RESEARCH ARTICLE

Regulatory effects of root pruning on leaf nutrients, photosynthesis, and growth of trees in a closed-canopy poplar plantation

Da-wei Jing1, Zhen-yu Du2*, Ming-you Wang3, Qing-hua Wang2, Hai-lin Ma2, Fang-chun Liu2, Bing-yao Ma2, Yu-feng Dong2

1 College of Resources Environment and Planning, Dezhou University, Dezhou, Shandong, China, 2 Institute of Resource and Environment, Shandong Academy of Forestry, Jinan, China, 3 College of Ecology and Garden Architecture, Dezhou University, Dezhou, Shandong, China

* zydu007@163.com

Abstract

A plantation of 5-year-old poplar Populus × euramericana cv. ‘Neva’ was used to study the regulatory effects of root pruning on nutrients, photosynthetic characteristics, and water-use efficiency (WUE) of leaves and growth rates of diameter at breast height (DBH; 1.3 m), tree height, and volume. Six root-pruning treatments were conducted with different combinations of intensity (at a distance of six, eight or ten times DBH from the trunk) and orientation (on two or four sides of the trees). Results showed that the N, P, K, photosynthetic rate, transpiration rate, and stomatal conductance of leaves were all significantly decreased by root pruning over the initial period following root pruning (30 days), but increased in the subsequent investigations. The values of the above indexes peaked in 8–2 treatment (i.e., eight times DBH distance on two sides). The leaf WUE in 8–2 treatment, and average growth rates of DBH, tree height and volume, were the highest among all treatments within 3 years of root pruning. The results indicated that the root pruning based on the appropriate selection of intensity and orientation had significant positive effects on leaf nutrients, photosynthesis, and growth of trees in a closed-canopy poplar plantation.

Introduction

Compared with other tree species, poplar has many characteristics that make it suitable for cultivating in plantations, enabling the production of large quantities of wood in short periods of time. These features consist of rapid growth, adaptability to diverse environmental conditions, and suitability for different silvicultural systems [1–3]. In China, poplars are usually cultivated at a density of 830–1100 trees per ha. With the increase of growth time, the lateral roots of poplar gradually expand and inevitably intermingle with those of neighboring trees after canopy closure. Interweaved roots inhibit the growth of poplar, and is beneficial to the spread of pests and diseases, and thus, significantly reduce root vitality during this period [4–5]. In addition, the amount of fine roots decreased when poplars were at the stage of canopy closing, thereby significantly suppressing the ability of the poplar root system to absorb water and nutrients [5–7]. Therefore, how to improve root system vitality in poplar closed-canopy plantations is particularly important for tree growth and, thus, the economic return of such plantations.
Root pruning is a common technique that can reduce the vegetative growth of fruit trees [8–9]. It not only assists in dwarfing, but also stimulates the emergence of new roots necessary to sustain growth [10]. Specifically, root pruning destroys the old growth balances of trees and changes their assimilation abilities, nutrient distributions, and hormone levels. The combined action of these elements can cause different effects on growth and fruit quality [9]. Root pruning is also used in poplar tree transplanting. The root system is trimmed to a manageable size (usually to a diameter of approximately 20 cm) after lifting in preparation for storage and planting the following spring [11]. Recent studies of Chinese winter jujube (Ziziphus jujuba Mill.) found that root pruning significantly stimulated the germination of a large number of new roots at the incision site, increased root vitality, and expanded the absorption area of the root, thereby significantly improving the nutrition conditions and photosynthetic characteristics of the leaves [12]. The results of a previous study showed that root pruning of poplar can also improve rhizosphere soil fertility at the stage of canopy closing [4]. However, less information is available on the effects of root pruning on the leaf nutrients and photosynthetic characteristics of poplar trees.

Water-use efficiency (WUE), measured as the biomass produced per unit transpiration, describes the relationship between water use and plant productivity. The basic physiological definition of leaf WUE is equal to the photosynthesis: transpiration ratio, also referred to as the transpiration efficiency [13]. If leaf-level transpiration and stomatal conductance are measured simultaneously, these measurements can be used to determine leaf WUE [14–15]. Under certain conditions, promoting root growth and increasing root weight can increase yield and drought resistance [16]. However, more roots might not be favorable in arid and semiarid areas. There is evidence that breeding of wheat varieties has unknowingly increased WUE by reducing the size of the root system [17–18]. However, no research has been reported regarding the effect of changing the size of the root system on WUE of closed-canopy poplar.

