

## Article

# Leaf removal Affects Maize Morphology and Grain Yield

Guangzhou Liu <sup>1</sup>, Yunshan Yang <sup>2</sup>, Wanmao Liu <sup>2</sup>, Xiaoxia Guo <sup>2</sup>, Jun Xue <sup>1</sup>, Ruizhi Xie <sup>1</sup>, Bo Ming <sup>1</sup>, Keru Wang <sup>1</sup>, Peng Hou <sup>1,\*</sup> and Shaokun Li <sup>1,\*</sup>

<sup>1</sup> Institute of Crop Sciences, Chinese Academy of Agricultural Sciences/Key Laboratory of Crop Physiology and Ecology, Ministry of Agriculture, Beijing 100081, China; liugz89@163.com (G.L.); xuejun5519@126.com (J.X.); xieruizhi@caas.cn (R.X.); obgnim@163.com (B.M.); wkeru01@163.com (K.W.)

<sup>2</sup> Oasis Ecology Agriculture of Xinjiang Construction Crops/The Center of Crop High-Yield Research, Shihezi 832003, China; 18093656011@189.cn (Y.Y.); 15026232318@163.com (W.L.); Gguoxiaoxia@163.com (X.G.)

\* Correspondence: houpeng@caas.cn (P.H.); Tel: 0086-10-82108595 (P.H.); lishaokun@caas.cn (S.L.); Tel: 0086-10-82108891 (S.L.),

Received: 8 February 2020; Accepted: 10 February 2020; Published: 13 February 2020

**Abstract** Increasing planting density is an important practice associated with increases in maize yield, but densely planted maize can suffer from poor light conditions. In our two-year field experiments, two morphologically different cultivars, ZD958 (less compact) and DH618 (more compact), were planted at 120,000 plants ha<sup>-1</sup> and 135,000 plants ha<sup>-1</sup>, respectively. We established different leaf area index (LAI) treatments by removing leaves three days after silking: (1) control, no leaves removed (D0); (2) the two uppermost leaves removed (D1); (3) the four uppermost leaves removed (D2); (4) the leaves below the third leaf below the ear removed (D3); (5) the leaves of D1 and D3 removed (D4); (6) the leaves of D2 and D3 removed (D5). Optimal leaf removal improved light distribution, increased photosynthetic capacity and the post-silking source-sink ratio, and thus the grain yield, with an average LAI of 5.9 (5.6 and 6.2 for ZD958 and DH618, respectively) for the highest yields in each year. Therefore, less-compact cultivars should have smaller or fewer topmost leaves or leaves below the ear that quickly senesce post-silking, so as to decrease leaf area and thus improve light distribution and photosynthetic capacity in the canopy under dense planting conditions. However, for more compact cultivars, leaves below the ear should senesce quickly after silking to reduce leaf respiration and improve the photosynthetic capacity of the remaining top residual leaves. In future maize cultivation, compact cultivars with optimal post-silking LAI should be adopted when planting densely.

**Keywords:** maize; high density; plant type; leaf removal; optimum LAI

## 1. Introduction

Maize is an important cereal crop that contributes to global food security [1,2]. By increasing planting density, an important measure that can increase maize yield per unit area [3,4], maize may more efficiently intercept and use solar radiation [5,6]. Some studies have shown that leaf area index (LAI) increases as plant density increases [7,8], causing decreased light transmission to the lower canopy leaves which, in turn accelerates their senescence [9–12] and causes yield loss [13–16]. However, a synthesis analysis in a previous study found that the LAI did not always increase with increasing yields [6]. A maize breeder claimed that the maximum LAI of erectophile maize should be 5.0–5.5 [17], while other studies have reported that 6.0 is the optimal LAI for maize [18,19]. More recent studies have observed maximum LAIs of more than 6.0 and even more than 8.0 in super

high-yield cultivation [7,20–22]. So, are higher LAIs better as density increases and what is the optimal LAI? The answers to those questions are yet unclear.

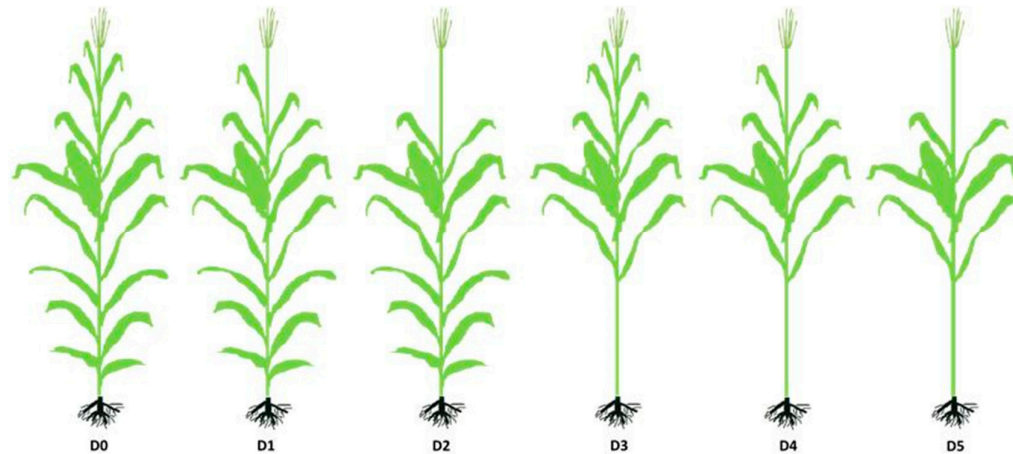
Crop canopy structure may be modified by partially removing vegetative organs to increase energy-use efficiency, especially that of solar radiation, and thus maximize yield [23–25]. Decreasing the LAI of densely planted maize can change the canopy structure, increase both the light intensity in the canopy and the photosynthetic capacity of the leaves, and thus increase the grain yield [15,16,26,27]. Changes in production capacity of source during grain filling are frequently accompanied by a marked change in stem weight as the supply of assimilates temporarily stored in the stem buffers the plant's demand for assimilates from the sink, which changes the source–sink ratio and the grain yield [15,26,28,29]. The source–sink ratio was defined as the aboveground biomass increase per kernel during a given period, and optimal reduction of leaf area can increase post-silking source–sink ratio and yield [15]. For example, Hao et al. [30] demonstrated that excising either 1/4 or 1/2 of all leaves on maize plants allowed more light into the field's canopy, thus allowing increased net photosynthesis and stomatal conductance in the remaining leaves at ear level. Some studies also reported that removal of the two uppermost leaves of maize plants increased grain yield [15,27]. Also, maize planted at a density of 10.0 plants m<sup>-2</sup>, showed significantly increased grain yield when the LAI was reduced from 8.2 to 5.5 following leaf removal [31]. Therefore, optimal reduction of the source could increase grain yield, which may be due to the fact that when the LAI exceeded 5.0, the maximum fraction of intercepted photosynthetically active radiation was 95% and canopy photosynthesis did not continuously increase [32,33]. However, most leaf removal studies have been conducted using lower planting densities (less than 105,000 plants ha<sup>-1</sup>) and with a maximum LAI of less than 7.0 [15,27,34,35]. Also, few of them have considered the effects of plant type.

