Biogeosciences Discuss., 12, 12091–12119, 2015
www.biogeosciences-discuss.net/12/12091/2015/
doi:10.5194/bgd-12-12091-2015

Biogeosciences Discussions
Open Access

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

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Received: 17 June 2015 – Accepted: 14 July 2015 – Published: 4 August 2015

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Published by Copernicus Publications on behalf of the European Geosciences Union.

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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 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 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 late Pleistocene Ice Complex (Rivkina et al., 2007; Rivkina and Kraev, 2008). 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 ob-

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served in samples from the late Pleistocene Ice Complex (Rivkina and Kraev, 2008). Similar results were obtained during experiments with radioactively labeled substrates (Rivkina et al., 2004, 2007, 2002), showing an absence of methanogenic activity in the late Pleistocene Ice Complex samples with this process evidently taking place in 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).

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 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 (lake sediments vs. sediments from the late Pleistocene Ice Complex). The results

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presented here will help to evaluate microbial community responses associated with permafrost thawing due to global warming.

2 Materials and methods

2.1 Sample collection and description

Samples were collected within the Kolyma–Indigirka Lowland in northeast Siberia (69°299 N, 156°599 E) 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.

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^{-1}, $\delta^{13}C = -85$ ‰ 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).

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2.2 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 × 100 cycle sequencing protocol.

2.3 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 and 19.7 Gb representing 131.7 M sequences with an average length of 150 bp for IC8, 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.

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2.4 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 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 $\mu g\,g^{-1}$ of wet sediment, in comparison to 0.37 $\mu g\,g^{-1}$ 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 7.0×10^7 for IC4 and 9.4×10^7 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. DNA from Archaea and Eukaryotes was more abundant in the IC4 sample compared to the IC8 sample, i.e., 2.4 vs. 1.3 and 1.0 vs. 0.9 %,

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respectively. While Bacteria were significantly more abundant than Eukaryotes, the diversity of the Eukaryotic organisms was higher. The number of detected species of Eukaryotic organisms including plants, algae, fungi, and protists was 3.4 and 2.6 times higher in comparison to Bacterial species detected in the IC4 and IC8 samples, respectively. The most abundant organisms 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 significantly dominant within IC8, whereas α-, β-, γ-, and δ- Proteobacteria ($P < 1e^{-15}$), Bacteroidetes ($P < 1e^{-15}$), Planctomycetes ($P < 1e^{-15}$), and Euryarchaeota ($P < 1e^{-15}$) significantly dominated within IC4.

3.2 Permafrost characteristics define the microbial community structure as evidenced 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^{-1}; 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 vs. 0.14 % in IC8), followed by *Methanoregula* (0.28 vs. 0.03 %), *Methanoculleus* (0.1 vs. 0.05 %), *Methanosphaerula* (0.1 vs. 0.03 %), *Methanospirillum*

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(0.1 vs. 0.03 %), *Methanosaeta* (0.1 vs. 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 vs. 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 vs. 7.7 % in IC8), which contains both methanotrophic and nitrogen-fixing bacteria. Alphaproteobacteria Type II methanotrophs (3.32 vs. 1.06 %) were represented by the genera *Methylocella* (0.53 vs. 0.14 %), *Methylosinus* (0.24 vs. 0.07 %), *Methylocystis* (0.22 vs. 0.06 %), and *Methylobacterium* (2.32 vs. 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 vs. 0.11 %), *Methylobacter* (0.12 vs. 0.05 %), and *Methylophaga* (0.02 vs. 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 vs. 0.16 %), *Methylobacillus* (0.07 vs. 0.08 %), *Methylovorus* (0.04 vs. 0.04 %), and *Methy-*