Our previous research on canopy-closed poplar trees reported that removing some roots at a distance of 8 times the diameter at breast height (DBH) from trunk on both inter-row sides of the trees improved tree growth and rhizosphere soil fertility [4–5]. However, it is currently unknown whether cutting roots of poplars on four sides at a certain distance from trunk will have improved results compared with pruning roots on two sides. In addition, a previous study that pruned roots only on two sides of poplar trees reported only preliminary data, lacking further exploration of the effects of root pruning on nutrition, photosynthesis, and WUE of leaves; therefore, it will be informative to clarify the regulatory effects of root pruning on closed-canopy poplar plantations.

In this study, we investigated the effects of root pruning on leaf nutrients, photosynthetic characteristics, WUE, and growth of closed-canopy poplars. We hypothesized that root pruning would improve photosynthetic performance and increase tree growth. The objective of this research was to determine the feasibility of implementing root pruning in plantations and then to select optimal root pruning measures to increase the growth of poplar at the stage of canopy closing.

**Materials and methods**

**Ethics statement**

This research did not involve human or other animal subjects. For plant collections, we collected the minimum number of specimens necessary to ensure that appropriate vouchers were obtained. The field studies did not involve endangered or protected species. Permission to work in a poplar plantation located in Ertun township was obtained through a cooperative agreement between Dezhou University and Dezhou Forestry Bureau.
Site description and plant material

The poplar plantation was located in Ertun township, Dezhou city, Shandong Province, north China (36˚24'N latitude, 115˚45'E longitude), which has a warm temperate zone continental monsoon climate with four distinct seasons, an annual average temperature of 14˚C, and average annual rainfall amount of 650–700 mm. The amounts of available nitrogen (N), phosphorus (P), and potassium (K) in the soil were 35.02, 14.21 and 86.17 mg kg⁻¹, respectively, and the organic matter content was 11.36 g kg⁻¹ [19]. Soil pH was 8.56 (1:2.5 soil/water suspension). N, P₂O₅, and K₂O had been carried out annually at rates of 205.36, 70.62, and 58.70 kg ha⁻¹, respectively. The fertilizers included urea, ammonium phosphate, and potassium chloride. The poplar ‘I-107’ (Populus × euramericana cv. ‘Neva’) had been planted 5 years previously at a spacing of 4 × 3 m. The experimental trees were uniform, and the average tree height and DBH (1.3 m) were 12.26 m and 11.25 cm, respectively. These trees are carefully managed and grown as short rotation (7–8 years) poplar mainly for pulpwood production.

Experimental design and root pruning treatments

The experiment involved a randomized complete block design with seven treatments of three replications each. A total 21 plots was established and each replication of every treatment contained a plot with 30 trees arrayed in five rows, but only the innermost 12 trees, which were identical to the plot mean, were used for detailed measurements. Shortly after the leaves had fully developed, seven treatments were applied to the poplar trees at the beginning of the growing season on April 18th, 2015 and the root-pruning treatments were carried out once during the experimental period. The treatments were as follows: (1) CK, the control, with no root pruning; (2) 6–2, removing the root system at a distance six times the DBH from the trunk (74.10 cm) on both inter-row sides of each poplar; (3) 6–4, removing the root system at a distance of six times the DBH from the trunk (74.10 cm) on four sides of each poplar; (4) 8–2, removing the root system at a distance of eight times the DBH from the trunk (98.80 cm) on both inter-row sides of each poplar; (5) 8–4, removing the root system at a distance of eight times the DBH from the trunk (98.80 cm) on four sides of each poplar; (6) 10–2, removing the root system at a distance of ten times the DBH from the trunk (123.50 cm) on both inter-row sides of each poplar; and (7) 10–4, removing the root system at a distance of ten times the DBH from the trunk (123.50 cm) on four sides of each poplar. Each root system was cut with a sharp metal spade to a depth of 30 cm and root pruning was conducted in a line that was either parallel or vertical to the tree row. The treated trees were managed in accordance with routine methods.

