The higher area-based photosynthesis in *Gossypium hirsutum* L. is mostly attributed to higher leaf thickness

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**Abstract**

There is a trade-off between leaf structures and photosynthesis physiology. While numerous reports have shown a difference in photosynthesis between *Gossypium hirsutum* L. (upland cotton) and *G. barbadense* L. (pima cotton), the potential contribution of leaf structures on this difference was not fully clarified. Here, we investigated the differences in area- and mass-based photosynthetic traits and leaf structures between upland and pima cottons. Our results showed that upland cotton had higher area-based net photosynthetic rate (*P*\textsubscript{area}) than that of pima cotton, which was attributed to the coordination of stomatal conductance (*g*s), area-based mesophyll conductance (*g*m\textsubscript{area}), and maximum carboxylation rate (*V*c\textsubscript{max-area}). *P*\textsubscript{area}, *g*s\textsubscript{area}, and *V*c\textsubscript{max-area} correlated positively with leaf mass per area. But upland and pima cotton had similar mass-based *P*\textsubscript{S} (*P*\textsubscript{max}, *g*m (*g*m\textsubscript{max}), *V*c\textsubscript{max} (*V*c\textsubscript{max-mass}), suggesting that they have a similar photosynthetic capacity of single cells. Consequently, the higher area-based values in upland cotton were only due to a higher leaf thickness (*T*\textsubscript{leaf}).

**Additional key words:** chlorophyll fluorescence; gas exchange; leaf density; leaf mass per area; nitrogen content.

**Introduction**

Leaf photosynthesis is determined by biochemical capacity (i.e., the maximum carboxylation rate, *V*c\textsubscript{max}, and the maximum electron transport rate, *J*\textsubscript{max}) and CO2 diffusion conductance from the atmosphere to chloroplasts, including stomatal conductance (*g*s) and mesophyll conductance (*g*m) (Flexas *et al*. 2007, 2008, 2012, 2016; Evans *et al*. 2009, Terashima *et al*. 2011). These traits should be tightly coordinated, thus maximizing photosynthetic efficiency (Wright *et al*. 2004a). The *g*s can be calculated relatively easily according to the concentration difference of water in and out of the stomata. But *in vivo* measurements of *g*m are not as straightforward as those of net photosynthetic rate (*P*\textsubscript{S}) and *g*s (Gago *et al*. 2016). Therefore, in the last decades, *g*m had been considered to be constant and infinite. Indeed, *g*m is variable and sufficiently small as to significantly limit photosynthesis to an extent similar to that known for *g*s (Flexas *et al*. 2006, 2008). Furthermore, it has been shown that stomatal, mesophyll conductance, and biochemical limitations to photosynthesis are of similar magnitude in leaves of angiosperm species (Flexas *et al*. 2016), but especially in crops (Nadal and Flexas 2018). Currently, there are three common *g*m estimation methods including the variable *J* method (Harley *et al*. 1992), the carbon isotope discrimination method (Evans *et al*. 1986), and the curve-fitting method (Ether and Livingston 2004, Sharkey *et al*. 2007, Sharkey 2016, Gu and Sun 2014), but they employ different assumptions and have their own shortcomings because of the very complex mesophyll diffusion process affected by many structural diffusion paths (e.g., leaf thickness, cell wall thickness, and chloroplast traits). Although a structures-based quantitative one-dimensional gas diffusion model of Niinemets and Reichstein (2003) as modified by Tosens *et al*. (2016) was also used to estimate *g*m, Parkhurst (1994) has indicated intercellular diffusion as fundamentally a three-dimensional process. Generally, the area-based photosynthetic parameters were often used to analyze the effects of leaf structures on photosynthetic traits, but some studies have shown that mass-based photosynthetic parameters can better reflect the photosynthetic capacity of single cells (Wostoby *et al*. 2013, Niinemets *et al*. 2015). Photosynthesis depends largely on CO2 concentration at the sites of carboxylation within chloroplasts (*C*); CO2 drawdown from the internal airspace to chloroplasts is a mesophyll-volume-weighted average.
(Niinemets et al. 2005, Niinemets and Sack 2006). Therefore, Niinemets and Sack (2006) indicated that the mass-based \( g_m \) (\( g_{m-mass} \)) is the true determinant of the volume-weighted average chloroplastic \( CO_2 \) concentration. Ellsworth et al. (2018) also proposed that modeled \( V_{max} \) and \( J_{max} \) normalized on a per-gram basis were effective in separating biochemical from anatomical effects on \( P_n \). Furthermore, the mass-based maximum \( P_n \) was more variable than the area-based maximum \( P_n \) in the global databases (Niinemets 1999, Wright et al. 2004b, Kattge et al. 2011), suggesting that the mass basis provides a more effective estimate of photosynthetic capacity for datasets with higher species coverage. An easily measurable leaf integrative structure trait, leaf mass per area (LMA), can be used to convert area-based photosynthetic parameters to mass-based ones. The relationships between area-based \( P_n \) (\( P_{area} \)) and LMA are often variable. This is partly because leaf thickness (\( T_{tot} \)) and leaf density (\( D_{tot} \)), which are the most important intrinsic drivers of LMA (Niinemets 2015), may influence leaf photosynthetic capacity in different ways (Niinemets 1999). But the relationship between mass-based \( P_n \) (\( P_{mass} \)) and LMA was found to be stronger than that between the \( P_{area} \) and LMA (Tosens et al. 2016). Numerous studies have indicated that low \( P_{mass} \) is associated with thick robust leaves and thus high LMA (Reich et al. 1997, Niinemets 2001, Wright et al. 2004b). Moreover, \( P_{mass} \) correlates positively with mass-based nitrogen content (\( N_{mass} \)), thus the leaves with high \( P_{mass} \) should have high nitrogen-use efficiency (Niinemets 2001, Wright et al. 2004a). The photosynthetic capacity per unit of nitrogen can be used to analyze the nitrogen investment proportion between anatomical structures and photosynthetic proteins. Consequently, the photosynthetic traits on the basis of mass should better reflect the trade-off between the physiological and structural characteristics of leaves (Westoby et al. 2013, Niinemets et al. 2015).

