EFFECT OF *PINUS DENSIFLORA* VAR. *ZHANGWUENSIS* SCION ON ROOT BIOMASS AND ARCHITECTURE OF *PINUS SYLVESTRIS* VAR. *MONGOLICA* ROOTSTOCK AFTER 25-YEAR GRAFTING

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Abstract. *Pinus densiflora* var. *zhangwuensis* is a natural hybrid in the genus *Pinus*, so the only suitable propagation method for this variety is grafting onto the *P. sylvestris* var *mongolica* rootstock (PR). This study was carried out in its clone plantation after 25-year grafting, located in Zhanggutai Town, Zhangwu County, Liaoning Province, China, and used the adjacent ungrafted *P. sylvestris* var. *mongolica* (UP) plantation of the same age as the control, to evaluate the influence of *P. densiflora* var. *zhangwuensis* scion (PS) on the root architecture of PR at long time scale based on the investigation of soil physicochemistry, tree and root growth dynamics through minirhizotron root ecology monitoring system, and the biomass distribution of different diameter sizes of roots between PR and UP by the full excavation method. The root length, root link number (RLN), average root link length (RLL) as well as specific root length (SRL) were determined, and fractal dimension and fractal abundance were calculated by regression equation method. The results showed that the root biomass distribution and architecture of PR has been affected by PS, and the RLN of medium roots, fractal dimension, and fractal abundance of PR were larger than UP ($p<0.05$), enabling its root system to have more branches, more complex topology, and stronger ability to absorb water and nutrient in the deep soil. Although the PR underwent beneficial changes in the root biomass distribution strategy and root architecture, some root indicators still remained unchanged, such as fine root biomass : total root biomass ratio, and the average SRL.
Keywords: *grafting, root growth dynamics, root architecture, fractal dimension, fractal abundance*

Introduction

Rootstock-scion interaction has been comprehensively reviewed recently in vegetables and horticultural plants (Rasool et al., 2020; Tsaballa et al., 2021). Rootstocks can affect scions in many aspects including yield and stress tolerance due to their different root characteristics and utilization ability of soil resources, and vice versa, scions can also affect the phenotypic characteristics (such as root elongation) of rootstocks due to different physiological properties (photosynthetic ability or stress resistance) (Koepke et al., 2013; Santarosa et al., 2016; Gautier et al., 2018). Studies on some plants (such as cherries and tomatoes) showed that sRNAs could be transported from the scion into the rootstock across the graft union (Zhao et al., 2020), and cyclophilin protein could also move from the scion to the rootstock through phloem and this transport accompanied by augmented auxin levels, eventually helped in promoting the root growth (Spiegelman et al., 2015). Heteroplastic grafting has always been adopted to construct asexual seed orchards or propagate superior conifer genotypes (Pérez-Luna et al., 2020), which has formed many large-area clone plantations. To maintain long-term stability of those

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plantations, it is necessary to explore the influence of scions on root characteristics of rootstocks at long time scale, but such research studies are limited, especially in conifers.

Roots play a crucial role in maintaining plant stability, water uptake, and nutrient assimilation (Lynch, 1995; Liao et al., 2019), and the fine roots mainly bear the absorption function, while the medium roots mainly have the water transmission and storage function, so the amount and distribution pattern of fine and medium root biomass will affect the overall water and nutrient balance of plants (Gu et al., 2017; Kirfel et al., 2017). The growth dynamics of fine roots can be monitored using minirhizotron technology (Iversen et al., 2012), and the root biomass and architecture can be revealed by full excavation method (Sun et al., 2022). The root architecture indices such as root length, root link number (RLN) and root link length (RLL) are also closely related to plant growth and resistance (Price et al., 1997), which determine the location of roots in soil space, spatial expansion capacity and access to resources (Malamy, 2005). Different orders of roots are typical fractal structures showing similar regularity in structure and function. Fractal parameters can well reflect the root architecture characteristics and have been applied in the research of crop roots (Grift et al., 2011), sandy shrubs (Yang et al., 2015) and conifer saplings (Dong et al., 2014) through box-counting method or X-ray computed tomography (Paya et al., 2015). But these methods often depend on root images including information of all different orders of roots, which are not suitable for huge root system of mature trees due to over-counting and under-counting of boxes, larger box-height, smaller number of grid sizes, and inappropriate line fitting method (Panigrahy et al., 2019). Thus, fractal parameters are still difficult to be applied in huge root system.

P. densiflora var. *zhangwuensis* is a natural hybrid of *P. densiflora* and *P. tabuliformis* (based on the evidence of RAPD) or *P. sylvestris* (based on the evidence of complete chloroplast genome) (Xia et al., 2021). Previous studies showed that, compared with the same-age *P. sylvestris* var. *mongolica*, *P. densiflora* var. *zhangwuensis* had higher chlorophyll and leaf water content, lower transpiration rate and higher photosynthetic rate (Li et al., 2003; Meng et al., 2012), higher antioxidant and osmoregulation ability under drought stress (Meng et al., 2010). In this study, it was used as scion, and *P. sylvestris* var. *mongolica* as rootstock, and the root characteristics of *P. sylvestris* var. *mongolica* rootstock (PR) and ungrafted *P. sylvestris* var. *mongolica* (UP) were compared after 25-year grafting. The aim is to answer the following questions: 1) Does *P. densiflora* var. *zhangwuensis* scion (PS) affect the root characteristics of PR at long time scales? What are the 2) differences and 3) similarities of the root characteristics between PR and UP?

