doi:10.1038/nature12375

Supplementary Tables

Supplementary Table 1. Composition of the bioreactor community on day 250 determined using 16S rRNA gene amplicon sequencing. Operational taxonomic units (OTUs) ≥1% relative abundance are shown.

OTUs	Percentage
Archaea; Euryarchaeota; Methanomicrobia; Methanosarcinales; ANME-2d	78.0%
Bacteria; Planctomycetes; Phycisphaerae; Phycisphaerales	5.8%
Bacteria; Planctomycetes; Kueneniae; Kueneniales	2.8%
Bacteria; Actinobacteria; Actinobacteria; koll13	2.1%
Bacteria; Chloroflexi; Anaerolineae; envOPS12	2.1%
Bacteria; Chlorobi; Ignavibacteria; Ignavibacteriales	2.0%
Bacteria; Chlorobi; OPB56	1.4%
Bacteria; Chloroflexi; Anaerolineae; S0208	1.0%
Bacteria; Chloroflexi; Anaerolineae; SJA-101	1.0%

Supplementary Table 2. Summary of metagenome assembly and single-cell genome mapping.

Metagenome	Number of raw Illumina reads	88,859,724
	Number of reads after trimming	73,165,513
	Reads in metagenomic contigs	52,200,776
	Assembled metagenome size (Mbp)	68.8
	N50 (bp)	6,325
	Maximum scaffold size (bp)	414,561
Single-cell genome	Number of single cell reads	31,254,040
	Percentage of reads mapped to metagenomic contigs	91.5%
	Percentage of reads mapped to *'M. nitroreducens'* genome (contigs > 1 kb)	90.2%

Supplementary Table 3. Taxonomic identification of genomes based on 16S rRNA genes present in each metagenomic bin. 16S rRNA genes were compared to the Greengenes database[1]. Bin IDs are consistent with Supplementary Fig. 4.

Bin ID	**Greengenes ID**	**Taxonomy**
1	12752	k__Bacteria;p__Actinobacteria;c__Actinobacteria; o__koll13
2	213866	k__Bacteria;p__Chlorobi;c__Ignavibacteria;o__Ignavibacteriales; f__Ignavibacteriaceae
3	242876	k__Archaea;p__Euryarchaeota;c__Methanomicrobia; o__Methanosarcinales
4	537668	k__Bacteria;p__Planctomycetes;c__Kueneniae; o__Kueneniales;f__Kueneniaceae
5	361409	k__Bacteria;p__Planctomycetes;c__Phycisphaerae; o__Phycisphaerales
6	185747	k__Bacteria;p__Acidobacteria;c__Sva0725; o__Sva0725
7	167480	k__Bacteria;p__Chloroflexi;c__Anaerolineae; o__envOPS12

Supplementary Table 4. *'M. nitroreducens'* gene expression analysis based on the number of fragments mapped per kilobase of gene length (FPKG) values. FPKG values of the ORFs in *'M. nitroreducens'* are listed on Sheet 1. The phylogenetic marker genes used by AMPHORA2[2] to check the completeness of the genome are listed on Sheet 2, and the housekeeping genes and median FPKG value used as baseline for metatranscriptomics is listed on Sheet 3 **(as separate Excel document available from Nature website).**

Supplementary Table 5. Comparison of the *'M. nitroreducens'* and ANME-1b genome[3].

	'M. nitroreducens'	**ANME-1b**
Size (base pairs)	3,198,961	3,398,838
Number of scaffolds	10	13 supercontigs and 17 fosmids
GC content (%)	43.22	43.21
Number of ORFs	3481	3578
rRNAs	3	3
tRNAs	46	35

Supplementary Table 6. Single-cell reads mapped against the *'M. nitroreducens' narGH* genes.

Genes	**Locus Tag**	**Median coverage**	**Min coverage**	**Max coverage**
Nitrate reductase alpha subunit	ANME2D_03460	91	45	147
Nitrate reductase beta subunit	ANME2D_03461	141	97	184

Supplementary Table 7. Correspondence analysis of codon usage for all *'M. nitroreducens'* ORFs. Codon usage was determined using codonw (http://codonw.sourceforge.net/) **(as separate Excel document available from Nature website).**

Supplementary Table 8. Fragments mapped per kilobase of gene length (FPKG) values for all *narG* (Sheet 1) and *narH* (Sheet 2) genes in metagenome **(as separate Excel document available from Nature website).**

Supplementary Table 9. *Kuenenia* population gene expression analysis based on the fragments mapped per kilobase of gene length (FPKG) values. FPKG values of key genes involved in anaerobic ammonium oxidation are listed on Sheet 1. Phylogenetic marker genes used by AMPHORA2[2] to check the completeness of the genome, the housekeeping genes and median FPKG value are listed on Sheet 2 **(as separate Excel document available from Nature website).**

Supplementary Table 10. Reaction rates and electron and mass balance calculation based on bioreactor performance data collected between days 230 and 290. The consumption rate of methane (rCH_4), ammonium (rNH_4^+), nitrate (rNO_3^-) and nitrite (rNO_2^-), and production rate of dinitrogen gas (rN_2-N) were determined from the respective measured concentrations through linear regression. Nitrogen balance was calculated as the difference between the measured dinitrogen gas production rate and the sum of the measured ammonium and nitrate consumption rates: $rN_2\text{-}N + (rNH_4^+ + rNO_3^-)$. The percentage error was calculated as the ratio between nitrogen balance error and the measured dinitrogen gas production rate: $(rN_2\text{-}N + (rNH_4^+ + rNO_3^-))/ rN_2\text{-}N$. Electron balance was calculated as the difference between the electrons required by the reduction of nitrate to dinitrogen gas and the electrons produced by the oxidation of ammonium to dinitrogen gas and methane to carbon dioxide: $-5 \times rNO_3^- + 3 \times rNH_4^+ + 8 \times rCH_4$. The percentage error was calculated as the ratio between electron balance error and electrons required by the reduction of nitrate to dinitrogen gas: $(-5 \times rNO_3^- + 3 \times rNH_4^+ + 8 \times rCH_4)/ -5 \times rNO_3^-$.

	Measurement (mmol/day)					Nitrogen balancing error		Electron balancing error	
						mmol/day	% error	mmol/day	% error
Day	rNH_4^+	rNO_2^-	rNO_3^-	rN_2-N	rCH_4	Nitrogen	Nitrogen	Electron	Electron
230-290	-3.33	0.00	-3.54	6.68	-1.06	-0.19	-2.8%	-0.77	-4.4%
stdev	0.14	0.00	0.14	0.36	0.13				

The nitrogen balance indicates that all nitrate and ammonium consumed were converted to dinitrogen gas. The electron balance indicates that electron supply (sources) matches electron consumption (sinks) in the bioreactor.

Nitrogen and carbon flux calculation for Figure 4a

Based on the following stoichiometry of the anammox reaction[4]:

$$1.32NO_2^- + NH_4^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^-$$

The oxidation of ammonium at 3.33 mmol d^{-1} (**Supplementary Table 10**) would consume nitrite at a rate of 4.40 mmol d^{-1} and produce nitrate at a rate of 0.87 mmol d^{-1}. The total nitrate reduction rate in the bioreactor is 4.41 mmol d^{-1} (= 3.54 mmol d^{-1} (Table 10) + 0.87 mmol d^{-1}). This is very close to the nitrite consumption rate of 4.40 mmol d^{-1} required by the annammox reaction, as calculated above.

