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# Interactive effects of nitrogen and phosphorus on the acclimation potential of foliage photosynthetic properties of cork oak, *Quercus suber,* to elevated atmospheric $CO_2$ concentrations

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**Abstract**

**Leaf gas-exchange and chemical composition were investigated in seedlings of *Quercus suber* L. grown for 21 months either at elevated (700 μmol mol$^{-1}$) or normal (350 μmol mol$^{-1}$) ambient atmospheric $CO_2$ concentrations, [$CO_2$], in a sandy nutrient-poor soil with either 'high' N (0.3 mol N m$^{-3}$ in the irrigation solution) or with 'low' N (0.05 mol N m$^{-3}$) and with a constant suboptimal concentration of the other macro- and micronutrients. Although elevated [$CO_2$] yielded the greatest total plant biomass in 'high' nitrogen treatment, it resulted in lower leaf nutrient concentrations in all cases, independent of the nutrient addition regime, and in greater nonstructural carbohydrate concentrations. By contrast, nitrogen treatment did not affect foliar N concentrations, but resulted in lower phosphorus concentrations, suggesting that under lower N, P use-efficiency in foliar biomass production was lower. Phosphorus deficiency was evident in all treatments, as photosynthesis became $CO_2$ insensitive at intercellular $CO_2$ concentrations larger than ≈ 300 μmol mol$^{-1}$, and net assimilation rates measured at an ambient [$CO_2$] of 350 μmol mol$^{-1}$ or at 700 μmol mol$^{-1}$ were not significantly different. Moreover, there was a positive correlation of foliar P with maximum Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase) carboxylase activity ($V_{cmax}$), which potentially limits photosynthesis at low [$CO_2$], and the capacities of photosynthetic electron transport ($J_{max}$) and phosphate utilization ($P_{max}$), which are potentially limiting at high [$CO_2$]. None of these potential limits was correlated with foliar nitrogen concentration, indicating that photosynthetic N use-efficiency was directly dependent on foliar P availability. Though the tendencies were towards lower capacities of potential limitations of photosynthesis in high [$CO_2$] grown specimens, the effects were statistically insignificant, because of (i) large within-treatment variability related to foliar P, and (ii) small decreases in P/N ratio with increasing [$CO_2$], resulting in balanced changes in other foliar compounds potentially limiting carbon acquisition. The results of the current study indicate that under P-deficiency, the down-regulation of excess biochemical capacities proceeds in a similar manner in leaves grown under normal and elevated [$CO_2$], and also that foliar P/N ratios for optimum photosynthesis are likely to increase with increasing growth $CO_2$ concentrations.**

*Symbols: A*, net assimilation rate (μmol m$^{-2}$ s$^{-1}$); $A_{max}$, light-saturated *A* (μmol m$^{-2}$ s$^{-1}$); α, initial quantum yield at saturating [$CO_2$] and for an incident *Q* (mol mol$^{-1}$); [$CO_2$], atmospheric $CO_2$ concentration (μmol mol$^{-1}$); $C_i$, intercellular $CO_2$ concentration (μmol mol$^{-1}$); $C_a$, $CO_2$ concentration in the gas-exchange cuvette (μmol mol$^{-1}$); $F_B$, fraction of leaf N in 'photoenergetics';

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$F_L$, fraction of leaf N in light harvesting; $F_R$, fraction of leaf N in Rubisco; Γ*, $CO_2$ compensation concentration in the absence of $R_d$ (μmol $mol^{-1}$); $J_{max}$*, capacity for photosynthetic electron transport; $J_{mc}$, capacity for photosynthetic electron transport per unit cytochrome f (mol $e^-$ [mol cyt f]$^{-1}$ $s^{-1}$); $K_c$, Michaelis-Menten constant for carboxylation (μmol $mol^{-1}$); $K_o$, Michaelis-Menten constant for oxygenation (mmol $mol^{-1}$); $M_A$, leaf dry mass per area (g $m^{-2}$); $O$, intercellular oxygen concentration (mmol $mol^{-1}$); [$P_i$], concentration of inorganic phosphate (mM); $P_{max}$*, capacity for phosphate utilization; $Q$, photosynthetically active quantum flux density (μmol $m^{-2}$ $s^{-1}$); $R_d$*, day respiration ($CO_2$ evolution from nonphotorespiratory processes continuing in the light); Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; RUBP, ribulose-1,5-bisphosphate; $T_l$, leaf temperature (°C); $U_{TPU}$*, rate of triose phosphate utilization; $V_{cmax}$*, maximum Rubisco carboxylase activity; $V_{cr}$, specific activity of Rubisco (μmol $CO_2$ [g Rubisco]$^{-1}$ $s^{-1}$]

*given in either μmol $m^{-2}$ $s^{-1}$ or in μmol $g^{-1}$ $s^{-1}$ as described in the text.

*Keywords:* nitrogen, nutrient imbalances, Rubisco, phosphorus nutrition, phosphate limitation, photosynthetic electron transport

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## Introduction

Reports of the influences of increased atmospheric $CO_2$ concentrations, [$CO_2$], on foliar photosynthesis and plant net productivity are contradictory. While a significant enhancement of growth has been demonstrated in the majority of studies, no or even negative effects of [$CO_2$] have been observed in others (Gunderson & Wullschleger 1994; Poorter *et al.* 1996). A variety of responses exist in photosynthetic acclimation to high [$CO_2$] as well (Sage 1994; Curtis 1996). According to model simulations, foliar resources are optimally partitioned when less nitrogen is invested in Rubisco, thus allowing greater allocation of N to other potentially limiting enzymes (Woodrow 1994; Medlyn 1996). Lowered investment in Rubisco brings about decreased photosynthetic rates at low [$CO_2$], but reallocation of N to other potentially limiting enzymes results in a greater photosynthetic carbon acquisition at high [$CO_2$]. For example, experimental studies with genetically engineered plants with a reduced amount of Rubisco indicate that enhanced N investment in other rate-limiting proteins at the expense of Rubisco leads to improved foliar nitrogen use-efficiency in high [$CO_2$] (Makino *et al.* 1997). Yet, the content of Rubisco is not necessarily optimized in wild plants (Sage *et al.* 1989, 1995; Socias *et al.* 1993; Makino 1994; Makino *et al.* 1997), suggesting that they are subject to additional selection pressures, which are partly linked to survival under environmental stress (Krapp *et al.* 1994).

Because increased supply of reduced carbon may give rise to enhanced nutrient limitations, there are key interactions between the mineral nutrition of plant, and foliar photosynthesis, plant growth, and biomass allocation under elevated atmospheric $CO_2$ concentration. Foliar nutrient concentrations commonly decline with increasing growth [$CO_2$] (Peet *et al.* 1986; Owensby *et al.* 1993; Curtis 1996; BassiriRad *et al.* 1997; Luo *et al.* 1997), which results in lower rates of net photosynthesis (Gunderson & Wullschleger 1994). In contrast, when enough nutrients are provided to maintain the balance between minerals and reduced carbon, foliar photosynthetic capacities do not necessarily decline with increasing growth [$CO_2$] (Radoglou *et al.* 1992; Sage 1994; Curtis 1996; Riviere-Rolland *et al.* 1996; Luo *et al.* 1997). The interactions between plant $CO_2$ responsiveness, nutrient availability, and nutrient allocation are further complicated by significant species-dependent attributes (Pettersson & McDonald 1994; Stock & Midgley 1995).

While studies on plant–nutrient relations often focus on N, since it is required in the largest quantities for photosynthetic function and is considered the nutrient most universally limiting carbon gain (Chapin *et al.* 1987; Schulze *et al.* 1994), phosphorus also plays a primary role in plant metabolism, and there is evidence that P and N use-efficiencies are tightly linked. A decline in the availability of one nutrient may lead to a less efficient use of the other (Lajtha & Klein 1988; Reich & Schoettle 1988; Reich *et al.* 1994; Raaimakers *et al.* 1995). From the viewpoint of photosynthetic adjustment to elevated [$CO_2$], the interaction between enhanced [$CO_2$] and P-deficiency deserves special attention, because low P may directly curb photosynthesis at higher $CO_2$ concentrations (Foyer & Spencer 1986; Brooks *et al.* 1988; Conroy *et al.* 1988; Sharkey & Vanderveer 1989). It is also important to consider that optimum concentrations of phosphate for photophosphorylation may be higher in elevated [$CO_2$], where large amounts of phosphate are bound to photosynthetic intermediates (Sharkey 1985, 1990). Thus,

P may become relatively more limiting than N with increasing atmospheric $CO_2$ concentration.

