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Informal Report

The Photosynthetic Response of the Perennial Ryegrass (*Lolium perenne*) in its Fifth Year of Free-air CO_2 Enrichment (FACE) at Eschikon, Switzerland.

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ABSTRACT.

Stands of Ryegrass (*Lolium perenne* L. cv.Bastion) were grown in the field at ambient or elevated (600 μmol mol^{-1}) [CO_2], high (560 kg Ha^{-1} y^{-1}) or low (140 kg Ha^{-1} y^{-1}) nitrogen addition and were harvested five times a year during the growing season. The plants were sown during 1992, additional plots being sown during 1995. These were in their fifth year and second year of growth respectively. Exposure to elevated [CO_2] was carried out with a Free-Air CO_2 Enrichment (FACE) system which provides the most 'realistic' system of fumigation currently available.

Elevated [CO_2] increased diurnal CO_2 uptake by between 40 to 83% whilst reducing stomatal conductance by between 1 and 38% in all of the 1992 grown plants measured at high [CO_2]. Analysis of the A/c_i response of 1992 grown plants showed no acclimation of the photosynthetic apparatus in response to elevated [CO_2] - both $V_{c,max}$ (a measure of the maximum *in vivo* rate of carboxylation) and J_{max} (a measure of the maximum capacity for the regeneration of RuBP) showed no significant change during any of the periods of regrowth. In contrast the leaves of 1995 grown plants, appeared to be experiencing an acclimatory change in their photosynthetic apparatus in response to elevated [CO_2]. However, this negative response seemed to be removed directly after a harvest when the source:sink balance had increased. The apparent lack of an acclimatory response after almost 5 years of growth at elevated [CO_2], suggests that *L. perenne* may be close to achieving the appropriate photosynthetic adjustments which would allow it to attain a significantly higher photosynthetic potential.

ABBREVIATIONS USED IN THE TEXT.

A = CO_2 uptake per unit leaf area (μmol m^{-2} s^{-1}); $\mathbf{c_a}$= CO_2 concentration in the air (μmol mol^{-1}); $\mathbf{c_i}$ = Intercellular CO_2 concentration (μmol mol^{-1}); **[CO_2]** = CO_2 concentration (μmol mol^{-1}); **FACE** = Free Air CO_2 Enrichment; $\mathbf{g_s}$ = stomatal conductance per unit leaf area (mmol m^{-2} s^{-1}); $\mathbf{J_{max}}$ = maximum RuBP regeneration capacity (μmol m^{-2} s^{-1}); **PPFD** = photosynthetic photon flux density (μmol m^{-2} s^{-1}); **RubisCO** = ribulose bisphosphate carboxylase oxygenase, **RuBP** = Ribulose bisphosphate; $\mathbf{V_{c,max}}$ = maximum rate of carboxylation (μmol m^{-2} s^{-1}).

TABLE OF CONTENTS.

1. **INTRODUCTION** 2

2. **METHODS AND MATERIALS** 10

2.1. Growth Conditions 10
2.2. Gas Exchange Measurements 13
2.2.1. Diurnal gas exchange measurements 14
2.2.2. A/c_i response measurements 16

3. **RESULTS** 19

3.1. Diurnal Gas Exchange Measurements 19
3.2. A/c_i Response of *Lolium perenne* 27
3.2.1. A/c_i response of 1992 grown stands 27
3.2.2. A/c_i response of 1995 grown stands 35

4. **DISCUSSION** 42

5. **ACKNOWLEDGEMENTS** 52

6. **REFERENCES** 53

APPENDIX A. Layout of the Swiss-FACE facility.

APPENDIX B. Combined infra-red gas analyser, leaf cuvette, and light source.

APPENDIX C. Equations for calculating gas exchange parameters.

APPENDIX D. Condition of 1992 FACE grown *L. perenne* stands during regrowth.

APPENDIX E. Micrometeorological data obtained over the measurement period.

Floppy Disk A. Raw data - Diurnal gas exchange measurements (1992 stands only).

Excel 7 files entitled ; Diur1 (measurements made on the 02/07/97)
Diur2 (measurements made on the 16/07/97)
Diur3 (measurements made on the 30/07/97)
Diur4 (measurements made on the 10/08/97)
Diur5 (measurements made on the 18/08/97)

Floppy Disk B. Raw data - A/c_i response measurements (1992 stands only).

Excel 7 files entitled ; Aci1 (measurements made on the 24/06/97)
Aci2 (measurements made on the 01/07/97)
Aci3 (measurements made on the 17/07/97)
Aci4 (measurements made on the 25/07/97)
Aci5 (measurements made on the 06/08/97)

1. INTRODUCTION.

The present day atmospheric [CO_2] (averaging around 360 μmol mol^{-1}) has increased by over 25% since the beginning of the industrial revolution (Hebeisen *et al.* 1997), mainly due to the burning of fossil fuels and changes in land use. Indeed such anthropogenic activities have added CO_2 to the atmosphere at a rate of 8 Gt of carbon per year (Bowes 1991). It has been predicted by the Intergovernmental Panel on Climate Change (IPCC) that under present anthropogenic CO_2 emissions, the atmospheric [CO_2] will double by the end of the next century (IPCC 1995).

Since CO_2 is a limiting substrate for plants using the C_3 photosynthetic pathway, it has been suggested that a rising atmospheric [CO_2] may have wide ranging effects on plant growth and development (Bowes 1991). In a geological and biological perspective, the rapidity of change in ambient [CO_2] may be more significant than the degree of change (Bowes 1993). Previously, significant changes in atmospheric [CO_2] have occurred over thousands or millions of years rather than decades. Indeed, trees 200 years old have encountered a 28% increase in atmospheric [CO_2] and are facing the prospect of a 100% increase during there life span (Bowes 1991). Ultimately the time span allowed for plants to respond and adapt to this rapid change in the atmospheric [CO_2] will be considerably foreshortened.

The current ambient CO_2/O_2 regime restricts most C_3 plants to only 60-70% of their photosynthetic potential. This is due to the kinetic constraints of ribulose bisphosphate carboxylase oxygenase (RubisCO), the enzyme which catalyses the first and major rate

limiting step in the Calvin cycle (Bowes 1993). Both CO_2 and O_2 compete to bind to the active sites of RubisCO, as the enzyme catalyses both the carboxylation and oxygenation of ribulose bisphosphate (RuBP). Consequently O_2 acts as a competitive inhibitor of the carboxylation of RuBP (Stirling *et. al.* 1997) and as a consequence RubisCO holds a pivotal position between the Calvin cycle and the photorespiratory carbon oxidation cycle.

The ambient [CO_2] is currently insufficient to saturate the carboxylation reaction of photosynthesis and both carboxylation and photorespiration occur (Nie *et. al.* 1995). The photorespiratory carbon oxidation cycle is seen to be a wasteful process as it can remove some 40-50% of the carbon plants assimilate (Bowes 1993; Nosberger *et. al.* 1996). Thus the [CO_2] in the atmosphere at present is normally limiting to photosynthesis in C_3 plants.

The net photosynthetic rate of C_3 species is initially stimulated after exposure to an elevated [CO_2] (Nosberger *et. al.* 1996; Stirling *et. al.* 1997). This primarily occurs because RubisCO has a low affinity for CO_2 relative to its current ambient concentration and through the suppression of the competitive oxygenation of RubisCO (Bowes 1993; Stirling *et. al.* 1997). Since photorespiration may depress photosynthesis by up to 50% substantial increases in the net photosynthetic rate of C_3 species may be possible in an atmosphere of double the current [CO_2] (Long 1991).

However, studies suggest that any initial stimulation under an elevated [CO_2] may be complicated over the long term by physiological adjustment of the photosynthetic apparatus termed down regulation or acclimation (e.g. Arp 1991; Bryant 1994; Long and Drake 1992; Ryle *et.al.* 1992; Williams 1995).

Acclimation of the photosynthetic apparatus has been attributed to a number of causes and underlying mechanisms such as; a decrease in photosynthetic enzymes (namely RubisCO), the accumulation of carbohydrates in leaves due to insufficient sink demand or by a repartitioning of nitrogen (N) away from RubisCO to other N pools within the plant (Stirling *et. al.* 1997).

Initially, the rate of CO_2 uptake increases in C_3 plants grown under an elevated [CO_2], altering the balance between supply and capacity to use carbohydrate. If the sink of an elevated [CO_2] grown plant becomes saturated, a sink being a growth or storage region of a plant that uses photosynthates, then an accumulation of carbohydrate may occur in the leaves. This could then feedback on the rate of photosynthesis, reducing it in proportion to the carbohydrate levels present (Arp 1991; Webber *et. al.* 1994). Indeed down regulation in the photosynthetic capacity of plants as a response to growth at an elevated [CO_2], may be necessary to restore the source-sink balance (Webber *et. al.* 1994).

A further inhibitory effect on carbon uptake may arise if a plants sink strength is low. Phosphate may become locked up with the intermediate products of the Calvin cycle and the inorganic phosphate (P_i) requirements, for the production of ATP, may not be met. ATP synthesis is therefore insufficient and RuBP regeneration and the activity of RubisCO, dictated by the ATP:ADP ratio, would be negatively effected (Pettersson and McDonald 1994; Webber *et. al.* 1994). The evidence, however, for P_i acting as a long term regulator of source:sink interactions is lacking and long term changes may require adjustment of the concentration of photosynthetic proteins, such as RubisCO (Webber *et. al.* 1994).

Several studies have associated acclimation of the photosynthetic capacity with a reduction in RubisCO (reviewed by Bowes 1991). A decrease in RubisCO protein of as much as 60% has been documented for some C_3 species (Sage *et. al.* 1989). In a N limiting situation this may reflect the reallocation of N resources away from RubisCO, allowing the plant to establish a balance within its photosynthetic apparatus. This would improve the plants efficiency and increase the pool of N available for investment into limiting processes, e.g. decreasing any sink limitations (Webber *et. al.* 1994).

The parameter values of an A/c_i curve provide a model for interpreting the biochemistry of photosynthesis in C_3 species. The initial slope of the dependence of CO_2 assimilation rate (A) upon the intercellular $[CO_2]$ (c_i) is interpreted as the carboxylation efficiency related to the amount of active RubisCO. The plateau is associated with the range of c_i for which A is limited by the regeneration rate of RuBP (Pettersson and McDonald 1994). Using such a model Webber *et. al.* (1994) illustrated three hypothetical patterns of acclimation for different scenarios of N supply and source-sink balance;

1) *Photosynthesis is strictly sink limited* - a decrease in both RubisCO activity and capacity for RuBP regeneration could be expected to reduce CO_2 uptake at elevated $[CO_2]$ to the rate achieved at the current $[CO_2]$.

2) *N supply is strictly limiting to plant growth* - decreasing the requirement for RubisCO at elevated $[CO_2]$ resulting in the reallocation of N away from RubisCO into RuBP regeneration.

3) *N supply is strictly limiting to plant growth* - decreasing the requirement for RubisCO at elevated [CO_2] resulting in the reallocation of N away from RubisCO and into to other plant organs, decreasing any sink limitations.

Whilst down regulation of the photosynthetic apparatus observed in laboratory studies could be a result of the use of small pots restricting root growth (Arp 1991), acclimation has also been observed in field studies (Bryant 1994). However, down regulation at an elevated [CO_2] is not always the case, for example a study by Arp and Drake (1991) into the long term effects of elevated CO_2 on a community of *Scirpus olneyi* showed no loss of photosynthetic capacity. While Pettersson and McDonald (1994) observed that A/c_i curves for small plants at optimal N nutrition were found to be similar in leaves of plants acclimated to present or elevated [CO_2].

The contrasting results observed in the study of acclimation to elevated [CO_2] suggest that down regulation may be species specific, due to growth conditions and environmental constraints, or both (Bowes 1993; Stirling *et. al.* 1997). Any response to increasing atmospheric [CO_2], will inevitably vary with several environmental factors such as temperature (Long 1991; Stirling *et. al.* 1997), water availability (Bowes 1993; Campbell *et. al.* 1997), light and especially nutrient availability (Pettersson and McDonald 1994).

Long and Drake (1992) found that acclimation of photosynthesis under elevated [CO_2] appeared to be most marked in species which were unable to use additional carbohydrate in respiration, growth or storage, i.e. were sink limited. Indeed a study by

Fischer *et. al.* (1997) observed that carbohydrate concentrations appear to increase in the source leaves of *Lolium perenne* at elevated [CO_2], particularly at low N supply, thus reflecting N-dependant sink limitations. While Ryle *et. al.* (1992) observed that the growth response of *Lolium* appeared to be sink limited at elevated [CO_2].

It has been suggested by Arp and Drake (1991) that new sinks for the utilisation or storage of increased carbohydrate could require years to develop in perennial species. The perennial ryegrass (*Lolium perenne*) may be one of a number of C_3 species which falls into this category of response under elevated [CO_2].

Determining the physiological behaviour of *L. perenne* at elevated [CO_2] is important as; it is dominant in many natural ecosystems (Nijs *et. al.* 1988), and provides the mainstay of lowland pasture production in temperate western Europe, and they or their hybrids are likely to continue to do so in the foreseeable future (Ryle *et al.* 1992). As the atmospheric [CO_2] continues to rise and agricultural practices become more intensified, it is crucial to investigate the interacting effects of elevated [CO_2] and agricultural management practices (Hebeisen *et al.* 1997). To optimise the productivity of this forage crop in a future high CO_2 world, management practices such as N fertilisation and harvesting regimes may well have to be modified.

Despite the ecological and economical significance of forage crops, little information exists on the long term response of grassland species in natural or managed communities, the majority of literature being devoted to arable and glasshouse crops.

Furthermore, the majority of studies into the response of plants to an elevated [CO_2] have involved various types of growth chamber or enclosure systems. These systems inevitably introduce unwanted artefacts into the experimental environment and lack the realism of natural field conditions. Indeed it has been claimed by Arp (1991) that acclimation of photosynthesis could well be an artefact caused by small experimental pots which restrict the sink size of plants.

The Free-Air CO_2 Enrichment (FACE) technology developed by Brookhaven National Laboratory allows large numbers of plants to be grown under elevated [CO_2] in the field under entirely natural conditions. It has been used in Switzerland as part of a joint 6 year study between the University of Essex and Swiss Federal Institute of Technology Zurich (ETHZ) looking into the effects of elevated [CO_2] on perennial ryegrass and white clover.

Studies conducted by Prof. S. Long (University of Essex) into the leaf photosynthesis of *L. perenne*, grown within the Swiss- FACE facility since 1992, have so far shown a sustained increase in leaf photosynthesis at high [CO_2], at both high and low nitrogen treatments. Simultaneously stomatal conductance appeared to show a sustained decrease. Acclimation (i.e. down regulation of RubisCO) was present before stands of *L. perenne* where harvested and was removed after a harvest, suggesting the loss was driven by a source-sink balance (Nosberger *et. al.* 1996)

The photosynthetic measurements of *L. perenne* reported in this dissertation were made by the author in 1997, as part of this 6 year study. Using the data obtained from these photosynthetic measurements the following hypotheses were tested:

1) After 5 years growth at elevated [CO_2] leaf photosynthesis has decreased, when measured at elevated [CO_2], to the level of leaves grown and measured at current ambient [CO_2]. This is also more likely at low than high N treatments. (Tested with diurnal measurements).

2) Acclimation of photosynthesis (decreased $V_{c,max}$ and/or J_{max}) to elevated [CO_2] will be more pronounced at low N and more pronounced before a harvest when the source:sink ratio is low than after a harvest when the source:sink ratio is high. (Tested with A/c_i measurements).

3) The photosynthetic response ($V_{c,max}$ and J_{max}) of *L. perenne* stands exposed to elevated [CO_2] for 2 years (1995 grown stands), will be similar to those grown under elevated [CO_2] for 5 years (1992 grown stands). (Tested with A/c_i measurements).

2. METHODS AND MATERIALS.

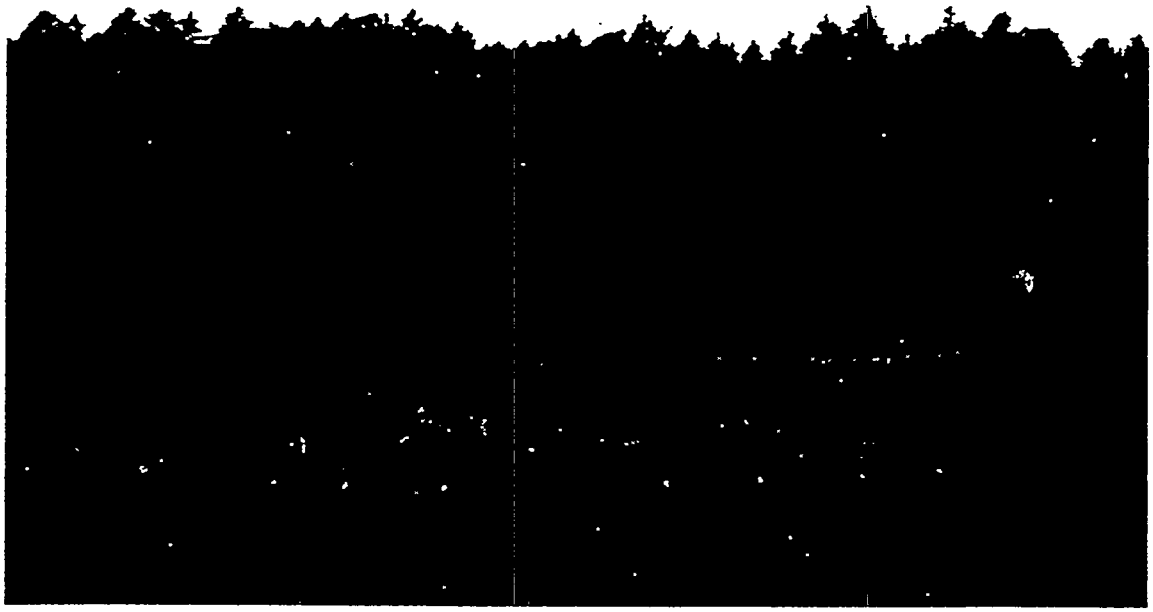
2.1. Growth Conditions.

Lolium perenne (cv. Bastion) plants were grown in pure stands within a Free-Air CO_2 Enrichment (FACE) system at elevated (600 μmol mol^{-1}) and ambient (360 μmol mol^{-1}) [CO_2]. The FACE system (developed by Brookhaven National Laboratories) is a chamberless system which provides controlled fumigation conditions in the field. It eliminates the potential of 'chamber effects' such as; changes in wind velocity, humidity, temperature, light quality and intensity, and soil variables (Hendrey and Kimball 1993) all of which can lead to unwanted perturbations within an experiment. Indeed the restricted rooting volume of plants grown within pots and soil bins used in growth chamber experiments, has been associated with the limitation of sinks for photosynthate (Arp 1991). The FACE system however, allows large numbers of field grown plants to be exposed to controlled [CO_2] in the field, while providing an exposure regime where only the chosen variables ([CO_2] and nitrogen availability) are altered, leaving other features of the environment unaltered and in a natural state.

The experimental site was located in Eschikon, 20 km north-west of Zurich Switzerland and included three 18 m-diameter FACE rings, and three 18 m-diameter control rings. A map showing the layout of the Swiss-FACE facility can be seen in Appendix A. Each FACE ring consisted of a circular array of vertical vent pipes surrounding experimental plots (see Figure 1). Multiple ports within these vent pipes allowed computer controlled

releases of CO_2 gas over the whole experimental area. By measuring wind speed and direction at the centre of the FACE array, CO_2 gas was always released up wind of the array and in proportion to wind speed in appropriate amounts determined by infra-red gas analysis measurement at the centre of each array. For a full technical description see Lewin *et al.* (1992) and Hendrey and Kimball (1993).

Figure 1. Swiss-FACE ring showing circular array of vertical vent pipes surrounding experimental plots.



Pure stands of *L. perenne* were sown in plots within the FACE and control rings during 1992, additional plots being sown during 1995. These were in their fifth year and second year of growth respectively. All the plots were harvested five times a year during the growing season, being cut to around 4 cm above ground level. This allowed

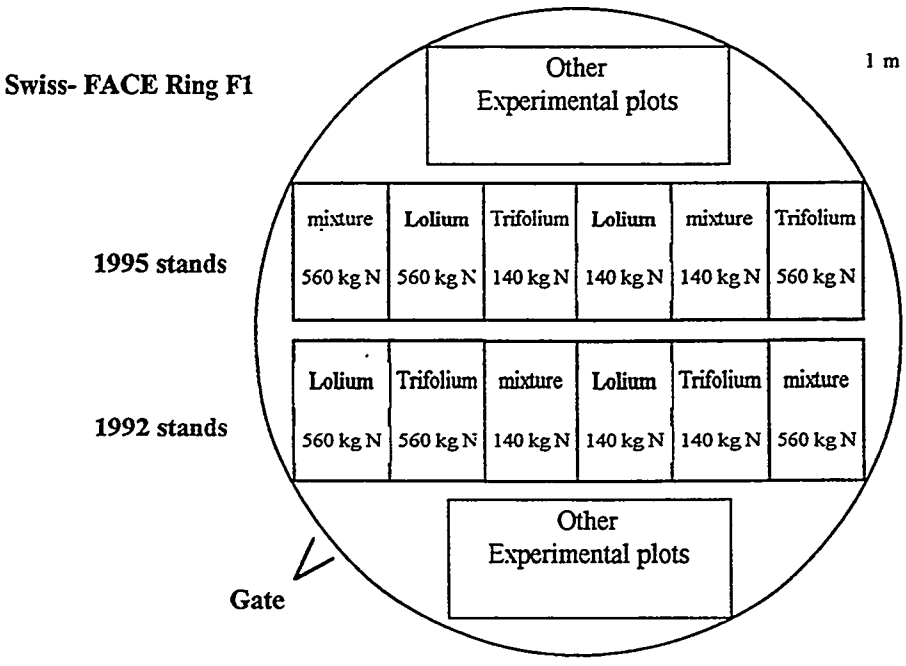
photosynthetic measurements to be taken during periods of regrowth and consequently at different source:sink ratios. The dates of each harvest can be seen in Table 1.

