Supplemental Information titles and legends

Data S1. Sequence alignment of denitrification enzymes in *Globobulimina*. Related to Figures 2–4. Multiple sequence alignment of representative protein sequences of *Globobulimina* Nrt, NirK and Nor (shown in bold) with homologs obtained from literature. Functional associated domains obtained by CD search of individual sequences are highlighted in different colours. **A)** Two types of Nrt with homologs from literature. The typical prokaryotic NarK superfamily domain is present in all of them. Note that although the Nterminus of Nrt II is incomplete it has an extended length in comparison to the other homologs (Nrt I: GloG15_CONTIG80510:375-855,926-2061(+); Nrt II: GloG15_CONTIG110211:243-1504,1578-2146(-)). **B)** Two representative NirK sequences and corresponding homologs from literature. The cupredoxin domains I and II are represented in grey and blue, respectively. Conserved copper ligand binding sites are highlighted in green. Furthermore, amino acid residues at predicted splice site positions on the corresponding genome contig sequences are shown with a black background, and nucleotides surrounding individual splice sites are mentioned above. Nucleotides flanking splice sites are shown by sequence outlines starting with the 3'-end of the exon before (i.e., exon|intron|exon). Splice sites were mapped from genomic splice sites prediction obtained for associated genomic contigs using BRAKER tool (GloG15_CONTIG58586 for NirK I and GloG15_CONTIG57448 for NirK II). One splice site shown in brackets relies on manual prediction based on the break in sequence similarity of the transcript in comparison to the genomic contig and presence of canonical nucleotides found and splice sites. (NirK I: GloT15_NODE_12185:141-2504(-); NirK II: GloT15_NODE_4197:1119-3623(+)). **C)** Two types of Nor with homologs from Nor and Nod candidates. The typical Cytochrome c oxidase domain superfamily domain is present in all homologs. Conserved amino acid residues of the catalytic site are highlighted in red for Nod candidates and in green for NorZ or both enzyme types. *Globobulimina* Nor exhibits typical conserved residues found for Nod (Nor I: GloG15_CONTIG49717:890-3289(+); Nor II: GloT15_NODE_10819:151-2556(+)

Data S2. Detailed phylogenies of denitrification enzymes identified in *Globobulimina*. Related to Figures 2–4. Complete phylogenies of denitrification enzymes identified in the current study and homologs from public databases. Colour coding of the terminal sequence names denote homologs obtained from genomics (GloG15: red) and transcriptomics (GloT14: green; GloT15: blue) assemblies or from public protein sequence databases (black). The background colour coding indicates different taxonomic groups. Different protein families are given at the tree branches. Note that sequences identified in *Globobulimina* can be

distinguished from associated bacteria by being binned into the *Globobulimina* draft genome (as indicated by #) and forming a clade with the corresponding transcripts obtained from transcriptome data. Original query sequences used to find homologs in sequencing assemblies are highlighted with asterisks (*). Numbers at the branches indicate bootstrap support (BS) relying on 100 replicates, and the scale bar refers to substitutions per side. Thick branches mark the root neighbourhood as inferred with MAD. The branches in the root neighbourhood were identified as following: i) branch ancestral deviations (r) were calculated with MAD, and the minimal value was determined (r_{min}), ii) the ambiguity index of branches in respect to rmin, calculated as AI = r_{min}/r_n, was inferred, iii) branches having an AI > 0.95 are considered as the root neighbourhood. **A)** Nitrate/nitrite transporter (Nrt) protein. The tree shows two diverse clades supported by *Globobulimina* genomics and transcriptomics. The other clades are only supported by genomics, indicating representation by associated bacteria only. Nrt shown in the current study represents the NNP family of transporters or NarK protein superfamily belonging to the MFS transporter family. The clade associated with *Globobulimina* clade I contains eukaryotic Nrt protein (e.g., Nrt2.1 and Nrt2.4 found in *Arabidopsis thaliana*; O82811 and Q9FJH8, respectively), while the clade containing *Globobulimina* clade II is nearly exclusively bacterial (e.g., containing NarK and NarU from *Escherichia coli*; accessions P10903 and P37758, respectively). Homologs of transcriptome assemblies that group with *Chlorella* and *Symbiodinium* are likely contamination as they were not sorted into the *Globobulimina* draft genome, and the corresponding transcript contigs are lowly represented. Rooting was performed using representatives of DHA14 and ACS family of MFS transporters as outgroup. **B)** Copper-containing nitrite reductase (NirK). The tree shows two monophyletic clades of *Globobulimina* homologs grouping with type Class II of NirK. Both of them group with a prokaryotic clade containing archaea, albeit the BS is only 62. Although eukaryotic homologs are present, they form a distinct cluster. Note that NirK homologs previously proposed in association with denitrification of endobiotic origin in other foraminifera species [S9,S11] (highlighted by black circles) do not cluster with any homolog (neither *Globobulimina* nor associated bacteria) identified in the current study. The tree was rooted with MAD. **C)** Cytochrome cd1-containing nitrite reductase (NirS). The tree shows no homologs of the *Globobulimina* draft genome indicating a sole representation by associated bacteria. The most abundant sequences are related to *Colwellia psychrerythraea*, although overall a diversity of taxa is observed. Note that NirS homologs previously proposed in association with denitrification of endobiotic origin in other foraminifera by Bernhard and colleagues (highlighted by black circles; [S9,S11]) do not group closely with any homolog identified in the current study. Rooting was performed by applying the MAD method. D) Nitric oxide reductase (Nor). The tree shows two distinct clades representing NorB and NorZ protein families. While NorB is restricted to homologs found in genomics and, therefore, represents

