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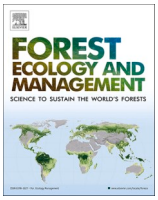

Comparisons of interspecies field performance of Fagaceae (*Castanea* and *Quercus*) planted in the southeastern United States with attention to soil fungal impacts on plant performance

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ABSTRACT

The loss of Fagaceae species is an increasing concern globally, including in North American where American chestnut (*Castanea dentata*) has been virtually eliminated by non-native pathogens, and oaks (*Quercus*) are experiencing widespread regeneration failures and declines. Tree improvement and breeding programs are producing trees for disease resistance or improved performance traits but require field testing to refine efforts. We established a study in 2015 on a xeric pitch pine (*Pinus rigida*) site in the Blue Ridge Mountains of North Carolina to regenerate American chestnut and interspecies hybrids (BC_3F_3) and the co-occurring species of white oak (*Q. alba*) through planting bare-root, quality-graded seedlings. Chinese chestnut (*C. mollissima*) was also tested as a control species. We used pedigreed seed sources from open-pollinated genetic families that were nursery grown (1–0 bareroot seedlings for chestnut, 2–0 bareroot seedlings for white oak) to maximize overall size and competitive ability. Though there was variability within and among plant families in performance, American chestnut and BC_3F_3 hybrids generally outperformed Chinese chestnut (at least 13 % taller) and white oak (at least 29 % taller) for the first three years, but intraspecies differences among genetic families were significant for nearly all traits tested. Initial seedling root morphology poorly explained field performance ($R^2 < 0.17$), but this relationship was significant for both white oak families and the only northern BC_3F_3 seed source. American chestnuts and BC_3F_3 hybrids had higher stem height to ground diameter ratios compared to white oak (at least 11 % greater), indicating that white oak likely concentrates more resources to root development while chestnut concentrates more resources to maintaining above-ground competitive advantages. Additionally, we investigated soil fungal communities, both pre- and post-tree establishment and tested if these fungal communities can be used to predict plant performance or health. Soil fungi did a poor job predicting plant performance. Our results indicate that co-occurring Fagaceae species can be established in restoration plantings using well developed quality seedlings on relatively xeric sites. Managers should use diverse seed sources to avoid planting poor performing families and expect that chestnuts bred for blight resistance will outcompete planted white oak, at least in the short-term.

1. Introduction

Fagaceae, such as oak (*Quercus*) and chestnut (*Castanea*), have had their populations drastically reduced and this reduction is a global concern due to changes in historical disturbance regimes, introductions of non-native pests and pathogens, climate change, and land use changes, among many other factors (Abrams, 2003; Dumroese et al., 2015; Rigling and Prospero, 2018; Thomas et al., 2002). Oak and

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chestnut species provide numerous ecosystem services such as hard mast, increased water yield, and habitat diversity for invertebrates and wildlife as well as serving local and global economic markets (Dey, 2014; Jacobs et al., 2013; Kerr and Evans, 1993; Luppold and Bumgardner, 2019). Notably, impending reductions in white oak (*Q. alba* L.) populations due to long-term regeneration failures (Abrams, 2003) has led to emerging research and interest in white oak sustainability and conservation, which includes cooperative hardwood initiatives and programs, as well as the formation of a Congressional caucus in the United States (Clark et al., 2022; Clark and Dey, 2022; Thomas et al., 2021). American chestnut restoration has a long history involving a multi-agency and multi-disciplinary approaches in direct response to widespread mortality caused by introduced pests (Clark et al., 2014; Diskin et al., 2006; Newhouse and Powell, 2021; Westbrook et al., 2020). Restoration of these two important co-occurring tree species requires active forest management facilitated by an understanding of ecological processes and relationships controlling outplanting success (Dumroese et al., 2015). Management for natural regeneration of white oaks should be the priority, but where this is not feasible, it may also require planting improved and/or locally adapted genetic material into silvicultural stands (Stanturf et al., 2014).

Global trade has facilitated the unintentional importation of pests and pathogens from non-native species that have historically impacted forested ecosystems, leading to ecological instability by altering community composition, canopy cover, understory vegetation, and soil microbial communities (Lovett et al., 2010, 2016). Notably here, Phytophthora root rot disease [causal organism *P. cinnamomi* Rands.] and chestnut blight *Cryphonectria parasitica* (Murrill) Barr [Ascomycota, Sordariomycetes, Cryphonectriaceae] have caused widespread species declines (Anagnostakis, 1995; Griffin and Elkins, 1986). Root rot disease was first noticed in North America in the 1850s, and has an exceptionally wide host range, including most chestnut and many oak species, causing devastating impacts in forestry, agriculture, and in nurseries (Anagnostakis, 1995; Balci et al., 2007; Dalgleish et al., 2016; Milburn and Gravatt, 1932; Westbrook et al., 2019). Root rot effects on oak have gone largely unreported and are poorly studied, but have contributed to major declines in Europe and North America (Haavik et al., 2015; Nagle et al., 2010).

Currently, the most effective long-term solution to these pathogens are breeding programs for disease resistance or reintroduction of plants into areas unfavorable for infection. For blight disease, Asian chestnut species [predominately Chinese chestnut (*C. mollissima* Blume)] serve as sources for disease resistance in backcross breeding programs whose goals are to develop genetically diverse populations that are phenotypically similar to the native American chestnut but incorporate Asian chestnut blight resistance traits (Anagnostakis, 2012; Burnham et al., 1986). The most advanced progeny currently available for testing is the third backcross generation (BC_3F_3) with intermediate resistance levels in 8-year old forest restoration field trials (Clark et al., 2019), although its parents exhibited relatively low blight resistance in orchard tests (Steiner et al., 2017).

Artificial regeneration through planting is a potential solution to combat species extirpations or population declines, but many knowledge gaps remain, particularly for North American Fagaceae. The majority of artificial regeneration research on oak species has been conducted with northern red oak (*Q. rubra* L.) (Dey et al., 2008), whereas research on white oak, particularly from pedigreed sources, is relatively sparse (*but see* Granger and Buckley, 2021; Kormanik et al., 2002; Weigel and Johnson, 1998). The information gap on natural regeneration methods for white oak is smaller (e.g., Schweitzer et al., 2019), but planted seedlings behave differently than those naturally regenerating (Clark et al., in review). Reintroduction trials of chestnut backcross hybrids are relatively new as they develop concurrent with availability of breeding lines to test for blight resistance (Hebard, 2001). Early results show divergence in survival, physiology, and growth related to breeding, genetics, and management (Bauman et al., 2014; Clark et al., 2016; Knapp et al., 2014; Skousen et al., 2018). Planting success of many tree species has been linked to nursery stock morphology, including root system size or structure (Dumroese et al., 2016), as has been shown specifically for white oak (Dey et al., 2008; Granger and Buckley, 2021; Kormanik et al., 2002) and American chestnut (Clark et al., 2016) in forest reintroduction trials. However, more research is needed as outcomes can differ based on local nursery soil microbial communities and planting site abiotic variables (Iverson et al., 2008) and soil pathogens (Coughlin et al., 2021). How these factors interact with host genetic material remains understudied. The role of soil microbial communities in establishment and performance of Fagaceae remains poorly studied as well. Soil microbial communities are key drivers of plant health (Trivedi et al., 2020) and there are often strong soil microbe by plant genetic interactions that affect plant performance (Brown et al., 2020; Busby et al., 2017). Emerging evidence suggests that endophytic fungi may play a particularly strong role in plant performance by modifying physiological factors such as nutrients, photosynthesis, and stress (Busby et al., 2022; Sarkar et al., 2019), even beyond the better studied mutualistic interactions of mycorrhizae. Despite the potential importance of soil fungi in mitigating plant health, there are limited field studies evaluating soil fungal communities associated with Fagaceae species, particularly for backcross hybrid chestnuts. In one study, healthy 7-year-old native American chestnut saplings had greater root biomass and ectomycorrhizal colonization rates than diseased trees (Bauman et al., 2018), even though the diseased and healthy trees had similar mycorrhizal communities overall.

Thus far, most chestnut research and programmatic efforts have focused on development of a blight resistant tree (Anagnostakis, 2012; Clark et al., 2014), whereas limited work has examined the performance of hybrids in restoration plantings (*but see* Bauman et al., 2018; Clark et al., 2016; Schaberg et al., 2022 as examples). To inform reforestation efforts, it is imperative that survival, growth, and ecological factors, not just disease resistance, are evaluated. White oak is a relatively slow-growing tree (Rogers, 1990), and as such, may recruit fewer saplings because of competition by understory shrubs (Walters et al., 2020) or for light (Dyderski and Jagodziński, 2018). However, this competition may be less important for mature late-successional trees (De Lombaerde et al., 2021), such as white oak. In contrast, American chestnut is often considered an extremely fast-growing tree but can also persist in shaded conditions for decades (Wang et al., 2013).

