Metagenomic analyses of the late Pleistocene permafrost – additional tools for reconstruction of environmental conditions

Elizaveta Rivkina[1], Lada Petrovskaya[2], Tatiana Vishnivetskaya[3], Kirill Krivushin[1], Lyubov Shmakova[1], Maria Tutukina[4,7], Arthur Meyers[3], Fyodor Kondrashov[4,5,6]

[1]Institute of Physicochemical and Biological Problems in Soil Science, Russian Academy of Sciences, Pushchino, Russia

[2]Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

[3]University of Tennessee, Center for Environmental Biotechnology, Knoxville, USA

[4] Bioinformatics and Genomics Programme, Centre for Genomic Regulation (CRG), Barcelona, Spain

[5] Universitat Pompeu Fabra (UPF), Barcelona, Spain

[6]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

[7]Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino, Russia

Corresponding author E-mail: elizaveta.rivkina@gmail.com

Institute of Physicochemical and Biological Problems in Soil Science, Pushchino, Moscow region, Russia, 142292

Key words: permafrost, Kolyma Lowland, methane, metagenome, methanogens, methanotrophs, late Pleistocene Ice Complex, lake sediments

Abstract

A comparative analysis of the metagenomes from two 30,000 year-old permafrost samples, one of lake-alluvial origin and the other from late Pleistocene Ice Complex sediments, revealed significant differences within microbial communities. The late Pleistocene Ice Complex sediments (which have been characterized by the absence of methane with lower values of redox-potential and Fe^{2+} content) showed both a low abundance of methanogenic archaea and enzymes from the carbon, nitrogen and sulfur cycles. The metagenomic and geochemical analyses described in the paper provide evidence that the formation of the sampled late Pleistocene Ice Complex sediments likely took place under much more aerobic conditions than lake-alluvial sediments.

1. Introduction

Permafrost, including constantly frozen sediments of the Arctic, is a unique subsurface complex environment where microorganisms retain viability over a long period of time, from thousands to millions of years (Gilichinsky and Rivkina 2011). The impact of climate change on permafrost stability has recently been discussed widely by the scientific community (Anthony et al 2014, Walter et al 2007, Zimov et al 2006). The permafrost deposits of the North-East Siberia, which did not thaw during the Holocene climatic optimum, have attracted particular interest, especially the late Pleistocene Ice Complex deposits (Yedoma Suite) that are widespread on the Kolyma-Indigirka lowland (152-162°E, 68-72°N) (Schirrmeister et al 2011). Earlier we found that the epigenetically (consecutively) frozen sediments of both lake and marine origin (independent of age) contain biogenic methane, whereas methane was either absent or present at trace concentrations in samples from the sincryogenic (simultaneously frozen) late Pleistocene Ice Complex (Rivkina et al 2007, Rivkina and Kraev 2008). However, low concentrations of methane have been detected in the Yedoma deposits located both far south in boreal forest (Brouchkov and Fukuda 2002) and near the Arctic coast in Lena River Delta (Bischoff et al 2013, Brouchkov and Fukuda 2002). The presence of biogenic methane in the Ice Complex deposits located near the coast of the Arctic Ocean was also confirmed by our research conducted in 2013 on the Gydan Peninsula (unpublished data).

Anaerobic microcosm incubation of thawed permafrost samples in a carbon dioxide- and hydrogen- enriched atmosphere showed methanogenic activity in epicryogenic sediments only, while this process was not observed in samples from the sincryogenic late Pleistocene Ice Complex (Rivkina and Kraev 2008). Similar results were obtained during experiments with radioactively labeled substrates (Rivkina et al 2004, Rivkina et al 2007, Rivkina et al

2002), showing an absence of methanogenic activity in the sincryogenic late Pleistocene Ice Complex samples with this process evidently taking place in epicryogenic sediments of lake or lake-alluvial origin. Moreover, only from the latter sediments methanogenic archaea were isolated in pure culture (Krivushin et al 2010, Rivkina et al 2007, Shcherbakova et al 2011). Viable methanogens were also isolated from the permafrost-affected soils located in the Lena River Delta region (Morozova et al, 2007, Wagner et al, 2013).

The specific distribution of methane and methane-producing microorganisms in permafrost raises number of questions; for example, why the sediments of the late Pleistocene Ice Complex in Kolyma–Indigirka Lowland do not contain methane or methanogenic activity. To answer this question, it is thought that the application of new methodologies such as metagenomic analyses is required (Graham et al 2012, Jansson and Tas 2014). Until recently, the determination of microbial diversity in low biomass environments, including permafrost, was problematic. The microbial cell abundances in the ancient permafrost is 10–100 times lower than that in the active layer samples, thereby resulting in low yields of the total community genomic DNA (gDNA) (Yergeau et al 2010). However, using appropriate DNA extraction kits (Vishnivetskaya et al 2014) and the whole-community genome amplification technique (Yergeau et al 2010), a sufficient amount of gDNA can be obtained for next-generation sequencing technologies, producing sequences on an unprecedented scale. Indeed, the first metagenomic analyses of permafrost samples became available recently. Specifically, the analyses of the metagenomes from active layer soil and two-meter deep permafrost samples collected in the Canadian High Arctic and Alaska identified signature genes responsible for hydrogenotrophic and acetoclastic methanogenesis, methylotrophic methane oxidation, nitrification, and carbohydrate degradation (Mackelprang et al 2011, Yergeau et al 2010).

Here we report results of the comparative metagenomic analyses of the two ancient permafrost samples similar in age (*ca.* 30,000 years old), however of different origins (epigenetically frozen lake sediments versus sincryogenic sediments from the late Pleistocene Ice Complex). The results based on comparative analyses of microbial communities from sediments of different origin may be used for prediction of responses associated with permafrost thawing due to global warming.

2. Materials and Methods

Sample collection and description

Samples were collected within the Kolyma-Indigirka Lowland in northeast Siberia (69°299N, 156°599E) during the summer field season of 2007 (Fig. 1A). Permafrost sediments were sampled using drilling equipment that operates without fluids and prevents down-hole contamination. The sampling technique was tested and described previously (Shi et al 1997). Briefly, the surfaces of the 20- to 30-cm-long cores were cleaned immediately by shaving melted layers out with an ethyl alcohol-sterilized knife and then the frozen internal part of the core was split into 5-cm-long segments; these were placed into sterile aluminum containers and kept frozen during storage in field and transportation to the Institute of Physicochemical and Biological Problems in Soil Science, Pushchino.

The physicochemical characteristics of the samples are presented in Table 1. Methane content was measured as described previously (Rivkina et al 2007, Rivkina and Kraev 2008).

Sample IC4 corresponded to the permafrost sediment of lake origin from the floodplain of the Ambolikha River, borehole DH-4/07, depth of 22.5 m (Fig. 1B). Total carbon concentration was ~1.1% (w/w). Methane content of this sample was 1.2 mmol/kg, ^{13}C=-85‰ indicative of biogenic origin. The radiocarbon age of this sample was 30,696 ± 394 years (J–5829) (Kraev et al 2013).

Sample IC8 represented a permafrost soil from the late Pleistocene Ice Complex (Omolon River), borehole DH-2/07, depth of 16 m. Total carbon concentration was ~1.1% (w/w). Methane levels in all samples tested from this borehole were non- detectable (Fig. 1B). The age of this sample was estimated to be ~32,000 years, based on the age determination for the same outcrop which was described recently (Legendre et al 2014).

2.1 DNA extraction and sequencing

In the laboratory, material from the inner part of the permafrost cores was subsampled aseptically for DNA isolation. The gDNA was extracted from eight replicates of ~0.5 g each using the PowerSoil® DNA Extraction Kit (MO BIO Laboratories, Inc., USA). Due to low yield, gDNAs from eight replicates were combined, then purified and concentrated using Genomic DNA Clean & Concentrator® Kit (Zymo Research Corporation, USA).

The gDNA sequencing libraries were prepared using NEBNext® reagents (New England BioLabs Inc., USA), according to protocol recommended by the manufacturer, having an estimated peak insert size of 150 nt. Metagenome sequencing was performed at the CRG Genomics Core Facility (Centre for Genomic Regulation, Barcelona, Spain) on an Illumina HiSeq 2000™ machine using Flow Cell v3 with TruSeq SBS v3 reagents and a 2 x 100 cycle sequencing protocol.

2.2 MG-RAST Analysis

Raw sequencing data, i.e., 19.8 Gb representing 143.7 M sequences with an average length of 138 bp for IC4 (4606864.3) and 19.7 Gb representing 131.7 M sequences with an average length of 150 bp for IC8 (4606865.3), were uploaded to the MG-RAST server (Meyer et al 2008) for gene calling and annotation. A total of 6.6% (IC4) and 3.4% (IC8) sequences failed to pass the quality control (QC) pipeline, whereas 0.3% of total sequences in both data sets were assigned to ribosomal RNA genes.

For functional assignment, protein sequences of putative ORF were searched against the M5NR non-redundant protein database (Wilke et al 2012) with an e-value threshold of $1e^{-5}$, minimum percentage identity of 60%, and minimum alignment length of 15 aa. The taxonomic assignments of Illumina reads were performed against M5NR and M5RNA databases at default parameters. The best-hit classification method was used in both cases for match assessment.

2.3 Comparative metagenomic data analyses

Statistical analyses of the metagenomes were performed in order to compare community composition and functional profiles of the permafrost samples. Statistical significance (P-value) was calculated using two-sided Fisher's exact test. The differences between proportions were analyzed in STAMP (Parks and Beiko 2010) using the Newcombe-Wilson method (Newcombe 1998) at a 95% confidence interval and with Storey's FDR correction (Storey and Tibshirani 2003, Storey et al 2004). Original data sets were deposited at the NCBI Sequence Read Archive (SRA) under the accession numbers SRX763249, SRX751044 (Krivushin et al 2015).

