Response to Reviewer 1

L6 – we suggest to change the sentence to 'a sequencing depth of up to 1.2×10^6 reads per sample'

The v6 region was the one provided by the Census of Deep Life (CoDL) sequencing program. This region has been rigorously tested by the CoDL and for maximizing capture of all known groups of bacteria and archaea the V6 region was targeted with a mixture of similar primers for each primer site. The sequence quality was also maximized by using a completely overlapping paired-end approach, for which the V6 region was perfect (described in Eren et al., 2013). More information about the bacterial and archaeal 16S rRNA gene v6 primers can be found at https://vamps.mbl.edu/resources/primers.php. This link will be added to the paper. In addition, the following two papers will be added to the reference list, because here the authors developed and used the protocol provided for the CoDL. The sequencer was a HiSeq1000.

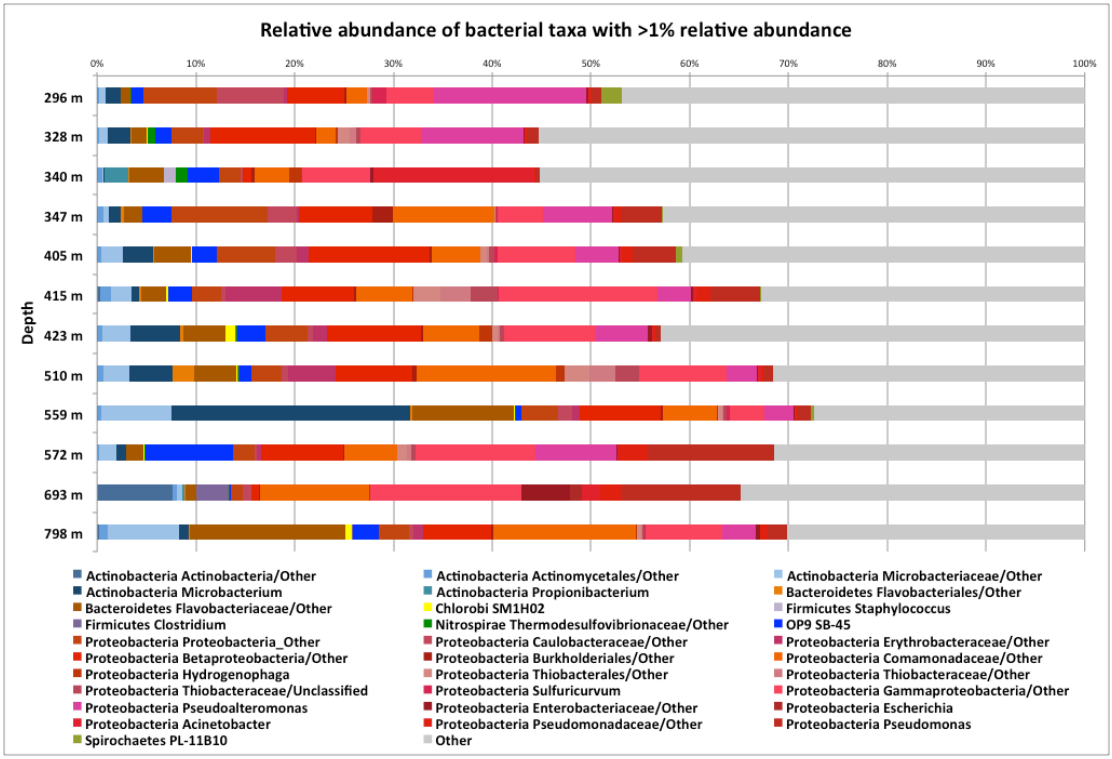
- Eren AM, Vineis JH, Morrison HG, Sogin ML. (2013). A filtering method to generate high quality short reads using Illumina paired-end technology. *PLoS One* **8**: e66643.