In this study, we investigate the divergences and similarities of field performance and soil fungal community dynamics among Fagaceae species American chestnut, interspecies hybrids (with Chinese chestnut included as a control), and white oak. Our research objectives align with forest management objectives on many public and private forest lands where restoration of Fagaceae species are desired. On the National Forests of North Carolina in particular, where this study takes place, restoration goals include promoting young forests, providing trees with exfoliating bark and crevices like white oak for bats (Order Chiroptera), and maintaining ecozones that include white oak as an important component (USDA USFS Nantahala and Pisgah National Forests Land Management Plan, 2022). We address important questions that affect restoration success: (1) do different chestnut hybrid lines or plant species exhibit differential growth and survivorship when outplanted onto a xeric site in the southeastern Blue Ridge Mountain region of the United States, (2) does nursery seedling morphology at the time of planting predict outplanting success, and (3) do soil fungal communities predict tree performances and if so, are initial soils or soils after plant establishment a better predictor of success?

2. Materials and methods

2.1. Planting site

The study site is located in Macon County, North Carolina, United States, on the Nantahala Ranger District of the Nantahala National

Forest (35.01° N; 83.35° W) and has been managed by the United States Department of Agriculture (USDA) Forest Service (FS). The planting site is located on a southwest (225°) facing slope (slopes average 20–30 % from horizontal) at approximately 850 m elevation above sea level. Soils are well drained, formed from residuum weathered from granite and gneiss, composed mostly of gravelly fine sandy loam, and are dominated by Edneyville-Chestnut and Evard-Cowee soil complexes. The site was dominated by pitch pine (*Pinus rigida* Mill.) in the overstory and mountain laurel (*Kalmia latifolia* L.) in the understory. White oak and scarlet oak (*Q. coccinea* L.) were the most common oak species prior to the harvest treatment. Site index for white oak was estimated to be 65 (base age 50) (Soil Survey Staff, 2022). The stand was harvested and prescribe-burned in October 2014 just prior to planting in March 2015. The post-harvest stand had no residual trees above DBH resulting in 0 m^2 per ha of basal area at the time of planting.

2.2. Experimental plant material

The experimental material consisted of seedlings grown from nuts of American chestnut (*Castanea dentata*), Chinese chestnut (*C. mollissima*), and various backcross hybrids or acorns of white oak (*Quercus alba*). Each nut/acorn was collected from open-pollinated orchard or wild trees (Table A1). Chestnuts were obtained from The American Chestnut Foundation (TACF) and the Connecticut Agricultural Experimental Station (CAES). White oak acorns were obtained from The University of Tennessee's Tree Improvement Program from two wild tree collections located in the Ridge and Valley province of east Tennessee. The Chinese chestnut was located on private property with limited pollen contamination (Paul Sisco, TACF, Asheville, NC, USA, personal communication) (Burnham et al., 1986). We will hereafter use 'species' to refer to American chestnut, Chinese chestnut, backcross chestnut hybrids, or white oak and 'families' to indicate different plant genetic backgrounds.

The backcross hybrids, hereafter 'BC_3F_3 hybrids' are theoretically 94 % *C. dentata* and 6 % C. *mollissima*, (Hebard, 2006), and were provided by TACF from their orchards in Meadowview, VA: families D22 (orchard identifier D-3–28-57), W3 (orchard identifier W-6–31-33), W4 (orchard identifier W-6–22-97, W5 (orchard identifier W-3–32-49), and W6 (orchard identifier W-1–31-60). The CAES hybrid (orchard identifier 4–75) is theoretically 90 % American chestnut with remaining 10 % a mix of Chinese chestnut, European chestnut (*C. sativa*), and Japanese chestnut (*C. crenata*) and was located in the CAES chestnut orchard in New Haven, CT (Anagnostakis, 2012). The seedlings were grown from nuts or acorns from each collection tree and were putative half-siblings (known mother, unknown father), hereafter referred to as 'family' (Table A1).

The chestnut seedlings were grown at the Indiana State Nursery in Vallonia, IN as 1–0 seedlings (grown one year in nursery seedbeds) as previously described (Reazin et al., 2019), and white oak seedlings were grown at the Tennessee State Nursery in Delano, TN as 2–0 seedlings (grown two years in nursery seedbeds). Family seed lots were sown at a density of 65 nuts per m^2 and separated by 0.5 m of unsown nursery bed space. After sowing, the beds were left uncovered and unmulched, as per standard nursery procedures, and arrival of fungal inoculum was not controlled. The seedlings from both nurseries were irrigated as needed and fertilized according to prescriptions to produce large, high-quality seedlings through continuous application of fertilizer during the 2014 growing season (cf. Kormanik et al., 1994). In February 2015, machine lifters were used to undercut seedlings' roots (25–30 cm depth) and loosen soil around the roots. At the Indiana nursery, chestnut seedlings were manually removed from the nursery beds, their roots were packed in sphagnum moss as per the nursery standard operating protocol to minimize seedling desiccation during transport, and seedlings were packed in poly-coated paper tree bags in cold storage until processed for planting. While we did not control for fungal inoculum in the sphagnum moss, we expect it to be uniform across the material, exposure short in duration, and an unlikely factor to explain divergence among the analyzed genetic families after planting at the field site. At the Tennessee nursery, white oak seedlings were manually removed from the nursery beds, roots were sprayed with a hydrogel slurry solution to prevent desiccation, and trees were packed in poly-coated paper tree bags in cold storage until processed for planting. After lifting, seedlings were visually graded based on overall seedling size and root system morphology to remove the smallest seedlings that would not be competitive after planting (Clark et al., 2000). Seedling size variability was relatively large within each family, which is typical for Fagaceae bareroot nursery seedlings (Clark et al., 2012, 2000).

2.3. Experimental design

Trees were planted on a 3.7 m spacing using KBC bars modified to increase bar width to 15 cm to accommodate larger seedlings. We planted 225 chestnut seedlings and 222 white oak seedlings (Table A1). We used stem volume (see Data Collection section) to distribute individual seedlings of a family within three equally sized but topographically distinct areas of the planting designated based on slope position (top slope, mid slope, and bottom slope). A total of 149 trees were planted in each area with a varying number of replications and replication sizes (see below). This was to ensure that size variability within a family was relatively balanced across the entire planting area to more robustly compare treatments (Pinto et al., 2011a). Ideally, replicates or blocks would be equally balanced, but that was not possible given the relatively large number of replicates and blocks in the study.

We used a resolvable incomplete block design with single tree plots and a nested treatment arrangement. Incomplete blocks were used to control for environmental variation that changed rapidly, thus requiring blocks with fewer experimental units than the number of treatments. Two to six incomplete blocks were grouped to form a complete replication. Twelve to 14 replicates were used within each of the three planting areas. Treatments (species or hybrid type and family nested within species or hybrid type), incomplete block, and replications were arranged using Proc Optex (SAS, SAS Institute, Cary NC) to maximize treatment information.

2.4. Tree performance data collection

Nursery data were collected just after seedlings were lifted. We measured seedlings for total height (nearest 1 cm) from the root collar to the top of the tallest terminal bud. The root collar is defined as the transition zone between the above-ground and below-ground portion of the stem at the ground-line of the seedling. We measured root-collar diameter (RCD, nearest 0.1 mm) and stem diameter at 3 cm below the terminal bud (Topdia, nearest 0.1 mm) using digital calipers. We counted the number of first-order lateral roots (FOLRs), defined as a lateral root stemming from the main tap root that is at least 1 mm at the proximal end. The same individual counted roots on all seedlings from both nurseries to reduce bias in FOLR counts. Measuring the proximal end of each lateral root to ensure it meets the minimum size requirement of 1 mm is impractical; therefore, the FOLR counts can be subjective if different individuals assess the root systems.

