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# Effects of Soil Water and Nitrogen on the Stand Volume of Four Hybrid *Populus tomentosa* Clones

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**Abstract:** With the aim of improving poplar timber production, a successive 8-year irrigation and fertilization factorial experiment with three blocks was designed to measure the response of *Populus tomentosa* stands to water and nitrogen in Huabei Plain, China. Specifically, we examined the responses of four *P. tomentosa* clones (*P. tomentosa* BT17, S86, B331, and 1316) to three irrigation levels (45%, 60%, and 75% above field capacity), as irrigation thresholds, and four N levels (0, 80, 160, and 240 g per plant). The results showed that both irrigation and nitrogen had significant effects in terms of improving clone stand volume. Further, we demonstrated positive interactions between irrigation and nitrogen. The stand volume increment of the four hybrid clones varied from  $104.53 \pm 19.84$  to  $191.35 \pm 30.56$  m³/ha in the descending order S86 > B331 > BT17 > 1316. With increasing irrigation level, the average stand volume of the four clones increased significantly from  $120.46 \pm 5.23$  to  $158.53 \pm 21.72$  m³/ha. When nitrogen level was increased from 0 to 240 g/plant, the average stand volume increment of the four clones increased from  $126.04 \pm 8.75$  to  $156.16 \pm 26.01$  m³/ha, respectively. Our results suggest that a comprehensive and specific management program is needed to improve poplar timber production.

**Keywords:** irrigation; nitrogen; *Populus tomentosa*; stand volume increment; clones; water and nitrogen interaction

## 1. Introduction

There is currently an increasing demand for timber, which is the only renewable source of the four traditional construction materials. China has a large demand for timber, with approximately 49.94 million cubic meters of timber being imported every year, making it the world's largest importer, and the second largest consumer, with a usage of 500 million cubic meters per year. The need for timber in the country will rise to 700 million cubic meters. It is estimated that, by 2050 [1–3], 75% of the industrial timber supply will be provided by planted forests, and of this supply, approximately 50% will be provided by fast-growing plantations. Among the fast-growing tree species, poplars have the largest planting area and timber yield in temperate regions of the world. In China alone [4], the area planted with poplar exceeds 8.5 million ha. Intensively cultivated poplar plantations generally require a comprehensive management program to yield more high-quality timber and biomass. In addition to site conditions and climate, the key factors that need to be considered in a comprehensive management program include species, irrigation, fertilizer, seedling quality, and planting density. It has been estimated that the worldwide average annual volume increment for poplar plantation is between 9 and 30 m<sup>3</sup>/ha/year among different clones, with that in China being less than 15 m<sup>3</sup>/ha/year [5–8].

Researchers in China are making efforts to improve timber production by establishing 20 national forest reserves in seven key areas.

Native to most of the Northern Hemisphere, the genus *Populus* is widely distributed in Asia, Europe, and North America [9]. Traditionally, the genus has been divided into six sections, and consists of a large number of subspecies and transient forms, owing to intra- and inter-species hybridization [10,11]. Hybrid clones of poplar trees are widely used as an important source to enhance timber and biomass production [5,12,13]. Particular attention has been paid to the performance of modern poplar hybrid clones growing in sub-optimal conditions in Central and Southern Italy [14–18]. In Denmark, research on the yield production of 36 poplar clones showed that the clones had differing productivity, with *Populus maximowiczii* × *trichocarpa* showing the highest yield and *Populus deltoides* the lowest yield [19]. China has been making progress in the breeding and cultivation of fast-growing poplars trees, for example, the fast-growing clones of *P. tomentosa* [4,20–23].

Poplars are distinguished by fast growth and easy vegetative propagation. The rapid growth depends on the availability of soil water and nutrients. In this regard, different clones may have differing water-use efficiencies in response to water stress [9,24,25]. Irrigation improves poplar plantation production [26–29], and is needed to ensure that intensive poplar plantations achieve fast growth in the sandy regions of the Huabei Plain, China, where the irrigation threshold varies from 50% to 70% of the field capacity [23–31].

