



# Article Effects of Root Pruning and Size on Growth Traits of Hybrid Poplar Seedlings

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Abstract: Selecting seedlings of varying sizes and effectively managing root pruning are key challenges in transplantation. However, the effects of seedling size and root pruning on transplantation outcomes are not fully understood. This study classified one-year-old Populus 'Beilinxiongzhu-01' seedlings into three size categories based on height: large (308.75  $\pm$  9.66 cm), medium  $(238.00 \pm 7.71 \text{ cm})$ , and small  $(138.92 \pm 7.18 \text{ cm})$ . In early March of the subsequent year, root pruning was applied with varying intensities based on root collar diameter: low (15 times), medium (7.5 times), and high (3.75 times). A control group without pruning was also included. Over the year, key phenological and morphological traits were monitored. The results showed that (1) root pruning significantly impacted the phenology of seedlings, accelerating root emergence, delaying early leaf phenology, increasing the dieback rate, and postponing end-of-season defoliation. Mortality and the rapid growth phase were not significantly affected. Larger seedlings exhibited earlier end-of-season defoliation and higher dieback rates early in the growing season, while smaller seedlings advanced in early leaf development. (2) Except under low or no pruning, root pruning reduced seedling height (H), diameter at breast height (DBH), and root collar diameter (RCD). However, across all treatments, these indicators remained higher in larger seedlings compared to smaller ones. Under medium- and high-intensity pruning, smaller seedlings exhibited higher relative growth rates and larger leaf areas than larger seedlings, with the reduction in these variables becoming more pronounced as seedlings increased in size. Notably, only larger seedlings demonstrated a reduction in maximum growth rate, suggesting greater vulnerability to root pruning. In summary, root pruning induced significant phenological and morphological differences across seedling sizes. While smaller seedlings showed some response to pruning, larger seedlings experienced more pronounced phenological disruptions and growth inhibition.

Keywords: root pruning; seedling size; morphological traits; phenology; Populus 'Beilinxiongzhu-01'

# 1. Introduction

With the advancement of modern cultivation techniques, humans can now actively produce multiple batches of seedlings by adjusting and simulating optimal growth conditions [1]. However, achieving uniformity in seedling specifications remains a significant challenge, even with these advanced methods. Seedling differentiation is common, even within the same batch of sown seedlings [2,3]. Additionally, variations in water and fertilizer management during different seedling periods, combined with external environmental factors, often result in seedlings of varying sizes [4,5]. Morphological differences become more pronounced when seedlings are sown at different times or obtained through asexual propagation. Research has indicated significant differences in height and survival rates of hybrid aspen seedlings sown at different times [6]. For instance, a sowing delay of less



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**Copyright:** © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). than two months may cause oak seedlings to lose height and biomass equivalent to a full year's growth [7]. Similarly, the best survival and height growth results were obtained from 1-year-old *Quercus* seedlings planted early [8]. Although research has shown that batches planted near the end of the growing season do not significantly differ, achieving optimal planting dates remains a significant challenge for plant factory sites and labor allocation [9]. Furthermore, variations in seedling morphology arising from various causes pose several challenges in afforestation [10]. Generally, large seedlings can significantly increase survival and establishment rates [11], but small seedlings are more adaptable under drought stress [12]. Therefore, it is essential to categorize and manage seedlings with clear morphological differences to enhance the efficiency and success of afforestation efforts [13,14].

The root system is a crucial organ for support and nutrition, vital to the plant's growth [15–17]. Ensuring superior root growth capacity is imperative for the success of forest restoration [18,19]. Despite the availability of several strategies to improve root quality during the seedling stage [15,19], preventing root damage during transplanting and subsequent care remains challenging [20,21]. Root damage can negatively impact the quick establishment of field plantations and reduce resistance to diseases and pests [21–23]. While some studies have shown that root damage can disrupt plants' physiological balance, leading to negative outcomes [24–26], including potential plant death under extreme stress conditions [27], others have suggested that controlled root damage might serve as an effective nursery management strategy to enhance plant growth and water-use efficiency [28]. In fact, the consequences of root injury largely depend on the severity of the damage [29].

However, the differential response to root damage intensity based on seedling size remains unclear. Some studies suggest that applying the same length of root pruning to root systems of varying sizes can lead to different outcomes [3]. Researchers argue that larger trees are more sensitive to root damage, making transplantation more difficult [30]. Similarly, previous studies support the idea that smaller trees recover more effectively after root damage, primarily because their less extensive root systems are easier to reestablish [31]. Additionally, smaller trees generally exhibit higher post-transplantation growth rates, which facilitates faster recovery and damage response [32–34]. On the other hand, some studies suggest that smaller trees may be more vulnerable during transplantation, possibly due to larger trees having more extensive root systems, stronger hydraulic conductivity, and more efficient transpiration regulation, which enhance survival and stress resistance under adverse conditions [35,36]. Despite this, most existing research has focused on a single level of damage intensity, with limited studies examining the responses of seedlings of different sizes to varying levels of root damage.

