Supporting Information

Figure S1 Ace Lake and surrounds in the Vestfold Hills. A) Schematic of the Vestfold Hills adapted from Gibson (Gibson, 1999). The Vestfold Hills region is a coastal, ice-free, Antarctic oasis approximately 400 km^2 in area that is remarkable for the presence of hundreds of lakes, ranging in salinity from fresh to hypersaline (Gibson, 1999). Most of the saline lakes were originally pockets of seawater and retain a marine-derived biota (Gibson, 1999; Roberts & McMinn, 1999; Zwartz *et al.,* 1998; Rankin *et al.,* 1999; Crommer *et al.,* 2005; Andrássy & Gibson, 2007). B) Aerial photograph of Ace Lake and surrounds. Ace Lake (68.473°S, 78.189°E) was formed at the end of the Quaternary ~12,000 yr BP, and was initially fresh (Rankin *et al.,* 1999; Crommer *et al.,* 2005; Coolen *et al.,* 2004; Coolen *et al.,* 2006). Seawater invaded the lake basin during an early Holocene sea level highstand (~7,000 yr BP) that resulted from Holocene sea level rise outstripping isostatic rebound resulting from reduction of mass of the polar ice cap through melting. The lake was reformed after subsequent sea level fall (due to continued isostatic rebound after stabilization of the global sea level) after ~5,000 yr BP, and has remained saline since (Rankin *et al.,* 1999; Crommer *et al.,* 2005; Coolen *et al.,* 2004; Coolen *et al.,* 2006). The lake is exposed to air temperatures ranging annually from approximately -40 to +10ºC, and the lake surface is ice covered for ~11 months of the year (some years not completely melting). Below the ice, the mixolimnion is mixed by brine exclusion during ice formation in winter, and wind turbulence occuring after the ice has melted may also contribute to mixing. The lake is cold ranging from ~0 °C in the mixolimnion to <3.5 °C in the monimolimnion. Supersaturated levels of oxygen are seasonally generated in the mixolimnion. The anoxic waters (14-23 m) support stable increasing gradients of salt (up to 4.3%), methane (saturated below 20 m, ~5mM) and H_2S (up to 8mM), and decreasing gradients of sulfate (essentially depleted below 19 m). The surface of Ace Lake is ~9 m above sea level, it has a maximum depth of 25 m, and is approximately 150 m from the nearest marine environment, Long Fjord. The drainage basin of the lake is small (35.5 ha), and consists of low rocky hills comprising Archaean gneisses with limited soil development. Ace Lake receives water from melt of snowbanks that develop in the lee of hills and boulders in winter, and direct capture of precipitation in summer. There is no evidence of groundwater inflow, and no surface or groundwater outflow, although the lake surface has been within ca 1 m of the sill separating the lake from Long Fjord since the 1980s. Few if any lichens or mosses occur within the drainage basin, and higher plants are absent. Adelie Penguins visit the area during summer, although none breed in the drainage basin. Snow Petrels and Wilson's storm-petrels have been recorded breeding, but only in low numbers (<10 pairs). C) Sampling site on Ace Lake, December 2006. D) Datasets, annotation and assembly metrics. E-H) Ace Lake depth-profiles December 2006. A Sonde probe (YSI model 6600) shown in Fig. S1C was used to record: (E) temperature (yellow, Celsius), pH (blue, pH units); (F) salinity (green, ppt); (G) dissolved oxygen (pink, % saturation); (H) turbidity (brown, NTU).

Figure S2 16S rRNA gene sequence analysis. The percentage composition of each clade in each sample is displayed as barplots in iTOL (Letunic & Bork, 2006) for the 0.1 μm (red), 0.8 μm (green) and 3.0 μm (blue) fractions. A) 5 m; B) 11.5 m; C) 12.7 m; D) 14 m; E) 18 m and F) 23 m. The proportion of each clade has been normalized for each size

fraction from each depth. The height of the bars represents the relative contribution of the clade and is comparable only within each size fraction from each depth. The SAR11 clade is the major contributor to the *Rhizobiales*.

Figure S3 18S rRNA and 16S-23S rRNA ITS sequence analysis. A) ARB neighbour joining phylogenetic reconstruction identifying the positions of 18S rRNA gene sequences retrieved from Ace Lake metagenome data. Number of gene copies relating to each organisms and the depth at which genes were identified are shown to the right. Microsporidia were used as the outgroup. "Ace" sequences shown in bold are from this study. All except four of the 18S rRNA gene sequences were retrieved from the mixolimnion; the four were from the oxycline and represented a novel clade of the Euglenozoa, the Symbiontida, which have been identified from marine anoxic environments, and contain epibiotic bacteria (Yubuki *et al.,* 2009). The proportion of eucarya in the mixolimnion estimated from reads and absolute numbers of rRNA genes was ~5% of the microbial population. This may over-estimate the number of eucarya as the rRNA counts have not been normalized for copy number which will be as low as a single copy for oligotrophic bacteria (*e.g.* SAR11 clade) compared with >10 copies for *Mesopedinella arctica* and >10,000 copies for dinoflagellates (*e.g. Akashiwo sanguinea*) (Zhu *et al.,* 2005). Eucaryal sequences in the mixolimnion represented eight different obligately or potentially phototrophic organisms of marine origin. These included three stramenopiles, two chlorophytes, the potentially karyokleptic coupling of alveolate *Myrionecta rubra* and the cryptophyte *Cryptophyta cryophila* (Johnson *et al.,* 2007), and the obligately heterotrophic metazoan, *Paralabidocera antarctica*. The data indicate that bacteria, and not eucarya, dominated the cellular population in the mixolimnion at the time of sampling. B) Association of SAR11 clade representatives determined from sequence variation of the 16S-23S rRNA ITS region. Ace lake contains ITS sequences associated with three distinct SAR11 clusters: SAR11 surface 1, SAR11 surface 3 Antarctic, and SAR11 deep (Brown & Fuhrman, 2005). SAR11 deep, contains sequences retrieved from deep (>50m – 3000m) marine samples (Field *et al.,* 1997). Sequences in purple have been retrieved from polar regions, while sequences in red have been retrieved from tropical regions.

Figure S4 Microbial cells and virus-like particles counted by epifluorescence microscopy. A) Counts were made for whole water samples of Ace-Lake as a function of depth. Error bars represent two standard deviations. No VLPs were observed at 12.7 m depth and the value reported represents the detection limit of the counting procedure (*i.e.* one cell detected in one field of view); the true number is likely to be lower than this. B) Microscopic analysis of different filter-size fractions of water samples from Ace Lake highlighting the efficacy of the size-fractionation protocol. The bar in each panel is 20 μm long. The absence of very small fluorescing VLPs in contrast to the presence of large numbers of small microbial cells of a single morphotype in all size fractions from the 12.7m depth is consistent with the absence of viral predation in this zone. The absence of larger filamentous cells in the smaller size fractions is evidence of the effectiveness of the size fractionation.

