The "Who" and "How" of Microbial Control over Soil Carbon Dynamics: a Multi –omics, Stable Isotope Probing, and Modeling Approach

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Project Goals

Our goal is to define the genomic basis of soil microbial carbon use efficiency (CUE) in the context of changing environments, and use this new knowledge to generate more realistic models of SOM decomposition. Our specific objectives are to: (1) Define genomic markers that indicate how terrestrial bacteria and fungi change CUE in response to environmental drivers of microbial activity; (2) Determine how well genomic markers identified in Objective 1 predict CUE in soils; and (3) Improve predictions of soil C dynamics by incorporating genomics-informed CUE estimates into ecosystem C models based on genomic features defined in Objective 1 and validated for soil in Objective 2. To scale from cells to ecosystems, we are studying CUE in pure cultures of bacteria and fungi, mock communities in artificial, organic matter-free soil, and field soil manipulated to elicit microbial community composition and CUE. With each level of complexity, we refine our understanding of the genomic and genetic basis of CUE using statistical and simulation models.

Abstract

Soils are the largest repository of organic carbon (C) in the terrestrial biosphere and represent an important source of CO_2 to the atmosphere. Microbes are key drivers of C cycling and exhibit a large range in C use efficiency (CUE), the fraction of substrate C incorporated into biomass per substrate assimilated. While small changes in CUE can have profound effects on soil CO_2 flux, our understanding of the intersection of genomic and abiotic factors driving soil CUE dynamics is still very limited. This research couples two culture collections of fungi and bacteria isolated from a long-term soil warming experiment at Harvard Forest, where soils have been heated 5°C above ambient for 26 years. We have over a decade of field measurements of C cycling and microbial community analyses using metagenomics and metatranscriptomics. Because microbial feedbacks to the environment are regulated at the organismic level, the foundation of this research is the physiology and genomics of CUE among isolates that we have determined to be either dominant in our soil system, sensitive to climate change factors, or both. Experiments using artificial soil, lab incubations of field-collected soil, and a field manipulation will validate potential omics-informed, novel genomic markers to be used as proxies for CUE in models.

Towards our first goal of defining genomic markers of CUE response to environmental change, we have been defining the physiological response of bacterial and fungal isolates to changes in

substrate and temperature in the lab. The CUE of fifteen soil bacteria covering six phyla is being evaluated under three temperatures and up to four C sources, with a similar study being conducted for fungi. Both bacterial and fungal isolates show diverse temperature responses, with some bacterial isolates showing reduced efficiency at higher temperatures while others showed no change or an increase. An exploratory analysis determined that bacterial genomes enriched in energy generation and metabolism tended to show a decrease in CUE on glucose at higher temperatures, while those relatively depleted in these genes tended to show an increase. This analysis was facilitated by computational tools generated by our group for automating steps in multigene phylogenetic tree production and will be followed up in the upcoming year with the development and calculation of metrics for metabolic complexity correlated with CUE.

For objective 2, we have finalized method development for measuring microbial growth and CUE in soil using a novel, substrate-free approach with ^{18}O-H$_2$O. This method was found to be more sensitive and interpretable than traditional ^{13}C approaches. Cultivation of select fungal and bacterial isolates and of simplified soil-derived microbial consortia in artificial soil shows a promising route for linking the liquid-culture-based evaluation of genomic markers of CUE sensitivity in natural soils. A qPCR-based approach is being developed to estimate fungal and bacterial biomass and growth in artificial soil, and we have begun to use it to evaluate CUE of bacteria under a range of temperatures and moistures. Expansion of results to natural soil communities and the consortia will enable us to independently validate genomic markers of efficiency identified in isolates.

In pursuit of objective 3, the data derived from the field and microbial laboratory studies will be used to improve model parameterization of terrestrial system responses to environmental change. Incorporating the range of CUE temperature sensitivities observed in bacterial isolates into the litter decomposition model DEMENT confirms that predicted soil C stock response to warming is highly sensitive to this parameter. Continued work to refine the "soil" version of this model with organo-mineral interactions will enable predictions of slower-cycling soil C pools. We will also incorporate the genomics-informed data on microbial CUE combined with field measurements from a long-term warming and warming × nitrogen study into a stoichiometrically-coupled, acclimating microbe-plant-soil model (SCAMPS) that aggregates microbial dynamics at the community-scale and compare these simulations with the guild-based microbial models (DEMENT and MIMICS).

Understanding the genes, metabolic pathways, and phylogenetic signals associated with microbial CUE that drive soil community responses to key global change drivers will provide a stronger basis for modeling soil C dynamics and climate-C feedbacks. Our research will establish a path by which new and extant -omics datasets may be integrated into ecosystem models. These new proxies should create greater power to infer the sensitivity of CUE to environmental dynamics and the tendency of CUE to change and adapt over long times.

Publications related to this research

1. <u>Grace Pold</u>, Erin M. Conlon, Marcel Huntemann, Manoj Pillay, Natalia Mikhailova, Dimitrios Stamatis, T.B.K. Reddy, Chris Daum, Nicole Shapiro, Nikos Kyrpides, Tanja Woyke, **Kristen M. DeAngelis**. (2018) "Genome sequence of *Verrucomicrobium sp.* strain GAS474, a novel bacterium isolated from soil." *ASM Genome Announcements*, in press.

2. <u>Grace Pold</u>, Marcel Huntemann, Manoj Pillay, Natalia Mikhailova, Dimitrios Stamatis, T.B.K. Reddy, Chris Daum, Nicole Shapiro, Nikos Kyrpides, Tanja Woyke, **Kristen M. DeAngelis**. (2018) "Draft genome sequences of three strains of a novel Rhizobiales species isolated from forest soil." *ASM Genome Announcements*, in press.

3. Jerry M Melillo, Serita D Frey, **Kristen M DeAngelis**, William Werner, Michael Bernard, F P Bowles, <u>Grace Pold,</u> Melissa A. Knorr, A Stuart Grandy (2017) "Long-term Pattern and Magnitude of Soil Carbon Feedback to the Climate System in a Warming World." *Science* 358 (6359): 101-105. doi:10.1126/science.aan2874.