In the experiments reported here, the commercially important Mediterranean evergreen sclerophyll *Quercus suber* was grown in environmentally controlled greenhouses with suboptimal constant P supply at two atmospheric $CO_2$ concentrations and with two contrasting nitrogen concentrations. We hypothesized that (i) foliage nutrient concentrations decline in response to elevated [$CO_2$], bringing about lower rates of photosynthesis, and (ii) that this is more significant in plants grown with low N supply. We also suggested that (iii) net photosynthetic rates are limited by P-supply at higher [$CO_2$] in both 'normal' and 'elevated' [$CO_2$] grown specimens, and (iv) that the P limitation is more severe with both higher N supply and higher [$CO_2$].

## Materials and methods

### *Plant growth: experimental design*

Seedlings of *Q. suber* with cotyledons ablated after germination were grown in a local nursery until March 1995. At this time, the seedlings were 5-months-old, and were transferred to two naturally lit greenhouses at the Instituto Superior de Agronomia, Lisbon, Portugal. Each greenhouse had two compartments ventilated with a homogeneous descending forced-air convection of 0.3 m $s^{-1}$, and with a $CO_2$ concentration of either 350 μmol $mol^{-1}$ ('normal') or 700 μmol $mol^{-1}$ ('elevated'). During the entire experiment, air temperature was held close to open-air conditions and relative humidity was kept around 50%. Maximum quantum flux densities above the plants were 1500 μmol $m^{-2}$ $s^{-1}$ in summer and 600 μmol $m^{-2}$ $s^{-1}$ in winter months, i.e. light was reduced by about 25% in comparison with the values observed above the greenhouses (Faria *et al.* 1996).

At the beginning of the experiment, each seedling was transplanted to one 8 L cylindrical container filled with a sandy infertile soil collected from a local nursery. The soil was mixed thoroughly and sieved (2 mm) before the pots were filled. To ameliorate possible environmental microheterogeneity, the containers were rotated weekly within the greenhouse compartments, and once per month between the greenhouses. Potential impacts of low moisture availabilities on $CO_2$ treatments were avoided by watering the containers to field capacity every two days. The irrigation solution contained either 0.15 mol $NH_4NO_3$ $m^{-3}$ ('high' N) or 0.025 mol $NH_4NO_3$ $m^{-3}$ ('low' N) with the balanced mixture (Proe & Millard 1995) of the other major nutrients – 0.03 $Na_2HPO_4$, 0.06 $K_2SO_4$, 0.035 $MgSO_4$ and 0.1 $CaCl_2$ (all in mol $m^{-3}$), and with the micronutrients – 0.2 $FeC_6H_5O_7$, 0.1 $MnSO_4$, 0.25 $H_3BO_3$, 0.01 $ZnSO_4$, and 0.01 $CuSO_4$ (all in mmol $m^{-3}$).

Both the $CO_2$ and N treatments were replicated by two greenhouses per treatment, and all measurements by 4–6 plants per treatment. All analyses were conducted with 7–8-month-old fully expanded leaves on plants grown at the respective experimental treatment for 21 months. At that time, the seedlings of the 'high' N/'elevated' [$CO_2$] treatment had significantly larger ($P < 0.01$) foliar biomass (18.4 g) and greater foliar area per plant (0.118 ± 0.01 $m^2$) than those in the other treatments (total foliar mass varying from 6.2 to 9.4 g, foliar area from 0.031 to 0.068 $m^2$) according to non-destructive analyses of foliar biomass and leaf area in 20 plants per treatment.

### *Chemical and morphological analyses*

Leaves adjacent to those for which assimilation parameters had been measured were collected. For carbohydrate and chlorophyll analyses, several disks of 0.45 $cm^2$ were punched between the major veins with a cork-borer, immediately frozen in liquid nitrogen and stored at –80 °C until analysed. Samples for carbohydrate analysis were collected both in the morning (08.00–09.00 hours) and in the afternoon (16.00–18.00 hours). Total N of leaf laminas was estimated by gas chromatography after combustion the sample at > 1000 °C in oxygen (elemental analyser CHN-O-Rapid, Foss Heraeus GmbH, Hanau, Germany), and total concentrations of Ca, K, Mg and P by inductively coupled plasma emission spectroscopy (Integra XMP, GBC Scientific Instruments, Melbourne, Australia) after digestion of pulverised leaves in 65% $HNO_3$. Chlorophyll (a + b) content was determined in 80% acetone from the extinction measurements at 661.6 and 644.8 nm with a UV-visible dual-beam spectrophotometer (U-2001, Hitachi Ltd, Tokyo, Japan) using the equations of Arnon (1949). An enzymatic method as described in Stitt *et al.* (1989) was used for determination of soluble carbohydrates (fructose, glucose, sucrose) and starch. Total nonstructural carbohydrate concentration was defined as the sum of soluble carbohydrates and starch. Leaf area was measured with a video-based planimeter (DIAS, ΔT-Devices Ltd, Cambridge, UK), leaves were weighed after oven-drying at 70 °C to a constant mass, and leaf dry mass per area ($M_A$) was calculated. Since average surface area per leaf ranged from 2.5 to 21.2 $cm^2$, pooled sample of two to five leaves was used for nutrient and $M_A$ analyses, whereas the leaf disks for chlorophyll and carbohydrate analyses were removed from a single leaf. The measurement error of the analytical procedures was always below 5%.

Initially, all physiological, chemical and structural variables were calculated also on a nonstructural carbohydrate-free dry mass as described in Niinemets (1995, 1997). However, because the concentrations of nonstruc-

tural carbohydrates were relatively low (cf. Results), no relationship with foliar physiological or structural parameters was significantly altered by this routine. Thus, only the variables calculated per unit total dry mass are presented here.

### *Gas-exchange measurements*

Net assimilation ($A$) vs. intercellular $CO_2$ ($C_i$), and $A$ vs. incident quantum flux density ($Q$) responses were determined in a feed-back control leaf gas-exchange system incorporating a controlled environment leaf cuvette and a humidity bypass for operating at low vapour pressure deficits (CMS-400 and CNF-400, Heinz Walz GmbH, Effeltrich, Germany). Vapour pressure deficit averaged (± SD) 18.2 ± 2.0 mmol $mol^{-1}$ for $A$ vs. $C_i$, and 17.9 ± 2.0 for light curves. Cuvette temperature was controlled at 25 °C, and the range in leaf temperature, measured by a thermocouple attached to the lower leaf surface, was within 1 °C in measurements for a single response curve. However, across all measurements, it varied between 24.4 and 27.5 °C for $C_i$, and 21.8–28.2 °C for light response curves (average ± SD = 25.7 ± 0.9 for both $C_i$ and light curves). Uniform illumination was provided with a fibre-optic light source (Fibre Illuminator-400, Heinz Walz GmbH, Effeltrich, Germany) containing a halogen lamp (Scholly Fiberoptic GmbH, Denzlingen, Germany) with a spectrum matched to mimic sunlight.

For $A$ vs. $C_i$ responses, cuvette $CO_2$ concentrations ($C_a$) were increased in steps starting from the lowest (50 μmol $mol^{-1}$) concentration. The leaf was held at each $C_a$ until steady-state values of net carbon assimilation were reached. On average, one $A$ vs. $C_i$ curve was obtained in 4 h. $Q$ was measured with a quantum sensor (LI-190SA, Li-Cor, Inc., Lincoln, Nebraska, USA), and averaged 1050 ± 35 μmol $m^{-2}$ $s^{-1}$.

Light response curves of $A$ were attained by increasing $Q$ in steps from 115 to 1600 μmol $m^{-2}$ $s^{-1}$, and steady-state values were recorded at each $Q$. For all studied plants, $A$ vs. $Q$ response was measured at a $C_a$ of 350 μmol $mol^{-1}$. To gain further insight into the possible limitations of photosynthesis, several light response curves were also measured at a $C_a$ of 700 μmol $mol^{-1}$ for each treatment. The measurements for an $A$ vs. $Q$ curve took on average 4 h. Gas-exchange parameters were calculated according to von Caemmerer & Farquhar (1981).