Figure S5 Analysis of trophic strategy according to the model of Lauro *et al.* (2009). The cluster location of the Ace Lake samples and of representative sequenced genomes are: Green (oligotrophic): *Pelagibacter ubique*, *Sphingopyxis alaskensis*, *Chlorobium chlorochromatii*, *Chlorobium phaeobacteroides*, *Chlorobium tepidum*, Ace Lake 12.7 m (Sanger, 454 0.1 μm, 0.8 μm); Blue (oligotrophic): *Planctomyces maris*, *Rhodopirellula baltica*, Ace Lake 23m (Sanger, 454 0.1 μm), Ace Lake 18 m (Sanger, 454 0.1 μm), Ace Lake 14 m (Sanger, 454 0.1 μm), Ace Lake 11.5 m (Sanger, 454 0.1 μm), Ace Lake 5 m (Sanger, 454 0.1 μm); Yellow (copiotrophic): *Flavobacterium johnsoniae*, *Kordia algicida*, *Pseudoalteromonas atlantica*, Ace Lake 23 m (454 0.8 μm, 3.0 μm), Ace Lake 18 m (454 0.8 μm, 3.0 μm), Ace Lake 14 m (454 0.8 μm, 3.0 μm), Ace Lake 12.7 m (454 3.0 μm), Ace Lake 11.5 m (454 0.8 μm, 3.0 μm), Ace Lake 5 m (454 0.8 μm, 3.0 μm); Cyan (copiotrophic): *Shewanella frigidimarina*, *Shewanella denitrificans*, *Marinomonas* sp. MWYL1; Red (copiotrophic): *Photobacterium angustum*, *Vibrio cholerae*, *Vibrio fischeri*, *Vibrio splendidus*.

Figure S6 Statistical (STAMP) analysis of normalised mass spectra from COG annotated proteins between each zone in Ace Lake. Proteins are shown grouped into COG categories. Only differences with corrected p-value < 0.05 and effect size <5 are displayed. A) Mixolimnion vs interface; B) Mixolimnion vs monimolimnion; C) Interface vs monimolimnion. Blue, mixolimnion; green, interface; orange, monimolimnion. COG category descriptions are: E, Amino acid transport and metabolism; G, Carbohydrate transport and metabolism; J, Translation, ribosomal structure and biogenesis; C, Energy production and conversion; P, Inorganic ion transport and metabolism; H, Co-enzyme transport and metabolism; O, Post-translational modification, protein turnover and chaperones; K, Transcription; L, Replication, recombination and repair; N, Cell motility.

Figure S7 High resolution heatmap plot and functional clustering of KEGG assignments for the predicted ORFs from the metagenomic reads for Ace lake samples. The heat-scale is the percentage of ORFs assigned to each individual KEGG pathway.

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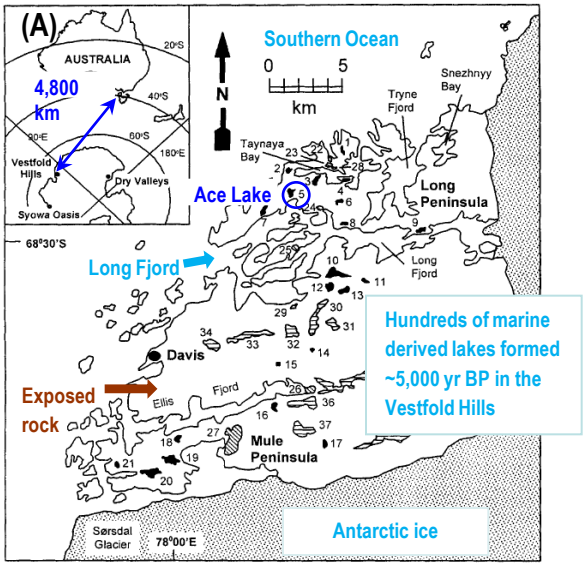
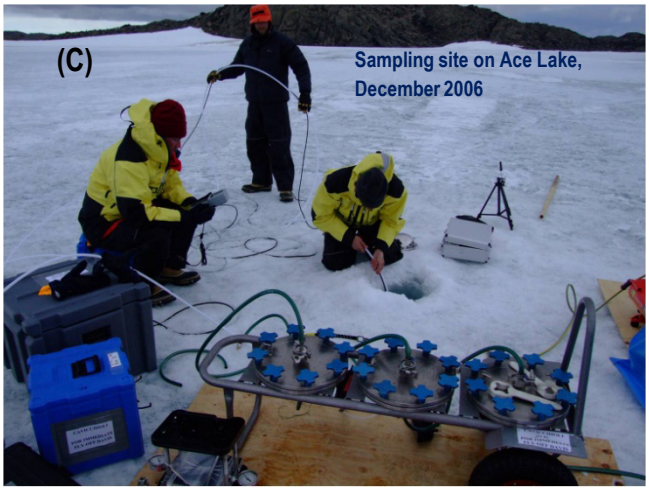
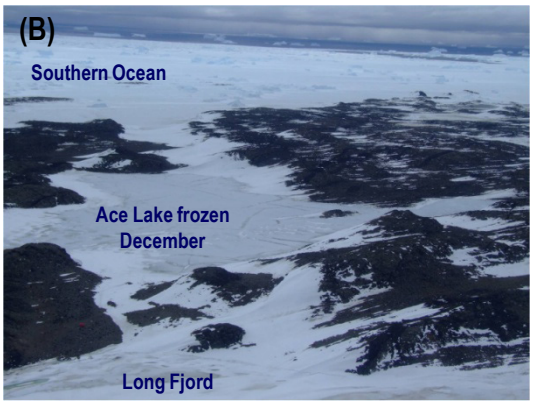
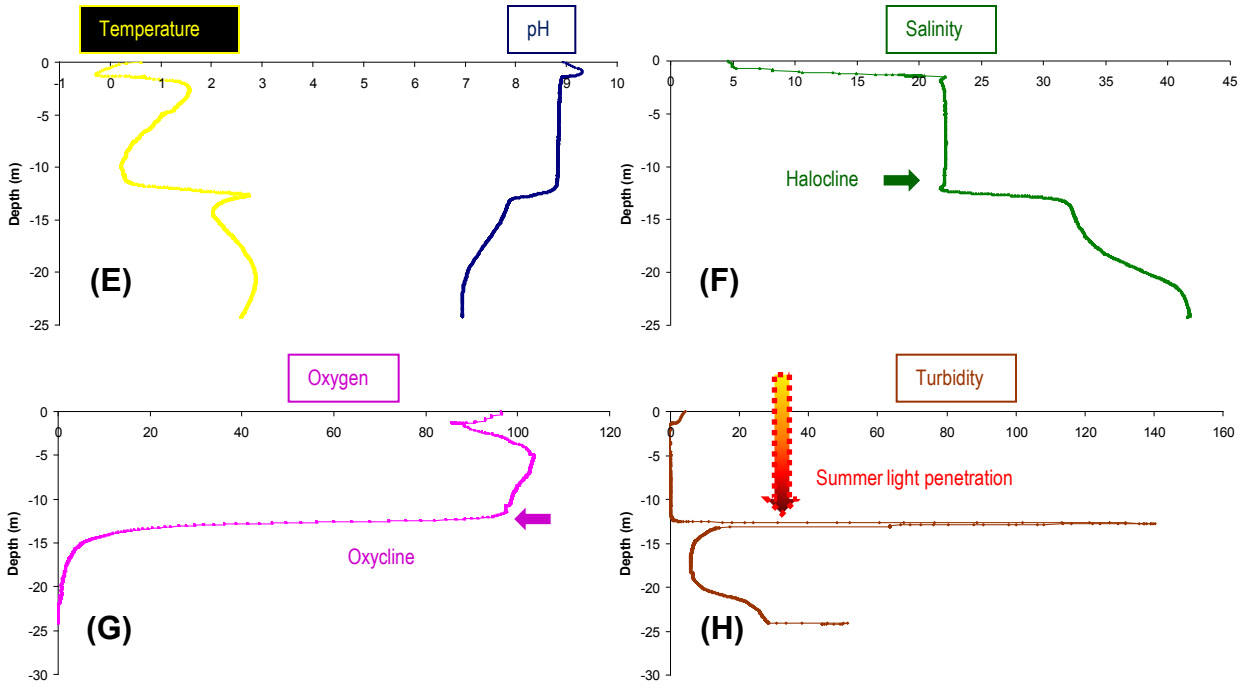