Table 1. Dates of harvest for *L. perenne* pure stands.

Harvest	May 12	June 16	July 21	August 25	Sept. 29

Within each ring both the 1992 and 1995 stands were also divided into high and low nitrogen plots, with applications of 560 and 140 kg Ha^{-1} y^{-1} of NH_4NO_3 respectively. The nitrogen fertiliser was applied after each harvest, proportional to expected yield. An example of a FACE ring showing the layout of plots can be seen in Figure 2.

Figure 2. The layout of various plots and treatments within a FACE ring.



Fumigation within the Swiss-FACE rings first began during the early part of 1993, a year after the 1992 stands were sown. This allowed the plants time to establish themselves. Since 1993, fumigation has taken place continuously throughout the growing season, beginning at sunrise and ceasing at sunset.

2.2. Gas Exchange Measurements.

Two open infra-red gas analysis systems (CIRAS-1, PP Systems, Hitchin, Herts.) attached to Parkinson Leaf Cuvettes (PLC-1, PP Systems, Hitchin, Herts.) were used during the experiment to measure gas exchange parameters (see Figure B1 in Appendix B). These were cross calibrated against a dual channel infra-red CO_2/H_2O analyser (LI-6262, LICOR Inc., Lincoln, Nebraska). This IRGA was in turn calibrated with a standard gas mixture of CO_2 in air (600±2 ppm, AGA Atiengessellscaft, Zurich, Switzerland).

The gas exchange parameters - CO_2 uptake (A); intercellular CO_2 concentration (c_i); stomatal conductance (g_s); and transpiration (E), were calculated by the CIRAS software according to the equations of Von Caemmerer and Farquhar (1981), see Long and Hallgren (1993) and Long *et al.* (1996). The basic equations can be seen in Appendix C.

Leaf area was obtained by measuring leaf width at each end of the leaf in the chamber. The average of the two numbers was then multiplied by the chamber length, assuming that the enclosed leaf area formed a trapezium. The chamber length was 4.6 cm. All the gas

exchange measurements were made on the oldest emerging leaves (i.e. the ligule had not yet emerged), halfway down from the tip of the leaf. The leaf was placed in the chamber so that the upper part of the leaf always faced upwards in the chamber.

2.2.1. Diurnal Gas Exchange Measurements.

To determine the diurnal photosynthetic response of *L. perenne* at elevated [CO_2], five sets of diurnal gas exchange measurements were made over a ten week period from 16 June to 22 August. The diurnal measurements were made on the 1992 grown stands only. During a set of diurnal measurements, five leaves per nitrogen plot were measured in each of the six rings every two hours from 06:00 am until 08:00 pm. The measurements were made at the appropriate growth [CO_2], 360 μmol mol^{-1} for control and 600 μmol mol^{-1} for FACE rings. The chamber was held horizontally, making sure that both the chamber and the attached quantum light sensor were not shaded.

The measurement procedure was as follows; the CIRAS was set to the appropriate growth [CO_2], and a healthy emerging leaf was selected and placed into the chamber. The surface area of the leaf was then calculated and entered into the CIRAS, the chamber was held horizontally and the measurement recorded once the A and gs values had stabilised. This procedure was then repeated on a new leaf.

Where possible a set of diurnal measurements were made; one week after a harvest, 2.5 weeks (mid-point) after a harvest and one week before the next harvest (Appendix D shows the condition of the stands during these intervals). However as clear dry days were

essential for diurnal measurements, the dates of the diurnal measurements were dependent on weather conditions. Wet and overcast days would limit the light levels, while wet leaves would prevent any accurate measurement of stomatal conductance. The dates on which sets of diurnal measurements were made in relation to the harvests can be seen in Table 2.

Table 2. Dates when sets of diurnal measurements were taken in relation to harvests

Diurnal	1	2	3	4	5
Date of Measurement	2 July	16 July	30 July	10 Aug	18 Aug
Days From a Harvest	16 after	5 before	9 after	20 after	7 before

To achieve the required number of measurements at each time point during the diurnal on the 2 July, two open infra-red gas analysis systems were used - CIRAS (A) and CIRAS (B). The sequence of measurements at each time point were as follows:

CIRAS (A)

06:00 sequence
FACE 2
Control 1
FACE 3

08:00 sequence
Control 2
FACE 1
Control 3

and so on.

CIRAS (B)

06:00 sequence
Control 2
FACE 1
Control 3

08:00 sequence
FACE 2
Control 1
FACE 3

The above sequence whereby FACE and Control rings are sampled simultaneously, was chosen to avoid any time bias toward one treatment within the sampling procedure. However, due to technical problems with CIRAS (B), only one system (CIRAS A) was available for the remaining sets of diurnal measurements. The sequence used at each time point on the 16 July, 30 July, 10 August and 18 August was C2, F1, C1, F2, F3, C3. While this was chosen partly for logistical reasons, it was also important that it avoided any time bias toward one treatment.

The total carbon uptake and stomatal conductance, over the whole day, was then estimated for each of the diurnal sets of measurements per nitrogen treatment per ring. These values were then tested using two factor analysis of variance to highlight any significant differences.

2.2.3. A/c_i Response Measurements.

To determine the response of leaf CO_2 uptake (A) to intercellular [CO_2] (c_i), leaves were sampled from the plots within each ring and measured in the laboratory. Determination of the A/c_i response requires constant conditions of light, temperature and humidity. Since the objective was to determine the maximum *in vivo* carboxylation ($V_{c,max}$) and RuBP regeneration capacity (J_{max}) of each leaf, it was also important to avoid water stress, photoinhibition and end-product inhibition, which could all develop in leaves in the field.

Leaves were therefore sampled by cutting the oldest emerging leaf from the stem under water to avoid the uptake of air into the xylem. The leaf was then placed into a plastic bag containing water and a damp tissue (to prevent the leaf drying out) and taken back to the laboratory for immediate measurement. This procedure produced rates of photosynthesis and stomatal conductance that were as great or greater than those observed in the field, suggesting that the sampling method had not damaged the leaves.

A/c_i measurements were made on one leaf per nitrogen treatment per ring. A quartz iodide light source was clipped over the leaf chamber to provide constant near saturating light at around 1000 μmol m^{-2} s^{-1} (see Figure B2 in Appendix B). The $[CO_2]$ in the chamber was altered in 7 steps, the direction dependant on the growth $[CO_2]$. For control grown plants the sequence was 360, 250, 150, 50, 600, 900, 1200 μmol mol^{-1} CO_2, while FACE grown plants were measured at 600, 360, 250, 150, 50, 900, 1200 μmol mol^{-1} CO_2. The sequence for FACE grown plants was employed to try to avoid unnaturally high RubisCO activation by CO_2.

The maximum *in vivo* carboxylation ($V_{c,max}$) and RuBP regeneration (J_{max}) of each leaf was then calculated by fitting the equations of Farquhar *et al.* (1980) in a computer software package. The $V_{c,max}$ and J_{max} data sets were tested using two factor analysis of variance to highlight any significant differences caused by CO_2 or nitrogen treatment.

Over the ten week period, five sets of A/c_i measurements were made for the 1992 stands of *L. perenne*. These were made where possible approximately 1 week after a harvest, 2.5 weeks after a harvest and 1 week before a harvest. Three sets of A/c_i measurements

for the 1995 stands of *L. perenne* are also included. These measurements were made along with a fellow student Paul Humphreys using the same equipment and methods as mentioned above. The inclusion of this data allows a comparison to be made between the 1992 and 1995 grown stands. The dates on which the measurements were made in relation to the harvests can be seen in Table 3.

Table 3. Dates when sets of A/c_i measurements were taken in relation to harvests.

1992 stands					
A/c_i measurements	24 June	1 July	17 July	25 July	6 August
Days from a harvest	8 after	15 after	4 before	4 after	16 after
1995 stands					
A/c_i measurements	24 June	-	19 July	29 July	-
Days from a harvest	8 after	-	2 before	8 after	-

3. RESULTS.

3.1. Diurnal Gas Exchange Measurements.

The diurnal measurements taken over five separate days in July and August all showed a consistent increase in assimilation rates and a decrease in stomatal conductance for 1992 stands of *L. perenne* growing at high [CO_2] (see Figures 3 to 7). The raw data for each set of diurnal measurements can be viewed as Excel 7 files on 'floppy disc A' held in the back cover of this dissertation. Although enhancement of photosynthesis occurred over the whole day, it was also marked at the beginning and end of each day. Table 4 shows the changes in A and g_s due to growth at high [CO_2] as a percentage change in total carbon uptake and total stomatal conductance over the whole of each diurnal.

Table 4. Percentage change in estimated total CO_2 uptake and total stomatal conductance over the whole day, for *L. perenne* due to growth at elevated [CO_2].

Date Measured	Days from harvest	Assimilation (A)		Stomatal Conductance (g_s)		Mean leaf Temperature (°C)
		High	Low	High	Low	
02/07/97	16 after	+51	+53	-23	-38	19
16/07/97	5 before	+53	+40	-30	-38	23
30/07/97	9 after	+42	+44	-17	-26	22.6
10/08/97	20 after	+57	+52	-15	-23	23
18/08/97	7 before	+65	+83	-1	-12	21

Figure 3. Diurnal assimilation rates and stomatal conductance for 1992 stands of *Lolium perenne* grown within FACE and Control rings.
Measured on the 2 July 1997 - 16 days after the 16 June harvest.

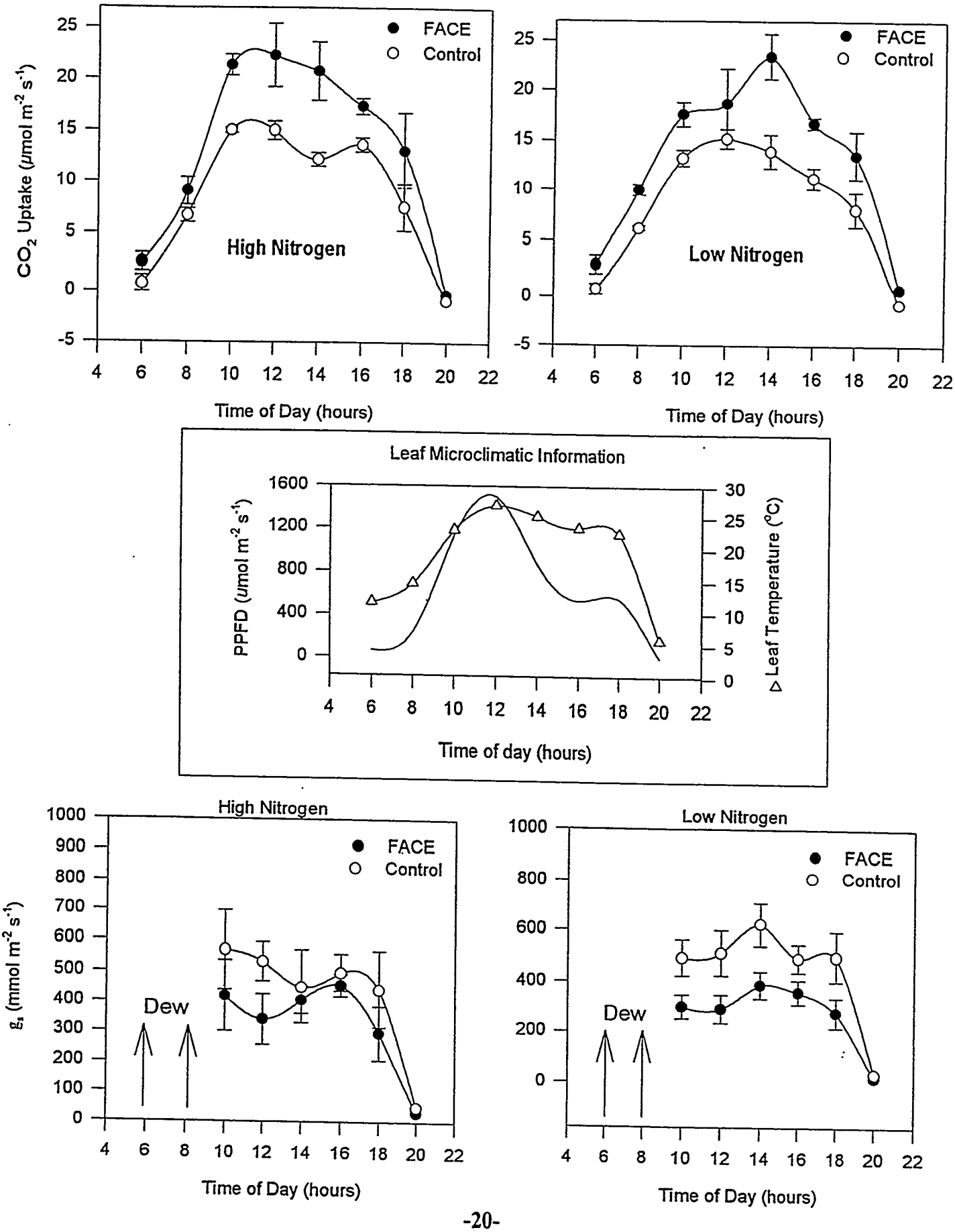
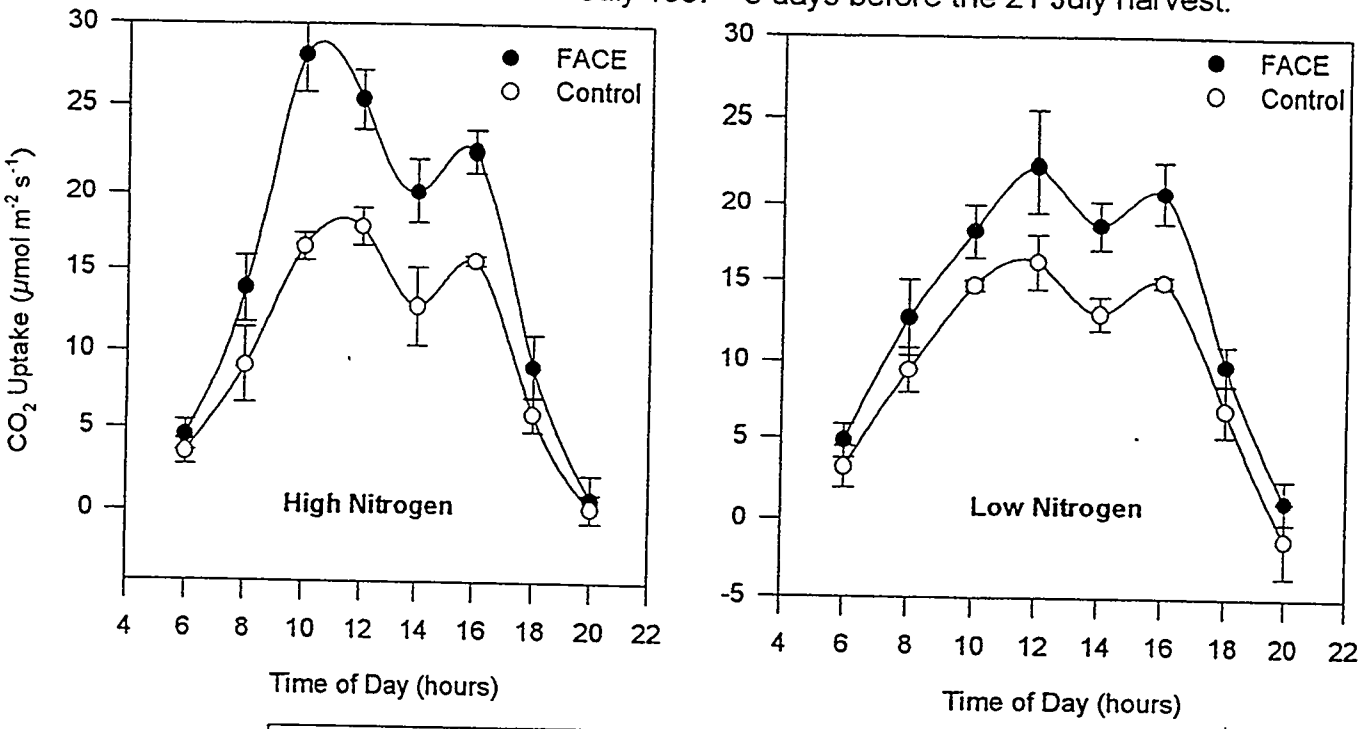
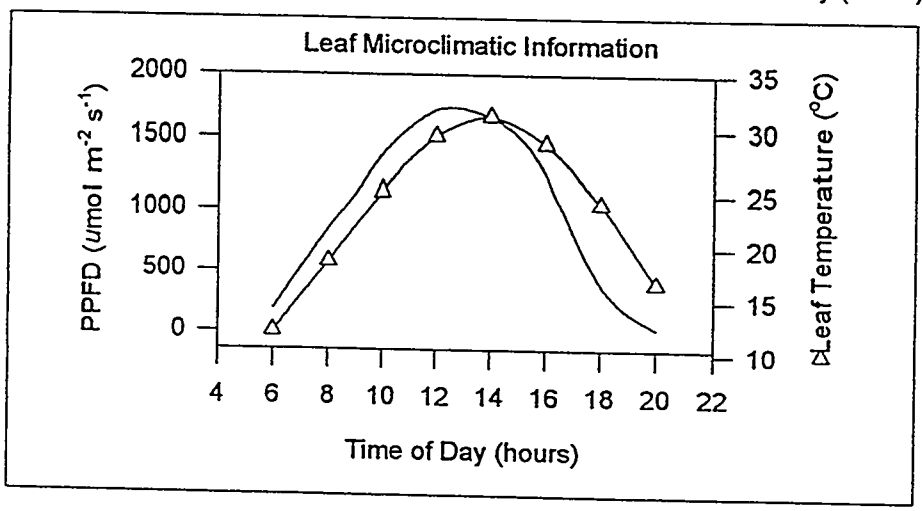


Figure 4. Diurnal assimilation rates and stomatal conductances for 1992 stands of *Lolium perenne* grown within FACE and Control rings.
Measured on the 16 July 1997 - 5 days before the 21 July harvest.





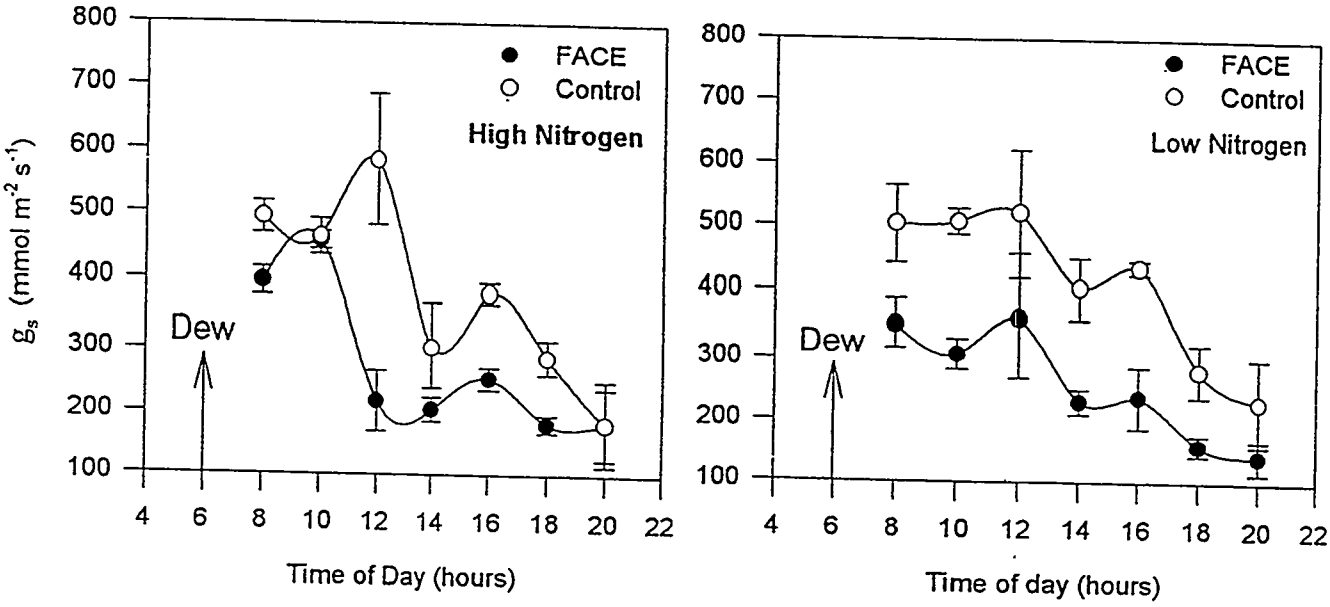


Figure 5. Diurnal assimilation rates and stomatal conductances for 1992 stands of *Lolium perenne* grown within FACE and Control rings.

Measured on 30 July 1997 - 9 days after the 21 July harvest.

