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Supplementary data for

Effects of Fe (II) on microbial communities and nitrogen transformation pathway of nitrogen and iron cycling in the Anammox process: kinetic, quantitative mechanism and metagenomic analysis

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China

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Table S1 Primers used for qPCR and thermal programs in this study.

Target prokaryote	Target gene	Sequence (5'-3') of parimer pairs	Annealing (°C)	Thermal program	Reference
Total bacteria	16S rRNA	341F: CCTACGGGAGGCAGCAG 518R: ATTACCGCGGCTGCTGG	60	5 min at 95°C, 40 cycles of 30 s at 95°C, 30 s at 60°C, and 40 s at 72°C	[1]
Anammox Bacteria	16S rRNA	Amx809f: GCCGTAAACGATGGGCACT Amx1066r: AACGTCTCACGACACGAGCTG	60	10min at 95°C, followed by 35 cycles of 60 s at 95°C, 60 s at 60°C, and 45 s at 72°C	[2]
AOA	*amoA*	amoAF: STAATGGTCTGGCTTAGACG amoAR: GCGGCCATCCATCTGTATGT	53	3 min at 94°C, followed by 40 cycles of 30 s at 94°C, 1 min at 53°C, and 1 min at72°C	[3]
AOB	*amoA*	amoAF: GGGGTTTCTACTGGTGGT amoAR: CCCCTCKGSAAAGCCTTCTTC	55	3 min at 94°C, followed by 40 cycles of 30 s at 94°C, 30 s at 55°C, and 45 s at 72°C	[4]
Denitrifying bacteria	nosz	nosZ1F: WCSYTGTTCMTCGACAGCCAG nosZ1R: ATGTCGATCARCTGVKCRTTYTC	63	5 min at 95°C, followed by 35 cycles of 60 s at 95°C, 60 s at 63°C, and 45 s at 72°C	[5]
Denitrifying bacteria	nirS	nirSnF: TACCACCCCGAGCCGCGCGT nirSnr: GCCGCCGTCRTGVAGGAA	63	5 min at 95°C, followed by 35 cycles of 60 s at 95°C, 60 s at 63°C, and 45 s at 72°C	[6]
Denitrifying bacteria	nirK	nirKF: ATYGGCGGVAYGGCGA nirKR: GCCTCGATCAGRTTRTGG	57	5 min at 95°C, followed by 35 cycles of 60 s at 95°C, 60 s at 57°C,and 45 s at 72°C	[6]
Dissimilarity nitrite reducing bacteria	narG	narG2F: CTCGAYCTGGTGGTYGA narG2R: TTYTCGTACCAGGTSGC	55	5 min at 95°C, followed by 35 cycles of 60 s at 95°C, 60 s at 55°C, and 45 s at 72°C	[7]
Dissimilarity nitrite reducing bacteria	napA	napA3F: CCCAATGCTCGCCACTG napA3R: CATGTTKGAGCCCCACAG	60	5 min at 95°C, followed by 35 cycles of 60 s at 95°C, 60 s at 60°C, and 45 s at 72°C	[7]
Dissimilarity nitrate reducing bacteria	nrfA	nrfA2F: CACGACAGCAAGACTGCCG nrfa2R: CCGGCACTTTCGAGCCC	60	5 min at 95°C, followed by 35 cycles of 60 s at 95°C, 60 s at 60°C, and 45 s at 72°C	[8]
Nitrite oxidizing	*nxrA*	F1norA: CAGACCGACGTGTGCGAAAG	57	Pre-heating at 50 °C for 2 min, pre-denaturation at 95 °C	[9]

