

Does photosynthetic acclimation to elevated CO₂ increase photosynthetic nitrogen-use efficiency?

A study of three native UK grassland species in open-top chambers

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Summary

1. The photosynthetic response to elevated CO₂ and nutrient stress was investigated in *Agrostis capillaris*, *Lolium perenne* and *Trifolium repens* grown in an open-top chamber facility for 2 years under two nutrient regimes. Acclimation was evaluated by measuring the response of light-saturated photosynthesis to changes in the substomatal CO₂ concentration.

2. Growth at elevated CO₂ resulted in reductions in apparent Rubisco activity *in vivo* in all three species, which were associated with reductions of total leaf nitrogen content on a unit area basis for *A. capillaris* and *L. perenne*. Despite this acclimation, photosynthesis was significantly higher at elevated CO₂ for *T. repens* and *A. capillaris*, the latter exhibiting the greatest increase of carbon uptake at the lowest nutrient supply.

3. The photosynthetic nitrogen-use efficiency (the rate of carbon assimilation per unit leaf nitrogen) increased at elevated CO₂, not purely owing to higher values of photosynthesis at elevated CO₂, but also as a result of lower leaf nitrogen contents.

4. Contrary to most previous studies, this investigation indicates that elevated CO₂ can stimulate photosynthesis under a severely limited nutrient supply. Changes in photosynthetic nitrogen-use efficiency may be a critical determinant of competition within low nutrient ecosystems and low input agricultural systems.

Key-words: Acclimation, climate change, elevated carbon dioxide, grassland, photosynthetic nitrogen-use efficiency

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Introduction

To quantify the response to elevated [CO₂] of different species, most studies have grown single plants in individual pots using controlled environments. Typically, plants are grown at elevated [CO₂] for short periods of weeks, free from sustained defoliation or nutrient stress (Amthor 1995). Although this may be essential in analysing the effect of a single environmental factor upon very specific physiological mechanisms, it is limited when analysing long-lived, multispecies perennial systems such as grasslands. Recent reviews have emphasized the need to supplement this research with field-based studies growing plants for more than one season. Short-term experiments may fail to detect important acclimatory responses evident in longer-term studies. This is especially relevant to agricultural and natural grassland communities, where longer-term management, such

as continual defoliation and nutrient availability, may be critical in determining interspecific responses to elevated [CO₂] in the longer term.

It is a common conclusion in the literature that plants growing under nutrient stress will respond much less to elevated [CO₂] in growth (Zangerl & Bazzaz 1984; Oberbauer *et al.* 1986) and carbon acquisition (Tissue, Thomas & Strain 1993; Körner *et al.* 1996; Nakano, Makino & Mae 1997). For example, Tissue *et al.* (1993) studying tree seedlings concluded that 'in native low nutrient soil conditions, Loblolly Pine is not expected to exhibit higher photosynthetic rates after long-term exposure to elevated CO₂'. The theoretical basis for this is unclear. Most studies refer to lack of growth and the resulting restriction of carbohydrate sinks at a limiting nitrogen supply. However, this hypothesis ignores the possibility that photosynthetic acclimation can lead to an

increase in photosynthetic nitrogen-use efficiency. Being nitrogen-limited does not necessarily mean that plants remain unresponsive to other parameters such as $[\text{CO}_2]$ and light (Lloyd & Farquhar 1996). At elevated $[\text{CO}_2]$, the rate of photosynthesis immediately increases, both because CO_2 is a competitive inhibitor of the oxygenation of RubP and therefore photorespiration is depressed, and because the current $[\text{CO}_2]$ is inadequate to saturate Rubisco. Elevated $[\text{CO}_2]$ therefore leads to increased substrate binding and increased velocity of carboxylation (Drake, González-Meler & Long 1997). Consequently, at elevated $[\text{CO}_2]$ the efficiency of carboxylation by Rubisco is increased and the subsequent requirement for the protein is reduced (Webber, Nie & Long 1994). As up to 30% of leaf nitrogen is allocated to Rubisco (Evans 1989), a significant fraction of leaf nitrogen can therefore be reallocated to other photosynthetic or non-photosynthetic processes at elevated $[\text{CO}_2]$ (Sage 1994).

Numerous studies have detected a decline in total leaf nitrogen content at elevated $[\text{CO}_2]$ (Tissue *et al.* 1993; Thomas, Lewis & Strain 1994; Rogers *et al.* 1996; Nakano *et al.* 1997). This implies a physiological adjustment conducive with a higher photosynthetic nitrogen-use efficiency, i.e. an increase in the rate of carbon assimilation per unit nitrogen in the foliage. However, the extent to which decreases in Rubisco can account for changes in foliar nitrogen is unclear (Conroy & Hocking 1993). An accumulation of carbon leading to a decrease in specific leaf area would also lower leaf nitrogen contents per unit dry mass (Wong 1990; Coleman, McConnaughay & Bazzaz 1993). However, Tissue *et al.* (1993) found a reduction in Rubisco content per unit leaf area in Loblolly Pine after long-term growth at elevated $[\text{CO}_2]$, corresponding to a reduction in total leaf nitrogen. The natural selection pressures for this optimization may be expected to be high. In low-nutrient, multispecies systems such as grasslands, differential responses in nitrogen-use efficiency at elevated $[\text{CO}_2]$ may be the key determinant of the relative competitive ability of species (Poorter, Remkes & Lambers 1990). This, however, is opposed to the expectation of others, that low nitrogen supply will decrease or eliminate the photosynthetic response to elevated $[\text{CO}_2]$ (Tissue *et al.* 1993; Körner *et al.* 1996; Nakano *et al.* 1997).

