

Supplementary Information

Phylogenetically and catabolically diverse diazotrophs reside in deep-sea cold seep sediments

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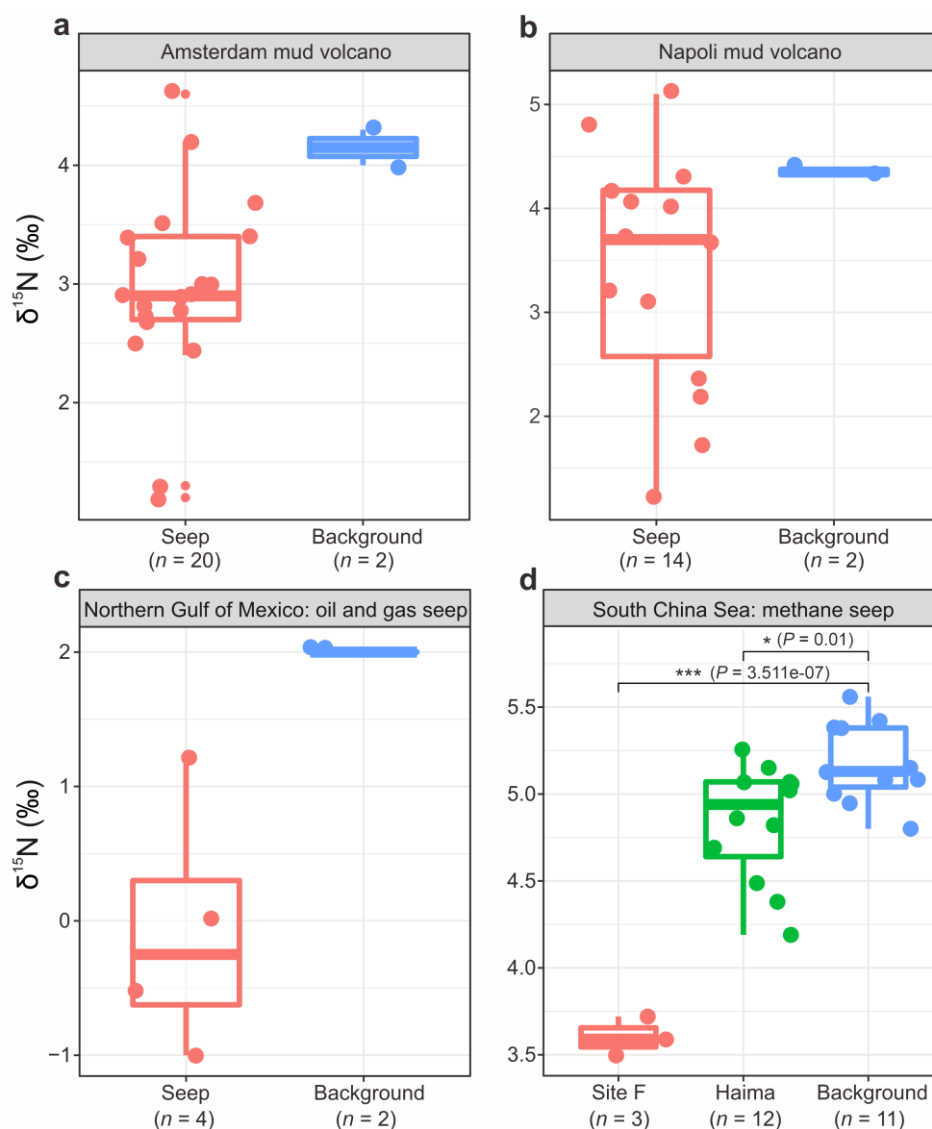
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Supplementary Notes

$\delta^{15}\text{N}$ records of bulk sediment organic matter from published literature

The nitrogenase reaction induces a large degree of nitrogen isotopic fractionation due to the preferred utilization of ^{14}N , resulting in ^{15}N depletion in sedimentary organic nitrogen¹⁻³. On average, lighter $\delta^{15}\text{N}$ values were observed in active seep sites than in reference areas lacking signatures of seepage (Supplementary Figure 1). These trends were significant in the sites where the sample size is sufficient for statistical tests to be performed (Site F and Haima; $P < 0.05$ and $P < 0.001$, two-sided Student's t-test, respectively). Overall, lighter sedimentary $\delta^{15}\text{N}$ values found at various types of cold seeps suggest nitrogen assimilation through microbial nitrogen fixation². It cannot be ruled out that external reduced nitrogen with low $\delta^{15}\text{N}$ signatures, for example from isotopically light ammonium, may have also contributed to these lower values⁴⁻⁶.

