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Is stimulation of leaf photosynthesis by elevated carbon dioxide concentration maintained in the long term? A test with *Lolium perenne* grown for 10 years at two nitrogen fertilization levels under *F*ree *A*ir *C*O$_2$ *E*nrichment (FACE)

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ABSTRACT

Photosynthesis is commonly stimulated in grasslands with experimental increases in atmospheric CO$_2$ concentration ([CO$_2$]), a physiological response that could significantly alter the future carbon cycle if it persists in the long term. Yet an acclimation of photosynthetic capacity suggested by theoretical models and short-term experiments could completely remove this effect of CO$_2$. Perennial ryegrass (*Lolium perenne* L. cv. Bastion) was grown under an elevated [CO$_2$] of 600 μmol mol^{-1} for 10 years using *F*ree *A*ir *C*O$_2$ *E*nrichment (*FACE*), with two contrasting nitrogen levels and abrupt changes in the source : sink ratio following periodic harvests. More than 3000 measurements characterized the response of leaf photosynthesis and stomatal conductance to elevated [CO$_2$] across each growing season for the duration of the experiment. Over the 10 years as a whole, growth at elevated [CO$_2$] resulted in a 43% higher rate of light-saturated leaf photosynthesis and a 36% increase in daily integral of leaf CO$_2$ uptake. Photosynthetic stimulation was maintained despite a 30% decrease in stomatal conductance and significant decreases in both the apparent, maximum carboxylation velocity ($V_{c,max}$) and the maximum rate of electron transport (J_{max}). Immediately prior to the periodic (every 4–8 weeks) cuts of the *L. perenne* stands, $V_{c,max}$ and J_{max}, were significantly lower in elevated than in ambient [CO$_2$] in the low-nitrogen treatment. This difference was smaller after the cut, suggesting a dependence upon the balance between the sources and sinks for carbon. In contrast with theoretical expectations and the results of shorter duration experiments, the present results provide no significant change in photosynthetic stimulation across a 10-year period, nor greater acclimation in $V_{c,max}$ and J_{max} in the later years in either nitrogen treatment.

Key-words: atmospheric change; Free Air Carbon dioxide Enrichment; global change; meta-analysis; pasture grass; photosynthesis; Rubisco.

Abbreviations: A, net leaf CO$_2$ uptake rate (μmol m^{-2} s^{-1}); A', daily integral of CO$_2$ fixation (mmol C m^{-2} d^{-1}); A_{sat}, light-saturated value of A (μmol m^{-2} s^{-1}); DAC, days after cut; *FACE*, free-air CO$_2$ enrichment; g_s, stomatal conductance to H$_2$O vapour (mmol m^{-2} s^{-1}); J_{max}, light-saturated rate of electron transport contributing to ribulose 1,5 bisphosphate regeneration (μmol m^{-2} s^{-1}); r, response ratio (rate in elevated [CO$_2$]/rate in ambient [CO$_2$]); Rubisco, ribulose 1,5 bisphosphate carboxylase-oxygenase; RuBP, ribulose 1,5 bisphosphate; $V_{c,max}$, maximum *in vivo* rate of ribulose 1,5 bisphosphate-saturated carboxylation (μmol m^{-2} s^{-1}).

INTRODUCTION

Grasslands are an important component of the global C budget, storing approximately 10% of global C stocks, and covering approximately 20% of the world's land area (FAO 1995; Parton *et al.* 1995). Increased knowledge of how these ecosystems will respond to a predicted doubling of atmospheric [CO$_2$] is critical to improving our understanding of the future effects of global change on the carbon cycle (Houghton *et al.* 2001).

In order to investigate how a managed grassland would respond to a step increase in [CO$_2$], a *F*ree *A*ir *C*arbon dioxide *E*nrichment (*FACE*) experiment was established over perennial ryegrass (*Lolium perenne* L. cv. Bastion) in Eschikon, Switzerland in 1993 (Swiss *FACE*). *Lolium*

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perenne is an important and often dominant component of temperate, managed grasslands and understanding its response to the effects of elevated [CO_2] is of global economic and environmental concern. Unlike other fumigation methodologies, *FACE* technology allows plants to be grown in elevated [CO_2] without altering the microclimate (Hendrey *et al*. 1999; McLeod & Long 1999). *Lolium. perenne*, like all pasture species, is adapted to defoliation, fertilization, and regrowth cycles that quickly alter carbon source and sink capacities. In the Swiss *FACE* experiment, *L. perenne* was managed as a frequently cut pasture crop at both low- and high-N supply (Hebeisen, Lüscher & Nösberger 1997a).

The mechanism underlying the stimulation of C_3 photosynthesis in elevated [CO_2] is understood (Long & Drake 1992), and an increase in photosynthetic CO_2 uptake rate (A) in elevated [CO_2] is well-documented (Bowes 1993; Drake, González Meier & Long 1997; Wand *et al*. 1999). Changes in the response of photosynthesis to elevated [CO_2] will impact 'down-stream' processes and to a large extent modulate responses at the whole plant and stand level. For this reason, measurements of A in studies examining the response of plants to growth in elevated [CO_2] are routinely made. However, in many studies, only a 'snapshot' view of A at a given point in time is reported and uncertainty over the long-term response of A remains.

