

Original Articles

Early root development of *Salix alba* and *Populus nigra* cuttings in a controlled nursery experiment

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Abstract

Vegetation-based stabilization approaches increasingly rely on living plant cuttings; however, root system development in woody cuttings remains poorly quantified, particularly with respect to species-specific differences during initial establishment. This study quantified early root system development in cuttings of white willow (*Salix alba*) and black poplar (*Populus nigra*) at 2, 4, and 6 months after planting, corresponding to the typical nursery phase prior to field transfer for stabilization applications. A total of 120 cuttings per species were grown in uniform loamy soil, and 30 cuttings per species were destructively sampled at each time point. Root number, total root length, midpoint diameter, root dry mass, root volume, root tissue density, and specific root length were measured, and the root volume-to-soil volume ratio was calculated to assess differences in root system traits between species. Across both species, root number, total root length, root volume, and root dry mass increased significantly with time. However, *S. alba* consistently exhibited greater root proliferation, greater root volume, higher specific root length, and greater root biomass accumulation than *P. nigra*, indicating more extensive early belowground development. Root tissue density was initially higher in *P. nigra* but became similar between species over time. Strong positive correlations among root number, root length, root volume, and root dry mass indicated coordinated root system expansion during establishment. Overall, the findings demonstrate clear species-specific differences in early root development, which are relevant to vegetation-based stabilization and riparian bioengineering applications.

Keywords: root functional traits, riparian woody species, root system development, *Populus nigra*, *Salix alba*.

Introduction

Human development and associated land-use modifications have substantially altered riparian ecosystems through changes in channel form, flow pathways, and sediment dynamics (Randall, 2015). These alterations accelerate soil erosion by disrupting the balance between erosive forces and ecosystem resistance mechanisms (Morgan & Rickson, 1995). Riparian zones are particularly vulnerable because they are frequently converted for agriculture, transportation, recreation, and settlement (Atesoglu et al., 2025). As a result, vegetation removal in these systems often increases erosion risk and reduces the natural stabilizing function of riparian buffers (Randall, 2015). Because riparian vegetation plays a central role in slope and riverbank stabilization, disturbances to these systems can substantially reduce ecosystem resistance to erosion and shallow bank failure (Kamath & van de Kuilen, 2026; Liu et al., 2013; Ngo et al., 2023). Vegetation contributes to stabilization through root reinforcement, reductions in pore-water pressure, and soil binding effects. Despite these benefits, conventional engineering approaches such as concrete revetments and rip-rap remain widely used (Zhang et al., 2018; Randall, 2015), partly because the quantitative understanding of vegetation-based stabilization remains limited, although interest in riparian bioengineering has increased in recent years (Kamath & van de Kuilen, 2026).

As interest in vegetation-based stabilization has increased, bioengineering techniques using living plant materials have emerged as practical approaches that operate in concert with natural processes (Chavez Torres et al., 2025; Deljouei et al., 2023). These techniques use vegetation or plant-derived materials as structural elements to control erosion and stabilize slopes, and empirical studies show that vegetation cover can reduce erosion rates and sediment yield relative to unvegetated surfaces (Zhang et al., 2018). Vegetation increases resistance to erosive forces by acting as a protective interface between soil and atmospheric drivers (Keybondori et al., 2025; Morgan & Rickson, 1995) and by reducing sediment delivery across a range of environmental systems (Ngo et al., 2023). The stabilizing influence of vegetation arises from the combined effects of above- and belowground components, with root systems playing a central role in soil reinforcement.

Roots contribute to soil stabilization through reinforcement, soil binding, and reductions in pore-water pressure (De Baets et al., 2008; Kamath et al., 2025; Liu et al., 2013). However, despite the increasing use of vegetation in stabilization efforts, quantitative and time-resolved evidence linking early root development to stabilization-relevant traits remains limited. Root traits such as total root length, lateral spread, and rooting depth are considered

key indicators of bioengineering performance, particularly during early establishment (Phillips et al., 2023). Because these traits vary with genetics, physiology, and environmental conditions (Ji et al., 2012; Deljouei et al., 2020), understanding early root system development is critical for evaluating species suitability in stabilization applications.

The Salicaceae family includes a diverse group of genera; however, in temperate riparian restoration and bioengineering, willows (*Salix* spp.) and poplars (*Populus* spp.) are among the most commonly used woody taxa because of their rapid growth, strong rooting capacity, and ease of vegetative propagation (Liu et al., 2013; Ngo et al., 2023). These genera are commonly used for streambank stabilization and short-rotation forestry systems (Frymark-Szymkowiak & Kieliszewska-Rokicka, 2023; Kamath et al., 2024; Kamath et al., 2025; Bhat et al., 2019; Dimitriou et al., 2009; Weih et al., 2009). However, stabilization does not occur immediately after planting because vegetation must first establish a reinforcing root matrix (Ishii, 2019). To accelerate this process, cuttings are widely used in bioengineering techniques such as live staking and vegetated structures (Morgan & Rickson, 1995; Rey & Labonne, 2015). Species within Salicaceae differ substantially in adventitious rooting capacity and early root proliferation, reflecting both genetic and physiological controls (Bannoud & Bellini, 2021). Willows (*Salix* spp.) generally initiate roots more rapidly and develop higher root densities during early establishment than poplars (*Populus* spp.) (Maldonado et al., 2022; Phillips et al., 2014). These differences suggest that species-specific rooting strategies may strongly influence early soil occupation and stabilization potential. Previous controlled studies have also compared the early root development of willow and poplar cuttings in pot or greenhouse systems. McIvor et al. (2014) showed that root initiation, total root length, and root dry mass were generally greater in *Salix* than in *Populus* clones, while root development was also influenced by soil bulk density. Zalesny and Bauer (2007) similarly evaluated the early development of *Populus* and *Salix* under controlled irrigation conditions, showing that early establishment can differ between genera and growing environments. Together, these studies show that pot-based experiments are useful for evaluating early rooting behavior, but they also indicate the need for species-specific and time-resolved assessments under defined nursery conditions.

Despite their widespread use, comparative quantitative studies of early root development in cuttings remain limited (Phillips et al., 2014; Phillips et al., 2023), particularly during the initial nursery establishment period. Accordingly, this study quantified early root system development in two widely used temperate riparian species—white willow (*Salix alba*) and black poplar (*Populus nigra*)—grown

from cuttings under controlled conditions. We evaluated temporal changes in root number, total root length, root volume, dry mass, and specific root length during the first six months after planting. We hypothesized that: (i) early root expansion would be driven primarily by coordinated increases in root number and total root length rather than by changes in root tissue density; and (ii) *S. alba* would exhibit greater root system size and soil occupation than *P. nigra* because of its higher adventitious rooting capacity and faster early proliferation.

