Biogeosciences Discuss., 12, C8527–C8529, 2015
www.biogeosciences-discuss.net/12/C8527/2015/

Biogeosciences
Discussions

Interactive comment on "Metagenomic analyses of the late Pleistocene permafrost – additional tools for reconstruction of environmental conditions" *by* E. Rivkina et al.

Anonymous Referee #3

Received and published: 16 December 2015

The manuscript describes a study of metagenomic data derived from 2 samples of Siberian permafrosts. One core sample was from the Ambolikha River containing methane at 1.2 mmol kg-1 (IC4), while the other core was from the Omolon River with no detectable methane (IC8). The section of the permafrost core where samples were taken was approximately 32,000 years old. The authors extracted DNA from 8 replicates using the Powersoil kit and subjected the DNA to Illumina sequencing of 150 nt fragments. Over 130 million reads were obtained for each sample, respectively. Less than 7% of sequences did not meet QA/QC and were discarded and the rest were analyzed by MG-RAST. The authors report similar microbial communities differing in abundance for the Illumina reads of 16S rRNA genes. These differences were

reported at fractions of a percent with respect to the overall community. The exceptions were the Methanogens and Proteobacteria for the IC4 and the Actinobacteria for IC8. Nearly all microbial phyla exhibited a factor of 3 difference in the reported 16S rRNA gene abundances between IC4 and IC8. Analysis of the functional genes reflected the enrichment of genes involved in methanogenesis or methanotrophy for IC4 along with genes for nitrate respiration. While the IC8 sample was enriched in nitrite reductase and sulfate adenyltransferase. Otherwise, most of the functional genes detected in the analysis were of roughly equal abundance. The authors suggest this type of analysis can be used to reconstruct the conditions when the permafrosts formed or predict the microbial response when permafrost thaws. These conclusions do not take into account that the microbial community may change during the 32,000 years due to subzero activity as the primary author has shown in her prior publications. That caveat should be mentioned in the conclusion. Furthermore, documenting the genetic potential provides some information on how many different genes may exist in a sample. However, whether those genes are ultimately expressed is an entirely different matter. Much more information would be needed before a predictive capability can be achieved with respect to microbial community function after permafrost thaw.

Specific questions for the authors:

A. It appears the eight subsamples from each core were extracted and combined into a single run on the Illumina flow cell for IC4 or IC8. Figure 1 indicates these pseudo-replicates were taken at roughly the same depth. Is that correct or were samples collected over a broader range, say up to 0.5 m?

B Unfortunately, most metagenome studies lack replication. How reproducible are the extractions and the Illumina sequencing of DNA in the authors' hands? What is the variability between samples? Can the authors indicate their DNA yield for all of the 8 pseudo-replicates? Were there differences in yield between the IC4 and IC8 samples after the Zymo Concentrator kit that can explain the observed changes in gene abundance?

C. Most of the microbial community differences are reported to 2 or 3 significant figures (such as page 12099 line 27 and 12100 line 7). Without replicates and an understanding of the variability within the analysis for these particular samples, it could be that all observed differences between IC4 and IC8 are artifactual. Until the authors can prove the ability to discern these small differences in samples, reporting to 3 significant figures is unwarranted.

D. I suspect that not all ORFs or 16S rRNA genes within the Illumina dataset were identified with a strong degree of confidence. What proportion of the ORFS that were detected are annotated as hypothetical proteins or very loosely affiliated with known genes? That data seems to have been eliminated from the manuscript and could represent a significant fraction of the reads.

Interactive comment on Biogeosciences Discuss., 12, 12091, 2015.