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Iotenera (0.06 vs. 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 vs. 0.5 %), *Sinorhizobium* (1.3 vs. 0.6 %), *Rhizobium* (0.82 vs. 0.4 %), *Rhodospirillum* (0.63 vs. 0.3 %), *Afipia* (0.4 vs. 0.12 %), *Azospirillum* (0.016 vs. 0.006 %), *Azorhizobium* (0.005 vs. 0.001 %) and *Azotobacter* (0.06 vs. 0.05 %), the γ-Proteobacteria nitrogen-fixing species. Other species involved in the nitrogen cycle from the *Hyphomicrobium* (2.5 vs. 0.23 %, capable of denitrification with methanol), *Nitrobacter* (1.9 vs. 0.6 %, capable of oxidizing nitrite into nitrate) and *Rhodopseudomonas* (3.8 vs. 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* ($\sim$ 0.1 %),*Nitrosospira* ($\sim$ 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 vs. 0.34 %) being the most abundant, followed by *Pirellula* (0.61 vs. 0.28 %), *Blastopirellula* (0.57 vs. 0.24 %), and *Isosphaera* (0.16 vs. 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.

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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 vs. 0.41 %), *Desulfatibacillum* (0.22 vs. 0.08 %), *Desulfococcus* (0.18 vs. 0.07 %), *Desulfobacterium* (0.14 vs. 0.04 %), *Desulfomicrobium* (0.11 vs. 0.06 %), and *Geobacter* (1.13 vs. 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 vs. 0.18 % in IC8) and the benzoate-degrading bacterium *Syntrophus* (0.62 vs. 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 SEED function level using profile scatter plot showed that the IC4 and IC8 metagenomes possess > 83 % similarity at the function level (Fig. S1). Functional analysis of the IC4 and IC8 metagenomes demonstrated that among the annotated protein sequences, the most abundant groups represented

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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).

3.3.1 Methane metabolism

KEGG analysis demonstrated that several functional genes directly related to methanogenesis are abundant in the IC4 metagenome (Fig. 4), including genes coding for F420-dependent methylene-H4 MPT reductase (EC 1.5.99.11), formylmethanofuran dehydrogenases (*fmd*) (EC 1.2.99.5), CoB-CoM heterodisulfide reductases (EC 1.8.98.1), F420-reducing hydrogenases (EC 1.12.98.1), and methylenetetrahydromethanopterin dehydrogenases (*mer*) (EC 1.5.99.9). All of these genes, with exception of F420-dependent methylene-H4 MPT reductase, were also present in the IC8 metagenome though in significantly lower amounts. For example, the gene coding for the enzyme catalyzing the last step of methanogenesis, methyl-coenzyme M reductase (MCR) (EC 2.8.4.1), was represented by 1333 hits in IC4 but only by nine hits in the IC8 metagenome.

3.3.2 Nitrogen metabolism

The abundance of genes associated with nitrogen metabolism was higher in the IC4 metagenome than in that of IC8 (2.2 vs. 1.7 %). The percentage of genes related to nitrogen metabolism detected in permafrost was slightly higher than the relative abundance (1.3 %) of genes responsible for nitrogen metabolism reported in metagenomes examined from glacier ice of the Northern Schneeferner (Simon et al., 2009). Notably, in the IC8 metagenome the gene *nifH* coding for a nitrogenase (EC 1.18.6.1), which performs nitrogen fixation, was represented only by seven features. These genes were associated mainly with Proteobacteria (43.7 %) represented by *Bradyrhizobium* sp.,

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Azoarcus sp., *Mesorhizobium loti*, and Actinobacteria (*Frankia* sp., 41 %). In the IC4 metagenome 90 features corresponding to *nifH* gene were detected, and these nitrogenase sequences were linked predominantly with Proteobacteria (33.5 %). Nitrogenase genes related to Firmicutes (primarily *Desulfitobacterium hafniense* and *Alkaliphilus metalliredigens*) and Actinobacteria (*Frankia* species) constituted 17.6 and 15 %, respectively. Interestingly, *nifH* genes of cyanobacterial origin (primarily linked to *Nostoc* species) were detected exclusively within the IC4 metagenome (12.9 %).

Genes connected with the denitrification processes, such as nitrate reductase (EC 1.7.99.4) and nitrite reductase (EC 1.7.2.1) were found in both metagenomes (Fig. 4). The nitrate reductase (*narG*, EC 1.7.99.4) sequences predominating within the IC4 metagenome came from more diverse phylogenetic groups in contrast to *narG* gene from IC8. By contrast, even though nitrite reductase (*nirS*) genes were significantly overrepresented in IC8, their presence was detected in similar phylogenetic groups in both metagenomes (Fig. 5). The sequences related to both nitrite reductase (EC 1.7.1.4) and nitric oxide reductase (EC 1.7.2.5) were found in similar phylogenetic groups in both metagenomes with prevalence in IC4 (Fig. 5).

