Fig. S1. Relative humidity and leaf-to-air vapour pressure deficit in the leaf cuvette during measurements of CO_2 response curves of plants grown at low (V1, open symbols) or high VPD (V2, filled symbols). Dashed lines indicate the relative humidity or VPD of the growth environment of the low VPD treatment (grey lines) and high VPD treatment (black lines). Data are shown as mean ± SE (n = 17-20, data of N treatments were averaged).

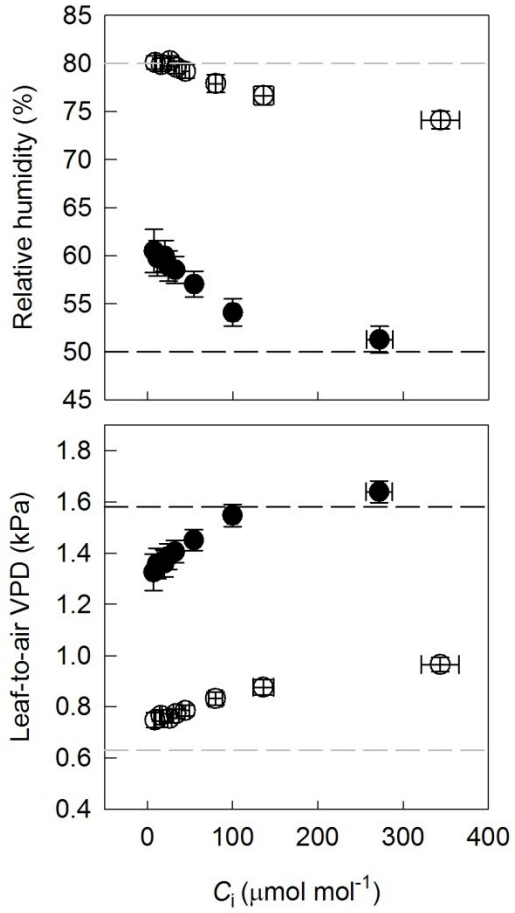

Fig. S2. Minimum mesophyll conductance to CO_2 ($g_{m\ min} = A/C_i$) and the estimated g_m in response to short-term variation of C_i under low (N1, circles) or high N supply (N2, triangles) combined with low (V1, open symbols, dushed lines) or high VPD (V2, filled symbols, solid lines). Operating conditions of gas exchange measurements were the same as conditions in growth chambers (leaf temperature 25 °C, PPFD 800 μmol m^{-2} s^{-1}, VPD 0.8 kPa for V1 and 1.6 kPa for V2). Data are shown as mean ± SE (n = 5-6).