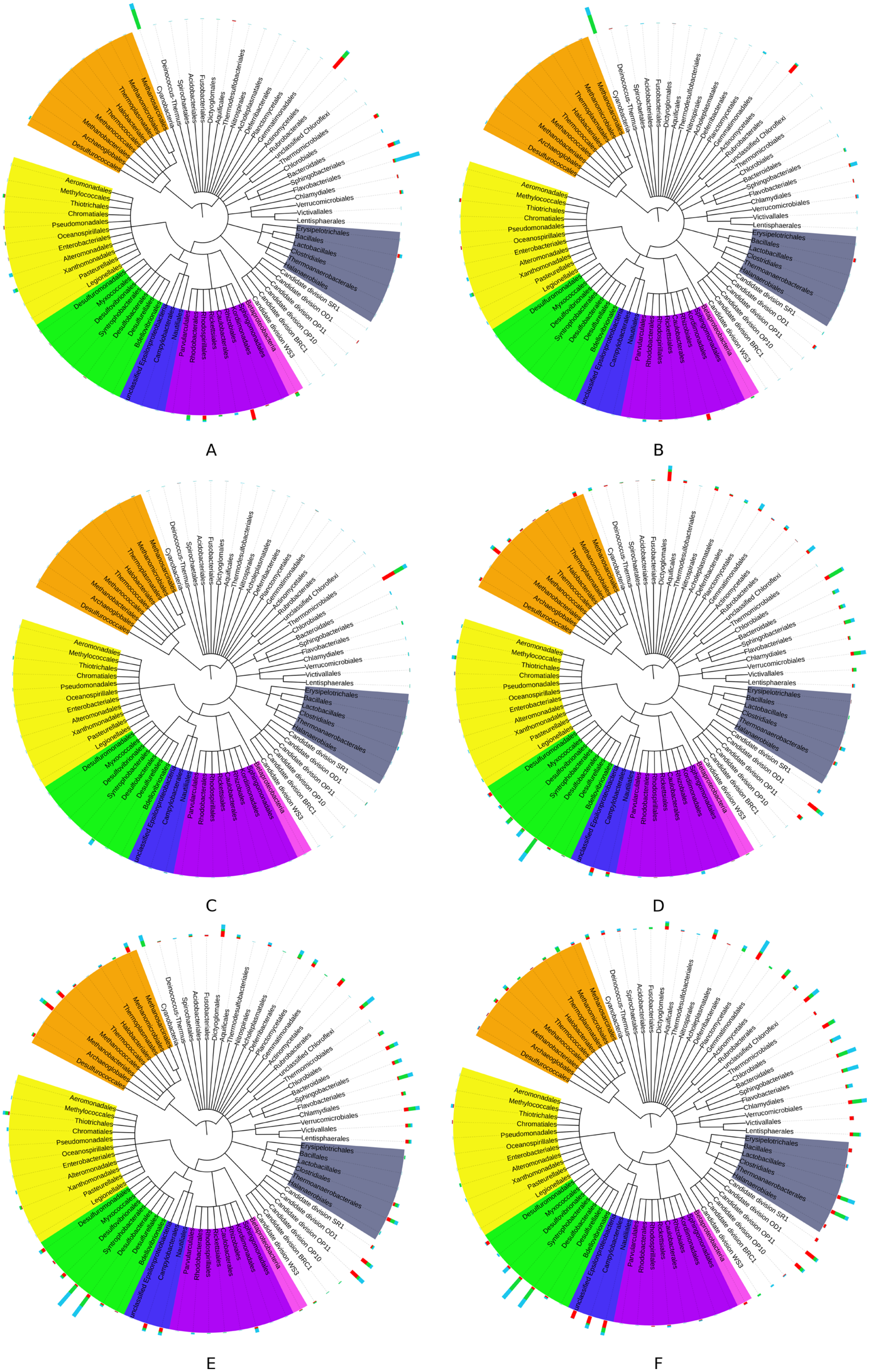




GOS Sample ID	Depth (m)	Size fractions	Trimmed reads	Predicted ORFs (from reads)	ORFs (from reads) with COG/KEGG assignments	Contigs over 5kb (reads in contigs)	Predicted ORFs (from contigs)	Peptide hits
GS232	5	Sanger	281,490	421,252	112,490 / 96,771	*	*	*
		454-0.1µm	539,536	638,757	109,511 / 103,201	2,809 (349,015)	45,281	5,728
		454-0.8µm	468,122	485,021	150,660 / 135,451	296 (66,743)	3,215	*
		454-3.0µm	160,835	138,191	24,920 / 22,240	33 (2,980)	353	*
GS231	11.5	Sanger	283,663	427,889	124,332 / 107,523	*	*	*
		454-0.1µm	523,650	608,671	99,175 / 95,266	2,814 (390,490)	47,987	3,213
		454-0.8µm	474,419	511,909	218,126 / 176,332	174 (161,891)	2,321	*
		454-3.0µm	373,226	307,850	57,961 / 51,915	64 (8,139)	766	*
GS230	12.7	Sanger	54,446	75,576	42,790 / 41,391	*	*	*
		454-0.1µm	442,389	492,995	201,203 / 227,726	88 (282,232)	3,039	12,718
		454-0.8µm	529,711	555,328	209,078 / 234,682	86 (313,550)	2,187	*
		454-3.0µm	208,272	215,741	80,925 / 77,340	75 (49,942)	1,792	*
GS229	14	Sanger	10,042	14,326	5,261 / 4,469	*	*	*
		454-0.1µm	413,992	458,942	100,045 / 88,300	228 (22,556)	2,443	3,427
		454-0.8µm	453,205	435,534	142,743 / 129,403	139 (45,083)	2,118	*
		454-3.0µm	291,065	301,580	105,756 / 89,188	31 (2,422)	262	*
GS228	18	Sanger	9,672	15,077	3,667 / 3,008	*	*	*
		454-0.1µm	362,490	389,077	51,312 / 44,290	29 (1,815)	260	725
		454-0.8µm	544,302	556,243	186,455 / 163,878	154 (14,806)	1,334	*
		454-3.0µm	278,846	287,423	95,876 / 81,161	2 (131)	15	*
GS227	23	Sanger	100,085	160,302	33,462 / 27,302	*	*	*
		454-0.1µm	482,527	547,170	84,257 / 73,074	1,136 (51,163)	12,339	1,602
		454-0.8µm	553,234	611,717	161,973 / 137,632	105 (7,904)	825	*
		454-3.0µm	264,160	270,188	85,596 / 72,206	6 (287)	48	*
TOTAL	---	---	8,103,379	8,926,759	2,487,574 / 2,283,749	8,269 (1,771,149)	126,585	27,413