Plant data were collected just after planting (year 0) and in years 1–3 following planting, unless otherwise noted. Total stem height (nearest 1 cm) and ground-line diameter (GLD) (nearest 0.1 mm) were measured on all seedlings in the dormant season after bud set was complete (October–March). Total height was measured as the vertical height from the base of the tree to the top of the tallest live bud. Total GLD was measured where the stem emerged from the litter layer using a digital or manual dial caliper. Stem volume (cm^3) was calculated following *Eq. (1)*.

$$Volume = \frac{2.5 \times \pi \times Topdia^2 + \left[(10HT - 30) \times \pi \times \left(\frac{RCD+Topdia}{4}\right)^2\right]}{1000} \quad (1)$$

Where HT is stem height, RCD is root-collar diameter, and Topdia is

the diameter of the stem 3 cm below the terminal bud.

Additionally, we recorded the presence or absence of natural chestnut blight cankers on live chestnut trees. Blight was identified as an ellipsoid–shaped canker on the stem that was sometimes sunken or slightly swollen and was sometimes accompanied by bark discoloration. We were conservative in our identification of natural blight cankers, and the cankers had to be accompanied by vertical cracking or fissuring of the bark with mycelial fans just below the bark surface (visible with a hand lens), and/or have orange stromata protruding through the bark surface (cf. Griffin and Elkins, 1986).

2.5. Data analyses for differential growth and survivorship

We calculated a ratio of height to RCD (for nursery data) and a ratio of height to GLD (for post-planting data), hereafter referred to as height:RCD or height:GLD, respectively. Height, RCD, and GLD were first standardized for each year after planting by subtracting the mean and dividing by the standard deviation, producing values with a mean of 0 and a standard deviation of 1. We added a constant of 10 to produce non-negative values and calculated the ratios by dividing height by RCD or height by GLD. Ratios greater than 1 were interpreted to represent trees that allocate more growth to their height than their stem diameter (taller, thinner trees) and ratios < 1 represented trees that allocate more growth to their stem diameter than their height (shorter, thicker trees).

We conducted analyses of variance (ANOVAs) to determine treatment effects on the dependent variables: survival, blight occurrence, height, GLD, and height:GLD for the year of planting (year 0) and growing seasons (years 1–3) after planting. We used general linear mixed models (Proc Mixed) to analyze survival and growth variables (height, diameter, and height:GLD). For binary survival (1 = alive, 0 = dead) and blight (1 = blight observed, 0 = blight not observed) data, we used the arcsine square-root transformation, and only present results from year 3. For all other dependent variables, year after planting (0–3) was included as a repeated measure, and we used an autoregressive covariance structure (Littell et al., 1998) in our analyses. Normality assumption of residuals was assumed if the Kolmogorov–Smirnov D-statistic was greater than 0.90. Homogeneity of variance assumptions were tested by examining plots of residual versus predicted values. If needed, unequal variance was added to the overall resistance model by using the 'Group' option in the 'Repeated' statement, and denominator degrees of freedom were adjusted using the Kenward–Roger method. A likelihood ratio test was used to test whether unequal variance or covariates were justified. For survival, height, and GLD models, we computed comparisons among least-square means using Tukey's mean separation and macros (Saxton, 1998) were used to more easily identify differences by assigning associated letters to the means. Means were reported with the associated standard error (e.g., ±x SE). The 'Slice' option in the 'Lsmeans' statement was used to test simple effects within interactions when significant.

Logistic regression (Proc Logistic) was used to determine whether the probability of survival was influenced by nursery seedling height, RCD, stem volume, number of FOLR, and height:RCD in combination with their species/hybrid type. Significant predictor variables were selected for inclusion in the final model and a Hosmer–Lemeshow goodness-of-fit test was used to test whether the logistic regression model accurately described the data. We used indicator variable regression (Proc GLM) to determine whether the seedlings' number of FOLR and species/hybrid type could be used to predict height and GLD in year 3.

2.6. Soil sampling

In all, had six families of the BC_3F_3 chestnut hybrids, far more than the other plant species (Table 2). To allow for a more balanced design, we only included two of the BC_3F_3 families (D22 and W3) for our soil analyses. For the included families, we randomly selected four representative plants to investigate if and how soil fungi may play a role in growth (chestnuts and oaks), plant survival (chestnuts and oaks), and blight occurrence (chestnuts only). We sampled soils both at the time of planting and 3 years post establishment, but only used 3-year old plant growth, survivor, and blight data for analyses. Soils were collected directly from planting locations and consisted of 10 soil probe subsamples, establishment soils were collected at the site of planting whereas third year soils were collected within 10 cm from the plant-soil interface to prevent disturbance of young roots. The subsamples were pooled per plant individual, mixed manually and placed into clean zip-top bags containing approximately 500 g of soil per sample. In total, we had four replicates for each tree family for each time point for a total of 64 soil samples. The soil samples were frozen (-20 °C) and shipped overnight on ice to Mississippi State University where they were frozen at −80 °C within 24 h of sampling and until further processing.

2.7. DNA extraction and metabarcoding sequencing

Total genomic DNA was extracted from three technical replicates of 0.25 g (fresh weight) of soils using PowerSoil DNA Isolation kit (MoBio Laboratory, Inc., Carlsbad, CA). The three technical replicates were pooled into one, DNA was quantified using Nanodrop 2000 Spectrophotometer (ThermoScientific, Waltham, MA), and DNA adjusted to a concentration of 2 ng/μL.

Metabarcoding libraries were constructed as described in Reazin et al., (2019). Briefly, the Internal Transcribed Spacer region 2 (ITS2) was amplified using a 2-step amplification procedure with the fungal specific primers fITS7 and ITS4 (Ihrmark et al., 2012; White et al., 1990). Primary PCR was conducted in duplicate in 50 μl reactions. PCRs consisted of 20 ng of template DNA, 200 μM of each dNTP, 1 μM of forward and reverse primer, 10 μl of Phusion 5x HF buffer which includes 1 unit of Phusion Green Hot Start II High-Fidelity DNA polymerase (ThermoScientific, Pittsburgh, PA, USA). The primary PCR cycling conditions were: 30 s denaturing at 98 °C, followed by 30 cycles of 10 s denaturing at 98 °C, 10 s annealing at 56 °C, 1 min extension at 72 °C, and final 5-minute extension at 72 °C. The duplicate amplicons were combined, and PCR products were cleaned using Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles (GE Healthcare, Little Chalfont Buckinghamshire, UK). Secondary PCRs were conducted to include 12 bp unique dual barcodes that were synthesized to be on the flanking ends of the fTIS7 and ITS4 primers and include 5 cycles of PCR using the same parameters as above followed by a second Sera-Mag clean-up.

Following purification, the amplicons were quantified with the ND2000 and 200 ng of each sample was pooled for sequencing. Using a

Table 1
Analysis of variance used to determine differences among species, family within species and year after planting in height, ground-line diameter (GLD) and height:GLD.

Source of Variation	Height		GLD		Height:GLD	
	F-statistic	P-value	F-statistic	P-value	F-statistic	P-value
Year	142.61	<0.0001	158.18	<0.0001	3.09	0.0261
Species	59.82	<0.0001	2.61	0.0726	196.6	<0.0001
Species*Year	19.28	<0.0001	15.54	<0.0001	14.97	<0.0001
Family(Species)	25.26	<0.0001	11.73	<0.0001	4.9	<0.0001
Family*Year(Species)	3.99	<0.0001	1	0.4638	8.66	<0.0001

Table 2
Height and ground-line diameter means for each family and year after planting. Means with the same letter within a year are not significantly different.

