Effects of sustained elevated CO_2 concentration and Nitrogen nutrition on wheat (*Triticum aestivum* L. cv Gamtoos)

THESIS

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By

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IN MEMORY OF MY UNCLE
MOSALA JOSEPH SEREMO
1953 – 1999

DEFINITION OF SYMBOLS

Symbol	**Unit**	**Definition**
A	μmol mol-1	Assimilation of CO_2 per unit leaf area
C_i	μmol mol-1	Intercellular carbon dioxide concentration
E	mmol m^{-2} s^{-1}	Transpiration rate
g_s	mol m^{-2} s^{-1}	Stomatal conductance
PPFD	μmol m^{-2} s^{-1}	Photosynthesis photon flux density
WUE	mmol mol^{-1}	Water use efficiency

DEFINITION OF ABRREVIATIONS

Abbreviation	Definition
CO_2	Carbon dioxide
[CO_2]	Carbon dioxide concentration
C/N	Carbon to nitrogen ratio
DAG	Days after germination
DW	Dry weight
mM	millimolar
NAR	Net assimilation rate
[N]	Nitrogen concentration
NSC	Non-structural carbohydrates
NUE	Nitrogen use efficiency
PCR	Photosynthetic carbon reduction cycle
PCO	Photorespiratory carbon oxidation cycle
P_i	Inorganic phosphate
rb	Boundary layer resistance
RGR	Relative growth rate
RH	Relative humidity
Rubisco	Ribulose bisphosphate carboxylase-oxygenase

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Abstract

There is consensus that high CO_2 results in enhanced growth and yield for most crop plants. However, most of these studies were carried out in the presence of adequate nutrients, which is also the case in agricultural systems (managed ecosystems). About 20% of the earth's land mass have sufficiently low levels of nutrients to cause some kind of stress to plants. On the other hand, elevated [CO_2] decreases foliar nutrient elements in plants and as a result partitioning of certain nutrient elements in plants is altered. Little data is available on the partitioning of most nutrient elements in plants, and this will definitely impact on growth and yield.

To investigate this, wheat (*Triticum aestivum* L. c.v. Gamtoos) was grown in controlled environment cabinets at 360 and 700 μmol $mol^{-1}CO_2$. The full Long-Ashton nutrient solution comprising of three-nitrogen concentrations ([N]) viz. (4,6 and 12 mM) was used to water plants everyday. The measurement of net assimilation rate (NAR), stomatal conductance (g_s), transpiration rate (E), water use efficiency (WUE), foliar [N], nitrogen use efficiency (NUE) and growth parameters (total plant biomass (TPB), total plant height (TPH), leaf area (LA), shoot and root dry weight) were made 7 days after germination (7 DAG) till the onset of flowering.

The increase in nitrogen supply in the order of 4, 6 and 12mM resulted in an increase in NAR, g_s , WUE and a decline in E under elevated [CO_2]. Under elevated [CO_2] NAR was observed to increase during the first two weeks

reaching its maximum at 14 DAG, thereafter followed by a decline reaching its maximum at 28 DAG. This was later followed by an increase at 35 DAG onwards. Under elevated [CO_2], NAR was increased significantly between the nitrogen regimes during the first (7-14 DAG) and the last two (35-42 DAG) weeks.

The response of assimilation as a function of internal [CO_2] (C_i), showed a decrease with age at ages 14, 28 and 35 DAG. This negatively affected the initial slope and the CO_2 saturated photosynthetic rates under all treatments. This suggest that acclimation may have been as a result of both stomatal and biochemical limitations.

All the photosynthetic pigment levels (chl_a, chl_b, chl_{a+b}, and C_{x+c}) increased with an increase in nitrogen supply from 4 to 6mM [N]. A 12mM [N] resulted in a significant decline in the photosynthetic pigment levels compared to a 6mM [N]. Chl_a remained higher than chl_b under all treatments. Also, NAR was seen to increase and decrease concomitantly with the photosynthetic pigment levels.

Foliar [N] was seen to decrease with an increase in nitrogen supply from 4 to 6 mM [N] under elevated [CO_2] and the effects were adverse under the 4mM [N]. Under the 6mM N regime foliar [N] was positively correlated to NAR for elevated [CO_2] grown plants. Similarly, E was positively correlated to foliar [N] under the same conditions.

Elevated CO_2 and increase in nitrogen supply had a pronounced effect on total plant height (TPH), total plant biomass (TPB), leaf area (LA), shoot and root dry weight and nitrogen use efficiency (NUE). The effects were more pronounced under a 6mM [N] as a result of high NUE. However, under 12mM [N] growth was not as expected as a result of lower NUE. Under all treatments shoot dry weight (SDW) was positively correlated to NUE.

Anatomical studies revealed that total leaf and midrib thickness was significantly increased with an increase in nitrogen supply under elevated CO_2 to support the larger leaf areas. There were no significant changes in the chloroplast ultrastructure as a result of the increase in nitrogen supply and CO_2 enrichment. Starch grain surface area was seen to decline with an increase in nitrogen under both ambient and elevated CO_2.

Elevated CO_2 and increase in nitrogen supply significantly increased total grain dry weight per plant by 47 and 46% respectively under 6 and 12mM [N]. In contrast, the increase was by about 21, 61 and 67% respectively under 4, 6 and 12mM [N] between the CO_2 regimes.

CHAPTER 1: INTRODUCTION

1.1 Atmospheric CO_2 levels – fact or fiction?

The world is currently awash with reports, symposia, conferences and dialogues on the potentially disastrous climatic effects such as increased atmospheric temperatures caused by rising levels of the atmospheric CO_2. On the other hand many popular press articles appear with the following type of headlines: 'The Silent Warm-up', 'CO_2 Threat',' What if planet earth becomes the Greenhouse?', 'Synthetic fuels - A peril to the atmosphere' and many more of this kind. The most important question to be asked is: Are these type of statements factual or are they just fiction? (Wittwer, 1985).

The increase in the global concentration of CO_2 has been carefully measured and documented from as early as 1957 (Vitousek, 1992). In the pre-industrial era, levels of CO_2 in the atmosphere were approximately 280 ppm (parts per million) with a subsequent increase of about 1.8 μmol mol^{-1} per annum (Watson, 1990; Hendry, 1992) (see Fig.1.1). These changes were caused primarily by fossil fuel combustion and secondarily by changes in land use (Vitousek, 1992). Land use change has resulted in anthropogenic releases of carbon dioxide in the atmosphere through the cement industry (and other industries that emit CO_2 as waste product) and by deforestation. The timing and the magnitude of fossil fuel combustion alone are more than sufficient to account for the global increases of carbon dioxide (Vitousek, 1992). Although terrestrial photosynthesis and oceanic dissolution take up substantial amounts of CO_2, the rate at which the increase is occurring still results in an imbalance (Taylor & Lloyd 1992, Bowes 1993). It is predicted that the

atmospheric carbon dioxide concentration will reach approximately 700 μmol mol^{-1} by the middle of the next century if the burning of fossil fuels is not reduced (see Vu *et al*., 1989; Newton, 1991; Coleman *et al*, 1993; Rogers & Dahlman 1993). The increased concentrations of carbon dioxide are expected to have substantial direct effects on plants, herbivores, and more importantly, whole ecosystems (Mooney *et al*., 1991; Bazaaz, 1990).

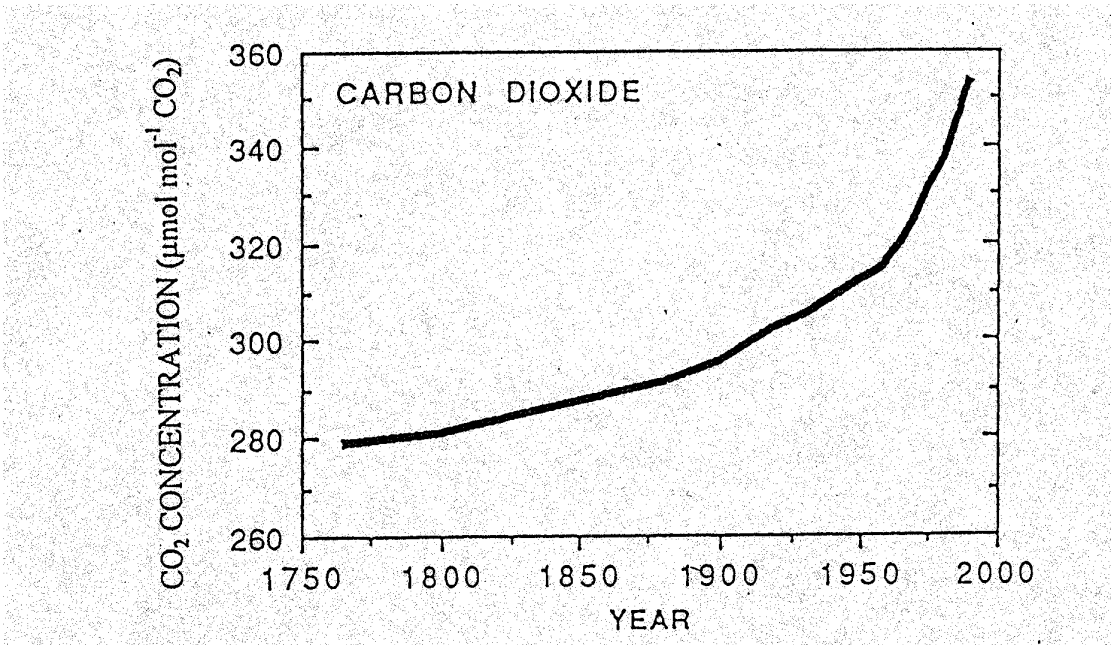


Fig.1.1 Shows global rise of [CO_2] over the last 200 years (Roeckener, 1992).

1.2 CO_2 increase is of great concern to science

The increasing levels of CO_2 in the atmosphere are of great concern to plant scientists. The main reason for this concern is that carbon is the first molecular link between the atmosphere and the biosphere, and secondly, increases in atmospheric carbon dioxide may affect the food chain. This has given rise to studies on both short-term (Bjorkman *et al.,* 1972; Grub & Machler, 1990) and long-term (Sage *et al*., 1989) exposure to elevated carbon dioxide concentration. From studies such as these, it was observed that the increasing carbon dioxide levels make more carbon available at the assimilation sites. This favours the photosynthetic carbon reduction cycle (PCR) over the photorespiratory carbon oxidation (PCO) cycle (Fig.1.2). This will result in greater photosynthetic rates and biomass accumulation in C_3 plants, which, at current ambient CO_2 concentrations, have relatively inefficient photosynthetic capacities (Keys 1986; Lawlor & Keys, 1993) (Fig.1.2).

Kimball (1983) and Cure and Acock (1986) demonstrated an increase in biomass productivity of about 33% on average on C_3 plant species, which were grown in 330 and 660 μmol mol^{-1} [CO_2]. Plants with the C_4 photosynthetic pathway do not respond to elevated CO_2 because they have a biochemical CO_2 concentrating mechanism within the leaf. Consequently, CO_2 fixation rates and growth are unaffected by increases in the atmospheric CO_2 concentration (Conroy, 1992).

Despite the advantages of exposure of plants to elevated CO_2, many experiments show that the potential for increased rates of carbon assimilation may not be realised by all C_3 species (eg. tomato; Besford *et al*., 1990). Idso and Kimball (1991) reported that leaves can respond to elevated CO_2 by reducing the rate of photosynthesis without changing leaf composition or photosynthetic capacity (often referred to as 'down regulation' or 'fine control'), or by decreasing the amounts of photosynthetic components resulting in a loss of photosynthetic capacity (so called 'acclimation' or 'coarse control') (Yelle *et al*.,1989; Sage *et al*., 1989; Stitt, 1991). The latter conditions were observed in cases where carbohydrates accumulate due to inadequate sink demand. As an example, lower sink accumulation in cereals may result from poor development of tillers under conditions of nutrient deficiency or high temperatures.

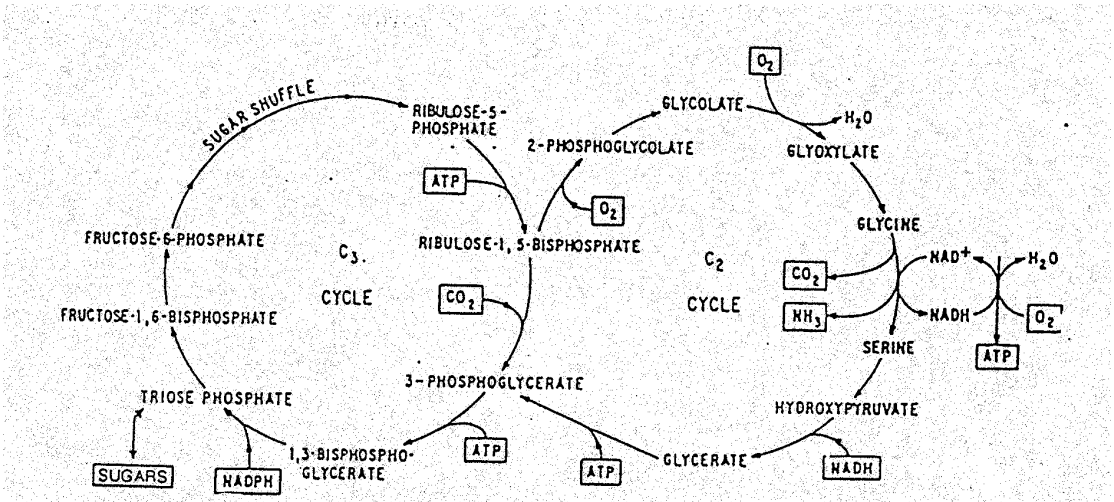


Fig.1.2 Diagram showing the photosynthetic carbon reduction (PCR, sometimes referred to as C_3 cycle) and photorespiratory carbon oxidation (PCO, sometimes referred as C_2 cycle) pathways. Rubisco catalyses both the carboxylation and oxygenation of RuBP, the initial reactions of the pathways (from Morell., 1992).

Although CO_2 enrichment results in higher photosynthetic rates and growth in the C_3 species, this must be coupled with adequate conditions. For example, temperature, the appropriate light intensity and nutrition (Lawlor & Mitchell, 1991; Bazzaz & Miao, 1993; Smolander & Lappi, 1984;Tissue & Ochel, 1987). For instance, low light intensities and higher temperatures affect the activity of rubisco by favouring ribulose-1,5-biphosphate oxygenase (RuBP-oase) over carboxylase (RuBP-case) (Badger ,1992). This could be a result of a change in the kinetic constraints for CO_2 and O_2 favouring reactions with O_2 (Edwards & Walker, 1983). Temperature increase, on the other hand, decreases affinity of CO_2 and O_2 for carboxylation and oxygenation reactions. Therefore, high photosynthetic rates can be maintained at high CO_2 levels within optimal temperatures (Long, 1994).

1.3 Effects of CO_2 enrichment on nutrients in the growth medium

Approximately 20% of the earth's land mass have sufficiently low levels of nutrients to cause some kind of stress to plants (Conroy, 1992 and references cited therein). Increases in atmospheric CO_2 concentration will result in changes in nutrient uptake from the soil, remobilization in the plant, or efficiency of utilisation by plants. This will influence fertiliser management both in agricultural and forestry systems, and nutrient cycling in natural ecosystems (Conroy, 1992). The nutrients most likely to be affected by high CO_2 in the growth medium are nitrogen (N) and phosphorus (P). These two nutrients provide an interesting contrast as they differ in their mobility in the soil, their metabolic function in the plant and their partitioning within the leaf (Conroy, 1992). The effects of insufficient nutrients will be manifested in

reduced growth of plants, since N and P have marked effects on photosynthetic rates, photosynthetic productivity, biomass and yield (Lawlor, Konturri & Young, 1989; Jacob & Lawlor, 1993).

1.4 Plant nutrition, elevated CO_2 and nutrient partitioning

According to Hocking and Meyer, (1991) increases in atmospheric [CO_2] will result in the dilution of the most agriculturally important nutrients, particularly nitrogen (N) and phosphorus (P) in the growth medium. This may alter the key biochemical reactions (PCR and PCO cycles), because phosphorus is central to the regulation of the PCR cycle and considerable amounts of N are tied up in the PCO cycle (Conroy & Hocking, 1993 & references therein). As a result many subsequent metabolic activities will also be affected. Equally important, this will affect the tissue concentrations of elements such as N in most crop plants (Hocking and Meyer ,1991).

As mentioned earlier, plants respond to elevated CO_2 positively, when adequate nutrients are present in the growth medium. A number of studies have been undertaken to elucidate the effects of elevated CO_2 on plant nutrition of both soil and hydroponically grown plants. Such studies have shown that more nutrients were required in the soil medium than in hydroponics, to achieve the same concentration of nutrients in the foliage (Robinson *et al*., 1991).

1.4.1 Hydroponics

Hydroponic growth allows increased nutrients mobility, which results in roots having greater access to nutrients. This increases nutrient concentration at

the surface of the roots (Conroy & Hocking, 1993). Hawkins and Lewis (1993) reported an increase in the growth of wheat grown under hydroponic conditions. They observed that growth was directly proportional to the increase in N concentration in the growth medium. Deignan and Lewis (1988) and Lewis *et al*., (1989) reported the same results when they used nitrate instead of ammonium as their source of N for hydroponically grown wheat. The advantage with hydroponics is the ease with which the underground plant matter is harvested: far less damage occurs than with soil grown plants.

1.4.2 Soil / sand culture

A number of studies have been carried out on a few C_3 crops, mostly cereal grasses cultured in soil (Delgado *et al*., 1994). These have shown that more nutrients were required, since nutrients are less mobile than in hydroponics. An increased supply of certain nutrients, like P, is important because it is virtually immobile, compared to nitrogen, in the soil. An increased addition of P assists in creating a diffusion gradient that will eventually lead to movement towards the roots and subsequent absorption (Conroy & Hocking, 1993). This is very important, especially for nutrients required in large amounts under elevated CO_2 conditions, of which P is an example (Conroy &Hocking, 1993).

1.5 Effects of elevated CO_2 on P-nutrition in plants

Low levels of phosphorus are limiting under elevated CO_2 concentration (Conroy & Hocking, 1993, Hocking & Meyer, 1991). This is because there is not adequate quantities of phosphorus for the synthesis of ATP, which drives the regeneration of RuBP, and this decreases CO_2 assimilation by the leaves (Jacob & Lawlor, 1991). Heldt *et al*., (1970) reported that an inadequate

supply of P_i prevents the efflux of triose–phosphates from chloroplasts and, therefore, exerts an effect on the synthesis of sucrose (Stitt *et al*., 1987). This affects the light saturated photosynthetic rate, A_{max} and the apparent quantum yield (ϕ) for CO_2 assimilation (i.e. a small amount of [P_i] in leaf water). The reduction of A_{max} and apparent quantum yield respectively was 24% and 44% (Jacob & Lawlor 1991). Elevated CO_2 increases the foliar concentration of phosphorus (Conroy, 1992).

1.6 Effects of elevated CO_2 on N - nutrition in plants

Unlike phosphorus, nitrogen appears to affect the growth of some plants differently. For instance, shoot growth of both cotton and wheat has been shown to increase with CO_2 enrichment at all but the lowest soil N concentrations (Rogers *et al*., 1993). These authors observed that the highest N treatment tended to suppress shoot growth. Wong, (1990) and Mjwara *et al*., (1996) reported similar results for cotton and soybean respectively. Conroy *et al*., (1992), reported an increase in the seedling dry weight of *Eucalyptus grandis* in response to high CO_2 at each rate of N and P added to the soil. This resulted in the decrease in foliar concentration of N and P.

Plants with the C_4 photosynthetic pathway do not respond to elevated CO_2 because they have a biochemical CO_2 concentrating mechanism within the leaf (Fig.1.3). Since Rubisco is not the primary acceptor of CO_2 in C_4 plants, these plants are not efficient users of nitrogen compared to the C_3 plants. Several authors have reported that maize grown at three different levels of N

did not respond to elevated CO_2 in a similar manner to C_3 plants (Wong, 1979; Hocking & Meyer, 1991).

1.7 Growth response to elevated CO_2 and nutrient concentration

Earlier studies (Akita & Tanaka, 1973) as well as current studies have shown that C_3 but not C_4 plants had substantial dry matter gains when exposed to elevated CO_2. Most of these studies were only on the effects of elevated CO_2 on plants. Investigators have observed increases in net photosynthesis, dry matter accumulation and yield of several field crop species in response to an increase in atmospheric CO_2 concentration (Imai & Murata, 1976; Gifford, 1977; Neals & Nicholls, 1978; Wong, 1979; Kramer,1981; Sionit *et al*., 1981). The more rapid growth rate and dry matter production of plants in a CO_2 enriched environment presumably result in faster depletion of mineral nutrients in the root media.

Under the conditions of CO_2 enrichment, adequate nutrient supply, appropriate temperature and light intensity most plants have shown increased number of tillers, increased number of leaves and increased grain yield (Acock & Allen, 1985; Newton, 1991; Bosac *et al*., 1995). High CO_2 was shown to increase the yield of 38 agricultural crops by 38% on average (Acock & Allen, 1985). However, contradictory responses have been reported as well. Scheidegger & Nosberger, (1984) found that leaf size was not affected by CO_2 enrichment in white clover. In addition, Garbutt and Bazzaz (1984) indicated that three herbaceous annual species used in their study did not respond to high CO_2 by increased yield, but rather showed decreases in

yield. Increased stem diameters have also been reported under elevated CO_2 conditions (Acock *et al*., 1985). The increase in stem diameters, which serve as large sinks, was reported as a contribution to increased photosynthetic rates in *Pinus radiata* c.v. Don (Conroy *et al*., 1990a).

All the morphological changes observed under the previously mentioned conditions may lead to increased total plant dry weight (Mauney *et al*., 1978; Cure & Acock, 1986; Idso & Idso, 1994; Wheeler *et al*., 1994). In contrast, Manderscheid & Weigel, (1995), reported that the total above ground biomass was not affected by CO_2 enrichment in barley, bean, maize and wheat respectively. Recently, Mjwara *et al*., (1996) reported positive results for non-nodulated bean plants c.v. Contender.

1.8 Effects of elevated CO_2 on stomatal density and conductance

Elevated CO_2 causes stomata to close, reducing the rate of transpiration and increasing the water use efficiency of the plant. This was suggested as a possible reason for the decrease, or loss of stimulation, in photosynthesis after long term exposure (Bowes, 1991; Kimball & Mauney, 1993; Knapp *et al*., 1994; Sage, 1994; Thomas *et al*., 1994; Samarankoon *et al*., 1995). Equally importantly, Woodward (1987) found a 40% reduction in stomatal density during the last 200 years by examining arboreal species stored in herbaria. This clearly indicates the effects of elevated CO_2 on stomatal density over the years and the impacts will surely be manifested through photosynthesis.

1.9 Structural responses to elevated CO_2 and nutrition

A few studies on the effects of elevated CO_2 on the leaf anatomy have been reported in the literature (see Siphugu, 1997 and references cited). When the CO_2 concentration of the atmosphere is increased above ambient levels, leaf morphology may be subjected to change (Thomas & Harvey, 1983). An observation made was the increase in leaf thickness (Thomas & Harvey, 1983; Vu *et al*., 1989), which was thought to be due to an increase in mesophyll thickness. Other observations such as reduced intercellular air space have been reported due to an apparent increase in the number of mesophyll cells (Vu *et al*., 1989). Thomas and Harvey (1983) argued that the effect of CO_2 enrichment on mesophyll thickness was species-dependent. Kutik *et al*., (1995) observed that the effects of elevated CO_2 on chloroplast is species-dependent. Thomas and Harvey (1983) reported an increase in the volume and density of chloroplast but, contrary to that, Robertson and Leech (1995) reported that elevated CO_2 did not affect the number of chloroplasts in relation to mesophyll cell size. Larger starch grains were reported in the elevated CO_2 grown plants (Mjwara, 1996; Siphugu, 1997; Vu *et al*., 1989). This scenario has been reported to decrease the rate of photosynthesis because the starch shields the photosynthetic machinery affecting the light harvesting complexes.

1.10 Why was *Triticum aestivum* L. cv. Gamtoos chosen as the experimental plant, and why controlled environment chambers as the plant culture facility?

Predictions that CO_2 will continue to rise into the next century have prompted a great deal of research into the effects of climate change on future crop production. Accurate predictions of the effects of elevated CO_2 on plants in future are solely dependent on the use of specific growth facilities. These facilities range from controlled environment cabinets to field open top chambers (Rogers & Dahlman, 1993). In this study high CO_2 controlled environment cabinets were used. The advantage of cabinets over open top experiments is that the stresses introduced into the experiments through uncontrolled humidity in open top chambers or greenhouse experiments, make it impossible to control relative humidity (RH) and saturation vapour pressure (SVP). For this reason we decided to utilise the controlled environment chambers in which SVP could be controlled better than half a percent.

In 1985, Strain and Cure proposed that crop plants be given priority as experimental plants, these are of immediate need or consumption by the people of the world. Wheat being the number one crop, was thus an easy choice.

In addition:

a) Wheat provides 60% of the calorific intake and 50% of the protein intake by the people of the world (Witter, 1985). In addition, although more wheat

is produced than any other crop (Briggle & Curtis, 1987), little is known about nutrient and photo-assimilate partitioning (Hocking and Meyer,1991; Cruz-Aguado *et al*.,1999) in this plant under elevated [CO_2]. On the other hand elevated [CO_2] is known to reduce foliar concentration of certain nutrient elements which may affect important compounds in plants ranging from essential amino acids to proteins in the case of wheat. For this reason therefore wheat requires an on going nutritional research.

b) Daignan and Lewis (1988), Cramer and Lewis (1993), Hawkins and Lewis (1993), have published excellent work on wheat (same cultivar). Although their work was done on hydroponics and under ambient [CO_2], they tested response of wheat c.v. Gamtoos to factors ranging from salinity stress to nutrient feeding in the form of either NO_3 or NH_4 or a combination of both as a source of nitrogen. They looked at both gas exchange analysis and growth parameters and wheat responded variably to all the treatments. They further tested the effects of artificial calcium feeding under salinity conditions. This study serves as a follow up to the latter studies in wheat c.v. Gamtoos raised in sand under sustained elevated [CO_2].

c) *T. aestivum* can be grown conveniently under confined conditions of controlled environment facilities such as the growth chambers used in this study. Controlled environment conditions provide a unique property in that the growth period is not seasonally limited, as is the case in nature and

enable multiple cultivation within a short space of time, as well as sufficient replication for both scientific verification and statistical purposes.

1.11 Research Objectives

Previous studies in our laboratory (Logie, 1990; Mjwara *et al*., 1996; Siphugu, 1997) on elevated CO_2 effects support the suggestion that plant species respond differently to elevated CO_2 (Newton,1991; Bowes, 1993; Rogers & Dahlman, 1993) by affecting growth and photosynthetic rates (Lawlor & Mitchell, 1991; Stitt, 1991; Baxter *et al*., 1994). Most of these studies have focused on the short–term effects of whole plant growth and enhanced photosynthesis without any emphasis on plant nutrition (Newton,1991; Lawlor & Mitchell,1991).

An understanding of the mechanisms underlying the accumulation response to elevated CO_2 concentration and nutrient supply to plants is essential in order to predict accurately the impact of the future global rises of atmospheric CO_2.

The control of photosynthesis by the nutrients is undoubtedly important for enhancing the productivity of cereal crops (Makino *et al*., 1984). The supply of N stands out as being particularly effective for the increase in photosynthetic activity and the N content of the leaf as well is correlated with leaf photosynthesis (Makino *et al*., 1984). Although earlier researchers have reported reductions of nitrogen (Wong, 1979; Wong & Osmond, 1991), there

is still a dearth of information on the interaction of nitrogen and elevated [CO_2].

On the other hand, large amounts of phosphorus are tied up in the PCR cycle and this supplies carbon skeletons for growth and to other sinks as well. Similarly, elevated CO_2 was reported to decrease the levels of foliar [P] (Conroy *et al*., 1992). Clearly, this justifies the need to monitor growth response under sustained elevated [CO_2] and changing nutrient regimes through various growth parameters in wheat. Consequently, the primary objectives of this study were:

i) To compare the effects of normal and elevated carbon dioxide concentration on the photosynthetic rates, relative growth rates (RGR), and grain yield of wheat,

ii) To investigate the response of wheat under different [N] whilst keeping P constant in ambient and elevated CO_2 grown plants and,

iii) To examine how anatomy and morphology are affected by a combination of the above factors.

iv) Nitrogen supply is the primary factor that affects photosynthesis adversely. This study provides an opportunity to investigate this hypothesis through a study of the various growth parameters (total, shoot and root dry weight).

CHAPTER 2: Materials and Methods

2.1 Growth medium

Sand was obtained from the river banks of the Palmiet river. It was acid washed in 10% Hydrochloric acid and thereafter rinsed in running tap water and finally with distilled water to get the pH of between 6.2 and 6.5.

2.2 Plant material

Wheat (*Triticum aestivum L.* c.v. Gamtoos) seeds were obtained as a gift from Mr Clive Browne of the East Cape Agricultural Co-operation, Paterson. Seeds were pre-germinated in petri dishes and thereafter sown in acid washed river sand in 5l plastic pots.

2.3 Plant culture

Seeds were germinated in either a Conviron Ef-7H or Conviron S-10H (Controlled Environments Ltd, Winnipeg, Canada) controlled environment cabinets. Following germination, healthy seedlings of the same length and stages of development were thinned from nine to four seedlings per pot. The plants were watered daily with Long-Ashton nutrient solution (Hewitt, 1966) (see Table2.1for formulation). The nitrogen source in the nutrient medium was $Ca(NO_3)$ and KNO_3. The various concentrations of N were prepared by changing amounts and sources of NO_3 as in Hewitt (1966) and as given in Appendix? In this study high nitrogen refers to 12mM, medium to 6mM and 4mM to low N supply. The various nutrient regimes used were; 4:12:2, 6:12:2 and 12:12:2 in mM for N:P:K respectively.