associated bacteria, NorZ contains a *Globobulimina*-associated clade supported by *Globobulimina* genomics as well as transcriptomics. Only one homologous transcript was found outside of this clade, but as it exhibited only a low level of transcription, it probably represents contamination. The tree was rooted using two cbb3 oxidases as outgroup. **E)** Prokaryotic periplasmic nitrate reductase (NapA). The tree shows only homologs from associated bacteria (i.e. genome data not allocated to the *Globobulimina* draft genome). The most represented genes group with homologs of *Vibrio* and *Photobacterium*. Although the clades at the top group with the NapA paralogs NarB and NasA, they were still included due to difficulties to clearly distinguish these paralogs from NapA orthologs. MAD rooting was applied, and paralogs were distinguished from orthologs using NarB and NasA outgroup sequences. Rooting of the final phylogeny is based on the MAD method. **F)** Membranebound nitrate reductase (NarG). The tree shows that only homologs in the genomics data were identified indicating the absence from the *Globobulimina* draft genome and sole representation of associated bacteria. Sequences form three clades. While automatic annotations from NCBI indicate a NarG function of all of them, only one clade contains protein sequences obtained from literature. The most abundant genes group with two sequences of Nitrospirae, although not closely. Rooting was performed by applying the MAD method. **G)** Nitrous oxide reductase (NosZ). Only the homologs from associated bacteria are represented. The most abundant genes cluster with *Colwellia psychrerythraea*. Rooting was performed by applying the MAD method. **H)** Eukaryotic assimilatory nitrate reductase (Nr) and paralogs. All Nr homologs of the *Globobulimina* draft genome form a clade with sulfite oxidases, albeit with a low BS of 40. The clade of Nr from literature is a sister clade to eukaryotic sulfite oxidases. Most other clades represent prokaryotic specific taxa as indicated by the absence of transcriptomics homologs and *Globobulimina* draft genome classification.

Three transcript sequences branch distantly with Nr proteins but are neither supported by genomics nor do they show the typical functional domain structure of Nr (See Figure S3). The tree was rooted using the MAD method. **I)–K)** *Globobulimina* Nrt, NirK and Nor phylogeny (respectively) including foraminifera homologs from public databases. The trees were rooted at the corresponding branches determined for phylogenies without homologs from public databases. The topology of foraminifera homologs derived from public databases is highlighted by arrows.

Table S4. Denitrification protein catalogue from literature and public databases. Related to Figures 2–4.

Table S5. IDs of homologs removed for phylogenetic reconstructions. Related to Figures 2–4.

