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Plants increase CO_2 uptake by assimilating nitrogen via the photorespiratory pathway

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Derivation of expressions for α_G and α_S

The rate of photorespiration is strongly dependent on C_c; over the range 0 – 200 Pa C_c the change in V_o is approximately 9-fold (Fig. S4). The rate of nitrogen assimilated and exported from the photo-respiratory pathway as glycine is determined by $N_G = \alpha_G V_o$, and that exported as serine by $N_S = \frac{2}{3}\alpha_S V_o$ (Fig. 1). A constant value of α_G or α_S would therefore correspond to a 9-fold difference in assimilated nitrogen across the chosen C_c range, resulting in an effective rate of N-assimilation that would be much higher than the CO_2 assimilation rate at a C_c of around Γ^*. Hence, it seems likely that there is a limit to the rate of nitrogen assimilation set by the maximum rate of *de novo* nitrogen supply to the chloroplast (defined here as N^{max}). This implies that α_G or α_S would decrease with increasing rates of photorespiration. Conversely, amino acid export from the photorespiratory pathway is likely to have an upper bound at low rates of photorespiration, determined by how well glycine export can compete with the decarboxylation via glycine decarboxylase and by how well the alternative use of serine can compete with serine:glyoxylate aminotransferase. We set this upper bound as α_G^{max} for the maximum proportion of carbon exported as glycine and as α_S^{max} for the maximum proportion of carbon exported as serine, where $0 < \alpha_G^{max} < 1$ and $0 < \alpha_S^{max} < 0.75$, with combined bounds of α_G^{max} and α_S^{max} of $0 \le \alpha_G^{max} + \frac{4}{3}\alpha_S^{max} \le 1$. The effective values α_G and α_S are modelled from N^{max}, α_G^{max} and α_S^{max}, so that the ratio of glycine to serine export stays constant and the following conditions are met: (1) α_G and α_S are always smaller than or equal to α_G^{max} and α_S^{max}, and (2) the amount of N exported as glycine and serine is not greater than N^{max}.

Since the flux of nitrogen assimilated into glycine equals $N_G = \alpha_G V_o$ and that into serine equals $N_S = \frac{2}{3}\alpha_S V_o$ (Fig. 1), we can estimate the proportion of nitrogen assimilated as glycine as

$$\beta = \frac{3\alpha_G^{max}}{3\alpha_G^{max} + 2\alpha_S^{max}} \quad . \tag{15}$$

Applying condition (2) to the equations above it follows that the flux of nitrogen assimilated as glycine cannot be larger than $N_G = N^{max}\beta$ and as serine not larger than $N_S = N^{max}(1-\beta)$. These equations correspond to

$$\alpha_G = \frac{N^{max}\beta}{V_o} \quad \text{or} \quad V_o = \frac{N^{max}\beta}{\alpha_G} \tag{S1}$$

and

$$\alpha_S = \frac{3N^{\max}(1-\beta)}{2V_o} \quad \text{or} \quad V_o = \frac{3N^{\max}(1-\beta)}{2\alpha_S} \ . \tag{S2}$$

Here, V_o can be calculated from equation (2) as

$$V_o = V_c \frac{O}{S_{c/o}C} = V_c \Phi \ . \tag{S3}$$

Under a **Rubisco limitation**, $V_c = W_c$ and therefore:

$$W_c = \frac{V_{c\max} C}{C + K_c(1 + O/K_o)} \tag{6}$$

and

$$V_o^c = \frac{V_{c\max} \dfrac{O}{S_{c/o}}}{C + K_c(1 + O/K_o)} \ . \tag{S4}$$

Since V_o^c is independent of the rate of nitrogen assimilation, we can write α_G and α_S as the minimum of the "competition-limited" maximum α and the N-supply-limited α described in equations (S1) and (S2):

$$\alpha_G = \min\left\{\alpha_G^{\max}, \frac{N^{\max}\beta}{V_o^c}\right\} \tag{16}$$

and

$$\alpha_S = \min\left\{\alpha_S^{\max}, \frac{3N^{\max}(1-\beta)}{2V_o^c}\right\} \ . \tag{17}$$

Equations (16) and (17) fulfil conditions (1) and (2) outlined above.