This study tests the hypothesis that growth at elevated $[\text{CO}_2]$ will result in a significant increase in photosynthetic nitrogen-use efficiency. Acclimation was evaluated by measuring the response of light saturated photosynthesis to changes in c_i (the A/c_i response). Critically, changes in this response can be indicative of a reallocation of resources involving the key processes of photosynthesis (Sage 1994). In parallel to gas-exchange, total leaf nitrogen content was analysed on a leaf area basis.

The three species selected for this study are representative of economically important and ubiquitous Lolio-Cynosuretum grasslands in lowland UK (MG 6

classification, Rodwell 1991) and differ critically in their response to mineral nitrogen. The primary component of these grasslands, *Lolium perenne* L. cv. Melle, is highly responsive to nitrogen fertilizer, whereas *Agrostis capillaris* L. and the nitrogen-fixing *Trifolium repens* L. cv. Haia show lesser degrees of response (Rodwell 1991). To allow maximum opportunity for photosynthetic acclimation, the photosynthetic response was analysed after a 2 year period at elevated $[\text{CO}_2]$.

Materials and methods

GROWTH CONDITIONS

This experiment was conducted using a field based CO_2 fumigation system at the Institute of Grassland and Environmental Research (IGER), North Wyke, UK. A series of 20 open-top chambers exposed three key grassland species to elevated $[\text{CO}_2]$ and sustained nutrient stress over 2 years. Chamber design and environmental control were adapted from Ashenden, Baxter & Rafarel (1992) and the experimental design was as described by Parsons, Atkinson & Wadge (1996). Briefly, each chamber consisted of a 1 m high by 1.2 m diameter cylinder of rigid, UV stabilized, clear plastic sheet (Corrulux, Jewson Ltd, Penlon Works, Bangor, Wales), mounted on a lightweight steel frame. Each chamber contained plastic boxes, 20 cm square \times 30 cm high, partially sunk into the ground to allow free drainage and minimize nutrient influx from the surrounding soil without decoupling the boxes from the thermal inertia of the soil. Each box was filled with sieved soil from adjacent areas. The soil was low in mineral nitrogen and phosphorus as a result of the previous agricultural management.

Seeds of *A. capillaris* (Trinity College, Dublin, Eire), *L. perenne* and *T. repens* were germinated in horticultural plug trays using John Innes seedling compost. After 2 weeks, 10 seedlings of each species were transplanted into separate boxes within each open-top chamber. For the following 3 weeks, seedling progress was monitored, and any mortalities were replaced.

Of 20 open-top chambers, 10 were maintained at $700 \mu\text{mol mol}^{-1} \text{CO}_2$ in air ($[\text{CO}_2]_{700}$), and 10 remained at an ambient CO_2 concentration of $360 \mu\text{mol mol}^{-1} \text{CO}_2$ ($[\text{CO}_2]_{360}$). Within each CO_2 treatment, the chambers were further divided by nutrient treatment. Half the chambers were provided with one application of $3.33 \text{ g m}^{-2} [\text{N}]$ each month, and $8.00 \text{ g m}^{-2} [\text{P}]$ and $8.00 \text{ g m}^{-2} [\text{K}]$ at the start of each growing season. No fertilizers were added to the other half. These nutrient regimes were denoted as 'moderate' and 'low' nutrient supply, respectively. The CO_2 treatment was applied from 3 weeks after seedling transplantation (on 6 September 1994). Plots were maintained close to field capacity and to simulate continuous grazing, all species were cut to maintain a sward height of 6 cm. Sward height was measured weekly and when necessary, cut weekly.

Leaf photosynthetic CO_2 uptake rate (A) was determined in response to changes in the intercellular $[CO_2]$ (c_i) by a portable, steady-state and feedback-control gas-exchange system (LI-6400, LI-COR, Lincoln, NE, USA). The analyser was calibrated prior to use for CO_2 , using a standard gas ($\pm 2\%$ tolerance, Lindegas, Stoke-on-Trent, UK) and for H_2O using a water vapour generator ($\pm 2.5\%$ tolerance, WG600, ADC, Hoddesdon, UK). A Peltier cooling system maintained leaf temperature at $25\text{ }^\circ\text{C}$ and VPD was controlled between 1 and 1.5 kPa . An integral LED light source provided saturating Q of $1300\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$.