Therefore, it is essential to observe seedlings of various morphological sizes and evaluate their responses to different intensities of root pruning, which is of significant importance for afforestation [2]. Poplar, a key species for afforestation in northern China, despite being genetically homogeneous when propagated vegetatively, shows significant morphological differences when produced in different batches. Currently, poplar seedlings are transplanted according to national standards and field practices using a uniform root span without considering size differences among seedlings. This uniform approach may hinder scientific seedling cultivation and affect afforestation outcomes. Most research on poplar root damage has primarily focused on mature tree transplantation or varying intensities of root damage on trees of the same size [37,38], with limited studies systematically addressing different seedling sizes. Therefore, our study selected seedlings from three growth periods, applied three levels of root pruning, and included a control group with no pruning. We make the following hypotheses: (1) Root pruning delays the primary phenological stages of seedlings, with the delay becoming more pronounced as the degree of pruning increases. Larger seedlings are more susceptible to phenological fluctuations following root pruning, while smaller seedlings show less sensitivity to these changes. (2) Slight root damage enhances leaf morphological traits and end-of-season characteristics, whereas moderate to severe root damage significantly reduces these traits, with the adverse effects on growth metrics becoming more pronounced as seedling size increases.

#### 2. Materials and Methods

#### 2.1. Seedling Preparation and Selection

The experimental materials consisted of hybrid Populus 'Beilin Xiongzhu-01' (P. alba imes*P. glandulosa*)  $\times$  (*P. tomentosa*  $\times$  *P. bolleana*) seedlings from different batches. Tissue culture and acclimatization were performed at the State-owned Populus Nursery in Guanxian County, Shandong Province, China. Based on preliminary observations and experimental design, three batches of tissue-cultured seedlings from 3 May, 23 June, and 20 July 2021 were selected for the batch difference seedling test, resulting in three populations with significant morphological differences. Once each batch of group-cultured seedlings completed the acclimatization process and reached a height of 25 cm, they were immediately transported to the Beijing Greenland Maintenance and Management Service Center. The seedlings underwent a 3-day acclimatization period in a greenhouse to mitigate transportation stress. Afterward, high-quality seedlings, selected based on uniformity and vigor, were removed from nutrient cups and transplanted to an outdoor nursery. Planting containers, supplied by Haining Ripple Zee Horticultural Factory, Zhejiang, China. were blow-molded gallon pots in sizes of 15 gallons (47 cm  $\times$  36.5 cm  $\times$  38.5 cm), 10 gallons (41.5 cm  $\times$  34 cm  $\times$  30.5 cm), and 3 gallons (28 cm  $\times$  23 cm  $\times$  25 cm), based on previous experimental observations. The growing medium consisted of a 3:1 (v:v) mixture of peat moss (Beijing Jing Sheng Flower Garden Co., Ltd., Beijing, China) and perlite (Langfang New Mass Filter Aid Co., Ltd., Langfang, China). After planting, the containers were covered with rigid sponge film centered around the root neck and were additionally covered during rainy days to prevent water infiltration. The seedlings were watered regularly, with increased frequency during drought conditions. To avoid edge effects, container positions were periodically rotated, and plastic film was laid on the ground to prevent root outgrowth. The seedlings were nurtured at the Beijing Greenland Maintenance and Management Service Center nursery until they became woody and defoliated at the end of the year.

On 10 March 2022, the height (H), diameter at breast height (DBH), and root collar diameter (RCD) of all poplar seedlings across the three batches were measured, revealing significant size differences among the seedling populations (Table 1).

Size/Index	H (cm)	DBH (mm)	RCD (mm)	H/DBH	H/RCD
Large	$308.75\pm9.66a$	$15.94\pm0.68a$	$28.47\pm0.61a$	$17.64\pm5.73b$	$9.85\pm3.22a$
Medium	$238.00\pm7.71b$	$11.07\pm0.49\mathrm{b}$	$24.20\pm0.43b$	$19.51\pm6.35a$	$8.94 \pm 2.91 \mathrm{b}$
Small	$138.92\pm7.18c$	$6.59\pm0.51c$	$14.87\pm0.29c$	$19.20\pm6.14a$	$8.52\pm2.73c$

Table 1. Initial parameters before planting.

Note: Data are mean  $\pm$  standard deviation (SD); different lowercase letters indicate significant differences (p < 0.05) for different seedling sizes.