Materials and methods

Study site

Study site is located in Zhanggutai Town, Zhangwu County, Liaoning Province, China, at 42.684~42.686°N, 122.573~122.575°E, with an altitude of 226.5 m. The average annual precipitation is 433 mm, the average evaporation is 1570 mm, the average annual temperature is 6.7°C, the frost-free period is 154 d. The strong wind frequently occurred in spring and winter, and the instantaneous maximum wind speed is 32 m/s, and the sandstorms blow up to more than 240 times with speed of more than 5 m/s per year (Deng et al., 2020).

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Grafted and ungrafted plantations

The *P. sylvestris* var. *mongolica* plantation with an area of 2.5 hm^2 was grown in a flat sandy land in early April 1992, and the primary plant spacing was 2.0 m×2.5 m. In 1995, PS were grafted onto the PR, forming 1.3 hm^2 clone plantation, and the remaining 1.2 hm^2 UP as the control (*Fig. 1*). The two plantations underwent the same management practices, with a thinning in 2012 increasing plant spacing to 4.0 m×5.0 m. Each plantation was divided into two blocks, and three 10 m×10 m permanent plots were set randomly in each block. The growth indices including tree height (TH) and diameter at breast height (DBH) of every tree in each plot were investigated in the year of 2000, 2010, and 2020, and one sample tree was selected per plot per investigation according to average TH and DBH used to determine biomass and collect root samples.

Figure 1. *Aerial photo of study site (left) and growth appearance of the two species (right). I: P. densiflora var. zhangwuensis clone plantation, II: Ungrafted P. sylvestris var. mongolica (UP) plantation. The small white square in the left figure represents the position of the sample plots in each plantation. Red arrow in the right figure indicates the graft junction of P. densiflora var. zhangwuensis*

Soil physicochemical properties

In beginning of the study, the sandy soil in study site was barren and arid, and the organic matter content of 0~100 cm soil layer was 0.646%, total nitrogen and total phosphorus were 0.017% and 0.019%, and pH value was 6.27. From top to bottom, the soil was made up of 0~50 cm gray sand layer, 50~150 cm black brown sand layer, and white sand layer. A hard crust layer existed in the white sand layer (about 250~300 cm underground), which was too hard to excavate with shovel or pick and had low organic matter and high particle content of above 0.25 mm (more than 60% of total soil particles) (*Fig. 2A*). The groundwater level was 3.6 m, and topsoil (0~40 cm layer) water content varied annually, from 4.0% to 8.0%.

At the last soil investigation (2020), the total nitrogen and total phosphorus in 0~100 cm soil layer increased to 0.032 ± 0.011% and 0.026 ± 0.009%. However, the soil was clearly acidified with pH values ranging from 5.3 to 5.9, leading to an increase in available phosphorus (AP) release and accumulation in the deep soil with rainfall, so the AP content in 150~300 cm layer was significantly higher than 0~100cm soil layer

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(*Fig. 2B*), whereas the available nitrogen and potassium decreased significantly with soil depth (*Fig. 2C*). The annual change of topsoil water content was further increased, from 1.5% to 9.5%, and the groundwater level decreased to 5 m.

