

Potassium nutrition recover impacts on stomatal, mesophyll and biochemical limitations to photosynthesis in *Carya cathayensis* and *Hickory illinoensis*

Chao Shen¹, Ruimin Huang¹, Yiquan Tang¹, Zhengjia Wang^{1*}

¹ State Key Laboratory of Subtropical Silviculture, Zhejiang A&F University, Lin'an, Hangzhou 311300, China

Abstract

Potassium (K) influences the photosynthesis process in a number of ways; However, the mechanism of photosynthetic response to the long-term supply of potassium is not yet clear. Concurrent measurements of gas exchange and chlorophyll fluorescence were made to investigate the effect of potassium nutrition on photosynthetic efficiency and stomatal conductance (g_s), mesophyll conductance (g_m) in Pecan (*Carya illinoensis* K.Koch) and Hickory (*Carya cathayensis* Sarg.) seedlings in a greenhouse. The results show that the photosynthetic capacity of Pecan and Hickory plants was not limited when the leaves had potassium concentrations $>1.4\%$ and 1.42% of dry weight. Most of limitation under potassium deficiency were dominated by MCL for Pecan and Hickory. Both cultivars showed remarkable improvement in S_L , MC_L , J and $V_{c,max}$ with additional K supplies. However, effect from potassium deficiency on photosynthesis in plant leaves was irreversible. All of S_L , MC_L , and B_L nearly half down with recovery K supply in both species. These results emphasize the important role of potassium on regulation of photosynthesis by three limitations.

Key words: *Carya cathayensis* Sarg., *Hickory illinoensis* K.Koch., potassium deficiency, recover, photosynthetic limitations.

Introduction

Pecan (*Hickory illinoensis* K.Koch), one of the world's efficient economic trees, can provides high quality dried fruit, good wood and other products. In China, pecan has been introduced for more than 100 years (Shi et al. 2013), and pecan have been grown in all parts of Zhejiang Province. Fruit trees are potassium sensitive crops, and their normal growth and development need adequate potassium supply. Potassium deficiency largely restricts the improvement of fruit quality. (Zhang 2016, Shen et al. 2017). However, the total potassium content of this region is low, resulting in less efficient potassium that can be absorbed and utilized by plants (Cong et al. 2016). Potassium content in plant leaves is closely related to photosynthesis (Wood et al. 2016), most

32 studies have focused on crops (Pettigrew et al. 2010, Lu et al. 2016, Sousa et al. 2010, Li et al.
33 2014), while few studies have been done on fruit trees.

34 Potassium is one of the three microelement of plant nutrition. It is different from nitrogen and
35 phosphorus, that exists mainly in the form of soluble inorganic salt in the cell fluid (Blevins et al.
36 1985), or adsorbed on the surface of the plasma colloid in the form of ions, and has no structural
37 purpose (Tester et al. 2001). K^+ is an activator of more than 60 enzymes in plants (Berg et al., 2010;
38 Hu et al., 2015; Wang and Wu, 2013), including enzymes that alter carbohydrate metabolism and
39 nitrogen metabolism, and promote protein and nucleic acid synthesis (Amtmann et al., 2008;
40 Maathuis 1997). Potassium (such as potassium nitrate, potassium chloride and potassium citrate)
41 is the main regulator of vacuole osmotic regulation in vacuoles (Hsiao and Läuchli 1986),
42 regulating cell water potential and turgor pressure, thus affecting plant stomatal opening and
43 closing movement(Jordan et al., 2008; Peiter 2011). Therefore, potassium deficiency affects
44 various plant metabolism and osmotic adjustment, resulting in decreased leaf area, plant thin,
45 yellow leaf wilting, ultimately inhibit the plant growth and yield formation. (Severtson et al.,
46 2016).

47 K^+ as the main regulator of guard cell permeability, its richness affects stomatal function
48 (Shavala, 2003; Lebaudy et al. 2008; Andrés et al. 2008), thereby affecting the exchange process
49 of the blade and outside the water and gas. Therefore, stomatal limitation was thought to be the
50 primary cause of the decrease in leaf photosynthetic rate due to potassium deficiency (Bednarz et,
51 al. 1998).But the research in apricot showed that leaf photosynthetic rate decreased significantly
52 under potassium starvation (Basile et al. 2003; Oosterhuis et al. 2014; Quentin et al. 2013; Flexas et
53 al. 2015), but the stomatal conductance is not affected, and the biochemical disorders caused by
54 inadequate supply of potassium is the main reason that limit the photosynthetic rate (Basile et al.
55 2003; Flexas et al. 2012). Potassium deficiency decreased leaf chlorophyll content, decreased
56 Rubisco activity, accelerate the generation of reactive oxygen species (ROS), may also lead to the
57 accumulation of photosynthetic products and feedback inhibition of leaf net photosynthetic rate
58 (Paul et al. 2003; Cakmak 2005; Araya et al., 2006; Battie-Laclau et al. 2013; Vislap et al. 2012).In
59 recent decades, with the in-depth research on the mesophyll conductance (Bernacchi et al., 2010;
60 Flexas et al., 2007; Galmés et al., 2007; Xiong et al., 2016), people gradually realize that
61 potassium plays an important role in the regulation of mesophyll conductance under potassium

deficiency leads to reduced mesophyll conductance on leaf photosynthetic rate limit as due to stomatal function limited (Song et al., 2011; Battie-Laclau et al., 2014). Thus, the dominant limiting factor of potassium deficiency on photosynthesis has a significant effect on the surface, this effect is based on the three factors in proportion to the size of the plant, but is regulated by internal features, including changes in the degree of stress and porosity, the resulting mesophyll layer and the physiological and biochemical characteristics.

Recently, a quantitative limitation analysis for the RuBP-limited phase of photosynthesis was proposed (Chen et al., 2013; Christian et al., 2014; Wang et al., 2015; Song et al., 2013; Wang et al., 2012). In this way, the quantitative analysis of photosynthesis limitation was conducted by combining stomatal conductance and mesophyll conductance (Song et al., 2011; Tosens et al., 2012), the total photosynthesis limitation of leaves can be divided into three components: $A_L = S_L + M_L + B_L$, S_L , M_L , B_L , stomata, mesophyll, and biochemical limitation, respectively. To date, most studies on quantitative analysis of photosynthetic restriction have focused on crops (Sagardoy et al., 2010; Pettigrew et al., 2010; Lu et al., 2016; Sousa et al., 2010), and most of them compare genetically modified species and genotypes with large variation in leaf structure. Nevertheless, the information related to the influence of nutrient deficiency on quantitative limitation analysis for photosynthesis is missing. Previous study showed a relationship between potassium and photosynthesis in plant leaves of Pecan and Brassica napus, but do not involve the effect of potassium deficiency time on photosynthesis of leaves. For this reasons, it is desirable to develop a better understanding of the mechanisms which K supply affects photosynthesis of leaves.