3. Results and Discussion

3.1 Community description

The gDNA yield was higher in IC8 sample, with an average of 0.5 μg g^{-1} of wet sediment, in comparison to 0.37 μg g^{-1} in the IC4 sample. Based on the metagenomics data (Angly et al 2009, Raes et al 2007) giving an average genome length of 4.7 Mb for the soil bacterial/archaeal population and an estimated weight of 4.05 fg (Ellenbroek and Cappenberg 1991) for a genome of this size, the theoretical level of the prokaryotic cell populations calculated from the total gDNA recovered were 7.0 x 10^7 for IC4 and 9.4 x 10^7

for IC8, including a reduction of the total cell population by the eukaryotic component equal to 25% (Raes et al 2007).

Analyses of metagenomes of the two permafrost samples showed that bacterial genes were dominant and 96.4% and 97.7% of sequences were assigned to the domain Bacteria in the IC4 and IC8 samples, respectively. Archaea were the second dominant domain followed by Eukaryotes, while viruses comprised only 0.06% in IC4 and 0.03% in IC8 samples. Archaea and Eukaryotes were more abundant in the IC4 sample compared to the IC8 sample, i.e., 2.4% versus 1.3% and 1.0 versus 0.9%, respectively. While Bacteria were significantly more abundant than Eukaryotes, the functional diversity was higher among the Eukaryotic organisms, which play essential roles in the biology and fertility of soils. A number of detected unparalleled genes of Eukaryotic organisms including plants, algae, fungi, and protists was 3.4 and 2.6 times higher in comparison to a number of Bacterial unparalleled genes detected in the IC4 and IC8 samples, respectively. The most abundant microorganisms were comprised of nine bacterial and one Archaeal phyla, with Proteobacteria being the most plentiful, followed by Actinobacteria, Firmicutes, Bacteroidetes, Chloroflexi, Planctomycetes, Euryarchaeota, Acidobacteria, Cyanobacteria, and Verrucomicrobia in that order (Fig. 2). The quantities of all other phyla were less than 1%. Sequences affiliated with Actinobacteria ($P < 1e^{-15}$), Firmicutes ($P < 1e^{-15}$), Chloroflexi ($P < 1e^{-15}$), Cyanobacteria ($P < 1e^{-15}$), Acidobacteria ($P < 1e^{-15}$), Verrucomicrobia ($P < 1e^{-15}$) were dominant within IC8, whereas α-, β-, γ-, and δ- Proteobacteria ($P < 1e^{-15}$), Bacteroidetes ($P < 1e^{-15}$), Planctomycetes ($P < 1e^{-15}$), and Euryarchaeota ($P < 1e^{-15}$) dominated within IC4. The microorganisms that are involved into methane formation and methane metabolism, for example methanogenic archaea (phylum Euryarchaeota) or methanotrophic and methylotrophic bacteria (phylum Proteobacteria), were more abundant in more reduced IC4 sample (Table 1). Microorganisms

without strong association to methane metabolism (e.g. phyla Actinobacteria and Chloroflexi) dominated in IC8.

3.2 Dependence of the microbial community structure on permafrost characteristics as detected from genus taxonomical level

3.2.1 Methanogenic community. As was shown in previous research (Kraev et al 2013, Legendre et al 2014), methane was not detected in the IC8 sample, but its level in the IC4 sample reached 1-2 mmol/kg; hence, one would expect to find a greater abundance of methanogenic species in the IC4 sample. Indeed, the phylum Euryarchaeota, which contains methanogens, was twice as prevalent in IC4 sample, and methanogenic Archaea constituted as much as 1.5% of the microbial communities in IC4 (compared to 0.5% in IC8). The most abundant methane-producing species was *Methanosarcina* (0.32% in IC4 versus 0.14% in IC8), followed by *Methanoregula* (0.28% versus 0.03%), *Methanoculleus* (0.1% versus 0.05%), *Methanosphaerula* (0.1% versus 0.03%), *Methanospirillum* (0.1% versus 0.03%), *Methanosaeta* (0.1% versus 0.03%). Twelve other methanogenic species were detected at <0.1% in IC4 and at an even lower percentage in IC8.

3.2.2 Methanotrophic bacteria. In the vicinity of the environments where methane is produced, methane-oxidizing (methanotrophic) bacteria can be found (Khmelenina et al 2002). Thus, we analyzed the presence of methanotrophic DNA in our data. To date two types of methane-oxidizing bacteria are recognized, Type I methanotrophs belonging to γ-Proteobacteria and type II methanotrophs from α-Proteobacteria. Indeed, the phylum Proteobacteria clearly dominated in the IC4 sample (50.0% versus 26.5% in IC8) with the α-Proteobacteria being more abundant in IC4 (32.7%) in comparison to IC8 (12.3%). On a more refined taxonomical scale the most abundant order of α-Proteobacteria was Rhizobiales (24.2% in IC4 versus 7.7% in IC8), which contains both methanotrophic and

nitrogen-fixing bacteria. Alphaproteobacteria Type II methanotrophs (3.32% versus 1.06%) were represented by the genera *Methylocella* (0.53% versus 0.14%), *Methylosinus* (0.24% versus 0.07%), *Methylocystis* (0.22% versus 0.06%), and *Methylobacterium* (2.32% versus 0.8%) in IC4 and IC8. The last genus (*Methylobacterium*) is a facultative methylotroph, however, some species are capable of growth on methane. The class γ-Proteobacteria was the most diverse Proteobacteria class (184 species); nonetheless, it was six-fold less plentiful in comparison to α-Proteobacteria. Gammaproteobacteria Type I methanotrophs, such as *Methylococcus* (0.13% versus 0.11%), *Methylobacter* (0.12% versus 0.05%), and *Methylophaga* (0.02% versus 0.01%) were again more abundant in the IC4 than in the IC8 sample. In this Siberian permafrost, Type II methanotrophs dominated over Type I methanotrophs, which is similar to methanotrophic bacteria abundance and diversity in Canadian high Arctic permafrost (Lau et al 2015). Methylotrophs, as well as the subset methanotrophs, play an essential role in the carbon cycle. Interestingly, obligate methylotrophic bacteria belonging to the β-Proteobacteria, such as *Methylibium* (0.17% versus 0.16%), *Methylobacillus* (0.07% versus 0.08%), *Methylovorus* (0.04% versus 0.04%), and *Methylotenera* (0.06% versus 0.05%), were detected in both the IC4 and IC8 samples at equally low levels.

3.2.3 Bacteria of nitrogen cycle. Another important metabolic process in an environment is the nitrogen cycle. Nitrogen-fixing bacteria were more abundant in the IC4 of the following genera: *Bradyrhizobium* (1.85% versus 0.5%), *Sinorhizobium* (1.3% versus 0.6%), *Rhizobium* (0.82% versus 0.4%), *Rhodospirillum* (0.63% versus 0.3%), *Afipia* (0.4% versus 0.12%), *Azospirillum* (0.016% versus 0.006%), *Azorhizobium* (0.005% versus 0.001%) and *Azotobacter* (0.06% versus 0.05%), the γ-Proteobacteria nitrogen-fixing species. Other species involved in the nitrogen cycle from the *Hyphomicrobium* (2.5% versus 0.23%, capable of denitrification with methanol), *Nitrobacter* (1.9% versus 0.6%, capable of

oxidizing nitrite into nitrate) and *Rhodopseudomonas* (3.8% versus 1.2%, capable of carbon dioxide and nitrogen fixation) genera were also more abundant in the IC4 sample. However, ammonia-oxidizing and nitrifying bacteria of the class β-Proteobacteria, such as *Nitrosomonas* (~0.1%), *Nitrosospira* (~0.17%), *Nitrosovibrio* (<0.001%), were detected in both samples at the similar level. Other bacteria involved in the nitrogen cycle are members of the phylum Planctomycetes, many of which conduct anaerobic ammonium oxidation or so-called "anammox" metabolism, a process of ammonia oxidation by nitrite involvement to yield nitrogen gas. Four planctomycetes genera were more abundant in IC4 compared to the IC8 sample with *Plantomyces* (0.63% versus 0.34%) being the most abundant, followed by *Pirellula* (0.61% versus 0.28%), *Blastopirellula* (0.57% versus 0.24%), and *Isosphaera* (0.16% versus 0.13%). Some planctomycetes, e.g., *Pirellula*, are able to live in environments with high inorganic sulfate concentrations (Glockner et al 2003). Nitrogen-fixing cyanobacteria slightly dominated in IC8 (0.82%) in comparison to IC4 (0.71%); however, the proportion of nitrogen-fixing to total amount of cyanobacteria was higher in the IC4 (55.5%) than in the IC8 (48.6%) sample. Another nitrogen-fixing bacterium dominating in IC8 (2.6%) in comparison to IC4 (0.8%) was the actinobacterium *Frankia*, which is characterized by the ability to engage in a symbiotic relationship with plants, producing nitrogen-fixing root nodules.

3.2.4 **Sulfate-reducing bacteria**. The δ-Proteobacteria were also more abundant in IC4 (5.8%) than in IC8 (4.5%). Sulfate-reducing bacteria, namely, *Desulfovibrio* (0.49% versus 0.41%), *Desulfatibacillum* (0.22% versus 0.08%), *Desulfococcus* (0.18% versus 0.07%), *Desulfobacterium* (0.14% versus 0.04%), *Desulfomicrobium* (0.11% versus 0.06%), and *Geobacter* (1.13% versus 0.82%) were more plentiful in the IC4 sample. Two species from the order Syntrophobacterales were more abundant in IC4; these included the strictly anaerobic, sulfate-reducing, propionate-degrading bacterium *Syntrophobacter* (0.33% versus

0.18% in IC8) and the benzoate-degrading bacterium *Syntrophus* (0.62% versus 0.13%). During growth on certain compounds, both of these organisms are known to form syntrophic associations with methanogens, e.g., *Methanospirillum hungateii*, facilitating methane production (Harmsen et al 1998, Jackson et al 1999). The sulfate-reducing bacterium *Desulfotomaculum* from the phylum Firmicutes was found at similar concentration in both samples (i.e., 0.32-0.33%). However, another sulfate-reducing Firmicutes genus, *Desulfitobacterium*, capable of using hydrogen gas as an electron donor at extremely low concentrations to facilitate sulfate reduction and methanogenesis (Villemur et al 2006) was twice as abundant in the IC4 (0.2%) than in the IC8 sample (0.1%). Sulfate-reducing *Thermodesulfovibrio* species from the Nitrospirae division were found at 0.09% and 0.06% in the IC4 and IC8 samples, respectively.