2.4 Growth conditions.

Plants were grown under two different CO_2 concentrations ([CO_2]) in the controlled environment cabinets (360 and 700 μmol.mol^{-1} CO_2) at a temperature regime of 25/20 ^{o}C day/night respectively and the photoperiod was set at 16hrs (Hawkins and Lewis, 1993). The two [CO_2] are referred to as ambient and elevated for 360 and 700 μmol mol^{-1}respectively. The maximum irradiance of photosynthetically active radiation (PAR, 400-700nm) obtainable was approximately 550 μmol. m^{-2} s^{-1} at the top of the plant canopy which was monitored with Li-85A Quantum Sensor (Li-Cor Inc., Nebraska, USA). Light was provided by a combination of fluorescent tubes (F48T 12.cw/ VH01500, Sylvania, USA) and frosted incandescent 40W Osram bulbs (Germany). The day/night relative humidity was set at 65/45% to maintain a constant saturated vapour pressure deficits in the cabinets.

The [CO_2] in both cabinets were monitored using a LCA-2 portable infra red gas analyser (Analytic Development Co. Ltd., Hoddesdon, Herts, UK) Plant growth conditions differed with the amount of CO_2 supplied to plants.

2.5 Leaf gas exchange measurements

Leaf gas exchange measurements were done by using two calibrated ADC 225MK3 CO_2 Infra-red gas analysers (IRGA) (Analytical Development Co. (ADC), Hoddesdon, UK) set up in differential mode and in open circuit (see Fig.2.1 for laboratory set up). Air was supplied via a positive pressure buffer drum, fed from outside the laboratory by a high-pressure pump. The flow rate

of air was kept constant at 350 ml per minute using a rotameter. In order to reduce interplant variability, all gas exchange measurements were performed on recently-expanded leaves of the same morphological age and position on plants from each CO_2 treatment. All measurements were made between 11h00 and 12h00 (i.e. 4-5 hrs into the light period).

2.6 A/Ci experiments

The A/C*i* curves were constructed using bottled CO_2 gas with less than 1% O_2 (Fedgas, Alrode, P.E., S.A.) balanced against nitrogen. Response of A to changes in C*i* was measured as described by Sage *et al*., (1989). Leaves were first exposed to their respective growth CO_2 concentrations (either 360 or 700 μmol $mol^{-1}CO_2$) until steady state conditions were achieved (usually between 10-15 min). An ADC GD-600 as diluter was used to reduce the [CO_2] in the leaf cuvette to approximately 0 μmol $mol^{-1}CO_2$, before the first measurements were taken. Subsequent readings were taken as the [CO_2] in the leaf cuvette was increased in predetermined steps to a maximum of 1000 μmol $mol^{-1}CO_2$.

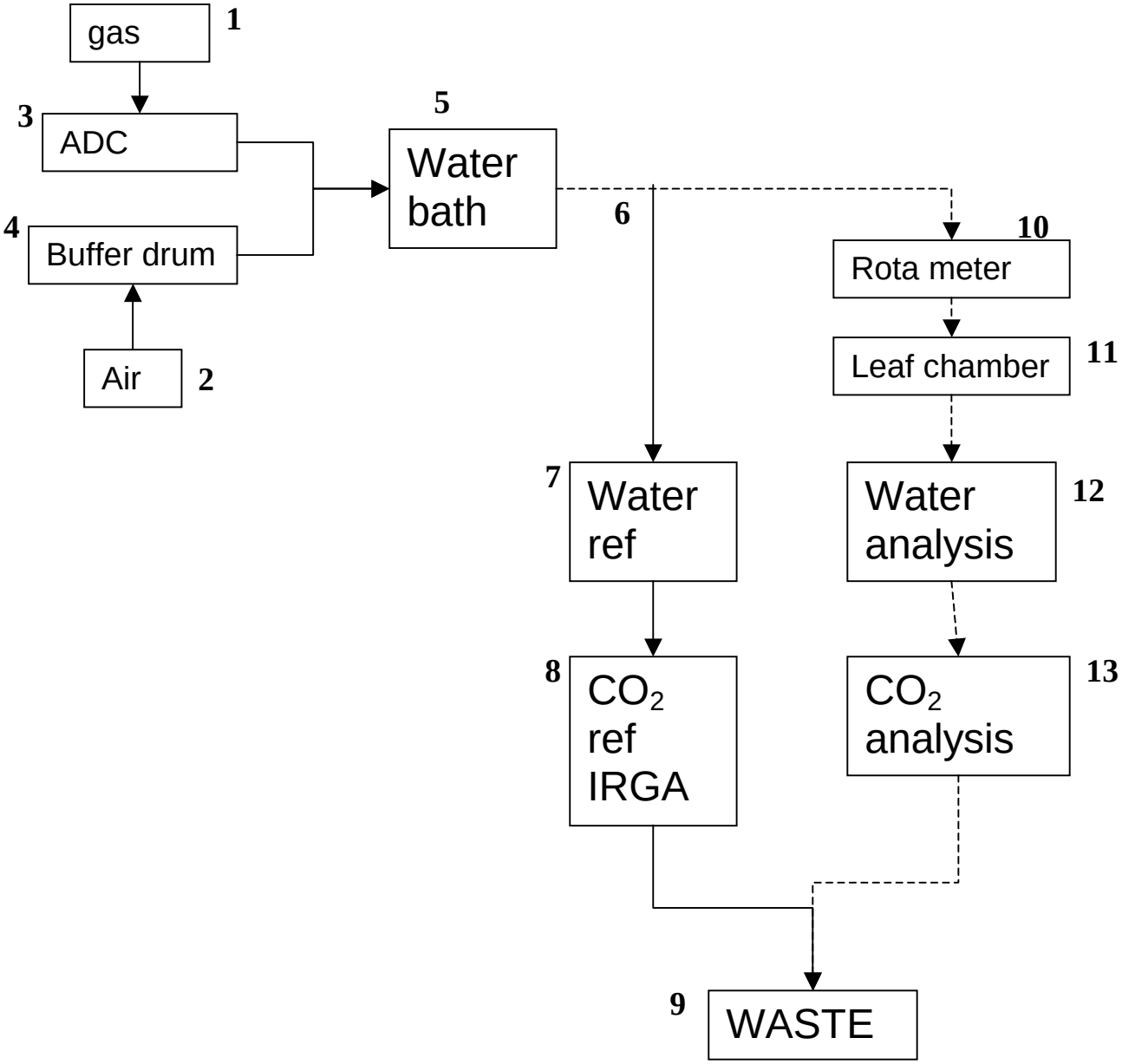


Fig.2.1 Laboratory systems setup. 1. Bottled gas supply; 2. External air supply; 3. ADC gas diluter; 4. Buffer drum; 5. Water bath for dew point temp.; 6. Air is split; 7. Water vapour reference IRGA; 8. CO_2 reference IRGA; 9. Waste; 10. Rotameter (for controlling air flow over the leaf); 11. Water jacketed leaf chamber; 12. Water vapour analysis IRGA; 13.CO_2 analysis IRGA. (See text for explanation).

2.7 Calculation of the boundary layer resistance

The procedure followed here is the one described by Parkinson (1985), whereby a double layer of filter paper of known area, moistened with distilled water and supported within a brass wire frame, was placed inside the cuvette. The filter paper was cut to approximate leaf shape. A K/J type thermocouple was placed inside the two pieces of filter paper and thermocouple voltages measured using a fluke 51 K/J thermometer. Magnesium perchlorate ($Mg(ClO)_4$) dried air was then passed into the cuvette at a flow rate of 360 ± 5 ml min^{-1}. The humidity of the air leaving the leaf cuvette was determined by the water vapour IRGA. All the measurements were taken at 25^{o} C. After the filter paper temperature has stabilised, the temperature was recorded together with the cuvette air temperature and airflow rate. The boundary layer resistance was then calculated using the following equation:

$$r_b = [(x_f - x_o) / (x_o - x_i)] / S/W$$

where, r_b = boundary layer resistance

x_i = humidity of air entering the cuvette

x_o = humidity of air leaving the cuvette

x_f = saturation humidity at the filter paper temperature

S = projected area of filter paper

W = mass flow rate of air

The boundary layer resistance was calculated at 0.573 ± 0.156

2.8 Light source and temperature control

A Phillips (SON-T) 400-watt high-pressure sodium lamp (Phillips, RSA) provided actinic light. This lamp has spectral qualities similar to sunlight. The lamp has maximum light intensity in excess of photosynthetic photon flux density (PPFD) of 2000 μmol m^{-2} s^{-1}. The deleterious effects of infra red radiation produced by the Son-T was reduced by the water-jacket surrounding the glass cuvette. To achieve the required light intensity, fine shade cloth frames were mounted under the light source, above the leaf chamber. The temperature of the leaf chamber was monitored using a Laudar RM-3 multitemp water circulator (Optolabor, Johannesburg, South Africa). The leaf temperature was measured with a K/J type thermocouple attached to the abaxial surface of the leaf inside the cuvette. Preliminary light response measurements indicated that the optimum light intensity for wheat was between 550 and 790 μmol m^{-2} s^{-1} PPFD at a leaf temperature of 25 ^{o}C.

2.9 Humidity Control

The air was humidified by passing it through distilled water at 8.0 ^{o}C, to acquire a humidity of (10.7mbar) this was important for the use of saturated water vapour pressures in the photosynthetic gas exchange calculations.

2.10 Photosynthetic data calculation

Photosynthetic data calculations were carried out using “Photocal” software (Copyright, Botha CEJ, 1990, Rhodes University, Grahamstown, S.A.) This software comprised of the equations of photosynthetic calculations described by von Caemmerer & Farquhar (1981) (see Appendix 2 for equations). The following parameters were calculated Net assimilation rate, stomatal conductance, transpiration rate, water use efficiency, apparent quantum efficiency and intercellular [CO_2].

2.11 Plant harvest and measurements

Plants were harvested 7 days after germination (DAG) and thereafter weekly for dry weight (DW) determination. The roots were thoroughly washed free of soil with deionised water and subsequently given a final rinse in distilled water. Single plants were then divided into root and shoot components and the fresh and dry weight of each measured. Dry weight (DW) was measured after the plants have been oven dried for 48hrs at 80^{o} C. Shoot: root ratios were expressed on a dry weight basis.

Various growth parameters such as leaf area (LA), dry weight (DW), and total plant height (TPH) were measured from 7 days post-germination then following the emergence of the first trifoliate leaf and subsequently every fifth day thereafter till flowering.

2.12 Tissue preparation and extraction for Nitrogen

Foliar total nitrogen was analysed using the Kjehldahl method (e.g. using methodology described by Hasses, 1971; Allen 1974). Sub-samples (about

0.100g) of dry leaf material were digested using a KB 12S Micro-Kjeldatherm, Kjeldahl digestion system (G. Gehardt & Co., KG Fabrik Fu Larburgerate, Federal Republic of Germany). About 1.5 g of Kjeldahl-Pak Se catalyst was added in each tube along with the sample and finally 2.5ml of conc H_2SO_4 was added in each tube. The samples were digested for approximately 35 min with the addition of H_2O_2 at 10-min intervals to flush down the system. The mixture was allowed to boil for further 20 min to boil away the remaining H_2O_2. The total digestion did not last for more than one hour to prevent possible loss of N due to the decomposition of the ammonium sulphate formed in the reaction (Allen et al., 1974). After completion of digestion, the mixture was quantitatively transferred to a steam-distillation apparatus after addition of 10ml H_2O distilled and 10ml of caustic/ hypo solution (prepared from 25 g sodium thiosulphate solution dissolved in 200 ml deionised H_2O and 400 g NaOH, made up to volume in a 1 litre volumetric flask).

2.13 Nitrogen determination

The titration of the distillate was carried out with standard 0.015 *M* HCl solution (12.5 ml conc. HCl to 10 litres of deionised H2O, standardised with 10 ml aliquots of 0.01 *M* Borax solution and two drops of screened indicator) to the grey end-point or the first tinge of pink in 250 Erlenmeyer flasks containing 1% boric acid indicator (0.2% methyl red solution and 0.2% methylene blue solution in 95% ethanol which was mixed in 2:1 ratio stored in an amber glass container). Blank determinations were performed on collected distillates in which the above procedure was followed without any leaf

material. Volumes of standard HCl solution were recorded, and after subtraction of the mean blank readings, foliar N content was calculated.

2.14 Determination of nitrogen use efficiency

Nitrogen use efficiency (NUE) was determined according to the method of Chapin and Van Cleve (1989). It is commonly defined as the amount of biomass produced per unit of nutrient. The most appropriate measure of NUE depends upon the question posed. NUE of the mature leaves may be the best indicator of the plant physiological responses to nutrient stress (Chapin and Van Cleve, 1989). Because only the analysis of foliar [N] was carried out in this study, NUE was calculated by dividing the shoot DW by foliar [N]. However the are other ways of determining NUE as suggested by Chapin and Van Cleve, (1989).

2.15 Chlorophyll and carotenoid extraction

Immediately after completion of gas exchange measurements, the same leaf tissue was used for pigment extraction. The leaves were quickly frozen in liquid N_2 thereafter homogenised to a pulp using a cold mortar and pestle containing ice cold 10ml of 80% acetone. The homogenates were then filtered through Whatman No.1 filter paper in a Buchner funnel, under vacuum and the residue was extracted with a further 5ml of 80% acetone until the tissue became white in colour. The extraction was performed in dim light to avoid photo-bleaching of chlorophylls and carotenoids (Lichtenthaler, 1987). The concentrations of chlorophylls and carotenoids were determined

spectrophotometrically against 80% acetone at 663, 646 and 470nm. The concentrations of chlorophylls and pigments were determined according to Lichtenthaler (1987) as follows:

$C_a = 12.21.A_{663} - 2.81.A_{646}$

$C_b = 20.13.A_{646} - 5.03.A_{663}$

$C_{a+b} = C_a + C_b$

$C_{x+c} = 1000.A_{470} - 3.27.C_a - 104.C_b / 229$

2.16 Microscopy

Leaf material from ambient and elevated CO_2 grown plants was examined at the light and electron microscopy level.

2.16.1 Electron Microscopy

2.16.1.1 Transmission Electron Microscopy (TEM)

The first fully mature leaves were harvested and dissected into small pieces of approximately 3x5mm. These tissues were immediately fixed in 2.5% glutaraldehyde in 0.2M cacodylate buffer (pH 7.2) at 4 ^{o} C for 12hrs. The material then was processed through an ethanol dehydration series followed by two changes in propylene oxide (1hr total). Leaf tissues were then embedded in Araldite Taab 812 resin. Ultrathin sections were cut using a diamond knife mounted on the RMC MT-7 (Research & Manufacturing Co. Inc., Tucson, Arizona) ultramicrotome. The sections were collected on 200 mesh copper grids and double-stained in uranyl acetate and lead citrate in

that order. Sections were viewed and photographed using a Joel 100-CX-2 (Tokyo, Japan) transmission electron microscope at an accelerated voltage of 80kV.

2.16.1.2 Scanning electron microscopy (SEM)

Leaf tissues were dissected and fixed in 2.5% glutaraldehyde as for TEM preparation. After the final change of the alcohol dehydration series, leaf sections were placed in an amylacetate series allowing amylacetate to infiltrate for 20min in each change. Subsequent to this specimens were then critical point dried for 1-2 hrs. Thereafter plant material was mounted on metal stubb and sputter coated with gold and viewed using a JEOL 840SEM.

2.17 Light microscopy

Leaf segments aged 25 DAG were fixed in FAA for 24 h. The leaf segments were then gently cut into smaller, more manageable pieces and dehydrated through an alcohol and tertiary butyl alcohol series. The material was then infiltrated with a number of changes of paraplast wax over three days, in an embedding oven at 60^{o}C. Blocks were mounted and trimmed and serial sections were cut at 15μm using a Leitz Wetzlar rotary microtome and stained safranin and Fast green. The sections were then mounted onto slides using Canada Balsam and oven dried at 37^{o}C for three weeks. The leaf sections were examined using a Zeiss universal microscope (Carl Zeiss (Pty) Ltd., Oberkochen, Germany) and photographed using an integral Zeiss MC-63 35 mm camera system mounted on the microscope.

2.18 Stomatal density determination

Scanning electron microscope images of leaf surfaces were used for stomatal density determination. Images were then recalled from digital storage and the total number of stomata were counted within the 0.32 mm^{-2} area. Stomata were counted for images of the abaxial and adaxial surfaces. Leaf surface area was determined using the image measurement software package, SigmaScan/Image. Version 1993 (Jandel Scientific, CA, USA). Because both the abaxial and the adaxial surfaces were involved in gas exchange measurements, weighted stomatal density had to be calculated using equation 2.1 below as described by El-Sharkawy et al (1985):

$$WSD = ADSD^2 + ABSD^2 / ADSD + ABSD \qquad \text{Equation 2.1}$$

Where, WSD is weighted stomatal density

ADSD is adaxial stomatal density, and

ABSD is abaxial stomatal density

2.19 Statistical analysis

All were applicable and unless otherwise stated. The results were subjected to one way analysis of variance (ANOVA) and Scheffe's multiple range test (95% confidence limits) using a computer software called Statgraphics (Manugistics, Inc. & Statistical Graphics Corporation, USA, 1993). Two way ANOVA analyses were performed to test for interactions where possible.

CHAPTER 3:

Gas exchange characteristics, photosynthetic pigments and foliar nitrogen concentration under ambient and elevated CO_2.

3.1 Introduction

As mentioned in Chapter 1, plants respond to elevated CO_2 concentration positively in the presence of adequate nutrients in the growth medium. This situation is likely to influence the carbon and nutrient biochemistry in the leaf, thereby changing nutrient concentrations (Conroy & Hocking, 1993). One commonly documented effect of elevated CO_2 concentration is a reduction in the nitrogen concentration of the plant tissues especially in the leaf (Coleman *et al*., 1993 and references therein). The relationship between photosynthesis and nitrogen nutrition has frequently been studied because of the high importance of N as a limiting factor of plant productivity (Wojcieska, 1994 and references therein). This is because the effects of reduced foliar [N] are manifested in photosynthesis. However, the physiological and biochemical mechanisms that suppress photosynthesis in plants growing under elevated [CO_2] have not been elucidated (Makino *et al*., 1997). The suppression of photosynthesis by CO_2 enrichment is according to the literature always

associated with a decrease in total leaf N content (Makino *et al*., 1997; Wojcieska, 1994 a, b; Besford *et al*., 1990). On the other hand, reduction of NAR under elevated [CO_2] has been attributed to high C/N ratios (particularly of starch) by Ehret and Jolliffe (1985b) and Delgado *et al*., (1994). Other workers have suggested that factors such as Rubisco content, its activity and / or regeneration capacity are responsible for acclimation (Rowland-Bamford *et al*., 1991; Bowes, 1993; Sage, 1994).

Rubisco availability and specific activity determines the rate at which CO_2 assimilation occurs. This is affected by foliar N concentration as well (Bowes, 1991). Mjwara *et al*., (1996) reported a decline in both foliar [N] and Rubisco. This is manifested in retarded plant growth and poor yield in terms of grain.

It has been demonstrated previously that elevated [CO_2] decreases transpiration rate and increases water use efficiency (Radoglou *et al*., 1992; and references therein). The magnitude of the response appears to depend on the species and on the environmental conditions. Elevated [CO_2] appears to influence stomatal opening and this affects stomatal conductance and water vapour exchange (Prior *et al*., 1991). Some workers have attributed decreased stomatal conductance to a decrease in stomatal density (Woodward & Bazzaz, 1988; Ceulemans & Mosseau, 1994; and references cited therein). Elsewhere, elevated CO_2 has been reported to have no significant effect on stomatal density by other workers (O'Leary & Knecht, 1981; Drake, 1992; Berryman, *et al*., 1994).

Sage *et al*., (1987) suggested a positive correlation between the chlorophyll content and foliar N content. Since N is the most important component of most proteins and enzymes and its decline may affect the synthesis of the photosynthetic pigments. The change in chlorophyll content affects CO_2 assimilation which later translates into less gain in biomass content or poor grain yield.

This chapter reports the investigations of the change in N concentration on gas exchange characteristics of elevated CO_2 grown wheat. The change in both foliar [N] and chlorophyll content of the plant is reported as well.

3.2 Results

3.2.1 Net assimilation rate

Measured net assimilation rate (NAR), increased continually from the beginning of the experimental period, reaching its maximum at 14 days after germination (DAG), thereafter decreased until 35 DAG where an increase in NAR was observed under elevated CO_2 treatments (Fig 3.1). This trend was observed under all the nitrogen regimes viz: 4,6 & 12 mM and elevated CO_2 grown plants (Fig. 3.1). Similar results have been reported by other researchers for barley, beans, wheat and rice (Siphugu, 1997; Mjwara *et al*., 1996; Delgado *et al*., 1994 and Conroy *et al*., 1994) respectively.

Under ambient [CO_2] measured NAR declined continuously from the beginning of the experimental period, reaching its maximum at 21 DAG and

later followed by a noticeable increase from 28 DAG onwards (Fig.3.1). At 21 DAG (4mM [N]) a net difference of 4.2 μmol m^{-2} s^{-1} (16.5 and 12.5) was recorded, resulting in the only difference between the two CO_2 regimes ($p<0.01$). Under 6 and 12mM [N] and ambient [CO_2], NAR increased continuously during the first two weeks (7-14DAG) reaching its maximum at 14 DAG and this was later followed by a decline at 21 DAG which was extended to 28 DAG under the 6mM nitrogen regime (Fig.3.1). Under 6mM N regime a net difference of 3.6 and 4.6 μmol m^{-2} s^{-1} was recorded respectively, resulting in a significant difference between the two CO_2 regimes.

High [CO_2] and increase in nitrogen supply from 4 to 6mM [N] resulted in an increase in NAR by about 26% on average during the first two weeks (7-14 DAG) (Fig 3.1). This was later followed by a noticeable decline of NAR by about 22% on average during the last four weeks (21-42 DAG) of the experimental period. The change in N supply from 4 to 6mM N regime resulted in significant differences in NAR between the two N regimes (see Table 3.1). Siphugu (1997) reported an increase by 28% and a decline by 40% at the same ages for barley using a commercial fertiliser. A further increase in N supply from 6 to 12 mM [N] under elevated [CO_2] increased NAR slightly by about 10% on average during the first two weeks (7-14 DAG) and by about 20% over the last four weeks of the experimental period (Fig 3.1). The change in NAR between the two nitrogen regimes was significant as a result of change in nitrogen supply (see Table 3.1).

Under ambient CO_2 NAR increased by about 15% when the N supply was increased from 4 to 6mM during the first two weeks (7-14DAG) of the experimental period. Measured NAR reduced by about 40% on average over the last two weeks (35-42DAG) of the experimental period, resulting in the only significant change in the last two weeks ($p<0.001$) (Fig 3.1). Increasing N supply from 6 to 12 mM [N] resulted in a noticeable reduction in NAR by about 0.8% on average over the first two weeks (7-14DAG). This was followed by a subsequent significant increase of about 63% on average over the last three weeks (28-42DAG) of the experimental period. The change in NAR as a result of high nitrogen was significant over the last four weeks (21-28; $p<0.001$ and 35-42DAG; $p<0.01$) (Table 3.1).

3.2.2 A / C_i responses

The response of the plot of assimilation as a function of intercellular [CO_2] (C_i) with respect to change in N supply are presented in Fig 3.2 to 3.4 at the ages of 14, 28 & 35 DAG. The general response pattern under all treatments was that assimilation was highest at 14 DAG, followed by a noticeable decline at 28 DAG and finally increasing at 35 DAG (Fig 3.2 to 3.4). Similar results were reported by Mjwara (1996) for soybean. An interesting response at 28 DAG was that assimilation of high CO_2 plants was lower than that of ambient CO_2 grown plants (Fig 3.2 to 3.4). At all growth stages under different N regimes wheat responded to increase in C_i by a rapid initial increase in NAR. Photosynthetic saturation was approximately 550 μmol mol^{-1} CO_2. The CO_2 saturated photosyntheic rates were significantly higher ($p<0.05$) under CO_2 enrichment at all ages but 28 DAG under all N treatments (Fig. 3.2; 3.3 &

3.4). A significant difference was also apparent over age under all nutrient treatments ($p<0.05$) (see Tables 3.2; 3.3 an 3.4). Under ambient CO_2 and with a 4mM N regime, assimilation increased on average by 3% from 14 to 28 DAG but was later followed by a decline of about 19% on average at 35 DAG (Fig.3.2). In contrast at the same age the 6mM N regime resulted in an initial decline of about 14% on average and later followed by an increase of about 11% on average at 35 DAG. The 12mM N regime resulted in a decrease in assimilation of about 18 and 27% for 28 and 35 DAG respectively.

On the other hand high CO_2 and 6mM N regime resulted in a decrease in assimilation by about 60, 0.11 and 25% on averages for 14, 28 and 35 DAG respectively. In contrast an increase in assimilation was apparent under the 12mM N regime. The increase was 6 and 29% on average for 14 and 35 DAG respectively. The decline was exacerbated under the 6mM N regime at 28 DAG. In contrast ambient CO_2 grown plants exhibited a decline of about 18, 16 and 32% on average for all ages under the 12mM N regime. However, age did not seem to have any effect on assimilation for the 6mM N regime plants resulting in increase of about 7, 42 & 23% on average for 14, 28 & 35 DAG respectively.

3.2.3 Stomatal conductance (g_s)

Stomatal conductance (g_s) responded differently under all treatments (Fig.3.6). Under 4mM [N] there was a noticeable increase in gs during the first two weeks under ambient and elevated [CO_2] (Fig.3.6). This was later followed by a decline in g_s under ambient CO_2 at 21 DAG, thereafter a steep

increase was observed from 28 DAG onwards under both CO_2 regimes. A net difference of 53 and 72 mol m^{-2} s^{-1} was recorded at 21 and 28 DAG resulting in the only significant difference between the two CO_2 regimes.

A continuous decline in g_s was observed under elevated CO_2 and 6mM [N] from the beginning of the experimental period to 21 DAG (Fig.3.6). This was followed by a slight increase at 28 DAG and finally a decline over the last two weeks. In contrast, under ambient [CO_2] g_s was observed to increase during the first two weeks (7-14 DAG) and later followed by a sharp decline until 28 DAG (Fig.3.6). This was followed by a slight increased at 35 DAG and eventually a decline at 42 DAG. A net a difference of 53 mol m^{-2} s^{-1} resulted in the only significant difference in gs between the CO_2 regimes over the experimental period (Fig.3.6).

Under the 12mM [N] and both CO_2 regimes there was an initial increase in g_s over the first two weeks (7-14 DAG) which was later followed by a decline from 21-35 DAG (Fig.3.6). An increase in g_s was observed at 42 DAG under both CO_2 regimes. The only significant difference recorded between the two CO_2 regimes was at 21 and 28 DAG with a net difference of 80 and 32 mol m^{-2} s^{-1} respectively.

At 14 DAG and under ambient [CO_2], average g_s was 190; 366 and 390 mol m^{-2} s^{-1} and under elevated [CO_2] 180; 215 and 322 mol m^{-2} s^{-1} were recorded for 4 ; 6 and 12 mM [N] respectively (Fig.3.6). The greatest significant differences between the nitrogen regimes were recorded at 7-14

and 35-42 DAG under ambient and elevated [CO_2](see Table 3.5). As a result under elevated CO_2 and 6mM [N] the % increase in gs was about 35% on average over the latter stages of the growth period. Under 12mM [N] and elevated CO_2 g_s was increased slightly by about 7.9% on average over the latter two weeks of the growth period.

Under ambient CO_2 and 6mM [N] g_s was increased by about 84% on average during the first two weeks, followed by a decrease of about 68% on average over the last two weeks (35-42DAG). A further increase in nitrogen supply to 12 mM reduced g_s by about 44% over the first three weeks compared to 6mM at the same age.

Responses of stomatal density to elevated CO_2 and N supply at 25 DAG are presented in Table 3.6. Stomatal density varied with the change in N supply and [CO_2] on either side of the leaves. The p values as a result of a change in N supply under ambient and elevateds [CO2] are depicted in Table 3.7. The general trend observed was that abaxial, adaxial stomata and WSD increased with the increase in N to 6mM N and this was followed by a noticeable decline at the highest N level. The trend was irrespective of the level of CO_2 supply. However, elevated CO_2 increased the number of stomata on either side per mm^{-2}. Ambient CO_2 and 6mM N level increased weighted stomatal density (WSD) by 9% and later followed by a decline of about 34% in WSD when N supply was increased to 12mM. Similarly high CO_2 and increase in N to 6mM N level increased WSD by over 100%. However a further increase in N (12mM) and high CO_2 decreased WSD by about 24%.

The increase in adaxial stomatal density in this study contrasts the results reported by O'Leary and Knecht (1981) for beans and Xu *et al*., (1994b) for soybean. Mjwara *et al*., (1996) also reported no significant differences in stomatal density for soybean between elevated and ambient CO_2 grown bean plants.

3.2.4 Transpiration (E)

Generally, transpiration rates exhibited a trend similar to that observed in g_s over time under all treatments indicating a close relationship between these two parameters (Fig.3.5). Under 4mM [N], there was a significant difference in E at 21 and 28 DAG between the two CO_2 regimes. As a result a net difference of 0.7 and 0.4 mmol m^{-2} s^{-1} respectively was measured at 21 and 28 DAG, resulting in the only significant difference in between the two CO_2 regimes.

Under ambient CO_2 an increase in nitrogen supply from 4 to 6mM [N] resulted in an increase in E of about 41% from 7 to 14 DAG and this was later followed by a decline of about 47% on average over the latter part of the experimental period (Fig. 3.5). Consequently, there was a significant change ($p<0.001$) in E between the two N regimes at 7 to 14 DAG and 35 to 42 DAG (see Table 3.5). A similar effect was observed under elevated [CO_2] between the two nitrogen regimes ($p<0.001$) (see Table 3.8). At 42 DAG under 6mM [N] there was a significant difference in E between the two CO_2 regimes as a result of a net difference of 1.08 mol m^{-2} s^{-1} (Fig.3.5). Mjwara (1996) and Siphugu (1997) reported the same results.

