Long Term Soil Productivity Methods

Site Description

The Fork Mountain Long-Term Soil Productivity Study (herein LTSP) is located in the central Appalachian Mountains near the town of Parsons, West Virginia (latitude 39°04' N, longitude 79°41' W) on Fork Mountain and was established in 1996. The LTSP has a southeast aspect with 15-31% slopes over an elevation of 798 to 847m (Adams *et al*., 2004). The purpose of the experiment is to measure the effects of base-cation depletion on forest productivity in response to acid rain deposition. As such, it was designed with four replicate plots of four treatments. The treatments are uncut and untreated control, whole-tree harvest control, whole-tree harvest plus addition of ammonium sulfate fertilizer, and whole-tree harvest plus the addition of ammonium sulfate fertilizer and dolomitic lime (Adams *et al*., 2004). The ammonium sulfate fertilization rate is equal to double the ambient rates of throughfall deposition (~15 kg kg N ha^{-1} yr^{-1} and ~17 kg S ha^{-1} yr^{-1}). The plot layout is a random square grid in which each plot is 0.2 ha with a 7.6m wide buffer strip between each plot.

Soil sampling

In June 2015 three 5-cm diameter soil cores were extracted from the four replicate whole-tree harvest control and whole-tree harvest ammonium sulfate treatment plots to a depth of 15 cm. These treatments were chosen for sampling as they are most synonymous with the reference and N fertilized watersheds at the Fernow. All samples were stored on ice and returned to West Virginia University for further processing. Within 24 hours of sampling, mineral soil samples were separated into bulk and rhizosphere fractions. Rhizosphere soil was operationally defined as soil that adhered to roots upon removal from the soil matrix, and was carefully subsampled using forceps and homogenized (*sensu* Phillips & Fahey, 2005). The remaining soil was considered

mineral bulk soil. After root and rhizosphere separation, soil was homogenized by sieving through a 2-mm mesh. A subsample of each soil sample and fraction was stored at -80° C for assays of enzyme activity. All fine roots (< 2 mm) were washed in deionized water and subsequently dried and weighed to determine standing root biomass. Subsamples of roots from each plot from the final sampling date were used to examine AM colonization and root morphology.

Extracellular enzyme activity

We assayed for activities of liginolytic oxidative enzymes, phenol oxidase and peroxidase, using a colorimetric microplate assay based on the hydrolysis of L-3,4-dihydroxyphenylalanine linked substrates (Saiya-Cork *et al*., 2002). These assays were performed under saturated substrate conditions wherein the concentration of enzyme limits activity.

Supplementary Figures:

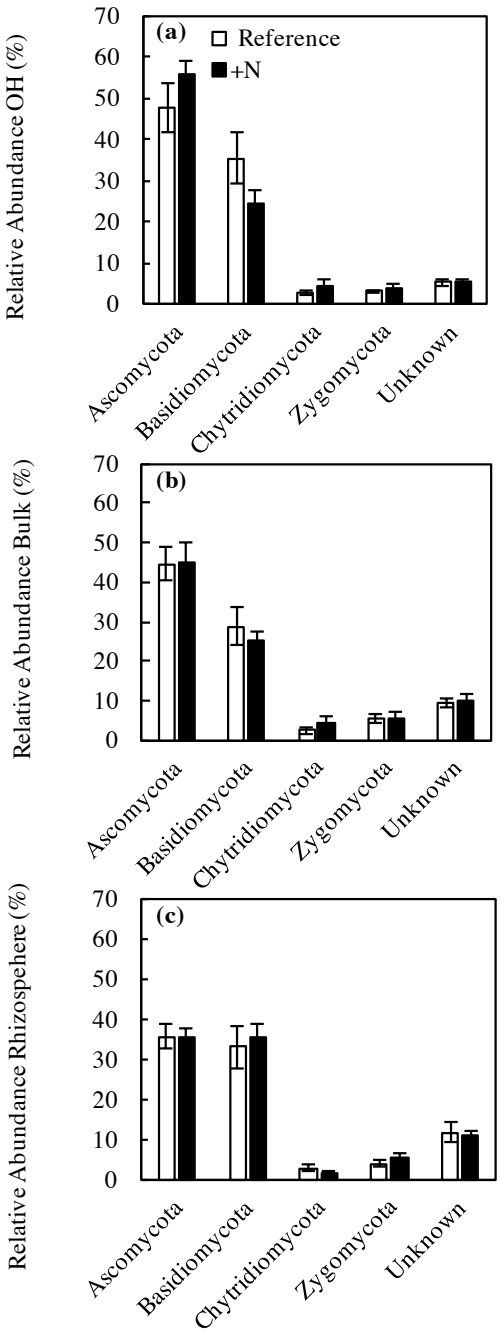

Figure S1. N fertilization does not alter the relative abundance of the 4 most common fungal phyla and unclassified phyla in OH, bulk, and rhizosphere soil fractions. Data presented are the watershed-level relative abundances (mean ±SE) for each soil fraction (a) organic horizon (b) bulk (c) rhizosphere soil across all plots (n=10 plots per watershed). There were no significant differences in phyla abundance between watersheds in any soil fraction. Data presented were measured in July 2015. AM fungi are included in the phylum *Glomeromycota* which for which relative abundance did not vary between watersheds. ECM fungi are represented in the phyla *Basidiomycota, Ascomycota, and Zygomycota*.

