

## **Following Soil Carbon Cycling under Reduced Precipitation in a California Annual Grassland**

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**Project Goals: Our project addresses both a fundamental understanding of carbon (C) cycling as mediated by multi-trophic interactions in the rhizosphere and the potential impacts of altered precipitation regimes on these interactions. Specifically, our work employs the use of stable isotopes to identify and quantify pathways of C-flow through multi-trophic interactions in the rhizosphere. Primary goals are to broaden knowledge of the roles played by multi-trophic interactions in terrestrial C cycling and to discover if drought alters the interactions and/or C cycling. This research will substantially expand our knowledge of soil microbial ecology, belowground food web, and terrestrial C cycling under a changing climate.**

The soil carbon (C) pool is larger than the atmospheric and terrestrial vegetation pools combined. Within soil C there exists a zone of dynamic biological activity and intense C turnover: the rhizosphere, the area of soil directly interfacing the root. Within the rhizosphere, there is a complex soil food web comprised of plant roots, fungi, bacteria, archaea, viruses and fauna that interact with each other and also utilize, transform, and transfer C from root exudates and root debris. Therefore, the food web interactions that occur within the rhizosphere influence whether C that is fixed via photosynthesis will return back to the atmosphere or remain in the soil for some period of time. While the concept of the soil food web is generally well recognized, the importance of food web pathways and their influence on soil C-cycling is much less known.

Climate models have predicted changes in the number of drought-impacted months in California, as in much of the rest of the world. Drought often reduces C efflux from terrestrial ecosystems, but the mechanisms underlying altered C cycling may be distinct for different periods after rainfall reduction. Understanding how reduced precipitation will impact food web dynamics and the subsequent effects on terrestrial C cycling is essential for anticipating future ecosystem responses. Accomplishing our research objectives requires: 1) Following the flow of carbon from the atmosphere through plants into different trophic levels of the soil food web in a field grassland ecosystem, and 2) Modifying the precipitation regime of the grassland ecosystem,

We hypothesize that under reduced precipitation, a higher proportion of plant derived C will be carried into soil by arbuscular mycorrhizal fungi (AMF), and mineralization via phage and faunal interactions will also be affected. To test these hypotheses, we constructed 16 “trenched”, rainout plots in an annual California grassland located at the Hopland Research and Extension Center (HREC) in Hopland, California. These plots contain a community of mixed California annual grasses and forbs. Precipitation inputs to these plots have been manipulated so that half receive a

50% reduction of the 65-year rainfall average, and the other half receive the full average rainfall amount. Two in-field  $^{13}\text{C}$  pulse labeling events were conducted in the Spring of 2018 to trace the pathways of C after it was fixed by the plant and delivered belowground in the form of exudates and fresh roots, and consumed by rhizosphere residents. Ecosystem respiration from the plots was estimated by measuring the overnight  $\text{CO}_2$  accumulation rate in the labeling chambers during both labeling events. Soils and plants were sampled at multiple time points following  $^{13}\text{C}$  pulse labeling to track the location and persistence of the recently fixed C. DNA and RNA have been extracted from rhizosphere soil for stable isotope probing (SIP) enabled-omic analyses. Together, these results will help elucidate how C travels through the rhizosphere food webs.

During the growing season, rainfall reduction significantly decreased soil moisture by 35% and ecosystem respiration by 20%. There was a significant difference in the amount of enrichment of rhizosphere-influenced soil between the  $^{13}\text{C}$  and  $^{12}\text{C}$  labeled samples. The  $^{13}\text{C}$  samples had a  $\delta^{13}\text{C}$  enrichment of 67‰ higher compared to the  $^{12}\text{C}$  samples. While  $^{13}\text{C}$  enrichment in rhizosphere-influenced soils was not affected by precipitation treatment, rhizosphere soil DNA was significantly  $^{13}\text{C}$  enriched, with an atom percent excess ranging from 22-30%. Individual organisms (nematodes, AMF hyphae, protists) drawn from the root zone were also enriched, up to 3500‰. In sum, this large-scale multi-day field  $^{13}\text{C}$  labeling of California annual plants successfully labeled belowground communities to a degree that will enable us to track the flow and fate of root C into and through multiple soil communities.

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