A further increase in nitrogen supply from 6 to 12mM [N] under ambient [CO_2] resulted in the only significant change in E at 35 to 42 DAG between the nitrogen regimes ($p<0.001$) (see Table 3.8). The change in nitrogen supply under elevated [CO_2] did not result in any significant difference in E over the growth period (see Table 3.8). Under 12 mM [N] no significant differences were reported between the two CO_2 regimes (Fig 3.5).

3.2.5 Water Use Efficiency (WUE)

Water use efficiency of elevated [CO_2] grown plants was higher than that of ambient [CO_2] grown plants for most of the growth period under all treatments (Fig.3.7). Under 4 and 6mM [N] and both CO_2 regimes an increase in WUE was observed over the first three weeks (7-21 DAG) followed by a sharp decline at 28 DAG. A steep increase was recorded from 35 DAG onwards under the 6mM [N]. In contrast to 6mM [N], there was an increase in WUE at 28 DAG under elevated [CO_2] and 4 mM [N], followed by a sharp decline at 35 DAG onwards. Under 4mM [N] a net difference of 0.0003 and 0.002 mmol mol^{-1} was recorded at 14 and 28 DAG respectively resulting in the only significant difference in WUE between the two CO_2 regimes (Fig. 3.7).

Under ambient [CO_2] an increase in nitrogen supply from 4 to 6mM [N] resulted in a significant change in WUE at 7 to 14 DAG and 35 to 42 DAG (see Table 3.9). In contrast, under elevated [CO_2] the only significant change in WUE recorded was at 35 to 42 DAG (Table 3.9). The 6mM [N] resulted in a

significant difference ($p<0.01$) in WUE at 28 and 42 DAG as a result of a net difference of 0.001 and 0.02 respectively (Fig.3.7).

Under 12mM [N] WUE exhibited an initial decline over the first two weeks (7-14 DAG), followed by a continuous increase, reaching its maximum at 35 DAG and eventually declining at 35 DAG (Fig.3.7). The only significant difference in WUE between the two CO_2 regimes was at 14 and 42 DAG with a net difference of 0.001 at both growth stages (Fig. 3.7). The only significant change recorded as result of increase in nitrogen supply from 6 to 12mM [N] was at 35 to 42 DAG under ambient [CO_2].

3.2.6 Photosynthetic pigments

The photosynthetic pigment {chlorophyll a (chl_a); chlorophyll b (chl_b); total chlorophyll content (chl_{a+b}) and carotenoids (C_{x+c})} levels were observed to follow the same trend as NAR over time under all treatments (see Fig.3.1 and Fig. 3.8; 3.9; 3.10). In addition, chl_a was always higher than chl_b under all treatments (Fig.3.8 and 3.9), confirming the results obtained by Mjwara (1996) and Siphugu (1997).

Under 4mM [N] no significant differences in chl_a levels were reported between the two CO_2 regimes over the growth period (Fig 3.8). However, an increase in nitrogen supply from 4 to 6mM [N] under ambient and elevated [CO_2] resulted in a significant increase in chl_a levels over the growth period (see Table 3.10). This increase between the two N regimes was by over 100% under ambient and elevated [CO_2]. Under the 6mM [N], a net difference of 2.2

and 1.9 μg per plant extract^{-1}at 14 and 35 DAG respectively, resulted in the only significant difference in chl_a levels between the CO_2 regimes (Fig. 3.8).

A further increase in N supply from 6 to 12 mM[N] resulted in a significant change in chl_a at 21 to 28 and 35 to 42 DAG under both CO_2 regimes (Table 3.10). There were no significant differences observed between the [CO_2] under the 12mM [N].

The change in chl_b levels between the nitrogen regimes were significant ($p<0.05$; $p<0.01$ and $p<0.001$) under ambient and elevated [CO_2]. (see Table 3.11). Under 4mM [N] significant difference in chl_b were recorded at 21 DAG between the CO_2 environments ($p<0.05$). In contrast, under 6mM [N] a significant difference was recorded at 35 DAG ($p<0.05$) (Fig.3.9). There were no significant changes in chl_b between the CO_2 environments as a result of 12mM [N].

Similarly, total chlorophyll content (chl_{a+b}) changed significantly by over 100% with a change in nitrogen supply (under ambient and elevated CO_2) resulting in the only significance between the nitrogen regimes (see Table 3.12). There were no significant differences in chl_{a+b} between the tow CO_2 regimes under 4 and 12mM [N] (Fig.3.10). However, under 6mM [N] a significant difference was recorded at 35 DAG due to a net difference of 2.3 μg per plant extract^{-1}.

Carotenoid levels (C_{x+c}) changed significantly with a change in nitrogen supply (Table 3.13). However, at 7 to 14; 21 to 28 and 35 to 42 DAG there

was no significant change as a result of 12mM [N] under elevated [CO_2]. Under 4mM a net difference of 0.11μg ml per plant extract^{-1} at 35 DAG resulted in the only significant difference between the two CO_2 regimes (Fig.3.11). Under 6mM [N] a net difference of 0.64 and 0.36 μg ml per plant extract-1at 25 and 35 DAG resulted in the only significant change in C_{x+c} between the two CO_2 regimes (Fig.3.11). There was no significant difference between the two CO_2 regimes under a 12mM [N].

3.2.7 Foliar nitrogen concentration

Under 4mM [N] foliar [N] (at ambient and elevated [CO_2]) was observed to increase from 7 to 14 DAG, reaching its maximum at 14 DAG subsequently followed by a sharp decline from 21 DAG onwards (Fig.3.12). This was followed by a slight increase in foliar [N] at 35 DAG and eventually by a decline at 42 DAG (Fig.3.12). Significant differences in foliar [N] between the CO_2 regimes were recorded at 7; 14; 21 and 28 DAG with net differences of 2.5; 0.6; 2.1 and 4.6 %mg g^{-1} DW of N.

There was a significant change in foliar [N] under ambient and elevated [CO_2] as a result of a change in nitrogen supplies from 4 to 6mM [N] (see Table 3.14). Under 6mM [N] an initial increase in foliar [N] during the first two weeks (7 to 14 DAG) was followed by a decline under elevated [CO_2] to levels far below those of ambient [CO_2]. As a result of this, large significant difference in foliar [N] were recorded at 7; 21; 28; 35 and 42 DAG ($p<0.001$) (Fig.3.12). This significant change was as a result of net differences of 1.79; 8.51; 13.29; 13.78 and 10.68 % mg g^{-1} DW of N respectively.

Similarly, under ambient and elevated [CO_2], increase in nitrogen supply from 6 to 12mM [N] resulted in a significant change in foliar [N] between the two nitrogen regimes (see Table 3.14), except during the first two weeks. As in the other two nitrogen regimes, there was a steep increase in foliar [N] during the first two weeks (7-14 DAG) followed by a decline under elevated [CO_2] from 21 DAG onwards, to levels below those of ambient [CO_2] (Fig.3.12). As a result of this, a net difference of 2.2; 1.6; and 1.4 % mg g^{-1} DW of nitrogen respectively was recorded at 14; 21 and 42 DAG resulting in the only significant differences in foliar [N] between the two CO_2regimes (Fig.3.12). Mjwara *et al*., (1996) reported an increase in foliar [N] during the first two weeks and thereafter a continuous decline until the end of the experimental period for soybeans. However, an interesting feature in this study is that under elevated [CO_2] at all nitrogen regimes foliar [N] was observed to increase and decrease concomitantly with transpiration (see Fig. 3.5 and 3.12).

3.3 Discussion

The results presented in this chapter suggest that the supply of nitrogen directly affects photosynthesis and related growth parameters, under the experimental conditions. Earlier studies suggested a number of factors that may lead to down-regulation or acclimation of photosynthesis (Sage, 1994). This response was mainly associated with the imbalance of source (leaves) and sink (storage organs). In this study, above ground plant matter (leaves, total plant height, and number of tillers) showed a continuous increase in

growth (sink) despite the decrease in NAR. This is consistent with the results reported by Mjwara *et al*., (1996). Definitely this rules out the imbalance between source and sink as the result of acclimation in this study. Although this is the case, in this study flowering started just 28 DAG for all treatments with elevated CO_2 grown plants flowering first. Coincidentally NAR was observed to increase at the same age and there was remobilization of photosynthetic pigments as well. This suggests that an increase in sink strength associated with the flower heads may have corrected the imbalance between the source and sink. The data thus suggest that NAR appears to may have been affected by source and sink relationship to a certain degree, supporting the earlier suggestion by Delgado *et al*., (1994) and Azcon-Bieto, (1983) for wheat.

Substantial amounts of N are invested in the most important but sluggish enzyme called Rubisco (Bowes 1991). This enzyme is an integral protein that functions in binding CO_2 and it is responsible for about 60% of the total protein in mature leaves (Besford *et al*., 1990; Wocjieska, 1994). Several studies were able to demonstrate that a decrease in foliar [N] decreased the amount and acivity of Rubisco (Besford *et al*., 1990; Wocjieska, 1994 and references cited therein). Sage *et al*., (1987) confirmed these results by showing a significant positive correlation between foliar [N] and NAR. As a result this decreased photosynthesis and translated into reduced growth. The results in this study show that foliar [N] decreased concomitantly with NAR for elevated CO_2 plants for all nutrient treatments. This had an adverse effect on NAR especially under the 6mM N regime (Fig 3.1). This clearly shows a very

delicate system where a slight decline in foliar [N] is manifested through NAR over time course in this study. This contention is strengthened by a significant positive correlation observed between foliar [N] and NAR under the 6mM N regime (Fig 3.13). The lowest and the highest N supplies resulted in a poor positive correlation as a result of N allocation to other limiting process (Fig 3.13). This is concordant with the results reported by Mjwara *et al*., (1996); Hocking & Meyer, (1991); Conroy *et al*., (1992) for plants grown under elevated CO_2. Wocjieska (1994) demonstrated the same relationship between NAR and foliar [N] in wheat and beans. The relatively low photosynthetic rates under the 12mM N regime may have been exacerbated by shading due to larger leaves as a result decreasing Rubisco's activity (Wojcieska, 1994). When light intensity is reduced, especially when plants are at the seedling stage, RuBPCase breakdown is facilitated resulting in acclimation. This was demonstrated by Thimann (1980) using eleven day old wheat plants resulting in a reduction in NAR due to a reduction in Rubisco.

In general the results for the A/C_i response are similar to those obtained by Mjwara (1996) for soybean grown under constant supply of N in the same laboratory. The A/C_i response describes a biphasic curvilinear progression consisting of the initial slope of which is indicative of the plant's photosynthetic capacity and closely which is correlated to Rubisco acitivity, and the second the slower rising phase which indicates limitations imposed by the rate of RuBP and Pi regeneration (von Caemmerer & Farquhar, 1981; Sharkey, 1985; Long *et al*., 1993 and Sage, 1994). The results in this study clearly indicate that at 14 DAG high CO_2 had little effect on Rubisco activity

and high assimilation rate at high Ci appears to be as a result of an increase in P_i regeneration capacity. However during the latter stages of growth, high CO_2 is showed to have effected decreasing RuBisco activity. Since the supply of P was high in this study, RuBP and P_i regeneration capacity may have not been suppressed, but rather impeded by a feedback mechanism, due to lowering of the sink strength. This contention is supported by a slight increase in assimilation rates at 35 DAG, which was concomitant with an increase in the sink strength at the flower heads mentioned previously. The responses reported here are very common for a wide range of C_3 species (Sage, 1994 and references cited therein).

It is widely reported that elevated [CO_2] induces reduced stomatal conductance in elevated CO_2 grown plants, due to partial closure of stomata induced by high CO_2 (Grodzinski, 1992; Lawlor; 1993). As a result CO_2 diffusion into the leaves may be severely affected. NAR and g_s increased and decreased concomitantly over time for elevated CO_2 grown plants under all nutrient treatments (Fig. 3.1 & 3.6). This suggests that stomatal conductance had an effect on NAR as illustrated by a significant positive correlation observed between NAR and g_s for 12mM N regime plants under high CO_2 (Fig. 3.13). This data suggests a high impact of g_s on NAR at 12mM N regime. On the contrary, the 6mM N regime resulted in a moderate but positive correlation between NAR and gs for high CO_2 plants (Fig. 3.13). The poor positive correlation between NAR and g_s for 4mM N grown plants may have been due to mesophyll limitations imposed by low protein levels that limit photosynthesis (Wojcieska, 1994 and references cited therein).

The trend in increase and decrease in WSD in this study resembles that of NAR, g_s, E and leaf area (Chapter 4; Fig. 4.2). This appears to suggest that WSD had a partial impact on NAR and related parameters. This is supported by the fact that under high CO_2 and 6mM N regime, NAR was higher because there were more stomata per mm^{-2} for diffusion of CO_2 into the leaf (Table 3.1). The low NAR than expected under the 12mM N regime might have been exacerbated by the low WSD. The increase in WSD in this study is consistent with the results of O'Leary and Knecht (1981) and Apel, (1989) and Berryman et al., (1994) for elevated CO_2 grown plants. This result contrasted with Woodward and Bazzaz (1988) who reported a decline in WSD for high CO_2 plants. Clearly this suggests that the response is species dependent.

Reduction in gs resulted in a decline in transpiration and a concomitant increase in WUE under all nutrient levels for elevated CO_2 grown plants (Fig. 3.5; 3.6; 3.7). This has been widely reported for most C_3 plants (Radoglou et al., 1992; Mjwara *et al*., 1996; Siphugu, 1997). Increased WUE under elevated CO_2 resulted in high growth rates for elevated CO_2 plants (Chapter 4). An interesting relationship was observed between foliar [N] and transpiration during the first three and the sixth weeks for all treatments (Fig 3.5 & 3.12). At these growth stages transpiration was observed to increase and decrease concomitantly with foliar [N]. This is supported by a strong positive correlation between foliar [N] and transpiration under the 6mM N regime for high CO_2 plants (Fig 3.15). The latter three weeks showed a further increase in foliar [N] of about 86% which coincided with remobilization of most parameters for an example chl content of the leaf, sink strength at the flower

heads and NAR at 35 DAG onwards. The poor positive correlation for the 4 and 12mM N regimes may have been because of the poor and excess N uptake (relative to soil content) effected by presence of other elements as suggested by Chapin (1980). This supports the notion that transpiration may have a partial role in nutrient uptake as suggested by Wong (1979).

Photosynthetic pigments (chl_a, chl_b, chl_{a+b}, and carotenoids) are the primary centres for photosynthesis. These comprise the light harvesting-chlorophyll protein complexes namely the PSI (chl_a & β-carotene) PSII (chl_b & carotenes). The reduction in photosynthetic pigments under the 4 and 12mM regimes resulted in lower NAR rates. This reduction may be subject to the availability of certain micro-nutrients (e.g. Fe and Mg) for elevated CO_2 grown bean plants as suggested by Mjwara *et al*.,(1996). Comparison of NAR and photosynthetic pigments exhibited a similar trend (Fig. 3.1,3.8 & 3.9). Again this shows a delicate system where a slight decline in photosynthetic pigments is manifested through NAR. A similar kind of relationship was observed between foliar [N] and photosynthetic pigments under the 4 and 6mM N regimes for both CO_2 treatments (Fig. 3.8; 3.9; 3.10; 3.11). This confirms a significant positive correlation obtained by Sage *et al*., (1987) between foliar [N] and total chlorophyll content in leaves. The higher NAR rates for the 6mM N regime were partially due to more photosynthetic pigments compared to the highest and lowest N regimes. Lower carotenoid levels under the 4 and 12mM regime affected NAR adversely as these pigments serve as additional light receptors under certain conditions (Sifermann-Harms, 1985).

Elevated CO_2 reduced foliar [N] to levels far below those of control under 6mM [N] as result affecting photosynthesis and pigment levels and NAR (Fig. 3.12). This effect appears to be as a result of N reallocation to other limiting processes resulting in higher NUE that translates into enhanced growth under elevated [CO_2]. Hocking and Meyer (1991) reported similar results for elevated [CO_2] grown wheat. On the other hand, Conroy (1992) warned that most of the discussion in the literature on acclimation to elevated [CO_2] has concentrated on Rubisco as a result ignoring photorespiration (PCO cycle) and associated nitrogen assimilation and amino acid synthesis. The flux of nitrogen through the PCO cycle can be up to ten times the rate of primary nitrogen assimilation (Wallsgrove *et al*., 1983). Thus, suppressing the PCO cycle due to CO_2 enrichment would reduce the flux of nitrogen through the cycle. This could not only lower the requirement for nitrogen in the leaves, but also alter amino acid balance in the leaf and other organs (Conroy, 1992), resulting in acclimation as observed in the present study. Although the data presented in this study points to high nitrogen use efficiency as a result of decline in foliar [N], the latter effect is not ruled out as having exacerbated low foliar [N] under elevated [CO_2].

A very strong correlation was obtained between NAR and foliar [N] for high CO_2 plants under the 6mM N regime (Fig. 3.14). Clearly, this result strengthens the contention that foliar [N] affects photosynthesis as this automatically indicates a decline in protein levels involved in photosynthesis.

3.4 Conclusions

Carbon dioxide enrichment and increase in [N] significantly increased NAR during the first two and the last week of the experiment. The latter increase appears to be mainly as a result of remobilization of photosynthetic pigments and proteins. The remobilization was mainly due to a change in source and sink capacities translating into higher photosynthetic rates under high CO_2 as reported by Delgado *et al*., (1994) and Arp (1991).

High CO_2 increased NAR and growth at the expense of foliar [N], confirms the results presented by Conroy and Hocking, (1993). Decline in foliar [N] negatively affected Rubisco amount and activity hence the lower NAR observed in this study. However, proteolysis as a result of leaf senescence and relatively low light intensity may have contributed in lowering both the amount and activity of Rubisco in this study. This may have impacted on proteins involved in harvesting light as confirmed by low photosynthetic pigments in this study under the low and high N treatments. In addition, elsewhere it was stated that little information is available on foliar concentration of most macro and micro-nutrients under elevated [CO_2]. Also, Chapin (1980) warned that in the soil solution absorption or nutrient uptake of one nutrient element maybe affected by the presence (quantities) of the others. Accumulation of data on this aspect may elucidate the mechanisms involved in reducing foliar [N] under elevated [CO_2].

In this study the data shows that low N supply affected NAR adversely and this is manifest in the growth analysis (see Chapter 4). In addition the 6mM N

regime provides a complex picture, were under optimal nutrient supply and high CO_2, NAR apparently affected by a combination of factors such as g_s, E, foliar [N] and pigment levels over time. Another interesting relationship that this study has unveiled, is there seems to be a relationship between foliar [N] and transpiration that still needs further investigation before a conclusion may be made. The A/C_i response in this study demonstrates that a concomitant decline in NAR with time under high CO_2 for all N treatments may have been due to mesophyll limitations (von Caemmerer and Farquhar, 1981 and Mjwara, 1996). Finally this study is in agreement with the hypothesis that foliar [N] affects photosynthesis adversely as observed before. This study clearly indicates that debate and research on the effects of high CO_2 remains a topic of great interest. The elucidation of the mechanisms affecting NAR in relation to reduction of foliar [N] must rank as a priority for future research. Additional studies are needed to elucidate complex interrelationships and limitations caused by elevated CO_2 on photosynthesis. The emphasis being on how plants utilise macro and micro-nutrients over the growth period till seed maturity.

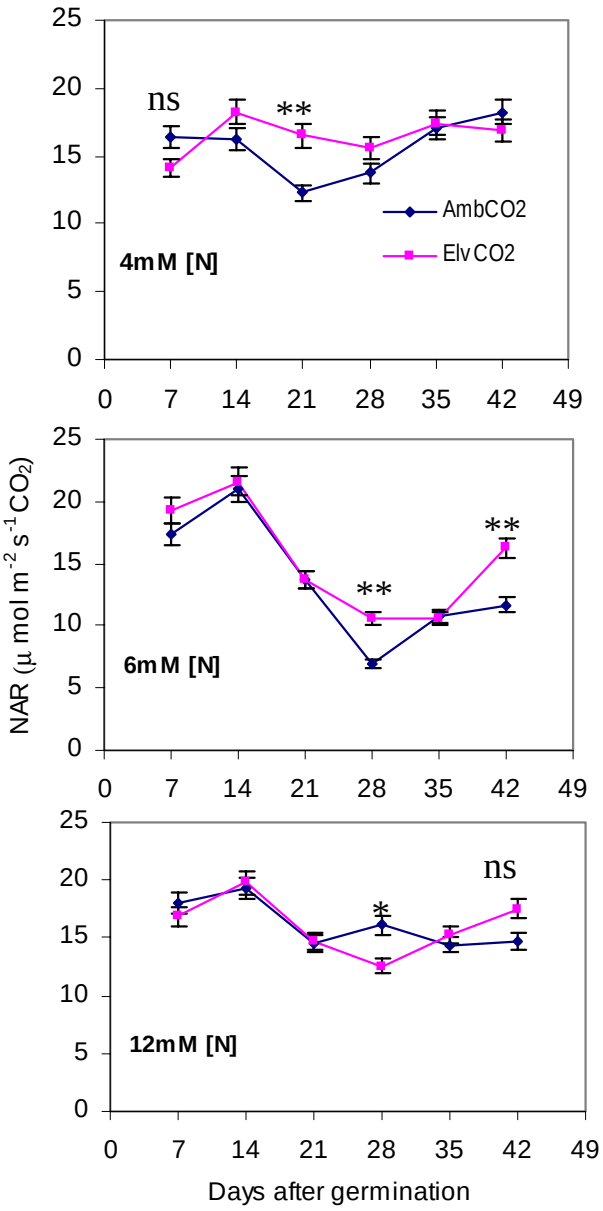


Figure 3.0Time course of NAR response measured at incident light of 790 μmol m^{-2} s^{-1} in wheat plants grown at 360 (AmbCO2) and 700 (ElvCO2) μmol $mol^{-1}CO_2$. Values are the means of six replicates per data point and error bars denote confidence limit where ns denotes not significant and *,** indicate significance at $p<0.05$ and $p<0.01$ respectively.

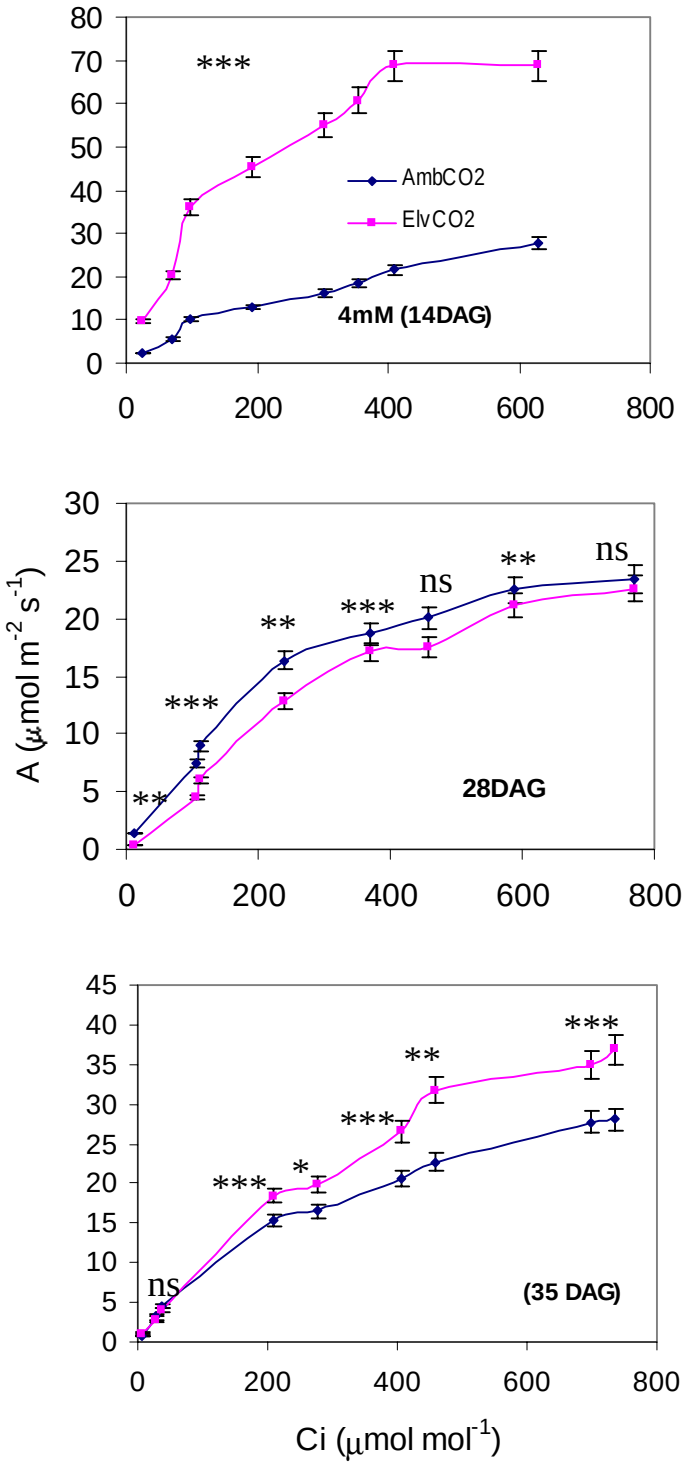
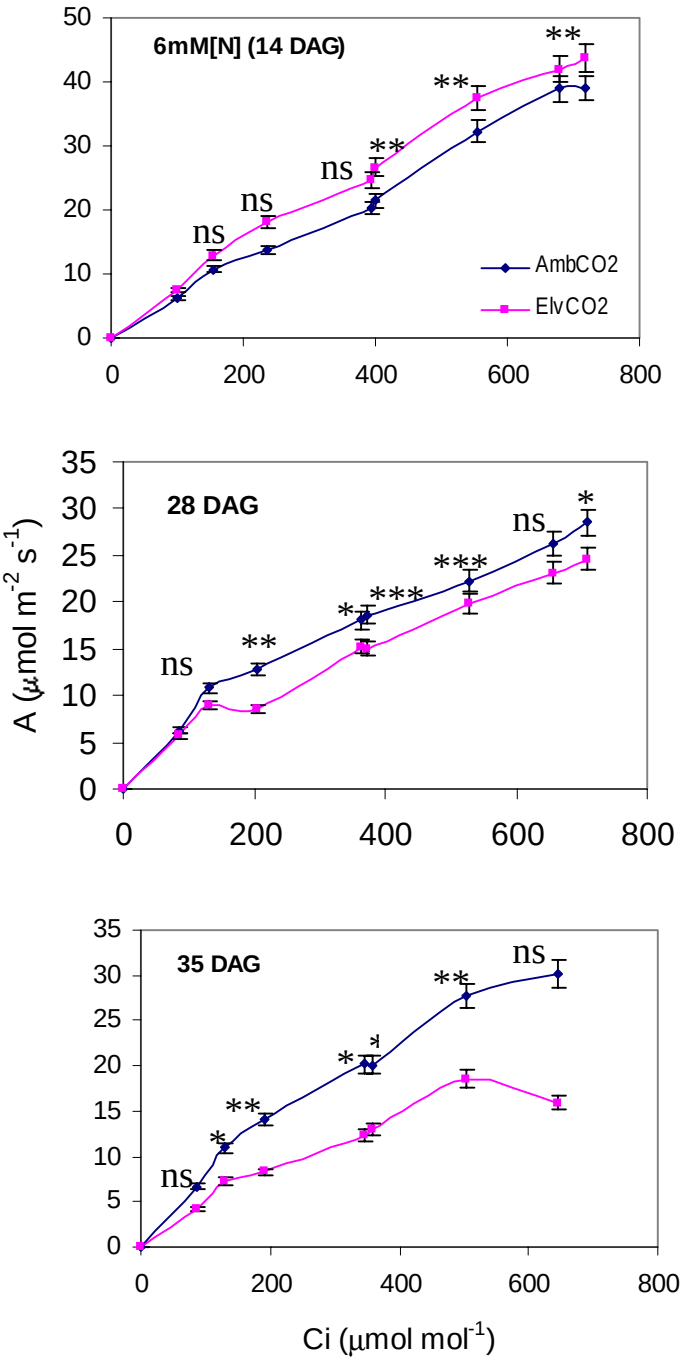


Figure 3.2 Assimilation as a function of intercellular [CO_2] (C_i) in wheat leaves grown at 360 (AmbCO2) and 700 (ElvCO2) µmol $mol^{-1}CO_2$. Measurements were obtained at three different growth stages 14, 28 and 35 DAG. Values are the means of three replicates and error denote confidence limit where ns, denotes not significant and *,**and *** indicate significance at $p<0.05$, $p<0.01$ and $p< 0.001$ respectively.



