

Complementarity increases production in genetic mixture of loblolly pine (*Pinus taeda* L.) throughout planted range

DAVID R. CARTER,^{1,†} TIMOTHY J. ALBAUGH,¹ OTÁVIO C. CAMPOE^{1D},² JAKE J. GROSSMAN^{1D},³
 RAFAEL A. RUBILAR,⁴ MATTHEW SUMNALL,¹ CHRISTOPHER A. MAIER,⁵ RACHEL L. COOK,⁶ AND
 THOMAS R. FOX⁷

¹Department of Forest Resources and Environmental Conservation, Virginia Tech, 228 Cheatham Hall, Blacksburg, Virginia 24061 USA

²Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil

³Arnold Arboretum of Harvard University, 1300 Centre Street, Boston, Massachusetts 02131 USA

⁴Cooperativa de Productividad Forestal, Departamento de Silvicultura, Facultad de Ciencias Forestales, Universidad de Concepción, Victoria 631, Casilla 160-C, Concepción, Chile

⁵USDA Forest Service, Southern Research Station, Research Triangle Park, North Carolina 27709 USA

⁶Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, North Carolina 27695 USA

⁷Rayonier, Forest Research Center, Yulee, Florida 32097 USA

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Abstract. Increased genotypic diversity has been associated with increased biomass production in short-rotation tree species. Increasing the genotypic diversity of loblolly pine (*Pinus taeda* L.) in an attempt to increase productivity has not been extensively studied nor tested operationally or over long durations (i.e., >7 yr). We used genetically mixed and pure rows of loblolly pine growing throughout its planted range—Virginia, North Carolina, and Brazil—to test the effects of genetic mixing on volume production. There were no significant effects of mixing rows compared to pure rows on uniformity or mortality. Under intensive silviculture, individual trees planted in mixed rows had approximately 7% greater volume than those in the pure rows (estimate = $0.015 \text{ m}^3/\text{tree} \pm 0.006$) in the final year of measurement—year 8 for Brazil and year 10 for North Carolina and Virginia. Scaling the increase in individual stem volume under mixed rows and intensive silviculture to $1235 \text{ stems ha}^{-1}$ would equate to an additional $1.85 \text{ m}^3 \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ in mean annual increment. Measuring the net biodiversity effect, our data suggest the positive growth response is driven by complementarity and not selection, meaning both genetic entries tend to grow larger when grown together. Additional trials are necessary to test the effects of mixing rows across large plots and to assess whether this increase is sustained throughout the rotation. If this increasing trend were to hold for intensively managed plantations, strategically mixing rows to increase productivity could be a valuable addition to an intensively managed plantation requiring relatively little added operational consideration to implement.

Key words: clonal forestry; genotypes; net biodiversity effect; selection; uniformity.

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† **E-mail:** davidcarter@vt.edu

INTRODUCTION

Decades of tree improvement have led to increased loblolly pine (*Pinus taeda* L.)

productivity in the southeastern United States (McKeand et al. 2006) and South America (Rubilar et al. 2018). These technological advancements have contributed to increasing mean

growth rates of loblolly pine across its planted range (i.e., its native range plus exotic locations outside of its native range). Intensively managed stands commonly yield $25 \text{ m}^3 \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ in the United States (Zhao et al. 2016) and $56 \text{ m}^3 \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ in Brazil (Leite et al. 2006), continuing toward the theoretical maximum of $75 \text{ m}^3 \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ (Farnum et al. 1983). Open-pollinated, full-sib families, and clonal trees propagated vegetatively by somatic embryogenesis have enabled resource managers to deploy phenotypically and genetically more uniform trees in forest plantations, increasing productivity (Bridgwater et al. 2005).

While there has been a substantial amount of research investigating interspecies competitive effects (Schnabel et al. 2019), competition is expected to be most severe between close genetic relatives (Cheplick and Kane 2004, Slingsby and Verboom 2006). Seemingly, as long as the deployment of more productive but less diverse genotypes has existed, so too has the concept of increasing productivity by increasing within-stand genetic diversity (Adams et al. 1973).

Genotypes bred for specific productivity-related phenotypes can potentially vary in resource utilization, biomass partitioning, phenology, or their effects on nutrient cycling (DeBell and Harrington 1993). There is the potential for productivity gains from mixing genotypes due to these morphological and physiological differences (Foster et al. 1998). There is also the potential for reductions in productivity resulting from decreases in stand-wide uniformity and increased inter-tree competition and mortality (Soares et al. 2016). Within-stand uniformity offers operational benefits, however, as uniform stands can be reasonably expected to respond to silvicultural inputs similarly. This advantage promotes the deployment of genetically uniform stands (Rezende et al. 2014).

While still not widespread in its implementation in forestry, some empirical studies testing the effects of intraspecies or intergenotypic diversity on productivity do exist for short-rotation species, that is, *Eucalyptus* spp., *Populus* spp., *Robinia* spp., *Paulownia* spp., and *Salix* spp. (e.g., DeBell and Harrington 1997, Brodie and DeBell 2004, Boyden et al. 2008, Hoeber et al. 2018, Pavan et al. 2019, Rezende et al. 2019, Schweier et al. 2019), as well as for *Pinus elliottii* and loblolly pine (Adams et al. 1973, Staudhammer

et al. 2009). These studies have yielded mixed results as productivity responses often depend on site, spacing, silvicultural inputs, and genotypic combination. However, substantial gains have been noted. For example, Foster et al. (1998) found some mixtures of *Populus* clones outperformed pure plots of the best-performing clones by as much as 27%. In *Eucalyptus* spp. plantations, mixing genotypes has resulted in mean annual increment gains of 7.7% (Rezende et al. 2019) and 4% to 13% (Pavan et al. 2019). Rarely, however, are these studies long-term (i.e., >7 yr), tested at operational scales or conducted with loblolly pine.