The ammonium oxidation pathway was represented by a few sequences related to hydroxylamine oxidase (EC 1.7.3.6) genes in known nitrifying bacteria such as *Nitrosomonas eutropha*, *Nitrosococcus oceani*, *Nitrosospira multiformis* and some others. Genes coding for ammonia monooxygenase (EC 1.14.99.39) were not detected in either metagenome by a search with its EC number. However, a search for ammonia monooxygenase using functional hierarchies such as KEGG orthologs yielded ten hits in IC4 and four hits in IC8, while SubSystems showed presence of 509 hits in IC4 and 324 hits in IC8. The ammonia monooxygenase sequences were annotated as methane monooxygenase (EC1.14.13.25). It should be noted that the particulate methane monooxygenase and ammonia monooxygenase are related and occur in both methanotrophs and ammonia oxidizers (Holmes et al., 1995). These enzymes have wide substrate specificity catalyzing the oxidation of various substrates including ammonia, methane, halogenated hydrocarbons, and aromatic molecules (Arp

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et al., 2002). Overall, the 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 (33 683 hits in IC4 metagenome and 28 557 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

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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).

4 Conclusions

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 paleotemperature 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 environ-

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mental processes involved in carbon, nitrogen and sulfur cycling) could be used for paleo-reconstructions.

In the current study, we demonstrated that the metagenomic analyses of permafrost communities could be also a central key for paleo-reconstruction of conditions under which the permafrost sediments were formed in nature. The late Pleistocene Ice Complex or Yedoma deposits are widely distributed in north-east Siberia and because of their wide occurrence on the Kolyma lowland, these deposits may play a significant role in climate warming, permafrost degradation and greenhouse gases emission. A question, which processes formed Yedoma has been under dispute in the last several decades. Several hypotheses have been proposed about the origin of the late Pleistocene Ice Complex, including eolian (Tomirdiaro et al., 1984; Tomirdiaro and Chernen'k'ii 1987), alluvial (Rozenbaum, 1981), and polygenetic (Konishchev and Kolesnikov, 1981; Sher et al., 1987) periods. Different opinions on the origin of these deposits have been summarized in the recent publications of Lutz Schirrmeister and co-authors (Schirrmeister et al., 2011, 2013). The researchers suggested that the ice rich syngenetic permafrost of the late Pleistocene Ice Complex was developed under a cold-arid climate at less hydromorphic conditions than the lake and lake-alluvial sediments. In general, Yedoma sediments have been characterized by the absence of methane (Rivkina et al., 2007; Rivkina and Kraev, 2008) and much lower values for redox-potential and iron (Fe^{2+}) content in comparison to permafrost layers of lake and lake-alluvial origin (Rivkina et al., 2006).

A comparison of the two late Pleistocene permafrost metagenomes of different genesis, IC4 and IC8, revealed differences in the composition of the microbial community that reflects the conditions under which these deposits were formed. These data uncovered significant distinctions in microbial community compositions between Yedoma and lake-alluvial sediments. The relatively low abundance of methanogenic archaea, restricted presence of enzymes from the carbon, nitrogen, and sulfur cycles, as well as the presence of methanotrophic bacteria could help explain the absence of methane in Yedoma deposits and provide evidence that the formation of these sediments took

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place under much more aerobic conditions. In other words, we may assume that during the late Pleistocene period, nearly 30 000 years ago, there existed various conditions that predetermined biogeochemical regimes and composition of microbial communities. The involvement of metagenomic analyses, along with geological and biogeochemical methods, may be useful for understanding not only how the permafrost microbial community will react to climate warming, but may become an additional instrument in paleo-reconstructions.

The Supplement related to this article is available online at doi:10.5194/bgd-12-12091-2015-supplement.

