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THE PHOTOSYNTHETIC ACCLIMATION OF *LOLIUM PERENNE* IN RESPONSE TO THREE YEARS GROWTH IN A FREE-AIR CO$_2$ ENRICHMENT (FACE) SYSTEM

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DEPARTMENT OF APPLIED SCIENCE

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by

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DISCLAIMER

THE PHOTOSYNTHETIC ACCLIMATION OF *LOLIUM PERENNE* IN RESPONSE TO THREE YEARS GROWTH IN A FREE-AIR CO_2 ENRICHMENT (FACE) SYSTEM.

BY GRAHAM J. HYMUS

Report of original research submitted in partial fulfilment of the requirements
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Dept of Biology, University of Essex; and Writtle College.

CONTENTS.

ABSTRACT.

Pure stands of Ryegrass (*Lolium perenne* L. cv Bastion) were in their third year of growth in the field, exposed to either ambient (355 µmol mol^{-1}), or elevated (600 µmol mol^{-1}) atmospheric CO_2 concentration. A Free-Air CO_2 Enrichment (FACE) system was used to maintain the elevated CO_2 concentration whilst limiting experimental constraints on the field conditions. The theoretically predicted increase in the net rates of CO_2 uptake per unit leaf area (A µmol mol^{-1}) as a consequence, primarily, of the suppression of photorespiration by CO_2 a competitive inhibitor of RubP oxygenation by Rubisco, was observed for the *Lolium perenne* studied. Also observed was a general decline in leaf evapotranspiration (E) consistent with observations of increased water use efficiency of crops grown in elevated CO_2. Enhancement of leaf A in the FACE grown *L. perenne* ranged from 26.51% to 44.95% over the course of a diurnal set of measurements. Whilst reductions in leaf E reached a maximum of 16.61% over the same diurnal course of measurements. The increase in A was reconciled with an absence of the commonly observed decline in $V_{c\ max}$ as a measure of the maximum *in vivo* carboxylation capacity of the primary carboxylasing enzyme Rubisco and J_{max} a measure of the maximum rate of electron transport. The manipulation of the source sink balance of the crop, stage of canopy regrowth or height in the canopy had no effect on the observation of a lack of response. The findings of this study will be interpreted with respect to the long term implications of C_3 crops being able to adapt physiologically to maximise the potential benefits conferred by growth in elevated CO_2.

Key words: *Lolium perenne,* elevated CO_2, photosynthesis, acclimation, Free-Air CO_2 Enrichment.

Abbreviations and symbols:

A = Net rate of CO_2 uptake per unit area (µmol mol^{-1}); **Ca** = Atmospheric CO_2 concentration (µmol CO_2 / mol air); **Ci** = Intercellular CO_2 concentration (µmol CO_2 / mol air); **E** = H_2O transpiration rate (mmol m^{-2} s^{-1}); **FACE** = Free-Air CO_2 Enrichment ; **J_{max}** = Maximum light saturated rate of electron transport (µmol m^{-2} s^{1}).; **Pi** = Inorganic phosphate; **PPFD** = Photosynthetic photon flux density (µmol m^{-2} s^{-1}); **Rubisco** = Ribulose-1,5 bisphosphate Carboxylase-Oxygenase; **RubP** = Ribulose-1,5 bisphosphate; **$V_{c\,max}$** = Maximum rate of carboxylation (µmol m^{-2} s^{-1}).

1. INTRODUCTION.

1:1. Climate change.

The exponential increase in the atmospheric carbon dioxide concentration (C_a), from pre-industrial revolution levels of 275 µmol mol^{-1}, to the current level of 345 µmol mol^{-1} (Lindzen 1993) has been facilitated primarily by anthropogenic emissions of CO_2. Namely, the burning of fossil fuels (5.4 Gt per annum) and deforestation (1.6 Gt per annum) (Idso 1989, Post *et al* 1990). Projections suggest that C_a will reach 530 and 700 umol mol^{-1} by 2050 and 2100 respectively (IPCC business as usual scenario) (Watson *et al* 1990). A concurrent rise in temperature of 3 °C and 4°C by 2050 and 2100 is also predicted (Watson *et al* 1990).

The implications for crop plants, of such increases in atmospheric CO_2 concentration. Concurrent with increases in temperature are considerable. With new balances between respiration (carbon release) and photosynthesis (carbon sequestration) dependent on the interactive nature of the increasing C_a and temperature (Long 1991). It is envisaged that fundamental to the crop plants performance will be the way in which its biochemical systems, geared towards coping with an atmosphere low in CO_2 can adapt (acclimate) to a higher C_a under existing genetic feedback mechanisms so that limiting resources like nitrogen are not wasted (Woodrow 1994).

1:2. Theoretical crop responses.

In the short term, increases in average crop yields for C_3 crops, of which Ryegrass is one, are expected to be in the region of 34% (Kimball 1983). These increases are largely attributable to substantial increases in the net rate of CO_2 uptake per unit area (A) (µmol mol^{-1}) (Long 1991). In turn being as a result of the supression of RubP oxygenation by Rubisco, by CO_2 a competative inhibitor (Webber *et al* 1994). Photorespiration is responsible for a decrease in A of 20% - 50% in temperate climates (Long 1985). In addition, provided RubP is in excess, there will be an expected increase in carboxylation velocity as present C_a does not saturate Rubisco (Stitt 1991).

In intact leaves, photosynthesis is usually limited by one of three processes.

1) The capacity of Rubisco to consume RubP in CO_2 fixation.

2) The capacity of the thylakoid membranes to supply ATP and NADPH for RubP regeneration.

3) The capacity of starch and sucrose to utilise triose phosphates and subsequently regenerate inorganic orthophosphate (Pi) for photosynthesis. (Sage *et al* 1994).

1:3. Acclimation.

With an elevated C_a the balance of control exerted on the plant by 1-3 above, appears to be upset and a new equilibrium sought at a new photosynthetic capacity (Stitt 1991). The primary cause of acclimation is deemed to be the inability of the plant to utilise the excess carbohydrate produced due to an inbalance between excess sources and insufficient sinks for carbohydrate (Arp 1991, Long & Drake 1992). Whilst the extreme consequences of this imbalance are the accumulation of carbohydrate, in the form of starch, in the chloroplast both damaging (Cave *et al* 1981) and changing its structure (Sionit *et al* 1984), these now seem to be rare events. More common appears to be the inability of the plant to utilise sucrose produced which will result in the reduction of inorganic phosphate (Pi) flux from the cytosol to the chloroplast and the inhibition of ATP and NADPH production and subsequently the rate of RubP regeneration. In addition evidence is pointing to the repression of specific genes via the accumulation of soluble carbohydrates leading to decreased levels of photosynthetic proteins (Webber *et al.* 1994).

The degree to which these theoretical and often observed initial stimulatory responses to elevated C_a will be maintained longer term is of paramount importance. Evidence increasingly suggests that physiological adjustment (Oechel & Strain 1985) and acclimation of the photosynthetic apparatus (Sage *et al* 1989, Beresford 1990) lead to downregulation of photosynthesis in C_3 crops in response to prolonged growth in elevated C_a (Delucia *et al* 1985, Cure & Acock 1986). Whilst acclimation is a commonly observed phenomenon of crop plants grown in controlled environments the relevence of these results to those that occur in the field are routinely questioned (Fischer & Aguilar 1976, Lawlor & Mitchell 1991). Indeed there is evidence that the downregulation of photosynthesis in controlled environments is an artefact of the constraints of the experimental system, particularly pot size (Arp 1991) and coupling of environmental growth parameters (Lawlor & Mitchell 1991).

The observed artefacts or "chamber effects" of plant growth in environments that are controlled to some degree commonly include changes in wind velocity, humidity, temperature, light quality or intensity and soil variables (Lewin *et al* 1994). In an attempt to negate such alterations in the microclimate around the plants compared to the natural environment . A Free-Air (chamberless) carbon dioxide enrichment (FACE) system has been developed by Brookhaven National Laboratory (BNL) to provide controlled fumigation conditions over a large area of field grown plants throughout the plants phenological development whilst minimising the aforementioned "chamber effects" (Lewin *et al* 1994).

As yet the few field relative to controlled environment experiments and the restricted number of crops studied, are quoted as reasons that conclusive hypotheses regarding field responses to elevated CO_2 cannot be formulated (Rozema *et al* 1993). Indeed results range from acclimation being achieved within three years on Arctic Tussock Grass (Oechel & Strain 1984), to the lack of downward acclimation observed over a four year study on a C_3 sedge (Arp and Drake 1991), whilst acclimation observed late in one growing season on cotton was attributed to earlier senescence (Radkin 1987). To a conclusion that there was a lack of acclimation in field grown crops in natural environments (Arp 1991, Sage *et al* 1994).

1:4. A / Ci response.

The major aid to quantifying the acclimatory response of crops to growth in elevated C_a is the construction of an A (Leaf net CO_2 uptake, Assimilation), Ci (Internal CO_2 concentration of the leaf) response curve. Derived from the equations of Farquhar *et al* (1980) the A / Ci response provides an *in vivo* measure of.

1) The maximum capacity of the leaf to regenterate RubP, a plateau or shallow slope at high Ci where photosynthesis is limited by the rate of regeneration of RubP.

2) A measure of the carboxylation efficiency of the leaf, an initial slope at low Ci determined by Rubisco activity, which will be RubP saturated.

Acclimation, defined for the purpose of this study as the downregulation of photosynthetic capacity, is characterised by a decreased carboxylation efficiency, commonly caused by a reduction in the amount / activity of Rubisco (Long & Drake

1992) and can be determined from changes in the initial slope of the A / Ci response curve. In practice upgrading of the photosynthetic capacity of a plant is also acclimation and highlights the great inter and intra species differences in photosynthetic reponse to elevated C_a.

The response of perennial systems to elevated C_a will be of particular importance in an elevated C_a world. Ecosystems where enhanced production will be expected to result from increased productivity and an extended growing season into winter months, as a consequence of concurrent rises in C_a and temperature (Ryle *et al* 1992). When considering perennial crops additional factors will determine their response to elevated C_a. In particular the allocation of photosynthates to roots and shoots, a trade off which represents the choice of maximising above ground growth in one year and possibly compromising growth in the next, or investing in a larger root system and underground carbohydrate storage for enhanced growth in subsequent growth periods. Increased tillering one year may compromise nitrogen sequestration in the soil in the next year.

1:5. *Lolium perenne.*

Given these considerations a study of Perennial Ryegrass, *Lolium perenne* was undertaken to ascertain it's photosynthetic response to prolonged growth of three years in elevated C_a in a FACE system (Lewin *et al* 1994). The responses observed could then be reconciled with generalised response of C_3 crops and prior work on Ryegrasses in a variety of experimental designs. A number of facts justify the time devoted to studying this crop. As the most useful and widely spread pasture grasses in many parts of the world several species of *Lolium* are of economic importance (Langer & Hill 1991). At present and for the forseeable future, Ryegrasses will provide the mainstay of lowland pasture production in temperate western Europe (Ryle *et al* 1992). Since reserves for the expansion of grassland are limited and since farmland tends to be reducing in industrialised areas emphasis must be placed on the monitoring of changes in grassland productivity and predict future changes . Adaption of management techniques to a higher CO_2 atmosphere may be required (Nijs *et al* 1988) particularly under low input conditions.

Recent experiments suggest that the responses of *Lolium perenne* to elevated C_a are consistent with those of other C_3 crops, with increases in A at both the single leaf (Nijs *et al* 1989, Ryle *et al* 1992, Gay & Hauck 1993) and canopy (Ryle *et al* 1992, Nijs & Impens 1993). Dry matter production also exhibited expected increases (Nijs *et al* 1988, Ryle *et al* 1992). Acclimatory modification of the photosynthetic apparatus also mirrors generalised C_3 responses, V_{cmax} and J_{max} values as *in vivo* measurements of the maximum velocity of carboxylation and maximum light saturated rate of electron transport respectively have been shown to decline in response to exposure to elevated C_a (Moon *et al* 1990). Contention at present seems to surround the response of *Lolium* to elevated C_a at low light intensities. Evidence tends to suggest that at lower light intensites leaf A decreases for *Lolium perenne* (Nijs *et al* 1989, Ryle *et al* 1992, Gay & Hauck 1993). Such results are inconsistent with the predictions of Long and Drake (1992) who predict that A should increase in elevated C_a even at low light intensities, and the work of Bryant (1994) where enhanced leaf A was achieved even at low light intensities. Consensus on this area is needed to be able to better determine whether the extension of the growing season for *Lolium perenne* into winter months will be a result of elevated C_a.

The implications of using different management strategies is mimicked in the F.A.C.E experiment by varying nitrogen treatments and cutting regimes. Again the effects of nitrogen availability on the response of crops to elevated C_a is in dispute. It is argued that a shortage of nitrogen to facilitate the growth of new sinks for carbohydrate assimilation will enhance downregulation (Wong 1979, Larigauderie 1988, Vessey *et al* 1990). In contrast, it is claimed that a loss of 30 % of Rubisco could be sustained by a plant before its photosynthetic performance is reduced (Long & Drake 1992), ultimately allowing the re-apportioning of nitrogen from Rubisco and mitigating the effects of nitrogen deficits (Sinclair 1992). Manipulation of the source sink balance by cutting should alleviate any photosynthetic downregulation and allow the higher carbon storage in the roots of Ryegrass stands to facilitate speedy development of a new canopy (Nijs & Impens 1993).

This study will, therefore attempt to address a number of key questions and evaluate whether or not the results validate or refute previous findings obtained in a number of experimental environments, for *Lolium perenne.*

1) Does prolonged growth in elevated C_a stimulate A throughout the course of a day and at different stages of growth. As inferred from diurnal course's of measurement.