Materials and methods

Plants materials and growth conditions

Two-year-old pecan seedlings (*Hickory illinoensis K.Koch*) were transplanted into 30.5 cm tall plastic pots with a top diameter of 25 cm, containing full-strength nutrient solution. The composition of the standard nutrient solution was as follows: 2.5 mM $\text{Ca}(\text{NO}_3)_2$, 0.5 mM $\text{Ca}(\text{H}_2\text{PO}_4)_2$, 1.0 mM MK_2SO_4 , 0.5 mM MgSO_4 , 12.5 μM H_3BO_3 , 1.0 μM MnSO_4 , 1.0 μM ZnSO_4 , 0.25 μM CuSO_4 , 0.1 μM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ and 10 μM EDTA-Fe. The seedlings were grown in a greenhouse with natural sunlight during the day. The mean daytime maximum and minimum temperatures in the greenhouse were 28 and 20°C, with a constant relative humidity of 60%. After

2 months, the composition of the nutrient solution was altered to one of three K concentrations:0, 2.0 and 5.0 mM K, respectively. In all cases, Ca(OH)₂ and HCl were used to adjust the pH of the nutrient solution to 5.7. The nutrient solution was changed every 7 days. All the treatments had 10 replicates with a completely random design.

Leaf gas exchange and fluorescence measurements

Measurements were made on the youngest fully expanded leaf from 6–8 randomly selected seedlings on the 60th day of the treatment, using leaves developed after the initiation of the K nutrition treatment. Leaf gas exchange and chlorophyll fluorescence were measured simultaneously using a portable infrared gas analyser system (Li-6400, Li-Cor, Lincoln, NE, USA) equipped with an integrated leaf chamber fluorometer (Li-6400-40) at a concentration of 380 $\mu\text{mol mol}^{-1}$ CO₂, 21% O₂ and 50% relative humidity. Leaf chamber temperature was maintained at 28 °C. All measurements were carried out at 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, with 90% red light and 10% blue light, which we previously determined to be just above light saturation for pecan seedlings. Once a steady state was reached (~20 min at a photosynthetic photon flux density (PPFD) of 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$), a CO₂ response curve (A–C_i curve) was performed. The ambient CO₂ concentration (C_a) was lowered stepwise from 380 to 50 $\mu\text{mol mol}^{-1}$, and then returned to 380 $\mu\text{mol mol}^{-1}$ to re-establish the initial steady-state value of photosynthesis. C_a was then increased stepwise from 380 to 1800 $\mu\text{mol mol}^{-1}$. At each C_a, photosynthesis was allowed to stabilize for 3–4 min until gas exchange was steady, so that each curve was completed in 35–50 min. Corrections for the leakage of CO₂ in and out of the Li-6400 leaf chamber, as described by Perez-Martin et al. (2009), were applied to all gas-exchange data.

The actual photochemical efficiency of photosystem II (Φ_{PSII}) was determined by measuring steady-state fluorescence (F_s) and maximum fluorescence during a light-saturating pulse (F'_m) following the procedure of Genty et al. (1989):

$$\Phi_{\text{PSII}} = (F'_m - F_s) / F'_m \quad (1)$$

The rate of electron transport estimated from chlorophyll fluorescence is given by the equation (Bilger and Björkman 1994)

$$J = \Phi_{\text{PSII}} \cdot \text{PPFD} \cdot \alpha \cdot \beta \quad (2)$$

where PPFD is the photosynthetic photon flux density, α is leaf absorptance and β is the proportion of quanta absorbed by photosystem II. $\alpha \cdot \beta$ was determined for each treatment from the

slope of the relationship between Φ_{PSII} and Φ_{CO_2} (i.e., the quantum efficiency of gross CO_2 fixation), which was obtained by varying light intensity under non-photo respiratory conditions in an atmosphere containing <1% O_2 (Valentini et al.1995).

Measurement of mitochondrial respiration rate in the light (R_d) and intercellular CO_2 compensation point (C_i^*)

R_d and C_i^* were determined according to the method of Laisk (1977). $A-C_i$ curves were measured using an open gas-exchange system (Li-6400, Li-Cor Inc.) equipped with an integrated light source (Li-6400-02) at three different photosynthetically active PPFDs (50, 200 and 500 $mmol\ m^{-2}\ s^{-1}$) at six different CO_2 levels ranging from 300 to 50 $mmol\ CO_2\ mol^{-1}$ air. The curves intersected at the point where A is the same at different PPFDs; therefore, A at that point represents R_d , and C_i represents C_i^* .

Estimation of g_m

From combined gas-exchange and chlorophyll fluorescence measurements, the mesophyll conductance for CO_2 (g_m) was estimated according to Harley et al. (1992) as

$$g_m = A / (C_i - \Gamma^* (J + 8(A + R_d)) / (J - 4(A + R_d))) \quad (3)$$

where A , C_i , R_d and J were determined as previously described for each treatment. Γ^* is the chloroplastic CO_2 photocompensation point calculated from the C_i^* and R_d measurements according to the method of Warren et al. (2007) using a simultaneous equation with g_m :

$$\Gamma^* = C_i^* + R_d / g_m \quad (4)$$

Equation (4) was then substituted into (3) and the value of g_m was found; then Γ^* was calculated. The value of Γ^* was found to be slightly higher for the K0-treated plants ($53.9 \pm 9.6\ \mu mol\ mol^{-1}$), compared with the four other treatments (47.3 ± 7.5 , 44.9 ± 7.8 , 44.7 ± 5.1 and $44.6 \pm 8.6\ \mu mol\ mol^{-1}$ for K1, K2, K3 and K4 treatments, respectively). Changes in Γ^* derived using the method of Laisk (1977) have been frequently observed under stress conditions such as drought (Galmés et al. 2007); therefore, we re-calculated g_m using the non-stressed Γ^* values ($44.6\ \mu mol\ mol^{-1}$), which is a reasonable assumption as Γ^* is an intrinsic property of Rubisco and thus varies only by a small amount within a species under different growing conditions. The CO_2 concentration in the chloroplast stroma (C_c) was calculated using the equation

$$C_c = C_i - A / g_m \quad (5)$$

Quantitative limitation analyses

The limitations (stomatal limitation, S_L ; the mesophyll conductance limitation, MC_L ; and the biochemical limitation, B_L) imposed by K deficiency on A were investigated following Grassi and Magnani (2010). Because the fluorescence derived linear electron transport rate (J) is tightly coupled with the maximum rate of Rubisco-catalysed carboxylation ($V_{c,max}$) (Galmés et al. 2007; Gallé et al. 2009), a minor modification was adopted when calculating B_L using J instead of $V_{c,max}$ (Gallé et al. 2009). Relative changes in light-saturated assimilation are expressed in terms of relative changes in stomatal, mesophyll conductance and biochemical capacity as showed in Eqn 6:

$$dA/A = S_L + MC_L + B_L = l_s \cdot dg_{sc}/g_{sc} + l_{mc} \cdot dg_m/g_m + l_b \cdot dJ/J \quad (6)$$

where l_s , l_{mc} and l_b are the corresponding relative limitations calculated as Eqns 7–9 and g_{sc} is stomatal conductance to CO_2 (gs/1.6).

