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Photosynthetic characteristics of cotton are enhanced by altering the timing of mulch film removal



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Abstract

Background: The photosynthetic parameters of cotton plants may be modified by the timing of film removal during their growing period. This study was undertaken during 2015–2017 in Xinjiang, China, to determine to what extent the film mulching removal time, 1 and 10 days before the first irrigation and 1 day before the second irrigation after seedling emergence, influenced cotton's photosynthetic characteristics. The control group (CK) was film-mulched throughout the growth stages.

Results: The results suggested the following: (1) Removing mulching-film within 50 days since seedling emergence had adverse effects on soil temperature and moisture. (2) Film-removal before the first or second irrigation after emergence improved the net photosynthetic rate in cotton's later flowering stage and its transpiration rate in mid and later flowering stages while enhancing the actual electron transport rate (ETR) and maximum electron transfer rate (ETR_{max}) between cotton photosystems I and II. (3) Film-removal treatment also increased cotton plants' tolerance to high irradiation after emergence, the trend was more pronounced in the early flowering stage in wetter years. (4) Leaf area index (LAI) of cotton was reduced in the film-removal treatment for which the least accumulation of dry matter occurred in a drought year (i.e., 2015). (5) Film removal caused a yield decrease in the dry year (2015), and the earlier the film was removed, the more seriously the yield decreased. Removing mulching film before the second irrigation could increase the yield of XLZ42 in the rainy year (2016) and the normal rainfall year (2017). Early film removal can increase the yield of XLZ45 in the rainy year (2016).

Conclusions: Collectively, our study's experimental results indicate that applying mulch film removal at an appropriate, targeted time after seedling emergence had no adverse effects on soil moisture and temperature, and improved the photosynthetic performance of cotton, thus increased cotton yield and fiber quality, but no significant difference was reached.

Keywords: Chlorophyll fluorescence, Gas exchange parameters, Lint yield, Removing mulch film, Soil temperature and moisture content

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Introduction

Xinjiang is a domestically and globally important cotton-growing region, where, since the early 1990s, the drip irrigation technique under mulch film (Hu and Li 2003) has been extensively used because it considerably increases cotton production there (Jian et al. 2007; Rao et al. 2016). The cotton-growing industry has expanded greatly: in 2014 the cotton acreage in Xinjiang (2.42×10^6 hm²) was six times its acreage in 1990 (Statistic Bureau of Xinjiang Uygur Autonomous Region, 1990–2014). The cotton yield of Xinjiang now accounts for > 87% of China's total cotton production and film mulching is now used in 85% of Xinjiang's cotton fields (Bai et al. 2015).

However, the continuous and widespread application of mulch has led to the problem of plastic film residues, which has reduced cotton production (Li 2016) and damaged farmland ecosystems (Dong et al. 2013; Nkwachukwu et al. 2013; Thompson et al. 2009; Adhikari et al. 2016). The abatement of residual film pollution is now an urgent issue impacting agricultural production in not only Xinjiang but also other arid and semi-arid regions. Yet it is difficult to convince cotton-growers to accept using a degradable mulch film as a substitute for common polyethylene film since it is currently more expensive. Instead, mechanically recycling the plastic film has become a common practice to reduce film residues. More specifically, removing the film only during the key growth stages of cotton lets the film increase soil temperature and conserve soil moisture before its removal time, thus facilitating film recycling while preserving the film's mechanical strength. This is an effective approach to reduce the pollution caused by such film residues.

Previous researches (Li et al. 2010; Su et al. 2011a, b; Xie et al. 2012; Zhang et al. 2016a, b) have mainly focused on how the removal of the cotton field's film layer changed soil temperature and cotton yield. Only a few studies have considered the effects of film-removal on the gas exchange characteristics of crop plants, such as maize (He et al. 1999; Yu et al. 2006; Zhang et al. 2016a) and tobacco (Wang et al. 2010; Yang et al. 2010). Surprisingly, such studies on leaf gas exchange and chlorophyll fluorescence parameters are generally scarce for cotton crops grown.

Photosynthesis is a vital physiological process that is sensitive to water conditions. Besides being affected by stomatal factors, this process is also affected by non-stomatal factors of leaves, such as their chlorophyll content and chloroplast functioning (Zhao et al. 2007). Water availability (Wang et al. 2006) and temperature (Andersson and Nilsson 2001; Nabi and Mullins 2008; Stone et al. 1999) are the top two crucial factors affecting crop yield in Xinjiang, which is best described as a desert-oasis agriculture region. Therefore, it is crucial to find out what effect the mulch removal has on soil temperature, moisture

content, and evaporation in the cotton field, and how cotton photosynthesis changes under this environment, to ensure Xinjiang cotton production.

Studying the photosynthetic characteristics of cotton plant populations at different film removal times can provide valuable knowledge to guide the best practices in cotton production. Here, our study objective was: (1) to investigate the variation in cotton leaf gas exchange and chlorophyll fluorescence parameters of cotton populations across key plant growth stages, and (2) to examine the influence of film removal times on cotton growth, as well as lint cotton yield and its fiber quality; and (3) to provide a scientific basis for reducing residual film pollution in cotton production areas.

Material and methods

Study area and design

The experimental research area was located in Shihezi, Xinjiang, China (44.3108°N, 85.986°E; elevation: 460 m). We established and replicated the field experiment yearly over 3 years (2015–2017) by using a split-plot experimental design with three replicates per treatment. The main plot level was two varieties of *Gossypium hirsutum* L. with different water sensitivity (XLZ 42 and XLZ 45), and the subplot level was treated with different film removal times. Three treatments of mulch film-removal time were applied: 10 days (T10) and 1 day (T1) before the first irrigation and 1 day (E1) before the second irrigation after seedling emergence, with one control group of film mulching present across growth stages (CK). Four subplots were randomly arranged in every main plot and replicated three times, amounting to 24 subplots in this experiment. Each subplot is 20 m long, 4.2 m wide and covers an area of 84 m². There were two plastic films, and 12 rows of cotton were planted in each subplot. The plant spacing of cotton was 10 cm and there were 2 400 cotton plants in each plot. The two varieties were arranged interspecifically in the field, such that, XLZ42 was on one film, but XLZ45 on the next one, with this alternation continued. No buffer space was used between adjacent subplots.

Cotton seeds were purchased at the local market. In 2015, these seeds were sown by cotton planters on 24 April; they germinated on 6 May and plants were harvested on 10 September. In 2016, both cotton varieties were likewise sown on 5 May, germinated on 16 May, and harvested on 26 September. In 2017, they were sown on 21 April, germinated on 28 April, and harvested on 6 September. The length of the seasonal cotton-growing period in 2015, 2016, and 2017 was 127, 134, and 131 days, respectively. Mulch film was removed manually at 19 (T10, May 25), 29 (T1, June 4), and 39 (E1, June 14) days after emergence in 2015; at 24 (T10, June 9), 34

(T1 June 19), 44 (E1, June 29) days after emergence in 2016; at 33 (T10, May 31), 43 (T1, June 10), 53 (E1, June 20) days after emergence in 2017.

The average air temperature, $\geq 10^\circ\text{C}$ active accumulated temperature, and precipitation from May to September, was 22.86°C , $3\ 014^\circ\text{C}$, and $94\ \text{mm}$ in 2015; 22.58°C , $3\ 165^\circ\text{C}$, $120.2\ \text{mm}$ in 2016; and 22.45°C , $3\ 413^\circ\text{C}$, and $96.5\ \text{mm}$ in 2017 (the meteorological data were obtained from the Shihezi Weather Bureau). The basic physical and chemical properties of soil in the experimental area, and the latter's cropping pattern and field management practices could refer to Yang *et al.* (2017).

Sample collection and determination

Soil temperature measurement and calculation

After the cotton sowing, MicroLite USB Loggers (Fourier Technologies Ltd. Rosh, Haayin, Israel) were buried in the middle of each wide and narrow row of XLZ42 in the four treatments at depths of 10 cm, 20 cm, and 30 cm (Fig. 1). So, in all, 24 data loggers were thus buried. Data was collected hourly; the daily average temperature was the mean value of 24 recorded values per day.

Accumulated soil temperature of different periods, similar to the accumulated air temperature, is the sum of daily average soil temperatures of different soil layers during different periods. Average soil temperature is the mean value of accumulated soil temperature during a given period. The daily soil temperature difference is the difference between the maximum and minimum daily temperatures. The accumulated soil temperature difference is the summed daily soil temperature difference during a given period. The average soil temperature difference is the mean value of accumulated soil temperature difference for a given period. For calculation methods refer to Chen (2005).

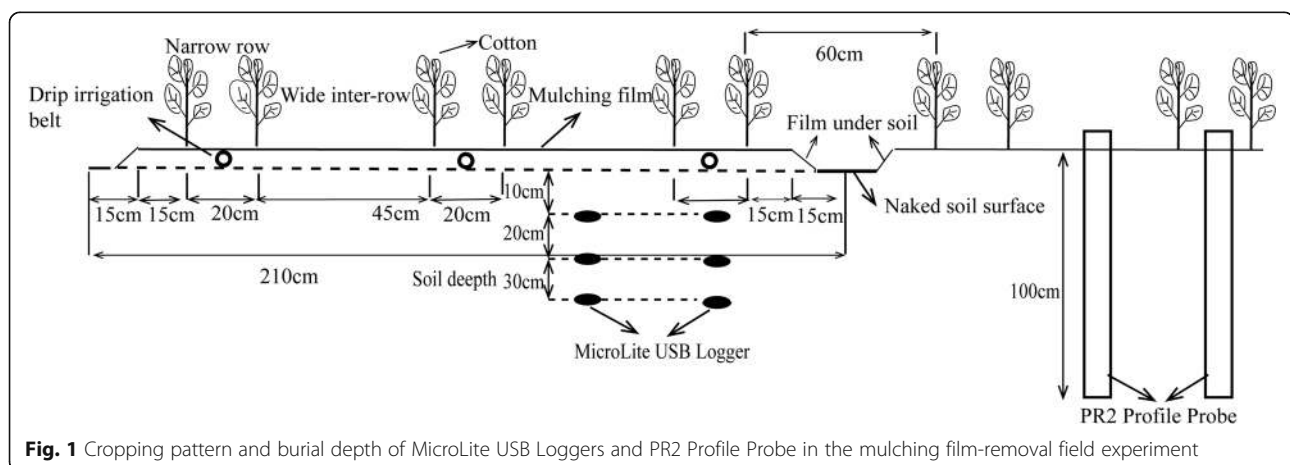
Measurement and calculation of soil moisture content

One week before the removal of film in 2017, a PR2 Profile Probe (Delta-T Devices Ltd., Burwell, Cambridge, UK) was buried in the middle of each of the wide and narrow rows of XLZ42 in the four treatments (Fig. 1); hence a total of eight probes were buried. During the 33–55 days since seedling emergence, the volumetric moisture contents of the 0–10, 10–20, 20–30, 30–40, 40–60, and 60–100 cm soil layers were monitored daily. After 55 days post-emergence these measurements were taken once every 4 days, plus an extra additional measurement made before and after each irrigation event.

Gas-exchange parameters of cotton

The Li-6 400 XT portable photosynthesis system (Li-Cor Inc., Lincoln, USA) was used to monitor the gas exchange parameters of cotton (XLZ42 and XLZ45) leaves at 5, 15, 25, 35, and 45 days after flowering. The standard leaf chamber ($2\ \text{cm} \times 3\ \text{cm}$) was used, and three plants were measured in this way per treatment combination (variety \times removal time; the same for below). Each treatment combination thus had replicated three times. In each case, the sampling time of measurement was between 12:00 and 14:00, when the it was clear and cloudless. The second leaf from top to bottom on the main stem was measured per plant. To reduce the error and ensure the consistency of these in situ measurements, we applied the method of Zhan (2014); briefly, approximately 100 leaves from 100 plants with uniform growth were first marked, with the same leaves measured each time.

