

Title Crown closure affects endophytic leaf mycobiome compositional dynamics over time in *Pseudotsuga menziesii* var. *menziesii*

Topic Summary

Tree canopies are home to communities of fungi that grow into the tree needle tissue without causing disease. These fungal communities can modulate nutrient uptake, stomatal conductance, and foliar disease severity. Research at the landscape and regional scales has shown that microclimate can impact variation in fungal community composition. The authors set out to determine how fungal community composition and diversity varied vertically in eight trees canopies using LiDAR to describe canopy closure, a measure of exposure level.

Where was the most variation in composition of the mycobiome observed?

- The greatest amount of mycobiome compositional variation was explained by the source tree. Genetic analysis of samples revealed 218 operational taxonomic units (OTUs). Of these, 3% were found on every tree and 3.67% (8 OTUs) had average relative abundance >1%.

How was the variation explained?

- Crown closure, the amount of cover provided by the tree's foliage above the sample height, was the only factor that explained unique components of variation. Therefore, it was used to categorize sample sites as exposed or unexposed.

What were the differences between exposed and unexposed sites?

- For exposed sites, OTU.1 (Mycosphaerellales, Dothideomycete) was the indicator taxon. For unexposed sites, OTU.2 and OTU.3 (Helotiales, Leotiomycetes) were indicator taxa. More taxa were associated with unexposed sites, which have greater crown closure, than with exposed sites.
- OTU diversity and richness increased with increasing crown closure. Richness also increased with increasing needle age class.

Did needle age impact the foliar fungal community?

- Crown closure affected fungal endophyte composition at all needle age classes, but was most prominent at classes A2 and A3.
- Needle age class was related to mycobiome composition, with older age classes having different community composition than younger age classes.
- Variation in community composition by needle age class differed between exposed and unexposed crown closure categories. In unexposed sites there was some retention of community composition between A3 and A4. Community composition retention was not found at any age class transition in exposed sites.

What further research would assist in future analysis of tree-canopy fungal communities

- In order to more readily compare studies of foliar fungi community composition in tree canopies, investigations should include measures of exposure, such as canopy closure, in addition to height and position in the canopy.
- The relationship between metrics of crown microclimate and within-crown LiDAR should be more fully investigated and described before this approach is widely-used.

Citation Gervers, K.A., Thomas, D.C., Roy, B.A., Spatafora, J.W., and Busby, P.E. (2022). Crown closure affects endophytic leaf mycobiome compositional dynamics over time in *Pseudotsuga menziesii* var. *menziesii*. *Fungal ecology* 57-58, 101155. <https://doi.org/10.1016/j.funeco.2022.101155>.

Keywords *Nothophaeocryptopus*, *Rhabdocline*, fungi, endosphere, old-growth, community ecology, ITS, metabarcoding, airborne LiDAR

