Supplementary materials for

Temperature-induced metabolic reconfiguration and community shifts reveal the mechanistic basis of cold inhibition on anammox consortia

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Text S1 Sample processing and sequencing.

Total genomic DNA extraction of microbial communities was performed in accordance with the instructions of E.Z.N.A.® soil DNA kit (Omega Bio-tek, Norcross, GA, USA). After DNA extraction was completed, the concentration and purity of DNA were detected, and the integrity of DNA was examined by 1% agarose gel electrophoresis. DNA was fragmented by Covaris M220 (Gene Company, China), and fragments of approximately 350 bp were screened for the construction of PE libraries. The library was established using NEXTFLEX Rapid DNA-Seq (Bioo Scientific, USA). Then metagenomic sequencing was performed using the Illumina NovaSeq™ X Plus (Illumina, USA) sequencing platform.

Table S1 Composition of the inorganic solutions and trace elements.

Type	Component	Purity	Manufacturer	Concentration
Inorganic solution (g/L)	NaH_2PO_4	AR	SCR	0.2
	$CaCl_2{\bullet}2H_2O$	AR	SCR	0.113
	$MgSO_4{\bullet}7H_2O$	AR	SCR	1.172
	$NaHCO_3$	AR	SCR	16.8
Trace elements I (g/L)	EDTA	AR	SCR	5
	$FeSO_4{\bullet}7H_2O$	AR	SCR	9.14
Trace elements II (g/L)	EDTA	AR	SCR	15
	$ZnSO_4{\bullet}7H_2O$	AR	SCR	0.43
	$CoCl_2{\bullet}6H_2O$	AR	SCR	0.24
	$MnCl_2{\bullet}4H_2O$	AR	SCR	0.99
	$CuSO_4{\bullet}5H_2O$	AR	SCR	0.25
	$NaMoO_4{\bullet}2H_2O$	AR	SCR	0.22
	$NiCl_2{\bullet}6H_2O$	AR	SCR	0.21
	H_3BO_3	AR	SCR	0.014

Table S2 Primers sequences and annealing temperatures used for gene quantification.

target DNA	Primer sequence (5'-3')	Annealing Temp. (°C)
hdh	F-GGTGGTTTGAGGGGTTCCA	55
	R-TATGGCGACCTCTGTGCATC	
*hzs*A	F-WTYGGKTATCARTATGTAG	55
	R-AAABGGYGAATCATARTGGC	
*nir*S	F-AAATTACTGGCCTCCAAGC	56
	R-TCCACAAGCAGGATGAGTC	
	R: TTYTCRTACCABGTBGC	

Table S3 The diversity index of sludge at different stages.

Sample	Chao	Shannon	Simpson
P1	19469	5.29	0.034
P2	19840	5.50	0.027
P3	18673	5.14	0.032

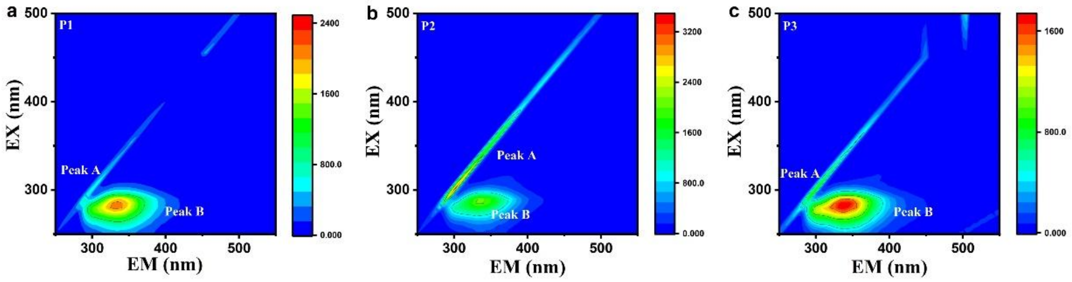

Fig. S1 Three-dimensional fluorescence spectrum of sludge EPS in stages P1 (a), P2 (b) and P3 (c).

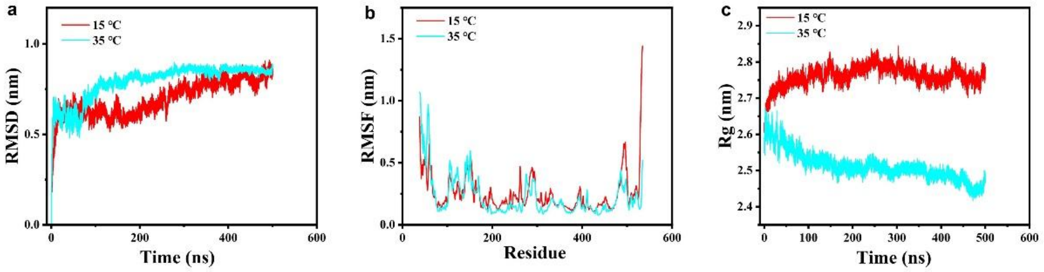

Fig. S2 Molecular dynamics simulation results of hydroxylamine oxidoreductase at 15°Cand 35°C. RMSD plots of protein and their corresponding apo forms over a 500 ns simulation (a). RMSF plots showing per-residue flexibility (b). Radius of gyration (Rg) plots reflecting overall structural compactness (c).

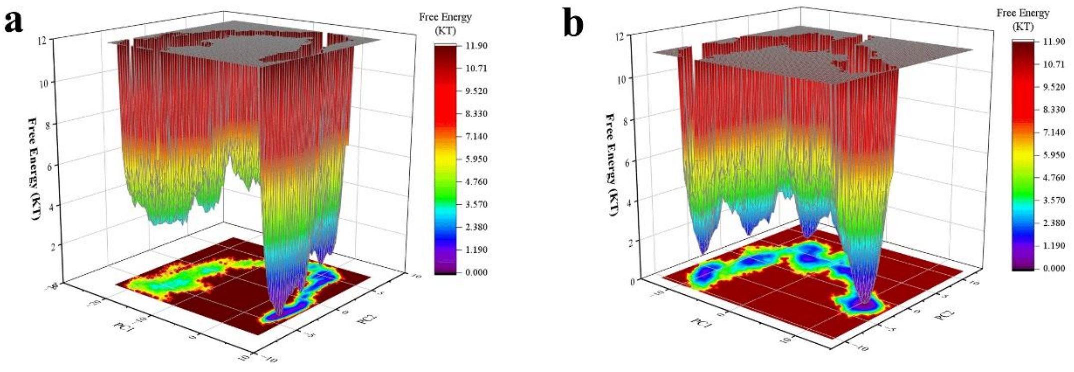

Fig. S3 Three-dimensional free energy landscape of protein based on PCA. 35℃ (a) and 15℃ (b).