$$l_s = (g_{tot}/g_{sc} \cdot \partial A / \partial C_c) / (g_{tot} + \partial A / \partial C_c) \quad (7)$$

$$l_m = (g_{tot}/g_m \cdot \partial A / \partial C_c) / (g_{tot} + \partial A / \partial C_c) \quad (8)$$

$$l_b = g_{tot} / (g_{tot} + \partial A / \partial C_c) \quad (9)$$

Where the g_{tot} is the total conductance to CO_2 from the leaf surface to carboxylation sites determined in Eqn 10. $\partial A / \partial C_c$ was calculated as the slope of A/C_c response curves over a C_c range of 50–100 $\mu\text{mol mol}^{-1}$ (Tomás et al. 2012).

$$g_{tot} = 1 / (1/g_{sc} + 1/g_m) \quad (10)$$

Then, the relative change of A, g_{sc} , g_m and J in Eqn 6 can be approximated by the following (Chen et al. 2013):

$$dA \approx (A_{max}^{ref} - A) / A_{max}^{ref} \quad (11)$$

$$dg_{sc}/g_{sc} \approx (g_{sc}^{ref} - g_{sc}) / g_{sc}^{ref} \quad (12)$$

$$dg_m/g_m \approx (g_m^{ref} - g_m) / g_m^{ref} \quad (13)$$

$$dJ/J \approx (J_{max}^{ref} - J) / J_{max}^{ref} \quad (14)$$

where A_{max}^{ref} , g_{sc}^{ref} , g_m^{ref} and J_{max}^{ref} are the reference values. Reference maximum values of net CO_2 assimilation rate, stomatal and mesophyll conductance and the rate of electron transport were obtained in +K treatments; therefore, its parameters were defined as standard.

Statistical analysis

Descriptive statistical analyses were used for the obtained parameters to assess the range of variability and standard error (SE). All data were subjected to a two-way analysis of variance

181 (ANOVA) with SPSS 18.0 software (SPSS, Chicago, IL, USA). The difference between mean
182 values was compared using Duncan's multiple range test at $P < 0.05$. Graphics and regression
183 analysis were performed using the GraphPad Prism 7.0 software (GraphPad, San Diego, CA).

184 **Results**

185 The leaf potassium (K) concentration (%), net CO₂ assimilation rate (A_N) and chloroplastic
186 CO₂ concentrations (C_C) of daily potassium supplied plants (control) remained mostly unchanged
187 throughout the experiment (Fig. 1a,b,d,e), but, slightly different between species, Pecan had a little
188 larger K and C_C but lower A_N compared with Hickory. After withholding potassium from plants,
189 K and A_N decreased progressively in two treatment (K0 and K2), reaching minimum values of 0.5%
190 and $< 5 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, respectively, while C_C increased in both two cultivars, with similar
191 trends during severe potassium stress (Fig. 3). Compare with K0 treatment, K, A_N and C_C of K2
192 recovered more quickly and closer to K5 (control, daily potassium supplied) throughout the
193 recovery period of potassium supply. K, A_N of K2 rose 41.24% and 26.98% after restoring
194 potassium supply 7 days after in Pecan, which were significantly larger than those (18.92% and
195 16.16%) in Hickory.

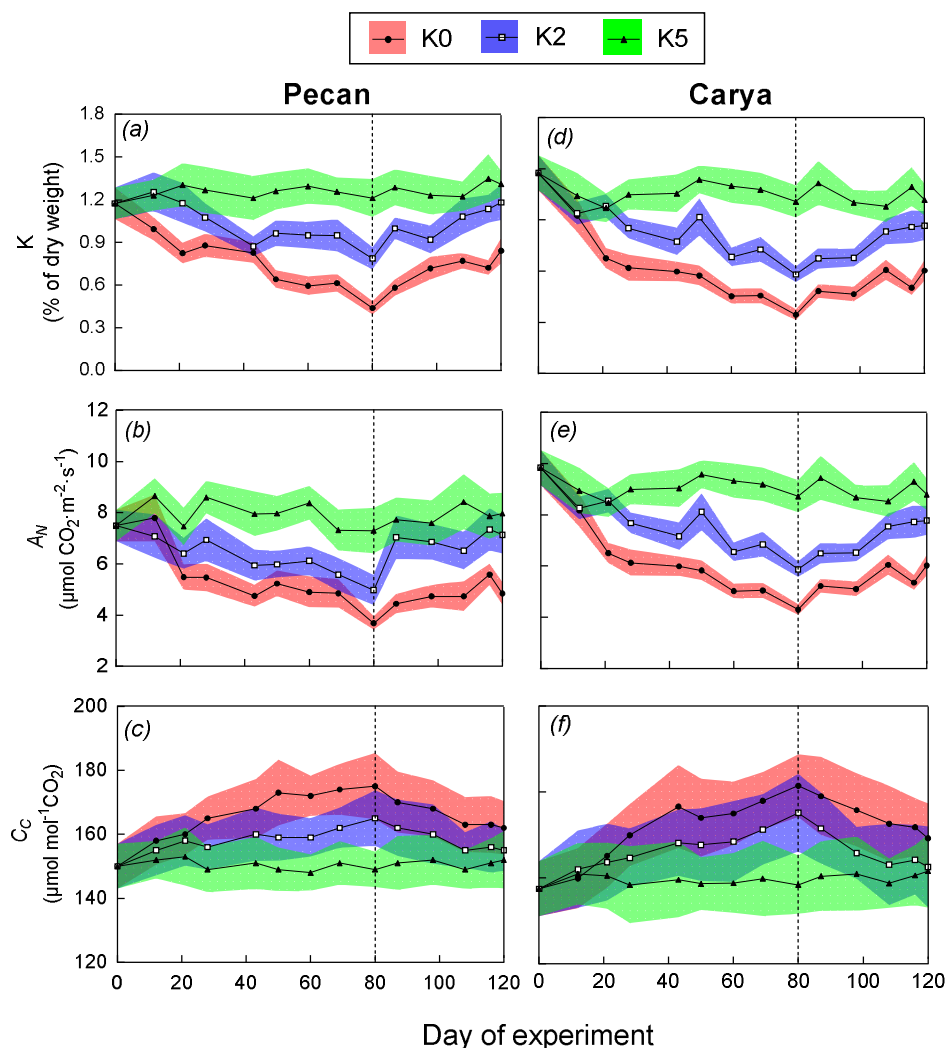


Fig.1. Leaf potassium percentage (%) of dry weight (K), net CO₂ assimilation rate (A_N), chloroplastic CO₂ concentrations (C_C) of Pecan and Hickory as affected by different K levels during seedling stage. The vertical dashed lines indicate the beginning of the beginning of recovering potassium supply. Data points represent means and standard errors of at least four replicates. The width of green, purple and red ribbons is the standard deviation.