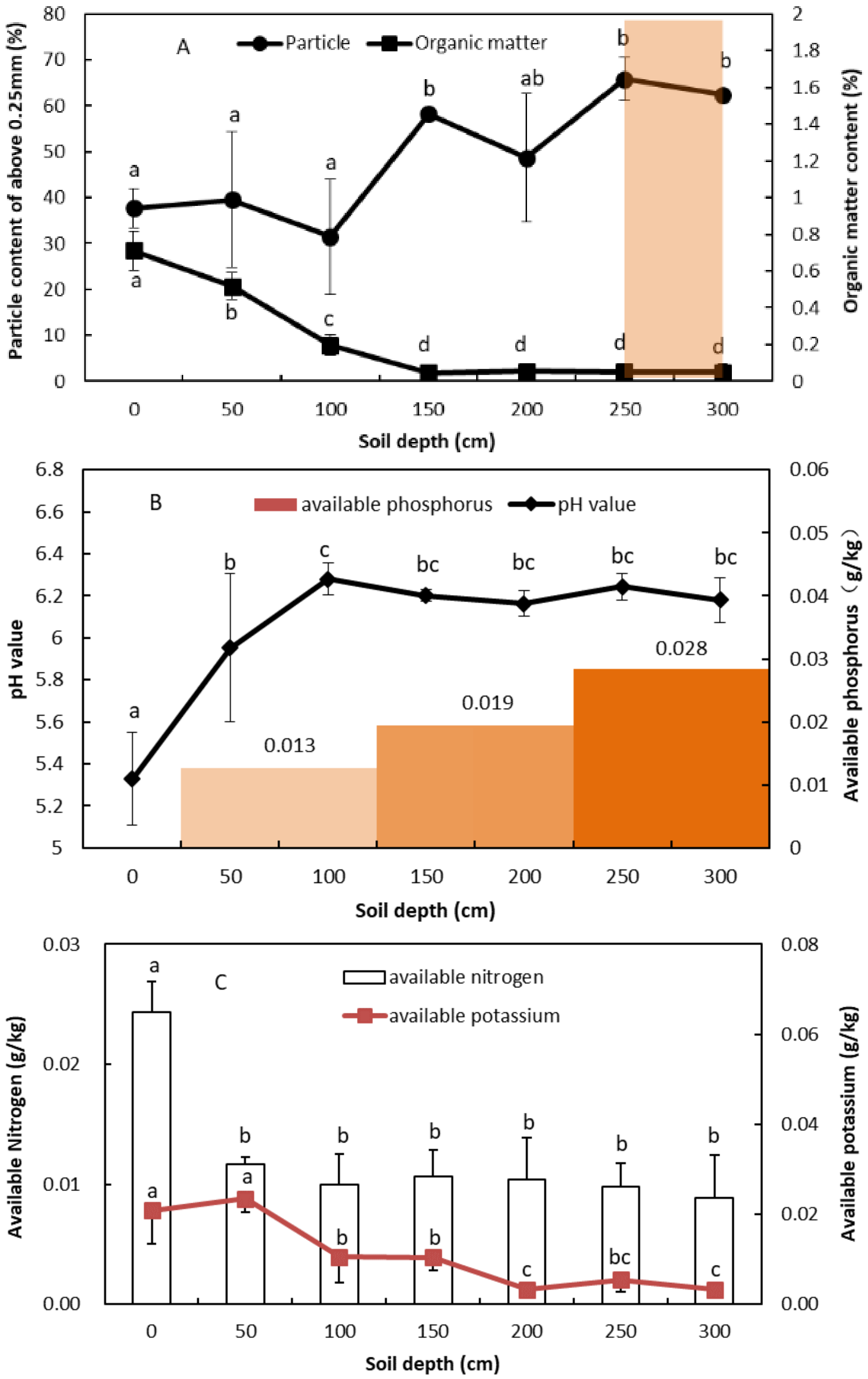

Figure 2. *Soil conditions of the studied plots. A: Particle content above 0.25mm and organic matter (shadow range is hard crust layer). B: pH value and available phosphorus content of different soil depths. C: Available nitrogen and potassium content of different soil depths. Data in A were investigated in 2000, and B,C in 2020*

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Root excavation and investigation of root biomass and architecture

In April 2019, 36 root tubes were buried in three directions (northeast, south and northwest; one tube each direction) for each sample tree with embedding angle of 45°. On July 20th, August 31st, and October 10th of 2019, the root growth dynamics of two species was investigated using BTC-100 minirhizotron root ecology monitoring system (Bartz company, USA). Firstly, the probe with ultraviolet light source was inserted into the tube to find the root that could be investigated. After determining the target root, the computer would record the tube number and tube length where the probe was located, so as to continuously investigate the same root at the next time point. Finally, the photos of the target roots in different periods were compared and analyzed.

Three full excavations were done to determine the root biomass of coarse roots (≥2.0 cm diameter), medium roots (0.2~2.0 cm) and fine roots (≤0.2 cm) in June of the year 2000, 2010 and 2020, respectively. At the last root investigation (2020), the 8 m long, 6 m wide and 4 m deep pits were manually excavated to ensure more than 98% of total roots could be excavated. To prevent the roots broken, the soil was soaked with water before excavation, and a sprinkling can was used to keep them wet. Photos were taken when the vertical profile of root system was excavated out, then the sample trees were cut down and all roots were collected.

The order of root was determined as follows: 1st order roots referred to the roots born from root collar, 2nd order roots were born from1st order roots, and so on. The diameter and length of coarse and medium roots were measured with electronic cursor caliper (Mitutoyo, Japan). The fine roots were taken back to the laboratory and evenly tiled in the root disc and scanned with an Epson V700 scanner at a resolution of 300 dpi. Then Win RHIZO 2003b (Regent Instruments, Quebec, Canada) was used for image analysis to determine root length and diameter indicators. The RLN was counted, which is the sum of the number of internal links (between two branch points) and external links (between branch point next to a certain root tip and the root tip), and the average RLL was calculated, which was the ratio of the total root length to the RLN (Shan et al., 2013). The root biomass in each soil layer was measured by coarse, medium, and fine root through oven drying method (Le Goff et al., 2001). Then, the specific root length (SRL, cm/g) was calculated, which was the ratio of the total root length to the total root biomass (Spek et al., 1994). The ratio of stem biomass to leaf biomass (stem : leaf ratio) and the ratio of root biomass to aboveground biomass (root : stem ratio) were also calculated.