Under a **RuBP-regeneration limitation**, $V_c = W_j$ and therefore:

$$W_j = \frac{J}{4 + (4 + 8\alpha_G + 4\alpha_S)\Phi} \tag{13}$$

and

$$V_o^j = \frac{J}{\dfrac{4}{\Phi} + (4 + 8\alpha_G + 4\alpha_S)} \ . \tag{S5}$$

Substituting equation (S2) for α_S and equation (S1) for V_o^j into equation (S5) results in

$$\frac{N^{\max}\beta}{\alpha_G} = \frac{J}{\frac{4}{\Phi} + 4 + \alpha_G\left(8 + 6\frac{1-\beta}{\beta}\right)},$$

which we can solve for α_G:

$$\alpha_G = \frac{4N^{\max}\beta\left(\frac{1}{\Phi}+1\right)}{J - N^{\max}\left(2\beta+6\right)}. \quad \text{(S6)}$$

Similarly, in equation (S5) we can substitute equation (S1) for α_G and equation (S2) for V_o^j and solve for α_S:

$$\alpha_S = \frac{6N^{\max}\left(1-\beta\right)\left(\frac{1}{\Phi}+1\right)}{J - N^{\max}\left(2\beta+6\right)}. \quad \text{(S7)}$$

Under a RuBP-regeneration limitation we can set expressions for α_G and α_S, equivalent to the parameterisation of α_G and α_S under a Rubisco limitation,

$$\alpha_G = \begin{cases} \min\left\{\alpha_G^{\max}, \dfrac{4N^{\max}\beta\left(\frac{1}{\Phi}+1\right)}{J - N^{\max}\left(2\beta+6\right)}\right\}, \text{ if } J > N^{\max}\left(2\beta+6\right) \\ \alpha_G^{\max}, \text{ if } J \le N^{\max}\left(2\beta+6\right) \end{cases} \quad \text{(18)}$$

and

$$\alpha_S = \begin{cases} \min\left\{\alpha_S^{\max}, \dfrac{6N^{\max}\left(1-\beta\right)\left(\frac{1}{\Phi}+1\right)}{J - N^{\max}\left(2\beta+6\right)}\right\}, \text{ if } J > N^{\max}\left(2\beta+6\right) \\ \alpha_S^{\max}, \text{ if } J \le N^{\max}\left(2\beta+6\right) \end{cases}. \quad \text{(19)}$$

Similarly, under a **TPU limitation**, $V_c = W_p$ is dependent on N-assimilation:

$$W_p = \frac{3T_P}{1 - 0.5\left(1 + 3\alpha_G + 4\alpha_S\right)\Phi} \quad \text{(14)}$$

and therefore, with equation (S3):

$$V_o^p = \frac{3T_p}{\frac{1}{\Phi} - 0.5\left(1 + 3\alpha_G + 4\alpha_S\right)} \ . \tag{S8}$$

As before, if the N-supply is limiting N-assimilation, we can substitute α_S for equation (S2) and V_o^p for equation (S1):

$$\frac{N^{\max}\beta}{\alpha_G} = \frac{3T_p}{\frac{1}{\Phi} - 0.5\left(1 + 3\alpha_G\left(1 + \frac{2(1-\beta)}{\beta}\right)\right)} \ , \tag{S9}$$

which we can solve for α_G to obtain:

$$\alpha_G = \frac{N^{\max}\beta\left(\frac{2}{\Phi} - 1\right)}{6T_p + 3N^{\max}\left(2 - \beta\right)} \ . \tag{S10}$$

