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JGI Small-Scale Metagenome Proposal

* all sections limited to 4000 characters
“Proposals must show clear DOE mission relevance in areas such as bioenergy-related plant-microbe interactions, carbon/nitrogen cycling and/or carbon sequestration processes in soils and sediments, and biogeochemical processes contributing to contaminant biotransformation and/or immobilization.”

Title: Microbial community composition and function throughout decomposition in temperate forests underlain by N-rich bedrock

Description: We propose to examine differences in soil microbial community composition and function a) across a series of six temperate forest sites in the northern California coast range that span a wide range in bedrock nitrogen (N) concentration and b) throughout leaf litter decomposition at three of these sites. We propose to generate 12 draft metagenomes and 110 16S rRNA amplicon sequences to support this work. Previous research by the Houlton lab group has found that bedrock can be a significant source of ecologically-available N, driving increased above-ground and soil carbon (C) storage in forests underlain by N-rich bedrock compared to forests lacking bedrock N inputs. Our group has conducted extensive sampling of forests across various lithologies in the California coast ranges, and has characterized a set of sites that serve as a “rock N gradient,” with rock N concentrations spanning from 50 mg N/kg bedrock to >1000 mg N/kg bedrock. Differences in bedrock N content between these sites drives differences in soil, foliage, and leaf litter chemistry (Figure 1) and accelerates soil N cycling processes such as N mineralization and free-living N fixation (Figure 2). Understanding the differences in soil microbial community composition and function associated with these differences in ecosystem chemistry and processes is central to an understanding of how soil N cycling and C storage may be influenced by bedrock N inputs.

The decomposition of dead plant biomass is of particular importance in soil N and C cycling. Belowground inputs of N from bedrock can plausibly affect decomposer microbial communities via changes in soil nutrient availability as well as via changes to leaf litter substrate chemistry. In order to examine differences in decomposition dynamics between sites of varying bedrock N inputs and differentiate the competing roles of soil fertility and leaf litter chemistry in shaping decomposer microbial communities, we conducted a reciprocal transplant experiment. Freshly abscised leaves were collected from three sites in our bedrock N gradient; one underlain by low N bedrock (<100 mg N/kg bedrock), one underlain by high N bedrock (700 mg N/kg bedrock), and one underlain by extremely high N bedrock (>1000 mg N/kg bedrock). We transferred leaves into mesh bags and allowed them to decompose for 100 days *in situ* (at the site that leaves were collected from) as well as at two common garden sites (one with the highest bedrock N content and one with the lowest bedrock N content). Leaf litter subsamples were collected after 1, 2, 7, 30, and 100 days of decomposition.

We are measuring mass loss, free-living N fixation, and chemical changes in these leaf litter samples throughout decomposition.

We propose to generate 12 draft metagenomes from this experiment: six from soils collected along our bedrock N gradient (including the three sites used for our decomposition experiment), three from the initial leaf litter samples from our decomposition experiment, and three from the final leaf litter samples (100 days of decomposition) that were decomposed *in situ*. To examine microbial community dynamics during decomposition with greater temporal resolution, as well as differentiate the roles of soil fertility and substrate chemistry in influencing microbial communities, we propose to generate 110 additional 16S rRNA amplicon sequences from the leaf litter subsamples decomposed *in situ* and at common garden sites collected after 1, 2, 7, 30, and 100 days of decomposition.

Justification: Although not widely recognized as an ecologically significant source of N until recently, global mass-balance modeling suggests that weathering of N from bedrock contributes 12.6-28.2 Tg N yr^{-1} to terrestrial ecosystems. In the northern California coast ranges, N-rich sedimentary bedrock supports forests with approximately 40% more above-ground biomass C and 60% more soil C than otherwise similar forests underlain by N-poor bedrock. Little is known about how this belowground N input affects ecosystem processes to result in this increase in C storage, or how it may affect other ecosystem N cycling processes, such as those that occur throughout decomposition. The Houlton group has conducted extensive sampling of forests underlain by N-rich bedrock in the California coast ranges, and we have found increasing N content in foliage, litter, and soil with increasing concentration of N in bedrock (Figure 1). We have also found evidence of accelerated N cycling in these forests, with significantly higher rates of both N mineralization in soils and leaf litter N fixation (Figure 2) in forests with high rock N content (>500 mg N/kg bedrock) vs. forests with low rock N content (<500 mg N/kg bedrock). Characterizing the feedbacks resulting in accelerated N cycling processes and increased plant-available N at sites with high bedrock N content has implications for future productivity and C storage in aboveground biomass and soil in these highly productive conifer forests.