The measured leaf parameters were as follows: Pn (photosynthetic rate, $\mu\text{mol}\ \text{CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), Trmmol (transpiration rate, $\text{mmol}\ \text{H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), Cond (conductance to H_2O , $\text{mmol}\ \text{H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), Ci (intercellular CO_2 concentration, $\mu\text{mol}\cdot\text{mol}^{-1}$), PAR (photosynthetic active



radiation, $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) and Ca (atmospheric CO_2 concentration, $\mu\text{mol} \cdot \text{mol}^{-1}$).

The stoma limit value (Ls) was calculated as $Ls = 1 - (Ci/Ca)$ (Berry and Downton 1982). Water use efficiency (WUE) and intrinsic WUE (WUEi) were calculated according to the equations of $WUE = Pn / Trmmol$ and $WUEi = Pn / Cond$ (Peñuelas *et al.* 1998). Light use efficiency (LUE) was calculated as $LUE = (Pn / PAR) \times 100\%$ (Li *et al.* 2014a, b).

Chlorophyll fluorescence parameters of cotton leaf

A PAM-2500 portable modulated chlorophyll fluorometer (Heinz Walz GmbH, Eichenring, Effeltrich, Germany) was used to quantify the chlorophyll fluorescence parameters of the leaf in same position of the sampled cotton plants. For each treatment combination three replicate plants were used. Each treatment combination thus had three replicates. Their measuring time was between 8:00 and 12:00 on the same day the gas exchange parameters were measured, with a 2030-B leaf clip used. Before their determination, the leaf was fully dark-adapted for about 30 min. We then set the measuring light intensity to $102 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and the actinic light intensity to $713 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. The time interval between the first saturating pulse and open actinic light was 40 s, with a 20-s time interval between each saturating pulse after turning on the actinic light (width of 310 S). The saturating pulse intensity in the quenching analysis was $17\,250 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Then, we transferred to the “Slow Kinetics Window” and started its automatic program to determine the slow-induction parameters. The following parameters were considered: F0 (original fluorescence), Fm (maximal fluorescence), F' (fluorescence at any time), Fm' (maximal Fluorescence at light adaptation), and F0' (minimal fluorescence at light adaptation). The remaining fluorescence parameters were calculated according to established methods: Fv/Fm was used to express the maximum photochemical quantum yield of photochemical system II (PS-II) (Kitajima and Butler 1975), Y (II) is the actual photochemical quantum yield of PS-II (Genty *et al.* 1989), qL is the coefficient of photochemical fluorescence quenching, assuming interconnected PS II antennae and lake model (Kramer *et al.* 2004), NPQ is the Stern-Volmer type non-photochemical fluorescence quenching (Bilger and Björkman 1990), Y (NO) is the quantum yield of non-light-induced non-photochemical fluorescence quenching (Kramer *et al.* 2004), and Y (NPQ) expressed the quantum yield of light-induced (i.e., ΔpH and zeaxanthin-dependent) non-photochemical fluorescence quenching (Kramer *et al.* 2004).

Fitting the light curve

To do this, we determined the relative electron transfer rate (rETR, $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) under different intensities of PAR (9, 65, 111, 205, 352, 570, 722, 921, 1 298, 1 796, and 2 139 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). Each level of PAR lasted 20 s, with three replicates used per treatment combination. We used Pamwin-3 (the operating software of the PAM-2500 device) to fit curves to this collected data. The fitting formula used was $ETR = PAR / (a \cdot PAR^2 + b \cdot PAR + c)$ (Eilers and Peeters 1988).

Fitting parameters consisted of an initial slope of the fast light curve (α , electrons photons⁻¹, conveying the efficiency of light energy utilization), the minimum saturating irradiance (I_k , in $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, corresponding to plant tolerance of intense light), and the maximum electron transfer rate (ETRmax, in $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). The calculation formulas for each parameter were $\alpha = 1/c$; $ETR_{\text{max}} = 1 / (b + 2 \cdot \sqrt{a \cdot c})$; $I_k = c / (b + 2 \cdot \sqrt{a \cdot c})$.

Dry matter accumulation, canopy structure, lint yield and fiber quality characters of cotton

Cotton plants—their shoots and roots in the 0–30 cm soil layer—were sampled in each subplot every 14 days from day 33 (2017), day 21 (2016) and day 35 (2015) since seedling emergence. The details of this sample collection and determination could refer to Yang *et al.* (2017). Further, an LAI-2200C plant canopy analyzer (Li-Cor Inc., Lincoln, USA) was used to measure and determine the leaf area index (LAI) of cotton, following Malone *et al.* (2002). LAI values were measured once between two wide rows and three narrow rows in each treatment, and the average value of these five values was used as the LAI values in this treatment. Yield and fiber quality characters of harvested cotton were measured according to Yang *et al.* (2017).

Data analysis

Data processing and figure drawing were performed with Microsoft Excel 2010 (Microsoft Corporation, Redmond, USA) and SigmaPlot 12.5 (Systat Software Inc., San Jose, USA), respectively.

The MANOVA (multi-factor analysis of variance) was carried out with univariate GLM (general linear model). The number of days after flowering ($df = 4$), film-removal time ($df = 3$), and cotton variety ($df = 1$) were used as fixed factors, and the different photosynthetic characteristics were used as the dependent variables, respectively, in each GLM. The fixed factors had significant effects on cotton's photosynthetic characteristics was examined by conducting multiple comparisons using LSD (least significant difference) tests at an alpha level = 0.05. Associations between net photosynthetic rate (Pn) and other gas exchange parameters in various treatment groups were investigated with Pearson

correlations ($n = 30$) in a two-tailed test (*, $P < 0.05$, **, $P < 0.01$). These analyses were carried out in SPSS 23.0 statistical software (International Business Machines Corp, Armonk, USA).

Dry matter accumulation of cotton was modeled using a logistic equation: $Y = K/(1 + \text{EXP}[a + bt])$. The method developed by Ming (2006) was used to calculate the following parameters: R_{max} represents the largest dry matter accumulation rate at T_{max} , which is the time at which cotton dry matter accumulation rate has reached its maximum; the weight of dry matter at T_{max} is given by W_{m} . The time point when rectilinear accumulation starts is recorded as t_1 and when it ends accumulation ends as t_2 ; hence $\Delta W_{t_2-t_1}$ represents dry matter accumulated from t_1 to t_2 . This analysis was performed in DPS 16.05 (Tang and Zhang 2012) using the Marquardt method.

Results

Soil temperature and moisture among film-removal treatments

Within 1 to 50 days after seedling emergence, the soil temperatures of all soil layers in the film-removal treatments were lower than those of CK. After the 50-day mark, however, the gap gradually narrowed until it reversed, becoming higher than under CK (Fig. 2). Soil average temperature, accumulated temperature, and mean temperature difference (Table 1) in the 0–30 cm soil layer during the entire growth period were highest in CK in 2015 (23.19 °C, 2 533 °C, and 5.94 °C, respectively) and T10 treatment in 2016 (22.93 °C, 2 583 °C, and 2.98 °C, respectively). In each experimental year, the accumulated temperature difference (Table 1) was the highest in CK, at 648 °C (2015) and 145 °C (2016).

From 37 days post-emergence to the first irrigation (i.e., 42 days since emergence), soil moisture content (V/V ; the same blow) in the 0–10 cm soil layer was highest in CK, while for the 10–20 cm soil layer it was the highest in the T10 treatment. In deeper soil (40–100 cm soil layer), the soil moisture content of CK was the highest among treatments. From the first to second irrigation (43–52 days after emergence), soil moisture content under CK in soil 0–30 cm deep increased slightly, but differences between treatments were not obvious. The rank order of moisture content in the 30–100 cm soil layer was thus $T1 > CK > T10$, and the deeper the soil layer, the greater the gap in moisture content found between treatments (Fig. 3).

After the second irrigation (53 days after emergence), the soil moisture content of CK was the greatest in the 0–60 cm soil layer, while that under the T1 treatment was the highest for the 60–100 cm layer. From the 20-cm depth mark and downward, T10 consistently had the lowest moisture content (Fig. 3).

Gas exchange parameters of film-removal treatments at different days since flowering

The net photosynthetic rate (P_n , Fig. 4a) of film-removal treatments exceeded that of CK at 45 days after flowering, while at the early flowering stage there were no significant differences among the treatments. Nonetheless, the film-removal treatment early in the flowering of XLZ45 in 2017 had a slightly lower P_n than that under CK, whereas other film-removal treatments had P_n values slightly greater than CK's.

At 5 days after flowering in 2017, relative to CK, the P_n (Fig. 4a), Cond (Fig. 4b) and Ci (Fig. 4c) values of XLZ45 under all film-removal treatments were lower but their L_s value was much higher (Fig. 4d). This indicated that the decreased photosynthetic rates of cotton plants in those film-removal treatments were driven by reductions in stomatal conductance.

However, at 45 days after flowering, the P_n (Fig. 4a), Cond (Fig. 4b) in 2016/2017 and Ci (Fig. 4c) in 2016 of CK were lower than those recorded in the T1 and T10 treatments, while the L_s value (Fig. 4d) of CK in 2016 was higher than those of T1 and T10 treatments; hence, this indicated that in the years with more rainfall (i.e., 2016), the net photosynthetic rate declined at later growth stages because of lower stomatal conductance. But in 2017, when rainfall was normal, the decreased net photosynthetic rate of CK was mainly caused by non-stomatal factors.

The MANOVA (Table S1) showed that different flowering days had a significant impact on P_n , Cond , Ci , and L_s , while treatments differed in their significant impacts on P_n (2016/2017), Cond (2016/2017), and Ci (2016), whereas the cotton variety growth had a significant impact only on P_n (2016/2017) and Cond (2016).

According to the correlation coefficients of P_n with other gas exchange parameters of different treatments in 2016 and 2017 (Table 2), the association between P_n and Cond or LUE of each treatment in 2016 was stronger than that in 2017, whereas P_n and WUE were more strongly associated in 2017 than those in 2016. This indicated that cotton's photosynthetic rate was mainly affected by light energy utilization rate and stomatal factors in the year (2016) with heavy rainfall, yet in a normal rainfall year (2017) soil water status has a greater influence on photosynthesis.

