Effects of Drought on Microbes Colonizing Lignocellulose

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INTRODUCTION

Much of the global carbon pool is tied up in soil organic compounds from plant litter, such as lignin, hemicelluloses, and celluloses (6;1). Litter decomposition is important to global carbon cycling, and may be impacted by climate change. Extracellular enzymes produced by microbes such as fungi and bacteria can break down lignocellulose to soluble subunits (1) which are then mineralized to CO2. Basidiomycetes are largely responsible for the breakdown of lignin (10), especially white rot and some brown rot fungi (3). Bacteria are known to play a more minor role in the breakdown of lignin. Three classes of lignin-degrading enzymes include laccase, manganese-dependent peroxidases, and lignin peroxidases (3). Laccase, especially, is considered necessary for efficient lignin breakdown (3;2).

Climate change may either increase or decrease the rate of decomposition by a variety of mechanisms, including by reducing soil moisture. The purpose of this study is to examine the effect of a simulated drought in boreal forest soil on the abundance of lignin-degrading microbes as well as to explore the correlation between the presence of these microbes and the decomposition rate. According to Schimel et al. (2007), drought may cause resource limitation for microbes, thus slowing down biogeochemical processes, including decomposition. Drought forces microbes to gather solutes, which are energetically expensive, in order to reduce the risk of dehydration and death (9). Bacteria use amino compounds as their primary solutes while fungi use polyols. Bacteria are thought to be less drought-tolerant in part because of their demand for nitrogen. My hypothesis is that the simulated drought treatment would decrease the abundance of lignin-degrading microbes, thus lowering the rate of decomposition.

METHODS

Samples were obtained from an ongoing study measuring mass loss of buried birch tongue depressors (BTDs) as an indicator of decomposition in control versus a simulated drought/rain out treatment within the Bonanza Creek Long Term Ecological Research (LTER) Site (Fig. 3). BTDs were buried in soil for 2 years and then removed, washed, weighed and stored frozen prior to this study (8). Total DNA was extracted from subsamples of BTDs using the MoBIO PowerSoil DNA extraction kit with a few modifications to the manufacturer's protocol (MoBIO, CA). Instead of vortexing in an adapter, samples were put in a bead beater at maximum speed for 30 seconds. DNA was eluted in 30 µL PCR grade water instead of 100 µL of solution C6. Bacterial biomass, fungal biomass and the quantity of laccase genes was determined using quantitative real-time PCR (qPCR). For standard curves for qPCR, purified PCR products were used after amplification using primers for bacterial 16S rRNA (on *Burkholderia xenovorans* LB400), fungal ITS (on *Saccharomyces cerevesiae*), and laccase genes (on DNA from nearby soil samples). PCR products were viewed by means of gel electrophoresis to verify amplification of target genes based on fragment size. qPCR was applied to quantify the number of copies of bacterial 16S rRNA genes, fungal ITS and laccase genes in each sample on the basis of BTD dry weight. The program JMP 7 was used for all statistical analyses (SAS). The two-sample t-test was used to compare the drought and control treatments for each type of gene. The bivariate analysis was used to investigate the correlation between the number of gene copies of each gene to percent mass loss of BTDs. Sample size was 26 BTDs. All qPCR reactions were performed in duplicates for samples and triplicates of standards.

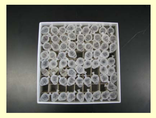


Fig. 1: DNA extracted from BTD subsamples

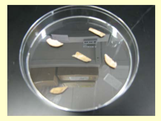


Fig. 2: Subsamples of a BTD

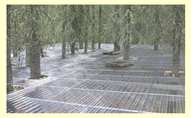
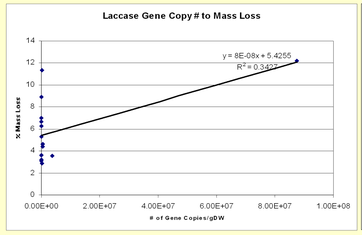


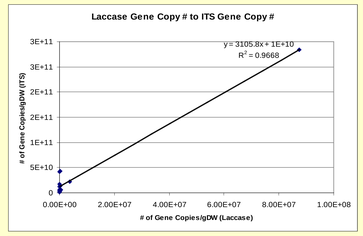
Fig. 3: Rainout shelters at Bonaza Creek LTER that simulate summer drought due to climate change

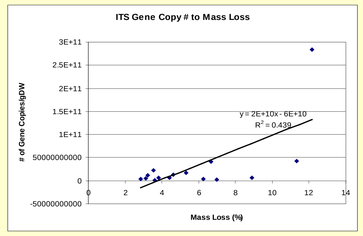
RESULTS

Two-sample t-tests were run to compare the control & drought treatments for each type of gene (laccase, bacterial 16S, fungal ITS, and bacterial:fungal ratio). None of these results were significant (16S p = 0.8; ITS p = 0.6; laccase p = 0.35; B:F p=0.98). When all data were considered, there was no significant correlation between bacteria, fungi or laccase gene copy number with BTD mass loss. However, within the drought treatment alone, the correlation between ITS gene copy number and BTD mass loss was significant, as well as laccase gene copy number to mass loss and laccase gene copy number to ITS gene copy number. After eliminating one outlying datapoint, however, these results became insignificant except for the ITS gene copy number to mass loss comparison. The outlier differed from other BTDs in that it was considerably softer and lighter in color.