Finally, solving equation (S8) for α_S by substituting equation (S1) for α_G and equation (S2) for V_o^p yields

$$\alpha_S = \frac{\frac{3}{2}N^{\max}\left(1-\beta\right)\left(\frac{2}{\Phi} - 1\right)}{6T_p + 3N^{\max}\left(2 - \beta\right)} \ . \tag{S11}$$

To fulfil conditions (1) and (2), we write α_G and α_S as the minimum of the "competition-limited" maximum α and the N-supply-limited α described in equations (S1) and (S2):

$$\alpha_G = \min\left\{\alpha_G^{\max}, \frac{N^{\max}\beta\left(\frac{2}{\Phi} - 1\right)}{6T_p + 3N^{\max}\left(2 - \beta\right)}\right\} \tag{20}$$

and

$$\alpha_S = \min\left\{\alpha_S^{\max}, \frac{\frac{3}{2}N^{\max}\left(1-\beta\right)\left(\frac{2}{\Phi} - 1\right)}{6T_p + 3N^{\max}\left(2 - \beta\right)}\right\} \ . \tag{21}$$

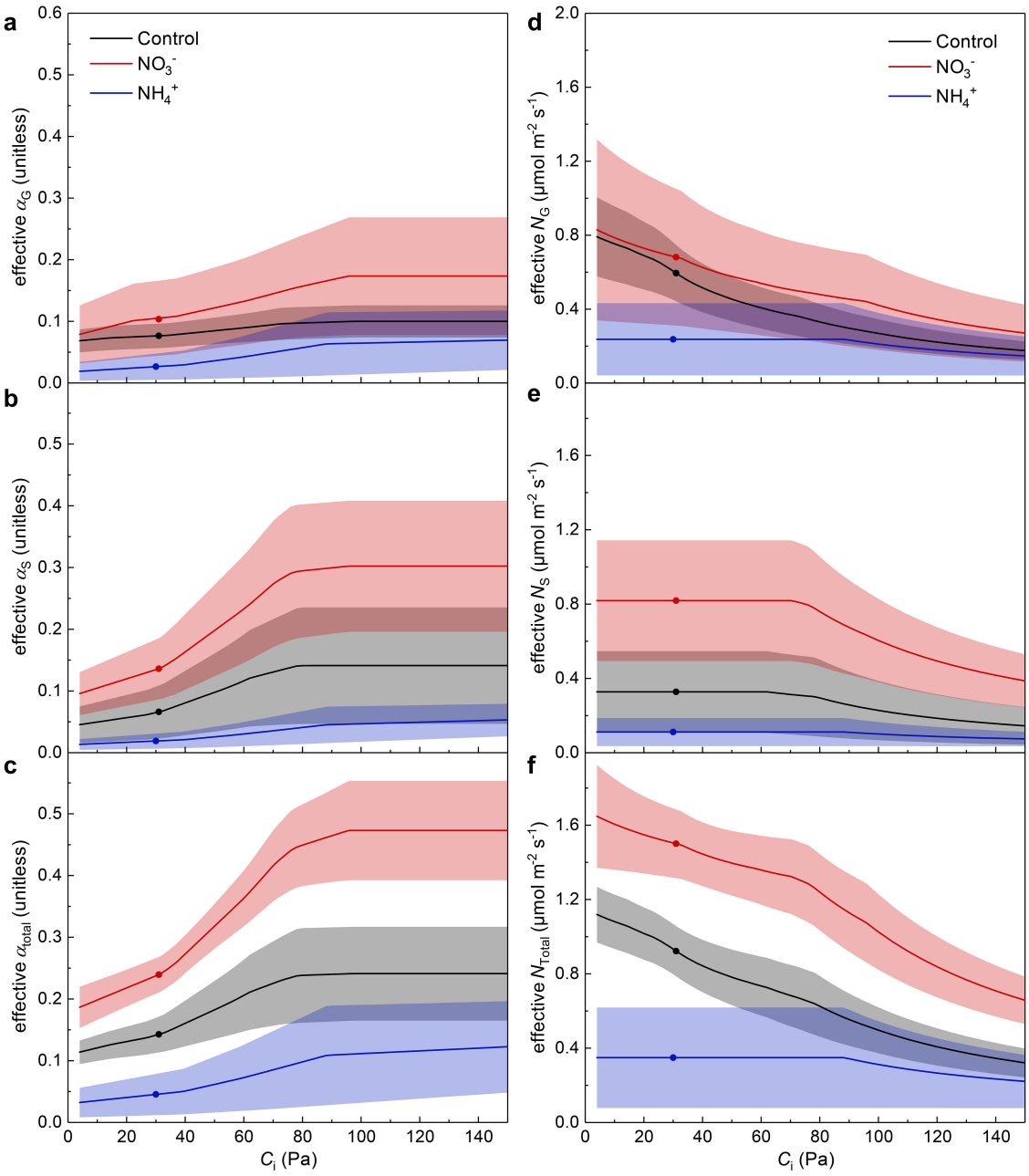

Figure S1 | CO_2 responses of the fraction of photorespiratory carbon exported as amino acids. Shown are carbon export as (**a**) glycine (α_G); (**b**) serine (α_S); and (**c**) the sum of both (α_{total}), as the average of the values fitted to individual A/C_i response curves from control plants (black), NO_3^--fertilised plants (red), and NH_4^+-fertilised plants. The corresponding rates of N assimilated as amino acids are shown for (**d**) glycine (N_G); (**e**) serine (N_S); and (**f**) the total rate of *de novo* N-assimilation via the photorespiratory pathway (N_{total}). Circles indicate the actual rates at an ambient CO_2 concentration of 40 Pa. While the allocation of N-assimilation to glycine *vs*. serine by the model depends very much on the quality of the data as well as the accuracy of the value used for $S_{c/o}$ (**a**, **b**, **d**, **e**), the effective α_{total} and N_{total} is much less sensitive to these factors (**c**, **f**). The A/C_i data that were used to derive the parameters in (**a**-**f**) are shown in Figure S2. n = 3 to 8 ± SE.

