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Influence of phosphorus and nitrogen on photosynthetic parameters and growth in *Vicia faba* L.

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Abstract

The influence of phosphorus (P) and nitrogen (N) supply on biomass, leaf area, photon saturated photosynthetic rate (P_{max}), quantum yield efficiency (α), intercellular CO_2 concentration (C_i), and carboxylation efficiency (CE) was investigated in *Vicia faba.* The influence of P on N accumulation, biomass, and leaf area production was also investigated. An increase in P supply was consistently associated with an increase in N accumulation and N productivity in terms of biomass and leaf area production. Furthermore, P increased the photosynthetic N use efficiency (NUE) in terms of P_{max} and α. An increase in P supply was also associated with an increase in CE and a decrease in C_i. Under variable daily meteorological conditions specific leaf nitrogen content (N_L), specific leaf phosphorus content (P_L), specific leaf area (δ_L), root mass fraction (R_f), P_{max}, and α remained constant for a given N and P 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. We tested also the hypothesis that P supply positively affects both N demand and photosynthetic NUE by influencing the upper limit of the asymptotic values for P_{max} and CE, and the lower limit for C_i in response to increasing N.

Additional key words: carboxylation efficiency; intercellular CO_2 concentration; leaf area; nitrogen use efficiency; photon saturated photosynthetic rate; quantum yield efficiency; root; stem.

Introduction

Jia and Gray (2003) showed that for *Vicia faba* a curvilinear relationship exists between leaf nitrogen content (N_L) and the photon saturated photosynthetic rate (P_{max}). A similar relation holds for the quantum yield efficiency (α). Carboxylation efficiency (CE) increases and intercellular CO_2 concentration (C_i) decreases in response to increasing N supply. With increasing N supply, P_{max}, CE, and C_i converged into asymptotic values. This study tests the hypothesis that P supply positively affects both N demand and photosynthetic N use efficiency (NUE) by influencing the upper limit of the asymptotic values for the above characters in response to increasing N supply.

Materials and methods

Growth conditions: The cold-hardy faba bean (*Vicia faba* L.) cv. Aquadulce Claudia (*Straathof Seed Group*) requiring approximately 130–150 d for crop development was used. Outdoor experimental pot trials were carried out as reported in Jia and Gray (2003). Faba bean seeds were planted in 15 cm diameter (area equivalent to

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Abbreviations: α – quantum yield efficiency; C_a – ambient CO_2 concentration; CE – carboxylation efficiency; C_i – intercellular CO_2 concentration; DM – dry mass; $[E_a]$ – content of activated ribulose-1,5-bisphosphate carboxylase/oxygenase; h – leaf thickness; k_{cat} – ribulose-1,5-bisphosphate carboxylase/oxygenase turnover rate; k_{cat}^{app} – P-dependent apparent catalytic constant for ribulose-1,5-bisphosphate carboxylase/oxygenase; Km_{appCO2} – apparent Km for CO_2 at varying O_2 concentrations; K_N – Michaelis constant for N; K_N^{app} – apparent specificity constant for N; K_{NP} – kinetic coefficient; K_P – Michaelis constant for P; M_r – the maintenance respiration; N_L – leaf nitrogen per area; N_{plt} – total plant elemental N; NUE – nitrogen use efficiency; P_G – gross photosynthetic rate; P_L – leaf phosphorus per m^2; P_{max} – photon saturated rate of photosynthesis; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; Qt – quantum; R_f – root mass fraction; R_G – growth respiration; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; V_{max} – the maximum velocity of ribulose-1,5-bisphosphate carboxylase/oxygenase activity; W – biomass; δ_L – specific leaf area; τ – the carboxylation constant.

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0.0177 m^2) pots. The pots were filled with sterile river sand. Sand was sterilized by autoclaving at 121 °C and 103.4 kPa for 3 h in metal buckets. Two seeds were planted in each pot and after germination one seedling per pot was selected, so as to give an initial population consisting of very similar 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 modification involved the application of nitrogen in the form KNO_3 at 10, 25, 50, 100, 250, and 500 g(N) m^{-3}. LP and HP supply rates were 0.05 and 1.60 mM P, respectively.