Species/Hybrid Type	Family	Year 3		Year 2		Year 1		Planting	
Height (cm)									
American chestnut	Pryor 18	174 (9.9)	ab	150 (9.3)	ab	121 (8.6)	abc	113 (8.3)	abce
	Pryor 43	192 (6.7)	a	176 (6.7)	a	148 (6.6)	a	128 (6.5)	a
CAES hybrid	4–75	164 (7.1)	ab	150 (6.9)	ab	133 (6.7)	ab	128 (6.7)	a
BC_3F_3 hybrid	D22	140 (5.2)	b	127 (4.8)	bc	100 (4.5)	c	77 (4.4)	ef
	W3	153 (7.1)	ab	142 (7.0)	abc	123 (6.9)	abc	106 (6.9)	abcef
	W4	188 (6.7)	a	160 (6.7)	a	119 (6.6)	abc	87 (6.6)	ef
	W5	169 (7.3)	ab	165 (6.9)	a	143 (6.6)	a	123 (6.6)	ab
	W6	175 (6.1)	a	148 (5.9)	ab	117 (5.8)	abc	90 (5.7)	bcef
Chinese chestnut	Princeton	132 (7.6)	bc	117 (7.6)	cd	102 (7.6)	bcd	88 (4.6)	cdef
White oak	AS	141 (3.5)	b	118 (3.4)	c	111 (3.4)	bc	118 (3.3)	ad
	ETN	108 (2.2)	c	89 (2.2)	d	79 (2.1)	d	79 (2.1)	f
GLD									
American chestnut	Pryor 18	27.5 (1.33)	ab	20.0 (1.24)	ab	15.3 (1.12)	ab	10.6 (1.06)	bce
	Pryor 43	29.5 (2.15)	ab	25.9 (2.14)	a	16.5 (2.12)	ab	11.3 (2.08)	cde
CAES hybrid	4–75	26.0 (1.09)	ab	21.4 (1.06)	a	14.9 (1.04)	ab	11.0 (1.03)	bce
BC_3F_3 hybrid	D22	24.6 (0.92)	b	20.1 (0.85)	ab	13.6 (0.79)	b	8.4 (0.76)	e
	W3	24.9 (1.45)	ab	21.5 (1.42)	ab	14.4 (1.40)	ab	10.2 (1.40)	bce
	W4	26.6 (26.6)	ab	22.1 (1.15)	a	15.1 (1.12)	ab	9.7 (1.12)	bce
	W5	24.6 (1.54)	ab	24.5 (1.47)	a	15.9 (1.40)	ab	11.4 (1.40)	bce
	W6	27.6 (1.36)	ab	22.6 (1.32)	a	14.6 (1.31)	ab	9.5 (1.27)	bce
Chinese chestnut	Princeton	25.1 (2.20)	ab	18.8 (2.20)	ab	14.5 (2.20)	ab	9.5 (2.20)	bce
White oak	AS	29.5 (0.74)	a	22.6 (0.73)	a	19.4 (0.72)	a	18.9 (0.71)	ad
	ETN	22.9 (0.50)	b	16.7 (0.49)	b	13.1 (0.48)	b	12.2 (0.48)	bc
Height:GLD									
American chestnut	Pryor 18	1.13 (0.03)	ab	1.13 (0.02)	a	1.11 (0.02)	a	1.11 (0.02)	ab
	Pryor 43	1.08 (0.02)	ab	1.10 (0.02)	a	1.09 (0.02)	a	1.08 (0.02)	abd
CAES hybrid	4–75	1.05 (0.01)	b	1.07 (0.01)	a	1.08 (0.01)	a	1.11 (0.01)	ac
BC_3F_3 hybrid	D22	1.09 (0.01)	ab	1.10 (0.01)	a	1.07 (0.01)	a	1.00 (0.01)	d
	W3	1.06 (0.02)	ab	1.08 (0.02)	a	1.09 (0.02)	a	1.06 (0.02)	abd
	W4	1.17 (0.02)	a	1.16 (0.02)	a	1.11 (0.02)	a	1.02 (0.02)	bcde
	W5	1.09 (0.02)	ab	1.13 (0.02)	a	1.13 (0.02)	a	1.12 (0.02)	ac
	W6	1.13 (0.02)	ab	1.12 (0.02)	a	1.09 (0.02)	a	1.02 (0.02)	bd
Chinese chestnut	Princeton	1.03 (0.03)	bc	1.03 (0.03)	ab	1.03 (0.03)	ab	1.00 (0.03)	bcde
White oak	AS	0.88 (0.01)	d	0.87 (0.01)	c	0.90 (0.01)	c	0.94 (0.01)	e
	ETN	0.95 (0.01)	c	0.94 (0.01)	b	0.94 (0.01)	b	0.94 (0.01)	e

NEBNext® DNA MasterMix for Illumina kit (New England Biolabs Inc., Ipswich, MA, USA) Illumina specific primers and adapters were ligated to the amplicons at the Integrated Genomics Facility at Kansas State University (Manhattan, KS, USA). The library was sequenced in one Illumina MiSeq reaction (300 bp PE; Illumina, San Diego, CA, USA). All data were submitted to the Sequence Read Archive (SRA) at the National Center for Biotechnology Information (NCBI) under the following accessions: BioProject PRJNA820875 and BioSample Runs SRR18548713-SRR1858776.

2.8. Sequence data analyses

Sequence data were processed using the program mothur (v.1.47.0; Schloss et al., 2009). Paired reads were contiged and screened to cull sequences that contained ambiguities and the ITS2 region was excised using the HMM-based program ITSx (v.1.1.3; Bengtsson-Palme et al., 2013) and reads without complete ITS2 regions were culled. Retained sequences were screened for chimeric properties using mothur-implemented VSEARCH (Rognes et al., 2016) and putative chimeras were removed. Sequences were then classified to best taxonomic placement using a Naïve Bayesian classifier (Wang et al., 2007) against a locally modified UNITE Species Hypothesis database (v.8; Nilsson et al., 2019) enriched to include increased representation of plants and other eukaryotic microbes to help identify non-fungal reads and all non-fungal reads were removed. Operational Taxonomic Units (OTUs) were demarcated using the mothur-embedded VSEARCH using abundance based greedy clustering (Rognes et al., 2016) at a threshold of 97 % and OTUs with a global count<10 were culled to reduced inclusion of spurious OTUs (Brown et al., 2015; Oliver et al., 2015).

2.9. Data analysis of soil fungal effects on tree survival, tree growth, and blight incidence

We aimed to assess if soil fungal communities influence or can explain plant survival, growth, and/or blight occurrence. To achieve this, we took all above ground growth data (e.g., height, GLD, etc.) across the three measured years (Table A1), and categorized them into discrete categories using hierarchal K-means clustering (Chestnut and Oak trees were clustered separately) whereby each individual tree would cluster into a group representing growth, such that there were a minimum of three experimental units per cluster; this resulted two growth clusters (Cluster 1 – trees with greater above ground growth; Cluster 2 – trees with lesser above ground growth); these clusters were used in downstream analyses. Additionally, blight occurrence was screened on chestnuts (see above) and categorized in a binary fashion (0 = not observed, 1 = present). Further, tree survival was also recorded in a similar binary fashion (0 = dead, 1 = alive).

To query fungal communities associated with each tree for the 2015 planting year and the 2018 sampling (3-years post establishment), we calculated average Bray-Curtis (BC; Bray and Curtis, 1957) dissimilarity and an abundance-based Sørensen index ($\widehat{L}_{abd}$; Chao et al., 2005) between each sample, which is robust to rare occurrences and large numbers of zero counts – common characteristics of OTU data. We calculated these indices using 1000 iterations at a subsampling depth of 15,000 sequences per sample and used the average values here (this depth was deemed appropriate based on estimates of Good's coverage, mean = 0.996). Further, the abundance based Sørensen matrix was used to generate loading axes using Nonmetric Multidimensional Scaling (NMDS), NMDS was optimally resolved using three axes (stress = 0.189, $R^2 = 0.854$) and these were used to test against tree survival (see below). Additionally, we identified fungal biomarkers that were differentially

abundant across growth clusters and/or blight occurrence for both years using LEfSe (Segata et al., 2011), and where significant, we identified putative functional guilds by querying genus-level identifies against the traits database FungalTraits (Põlme et al., 2020).

To investigate if fungal communities are more or less similar when associated with growth performance clusters (chestnut and oaks) or blight occurrence (chestnuts), we used a pairwise comparison approach of BC and $\widehat{L}_{abd}$ dissimilarity values. Similar frameworks have been used to query community responses to disease states (Wei et al., 2019) and biogeography (Brown and Jumpponen, 2019). Dissimilarity values between samples that belong to different growth clusters or blight occurrence states were compiled for soils at planting (2015) and three years post planting (2018). Mann-Whitney U tests were used to test if medians differed between 2015 and 2018 using Monte Carlo permutations (9999 permutations) and Monte Carlo based p-values were calculated; additionally, Z-score based effect sizes were calculated (η^2). Using NMDS axes loading scores as independent variables, Logistic Regression was conducted to assess if the probability of chestnut survival was influenced by soil fungal communities for either 2015 or 2018 sampling timepoints. Since for each sampling point, we had data for only eight individual oak plants, we omit logistic regression analyses for oaks as we have too few samples to be confident in the robustness of obtained results.

2.10. Statistics

All statistics were conducted using a combination of mothur (v.1.47.0; Schloss et al., 2009), JMP Pro (v.15; SAS Institute, Cary, NC, USA), SAS (v 9.4; SAS Institute, Cary, NC, USA), and PAST 4 (v.4.09; Hammer et al., 2001).