*Gossypium hirsutum* L. (upland) and *G. barbadense* L. (pima) are the most important fiber crops grown worldwide in more than 50 countries and play an important role in the global economy. They have obvious yield difference, fiber quality difference, and plant morphological difference. In previous works, it has been shown that upland cotton has higher photosynthesis than that of pima cotton due to larger \( g_s \) and much more photosynthetic tissues reflected by higher palisade thickness (Zhang et al. 2011). But these data are from area-based photosynthetic physiological analysis. At present, no studies analyzed the photosynthetic physiological and structural trading between these two species. The aims of the study were (1) to determine if \( g_m \) also plays an important role in the difference of photosynthesis between upland and pima cotton; (2) to reveal if there is trade-off between mass-based photosynthetic traits and leaf structures in upland and pima cotton.

**Materials and methods**

**Plant materials**: Four representative cotton cultivars (upland cotton – *Gossypium hirsutum* L. cv. Xinluzao 45 and Xinluzao 33 and pima cotton – *Gossypium barbadense* L. cv. Xinhai 28 and Xinhai 25) were used in this study. The experiment was conducted in an experimental field (a clay loam) of Shihezi Agricultural College, Shihezi University, Xinjiang, China (45°19'N, 86°03'E) in 2015. Seeds were sown on 21 April, 2015, in rows 12 cm apart at a plant density of \( 1.8 \times 10^3 \) ha\(^{-1} \). Cottons were drip irrigated according to local field irrigation level (495 mm). The plots were fertilized before sowing with 240 kg(N) ha\(^{-1} \) (urea), 170 kg(P\(_2\)O\(_5\)) ha\(^{-1} \) \( \left[(NH_4)_2PO_4\right] \), and 1,500 kg ha\(^{-1} \) of organic fertilizer [235 g(organic matter) kg\(^{-1} \), 18 g(total N) kg\(^{-1} \), 14 g(total P) kg\(^{-1} \), and 22 g(total K) kg\(^{-1} \)]. An additional 120 kg(N) ha\(^{-1} \) (urea) was applied by drip irrigation during the growing season. Weeds and pests were controlled in the field using standard management practices. Measurements were conducted on the topmost fully expanded leaf on the main stem of the cotton selected at random at the boll formation stage (about 80 d after sowing). Meteorological data during the growing season are shown in Fig. 1.