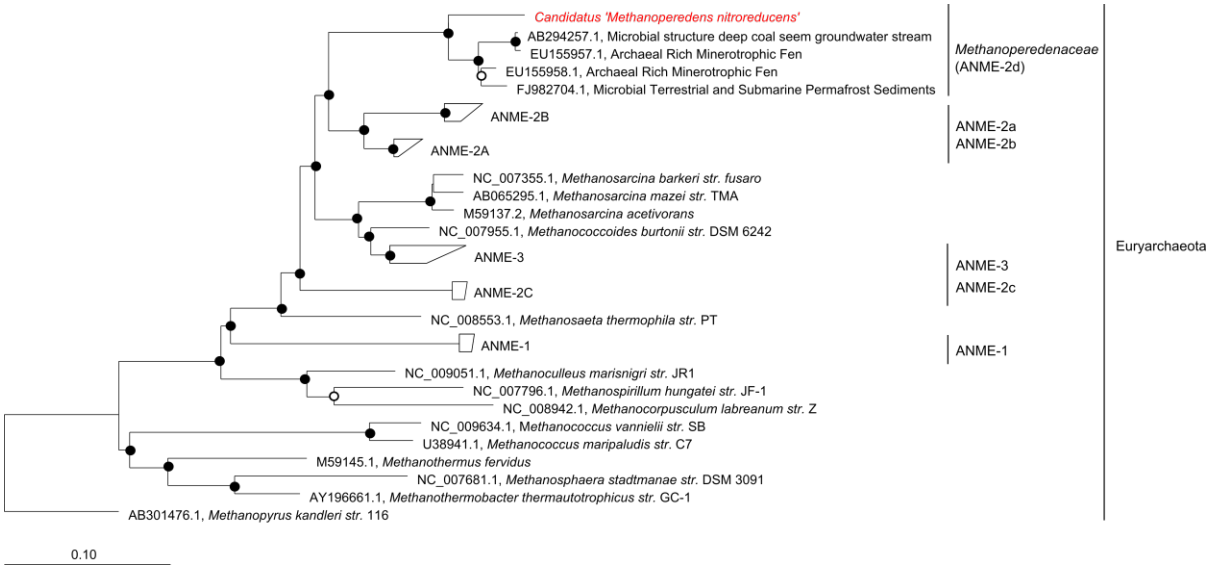
Conversely, the reduction of nitrate to nitrite at a rate of 4.41 mmol d^{-1} with methane as the electron donor would require a methane consumption rate of 1.10 mmol d^{-1} (=4.41/4, referring to Equation 1 in the manuscript). This is very close to the observed methane consumption rate of 1.06 ± 0.13 mmol d^{-1}. This indicates that methane is not have been used for nitrite reduction to dinitrogen gas.

The above nitrogen and electron balance strongly suggests that all methane consumed in the bioreactor was used for nitrate reduction by *'M. nitroreducens'*, and all nitrite produced from nitrate reduction was consumed by anammox organisms, giving rise to the conceptual reaction model in the bioreactor (**Fig. 4a**).

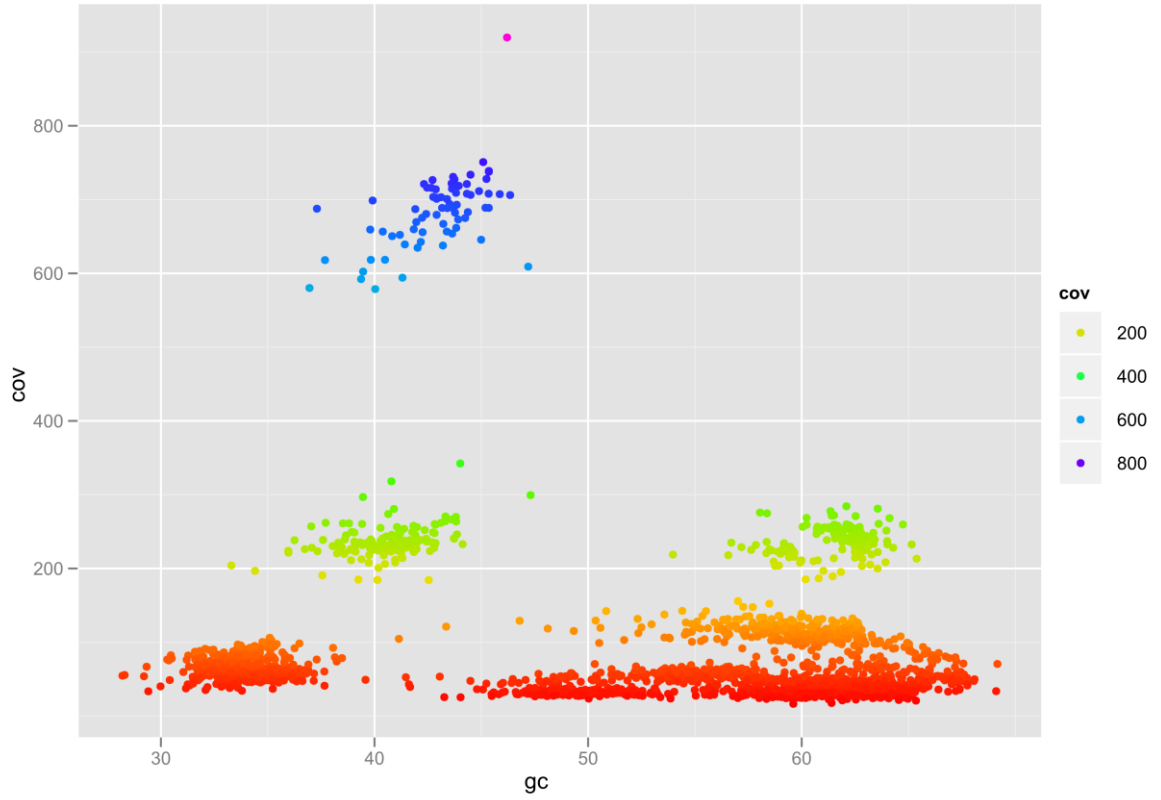
Comparison of AOM and anammox rates in this study with literature

Using quantitative PCR, the cell density in our bioreactor was estimated to be ~1.75 x 10^9 cells ml^{-1}, which would equate to 2.4 x 10^{11} anammox cells (~3%) and 6.2 x 10^{12} ANME-2d cells (~78%). Thus, the specific cell rate for anammox is 14 fmol ammonium per anammox cell per day, which is comparable to the reported anammox rate (2–20 fmol ammonium per cell per day in laboratory bioreactors[5]). The specific cell rate for AOM is 0.17 fmol methane per ANME-2d cell per day. This is comparable to the reported sulfate-dependant AOM (0.7 fmol CH_4 per cell per day[6]).

