

## Article

# Effects of Water and Nitrogen Regulation on Cotton Growth and Hydraulic Lift under Dry Topsoil Conditions

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**Abstract:** Dry topsoil and relatively moist subsoil can occur in specific areas and times, limiting plant growth but creating conditions for hydraulic lift (HL). There is a lack of a rational water and nitrogen (N) strategy to improve cotton growth and maintain HL. This study investigated the effects of three topsoil water conditions ( $W_{0.6}$ : 60–70%,  $W_{0.5}$ : 50–60%, and  $W_{0.4}$ : 40–50% of field capacity) and three N rates ( $N_{120}$ -120,  $N_{240}$ -240, and  $N_{360}$ -360 kg N ha<sup>-1</sup>) plus one control treatment on cotton growth and HL under dry topsoil conditions in 2020 and 2021. The results showed that plant height and leaf area increased with increasing N rate, but the differences among topsoil water conditions were relatively small, except for leaf area in 2021. The HL water amount of all treatments increased gradually and then continued to decline during the observation period. There was a trend that the drier the topsoil or the more N applied, the greater the amount of HL water. Additionally, topsoil water conditions and N rate significantly affected the total HL water amount and root morphological characteristics (root length, surface area, and volume). Seed and lint cotton yield tended to decrease with increasing topsoil dryness at  $N_{240}$  or  $N_{360}$ , except for lint yield in 2021, or with decreasing N rate, especially under  $W_{0.6}$ . As topsoil became drier, the total evapotranspiration (ET) decreased, while with the increase in N rate, ET showed small differences. Water use efficiency increased with a higher N rate, while N partial factor productivity (PPFN) did the opposite. Furthermore, the PFPN under  $W_{0.4}$  was significantly lower than that under  $W_{0.6}$  at  $N_{240}$  or  $N_{120}$ . These findings could be useful for promoting the utilization of deep water and achieving sustainable agricultural development.

**Keywords:** dry topsoil; water regulation; N application; hydraulic lift; cotton yield

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## 1. Introduction

Climate change, arising from natural causes, human activities, and land use changes, can lead to a range of adverse impacts, including global warming [1], which is reflected in the current trend of an increase of 2 to 3 °C or at best, slightly less than 2 °C [2]. Rising air temperatures would bring about hot and dry conditions, potentially threatening global crop production [3]. In specific areas and times, due to high land surface temperature, low precipitation, and high evapotranspiration (ET), the topsoil may face dryness or even drought [4–6], while the subsoil would be relatively moist [7]. In this particular case, cotton, as the most important fiber crop and a major source of seed oil and protein around the world [8,9], can survive by extending its roots into the moist subsoil to absorb water. However, the topsoil layer often accumulates a large amount of nutrients required for plant growth [10]. When the topsoil layer is under dry conditions, the vitality of related microorganisms decreases, the ion mobility is weakened, and a serious spatial mismatch of water and nutrient profiles is formed.

Soil water restriction and low soil nitrogen (N) concentration are increasingly serious problems [11], and water and N supply and regulation can play a critical role in cotton

growth and development. It was found that among all treatments with different drought stress (367, 618, and 917 mm) and N application levels (0, 150, 225, and 300 kg N ha<sup>-1</sup>), the N application of 225 kg N ha<sup>-1</sup> with moderate drought treatment of 618 mm would achieve the highest yield and high N use efficiency [12]. Hou et al. [13] reported that the optimal dry matter, seed cotton yield, N recovery efficiency, and total N accumulation were obtained in treatment where irrigation was 100% of crop ET and N application was 350 kg N ha<sup>-1</sup>. Zhang et al. [11] evaluated the effects of four irrigation schedules and two dressing ratios and concluded that increased water consumption in deep soil layers resulted in increased seed cotton yield and water use efficiency (*WUE*). However, few studies have been conducted on the rational regulation of soil water and N in the topsoil layer to improve cotton growth under the condition of relatively moist subsoil.

Particularly, in situations where topsoil is dry and subsoil is moist, the plant root system has the potential to passively transfer water upward according to the water potential gradient, which is known as hydraulic lift (HL) and has been found in many woody plants including cotton [14–18]. This involves water redistribution within the plant and water release from roots to topsoil and usually occurs at night when plant transpiration rates are low. The addition of water to topsoil through HL could reduce the rate of water consumption, delay the embolism of shallow roots, and enhance root survival on the one hand, and on the other hand, could increase the rate of ion diffusion to roots, improve the nutritional status during arid periods and extend the growing season of crops [19–21]. It has been reported that HL was driven by the difference in soil water potential between the upper and lower layers, and different fertilizer strategies also impacted HL [22]. Thus, maintaining relatively high HL in cotton also needs to be considered when regulating water and N in the topsoil layer.

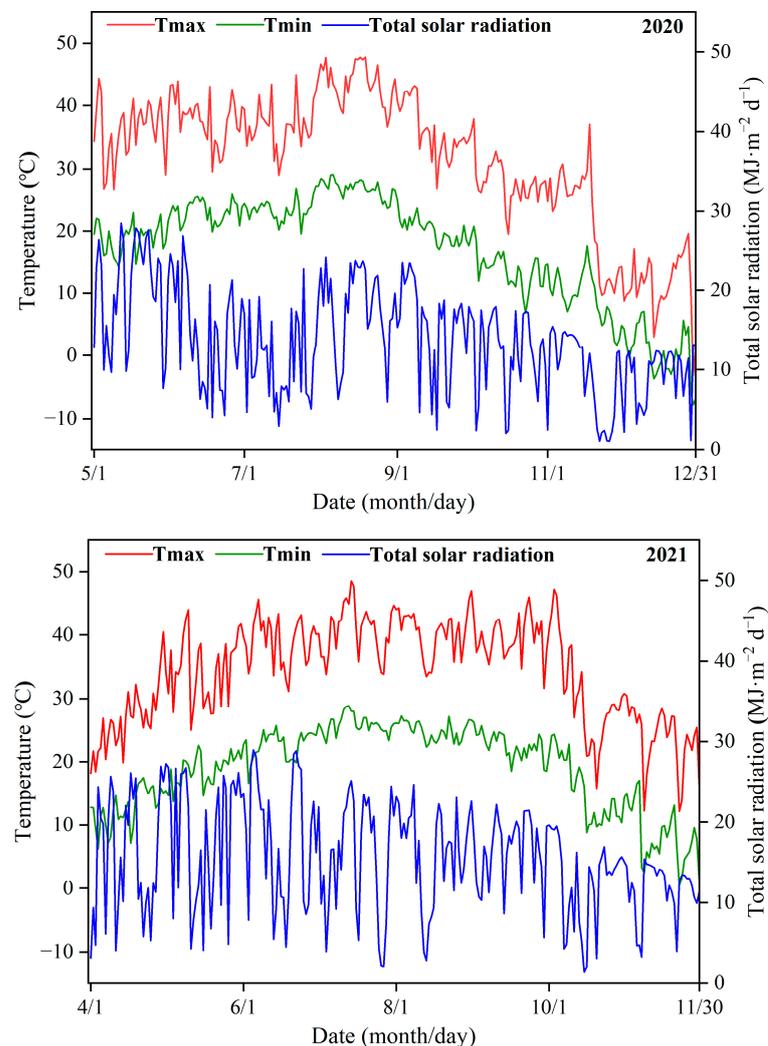
This study aimed to analyze the effects of water and N regulation on cotton growth and HL under dry topsoil conditions using the specially constructed split-root system. The specific objectives were to (1) illustrate the growth dynamics of cotton plants, including plant height, leaf area, leaf photosynthetic characteristics, and root morphological characteristics; (2) investigate the changes in the amount of water from root HL at different periods and the HL water amount for the whole test period; and (3) explore the yield, *WUE*, and N use efficiency of cotton in response to different water and N levels in topsoil. This research will contribute to our understanding of how to rationally regulate water and N in dry topsoil layers to promote cotton growth and maintain high HL.

## 2. Materials and Methods

### 2.1. Experimental Site

The experiment was conducted in a plastic greenhouse for two successive years (2020–2021) at the campus of Hohai University, Nanjing, China (31°57' N, 118°50' E). The site has a subtropical and humid monsoon climate, with an average annual temperature and rainfall of 15.7 °C and 1061.1 mm, respectively. An automatic weather station (FT-XY09, Weifang, China) was installed in the rain-sheltered greenhouse, recording meteorological parameters such as temperature, relative humidity, and total solar radiation at intervals of 2 min. The daily values of maximum and minimum air temperature and total solar radiation during the experiment are illustrated in Figure 1. The initial topsoil (0–30 cm) at the experimental site was classified as clay loam according to the World Reference Base (WRB) 2022 [23], with available N, P, and K of 16.2, 9.9 and 20.4 mg kg<sup>-1</sup>, respectively. Soil-available N content was determined photometrically from on-site KCl extractions using a continuous flow analyzer (SAN Plus, Skalar, Breda, The Netherlands) [24]. Soil-available P content was determined with NaHCO<sub>3</sub> extraction and measured using the continuous flow analyzer (Auto Analyzer-III, Bran Luebbe, Norderstedt, Germany), and soil-available K content was determined with NH<sub>4</sub>OAc extraction and measured using an atomic absorption spectrophotometer (AA370MC, Yiyou, Shanghai, China) [25]. Soil organic carbon (SOC), measured via wet oxidation with KCr<sub>2</sub>O<sub>7</sub> + H<sub>2</sub>SO<sub>4</sub> and titrated with

$\text{FeSO}_4$  [26], was  $12.43 \text{ g kg}^{-1}$ , and soil total N, measured using the Kjeldahl method [26], was  $0.98 \text{ g kg}^{-1}$ . The soil bulk density was  $1.34 \text{ g}\cdot\text{cm}^{-3}$ .



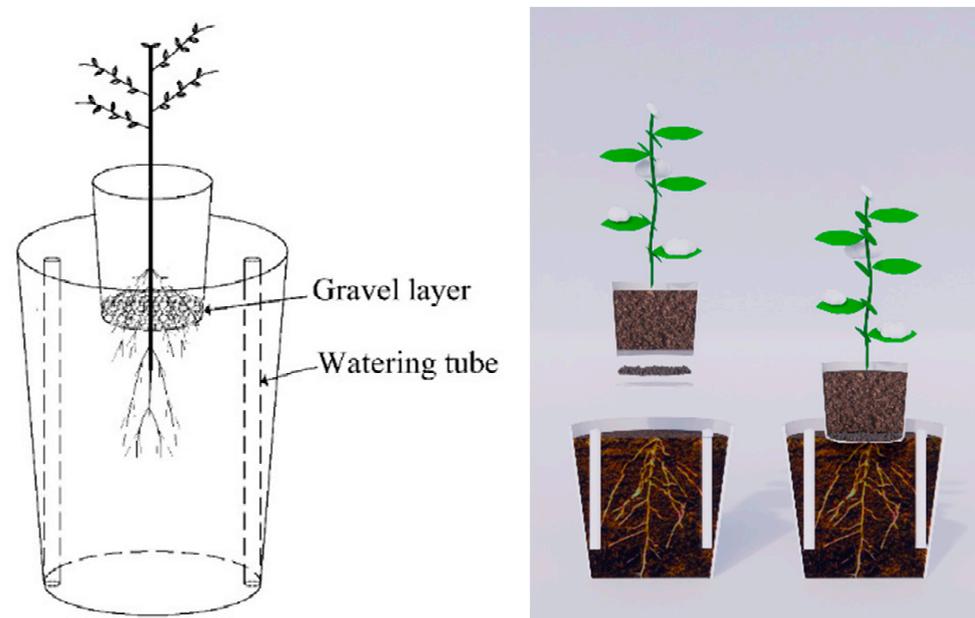
**Figure 1.** Daily maximum (Tmax) and minimum (Tmin) air temperature and total solar radiation during the cotton-growing season in 2020 and 2021.

