Title: Soil fungal community responses to long term elevated CO_2 and N deposition conditions in a temperate pine forest (DOE Duke Forest FACE site).

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Project goals: The simultaneous increase of atmospheric CO_2 and nitrogen (N) deposition to forest ecosystems is predicted to alter plant productivity and, consequently, to change the amount and quality of above and belowground carbon entering forest soils. It is not known how such changes will impact the composition and function of soil microbial communities, particularly fungal communities, that play a key role in degrading complex carbon. Our recent studies explored the composition of soil fungal biomass after ten years of elevated CO_2 conditions, and under five years of combined elevated CO_2 and increased soil N conditions, in a temperate pine forest (DOE Duke Forest FACE site). We used a combination of DNA- and RNA-based surveys and sequencing of target genes to compared features of the resident and active soil fungal community.

Ecological studies: First, we conducted a comparative study of fungal cellobiohydrolase I genes (*cbh*I), representing the resident (DNA-based) and expressed (cDNA-based) communities in surface soil (0-10 cm depth) across the elevated CO_2 and N-fertilization treatments at the Duke Forest FACE site. Our study demonstrated that the richness and composition of the soil cellulolytic fungal community was distinct between the DNA- and cDNA-based gene surveys. Richness or composition of the cellobiohydrolase-containing fungal community was not altered by elevated CO_2 and/or N-fertilization conditions relative to the ambient controls. The soil fungal community was dominated by members of the Basidiomycota that have minimal or no representation in current sequence databases.

Second, we conducted seasonal surveys of the fungal *cbhI* gene and the fungal LSU gene (phylogenetic marker) across the treatments at the Duke Forest FACE site, over two years and five seasonal time points to determine how fungal community responses to climate change parameters may vary with season. In all seasons, soil fungal community richness (LSU gene) was decreased in elevated CO_2 plots compared to ambient plots. In both ambient and elevated CO_2 plots, N fertilization increased richness in spring and summer sampling points indicating that some taxa are nitrogen limited at this site during these seasons. In contrast, we did not detect a change in *cbhI* richness that correlated with season.

Third, we conducted an intensive LSU sequencing survey of soil fungal communities present in forest floor and in underlying soil to determine the response to elevated CO_2 and N deposition treatments manifest across soil depths of a few cm (forest

floor, 0-2 cm, 2-5 cm, 5-10 cm). Soil chemistry and fungal community (LSU gene) richness and composition differed significantly across shallow changes in soil depth. Fungal community response to elevated CO_2, N deposition, and the combined treatment was also highly stratified by depth, illustrating that fungal community roles in this forest ecosystem cannot be accurately predicted using bulk, homogenized samples typically employed in large field studies.

Method development and database resources: Defining the factors underpinning methodological biases is required to optimize the design of gene expression studies in soils. As a prerequisite to the ecological studies described above, we compared the richness and composition of the fungal *cbhI* gene in forest floor and underlying soil, that could be captured using two cDNA preparation methods and two different cDNA priming methods. Richness, composition and reproducibility of gene expression profiles of the fungal *cbhI* gene were examined when amplified from sscDNA, or from dscDNA synthesized using SMART PCR. In the dscDNA libraries from soil or litter samples, richness was significantly reduced and the composition was altered relative to sscDNA libraries. Library composition was significantly more reproducible among replicate sscDNA libraries than among parallel dscDNA libraries from litter. We also performed comparative richness and compositional analyses of the fungal *cbhI* gene amplified from soil cDNAs that had been generated using either oligo(dT) primers or random hexamers. Our results demonstrated that similar *cbh*I richness and composition were recovered using either priming method.

To support taxonomic interpretation of the soil fungal community sequences obtained in the ecological studies, we established a taxonomic database for the *cbhI* gene, and for the fungal LSU gene. The accuracy of the LSU database using multiple sequence lengths was determined using naïve Bayesian classifier and BLAST approaches. These classification resources are publicly available to the scientific community through the Ribosomal Database Project.

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