Fractal parameter estimation

Based on the average diameter of 1st order roots, three sample roots were selected for each sample tree in the northeast (NE), south (S) and northwest (NW) directions. Then the diameter (d) at all the branch points of the sample root and the corresponding total root length of all roots below each branch point (Lp) were investigated, and the correlation formulas (*Eq.1~Eq.3*) were based on the improved McMahon and Kronauer method, and the root fractal dimension was estimated with SPSS19.0 software (IBM, USA) (Meng et al., 2011).

$$d = \gamma(Lp+L_0)^{\beta} \qquad \text{(Eq.1)}$$

where γ and L_0 were the constant, and β was fractal dimension.

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Eq.1 was transformed to *Eq.2* when estimating the parameters through a two-step regression method. Step 1: L_0 was estimated based on the linear relationship between *d* and *Lp* according to *Eq.3*, and the fitness (R^2) of the formula should be above 0.3. Step 2: The estimated L_0 was put into the *Eq.2* to estimate the fractal dimension (β) and fractal abundance (lnγ).

$$\ln d = \beta \ln(Lp + L_0) + \ln\gamma \quad \text{(Eq.2)}$$

where – lnγ was fractal abundance.

$$d = kLp + kL_0 \quad \text{(Eq.3)}$$

where k and kL_0 were the slope and intercept of trend line.

Data processing

All data were statistically analyzed by SPSS 19.0 software. One-way ANOVA was performed for inter-group data of growth amount, biomass, and root architecture parameters. The means were compared at the least significant difference LSD test at 5% probability. Excel 2019 was used for drawing figures.

Results

Growth comparison between the two plantations

As shown in *Table 1*, the growth indices of *P. densiflora* var. *zhangwensis* at different ages were higher than the same-age UP. After 25-year grafting, its TH, DBH and individual volume were 1.12, 1.44 and 2.06 times higher than those of UP, respectively. The variation coefficients of TH, DBH and individual volume of *P. densiflora* var. *zhangwuensis* clone plantation was smaller, indicating that it had smaller growth dispersion degree and more consistent growth among individual trees due to the characteristics of high repeatability in grafted clone (Zheng et al., 2015).

Table 1. *Growth comparison between P. densiflora var. zhangwuensis and UP*

Age (year)	Species	TH (m)		DBH (cm)		Individual volume (m^3)	
		Mean±SD	Variation coefficient	Mean±SD	Variation coefficient	Mean±SD	Variation coefficient
3+5	*P. densiflora* var. *zhangwuensis*	2.84±0.29a	0.102	7.86±0.79a[1]	0.101	——	——
8	UP	1.56±0.29b	0.186	5.37±1.02b[1]	0.190	——	——
3+15	*P. densiflora* var. *zhangwuensis*	6.08±0.26a	0.042	11.40±0.92a	0.081	0.038±0.006a	0.160
18	UP	3.76±0.47b	0.126	6.98±1.03b	0.147	0.011±0.004b	0.330
3+25	*P. densiflora* var. *zhangwuensis*	7.96±0.43a	0.054	16.76±2.54a	0.152	0.097±0.030a	0.309
28	UP	7.12±0.82b	0.115	11.65±2.75b	0.236	0.047±0.023b	0.489

Different lowercase letters indicated significant difference between different species (n=30), and the variation coefficient was standard deviation divided by mean value. In the first column of this table, the age of *P. densiflora* var. *zhangwuensis* was the sum of the age of rootstock when grafting and age after grafting, which was the same age as the age of UP. Subscript 1 indicated the data was ground diameter

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Figure 3 showed that in the shallower soil layer (20-60 cm), *P. densiflora* var. *zhangwuensis* had formed branch roots when investigated in July, and new branch roots were still formed in autumn, while roots of UP focused on elongation and thickening growth, and rarely produced branch roots. In deeper layer, the roots of *P. densiflora* var. *zhangwuensis* elongated and formed many branches in late summer, and these newly formed branches did not die in autumn. However, the roots of UP elongated without branches in summer, and produced branches only in autumn, furthermore the majority of its branches died rapidly.