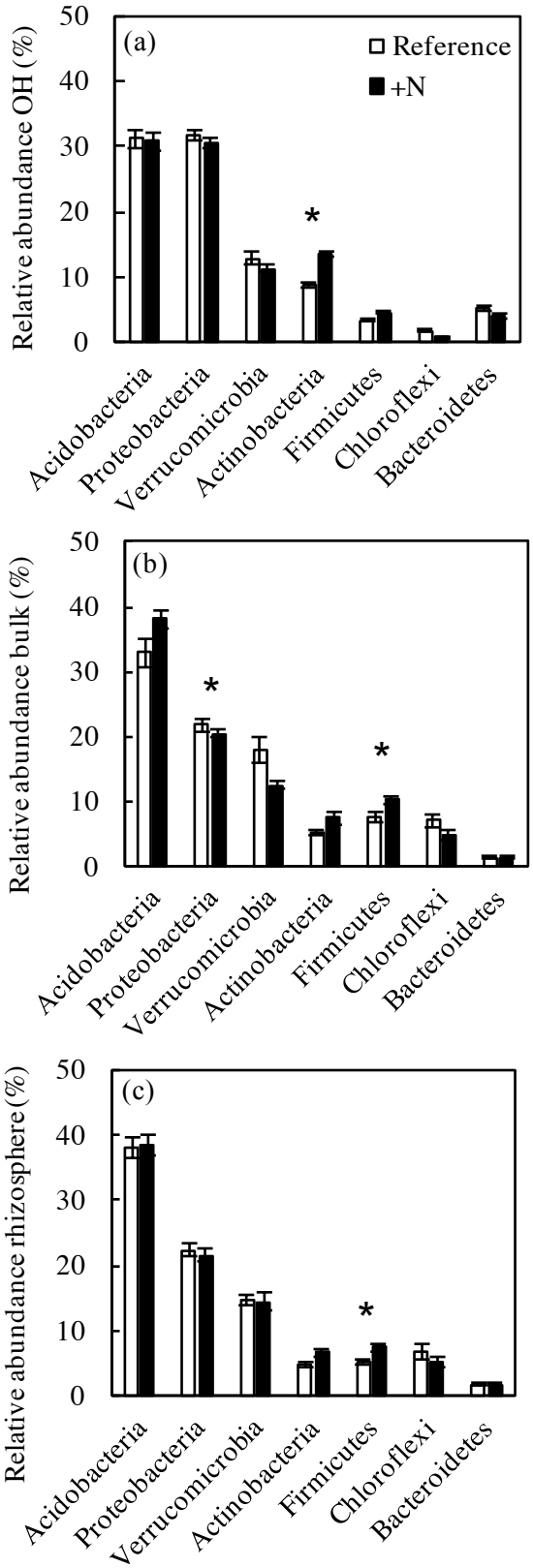

Figure S2. Response of dominant bacterial phyla to N fertilization. Data presented are the watershed-level relative abundances (mean ±SE) for each soil fraction (a) organic horizon (b) bulk (c) rhizosphere soil across all plots (n=10 plots per watershed). Asterisks indicate significant differences between treatments ($p<0.05$). Data presented were measured in July 2015.