Method

Study site and species. The study was conducted in a local open-air nursery in Aligudarz, Lorestan Province, Iran (33°21'8.7" N, 49°47'35.9" E; 2,100 m a.s.l.). The broader study region (33°24' N, 49°43' E; approximately 2,022 m a.s.l.) is mountainous and characterized by a Mediterranean climate. Long-term climatic records indicate mean maximum and minimum temperatures of 34.3 °C and 5.7 °C, respectively. Mean annual precipitation is approximately 388 mm, with a maximum recorded 24-h rainfall of 72 mm. Two woody species commonly cultivated in the region—*Salix alba* clone 84/22 and *Populus nigra* clone 62/154 (Figure S1)—were selected because of their wide distribution and frequent use in agroforestry, restoration, and bioengineering applications.

Experimental design and planting. The experiment consisted of two species (*P. nigra* and *S. alba*) and three sampling periods (2, 4, and 6 months), with independent destructive sampling at each harvest. A pot-based design was used, in which each pot contained one centrally planted cutting and served as one replicate (Freschet et al., 2021). Pots were truncated cones (22 cm upper diameter, 18 cm base diameter, 18 cm height; volume = 5,674 cm³) filled with uniform loamy soil classified using the USDA Soil Texture Triangle (Gee & Bauder, 1986). Although the total pot capacity was 5,674 cm³, pots were filled to approximately 85% of their capacity. Therefore, the effective soil volume available for root growth was estimated to be 4,823 cm³ per pot, and this value was used to calculate the root volume ratio (RVR).

A total of 120 one-year-old cuttings per species (~50 cm in length and ~1 cm in diameter) were prepared (Randall, 2015; Rey & Labonne, 2015), including 30 additional cuttings per species to compensate for potential establishment failure or mortality. Cuttings were collected from multiple parent plants, although individual source trees were not tracked. Prior to planting, all cuttings were soaked overnight in deionized (DI) water to ensure hydration and then immersed for 20 min in a 2% thiophanate-methyl fungicide

solution (Topsin M). After this pre-planting treatment, the cuttings were planted immediately in the prepared pots. Pots were irrigated every three days throughout the experiment. The experiment was established in May, approximately 45 days after the beginning of the growing season in the Mediterranean region of Iran. Therefore, the first harvest, conducted 2 months after planting, corresponded to approximately 105 days after the start of the regional growing season. At each sampling period, 30 independent replicates per species were randomly selected and harvested.

Sampling and root measurements. Harvests were conducted 2, 4, and 6 months after planting, corresponding to early root initiation, active expansion, and early structural development. The 6-month period represents the typical nursery establishment phase for these species before the potential transfer of rooted cuttings to field sites in temperate and Mediterranean regions. However, field transplantation was not conducted in the present experiment; the study focused on root development during the nursery phase. At each harvest, root systems were extracted after the soil had been saturated and carefully washed free of soil using gentle water pressure (Figure S2).

For each root system, total root number, root segment length, midpoint diameter, and root dry mass were measured (Azaryan et al., 2025; Freschet et al., 2021). Root segments were defined as portions of roots between two branching points, and all visible roots were included without classification by root order. Root length was measured to the nearest millimeter using a ruler, and root diameter was measured using a digital caliper (± 0.01 mm). Root volume was estimated by approximating each segment as a cylinder with a circular cross-section, using its midpoint diameter, and summing the volumes of all segments across the entire root system (Liu et al., 2013). RVR (%) was calculated by dividing total root volume by the estimated effective soil volume in each pot (4,823 cm³) and multiplied the resulting value by 100. In this study, RVR was used as a pot-scale indicator of relative soil occupation by roots. Root tissue density (RTD; g cm⁻³) was calculated as root dry mass divided by root volume (Birouste et al., 2014).

Roots were oven-dried at 80 °C for 24 h and weighed to the nearest 0.01 g. Specific root length (SRL; m g⁻¹) was calculated as total root length divided by root dry mass (Azaryan et al., 2025; Freschet et al., 2021).

Statistical analysis. For each root trait, a two-way factorial analysis of variance (ANOVA) was used to test the effects of species, sampling time, and their interaction. The species factor had two levels, *P. nigra* and *S. alba*, and the sampling-time factor had three

levels, corresponding to 2, 4, and 6 months after planting. Because independent destructive sampling was conducted at each harvest, sampling time was treated as an independent factor rather than a repeated measure.

Prior to ANOVA, model assumptions were evaluated separately for each root trait. Normality of residuals was assessed using the Shapiro–Wilk test, and homogeneity of variance among species × sampling time combinations was evaluated using Levene’s test. For traits that showed right-skewed distributions and/or heterogeneous variances, a $\log(x + 1)$ transformation was applied before conducting parametric analyses. Statistical tests were performed on transformed data when transformation was required, while untransformed values were presented in figures and descriptive summaries to facilitate biological interpretation.

When significant main effects or species × sampling time interactions were detected, Tukey’s post hoc test was used for pairwise comparisons. For significant interactions, post hoc comparisons were conducted among species × sampling time combinations. Statistical significance was assessed at $P < 0.05$. Pearson correlation analysis was used to quantify relationships among root traits within each species using data pooled across sampling periods. All analyses were conducted using parametric statistical procedures after model assumptions had been checked and, when necessary, data transformations had been applied.

Results

Descriptive statistics, including mean, standard error (SE), minimum, and maximum values for each root trait by species and sampling time, are provided in Supplementary Table S1.

Total root number. Two-way factorial ANOVA showed significant effects of species, sampling time, and their interaction on total root number (species: $F_{1,174} = 190.81$, $P < 0.001$; sampling time: $F_{2,174} = 189.95$, $P < 0.001$; species × sampling time: $F_{2,174} = 52.25$, $P < 0.001$; Figure 1; Table S1). Mean total root number increased over time in both species, but the magnitude of increase differed strongly between *P. nigra* and *S. alba* (Figure 1). Early values at 2 months were relatively low and did not differ significantly from *P. nigra* at 4 months. By 6 months, *P. nigra* increased significantly, reaching a level comparable to *S. alba* at 4 months. However, *S. alba* continued to increase sharply and showed the highest value among all species × sampling time combinations at 6 months. This pattern is also visible in representative washed root systems, which show limited early root development at 2 months and substantially greater root proliferation by 6 months, particularly in *S. alba* (Figure 1).