3. Results

3.1. Nursery seedling quality

All seedling species were relatively large at outplanting, averaging more than 80 cm in height, 10.1 mm in RCD, 8 FOLR (Table A1). Overall, the American chestnut family Pryor 24 seedlings were largest (average height of 173 cm at year three), whereas white oak family ETN seedlings were smallest (average height of 108 cm at year three). Range in seedling size at planting was broad within each family, particularly for FOLR that ranged from 0 to 35 in the ETN white oak family alone.

3.2. Seedling performance

Across all species, third-year survival rate was 85 % (SE = 2 %) and trees grew an average of 43 cm in height (SE = 2.2 cm) and 13.3 mm in GLD (SE = 0.45 mm) in three years. Differences in survival were marginally significant among species (F = 2.16, P = 0.0926) and significant among families (F = 2.58, P = 0.0132). All families had greater than 91 % survival rate, except for the American chestnut family Pryor 18 (69 %) and a BC_3F_3 hybrid D22 (75 %) (Fig. 1). For chestnuts, third-year blight incidence depended on species (F = 4.77, P = 0.0100) and family within species (F = 2.38, P = 0.0331). The American chestnut had the highest blight incidence (8 %) and the BC_3F_3 hybrids had < 1 % blight incidence. In contrast, we observed no blight in the Chinese chestnut three years after outplanting. Two American chestnut families differed in blight incidence: with Pryor 18 having 22 % blight and Pryor 43 having < 1 % (P = 0.0054) in the first three years of growth. The BC_3F_3 hybrid families did not differ significantly from each other.

Seedling height depended on species, family, and on their interactions with time (Table 1). Chinese chestnut and white oak were generally smaller than the American chestnut across the three years and the hybrids were generally tallest (Fig. 2). Family D22 seedlings were shorter than other BC_3F_3 hybrids across all years. All families increased in height and GLD from the time of planting to the third year (P < 0.001). The hybrid family W4 grew most – more than 100 cm in three years. The two American chestnut families (Pryor 18 and Pryor 43) were similar in height and GLD growth across the years, whereas the two white oak families differed in height and GLD growth (Table 2).

The height:GLD depended on species, family, and interactions with time (Table 1). Within each year, chestnut species and hybrids had height:GLD values greater than 1 indicating that the chestnut seedlings were generally taller and thinner than white oak seedlings (Fig. 3). BC_3F_3 hybrid families W4, W6, and D22 seedlings increased in height:

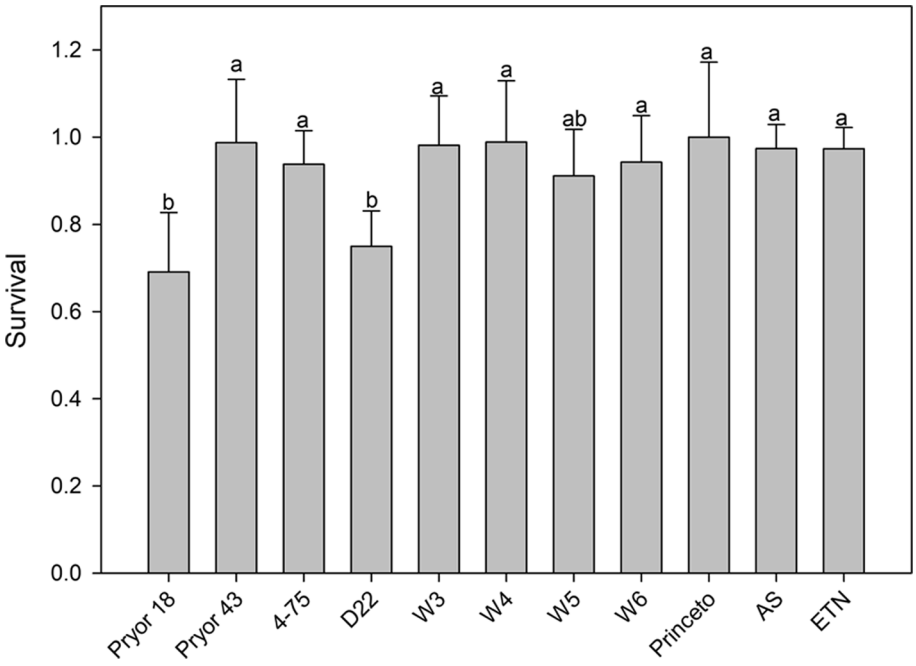

Fig. 1. Third-year survival and associated standard error bars for each family (refer to Table A1 for family descriptions; Pryor 18 and Pryor 43 are American chestnuts, 4–75 is a CAES hybrid, D22, W3, W4, W5, and W6 are BC_3F_3 hybrids, Princeton is a Chinese chestnut, and AS and ETN are white oak). Means with the same letter are not significantly different (P ≥ 0.05).

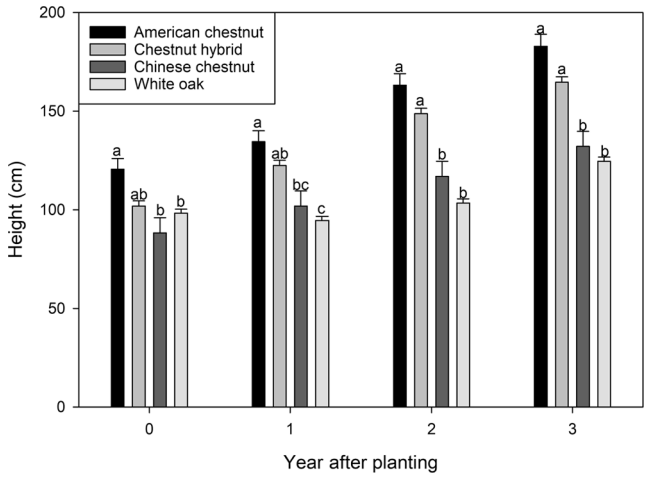

Fig. 2. Mean height and associated standard errors of each species (family information combined) each year after planting. Mean values with the same letter within a year are not significantly different.

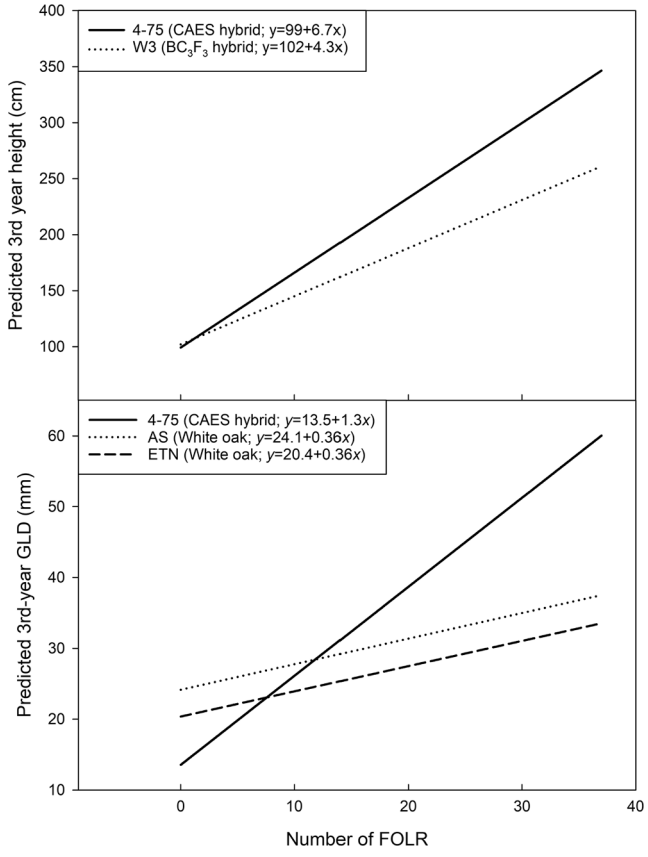

Fig. 3. Linear regression of height (top graph; $R^2 = 0.17$) and ground-line diameter (GLD; bottom graph; $R^2 = 0.17$) versus number of first-order lateral roots (FOLR) for families with significant relationships ($P < 0.05$).

GLD over time ($P < 0.0001$), whereas BC_3F_3 hybrid family W5 and CAES hybrid family 4–75 seedlings decreased in height:GLD over time ($P < 0.0001$). American chestnut families did not differ from each other in height:GLD at any point in time (Table 2) or change in ratios from year to year ($P = 0.48$). The two white oak families had similar height:GLD at the time of outplanting but diverged over time: family AS had greater height:GLD than family ETN after outplanting (Table 2).