After withholding potassium from plants, Mesophyll conductance for CO₂ (g_m), stomatal conductance for CO₂ (g_s), electron transport rate (J) and maximum velocity of carboxylation (V_{c,max}) decreased progressively in all treatments of Pecan and Hickory, reaching minimum values of 0.0265 mol CO₂·m⁻²·s⁻¹, 0.0457 mol·m⁻²·s⁻¹, 99.94 μmol e⁻¹·m⁻²·s⁻¹ and 47.5904 μmol CO₂·m⁻²·s⁻¹ after severe potassium stress for 80d, respectively. After recovering potassium supply, the large restoration of g_m, g_s, J and V_{c,max} were observed in the K0 and K2 treatments in two cultivations. The g_m of K0 and K2 recovered 55.16% and 71.54% to control values (0.09684 mol CO₂·m⁻²·s⁻¹) of Pecan (Fig. 2a), respectively; g_m of K0 and K2 recovered 55.65% and 81.93% to control values

211 (0.09938 mol CO₂·m⁻²·s⁻¹) of Hickory (Fig. 2b), respectively; gs recovered 76.80% and 90.64% to
 212 control values (0.1082 mol·m⁻²·s⁻¹) of Hickory (Fig. 2f) respectively, which were nearly 10
 213 percentage points more than of Pecan. The restoration of *J* and *V_{c,max}* under both K0 and K2
 214 treatments were reached to control values after recovering potassium supply for 40d (Fig. 2c, d, g
 215 h), respectively. However, slightly different levels of recovery time of *g_m*, *g_s* and *V_{c,max}* were
 216 observed in K2 treatments, with the three photosynthetic parameters show faster recovery speed
 217 after recovering potassium supply. Under K5 treatment, *g_m*, *g_s*, *J* and *V_{c,max}* of Pecan and Hickory
 218 almost unaltered throughout the experiment, while those photosynthetic parameters of Hickory
 219 were slightly larger than Pecan.

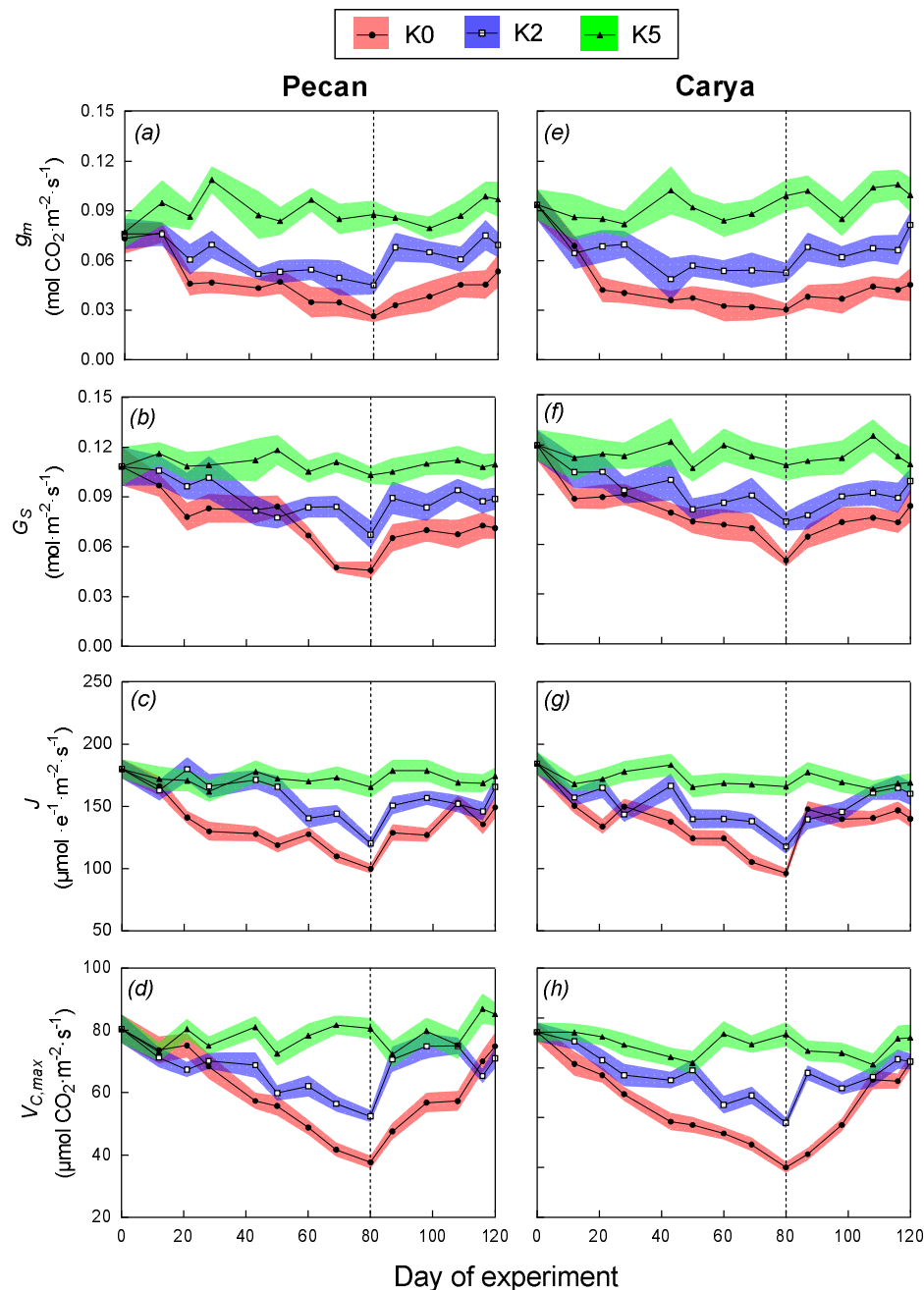
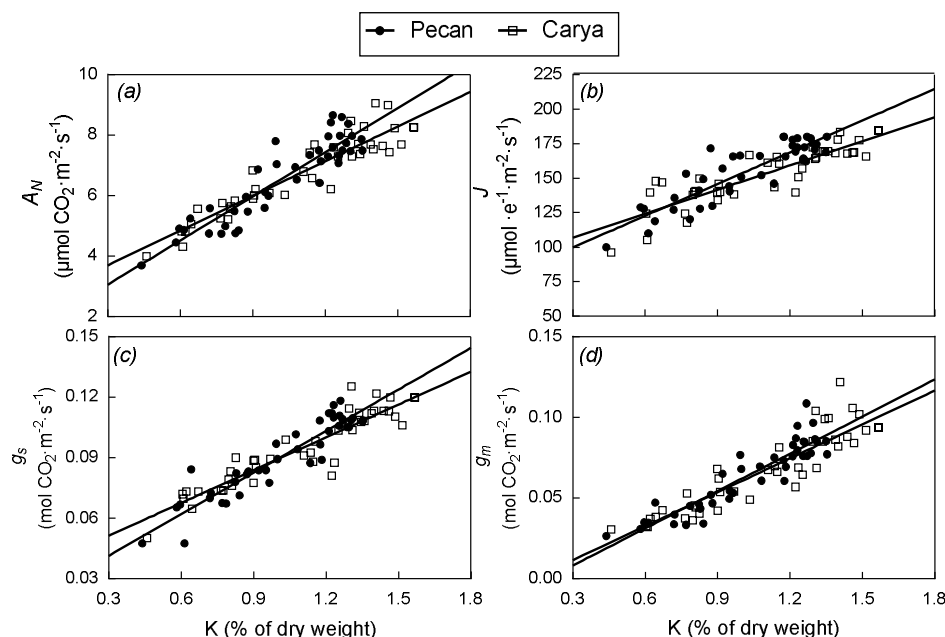