Author contributions. E. Rivkina, L. Petrovskaya and F. Kondrashov designed research; L. Shmakova, M. Tutukina, L. Petrovskaya, E. Rivkina performed research; L. Shmakova and M. Tutukina extracted DNA; F. Kondrashov performed DNA sequencing; E. Rivkina, L. Petrovskaya, K. Krivushin, T. Vishnivetskaya, A. Meyers analyzed data; E. Rivkina and F. Kondrashov contributed new reagents and analytical tools; E. Rivkina, L. Petrovskaya, T. Vishnivetskaya wrote the paper.

Acknowledgements. This work was supported by grants from the Russian Scientific Fund (14-14-01115) to E. Rivkina; from the National Science Foundation (DEB-1 442 262) to T. Vishnivetskaya; and by HHMI International Early Career Scientist Program (55 007 424), the EMBO Young Investigator Programme, MINECO (BFU2012-31329 and Sev-2012-0208), AGAUR program (2014 SGR 0974) to F. Kondrashov.

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Table 1. Number of sequences showing homologies to genes associated with KEGG pathways within the IC4 and IC8 metagenomes. Data were generated by searching in Hierarchical classification table based upon SubSystems annotation source.

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	24 601	3982
EC 1.2.99.5	Formylmethanofuran dehydrogenase	10 757	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	11 405
EC 1.7.2.5[a]	Nitric-oxide reductase	3440	1838
EC 1.7.1.4	Nitrite reductase [NAD(P)H] small subunit	20 713	13 041
EC 1.7.99.4	Respiratory nitrate reductase alpha chain narG	36 476	28 169
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	11 165
EC 1.8.7.1	Ferredoxin–sulfite reductase	2587	8920
EC 2.7.7.4	Sulfate adenylyltransferase subunit 2	15 449	23 435
EC 2.7.1.25	Adenylylsulfate kinase	22 898	22 418
EC 1.8.3.1	Sulfite oxidase	14 353	13 968
	Stress response		
EC 1.11.1.6	Catalase	74 622	41 693
EC 1.15.1.1	Manganese superoxide dismutase	8922	13 302
EC 1.11.1.7	Peroxidase	35 552	19 698
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	25 244	18 018
EC 1.2.1.8	Betaine-aldehyde dehydrogenase	10 022	12 782
NR	Chaperone protein DnaK	78 365	89 944
NR	Interacting protein DnaJ	32 897	45 343

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

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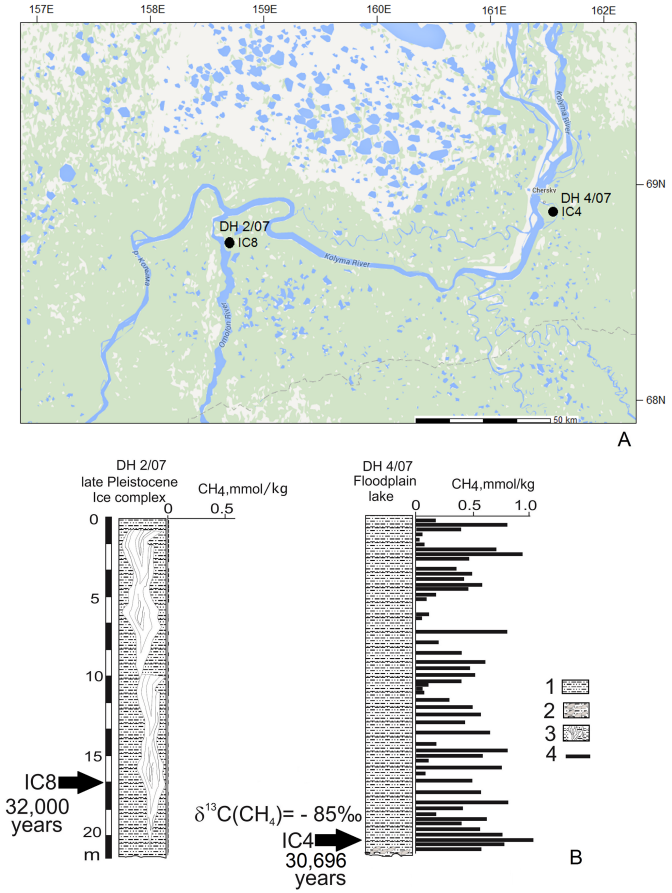

Figure 1. Location of sampling sites on the Kolyma lowland **(a)**, and position of samples IC4 and IC8 in drilling holes **(b)**.

