

## Supplementary data

### **Enhanced long-term nitrogen removal by organotrophic anammox bacteria under different C/N ratio constrains: quantitative molecular mechanism and microbial community dynamics**

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### **4. References**

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

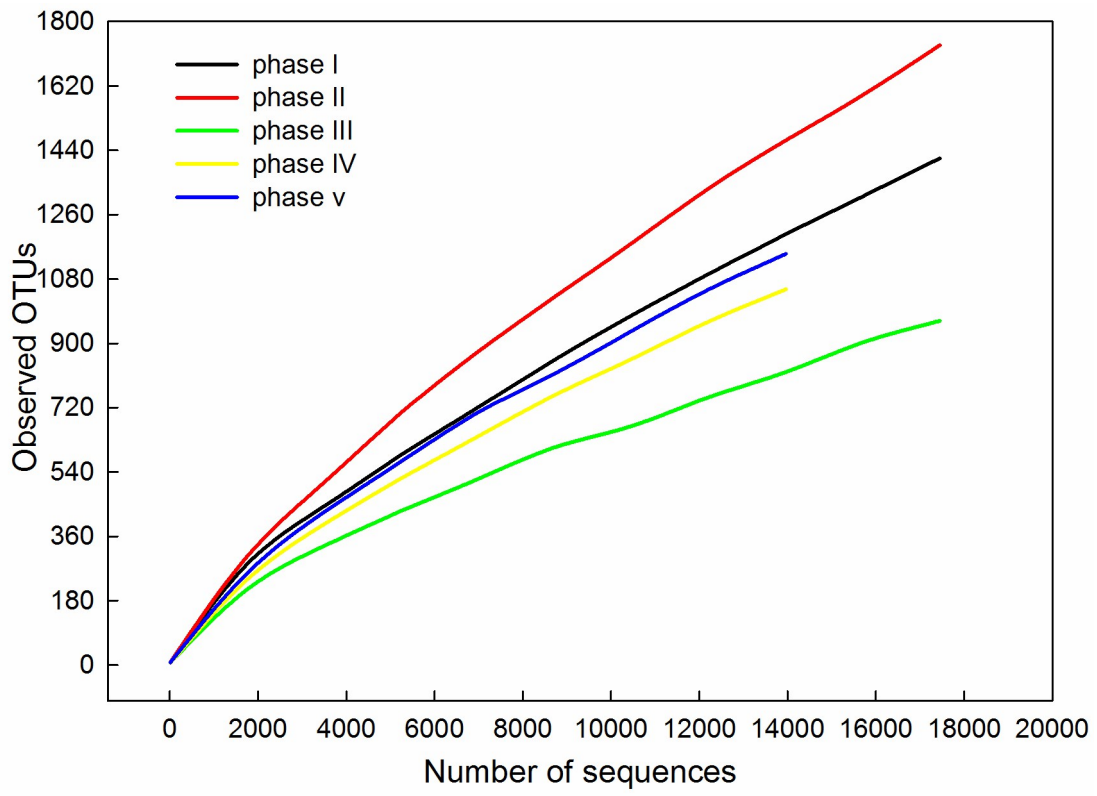
Target prokaryote	Target gene	Sequence (5'-3') of primer pairs	Annealing (°C)	Thermal program	Reference
Total bacteria	16S rRNA	341F: CCTACGGGAGGCAGCAG 518R: ATTACCGGGCTGCTGG	60	5 min at 95°C, 40 cycles of 30 s at 95°C, 30 s at 60°C, and 40 s at 72°C	<sup>1</sup>
Anammox Bacteria	16S rRNA	Amx809f: GCCGTAAACGATGGGCACT 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	<sup>2</sup>
AOA	<i>amoA</i>	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 at 72°C	<sup>3</sup>
AOB	<i>amoA</i>	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	<sup>4</sup>
Denitrifying bacteria	<i>nosZ</i>	nosZ1F: WCSYTGTTTCMTCGACAGCCAG 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	<sup>5</sup>
Denitrifying bacteria	<i>nirS</i>	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	<sup>6</sup>
Denitrifying bacteria	<i>nirK</i>	nirKF: ATYGGCGGVAYGGCGA nirKR: GCCTCGATCAGRTRTRTGG	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	<sup>6</sup>
Dissimilarity nitrite reducing bacteria	<i>narG</i>	narG2F: CTCGAYCTGGTGGTYGA narG2R: TTYTCGTACCAGGTS GC	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	<sup>7</sup>
Dissimilarity nitrite reducing bacteria	<i>napA</i>	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	<sup>7</sup>
Dissimilarity nitrate reducing bacteria	<i>nrfA</i>	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	<sup>8</sup>
Nitrite oxidizing	<i>nxrA</i>	F1norA: CAGACCGACGTGTGCGAAAG	57	Pre-heating at 50 °C for 2 min, pre-denaturation at 95 °C	<sup>9</sup>