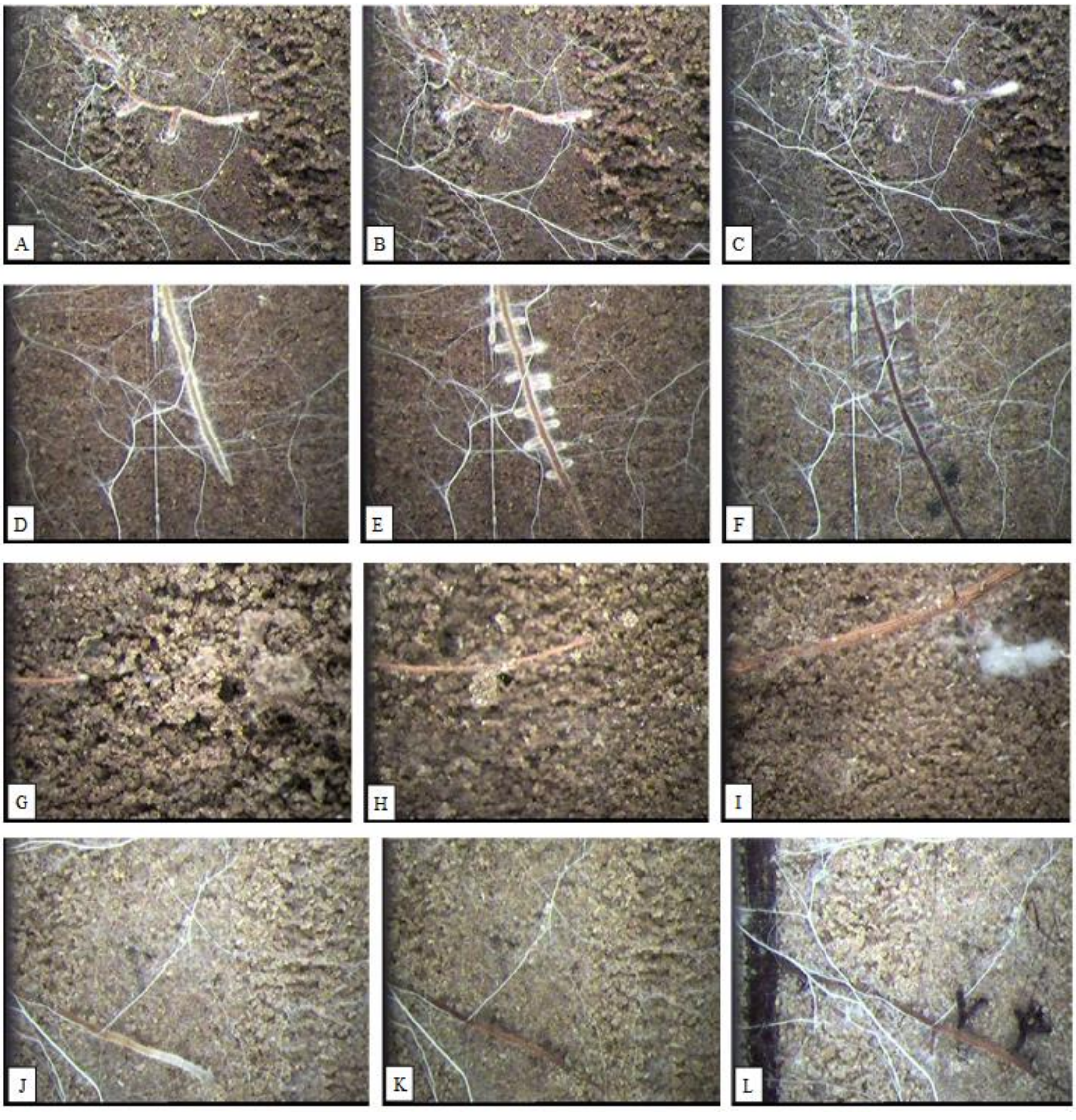

Figure 3. *The root growth dynamics of two species in shallow(20-60cm) and deep(60-100cm) soil layers investigated using minirhizotron root ecology monitoring system on July 20th (Shallow layer: A: P. densiflora var. zhangwensis; G:UP. Deep layer: D: P. densiflora var. zhangwensis; J:UP.), August 31st (Shallow layer: B: P. densiflora var. zhangwensis; H:UP. Deep layer: E: P. densiflora var. zhangwensis; K:UP.), and October 10th (Shallow layer:C: P. densiflora var. zhangwensis; I:UP. Deep layer: F: P. densiflora var. zhangwensis; L:UP.) of 2019. Black branching roots in L were dead*

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Biomass comparison of roots in different sizes between PR and UP

Since *P. densiflora* var. *zhangwuensis* was grafted onto *P. sylvestris* var. *mongolica*, its roots actually born from PR. After 25-year grafting, the root biomass of PR was 17.1 kg, 3.24 times that of UP and occupied 24.8% of TB of *P. densiflora* var. *zhangwuensis* (69.09 kg). However, root biomass of UP only occupied 15.6% of its TB (33.88 kg). The coarse root biomass of PR was about 9.20 kg and 2.75 times higher than UP, but the proportion of coarse root biomass to the total root biomass (TRB) was less than UP by 9.6% (*Fig. 4*). The medium root biomass in PR was 7.28 kg and significantly higher than UP (1.74 kg), and the proportion of medium root biomass to TRB was also more than UP by 9.6%. The fine root biomass of PR was about 0.65 kg and 3.25 times higher than UP, furthermore, the fine root biomass : TRB ratio for both were the same (3.8%).