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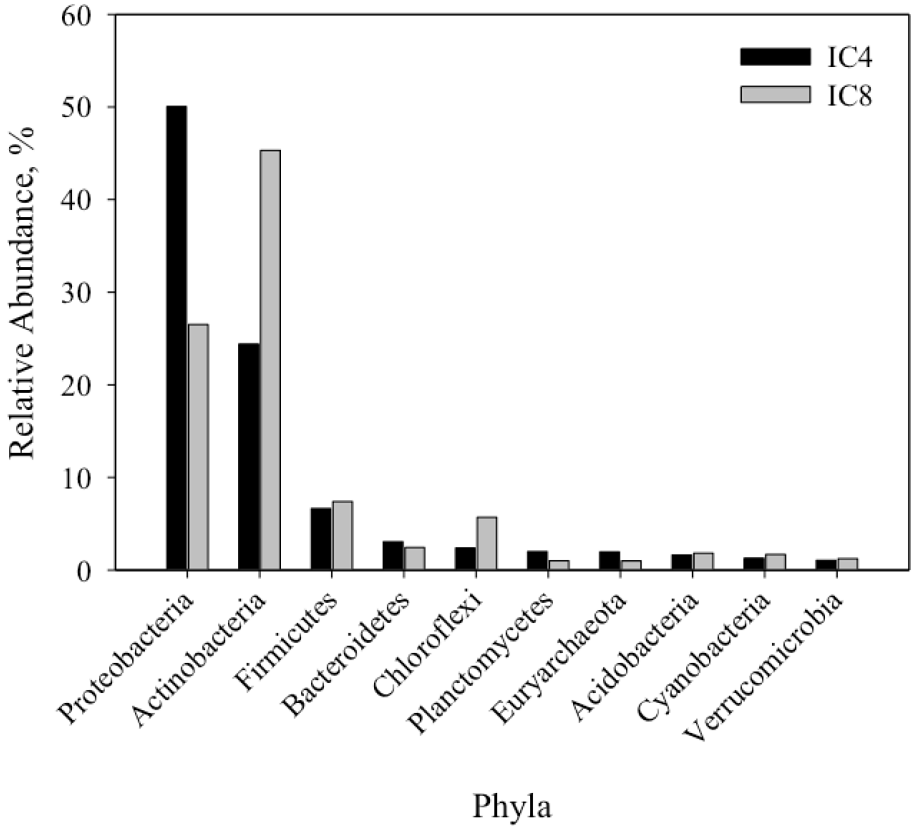

Figure 2. Community analyses of the IC4 and IC8 metagenomes at phylum level. The phyla present in metagenomes at a > 1 % level are shown.