Data collection and determination

For all treatments, mature leaves were sampled during late May, 2015 (the start of the fast-growth period); mid-October, 2015 (the end of the fast-growth period); late May, 2016; mid-October, 2016; late May, 2017; and mid-October, 2017. At each sample date, nine representative samples from every tree were collected for each treatment; the samples were selected from mature leaves on the sunny side of each tree. A portable gas exchange system (LI-6400, LI-COR, Nebraska, US) was used to determine the net photosynthetic rate (Pₙ), transpiration rate (Tᵣ), and stomatal conductance (gₛ) from 09:00 h to 11:00 h. Instantaneous leaf WUE was calculated as the ratio of Pₙ to Tᵣ (Pₙ/Tᵣ) according to Wong et al. [20] and Ren et al. [21]. The leaves sampled were quickly taken back to the lab, which was placed in an oven at 105˚C for 15 min, then dried at 80˚C and ground through a 30-mesh screen for the determination of N, P, and K concentrations. Leaf N concentration was measured using the H₂SO₄–H₂O₂– distillation method, P concentration using the vanadium molybdenum yellow colorimetric method, and K concentration using flame photometric method [19].
Tree height and DBH were measured by the tangent method [22] using a ruler with 0.5-mm accuracy at the beginning of experiment (April 18th, 2015) and at the end of the short rotation period (October 18th, 2017). The tree volume was calculated using Eq 1:

\[ V = \frac{3.14d^2hf}{4} (f = 0.42) \]

where \( d \) and \( h \) represent DBH (cm) and tree height (m), respectively [4]. Then the average growth rates of DBH, tree height, and tree volume were calculated according to the method of Meng [23].

Additionally, all the fine roots less than 2 mm diameter in the root distribution zone with the depth of 0–40 cm for all treatments were collected from the soil at the last sample time (mid-October, 2017), according to the method of Du et al. [24]. After scanning, they were processed with WinRHIZO (Regents Instruments Inc. Quebec, Canada) to obtain surface area. Then, fine roots were dried in an oven at 80˚C for 48 hours and weighed.

**Statistical analysis**

The data were analyzed as a completely randomized design. An analysis of variance (ANOVA) was carried out to evaluate the effects of root-pruning treatment on leaf nutrients, photosynthetic characteristics, WUE, and growth of poplar at each measurement date. When the ANOVA analysis revealed significant differences between treatments, the least significant difference (LSD) test was conducted to detect differences between individual treatment-level means. All statistical analyses were performed at a significant level of \( P < 0.05 \). ANOVA and multiple comparisons were performed using SPSS software (version 22.0; SPSS Inc., Chicago, IL, USA). All results in Figs 1–3 and Tables 1–4 are given as the means of three replicates.

**Results**

**N, P, K concentrations in the leaves**

The effects of different treatments on the N, P and K concentrations in the poplar leaves are shown in Fig 1. During late May 2015, 30 days after root pruning, the leaf N, P and K concentrations significantly decreased following root pruning compared with CK, the largest decline occurring in treatments 6–2 and 6–4 followed by 8–2 and 8–4, whereas the smallest decline occurred in the 10–2 and 10–4 treatments. The N and P concentrations in the 8–2 treatment were significantly increased over CK, and were also higher compared with the other treatments at the subsequent survey dates. Similarly, the K concentration in the 8–2 treatment also increased between the 2nd and 5th survey dates. However, the N and P concentrations in the 8–4 treatment were not significantly different from CK at the 2nd and 3rd survey dates, but were significantly increased compared with CK in the following survey. The 10–2 treatment increased the N and P concentrations compared with CK at the 2nd and 3rd survey dates, but did not differ significantly from CK from the 4th to 6th survey dates. However, the K concentration was significantly higher than that of CK from the 2nd to 4th survey dates. The N, P, and K concentrations in the 10–4 treatment were higher compared with CK from the 2nd to 5th survey dates, whereas the N, P and K concentrations in the 6–2 and 6–4 treatments were significantly decreased compared with CK, with the lowest concentrations occurring in the 6–4 treatment. The results indicated that different root-pruning treatments had varied effects on the concentrations of N, P and K in poplar leaves. Among all root-pruning treatments, the 8–2 treatment had more positive effects on the nutritional status of poplar tree leaves.