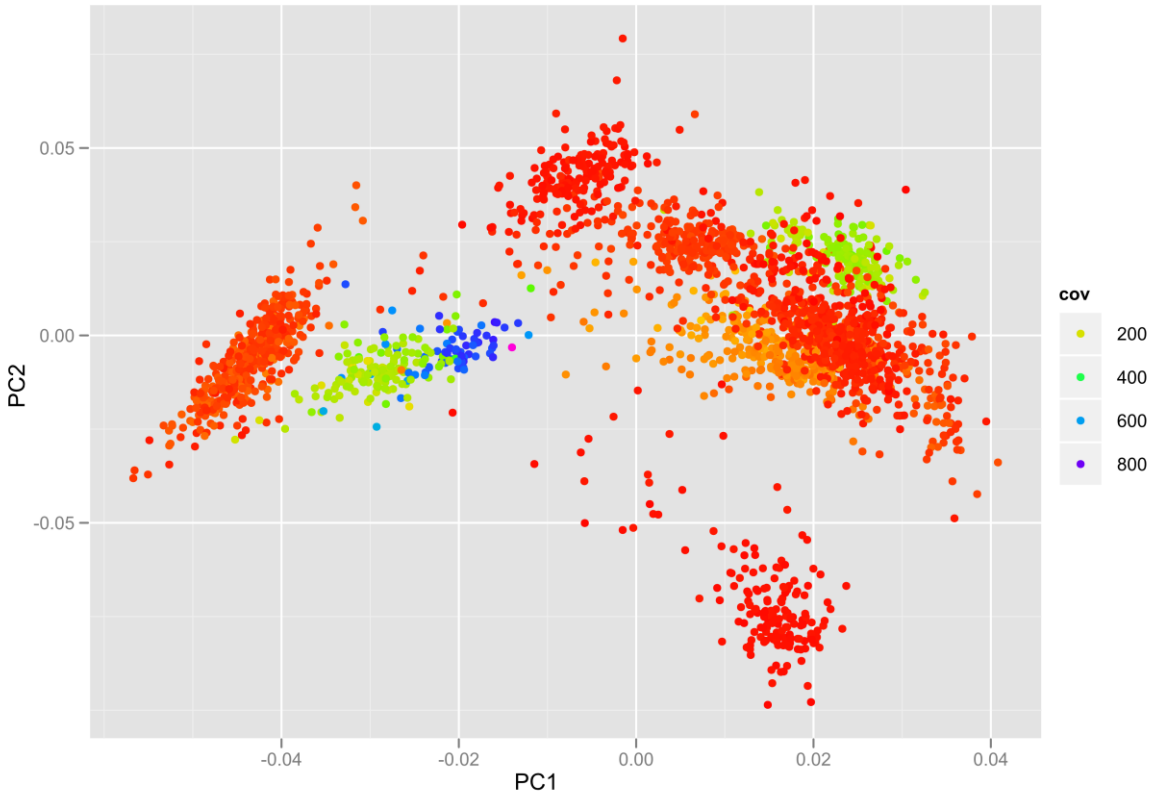
Supplementary Figures



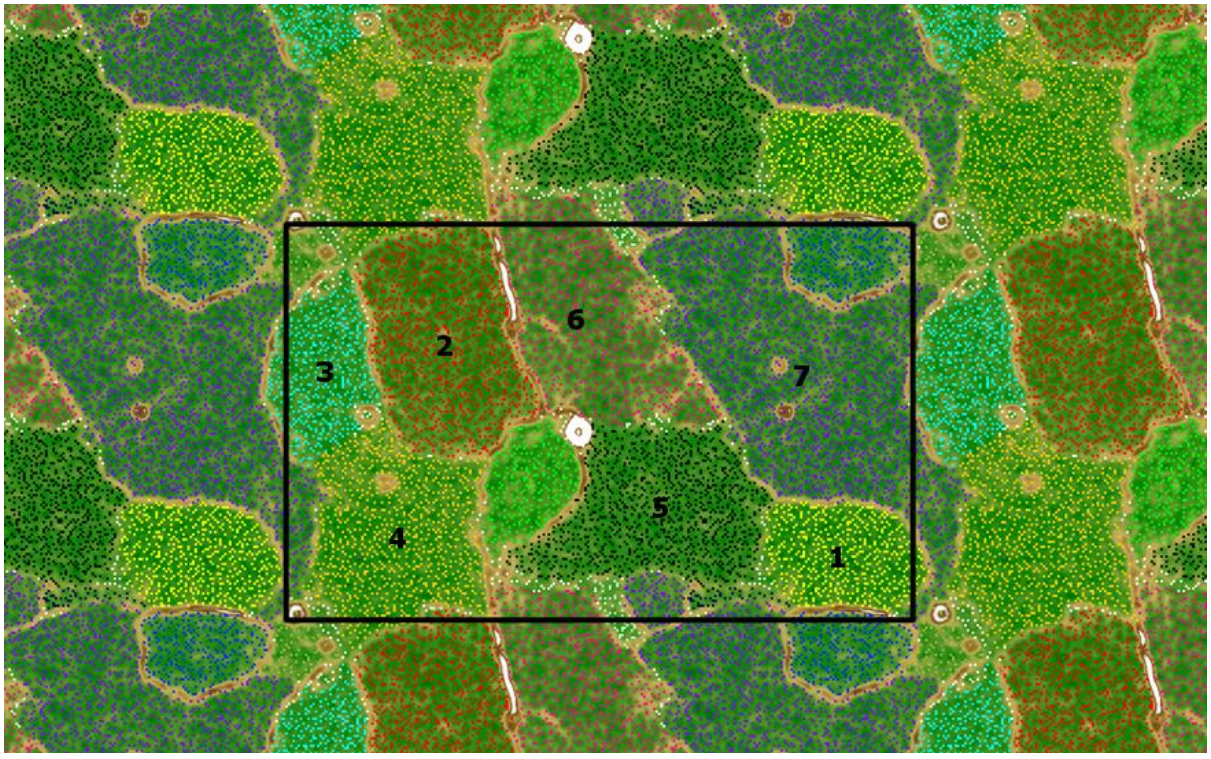
Supplementary Fig 1. Phylogenetic tree of ANME lineages. The 16S rRNA gene tree was generated using the maximum-likelihood method with 100 bootstraps. Bootstrap values are indicated at the nodes (solid circles, >75%; open circles, >50%; no circles, <50%). Scale bars are equal to 0.1 changes per site.