Results
'occurring' will be changed to 'detected'. In the previous section on p11 (L7-14) we present the most common bacterial and archaeal groups where the relative abundance of the reported genera (or equivalent group) was at least 1% of the sequence reads in any sample. 31 bacterial genera and 15 archaeal genera belonged to these 'most common' taxa. In the core community we have not set a threshold, but report the taxa that were detected in all samples, no matter how few the sequence reads were per taxon. We did not look at this data on OTU level, but only on genus level so we did not have any single sequence groups. In section 3.5, L4, the rare biosphere is considered the microbial taxa that were sporadically detected in only some of the samples, but not in all samples. This will be added to the text as; 'These groups represented the rare biosphere, i.e. taxa that were only detected at low relative abundance in some of the samples, but not in other'. Two different representations of these results are shown in the figures below for Bacteria and Archaea separately. Now all the 'not common' taxa are presented as 'Other'. Would one of these be easier to look at, maybe?

I tried to present the relative abundance data of the most common taxa as figures instead of tables (Tables 3 and 4). Two different representations in Figure 1 and 2. are presented below.

Relative abundance of the most common bacterial taxa:

		296 m	328 m	340 m	347 m	405 m	415 m	423 m	510 m	559 m	572 m	693 m	798 m
Actinobacteria	Actinobacteria/Other	0.04%	0.02%	0.09%	0.05%	0.09%	0.34%	0.09%	0.10%	0.09%	0.04%	7.63%	0.18%
	Actinomycetales/Other	0.17%	0.14%	0.45%	0.63%	0.28%	1.09%	0.43%	0.52%	0.35%	0.22%	0.41%	0.85%
	Microbacteriaceae/Other	0.63%	0.87%	0.10%	0.50%	2.27%	2.03%	2.88%	2.69%	7.07%	1.68%	0.61%	7.27%
	Microbacterium	1.56%	2.32%	0.10%	1.17%	3.08%	0.79%	5.01%	4.35%	24.23%	1.01%	0.02%	0.97%
	Propionibacterium	0.00%	0.00%	2.44%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.21%	0.00%
Bacteroidetes	Flavobacteriales/Other	0.05%	0.08%	0.09%	0.39%	0.05%	0.17%	0.31%	2.18%	0.21%	0.03%	0.06%	0.07%
	Flavobacteriaceae/Other	0.90%	1.58%	3.46%	1.81%	3.68%	2.58%	4.30%	4.24%	10.28%	1.70%	1.10%	15.76%
Chlorobi	SM1H02	0.04%	0.11%		0.01%	0.12%	0.17%	1.00%	0.16%	0.08%	0.07%		0.70%
Firmicutes	Staphylococcus	0.01%	0.01%	1.24%	0.01%	0.01%	0.01%	0.01%	0.00%	0.00%	0.01%	0.03%	0.01%
	Clostridium	0.00%	0.00%	0.01%	0.00%	0.02%	0.02%	0.02%	0.07%	0.01%	0.01%	3.27%	0.04%
Nitrospirae	Thermodesulfovibrionaceae/Other	0.08%	0.73%	1.23%	0.03%	0.04%	0.03%	0.12%	0.09%	0.06%	0.14%	0.10%	0.02%
OP9	SB-45	1.23%	1.62%	3.12%	2.92%	2.52%	2.41%	2.93%	1.25%	0.62%	8.91%	0.07%	2.66%
Proteobacteria	Proteobacteria_Other	7.43%	3.18%	2.21%	9.70%	5.81%	2.94%	4.24%	3.00%	3.70%	2.10%	1.19%	3.01%
	Caulobacteraceae/Other	6.72%	0.17%	0.23%	3.00%	2.22%	0.38%	0.51%	0.70%	1.34%	0.20%	0.96%	0.49%
	Erythrobacteraceae/Other	0.41%	0.58%	0.03%	0.21%	1.27%	5.73%	1.46%	4.75%	0.78%	0.45%	0.00%	0.99%
	Betaproteobacteria/Other	5.71%	10.64%	0.80%	7.38%	12.25%	7.35%	9.48%	7.76%	8.24%	8.39%	0.69%	6.87%
	Burkholderiales/Other	0.28%	0.09%	0.30%	2.11%	0.20%	0.22%	0.19%	0.50%	0.19%	0.05%	0.17%	0.18%
	Comamonadaceae/Other	2.08%	2.03%	3.58%	10.29%	4.89%	5.65%	5.69%	14.04%	5.44%	5.40%	11.06%	14.48%
	Hydrogenophaga	0.03%	0.14%	1.21%	0.06%	0.03%	0.12%	1.33%	0.95%	0.15%	0.01%	0.08%	0.11%
	Thiobacterales/Other	0.24%	1.26%	0.00%	0.04%	0.62%	2.78%	0.59%	2.46%	0.44%	0.95%	0.00%	0.38%
	Thiobacteraceae/Other	0.06%	0.59%	0.00%	0.03%	0.19%	3.05%	0.19%	2.65%	0.13%	0.48%		0.14%
	Thiobacteraceae/Unclassified	0.19%	0.45%	0.00%	0.04%	0.52%	2.75%	0.41%	2.38%	0.35%	0.36%		0.28%
	Sulfuricurvum	1.44%	0.02%	0.07%	0.16%	0.41%	0.04%	0.05%	0.04%	0.29%	0.01%	0.02%	0.03%
	Gammaproteobacteria/Other	4.84%	6.12%	6.78%	4.56%	7.82%	16.08%	9.28%	8.79%	3.49%	12.17%	15.27%	7.84%
	Pseudoalteromonas	15.34%	10.42%	0.13%	7.08%	4.45%	3.44%	5.20%	3.07%	2.95%	8.22%	0.00%	3.40%
	Enterobacteriaceae/Other	0.22%	0.15%	0.31%	0.10%	0.09%	0.18%	0.49%	0.15%	0.06%	0.12%	4.98%	0.34%
	Escherichia	0.00%		0.02%		0.00%	0.00%	0.00%	0.00%			1.15%	0.00%
	Acinetobacter	0.13%	0.06%	16.27%	0.06%	0.18%	0.37%	0.10%	0.28%	0.10%	0.04%	1.89%	0.07%
	Pseudomonadaceae/Other	0.23%	0.28%	0.10%	0.69%	1.15%	1.42%	0.22%	0.26%	0.31%	2.97%	2.17%	0.76%
	Pseudomonas	1.00%	0.99%	0.49%	4.14%	4.31%	5.03%	0.53%	0.96%	1.24%	12.80%	12.06%	1.92%
Spirochaetes	PL-11B10	2.03%	0.04%	0.00%	0.09%	0.63%	0.05%	0.07%	0.05%	0.36%	0.02%		0.05%
Other		46.89%	55.30%	55.14%	42.73%	40.79%	32.78%	42.89%	31.54%	27.41%	31.45%	34.81%	30.11%