Fig.2. Mesophyll conductance for CO_2 (g_m), stomatal conductance for CO_2 (g_s), electron transport rate (J) and maximum velocity of carboxylation ($V_{c,\max}$) of Pecan and Hickory as affected by different K levels during seedling stage. The vertical dashed lines indicate the beginning of recovering potassium supply. Data points represent means and standard errors of at least four replicates. The width of green, purple and red ribbons is the standard deviation.

229 When plotting all K content (consisting all of K5, K2 and K0 under potassium stress and
230 recover, respectively) against the corresponding calculated A_N , J , g_s and g_m , highly significant
231 positive correlation relationships were obtained pooling potassium supply and potassium stress
232 data together, although two different functions were derived for the Pecan and Hickory (Fig. 3a-d).
233 In these four figures, slightly steep slope was determined for the Pecan data set, however, less
234 clear difference was observed between two cultivars. Moreover, K content on A_N , J , g_s and g_m
235 values resulted in almost similar slopes of linear regression for both two cultivars, respectively.



236
237 Fig.3. The relationships between leaf potassium percentage(%) of dry weight (K) and net CO₂
238 assimilation rate (A_N), electron transport rates (J), stomatal conductance for CO₂ (g_s) and
239 mesophyll conductance for CO₂ (g_m) derived from data of the whole experimental periods. Circles
240 and diamonds denote Pecan and Hickory data, respectively. Data points represent means and
241 standard errors of at least four replicates.

242
243 The A_N correlated negatively with chloroplastic CO₂ concentrations (C_C), while, A_N in
244 Hickory was slightly higher Pecan. When plotting all A_N (consisting all of K5, K2 and K0 under
245 potassium stress and recover potassium, respectively) against the corresponding calculated J and
246 g_m , highly significant positive correlation relationships were obtained pooling potassium supply
247 and potassium stress data together, although two different functions were derived for the Pecan
248 and Hickory (Fig. 4b,c). In addition, A_N on J and g_m values resulted in almost similar slopes of
249 linear regression for both two cultivars, respectively.

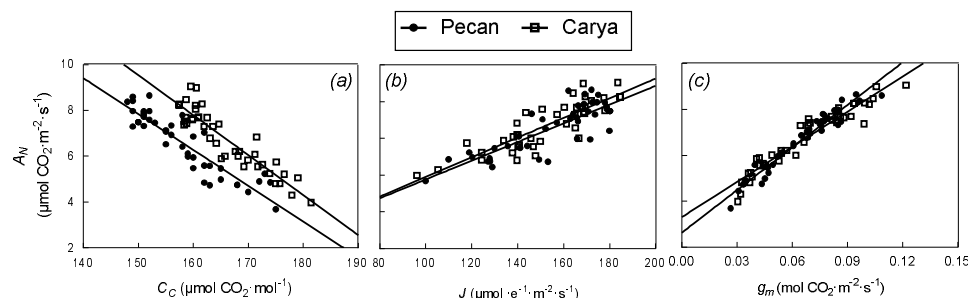


Fig.4. The relationships between leaf potassium percentage (%) of dry weight (K) and net CO₂ assimilation rate (A_N), electron transport rates (J), stomatal conductance for CO₂ (g_s) and mesophyll conductance for CO₂ (g_m) derived from data of the whole experimental periods. Circles and diamonds denote Pecan and Hickory data, respectively. Data points represent means and standard errors of at least four replicates.

When analyzing the effects of potassium on photosynthesis different indicators of stress intensity can be used. In order to enhance the comparability of our data with other experiment, we, therefore expressed relative limitations in terms of both of both K (% of dry weight) (Fig. 5) and day of experiment (Fig. 6). what the stress index adopted, small differences between Pecan and Hickory. With increasing potassium stress intensity MC_L , B_L and S_L increased significantly, and, the increase rate of MC_L is greater than that of B_L and S_L . At mild-to-moderate potassium stress levels (corresponding to values of K >0.9% of dry weight), about half of the decline in A_N was attributable to mesophyll resistance.