Leaf litter decomposition is a critical process in forest N and C cycling. As decomposer microorganisms break down organic compounds and respire carbon dioxide, they also transform N into various forms contribute to organic matter stabilization. An understanding of soil microbial communities is thus central in order to understand C storage in soils as well as soil N cycling dynamics, both of which have implications for global climate change. The balance between microbial respiration and C stabilization dictate whether forest soils behave as a C sink or a C source; similarly, the relative rates of microbially-mediated N transformation processes such as N fixation, mineralization, and denitrification dictate N availability for plant uptake as well as influence N losses through nitrate leaching and nitrous oxide emissions, which are of concern for both human health and global climate change. Despite the importance of understanding the microbial communities involved in decomposition, few studies have

been conducted to date on changes in bacterial community composition and function throughout decomposition, especially in forest soils

In order to understand how these changes in ecosystem chemistry and processes may affect the soil microbial communities involved in N cycling, we have collected and extracted DNA from soils across a series of forested sites in the northern California coast ranges where we have previously conducted extensive sampling. These sites serve as a “rock N gradient” with rock N concentrations spanning from 50 mg N/kg bedrock to >1000 mg N/kg bedrock. At three of these sites, we have established a leaf litter decomposition experiment and are examining mass loss, free-living N fixation, and chemical changes throughout decomposition. Because rock N inputs influence both soil chemistry and litter quality we expect to find differences in soil microbial communities and decomposition dynamics between sites underlain by N-rich vs. N-poor lithologies. A study microbial community composition and function in parallel with the other biogeochemical variables we are measuring in this experiment will provide insight into the link between microbial communities and larger-scale biogeochemical function and C storage. Furthermore, our sampling scheme (samples collected after 1, 2, 7, 30, and 100 days of decomposition) allows for the examination of rapid changes in microbial community composition during early decomposition.

Utilization: Draft metagenomes will be compared between sites across our rock N gradient, as well as for both beginning and end of the decomposition experiment within individual sites. 16S rRNA amplicon sequencing will be used to examine microbial community changes throughout decomposition with greater temporal resolution (days 1, 2, 7, 30, and 100 of decomposition) decomposed *in situ* and at the common garden sites.

Microbial community structure will be compared between samples using non-metric multidimensional scaling (NMDS). Richness, evenness, and Shannon’s diversity index will be compared between samples. PERMANOVA and Mantel tests will be used to determine significant correlations between community composition and other biogeochemical parameters measured in this experiment or previously measured at these sites (mass loss rate, litter chemistry throughout decomposition, N fixation rate throughout decomposition, soil pH, soil chemistry and stable isotopic ratios, foliar chemistry and stable isotopic ratios). The abundances of key functional genes involved in C and N cycling will also be compared between metagenomes.

The Houlton lab group has extensive experience in measuring temperate forest biogeochemistry. Additionally, we collaborate with the soil microbiology lab within the department of Land, Air, and Water Resources at UC Davis, whose members have extensive experience analyzing soil metagenomic and 16S amplicon data. These complementary skills make our research group poised to successfully connect microbial metagenomics and 16S rRNA amplicon data to biogeochemical data, providing insight into critical biogeochemical processes and the responsible microorganisms.

Community Interest: This research will benefit a wide variety of scientific communities. The community of soil microbial ecology researchers is rapidly growing and many are interested in understanding changes in microbial community composition and function throughout decomposition; not much research has been conducted to date in this area. There is a growing interest in including microbial community data in biogeochemical models on both an ecosystem and a global scale, and this research will help inform such modeling efforts.

Bedrock as a source of ecosystem-available N is a relatively new paradigm and the ways in which bedrock N inputs influence ecosystem N cycling remain largely unknown. Our group's initial modeling efforts suggest a total global flux of N from bedrock of 12.6-28.2 Tg N yr^{-1}, rivaling biological N fixation inputs in many areas; thus, understanding how this significant input of N affects the soil microbial community involved in C and N cycling is of utmost importance for the wider ecology and biogeochemistry research community.

DOE Mission: This research is directly connected to two DOE missions: carbon cycling and biogeochemistry. Our group has previously demonstrated increased aboveground and belowground C storage in forests receiving significant inputs of N from bedrock. However, the mechanisms by which this belowground N input affects ecosystem processes resulting in this increased C storage, or how bedrock N inputs may affect other ecosystem N cycling processes remain unknown. The decomposition and stabilization of dead plant biomass drives long-term C storage in forest soils. Therefore, it is critical to understand changes to microbial communities that occur throughout decomposition, differences in decomposing communities driven by soil and leaf litter chemistry, and how these differences are related to decomposition rates and soil C storage. Further, this research will inform an understanding of how N-rich bedrock may influence stabilization of C during decomposition via ecosystem feedbacks affecting microbial communities.