## 2.2. Experimental Setup

### 2.2.1. Split-Root System

A split-root system consisted of two compartments for roots (a 26 cm top diameter, 23 cm bottom diameter, 22 cm deep upper compartment, and a 48 cm top diameter, 36 cm bottom diameter, and 45 cm deep lower compartment) was specially constructed for this study (Figure 2). To cut off capillary water transmission between the soil of the upper and lower compartments and ensure that the roots penetrate successfully into the soil of the lower compartment, round holes of 1 cm in diameter were punched evenly in the bottom of the upper compartment, and above the bottom, a 1.5 cm thick layer of gravel with a grain size of 1–2 cm was uniformly filled. Then, the upper compartment was filled with 20 cm of sieved dry topsoil (0–30 cm) from the experimental site (i.e., topsoil layer) and the lower compartment was filled with 40 cm of the same dry soil (i.e., subsoil layer), both of which had initial bulk density, field capacity, and saturated moisture content of  $1.30 \text{ g}\cdot\text{cm}^{-3}$ , 27.37% (*w/w*), and 39.23 (*w/w*), respectively. During the filling process, two 35 cm long, 30 mm inner-diameter mesh hollow steel pipes (i.e., watering tube) wrapped with water-permeable gauze on the outside were buried vertically and symmetrically near the outer edges of the lower compartment to irrigate the subsoil evenly. And the bare

soil surface of each lower compartment and the watering tube (when not irrigated) were covered with white polyethylene plastic film to reduce soil evaporation.



**Figure 2.** Diagram of the specially constructed split-root system used in this study.

### 2.2.2. Experimental Design

The completely randomized experiment was adopted for the cotton cultivation in a greenhouse and consisted of three topsoil water conditions (soil water content at 60–70%, 50–60%, and 40–50% of field capacity, designated as  $W_{0.6}$ ,  $W_{0.5}$ , and  $W_{0.4}$ , respectively) and three N rates (conventional farmers' fertilization of  $360 \text{ kg N} \cdot \text{ha}^{-1}$ ; one-third N reduction at  $240 \text{ kg N} \cdot \text{ha}^{-1}$ ; and two-thirds N reduction at  $120 \text{ kg N} \cdot \text{ha}^{-1}$ ; designated as  $N_{360}$ ,  $N_{240}$ , and  $N_{120}$ , respectively). Meanwhile, an additional control treatment (CK) was set up with the soil water content controlled at 60–70% of field capacity and no N fertilizer applied. The N fertilizer used was urea, which was uniformly mixed into topsoil before transplanting. All treatments were set up for the topsoil layer with three replications (30 in total). For subsoil, the soil water content was controlled at 70 to 80% of field capacity until harvest, and no more N fertilizer was applied. In order to ensure the survival of cotton seedlings after transplanting, the topsoil water was kept at 70–80% of field capacity during the seedling stage, while the water treatment was started at the end of the seedling period.

### 2.2.3. Plant Management

The cotton (*Gossypium hirsutum* L. cv. Zhongmian 117) was sown in peat pots on 10 May 2020 and 18 April 2021 in the greenhouse, respectively. The relatively late sowing of cotton in 2020 was mainly due to the coronavirus pandemic. At the four-leaf stage, the cotton seedlings with good growth and consistent traits were selected and transplanted into topsoil one plant per split-root system on 20 June 2020 and 29 May 2021, and the cotton was harvested on 16 December 2020 and 20 November 2021, respectively. Prior to transplanting, a phosphorus fertilizer ( $\text{Ca}(\text{H}_2\text{PO}_4)_2$ ) at the rate of  $180 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  and a potassium fertilizer ( $\text{K}_2\text{SO}_4$ ) at the rate of  $180 \text{ kg K}_2\text{O} \text{ ha}^{-1}$  were mixed evenly into topsoil as a base fertilizer, and the corresponding N fertilizer was also applied. Other agronomic practices, such as weed control and pesticide application, were the same as the local traditional cotton cultivation, but cotton was not topped in either growing season.

### 2.3. Measurements

#### 2.3.1. Soil Water and HL Water

During the experiment, the soil water content of topsoil and subsoil was measured every 2–3 d using a calibrated TDR 350 soil moisture meter (Spectrum Technologies, Inc., Aurora, IL, USA), and irrigation was applied only when soil water reached the low thresholds. The TDR was fitted with a pair of probes with a diameter of 0.5 cm and a spacing of 3 cm. We used 12 cm long and 20 cm long probes to measure the soil water content of the upper and lower compartments, respectively. When measuring, a narrow trench was first dug, then the probes were inserted vertically into the tested soil. The measurements were made at different locations. For HL, the nocturnal variation of the volumetric water content of the topsoil (at 8:00 p.m. and 6:00 a.m. of the following day) was monitored approximately every five days by taking multiple measurements at multiple points using a TDR 350 soil moisture meter, as an indirect way of calculating the amount of water lifted and released by roots at night. It was noted that the monitoring of HL did not start until the intermediate period of cotton growth. This was because, on the one hand, in this experiment, the roots of the cotton plants needed to penetrate through the gravel layer and the holes in the bottom of the upper compartment before they could enter the subsoil, which often took longer, as obtained from the destructive pre-test. In addition, we hypothesized that root HL would occur only when the cotton plant was established to a certain stage. This meant that the roots should be elongated to a longer length in the lower compartment in this case. The amount of water released by roots in the topsoil was calculated using the following formula:

$$G = \Delta W \quad (1)$$

where  $G$  is the amount of HL water, mm, and  $\Delta W$  is the variation of soil water in the root zone between 6:00 a.m. on the next day and 8:00 p.m. on the previous day, mm. Nighttime soil evaporation and plant transpiration were negligible because they were usually small.

#### 2.3.2. Growth Dynamics

The plant height and leaf area were recorded about once a week. In addition, leaf photosynthetic characteristics were performed on the third fully expanded leaf at 10:00 a.m. on a clear and cloudless day at the flowering stage (11 August) in 2020 and at the flowering stage (21 July) in 2021, including the net photosynthetic rate ( $P_n$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and transpiration rate ( $T_r$ ,  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ). Measurements were conducted using an LI-6800 photosynthesis system with photosynthetic active radiation of  $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , a flow rate of  $500 \mu\text{mol s}^{-1}$ , and a  $\text{CO}_2$  concentration of  $400 \mu\text{mol mol}^{-1}$ , respectively.

After the cotton harvest, we collected cotton roots from the soil of the split-root system. Then, the collected roots were washed slowly, air dried, and scanned using the Epson Perfection 4990 Photo scanner. The scan images of root morphology were analyzed using the WinRHIZO software (WinRHIZO Pro 2012b, Regent Instruments, Québec, QC, Canada) to obtain the total root length, total root surface area, and total root volume.

#### 2.3.3. Yield and Water-N Utilization

At the boll opening stage, the seed cotton was harvested immediately by hand-picking, weighed to obtain seed cotton yield, and then partially ginned to determine the lint percentage. The lint yield was determined by multiplying the average lint percentage by the respective seed cotton yield [27].

$WUE$  was determined as follows [28]:

$$WUE = Y/ET \quad (2)$$

where  $Y$  is the seed cotton yield ( $\text{g plant}^{-1}$ ), and  $ET$  is the total evapotranspiration for the whole season (mm). Since the experiment was conducted with pot planting in a greenhouse sheltered from the rain, there was no effective precipitation, the upward capillary flow into

the root zone, the runoff, and the downward drainage. According to the water balance equation, the  $ET$  for cotton plants can be simplified as follows [29]:

$$ET = I + \Delta W \quad (3)$$

where  $I$  is the cumulative irrigation depth, mm, and  $\Delta W$  is the variation of soil water in the root zone between the two soil water measurements, mm.

Nitrogen partial factor productivity ( $PFPN$ ) was determined as follows [30]:

$$PFPN = Y/T_N \quad (4)$$

where  $Y$  is the seed cotton yield ( $\text{g plant}^{-1}$ ), and  $T_N$  is the total application of the N fertilizer ( $\text{g plant}^{-1}$ ).

#### 2.4. Statistical Analysis

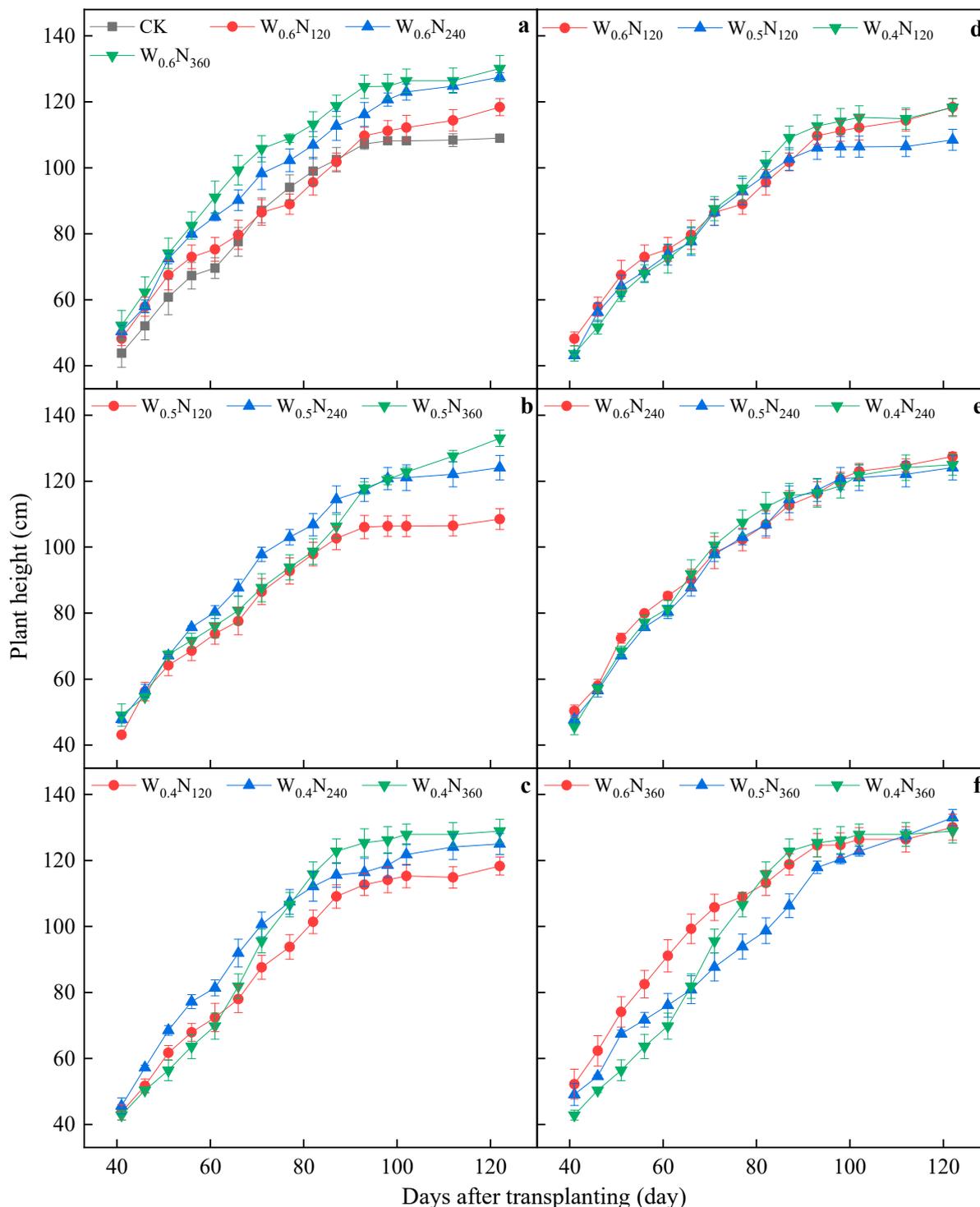
A three-way analysis of variance (ANOVA) was performed for the year, topsoil water condition, and N rate, including two- and three-way interactions. A one-way ANOVA and the least significant difference test (LSD) were used to test for significant differences between treatments in the same year. Statistics were performed using SPSS software (version 23.0; IBM SPSS Statistics, Chicago, IL, USA), and the tests were conducted at an alpha level of 0.05. All data were expressed as mean  $\pm$  standard error ( $n = 3$ ), and all figures were drawn using Origin 9.1 software (OriginLab Corporation, Northampton, MA, USA).