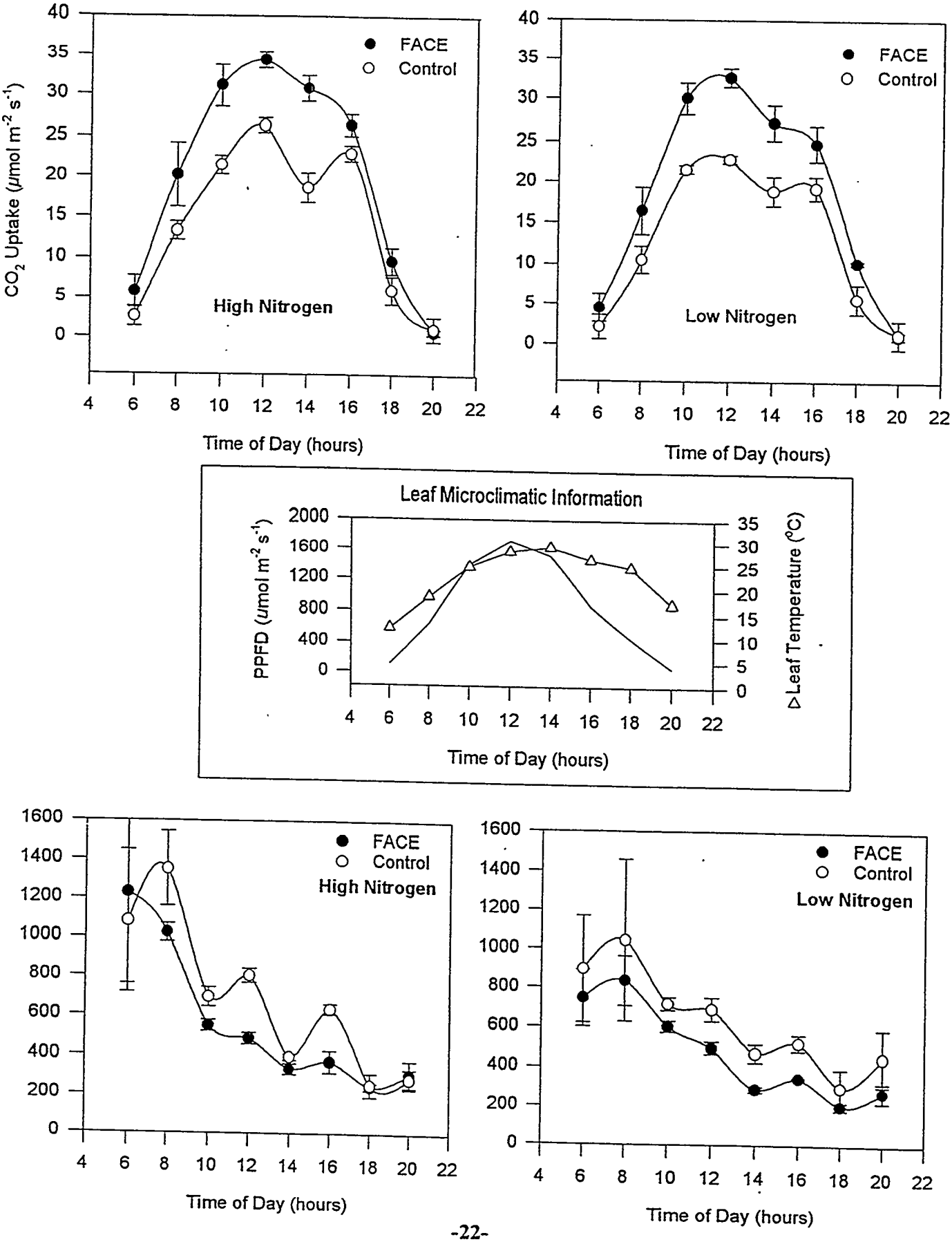
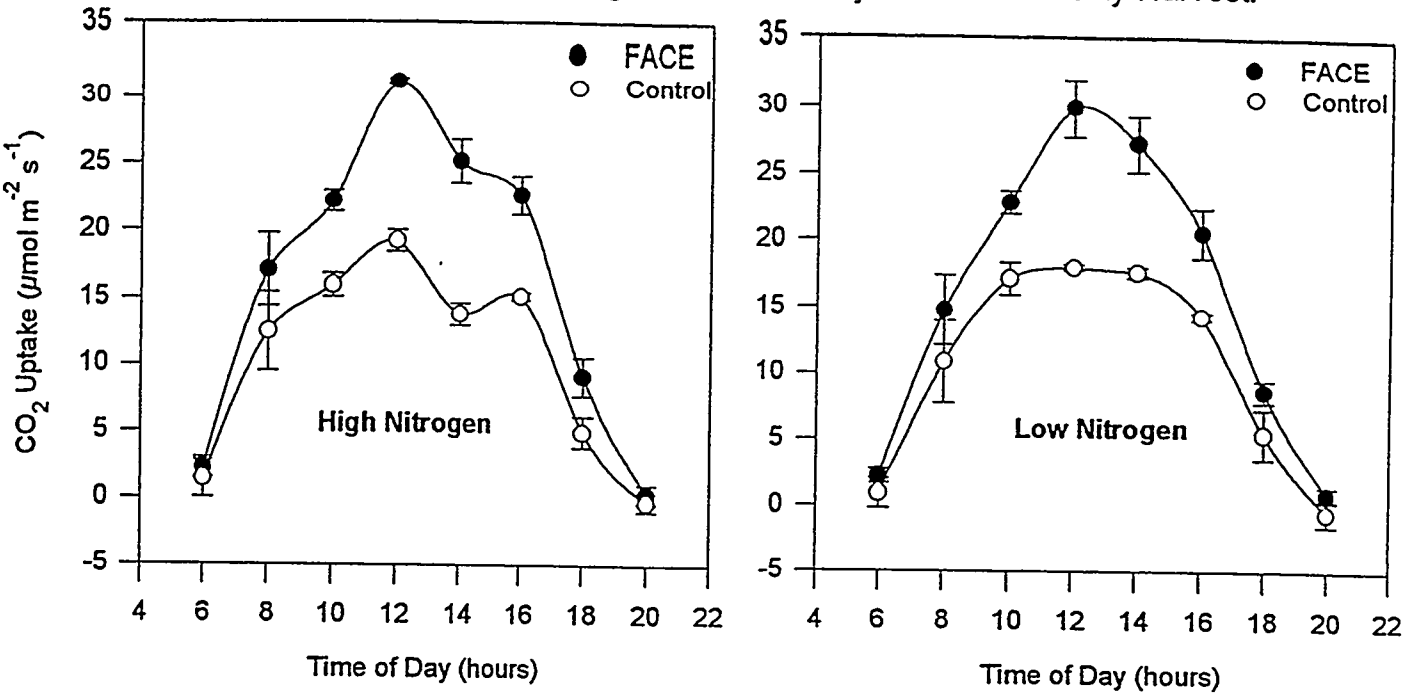
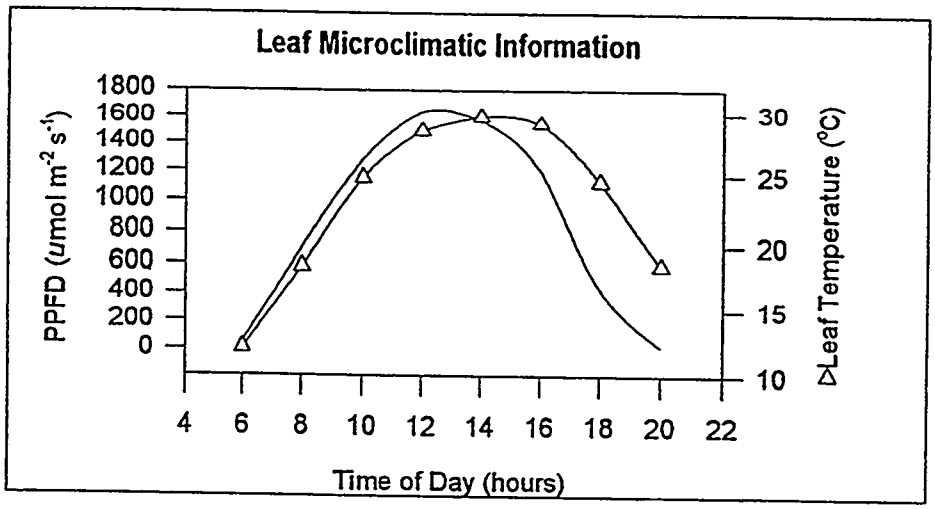


Figure 6. Diurnal Assimilation Rates and Stomatal Conductance For 1992 Stands of *Lolium perenne* Grown Within FACE and Control Rings.

Measured on 10 August 1997 - 20 days after the 21 July Harvest.





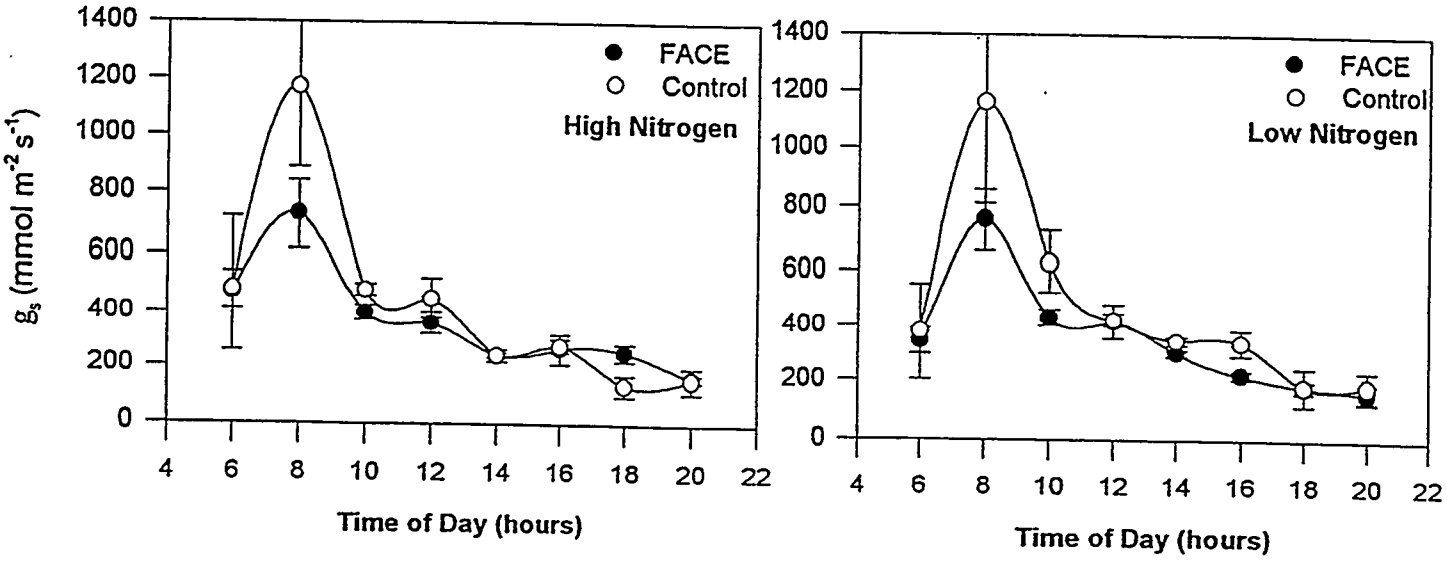
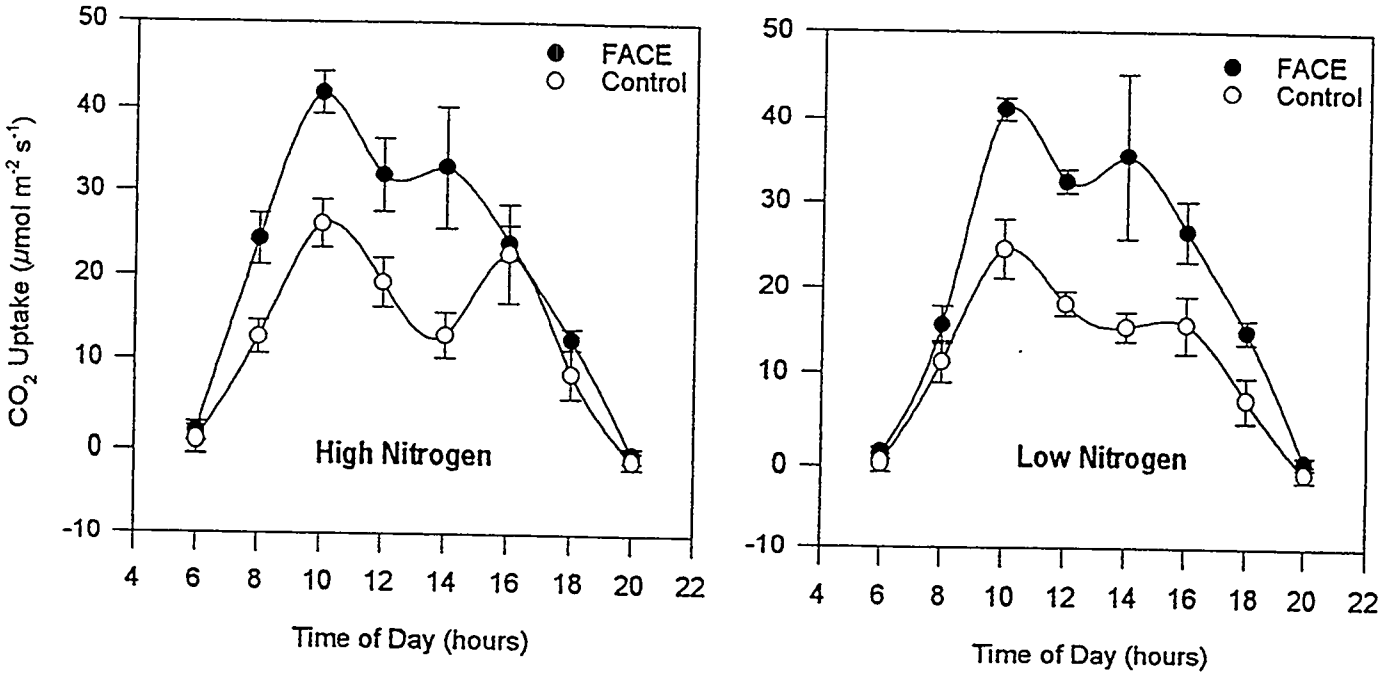
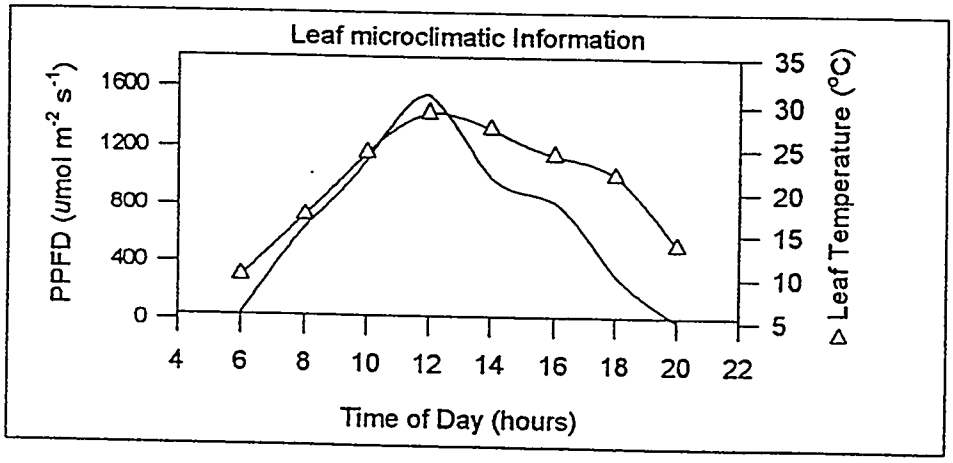
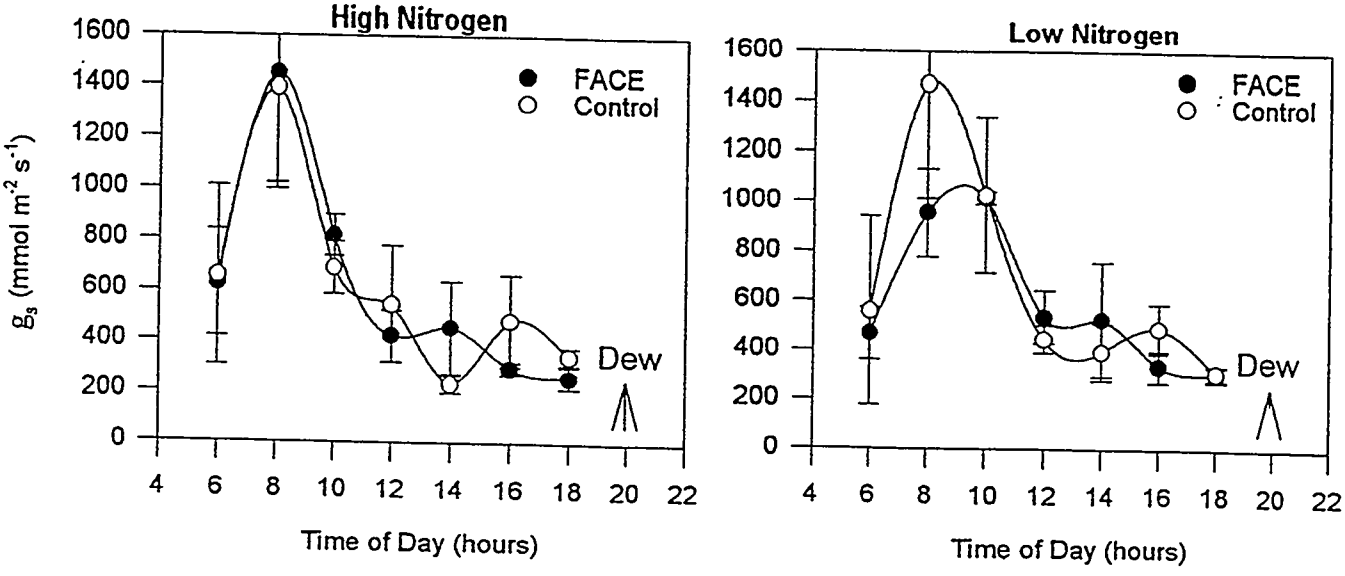


Figure 7. Diurnal assimilation rates and stomatal conductance for 1992 stands of *Lolium perenne* grown within FACE and control rings.

Measured on 18 August 1997 - 7 Days before the 25 August Harvest.







Total carbon uptake over the whole day, increased for plants growing at high [CO_2] at both nitrogen treatments during all periods of regrowth. Stimulation ranged from 40 to 83%. This enhancement of photosynthesis was significant, at the 5% significance level, at all periods of regrowth (see Table 5).

Table 5. Significance levels of variation in total carbon uptake (A) and stomatal conductance (g_s) over the whole day, due to [CO_2] and nitrogen treatment.

	Source of variation	2 July	16 July	30 July	10 August	18 August
A	CO_2	*P<0.05; F=57.7*	*P<0.05;F=47.3*	*P<0.05; F=210.8*	*P<0.05; F=59.5*	*P<0.05; F=101.8*
	Nitrogen	*P=0.54;F=0.41*	*P=0.09; F=3.5*	*P<0.05; F=12.6*	*P=0.8;F=0.06*	*P=0.4; F=0.66*
	Interaction	*P=0.9; F=0.02*	*P=0.31; F=1.15*	*P=0.78; F=0.07*	*P=0.76; F=0.09*	*P=0.5; F=0.45*
g_s	CO_2	*P<0.05; F=5.78*	*P<0.05;F=38.7*	*P<0.05;F=8.6*	*P<0.05; F=10.2*	*P=0.49; F=0.52*
	Nitrogen	*P=0.79; F=0.07*	*P=0.78; F=0.08*	*P=0.18; F=2.2*	*P=0.7; F=0.14*	*P=0.84; F=0.05*
	Interaction	*P=0.54; F=0.41*	*P=0.37; F=0.9*	*P=0.66; F=0.2*	*P=0.48; F=0.56*	*P=0.53; F=0.43*

The 13% difference in stimulation between nitrogen treatments on the 16 July (5 days before a harvest) and the 18% difference on the 18 August (7 days before a harvest) seen in Table 4, were not significant. Although a significant difference in stimulation occurred between nitrogen plots on the 30 July (9 days after a harvest).

The large stimulation in total carbon uptake on the 18 August (see Table 4) was not associated with a rise in mean leaf temperature, in comparison to the temperatures on the other measurement dates. Micrometeorological data also indicates that the air temperature on the 18 August was also not high in comparison. Micrometeorological data can be seen in Appendix E.

Total stomatal conductance over the whole day was reduced in FACE grown plants at all periods of regrowth (Figures 3 to 7). Note, g_s means were removed from time points where dew or dampness on the leaves may have affected the measurements. The percentage change over the whole day due to growth at high [CO_2] ranged from -1% to -38%, with larger percentage reductions in the low nitrogen plots.

The reductions in total g_s were significant on all of the measurement dates except on the 18 August where the percentage change was considerably smaller at both nitrogen treatments in comparison to the four other dates. Although the percentage reductions seemed to be greater in the low nitrogen plots, this was not statistically significant on any of the measurement dates.

The smaller percentage reductions and lack of a statistical difference between FACE and Control g_s values on the 18 August, may be explained by the drought conditions leading up to the 18 August (Appendix E). Cumulative effects of higher transpiration rates in ambient grown plants on the other dates, may have reduced the soil moisture in the control plots. This may have led to more drastic water conservation measures in the ambient grown plants on the 18 August. Indeed, the g_s values seen in Figures 6 and 7 are typical of drought conditions, where g_s is high during the early hours of the day dropping off dramatically as temperature and light increases.

3.2. A/c_i response of *Lolium perenne.*

3.2.1. A/c_i response of 1992 grown stands.

The A/c_i response measurements obtained for 1992 grown stands of *L. perenne* over the ten week period, can be seen in Figures 8 -12. The raw data for each set of A/C_i measurements can be viewed as Excel 7 files on 'floppy disc B' held in the back cover of this dissertation. The mean $V_{c,max}$ and J_{max} values calculated from these curves, can be seen in Figures 13 and 14 respectfully.