The net biodiversity effect, where system productivity increases with increasing species richness, has been studied extensively in mixed species and, less so, in mixed genotype forests. The net biodiversity effect is calculated by subtracting observed yield from expected yield (Loreau and Hector 2001) and is a way of quantifying the potential yield benefits from diversity. The effect is attributed to two mechanisms: selection and complementarity (Vandermeer 1989, Tilman et al. 1997, Loreau and Hector 2001). Selection effects can be positive when a highly productive species disproportionately increases stand-wide productivity. Complementarity occurs under niche differentiation, also called resource partitioning (Pimentel 1968, Tilman 1982), or facilitation. Niche differentiation is said to take place when functional diversity among species or genotypes allows for stratification of resource use over space and time, reducing negative competition effects. Facilitation can occur when one species or genotype improves growing conditions for another. The degree to which these differences may influence productivity in loblolly pine has not been extensively studied. The proper deployment of elite genetics in intensively managed plantations requires an understanding of the potential interactions between genotypes and silvicultural inputs, spacing, and site conditions (Burdon 2001, Binkley et al. 2017).

We measured the uniformity, mortality, and individual tree volume of mid-rotation (8 and 10 yr old) loblolly pine trees in mixed (i.e., two adjacent rows of different genetics) and pure rows (i.e., two adjacent rows of the same genetics). We expected (1) greater productivity under

mixed rows than pure rows; (2) differences to not be driven by increased non-uniformity or greater mortality in the mixed rows; and (3) increases in productivity to be associated with complementarity and not selection.

METHODS

Study sites

This study has three installations, two in the United States (Virginia Piedmont, North Carolina Coastal Plain) that were established in 2009 and one in Brazil (Paraná State) that was established in 2011. These three locations were selected to form a gradient of productivity within the planted range of loblolly pine. The Virginia Piedmont site represents the lower end of loblolly pine productivity due the cold temperatures found here. The soil at this installation is a well-drained Fairview series (fine, kaolinitic, mesic Typic Kanhapludults). The North Carolina Coastal Plain site represents typical conditions for loblolly pine planted in the southern United States. The soil at this installation is a somewhat poorly to poorly drained Rains series (fine-loamy, siliceous, semiactive, thermic Typic Paleaquults). The Paraná State site in Brazil was selected to represent the upper end of productivity for loblolly pine in the world, as growth rates that are twice those found in the United States have been documented here (Cubbage et al. 2007). Soils at this site are well-drained Inceptisols and Hapludults.

Experimental design

The data for the analysis were extracted from a split-split plot design (Appendix S1: Tables S1–S3 and Fig. S1). Six genetic entries were grown at three spacings: low, medium, and high. In the U.S. sites, the spacings resulted in 618 trees per hectare (TPH), 1235 TPH, 1854 TPH. In Brazil, the spacings were slightly different, producing densities of 618 TPH, 1235 TPH, and 1853 TPH. The main plot treatment was two levels of silviculture (operational or intensive; described later), the split-plot treatment was six different genotype entries, and the split-split plot treatment was three planting densities. Each plot had 81 or 63 trees in a 9×9 or 9×7 row configuration, respectively. Large, 20-m buffers of several planted rows were established between the main

plots (two levels of silvicultural intensity; described later). There was no buffer zone between plots within each block. The stands had reached crown closure by, at least, the final year of measurements used in this analysis.

The six genetic entries (genotypes) provided by Arborgen were all sourced from the Atlantic Coastal Plain. The entries were 4 different clones (henceforth referred to as 1, 2, 3, 4), one control-pollinated family (henceforth referred to as 5), one open-pollinated family (henceforth referred to as 6), and, in Brazil-only, there was an additional clone tested (henceforth referred to as 8; the designation of 8 matches internal records of the study. There is no entry 7 in this study.). In North Carolina and Virginia, the open-pollinated and control-pollinated seedlings were bareroot stock while all varietal material was containerized. In Brazil, all seedlings were containerized. Seedlings were hand-planted at both North American installations in 2009 and planted in 2011 in Brazil. Seedlings were all 6–8 months old and approximately 15–20 cm tall when planted.

There were two levels to the silviculture factor: operational and intensive. Operational silviculture consisted of regionally common applications of site preparation and intermediate treatments over a typical rotation for loblolly pine. Intensive silviculture consisted of practices intended to approach the maximum growth rate of loblolly pine by ameliorating any nutrient deficiencies through more and frequent weed control applications early in the rotation and regular fertilization. The silviculture applied within each treatment level (operational and intensive) varied slightly to match site conditions, particularly drainage. Specifics on treatment differences can be found in Vickers et al. (2011).

Data for the following analyses were extracted from this experimental design to test the effects of genetic mixing on productivity. Mixed rows consisted of two rows of trees found at the interface where two different genotypes met in the split-split plots. At these interfaces, spacings, silvicultural intensities, and site were the same. Pure row analogues of each of the genotypes in the mixed rows were selected from the same respective split-split plots with a two-tree buffer between the mixed and pure rows. This selection of pure analogues was done to attempt to capture any underlying gradient that may also be

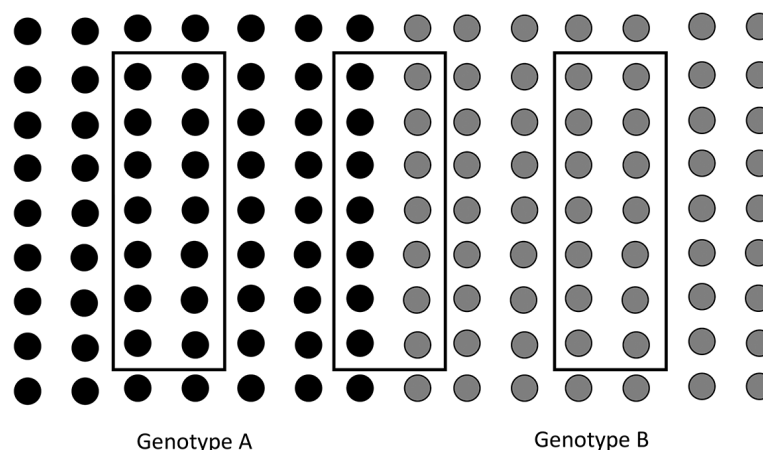


Fig. 1. Data were collected from an existing experiment using this plot layout. Black and gray circles represent different genotypes. The rectangles represent the trees that were selected for analysis.

influencing the mixed rows (Fig. 1). Trees on the ends of rows were omitted from the dataset to remove any influences adjacent plots may have on the growth of these trees, resulting in 7-tree rows in the 9×9 configuration and 5-tree rows in the 7×9 configuration. All trees in this experiment were permanently tagged and inventoried annually.