To achieve a high rate of biomass production, plants not only need to gain access to sufficient soil water and nitrogen but also to utilize these efficiently [32,33]. The characteristic light requirement and growth traits of different tree species determine their respective rates of nutrient uptake [34]. Poplar plantations are typically established on fertile sites, such as riparian areas [35]. Over a 6-year period, a hybrid poplar (*Populus* spp.) clone growing in the riparian zone of four southern Quebec (Canada) agro ecosystems accumulated N and P as high as 770 kg/ha and 82 kg/ha, respectively [36]. The 6-year cumulative fertilization rate, ranging from 0 to 1600 N kg/ha and 0 to 700 P kg/ha, improved leaf area index and growth [37]. Extensive site-specific data show that nitrogen can significantly improve the biomass and timber yield of poplar plantations [5,38–40]. A combination application of urea (460 g), calcium phosphate (75 g), and potassium sulfate (75 g) per tree was shown to improve the stand volume of a P. tomentosa plantation by 142% compared with the control [41]. P. tomentosa takes up both NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>, although NO<sub>3</sub><sup>-</sup> is taken up with higher efficiency [42]. Fertilization form and quantity also have an effect on soil nutrients, which further affects the abundance, diversity, and composition of soil fauna across an age-sequence of poplar plantations (i.e., 4-, 9-, and 20-year-old) in the coastal region of eastern China [43]. Although there are few studies that have focused on the effects of water and nitrogen interactions on poplar growth, accumulating data on other crops indicate that the interaction of water and nitrogen has a significant influence on growth [44–46].

With a view toward large-scale poplar timber production, an 8-year irrigation and fertilization manipulation experiment, currently in progress in the central region of Huabei Plain, China, has provided an opportunity to measure the response of poplar plantation stand volume to water and nitrogen. Our objectives in the present study were as follows: (1) to determine whether different clones have different responses to irrigation and nitrogen; (2) to examine the effect of irrigation and nitrogen on poplar stand volume; and (3) to analyze the interactions among clones, irrigation level, and nitrogen application rate. We anticipate that our findings will provide information that will help to guide the initial steps in improving timber production through clone selection, irrigation, and fertilization.

### 2. Materials and Methods

Huayang Forest Tree Nursery is located in the central region of Huabei Great Plain  $(36^{\circ}50'-37^{\circ}47' \text{ N}, 113^{\circ}52'-115^{\circ}49' \text{ E})$ . The elevation of the area ranges from 30 to 50 m and the monthly mean lowest and highest temperatures are -2.5 °C in January and 27.0 °C in July. The average annual precipitation is approximately 497.7 mm, with 70% of the rain falling between July and September. Annual sunshine duration is estimated to be 2575 h, and the number of frost-free days is 198 days.

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As one of the key plantations for hybrid poplar tree breeding and large-scale timber production designated by the National Forestry Bureau of China, 70 ha of poplar stands were established in Spring 2007 by planting dormant 2-year-old bare-rooted seedlings developed from stem cuttings (of four *P. tomentosa* hybrid clones—*P. tomentosa* 1316, *P. tomentosa* BT17, *P. tomentosa* B331, and *P. tomentosa* S86 ( $\bigcirc$  *P. tomentosa*  $\times$  *P. bolleana*:  $\bigcirc$  *P. alba*  $\times$  *P. glandulosa*)—with spacing of 4 m  $\times$  3 m. The average stem height of the cuttings was 5.8 m and the average base diameter was 6.1 cm.

In the spring of 2008, a chronic irrigation and nitrogen application experiment was started on stands of the four *P. tomentosa* hybrids, with a view toward large-scale poplar timber production. Three irrigation levels were applied to maintain the soil water content at 45%, 60%, and 75% of field capacity (FC) (representing W45, W60, and W75, respectively) by furrow irrigation. There were four different levels of annual nitrogen application (N0 = 0 g, N80 = 80 g, N160 = 160 g, and N240 = 240 g per plant) or 0 kg/ha, 66.64 kg/ha, 133.28 kg/ha, and 199.92 kg/ha, respectively.

A factorial experimental design was used with three irrigation levels, four nitrogen levels, and three blocks (replicates) to control for environmental heterogeneity (36 plots). Each plot was further split into four subplots to arrange for four clones. Each clone was arranged in two rows with each row 4 trees, so there were eight trees per clone (spacing:  $4 \text{ m} \times 3 \text{ m}$ ). Therefore, the present study was conducted with a total of 144 subplots and 1152 trees. Two guard rows of *Platanus orientalis* (spacing  $4 \text{ m} \times 3 \text{ m}$ ) were planted around the entire perimeter of each block to reduce edge effects. The experiment site was located in (part of) the stand.