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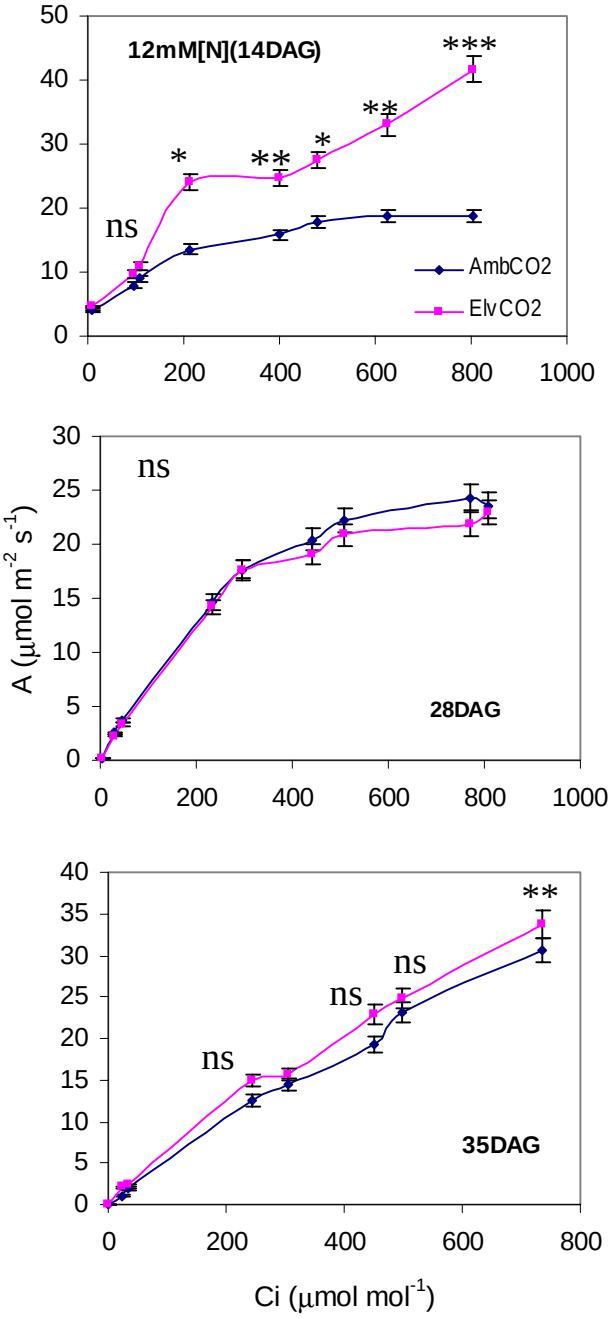


Figure 3.4 Assimilation as a function of intercellular [CO_2] (C_i) in wheat leaves at 360 (AmbCO2) and 700 (ElvCO2) µmol mol^{-1}CO_2. Measurements were obtained at three different growth stages 14, 28 and 35 DAG respectively and error bars denote confidence limit were ns denotes not significant and *,** and *** indicates significance at $p<0.05$, $p< 0.01$ and $p< 0.001$ respectively.

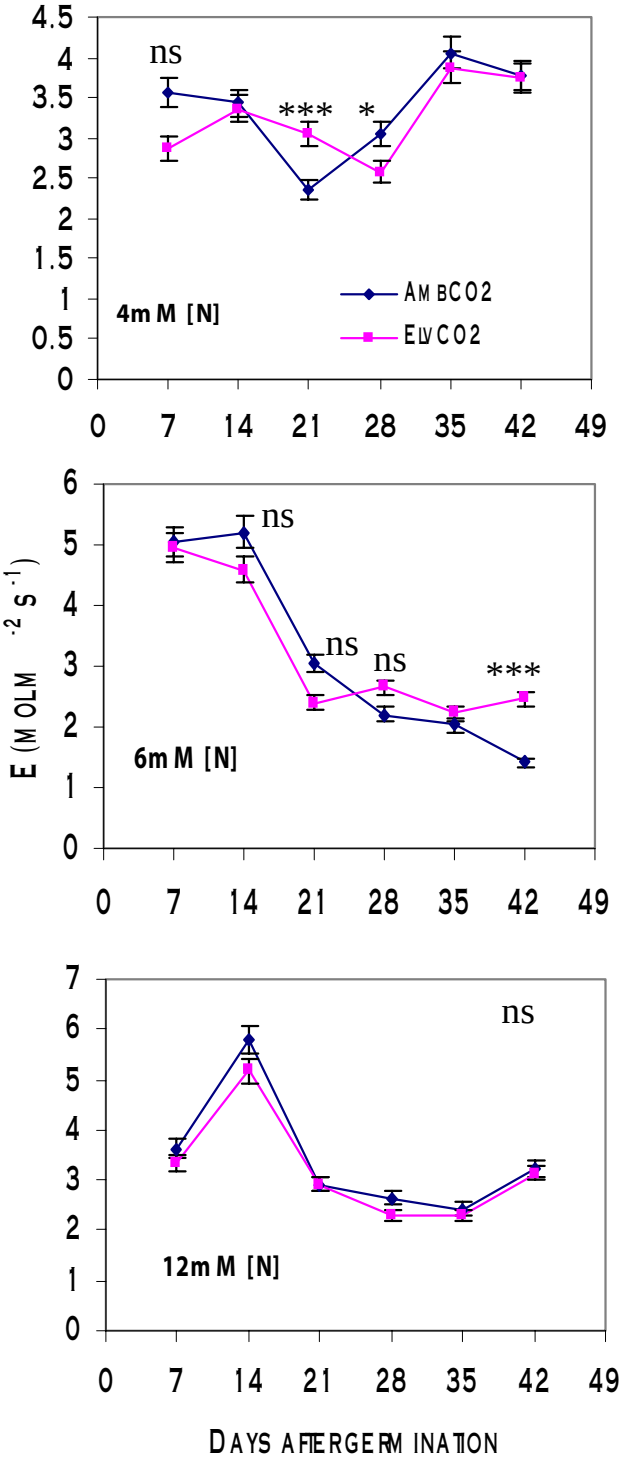


Figure 3.5 Times course of the effects of [CO_2] and N supply on E of wheat plants grown at 360 (AmbCO2) and 700 (ElvCO2) µmol $mol^{-1}CO_2$. Values are the means of six replicates per data point and error bars denote confidence limit where ns denotes not significant and *, ** and *** indicate significance at $p< 0.05$, $p< 0.01$ and $p< 0.001$ respectively.

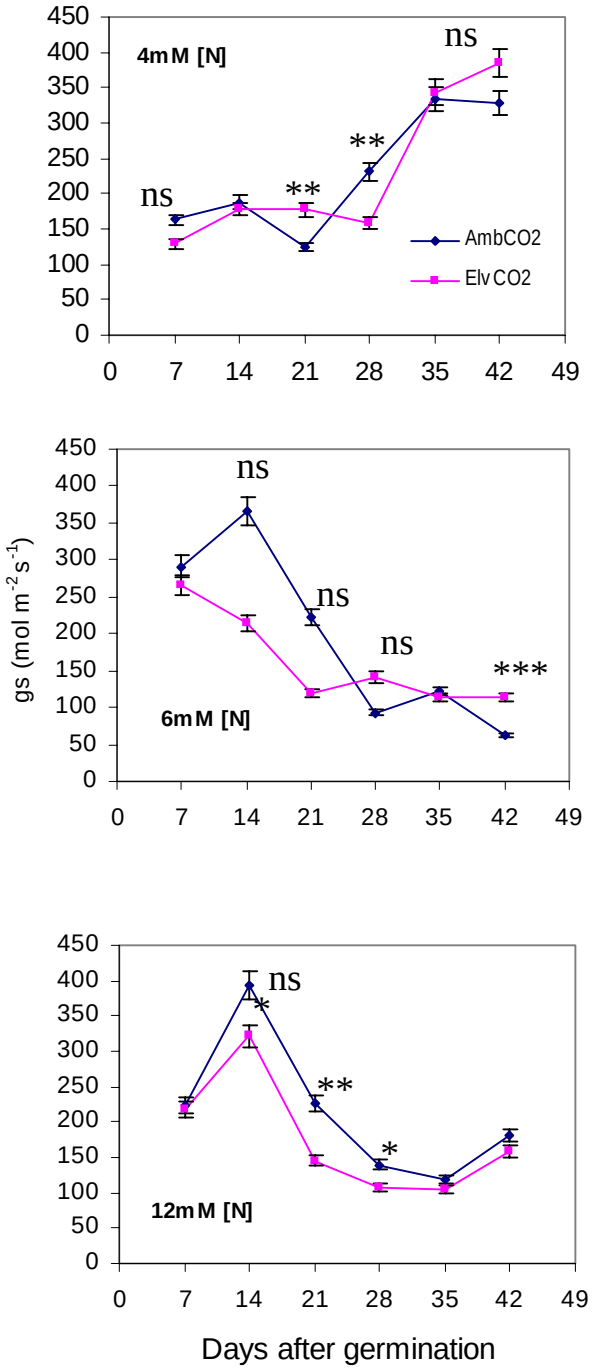


Figure 3.6 Time course of gs response measured at incident light of 790 µmol m^{-2} s^{-1} in wheat leaves grown at 360 (AmbCO2) and (ElvCO2) µmol $mol^{-1}CO_2$. Values are the means of three replicates per data point and error bars denote confidence limit where ns denotes not significant and *, ** and *** indicate significance at $p< 0.05$, $p< 0.01$ and $p< 0.001$ respectively

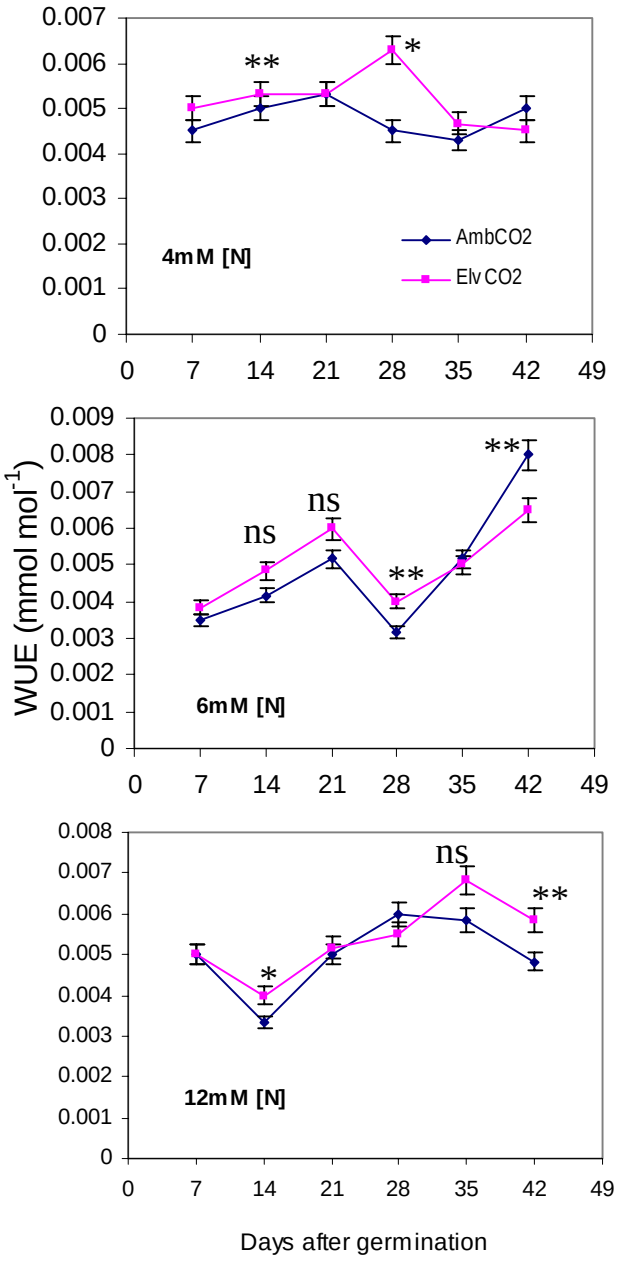


Figure 3.6 Time course of gs response measured at incident light of 790 μmol m^{-2} s^{-1}in wheat leaves grown at 360 (AmbCO2) and 700 (ElvCO2) umol $mol^{-1}CO_2$. Values are the means of six replicates per data point and error bars denote confidence limit where ns denotes not significance and *, ** and *** indicate significance at $p< 0.05$, $p< 0.01$ and $p< 0.001$ respectively.

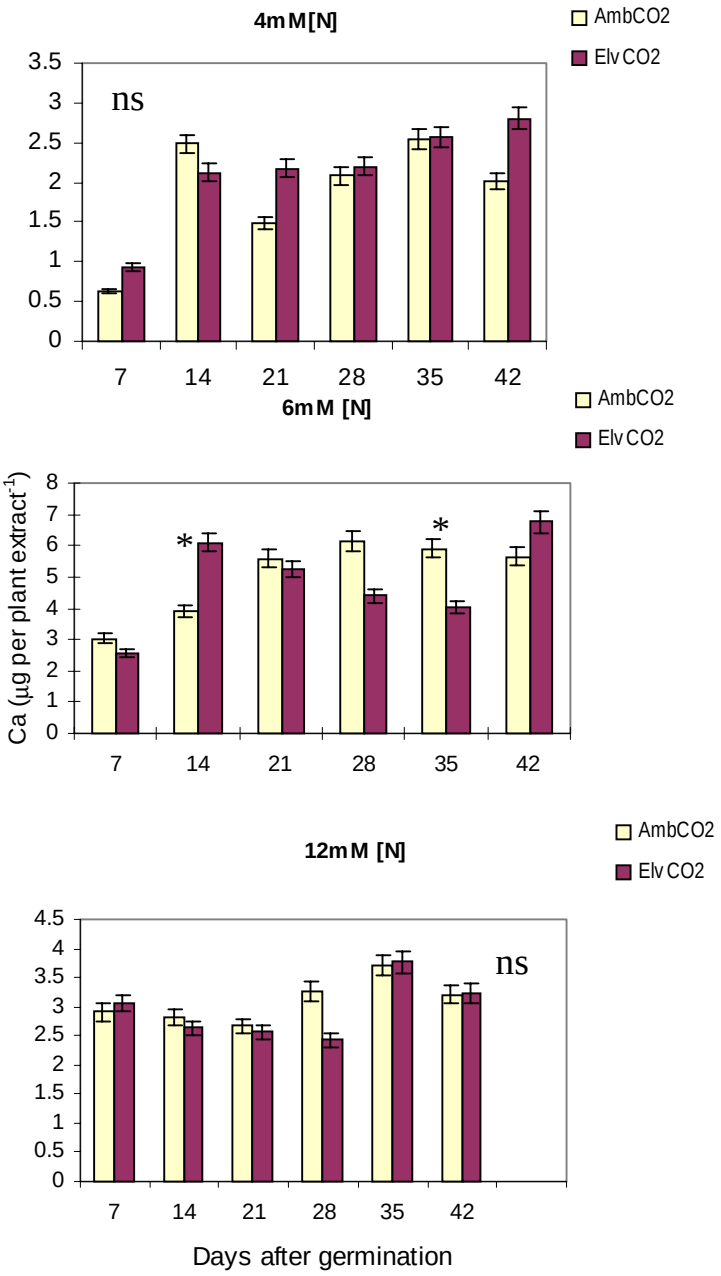


Figure 3.8 Changes in chlorophyll a content at various stages of development in wheat plants grown at 360 (open bars) and 700 (shaded bars) µmol $mol^{-1}CO_2$. Values are the means of three replicates per data point and the error bars denote confidence limit where ns denotes not significant, and * indicate significance at $p<0.05$.

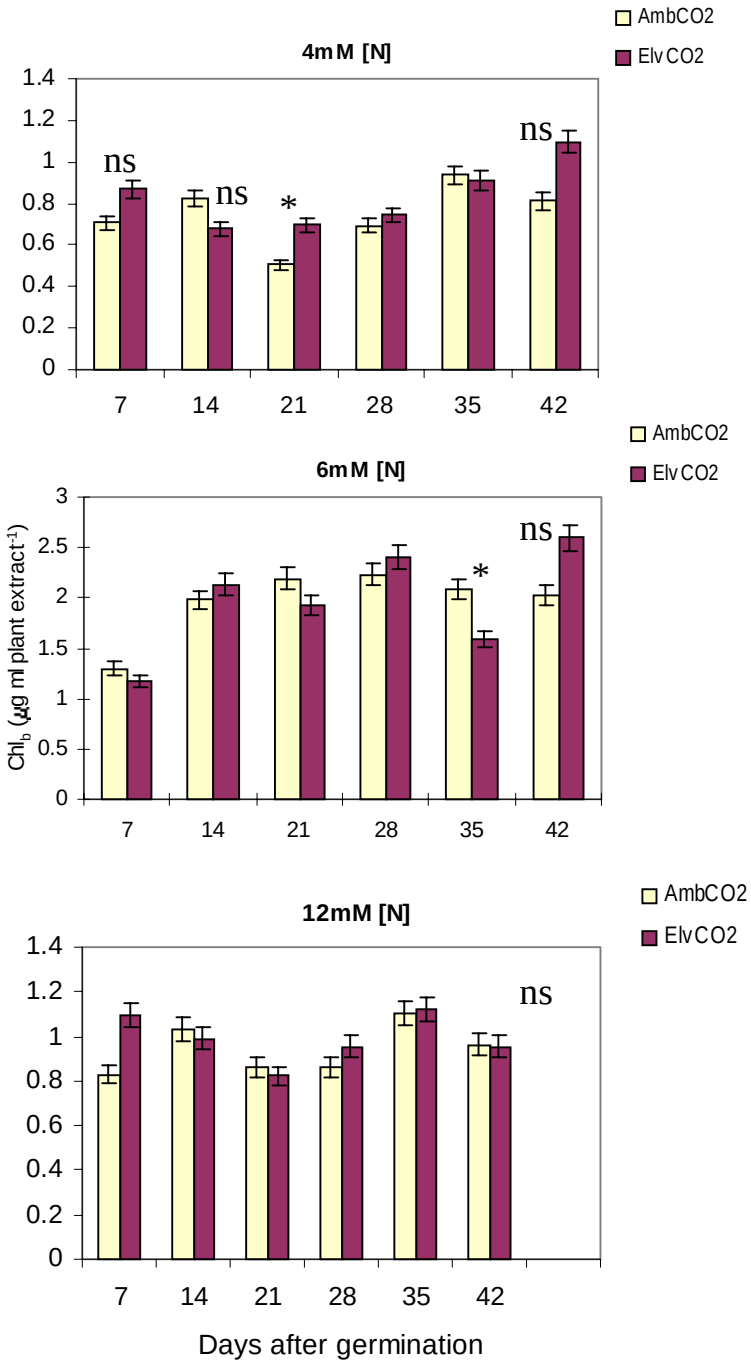


Figure 3.9 Changes in chlorophyll b content at various stages of development in wheat plants grown at 360 (open bars) 700 (shaded bars) µmol $mol^{-1}CO_2$. Values are the means of three replicates and the error bars denote confidence limit where ns denotes not significant and * indicates significance at $p< 0.05$.

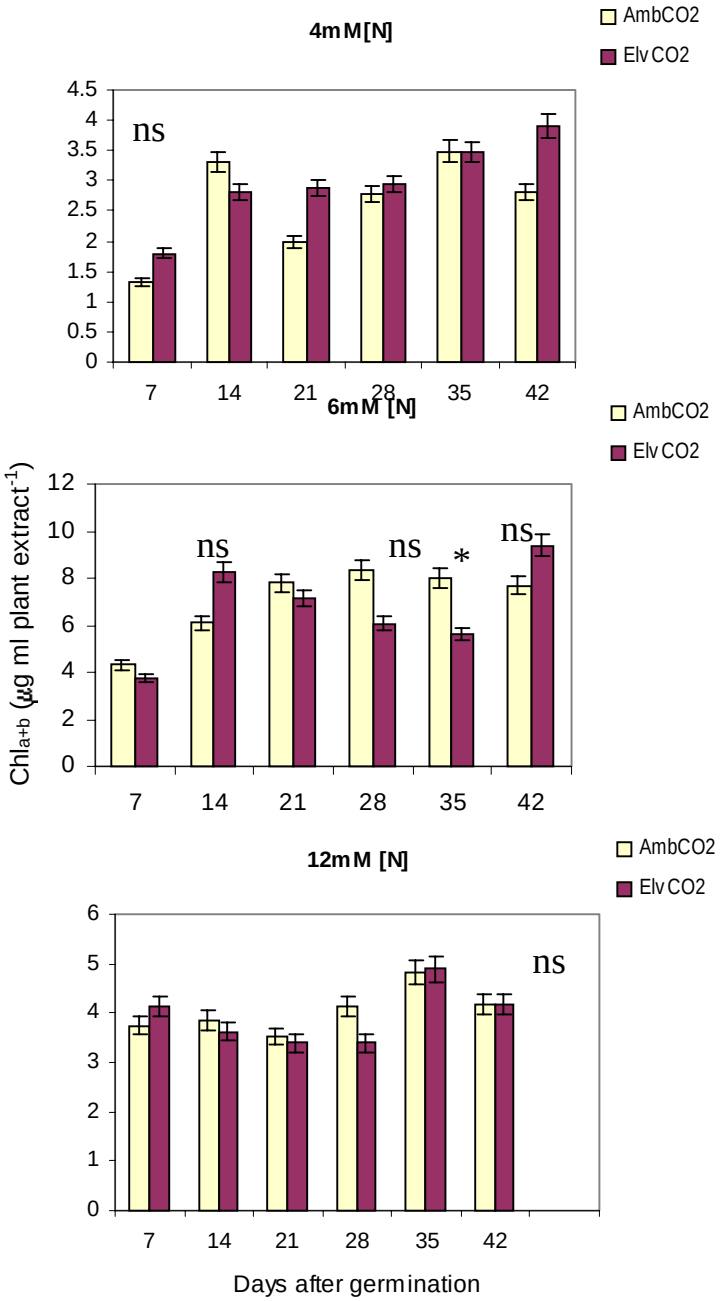


Figure 3.10 Changes in total chlorophyll (a+b) content in wheat plants grown at 360 (open bars) and 700 (shaded bars) umol $mol^{-1}CO_2$. Values are the means of three replicates and error bars denote confidence limit where ns denotes not significant and * indicates significance at $p< 0.05$.

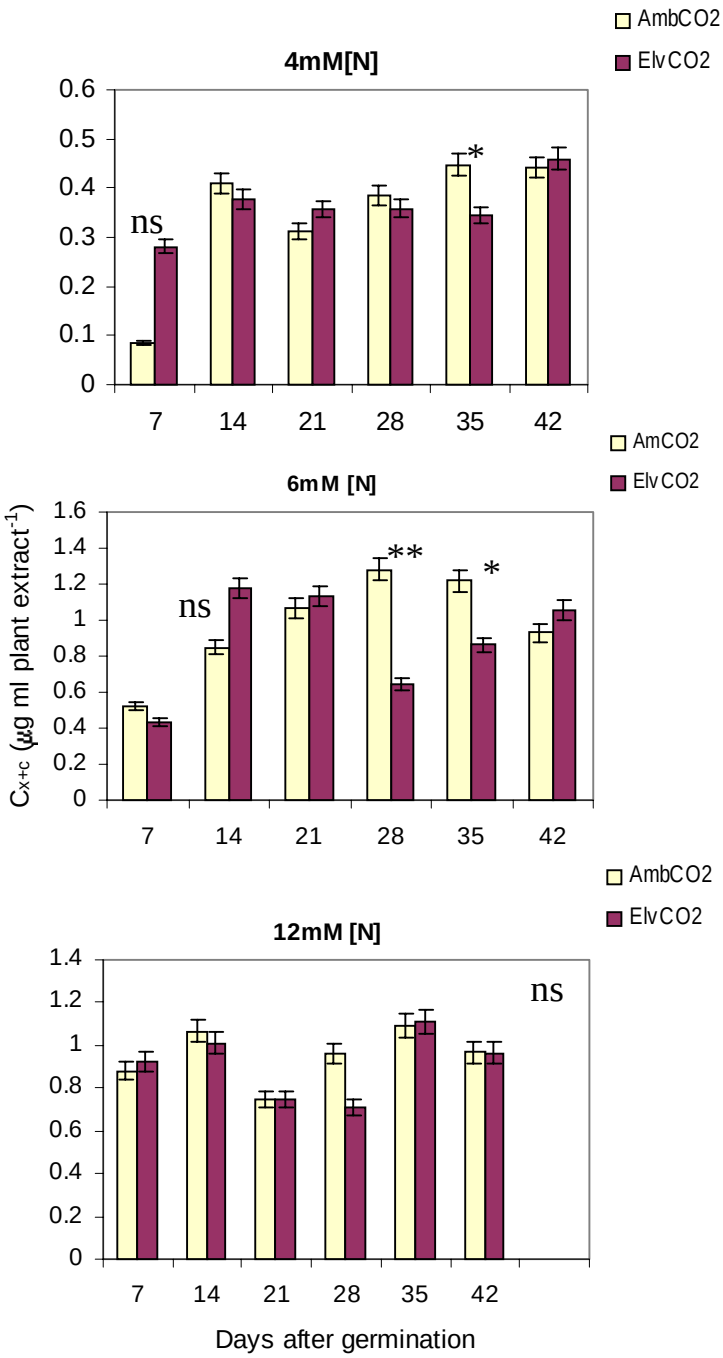


Figure 3.11 Changes in total carotenoid content at various stages of development in wheat plants grown at 360 (open bars) and 700 (shaded bars) µmol $mol^{-1}CO_2$. Values are the means of three replicates and error bars denote confidence limit where ns not significant and * , ** indicate significance at $p< 0.05$ and $p< 0.01$ respectively.

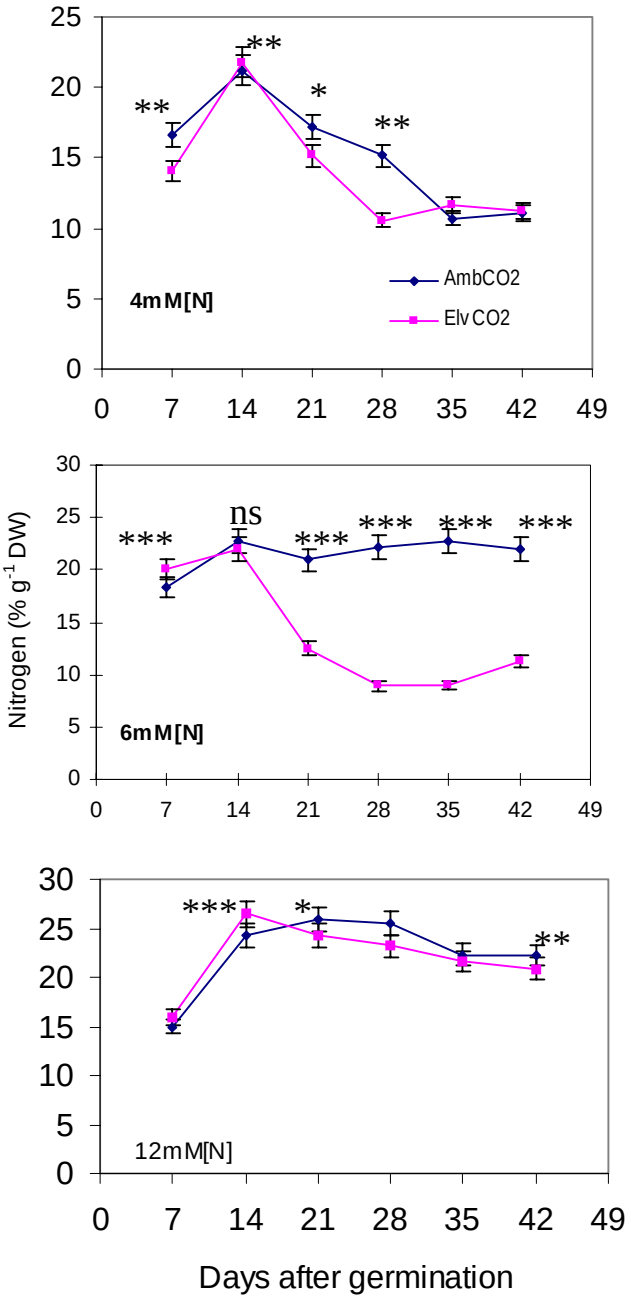


Figure 3.12 Changes in leaf nitrogen content in wheat plants grown at 360 (AmbCO2) and 700 (ElvCO2) µmol mol^{-1}CO_2. Values are the means of three replicates and error bars denote confidence limit where ns denotes not significant and *, ** and *** indicate significance at $p< 0.05$, $p< 0.01$ and $p< 0.001$ respectively.

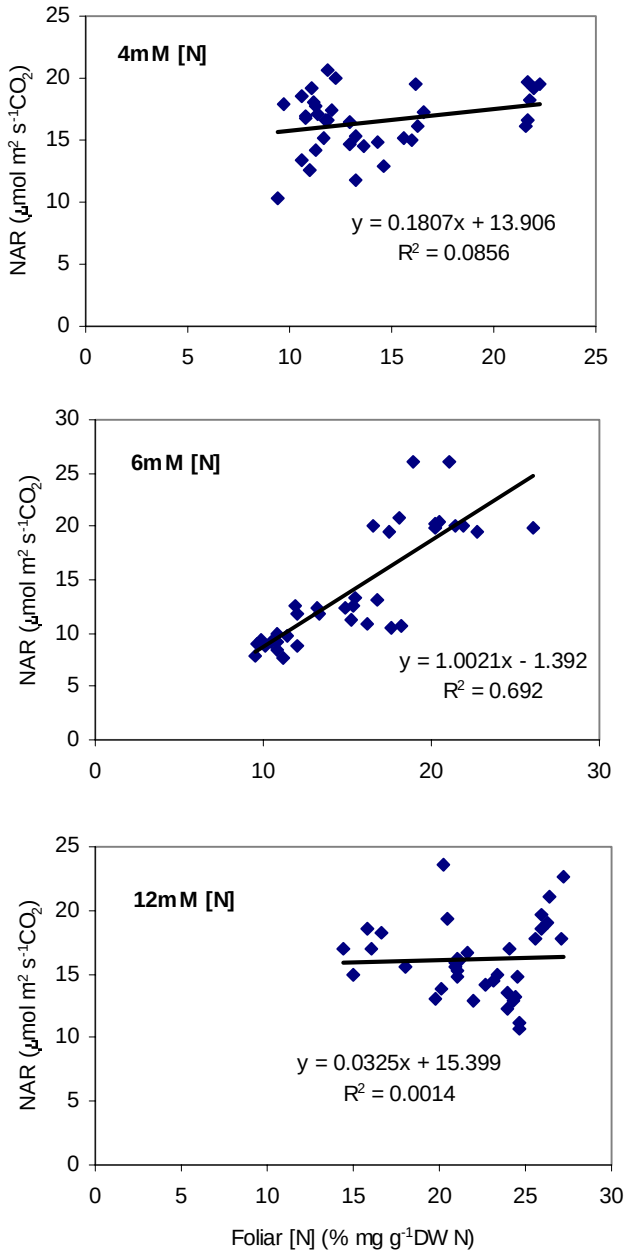


Figure 3.13 Relationship between net assimilation rate (NAR) and foliar nitrogen concentration (foliar [N]) under 700 µmol mol^{-1}CO_2.