The Swiss *FACE* experiment has provided a unique opportunity to examine the long-term response of photosynthesis in a managed pasture to elevated [CO_2]. Nitrogen treatments and different management practices provided a further opportunity to study the combined effects of elevated [CO_2] and alterations in C and N source : sink relations. The loss of photosynthetic capacity in elevated [CO_2], largely attributable to a loss of Rubisco (Rogers & Humphries 2000), is observed in the field (Osborne *et al*. 1998; Rogers *et al*. 1998; Davey *et al*. 1999; Hymus *et al*. 2002; Rogers & Ellsworth 2002), and is more readily observed when plants are grown with a low N supply or in conditions where growth may become sink-limited (Farrar & Williams 1991; Rogers *et al*. 1998). Woodrow (1994) demonstrated that because photosynthetic efficiency is enhanced in elevated [CO_2], plants could attain the same levels of A as plants grown in current [CO_2] with less Rubisco, suggesting that acclimation may not preclude stimulation in A. The prevailing view is that perennial systems will respond to elevated [CO_2] in the short term, but that the response for grasslands will be short-lived (Roumet *et al*. 2000); Luo & Reynolds (1999) suggested a period of 9.6 years for high- and low-productivity grasslands. We examined these theories in the Swiss *FACE* experiment and tested the following predictions: (1) stimulation of photosynthesis is maintained throughout the duration of the experiment; (2) acclimation of photosynthesis, observed as reduced carboxylation capacity, does not negate a stimulation of photosynthetic C uptake in elevated [CO_2]; (3) acclimation of photosynthesis will be more pronounced under N-limiting conditions and under conditions where the source : sink ratio is high; and (4) increasing the relative sink strength of the swards by periodic harvests will alleviate photosynthetic acclimation.

MATERIALS AND METHODS

Experimental site and plant growth conditions

The Swiss *FACE* site was located at Eschikon, Switzerland (8°41′ E, 47°27′ N), 550 m above sea level. Three blocks, each consisting of two, 18-m-diameter, circular rings, one fumigated to 600 μmol mol^{-1} [CO_2] and one control (non-fumigated 360 μmol mol^{-1}), were established in 1993. The fumigated and control areas were at least 100 m apart to prevent contamination in control areas. The CO_2 enrichment lasted for the entire growing period, from March to November Fumigation began when mean air temperature reached a threshold of 5 °C, and ended when air temperatures were below that threshold. The pasture is dormant during the period of the year when temperatures are below 5 °C and often is covered by snow. The site and experiment have been described in detail previously (Zanetti *et al*. 1996; Hebeisen *et al*. 1997b).

Lolium perenne L. cv. Bastion was sown in monocultures (2.8 m × 1.9 m plots with 3.2 g seed m^{-2}) in August 1992. From 1993 to 1995, swards that were formed from the sowing were either cut frequently (six times per year in 1993, eight times in 1994, 1995) or infrequently (four times). Because the latter swards deteriorated, the infrequently cut plots were re-seeded in 1996. All plots were cut five times per year from 1996 to 2002. Swards were always cut to a height of 5 cm, simulating the levels used in harvesting of a grass crop. Within each CO_2 treatment, *L. perenne* monocultures were grown under both low and high N fertilization treatments, applied as NH_4NO_3. The low N treatment was 10 g m^{-2} year^{-1} in 1993 and 14 g m^{-2} year^{-1} from 1994 to 2002, whereas the high N treatment was 42 g m^{-2} year^{-1} in 1993, and 56 g m^{-2} year^{-1} thereafter. The procedure for N fertilization application has been described in detail in Zanetti *et al*. (1996) and Daepp *et al*. (2000).

Photosynthetic gas exchange

Measurements of leaf gas exchange were made *in situ* every 2 to 3 h through the diurnal period (from dawn until after dusk), using an open gas-exchange system incorporating a CO_2/H_2O vapour IR gas analyser (version 1.4, CIRAS 1: PP Systems, Hitchin, UK; version LCA 4: ADC Ltd, Hoddesdon UK; or Li-6400: LiCor Inc., Lincoln, NE, USA). Measurements of A were taken at growth [CO_2] (360 μmol mol^{-1} for control and 600 μmol mol^{-1} for *FACE*) on fully expanded leaves at the top of the canopy. Leaves were oriented approximately horizontally so the leaf cuvette was maintained in a horizontal position. The cuvette was clamped onto the leaf, 5 cm from the axis. These precautions minimized the effect of leaf angle on light (Q) and developmental variation between leaves. All measurements were taken at incident Q, temperature, and vapour pressure deficit. Table 1 specifies the environmental

Table 1. The dates of diurnal photosynthesis measurements and the meteorological data, daily mean temperature, total daily global radiation, and precipitation describing those dates are listed. The cutting regime (number of harvests per season) of the measured plot, the number of days following the preceding cut, and the percentage change in A' are described

Date	Temperature (°C)	Global radiation (MJ m^{-2})	Precipitation (mm)	Cutting regime	Days after cut	% change in A' High N	% change in A' Low N
2 August 1993	20.5	24.50	0	6	19	30.7	38.4
23 June 1994	19.9	27.06	0	8	10	36.5	37.5
25 June 1994	21.6	24.91	0.2	4	40	−0.3	−12.3
22 July 1994	20.8	25.79	0	4	10	50.0	34.2
28 July 1994	21.8	21.36	15.0	8	16	66.9	100.4
24 May 1995	19.4	21.46	0	8	9	36.0	42.9
20 June 1995	20.8	28.00	0	8	8	27.5	41.6
9 July 1995	22.6	26.54	0	8	27	47.4	49.9
18 October 1995	13.0	8.59	0.5	8	16	54.0	50.7
2 July 1996	12.5	9.74	0	5	8	49.2	46.1
14 July 1996	20.6	26.13	0	5	20	60.5	52.0
25 June 1997	12.7	18.85	0	5	11	30.5	34.4
2 July 1997	17.2	22.84	1.8	5	16	49.2	46.1
9 July 1997	17.7	28.74	0	5	23	42.3	28.2
12 July 1997	18.6	23.06	0	5	26	39.9	33.4
16 July 1997	18.5	28.25	0	5	30	52.8	39.1
30 July 1997	19.9	26.39	0	5	9	40.0	41.4
10 August 1997	20.1	26.37	0	5	20	60.5	52.0
19 August 1997	18.6	20.47	0	5	29	50.2	37.2
23 September 2000	11.8	16.35	0	5	26	43.2	27.6
28 April 2001	11.6	18.01	3.5	5	B*	16.8	4.2
24 May 2001	19.4	21.46	0	5	13	27.9	33.0
9 May 2002	15.9	19.05	0	5	B	25.0	30.5
20 May 2002	14.3	29.59	0	5	7	19.2	39.2

