

Supplementary Material

Biogeographic shifts in the microbial co-occurrence network features of three domains across complex environmental gradients in subtropical coastal waters

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The supplementary material contains:

- 8 Figures
- 3 Tables

Supplementary Figures

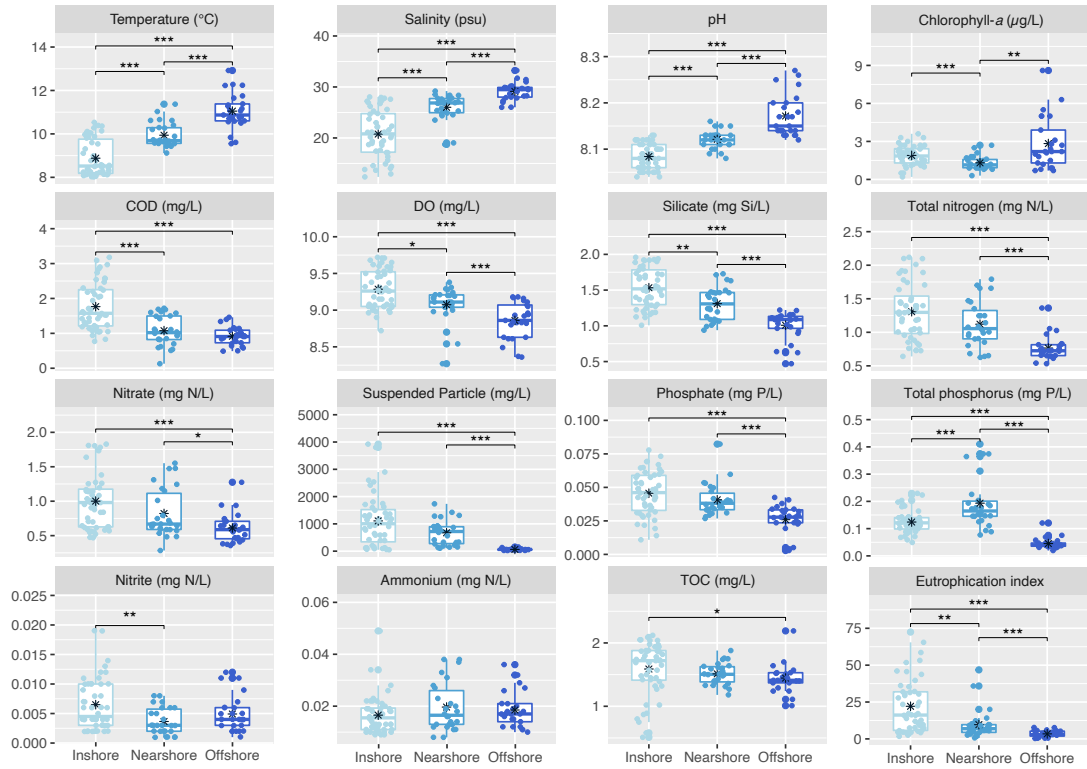


Figure S1. Boxplots showing the changes of environmental factors from inshore to offshore zones. COD, chemical oxygen demand; DO, dissolved oxygen; TOC, total organic carbon. The lower and upper hinges of the boxes correspond to the 25th and 75th percentiles. The lines and asterisks (*) in the boxes correspond to median and mean. The upper whisker extends from the hinge to the value no further than 1.5IQR (inter-quartile range). The lower whisker extends from the hinge to the smallest value at most 1.5IQR. Data beyond the end of the whiskers are “outlying” points (multiple comparisons after Kruskal-Wallis test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

