



Can Substituting Biochar for Peat Maintain Southern Pine Seedling Quality and Reduce Nursery Carbon Emissions?

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Abstract

Peat extraction for nursery substrates contributes to carbon emissions and peatland destruction, highlighting the need for sustainable alternatives such as biochar. In this study, we evaluated how biochar substitution of peat (0–100% by volume) combined with four fertilizer rates (0, 0.5×, 1×(standard), 2×) affected seed germination, seedling survival, total seedling biomass, root-to-shoot biomass ratio (RSR), and CO₂ flux rates during containerized seedling production of three primary southern pine species (*Pinus taeda* (loblolly pine), *Pinus palustris* (longleaf pine), and *Pinus elliottii* (slash pine)). Seed germination differed among species, with slash pine showing the highest rate (85.6% germination), followed by loblolly (82.1%) and longleaf pine (66.1%), while biochar had no detectable effect ($p=0.0618$). Seedling survival followed a similar pattern, with longleaf pine exhibiting the lowest survival (36–44% survival) and loblolly the highest (68–92%). Partial biochar substitution (25–50% biochar levels) supported seedling survival comparable to peat alone, whereas 100% biochar level significantly reduced survival ($p<0.05$). Total seedling biomass responded strongly to fertilizer and high biochar levels. Fertilizer increased biomass in loblolly and slash pine, peaking under the standard rate, whereas high biochar levels (100%) reduced growth, particularly in these species. Longleaf pine seedling biomass increased modestly under the 0.5× and standard fertilization rates and 25% biochar level. RSR responded to fertilizer, while biochar had no effect. CO₂ flux rate declined only at 100% biochar level and increased with fertilizer ($p<0.001$), remaining lower only under no fertilizer. These findings indicate that partial peat substitution with 25–50% biochar may sustain seedling quality, whereas complete substitution impairs growth. Responses were species-specific, underscoring the need for tailored substrate and nutrient management for sustainable nursery production.

Keywords *Pinus taeda* L · *Pinus palustris* Mill. · *Pinus elliottii* Engelm. · Carbon sequestration · Climate-smart forestry · Forest resilience

Introduction

The increasing concentration of greenhouse gases (GHGs) in the atmosphere is a critical driver of global climate change, prompting urgent efforts to reduce carbon emissions across multiple sectors (Verheggen et al. 2014). An important anthropogenic contributor to carbon emissions is land-use change, including degradation and mining of peatlands, which, when undisturbed, serve as major carbon sinks (Hansen et al. 1998). Peat extraction for horticultural and forestry nursery substrate not only releases stored carbon but also causes ecosystem disruption. The United States uses approx. 2 Tg of peat annually, with around 80% imported primarily from Canada (Di Lonardo et al. 2017; Brioché 2022). However, political and economic uncertainties, such as volatile tariff policies and potential export bans on peat from Canada, threaten the stability of the peat supply chain. In addition, container seedling production in the southern United States represents a large and growing industry (Starkey et al. 2015; Meng et al. 2019). Together, these environmental and supply-chain pressures highlight the need for alternative nursery substrates that reduce reliance on peat and maintain seedling quality (Mariotti et al. 2023; Simiele et al. 2022).

Biochar, a carbon-rich product produced through the slow pyrolysis of biomass, is increasingly viewed as a promising substitute for peat in nursery substrate (Di Lonardo et al. 2017; Leopard et al. 2025). This is especially true in the southern United States, where frequent windstorms and insect infestations generate large amounts of forest debris (Vogt et al. 2020). Such biomass can be converted into an affordable biochar, reducing carbon emissions in the process. Due to its porous structure, high cation exchange capacity (CEC), and strong water retention, biochar can replicate many of the beneficial physical and chemical properties of peat (Steiner and Harttung 2014; Huang et al. 2019; Dumroese et al. 2020). However, adding biochar to substrates can significantly change substrate pH, bulk density, and air-filled porosity—especially when applied at higher rates—making it essential to maintain proper cultural practices to ensure healthy seedling growth (Dumroese et al. 2018; Guo et al. 2018). Biochar is also more resistant to chemical and microbial breakdown than peat, which could lead to lower emissions from nursery production systems (Hashemi et al. 2024; Bolan et al. 2023). Counteracting these benefits, nursery operations often rely on frequent irrigation and fertilization (South and Nadel 2020; Sainju et al. 2008; Jassal et al. 2011), which can influence carbon fluxes (Leopard et al. 2025). This underscores the need to evaluate biochar within the framework of standard nursery fertilization and irrigation practices.

The origin, particle size distribution, and the proportion of biochar replacing peat in nursery substrates are critical factors that can significantly influence the quality and growth of seedlings. Dumroese et al. (2018) substituted up to 75% of peat volume with three biochar types (powdered biochar, pyrolyzed softwood pellets, and composite wood–biochar pellets) under two nitrogen fertilization regimes in *Pinus ponderosa* (ponderosa pine). They found that peat could be substituted with 25% powdered biochar and up to 50% pelletized biochar without

compromising seedling morphology. Several other studies on container-grown seedlings also demonstrated biochar's potential to reduce peat usage (Yu et al. 2023; Guo et al. 2018; Huang et al. 2019) and improve seedling establishment, overall growth, and total biomass accumulation (Ali et al. 2021; Das et al. 2020). In contrast, in other studies, biochar additions resulted in little to no improvement in seedling survival or growth (Griego et al. 2026; Sarauer and Coleman 2019). High application rates or biochar types with unfavorable properties (e.g., extreme pH, low nutrient content, or unsuitable particle size) can even impair seedling quality (Atkinson et al. 2010; Thomas et al. 2013). Changes in peat-based substrate physical structure caused by the addition of biochar could also influence relative seedling root and shoot biomass allocations (i.e., root-shoot biomass ratio (RSR)) as well as total biomass (Boudreault et al. 2023; Griego et al. 2026). Under reduced nutrient availability, seedlings tend to allocate relatively more biomass into roots than into shoots (Razaq et al. 2017), indicating that biochar-mediated changes in substrate structure and nutrients could influence patterns of carbon partitioning during nursery seedling production.