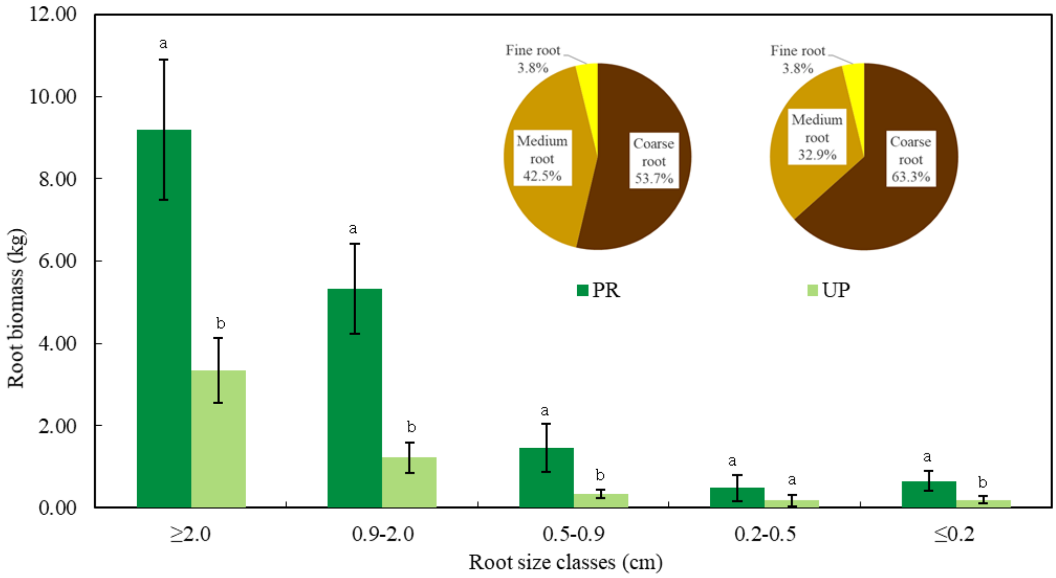

Figure 4. *Biomass of different diameter sizes of roots in PR and UP. The two pie charts in the figure showed the proportion of different sizes of roots' biomass to the total root biomass in PR and UP, respectively*

Biomass comparison of roots in different soil layers between PR and UP

In 0~120 cm soil layer, the TRB of PR was significantly higher than UP, especially it was 37.2 times higher in 60~80 cm soil layer. In the range of 0~100 soil depth, the fine root biomass of PR in all layers were significantly higher than UP (except in 40~60 cm layer, p=0.055, close to significant difference), especially in 80~100 cm soil layer, which was 5.7 times that of UP (*Table 2*). Most of fine roots of UP distributed in 0~40 cm soil surface layer, accounting for more than half of its total fine root biomass, and the remaining fine roots only distributed in 40~100 cm soil layer, and no fine roots distributed in 100~160cm soil layer. However, fine roots of PR distributed vertically in larger range from 0 to 160 cm soil layers. Its fine root biomass in 0~40 cm soil layer only occupied 35.6% of its total fine root biomass, and the remaining distributed in deep soil from 40 to 160 cm (*Table 2*).

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Table 2. *Vertical distribution of root biomass of PR and UP (Net weight, g)*

Soil depth (cm)	PR		UP	
	Total root biomass	Fine root biomass	Total root biomass	Fine root biomass
0~20	5198.83±825.71aA	164.79±49.52aA	2460.54±481.33bA	62.88±25.27bA
20~40	1601.85±254.42aC	66.47±20.01aA	1736.44±339.69aA	38.86±16.70bA
40~60	2787.89±442.79aB	65.13±19.58aA	155.39±30.40bB	24.33±8.96aA
60~80	4441.63±705.45aA	155.63±46.76aA	119.40±23.36bBC	48.34±18.98bA
80~100	2221.82±352.88aBC	137.90±41.43aA	98.49±19.27bBCD	24.01±9.42bA
100~120	847.66±134.63aD	23.46±7.05B	72.38±16.18bCD	——
120~140	22.32±6.71aE	22.32±6.71B	66.78±13.06bD	——
140~160	14.30±4.30aE	14.30±4.30B	69.05±11.49bCD	——
280~300	——	——	75.32±16.35CD	1.58±0.64B

The data in the table were the mean ± standard deviation, n=3. Different lowercase letters indicated significant differences between PR and UP, and having no same uppercase letters indicated significant differences among different soil layers for PR or UP

UP always had a root (nearly 4 m in length) that could pass through the hard crust in 250~300 cm soil layer, but the fine root biomass in this layer was very small, mean value only 1.58 g, accounting for approximately 0.8% of its total fine root biomass. However, PR had no roots passing through the hard crust.

Comparison of root architecture between PR and UP

As shown in *Table 3*, the total root length of PR was 2492.9 m and 4.24 times higher than UP, and its root length of all sizes were also higher, especially medium root length was significantly higher. In all medium roots, the root length of PR was 5.46 (for diameter 0.2~0.5 cm roots), 5.67 (for diameter 0.5~0.9 cm roots) and 4.72 (for diameter 0.9~2.0 cm roots) times that of UP. Furthermore, the medium root length occupied 70.7% of the total root length in PR, but it was only 57.4% of the total root length in UP.