Total root length. Two-way factorial ANOVA showed significant effects of species, sampling time, and their interaction on total root length (species: $F_{1,174} = 142.41$, $P < 0.001$; sampling time: $F_{2,174} = 242.48$, $P < 0.001$; species × sampling time: $F_{2,174} = 32.09$, $P < 0.001$; Figure 2; Table S1). Mean total root length increased over time in both species, but the magnitude of increase differed between *P. nigra* and *S. alba*. At 2 months, both species showed low values and did not differ significantly from *P. nigra* at 4 months. At 4 months, *S. alba* showed a marked increase and was statistically similar to *P. nigra* at 6 months. By 6 months, *S. alba* reached the highest value among all species × sampling time combination.

Specific root length. Two-way factorial ANOVA showed no significant main effect of species or sampling time on SRL (species: $F_{1,174} = 0.72$, $P = 0.397$; sampling time: $F_{2,174} = 1.04$, $P = 0.354$; Figure 3; Table S1). However, the species × sampling time interaction was significant ($F_{2,174} = 3.75$, $P = 0.025$). Mean values in *P. nigra* remained relatively stable across the three sampling times, whereas *S. alba* showed a decrease at 4 months followed by an increase at 6 months. Nevertheless, Tukey’s post hoc comparisons did not detect significant differences among individual species × sampling time combinations (Figure 3).

Root dry mass. Two-way factorial ANOVA showed significant effects of species, sampling time, and their interaction on root dry mass (species: $F_{1,174} = 9.50$, $P = 0.002$; sampling time: $F_{2,174} = 79.17$, $P < 0.001$; species × sampling time: $F_{2,174} = 3.24$, $P = 0.042$; Figure 4; Table S1). Mean root dry mass increased over time in both species, but the temporal pattern differed between *P. nigra* and *S. alba* (Figure 4). At 2 months, both species showed low values and did not differ significantly (Figure 4). At 4 months, *S. alba* showed significantly higher root dry mass than *P. nigra*. By 6 months, both species reached the highest values and did not differ significantly from each other (Figure 4).

Total root volume. Two-way factorial ANOVA showed significant effects of species, sampling time, and their interaction on total root volume (species: $F_{1,174} = 66.91$, $P < 0.001$; sampling time: $F_{2,174} = 133.13$, $P < 0.001$; species × sampling time: $F_{2,174} = 9.14$, $P < 0.001$; Figure 5). Mean total root volume increased over time in both species, but the magnitude and timing of increase differed between *P. nigra* and *S. alba* (Figure 5; Table S1). At 2 months, both species had low values and did not differ significantly. At 4 months, *S. alba* showed a marked increase and was significantly higher than the early-stage groups, whereas *P. nigra* remained comparatively low (Figure 5). By 6 months, *P. nigra* increased to a level similar to *S. alba* at 4 months, while *S. alba* continued to increase and showed

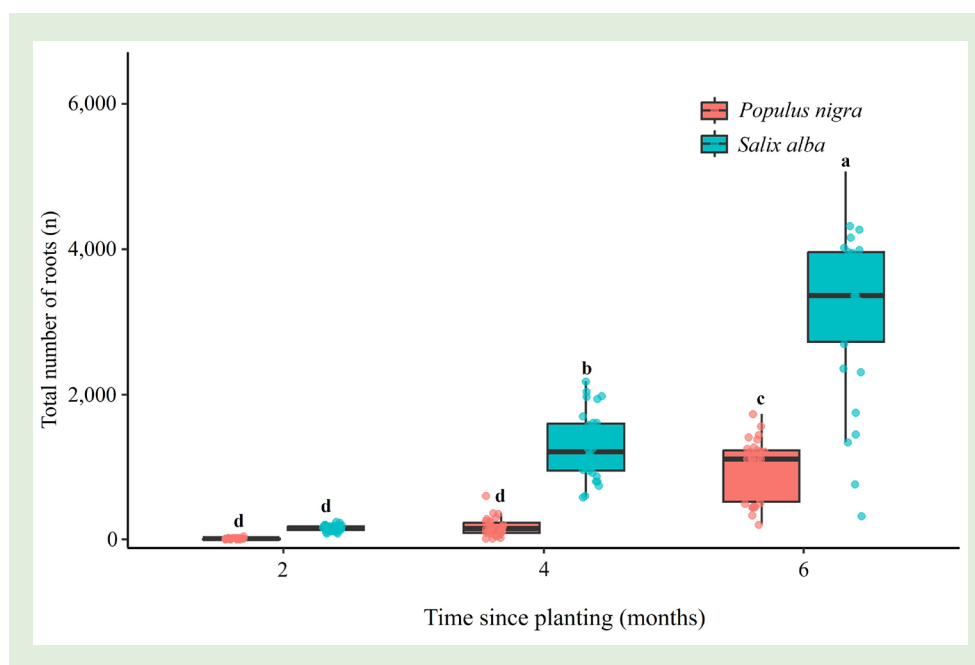


Figure 1. Total root number of *Populus nigra* and *Salix alba* cuttings at 2, 4, and 6 months after planting (n = 30 per species × sampling time combination). Boxes represent the interquartile range, central horizontal lines indicate medians, whiskers show values within 1.5 × the interquartile range, and points represent individual observations. Different letters indicate significant differences among species × sampling time combinations based on Tukey's post hoc test following two-way factorial ANOVA (Tukey test, $P < 0.05$).