### *Curve fitting: derivation of model parameters*

According to the leaf photosynthesis model of Farquhar (Farquhar *et al.* 1980; Farquhar & von Caemmerer 1982) as modified by Sharkey (1985), ribulose-1,5-bisphosphate (RUBP) carboxylation is limited by either Rubisco activity, or the regeneration of RUBP or the stromal concentration of inorganic phosphate, [$P_i$]. Chloroplastic RUBP concentration generally scales with the rate of photosynthetic electron transport, which provides ATP and reductive equivalents for RUBP regeneration (Farquhar *et al.* 1980). Phosphate availability may become limiting in conditions of high light and [$CO_2$] when the reactions of starch and sucrose synthesis releasing phosphate do not keep up with phosphate sequestration into triose phosphates (Sharkey 1985; Laisk & Walker 1986). However, low [$P_i$] may also curb ATP synthesis, and thereby reduce the rate of RUBP production even in conditions where the potential capacity for electron transport would not appear limiting. Although each potentially limiting component is suggested to be down-regulated to match the overall capacity over both the short- (Sage 1990) and long-term (von Caemmerer & Farquhar 1984; Farquhar *et al.* 1989), excess electron transport potentials have been observed in the leaves carrying out photosynthesis at high light and $CO_2$ (Stitt 1986). To get a more realistic estimate of the capacities of potential limitations, we used both $A$ vs. $C_i$ and $A$ vs. $Q$ response curves to derive the estimates of maximum Rubisco carboxylase activity ($V_{cmax}$), maximum rates of photosynthetic electron transport ($J_{max}$), and phosphate consumption ($P_{max}$) as described in Appendix I. $V_{cmax}$ and $J_{max}$ were standardized to a common temperature of 25 °C using the temperature dependencies for the specific activity of Rubisco and maximum rate of electron transport per unit cytochrome f as described in Niinemets & Tenhunen (1997). The same temperature performance as for Rubisco was assumed for $P_{max}$. An example of the curve fitting is given in Fig. 1.

### *Nitrogen investments in Rubisco, photoenergetics and light harvesting*

To separate the physiological and morphological sources of variation in $V_{cmax}$, it is written as (Niinemets & Tenhunen 1997):

$$V_{cmax} = 6.25 V_{cr} M_A F_R N_m, \quad (1)$$

where $F_R$ is the fraction of leaf N in Rubisco, $V_{cr}$ the specific activity of Rubisco (the maximum rate of RUBP carboxylation per unit Rubisco protein), $M_A$ leaf dry mass per area (g $m^{-2}$), and 6.25 (g Rubisco [g N in Rubisco]$^{-1}$) converts nitrogen content to protein content. $V_{cr}$ is equal to 20.5 μmol $CO_2$ (g Rubisco)$^{-1}$ $s^{-1}$ at 25 °C for purified enzyme from *Spinacia oleracea* (Jordan & Ogren 1984; see also Niinemets & Tenhunen 1997).

$J_{max}$ is expressed similarly:

$$J_{max} = 8.06 J_{mc} M_A F_B N_m, \quad (2)$$

where $J_{mc}$ is the capacity of electron transport per unit

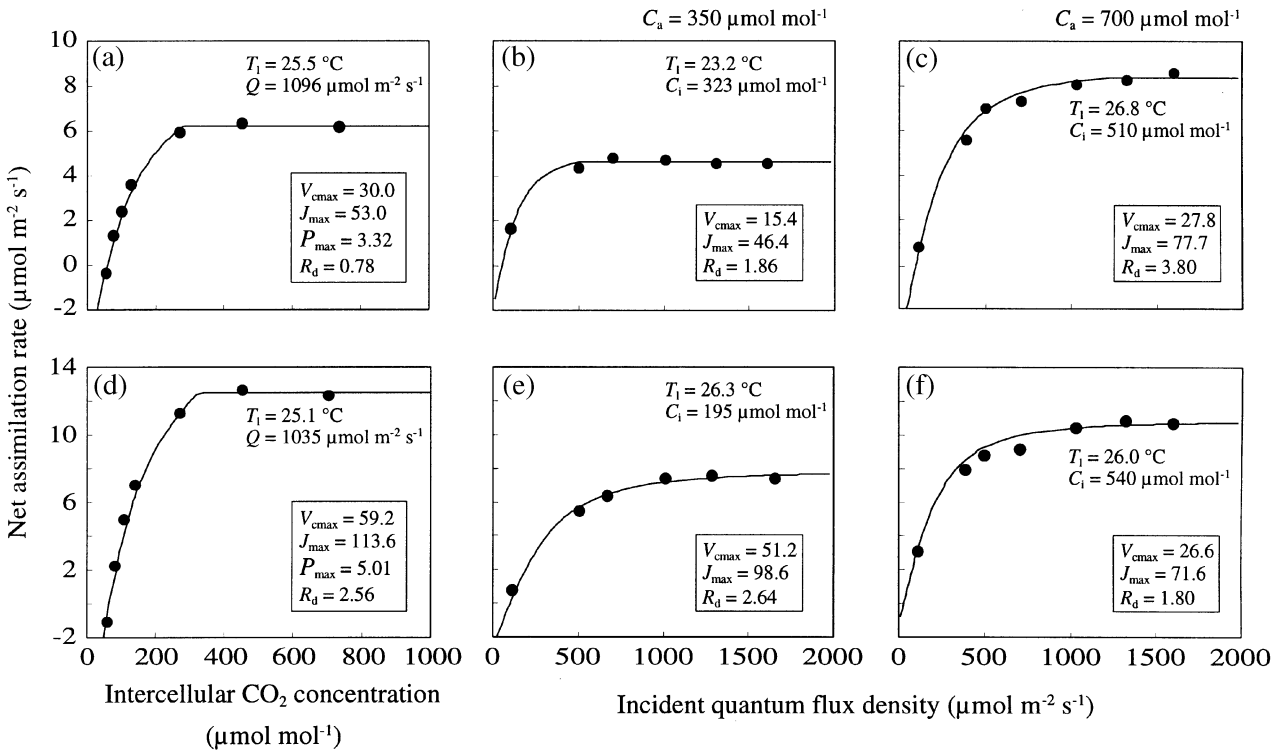


**Fig. 1** Examples of net assimilation ($A$) vs. intercellular $CO_2$ concentration ($C_i$) response curves (panels a and d), and $A$ vs. incident quantum flux density ($Q$) response curves at ambient $CO_2$ concentrations ($C_a$) of 350 µmol mol$^{-1}$ (panels b and e) and 700 µmol mol$^{-1}$ (panels c and f). All depicted dependencies are from 'high' N treatment, where the plants were irrigated with nutrient solution containing 0.3 mol N m$^{-3}$; a representative specimen grown under 'normal' [$CO_2$] (350 µmol mol$^{-1}$; panels a–c), and one grown under 'elevated' [$CO_2$] regime (700 µmol mol$^{-1}$; panels d–f) were selected. Each curve was measured in a different leaf. Points are measured values, the curves represent an estimate of the photosynthesis model (Farquhar & von Caemmerer 1982; Sharkey 1985) with the depicted biochemical parameters, and for average values calculated for all data points per curve of leaf temperature ($T_l$) and $Q$ for $A$ vs. $C_i$ curves, and $T_l$ and $C_i$ for $A$ vs. $Q$ curves. $V_{cmax}$ (maximum rate of Rubisco carboxylase activity), $J_{max}$ (maximum rate of photosynthetic electron transport), $P_{max}$ (maximum rate of phosphate consumption in triose phosphate production), and $R_d$ (rate of mitochondrial respiration in the light) are all in µmol m$^{-2}$ s$^{-1}$.

cytochrome f (mol electrons [mol cyt f]$^{-1}$ s$^{-1}$) and $F_B$ the fraction of leaf nitrogen in 'photoenergetics'. The index $F_B$ reflects the changes in the content of cytochrome f, ferredoxin NADP reductase, and the coupling factor, and considers that an investment of one g N in photoenergetics is equivalent to 8.06 µmol cyt f. We assume that the rate-limiting step of thylakoid electron transport at light saturation resides between the two photosystems (e.g. Foyer 1993; Harbinson 1994). $J_{mc}$ is equal to 156 mol e$^-$ (mol cyt f)$^{-1}$ s$^{-1}$ at 25 °C (Niinemets & Tenhunen 1997). The fraction of leaf N invested in light harvesting ($F_L$) is calculated from leaf chlorophyll concentration along with information on the stoichiometry of thylakoid proteins as described in Niinemets & Tenhunen (1997).

### *Data analyses*

Since there was no significant main effect and no interactive effects of different greenhouses, two-way analysis of variance with nitrogen and [$CO_2$] as main effects was used to separate the treatment means. To reduce the error variance, we tried to estimate all chemical and morphological variables as well as foliar gas-exchange from a few closely positioned leaves on the same branch. However, in several instances, the foliar material did not suffice for all analyses, resulting in imbalanced data; this was handled by using Type III Sum of Squares (SS) instead of Type I SS (Potvin 1993), or by replacing the missing cell by an average value of all cells per treatment. Both methods resulted in identical conclusions with respect to the significance of the treatment effect. Regression analysis was used to detect the influences of plant variability in P and N on foliar photosynthetic variables as well as on foliage chlorophyll concentration. The significance of treatment effects on regressions was checked by covariation analysis using a common slope model. All relationships were considered significant at $P < 0.10$ (SAS Institute Inc. 1990).

