

SOIL FUNGAL RESPONSE INTERACTIONS WITH SEEDLING STOCK AGE ACROSS WHITE OAK (*QUERCUS ALBA*) OUTPLANTINGS IN KENTUCKY, TENNESSEE, AND ALABAMA, USA

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PURPOSE AND SCOPE

Integration of soil fungal communities into white oak artificial regeneration studies can provide insight into growth success (Brown et al. 2022). Yet, little work has been conducted to understand the interrelationships between soil fungi, white oak outplanting success, and how this might be influenced by planting location and the age of seedling stock. The goal of this study was to investigate how white oak planting practices influence soil fungal dynamics. We implemented white oak outplanting experiments across three national forest sites (Daniel Boone National Forest [DBNF], KY; Cherokee National Forest [CNF], TN, and Bankhead National Forest [BNF], AL) in the Southeastern United States. These experiments included multiple white oak genetic families (half-siblings) that consisted of different seedling ages (1-0 and 2-0) but were planted concurrently.

METHODS AND APPROACH

As part of a larger study (planted February 2023), six trees from each genotype and seedling age were selected from three blocks in CNF and BNF and six blocks in DBNF and root associated soils (5-cm depth) were collected twice during the first growing season (April and July 2023). Soil genomic DNA was extracted and metabarcoding of the fungal ITS2 region of the rDNA operon was conducted and sequenced using Illumina MiSeq. Sequences were processed the program mothur (Schloss et al. 2009). We estimated relative operational taxonomic unit (OTU) richness and Shannon's diversity (2,000 sequences per sample for 1,000 iterations). We tested if richness and diversity were impacted by outplanting locations and seedling stock age by using a two-way analysis of variance (ANOVA) separately for each of the sampling events (April and July). We used

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permutational multivariate ANOVA (PerMANOVA) to test if fungal community structure (Jaccard similarity index) differed between location, stock age, and their interaction.

FINDINGS AND IMPLICATIONS

We found that fungal OTU richness and diversity was significantly different in our model for April sampling ($F_{5,187} = 5.65, P < 0.001$ and $F_{5,187} = 3.77, P = 0.003$, respectively; table 1) with location being the only significant factor (stock age and stock age x location being nonsignificant). Bankhead National Forest samples had on average 20.3 and 33.5 percent more OTUs than DBNF and CNF, respectively. For July sampling, approximately 6 months after planting, fungal OTU richness and diversity responded to our model ($F_{5,264} = 2.38, P = 0.041$ and $F_{5,264} = 5.81, P < 0.001$, respectively). Seedling stock age was the only significant factor for richness with 1-0 seedling having on average 18.5 percent more OTUs than 2-0 seedlings. Diversity, stock age, location, and stock age x location interaction all differed in our model. Further, our PerMANOVA test indicated that fungal community structure differed across locations ($F_{2,268} = 12.617, P < 0.001$), and by seedling stock age ($F_{1,268} = 12.617, P = 0.011$), but not by the location x stock age interaction ($F_{2,268} = 1.093, P = 0.161$).

Table 1—Mean $\pm$ standard error values for fungal operational taxonomic unit richness and Shannon’s diversity (H') estimates of white oak-associated soil fungi

Model factor		Sampling	Richness	Diversity (H')
Location	Bankhead National Forest, AL	April	137.91 $\pm$ 4.46A	3.19 $\pm$ 0.05A
	Cherokee National Forest, TN	April	102.81 $\pm$ 6.12B	2.85 $\pm$ 0.07B
	Daniel Boone National Forest, KY	April	114.26 $\pm$ 3.88B	3.01 $\pm$ 0.04B
Seedling stock type	1-0	April	122.34 $\pm$ 3.91	3.08 $\pm$ 0.05
	2-0	April	118.47 $\pm$ 4.11	3.01 $\pm$ 0.05
Location	Bankhead National Forest, AL	July	135.94 $\pm$ 5.11	3.27 $\pm$ 0.06A
	Cherokee National Forest, TN	July	133.81 $\pm$ 6.12	3.02 $\pm$ 0.07B
	Daniel Boone National Forest, KY	July	135.94 $\pm$ 7.27	2.99 $\pm$ 0.08B
Seedling stock type	1-0	July	143.76 $\pm$ 6.06A	3.27 $\pm$ 0.07A
	2-0	July	126.49 $\pm$ 6.50B	3.07 $\pm$ 0.08B

Data are from 2023 outplantings.

Different letters represent significant differences for main factors according to Tukey HSD post hoc results; interaction terms not shown.

These findings suggest that white oak outplanting management strategies directly influence plant-associated soil fungal dynamics. This and previous work which showed little soil fungal differences in white oak soils (Brown et al. 2023) suggests that much more work is needed to understand drivers of oak-associated soil fungal dynamics. However, coupled plant management strategies and fungal community analyses can be an additional tool for white oak reforestation success.

LITERATURE CITED

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