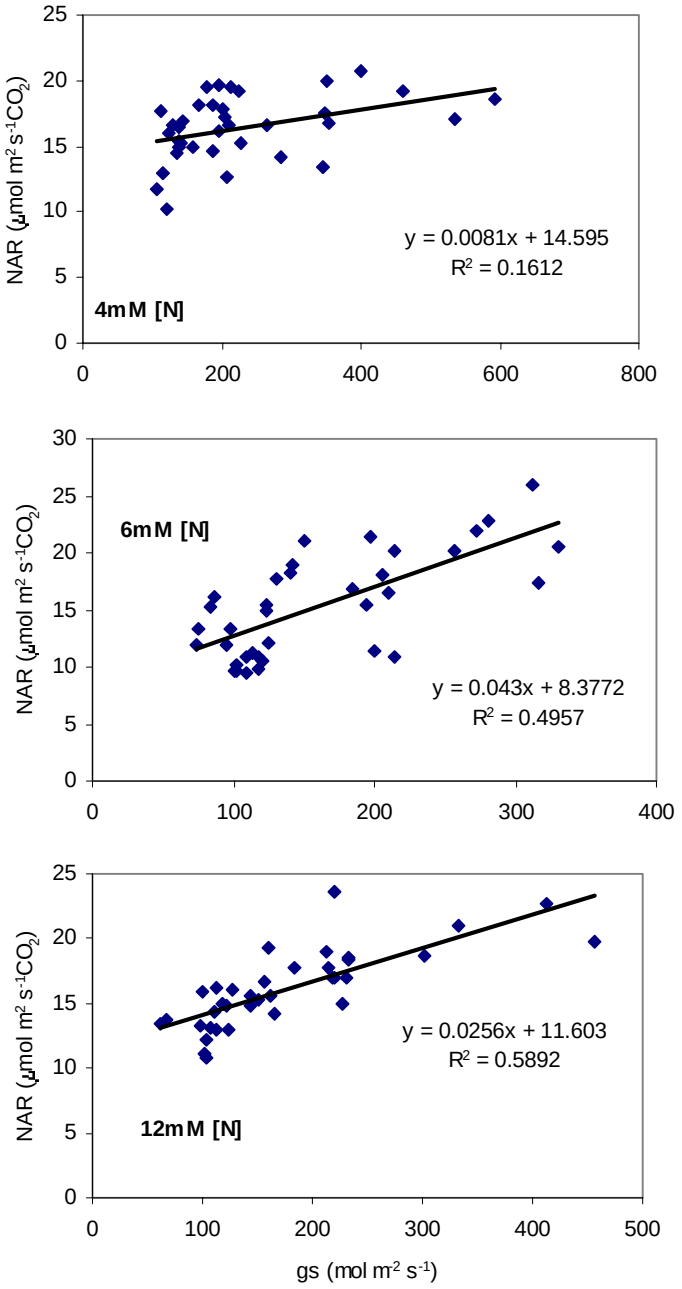


Figure 3.14 Relationship between NAR and gs in wheat leaves at 700 mol mol-1CO_2.

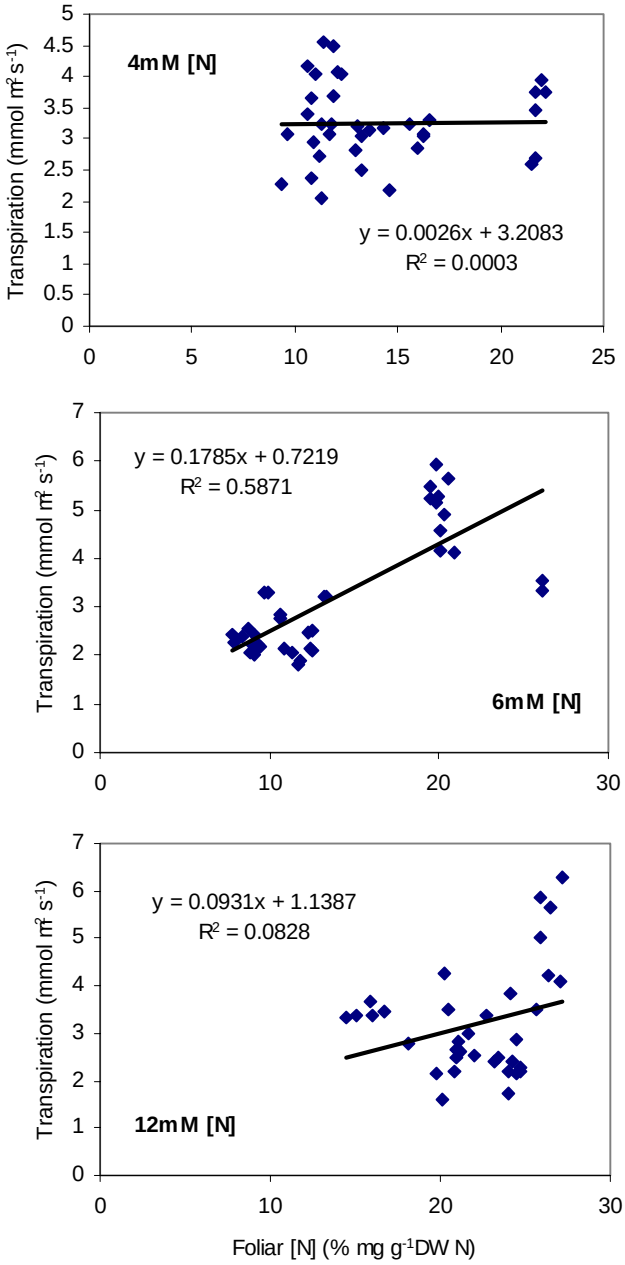


Figure 3.15 Relationship between transpiration and foliar nitrogen concentration (foliar [N]) at 700 µmol mol^{-1}CO_2.

Table 3.1 Table 4.7. Shows a comparison of the effect of changing [N] from 4 to 6mM (4 – 6), and 6 to 12mM (6 – 12) growth regimes on net assimilation rate (NAR).

$[CO_2]$ (μmol $mol^{-1}CO_2$)	ΔN (mM)	DAG	Stats
360	4 – 6 4 – 6 4 - 6	7 – 14 21 – 28 35 – 42	P< 0.084 P< 0.089 P< 0.000
360	6 – 12 6 – 12 6 – 12	7 – 14 21 – 28 35 – 42	P< 0.674 P<0.004 P< 0.001
700	4 – 6 4 – 6 4 – 6	7 – 14 21 – 28 35 – 42	P< 0.001 P< 0.001 P< 0.003
700	6 – 12 6 – 12 6 - 12	7 – 14 21 – 28 35 – 42	P< 0.042 P< 0.115 P< 0.020

Table 3.2 Shows a comparison of the effect of changing [N] from 4 to 6mM (4 – 6), and 6 to 12mM (6 – 12) growth regimes on A/C_i 1.

$[CO_2]$ **(μmol $mol^{-1}CO_2$)**	**Age**	**Slope**	**Stats**
360	14 – 28	1 – 4 5 – 8	P< 0.733 P< 0.960
360	28 – 35	1 – 4 5 – 8	P< 0.283 P< 0.609

700	14 – 28	1 – 4 5 – 8	P< 0.000 P< 0.000
700	28 – 35	1 – 4 5 - 8	P< 0.794 P< 0.000

Table 3.3 Shows a comparison of the effect of changing [N] from 4 to 6mM (4 – 6), and 6 to 12mM (6 – 12) growth regimes on A/C_i 2.

$[CO_2]$ (μmol $mol^{-1}CO_2$)	AGE	Slope	Stats
360	14 – 28	1 – 4 5 – 8	P< 0.822 P < 0.012
360	28 – 35	1 – 4 5 – 8	P< 0.834 P< 0.079
700	14 – 28	1 – 4 5 – 8	P< 0.218 P< 0.000
700	28 – 35	1 – 4 5 - 8	P< 0.562 P< 0.117

Table 3.4 Shows a comparison of the effect of changing [N] from 4 to 6mM (4 – 6), and 6 to 12mM (6 – 12) growth regimes on A/C_i 3.

$[CO_2]$ (μmol $mol^{-1}CO_2$)	**AGE**	**Slope**	**Stats**
360	14 – 28	1 – 4 5 – 8	P< 0.112 P< 0.007
360	28 – 35	1 – 4 5 – 8	P< 0.547 P< 0.709
700	14 – 28	1 – 4 5 – 8	P< 0.032 P< 0.000
700	28 – 35	1 – 4 5 - 8	P< 0.943 P< 0.042

Table 3.5 Shows a comparison of the effect of changing [N] from 4 to 6mM (4 – 6), and 6 to 12mM (6 – 12) growth regimes on stomatal conductance (gs).

$[CO_2]$ ($\mu mol\ mol^{-1}CO_2$)	ΔN (mM)	DAG	Stats
360	4 – 6 4 – 6 4 – 6	7 – 14 21 – 28 35 – 42	P< 0.014 P< 0.617 P< 0.000
360	6 – 12 6 – 12 6 – 12	7 – 14 21 – 28 35 – 42	P< 0.7768 P< 0.519 P< 0.133
700	4 – 6 4 – 6 4 – 6	7 – 14 21 – 28 35 – 42	P< 0.000 P< 0.047 P< 0.000
700	6 – 12 6 – 12 6 - 12	7 – 14 21 – 28 35 - 42	P< 0.369 P< 0.856 P< 0.150

Table 3.6 Responses of stomatal density to [CO2] and N supply. Results are means of three replicates (ns indicates not significant and * and ** indicate significance at p<0.05 and p<0.01 repsectively between ambient and elevated CO_2 for each N regime).

$[CO_2]$ ($\mu mol\ mol^{-1}$)	[N] (mM)	Abaxial stomata (mm^{-2})	Adaxial stomata (mm^{-2})	WSD (mm^{-2})
360	4	8.5 ± 4.63	12.33 ± 2.36	11.40 ± 0.61
700	4	8.67 ± 0.65^{ns}	7.67 ± 0.65^{ns}	8.22 ± 0.31^{ns}
360	6	13.50 ± 0.98	10.83 ± 2.79	12.39 ± 1.41
700	6	17.50 ± 1.49**	16.67 ± 4.28^{ns}	17.41 ± 1.72**
360	12	9.67 ± 4.39	7.67 ± 3.22	8.18 ± 4.23
700	12	11.67 ± 6.26^{ns}	13.5 ± 1.96*	13.17 ± 2.85^{ns}

Table 3.7: Shows changes in stomatal density as a result of a change in N supply and [CO_2].

[CO_2] (μmol mol^{-1})	[N] (mM)	Abaxial stomata (mm^{-2})	Adaxial stomata (mm^{-2})	WSD (mm^{-2})
360	4 - 6	P < 0.107	P < 0.466	P < 0.275
360	6 - 12	P < 0.170	P < 0.219	P < 0.137
700	4 - 6	P < 0.150	P < 0.258	P < 0.067
700	6 - 12	P < 0.171	P < 0.219	P < 0.137

Table 3.8 Shows a comparison of the effect of changing [N] from 4 to 6mM (4 – 6), and 6 to 12mM (6 – 12) growth regimes on transpiration rate (E).

[CO_2] (μmol $mol^{-1}CO_2$)	ΔN (mM)	DAG	Stats
360	4 – 6 4 – 6 4 – 6	7 – 14 21 – 28 35 – 42	P< 0.001 P< 0.831 P< 0.000
360	6 – 12 6 – 12 6 – 12	7 – 14 21 –28 35 – 42	P< 0.409 P< 0.659 P< 0.001
700	4 – 6 4 – 6 4 – 6	7 – 14 21 – 28 35 – 42	P< 0.000 P< 0.160 P< 0.000
700	6 – 12 6 – 12 6 - 12	7 – 14 21 – 28 35 - 42	P< 0.219 P< 0.756 P< 0.085

Table 3.9 Shows a comparison of the effect of changing [N] from 4 to 6mM (4 – 6), and 6 to 12mM (6 – 12) growth regimes on water use efficiency (WUE).

$[CO_2]$ ($\mu mol\ mol^{-1}CO_2$)	ΔN (mM)	DAG	Stats
360	4 – 6 4 – 6 4 – 6	7 – 14 21 – 28 35 – 42	P< 0.007 P< 0.184 P< 0.000
360	6 – 12 6 – 12 6 – 12	7 – 14 21 – 28 35 – 42	P< 0.319 P< 0.014 P< 0.020
700	4 – 6 4 – 6 4 – 6	7 – 14 21 – 28 35 – 42	P< 0.104 P< 0.104 P< 0.020
700	6 – 12 6 – 12 6 - 12	7 – 14 21 – 28 35 - 42	P< 0.769 P< 0.324 P< 0.260

Table 3.10 Shows a comparison of the effect of changing [N] from 4 to 6mM (4 – 6), and 6 to 12mM (6 – 12) growth regimes on chlorophyll a (chl_a).

$[CO_2]$ ($\mu mol\ mol^{-1}CO_2$)	ΔN (mM)	DAG	Stats
360	4 – 6 4 – 6 4 – 6	7 – 14 21 – 28 35 – 42	P< 0.007 P< 0.000 P< 0.000
360	6 – 12 6 – 12 6 – 12	7 – 14 21 – 28 35 – 42	P< 0.157 P< 0.000 P< 0.000
700	4 – 6 4 – 6 4 – 6	7 – 14 21 – 28 35 – 42	P< 0.008 P< 0.000 P< 0.003
700	6 – 12 6 – 12 6 – 12	7 – 14 21 – 28 35 - 42	P< 0.100 P< 0.000 P< 0.024

Table 3.11 Shows a comparison of the effect of changing [N] from 4 to 6mM (4 – 6), and 6 to 12mM (6 – 12) growth regimes on chlorophyll b (chl_b).

$[CO_2]$ ($\mu mol\ mol^{-1}CO_2$)	ΔN (mM)	DAG	Stats
360	4 – 6 4 – 6 4 – 6	7 – 14 21 – 28 35 – 42	P< 0.001 P< 0.000 P< 0.000
360	6 – 12 6 – 12 6 – 12	7 – 14 21 – 28 35 – 42	P< 0.005 P< 0.000 P< 0.000
700	4 – 6 4 – 6 4 – 6	7 – 14 21 – 28 35 – 42	P< 0.004 P< 0.000 P< 0.003
700	6 – 12 6 – 12 6 - 12	7 – 14 21 – 28 35 - 42	P< 0.023 P< 0.001 P< 0.004

Table 3.12 Shows a comparison of the effect of changing [N] from 4 to 6mM (4 – 6), and 6 to 12mM (6 – 12) growth regimes on total chlorophyll content (chl_{a+b}).

$[CO_2]$ ($\mu mol\ mol^{-1}CO_2$)	ΔN (mM)	DAG	Stats
360	4 – 6 4 – 6 4 – 6	7 – 14 21 – 28 35 – 42	P< 0.004 P< 0.000 P< 0.000
360	6 – 12 6 – 12 6 – 12	7 – 14 21 – 28 35 – 42	P< 0.058 P< 0.000 P< 0.000
700	4 – 6 4 – 6 4 – 6	7 – 14 21 – 28 35 – 42	P< 0.006 P< 0.000 P< 0.003
700	6 – 12 6 – 12 6 - 12	7 – 14 21 – 28 35 - 42	P< 0.071 P< 0.000 P<0.013

Table 3.13 Shows a comparison of the effect of changing [N] from 4 to 6mM (4 – 6), and 6 to 12mM (6 – 12) growth regimes on carotenoids (C_{x+c}).

$[CO_2]$ ($\mu mol\ mol^{-1}CO_2$)	ΔN (mM)	DAG	Stats
360	4 – 6 4 – 6 4- 6	7 – 14 21 – 28 35 – 42	P< 0.007 P< 0.000 P< 0.000
360	6 – 12 6 – 12 6 – 12	7 – 14 21- 28 35 – 42	P< 0.038 P< 0.021 P< 0.648
700	4 – 6 4 – 6 4 – 6	7 – 12 21 – 28 35 – 42	P< 0.024 P< 0.003 P< 0.000
700	6 – 12 6 – 12 6 - 12	7 – 14 21 – 28 35 - 42	P< 0.392 P< 0.270 P<0.447

Table 3.14 Shows a comparison of the effect of changing [N] from 4 to 6mM (4 – 6), and 6 to 12mM (6 – 12) growth regimes on foliar [N].

$[CO_2]$ ($\mu mol\ mol^{-1}CO_2$)	ΔN (mM)	DAG	Stats
360	4 – 6 4 – 6 4 – 6	7 – 14 21 – 28 35 – 42	P< 0.130 P< 0.000 P< 0.000
360	6 – 12 6 – 12 6 – 12	7 – 14 21- 28 35 – 42	P< 0.596 P< 0.000 P< 0.055
700	4 – 6 4 – 6 4 – 6	7 – 12 21 – 28 35 – 42	P< 0.033 P< 0.039 P< 0.008
700	6 – 12 6 – 12 6 - 12	7 – 14 21 – 28 35 - 42	P< 0.921 P< 0.000 P<0.000

Chapter 4: Grain yield and growth response of wheat to [CO_2] and change in N-nutrition.

4.1 Introduction

Environmental conditions interact with plant genetic potential resulting in growth and yield in plants (Sionit *et al*., 1981). Among other factors, plant productivity is affected by atmospheric CO_2 concentration and nutrient supply (Sionit *et al*., 1981). As mentioned earlier NAR usually increases in plants grown under elevated [CO_2] in combination with adequate nutrient supply. High photosynthetic rates translate into faster growth rates and increased dry matter production (Imai & Murata 1976; Gifford, 1977; Sionit *et al*., 1980). Increase in biomass production as a result of CO_2 enrichment has been reported for a large number of crop species (see Kimball, 1983 and references cited therein). These studies (Kimball, 1983) were conducted under adequate nutrient supply, which is also the case in agricultural systems. As a result, little information is available on CO_2 enrichment and plant nutrition more especially on growth parameters (Conroy *et al*., 1992).

It has been established that N is the most limiting nutrient for maximum yield or plant productivity, more especially dry matter production (Wojcieska, 1994, and references cited therein). This is because N is a major component of RuBisco, the enzyme that drives photosynthesis. CO_2 enrichment together with high N levels increases dry matter production and total leaf area. As observed before, the likely growth parameters to be affected are the leaves, tiller numbers, shoot and root dry weight and total plant height and root: shoot

ratios. The latter parameter is of great significance, because it relates to the ability of the plant to utilise the nutrients in its environment.

Information about the qualitative and quantitative changes in plant organs caused by elevated CO_2 is essential as this information can be used effectively by the grower for the regulation of the greenhouse CO_2 content in order to balance quality and yield in crops. This is important as certain crops and other plants of economic importance are grown under greenhouse conditions (Enoch & Zieslin, 1988).

4.2 Previous studies on growth response to high CO_2

As mentioned earlier, plant communities respond differently to elevated CO_2. This response is manifest through growth, and affects various plant parts differently for different species. As a result, various responses have been reported on the effects of CO_2 enrichment on the above ground biomass. For example high CO_2 was reported to decrease total leaf area by 25% with a corresponding decrease in total shoot weight (Mousseau & Enoch 1989) in sweet chestnut seedlings (*Castanea sativa* Mill). In contrast there are reports which show no effects on leaf growth and total above ground mass production (Ehret & Jolliffe, 1985; Stulen and den Hertog, 1993). At this time, there is no consensus on growth response of roots to elevated CO_2. The literature contains reports in which an increase, no response or decrease have been noted (Cure & Acock, 1986; Rogers *et al*., 1994; Stulen and den Hertog, 1993). As any effect on root growth, automatically affects root:shoot ratios,

dry matter partitioning primarily induced by elevated CO_2, has to be considered as an important factor in any yield analysis.

There is a great deal of information available on the response of above ground matter to elevated CO_2 as opposed to root growth response (Kimball, 1983; Enoch & Zieslien 1988; Hocking & Meyer, 1991; Mjwara *et al*.,1996; Sitt & Krapp,1999). Clearly, roots have an important role in plant growth as they anchor the plant and function in providing nutrients required by the aerial parts of the plants for growth.

Most of the information acquired from the previous studies, have emphasised the effects of elevated CO_2 only. Little information is available on growth response to both [CO_2] and nutrient supply (Hocking & Meyer, 1991). This chapter describes the overall growth response of wheat c.v. Gamtoos to two [CO_2] and changing nitrogen supply under controlled environment conditions.

4.3 Results

4.3.1 Total plant height (TPH) and Leaf Area (LA)

Overall, wheat plants exhibited enhanced growth of above ground parts. Total plant height (TPH), exhibited an initial curvilinear growth pattern during the first three weeks followed by a steep increase reaching its maximum at 42 DAG under all treatments for ambient and elevated CO_2 grown plants (Fig. 4.1). TPH remained higher over time for elevated CO_2 grown plants under all the N regimes, confirming the result obtained by Hocking and Meyer (1991)

and Mjwara *et al*., (1996) for elevated [CO_2] grown wheat and soybean respectively.

Under ambient [CO_2] and at 14 DAG average TPH was 35; 33 and 34 cm under 4, 6 and 12mM [N] and marginally 38; 35 and 38 cm under elevated [CO_2] for 4, 6 and 12mM [N] respectively (Fig.4.1). The greatest significant differences between the two CO_2 environments were recorded at 42 DAG with large increases in TPH under elevated [CO_2] compared with ambient [CO_2]. For an example under 4m[N] a net difference of about 19 cm, 13.7 cm for 6mM [N] and 6.3 cm under 12mM [N] were recorded (Fig.4.1). The latter net difference resulted in significant differences in TPH between the CO_2 environments under each nitrogen regime (Fig.4.1).

Because the growth pattern in TPH was similar under all treatments over the growth period, statistical comparison between the nitrogen regimes was carried out on average at three-week interval (i.e. 7-21 and 28-35 DAG). Under ambient [CO_2] increase in nitrogen supply from 4 to 6mM [N] regime resulted in a change that was statistically significant between the two nitrogen regimes over the growth the last three weeks (28-42 DAG) (see Table 4.1). The former first three weeks did not result in any significant difference between the two nitrogen regimes (Table 4.1). A further increase in nitrogen supply from 6 to 12mM [N] did not result in any significant change in TPH over the growth period between the two nitrogen regimes (Table 4.1).

Elevated [CO_2] and increase in nitrogen supplies from 4 to 6mM [N] did not result in any significant change in TPH over time between the two nitrogen regimes (Table 4.1). Ceulemans and Mousseau (1994) who also reported no change in TPH in poplar plants grown under elevated [CO_2]. Similarly, a further increase in nitrogen supply from 6 to 12mM [N] resulted in a change in TPH that was not significant between the two nitrogen regimes (Table 4.1). Hocking and Meyer (1991) reported similar results for wheat under ambient and elevated [CO_2] for maize and wheat at the highest nitrogen level in their study.

Leaf Area (LA) exhibited a similar growth pattern to TPH over time (Fig.4.2). Overall elevated [CO_2] grown plants were higher than ambient [CO_2] grown plants with the exception of the 4 and 6mM [N] grown plants onwards (Fig4.2). However for 4 and 6mM N regimes elevated CO_2 grown plants showed a decline in L A at 28 DAG onwards and the ambient grown plants followed later (Fig. 4.2). The net differences in LA between the [CO_2] at 35 and 42 DAG were respectively 4.0 and 5.0 cm^2 under 4mM [N] and 22 and 22.3 cm^2 under 6mM [N](Fig.4.2). Elevated [CO_2] plants had higher LA compared to ambient [CO_2] grown plants over the growth period as seen in Fig. 4.2. In contrast, under 12mM [N] the latter scenario was reversed at 35 and 42 DAG resulting in net differences of 11 and 1.5 cm^2 respectively with the latter being insignificant as seen in Fig 4.2.

Under ambient [CO_2] increase in nitrogen supply from 4 to 6mM [N] resulted in a significant change in LA between the two nitrogen regimes during the last three weeks (28-42 DAG) ($p<0.001$; see Table 4.2). The overall percentage

increase on average was 67% over the growth period. A further boost in nitrogen supply from 6 to 12mM [N] did not result in any significant differences between the nitrogen regimes over the growth period ($p<0.05$) (Table 4.2). Leaf growth was reduced by 7% on average over the growth period as a result of 12mM [N].

A combination of high [CO_2] and increase in nitrogen supplies from 6 to 12mM [N] did not result in any significant changes in LA between the two nitrogen regimes over the growth period (Table 4.2). A further boost in nitrogen supply from 6 to 12mM [N] resulted in the only significant change in LA over the last three weeks of the growth period (see Table 4.2).

4.3.2 Total plant biomass (TPB)

Elevated CO_2 results in the production of plants with higher mass, due to the production of increased plant dry matter. Under 4mM [N] the only significant difference in TPB between the CO_2 regimes occurred at 28 DAG due to a net difference of 790mg (1605.1 & 815.03 mg) (Fig.4.3). However under ambient [CO_2] the increase in N supply from 4 to 6mM [N] resulted in a significant change in TPB from 28 DAG onwards (see Table 4.3).

In contrast, large significant differences in TPB were observed between the two CO_2 environments from 21 DAG onwards as a result of a 6mM [N]. The net differences recorded were 827; 1738; 2518 and 4417mg at 21; 28; 35 and 42 DAG respectively (Fig.4.3). Consequently, there was a large significant

change in TPB as a result of a change in nitrogen supplies (from 4-6mM) (see Table 4.3).

The observed significant difference in TPB between the [CO_2] under 6mM [N] was reversed under 12mM [N] over the growth period (Fig.4.3). However, the increase in nitrogen supplies from 6 to 12mM [N] resulted in a large significant difference in TPB ($p<0.01$) under ambient and elevated [CO_2] at 28 to 42 DAG (Table 4.3). The increase in TPB under elevated [CO_2] and 12mM [N] was reduced by about 50% over the growth period (7-42 DAG). The results presented here are in harmony with those of Hocking and Meyer (1991), El Kohen *et al*., (1993) and Ferris and Taylor (1993).

Shoot dry weight (SDW): Fig. 4.4 depicts shoot dry weight of elevated and ambient CO_2 grown wheat under changing N supply. SDW exhibited a growth pattern similar to that observed in TPB under all treatments (Fig.4.4). Under 4mM [N] the only significant difference ($p<0.001$) in SDW between the CO_2 regimes was at 28 DAG with a net difference of 356.2 mg (909.2 & 553 mg).

Increase in nitrogen supplies from 4 to 6mM [N] under ambient [CO_2] did not result in any significant change in SDW over the growth period (Table 4.4). A significant difference ($p<0.05$) was recorded between the two nitrogen regimes (4 and 6mM) as a result of CO_2 enrichment during the latter part of the growth period (28-42 DAG) (Table 4.4). A 6mM [N] resulted in a large significant difference in SDW between the two CO_2 regimes with a maximum difference at 42 DAG (Fig.4.4). Net differences of 321; 523; 1172 and 2465

mg were recorded respectively at 21; 28; 35 and 42 DAG (Fig4.4). Consequently elevated [CO_2] and 6mM [N] increased SDW by about 86% on average over the experimental period.

A further increase in nitrogen supplies from 6 to 12mM [N] did not result in any significant changes in SDW between the two nitrogen regimes under elevated [CO_2]. In contrast, under ambient [CO_2] a significant difference was observed at 28 to 42 DAG (Table 4.4). SDW was increased by about 100% as a result of 12mM [N] under ambient [CO_2]. No significant differences in SDW were observed between the two CO_2 regimes over the growth period, however overall elevated [CO_2] grown plants had higher SDW compared to ambient [CO_2] grown plants (Fig.4.4).

Root dry weight (RDW): RDW exhibited a similar growth pattern to SDW with elevated [CO_2] having higher RDW than ambient [CO_2] grown plants but this scenario was reversed under 12mM [N] (Fig.4.5). Under 4mM [N] no significant difference was observed between the CO_2 regimes.

Increase in nitrogen supplies from 4 to 6mM [N] under ambient and elevated [CO_2] resulted in a significant change in RDW between the two nitrogen regimes (see Table 4.5). RDW increased by over 100% on average over the growth period as a result of 6mM [N] under ambient and elevated [CO_2]. Under a 6mM [N], elevated [CO_2] grown plants exhibited a steep increase in RDW resulting in a large significant difference in RDW between the two CO_2 regimes especially at 41 DAG (Fig. 4.5). For an example, net differences of

505; 1189; 1263 and 1889 mg were recorded respectively at 21; 28; 35 and 42 DAG between the two CO_2 environments (Fig 4.5).

Under ambient [CO_2] a further increase in nitrogen supply from 6 to 12mM [N] resulted in a significant change in RDW at 7 to 21 DAG between the two nitrogen regimes (Table 4.5). In contrast, elevated [CO_2] resulted in a large significant difference ($p<0.001$) in RDW as a result of a change in nitrogen (6-12mM) over the growth period (Table 4.5). Consequently, RDW was increased by about 71% over the experimental period. Under a 12mM [N] a comparison between the two CO_2 regimes did not result in any significant differences in RDW but at 35 DAG ($p<0.05$). Similar results were obtained by Rogers *et al*., (1980); Sionit *et al*., (1981) and Hocking and Meyer (1991) for soybean and wheat respectively.

Root to shoot ratios (R:S): R:S exhibited different growth patterns over time depending on N supply and CO_2 concentration (Fig. 4.6). Under 4mM [N] and elevated [CO_2] there was a noticeable decline in R:S at 14 DAG, thereafter followed by a steep increase from 21 to 28 DAG eventually reaching its maximum at 42 DAG. In contrast ambient [CO_2] grown plants exhibited a slight increase in R:S at 14 DAG which was later followed by a steep increase form 28 DAG onwards (Fig.4.6). Consequently a net difference of 0.081mg was recorded at 14 DAG resulting in the only significant difference between the two CO_2 regimes ($p<0.001$).