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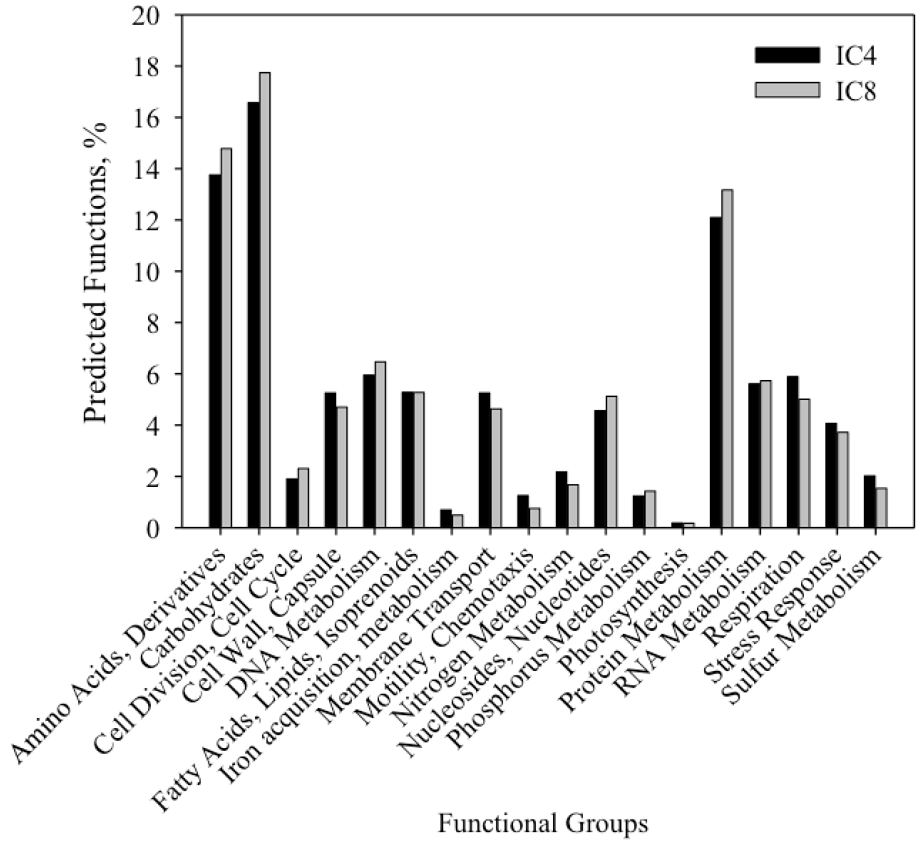

Figure 3. Percentage of gene sequences associated with different functions in the annotated protein sequences within IC4 and IC8 metagenomes.

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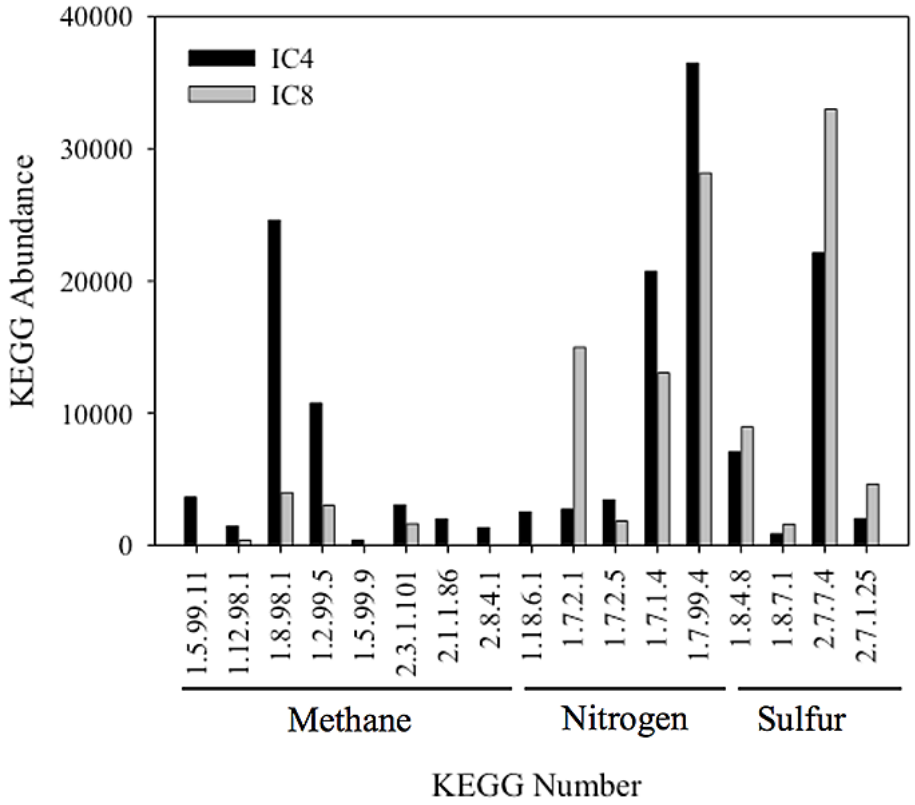

Figure 4. KEGG abundance of the selected functional gene sequences found within IC4 and IC8 metagenomes. Genes found in low abundance were not included in the figure. Genes from methane, nitrogen, and sulfur metabolic pathways are underlined. The enzyme name that corresponds to each KEGG number is given in Table 1.