#### 2.2. Root Pruning Procedure

On 18 March 2022, 480 uniform and disease-free seedlings were selected from each batch based on measured data for root pruning treatment. Root pruning was performed according to the seedlings' root collar diameter (RCD): 15 times the RCD (P1), 7.5 times the RCD (P2), and 3.75 times the RCD (P3), with a pruning depth of 20 cm. A control group (CK) with no root pruning was included. The experiment involved four treatments per batch, with three replicates per treatment and 40 seedlings per replicate, resulting in a total of 1440 seedlings across the three batches. After root pruning, all seedlings were uniformly transplanted into 20-gallon pots (50 cm  $\times$  42 cm  $\times$  45 cm) for observation. To minimize edge effects, the container positions were regularly rotated, and plastic film was laid on the ground to prevent root outgrowth. The pots were covered with rainproof plastic sheeting to protect against rainfall, and the seedlings were watered twice a week to maintain adequate moisture in the substrate.

#### 2.3. Indicator Measurements

#### 2.3.1. Early Planting Performance and Key Phenology

Before transplantation, we observed varying degrees of dieback in seedlings of all sizes. Only seedlings without significant dieback were selected for transplantation. One month post-transplantation, survival rates were assessed, and any new occurrences of dieback were monitored. A follow-up observation in May confirmed that there was no further increase in dieback. Dieback was quantified for seedlings where the affected length exceeded 1% of the total seedling height, with a maximum observed dieback length not exceeding 10%. The dieback rate was calculated as the number of seedlings exhibiting dieback divided by the total number of seedlings, expressed as a percentage. Starting from March 18, the date of root pruning, phenological changes were monitored every other day until full leaf expansion was achieved. The monitored stages included bud burst, leaf unfolding initiation, full leaf unfolding, and mid-leaf fall. Additionally, root emergence phenology (new root growth stage) was observed by excavating the root system every two days until more than five new roots were visible. Morphological indicators were used to fit four-parameter logistic growth curves to estimate the onset (RGO), cessation (RGC), and duration (RGD) of the rapid growth period for height (H), diameter at breast height (DBH), and root collar diameter (RCD), denoted as RGO.H, RGC.H, RGD.H, RGO.DBH, RGC.DBH, RGD.DBH, RGO.RCD, RGC.RCD, and RGD.RCD, respectively.

# 2.3.2. Responses of H, DBH, and RCD

Immediately after transplantation, we measured the height (H), diameter at breast height (DBH), and root collar diameter (RCD) of all treated seedlings. Although these post-transplant measurements showed a slight reduction compared to pre-transplant values, the changes were minimal and not statistically significant. Morphological indicators were subsequently measured monthly from March through October. For each assessment, ten seedlings were randomly selected from each replicate, and the average value was calculated to represent each treatment group. Using the collected measurements, we computed the following ratios: height to diameter at breast height (H/DBH), height to root collar diameter (H/RCD), and diameter at breast height to root collar diameter (DBH/RCD). Additionally, we calculated the relative growth rates for height (RGR.H = (H<sub>end</sub> – H<sub>initial</sub>)/ $\Delta$ t), diameter at breast height (RGR.DBH = (DBH<sub>end</sub> – DBH<sub>initial</sub>)/ $\Delta$ t), and root collar diameter (RGR.RCD = (RCD<sub>end</sub> – RCD<sub>initial</sub>)/ $\Delta$ t), where  $\Delta$ t represents the one-year interval. These data were then used to fit a four-parameter logistic growth model for H, DBH, and RCD, from which we derived the maximum growth rates (MGR) for each parameter: MGR.H for height, MGR.DBH for DBH, and MGR.RCD for RCD.

### 2.4. Statistical Analysis

The dieback rate was first subjected to an arcsine transformation before data analysis. Growth curves for height (H), diameter at breast height (DBH), and root collar diameter (RCD) were fitted using a four-parameter logistic growth model based on monthly observation data. The days on which the maximum growth rate occurred, along with the corresponding maximum growth rates, were calculated by identifying the points where the second derivative equaled zero. The onset and cessation of the rapid growth periods were determined by locating the points where the third derivative equaled zero. A two-way ANOVA was performed for all indicators, with preliminary tests conducted to assess normality and homogeneity of variance. Data transformation was applied when necessary. Multiple comparisons were carried out using the Tukey method, with a significance threshold of p < 0.05. All statistical analyses were conducted using R software (version 4.4.0). Graphs were generated with OriginPro (Learning Edition), and PCA and plotting were performed using R.

#### 3. Results

3.1. Key Phenological Traits

3.1.1. Survival Rate and Dieback

In this experiment, no seedling mortality was observed. Seedling size, root pruning intensity, and their interaction had a significant impact on the dieback rate (Table S1). Under no-pruning conditions, small seedlings exhibited only slight dieback. However, as root pruning intensity increased, the dieback rate rose significantly across all seedling sizes. Large seedlings were particularly sensitive to pruning, with any level of pruning significantly increasing the dieback rate, and medium to high pruning intensities led to especially pronounced effects, with dieback rates exceeding 30%. Medium-sized seedlings showed significant increases in dieback rate at a medium pruning intensity, while small seedlings exhibited significant increases only at a high pruning intensity (Table 2).