Nutrients: A modified Long-Ashton nutrient mixture was used to supply the non-nitrogen micro- and macronutrients. For the micro-nutrients, 100× stock solutions were made up as follows [kg m^{-3}]: $MgSO_4 \times 7\,H_2O$ 36.900, $MnSO_4 \times H_2O$ 0.223, $CuSO_4 \times 5\,H_2O$ 0.024, $ZnSO_4 \times 7\,H_2O$ 0.029, H_3BO_3 0.019, $(NH_4)_6Mo_7O_{24} \times 4\,H_2O$ 0.004, $CoSO_4 \times 7\,H_2O$ 0.003, NaCl 0.585. For the macro-nutrients 100× stock solutions were made up as follows [kg m^{-3}]: $CaCl_2$ 50.00, K_2SO_4 21.75, FeEDTA 3.00. After adding the appropriate quantity of N and P, 10 cm^3 of the stock solutions were added. The pH of the nutrient solutions were then adjusted to pH 7.0 and made up to 1 000 cm^3 with distilled water before being applied to the pots. Because of the low water holding capacity due to the coarse texture of the river sand in the pots, it was necessary to water the plants every 2 d with 150 cm^3 of a modified Long-Ashton nutrient solution. Inclusion of 0.218 kg m^{-3} K_2SO_4 to all treatments meant that K supply, while abundant to the high N treatments, was not limiting with regard to the low N treatments.

Biomass analysis: Dry mass (DM) and photosynthetic measurements were determined at 38 (T_1) and 45 (T_2) d after planting (DAP), respectively. After measuring the photosynthetic rate, the plants were harvested for biomass and total N content determination. The plants were divided into leaf, stem, and root, respectively. The roots were rinsed carefully with tap water to remove sand. Before drying, leaf area was determined with an area meter *Li-Cor 3100* (*LiCor*, Lincolin, 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. DM was determined using electronic balance (model *JP$_2$-3000*, *Chyo Balance Corporation*, Kyoto, Japan). Dried plant material was milled to sub-samples for total N and total P determinations.

Tissue N and P analysis: After the determination of DM of leaves, stems, and roots, tissues were milled and analysed for N and P contents. Sub-samples (0.100±0.001 g) were digested in a hydrogen peroxide-sulphuric acid digestion mixture by the Kjeldahl procedure followed by standard colorimetric assays (Anderson and Ingram 1993).

Gas exchange measurements were done using the portable *CIRAS-1, PP IR* gas analysis system at 38 and 45 DAP. For the estimation of P_{max} and α, the *ADC* infrared gas analyser *225-2B-SS* (*Analytical Development Co.*, Hoddesdon, UK) on differential mode was used. Leaf surfaces were exposed to photosynthetic photon flux density (PPFD) of 75–2 000 µmol(quantum) m^{-2} s^{-1} by adjusting the height of a 400 W halide (*Power Star HQI*) lamp above the leaf surface. PPFD at the leaf surface was measured with a *Li-COR LI-188B* quantum meter. Net photosynthetic rate (P_N) was measured on the youngest fully expanded leaves. The trifoliate leaflet was clamped into and sealed in a *Perspex* cuvette leaf chamber which allowed a leaf area of 0.63 cm^2 to be irradiated. The cuvette chamber was surrounded by a water jacket connected to a temperature regulated water bath *SS-CD-5* (*Specht Scientific*, Johannesburg, SA). Leaf temperature was monitored with a thermocouple *BAT-12* (*Bailey Instruments*, Saddlebrook, NJ, USA) touching the underside of the lamina within the leaf chamber. The leaf chamber or cuvette was surrounded by a water jacket and temperature was maintained at 25 °C. Average barometric pressure during photosynthetic measurements was 83.5 kPa.

Experimental design and statistical analysis: The experiment was a randomized complete block with four replicates for each harvest interval, with combinations of treatment factors (4×6 factorial) randomly assigned to pots in the block. The various treatments were six different N supply rates [10, 25, 50, 100, 250, and 500 g(N) m^{-3}] applied with two different P supply rates (0.05 mM and 1.60 mM P). A replicate consisted of one plant per container. Dry mass, leaf area, and N and P contents were analysed by three-way ANOVA for main effects and interacting ional effects (Zar 1984). The significance of the differences in variables as a result of the interactions between factors A (N supply concentration), B (P supply concentration), and C (harvest dates, T_1 and T_2) were tested. For the multi-factorial experiment the sources of variation were subdivided into three main effects: A (effect of N supply concentration); B (effect of P supply concentration); C (harvest intervals); three first-order interactions, A×B, A×C, and B×C; and one second-order interaction, A×B×C. Multiple comparisons of means were performed by the Tukey's HSD test ($p<0.05$) after performing three-way ANOVA with residual estimation. The *Statistica* version *6.0* package was used for statistical analysis.