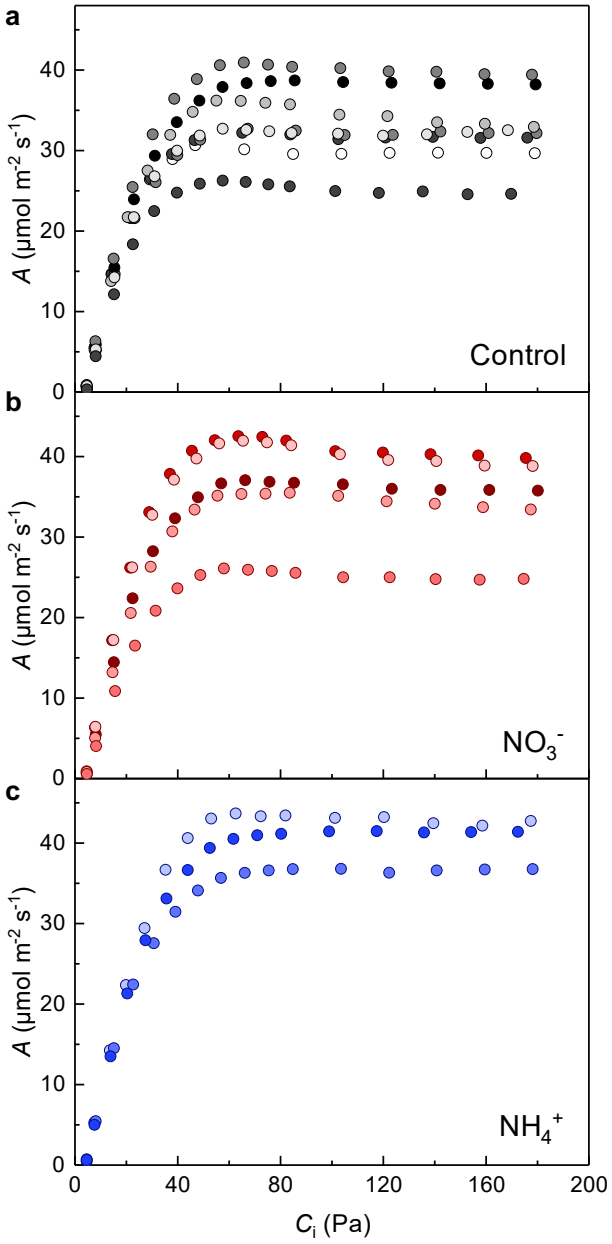

Figure S2 | A/C_i response curves measured on plants exposed to different fertilisation regimes. Shown are measurements on untreated control plants (**a**), NO_3^--fertilised plants (**b**), and NH_4^+-fertilised plants (**c**). Symbols of different shading correspond to measurements on different plants.

