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SUPPLEMENTARY MATERIAL

Exploring archaeal and bacterial diversity and co-occurrence in Lake Geneva

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Sequencing results

Following sequencing, each raw data file (R1 forward and R2 reverse sequence) contained 55,679,272 reads. After merging, we obtained 49,545,789 paired end reads. At this step, 11% of reads were lost, and the median read length was 437 bp. After demultiplexing, and after a first dereplication, the number of reads reached 11,079,438, with a median length of 377 pb. The second dereplication of reads after reuniting them in one file gave 6,861,908 reads. The clustering process yielded 127,052 representative sequences, including 84.3% chimeric sequences and 15.3% non-chimeric sequences. However, when considering abundance into account, this corresponded to 1.3% chimeras and 98.7% non-chimeras. The median read length after the clustering was 376 pb.

For the archaeal OTU table, we obtained with the default filter, 631 OTUs and 2,868,729 reads. After applying the stringent filter, this number fell down to 194 OTUs and 2,862,662 reads. In addition, only 112 OTUs (2,858,335 reads) were common to both replicates. After applying all these filters, we only lost 0.4% of archaeal reads. Overall, 5 phyla and 7 classes were detected after taxonomic assignment. Woesearchaeia (Nanoarchaeaeota) had the highest number of OTUs (*i.e.*, 91), but was classed second regarding the number of reads that was 67,646. Nitrososphaeria

(Thaumarchaeota) followed with 11 OTUs, corresponding however to 2,790,583 reads. For Methanomicrobia (Euryarchaeota), Iainarchaeia (Diapherotrites), Bathyarchaeia (Crenarchaeota), Methanobacteria (Euryarchaeota), and Micrarchaeia (Diapherotrites) the number of OTUs varied between 1 and 3, with 3 to 43 reads (Supplementary Fig. S2 and S3).

For the bacterial OTU table, the “default” step gave 7,987 bacterial OTUs and 29,846,037 reads. After applying the “stringent” step we obtained 3,712 OTUs and 29,502,677 reads. This step resulted in a loss of only 1.15% in read number. The “shared” filter yielded 2,674 OTUs and 29,383,517 reads. Only 0.4% of reads were lost between these two steps, for a total loss of 1.55%. In general, 41 bacterial phyla and 110 classes were assigned according to the pipeline. Alphaproteobacteria (Proteobacteria) yield the highest number of OTUs (*i.e.*, 411) (Supplementary Fig. S6), followed by Bacteroidetes (Bacteroidia) (*i.e.*, 400). The Gammaproteobacteria (Proteobacteria) class followed with 381 OTUs, the Deltaproteobacteria (Proteobacteria) with 223 OTUs, the Planctomycetacia (Planctomycetes) with 122 OTUs, and the Oxyphotobacteria (Cyanobacteria) with 110 OTUs. Actinobacteria was characterized by the highest number of reads, *i.e.*, 8,948,606. It was followed by the Oxyphotobacteria (Cyanobacteria) with 4,699,878 reads, the Gammaproteobacteria (Proteobacteria) with 2,220,792 reads, the Anaerolineae (Chloroflexi) with 2,209,038 reads, the Bacteroidetes (Bacteroidia) with 2,076,034 reads and the Alphaproteobacteria (Proteobacteria) with 1,955,836 reads. 48 bacterial classes had less than 1,000 reads (Supplementary Fig. S7).