Results

Summary of ANOVA: Each of the treatment factors (A, B, C), when considered individually, had significant effects on different plant variables (Table 1). In relation to their combined effects, the three-way ANOVA results showed that the majority of first-order interactions had significant effects on all plant variables. The combined effects of the three factors on N and P tissue contents, specific leaf area (δ_L), root: biomass ratio (R_f), plant DM, leaf area, total N and P accumulation, are shown in Figs. 1 to 3. The effect of factor C or time on the intensive variables such as tissue N content, tissue P content, δ_L, and R_f are given in Figs. 1 and 2. In relation to second-order interactions, there were no significant differences. With regard to the effects arising from the interaction of factors, P was consistently associated with a significant increase in plant N accumulation and NUE in relation to biomass production and photosynthesis.

Table 1. The significance of differences in variables as a result of the interactions among factors A [10, 25, 50, 100, 250, and 500 g(N) m^{-3}], B (P supplies 0.05 mM and 1.60 mM), and C (harvest intervals T_1 and T_2). Biomass (DM), leaf area, total N accumulation, total P accumulation, specific leaf area (δ_l), root fraction (R_f), and elemental N and P contents of leaves, roots, and stems were analyzed by three ways ANOVA with residual estimation, for main effects and interaction effects. *, **, *** indicate effects that are significant at $p<0.05$, 0.01, and 0.001, respectively; ns = not significant at $p<0.05$.

Source of variance	A	B	C	A×B	A×C	B×C	A×B×C
Degree of freedom	5	1	1	3	3	3	3
Leaf N [g kg^{-1}(DM)]	***	***	ns	***	ns	ns	ns
N_L [g(N) m^{-2}]	***	***	ns	***	ns	ns	ns
Stem N [g kg^{-1}(DM)]	***	***	ns	***	ns	ns	ns
Root N [g kg^{-1}(DM)]	***	***	ns	***	ns	ns	ns
Leaf P [g kg^{-1}(DM)]	***	***	ns	***	ns	ns	ns
P_L [g(P) m^{-2}]	***	***	ns	**	ns	ns	ns
Stem P [g kg^{-1}(DM)]	***	***	ns	***	ns	ns	ns
Root P [g kg^{-1}(DM)]	***	***	ns	**	ns	ns	ns
Total N [mg(N) $plant^{-1}$]	***	***	***	***	***	***	**
Total P [mg(P) $plant^{-1}$]	***	***	***	***	Ns	***	ns
Biomass [g $plant^{-1}$]	***	***	***	***	**	*	ns
Leaf area [cm^2 $plant^{-1}$]	***	***	***	***	*	ns	ns
δ_L [m^2 kg^{-1}]	*	***	ns	ns	ns	ns	ns
Root fraction (R_f)	***	***	ns	ns	ns	ns	ns
Photosynthesis (P_{max})	***	***	ns	ns	ns	ns	ns

Effects of P supply on plant N accumulation: While within treatments there were significant differences in the accumulation of total elemental N and P over the interval $T_2 - T_1$, there were no significant differences in the contents of elemental N and P over this interval (Figs. 1 and 3). It is not certain what precisely determines the plant demand for N (Grindlay 1997). If the amount of N accumulated reflects the plant demand for N, then the results indicated that P supply was a major factor governing the demand for N (Table 1, Figs. 1 and 3). It is not certain how P influences N demand. An increase in the plant total transpiration surface area will tend to increase the flux of water through the soil-plant-atmosphere continuum and increase the flux of nitrate to the root system. This could be a possible explanation for an indirect effect of P supply on nitrate uptake. Increasing P supply was also consistently associated with an increase in N productivity in terms of biomass and leaf area production (Fig. 3).

The effects of N supply on plant P accumulation: There was no significant difference in plant P contents within treatments over the harvest intervals (Table 1 and Fig. 1). The level of N supply was also a major factor governing the amount of P accumulation in growing plants (Fig. 3). While total P accumulation increased with N supply, the associated increases in DM production (Fig. 3) had a dilution effect on P content (Fig. 1). It was not certain whether the increase in total P accumulation in response to N supply could also be explained as an indirect consequence of increased transpiration water fluxes delivering P to the plant root system.