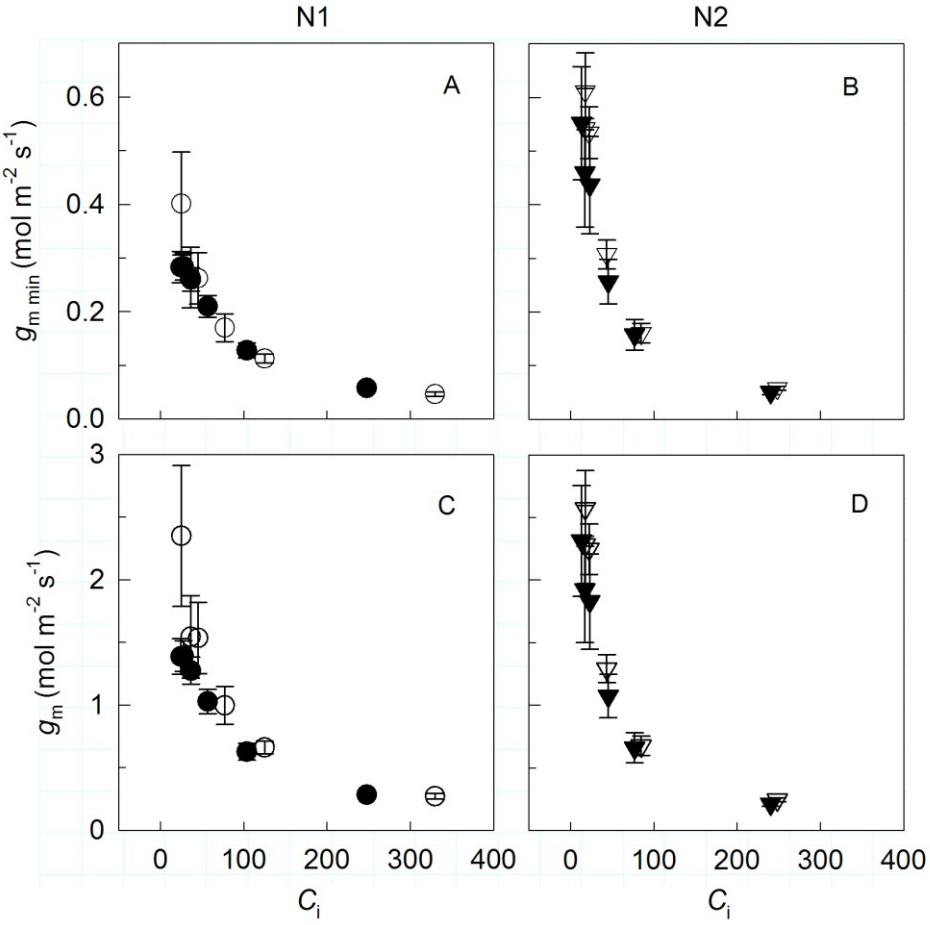

Fig. S3. Photosynthetic light response curves of leaves of *C. squarrosa* grown under low (N1, circles) or high N supply (N2, triangles) combined with low (V1, open symbols) or high VPD (V2, filled symbols). Operating conditions of gas exchange measurements were the same as conditions in growth chambers (leaf temperature 25 °C, [CO_2] 390 μmol mol^{-1}, VPD 0.8 kPa for V1 and 1.6 kPa for V2). Data are shown as mean ± SE (n = 3-4).

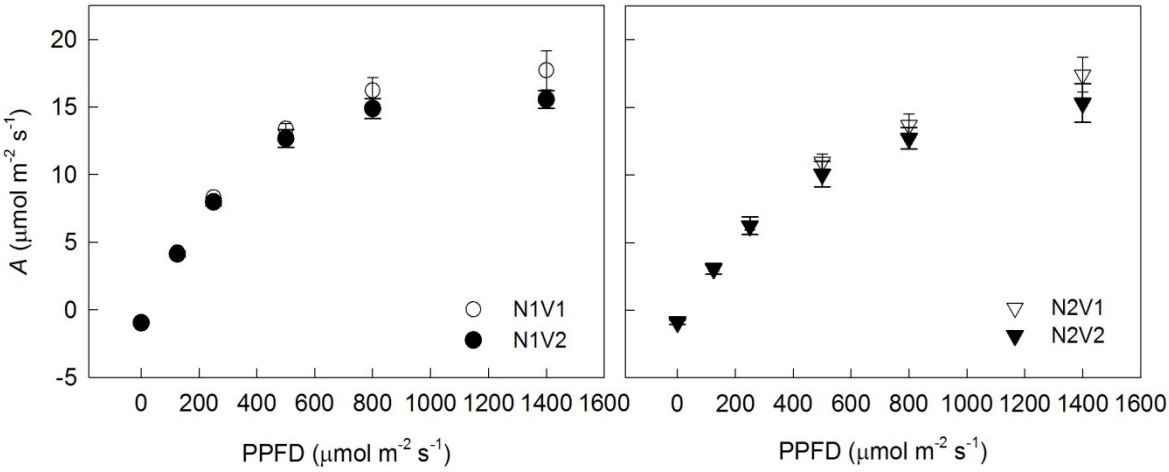

Fig. S4. PEP carboxylation rate (V_p, squares) and Rubisco carboxylation rate (V_c, triangles) in response to short-term variation of intercellular CO_2 (C_i) under low (N1, panel A) or high N supply (N2, panel B) combined with low (V1, open symbols, dushed lines) or high VPD (V2, filled symbols, solid lines). V_p and V_c were estimated according to equations in von Caemmerer and Furbank (1999): $V_p = A + 0.5R_L/(1 - \phi)$; $V_c = A + R_L + 0.5V_o = (A + R_L)/(1 - 0.5V_o/V_c)$. For determination of V_o/V_c see 'Materials and Methods'. Operating conditions of gas exchange measurements were the same as conditions in growth chambers (leaf temperature 25 °C, PPFD 800 µmol m^{-2} s^{-1}, VPD 0.8 kPa for V1 and 1.6 kPa for V2). Data are shown as the mean ± SE (n = 5-6). The regressions were fitted using a function of $y = y_0 + a\ Ln\ (x)$ for V_p, and using a function of $y = a\ (1\text{-}exp(-bx))$ for V_c , all regressions have $r^2 > 0.9$.