Uniformity was calculated by assigning trees to pairs along the short-side of their rectangular grouping and dividing the small stem by the large stem to get a value of 1 or less. The lower the value, the less uniform the pair. This calculation of uniformity was intended to target the treatment differences driven by mixing genotypes by comparing the size of these trees to their nearest, non-matching pair. Mortality was noted during each annual inventory. Volume was derived from measurements of stem diameter at breast height and stem height using equations from Tasissa et al. (1997).

Plots in which numerous, individual genetic entries are grown together (hereafter referred to as “single-tree plots”) were established at the North Carolina and Brazil sites, adjacent to the previously described study. All single-tree plots were spaced at 1235 TPH and planted in 2009 in North Carolina and 2011 in Brazil. In North Carolina, 63 varieties were used and, in Brazil, 36 entries were tested. Two levels of silvicultural treatments were used and matched the previously described study: operational and intensive.

There were five replications of each silvicultural intensity at each site.

We utilized the equations described in Loreau and Hector (2001) to partition the effects of selection and complementarity on yield. The sum of these effects is the net biodiversity effect. For these metrics, the plot-level (Fig. 1) means of mixed and pure conditions are compared to separate any potential mechanism that may be driving positive or negative effects of mixing genotypes.

Analysis

Mixed-effects type III analyses of variance (ANOVAs) with Satterthwaite’s method of uniformity, mortality, and individual stem volume were fit using the lmer function in the lme4 package in R v.3.5 (Bates et al. 2015, R Core Team 2017). *P*-values derived from using the lmer function were computed using the lmerTest package in R (Kuznetsova et al. 2017). Composition (mixed or pure), site (Virginia, North Carolina, and Brazil), spacing (618, 1235, and 1854 TPH), and silviculture (operational and intensive) were used as fixed effects. Block and the orientation of the interface in which blocks met (east–west, e.g., Fig. 1, or north–south example not shown) were used as random effects (Eq. 1). The inclusion of random effects in the models was informed by comparing Akaike information criterion values among candidate models. The ANOVA for mortality was fit using the glmer function in the lme4

package (Bates et al. 2015) included a binomial error distribution with a logit link. Post hoc comparisons were made using the least squares means (LS means) function in the lsmeans package (Lenth 2016). Post hoc comparisons were made within site when site was included in a significant interaction.

Mixed-effects type III analyses of covariance (ANCOVAs) were used to test differences in uniformity and volume using lme4 (Bates et al. 2015). Additional fixed and random effects terms were added to the aforementioned terms to test for influence on respective response variables. Year since treatment (YST) was used as a covariate to test for differences over time. When using YST as a covariate, comparisons were made within year, when significant, and individual tree IDs were used as a random effect to account for repeated measures, along with block and orientation. The individual tree volume of one tree in a genetic combination was used as a covariate to test its influence on the growth of its pair in the final year of measurement. Additionally, to elucidate any potential effect of mortality and competition on volume, the number of living neighbors immediately adjacent to each individual tree (i.e., the competitive neighborhood), up to a possible eight, was included. Block, orientation, and spacing were used as random effects for this model.

The influences of individual genetic entries and their combinations on volume growth were assessed. These type III mixed-effects ANOVAs tested a site $\times$ composition $\times$ genotype (individual and combination, respectively) interaction with spacing, block, and orientation as random effects using the lme4 package (Bates et al. 2015), as before. Lastly, volumes in the single-tree plots were compared to the volumes in pure blocks with 1235 TPH to test the effects of plot-level mixing on productivity. Only composition, site (North Carolina and Brazil, only), and silviculture were tested. Spacing was the same in all single-tree plots: 1235 TPH. Block was used as a random effect. The term plot was used as a random effect to account for repeated measures when assessing trends over time. To conserve space, results of post hoc comparisons are reported in Appendix S2.

To determine the respective effects of selection and complementarity on the net biodiversity

effect, three separate analyses were conducted. Selection and complementarity were used to predict a net biodiversity effect in a linear regression model. The net biodiversity effect, selection effect, and complementarity effect were evaluated in a one-sample t-test to see if their respective effects on productivity were different than zero, or, in other words, different from pure rows. These values were compared among both silvicultural intensities and within each silvicultural intensity. Lastly, a mixed-effects ANOVA tested the silviculture $\times$ genetic entry interaction with spacing and site as random effects.

All mixed-effects models used took this general form:

$$y_{ijk} = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \dots + u_j z_k + \varepsilon_i \quad (1)$$

where y is the dependent variable, β_0 is the intercept, β_n are the coefficients of the fixed effects, x_n are the observed values, u and z are the random effects, and ε is residuals.

The effect of composition on the response variables was the primary interest of the study. Therefore, only main effects and interactions including this term are analyzed in post hoc tests and discussed. Unless assessing trends, only data from the final year of measurement—year 8 in Brazil and year 10 in Virginia and North Carolina were tested. The alpha for all tests was set at $P < 0.05$.

RESULTS

Uniformity

The only significant interaction including composition (mixed or pure) influencing uniformity in the final year of measurement was composition $\times$ site $\times$ spacing ($P = 0.003$; Table 1). Within site, the only significant difference among comparisons was the 618 TPH density in the mixed composition possessing greater uniformity than the 1235 TPH density in the pure composition (0.11 ± 0.04 SE; $P = 0.04$; Appendix S2: Table S1).

Mixed and pure row uniformities did not vary within years in the operational and intensive silviculture treatments ($P = 0.9$; Fig. 2). The mixed row uniformity over the duration of the experiment, however, was lower than the pure row (estimate = -0.01 ± 0.006 SE; $P = 0.03$), but the composition $\times$ time interaction was not significant ($P = 0.35$).

Table 1. ANOVA results for three response variables: final measurement year values (year 8 for Brazil, year 10 for Virginia and North Carolina) for uniformity, mortality, and volume of individual trees.

