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Interrelationships between nitrogen supply and photosynthetic parameters in *Vicia faba* L.

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Abstract

We determined for *Vicia faba* L the influence of nitrogen uptake and accumulation on the values of photon saturated net photosynthetic rate (P_{Nmax}), quantum yield efficiency (α), intercellular CO_2 concentration (C_i), and carboxylation efficiency (C_e). As leaf nitrogen content (N_L) increased, the α converged onto a maximum asymptotic value of 0.0664±0.0049 µmol(CO_2) µmol(quantum)$^{-1}$. Also, as N_L increased the C_i value fell to an asymptotic minimum of 115.80±1.59 µmol mol^{-1}, and C_e converged onto a maximum asymptotic value of 1.645±0.054 µmol(CO_2) m^{-2} s^{-1} Pa^{-1} and declined to zero at a N_L-intercept equal to 0.596±0.096 g(N) m^{-2}. α fell to zero for an N_L-intercept of 0.660±0.052 g(N) m^{-2}. As N_L increased, the value of P_{Nmax} converged onto a maximum asymptotic value of 33.400±2.563 µmol(CO_2) m^{-2} s^{-1}. P_N fell to zero for an N_L-intercept of 0.710±0.035 g(N) m^{-2}. Under variable daily meteorological conditions the values for N_L, specific leaf area (δ_L), root mass fraction (R_f), P_{Nmax}, and α remained constant for a given N supply. A monotonic decline in the steady-state value of R_f occurred with increasing N supply. δ_L increased with increasing N supply or with increasing N_L.

Additional key words: carboxylation efficiency; faba bean; intercellular CO_2 concentration; net photosynthetic rate; quantum yield efficiency; root mass fraction.

Introduction

In general, the overall relationship between the photosynthetic rate and leaf nitrogen content (N_L) follows a curvilinear pattern, with the linear portion of the curve originating from a positive N_L-intercept (Schmitt and Edwards 1981, Sage and Pearcy 1987, Sinclair and Horie 1989, Meinzer and Zhu 1998). As N_L increases, the photosynthetic rate converges onto an asymptotic maximum value. This curvilinear relationship arises as a direct consequence of various rate limiting processes which play a role in setting the upper limit for the maximum possible values obtainable for photosynthetic parameters such as the photon-saturated net rate of photosynthesis (P_{Nmax}) and the quantum yield efficiency (α) as N_L increases. For example, the maximum value for P_{Nmax} is directly proportional to the flux of CO_2 from the atmosphere. The rate of CO_2 diffusion into the leaf is directly proportional to the atmosphere-leaf CO_2 concentration gradient, the stomatal conductance, the mesophyll conductance, and the carboxylation efficiency of the leaf (Charles-Edwards 1978, Fisher *et al.* 1981, Evans and Caemmerer 1996, Katul *et al.* 2000). The carboxylation efficiency (C_e) is dependent on the concentration of the various enzyme catalytic sites available for the different reactions involved in photosynthetic metabolism. Theoretically the upper limit to the maximum value for P_{Nmax} which also determines the plant potential photosynthetic capacity, is proportional to the number of active catalytic sites in chloroplasts that are involved in the reductive assimilation of CO_2. The total number of these catalytic sites involved in CO_2 assimilation is dependent on the proportion of the total N_L allocated to the chloroplasts.

The asymptotic relationship between P_{Nmax} or α and N_L indicates that as the number of catalytic units increases, other processes become in turn rate limiting. As N_L increases, these processes will in turn fix the upper limit of the plant N efficiency for biomass production. In this respect, the rate of carbon dioxide diffusion into the leaf will be a factor limiting the plant N dependent

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Abbreviations: C_a – ambient CO_2 concentration; C_e – carboxylation efficiency; C_i – intercellular CO_2 concentration; N_c – tissue nitrogen concentration; N_L – leaf nitrogen content; N_s – nitrogen supply concentration; N_t – total elemental nitrogen accumulated by the plant; P_{Nmax} – photon-saturated rate of photosynthesis; R_f – root mass fraction; r_m – mesophyll resistance; α – quantum yield efficiency; δ_L – specific leaf area; μ_w – specific growth rate.