Table 2. Dieback rate at the early stage of planting.

Treatment	СК	P1	P2	P3
Large Middle Small	$\begin{array}{l} 0.00\% \pm 0.00\% cB \\ 0.00\% \pm 0.00\% bB \\ 3.33\% \pm 2.89\% bA \end{array}$	$\begin{array}{l} 3.33\% \pm 2.89\% bA \\ 1.67\% \pm 1.44\% bA \\ 1.67\% \pm 1.44\% bA \end{array}$	$\begin{array}{c} 31.67\% \pm 5.77\% aA \\ 15.00\% \pm 5.00\% aB \\ 6.67\% \pm 2.89\% aC \end{array}$	$\begin{array}{l} 33.33\% \pm 2.89\% aA \\ 20.00\% \pm 5.00\% aB \\ 15.00\% \pm 5.00\% aB \end{array}$

Note: Data are mean  $\pm$  standard deviation (SD); different lowercase letters indicate significant differences (p < 0.05) for different root pruning intensities at the same seedling size, and different uppercase letters indicate significant differences (p < 0.05) for different seedling sizes at the same root pruning intensity.

#### 3.1.2. Key Leaf Traits and Root Emergence

Overall, the treatments did not significantly affect the bud burst stage. However, seedling size and root pruning intensity had significant effects on both the full leaf unfolding stage and the leaf unfolding initiation stage, although their interaction did not show a significant effect. The new root growth stage responded significantly only to root pruning, while seedling size, root pruning intensity, and their interaction significantly influenced the mid-leaf fall stage (Table S2).

As seedling size increased, the leaf unfolding initiation stage was significantly delayed by 0.6 to 3.3 days, and the full leaf unfolding stage was delayed by 0 to 2.3 days. In contrast, the mid-leaf fall stage was significantly advanced by 3.0 to 7.0 days as seedling size increased. With increasing root pruning intensity, both the leaf unfolding initiation stage and the full leaf unfolding stage exhibited noticeable phenological delays. Specifically, the leaf unfolding initiation stage was delayed by 3.0 to 3.3 days and the full leaf unfolding stage by 1.6 to 3.6 days. Similarly, the mid-leaf fall stage was delayed by 1.0 to 3.6 days. Conversely, the new root growth stage was notably advanced by 14 to 19.6 days with increased root pruning intensity. No significant differences were observed in the leaf unfolding stage followed a similar trend, though the delay was less pronounced. Due to the interaction effect, the mid-leaf fall stage showed significant differences in large and medium seedlings under medium to high pruning intensity, but this difference was not observed in small seedlings (Figure 1).

#### 3.1.3. Key Stem Phenological Traits

The curves fitted by the four-parameter logistic model demonstrated a high degree of fit, with R<sup>2</sup> values ranging from 0.9911 to 0.9042 (Table S2). The slope of these curves indicated that the growth trajectories of seedlings with pruned roots were more gradual compared to those of the control group (Figure 2). Significant differences were observed in the onset (RGO.DBH), cessation (RGC.DBH), and duration of the rapid growth period only in response to seedling size, specifically for DBH (Table S3). All other phenological indicators showed no significant differences (Figure 3).



**Figure 1.** Effects of root pruning intensities and seedling sizes on aspen leaf phenology and root emergence. The intensity of root pruning is indicated as follows: CK: dark blue, P1: light blue, P2: orange, P3: dark red. The phenological stages are indicated as follows: solid circle: bud burst stage, solid triangle: full leaf unfolding stage, solid square: leaf unfolding initiation stage, solid rhombus: mid-leaf fall stage, and hollow circle: new root growth stage. The dotted line connects the leaf-related indicators, and independent lines are the root indicators. Different lowercase letters indicate significant differences (p < 0.05) for different root pruning intensities at the same seedling size, and different uppercase letters indicate significant differences (p < 0.05) for different seedling sizes at the same root pruning intensity.

# 3.2. Morphological Traits

# 3.2.1. Leaf Traits and Stem Growth Rate during the Rapid Growth Period

Seedling size, root pruning intensity, and their interaction had significant effects on leaf area. Leaf thickness and the maximum growth rate of height (H) were significantly influenced by pruning intensity, while the maximum growth rate of diameter at breast height (DBH) responded significantly to pruning intensity, seedling size, and their interaction. The maximum growth rate of root collar diameter (RCD) was significantly affected only by seedling size (Table S4).