Relative abundance of the most common archaeal taxa:

		296 m	328 m	340 m	347 m	405 m	415 m	423 m	510 m	559 m	572 m	693 m	798 m
Crenarchaeota	Other	0.63%	1.26%	2.30%	1.35%	0.40%	2.70%	0.89%	1.97%	0.59%	0.23%	3.46%	5.53%
	Thaumarchaeota/Other	2.64%	1.54%	2.56%	2.31%	2.31%	11.78%	8.31%	9.92%	10.59%	0.48%	2.79%	0.02%
Euryarchaeota	Other	20.35%	14.12%	41.59%	20.43%	25.85%	28.86%	12.89%	29.18%	25.60%	63.34%	26.61%	30.92%
	Methanobacteriales/Other	0.14%	0.08%	0.45%	0.21%	0.06%	1.16%	0.06%	0.39%	0.53%	0.10%	3.12%	3.08%
	MSBL1/Other	0.05%	0.02%	0.42%	0.06%	0.01%	0.43%	0.02%	0.06%	0.16%	0.07%	0.80%	1.38%
	SAGMEG-1	0.17%	0.12%	0.67%	0.67%	0.09%	1.44%	0.15%	0.29%	0.69%	0.13%	4.38%	6.71%
	Methanobacteriaceae/Other	0.11%	0.26%	0.76%	0.13%	0.31%	0.40%	0.17%	2.38%	0.57%	0.55%	0.90%	0.19%
	Methanobacteiaceae/Unclassified	0.02%	0.01%	0.30%	0.04%	0.04%	0.01%	0.05%	7.64%	0.08%	0.27%	0.20%	0.06%