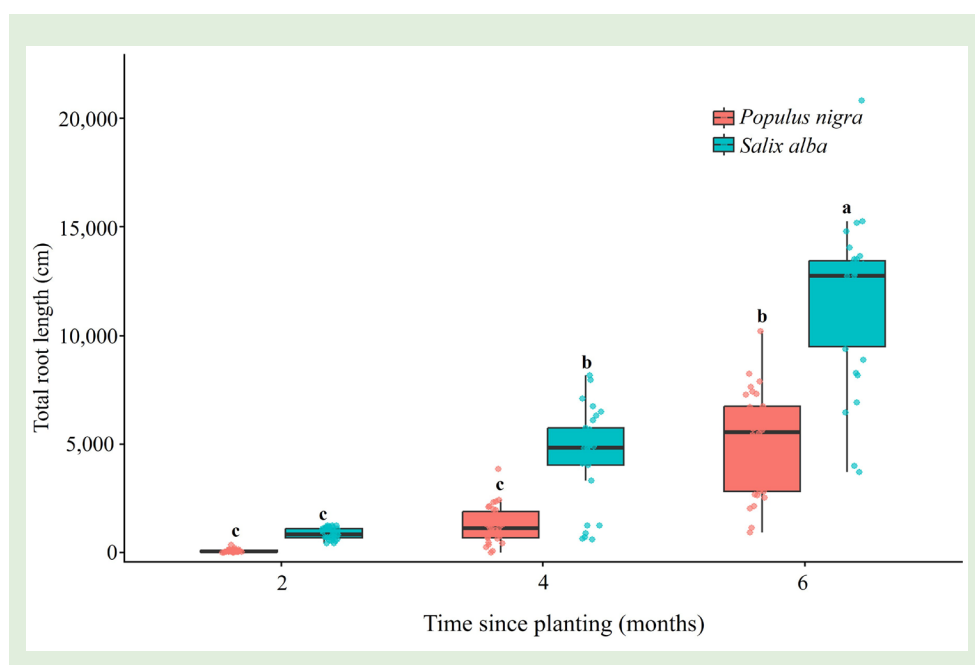


Figure 2. Total root length of *Populus nigra* and *Salix alba* cuttings at 2, 4, and 6 months after planting (n = 30 per species × sampling time combination). Boxes represent the interquartile range, central horizontal lines indicate medians, whiskers show values within 1.5 × the interquartile range, and points represent individual observations. Different letters indicate significant differences among species × sampling time combinations based on Tukey's post hoc test following two-way factorial ANOVA (Tukey test, $P < 0.05$).

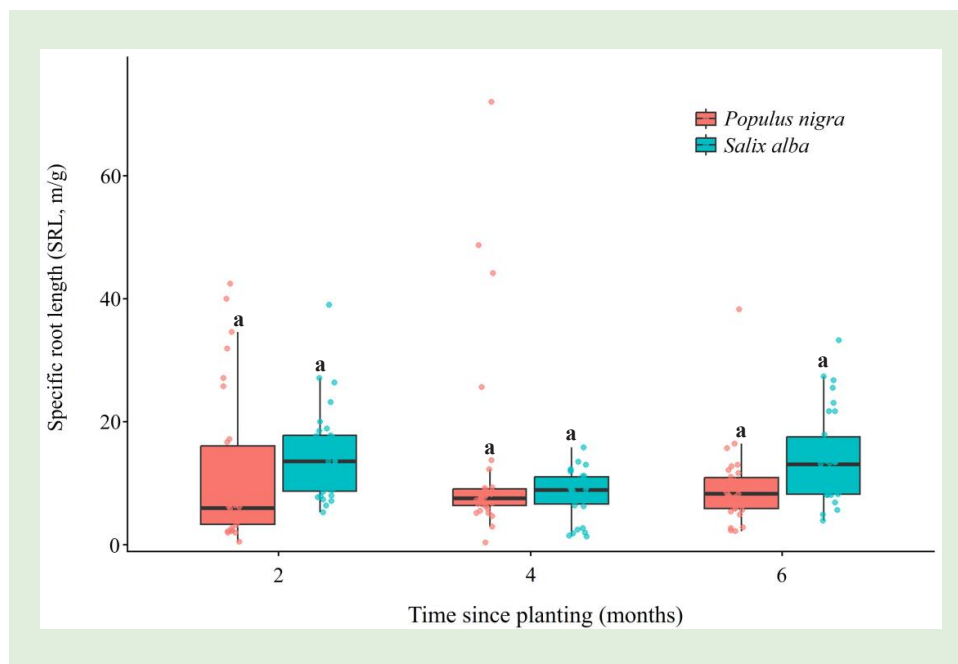


Figure 3. Specific root length (SRL) of *Populus nigra* and *Salix alba* cuttings at 2, 4, and 6 months after planting ($n = 30$ per species × sampling time combination). Boxes represent the interquartile range, central horizontal lines indicate medians, whiskers show values within $1.5 \times$ the interquartile range, and points represent individual observations. Tukey's post hoc comparisons following two-way factorial ANOVA showed no significant differences among species × sampling time combinations ($P < 0.05$); therefore, all groups share the same letter.

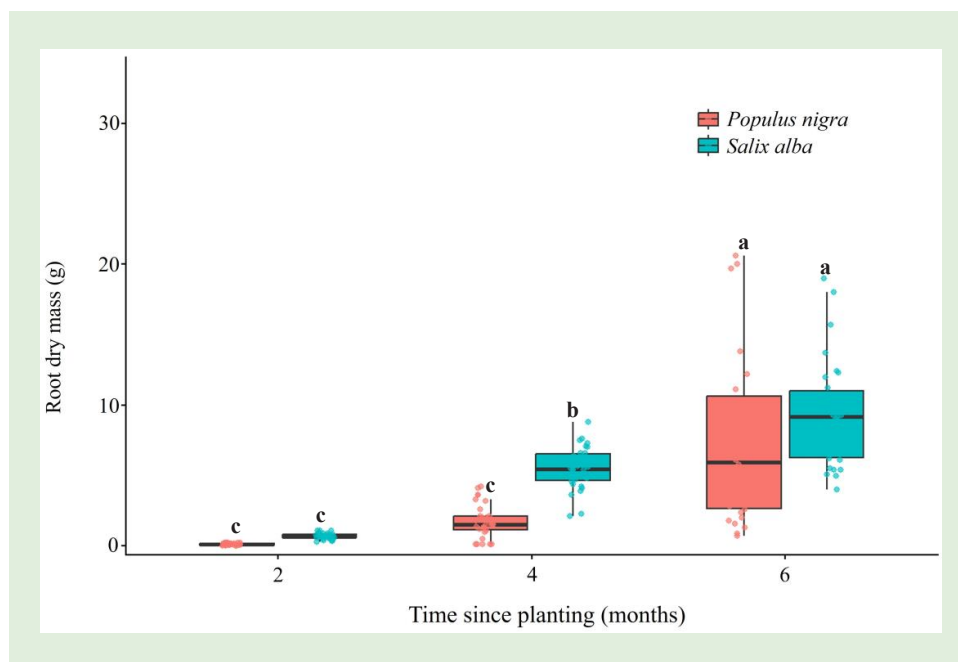


Figure 4. Root dry mass of *Populus nigra* and *Salix alba* cuttings at 2, 4, and 6 months after planting ($n = 30$ per species × sampling time combination). Boxes represent the interquartile range, central horizontal lines indicate medians, whiskers show values within $1.5 \times$ the interquartile range, and points represent individual observations. Different letters indicate significant differences among species × sampling time combinations based on Tukey's post hoc test following two-way factorial ANOVA (Tukey test, $P < 0.05$).

the highest value among all species × sampling time combinations (Figure 5). The root volume ratio (RVR) increased progressively through time in both species and remained consistently greater in *S. alba* than in *P. nigra*. RVR values at 6 months reached 0.111% in *P. nigra* and 0.201% in *S. alba*.