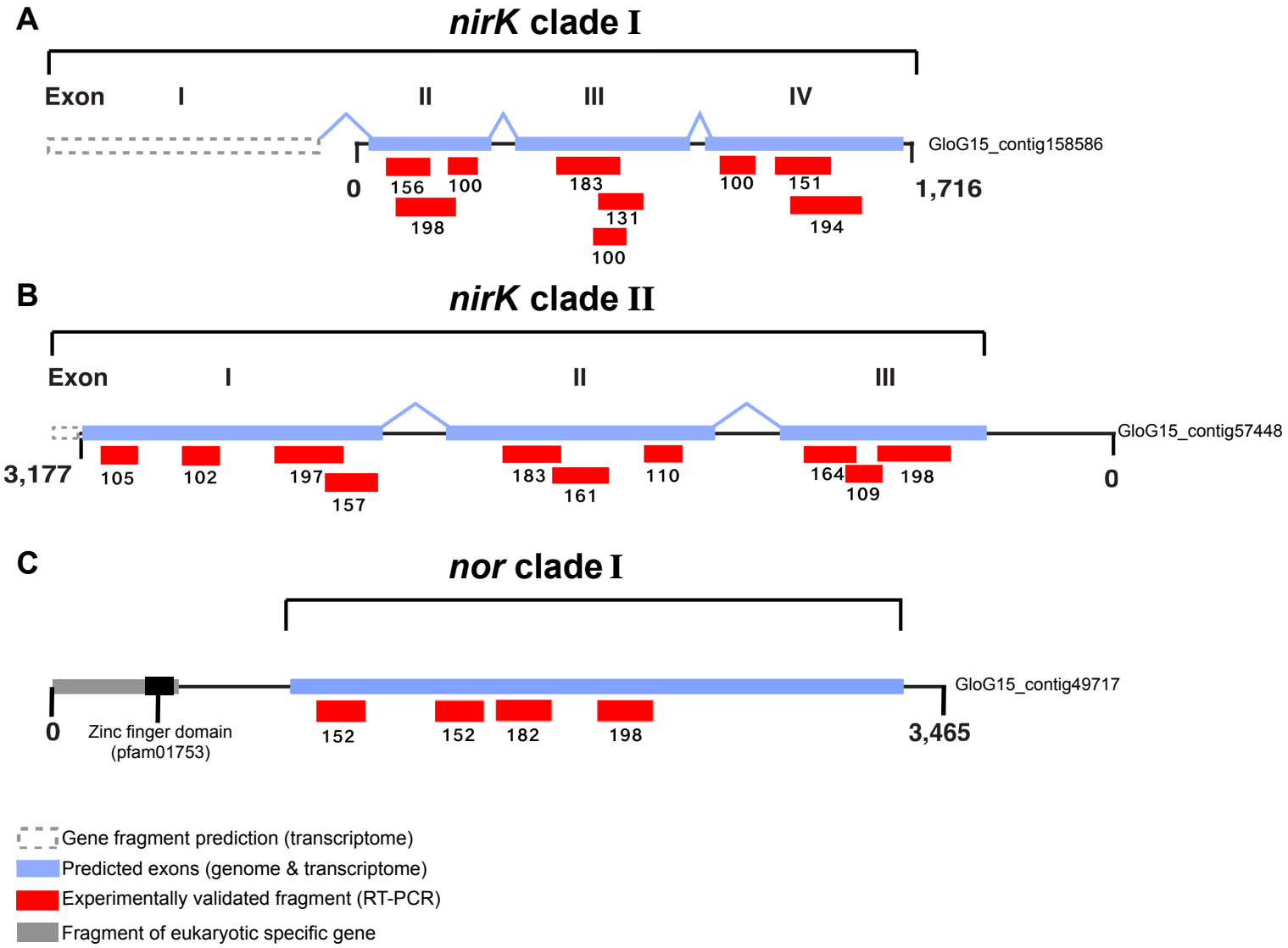

Figure S1. Validation of denitrifying gene sequences. Related to Figures 3–4. Schematic representation of validated gene fragments (red) and their correpsonding size (numbers below fragments) for **A)** *nirK* clade I, **B)** *nirK* clade II and **C)** *nor* clade I.