All photosynthesis measurements were conducted between 5 and 9 August 1996, nearly 2 years after initial exposure to elevated $[CO_2]$, and *c.* 10–14 days after the last cut. Gas exchange was conducted on intact leaves within the open-top chambers. Five replicate measurements were completed for each species and treatment with chambers rather than leaves as the replicate measure to avoid pseudo-replication. The youngest fully expanded leaves were selected for gas analysis. In grasses, this was determined by emergence of a ligule on vegetative tillers. For *T. repens*, the largest leaflets with no signs of senescence were sampled; the high frequency of the cutting regime ensured a maximum limit to leaf age. To further minimize variation in the age of tissue measured in the grasses, measurements were made only on the mid-section of the laminae.

Photosynthesis was induced at the growth $[CO_2]$. The reference $[CO_2]$ was reduced stepwise to a lower concentration of $50\text{ }\mu\text{mol mol}^{-1}$ and then increased stepwise to an upper limit of $1300\text{ }\mu\text{mol mol}^{-1}$. Each stepwise measurement was completed within 1–2 min to minimize alteration to the activation state of Rubisco. Real-time calculation of A and c_i followed the equations of von Caemmerer & Farquhar (1981).

The parameters describing the response of A to c_i , $V_{c,\text{max}}$ and J_{max} , were calculated by fitting the equations of Farquhar, von Caemmerer & Berry (1980) and by maximum likelihood regression (Sigmaplot, Jandel Scientific, Erkrath, Germany) following the method of Wullschlegel (1993). $V_{c,\text{max}}$ and J_{max} were calculated from different phases of the A/c_i response; $V_{c,\text{max}}$ was determined from points at low c_i visually judged to be below the inflexion of the A/c_i plot and J_{max} from values judged to be above the inflexion.

LEAF NITROGEN DETERMINATION

On completion of leaf gas-exchange measurement, the leaf section was cut and dried to constant mass at $80\text{ }^\circ\text{C}$. Each individual leaf sample was ground to a fine powder and total leaf nitrogen was determined by combustion and then thermal conductivity separation in an elemental analyser (PE 2400 Series II CHNS/O Analyser, Perkin Elmer, Norwalk, CT, USA), previously calibrated with acetanilide standards.

Results

PHOTOSYNTHETIC RESPONSE

Growth at $[CO_2]_{700}$ for *A. capillaris* resulted in a stimulation of A_{sat} regardless of nutrient supply (Figs 1 and 2, Table 1). This increase in carbon fixation at $[CO_2]_{700}$ was proportionally greater at 'low' (38%) than 'moderate' nutrient supply (12%) (Fig. 2, Table 1). Although A_{sat} was stimulated, there were significant reductions in $V_{c,\text{max}}$ of 27 and 25% at 'low' and 'moderate' nutrient supply, respectively (Fig. 2). Growth $[CO_2]$ did not significantly decrease J_{max} (Fig. 2, Table 1). Figure 1 indicates that the operating point of A_{sat} had moved towards RuBP regeneration-limited and away from Rubisco-limited photosynthesis under elevated $[CO_2]$. Nutrient supply positively affected photosynthesis, significant increases were detected in $V_{c,\text{max}}$ and A_{sat} at 'moderate' nutrient supply (Fig. 2, Table 1).

Growth of *L. perenne* at $[CO_2]_{700}$ led to marked photosynthetic acclimation at 'low' and 'moderate' nutrient supply. Reductions in $V_{c,\text{max}}$ of 39% at 'low' nutrient supply resulted in a lack of stimulation of photosynthesis at $[CO_2]_{700}$ when compared to plants grown and measured at $[CO_2]_{360}$ (Fig. 2, Table 1). Figure 1 suggests that the operating point had partially moved towards J_{max} -limited photosynthesis. However, the A/c_i response indicates that photosynthesis is still largely limited by $V_{c,\text{max}}$ at $[CO_2]_{700}$. No parallel significant decreases were detected in J_{max} at $[CO_2]_{700}$ at either 'low' or 'moderate' nutrient supply (Fig. 2, Table 1). A_{sat} , $V_{c,\text{max}}$ and J_{max} increased with increased nutrient supply.

Trifolium repens exhibited significant decreases in $V_{c,\text{max}}$ at $[CO_2]_{700}$ at both 'low' (26%) and 'moderate' (29%) nutrient supply (Fig. 2, Table 1). However, no parallel decreases were apparent in J_{max} . Despite acclimation mediated through $V_{c,\text{max}}$, A_{sat} had increased by 74 and 25% at 'low' and 'moderate' nutrient supply, respectively (Fig. 2). This long-term increase of A_{sat} had not declined from the initial stimulation at $[CO_2]_{700}$, i.e. the photosynthetic rate at $[CO_2]_{700}$ was the same for plants grown at $[CO_2]_{700}$ and $[CO_2]_{360}$. Photosynthesis at $[CO_2]_{700}$ was co-limited between $V_{c,\text{max}}$ and J_{max} , whereas at $[CO_2]_{360}$, $V_{c,\text{max}}$ seemed to be the sole limiting process (Fig. 1). Nutrient supply had no significant effect on J_{max} ; however, both $V_{c,\text{max}}$ and A_{sat} were increased at 'moderate' nutrient supply (Fig. 2, Table 1).