Root tissue density. Two-way factorial ANOVA showed significant effects of species, sampling time, and their interaction on RTD (species: $F_{1,174} = 11.82$, $P = 0.0007$; sampling time: $F_{2,174} = 6.10$, $P = 0.0028$; species × sampling time: $F_{2,174} = 4.02$, $P = 0.0196$; Figure 6; Table S1). The significant interaction indicated that the temporal pattern of RTD differed between *P. nigra* and *S. alba* (Figure 6). In *P. nigra*, mean RTD was highest at 2 months and declined thereafter, whereas *S. alba* maintained comparatively low values across all sampling times (Figure 6). Tukey's post hoc test showed that *P. nigra* at 2 months had significantly higher values than the low-value groups, while *P. nigra* at 4 months was intermediate and not significantly different from either the highest or lowest groups.

Species-specific correlations among root system traits. Pearson correlation analysis revealed strong positive relationships among total root number, total root length, root dry mass, and total root

volume in both *S. alba* and *P. nigra* (Figure 7). In contrast, SRL exhibited weaker relationships with other root traits and was negatively associated with root dry mass. Overall, correlation patterns were broadly similar between species.

Discussion

Rapid early root system expansion in cuttings. Both *S. alba* and *P. nigra* established rapidly from unrooted cuttings, as showed by consistent increases in total root number, total root length, root dry mass, and total root volume across the 2-, 4-, and 6-month sampling periods. Root development progressed from initial emergence at 2 months to substantial expansion and biomass accumulation at later stages (Table S1), indicating strong belowground establishment during the nursery phase. Significant species × sampling time interactions for most size-related traits further indicated that the species differed not only in overall root production but also in their developmental trajectories. This temporal pattern is relevant to bioengineering because increasing root abundance and biomass can enhance soil anchorage and reinforcement (De Baets et al., 2008). The results also provide time-resolved evidence of root development during a period that is critical for the establishment of stabilization cuttings.

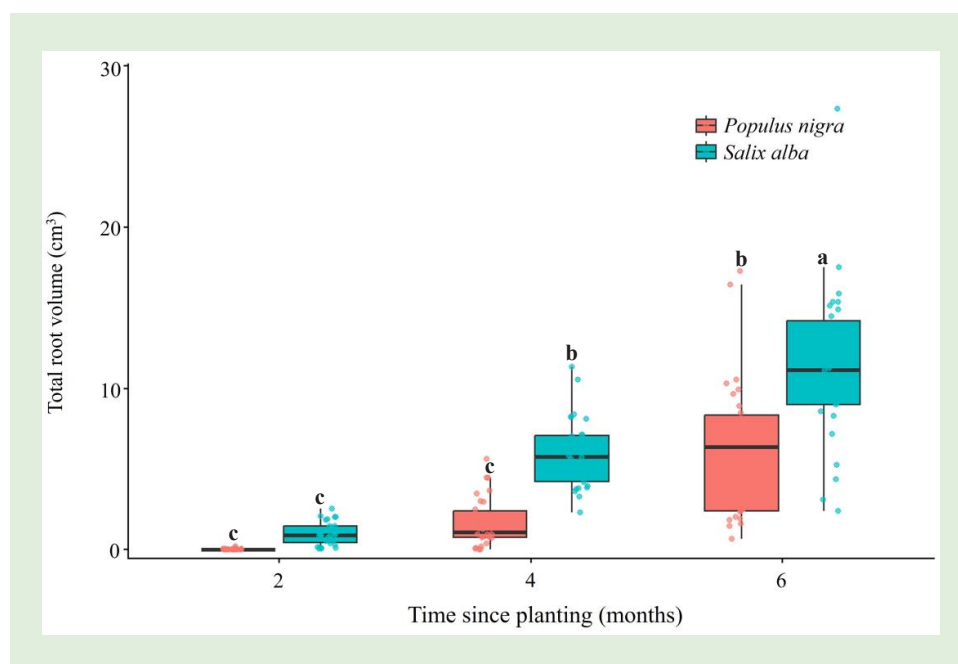


Figure 5. Total root volume of *Populus nigra* and *Salix alba* cuttings at 2, 4, and 6 months after planting ($n = 30$ per species × sampling time combination). Boxes represent the interquartile range, central horizontal lines indicate medians, whiskers show values within $1.5 \times$ the interquartile range, and points represent individual observations. Different letters indicate significant differences among species × sampling time combinations based on Tukey's post hoc test following two-way factorial ANOVA (Tukey test, $P < 0.05$).