The site soil is an entisol developed from a thick uniform sandy loam alluvial soil. Soil pH is 8.6 and bulk density is 1.43 g/cm $^3$ . The basic physical and chemical properties of the soil at the experimental site are shown in Table 1.

| Organic Mat | tter Total N<br>g/kg | Olsen P<br>mg/kg | Available K<br>mg/kg | Field Capacity | Total Porosity |
|-------------|----------------------|------------------|----------------------|----------------|----------------|
| 8.6         | 0.58                 | 8.09             | 90                   | 26             | 46.7           |

Table 1. Soil physical and chemical characteristics at the experimental site.

Phosphorus and potassium fertilizers have been applied annually as 66.64 kg/ha (80 g/plant) of  $P_2O_5$  and 33.32 kg/ha (40 g/plant) of  $K_2O$  in each experimental plot. Urea, phosphate ammonium, and potassium sulfate serve as the nitrogen, phosphorus, and potassium sources; they are applied at the end of April in amounts of 1/3, 1/2, and 1/3, respectively. The remaining fertilizer is applied during the middle of June each year. Fertilizers were applied to four corner pits sited 50 cm from the base trunk of each tree. Each pit was 20–30 cm deep from the top soil. After fertilizer application, the pits were covered with soil and irrigated according to the experimental design. In order to determine whether irrigation was required, we used an ML2x soil moisture measuring instrument to monitor soil moisture content.

At the end of the 8th growing season (late November 2014), the diameter at breast height (DBH) and height of all the 1152 trees in the 36 plots were measured. The stem volume of each tree was calculated using the following equation from a standard issued by the People's Republic of China Ministry of Agriculture and Forestry Department (LY208-77):

$$V_{\text{stem}} = 0.000065678245 \cdot D^{1.9410626} \cdot H^{0.84929086}, \tag{1}$$

where  $V_{\text{stem}}$  is the individual tree volume in  $m^3$ ; H is the tree height in m; and D is the diameter at breast height in cm.

The average stem volume (average  $V_{\text{stem}}$ ) of each subplot was calculated by accumulation  $V_{\text{stem}}$  divided by the number of trees in each subplot. The stand volume of each subplot was calculated using the following equation:

$$V_{stand} = average V_{stem} \times N_{tree},$$
 (2)

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where  $V_{stand}$  is the stand volume in m<sup>3</sup>/ha; and  $N_{tree}$  is the number of trees per hectare ( $N_{tree}$  = 833). Stand volume increments of the four *Populus tomentosa* clones were calculated by using the Stand volume of October 2014 minus the Stand volume of April 2007.

We used SPSS 17.0 statistical analysis functions to calculate the mean and standard deviation of tree height and DBH in each plot. Initially, the data were tested for normality using the Shapiro–Wilk test. A three-way analysis of variance was performed on stand volume, irrigation and nitrogen as the two whole-plot factors and clone as the subplot factor. Where significant effects were detected, pairwise comparisons for post-hoc determination of significant differences between means among levels of irrigation, nitrogen treatments were performed, using Tukey's honestly significant difference (HSD) test.

#### 3. Results

The stand volume of the four hybrid clones varied from  $70.97 \pm 6.31$  to  $243.97 \pm 22.34$  m<sup>3</sup>/ha, in the descending order S86 > B331 > BT17 > 1316 (Table 2). Three-way ANOVA showed that clone, irrigation, and nitrogen had significant effects on stand volume increment (Table 3). Significant interactions between water and nitrogen were observed (Table 3, F = 27.025, P < 0.05 Table 4).

**Table 2.** Stand volume increment of four *Populus tomentosa* clones in October 2014 in relation to irrigation and nitrogen application since 2007. W45, W60, and W75 indicate plots in which the soil water contents were maintained at 45%, 60%, and 75% above field capacity, respectively. N0, N80, N160, and N240 indicate plots in which 0, 80, 160, and 240 g nitrogen per plant, respectively, was applied. Means and standard errors are shown (n = 3).