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	
Methanogen bacteria	mcrA	mcrAme1f: GCMATGCARATHGGWATGTC mcrAme3r: TGTGTGAASCCKACDCCACC	54	10 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	10
Sulfate reducing bacteria	dsrA	dsr1F: ACSCACTGGAAGCACGGCGG dsrR: GTGGMRCCTGCAKRTTGG	63	10 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	11

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

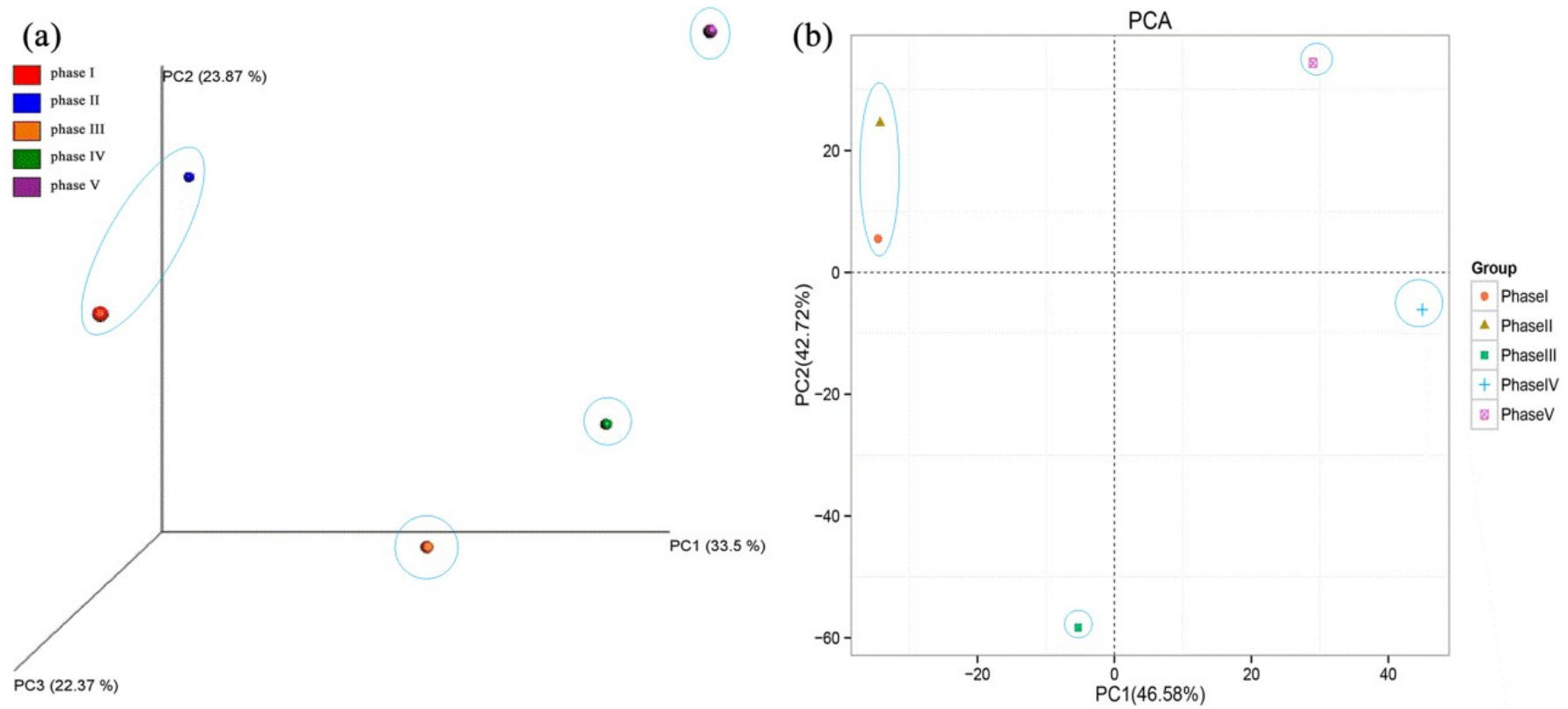
Sample ID	Raw reads	Effective reads	OTUs	Good's coverage	Shannon	Chao 1	ACE	Simpson
phase I	24,789	17,454	1412	0.99	5.68	5273.04	5623.46	0.93
phase II	25,514	17,724	1750	0.99	6.05	6630.98	7130.23	0.95
phase III	29,110	21,586	1112	0.99	5.05	3504.92	3842.40	0.88
phase IV	21,284	15,453	1131	0.99	4.70	3456.78	3789.32	0.86
phase V	21,172	14,505	1181	0.99	5.35	3774.60	4294.51	0.91