*B: Diurnal response was measured before the first cut of the season.

conditions of the 25 d when diurnal photosynthesis measurements were taken and Fig. 1 shows typical diurnal response curves.

The response of A to changes in intercellular CO_2 concentration (c_i) was measured under saturating or near-saturating light (750–1250 μmol m^{-2} s^{-1}) on 78 different occasions covering the duration of the experiment (Fig. 2). Measurements were taken on attached leaves in the field before 1300 h and/or on overcast days (see Rogers *et al.* 1998), or in the laboratory on leaves cut, under water, before dawn on the day that they were measured. This ensured that the A/c_i responses reflected the potential photosynthesis on the day of measurement, and were not affected by transient decreases that may result during the day due to photo-inhibition, water stress, or feedback inhibition due to carbohydrate accumulation and cytosolic P_i limitation. The maximum RuBP-saturated rate of carboxylation *in vivo* ($V_{c,max}$) and the light-saturated potential rate of electron transport (J_{max}) were calculated by fitting the equations of Farquhar, von Caemmerer & Berry (1980), following the procedure of Wullschleger (1993). Rates of CO_2 uptake at the growth CO_2 concentration equalled or exceeded the peak rates recorded at that temperature *in situ*, suggesting the procedures did not cause any loss of photosynthetic capacity. In measurements where temperature varied significantly, estimates of $V_{c,max}$ and J_{max} were corrected to 25 °C, following the equations of McMurtrie & Wang (1993).

Statistical analysis

Gas exchange technologies, management practices, investigators, and even the swards changed over the 10 years of the experiment. Therefore we considered each investigator's work to be independent, and suited to meta-analytic approach. Meta-analytic techniques provided a means to deal with heterogeneous sampling and have already been adapted for elevated [CO_2] research (Curtis & Wang 1998; Medlyn *et al.* 1999, 2001; Kerstiens 2001; Ainsworth *et al.* 2002). The effect of growth at elevated [CO_2] on the daily integral of CO_2 fixation (A'); stomatal conductance (g_s) measured at midday in the field under incident light, temperature, and vapour pressure deficit conditions; light-saturated net CO_2 assimilation rate (A_{sat}) extrapolated from A/c_i response curves; apparent *in vivo* Rubisco activity ($V_{c,max}$); the light-saturated potential rate of electron transport (J_{max}); and the ratio of $V_{c,max}/J_{max}$ was reviewed using meta-analytic procedures. Together, the independent measurements comprising this meta-analysis form the most comprehensive, contiguous data set of photosynthetic responses for any species exposed to long-term CO_2 enrichment.