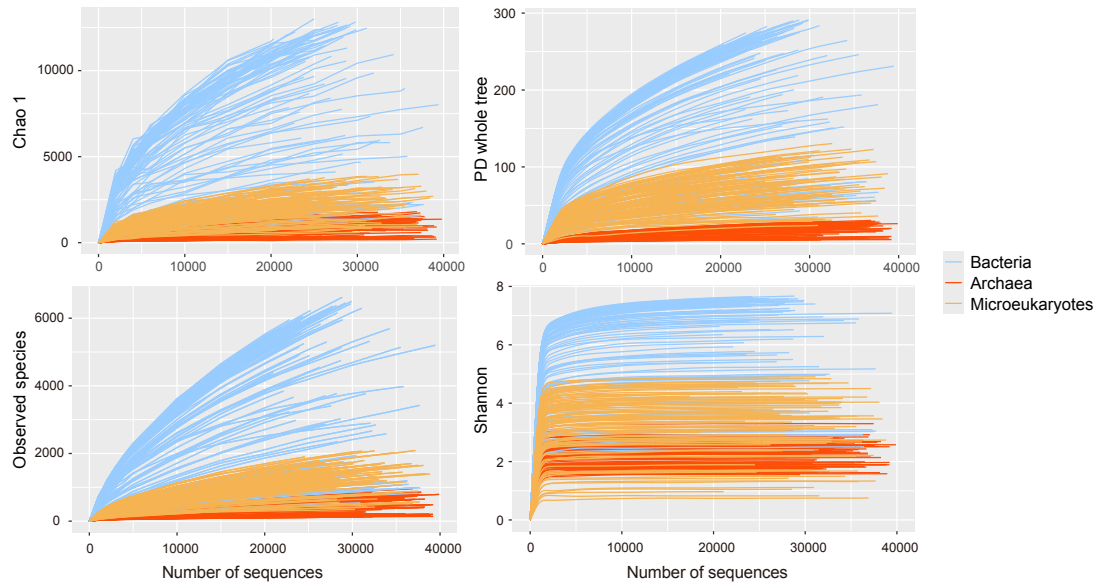


Figure S2. Rarefaction curves showing α -diversity indices (including Chao 1, observed species, phylogenetic diversity (PD), and the Shannon-Wiener index) of each sample at different sequencing depths.

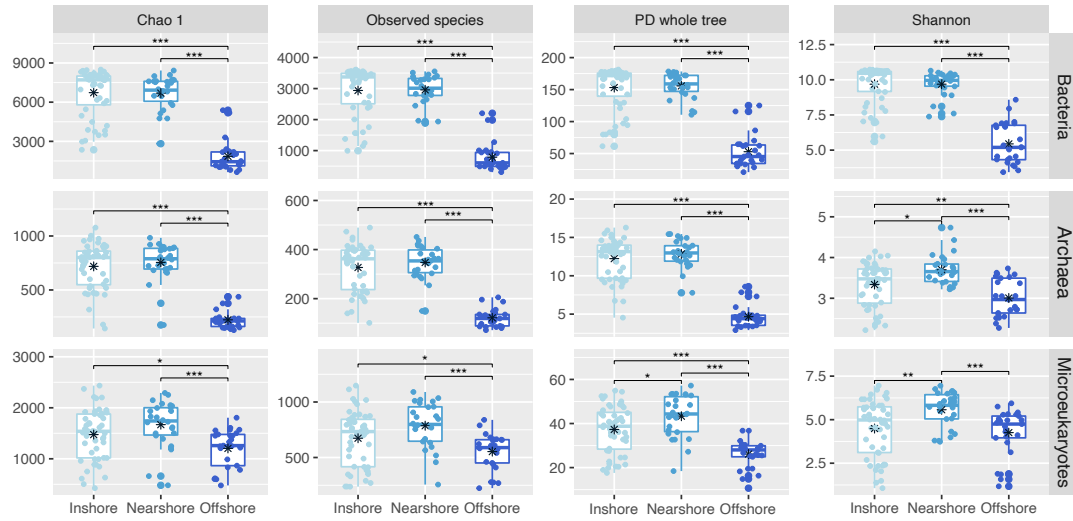


Figure S3. Boxplots showing the changes of α -diversity indices (including Chao 1, observed species, phylogenetic diversity (PD), and Shannon-Wiener index) of Bacteria, Archaea, and Microeukaryotes from inshore to offshore zones (multiple comparisons after Kruskal-Wallis test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