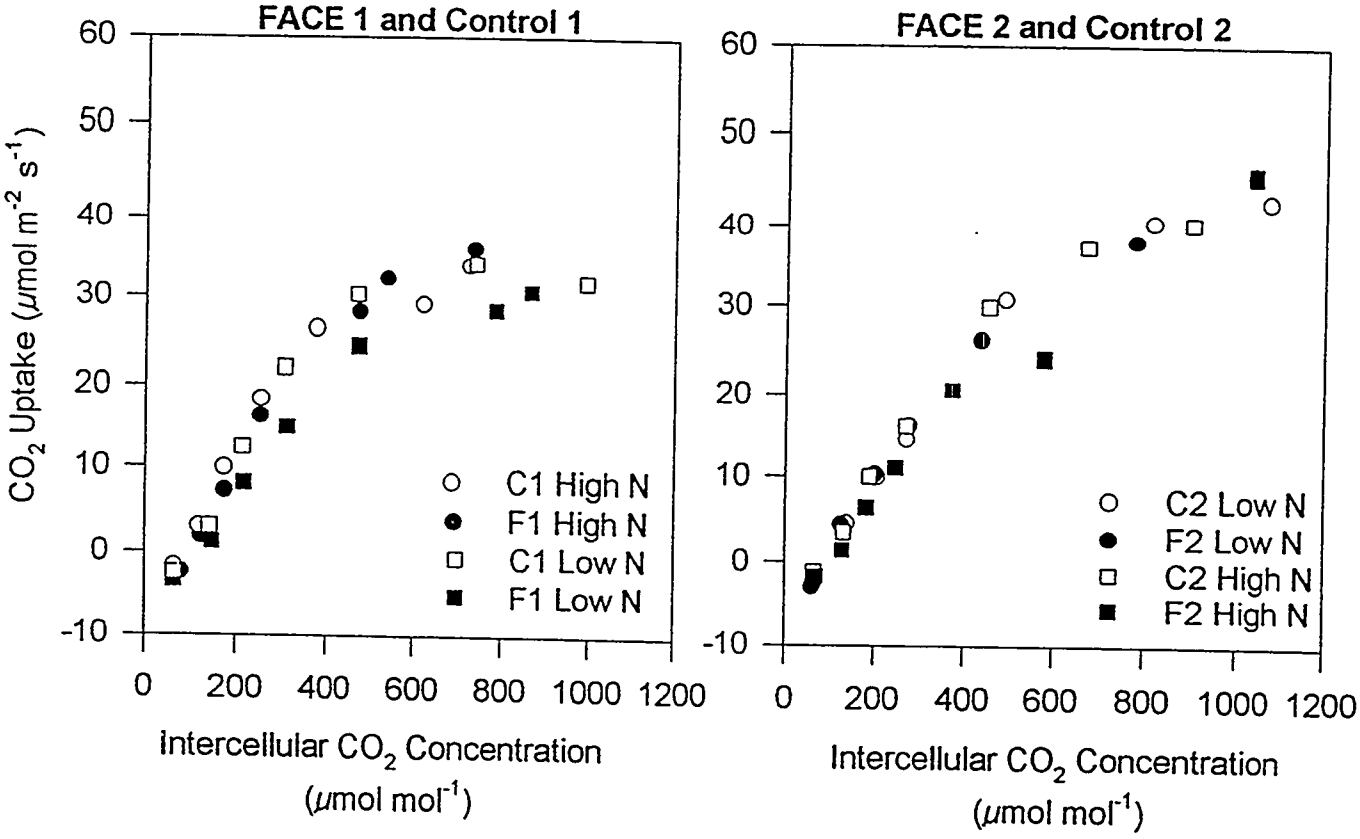
The trend seen in Figure 13 at each CO_2 and nitrogen treatment, where $V_{c,max}$ values are lower before a harvest, increasing again after a harvest, reflects that the maximum rate of carboxylation may be driven by the source-sink balance. It was therefore presumed that at elevated $[CO_2]$ a decrease in $V_{c,max}$ values would occur (and be more pronounced) before a harvest when the source:sink ratio was low. However, there was no significant effect of either $[CO_2]$ or nitrogen treatment on the $V_{c,max}$ values at any of the periods of regrowth (see Table 6).

Table 6. Significance levels of variation in $V_{c,max}$ and J_{max} due to $[CO_2]$ and N- treatment.

	Source of variation	24 June	1 July	17 July	25 July	6 August
V_{cmax}	N Treatment	*P=0.22; F=1.75*	*P=0.78; F=0.09*	*P=0.44; F0.65*	*P=0.55; F=0.40*	*P=0.51; F=0.49*
	CO_2 Treatment	*P=0.65; F=0.22*	*P=0.39; F=0.84*	*P=0.21; F=1.84*	*P=0.65; F=0.22*	*P=0.55; F=0.38*
	Interaction	*P=0.95; F=0*	*P=0.41; F=0.76*	*P=0.83; F=0.05*	*P=0.31; F=1.17*	*P=0.60; F=0.30*
J_{max}	N Treatment	*P=0.88; F=0.02*	*P=0.879; F=0.025*	*P=0.13; F=2.82*	*P=0.28; F=1.32*	*P=0.24; F=1.61*
	CO_2 Treatment	*P=0.25; F=1.56*	*P=0.828; F=0.05*	*P=0.29; F1.26*	*P=0.83; F=0.05*	*P=0.66; F=0.22*
	Interaction	*P=0.82; F=0.05*	*P<0.05; F=5.416*	*P=0.27; F=1.40*	*P=0.32; F=1.13*	*P=0.42; F=0.73*

Figure 6. A/C_i response of *Lolium perenne* 1992 stands, grown in FACE and Control rings.

Measurements made on the 24 June 1997 - 8 days after the 16 June harvest.



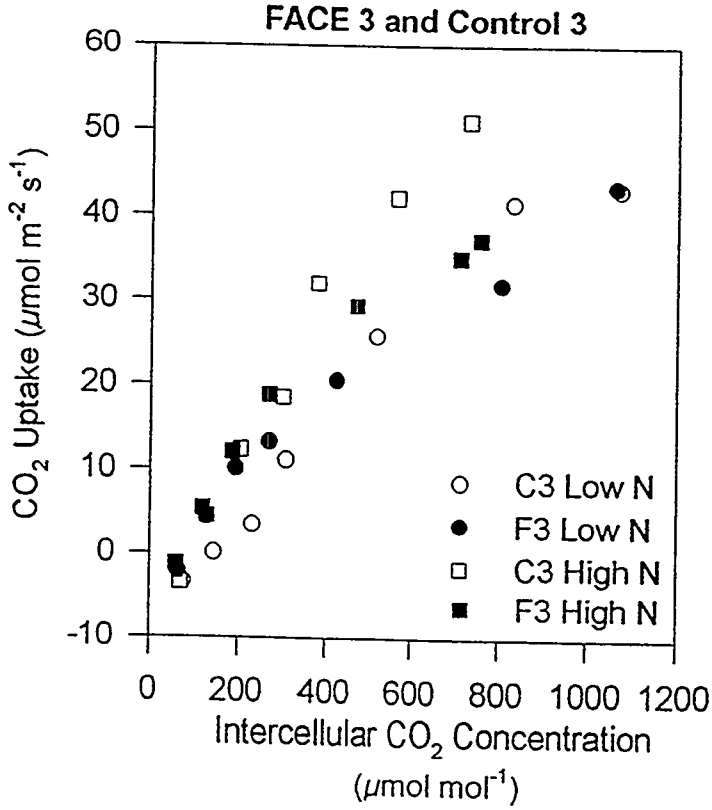
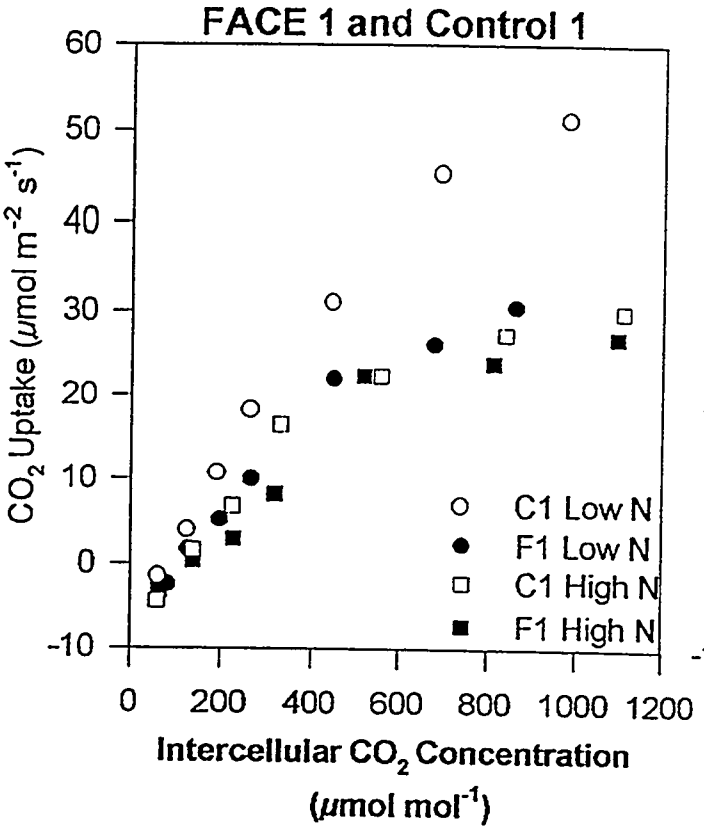
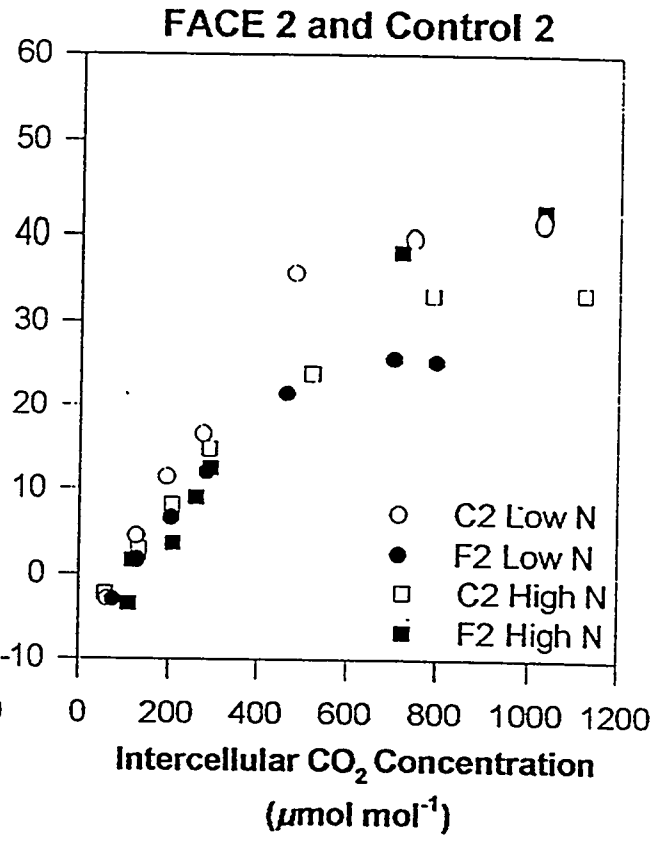
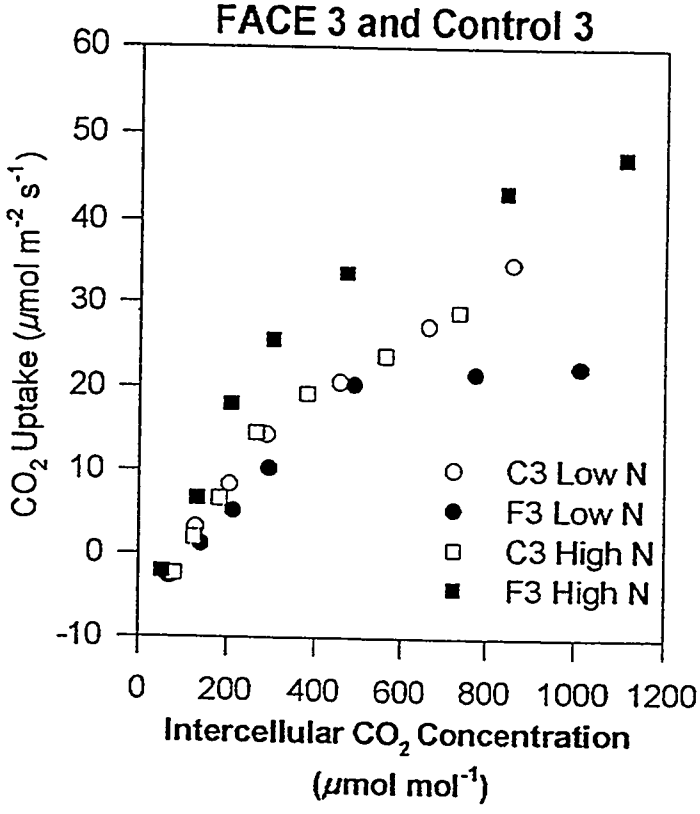


Figure 9. A/C_i response of *Lolium perenne* 1992 stands, grown in FACE and Control rings.

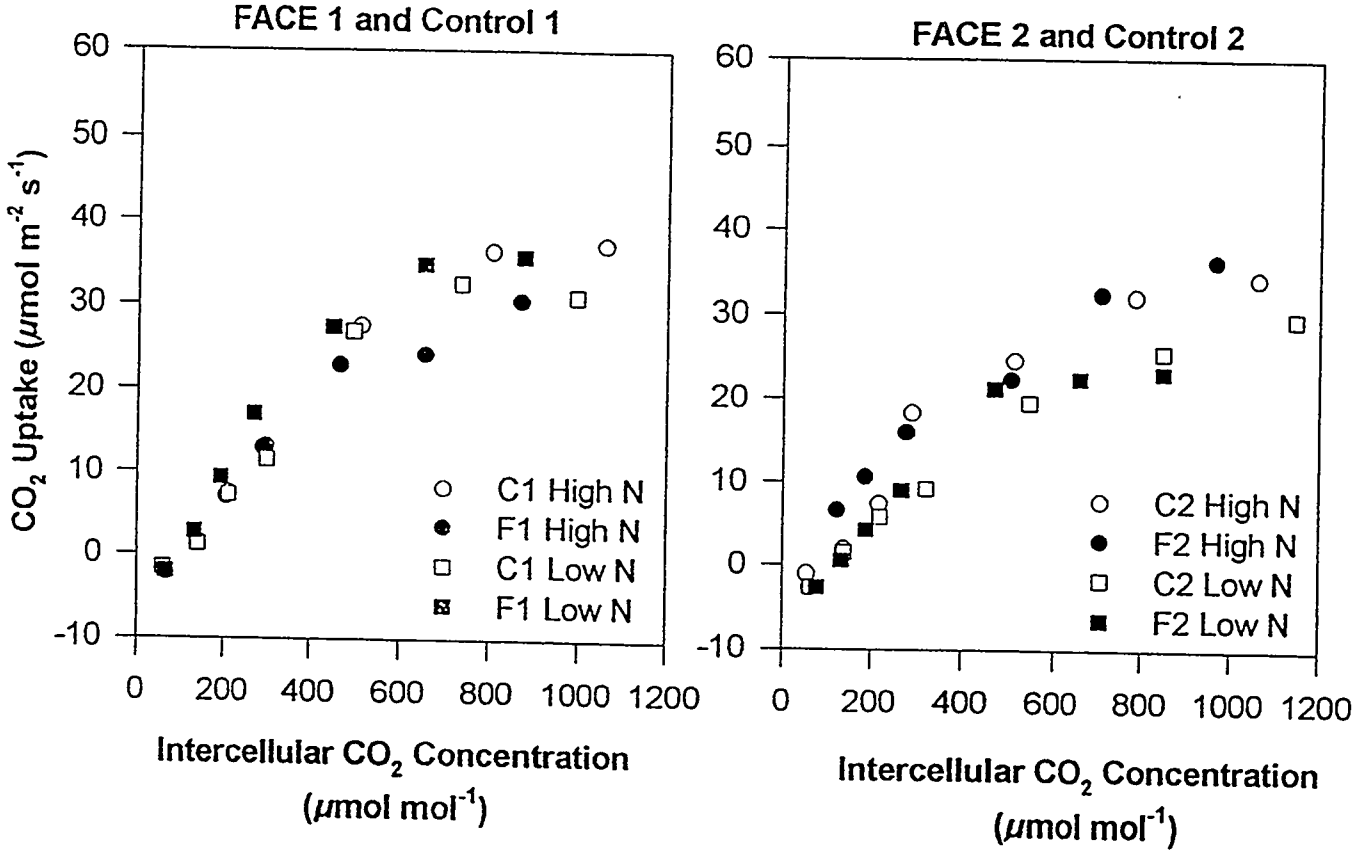
Measurements made on the 1 July 1997 - 15 days after the 16 June harvest.







grown in FACE and Control rings.
Measurements made on the 17 July 1997 - 4 days before the 21 July harvest.



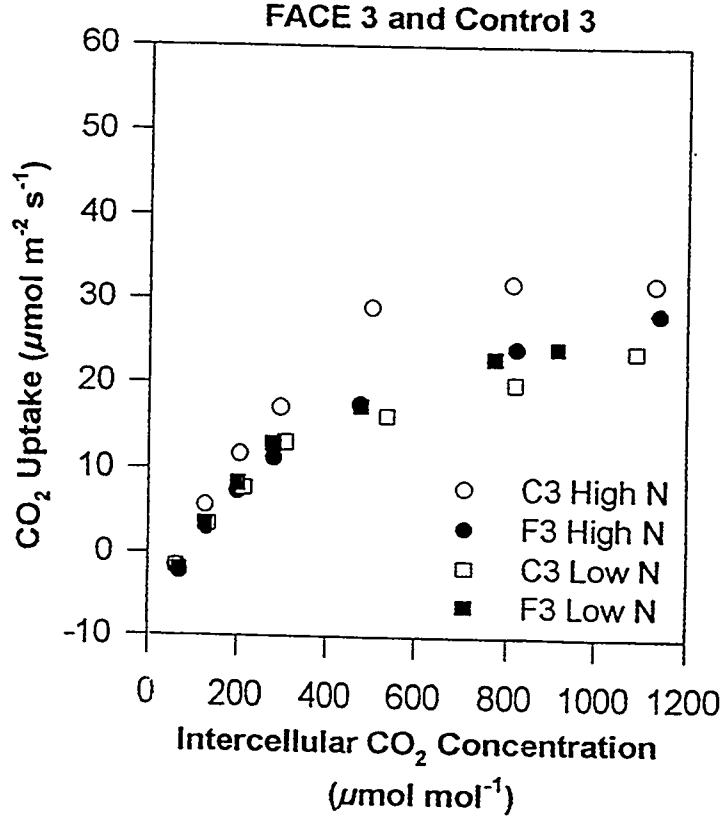
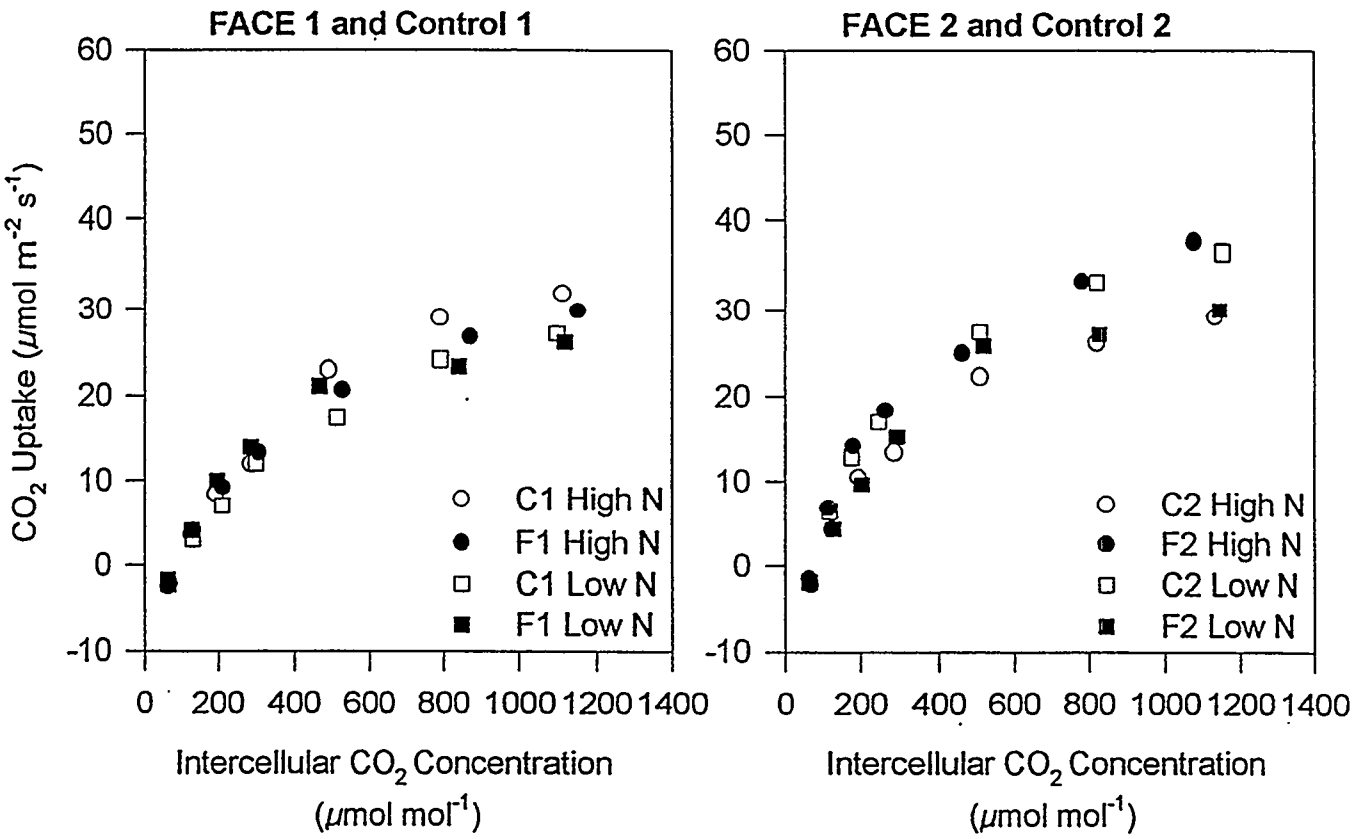


Figure 11. A/c_i response of *Lolium perenne* 1992 stands, grown in FACE and Control rings.
Measurements made on the 25 July 1997 - 4 days after 21 July harvest.