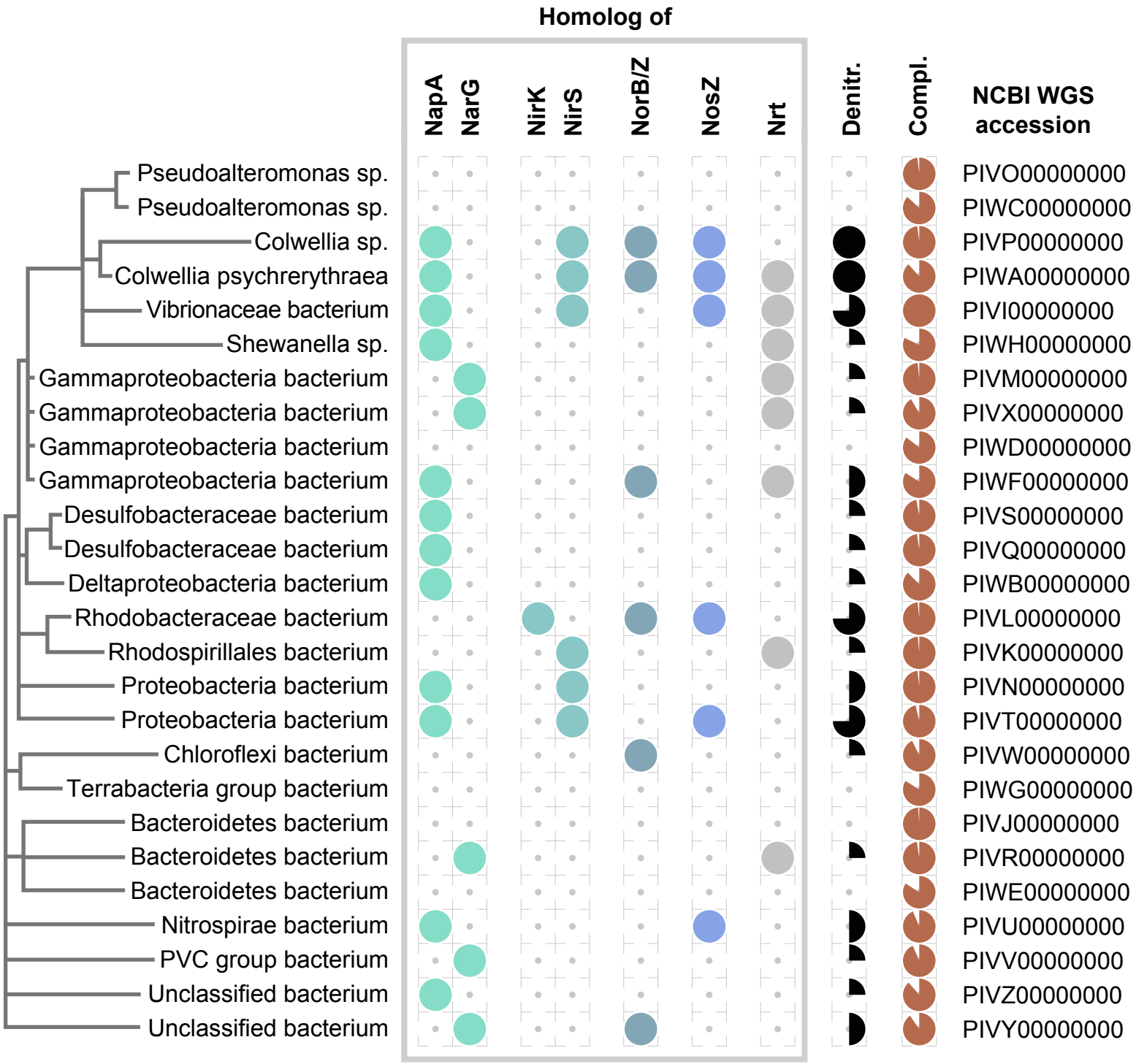

Figure S2. Distribution of denitrification enzymes in *Globobulimina* associated bacterial draft genomes. Related to Figure 5. Presence/absence of denitrification enzyme homologs in different draft genome obtained from genomics data. The proportion of covered denitrification steps and predicted genome completeness is indicated in the "Denitr." and "Compl." columns, respectively. Represented proteins refer to those protein sequences determined by phylogenies and shown in Data S2. The only exception is the NapA homolog of Colwellia psychrerythraea (GloG15_CONTIG22649:3-230(-)). This homolog was replaced during protein clustering to reduce redundancy by GloG15_CONTIG63497:3-2366(-) in Data S2 and is 99% identical to the former.