**Leaf photosynthesis**

As shown in Tables 1–3, the net photosynthetic rate \( (P_n) \), transpiration rate \( (T_r) \), and stomatal conductance \( (g_s) \) in poplar were significantly decreased compared with CK 1 month after root
pruning, with the largest decline occurring in the 6–4 treatment and the smallest in the 10–2 treatment. $P_n$ and $g_s$ showed consistent seasonal variation and were significantly higher in the 8–2 treatment than in the other treatments. Compared with CK, root pruning in the 8–4 and 10–4 treatments enhanced $P_n$ and $g_s$, whereas these were decreased in the 6–2 and 6–4 treatments, especially in the latter. As shown in Table 2, $T_r$ was also the highest in the 8–2 treatment, and $T_r$ in the 8–4 treatment was significantly increased compared with CK in the 3rd year after root pruning (i.e., in 2017). Even though $T_r$ in the 10–2 treatment was significantly higher than in CK at the 2nd and 3rd survey dates, no differences were found in the following survey dates. The aforementioned results showed that root pruning had significant effects on the photosynthetic characteristics of poplar leaves, which was closely related to the severity of root pruning.

**WUE of leaves**

As shown in Fig 2, the WUE of leaves in the 8–2, 8–4, 10–2 and 10–4 treatments significantly increased over CK 1 month after root pruning, whereas it was significantly decreased in the 6–2 and 6–4 treatments. The largest decline in WUE occurred in the 6–4 treatment.
subsequent survey dates, the WUE among all treatments was highest in the 8–2 treatment. Compared with CK, WUE was significantly higher in the 10–4 and 8–4 treatments. There was no difference in WUE between the 10–2 treatment and CK in the 3rd year after root pruning.

Table 1. Effects of root pruning on photosynthetic rate of poplar leaves (mean ± SD).

| Treatment | Net photosynthetic rate (μmol·m⁻²·s⁻¹) |
|-----------|--------------------------------------|
|           | 2015–05 | 2015–10 | 2016–05 | 2016–10 | 2017–05 | 2017–10 |
| CK        | 11.49±0.58a | 7.02±0.29c | 11.26±0.51d | 7.47±0.35cd | 11.78±0.37c | 7.82±0.29cd |
| 6–2       | 6.97±0.52d | 6.45±0.33c | 10.18±0.46e | 6.84±0.28de | 10.93±0.41d | 7.21±0.27de |
| 6–4       | 6.46±0.47d | 5.62±0.40d | 9.29±0.37f | 6.25±0.31e | 10.85±0.45d | 7.18±0.32e |
| 8–2       | 9.61±0.39b | 8.86±0.32a | 14.81±0.43a | 9.89±0.65a | 15.39±0.55a | 10.22±0.41a |
| 8–4       | 8.75±0.36c | 7.65±0.31b | 12.68±0.35c | 7.96±0.33bc | 13.87±0.49b | 9.35±0.41b |
| 10–2      | 10.22±0.42b | 8.19±0.33b | 13.52±0.42b | 8.02±0.42bc | 12.05±0.38c | 7.96±0.39c |
| 10–4      | 9.81±0.55b | 8.23±0.41b | 13.59±0.41b | 8.57±0.38b | 13.96±0.57b | 9.29±0.42b |

Note: Different letters indicate significant differences among treatments at $P<0.05$ by LSD.

https://doi.org/10.1371/journal.pone.0197515.t001
However, leaf WUE in the 6–2 and 6–4 treatments was lower compared with CK within 3 years after root pruning, with the lowest value being found in the 6–4 treatment. Although there was an obvious regulatory effect of root pruning on the WUE of poplar leaves, not all root pruning treatments improved WUE.

Growth rates of DBH, tree height, and volume

The effects of root pruning on growth rates of DBH, height, and volume of poplar trees in over consecutive years are detailed in Table 4. The average growth rates of DBH, tree height, and tree volume showed consistent variation within treatments, with the overall order of measurements as follows: 8–2 > 10–4 > 8–4 > 10–2 > CK > 6–2 > 6–4. For average growth rates of DBH, tree height, and volume, the highest values all occurred in the 8–2 treatment, increasing by 46.31%, 39.19%, and 32.56%, respectively, compared with CK. However, the average growth rates of DBH and volume in the 6–4 treatment were lower than those in CK. Therefore, different root pruning treatments had significantly different effects on poplar growth.