### 3. Results

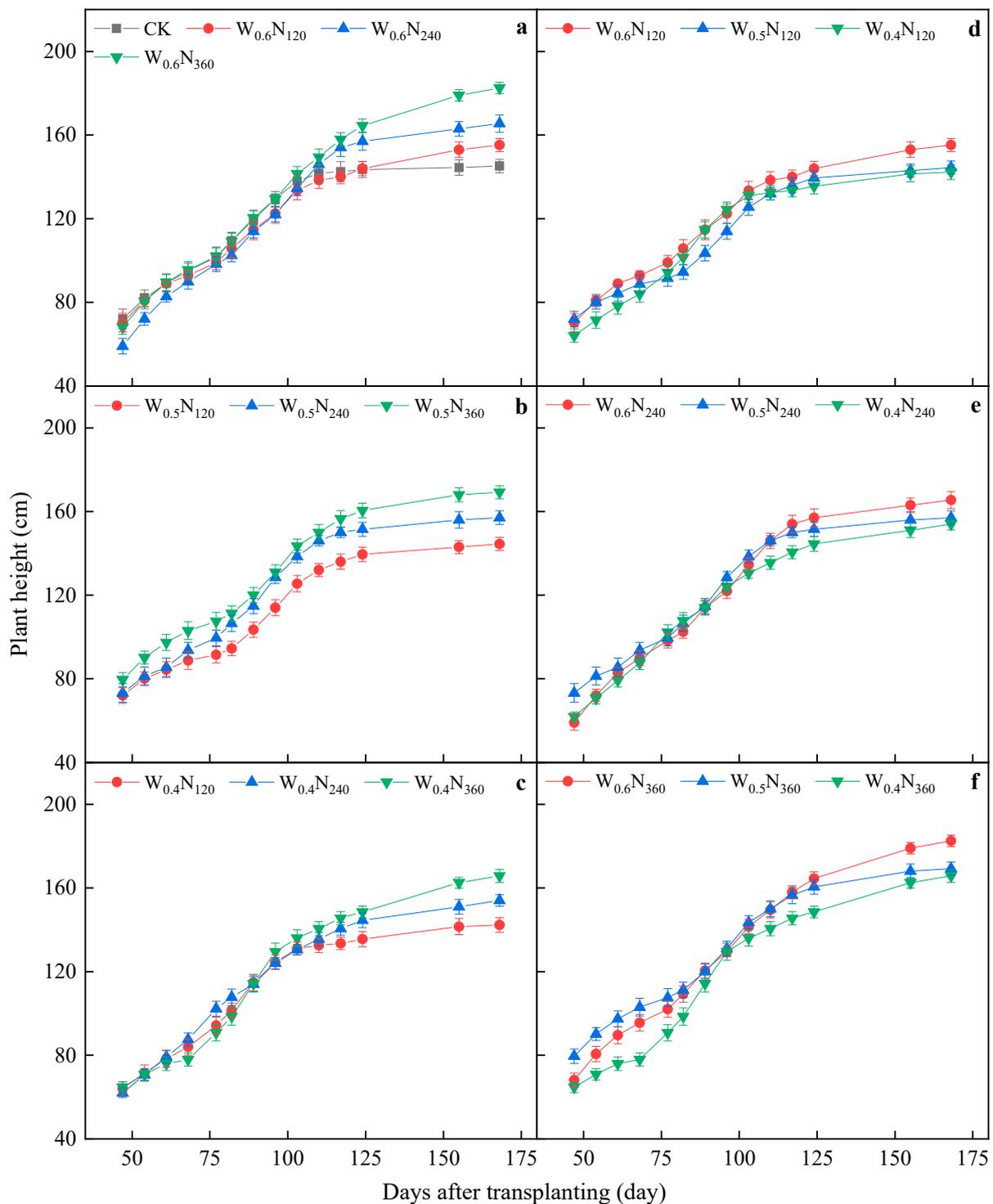
#### 3.1. Growth Parameters

Figure 3 illustrates the dynamics of cotton plant height over time in 2020. When the topsoil layer was under  $W_{0.6}$ , the plant height of cotton basically increased with the increase in N application rate, and the growth rate of the CK and  $N_{120}$  slowed down after 61 d while that of  $N_{240}$  and  $N_{360}$  slowed down after 71 d. When the topsoil layer was under  $W_{0.5}$ , the growth rate of plant height was greater at  $N_{240}$  than at  $N_{120}$  and  $N_{360}$  from 41 to 82 d, but after 82 d,  $N_{360}$  accelerated and finally exceeded  $N_{240}$ . This pattern of change was more notable under  $W_{0.4}$ . Unlike the significant differences among N application rates under the same topsoil water conditions, the differences among topsoil water conditions at the same N application rate were not significant, except for  $N_{360}$ . At  $N_{360}$ , the plant height of  $W_{0.6}$  was greater than that of  $W_{0.5}$  and  $W_{0.4}$  until 82 d after transplanting, whereas  $W_{0.4}$  showed a substantial increase in growth rate from about 61 d and finally arrived at the same plant height as  $W_{0.6}$  and  $W_{0.5}$ . The dynamics of cotton plant height over time in 2021 (Figure 4) were broadly similar to those in 2020, but with some differences, i.e., the early plant height was slightly higher under  $W_{0.5}$  than under  $W_{0.6}$  at  $N_{240}$  and  $N_{360}$ , and there was a tendency that the drier the topsoil layer, the lower the final plant height.

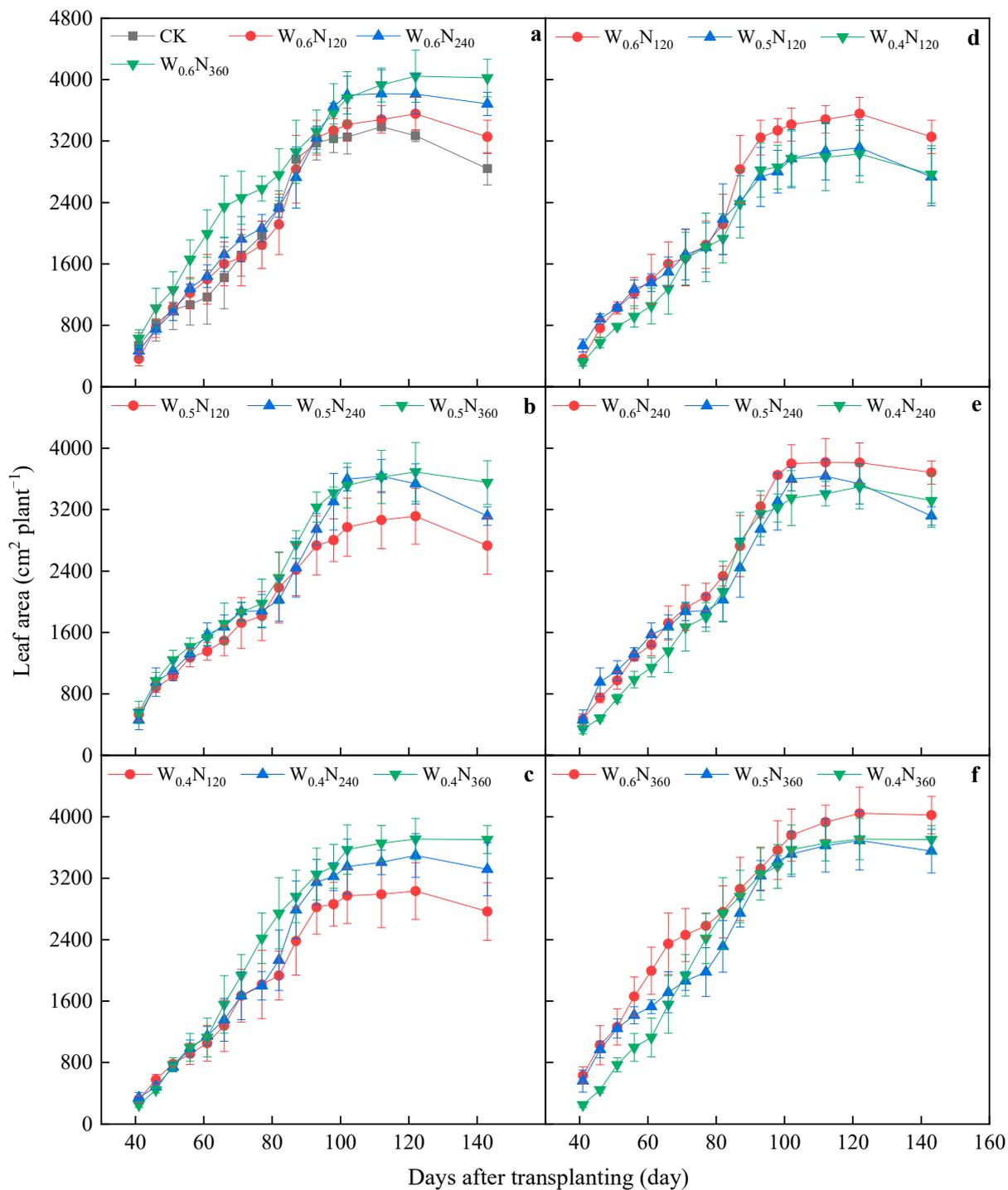
Figure 5 presents the dynamics of the cotton leaf area in 2020. When the topsoil layer was under  $W_{0.6}$ , the leaf area grew fastest at  $N_{360}$  during the first 66 d and then slowed down, and at this time, the cotton leaf area at CK,  $N_{120}$ , and  $N_{240}$  began to increase rapidly and reached the same value as that of  $N_{360}$  near 90 d, after which the leaf area of  $N_{240}$  and  $N_{360}$  continued to show a significant increase. In contrast, the leaf area divergence across N rates under  $W_{0.5}$  and  $W_{0.4}$  appeared relatively late, which was consistent with the dynamics of cotton plant height in 2020 (Figure 3a–c), but the relative magnitude of leaf area across treatments was the same as under  $W_{0.6}$ . The leaf area of  $W_{0.6}$  was normally larger than that of  $W_{0.5}$  and  $W_{0.4}$ , and the leaf area divergence across topsoil water conditions at  $N_{120}$  and  $N_{240}$  appeared later than at  $N_{360}$ . For the dynamics of the cotton leaf area in 2021 (Figure 6), under the same topsoil water condition, the leaf area size of each N rate showed  $CK < N_{120} < N_{240} < N_{360}$ , and at the same N rate, the leaf area of  $W_{0.6}$  was normally larger than that of  $W_{0.5}$  and  $W_{0.4}$ , especially in the later stage of the experiment.