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capacity for biomass production (Evans and Caemmerer 1996, Katul *et al.* 2000). Other transport processes such as the rate of phosphate recycling between the chloroplast and cytosol also play a prominent role in fixing the upper limit of the plant capacity for biomass production (Cockburn *et al.* 1967a,b, Usuda and Edwards 1982, Pradet and Raymond 1983, Mächler *et al.* 1984, Rao and Terry 1989, 1995, Rao *et al.* 1989a,b).

If the concentration of nitrogen supply (N_s) to the roots remains constant while the plant exponentially grows, then steady-state contents of N within the plant can only be maintained if the capacity for N accumulation increases exponentially during plant growth (Hirsose 1986, Garnier *et al.* 1989). In this context, the term balance exponential growth has been used to describe the situation where under constant conditions of nutrient supply, moisture availability, and saturating irradiance, all extensive variables (*e.g.* plant biomass and leaf area) increase exponentially at a constant specific growth rate (μ_w). Under these conditions all variables that are either ratios or rates (*e.g.* plant N concentration) remain constant (Thornley 1998). Under balanced exponential growth, it is expected that μ_w or specific photosynthetic rate would be determined by the plant steady-state N_c. This was an important consideration in this study. Under balanced exponential growth, it would also be expected that ratios such as the root mass fraction (R_f) and the specific leaf area (δ_L) would remain constant. Information on these predictions does not exist for *Vicia faba*. For *V. faba* there are also no reports on how N_L influences P_{Nmax} and α. Therefore the objectives of this study were: (*1*) determination of the asymptotic values and associated intercept values for P_{Nmax} and α with respect to $N_{L;}$ (*2*) provision of information on the minimum value of N_L below which P_{Nmax} and α equal zero; (*3*) establishing whether intensive variables or ratios such as P_{Nmax}, α, N_c, N_L, δ_L, and R_f remain constant under steady-state N_s. The latter information will help decide whether these intensive variables or ratios can be treated as unchanging plant growth parameters whose values are governed by the steady-state N_s.

Materials and methods

Plants: We used the cold-hardy *Vicia faba* L. cv. Aquadulce Claudia. Early spring or autumn planted cold-hardy cultivars require approximately 130-150 d for crop development, while over-wintering cultivars require approximately 240 d to reach dry seed maturity. Faba beans germinate and grow well in cool soil. For out-door experimental pot trials seeds were sown on the 23rd February and all measurements were done in April. During April, the daily average extraterrestrial global irradiance was 28.7 MJ m^{-2}, average photoperiod 11.99 h, average daily maximum temperature 21.6 °C, average daily minimum temperature 9.1 °C, and average A-pan evaporation 6.3 mm d^{-1}.

Faba bean seeds were planted in 1 600 cm^3 pots (150 mm diameter) filled with autoclaved river sand (medium grained with sieve mesh 2.5 mm). Two seeds were planted in each pot and after germination one seedling per pot was selected so as to give an initial uniform population of seedlings. All pots were watered every second day with tap water until emergence of cotyledons. Once the seedlings had emerged, they were watered every second day with a modified Long Ashton nutrient solution. The modifications involved the application of the nitrogen in the form KNO_3 at the following concentrations of N: 0, 0.714, 1.786, 3.571, 7.143, 17.857, and 35.714 mM N [equivalent to 0, 10, 25, 50, 100, 250, and 500 g(N) m^{-3}]. All zero nitrogen treatments were watered using N-free Long Ashton nutrient solution. Dry biomass, nitrogen, and photosynthesis were measured for three harvest intervals: 44 (T_1, 8th April), 51 (T_2, 15th April), and 58 (T_3, 22nd April) d after planting (DAP).

Biomass determination: At each harvesting interval, the roots were rinsed carefully with tap water to remove sand. The plants were divided into leaf, stem, and root components, respectively. Before drying, leaf area was measured using a *Li-Cor 3100* area meter (*Li-Cor*, Lincoln, NE, USA). The leaves, stems, and roots were dried in the oven at 105 °C for 15 min and then at 65 °C for 3 d for dry mass determinations.