**Figure S1**



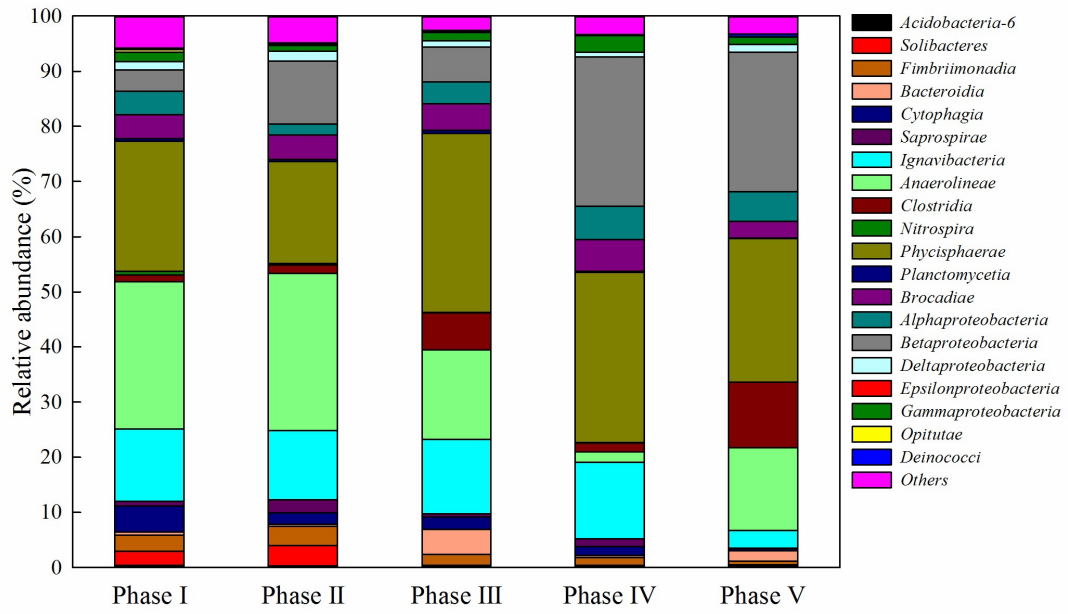
**Figure S1** Rarefaction curves base on MiSeq sequencing of bacterial communities in different phases. The OTUs were defined by 3% distances.