**Table 1** Means ± SE of foliar structural and chemical variables and *P*-values for the significance of the treatment effect (two-way ANOVA)
(a) Foliage mineral concentrations

| | Treatment[1] | | | | | *P*-value[3] | | |
|---|---|---|---|---|---|---|---|---|
| Variable | 350/high N | 350/low N | 700/high N | 700/low N | Field[2] | $CO_2$ | N | $CO_2 \times N$ |
| Nitrogen (mmol $g^{-1}$) | 1.41 ± 0.06 | 1.19 ± 0.08 | 1.10 ± 0.09 | 1.10 ± 0.05 | 1.42 | <0.01 | 0.13 | 0.14 |
| Phosphorus (μmol $g^{-1}$) | 23.1 ± 5.3 | 40.5 ± 8.7 | 10.9 ± 2.1 | 24.2 ± 3.2 | 35.48 | <0.02 | <0.01 | 0.70 |
| P/N (mmol $mol^{-1}$) | 17.2 ± 5.0 | 34.2 ± 7.9 | 9.7 ± 1.5 | 22.5 ± 3.4 | 25.0 | <0.06 | <0.01 | 0.67 |
| Calcium (μmol $g^{-1}$) | 213 ± 20 | 266 ± 31 | 212 ± 27 | 180 ± 21 | 128 ± 4 | 0.10 | 0.69 | 0.10 |
| Magnesium (μmol $g^{-1}$) | 40.9 ± 4.5 | 43.9 ± 6.3 | 32.9 ± 2.2 | 28.4 ± 5.5 | 53.5 ± 4.1 | <0.02 | 0.87 | 0.44 |
| Potassium (μmol $g^{-1}$) | 152 ± 17 | 176 ± 19 | 118 ± 4 | 137 ± 13 | 123 ± 10 | <0.02 | 0.15 | 0.87 |

(b) Leaf dry mass per area, and foliage carbohydrate and chlorophyll concentrations

| | Treatment | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|
| Variable | 350/high N | 350/low N | 700/high N | 700/low N | $CO_2$ | N | $CO_2 \times N$ |
| Lamina dry mass per area (g $m^{-2}$) | 129.3 ± 3.8 | 127.1 ± 7.1 | 130.4 ± 4.7 | 124.3 ± 5.3 | 0.86 | 0.42 | 0.72 |
| Soluble carbohydrates (%)[4] | 5.70 ± 0.72 | 5.02 ± 0.36 | 5.62 ± 0.24 | 6.97 ± 1.11 | 0.17 | 0.61 | 0.13 |
| Starch (%) | 1.36 ± 0.50 | 1.22 ± 0.19 | 2.40 ± 0.61 | 3.00 ± 0.45 | <0.02 | 0.67 | 0.49 |
| Chlorophyll (a + b; μmol $g^{-1}$) | 3.74 ± 0.23 | 3.20 ± 0.13 | 3.11 ± 0.30 | 2.85 ± 0.15 | 0.11 | <0.06 | 0.21 |
| Chlorophyll a/b ratio | 2.51 ± 0.08 | 2.44 ± 0.08 | 2.50 ± 0.09 | 2.43 ± 0.05 | 0.89 | 0.41 | 0.99 |
| Chl(a + b)/N (mmol $mol^{-1}$) | 2.83 ± 0.16 | 2.67 ± 0.22 | 2.83 ± 0.61 | 2.77 ± 0.21 | 0.76 | 0.51 | 0.76 |

[1] Plants were grown at an ambient $CO_2$ concentration, [$CO_2$], of either 350 or 700 μmol $mol^{-1}$, and with irrigation every two days with N-rich nutrient solution (0.3 mol $m^{-3}$, 'high' N) or with N-deficient solution (0.05 mol N $m^{-3}$, 'low' N) to soil field capacity.
[2] Leaf material of mature trees was collected from the upper well-illuminated canopy from two natural habitats of *Q. suber*, whereas only the trees close to the seed source of the plants used in the $CO_2$ and N treatments were sampled. Field values were not included in the statistical comparisons.
[3] Number of degrees of freedom (d.f.) = 1 for each factor, and 21 for the residual variance.
[4] Soluble carbohydrate and starch values are given for only the samples taken during morning hours (08.00–09.00 hours), because total nonstructural carbohydrate concentration (starch + sugars) of the afternoon samples (16.00–18.00 hours) was strongly correlated with that of the morning samples ($r^2 = 0.79$, $P < 0.001$). However, afternoon samples contained on average 17% more nonstructural carbohydrates than those taken in the morning.

## Results

### *Chemical composition and morphology*

Neither [$CO_2$] nor nitrogen treatment influenced lamina dry mass per area, but foliar nutrient concentrations consistently decreased with increasing atmospheric $CO_2$ concentration (Table 1). There was also a decrease in P/N molar ratio with increasing growth [$CO_2$], indicating that P became increasingly more limiting with increasing carbon availability (Table 1). Enhanced N availability did not increase lamina N concentrations, but resulted in lower P concentrations and lower P/N ratio (Table 1), suggesting that improved N nutrition caused a greater P deficiency. P/N ratio observed in all treatments, except for 'high' N/'high' [$CO_2$] treatment, was comparable to that in plants growing in the field (Table 1), yet, both P concentrations and P/N ratios were low. Given that foliar P/N ratios < 32 mmol $mol^{-1}$ are indicative of P-limited growth (Wassen *et al.* 1995), they were in the limiting range for the field data and for all potted plants, except for 'low' N/'low' [$CO_2$] treatment.

Leaf chlorophyll (a + b) concentrations increased linearly with increasing N in the leaves (Fig. 2a), and Chl concentrations were also positively affected by N treatment (Table 1). Chl concentration as well as Chl/N ratio were independent of foliar P concentrations (Fig. 2b, c); however, Chl/N ratio decreased with increasing N concentration ($r^2 = 0.22$, $P < 0.06$), suggesting that the gain in Chl with N was progressively less at higher N concentrations. Foliar nitrogen investment in light harvesting ($F_L$) followed the same patterns as Chl/N ratio (data not shown).

### *Foliar photosynthesis parameters*

Independent of treatment, maximum net assimilation rates ($A_{max}$) measured at an ambient [$CO_2$] of 350 μmol $mol^{-1}$ or at 700 μmol $mol^{-1}$ (Table 2) were also

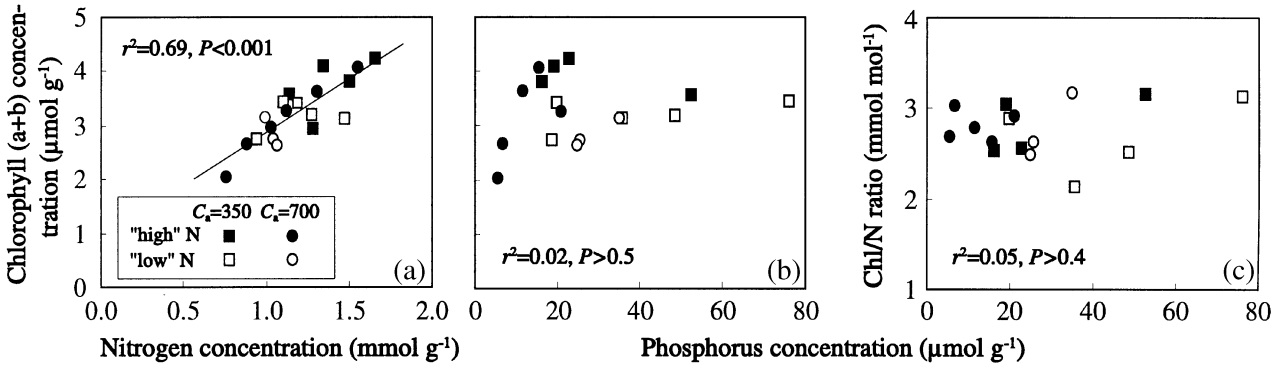


**Fig. 2** Correlation of foliar chlorophyll (a + b) concentration with nitrogen (a) and phosphorus (b), and chlorophyll to nitrogen molar ratio with phosphorus concentration (c). ■, 'high' N, growth $CO_2$ concentration ($C_a^g$) = 350 μmol $mol^{-1}$; □, 'low' N, $C_a^g$ = 350; ●, 'high' N, $C_a^g$ = 700; ○, 'low' N, $C_a^g$ = 700. 'High' N plants were irrigated with a nutrient solution containing 0.3 mol N $m^{-3}$, the nutrient solution contained 0.05 mol N $m^{-3}$ in 'low' N plants. $r^2$–s and $P$-values are given for linear regressions.