	Methanobacterium	0.05%	0.07%	3.65%	0.05%	0.06%	0.13%	0.06%	0.24%	0.18%	0.70%	0.02%	0.24%
	Methanomicrobia/Other	3.41%	5.07%	0.66%	2.87%	2.25%	1.41%	3.13%	2.02%	2.81%	0.68%	1.91%	3.97%
	Methanosarcinales/Other	10.72%	13.29%	3.59%	10.14%	11.93%	2.67%	10.37%	2.87%	6.54%	2.33%	5.75%	0.88%
	ANME-2D	42.90%	50.29%	21.65%	43.21%	37.68%	9.09%	56.11%	19.99%	27.00%	18.45%	10.62%	4.88%
	E2/Other	11.37%	3.34%	3.95%	3.83%	4.59%	21.23%	1.94%	5.40%	13.61%	4.36%	6.16%	2.84%
	Marine group II/Other	0.38%	0.15%	0.25%	0.35%	0.38%	0.75%	0.15%	3.31%	1.53%	0.05%		
	Marine group II/Unclassified	0.02%	0.22%	0.69%	1.60%	0.14%	0.49%	0.23%	0.71%	0.05%	0.07%		0.01%
Other		7.05%	10.15%	16.52%	12.74%	13.90%	17.45%	5.45%	13.64%	9.46%	8.19%	33.28%	39.28%

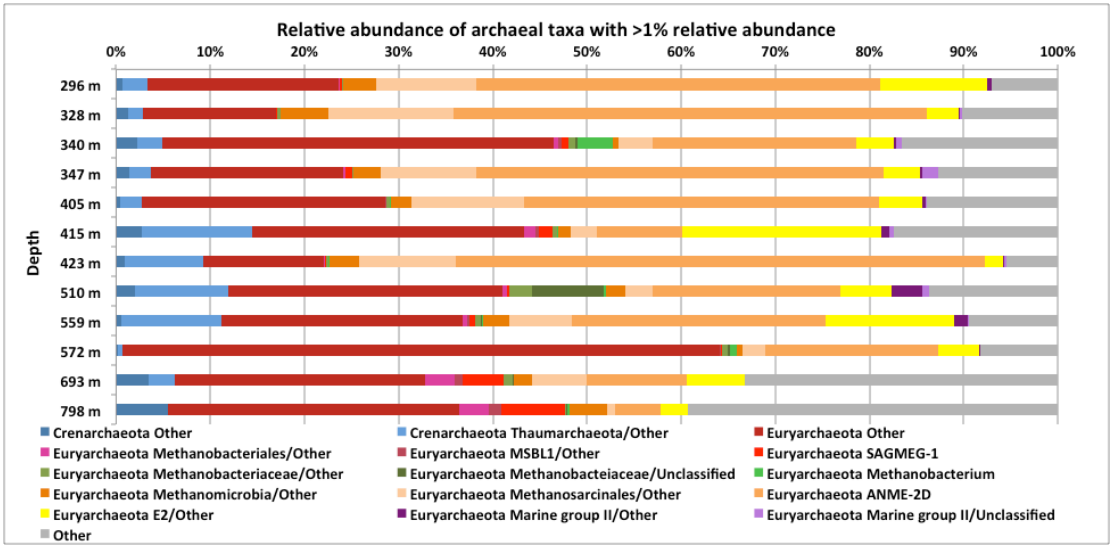
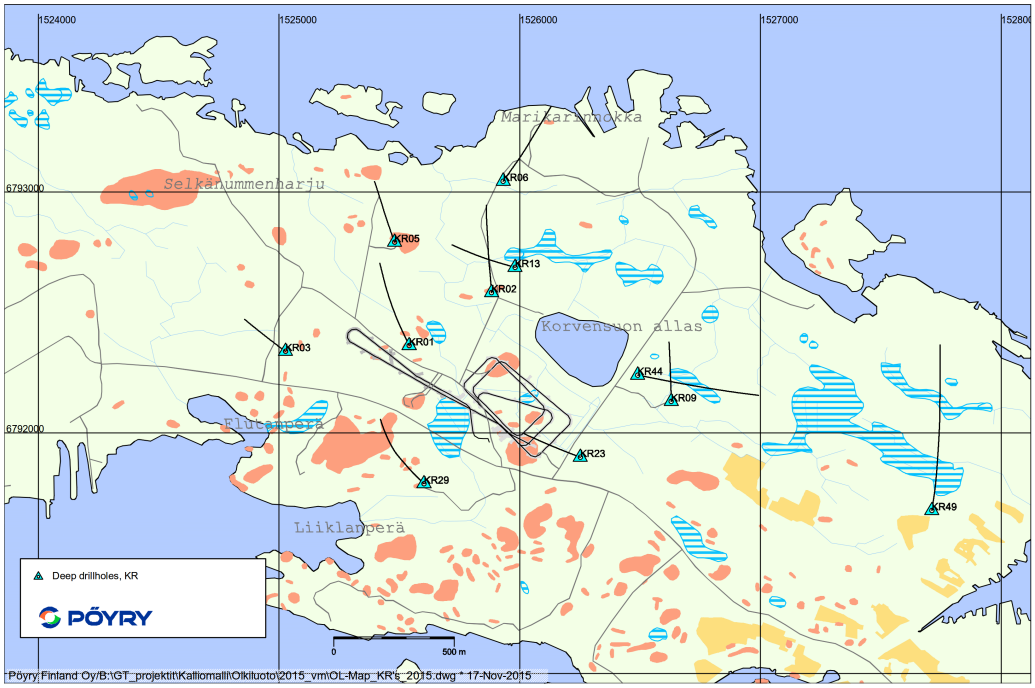