(D)




A
B
C
D
E
F
Methanosarcinales
Methanomicrobiales
Thermoplasmatales
Halobacteriales
Thermococcales
Methanococcales
Methanobacteriales
Archaeoglobales
Desulfurococcales
Deinococcus-Thermus
Cyanobacteria
Spirochaetales
Acidobacteriales
Fusobacteriales
Dictyoglomales
Aquificales
Thermodesulfobacteriales
Nitrospirales
Acholeplasmatales
Deferribacterales
Planctomycetales
Gemmatimonadales
Actinomycetales
Rubrobacterales
unclassified Chloroflexi
Thermomicrobiales
Chlorobiales
Bacteroidales
Sphingobacteriales
Flavobacteriales
Chlamydiales
Verrucomicrobiales
Victivallales
Lentisphaerales
Erysipelotrichales
Bacillales
Lactobacillales
Clostridiales
Thermoanaerobacterales
Halanaerobiales
Candidate division SR1
Candidate division OD1
Candidate division OP11
Candidate division OP10
Candidate division BRC1
Candidate division WS3
Betaproteobacteria
Sphingomonadales
Rhizobiales
Caulobacterales
Rickettsiales
Rhodospirillales
Rhodobacterales
Parvularculales
Nautiliales
Campylobacterales
unclassified Epsilonproteobacteria
Bdellovibrionales
Desulfurellales
Desulfobacterales
Syntrophobacterales
Desulfovibrionales
Myxococcales
Desulfuromonadales
Legionellales
Pasteurellales
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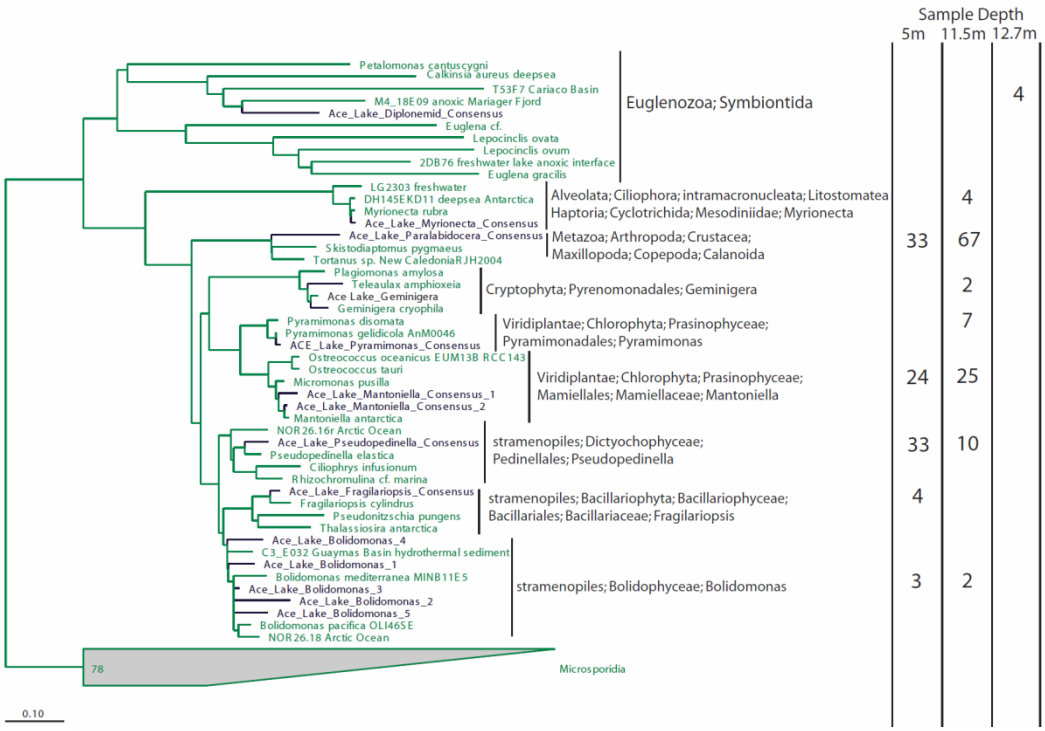
Sample Depth
5m 11.5m 12.7m
Petalomonas cantuscygni
Calkinsia aureus deepsea
T53F7 Cariaco Basin
M4_18E09 anoxic Mariager Fjord
Ace_Lake_Diplonemid_Consensus
Euglena cf.
Lepocinclis ovata
Lepocinclis ovum
2DB76 freshwater lake anoxic interface
Euglena gracilis
Euglenozoa; Symbiontida
4
LG2303 freshwater
DH145EKD11 deepsea Antarctica
Myrionecta rubra
Ace_Lake_Myrionecta_Consensus
Alveolata; Ciliophora; intramacronucleata; Litostomatea
Haptoria; Cyclotrichida; Mesodiniidae; Myrionecta
4
Ace_Lake_Paralabidocera_Consensus
Skistodiaptomus pygmaeus
Tortanus sp. New CaledoniaRJH2004
Metazoa; Arthropoda; Crustacea;
Maxillopoda; Copepoda; Calanoida
33
67
Plagiomonas amylosa
Teleaulax amphioxeia
Ace Lake_Geminigera
Geminigera cryophila
Cryptophyta; Pyrenomonadales; Geminigera
2
Pyramimonas disomata
Pyramimonas gelidicola AnM0046
ACE_Lake_Pyramimonas_Consensus
Viridiplantae; Chlorophyta; Prasinophyceae;
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7
Ostreococcus oceanicus EUM13B RCC143
Ostreococcus tauri
Micromonas pusilla
Ace_Lake_Mantoniella_Consensus_1
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25
NOR26.16r Arctic Ocean
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10
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4
Ace_Lake_Bolidomonas_4
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Ace_Lake_Bolidomonas_1
Bolidomonas mediterranea MINB11E5
Ace_Lake_Bolidomonas_3
Ace_Lake_Bolidomonas_2
Ace_Lake_Bolidomonas_5
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3
2
78
Microsporidia
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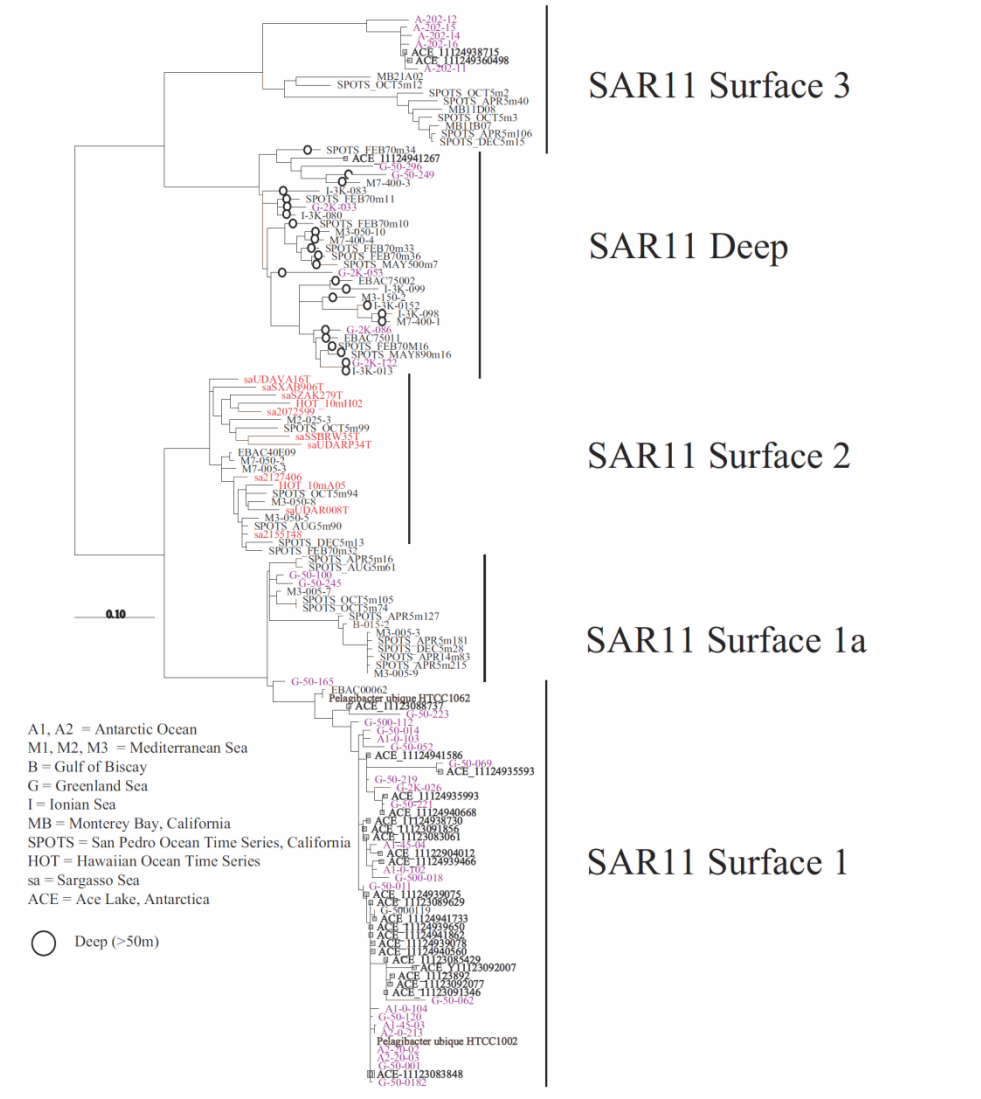
(A)