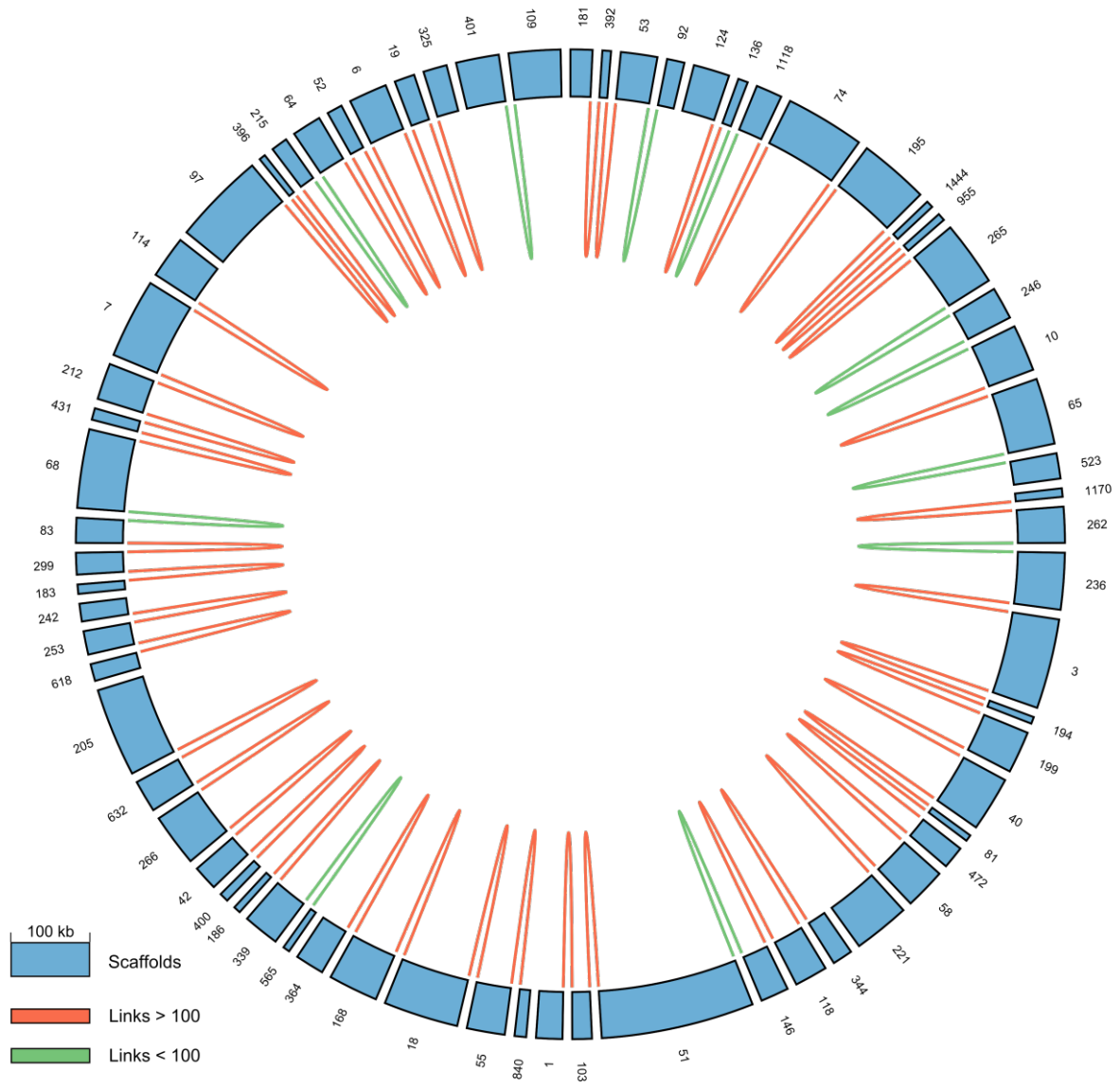
Supplementary Fig 2. Binning of the metagenome contigs based on coverage and GC content. Each dot represents a contig that is coloured based on its coverage in the metagenome. Only contigs ≥ 5kb are shown. The blue dots correspond to the *'M. nitroreducens'* genome, and were validated by 16S rRNA gene and single copy gene analysis.



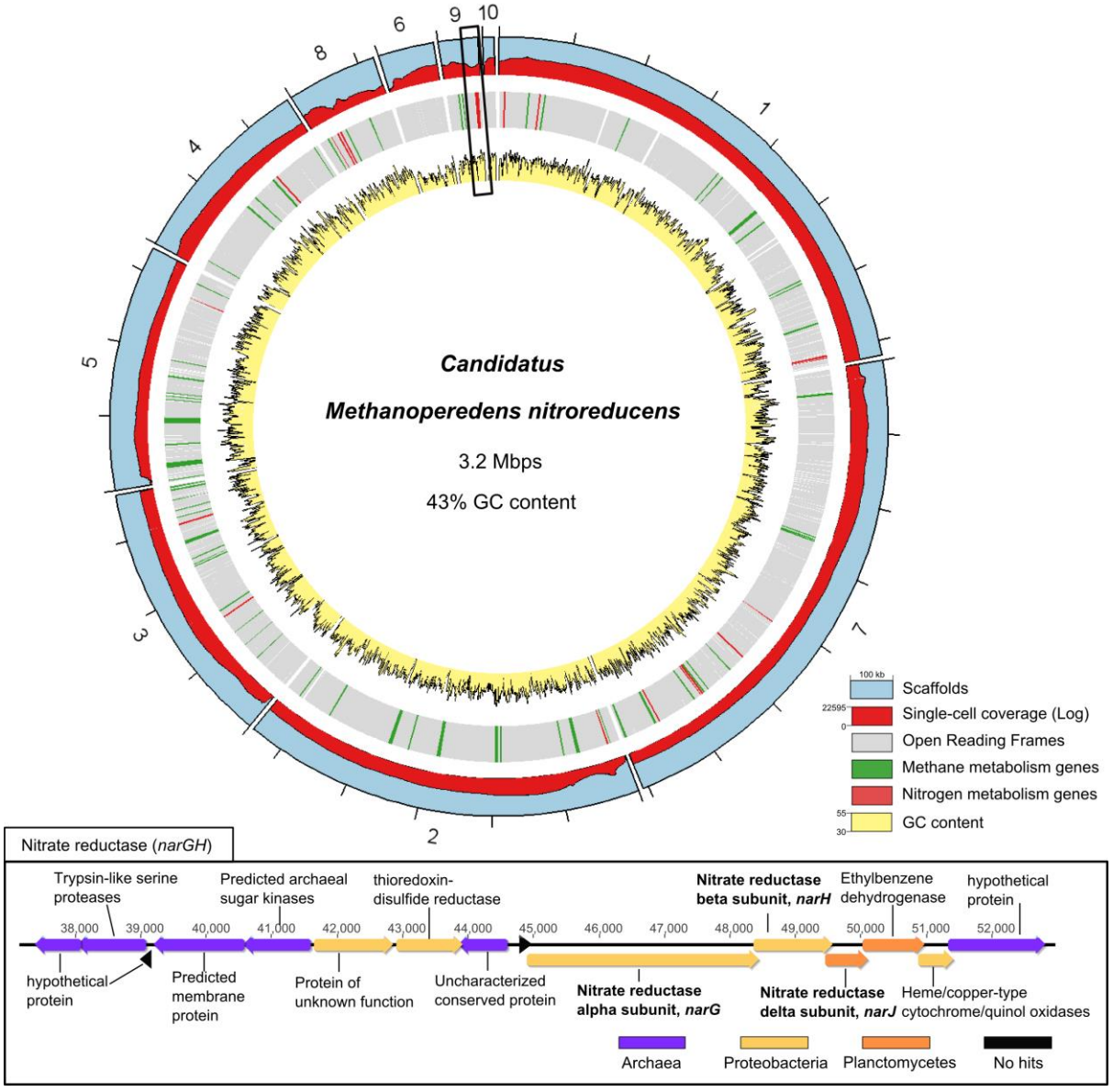
Supplementary Fig 3. Binning of metagenome contigs based on principle component analysis of contig tetranucleotide frequencies. Each dot represents a contig that is coloured based on its coverage. Only contigs ≥ 5kb are shown.