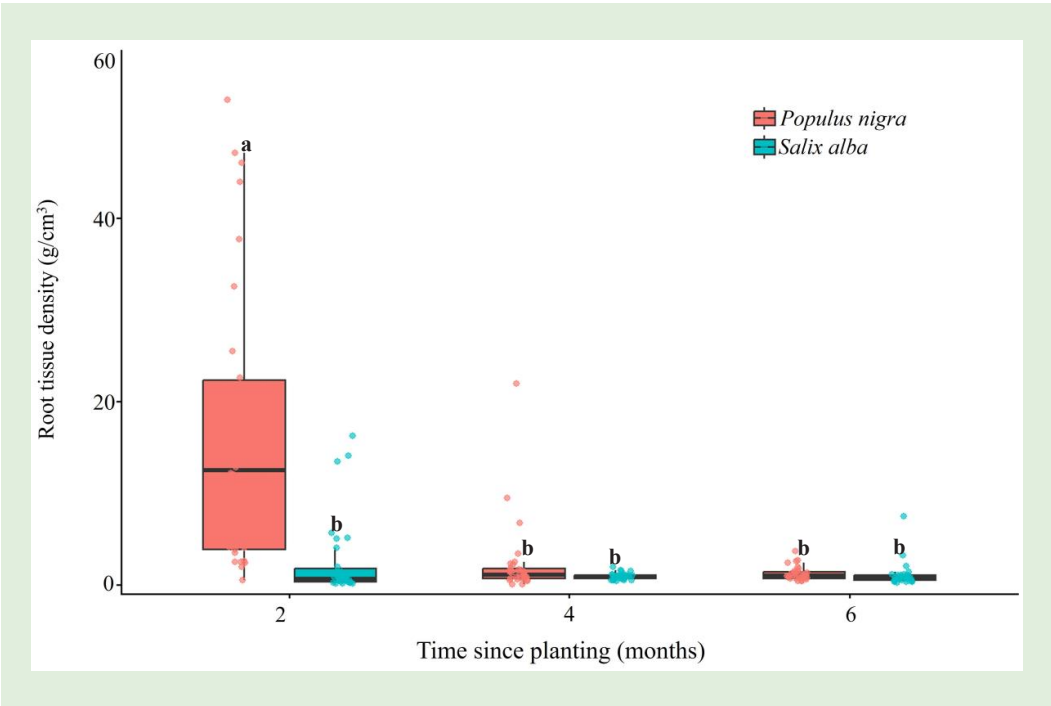


Figure 6. Root tissue density (RTD) of *Populus nigra* and *Salix alba* cuttings at 2, 4, and 6 months after planting (n = 30 per species × sampling time combination). Boxes represent the interquartile range, central horizontal lines indicate medians, whiskers show values within 1.5 × the interquartile range, and points represent individual observations. Different letters indicate significant differences among species × sampling time combinations based on Tukey’s post hoc test following two-way factorial ANOVA (Tukey test, P < 0.05).

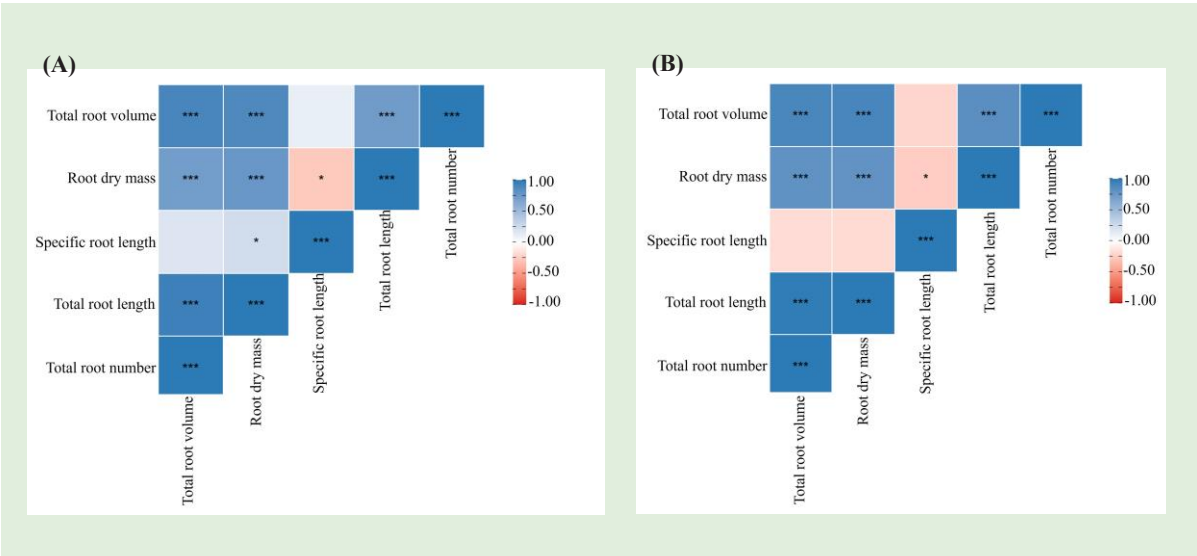


Figure 7. Pearson correlation matrices among root traits for (A) *Salix alba* and (B) *Populus nigra*. Color intensity indicates correlation strength, and asterisks indicate significance levels (*P < 0.05, **P < 0.01, ***P < 0.001).

Previous studies have similarly shown that the timing and magnitude of early root growth influence reinforcement functions (Phillips et al., 2014; Phillips et al., 2023), while experiments with hybrid poplars have demonstrated substantial adventitious root development under favorable moisture conditions (Krabel et al., 2015).

No cutting mortality occurred during the experiment, indicating a high establishment capacity under the non-limiting moisture conditions provided in the nursery. However, this result does not necessarily predict survival after field transplantation, where moisture limitation, prolonged saturation, and other environmental stressors may constrain establishment. Similar responses of *S. alba* and *P. nigra* to variations in moisture availability have been reported previously (Rey & Labonne, 2015; Randall, 2015; Mc Carthy et al., 2018). Mechanical reinforcement has also been shown to increase with root abundance and overall root system development in cuttings (Liu et al., 2014). Thus, the observed root expansion suggests that both species can rapidly develop stabilization-relevant root systems under favorable conditions, although their performance under field conditions requires further evaluation.

Species differences in root system size and soil occupation. Although both species exhibited strong early rooting, *S. alba* consistently developed a more extensive root system and occupied a greater proportion of the available soil volume than *P. nigra*. By six months, *S. alba* produced ~3.4× more roots, ~2.3× greater total root length, and ~1.8× greater total root volume than *P. nigra*, indicating greater adventitious rooting vigor and more sustained proliferation during nursery establishment. Similar patterns of stronger early belowground development in *S. alba* have been reported under nursery and bioengineering conditions (Phillips et al., 2014; Sulaiman, 2006). These findings are also consistent with McIvor et al. (2014), who reported that root initiation, total root length, and root dry mass were generally greater in *Salix* than in *Populus* cuttings, although variation occurred among clones within each genus. Zalesny and Bauer (2007) similarly showed that the early development of these genera varies under controlled growing conditions. Together, these studies support the interpretation that *S. alba* and *P. nigra* follow contrasting early rooting strategies, with *S. alba* achieving faster soil occupation during nursery establishment. The greater root proliferation of *S. alba* may increase root surface area and root–soil contact, potentially improving the binding of fine riverbank sediments. However, this functional interpretation cannot be confirmed directly because root diameter-class distribution and spatial root arrangement were not quantified. Species-specific phenology may also have contributed to the observed differences, as the timing of experiment establishment could have favored earlier physiological activity and

root initiation in *S. alba*. More generally, the two species may differ in biomass-allocation and root-morphology strategies: *S. alba* may prioritize the rapid production of numerous finer roots, whereas *P. nigra* may allocate proportionally more biomass to thicker roots during later establishment. Because aboveground biomass, root length by diameter class, root exudation, and three-dimensional root distribution were not measured, these mechanisms remain plausible explanations rather than direct findings.