Supplementary Figures



Supplementary Figure 1. A literature summary of $\delta^{15}\text{N}$ records of bulk sediment organic matter from five active cold-seep sites. Cold seep sediments are compared to nearby background sites where no seepage signals were detected. Nitrogen stable isotope values are reported in per mil (‰) relative to atmospheric N_2 (air). n values refer to the number of biologically independent samples for statistics analysis. Asterisks indicate statistically significant differences between cold seep and background groups (two-sided Student's t-test; * for $P < 0.05$, ** for $P < 0.01$ and *** for $P < 0.001$). Boxplot components: center line, median values; box limits, upper and lower quartiles; whiskers, 1.5× interquartile range; points, outliers. Additional details on $\delta^{15}\text{N}$ values, cold seep sites and references are presented in Supplementary Data 11.

		MgATP Binding	Cys97 Arg100	Cys132
Rhodopseudomonas_palustris_BisB5 YP_568212.1	I NifH	IAFYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
Rhodospirillum_centenum_SW YP_002299844.1		IAFYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
Rhodospirillum_rubrum WP_011388765.1		IAFYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
Chlorobium_phaeobacteroides_BS1 YP_001960150.1		IAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
Desulfobulbus_propionicus_1pr3_DSM_2032 WP_015725710.1	II NifH	VAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
Desulfocapsa_sulfexigens WP_015402616.1		VAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
HMR23_11 00880		-----	PGVGCAGRGVIT	GDVVCGGFA
SF_Co_bin_98 01		VAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
SF_GA-4_7 00183		IAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
HMR17_21 01025		IAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
HMR_13C2018 007		IAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
SF_Co_bin_152 0		IAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
SF_Co_bin_125 0		IAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
SMM_sbin_10 003		IAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
HMR_13C1559 005		IAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
SB_cbin_23 0127		IAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
SB_sbin_7_50 00		IAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
WGoM_cbin_51 00		IAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
HM_cbin_48 0236		IAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
HMR_5C828 01330		VAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
WGoM_cbin_8 018		IAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
Rhodopseudomonas_palustris_DX-1 YP_004109434.1	III VnfH & AnfH	VAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
Rhodomicrobium_vannielii_ATCC_17110 YP_004013647.1		VAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
Methanothermobacter_marburgensis YP_003849068.1		IGIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
SB_sbin_5_11 00		IGIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
SB_sbin_5_3 003		IGIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
WGoM_cbin_46 00		IAFYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
SF_Co_bin_55 01	MSL NifH	IAFYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
HMR23_7 00565		VAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
HMR26_13 00857		VAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
SF_HDNA3_18 000		-----	PGVVCAGRGVIT	GDVVCGGFA
MS_sbin_11 0098		VAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
HM_cbin_52 0036		IAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
SB_sbin_5_18 00		VAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
SB_sbin_6_17 00		VAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
HM_sbin_21_2 01		IAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
HMR20_26 00145	VII NifH	-----	-----	-----
JL_TianS7_2 008		IAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
SB_sbin_4_35 01		IAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
SB_sbin_7_17 01		IAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
HY4_Co_17 00502		-----	PGVGCAGRGVIT	GDVVCGGFA
SMM_sbin_21 001		IAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
SB_sbin_8_44 00	IV-A NifH	IAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
SB_sbin_3_3 010		IAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
Endomicrobium_proavitum AKL98072.1		IAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
Paenibacillus_riograndensis WP_020429374.1	IV-D CfbC	IAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
Clostridium_ljungdahlii WP_063557083.1		IAIYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
Methanopyrus_kandleri WP_011019784.1		IAVYGKGGIGKST	PGVGCAGRGVIT	GDVVCGGFA
Methanosarcina_acetivorans WP_011023535.1	V BchX & ChlL	VAIYGKGGIGKSS	PGIGCAGRGVIT	GDIVCGGFV
Methanosarcina_mazei_Go1 WP_011032466.1		IAIYGKGGIGKSS	PGIGCAGRGVIT	GDIVCGGFV
Rhodospirillum_rubrum WP_011390728.1		IAIYGKGGIGKSF	VGRGCGGRGIIH	GDVVCGGFG
Rhodobacter_capsulatus WP_013066431.1		IAIYGKGGIGKSF	VGRGCGGRGIIH	GDVVCGGFG
Rhodobacter_capsulatus WP_013066406.1		FSVYGKGGIGKST	AGTGCGRGYVVGQ	GDVVCGGFA