**Figure S2**



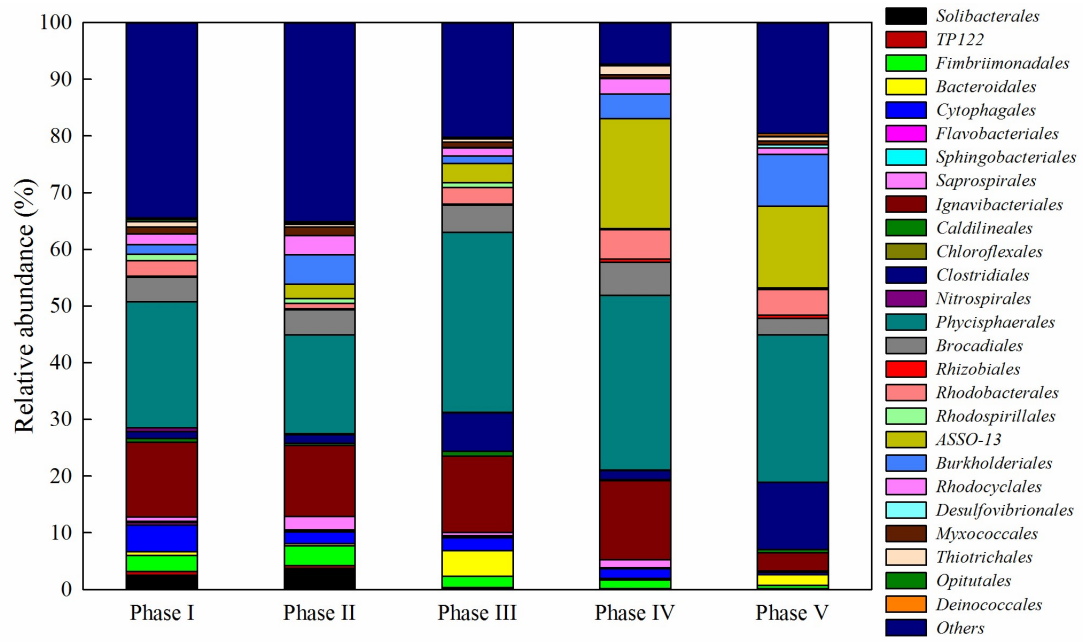
**Figure S2** Beta diversity for five samples. (a) 3-D PCoA analysis; (b) 2-D PCoA analysis.