The significant species × sampling time interactions observed for total root number, total root length, total root volume, and root dry mass indicated that differences between the species changed throughout establishment. The advantage of *S. alba* was particularly pronounced during the intermediate and later sampling periods. In contrast, *P. nigra* generally showed slower initial development, although its root dry mass increased substantially by six months and became statistically similar to that of *S. alba*. Thus, *S. alba* achieved more rapid early soil occupation, whereas *P. nigra* required more time to accumulate comparable belowground biomass under the nursery conditions.

Differences in soil occupation were also reflected in RVR. Although RVR values remained low during these early stages, *S. alba* consistently exhibited greater RVR than *P. nigra*, indicating that its roots occupied a larger proportion of the effective soil volume. Greater root occupancy may enhance root–soil contact and promote the development of a reinforcing root–soil matrix important for stabilization. Similar interpretations of RVR and related root-volume metrics have been reported in bioengineering studies (Karimi et al., 2022; Phan et al., 2021).

The observed advantage of *S. alba* is consistent with the broader understanding that adventitious rooting capacity is strongly species- and genotype-dependent (Zhao et al., 2014). Rooting responses in *P. nigra* are also sensitive to environmental conditions, which may lead to context-dependent establishment performance. Under the controlled conditions of this study, *S. alba* exhibited greater early rooting vigor and soil occupation. However, these results represent comparative responses under nursery conditions rather than direct predictions of field performance, consistent with previous stabilization research on *P. nigra* root distribution (Ngo et al., 2023).

The practical relevance of these differences depends on the planting material and establishment strategy. For direct planting of unrooted cuttings, rapid adventitious root initiation is essential because survival and initial stabilization depend on the ability to form roots under prevailing soil-moisture conditions. The stronger early proliferation of *S. alba* may therefore be advantageous where rapid belowground establishment is required. For nursery-produced rooted cuttings, the measurements at 2, 4, and 6 months indicate

how root system development progresses before outplanting and can help inform the timing of field transfer. When larger plants with developed shoots and foliage are used, however, root–shoot balance, transpiration demand, transplant stress, planting season, and compatibility between stock type and site conditions must also be considered. These considerations are consistent with the broader target-plant concept, which emphasizes that field performance depends not only on nursery root development but also on stock-type attributes, planting window, and site limitations (Grossnickle & MacDonald, 2026).

Trait coordination and functional differentiation during early establishment. Correlation analysis revealed strong coordination among total root number, total root length, root dry mass, and total root volume in both *S. alba* and *P. nigra*, indicating that increases in root proliferation were accompanied by corresponding increases in root system size and biomass. Similar coordination among root size-related traits has been reported in young trees and seedlings, where rapid root expansion supports anchorage and resource acquisition (Freschet et al., 2021; Wells & Eissenstat, 2002). The strong relationship between root number and total root length identifies root proliferation as an important driver of early root system expansion, whereas the weaker relationship between root number and root volume likely reflects the additional influence of root diameter on volume. From a bioengineering perspective, root number and length primarily influence soil occupation and root–soil contact, whereas root volume is more closely associated with reinforcement capacity (De Baets et al., 2008). Collectively, these relationships indicate that early root development was characterized primarily by spatial expansion, followed by greater biomass accumulation and structural development.

In contrast, SRL exhibited weak or non-significant correlations with root number and root volume and was negatively associated with root dry mass, indicating that variation in SRL was largely independent of overall root system magnitude. Consistent with this interpretation, SRL showed no significant main effects of species or sampling time, and Tukey comparisons did not detect significant differences among species × sampling time combinations, despite the significant interaction detected by the factorial analysis. Previous studies similarly describe SRL as a distinct functional trait rather than a direct indicator of root system size during early development (Beatrice et al., 2024; Freschet et al., 2021; Kramer-Walter et al., 2016). Although *S. alba* exhibited slightly higher SRL than *P. nigra*, this difference was minor relative to the pronounced interspecific differences in root number, length, volume, and biomass. Thus, the observed differences in stabilization-relevant development were

driven primarily by root system size and soil occupation rather than by SRL, consistent with previous stabilization studies emphasizing root distribution and structural development (De Baets et al., 2008; Phillips et al., 2023).

The negative relationship between SRL and root dry mass may indicate variation in root construction strategy, but it should be interpreted cautiously because root length was not quantified separately among diameter classes. Consequently, the present data cannot determine whether the species differed in diameter-specific biomass allocation or structural thickening. SRL can also be strongly influenced by growing conditions, including physical constraints such as soil bulk density, which may differ substantially between pot and field environments. The observed pattern is broadly consistent with root economics frameworks, in which higher SRL is associated with a more acquisitive strategy and lower SRL with greater structural investment (Kramer-Walter et al., 2016; Reich et al., 2014). Nevertheless, SRL should be interpreted here as a pot-based indicator of root morphology rather than direct evidence of field root architecture. Moreover, in bioengineering applications, reinforcement generally depends more strongly on overall root system dimensions and volume than on fine-root efficiency alone (De Baets et al., 2008).

RTD showed a different pattern from the size-related traits. Rather than increasing over time, RTD was highest in *P. nigra* at 2 months and declined thereafter, while *S. alba* maintained comparatively low RTD values across sampling times. This pattern may indicate that *P. nigra* initially produced fewer but relatively dense roots, followed by an increasing contribution of lower-density lateral roots as the root system expanded. In contrast, *S. alba* exhibited rapid proliferation from the earliest sampling period, resulting in consistently lower RTD and a larger overall root system. The convergence of RTD between the species over time indicates that the initial difference reflected contrasting developmental timing rather than a persistent interspecific difference in tissue density. Overall, the stronger early development of *S. alba* was associated with greater root number, length, volume, and biomass, demonstrating that root system expansion and soil occupation were more important than high tissue density alone.

Study limitations and future research. This study provides a controlled temporal assessment of early root development in *S. alba* and *P. nigra* cuttings over 2, 4, and 6 months, a period that corresponds to the main nursery establishment phase before typical field transfer. By repeatedly measuring below-ground traits during this early developmental window, the study offers useful information on species-specific rooting patterns that are relevant for nursery production, restoration planning, and soil bioengineering applications.