not significantly different ($P > 0.3$, two-way covariation analysis with $A$ at 350 μmol $mol^{-1}$ as dependent variable, N and [$CO_2$] as main effects and $A$ at 700 μmol $mol^{-1}$ as covariate). According to two-way covariation analyses, neither N nor [$CO_2$] treatment altered the correlations between $A_{max}$ and stomatal conductance measured at either a $C_a$ of 350 μmol $mol^{-1}$ (Fig. 3a; $P > 0.25$) or at 700 μmol $mol^{-1}$ (Fig. 3b, $P > 0.8$). Leaf photosynthesis at $C_a$ = 350 was also not significantly different between the treatments. However, at $C_a$ = 700, low [$CO_2$] grown plants had significantly higher net assimilation rates (Table 2), suggesting that either photosynthetic electron transport or phosphate availability limited assimilation to a greater extent in high [$CO_2$] grown plants, since stomatal constraints were similar between the treatments (Table 2). Yet, the analysis of $A$ vs. $C_i$ and $A$ vs. $Q$ response curves gave equivocal support to this hypothesis. According to $A$ vs. $C_i$ curves, [$CO_2$] treatment did not alter the various biochemical limitations to differing extents (Table 2), and there was a co-ordination between all components of the photosynthetic machinery (Fig. 4). However, electron transport capacities calculated from $C_i$-response curves were suspect, because of the limitation arising from phosphate availability at higher $C_i$-s (Fig. 1a, d). The assumption that we did not obtain 'true' electron transport capacities from $A$ vs. $C_i$ curves was backed by low $J_{max}/V_{cmax}$ ratios calculated from these curves (Table 2, Fig. 4b, see Leuning 1997). When both $J_{max}$ and $V_{cmax}$ were computed from the light response curves measured at $C_a$ = 350, their ratio was more realistic (Table 2, Fig. 4c), yet, the relationship between $J_{max}$ and $V_{cmax}$ was very similar for all treatments (Fig. 4c), and the treatments did also not differ significantly according to ANOVA (Table 2). This was different for $J_{max}$ calculated from light curves measured at $C_a$ = 700, where the elevated [$CO_2$] treatment resulted in significantly lower $J_{max}$. As may be suggested from high $J_{max}/V_{cmax}$ ratios calculated from $A$ vs. $Q$ curves at $C_a$ = 700 (Table 2, Fig. 4c), photosynthesis was likely to be curtailed by phosphate availability rather than by Rubisco at saturating light intensities and at $C_a$-s around 600–700 (see also Fig. 1a, d).

The intercept of $J_{max}$ vs. $V_{cmax}$ relationships was generally small, and not significantly different from zero (Fig. 4b, c). However, $P_{max}$ vs. $V_{cmax}$ relationship had a significant positive intercept (Fig. 4a), suggesting that $P_{max}$ to $V_{cmax}$ ratio should decrease with increasing $V_{cmax}$. The expected pattern was indeed observed in the current data set ($r^2$ = 0.70, $P < 0.001$).

### *Effects of N and P on photosynthesis*

Improved N nutrition had a negative impact on both the fractional nitrogen investments in Rubisco and 'photoenergetics' (Table 2). At the same time, foliar N concentrations were not related to $V_{cmax}$ (Fig. 5a), $P_{max}$ (Fig. 5c), and $J_{max}$ ($r^2$ = 0.14, $P > 0.16$), or to either nitrogen investments in Rubisco ($F_R$) or 'photoenergetics' ($F_B$; $P > 0.4$ for both). These relationships proposed that lower foliar P availability, which resulted from enhanced nitrogen nutrition (Table 1), may have decreased the nitrogen investment in photosynthetic compounds. Both $F_R$ (Fig. 5b) and $F_B$ ($r^2$ = 0.29, $P < 0.05$) increased with increasing foliar P concentrations. There were also positive correlations of $V_{cmax}$ (Fig. 5b), $P_{max}$ (Fig. 5d) and $J_{max}$ ($r^2$ = 0.30, $P < 0.05$) with foliar P, whereas these correlations were similar for all treatments (Fig. 5).

## Discussion

### *Response of foliage morphology and nutrient content to [$CO_2$]*

Leaf dry mass per area ($M_A$) consistently increases with increasing [$CO_2$] (Sage *et al.* 1989; Sicher *et al.* 1995; Curtis

**Table 2** Parameters of the biochemical photosynthesis model ($V_{cmax}$, $J_{max}$, $P_{max}$, all in μmol $g^{-1}$ $s^{-1}$), and estimates of foliar light-saturated net assimilation rate ($A_{max}$, μmol $m^{-2}$ $s^{-1}$), day respiration rate ($R_d$, μmol $g^{-1}$ $s^{-1}$) and stomatal conductance to $H_2O$ (mmol $m^{-2}$ $s^{-1}$) at low (350 μmol $mol^{-1}$) and high (700 μmol $mol^{-1}$) ambient [$CO_2$]: averages and results of two-way ANOVA

| Variable[1] | Treatment | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|
| | 350/high N | 350/low N | 700/high N | 700/low N | $CO_2$ | N | $CO_2 \times N$ |
| $A_{max}$ ($C_a$ = 350)[2] | 9.6 ± 1.2 | 7.97 ± 0.41 | 6.5 ± 1.3 | 7.6 ± 1.4 | 0.18 | 0.83 | 0.30 |
| Stomatal conductance to $H_2O$ ($C_a$ = 350) | 93 ± 19 | 108 ± 14 | 71.2 ± 6.0 | 99 ± 15 | 0.52 | 0.50 | 0.34 |
| $A_{max}$ ($C_a$ = 600 – 700) | 9.2 ± 1.2 | 11.2 ± 1.0 | 7.7 ± 1.0 | 7.29 ± 0.54 | <0.02 | 0.61 | 0.21 |
| Stomatal conductance to $H_2O$ ($C_a$ = 600 – 700) | 59.7 ± 2.9 | 70.0 ± 6.6 | 58 ± 12 | 51.5 ± 2.6 | 0.23 | 0.91 | 0.31 |
| $V_{cmax}$ from *A* vs. $C_i$ curves | 0.461 ± 0.084 | 0.464 ± 0.034 | 0.343 ± 0.059 | 0.425 ± 0.083 | 0.27 | 0.54 | 0.57 |
| Fraction of leaf N in Rubisco (eqn 1) | 0.169 ± 0.033 | 0.216 ± 0.019 | 0.161 ± 0.016 | 0.217 ± 0.031 | 0.89 | <0.07 | 0.87 |
| $J_{max}$ from *A* vs. $C_i$ curves | 0.89 ± 0.17 | 0.844 ± 0.049 | 0.65 ± 0.10 | 0.78 ± 0.13 | 0.22 | 0.72 | 0.46 |
| Fraction of leaf N in 'photoenergetics' (eqn 2) | 0.0334 ± 0.0066 | 0.0404 ± 0.0032 | 0.0312 ± 0.0032 | 0.0410 ± 0.0045 | 0.87 | <0.09 | 0.77 |
| $J_{max}/V_{cmax}$ ratio from *A* vs. $C_i$ curves | 1.92 ± 0.11 | 1.83 ± 0.05 | 1.89 ± 0.03 | 1.86 ± 0.05 | 0.96 | 0.37 | 0.62 |
| $P_{max}$ | 0.0288 ± 0.0036 | 0.0313 ± 0.0019 | 0.0232 ± 0.0025 | 0.0297 ± 0.0029 | 0.22 | 0.14 | 0.49 |
| $R_d$ | 0.0093 ± 0.0019 | 0.0157 ± 0.0025 | 0.0118 ± 0.0034 | 0.0184 ± 0.0020 | 0.32 | <0.03 | 0.98 |
| $J_{max}$ from *A* vs. *Q* curves at ($C_a$ = 350) | 1.08 ± 0.44 | 0.634 ± 0.076 | 0.550 ± 0.154 | 0.620 ± 0.098 | 0.33 | 0.49 | 0.36 |
| $J_{max}/V_{cmax}$ from *A* vs. *Q* curves ($C_a$ = 350) | 2.61 ± 0.10 | 2.45 ± 0.045 | 2.39 ± 0.12 | 2.44 ± 0.11 | 0.30 | 0.65 | 0.35 |
| $J_{max}$ from *A* vs. *Q* curves ($C_a$ = 700) | 0.695 ± 0.11 | n.d.[3] | 0.482 ± 0.07 | 0.488 ± 0.05 | <0.03 | nd. | nd. |
| $J_{max}/V_{cmax}$ from *A* vs. *Q* curves ($C_a$ = 700) | 3.29 ± 0.02 | n.d. | 3.17 ± 0.08 | 3.16 ± 0.07 | 0.24 | nd. | nd. |

[1]See Fig. 1 for the explanation of the symbols. $C_a$ is in μmol $mol^{-1}$, and various ratios are unitless. $J_{max}$, $V_{cmax}$, $P_{max}$ and $R_d$ were standardized to 25 °C as described in the Appendix I.
[2]Assimilation rates at a $C_a$ of 350 μmol $mol^{-1}$ are maximum values obtained from the light response curves, and these are paired by respective values of stomatal conductance. *A*-s at a $C_a$ of 700 μmol $mol^{-1}$ were similarly attained from the light-response curves at $C_a$ = 700, but values from $CO_2$-response curves measured at $C_a$-s 600–700 μmol $mol^{-1}$, and a *Q* of about 1000 μmol $m^{-2}$ $s^{-1}$ were also included. As the light-response curves demonstrated (Fig. 1c, f), *A* was essentially light-saturated in these conditions.
[3]n.d., not determined, one-way ANOVA with $CO_2$ as main effect was conducted with 'high' N specimen.
*P*-values are provided for the significance of the zero hypothesis that the treatment had no effect. Treatment codes as in Table 1. d.f. = 1 for each factor, and 13 for the parameters of biochemical photosynthesis model as well as for photosynthesis measurements.