SAR11 Surface 3
SAR11 Deep
SAR11 Surface 2
SAR11 Surface 1a
SAR11 Surface 1
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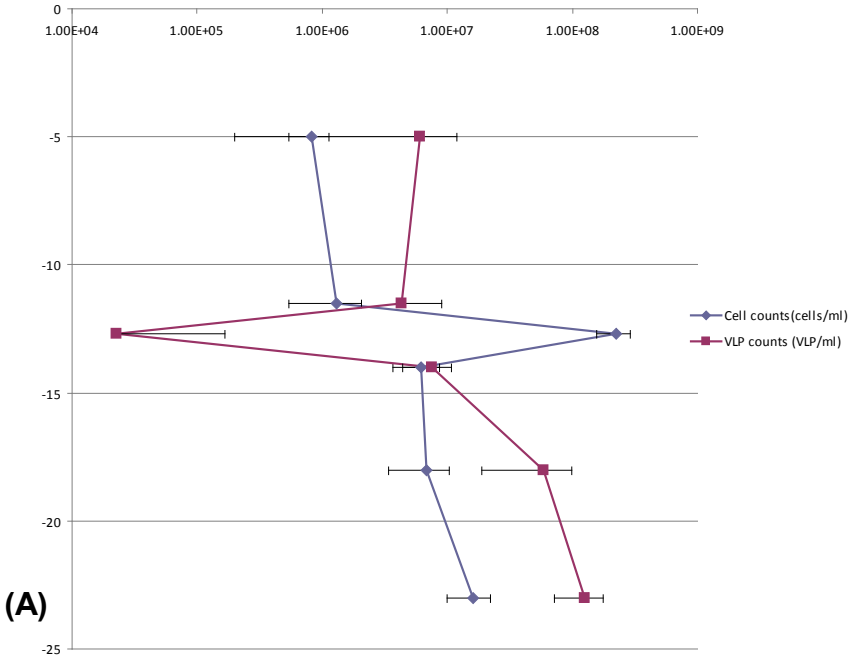
A1, A2 = Antarctic Ocean
M1, M2, M3 = Mediterranean Sea
B = Gulf of Biscay
G = Greenland Sea
I = Ionian Sea
MB = Monterey Bay, California
SPOTS = San Pedro Ocean Time Series, California
HOT = Hawaiian Ocean Time Series
sa = Sargasso Sea
ACE = Ace Lake, Antarctica
Deep (>50m)
Pelagibacter ubique HTCC1062

(B)