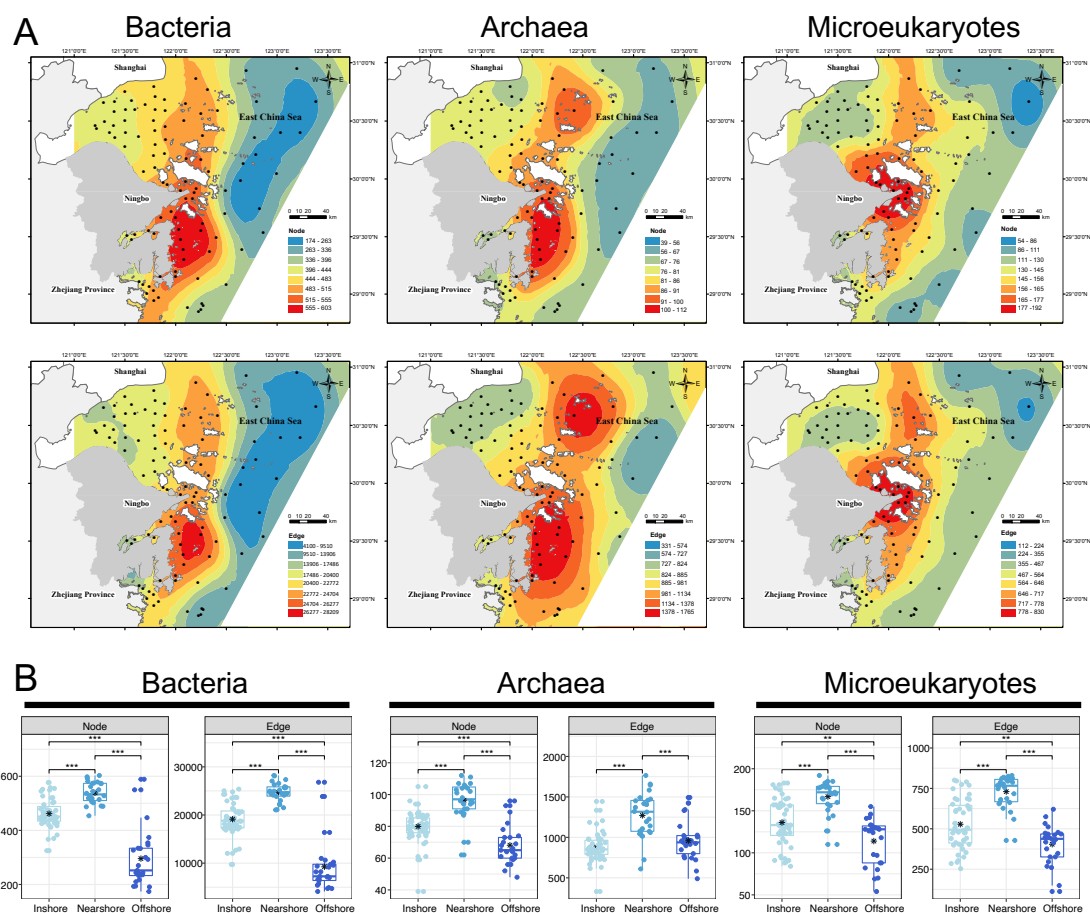


Figure S4. Geographic patterns of topological features (the number of nodes and edges) for intra-domain sub-networks of three microbial domains. (A) Kriging maps visualizing the spatial distribution of the number of nodes and edges across the sampling zones. (B) Boxplots showing the differences of these two topological features in inshore, nearshore and offshore zones (multiple comparisons after Kruskal-Wallis test, $** P < 0.01$, $*** P < 0.001$).