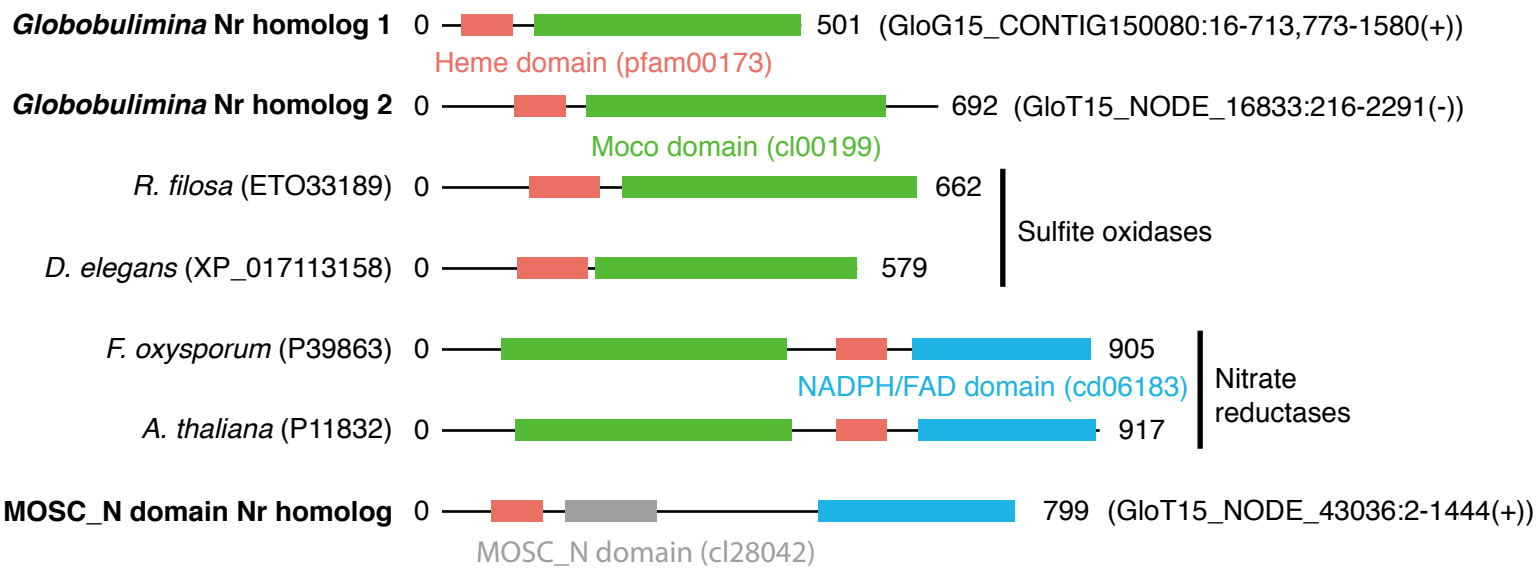

Figure S3. Comparison of protein domain structure of *Globobulimina* Nr homologs, Nr and sulfite oxidase proteins. **Related to Figure 5**. Conserved domain family are highlighted on protein sequences as identified by CD-search. Names and accessions of domains are given in corresponding colour. Domain structure of sulfite reductases and eukaryotic assimilatory nitrate reductases (Nr) differ substantially and the two representative homologs of *Globobulimina* clearly resemble the former. Names of sequences referring to the current study are shown in bold. An additional domain structure containing a 'MOSC_N' domain was observed for an individual cluster of Globobulimina homologs. It is only represented by transcriptome data, hence encoding in the genome of *Globobulimina* is not supported.