| Irrigation | Nitrogen | BT17               | B331               | S86                | 1316               |  |
|------------|----------|--------------------|--------------------|--------------------|--------------------|--|
|            | N0       | $122.14 \pm 7.67$  | $137.98 \pm 5.05$  | $147.66 \pm 9.08$  | $70.97 \pm 6.31$   |  |
| TA745      | N80      | $116.15 \pm 6.08$  | $122.75 \pm 11.09$ | $158.38 \pm 8.12$  | $80.91 \pm 5.68$   |  |
| W45        | N160     | $116.33 \pm 8.67$  | $135.91 \pm 1.74$  | $163.82 \pm 5.96$  | $84.58 \pm 6.2$    |  |
|            | N240     | $131.38 \pm 8.24$  | $130.23\pm2.1$     | $172.12 \pm 10.82$ | $88.41 \pm 9.67$   |  |
|            | N0       | $114.25 \pm 11.91$ | $145.11 \pm 17.53$ | $177.2 \pm 19.44$  | $111.08 \pm 11.48$ |  |
| TATEO      | N80      | $134.86 \pm 7.29$  | $149.21 \pm 10.72$ | $201.36 \pm 19.46$ | $107.74 \pm 8.97$  |  |
| W60        | N160     | $140.44 \pm 4.15$  | $164.53 \pm 13.84$ | $173.47 \pm 7.53$  | $126.6 \pm 3.29$   |  |
|            | N240     | $154.7\pm10.83$    | $176.39 \pm 9.55$  | $202.85 \pm 23.72$ | $118.24\pm2.24$    |  |
|            | N0       | $113.92 \pm 8.65$  | $142.29 \pm 13.06$ | $217.27 \pm 20.39$ | $94.23 \pm 9.57$   |  |
| W75        | N80      | $148.6\pm16.16$    | $173.84 \pm 25.62$ | $204.5 \pm 6.03$   | $127.8 \pm 16.02$  |  |
|            | N160     | $158.11 \pm 13.22$ | $174.16 \pm 7.28$  | $233.59 \pm 19.97$ | $119.74 \pm 34.56$ |  |
|            | N240     | $172.2 \pm 11.43$  | $183.85 \pm 24.28$ | $243.97 \pm 22.34$ | $124.08 \pm 19.33$ |  |

**Table 3.** Analysis of variance (ANOVA) summaries for stand volume increment in October 2014 in relation to irrigation, nitrogen application clone, block since 2007.

| Source of Variation                            | df | F Value | Sig.      |
|------------------------------------------------|----|---------|-----------|
| Model                                          | 49 | 16.404  | <0.010 ** |
| Block                                          | 2  | 1.096   | 0.339     |
| Water                                          | 2  | 84.268  | <0.010 ** |
| Nitrogen                                       | 3  | 27.025  | <0.010 ** |
| Water × Nitrogen                               | 6  | 3.913   | <0.010 ** |
| Error1(Block $\times$ Water $\times$ Nitrogen) | 22 |         |           |
| Main                                           | 35 |         |           |
| Clone                                          | 3  | 163.627 | <0.010 ** |
| Clone $\times$ Water                           | 6  | 1.317   | 0.257     |
| Clone $\times$ Nitrogen                        | 9  | 1.058   | 0.401     |

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|-----|---|---|-----|----|-----|
|     |   |   |     |    |     |

| Source of Variation                    | df  | F Value | Sig.  |
|----------------------------------------|-----|---------|-------|
| Clone $\times$ Nitrogen $\times$ Water | 18  | 1.123   | 0.343 |
| Error2(subplot)                        | 72  |         |       |
| Split                                  | 108 |         |       |
| Total                                  | 143 |         |       |

Stand volume increment of the four *Populus tomentosa* clones from April 2007 to October 2014. Stand volume increment is the average of each subplot (4 nitrogen  $\times$  3 water  $\times$  3 block  $\times$  4 clone) of each clone. \* significance level P < 0.05, \*\* significance level P < 0.01.

**Table 4.** Tukey's honestly significant difference (HSD) test results of the means of stand volume increments under different water and nitrogen treatments for each clone.

| Treatment | BT17 | B331 | S86  | 1316 |
|-----------|------|------|------|------|
| W45N0     | Cd   | cd   | e    | e    |
| W45N80    | D    | d    | e    | de   |
| W45N160   | Bcd  | bcd  | e    | de   |
| W45N240   | Cd   | cd   | de   | cde  |
| W60N0     | Cd   | cd   | cde  | abcd |
| W60N80    | Abcd | abcd | abcd | abcd |
| W60N160   | Abc  | ab   | abc  | abcd |
| W60N240   | Abc  | abcd | bcde | bcde |
| W75N0     | Cd   | cd   | bcde | abcd |
| W75N80    | Abc  | a    | abc  | ab   |
| W75N160   | Ab   | a    | a    | a    |
| W75N240   | A    | abcd | ab   | abcd |

Different letters indicate a significant difference between water  $\times$  nitrogen treatments for each clone, P < 0.05.