Building upon this foundation, we conducted this study on integrating biochar into container nursery seedling production of three primary southern pine species, viz., *Pinus taeda* (loblolly pine), *Pinus palustris* (longleaf pine), and *Pinus elliottii* (slash pine). Specifically, we aimed to quantify the effects of biochar and fertilizer rates on (1) seed germination, (2) seedling survival, (3) total biomass of seedlings, (4) biomass allocation to roots and shoots (RSR), and (5) CO₂ flux rates.

Overall, we hypothesized that intermediate levels of biochar substitution (25–50% by volume) would provide optimal conditions for germination, survival, and growth, and that increasing biochar content would reduce overall CO₂ flux rates. Conversely, increasing fertilizer rates would increase CO₂ flux rates. We expected that total biomass and RSR would be significantly affected by treatment, with increasing biochar levels leading to higher RSR. The findings of the study provide insights into the potential of biochar to reduce peat use and carbon emissions from pine nursery operations in the southern United States and potentially in similar systems elsewhere.

Methods

Experimental Design and Growing Conditions

To test our hypotheses, we grew the three pine species (i.e., loblolly, slash, and longleaf pines) using four rates of biochar mixed with peat in combination with four rates of fertilizer, replicated three times (described below). Seedlings were grown in a greenhouse at Auburn University's Southern Forest Nursery Management Cooperative (SFNMC) between April and December 2024, with an air temp maintained near 22 °C. Our container is commonly used to grow pine seedlings in the southern United States (FT45, 45-cavity trays). Each tray has six rows of cavities, alternating between seven and eight cavities per row.

Containers were filled with one of four peat–biochar mixtures. Biochar was sourced from Ashwood Trinity (Auburn, AL, USA) (<https://www.ashwoodtri>

nity.com), a local producer using pine biomass residue. The biochar's particle size distribution was 0–2 mm=30.8%, 2–4 mm=40.2%, 4–8 mm=24.0%, and 8–16 mm=4.9%. It contained 85.9% carbon, 6.7% ash, and 0.86% nitrogen, with total phosphorus and potassium concentrations of 790 mg kg⁻¹ and 2445 mg kg⁻¹, respectively. Inorganic nitrogen concentrations were 96.6 mg kg⁻¹ ammonium (NH₄⁺) and 0.6 mg kg⁻¹ nitrate (NO₃⁻). Additional substrate properties, including pH, bulk density, and nutrient content, are provided in Table 1.

Using this biochar, we prepared four peat–biochar mixtures using a cement mixer: 0% (control), 25%, 50%, and 100% biochar added by volume to conventional peat-based growing substrate. The peat-based growing substrate used was Sun Gro Horticulture (Sun Gro Horticulture Canada Ltd., Mississauga, ON, Canada), which contained 75–85% sphagnum peat moss, perlite, dolomite, and 0.25% silicon dioxide. Substrate samples were collected from each peat–biochar mix (n=3) immediately after mixing and before sowing for testing.

These four biochar mixtures were used to fill the containers that were assigned to one of four fertilizer rates (described below); thus, there were 16 treatment combinations. Each treatment combination was replicated three times (except the slash pine 100% biochar * 2×fertilizer treatment combination, which was omitted due to an error), resulting in a total of 48 trays. Each replicate of 16 treatment combinations constituted a block. Trays were maintained in fixed positions within blocks throughout the experiment, with blocking used to account for potential spatial gradients across the growing area.

Into each tray, for each species, one locally sourced, stratified seed was sown into each of 15 cavities (i.e., two rows). Species were arranged consistently within each

Table 1 Physical and chemical properties of the peat-biochar mixtures at volumetric proportions of 0, 25, 50, and 100% biochar

	Biochar Level			
	0%	25%	50%	100%
pH	6.77	7.22	7.59	8.91
Bulk Density (kg/m ³)	128.1	144.2	160.2	191.6
Electrical Conductance (mmhos/cm)	1.05	0.70	0.64	0.78
Soluble Salts (ppm)	735	490	448	546
Nitrate-Nitrogen (ppm)	<0.05	0.08	0.06	<0.05
Phosphorus (ppm)	9.6	2.7	1.9	0.4
Potassium (ppm)	84.0	83.5	85.1	138.6
Magnesium (ppm)	46.7	17.4	10.9	5.2
Calcium (ppm)	100.2	32.1	21.8	4.1
Boron (ppm)	<0.05	<0.05	<0.05	<0.05
Copper (ppm)	0.14	0.06	0.05	<0.05
Iron (ppm)	<0.05	0.07	0.09	0.24
Manganese (ppm)	<0.05	<0.05	<0.05	<0.05
Zinc (ppm)	0.08	0.04	0.02	<0.05
Sodium (ppm)	43.0	46.5	52.9	85.0
Aluminum (ppm)	<0.05	0.12	0.16	0.52

tray in adjacent two-row groupings, with rows assigned in the same order across all trays. Seeds were soaked in aerated water for 24 h, lightly dried to remove surface moisture, and then stratified at 3 °C and staggered to ensure all species were sown concurrently in early April 2024.

Fertilization and Irrigation

We began fertilizer treatments on April 15, 2024. The four fertilizer rates were 0, 0.5×, 1×(standard), and 2×. Every four days, we applied a liquid 20–10–20 (N-P₂O₅-K₂O) fertilizer (EZ-GRO, ~1 g mL⁻¹; Abbotsford, British Columbia, Canada) at 0, 105, 211, and 422 ppm N (i.e., 0, 0.5×, 1×, and 2×, respectively). The twelve trays corresponding to a specific fertilizer rate received 3.8 L of fertigation per application, delivering approximately 0, 0.5, 1, and 2 mg N per seedling per application, respectively (Jackson et al. 2012). Fertigation continued until December 7, 2024.