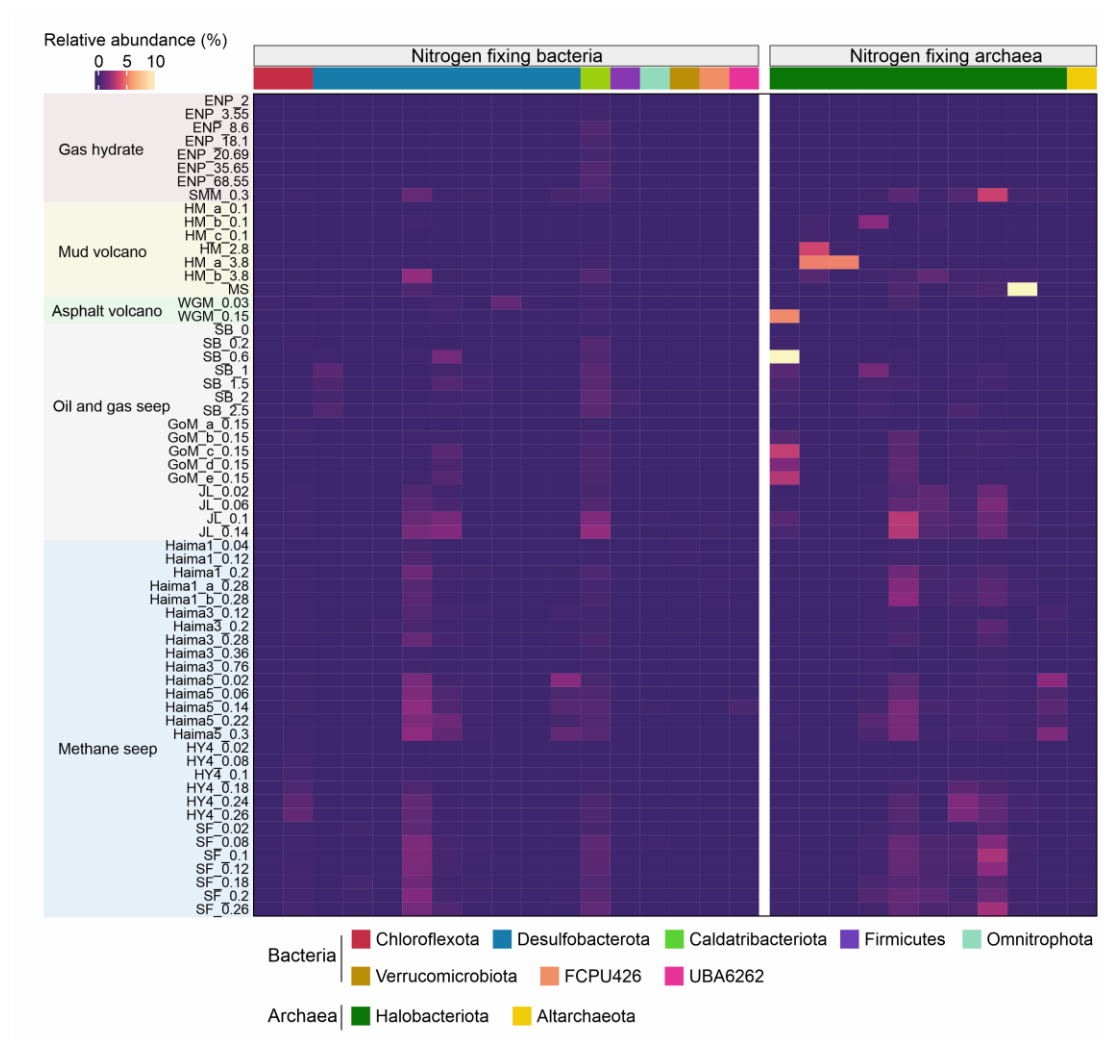
Supplementary Figure 2. NifH superfamily amino acid alignment. Pairwise alignments of NifH superfamily sequences are shown in the region of active site residues responsible for MgATP binding and hydrolysis Fe4-S4 iron sulfur cluster binding. MSL: *Methanosarcina*-like group.