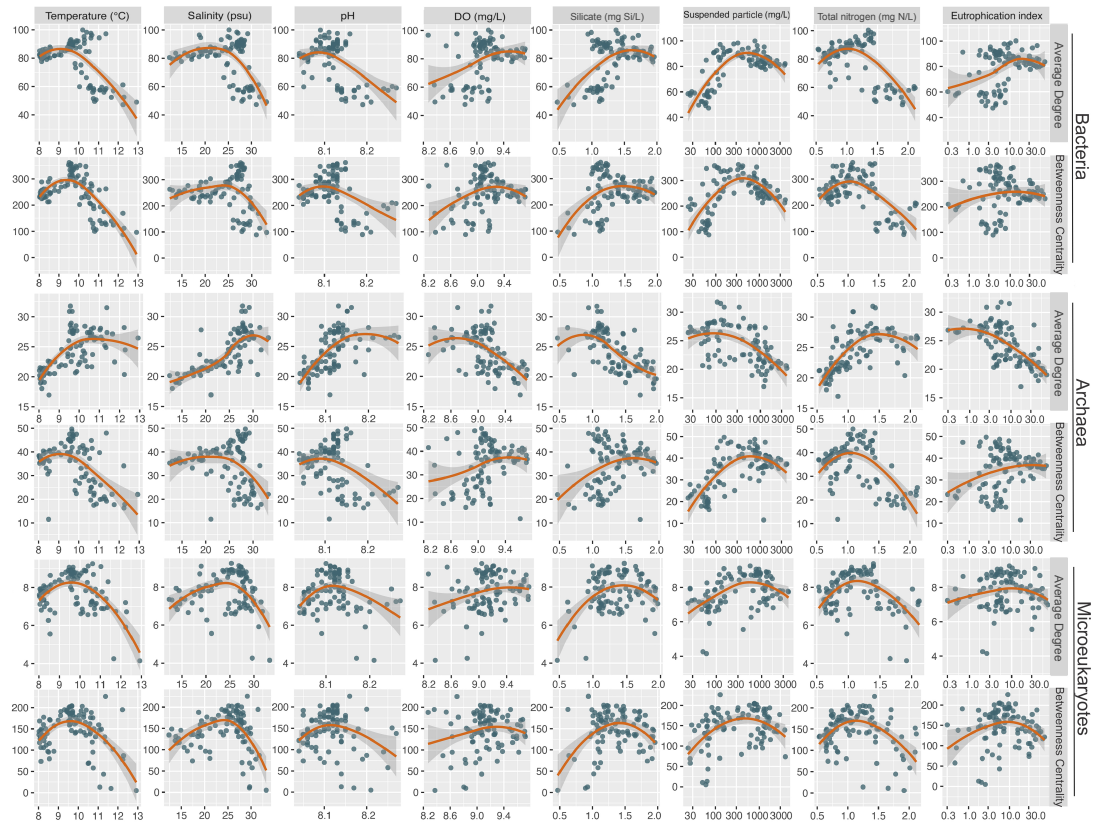


Figure S5. Scatter diagrams showing the relationships between topological features (average degree and betweenness centrality) of co-occurrence networks for three domains and environmental factors across the sampling zones. Environmental factors without significant gradients across inshore-nearshore-offshore zones (including chlorophyll-*a*, total phosphorus, nitrite, ammonium, and TOC) were not included in this analysis. Other factors involved in the calculation of eutrophication index (including COD, phosphate, and nitrate) were also excluded.