All seedling containers were irrigated daily, four times per day for five minutes each, using a mist spray system. Based on system calibration and typical flow rates, this delivered roughly 4 mm of water per irrigation event. Irrigation frequency and duration were maintained consistently throughout the eight-month experiment, from sowing in early April until the final measurements in December. Fertigation was applied separately, typically one to two hours after the first irrigation event each day. A fixed irrigation regime was maintained in our study, although operational nurseries may adjust irrigation as needed. Uniform irrigation was applied across all treatments despite known influences of biochar particle size and porosity on water-holding capacity (Adhikari et al. 2023).

Seedling Measurements

We recorded germination (%) one month after sowing as the proportion of sown seeds that successfully emerged. Seedling survival rate was recorded four months after sowing (August 28) as the proportion of germinated seedlings that remained alive. We harvested seedlings seven months after sowing (December 9) to determine total biomass and root-to-shoot biomass ratio (RSR) of seedlings. RSR was calculated as the dry mass of roots divided by the dry mass of shoots after drying each component at 70 °C for 72 h. For each treatment combination, total biomass and RSR were calculated as the mean of up to ten dried seedlings (or all surviving seedlings when fewer than ten remained), and further analyses were conducted on these treatment-level means.

CO₂ flux rates were measured three months after sowing over three weeks in July 2024. During each weekly measurement period, one tray per treatment combination was randomly selected from the three replicate blocks, and up to 10 surviving seedlings per species within each tray (maximum of 30 seedlings per tray) were used for dark respiration measurements. When more than 10 seedlings of a species were present, excess individuals were randomly removed from their cavities during measurement to maintain equal representation and then returned immediately afterward;

when fewer than 10 were present, all available seedlings were included. Cavities without seedlings had their media removed and remained empty. Measurements were conducted concurrently for all species within each selected tray, and the same trays and seedlings were repeatedly measured across all sampling days and weeks. For each weekly fertilization cycle, CO₂ flux was measured beginning 20–30 min after fertilizer application (day 1) and continued daily for three additional days, resulting in four consecutive days of measurements per week. This schedule was repeated over three consecutive weeks using the same trays and seedlings, yielding three temporal replicates of measurements. Seedlings were placed in a closed custom-built gas flux chamber connected to a LI-COR trace gas analyzer (LI-830 CO₂ Gas Analyzer, LI-COR, Inc., Lincoln, NE, USA), following Leopard et al. (2025). Dark respiration was measured over approximately 15 min under dark conditions to prevent chamber heating and photosynthetic interference. Flux rates were calculated using regression analysis of CO₂ concentration over time and represent total chamber respiration, including plant (autotrophic) and substrate microbial (heterotrophic) components.

Statistical Analyses

Data were analyzed using a combination of descriptive and inferential statistics. Key variables, including germination, total biomass, RSR, and seedling survival, were summarized descriptively. Germination and seedling survival were analyzed using two-way ANOVA with species and biochar level or species and fertilizer rate as factors, as appropriate. Total biomass and RSR of seedlings were analyzed both across species and within species to capture species-specific responses. Post-hoc comparisons were made using Tukey's HSD. The CO₂ flux data ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) were analyzed using repeated-measures ANOVA to account for temporal measurements within trays and blocks, with fertilizer and biochar as fixed factors and block as a random factor. Day of measurement was included as a repeating factor, and interactions among factors were tested.

All analyses were performed using R Studio, with Shapiro–Wilk and Levene's tests to ensure model assumptions of normality and homoscedasticity. Significance was set at $\alpha=0.05$.

Results

Germination and Survival

Species significantly affected germination ($p<0.05$), whereas biochar level ($p>0.05$) and the species $\times$ biochar interaction ($p>0.05$) were not significant. Germination of loblolly pine ($82.1 \pm 5.2\%$) and slash pine ($85.6 \pm 1.9\%$) did not differ ($p>0.05$) and were greater than that of longleaf pine ($66.1 \pm 4.4\%$) ($p<0.05$). Across biochar levels, germination ranged from 72% at 0% biochar to 80% at 100% biochar.

The fertilizer×biochar interaction was significant for seedling survival (Table 2), with post hoc comparisons indicating that the 2× fertilizer consistently reduced survival relative to lower fertilizer rates at multiple biochar levels. Specifically, survival in 2× fertilizer seedlings was lower than 0.5× at 0% ($p < 0.01$), 25% ($p < 0.05$), and 100% ($p < 0.05$) biochar, and also lower than 1× at 25% biochar ($p < 0.05$). All other fertilizer×biochar combinations were not significantly different.

For fertilizer, mean survival was highest (73%) for seedlings receiving the 0.5× fertilizer treatment and lowest (55%) for those receiving the 2× fertilizer treatment ($p < 0.01$; 95% CI: −0.300 to −0.056) (Fig. 1). Survival for the no-fertilizer (67%) and 1× (63%) treatments was similar ($p > 0.05$) and intermediate to the 0.5× and 2× fertilizer treatments.