		P-cluster ligand			FeMoco ligand	
		Cys62	Cys88	Cys154	Cys275	His 442
Rhodopseudomonas palustris_BisB5 YP_568213.1	I NifD	G C A Y A G	P V G C G Q	Q S E C P I	L H C Y R S	R Q M H S W
Rhodospirillum centenum_SW YP_002299843		G C A Y A G	P V G C G H	Q S E C P I	I H C Y R S	R Q M H S W
Rhodospirillum rubrum WP_011388766.1		G C A Y A G	P V G C G Q	Q S E C P I	I H C Y R S	R Q M H S W
Chlorobium phaeobacteroides_BS1 YP_001960154.1	II NifD	P A K T C G	S Q G C C S	H S T C L S	F E L K S T	R V G H S Y
Desulfotulbus propionicus 1pr3 DSM_2032 WP_015725713.1		G C T Y A G	P I G C S F	F A T C P V	V M C H R S	K Q L H S Y
Desulfocapsa sulfexigens WP_015402619.1		G C T Y A G	P I G C S F	F A T C P V	V M C H R S	K Q L H S Y
HMR_5C828 01333		G C C Y A G	P V G C A Y	F A T C P V	V M C H R S	K Q L H S Y
WGoM_cbin_8 017		-----	-----	-----	-----	K Q L H S Y
HM_cbin_48 0236		G C C Y A G	P I G C G F	-----	-----	-----
WGoM_cbin_51 00		G C C Y A G	P I G C G F	F A T C P V	I M C Y G S	K Q L H S Y
SB_sbin_7_50 00		G C C Y A G	P V G C G F	F A T C P V	-----	-----
SB_cbin_23 0127		G C C Y A G	P I G C G F	F A T C P V	-----	-----
HMR_13C1559 005		G C C Y A G	P I G C G F	F A T C P V	I M C H R S	K Q L H S Y
SMM_sbin_10 003		G C C Y A G	P I G C G F	F A T C P V	I M C H R S	K Q L H S Y
SF_Co_bin_152 0		G C C Y A G	P I G C G F	F S T C P V	I M C H R S	-----
SF_Co_bin_125 0		G C C Y A G	P I G C G F	F S T C P V	I M C H R S	K Q L H S Y
HMR_13C2018 007		G C C Y A G	P I G C G F	F A T C P V	I M C H R S	K Q L H S Y
HMR17_21 01022		G C T Y A G	P I G C S F	F S T C P V	V Q C H R S	-----
SF_GA-4_7 00186		G C T Y A G	P I G C S F	F A T C P V	V M C H R S	K Q L H S Y
SF_Co_bin_98 01		G C T Y A G	P M G C S F	F A T C P V	V M C H R S	K Q L H S Y
HMR23_11 00877		G C T Y A G	P I G C A F	F A T C P V	V M C H R S	K Q L H S Y
Rhodopseudomonas palustris_DX-1 YP_004109435.1	III VnfD & AnfD	G C A Y C G	P V G C T Y	Y Q T C A T	L E C A R S	L N A H A Y
Rhodomicrobium vannielii_ATCC_17100 YP_004013646.1		G C A Y C G	P V G C T Y	Y Q T C A T	L E C A R S	L N A H A Y
Methanothermobacter marburgensis YP_003849071.1		G C A F A G	P V G C T A	Y A T C T T	V R C Q R S	I L I H S Y
SB_sbin_5_11 00		G C A F A G	P A G C A W	Y T T C T S	L H C Q R S	V N S H S Y
SB_sbin_5_3 003		G C A F A G	P A G C A W	Y T T C T S	L H C Q R S	V N S H S Y