None of the nursery variables (height, RCD, stem volume, number of FOLR, height:RCD) successfully predicted survival in the logistic regression analyses (Wald estimate = 1.3, $P = 0.26$). In the indicator regression analysis, the number of FOLR interacted with family to explain the variation in third-year height ($F = 3.16$, $P = 0.0007$, $R^2 = 0.17$) and third year GLD ($F = 2.00$, $P = 0.0329$, $R^2 = 0.17$), but explanatory power was low so these results should be interpreted with caution. The FOLR number and third-year height correlated positively only for the hybrid families 4–75 and W3 whereas these correlations for other families were non-significant. For GLD, only hybrid family 4–75 and both white oak families significantly and positively correlated with the FOLR number (Fig. 3). The CAES hybrid family 4–75 had the strongest correlations between number of FOLR at the time of planting and third-year height and GLD, where an increase of one in FOLR increased height by 7 cm and GLD by 1.3 mm.

3.3. Fungal communities

After sequence quality control, OTU demarcation, and post-OTU processing, we detected 1022 total OTUs (a total of 3,887,607 sequences). OTUs were dominated by taxa assigned to the phyla Ascomycota (618 OTUs, 60.4 % of all OTUs), Basidiomycota (249 OTUs, 24.3 %), and Mortierellomycota (36 OTUs, 3.5 %), with high representation of the families Herpotrichiellaceae (92 OTUs, 9.0 % of all OTUs), Mortierellaceae (36 OTUs, 3.5 %), and Trichocomaceae (23 OTUs, 2.3 %).

LEfSe-identified biomarker taxa, which indicates that a particular OTUs is overrepresented within a treatment and have greater than 0.1 % total relative abundance, included 14 biomarker OTUs for either blight presence or absence (chestnuts), five for the 2015 sample (soils prior to planting) and nine for the 2018 samples (Table 3). Among these, several putative saprotrophs were overrepresented in samples with blight occurrence. For the 2015 samples, these included OTU0042 (*Mortierella pulchella*), OTU0133 (*Pholiota chocenesis*), and OTU0162 (*Talaromyces ramulosus*), and for the 2018 samples, OTU0076 (*Mortierella horticola*), OTU0085 (*Brachysporium* sp.), OTU116 (*Rectipilis davidii*), and OTU0120 (*Melanchlenus eumetabolus*). Taxa that were biomarkers for plant performance includes one biomarker for chestnuts in 2015 (Growth Cluster 1 – better growth), five for oaks in 2015 (Growth Cluster 2 –poorer growth) including the plant pathogens *Mycosphaerella tassiana* and *Scleroconidioma sphagnicola*, and six for the 2018 chestnut samples (two for Growth Cluster 1 and four for Growth Cluster 2), and six for the 2018 oak samples (two for Growth Cluster 1 and four for Growth Cluster 2) (Table 3).

Analyses of community dissimilarities between samples that have different responses (different growth clusters or different blight occurrence) indicate that 2018 samples (soils three years post planting) were more dissimilar than 2015 samples (soils at planting) for both chestnuts and oaks (Fig. 4). This indicates that fungal communities were more similar and homogeneous between differentially performing trees and between trees that developed blight symptoms in pre-planting soils than they were after three years of interaction with establishing plants, suggesting fungal community filtering by plant hosts. This was true for both growth and blight occurrence, and for both Bray-Curtis and abundance-based Sørenson dissimilarities, but more pronounced for abundance-based Sørenson dissimilarity based on calculated effect sizes and had the following results: BC between growth clusters for chestnuts ($U = 4010$, $P < 0.001$, $\eta^2 = 0.131$), BC for growth clusters for oaks ($U = 27$, $P < 0.001$, $\eta^2 = 0.445$), BC for blight occurrence ($U = 460$, $P < 0.001$, $\eta^2 = 0.206$), $\widehat{L}_{abd}$ for growth clusters for chestnuts ($U = 977$, $P < 0.001$, $\eta^2 = 0.548$), $\widehat{L}_{abd}$ for growth clusters for oaks ($U = 0$, $P < 0.001$, $\eta^2 = 0.743$), and $\widehat{L}_{abd}$ for blight occurrence ($U = 195$, $P < 0.001$, $\eta^2 = 0.477$). Further, none of the NMDS axes for either year predicted chestnut plant survival well in our logistic regression analyses (2015: Axis 1 - $\chi^2 = 0.004$, $P = 0.9448$, Axis 2 - $\chi^2 = 1.015$, $P = 0.3135$, Axis 3 - $\chi^2 = 1.550$, $P = 0.2131$; 2018: Axis 1 - $\chi^2 = 0.234$, $P = 0.8782$, Axis 2 - $\chi^2 = 0.532$, $P =$

Table 3
List of biomarker OTUs (LEfSe analyses) from plant associated soils that are overrepresented for either Blight Occurrences, or Plant Growth (Growth Cluster 1 (better growth) or 2 (poorer growth)) for 2015 (soils at planting) or 2018 (3-years post planting). Only biomarker OTUs that are greater than 0.1 % relative abundance for either year are included. Presented are OTU label, associated condition, Linear Discriminate Analysis (LDA) effect sizes, P-value, OTU taxon identity (lowest level of certainty) and functional guild (where available; EcM - Ectomycorrhizal).

OTU ID	Treatment	LDA	P-value	Taxonomy	Functional Guild
Biomarker of Blight Condition 2015 (planting)					
Otu0042	Blight Occurrence	4.211	0.047	*Mortierella pulchella*	Saprotroph (soil)
Otu0063	Blight Occurrence	3.076	0.047	Herpotrichiellaceae sp.	
Otu0090	Blight Occurrence	2.311	0.031	Herpotrichiellaceae sp.	
Otu0133	Blight Occurrence	2.837	0.028	*Pholiota chocenensis*	Saprotroph (wood)
Otu0162	Blight Occurrence	3.247	0.013	*Talaromyces ramulosus*	Saprotroph
Biomarker for Growth Cluster 2015 (Chestnut – at planting)					
Otu0030	Growth Cluster 1	3.188	0.033	*Exophiala xenobiotica*	Animal Parasite
Biomarker for Growth Cluster 2015 (Oak – at planting)					
Otu0026	Growth Cluster 2	3.445	0.025	*Exophiala xenobiotica*	Animal Parasite
Otu0030	Growth Cluster 2	3.365	0.025	*Mycosphaerella tassiana*	Plant Pathogen
Otu0118	Growth Cluster 2	2.186	0.047	Helotiales sp.	
Otu0126	Growth Cluster 2	2.428	0.024	*Hormonema macrosporum*	Saprotroph
Otu0131	Growth Cluster 2	2.821	0.025	*Scleroconidioma sphagnicola*	Plant Pathogen
Biomarker of Blight Condition 2018					
Otu0032	Blight Occurrence	3.819	0.019	Helotiales sp	
Otu0043	Blight Occurrence	4.598	0.031	*Rhizopogon* sp.	EcM
Otu0076	Blight Occurrence	3.752	0.046	*Mortierella horticola*	Saprotroph (soil)
Otu0085	No Blight	3.256	0.038	*Brachysporium* sp.	Saprotroph
Otu0092	Blight Occurrence	3.962	0.012	Herpotrichiellaceae sp.	
Otu0113	Blight Occurrence	2.451	0.035	Ascomycota sp.	
Otu0116	No Blight	3.049	0.045	*Rectipilus davidii*	Saprotroph (wood)
Otu0120	Blight Occurrence	3.402	0.036	*Melanchlenus eumetabolus*	Saprotroph
Otu0142	Blight Occurrence	2.631	0.019	*Sebacina* sp.	EcM
Biomarker for Growth Cluster 2018 (Chestnut)					
Otu0045	Growth Cluster 1	3.708	0.011	GS34 sp.	
Otu0051	Growth Cluster 2	3.348	0.025	*Sphaerobolus* sp.	Saprotroph (wood)
Otu0092	Growth Cluster 2	3.126	0.006	Herpotrichiellaceae sp.	
Otu0095	Growth Cluster 2	3.190	0.004	Agaricomycetes sp.	
Otu0108	Growth Cluster 1	3.820	0.038	Herpotrichiellaceae sp.	
Otu0186	Growth Cluster 2	2.248	0.006	*Clavaria* sp.	Saprotroph (soil)
Biomarker for Growth Cluster 2018 (Oak)					
Otu0055	Growth Cluster 2	2.920	0.043	Herpotrichiellaceae sp.	
Otu0063	Growth Cluster 1	2.666	0.043	Herpotrichiellaceae sp.	
Otu0074	Growth Cluster 2	3.149	0.047	*Cortinarius* sp.	EcM
Otu0092	Growth Cluster 2	3.946	0.017	*Clavaria* sp.	Saprotroph (soil)
Otu0117		2.970	0.047	*Cenococcum* sp.	EcM
Otu0127	Growth Cluster 1 Growth Cluster 2	3.343	0.042	Chaetothyriales *sp.*	

0.4657, Axis 3 - $\chi^2 = 0.101$, P = 0.7497).