 

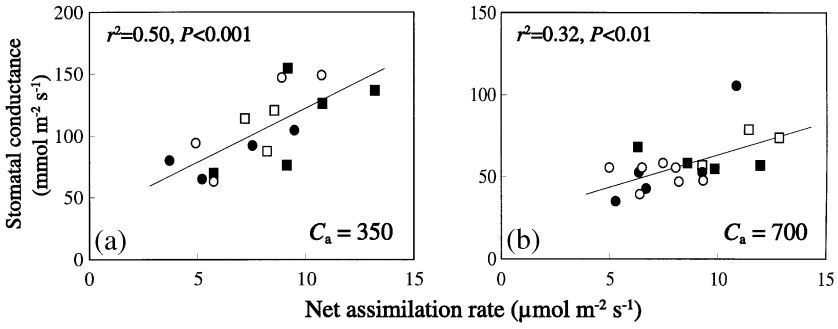


**Fig. 3** Correlations between stomatal conductance for water vapour and net assimilation rate measured at an ambient $CO_2$ concentration of 350 μmol $mol^{-1}$ (a) or of 600–700 μmol $mol^{-1}$ (b). Data on panel (a) were derived from photosynthesis vs. light response curves, the highest value of $A$ and the paired value of stomatal conductance for each curve are depicted. $A$ vs. $Q$ response curves measured at a $C_a$ of 700 μmol $mol^{-1}$ as well as values from $A$ vs. $C_i$ curves at $C_a$-s of 600–700 μmol $mol^{-1}$ were included in panel (b). Symbols as in Fig. 2.

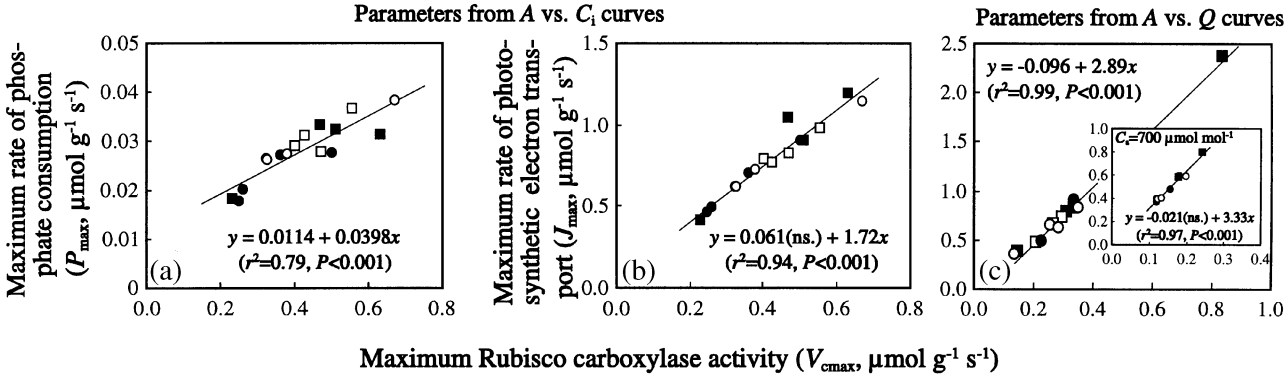


**Fig. 4** Interrelationships between the capacities for phosphate consumption ($P_{max}$, panel a), photosynthetic electron transport ($J_{max}$, panels b and c) and maximum carboxylase activity of Rubisco ($V_{cmax}$). $P_{max}$ was estimated from $A$ vs. $C_i$ curves, $J_{max}$ and $V_{cmax}$ from both $A$ vs. $C_i$ (panel b) and $A$ vs. $Q$ curves at $C_a$-s of 350 μmol $mol^{-1}$ (panel C), and 700 μmol $mol^{-1}$ (inset in panel c). $J_{max}$ and $V_{cmax}$ were standardized to 25 °C using the temperature dependencies for Rubisco activity and electron transport assumed constant for C3 species (Niinemets & Tenhunen 1997), for $P_{max}$, the same temperature behaviour as for Rubisco specific activity was assumed. Symbols as in Fig. 2. Intercepts denoted as ns. are not significantly different from zero ($P > 0.5$).

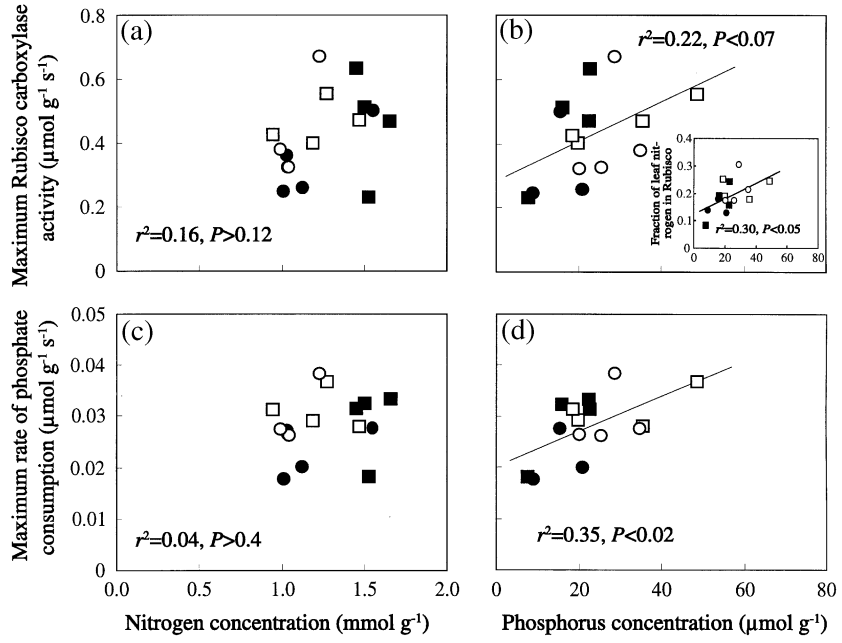


**Fig. 5** Effects of leaf lamina nitrogen (a, c) and phosphorus (b, d) concentrations on $V_{cmax}$ (a, b) and $P_{max}$ (c, d) determined from $A$ vs. $C_i$ curves. Inset in panel (b) demonstrates the correlation between the fraction of foliar nitrogen in Rubisco ($F_R$, eqn 1) and phosphorus concentration. Relationships with $J_{max}$ and nitrogen investment in 'photoenergetics' (eqn 2) were qualitatively identical to those with $V_{cmax}$ and $F_R$ (see text), and have therefore not been depicted. Symbols as in Fig. 2.

1996; Luo *et al.* 1997). This was observed in *Q. suber* after a relatively short-term exposure to elevated [$CO_2$] (Damesin *et al.* 1996), but was not evident after a more extensive exposure time (Faria *et al.* 1996). Higher foliar soluble carbohydrate and starch concentrations at high [$CO_2$] (Peet *et al.* 1986; Yelle *et al.* 1989; Sicher *et al.* 1995; Poorter *et al.* 1997) generally play an important part in the changes of $M_A$ with growth [$CO_2$]. Yet, in the current study, the concentrations of nonstructural carbohydrates were low (Table 1), and did not produce any significant variability in $M_A$. Low carbohydrate concentrations and only minor changes in $M_A$ in response to elevated [$CO_2$] have been documented for the related evergreen sclerophyll *Quercus ilex* (Körner & Miglietta 1994; Chaves *et al.* 1995) and low starch concentrations in *Q. suber* (Faria *et al.* 1996).