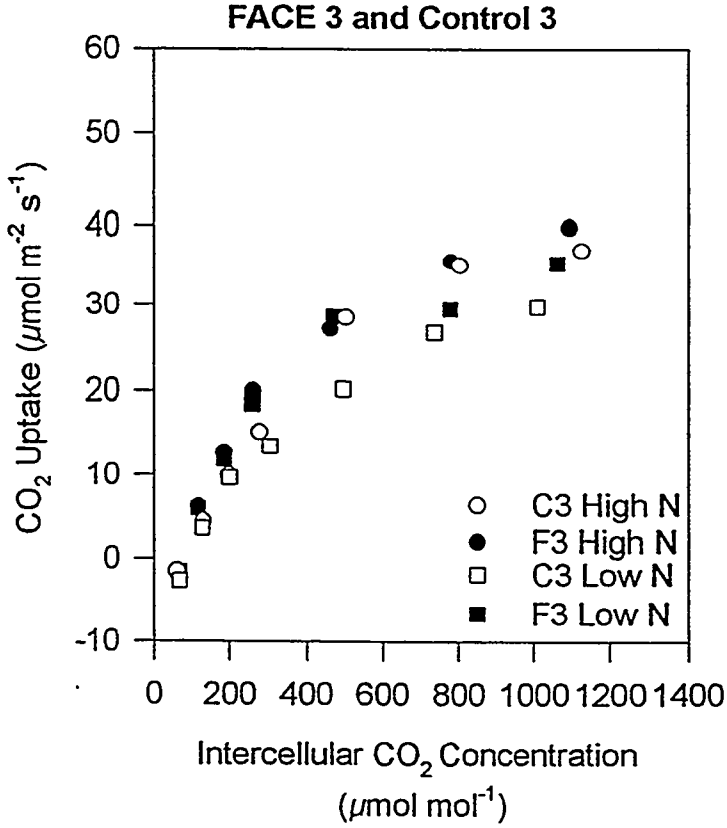
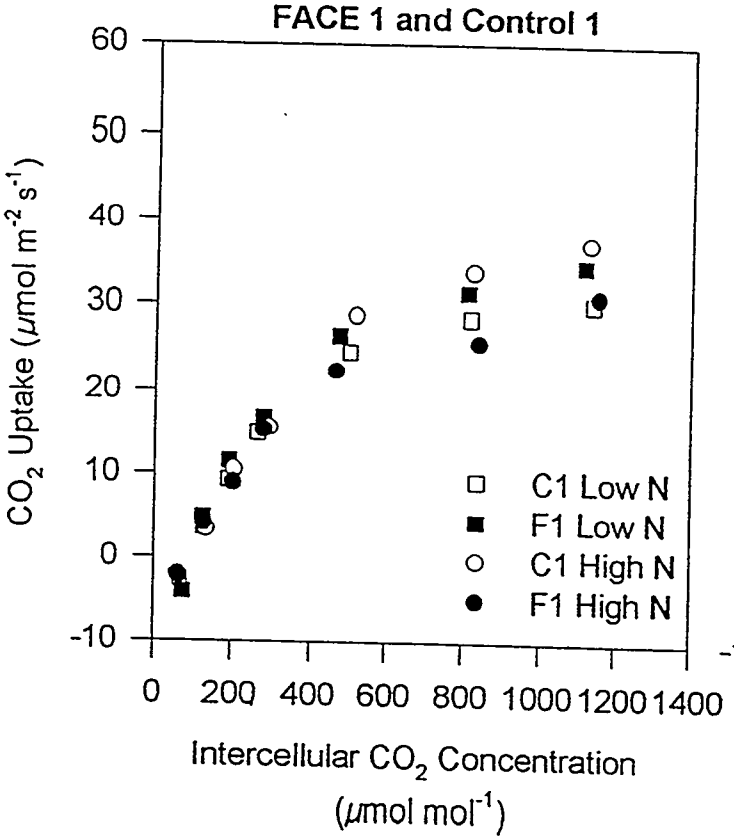
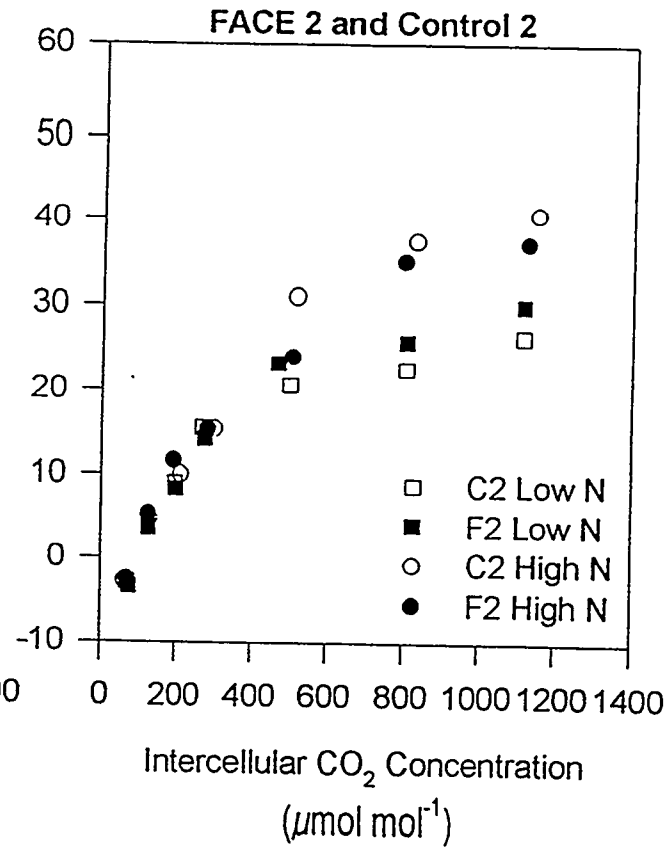


Figure 12. A/c_i response of *Lolium perenne* 1992 stands, grown in FACE and Control rings.
Measurements made on the 6 August 1997 - 16 days after the 21 July harvest.





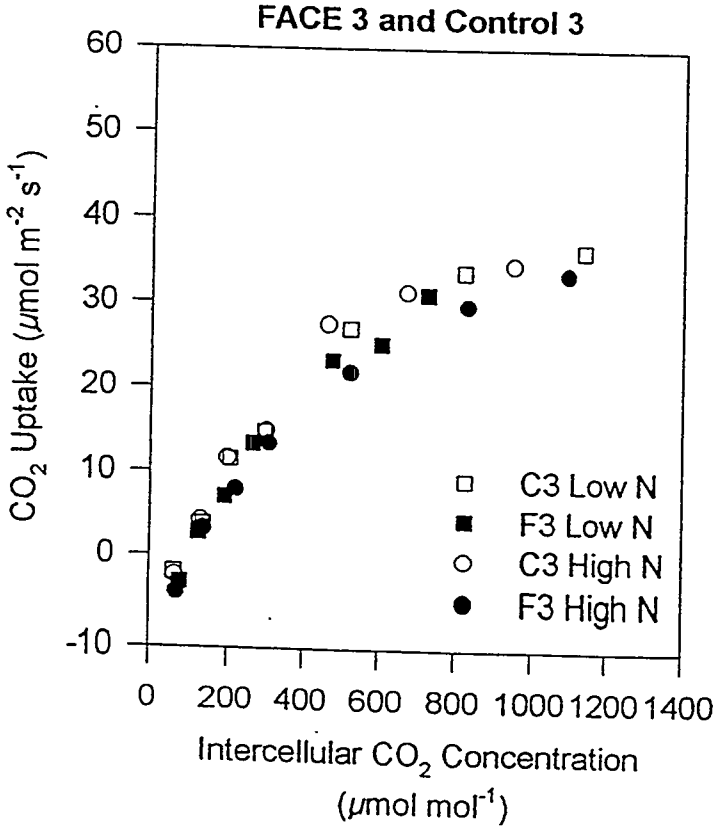


Figure 13. Variation in $V_{c,max}$ during periods of regrowth for 1992 stands of *L. perenne.*

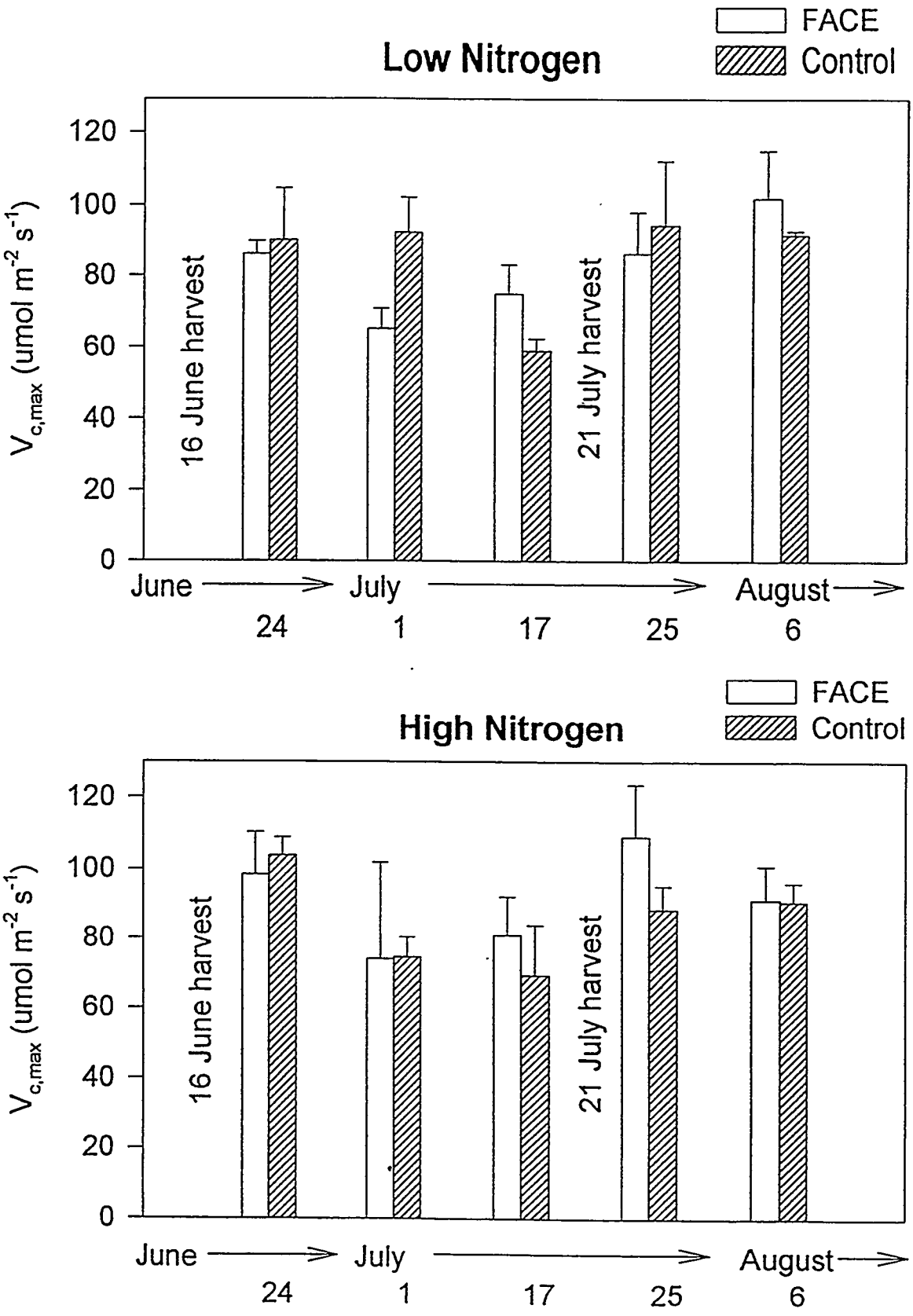
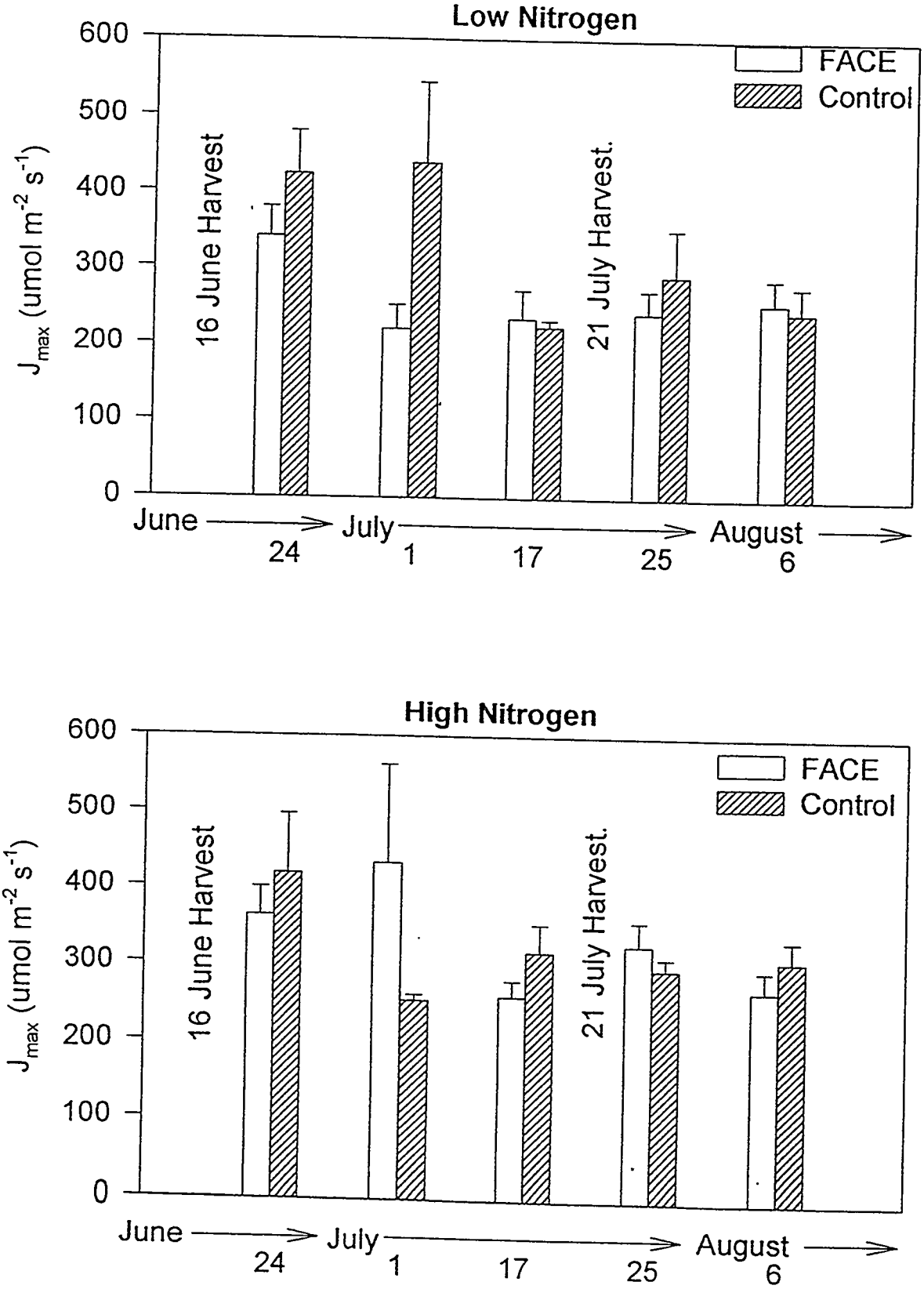


Figure 14. Variation in J_{max} during periods of regrowth for 1992 stands of *L. perenne.*



A similar trend was also seen in Figure 14 for the J_{max} values at both CO_2 and nitrogen treatments, with no significant effect of either [CO_2] or nitrogen treatment recorded for any of the periods of regrowth, except on the 1 July. On the 1 July the significance levels of variation show a significant difference between FACE and control J_{max} values due to the interactions of both [CO_2] and nitrogen treatment. This suggests that on the 1 July the maximum RuBP regeneration capacity in the low nitrogen FACE plots was significantly lower than that of the ambient plants, while in the high nitrogen FACE plots the reverse was true.

3.2.2. A/c_i response of 1995 grown stands.

The A/c_i response measurements obtained for 1995 grown stands of *L. perenne* over the ten week period, can be seen in Figures 15 -17. The mean $V_{c,max}$ and J_{max} values calculated from these curves, can be seen in Figures 18 and 19 respectfully:

The effect of both [CO_2] and nitrogen treatment upon $V_{c,max}$ values on the 24 June were significant, see Table 7 below. The $V_{c,max}$ values on this date, shown in Figure 18, were significantly lower for FACE grown plants and significantly lower in the low nitrogen plots.

Although a significant difference was expected on the 19 July 2 days before a harvest, when the source:sink ratio would be low, there was no significant effects from either [CO_2] or nitrogen treatment on this date. There was also no significant effect of [CO_2]

Figure 15. A/c_i response of *L. perenne* 1995 stands, grown in FACE and Control rings.

Measurements made on 24 June 1997 - 8 days after 16 June harvest.

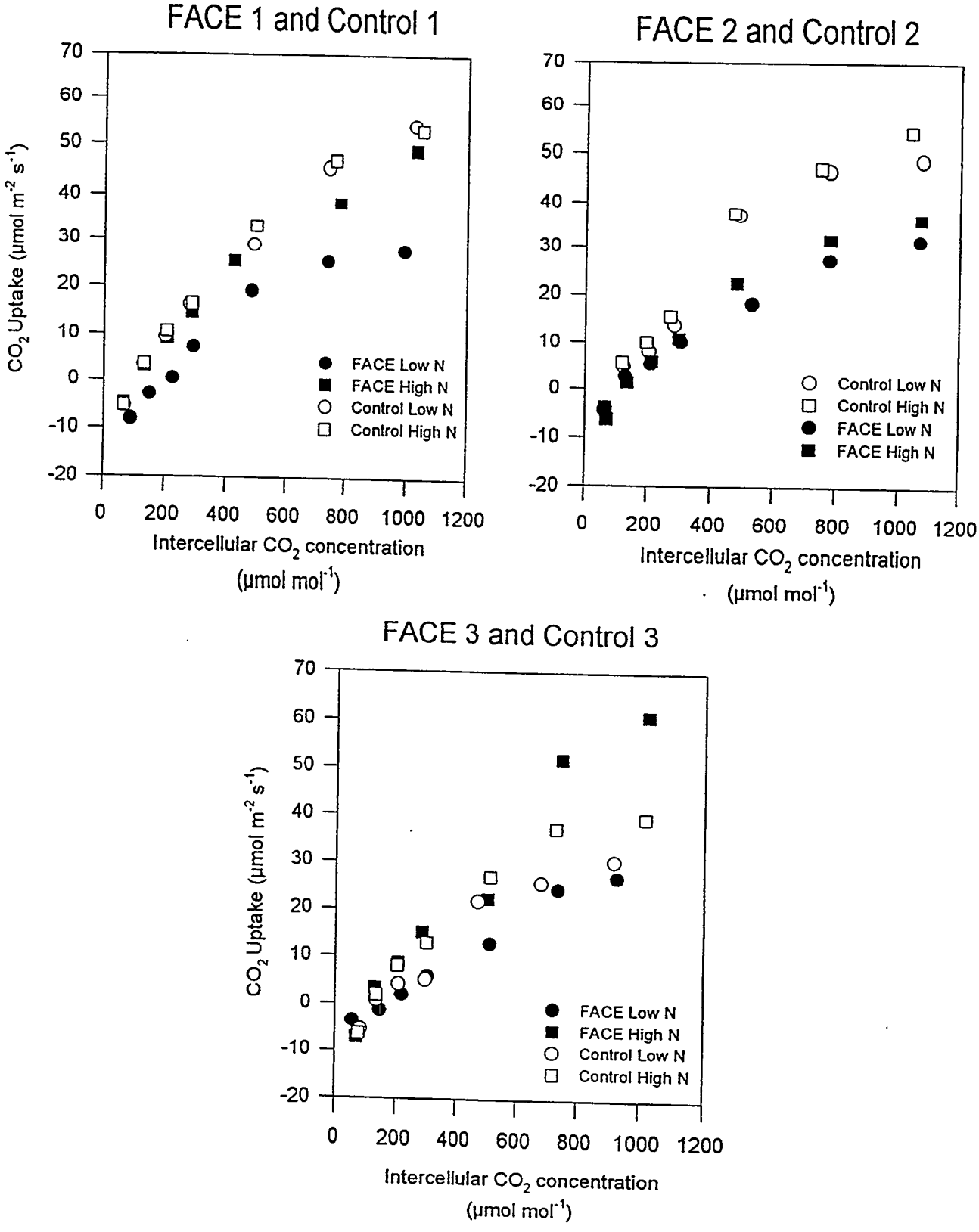
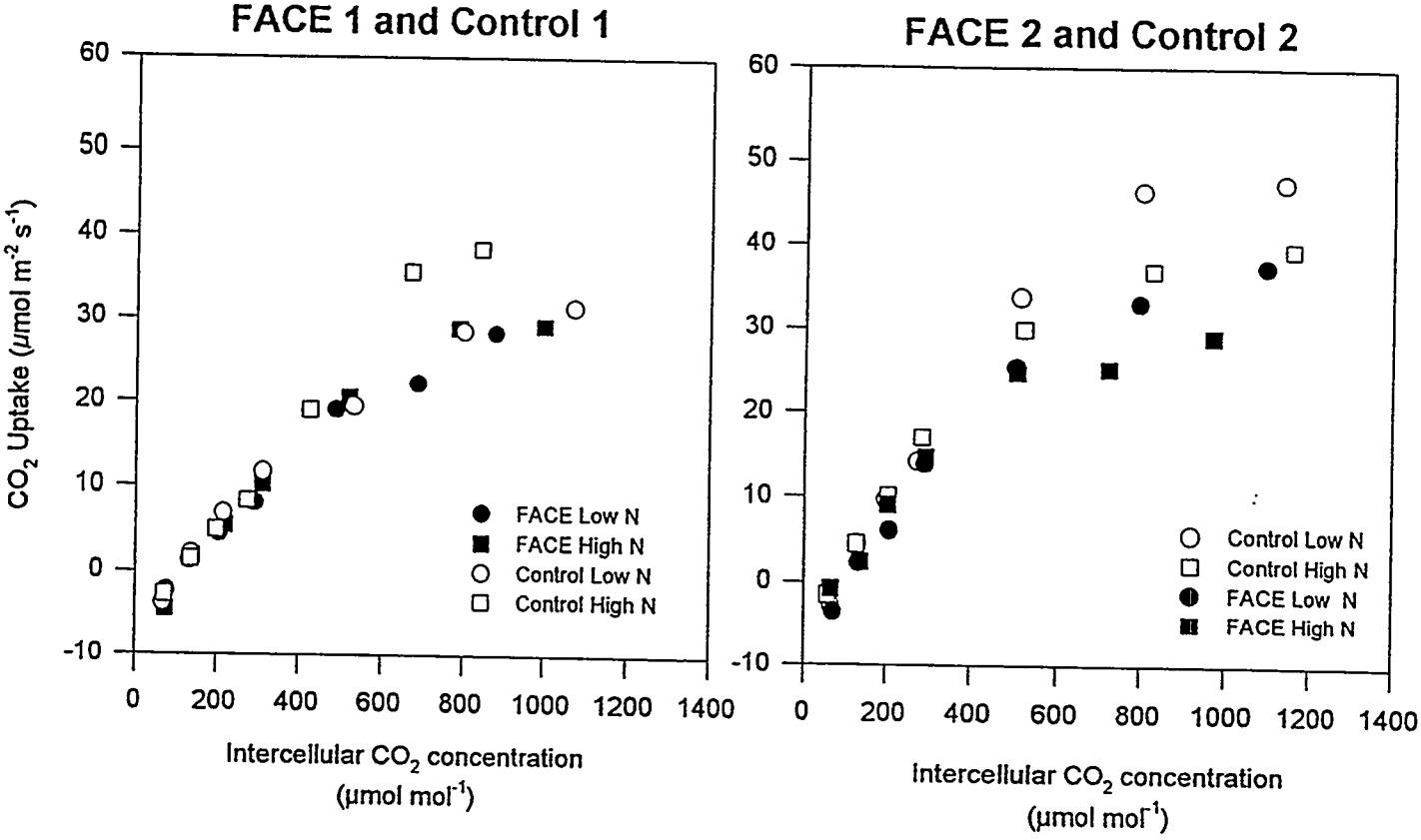


Figure 16. A/c_i response of *L. perenne* 1995 stands, grown in FACE and Control rings.