Table 2 ANOVA summary of seedling survival showing the effects of fertilizer application rate, biochar level, and species, including their interactions. Significance is indicated as ***, $p \leq 0.001$; **, $p \leq 0.01$; *, $p \leq 0.05$; ns, not significant

	Df	Sum Sq	Mean Sq	F-Value	P-Value	Significance
Fertilizer Rate	3	0.577	0.1925	5.177	< 0.01	**
Biochar Level	3	0.396	0.1321	3.552	< 0.05	*
Species	2	4.855	2.4273	65.280	< 0.001	***
Fertilizer Rate × Biochar Level	9	0.919	0.1021	2.747	< 0.01	**
Fertilizer Rate × Species	6	0.226	0.0376	1.012	> 0.05	ns
Biochar Level × Species	6	0.221	0.0368	0.990	> 0.05	ns
Fertilizer Rate × Biochar Level × Species	17	0.626	0.0368	0.990	> 0.05	ns
Residuals	91	3.384	0.0372			

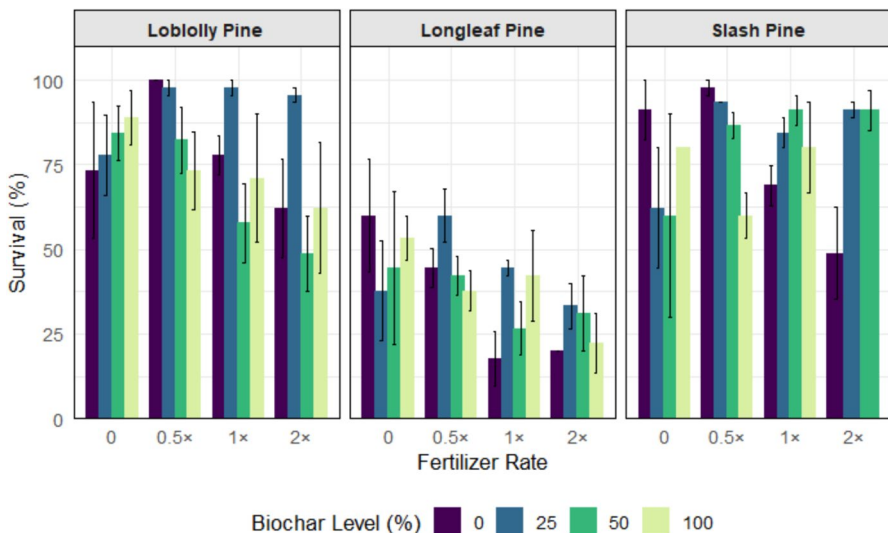


Fig. 1 Survival (%) of pine seedlings across fertilizer application rates and biochar substitution levels, shown separately for loblolly pine, longleaf pine, and slash pine. 1× represents the standard rate of fertilizer application in our study (equivalent to ~1 mg N/seedling applied once every 4 days). Error bars represent the standard error of the mean. Absence of error bar represents a lack of variation within replications

For biochar, survival was similar among treatments of $\leq 50\%$ biochar levels ($p > 0.05$). Survival in the 25% biochar treatment was approximately 15 percentage points higher than in the 100% biochar treatment ($p < 0.05$), indicating reduced survival at full biochar substitution.

Across species, survival declined at the 100% biochar level. Mean survival in the 100% biochar treatment was 15 percentage points lower than in the 25% biochar treatment ($p < 0.05$; 95% CI ± 0.063), while survival at moderate substitution levels (25–50%) did not differ from the control ($p > 0.05$) (Fig. 1). Longleaf pine survival was consistently low (18–60%) and unaffected by treatment combinations. In contrast, loblolly and slash pine were more responsive: both species achieved near-complete survival ($\geq 93\%$) at $0.5 \times$ fertilizer with low to moderate biochar, but survival declined sharply (49–62%) under $2 \times$ fertilizer, particularly at 0–50% biochar ($p < 0.05$). At 100% biochar, slash pine survival dropped to 60% under $0.5 \times$ fertilizer but remained higher (80%) with no or $1 \times$ fertilizer.

CO₂ Flux Rate

The fertilizer $\times$ biochar interaction was significant for CO₂ flux rates (Table 3), with the magnitude of the fertilizer response varying across biochar levels. Across low to moderate biochar level (0–50%), fertilized treatments consistently increased respiration relative to unfertilized controls. For example, $1 \times$ and $2 \times$ fertilizer at 0% biochar increased flux by 0.029 – $0.046 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ($p < 0.01$), and similar positive effects were observed at 25–50% biochar. Fertilizer rate and biochar level also had significant main effects, whereas Day and all interactions involving Day as a factor were not significant.

For fertilizer, CO₂ flux rates were lowest in the no-fertilizer treatment across all biochar levels. Relative to the $1 \times$ fertilizer treatment, flux rates were reduced by approximately 28% under no-fertilizer conditions ($p < 0.001$; Fig. 2). CO₂ flux did not differ significantly between the $0.5 \times$ and $1 \times$ treatments ($p > 0.05$), nor between the $1 \times$ and $2 \times$ treatments ($p > 0.05$).

Table 3 Summary of main effects and interactions for CO₂ flux rates ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) from analysis of variance (ANOVA). Factors included biochar level, fertilizer level, and sampling Day. Significance is indicated as ***, $p \leq 0.001$; **, $p \leq 0.01$; *, $p \leq 0.05$; ns, not significant

	Df	Sum Sq	Mean Sq	F-Value	P-Value	Significance
Fertilizer Rate	3	0.02719	0.00906	38	< 0.001	***
Biochar Level	3	0.00946	0.00315	13.215	< 0.001	***
Day	3	0.00143	0.00048	2.005	> 0.05	ns
Fertilizer Rate $\times$ Biochar Level	9	0.00526	0.00059	2.452	< 0.05	*
Fertilizer Rate $\times$ Day	9	0.00365	0.00041	1.698	> 0.05	ns
Biochar Level $\times$ Day	9	0.00146	0.00016	0.68	> 0.05	ns
Fertilizer Rate $\times$ Biochar Level $\times$ Day	27	0.00483	0.00018	0.75	> 0.05	ns
Residuals	112	0.02673	0.00024			