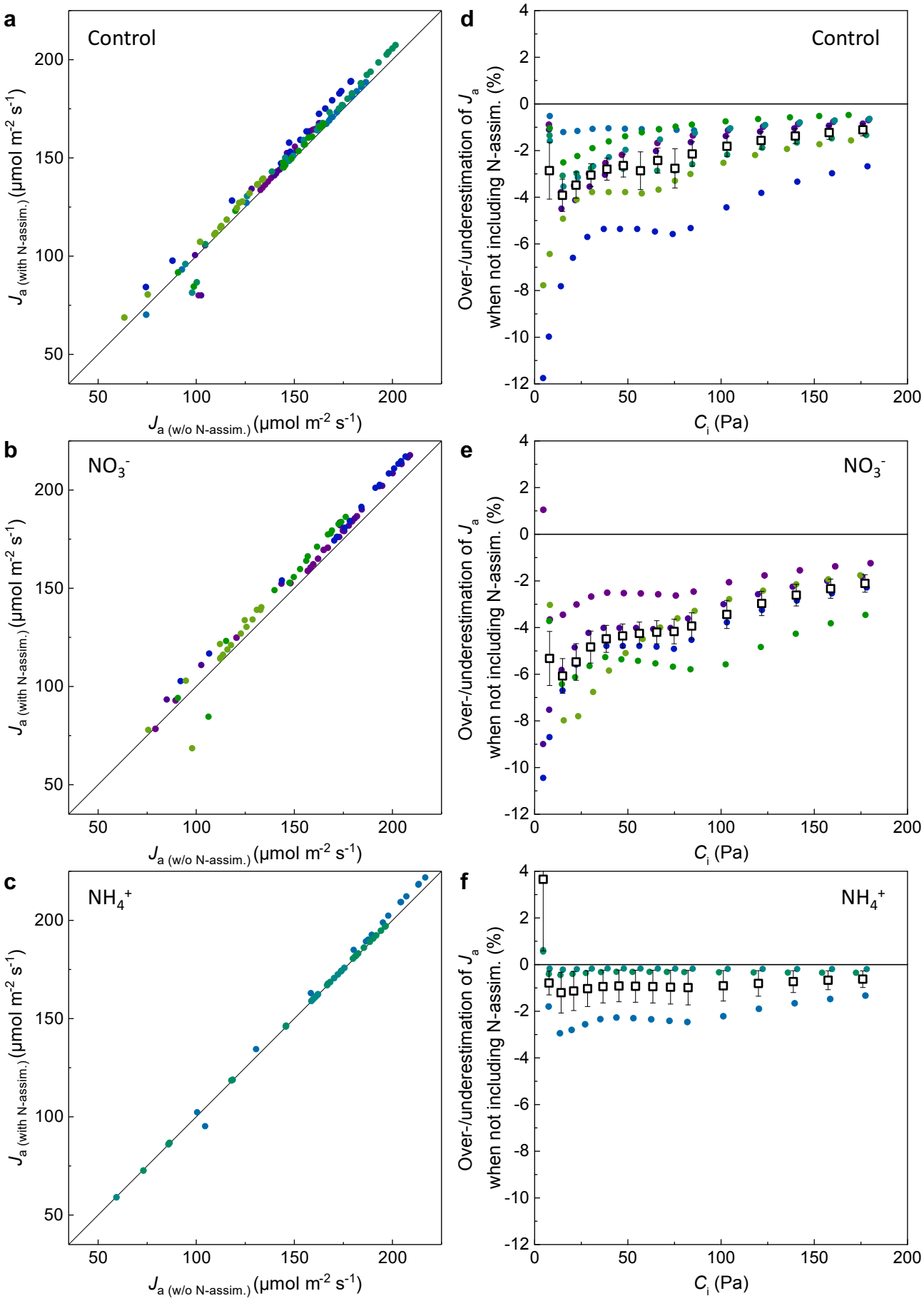
a
Control
$J_{a\ (with\ N\text{-}assim.)}$ (μmol m^{-2} s^{-1})
$J_{a\ (w/o\ N\text{-}assim.)}$ (μmol m^{-2} s^{-1})
b
NO_3^-
c
NH_4^+
d
Control
Over-/underestimation of J_a when not including N-assim. (%)
C_i (Pa)
e
NO_3^-
f
NH_4^+