Under ambient [CO_2] an increase in nitrogen supply from 4 to 6mM [N] resulted in a significant change in R:S over the growth period (Table 4.6). In contrast, under elevated [CO_2] the only significant difference ($p<0.05$) was observed during the first three weeks (Table 4.6). As seen if Fig 4.6, under elevated [CO_2] and 6mM [N] there was an initial decline in R:S at 7 to 14 DAG and later followed by a steep increase reaching maximum at 28 DAG and eventually declining to levels almost of ambient [CO_2]. A net difference of 0.51; 1.19; 1.42 and 0.83 mg were recorded respectively at 7; 21; 28 and 35 DAG, resulting in significant differences between the two CO_2 regimes under 6mM [N]. R:S was increased by about 62% on average as a result of CO_2 enrichment and 6mM [N] compared to 4mM [N].

Increase in nitrogen supply from 6 to 12mM [N] resulted in the only significant change ($p<0.01$) in R:S between the two nitrogen regimes during the first two weeks (Table 4.6). A comparison of between the two nitrogen regimes under elevated [CO_2] showed a significant change in R:S over the experimental period ($p<0.001$; Table 4.6). Under a 12mM [N], there was a decline in R:S during the first three weeks (7-21 DAG) in the two CO_2 environments (Fig.4.6). A slight increase and decrease was observed at 28 DAG under elevated and ambient [CO_2] respectively. In the last two weeks a sharp increase and slight decline was observed under ambient and elevated [CO_2] resulting in the only significant difference between the two CO_2 regimes. In contrast to 6mM [N] R:S of ambient [CO_2] grown plants was higher than that of elevated [CO_2] grown plants (Fig.4.6). As a result R:S was reduced by 64% on average over the growth period under ambient and elevated [CO_2] as

a result of 12mM [N].Sionit *et al*., (1981) reported a significant decrease in R:S with increasing nutrient level under high [CO_2]. Similar results were obtained by Raper *et al*., (1977) at high nutrient level for soybean.

Relative growth rates (RGR): RGR of plants grown under the 4 & 12mM [N] exhibited a continuous decline over the growth period for both CO_2 environments (Fig. 4.7). Under 4mM [N] and ambient [CO_2] there was a decline in RGR over the first three weeks and later followed by a slight increase from 28 DAG onwards (Fig4.7). In contrast, under elevated [CO_2] an initial increase in RGR was followed by a decline from 21 DAG onwards resulting in significant differences at 7; 14 and 28 DAG. Under 12mM [N] RGR was observed to decline continuously over the experimental period resulting with no significant differences between the two CO_2 environments.

The 6mM N regime plants exhibited a different pattern of growth which was an increase during the first two weeks then followed by a noticeable decline in the next two weeks and finally a slight increase became apparent at 35 DAG onwards for high CO_2 plants. The results presented here are consistent with those of Rogers *et al*., (1986), Mjwara *et al*., (1996) and Siphugu, (1997).

Nitrogen Use Efficiency (NUE): Under all treatments NUE exhibited an initial slight increase over the first three weeks (7-14 DAG) and later followed by steep increase from 28 DAG onwards (Fig.4.8). NUE of elevated [CO_2] grown plants was higher than that of ambient CO_2 grown plants over the experimental period under all treatments (Fig.4.8). Under 4mM [N] the only

significant difference observed between the CO_2 environments was at 14 and 28 DAG with NUE reaching its maximum at 42 DAG.

Increase in nitrogen supply from 4 to 6mM [N] resulted in a large significant change in NUE under elevated [CO_2] between the two nitrogen regimes from 28 to 42 DAG (Table 4.7). A similar response was observed under ambient [CO_2] between 4 and 6mM [N]. Under 6mM [N], as with other growth parameters a large significant difference in NUE was observed between the two CO_2 regimes with the largest net difference of 26.5% mg g^{-1} DW of N at 42 DAG. NUE was increased by about 86% on average over the growth period as a result of CO_2 enrichment and 6mM [N].

A further increase in nitrogen supply from 6 to 12mM [N] reversed the scenario observed under the 6mM [N]. Consequently, there was a significant change in NUE between the two nitrogen regimes (Table 4.7). Under 12mM [N] the only significant difference in NUE observed between the two CO_2 regimes was at 14 DAG ($p<0.05$) with NUE reaching its maximum at 42 DAG under elevated [CO_2]. NUE was increased by about 77% on average over the growth period as a result of CO_2 enrichment and 12mM [N]. Overall the data presented here supports the findings of Hocking and Meyer (1991) who reported high NUE for wheat under elevated [CO_2] growth conditions.

4.3.3 Grain yield

[N] and elevated [CO_2] (Table 4.8, 4.9 & 4.10) affected grain yield. The most important components of grain yield viz. the number of grains per plant and

the total grain dry weight per plant increased with increasing [CO_2] and N supply. This resulted in significant differences between the two CO_2 environments under the 6 and 12mM N regimes for these two components ($p<0.05$). Under 4mM N, there were no significant differences in grain yield or number of grains per plant between the two CO_2 environments. In contrast, the number of grains per head and the total dry weight per grain were significantly different between the two CO_2 environments (Table 4.8).

Under ambient CO_2 total number of grains per plant shows an increase with an increase in [N] (61.83; 73.17 and 150 respectively for 4, 6 and 12mM N regimes; see Table 4.8 - 4.11). The percentage increase was 18 and over 100% for 6 and 12mM N regimes respectively. This increase in total number of grains per plant was offset by a decline in the number of grains per head (38.3; 28.5 and 35.4 respectively for 4,6 and 12mM N regimes). As a result, the percentage decrease was 26, and 24% respectively for 6 and 12mM N regimes respectively (Table 4.9 & 4.10). Total DW per grain declined with an increase in N supply by 34.6, 20.6 and 24.2 mg respectively for 4,6 and 12mM N regimes. This resulted in a percentage decrease and increase of 40 and 18 for 6 and 12mM N regimes respectively. As a result there was a statistically significant difference between the 4 and 6mM N regimes ($p<0.05$; Table 4.11).

Elevated [CO_2] and increase in N supply decreased the number of grains per head by 47.2 and 36.3 for 4 and 6mM N regimes and later followed by an increase by about 36.44 in number. The decrease from 4 to 6mM was

significant ($p<0.05$) as opposed to the increase from 6 to 12mM, which was not significant ($p>0.05$). Garbut and Bazzaz (1984) also reported a slight decline in the number of seeds in *Datura stramonium*. A similar trend was observed for total DW per grain resulting in significant differences between the three N regimes ($p<0.05$) (Table 4.8 - 4.11). The number of grains per plant increased directly proportional to N supply by values of 69.5, 117 and 174.5 for 4,6 and 12mM N regimes respectively. The percentage increase was significant between the N regimes. High CO_2 and increase in N supply significantly increased total grain DW per plant between the N regimes (Table 4.8 - 4.11), confirming the results of Mjwara (1996). Siphugu (1997) reported a decline of about 50% in grain yield for elevated CO_2 grown barley. He also reported no effect on the total DW per individual grain as a result of CO_2 enrichment.

4.4 Discussion

Plants grown under elevated CO_2 showed enhanced overall above ground growth, compared with ambient CO_2 grown plants. The present study shows that elevated CO_2 and an increase in [N] increased TPH. The slight increase in TPH for the high N regimes under elevated CO_2 was as a result of the production of more tillers per plant, and the possible diversion carbon skeletons to the formation of more structural material in the new tillers. As a result, increase in both TPH and tillering induced more leaves per plant and this resulted in an increase in sink strength. However as seen in Chapter 3 (Fig. 3.1), NAR declined despite the increase in sink strength. TPH increased with the increase in N supply despite the decline in foliar [N] under elevated

[CO_2]. Clearly, this shows that high CO_2 grown plants use N more efficiently for growth as indicated by the TPH data. Stem diameters were increased proportionally to the increase in N supply under elevated CO_2.

Leaf Area: As large amounts of P are trapped in the PCR cycle (Conroy and Hocking, 1993) and as a result, more carbon passes through this cycle. Nitrogen on the other hand is known to limit growth when supplied in low quantities (Wojcieska, 1994). An increase in both N and P should therefore result in increased leaf area. This is what was observed in this study especially during the first four weeks of the experimental period, for high CO_2 grown plants, under all N regimes. The observed unusual decrease in leaf areas during the last two weeks of the experimental period coincided with a decline in foliar [N] in this study (Fig 3.12 and 4.2). Clearly, this indicates the effects of deficiency or N limitation on leaf growth as observed from decreased foliar [N] (Chapter 3; Fig.3.12). This argument is strengthened by the sustained larger leaf area through out the growth period due to higher foliar [N] under a 12mM [N] (Chapter 3; Fig.3.12). The flower formation stage, may have resulted N reallocation away from leaf growth other source demands. However, overall relatively larger leaf areas were observed under the three N treatments especially under ambient CO_2. The larger leaf areas are due to a high supply in P (Fredeen *et al*., 1989).

The increase in TPH and LA definitely resulted in enhanced dry matter production or accumulation in plants as a result [CO_2] enrichment than did ambient [CO_2] grown plants. As a result dry matter partitioning between the

shoots and roots was altered under the three nitrogen regimes in this study. For an example under 6mM [N] and elevated [CO_2], enhanced TPB and SDW were more pronounced than under ambient [CO_2] as compared to the other two nitrogen regimes. This is not unexpected due to the enhanced NUE under elevated [CO_2] (Fig.4.8). Foliar [N] was the lowest under the 6mM [N] plants confirming the result that high [CO_2] and NUE are responsible for this response. To strengthen this contention a significant positive correlation between SDW and NUE was established (see Fig. 4.9).

Nitrogen stressed plants (4mM [N]) did not reduce the relative magnitude of the growth response of wheat to CO_2 enrichment and as a result N stressed plants had relatively equal dry matter gain as the 12mM [N] plants. This result is in contrast with a number of studies that have shown little effects on dry matter accumulation when the plants were N stressed as a result of CO_2 enrichment (Goudrian & de Ruiter, 1983; Sionit *et al*., 1983). However, the data presented in this study is in agreement with Wong (1979), Hocking and Meyer (1991) and Norby *et al*., (1986) who found little effect of N stress on increase in dry matter accumulation as a result of CO_2 enrichment in cotton, *Quercus alba* and *X. occidentale* respectively. A reason for this descripient result maybe the level of the nutrient stress imposed on the plant.

The proportionally larger RDW of the CO_2 enriched plants would enable them to exploit a greater volume of the soil for nutrient uptake, a character that may be important as the CO_2 enriched plants exhibit lower foliar [N], than those of ambient [CO_2] (Hocking and Meyer, 1991). This resulted in a higher R:S

especially under the 6mM [N]. Similarly the higher R:S under 6mM [N] and elevated [CO_2] is attributed to higher NUE. Elevated [CO_2] had no effect on the R:S under 12mM [N] due to reduced NUE. This is consistent with findings of other researchers (Sionit *et al*., 1981; Wojcieska, 1994). The lower R:S under the 12mM [N] may have been exacerbated by reduced root growth under supra optimal supply of nutrients especially nitrogen as suggested by (Marschner, 1995).

In summary, leaves can be considered to be the primary if not the most important organs of the plant, as they are the principal site of N metabolism and subsequent translocation of these products to other parts of the plants. Shoot NUE is therefore a measure of how efficient can the plant use up N. Increase in SDW was positively correlated to NUE under all the nitrogen regimes (Fig.4.9). The more efficient use of the CO_2 enriched wheat plants, probably resulted from a decrease in the Rubisco content of their leaves (Wong,1979; Allen *et al*., 1987) which would enable the nitrogen to be channelled into other regions of the plant (Hocking and Meyer, 1991). This contention is strengthened by a decrease in foliar [N] under the 6mM N regime (Ch3 Fig. 3.10).

The results in this study clearly demonstrate that despite an increase in total plant dry weight, the relative growth rate (RGR) decreases over the growth period (Fig. 4.7). The higher RGR observed during the first two weeks can be explained by an initial growth stimulation by elevated CO_2 due to increased

NAR for example. Similar results have been reported for rice, as well as for other plants (see references cited by Makino *et al*.,1997).

However an interesting observation in this study was that RGR picked up again from 35 DAG onwards under the 4 and 6mM N regimes for elevated CO_2 plants. This again coincided with the increase in NAR at the same age under the same conditions (Ch3 Fig. 3.1), which can be attributed to an increase in sink strength during flower formation as well as tillering, which also contributed to enhanced dry matter production. This supports the observation that RGR is stimulated by an increase in NAR (Makino *et al*., 1997).

The increase in tillering under elevated CO_2 for all nutrient treatments obviously resulted in an increase in the number of heads per plant, and for the significant increase in total number and weight of seeds per plant. The was more pronounced under elevated CO_2, for all the nutrient levels used in this study, confirming the results obtained by Sionit *et al*., (1981). Whilst there was a decrease in the number of grains per head and their total dry weight per grain for the 6 and 12mM N levels, this was compensated for an increase in the number of grains and total grain dry weight per plant coupled with larger grain size. In simple terms, the fewer the number of seeds per head, the larger the size and mass of each grain. Beyond 35 DAG most of the foliar [N] is used in grain filling. Thus, the lower foliar [N] under the 4 and 6mM [N] beyond 35 DAG, correlated with the lower total grain weight per plant, compared to the 12mM [N].

These results direct contrast to those of Fischer *et al*., (1976) for field grown wheat. The authors reported a decrease in yield for elevated CO_2 plants and an increase for ambient grown plants. Possibly, this was associated with the genetic potential of the cultivar they used (cultivar 'Yecora 70'). In our laboratory prior to this study Siphugu (1997) reported a 50% decline in grain yield for barley and this may have been partly due to nutrition.

4.5 General morphological observations

The 4mM N grown plants showed chlorosis at the leaf apex from 14DAG onwards for both CO_2 environments. The beginning of flower formation was just after 28 and 25 DAG for high and low CO_2 environments respectively. Anthesis started at 35 DAG in elevated CO_2 and at 45 DAG for ambient grown plants. Similarly flower formation under the 6mM N regime occured at the same time as in 4mM N regime. However, Anthesis occurred at 40 & 48 DAG for high and low CO_2 respectively under the 6mM N regime. Chlorosis only occurred in the older leaves, possibly as a result of leaf senescence. Flowering occurred at 49 and 55 DAG for high and low CO_2 respectively under the 12mM N regime. Chlorosis was not apparent in the leaves and plants were very green in appearance compared to plants in the other two N regimes. Elevated CO_2 appears to accelerate the growth of plants to maturity as observed during grain production. A combination of high CO_2 and the N regimes used in this study appear to have affected anthesis as mentioned above.

4.6 Conclusion

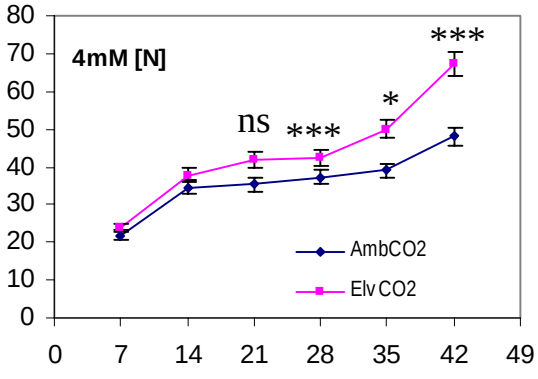
Overall, the data presented in this study supports the hypothesis that increase in atmospheric [CO_2] changes the allocation of dry matter and foliar [N] as a result affecting plant productivity. In the present study, increase in [CO_2] and [N] result in higher NUE which translates into enhanced TPH, dry weight (root, shoot and total plant boimass), R:S and grain yield.

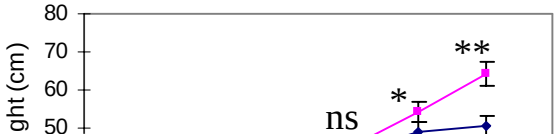
The data presented in this study clearly points to high NUE as a result of the observed plant growth. This result may be altered pending data on soluble non-structural carbohydrates (NSC), as these are reported to increase with a concomitant decline in nitrogen content in both leaves and reproductive structures of plants under elevated [CO_2]. In addition this result may also impact on soluble protein and amino acid compounds pool (Mjwara, 1996).

The higher RDW especially under the 6mM [N] and elevated [CO_2], was as a result of more tillers as these tend to develop their roots. Although it has been argued by Vessey *et al*., (1990) that the increased R:S often found in high [CO_2] grown plants (Sionit *et al*.,1981) indicate that they are nitrogen deficient. The present study showed no indication of nitrogen deficiency under elevated [CO_2] as there was little growth response when nitrogen was increased from 6 to 12mM [N]. This could practically mean that under high [CO_2] the nitrogen level for optimal for optimal growth is somewhere between 6 and 12mM [N] under the experimental conditions in this study.

It has been established that during seed or grain filling, N and C are essential in ensuring proper development of the seed. Final seed yields often depend on the proteolysis of stored foliar N and its translocation to the seed (Dalling *et al*.,1975; Huffaker, 1982). In the present study, the reduction in yield components under 4 and 6mM [N] was definitely as a result of reduced foliar [N]. Leaf senescence is not ruled out as a result of accelerated growth under elevated. This calls for the monitoring of all the physiological activities taking place in the flag leaves as these act as the main sources of the sinks for the grain.

The present study suggests that a rise in the atmospheric [CO_2] will definitely require a proportional increase in nitrogen fertiliser, in order to maintain optimal NAR that will eventually translate into enhanced growth and yield. However good yield needs reassessment as well as this has to be coupled with quality grain in terms of protein content. Furthermore high nitrogen requirement under elevated [CO_2] will have implications on fertiliser management as well as these might affect nutrient uptake. Clearly, the interactive effects of elevated [CO_2] and plant nutrition remain a topic of great interest, especially because there is inadequate information about the physiological processes limiting yield (Cruz-Aguado *et al*.,1999).





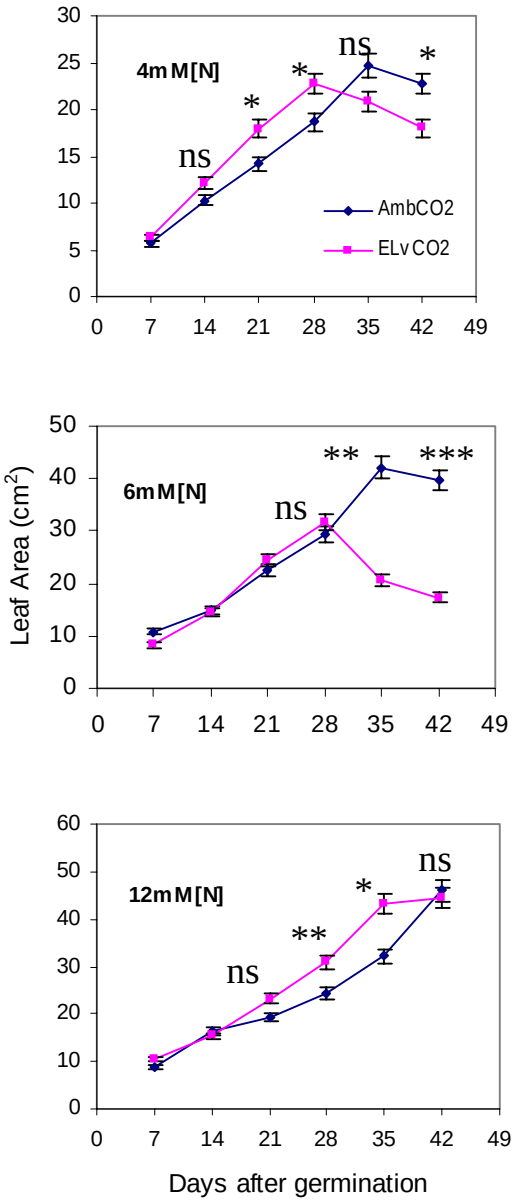


Figure 4.2 Changes in leaf area (LA) in wheat plants grown at 360 (AmbCO2) and 700 (ElvCO2) µmol $mol^{-1}CO_2$ and three N regimes. Values are the means of three replicates per data point and error bars denote confidence limit where ns denotes not significant and *, ** and *** indicates significance at $p< 0.05$, $p< 0.01$ and $p< 0.001$ respectively.

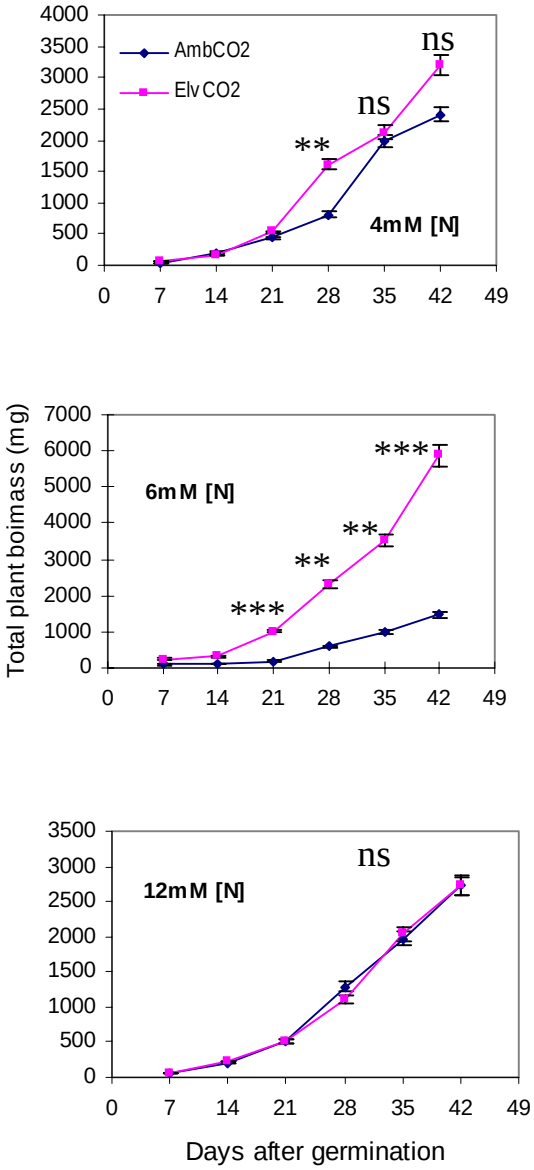


Figure 4.3 Shows total plant biomass (TPB) in response to [CO_2] and change in N supply. Values are the means of three replicates per data point and error bars denote confidence limit where ns denotes not significant and ** and ** indicate significant difference at $p< 0.01$ and $p< 0.001$ respectively.

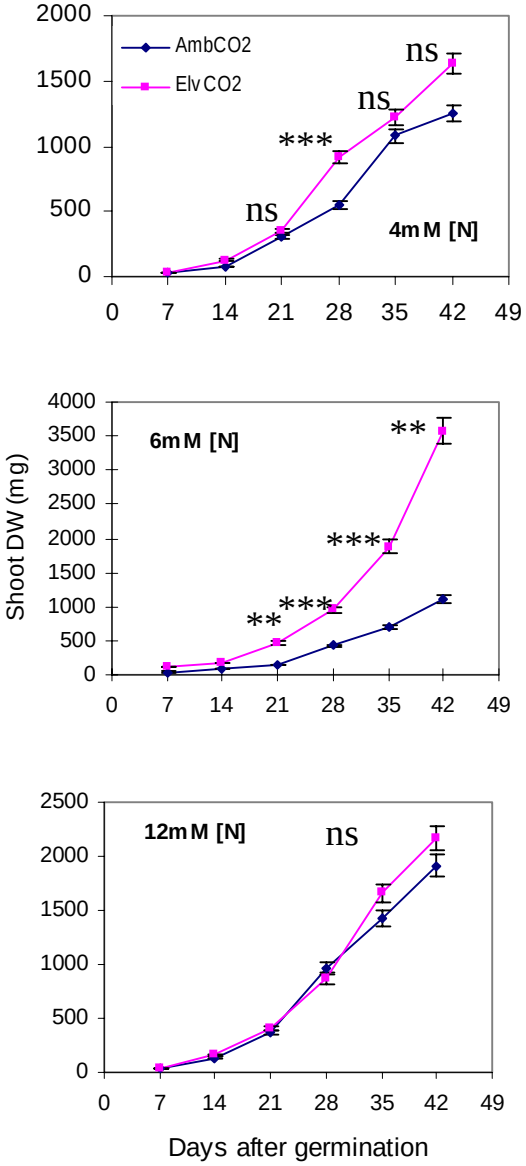


Figure 4.4 Shows shoot dry weight (SDW) of wheat plants in response to [CO_2] and change in N supply over time. Values are the means of three replicates per data point and error bars denote confidence limit where ns denotes not significant and ** and *** indicate significant difference at $p< 0.01$ and $p< 0.001$ respectively.

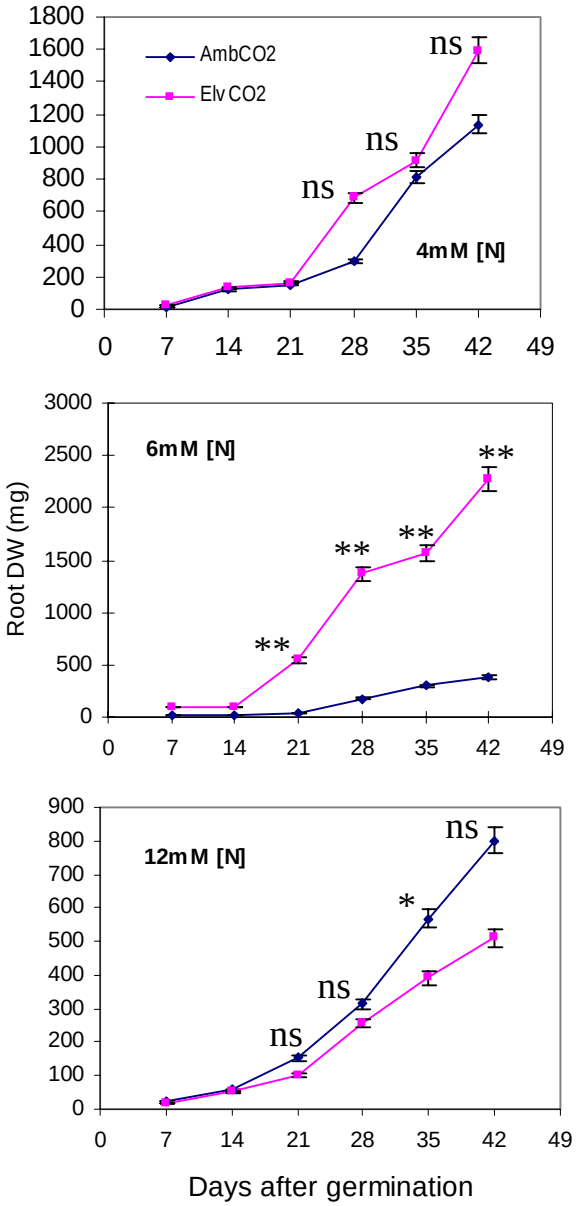


Figure 4.5 Shows root dry weight (RDW) response of wheat plants to [CO_2] and change in N supply. Values are the means of three replicates per data point error bars denote confidence limit where ns denotes not significance and * and ** indicate significance at $p< 0.05$ and $p< 0.01$ respectively.

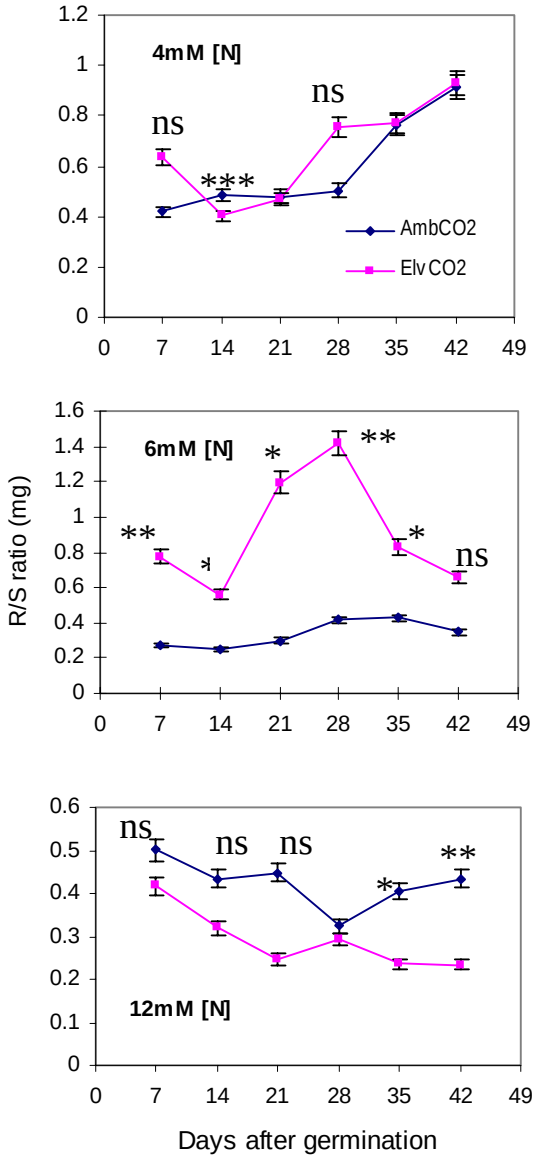


Figure 4.6 Shows root:shoot ratio (R:S) of wheat plants in response to [CO_2] and N supply. Values are the means of three replicates per data point error bars denote confidence limit where ns denotes not significant and *, ** and *** indicate significant difference at $p< 0.05$, $p< 0.01$ and $p<0.001$ respectively.