2) Does prolonged growth in elevated C_a induce an acclimatory response in the photosynthetic apparatus in the field, as inferred from the construction of the A/Ci response curve. Three experiments have been designed to allow acclimation to be studied .1) Measurements will be taken at varying times through the regrowth cycle in all plots, as well as, 2) on plots of *L.perenne* cut to two different heights within each

low nitrogen plot. This will allow the effects of changing source sink balances to be studied at a single point in time. Removing the effects of variable climate conditions from the equation. The low nitrogen plots only have been chosen as it is believed that acclimation is most likely to be apparent in these plots.3) The interaction between leaf age, shading and acclimation will be addressed by taking measurements on the same leaves at two points through the regrowth cycle.

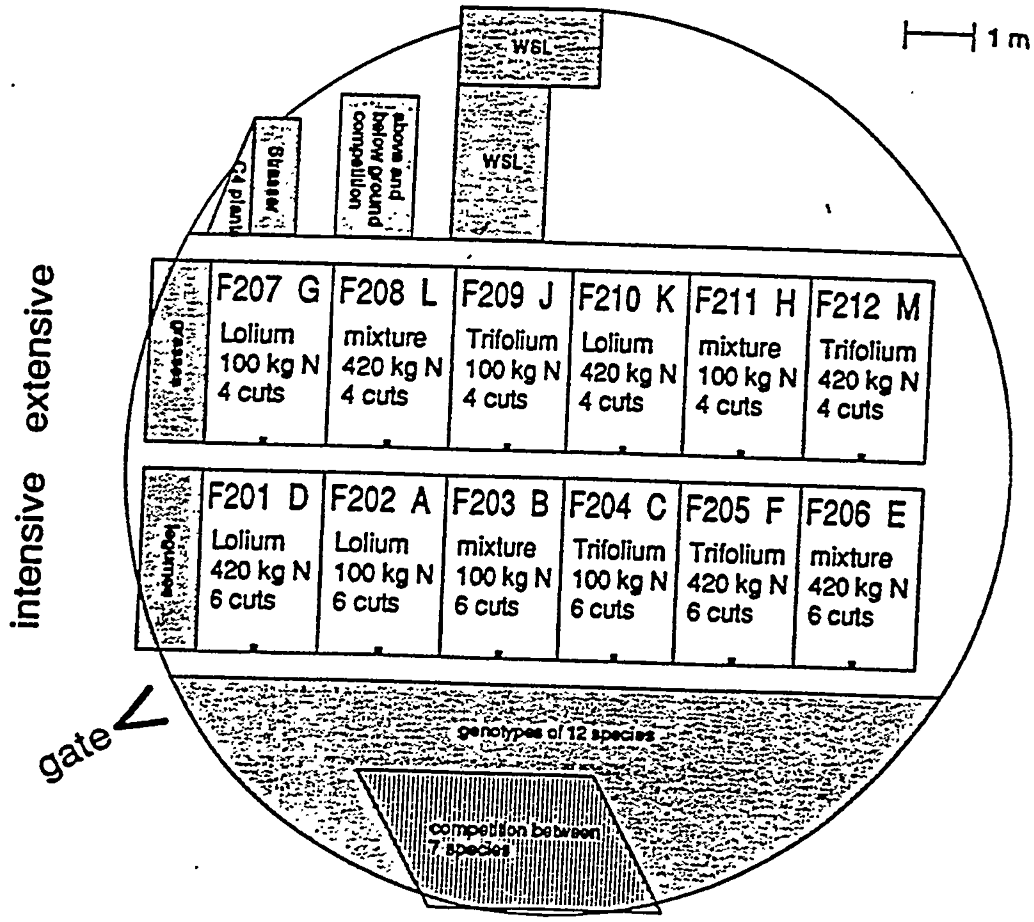
3) What are the implications for 1 and 2 above of variations in cutting regimes and nitrogen treatments, as methods of altering the source sink balance.

2. Materials and Methods.

2:1 The Site.

The ETHZ Eshikon research site utilises the Free Air Carbon Dioxide Enrichment (F.A.C.E.) system developed by Brookhaven National Laboratory. (Hendrey *et al* 1993,Lewin *et al* 1994). The FACE system at Eschikon consists of a 14 m diameter toroidal plenum below the ground. 32 vertical vent pipes protrude from below ground from the plenum at regular intervals. From these pipes the release of CO_2 is controlled by a central computer and governed by wind speed, wind direction and air temperature to maintain an elevated C_a of 600μmol mol^{-1} within the FACE rings. There are six rings paired within three blocks, three control at ambient C_a (355 μmol mol^{-1}) and three FACE at elevated Ca (600 μmol mol^{-1}) . Figure 1 details the layout of one of the six rings.

FIGURE 1.



Fumigation of the rings commenced in March this year and is scheduled to end in November and is switched on when leaf temperatures exceed 8°C. Elevated C_a was maintained within 10% of the target CO_2 concentrations (540-660 µmol mol^{-1}) for 89 - 94% of the time, measured over one minute integrals during 1994 (Nagy *et al* 1995).

Perennial Ryegrass *Lolium perenne* L (cv Bastion) is in its third year of growth in pure stands in each of the six rings.Variation in growing conditions is achieved by the interaction of three main variables.

1) CO_2 level control (355 µmol mol^{-1}) or FACE (600 µmol mol^{-1})
2) Level of Nitrogen application (140 or 540 kg / N ha), in the form of NH_4 NO_3.
3) Cutting regime (8 cuts during the growing season). Every 4 weeks commencing 24.3.95 and ending 2.11.95.

Additional variants influencing growth of the crop are provided by fluctuations in atmospheric and meteorological conditions.

2:2. Materials.

Data collected consisted exclusively of leaf gas analysis measurements, obtained non destructively, from which the main variables A and C_i were derived based on the equuations of Von Caemmerer & Farquar (1981) and Harley (1992) to standardise to 25 °C. Photosynthesis was measured using an Open Combined Infra-Red Gas Analysis System (CIRAS 1 model 1.4, PP Systems ,Hitchin , Herts UK). in conjunction with a Parkinson Leaf Cuvette (Model 1.1, PP Systems, Hitchin, Herts UK). Weekly calibration of the CIRAS was made for CO_2 at 600 µmol mol^{-1} and water vapour at 10 mb. CO_2 calibration was made against a gravimetric standard CO_2 concentration (Carbagas Zurich), whilst a known humidity was generated with a water vapour generator (Type WE 600, Analytical Development Co, Hoddesden UK).

Whilst leaf gas exchange measurements were always made with the upper leaf surface displayed in the top of the leaf chamber, the type of leaf chosen and the position along the leaf at which measurements were taken differed and depended on the experiment being conducted. For most of the sampling period the plants were in the vegetative stage of growth, it was only towards the end of the cutting period that some entered the reproductive stage of growth. Care was taken not to sample these plants where substantial non photosynthesising sinks had developed. The selection and measurement criteria will therefore be discussed at the appropriate place. Fundamental to analysis was the estimation of leaf area in the circular leaf cuvette of 1.8 cm diameter. This was achieved by multiplying the width of the leaf by the diameter of the chamber and assuming a rectangle was formed. This assumption results in the overestimation of leaf area to a tiny degree. Which was considered negligable when compared to the ability of the CIRAS to only accept leaf areas calculated to 1 mm^2 accuracy.

For the construction of A/Ci curves saturating photosynthetic photon flux densities were required, so a quartz iodide lamp was attatched to the upper surface of the leaf chamber to provide a constant light saturating photon flux density of 750 µmol $m^{-2}\ s^{-1}$.

2:3. Methods.

2:3:1. Diurnal Measurements.

Gas analysis measurements were performed every two hours hours in each of the six rings to determine the diurnal course of, photosynthetic leaf CO_2 uptake per unit area (A). This was done in 2 hour runs between 6.00 and 18.00 hrs for both the low and high nitrogen *Lolium* plots for the 8 cut regime on the 24th May and the 20th June, whilst on the 9th July the last run started at 20.00 hrs. For both nitrogen treatments in each ring three leaves were sampled every two hours. Measurements were made at the growth C_a (either 355 or 600 µmol mol^{-1}) and in the climatic conditions prevailing at the time, the only restriction on measurments was enforced by rain, yet days with a minimum of cloud cover were considered preferable.

Leaves chosen for the measurements were consistently the youngest fully expanded leaf, namely those leaves nearest to the top of the canopy bearing a ligule. Measurements were made half way along these leaves with the leaf cuvette always held horizontally parallel to the ground. Table 1 details the days when measurements were taken whilst the rings were sampled in the following order, C2, F1, C1, F2, F3, C3. See Appendix 1 for ring locations.

TABLE 1. Dates of diurnal measurements, nitrogen treatments sampled and the canopy regrowth status.

	DATE.	NITROGEN	DAYS FROM CUT
DIURNAL 1	24th MAY 1995	HIGH & LOW	9 AFTER
DIURNAL 2	20th JUNE 1995	HIGH & LOW	8 AFTER
DIURNAL 3	9th JULY 1995	HIGH & LOW	1 BEFORE

A two way anova with replication was used to statistically test the results obtained during the midday hours of peak insolation and temperature, between 11.00 hrs and 15.00 hrs. Compared with the results gained earlier in the morning and in the evening.

2:3:2. A / C_i determination.

The construction of light saturated leaf CO_2 uptake per unit area (A_{sat}) to intercellular CO_2 concentration (C_i) plots was made by gas analysis measurements. For both the low and high nitrogen treatments of the eight cut regime A / C_i curves were constructed for two leaves in each treatment in all six rings from a sequence of six C_a levels.

For ambient plots the sequence was : 355, 250, 150, 50, 600, 900 µmol mol^{-1}.

For F.A.C.E plots the sequence was : 600, 355, 250, 150, 50, 900 µmol mol^{-1}.

Adherance to these sequences was designed to negate any problems associated with the activation state of Rubisco being raised above natirally occuring levels. Measuerments were taken at C_a concentrations within + or - 40 umol mol^{-1} of the above stated values except at the growth C_a when a divergence of only + or - 15 umol mol^{-1} was permitted. For all measurements photosynthetic photon flux densities that were light saturating were used and measurements were concluded before 15.00 hrs to reduce the possibility of feedback inhibition of photosynthesis by carbohydrate accumulation and cytosolic inorganic phosphate limitation. The criteria for leaf sampling was the same as that already detailed for the Diurnal measurements (p 14). Four sets of measurements were obtained in total, one set was made either side of the cuts on the 12th June and the 10th July. This enabled analysis before and after the two cuts as well as during the regrowth period between the cuts.

From the plots sampled, values of V_{cmax} and J_{max} were fitted. These values represent *in vivo* quantifications of the maximum rate of carboxylation (μmol m^{-2} s^{-1}) and hence Rubisco activity (V_{cmax}) and the maximum light saturated rate of electron transport (μmol m^{-2} s^{-1}) or the maximum capacity for RubP regeneration (J_{max}). These values were achieved by fitting the data of McMurtrie & Wang (1993) to the A_{sat} values achieved.

Three way analysis of variance was performed on the V_{cmax} and J_{max} values obtained from each individual A/Ci curve obtained to highlight any significant differences in those values caused by CO_2, nitrogen and the date (before or after cutting).

Dates of experiments: 22/23 May, 6/11 June, 19/21 June, & 1 July.

2:3:3. Diurnal A / C_i determination.

An attempt to detail the diurnal nature of fluctuating V_{cmax} and J_{max} values was made by constructing A / C_i curves for two rings, one F.A.C.E and one ambient in the time window between 15.00 and 17.00 hrs when feedback inhibition of photosynthesis would be expected to excert an influence on the V_{cmax} and J_{max} values achieved. The values gained from the two rings sampled in the afternoon were compared to the values achieved in the morning in the same rings and on the same two tagged leaves in both the low and high nitrogen 8 cut regime plots. Leaf selection and the sequences of

C_a exposure levels to construct the A / C_i responses have already been detailed (pgs 14 & 15).

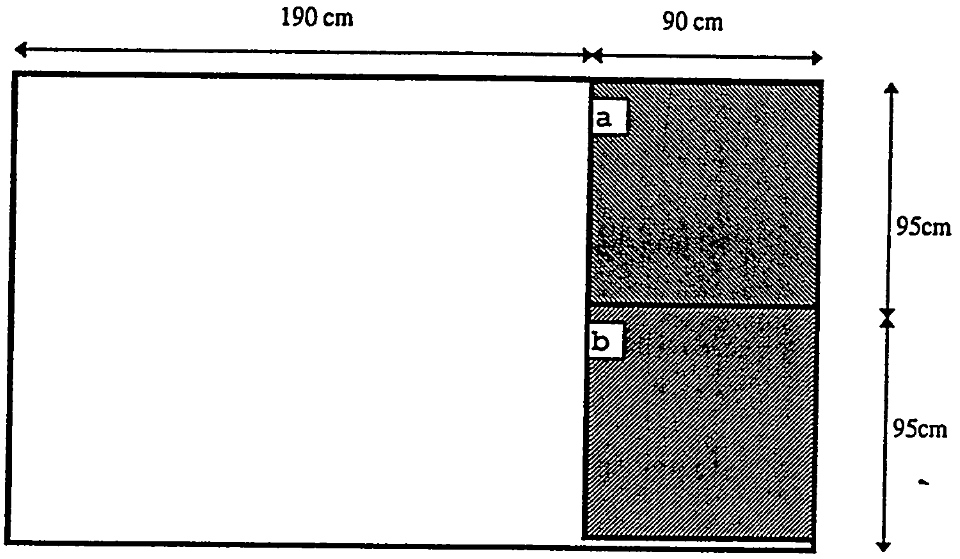
Dates of experiments: 23 May, & 26 June.

2:3:4. Manipulation Experiment.

The size of the area cut provided a central area 30 by 35 cm's for sampling where border and shade effects would be at a minimum. (Figure 2).