3.3 Similarities and dissimilarities in the microbial communities based on functional annotation

In contrast to the taxonomical assignment, the functional annotation of the metagenomes exhibited a similar structure for the microbial communities. A comparison of the metagenomes at the function level using the SEED (Overbeek et al 2005) genome annotations and profile scatter plot showed that the IC4 and IC8 metagenomes possess >83% similarity at the function level (Fig. 1S). Functional analysis of the IC4 and IC8 metagenomes demonstrated that among the annotated protein sequences, the most abundant groups represented housekeeping functions, including carbohydrate metabolism (16.6% and 17.7%), amino acid biosynthesis (13.8% and 14.8%), and vitamin and protein metabolism (12.1% and 13.2%). Approximately 4.1% and 3.7% of annotated reads were related to stress response including a high proportion of oxidative stress- (52% and 48%) and osmotic stress- (13% and 12%) related sequences (Fig. 3).

283 **3.3.1 Methane metabolism.** Analyses using KEGG (Kyoto Encyclopedia of Genes and
284 Genomes) database (Kanehisa and Goto 2000) demonstrated that several functional genes
285 directly related to methanogenesis are abundant in the IC4 metagenome (Fig. 4), including
286 genes coding for F420-dependent methylene-H4 MPT reductase (EC 1.5.99.11),
287 formylmethanofuran dehydrogenases (*fmd*) (EC 1.2.99.5), CoB-CoM heterodisulfide
288 reductases (EC 1.8.98.1), F420-reducing hydrogenases (EC 1.12.98.1), and
289 methylenetetrahydromethanopterin dehydrogenases (*mer*) (EC 1.5.99.9). All of these genes,
290 with exception of F420-dependent methylene-H4 MPT reductase, were also present in the
291 IC8 metagenome though in lower amounts. For example, the gene coding for the enzyme
292 catalyzing the last step of methanogenesis, methyl-coenzyme M reductase (MCR) (EC
293 2.8.4.1), was represented by 1333 hits in IC4 but only by nine hits in the IC8 metagenome.
294 **3.3.2 Nitrogen metabolism.** The abundance of genes associated with nitrogen metabolism
295 was higher in the IC4 metagenome than in that of IC8 (2.2% versus 1.7%). The percentage
296 of genes related to nitrogen metabolism detected in permafrost was slightly higher than the
297 relative abundance (1.3%) of genes responsible for nitrogen metabolism reported in
298 metagenomes examined from glacier ice of the Northern Schneeferner (Simon et al 2009).
299 Notably, in the IC8 metagenome the gene *nifH* coding for a nitrogenase (EC 1.18.6.1), which
300 performs nitrogen fixation, was represented only by seven sequences. These genes were
301 associated mainly with Proteobacteria (43.7%) represented by *Bradyrhizobium* sp., *Azoarcus*
302 sp., *Mesorhizobium loti*, and Actinobacteria (*Frankia* sp., 41%). In the IC4 metagenome 90
303 features corresponding to *nifH* gene were detected, and these nitrogenase sequences were
304 linked predominantly with Proteobacteria (33.5%). Nitrogenase genes related to Firmicutes
305 (primarily *Desulfitobacterium hafniense* and *Alkaliphilus metalliredigens*) and
306 Actinobacteria (*Frankia* species) constituted 17.6% and 15%, respectively. Interestingly,

307 *nifH* genes of cyanobacterial origin (primarily linked to *Nostoc* species) were detected
308 exclusively within the IC4 metagenome (12.9%).
309 Genes connected with the denitrification processes, such as nitrate reductase (EC 1.7.99.4)
310 and nitrite reductase (EC 1.7.2.1) were found in both metagenomes (Fig. 4). The nitrate
311 reductase (*narG*, EC 1.7.99.4) sequences predominating within the IC4 metagenome came
312 from more diverse phylogenetic groups in contrast to *narG* gene from IC8. By contrast, even
313 though nitrite reductase (*nirS*) genes were significantly overrepresented in IC8, their
314 presence was detected in similar phylogenetic groups in both metagenomes (Fig. 5). The
315 sequences related to both nitrite reductase (EC 1.7.1.4) and nitric oxide reductase (EC
316 1.7.2.5) were found in similar phylogenetic groups in both metagenomes with prevalence in
317 IC4 (Fig. 5).
318 The ammonium oxidation pathway was represented by a few sequences related to
319 hydroxylamine oxidase (EC 1.7.3.6) genes in known nitrifying bacteria such as
320 *Nitrosomonas eutropha*, *Nitrosococcus oceani*, *Nitrosospira multiformis* and some others.
321 Genes coding for ammonia monooxygenase (EC 1.14.99.39) were not detected in either
322 metagenome by a search with its EC number. However, a search for ammonia
323 monooxygenase using functional hierarchies such as KEGG orthologs yielded ten hits in IC4
324 and four hits in IC8, while SubSystems annotation approach (Storey et al 2004) showed
325 presence of 509 hits in IC4 and 324 hits in IC8. The ammonia monooxygenase sequences
326 were annotated as methane monooxygenase (EC1.14.13.25). It should be noted that the
327 particulate methane monooxygenase and ammonia monooxygenase are related and occur in
328 both methanotrophs and ammonia oxidizers (Holmes et al 1995). These enzymes have wide
329 substrate specificity catalyzing the oxidation of various substrates including ammonia,
330 methane, halogenated hydrocarbons, and aromatic molecules (Arp et al 2002). Overall, the
331 low abundance of ammonia-oxidizers presumably represents the rare utilization of this

pathway by permafrost bacteria in studied environments. Similar results were also reported for metagenomes from other cold environments, e.g., high Arctic hypersaline subzero spring (Lay et al 2013) and Arctic snow packs (Larose et al 2013).

3.3.3 Sulfur metabolism. Sequences associated with sulfur metabolism were present in both IC4 and IC8 metagenomes and related to both reduction and oxidation (Fig. 6). Genes coding for sulfate reduction were more abundant in the IC8 metagenome including genes for sulfate adenylyltransferase (EC 2.7.7.4), phosphoadenylyl-sulfate reductase (EC 1.8.4.8), and ferredoxin-sulfite reductase (EC 1.8.7.1). Taxonomic distribution of associated species was similar in both metagenomes with the exception of sulfate adenylyltransferase, which was represented in IC4 by sequences related mainly to Proteobacteria and Actinobacteria. In the IC8 sample this gene was of more diverse phylogenetic origin (Fig. 6). A few sulfur oxidation genes detected were associated with *Renibacterium salmoninarum* and *Gordonia bronchialis* in IC8 and *Mycobacterium* species and *Sinorhizobium meliloti* in IC4.

3.3.4 **Stress response.** Genes associated with stress response were detected in both of the metagenomes (33683 hits in IC4 metagenome and 28557 in IC8). The three most abundant groups present corresponded to oxidative stress, heat shock, and osmotic stress response genes. Sequences related to oxidative stress originated principally from Proteobacteria, Actinobacteria and Firmicutes (5148 features in IC4 and 3832 features in IC8) and included genes for catalase (EC 1.11.1.6), peroxidase (EC 1.11.1.7), and different superoxide dismutases (EC 1.15.1.1.). Their occurrence is presumably explained by increased oxygen solubility at low temperatures and associated increase of reactive oxygen species concentration (Chattopadhyay 2006). Sequences related to osmotic stress were represented by the genes involved in the synthesis and uptake of compatible solutes including choline, betaine, periplasmic glucan, and ectoine. Genes for osmoprotectant ABC transporters were also detected. Choline dehydrogenase (EC 1.1.99.1) (222 features in IC4

and 213 features in IC8 mainly from Proteobacteria and Actinobacteria) and betaine-aldehyde dehydrogenase (EC 1.2.1.8) (166 features in IC4 and 186 features in IC8 from Proteobacteria, Actinobacteria and Firmicutes) were the most abundant enzymes of this class. This emphasizes the importance of betaine osmolyte for the osmoprotection of members in microbial communities from subfreezing environments. The genes encoded the heat shock proteins were mainly represented by the chaperone protein DnaK (816 hits in IC4 and 54 in IC8) and its interacting protein DnaJ (759 hits in IC4 and 67 in IC8). These proteins are among the most plentiful chaperons in the bacterial cell and often prevalent in microorganisms from cold environments (D'Amico et al 2006).

3.4. Implication of metagenomic sequencing data for the analysis of the samples origin and evolution

The application of biological markers for paleo-reconstructions in various environmental sites has been used occasionally. For example, utilization of lipid analyses for petroleum reservoirs formation and maturation (Seifert and Moldowan 1981); analysis of fossil chironomid assemblages in the Holocene lake-sediment cores (south-central Alaska) for evaluating anthropogenic climatic changes and quantitative paleo-temperature reconstructions (Clegg et al 2010); and analyses of fossil ostracodal assemblages from the Arctic seas for reconstruction of coastline and interpretation of environmental differences in Arctic areas (Stepanova et al 2010). To track the occurrence and distribution of microorganisms in the environment, the gDNA and DNA fragments amplified with PCR can be employed as biological markers. Therefore, we anticipated that integration of the next generation sequencing capabilities and approaches of microbial ecology (such as linking microbial community composition and environmental processes involved in carbon, nitrogen and sulfur cycling) could be used for paleo-reconstructions.