Figure 3 – I'm not sure I understand what you mean. The depth of each sample is given in the sample names in this figure and all the chemistry that the calculations are based on is given in Table 1. This sample is chemically very different from the other deep samples in this study. There is a peak in the concentration of organic carbon (NPOC) and sulphate, which are more typical for the samples from lesser depth, while at the same time the salinity of the sample is high, which is typical for the deeper samples. I could increase the font size of the sample names, if that helps? I also have a map of the Olkiluoto site showing the positions of all the boreholes we sampled for this study and the map can be included in the paper (Fig3).



Map of Olkiluoto. The boreholes used in this study are marked with a turquoise triangle and the attached black line depicts the direction of the borehole. (with courtesy of Pöyry Oy, Nov 17th, 2015 by Eemeli Hurmerinta)

The samples were collected over several years. This is shown in Table 1 (first row).

P13, L1=> language check: This sentence will be simplified to '42 bacterial and 59 archaeal genera correlated significantly with other microbial genera.'

Figure 4. The use of 'communities' will be revised and the whole figure will be recalculated.

The PICRUSt analysis was done on the total communities in the different samples, but the co-occurrence data in Fig 4 displays only how the different co-occurring taxa connect with each other. In other words, in Fig 4 only taxa with strong positive correlation to other taxa are shown. This is not a representation showing the individual samples, only microbial groups that hang together.

The image does not *per se* show depth or location, or samples, but instead the interactions between different microbial groups regardless of sample. The microbial taxa are grouped according to how well they correlate to each other. Taxa always occurring together are clustered together in the network and taxa never occurring together fall into different groups. Each tightly correlating group has its own color for easier viewing. The bigger the dot for each taxon is the more numerous are the number of taxa the specific taxon correlates with. We found seven distinct correlation groups that all have their own color in the graph. The geochemical parameters that the members of each groups correlated with most are indicated in red text by each group. This data is obtained from the Pearson correlation calculations that are presented in Tables 5-8.