As seedling size increased, leaf area gradually increased under the control treatment and slight pruning conditions. However, under moderate to high pruning intensity, leaf area significantly decreased. Seedling size had no significant effect on leaf thickness, but leaf thickness decreased with increasing pruning intensity. Except in small seedlings, where no significant difference was observed, larger seedlings exhibited a reduction in the maximum growth rates of height and DBH as pruning intensity increased. The increment in root collar diameter decreased with increasing seedling size under control treatment and slight pruning, but no significant differences were observed under moderate to high pruning intensity (Figure 4). As pruning intensity increased, leaf area significantly decreased, with this trend being more pronounced in larger seedlings. Under moderate to high pruning intensity, leaf area decreased by 76.3% to 89.0% in large seedlings, 48.8% to 59.1% in medium seedlings, and 28.8% to 37.5% in small seedlings compared to the control treatments.



**Figure 2.** Dynamics of root pruning intensities and seedling sizes on the growth of height, diameter at breast height, root collar diameter, and the fitted logistic growth curves. In logistic growth curves, the intensity of root pruning is indicated as follows: CK: dark blue, P1: light blue, P2: orange, P3: dark red. In measured points, root pruning intensity is indicated as follows: CK: solid circle, P1: hollow circle, P2: upper half-solid and lower half-hollow circle, P3: upper half-hollow and lower half-solid circle. Numbers 1–3 denote the height (H) growth of large, medium, and small seedlings, 4–6 denote the diameter at breast height (DBH) growth of large, medium and small seedlings, 7–9 denote the root collar diameter (RCD) growth of large, medium and small seedlings, and the dashed line denotes the initial height of planting for the corresponding size, with the initial date of planting being 18 March.



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**Figure 3.** Effects of root pruning intensities and seedling sizes on growth onset and cessation days, and durations of the rapid growth period for H, DBH, and RCD. Root pruning intensity is indicated as follows: CK: dark blue, P1: light blue, P2: orange, P3: dark red. Phenology: rapid growth onset: solid squares, rapid growth cessation: solid triangles. Numbers 1–3 represent a fast-growing phenology for H, DBH, and RCD, respectively, and the dashed line represents the corresponding rapid growth duration.



**Figure 4.** Effects of root pruning intensities and seedling sizes on leaf traits and stem growth rate of during rapid growth. Root pruning intensity is indicated as follows: CK: dark blue, P1: light blue, P2: orange, and P3: dark red. Figure 4(1) indicates leaf area, Figure 4(2) indicates leaf thickness, Figure 4(3) indicates maximum growth rate of height (MGR.H), Figure 4(4) indicates maximum growth rate of diameter at breast height (MGR.DBH), and Figure 4(5) indicates maximum growth rate of root collar diameter (MGR.RCD). Different lowercase letters indicate significant differences (p < 0.05) for different root pruning intensities at the same seedling size, and different uppercase letters indicate significant differences (p < 0.05) for different seedling sizes at the same root pruning intensity.

# 3.2.2. End-of-Season Growth Traits

Seedling size, root pruning intensity, and their interaction had significant effects on H, DBH, H/DBH, H/RCD, DBH/RCD, RGR.H, RGR.DBH, and RGR.RCD. Changes in RCD were significantly influenced by seedling size and root pruning intensity, but their interaction showed no significant effect (Table S5).

Overall, the slight root pruning treatment produced results similar to the control group across various indicators. Although some indicators slightly decreased, none showed

significant differences. The major changes were observed in the medium and high pruning treatments. As seedling size increased, H, DBH, RCD, H/RCD, and DBH/RCD all gradually increased, while the increment in RCD gradually decreased. In the control and slight pruning treatments, the increments in H and DBH increased with seedling size, but under medium to high pruning intensity, these increments showed a decreasing trend. H/DBH did not exhibit a consistent pattern of change. With increasing pruning intensity, H, DBH, RCD, and their increments, as well as H/RCD and DBH/RCD, all gradually decreased. However, in large seedlings subjected to high pruning intensity, H/DBH was significantly higher than in other treatments. In other seedling sizes, H/DBH generally decreased with increasing pruning intensity (Figure 5).



**Figure 5.** Effects of root pruning intensities and seedling sizes on tree height, diameter at breast height, root collar diameter, relative growth rate of tree height, relative growth rate of diameter at breast height, relative growth rate of root collar diameter, height/diameter at breast height, height/root collar diameter, diameter at breast height/root collar diameter, diameter at breast height/root collar diameter at the end of the season. Root pruning intensity is indicated as follows: CK: dark blue, P1: light blue, P2: orange, and P3: dark red. Numbers 1–9 in order indicate height (H), diameter at breast height (DBH), root collar diameter (RCD), relative growth rate of tree height, relative growth rate of diameter at breast height, relative growth rate of root collar diameter, height/diameter at breast height (DBH), root collar diameter (RCD), relative growth rate of tree height, relative growth rate of diameter at breast height (H/RCD), diameter at breast height/root collar diameter (DBH/RCD). Different lowercase letters indicate significant differences (p < 0.05) for different root pruning intensities at the same seedling sizes at the same root pruning intensity.