After allowing a regrowth period of between 3 and 5 days measurements were taken on two leaves in the cut and uncut section of each of the six rings (NB for the measurements on the 6th of June only four rings were sampled due to constraints imposed in the other two rings by other experiments, they were C2, F2, C3, F3).

FIGURE 1. Low nitrogen 8 cut *L.perenne* plot. Shaded areas denote areas cut in manipulation experiment. Area a cut on 2nd June, area b cut on 30th June.



The leaves chosen for the measurements were chosen in a way that aimed to ensure uniformity of age. This involved taking measurements on leaves that were not fully developed, (None were in the cut section) 5 cm's from the leaf juncture with the stems high in the canopy as possible for the uncut plot. A / C_i curves were then constructed in the aforementioned manner. An assumption that rates of leaf expansion were uniform was fundamental to sampling leaves of the same age, in reality rates of expansion may differ.

Dates of experiments: 8 June & 5 July.

2:3:5. Canopy Effects on A / C_i response.

In an effort to assess the implications of shading and leaf age on the A/Ci response of *Lolium perenne*, A/Ci curves were constructed at two time periods during canopy regrowth, firstly eight and then fourteen days into the regrowth period. The leaves used for the initial A/Ci measurements were tagged to allow recognition and re-measurement on the two subsequent measurement days, Whilst also ensuring that the leaves sampled were of the same age.

Dates of experiment: 19/21 June & 27/28 June.

3. Results

3:1. Diurnal Measurements.

Studies of the diurnal nature of CO_2 and water vapour exchange undertaken on three separate days throughout June and July show substantial increases in A and slightly more variable, yet generally decreases in E for *L.perenne* grown at elevated C_a with respect to that grown at ambient C_a (Table 3 , & Figs 3-8).

TABLE 3. Relative enhancement (%) of A and E for FACE grown *L.perenne* over controls, over a diurnal course of measurements. On three separate days and two nitrogen treatments.

		A % Enhancement		E % Enhancement	
DATE	Days from cut	HIGH N	LOW N	HIGH N	LOW N
24.5.95	+9	30.51	39.35	-13.40	-15.14
20.6.95	+8	26.51	38.14	-8.84	-10.03
9.7.95	-1	33.05	44.95	+4.98	-16.01

A stimulation in A was recorded in the elevated plots during every two hour period and in both nitrogen treatments over the course of the measurement days. This stimulation was irrespective of the period into regrowth of the crop. Expressed as a percentage over the whole study period this stimulation in A ranged from 26.51 % to 44.95 %.

Greatest total enhancement was shown to correspond to periods of the highest Photosynthetic photon flux densities (PPFD) and higher temperatures. These conditions occurred between 11:00 hrs and 15:00 hrs, a period of time when there was a significant difference between A in the FACE and control plots (Table 4). During the

FIGURE 3. Diurnal Assimilation and Evapotranspiration rates for Control and FACE grown *L.perenne*. Low nitrogen treatment 23.5.95, 9 Days after the cut.

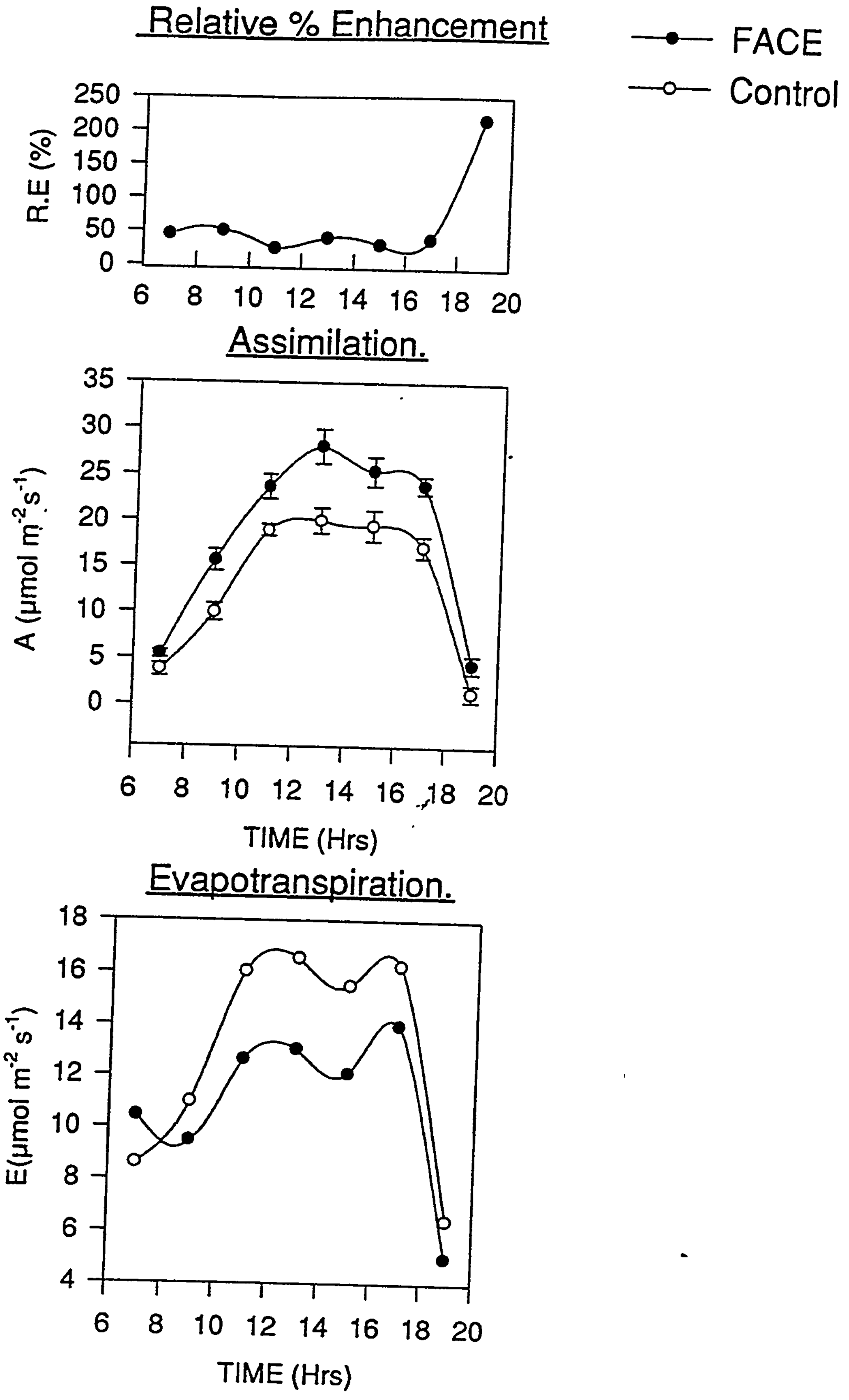
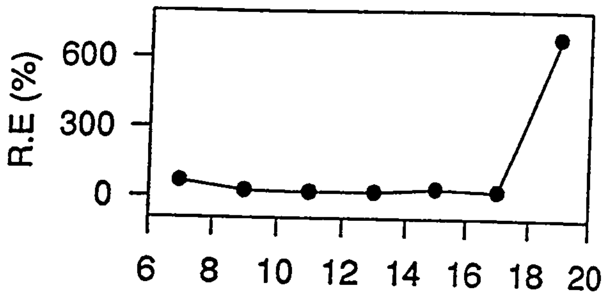
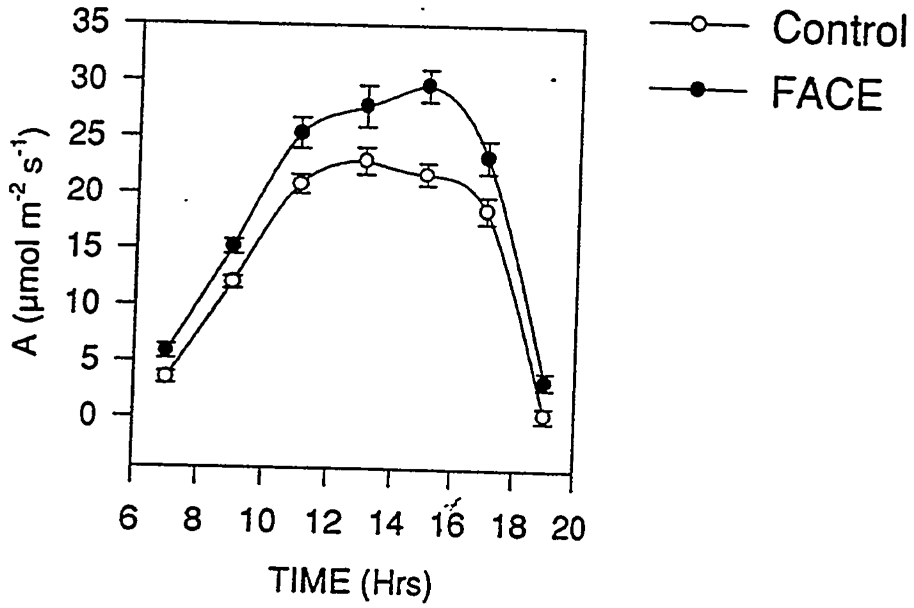


FIGURE 4. Diurnal Assimilation and Evapotranspiration rates for Control and FACE grown L.perenne. High nitrogen treatment 23.5.95, 9 Days after the cut.

Relative % Enhancement of A.



Assimilation (A).



Evapotranspiration (E).

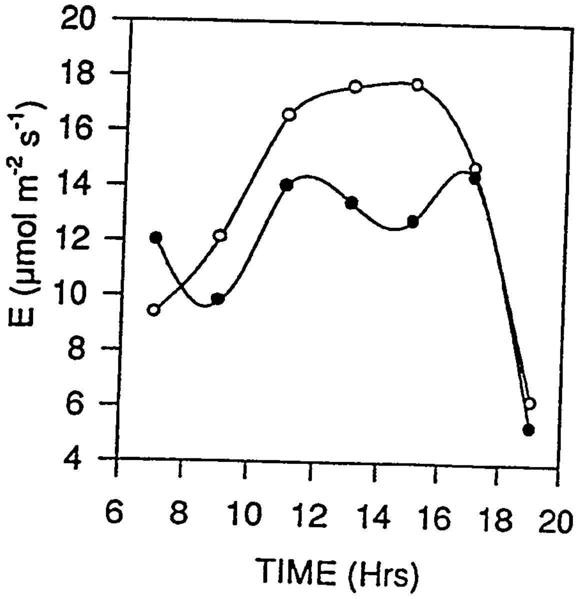


FIGURE 5. Diurnal Assimilation and Evapotranspiration rates for Control and FACE grown *L.perenne*. Low nitrogen treatment. 20.6.95 8 Days after cut.