Discussion

*Populus × euramericana* cv. 'Neva' is one of the most suitable species for afforestation and woody production in arid and semiarid areas of China [5]. The successful establishment and rapid growth of the trees depend on the fast growth and nutrient-absorbing ability of the root system. Previous preliminary studies on winter jujube and poplar illustrated that different intensities of root pruning have disparate effects on tree nutrition [4, 12]; however, these studies only pruned

| Treatment | Stomatal conductance (mmol·m⁻²·s⁻¹) |
|-----------|-------------------------------------|
|           | 2015–05   | 2015–10   | 2016–05   | 2016–10   | 2017–05   | 2017–10   |
| CK        | 162±5a    | 85±7c     | 168±9c    | 105±6de   | 190±9c    | 118±5c    |
| 6–2       | 86±7e     | 70±9d     | 149±7d    | 89±7e     | 163±8d    | 96±10d    |
| 6–4       | 83±9e     | 68±6d     | 117±12e   | 71±9f     | 159±10d   | 93±13d    |
| 8–2       | 119±8c    | 122±7a    | 236±11a   | 159±5a    | 268±12a   | 166±9a    |
| 8–4       | 103±6d    | 102±6b    | 176±10c   | 123±15bc  | 239±9b    | 145±8b    |
| 10–2      | 144±10b   | 118±9a    | 212±9b    | 121±12cd  | 198±14c   | 121±12c   |
| 10–4      | 137±8b    | 103±5b    | 208±7b    | 140±10b   | 230±8b    | 148±7b    |

Note: Different letters indicate significant differences among treatments at P<0.05 by LSD.

https://doi.org/10.1371/journal.pone.0197515.t003
### Table 4. Effects of root pruning on growth rates of DBH, height and volume (mean ± SD).

| Treatment | DBH/cm | Tree height/m | Volume ($10^3$/m³) | DBH growth rate/ % | Tree height growth rate/ % | Volume growth rate/ % |
|-----------|--------|---------------|---------------------|--------------------|--------------------------|----------------------|
|           | 2015–05 | 2017–10 | 2015–05 | 2017–10 | 2015–05 | 2017–10 | 2015–05 | 2017–10 | 2015–05 | 2017–10 | 2015–05 | 2017–10 |
| CK        | 10.82±0.27a | 15.92±0.16d | 12.68±0.15a | 18.03±0.15b | 4.89±0.31a | 15.07±0.42d | 12.72±0.61d | 11.61±0.33d | 33.97±0.86d |
| 6–2       | 11.09±0.15a | 15.28±0.24e | 12.52±0.18a | 17.69±0.10c | 5.08±0.26a | 13.62±0.53e | 10.59±0.26e | 11.41±0.27d | 30.46±0.58e |
| 6–4       | 10.96±0.20a | 14.51±0.40f | 11.29±0.80a | 15.02±0.25e | 4.47±0.85a | 10.43±0.71f | 9.29±0.30f | 9.45±0.39e | 26.65±0.89f |
| 8–2       | 11.21±0.18a | 19.89±0.16a | 12.17±0.12a | 19.96±0.58a | 5.04±0.20a | 26.03±0.65a | 18.61±0.58a | 16.16±0.75a | 45.03±0.81a |
| 8–4       | 11.33±0.25a | 17.42±0.72c | 12.25±0.38a | 18.19±0.14b | 5.18±0.29a | 18.20±0.31c | 14.12±0.36c | 13.01±0.64c | 37.10±1.02c |
| 10–2      | 10.81±0.29a | 15.88±0.35d | 11.96±0.76a | 17.13±0.21d | 4.61±0.65a | 14.24±0.59d | 12.66±0.52d | 11.85±0.22d | 34.07±0.55d |
| 10–4      | 12.02±0.96a | 19.47±0.21b | 12.85±0.82a | 19.89±0.68a | 6.12±0.93a | 24.86±0.77b | 15.77±0.65b | 14.34±0.28b | 40.32±0.63b |

Note: Different letters indicate significant differences among treatments at P<0.05 by LSD.

https://doi.org/10.1371/journal.pone.0197515.t004

The roots on two sides of each tree. In the current study, comprehensive methods of root pruning were implemented by combining three intensities (six, eight, or ten times the DBH from the trunk) and two orientations (two or four sides). The results showed that the N, P and K concentrations of leaves in all treatments decreased in the short term (30 days) following root pruning. This could be because some of the absorptive roots of the trees were removed by pruning, thereby significantly weakening the absorptive capacity of the root system and resulting in a rapid decrease in nutrient uptake [9]. On subsequent survey dates, the N and P concentrations of leaves in the 8–2 treatment had increased and were significantly higher compared with other treatments, which was in part agreement with a previous study [4]. Compared with the 8–4 treatment, the same strength of root pruning on two sides of the tree in the 8–2 treatment not only stimulated the growth of new roots [5], but also increased the absorption area of the roots (Fig 3), which subsequently increased the nutrient uptake [9, 25].