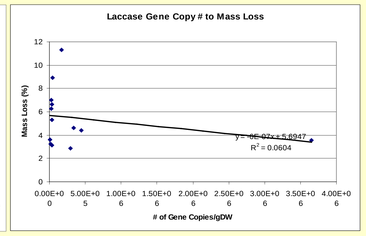
Before Outlier Was Removed

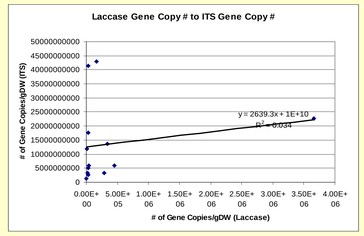


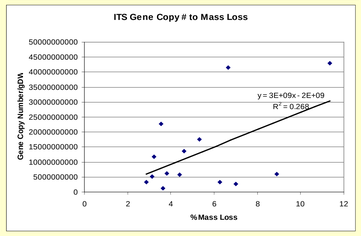


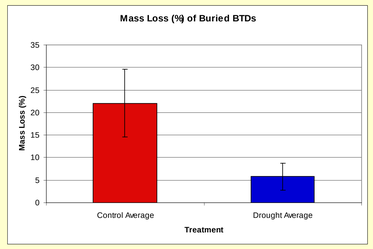


After Outlier Was Removed









DISCUSSION

There was not a significant difference in the number of laccase genes or biomass of bacteria or fungi or B:F ratio in the different treatments. This does not support the original hypothesis that drought would decrease the abundance of microbes and thus the number of gene copies. This suggests that the simulated drought has little detectable effect on the number of copies of each of the genes or the B:F ratio. One reason for this may be the fact that the BTDs were washed gently prior to weighing. Although lignocellulose degraders are generally thought to be tightly bound to surfaces, washing would have removed much of the loosely-associated biomass. Also, it is possible that both control and drought treatments were colonized by the same number of microbes but that they functioned at much lower rates due to drought. Another explanation may be that although the total number of bacteria and fungi were similar, the community composition may have differed so that there were fewer lignocellulolytic organisms present in the drought treatment.

It was expected that fungal biomass would correlate to mass loss of BTDs. However, in the control, the abundance of the different genes was not correlated with mass loss, while in the drought treatment, the abundance of the fungal ITS gene did correlated with mass loss. An outlier that was excluded from the analysis had high levels of laccase and ITS gene copy numbers, and was obtained from an unusually soft and light-colored BTD sample from the drought treatment. This BTD may be worthy of further study for the presence of white-rot fungi, and also may be an indicator of spatial heterogeneity at the site since other samples buried within 0.2 m of this sample were dissimilar.

LITERATURE CITED

1. Amatangelo, Raab, Stewart, Waldrop, Neff, Vitousek. (in process). Plant lignin influences microbial growth and decomposition dynamics.
2. Blackwood, C. B., M. P. Waldrop, D. R. Zak, R. L. Sinsabaugh. 2007. Molecular analysis of fungal communities and laccase genes in decomposing litter reveals differences among forest types but no impact of nitrogen deposition. Environmental Microbiology 9:1306-1316
3. D'Souza, T. M., K. Boominathan, C. A. Reddy. 1996. Isolation of laccase gene-specific sequences from white rot and brown rot fungi by PCR. Applied and Environmental Microbiology 62:3739-3744.
4. Fierer, N., J. A. Jackson, R. Vilgalys, R. B. Jackson. 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Applied and Environmental Microbiology 71:4117-4120.
5. Lee, C.C., D. W. S. Wong, G. H. Robertson. 2001. Cloning and characterization of two cellulase genes from *Lentinula edodes*. FEMS Microbiology Letters 205:355-360.
6. Luis, P., G. Walther, H. Kellner, F. Martin, F. Buscot. 2004. Diversity of laccase genes from basidiomycetes in a forest soil. Soil Biology & Biochemistry 36:1025-1036.
7. Ohtoko, K., M. Ohkuma, S. Moriya, T. Inoue, R. Usami, T. Kudo. 2000. Diverse genes of cellulase homologues of glycosyl hydrolase family 45 from the symbiotic protists in the hindgut of the termite *Reticulitermes speratus*. Extremophiles 4:343-349.
8. Runck, S., D. Valentine, J. Yarie. Sensitivity of soil organic carbon dynamics to long-term throughfall exclusion in interior Alaska. ASA-CSSA-SSSA 2006 International Meetings, Indianapolis, IN. Nov. 12-16, 2006.
9. Schimel, J, T Balser, M Wallenstein. 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88:1386-1394.
10. Stuardo, M., M. Vásquez, R. Vicuña, B. González. 2004. Molecular approach for analysis of model fungal genes encoding ligninolytic peroxidases in wood-decaying soil systems. Letters in Applied Microbiology 38:43-4

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