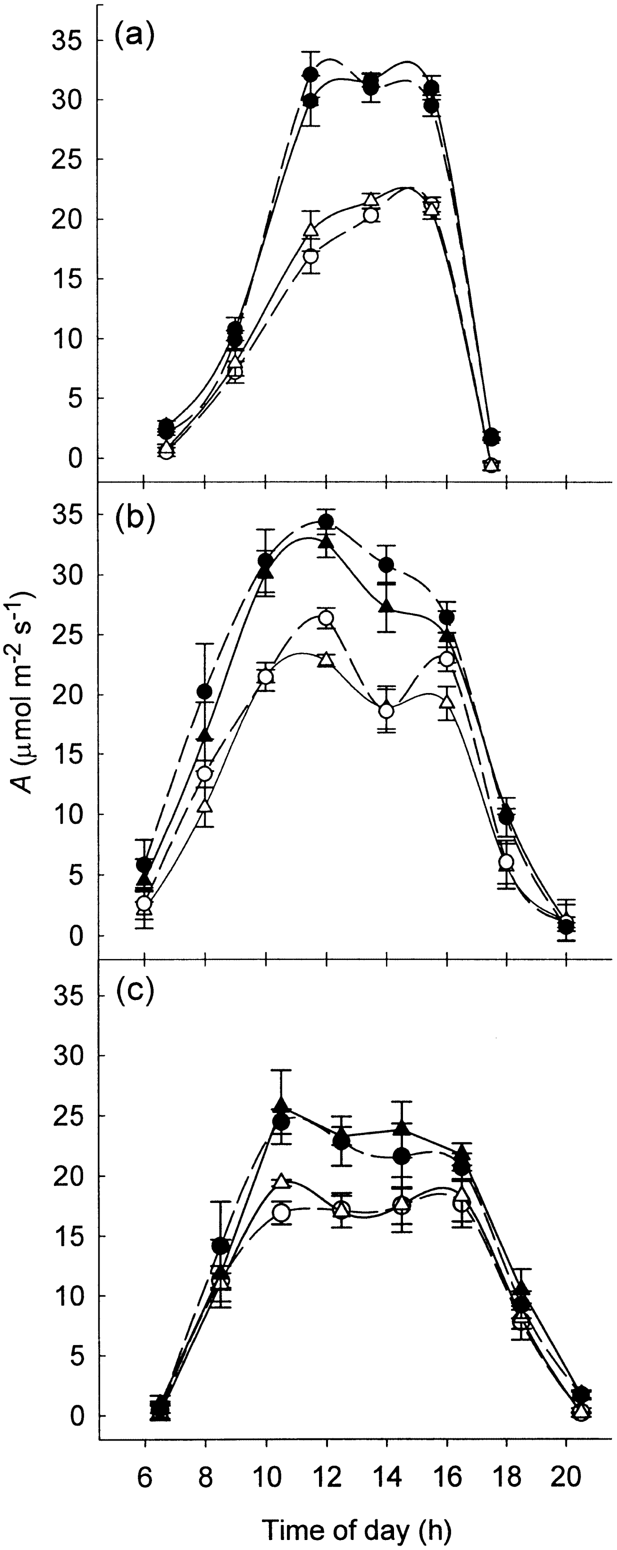

Figure 1. Diurnal time course of the rate of leaf CO_2 uptake (A) in *Lolium perenne* grown at 360 (○, △) and 600 mmol mol^{-1} (●, ▲) with two N fertilization treatments of 14 (△, ▲) and 56 (○, ●) g N m^{-2} $year^{-1}$. Plants were measured on (a) 18 October 1995; (b) 30 July 1997; and (c) 9 May 2002. Meteorological data describing the dates of measurement is given in Table 1.

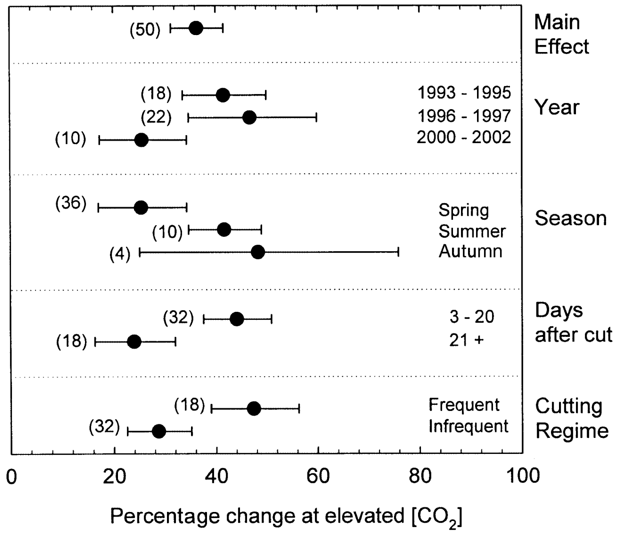

Figure 2. The percentage change of daily integrated carbon assimilation with growth at elevated [CO_2]. Sample sizes (n) for each categorical treatment are given in parenthesis. The mean effect sizes (r) surrounded by 95% confidence intervals are shown.

The natural log of the response ratio [$\ln(r) = \ln(\overline{X}_e) - \ln(\overline{X}_a)$]) was used as the metric in all of our analyses, where r is the mean value in elevated [CO_2]/mean value in ambient [CO_2] (Hedges, Gurevitch & Curtis 1999; Rosenberg, Adams & Gurevitch 2000). The meta-analysis procedure followed the techniques of Curtis & Wang (1998), using the statistical software, MetaWin (Rosenberg *et al.* 2000). A mixed-model analysis was used, based on the assumption of random variation in effect sizes between individual measurements. A weighted parametric analysis was used (Gurevitch & Hedges 1999), and each individual observation or response was weighted by the reciprocal of the mixed-model variance, which is the sum of the natural log of the response ratio and the pooled within-class variance (Hedges *et al.* 1999). A response to elevated [CO_2] was considered significant if a 95% confidence interval for the natural log of a given response variable did not overlap with zero, where zero represents no change in elevated [CO_2].

The mean parameter values for both ambient and elevated [CO_2] treatments and standard deviations around those values were extracted from the original data files stored for each year of the experiment. This information was required in order to weight the analysis according to the sampling levels (see Curtis & Wang 1998). To address our four predictions, we examined the effect of N fertilization level, cutting regime, the number of days after cut (DAC), and timing with respect to month, season, and year. Two levels of N fertilization, two cutting regimes (frequent and infrequent), two discrete levels of DAC (1–20 d and 21 or more days), three climatological seasons according to mean temperature (March to May, Spring; June to August, Summer; September to November, Autumn), and three categories dividing years of the experiment (early, 1993–95; middle, 1996–97; late, 2000–02) were included in the analysis. Years were divided, as indicated, based on changes in the N status of the plots (Daepp *et al.* 2000). Input to the high N plots overcame an initial limitation of N on the yield response after 1995 (Daepp *et al.* 2000). The late years of the experiment were divided into a separate category because no photosynthesis measurements were taken in 1998 and 1999.

RESULTS

Photosynthesis *in situ*

In situ measurements of the diurnal course of CO$_2$ uptake (A) were made on 25 d over the 10 years of the Swiss FACE experiment (Table 1, Fig. 1). Growth at elevated [CO$_2$] resulted in a 36% stimulation in A', from an average 644 mmol m^{-2} d^{-1} in ambient [CO$_2$] to 893 mmol m^{-2} d^{-1} in elevated [CO$_2$]. Coincidentally, this average stimulation is almost identical to the 38% increase seen on the first day of measurements in August 1993 and the 39% stimulation on the last day of measurements in May 2002 (Table 1). There was significant variation in the percentage stimulation of A' at elevated [CO$_2$] during different years and seasons (Table 2, Fig. 2). From 1993 to 1997, the percentage stimulation of A' was over 40%; by 2000–02, the percentage stimulation was 25%. However, all but two of the diurnal measurements in 2000–02 were in the spring. Over the experiment as a whole, the stimulation of A' with growth in elevated [CO$_2$] was just 25% in the spring, which is significantly less than the 41% stimulation in the summer and 48% stimulation in the autumn (Fig. 2).