In the 8–4 and 10–4 treatments, root pruning was conducted on four sides; thus, the damage to the roots was much bigger. However, the nutrient concentrations of leaves still significantly increased compared with those in CK during the later stages of the experiment. This might be because, even though the increase in poplar growth had a dilution effect on nutrient concentrations of the leaves, a large number of new roots gradually sprouted on the root incisions on all four sides of the trees over time and absorbed a large amount of nutrients from soil, resulting in the increase in leaf nutrient concentrations in these treatments (Figs 1 and 3). This also suggests that new roots growing from root incisions were able to strongly absorb soil nutrients. Root pruning in the 10–2 treatment was carried out relatively far from the trunk, which inflicted less damage to the root system and shortened the recovery time of the root; thus, the nutrient content of the leaves increased rapidly compared with CK [4, 26]. However, because the stimulation effect of root pruning on the incision was significantly reduced, the amount of new roots and the root absorption area were less compared with other treatments [25, 27]; thus, the incremental change in nutrient content in the leaves was not significant during the later stages of the study. However, in the 6–2 and 6–4 treatments, root pruning occurred closest to the trunk, which resulted in the most damage to the root system, especially in the 6–4 treatment. This was also possibly correlated with the fact that, because the roots were shorter following pruning, the volume of soil the trees had access to was limited. Thus, the root recovery time was prolonged and their nutrient uptake would have lagged behind in other treatments. This suggested that the intensity and orientation of root pruning have a decisive role in the leaf nutrition of poplar trees.

Plants are not able to absorb as much water and nutrients within a certain period of time after root pruning, mainly because the latter initially decreases the root surface area. With an increase
in root-pruning intensity, the net photosynthetic rate, transpiration rate, and stomatal conductance correspondingly decrease [11–12]. Similar conclusions were drawn from the results from the first survey date in the current study. It might be that roots and leaves are able to communicate via signaling molecules [28] that might result in partial stomatal closure and, thus, a decrease in stomatal conductance and transpiration rate, in leaves when the roots have been fully or partially removed [29]. Root pruning caused an initial decrease in the transpiration rate, which was the direct cause of the decrease in the net photosynthetic rate in the current study. However, with the emergence of new roots, an increase in photosynthetic parameters was observed for the 8–2, 8–4, 10–2, and 10–4 treatments from the 2nd to 5th survey dates compared with CK. These findings are consistent with those obtained in previous studies [12, 29].

The ratio of photosynthesis to transpiration is an indicator of the water use efficiency of leaves [15–16, 21]. The experimental results showed that gs in the 8–2, 8–4, 10–2 and 10–4 treatments was decreased at 30 days after root pruning, as were the net photosynthetic rate and transpiration rate. In these treatments, the leaf transpiration rate decreased faster than the net photosynthetic rate, so that WUE increased, and was significantly higher than that of CK. This might be because the decline in stomatal conductance had less influence on photosynthesis than it had on transpiration [12]. Transpiration rate has a strong dependence on the stoma, thus partial stomatal closure would have been conducive to improving the WUE of the leaves. On the subsequent survey dates, the net photosynthetic rate, transpiration rate, and stomatal conductance in the 8–2, 8–4 and 10–4 treatments were all higher than those of CK. In these treatments, the WUE was also higher compared with CK, probably because of a faster increase in net photosynthetic rate than transpiration rate, along with the increase in stomatal conductance as a result of root pruning. High WUE is beneficial for enhancing the total production of plants under variable soil water content as an adaptation to water shortage [2]. However, the current results indicated that WUE in the 6–2 and 6–4 treatments was lower than CK during the 3 years after root pruning, especially in the 6–4 treatment, suggesting that excessive root pruning would reduce the WUE because of irrecoverable damage to the root system.