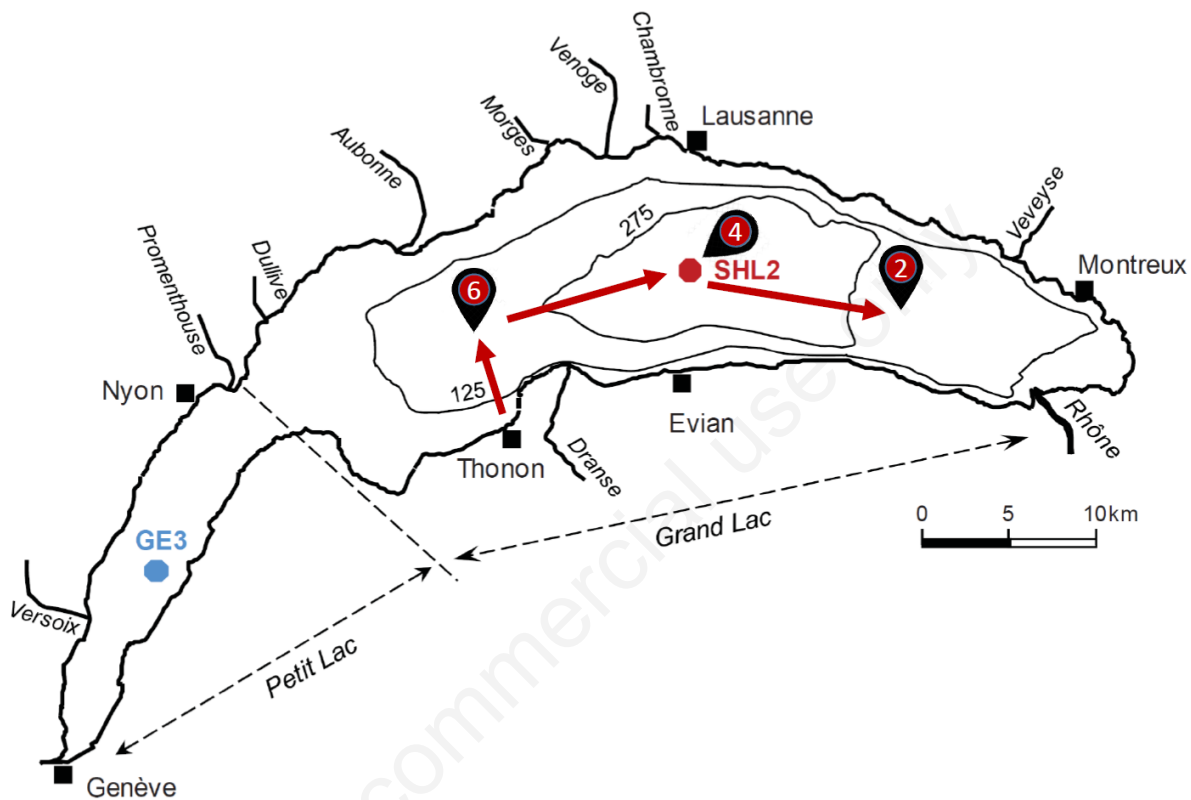


Fig. S1. Map of Lake Geneva and coordinates of the different sampling points selected during TRANSLEM: Site 2 (N 46° 26.206 / E 006° 46.848), Site 4-SHL2 (N 46° 27.207 / E 006° 35.654) and Site 6 (N 46° 25.061 / E 006° 24.957).

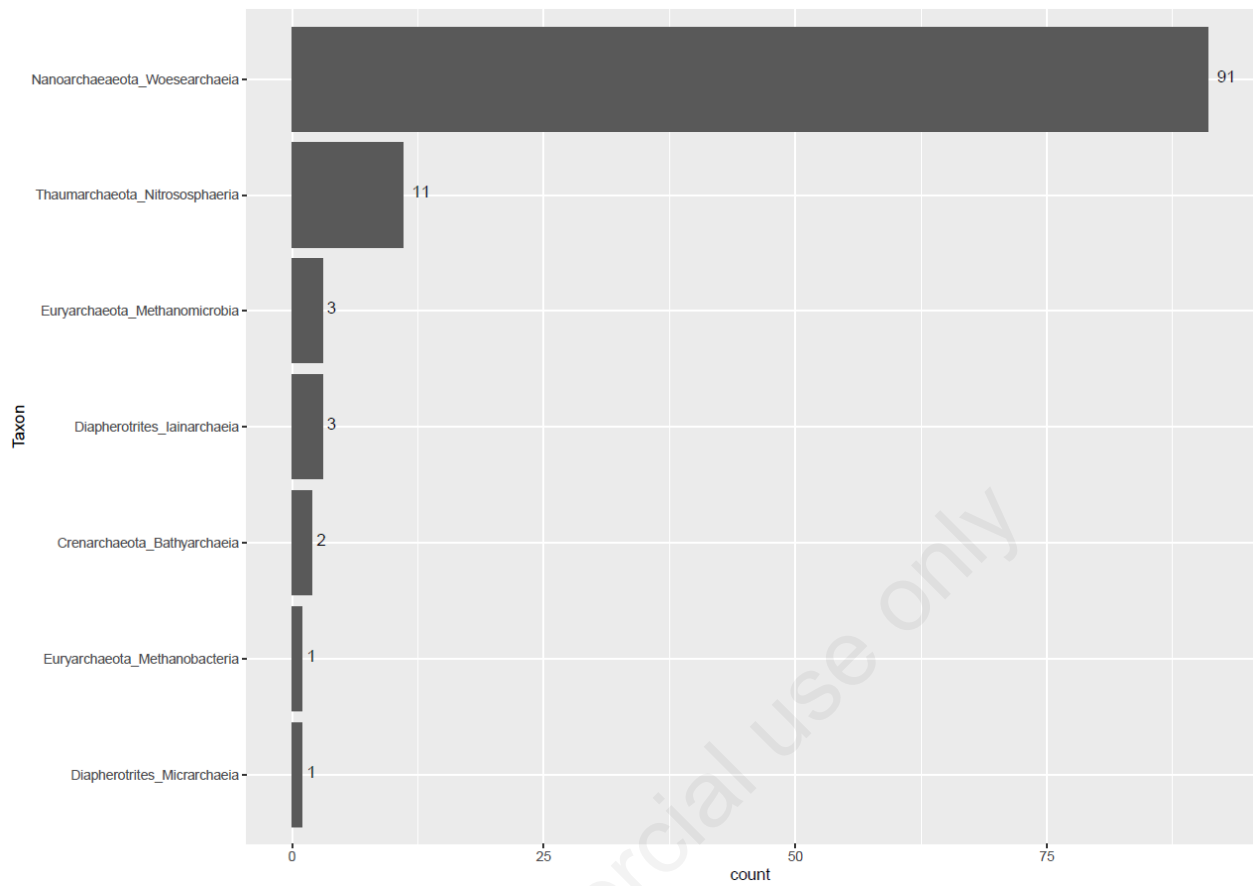


Fig. S2. Number of OTUs per archaeal class.