**Leaf gas exchange and Chl fluorescence parameters** were measured simultaneously using an open gas-exchange system (Li-6400, Li-Cor, Inc., Lincoln, NE, USA) connected to leaf fluorometer chamber (2 cm\(^2\), Li-6400-40, Li-Cor, Inc., Lincoln, NE, USA). At least three \( CO_2 \)-response curves were measured per cultivars using light-adapted mature leaves. Leaf temperature was set to 30°C. The photosynthesis measurements started at a \( CO_2 \) concentration surrounding the leaf (\( C_i \)) of 400 \( \mu \)mol mol\(^{-1} \) and a saturating PPFD of 2,000 μmol m\(^{-2} \) s\(^{-1} \). Once steady state was reached (usually 20 min after clamping the leaf), data were recorded. Immediately after that, the air inlet pipe was replaced with medical gas bag with 2% O\(_2\) and 98% N\(_2\), and a \( CO_2 \)-response curve (\( P_n/C_i \); \( C_i \) – the intercellular \( CO_2 \) concentration) was performed. After that, Li-COR inlet was disconnected from N\(_2\) medical gas bag (i.e., air with 21% O\(_2\) was supplied again to the plant). After reaching steady state, another \( P_s/C_i \) curve was performed. Gas exchange and Chl fluorescence were first measured at 400 \( \mu \)mol mol\(^{-1} \), then \( C_i \) was decreased.

**Fig. 1.** Daily maximum (open circles) and minimum (solid circles) air temperature and precipitation (bars) during the growing season at the experimental field.
stepwise until 50 μmol mol\(^{-1}\) upon completion of measurements at low \(C_n\), this was returned to 400 μmol mol\(^{-1}\) to restore the original \(P_n\). Then, \(C_i\) was increased stepwise to complete the curve. The number of different \(C_i\) values used for the curves was 12, and the time interval between two consecutive measurements at different \(C_i\) was restricted to 2–4 min, so that each curve was completed in 30–50 min. Leakage of CO\(_2\) into and out the leaf cuvette was determined with photosynthetically inactive leaves of each species enclosed in the leaf chamber (obtained by heating the leaves until no variable Chl fluorescence was observed), and used to correct measured leaf fluxes (Flexas et al. 2007). The steady-state fluorescence (\(F_s\)) and maximum fluorescence during the multiphasic saturation pulse flash [a light-saturating pulse at the end of phase 1 was ca. 8,000 μmol(photon) m\(^{-2}\) s\(^{-1}\)] (\(F_{s0}\)) were estimated, and the actual photochemical efficiency of PSII (\(\Phi_{PSII}\)) was calculated as \(\left(\frac{F'_{s0} - F_{s0}}{F_{s0}}\right)\) (Genty et al. 1989). The electron transport rate (\(J_{n0}\)) was then calculated as \(\Phi_{PSII} \times \alpha \times \beta \times PPFD\), where \(\alpha\) is leaf absorptance and \(\beta\) reflects the partitioning of absorbed quanta between PSI and PSII. The term \(\alpha\) was assumed to be 0.85 and \(\beta\) assumed value was 0.5. Numerous studies have shown that the estimation of \(J_{n0}\) is affected by PSI and the signal-to-noise ratio in the determination of \(F'_{s0}\) at high light. To overcome the uncertainties, electron transport from gas exchange (\(J_{n0}\)) under 2% \(O_2\) conditions was used to calibrate \(J_{n0}\) (see Pons et al. 2009 for details).