To understand the effects of leaf removal on maize grain yield and clearly show the optimal LAI, we selected two morphologically different maize hybrids for a leaf removal experiment using a higher planting density at Xinjiang, China, where both the maize grain yield and the planting density are the highest in China. Our work, which examined the influences of leaf removal on two morphologically different types of maize plants, aids optimal LAI maize breeding, thus improving future higher density, maize cultivation.

## 2. Materials and Methods

### 2.1. Site and Experiment Design

We conducted field experiments at Qitai Farm (89°48'22" E, 43°49'27" N) in Xinjiang, China in 2017 and 2018 in an alternating narrow-wide-row planting pattern, where the narrow row was 0.4 m and the wide row was 0.7 m [6,36]. We selected a less compact maize cultivar ZD958 and a more compact cultivar DH618, the former having been widely planted in China and the latter having held the highest yield record, also in China [6]. Since DH618 is more compact than ZD958, it is more density-resistant than ZD958, so ZD958 and DH618 were sown at 120,000 and 135,000 plants ha<sup>-1</sup>, respectively. To create populations with different LAIs, different numbers and patterns of leaves were removed three days after silking (Figure 1), as follows: (1) control, no leaves removed (D0); (2) the two uppermost leaves removed (D1); (3) the four uppermost leaves removed (D2); (4) the leaves below the third leaf below the ear removed (D3); (5) the two uppermost leaves and the leaves below the third leaf below the ear removed (D4); and (6) the four uppermost leaves and the leaves below the third leaf below the ear removed (D5). We used a split-plot design with cultivars as the main plots and leaf-removal treatment groups as sub-plots. Individual sub-plot was 10 m long by 2.75 m width with five rows and an average row space of 0.55 m ((0.7 + 0.4)/2), and all plots were arranged in a completely randomized design with three replications.



**Figure 1.** Schematic diagram of different leaf removal treatments. D0 was the control with no leaves removed, D1 with the two uppermost leaves removed, D2 with the four uppermost leaves removed, D3 with the leaves below the third leaf below the ear removed, D4 with the two uppermost leaves and the leaves below the third leaf below the ear removed, D5 with the four uppermost leaves and the leaves below the third leaf below the ear removed.

We sowed seeds in mid-April and harvested plants in mid-October. Soil (0 to 60 cm depth) physicochemical properties and field management protocols were mentioned in our previous study [36].

## 2.2. Sampling and Measurement

At four days after silking and at physiological maturity, we collected three uniform and representative plants from the central rows of each plot and measured leaf length (L) and maximum leaf width (W) of each green leaf to calculate leaf area (LA):  $LA = 0.75 \times L \times W$ . The leaf area index (LAI) was calculated as follows:  $LAI = \text{Total LA per plant} \times N/S$ , where N is the number of plants within a unit area of land and S is the unit area of land. The plants were then separated into stalk (tassels were included with the stalk), leaf, sheath, cob, husk, and grain and all separated components were oven-dried at 85 °C to a constant weight and then those weights were recorded.

$$\text{Dry matter post-silking} = \text{Dry matter at maturity} - \text{Dry matter at silking} \quad (1)$$

$$\text{Harvest index (HI)} = \text{Grain yield/Dry matter at maturity} \quad (2)$$

The post-silking source–sink ratio was defined as the aboveground biomass increase per kernel during a given period [15]. We defined the biomass increase as the difference in plant biomass between physiological maturity and four days after silking. We counted kernel number per plant at maturity of ten selected ears.

Intercepted photosynthetically active radiation (IPAR) was measured above the canopy and transmitted PAR (TPAR) was measured with a diagonal orientation across the wide row and narrow row widths at the bottom (below the lowest green leaves) and at the ear above the ground with six replications, respectively. Photosynthetically active radiation measured in the wide and narrow row widths were averaged in each layer. In 2017 and 2018, fractions of intercepted solar radiation were measured using a line quantum sensor (SunScan, Delta-T, Cambridge, UK) at 1100–1300 on clear days after the leaves had been removed.

$$\text{Light transmission} = \frac{\text{TPAR}}{\text{IPAR}} \times 100\% \quad (3)$$

Photosynthesis and the related physiological variables were measured 40 days after leaf removal and an ear leaf from each of four uniform and representative plants per plot was chosen for

photosynthesis rate (Pn) and stomatal conductance (Cn) measurement in 2018. We performed the measurements using a portable photosynthesis system (Li-Cor 6400, Li-Cor Inc., Lincoln, NE, USA) with a controlled light density of  $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The  $\text{CO}_2$  concentration in the leaf chamber was controlled at  $400 \mu\text{mol CO}_2 (\text{mol air})^{-1}$  [37], the ambient temperature was  $24\text{--}26^\circ\text{C}$ .

When the maize plants were physiologically mature, we harvested grain from 5 m of two successive rows in the centers of each plot to calculate the final yield, with three replications. Using 10 selected ears from each replication according to the average ear weight, we counted numbers of kernel rows per ear and number of kernels per row (according to the average weight per ear) to calculate kernel number per ear (KNP) as follows:  $\text{KNP} = \text{kernel rows per ear} \times \text{kernel number per row}$ . Then the 10 ears were threshed and grain moisture contents were tested with a portable moisture meter (PM8188, Kett, Japan). Finally, the grain yield and thousand kernel weights (TKW) were calculated at 14.0% moisture.

### 2.3. Statistical Analysis

After leaf removal, the differences of LAI and other indices (grain yield, yield component, dry matter accumulation, Pn, Cn, HI, post-silking source–sink ratio) between different treatments were tested by using one-way analysis of variance (ANOVA) with the Duncan test at a 5% significance level. We conducted univariate analyses to examine interactions with grain yield and yield components as dependent variables and the year, cultivar, and leaf removal as independent variables. All data analysis was conducted by using SPSS v. 21.0 (IBM Inc., Armonk, NY, USA).