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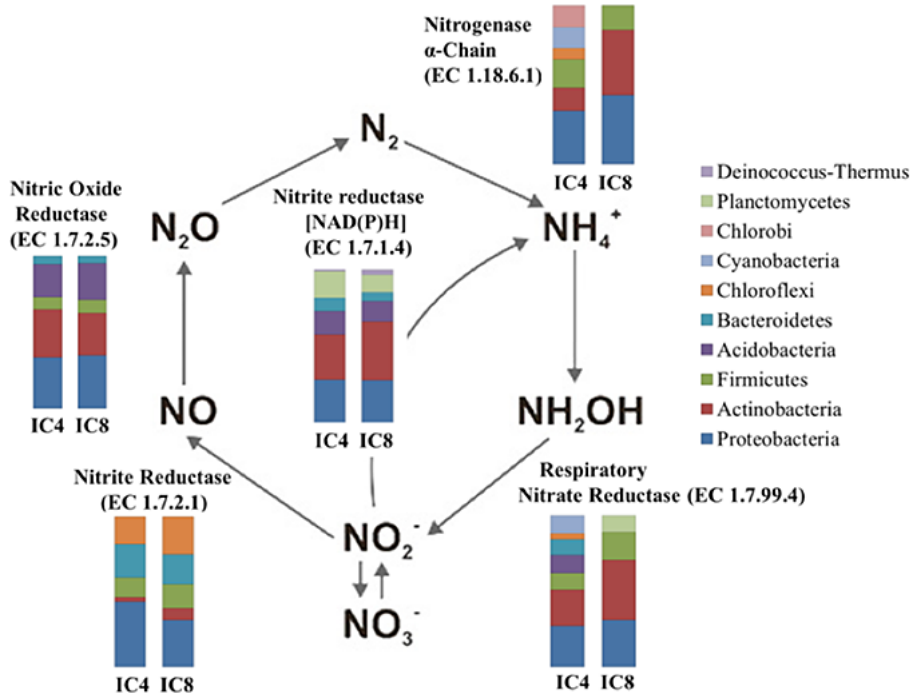

Figure 5. Phylogenetic distribution of the sequences related to nitrogen metabolism within the IC4 and IC8 metagenomes.

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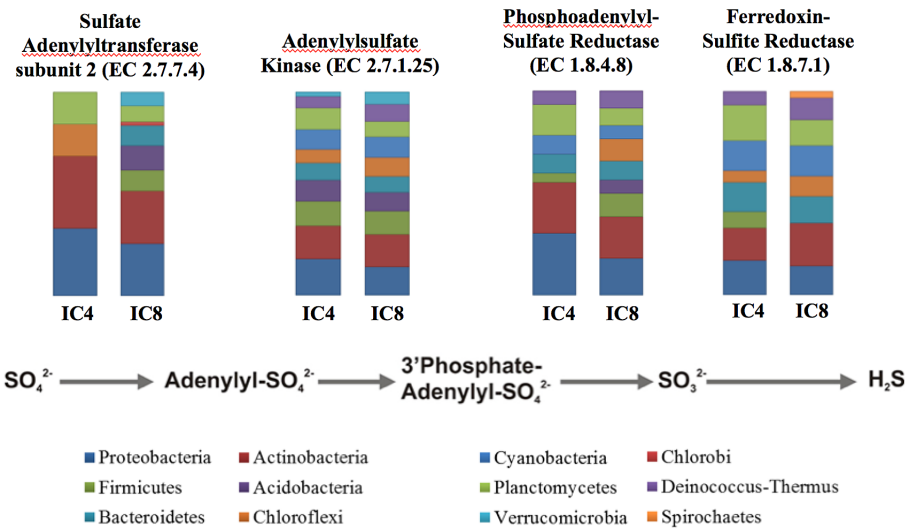

Figure 6. Phylogenetic distribution of the sequences related to sulfur metabolism (sulfur reduction) within the IC4 and IC8 metagenomes.

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