Partial defoliation abruptly decreased the source : sink ratio of the plants and significantly increased the stimulation of A' in elevated [CO$_2$] (Table 1, Fig. 2). In the first 20 d following a cut, stimulation of A' with growth at elevated [CO$_2$] was 44%. However, with continued regrowth, stimulation in A' diminished. After 21+ days following a cut, the percentage stimulation in A' was just 23%. Cutting frequency also significantly affected the percentage change in A' with growth at elevated [CO$_2$]. Plants that were frequently harvested had a 47% increase in A', but plants that were infrequently cut had only a 29% increase (Fig. 2). However, when the infrequently cut plants were measured within the first 20 d following a cut, the stimulation of A' was 38%.

Table 2. Between-group heterogeneity (Q_b) for CO$_2$ effect size across categorical variables for daily integrated CO$_2$ uptake (A'), stomatal conductance (g_s), light-saturated CO$_2$ uptake rate (A_{sat}), maximum velocity of Rubisco carboxylation ($V_{c,max}$), maximum rate of electron transport (J_{max}), and $V_{c,max}/J_{max}$. Each response variable was represented by k independent evaluations

Variable	k	Nitrogen	Cutting regime	Days after cut	Month	Year
A'	50	1.00	13.66**	15.99**	12.38*	12.65*
g_s	39	0.83	5.40	2.16	6.24	6.71*
A_{sat}	43	0.69	3.38	50.70**	0.83	11.32*
$V_{c,max}$	86	8.77*	2.89	8.16*	5.54	7.32
J_{max}	66	1.08	2.54	1.96	1.16	1.89
$V_{c,max}/J_{max}$	59	2.20	0.03	2.23	0.70	3.22

*P <0.05, **P <0.01.

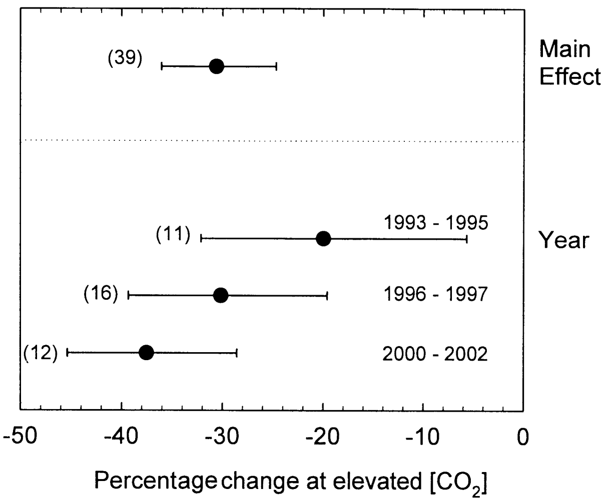

Figure 3. The percentage change in stomatal conductance (g_s) with growth at elevated [CO$_2$]. Sample sizes (n) for each categorical treatment are given in parenthesis. The mean effect sizes (r) surrounded by 95% confidence intervals are shown.

Daily carbon assimilation was significantly increased in *L. perenne* grown at elevated [CO$_2$] despite a significant 31% decrease in g_s (Fig. 3). Average g_s was 621 mmol m^{-2} s^{-1} in ambient [CO$_2$] and 442 mmol m^{-2} s^{-1} in elevated [CO$_2$] when averaged over all measurement periods. The percentage change in g_s was significantly affected by year of measurement (Table 2, Fig. 3). During the first 3 years of the experiment, g_s was reduced by 20%, with a further reduction to 30% from 1996 to 1997, and to nearly 40% in 2000–02.

A/*c*$_i$ response

The response of A to changing c_i was measured on over 900 leaves over the duration of the experiment in order to define the light-saturated rate of A at growth [CO$_2$] (A_{sat}), $V_{c,max}$ and J_{max} (Fig. 4). Growth at elevated [CO$_2$] resulted in an average 43% stimulation of A_{sat} (Fig. 5) from a mean of 17.5 μmol m^{-2} s^{-1} in ambient [CO$_2$] to 24.4 μmol m^{-2} s^{-1} in elevated [CO$_2$]. The percentage change in A_{sat} was significantly altered during different years of the experiment (Table 2, Fig. 5). During the first 3 years (1993–95), stimulation of A_{sat} was just 30%, but from 1996 to 1997, stimulation of A_{sat} in elevated [CO$_2$] was over 50%. This contrasts with the results previously described for A', where stimulation was greatest in the early years of the experiment. The difference in percentage change at elevated [CO$_2$] between the years arises from differences in measured values of A_{sat} in ambient [CO$_2$]. From 1993 to 1995, the mean value of A_{sat} for control plots was 18.8 μmol m^{-2} s^{-1}; however, from 1996 to 1997, the mean A_{sat} for control plots was 15.6 μmol m^{-2} s^{-1}. During both measurement periods, the