## 3. Results

### 3.1. Grain Yield and Yield Components

Table 1 shows the yield and yield components of ZD958 and DH618 after leaf removal in 2017 and 2018. In treatment D0, DH618's grain yield was higher than that of ZD958 in both years. In 2017, ZD958 obtained its highest grain yield in D4 and it was significantly greater than that in D0, but its KNP and TKW in D4 that year did not significantly increase after leaf removal and its ear number per ha in D4 was 7.1% higher than in D0. In 2017, cultivar DH618's highest grain yield was in D3 and it was significantly greater than its yield in D0; its KNP in D3 decreased by 7.1%; and ear number and TKW in D3 increased by 3.9% and 6.2%, respectively, compared to D0.

In 2018, cultivars ZD958 and DH618 obtained their highest yields in D3 and D4, respectively; however, they were not significantly different than yields in D0. Also, ear number, KNP, and TKW of ZD958 in D3 and of DH618 in D4 were not significantly different than those measures in D0.

The interactions among year, cultivar, and leaf removal treatment are shown in Table 1; however, there was no significant interaction on grain yield, but there were partial interactions on yield components. In addition, there was significant influence on grain yield and yield components between years, cultivars, and leaf removals, respectively. Therefore, we analyzed the effects of leaf removal on the two cultivars independently.

**Table 1.** Yield and yield components of ZD958 and DH618 under different treatments in 2017 and 2018.

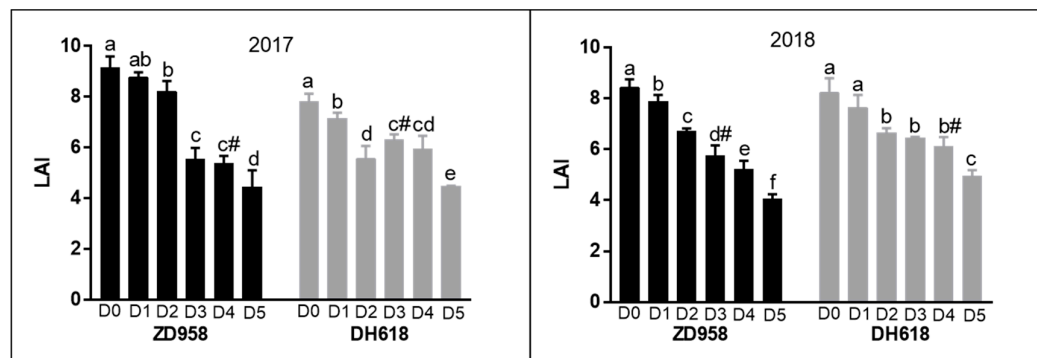
Year	Cultivar	Treatment	Yield ( $\text{Mg ha}^{-1}$ )	Ear Number ( $10^4 \text{ ears ha}^{-1}$ )	KNP	TKW (g)
2017	ZD958	D0	$19.4 \pm 0.7\text{b}$	$11.3 \pm 0.0\text{a}$	$469 \pm 15\text{ab}$	$382.5 \pm 16.9\text{a}$
		D1	$20.0 \pm 1.4\text{ab}$	$11.7 \pm 1.2\text{a}$	$530 \pm 80\text{a}$	$358.6 \pm 13.4\text{a}$
		D2	$18.3 \pm 0.4\text{b}$	$12.1 \pm 0.5\text{a}$	$450 \pm 5\text{b}$	$369.3 \pm 6.0\text{a}$
		D3	$19.0 \pm 1.7\text{b}$	$11.6 \pm 0.6\text{a}$	$475 \pm 20\text{ab}$	$364.4 \pm 13.6\text{a}$
		D4	$21.5 \pm 0.7\text{a}$	$12.1 \pm 0.6\text{a}$	$445 \pm 18\text{b}$	$371.8 \pm 10.6\text{a}$
		D5	$19.0 \pm 1.2\text{b}$	$12.4 \pm 0.3\text{a}$	$409 \pm 18\text{b}$	$375.2 \pm 11.7\text{a}$
	DH618	D0	$19.8 \pm 0.6\text{b}$	$12.8 \pm 0.2\text{a}$	$424 \pm 7\text{a}$	$401.3 \pm 6.9\text{c}$
		D1	$20.5 \pm 0.6\text{ab}$	$13.2 \pm 0.5\text{a}$	$356 \pm 30\text{bc}$	$457.3 \pm 14.4\text{a}$

2018	ZD958	D2	19.7 ± 2.4b	13.1 ± 1.5a	355 ± 17bc	453.9 ± 15.9ab
		D3	22.1 ± 0.1a	13.3 ± 0.9a	394 ± 18ab	426.3 ± 16.5bc
		D4	20.9 ± 0.7ab	13.4 ± 0.3a	357 ± 19bc	441.8 ± 5.1ab
		D5	18.9 ± 0.7b	13.2 ± 0.3a	340 ± 32c	442.1 ± 22.2ab
		D0	17.6 ± 2.8ab	9.6 ± 1.3abc	493 ± 42a	368.4 ± 18.7a
		D1	18.6 ± 1.7a	8.1 ± 0.1c	535 ± 10a	390.4 ± 31.3a
		D2	17.8 ± 2.1a	9.5 ± 1.0abc	501 ± 13a	359.8 ± 12.6a
		D3	19.3 ± 1.9a	8.8 ± 0.5bc	502 ± 23a	365.1 ± 12.6a
	DH618	D4	17.8 ± 1.0a	10.3 ± 0.7ab	501 ± 47a	332.3 ± 1.0a
		D5	15.1 ± 0.5b	10.7 ± 0.6a	496 ± 32a	363.0 ± 68.1a
		D0	19.4 ± 2.1a	10.7 ± 0.2ab	453 ± 56a	405.4 ± 5.0b
		D1	18.7 ± 1.4a	11.6 ± 0.7ab	404 ± 44ab	413.7 ± 15.2b
		D2	17.5 ± 1.3a	12.8 ± 0.1a	385 ± 3ab	400.8 ± 19.4b
		D3	18.9 ± 0.9a	12.4 ± 0.9a	394 ± 9ab	401.6 ± 15.1b
		D4	19.8 ± 1.2a	10.3 ± 0.3b	449 ± 21ab	403.8 ± 10.2b
		D5	17.8 ± 0.9a	12.7 ± 2.3a	369 ± 68b	488.9 ± 60.9a
Source of variation						
Year (Y)		**	**	**	*	
Cultivar (C)		**	**	**	**	
Leaf removal (L)		**	**	**	*	
Y × C		ns	*	ns	ns	
Y × L		ns	ns	ns	ns	
C × L		ns	*	**	*	

Values were means ± standard deviation (STD). Within each year, different lowercase letters of the same cultivar in the same column showed the significant difference between each datum at  $P < 0.05$ . KNP, kernel number per ear; TKW, thousand kernel weight. \* Significant at  $P \leq 0.05$ ; \*\* Significant at  $P \leq 0.01$ ; ns, non-significant.

