

Investigation into the Stabilization of Soil Organic Matter by Microbes

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A better understanding of below ground carbon (C) flux is of fundamental importance to predict how changing climate will influence the C balance of forest (and other) ecosystems [1]. The root system of higher plants is associated not only with soil environment composed of inorganic and organic matter, but also with a vast community of metabolically active microorganisms. Rhizosphere is the zone of soil immediately surrounding the plant roots, with the microbial population considerably higher than that of root free soil environment. Soil organic carbon pools are often defined either as labile or as recalcitrant, referring to its stability against decomposition of soil organic matter (SOM). We studied the microbial role in production and stabilization of SOM in laboratory setup of column-grown *Pinus resinosa* mesocosm systems [2], by (a) imaging by light and electron microscopy, with (b) high resolution chemical analysis by Fourier transform ion cyclotron resonance-mass spectroscopy (FTICR-MS), and (c) crystallographic X-ray analyses of the microbially-induced mineral weathering, to determine SOM resistance to decomposing activities.

Our main area of interest was to characterize the stabilization of microbially-produced extracellular polymeric substances (EPS), a remarkable dynamic entity that plays critical functional role in a wide variety of geomicrobial processes in soil. EPS is primarily associated with physical adhesiveness, therefore with biofilm formation, cell adhesion to solid surfaces, with creation of protective microhabitats against adverse environmental conditions, and facilitating mineral aggregation. Additionally, due to its immense absorptive capacity, EPS is capable of binding, accumulating, and sequestering dissolved organic matter and metals from the environment and it is consequently able to influence a wide range of biogeochemical processes. These include the dissolution and precipitation of minerals, as well as redox and/or complexation reactions. Generally composed of microbially-secreted heterogeneous combinations of high-molecular-weight polysaccharides, lipids, phosphate, proteins, and nucleic acids, EPS mass can consist of up to 95% of bound and unbound water. Due to this extreme hydration, EPS is among the most difficult biological structures to preserve and characterize in its native state and presents a major challenge for obtaining accurate high-resolution images via electron microscopy [3].

As a field follow-up study with a goal to relate our laboratory tree columns system to a more natural setup, we implanted mesh bags filled with biotite as used in the designed soil in the previous lab mesocosms experiment [2, 4]. The bags were placed in the rhizospheric zone of *Pinus ponderosa* young seedlings in Wenatchee national forest, following the experimental setup [5]. The material was allowed to incubate with the natural inoculum of microbial community present in the soil over period of 6 months. After that, the bags were removed, and the material was imaged and analyzed for microbial presence and specific associations with minerals, characterized for organic carbon compounds, for microbially-induced mineral weathering, and for microbial community genetic signatures. These results were then related for recurring features in both the laboratory column experiments and the field study.

The above scheme of imaging and analytical techniques will serve to characterize the rhizosphere interactions resulting in the environmental processes such as soil organic matter persistence in ecosystems, relevant to carbon sequestration, and its stability to conditions applicable to responses to global change.

References:

- [1] Litton, C.M., Giardina, C.P., 2008. "Below-ground carbon flux and partitioning: global patterns and response to temperature". *Functional Ecology* 22, 941-954.
- [2] Dohnalkova A, et al., 2014. "Correlative Imaging and Analyses of Soil Organic Matter in the Rhizosphere". *Microscopy and Microanalysis*, (20), S3, 1192–1193
- [3] Dohnalkova A, et al., 2011. "Imaging Hydrated Microbial Extracellular Polymers: Comparative Analysis by Electron Microscopy." *Applied and Environmental Microbiology* 77(4):1254-1262.
- [4] Balogh-Brunstad, Z., C.K. Keller, R.A. Gill, B.T. Bormann, and C.Y. Li, 2008b. "The effect of bacteria and fungi on chemical weathering and chemical denudation fluxes in pine growth experiments." *Biogeochem.* doi: 10.1007/s/10533-008-9202.
- [5] Wallander H. et al., 2013. "Evaluation of methods to estimate production, biomass and turnover of ectomycorrhizal mycelium in forests soils e A review" *Soil Biology & Biochemistry* 57, 1034e1047
- [6] This research was performed at the Environmental Molecular Sciences Laboratory (EMSL), a national scientific sponsored by the Department of Energy's Office of Biological and Environmental Research, located at PNNL.

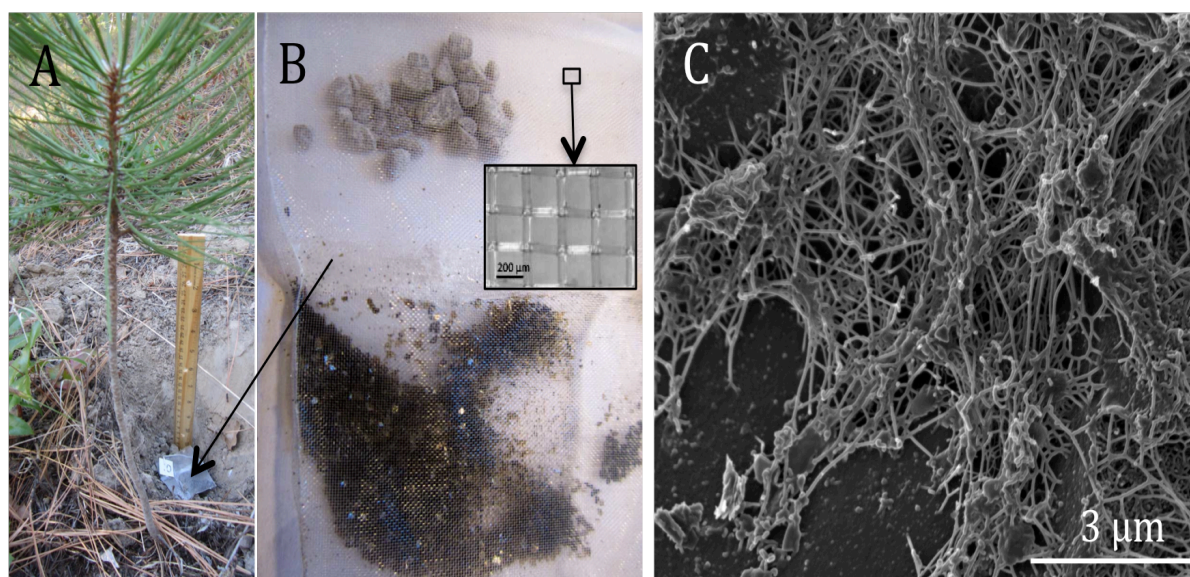


Figure 1. A - Mesh bag field experimental setup for microbial inoculation of soil minerals in ponderosa pine ecosystem. B – detail of the 200 μm nylon mesh bag containing biotite minerals. C – SEM image of resulting microbial EPS deposited on biotite. The intricate network of stabilized residual carbohydrates-based polymers maintain their integrity even after solvent extraction and dehydration.