Effects of N and P supply on plant parameters: Figs. 1 and 2 show that under a regime of constant nutrient supply, intensive variables such as tissue N and P contents, δ_L, and R_f attained steady-state values. Both R_f and δ_L declined slightly with increasing N supply (Fig. 2). These slight declines in both R_f and δ_L as the plant N content increased were significantly different at the 5 % level.

There were no significant differences at the 5 % level among the harvest intervals for the N_L and P_L within treatments, indicating maintenance of steady-state values for specific leaf N and P content. N_L and P_L remained

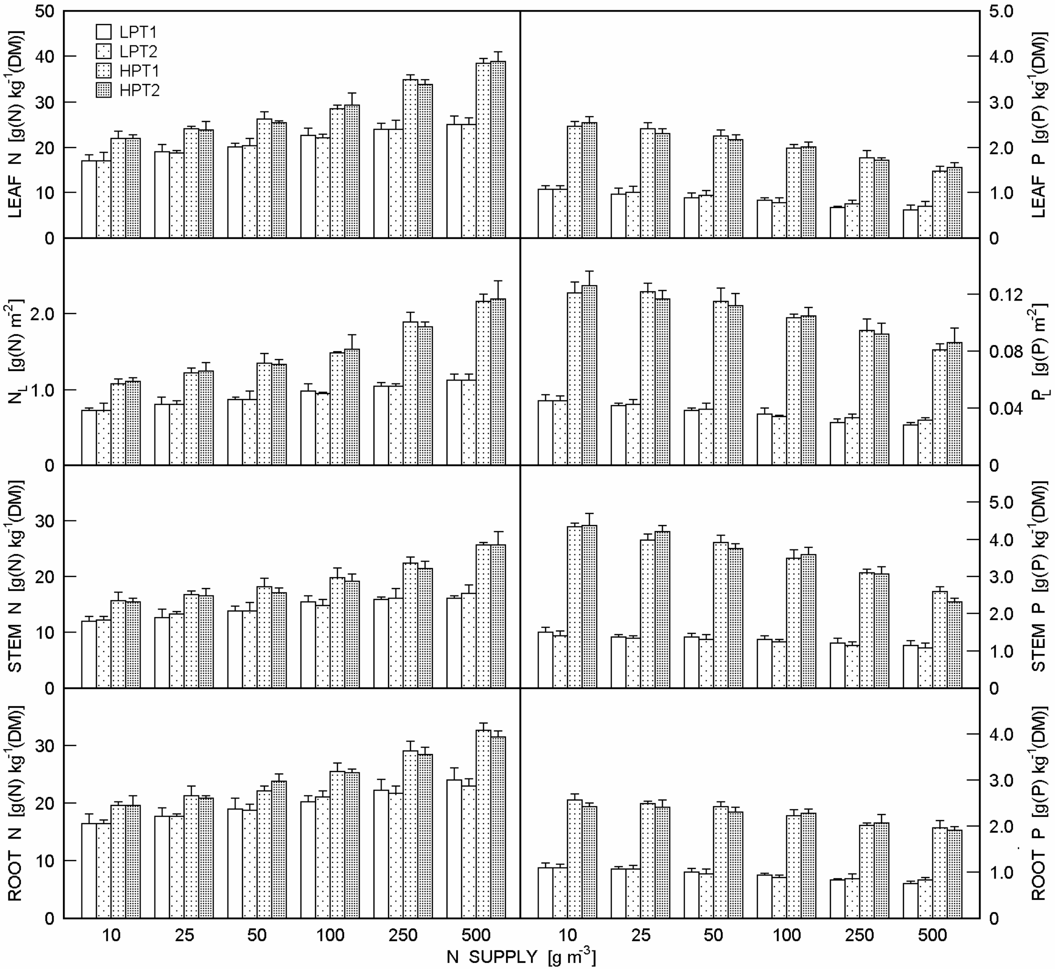

Fig. 1. N and P contents [g kg^{-1}(DM)] of leaf, stem, root, and specific leaf N or P in *Vicia faba* as influenced by N supply [10, 25, 50, 100, 250, and 500 g(N) m^{-3}] and P supply (LP and HP) at harvest intervals T_1 and T_2. *Vertical bars* represent SE (*n* = 4) of the means. Tukey's HSD test ($p<0.05$) after performing three ways ANOVA with residual estimation indicated significant differences among the N and P treatments, but no differences within treatments over harvest intervals.

constant while DM and leaf area increased exponentially. Thus both N and P uptake rates had also to increase exponentially during plant growth, even if the N and P supply remained constant.

