Plant roots provide the primary source of carbon for stabilized organic C in soil. In addition, living roots and their exudates exert control over the microorganisms mediating decomposition of complex soil carbon compounds. Over time, living roots become root debris and undergo decomposition by soil microbes. It is now understood that most plant C is utilized or transformed by soil microorganisms en route to stabilization. Hence the composition of microbial communities that mediate decomposition and transformation of root C is critical, as are the metabolic capabilities of these communities. *The change in composition and function of the C-transforming microbial communities over time in effect defines the biological component of soil C stabilization.* Our research was designed to test 3 general hypotheses; the first two hypotheses are discussed first:

H1: ***Root-exudate interactions with soil microbial populations results in the expression of enzymatic capacities for macromolecular, complex carbon decomposition.***

H2: ***Microbial communities surrounding roots undergo taxonomic succession linked to functional gene activities as roots grow, mature, and decompose in soil.***

Over the term of the project we made significant progress in 1) quantifying the temporal pattern of root interactions with the soil decomposing community and 2) characterizing the role of root exudates in mediating these interactions. Roots can stimulate or suppress rates of litter decomposition by mechanisms that are poorly understood. Decomposition of ^{13}C-labeled (90 atom %) root litter was followed in the presence and absence of *Avena barbata roots.* Total CO_2 and $^{13}CO_2$ flux rates were followed for about 10 months. After 2.5 months, plant shoots were clipped and a dry season imposed for 3 months. Plant soil systems were then rewet and analysis of CO_2 & $^{13}CO_2$ flux rates continued. The presence of roots reduced the rate of ^{13}C –labeled litter decomposition; in contrast, the presence of live roots increased the rate of total CO_2 production. These patterns had little dependence on the availability of N in the soils. Reduced rates of litter decomposition resulted from altered profiles of functional genes responsible for decomposition of macromolecular compounds and occurred in concert with higher relative abundances of gene responsible for utilization of small MW compounds known to occur in *Avena sp*. root exudates. In addition reduced water content of rhizosphere soils resulted in higher abundances of genes associated with microbial water stress; water stress was also likely a factor depressing rates of litter decomposition near grass roots.

In collaboration with Trent Nothern at LBNL, metabalomic profiles of ^{13}C-labeled Avena root –derived compounds were developed from sterile roots in hydroponic systems as well as roots growing in biologically complex soil. GC-MS analysis of sterile root solutions generated a library of compounds (including sugars, organic acids, fatty acids, amino acids, carboxylic acids, sterols, glycerol, among others) to be used in interpretation of more complex root-soil systems. TOF/TOF analysis of NIMS chips yielded spatially explicit patters of root-originating labeled C near roots in soil.

In order to connect root carbon inputs to the rates of decomposition by rhizosphere microbial communities, we grew plants under $^{13}CO_2$ and $^{12}CO_2$ in the presence and absence of ^{13}C-labeled litter and ^{12}C- litter. Functional gene transcripts were analyzed using GeoChip arrays in order to provide the

temporal resolution and replication necessary for Random Matrix Analysis. We also identified and isolated the C-cycling transcriptome associated with the consumption of labeled root exudates as well as labeled root litter. Additionally we began identification of the key microbial players by ^{13}C- CHIP-SIP using "*rhizochips*" developed from our previous analysis of rhizosphere communities associated with *A. barbata* roots in this soil. Stabilization of both live-root-derived C and root-litter-derived was followed using standard soil density fractionation methods to characterize the degree of stabilization ^{13}C in the microcosm soil from this experiment. Density fractionation of the soil allowed us to follow the stabilization of the ^{13}C into three operationally defined fractions: the free light fraction, occluded light fraction, and heavy fraction. As decomposition and stabilization progressed, root C moved from the free light fraction to the occluded light fraction and mineral-bound heavy fraction. The functional gene microarrays analyzing transcriptional activity contained probes from numerous genes involved in microbial mediation of C (as well as N and S) cycling. Information on transcriptional activity enabled us to identify and associate enzymatic processes involved in the breakdown and subsequent stabilization of organic C into defined fractions over time.

H3: ***Elevated CO_2 accelerates the sequential expression of functional genes, which translates into altered rates of decomposition and C-stabilization.***

Our 3rd major experiment tracked the functional and compositional succession of microbial communities from the early stage of utilization of root exudates through C stabilization under ambient and elevated CO_2 for two growing seasons. We constructed and used 16 fully-instrumented labeling chambers for the elevated and ambient CO_2 experiments. Exposure of plants to one and two seasons of elevated CO_2 increased the root biomass and the amount of plant C remaining in soil after the growing seasons. Exposure to elevated CO_2 also altered the profile of functional genes present in rhizosphere soil as indicated by functional gene array analysis. Interestingly however, elevated CO_2 did not alter the composition of the bacterial or fungal communities associated with *Avena sp* roots.

Publications:

Herman, Donald, Mary Firestone, Erin Nuccio, Angela Hodge. 2012. Interactions between an arbuscular mycorrhizal fungus and a soil microbial community mediating litter decomposition. FEMS Microbiology Ecology. FEMS Microbiology Ecology. 80:236-247.

Nuccio, Erin E., Angela Hodge, Jennifer Pett-Ridge, Donald J. Herman, Peter K. Weber, and Mary K. Firestone. 2013. An arbuscular mycorrhizal fungus significantly modifies the soil bacterial community and nitrogen cycling during litter decomposition. Environmental Microbiol. 15:1870-1881. With cover image

Blazewicz, S.J., R.L. Barnard, R.A. Daly, M.K. Firestone. 2013. Evaluating rRNA as an indicator of microbial activity in environmental communities: limitations and uses. ISME J. 7:2061-2068.

Keiluweit, M., J.J. Bougoure, J. Pett-Ridge, P.K. Weber, P.S. Nico, M. Kleber. Root exudates counteract mineral control on soil carbon turnover. 2015. Nature Climate Change, *in press.*

Shi, S., Nuccio, E., Herman, D. J., Rijkers, R., Estera, K., Li, J., Nune da Rocha, U., He, Z., Pett-Ridge, J., Brodie, E. L., Zhou, J., and Firestone, M. (In review) Successional trajectories of rhizosphere bacterial communities repeat in consecutive seasons. mBio.

Shi, S., Herman,,D.J., Pett-Ridge, J., He, Z, Wu, L., Zhou, J. and Firestone, M.K. (In review) Plant roots alter microbial functional capacity for mediation of decomposition. Ecology Letts.