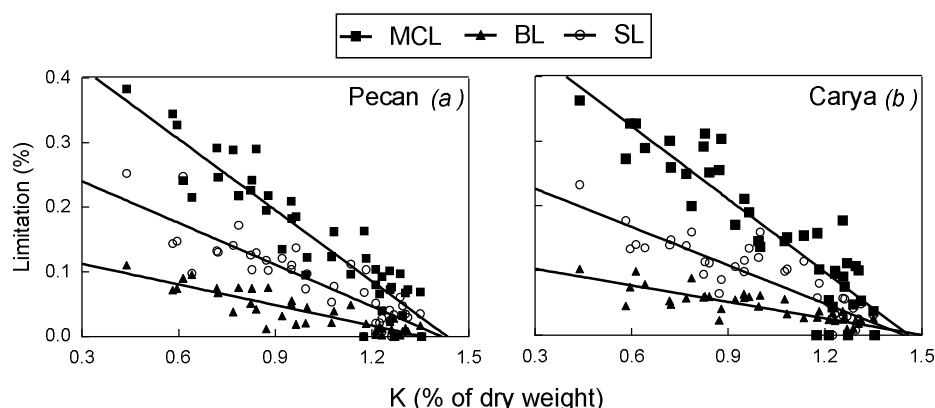


Fig.5. The relationships between photosynthetic limitations and leaf potassium percentage (%) of dry weight (K) of Pecan (a) and Hickory (b). S_L , MC_L , B_L denote stomatal, mesophyll conductance and biochemical limitation respectively. Each point in the same shape represents a calculation (42 values were calculated for each limitation)

Quantitative limitation analysis of photosynthesis underlined the above-described changes during potassium stress and recovery after subsequent recover potassium. In all three experiments,

MC_L play a major role of the total limitation under severe potassium stress. In K0 and K2 treatments, MC_L made up >40% and >50% of the total limitation under severe potassium stress in Pecan and Hickory, while S_L accounted for only up to 20%. Furthermore, B_L did not exceed 10% of the total limitation. As already observed for the A_N, g_s, and g_m data during stress and recovery, almost no limitation of S_L and B_L during potassium stress and after recover potassium (Fig. 6c,f). Limitation of photosynthetic recovery of the K0 and K2 plants was mainly driven by a still high MC_L and somewhat lower S_L and B_L (Fig. 5a, b, d, e). The delayed recovery of photosynthesis in the K0 plants was mainly due to a maintained high proportion of MCL and SL (Fig. 5d) during several days of recover potassium (Fig. 6a, d), while SL contributed only partially to the total limitation in the initial phase of re-watering. The recovery of photosynthesis in the K2 plants is due to a rapidly decreasing MCL during the recover potassium period, while SL is nearly equaled MCL in the later phase of recover potassium (Fig. 6b, e).

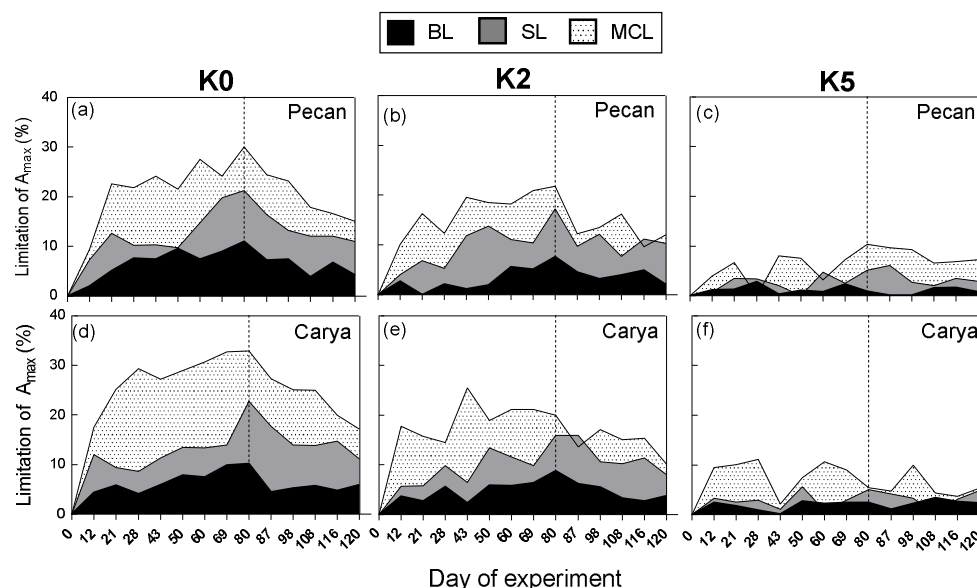


Fig.6. Quantitative limitation analysis of photosynthetic CO₂ assimilation during potassium stress and subsequently recovering. The shaded areas represent the percentage of stomatal (SL), mesophyll (M_L), and biochemical (B_L) limitation based on control values of A_N, g_s, g_m, and V_{c,max} and J. Calculations were done with mean values of at least five measurements per treatment and day. The vertical dashed lines indicate the beginning of the beginning of recovering potassium supply. Data points represent means and standard errors of at least four replicates.

Discussion

In the present study, the same experimental design was applied to Brassica napus under four different potassium treatments, followed by a potassium stress period and a recover after recover potassium. The most important difference between the four potassium treatments was observed: the rate of photosynthetic recovery was the slowest under K0 treatment and the quickest under K2

296 treatment of Pecan. Then, extremely low photosynthetic limitation in plant leaves of Pecan and
297 Hickory under K5 treatment. A positive relationship between K supply and A_N had reported in
298 numerous previous studies. However, photosynthesis rates may not have changed as a response to
299 K treatment due to relatively higher leaf K concentration, which were far more than the values
300 (1.04% and 1.28 % of dry weight) proposed by Gierth (2007) and Zhifeng Lu (2016) respectively,
301 as are the values (1.4 % and 1.42%) in this study.

302 Withholding potassium resulted in a closure of stomata, which was accompanied by a marked
303 decrease of net photosynthesis (A_N) in K0 and K2 treatment of Pecan and Hickory (Fig. 2).
304 Throughout periods of potassium stress imposition, stomatal conductance (g_s) and A_N followed
305 the same course, indicating a strong correlation between them, which has been shown elsewhere
306 (Medrano et al., 2002). Moreover, plants under K5 treatment displayed an inequable course for
307 stomatal conductance (g_s) of Pecan and Hickory. In addition, mesophyll conductance (g_m) showed
308 a similar trend with stomatal conductance (g_s) under all three potassium supply in Pecan and
309 Hickory. These results are in line with previous studies, where a decrease of g_m has been observed
310 during potassium stress (Song et al., 2011; Lu et al., 2016; Wang et al., 2012; Battie-Laclau et al.,
311 2014). The rapid restoration of J and V_{cmax} to control values during prolonged recover potassium,
312 while the restoration of g_s and g_m could not reach the level of the control value, especially under
313 K0 stress of Pecan and Hickory. These results are indicating restored J and V_{cmax} during prolonged
314 recover potassium presumably facilitated photosynthetic recovery after the beginning of
315 re-watering, because A_N and J , V_{cmax} were immediately restored to control values within the initial
316 phase.