### 3.2. Maximum Leaf Area Index (LAI)

In 2017, the LAIs of ZD958 in D0, D1, D2, D3, D4, and D5 were 9.1, 8.8, 8.2, 5.6, 5.4, and 4.5, respectively, and were 7.8, 7.2, 5.6, 6.3, 5.9, and 4.5, respectively, for DH618 (Figure 2). In 2018, the LAIs (D0, D1, D2, D3, D4, and D5) were 8.4, 7.9, 6.7, 5.8, 5.2, and 4.1, respectively, for ZD958 and were 8.2, 7.6, 6.5, 6.7, 6.1, and 4.9, respectively, for DH618. The LAIs of ZD958 and DH618 were 5.4 and 6.3, respectively, when the highest grain yield was obtained in 2017, but LAIs were significantly less than those in D0. The LAIs of ZD958 and DH618 were 5.8 and 6.1, respectively, when the highest grain yields were obtained in 2018, but they were significantly less than the LAIs in D0. Leaf area indexes were similar (5.4 and 5.8 for ZD958, 6.3 and 6.1 for DH618) when the highest yield were obtained in 2017 and 2018 for both cultivars.



**Figure 2.** Leaf area index (LAI) of maize cultivars ZD958 and DH618 under different treatments at silking in 2017 and 2018. Within each year, different lowercase letters of the same cultivar above the

columns showed the significant difference between each treatment at  $P < 0.05$ . # denotes treatments with highest grain yield.

### 3.3. Dry Matter Accumulation and Post-Silking Source–Sink Ratio

In 2017, ZD958's accumulated dry matter at maturity in D4 was not significantly different than that in D0, but dry matter during both post-silking and the HI in D4 increased significantly compared to those measures in D0 (Table 2). That year 2017 in D3, DH618's accumulated dry matter at maturity and during post-silking, and its HI, were not significantly different with those measures in D0. Dry matter accumulation at maturity and during post-silking, as well as the HI, of ZD958 in D3 and DH618 in D4 increased in 2018; however, they were not significantly different with those measures in D0. Optimal leaf removal can increase the post-silking source-sink ratio significantly in 2017, while not in 2018. Furthermore, we also found that extremely lowered LAI caused by excessive leaf removal may limit dry matter accumulation during post-silking, which further exacerbates low dry matter accumulation at maturity. In addition, ZD958's HI increased significantly after leaf removal in 2017. Even though there was no significant difference between the ZD958's HIs in D3 and D0 in 2018, the HI still increased by 5.4% after leaf removal. However, there was no significant increase in DH618's HIs in 2017 and 2018. Therefore, cultivar ZD958 was more affected by leaf removal than was DH618.

**Table 2.** Dry-matter accumulation at three different development stages, harvest index (HI) and post-silking source–sink ratio of maize cultivars ZD958 and DH618 in different treatments in 2017 and 2018.

Year	Cultivar	Treatment	Dry Matter (Mg ha <sup>−1</sup> )			HI	Source–sink Ratio (g/kernel)
			Silking	Post-silking	Maturity		
2017	ZD958	D0	19.8 ± 1.5a	15.0 ± 2.8cd	34.7 ± 1.5a	0.47 ± 0.02b	0.27 ± 0.01d
		D1	19.2 ± 2.3a	13.3 ± 2.3d	32.5 ± 0.0b	0.53 ± 0.01a	0.21 ± 0.03e
		D2	16.9 ± 1.5a	17.4 ± 1.5bc	34.3 ± 0.1a	0.53 ± 0.01a	0.32 ± 0.00c
		D3	16.9 ± 1.7a	18.5 ± 1.3b	35.4 ± 1.2a	0.56 ± 0.01a	0.32 ± 0.01c
		D4	13.9 ± 0.8b	21.9 ± 0.9a	35.8 ± 0.4a	0.55 ± 0.04a	0.41 ± 0.02a
	DH618	D5	13.6 ± 0.6b	17.9 ± 0.5bc	31.5 ± 0.5b	0.56 ± 0.01a	0.36 ± 0.02b
		D0	17.9 ± 0.6a	20.5 ± 0.5bc	38.3 ± 0.5a	0.53 ± 0.01a	0.36 ± 0.01c
		D1	16.0 ± 0.9b	19.9 ± 2.7bc	35.9 ± 3.6a	0.54 ± 0.06a	0.41 ± 0.04b
		D2	15.6 ± 1.3b	23.5 ± 1.2a	39.2 ± 0.2a	0.54 ± 0.01a	0.49 ± 0.02a
		D3	15.3 ± 0.2b	22.4 ± 0.5ab	37.7 ± 0.3a	0.55 ± 0.01a	0.42 ± 0.02b
	ZD958	D4	13.4 ± 0.3c	24.0 ± 1.0a	37.4 ± 1.2a	0.52 ± 0.02a	0.50 ± 0.03a
		D5	13.3 ± 0.8c	18.5 ± 2.1c	31.8 ± 1.7b	0.53 ± 0.01a	0.40 ± 0.04bc
		D0	14.3 ± 1.1a	23.8 ± 1.3a	38.0 ± 2.2a	0.56 ± 0.00a	0.40 ± 0.04a
		D1	14.0 ± 1.0a	20.3 ± 0.7ab	34.3 ± 5.1ab	0.56 ± 0.02a	0.32 ± 0.01bc
		D2	13.4 ± 1.0ab	17.6 ± 2.6bc	31.0 ± 1.9b	0.56 ± 0.02a	0.29 ± 0.01c
2018	DH618	D3	12.9 ± 0.9ab	25.6 ± 3.9a	38.4 ± 3.1a	0.59 ± 0.02a	0.42 ± 0.02a
		D4	12.6 ± 0.9ab	20.7 ± 1.7abc	33.3 ± 0.9ab	0.57 ± 0.02a	0.34 ± 0.03b
		D5	12.0 ± 0.9b	16.6 ± 2.8c	28.6 ± 2.0b	0.57 ± 0.04a	0.28 ± 0.02c
		D0	15.0 ± 1.1a	29.6 ± 2.0a	44.6 ± 2.5ab	0.54 ± 0.00b	0.48 ± 0.06ab
		D1	14.7 ± 1.1a	25.5 ± 0.9b	40.2 ± 0.1b	0.54 ± 0.01b	0.47 ± 0.05ab
	ZD958	D2	13.9 ± 1.0a	16.2 ± 2.2d	30.1 ± 3.5d	0.51 ± 0.00c	0.31 ± 0.00c
		D3	14.2 ± 1.1a	25.3 ± 1.7b	39.5 ± 2.5b	0.56 ± 0.01ab	0.48 ± 0.01ab
		D4	13.8 ± 1.0a	32.0 ± 1.2a	45.8 ± 0.3a	0.55 ± 0.01b	0.53 ± 0.02a
		D5	13.1 ± 0.9a	20.7 ± 1.5c	33.8 ± 1.4c	0.57 ± 0.00a	0.42 ± 0.03b

Values were means ± STD. Within each year, different lowercase letters of the same cultivar in the same column showed the significant difference between each datum at  $P < 0.05$ .

