

**Title: Linking microbial functional traits and organic matter chemistry to identify the pathways of carbon loss in response to warming through the soil profile**

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**Project Websites:** <https://eesa.lbl.gov/projects/terrestrial-ecosystem-science/>  
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**Project Abstract:** The microbial mechanisms controlling soil organic matter (SOM) decomposition and its responses to warming are poorly understood, particularly in subsoils, which contain over 50% of global soil carbon. Our prior work has shown that 4.5 years of whole-profile soil warming at a coniferous forest consistently stimulated CO<sub>2</sub> emissions and microbial growth rates at depths down to 1 m, while it decreased plant inputs. Warming only led to net carbon losses from subsoils, although depth-dependent changes in microbiome composition, SOM chemistry, and decomposition of distinct SOM fractions suggests that the microbial mechanisms driving SOM transformation and carbon fluxes are depth-dependent. Microbiomes in deeper soils have different functional traits than those at the surface, namely lower growth rates, carbon use efficiencies and ability to degrade complex carbohydrates, and exoenzymes with different kinetic properties.

We hypothesized that interactions between thermodynamic constraints dependent on SOM composition and microbial life strategies results in CO<sub>2</sub> emissions driven by distinct metabolisms and mechanisms of response to warming through the soil profile. To evaluate this, we are investigating the dynamics of microbial trait distributions and expression, and SOM composition in our whole-soil profile warming experiment using a multi-omics approach. Initial results from 21T-FT-ICR-MS showed that average oxidation states of carbon decreases with soil depth, with a predominance of lignins, tannins and condensed hydrocarbons at the surface, and lipids, proteins, amino-sugars and unsaturated hydrocarbons in deeper soils. After six months, warming led to depletion of more oxidized carbon compounds in surface soils and to their enrichment at mid-depths. However, 4.5 years of warming led to an increase in more oxidized compounds across all depths, possibly due to accumulation of tannins and other aromatic compounds, rather than more easily degradable carbohydrates. Interestingly, ethanol and methanol were the most abundant compounds quantified by <sup>1</sup>H-NMR, with relatively high concentrations through the soil profile, whereas those of more thermodynamically favorable compounds (glucose, acetate, and glycerol) declined sharply with depth. Consistently, we identified enzymes involved in alcohol, including

methanol, metabolism in the metaproteomes, as well as in carbon monoxide, carboxylic acid, and alkane oxidation, further supporting a role for simple carbon compounds in carbon fluxes, beyond biomass depolymerization.

These results suggest that variation in SOM composition with depth and during warming may constrain the metabolisms driving carbon fluxes, and that feedbacks between different microbial life strategies and substrate-dependent thermodynamics may be key regulators of carbon budgets in response to warming through the soil profile.