As discussed in the Introduction, foliar nutrient concentrations characteristically decline with increasing [$CO_2$] as was also seen in the present study conducted under suboptimal nutrient supply (Table 1). A dilution effect of increased carbohydrate concentration has been considered to be partly responsible for such decreases (Wong 1990). Though nonstructural carbohydrates also diluted nutrients in our experiments, all foliar chemical, morphological and physiological variables expressed on a nonstructural carbohydrate free dry mass behaved exactly the way as did those expressed on a total dry mass.

### *Interaction of mineral nutrition with [$CO_2$]: implications for growth*

Apart from the carbohydrate effects, foliar nutrient levels may decline because the plants grown more quickly at high [$CO_2$] with greater biomass in each compartment have higher nutrient requirements and accordingly, may suffer from greater nutrient stresses at a common addition rate of minerals. Such nutrient limitations arising from enhanced availability of carbon may curtail the positive effects of [$CO_2$] on plant growth (cf. Introduction). In the previous studies, elevated [$CO_2$] resulted in greater growth rates in both 4- and 6-month-old seedlings of *Q. suber* (Damesin *et al.* 1996; Pereira & Chaves 1997), but the enhanced growth levelled off during the second growing season, probably as a result of nutrient limitation (Pereira & Chaves 1997). The assumption of interactive influences of nutrient availability with [$CO_2$] treatment was confirmed in our experiments, where 26-months-old saplings grown at respective treatments for 21 months were the largest in 'high' N/elevated [$CO_2$] regime, but not significantly different between the other treatments (cf. Methods), indicating that enhanced N nutrition may crucially improve the responsiveness of *Q. suber* growth to elevated [$CO_2$].

Despite increased total foliar area and plant biomass at greater N availability, there was an insignificant effect of the N treatments on foliar N concentration (Table 1). This is not surprising in light of other data demonstrating that enhanced N availability first of all speeds up foliage area production (Evans 1983; Thompson *et al.* 1988; Vose & Allen 1988; Pereira *et al.* 1994; van den Boogaard *et al.* 1995) and that changes in nitrogen concentration are of secondary importance, e.g. total N in foliage varied by more than 21-fold between high and low nutrient treatments at high irradiance in seedlings of *Flindersia brayleyana*, while foliar N concentrations varied by only 2.5-fold (Thompson *et al.* 1988). From another perspective, given that improved N-nutrition resulted in a relatively more limited phosphorus supply (Table 1), low sensitivity of foliar N concentration with respect to N-fertilization may partly be explained by the necessity to maintain a balance between foliar nitrogen and phosphorus, possibly accomplished by lowered N uptake with suboptimal P.

### *Differential effects of [$CO_2$] on nutrient acquisition: a whole plant perspective of P vs. N uptake*

We found that P/N ratio declined with increasing growth [$CO_2$] concentration (Table 1). In general, changes in N vs. P uptake in response to [$CO_2$] treatment are not necessarily co-ordinated (Norby *et al.* 1986; van Vuuren *et al.* 1997). This is because the acquisition of relatively immobile phosphate is strongly related to plant ability to build an extensive root system exploring large soil volumes, but effective capture of mobile nitrate and ammonia relies less heavily on extensive root systems (Sands & Mulligan 1990). Thus, below-ground resource capture physiology as well as [$CO_2$]-responsiveness of biomass partitioning between above-and below-ground plant compartments largely determine how the uptake of P relative to N changes in response to an increase in growth [$CO_2$]. According to recent investigations, the genetic potentials of species may play a major role here: growth at high [$CO_2$] resulted in a greater proportion of plant biomass in roots as well as greater nutrient uptake rates per unit root mass in a grass *Bouteloua eriopoda*, but in decreased root fractions and only moderately altered uptake capacity per unit root mass in the shrubs *Larrea tridentata* and *Prosopis glandulosa* (BassiriRad *et al.* 1997). This difference led to greater foliar N and P concentrations in the elevated [$CO_2$] treatment for the grass species, but lowered nutrient concentrations in shrub foliage (BassiriRad *et al.* 1997). In *Quercus alba* (Norby *et al.* 1986) and *Triticum aestivum* (van Vuuren *et al.* 1997), elevated [$CO_2$] increased the fractional biomass investment in roots and resulted in greater foliar P concentrations in the high [$CO_2$] treatment, but foliar N concentrations were greater in low as opposed to the high [$CO_2$] treatment. The fractional biomass investment in roots is relatively

[$CO_2$]-insensitive or even declines in response to elevated [$CO_2$] in *Q. suber* (Damesin *et al.* 1996). Thus, we suggest that conservative investment patterns in roots vs. aboveground biomass in *Q. suber* provides the explanation for decreasing P/N ratio with increasing growth [$CO_2$] in this species (Table 1).

### *Role of phosphate limitation in photosynthetic responses to high [$CO_2$]*

The potential limitation of photosynthesis arising from the deficiency of stromal phosphate or from the inability of the reactions of sucrose and starch synthesis to keep up with the Calvin cycle, have been dismissed in recent physiological growth models, which include feedbacks that result from nutrient deprivation (Kirschbaum *et al.* 1994; Lloyd & Farquhar 1996). Yet, for optimal partitioning of foliar nitrogen between the components of photosynthetic apparatus under conditions of elevated [$CO_2$], the enzymatic capacities for starch and sucrose synthesis should increase at the expense of Rubisco (Woodrow 1994). Since there is some evidence that an up-regulation of these capacities occurs under high growth [$CO_2$] regime (Sage *et al.* 1989; Sage 1994; Riviere-Rolland *et al.* 1996), it has been assumed that starch and sucrose synthesis adjust to changed environmental conditions such that triose phosphate utilization does not play a role as a limitation over the long-term (Medlyn 1996). However, the validity of such assumptions is seriously handicapped by other reports indicating that these enzymatic capacities remain the same or even decrease in response to elevated [$CO_2$] (Peet *et al.* 1986; Socias *et al.* 1993; Sage 1994).

Over the short-term, when the reactions of sucrose and starch synthesis releasing phosphate fail to keep up with the Calvin cycle, photosynthesis becomes constrained by limited ATP supply due to low stromal phosphate concentrations (Sharkey *et al.* 1986). However, there is generally a large vacuolar pool of phosphate, and on a time-scale of hours, the changes in cytoplasmic [$P_i$] may be buffered significantly by phosphate exchange between cytoplasm and vacuole (Rebeille *et al.* 1983; Woodrow *et al.* 1984; Foyer & Spencer 1986). In general, stromal phosphate concentration is maintained just above the level required to saturate photophosphorylation in intensively photosynthesizing chloroplasts (Robinson & Giersch 1987). Yet, the vacuolar pools are depleted under conditions of extreme phosphate deficiency (Rebeille *et al.* 1983; Foyer & Spencer 1986; Sharkey & Vanderveer 1989), leading to P-limited photosynthesis (Foyer & Spencer 1986; Brooks *et al.* 1988; Sharkey & Vanderveer 1989; Jacob & Lawlor 1993). In contrast to the situation where low turnover of phosphate in starch and sucrose synthesis restricts carbon assimilation, the pools of photosynthetic intermediates are low in P-deficient leaves, indicating that there is simply not enough inorganic phosphate to fuel the existing enzymatic capacities (Brooks *et al.* 1988). It is important to grasp here that improved N nutrition may increase the enzymatic capacities, but it cannot alleviate the limitation arising when phosphate as substrate is missing. Although foliar P/N ratio was above that considered limiting for growth in the case of 'normal' [$CO_2$]/'low' N treatment, P-limited photosynthesis was seen in all treatments (cf. Fig. 1a, d), and was the major reason why the assimilation rates differed little between the treatments (Table 2, Figs 3, 5). It is not possible to determine from this experiment whether P-limitation resulted from low enzymatic capacities or from low [$P_i$] in all treatments. Nevertheless, our results are consistent with the hypothesis that optimal P/N ratio is higher in higher atmospheric $CO_2$ concentrations.

Elevated [$CO_2$] reduced foliar P levels (Table 1), raising the question why $P_{max}$ calculated from $A$ vs. $C_i$ response curves (Fig. 1a, d) did not differ between [$CO_2$] treatments. We calculated $P_{max}$ under the conservative assumption that 3/4 of C removed as glycollate from the chloroplast reenters as triose phosphate (Harley & Sharkey 1991). However, recent research demonstrates that this fraction increases with increasing P deficiency (Kondracka & Rychter 1997), allowing greater rate of carbon assimilation with the same $P_{max}$ (see Harley & Sharkey 1991). Since it is likely that this fraction of glycollate carbon remains in the cytosol in the form of amino acids (Harley & Sharkey 1991; Kondracka & Rychter 1997), this enhanced capacity should depend on nitrogen availability for amino acid synthesis. Thus, higher phosphate-turnover in both elevated [$CO_2$] and 'high' N plants may provide an explanation why calculated $P_{max}$ was constant for all treatments irrespective of varying P/N ratios (Table 2).