**Estimation of mesophyll conductance, \(g_m\), by gas exchange and Chl fluorescence and by the \(P_n/C_i\) curve fitting:** Mesophyll conductance (\(g_m\)) was estimated according to the method of Harley et al. (1992), as follows:

\[
g_m = \frac{P_n}{C_i} \times \frac{\left(\frac{J_{n0}}{4} + 8 \times (P_n + R_d)\right)}{J_{n0} + 4 \times (F_{s0} + R_d)} \tag{1}
\]

where \(P_n\) and \(C_i\) were taken from gas-exchange measurements at saturating light. \(\Gamma^*\) is the CO\(_2\)-compensation point in the absence of light respiration, and \(R_d\) is day respiration. \(\Gamma^*\) was taken according to Bernacchi et al. (2002) \([\Gamma^* = \text{EXP}(13.49 - 24,460/8.314/(273.15 + T_i))\)\], where \(T_i\) is the leaf temperature in °C. \(R_d\) was assumed to be 0.5 times of the measured dark respiration (\(R_d = R_i/2\)) (Piel et al. 2002, Niinemets et al. 2005). \(R_d\) was determined by gas-exchange measurement (Li-6400) after plants had been dark-adapted for more than half an hour in the evening. The maximum ribulose-1,5-bisphosphate carboxylation (\(V_{max}\)) and maximum electron transport rate (\(J_{m0}\)) were calculated from the \(P_n/C_i\) curves, using the Rubisco kinetic constants and their temperature dependencies described by Bernacchi et al. (2002). The model of Farquhar et al. (1980) was fitted to the data by applying iterative curve-fitting (minimum least-square difference) using the Solver tool of Microsoft Excel (Sharkey 2016). Meanwhile, an alternative estimate of \(g_m\) was obtained by the curve-fitting method introduced by Sharkey (2016). This method is based on changes in the curvature of \(P_n\) vs. \(C_i\) response curves due to a finite \(g_m\). By nonlinear curve fitting minimizing the sum of squared model deviations from the data, \(g_m\) can be estimated from observed data. The same leaves were used for estimation of \(g_m\) by the methods of Sharkey (2016) and Harley et al. (1992).

**Relative limitation analyses on \(P_n\):** According to Grassi and Magnani (2005), relative stomatal limitation (\(l_s\)), mesophyll limitation (\(l_m\)), and biochemical limitation (\(l_b\)) were investigated, respectively, in the cotton leaves. The quantitative changes in light-saturated assimilation can be expressed in terms of parallel changes in stomatal and mesophyll conductance and in biochemical capacity as follows:

\[
l_s = \frac{\frac{\partial P_n}{\partial C_i}}{\frac{\partial P_n}{\partial C_i}} \left(\frac{g_s}{g_{tot}}\right) \tag{2}
\]

\[
l_m = \frac{\frac{\partial P_n}{\partial C_i}}{\frac{\partial P_n}{\partial C_i}} \left(\frac{g_m}{g_{tot}}\right) \tag{3}
\]

\[
l_b = \frac{\frac{\partial P_n}{\partial C_i}}{\frac{\partial P_n}{\partial C_i}} \left(\frac{g_b}{g_{tot}}\right) \tag{4}
\]

where \(g_s\) is total conductance to CO\(_2\) between the leaf surface and the sites of carboxylation (\(1/g_s = 1/g_g + 1/g_m\)); \(l_s\), \(l_m\), and \(l_b\) are the corresponding relative limitations (\(0 < l_i < 1\); \(i = s, m, b\); \(l_s + l_m + l_b = 1\)). \(\partial P_n/\partial C_i\) was calculated as the slope of \(P_n/C_i\) response curves over a \(C_i\) range of 50–100 μmol mol\(^{-1}\) (Tomás et al. 2013).

