										Random networks ^d		
System	No. of original OTUs ^a	S _t	Network size (n) ^b	r of scale free ^c (significance)	Avg degree	Avg path distance	Avg clustering coefficient	Modularity (No. of modules)	Transitivity (Trans)	Avg path distance ± SD	Avg clustering coefficient ± SD	Avg modularity ± SD
AD	469	0.850	219	0.988 (< 0.001)	3.187	5.290	0.087 ^e	0.718 ^e (26)	0.179 ^e	4.066 ± 0.064	0.023 ± 0.007	0.564 ± 0.008
WB	1526	0.940	1065	0.951 (< 0.001)	3.917	10.975	0.109 ^e	0.918 ^e (91)	0.141 ^e	4.878 ± 0.024	0.005 ± 0.001	0.526 ± 0.004
SB	1676	0.930	849	0.984 (< 0.001)	4.141	6.027 ^e	0.147	0.796 (115)	0.192	4.211 ± 0.033	0.015 ± 0.003	0.495 ± 0.004

^a The number of OTUs originally used for network construction using the RMT-based approach.

^b The number of nodes in a network.

^c The correlation coefficient (*r*) of the linear relationship in $P(k) \sim \gamma \cdot k$, where $P(k)$ is the fraction of connectivity *k* and γ is a constant.

^d The random networks were generated by rewiring all of the links of a network with the identical numbers of nodes and links to the corresponding empirical network.

^e Significant difference ($P < 0.001$) between the networks of OD and MBR.