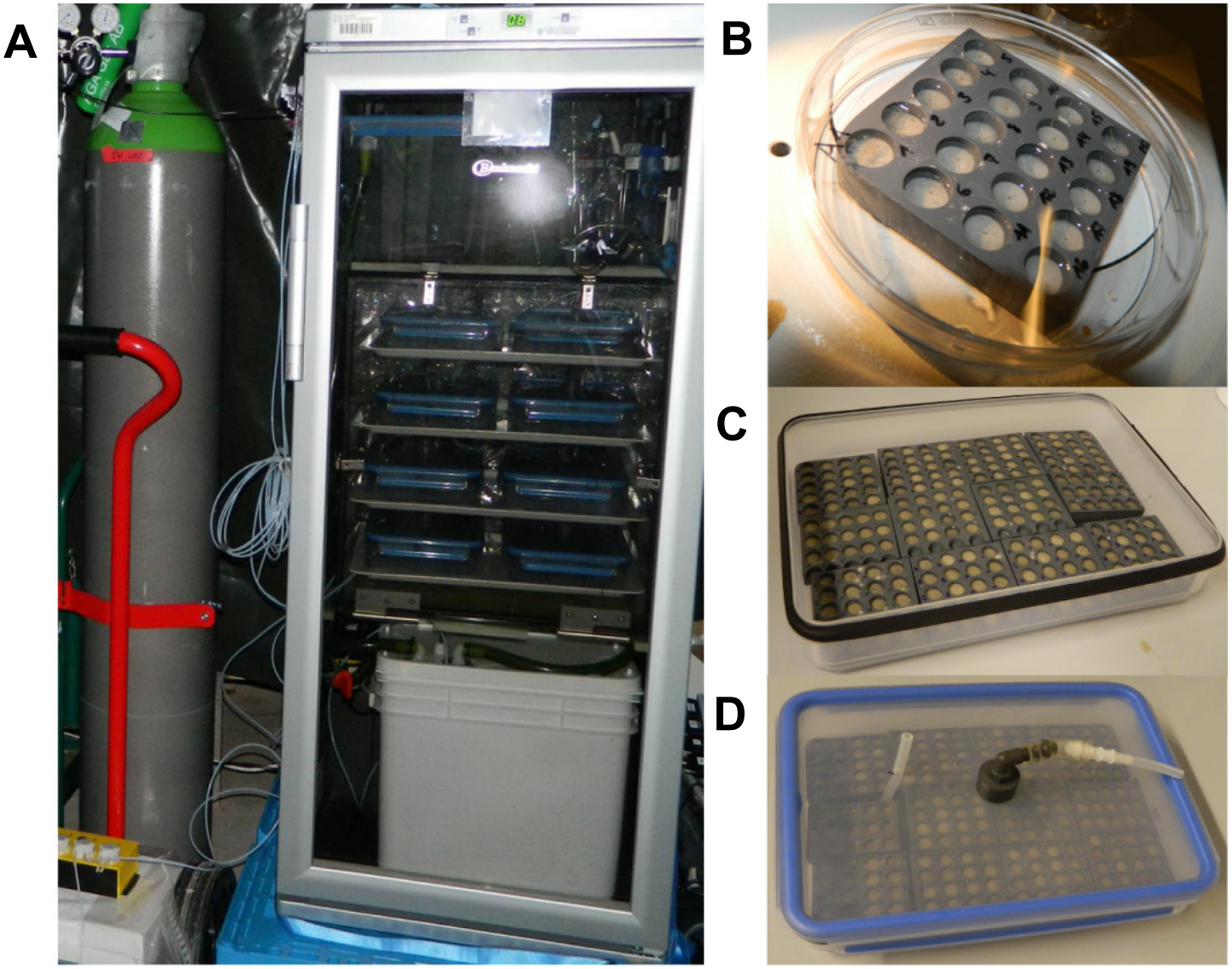

Figure S4. Description of the incubation system. Related to STAR Methods. A) Picked foraminifera were placed in PVC well plate where each well contained sterile quartz sand and one individual. **B)** Well plates were placed in culturing vessels that were filled with equilibrated sterile 30 psu, 20 µmol l-1 NO_3^- ASW **(C–D)**. These culturing vessels were then incorporated to one of two culturing systems assembled to reproduce Alsbäck Deep environmental conditions. One system was dedicated to natural oxygen concentration of the Alsbäck Deep (125 µ mol/l) during summer whereas the second system was completely drawn down of oxygen (<10 µmol/l). Each system was filled with sterile ASW and sparged to oxygen concentration of interests with N_2, CO_2 and O_2 pre-mixed gas (AGA Gas A; gas entries indicated by red arrows) before and during the length of the experiments. Culturing vessels were only added once systems were equilibrated and stable. The bioinformatics analysis of transcriptomes from the two incubation systems did not reveal patterns of significantly different transcript abundances for Nrt, NirK or Nor genes (Using R software package edgeR). Consequently, the genomics and transcriptomics data from the different conditions was merged with the ambient samples for further analysis.

Replicate	Species	Number of specimens	Mean denitrification rate[1]	$1\sigma_{sd}$[1]	$1\sigma_{sem}$[1]	N[2]
1	*G. turgida*	4	250	10.8	4.8	5
2	*G. turgida*	4	63	3.3	1.7	4
3	*G. turgida*	3	220	3.3	1.2	8
4	*G. turgida*	5	28	0.5	0.2	4
5	*G. turgida*	5	1712	10.8	4.8	5
6	*G. turgida*	5	105	1.6	0.7	5
7	*G. turgida*	5	75	2.5	1.2	4
8	*G. turgida*	5	29	1.8	0.9	4
1	*G. auriculata*	4	99	3.3	1.5	5
2	*G. auriculata*	4	46.9	1.0	0.4	5
3	*G. auriculata*	5	124	4.0	1.8	5
4	*G. auriculata*	5	29	0.6	0.3	5

[1] pmol individual^{-1} day^{-1}

[2] number of measurements

Table S1. Denitrification rate measurements for *Globobulimina* spp. Related to Figure 1.