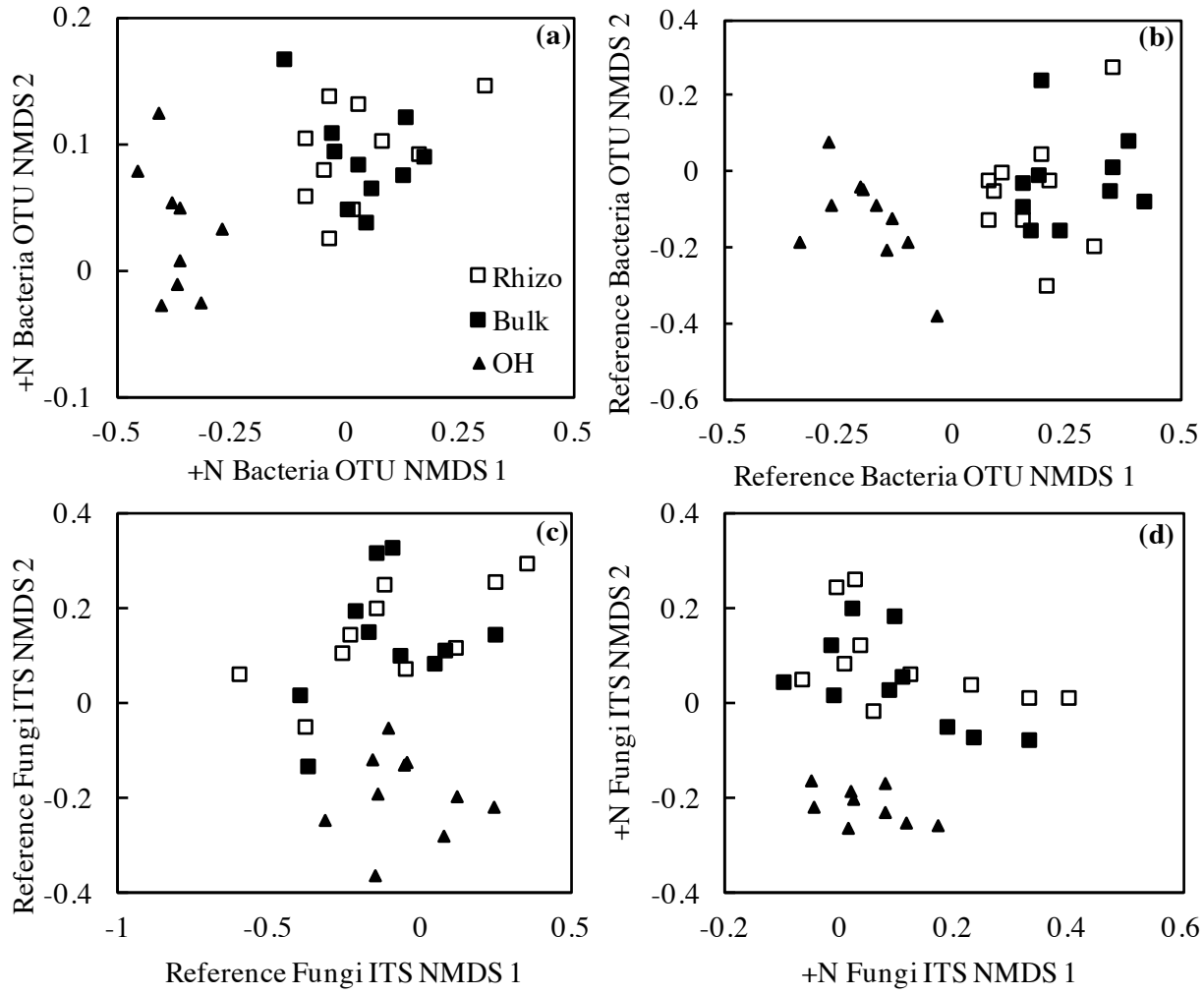

Figure S3. Bacterial and fungal community composition did not vary between bulk and rhizosphere soil within either the reference or N fertilized watershed. OH horizon varied from bulk and rhizosphere communities for fungi and bacteria in both watersheds. Data presented were gathered in July 2015 (n=10 plots per watershed x 3 soil horizons).