Table 3. *Comparison of root architecture indices for different diameter sizes of roots between PR and UP*

Treatment	Index	≥2.0 cm	0.9~2 cm	0.5~0.9 cm	0.2~0.5 cm	≤0.2 cm
PR	Total root length(m)	326.2±103.2a	608.0±187.9a	482.2±141.4a	673.8±253.5a	402.7±171.6a
	Proportion (%)	13.1	24.4	19.3	27.0	16.2
	RLN	384±158a	2620±1010a	5542±2240a	23589±10428a	34596±14168a
	Proportion (%)	0.6	3.9	8.3	35.3	51.8
	Average RLL(cm)	90.7±14.1a	23.8±1.7a	9.2±1.2a	3.0±0.3a	1.2±0.1a
	SRL(cm/g)	3.4±0.8a	11.3±2.8a	35.4±16.2a	160.0±80.4a	77.7±64.4a
UP	Total root length(m)	108.0±66.3a	128.9±78.2b	85.1±11.9b	123.3±33.5b	142.1±76.5a
	Proportion (%)	18.4	21.9	14.5	21.0	24.2
	RLN	105±66a	330±171b	555±208b	1877±729b	10274±5841a
	Proportion (%)	0.8	2.5	4.2	14.3	78.2
	Average RLL(cm)	104.7±3.4a	37.9±9.0a	18.0±7.2a	7.3±2.1b	1.5±0.3a
	SRL(cm/g)	3.4±2.9a	9.6±5.5a	25.8±3.0a	142.3±160.9a	99.0±108.3a

The data in the table were the mean ± standard deviation, n=3. Different lowercase letters indicated significant differences between PR and UP

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The total RLN of PR was 66731, and 5.08 times that of UP, especially RLN in medium roots was significantly higher. In all medium roots, the RLN of PR was 12.57 (for diameter 0.2~0.5 cm roots), 9.99 (for diameter 0.5~0.9 cm roots) and 7.94 (for diameter 0.9~2.0 cm roots) times that of UP, respectively. In addition, in PR, the ratio of RLN of medium roots to the total RLN was 47.5%, which was higher than that in UP. The average RLL showed no significant difference between PR and UP, except for diameter 0.2~0.5 cm roots, where the RLL of UP is longer than that of PR, which may be caused by the premature death of its branch root (*Fig. 3*). In addition, there was no significant difference in SRL of different sizes of roots between PR and UP.

For all roots investigated, the highest root order was 8^{th} order for *P. densiflora* var. *zhangwuensis*, and 7^{th} order for UP, while the lowest root order was 5^{th} order for the former, and 4^{th} order for the later. The number of 2^{nd} order roots per 1^{st} order root was 103.8±36.2 for *P. densiflora* var. *zhangwuensis*, which was 2.86 times that for UP (36.3±24.1). Both PR and UP roots had fractal characteristics, and the fractal dimension and fractal abundance of PR roots were 1.741 ± 0.170 and 8.138 ± 1.195, respectively, which were significantly higher than UP by 1.18 and 1.27 folds, respectively (*Table 4*).

Table 4. *Fractal parameters of root system of PR and UP*

Sample tree	Sample root	PR		UP	
		Fractal dimension	Fractal abundance	Fractal dimension	Fractal abundance
NO.1	NE	1.773	8.357	1.466	6.377
	NW	1.587	7.203	1.734	8.165
	S	1.923	9.425	1.631	6.567
NO.2	NE	1.530	5.829	1.172	4.373
	NW	1.921	9.823	1.162	5.330
	S	1.834	9.003	1.746	7.420
NO.3	NE	1.554	7.722	1.382	5.699
	NW	1.571	7.197	1.355	5.799
	S	1.972	8.682	1.580	7.891
Mean ± standard deviation		1.741±0.170a	8.138±1.195a	1.470±0.104b	6.402±0.544b

Different lowercase letters indicated significant differences between PR and UP

Discussion

Limited data are available regarding the effects of grafting on root system in grafted trees at long time scale (Fassio et al., 2016), and changes in proportion of different sizes of roots after grafting can reflect the biomass allocation strategy (Blume-Werry et al., 2018). Our study found that, after 25-year grafting, the root biomass of PR did increase compared with UP at the same age, the coarse root biomass ratio of PR decreased by nearly 10%, and its medium root biomass ratio increased by nearly 10%, indicated that PR has reduced the coarse root biomass to improve the medium root biomass, but remained the fine root biomass allocation ratio unchanged.