Variable	df	Uniformity		Mortality		Volume	
		F	P	F	P	F	P
Composition (C)	1, 928	0.05	0.82	1.8	0.18	0.9	0.34
Site (S)	2, 50	6.3	0.00	16.6	0.00	21.3	<0.01
Spacing (P)	2, 51	1.6	0.22	4.6	0.00	27.0	<0.01
Silviculture (V)	1, 51	0.2	0.68	11.3	0.00	21.9	<0.01
C × S	2, 926	0.1	0.92	2.3	0.10	0.2	0.86
C × P	2, 928	1.2	0.30	0.5	0.61	2.2	0.11
S × P	4, 50	1.4	0.24	2.5	0.04	1.9	0.13
C × V	1, 959	0.1	0.74	0.7	0.41	6.9	<0.01
S × V	2, 49	0.8	0.44	3.1	0.04	1.4	0.26
P × V	2, 51	0.6	0.53	1.8	0.17	0.8	0.44
C × S × P	4, 926	3.9	0.00	0.6	0.64	0.7	0.61
C × S × V	2, 865	0.3	0.77	1.0	0.38	0.1	0.94
C × P × V	2, 959	0.6	0.55	1.6	0.20	0.1	0.95
S × P × V	4, 49	1.5	0.21	2.6	0.03	0.6	0.69
C × S × P × V	4, 865	1.8	0.12	0.4	0.80	1.0	0.40

Note: P values equal to or less than 0.05 appear in boldface.

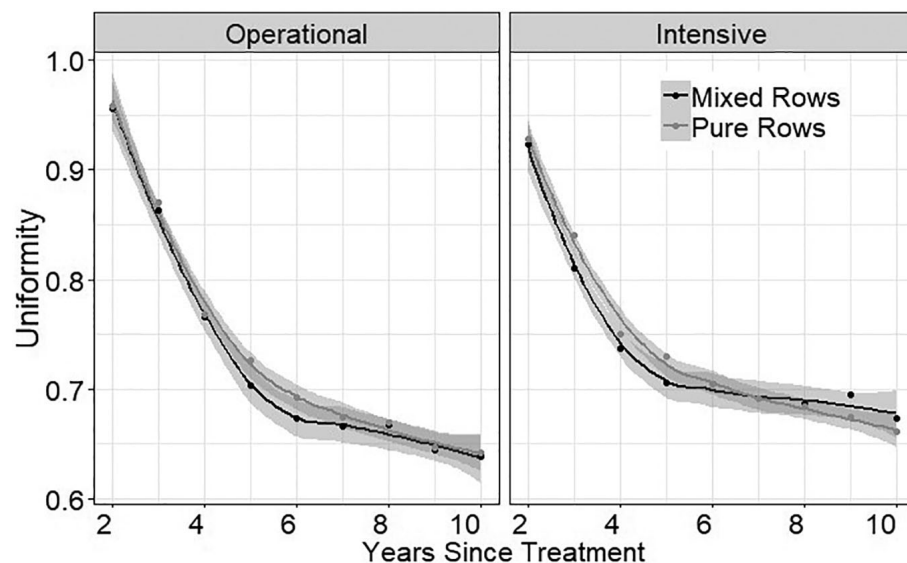


Fig. 2. Uniformity of the genetically mixed and pure rows over the duration of the experiment under operational and intensive silviculture. Uniformity is a measure of the larger tree in a pair divided by the smaller tree in pair. A uniformity value of 1 would mean both trees possessed the same volume.

There were no significant terms predicting the volume of tree A in a pair in the volume-of-tree-B × composition × site × spacing × silviculture interaction that included volume of tree B and composition ($P > 0.05$) for the final year of measurement. Marginal R^2 values of the volume of

tree B predicting the volume of tree A in the final year of measurement among sites and silvicultural intensities were all positive and weak ($R^2 < 0.07$; Fig. 3). Overall, R^2 values were greater under the intensive treatment and in pure rows.

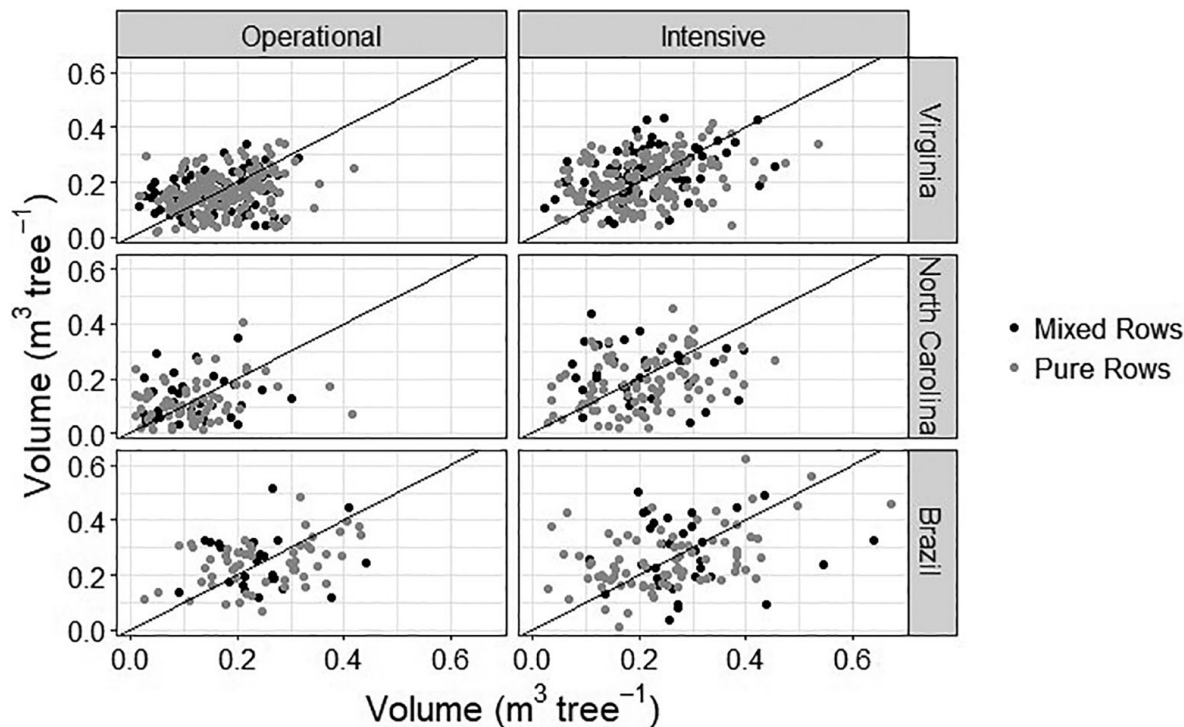


Fig. 3. Volumes of individual trees predicting the volume of their specific pair in mixed and pure rows. The solid line is a 1:1 line. Points deviating from this line represent individual trees that are less similar than their neighbor in terms of stem volume.