Tissue N content analysis: After the determinations of dry mass, the dried leaves, stems, and roots were milled for N analysis; 0.100±0.001 g milled plant material were digested by the Kjeldahl procedure. The total N content of the digests was determined using colorimetric assays (Dorich and Nelson 1983, Anderson and Ingram 1993). For the Kjeldahl digestion mixture, 0.42 g of selenium powder and 14 g lithium sulphate was added to 350 cm^3 of 30 % hydrogen peroxide, and 450 cm^3 of concentrated sulphuric acid was added slowly to the mixture that was kept cool in an ice bath.

For the colorimetric determination of ammonium nitrogen, two reagent solutions were used. The first reagent solution was made up of 34 g sodium carbonate, 25 g sodium citrate, and 25 g sodium tartarate dissolved together in 750 cm^3 H_2O. To this mixture 0.12 g sodium nitroprusside was added and when dissolved the solution was made up to 1 000 cm^3 with H_2O. The second reagent solution was made of 30 g sodium hydroxide dissolved in 750 cm^3 H_2O. After cooling, 10 cm^3 of sodium hypochlorite was added and the solution was brought up to 1 000 cm^3. For the ammonium assay, a 0.1 cm^3 ammonium sample was added to a 25 cm^3 Erlenmeyer flask and 5.0 cm^3 of both the first and second reagent solutions were added. After 1 h the absorbance was measured at 665 nm.

Gas exchange measurements: Photosynthetic gas exchange rates were determined at the three harvest intervals on equal-aged cohorts. All plants were fully acclimated to full sunlight. For the measurements of C_i and C_e, the portable *CIRAS-1*, *PP IR* gas analysis system (*PP system*, Hitchin, Hertfordshire, U.K.) was used. For the estimation of P_{Nmax} and α, an *ADC* infra-red gas analyser (IRGA) *225-2B-SS* (*Analytical Development*, Hoddesdon, Hertfordshire, England) on differential mode was used. These values were measured at 44, 51, and 58 DAP. Leaf surfaces were exposed to photosynthetically active photon flux densities (PPFD) ranging from 75 to 1 800 μmol(quantum) m^{-2} s^{-1} by adjusting the height of a 400 W halide (*Power Star HQ1*) lamp above the leaf surface. The PPFD at the leaf surface was measured with a *LI-188B* quantum meter. P_N was measured on the youngest fully expanded leaves. The trifoliate leaflet was clamped into and sealed in a *Perspex* leaf chamber that allowed the irradiation of 0.63 cm^2 of leaf area. The chamber was surrounded by a water jacket connected to a temperature regulated water bath water (*SS-CD-5*, *Specht Scientific*, Johannesburg, SA). Leaf temperature was continuously monitored by means of a thermocouple (Model *BAT-12*, *Bailey Instruments*, Saddlebrook, NJ, USA) touching the underside of the lamina within the leaf chamber. The leaf temperature was maintained at 25 °C for all PPFDs. Average barometric pressure during photosynthetic measurements was 83.5 kPa.

Experimental design and statistical analysis: The experiment involved a completely randomised 3×7 factorial design. The experimental factors were three different harvest intervals (T_1, T_2, and T_3) and seven N treatments. A replicate consisted of one faba bean plant per container. The appropriate data were analysed using *ANOVA*. A Tukey's multiple comparison test was performed to determine which treatments differed. When appropriate, means±SE (standard error) were calculated, and when the F ratio was significant, the least significant differences were evaluated by the Tukey HSD-test to examine differences among the harvesting intervals ($T_{1,}$ T_2, and T_3) in each treatment. *SYSTAT* version *8.0* was used for all data analyses.

Results

Effects of N supply on biomass production and N accumulation: For each nitrogen supply concentration (N_s) the nitrogen contents (N_c) in leaves, stems, and roots remained constant over the three harvest intervals (Fig. 1). While the total quantity of elemental N accumulated per plant (N_t) increased with increasing N_s (Fig. 3), N_c for the different tissue components always remained constant over time for each N_s (Fig. 1). Hence the plant capacity to accumulate N increased exponentially or kept pace with the rate of biomass production. Also under constant N_s, the specific leaf nitrogen (N_L), specific leaf area (δ_L), root mass fraction (R_f), and P_{Nmax} all remained constant over the three harvest intervals (Fig. 2). All these results are consistent with the balanced exponential growth hypothesis. Increasing N_s promoted increases in the production of total biomass, total plant leaf area, and total accumulation of nitrogen (Fig. 3).