## 3.3. PCA and Correlation Analysis

Principal component analysis (PCA) revealed that the first principal component (Dim1) accounted for 46.3% of the total variance, while the second principal component (Dim2) accounted for 33.4% of the total variance. Growth indicators contributed significantly to Dim1, underscoring their importance in explaining the overall variation. Phenological indicators contributed significantly to Dim2, highlighting the role of these phenological stages in physiological variation (Figure 6). The correlation matrix further illustrated the relationships between phenological indicators and growth traits (Figure 7).

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**Figure 6.** PCA loading plot. Arrow direction and length indicate the contribution of each variable to the principal components: RGR/RCD, MGR/RCD, RGR/DBH, MGR/DBH, MGR.H, RGR.H, H/DBH, H/RCD, DBH/RCD, H, DBH, RCD, leaf area, leaf thickness, dieback, leaf unfolding initiation stage, full leaf unfolding stage, mid-leaf fall stage, and new root growth stage. Color intensity reflects the degree of contribution to Dim1 (66.3%) and Dim2 (23.4%).



**Figure 7.** Correlation matrix of growth and phenology indicators. Colors indicate the strength and direction of the correlations: red represents positive correlations, blue represents negative correlations, and the intensity of the color reflects the magnitude of the correlation. The numerical values in each cell represent the Pearson correlation coefficients (r) between the corresponding variables. The variables include leaf unfolding initiation stage, full leaf unfolding stage, mid-leaf fall stage, new root growth stage, dieback, H, DBH, RCD, H/DBH, H/RCD, DBH/RCD, RGR.H, MGR.H, RGR.DBH, MGR.DBH, RGR/RCD, leaf area, and leaf thickness.

# 4. Discussion

## 4.1. Effects of Root Pruning Intensity and Seedling Sizes on Key Phenological Traits

This study reveals significant differences in the mid-leaf fall stage at the end of the growing season between seedlings of different sizes under root pruning conditions. However, during the early phenological stages post-planting, these differences were not pronounced, indicating that the response was primarily driven by a single factor. These findings lend support to our first hypothesis.

Regarding bud burst, the results indicate that none of the treatments significantly affected its timing. Previous studies have consistently shown that smaller trees tend to initiate bud bursts earlier than larger ones [39]. However, some researchers have posited that the relationship between tree size and bud burst timing is non-linear, with the difference between saplings and small trees being less marked than that between small and large trees [40]. The relatively minor size differences among seedlings in our study might account for the absence of discernible differences in bud burst timing. The direct impact of mechanical root damage on bud burst remains underexplored. However, existing evidence suggests that the early stages of ecological dormancy are not heavily dependent on nutrient supply from distal roots [41]. Research on Populus species has shown that vessel differentiation occurs almost concurrently with bud bursts, implying that nutrient reserves within the buds play a more critical role than nutrient supply from distant roots [42]. Xie (2018) observed in grapevines that the initial sign of bud development is an increase in bud water content, occurring prior to the establishment of vascular connections between the bud and cane [43]. These findings suggest that early bud development primarily relies on internal resources, rendering the impact of root damage on bud phenology less significant.

As bud development progresses, the formation of vascular bundles enables the roots to supply substantial water and nutrients necessary for leaf expansion and growth [42,43]. Our study demonstrates that root damage significantly delays the leaf unfolding initiation stage and full leaf unfolding stage in spring. Unlike bud burst, post-budburst leaf growth requires greater nutrients and water, which are transported from other organs via the vascular system [41]. Consequently, the reduced water potential and impaired nutrient uptake due to root damage adversely affected leaf expansion [44,45]. The influence of tree size on leaf appearance mirrored the patterns observed in bud burst; first-year seedlings generally leaf out earlier, with leaf appearance progressively delayed in larger trees [46]. Our findings corroborate this, as smaller trees exhibited significantly earlier leaf expansion than larger ones. This may be attributable to the lower thermal sum required for smaller trees to leaf out [47] and, ecologically, to the necessity for smaller seedlings in a forest to leaf out earlier to secure adequate sunlight for carbon acquisition [40].