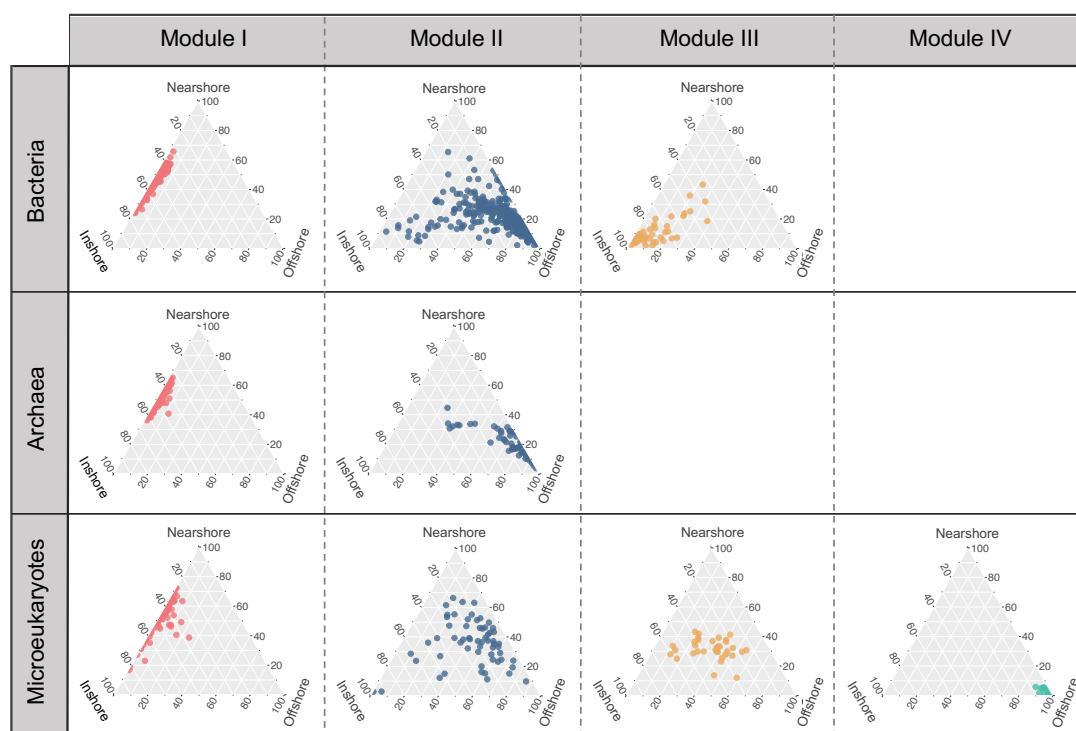


Figure S6. Ternary plots showing the distribution patterns of each module in the intra-domain co-occurrence networks for Bacteria, Archaea, and Microeukaryotes in different zones (inshore, nearshore and offshore).

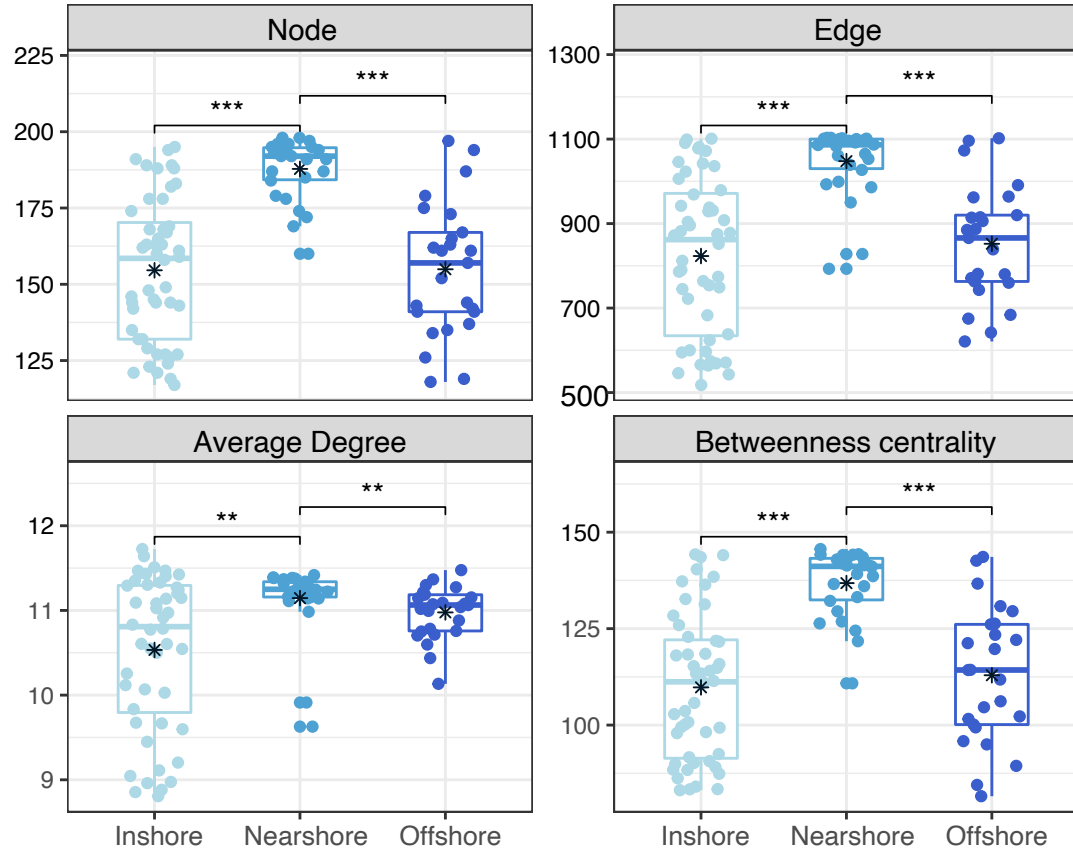


Figure S7. Boxplots showing the differences in topological features (the number of nodes and edges, degree, and betweenness centrality) of inter-domain sub-networks for three microbial communities in the inshore, nearshore and offshore zones (multiple comparisons after Kruskal-Wallis test, ** $P < 0.01$, *** $P < 0.001$).