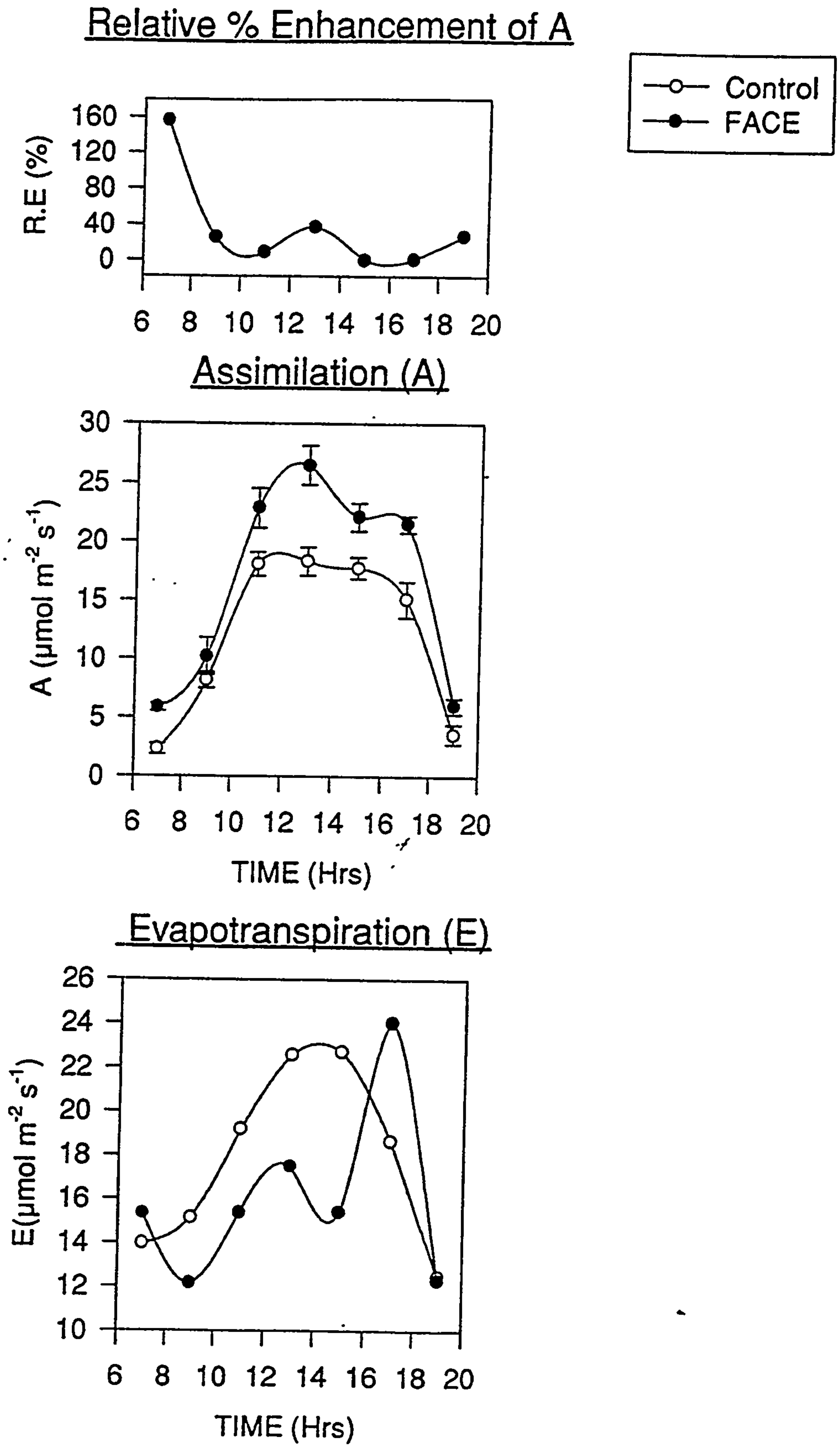
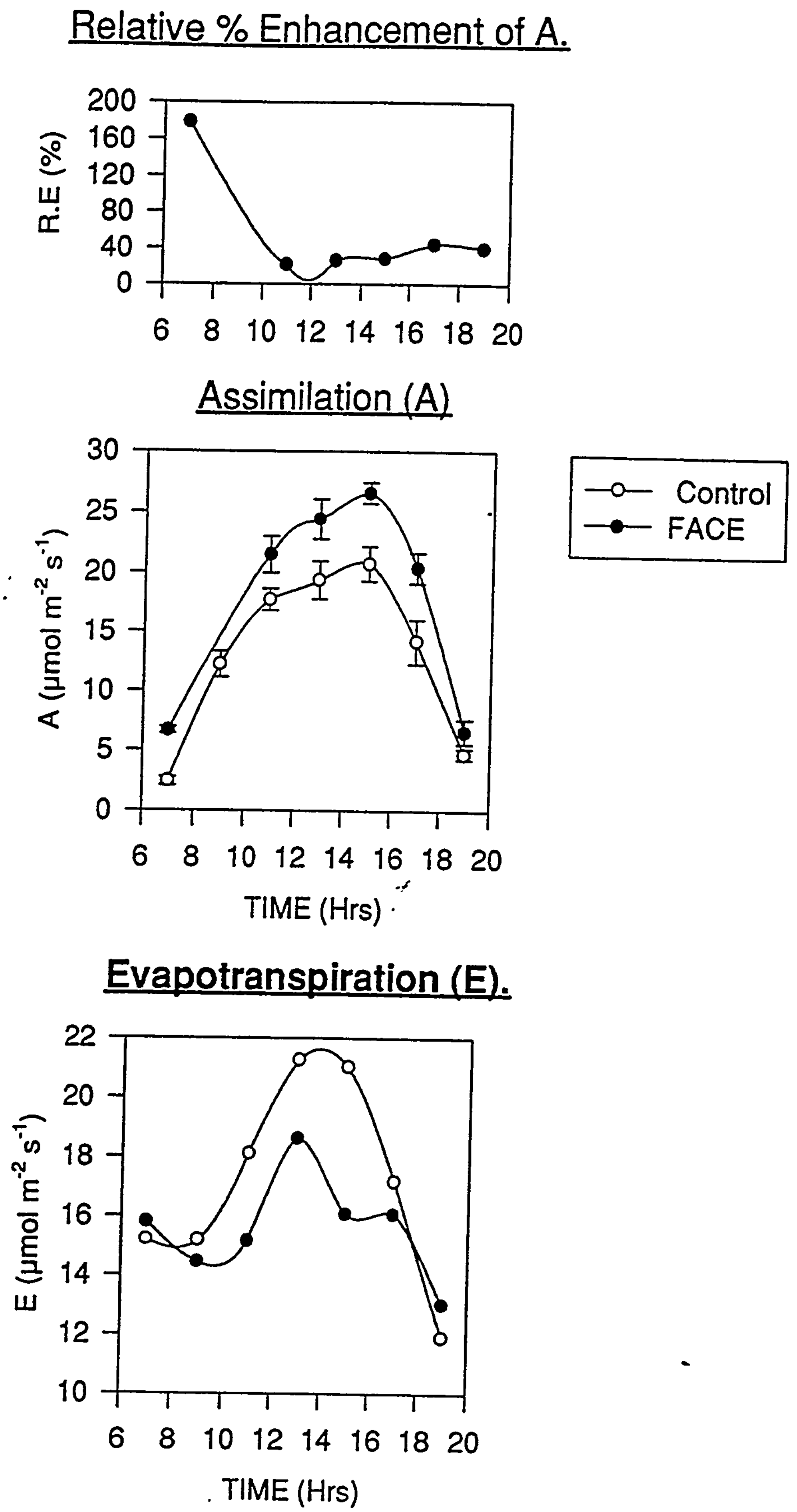


FIGURE 6. Diurnal Assimilation and Evapotranspiration rates for Control and FACE grown *L.perenne.* High nitrogen treatment. 20.6.95 8 Days after cut.

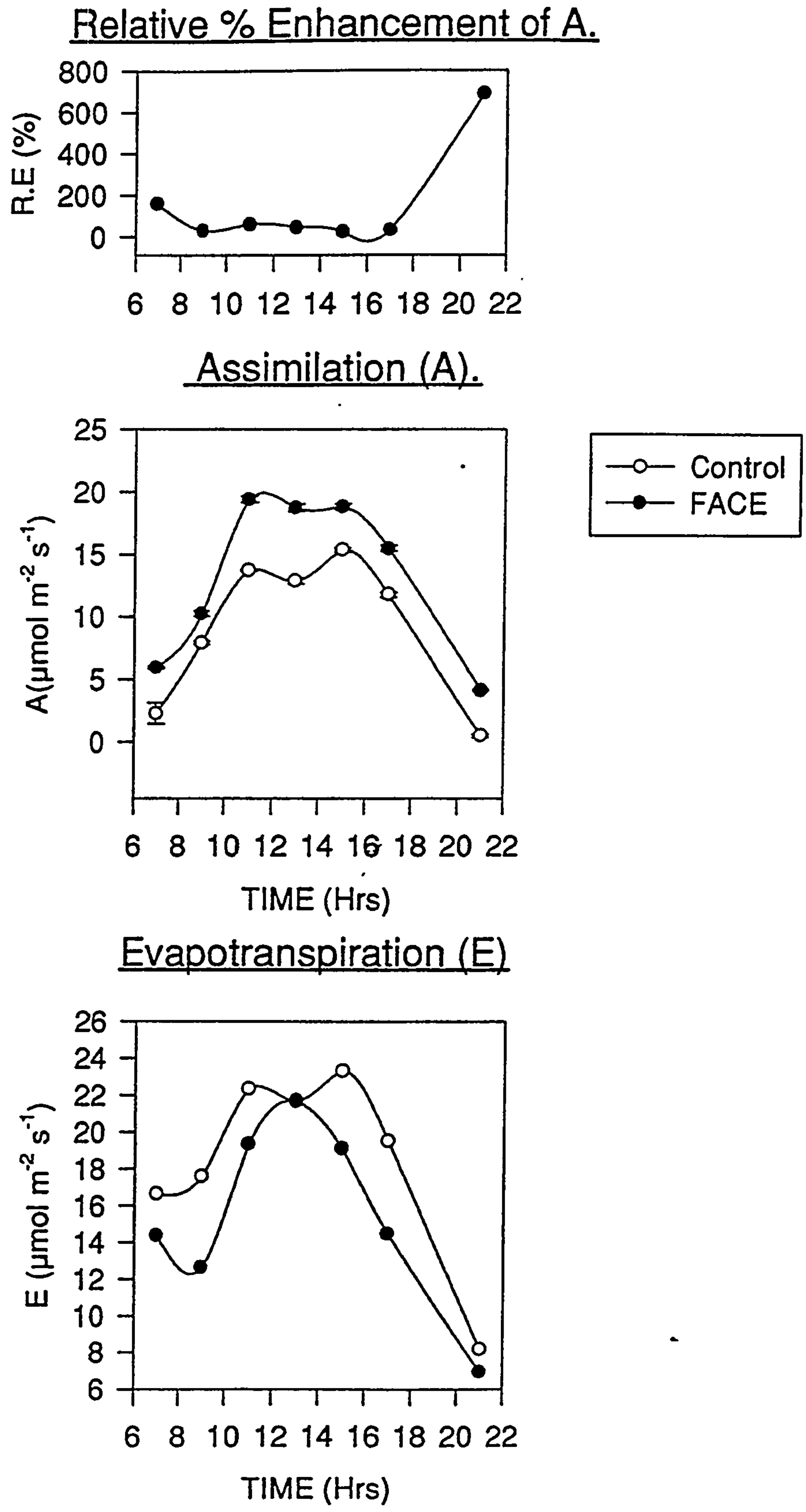


Relative % Enhancement of A.

Assimilation (A)

Evapotranspiration (E).

FIGURE 7. Diurnal Assimilation and Evapotranspiration rates for Control and FACE grown *L. perenne.* Low nitrogen treatment. 9.7.95. 1 Day before cut.

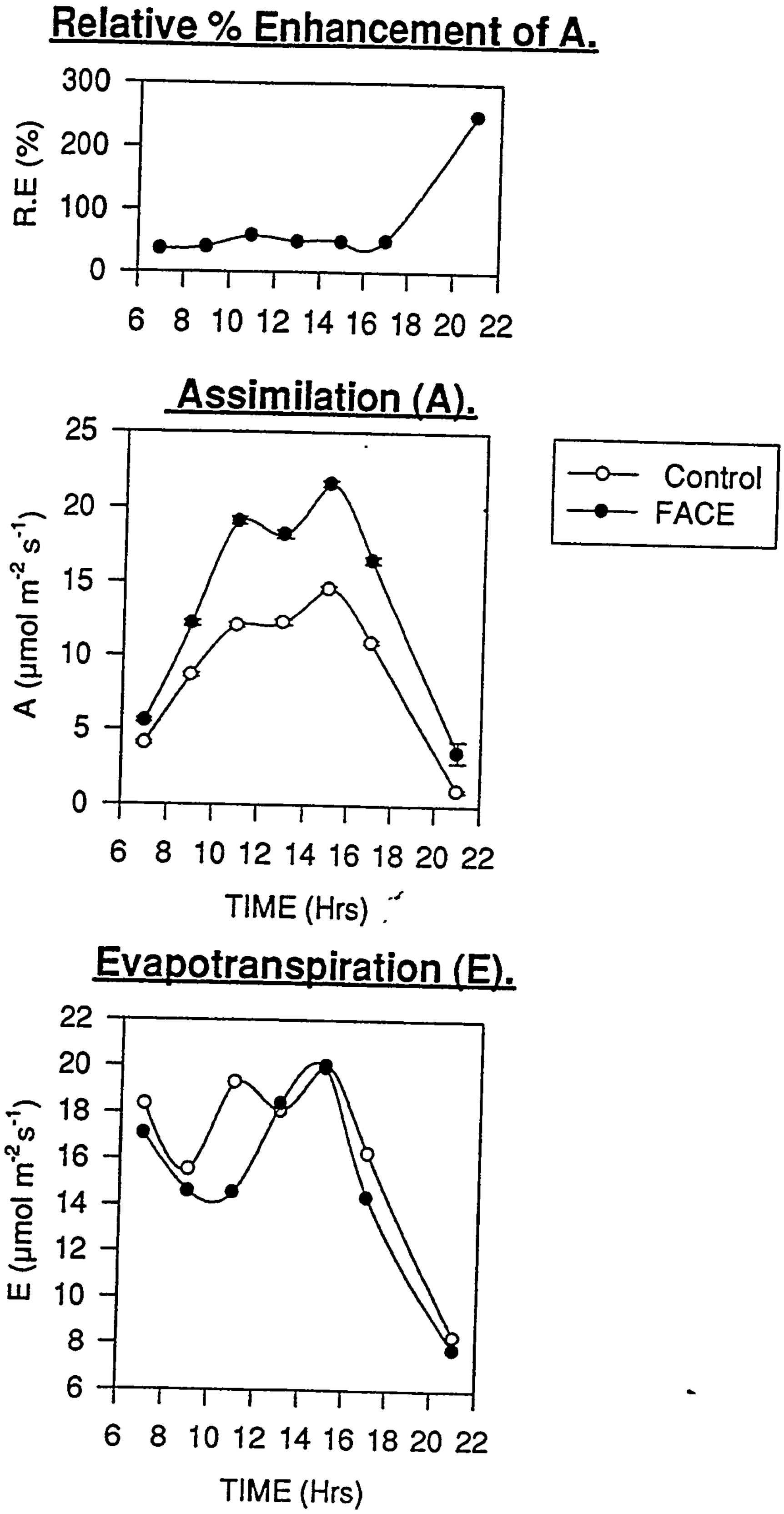


Relative % Enhancement of A.

Assimilation (A).

Evapotranspiration (E)

FIGURE 8. Diurnal Assimilation and Evapotranspiration rates for Control and FACE grown *L. perenne*. High nitrogen treatment. 9.7.95. 1 Day before cut.



Relative % Enhancement of A.

Assimilation (A).

Evapotranspiration (E).

hours of measurement preceding and following this time period when temperatures and PPFD were lower, a stimulation of A was still evidenced in the FACE plots on each occasion yet the stimulation was not significant (Table 5).