Chlorophyll fluorescence parameters of cotton leaves among film-removal treatments at different days since flowering

Influence of mulch film removal on the maximum photochemical quantum yield of PS-II (Fv/Fm) and actual photochemical quantum yield of PS-II (Y (II))

In the early stage of flowering (i.e., 5 days post-flowering), F_v/F_m of CK was the highest. As the cotton

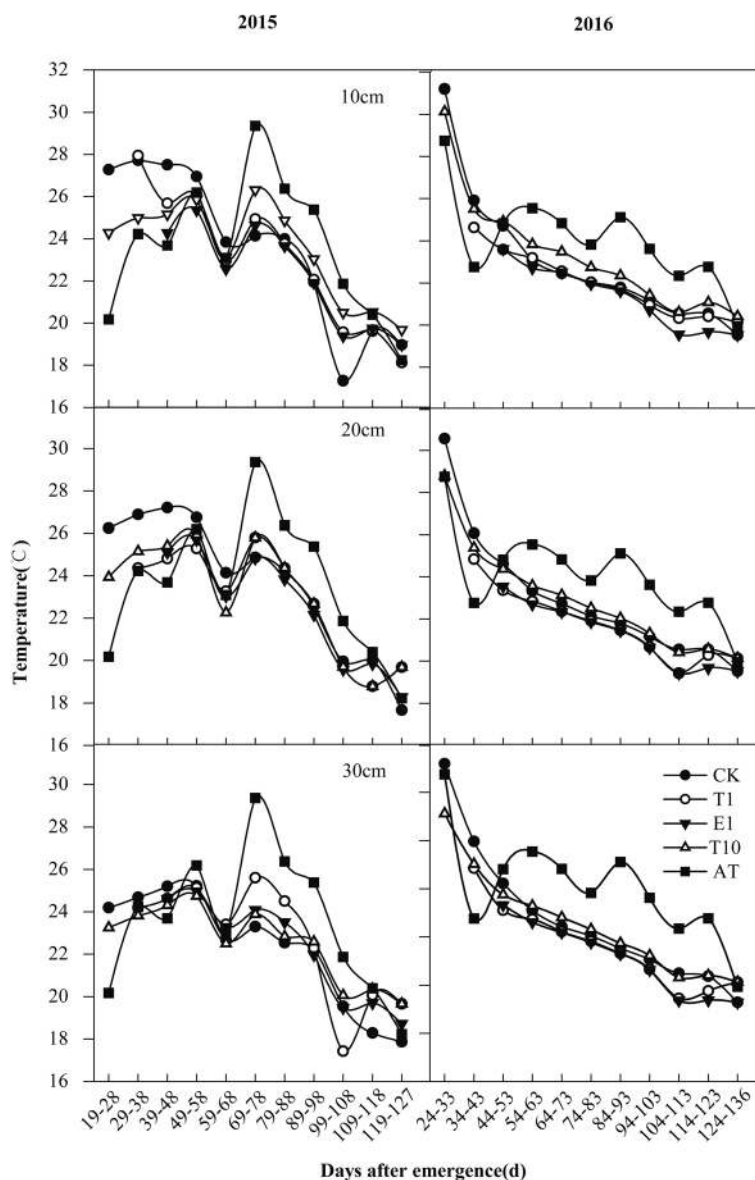


Fig. 2 Daily average soil temperature (°C) variation in various treatment groups: 1 and 10 days before the first irrigation (T1, T10, respectively) and 1 day before the second irrigation (E1) after seedling emergence, with one control group of film mulching present across cotton plant growth stages (CK) and soil depth layers (10, 20, and 30 cm) across growth stages during 2015–2016. AT is air temperature. A and D represent the removal of mulch 10 days before the first irrigation on May 25, 2015 and June 9, 2016, respectively. B and E represent the removal of mulching film on June 4, 2015 and June 19, 2016, respectively, one day before the first irrigation. C and F represent the removal of mulching film on June 14, 2015 and June 29, 2016, respectively, one day before the second irrigation

grew, the gap in Fv/Fm values between the CK and film-removal treatments gradually narrowed, with Fv/Fm under the film-removal treatments eventually surpassing that of CK. However, at 45 days post-flowering, except for Fv/Fm of XLZ42 plants in 2017 being significantly higher than CK, the Fv/Fm values for the film-removal treatment of different cotton varieties were all lower than CK's (Fig. 5). The MANOVA showed that only the number of days since flowering

in either year had a significant impact on Fv/Fm, whereas it was similar among film-removal treatments (Table S1).

In 2017, removing the film mulching reduced the Y (II) value at the early flowering stage, then increased significantly, especially in the late growth stages; the Y (II) value increased obviously when film was removed earliest (T10). However, in the heavy rainfall year (2016), removing the film significantly increase Y (II) at the early

Table 1 Soil average temperature, accumulated temperature, average temperature difference, and accumulated temperature difference in various treatment groups: 1 and 10 days before the first irrigation (T1, T10, respectively) and 1 day before the second irrigation (E1) after seedling emergence, with one control group of film mulching present across growth stages (CK) for three soil depth layers (10, 20, and 30 cm) during 2015–2016

Item	Year	Soil layer depth /cm	CK	T1	E1	T10	Air Temperature /°C
Average temperature /°C	2015	10	23.56	23.08	22.27	23.48	23.54
		20	23.68	22.87	22.49	23.06	
		30	22.34	22.69	22.26	22.54	
	2016	10	22.99	21.95	21.32	23.31	24.03
		20	23.00	21.72	21.24	22.93	
		30	22.70	21.60	21.09	22.55	
Accumulated temperature /°C	2015	10	2 572.90	2 562.80	2 535.62	2 563.53	2 571.68
		20	2 587.03	2 529.05	2 536.32	2 517.27	
		30	2 439.18	2 491.04	2 472.97	2 459.96	
	2016	10	2 587.76	2 570.10	2 547.73	2 625.77	2 703.08
		20	2 588.33	2 536.72	2 532.49	2 582.41	
		30	2 554.44	2 509.85	2 501.04	2 541.30	
Average temperature difference /°C	2015	10	9.79	6.46	6.18	8.38	13.34
		20	5.58	4.59	4.22	5.28	
		30	2.45	2.30	2.22	2.45	
	2016	10	3.76	2.83	2.94	5.78	13.33
		20	2.85	1.56	1.74	2.33	
		30	1.28	0.84	0.81	0.85	
Accumulated temperature difference /°C	2015	10	1 068.56	749.50	824.28	912.97	1 452.60
		20	609.13	524.83	538.85	571.94	
		30	265.95	265.55	271.44	265.98	
	2016	10	419.60	378.80	430.62	655.84	1 514.70
		20	319.50	217.42	264.24	258.89	
		30	144.81	114.12	124.48	96.89	

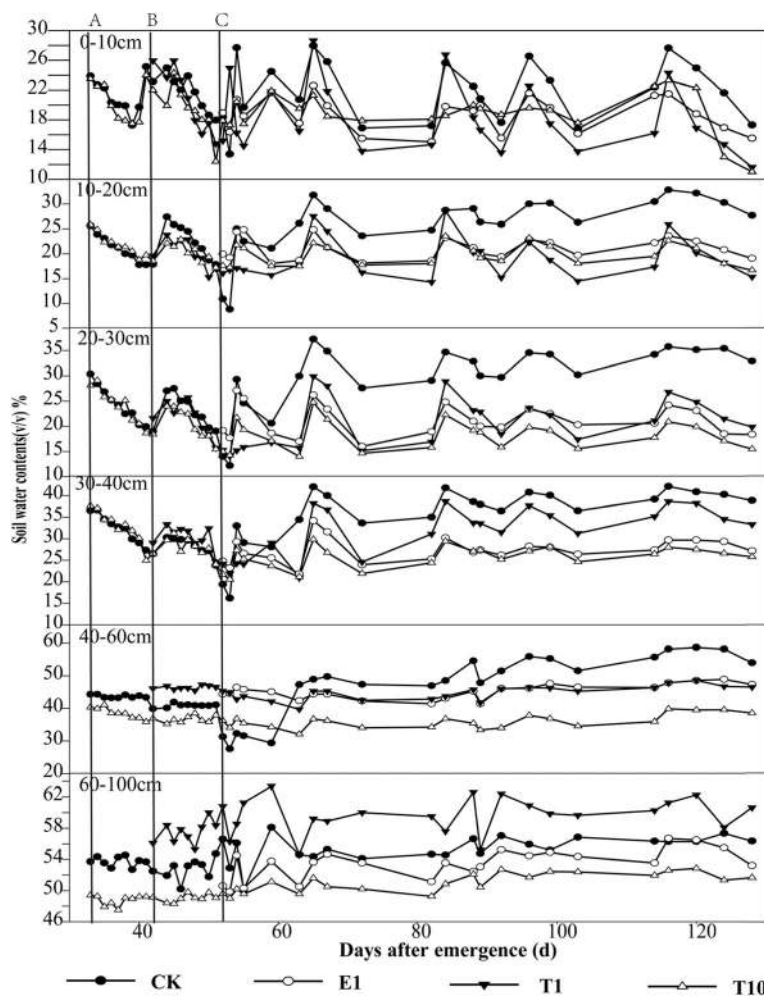


Fig. 3 Soil volume moisture content variation of different soil layer at 33–128 days after emergence in 2017 in various treatment groups: 1 and 10 days before the first irrigation (T1, T10, respectively) and 1 day before the second irrigation (E1) after seedling emergence, with one control group of film mulching present across cotton plant growth stages (CK). A, B, and C represent removal of mulch on May 31, June 10, and June 20, 2017, i.e., 10 days, 1 day before the first irrigation, and one day before the second irrigation, respectively

flowering stages. As cotton reproduction proceeded, the gap in Y (II) values between the film-removal treatments and CK gradually narrowed (Fig. 5). In sum, in 2017, both the number of days after flowering and the timing of mulch removal significantly impacted Y (II). In 2016, only the latter had a significant impact on Y (II).

Influence of mulch film removal on photochemical fluorescence quenching assuming interconnected PS-II antennae (qL) and stern-Volmer type non-photochemical fluorescence quenching (NPQ)

The qL (Fig. 6) value represents the level of electron transfer activity. In the year with more rainfall (2016), removing the plastic film significantly enhanced the qL of PS-II in the early stage of flowering (5 days post-flowering), but earlier film-removal (T1 and T10 treatments) weakened electron transfer activity of PS-II in

the later growth stage. The qL under the E1 treatment was strongest during the whole cotton growth period. In the normal rainfall year (2017), removal of the film promoted electron transfer activity of PS-II of XLZ42 earlier in the flowering (i.e., 1–15 days post-flowering) but that of XLZ45 in mid- and later stages of flowering (i.e., 25–45 days post-flowering). At 45 days after flowering, qL of PS-II in the T10 treatment was the highest.

In the late flowering stage (45 days post-flowering), among treatments, the NPQ (Fig. 6) of T10 was the lowest. However, in the early flowering stage, the NPQ of all three film-removal treatments exceeded that of CK in 2017, while the opposite occurred in 2016.

Different treatments, cotton varieties, and days after flowering had no significant influence on NPQ in 2016. Yet different treatments significantly affected qL, in that there were significant differences between E1 or T10 and CK. In

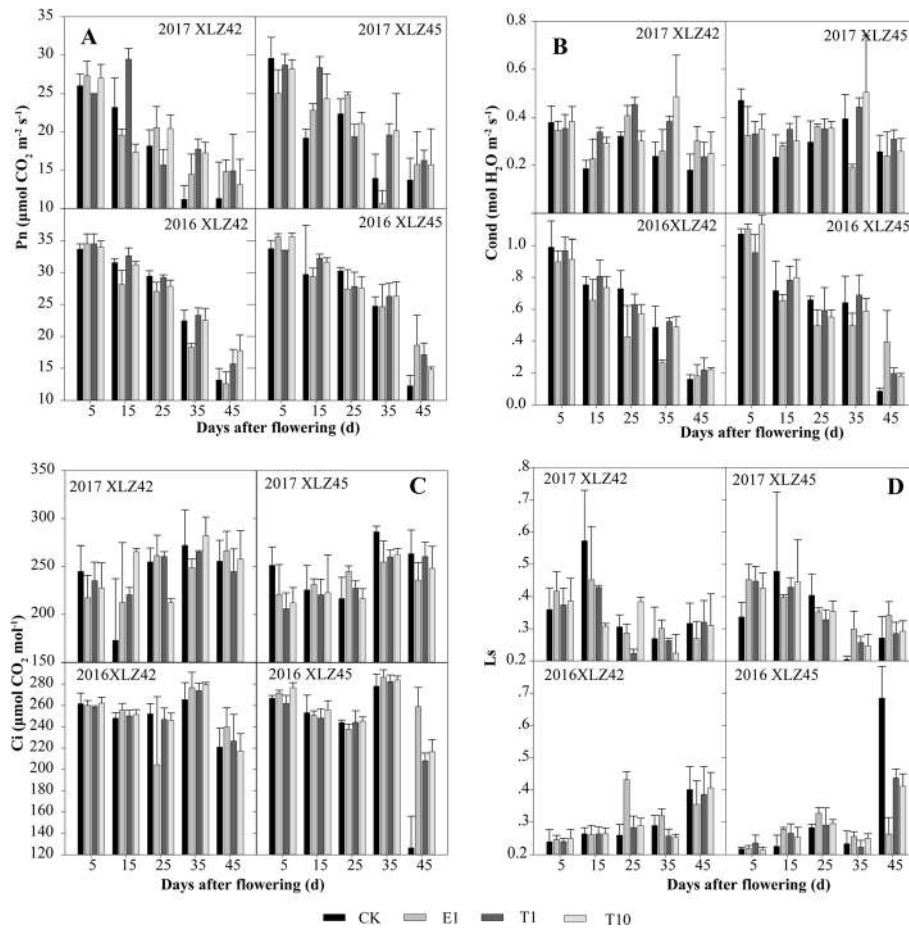


Fig. 4 Gas exchange parameters of cotton varieties (XLZ42 and XLZ45) at different days since flowering in various treatment groups: 1 (T1) and 10 days (T10) before the first irrigation, respectively, and 1 day before the second irrigation (E1) after seedling emergence during 2016–2017. The control group (CK) had film mulching throughout the growth stages. Bars are means \pm standard deviation ($n = 3$). Pn, photosynthetic rate; Cond, conductance to H₂O; Ci, intercellular CO₂ concentration; Ls, stoma limit value