4. Discussion

4.1. Comparative successfulness of hardwood reforestation

Our results demonstrate successful hardwood reforestation during the critical stand establishment and development phase by interplanting two foundational species, white oak and American chestnut. Nearly all chestnut and white oak families had greater than 90 percent survival rate and grew relatively fast (growing 23–101 cm in height and 11–18 mm in GLD three years). Although not empirically measured, we observed that the majority of planted trees were in competitive canopy positions relative to the naturally regenerating hardwood and pine species, an important factor that will determine future reforestation success (Dey et al., 2008; Spetich et al., 2002). We attribute the early success to using large, high-quality, competitive nursery seedlings from predominately locally adapted sources (Dumroese et al., 2016) and planting on a site with abiotic characteristics and silvicultural prescriptions that have been described as suitable for these species (e.g., well-drained soils, open-canopy conditions, reduced competition from prescribed fire) (Rogers, 1990; Russell, 1987; Wang et al., 2013; Weigel and Johnson, 1998). However, information gleaned from our study is relatively novel, and inferences should consider local stand conditions (e.g., open canopy, relatively xeric site) and seedling characteristics (relatively large 1–0 or 2–0 bare-root seedlings from pedigreed sources), particularly when extrapolating results to inform management decisions.

Oak regeneration by planting has had relatively few successes in upland hardwood stands, even in those with marginal productivity like our planting site (Dey et al., 2008; Johnson, 1984; Pope, 1993). Research on white oak artificial regeneration is relatively sparse, particularly using quality-grown graded seedlings. We report similar survival and slightly greater height growth than in a study in Tennessee that used graded 1–0 planting stock on a moderately productive and open canopy site (Granger and Buckley, 2021). The early white oak planting success in our study compared to other similar efforts supports recommendations that oak seedling performance may be improved by using larger seedlings from locally adapted sources planted into canopy openings with more than 30–50 percent full sunlight (Dey et al., 2008; Kormanik et al., 2002).

Reintroduction of American chestnuts bred for blight resistance and analysis of their growth and success outside of orchard plantings is relatively novel (Bauman et al., 2014; Clark et al., 2014; Schaberg et al., 2022). Research over the last two decades suggests that American or hybrid chestnuts from a variety of seed sources and stock types are capable of relatively fast growth and high survival, especially in the early years after planting on a wide range of forest site types and silvicultural conditions (Clark et al., 2016, 2012; Pinchot et al., 2020; Rhoades et al., 2009; Schaberg et al., 2022; Thomas-Van Gundy et al., 2017). Growth and survival of the BC_3F_3 hybrids and American chestnut seedlings planted on coal mine reclamation sites were lower than reported in our study, even after five growing seasons (Bauman et al., 2013, 2014), likely due to harsher soil conditions that limited nutrient availability and beneficial soil microbes. Here, we observed greater survival and similar three-year height and GLD growth to large-size backcross chestnut seedlings planted on more productive sites in the

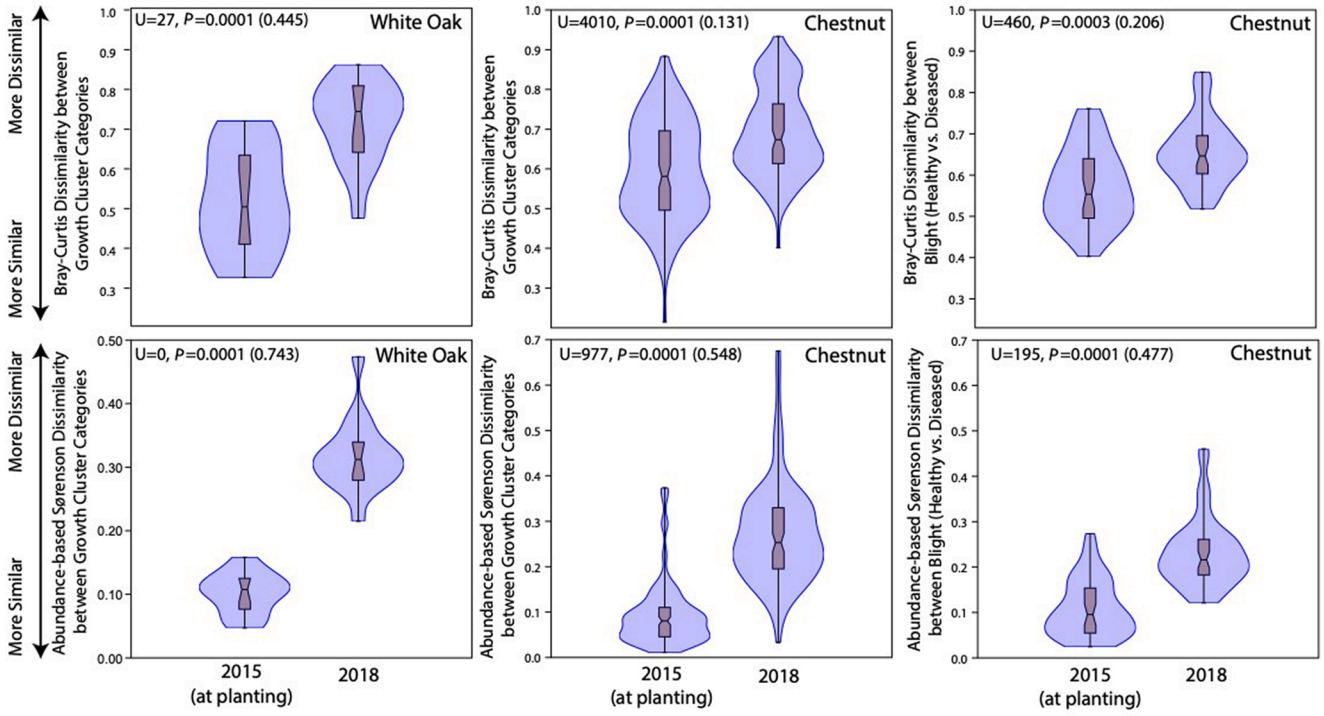

Fig. 4. Changes in pairwise community dissimilarity (Bray-Curtis and Abundance-based Sørenson ($\widehat{L}_{abd}$)) between White Oak (left) and Chestnut (center) trees that differ in growth (Growth Cluster 1 vs 2) or observed occurrence of Chestnut Blight (Healthy vs Diseased; right). The violin plots represent dissimilarities with box and whiskers plots inset with median, interquartile range and extreme values. Also presented are Mann-Whitney U tests statistics testing if years differ, P-values, and effect sizes (η^2) parenthetically.

same physiographic region (Clark et al., 2016), but slower height growth than backcross seedlings planted in shelterwood harvests on mesic sites in West Virginia (Thomas-Van Gundy et al., 2017). Interestingly, our three-year survival and growth rates were similar to backcross chestnut seedlings with a similar seed source (4–75 was common in both studies,) outplanted in xeric sites in northern Pennsylvania and initially grown in the same commercial nursery in the same year (Pinchot et al., 2020). While the families vary, American chestnut survival here was similar but growth was slower than that of smaller pure American chestnuts planted on moderate to productive sites in a nearby-two-age shelterwood stands (Clark et al., 2012). Collectively, these results indicate that chestnut restoration may be facilitated by chestnut adaptability to a relatively wide range of site conditions and seedling stock types or sizes. However, it is good to bear in mind that restoration is currently mostly limited by blight resistance (Westbrook et al., 2019). White oak restoration will require more deliberate use of quality-graded seedlings coupled with selection of appropriate sites where competition intensity may be relaxed (Dey et al., 2008), such as lower productivity sites similar to this study.