WGoM_cbin_46 00	MSL NifD	G C A F A G	P V G C A W	Y T T C T T	V H C Q R S	V N S H S Y
SF_Co_bin_55 01		G C A F A G	P V G C A W	F T T C T T	V H C Q R S	V N S H S Y
SB_sbin_5_18 00		G C A F A G	P I G C A Y	F A T C P V	L M C H R S	R Q I H S Y
SB_sbin_6_17 00		G C A F A G	P I G C A Y	F A T C P V	L M C H R S	R Q I H S Y
HM_sbin_21_2 01		G C A F A G	P I G C A Y	F A T C P I	L L C H R S	R Q M H S Y
HM_cbin_52 0036		G C A F A G	P I G C A Y	S A T C P V	L L C H R S	R Q I H S Y
MS_sbin_11 0098		G C T F A G	P I G C S Y	F A T C P V	L L C H R S	R Q I H S Y
HMR23_7 00568	VII NifD	G C S Y A G	P I G C G Y	F A T C P V	L L C H R S	R Q I H S Y
SF_HDNA3_18 000		G C S Y A G	P I G C G Y	F A T C P V	L L C H R S	R Q I H S Y
HMR26_13 00630		-----	-----	-----	-----	R Q I H S Y
SB_sbin_3_3 010		G C T Y A G	P I G C A Y	Y A T C A I	M H C Q R S	V N G H A Y
SB_sbin_4_35 01		G C T Y A G	P I G C A Y	Y A T C V I	M H C Q R S	V N G H A Y
SB_sbin_7_17 01		G C T Y A G	P I G C A Y	Y A T C V I	M H C Q R S	V N G H A Y
SB_sbin_8_44 00		G C T F A G	P I G C A Y	Y A T C V I	M H C Q R S	V N G H A Y
HY4_Co_17 00505	IV-A NifD	G C T Y A G	P I G C A Y	Y A T C V I	M H C Q R S	V N G H A Y
HMR20_26 00142		G C T Y A G	P I G C A Y	Y A T C V I	M H C Q R S	V N G H A Y
JL_TianS7_2 008		G C T Y A G	P I G C A Y	Y A T C V I	-----	-----
SMM_sbin_21 001	IV-D CfbD	G C T Y A G	P I G C A F	Y A S C V I	M H C Q R S	V N G H S Y
Paenibacillus riograndensis WP_020429361.1		Q C M G C S	P V G C A G	T T S C A S	Q I C P T L	L L I G D E
Endomicrobium proavitum AKL98064.1		Q C L G C S	P V G C A G	T T T C A S	Q I C S T L	L L I G D E
Clostridium ljungdahlii WP_063557082.1	V BchY & ChlL	Q C T E C S	P L G C S A	T T S C A S	V C P T L	L L I G D E
Methanopyrus kandleri WP_011019753.1		P G P I P A	P K G C C F	V G T C V S	-- G K R G	-----
Methanosarcina acetivorans WP_011023536.1		P S S I V A	P P G C S F	V G T C A S	A L G K K E	-----
Methanosarcina mazei Go1 WP_011032467.1		P S S I V A	P P G C S F	V G T C A S	A L G K K E	-----
Rhodobacter capsulatus WP_013066409.1		F C G L T S	S R T C A H	V G S C P S	L A Q P E L	K W A I E L
Rhodobacter capsulatus WP_013066432.1		M C P A F G	S A C C V Y	I N L C V P	A I H --	L Y F T N L
Rhodospirillum rubrum WP_014626485.1		M C P A F G	S A C C V Y	T N L C V P	A I H --	L Y F T N L