Clone S86 had highest timber production, yielding 86.82 m<sup>3</sup> more timber than clone 1316, followed by clone BT17 and clone B331, which yielded 38.33 m<sup>3</sup> and 56.09 m<sup>3</sup> more timber, respectively, than 1316 (Table 2).

Under different irrigation levels: 45%, 60%, and 75% of the FC, the average stand volume increments of the four clones were 120.46, 149.37, and 158.53 m³/ha, respectively (Table 2). The stand volume increased by 23.95% and 31.60% when the irrigation level was increased from 45% to 60% and 75% above the FC, respectively. Tukey's HSD test between means of stand volume increment under different irrigation levels were performed (Table 5). Under W75, the average stand volume increments of BT17 clone were significantly higher than that of the W45 level; W60 showed no significant difference to both W75 and W45. The average stand volume increments of B331, S86, and 1316 have no significant difference on W75 and W60, but had significant difference on W45.

**Table 5.** Tukey's HSD significant difference test results of the means of stand volume increments under different irrigation levels for each clone.

| Irrigation | BT17 | B331 | S86 | 1316 |
|------------|------|------|-----|------|
| W45        | b    | b    | b   | b    |
| W60        | ab   | a    | a   | a    |
| W75        | a    | a    | a   | a    |

Data collected from 2007 April to 2014 October, Different letters indicate a significant difference between water  $\times$  nitrogen treatments for each clone, P < 0.05.

Nitrogen application significantly increased the stand volume of the four *P. tomentosa* clones (Tables 2, 3 and 6). Compared with the control (0 g N/plant), the average stand volume increment of the four clones increased significantly from 126.04 to 140.56, 148.47, and 156.16 m<sup>3</sup>/ha with the

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application of 80, 160, and 240 g N/plant, respectively (Table 2). These values represent an increase in stand volume of 11.52%, 17.80%, and 23.90%, respectively. Tukey's HSD test between means of stand volume increment among levels of irrigation showed that different clones had different responses to the nitrogen rate (Table 6). Under N240, the average stand volume increments of S86 were significantly higher than that of the other three nitrogen levels, while 1316 showed no significant response to nitrogen rate among N0 to N240.

**Table 6.** Significant difference test results between the average stand volume increments under different nitrogen levels for each clone.

| Nitrogen Application | BT17 | B331 | S86 | 1316 |
|----------------------|------|------|-----|------|
| N0                   | b    | b    | b   | a    |
| N80                  | a    | ab   | b   | a    |
| N160                 | a    | a    | b   | a    |
| N240                 | a    | ab   | a   | a    |

Data collected from 2007 April to 2014 October. Different letters indicate a significant difference between water  $\times$  nitrogen treatments for each clone, P < 0.05.

Different combinations of water and nitrogen showed different volume increments for each clone; we can obviously see (Table 4) that the three clones (except B331) of the W75N240 combination were significantly higher than those of the W45N0 combination; only W75N240 for clone B331 showed no significance to other combinations of water and nitrogen application. W75N240 was significantly higher than that of W75N0, W60N0, W45N240, W45N160, W45N80, W45N0 for BT17. W75N80 was significantly higher than that of W75N0, W60N0, W45N240, W45N160, W45N80, W45N0 for clone B331. W75N240 was significantly higher than that of W45N240, W45N160, W45N80, W45N0 for clone S86. W75N160 was significantly higher than that of W45N240, W45N160, W45N80, W45N0 for clone 1316 The data show that (Table 2) W75N240 for clone S86 had the maximum increment of volume, which was 3.43 times that of W45N0 which showed the minimum increment of volume for clone 1316.