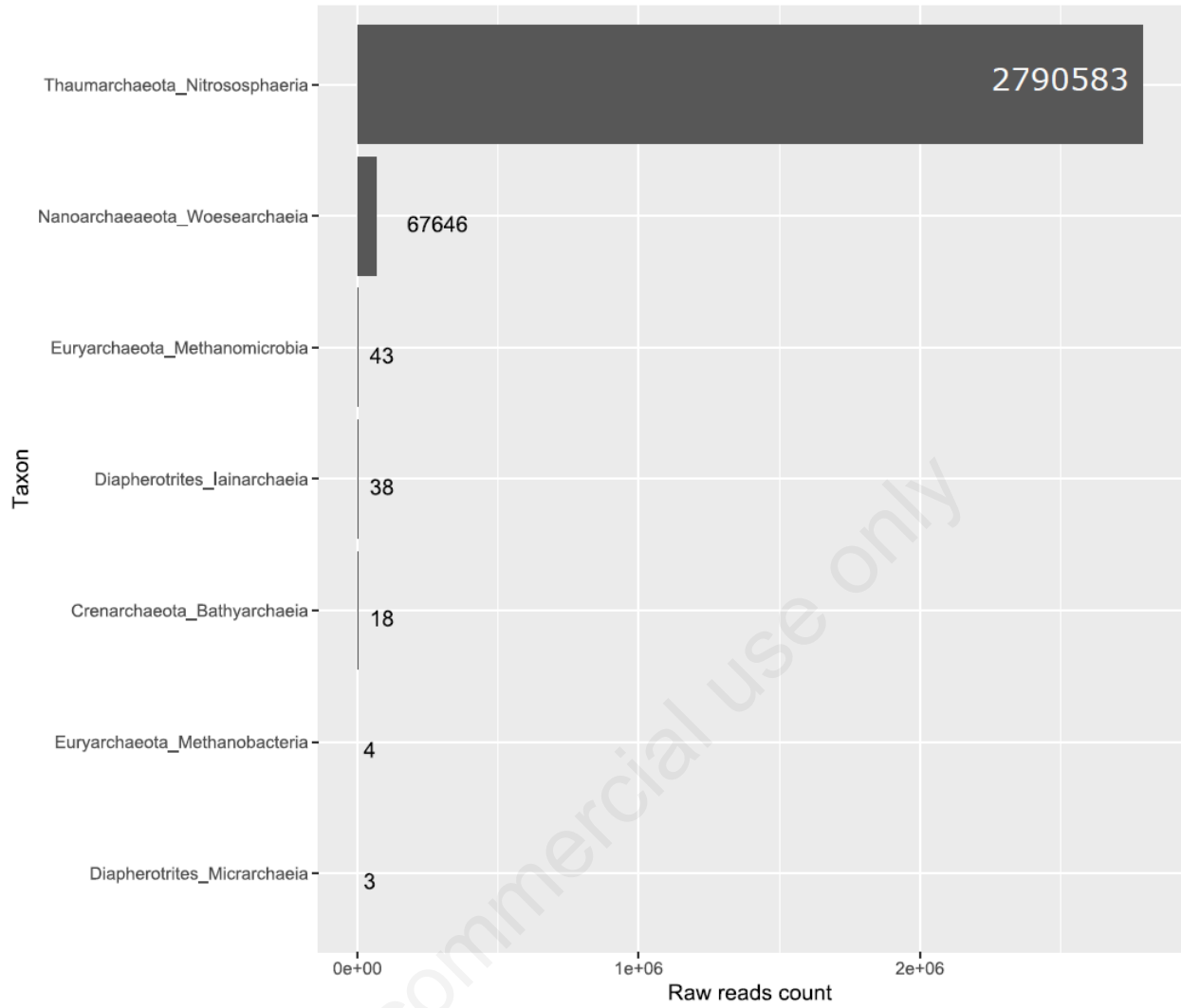


Fig. S3. Number of raw reads per archaeal class.