LEAF NITROGEN AND PHOTOSYNTHESIS

Leaf nitrogen content on an area basis was significantly lower with growth at $[CO_2]_{700}$ for *A. capillaris* and *L. perenne* (Table 2). *Agrostis capillaris* exhibited a 23 and 16% reduction of leaf nitrogen at $[CO_2]_{700}$ at 'low' and 'moderate' nutrient supply, respectively. For *L. perenne* a 29 and 17% decrease in leaf nitrogen at $[CO_2]_{700}$ was apparent at 'low' and 'moderate' nutrient

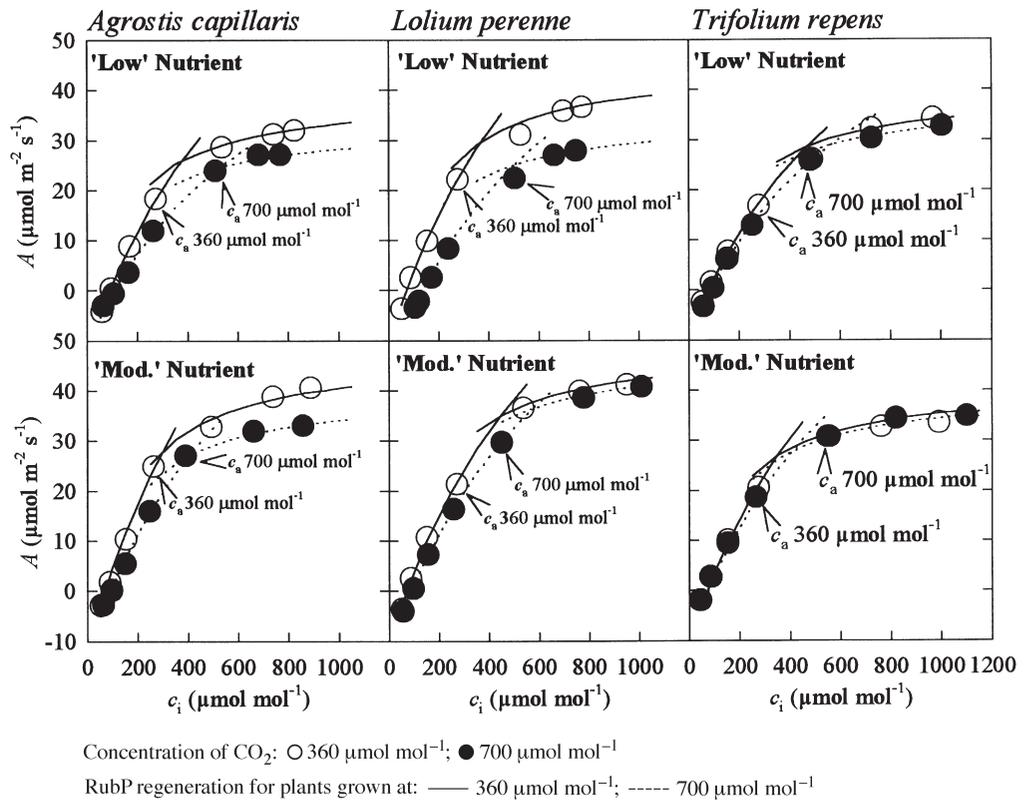


Fig. 1. Representative plots of A/c_i response curves for *A. capillaris*, *L. perenne* and *T. repens* grown under a factorial combination of two concentrations of CO₂ at 360 and 700 μmol mol⁻¹ and two nutrient treatments (low and moderate). The modelled values of the maximum RubP-saturated rate of carboxylation *in vivo* ($V_{c,max}$) and the maximum *in vivo* rate of electron transport contributing to RubP regeneration (J_{max}) for 360 and 700 μmol mol⁻¹ CO₂ grown plants are fitted for each plot. The arrows on each graph indicate the point at which A_{sat} (the light saturated rate of CO₂ uptake) operates at the growth [CO₂]. Mean values of A_{sat} , $V_{c,max}$ and J_{max} with full statistical analysis are shown in Fig. 2 and Table 1.

supply, respectively. *Trifolium repens* failed to exhibit any significant changes in leaf nitrogen with growth at [CO₂]₇₀₀.

Photosynthetic nitrogen-use efficiency ($PNUE$) was calculated by dividing A_{sat} at the growth [CO₂] by leaf nitrogen content. $PNUE$ significantly increased at [CO₂]₇₀₀ for all three species (Table 2). For example, a 62 and 27% increase in $PNUE$ was evident in *A. capillaris* at [CO₂]₇₀₀ at a 'low' and 'moderate' nutrient supply, respectively. Nutrient supply had no significant effect on $PNUE$. There were significant linear relationships between $V_{c,max}$, J_{max} and total leaf nitrogen content for *A. capillaris* and *L. perenne* (Fig. 3, Table 3). There were no apparent differences between the slopes of regressions for plants grown at [CO₂]₃₆₀ and [CO₂]₇₀₀. No linear relationships were detected for *T. repens* and therefore have been omitted.