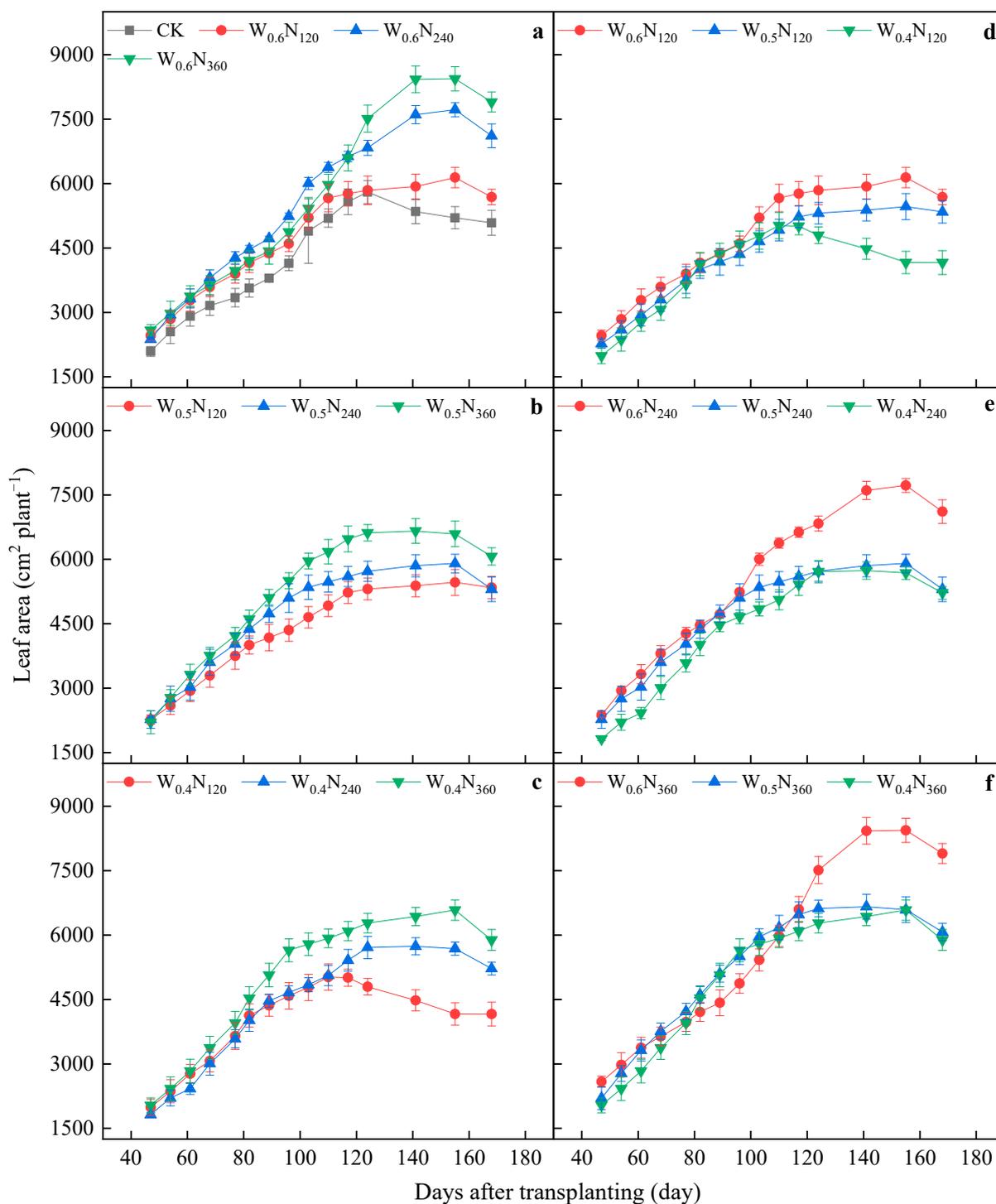
**Figure 3.** The dynamics of cotton plant height over time for different nitrogen application rates under the same topsoil water condition (a–c) and for different topsoil water conditions at the same nitrogen application rate (d–f) in 2020. Note: CK means soil water content controlled at 60–70% of field capacity and no nitrogen fertilizer applied.  $W_{0.6}$ ,  $W_{0.5}$ , and  $W_{0.4}$  mean topsoil water condition at 60–70%, 50–60%, and 40–50% of field capacity, respectively.  $N_{120}$ ,  $N_{240}$ , and  $N_{360}$  mean nitrogen rates of 120 kg·N ha<sup>-1</sup>, 240 kg·N ha<sup>-1</sup>, and 360 kg N·ha<sup>-1</sup>, respectively.



**Figure 4.** The dynamics of cotton plant height over time for different nitrogen application rates under the same topsoil water condition (a–c) and for different topsoil water conditions at the same nitrogen application rate (d–f) in 2021. Note: CK means soil water content controlled at 60–70% of field capacity and no nitrogen fertilizer applied.  $W_{0.6}$ ,  $W_{0.5}$ , and  $W_{0.4}$  mean topsoil water condition at 60–70%, 50–60%, and 40–50% of field capacity, respectively.  $N_{120}$ ,  $N_{240}$ , and  $N_{360}$  mean nitrogen rates of 120 kg·N ha<sup>-1</sup>, 240 kg·N ha<sup>-1</sup>, and 360 kg N·ha<sup>-1</sup>, respectively.



**Figure 5.** The dynamics of cotton leaf area over time for different nitrogen application rates under the same topsoil water condition (a–c) and for different topsoil water conditions at the same nitrogen application rate (d–f) in 2020. Note: CK means soil water content controlled at 60–70% of field capacity and no nitrogen fertilizer applied. W<sub>0.6</sub>, W<sub>0.5</sub>, and W<sub>0.4</sub> mean topsoil water condition at 60–70%, 50–60%, and 40–50% of field capacity, respectively. N<sub>120</sub>, N<sub>240</sub>, and N<sub>360</sub> mean nitrogen rates of 120 kg·N ha<sup>-1</sup>, 240 kg·N ha<sup>-1</sup>, and 360 kg N·ha<sup>-1</sup>, respectively.



**Figure 6.** The dynamics of cotton leaf area over time for different nitrogen application rates under the same topsoil water condition (a–c) and for different topsoil water conditions at the same nitrogen application rate (d–f) in 2021. Note: CK means soil water content controlled at 60–70% of field capacity and no nitrogen fertilizer applied.  $W_{0.6}$ ,  $W_{0.5}$ , and  $W_{0.4}$  mean topsoil water condition at 60–70%, 50–60%, and 40–50% of field capacity, respectively.  $N_{120}$ ,  $N_{240}$ , and  $N_{360}$  mean nitrogen rates of 120 kg·N ha<sup>-1</sup>, 240 kg·N ha<sup>-1</sup>, and 360 kg·N ha<sup>-1</sup>, respectively.

According to Table 1, the effects of topsoil water condition, N rate, and their interaction on  $P_n$  and  $T_r$  at the flowering stage were not significant. However, the effect of the year on  $P_n$  or  $T_r$  was significant. This was mainly due to the large difference in sowing and transplanting dates between the two years of the trial. Trends in plant height and leaf area in both years also indicated that the difference between these two growing seasons was very large.

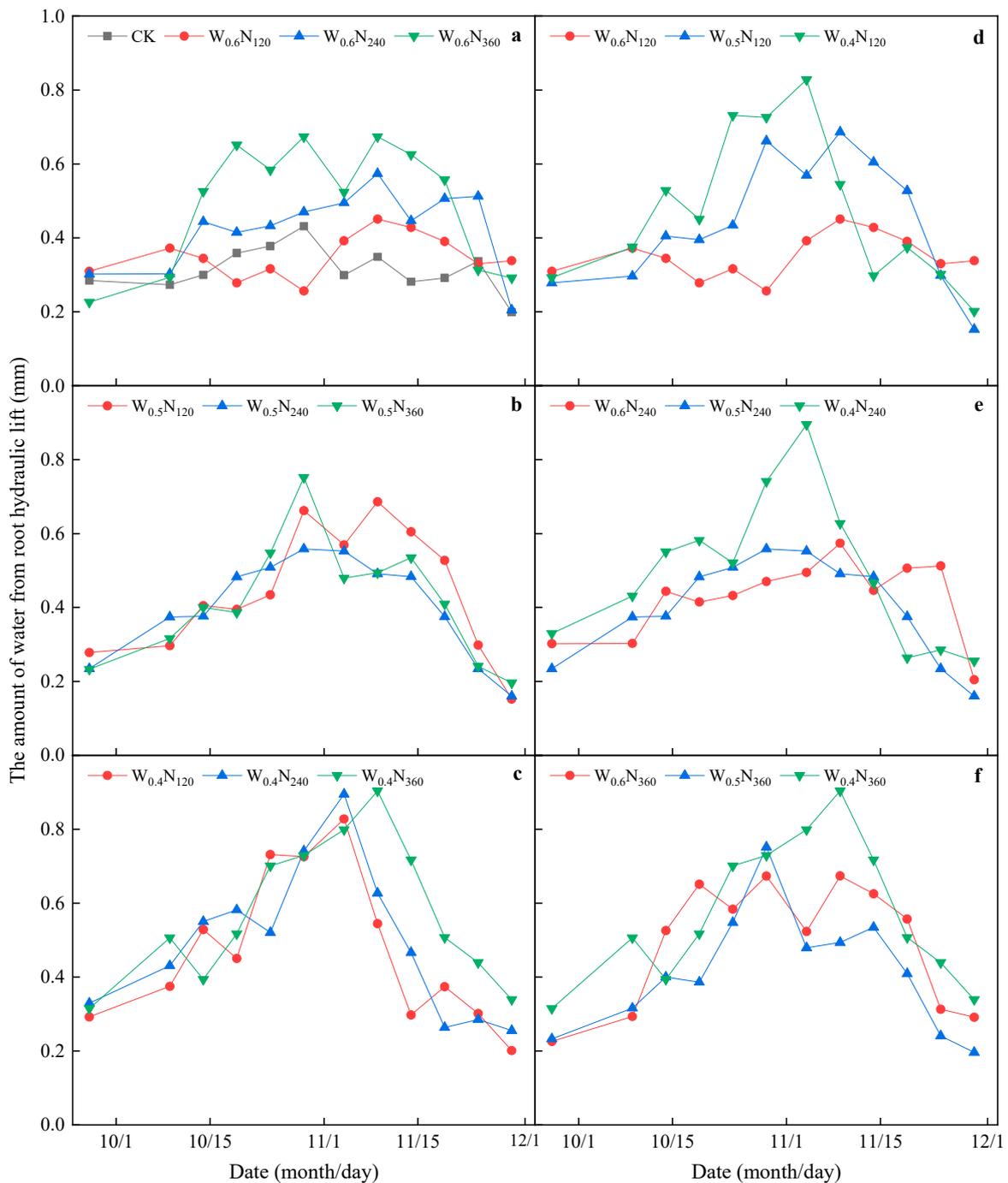
**Table 1.** Photosynthetic characteristics of leaves at the flowering stage for treatments in 2020 and 2021, presented as a mean  $\pm$  S.E. (n = 3).