Figure S3 (previous page) | Model comparison of rates of electron transport estimated from gas exchange measurements. Comparison of J_a calculated according to the standard FvCB model ($J_{a\ (w/o\ N\text{-}assim.)}$) and according to our new model that includes the electron requirement for nitrate reduction associated with photorespiratory nitrogen assimilation ($J_{a\ (with\ N\text{-}assim.)}$). (**a**) Plants measured before fertilisation (control); plants measured two days after fed with 0.5L of a solution containing 25mM nitrogen in the form of KNO_3 (**b**), or in the form of $(NH_4)_2SO_4$ (**c**). Symbols of different colour represent different individual plants. J_a is generally larger when N-assimilation is included, except for some values measured at low CO_2 concentrations. This is a consequence of $\Gamma^*_{\alpha_G}$ changing with α_G, which becomes significant at CO_2 concentrations close to the compensation point. (**d**)-(**f**) Over- or underestimation of J_a when N-assimilation is not accounted for, relative to values calculated with equation (9) that account for the electrons required for nitrate reduction. Symbols of different colour correspond to different individual plants, open squares represent average values for each treatment. Values shown are restricted to the range of +4 to -12% for clarity, which excludes some values close to $\Gamma^*_{\alpha_G}$. Ignoring N-assimilation has a negligible impact on the estimated J_a when N-assimilation is small (**f**). However, when plants actively assimilate N, such as in plants fertilised with nitrate, more than 10% of photosynthetic electron transport (with an average of 2-4% in unfertilised plants (**d**) and 3-6% in nitrate-fertilised plants (**e**)) can go towards N-assimilation.