317 Lots of previous studies have shown total photosynthetic dramatically decreased with
318 decreasing K supply. (Bednars et al. 1998; Jin et al. 2011; Wang et al. 2012; Battie-laclau et
319 al. 2014; Erel et al. 2015). And the results of quantitative analysis revealed that the three
320 components contributing to total photosynthetic limitation, namely, S_L , MC_L , and B_L varied at
321 varying K concentrations. The decline of P_n with the decrease of potassium concentration in
322 leaves, while all of S_L , B_L and MC_L were decreased, B_L was markedly lower and MC_L was higher,
323 especially at lower treatment in both cultivars (Fig. 6). This can be attributed to prioritization of
324 allocation of excess K to the cytosol for metabolic activity rather than to reduce the transmission
325 resistance of CO_2 in the chloroplast. (Reich et al., 1997; Pettigrew 1999; Zhao et al., 2001;
326 Battie-Laclau et al., 2013; Tomás et al., 2016). Then our quantitative limitation analysis showed
327 that S_L was always higher than B_L under three treatments in both species. This pattern of response
328 is consistent with that decreed by other authors (Tanaka et al., 2005; Christian et al., 2014; Peiter et
329 al., 2011). Although several studies have reported that stomatal closure (S_L) plays by far the main

role in the decline of photosynthesis, even at rather severe levels of potassium stress (Bednarsz 1998; shavala ,2003; Lebaudy ,208; Andrés, 2014), but our study draw different conclusions that MC_L is the dominant factor in the AN reduction rather than the S_L in both species. This striking discrepancy is explained by the fact that potassium stress increases the transmission distance of CO_2 in the chloroplast (Zhao et al., 2001; Battie-Laclau et al., 2014).

An even clear picture of the potassium effects emerges when the different limitations are plotted against potassium stress and subsequent recovering (Fig. 6). In both species, all the limitations increased with increasing potassium stress and duration of treatment, but their relative contribution changed. Moreover, in the early stage of potassium treatment (0-21d), all the three limitations under K_0 and K_2 rapidly risen. In the middle and late period (21-80d), all the limitations maintained their high level and changed little under K_0 treatment, this could be the acclimation of plants to severe potassium deficiency, a similar situation had been appeared in the plant drought test (Galle et al., 2009). After recover potassium, obvious decline could be observed of all the three limitations under K_0 and K_2 treatment in both species. However, S_L and MC_L were not hopely recovered to the control level (K_5), they remain at a high level until the end of the experiment. The most likely explanation for this discrepancy might be derived from the effect of potassium stress on the irreversible structural effects of plant leaves. In this sense, potassium deficiency induced the increase of with leaf dry mass per area (MA)(Reich et al., 1997; Pettigrew, 1999; Zhao et al., 2001; Song et al., 2011), reducing the leaves thickness and the volume of cell gap in the leaves, thus reducing the gas conduction ability of CO_2 (Battie-Laclau et al., 2014).

In conclusion, A_N of Pecan and Craya plants declined by increasing S_L , MC_L , and B_L under prolonged severe potassium stress. Pecan, needed K (1.4% of dry weight less than that of Hickory) to avoid the decline of A_N . All of S_L , MC_L , and B_L had a sharp decline under K_0 and K_5 treatment in both species. In summary, the present study strongly reinforces the important role of g_s , g_m , J and $V_{c,max}$ during recovery from potassium stress induced inhibition of photosynthesis, and shows for the first time that such a role depends on the Long-term processing.

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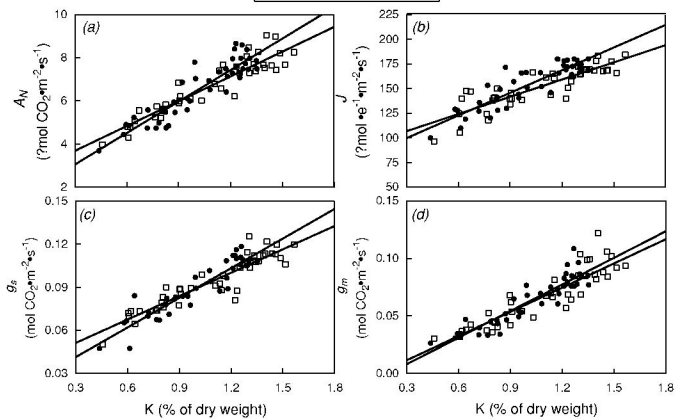
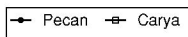
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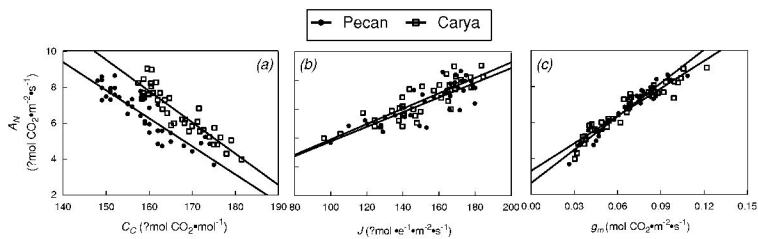
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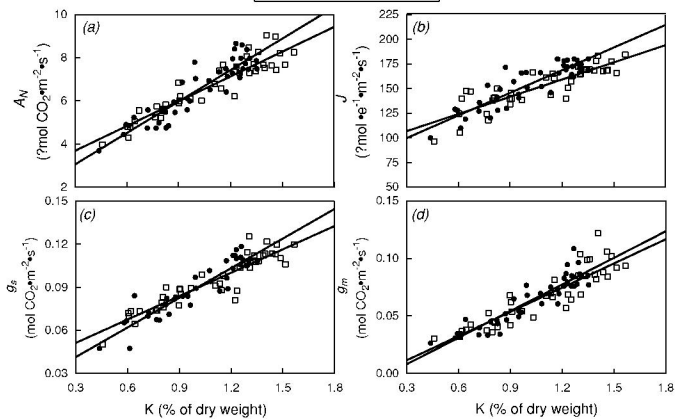
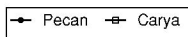
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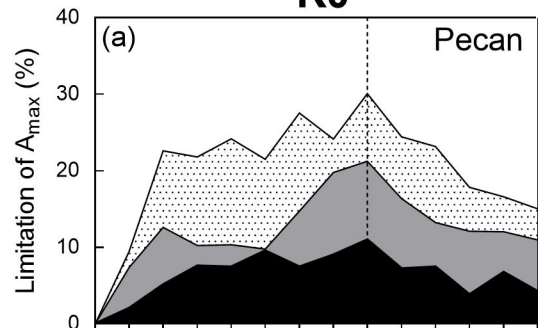




