

**Title:** Meta-omic, Enzymatic and Soil Chemical Measures Identify Surface Soil Decomposition Processes as Influenced by N Amendment in a Temperate Pine Forest

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<http://www.lanl.gov/org/padste/adcles/bioscience/bioenergy-biome-sciences/soil-carbon.php>  
<http://www.lanl.gov/org/padste/adcles/bioscience/bioenergy-biome-sciences/environmental-microbiology.php>

**Project Goals:** The LANL Genomic Science SFA is focused on microbial communities in surface soil horizons and their functional processes that influence soil carbon storage and release. The SFA examines soil carbon cycling under conditions of environmental change to understand the metabolic and ecological roles of fungi and bacteria in surface soils in two important temperate ecosystems – forests and arid grass/shrub lands. In both biomes fungal and bacterial biomass is concentrated in shallow surface soil strata where C and N cycling are major processes. Advancing fundamental knowledge of soil communities within the context of altered environmental regimens will improve our ability to predict and possibly manage ecosystem contributions to global climate. This involves discovery of fundamental principles at different scales that influence the organization, interactions, and response of soil communities.

Anthropogenic N deposition is a chronic and increasing condition in temperate regions that may strongly influence C cycling dynamics. Our goal is to provide an understanding of the active and responsive components of temperate forest surface soils that contribute to C cycling and their collective responses to environmental change. This poster describes the current status of our field experiments in a temperate pine forest (Duke forest, NC) after eleven years of N fertilization (annual application of ammonium nitrate pellets, 11.2 g N m<sup>-2</sup>). To link microbial community structure with functions in response to N fertilization, we measured total, fungal and bacterial biomass (by rRNA gene qPCR); fungal and bacterial community richness, diversity and composition (by MiSeq rRNA gene sequencing); activities of seven exoenzymes in the total community measured in soil; and collective metatranscriptome gene expression of the bacterial, archaeal, and fungal communities. The field site was extensively sampled with at least 10 field replicates for each of three soil horizons (Oa, Oe, and A horizons to 10 cm depth), in each treatment plot. We present correlations among these datasets and with soil chemistry measures, and will present our inferences about the resident fungal and bacterial communities, their enzyme activities, and local geochemistry across shallow soil horizons in the pine forest.

All soil chemistry measures except pH and nitrate declined with depth over 10 cm. This included organic matter, P, K, metal ions, total N and total C. The long term N amendment depressed soil pH, but increased nitrate, P, K, Fe and Mn concentration in each horizon. Biomass, measured as extractable DNA, decreased significantly with depth and with N-amendment. Bacteria SSU rRNA gene copy number followed the same trend. Fungal SSU RNA gene copy number dropped

an order of magnitude across a few cm depth with no effect of N-amendment. Hydrolase activities per g soil declined sharply with soil depth, largely in response to declining SOC. In contrast, oxidase activities increased with soil depth on both a dry mass and SOC basis. A vector analysis of hydrolytic EEA responses to N treatment showed significant increase in vector length, indicating a relative increase in C limitation, and vector angle, indicating increased P limitation relative to N. For all horizons, N treatment suppressed phenol oxidase (POX) activity relative to B-galactosidase (BG) activity. As a result, the BG/POX ratio increased by 43%, 184%, and 55%, for Oa, Oe and A horizons, respectively. This trend was similar to that for the hydrolytic C:N activity ratio BG(NAG+LAP), suggesting that increased N availability also reduced the potential for N mining from recalcitrant SOM. Carbon use efficiency (CUE) increased significantly with soil depth and decreased with N treatment.

Bacterial community richness and diversity were negatively and significantly reduced, and bacterial community composition was altered, in N fertilized plots at all three depths. In contrast, the fungal community richness and diversity were unaffected by N fertilization in the top two depth intervals, and were only enriched in the A horizon. The fungal community composition was significantly different across soil depth and with N fertilization. Twenty-seven of the thirty most abundant OTUs were Basidiomycota. Eight genera were significantly, positively affected by the N fertilization and eight genera were negatively affected; all were Basidiomycota genera that function as ectomycorrhizal associates with pine or as wood rotters.

In summary, across a 10 cm soil depth column, (i) all of the soil chemistry measures were statistically different with depth. Correspondingly, (ii) the profile of enzyme activity, including enzymes responsible for C, N and P acquisition and oxidative decomposition, differed with soil depth. In response to N amendment, patterns of enzyme activity suggest a shift to greater C and P acquisition activity, and a reduced potential for mining C from recalcitrant organic matter and N acquisition activities. In the metatranscriptomes, (iii) transcripts representing these enzyme activities showed similar depth and N-response trends, and general patterns of CAZyme transcripts shifted across depth and with N fertilization. Both fungal and bacterial transcripts contributed to the observed transcriptome patterns. (iv) Fungal and bacterial community biomass was reduced with soil depth and with N fertilization, and the composition of both communities shifted with N fertilization at each depth interval.

The concerted changes in soil chemistry, microbial biomass, gene expression and enzyme activities across the shallow depth gradient, and in response to N fertilization, illustrate the dramatic impact that long-term N amendment may have on C cycling and storage processes in surface soils of temperate forests. The results presented here illustrate how important it is to select appropriate sampling sites and depths when trying to map local soil processes, and when defining soil traits that may be useful for establishing soil processes. Forests deposit considerable organic matter to the soil surface; the extent to which this organic matter is decomposed or stored in the soil as fixed carbon is an important parameter to understand when predicting responses of natural forest ecosystems to climate changes.

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