**Light microscopy:** After the gas-exchange and Chl fluorescence measurements, sections of 2 × 2 mm were cut between the main veins and subjected to microscopic analysis. Leaf samples were fixed by infiltration of 4% glutaraldehyde and 3% paraformaldehyde in phosphate buffer (0.1 mol L\(^{-1}\), pH 7.2) under vacuum. Leaf samples were fixed again in 1% osmium tetroxide overnight and dehydrated in a graded acetone series and embedded in Spurr’s resin. Semi-thin (1 μm) cross-sections were prepared with an ultramicrotome (Leica Ultracut R). Semi-thin cross-sections for light microscopy were stained with 0.5% toluidine blue and observed under light microscope with a digital camera (BH-2, Olympus). Leaf thickness (\(T_{leaf}\)) the thickness of palisade and spongy tissue layers were obtained and six different positions were measured in each sample.

**The mass per area and nitrogen content:** Leaf mass per unit area (LMA) is the ratio of dry mass and leaf area. Dry mass was determined from oven-dried certain area of leaf discs after 48 h at ca. 80°C. Dividing LMA by leaf thickness is defined as leaf density.

For the measurement of the nitrogen content, leaves were harvested on the same day. Total nitrogen content of the dried tissues was determined according to the micro-Kjeldahl method (Schuman et al. 1972).
**Results**

The daily minimum and maximum temperature and precipitation during the growing season at the experimental field is shown in Fig. 1; we conducted this experiment in mid-July. The upland cotton had higher area-based net photosynthetic rate ($P_{area}$), mesophyll conductance ($g_{area}$), maximum carboxylation rate ($V_{cmax-area}$), and maximum electron transport rate ($d_{m-area}$) than that of pima cotton (Table 1). Pooling all the data, $P_{area}$ positively correlated with $g_{area}$, $g_{mass}$, and $V_{cmax-area}$. Moreover, there was close relationship between $g_{mass}$ and $V_{cmax-area}$ and the drawdown in CO$_2$ between substomatal cavities and the sites of carboxylation within chloroplasts (C-C), respectively (Fig. 3). A quantitative limitation analysis following Grassi and Magnani (2005) revealed different weights for each potential limitation (Fig. 4). Only the relative mesophyll limitation ($l_m$) dominated in pima cotton, but $l_m$ and biochemical limitation ($l_b$) appear to co-limit photosynthesis in upland cotton.

From the analysis of leaf structure (Table 2), upland cotton had higher leaf mass per area (LMA) contributed by higher leaf thickness ($T_{leaf}$) than that of pima cotton. Palisade thickness ($T_p$) and spongy thickness ($T_s$) in upland cotton were also larger than those in pima cotton. LMA was positively correlated with $P_{area}$, $g_{mass}$, and $V_{cmax-area}$ (Fig. 5). Although upland cotton had higher area-based nitrogen content ($N_{area}$) than that of pima cotton, there was no significant difference in mass-based nitrogen content ($N_{mass}$) between upland and pima cotton. Furthermore, interestingly, we converted the area-based photosynthetic parameters into mass-based ones according to available leaf mass per area (LMA) and found there was no significant difference in $P_{mass}$, $g_{mass}$, and $V_{cmax-mass}$ between upland and pima cotton (Table 1). Also, pooling all of the data, a closer relationship between $T_{leaf}$ and LMA was observed than that between $D_{leaf}$ and LMA (Fig. 6), which was also shown clearly in Fig. 7.

**Discussion**

Area-based net photosynthetic rate ($P_{area}$) was significantly higher in upland cotton than that in pima cotton (Table 1), which is consistent with other studies (Zhang et al. 2011). Zhang et al. (2011) have shown that stomatal conductance ($g_s$) mainly contributed to the difference in $P_{area}$ between upland and pima cotton (see also Fig. 2A). But in this study, we found that the low internal CO$_2$ diffusion, i.e., area-based mesophyll conductance ($g_{mass}$) was also an important factor causing a low $P_{area}$ in pima cotton (Table 1, species at the 0.01 and 0.001 probability level, respectively. ** indicates no significant differences among species.