The taxonomical designations in Figure 4 are on genus level. The fact that some are given as family or other is simply because the databases for some uncultured groups do not go deeper than that. If you look at Supplement tables 1 and 2 where the whole dataset is presented you can see that the classification is done to the level as close to genus as possible. For example, in Supplement table 1, Phylum AC1, we found AC1 sequences that fell into two different groups. One group can't be classified to other than AC1 (Unclassified) and the other group was classified to be B04R032, but no more detailed classification was available. This means that the result we got read p_AC1; c_ B04R032; o_; f_; g_; and we cleaned it up for clarity.

I'll see what I can do about the size of Fig 4. This is all that was allowed in this version of the type set. If no other is possible I'll turn the figure 90° and change the fonts.

However, the whole figure will be revised due to the fact that uncorrected significance values have been used. This means that the data will be recalculated for the network analysis and a new figure will be presented.

Rouhg – we mean a not very fine-scaled estimation, not inaccurate. Meant more as an approximation. *Rough prediction' will be changed to 'approximate estimation'. Langille et al. (2013) describes the NSTI as 'the sum of phylogenetic distances for each organism in the OTU table to its nearest relative with a sequenced reference genome, measured in terms of substitutions per site in the 16S rRNA gene and weighted by the frequency of that organism in the OTU table'. If the NSTI value is given as 0.282 it means that the test subject shares about 78% 16S rRNA gene similarity to its nearest sequenced match, i.e. not very close. It is close enough, however, to be able to say that this uncultured putative methanogen has methane metabolism and that it probably uses, say, methanol for its methanogenesis, because all the closest relatives do so and it falls within a bigger archaeal cluster that all use methanol. Whether this is the case in reality is not sure, of course, but here we are trying to link taxonomical data from uncultured communities to what is known for cultured and tested species. I agree that metagenomic analysis would be better, and we have done a few. The metagenomes show quite the same as the PICRUSt analyses, but the metagenomes also suffer from uncertainty, since we get good matches only to properly annotated and well-known species. Agreed, the sequence data and predicted gene data etc would be obtained. Nevertheless, the biomass obtained from the collected samples is very low and they were not originally collected for metagenomic analysis. The DNA yield is low, which means that the DNA needs to be amplified before sequencing (or at least had to be, then). These samples were also a part of the Census of Deep Life sequencing effort and the data is here published if the name of this sequencing project and we tried to get out as much as possible from the data. Now we can continue to prove these estimations right or wrong.

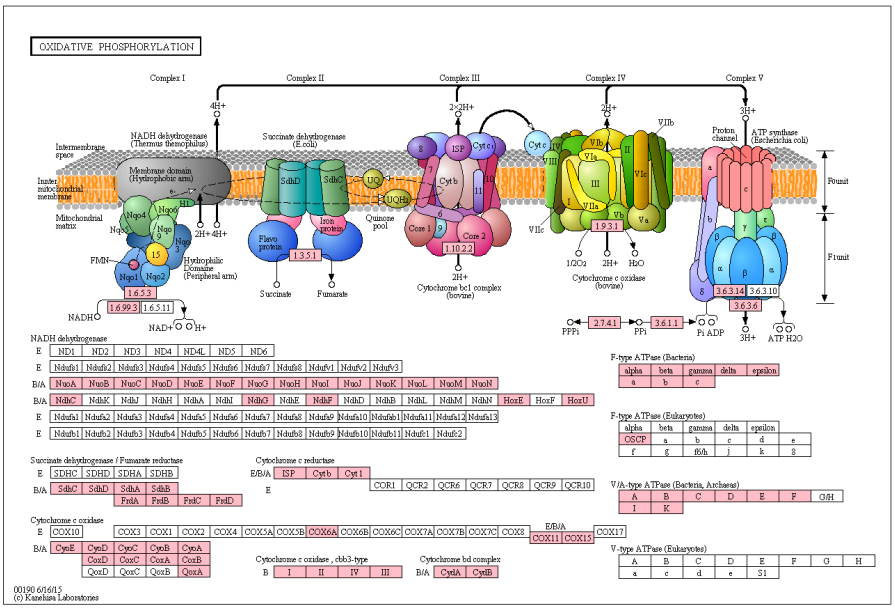
The NSTI of environmental samples is higher than that of the human microbiome, which is stated in Langille et al. (2013). This can be added to the text with the explanation that a higher NSTI value means less accuracy.