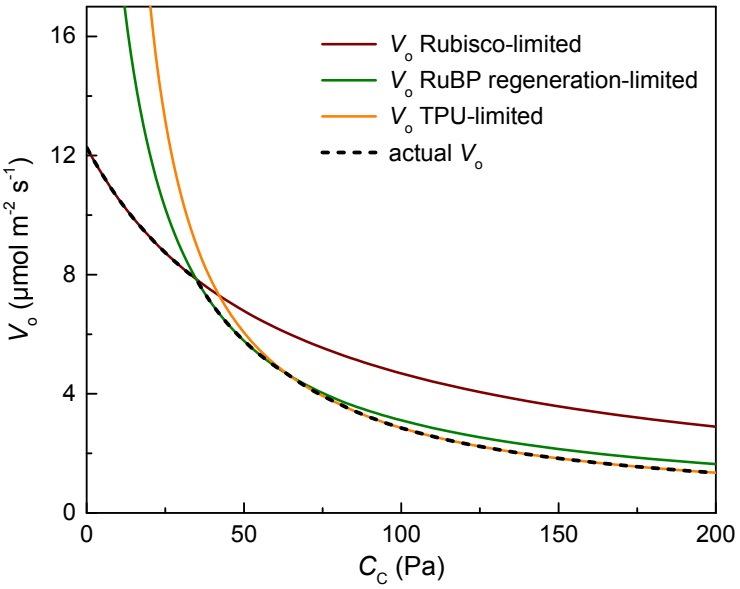

Figure S4 | Modelled rates of RuBP oxygenation (V_o). The graph shows values expected under a Rubisco limitation (dark red), RuBP-regeneration limitation (green), and a TPU limitation (orange) in response to changes in the chloroplastic CO_2 concentration (C_c). The effective rate of V_o accounting for the change in limitations along the CO_2 gradient is plotted as a dashed black line. Parameter values used to model the responses are the same as in Figure 3a.

Table S1 | Results from individual A/C_i curves fitted with the photosynthesis model incorporating N-assimilation. In addition to the upper bounds of the model parameters α_G^{max}, α_S^{max}, and N^{max} the effective values at an ambient CO_2 concentration of 40 Pa are given. Also calculated is the ratio of N assimilated per C assimilated (mol mol^{-1}). In the control treatment, corresponding most closely to the average condition the sunflower plants would experience throughout their growth period, the model indicates that N is assimilated via the photorespiratory pathway at a rate of 3.4% of the rate of *A*. Given an average C/N ratio of sunflower plants of around 10[Ref 1], these results indicate that, on average, approximately 1/3 of the plant N is assimilated via the photorespiratory pathway. In the presence of NO_3^-, the rate of photorespiratory N-assimilation increases to 5.5% of the rate of *A*, indicating that the plant has the ability to substantially increase this N-assimilation capacity when nitrogen becomes available. n = 3 to 8 ± SE. The letters indicate the statistical ranking among the treatments using a one-way ANOVA with a post-hoc Tukey-Kramer test (different letters indicate differences at $P < 0.05$).

Treatment	**Control**	**NO_3^-**	**NH_4^+**
Upper bounds of parameters			
α_G^{max}	0.100 ± 0.026 [a]	0.171 ± 0.094 [a]	0.088 ± 0.039 [a]
α_S^{max}	0.141 ± 0.094 [a]	0.302 ± 0.106 [a]	0.077 ± 0.021 [a]
N^{max}	1.17 ± 0.16 [a,b]	1.70 ± 0.32 [a]	0.35 ± 0.27 [b]
Effective values at ambient CO_2 (40 Pa)			
α_G	0.077 ± 0.020 [a]	0.103 ± 0.060 [a]	0.026 ± 0.021 [a]
α_S	0.066 ± 0.043 [a]	0.136 ± 0.049 [a]	0.019 ± 0.012 [a]
α_{total}	0.143 ± 0.029 [a,b]	0.239 ± 0.029 [a]	0.045 ± 0.033 [b]
N_G	0.59 ± 0.15 [a]	0.68 ± 0.37 [a]	0.24 ± 0.20 [a]
N_S	0.33 ± 0.22 [a]	0.82 ± 0.32 [a]	0.11 ± 0.08 [a]
N_{total}	0.92 ± 0.14 [a,b]	1.50 ± 0.18 [a]	0.35 ± 0.27 [b]
A	27.14 ± 0.97 [a]	28.25 ± 2.26 [a]	28.30 ± 0.58 [a]
mol N/mol C assimilated	0.034 ± 0.005 [a,b]	0.055 ± 0.010 [a]	0.012 ± 0.009 [b]

References

1 de la Mata, L., Cabello, P., de la Haba, P. & Agüera, E. Growth under elevated atmospheric CO_2 concentration accelerates leaf senescence in sunflower (*Helianthus annuus* L.) plants. *J Plant Physiol* **169**, 1392-1400, doi:10.1016/j.jplph.2012.05.024 (2012).