Even though both total DM and leaf area production increased significantly in response to P under increasing N supply rates, the values for R_f and δ_L remained constant over the harvest intervals. The δ_L declined slightly with increasing N_L. These results are consistent with the balanced exponential growth hypothesis of Thornley (1998). This hypothesis predicts that under constant nutrient supply, non-limiting moisture availability, and saturating irradiance all extensive variables (*e.g.* plant DM and leaf area) increase exponentially at a constant specific growth rate. Also, under these conditions all intensive variables that are either ratios or rates (*e.g.* N_L, P_L, δ_L, and R_f) remained constant. The results corroborate the balance growth hypothesis and consequently the intensive variables R_f, δ_L, N_L, and P_L behave as plant parameters. The values of these parameters would be dependent on the amounts of N and P supplied to the plant. The balanced growth hypothesis should also hold for intensive variables such as the content of activated ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO). Thus for a given constant supply of N and P the content of activated RuBPCO should remain constant. This conclusion was important for analyzing the relationship between N supply and photosynthetic activity, and the role that P may play in this relationship [see Eqs. (1) to (10)].

Effect of N and P on CE and C_i: A rectangular hyperbolic relationship was found between CE and N supply for both LP and HP plants (Fig. 4). HP plant had a higher asymptotic CE value and N_L-intercept than LP plants (Table 2). NUE in relation to CE has been defined as the initial slope of CE *versus* N_L. HP plants had a higher value than LP plants (Table 2). As N supply increased, C_i declined exponentially to 116.1 cm^3 m^{-3} for HP plants and 131.9 cm^3 m^{-3} for low P plants (Fig. 4). While plant water status and stomatal conductance are fundamental in determining the upper limits of the atmosphere–leaf CO_2 gradient (C_a–C_i), this gradient was also influenced by the proportion of P to N supply.

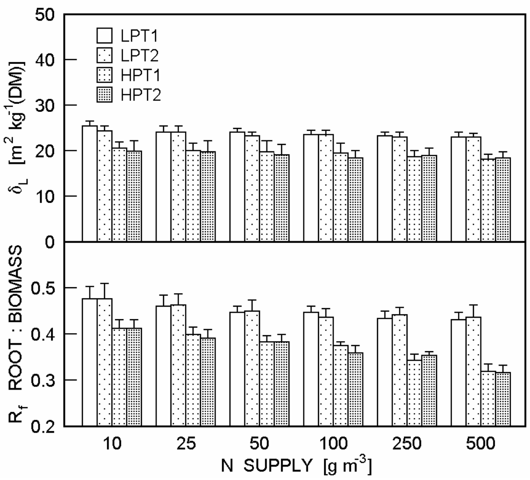

Fig. 2. Specific leaf area and root fraction of *Vicia faba* as influenced by N supply (10, 25, 50, 100, 250, and 500 g N m^{-3}) and P supply (LP and HP) at harvest intervals T_1 and T_2. *Vertical bars* represent SE (n = 4) of the means. Tukey's HSD test (p<0.05) after performing three ways ANOVA with residual estimation indicated significant differences among the N and P treatments, but no differences within treatments over harvest intervals.

CE gives an indirect indication of the content of activated RuBPCO [see Eqs. (3) and (4)]. Hence N supply influences the content of activated RuBPCO. The content of activated RuBPCO converges in a curvilinear fashion to an asymptotic value with increasing N supply; and the asymptotic content of activated RuBPCO in relation to N supply is sensitive to P. P supply can modulate the content of activated RuBPCO either directly (Marcus and Gurevitz 2000) or indirectly (Cockburn *et al.* 1967a,b, Usuda and Edwards 1982, Mächler *et al.* 1984, Rao and Terry 1989, 1995, Rao *et al.* 1989, 1990, Usuda and Shimogawara 1991, Pieters *et al.* 2001).