Effects of N_s on R_f, δ_L, and N_L: Both R_f and δ_L declined slightly with increasing N_s (Fig. 2). However, these slightly declining differences in R_f and δ_L in response to increasing N_s were significantly different at the 5 % level. There were no significant differences at the 5 % level among three harvest intervals within the specific N treatments. Even though both total biomass and leaf area production increased significantly with increasing N_s, the values for R_f and δ_L remained constant over the three harvest intervals. δ_L also declined slightly with increasing N_L. N_L expressed on a leaf area basis also remained constant over time for a given steady state N_s (no significant differences at the 5 % level among three harvest intervals within the N_L treatments). These results are consistent with the balanced exponential growth hypothesis that predicts that if N_s is constant then intensive variables

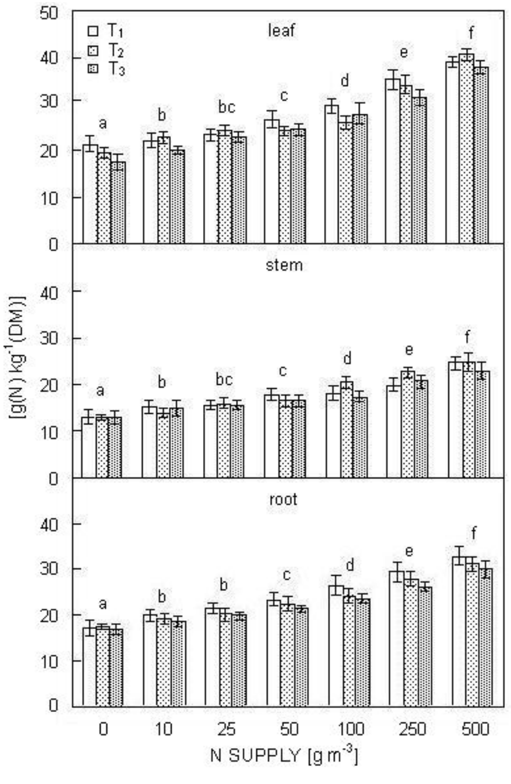

Fig. 1. Steady-state leaf, stem, and root N contents (N_c) at the three harvests (T_1, T_2, and T_3) in response to different N supply (0, 10, 25, 50, 100, 250, and 500 g m^{-3}). *ANOVA* results for tissue N_c did not indicate significant differences (at the 5 % level) among the harvest intervals within the individual N treatments, but the differences among the N treatments were significant. *Different letters* indicate significant difference among treatments assessed by Tukey HSD-test ($p < 0.05$).

