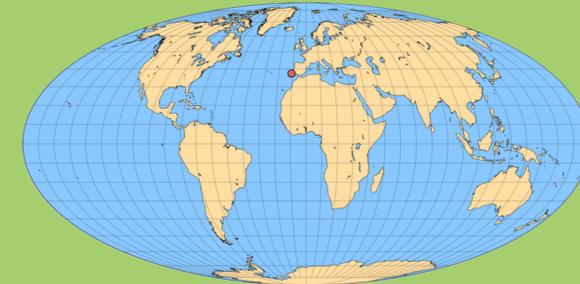


Molecular Insights into Microbial Ecology of the Iberian Margin Deep Biosphere

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Abstract

The marine deep sedimentary biosphere covers the majority of the global seafloor. It is an important global reservoir of organic carbon, minerals, and trace metals and sustains an extant microbial biosphere living off of these materials. How active these populations are is subject to much debate with some estimates of population turnover times of $>10^5$ years. Recent work involving successful extraction and sequencing of mRNA transcripts, commonly considered a sign of active life due to relative instability of RNA vs. DNA in the environment, has re-invigorated the notion of studying the active deep biosphere. However, preservation potential of RNA in deep biosphere environments, particularly clay-rich sediments, has not been explicitly identified.

Here, we present bacteria and eukaryote diversity via illumina sequencing of 16S and 18S rRNA amplicons, as well as initial results of metagenomics analysis, from 6 depths down-core at IODP site U1385 ranging from 10 to 123 meters below seafloor (mbsf). Eukaryotes were examined from the sedimentary DNA and RNA pools. The DNA-based eukaryote signal drops out past 30 mbsf but the RNA-based signal persists to depth. The RNA signal shows a much higher abundance of fungi, a group more likely to be active compared to the *Alveolates* and *Rhizaria* detected via DNA. However, abundance of *Viridiplantae*, a group containing flowering plants, roughly mirrors shipboard pollen spore counts, indicating preservation potential of RNA on geological timescales. Bacterial diversity down-core at the phylum level is relatively low, specifically at depth, where the population is dominated by uncultivated lineages *CD12* and *JS1*. Genomic features of this group are reflected in the metagenomic datasets obtained from the same DNA pools as the amplicons. On-going work is attempting to isolate individual genomes of these ubiquitous yet uncultured groups using cutting-edge metagenomic binning algorithms.