### Table 1. Area- and mass-based net photosynthetic rate ($P_{area}$ and $P_{mass}$), stomatal conductance ($g_s$), area- and mass-based mesophyll conductance ($g_{area}$ and $g_{mass}$), area- and mass-based maximum carboxylation rate ($V_{cmax-area}$ and $V_{cmax-mass}$), and maximum electron transport rate ($d_{m-area}$ and $d_{m-mass}$) estimated by two independent methods; using gas-exchange plus fluorescence measurements following Harley et al. (1992), area-based maximum electron transport rate ($d_{m-area}$), and $V_{cmax-area}$ expressed on the basis of nitrogen content ($V_{cmax-N}$) in upland and pima cotton. Values are means ± SE of three replicates. Different letters indicate significant differences at the 0.05 probability level. Data are presented as the means ± standard errors of three replicates. Interrelations between variables by means of linear regression analysis were investigated.

<table>
<thead>
<tr>
<th>Species (V-value)</th>
<th>$P_{area}$</th>
<th>$P_{mass}$</th>
<th>$g_s$</th>
<th>$g_{area}$</th>
<th>$g_{mass}$</th>
<th>$V_{cmax-area}$</th>
<th>$V_{cmax-mass}$</th>
<th>$d_{m-area}$</th>
<th>$d_{m-mass}$</th>
<th>$V_{cmax-N}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upland-45</td>
<td>372.3 ± 3.85</td>
<td>35.88 ± 1.49</td>
<td>3.97 ± 0.14</td>
<td>5.37 ± 0.04</td>
<td>4.00 ± 0.04</td>
<td>3.12 ± 0.04</td>
<td>2.21 ± 0.04</td>
<td>3.10 ± 0.04</td>
<td>4.00 ± 0.04</td>
<td>3.89 ± 0.19</td>
</tr>
<tr>
<td>Upland-13</td>
<td>35.88 ± 1.49</td>
<td>29.46 ± 1.85</td>
<td>3.89 ± 0.03</td>
<td>3.31 ± 0.04</td>
<td>3.11 ± 0.03</td>
<td>2.21 ± 0.04</td>
<td>2.30 ± 0.04</td>
<td>3.31 ± 0.04</td>
<td>3.11 ± 0.03</td>
<td>3.31 ± 0.04</td>
</tr>
<tr>
<td>Pima-25</td>
<td>27.43 ± 1.04</td>
<td>252.7 ± 15.70</td>
<td>3.31 ± 0.04</td>
<td>3.09 ± 0.04</td>
<td>2.87 ± 0.04</td>
<td>2.21 ± 0.04</td>
<td>2.30 ± 0.04</td>
<td>3.09 ± 0.04</td>
<td>2.87 ± 0.04</td>
<td>2.59 ± 0.12</td>
</tr>
<tr>
<td>Pima-25</td>
<td>252.7 ± 15.70</td>
<td>297.0 ± 28.92</td>
<td>3.31 ± 0.04</td>
<td>3.09 ± 0.04</td>
<td>2.87 ± 0.04</td>
<td>2.21 ± 0.04</td>
<td>2.30 ± 0.04</td>
<td>3.09 ± 0.04</td>
<td>2.87 ± 0.04</td>
<td>2.59 ± 0.12</td>
</tr>
</tbody>
</table>

**Statistical analysis** was performed with SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). All data were tested by analysis of variance (ANOVA). The significance of differences between treatment means were separated by using Student-Newman-Keuls (S-N-K) test at the 0.05 probability level. Data are presented as the means ± standard errors of three replicates. Interrelations between variables by means of linear regression analysis were investigated.
Ellsworth et al. (2018) indicated that the drawdown in CO$_2$ between substomatal cavities and the sites of carboxylation within chloroplasts ($C_i$-$C_c$) should always be estimated to assess the influence of $g_m$-area on $P_{area}$. There was a negative correlation between $C_i$-$C_c$ and $g_m$-area (Fig. 3), suggesting that upland cotton leaves with greater $g_m$-area do have lower mesophyll diffusion limitations of photosynthesis (Niinemets and Sack 2006, Warren and Adams 2006, Warren 2008) and then higher CO$_2$ concentration at the sites of carboxylation within chloroplasts ($C_c$) and $P_N$. Besides the CO$_2$ diffusional limitation, $P_{area}$ correlated strongly with the area-based maximum carboxylation rate ($V_{max-area}$) (Fig. 2C) that can reflect biochemical capacity just like Rubisco activity (Flexas et al. 2014). Study has shown that Rubisco activity was regulated by $C_c$ (Galmés et al. 2011), and $V_{max-area}$ was closely related with $g_m$-area in this study, suggesting that the CO$_2$ diffusion limitation and biochemical limitation tightly co-regulated photosynthesis. A quantitative limitation analysis following Grassi and Magnani (2005) revealed different weights for each potential limitation (Fig. 4). Only the relative mesophyll limitation ($l_m$) dominated in pima cotton, but $l_m$ and biochemical limitation ($l_b$) appeared to co-limit photosynthesis in upland cotton. Indeed, from this limitation weight analysis, none of these three limitations can be ignored in setting the differences between upland and pima cotton.