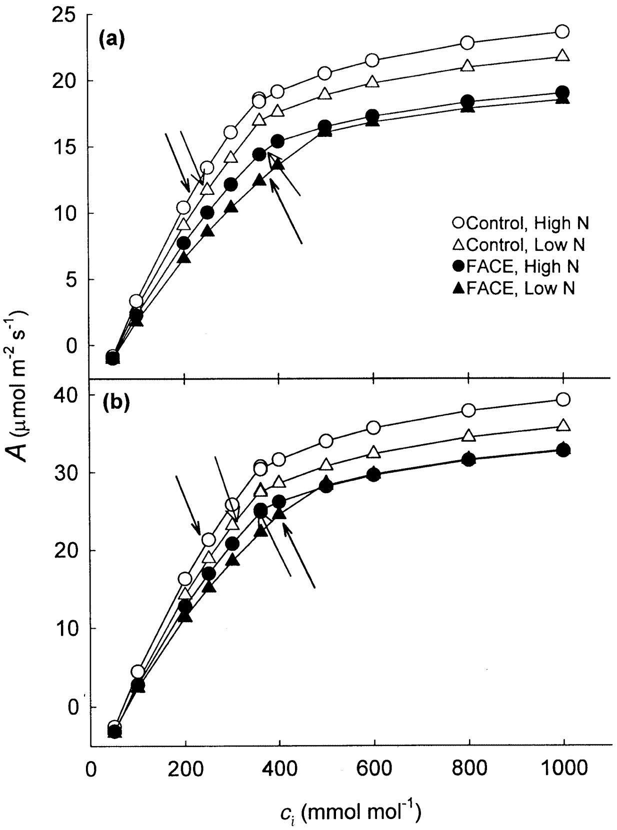

Figure 4. The response of light-saturated carbon assimilation (A) to changes in intracellular carbon dioxide concentration (c_i) for two of the 78 measurement dates. Arrows represent the operating point of photosynthesis. Plants were measured on (a) 9 August 1993 and (b) 28 April 2001.

mean A_{sat} for FACE plots was 24.0 μmol m^{-2} s^{-1}. Thus, the variation in A_{sat} between years does not appear to be caused by any physiological or biochemical changes in grasses grown at elevated [CO$_2$].

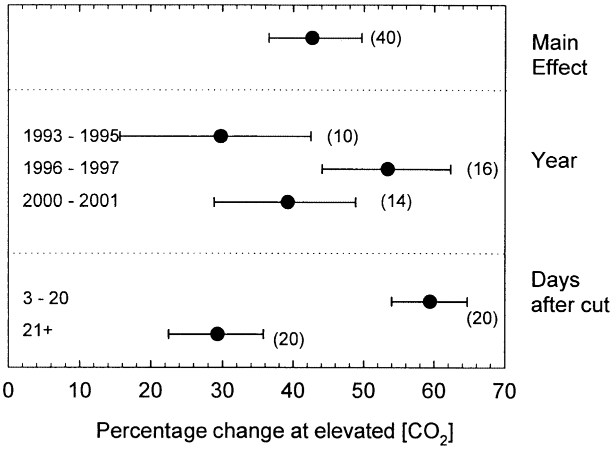

Figure 5. The percentage change of light-saturated CO$_2$ uptake (A_{sat}) with growth at elevated [CO$_2$]. Sample sizes (n) for analyses are shown in parenthesis. The mean effect (r) surrounded by 95% confidence interval is shown.

Cutting the swards had a large effect on the percentage response of A_{sat} in elevated [CO$_2$] (Fig. 5). Initially following a cut (3–20 d after cut), A_{sat} was stimulated by 59%, but 21+ days following a cut, stimulation was reduced to just 29%. This highly significant effect of partial defoliation on A_{sat} (Table 2) follows the same trend described for A', where the initial large stimulation was reduced coincident with leaf area development and increased photosynthate supply in ageing swards.

The A/c_i response curve analysis revealed that photosynthesis was largely limited by $V_{c,max}$ in both ambient and elevated [CO$_2$] (Fig. 4). Growth at elevated [CO$_2$] resulted in an average 18% decrease in $V_{c,max}$; however, N fertilization level significantly affected the percentage response (Table 2, Fig. 6a). Under high N conditions, $V_{c,max}$ was reduced by 12% and under low N conditions, $V_{c,max}$ was reduced by 23% (Fig. 6a). Similarly, days after cut significantly affected the response (Table 2). In the first 20 d after a cut, $V_{c,max}$ was reduced by 14%; thereafter, the percentage reduction increased to 25% (Fig. 6a). There was a significant interaction between N fertilization and days after cut. Under high N conditions in the first 20 d following a cut, no significant reduction in $V_{c,max}$ was detected; however,

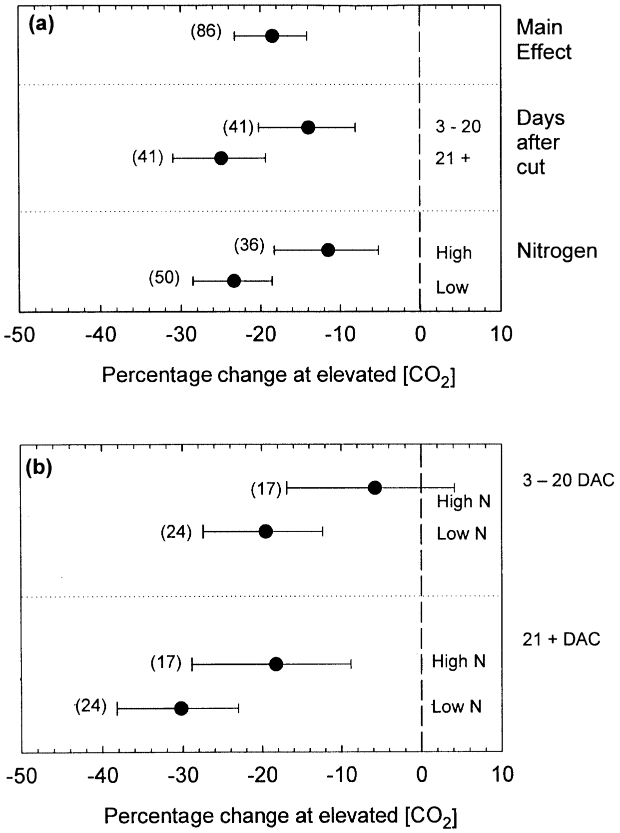

Figure 6. The percentage change of $V_{c,max}$ with growth at elevated [CO$_2$]. (a) Main effect when all data are considered, as well as the categorical effects of days after cut (DAC) and nitrogen fertilization level. (b) Interactive effects of DAC and N. Sample sizes (n) are given in parenthesis. Mean response ratio (r) surrounded by 95% confidence interval is shown.