Table 2 Correlation coefficients of net photosynthetic rate (Pn) and other gas exchange parameters in various treatment groups: 1 and 10 days before the first irrigation (respectively T1, T10) and 1 day before the second irrigation (E1) after seedling emergence during 2016–2017 ($n = 30$)

Year	Treatment groups	Cond	Ci	Trmmol	WUE	WUEi	Ls	LUE
2016	CK	0.926**	0.433*	0.919**	0.021	-0.654**	-0.589**	0.971**
	E1	0.882**	-0.011	0.809**	0.192	-0.461*	-0.302	0.966**
	T1	0.938**	0.541**	0.830**	0.218	-0.855**	-0.784**	0.934**
	T10	0.939**	0.553**	0.875**	0.193	-0.872**	-0.801**	0.942**
2017	CK	0.478**	-0.331	0.590**	0.411*	0.305	0.358*	0.807**
	E1	0.480**	-0.516**	0.655**	0.449*	0.497**	0.559**	0.899**
	T1	0.094	-0.768**	0.283	0.775**	0.801**	0.825**	0.889**
	T10	0.432*	-0.453*	0.757**	0.376*	0.328	0.423*	0.871**

Note: Pearson correlations were used. * Significant at the 0.05 probability level (two tailed). ** Significant at the 0.01 probability level (two tailed)
 Cond Conductance to H₂O, Ci Intercellular CO₂ concentration, Trmmol Transpiration rate, WUE Water use efficiency, WUEi Intrinsic WUE, Ls Limiting value of stomata, LUE Light use efficiency

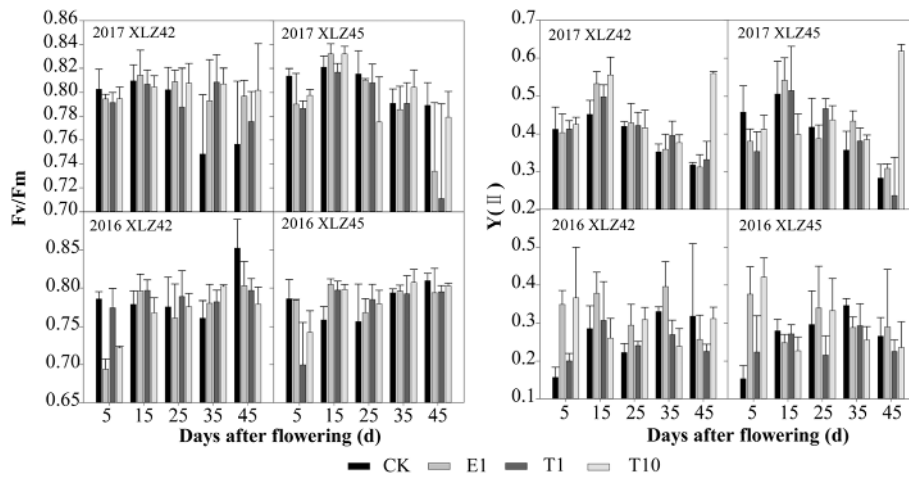


Fig. 5 Maximum photochemical quantum yield of PS-II (Fv/Fm) and actual photochemical quantum yield of PS-II (Y (II)) of cotton varieties (XLZ42 and XLZ45) at different days since flowering in various treatment groups: 1 and 10 days before the first irrigation (T1, T10, respectively) and 1 day before the second irrigation (E1) after seedling emergence, during 2016–2017. The control group (CK) had film mulching throughout the growth stages. Bars are means ± standard deviation (n = 3)

2017, only days after flowering had a significant impact on NPQ, and NPQ was similar among treatments. However, different treatments and days after flowering significantly influenced qL, with that of T10 differing considerably from the other three film-removal treatments.

Influence of mulch film removal on the quantum yield of light-induced (Y (NPQ)) and non-light induced(Y (NO)) non-photochemical fluorescence quenching of cotton leaves

At the early stage of flowering (5 days post-flowering), the values of Y (NPQ) (Fig. 7) of the three film-removal

treatments in 2017 and the CK treatment in 2016 were greatest. This suggested cotton plants were stressed in each treatment and protected themselves by heat dissipation.

The Y (NO) value (Fig. 7) is an index of photic injury. In the early flowering stage, the film-removal treatment of XLZ42 (i.e., with the lower Y (NO) value), which tolerates the drought stress better (mainly via heat dissipation to avoid the photic injury), although XLZ45 (with the higher Y (NO) value) has poor drought tolerance, nonetheless it tried to protect itself from heat dissipation, but film-removal treatment still received the photic injury. By

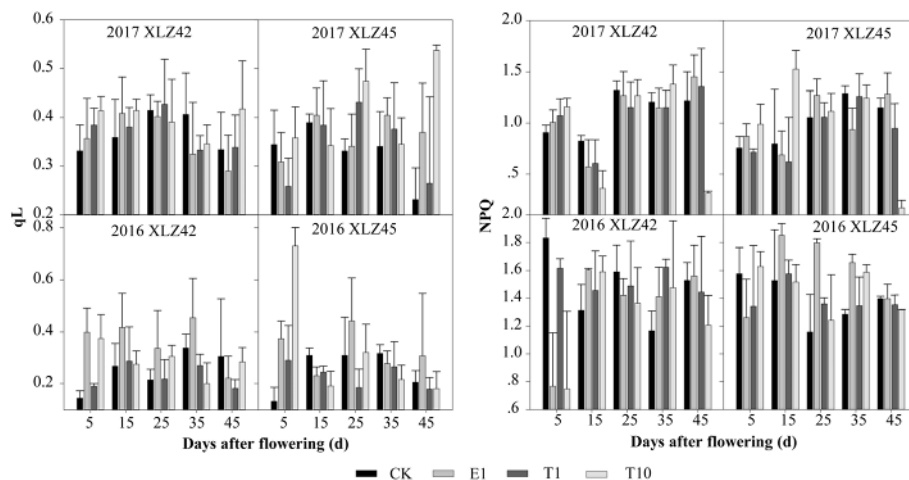
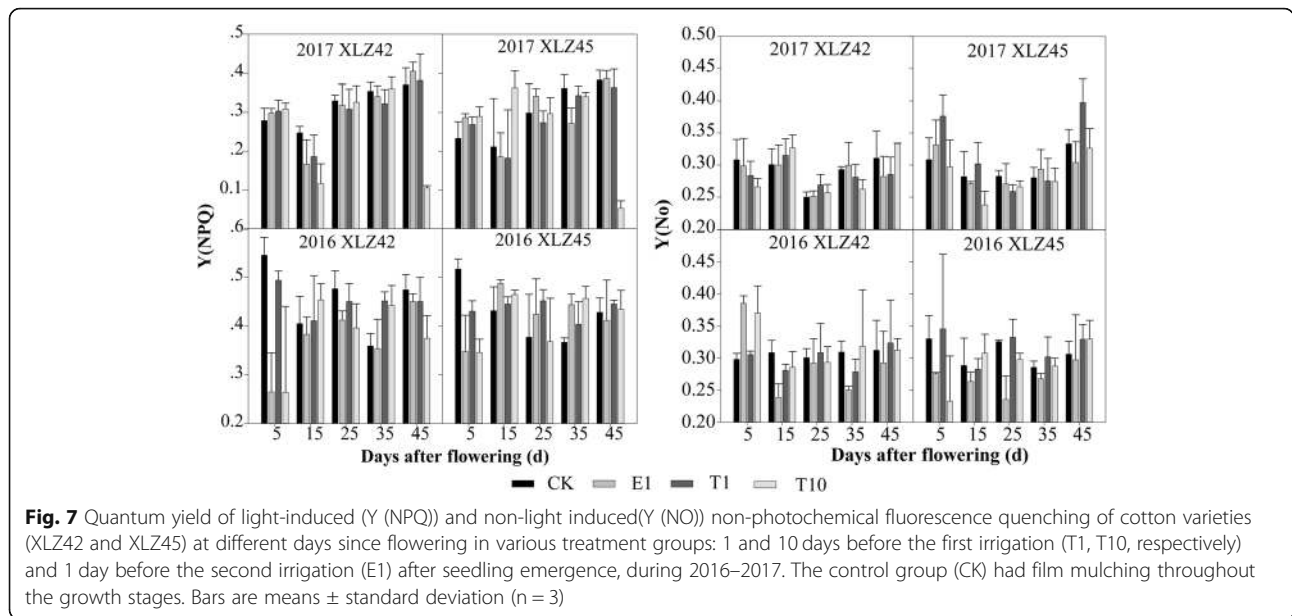


Fig. 6 Coefficient of photochemical fluorescence quenching assuming an interconnected PS-II antennae (qL) and Stern-Volmer type non-photochemical fluorescence quenching (NPQ) of cotton varieties (XLZ42 and XLZ45), at different days since flowering in various treatment groups: 1 and 10 days before the first irrigation (T1, T10, respectively) and 1 day before the second irrigation (E1) after seedling emergence, during 2016–2017. The control group (CK) had film mulching throughout the growth stages. Bars are means ± standard deviation (n = 3)



contrast, in 2016 the lowest values of XLZ42 and XLZ45 were in the CK and T10 treatments, respectively. At the 45 days after flowering, T10 exhibited the lowest light protection capacity and the greatest photic injury. Multivariate analysis revealed that different treatments in 2016 and 2017 and days after flowering (2017) had a significant impact on $Y(NPQ)$, while only days after flowering significantly affected $Y(NO)$.

Effects of mulch film removal on energy conversion of cotton leaves in different growth stages

In 2017, except for the highest proportion of energy entering the photochemical process (P , the actual photochemical quantum yield of PS-II) in the T10 treatment of XLZ45 at 45 days after flowering, the P value of other treatments showed a unimodal curve change, with the highest proportion occurring in the first 15 days since flowering. This indicated that in the normal year of rainfall, light energy absorbed at the early flowering stage is mainly shunted into photochemical reactions, but at the later flowering stage it mainly lost through thermal dissipation to avoid damage to cotton’s photosynthetic mechanism. In 2016, the proportions of heat dissipation (D) under the four treatments were higher among years, changing little during the whole growth period. The P value of CK at 5 days after flowering was significantly lower than those of other treatments. Thus further suggested that the activity of the PS-II photochemical reaction center of CK plants in their early flowering stage was lower in 2016 than that in 2017, with most excess light energy absorbed dissipated via heat dissipation and a few parts entering the photochemistry processes (Fig. 8)

At 45 days after flowering, the P value of the T10 treatment was highest, and more obviously in 2017. This suggested that the earlier the film was removed, the sooner the drought stress, the more of which can increase the actual photochemical quantum yield of PS-II, making this trend is more obvious in the dry year (2015) performance of cotton.