**Figure S3a**

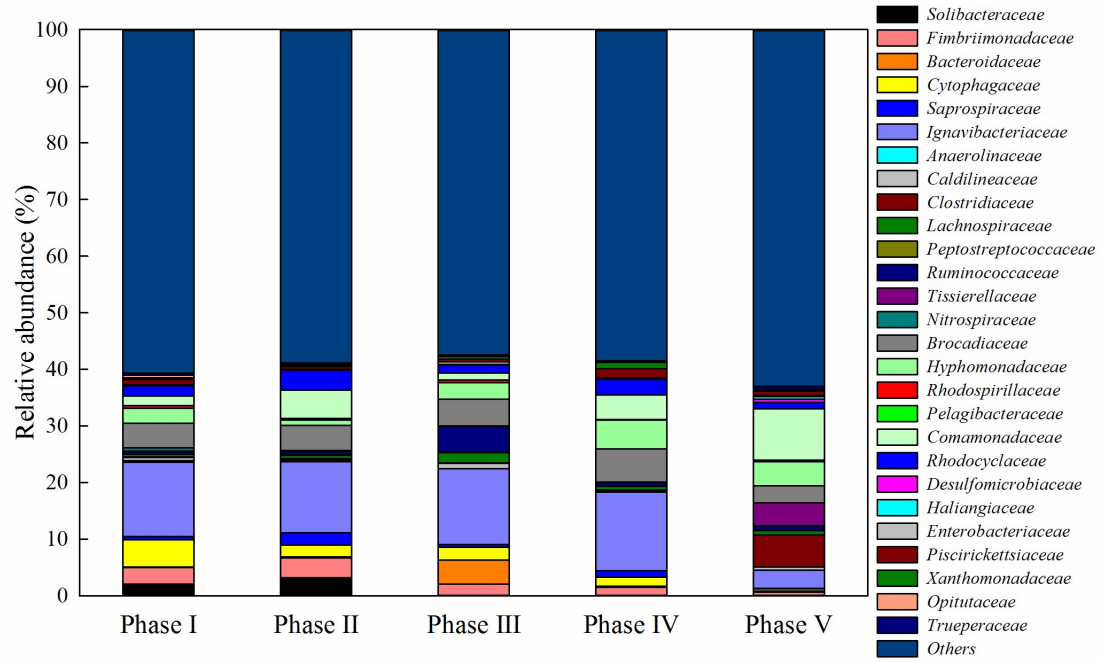




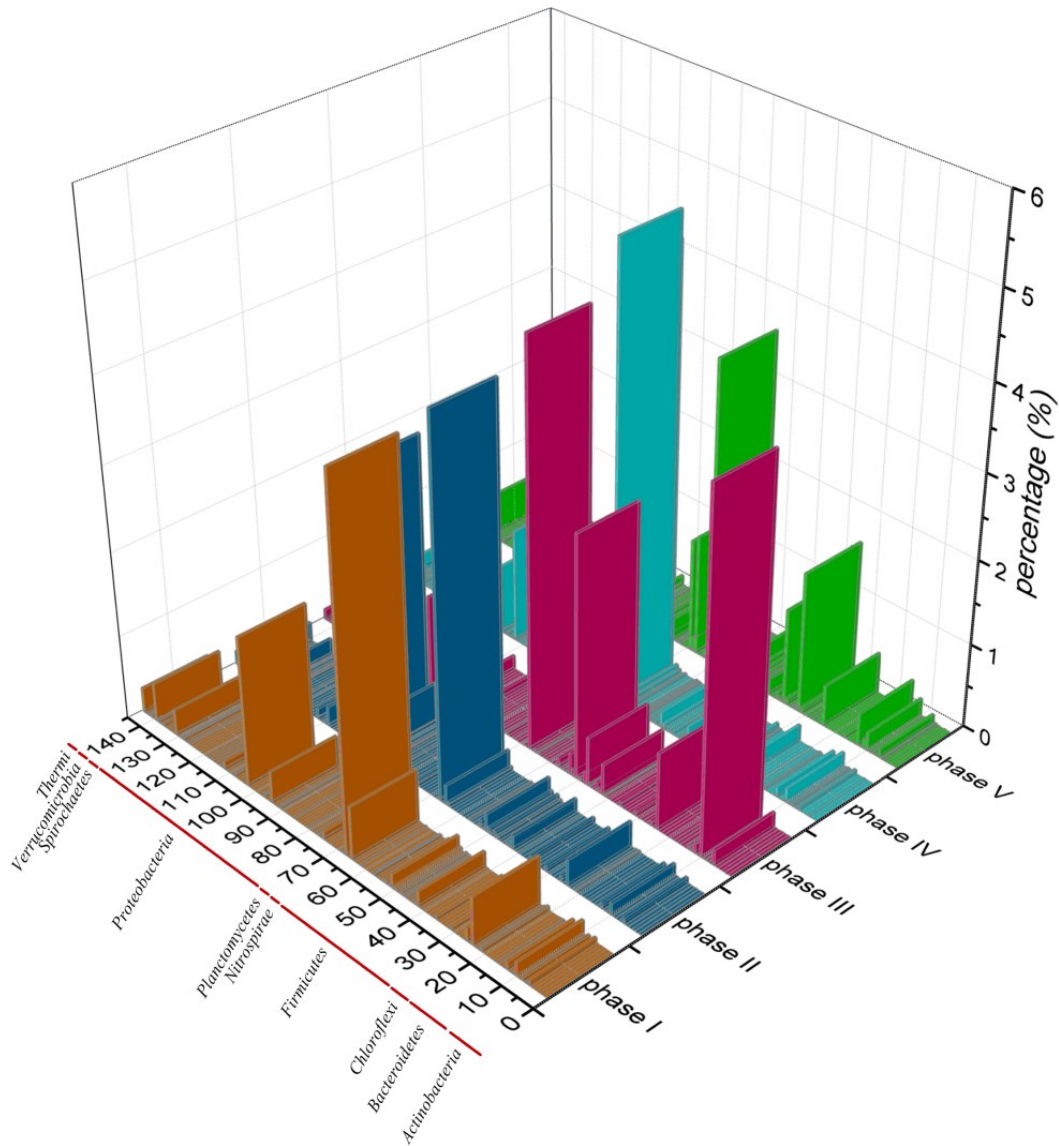
**Figure S3b**



**Figure S3c**



**Figure S3d**



**Figure S3** 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  $> 0.5\%$  frequency in at least one sample. Others refer to the taxa with their maximum abundance  $< 0.5\%$  in any sample.

## References

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