Figures

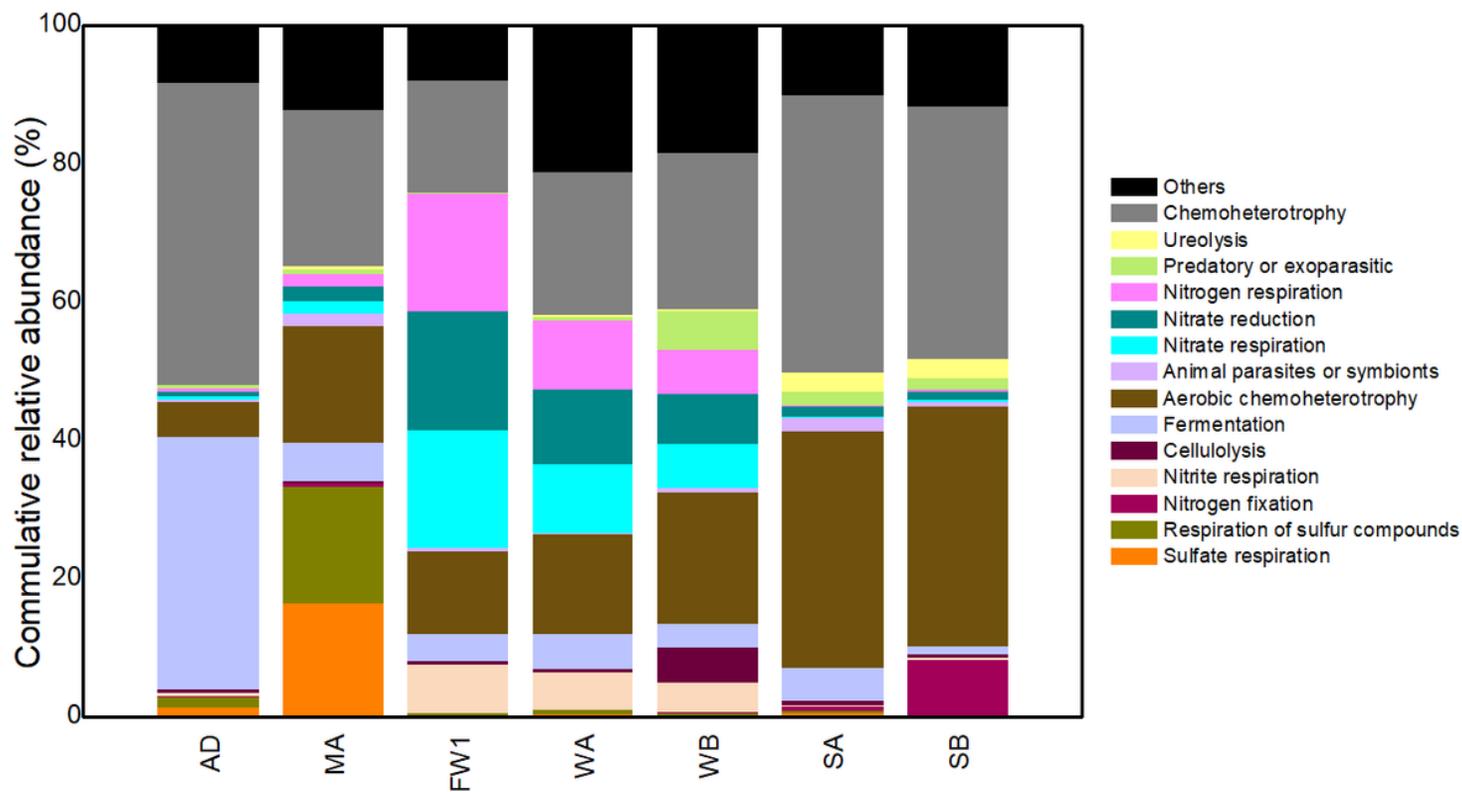


Figure 1

Relative abundance distribution of community functions in each dataset based on average value.

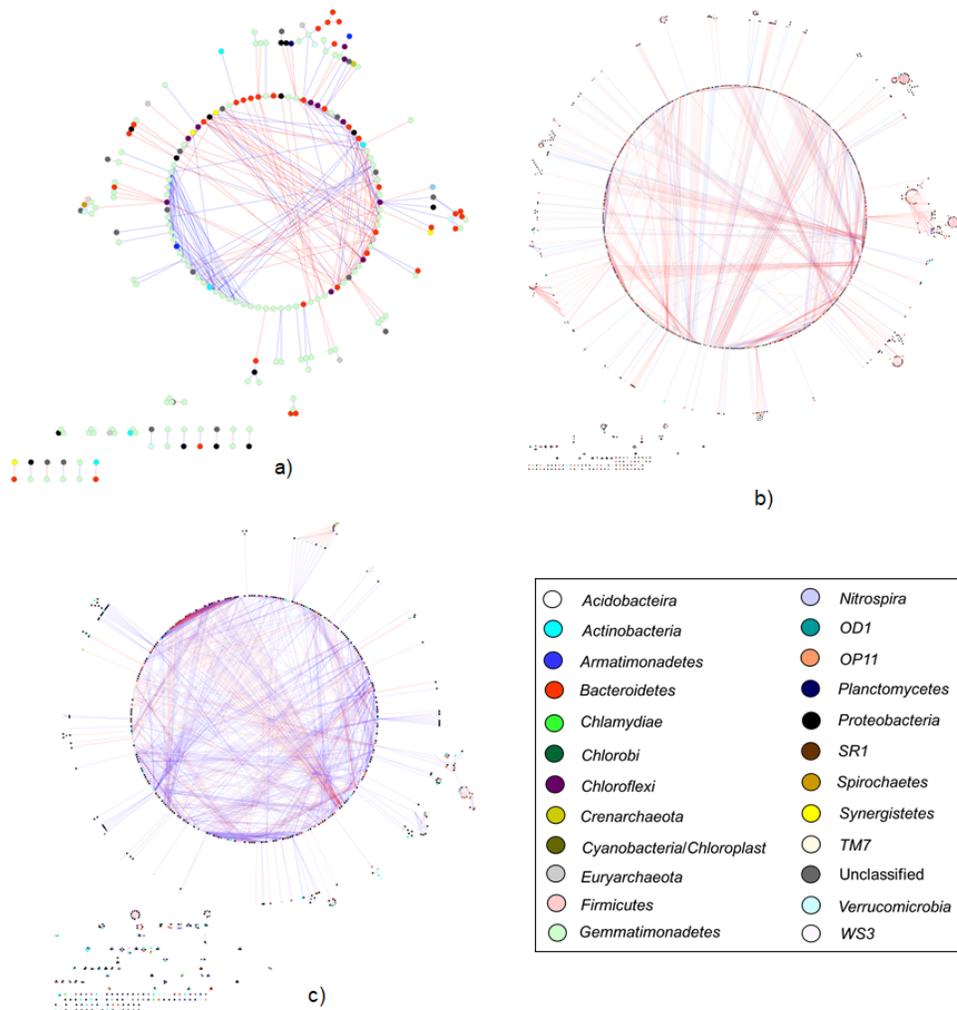


Figure 2
Correlations of the nodes in the (a) AD, (b) WB and (c) SB networks. Colors of the nodes indicate different phylum. A blue line indicates a positive interaction between two nodes, and a red line indicates a negative one.

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