

Unearthing the Active Microbes, Viruses and Metabolites in Dynamic-Redox Tropical Soils with Quantitative SIP and Metagenomics

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Project Goals: This Early Career research examines the genomic potential and activity of tropical soil microorganisms as they experience shifts in soil temperature, moisture, depth and oxygen availability. Associated fluctuations in redox potential are proximal controls of mineral-organic matter interactions in humid tropical soils. By tracking the degradation and fate of organic ¹³C labeled compounds during shifts in soil redox status, this work will improve our understanding of microbial metabolic flexibility, and how microbial processes affect the fate of organic carbon in wet tropical systems. The mechanistic understanding produced by this research will also improve the predictive capacity of mathematical models that forecast future tropical soil carbon balance.

In soils, anaerobic processes are dynamic and widespread—even in bulk-oxygenated upland environments—and exist within soil aggregates, near perched water tables, and in zones of abundant labile C. However, the drivers and dynamics of these anoxic volumes remain poorly constrained, particularly in wet tropical soils where we know little about the metabolic capacities of soil microorganisms, and the mineral-organic matter relationships that regulate many aspects of soil C cycling. Using a 44 day redox manipulation experiment with soils from the Luquillo Experimental Forest, Puerto Rico, we examined patterns of tropical soil microorganisms and metabolites when soils were exposed to different redox regimes - static oxic, static anoxic, high frequency redox fluctuation (4 days oxic, 4 days anoxic), or low frequency redox fluctuation (8 days oxic, 4 days anoxic). Replicate microcosms were harvested throughout the incubation to measure the impact of redox condition on microbial community structure and activity, organic matter turnover, and soil metabolites. An addition of ¹³C enriched plant biomass allowed us to distinguish decomposition of fresh plant litter vs native organic matter and conduct Stable Isotope Probing (SIP) with genome resolved metagenomics and viromics to identify active microorganisms and their viruses.

Our amplicon data show that bacterial and fungal community composition in the two fluctuating redox treatments was indistinguishable from the native soil community, while the static redox communities were distinct, suggesting the microbes in these soils are highly adapted to dynamic redox conditions. Using differential abundance analysis, we found that fluctuating redox enriched for relatively more bacterial and fungal taxa –compared to the static redox conditions. However, the anoxic treatment had a distinct iron-cycling microbial community relative to the other treatments. The majority of taxa adapted a facultative strategy in the first weeks of the incubation (when litter decomposition activity was highest) –suggesting they maintained mechanisms to tolerate inhospitable redox periods. However, by the end of the experiment, labile

C had become limiting, and obligate anaerobes had increased in their relative abundance. Using ^{13}C quantitative SIP of over 1100 16S rRNA libraries, coupled to CO_2 and DOC measurements, we measured reduced microbial carbon use efficiency (CUE) under static redox conditions compared to fluctuating redox conditions. SOM-C respiration was highest under static oxic conditions, litter-C respiration was highest under static anoxic conditions, and litter-C assimilation was highest under fluctuating redox conditions. Intriguingly, some taxa remained active (i.e., assimilating litter-derived C) under all redox conditions, and abundance was not always correlated with activity; many relatively 'rare' taxa assimilated a high ratio of litter C.

We also analyzed 95 metagenomes (85 SIP fractions and 10 bulk samples) from our 4 redox treatments, using sequences generated by the JGI (22 billion reads and over 3.3 trillion base pairs). Metagenome assemblies produced over 6,000 genome bins (MAGs), and co-assemblies produced 326 medium-to-high-quality MAGs from ^{13}C enriched DNA fractions. Over the 44 day incubation, we saw large differences in the active (^{13}C enriched MAGs) from the static anoxic vs static oxic soil, and a strong response in the Fe-reducer community. Overall, the fluctuating soil MAGs had generally more ^{13}C incorporation, particularly in Proteobacteria and Actinobacteria. In the static anoxic soils, we only observed ^{13}C incorporation by Bacteroidetes and Firmicutes.

Viruses were detected with VirSorter and VirFinder and clustered into viral populations (vOTUs). Active vOTUs were identified as those present in ^{13}C samples but not the paired ^{12}C sample. We recovered nearly 48,000 viruses clustering into 640 vOTUs >10 kb. SIP-fractionated samples recovered ~6% vOTUs that were not observed in the un-fractionated bulk samples. Viral diversity was highest in the oxic samples and decreased as follows: oxic>high frequency>low frequency>anoxic. Beta diversity suggests fluctuation between oxic and anoxic conditions had the largest impact on the active viral community structure. Only 27% of the vOTUs were active, with 33% active in all treatments, and 16% only active in the anoxic samples. Nearly 21,000 genes were predicted from the vOTUs, 15% of which had a known function and of the 85% of known genes, 65% were novel. From the 15% of known genes, 10% were structural, 86% were genes involved in replication, and 4% were putative auxiliary metabolic genes (AMGs). We categorized the AMGs into two distinct groups: host survival and C cycling. Host survival genes included oxidative stress, sporulation, heat shock, whereas C cycling genes included central C metabolism and CAZy enzymes. Almost 30% of the vOTUs were linked to the 326 MAGs across four phyla (Acidobacteria, Actinobacteria, Proteobacteria, and Bacteroidetes).

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