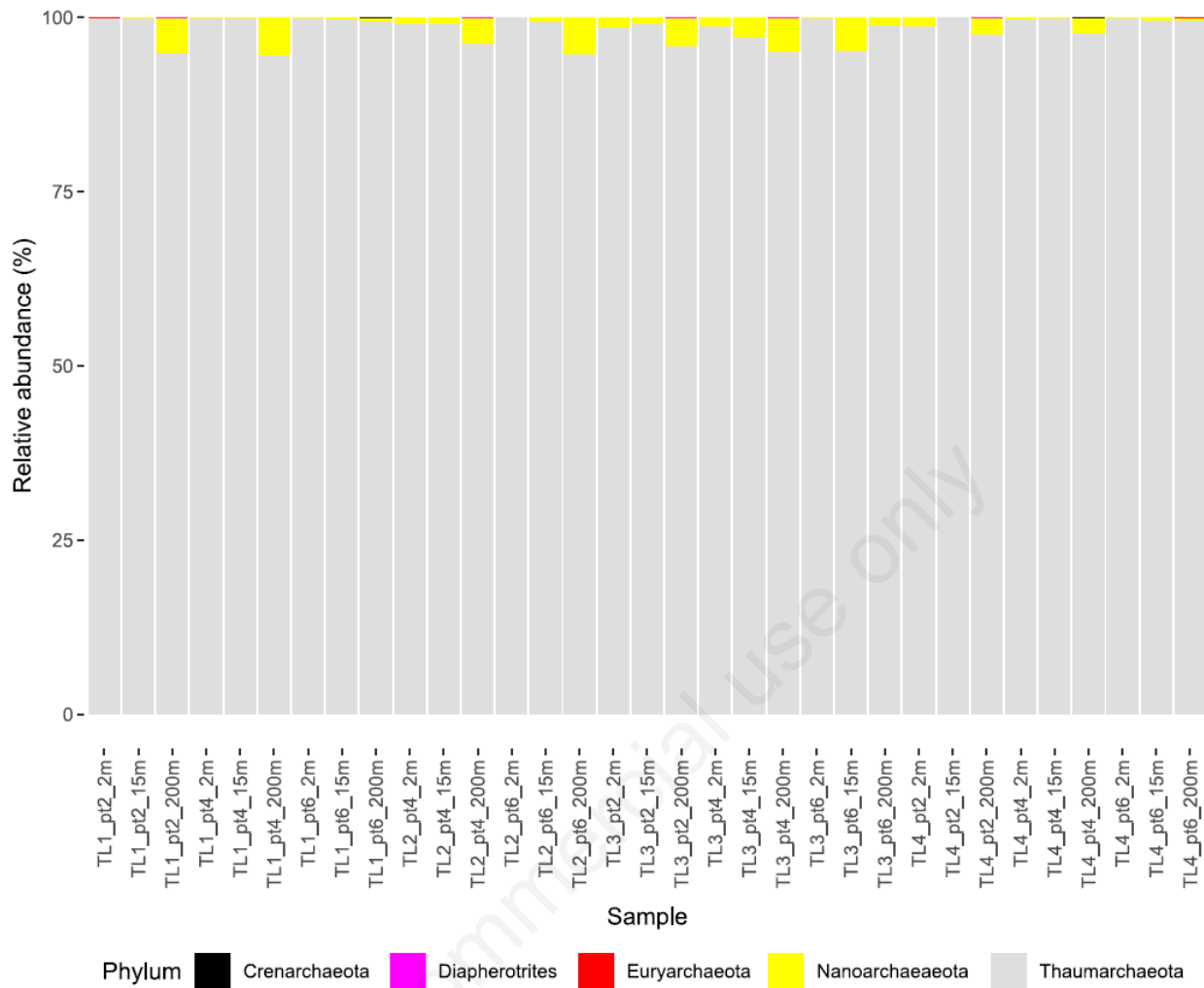


Fig. S4. Stacked histogram of archaeal phylum. Phylum abundances are represented by reads relative abundance per sample. Thaumarchaeota is dominant in all samples.

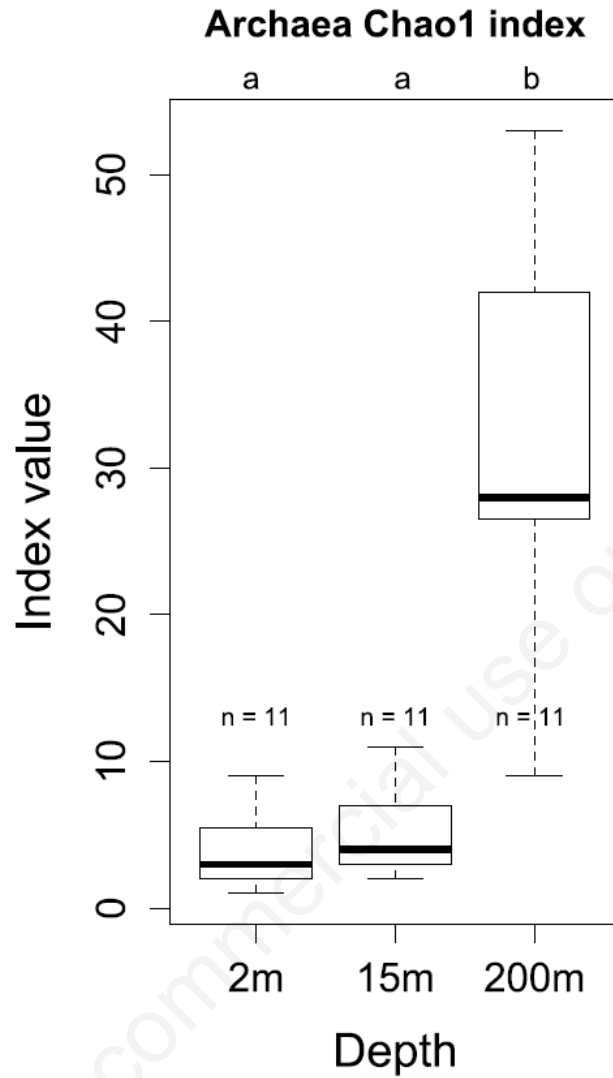


Fig. S5. Chao1 richness index for archaea showing significant differences between depths. N, number of samples; a, b, significant differences.