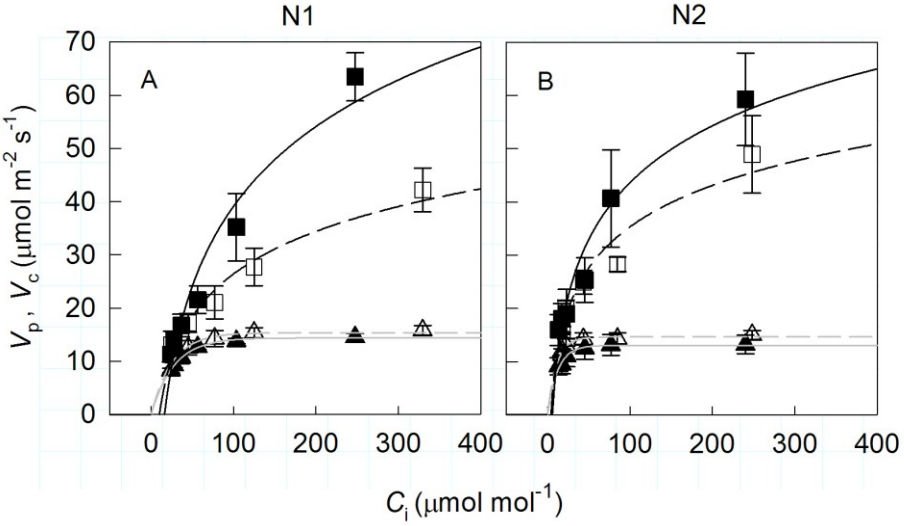

Fig. S5. Dynamic changes of bundle-sheath leakiness in response to short-term variation of C_i under low (N1, circles) or high N supply (N2, triangles) combined with low (V1, open symbols, dushed lines) or high VPD (V2, filled symbols, solid lines). Bundle-sheath leakiness was calculated using different models of ^{13}C discrimination: the first model (panel A and B) assumes a constant gm/gmmin ratio across C_i levels, g_m at ambient CO_2 is 0.45 mol m^{-2} s^{-1}; the 2rd model (panel C and D) assumes infinite g_m across C_i levels; the 3rd model (panel E and F) assumes g_m = infinite, CO_2 and HCO_3^- are not at equilibrium, and $V_p/V_h = 0.23$; the 4th model assumes g_m = infinite, $C_{bs}/(C_{bs} - C_m) = 1$, and $C_m/(C_{bs} - C_m) = 0$. Operating conditions of gas exchange measurements were the same as conditions in growth chambers (leaf temperature 25 °C, PPFD 800 μmol m^{-2} s^{-1}, VPD 0.8 kPa for V1 and 1.6 kPa for V2). Data are shown as mean ± SE (n = 5-6).