Measurements made on the 19 July 1997 - 2 days before 21 July harvest



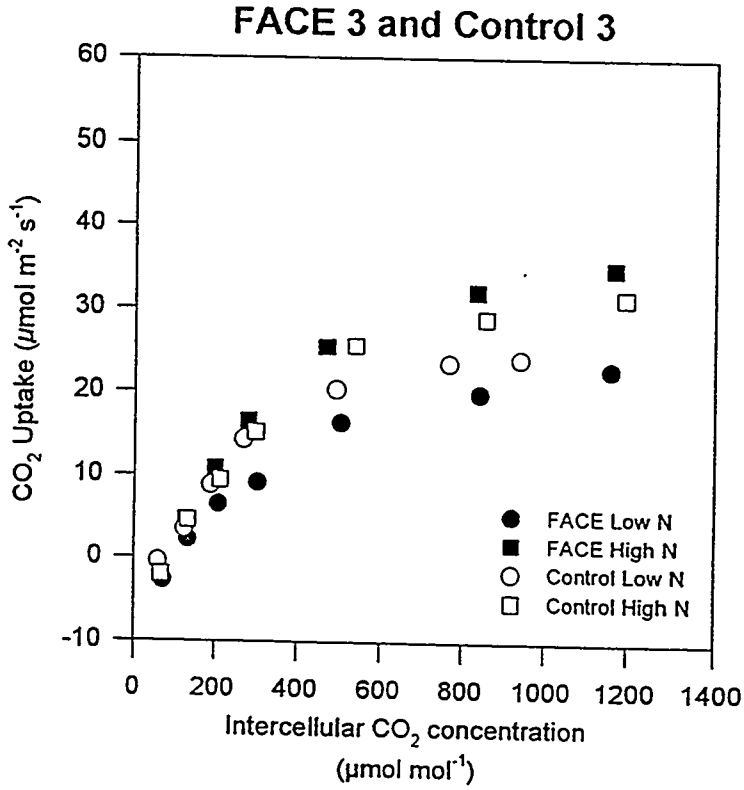
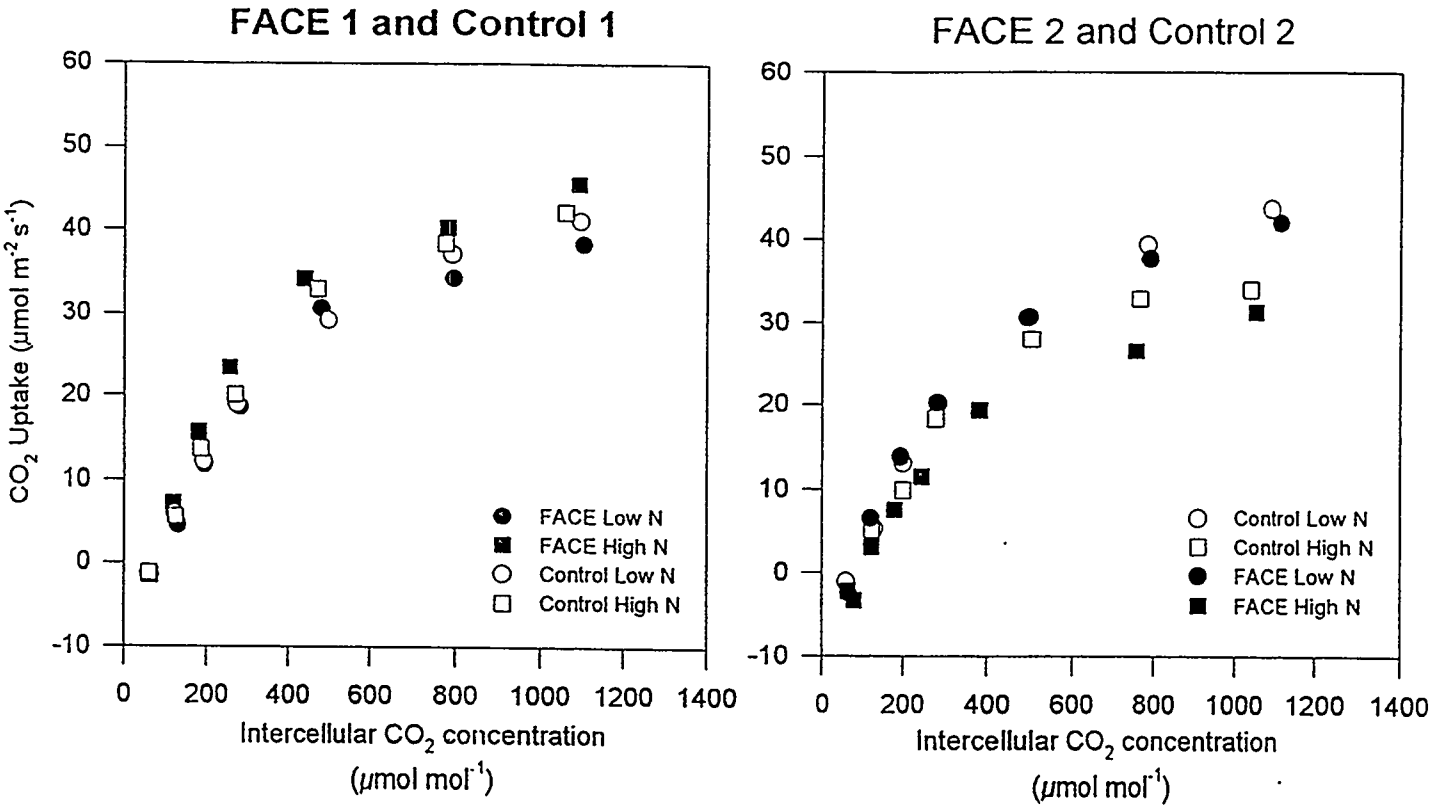


Figure 17. A/c_i response of *L. perenne* 1995 stands, grown in FACE and Control rings.

Measurements made on the 29 July 1997 - 8 days after 21 July harvest



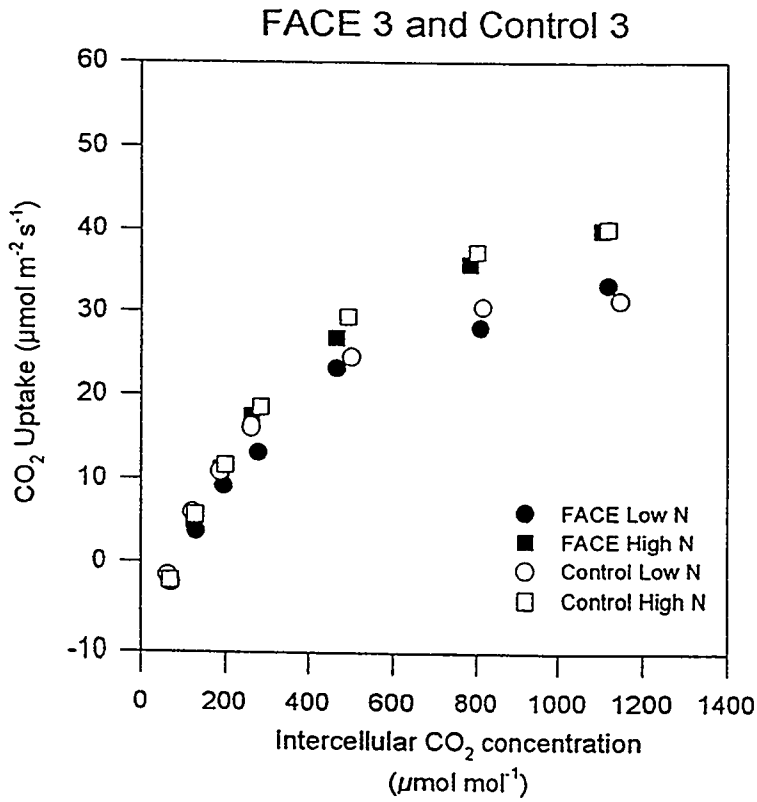


Figure 18. Variation in $V_{c,max}$ during periods of regrowth for 1995 stands of *L. perenne*.

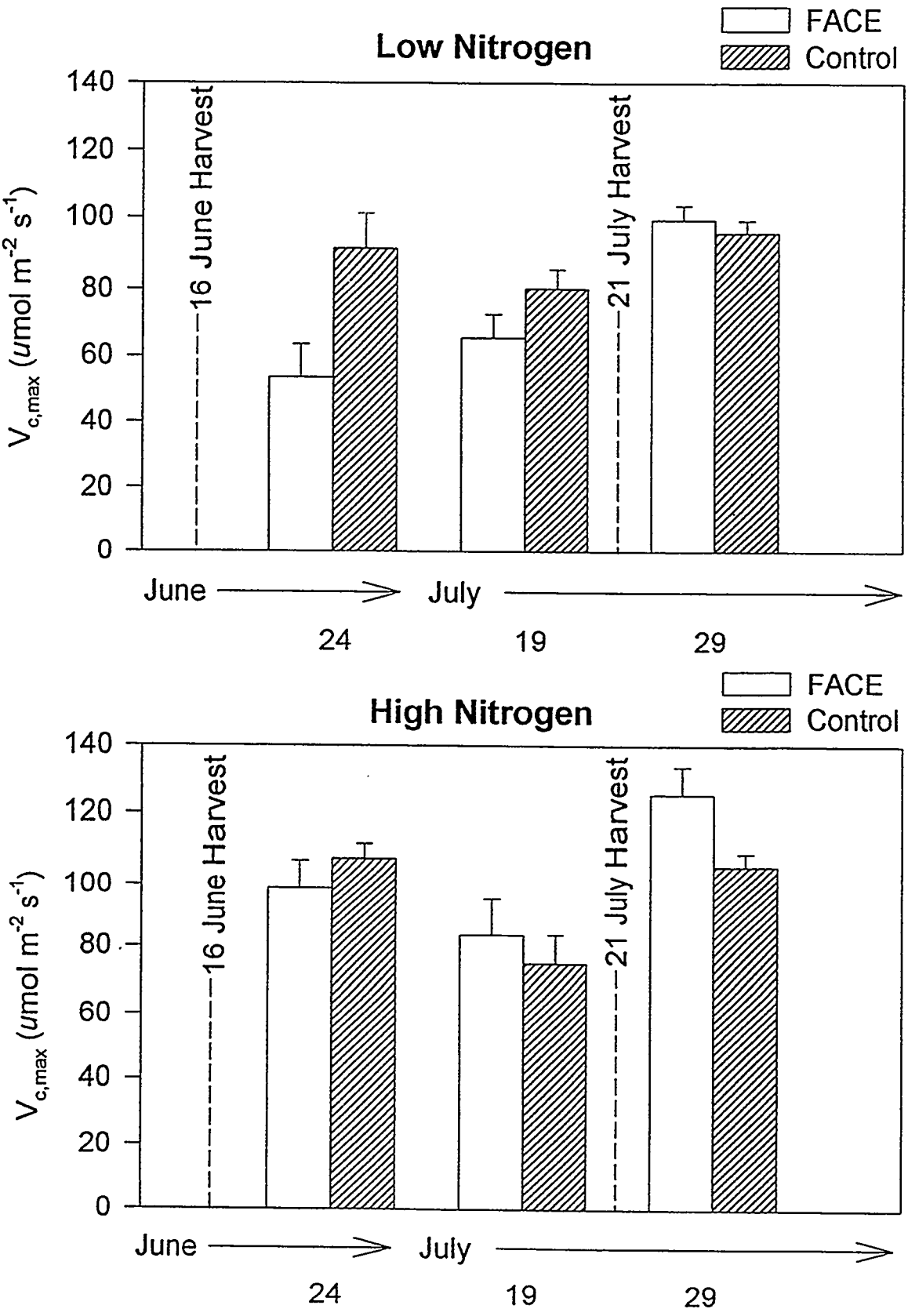
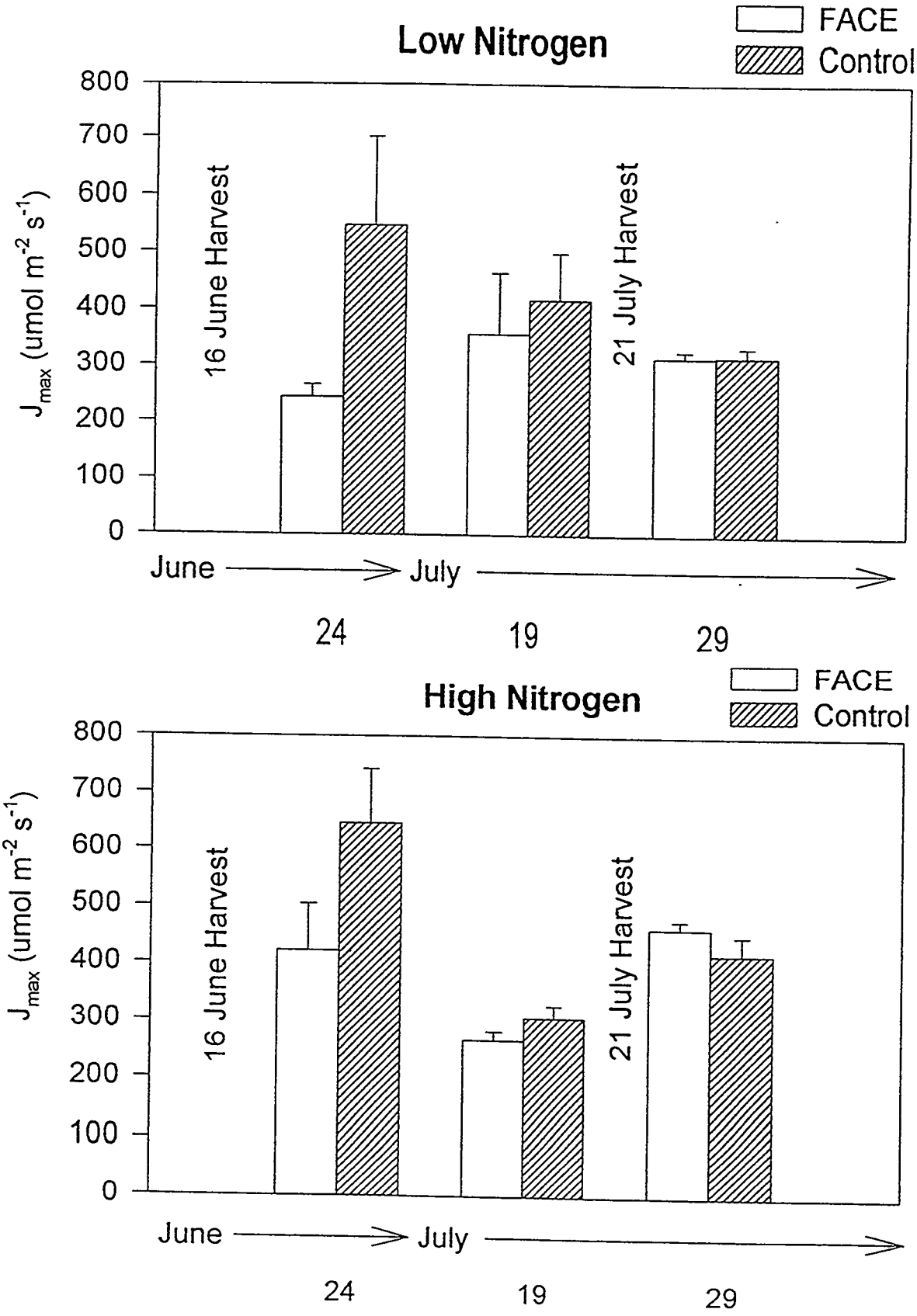


Figure 19. Variation in J_{max} during periods of regrowth for 1995 stands of *L. perenne.*



on the 29 July. However, $V_{c,max}$ values at both [CO_2] were significantly lower in the low nitrogen plots on this date.

Table 7. Significance levels of variation in $V_{c,max}$ and J_{max} due to [CO_2] and nitrogen treatments.

	Source of variation	24 June	19 July	29 July
V_{cmax}	CO_2 Treatment	*P<0.05; F=7.7*	*P=0.72; F=0.14*	*P=0.06; F=4.6*
	N Treatment	*P<0.05; F=13.5*	*P=0.45; F=0.62*	*P<0.05; F=11.65*
	Interaction	*P=0.13; F=2.9*	*P=0.21; F=1.82*	*P=0.17; F=2.23*
J_{max}	CO_2 Treatment	*P<0.05; F=8.13*	*P=0.48; F=0.54*	*P=0.32; F=1.13*
	N Treatment	*P=0.57; F=0.34*	*P=0.18; F=2.14*	*P<0.05; F=38.87*
	Interaction	*P=0.8; F=0.06*	*P=0.89; F=0.06*	*P=0.29; F=1.28*

The effect of [CO_2] on J_{max} values was also significant on the 24 June, with J_{max} being significantly lower in FACE grown plants. However, the effect of nitrogen treatments on J_{max} was not significant except on the 29 July, when J_{max} was significantly lower in the low nitrogen plots for both FACE and ambient grown plants.

4. DISCUSSION

The results of the diurnal measurements for the 1992 stands of *L. perenne* revealed a clear and consistent increase in leaf A and a consistent decrease in leaf g_s values for all the plants grown and measured at high [CO_2]. This corresponds to studies into *L. perenne* stands conducted at the Swiss-FACE facility in previous years (Bryant 1994; Nosberger *et. al.* 1996), and follows the expected response of C_3 species to elevated [CO_2] (Gay and Hauck 1994; Ryle *et. al.* 1992).

Enhancement of leaf photosynthesis was significant ($p<0.05$) at both N treatments and all periods of regrowth (Table 5), the percentage changes due to growth at high [CO_2] ranging from 40 to 83% (Table 4). While enhancement of photosynthesis occurred over the whole day, it was also marked at the beginning and end of each day, when the PPFD levels were low. This corresponds to the findings of Gay and Hauck (1994) into *Lolium temulentum* under low PPFD, but is in contrast to Ryle *et. al.* (1992) who suggested that enhancement of A in perennial ryegrass at high [CO_2] may only occur at high PPFD levels.

The observed enhancement of A at elevated [CO_2], was presumably related to the inhibition of RuBP oxygenation, which reduced the burden of photorespiration in FACE grown plants. The large stimulation in A observed on the 18 August at both N treatments, and to a lesser extent the stimulation on the 4 other dates, may also be associated with temperature interactions. Temperature is an important factor which can influence the response of plants growing at elevated [CO_2] (Long 1991). High

temperatures can alter the CO_2/O_2 specificity of RubisCO in favour of oxygenase. For plants growing under an ambient $[CO_2]$, this can result in a higher burden of photorespiration. FACE grown plants, in contrast, are grown under high $[CO_2]$ with a CO_2/O_2 regime which counteracts the competitive oxygenation of RubisCO (Bowes 1993). Therefore the temperature optimum of photosynthesis for FACE grown plants is much higher than their ambient grown counterparts. Any increase in temperature could therefore reduce the rates of photosynthesis in plants grown at the ambient $[CO_2]$, while FACE grown plants would be largely unaffected.