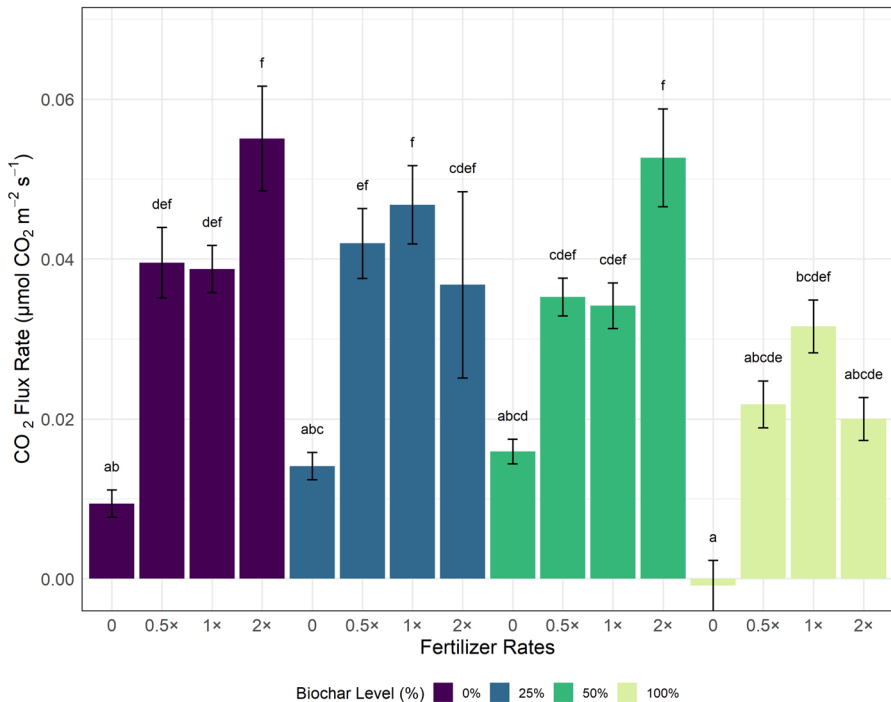


Fig. 2 Interactive effects of biochar level and fertilizer application rate on CO₂ flux rate. Error bars represent the standard error of the mean. 1× represents the standard rate of fertilizer application in our study (equivalent to ~1 mg N/seedling applied once every 4 days)

For biochar, CO₂ flux rates declined with increasing biochar proportion (Fig. 3). The 100% biochar treatment exhibited significantly lower flux than the 0%, 25%, and 50% treatments ($p < 0.001$), with reductions of approximately 16–18% relative to the 0% and 25% treatments. No differences were observed among the 0%, 25%, and 50% biochar levels ($p > 0.05$).

Seedling Biomass and Biomass Allocation

Total biomass of seedlings varied within species in response to fertilizer and biochar treatments (Fig. 4). In loblolly pine, total biomass was significantly affected by both fertilizer ($p < 0.001$) and biochar ($p < 0.01$). Total biomass increased strongly with fertilizer application, rising from 0.15 g per seedling in the unfertilized treatment to 1.80 g under the 1× fertilizer rate, followed by a slight decline under the 2× treatment. Increasing biochar generally reduced total biomass, with mean total biomass declining from 1.66 g per seedling at 0% biochar to 0.53 g per seedling at 100% biochar. In longleaf pine, neither fertilizer nor biochar significantly affected

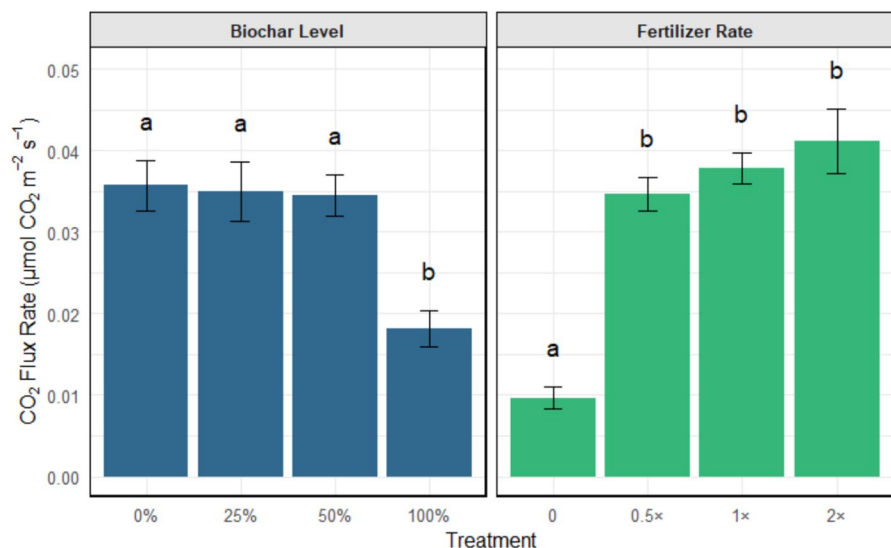


Fig. 3 CO₂ flux rate (μmol CO₂ m⁻² s⁻¹) by biochar level (left) and fertilizer application rate (right). 1× represents the standard rate of fertilizer application in our study (equivalent to ~1 mg N/seedling applied once every 4 days). Error bars represent the standard error of the mean. Letters above bars indicate statistically significant differences within each biochar level or within each fertilizer rate