Rapid light curve of cotton leaf in different film-removal treatments at different days after flowering

The rapid light curve directly conveys changes in the electron transfer activity of photoreaction system under different light intensity conditions. By fitting this curve to our data for cotton can be used to gauge the maximum electron transfer rate (ETRmax, Table 3), light energy utilization efficiency (α , Table 4), and the tolerance degree to strong light (I_k , Table 5) of the plant’s photoreaction system.

In the rainy year (2016), the removal of film improved both the ETR (Fig. 9) and ETRmax (Table 3) of cotton in all growth periods, especially in its early flowering stage. In the normal rainfall year (2017), however, it increased ETR (Fig. 9) and ETRmax (Table 3) in the mid flowering stage (15–25 days post-flowering). Film-removal treatments improved the light energy utilization efficiency in the early flowering stage but it was adversely affected in the mid flowering stage in the normal rainfall year (2017) (Table 4); in other plant growth periods it improved the light energy use efficiency of cotton (Table 3). The ability of plants to withstand strong light (Table 5) can be improved by removing the film at suitable periods, namely before the first irrigation in a rainy year, but before the second irrigation in normal rainfall years.

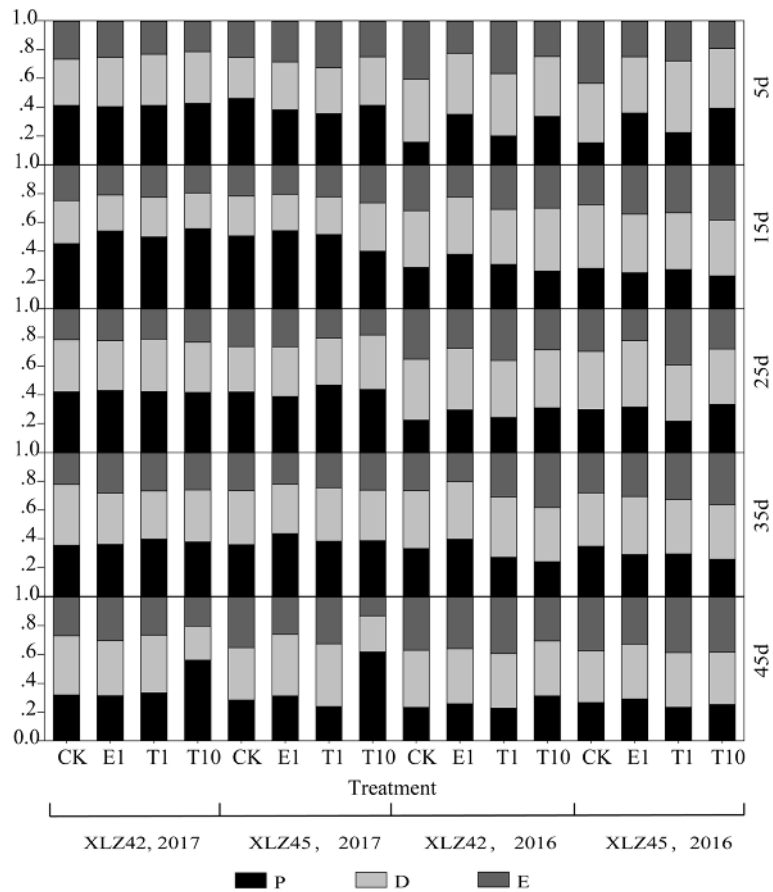


Fig. 8 Absorbed light dissipation of two cotton varieties (XLZ42 and XLZ45) at different days since flowering in various treatment groups: 1 and 10 days before the first irrigation (T1, T10, respectively) and 1 day before the second irrigation (E1) after seedling emergence, during 2016–2017. The control group (CK) had film mulching throughout the growth stages. D, portion of absorption light energy lost via the PS-II antenna pigment; P, actual photochemical quantum yield of PS-II; E, portion of absorption light energy which cannot enter the photochemical process and cannot be lost through the antenna pigment

The MANOVA indicated that the number of days after flowering (2016/2017) (Table S2, S3) and different film-removal treatments (2016) (Table S2) had significant impacts on the ETRmax and Ik values of cotton, but in 2017 (Table S3) only days after flowering significantly influenced the α value.

Population-level physiological parameters of cotton among film-removal treatments at different days since flowering

As Fig. 10 shows, the leaf area index (LAI) of each treatment followed a unimodal curve of change. At the initial growth stage, leaf area increased most quickly, almost linearly. At different growth stages during the three years, the LAI of CK plants was generally the highest among treatments. Apart from the LAI of XLZ45 under the E1 treatment in 2017 (4.40) being highest, larger values were found in CK for both cotton varieties: 4.75 (XLZ42 in 2017), 6.42 (XLZ45 in 2016), 5.93 (XLZ42 in 2016), and 4.60 (XLZ42 in 2015). Higher LAIs of CK

plants were beneficial for promoting their dry matter accumulation.

The trend in cotton dry matter accumulation in the film-removal treatments followed an S-shaped curve (Fig. 11). As cotton grew in size, its dry matter accumulation increased, but the rates of accumulation clearly varied among growth stages.

Table 6 showed that, in addition to 2015, all film-removal treatments promoted dry matter accumulation and the maximum dry matter accumulation under the film-removal treatments were greatest overall. Specifically, Tmax appeared earlier with film removed than that in CK, as did the linear accumulation, while the linear accumulation time (t_2-t_1) was longer with a larger $\Delta W_{t_2-t_1}$ as well.

Yield and fiber quality of cotton among film-removal treatments

The effect of removing the mulch film on yield was related to climatic conditions in different years. In the drought year (2015), it reduced the yield of cotton whereas increased the yield in other years. Fiber quality

Table 3 Maximum electron transfer rate (ETR_{max}, $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) of two cotton varieties (XLZ42 and XLZ45) at different days after flowering in various treatment groups: Mulch film was removed at 1 and 10 days before the first irrigation (respectively T1(June 19, 2016 and June 10, 2017), T10(June 9, 2016 and May 31, 2017)) and 1 day before the second irrigation (E1(June 29, 2016 and June 20, 2017)) after seedling emergence, during 2016–2017. The control group (CK) had film mulching throughout the growth stages. Values are means \pm standard deviation (n = 3)

Year	Varieties	Treatments	Days after flowering /d				
			5	15	25	35	45
2017	XLZ42	CK	112.80 \pm 13.49	203.43 \pm 68.76	350.87 \pm 102.40	216.07 \pm 34.76	158.63 \pm 30.01
		E1	114.10 \pm 12.65	242.00 \pm 7.50	273.80 \pm 57.09	269.97 \pm 98.73	163.37 \pm 28.63
		T1	133.70 \pm 31.40	339.75 \pm 30.19	312.80 \pm 31.68	221.03 \pm 37.50	175.90 \pm 12.85
		T10	142.27 \pm 12.95	293.57 \pm 49.84	315.50 \pm 3.38	226.53 \pm 65.22	153.80 \pm 43.27
	XLZ45	CK	220.27 \pm 93.04	202.70 \pm 66.08	260.20 \pm 32.46	244.67 \pm 72.55	140.07 \pm 15.14
		E1	87.30 \pm 5.91	261.17 \pm 54.77	279.23 \pm 26.5	262.30 \pm 35.13	166.83 \pm 12.39
		T1	94.07 \pm 23.07	223.90 \pm 69.86	312.17 \pm 34.04	254.07 \pm 66.68	118.20 \pm 65.06
		T10	122.67 \pm 10.36	360.77 \pm 96.89	320.53 \pm 14.02	209.70 \pm 28.74	160.87 \pm 37.17
2016	XLZ42	CK	134.20 \pm 22.29	209.40 \pm 12.87	185.95 \pm 7.00	258.90 \pm 7.35	240.20 \pm 160.94
		E1	586.27 \pm 129.82	561.13 \pm 352.30	331.10 \pm 8.02	294.40 \pm 22.88	231.97 \pm 31.76
		T1	146.10 \pm 45.72	365.37 \pm 180.43	182.35 \pm 19.73	223.55 \pm 5.87	209.85 \pm 18.60
		T10	434.75 \pm 22.98	375.33 \pm 176.71	248.30 \pm 16.03	204.15 \pm 25.39	244.35 \pm 1.34
	XLZ45	CK	151.97 \pm 7.60	289.90 \pm 26.02	254.90 \pm 30.21	259.25 \pm 0.49	220.85 \pm 15.49
		E1	381.60 \pm 77.64	209.93 \pm 6.67	243.90 \pm 35.50	243.05 \pm 57.77	304.75 \pm 10.25
		T1	222.53 \pm 51.70	517.50 \pm 25.74	207.60 \pm 25.81	248.95 \pm 8.41	197.90 \pm 31.68
		T10	475.33 \pm 197.34	286.53 \pm 83.56	307.40 \pm 26.02	226.05 \pm 16.33	195.20 \pm 64.06

Table 4 The initial slope of the fast light curve (α , electrons photons⁻¹) of two cotton varieties (XLZ42 and XLZ45) at different days after flowering in various treatment groups: Mulch film was removed at 1 and 10 days before the first irrigation (respectively T1(June 19, 2016 and June 10, 2017), T10(June 9, 2016 and May 31, 2017)) and 1 day before the second irrigation (E1(June 29, 2016 and June 20, 2017)) after seedling emergence, during 2016–2017. The control group (CK) had film mulching throughout the growth stages. Values are means \pm standard deviation (n = 3)

Year	Varieties	Treatments	Days after flowering /d				
			5	15	25	35	45
2017	XLZ42	CK	0.22 \pm 0.01	0.28 \pm 0.03	0.30 \pm 0.01	0.27 \pm 0.04	0.28 \pm 0.02
		E1	0.27 \pm 0.06	0.30 \pm 0.01	0.29 \pm 0.01	0.29 \pm 0.03	0.28 \pm 0.00
		T1	0.25 \pm 0.04	0.29 \pm 0.02	0.23 \pm 0.02	0.31 \pm 0.02	0.28 \pm 0.01
		T10	0.28 \pm 0.01	0.29 \pm 0.01	0.28 \pm 0.01	0.32 \pm 0.03	0.32 \pm 0.02
	XLZ45	CK	0.21 \pm 0.03	0.25 \pm 0.04	0.29 \pm 0.01	0.29 \pm 0.03	0.31 \pm 0.03
		E1	0.19 \pm 0.01	0.30 \pm 0.00	0.25 \pm 0.03	0.26 \pm 0.02	0.27 \pm 0.03
		T1	0.12 \pm 0.07	0.32 \pm 0.00	0.24 \pm 0.06	0.30 \pm 0.04	0.27 \pm 0.06
		T10	0.29 \pm 0.02	0.25 \pm 0.01	0.26 \pm 0.02	0.31 \pm 0.02	0.33 \pm 0.00
2016	XLZ42	CK	0.23 \pm 0.04	0.25 \pm 0.02	0.29 \pm 0.01	0.26 \pm 0.02	0.29 \pm 0.02
		E1	0.26 \pm 0.02	0.25 \pm 0.01	0.24 \pm 0.01	0.23 \pm 0.01	0.27 \pm 0.05
		T1	0.23 \pm 0.03	0.29 \pm 0.05	0.28 \pm 0.02	0.24 \pm 0.02	0.27 \pm 0.01
		T10	0.26 \pm 0.00	0.28 \pm 0.01	0.28 \pm 0.02	0.26 \pm 0.01	0.29 \pm 0.00
	XLZ45	CK	0.26 \pm 0.02	0.26 \pm 0.01	0.27 \pm 0.01	0.27 \pm 0.02	0.29 \pm 0.00
		E1	0.30 \pm 0.02	0.26 \pm 0.01	0.24 \pm 0.01	0.27 \pm 0.00	0.27 \pm 0.00
		T1	0.27 \pm 0.07	0.28 \pm 0.01	0.27 \pm 0.01	0.27 \pm 0.01	0.29 \pm 0.04
		T10	0.28 \pm 0.01	0.27 \pm 0.02	0.26 \pm 0.01	0.27 \pm 0.01	0.30 \pm 0.01