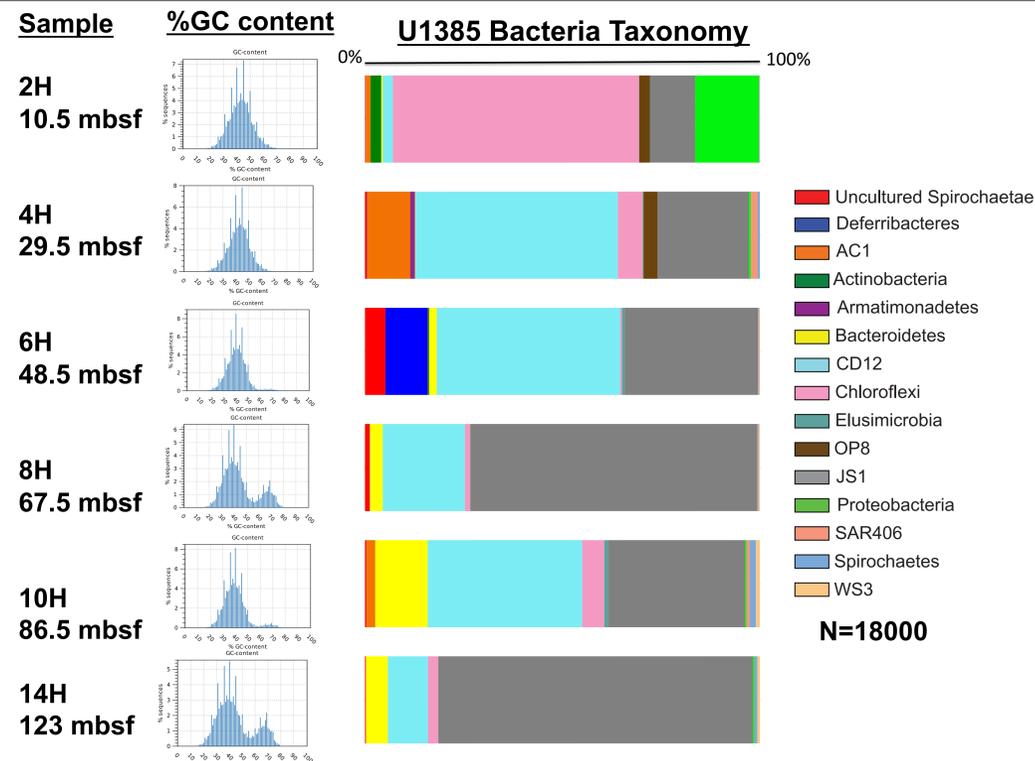


Figure 3: Taxonomic profile of bacterial populations down-core at site U1385. To the left of the barchart is the %GC profile of the metagenome corresponding to each depth. Note the appearance and disappearance of the second, higher %GC peak that mirrors the fluctuation in *JS1* abundance. Recent advances in metagenome binning and assembly strategies will allow us to take advantage of high representation of uncultivated lineages in metagenomic data with the aim of constructing full genomes without the need to isolate these organisms.

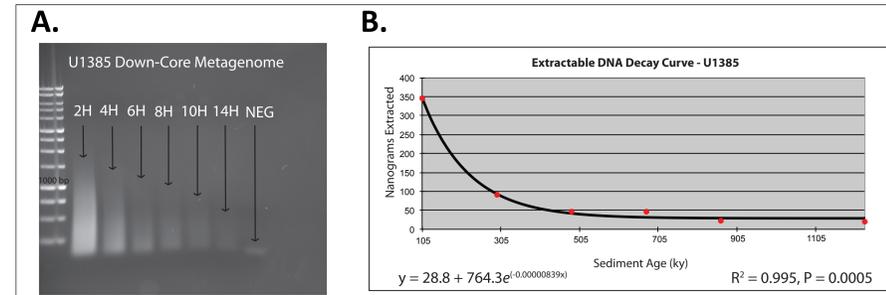


Figure 4: *Metagenome vs. Paleome?* A down-core metagenome was generated for site U1385 by adding 2 nanograms genomic DNA to the Sigma Genomeplex Whole Genome Amplification kit. Amount and size of amplifiable gDNA decreased down-core (A), mirroring the trend seen in extractable DNA from 10 grams of sediment per depth (B).

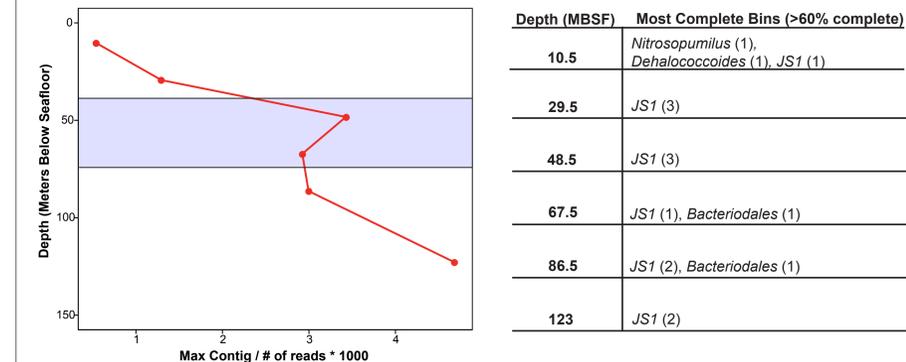


Figure 5: Maximum contig size relative to read count per depth. A linearly decreasing trend is disrupted at the sulfate-methane transition zone (blue shaded) where a more clonal population adapted to this environment would be expected.

Depth (MBSF)	Most Complete Bins (>60% complete)
10.5	<i>Nitrosopumilus</i> (1), <i>Dehalococcoides</i> (1), <i>JS1</i> (1)
29.5	<i>JS1</i> (3)
48.5	<i>JS1</i> (3)
67.5	<i>JS1</i> (1), <i>Bacteroidales</i> (1)
86.5	<i>JS1</i> (2), <i>Bacteroidales</i> (1)
123	<i>JS1</i> (2)

Table 1: Genomic fragments matching to particular microbial groups are 'binned' through *Maxbin* in an attempt to construct near-complete genomes of uncultivated phyla. At U1385, the cosmopolitan yet uncultured *JS1* group is highly represented.