382 The late Pleistocene Ice Complex or Yedoma deposits are widely distributed in north-east
383 Siberia and because of their wide occurrence on the Kolyma lowland, these deposits may
384 play a significant role in climate warming, permafrost degradation and greenhouse gases
385 emission. A question, which processes formed Yedoma has been under dispute in the last
386 several decades. Several hypotheses have been proposed about the origin of the late
387 Pleistocene Ice Complex, including eolian (Tomirdiaro et al 1984, Tomirdiaro and
388 Chernen'k'ii 1987), alluvial (Rozenbaum 1981), and polygenetic (Konishchev and
389 Kolesnikov 1981, Sher et al 1987) formation. Different opinions on the origin of these
390 deposits have been summarized in the recent publications of Lutz Schirrmeister and co-
391 authors (Schirrmeister et al 2011, Schirrmeister et al 2013). The researchers suggested that
392 the ice rich syngenetic permafrost of the late Pleistocene Ice Complex was developed under
393 a cold-arid climate at less hydromorphic conditions than the lake and lake-alluvial
394 sediments. In general, Yedoma sediments have been characterized by the absence of methane
395 (Rivkina et al 2007, Rivkina and Kraev 2008) and much lower redox-potential and iron
396 (Fe^{2+}) content in comparison to permafrost layers of lake and lake-alluvial origin (Rivkina et
397 al 2006).
398 A comparison of the two late Pleistocene permafrost metagenomes of different genesis, IC4
399 and IC8, revealed differences in the composition of the microbial community that reflects
400 the conditions under which these deposits were formed. These data uncovered significant
401 distinctions in microbial community compositions between Yedoma and lake-alluvial
402 sediments. The relatively low abundance of methanogenic archaea, limited presence of
403 enzymes from the carbon, nitrogen, and sulfur cycles, as well as the presence of
404 methanotrophic bacteria could explain the absence of methane in Yedoma deposits and
405 provide evidence that the formation of these sediments took place under much more aerobic
406 conditions. In other words, we may assume that during the late Pleistocene period, nearly

30,000 years ago, different environmental conditions predetermined biogeochemical regimes and composition of microbial communities of the studied samples. Similarly to our study the recent survey of soil taxonomic, phylogenetic, and functional diversity have demonstrated that metagenomic approaches can be used to build a predictive understanding of variations in microbial diversity and functions across terrestrial biomes (Fierer et al 2012). Thus, the involvement of metagenomic analyses, along with geological and biogeochemical methods, may be used for characterization of the permafrost microbial community, its contribution during climate warming and permafrost thawing, as well as it may become an additional instrument in paleo-reconstructions.

4. Conclusions

In the current study, we performed a comparative analysis of the two permafrost samples isolated from lake sediments and ice complex with utilization of metagenomic sequencing approach. We have shown that the presence of methane in one sample and its absence in the second are associated with the prevalence of the certain groups of microorganisms and their corresponding genes. The possible scenario of the development of both Yedoma and lake sediments can be proposed on the basis of these findings. Analysis of metagenome from IC4, a sample of the lake sediments, demonstrated occurrence of many physicochemical reactions such as denitrification, iron reduction and sulfate reduction, which could reduce environmental redox potential and ultimately create favorable conditions for development of methanogenic community and methanogenesis. As reflected from the composition of IC8 metagenome, the sporadic occurrence of such physicochemical reactions brought to deficiency of methanogenic activity and lack of biogenic methane in the late Pleistocene ice complex on Kolyma-Indigirka lowland.

The obtained results demonstrate that the metagenomic analysis of permafrost microbial communities can represent a valuable instrument for paleo-reconstruction of conditions under which the permafrost sediments were formed in geological perspective.

Author contribution:

E.R., L.P. and F.K. designed research; L.S., M.T., L.P., E.R. performed research; L.S. and M.T. extracted DNA; F.K. performed DNA sequencing; E.R., L.P., K.K., T.V., A.M. analyzed data; E.R. and F.K. contributed new reagents and analytical tools; E.R., L.P., T.V. wrote the paper.

Acknowledgements

This work was supported by grants from the Russian Scientific Fund (14-14-01115) to E.R.; from the National Science Foundation (DEB-1442262) to T.V.; and by HHMI International Early Career Scientist Program (55007424), the EMBO Young Investigator Programme, MINECO (BFU2012-31329 and Sev-2012-0208), AGAUR program (2014 SGR 0974) to F.K. The Russian Scientific Fund (14-14-01115) support was allocated for sample collection, gDNA isolation, and analysis of metagenomic data.

448 **References**

449 Angly, F. E., Willner, D., Prieto-Davó, A., Edwards, R. A., Schmieder, R., Vega-Thurber, R.,
450 Antonopoulos, D. A., Barott, K., Cottrell, M. T., Desnues, C., Dinsdale, E. A., Furlan,
451 M.,Haynes, M., Henn, M. R., Hu, Y., Kirchman, D. L., McDole, T., McPherson, J. D., Meyer,
452 F., Miller, R. M., Mundt, E., Naviaux, R. K., Rodriguez-Mueller, B., Stevens, R., Wegley, L.,
453 Zhang, L., Zhu, B., and Rohwer, F.: The GAAS metagenomic tool and its estimations of
454 viraland microbial average genome size in four major biomes, PLoS Computational Biology,
455 5,e1000593, doi:10.1371/journal.pcbi.1000593, 2009.
456
457 Anthony Walter, K. M., Zimov, S. A., Grosse, G., Jones, M. C., Anthony, P. M., Chapin III, F.
458 S., Finlay, J. C., Mack, M. C., Davydov, 5 S., Frenzel, P., and Frolking, S.: A shift of
459 thermokarst lakes from carbon sources to sinks during the Holocene epoch, Nature, 511, 452–
460 456, 2014.
461
462 Arp DJ, Sayavedra-Soto LA, Hommes NG:Molecular biology and biochemistry of ammonia
463 oxidation by *Nitrosomonas europaea*. Arch Microbiol 178, 250-255, 2002.
464
465 Bischoff, J., Mangelsdorf , K., Gattinger , A., Schloter, M., Kurchatova, A., Herzschuh, U.,
466 Wagner, D.: Response of methanogenic archaea to Late Pleistocene and Holocene climate
467 changes in the Siberian Arctic, Global Biogeochemical Cycle. 27, 305–317, 2013.
468
469 Brouchkov A., Fukuda M.: Preliminary Measurements on Methane Content in Permafrost,
470 Central Yakutia, and some Experimental Data 21. Permafrost and Periglacial Processes, 13,
471 187–197, 2002.
472
473 Chattopadhyay M.K. : Mechanism of bacterial adaptation to low temperature. Journal of
474 biosciences*,* 31**,** 157-165, 2006.
475
476 Clegg, B. F., Clarke, G. H., Chipman, M. L., Chou, M., Walker, I. R., Tinner, W., and Hu F.
477 S.:Six millennia of summer temperature variation based on midge analysis of lake sediments
478 from Alaska, Quaternary Sci. Rev., 29, 3308–3316, 2010.
479

480 D'Amico S, Collins T, Marx JC, Feller G, Gerday C.: Psychrophilic microorganisms:
481 challenges for life. EMBO Rep, 7, 385-389, 2006.
482
483 Ellenbroek, F. M. and Cappenberg, T.E.: DNA-synthesis and tritiated-thymidine incorporation
484 by heterotrophic fresh-water bacteria in continuous culture, Appl. Environ. Microb., 57,
485 1675–1682, 1991.
486
487 Fierer N., Leff J.W., Adams B.J., Nielsen U.N., Bates S.T., Lauber C.L., Owens, S., Gilbert
488 J.A., Caporaso J.G.: Cross-biome metagenomic analyses of soil microbial communities and
489 their functional attributes, Proc Natl Acad Sci USA,109, 21390-21395, 2012.
490
491 Gilichinsky, D. and Rivkina, E.: Permafrost microbiology, in: Encyclopedia of Geobiology,
492 edited by: Reitner, J. and Thiel, V., Springer, Dordrecht, the Netherlands, 726–732, 2011.
493
494 Glockner, F. O., Kube, M., Bauer, M., Teeling, H., Lombardot, T., and Ludwig, W., Gade,
495 D.,Beck, A., Borzym, K., Heitmann, K., Rabus, R., Schlesner, H., Amann, R., and
496 Reinhardt,R.: Complete genome sequence of the marine planctomycete Pirellula sp. strain 1,
497 P. Natl.Acad. Sci. USA, 100, 8298–8303, 2003.
498
499 Graham, D. E., Wallenstein, M. D., Vishnivetskaya, T. A., Waldrop, M. P., Phelps, T. J.,
500 Pfiffner, S. M., Onstott, T. C., Whyte, L. G., Rivkina, E. M., Gilichinsky, D. A., Elias, D. A.,
501 Mackelprang, R., VerBerkmoes, N. C., Hettich, R. L., Wagner, D., Wullschleger, S. D., and
502 Jansson, J. K.: Microbes in thawing permafrost: the unknown variable in the climate change
503 equation, ISME J., 6, 709–712, 2012.
504
505 Harmsen, H. J. M., Van Kuijk, B. L. M., Plugge, C. M., Akkermans, A. D. L., De Vos, W. M.,
506 and Stams, A. J. M.: *Syntrophobacter fumaroxidans* sp. nov., a syntrophic propionate-
507 degrading sulfate-reducing bacterium, Int. J. Syst. Bacteriol., 48, 1383–1387, 1998.
508
509 Holmes, A. J., Costello, A., Lidstrom, M. E., and Murrell, J. C.: Evidence that
510 particulatemethane monooxygenase and ammonia monooxygenase may be evolutionarily
511 relatedFEMS Microbiol. Lett., 132, 203–208, 1995.
512

513 Jackson, B. E., Bhupathiraju, V. K., Tanner, R. S., Woese, C. R., and McInerney, M. J.:
514 Syntrophus aciditrophicus sp. nov., a new anaerobic bacterium that degrades fatty acids
515 andbenzoate in syntrophic association with hydrogen-using microorganisms, Arch.
516 Microbiol.,171, 107–114, 1999.
517
518 Jansson, J. K. and Tas, N.: The microbial ecology of permafrost, Nature. Rev. Microbiol., 12,
519 414–425, 2014.
520
521 Kanehisa M., Goto S. : KEGG: Kyoto Encyclopedia of Genes and Genomes, Nucleic Acids
522 Res, 28**,** 27-30, 2000.
523
524 Khmelenina, V. N., Makutina, V. A., Kalyuzhnaya, M. G., Rivkina, E. M., Gilichinsky, D.
525 A.,and Trotsenko, Y.: Discovery of viable methanotrophic bacteria in permafrost sediments of
526 northeast Siberia, Dokl. Biol. Sci., 384, 235–237, 2002.
527
528 Konishchev, V. N. and Kolesnikov, S. F.: Specialities of structure and composition of late
529 Cenozoic deposits in the section of Oyogossky Yar, in: Problems of Cryolithology, MGU
530 Publishing, Moscow, 107–117, 1981 (in Russian).
531
532 Kraev, G. N., Schultze, E. D., and Rivkina, E. M.: Cryogenesis as a factor of methane
533 distribution in layers of permafrost, Dokl. Earth Sci., 451, 882–885, 2013.
534
535 Krivushin, K. V., Shcherbakova, V. A., Petrovskaya, L. E., and Rivkina, E. M.:
536 Methanobacterium veterum sp nov., from ancient Siberian permafrost, Int. J. Syst. Evol.
537 Micr., 60, 455–459, 2010.
538
539 Krivushin, K., Kondrashov, F., Shmakova, L., Tutukina, M., Petrovskaya, L., and Rivkina, E.:
540 Two metagenomes from Late Pleistocene northeast Siberian permafrost, Genome
541 Announcements, 3, e01380-14, doi:10.1128/genomeA.01380-14, 2015.
542
543 Larose, C., Dommergue, A., and Vogel, J. G.: Microbial nitrogen cycling in Arctic snowpacks,
544 Environ. Res. Lett., 8, 035004, 2013.

