Linking Activity to Phylogeny in Groundwater/Soil Ecosystems

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ENIGMA (http://enigma.lbl.gov/)

Project Goals: ENIGMA (Ecosystems and Networks Integrated with Genes and Molecular Assemblies) uses a systems biology approach to understand the interaction between microbial communities and the ecosystems that they inhabit. To link genetic, ecological, and environmental factors to the structure and function of microbial communities, ENIGMA integrates and develops laboratory, field, and computational methods. A fundamental goal in the field of microbial ecology is to link the activity of specific microorganisms to processes occurring within an ecosystem. This project aims to identify the drivers of community structure and succession by quantifying activity and identifying the metabolically active fraction of microbial communities from both pristine and contaminated groundwater and sediment from the Field Research Center (FRC) at Oak Ridge National Laboratory (ORNL).

It is broadly accepted that free-living and attached communities have distinct microbial community compositions; however, due to sampling constraints there are significantly fewer studies that have simultaneously looked at the activity of microorganisms in both the sediment and groundwater fractions. Historically, it has been shown that not only are there greater densities of total cells (90-99.99% of the microbial biomass in porous aquifers) but there are also higher proportions of active cells associated with sediment compared to free-living groundwater cells. An explanation for differences in activities between attached and free-living populations is likely due to differences in the densities of cell abundances. However, it remains unresolved whether free-living cells in porous subsurface habitats are in fact metabolically slower or if the explanation for these differences in activities is merely based upon numbers respective to a given volume. Recently, it has been proposed that microbial competition selects against rapid growth in attached populations and that bulk-phase populations have faster growth rates . These findings offer a unique and contradictory perspective as to the role of free-living organisms compared to sediment biofilms which alter our current understanding of dispersal and colonization in porous environments as well as the distribution of microbial activities.

Using a combination of complementary culture-independent methods, activity rates and the active microbial assemblages were determined for groundwater and sediment associated cells from pristine and contaminated aquifers. Bioorthogonal non-canonical amino acid tagging (BONCAT) and Propidium Monoazide (PMA) were used to differentiate the contributions between different DNA pools (DNA from viable cells with intact cellular membranes versus extracellular or "relic" DNA) for two groundwater wells representing geochemical extremes. BONCAT samples were microscopically evaluated and sorted for amplicon sequencing (BONCAT-Seq). *Pseudomonadacea* and *Comamonadaceae* were the dominant

active assemblages for pristine groundwater, while *Xanthomonadaceae* and *Nocardiaceae* dominated contaminated groundwater. A greater diversity of active organisms was observed in background sites. For pristine wells, 1,268 OTUs were observed on average, with between 8.5 and 26% of identified OTUs being translationally active for 24 and 72 h of incubation, respectively. On average 346 OTUs were observed for contaminated groundwater, with between 60 and 66% of observed OTUs being active for 24 and 72 h, respectively.

With PMA analyses, the pristine groundwater showed average higher richness (4,958 OTUs) than contaminated groundwater (3,886 OTUs). For communities captured on 0.2 μM filters, OTU richness was similar with and without PMA treatment for pristine groundwater, and these results indicated that most sampled populations captured within this fraction did not have compromised cellular membranes and were viable. A similar trend was observed for contaminated groundwater, and OTU richness was similar between PMA treated and non-treated samples. Ordination analysis demonstrated that samples formed tight clusters that were primarily separated by well and secondarily by filter size. These results corroborated BONCAT analyses in that a significant portion of groundwater populations appear to be viable in terms of non-compromised membranes for both pristine and contaminated groundwater.

In pristine wells activity on a per cell basis was two to three-fold greater for planktonic cells compared to particle associated organisms, with small cells (<0.1um) contributing up to 19% of total activity. Conversely, in contaminated samples, activity was greater for sediment associated cells. We observed two to three orders of magnitude lower cell specific growth for sediment associated cells compared to planktonic groundwater cells. However, combining activity measurements, cellular abundances, porosity, and the degree of saturation, the biological activity of planktonic groundwater cells and sediment associated cells in a cubic meter of the saturated subsurface was estimated. The activity estimated to corresponding sediment associated cells accounted for up to 99% of the activity within a cubic meter of the saturated subsurface. Using a combination of methods, we show that the majority of planktonic populations in pristine aquifers are highly active and consist of intact cells. While attached populations have slower rates on a per cell basis, the sediment biofilms are responsible for the majority of the activity within a shallow aquifer.

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