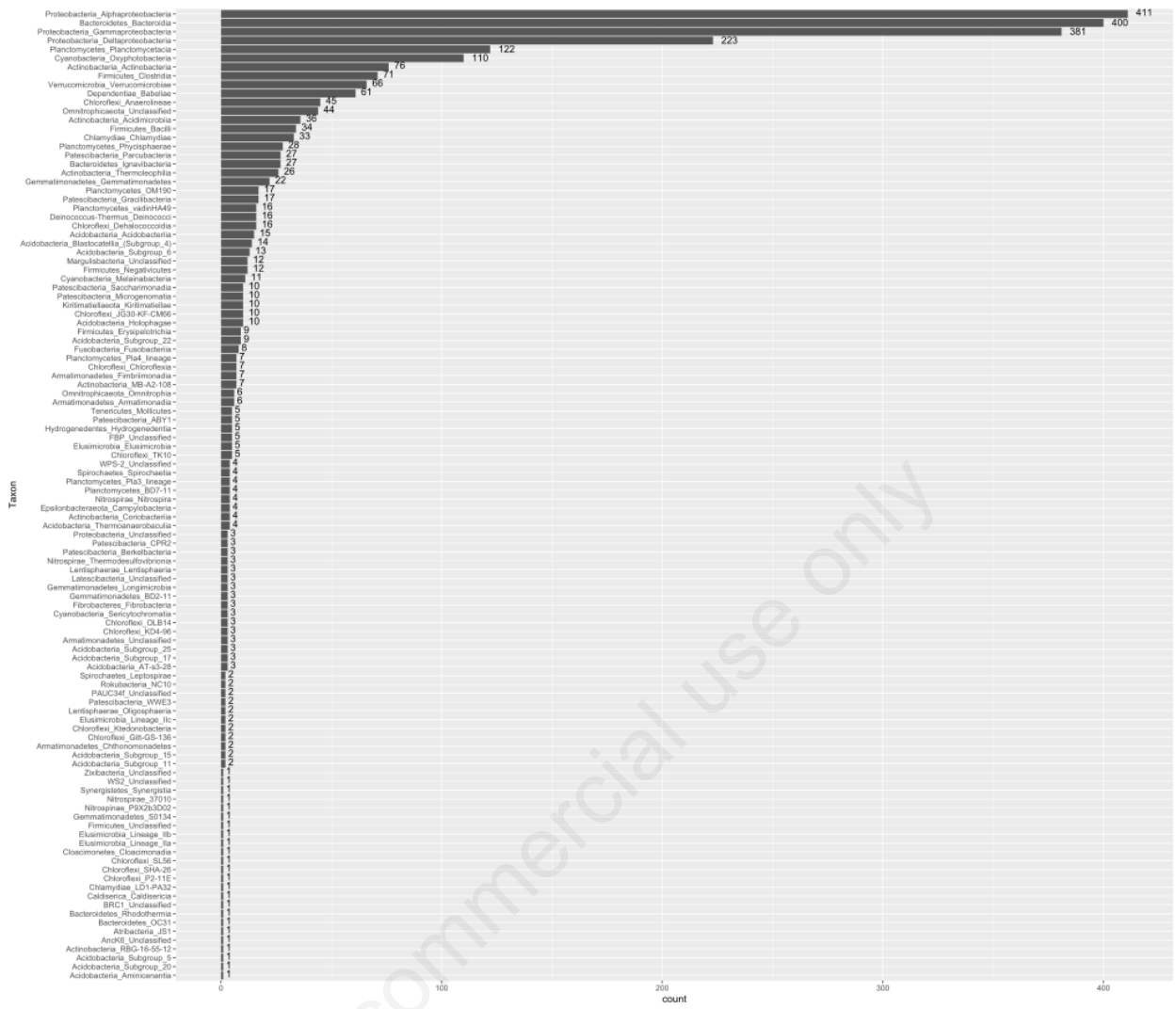


Fig. S6. Number of OTUs per bacterial class.



Fig. S7. Number of raw reads per bacterial class.

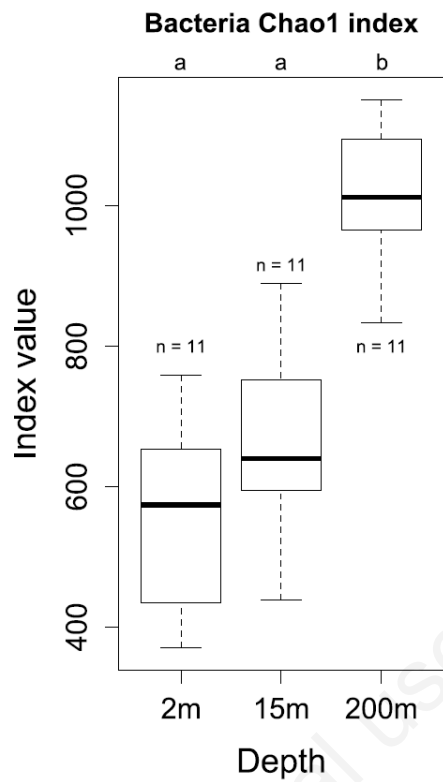


Fig. S8. Chao1 richness index for bacteria showing significant differences between depths. N, number of samples; a and b show where the differences are.