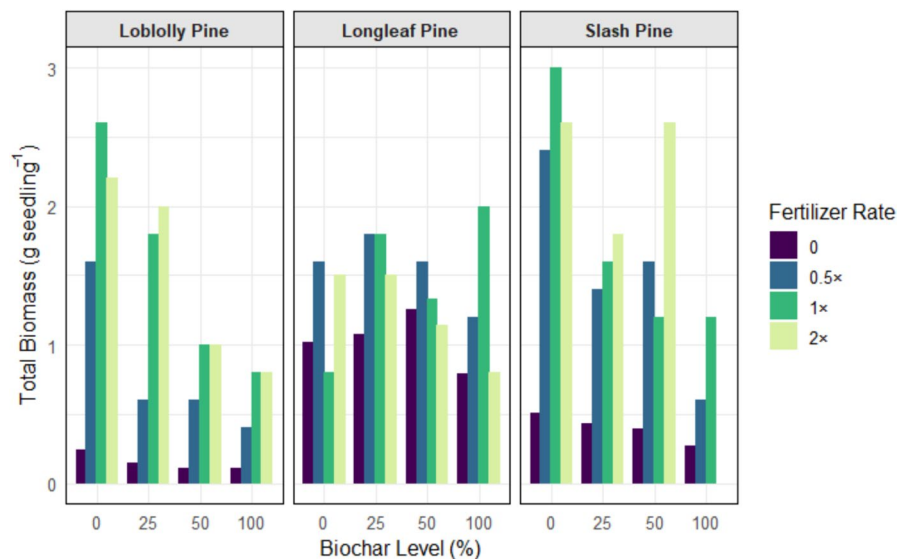


Fig. 4 Total biomass per seedling of loblolly, longleaf, and slash pine under varying fertilizer and biochar treatment combinations. 1× represents the standard rate of fertilizer application in our study (equivalent to ~1 mg N/seedling applied once every 4 days)

total biomass ($p > 0.05$); however, total biomass showed modest trends, with higher values under low to moderate fertilizer rates (1.04–1.55 g per seedling) and a peak at 25% biochar (1.54 g), followed by slight declines at higher biochar levels.

In slash pine, both fertilizer and biochar significantly influenced biomass ($p < 0.05$). Fertilizer increased biomass from 0.40 g per seedling in the unfertilized treatment to approximately 1.75 g under both the 1× and 2× fertilizer rates. Biochar effects were most pronounced at higher application levels, with biomass declining sharply at 100% biochar (0.52 g), while intermediate biochar levels (25–50%) maintained higher biomass (1.31–1.45 g). Overall, fertilizer consistently increased total seedling biomass across species, whereas high biochar levels tended to reduce biomass, particularly in loblolly and slash pine.

RSR differed significantly among species ($p < 0.001$; Table 4). Because species effects were pronounced, subsequent analyses were conducted within species to examine fertilizer and biochar influences.

In loblolly pine, fertilizer produced modest changes in RSR (Fig. 5). Mean RSR declined with increasing fertilizer, from approximately 0.45 under the 0.5× to 0.41 under the 2×. The 2× fertilizer treatment resulted in significantly lower RSR values than the 0.5× treatment ($p < 0.05$), while the difference between the 1× and 0.5× rates approached significance ($p = 0.052$). All other fertilizer comparisons were not statistically significant. Biochar did not significantly influence RSR in loblolly pine, and mean RSR values were similar across all biochar substitution levels ($p > 0.05$).

Fertilizer strongly affected RSR in longleaf pine ($p < 0.001$; Fig. 5). Mean RSR ranged from 0.44–0.46 under 0, 0.5×, and 1× fertilizer rates and increased to 0.52 under the 2×. Tukey's post hoc tests indicated that seedlings receiving the 2× fertilizer treatment had significantly higher RSR than those in all other fertilizer treatments ($p < 0.01$), whereas differences among the 0, 0.5×, and 1× treatments were not significant. Biochar had no effect on RSR, with mean values remaining consistent across all substitution levels ($p > 0.98$ for all comparisons).

Fertilizer did not significantly affect RSR in slash pine ($p > 0.05$; Fig. 5). Mean RSR ranged from 0.50 under no fertilizer and 0.5× rates to 0.48 under the 2× rate. Tukey's post-hoc comparisons indicated that none of the pairwise differences among fertilizer rates were statistically significant. Biochar also did not affect RSR, with mean values remaining similar across all substitution levels ($p > 0.05$ for all comparisons).

Table 4 Summary of ANOVA results for root-to-shoot biomass ratio (RSR) across species, fertilizer application rates, and biochar levels. The table presents main and interaction effects. Significance is indicated as ***, $p \leq 0.001$; **, $p \leq 0.01$; *, $p \leq 0.05$; ns, not significant

	Df	Sum Sq	Mean Sq	F-Value	P-Value	Significance
Species	2	0.02070	0.01035	10.373	< 0.001	***
Fertilizer Rate	3	0.00403	0.00134	1.345	> 0.05	ns
Biochar Level	3	0.00186	0.00062	0.620	> 0.05	ns
Residuals	39	0.03892	0.001			

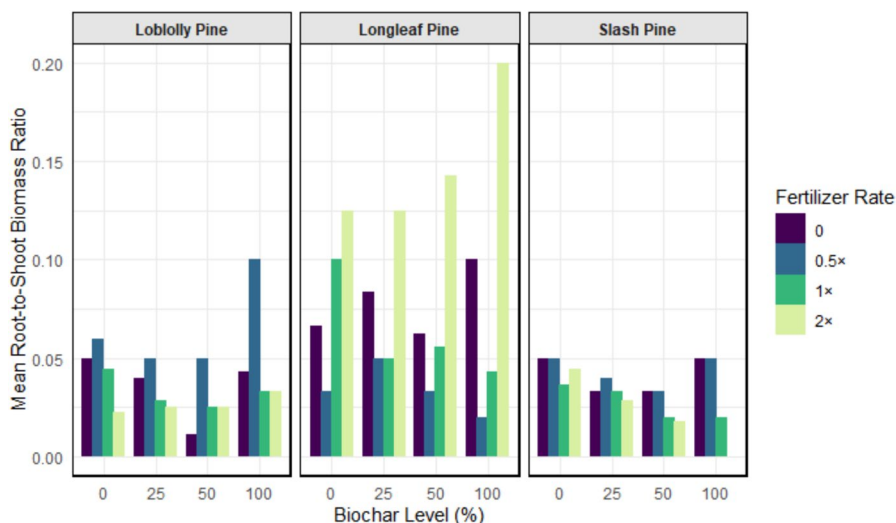


Fig. 5 Mean root-to-shoot biomass ratio (RSR) in response to biochar level and fertilizer application rate across three pine species. 1× represents the standard rate of fertilizer application in our study (equivalent to ~1 mg N/seedling applied once every 4 days)

Discussion

We hypothesized that moderate levels of biochar substitution (25–50% by volume) would provide optimal conditions for germination and growth, whereas higher concentrations might reduce seedling growth and alter root and shoot development due to shifts in substrate properties. Across all species, biochar substitution did not significantly influence germination within species, indicating that seedling emergence was largely insensitive to biochar amendment under the conditions tested. Observed differences in germination among species are consistent with well-documented interspecific variation in pine seed dormancy and early growth phenology (Barnett 1979). Thus, while species-level variation shaped emergence patterns, substrate amendment alone did not constrain initial germination, suggesting that biochar effects, if present, emerge after establishment rather than during seedling initiation.

