

**Supplementary Methods S1: model of  $^{13}\text{C}$  discrimination in  $\text{C}_4$  species**

The *theoretical* model for photosynthetic discrimination against  $^{13}\text{C}$  is (Farquhar and Cernusak, 2012):

$$\Delta_{\text{theo}}^{13} = \frac{1}{1-t} \left[ a_b \frac{C_a - C_L}{C_a} + a_s \frac{C_L - C_i}{C_a} \right] + \frac{1+t}{1-t} \left[ a_m \frac{C_i - C_m}{C_a} + \frac{b_4 + \phi \left( \frac{b_3 C_{\text{bs}} - s}{C_{\text{bs}} - C_m} \right) C_m}{1 + \frac{\phi C_m}{C_{\text{bs}} - C_m}} \frac{C_m}{C_a} \right] \quad \text{Eqn S1.1}$$

where  $C_a$ ,  $C_L$  ( $C_L = C_a - \frac{1.37A}{g_{\text{bl}}}$ , with  $g_{\text{bl}}$  = boundary layer conductance),  $C_i$ , and  $C_{\text{bs}}$

( $C_{\text{bs}} = \frac{\gamma^* O_s + K_c \left( 1 + \frac{O_s}{K_o} \right) \left( \frac{A + R_d}{V_{\text{cmax}}} \right)}{1 - \frac{A + R_d}{V_{\text{cmax}}}}$ , von Caemmerer 2000) are the  $p\text{CO}_2$  in the chamber air, at

the leaf surface, inside the leaf, and in the bundle-sheath cells respectively. The  $t$

( $t = \frac{\alpha_{\text{ac}} E}{2g_{\text{ac}}}$ , Farquhar & Cernusak, 2012) is the ternary correction coefficient where  $E$  is

measured transpiration rate,  $g_{\text{ac}}$  is the conductance to diffusion of  $\text{CO}_2$  in air and

$\alpha_{\text{ac}} = 1 + \bar{a}$ , where  $\bar{a}$  ( $= \frac{a_b(C_a - C_L) + a_s(C_L - C_i)}{C_a - C_i}$ ) is the fractionation for combined  $^{13}\text{C}$

diffusion across the stomata and boundary layer. The  $a_b$ ,  $a_s$ ,  $a_m$ , and  $s$  are the  $^{13}\text{C}$

fractionation during diffusion through the leaf boundary layer (2.9‰), diffusion in air

(4.4‰), liquid phase diffusion and dissolution (1.8‰), and leakage of  $\text{CO}_2$  out of the

bundle-sheath cells (1.8‰), respectively. The  $b_3$  ( $^{13}\text{C}$  fractionation during carboxylation

by Rubisco) and  $b_4$  (net fractionation by  $\text{CO}_2$  dissolution, hydration and PEPc) are

calculated as (Farquhar, 1983):

$$b_3 \cong b'_3 - \frac{eR_d}{V_c} - \frac{0.5fV_o}{V_c} \quad \text{Eqn S1.2}$$

$$b_4 = b'_4 \left( 1 - \frac{V_p}{V_h} \right) + (e_s + h) \frac{V_p}{V_h} - \frac{eR_m}{V_p} \quad \text{Eqn S1.3}$$

where  $b'_3 = 30\text{‰}$ ;  $b'_4 = \left( \frac{-9.483 \cdot 1000}{273 + T} + 23.89 + 2.2 \right) \text{‰}$  ( $T$  is leaf temperature in Celsius);  $f$ ,  $h$ ,

$e_s$  and  $e$ , are the  $^{13}\text{C}$  fractionations during photorespiration (11.6‰, Lanigan *et al.*, 2008),

catalysed  $\text{CO}_2$  hydration (1.1‰, Cousins *et al.*, 2006),  $\text{CO}_2$  dissolution (1.1‰, O'Leary,

1984), and decarboxylation, respectively. The  $e = e_{\text{Rd}} + e^*$ , where  $e^* = \delta_a^{13} - \Delta_{\text{obs}} -$

$\delta_{\text{substrate}}^{13}$  (Wingate *et al.*, 2007; Cernusak *et al.*, 2013). We used  $e_{\text{Rd}} = 0$  and  $e^* \approx \delta_a^{13} -$

(-8), which resulted in average values of 2.0 and 1.9‰ for *Setaria* and *Zea*, respectively.

Rubisco carboxylation ( $V_c$ ), and Rubisco oxygenation ( $V_o$ ) rates are calculated as  $V_c$

$$= \frac{C_{bs}V_{cmax}}{C_{bs}+K_C\left(1+\frac{O_s}{K_O}\right)}, \text{ and } V_o = V_c \frac{2\Gamma^*}{C_{bs}}, \text{ respectively, with } \Gamma^* = O_s\gamma^* \text{ (von Caemmerer, 2000).}$$

Hydration rate ( $V_h$ ) is  $C_m \cdot K_{CA}$  where  $K_{CA}$  is the rate constant of CA for  $CO_2$  and PEP

$$\text{carboxylation rate } (V_p) \text{ is } V_p = \left\{ \begin{array}{l} \text{CA saturated} \rightarrow \frac{C_m V_{pmax}}{C_m + K_p} \\ \text{CA limited} \rightarrow \frac{[HCO_3^-] V_{pmax}}{[HCO_3^-] + K_p} \end{array} \right\}.$$

## References

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