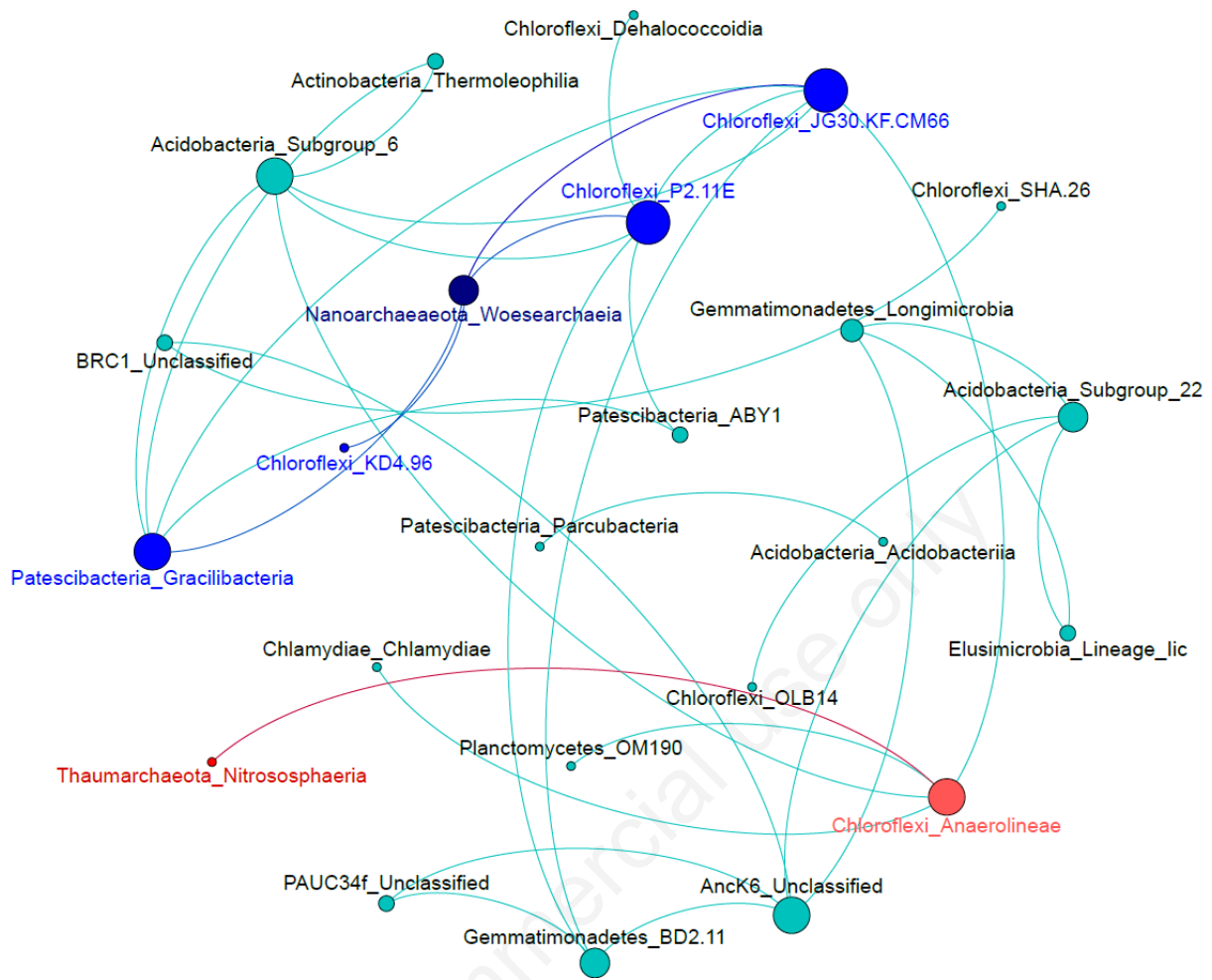


Fig. S9. Co-occurrence network between archaeal and bacterial phyla. Only phylum that passed the cutoff (correlation coefficient ≥ 0.9 and $p < 0.01$) are shown.