Effect of P on the response of P_{max} to N: Fig. 4 shows the impact of P treatment on the asymptotic values for P_{max} in relation to N supply. Under HP and LP, P_{max} increased in a curvilinear fashion with N supply (Fig. 4). However, higher P_{max} values were achieved in the HP plants. The results in Figs. 3 and 4 are consistent with a P sensitive link between N supply, photosynthetic activity, and plant growth.

Table 2 shows the relationship between P_{max} and N_L. The asymptotic value for P_{max} *versus* N_L and the N_L-intercept value for P_{max} = 0 were calculated using the empirical equation of Sinclair and Horie (1989). Plants of the HP treatment had a higher asymptotic value for P_{max} compared with LP plants. However, for the HP plants the photosynthetic NUE (calculated as the initial slope of the P_{max} *versus* N_L plot) was less than 50 % of the value for the LP plants. Also the P_{max} *versus* N_L-intercept value for the HP plants was considerably higher than the value for the LP plants (Table 2).

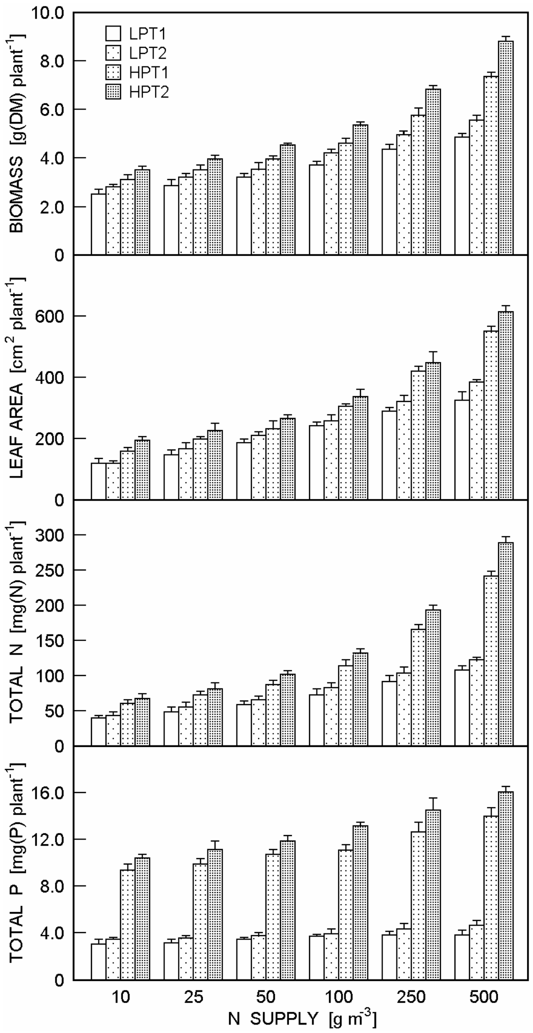

Fig. 3. Biomass, leaf area, total elemental N and P of *Vicia faba* as influenced by N supply [10, 25, 50, 100, 250, and 500 g(N) m^{-3}] and P supply (LP and HP) at harvest intervals T_1 and T_2. *Vertical bars* represent SE (n = 4) of the means. Tukey's HSD test (p<0.05) after performing three ways ANOVA with residual estimation indicated significant differences among the N and P treatments, but no differences within treatments over harvest intervals.

Effect of P on the response of α to N: Under both HP and LP, α also increased in a curvilinear fashion with respect N supply (Fig. 4). The curvilinear relationship between α and N_L for faba bean was very similar to the one reported for soybean, wheat, and maize by Sinclair and Horie (1989). The empirical equation of Sinclair and Horie (1989) was also used for generating the asymptotic and intercept values for α *versus* N_L. With increasing N_L the value for α converges onto a maximum asymptotic a value. Plants receiving the HP treatment had a higher asymptotic value for α value compared to the LP plants

Table 2. The effect of low P (0.05 mM, LP) and high P (1.60 mM, HP) treatments on photosynthetic parameters in response to N supply. Q: quantum.