### 3.4. Light Distribution

In 2017, light transmission at the bottom green leaves of ZD958 increased significantly in D4, compared to that in D0; however, there was no significant difference between D3 (treatment with highest grain yield) and D0 for DH618 (Table 3). However, that same year, light transmission at the ear, between D4 and D0 for ZD958 and between D3 and D0 of DH618, did not differ significantly. In 2018, light transmission at the bottoms of ZD958 in D3 and of DH618 in D4 increased significantly compared to that in D0 of both cultivars. Light transmission at the ear for ZD958 in D3 had also increased significantly; however, there was no significant difference between D4 and D0 for DH618. In addition, although light transmission was high in both years at both the bottoms and at the ears in D5 for both cultivars, the grain yield was not the highest in D5, a result that may be attributed to low light interception at the canopy. In summary, the influences of leaf removal on light transmission on cultivar ZD958 was greater than that on DH618.

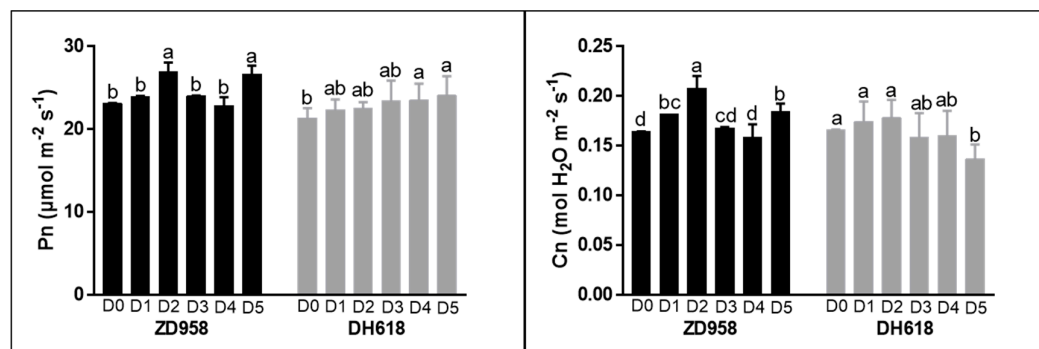
**Table 3.** Light transmission measured at the lowest green leaves and at the ears of cultivars ZD958 and DH618 under different treatments in 2017 and 2018.

Year		2017		2018	
Transmission (%)		Bottom	Ear	Bottom	Ear
ZD958	D0	0.6 ± 0.2b	3.6 ± 0.2b	1.1 ± 0.4c	9.4 ± 3.9b
	D1	0.2 ± 0.0b	8.5 ± 2.6ab	3.2 ± 0.1b	16.4 ± 2.3a
	D2	0.7 ± 0.0b	8.9 ± 1.9ab	3.6 ± 1.4b	21.4 ± 8.0a
	D3	5.3 ± 1.7a	9.3 ± 1.4ab	7.1 ± 2.9b	13.9 ± 3.7a
	D4	8.3 ± 0.9a	13.3 ± 4.2ab	7.2 ± 3.4b	16.0 ± 1.0a
	D5	7.9 ± 2.1a	14.1 ± 3.8a	20.0 ± 2.5a	21.1 ± 1.8a
DH618	D0	0.5 ± 0.2b	2.7 ± 0.6b	1.1 ± 0.6b	14.3 ± 2.5a
	D1	0.6 ± 0.2b	6.3 ± 1.7ab	0.8 ± 0.3b	17.7 ± 4.4a
	D2	2.5 ± 0.8b	10.8 ± 1.4a	0.7 ± 0.3b	21.3 ± 2.7a
	D3	1.7 ± 0.3b	3.6 ± 1.0ab	6.2 ± 2.2a	14.8 ± 0.9a
	D4	2.8 ± 0.9ab	8.2 ± 1.4ab	8.4 ± 3.2a	18.7 ± 3.0a
	D5	4.7 ± 2.1a	9.3 ± 3.9ab	10.8 ± 6.5a	22.6 ± 8.3a

Values were means ± STD. Within each year, different lowercase letters of the same cultivar in the same column showed the significant difference between each datum at  $P < 0.05$ .

### 3.5. Photosynthetic Rate and Stomatal Conductance

ZD958's Pn in treatments D2 and D5, in which the topmost four leaves were removed, increased significantly compared to that in D0 (Figure 3). Similarly, DH618's Pn increased significantly in D4 and D5 compared to its Pn in D0. The Pns of ZD958 in D2 and D5 were 16.5% and 15.6% greater, respectively, than in D0, and the Pns of DH618 in D4 and D5 were 9.8% and 12.6% greater, respectively, than in D0. Again, the influence of leaf removal on cultivar ZD958 was greater than it was on DH618.



**Figure 3.** Ear leaf photosynthetic rate (Pn) and stomatal conductance (Cn) at the grain filling stage of maize cultivars ZD958 and DH618 after six leaf removal treatments in 2018. Different lowercase



letters of the same cultivar above the columns showed the significant difference between each treatment at  $P < 0.05$ .

The stomatal conductances (Cns) of ZD958 increased significantly when the topmost four leaves were removed in treatments D2 and D5, compared to D0 (Figure 3). However, DH618's Cns were not significantly different between treatments, except for D5, which was significantly less than D0. Cns of both ZD958 and DH618 in D5 were significantly less than in D2, in which the leaves below the third leaf under the ear leaf were simultaneously removed. Therefore, a very low LAI may have adverse effects on Cn. Yet, when we compared Cn responses of both ZD958 and DH618 to the leaf removal treatments in D2 and D5, cultivar ZD958 was more affected by leaf removal than was cultivar DH618.