Images

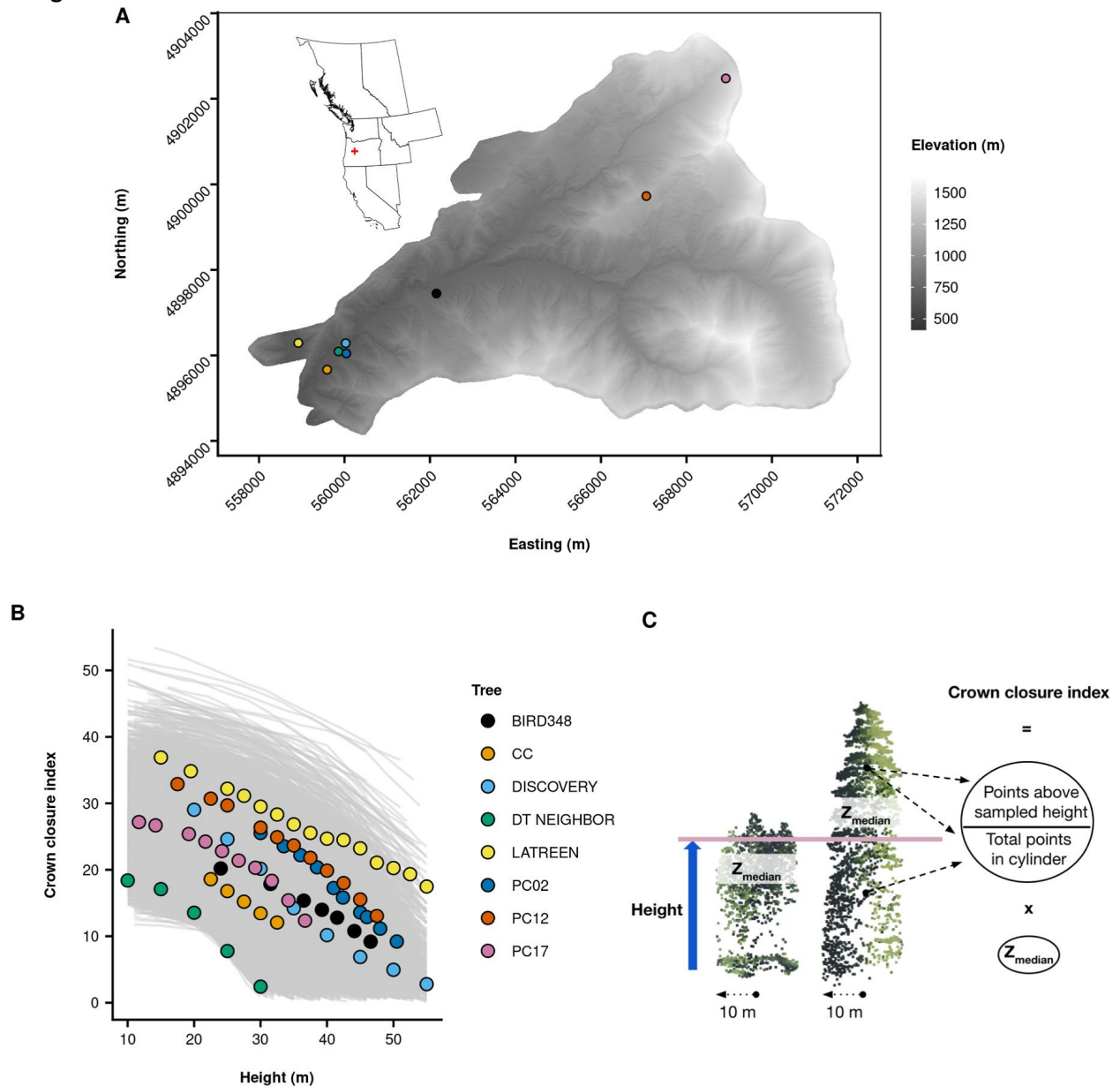


Fig. 1 from paper. Locations of the H.J. Andrews Experimental Forest (HJA) and the climbed and sampled trees (A). The inset and the red cross indicate the center position of HJA within the North American Pacific Northwest. The positions of sampled trees have been jittered (± 150 m) to avoid overlap. The map is based on a digital terrain model from Spies, 2016. Relationship between height in crown and crown closure (B). Grey lines show data from point clouds associated with 5828 trees randomly sampled across HJA (each with randomly sampled crown heights), while colored lines and points represent point clouds associated with trees that were climbed and sampled as part of this study. Point cloud cylinders are centered on the treetop of a focal tree, but include all point returns within a 10 m radius (C). Although the same vertical height can be sampled in two different trees, these heights can be associated with different crown closure indices.