Under P-deficiency conditions, plant resources are optimally distributed if plant P and N concentrations are in a balance such that photosynthetic carbon acquisition is colimited by both P and N. Although Rubisco activity was down-regulated in response to insufficient P supply, $P_{max}/V_{cmax}$ ratio decreased with increasing $V_{cmax}$, hinting at an excessive capacity of Rubisco at higher [$CO_2$] concentrations. Furthermore, chlorophyll fluorescence studies (Jacob & Lawlor 1993), measurements of uncoupled whole-chain electron transport rates (Brooks 1986), and manipulations with leaf phosphate status by feeding phosphate (Brooks *et al.* 1988) indicate that depending on environmental conditions there might be a surplus electron transport capacity in P-deficient leaves. This was also seen in the current study, where $J_{max}$ relative to $V_{cmax}$ calculated from $A$ vs. $C_i$ curves was considerably lower than that calculated from light-response curves (Fig. 4). Nevertheless, $V_{cmax}$ and $J_{max}$ were similar between the treatments (Table 2), and collect-

ively, the results of the current study suggest that P-deficiency leads to a down-regulation of plant photosynthetic potentials at high [$CO_2$] in both normal and high [$CO_2$] grown plants.

## Conclusion: potential influences of elevated [$CO_2$] on nutrient-limited ecosystems

The interaction effects of [$CO_2$] with mineral nutrition should be of great relevance in Mediterranean-type shrublands, where in addition to water, growth is typically limited by nutrient availability (di Castri 1981). There is a general consensus that in current climatic conditions South-African and Australian Mediterranean communities are mostly limited by P, e.g., foliar P/N ratios as low as 10 mmol $mol^{-1}$ have been measured for Australian mallee ecosystems (Specht 1969). However, soil P availabilities relative to N are also low in many European sclerophyll forests (di Castri 1981). In a number of evergreen *Quercus ilex* ecosystems, P/N ratio (mmol $mol^{-1}$) of young foliage – 32.9 ± 1.4 (± SE, range 24–42; Canadell & Vilà 1992), 30.9 (Mayor & Rodà 1992) or 26.9 ± 0.7 (Rapp *et al.* 1992) – is just above or below the threshold considered limiting (32 mmol $mol^{-1}$ according to Wassen *et al.* 1995). These, and the data of the current study ('field' data in Table 1) collectively indicate that P-limitations play currently an important role in European sclerophyll forest ecosystems as well. Because more P will be bound up in leaf sugar phosphates, and plants are generally larger at elevated [$CO_2$], P requirements for both photosynthesis and growth are greater at higher [$CO_2$]. Given greater P requirement, and that there are interactions between P nutrition and [$CO_2$], we conclude that (i) plant P requirements increase relative to N requirements more with increasing [$CO_2$], and that (ii) in many ecosystems – like European Mediterranean-type communities – where plant N and P supply is just in balance or which are slightly P-limited at current ambient $CO_2$ concentrations, plant productivity may become seriously P-constrained as atmospheric $CO_2$ concentrations increase.

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## Appendix

### *Calculation of maximum Rubisco carboxylase activity ($V_{cmax}$), and the capacities for photosynthetic electron transport ($J_{max}$), and phosphate consumption ($P_{max}$) in Q. suber*

*1 Parameter derivation from net assimilation (A) vs. intercellular $CO_2$ ($C_i$) response curves.* An estimate of $V_{cmax}$ and day respiration ($R_d$, $CO_2$ evolution from nonphotorespiratory processes continuing in the light) was derived from the initial portion ($C_i \approx 50$–$180$ μmol mol$^{-1}$) of an $A$ vs. $C_i$ curve (Fig. 1a, d). First, slope and intercept ($A = kC_i + i$) were calculated for each combination of points in the Rubisco limited part of the $A$ vs. $C_i$ curve. Given that the slope is equivalent to the first derivative of the equation of leaf photosynthesis when Rubisco limits carboxylation (Farquhar & von Caemmerer 1982), $V_{cmax}$ was calculated for each slope as:

$$V_{c\,max} = k\,\frac{[C_i + K_c\,(1 + O\,/\,K_o)]^2}{\Gamma^* + K_c\,(1 + O\,/\,K_o)}, \qquad \text{(A1)}$$

where $K_c$ and $K_o$ are Michaelis–Menten constants for carboxylation and oxygenation, respectively, $O$ is intercellular oxygen concentration, and $\Gamma^*$ is $CO_2$ compensation concentration in the absence of mitochondrial respiration (Laisk 1977). $R_d$ was found as:

$$R_d = V_{cmax}\,\frac{C_i - \Gamma^*}{C_i + K_c\,(1 + O\,/\,K_o)} - (k \cdot C_i + i). \qquad \text{(A2)}$$

The average of the two highest calculated values of $V_{cmax}$ with their average $R_d$ was used in the following calculations.

Since $A$ exhibited $CO_2$ insensitivity or reversed $CO_2$ sensitivity at $C_i$-s larger than 250–400 μmol mol$^{-1}$, $A$ was limited by inorganic phosphate, [$P_i$], release from starch and sucrose synthesizing pathways (Sharkey 1985) at higher $C_i$. Given that under such conditions, [$P_i$] release must equal its consumption, and accordingly the rate of triose phosphate utilization ($U_{TPU}$), $P_{max}$ was calculated as (Sharkey 1985) $U_{TPU} = P_{max} = (A + R_d)/3$ for each point in the $CO_2$ curve exhibiting phosphate limitation, and from these estimates, an average value was calculated for the phosphate limited region (Fig. 1a, d). The $P_{max}$ calculations base on an

assumption that 3/4 of the carbon lost as glycollate returns to the chloroplasts as glycerate. Although the model simulations demonstrate that the fraction of carbon lost from the Calvin cycle due to photorespiration must be larger to explain the reverse $CO_2$ sensitivity of net assimilation (Harley & Sharkey 1991), the fits to our data were only moderately improved by adjusting $P_{max}$ values by assuming various carbon fractions to return from the photorespiratory cycle. Of course, when limited by phosphate availability in the stroma and cytoplasm, $P_{max}$ derived from phosphate-limited photosynthesis at high light and $C_i$ is only an estimate for the potential capacity for phosphate consumption. An estimate of $J_{max}$ was obtained from the points measured at $C_i$-s $>$ 200 μmol mol$^{-1}$, but which still exhibited $CO_2$ sensitivity. We assumed that RUBP regeneration is ATP-limited, and that the additional ATP to balance NADPH production is generated by pseudocyclic electron flow to $O_2$ in the Mehler reaction (Farquhar & von Caemmerer 1982). Initial quantum yield at saturating [$CO_2$] and for an incident $Q$ (α), necessary to calculate $J_{max}$ backwards from Smith equation (Tenhunen *et al.* 1976), was assumed to depend on leaf absorptance only, and was calculated from leaf chlorophyll (a + b) content per area according to an empirical equation (Niinemets & Tenhunen 1997).

*2 Parameter derivation from net assimilation vs. light response curves.* The $A$ value measured at the lowest $Q$ along with the information of quantum yield was used to compute $R_d$. Theoretical quantum yield at an ambient [$CO_2$] was computed according to Farquhar & von Caemmerer (1982), whereas leaf absorptance, necessary to calculate absorbed $Q$, was found from leaf chlorophyll per area as in the analysis of $A$ vs. $C_i$ curves. The rate of electron transport was found as:

$$J = \frac{(A + R_d)(4.5C_i + 10.5\Gamma^*)}{C_i = \Gamma^*)}, \quad \text{(A3)}$$

and $J_{max}$ from the Smith equation for each point exhibiting light-sensitive photosynthesis:

$$J_{max} = \frac{\alpha \cdot Q \cdot J}{\sqrt{\alpha^2 Q^2 - J^2}}. \quad \text{(A4)}$$

Since leaf temperature generally increased with increasing $Q$, $R_d$ was adjusted for each light level by an exponential equation (Niinemets & Tenhunen 1997) using an activation energy of 41.02 kJ mol$^{-1}$ (Tenhunen *et al.* 1987). $V_{cmax}$ was computed from the saturated part of the light curve, and again, a value was calculated for each data point. $J_{max}$ and $V_{cmax}$ were defined as the averages of the three highest estimates (Fig. 1). All estimates of $J_{max}$ and $V_{cmax}$ were standardized to 25 °C with the equations given in Niinemets & Tenhunen (1997) before the averages were calculated. The analysis was basically identical for light-response curves measured at $C_a$-s of 350 and 700 μmol mol$^{-1}$, however, RUBP carboxylation rate in the saturated part of the response curve was likely to be limited by phosphate availability rather than by Rubisco at the higher $C_a$ (cf. Fig. 1a, d).