Lau, M. C. Y., Stackhouse, B. T., Layton, A. C., Chauhan, A., Vishnivetskaya, T. A., Chourey,K., Ronholm, J., Mykytczuk, N. C. S., Bennett, P. C., Lamarche-Gagnon, G., Burton, N., Pollard, W. H., Omelon, C. R., Medvigy, D. M., Hettich, R. L., Pfi ner, S. M., Whyte, L. G., and Onstott, T. C.: An active atmospheric methane sink in high Arctic mineral cryosols, ISME J., ., 9, 1880–1891, 2015.

Lay C.Y., Mykytczuk N.C., Yergeau E., Lamarche-Gagnon G., Greer C.W., Whyte L.G.: Defining the functional potential and active community members of a sediment microbial community in a high-arctic hypersaline subzero spring, Appl. Environ. Microb., 79, 3637–3648, 2013.

Legendre, M., Bartoli, J., Shmakova, L., Jeudy, S., Labadie, K., Adrait, A., Lescot, M., Poirot,O., Bertaux, L., Bruley, C., Couté, Y., Rivkina, E., Abergel, C., and Claverie, J-M.: Thirty thousand-year-old distant relative of giant icosahedral DNA viruses with a pandoravirus morphology, P. Natl. Acad. Sci. USA, 111, 4274–4279, 2014.

Mackelprang, R., Waldrop, M. P., DeAngelis, K. M., David, M. M., Chavarria, K. L., Blazewicz,S. J., Rubin, E. M., and Jansson, J. K.: Metagenomic analysis of a permafrost microbialcommunity reveals a rapid response to thaw, Nature, 480, 368–371, 2011.

Meyer, F., Paarmann, D., D'Souza, M., Olson, R., Glass, E., Kubal, M., Paczian, T., Rodriguez, A., Stevens, R., Wilke, A., Wilkening, J., and Edwards, R. A.: The metagenomics RAST server – a public resource for the automatic phylogenetic and functional analysis of metagenomes, BMC Bioinformatics, 9, 386, doi:10.1186/1471-2105-9-386, 2008.

Newcombe, R. G.: Two-sided confidence intervals for the single proportion: comparison of seven methods, Statistics in Medicine, 17, 857–872, 1998.

Overbeek, R., , Begley, T., Butler, R.M., Choudhuri, J.V., Chuang, H-Yu., Cohoon, M., Vale ´rie de Cre´cy-Lagard, Diaz N., Disz, T., Edwards, R., Fonstein, M., Frank, E.D., Gerdes, S., Glass,E.M., Goesmann, A., Hanson, A. Iwata-Reuy, D., Jensen, R., Jamshidi, N., Krause L., Kuba, M., Larsen, L., Linke, B., McHardy, A.C., Meyer, F., Neuweger, H., Olsen, G., Olson,

R., Osterman, A., Portnoy, V., Pusch, G.D., Rodionov, D.A., Ruckert, C., Steiner, J., Stevens, R., Thiele, I., Vassieva, O., Ye,Yu., Zagnitko, O. and Vonstein, V. : The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes. Nucleic Acids Res, 33, 5691-5702, 2005.

Parks, D. H. and Beiko, R. G.: Identifying biologically relevant di erences between metagenomic communities, Bioinformatics, 26, 715–721, 2010.

Raes, J., Korbel, J. O., Lercher, M. J., von Mering, C., and Bork, P.: Prediction of effective genome size in metagenomic samples, Genome. Biol., 8, R10, doi:10.1186/gb-2007-8-1-r10, 2007.

Rivkina, E. and Kraev, G.: Permafrost degradation and influx of biogeogases into the atmosphere, Ninth International Conference on Permafrost, Fairbanks, USA, University of Alaska Fairbanks, 29 June–03 July, 1499–1504, 2008.

Rivkina, E., Laurinavichius, K., McGrath, J., Tiedje, J., Shcherbakova, V., and Gilichinsky, D.: Microbial life in permafrost, Adv. Space Res., 33, 1215–1221, 2004.

Rivkina, E., Kraev, G., Krivushin, K., Laurinavichius, K., Fyodorov-Davydov, D., Kholodov, A.,Shcherbakova, V., and Gilichinsky, D.: Methane in permafrost of northeastern Arctic, Earth Cryosphere (Russian), 10, 23–41, 2006.

Rivkina, E., Shcherbakova, V., Laurinavichius, K., Petrovskaya, L., Krivushin, K., Kraev, G.,Pecheritsina. S., and Gilichinsky, D.: Biogeochemistry of methane and methanogenic archaeain permafrost, FEMS Microbiol. Ecol., 61, 1–15, 2007.

Rozenbaum, G. E.: Special features of lithogenesis of the alluvial planes in the Eastern Subarctic as related to the problem of the Ice (Yedoma) Complex, in: Problems of Cryolithology, MSU Press, Moscow, 87–100, 1981 (in Russian).

Schirrmeister, L., Kunitsky, V., Grosse, G., Wetterich, S., Meyer, H., Schwamborn, G., Babiy, O., Derevyagin, 5 A., and Siegert, C.: Sedimentary characteristics and origin of the Late

610 Pleistocene Ice Complex on North-East Siberian Arctic coastal lowlands and islands – a
611 review, Quatern. Int., 241, 3–25, 2011.
612
613 Schirrmeister, L., Froese, D., Tumskoy, V., Grosse, G., and Wetterich, S.: Yedoma: late
614 Pleistocene ice-rich syngenetic permafrost of Beringia, in: Encyclopedia of Quaternary
615 Science, 2nd edn., edited by: Elias, S., Mock, C., and Murton, J., Elsevier, Amsterdam, 542–
616 552, 2013.
617
618 Seifert, W. K. and Moldowan, J. M.: Paleoreconstruction by biological markers, Geochim.
619 Cosmochim.Ac., 45, 783–794, 1981.
620
621 Shcherbakova, V., Rivkina, E., Pecheritsyna, S., Laurinavichius, K., Suzina, N., and
622 Gilichinsky, D.: Methanobacterium arcticum sp. nov., a methanogenic archaeon from
623 Holocene Arctic permafrost, Int. J. Syst. Evol. Micr., 61, 144–147, 2011.
624
625 Sher, A., Kaplina, T., and Ovander, M.: Unified regional stratigraphic chart for the Quaternary
626 deposits in the Yana-Kolyma Lowland and its mountainous surroundings, Explanatory Note,
627 Decisions of Interdepartmental Stratigraphic Conference on the Quaternary of the Eastern
628 USSR, Magadan, 1982, USSR Academy of Sciences, Far-Eastern Branch, North-
629 EasternComplex Research Institute, Magadan, USSR, 29–69, 1987 (in Russian).
630
631 Shi, T., Reeves, R. H., Gilichinsky, D. A., and Friedmann, E. I.: Characterization of viable
632 bacteria from Siberian permafrost by 16S rDNA sequencing, Microb. Ecol., 33, 169–179,
633 1997.
634
635 Simon, C., Wiezer, A., Strittmatter, A. W., and Daniel, R.: Phylogenetic diversity and
636 metabolic potential revealed in a glacier ice metagenome, Appl. Environ. Microb., 75, 7519–
637 7526, 2009.
638
639 Stepanova, A. Y., Taldenkova, E. E., and Bauch, H. A.: Arctic quaternary ostracods and their
640 use in paleoreconstructions, Paleontol. J., 44, 41–48, 2010.
641

642 Storey, J. D. and Tibshirani, R.: Statistical significance for genome wide studies, P. Nat.
643 Acad.Sci. USA, 100, 9440–9445, 2003.
644
645 Storey, J. D., Taylor, J. E., and Siegmund, D.: Strong control, conservative point estimation
646 and simultaneous conservative consistency of false discovery rates: a unified approach, J.
647 Roy.Stat. Soc. B, 66, 187–205, 2004.
648
649 Tomirdiaro, S. V. and Chernen'k'ii, B. I.: Cryogenic Deposits of East Arctic and Sub Arctic,
650 ANSSSR Far-East-Science Center, Magadan, 1987.
651
652 Tomirdiaro, S. V., Arslanov, K. A., Chernen'kii, B. I., Tertychnaya, T. V., and Prokhorova, T.
653 N.: New data on formation of loess-ice sequences in Northern Yakutia and ecological
654 conditionsof mammoth fauna in the Arctic during the late Pleistocene, Reports Academy of
655 Sciences USSR 278, 1446–1449, 1984 (in Russian).
656
657 Villemur, R., Lanthier, M., Beaudet, R., and Lepine, F.: The desulfitobacterium genus,
658 FEMSMicrobiol. Rev., 30, 706–733, 2006.
659
660 Vishnivetskaya, T. A., Layton, A. C., Lau, M. C. Y., Chauhan, A., Cheng, K. R., Meyers, A. J.,
661 Murphy, J. R., Rogers, A.W., Saarunya, G. S.,Williams, D. E., Pfi ner, S. M., Biggersta , J.
662 P.,Stackhouse, B. T., Phelps, T. J., Whyte, L., Sayler G. S., and Onstott, T. C.: Commercial
663 DNA extraction kits impact observed microbial community composition in permafrost
664 samples, FEMS Microbiol. Ecol., 87, 217–230, 2014.
665
666 Walter, K. M., Edwards, M. E., Grosse, G., Zimov, S. A., and Chapin, F. S.: Thermokarst lakes
667 as a source of atmospheric CH_4 during the last deglaciation, Science, 318, 633–636, 2007.
668
669 Wilke, A., Harrison, T., Wilkening, J., Field, D., Glass, E. M., Kyrpides, N., Mavrommatis,
670 K.,and Meyer, F.: The M5nr: a novel non-redundant database containing protein sequences
671 and annotations from multiple sources and associated tools, BMC Bioinformatics, 13,
672 141,doi:10.1186/1471-2105-13-141, 2012.
673

674 Yergeau, E., Hogues, H., Whyte, L. G., and Greer, C. W.: The functional potential of high
675 Arctic permafrost revealed by metagenomic sequencing, qPCR and microarray analyses,
676 ISME J.,4, 1–9, 2010.
677
678 Zimov, S. A., Schuur, E. A. G., and Chapin, F. S.: Permafrost and the global carbon budget,
679 Science, 312, 1612–1613, 2006.