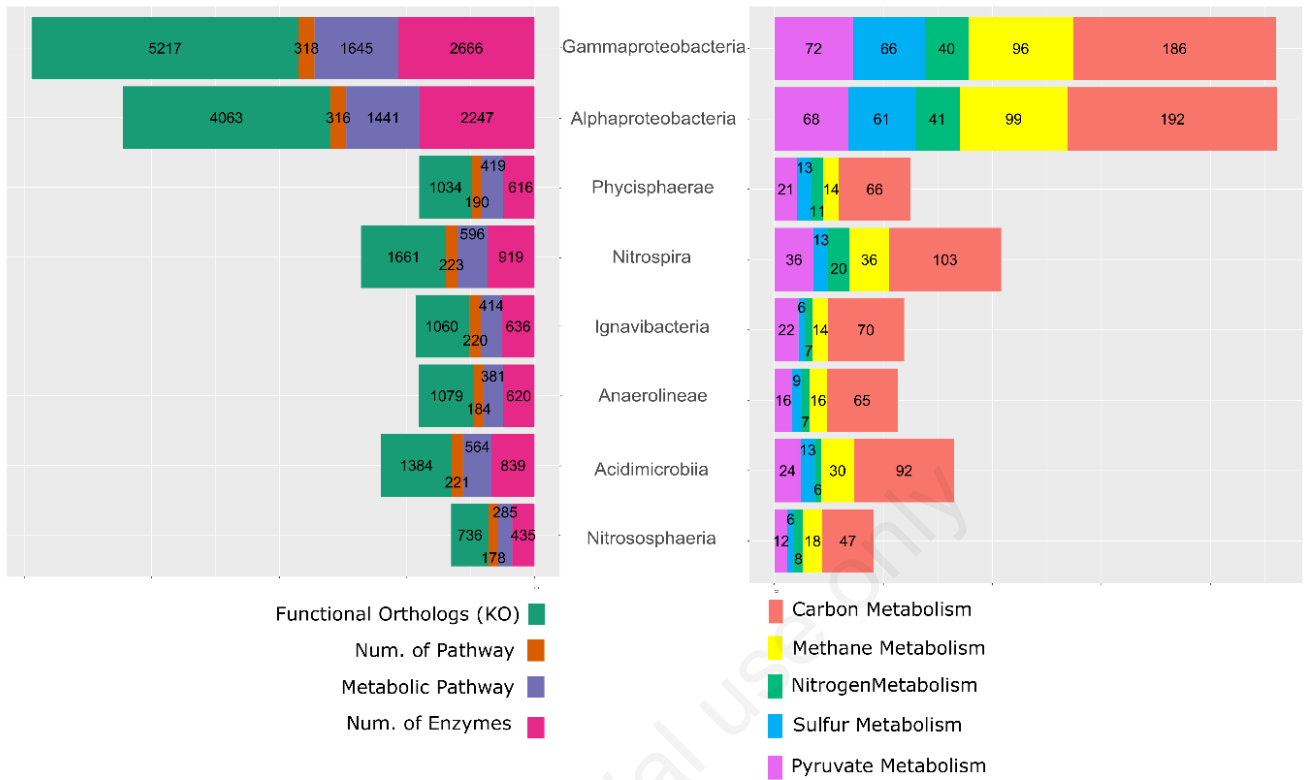


Fig. S10. Functional prediction based on PanFP software of the archaeal OTU 2 Nitrososphaeria and its bacteria associates showed in the co-occurrence network (Fig. 6). The archaea Nitrososphaeria is shown to possess less functional orthologs and pathway than its bacterial associates except for the nitrogen and methane metabolism.

Pairwise Intersections

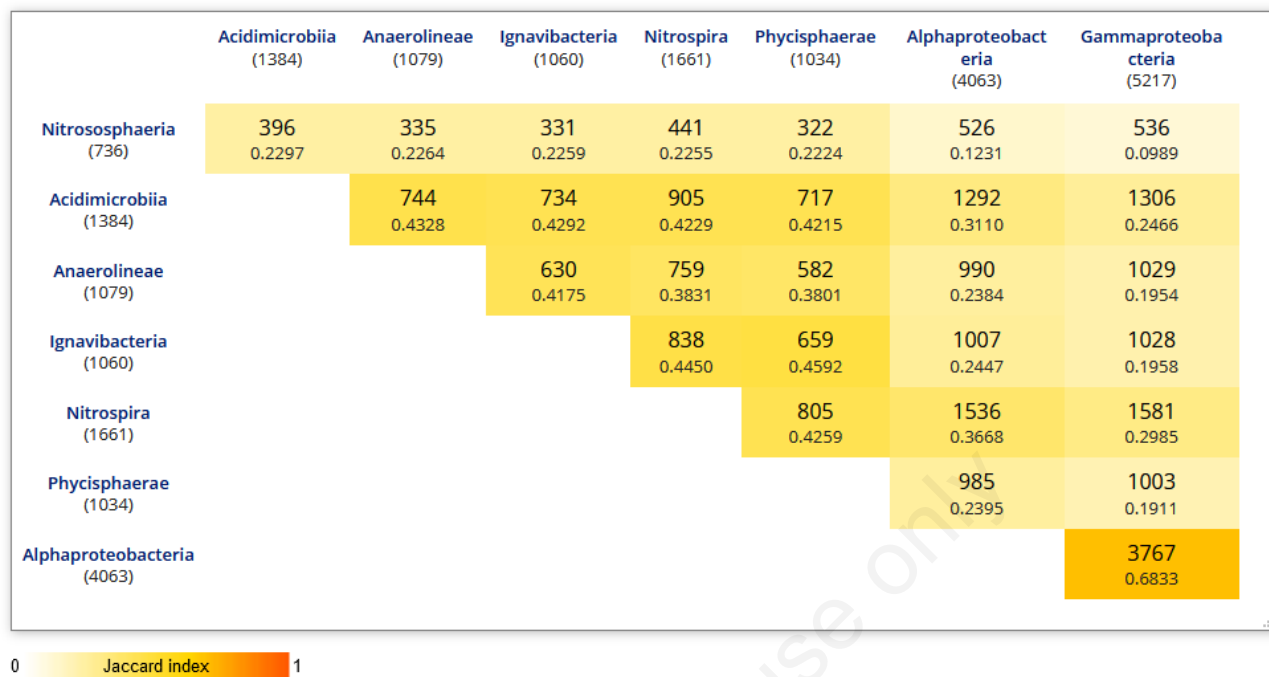


Fig. S11. Pairwise intersection comparison between the numbers of KOs generated *via* PanFP software. The presented class are from the co-occurrence network of Fig. 6.

Tab. S1. Results of the statistical analyses on richness and diversity indices for archaeal OTUs table.

Chao1	Site	Month	Depth
Kruskal p-value	0.5268	0.3121	1.859 ⁻⁰⁵ ***
Dunn test			2 m ≠ 200 m (0.0000) 15 m ≠ 200 m (0.0006) 2 m = 15 m (0.5)
Pielou	Site	Month	Depth
Kruskal p-value	0.4466	0.01758*	0.6221
Dunn test		TL1 ≠ TL3 (0.0084*)	
Shannon	Site	Month	Depth
Kruskal p-value	0.3802	0.1672	0.006761
Dunn test			2 m ≠ 200 m (0.0028) 15 m = 200 m (0.0605) 2 m = 15 m (0.4)
Simpson/ InvSimpson	Site	Month	Depth
Kruskal p-value	0.3579	0.1792	0.006761***
Dunn			2 m ≠ 200 m (0.0028) 15 m = 200 m (0.0605) 2 m = 15 m (0.4)

Tab. S2- Anosim and Adonis results performed on the Bray-Curtis dissimilarity matrix generated from the archaeal OTUs table in order to validate the NMDS graph.