Discussion

Previous studies have concluded that a non-limiting nutrient supply is a prerequisite for the stimulation of photosynthesis and growth at elevated [CO₂] (Tissue et al. 1993; Bowler & Press 1996; Körner et al. 1996; Nakano et al. 1997). With regard to leaf photosynthesis this is not necessarily the case. All species in this

study exhibited photosynthetic acclimation to elevated [CO₂]. However, despite large decreases in $V_{c,max}$ apparent in *A. capillaris* and *T. repens*, A_{sat} was still generally higher at [CO₂]₇₀₀. This reflects the fact that increasing [CO₂] moves control of photosynthesis away from limitation by Rubisco towards RubP-regeneration (Woodrow 1994). For both species, analysis of the A/c_i response (Fig. 1) indicates that $V_{c,max}$ was limiting at ambient but not at elevated [CO₂]. As the [CO₂] increased, *in vivo* Rubisco activity was reduced without necessarily reducing carbon fixation at [CO₂]₇₀₀. At elevated [CO₂] the stimulation of photosynthesis in *A. capillaris* was greater at 'low' rather than 'moderate' nutrient supply.

Elevated [CO₂] did not lead to an increase in the photosynthetic rate of *L. perenne* at 'low' nutrient supply. Initially, this suggests that photosynthesis had been limited by nutrient supply rather than [CO₂]. However, the same rate of CO₂ uptake was achieved with a significant reduction in leaf nitrogen content (Table 2). At [CO₂]₇₀₀ the requirement of photosynthesis for leaf nitrogen had been reduced. For *L. perenne* growing at [CO₂]₇₀₀ under a 'low' nutrient supply, the decreases in $V_{c,max}$ and J_{max} could allow a reallocation of 15 and 7% of the total leaf nitrogen content, respectively (Evans 1989; Hikosaka & Terashima 1995).

