Microbial Analysis Methods

DNA was extracted from 1L water obtained prior to oil additions and 1 month after the additions to facilitate microbial community analysis. Filtered (0.22 µm) water samples were extracted according to Ausubel et al. (1995). The gDNA was eluted in Ambion nuclease-free water and kept at -20°C. The total RNA samples were eluted in Ambion nuclease-free water and kept at -80°C. The gDNA was treated using RNAse If (New England Biolabs, Ipswich MA). Treated DNA was purified using the QIAExII kit (Qiagen) eluted in water, and quantified by picogreen fluorescence (Quant-iT™ PicoGreen™ dsDNA Assay Kit (ThermoFisher Scientific, Waltham MA).

Taxonomic 16S and 18S rRNA gene amplifications were performed on all the gDNA samples. Bacterial communities, including eubacteria and archaea, were targeted using the primer set 515F (5′- GTGCCAGCMGCCGCGGTAA-3′) / 806R (5′- GGACTACHVGGGTWTCTAAT-3′). The 18S rRNA gene region of the eukaryotic ribosomal DNA was amplified using the primer set 565F (5′- CCAGCASCYGCGGTAATTCC-3′) / 948R (5′- ACTTTCGTTCTTGATYRA-3′). Preparation of sequencing libraries and sequencing were as described (Schreiber et al. 2019). After quality control procedures had been applied, pyrotag reads were clustered into operational taxonomic units (OTU) with methods cd-hit (Caporaso et al. 2012) and usearch (Li and Godzik 2006). Each OTU was assigned a taxonomic classification using Basic Local Alignment Search Tool nucleotide (BLASTN) of the representative sequences to the Silva database (Edgar 2010). Phylogenetic trees were constructed (Gurevich et al. 2013) with 100 bootstraps of the nucleotide substitution model (Edgar 2004). Sequences for closely related microbial species and environmental clones were found by BLASTN to the National Center for Biotechnology Information (NCBI) refseq and nucleotide databases (Hasegawa et al.

1985), respectively. Alpha and beta diversity analyses were applied to study and compare the structure of microbial communities and analyze significant differences through time for each of the enclosures.

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