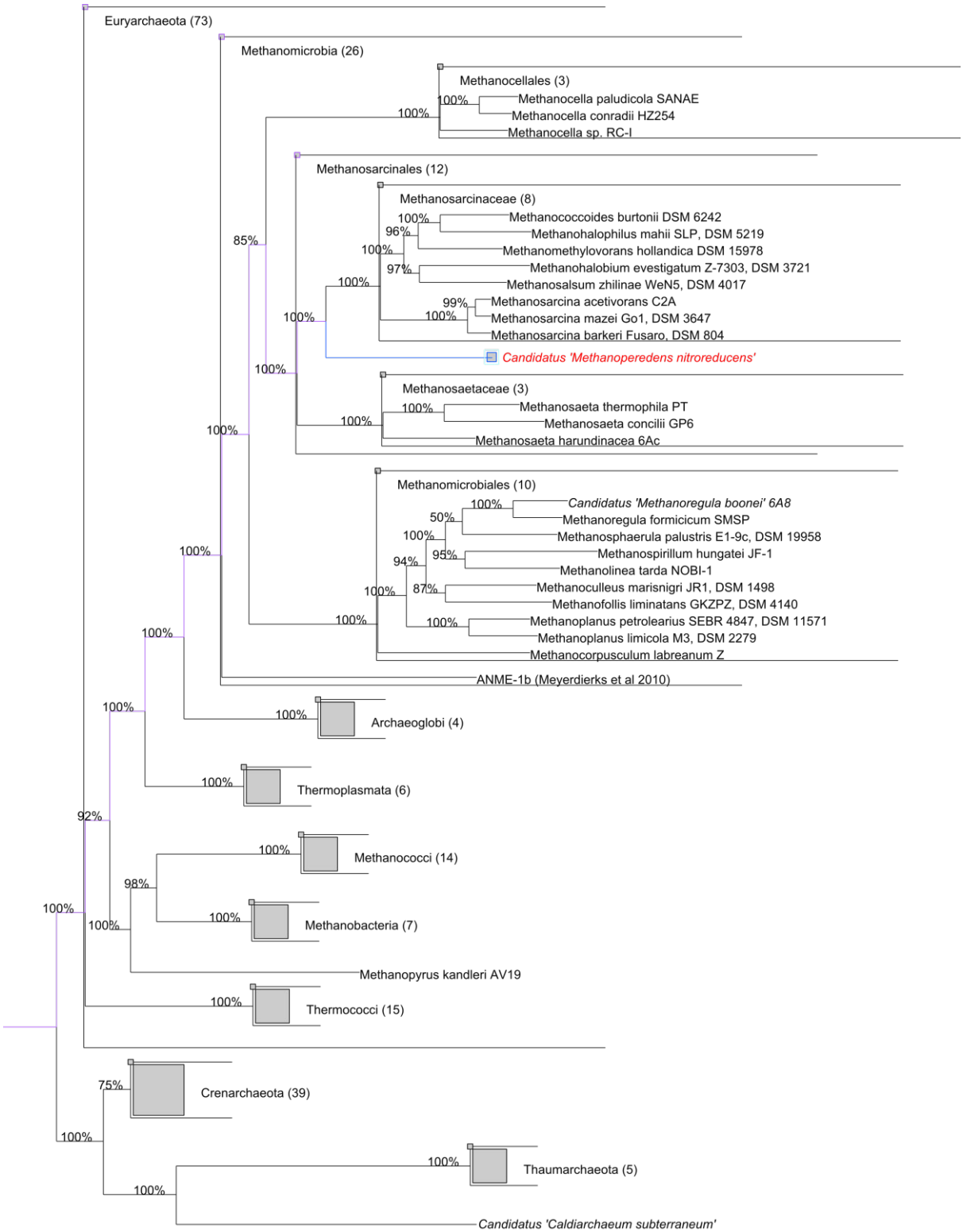
Supplementary Fig 4. Emergent self-organising map (ESOM) of metagenome contigs (≥ 5kb contigs with 5-kb window size) based on tetranucleotide frequencies. Each point is coloured based on its putative genome bin. The numbers indicate different genome assignments. Bins were identified as (class level): **(1)** *Actinobacteria*, **(2)** I*gnavibacteria*, **(3)** *'M. nitroreducens'*, **(4)** *Kuenenia*, **(5)** *Phycisphaerae*, **(6)** Sva0725 (*Acidobacteria*), **(7)** *Anaerolinea*.



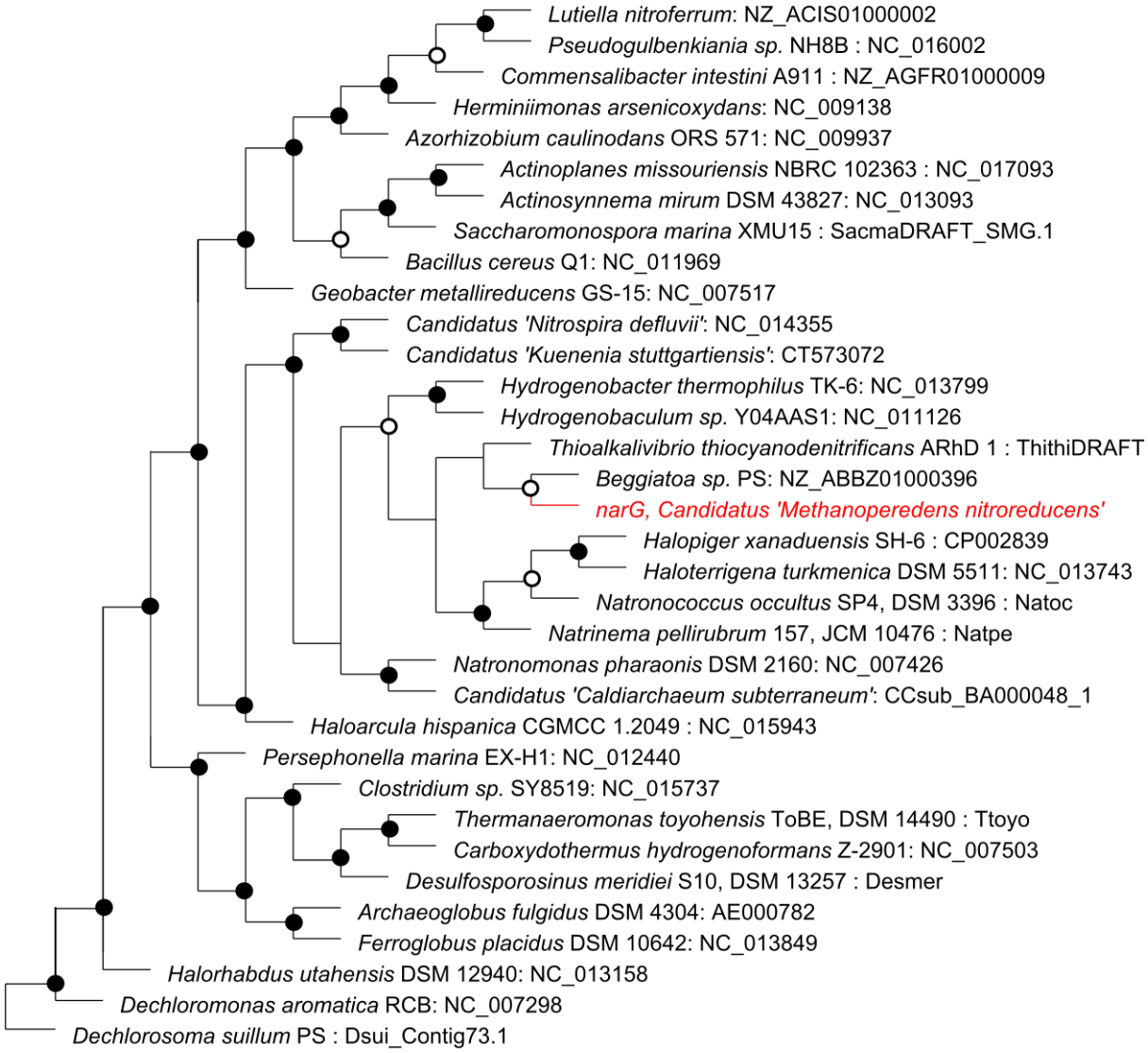
Supplementary Fig 5. Scaffolding of *'M. nitroreducens'* genome using 3kb mate-pair library information. Blue rectangular blocks represent *'M. nitroreducens'* contigs identified using GC content, read coverage and tetranucleotide frequencies. Coloured lines linking contigs indicate the number of mate-pairs spanning the gap between the contigs. Red lines indicate more than 100 mate-pairs linking the contigs, and green lines indicate less than 100 mate-pairs linking the contigs. Mate-pair read mapping carried out using bwa v.0.6.2[7] with default settings.