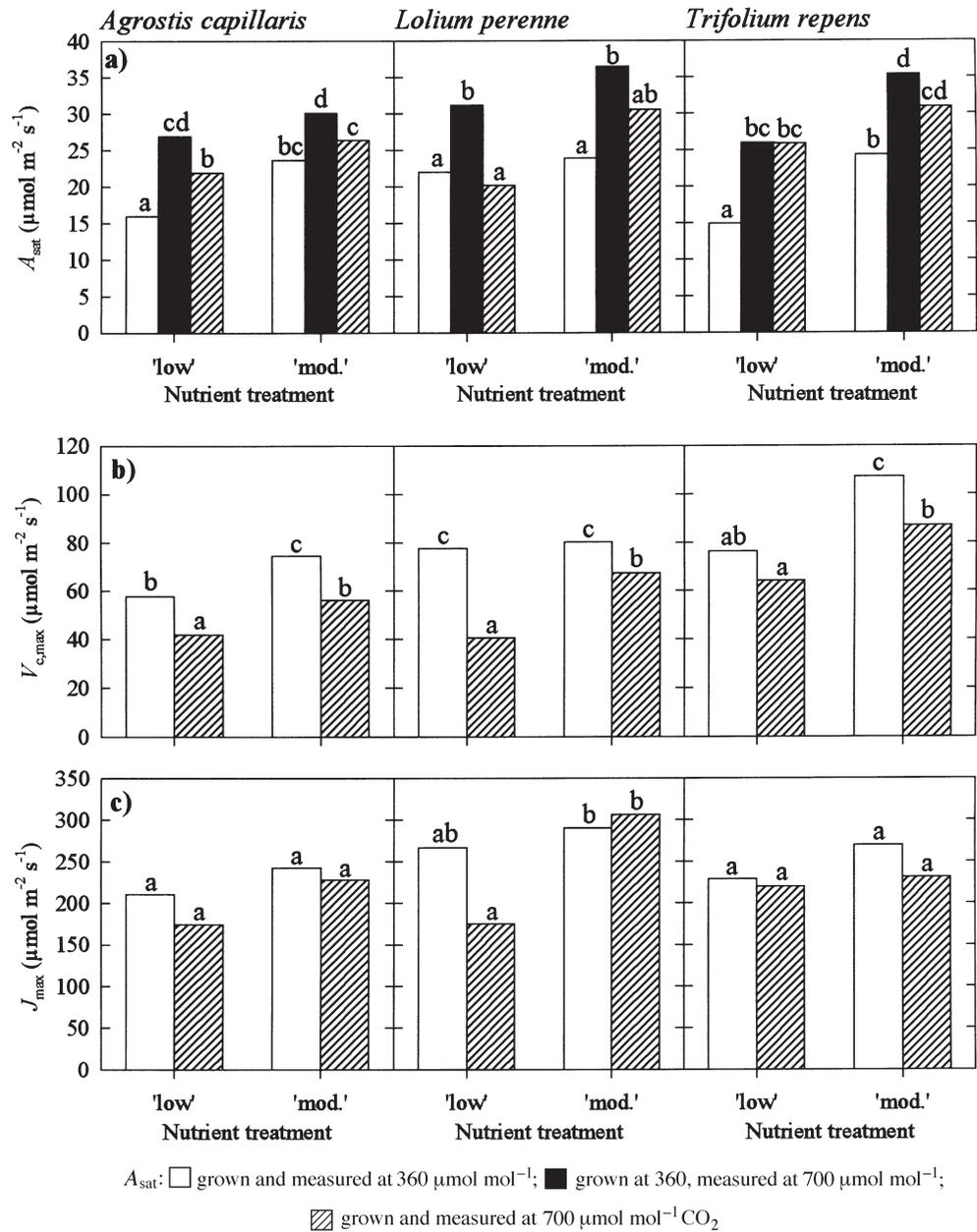


Fig. 2. The effects of elevated $[CO_2]$ and nutrient supply on A_{sat} , $V_{c,max}$ and J_{max} in *A. capillaris*, *L. perenne* and *T. repens*. Plants were grown under a factorial combination of two concentrations of CO_2 and two nutrient treatments. Mean values of: (a) the light saturated rate of CO_2 uptake (A_{sat}); (b) the maximum RubP-saturated rate of carboxylation *in vivo* ($V_{c,max}$); (c) the maximum *in vivo* rate of electron transport contributing to RubP regeneration (J_{max}). The response of A_{sat} was measured at 360 and $700 \mu\text{mol mol}^{-1}$ for plants grown at 360 and at $700 \mu\text{mol mol}^{-1}$ for plants grown at $700 \mu\text{mol mol}^{-1} CO_2$. This indicates both the immediate and long-term response of A_{sat} to elevated CO_2 . $V_{c,max}$ and J_{max} are shown for 360 and $700 \mu\text{mol mol}^{-1}$ grown plants. Results for a two-way analysis of variance ($[CO_2]$, nutrients) using five replicate chamber measurements are given in Table 1. Significant differences between means were located by Tukey tests. Means labelled with the same letter are not significantly different ($P < 0.05$).

This 'release' may allow more nitrogen to be available for allocation into other processes such as growth. Net primary production at elevated $[CO_2]$ for *L. perenne* showed an increase in growth of 35% at 'low' nutrient supply (Parsons *et al.* 1996). Although absolute growth rates were higher at 'moderate' nutrient supply, the relative increase in growth was threefold higher under 'low' nutrient conditions.

Leaf nitrogen was a good predictor of both $V_{c,max}$ and J_{max} in *A. capillaris* and *L. perenne* (Fig. 3, Table 3).

The significance of the linear relationship (Table 3) suggested that changes in leaf nitrogen content strictly paralleled nitrogen investment in Rubisco, enzymes of the Calvin cycle and thylakoid membrane components of electron transport as expected from the large amounts of nitrogen invested in the photosynthetic systems (Evans 1989; Hikosaka & Terashima 1995).

All three species exhibited significant increases in $PNUE$ with growth at $[CO_2]_{700}$. However, these increases in $PNUE$ were associated with three very

Table 1. Results of two-way ANOVA examining the effects of growth at elevated [CO₂] and nutrient treatment upon, the light saturated rate of CO₂ uptake (A_{sat}) at the growth [CO₂], the maximum RubP-saturated rate of carboxylation *in vivo* ($V_{\text{c,max}}$) and the maximum *in vivo* rate of electron transport contributing to RubP regeneration (J_{max}). Significant results ($P < 0.05$) are in bold type

Species	Parameter	[CO ₂] ($F_{1,16}, P$)	Nutrients ($F_{1,16}, P$)	[CO ₂] × nutrients ($F_{1,16}, P$)
<i>A. capillaris</i>	$A_{\text{sat}}[\text{Growth}]$	5.27, 0.036	10.2, 0.006	0.757, 0.397
	$V_{\text{c,max}}$	7.75, 0.013	6.49, 0.022	0.044, 0.837
	J_{max}	1.05, 0.320	3.03, 0.101	0.212, 0.652
<i>L. perenne</i>	$A_{\text{sat}}[\text{Growth}]$	2.47, 0.136	4.50, 0.050	2.44, 0.138
	$V_{\text{c,max}}$	19.1, < 0.001	10.0, 0.006	6.64, 0.020
	J_{max}	1.57, 0.229	6.42, 0.022	3.24, 0.095
<i>T. repens</i>	$A_{\text{sat}}[\text{Growth}]$	9.26, 0.008	4.54, 0.049	0.081, 0.780
	$V_{\text{c,max}}$	4.78, 0.044	13.2, 0.002	0.293, 0.596
	J_{max}	1.44, 0.248	1.72, 0.208	0.600, 0.450