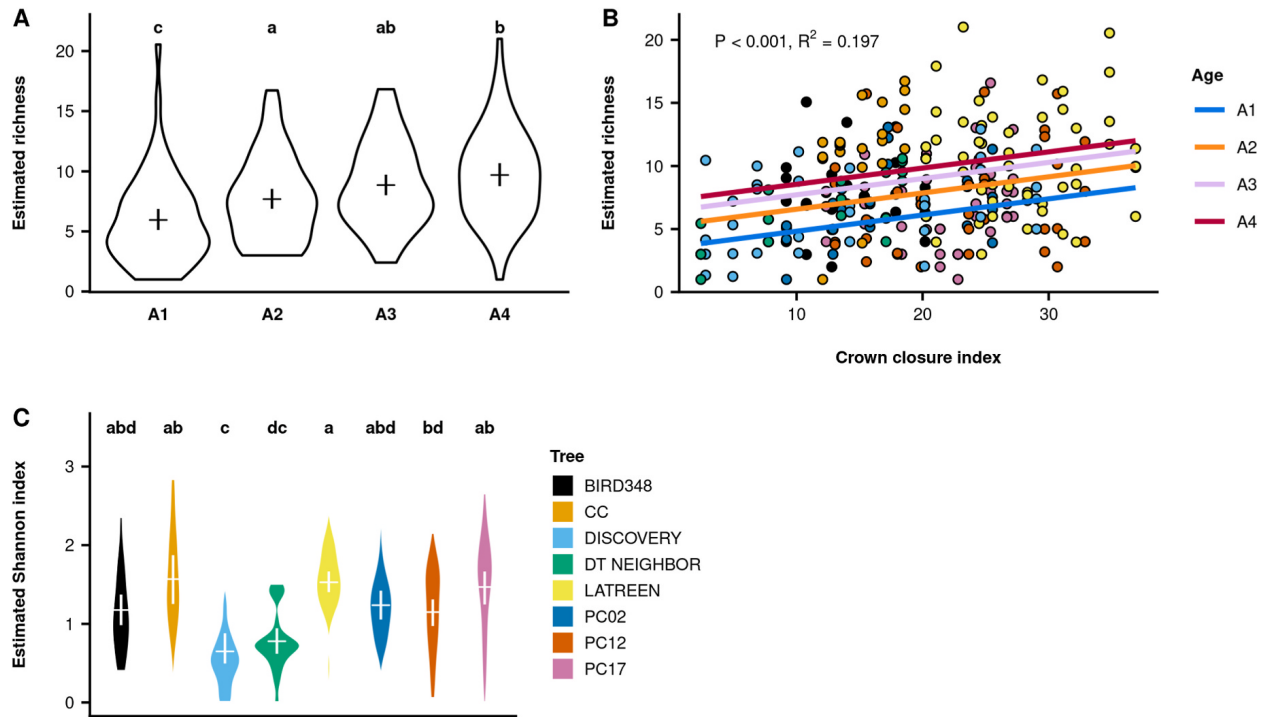


Fig. 4 from paper. Estimates of OTU richness or the Shannon index of diversity associated with needle age (A), crown closure index (B), and tree (C). Estimates were obtained for each sample via interpolation or extrapolation to a depth of 1000 reads. Violin plots illustrate the distribution of richness and Shannon index diversity estimates for each grouping. Letters for (A) and (C) indicate significant difference groups from Tukey HSD *post-hoc* comparisons (alpha = 0.05) among levels of each factor after performing ANOVA F-tests. Horizontal lines delineate the bootstrapped means of estimates, while vertical lines correspond to bootstrapped 95% confidence intervals (n = 999).