#### 4. Discussion

When planted at high density, erect maize plant types improve light conditions in the canopy, thus improving grain yield [6,36,38]. Decreasing the LAI of densely planted maize can also increase light intensity in the canopy and the photosynthetic capacity of the leaves, again boosting grain yield [15,16,26]. In addition, other studies have reported that partially removing vegetative organs can modify canopy structure, increase the corn plants' light energy-use efficiency, and maximize grain yields [16,23,24]. In this study, the highest grain yields in 2017 were 21.5 Mg ha<sup>-1</sup> for ZD958 in D4 (LAI = 5.4) and 22.1 Mg ha<sup>-1</sup> for DH618 in D3 (LAI = 6.3). Then, in 2018, the highest yields were 19.3 Mg ha<sup>-1</sup> for ZD958 in D3 (LAI = 5.8) and 19.8 Mg ha<sup>-1</sup> for DH618 in D4 (LAI = 6.1), with an average LAI of 5.6 and 6.2 for the highest yields of ZD958 and DH618, respectively, in the two years combined. In both years, average yield increases were greater for ZD958 than for the more compact DH618 (Table 1), indicating that leaf removal had a greater influence on the less compact hybrid. However, excessively low LAI may not contribute to grain yields because of a weak sink (lower KNP), as shown in D5 (Table 1) and in accordance with Liu et al. [15,27].

The yield components were important for the final grain yield [39]. Different cultivars had different responses to plant density by adjusting yield components. For example, the number of kernels per row and kernel weight were important under low plant population, while number of rows per ear and number of kernels per ear were important for a high plant population [40]. However, in the present study, the yield components had no significant changes compared to D0, therefore, the increase in yield may be the result of these small non-significant changes.

Dry-matter production has been closely related to photosynthesis capacity, especially post-silking dry matter accumulation [20,41]. Also, leaf removal can increase both total dry-matter accumulation, again especially post-silking dry matter accumulation, and the post-silking source-sink ratio, and thus also grain yield [15,27]. Optimal reduction of leaf area can increase post-silking source-sink ratio and canopy apparent photosynthesis [15]. In our study, total dry-matter production for the two cultivars in the treatments in which they had their highest grain yields were not significantly higher than that in the control treatments (D0), however, the source-sink ratio significantly increased compared with D0 (Table 2), which was consistent with a previous study [15]. In addition, harvest index was also important for grain yield [42], ZD958's HI increased more than DH618's after optimal leaf removal in 2017, however, the HI of DH618 had not increased significantly in the highest yield treatment in the two experimental years, incompletely agreeing with Liu et al. [15], which may be due to the different influence of leaf removal on morphologically different cultivars.

Leaves at the middle strata of the maize canopy contribute more photosynthates to grain than do other leaves [43], however, leaves at the lower strata may suffer from weak light condition and increase the consumption of respiration which was related to leaf age [44], therefore, the highest yield in the leaf removal treatment (D3 or D4) maybe due to the removal of the lower strata leaves after silking. The middle leaves are the main functional leaves for dry-matter production, and they obtain more solar radiation when the leaves in the upper canopy are upright [45]. Studies have also shown that smaller individual leaf area per plant [46] or partially removing vegetative organs can



improve crop structure and raise corn plant energy-use efficiency [23,24]. We found that light transmission increased significantly after leaf removal, but ZD958's incremental was greater than that of DH618, thus demonstrating a greater effect of leaf removal on the less compact ZD958 cultivar (Table 3). In addition, excessively lower LAI may result in the loss of light intercepted by the canopy, as in treatment D5, which agrees with Liu et al. [15]. Hao et al. [30] found that as light distribution improved after leaf removal, the Pn and Cn of the remaining ear leaves also increased. In our study, ZD958's Pn and Cn increased significantly in treatments D2 and D5, in which the four topmost leaves were removed. We also found that the Pn and Cn of the ear leaf in ZD958 had not significantly increased in the highest yield treatment, however, the Pn of DH618 had increased significantly, which was not consistent with the study of Liu et al. [15]. Therefore, leaf removal had a different influence on the Pn and Cn of the ear leaf, in order to clarify the mechanism of yield increase by leaf removal, further study should consider the influence of leaf removal on canopy-level photosynthesis. However, excessive leaf removal did not increase the grain yield, which in this study was mainly related to the number of leaves left above the ear leaf. Cultivar ZD958 had about six leaves above the ear leaf, which was seven in DH618. Removal of both the topmost two leaves and the leaves below the third leaf below the ear leaf (D4) in 2017, and removal of the leaves below the third leaf below the ear leaf (D3) in 2018, significantly increased ZD958's grain yield, which was in D3 in 2017 and D4 in 2018 for DH618. Therefore, leaf removal below the ear for both more and less compact cultivars benefits grain yield, and those leaves would senesce gradually anyway because of poor light conditions in high density planting [47].

A maize breeder once stated that the maximum LAI of erect maize should be 5.0–5.5 [17], and also a study indicated that the optimal LAI for high-yield maize was 4.5 under a density of no more than 75,000 plants m<sup>-2</sup> [48], while subsequent studies have reported that the optimal LAI for maize is 6.0 [18,19], because when the LAI exceeds 5.0, the maximum fraction of IPAR was 95% and canopy photosynthesis will not continuously increase by using maize hybrids [32,33]. We found that after leaf removal the highest grain yields were obtained when the LAIs of ZD958 and DH618 were 5.4 and 6.3, respectively, in 2017, and 5.8 and 6.1, respectively, in 2018 (Figure 2) with an average LAI of 5.9. Although we used high planting densities, the optimal LAI changed little, a result similar to those of previous studies that showed that leaf redundancy occurred during recent dense plantings of maize [6,27]. Therefore, optimizing the LAI, neither too high nor too low, is beneficial to grain yield.

## 5. Conclusions

Optimal leaf removal improved light distribution, increased photosynthetic capacity and the post-silking source–sink ratio, and thus the grain yield, with an average LAI of 5.9 (5.6 and 6.2 for ZD958 and DH618, respectively) for the highest yields in each year. Therefore, optimizing the LAI is beneficial to grain yield. In future maize breeding, less compact cultivars with less upright leaves in the upper canopy should have smaller or fewer topmost leaves or leaves below ear senesce quickly after silking to decrease leaf area. This will be beneficial to improve both light distribution and photosynthetic capacity in the canopy and thus improve grain yield. However, for more compact cultivars with more upright leaves in the upper canopy, leaves below the ear should senesce quickly after silking to reduce leaf respiration and improve photosynthetic capacity of the remaining top residual leaves. In future maize cultivation, compact cultivars with optimal post-silking LAI should be used when planting densely.