Treatment	Net Photosynthetic Rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )		Transpiration Rate ( $\text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ )	
	2020	2021	2020	2021
CK	20.47 $\pm$ 3.71 a	17.97 $\pm$ 0.33 bc	17.23 $\pm$ 3.77 a	12.41 $\pm$ 0.59 a
W <sub>0.6</sub> N <sub>120</sub>	21.23 $\pm$ 1.90 a	19.37 $\pm$ 1.76 abc	20.11 $\pm$ 0.61 a	13.16 $\pm$ 1.66 a
W <sub>0.6</sub> N <sub>240</sub>	19.46 $\pm$ 2.21 a	18.81 $\pm$ 0.93 abc	18.82 $\pm$ 3.40 a	13.57 $\pm$ 0.64 a
W <sub>0.6</sub> N <sub>360</sub>	22.57 $\pm$ 0.45 a	20.69 $\pm$ 0.50 ab	22.41 $\pm$ 1.53 a	14.35 $\pm$ 0.11 a
W <sub>0.5</sub> N <sub>120</sub>	21.40 $\pm$ 1.37 a	20.84 $\pm$ 0.33 a	20.54 $\pm$ 0.58 a	12.45 $\pm$ 0.26 a
W <sub>0.5</sub> N <sub>240</sub>	20.65 $\pm$ 1.26 a	21.15 $\pm$ 1.50 a	20.92 $\pm$ 1.78 a	12.57 $\pm$ 1.22 a
W <sub>0.5</sub> N <sub>360</sub>	23.09 $\pm$ 0.98 a	20.27 $\pm$ 0.52 abc	22.54 $\pm$ 1.33 a	13.47 $\pm$ 0.23 a
W <sub>0.4</sub> N <sub>120</sub>	21.57 $\pm$ 1.04 a	18.67 $\pm$ 1.19 abc	20.97 $\pm$ 1.37 a	13.61 $\pm$ 1.82 a
W <sub>0.4</sub> N <sub>240</sub>	21.84 $\pm$ 0.31 a	19.41 $\pm$ 0.11 abc	23.02 $\pm$ 1.34 a	14.22 $\pm$ 1.93 a
W <sub>0.4</sub> N <sub>360</sub>	23.61 $\pm$ 1.85 a	17.80 $\pm$ 0.63 c	22.67 $\pm$ 1.32 a	12.47 $\pm$ 0.92 a
Year	**		**	
Water	ns		ns	
Nitrogen	ns		ns	
Year $\times$ Water	ns		ns	
Year $\times$ Nitrogen	ns		ns	
Water $\times$ Nitrogen	ns		ns	
Year $\times$ Water $\times$ Nitrogen	ns		ns	

Note: Different letters mean significant differences ( $p < 0.05$ ). \*\* and ns mean  $p < 0.01$  and no significant difference, respectively. CK means soil water content controlled at 60–70% of field capacity and no nitrogen fertilizer applied. W<sub>0.6</sub>, W<sub>0.5</sub>, and W<sub>0.4</sub> mean topsoil water condition at 60–70%, 50–60%, and 40–50% of field capacity, respectively. N<sub>120</sub>, N<sub>240</sub>, and N<sub>360</sub> mean nitrogen rates of 120 kg·N ha<sup>-1</sup>, 240 kg·N ha<sup>-1</sup>, and 360 kg·N ha<sup>-1</sup>, respectively.

### 3.2. Root Hydraulic Lift and Root Morphological Characteristics

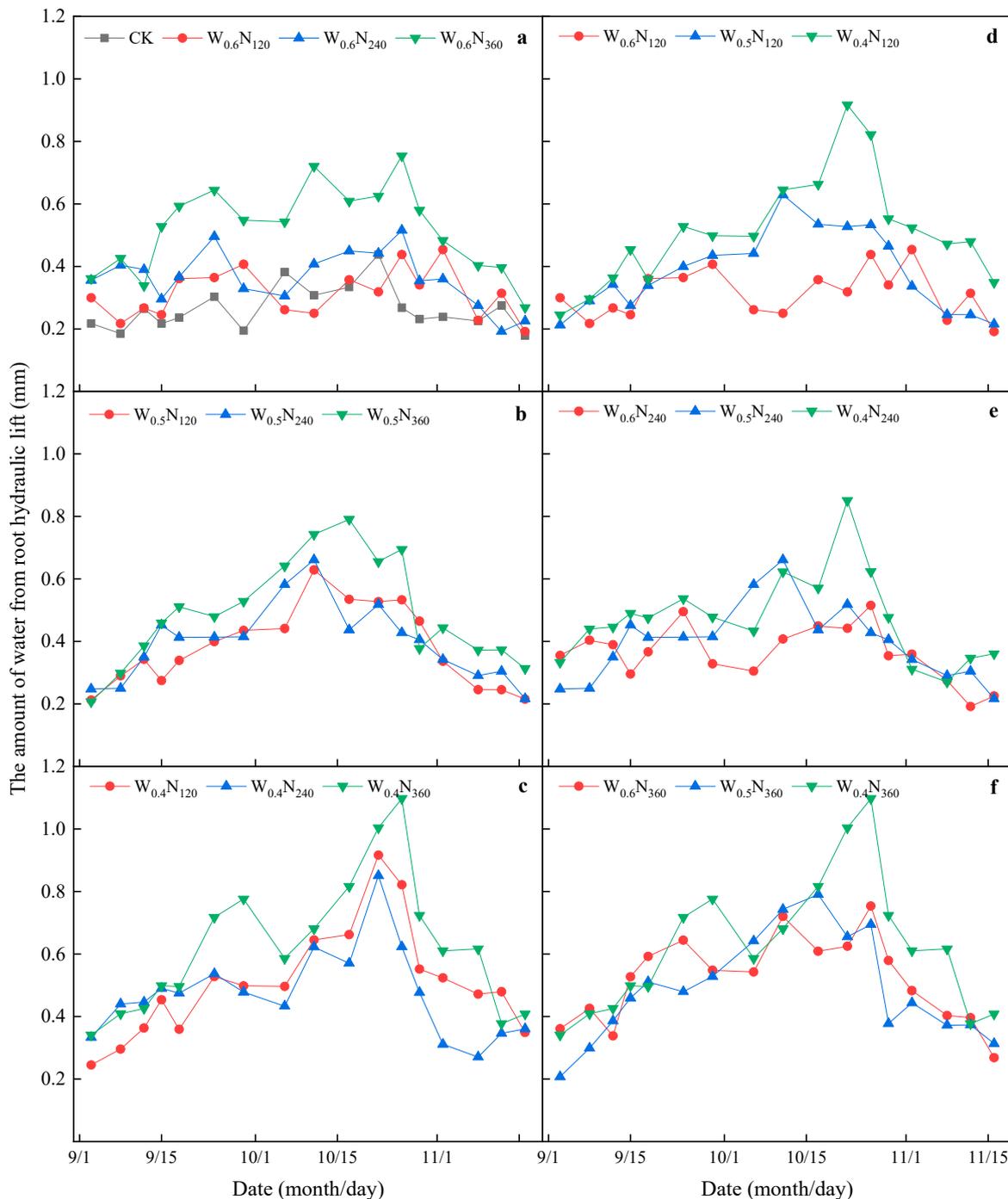
Figure 7 displays the changes in the amount of water from HL during the observation period in 2020. Generally, the HL water amount of all treatments increased gradually and then continued to decline. When the topsoil layer was under W<sub>0.6</sub>, there was a trend that the more N applied, the more the HL water amount except for CK in the mid-observation period (Figure 7a). In contrast, relatively high variability of HL water amount during the observation period was shown at N<sub>120</sub> and N<sub>360</sub> under W<sub>0.5</sub>, but the difference among N rates was small under W<sub>0.5</sub> (Figure 7b). The greatest magnitude of changes in HL water amount of each N rate appeared under W<sub>0.4</sub>, and the order of HL water amount was roughly N<sub>120</sub> < N<sub>240</sub> < N<sub>360</sub> (Figure 7c). For the variation of HL water amount under different topsoil water conditions (see Figure 7d–f), there was a trend that the drier the topsoil at N<sub>120</sub> and N<sub>240</sub>, the greater the amount of HL water in the early stage of observation, and at N<sub>360</sub> the HL water amount was generally high under all topsoil water conditions.



**Figure 7.** Changes in the amount of water from root hydraulic lift at different periods for different nitrogen application rates under the same topsoil water condition (a–c) and for different topsoil water conditions at the same nitrogen application rate (d–f) in 2020. Note: CK means soil water content controlled at 60–70% of field capacity and no nitrogen fertilizer applied.  $W_{0.6}$ ,  $W_{0.5}$ , and  $W_{0.4}$  mean topsoil water condition at 60–70%, 50–60%, and 40–50% of field capacity, respectively.  $N_{120}$ ,  $N_{240}$ , and  $N_{360}$  mean nitrogen rates of 120 kg·N ha<sup>-1</sup>, 240 kg·N ha<sup>-1</sup>, and 360 kg·N ha<sup>-1</sup>, respectively.

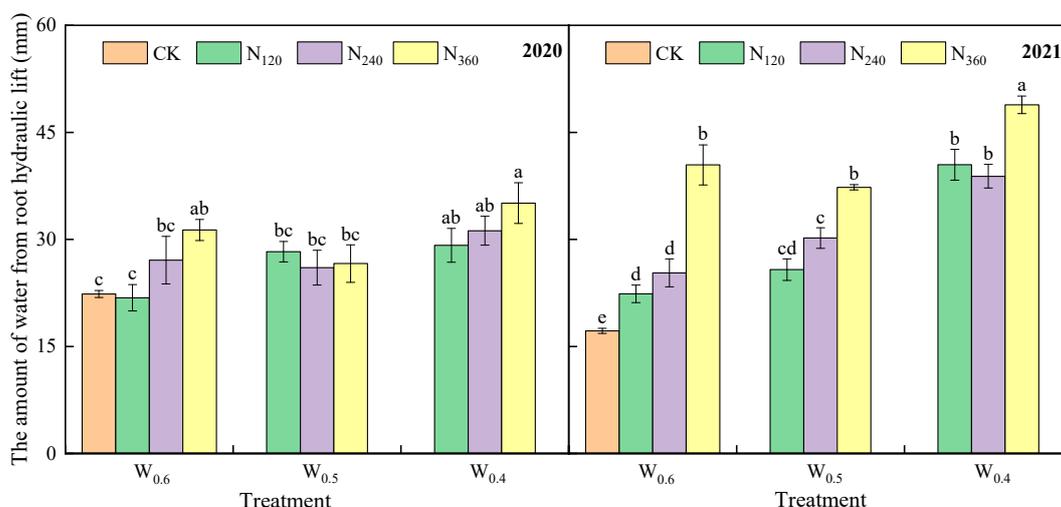
Data in Figure 8 suggest that changes in HL water amounts were approximately similar to those in 2020 throughout the test period. Differently, under  $W_{0.4}$ , the order of HL water amount was roughly  $N_{120} < N_{240} < N_{360}$  in the early stage of observation and  $N_{240} < N_{120} < N_{360}$  in the late stage of observation, and the HL was significantly enhanced at  $N_{360}$  (Figure 8c). In addition, the amount of HL water under different topsoil water

conditions at the same N rate did not differ much at the beginning and the end of the test, and the highest amount of HL water at any N rate was gained under  $W_{0.4}$  at the middle of the test. It was noted that at the same N rate, the HL water amount peaked later under  $W_{0.4}$  than  $W_{0.6}$  and  $W_{0.5}$ , which may be due to the fact that a higher degree of dryness in the topsoil layer had a stronger inhibitory effect on shallow root growth and water release.