Table 5 The minimum saturating irradiance (I_k , $\mu\text{mol m}^{-2}\text{s}^{-1}$) of two cotton varieties (XLZ42 and XLZ45) at different days after flowering in various treatment groups: Mulch film was removed at 1 and 10 days before the first irrigation (respectively T1(June 19, 2016 and June 10, 2017), T10(June 9, 2016 and May 31, 2017)) and 1 day before the second irrigation (E1(June 29, 2016 and June 20, 2017)) after seedling emergence, during 2016–2017. The control group (CK) had film mulching throughout the growth stages. Values are means \pm standard deviation ($n = 3$)

Year	Varieties	Treatments	Days after flowering /d				
			5	15	25	35	45
2017	XLZ42	CK	510.67 \pm 65.80	749.80 \pm 337.86	1 172.50 \pm 325.98	829.47 \pm 201.06	579.63 \pm 166.11
		E1	431.80 \pm 100.66	821.00 \pm 48.93	943.80 \pm 167.16	960.87 \pm 401.76	590.20 \pm 108.71
		T1	523.00 \pm 33.09	1 165.75 \pm 14.21	1 351.35 \pm 9.97	711.43 \pm 92.12	632.17 \pm 36.58
		T10	514.93 \pm 45.01	1 022.97 \pm 208.47	1 130.20 \pm 44.86	725.07 \pm 252.24	493.45 \pm 174.58
	XLZ45	CK	1 019.90 \pm 319.56	800.50 \pm 197.89	885.73 \pm 131.50	860.73 \pm 293.51	458.63 \pm 85.00
		E1	460.83 \pm 64.73	884.40 \pm 189.65	1 133.30 \pm 217.56	1 001.27 \pm 198.83	619.10 \pm 38.70
		T1	1 324.60 \pm 1 356.12	709.20 \pm 232.78	1 361.77 \pm 496.88	867.97 \pm 283.80	456.87 \pm 302.19
		T10	420.97 \pm 22.18	1 455.90 \pm 364.75	1 213.97 \pm 118.44	670.40 \pm 123.69	489.37 \pm 109.52
2016	XLZ42	CK	612.40 \pm 40.79	837.35 \pm 109.11	640.80 \pm 42.43	993.45 \pm 95.81	854.00 \pm 627.91
		E1	2 326.30 \pm 663.48	2 164.93 \pm 1 232.72	1 398.40 \pm 120.35	1 285.87 \pm 119.02	977.73 \pm 27.21
		T1	653.57 \pm 258.24	1 352.43 \pm 778.42	653.15 \pm 116.46	919.95 \pm 101.75	786.25 \pm 38.68
		T10	1 642.95 \pm 80.26	1 322.40 \pm 577.78	902.20 \pm 56.03	781.60 \pm 58.27	849.45 \pm 6.43
	XLZ45	CK	578.33 \pm 32.83	1 105.70 \pm 44.69	959.50 \pm 56.21	966.95 \pm 77.29	767.25 \pm 48.72
		E1	1 281.40 \pm 333.75	808.73 \pm 38.04	1 011.65 \pm 86.9	885.55 \pm 211.35	1 132.15 \pm 34.15
		T1	833.80 \pm 219.55	1877.15 \pm 4.45	774.70 \pm 42.35	912.05 \pm 8.56	708.40 \pm 209.87
		T10	1 696.00 \pm 615.06	1 045.90 \pm 257.11	1 170.90 \pm 39.74	832.25 \pm 106.42	656.35 \pm 237.38

was also improved, albeit to a certain extent, and the earlier the film removal, the more pronounced was this trend. The lint yield and fiber quality showed no statistical difference between treatments (Table 7).

Discussion

Influence of film-removal time on soil temperature, moisture, and cotton growth

Film mulching mainly functions by increasing soil temperature and promoting plants' growth and development early in ontogeny (Braunack *et al.* 2015; Farrell and Gilliland 2011; O'Loughlin *et al.* 2015; Ramakrishna *et al.* 2006; Wang *et al.* 2016). Compared with uncovered soil of the cotton field, the temperature of film-covered soil increased by 1–3 °C from sowing time in spring to tasseling stages (Liu *et al.* 2014a, b; Su *et al.* 2011a); however, no significant differences in soil temperature between film-covered and film-removed groups were found for summer-sown sweet potatoes (Hou *et al.* 2015). In our study, we found the soil temperature increase by the mulching film could be maintained for ca. 50 d. Within 50 d after cotton seedling emergence, film removal lowers soil temperature. From then on, the difference in soil temperature among layers between film-removal groups and CK narrowed: generally, with the film removed and

the closer to the surface soil layer, the greater was the difference (Fig. 2).

As cotton grows, temperature becomes less of a dominant limiting factor, such that long-term film mulching can lead to excessive soil temperatures and poor soil permeability during late growth stages (Jiang 2011; Kwabiah 2005; Li *et al.* 2014a, b; Wang *et al.* 2009). This can interfere with root respiration, affecting plant development and leading to detrimental impacts on crop yield and quality (Jiang 2011; Kwabiah 2005; Li *et al.* 2014a, b; Wang *et al.* 2009). For a given species, removing the film from the ground at the appropriate growth stage could effectively reduce soil temperature, enhance root system activity, and optimize the distribution of photosynthetic products. Doing so would also help prevent crop prematurity and improve yield (Al-Assir *et al.* 1991; Jiang *et al.* 2012; Kwon *et al.* 2011). This study showed that film-removal treatment increased the yield, with fiber quality also partly enhanced in the wet year (2016) (Table 7).

Mulching can reduce soil water consumption and increase water use efficiency, which is conducive to improved crop yields (Kader *et al.* 2017). However, some studies have shown that mulch can lead to increased water consumption of crops while promoting crop growth that need to absorb more water from

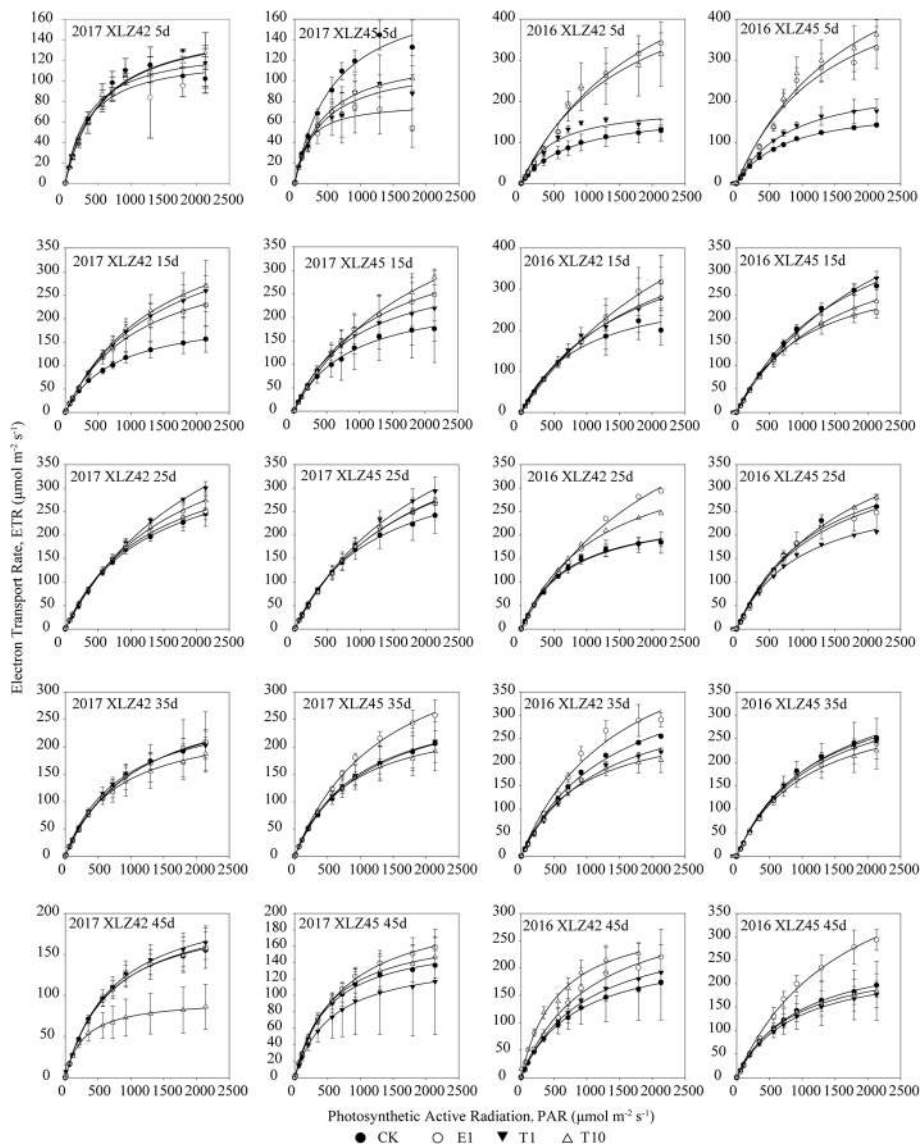


Fig. 9 Rapid light curves of two cotton varieties (XLZ42 and XLZ45) at different days since flowering in various treatment groups: 1 and 10 days before the first irrigation (respectively T1, T10) and 1 day before the second irrigation (E1) after seedling emergence, during 2016–2017. The control group (CK) had film mulching throughout the growth stages. Bars are means \pm standard deviation ($n = 3$)

the deeper soil layers (Liu *et al.* 2014a, b), resulting in excessive water consumption in the deeper layers of the soil, which leading to reduced water storage in deep soil layers (Sun *et al.* 2014). Removal the mulching film could significantly reduce soil moisture content before cotton plants begin to flower (Li *et al.* 2016; Zhang *et al.* 2016a, b), whereas no such effects occurred when it was applied after florescence (Li *et al.* 2016). More than 20 years ago, Xia and Zhang (1994) showed that film removal before irrigation led to a soil moisture content of the 0–35 cm soil layer that was 18.2% lower, on average, up 30% lower than under constant mulching. Our study

also indicated that, in 2017, the 0–60 cm soil layer treated with mulch had a higher moisture content, but deeper soil (60–100 cm layer) under T1 treatment had the greatest moisture (Fig. 3). The reason for the above experimental results may be that film mulching can reduce the evaporation of soil moisture caused by direct sunlight, so the water content of 0–60 cm soil layer is higher. However, the growth of cotton under removing film treatment was not as vigorous as that under mulching film treatment, and the absorption of deep soil moisture was less, so the moisture content in 60–100 cm soil layer of removing film treatment is higher.