However, the micrometeorological data (Appendix E) and leaf temperatures (Table 4) suggest that the large enhancements in A observed on the 18 August are unlikely to be a result of temperature interactions. A more likely explanation is that the large increases in A are related to the drought conditions which existed prior to and during the measurements on this date (Appendix E). Indeed leaf g_s values recorded on the 10 August and 18 August (Figures 6 and 7 respectfully) are typical of drought conditions, where g_s is high during the early hours of the day, dropping off dramatically as temperature and light increase.

Water availability is an important factor which may effect the response of plants at high $[CO_2]$ (Campbell *et. al.* 1997). The cumulative effects of higher g_s values for plants grown at ambient $[CO_2]$ and presumably the cumulative effects of higher transpiration rates as a consequence, may have reduced the soil moisture within the control plots (relative to the FACE plots) leading up to the 18 August. A lower soil moisture content and the continuing drought conditions, may be resulting in the partial closure of stomata within the

leaves of ambient grown plants on the 18 August. The consequences of this would be an inhibitory effect on the supply of CO_2 to the chloroplast. Indeed on the 18 August no significant difference existed between FACE and control g_s values. This suggests that either; the g_s of ambient grown plants was reduced (below the level which may have been expected) due to the drought conditions, or that the stomata of FACE grown plants were able to remain fully open in comparison to the ambient grown plants. It has been reported that the water use efficiency of C_3 plants may be improved under elevated [CO_2] (Bowes 1993) allowing the stomata to remain open, even under conditions of water stress. As a result, CO_2 uptake is maintained at near to normal values and FACE grown plants are more able to withstand drought stress, delaying its onset.

The effects of time after harvesting on the photosynthesis of FACE-grown plants can be seen in the mean A values at high N, before and after the 21 July harvest. On the 16 July (5 days before the harvest) the mean diurnal A in FACE grown plants at high N was 15.6 $\mu mol\ m^{-2}\ s^{-1}$, while on the 30 July (9 days after the harvest) the equivalent value was 20 $\mu mol\ m^{-2}\ s^{-1}$. This could be associated with the source:sink balance which would be higher after a harvest. The plant would be in its youngest stages of growth responding to elevated [CO_2] to a greater extent than before a harvest, when its source:sink balance would be much lower.

The effect of N treatment on diurnal A at elevated [CO_2] was only significant on the 30 July (9 days after the harvest), when A was greater in the high N plots (Table 5). As a harvest was carried out on the 21 July, and the peaks of A on the 16 July (5 days before the harvest, Figure 4) were also greater at the high N plots, this may reflect some N-

dependant sink limitations. Gorissen *et. al.* (1995) suggested that N availability may be important for stimulating effects of elevated [CO_2] on the root growth and shoot yield of *L. perenne* and therefore sink strength. Presumably the plants growing in the high N plots may be producing larger sinks before and after a harvest reducing any feedback inhibition of photosynthesis. The denser canopies in high N plots appear to indicate higher shoot yields (Appendix D).

With the exception of the diurnal measurements made on 16 July and 30 July, there was little difference in the diurnal A enhancement at elevated [CO_2] between N treatments. Bryant (1994) in a previous study into *L. perenne* suggested that leaves developing under lower PPFD in the considerably denser canopies of the high N plots may not have been reaching their full photosynthetic potential. Whereas leaves in the much less dense canopies of the low N plots were operating much closer to their full potential. The denser canopies in the high N plots (Appendix D) suggest that this could well be the case.

The rates of leaf g_s showed a significant decrease, with the obvious exception on the 18 August (Table 5). The reduced g_s values under elevated [CO_2] may be attributed to the partial closure of stomata resulting in a constant c_i/c_a ratio of 0.75 for *L. perenne* at elevated [CO_2] (Gay and Hauck 1994; Bryant 1994). The percentage reduction in g_s values for FACE grown plants appeared to be greater in the low N plots (Table 4), but this was only significant on the 16 July. Greater reductions in g_s at low N could be explained by reduced growth, leading to a higher stomatal density per unit leaf area. However, stomatal measurements would be needed before any definite conclusions could be drawn.

The A/c_i response curves obtained from the measurement of 1992 stands of *L. perenne* (Figures 8-12) appear to be similar in the leaves of plants acclimated to present or elevated $[CO_2]$. Indeed there was no significant effect of either $[CO_2]$ or N treatment on the $V_{c,max}$ values, which represent the maximum rate of carboxylation, for any of the periods of regrowth (Table 6). Furthermore, under no conditions did J_{max}, which represents a measure of the maximum capacity for the regeneration of RuBP, differ significantly between FACE and control plants. This suggests that after almost 5 years of growth at elevated $[CO_2]$, the leaves of *L. perenne* do not appear to be experiencing a negative acclimatory response to their photosynthetic apparatus.

This is in sharp contrast to previous studies into the acclimatory effect of elevated $[CO_2]$ on *L. perenne* growing within the Swiss-FACE facility. Such a study reported by Bryant (1994) observed considerable acclimation of the photosynthetic apparatus in response to elevated $[CO_2]$, where $V_{c,max}$ declined by 35%, but J_{max} showed no significant change. Indeed, Nosberger *et. al.* (1996) reported that after 3 years of growth at elevated $[CO_2]$ a loss of RubisCO was present before a harvest and was removed after a harvest, suggesting the loss was driven by source:sink balance.

The trend seen in Figure 13, appears to suggest that the $V_{c,max}$ values declined before a harvest, increasing again after a harvest, reflecting that the maximum rate of carboxylation may be driven by source-sink balance. A similar trend for J_{max} can also be seen in Figure 14. However, it was presuppose that at elevated $[CO_2]$ a <u>significant</u> decrease in $V_{c,max}$ (and possibly J_{max}) would occur, being more pronounced before a harvest (when the

source:sink ratio was low) especially at low N. Nevertheless, after 5 years of growth at elevated [CO_2] this does not seem to be the case. This is surprising in view of the sparse canopy development at low N grown *L. perenne* stands (Appendix D), where restricted sink size may have been expected to lead to feedback inhibition of photosynthesis (Arp 1991)

The significant variation in J_{max} due to the interactions of both [CO_2] and N treatment seen on the 1 July (Table 6 and Figure 14), suggest that the maximum rate of RuBP regeneration was significantly reduced in the low N FACE plots. This could be related to transient P_i limitations due to the inhibition of sucrose synthesis, as the sparse canopies seen in the low N plots could be reflecting lower sink strength. The $V_{c,max}$ values on the 1 July also appears to decline in the low N Face plots (Figure 13). However, P_i limitations have also been observed following a sudden increase in [CO_2] at low temperature (Sage *et. al.* 1990). A combination of factors, from the effects of leaf sampling to high c_a's used during A/c_i measurements, may therefore have also contributed to the reduced J_{max} values seen on the 1 July.

The apparent lack of acclimation after 5 years growth at elevated [CO_2] could be due to a number of reasons. Gay and Hauck (1994) observed no acclimatory response in *L. temulentum* to elevated [CO_2] and suggested that this was due to the fact that the plants were still growing at the end of the experiment and were at no stage sink limited. Although the diurnal measurements indicated some sink limitations before a harvest, the plants may still have been growing and any sink limitations may not have been sufficient to induce feedback inhibition and thus acclimation. However, analysis of photosynthates

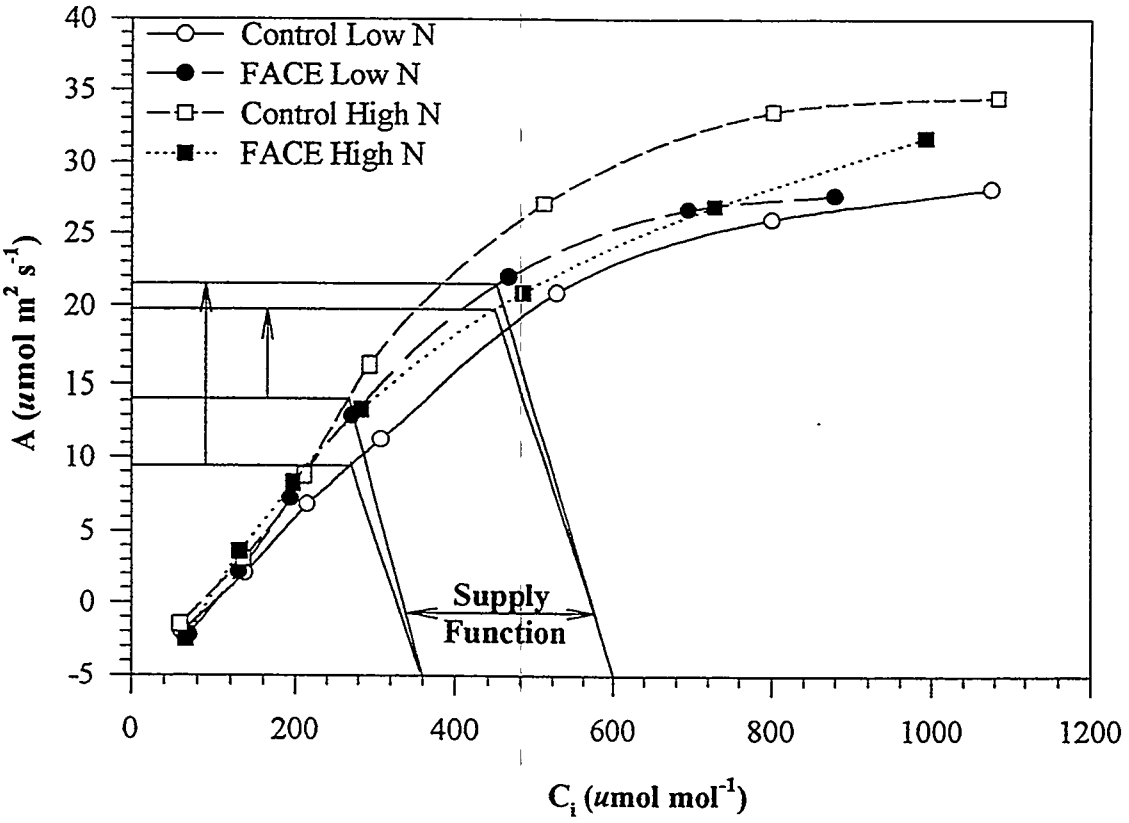
within leaves and other storage regions of the plants would be required, along with further A/c_i measurements, to establish whether this was the case.

Another possibility could be that after nearly 5 years of growth under elevated [CO_2], the acclimatory response initially seen in *L. perenne* has been partially removed as the plant has begun to adapt. Indeed Arp and Drake (1991) suggested a period of up to 4 years for the realisation of the perfect acclimatory response in field grown plants. During acclimation to elevated [CO_2], the capacity of RuBP carboxylation is down regulated (reduced $V_{c,max}$), as it is a non-limiting process at elevated [CO_2]. In a sink limited situation this would allow the plant to create a balance between the newly limiting processes of either P_i or RuBP regeneration. It has been suggested that the next stage, whereby resources are reallocated away from the down regulated, non-limiting components to the limiting components, may require years in perennial species (Long and Drake 1992). It may therefore have taken *L. perenne* 5 years to begin to realign the capacities of the limiting and non-limiting processes, whereby the development of stronger sinks (for example in the roots) has allowed them to partially remove an acclimatory effect at elevated [CO_2]. However, to test these assumption further A/c_i measurements would be required directly before and after a harvest, along with the analysis of leaf N, before any firm conclusion can be made.

Figure 20 illustrates how the considerable stimulation of photosynthesis at elevated [CO_2] observed in the diurnal sets of measurements is consistent with the similar rates of carboxylation and RuBP regeneration capacities of ambient and FACE grown plants. From mean A/c_i values plotted, it can be seen that even with similar carboxylation

efficiencies and RuBP regeneration rates the c_i's obtained at c_a 600 µmol mol^{-1} are sufficiently high to produce higher A values than those produced at the c_i corresponding to c_a 360 µmol mol^{-1}. This assumes a constant c_i/c_a ratio for *L. perenne* of 0.75 (Gay and Hauck 1994; Bryant 1994).

Figure 20. Mean A/c_i plot for *L. perenne*, measurements made on 1 July 1997.



In comparison, the A/c_i response curves obtained from measurements of 1995 grown stands of *L. perenne* (Figures 15-17), appear to suggest an acclimatory change in the photosynthetic apparatus in response to elevated [CO_2]. This negative response seems to be removed directly after the 21 July harvest. However, although this trend of down regulation can be seen in $V_{c,max}$ values especially at low N, the effect of [CO_2] was only

significant on the 24 June 8 days after a harvest. Nevertheless it can be clearly seen in Figure 18 that the $V_{c,max}$ values for FACE grown plants at low N on the 19 July (2 days before a harvest) had fallen below those of ambient grown plants. A similar trend can be seen in (Figure 19), with J_{max} values for FACE grown plants falling below those of ambient grown plants before a harvest and recovering after a harvest. However, the effect of $[CO_2]$ on J_{max} were also only significant on the 24 June.

The trends seen in both $V_{c,max}$ and J_{max} in Figures 18 and 19, appear to suggest that down regulation of the photosynthetic apparatus is taking place before the harvests (when the source:sink balance is low), and is being removed after the harvest (when the source:sink balance is increased). It is possible that the analysis of variance package on Excel 7, which was used to test the significance in variation, may be missing some small significant differences between $[CO_2]$ treatments. Based on these assumptions, it is suggested that the 1995 grown stands of *L. perenne* are undergoing a negative acclimatory change (although small) which may be driven by source:sink balance.

This follows the pattern seen in previous studies into *L. perenne* after 2 to 3 years growth at elevated $[CO_2]$ (Bryant 1994; Nosberger *et. al.* 1996) and is in direct contrast to the response observed for the 1992 stands of *L. perenne* after 5 years growth at elevated $[CO_2]$. Again further A/c_i measurements are required before any definite conclusions can be drawn from the above assumptions.

In conclusion, the diurnal gas exchange measurements suggest that Hypothesis 1 can be rejected. As after 5 years of growth leaf photosynthesis had not decreased, when measured at elevated [CO_2], to the level of leaves grown and measured at current ambient [CO_2], at either N treatment.

Hypothesis 2 can also be rejected for the 1992 grown stands of *L. perenne.* As acclimation of photosynthesis was absent at all periods of regrowth and N treatment. However, Hypothesis 2 is accepted for the 1995 grown stands of *L. perenne.* As acclimation of photosynthesis to elevated [CO_2] (although small) was more pronounced at low N before a harvest.

While hypothesis 3 can be rejected as the photosynthetic response of *L. perenne* exposed to elevated [CO_2] for 2 years showed a small negative acclimatory response, while *L. perenne* exposed to elevated [CO_2] for 5 years showed no acclimatory response.

Clearly, the cutting of ryegrass stands may temporarily help to alleviate sink saturation, while N availability may also increase the potential for sink growth. The apparent removal of an acclimatory response after almost 5 years of growth at elevated [CO_2], in this realistic open-field situation, suggests that the ryegrass *L. perenne* may be closer to achieving the appropriate photosynthetic adjustments which may eventually allow it to attain a significantly higher photosynthetic potential.

5. ACKNOWLEDGEMENTS.

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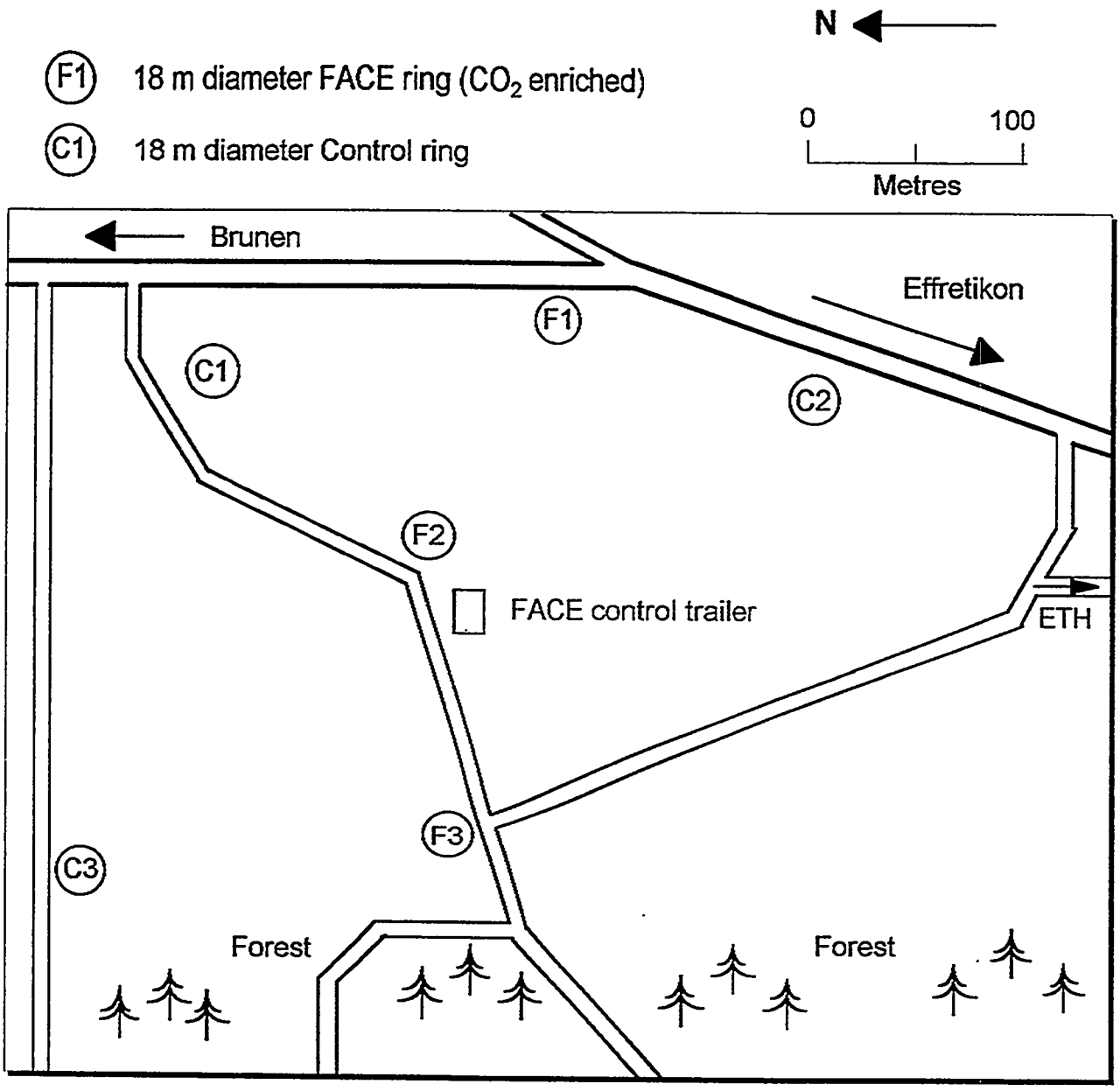
Appendix A.

Layout of the FACE facility at the ETH field station, Eschikon, Switzerland.



Appendix B.

Figure B1. Infra-red gas analysis system (CIRAS-1 PP systems, Hitch., Herts.) attached to a Parkinson Leaf Cuvette (PLC-1, PP Systems, Hitch., Herts.)