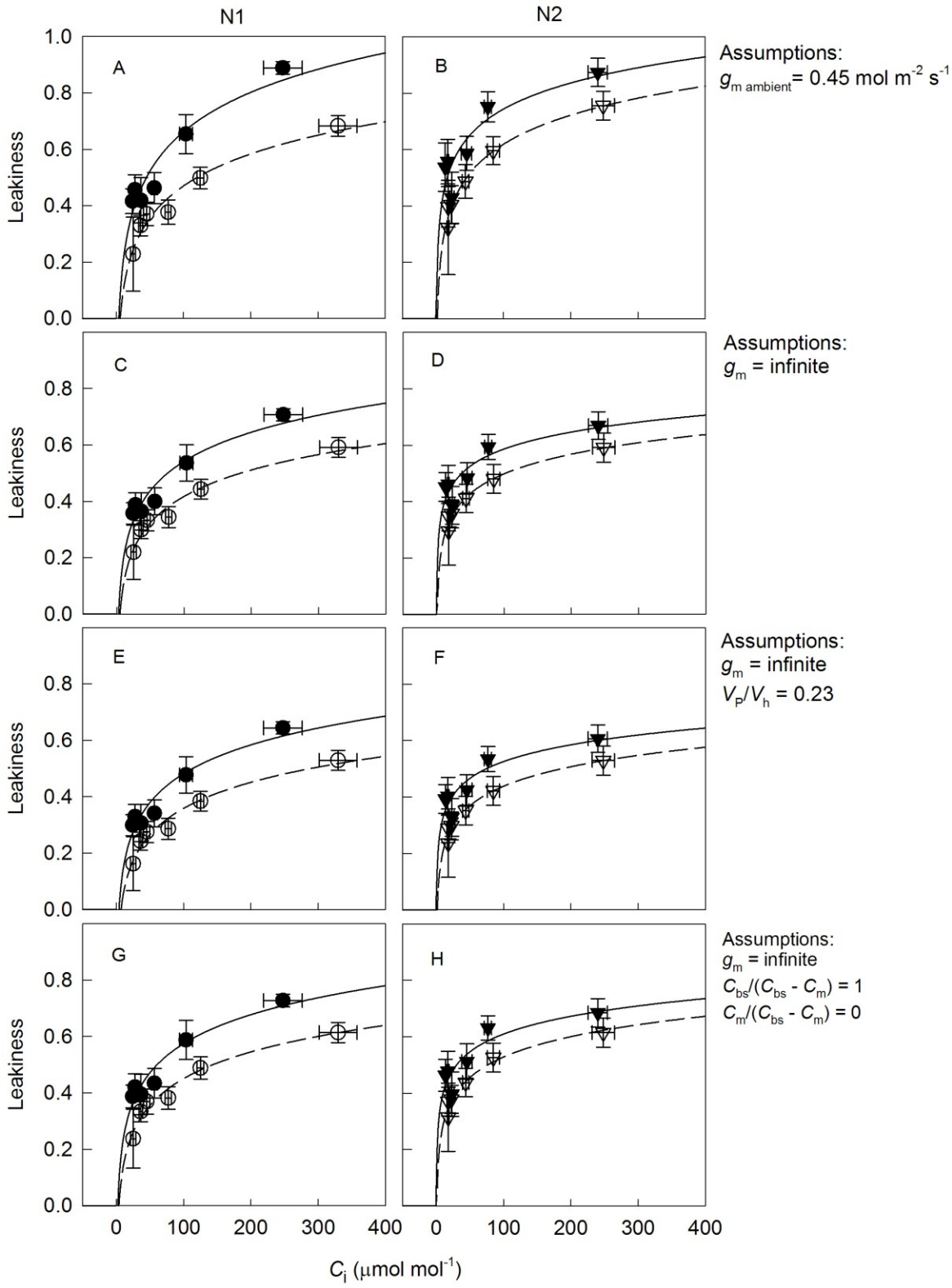
N1
N2
A
B
C
D
E
F
G
H
Leakiness
0.0
0.2
0.4
0.6
0.8
1.0
0
100
200
300
400
C_i (μmol mol^{-1})
Assumptions:
$g_{m\ ambient}$= 0.45 mol m^{-2} s^{-1}
Assumptions:
g_m = infinite
Assumptions:
g_m = infinite
V_P/V_h = 0.23
Assumptions:
g_m = infinite
$C_{bs}/(C_{bs} - C_m)$ = 1
$C_m/(C_{bs} - C_m)$ = 0

Fig. S6. Correlations between WUE_i and bundle-sheath leakiness of *C. squarrosa*. Data were obtained during short-term change of CO_2 concentration, each symbol represents mean ± standard error (n = 5-6). Symbols distinguish plants grown under low (N1, circles) or high N supply (N2, triangles) combined with low (V1, open symbols) or high VPD (V2, filled symbols). Operating conditions of gas exchange measurements were the same as conditions in growth chambers (leaf temperature 25 °C, PPFD 800 µmol m^{-2} s^{-1}, VPD 0.8 kPa for V1 and 1.6 kPa for V2).