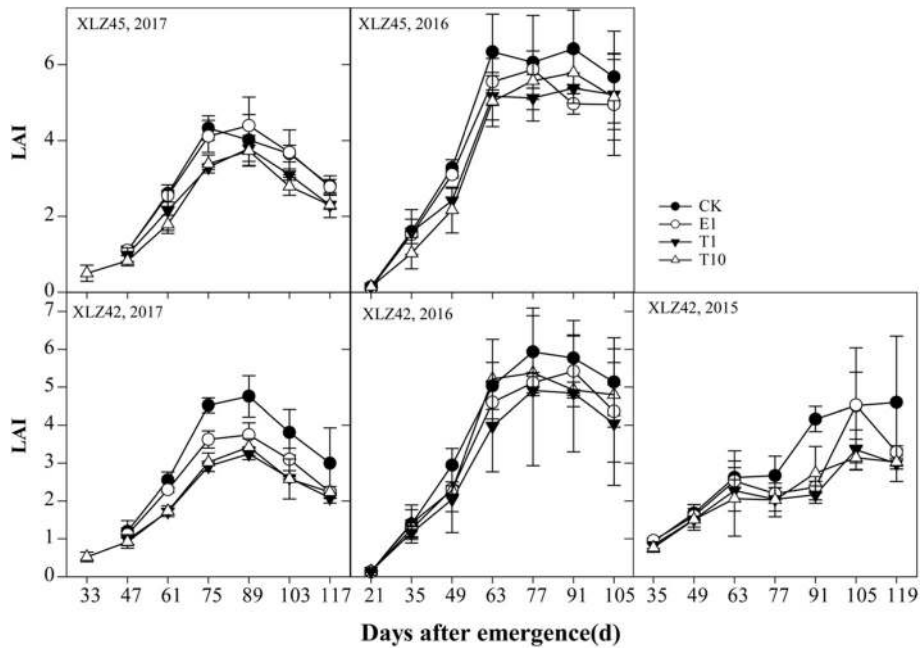


Fig. 10 Leaf area index (LAI) variation of two cotton varieties (XLZ42 and XLZ45) in various treatment groups: 1 and 10 days before the first irrigation (T1, T10, respectively) and 1 day before the second irrigation (E1) after seedling emergence, during 2015–2017. The control group (CK) had film mulching throughout the growth stages. Bars are means \pm standard deviation ($n = 3$)

Influence of film-removal timing on gas exchange parameters of cotton leaf

In the early growth stage of cotton, mulching has water-saving and temperature-raising effects, which shortens the growing season of cotton. After starting to irrigate

the cotton, with higher temperatures and irrigation amounts, continuous mulching may have adverse effects on the improved soil conditions, root development, and photosynthetic performance of targeted plants (Du *et al.* 1989). Covering the soil with mulching film throughout

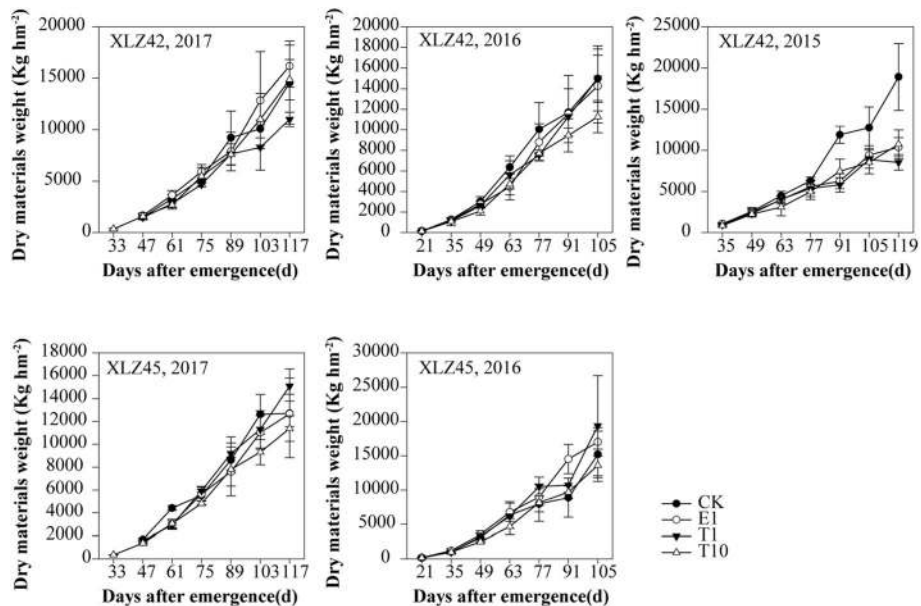


Fig. 11 Dry matter accumulation of two cotton varieties (XLZ42 and XLZ45) in various treatment groups: 1 and 10 days before the first irrigation (T1, T10, respectively) and 1 day before the second irrigation (E1) after seedling emergence, during 2015–2017. The control group (CK) had film mulching throughout the growth stages. Bars are means \pm standard deviation ($n = 3$)

Table 6 Parameters for the logistic equation of two cotton varieties' (XLZ42 and XLZ45) dry matter accumulation in various treatments groups: Mulch film was removed at 1 and 10 days before the first irrigation (T1(June 4, 2015, June 19, 2016 and June 10, 2017), T10(May 25, 2015, June 9, 2016 and May 31, 2017), respectively) and 1 day before the second irrigation (E1(June 14, 2015, June 29, 2016 and June 20, 2017)) after seedling emergence, during 2015–2017. The control group (CK) was film mulched throughout the growth stage

Varieties and Years	Treatments	K	a	b	R ²	Tmax /d	t1 /d	t2 /d	Rmax / (kg·ha ⁻¹ ·d ⁻¹)	Wm / (kg·ha ⁻¹)	ΔWt2-t1 / (kg·ha ⁻¹)
XLZ42, 2015	CK	28 448.19	4.5183	-0.0440	0.9813**	103	73	133	313.14	14 224.10	8 207.30
	E1	13 546.26	3.6292	-0.0416	0.9780**	87	56	119	140.86	67 73.13	3 908.10
	T1	98 03.42	3.7293	-0.0505	0.9566**	74	48	100	123.86	49 01.71	2 828.29
	T10	13 098.11	4.1369	-0.0478	0.9955**	87	59	114	156.55	65 49.06	3 778.81
XLZ42, 2016	CK	15 233.50	4.8979	-0.0732	0.9866**	67	49	85	278.86	7 616.75	4 394.86
	E1	15 730.68	4.9953	-0.0686	0.9889**	73	54	92	269.84	7 865.34	4 538.30
	T1	18 790.06	4.4678	-0.0554	0.9953**	81	57	104	260.43	9 395.03	5 420.93
	T10	11 573.49	5.2355	-0.0786	0.9933**	67	50	83	227.49	5 786.75	3 338.95
XLZ45, 2016	CK	26 514.86	3.9347	-0.0396	0.9467**	99	66	133	262.48	13 257.43	7 649.54
	E1	19 615.03	4.7176	-0.0635	0.9969**	74	54	95	311.55	9 807.51	5 658.93
	T1	30 025.95	4.2929	-0.0460	0.9612**	93	65	122	345.06	15 012.98	8 662.49
	T10	15 532.38	4.5283	-0.0597	0.9794**	76	54	98	231.63	7 766.19	4 481.09
XLZ42, 2017	CK	19 068.20	4.3338	-0.0457	0.9728**	95	66	124	217.82	9 534.10	5 501.17
	E1	24 279.73	4.5090	-0.0443	0.9943**	102	72	132	268.67	12 139.86	7 004.70
	T1	12 510.99	4.3294	-0.0516	0.9790**	84	58	109	161.48	6 255.49	3 609.42
	T10	20 762.82	4.7209	-0.0476	0.9964**	99	72	127	247.01	10 381.41	5 990.07
XLZ45, 2017	CK	14 775.97	4.5932	-0.0572	0.9719**	80	57	103	211.11	7 387.99	4 262.87
	E1	14 817.38	4.6920	-0.0551	0.9969**	85	61	109	204.16	7 408.69	4 274.82
	T1	18 182.35	4.6752	-0.0520	0.9915**	90	65	115	236.46	9 091.17	5 245.61
	T10	12 452.72	4.8122	-0.0592	0.9940**	81	59	104	184.18	6 226.36	3 592.61

Note: k, a, and b are equation coefficients

Tmax, the time when the dry matter accumulation rate reached a maximum

t1, starting time of linear accumulation

t2, end time of linear accumulation

Rmax, the maximum accumulation rate

Wm, dry matter weight at the time when the dry matter accumulation rate reached a maximum

ΔWt2-t1, dry matter accumulation from t1 to t2. R2, Correlation Index

* Significant at the 0.05 probability level. ** Significant at the 0.01 probability level

Table 7 Yield and fiber quality characteristics of two cotton varieties (XLZ42 and XLZ45) in different treatment groups: Mulch film was removed at 1 and 10 days before the first irrigation (T1(June 4, 2015, June 19, 2016 and June 10, 2017), T10(May 25, 2015, June 9, 2016 and May 31, 2017), respectively) and 1 day before the second irrigation (E1(June 14, 2015, June 29, 2016 and June 20, 2017)) after seedling emergence, during 2015–2017. The control group (CK) was film mulched throughout the growth stage.