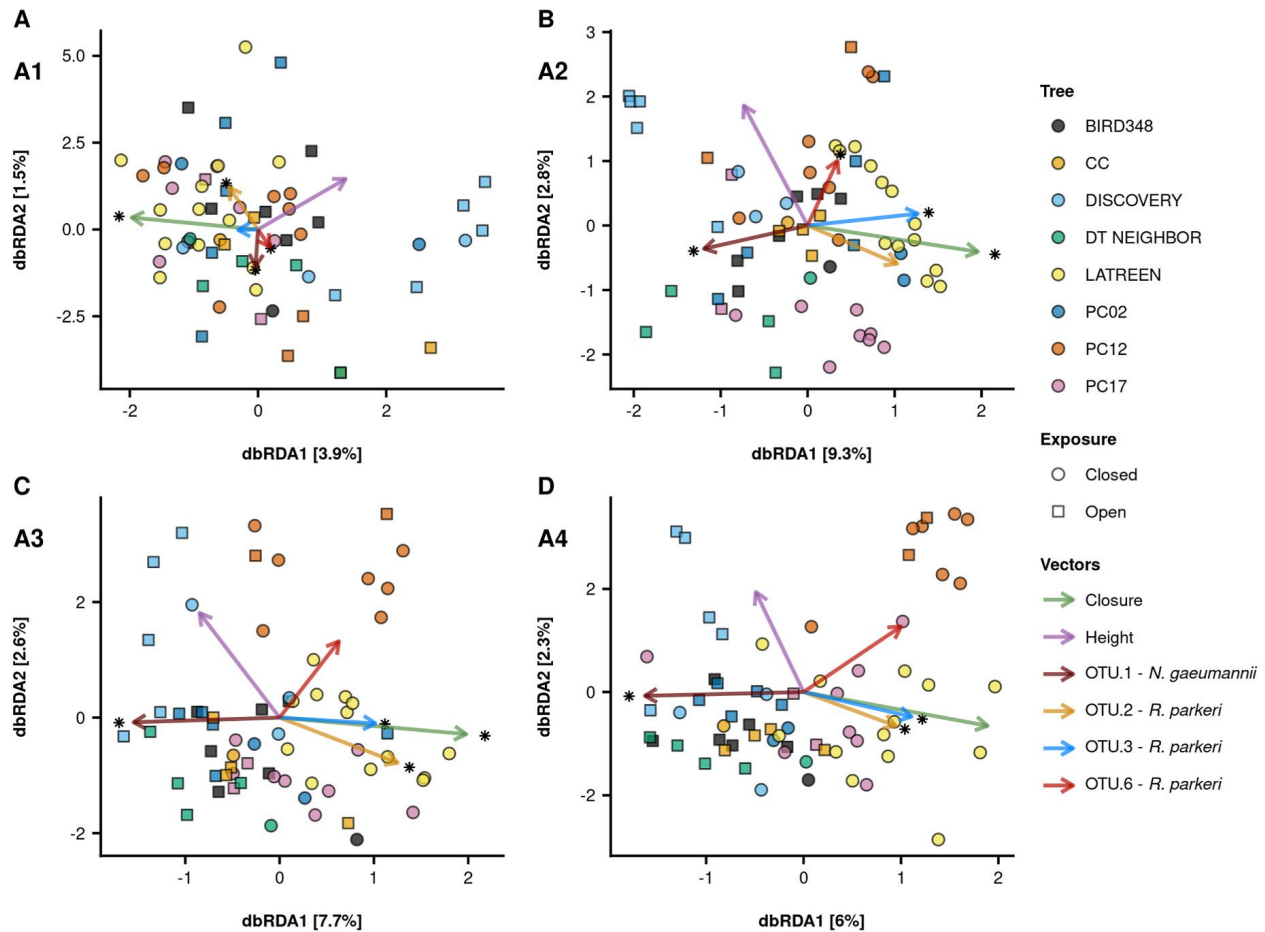


Fig. 3 from paper. Distance-based redundancy analyses (dbRDA) of Bray-Curtis dissimilarities for each needle age subset, constrained on height and crown closure. OTU vectors demonstrate correlations between ordination axes and untransformed OTU relative abundances, as assessed with the *envfit* function from the *vegan* R package, while crown variable vectors are extracted directly from the dbRDA biplot scores. Asterisks are associated with significant vectors ($\alpha = 0.05$), longer vectors signify stronger correlations, but vector length should only be compared within crown variable and OTU vectors. Vector significance was assessed with 999 permutations, treating “tree” as a permutation stratum; tests involving crown variables were marginal pseudo-F tests.