**Figure 8.** Changes in the amount of water from root hydraulic lift at different periods for different nitrogen application rates under the same topsoil water condition (a–c) and for different topsoil water conditions at the same nitrogen application rate (d–f) in 2021. Note: CK means soil water content controlled at 60–70% of field capacity and no nitrogen fertilizer applied.  $W_{0.6}$ ,  $W_{0.5}$ , and  $W_{0.4}$  mean topsoil water condition at 60–70%, 50–60%, and 40–50% of field capacity, respectively.  $N_{120}$ ,  $N_{240}$ , and  $N_{360}$  mean nitrogen rates of 120 kg·N ha<sup>-1</sup>, 240 kg·N ha<sup>-1</sup>, and 360 kg N·ha<sup>-1</sup>, respectively.

By linearly interpolating the amount of HL water measured on each two adjacent time points, the amount of HL water for the whole test period was roughly calculated (Figure 9). The results of the two-year experiment showed that the effects of year, topsoil water condition, N rate, year × water, year × N rate, and water × N rate on the total HL water amount were significant (Table 2). When the same N rate was applied, the highest total HL water was obtained under  $W_{0.4}$ , and the amount of total HL water basically showed an increasing trend with the increase in topsoil dryness. In 2020, when the topsoil water condition was under  $W_{0.4}$  or  $W_{0.6}$ , the amount of total HL water showed a decreasing trend as the N rate decreased, but there was no difference among different N rates under  $W_{0.5}$ . In 2021, the amount of total HL water at  $N_{360}$  was the highest under all topsoil water conditions, but no significant difference was found between the amount of total HL water at  $N_{120}$  and  $N_{240}$ .



**Figure 9.** The amount of water from root hydraulic lift throughout the test period of cotton in 2020 and 2021. Note: Different letters mean significant differences ( $p < 0.05$ ). CK means soil water content controlled at 60–70% of field capacity and no nitrogen fertilizer applied.  $W_{0.6}$ ,  $W_{0.5}$ , and  $W_{0.4}$  mean topsoil water condition at 60–70%, 50–60%, and 40–50% of field capacity, respectively.  $N_{120}$ ,  $N_{240}$ , and  $N_{360}$  mean nitrogen rates of 120 kg·N ha<sup>-1</sup>, 240 kg·N ha<sup>-1</sup>, and 360 kg N·ha<sup>-1</sup>, respectively.

**Table 2.** Significance levels ( $p$ -values) of the effects of topsoil water condition and N rate on the amount of water from root hydraulic lift.

Treatment	The Amount of Water from Root Hydraulic Lift
Year	**
Water	**
Nitrogen	**
Year × Water	**
Year × Nitrogen	**
Water × Nitrogen	*
Year × Water × Nitrogen	ns

Note: \*, \*\*, and ns mean  $p < 0.05$ ,  $p < 0.01$ , and no significant difference, respectively.

Roots are the most active organs in plants for absorbing water and nutrients. The root morphology characteristics, including total root length, root surface area, and root volume, would change in response to changes in the external growing environment. The effects of topsoil water condition, N rate, year × water, and water × N rate on root length were significant (Table 3). When the N rate was  $N_{120}$ , the topsoil water condition did not affect root length, but when the N rate was  $N_{240}$  or  $N_{360}$ , the total root length decreased as the topsoil water condition became poor. In 2020, under any given designed topsoil water condition, total root length did not change depending on the N rate except for promotion

by  $W_{0.6}N_{360}$  relative to CK. However, in 2021, root length at  $N_{240}$  or  $N_{360}$  was significantly improved compared to root length at  $N_{120}$  or CK when  $W_{0.6}$  was applied.

Year, topsoil water condition, and N rate had significant effects on root surface area (Table 3). When the topsoil water condition was the same, there was no difference in root surface area among the three N rates in 2020. When the N rate was  $N_{120}$ , the root surface area of  $W_{0.6}$  was significantly greater than those of  $W_{0.5}$  and  $W_{0.4}$ , and when the N rate was  $N_{240}$ , the root surface area of  $W_{0.4}$  was significantly lower than that of  $W_{0.6}$ . In 2021, when  $W_{0.6}$  was applied, root surface area increased with increasing N rate, but no difference was found under  $W_{0.5}$  or  $W_{0.4}$ . At  $N_{360}$ , the root surface area under  $W_{0.6}$  was significantly higher than those under  $W_{0.5}$  and  $W_{0.4}$ . And when the N rate was  $N_{240}$ , the root surface area of  $W_{0.4}$  was significantly lower than that of  $W_{0.6}$ .

The effects of topsoil water condition and N rate on root volume were significant (Table 3). When topsoil water condition was the same, there was no significant difference among the three N rates except for a greater root volume of  $W_{0.4}N_{360}$  relative to  $W_{0.4}N_{120}$ , and when the N rate was the same, there was no significant difference among three topsoil water conditions in 2020. In 2021, the root volume at  $N_{360}$  was greater than those at  $N_{120}$  and  $N_{240}$  under  $W_{0.6}$ , and the root volume at  $N_{360}$  was greater than that at  $N_{120}$  under  $W_{0.5}$ . The root volume of  $W_{0.6}N_{360}$  was the greatest.

### 3.3. Yield, WUE, and PFPN

Year, topsoil water condition, N rate, year  $\times$  water, water  $\times$  N rate, and year  $\times$  water  $\times$  N rate had significant effects on seed cotton yield (Table 4). In 2020, under  $W_{0.6}$ , seed cotton yield increased with the increasing N rate, but under  $W_{0.5}$  and  $W_{0.4}$ , there was no significant difference in seed cotton yield among all N rates. However, in 2021, seed cotton yield increased with the increasing N rate under all topsoil water conditions, and seed cotton yield at  $N_{360}$  was significantly higher than at  $N_{120}$  and  $N_{240}$  under  $W_{0.6}$  and  $W_{0.5}$  while seed cotton yield at  $N_{360}$  and  $N_{240}$  were significantly higher than at  $N_{120}$  under  $W_{0.4}$ . In addition, seed cotton yield tended to decrease with increasing topsoil dryness at  $N_{240}$  and  $N_{360}$  in 2020 and  $N_{360}$  in 2021.

**Table 3.** Total root length, root surface area, and root volume for treatments in 2020 and 2021 presented as a mean  $\pm$  S.E. (n = 3).

Treatment	Root Length (cm)		Root Surface Area (cm <sup>2</sup> )		Root Volume (cm <sup>3</sup> )	
	2020	2021	2020	2021	2020	2021
CK	832.84 $\pm$ 53.85 bcd	828.10 $\pm$ 8.49 cd	468.45 $\pm$ 24.89 ab	453.49 $\pm$ 12.24 bc	16.06 $\pm$ 0.70 d	17.10 $\pm$ 0.87 d
W <sub>0.6</sub> N <sub>120</sub>	878.41 $\pm$ 50.31 abc	758.85 $\pm$ 2.36 cdef	545.32 $\pm$ 33.33 a	429.11 $\pm$ 15.31 cd	19.41 $\pm$ 0.84 abc	19.58 $\pm$ 1.39 bc
W <sub>0.6</sub> N <sub>240</sub>	926.13 $\pm$ 36.24 ab	1025.06 $\pm$ 39.52 ab	546.20 $\pm$ 45.62 a	506.83 $\pm$ 19.03 ab	18.89 $\pm$ 0.74 abc	20.40 $\pm$ 0.67 b
W <sub>0.6</sub> N <sub>360</sub>	973.00 $\pm$ 28.11 a	1070.28 $\pm$ 47.72 a	550.22 $\pm$ 19.32 a	516.71 $\pm$ 30.52 a	21.00 $\pm$ 1.30 abc	23.51 $\pm$ 0.19 a
W <sub>0.5</sub> N <sub>120</sub>	805.94 $\pm$ 37.17 cd	806.19 $\pm$ 80.45 cde	457.97 $\pm$ 31.28 b	409.54 $\pm$ 32.44 cd	18.22 $\pm$ 0.87 bcd	17.00 $\pm$ 0.94 d
W <sub>0.5</sub> N <sub>240</sub>	848.83 $\pm$ 16.09 bcd	885.51 $\pm$ 27.97 bc	483.81 $\pm$ 37.30 ab	459.45 $\pm$ 8.93 abc	17.42 $\pm$ 0.62 cd	19.13 $\pm$ 0.10 bcd
W <sub>0.5</sub> N <sub>360</sub>	819.91 $\pm$ 7.68 cd	768.92 $\pm$ 4.56 cdef	489.50 $\pm$ 11.75 ab	433.62 $\pm$ 6.65 cd	19.68 $\pm$ 0.54 abc	20.11 $\pm$ 0.19 b
W <sub>0.4</sub> N <sub>120</sub>	788.33 $\pm$ 41.90 cd	654.27 $\pm$ 11.47 f	458.66 $\pm$ 19.88 b	387.83 $\pm$ 18.39 d	17.42 $\pm$ 0.66 cd	18.30 $\pm$ 0.69 bcd
W <sub>0.4</sub> N <sub>240</sub>	765.68 $\pm$ 17.06 d	709.97 $\pm$ 106.43 def	438.10 $\pm$ 22.94 b	403.08 $\pm$ 34.31 cd	17.65 $\pm$ 0.36 bcd	17.91 $\pm$ 0.78 cd
W <sub>0.4</sub> N <sub>360</sub>	829.85 $\pm$ 27.23 bcd	669.36 $\pm$ 1.72 ef	503.34 $\pm$ 9.45 ab	401.45 $\pm$ 1.12 cd	20.00 $\pm$ 1.12 ab	19.71 $\pm$ 0.08 bc
Year	ns		**		ns	
Water	**		**		**	
Nitrogen	**		**		**	
Year $\times$ Water	*		ns		ns	
Year $\times$ Nitrogen	ns		ns		ns	
Water $\times$ Nitrogen	**		ns		ns	
Year $\times$ Water $\times$ Nitrogen	ns		ns		ns	

Note: Different letters mean significant differences ( $p < 0.05$ ). \*, \*\*, and ns mean  $p < 0.05$ ,  $p < 0.01$ , and no significant difference, respectively. Note: CK means soil water content controlled at 60–70% of field capacity and no nitrogen fertilizer applied. W<sub>0.6</sub>, W<sub>0.5</sub>, and W<sub>0.4</sub> mean topsoil water condition at 60–70%, 50–60%, and 40–50% of field capacity, respectively. N<sub>120</sub>, N<sub>240</sub>, and N<sub>360</sub> mean nitrogen rates of 120 kg·N ha<sup>-1</sup>, 240 kg·N ha<sup>-1</sup>, and 360 kg·N ha<sup>-1</sup>, respectively.

**Table 4.** Seed cotton yield, lint cotton yield, and lint percentage for treatments in 2020 and 2021 presented as a mean  $\pm$  S.E. (n = 3).