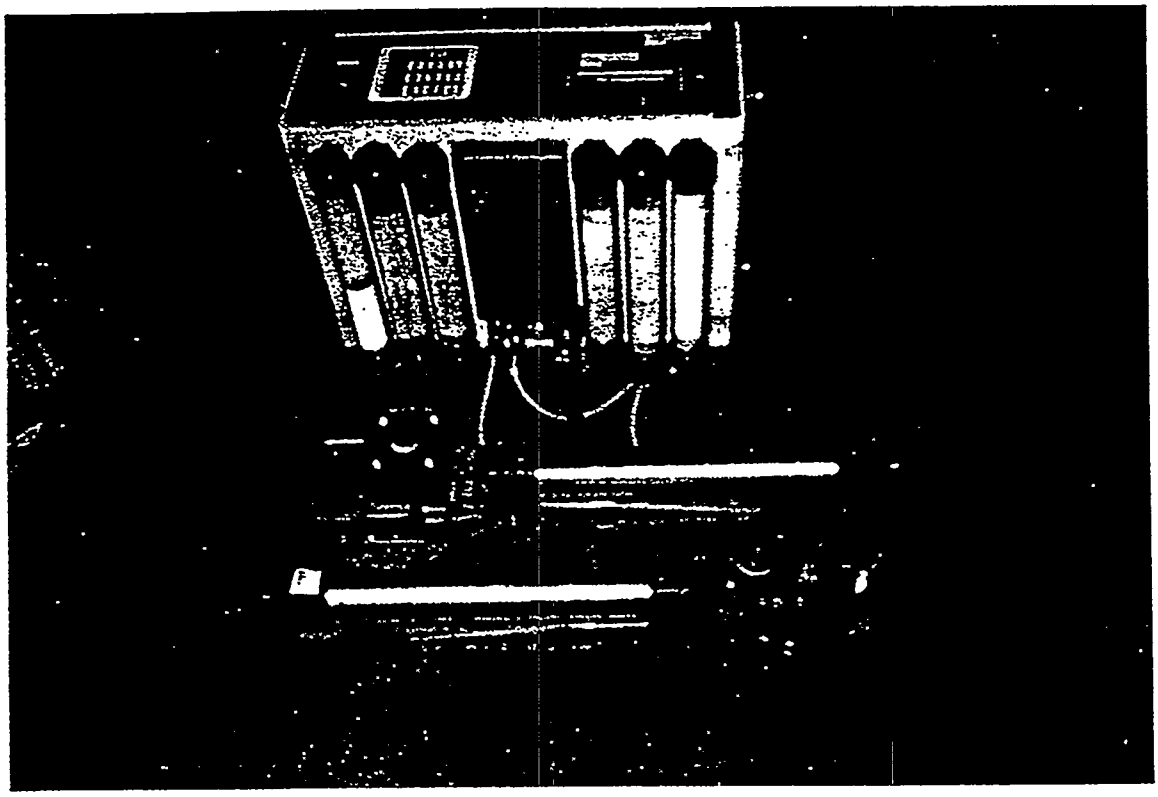
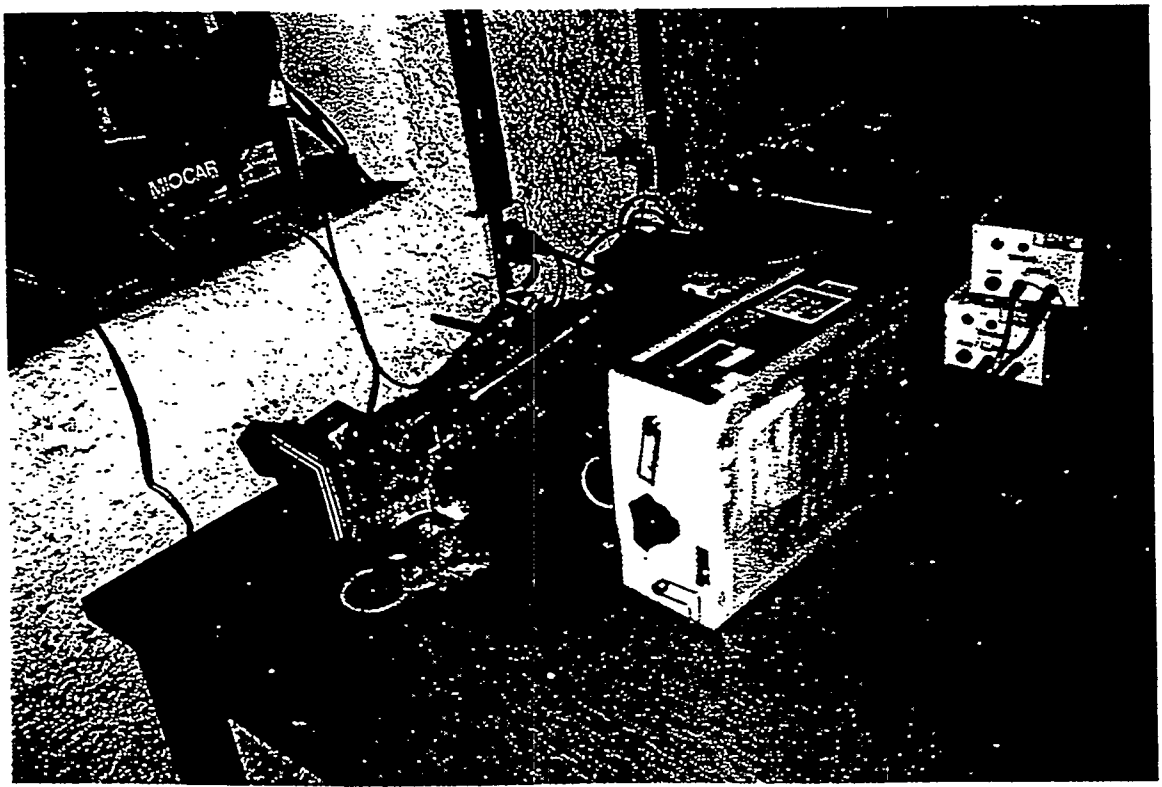


Figure B2. Parkinson Leaf Cuvette (PLC-1, PP Systems, Hitch., Herts.) with quartz iodide light source clipped over the leaf chamber



Appendix C.

Basic equations used to obtain gas exchange parameters, according to Von Caemmerer and Farquhar (1981), taken from Long and Hallgren (1993) and Long *et al.* (1996).

- **Net rate of CO_2 uptake (A) per unit leaf area.**

$$A = \frac{u(c_e - c_o)}{s}$$

Where **u** = flow of air entering the chamber (mol s^{-1})

c_e = $[CO_2]$ at the entrance of the chamber (μmol mol^{-1})

c_o = $[CO_2]$ at the outlet of the chamber (μmol mol^{-1})

s = surface area of the leaf (m^2)

- **Transpiration rate (E)**

$$E = \frac{(e_o - e_i)}{(P - e_o)}.u$$

Where e_o = humidity of air in the chamber (mbar)

e_i = humidity of the air entering the chamber (mbar)

P = atmospheric pressure (mbar)

- **Stomatal conductance to CO_2 (g_s).**

$$g_s = \frac{1}{r_1 \times 1.61}$$

Where **1.61** = ratio of diffusivity for water vapour/air and CO_2/air

r_1 = leaf stomatal resistance to water (m^{-2} s mol^{-1})

$$r_1 = \frac{\frac{(e_i - e_o)}{(e_o - e_i)} \times \frac{(P - e_o)}{P}}{u} - r_b(H_2O)$$

Where $r_b(H_2O)$ = boundary layer resistance for water vapour (m^2 s mol^{-1})

$$r_b(H_2O) = \frac{E}{W_{s[T]}/P - W_a/P}$$

Where $W_{s[T]}$ = water vapour concentration in saturated air at temperature T

W_a = water vapour in bulk air

- **Intercellular CO_2 concentration (c_i).**

$$c_i = \frac{(g_t - E/2).C_a - A}{g_t + E/2}$$

Where C_a = $[CO_2]$ in the air (µmol mol^{-1})

g_t = total conductance to CO_2 diffusion (mmol m^{-2} s^{-1})

$$g_t = \frac{g_s \times g_b}{g_s + g_b}$$

Where g_b = boundary layer conductance to CO_2 (mol m^{-2} s^{-1})

$$g_b = \frac{1}{r_b(H_2O) \times 1.37}$$

Where **1.37** = ratio of diffusivity in the boundary layer.

Appendix D.

The condition of FACE grown *Lolium perenne* stands during periods of regrowth after a harvest, at high and low nitrogen treatments.

Figure D1. Low nitrogen plots - 1 week after a harvest

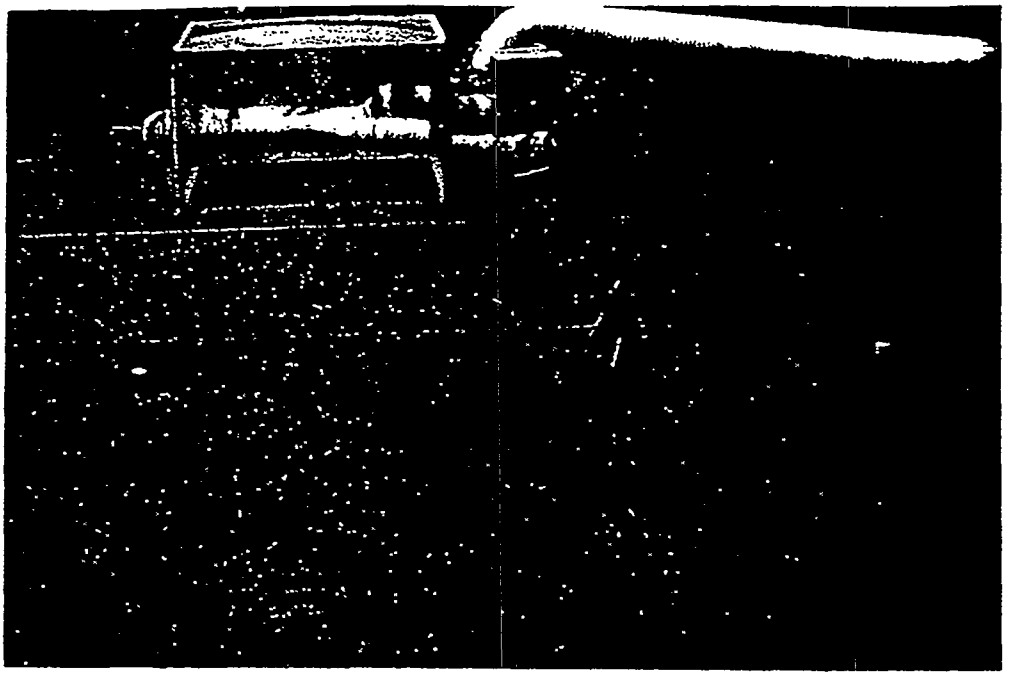


Figure D2. High nitrogen plots - 1 week after a harvest.

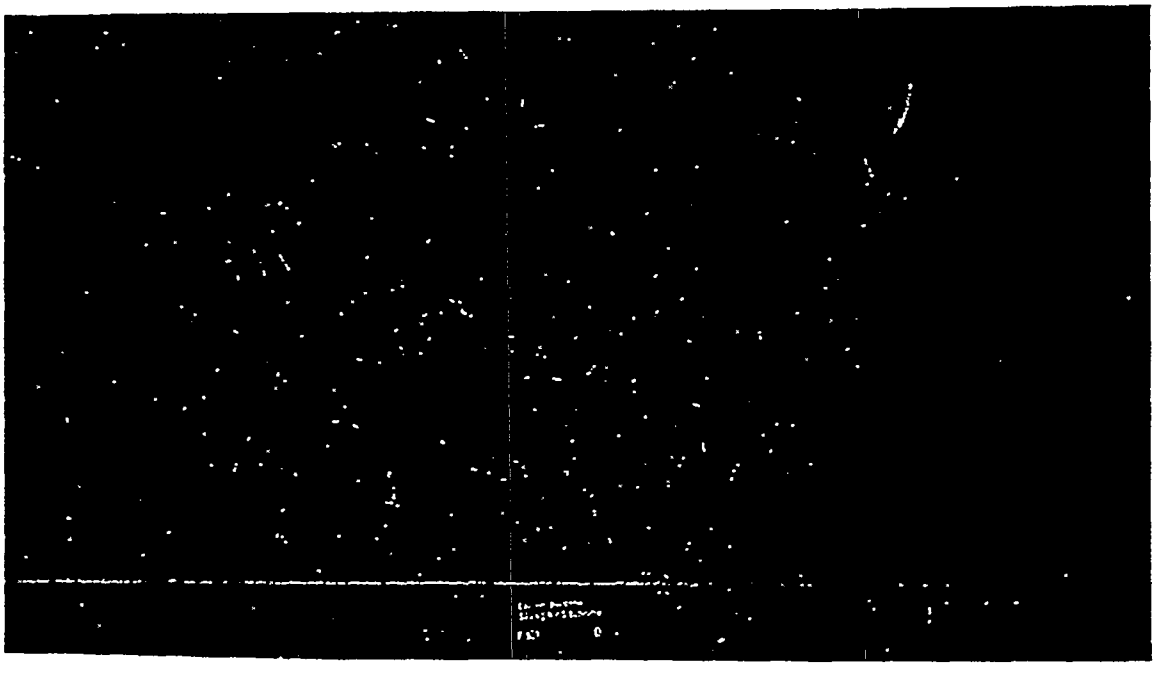


Figure D3. *L. perenne* low nitrogen plots - 2.5 weeks after a harvest.

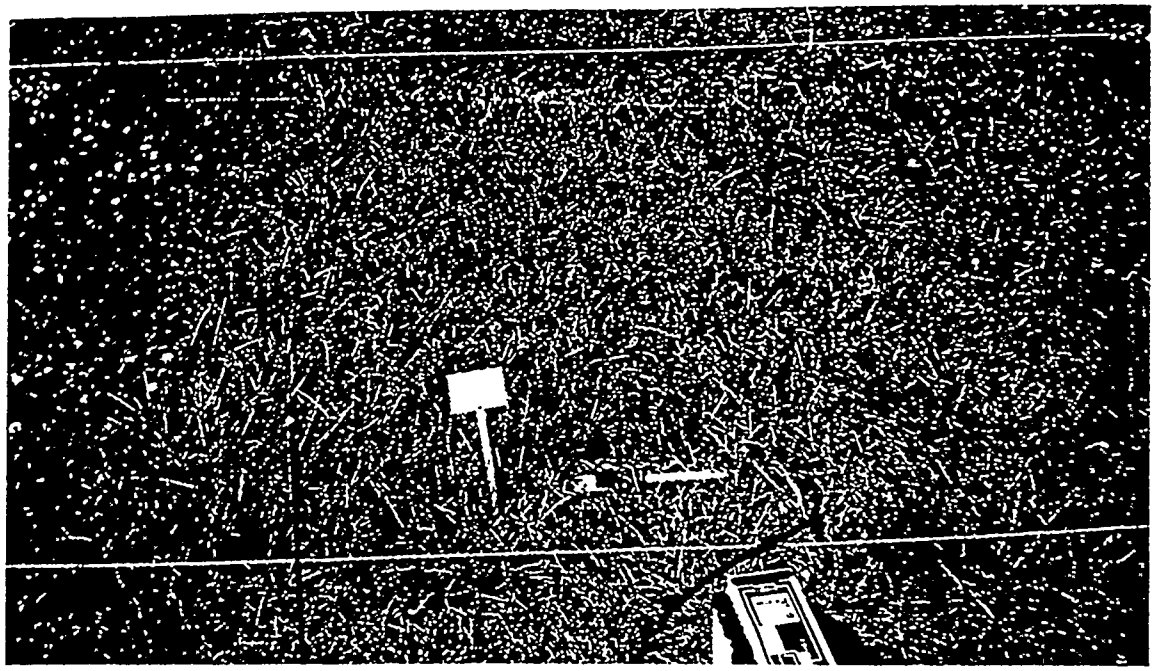


Figure D4. *L. perenne* high nitrogen plots - 2.5 weeks after a harvest.

Figure D5. *L. perenne* low nitrogen plots - 1 week before a harvest.

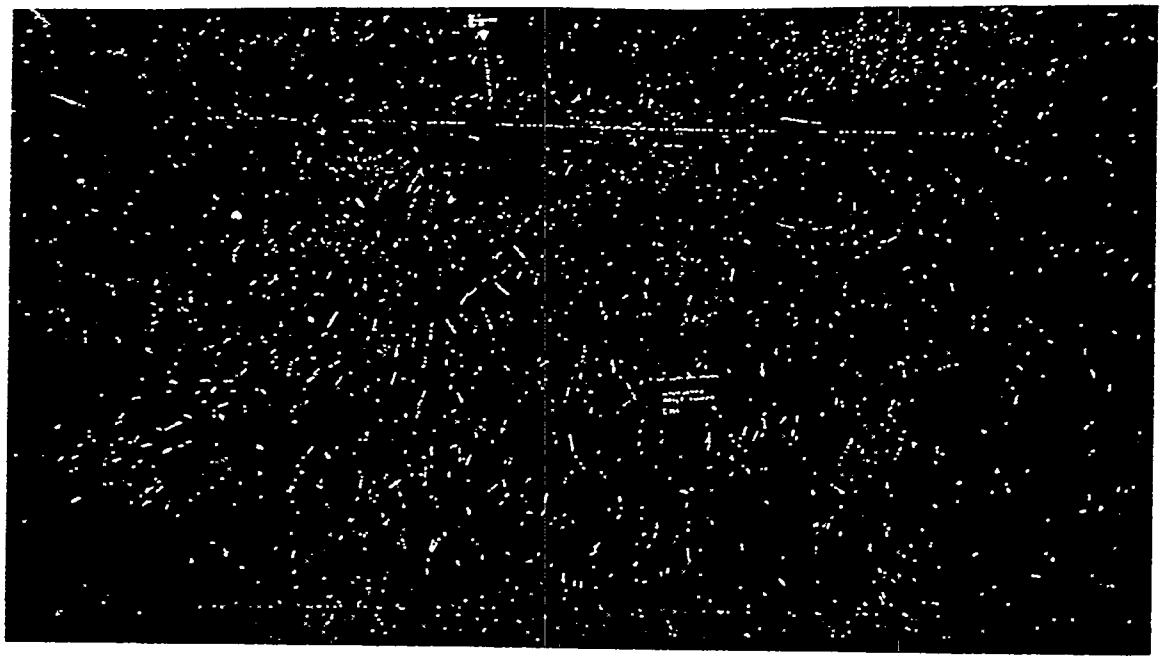
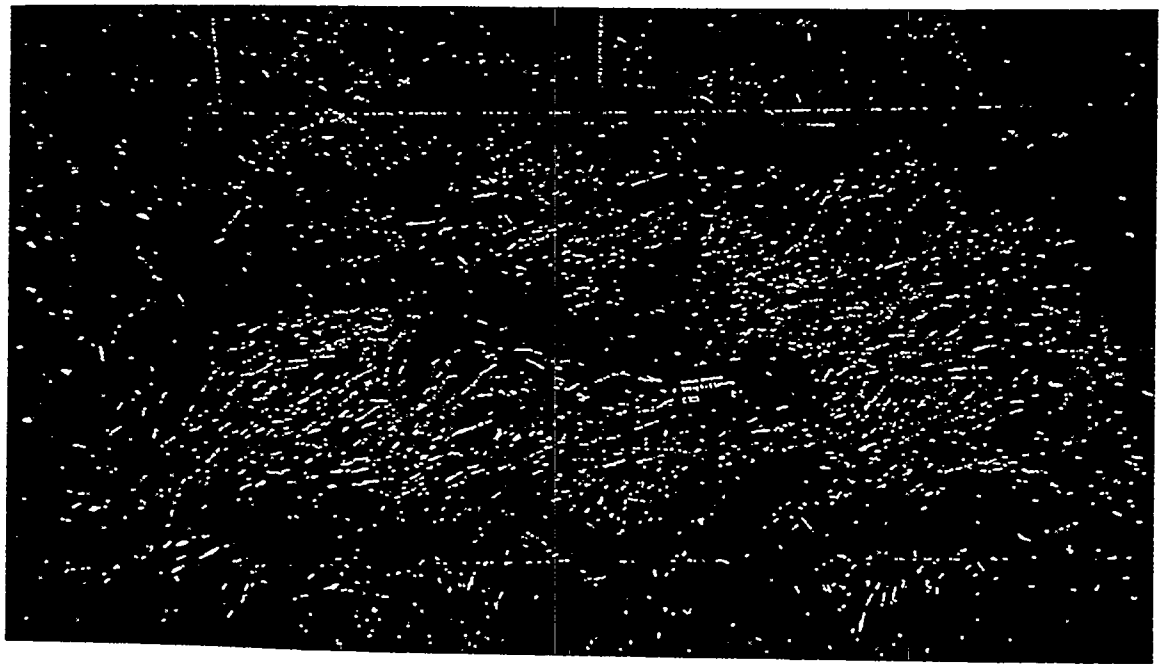


Figure D6. *L. perenne* high nitrogen plots - 1 week before a harvest.



Appendix E.

Micrometeorological Data from ETHZ Switzerland.

Date	Rain mm	Irradiance global MJ/m^2	Air Temp. at 2 m (°C)	Soil Temp. at 50 cm (°C)
14-Jun-97	18	-	15.36	15.68
15-Jun-97	16	-	16.16	15.59
16-Jun-97		-	16.32	15.64
17-Jun-97	2	-	16.14	15.74
18-Jun-97	9	-	14.26	15.8
19-Jun-97	6	-	11.43	15.68.
20-Jun-97	6	-	13.02	15.36
21-Jun-97	6	-	12.65	15.18
22-Jun-97	22	12.07	10.67	14.98
23-Jun-97	10	15.28	12.42	14.68
24-Jun-97	2	11.7	10.94	14.57
25-Jun-97	13	17.89	12.72	14.5
26-Jun-97	5	13.13	13.11	14.53
27-Jun-97		5.42	11.79	14.56
28-Jun-97	3	20.82	15.8	14.48
29-Jun-97	10	12.44	16.16	14.66
30-Jun-97	9	16.55	12.87	14.85
1-Jul-97		23.13	15.33	14.36
2-Jul-97		22.76	17.22	14.53
3-Jul-97	2	14.46	15.07	14.76
4-Jul-97	22	11.41	13.24	14.83
5-Jul-97	21	5.98	11.28	14.39
6-Jul-97	27	12.22	12.29	14.17
7-Jul-97	2	19.42	13.66	14.1
8-Jul-97		26.83	16.06	14.3
9-Jul-97		28.53	17.69	14.78
10-Jul-97		25.83	17.32	15.79
11-Jul-97		18.19	16.79	16.45
12-Jul-97		22.96	18.58	16.99
13-Jul-97		26.16	20.11	17.6
14-Jul-97		14.23	17.73	18.01
15-Jul-97	22	25.05	17.35	17.98
16-Jul-97		28.05	18.54	18.36
17-Jul-97		13.44	18.26	18.5
18-Jul-97	25	10.45	13.03	17.93
19-Jul-97	24	5.32	12.35	17.03
20-Jul-97	4	17.59	14.05	16.71
21-Jul-97		24.7	14.61	16.92
22-Jul-97		27.93	17.14	17.38
23-Jul-97	9	11.31	16.29	17.65
24-Jul-97		22.46	19.2	18
25-Jul-97	13	7.86	15.08	18.32
26-Jul-97		15.43	15.64	17.79
27-Jul-97		22.21	18.06	17.8
28-Jul-97		24.43	18.72	18.24
29-Jul-97		22.31	18.62	18.37
30-Jul-97		26.22	19.92	18.6
31-Jul-97		12.1	17.33	18.54
1-Aug-97	13	4.46	11.81	17.89
2-Aug-97	6	6.18	14.7	17.27
3-Aug-97		22.18	18.03	17.5
4-Aug-97		25.59	21.04	18.08
5-Aug-97		24.28	22.32	18.68
6-Aug-97		8.64	19.17	18.93
7-Aug-97		17.32	19.61	18.78
8-Aug-97		14.62	18.11	18.84
9-Aug-97		23.93	18.91	18.8
10-Aug-97		26.21	20.12	18.77
11-Aug-97		24.79	20.6	18.88
12-Aug-97		22.72	21.44	19.01
13-Aug-97		18.78	21.02	19.1
14-Aug-97		18.5	21.33	19.18
15-Aug-97		22.46	21.62	19.19
16-Aug-97		21.6	20.01	19.3
17-Aug-97		12.11	18.55	19.2
18-Aug-97		20.44	18.56	18.72
19-Aug-97		21.71	18.82	18.55
20-Aug-97		21.3	19.71	18.51