In contrast to the minimal effects observed during germination, treatment influences became more pronounced when evaluating post-germination survival. Seedling survival was influenced by species, biochar level, fertilizer rate, and their interactions. Survival declined significantly in the 100% biochar treatment, likely due to limited nutrient availability and shifts in substrate properties, including elevated pH (8.91) and very low phosphorus content (0.4 ppm) relative to lower biochar levels. In contrast, 25% and 50% biochar did not reduce survival relative to the control, indicating that partial substitution of peat by biochar is feasible without compromising seedling survival. Fertilizer rate significantly affected survival. Seedlings receiving the standard fertilizer rate had higher survival than unfertilized seedlings, whereas those receiving the 2× fertilizer rate experienced a reduction of 18

percentage points, suggesting possible nutrient stress or toxicity at excessive fertilization levels (Wallace 1994; Kulkarni & Goswami 2019). These findings align with previous work showing that over-fertilization in container nursery systems can lead to osmotic stress, root damage, and reduced water uptake (Bumgarner et al. 2015). Collectively, these patterns indicate that survival responses were shaped both by substrate composition and fertilizer rate, with extreme biochar and fertilizer treatment levels imposing the strongest constraints.

Although overall survival patterns reflected treatment intensity, responses were not uniform across species. Longleaf pine exhibited consistently lower survival across treatments, particularly at higher biochar substitution levels. Although substrate physical properties such as porosity and air-filled pore space were not measured directly, previous studies indicate that high biochar application rates can increase microporosity and water retention while reducing air-filled porosity (Matt et al. 2018), potentially constraining gas exchange for species with early, extensive tap root development such as longleaf pine. Alternatively, reduced survival in longleaf pine may reflect phenological differences and competitive interactions, as slower early shoot development could increase susceptibility to light competition from faster-growing cohorts within shared containers. This interpretation is consistent with known life-history differences among southern pines, in which longleaf pine invests heavily in early root development at the expense of rapid shoot elongation. These explanations are not mutually exclusive and warrant further investigation. Taken together, the reduced survival of longleaf pine under high biochar levels suggests that species-specific rooting strategies may mediate tolerance to altered substrate structure. Consistent with these demographic responses, substrate amendment also altered system dark respiration.

CO₂ flux rates were partially consistent with our predictions. Seedling trays containing 100% biochar exhibited significantly lower CO₂ flux rates than all other treatments, indicating that complete substitution of peat limits biological activity. In contrast, 25% and 50% biochar treatments did not differ from the control, suggesting that moderate substitution has minimal influence on carbon flux under the nursery conditions of this study. The reduced CO₂ flux at 100% biochar likely reflects the material's chemical stability and low availability of easily decomposable carbon for microbes (Wang et al. 2015), with smaller seedlings in this treatment further contributing to lower metabolic demand. Although this study did not include seedling-free controls, our previous work (Leopard et al. 2025) using the same substrate without seedlings showed that microbial respiration alone contributes substantially to CO₂ flux, and high biochar levels ($\geq 75\%$) suppressed microbial emissions to near zero, supporting our interpretation.

While freshly added biochar can briefly stimulate microbial activity through the release of soluble carbon compounds, our measurements occurred after three months, by which time much of this labile carbon could have been depleted (Ouyang et al. 2014). Thus, substantial biochar substitutions could possibly reduce system-level dark respiration, whereas moderate incorporation maintained functional equivalence with conventional peat-based substrates. Any transient stimulation likely diminished over time, contributing to the lower CO₂ flux observed at high substitution rates. These patterns are consistent with reports that high biochar levels

suppress substrate CO₂ flux rates over time due to nutrient limitation and microbial shifts (Shoudho et al. 2024). In contrast, field studies have shown that moderate biochar substitution can stimulate microbial activity and enhance nutrient availability, although such effects are context-dependent and may differ from controlled nursery conditions (Curcio et al. 2025).

Nutrient supply further modified dark respiration CO₂ flux rates. Fertilizer strongly increased CO₂ flux rates, consistent with enhanced root and microbial activity under greater nutrient supply. However, doubling the fertilizer rate did not further increase CO₂ flux relative to the 1× rate, suggesting a threshold beyond which additional nutrients do not promote higher metabolic activity (Sebilo et al. 2013). The suppressive effects of high biochar on CO₂ flux were most evident under unfertilized conditions, indicating that adequate nutrient supply can partially offset reductions in system-level dark respiration associated with extensive peat substitution.