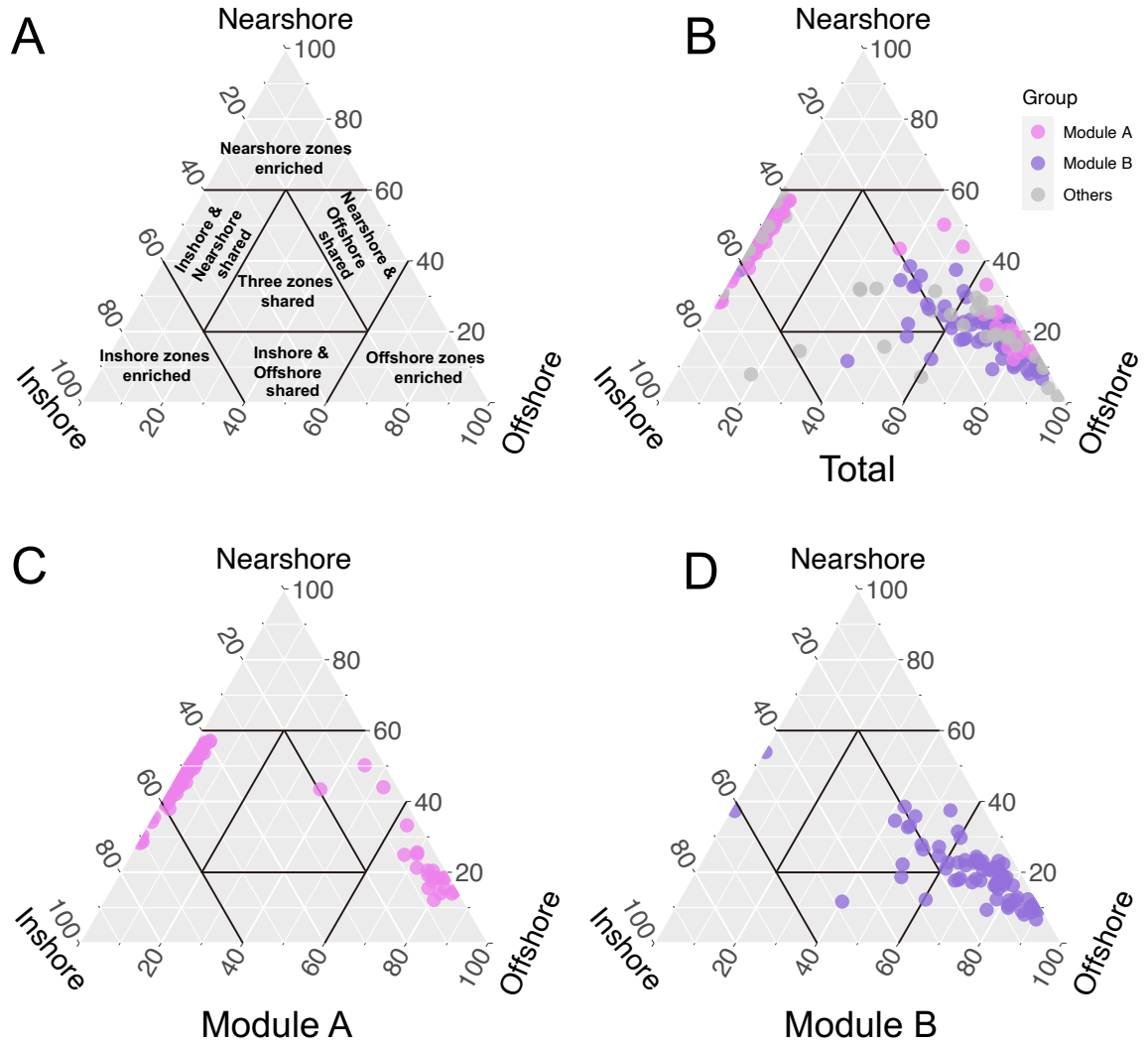


Figure S8. Ternary plots showing the distribution patterns of all OTUs (B) in the inter-domain co-occurrence network and the OTUs in Module A (C) and Module B (D) in different zones (inshore, nearshore and offshore). The schematic diagram (A) shows the OTUs categories, and the bar chart in Fig. 4C summarizes the proportion of OTUs belonging to the corresponding category in the entire network and in each module.

Supplementary Tables

Table S1. Information for primers used in this study.

	Primers	Reference	Primer sequence (5'- 3')	Target gene	Target fragment	PCR reaction condition
Bacteria	338F	(Dennis et al. 2013)	ACTCCTACGGGAGGCAGCA	16S rRNA	V3-V4	initial denaturation at 95°C for 3 min, followed by 27 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s, with a final extension at 72°C for 10 min
	806R		GGACTACHVGGGTWTCTAAT			
Archaea	Arch524F	(Casamayor et al. 2002)	TGYCAGCCGCCGCGGTAA	16S rRNA	V4-V5	initial denaturation at 95°C for 3 min, followed by 32 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s, with a final extension at 72°C for 10 min
	Arch958R		YCCGGCGTTGAVTCCAATT			
Eukaryotes	3NDF	(Zhang et al. 2018)	GGCAAGTCTGGTGCCAG	18S rRNA	V4	initial denaturation at 95°C for 3 min, followed by 32 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min, with a final extension at 72°C for 10 min
	V4_euk_R2		ACGGTATCT(AG)ATC(AG)TCTTCG			

Table S2. Description of topological features.

Level	Topological features	Description (Berry and Widder 2014; Greenblum et al. 2012; Ma et al. 2016)
Node-level	Degree	The number of adjacent edges.
	Betweenness centrality	The number of the shortest paths going through a node, refers to the potential influence of a particular node on the connections of other nodes.
	Clustering coefficient	The probability that the adjacent nodes of a node are connected.
	Average shortest path length	The average length of the shortest path of this node to any other nodes.
Network-level	Average degree	The average connections of each node with another unique node in the network.