	Asymptotic LP value	HP value	N_L-intercept LP value	HP value	Initial slope LP value	HP value
P_{max}×N_L	20.30±0.79 μmol(CO_2) m^{-2} s^{-1}	30.82±0.87 μmol(CO_2) m^{-2} s^{-1}	0.69±0.01 g(N) m^{-2}	0.80±0.02 g(N) m^{-2}	46.40±5.20 μmol(CO_2) m^{-2} s^{-1}[g(N) m^{-2}]$^{-1}$	19.84±1.75 μmol(CO_2) m^{-2} s^{-1}[g(N) m^{-2}]$^{-1}$
α×N_L	0.0249±0.0014 μmol(CO_2) μmol(Q)$^{-1}$	0.0909±0.0018 μmol(CO_2) μmol(Q)$^{-1}$	0.69±0.01 g(N) m^{-2}	0.98±0.02 g(N) m^{-2}	0.068±0.012 μmol(CO_2) $m^{-2}$$s^{-1}$[g(N) m^{-2}]$^{-1}$	0.168±0.005 μmol(CO_2) m^{-2} s^{-1}[g(N) m^{-2}]$^{-1}$
C_e×N_L	0.0489±0.0005 μmol(CO_2) m^{-2} s^{-1} [cm^3(CO_2) m^{-3}]$^{-1}$	0.0510±0.0005 μmol(CO_2) m^{-2} s^{-1} [cm^3(CO_2) m^{-3}]$^{-1}$	0.55±0.01 g(N) m^{-2}	0.83±0.01 g(N) m^{-2}	0.032±0.004 μmol(CO_2) m^{-2} s^{-1} [cm^3(CO_2) m^{-3}]$^{-1}$ [g(N) m^{-2}]$^{-1}$	0.050±0.007 μmol(CO_2) m^{-2} s^{-1} [cm^3(CO_2) m^{-3}]$^{-1}$ [g(N) m^{-2}]$^{-1}$

(Table 2). Quantum yield efficiencies fell to zero as the N_L value approached the critical value corresponding to the N_L-intercept. This critical N_L value or N_L-intercept value for the HP plants was considerably higher than the value for LP plants. Radiation NUE defined in terms of the gradient with respect to the linear portion of α *versus* N_L was higher for HP plants (Table 2). For a given N and P supply rate α remained constant over the two harvest intervals (data not shown). This is consistent with the hypothesis that photosynthetic parameters should remain constant when N and P supply rates are kept constant.

Discussion

Photosynthetic activity is limited by both N and P supply. During plant growth P modulates the influence of N supply on photosynthetic activity. Under balanced exponential growth the relationship among variables such as plant growth rate, rate of N accumulation, and P_N may be formulated as follows:

$$\frac{1}{W}\frac{dW}{dt}=\frac{1}{N_{plt}}\frac{dN}{dt}=\frac{1}{W}\left[\frac{P_G\,L}{\delta_L}-R_M\,W-R_G W\right]= =P_N\left(1-R_f\right) \qquad (1)$$

where W is plant biomass, N_{plt} is total plant elemental N, P_G is gross photosynthetic rate, L is leaf mass, δ_L is the specific leaf area, R_M is the maintenance respiration, R_G is the growth respiration. Under saturating irradiance, P_N tends to P_{max} and for a given fixed stomatal and mesophyll diffusion constant, P_{max} can be defined as follows (Charles-Edwards 1978, Fisher *et al.* 1981, Evans and Caemmerer 1996, Katul *et al.* 2000):

$$P_{max}=h\tau[C_a-C_i]=CE[C_a-C_i] \qquad (2)$$

where h is leaf thickness, τ is the carboxylation constant [s^{-1}], CE = h τ is the carboxylation efficiency, C_a is the ambient CO_2 concentration, and C_i is the intercellular CO_2 concentration. When irradiance is not limiting the relationship between the carboxylation efficiency, active RuBPCO content and P_{max} can be defined. For example, CE can be expressed as function of the maximum velocity of RuBPCO activity (V_{max}) or P_{max} as follows:

$$CE=\frac{V_{max}}{C_i-Km_{appCO_2}}=\frac{P_{max}}{[C_a-C_i]} \qquad (3)$$

where Km_{appCO_2} is the apparent Km for CO_2 at varying O_2 concentrations. Carboxylation efficiency is proportional to the content of activated RuBPCO catalytic sites, E_a, and the latter can be expressed as follows:

$$E_a=\frac{V_{max}}{k_{cat}}=\frac{P_{max}\,k_{CO_2}}{k_{cat}} \qquad (4)$$

where k_{cat} represents the RuBPCO turnover rate (3.3 s^{-1}) and k_{CO_2} has been defined as follows:

$$k_{CO_2}=\frac{\lfloor C_i+Km_{appCO_2}\rfloor}{[C_a-C_i]} \qquad (5)$$

From the above equations the relationship between E_a and the rate of N accumulation may be formulated as follows:

$$\frac{dN}{dt}=N_{plt}P_{max}\left(1-R_f\right)=N_{plt}\,\frac{E_a k_{cat}\left(1-R_f\right)}{k_{co_2}} \qquad (6)$$