These respiratory responses were paralleled by patterns in seedling growth, suggesting that treatment-driven shifts in metabolic activity were reflected in biomass accumulation. Total seedling biomass was strongly influenced by fertilizer in loblolly and slash pine. In loblolly pine, biomass increased sharply with fertilizer, peaking under the 1× rate before slightly declining at 2×, indicating that moderate nutrient addition stimulated early growth whereas excessive fertilization may have imposed physiological stress or nutrient imbalance. These responses are consistent with previous studies showing that fertilization enhances photosynthetic capacity and provides additional nitrogen for leaf and shoot development, resulting in greater total dry weight (Gough et al. 2004; King et al. 2006). Longleaf pine exhibited more modest biomass increases at low to moderate fertilizer rates, aligning with findings that supplemental nitrogen promotes seedling growth traits such as needle length and root collar thickness while reflecting this species' comparatively conservative early growth strategy (Dumroese et al. 2005; Jackson et al. 2007). Slash pine responded similarly to loblolly pine, with biomass increasing under 1× but showing limited additional gains at the 2× fertilizer rate, suggesting that growth responses plateaued at elevated nutrient inputs, consistent with the reduced survival observed under excessive fertilization.

Whereas fertilizer exerted the strongest influence on biomass accumulation, biochar effects were primarily evident at higher substitution levels. In loblolly pine, high biochar (100%) reduced biomass, while lower levels had smaller or negligible effects. Slash pine showed the highest biomass at intermediate biochar levels (25–50%), with a sharp decline at 100% biochar. These patterns are consistent with previous work in pine and other species showing that small biochar substitution can enhance growth, likely by improving nutrient availability or substrate properties, whereas excessive biochar can reduce growth, potentially through nutrient immobilization or altered water retention (Robertson et al. 2012; Köster et al. 2021). Longleaf pine biomass was only modestly affected by biochar, peaking at 25% amendment before declining at higher levels. Cumulatively, these findings indicate that fertilizer acts as the primary driver of early biomass accumulation, whereas biochar effects are dose-dependent, with low substitution levels (25–50%) neutral to beneficial and complete peat substitution inhibitory for loblolly and slash pine.

RSR was primarily driven by species identity rather than substrate or fertilization treatments. Longleaf pine exhibited substantially higher RSR values than loblolly or slash pine, consistent with its characteristic early allocation to belowground biomass as an adaptation to fire-prone ecosystems (Jin et al. 2019). Within species, fertilizer influenced RSR in longleaf pine, with the highest fertilizer rates producing increased root allocation relative to shoots, while loblolly pine showed a slight decrease in RSR under the highest fertilizer level, and slash pine exhibited minimal changes. These patterns are consistent with prior work in longleaf pine, where initial nitrogen application enhanced shoot biomass, but subsequent fertilization promoted a shift toward root growth as seedlings matured (Jackson et al. 2010, 2012). In contrast, RSR in loblolly and slash pine appears less sensitive to short-term nutrient additions, highlighting species-specific allocation strategies. Biochar did not significantly influence RSR in any species, suggesting that short-term variation in substrate composition is less influential than genetic and developmental factors in determining biomass allocation. These findings indicate that while longleaf pine may adjust biomass allocation in response to fertilization, young pine seedlings' RSR is largely conserved across substrate and moderate treatment differences, consistent with other nursery studies (Zou et al. 2021).

Together, these results indicate that moderate biochar substitution ($\leq 50\%$ by volume) can substitute peat in nursery substrates without adversely affecting seedling germination, survival, CO_2 flux rates, or biomass allocation, consistent with previous work in pine seedlings (Griego et al. 2026) and containerized production systems (Alvarez et al. 2018). By contrast, full substitution (100% biochar) reduced both survival and system dark respiration, likely due to nutrient limitations and altered substrate physical properties, while excessive fertilizer similarly reduced survival, emphasizing the need for balanced nutrient management in biochar-amended substrate. These patterns underscore the importance of aligning substrate and nutrient regimes with species-specific growth strategies. Longleaf pine, in particular, may require more tailored management due to its inherently slower early development and higher root allocation.

Conclusion

We expected that moderate levels of biochar would enhance seedling quality and reduce carbon fluxes, while fertilizer would increase growth and survival. Our results partially supported these predictions. CO_2 flux declined only under complete peat substitution (100% biochar), indicating that moderate biochar substitution preserves functional system-level respiration. Seedling survival decreased under excessive fertilizer and 100% biochar, highlighting potential nutrient stress or substrate limitations at high amendment levels. Total seedling biomass increased with fertilizer but was reduced under full biochar substitution, whereas RSR responded to fertilizer in a species-specific manner but was largely unaffected by biochar. Overall, results indicate that moderate biochar substitution (25–50% by volume) can maintain seedling quality, whereas excessive biochar or fertilization may compromise it. These outcomes underscore the importance of balanced nutrient management and species-specific substrate planning in container systems.

Although container-based seedling studies provide valuable insights into short-term seedling responses, linking these results to long-term field performance is essential for a comprehensive understanding of biochar's broader impacts. Additionally, the impact of biochar is significantly shaped by variables such as feedstock origin, pyrolysis temperature, and the physical and chemical characteristics of the substrate, complicating the extrapolation of results across different species and systems. Future research should evaluate seedling quality using biochar derived from diverse sources, monitor the post-outplanting performance of seedlings cultivated in biochar-amended substrates, investigate the mechanisms influencing CO₂ flux, survival, and biomass allocation, and analyze the economic viability of substituting biochar in nursery production systems.

Abbreviations RSR: Root-to-shoot biomass ratio; CO₂: Carbon Dioxide; CEC: Cation Exchange Capacity; SE: Standard Error; ANOVA: Analysis of Variance

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Authors' contributions J.L. conducted the methodology, investigation, formal analysis, and wrote the original draft. A.S. contributed to conceptualization, methodology, investigation, formal analysis, review and editing, supervision, and funding acquisition. A.M. contributed to conceptualization, and review and editing. C.D. contributed to the review and editing. R.C. contributed to the review and editing. J.V. contributed to conceptualization, methodology, equipment, and review and editing.

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Data availability The datasets generated and analyzed during the current study are available in the supplementary material. The R scripts used for statistical analyses will be made publicly available upon publication and can be accessed via a GitHub repository at URL <https://github.com/JohnWLeopard/biochartree>.

Declarations

Competing interests The authors declare no competing interests.

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