	Betweenness centrality	The mean of betweenness centrality values for all nodes.
	Graph density	The intensity of connections among nodes.
	Clustering coefficient	The degree to which the nodes tend to cluster together.
	Average path length	The average network distance between all pairs of nodes.

Table S3. Detailed information about the network hubs, module hubs and connectors for the inter-domain co-occurrence network of three microbial communities.

	Node ID	Module name	Z_i	P_i	Domain	Taxonomy
Network hubs	Arc_11	B	2.83	0.63	Archaea	p_Thermoplasmatota;c_Poseidoniia;o_Poseidoniales;f_Poseidoniaceae;g_Poseidonia;s_Poseidonia sp002494645
	Arc_14	B	3.22	0.62	Archaea	p_Thermoproteota;c_Nitrososphaeria;o_Nitrososphaerales;f_Nitrosopumilaceae;g_Nitrosopumilus;s_Nitrosopumilus sp905612485
	Arc_21	Others	2.92	0.66	Archaea	p_Thermoplasmatota;c_Poseidoniia;o_Poseidoniales;f_Thalassarchaeaceae;g_MGIib-O1;s_MGIib-O1 sp002457555
Module hubs	Arc_10	A	4.49	0.50	Archaea	p_Thermoproteota;c_Nitrososphaeria;o_Nitrososphaerales;f_Nitrosopumilaceae;g_Nitrosopelagicus
	Arc_6	A	3.97	0.60	Archaea	p_Thermoproteota;c_Nitrososphaeria;o_Nitrososphaerales;f_Nitrosopumilaceae;g_JACEMX01;s_JACEMX01 sp013867335
	Arc_8	A	4.75	0.51	Archaea	p_Thermoproteota;c_Nitrososphaeria;o_Nitrososphaerales;f_Nitrosopumilaceae;g_Nitrosopumilus
	Arc_5	B	2.83	0.57	Archaea	p_Thermoproteota;c_Nitrososphaeria;o_Nitrososphaerales;f_Nitrosopumilaceae;g_Nitrosopumilus;s_Nitrosopumilus sp905612485
	Arc_9	B	4.37	0.61	Archaea	p_Thermoproteota;c_Nitrososphaeria;o_Nitrososphaerales;f_Nitrosopumilaceae;g_Nitrosopumilus
Connectors	Arc_17	A	1.90	0.66	Archaea	p_Thermoproteota;c_Nitrososphaeria;o_Nitrososphaerales;f_Nitrosopumilaceae;g_Nitrosopumilus
	Arc_46	A	0.61	0.63	Archaea	p_Thermoplasmatota;c_Poseidoniia;o_Poseidoniales;f_Thalassarchaeaceae;g_MGIib-O1;s_MGIib-O1 sp002457555
	Bac_1	A	-0.17	0.63	Bacteria	p_Proteobacteria;c_Gammaproteobacteria;o_Enterobacteriales;f_Alteromonadaceae;g_Pseudoalteromonas;s_Pseudoalteromonas sp002964705