U1385 Eukaryote Taxonomy

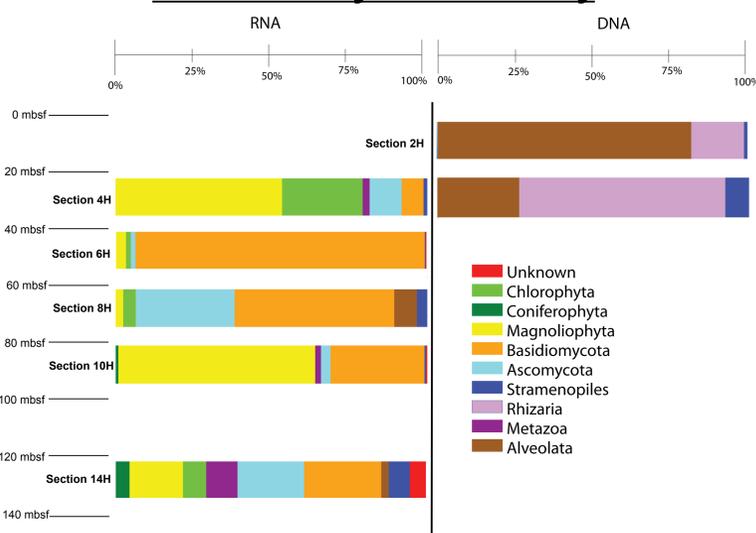


Figure 1: Comparison of microbial eukaryotes via 18S rRNA sequencing down-core at site U1385. The DNA-based signal drops out below 30 mbsf but RNA-based signal persists to 123 mbsf. Fungi and land-plant pollen spores predominate in the RNA pool. Fungi are likely to be an active member of the sediment column but the *Viridiplantae* signal indicates preservation of RNA from the early Pleistocene. The DNA-based signal is dominated by typical marine picoeukaryotes such as *dinoflagellates* and *foraminifera* and represents a preserved water-column signal that may 'swamp out' detection of active fungi and preserved pollen-spores seen in the more sensitive RNA-based analysis. The sequencing depth per sample is N=2600 for RNA, and N=4400 for DNA.

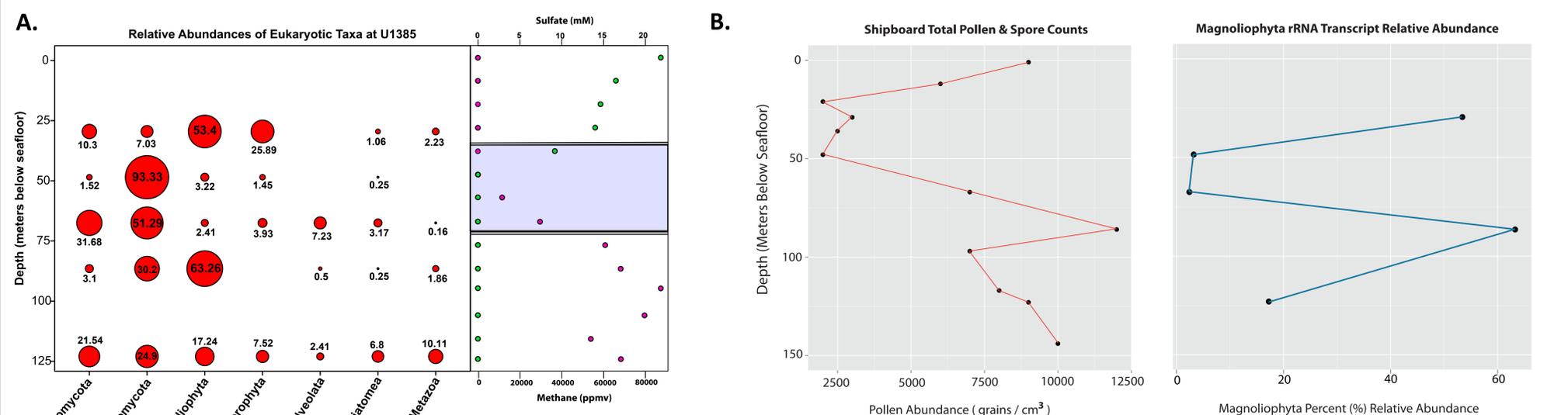


Figure 2: In the RNA-based study, fungi (specifically *Basidiomycota* and *Ascomycota*) and land-based plants (specifically *Magnoliophyta*) represent the most abundant eukaryotes detected (Fig. 2A). The molecular *Magnoliophyta* signal does not appear to be a contaminant as the general down-core trend mirrors that of shipboard pollen and spore counts (Fig. 2B). This indicates geological timescale preservation of RNA in the clay-rich sediments of the Iberian Margin. It cannot be determined from this data which fraction of the fungi are active and which are preserved. Further RNA-based work, such as down-core metatranscriptomics, would help toward that aim but attempts at generating this type of data have proven extremely difficult.