different patterns of acclimatory response. In *A. capillaris*, the increase in *PNUE* resulted from a concurrent increase in A_{sat} and decrease in leaf nitrogen content. In comparison, the increased *PNUE* for *L. perenne* at [CO₂]₇₀₀ and 'low' nutrient supply was entirely owing to a lower leaf nitrogen content, as no increase in A_{sat} was apparent. The reverse pattern was exhibited by *T. repens*, for which an increase in *PNUE* was solely dependent on a higher A_{sat} with growth at [CO₂]₇₀₀. By simultaneously reducing leaf nitrogen content and increasing photosynthesis, it could be perceived that *A. capillaris* showed the most 'economical' response, indicative of an optimal physiological adjustment to growth at [CO₂]₇₀₀.

Increases in *PNUE* have been detected in other long-term field studies at elevated [CO₂]. After 8 years at elevated [CO₂], the sedge *Scirpus olneyi* exhibited an 18% reduction in foliar nitrogen, while photosynthesis remained stimulated (Drake *et al.* 1996). In a 4 year study of a native Australian grass, photosynthetic nitrogen-use efficiency increased at elevated [CO₂] despite low availability of soil nitrogen (Lutze 1996).

Trifolium repens failed to exhibit any correlation between $V_{\text{c,max}}$ and J_{max} with leaf nitrogen content. This root nodulated species had higher leaf nitrogen concentrations owing to nitrogen-fixing capabilities. This suggests that a high photosynthetic nitrogen-use

Table 2. The effects of elevated [CO₂] and nutrient supply upon (a) nitrogen content on a unit area basis and (b) *PNUE* measured at the growth [CO₂]. Results of a two-way ANOVA are indicated for each species and significant results are in bold type. Significant differences between means were located by Tukey tests. Means labelled with the same letter are not significantly different ($P < 0.05$)

(a)	Total leaf nitrogen (mmol m ⁻²)				Statistical analysis		
	'Low' nutrients		'Moderate' nutrients		[CO ₂] ($F_{1,16}, P$)	Nutrient supply ($F_{1,16}, P$)	[CO ₂] × N ($F_{1,16}, P$)
	360	700	360	700			
<i>A. capillaris</i>	96.6 b	74.4 a	118 c	98.8 b	4.51, 0.050	5.52, 0.032	0.021, 0.871
<i>L. perenne</i>	107 b	76.0 a	126 b	104 b	4.74, 0.045	3.09, 0.098	0.075, 0.0788
<i>T. repens</i>	133 a	136 a	128 a	121 a	0.056, 0.815	1.53, 0.233	0.376, 0.549

(b)	<i>PNUE</i> (mmol CO ₂ mol N ⁻¹ s ⁻¹)				Statistical analysis		
	'Low' nutrients		'Moderate' nutrients		[CO ₂] ($F_{1,16}, P$)	Nutrient supply ($F_{1,16}, P$)	[CO ₂] × N ($F_{1,16}, P$)
	360	700	360	700			
<i>A. capillaris</i>	0.183 a	0.296 b	0.212 a	0.269 b	23.2, < 0.001	0.003, 0.958	2.57, 0.129
<i>L. perenne</i>	0.194 a	0.280 b	0.193 a	0.297 b	21.7, < 0.001	1.69, 0.687	0.199, 0.661
<i>T. repens</i>	0.126 a	0.366 b	0.185 a	0.307 b	17.8, 0.001	< 0.001 , 0.995	1.89, 0.188