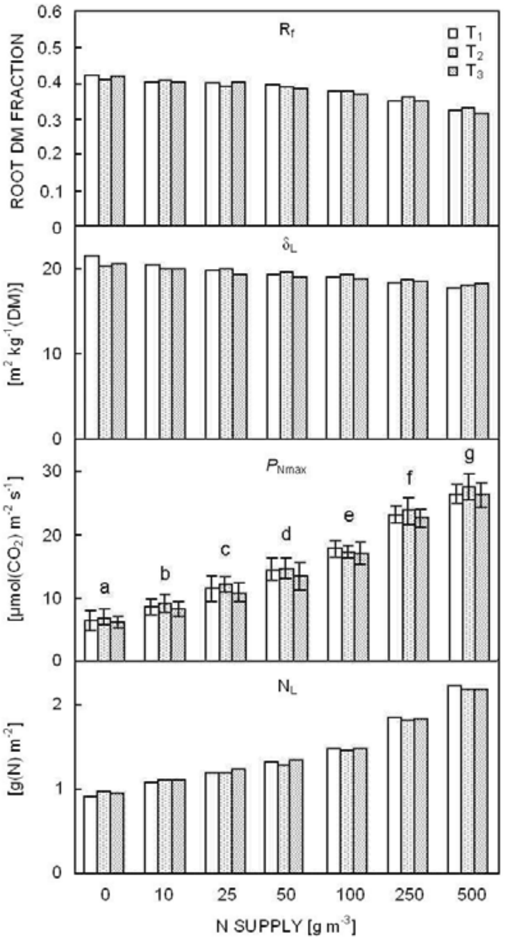

Fig. 2. The influence of increasing N supply on root dry mass fraction (R_f), specific leaf area (δ_L), photon-saturated net photosynthetic rate (P_{Nmax}), and specific leaf N content (N_L) at the three harvest intervals (T_1, T_2, and T_3). *ANOVA* results did not indicate significant differences (at the 5 % level) among the harvest intervals for individual N treatments, but the differences among the N treatments were significant. For P_{Nmax} results, *different letters* indicate significant difference among treatments assessed by Tukey HSD-test ($p < 0.05$).

such as R_f, δ_L, and N_L will also remain constant during plant growth and the capacity to accumulate N also increases exponentially. Therefore in modelling N uptake during plant growth R_f, δ_L, and N_L should be used as parameters.

α *versus* N_s: How does leaf N_c affect α, given the fact that the total leaf area per plant and the total number of catalytic units per unit leaf area increases markedly with increasing N_s (Fig. 3)? For *V. faba*, α showed a curvilinear response to increasing N_L (Fig. 4). The empirical equation of Sinclair and Horie (1989) was used for generating the asymptotic value for α and the N_L-intercept value for α = 0. With increasing N_L, α converged onto a maximum asymptotic value of 0.0664±0.0049 $\mu mol(CO_2)$ $\mu mol(quantum)^{-1}$. α fell to zero for N_L-intercept of below 0.6600±0.0515 $g(N) m^{-2}$. For a given N_s, α remained constant over all three harvest intervals (values not shown); this confirms the validity of the premise that a photosynthetic parameter such as α will also remain constant under steady-state or constant N_s. The curvilinear relationship between α and N_L for faba bean was quantitatively very similar to those reported for soybean, wheat, and maize by Sinclair and Horie (1989).

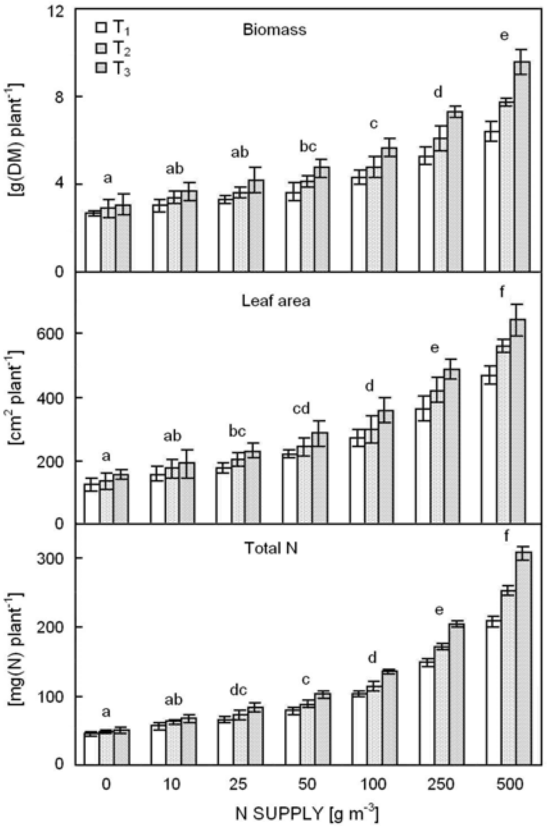

Fig. 3. The influence of increasing N supply on total biomass production, leaf area production, and total plant N accumulation (N_t) measured at the three harvests (T_1, T_2, and T_3). *ANOVA* for all the results indicated (*1*) significant differences (at the 5 % level) among the N treatments, and (*2*) significant differences among the harvest intervals within the N treatments. *Different letters* indicate significant difference among treatments assessed by Tukey HSD-test ($p < 0.05$).