#### 4. Discussion and Conclusions

Clone selection is very important for optimizing wood or biomass production under different site conditions [2,3,8,47]. The wide variety of poplar clones has generally adapted to become competitive in different environments. Our results showed that the stand volume increments of the four examined clones have varied in response to 8 years of continued irrigation and nitrogen application (Tables 2 and 4–6). Clone S86 has shown the highest response to irrigation and nitrogen, whereas clone 1316 has shown the lowest response. This may be related to the adaptive difference of water and fertilizer factors for clones. The results also showed that clones S86 were the fastest growing among the four clones in the region under long-term water and nitrogen conditions, while the 1316 clones were the slowest among the four clones. According to Table 3, the results showed that clones have significant effects on the increment of volume, and it is considered that there are differences among clones, which is consistent with the results of many studies [2,3,8,47,48].

Poplars are among the fastest growing trees in temperate latitudes, and their cultivation can lead to high biomass production under adequate conditions. This high productivity is associated with high water requirements, and consequently productivity may be strongly limited by water availability. Since soil water can exert an important control on poplar growth [49,50], irrigation has accordingly been widely used to increase the productivity of poplar plantations [26,28,29]. Fast-growing hybrid poplar plantations are known to have high water requirements, as their potential growth is highly dependent on the amount of available soil water [51]. Our results showed that irrigation to maintain soil moisture of 75% of FC or higher resulted in the highest stand volumes increment of all four examined clones. This result also highlights the inherent principle of diminishing marginal productivity. It further indicates that the four examined P. tomentosa hybrid clones have a preference for high soil moisture.

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These new findings corroborate and extend our previous findings in the region [52,53]. Irrigation at 65% FC may be a suitable irrigation threshold for young Poplar stands [54], and in this regard, determination of irrigation thresholds should take into consideration the factors of clone, soil moisture, stand age, and planting density. In the results of this study, we believe that the level of field irrigation maintained above 60% was better for the growth of poplar in these areas, while above 75% may be the best, which is consistent with Ren Zhong xiu's [53] research conclusions. Either nitrogen application or nitrogen availability has been reported to be effective in increasing Poplar biomass and yield, in both China and other parts of the world, although the nitrogen use efficiency may vary [41,48,55–59]. On the basis of the averages for the four examined clones, our results showed that nitrogen application increased stand volume by 1.01–2.74 m<sup>3</sup>/kg N, with application of 300 kg/ha yielding the highest volume. The recommended nitrogen application rate is approximately 200-400 kg/ha in both the Huabei and Jianghan plains, which have different nitrogen availability and poplar stand characteristics. Our results thus appear to be representative of those annual nitrogen application rates obtained in the region. We further identified the clone-specific relationships between nitrogen and stand volume. Our work may thus provide new data for determining site and clone-specific nitrogen application. Fan Wei [60] reported that application of 200 kgN/ha to two-year-old poplar seedlings of four clones increased DBH, tree height, and volume by 8.5%, 10.2% and 21.2 respectively, in the Yellow River alluvial plain. Li Su Yan [61] reported 350 kg nitrogen application per hectare was preferable for 3–4-year-old poplar with no irrigation. Xi Ben Ye recommended 192 kg per hectare nitrogen by using drip fertigation for 3-5-year-old young poplar stand [62]. When recommending the annual nitrogen rate for poplar plantation, factors that should be considered include stand condition, target tree, and stand age, so as to achieve site-specific management.

Our results also revealed positive interactions between irrigation and nitrogen application in improving the volume increment in a poplar stand. Wang Li et al. [26] have reported the same result based on their study of 1-year-old poplar seedlings. High nitrogen application and high soil moisture maintained by irrigation increased stand volume, with clone S86 showing the highest average annual increment in stand volume of 23.42 m³/ha, followed by clones B331, BT17, and 1316 with average annual increments of 18.63, 16.41, and 12.57 m³/ha, respectively. Researchers from Canada have also identified that five different 8-year-old hybrid poplar clones have different yields in eight regions (Quebec) with different climatic and soil fertility gradients [3]. In China, the average yield of poplar stands is between 9.0 and 30.0 m³/ha [5–8], depending on site, species, and stand characteristics. Our results provide further evidence of the need for a comprehensive and specific management program to improve poplar timber production.

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**Author Contributions:** Li shui Nie and Lian Jun Song conceived and designed the experiments; Jia Lei Zhu, Lian Jun Song, and Ju Tian performed the experiments; Jia Lei Zhu, Hui Juan Bo, Xuan Li, Ju Tian, and Jiang Wang collected samples and analyzed the data; Jia Lei Zhu wrote the paper.

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