680 Table 1. Physicochemical characteristic of two permafrost soils
681
682

Samples	Grain-size distribution			Ice content	Eh	C_{tot}	Water extract (1:5)								
	Sand	Silt	Clay				Dry residue	pH	HCO_3^-	Cl^-	SO_4^{2-}	Ca^{2+}	Mg^{2+}	K^+	Na^+
	%			%	mV	%	%	Meq/100g dry soil							
IC4	79.44	15.84	4.72	17.5	45	1.1	0.130	7.22	0.12	1.40	0.14	0.32	0.42	0.06	1.33
IC8	40.72	52.72	6.56	27.6	167	1.2	0.135	8.16	0.57	0.32	0.48	0.75	0.35	0.02	0.56

683

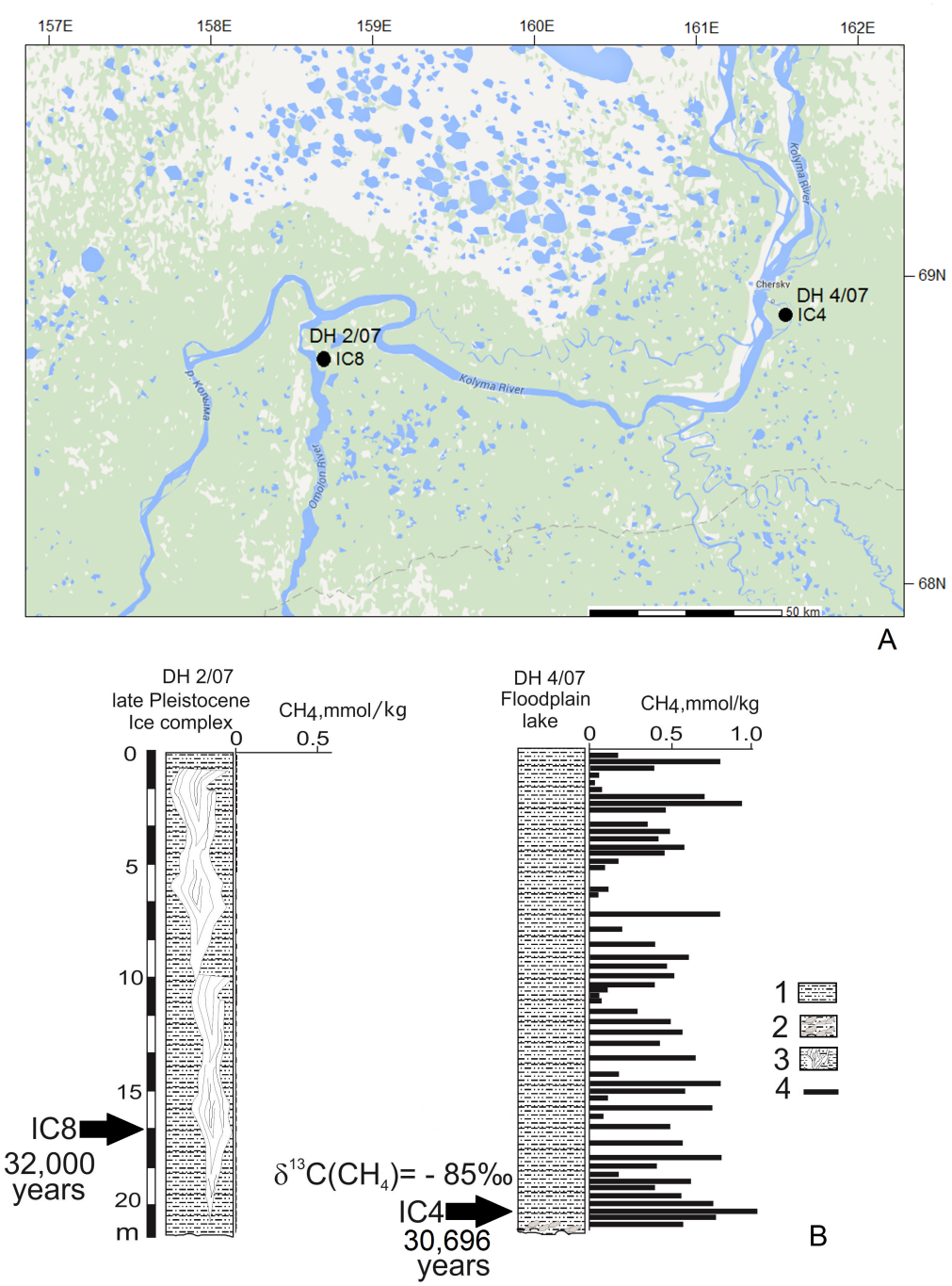
684 **Table 2.** Number of sequences showing homologies to genes associated with KEGG pathways
685 within the IC4 and IC8 metagenomes. Data were generated by searching in Hierarchical
686 classification table based upon SubSystems annotation source.
687

Enzyme Nomenclature	Function *and Enzyme Profile*	IC4	IC8
	Methane metabolism		
EC 1.5.99.11	F420-dependent methylene-H4 MPT reductase	3649	0
EC 1.12.98.1	Coenzyme F420 hydrogenase	1441	370
EC 1.8.98.1	CoB-CoM heterodisulfide reductase	24601	3982
EC 1.2.99.5	Formylmethanofuran dehydrogenase	10757	3012
EC 1.5.99.9	Methylenetetrahydromethanopterin dehydrogenases	370	3
EC 2.8.4.1	Methyl-coenzyme M reductase	1333	9
EC 2.3.1.101	Formylmethanofuran--tetrahydromethanopterin N-formyltransferase	3056	1642
EC 2.1.1.86	Tetrahydromethanopterin S-methyltransferase	1997	9
	Nitrogen metabolism		
EC 1.18.6.1	Nitrogenase (molybdenum-iron) alpha chain nifH	2519	42
EC 1.7.2.1	Copper-containing nitrite reductase	1824	11405
EC 1.7.2.5[a]	Nitric-oxide reductase	3440	1838
EC 1.7.1.4	Nitrite reductase [NAD(P)H] small subunit	20713	13041

EC 1.7.99.4	Respiratory nitrate reductase alpha chain narG	36476	28169
EC	Ammonia monooxygenase	509	324
EC 1.7.3.4	Hydroxylamine oxidase	517	103
EC 1.7.99.6	Nitrous oxide reductase	61	46
	Sulfur metabolism		
EC 1.8.4.8	Phosphoadenylyl-sulfate reductase [thioredoxin]	7521	11165
EC 1.8.7.1	Ferredoxin--sulfite reductase	2587	8920
EC 2.7.7.4	Sulfate adenylyltransferase subunit 2	15449	23435
EC 2.7.1.25	Adenylylsulfate kinase	22898	22418
EC 1.8.3.1	Sulfite oxidase	14353	13968
	Stress response		
EC 1.11.1.6	Catalase	74622	41693
EC 1.15.1.1	Manganese superoxide dismutase	8922	13302
EC 1.11.1.7	Peroxidase	35552	19698
EC 1.15.1.1	Superoxide dismutase [Cu-Zn] precursor	2855	1327
EC 1.15.1.1	Superoxide dismutase [Fe]	7221	3109
EC 1.1.99.1	Choline dehydrogenase	25244	18018
EC 1.2.1.8	Betaine-aldehyde dehydrogenase	10022	12782
NR	Chaperone protein DnaK	78365	89944
NR	Interacting protein DnaJ	32897	45343

688

689 [a]The current KEGG number has been created for enzyme EC 1.7.99.7.