Research on Douglas fir has indicated that trees rely on photosynthetic products obtained after leaf expansion to initiate new root growth [48], aligning with our observations. In our study, undamaged roots initiated new root growth only after leaf expansion, while root-damaged treatments exhibited earlier root growth, nearly concurrent with initial leaf expansion. This premature root growth may result from damage, as previous studies have suggested that root sprouting may be an adaptive response to severe and recurrent damage, with root bud formation often triggered by root injury or breakage [49]. Damaged roots are more likely to form callus, adventitious buds, and new root buds than undamaged roots, mainly when damage occurs in spring, where sprouting levels increase significantly [50]. Research on apple trees has demonstrated that the primary sources of carbohydrates and nitrogen for root sprouting are nutrients accumulated in the previous year rather than those absorbed from the environment [51]. Thus, stress-induced root growth may elevate the overall nutrient demand of the plant, exacerbating resource deficits during early growth stages reliant on local nutrient reserves. Accumulated stresses could have a compounding effect, progressively weakening the tree [52], which may also contribute to the delayed leaf phenology.

As growth intensified, dieback was observed at the shoot tips. Previous studies on root freezing injury or drought damage have reported similar dieback phenomena, attributing

them to hydraulic failure and carbon starvation caused by root damage [53,54]. Larger trees typically possess more extensive root systems, stronger hydraulic conductivity, and superior anatomical features, coupled with more efficient transpiration regulation, which enhances their survival and resilience to environmental stress [35,36].

Research on suburban trees has shown that smaller trees are more susceptible to dieback compared to larger ones. A regression analysis indicated that for every meter increase in tree height, crown dieback decreased by 0.8%. This suggests that larger trees may possess some resistance to dieback [55]. This finding aligns with our control group results, where smaller seedlings exhibited significantly more dieback than larger ones under non-stress conditions. However, when root systems were severely damaged, larger trees showed more pronounced dieback. Some studies show that when these critical structures are severely compromised, the inherent advantages of larger trees may amplify the negative effects, leading to more pronounced dieback [36]. The higher physiological and nutritional demands of larger trees may not be met when root systems are damaged, rendering them more vulnerable to dieback [35]. Other studies have also underscored the heightened sensitivity of larger trees to stress. For instance, research on Abies alba has demonstrated that larger trees are more sensitive to elevated temperatures and drought conditions than smaller ones, resulting in higher rates of decline and mortality [56]. In our study, dieback was observed only within the first month post-transplantation, with no further dieback observed subsequently, and no seedling mortality was recorded, in contrast to previous transplantation studies [11,57]. This may be attributed to the ample water provided in our experiment, which mitigated severe transplant shock [16,17].

Regarding the rapid growth phase during the growing season, we found that apart from some treatments slightly advancing the timing of DBH, the treatments did not significantly affect the initiation date or duration of height and basal diameter growth. Studies on temperate and boreal forest trees have demonstrated that temperature is the critical factor determining the onset of the rapid growth phase, particularly in early spring and summer. Even under stress conditions, temperature and photoperiod remain the dominant factors controlling growth initiation, while stress primarily reduces the growth rate [58]. This finding aligns with our study on the maximum growth rate during the rapid growth phase, where stress significantly reduced the maximum growth rate of H and DBH.

Studies have shown that shortening photoperiods and decreasing temperatures are the principal drivers of leaf senescence in autumn [59]. Due to their varying water and nutrient requirements, trees of different sizes may exhibit differences in their responses to these environmental changes [60]. However, no consensus remains on how tree size influences autumn leaf senescence. Some studies suggest that smaller trees may shed their leaves earlier, as larger trees generally possess greater photosynthetic capacity and resource accumulation, enabling them to retain their leaves longer into the fall [61]. Conversely, other studies have found that larger trees may shed their leaves earlier, with research on the leaf lifespan of Taiwanese hardwood species indicating that smaller understory seedlings generally have longer leaf lifespans [62]. Our findings support the latter view, showing that smaller trees retained their leaves longer in the fall, possibly due to reduced light availability and developmental stage. Furthermore, some studies suggest that higher growth rates during the growing season may precipitate premature leaf senescence in autumn [63]. Our study supports this perspective, as larger trees with higher growth rates and larger leaf areas exhibited earlier leaf senescence, while root damage reduced growth rates and delayed leaf senescence. This may be because increased photosynthetic activity speeds up the growing season, resulting in earlier leaf senescence. In another aspect, it is widely believed that stress accelerates leaf senescence, thereby advancing the end of the growing season [64]. However, our study demonstrated that root-damaged treatments delayed leaf senescence, possibly due to impaired sugar transport from leaves to sink organs, thus postponing the senescence process [65]. Additionally, root damage occurred early in the season. After a recovery period, this phenomenon may resemble the delayed leaf senescence observed in Quercus petraea seedlings following rewatering after severe

drought. This delay in leaf senescence may compensate for productivity losses incurred during stress, with ROS and ABA potentially influencing the timing of leaf senescence during stress recovery [66].

# 4.2. Effects of Root Pruning Intensity and Seedling Sizes on Morphological Traits

Our study found that leaf thickness only responded to root pruning, RCD showed no significant interaction, and all other morphological traits exhibited significant differences in response to root damage across different tree sizes, addressing our second hypothesis.