**Author Contributions:** All authors have read and agree to the published version of the manuscript. Conceptualization, P.H.; methodology, P.H. and G.L.; validation, H.P. and S.L.; formal analysis, G.L., P.H., J.X., B.M., R.X., K.W., S.L.; investigation, G.L., Y.Y., W.L., X.G.; resources, P.H., K.W., S.L.; data curation, G.L.; writing—original draft preparation, G.L.; writing—review and editing, G.L. and P.H.; visualization, G.L., P.H.; supervision, S.L. and P.H.; project administration, P.H.; funding acquisition, P.H. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the National Key Research and Development Program of China (2016YFD0300110 and 2016YFD0300101), the National Natural Science Foundation of China (31871558, 31501266, and 31371575), and the National Basic Research Program of China (973, Program 2015CB150401).

**Acknowledgments:** The authors thank the anonymous reviewers for their valuable comments and suggestions. The first author also thanks all the other co-authors' support and assistance all the time.

**Conflicts of Interest:** The authors declare that there are no conflicts of interest.

**Abbreviations:** LAI: leaf area index; KNP, kernel number per ear; TKW, thousand kernel weight; IPAR, intercepted photosynthetically active radiation; TPAR, transmitted photosynthetically active radiation; Pn, photosynthesis rate; Cn, stomatal conductance; HI, harvest index

## References

1. Grassini, P.; Thorburn, J.; Burr, C.; Cassman, K.G. High-yield irrigated maize in the western US Corn Belt I. On-farm yield, yield potential, and impact of agronomic practices. *Field Crop. Res.* **2011**, *120*, 142–150.
2. Gandhi, V.P.; Zhou, Z. Food demand and the food security challenge with rapid economic growth in the emerging economies of India and China. *Food Res. Int.* **2014**, *63*, 108–124.
3. Long, S.P.; Zhu, X.G.; Naidu, S.L.; Ort, D.R. Can improvement in photosynthesis increase crop yields? *Plant Cell Environ.* **2006**, *29*, 315–330.
4. Meng, Q.F.; Hou, P.; Wu, L.; Chen, X.P.; Cui, Z.L.; Zhang, F.S. Understanding production potentials and yield gaps in intensive maize production in China. *Field Crop. Res.* **2013**, *143*, 91–97.
5. Tokatlidis, I.S.; Koutroubas, S.D. A review of maize hybrids' dependence on high plant populations and its implications for crop yield stability. *Field Crop. Res.* **2004**, *88*, 103–114.
6. Liu, G.Z.; Hou, P.; Xie, R.Z.; Ming, B.; Wang, K.R.; Xu, W.J.; Liu, W.M.; Yang, Y.S.; Li, S.K. Canopy characteristics of high-yield maize with yield potential of 22.5 Mg ha<sup>-1</sup>. *Field Crop. Res.* **2017a**, *213*, 221–230.
7. Xu, W.J.; Liu, C.W.; Wang, K.R.; Xie, R.Z.; Ming, B.; Wang, Y.H.; Zhang, G.Q.; Liu, G.Z.; Zhao, R.L.; Fan, P.P.; Li, S.K.; Hou, P. Adjusting maize plant density to different climatic conditions across a large longitudinal distance in China. *Field Crop. Res.* **2017**, *212*, 126–134.
8. Xue, J.; Gou, L.; Shi, Z.G.; Zhao, Y.S.; Zhang, W.F. Effect of leaf removal on photosynthetically active radiation distribution in maize canopy and stalk strength. *J. Integr. Agr.* **2017**, *16*, 85–96.
9. Christensen, L.E.; Below, F.E.; Hageman, R.H. The effect of ear removal on senescence and metabolism of maize. *Plant physiol.* **1981**, *68*, 1180–1185.
10. Maddonni, G.A.; Otegui, M.E. Intra-specific competition in maize: Early establishment of hierarchies among plants affects final kernel set. *Field Crop. Res.* **2004**, *85*, 1–13.
11. Huang, S.B.; Gao, Y.B.; Li, Y.B.; Tao, H.B.; Wang, P. Influence of plant architecture on maize physiology and yield in the Heilonggang River valley. *Crop J.* **2017**, *5*, 52–62.
12. Li, R.F.; Liu, P.; Yang, Q.L.; Ren, H.; Dong, S.T.; Zhang, J.W.; Zhao, B. Effects of lower leaf senescence on carbon and nitrogen distribution and yield formation in maize (*Zea mays* L.) with high planting density. *Acta Agron. Sin.* **2018**, *44*, 1032–1042.
13. Boomsma, C.R.; Santini, J.B.; Tollenaar, M.; Vyn, T.J. Maize morphophysiological responses to intense crowding and low nitrogen availability: an analysis and review. *Agron. J.* **2009**, *101*, 1426–1452.
14. Timlin, D.J.; Fleisher, D.H. Plant density and leaf area index effects on the distribution of light transmittance to the soil surface in maize. *Agron. J.* **2015**, *106*, 1828–1837.
15. Liu, T.N.; Gu, L.M.; Dong, S.T.; Zhang, J.W.; Liu, P.; Zhao, B. Optimum leaf removal increases canopy apparent photosynthesis, <sup>13</sup>C-photosynthate distribution and grain yield of maize crops grown at high density. *Field Crop. Res.* **2015**, *170*, 32–39.
16. Srinivasan, V.; Kumar, P.; Long, S.P. Decreasing, not increasing, leaf area will raise crop yields under global atmospheric change. *Global Change Biol.* **2017**, *23*, 1626–1635.
17. Li, D.H. Review and prospect in compact plant type breeding in maize. *Crops* **2000**, 1–5.
18. Kiniry, J.R.; Bean, B.; Xie, Y.; Chen, P.Y. Maize yield potential: critical processes and simulation modeling in a high-yielding environment. *Agr. Syst.* **2004**, *82*, 45–56.
19. Wang, X.P.; Li, B.G.; Guo, Y.; Zhai, Z.X. Measurement and analysis of the 3D spatial distribution of photosynthetically active radiation in maize canopy. *Acta Agron. Sin.* **2004**, *30*, 568–576.
20. Ma, Y.H.; Xue, J.Q.; Zhang, R.H.; Zhang, L.C.; Hao, Y.; Sun, J. Relationship between dry matter accumulation and distribution to yield of different maize cultivars. *Guangdong Agr. Sci.* **2010**, 36–40.