bacteria		R1norA: TCYACAAGGAACGGAAGGTC		for 10 min, denaturation at 95 °C for 15 s, annealing at 57 °C for 30 s, and extension at 72 °C for 30 s	
Acidimicrobium	16S rRNA	Amf995: CTCTGCGGCTTTTCCCTCCATG Uni-907R-RC: AAACTCAAAKGAATTGACGG	52	Pre-heating at 50 °C for 2 min, pre-denaturation at 95 °C for 10 min, denaturation at 95 °C for 15 s, annealing at 52 °C for 45 s, and extension at 72 °C for 45 s	[10]
Ferrovum	16S rRNA	Ferrovum643F: ACAGACTCTAGCTTGCCAGT Uni-338F-RC: ACTCCTACGGGAGGCAGC	57	Pre-heating at 50 °C for 2 min, pre-denaturation at 95 °C for 10 min, denaturation at 95 °C for 15 s, annealing at 57 °C for 45 s, and extension at 72 °C for 45 s	[10]
Albidiferax	16S rRNA	RdoR-RC: GACCTGCATTTGTGACTGYA Uni-907R: CCGTCAATTCMTTTGAGTTT	52	Pre-heating at 50 °C for 2 min, pre-denaturation at 95 °C for 10 min, denaturation at 95 °C for 15 s, annealing at 52 °C for 45 s, and extension at 72 °C for 45 s	[10]
Geobacter	16S rRNA	Geo561F: GCGTGTAGGCGGTTTBTTAA Geo858R: TCAATACCCGCAACACCTAG	57	Pre-heating at 50 °C for 2 min, pre-denaturation at 95 °C for 10 min, denaturation at 95 °C for 15 s, annealing at 57 °C for 45 s, and extension at 72 °C for 45 s	[10]
Acidiphilium	16S rRNA	ACD840: CGACACTGAAGTGCTAAGC Uni-338F-RC: ACTCCTACGGGAGGCAGC	61	Pre-heating at 50 °C for 2 min, pre-denaturation at 95 °C for 10 min, denaturation at 95 °C for 15 s, annealing at 61 °C for 45 s, and extension at 72 °C for 45 s	[11]

Table S2 Raw and effective reads, plus numbers of OTUs, Good's coverage, Shannon, Chao1, ACE, and Simpson of five phases.

Sample ID	Raw reads	Effective reads	OTUs	Good's coverage	Shannon	Chao 1	ACE	Simpson
phase I	19527	18283	152	0.999562	3.36	161	154	0.0749
phase II	19051	18041	145	0.999612	3.08	149	147	0.1401
phase III	15616	13425	146	0.999628	3.16	147	147	0.1078
phase IV	13239	12125	132	0.998103	2.26	148	148	0.2605
phase V	19925	17130	230	0.99784	3.51	260	259	0.0733