TABLE 4. Significant levels of variation in A due to CO_2 and Nitrogen **between 11:00 & 15:00**

VARIABLES	25th MAY 1995	20th JUNE 1995	9th JULY 1995
Nitrogen	P=0.052, F=33.45	P=0.50, F=0.51	P=0.84, F=0.044
CO_2	**P<0.05, F=5.20**	**P<0.05, F=23.47**	**P<0.05, F=59.32**
Interaction	P=0.982, F=0.001	P=0.695, F=0.166	P=0.31, F=1.86
n	3	3	3

TABLE 5. Significant levels of variation in A due to CO_2 and Nitrogen **before 11:00 and after 15:00**

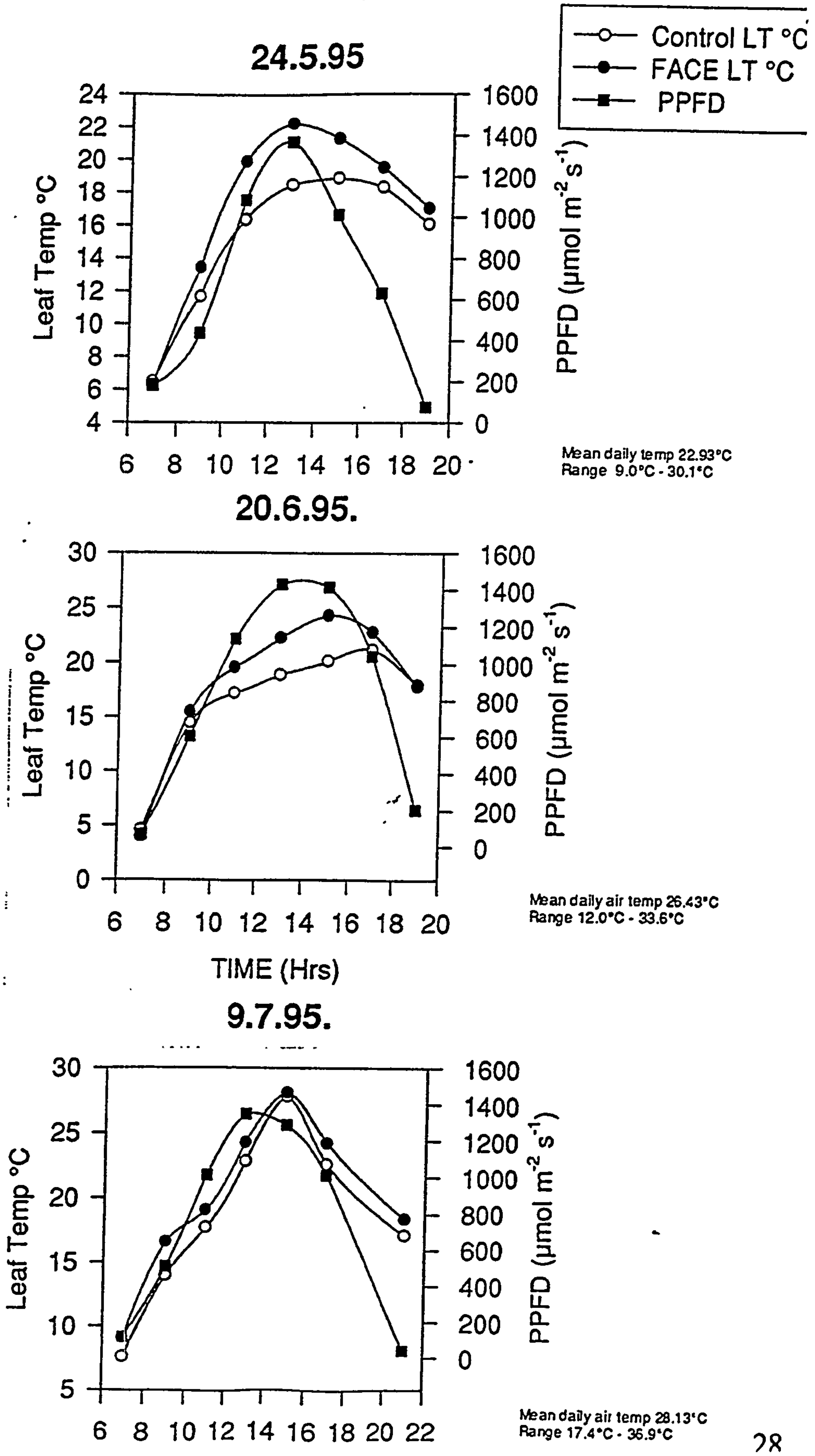
VARIABLES	25th MAY 1995	20th JUNE 1995	9th JULY 1995
Nitrogen	P=0.874, F=0.026	P=0.986, F=0.0003	P=0.831, F=0.047
CO_2	P=0.363, F=0.893	P=0.384, F=0.815	P=0.224, F=1.643
Interaction	P=0.853, F=0.036	P=0.915, F=0.012	P=0.991, F=0.0001
n	4	4	4

During neither of the two time periods on any of the dates was there a significant effect of nitrogen treatment on the stimulation of A in the FACE plots (Tables 4 & 5), Indeed in both the FACE and control plots there was little difference between peak A values and mean diurnal A values between nitrogen treatments . Not withstanding this fact on all three measurement days the greatest percentage enhancement of A was between the low nitrogen control and FACE plots (Table 3 Figs 3, 5 & 7), the largest being 44.95% on the 9th July. This day also saw the highest percentage enhancement between the high nitrogen FACE and control plots, 33.05 %. The 9th July was 27 days into the regrowth period, a time when some degree of sink limitation to photosynthesis would be expected compared to the previous two measurement days when the swards were less than 10 days into their regrowth. Whilst the percentage enhancement was greatest for both nitrogen treatments on the 9th July the actual values of A obtained were substantially lower than on the other two dates. The average diurnal value of A gained on the 9th July was 26.6% lower and 17.89 % lower than the values observed in the control and FACE plots respectively on the 20th June and 32.8 % and 36.4 % lower than the values gained on the 23rd May in the control and FACE plots.

As well as being further into the regrowth period the 9th July experienced the highest mean daily and maximum air and leaf temperatures, as well as PPFD comparable to the other two measurement days. (Figs 9) As a consequence it is likely that photoinhibition and carbohydrate feedback were limiting photosynthesis to some degree as well as longer term sink limitation.

With the exception of the low nitrogen treatment on the 9th July, *L.perenne* grown in the FACE plots experienced reduced rates of evapotranspiration expressed as a percentage over the course of the day (Table 3). These reductions ranged from 8.84 to 16.01% and were uniformly higher in the low nitrogen treatments, whilst also highest between 11:00 and 15:00 hrs when insolation and temperature were at there

FIGURE 9. Microclimate data for the three diurnal measurement days. Control and FACE leaf temperatures and PPFD.



Mean daily temp 22.93°C
Range 9.0°C - 30.1°C

Mean daily air temp 26.43°C
Range 12.0°C - 33.6°C

Mean daily air temp 28.13°C
Range 17.4°C - 36.9°C

highest levels, in both nitrogen treatments (Figs 3-8). A time period when growth in elevated C_a seemed to confer a clear advantage in terms of water conservation . Only on the 9th July was this not the case when the aforementioned weather conditions and possibly supraoptimal temperatures, seemed to have induced stomatal closure in the Control plots (Figs 7 & 8) reducing midday E to levels of the FACE grown plants.

A manifestation of these reduced rates of evapotranspiration from the FACE grown *L. perenne* is a consistent increase in the leaf temperatures of the FACE plots over the whole measurement period on each of the measurement days. This is attributable to the reduced rate of latent heat loss through evaporation for the FACE leaves and is again greatest during the 11:00 to 15:00 hrs time period. Except on the 9th July when the aforementioned midday early afternoon stomatal closure has reduced latent heat loss in the Control leaves (Fig 9).

3:2. A/Ci Results.

Values of $V_{c\,max}$ as indications of Rubisco activity and carboxylation efficiency were shown to increase significantly after a cut on two separate occasions in both control and FACE rings at both low and high nitrogen treatments (Table 6, Fig 10). Whilst the differences in $V_{c\,max}$ were significant between the days before and after both cuts, the increase was far smaller numerically after the second cut in both control and FACE low and high nitrogen treatments (Fig 10). The significance of the $V_{c\,max}$ values before and after the cut is not mirrored by any significant differences in $V_{c\,max}$ attributable to the effects of CO_2 treatment (TABLE 6). Only with respect to nitrogen treatments for measurements around the first cut is another significant difference in $V_{c\,max}$ values observed (Table 6).

FIGURE 10. Variations in Vc max , Pre, Post and Intra cuts for Control and FACE grown *L.perenne*, eight cut regime. a) Low Nitrogen, b) High Nitrogen.

