

Activity and Physiology of Uncultured Microorganisms from the Ocean Surface to Seafloor

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Our knowledge of the phylogenetic diversity of marine microbes and their genomic content has expanded significantly in recent years. However, our understanding of marine microbial activity, including its rates, distribution, and physicochemical controls, has lagged behind. The microbial ecology of the deep sea (>200 m water depth) remains particularly poorly characterized, despite covering approximately two thirds of the planet and hosting about 55% of all marine microbes. For example, we find in the northeastern Pacific Ocean the average 16S rRNA gene percent identity to sequences in the NCBI reference database is <90% below 150 m, and <85% below 1000 m (weighted by abundance). Deep-sea microorganisms have the potential to play important roles in biogeochemical cycling, including producing and consuming greenhouse gases (e.g., CO₂ and N₂O), attenuating the biological pump via remineralization of photosynthetically-derived organic matter, and producing, storing, and transporting nutrients that fuel surface waters in upwelling regions. However, the low cell density and slow growth rates in the deep sea make it particularly hard to measure microbial activity. Nanoscale secondary ion mass spectrometry (nanoSIMS) offers a quantitative and sensitive single-cell approach to characterizing the anabolic activity of uncultured microbes, and is particularly well suited to studying low activity, low biomass samples. Here, we use nanoSIMS to analyze microbial activity and physiology throughout the marine water column in the northeastern Pacific Ocean (50 – 4000 m water depth). Using stable isotope incubations with a variety of organic and inorganic carbon and nitrogen substrates we assess growth rates, nitrogen preferences, C/N relative use efficiency, and specific metabolic capabilities in whole communities and specific cells identified via fluorescence *in situ* hybridization. Our results provide a window into the role uncultured marine microorganisms play in biogeochemical cycles and suggest that the microorganisms in the pelagic deep sea are more active than previously realized.