In addition, the average growth rate of the tree volume in the 8–2 treatment was the highest among all treatments, which could be related to the improved leaf nutrient content and higher photosynthetic productivity and WUE of the leaves. Also, the 10–4 and 8–4 treatments increased the growth rate of tree volume compared with CK, which indicated that root pruning on all four sides resulted in significant harm to the trees initially, but possibly increased the nutrient absorption capacity of the root system at later survey dates. No significant differences in volume growth rate were found between the 10–2 treatment and CK. However, both the 6–2 and 6–4 treatments decreased the growth rate of tree volume compared with CK. These results further confirmed that the strength and orientation of root pruning were of great importance for regulating the growth of poplar trees. Furthermore, the experimental area is windy in spring, but the wind is usually no stronger than force 6 (strong breeze); thus, the pruned trees never encountered the problem of windthrow.

**Conclusions**

In conclusion, it is evident from this study that the application of root pruning had significant regulation effects on the nutrient content, photosynthetic characteristics, and WUE of poplar leaves, as well as on tree growth at the stage of canopy closing during the later stages of a short rotation. The selection of root-pruning intensity and orientation plays a decisive role in the effectiveness of this technique for the management of poplar plantations.
Acknowledgments

We are grateful to the anonymous reviewers for helpful comments and English corrections on the manuscript.

Author Contributions

Data curation: Da-wei Jing.

Formal analysis: Da-wei Jing, Zhen-yu Du, Ming-you Wang, Fang-chun Liu.

Funding acquisition: Da-wei Jing.

Investigation: Da-wei Jing, Zhen-yu Du, Ming-you Wang, Qing-hua Wang, Hai-lin Ma, Fang-chun Liu, Bing-yao Ma, Yu-feng Dong.

Methodology: Da-wei Jing, Ming-you Wang.

Project administration: Da-wei Jing.

Resources: Da-wei Jing.

Supervision: Ming-you Wang.

Writing – original draft: Da-wei Jing.

Writing – review & editing: Da-wei Jing, Zhen-yu Du.

References

1. Fang S, Xu X, Lu S, Tang L. Growth dynamics and biomass production in short-rotation poplar plantations: 6-year results from three clones at four spacings. Biomass Bioenergy. 1999; 17: 415–425.

2. Han X, Tang S, An Y, Zheng DC, Xia XL, Yin WL. Overexpression of the poplar NF-YB7 transcription factor confers drought tolerance and improves water-use efficiency in Arabidopsis. Journal of Experimental Botany. 2013; 64: 4589–4601. https://doi.org/10.1093/jxb/ert262 PMID: 24006421

3. Desrochers A, Maurin V, Tarroux E. Production and role of epicormic shoots in pruned hybrid poplar: effects of clone, pruning season and intensity. Annals of Forest Science. 2015; 72: 425–434.

4. Du ZY, Xing SJ, Ma BY, Liu FC, Ma HL, Wang QH. Effects of root pruning on the growth and rhizosphere soil characteristics of short-rotation closed-canopy poplar. Forest Systems. 2012; 21: 236–246.

5. Jing DW, Liu FC, Wang MY, Ma HL, Du ZY, Ma BY, et al. Effects of root pruning on the physicochemical properties and microbial activities of poplar rhizosphere soil. PLoS One. 2017; 12: e0187685. https://doi.org/10.1371/journal.pone.0187685 PMID: 29117215

6. Briggs NA, Kuehne C, Kohnle U, Bauhus J. Root system response of naturally regenerated Douglas-fir (Pseudotsuga menziesii) after complete overstory removal. Canadian Journal of Forest Research. 2012; 42: 1858–1864.

7. Dobrowolska D, Hein S, Oosterbaan A, Wagner S, Clark J, Skovsgaard JP. A review of European ash (Fraxinus excelsior L.): implications for silviculture. Forestry. 2011; 84: 133–148.

8. Asín LS, Montserrat R. Effect of paclobutrazol, prohexadione-Ca, deficit irrigation, summer pruning and root pruning on shoot growth, yield, and return bloom, in a Blanquilla, pear orchard. Scientia Horticulturae. 2007; 113: 142–148.

9. Yang S, Xing S, Liu C, Du Z, Wang H, Xu Y. Effects of root pruning on the vegetative growth and fruit quality of Zhanhuadongzao trees. Hort Sci. 2010; 37:14–21.

10. Yang SJ, Du ZY, Yu Y, Zhang ZL, Sun XY, Xing SJ. Effects of root pruning on physico-chemical characteristics and biological properties of winter jujube rhizosphere soil. Plant Soil Environment. 2011; 57: 493–498.