>Sorites sp. Nrt (CAMPEP_0114658376) MCVQSAEGVTFAVVPFVRPNSVGPVAGIVGAGGNVGAMIFAFALFAYVPDNLSYEA AWFILAAFVFIASFSCLLISFTKEETAIANEKMKEIKTDDTVKADDGETTKLTTDAESV VTTSSAGNLTTSPSEGNQSNHNGIEMADL
>Ammonia sp. 'North Sea' Nrt* (CAMPEP_0197046718) METNPNTPKRLLRVGGISVNAHNKAVQFSIFSKCGSFHVSNFWITTVSFWICFFVWF GNLNLLPWITKDDNYGIPLSTAQKSLSATLLTLSTTIFRVIIGDLADKIGSRYCYLLILVC SFIPVCALSFISSPTAYVVLNFFIGIVGASFVITEYHTTQFYVDRLVGLANATAAGWGN FGGGCAAIFMPFLATTVESKYGVNPWRYITFCSSLLLIIPMILYYYFAVDTVNGNLNY QSMVTRKKLLDFSVLWQTVKDSRTWILFAVYMACFGVEITVISFLQEYFRDTYGLSP DRAGLYVFMFSCLNLFARSVGGYGSDILYKKYSIQGRVYILVLTLLAESIFLLIFAFSSF NLGYCVVILLFFSFYVQCAEGITYAIVPFVKIGENKNIGPIYGIVAAGGNAGSVLFAST VFAFTPNVCEQPQLGCERNTISYQTAFIILAMFVGLVAFLSLCIKFTAKEIREADELLM SFLKKHHRAIYGQHEEQHAEQQQTPDTNNNNNSNNNNNNTENAQ
>Rosalina sp. Nrt (CAMPEP_0201568782) MVSNRVVGFAQAITAGFGNFGGGCANSVMPLLLYHFDLSWRVYITILSVILIILAIIYYF GTDDVFQPDNNNLNVKDNNNISSPNQSTTNDNDQAILLQHEESGSISSNNHPHNSN RKFSSSDIDTITAPTHIRGSFVETICDYRVW
>Elphidium margaritaceum Nrt (CAMPEP_0202730370) MPTLARVIENTNDYNAWRVITFASGLLLIIPLCLYYYFAVDTIEGNLDYKTMLTRQKLL DFSVLKATLRDRRTWILFVVYMICFGVEITVISFMQDYLRDTFNVDNTTAGLIVFVFS CLNLFARSVGGGSSDVLYRKFGIQGRVYILFLVLIAEAIFLFIFGFSDFSLGYSIFVLLF FSFYVQVAEGVTYAIVPFVKIGVHKKIGPIYGIVAAGGNFGSFLFSGTLFYLTPNSQSL ISYRLAFVILSLIIFLGAFTTLKIKFTAKEIRDADELLTSFLKVHESTLTMPPSPSPTKPV TEAAVX
>Ammonia sp. 'Camargue' Nrt fragment 1* SRR2003283.150025:3-380(+) SRERYILVLTLLAESIFLLIFAFSSFNLGYCVVILLFFSFYVQCAEGITYAIVPFVKIGEN KNIGPIYGIVAAGGNAGSVLFASTVFAFTPNVCEQPQLGCERIRSAIRLRSLSCRCLS VWSSFL*
> Ammonia sp. 'Camargue' Nrt fragment 2* SRR2003283.72844:476-3(-) GLFICRGHPDHTRRLYVVRPVLVSILLASCLWFAVVVITVLMMKYLLCCNYLSSTKQS VQRTFTILSMKHTWIMAYLYTTFSGFIGFSASFPVLIKQFYPNVNELHYAWLGPFVAS AIRPFGGWLSDKLGGARVTHWTIVVKVVTVIAVGLVIMTLAA
> Ammonia sp. 'Camargue' Nrt fragment 3* Assembled from multiple SRR2003283 reads XXLSPVCLGSLRRAARDYAQKQASVRVVDEYDEPNGAINMHDEDDFAASPKTNAA TDDMKNDPDLGEPSNNEPLKSHTNTHTTTASRNRSPVCIHTFQAHTNIVAIYHFWSV TESDTDFWRQYGQSVATKNLWISIGNLTLAFAVWIIWSIVVVLLFTLAA
>Bulimina marginata Nrt fragment SRR2003397.185677:2-451(+) QPRVYTILMLGFFSLYCQIAQGIVFAIIPGLPHQRYIGLIMGLIASGGNFGAIMGSFFVF YNVPNLIHYQTAWAICAIFVFVASFSVLFIKFTKKXIDEALWTKSNDLFSIIVQQPKKSG NATRRIYQDSLFIDIYRIKLFTPFFLIFC*
>Nonionellina sp. Nrt fragment 1 SRR2003403.1266:546-1(-) EYLTLEVRIGGWSSRRIKNLVSLKKVILASIDRLSAFPVAGMALVTVHRLYHCIFIDWY CWCQSFVITQYHTTAMFAGKTVGTANATSAGWGNFGGGCANFFMPLLYFHFKDTY ELSNSTAWRLCMIIPGLSYIVMSVVYWVFTVNKPGSDVQKEKPDGIGLDQNPKKAE ERLCLKSDSRG*
>Nonionellina sp. Nrt fragment 2 SRR2003403.26623:325-2(-) MFAGKTVGTANATSAGWGNFGGGCANFFMPLLYFHFKDTYELSNSTAWRLCMIIP GLSYIVMSVVYWVFTVNKPGSDVQKEKPDGIGLDQKPQKGGGASVFKVGQPRL
>Brizalina sp. NirK fragment SRR2003388.68811:22-213(+) MMVVGFHLVEVVMEYLSMKDSHVQTVLFNADGTDIEFVSHFMRDYTTPTIDTSHSK FPFQFHL
>Rosalina sp. NirK fragment 1 Assembled from MMETSP0190 reads ELEAGHTFEFFTYNATIPGPPIRVRVGDWIDLT
>Rosalina sp. NirK fragment 2 Assembled from MMETSP0190 reads SPQQNIQTTTVPPGGCAVTDWRHDTPGQF
>Brizalina sp. Nor fragment 1 Assembled from SRR2003388 reads VCLGSLRRAARSTTEYDGGILSKKAAERAXYIAVMLFSXLPPLVXDIIFIGXPNQQVXL HXVQHLVPRKXFHXYYXHLIRGKYIQMNEKGTKLNKRKENQQFIMKEVWLFVLDVN FWNIFGAGILGSFINLPVVNYYMHSTYLTGNHAHSAMWGVKGNIALAGMLFCMQHS IKKNHGVLNXYLLHFGHLMVVLHXXCFMFIPIGLFHMYVTMDQGLWAARNHQMQTS QAYQLLSKGRAIGGHLFLWDGLFPLVYFTCSRYFHLKPTTKIMVMQNINVRG
>Brizalina sp. Nor fragment 2 Assembled from SRR2003388 reads SRGRDIXSIYHRNCSILYREMGSAADVAAERATYIAVMLFFLTATIGVGHNFYWIAKP TGVIALGSAFSTTQVIPLILLTFNAWKYIQMNEKAQIEQKKGNQQFIMKEVWLFVLGV NFWNIFGAGILGSFINLPVVNYYMHSTYLTGNHAHSAMWGVKGNIALAGMLFCMQH SNKKNHGHQNXYLLQFWSFNGGIALMMFLCLFQLVLFHMYVTMDQG

*Different *Ammonia* sp. isolates are distinguished by adding unique isolation source names

Table S2. Foraminifera protein sequences derived from public database data. Related to Figures 2–4.