21. Roedel, V.R.J.; Couter, J.A. Agronomic responses of corn to planting date and plant density. *Agron. J.* **2011**, *103*, 1414–1422.
22. Yang, D.G.; Zhao, W.; Qin, D.L.; Liu, F.Y.; Zhang, Q.; Guan, Y.; Yang, K.J. Yield and canopy structure of maize under the condition of high yield cultivation. *J. Maize Sci.* **2016**, *24*, 129–135.
23. Sheng, C.F. Growth tediousness as an explanation of over compensation of crops for insect feeding. *J. Appl. Eco.* **1990**, *1*, 26–30.
24. Zhu, G.X.; Midmore, D.J.; Radford, B.J.; Yule, D.F. Effect of timing of defoliation on wheat (*Triticum aestivum*) central Queensland. 1. Crop response and yield. *Field Crop. Res.* **2004**, *88*, 211–226.
25. Chen, Y.Z.; Kong, X.Q.; Dong, H.Z. Removal of early fruiting branches impacts leaf senescence and yield by altering the sink/source ratio of field-grown cotton. *Field Crop. Res.* **2018**, *216*, 10–21.
26. Tollenaar, M.; Daynard, T.B. Effect of defoliation on kernel development in maize. *Can. J. Plant Sci.* **1978**, *58*, 207–212.
27. Liu, T.N.; Huang, R.D.; Cai, T.; Han, Q.F.; Dong, S.T. Optimum leaf removal increases nitrogen accumulation in kernels of maize grown at high density. *Sci. Rep.* **2017b**, *7*, 39601.
28. Tollenaar, M. Sink-source relationships during reproductive development in maize. A review. *Maydica* **1977**, *22*, 49–75.
29. Uhart, S.A.; Andrade, F.H. Nitrogen and carbon accumulation and remobilization during grain filling in maize under different source/sink ratios. *Crop Sci.* **1995**, *35*, 183–190.
30. Hao, M.B.; Wang, K.J.; Dong, S.T.; Zhang, J.W.; Li, D.H.; Liu, P.; Yang J.S.; Liu, J.G. Leaf redundancy of high-yielding maize (*Zea mays* L.) and its effects on maize yield and photosynthesis. *Chinese J. Appl. Ecol.* **2010**, *21*, 344–350.
31. Liu, Z.Q.; Xu, X.M.; Shen, H.B.; Sun, J.H.; Shen, F.Y.; Che, S.P.; Wang S.Y.; Lu, L. P. Effects of leaf cutting treatment to maize yield under the high plant population cultivation condition. *J. Maize Sci.* **2009**, *17*, 74–75, 81.
32. Maddonni, G.A.; Otegui, M.E. Leaf area, light interception, and crop development in maize. *Field Crop. Res.* **1996**, *48*, 81–87.
33. Stewart, D.; Costa, C.; Dwyer, L.; Smith, D.; Hamilton, R.; Ma, B. Canopy structure, light interception, and photosynthesis in maize. *Agron. J.* **2003**, *95*, 1465–1474.
34. Subedi, K.D.; Ma, B.L. Ear position, leaf area and contribution of individual leaves to grain yield in conventional and leafy maize hybrids. *Crop Sci.* **2005**, *45*, 2246–2257.
35. Wei, S.S.; Wang, X.Y.; Jiang, D.; Dong, S.T. Physiological and proteome studies of maize (*Zea mays* L.) in response to leaf removal under high plant density. *BMC Plant Biol.* **2018**, *18*, 378.
36. Liu, G.Z.; Hou, P.; Xie, R.Z.; Ming, B.; Wang, K.R.; Liu, W.M.; Yang Y.S.; Xu W.J.; Chen J.L.; Li, S.K. Nitrogen uptake and response to radiation distribution in the canopy of high-yield maize. *Crop Sci.* **2019**, *59*, 1236–1247.
37. Mu, X.H.; Chen, Q.W.; Chen, F.J.; Yuan, L.X.; Mi, G.H. Within-leaf nitrogen allocation in allocation in adaptation to low nitrogen supply in maize during grain-filling stage. *Front. Plant Sci.* **2016**, *7*, 699.
38. Ma, D.L.; Xie, R.Z.; Niu, X.K.; Li, S.K.; Long, H.L.; Liu, Y.E. Changes in the morphological traits of maize genotypes in China between the 1950 and 2000. *Eur. J. Agron.* **2014**, *58*, 1–10.
39. Hemingway, J.S. Effects of population density on yield of maize. *East Afric. Agri. J.* **1957**, *22*, 199–202.
40. Milander, L.; Jukic, Z.; Mason, S.; Glausha, T.; Kmail, Z. Plant population influence on maize yield components in Croatia and Nebraska. *Crop Sci.* **2016**, *56*, 2742–2750.
41. Zhou, B.Y.; Yue, Y.; Sun, X.F.; Wang, X.B.; Wang, Z.M.; Ma, W.; Zhao, M. Maize grain yield and dry matter production responses to variation in weather conditions. *Agron. J.* **2016**, *108*, 196–204.
42. Rivera-Amado, C.; Trujillo-Negrellos, E.; Molero, G.; Reynolds, M.P.; Sylvester-Bradley, R.; Foulkes, M.J. Optimizing dry-matter partitioning for increased spike growth, grain number and harvest index in spring wheat. *Field Crop. Res.* **2019**, *240*, 154–167.
43. Zhao, K.F. Effect of the leaves of different positions in maize on the corn yield and the photosynthetic properties of those leaves after the growing out of the female flowers. *Acta Agron. Sin.* **1981**, *7*, 259–266.
44. Stirling, C.M.; Aguilera, C.; Baker, N.R.; Long, S.P. Changes in the photosynthetic light response curve during leaf development of field grown maize with implications for modelling canopy photosynthesis. *Photosynth. Res.* **1994**, *42*, 217–225.
45. Austin, R.B.; Ford, M.A.; Edrich, J.A.; Hooper, B.E. Some effects of leaf posture on photosynthesis and yield in wheat. *Ann. Appl. Biol.* **1976**, *83*, 425–446.

46. Lambert, R.J.; Mansfield, B.D.; Mumm, R.H. Effect of leaf area on maize productivity. *Maydica* **2014**, *59*, 58–64.
47. Liu, T.N.; Xu, C.L.; Gu, L.M.; Dong, S.T. Effects of leaf removal on canopy apparent photosynthesis and individual leaf photosynthetic characteristics in summer maize under high plant density. *Acta Agron. Sin.* **2014**, *40*, 143–153.
48. Wang, Z.; Wu, Z.H.; Xu, K.Z. Relationship between leaf area index and photosynthetic rate of maize canopies. *J. Jilin Agri. Univer.* **2001**, *23*, 9–12, 16.



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).