

Natural Organic Matter Dynamics and ExoMetabolomics for Microbial Cultivation from the Shallow Subsurface at the Oak Ridge FRC

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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. Natural organic matter (NOM) is central to microbial food webs and microbially mediated NOM transformations determine much of the carbon (C) flux in subsurface environments. However, little is known about the molecular signatures from different C pools, the microbial activities/populations that regulate NOM turnover, nor the exometabolic markers for this activity. The goal of this project is to study the interactions between NOM (extracted from the field site) and native microbial communities present in uncontaminated and contaminated environments at Oak Ridge Field Research Center, TN.

Water-soluble NOM was extracted from sediment samples collected from two background uncontaminated sites. The amount of inorganic C in extracted NOM decreased significantly with depth. Extracted NOM was used as the sole source of carbon in controlled lab incubations. Lignin, lipid, and protein levels were similar for the different depth intervals, while the relative proportions of carbohydrate, tannin, and amino sugars declined with depth and condensed aromatics increased with depth. The nitrogen content of the extractable sediment NOM compounds varied between 13 to 28%, and the deeper sediment intervals contained more S than surface intervals. Groundwater NOM was compared to sediment-extracted NOM for an uncontaminated and contaminated well (well 106 v. 271). For groundwater, the NOM is dominated by amino acid-like fluorescence, and the fluorescence is approximately 60-fold greater in the contaminated well. The spectra also indicate the formation of more recalcitrant compounds in the sampled uncontaminated groundwater. For the sediment-extracted NOM, we used the humification index (HIX) as a measure of the complexity and the condensed (aromatic) nature of the NOM. The HIX indicates a drastic decrease in humic-like fluorescence for depths >2m for both cores compared to the shallower depths (<2m). The deeper depths had mainly amino acid-like fluorescence. NOM was extracted from the uncontaminated samples and incubated with groundwater as the inoculum. We monitored the trajectory of microbial biomass, respiration, community structure and activity over the course of the incubation. To document changes in organic matter chemistry, we applied high-resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) and soft X-ray absorption spectroscopy (sXAS). Together, these analyses provided a greater view of NOM degradation by indigenous

microbes. Transformation of NOM continued even after depletion of the labile C pool, and microbial populations shifted from presumptive copiotrophic to presumptive oligotrophic microorganisms that most likely possess greater affinity for diverse non-labile carbon. The C pool shifted during incubation, the proportion of lignin in cultures increased while overall protein levels declined. In addition, Geochip was used to identify the changes of microbial communities and expression of functional genes during transformation of the NOM, and putative gene sequences associated with chitin and lignin degradation increased after the depletion of more labile carbon. Furthermore, exometabolomic methods were developed to characterize ninety-six metabolites from sediment samples that included amino acids, carbohydrates (acid/alcohol), carboxylic acids, and nucleosides. The extraction procedure focused specifically on potentially accessible for microbial metabolites that may be present in sediment/soils. The water extractable organic carbon metabolites (WEOCs), were first qualitatively characterized using both liquid chromatography mass spectrometry (LC/MS) and gas chromatography mass spectrometry (GC/MS). Application of these complementary technologies provided orthogonal confirmation of metabolite identification while also expanding the analytical scope of the study. From these data, a subset of metabolites was selected for absolute quantification to assist in formulating a set of defined media that approximate the composition and quantity of metabolites within the sediments. One composition was demonstrated to support the growth of 25 phylogenetically diverse isolates out of 30 tested from the Oak Ridge Field Research Center (ORFRC), and detailed time series characterization of the substrate preferences are underway. Thus, the characterization of NOM and exometabolites in groundwater and sediment is described and then used to construct a defined medium for use with indigenous populations for the assessment of substrate utilization and microbial interactions.

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