Table 2. Summary of mortality across sites among the pure and mixed rows.

Site	Pure						Mixed					
	Operational			Intensive			Operational			Intensive		
	No. dead	Total no. trees	Mortality rate (%)	No. dead	Total no. trees	Mortality rate (%)	No. dead	Total no. trees	Mortality rate (%)	No. dead	Total no. trees	Mortality rate (%)
Brazil	4	112	3.6	7	168	4.2	5	56	8.9	8	84	9.5
North Carolina	16	156	10.3	47	256	18.4	6	78	7.7	34	128	26.6
Virginia	37	420	8.8	43	364	11.8	18	210	8.6	20	182	11.0

Mortality

Composition did not have a significant influence on mortality ($P > 0.05$; Tables 1, 2). In general, mortality varied equally among sites between mixed and pure row compositions (Table 2).

In the neighborhood analysis, the composition term was not included in any significant three-way interactions nor the four-way interaction term (Table 3). The two-way interaction of composition

and the number of living neighbors was marginally significant ($P = 0.06$; Table 3; Fig. 4; Appendix S2: Table S2). The volume of individual trees in mixed rows was greater than pure rows when all eight neighboring trees were alive (estimate = $0.015 \text{ m}^3/\text{tree} \pm 0.006 \text{ SE}$; Fig. 4).

Volume

There was a significant composition $\times$ silviculture interaction influencing individual tree

Table 3. ANCOVA results for individual tree volume at the final measurement year (year 8 for Brazil, year 10 for Virginia and North Carolina).

Variable	df	F	P
No. living neighbors (N)	5, 1902	1.2	0.31
Composition (C)	1, 1902	1.5	0.22
Site (S)	2, 1902	17.1	<0.01
Silviculture (V)	1, 1902	10.5	<0.01
N × C	5, 1902	2.1	0.06
N × S	8, 1902	0.7	0.71
C × S	2, 1902	0.2	0.83
N × V	4, 1902	0.5	0.76
C × V	1, 1902	4.3	0.04
S × V	2, 1902	11.6	<0.01
N × C × S	7, 1902	0.9	0.54
N × C × V	3, 1902	0.6	0.61
N × S × V	5, 1902	2.7	0.02
C × S × V	2, 1902	0.2	0.78
N × C × S × V	5, 1902	0.6	0.69

Note: P values equal to or less than 0.05 appear in boldface.

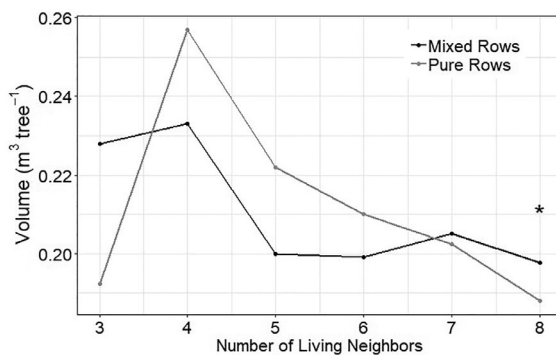


Fig. 4. Individual tree volume among mixed and pure rows over the recorded number of living neighbors immediately adjacent to the focal tree in the final year of measurement (year 8 for Brazil and year 10 for North Carolina and Virginia). Asterisk denotes significant difference within the level of number of living neighbors.

volume in the final year of measurement ($P < 0.01$; Table 1). Volumes in the intensively treated pure and mixed rows were both greater ($P < 0.01$) than their respective operational analogues, with differences ranging from 0.036 to 0.058 m³/tree. Notably, the pure and mixed rows under the operational silviculture treatment did not differ in final year volume (estimate = -0.007 m³/tree $\pm$ 0.006 SE; $P = 0.65$). Finally, under the

intensive silviculture treatment, the individual trees planted in mixed rows had approximately 7% greater volume than those in the pure rows (estimate = 0.015 m³/tree $\pm$ 0.006 SE; $P = 0.05$). This difference is the result of an increasing departure from volume growth in the pure rows over time under intensive treatment (Fig. 5; Appendix S2: Table S3).

When including years since treatment as a factor in the ANCOVA, composition was included in two significant three-way interactions: composition $\times$ spacing $\times$ years since treatment ($P = 0.04$; Table 4; Appendix S2: Table S4) and composition $\times$ silviculture $\times$ years since treatment ($P < 0.01$; Table 4; Appendix S2: Table S5). Pairwise comparisons within spacing and years since treatment showed marginal differences in volume manifesting in year 8. Here, the mixed rows had greater volume in the mid- (estimate = 0.007 m³/tree $\pm$ 0.004 SE; $P = 0.06$) and low-spacings (estimate = 0.007 m³/tree $\pm$ 0.004; $P = 0.08$). Similarly, pairwise comparisons within silviculture and years since treatment showed significant differences in volume starting to manifest by year 7, in which mixed rows possessed greater volume than pure rows under intensive silviculture (estimate = 0.009 m³/tree $\pm$ 0.003 SE; $P < 0.01$). These differences were sustained in North Carolina and Virginia in year 10.

Additionally, we found plot-wide volume did not differ significantly between the mixed, single-tree plots and pure plots in Brazil and North Carolina in the final year of measurement (Table 5); the only sites for which we have these data. At 1235 TPH (the spacing used in all single-tree plots) and averaged across site, silviculture, and genetic entry, final year of measurement single-tree plot volume was 226.7 m³/ha $\pm$ 12.0 SE and block plot volume was 220.4 m³/ha $\pm$ 8.6 SE. Among plots, the coefficient of variation of the single-tree plots was 5% and 4% in the block plots.