There is always a trade-off between photosynthetic physiological and structural characteristics as reported by most studies. Leaves with higher leaf mass per area (LMA) tend to have higher investment in nonphotosynthetic tissue and this leads to lower photosynthetic efficiency (Niinemets et al. 2009a, Hassiotou et al. 2010). Meanwhile,
RELATIONSHIP BETWEEN PHOTOSYNTHESIS AND LEAF STRUCTURES

LMA has been suggested to negatively affect the $g_{m-area}$ and limit area-based photosynthesis (Flexas et al. 2008). However, in this study, LMA was positively correlated with physiological traits (i.e., $P_{area}$, $g_{m-area}$, $V_{cmax-area}$) (Fig. 5). It is well established that LMA is an integrative trait of leaf structural characteristics and the product of leaf density ($D_{leaf}$) and thickness ($T_{leaf}$) (Poorter et al. 2009, Niinemets et al. 2015). Our results showed that the variation in LMA is primarily driven by variations in $T_{leaf}$ and to a lesser degree in $D_{leaf}$ (Fig. 6). It is likely that upland cotton with higher $T_{leaf}$ had more Rubisco carboxylation sites (Flexas et al. 2014) and/or higher surface of chloroplasts exposed to intercellular airspace ($S_c/S_i$) (Hanba et al. 1999, 2002; Terashima et al. 2006, Peguero-Pina et al. 2016), thereby increasing photosynthetic physiological traits.

Generally, photosynthetic characteristics and CO$_2$ diffusion properties were measured on the basis of leaf area, but it is reported that photosynthetic process inside the leaves is based on a three-dimensional structure (Parkhurst 1994, Niinemets et al. 2009b). The mass-based photosynthetic traits can be better used to measure the trade-off between the physiological and structural characteristics of leaves (Niinemets et al. 2015). The trait values on the mass basis can be calculated according to available LMA. Interestingly, it was documented that

<table>
<thead>
<tr>
<th>Species</th>
<th>LMA [g m$^{-2}$]</th>
<th>$T_{leaf}$ [μm]</th>
<th>$D_{leaf}$ [g cm$^{-3}$]</th>
<th>$T_{p}$ [μm]</th>
<th>$T_{s}$ [μm]</th>
<th>$N_{area}$ [%]</th>
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<tbody>
<tr>
<td>Upland-45</td>
<td>101.58 ± 1.37A</td>
<td>432.63 ± 10.98B</td>
<td>0.236 ± 0.01C</td>
<td>199.44 ± 6.39D</td>
<td>191.19 ± 13.85E</td>
<td>5.11 ± 0.17C</td>
<td>5.20 ± 0.06E</td>
</tr>
<tr>
<td>Upland-33</td>
<td>100.02 ± 0.59A</td>
<td>404.95 ± 7.70B</td>
<td>0.245 ± 0.00B</td>
<td>175.95 ± 4.02B</td>
<td>184.61 ± 7.79E</td>
<td>5.57 ± 0.36C</td>
<td>5.53 ± 0.05E</td>
</tr>
<tr>
<td>Pima-28</td>
<td>79.02 ± 2.70A</td>
<td>266.92 ± 1.58B</td>
<td>0.293 ± 0.00B</td>
<td>115.54 ± 2.24C</td>
<td>103.83 ± 7.59D</td>
<td>4.76 ± 1.66C</td>
<td>3.72 ± 0.09A</td>
</tr>
<tr>
<td>Pima-25</td>
<td>74.43 ± 3.08A</td>
<td>269.60 ± 1.66B</td>
<td>0.275 ± 0.00B</td>
<td>107.27 ± 5.81C</td>
<td>122.98 ± 9.07B</td>
<td>5.56 ± 0.11C</td>
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</tr>
<tr>
<td>Pima</td>
<td>74.43 ± 3.08A</td>
<td>269.60 ± 1.66B</td>
<td>0.275 ± 0.00B</td>
<td>107.27 ± 5.81C</td>
<td>122.98 ± 9.07B</td>
<td>5.56 ± 0.11C</td>
<td>4.12 ± 0.10B</td>
</tr>
</tbody>
</table>