This experiment will also contribute to an understanding of the soil microbial communities involved in the N cycle. Microbes are responsible for most soil N cycling processes, including internal N transformations (nitrification, mineralization) as well as N inputs (N fixation) and losses (denitrification). An understanding of how microbial community composition, especially functional group abundance, differs across our rock N gradient and changes throughout decomposition is essential for understanding differences in the N cycle due to bedrock N inputs as well as changes to the N cycle throughout decomposition. This has implications for plant N availability, which influences future aboveground productivity, C storage both above and belowground. The forest N cycle can also directly affect global climate change via losses of N as nitrous oxide, a potent greenhouse gas.

Sample Preparation: We have already collected >90% of the soil and leaf litter samples for this project and have extracted DNA using MoBio PowerSoil DNA extraction kits and stored at -20°C. We have previously obtained high-quality genomic

DNA using this extraction protocol. DNA samples will be quantified using the Qubit fluorimeter. DNA samples could be ready for shipment to JGI by late January.

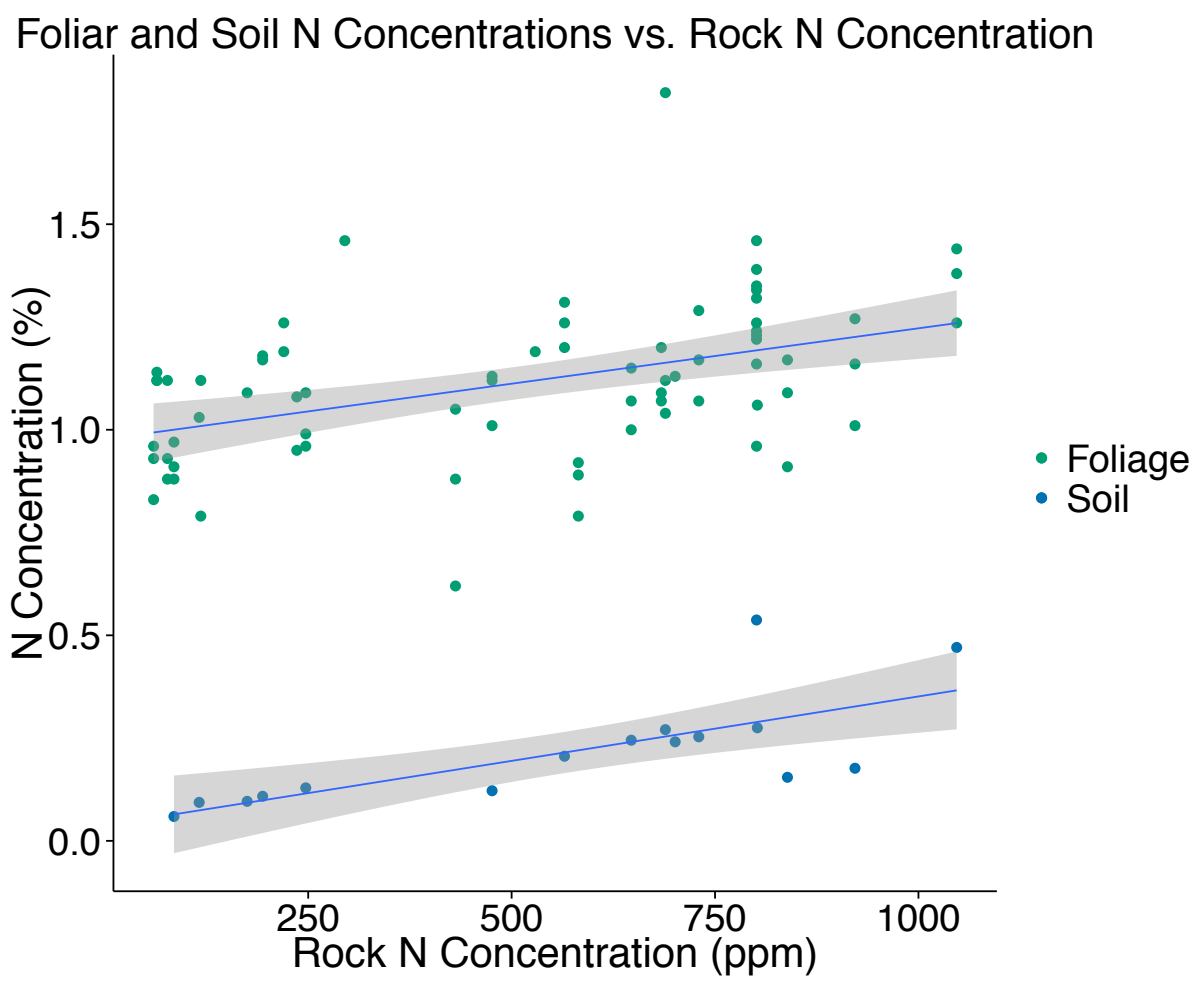

Figure 1: Linear regression of foliar N concentrations (green circles) and soil N concentrations (blue circles) against bedrock N concentration (in ppm N). Both foliar N and soil N are significantly correlated with rock N concentration (foliar R^2=0.1843, p<0.001, soil R^2=0.5039, p=0.001).