P_{Nmax} *versus* N_s: Again it may be asked: How does N_L affect P_{Nmax}, given the fact that the total leaf area per plant and the total number of catalytic units per unit leaf area increases markedly with increasing N_s? P_{Nmax} increased in a curvilinear fashion with respect to N_L (Fig. 4) and the empirical equation of Sinclair and Horie (1989) was used for deriving the asymptotic value for P_{Nmax} and the N_L-intercept value for P_{Nmax} = 0. With increasing leaf N_c, P_{Nmax} converged onto a maximum asymptotic value of 33.400±2.563 $\mu mol(CO_2)$ m^{-2} s^{-1}. Maximum asymptotic values with increasing N_L measured for maize, soybean, and rice were 57, 36, and 34 $\mu mol(CO_2)$ m^{-2} s^{-1}, respectively (Sinclair and Horie 1989).

***C_i* and *C_e* *versus* N_s**: In *V. faba* as N_L increased, C_i declined exponentially at an exponential of 3.53±0.29 µmol mol^{-1} [g(N) m^{-2}]$^{-1}$ to an asymptotic minimum C_i of 115.80±1.59 µmol mol^{-1}. A strongly rectangular hyperbolic relationship was observed between C_e and N_L (Fig. 4) with a maximum C_e asymptotic value of 1.645±0.054 µmol(CO_2) m^{-2} s^{-1} Pa(CO_2)$^{-1}$ and with an N_L-intercept equal to 0.596±0.096 g(N) m^{-2}.

Discussion

Relationship between α and N_L: In this study α with respect to increasing N_L reached a maximum of 0.0664±0.0049 µmol(CO_2) µmol(quantum)$^{-1}$. Meinzer and Zhu (1998) reported that α for CO_2 uptake in sugarcane increased linearly from 0.042 to 0.075 µmol(CO_2) µmol(quantum)$^{-1}$ with leaf N_c. Approximately doubling as leaf N_c, it was increased from 0.63 to 1.54 g m^{-2}. This dependence of α on leaf N_c was similar for different sugarcane clones. Hence any dynamic model of leaf photosynthesis should not assume that the value of α is not influenced by tissue N_c. Other α measurements for CO_2 uptake [measured at leaf temperatures of 30 °C, 330 cm^3(CO_2) m^{-3}, and 21 kPa(O_2)] in various monocot and dicot plants, with different photosynthetic pathways, give the following range of α values [µmol(CO_2) µmol(quantum)$^{-1}$]: C_3 dicots, 0.052±0.001; C_3 grasses, 0.053±0.001; C_4 (NAD-ME) dicots, 0.053±0.001; C_4 (NAD-ME) grasses, 0.060±0.001; C_4 (PCK) grasses, 0.060±0.002; C_4 (NADP-ME) dicots, 0.061±0.002; C_3 (NADP-ME) grasses, 0.065±0.001; C_4 (ME-MIX) dicot, 0.057 (Pearcy and Ehleringer 1984). Ehleringer and Björkman (1977) found α value for C_3 photosynthesis of 0.081 µmol(CO_2) µmol(quantum)$^{-1}$ when oxygenase activity was suppressed (low O_2 or high CO_2 concentrations). At a leaf temperature of 20 °C, α of 0.066 and 0.044 µmol(CO_2) µmol(quantum)$^{-1}$ was found for field grown ryegrass and clover, respectively (unpublished data of Papadopoulos). It is thus reasonable to assume that depending on leaf N_c, α for CO_2 uptake in C_3 and C_4 plants can range from 0.016 to 0.075 µmol(CO_2) µmol(quantum)$^{-1}$.

Relationship between *P_{Nmax}* and N_L: For faba bean, the N_L-intercept for P_{Nmax} was 0.7100±0.0345 g(N) m^{-2}. In other studies the linear response of P_{Nmax} to N_L rose from: zero at 1.0 g(N) m^{-2} to about 2.4 g(N) m^{-2} for soybean, zero at 0.3–1.6 g(N) m^{-2} for rice, and 0 at 0.2–0.6 g(N) m^{-2} for maize (Sinclair and Horie 1989). In *Chenopodium album* (C_3) and *Amaranthus retroflexus* (C_4), the P_{Nmax} intercept of the N_L axis ranged between 0.64–0.78 g(N) m^{-2} (Sage and Pearcy 1987), for a given N supply rate the values for the corresponding P_{Nmax} remained constant over all three harvest intervals (Fig. 2). This confirms the validity of the premise that a photosynthetic parameter such as P_{Nmax} will also remain constant under steady-state N_s.