Fig. 5. The relationships between leaf mass per area (LMA) and area-based net photosynthetic rate ($P_{area}$) (A), mesophyll conductance ($g_{m-area}$) (B), and the maximum carboxylation rate ($V_{cmax-area}$) (C), respectively, in upland (solid circles) and pima cotton (open circles). The solid lines are the linear regressions; ** and *** indicate significance at the 0.01 and 0.001 probability level, respectively.

Fig. 6. The relationships between leaf mass per area (LMA) and leaf thickness ($T_{leaf}$) (A) and leaf density ($D_{leaf}$) (B), respectively, in upland (solid circles) and pima cotton (open circles). The solid lines are the linear regressions; ** and *** indicate significance at the 0.01 and 0.001 probability level, respectively.
mass-based $V_{\text{cmax}}$ ($V_{\text{cmax-mass}}$) is more effective in separating biochemical from anatomical effects on $P_N$ than $V_{\text{cmax-area}}$ (Ellsworth et al. 2018); $g_{\text{m-mass}}$ is more strongly related to photosynthetic tissue volume than to the area; the $P_{\text{mass}}$ is the key player in the trade-off between the physiological and structural characteristics of leaves (Westoby et al. 2013, Niinemets et al. 2015). In our study, no difference in $P_{\text{mass}}, g_{\text{m-mass}},$ and $V_{\text{cmax-mass}}$ between upland and pima cotton was observed, indicating that both of upland and pima cotton had the similar photosynthetic capacity of single cells. Moreover, there was no significant difference in nitrogen investment. A similar carboxylation capacity per unit of nitrogen ($V_{\text{cmax}}/N$) suggests that upland and pima cotton divert a similar proportion of their N to nonphotosynthetic compounds. Therefore, the higher trait values on the basis of area in upland cotton were only due to higher $T_{\text{leaf}}$ or LMA. This was inconsistent with the change rule of data in the global databases, but keeping in mind that only four species were studied and these belonged to the trade-off within species that has not been proved to be consistent with the laws along species.

**Conclusion:** Upland cotton had higher area-based net photosynthetic rate ($P_{\text{area}}$) than that of pima cotton, which was attributed to the coordination of area-based stomatal conductance ($g_{\text{area}}$), mesophyll conductance ($g_{\text{m-area}}$), and biochemical capacity (i.e., $V_{\text{cmax-area}}$ and $J_{\text{max-area}}$). $P_{\text{area}}, g_{\text{m-area}},$ and $V_{\text{cmax-area}}$ correlated positively with leaf mass per area (LMA). But there was no difference in mass-based $P_N$ ($P_{\text{mass}}$), $g_{\text{m-mass}},$ and $V_{\text{cmax}}$ ($V_{\text{cmax-mass}}$) between upland and pima cotton, suggesting that upland and pima cotton had a similar photosynthetic capacity of single cells. Therefore, we can conclude that the higher trait values on the basis of area in upland cotton were only due to higher $T_{\text{leaf}}$ or LMA.

**References**


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