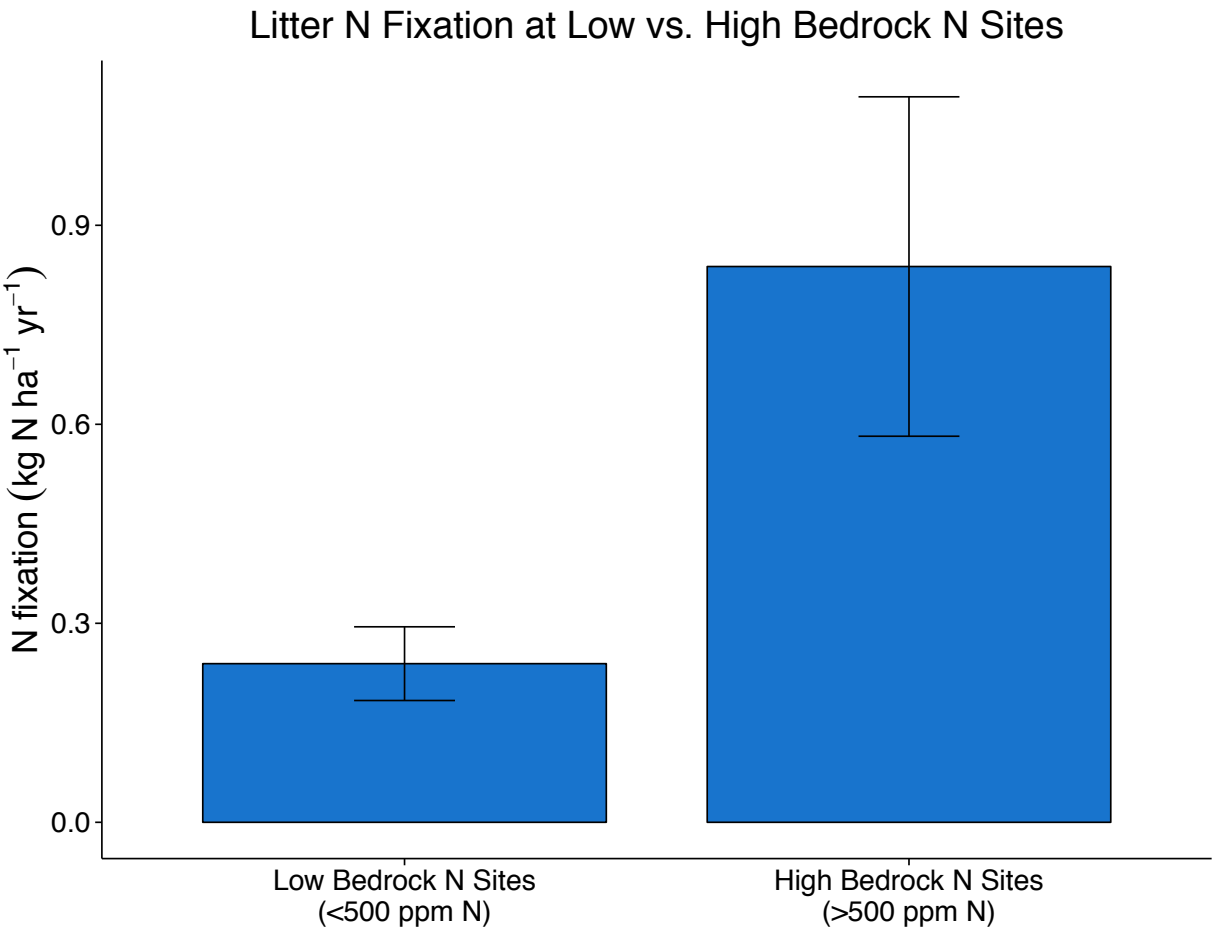

Figure 2. Mean litter N fixation (kg N ha^{-1} yr^{-1}) ± SE at low bedrock N (<500 ppm N) vs. high bedrock N (>500 ppm) sites. The mean litter N fixation rate (at high bedrock N sites (0.84 kg N ha^{-1} yr^{-1}) is significantly higher than the mean N fixation rate at low bedrock N sites (0.23 kg N ha^{-1} yr^{-1}, p=0.02).