Differences among genetic entries

Complementarity was significant predicting the net biodiversity effect (NBE) across both silvicultural intensities (estimate = 0.99 $\pm$ 0.02; $P < 0.01$) and among each intensity when tested separately (operational NBE = -0.001 [$\pm$ 0.001 SE] + 0.97 [$\pm$ 0.024 SE] β_1 , $P < 0.01$ for β_1 ; intensive NBE = -0.002 [$\pm$ 0.001 SE] + 1.00 [$\pm$ 0.021

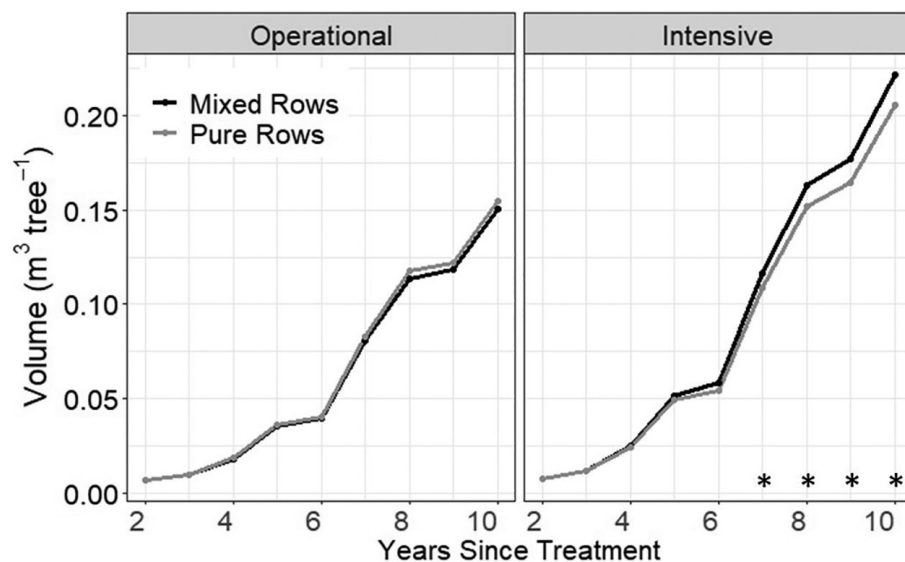


Fig. 5. Comparing individual tree volumes between the operational and intensive silviculture treatments between mixed and pure rows over time. Note that the final measurement year for Brazil is year 8 and is year 10 for North Carolina and Virginia. Asterisks denote significant differences between compositions within years.

SE] β_1 , $P < 0.01$ for β_1), while there was no relationship between selection and the net biodiversity effect (operational NBE = $-0.01 [\pm 0.006 \text{ SE}] - 0.86 [\pm 1.68 \text{ SE}]\beta_1$; $P = 0.61$ for β_1 ; intensive $0.01 + 1.02 [\pm 1.73 \text{ SE}]\beta_1$; $P = 0.56$ for β_1 ; Fig. 6). Overall, the selection effect was significantly less than zero ($P < 0.01$) while the complementarity effect was not significantly different from zero ($P = 0.57$). Under intensive silviculture, however, the selection effect remained significantly less than zero ($P < 0.01$; 95% CI = -0.05 to $-0.008 \text{ m}^3/\text{tree}$) while the complementarity effect was marginally greater than zero ($P = 0.09$; 95% CI = -0.03 to $0.36 \text{ m}^3/\text{tree}$). When testing the factors of genetic-entry combination and silvicultural intensity, no factors were significant. In general, however, selection effects among genetic entries were generally zero or slightly below zero, while complementarity effects ranged from positive to negative (Fig. 7). Selection effects were an order of magnitude less than complementarity effects.

DISCUSSION

Overall, averaging across sites, spacings, and genotypic combinations, mixed rows under intensive silviculture possessed significantly

greater individual tree volumes than pure rows by age 10 in Virginia and North Carolina and age 8 in Brazil—the most recent years of measurement for these trees (estimate = $0.015 \text{ m}^3/\text{tree} \pm 0.006 \text{ SE}$). Scaled to a per hectare basis, at 1235 TPH this would total an additional $18.5 \text{ m}^3/\text{ha}$ by age 8 in Brazil and age 10 in North Carolina and Virginia. Conservatively, averaged over 10 yr, this would equal $1.85 \text{ m}^3 \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$, which would equal an additional $46.3 \text{ m}^3/\text{ha}$ after 25 yr—a typical rotation length for intensively managed loblolly pine. For context, a growth response of $1.85 \text{ m}^3 \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ in volume is approximately 50% of what is expected from a nitrogen and phosphorus fertilization treatment in loblolly pine stands in the southeastern United States (Fox et al. 2007). Our data suggest the likely mechanism driving this relationship is a form of complementarity: the different genetic entries are partitioning growing space and utilizing resources in different spatial locations or at different times.

In general, uniformity was not impacted negatively under mixed rows (Figs. 2, 3). While non-uniformity could result in greater mortality or high reductions in growth for particular genetic-entry combinations, on average these consequences have yet to manifest despite that these

Table 4. ANOVA results for factors influencing volume over time.

Variable	df	F	P
Composition (C)	1, 1990	3.2	0.07
Site (S)	2, 25	110.0	<0.01
Spacing (P)	2, 39	19.2	<0.01
Silviculture (V)	1, 40	30.4	<0.01
Years since treatment (Y)	8, 13675	5376.7	<0.01
C × S	2, 2339	0.5	0.62
C × P	2, 2009	0.8	0.47
S × P	4, 38	3.8	0.01
C × V	1, 1990	13.3	<0.01
S × V	2, 38	1.9	0.17
P × V	2, 40	0.8	0.47
C × Y	8, 13677	1.7	0.08
S × Y	13, 13675	318.6	<0.01
P × Y	16, 13676	108.1	<0.01
V × Y	8, 13675	96.9	<0.01
C × S × P	4, 2423	0.4	0.84
C × S × V	2, 2339	1.8	0.17
C × P × V	2, 2009	1.6	0.20
S × P × V	4, 36	0.3	0.88
C × S × Y	13, 13672	0.3	0.99
C × P × Y	16, 13678	1.7	0.04
S × P × Y	26, 13670	18.0	<0.01
C × V × Y	8, 13677	3.0	<0.01
S × V × Y	13, 13670	7.3	<0.01
P × V × Y	16, 13676	2.0	0.01
C × S × P × V	4, 2423	0.5	0.73
C × S × P × Y	26, 163673	0.5	0.98
C × S × V × Y	13, 13672	0.4	0.95
C × P × V × Y	16, 13678	0.6	0.91
S × P × V × Y	26, 13671	3.0	<0.01
C × S × P × V × Y	26, 13673	0.6	0.92

Note: P values equal to or less than 0.05 appear in boldface.

Table 5. ANOVA results for plot-wide volume at the final measurement year (year 8 for Brazil, year 10 for North Carolina).