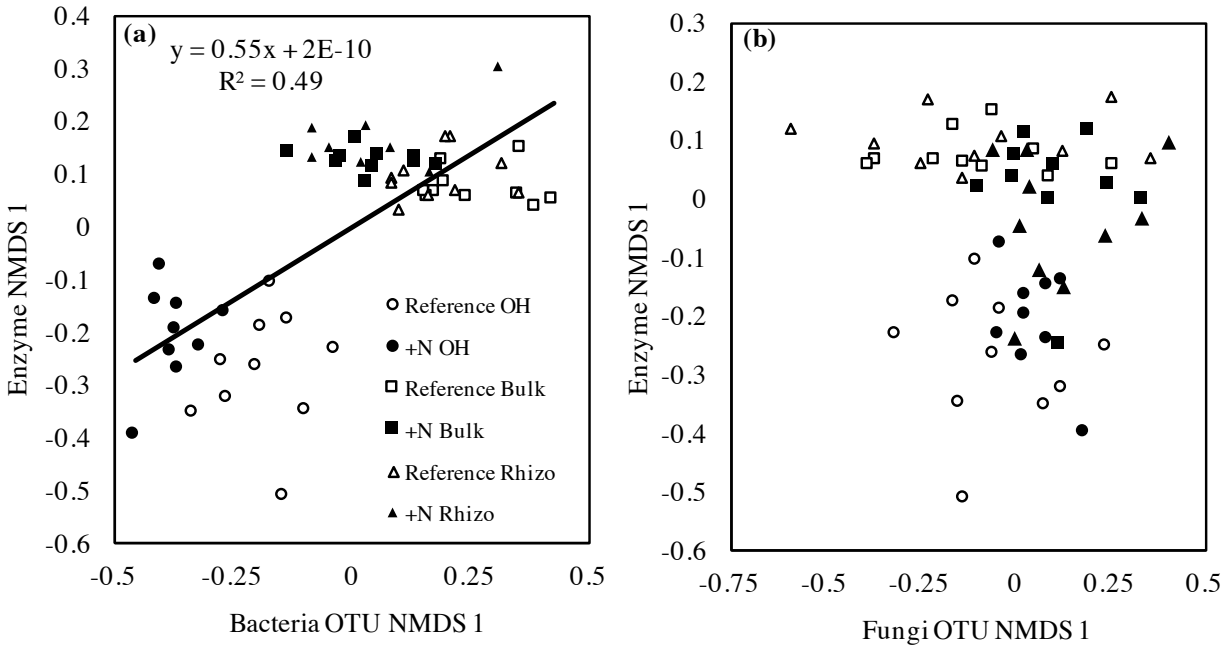

Figure S4. Bacterial community composition is significantly correlated with the first NMDS axis of enzyme activity across OH, bulk, and rhizosphere soil fractions (a). There is no relationship between fungal community composition and enzyme activity. Data presented are from microbial community analysis of each soil horizon between both watersheds (n=10 plots per watershed x 3 soil horizons).

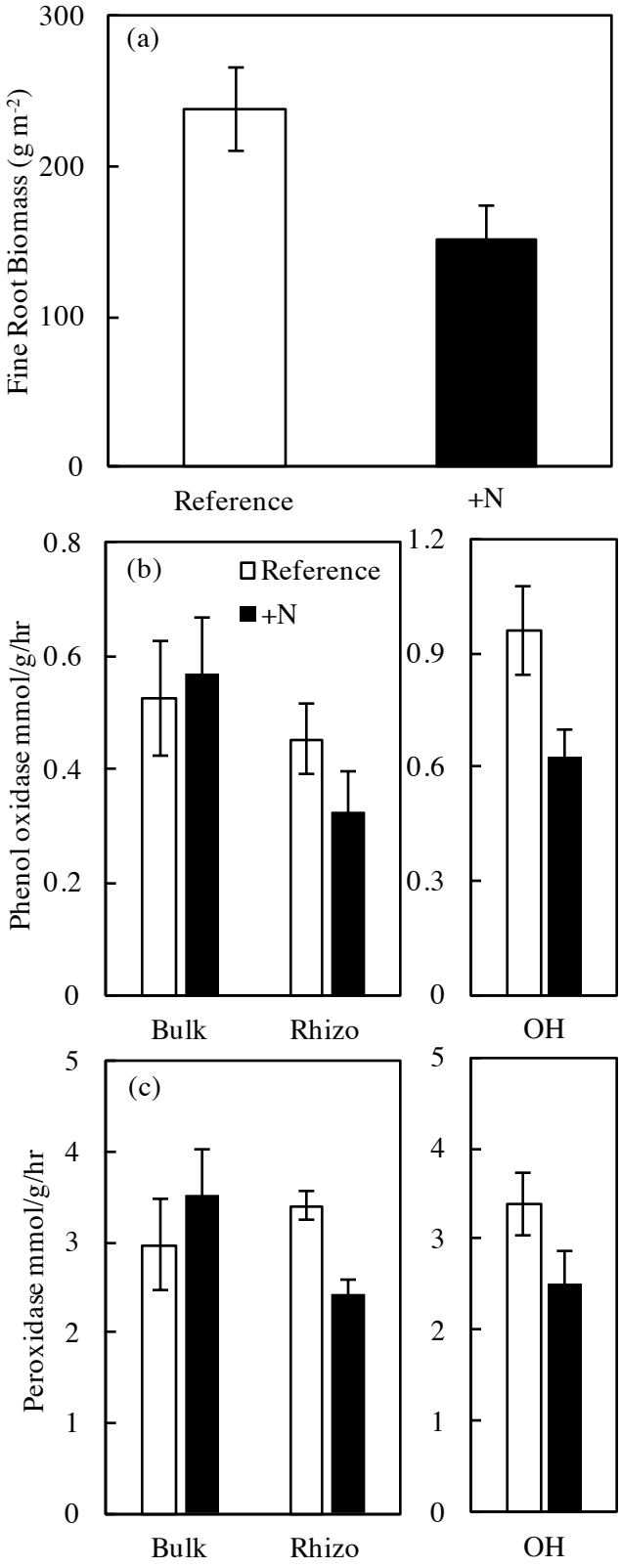

Figure S5. Fine root biomass (a), phenol oxidase (b), and peroxidase (c) responses to N-fertilization at the replicated Long-Term Soil Productivity experiment near the Fernow are similar to the watershed scale pseudo-replicated study. Data presented were gathered in June 2015 (n=6 replicate plots per treatment).