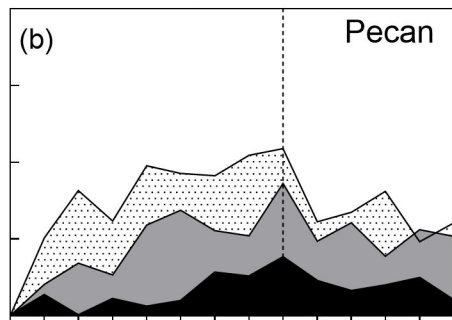




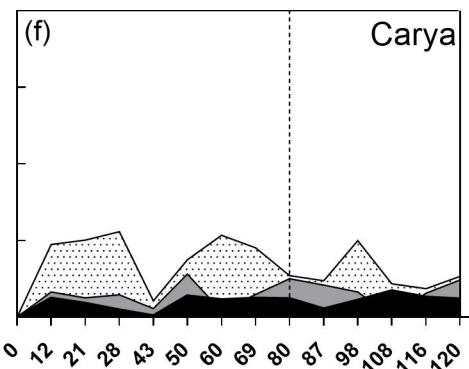
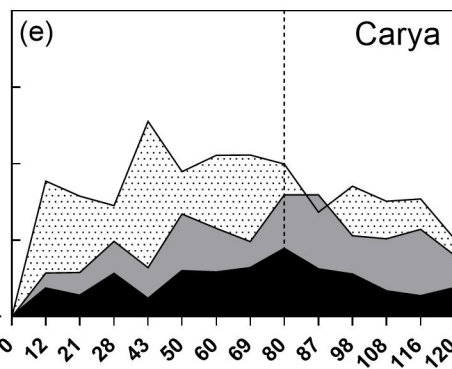
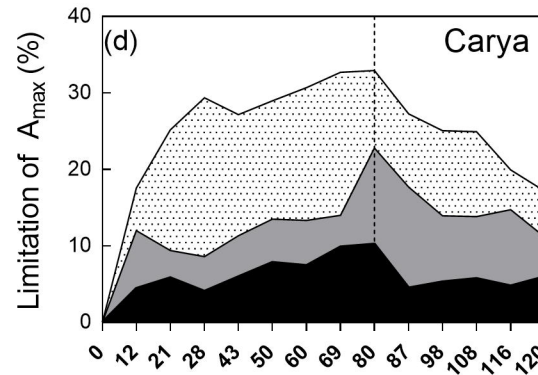
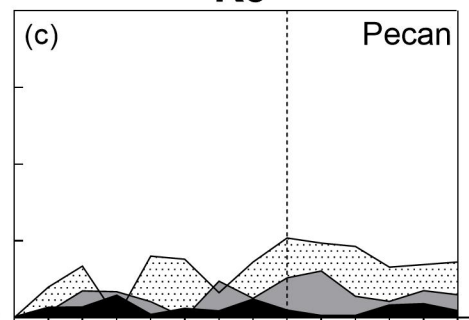
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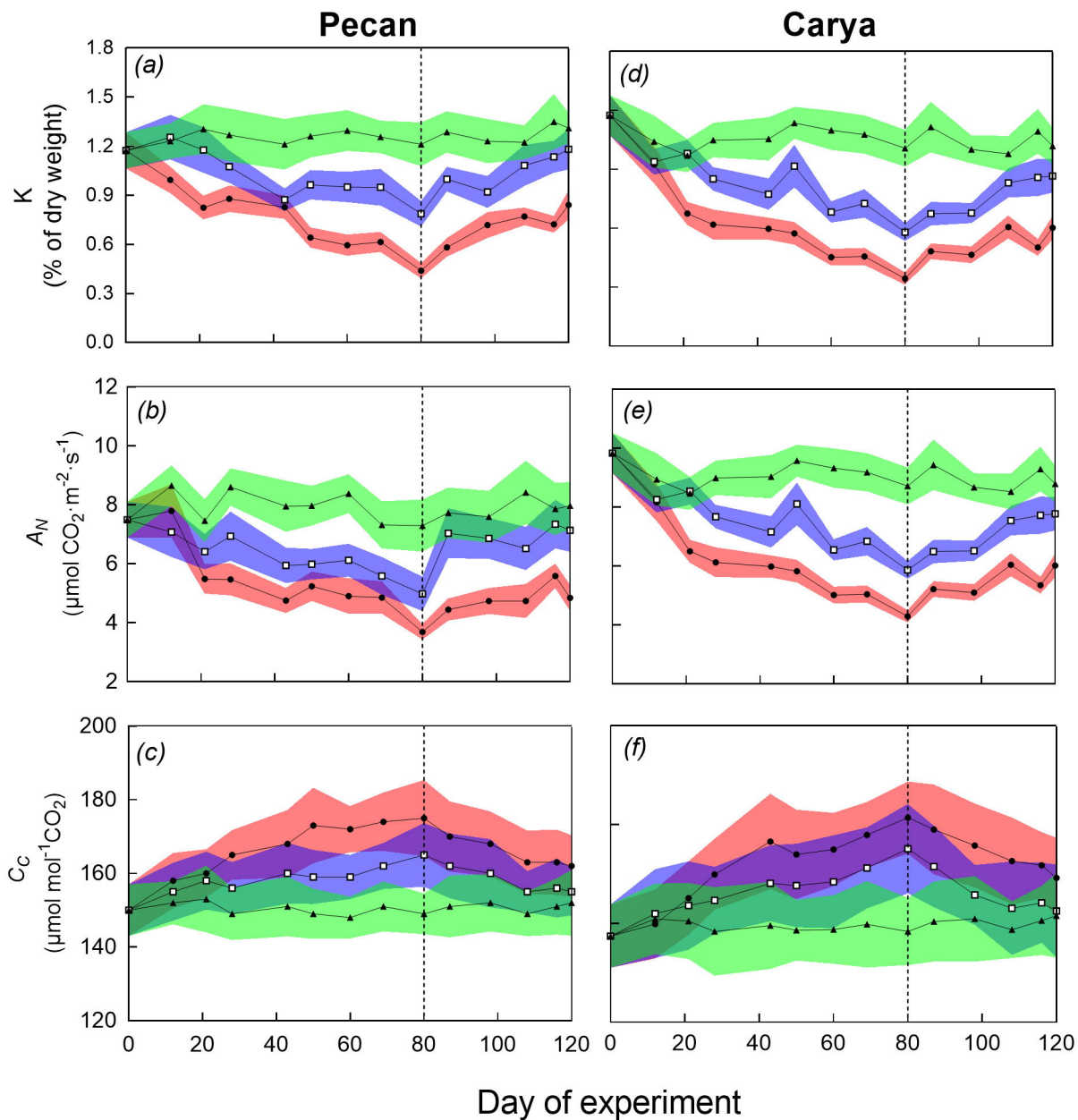
K2



K5



Day of experiment





Pecan

Carya