Figure S1

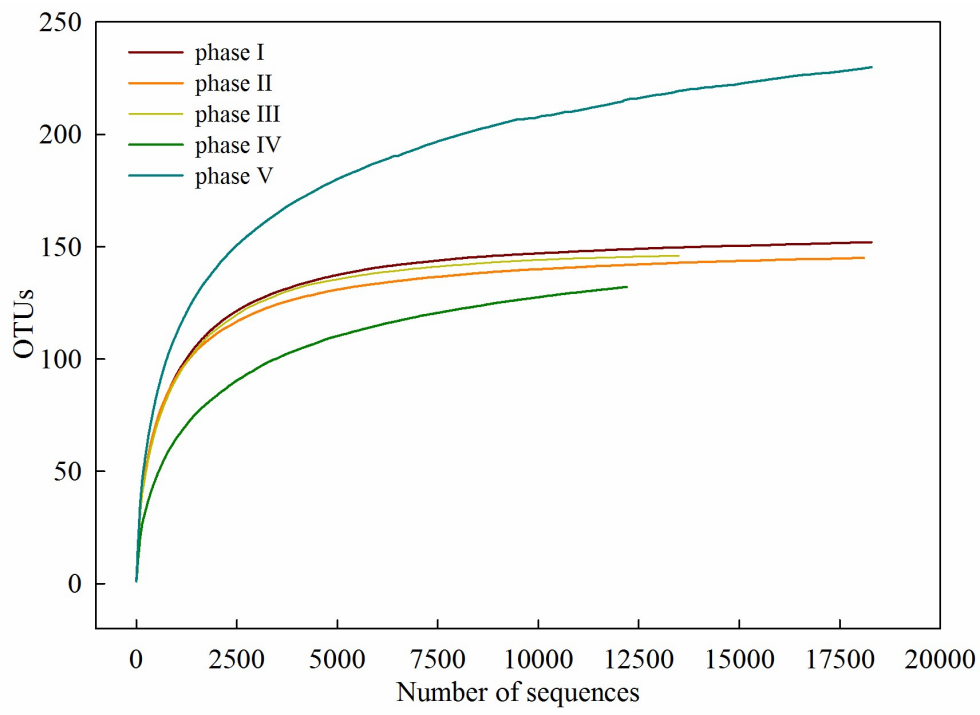

Figure S1 Rarefaction curves base on MiSeq pyrosequencing of bacterial communities in different phases. The OTUs were defined by 3% distances.

Figure S2a

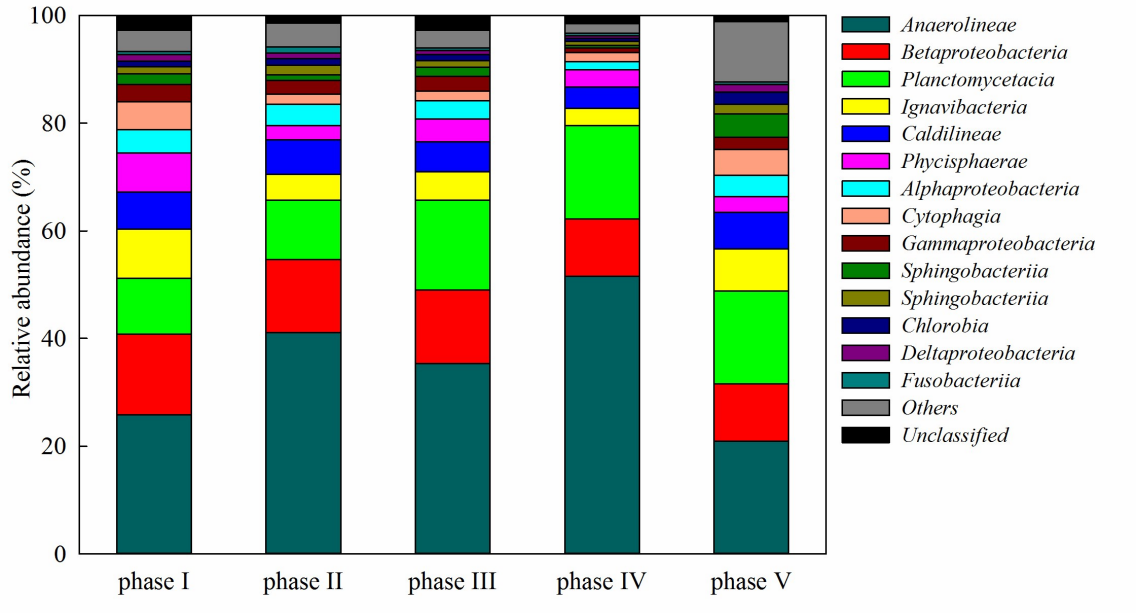
100
80
60
40
20
0
Relative abundance (%)
phase I
phase II
phase III
phase IV
phase V
Anaerolineae
Betaproteobacteria
Planctomycetacia
Ignavibacteria
Caldilineae
Phycisphaerae
Alphaproteobacteria
Cytophagia
Gammaproteobacteria
Sphingobacteriia
Sphingobacteriia
Chlorobia
Deltaproteobacteria
Fusobacteriia
Others
Unclassified