11. Desrochers A, Tremblay F. The effect of root and shoot pruning on early growth of hybrid poplars. Forest Ecology and Management. 2009; 258: 2062–2067.

12. Yang SJ, Du ZY, Yu Y, Che YY, Yuan CH, Xing SJ. Effect of root pruning on competitive ability in Chinese jujube tree. Fruits. 2012; 67:429–437.
13. Karaba A, Dixit S, Greco R, Aharoni A, Trijatmiko KR, Marsch-Martinez N, et al. Improvement of water use efficiency in rice by expression of HARDY, an Arabidopsis drought and salt tolerance gene. Proceedings of the National Academy of Sciences, USA. 2007; 104: 15270–15275.

14. Cao X, Jia JB, Li H, Li MC, Luo J, Liang ZS, et al. Photosynthesis, water use efficiency and stable carbon isotope composition are associated with anatomical properties of leaf and xylem in six poplar species. Plant Biol. 2012; 14: 612–620. https://doi.org/10.1111/j.1438-8677.2011.00531.x PMID: 22188382

15. Lüttchwager D, Ewald D, Alía LA. Comparative examinations of gas exchange and biometric parameters of eight fast-growing poplar clones. Acta Physiologica Plantarum. 2015; 37: 214.

16. Larchevêque M, Maurel M, Desrocchers A, Larocque GR. How does drought tolerance compare between two improved hybrids of balsam poplar and an unimproved native species? Tree Physiology. 2011; 31: 240–249. https://doi.org/10.1093/treephys/tpr011 PMID: 21444373

17. Fan XW, Li FM, Xiong YC, An LZ, Long RJ. The cooperative relation between non-hydraulic root signals and osmotic adjustment under water stress improves grain formation for spring wheat varieties. Physiologia Plantarum. 2008; 132: 283–292. https://doi.org/10.1111/j.1399-3054.2007.01007.x PMID: 18275460

18. Ma SC, Li FM, Yang SJ, Li CX, Xu BC, Zhang XC. Effects of root pruning on non-hydraulic root-sourced signal, drought tolerance and water use efficiency of winter wheat. Journal of Integrative Agriculture. 2013; 12: 989–998.

19. Lu RK. Analytical methods for soil and agro-chemistry. China Agricultural Science and Technology Publishing House, Beijing, China. 1999; 18–99.

20. Wong SC, Cowan IR, Farquhar GD. Leaf conductance in relation to assimilation in Eucalyptus pauciflora Sieb. ex Spreng: Influence of irradiance and partial pressure of carbon dioxide. Plant Physiology. 1978; 62: 670–674. PMID: 16660580

21. Ren BB, Wang M, Chen YP, Sun GM, Li Y, Shen QR, et al. Water absorption is affected by the nitrogen supply to rice plants. Plant Soil. 2015; 396:397–410.

22. Bragg DC. An improved tree height measurement technique tested on mature southern pines. Southern Journal of Applied Forestry. 2008; 32: 38–43.

23. Meng XY. Forest measurement. China Forestry Press, Beijing. 1996; 158–204.

24. Du ZY, Wang QH, Xing SJ, Liu FC, Ma BY, Ma HL, et al. Fine root distribution, characteristics and rhizosphere soil properties in a mixed stand of Robinia pseudoacacia and Fraxinus velutina in a saline soil. Silva Fennica. 2013; 47: article id 970.

25. Zhao XY, Zheng HQ, Li SW, Yang CP, Jiang J, Liu GF. The rooting of poplar cuttings: a review. New Forests. 2014; 45:21–34.

26. Krabel D, Meyer M, Solger A, Müller R, Carvalho P, Foulkes J. Early root and aboveground biomass development of hybrid poplars (Populus spp.) under drought conditions. Canadian Journal of Forest Research. 2015; 45: 1289–1298.

27. Tilki F, Apletken CU. Germination and seedling growth of Quercus vulcanica: effects of stratification, desiccation, radicle pruning, and season of sowing. New Forests. 2006; 32:243–251.

28. Yang HQ, Jie YL, Li J. The stress messenger from roots and its production and transport in plant. Chinese Bulletin of Botany. 2002; 18: 56–62.

29. Zhao QZ, Qiao JF, Liu H, Tian ZQ. Relationship between root and leaf photosynthetic characteristic in rice. Scientia Agricultura Sinica. 2007; 40:1064–1068.