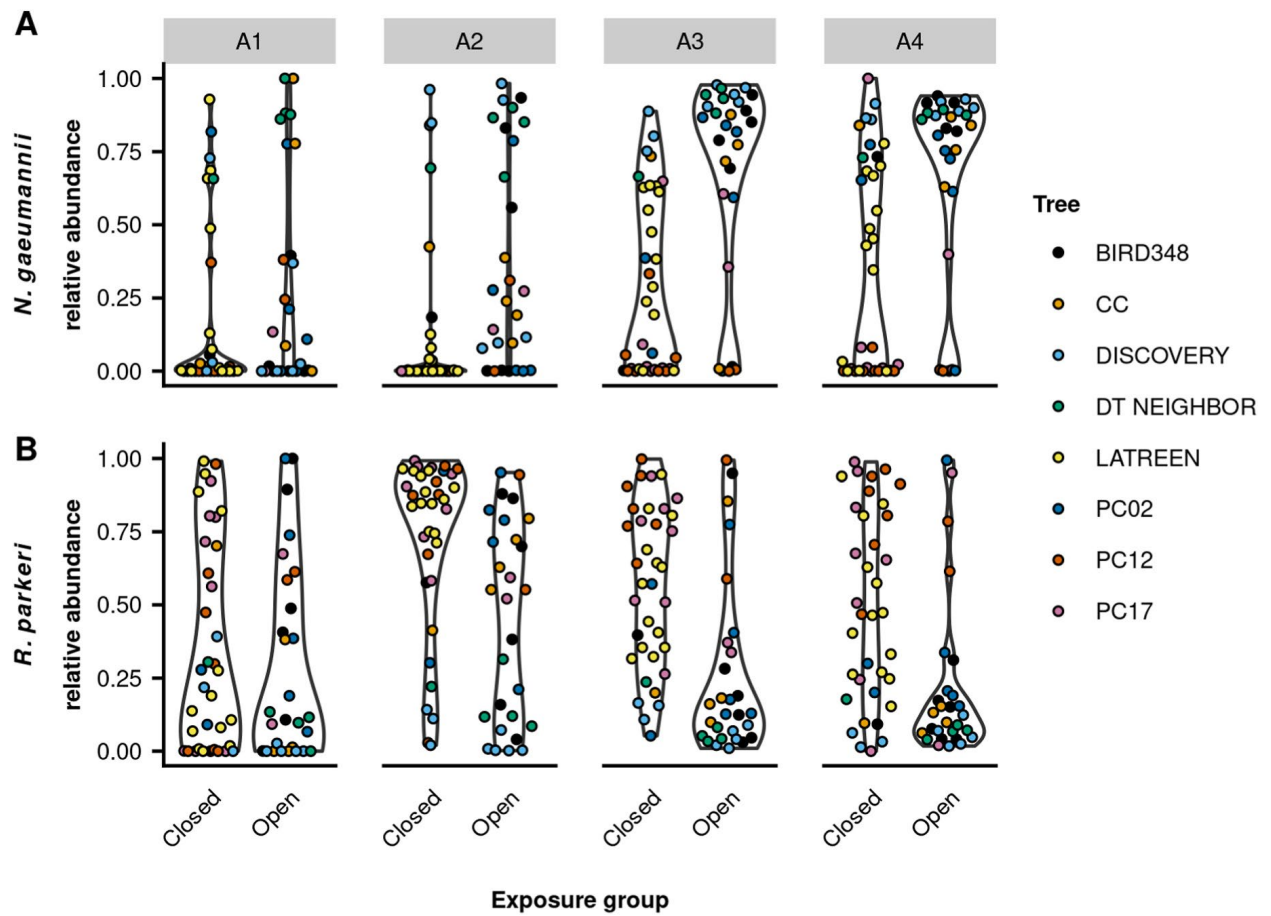


Fig. 6 from paper. Relative abundances of OTU.1 (identified to *Nothophaeocryptopus gaeumannii*) (A) and OTU.2, OTU.3, and OTU.6 (identified to *Rhabdocline parkeri*) (B) in each exposure group and needle age class.