Figure S2b

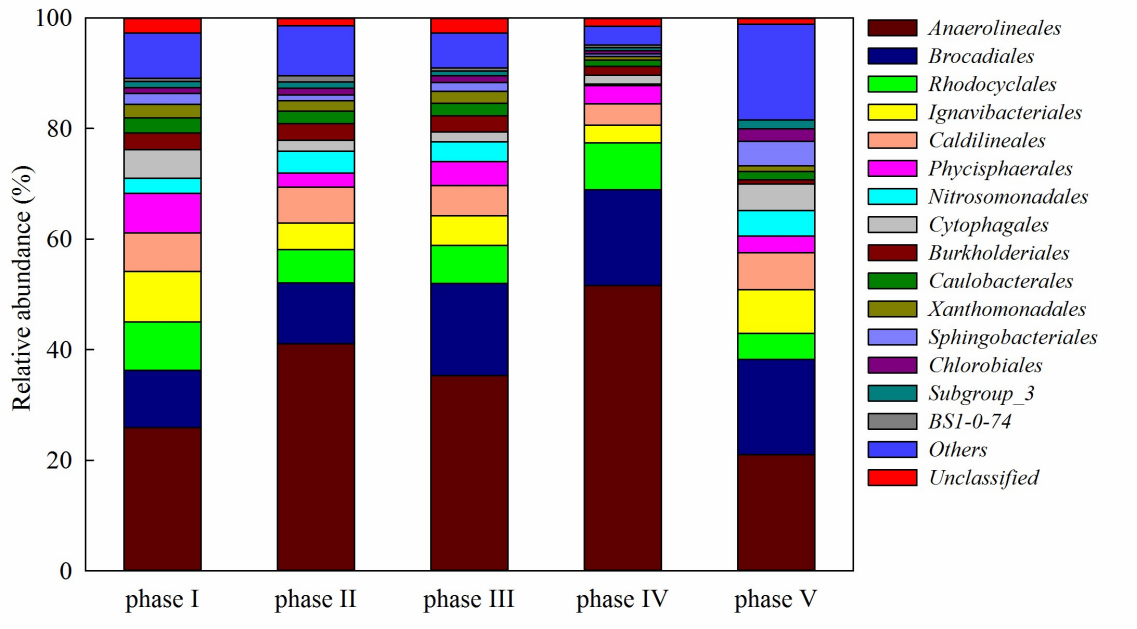
Relative abundance (%)
100
80
60
40
20
0
phase I
phase II
phase III
phase IV
phase V
Anaerolineales
Brocadiales
Rhodocyclales
Ignavibacteriales
Caldilineales
Phycisphaerales
Nitrosomonadales
Cytophagales
Burkholderiales
Caulobacterales
Xanthomonadales
Sphingobacteriales
Chlorobiales
Subgroup_3
BS1-0-74
Others
Unclassified

Figure S2c

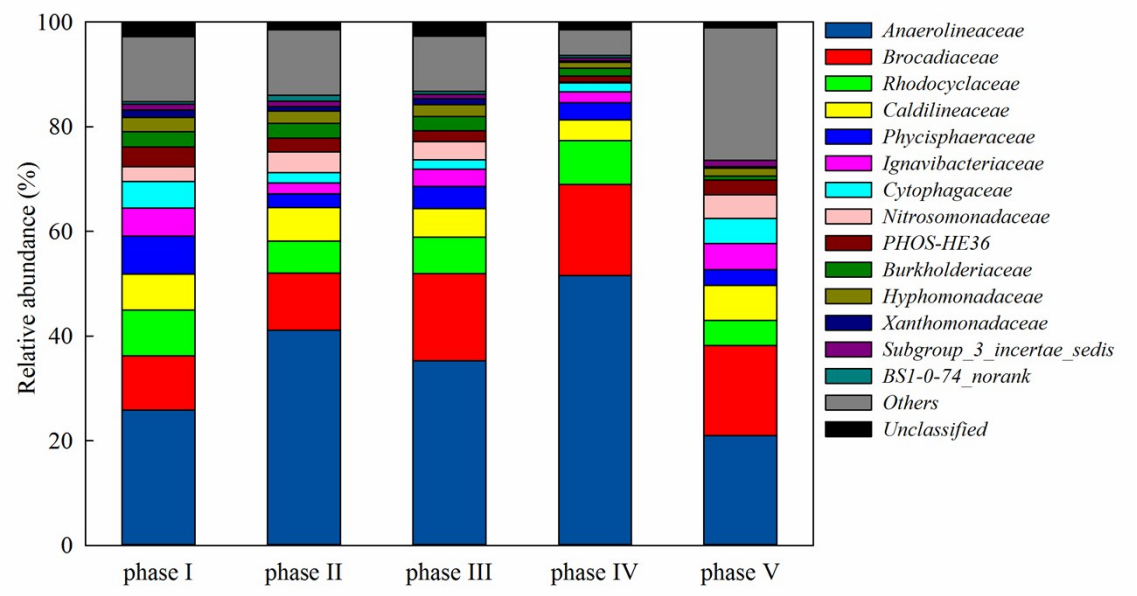
100
80
60
40
20
0
Relative abundance (%)
phase I
phase II
phase III
phase IV
phase V
Anaerolineaceae
Brocadiaceae
Rhodocyclaceae
Caldilineaceae
Phycisphaeraceae
Ignavibacteriaceae
Cytophagaceae
Nitrosomonadaceae
PHOS-HE36
Burkholderiaceae
Hyphomonadaceae
Xanthomonadaceae
Subgroup_3_incertae_sedis
BS1-0-74_norank
Others
Unclassified

