Title: **Microbial Response to Root Substrate Addition is Depth Dependent**

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Project Websites: https://eesa.lbl.gov/projects/terrestrial-ecosystem-science/

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Project Abstract:

The subsoil (<30 cm) contains almost half of the soil organic carbon (SOC) stock in the top meter of soil (Jobbágy & Jackson 2000, Balesdent et al. 2018), yet most studies only focus on surface layers (Yost & Hartemink 2020). Subsoil temperatures are projected to increase at a similar rate to air and surface soil temperatures (Soong et al. 2020), which can make this deep, relatively stable SOC pool vulnerable to increased microbial decomposition. Nonetheless, there is still no clear consensus on the factors that drive warming-induced changes in SOC stocks (van Gestel et al.2018). To understand the capacity of subsoil to store SOC with climate change, we must understand how decomposition changes with depth and warming.

We examined *in situ* litter decomposition by adding ^{13}C-labelled fine roots to surface (10-14 cm), mid- (40-44 cm), and deep soil layers (85-89 cm) of a coniferous temperate forest in soil classified as alfisols. At our site, we have warmed soil by 4°C relative to control temperatures to 100 cm since 2013. We recovered the root-derived C in bulk soil and microbial phospholipid fatty acids (PLFA) after 1 and 3 years of incubation. The overall recovered litter fraction in bulk soil significantly decreased by 60% in the surface and by 90% in the mid- and deep soil layers from 2017 to 2019, indicating that microbes in the subsoil relied on the added root-C more than surface microbes. Overall, total PLFA significantly decreased with depth (p-value <.0001), corroborating previous work at our site showing a significant decrease in microbial biomass with depth (Zosso et al. 2021). Though the root addition treatment did not significantly change total PLFA, it was on average higher at 85-89 cm relative to the disturbance control. Root addition significantly increased the relative abundance of fungi at 85-89 cm relative to the rest of the soil profile, contrasting previous findings that fungal biomarkers that relied on the added root substrate were less abundant at depth (Hicks Pries et al. 2018). Microbes in subsoil preferentially used the added substrate during the incubation period, leading to an increase in the fungal relative abundance at

depth. Our results suggest that particulate SOC in subsoil could be vulnerable to increased microbial decomposition, which can be exacerbated by warmer soil temperatures (Soong et al. 2021).

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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 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_2 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_2 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 ^{1}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.

Title: **Association Between Soil Organic Carbon and Calcium in Acidic Grassland Soils from Point Reyes National Seashore, CA**

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Project Abstract: Organo-mineral association and complexation processes are important for the retention and accumulation of soil organic carbon (SOC). Most of the research into these processes has focused on the biogeochemical interactions between SOC and Fe or Al, largely overlooking a role of Ca. Recent studies have demonstrated a strong link between calcium (Ca) and soil organic carbon (SOC) in a range of soil types (Rasmussen et al., 2018; Rowley et al., 2021; Yang et al., 2020), but a current paradigm suggests that Ca-mediated accumulation of SOC is predominantly constrained to soils with carbonates (pH > 6; Rowley et al., 2018).

To investigate the role of Ca in SOC accumulation of acidic soils, we combined classical characterisation and synchrotron-based spectro-microscopic methods to analyse three soil cores taken from grasslands with a pH gradient (soil pH 3.8 - 5.3) at the Point Reyes National Seashore, California. Bulk soil samples were characterised in detail, measuring classical soil physical (texture), mineralogical (X-ray diffraction), and chemical properties (pH, SOC, exchangeable and total elements). Subsequently, bulk Ca K-edge spectra were obtained for the core samples at three depths. We also used microprobe-coupled μ-X-ray absorption spectroscopy (μ-XANES; Ca K-edge) and STXM (Ca L-edge and C K-edge) to investigate the bonding environment of Ca and C in our samples, and their association with other elements (Al, Fe, K, Mg, Na, P, S, and Si).

Both the Ca and SOC content were high in our samples and were correlated in multivariate analyses of our standard characterisation dataset. Linear combination fitting of the bulk Ca K-edge XANES data revealed that Ca was predominantly associated with organic matter at our site. Additionally, STXM analysis showed that Ca had a strong spatial correlation with C, presenting a

higher spatial correlation than C with Fe. In characterising the SOC, we demonstrated that C associated with Ca had higher peaks in the alkyl and aromatic regions of the C K-edge spectra, relative to C associated with Fe or Fe-Ca-C. These spectral features are consistent with published spectra of C with a lignin-like nature and were observed in samples obtained from up to 70 cm depths. It therefore seems that Ca-C association is linked to the preservation of more plant-like products, even at depth in acidic grassland soils with no carbonate, potentially challenging existing paradigms (Rowley et al., 2018) that these Ca-mediated processes are only found in soils with a pH > 6.5.

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