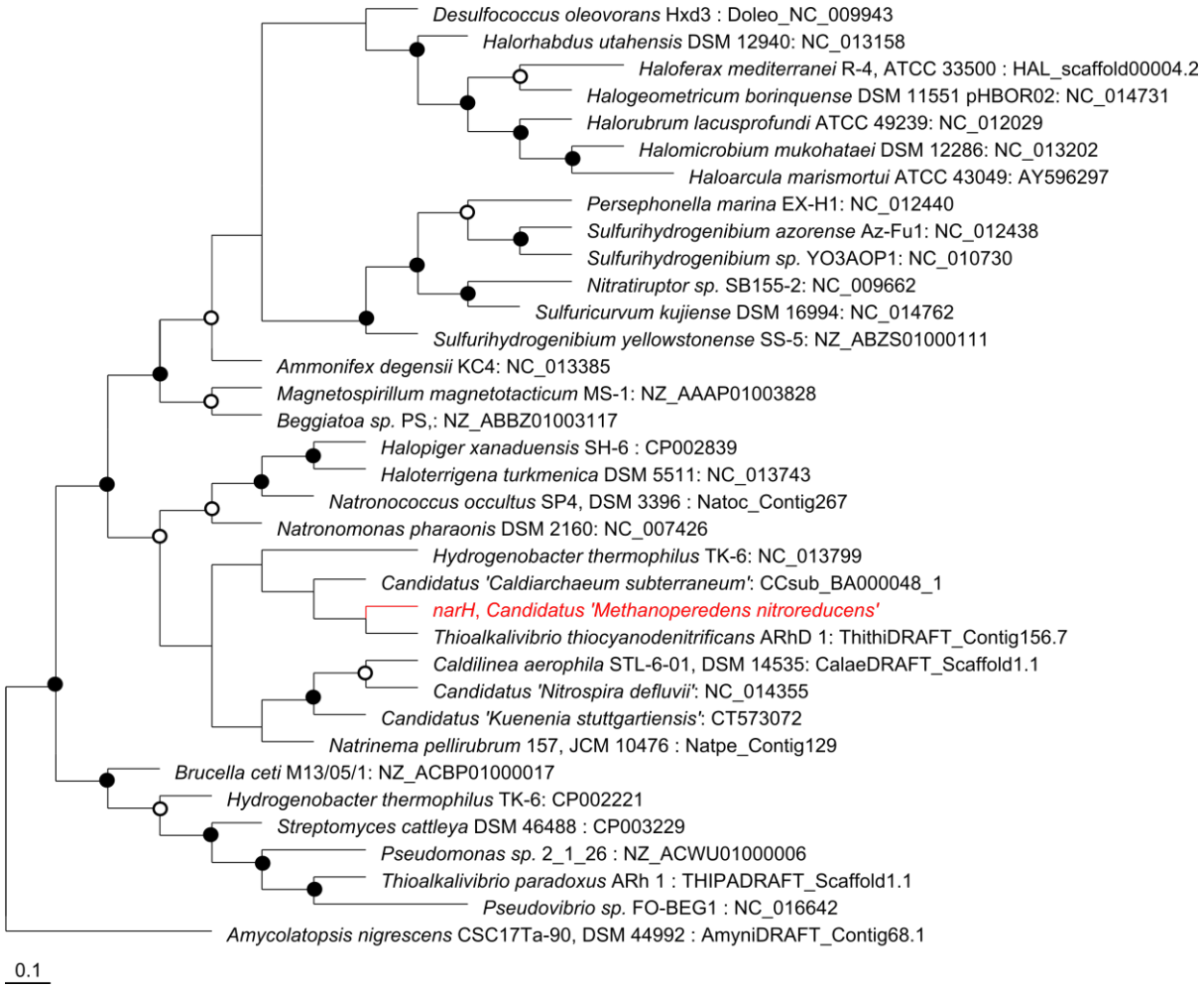
Supplementary Fig 6. *'M. nitroreducens'* genome and transcriptome. From the outermost to the innermost ring; the first ring (blue) represents the 10 scaffolds of the reconstructed genome (ordered by size). The red plot within the scaffold segments represents the log of the median single-cell coverage across 3205 non-overlapping 1 kb windows. Only 7 non-consecutive windows have no coverage. The second ring shows the open reading frames (grey) including genes for reverse methanogenesis (green) and nitrogen metabolism (red). The innermost yellow ring shows the GC skew. The box corresponds to laterally transferred *narGH* genes (yellow) that are found in the context of host archaeal genes (purple).