The vertical distribution of plant roots (especially fine roots) reflects the range of water and nutrients that plant can utilize (Wang et al., 2021). Long-term grafting had made root vertical distribution range enlarged, and more fine roots of PR occupied in 40~160 cm soil layer, which could effectively use the water and nutrients (especially AP) in deep soil layer and keep above-ground *P. densiflora* var. *zhangwuensis* in water and nutrient

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balance for a long time to maintain large growth and biomass (*Table 1, Fig. 4*). However, almost all fine roots (99.2%) of UP distributed in 0~100 cm soil layer, especially more than 50% fine roots distributed in topsoil, which was consistent with the findings of Zhang et al. (2021), indicating that UP was more suitable to use water and nutrients in topsoil. This strategy contributed to its low survival at young tree stage in dry years, but for mature trees, water demand increased rapidly, and this strategy has exposed the shortcoming of insufficient water and AP supply. Unsteady supply of water and AP during growing season was the reason for decline of mature *P. sylvestris* var. *mongolica* plantations in Horqin sandy land (Zhu et al., 2005; Wei et al., 2013).

The appropriate root architecture can make plants pass through water and nutrient deficit (Malamy, 2005), and then resist various natural disasters such as drought and windthrow. In some fruit tree varieties, the effect of scions on root architecture of rootstocks has been reported (Koizumi et al., 2008; Li et al., 2016), but few such reports in conifers. Our study found that the RLN of PR after long-term grafting was higher than UP, especially the RLN of medium roots increased significantly, indicating more root branching (proved by *Fig. 3*) and more complicated topological structure (higher fractal dimension), so the ability of PR to obtain water and nutrient in sandy soil was enhanced. Liese et al. (2017) also found that branching of lower order roots (medium roots) could be considered a leading root trait of the plant economics spectrum of temperate trees, since it related to the mycorrhizal association type and belowground resource exploitation. The larger fractal dimension often indicated more root branching and complex topology, while the greater fractal abundance indicated larger expansion of roots in the soil (Song et al., 2015) and improvement in fractal abundance can be achieved by increasing RLL or RLN (Hauck et al., 2015). The root fractal abundance of PR was significantly higher ($p<0.05$), reflecting the larger expansion of its roots in the sandy soil after long-term grafting. The average RLL of PR was not significantly different from UP, even smaller than UP for 0.2-0.5cm roots, so the volume expansion of PR roots was not achieved by increasing RLL, but by increasing total RLN mainly due to the increase in the number of medium root branches. The larger expansion volume of PR roots also increased the windthrow resistance of trees and enhanced the stability of *P. densiflora* var. *zhangwuensis* clone plantations in the sandy land with strong wind. Grafting did not alter SRL of PR, suggesting that this trait was determined by genetic properties. Previous studies have shown that SRL were greatly influenced by hereditary (Bingham et al., 2011) and had the species-specific characteristics (Pan et al., 2018). Since the roots of both studied plantations originated from only one species (*P. sylvestris* var. *mongolica*), it is understandable that no significant difference in SRL.

Unlike PR, UP tends to expand root range by increasing RLL, the most extreme example of which is to invest more biomass into one 1^{st} root to promote its elongation passing through the hard crust with very few fine roots (*Table 2*), and it may be induced by the increased AP in the hard crust layer (*Fig. 2*). The study site is located in a typical P deficiency region, which is lower than the global level (Khan et al., 2021). The effect of P deficiency on root architecture has been reported, various plants can change their root morphology and architecture to improve the tolerance to phosphorus stress, such as by increasing RLN, RLL, and root-stem ratio (Yu et al., 2018; Fletcher et al., 2020). In our study, UP has shallow root properties due to most of its root biomass distributing in topsoil where P is lacking. In response to this stress, UP has adopted the strategy of enhancing gravitropic growth of one 1^{st} root to use the deep soil P elements. This strategy appears to be inadequate to alter P deficiency due to the small fine root biomass in hard

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crust. However, PR under the long-term influence of PS with high photosynthetic capacity prolonged the survival time of fine roots and made most of them evolve into permanent medium roots and expanded the root range by increasing the number of medium roots and total root length and fine root biomass. PR adopted the above strategy to increase P absorption, instead of the strategy letting one 1st root pass through the hard crust.

Conclusion

After 25-year grafting, under the long-term induction of PS, the PR underwent beneficial changes in root biomass distribution and root architecture, but some root traits still remained unchanged. The total root length and root biomass of PR were increased significantly, and the biomass allocation strategy was also changed, mainly manifested in the increase of the medium root biomass ratio by reducing coarse root biomass ratio. The RLN, fractal dimension, and fractal abundance of PR were larger than UP, indicating that its roots had many branches, more complex topology, and larger expansion volume in the soil. Long-term grafting changed the root characteristics of *P. sylvestris* var. *mongolica* when used as a rootstock, made it abandon the strategy of rapid gravitropic growth of individual 1st order root, and significantly expand the vertical fine root range to better utilize the water and nutrients in the deep soil. The above characteristics would ensure *P. densiflora* var. *zhangwuensis* clonal plantations grafted by PR more suitable for growth in windy and sandy areas with long-term stability.

On the contrary, the allocation strategy of the fine root biomass of PR remained unchanged, showing no change in the fine root biomass : TRB ratio. In addition, the average SRL were not affected by long-term grafting, and had relative genetic stability.

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Conflict of interest. The authors declare that they have no conflict of interest.

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