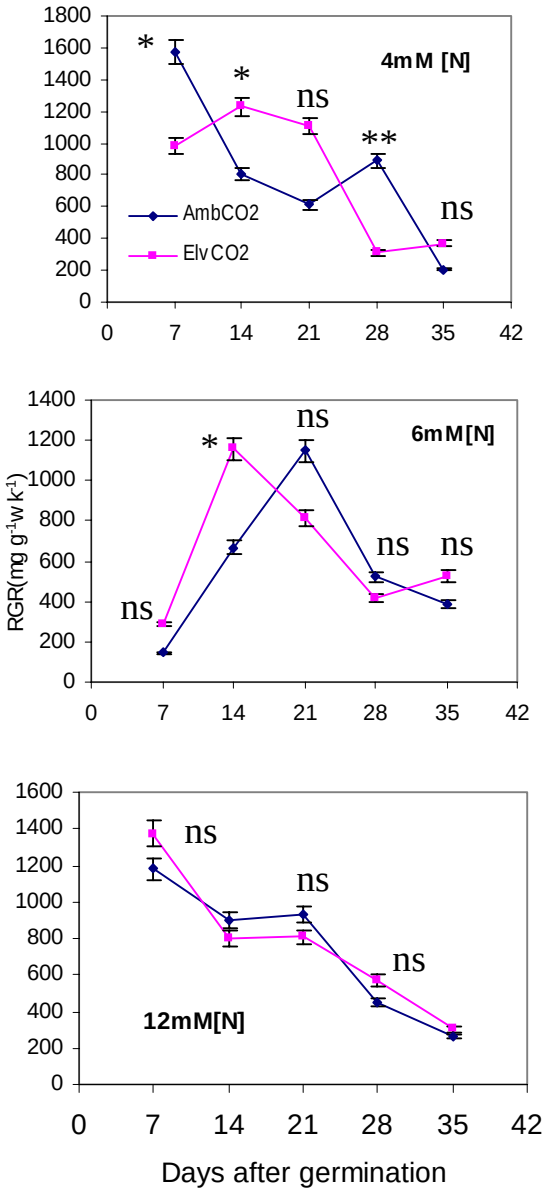


Figure 4.7 Shows relative growth rate (RGR) of wheat plants in response to [CO_2] and change in N supply. Values are the means of three replicates and error bars denote confidence limit where ns denotes not significant and * and ** indicate significant difference at $p< 0.05$ and $p< 0.01$ respectively.

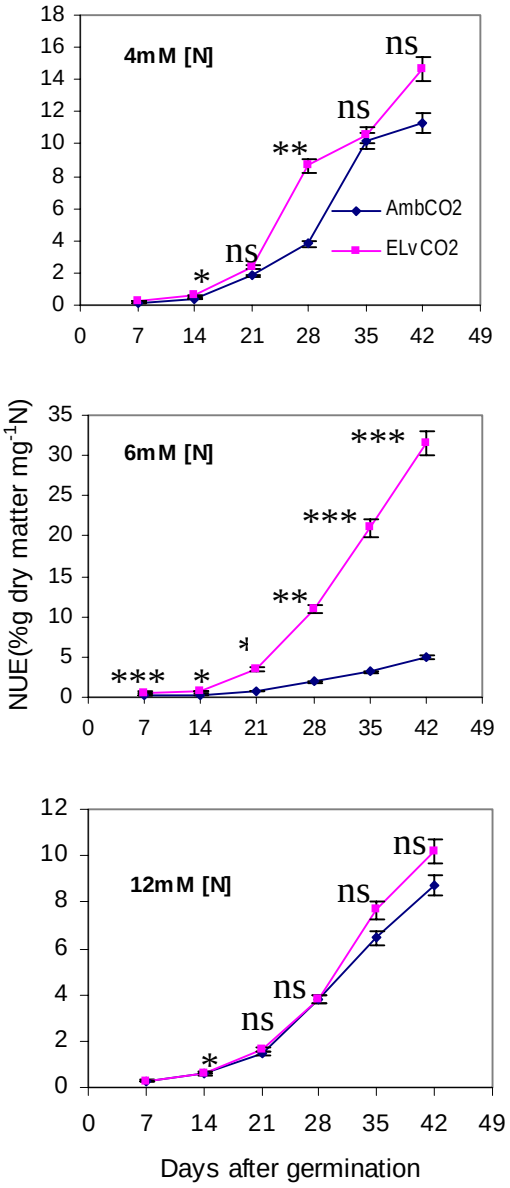


Figure 4.8 Effects of [CO_2] and N supply on nitrogen use efficiency (NUE) of wheat plants grown at 360 (AmbCO2) and 700 (ElvCO2) µmol $mol^{-1}CO_2$. Values are the means of three replicates per data point and error bars denote confidence limit where ns denotes not significant and *, ** and *** indicate significance at $p< 0.05$, $p< 0.01$ and $p< 0.001$ respectively.

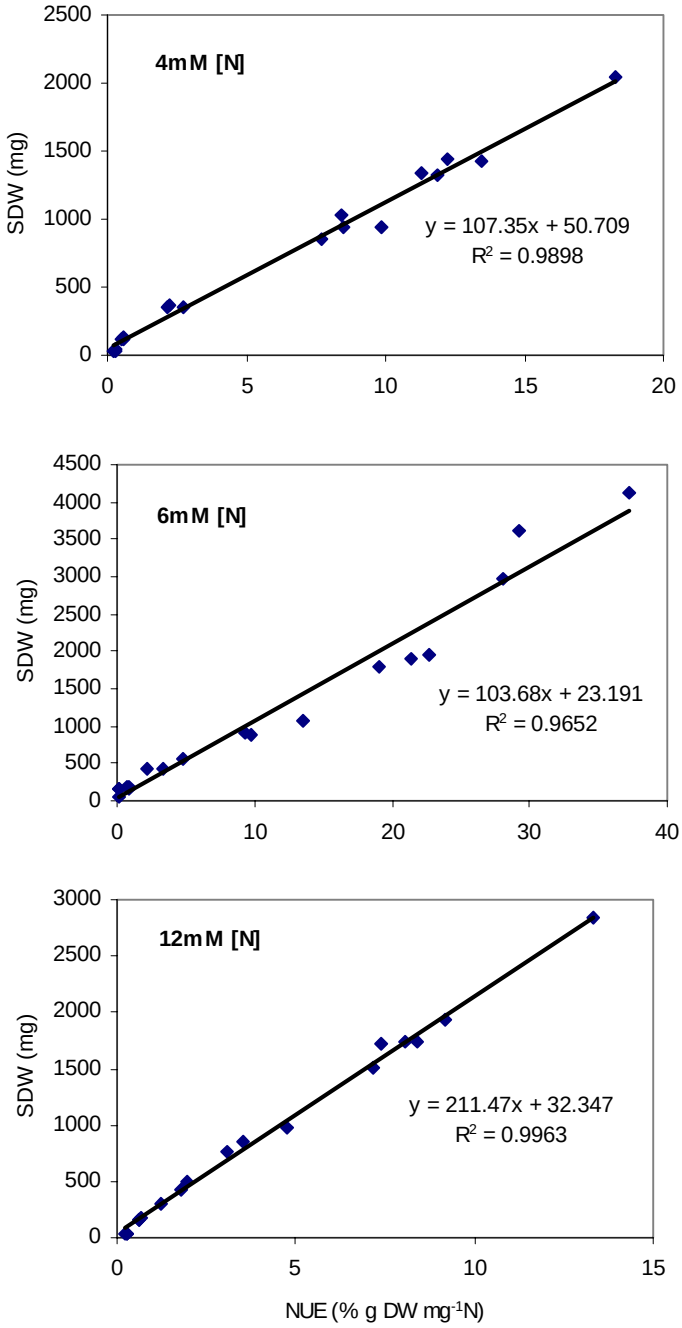


Figure 4.9 Relationship between nitrogen use efficiency (NUE) and shoot dry weight (SDW) at 700 µmol mol^{-1}CO_2.

Table 4.1. Shows a comparison of the effect of changing [N] from 4 to 6mM (4 – 6), and 6 to 12mM (6 – 12) growth regimes on total plant height.

$[CO_2]$ (μmol $mol^{-1}CO_2$)	ΔN (mM)	DAG	Stats
360	4 – 6 4 – 6	7 – 21 28 – 42	P< 0.785 P< 0.014
360	6 – 12 6 – 12	7 – 21 28 – 42	P< 0.306 P< 0.803
700	4 – 6 4 – 6	7 – 21 28 – 42	P< 0.365 P< 0.832
700	6 – 12 6 - 12	7 – 21 28 - 42	P< 0.237 P< 0.969

Table 4.2. Shows a comparison of the effect of changing [N] from 4 to 6mM (4 – 6), and 6 to 12mM (6 – 12) growth regimes on leaf area (LA).

$[CO_2]$ (μmol $mol^{-1}CO_2$)	ΔN (mM)	DAG	Stats
360	4 – 6 4 – 6	7 – 21 28 – 42	P< 0.353 P< 0.000
360	6 – 12 6 - 12	7 – 21 28 – 42	P< 0.487 P< 0.511
700	4 – 6 4 – 6	7 – 21 28 – 42	P< 0.271 P< 0.295
700	6 – 12 6 - 12	7 – 21 28 - 42	P< 0.854 P< 0.000

Table 4.3. Shows a comparison of the effect of changing [N] from 4 to 6mM (4 – 6), and 6 to 12mM (6 – 12) growth regimes on total plant biomass (TPB).

$[CO_2]$ (μmol $mol^{-1}CO_2$)	ΔN (mM)	DAG	Stats
360	4 – 6 4 – 6	7 – 21 28 – 42	P< 0.123 P< 0.021
360	6 – 12 6 – 12	7 – 21 28 – 42	P< 0.071 P< 0.003
700	4- 6 4 – 6	7 – 21 28 – 42	P< 0.075 P< 0.023
700	6 – 12 6 - 12	7 – 21 28 - 42	P< 0.155 P< 0.005

Table 4.4. Shows a comparison of the effect of changing [N] from 4 to 6mM (4 – 6), and 6 to 12mM (6 – 12) growth regimes on Shoot Dry Weight (SDW).

$[CO_2]$ (μmol $mol^{-1}CO_2$)	ΔN (mM)	DAG	Stats
360	4 – 6 4 – 6	7 – 21 28 – 42	P< 0.324 P< 0.179
360	6 – 12 6 – 12	7 – 21 28 – 42	P< 0.107 P< 0.004
700	4 – 6 4 – 6	7 – 21 28 – 42	P< 0.271 P< 0.050
700	6 – 12 6 - 12	7 – 21 28 - 42	P< 0.619 P< 0.124

Table 4.5. Shows a comparison of the effect of changing [N] from 4 to 6mM (4 – 6), and 6 to 12mM (6 – 12) growth regimes on root dry weight (RDW).

$[CO_2]$ (μmol $mol^{-1}CO_2$)	ΔN (mM)	DAG	Stats
360	4 – 6 4 – 6	7 – 21 28 – 42	P< 0.014 P< 0.002
360	6 – 12 6 – 12	7 – 21 28 – 42	P< 0.022 P< 0.006
700	4 – 6 4 – 6	7 – 21 28 – 42	P< 0.056 P< 0.031
700	6 – 12 6 - 12	7 – 21 28 - 42	P< 0.091 P< 0.000

Table 4.6. Shows a comparison of the effect of changing [N] from 4 to 6mM (4 – 6), and 6 to 12mM (6 – 12) growth regimes on root:shoot (R:S).

$[CO_2]$ (μmol $mol^{-1}CO_2$)	ΔN (mM)	DAG	Stats
360	4 – 6 4 – 6	7 – 21 28 – 42	P< 0.009 P< 0.003
360	6 – 12 6 – 12	7 – 21 28 – 42	P< 0.006 P< 0.807
700	4 – 6 4 – 6	7 – 21 28 – 42	P< 0.016 P< 0.364
700	6 – 12 6 - 12	7 – 21 28 - 42	P< 0.000 P< 0.000

Table 4.7. Shows a comparison of the effect of changing [N] from 4 to 6mM (4 – 6), and 6 to 12mM (6 – 12) growth regimes on nitrogen use efficiency (NUE).

$[CO_2]$ (μmol $mol^{-1}CO_2$)	ΔN (mM)	DAG	Stats
360	4 – 6 4 – 6	7 – 21 28 – 42	P< 0.171 P< 0.001
360	6 – 12 6 – 12	7 – 21 28 – 42	P< 0.085 P< 0.048
700	4 – 6 4 – 6	7 – 21 28 – 42	P< 0.521 P< 0.009
700	6 – 12 6 - 12	7 – 21 28 - 42	P< 0.297 P< 0.000

Chapter 5: Structural responses of wheat to high CO_2 and change in N supply.

5.1 Introduction

The preceding chapters have shown that whilst there is a considerable amount of information on the response of plants to both N supply and elevated [CO_2] on photosynthetic rates and about plant morphological responses, few researchers have explored the structural responses to elevated CO_2, therefore little information is available.

A study of structural changes induced by elevated [CO_2] is very important, as leaves are the first organs to interact directly with CO_2. Increase in atmospheric [CO_2] levels have been reported to increase photosynthetic rates of C_3 plants (Sage, 1994). As a result of this, plant organs such as the leaf increase in size dramatically (Mjwara *et al*., 1996; and references cited therein). This can lead to changes in leaf anatomy, which manifests itself through an increase in mesophyll area, overall leaf thickness, increased epidermal thickness and a reduction in intercellular air spaces.

Leaf growth and formation of the photosynthetic apparatus is very dependent on N supply (Kutik *et al*., 1995). A deficiency in N leads to restricted growth and yield and must eventually reduce photosynthetic competence associated with loss of components. On the other hand, assimilates may accumulate because reduced growth and respiration, which means the plant may not use

all the assimilated carbon (Kutik *et al*., 1995). Thus substantial change in basic biochemistry must occur under elevated [CO_2].

This chapter focuses on a comparison of the ultrastructure of the mesophyll chloroplast in response to [CO_2] and change in N supply.

5.2 Previous studies on anatomical responses to elevated CO_2

Previous studies on structural responses to elevated CO_2 have provided variable results for different plant species. High CO_2 appears to increase the leaf area of most plant species (Sionit *et al*., 1981; Mjwara *et al*., 1996; Enoch & Zieslin, 1988). The increase in leaf area relates to an increase in epidermal cell number and, as a result affecting stomatal density (Penuelas & Matamala, 1990; Mjwara *et al*., 1996). Stomatal density may impact on photosynthesis directly by either increasing or decreasing it. Furthermore the mesophyll cell area was observed to increase due to increase in leaf thickness (Thomas & Harvey, 1983; Vu *et al*., 1989).

Chloroplast ultrastructure may respond differently to [CO_2] for various plant species (Kutik *et al*., 1995). Increases in chloroplast numbers in mesophyll cells were reported by Thomas and Harvey (1983) for four plants species, whilst Robertson and Leech (1995) reported no effect on chlorophyll numbers in wheat mesophyll cells, in their study.

Ultrastructurally, chloroplasts showed an increase in number of starch grains and size inside the chloroplast due to high [CO_2] (Cave *et al*., 1981; Wulf &

Strain, 1981; Thomas & Harvey, 1983; Ehret & Jolliffe, 1985; Vu *et al*., 1989). Kutik *et al*., (1995) reported no effect on cross sectional area of chloroplasts they studied (*Beta vulgaris*). In contrast, Robertson and Leech (1995) reported no reduction in starch accumulation as a result of CO_2 enrichment.

5.3 Results

5.3.1 Leaf thickness

Figs 5.1-5.3 show cross-sections of mature leaves of wheat respectively, grown under 4,6 and 12mM N regimes and ambient and elevated [CO_2]. The most visible result from Figs 5.1-5.2 is the enormous reduction of intercellular spaces under elevated [CO_2]. This may be attributed to an increase in the number of mesophyll cells resulting in the cells so closely packed within the tissue. In contrast, Fig. 5.3 shows reduced number of intercellular air spaces under ambient [CO_2] and more under elevated [CO_2]. In addition, under 12mM N regime and elevated [CO_2] leaf thickness was larger.

Table 5.1 depicts the results of the change in leaf thickness in response to [CO_2] and N supply at 25 DAG. Under 4 and 6mM [N] there were no significant difference in abaxial and adaxial epidermis, total leaf thickness and mesophyll thickness between the CO_2 environments (Table 5.1). There was a difference in midrib thickness as a result of CO_2enrichment ($p<0.001$). In contrast, under 12mM [N], there was a significant change in abaxial epidermal thickness (cell), total leaf thickness, mesophyll and midrib thickness between the two CO_2 environments (Table 5.1).

A combination of ambient [CO_2] and 6mM N regime decreased leaf thickness by 0.49% on average from 128.1 ± 12.4 μm to 127.5 ± 13.42 μm. A further increase in N supply (12mM) resulted in a decrease of about 20% on average from 127.5 ± 13.2 μm to 101.1 ± 8.49 μm. A similar trend was observed in midrib thickness where the decrease in the latter parameter was 317 ± 4.38, 290 ± 8.00 and 195 ± 12.6 μm respectively under 4, 6 and 12mM [N] (Table 5.1). Mesophyll thickness increased by about 12% (80.6 ± 6.12 to 90.0 ± 12.0 μm) on average under the 6mM N regime and this was followed by a decline of about 20% (90 ± 12 to 71.75 ± 11.13 μm) on average under the 12mM N regime. Abaxial epidermal thickness declined with the increase in N supply by about 7 (20 to 18.8 ± 5.8 μm) and 25% (18.8 ± 5.8 to 15 ± 2 μm) on average respectively for 6 and 12 mM N regimes.

Under ambient [CO_2] no significant changes in abaxial and adaxial epidermis, total leaf thickness and mesophyll thickness were observed as a result of a change in nitrogen supplies from 4 to 6mM [N] (see Table 5.3). However there was a significant change in adaxial epidermis, total leaf thickness, mesophyll and midrib thickness as a result of a change in nitrogen from 6 to 12mM [N].

In contrast high [CO_2] and increase in N supply, resulted in increase in leaf thickness of about 7 and 13% on average respectively for 6 and 12mM N regimes (Table 5.1). This increase was lower than that reported by Siphugu (1997) for barley, which was 21% on average at the same age. Thomas and Harvey (1983) also reported an increase in leaf thickness of three C_3 species

(see reference for species names) due to high [CO_2]. Similarly, mesophyll and midrib thickness were increased by about 27 and over 100% on average respectively under high [CO_2]. Elevated [CO_2] and increase in N supply resulted in an increase in abaxial epidermal thickness by 10 and 13% on average for 6 and 12mM N regimes respectively (Table 5.1). This was far below the results reported by Siphugu (1997). Mesophyll chloroplasts appeared denser for high [CO_2] grown plants under all the nitrogen treatments and this is in agreement with the results of Thomas and Harvey (1983).

Under elevated [CO_2] no significant changes in all the leaf thickness components between the nitrogen treatments, except for the midrib thickness under a 12mM [N] ($p<0.001$; Table 5.1).

5.3.2 Chloroplast morphology

A change in chloroplast morphology in response to [CO_2] and N supply was apparent in wheat leaves. Under all the nitrogen treatments, the changes in chloroplast length, width and surface area between the CO_2 regimes were not significant at the 5% level of significant ($p>0.05$; Table 5.4).

Ambient CO_2 and the 6mM N regime resulted in a 15% (from 7.87 ± 0.76 to 6.69 ± 0.87 μm) reduction on average in chloroplast length and this was later followed by a noticeable increase of about 19% (from 6.69 ± 0.87 to 7.97 ± 1.05 μm) under the 12mM N regime. Siphugu (1997) reported a large increase of about 29 % on average in chloroplast length for one barley

cultivar (c.v. Stirling) under adequate nutrient supply. The increase in chloroplast width was 22 (from 2.45 ± 0.71 to 2.99 ± 0.57 μm) and 0.67% (from 2.99 ± 0.57 to 3.01 ± 0.91 μm) on average for 6 and 12 mM N regime respectively. Siphugu (1997) also reported an increase of about 24 and 29% on average in chloroplast width for two barley cultivars in his study. The chloroplast surface area increased with the increase in N supply by 4 (from 19.5 ± 6.8 to 20.9 ± 5.9 μm^2) and 16% (20.9 ± 5.9 to 23.5 ± 6.0 μm^2) on average for 6 and 12 mM N regimes respectively.

Under ambient [CO_2] the changes in chloroplast length, width and surface area were not statistically significant as a result of a change in nitrogen supplies (Table 5.4).

Similarly CO_2 enrichment resulted in a decline in chloroplast length by about 28% (9.31 ± 0.14 to 6.72 ± 1.33 μm) on average from 4 to 6mM [N] and, this was later followed by an increase of about 14% (6.72 ± 1.33 to 7.63 ± 1.99 μm) on average under the 12mM N regime. High CO_2 and increase in N supply increased chloroplast width by about 17 (3.07 ± 0.64 to 3.58 ± 1.14 μm) and 18% (3.6 ± 1.14 to 4.23 ± 0.63 μm) on average respectively, for 6 and 12mM N regimes. In contrast, the ambient [CO_2] chloroplast surface area declined by about 14% (28.5 ± 6.0 to 24.5 ± 11.3 μm^2) on average and increased by about 35% (24.5 ± 11.3 to 33 ± 11.9 μm^2) on average respectively for the 6 and 12mM N regime.

Under elevated [CO_2] the changes in chl length were statistically significant as a result of a change in nitrogen supplies from 4 to 6mM [N] (Table 5.4). A

further increase in nitrogen supplies to 12mM [N] did not result in any significant changes in chl length, width and surface area (Table 5.4).

5.3.3 Chloroplast ultrastructure

Fig. 5.4 - 5.6 are electron micrographs of mesophyll cell chloroplast of mature first fully developed leaves of plants grown under normal and high CO_2 at three different N regimes (4,6 and 12mM). Ambient CO_2 resulted in a decrease in length of starch grains by about 27 (0.81 ± 0.56 to 0.59 ± 0.21 μm) and 7% (0.59 ± 0.21 to 0.55 ± 0.15 μm) on average for 6 and 12mM N regimes. A similar pattern was observed for the starch grain surface area, which resulted in a reduction of about 70% on average throughout (Table 5.2). On the contrary starch grain width declined by about 48% (from 0.4 ± 0.29 to 0.21 ± 0.15 μm) on average under the 6mM N regime and this was later followed by a noticeable increase of about 19% (from 0.21 ± 0.15 to 0.25 ± 0.09 μm) on average. Fig 5.6 shows smaller but many starch grains scattered in the chloroplast under ambient [CO_2] whereas Fig.5.4 shows larger but few starch grains for the 4mM N grown plants. Interference of starch grains with the thylakoids was not apparent.

Elevated CO_2 decreased starch grain lengths and widths by about 60 and 27% and 36 and 25% respectively for 6 and 12mM N regime plants (Table 5.2). This effected a significant ($p<0.01$) decrease in the grain surface area as a result of an increase in N supply from 4 to 6mM [N] (see Table 5.4). This decrease was by 74 (from 0.66 ± 0.21 to 0.17 ± 0.11μm^2) and 47% (0.17 ± 0.11 to 0.09 ± 0.03 μm^2) on average respectively for 6 and 12mM N regimes.

These results are in contrast with those of Robertson and Leech (1995) for wheat c.v. Hereward grown under high CO_2. The accumulation of starch as a consequence of high CO_2 have been previously reported by many workers (Cave *et al.,* 1981; Wulf and Strain, 1982; Ehret and Jolliff, 1985; Kutik *et al*., 1995). Interference of starch grains with the thylakoids or any other additional alterations in ultrastructure were not apparent. This is in harmony with the results obtained by Vu *et al*., (1989) who reported no effects of high CO_2 on chloroplast ultrastructure.

5.4 Discussion

The results presented here clearly demonstrate that wheat leaves respond both anatomically and morphologically to [CO_2] and N supply (Table 5.3). Leaf thickness increments for high [CO_2] obtained in this study was dissimilar to those reported for phytotron chamber grown species (Thomas and Harvey, 1983 and references cited therein). High [CO_2] did not significantly increase leaf thickness and its components (Table 5.1) between the two CO_2 environments for 4 and 6 mM N regimes. However, 12mM [N] resulted in significant changes in leaf thickness and its components between the two CO_2 environments (Table 5.1).

At 25 DAG, the age at which leaves were harvested for anatomical and ultrastructural analysis, corresponded with a low foliar [N] under elevated [CO_2] as opposed to ambient [CO_2] at the same age under all nitrogen regimes (see Chapter 3; Fig.3.12). The fact that midrib thickness was higher under ambient than elevated [CO_2] leaves under 4mM [N], suggests suppressed growth as a result of nitrogen stress and nitrogen reallocation as

a result of a change in sink under 6mM [N]. This contention is strengthened when the 12mM [N] and elevated [CO_2] (25 DAG) data are considered. Here, plants had relatively higher foliar [N] consequently and this correlated with larger midrib thickness than in the other two nitrogen regimes. This resulted in significant differences ($p<0.001$) in midrib thickness between the two CO_2 regimes under all nitrogen treatments (Table 5.1).

Although mesophyll thickness was measured to increase under 4mM [N] and elevated [CO_2] compared to a decline under 6mM [N] and elevated [CO_2], these changes (between the CO_2 regimes) were not significant ($p>0.05$). However, under 12mM [N] and elevated [CO_2] mesophyll thickness was larger ($p<0.01$), strengthening the argument that N was inadequate under 4 and 6mM [N]. This result adversely affected total leaf thickness negatively under 4 and 6mM [N] but the effect was positive as a result of 12mM [N] and elevated [CO_2]. The primary source of this result is acclimation of NAR as this reduces CO_2 assimilation, which translates into reduced growth.

Phosphorus supply at optimal and supra-optimal levels, has been reported to enhance leaf area, possibly as a result of more carbon passing through the PCR cycle (Fredeen *et al*., 1989 and references therein). As a result larger leaf areas provide additional sinks for carbon skeletons. Similarly, in the present study leaf growth increased with an increase in nitrogen supply under elevated [CO_2]. As a result additional supportive tissue was formed in the midrib region to support the longer leaf blades, possibly for the correct orientation for light reception.

Leaf epidermal cells have been reported to affect stomatal density, by reducing or increasing the number of stomata on either side of the leaf (Penuelas & Matamala, 1990). They suggested that elevated [CO_2] enlarges epidermal cells, which later translates into increased leaf areas, with a corresponding decline in stomatal density. Thus the apparent changes in stomatal density may disappear if epidermal cell expansions are considered (Penuelas and Matamala, 1990). In this study, only the adaxial epidermal cell enlargement affected the number of stomata on the adaxial side of the leaf under high [CO_2] (Table 5.1). Based on the results obtained in this survey I therefore suggest that an increase in epidermal cell size affects stomatal density. The observed further decline in both stomatal density and and leaf epidermal thickness under ambient CO_2 is probably because of the already higher leaf areas under the 12mM N regime.

The positioning and shape of the mesophyll chloroplast within the mesophyll cells of wheat leaves were perturbed by elevated CO_2 growth conditions. This is in contrast to Robertson and Leech (1995) who reported that the number, positioning and shape of the chloroplast within the mesophyll cells of young chloroplast were not disturbed by high CO_2. The shape of chloroplasts affected the chloroplast surface area under all-nutrient levels and high CO_2.

Starch accumulation in plants grown under high [CO_2] have been singled out as the main cause of chloroplast disruption, including the deformation of the membranous systems of the thylakoids (Cave *et al*., 1981). This invariably

leads to adverse effects on photosynthetic machinery which in turn results in reduced NAR (Ehret and Jolliffe, 1985; Cure *et al*., 1991). In this study starch grain surface area was observed to decline (Table 5.2). This trend became more apparent as N supply was increased under both CO_2 environments. As a result no disruptions of the thylakoid membranes by the larger starch grains was apparent. This was despite the reduction in NAR in these plants (Chapter 3 Fig.3.1). As there is still debate on this issue, as a result of the absence of correlation between starch accumulation and NAR in leaves (Gucci *et al*., 1991; Vu *et al*., 1989), this suggests the involvement of other factors in down regulation of NAR (Sage, 1994).

In addition the reduction in starch grains with an increase in nitrogen supply is attributed to the source and sink capacities, as these play an important role in determining the extent of starch storage during leaf development (Robertson and Leech, 1995). For an example, under elevated [CO_2] rapidly growing plants are “source limited” (Robertson and Leech, 1995 and references therein), therefore additional carbohydrate resulting from enhanced CO_2 assimilation is immediately utilised for growth. These results in larger leaf areas that alter the ratio of non-structural carbohydrates (NSC) to structural carbohydrates (SC) hence the decline in starch grains in the chloroplasts. This contention is strengthened by the fact that leaves (for analysis of anatomy and ultrastructure) were harvested at 25 DAG which coincided with the flower formation stage. This may be another reason that exacerbated a decline in starch grain size.

5.5 Conclusion

The results presented in this study indicate that high [CO_2] and nitrogen supply affected leaf morphology and structure. The higher mesophyll density can be implicated in the increased photosynthesis under all nitrogen treatments for high CO_2 plants (see micrographs). Relatively higher NAR resulted in enhanced growth (especially leaves) resulting in more carbon sinks corresponding to the disappearance or reduction of starch grains (as a result of increased mesophyll cells with more chloroplasts). A further study of the chloroplast ultrastructure and thylakoid contents under high [CO_2] and changing nutrient supply can further elucidate sources of acclimation and down regulation of NAR. Thus far, in this study the changes were more physiological than ultrastructurally.

Larger midrib thickness appears to have affected leaf epidermal thickness negatively. In order to fully understand the changes in leaf anatomy in this study, a revision of leaf development over the growth period under high [CO_2] and changing nutrient supplies is imperative.

In addition the use of carbon isotopes can assist in identifying the changes in the source and sink capacities as well as identifying increased sink capacity as a result of manipulation in nutrient supplies. This is important as there is little information available on photoassimilate partitioning in wheat (Cruz-Aguado, 1999) especially for elevated [CO_2] grown plants, over the growth period till seed maturity. The results reported here support other workers and add to the little information as stated elsewhere in this chapter. This will not

only provide answers to the changes in sink and source capacities but also elucidate the impact of the latter factors on NAR. Clearly, there is still room for debate about the effects of elevated [CO_2] and nutrient on plant development over the growth period.

Table 5.1 Shows leaf thickness in response to [CO_2] and N supply. Values are the means of three replicates, ns indicates not significant and *,** and *** indicate significance at $p<0.05$, $p<0.01$ and $p<0.001$ respectively between ambient and elevated CO_2 for each N regime.