Table 9 presents the percentage of predicted genes from each sample that is connected to a specific metabolic pathway. Of course, some genes (or enzymes) function in many pathways, but the ones presented in Table 9 were the best covered ones. The table shows the distribution of predicted genes. From these 'whole pathways' were extracted simply by checking if a gene (or actually KO number) was present in a sample and how abundant that gene was in the sample. In the discussion only whole pathways are included.

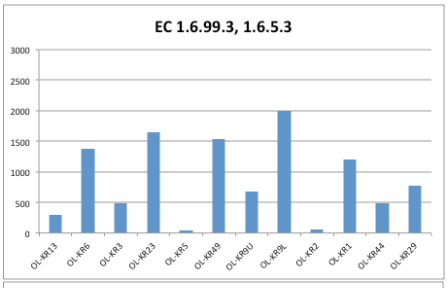
ANME-2D – well, this is a problem, because the nitrate level is below the detection limit of the assay. Ntot and ammonia could be measured, but not nitrate or nitrite. This could mean that nitrate is a rate limiting factor for the ANME-2D or that the ANME-2D are using the nitrate released from a possible ammonia oxidation process immediately. Or, the AMNE-2D are either doing something else or could be using sulphate as electron acceptor. However, sulphate for ANME-2D has not yet been shown to function as TEA, but it is possible.

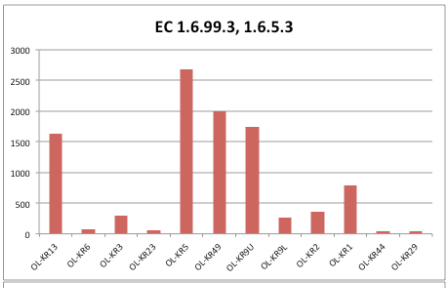
The NSTI values will be changed from 'great' to 'high'

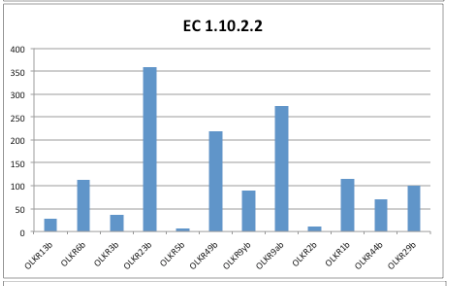
Oxidative phosphorylation was determined based on 114 KO numbers detected in the predicted genes, which occur in this pathway. In the figures below the predicted KO numbers were plotted on the Kegg map for oxidative phosphorylation, pink means hit, and the number of hits belonging to the EC numbers of the different enzyme complexes are displayed separately for the bacteria and the archaea.