As the reaction rates of the Calvin cycle are controlled by P recycling, P supply to the plant modulates the content of activated RuBPCO, P_{max} may then in relation to N and P supply be expressed as follows:

$$P_{max}=\frac{1}{N_{plt}\left(1-R_f\right)}\frac{k_{cat}^{app}K_N^{app}E_a N}{\left(k_{cat}^{app}+K_N^{app}N\right)} \qquad (7)$$

where

$$k_{cat}^{app}=\frac{k_{cat}K_P P}{k_{cat}+K_p P} \qquad (8)$$

has been defined as the P dependent apparent catalytic constant for Rubisco and K_P represents the Michaelis constant for P; and

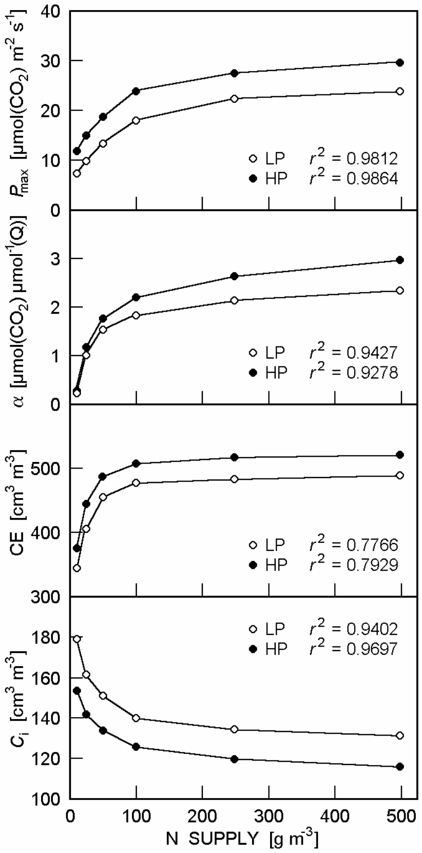

Fig. 4. Irradiance saturated photosynthetic rate (P_{max}), quantum yield efficiency (α), internal CO$_2$ concentration (C_i), and carboxylation efficiency (CE) of *Vicia faba* as influenced by N supply [10, 25, 50, 100, 250, and 500 g(N) m^{-3}] and P supply (LP and HP). r^2 estimated for natural log of the plot.

$$K_N^{app} = \frac{K_N K_{NP} P}{K_N + K_{NP} P} \qquad (9)$$

has been defined as the apparent specificity constant for N. In Eq. (9) K_N represents the Michaelis constant for N and K_{NP} represents a kinetic coefficient. Substituting Eq. (7) for P_{max} into Eq. (6) gives the following relationship for N, P, and E_a:

$$\frac{dN}{dt} = \frac{k_{cat}^{app} K_N^{app} E_a N}{\left(k_{cat}^{app} + K_N^{app} N\right)} \qquad (10)$$

Finally, with regard to photosynthetic activity, the results of this investigation show that P supply determines the upper limit of the asymptotic values for P_{max}, and CE in relation to N supply; and Eqs. (1) to (10) give a possible kinetic description of these results.

In conclusion, we found a tight coupling between the effects of P and N on photosynthesis and plant growth. A decline in the supply of N (Paul and Driscoll 1997) or P (Pieters *et al.* 2001) results in an immediate decline in photosynthetic activity. This study was focused primarily on the influence of N and P on carbon source dynamics, but increasing N and P supply also stimulates photosynthetic activity by increasing down-stream utilization of Calvin cycle end-products (Paul and Pellny 2003). Sugars alone probably do not mediate sink regulation of photosynthesis (Paul and Foyer 2001). Rather it is the whole plant nutrient balance in the form of the C : N : P supply ratios that mediates both source and sink regulation of photosynthesis.

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