0
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1.00E+05
1.00E+06
1.00E+07
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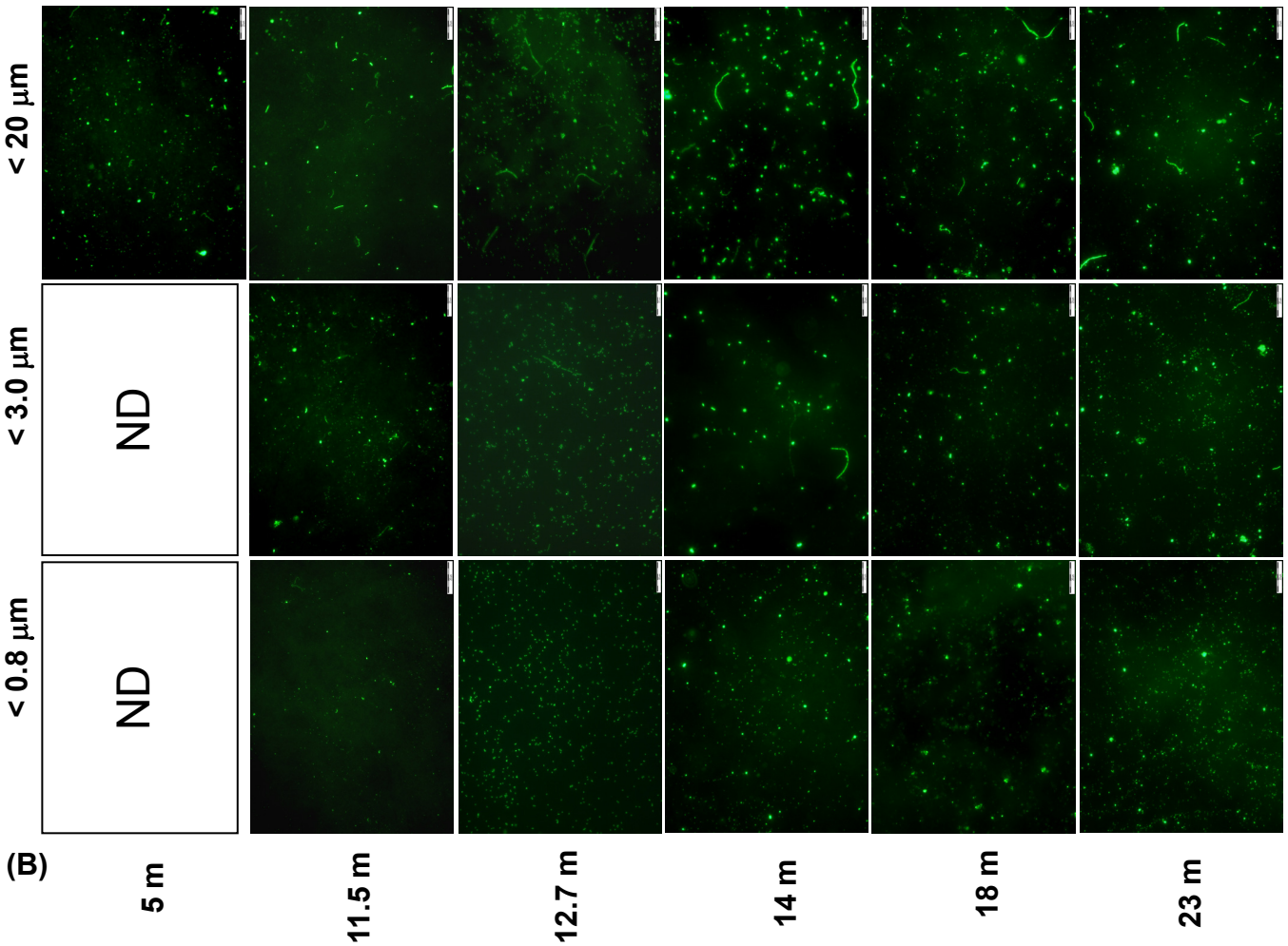
-5
-10
-15
-20
-25
Cell counts(cells/ml)
VLP counts (VLP/ml)

(A)


< 20 µm
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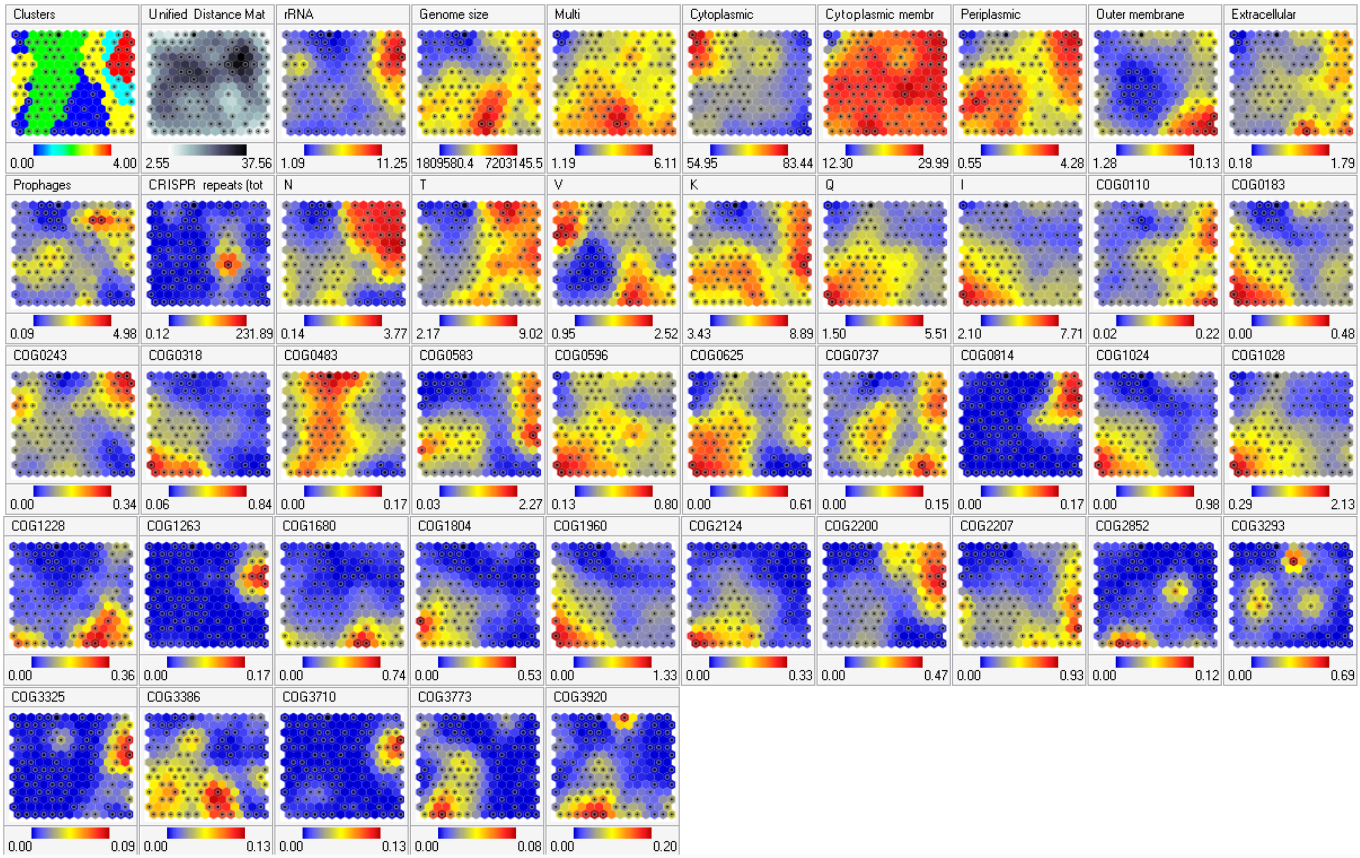
ND
ND
5 m
11.5 m
12.7 m
14 m
18 m
23 m