Supplementary Figure 3. NifD superfamily amino acid alignment. Pairwise alignments of NifD superfamily sequences are shown in the region of active site residues responsible for coordination of the P-cluster and FeMo-cofactor within the molybdenum nitrogenase subunit NifD, and substrate bound to the FeMo-cofactor. MSL: *Methanosarcina*-like group.

		P-cluster ligand			
		Cys70	Cys95	Cys153	
Rhodopseudomonas_palustris_BisB5 YP_568214.1	I NifK	PAKACQPLGAVF	HGSQGCVAYY-R	VSTTCMAEVI	
Rhodospirillum_centenum_SW YP_002299842.1		PVKACQPLGAVF	HGSQGCVAYY-R	VSTTCMAEVI	
Rhodospirillum_rubrum WP_011388767.1		PNKACQPLGALF	HGSQGCVAAYF-R	VCTSCMAEVI	
Chlorobium_phaeobacteroides_BS1 YP_001960153.1	II NifK	SQRGCSYAGCKG	HGPIGCSFYA--	IFSTCPVGLI	
Desulfobulbus_propionicus_1pr3_DSM_2032 WP_015725714.1		PAKTCQPIGAMY	HGSQGCCAYH-R	VHTTCLSETI	
Desulfocapsa_sulfixigens WP_015402620.1		PAKTCQPIGAMY	HGSQGCCAYH-R	VHTTCLSETI	
HMR23_11 00876		PAKTCQPIGAMY	HGSQGCCAYH-R	-----	
SF_Co_bin_98 01		PAKTCQPIGAMY	HGSQGCCAYH-R	VHTTCLSETI	
SF_GA-4_7 00187		PAKTCQPIGAMY	HGSQGCCAYH-R	VHTTCLSETI	
HMR17_21 00178		PAKTCQPIGAMY	HGSQGCCAYH-R	VHTTCLSETI	
HMR_13C2018 007		ACKVCAPLGASL	HGSQGCATYI-R	IATTCCLSETI	
SF_Co_bin_125 0		PAKACQPIGAMY	HGSQGCCAYH-R	VHTTCLSETI	
SMM_sbin_10 003		PAKTCQPIGAMY	HGSQGCCAYH-R	VHTTCLSETI	
HMR_13C1559 005		PAKTCQPIGAMY	HGSQGCYSYH-R	VHTTCLSETI	
SB_cbin_23 0127		PAKTCQPIGAMY	HGSQGCYSYH-R	VHTTCLSETI	
WGoM_cbin_51 00	III VnfK & AnfK	PAKTCQPIGAMY	HGSQGCYAYH-R	VHTTCLSETI	
HM_cbin_48 0226		PAKTCQPIGAMY	HGSQGCYAYH-R	VHTTCLSETI	
WGoM_cbin_8 017		PAKTCQPIGAMY	HGSQGCYAYH-R	VHTTCLSETI	
HMR_5C828 01334		PAKTCQPIGAIIY	HGAQGCCAYH-R	IHTTCLSETI	
Rhodopseudomonas_palustris_DX-1 YP_004109437.1		PIFTCQPAQAQY	HGGQGCVMFV-R	IIITCSTEVI	
Rhodomicrobium_vannielii_ATCC_17100 YP_004013644.1		PIFTCQPAQAQF	HGGQGCVMFV-R	IIITCSTEVI	
Methanothermobacter_marburgensis YP_003849072.1		PLVTCQPFAGMF	HGSQGCSTFV-R	IVTTCSEII	
SB_sbin_5_11 00		PGRICMPFGAMW	QGAQGCATYP-R	VITVCASEII	
SB_sbin_5_3 003		PGRICMPFGAMW	QGAQGCCTTYP-R	IIITVCSEII	
WGoM_cbin_46 00		PGRICMPLGAML	QGAQGCCTTYP-R	VVITTCSEII	
SF_Co_bin_55 01		PGNTCMPFGAMW	QGAQGCATYP-R	VVITTCSEII	
HMR23_7 00569	MSL NifK	PAKICMPIGAAF	HGSQGCCLSYL-R	VHTTCVAETI	
HMR26_13 00631		PAKICMPIGAAF	HGSQGCCLSYL-R	VHTTCVAETI	
SF_HDNA3_18 000		PAKICMPIGAAF	HGSQGCCLSYL-R	VHTTCVAETI	
MS_sbin_11 0098		PAKICMPIGASF	HGSQGCCLSYL-R	VHTTCVAETI	
HM_cbin_52 0037		PAKICQPIGAVY	HGSQGCCLSYL-R	IHTTCVAETI	
SB_sbin_5_18 00		PAKICMPIGATL	HGSQGCCLSYL-R	VHTTCVAETI	
SB_sbin_6_17 00		PAKICMPIGATL	HGSQGCCLSYL-R	VHTTCVAETI	
HM_sbin_21_2 01		PAKICQPIGAEL	HGSQGCCLSYL-R	IHTTCSEII	
SB_sbin_3_3 010	VII NifK	PAKMCQPIGAMY	HGSQGCATYPMR	VITTCCLSETI	
SB_sbin_4_35 01		PGMLCQPIGAMQ	HGSQGCAYPMR	VITTCCLSETI	
SB_sbin_7_17 01		PGILCQPIGAMQ	HGSQGCAYPMR	VITTCCLSETI	
HY4_Co_17 00506		PGMLCQPIGAMQ	HGSQGCAYPMR	VITTCCLSETI	
SB_sbin_8_44 00		PGMLCQPIGAMQ	HGSQGCAYPMR	VITTCCLSETI	
HMR20_26 00141		PGMLCQPIGAMQ	HGSQGCAYPMR	VITTCCLSETI	
SMM_sbin_21 001		PGMICQPIGAMQ	HGSQGCAYPMR	VITTCCLSETI	
Endomicrobium_proavitum AKL98065.1	IV-A NifK	QRFMC-AIGAIQ	HSGPGCGTMV-Q	ILTGCTSAIV	
Paenibacillus_riograndensis WP_046504163.1		PRYSC-ALGVQQ	HAGPGCSTKI-H	VLTGCTSDIV	
Clostridium_ljungdahlii WP_063557118.1		PRHFC-ALGQQQ	HSGPGCSYKLFER	VLTGCTADIV	
Rhodobacter_capsulatus WP_013066408.1	V BchZ & ChlB	TYEGPPHVGAMR	HGPGGDTYA--D	VALTCTAELL	
Rhodospirillum_rubrum WP_011390726.1		HDRAGGYWGAVY	DGPVGCENLPVT	VVTGSIAEMI	
Rhodobacter_capsulatus WP_013066433.1		HDRAGGYWGAVY	DGPVGCENLPVT	VVTGSIAEMI	