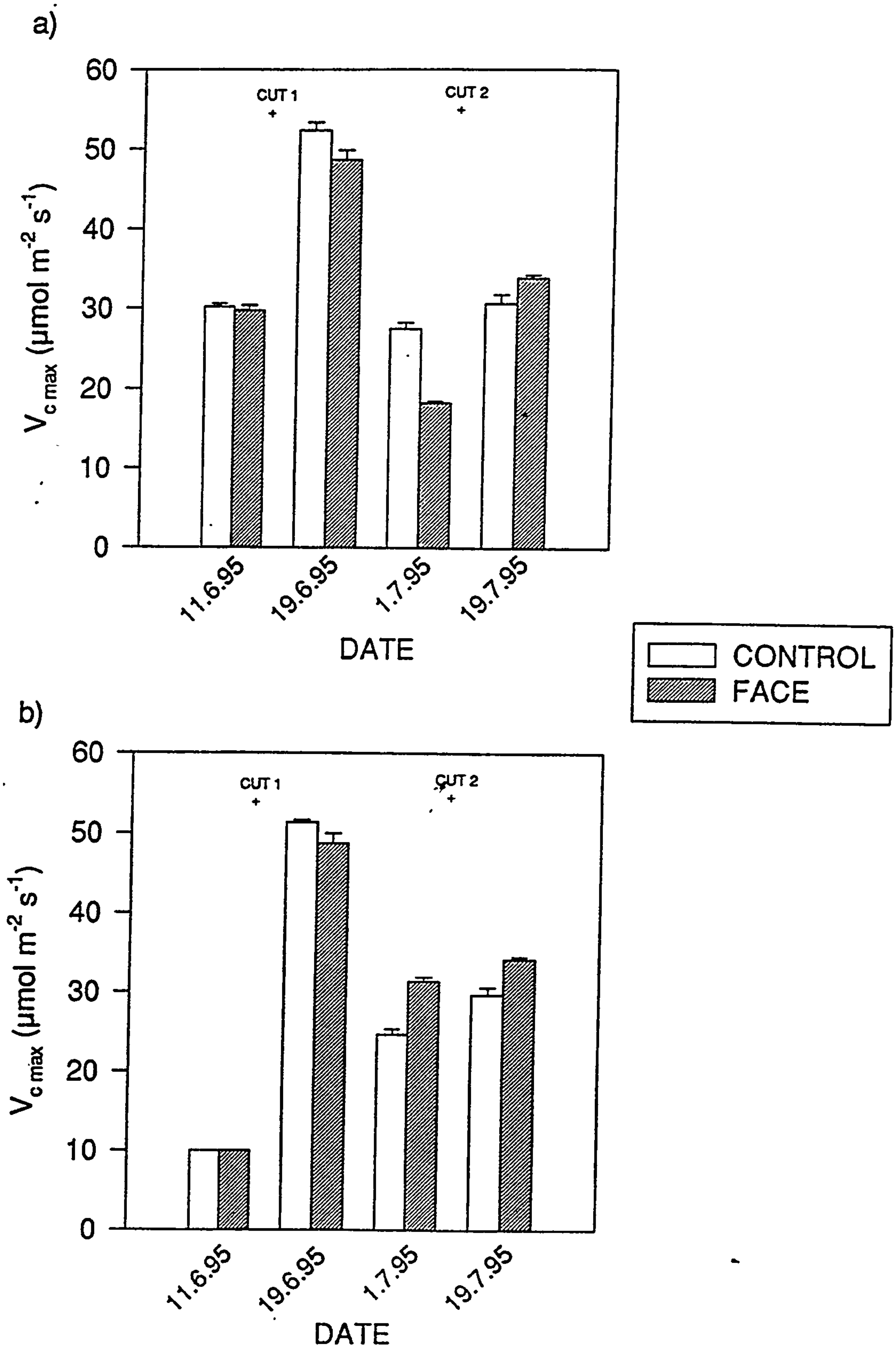


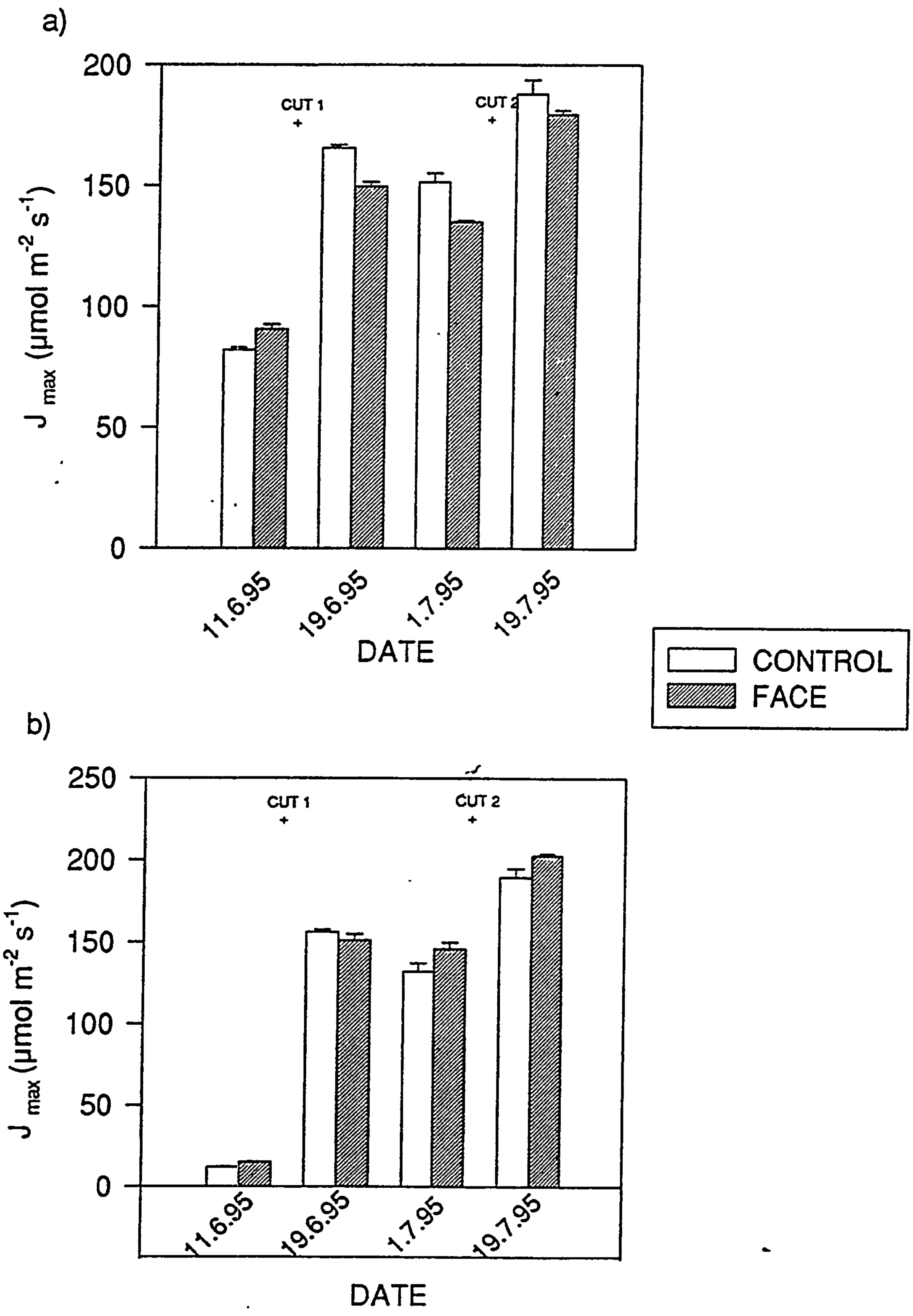
TABLE 6. Significant variation in V_{cmax} and J_{max} values due to CO_2 nitrogen treatment and measurement day pre or post cut.

VARIABLES	CUT 1 V_{cmax}	CUT 2 V_{cmax}	CUT 1 J_{max}	CUT 2 J_{max}
Day	**$P<0.05, F=90.2$**	**$P<0.05, F=11.2$**	**$P<0.05, F=337$**	**$P<0.05, F=14$**
CO_2	$P=0.53, F=0.40$	$P=0.42, F=0.67$	$P=0.96, F=0.00$	$P=0.99, F=0.0$
Day*CO_2	$P=0.65, F=0.21$	$P=0.33, F=1.01$	$P=0.43, F=0.63$	$P=0.9, F=0.02$
Nitrogen	**$P<0.05, F=7.06$**	$P=0.21, F=1.63$	**$P<0.05, F=21.7$**	$P=0.85, F=0.04$
Day*Nitrogen	**$P<0.05, F=9.23$**	$P=0.21, F=1.62$	**$P<0.05, F=40.6$**	$P=0.52, F=0.4$
CO_2*Nitrogen	$P=9.0, F=0.01$	$P=0.43, F=4.39$	$P=0.28, F=1.20$	$P=0.29, F=1.1$
Day*CO_2*N	$P=0.97, F=0.00$	$P=0.07, F=3.56$	$P=0.21, F=1.63$	$P=0.86, F=0.03$
n	6	4	6	6

The effect of cutting is just as pronounced for J_{max} which represents the maximum rate of electron transport and as such RubP regeneration. Again there was a highly significant increase in J_{max} values after each cut in both control and FACE plots and in both nitrogen treatments (Table 6, Fig 11). Again the increase was greatest after the first cut. Whilst there was no significant difference in V_{cmax} values attributable to CO_2 treatment. There was again a significant effect of nitrogen before and after the first cut.

Whilst CO_2 treatments confer no significant differences in either V_{cmax} or J_{max}, there is a significant effect of nitrogen before and after the first cuts for both V_{cmax} and J_{max}. The effect of nitrogen treatment appears enhanced when studying the regrowth period between the two cuts, from 19th June to 1st July. In the low nitrogen treatment

FIGURE 11. Variations in J max , Pre, Post and Intra cuts for Control and FACE grown *L.perenne*, eight cut regime. a) Low Nitrogen, b) High Nitrogen.



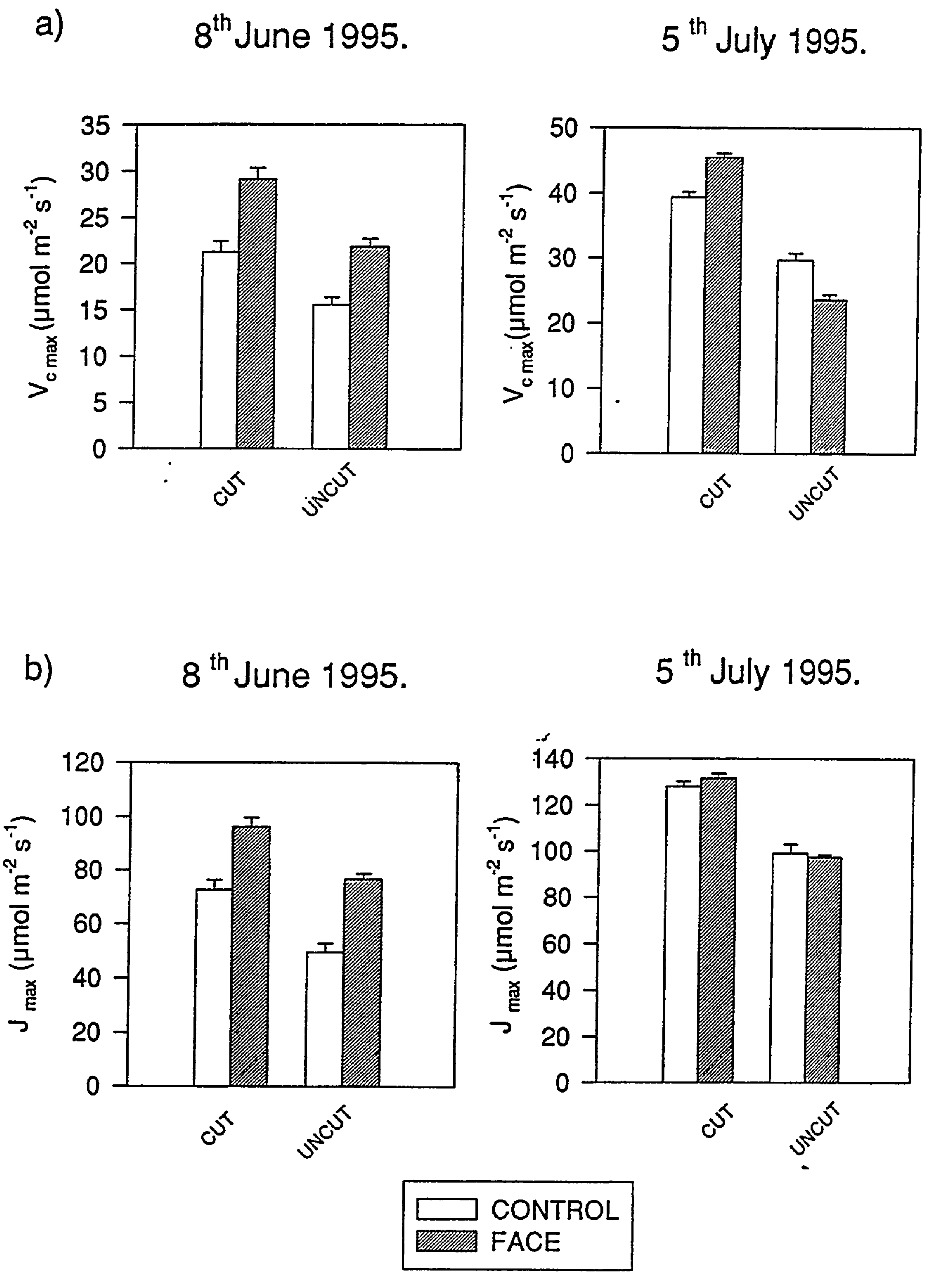
both V_{cmax} and J_{max} values are clearly lower in the face rings than the control rings. Immediately after the first cut V_{cmax} is 7% lower in the FACE rings than the control ring, prior to the second cut this figure rises to 33.58%. The same reduction is evidenced in the low nitrogen treatment for J_{max} values where the initial reduction in the FACE plots of 9.62% rises to 10.87% prior to the second cut (Fig 11). This effect is not observed in the high nitrogen treatments where V_{cmax} and J_{max} increase in the FACE plots relative to the control plots through the regrowth period.

3:3. Manipulation Experiment results.

The results of the manipulation experiment seem to confirm the findings of the A / Ci experiments. For both V_{cmax} and J_{max} values there was a significant difference in the values obtained in the uncut and cut plots during the second experiment on the 5th July 1995 when all six rings were sampled. With a significant enhancement of both sets of values occurring in both FACE and control plots in the cut areas. (Table 7, Fig 12). Also in agreement with the A / Ci findings is the lack of significant effect of CO_2 treatment on the results obtained on either day (Table 7, Fig 12).

Analysis of the relative enhancements of V_{cmax} and J_{max} between the two cutting treatments on both experimental dates also showed a lack of significant CO_2 effect (Table 8). In fact, the results clearly show that the enhancement of V_{cmax} and J_{max} in the cut FACE plots over the control plots is mirrored in the uncut plots where in experiment 1 the enhancement remains and in experiment 2 where the reductions are not significant (Fig 12). Given that this experiment was conducted solely in the low nitrogen plots it would be here where acclimatory downregulation of photosynthesis in

FIGURE 12. Variations in a) Vc max & b) J max for *L. perenne* measured on the same day, for a cut plot 5 days into regrowth and an uncut plot 22 days into regrowth. In low nitrogen Control and FACE plots.



response to sink saturation would be expected to be most evident.

TABLE 7. Significance levels of variation in V_{cmax} and J_{max} due to CO_2 and cutting on two days. Experiment 1, 8th June 1995. Experiment 2, 5th July 1995.

VARIABLES	V_{cmax} Expt 1	V_{cmax} Expt 2	J_{max} Expt 1	J_{max} Expt 2
CO_2	P=0.14, F=2.57	P=0.98, F=0.00	P=0.07, F=4.08	P=0.94,F=0.01
Cut/Uncut	P=1.33, F=2.59	P<0.05, F=14.3	P=0.12, F=2.87	P<0.05, F=6.8
Interaction	P=0.91,F=0.01	P=0.16, F=2.16	P=0.89, F=0.02	P=0.84,F=0.04
n	4	6	4	6

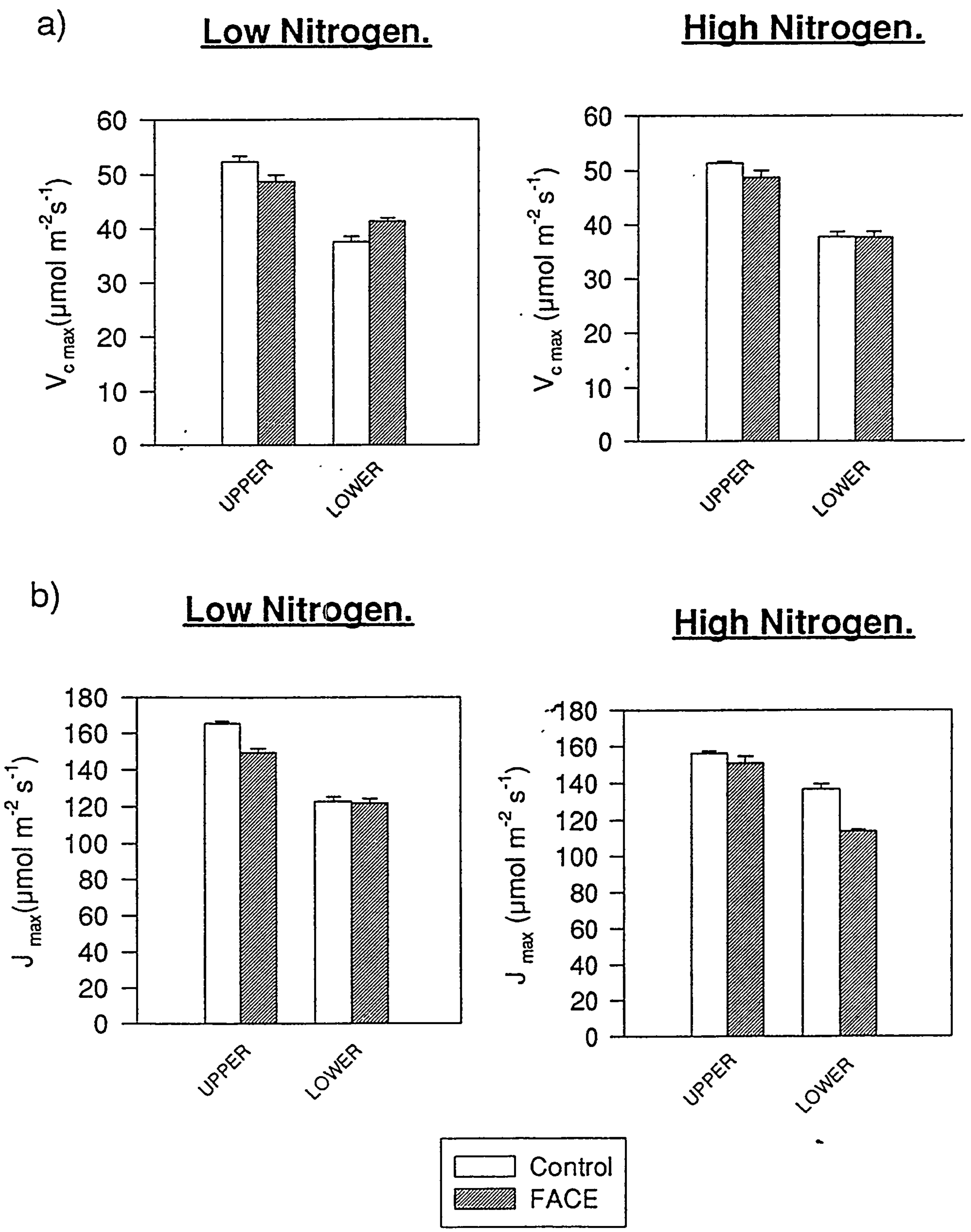
TABLE 8. Significant levels of variation in the relative enhancement of V_{cmax} and J_{max} due to CO_2 and cutting on two days (As in Table 7).