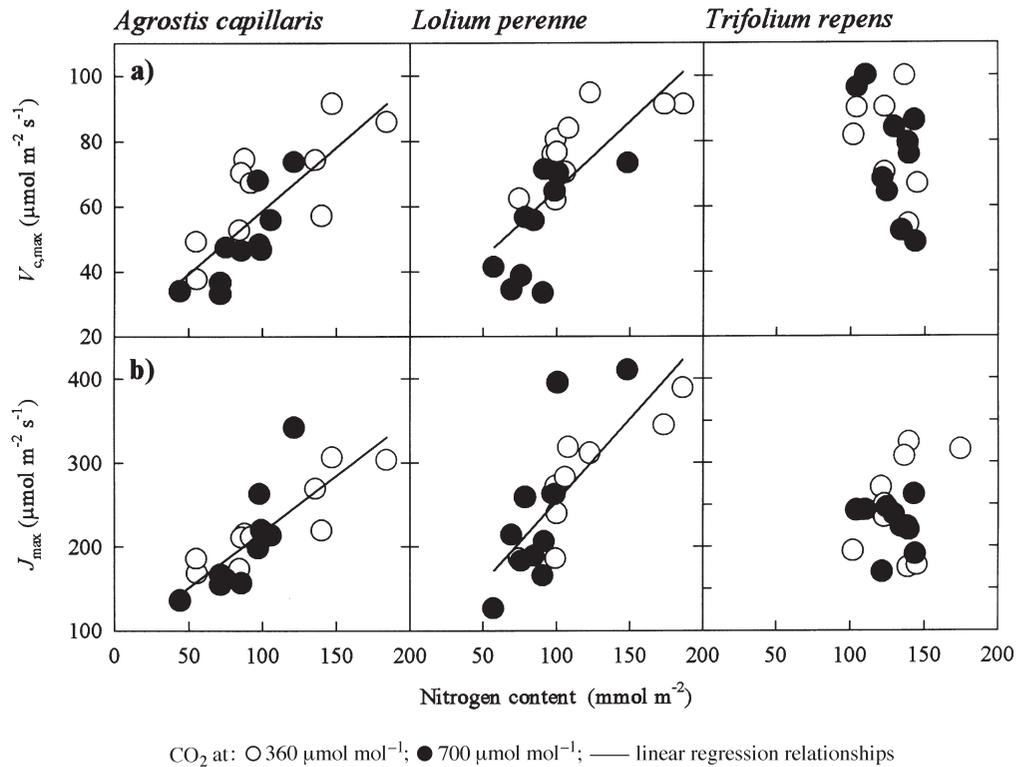


Fig. 3. Relationships between $V_{c,max}$, J_{max} and total leaf nitrogen content. Plants were grown under a factorial combination of two concentrations of CO_2 360 and 700 $\mu\text{mol mol}^{-1}$ and two nutrient treatments. Each point represents an individual leaf; (a) the maximum RubP-saturated rate of carboxylation *in vivo* ($V_{c,max}$); (b) the maximum *in vivo* rate of electron transport contributing to RubP regeneration (J_{max}). Both nutrient treatments were pooled. Linear regression relationships were fitted by the least squares method for $V_{c,max}$ and J_{max} against leaf nitrogen separately for 360 and 700 $\mu\text{mol mol}^{-1}$ CO_2 grown plants (Table 3). There were no significant differences between the slopes of the regressions for 360 and 700 $\mu\text{mol mol}^{-1}$ CO_2 grown plants (Table 3) therefore both $[CO_2]$ treatments in this figure have been pooled. Regressions analysed were non-significant for *T. repens* and have therefore been omitted.

efficiency may be less critical to growth than for the two grass species, although *T. repens* did exhibit higher photosynthetic rates at $[CO_2]_{700}$. It is possible in *T. repens*, that the photosynthetic acclimation at $[CO_2]_{700}$ was owing to phosphate supply (Barret & Gifford 1995). However, the degree of photosynthetic

acclimation was not reduced by the applied phosphate in the 'moderate' nutrient treatment.

The data support the hypothesis that growth at elevated $[CO_2]$ can increase *PNUE*. Lower leaf nitrogen contents sustain significantly higher rates of carbon uptake. This was apparent at both 'moderate' and 'low'

Table 3. Regression analysis of the relationship between $V_{c,max}$ and J_{max} with total leaf nitrogen content for *A. capillaris* and *L. perenne*. The maximum RubP-saturated rate of carboxylation *in vivo* ($V_{c,max}$) and the maximum *in vivo* rate of electron transport contributing to RuBP regeneration (J_{max}) were plotted as a function of leaf nitrogen content. Least squares linear regressions were fitted to each plot separately for $[CO_2]_{360}$ and $[CO_2]_{700}$ grown plants. The differences between the slopes ($\text{mmol } CO_2 \text{ mol } N^{-1} \text{ s}^{-1}$) for $[CO_2]_{360}$ and $[CO_2]_{700}$ grown plants were tested using the method of Fowler & Cohen (1990). Significant results ($P < 0.05$) are in bold type

Species	A/c_i parameter	Growth $[CO_2]$ ($\mu\text{mol mol}^{-1}$)	Significance of regression line T_8, P	Coefficient of determination (r^2)	Slope ($\text{mmol } CO_2 \text{ mol } N^{-1} \text{ s}^{-1}$)	Difference between slopes (b) T_{16}, P
<i>Agrostis capillaris</i>	$V_{c,max}$	360	3.23, 0.012	0.566	= 0.297	1.40, > 0.100
		700	4.24, 0.003	0.691	= 0.513	
	J_{max}	360	5.64, < 0.001	0.800	= 1.05	1.45, > 0.100
		700	4.54, 0.002	0.721	= 2.41	
<i>Lolium perenne</i>	$V_{c,max}$	360	3.10, 0.015	0.546	= 0.242	1.06, > 0.100
		700	2.65, 0.030	0.465	= 0.437	
	J_{max}	360	4.70, 0.002	0.734	= 1.57	1.73, > 0.100
		700	3.82, 0.005	0.648	= 3.07	

nutrient supply. This study suggests that elevated [CO₂] will stimulate photosynthesis under a 'low' nutrient supply. Differential increases in photosynthetic nitrogen-use efficiency may be a critical determinant of competition within low-nutrient ecosystems and low-input agricultural systems. Furthermore, rather than limiting the photosynthetic response to elevated [CO₂], limiting nutrient conditions could lead to the most significant stimulation in carbon acquisition.

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