Variable	df	F	P
Composition (C)	1, 219	0.01	0.94
Site (S)	1, 219	168.7	<0.01
Silviculture (V)	1, 219	14.2	<0.01
C × S	1, 219	0.5	0.5
C × V	1, 219	0.01	0.91
S × V	1, 219	0.01	0.94
C × S × V	1, 219	2.0	0.15

Notes: There were no single-tree plots in Virginia. P values equal to or less than 0.05 appear in boldface.

trees have been in competition with each other in the crown closure stage of development for, at least, two years. In the single-tree plots, where

non-uniformity due to genetic mixing would be even greater than the mixed rows, significant differences in productivity were not observed and CV was only 1% greater in the mixed, single-tree plots than the pure blocks. Staudhammer et al. (2009), however, found single-tree plots of slash and loblolly pine to only benefit a minority of the genotypes grown in mixture. There may be a threshold where productivity gains from mixing genetics are met and beyond which increased non-uniformity results in mortality or decreased productivity. Furthermore, the gains from genetic mixing may only result when complementary pairs are deployed together, as evidenced by this study, and dissipate when whole stands are composed of randomly assorted genotypes.

Overall, decreasing uniformity does not explain the greater individual tree volumes in the mixed rows; in other words, we found little evidence of selection influencing productivity. It is commonly believed that mixing genotypes by row will result in the faster growing genotype dominating over its slower growing neighbor (i.e., selection), increasing mortality or the need to thin earlier. To the contrary, the documented patterns in the net biodiversity effect are driven almost entirely by complementarity, with negligible negative contributions from selection. This supports the hypothesis that the increase in volume under the mixed rows is due to genetic mixtures benefiting from some form of complementarity when grown together. Similarly, Boyden et al. (2008) found seed-origin *Eucalyptus* had reduced competitive interactions relative to clone-origin, supporting the resource partitioning hypothesis among mixed genetics.

The mechanism for complementarity is not clear, however. Greater genotypic diversity has been associated with increasing microbe-mediated ecosystem function (Purahong et al. 2016). Additionally, Drum et al. (2019) found different loblolly pine families to respond differently to silvicultural intensities in terms of belowground biomass accumulation. If this is also true among the genetic entries tested in this study, these differences may allow for spatial partitioning of belowground growing space, increasing growth rates. Low genotypic diversity can result in greater inter-tree competition and reduced productivity (Boyden et al. 2008), however, more

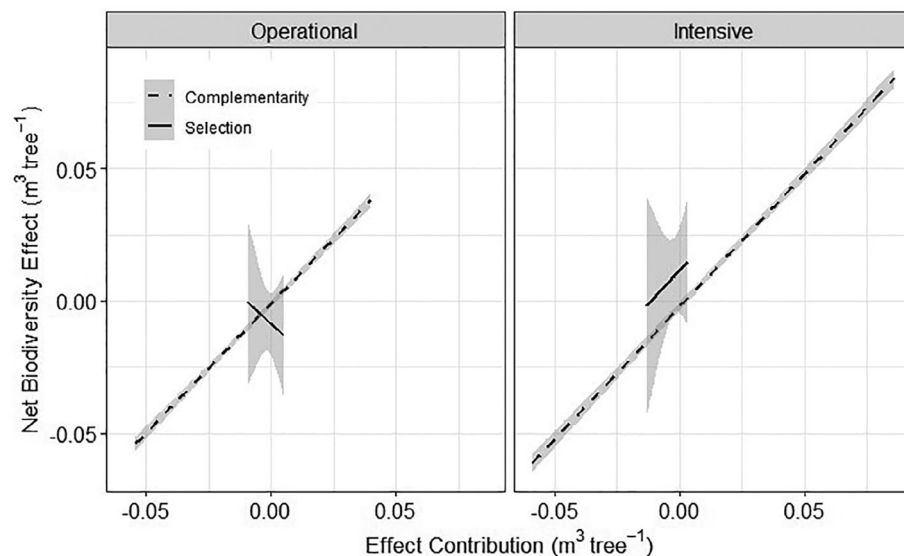


Fig. 6. Relationship among complementarity, selection, and the net biodiversity effect, as computed by Loreau and Hector (2001), among the two silvicultural intensities with standard error estimates in the shaded region.

testing would need to be done to determine the mechanism. The volume of the trees in the operational treatment has surpassed those in the intensive treatment in year 7: when increased productivity in mixed rows was first measured in the intensive treatment. If these differences in growth were driven strictly by size, we would have expected the mixed rows in the operational treatment to have started to show growth gains relative to the pure rows under this silviculture. As found by Drum et al. (2019), the genetic entries could be responding differently belowground to greater fertilization rates under the intensive silviculture, increasing belowground biomass partitioning differences. Belowground biomasses were not measured in this study. Similarly, either independently or in combination with the aforementioned mechanism, belowground biomass differences could facilitate greater resource utilization, enabling both trees to grow larger.

Further evidence of complementarity was found in analyzing the effect of living neighbors on productivity of individual trees. Individual trees in mixed rows possessed greater volume with all eight living neighbors than pure rows under the same conditions. This indicates that competitive effects on tree growth are lessened in mixed rows, where 38% of competitors have

different genetics. This reduction in competitive impacts on growth as a result mixing could have implications for planting densities and thinning regimes. If negative competitive interactions are delayed, planting densities could be increased and thinnings could occur later or be done less intensely. The trees in this experiment will not be thinned to monitor the carrying capacities across sites and will be monitored to test the effect of mixing on inter-tree competition and mortality.

The selection effect was fairly consistently negative and relatively small, while the complementarity effect ranged from relatively large to relatively small (95% CI = -0.03 to 0.36 m³/tree). This difference in the effect of complementarity among genetic entries will enable targeting a potential mechanism, aiding in informing why particular pairs are complementary when grown together. Furthermore, if whole stands were grown utilizing those pairs that were highly complementary, the result may be a greater gain than the average reported here across all genetic entries.