Figure S2d

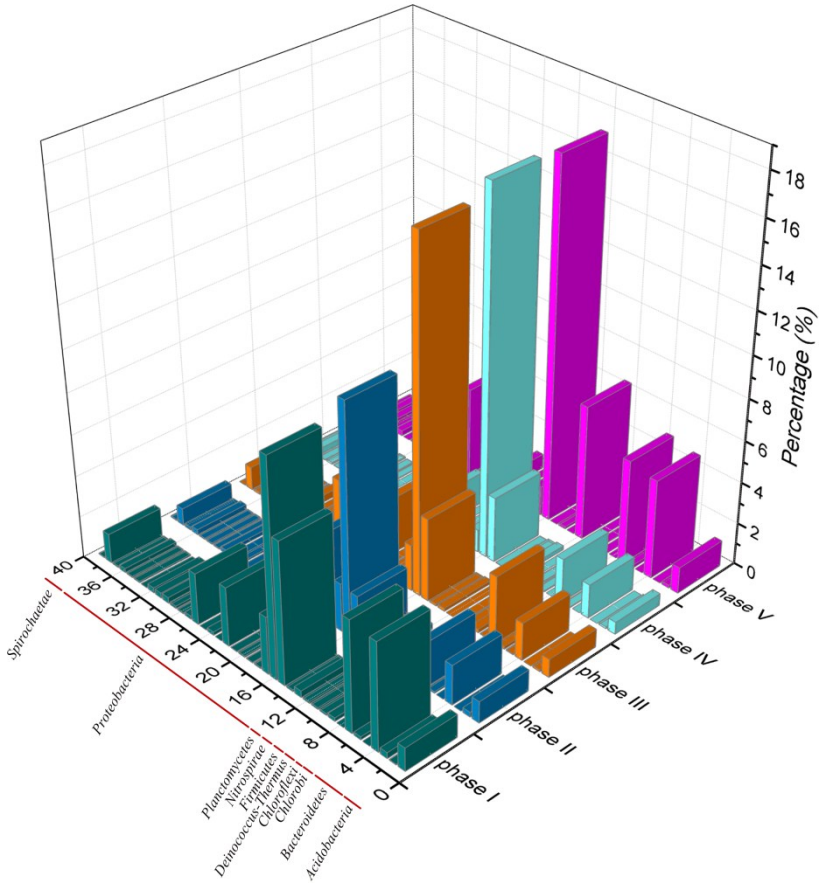

Figure S2 Distributions of bacteria in five phases at different taxonomy level. (a) At class level; (b) at order level; (c) at family level; (d) at genus level. Taxa represented occurred at >1% frequency in at least one sample. Others refer to the taxa with their maximum abundance <1% in any sample.

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