Bac_24	A	-0.17	0.67	Bacteria	p_Proteobacteria;c_Gammaproteobacteria;o_Woeseiales;f_Woeseiaceae;g_UBA1847;s_UBA1847 sp013042375
Bac_11	B	0.40	0.64	Bacteria	p_Actinobacteriota;c_Acidimicrobiia;o_Actinomarinales;f_Actinomarinaceae;g_Actinomarina
Bac_152	B	0.15	0.62	Bacteria	p_Proteobacteria;c_Alphaproteobacteria;o_Rhodobacterales;f_Rhodobacteraceae;g_LGRT01;s_LGR T01 sp001642945
Bac_22	B	-0.62	0.78	Bacteria	p_Proteobacteria;c_Alphaproteobacteria;o_Rhodobacterales;f_Rhodobacteraceae;g_CABZJG01
Bac_44	B	-0.75	0.67	Bacteria	p_Proteobacteria;c_Gammaproteobacteria;o_Burkholderiales;f_Methylophilaceae
Bac_45	B	0.53	0.64	Bacteria	p_Proteobacteria;c_Gammaproteobacteria
Bac_46	B	-0.62	0.63	Bacteria	p_Proteobacteria;c_Alphaproteobacteria;o_Puniceispirillales;f_AAA536-G10;g_AAA536-G10
Bac_8	B	-0.11	0.71	Bacteria	p_Actinobacteriota;c_Acidimicrobiia;o_Acidimicrobiales
Bac_103	Others	0.43	0.65	Bacteria	p_Proteobacteria;c_Alphaproteobacteria;o_Thalassobaculales;f_Thalassobaculaceae;g_UBA7326;s_ UBA7326 sp003212315
Bac_219	Others	-0.63	0.63	Bacteria	p_Nitrospinota;c_Nitrospina;o_Nitrospinales;f_Nitrospinaceae
Bac_26	Others	1.14	0.65	Bacteria	p_Marinisomatota;c_Marinisomatia;o_Marinisomatales;f_Marinisomataceae;g_Marinisoma
Bac_36	Others	0.43	0.65	Bacteria	p_Proteobacteria;c_Gammaproteobacteria;o_SAR86;f_D2472;g_VMDH01;s_VMDH01 sp008080945
Bac_84	Others	-0.63	0.67	Bacteria	p_Proteobacteria;c_Gammaproteobacteria
Euk_55	Others	0.43	0.64	Eukaryota	D_1_TSAR;D_2_Alveolata;D_3_Dinoflagellata;D_4_Syndiniales;D_5_Dino-Group-II;D_6_Dino- Group-II-Clade-14; D_7_Dino-Group-II-Clade-14_X; D_8_Dino-Group-II-Clade-14_X_sp.
Euk_8	Others	1.50	0.65	Eukaryota	D_1_TSAR;D_2_Alveolata;D_3_Ciliophora;D_4_Ciliophora_X;D_5_Ciliophora_XX;D_6_Ciliopho ra_XXX; D_7_Mesodinium; D_8_Mesodinium_sp.

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