	Site	Month	Depth
Anosim p-value	0.227	0.137	0.001***
Adonis p-value	0.592	0.26	0.003***

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Tab. S3- Simper test results and significance test analysis for archaeal OTUs table.

Group		Most influential OTU	Kruskal-Wallis (p-value)	Simper cumulative contributions	Average abundances in each compared treatment	Average dissimilarity between the two treatment
Month	February – June	2_Thaumarchaeota_Nitroso sphaeria	0.1792	48%	February > June	2%
	February – August	2_Thaumarchaeota_Nitroso sphaeria	0.1792	45%	February > August	3%
	February - November	2_Thaumarchaeota_Nitroso sphaeria	0.1792	46%	February < November	2%
	June - August	2_Thaumarchaeota_Nitroso sphaeria	0.1792	43%	June > August	3%
	June - November	2_Thaumarchaeota_Nitroso sphaeria	0.1792	45%	June < November	2%
	August - November	2_Thaumarchaeota_Nitroso sphaeria	0.1792	43%	August < November	3%
Site	pt2 - pt4	2_Thaumarchaeota_Nitroso sphaeria	0.3579	43%	pt2 = pt4	3%
	pt2 – pt6	2_Thaumarchaeota_Nitroso sphaeria	0.3579	45%	pt2 > pt6	2%
	pt4 – pt6	2_Thaumarchaeota_Nitroso sphaeria	0.3579	46%	pt4 > pt6	2%
Depth	2m – 15m	2_Thaumarchaeota_Nitroso sphaeria	0.006761***	45%	2m > 15m	2%
	2m – 200m	2_Thaumarchaeota_Nitroso sphaeria	0.006761***	47%	2m > 200m	3%
	15m – 200m	2_Thaumarchaeota_Nitroso sphaeria	0.006761***	40%	15m > 200m	3%

Tab. S4. Results of the statistical analyses on richness and diversity indices for bacterial OTUs table.

Chao1	Site	Month	Depth
Anova p-value	0.619	0.5945	7.71 ⁻¹⁰ ***
Tukey multiple comparisons test			2 m ≠ 200 m (0.000000) 15 m ≠ 200 m (0.0000003) 2 m = 15 m (0.07)
Pielou	Site	Month	Depth
Anova p-value	0.8347	0.4889	4.706 ⁻⁰⁶ ***
Tukey multiple comparisons test			2 m ≠ 200 m (0.000004) 15 m ≠ 200 m (0.0006579) 2 m = 15 m
Shannon	Site	Month	Depth
Anova p-value	0.8597	0.4149	4.322 ⁻⁰⁸ ***
Tukey multiple comparisons test			2 m ≠ 200 m (0.000000) 15 m ≠ 200 m (0.0000210) 2 m = 15 m (0.07)
Simpson	Site	Month	Depth
Anova p-value	0.9225	0.6342	4.757 ⁻⁰⁸ ***
Tukey multiple comparisons test			2 m ≠ 200 m (0.000016) 15 m ≠ 200 m (0.000001) 2 m = 15 m (0.6)
InvSimpson	Site	Month	Depth
Kruskal p-value	0.916	0.6285	2.335 ⁻⁰⁵ ***
Dunn test			2 m ≠ 200 m (0.0000) 15 m ≠ 200 m (0.0003) 2 m = 15 m (0.8)

Tab. S5. Anosim and Adonis results performed on the Bray-Curtis dissimilarity matrix generated from the bacterial OTUs table.

	Site	Month	Depth
Anosim p-value	0.982	0.006 ***	0.001 ***
Adonis p-value	0.975	0.011 **	0.001 ***

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Tab. S6. Simper test results with differences tested by statistical analysis for bacterial OTUs table.

Group		Most influential OTUs	Kruskal-Wallis (p-value)	Simper cumulative contributions	Average abundances in each compared treatment	Average dissimilarity between the two treatment
Month	February – June	5_Cyanobacteria_Oxyphoto bacteria	0.0003058 ***	14%	February > June	55%
	February – August	5_Cyanobacteria_Oxyphoto bacteria	0.0003058 ***	14%	February < August	54%
	February - November	5_Cyanobacteria_Oxyphoto bacteria	0.0003058 ***	15%	February < November	49%
	June - August	3_Cyanobacteria_Oxyphoto bacteria	0.3754	9%	June < August	50%
	June - November	4 Chloroflexi Anaerolineae	0.1434	8%	June < November	51%
	August - November	4 Chloroflexi Anaerolineae	0.1434	8%	August < November	48%
Site	pt2 - pt4	5_Cyanobacteria_Oxyphoto bacteria	0.4996	9%	pt2 > pt4	47%
	pt2 – pt6	5_Cyanobacteria_Oxyphoto bacteria	0.4996	8%	pt2 > pt6	48%
	pt4 – pt6	4 Chloroflexi Anaerolineae	0.6618	8%	pt4 > pt6	49%
Depth	2m – 15m	5_Cyanobacteria_Oxyphoto bacteria	0.8098	13%	2m > 15m	39%
	2m – 200m	4 Chloroflexi Anaerolineae	1.714 ⁻⁰⁵ ***	11%	2m < 200m	65%
	15m – 200m	4 Chloroflexi Anaerolineae	1.714 ⁻⁰⁵ ***	12%	15m > 200m	62%