Relationship between *C_i*, *C_e*, and N_L: The steady-state functional C_i/C_a ratio is not only determined by plant water status and stomatal conductance, the C_i/C_a ratio is also influenced by N_L. With regard to benchmarking C_i values, it is generally accepted that at 25 °C, under saturating PPFD and ambient CO_2, C_i is approximately 100 µmol mol^{-1} for C_4 species and 250 µmol mol^{-1} for C_3 species. The CO_2 compensation concentration (Γ) is close to 0 for C_4 *versus* 5.0 µmol mol^{-1} for C_3 species (Evans and Caemmerer 1996). C_i values for C_3 plants such as *Pinus pinaster* range from 103 to 266 µmol mol^{-1} (Warren *et al*. 2000, Warren and Adams 2001).

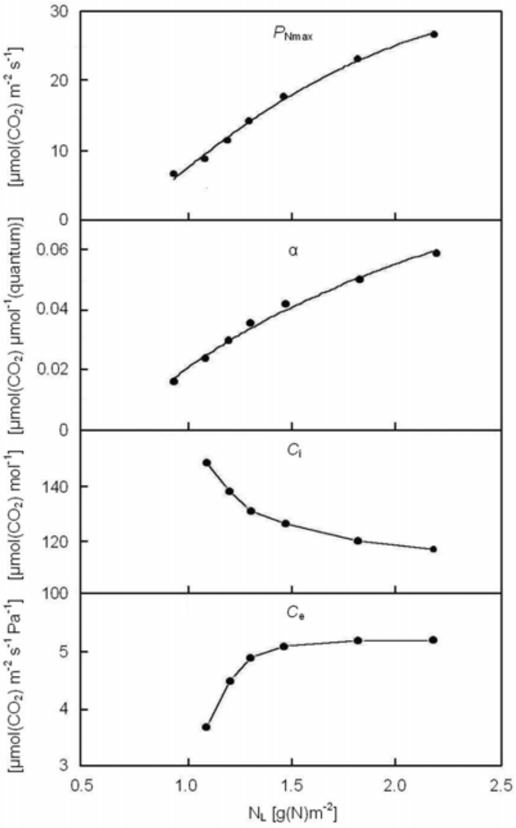

Fig. 4. The influence of increasing specific leaf nitrogen (N_L) on photon saturated CO_2 assimilation rate (P_{Nmax}), quantum yield efficiency (α), leaf intercellular CO_2 concentration (C_i), and the carboxylation efficiency (C_e).

C_e has been expressed as the reciprocal of mesophyll resistance r_m (Edwards and Walker 1983). The following range of r_m values [s cm^{-1}] have been reported for C_3 plants: *Glycine max* 2.0–3.0, *Atriplex hastata* 2.6, *Phaseolus* spp. 2.6, *Triticum aestivum* 2.8, *Solanum tuberosum* 5.4, and *Medicago sativa* 2.8. Thus the carboxylation efficiencies for the above C_3 plants range between 0.002–0.005 m s^{-1}. In other experiments the following carboxylation efficiencies [m s^{-1}] have been reported for C_3 plants: *Helianthus* 0.0063, *Xanthium* 0.0048, *Vigna* 0.0036–0.0047, *Tilia* 0.0035, *Ficus*

0.0031, and *Citrus* 0.0018–0.0024 (Laisk and Loreto 1996).

Assuming a temperature of 20 °C and air pressure of 101.315 Pa [under these conditions CO_2 per 1 000 cm^3 = 41.6×10^{-6} mol(CO_2) m^{-3} = 0.101 Pa(CO_2)] the range of C_e values reported for various C_3 plants can be recalculated to give the following equivalent values of 0.741–2.595 mol(CO_2) m^{-2} s^{-1} Pa(CO_2)$^{-1}$. In general, C_e ranged between 1.046–1.478 μmol(CO_2) m^{-2} s^{-1} Pa(CO_2)$^{-1}$ for C_3 *Cyperus* species and between 2.529–4.123 μmol(CO_2) m^{-2} s^{-1} Pa(CO_2)$^{-1}$ for C_4 *Cyperus* species (Li 1993). Thus the C_e values estimated for faba bean fell within the C_3 benchmark range of values.

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