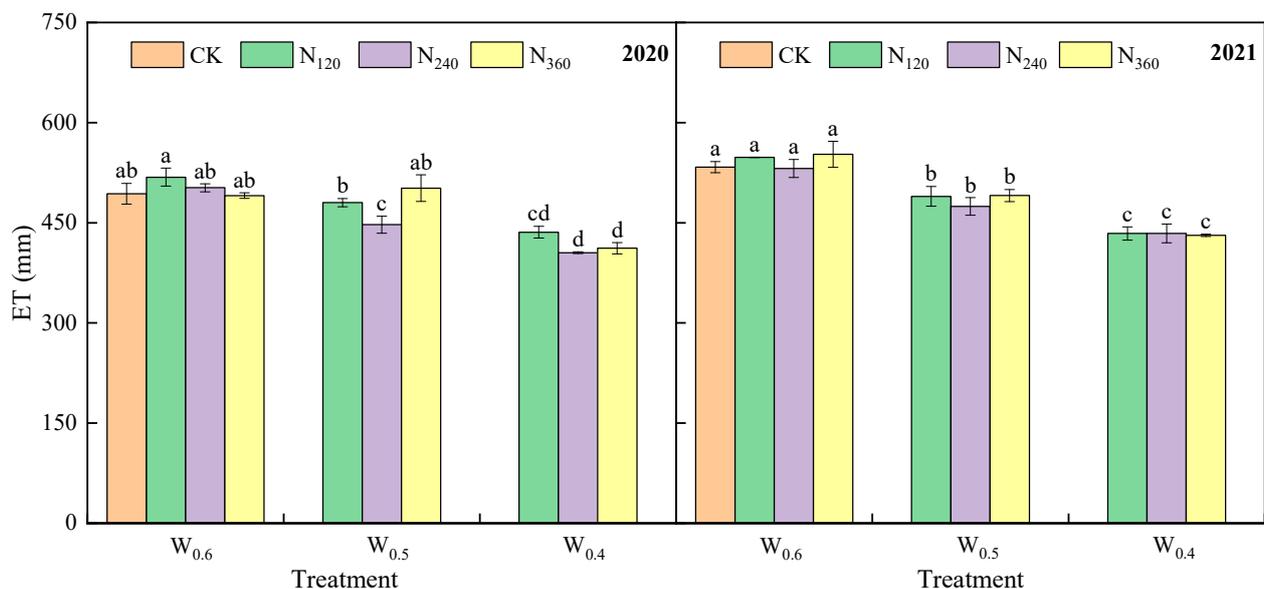
Treatment	Seed Cotton Yield (g·plant <sup>-1</sup> )		Lint Cotton Yield (g·plant <sup>-1</sup> )		Lint Percentage (%)	
	2020	2021	2020	2021	2020	2021
CK	60.92 $\pm$ 3.30 d	67.51 $\pm$ 3.87 fg	23.45 $\pm$ 1.27 e	28.66 $\pm$ 1.52 ef	38.50 $\pm$ 0.01 a	42.47 $\pm$ 0.18 a
W <sub>0.6</sub> N <sub>120</sub>	70.92 $\pm$ 0.72 cd	73.82 $\pm$ 0.12 efg	26.47 $\pm$ 0.94 de	30.85 $\pm$ 0.09 def	37.31 $\pm$ 1.02 a	41.80 $\pm$ 0.06 ab
W <sub>0.6</sub> N <sub>240</sub>	85.17 $\pm$ 3.37 ab	82.00 $\pm$ 6.85 cde	34.24 $\pm$ 1.95 ab	33.22 $\pm$ 2.16 bcd	40.15 $\pm$ 0.71 a	40.63 $\pm$ 0.76 abc
W <sub>0.6</sub> N <sub>360</sub>	96.67 $\pm$ 5.26 a	109.37 $\pm$ 1.67 a	38.32 $\pm$ 0.80 a	41.39 $\pm$ 0.19 a	39.80 $\pm$ 1.49 a	37.85 $\pm$ 0.40 d
W <sub>0.5</sub> N <sub>120</sub>	66.40 $\pm$ 6.70 cd	76.64 $\pm$ 2.78 def	26.26 $\pm$ 2.11 de	30.29 $\pm$ 1.02 def	39.74 $\pm$ 1.23 a	39.52 $\pm$ 0.11 cd
W <sub>0.5</sub> N <sub>240</sub>	77.63 $\pm$ 5.02 bc	78.69 $\pm$ 0.18 de	28.66 $\pm$ 2.14 cd	33.33 $\pm$ 0.58 bcd	36.89 $\pm$ 0.76 a	42.35 $\pm$ 0.83 a
W <sub>0.5</sub> N <sub>360</sub>	77.56 $\pm$ 3.55 bc	94.63 $\pm$ 2.65 b	31.51 $\pm$ 2.43 bc	37.55 $\pm$ 1.50 ab	40.52 $\pm$ 1.40 a	39.65 $\pm$ 0.47 bcd
W <sub>0.4</sub> N <sub>120</sub>	64.66 $\pm$ 2.27 d	67.07 $\pm$ 1.87 g	25.19 $\pm$ 1.27 de	27.39 $\pm$ 0.80 f	38.92 $\pm$ 0.58 a	40.83 $\pm$ 0.05 abc
W <sub>0.4</sub> N <sub>240</sub>	60.02 $\pm$ 4.05 d	85.13 $\pm$ 3.85 bcd	24.08 $\pm$ 0.40 e	32.77 $\pm$ 3.12 cde	40.51 $\pm$ 2.93 a	38.32 $\pm$ 1.93 d
W <sub>0.4</sub> N <sub>360</sub>	71.95 $\pm$ 4.10 cd	90.41 $\pm$ 2.60 bc	27.34 $\pm$ 0.54 cde	37.03 $\pm$ 1.34 abc	38.16 $\pm$ 1.43 a	40.94 $\pm$ 0.30 abc
Year	**		**		**	
Water	**		**		ns	
Nitrogen	**		**		ns	
Year $\times$ Water	*		*		ns	
Year $\times$ Nitrogen	ns		ns		ns	
Water $\times$ Nitrogen	*		ns		ns	
Year $\times$ Water $\times$ Nitrogen	*		ns		**	

Note: Different letters mean significant differences ( $p < 0.05$ ). \*, \*\*, and ns mean  $p < 0.05$ ,  $p < 0.01$ , and no significant difference, respectively. Note: CK means soil water content controlled at 60–70% of field capacity and no nitrogen fertilizer applied. W<sub>0.6</sub>, W<sub>0.5</sub>, and W<sub>0.4</sub> mean topsoil water condition at 60–70%, 50–60%, and 40–50% of field capacity, respectively. N<sub>120</sub>, N<sub>240</sub>, and N<sub>360</sub> mean nitrogen rates of 120 kg·N ha<sup>-1</sup>, 240 kg·N ha<sup>-1</sup>, and 360 kg N·ha<sup>-1</sup>, respectively.

The effects of year, topsoil water condition, N rate, and year  $\times$  water on lint cotton yield were significant (Table 4). In 2020, lint cotton yield tended to decrease as topsoil became drier except at  $N_{120}$ . In addition, under  $W_{0.6}$  and  $W_{0.5}$ , lint cotton yield increased with the increasing N rate, but under  $W_{0.4}$ , there was no significant difference among all N rates, and the lint cotton yield of all treatments was higher than that of CK. In 2021, lint cotton yield was less influenced by topsoil water conditions, but there was an obvious increasing trend in lint cotton yield as the N rate increased under all topsoil conditions. The highest lint cotton yield was obtained with  $W_{0.6}N_{360}$ , and the lowest was roughly obtained with CK or  $W_{0.4}N_{120}$ .

Lint percentage was not significantly affected by topsoil water conditions or N rate (Table 4). Cotton lint percentage in 2020 and 2021 ranged from 36.89% to 42.48%. When the  $W_{0.5}N_{240}$  or CK was applied, the lint percentage achieved the highest value in 2021. However, in 2020, there was no significant difference among treatments. It appeared that the differences in lint percentage among treatments had little effect on lint cotton yield.

The ET in the full life cycle of cotton in 2020 and 2021 were examined to analyze the effect of water and N regulation on cotton ET under dry topsoil conditions, as summarized in Figure 10. Overall, year, topsoil water condition, and N rate significantly affected cotton ET, whereas the interaction between topsoil water condition and N rate showed a nonsignificant effect on ET (Table 5). In 2020, at a given designed N rate, ET decreased with the increasing topsoil dryness, and under  $W_{0.6}$  and  $W_{0.4}$ , the differences among N rates in ET were not significant, whereas under  $W_{0.5}$ , ET at  $N_{240}$  was significantly lower than those at  $N_{120}$  and  $N_{360}$ . In 2021, as the topsoil became drier, the ET throughout the growth stages showed an obvious decreasing trend, but with the increase in the N rate, the differences in the ET of cotton were relatively small.



**Figure 10.** The total evapotranspiration (ET) of cotton in 2020 and 2021. Note: Different letters mean significant differences ( $p < 0.05$ ). CK means soil water content controlled at 60–70% of field capacity and no nitrogen fertilizer applied.  $W_{0.6}$ ,  $W_{0.5}$ , and  $W_{0.4}$  mean topsoil water condition at 60–70%, 50–60%, and 40–50% of field capacity, respectively.  $N_{120}$ ,  $N_{240}$ , and  $N_{360}$  mean nitrogen rates of 120 kg·N ha<sup>-1</sup>, 240 kg·N ha<sup>-1</sup>, and 360 kg·N ha<sup>-1</sup>, respectively.

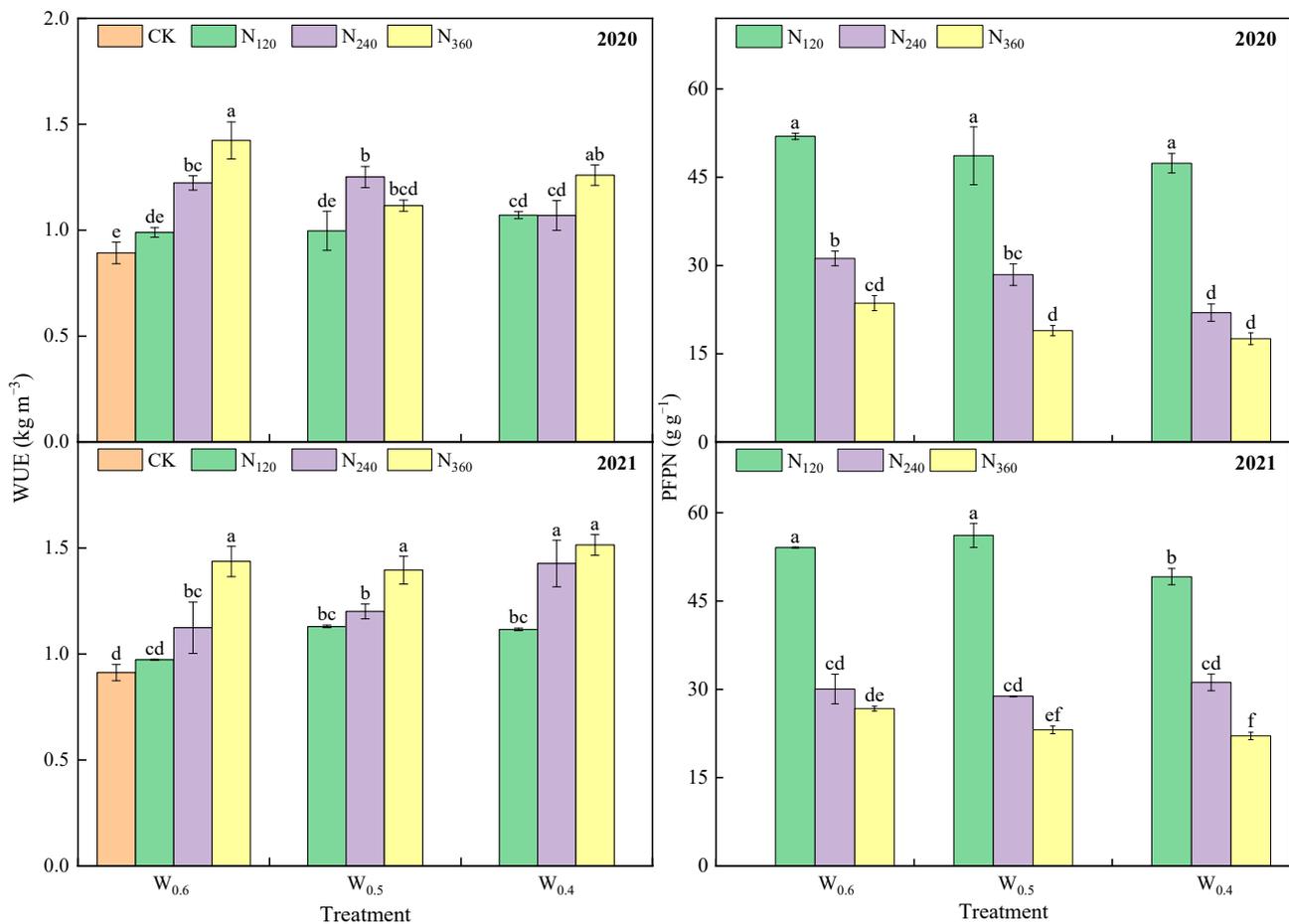
Since *WUE* and *PPFN* reflect the relationship between crop consumption of water and N fertilizer and yield, the *WUE* and *PPFN* for treatments in 2020 and 2021 were investigated and compared (see Figure 11). The effects of year, N rate, year  $\times$  water, water  $\times$  N rate, and year  $\times$  water  $\times$  N rate on *WUE* were significant (Table 5). When the  $W_{0.6}$  was applied, cotton *WUE* trended upward with more N rate in topsoil in 2020. This trend was obvious under any given designed topsoil water condition in 2021. The increase in N rate under