(B)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Unified Distance Mat
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Genome size
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Cytoplasmic
54.95 83.44
Cytoplasmic membr
12.30 29.99
Periplasmic
0.55 4.28
Outer membrane
1.28 10.13
Extracellular
0.18 1.79
Prophages
0.09 4.98
CRISPR repeats (tot
0.12 231.89
N
0.14 3.77
T
2.17 9.02
V
0.95 2.52
K
3.43 8.89
Q
1.50 5.51
I
2.10 7.71
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0.00 0.17
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0.00 0.74
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0.00 0.53
COG1960
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0.00 0.33
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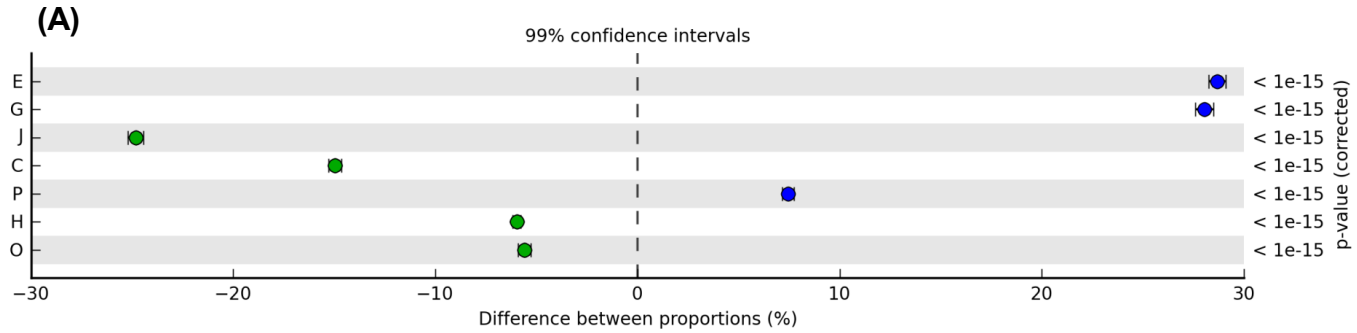
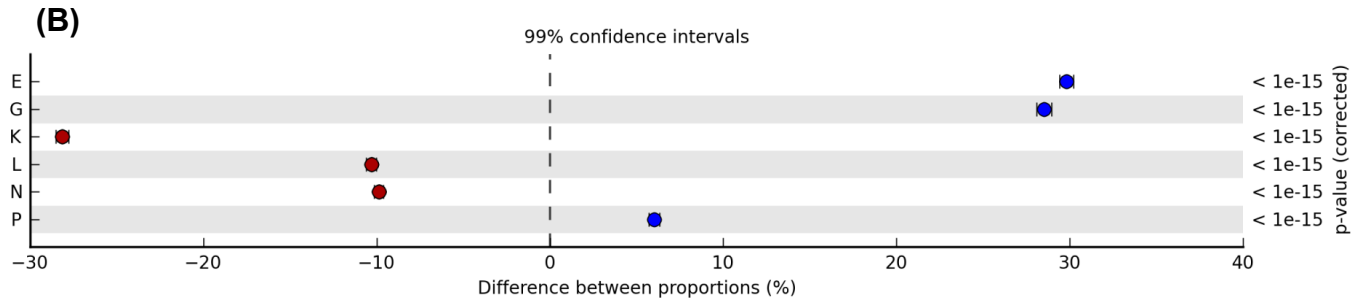
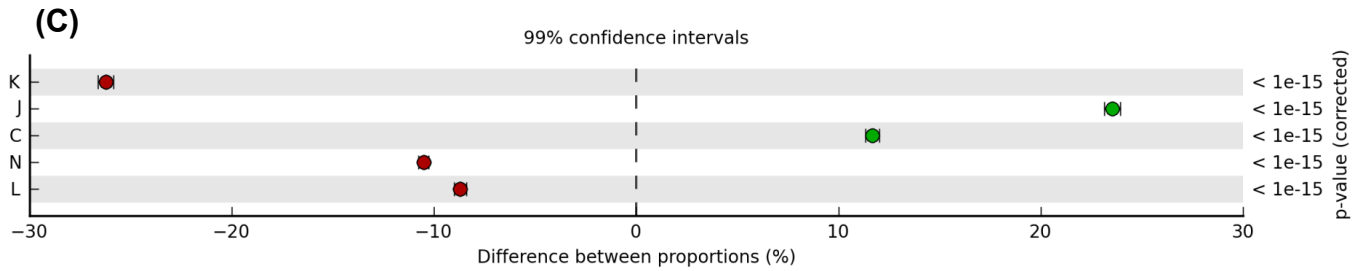
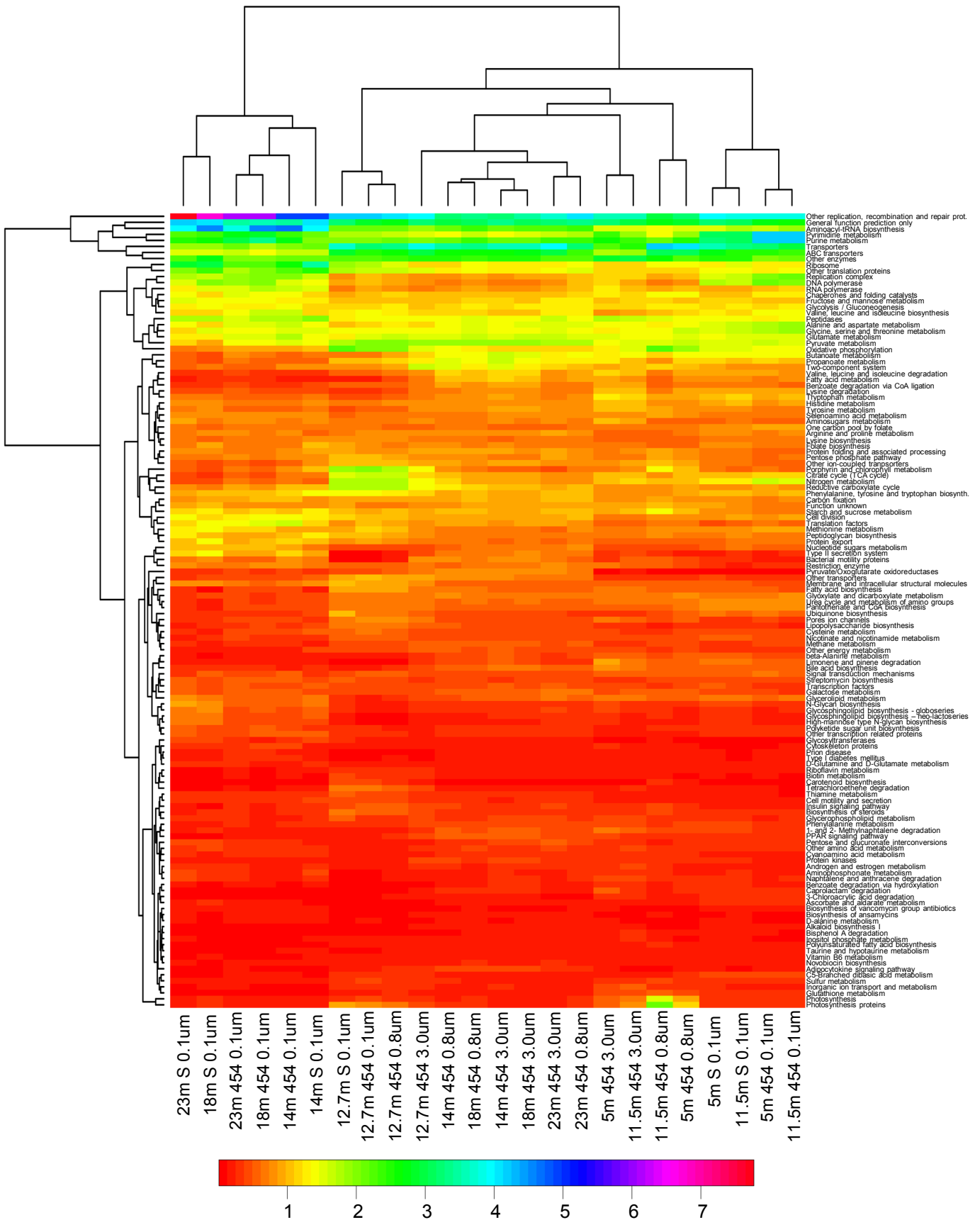
0.00 0.20