4.2. Effects of nursery seedling characteristics

Qualitative measures of nursery seedling size can influence planting outcomes of oak (Clark et al., 2015; Granger and Buckley, 2021; Kormanik et al., 1997) and chestnut (Clark et al., 2016, 2010), but few studies have analyzed the effect of quantitative nursery variables to field performance. In this study, none of the measured nursery morphological variables (height, RCD, stem volume, number of FOLR, and height:RCD) explained seedling survival - a result contrary to previous studies with oak (Dey and Parker, 1997; Thompson and Schultz, 1995). Seedling survival was relatively high across this planting (75–97 %, depending on family; mean of 85 %) indicating that other factors, such as unmeasured microsite variables or seedling physiological attributes, may be contributing more to variation in survival than nursery morphology (Jacobs et al., 2005). The number of FOLR was a stronger predictor of third-year height and GLD than it was for tree survival, but its explanatory power was relatively weak ($R^2 = 0.17$) and varied by family. Previous studies have identified that root system morphology is important in early outplanting success for many species, including oak (Jacobs et al., 2005; Kormanik et al., 2002; Thompson and Schultz, 1995; Ward et al., 2000), although one study reported a non-significant relationship between FOLR number and planting success with 1–0 chestnut hybrids (Clark et al., 2010). We used quality-graded seedlings that reduced the variability typical for bare-root Fagaceae seedlings used in similar plantings (Clark et al., 2012, 2000; Clark and Schlarbaum, 2018). This may have weakened the observable relationships among nursery seedling morphology, planting survival, and growth of the various families (Pinto et al., 2011b).

Some hybrid families, particularly the CAES family 4–75, and both white oak families had weak positive but significant relationships between FOLR and third-year height and GLD, indicating that some chestnut hybrid families, particularly those from a northern seed source, and locally adapted white oaks may more strongly depend on root system morphology for growth after planting. Jacobs and others (2005) observed similar, albeit stronger, relationships between FOLR and second year height and diameter growth than our study for smaller white oak 1–0 bareroot seedlings from non-pedigreed sources in Indiana.

4.3. Breeding and genetic differentiation in early field performance

Breeding and genetic effects on survival and growth has been previously demonstrated in field plantings of American chestnut (Clark et al., 2016; Thomas-Van Gundy et al., 2017) and provenance tests of oaks (Kriebel et al., 1988), including white oak (Huang et al., 2016). However, we are unaware of any previous studies that have tested white oak genetic effects in silvicultural plantings. We found evidence for differentiation between the two white oak families that we tested. Results comparing white oak to chestnut species included here should be

interpreted with caution as the white oaks were older planting stock (2–0) and from a different nursery than the chestnuts (1–0). These factors may confound our results, particularly early in the seedling development in the field. Regardless, our results and prior studies clearly indicate that genetic background and seed source influence outcomes, and managers would likely benefit from using diverse and/or improved seed sources to moderate family effects.

Blight incidence in this study was comparable to those previously reported: blight was relatively rare in Chinese chestnut and hybrids and relatively sparse in families except for American chestnut (Clark et al., 2016; Pinchot et al., 2020). However, our blight incidence data were collected for only three-year old plants, and blight is likely to dramatically increase over time. Our results corroborate a previous study, in which American chestnut families differentiated in early blight incidence and had greater occurrences than hybrids and the Chinese chestnut (Clark et al., 2016). Similar to other studies (Clark et al., 2016; Thomas-Van Gundy et al., 2017), Chinese chestnut grew slower than American chestnut and hybrids, and pure American chestnut grew slightly faster, albeit not always significantly, than hybrids. The slower growth of some BC_3F_3 hybrid trees may be due to polygenic inheritance of blight resistance – BC_3F_3 hybrid parents inherit 17 percent of their genome from Chinese chestnut (Westbrook et al., 2019), potentially leading to an intermediate performance phenotype of these offspring. Family differences in growth among hybrids have been reported in other studies and should preferably be evaluated across multiple sites (Clark et al., 2016; Thomas-Van Gundy et al., 2017), an issue beyond the scope of this study. The relatively good growth of the CAES hybrid family indicates that this northern seed source performs similar to the more southern BC_3F_3 hybrids, a finding that may inform future assisted migration to mitigate effects of climate change (Dumroese et al., 2015).

The two white oak families demonstrated a growth form over all three years to favor development of GLD over height (height:GLD < 1), particularly for the AS family whose height:GLD declined over time. American chestnuts, Chinese chestnut, and hybrids exhibited growth forms that favored height development over GLD (height:GLD greater than 1), and by year 3, two of the hybrid families (CAES family 4–75 and BC_3F_3 family W4) differentiated from each other in their height:GLD. If we use GLD as a surrogate for below-ground development (Dey and Parker, 1997), our results indicate that white oak may allocate more resources into the root system development than above-ground stem development, an attribute that contributes towards its shade tolerance. American chestnut and hybrids may grow taller to quickly establish canopy dominance, a trait that has been described in historical literature (Ashe, 1911) and in recent field trials (Clark et al., 2016; Thomas-Van Gundy et al., 2017). Family differences in overall size and growth form can inform future breeding initiatives or tree improvement programs to select for desirable or specific growth traits.

4.4. Soil fungal community impacts on growth and blight

Reforestation success is impacted by many factors including climate (MacKenzie and Mahony, 2021), edaphic characteristics (Günter et al., 2009; Pinto et al., 2011b), and plant growth qualities and physiology (Brancalion and Holl, 2020; Duryea and McClain, 1984), among many others. One of the main goals of this work was to investigate if existing soil fungal communities can be used to predict reforestation success. If so, this previously uninvestigated component could be used to inform location selection for reforestation efforts. While there have been studies monitoring fungal communities post-planting (Cavagnaro et al., 2016; Kałucka and Jagodziński, 2016), or using mycorrhizal fungal innocula to improve planting success (Holste and Kobe, 2017; Manaut et al., 2015; Menkis et al., 2007), to our knowledge, investigations of existing soil fungal communities have been overlooked in search of potential predictors to explain planting success. Here, fungal communities poorly predicted chestnut growth and post-planting success. We were unable to test if soil fungi could be used to predict oak success because too few oak-associated soils were available. However, qualitatively the available data suggest that fungal communities may not succeed in predicting oak performance either. While additional work is needed to confirm, especially on a larger scale, our data do not provide strong evidence to suggest that soil fungal monitoring is a viable tool for reforestation site selection.

Despite this, we do find evidence for modulation of soil fungal communities and individual fungal taxa by plants that differ in growth and blight occurrence. There were differences in soil community dissimilarities after three years of growth between (1) oaks and chestnuts that differed in their growth during the first three years after outplanting and (2) chestnuts that differed in their blight occurrence. This, in addition to several biomarker taxa observed for growth categories and blight occurrences, demonstrates that planted tree performance and/or above-ground disease can alter fungal communities below ground. However, more research is required to understand how these alterations may relate to future growth and survival in reforestation efforts.

4.5. Conclusions

Restoration during the stand establishment phase of development was successful using artificially regenerated Fagaceae species using high-quality seedlings at the time of planting coupled with appropriate site selection and silvicultural manipulations to create open stand conditions. The number of FOLR at the time of planting was not strongly related to planting survival or growth after three growing seasons, particularly for most of the chestnut families tested, but these trends may change over time as trees are challenged by drought stress and competition. White oak seedlings were more reliant on initial root system morphology than American and Chinese chestnut and chestnut BC_3F_3 hybrids and continued to allocate more resources to their below-ground structures, as inferred from a lower height:GLD ratio. Differentiations in growth and growth ratios among pedigreed families within oak and chestnut species provides evidence that managers should ensure interspecies diversity to avoid the possibility of planting only poor-performing families. We tested one northern seed source that performed exceptionally well, indicating that chestnuts bred for blight resistance have the potential for climate change adaptation, at least in the short-term. We were unable, however, to demonstrate the utility of soil fungal community monitoring to predict plant performances indicating that soil fungal reconnaissance may not be useful in selection reforestation sites, but we do demonstrate that plant performance and disease states can modulate soil fungi to an extent.

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CRediT authorship contribution statement

Shawn P. Brown: Methodology, Formal analysis, Writing – original draft, Visualization. **Stacy L. Clark:** Conceptualization, Methodology, Resources, Formal analysis, Writing – original draft, Visualization. **Emerald Ford:** Investigation, Writing – review & editing. **Ari Jumpponen:** Investigation, Resources, Writing – review & editing. **Arnold M. Saxton:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Scott E. Schlarbaum:** Conceptualization, Methodology, Resources, Writing – review & editing. **Richard Baird:** Methodology, Writing – original draft, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2022.120569.

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