It is generally accepted that reducing leaf area and increasing leaf thickness are coordinated strategies by which plants mitigate stress [67,68]. By minimizing leaf area, plants can reduce overall water loss, while increased leaf thickness enhances water retention capacity. However, our study found that root damage significantly reduced leaf thickness, likely due to more severe damage leading to extreme nutrient deficiency. This nutritional deficit may compel plants to adopt a short-term survival strategy by reducing investment in leaf development [69].

Our findings also demonstrated that except for mild damage, H, DBH, and RCD at the end of the growing season declined with increasing damage intensity, accompanied by a corresponding decrease in relative growth rate, consistent with previous studies [2,70,71]. Root damage significantly impaired the water and nutrient absorption capacity, inhibiting growth [24]. Additionally, trees experiencing root damage may prioritize resource reallocation to sustain survival, which could further suppress stem growth [72]. Some studies have suggested that mild damage can stimulate growth, as trees may enhance nutrient absorption and utilization efficiency to support physiological repair and growth, thereby partially offsetting the negative effects of damage [70]. This adaptive mechanism enables trees to maintain ecological function to some extent [73]. However, our study found no significant difference between mild damage and the control group, indicating that root damage disrupts the original balance without promoting plant growth.

Furthermore, as the intensity of damage increased, larger trees exhibited more pronounced growth inhibition across various growth indicators, including relative increment, maximum growth rate during the rapid growth phase, and leaf area. Previous studies have supported the theory that smaller trees display greater resilience to root damage, likely due to their advantage in recovery and rapid response following transplantation [30,32,33,74]. Smaller seedlings, compared to larger ones, suffer less severe root damage and can reestablish their root systems more readily [31], resulting in relatively lower stress during transplantation [32]. Moreover, post-transplantation, smaller trees generally exhibit higher growth rates than larger ones [32–34]. Our study also found that under moderate to severe treatments, smaller trees had a significantly higher relative growth rate than larger trees. However, some studies in the literature indicate that final growth indicators positively correlate with initial size, with larger trees consistently outperforming smaller ones across all treatments [75]. Our study also confirmed this, where larger trees maintained their advantage in H, DBH, and RCD over smaller trees, regardless of damage intensity. Previous studies have suggested that although larger trees exhibit lower growth rates than smaller ones, their absolute growth remains higher, preserving their size advantage [2,11]. Ultimately, the height increment in and final stem dry weight of larger seedlings significantly outperformed those of smaller seedlings [2]. This suggests that regardless of the growth rate of smaller seedlings, the initial size disparity prevents them from surpassing larger seedlings in absolute height [76].

By analyzing phenological and growth response patterns, we observed that the effects of treatment became increasingly apparent over time. In the early stages of phenological development, plant responses were predominantly influenced by a single factor. As dieback emerged, the interaction of two effects began to manifest, persisting through the end of the season. This suggests that plants undergo a stress-recovery process in response to damage, with the effects of treatment becoming progressively amplified. PCA and correlation analyses supported these observations.

# 5. Conclusions

Most previous studies have primarily focused on the effects of different intensities of root damage on seedlings of the same size, often overlooking the differences in how seedlings of various sizes respond to damage. Our research found that root damage leads to phenological and morphological differences in seedlings of different sizes. Except for slight damage, the adverse effects intensify with increasing root damage severity, and larger seedlings exhibit a more pronounced response under the same root damage treatment, showing greater phenological fluctuations, lower relative growth rates, and smaller leaf areas. However, by the end of the season, larger seedlings still maintained their initial morphological advantage. Based on phenological and morphological indicators, we recommend minimizing root damage during poplar transplantation. If damage is unavoidable and water supply is sufficient, larger seedlings are preferable under mild to moderate root damage. Only under severe damage should careful consideration be given to the potential reduction in stability and resistance associated with using larger seedlings.

**Supplementary Materials:** The following supporting information can be downloaded at https://www. mdpi.com/article/10.3390/f15101770/s1, Table S1. ANOVA results (*F* and *p*-values) for the effects of root pruning intensities and seedling sizes on dieback rate, leaf phenology, and root emergence at the early establishment stage. Table S2. Four-parameter logistic growth models and *R*<sup>2</sup>; for height, diameter at breast height, and root collar diameter by root pruning intensities and sapling sizes. Table S3. ANOVA results (*F* and *p*-values) for the effects of root pruning intensities and seedling sizes on stem phenology. Table S4. ANOVA results (*F* and *p*-values) for the effects of root pruning intensities and seedling sizes on leaf traits and stem growth rates during the rapid growth period. Table S5. ANOVA results (*F* and *p*-values) for the effects of root pruning intensities and seedling sizes on end-of-season growth indicators.

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