VARIABLE	V_{cmax} EXPT 1	V_{cmax} EXPT 2	J_{max} EXPT 1	J_{max} EXPT 2
CO_2	P=0.74, F=0.07	P=0.18, F=2.19	P=0.43, F=0.72	P=0.55,F=0.39
n	4	6	4	6

3:4. Canopy Experiment Results.

As would be expected there was a significant downregulation of both V_{cmax} and J_{max} values in both the control and FACE plots when the leaves were older and positioned lower in the canopy, measured on the 27th June 1995, in contrast to the initial measurements taken on the 19th June 1995.. This effect was observed in both the low and high nitrogen treatments. (Table 9, Fig 13). The results do not show any significant influence of CO_2 treatment (Table 9). Further analysis of the relative declines in V_{cmax} and J_{max} of the leaves on the second measurements day compared to

FIGURE 13. Variations in a) Vc max & b) J max measured on two occasions on the same leaves. When the leaves were in the upper canopy 7 days into regrowth . Then 8 days later when the same leaves are in the lower canopy.



the first again shows no significant influence of CO_2 treatment or nitrogen treatment on the values obtained. (Table 10).

TABLE 9. Significant variations in V_{cmax} and J_{max} due to CO_2 and leaf age / canopy position for two nitrogen treatments.

VARIABLES	V_{cmax} Low N	J_{max} Low N	V_{cmax} High N	J_{max} High N
CO_2	P=0.98, F=0.00	P=0.44; F=0.63	P=0.76, F=0.09	P=0.27, F=1.3
Leaf Age	P<0.05, F=5.4	P<0.05, F=10.9	P<0.05, F=7.6	P<0.05, F=5.2
Interaction	P=0.45, F=0.59	P=0.49, F=0.49	P=0.78, F=0.08	P=0.48, F=0.5
n	6	6	6	6

TABLE 10. Significant variations in the relative percentage decline of V_{cmax} and J_{max} due to CO_2 and

nitrogen for the same leaves through canopy regrowth.

VARIABLES	V_{cmax}	J_{max}
CO_2	P=0.28, F=1.26	P=0.83, F=0.05
Nitrogen	P=0.94, F=0.01	P=0.51, F=0.46
Interaction	P=0.98, F=0.00	P=0.52, F=0.42
n	6	6

The only indications of an interactive effect of CO_2 and nitrogen treatments are when the relative percentage declines in the $V_{c\ max}$ and J_{max} values are calculated based on average figures from the leaves sampled. Then we see higher percentage decreases in the FACE treatments than the control treatments for each combination of variables except high nitrogen J_{max} values . This analysis also suggests that for the FACE treatments downregulation is higher in the high nitrogen treatment. (Table 11).

TABLE 11. Percentage declines in average $V_{c\,max}$ and J_{max} values for two CO_2 and two nitrogen treatments.

	$V_{c\,max}$ FACE	$V_{c\,max}$ Control	J_{max} FACE	J_{max} Control
Low Nitrogen	15.25	28.32	18.55	25.80
High Nitrogen	22.0	26.48	24.63	12.50

3:5. Diurnal A/Ci results.

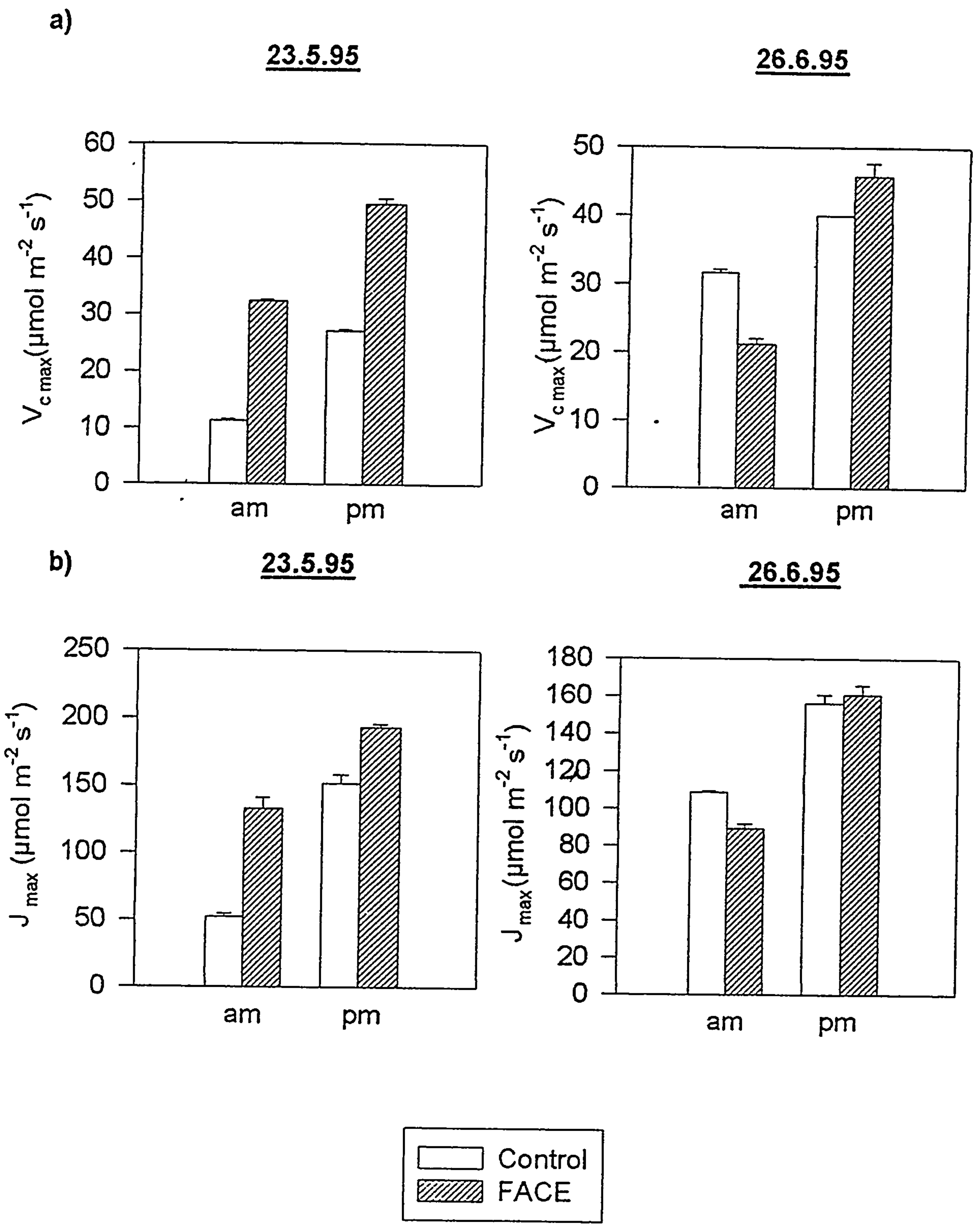
The results of this experiment show a significant difference in both $V_{c\,max}$ and J_{max} values obtained before 08:00 hrs and after 15:00 hrs on both the experiment days (23rd May & 26th June). On both days there was a significant increase in the values obtained for both control and FACE plots (Table 12, Fig 14)

TABLE 12. Significant levels of variation in $V_{c\,max}$ and J_{max} between two time periods. Before **08:00 and after 15:00 hrs** on two separate measurement days .

Variables	$V_{c\,max}$ 23.5.95	J_{max} 23.5.95	$V_{c\,max}$ 26.6.95	J_{max} 26.6.95
CO_2	P=0.62,F=0.26	P=0.64,F=0.23	**P<0.05,F=95.6**	**P<0.05,F=16.36**
Time	**P<0.05,F=12.5**	**P<0.05,F=15.65**	**P<0.05,F=55.3**	**P<0.05,F=27.91**
Interaction	P=0.11,F=3.05	P=0.43,F=0.66	P=0.76,F=0.09	P=0.22,F=1.69
n	4	4	4	4

The results also showed a significant effect of CO_2 on the 26th June with a significant increase in $V_{c\,max}$ and J_{max} in the FACE plots over the increase in the control plots (Table 12). Indeed the enhancement of the average $V_{c\,max}$ and J_{max} values in the control plots on the 26th June was 26.44% and 43.28% respectively. In the FACE plots the enhancement was 116.71% for $V_{c\,max}$ and 80.23 % for J_{max}. On the 23rd May the

FIGURE 14. Variations in a) $V_{c\,max}$ and b) J_{max} measured on the same leaves at two times of the day. Before 08:00 hrs (am) and after 15:00 hrs (pm).



enhancement was greater in the control plots, $V_{c\,max}$ values increased by 139 % in the afternoon in the control plots whilst J_{max} increased by 190%. Corresponding increases in the FACE plots were 53.1 % for $V_{c\,max}$ and 45.17% for J_{max}.

4. Discussion.

The enhancement of A observed during the diurnal set of experiments on *L.perenne* grown in elevated C_a, ranged from 26.51% to 44.95% over the course of a diurnal set of measurements. Such findings are in agreement with previous work on *L.perenne* single leaves (Nijs *et al* 1989, Ryle *et al* 1992,Gay & Hauck 1993). They also correspond with the normal and expected pattern of C_3 crops photosynthesising in elevated C_a (Kimbal 1983, Long 1985). Indeed the "Clear and Unequivocal" enhancement of A described by Ryle *et al* (1992) could equally well describe this studies findings.

Over the course of the three day's measurements the largest and significant increases in A between control and FACE rings corresponded to the period of the day when temperatures were at their highest. Whilst in ambient C_a there is a reduction in carboxylation relative to oxygenation as temperature rises, as a consequence of the decreased specificity of Rubisco for CO_2 and the reduced solubility of CO_2 (Jorden & Ogren 1984). These effects are offset in elevated C_a. As the key effect of increased C_a is increased competitive inhibition of RubP oxygenation (Long 1991, Kirshbaum 1994), it follows that proportionate increases in photosynthesis resulting from elevated C_a will rise with temperature. This study confirms these predictions and is in agreement with other findings demonstrating this principle (Sage & Sharkey 1987). The increasing burden of oxygenation in plants grown in ambient C_a is not mirrored in FACE grown plants whose temperature optimum of photosynthesis also shifts upwards (Sage & Sharkey 1987).

A related discovery of this study is the consistent increase in the temperature of the leaves of the FACE grown *L.perenne* over and above those grown in the control plots. This effect has been predicted by Long (1985) and is attributed to a reduction in evaporative latent heat loss from leaves and plants grown in elevated C_a. A consequence of commonly observed decreases in stomatal conductance (Valle *et al* 1985, Idso *et al* 1987). This effect was observed previously by Bryant (1994) for *L. perenne*, and would be expected to confer the aforementioned benefits for plants photosynthesising in elevated C_a at higher temperatures even if the air temperature does not increase.

Whilst not significant this study observed consistent enhancement of A during periods of the day when PPFD and temperature were lower. Namely the beginning and end of the sunlight hours of the day, mirroring findings the previous year on the same crop (Bryant 1994). This finding is in agreement with the general predictions of Long & Drake (1992) that stimulation of photosynthesis will occur even at lower light intensities, but is in contrast to previous observations on *L. perenne* (Nijs *et al* 1989, Ryle *et al* 1992). Nijs *et al* (1989) site the increased structural components associated with growth in elevated C_a as a disadvantage at lower irradience but of far greater benefit at higher irradience.

This study revealed no significant differences in A as a result of differing nitrogen treatment levels or nitrogen CO_2 interaction. With little differences in maximum A values achieved between FACE and control plots on any of the days.

Trends observed over the three day's measurements suggest that percentage enhancement of A in FACE plots over control plots was consistently greater in the low nitrogen treatment. Such a finding is juxtaposed to the theory that nitrogen supply, if sub-optimal, will be insufficient to maintain plant growth at a level where it can utilise the additional carbohydrate produced by plant growing in elevated C_a (Wong1979, Larigauderie *et al* 1988, Vessey *et al* 1990). The end result being sink limitation and carbohydrate accumulation leading to feedback inhibition of photosynthesis (Mauney *et al* 1979, Clough *et al* 1981, Ehret & Jolliffe 1985). These results tend to support the claims of Sinclair (1992) that a far more complex interactive relationship exists between nitrogen fertilisation and $C0_2$. The absence of any downregulation of A in the low nitrogen plots has been observed previously by Sage *et al* (1990). Analysis of the A/Ci response curve has revealed that at elevated C_a, where Rubisco levels are in excess of those required to maintain photosynthetic performance, up to 30 % could be re-apportioned without any reduction in photosynthetic capacity (Long & Drake 1992) . These results contrast those on the same crop the previous year when the enhancement of A was greater in the high nitrogen treatment (Bryant 1994). It was speculated last year that this was due to the denser high nitrogen canopy not reaching its full photosynthetic potential. For the measurements taken this year on the 24th May and 20th June when the canopies were only 9 and 8 days respectively into regrowth such a proposal would not seem valid. At low light levels or low temperature any depression of photosynthetic performance resulting from sub-optimal nutrient supply would be expected to be reduced (Bunce 1992).