It is important to note that while these differences are tested in more operational settings than what can be found in the literature, these combinations are still not truly operationally deployed. Currently, our data show that the differences in growth between mixed and pure

rows under intensive management are continuing to deviate which may result in greater differences by the end of the rotation (Fig. 5). Conversely, we do not know whether these

growth responses may stabilize, dissipate, or become negative (Albaugh et al. 2015), justifying further monitoring. Additional testing investigating the effects on productivity of

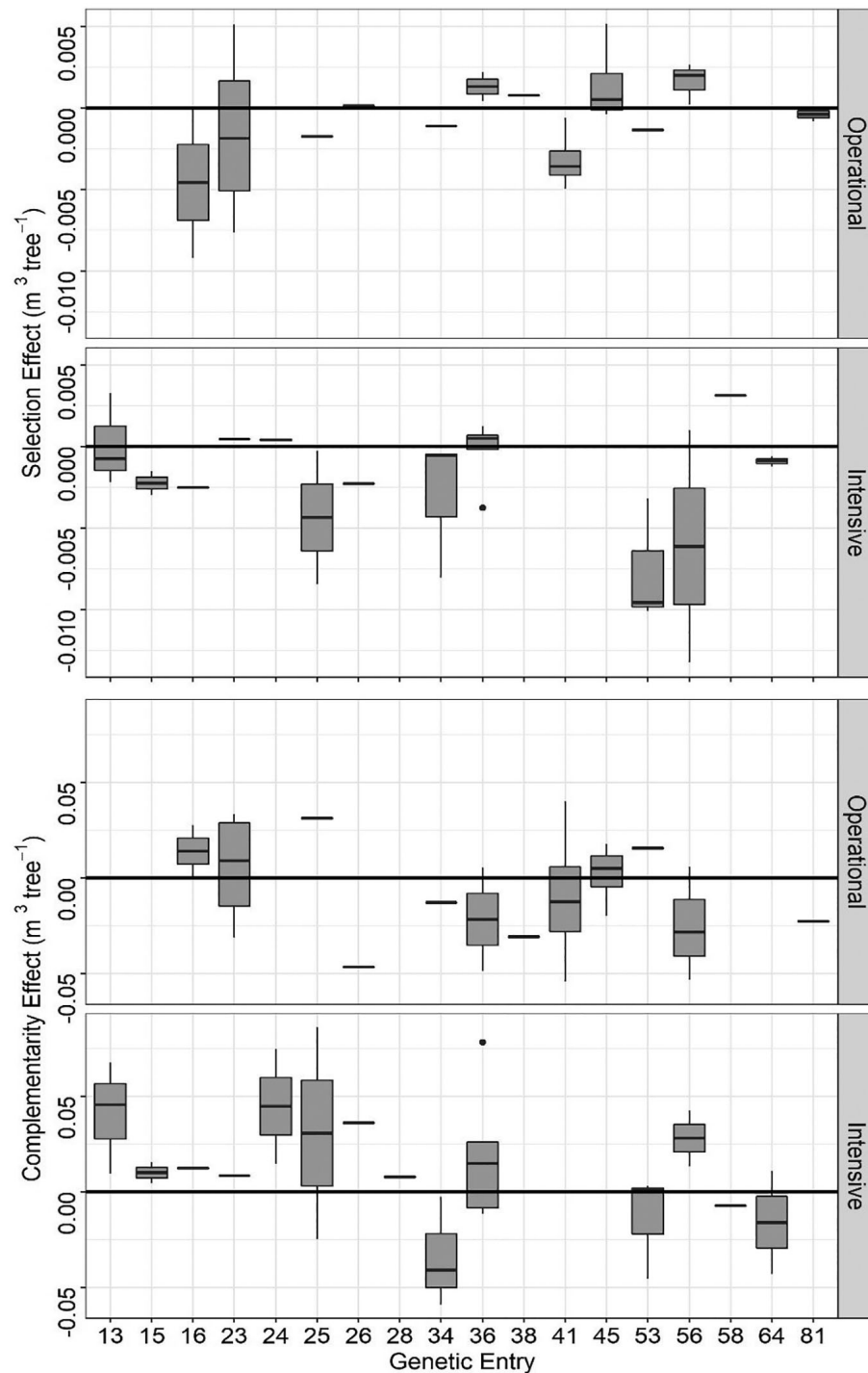


Fig. 7. Selection and complementarity effects among the two silvicultural intensities as computed by Loreau

(Fig. 7. Continued)

and Hector (2001) in the final year of measurement for genetic mixtures under operational and intensive silviculture. Means are reported with $\pm$ SE and are averaged over site (Virginia, North Carolina, and Brazil) and spacing (618, 1235, and 1854 trees per hectare). Means approaching the zero-line are genetic combinations which show no net gain from complementarity or selection. Those below the line are the genetic combinations that show a negative complementarity or selection effect relative to their pure row analogues. Those above the line are the genetic combinations that show a positive complementarity or selection effect relative to their pure analogues. Genetic entry is referencing which two genetic entries are growing side-by-side in the mixed rows (i.e., 13 equals genetic entry 1 and genetic entry 3 growing together). In box-and-whisker plots, the boxes represent the interquartile range, the median is the horizontal line inside the box, and the whiskers are the highest and lowest observation.

growing certain genetic entries together and the mechanism underlying potential gains needs to be done to potentially increase productivity and resource utilization, as well as knowledgably deploy certain genetic mixtures.

MANAGEMENT IMPLICATIONS

This study provided evidence that alternating genetic entries by row may allow for increased stand-wide productivity of approximately $1.85 \text{ m}^3 \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$. If the trends measured here were to hold across additional sites in stand-wide deployment, a mixed system may increase productivity while relaxing the strain on operations compared to a pure system. Intra-genotypic competition in clonal stands is likely reduced when a complementary genotype is co-planted. Trees may be able to remain in competition for longer under mixed systems—as indicated by the analysis of the number of living neighbors—meaning thinning could be delayed as competition-based mortality may be reduced in mixed plantations. Mixing genetic entries may also allow for greater deployment of costly, elite genetics. Phenotypically lesser genetic entries could be placed in rows anticipated for removal in mechanical thinning operations but used to maintain spacing until that time. Retained rows would be composed of more elite genetics expected to grow into more high-value products. The growth increase from mixing complementary genotypes may also aid to offset the cost of this planting stock. Mixtures of genetic entries may also indirectly increase production by decreasing the incidence and severity of pests

and disease (Marcatti et al. 2017). This deliberate deployment of genetic entries would allow for a reduction in growing stock expenses while, given what data from this study suggest, not sacrificing stand-wide productivity.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3279/full>