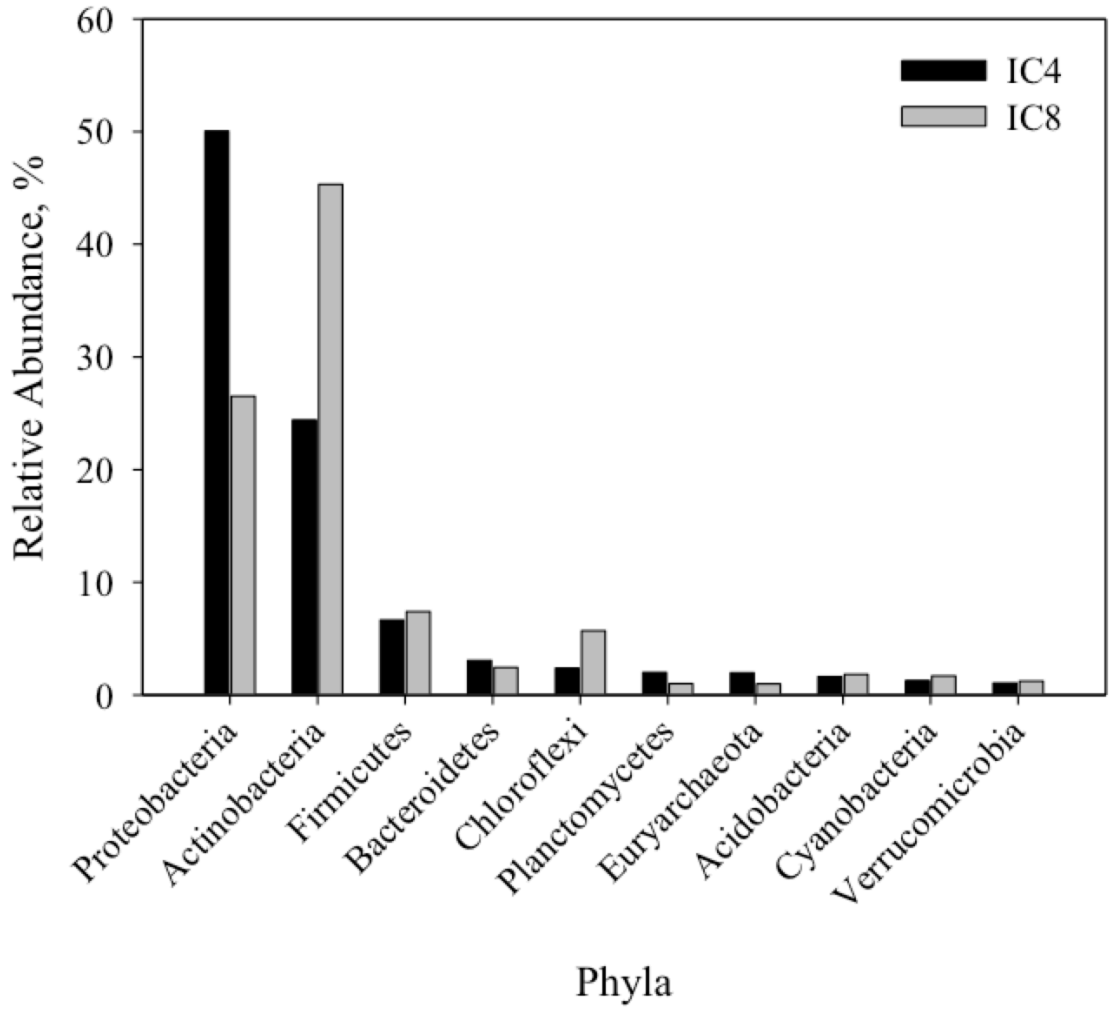


690

691 Fig. 1. Location of sampling sites on the Kolyma lowland (A). The position of samples IC4 and IC8
692 in drilling holes and methane content from different depth of these boreholes (B).

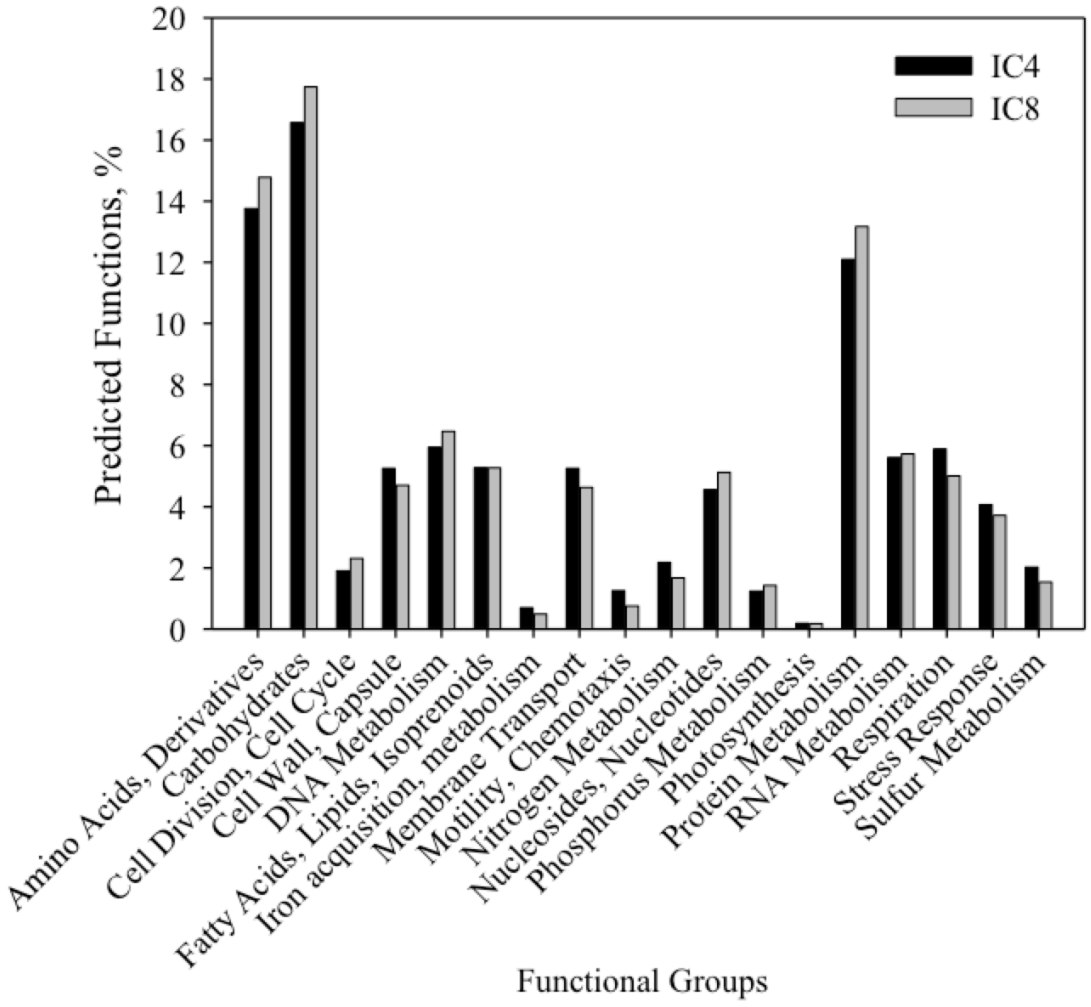
693

694



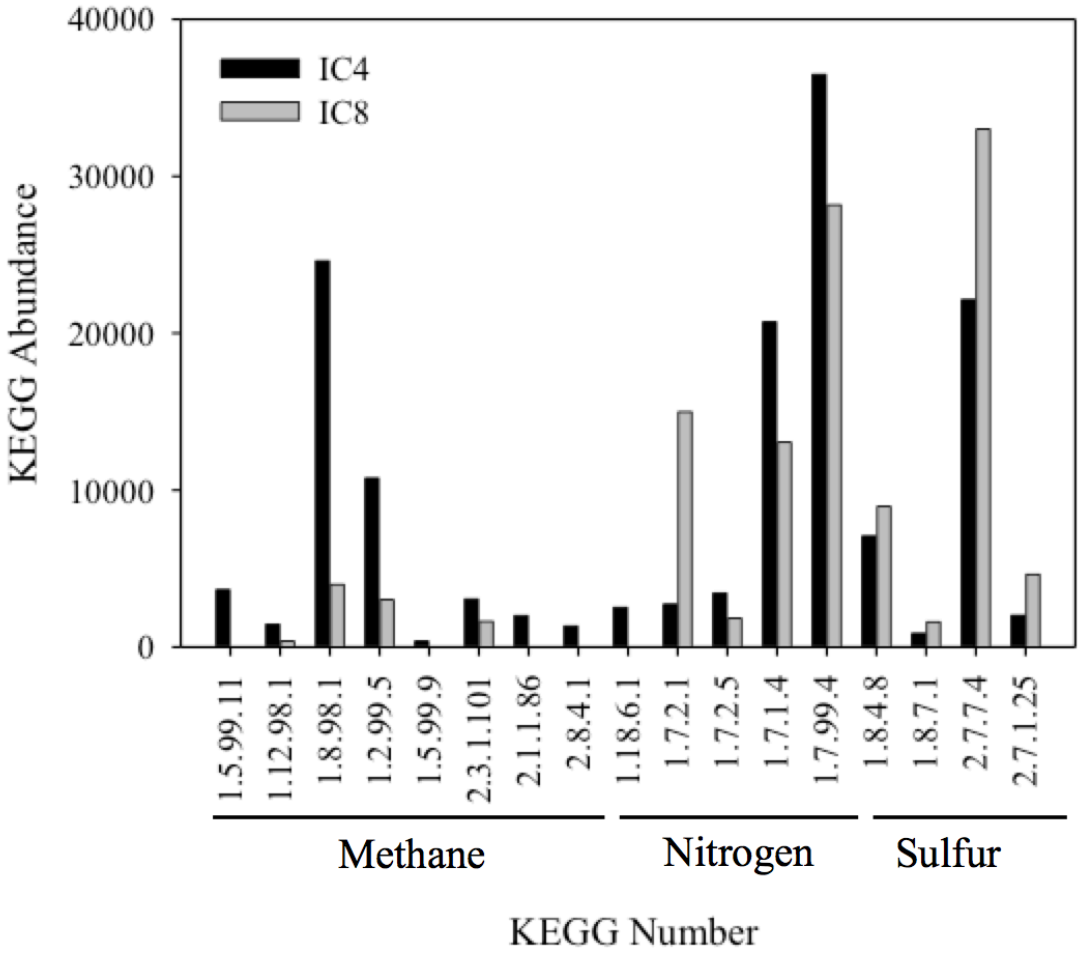
695

696 Fig. 2. Community analyses of the IC4 and IC8 metagenomes at phylum level. The phyla present in
697 metagenomes at a >1% level are shown.