Supplementary Figure 4. NifK superfamily amino acid alignment. Pairwise alignments of NifK superfamily sequences are shown in the region of active site residues responsible for coordination of the P-cluster within the molybdenum nitrogenase subunit NifK. MSL: *Methanosarcina*-like group.



Supplementary Figure 5. Relative abundance of dereplicated nitrogen-fixing MAGs across various cold seep sediment samples. Details on the abundance of nitrogen-fixing MAGs can be found in Supplementary Data 5.

References

1. Zhang, X., Sigman, D.M., Morel, F.M. & Kraepiel, A.M. Nitrogen isotope fractionation by alternative nitrogenases and past ocean anoxia. *Proc Natl Acad Sci U S A* **111**, 4782-4787 (2014).
2. Wada, E. & Hattori, A. Natural abundance of ^{15}N in particulate organic matter in the North Pacific Ocean. *Geochim Cosmochim Acta* **40**, 249-251 (1976).
3. Zerkle, A.L., Junium, C.K., Canfield, D.E. & House, C.H. Production of ^{15}N -depleted biomass during cyanobacterial N_2 -fixation at high Fe concentrations. *J Geophys Res* **113** (2008).
4. Philippi, M. et al. Purple sulfur bacteria fix N_2 via molybdenum-nitrogenase in a low molybdenum Proterozoic ocean analogue. *Nat Commun* **12**, 4774 (2021).
5. Vo, J., Inwood, W., Hayes, J.M. & Kustu, S. Mechanism for nitrogen isotope fractionation during ammonium assimilation by *Escherichia coli* K12. *Proc Natl Acad Sci U S A* **110**, 8696-8701 (2013).
6. Hu, Y. et al. A prominent isotopic fingerprint of nitrogen uptake by anaerobic methanotrophic archaea. *Chem Geol* **558**, 119972 (2020).