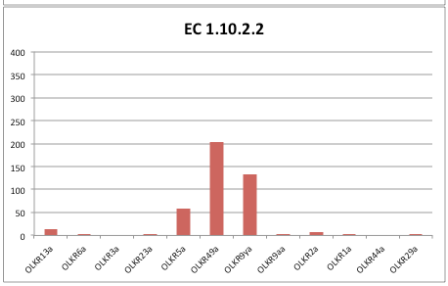


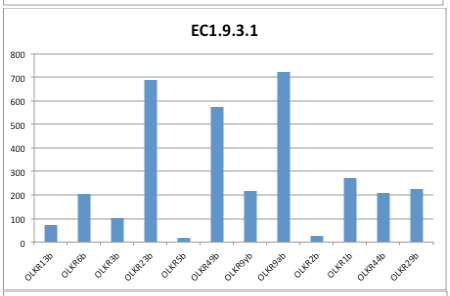
Bacteria Archaea

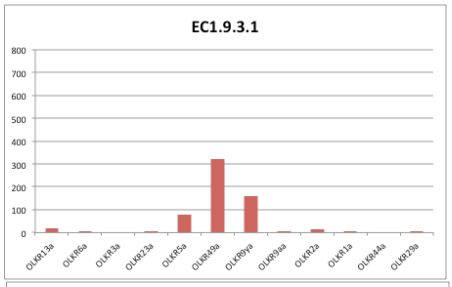

EC 1.6.99.3, 1.6.5.3
3000
2500
2000
1500
1000
500
0
OL-KR13
OL-KR6
OL-KR3
OL-KR23
OL-KR5
OL-KR49
OL-KR9U
OL-KR9L
OL-KR2
OL-KR1
OL-KR44
OL-KR29

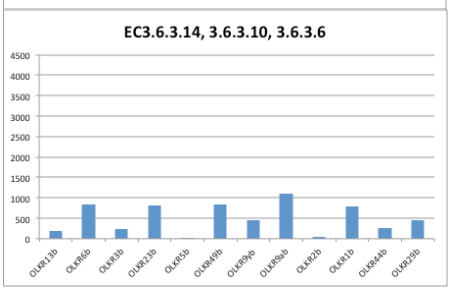

EC 1.6.99.3, 1.6.5.3
3000
2500
2000
1500
1000
500
0
OL-KR13
OL-KR6
OL-KR3
OL-KR23
OL-KR5
OL-KR49
OL-KR9U
OL-KR9L
OL-KR2
OL-KR1
OL-KR44
OL-KR29

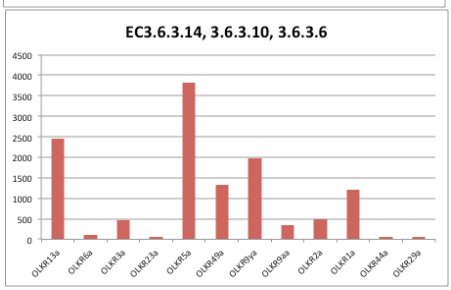

EC 1.10.2.2
400
350
300
250
200
150
100
50
0
OLKR13b
OLKR6b
OLKR3b
OLKR23b
OLKR5b
OLKR49b
OLKR9yb
OLKR9ab
OLKR2b
OLKR1b
OLKR44b
OLKR29b


EC 1.10.2.2
400
350
300
250
200
150
100
50
0
OLKR13a
OLKR6a
OLKR3a
OLKR23a
OLKR5a
OLKR49a
OLKR9ya
OLKR9aa
OLKR2a
OLKR1a
OLKR44a
OLKR29a


EC1.9.3.1
800
700
600
500
400
300
200
100
0
OLKR13b
OLKR6b
OLKR3b
OLKR23b
OLKR5b
OLKR49b
OLKR9yb
OLKR9ab
OLKR2b
OLKR1b
OLKR44b
OLKR29b


EC1.9.3.1
800
700
600
500
400
300
200
100
0
OLKR13a
OLKR6a
OLKR3a
OLKR23a
OLKR5a
OLKR49a
OLKR9ya
OLKR9aa
OLKR2a
OLKR1a
OLKR44a
OLKR29a


EC3.6.3.14, 3.6.3.10, 3.6.3.6
4500
4000
3500
3000
2500
2000
1500
1000
500
0
OLKR13b
OLKR6b
OLKR3b
OLKR23b
OLKR5b
OLKR49b
OLKR9yb
OLKR9ab
OLKR2b
OLKR1b
OLKR44b
OLKR29b


EC3.6.3.14, 3.6.3.10, 3.6.3.6
4500
4000
3500
3000
2500
2000
1500
1000
500
0
OLKR13a
OLKR6a
OLKR3a
OLKR23a
OLKR5a
OLKR49a
OLKR9ya
OLKR9aa
OLKR2a
OLKR1a
OLKR44a
OLKR29a