Gene	Name	Fragment length	Forward primers (5' to 3')	Reverse primers (5' to 3')
18S	14F1 - B	≈1200	AAGGGCACCACAAGAACGC	TGATCCTTCTGCAGCTTCACCTAC
nirK clade I	E2_a	100	TGACATCAAGGAGCGGTACG	GATGCTGCCCACCGTAGTAA
	E2_b	156	GGACCACATCGCCAAAGGAA	GGCGTAGGTCGGTTGAACTT
	E2_c	198	GGACCACATCGCCAAAGGA	TCGTACCGCTCCTTGATGTC
	E3_a	183	GCAGATTGGCGGCATGATAC	ACCATATCGATCGCGGTCAC
	E3_b	131	CGGGTGCAAAGAAGGAAACG	GGAGTATGCAAAGTCGTCGC
	E3_c	100	TTGTCGGGTGCAAAGAAGGA	ATACGCAATCATCGCCACCT
	E4_a	151	AATAGTGTGGATGGTGGCGG	GAGTGCCATAATCGCGGAGA
	E4_b	100	TGTCGCGGATCCTATGCTTG	GACGTCCCCGACATTAACGA
	E4_c	194	TGGATAGGTTTTGGTCGCGG	CCATCAGCAACACTCACCGA
nirK clade II	E1_a	105	TCAGTGGCGAATCCACCAAA	ACAGGTGGCGGTTAAAAGCA
	E1_b	102	AACGGCAAAGGAGTCACAGT	TTCAATTTCGCCGGTTGTGC
	E1_c	157	CGAGTTCGCATGAACACAGC	GAGATCCATCCACTCGCACA
	E1_d	197	ACACACCGGGTCACCTTAAC	CCGCTCATTGCGTGGAAATC
	E2_a	183	TGATGTTTAACGGTGCCCCA	TGTTTTGCTGTGGCGATGTG
	E2_b	161	ACACATCGCCACAGCAAAAC	GTCGCAGGCTCGAATAAAGC
	E2_c	110	GACTGCGTGTTTTGTCCGTG	TCCGTCCGCAGACTACATTG
	E3_a	198	CGCAGATGGCAGTGATGTTG	ACGTACGTCAAAGCATCCGA
	E3_b	109	GTAAAGTGAGTGGCGGTGGT	TCACTGCCATCTGCGTTGAA
	E3_c	168	GGAATGCGTCAGATGGAGGT	ACCACCGCCACTCACTTTAC
nor clade I	E1_a	152	TTGGGGAAGTGGCGCATTAT	GCCATCCCCTAAGAAGGAGC
	E1_b	152	CAGGATTTGGAGACTGGCGT	ATGTAACAGGCCGAGAAGCC
	E1_c	198	ATCCAAATGCGCGAGAAAGC	GGCATGAGCATGATTGCCAG
	E1_d	182	TGGTTCTTTGTGGCTTGGGT	TACGCCATTTCGCCCTGAAT

Table S3. PCR and RT-PCR primers for the rRNA gene 18S, Nor, Nir clade I and clade II. Related to Figure 1 and Figures 3–4.

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Data S1


A
Nrt I
A. thaliana (O82811)
A. nidulans (P22152)
Nrt II
E. coli (P10903)
B. subtilis (P46907)
NarK superfamily (cl27296)
Nrt I: GloG15_CONTIG80510:375-855,926-2061(+)
Nrt II: GloG15_CONTIG110211:243-1504,1578-2146(-)
B
NirK I
NirK II
H. marismortui (CAB93142)
G. thermodenitrificans (4YSA_A)
A. cycloclastes (P25006)
A. faecalis (P38501)
Cupredoxin domain I (cd11020)
Cupredoxin domain II (cd04208)
Conserved copper ligand binding sites (Site 1 or 2)
NirK I: GloT15_NODE_12185:141-2504(-)
NirK II: GloT15_NODE_4197:1119-3623(+)
C
Nor I
Nor II
'Ca. M. oxyfera' (CBE69496)
γ-proteobacterium HdN1 (WP_013260353)
M. ruestringensis (WP_014034207)
'Ca. M. oxyfera' (CBE68939)
N. gonorrhoeae (WP_003691589)
G. stearothermophilus (3AYF_A)
Cytochrome c oxidase domain (pfam00115)
Conserved NorZ residues of catalytic site
Conserved Nod residues of catalytic site
Nor I: GloG15_CONTIG49717:890-3289(+)
Nor II: GloT15_NODE_10819:151-2556(+)

Data S2