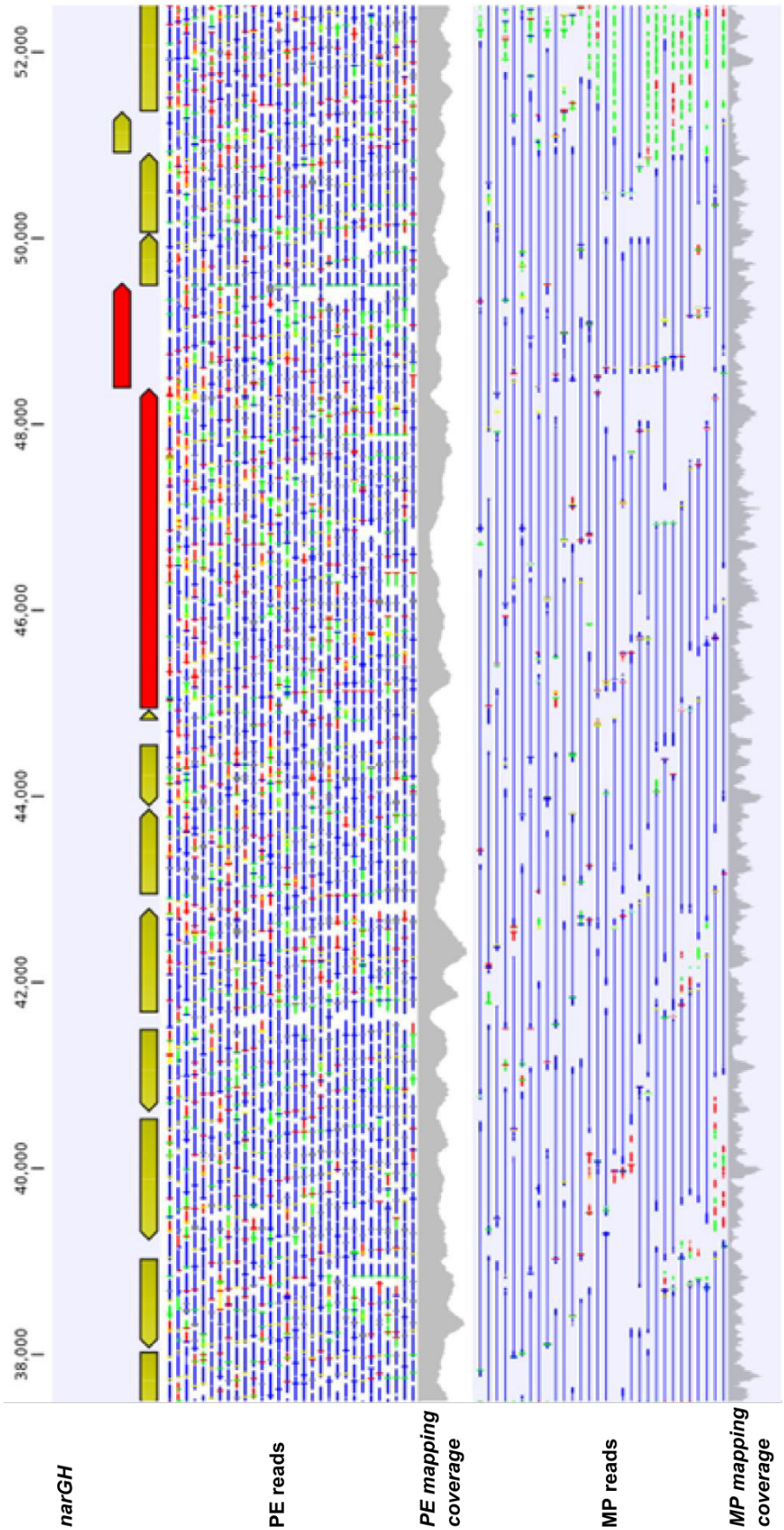
Supplementary Fig 7. Archaeal genome tree of archaeal based on 38 essential single-copy gene markers from Phylosift (A. Darling, H. Bik, G. Jospin, J. A. Eisen. Manuscript in preparation). The tree shows that *'M. nitroreducens'* is closely related to the *Methanosarcinaceae* family. The ANME-1 partial genome[3] is more closely related to the *Methanomicrobiales* family. Both *'M. nitroreducens'* and ANME-1 are within the *Methanosarcinales* order.



Supplementary Fig 8. Phylogenetic tree of *narG* sequences generated using maximum parsimony methods (Phylip PROTPARS) in ARB. Bootstrap values are indicated at the nodes (solid circles, >75%; open circles, >50%; no circles, <50%). Scale bars are equal to 0.1 changes per site.

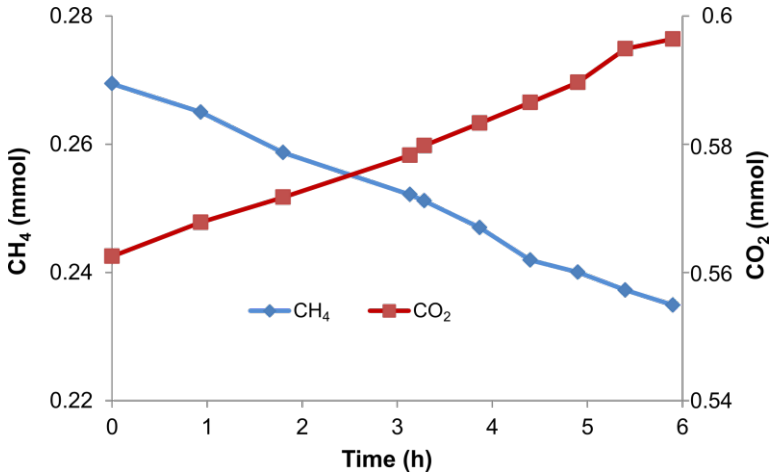


Supplementary Fig 9. Phylogenetic tree of *narH* sequences generated using maximum parsimony methods (Phylip PROTPARS) in ARB. Bootstrap values are indicated at the nodes (solid circles, >75%; open circles, >50%; no circles, <50%). Scale bars are equal to 0.1 changes per site.