under low N conditions, $V_{c,max}$ was reduced by 20% (Fig. 6b). After 21 d or more following a cut, there was a significant reduction in $V_{c,max}$ under both high and low N fertilization conditions (Fig. 6b). The time-dependent reduction in $V_{c,max}$ coincides with reductions in the percentage stimulation in both A_{sat} and A'.

The percentage change in elevated [CO$_2$] in J_{max} was less than the change in $V_{c,max}$. Growth at elevated [CO$_2$] resulted in a 9% decrease in J_{max} and no categorical variables significantly altered this response (Table 2). The interaction between days after cut and N fertilization followed the same general trend as previously described for $V_{c,max}$ (Fig. 7). Under high N conditions in the first 20 d following a cut, no reduction in J_{max} was apparent. However, in low N conditions, there was a significant 10% reduction in J_{max} with growth at elevated [CO$_2$] (Fig. 7). Three weeks or more following a cut, the percentage reduction in J_{max} with growth at elevated [CO$_2$] under both high and low N conditions increased, but the confidence intervals around those means increased as the sample size was reduced.

The ratio of $V_{c,max}/J_{max}$ was unchanged by growth at elevated [CO$_2$] except for plants grown under low N conditions, 3 weeks or more following a cut, in which the ratio was reduced by 20% (Fig. 8). A significant reduction in the ratio represents a shift of resources away from Rubisco and coincides with the period of greatest acclimation of both $V_{c,max}$ and J_{max} (Figs 6b & 7).

DISCUSSION

We report 10 years of photosynthetic data for the longest, continuously running *FACE* experiment, to date. Photosynthetic carbon assimilation of *L. perenne* was stimulated continuously with growth at elevated [CO$_2$], despite significant transient decreases in apparent *in vivo* Rubisco activity ($V_{c,max}$) and electron transport contributing to RubP regeneration (J_{max}), and a long-term decline in stomatal conductance (g_s). Photosynthetic acclimation, as indicated by significant decreases in $V_{c,max}$ in response to growth at elevated [CO$_2$], was dependent upon the carbon-sink status of the plants and was strongest under conditions of high source : sink ratio and low N fertilization. Increased sink strength of the swards following harvest completely alleviated photosynthetic acclimation in plants grown under high N fertilization. It did not totally alleviate acclimation in plants grown under low N conditions. These findings support the general hypothesis of Long & Drake (1992) and Drake *et al.* (1997), and the observations of Rogers *et al.* (1998) based on a single harvest of *L. perenne*. This is, that a decrease in Rubisco activity is accentuated under conditions of low sink/source activity, especially when a low nitrogen supply may limit the capacity for generating additional sinks.

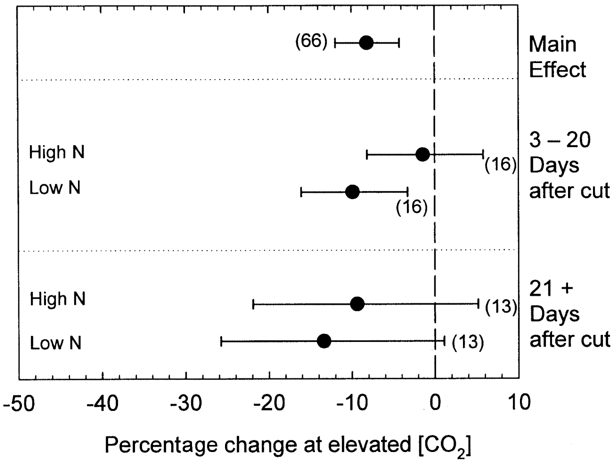

Figure 7. The percentage change of J_{max} with growth at elevated [CO$_2$]. Sample sizes (n) for analyses are shown in parenthesis. The mean effect sizes (r) surrounded by 95% confidence intervals are shown.

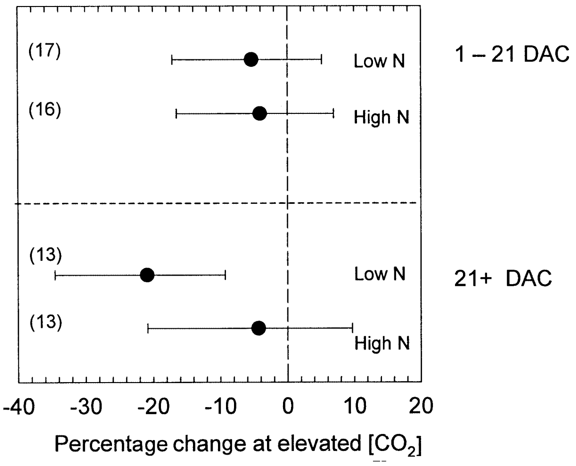

Figure 8. The percentage change in the $V_{c,max}/J_{max}$ ratio with growth at elevated [CO$_2$]. Sample sizes (n) for analyses are shown in parenthesis. The mean effect sizes (r) surrounded by 95% confidence intervals are shown.