(A)
99% confidence intervals
E
G
J
C
P
H
O
< 1e-15
< 1e-15
< 1e-15
< 1e-15
< 1e-15
< 1e-15
< 1e-15
p-value (corrected)
−30
−20
−10
0
10
20
30
Difference between proportions (%)


(B)
99% confidence intervals
E
G
K
L
N
P
< 1e-15
< 1e-15
< 1e-15
< 1e-15
< 1e-15
< 1e-15
p-value (corrected)
−30
−20
−10
0
10
20
30
40
Difference between proportions (%)


(C)
99% confidence intervals
K
J
C
N
L
< 1e-15
< 1e-15
< 1e-15
< 1e-15
< 1e-15
p-value (corrected)
−30
−20
−10
0
10
20
30
Difference between proportions (%)


Other replication, recombination and repair prot.
General function prediction only
Aminoacyl-tRNA biosynthesis
Pyrimidine metabolism
Purine metabolism
Transporters
ABC transporters
Other enzymes
Ribosome
Other translation proteins
Replication complex
DNA polymerase
RNA polymerase
Chaperones and folding catalysts
Fructose and mannose metabolism
Glycolysis / Gluconeogenesis
Valine, leucine and isoleucine biosynthesis
Peptidases
Alanine and aspartate metabolism
Glycine, serine and threonine metabolism
Glutamate metabolism
Pyruvate metabolism
Oxidative phosphorylation
Butanoate metabolism
Propanoate metabolism
Two-component system
Valine, leucine and isoleucine degradation
Fatty acid metabolism
Benzoate degradation via CoA ligation
Lysine degradation
Tryptophan metabolism
Histidine metabolism
Tyrosine metabolism
Selenoamino acid metabolism
Aminosugars metabolism
One carbon pool by folate
Arginine and proline metabolism
Lysine biosynthesis
Folate biosynthesis
Protein folding and associated processing
Pentose phosphate pathway
Other ion-coupled transporters
Porphyrin and chlorophyll metabolism
Citrate cycle (TCA cycle)
Nitrogen metabolism
Reductive carboxylate cycle
Phenylalanine, tyrosine and tryptophan biosynth.
Carbon fixation
Function unknown
Starch and sucrose metabolism
Cell division
Translation factors
Methionine metabolism
Peptidoglycan biosynthesis
Protein export
Nucleotide sugars metabolism
Type II secretion system
Bacterial motility proteins
Restriction enzyme
Pyruvate/Oxoglutarate oxidoreductases
Other transporters
Membrane and intracellular structural molecules
Fatty acid biosynthesis
Glyoxylate and dicarboxylate metabolism
Urea cycle and metabolism of amino groups
Pantothenate and CoA biosynthesis
Ubiquinone biosynthesis
Pores ion channels
Lipopolysaccharide biosynthesis
Cysteine metabolism
Nicotinate and nicotinamide metabolism
Methane metabolism
Other energy metabolism
beta-Alanine metabolism
Limonene and pinene degradation
Bile acid biosynthesis
Signal transduction mechanisms
Streptomycin biosynthesis
Transcription factors
Galactose metabolism
Glycerolipid metabolism
N-Glycan biosynthesis
Glycosphingolipid biosynthesis - globoseries
Glycosphingolipid biosynthesis - lacto-neolactoseries
High-mannose type N-glycan biosynthesis
Polyketide sugar unit biosynthesis
Other transcription related proteins
Glycosyltransferases
Cytoskeleton proteins
Prion disease
Type I diabetes mellitus
D-Glutamine and D-Glutamate metabolism
Riboflavin metabolism
Biotin metabolism
Carotenoid biosynthesis
Tetrachloroethene degradation
Thiamine metabolism
Cell motility and secretion
Insulin signaling pathway
Biosynthesis of steroids
Glycerophospholipid metabolism
Phenylalanine metabolism
1- and 2- Methylnaphtalene degradation
PPAR signaling pathway
Pentose and glucuronate interconversions
Other amino acid metabolism
Cyanoamino acid metabolism
Protein kinases
Androgen and estrogen metabolism
Aminophosphonate metabolism
Naphtalene and anthracene degradation
Benzoate degradation via hydroxylation
Caprolactam degradation
3-Chloroacrylic acid degradation
Ascorbate and aldarate metabolism
Biosynthesis of vancomycin group antibiotics
Biosynthesis of ansamycins
D-alanine metabolism
Alkaloid biosynthesis I
Bisphenol A degradation
Inositol phosphate metabolism
Polyunsaturated fatty acid biosynthesis
Taurine and hypotaurine metabolism
Vitamin B6 metabolism
Novobiocin biosynthesis
Adipocytokine signaling pathway
C5-Branched dibasic acid metabolism
Sulfur metabolism
Inorganic ion transport and metabolism
Glutathione metabolism
Photosynthesis
Photosynthesis proteins
23m S 0.1um
18m S 0.1um
23m 454 0.1um
18m 454 0.1um
14m 454 0.1um
14m S 0.1um
12.7m S 0.1um
12.7m 454 0.1um
12.7m 454 0.8um
12.7m 454 3.0um
14m 454 0.8um
18m 454 0.8um
14m 454 3.0um
18m 454 3.0um
23m 454 3.0um
23m 454 0.8um
5m 454 3.0um
11.5m 454 3.0um
11.5m 454 0.8um
5m 454 0.8um
5m S 0.1um
11.5m S 0.1um
5m 454 0.1um
11.5m 454 0.1um
1
2
3
4
5
6
7