Varieties/Years	Treatments	Number of plants harvested / (10 ⁴ plants·hm ⁻²)	Boll number on a single plant	Single boll weight /g	Estimated yield of lint cotton / (kg·hm ⁻²)	UHML /mm	Mic	Str / (g·tex ⁻¹)	Elg /%	SFI /%	UI /%
XLZ42, 2015	CK	20.87 ± 1.13a	5.54 ± 1.55a	5.17 ± 0.17a	1914.23 ± 734.23a	27.78 ± 0.94a	5.09 ± 0.07a	29.80 ± 2.00a	6.50 ± 0.14a	7.23 ± 0.41a	85.38 ± 1.29a
	E1	19.61 ± 0.92a	5.00 ± 1.36ab	5.00 ± 0.48a	1 558.63 ± 239.84a	27.24 ± 0.73a	5.07 ± 0.13a	31.23 ± 1.50a	6.53 ± 0.10a	7.45 ± 0.10a	85.05 ± 1.23a
	T1	18.79 ± 1.57a	4.92 ± 0.89ab	5.30 ± 0.37a	1715.35 ± 582.44a	26.76 ± 0.88a	5.10 ± 0.05a	29.55 ± 2.07a	6.40 ± 0.14a	7.40 ± 0.14a	85.15 ± 0.53a
	T10	19.67 ± 1.55a	4.68 ± 0.96b	4.97 ± 0.39a	1 539.82 ± 528.93a	27.06 ± 0.66a	5.21 ± 0.16a	29.28 ± 1.58a	6.43 ± 0.17a	7.23 ± 0.43a	85.55 ± 1.02a
XLZ42, 2016	CK	12.14 ± 2.74a	6.76 ± 0.24a	5.93 ± 0.15a	1 585.55 ± 265.56a	28.15 ± 0.54a	4.30 ± 0.19a	28.83 ± 1.06ab	7.10 ± 0.10a	7.10 ± 0.00a	85.57 ± 0.15a
	E1	12.20 ± 1.09a	7.24 ± 0.40a	6.03 ± 0.08a	1 631.69 ± 293.12a	27.86 ± 0.16a	4.31 ± 0.19a	27.67 ± 0.67b	7.03 ± 0.21a	7.40 ± 0.40a	85.00 ± 0.85a
	T1	12.50 ± 0.61a	7.38 ± 1.24a	5.87 ± 0.28a	1 586.81 ± 163.05a	28.13 ± 0.11a	4.25 ± 0.27a	29.40 ± 1.40a	7.03 ± 0.06a	7.43 ± 0.32a	84.83 ± 0.74a
	T10	11.13 ± 1.13a	7.05 ± 1.48a	5.98 ± 0.38a	1 609.93 ± 236.60a	28.30 ± 0.65a	4.57 ± 0.24a	28.90 ± 0.44ab	7.03 ± 0.15a	7.07 ± 0.15a	85.63 ± 0.40a
XLZ45, 2016	CK	11.44 ± 4.30a	8.13 ± 1.47a	6.35 ± 0.33a	1 558.84 ± 141.97a	29.85 ± 0.38a	3.92 ± 0.20a	30.77 ± 0.45a	7.53 ± 0.21a	6.83 ± 0.06a	86.20 ± 0.89a
	E1	14.03 ± 1.60a	7.82 ± 2.25a	5.98 ± 0.14a	1 484.72 ± 171.66a	29.58 ± 0.57a	3.91 ± 0.23a	30.57 ± 1.33a	7.53 ± 0.15a	7.00 ± 0.26a	85.47 ± 0.67a
	T1	14.22 ± 0.53a	6.43 ± 0.49a	6.13 ± 0.47a	1 609.83 ± 206.19a	30.04 ± 0.67a	3.76 ± 0.44a	31.33 ± 1.20a	7.70 ± 0.00a	6.83 ± 0.15a	86.17 ± 0.55a
	T10	11.42 ± 3.26a	7.45 ± 1.32a	6.21 ± 0.41a	1 602.97 ± 208.30a	29.61 ± 0.06a	3.99 ± 0.15a	31.00 ± 0.30a	7.50 ± 0.10a	7.27 ± 0.32a	84.60 ± 1.04a
XLZ42, 2017	CK	14.23 ± 1.03a	8.83 ± 1.65a	4.77 ± 0.19a	2014.12 ± 200.67ab	27.84 ± 0.63a	4.42 ± 0.28a	29.53 ± 1.21a	6.80 ± 0.10a	7.03 ± 0.49a	86.13 ± 1.25a
	E1	14.86 ± 0.71a	9.43 ± 1.59a	5.04 ± 0.36a	2 257.29 ± 248.48a	27.43 ± 0.15a	4.36 ± 0.14a	28.57 ± 0.32a	6.77 ± 0.06a	7.37 ± 0.15a	85.10 ± 0.26a
	T1	13.74 ± 1.19a	8.40 ± 0.79a	4.98 ± 0.30a	2056.67 ± 22.40ab	28.53 ± 0.37a	4.41 ± 0.30a	30.27 ± 0.85a	6.90 ± 0.00a	7.30 ± 0.30a	85.03 ± 0.76a
	T10	14.67 ± 1.40a	8.37 ± 2.06a	4.63 ± 0.16a	1869.94 ± 155.84b	26.53 ± 0.64a	4.39 ± 0.10a	28.80 ± 1.25a	6.73 ± 0.12a	7.67 ± 0.91a	84.80 ± 1.25a
XLZ45, 2017	CK	15.75 ± 0.64a	9.43 ± 0.59a	5.36 ± 0.15a	2 177.05 ± 166.17a	27.47 ± 1.01a	4.27 ± 0.07a	29.57 ± 0.70a	6.83 ± 0.15a	7.73 ± 1.07a	84.80 ± 1.90a
	E1	14.63 ± 0.64a	9.53 ± 0.95a	5.03 ± 0.26a	2 243.54 ± 389.42a	27.20 ± 1.11a	4.41 ± 0.30a	28.80 ± 2.77a	6.70 ± 0.10a	7.57 ± 0.65a	84.90 ± 1.08a
	T1	15.13 ± 0.62a	7.93 ± 0.87a	4.99 ± 0.31a	2 137.70 ± 271.74a	27.39 ± 0.35a	4.45 ± 0.25a	29.33 ± 0.95a	6.77 ± 0.15a	7.80 ± 0.70a	84.33 ± 1.17a
	T10	14.92 ± 0.15a	7.73 ± 0.51a	5.07 ± 0.28a	2 154.97 ± 175.75a	29.09 ± 0.53a	4.52 ± 0.06a	31.93 ± 0.83a	6.97 ± 0.06a	7.00 ± 0.10a	85.63 ± 0.25a

Note: Values are means ± SD (n = 3). Within a column, values with different lowercase letters are significantly different at the P < 0.05 level according to LSD among treatment groups in the same year; different capital letters indicate significant difference at the P < 0.01 level according to LSD among treatment groups in the same year

UHML Upper-half mean length, Mic Micronaire reading, Str Specific breaking strength, Elg Elongation percentage, SFI Short fiber index, UI Uniformity index

the growth period has been shown to cause rapid declines in net photosynthetic rate and chlorophyll content of tobacco (Wang et al. 2010; Yang et al. 2010), tomato (Wang et al. 2004), beet (Cai et al. 1988), cabbage (Zhang et al. 1995) and other crops, accelerating their diminished photosynthetic function in later growth stages. However, removal of mulch at the proper time improved photosynthetic functioning of both tobacco (Wang et al. 2010) and maize (He et al. 1999; Yu et al. 2006; Zhang et al. 2016a, b), which increased the accumulation of photosynthetic products, and alleviated the phenomenon of premature aging in these crop plants.

The results of our study also indicated that film removal could increase the Pn (Fig. 4a) in and Cond (Fig. 4b) in the late flowering stage. This may be because removing the mulch at later growth stages can create a favorable soil temperature and water environment and can promote photosynthesis.

Influence of film-removal timing on chlorophyll fluorescence parameters of cotton leaves

Chlorophyll fluorescence is closely related to each reaction in the process of photosynthesis, so how environmental change affects it may be shown by correlated changes in key fluorescence parameters (Chen et al. 2006). Just a few studies, from China and abroad, have investigated film-removal effects on chlorophyll fluorescence parameters at different growth stages. For example, the removal of mulching film at early growth stages caused different degrees of drought stress (Zhang et al. 2016a, b). In contrast to these, many studies (Boussadia et al. 2008; Mishra et al. 2012; Nankishore and Farrell 2016) worldwide have reported on how drought affects chlorophyll fluorescence parameters of crop plants.

Relevant studies have shown that Fv/Fm can be reliably used as a relative index for detecting drought-resistant crops (Mishra et al. 2012; Nankishore and Farrell 2016; Zhang et al. 2003), and it can quickly and accurately capture the water status of cotton leaves during drought stress (Xue et al. 2013). Under severe drought conditions, the Fv/Fm values of leaves from cotton (Liu et al. 2008; Tang et al. 2007; Xie et al. 2015), *Trigonella foenum-graecum* (Baghbani-Arani et al. 2017), tulips (Miao et al. 2015), and olive trees (Boussadia et al. 2008) are known to decrease considerably. In this study, we found that film-removal treatment caused a severe drought stress on cotton in the early flowering stage, which led to the decrease of Fv/Fm value (Fig. 5).

Mild drought is beneficial for increasing the opened proportion of the PS II reaction center, so more light energy is used to promote photosynthetic electron transport (Zhao et al. 2007), thereby improving the latter's ability. For example, ETR of *Prunus persica*

(L.) Batsch var. *silver king* was significantly improved after water stress induction (Osório et al. 2006). But the ETR of cotton (Deeba et al. 2012) decreases under severe drought conditions, and when its leaf water potential drops below -3Mpa , the ETR was reduced by more than 80% (Gleason et al. 2017). Earlier, Ogaya and Peñuelas (2003) had found that severe drought treatments caused a slight decrease in ETR values of both *Quercus ilex* and *Phillyrea latifolia*, whereas Snider et al. (2013) believe cotton's ETR is not affected by drought. Our results showed that removing the mulching film before irrigation in a rainy year (2016) could improve the ETR (Fig. 9) and the ETRmax (Table 3) by drought stress training, especially during the early flowering stage. But in a normal rainfall year (2017), film removal caused more severe drought and reduced ETR (Fig. 9) and ETRmax (Table 3) early in the flowering stage.

Under mild drought stress, the Fv/Fm, Y (II), and qL values of cotton plants can increase with prolonged stress (Xu et al. 2017). Drought stress also increased the NPQ of cotton (Liu et al. 2008) and olive (Boussadia et al. 2008) plants, and also decreased the qL value of rice (Pieters and Souki 2005).

Here, we found the Fv/Fm value of cotton under the film-removal treatments was generally higher after the hardening of certain drought stress in the mid-stage of flowering (Fig. 5). Removing the film improved the Y (II) values (Fig. 5) in the mid flowering stage in 2017 (i.e., 15 to 45 days since flowering). In 2017, the NPQ of film-removal treatments was higher than CK at the early flowering stage (Fig. 6). Our results suggest film removal can increase the qL value of cotton in rainy years, but in normal rainfall years, it would increase drought-resistant varieties' qL value. In the late flowering stage, the qL value of film-removal treatments was reduced in the years with heavy rainfall, while the opposite likely occurs true in years with normal rainfall.

Conclusion

To improve soil temperature and conserve soil moisture content in cotton plants, this effect of film mulching should be maintained for at least 50 days after seedlings emerge. Removing the film before this will seriously affect cotton growth and development. The benefits of timed removal of the film, whether before the first or second irrigation after emergence, should be decided according to the climate of a given year. It is beneficial for promoting photosynthesis in the late flowering stage of cotton after early drought stress induction, and increasing the cotton yield and fiber quality to a certain extent, but no significant difference was reached.

Abbreviations

$\Delta Wt-t_1$: Dry matter accumulation from t_1 to t_2 ; Ca: Atmospheric CO_2 concentration; Ci: Intercellular CO_2 concentration; Cond: Conductance to H_2O ; D: Portion of absorption light energy lost via the PS-II antenna pigment; E: Portion of absorption light energy which cannot enter the photochemical process and cannot be lost through the antenna pigment; Elg: Elongation percentage; ETR: Actual electron transport rate; ETR_{max}: Maximum electron transfer rate; F: Fluorescence at any time; F₀: Original fluorescence; F_{0'}: Minimal fluorescence at light adaptation; F_m: Maximal fluorescence; F_m' : Maximal Fluorescence at light adaptation; F_v/F_m: The maximum photochemical quantum yield of PS-II; GLM: General linear model; I_k: The minimum saturating irradiance (corresponding to plant tolerance of intense light); LAI: Leaf area index; Ls: The stoma limit value; LUE: Light use efficiency; MANOVA: Multi-factor analysis of variance; Mic: Micronaire reading; NPQ: The Stern-Volmer type non-photochemical fluorescence quenching; P: Actual photochemical quantum yield of PS-II; PAR: Photosynthetic active radiation; Pn: Photosynthetic rate; PS-II: Photoreaction system II; qL: The coefficient of photochemical fluorescence quenching, assuming interconnected PS II antennae and lake model; R^2 : Correlation Index; rETR: The relative electron transfer rate; R_{max}: The maximum accumulation rate; SFI: Short fiber index; Str: Specific breaking strength; t₁: Starting time of linear accumulation; t₂: End time of linear accumulation; T_{max}: The time when the dry matter accumulation rate reached a maximum; UHML: Upper-half mean length; UI: Uniformity index; W_m: Dry matter weight at the time when the dry matter accumulation rate reached a maximum; WUE: Water use efficiency; WUE_i: Intrinsic WUE; Y (II): The actual photochemical quantum yield of PS-II; Y (NO): The quantum yield of non-light-induced non-photochemical fluorescence quenching; Y (NPQ): The quantum yield of light-induced (i.e., ΔpH and zeaxanthin-dependent) non-photochemical fluorescence quenching; α : An initial slope of the fast light curve (conveying the efficiency of light energy utilization).

Supplementary Information

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Additional file 1: Table S1. F-value of MANOVA of the gas exchange parameters in 2016 and 2017. **Table S2.** F-value of MANOVA of the chlorophyll fluorescence parameters in 2016. **Table S3.** F-value of MANOVA of the chlorophyll fluorescence parameters in 2017.

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Authors' contributions

Yang XK designed the study, Zhang ZQ wrote the main manuscript text and prepared all figures. Zhang L, Tian HY, and Niu Y carried out the experimental work and analyzed the data. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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