$W_{0.6}$  increased the *WUE* of cotton, which increased by 10.77%, 36.98%, and 59.45% in 2020 and increased by 6.59%, 23.08%, and 58.24% in 2021 at  $N_{120}$ ,  $N_{240}$ , and  $N_{360}$ , respectively, compared to CK.

**Table 5.** Significance levels (*p*-values) of the effects of topsoil water condition and N rate on the ET, *WUE*, and *PPFN*.

Treatment	ET	<i>WUE</i>	<i>PPFN</i>
Year	**	**	**
Water	**	ns	**
Nitrogen	*	**	**
Year × Water	ns	**	ns
Year × Nitrogen	ns	ns	ns
Water × Nitrogen	ns	*	ns
Year × Water × Nitrogen	ns	*	ns

Note: \*, \*\*, and ns mean  $p < 0.05$ ,  $p < 0.01$ , and no significant difference, respectively.



**Figure 11.** Water use efficiency (*WUE*) and nitrogen partial factor productivity (*PPFN*) of cotton in 2020 and 2021. Note: Different letters mean significant differences ( $p < 0.05$ ). CK means soil water content controlled at 60–70% of field capacity and no nitrogen fertilizer applied.  $W_{0.6}$ ,  $W_{0.5}$ , and  $W_{0.4}$  mean topsoil water condition at 60–70%, 50–60%, and 40–50% of field capacity, respectively.  $N_{120}$ ,  $N_{240}$ , and  $N_{360}$  mean nitrogen rates of 120 kg·N ha<sup>-1</sup>, 240 kg·N ha<sup>-1</sup>, and 360 kg N·ha<sup>-1</sup>, respectively.

Year, topsoil water condition, and N rate had significant effects on *PPFN* (Table 5). In terms of N rate, the *PPFN* data reported the exact opposite of the *WUE* data, i.e., a decreasing trend with an increasing N rate. In 2020, the effect of topsoil water conditions

on *PPFN* was not significant at N120 or N360, but at N240, the *PPFN* under  $W_{0.4}$  was significantly lower than those under  $W_{0.6}$  and  $W_{0.5}$ . However, in 2021, the effect of topsoil water condition on *PPFN* was not significant at N240, but at N120, the *PPFN* under  $W_{0.4}$  was significantly lower than those under  $W_{0.6}$  and  $W_{0.5}$ , and at N360, the *PPFN* under  $W_{0.4}$  was significantly lower than that under  $W_{0.6}$ .

#### 4. Discussion

In areas or periods of low precipitation, the topsoil layer would sometimes saturate (because of precipitation or irrigation) and always dry up (because of heat and wind) compared to the subsoil layer [7]. This study investigated the effects of water and N regulation on cotton growth and HL under dry topsoil conditions using the specially constructed split-root system.

Understanding the growth dynamics of cotton plants is essential for balancing water conservation and N reduction as well as root HL enhancement. The results showed that cotton plant height and leaf area increased with increasing N application under the same topsoil water condition, but relatively small differences existed among topsoil water conditions at the same N application rate except for leaf area in 2021 (Figures 3–6). These may be due to the fact that the N content of the topsoil increased with the increase in N rate, which promoted the growth and development of early root tips and N uptake and utilization by shallow roots [31], while the subsoil layer set up in this study was deeper than the topsoil layer, somewhat mitigating the effects of topsoil dryness on cotton. Particularly, the sharp increase in plant height at N<sub>360</sub> and the leaf area divergence across N rates under  $W_{0.5}$  or  $W_{0.4}$  appeared later, but the plant height and leaf area of N<sub>360</sub> finally became the highest. These may be related to the inhibited N uptake resulting from declined root productivity in topsoil from higher N application under severe water deficit conditions in the early stages [32], and the relief of restricted root systems and the improvement of topsoil water condition via HL when cotton roots rooted into moist subsoil at a later stage. The latter may also explain why the plant height and leaf area of  $W_{0.4}N_{360}$  were first lower than those of  $W_{0.6}N_{360}$  and  $W_{0.5}N_{360}$  and then gradually increased to nearly the same plant height and leaf area.

As a desirable process, HL would improve the water adsorption efficiency in deep roots, provide additional water to enhance plant transpiration rates, and increase the rate of N mineralization in topsoil [19,20]. HL is influenced by differences in water potential between root and soil systems, and any force driving differences in water potential, such as the depth and vertical distribution of roots, may alter HL [18,33]. In this study, as the root system entered the moist subsoil at progressively longer depths and greater densities, the amount of water lifted hydraulically by the deep roots became greater, promoting water release from shallow roots to the drier topsoil and growth of shallow roots. On the contrary, in the later stages of cotton growth, deep roots began to senesce and draw relatively less water from the subsoil, limiting water release and the viability of shallow roots [16]. In turn, gaps may form in the root–soil interface, further compromising water release. However, it was reasonable that the senescence of shallow roots and a decrease in new root growth could also reduce net outward flow [21].

Prior work has observed that root HL occurred at night when the water content of the center compartment was reduced to a certain level, and the drier the soil, the greater the amount of water lifted [15]. In contrast to the lateral water transfer from the root system, the present study investigated the vertical water transfer and obtained the same conclusion. In addition, it was shown that the more N applied, the greater the HL water amount, which was in general agreement with the study of Shen and Li [34]. Briefly, both mentioned results were related to the larger water potential difference between the topsoil and the cotton shallow root system. Additionally, the morphological characteristics of the root system as affected by water conditions and N rate can also affect HL. It was reported that micro-meteorological conditions such as air temperature, relative humidity, and the vapor pressure deficit also influenced HL [20]. The combined effects of water and N treatments,

dynamics of root development, and micro-meteorological factors are complex, which may have contributed to some fluctuations in the observed results (Figures 7 and 8). Although the later monitoring of HL and the neglect of nighttime evapotranspiration may have resulted in the underestimated amount of HL water throughout the test period relative to actual values, the rough estimate indicated that topsoil water condition and N rate both had significant effects on total HL water amount, suggesting that potential for cotton root HL can be regulated by optimizing water and N supply. Further, control of the relative depths of the dry topsoil layer and the moist subsoil layer may also have an impact on the amount of HL water. This needs to be explored in the future.

In this study, the significantly lower cotton yields in 2020 than in 2021 should be related to the delay in sowing and transplanting cotton because of the coronavirus pandemic. For treatments, our current findings that seed cotton yield and lint cotton yield tended to decrease with increasing dryness and increase with higher N rate were compatible with the common sense that cotton yields are strongly influenced by water and N availability, and yields can increase with increasing water and N supply within a certain range [35]. Significantly, the seed and lint cotton yield of  $W_{0.6}N_{360}$  in 2020 and 2021 were significantly higher than that of  $W_{0.5}N_{360}$  or  $W_{0.4}N_{360}$ , but the differences between  $W_{0.5}N_{360}$  and  $W_{0.4}N_{360}$  were not significant except for lint cotton yield in 2021. These insignificant differences between  $W_{0.5}N_{360}$  and  $W_{0.4}N_{360}$  can be partly attributed to the promotion of cotton growth and yield formation by the relatively large amount of HL water of  $W_{0.4}N_{360}$  and partly to the compensation of the negative effect of dryness by a deeper and moist subsoil layer. After all, an increased HL water amount may mean more effective replenishment of topsoil water.

The ET throughout all growth stages showed a clear decreasing trend with increasing dryness in topsoil and small differences among N rates. The *WUE* and *PFPN* data obtained under different irrigation and N levels could be used to guide irrigation and fertilization management. It was shown that the *WUE* of cotton increased with more severe dryness or more N rate in topsoil, which was supported by various reports [36–40], while *PFPN* did the opposite. Our measured *WUE* was relatively high in comparison with these findings, which may be related to the less soil water evaporation from the dry topsoil layer, the facilitation of HL by the moist subsoil layer, and the promoted transfer of water to crop water use (more transpiration) [41] achieved by water storage in the topsoil layer [42]. Rational N application would be beneficial to the environment, and improving N use efficiency is important [43]. The decrease in *PFPN* with increasing N rate or dryness in topsoil was easy to understand when combined with the definition of *PFPN* and the response of seed cotton yield to water and N treatments. It was noted that although the *PFPN* was much lower at  $N_{240}$  and  $N_{360}$  compared to at  $N_{120}$  (Figure 11), the  $N_{240}$  and  $N_{360}$  were a great help for the HL of cotton roots on the whole, especially the  $N_{360}$  (Figures 7–9). Therefore, a balance should be struck between the contribution of increased N fertilization to HL enhancement and the resulting reduction in *PFPN*, while the economic and environmental impacts of N fertilizer losses should also be considered [44].

In addition, it should be noted that this experiment was conducted in a plastic greenhouse, which may be different from the field environment. Another limitation, given some differences in plant management practices between the two years in this study, is that this study was only a two-year continuous experiment. It is necessary to continue the experiment for several more years.

## 5. Conclusions

Cotton plant height and leaf area increased with increasing N rate, but the differences among topsoil water conditions were relatively small, except for leaf area in 2021. The sharp increase in plant height at  $N_{360}$  and the leaf area divergence across N rates under  $W_{0.5}$  or  $W_{0.4}$  appeared later. During the observation period, the HL water amount of all treatments increased gradually and then continued to decline. There was a trend that the drier the topsoil or, the more N applied, the greater the amount of HL water, and the topsoil water condition and N rate both had significant effects on the total HL water amount and

root morphological characteristics (root length, root surface area, and root volume). Seed cotton yield and lint cotton yield tended to decrease with increasing dryness at N<sub>240</sub> or N<sub>360</sub>, except for lint yield in 2021 and increased with higher N rates, especially under W<sub>0.6</sub>. Lint percentage was not significantly affected by topsoil water condition or N rate. As the topsoil became drier, the ET throughout the whole growth stages showed a decreasing trend, while with the increase in N rate, ET showed small differences. The WUE of cotton increased with more N rate in topsoil, while PFPN did the opposite. Moreover, the PFPN under W<sub>0.4</sub> was significantly lower than that under W<sub>0.6</sub> at N<sub>240</sub> or N<sub>120</sub>.

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