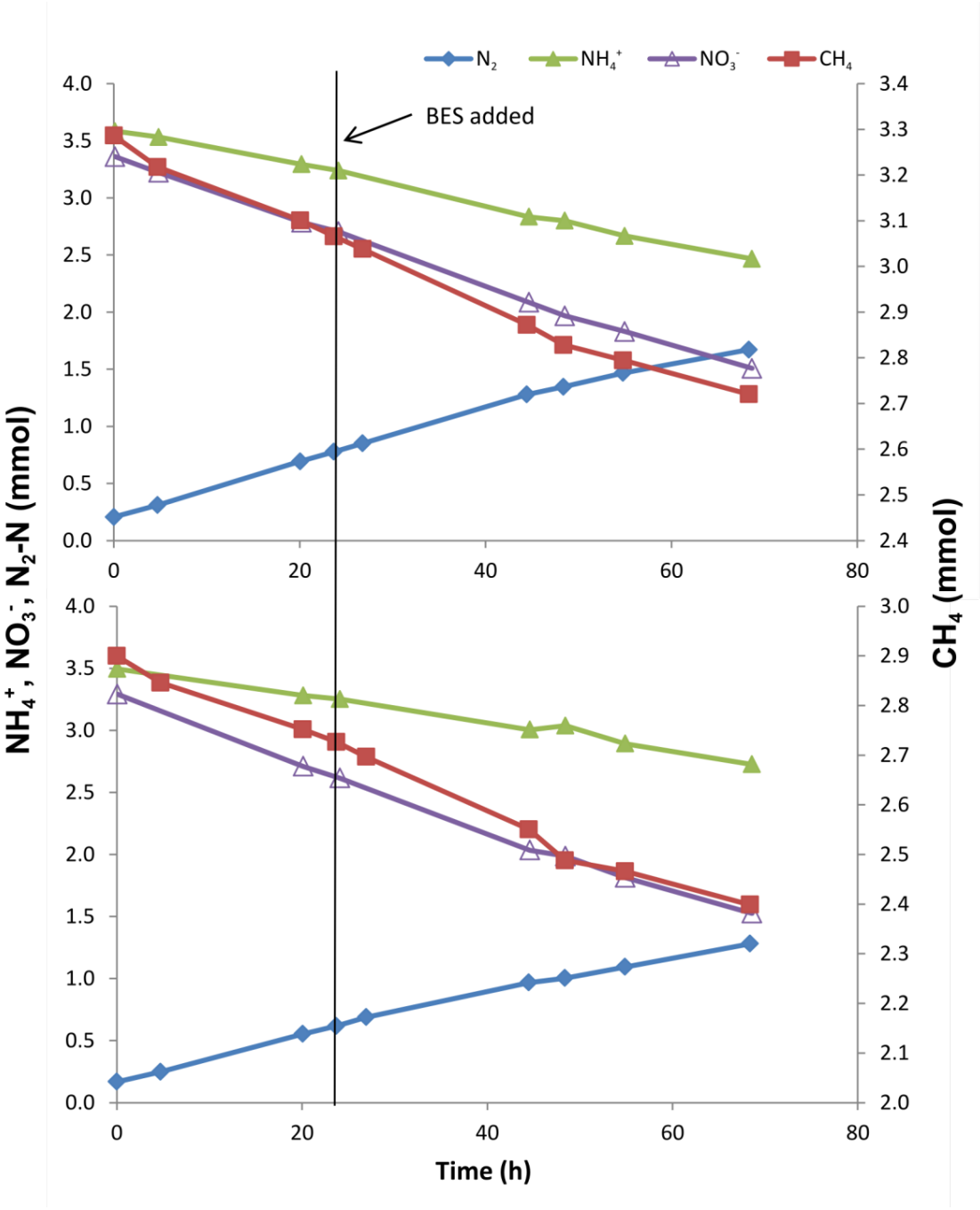


Supplementary Fig 10. Mapping of paired-end and mate-pair reads to laterally transferred region of *narGH* on *'M. nitroreducens'* scaffolds.

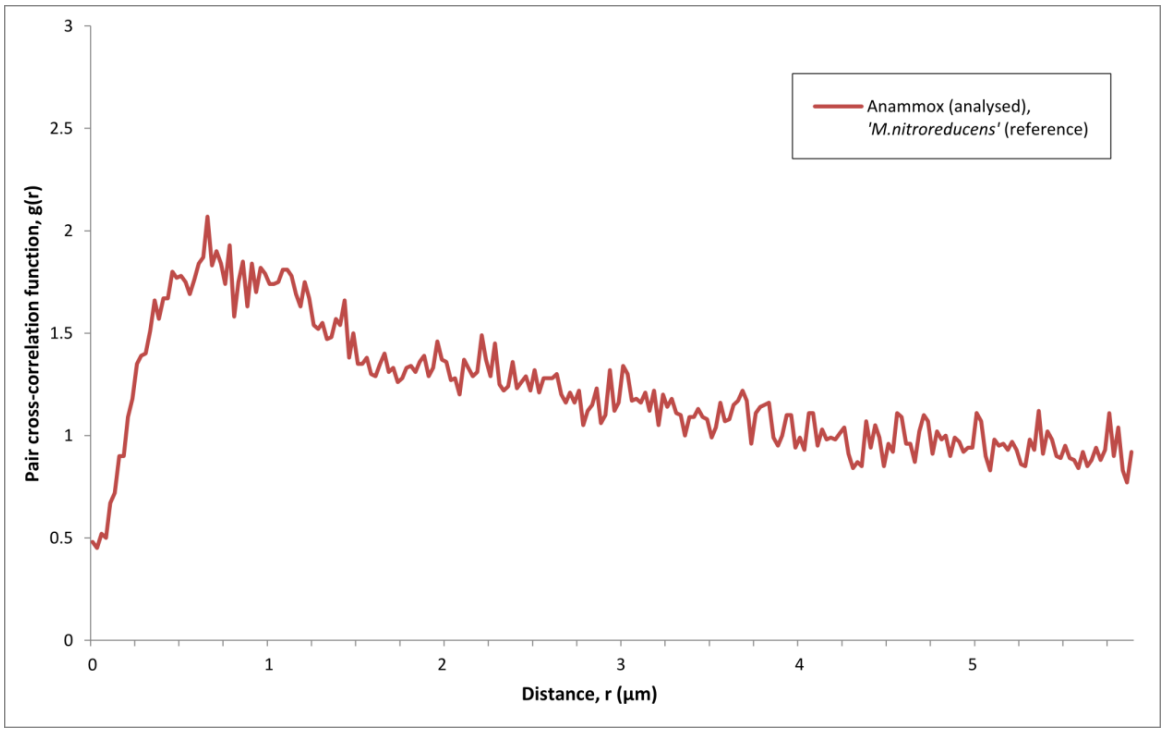
Supplementary Fig 11. Codon usage analysis for genes in the *'M. nitroreducens'* genome. Each dot represents a gene. The y-axis represents Axis 1 from the correspondence analysis on relative synonymous codon usage (RSCU) while the x-axis represents the G+C content at the 3rd codon position in the nucleotide sequence (GC3s). Yellow dots represent genes with typical codon usage, while red dots represent genes with atypical codon usage. The blue dots represent the *narGH* genes, which have codon usage on the boundary typical/atypical genes. Only genes ≥ 50 amino acids are shown.



Supplementary Fig 12. Data from the isotope labeling batch tests demonstrating conversion of CH_4 (total) to CO_2.



Supplementary Fig 13. BES inhibition tests performed between days 301 and 305. For each test, 260 ml biomass was transferred from the bioreactor into a 330 ml batch vessel. Twenty-four hours after the experiments started, BES was added to the batch vessels to reach concentrations of 20 mM (upper panel) and 50 mM (lower panel), respectively. No clear reduction of the methane oxidation rate or other reaction rates was observed.



Supplementary Fig 14. Plot of the pair cross-correlation function versus distance separating *'M. nitroreducens'* and anammox populations calculated using the Linear Dipole Algorithm in DAIME[8]. The peak between 0.5 – 1.5 μm (g(r) > 1.5) indicates that there is a strong co-aggregation signal suggesting co-localisation of *'M. nitroreducens'* and anammox populations.

References

1 McDonald, D. *et al*. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J* **6**, 610-618, doi:http://www.nature.com/ismej/journal/v6/n3/suppinfo/ismej2011139s1.html (2012).

2 Wu, M. & Scott, A. J. Phylogenomic analysis of bacterial and archaeal sequences with AMPHORA2. *Bioinformatics* **28**, 1033-1034, doi:10.1093/bioinformatics/bts079 (2012).

3 Meyerdierks, A. *et al*. Metagenome and mRNA expression analyses of anaerobic methanotrophic archaea of the ANME-1 group. *Environ Microbiol* **12**, 422-439, doi:10.1111/j.1462-2920.2009.02083.x (2010).

4 Kuenen, J. G. Anammox bacteria: from discovery to application. *Nat Rev Micro* **6**, 320-326 (2008).

5 Strous, M. *et al*. Missing lithotroph identified as new planctomycete. *Nature* **400**, 446-449 (1999).

6 Nauhaus, K., Treude, T., Boetius, A. & Krüger, M. Environmental regulation of the anaerobic oxidation of methane: a comparison of ANME-I and ANME-II communities. *Environ. Microbiol.* **7**, 98-106, doi:10.1111/j.1462-2920.2004.00669.x (2005).

7 Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* **25**, 1754-1760, doi:10.1093/bioinformatics/btp324 (2009).

8 Daims, H., Lücker, S. & Wagner, M. daime, a novel image analysis program for microbial ecology and biofilm research. *Environ. Microbiol.* **8**, 200-213, doi:10.1111/j.1462-2920.2005.00880.x (2006).