698

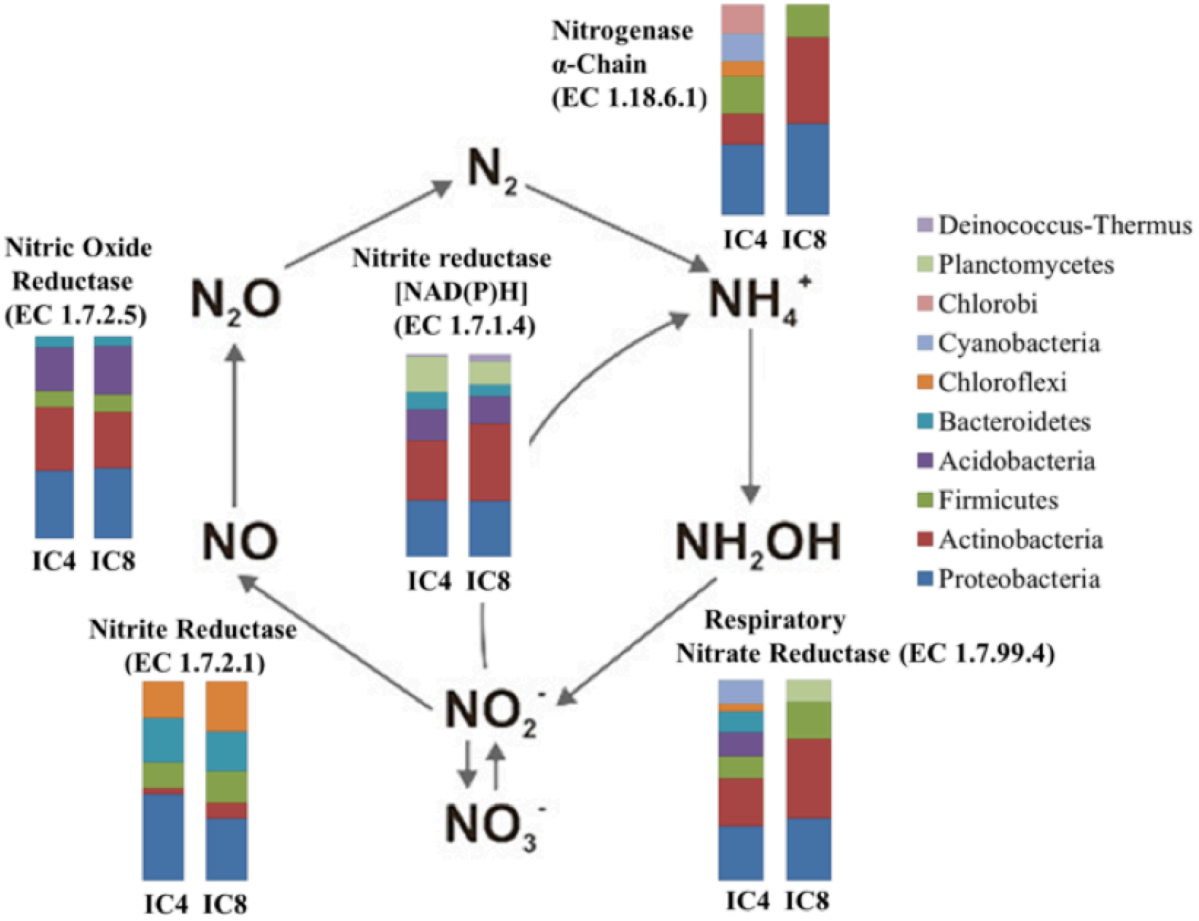
699 Fig. 3. Percentage of gene sequences associated with different functions in the annotated protein
700 sequences within IC4 and IC8 metagenomes.



701

702 Fig. 4. KEGG abundance of the selected functional gene sequences found within IC4 and IC8
703 metagenomes. Genes found in low abundance were not included in the figure. Genes from methane,
704 nitrogen, and sulfur metabolic pathways are underlined. The enzyme name that corresponds to each
705 KEGG number is given in Table 2.

706



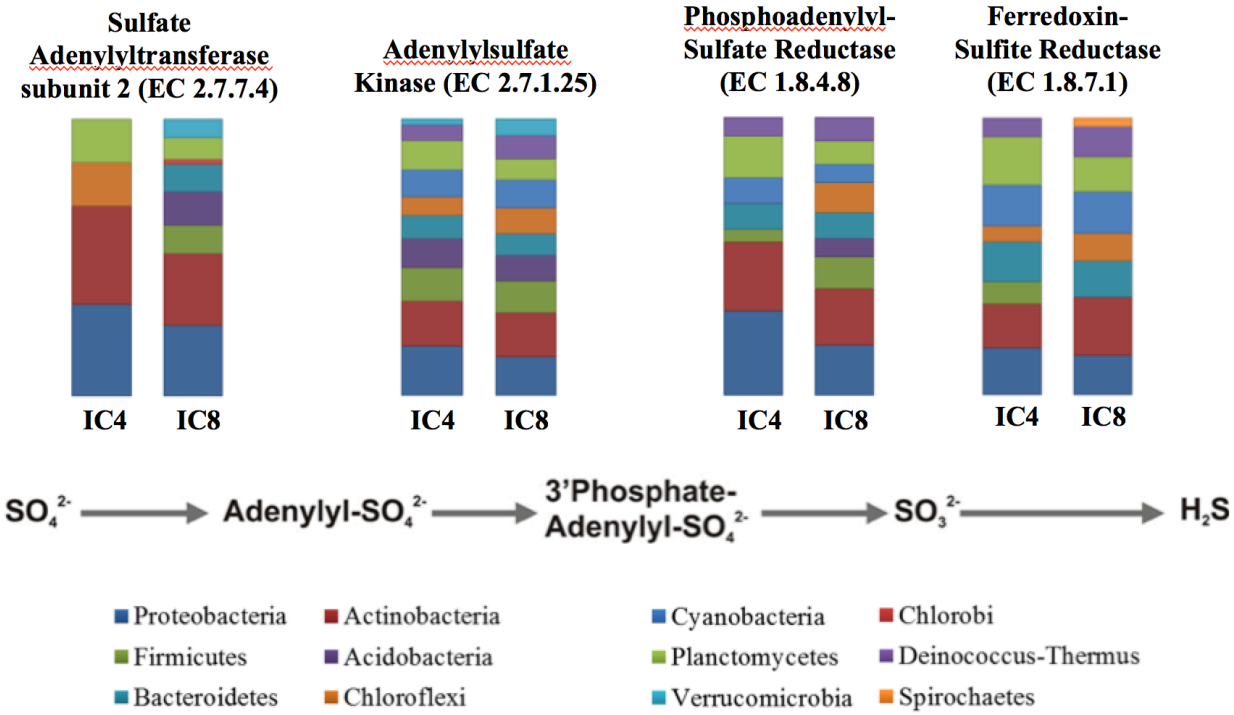
707

708

709 Fig 5. Phylogenetic distribution of the sequences related to nitrogen metabolism within the IC4 and

710 IC8 metagenomes.

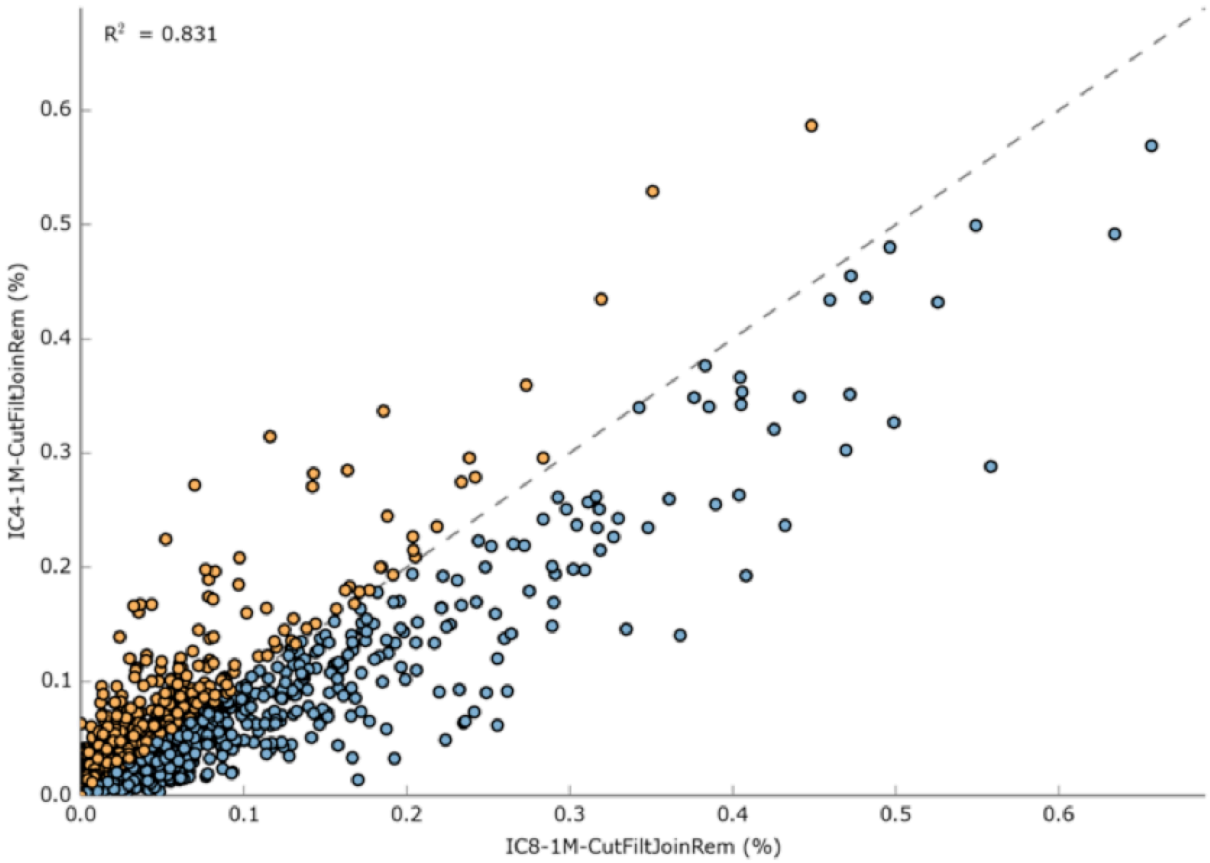
711



712

713 Fig. 6. Phylogenetic distribution of the sequences related to sulfur metabolism (sulfur reduction)

714 within the IC4 and IC8 metagenomes.



715

716 Fig. 1S. Profile scatter plot indicating the similarity of the metagenomes based on the SEED
717 functional annotation.

718

719