However, several limitations should be acknowledged. First, the experiment was conducted under nursery conditions using pots with uniform soil, and therefore the results may not fully represent root development under heterogeneous field conditions, where soil depth, compaction, moisture variability, slope position, and competition may influence rooting performance. This limitation is important because previous greenhouse work has shown that soil physical conditions, including bulk density, can alter root development and the proportion of thinner roots in *Salix* and *Populus* cuttings. Second, aboveground growth traits, such as shoot height, stem diameter, leaf area, and shoot biomass, were not measured. As a result, relationships between root development and shoot growth could not be evaluated in the present dataset. Third, detailed root diameter-class distribution, diameter-specific root length, root spatial arrangement within pots, and root mechanical properties, such as tensile strength and root pullout resistance, were not measured. Therefore, although root number, length, biomass, and volume provide useful indicators of early soil occupation, the present study cannot fully quantify diameter-specific root length distribution, pot-boundary root tracking, or mechanical reinforcement capacity. In addition, RVR should be interpreted as a container-based index because root growth was restricted by the fixed soil volume of the pots. Under field conditions, roots of *S. alba* and *P. nigra* may extend laterally and vertically beyond the initial planting zone, so field-scale root occupancy and reinforcement capacity may differ substantially from the RVR values observed in this pot experiment. Future studies should combine temporal measurements of root and shoot traits with root diameter distribution, root spatial mapping, direct measurements of root strength, and field-based trials to better understand root–shoot coordination, establishment success, and functional stabilization performance after transfer from nursery to field conditions.

Conclusions

This study evaluated early belowground development in two temperate riparian bioengineering species, *S. alba* and *P. nigra*, to identify species-level differences relevant to soil and streambank stabilization during the nursery establishment phase. Over six months, both species exhibited rapid root system expansion; however, *S. alba* consistently developed larger root systems than *P. nigra* across traits associated with soil occupation and potential reinforcement, including total root number, total root length, total root volume, and root dry mass. These findings indicate greater early belowground development in *S. alba* under the controlled nursery conditions of this experiment. Root growth in both species approached the volumetric

limits of the experimental pots, highlighting the intensity of early root proliferation while constraining the study period to six months.

The practical implications of these results depend on the type of planting material used in the field. For direct planting of unrooted cuttings, rapid adventitious root initiation is critical for early survival and stabilization. For nursery-grown rooted cuttings, the 2-, 4-, and 6-month results provide useful information on the time required to develop a functional root system before outplanting. If complete plants with developed shoots and leaves are used, additional factors such as root–shoot balance, transpiration demand, planting stress, and site moisture conditions should also be considered. Accordingly, *S. alba* may be a suitable candidate for bioengineering applications requiring rapid early belowground establishment, while *P. nigra* remains a viable alternative under appropriate site conditions. Because this study did not include field transplantation, the findings should be interpreted comparatively rather than as direct predictors of field performance. Future studies should evaluate rooting depth, persistence, root–shoot coordination, and mechanical reinforcement capacity under field conditions and across different planting material types.

Authors contribution

EA, AD: conceptualization of the work, formal analysis, supervision, interpretation of results, and writing, and review. SMMS: formal analysis, interpretation of results, and writing, and review. SS, VE: writing and review. SAH, MVM: review.

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Supporting information

Table S1. Descriptive statistics of root traits of *Populus nigra* and *Salix alba* cuttings at 2, 4, and 6 months after planting (n = 30 per species × sampling time point)

Root trait	Species	Months after planting	Mean ± SE	Min	Max
Total root number (n)	<i>P. nigra</i>	2	12.67 ± 1.42	4	41
		4	170.83 ± 22.44	10	600
		6	942.57 ± 74.58	200	1,728
	<i>S. alba</i>	2	157.68 ± 7.44	82	245
		4	1,285.70 ± 80.39	583	2,181
		6	3,224.70 ± 255.77	321	6,098
Total root length (m)	<i>P. nigra</i>	2	0.95 ± 0.14	0.13	3.84
		4	13.05 ± 1.53	0.30	38.65
		6	50.03 ± 4.29	9.44	102.10
	<i>S. alba</i>	2	8.94 ± 0.47	4.40	12.73
		4	45.22 ± 3.82	6.21	81.56
		6	115.86 ± 6.38	37.09	208.14
Specific root length (m g ⁻¹)	<i>P. nigra</i>	2	11.51 ± 2.21	0.56	42.45
		4	12.64 ± 2.78	0.41	72.07
		6	9.40 ± 1.17	2.24	38.30
	<i>S. alba</i>	2	14.61 ± 1.34	5.34	39.01
		4	8.38 ± 0.70	1.37	15.84
		6	14.23 ± 1.33	3.94	33.24
Root dry mass (g)	<i>P. nigra</i>	2	0.13 ± 0.01	0.01	0.25
		4	1.75 ± 0.21	0.10	4.20
		6	8.53 ± 1.48	0.70	31.50
	<i>S. alba</i>	2	0.69 ± 0.04	0.28	1.12
		4	5.49 ± 0.27	2.10	8.80
		6	9.43 ± 0.67	4.00	19.00
Total root volume (cm ³)	<i>P. nigra</i>	2	0.024 ± 0.008	0.001	0.21
		4	1.64 ± 0.27	0.01	5.63
		6	6.31 ± 0.75	0.64	17.29
	<i>S. alba</i>	2	0.97 ± 0.13	0.04	2.53
		4	5.94 ± 0.38	2.30	11.33
		6	11.41 ± 0.87	2.40	27.36
Root tissue density (g cm ⁻³)	<i>P. nigra</i>	2	16.81 ± 2.79	0.57	52.89
		4	7.48 ± 5.19	0.11	159.08
		6	1.30 ± 0.14	0.43	3.78
	<i>S. alba</i>	2	2.65 ± 0.78	0.22	16.27
		4	1.00 ± 0.07	0.52	1.98
		6	1.13 ± 0.24	0.33	7.51



Figure S1. Representative nursery pots showing early shoot emergence and establishment of cuttings during the early nursery phase. Images show (A) *Populus nigra* and (B) *Salix alba* cuttings grown under controlled pot conditions. Photographs were taken approximately 2 months after planting.



Figure S2. Representative washed root systems of *Populus nigra* and *Salix alba* cuttings at 2 and 6 months after planting. Panels show (A) *P. nigra* at 2 months; (B) *S. alba* at 2 months; (C) *P. nigra* at 6 months; (D) *S. alba* at 6 months.