Fertilization on Ectomycorrhizal Community Composition and Enzymatic Activity

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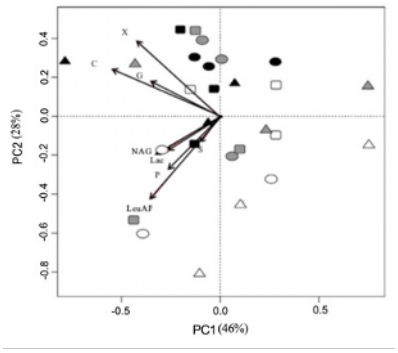
Results

For characterization of ectomycorrhizas, soil samples were thawed, soaked in tap water, gently shake, and then washed over a 2 mm sieve. Mineral and forest floor subsamples were combined. Forty independent ECM root tips were randomly selected per soil sample. In cases where fewer than 40 root tips were available, all root tips were characterized. Each tip or system was examined in detail under dissecting and compound microscopes and described according to Agerer (1987-200) and Goodman et al. (1996). Two of the most turgid and light-coloured tips from each morphotype were frozen at -80 degrees Celsius (Lee et al., 2007) for molecular analyses.

Overall, 38 fungal OUTs were identified in this study, with considerable amount of overlap in the taxa of ECM fungi detected among sites and among treatments at each site. At Crow Creek, 23 ECM fungal OTUs were observed; 12 of these were found in plots of all three treatments. At Hand Lake, 23 ECM fungal OUT's were observed; 16 of these were observed across all three treatments. At Lodi Lake 18 ECM fungal OTUs were detected, with 7 of these found in all three treatments. A total of 15 OTUs were common to all three sites. As is common in studies of ECM roots, sample-based rarefaction curves of ECM fungal OTUs for each treatment at each site did not reach asymptotes (Fig. 1.4), indicating that sampling did not detect all fungal OTUs present at each treatment for each site. Some rare taxa would have been missed during sampling.

Figure 2: Principal components analysis of extracellular mcyorrhizoplane enzyme activities from root tips harvested from the forest floor, averaged by plot (three plots per treatment) for three interior spruce stands (ovals – Crow Creek, rectangles – Hand Lake, triangles – Lodi Lake, fertilized annually (black symbols), every six years (gray symbols) or left unfertilized (open symbols).



There was an indication that the ECM fungal communities found on sprue roots differed among sites (P = 0.055, PERMANOVA), but were not affected by long-term fertilization. No fertilization effects were detected across sites (P = 0.4, PERMANOVA with treatments nested within site), nor for each site considered separately (P > 0.05; Fig. 1.3). Because some soil samples contained fewer than 40 root tips and some from Lodi Lake did not contain any root tips, we calculated both extrapolated and rarefied richness estimators of ECM fungal OTUs per plot. Richness estimators did not differ among treatments at individual sites or

across sites (Table 1.1). likewise, fungal diversity was not affected by fertilization (Table 1.2). Between 99% and 100% of root tips were ectomycorrhizal at all sites.

Six ECM fungal genera dominated (i.e., >5% of ectomycorrhizas identified per plot) samples collected from the spruce stands: Amphinema, Cenococcum, Cortinarius, Lactarius, Piloderma and Tylospora. No significant effect of fertilization was detected on the relative abundance of any of the dominant types of ectomycorrhizas across sites (Fig. 1). Furthermore, no relationship was apparent between the total amount of N + P + K applied per plot and the relative abundance of any of the taxa across sites. When sites were considered separately, Lactarius spp. mycorrhizas increased in relative abundance under annual fertilization and Cortinarius mycorrhizas decreased under periodic fertilization at Lodi Lake (Fig. 1). At Hand Lake, Tylospora spp. mycorrhizas, increased in relative abundance with annual fertilization, and there was a marginal negative effect on Cenococcum with periodic fertilization.

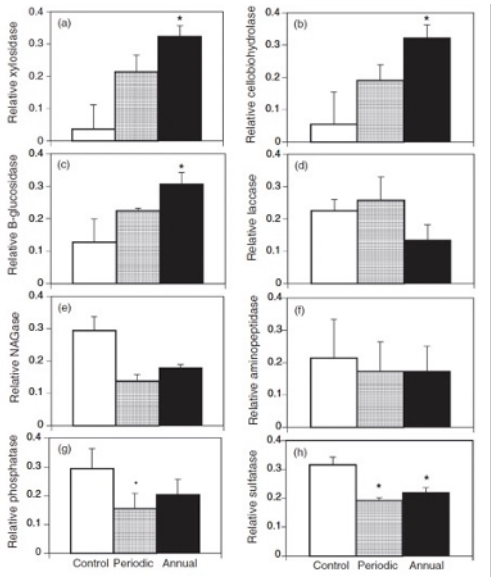
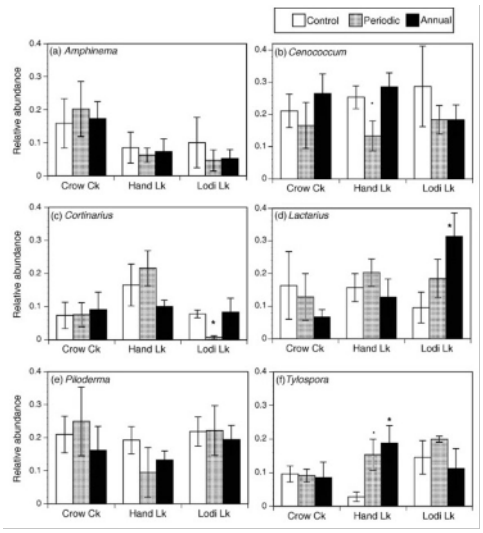


Figure 3: Extracellular mycorrhizoplane activities of (a) xylosidase, (b) cellobiohydrolase, (c) gucosidase, (d) acetylgucosaminidase, (f) leucine aminopeptidase, (g) phosphomonoesterase, and (h) sulfatase from root tips harvested from the forest floor of three interior spruce sites, standardized by dividing the activities per mm per tip by the average for each enzyme for each site, and then averaged by plot.

Across sites, the combination of activities of the eight mycorrhizoplane enzymes was affected by fertilizer application (permutational MANOVA, P = 0.049). The enzyme profiles of annually fertilized plots tended to be located on the negative side of Axis PC1 of the Principal Components Analysis, which explained 46% of the variation, and at the positive end of Axis PC2, which explained 28% of the variation (Fig. 2). By contrast, activities of the remaining enzymes were negatively correlated with both axes (Fig. 2). Consequently, indices of potential C:N, C:P, and C:S acquisition activities from soil were higher in fertilized plots (Table 1).



Application of a balanced fertilizer every six years or annually, had no detectable effect on overall ECM fungal community structure at three interior spruce forests. A few dominant genera decreased or increased in relative abundance with fertilization at single sites, but no consistent positive or negative effect on a genus was observed across sites. Furthermore, our prediction of reduced fungal richness and diversity was not seen. By contrast, fertilization affected mycorrhiza-associated exoenzymes as expected: a general increase in plant cell-wall degrading enzymes and a decrease in some of the enzymes that release soluble mineral nutrients.