These results evidence a clear decline in A as regrowth continues (9th July) with maximum values of A obtained noticeably lower than the A values attained on the measurement days immediately after the cut (24th May & 20th June) . These findings are consistent with the findings that the maximum rate of individual leaf photosynthesis of successive youngest fully expanded leaves of *L. perenne* declines as the vegetative sward develops (Leafe *et al* 1976, Sheehy 1977). Whilst supporting the notion of Nijs & Impens (1993) that the effective use of cutting as a management tool could yield higher increases in seasonal production in conditions of elevated C_a when the Ryegrass stands would be in the younger stages of growth, when response to CO_2 is largest. At the same time the enhancement of A in the FACE plots in both nitrogen treatments was higher than the previous two days. Current thought would lead us to believe that the further into the regrowth period the crop is, the greater will be the effects of photosynthetic disruptions as a consequence of carbohydrate feedback inhibition in the FACE plots. Closer inspection of the results points to a far greater downregulation of A in the control plots being the cause of this enhancement not an increase in A in the FACE plots. The downregulation of A between the 24th May and the 9th July in the control plots was 26.6 % compared to 17.89% in the FACE plots. Compared to the 20th June the downregulation in the control plots was 32.8% whilst in the FACE plots it was 26.4 %. This greater downregulation in the control plots is a likely consequence of the supra-optimal temperature experienced on the 9th July and the midday stomatal closure more evident in the control plots on that day.

The increased water use efficiency of plants grown in elevated CO_2 would seemingly explain this phenomenon. Evapotranspiration was consistently, yet not exclusively lower in the FACE plots than the levels in the control plots on the three measurement days . Proposals that plant water use efficiency commonly increases with elevated C_a (Morison 1993), largely as a result of reductions in stomatal aperture (Morison 1987), would seemingly explain the lower rates of evapotranspiration observed in this study for *L. perenne* grown in elevated C_a. Reduced daytime evapotranspiration is but one factor affecting the water use efficiency of a crop, water use efficiency that has been observed to increase in near unity to increasing C_a in *L. perenne* (Nijs *et al* 1989).

The increased temperature optimum for photosynthesis and improved water use efficiency predicted and observed for C_3 crops such as *L. perenne* would seemingly equip such crops well for predicted atmospheric changes. Unfortunately any further terrestrial or atmospheric feedback effects associated with increased temperature and atmospheric CO_2 increase could negate such benefits.

Given that the *L. perenne* crop studied was in its third year of growth in elevated C_a the results obtained provide a firm basis for determining the extent to which the long term acclimatory response (defined on pg 6) will negate the substantial sort term increases in photosynthesis by C_3 plants growing in elevated C_a. From the results obtained by this study it would have to be concluded that there is little evidence to suggest that after three years of growth in elevated C_a of 600 µmol mol^{-1} CO_2 that the photosynthetic capacity of plants grown in elevated C_a has downregulated with respect to plants grown in ambient C_a. Such a conclusion would appear to be in agreement with the findings of Arp (1991) who suggested that downregulation of the photosynthetic capacity of crops in response to growth in elevated C_a was primarily an artefact of experimental constraints. It is also in agreement with Sage (1994) who reviewed field studies since 1986 to conclude that there was a lack of acclimation of field grown crops to elevated C_a. At the same time this study is at odds with previous work on *L. perenne* which showed acclimation in response to long term growth in elevated C_a (Moon *et al* 1990, Ryle *et al* 1992), and indeed with work on the same crop 1 year previously that showed "Clear evidence of acclimation" (Bryant 1994). The controlled environments in which the experiments of Ryle *et al* (1992) and Moon *et al* (1990) were conducted may explain the apparent contradiction of the results.

The results of the 4 A / Ci experiments conducted either side of the two cuts showed no significant differences in values of $V_{c\ max}$ and J_{max} obtained as a result of growth in different CO_2 treatments. In contrast the advent of cutting greatly relieved the pre-cut depression of both $V_{c\ max}$ and J_{max} values, significantly increasing them for both CO_2 treatments. Depression would have been brought on by a combination of increasing leaf age and sink limitation. This was relieved by cutting and countered by the post cut need to ensure quick and effective re-establishment of the canopy.

Whilst there was not a significant CO_2 effect there was a substantial downregulation of $V_{c\ max}$ and to a lesser extent J_{max} values in the low nitrogen treatments on the 1st July for the FACE rings with respect to the control rings. Such a discovery if it were significant would be consistent with the aforementioned theory (pg 9) that when nitrogen supply or translocation are insufficient to support the growth rate of the plant, leaf nitrogen amounts start to fall below those levels associated with more optimal supplies of nitrogen with a corresponding decrease in photosynthetic capacity (Evans 1989).

These findings appear to be enforced by the results of the manipulation experiment conducted in the low nitrogen plots only, where theory would suggest that acclimation will be most extreme. Values of $V_{c\ max}$ and J_{max} obtained in both the cut and uncut plot were at no time significantly different as a response to growth in different CO_2 treatments or as a result of an interaction between CO_2 and the different cut status's of the plots. This conclusion was reached based on the actual values of $V_{c\ max}$ and J_{max} obtained and on analysis of the relative enhancement of the two sets of values between the cut and uncut plots. At the same time significant differences in $V_{c\ max}$ and J_{max} were obtained as a consequence of the manipulation of the source sink balance. As would be expected and was evidenced in the previously described A / Ci experiments. The removal of sink limitation in the cut sections of the plots used where measurements were taken 5 days into regrowth exhibited significantly higher values of $V_{c\ max}$ and J_{max} than the areas of the plot that were 23 days into the regrowth cycle, on the 5th July when all six rings were sampled. On the 8th June when only four rings were sampled the results did not achieve significance.

The canopy experiment allowed the way in which leaf ageing and shading affect acclimation to be studied. As canopy regrowth advances the initially measured young leaves at the top of the canopy will age and become shaded to some extent as they migrate down through the canopy profile. As well as the photosynthetic performance of individual leaves of *L.perenne* declining with age (Leafe *et al* 1976, Sheehy 1977), where light is limiting the photosynthetic capacity of the shaded leaves photosynthesis will be most limited by the supply of light dependent ATP and NADPH for RubP regeneration . Whilst Rubisco levels or activity will be in excess. A situation that mirrors that in elevated CO_2 . It has been proposed by Evans (1989) that such an excess of Rubisco in a shaded lower canopy will be countered by plants, by adapting and re-apportioning more leaf nitrogen into the thylakoids and chlorophyll away from Rubisco. Whilst the results of the canopy experiment show significant declines in both $V_{c\ max}$ and J_{max} of single leaves as canopy regrowth advances there was again no significant effect of CO_2 treatment on the values obtained. Whilst this would seem to mirror the findings so far that the FACE grown *L. perenne* is exhibiting no signs of acclimation, for this experiment we must consider the structure of the ryegrass canopy. It has been described how the combination of the low leaf area index in the top strata of the canopy and the vertical distribution of the youngest leaves (extinction co-eff (k) = 0.5) means that photosynthetically active radiation can penetrate deeply into the crop stand and canopy photosynthesis can be spread over a large area of leaf (Hay & Walker 1989). This would limit the reductions in $V_{c\ max}$ and J_{max} values in both the FACE and control plots as shading would not be expected to be such a consideration. If we also consider the fact that the results show higher downregulation of $V_{c\ max}$ and J_{max} in the low nitrogen plots for the control grown plants whilst down regulation is higher in the high nitrogen treatment in the FACE plots. We can speculate that in the control plots leaf age is more of a determining factor than shading that would be expected to be minimal in the low density low nitrogen canopy. Whilst the higher downregulation in the high nitrogen treatments of the FACE plots supports the view that the interactive effects of elevated CO_2, shade and high nitrogen have resulted in excess Rubisco levels that is re-apportioned, or its activity reduced as the canopy develops.

The final experiment designed to test for acclimation in the FACE plots was the diurnal A / Ci experiment. For the two experiments conducted on the 23rd May and the 26th June the expected downregulation of the $V_{c\,max}$ and J_{max} values in response to carbohydrate feedback between 15:00 hrs and 17:00 hrs did not occur. In fact there was a statistically significant enhancement of $V_{c\,max}$ and J_{max} on both measurement days in the afternoon measurement period. This increase was higher in the FACE plots for both sets of values on both days, significantly so for the measurements on the 23rd May. It must be borne in mind that as well as feedback inhibition of photosynthesis the plants used would potentially have been subject to some magnitude of photoinhibition. A phenomenon that plants grown in elevated C_a potentially have some protection against possibly giving some explanation for the greater enhancement of $V_{c\,max}$ and J_{max} observed in the FACE plots. Whilst there appears to have been no evidence of carbohydrate feedback inhibition on these two measurement days exposure of the leaf in the leaf chamber to a very low O_2 concentration of 1% would evidence inorganic phosphate (Pi) limitation as a horizontal line on the A / Ci response curve at higher Ci values.

5. Conclusion.

It is clearly possible to reconcile the substantial stimulation of A during the diurnal sets of measurements for *L.perenne* grown in the FACE rings with the apparent lack of acclimation of the photosynthetic apparatus of *L. perenne* to the long term exposure to elevated C_a. Studies have suggested that typically plants will undergo a succession of adaptive responses of the photosynthetic mechanism to growth in elevated C_a. Initially there will be a downregulation of the non limiting processes at high C_a, namely RubP carboxylation , to create a balance with the limiting process RubP regeneration (Long & Drake 1992). Next it is proposed that these resources released by this downregulation , namely leaf nitrogen, will be re-apportioned to the limiting processes of photosynthesis at elevated C_a (RubP regeneration). The period of time this takes to occur is of paramount importance for perennial crops.

Whilst more work is needed to firmly establish whether such a complete acclimation of the photosynthetic apparatus occurs. It could be suggested from this data that after three years of growth in elevated CO_2 that the crop of *L. perenne* studied is approaching if not at the stage where this re-apportioning of the resources of the non limiting processes to the limiting processes has been achieved. The fact that this study has observed no significant downregulation of J_{max} values, in FACE grown *L.perenne*, with respect to control plots, would support such a claim that the initially limiting capacity for RubP regeneration has now been upgraded. Fundamental to such a transformation would be the ability of the crop to overcome long term sink limitation. The restriction of tillering ability of *L. perenne* at high light intensities (low red : far red radiation ratio) (Deregibus *et al* 1983) and the fact that *L.perenne* is know to partition relatively more of additional photosynthate produced in elevated C_a into storage rather than growth of new sinks (Wardlaw 1982), have been cited as factors limiting the ability of *L. perenne* to generate new sinks. What may be more pertinent is the fact that after three years of growth in elevated C_a *L. perenne* may have generated a root system large enough that it does not experience prolonged sink limitation. This area of study revealing root responses to elevated C_a of *L .perenne* would perhaps enforce or dispel such a claim.

Whatever the causes, this study has evidenced a prolonged, considerable enhancement of photosynthesis and a lack of acclimatory downregulation of photosynthetic capacity of *L. perenne* grown in elevated C_a. These factors suggest that over the longer term, in a future high CO_2 world, seasonal enhancement in productivity could be attained, as could extensions of the growing season for *Lolium perenne*.

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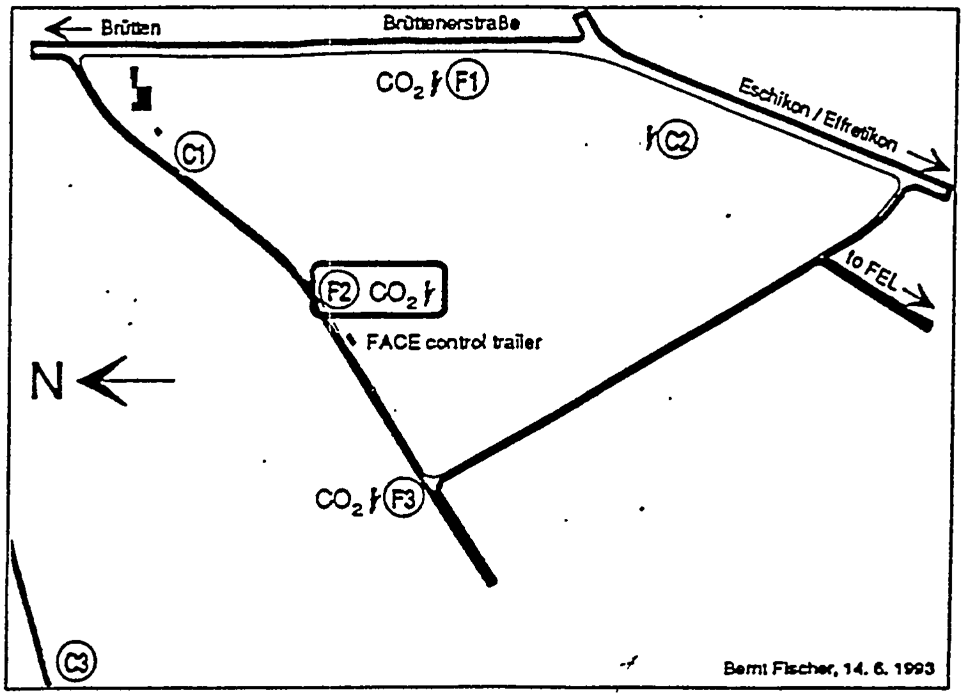
APPENDIX 1.


Brütten
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CO2 F1
C1
C2
Eschikon / Elfretikon
to FEL
F2 CO2
FACE control trailer
N
CO2 F3
C3
Bernt Fischer, 14. 6. 1993

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