Sustained stimulation of photosynthetic carbon uptake

Over 10 years of growth at elevated [CO$_2$], stimulation of daily integrated carbon uptake was 36%. The mean stimulation in light-saturated photosynthesis was slightly higher, at 43%. This difference is consistent with theoretical expectations. Growth at elevated [CO$_2$] increases photosynthesis at all light levels by competitively inhibiting the Rubisco-catalyzed oxygenation. However, in saturating light, a further increase in photosynthesis results, because the enzyme Rubisco is not CO$_2$-saturated at the current atmospheric concentration (Long 1991). The daily integral of photosynthesis includes periods of the day when photosynthesis will be light-limited as well as periods when it will be light-saturated. The degree of stimulation reported here for *L. perenne* is slightly higher than the 33% average stimulation in photosynthesis reported for prior studies of C$_3$ grasses grown under elevated [CO$_2$] (Wand *et al.* 1999). A significant stimulation of carbon uptake was maintained for the duration of the *FACE* experiment; however, the degree of stimulation was lower in the spring than in the summer. Again this is consistent with theoretical expectation, where because of the differing sensitivities of Rubisco oxygenase and carboxylase activity, the proportionate stimulation of photosynthesis by a given increase in [CO_2] will rise with temperature (Long 1991). Because the majority of measurements during the last 3 years of the experiment were taken in the spring, stimulation of A' was significantly less from 2000 to 2002 than in the early years of the experiment. It is also possible that during spring, temperatures were low enough to restrict growth of the swards (Newton *et al.* 1994; Clark *et al.* 1995), leading to conditions of source–sink imbalance. Indeed, analysis of leaves in early spring 2001 and 2002 before the first harvest of the season, revealed high levels of carbohydrates in source leaves, especially under low N and high CO_2 conditions (Ainsworth, unpublished results).

In grasslands, defoliation leads to a decline in photosynthetic C gain due to a reduction in the photosynthetically active leaf area. Carbohydrate reserves are depleted and potentially limit regrowth at severe defoliation (Richards 1993). Elevated [CO_2] may consequently stimulate regrowth through an increase in the availability of newly fixed and stored carbon (Nijs, Impens & Behaeghe 1988). Our analysis revealed that during the Swiss *FACE* experiment, the percentage increase in photosynthetic carbon uptake in the first 20 d following a harvest (45%) was nearly double the percentage increase later in the regrowth cycle (23%). The large positive effect on photosynthesis immediately following harvest coincides with an increase in total dry matter and leaf area (Suter, Nösberger & Lüscher 2001). Towards the end of a regrowth period, as photosynthetic stimulation was reduced, carbohydrates accumulated in source leaves, especially under low N and high [CO_2] (Fischer *et al.* 1997; Rogers *et al.* 1998; Isopp *et al.* 2000). Specific leaf area and apparent nocturnal carbohydrate export also decreased late in a regrowth cycle (Hebeisen *et al.* 1997a, Fischer *et al.* 1997). Consequently, late in the regrowth cycle, total dry matter and leaf area failed to show any net increase with growth at elevated [CO_2], and tiller number decreased (Suter *et al.* 2001).

Acclimation of photosynthesis to elevated [CO_2]

The photosynthetic capacity of *L. perenne* was clearly decreased by elevated [CO_2]. The value of $V_{c,max}$ was reduced significantly by 18% (Fig. 6) and J_{max} was reduced by 9% (Fig. 7). From 1995 to 1998, elevated [CO_2] caused an approximate 15% reduction in leaf N concentration (Hartwig *et al.* 2002). There was no significant acclimation of photosynthesis in *L. perenne* early in the Swiss *FACE* experiment under high N fertilization and immediately following defoliation in low N conditions (Rogers *et al.* 1998). However, analysis of the entire 10 years of data from the *FACE* experiment revealed that acclimation could occur under both high N and low N fertilization conditions, but the degree of acclimation was significantly less under high N conditions (Fig. 6). Acclimation was completely ameliorated in plants grown under high N conditions immediately following a harvest (Fig. 6), but there was still significant down-regulation of $V_{c,max}$ in the first 3 weeks following harvest in plants grown under low N. During that period there was also a reduction in the $V_{c,max}/J_{max}$ ratio (Fig. 8). Isopp *et al.* (2000) found that total leaf protein was reduced in *L. perenne* grown under low N and elevated [CO_2] immediately following a cut, but there was no reduction in plants grown under high N and elevated [CO_2]. Suter *et al.* (2001) suggested that there was little or no capacity to use additional C under elevated [CO_2] at the end of the regrowth cycle in *L. perenne* grown under high N, since neither leaf area, total dry matter nor yield showed any increase over control. These results strongly support the hypothesis that acclimation of photosynthesis to growth at elevated [CO_2] is due to limitation of the development of sinks for photo-assimilate.

CONCLUSIONS

A significant stimulation of both light-saturated photosynthesis and daily integrals of photosynthesis was maintained throughout the 10 year life of a *L. perenne* sward managed as a herbage crop and grown in the open under elevated [CO_2]. The stimulation was maximal following harvest, at the warmest times of year and with a high supply of nitrogen, but decreased progressively during regrowth following each harvest. This was associated with a loss of apparent *in vivo* Rubisco activity and was most pronounced in the low nitrogen treatment. The results were consistent with the hypothesis that acclimation of photosynthetic capacity in response to growth at elevated [CO_2] depends on the availability of sinks for the additional carbon and that ability to form new sinks is limited by nitrogen. Stomatal conductance was significantly lower throughout the 10 years in elevated [CO_2]. This open-air field experiment provides no support for the prediction that stimulation of photosynthesis under elevated [CO_2] is a transient phenomenon.

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