[CO_2] μmol mol^{-1} CO_2	[N] (mM)	Adaxial epidermis (μm)	Abaxial epidermis (μm)	Total leaf thickness (μm)	Mesophyll thickness (μm)	Mid rib thickness (μm)
360	4	21 ± 3	20 ± 0	128 ± 12	81 ± 6	318 ± 9
700	4	19 ± 4^{ns}	17 ± 3^{ns}	114 ± 17^{ns}	85 ± 7^{ns}	$220 \pm 25^{***}$
360	6	24 ± 6	19 ± 6	128 ± 13	90 ± 12	290 ± 8
700	6	21 ± 4^{ns}	19 ± 6^{ns}	123 ± 7^{ns}	82 ± 9^{ns}	$190 \pm 29^{***}$
360	12	14 ± 1	15 ± 2	101 ± 8	72 ± 11	195 ± 13
700	12	16 ± 2^{ns}	$21 \pm 4^{*}$	$138 \pm 17^{**}$	$104 \pm 23^{**}$	$448 \pm 9^{***}$

Table 5.2 Shows chloroplast ultrastructural and starch grain sizes in response to [CO_2] and N supply. Values are the means of three replicates, ns indicates not significant and *,** and *** indicate significance at p<0.05, p<0.01 and p<0.001 respectively between ambient and elevated CO_2 for each N regime.

[CO_2] (μmol mol^{-1} CO_2)	[N] (mM)	Chl length (μm)	Chl width (μm)	Chl surface area (μm^2)	Starch grain length (μm)	Starch grain width (μm)	Starch grain surface area (μm^2)
360	4	8.9 ± 0.8	2.5 ± 0.7	19.5 ± 6.8	0.8 ± 0.6	0.4 ± 0.3	0.45 ± 0.5
700	4	9.3 ± 0.1**	3.1 ± 0.6^{ns}	29 ± 6^{ns}	1.5 ± 0.5^{ns}	0.4 ± 0.1^{ns}	0.7 ± 0.2^{ns}
360	6	6.7 ± 0.9	2.9 ± 0.6	20 ± 5.9	0.6 ± 0.2	0.21 ± 0.2	0.14 ± 0.1
700	6	6.7 ± 1.3^{ns}	3.6 ± 1.1^{ns}	25 ± 11^{ns}	0.6 ± 0.3^{ns}	0.28 ± 0.1^{ns}	0.17 ± 0.1^{ns}
360	12	7.9 ± 1.1	3.01 ± 0.91	24 ± 6	0.6 ± 0.2	0.25 ± 0.1	0.14 ± 0.1
700	12	7.6 ± 2^{ns}	4.2 ± 0.6^{ns}	33 ± 12^{ns}	0.43 ± 0.1^{ns}	0.21 ± 0.04^{ns}	0.09 ± 0.03^{ns}

Table 5.3: Shows comparison of the effects of changing [N] from 4 to 6mM (4 – 6), and 6 to 12mM (6 – 12) growth regimes on leaf thickness.

[CO_2] µmol mol^{-1} CO_2	[N] (mM)	Adaxial epidermal thickness (µm)	Abaxial epidermal thickness (µm)	Total leaf thickness (µm)	Mesophyll thickness (µm)	Mid rib thickness (µm)
360	4 – 6	P < 0.488	P < 0.689	P < 0.949	P < 0.223	P < 0.005
360	6 - 12	P < 0.022	P < 0.278	P < 0.017	P < 0.0716	P < 0.000
700	4 - 6	P < 0.654	P < 0.387	P < 0.422	P < 0.598	P < 0.176
700	6 - 12	P < 0.656	P < 0.356	P < 0.155	P < 0.138	P < 0.000

Table 5.4: Shows comparison of the effects of changing [N] from 4 to 6mM (4 – 6), and 6 to 12mM (6 – 12) growth regimes on chloroplast ultrastructure.

[CO_2] (µmol mol^{-1} CO_2)	[N] (mM)	Chl length (µm)	Chl width (µm)	Chl surface area (μm^2)	Starch grain length (µm)	Starch grain width (µm)	Starch grain surface area (μm^2)
360	4 – 6	P < 0.944	P < 0.294	P < 0.868	P < 0.506	P < 0.284	P < 0.247
360	6 - 12	P < 0.119	P < 0.967	P < 0.477	P < 0.753	P < 0.631	P < 0.988
700	4 - 6	P < 0.010	P < 0.485	P < 0.561	P < 0.017	P < 0.023	P < 0.006
700	6 - 12	P < 0.484	P < 0.387	P < 0.347	P < 0.336	P < 0.159	P < 0.210

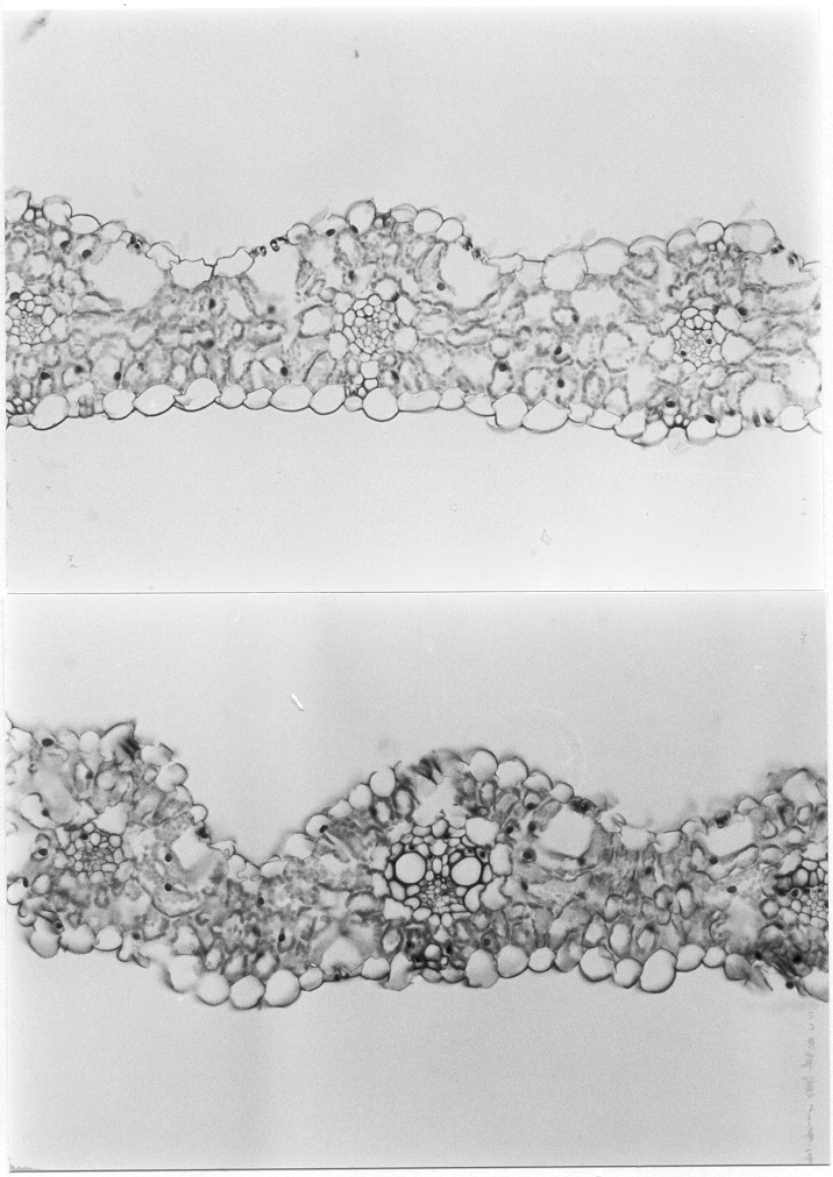


Fig.5.1 Cross-section of mature first fully developed leaves of wheat cv Gamtoos grown under ambient (A) or elevated (B) [CO_2] and a 4mM N regime. Plants were 25 DAG.

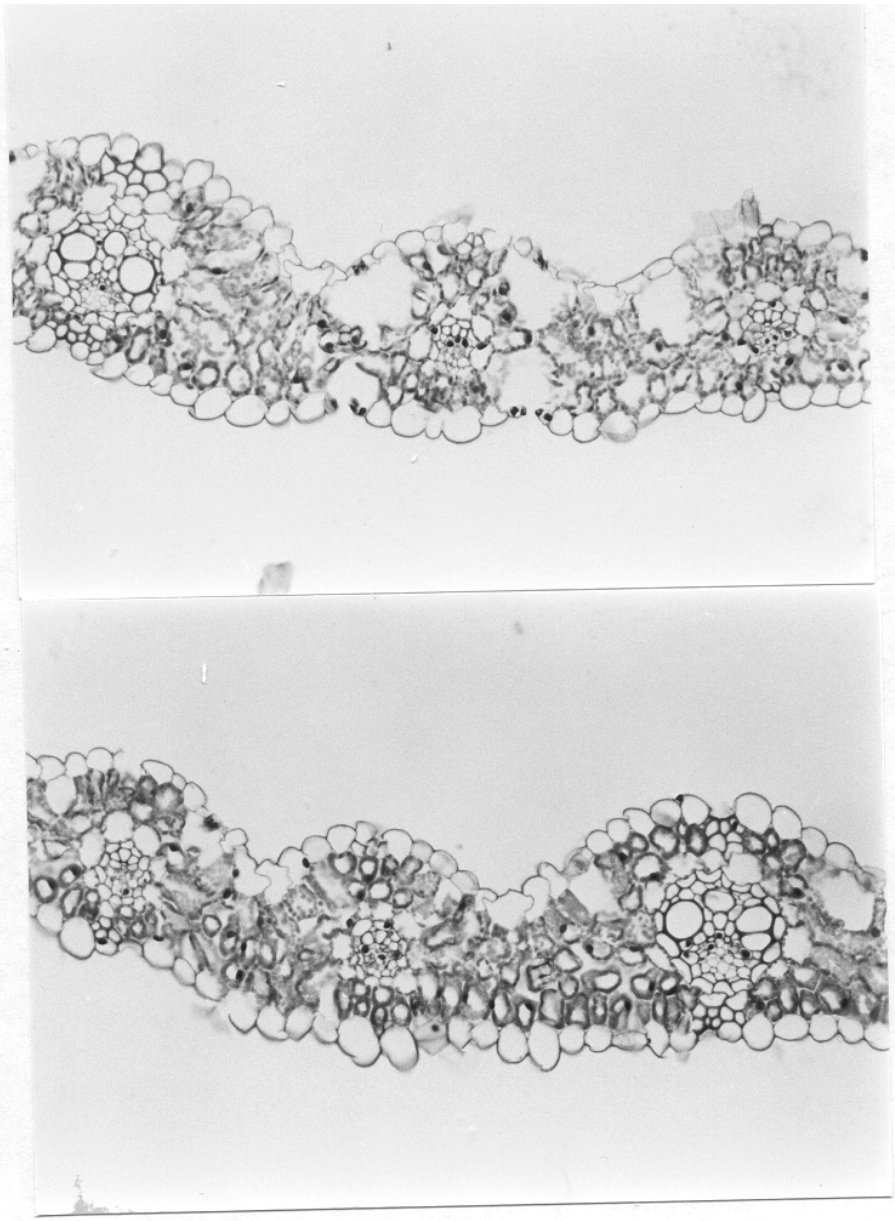


Fig.5.2 Cross-section of mature first fully developed leaves of wheat cv Gamtoos grown under ambient (A) or elevated (B) [CO_2] and a 6mM N regime. Plants were 25 DAG.

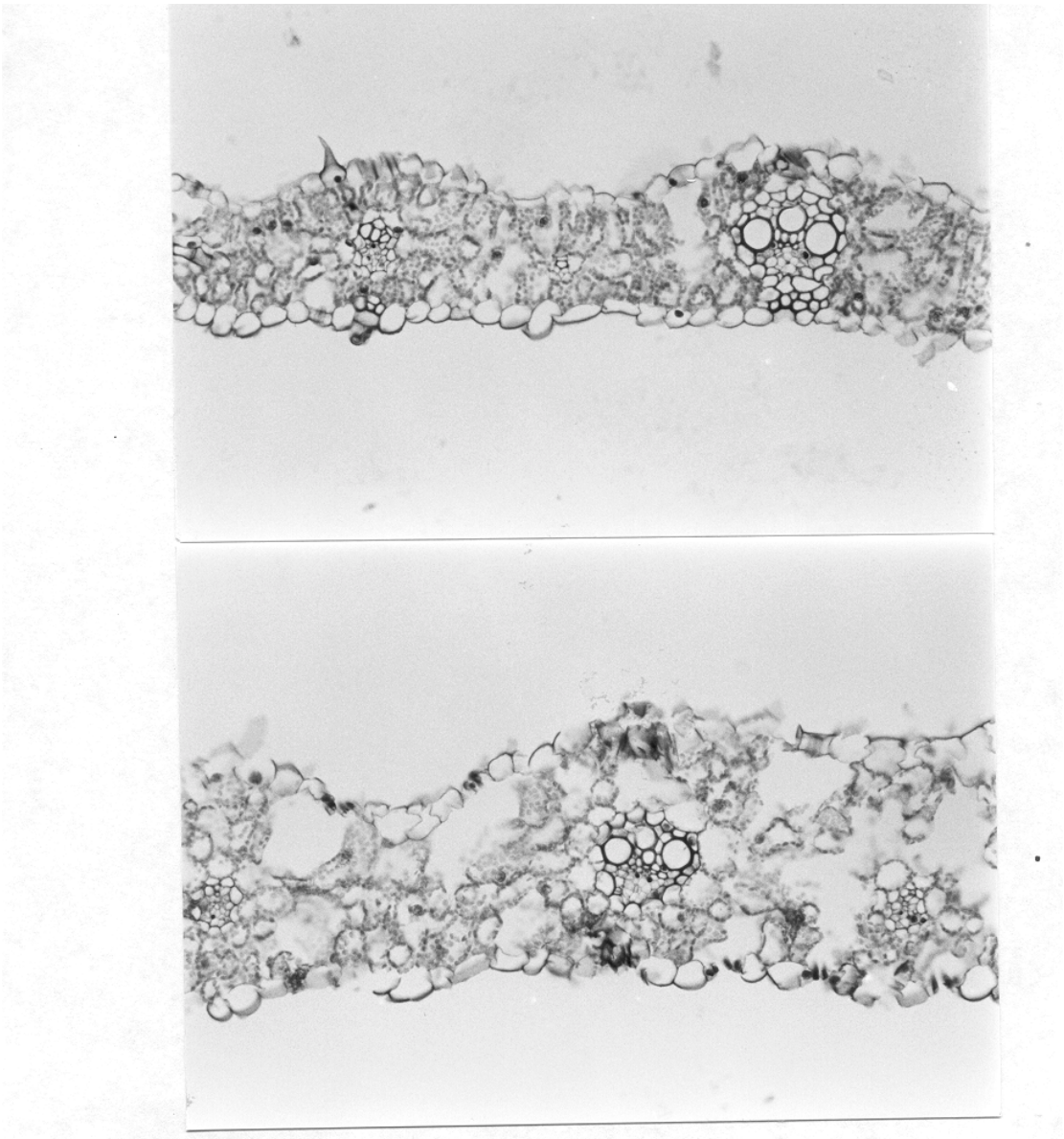


Fig.5.3 Cross-section of mature first fully developed leaves of wheat cv Gamtoos grown under ambient (A) or elevated (B) [CO_2] and a 12mM N regime. Plants were 25 DAG.

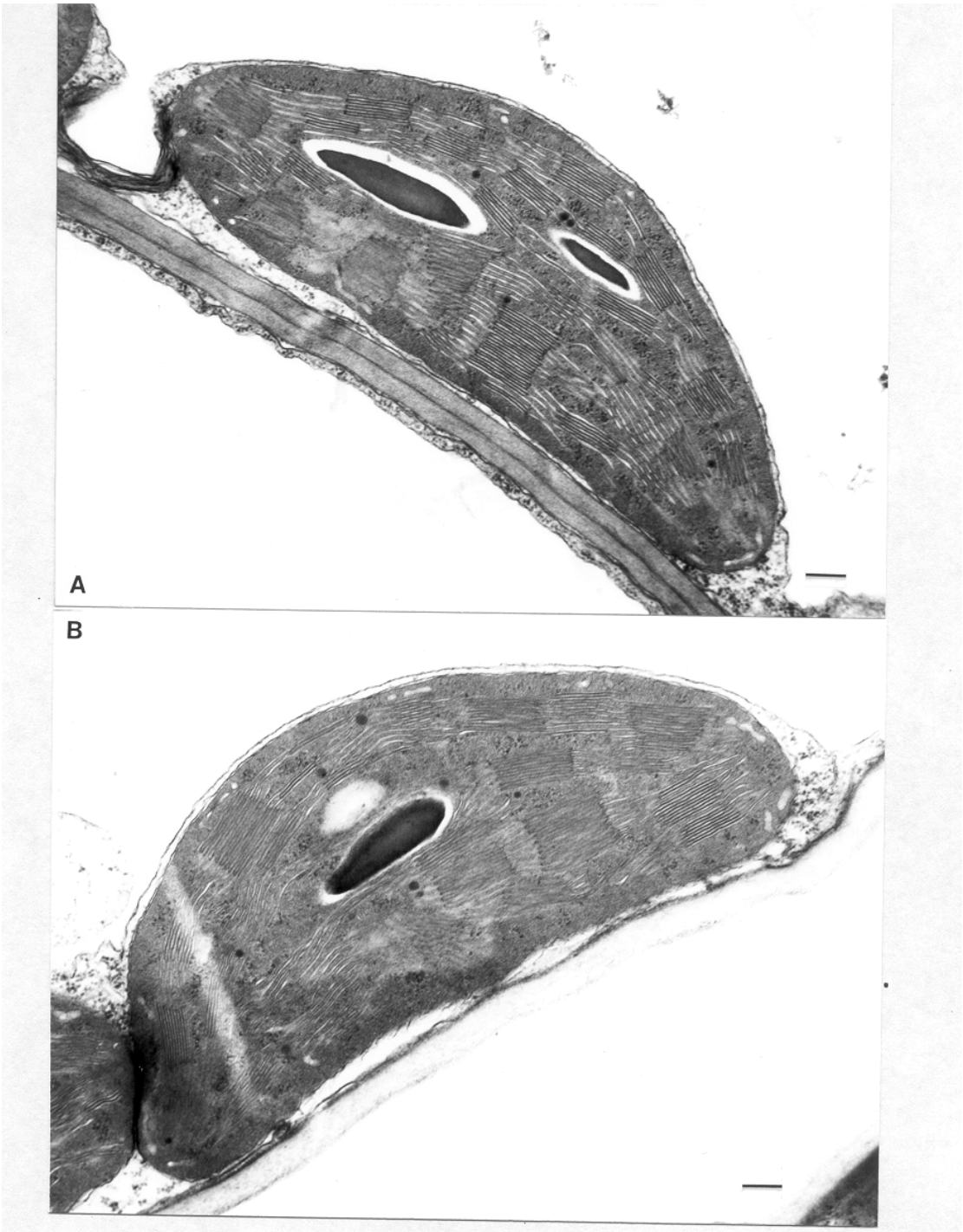


Fig.5.4 Electron micrographs of mesophyll cell chloroplasts of wheat cv Gamtoos for plants grown under ambient (A) or elevated (B) [CO_2] and a 4mM N regime. Plants were 25 DAG.

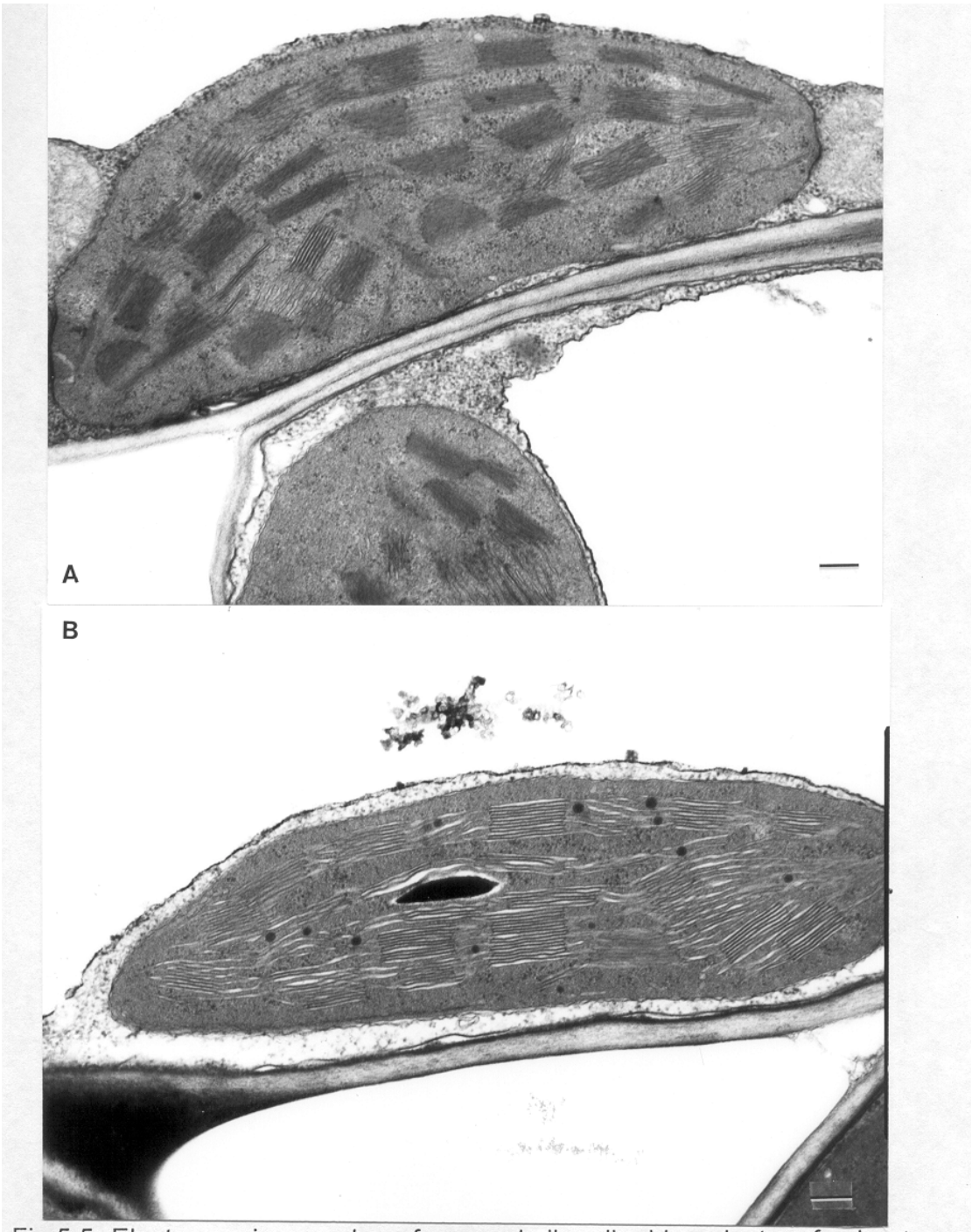


Fig.5.5 Electron micrographs of mesophyll cell chloroplasts of wheat cv Gamtoos for plants grown under ambient (A) or elevated (B) [CO_2] and a 6mM N regime. Plants were 25 DAG.

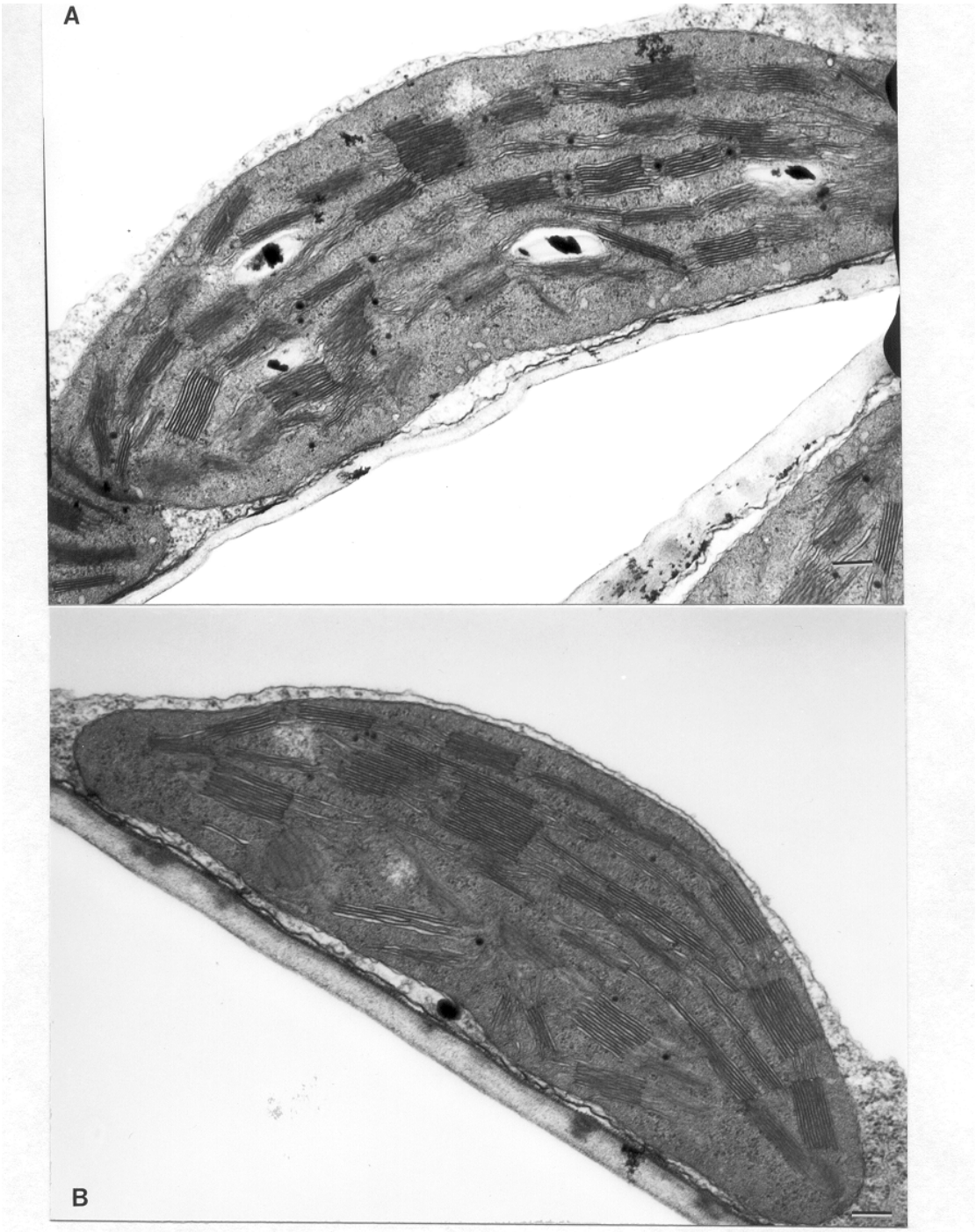


Fig.5.6 Electron micrographs of mesophyll cell chloroplasts of wheat cv Gamtoos for plants grown under ambient (A) or elevated (B) [CO_2] and a 12mM N regime. Plants were 25 DAG.

Chapter 6: General discussion

6.1 General discussion

Evidence has increased over the years to convince even the hardest sceptics that global atmospheric [CO_2] is increasing annually (Conroy, 1992). Locally South Africa is responsible for about 1.6% of greenhouse emissions, which makes it the largest source of emissions in Africa and the 18th largest in the world (Mail & Guardian, 1997). This scenario is expected to result in a change in the climatic conditions of this region. The resultant effect will be a change in nutrient availability, demand and use efficiency by plants.

Equally importantly, there will be a change in nutrient availability in the growth medium. On the other hand high [CO_2] has been implicated in the decrease of foliar concentrations of certain important nutrient elements due to high use efficiency of the nutrient elements (Conroy and Hocking, 1993). A combination of this and other factors such as sink and source capacities have been suggested as the cause of acclimation or down regulation (Arp, 1990; Sage, 1994). In this chapter, I have attempted to summarise the effects of sustained elevated [CO_2] on wheat at different N regimes as well as integrating the factors that affect photosynthesis and growth as described in the previous chapters.

6.2 The role of controlled environment (CE) chambers

Field conditions are far too dynamic to enable the researcher to accurately track the exact influence on the change in physiological and biochemical processes in plants. Equally importantly, modelling requires exact control of

the environmental conditions which plants are subjected to. For predictive purposes, outdoor studies with regulated environments under natural solar irradiance, and using individual plant canopies, and even whole plants communities for one or more growth seasons, are to be welcomed (Bowes, 1991). However, such studies do not always provide mechanistic answers to the physiological and biochemical acclimation, and consequently there continues to be a place for growth chamber and short term experiments (Bowes, 1991).

6.3 Photosynthesis and nitrogen nutrition

Photosynthesis is arguably, the most important reaction in the world as it serves as the primary basis of life for most organisms. Carbon dioxide serves as a substrate for photosynthesis and, as such, over half of the global uptake of carbon is via this process (Bowes, 1991). Autotrophic organisms process CO_2 via the photosynthetic carbon reduction cycle (PCR) specifically through the initial carboxylation enzyme of this cycle known as ribulose-biphosphate carboxylas-oxygenase (Rubisco) (Bowes, 1991).

Rubisco is a large, sluggish enzyme, requiring substantial amounts of a plant's resource, particularly nitrogen. It is the major leaf protein of the C_3 plants, consisting of 30 – 50% of the soluble protein (Bowes, 1991). In addition large amounts of nitrogen are tied up in the photooxidation cycle (PCO) and the respiratory rate has been correlated to tissue nitrogen content (Amthor, 1989). Consequently, a high nitrogen (protein) content of plant

tissue might mean a large amount of respiratory enzymes, giving rise to high rates of respiration (Amthor, 1989 and references therein).

The data in the present study clearly shows that photosynthesis is directly proportional to an increase in nitrogen supply and [CO_2]. Larger significant differences in NAR were observed when N was increased from 4 to 6mM [N] under elevated [CO_2]. This was facilitated by a larger significant difference in stomatal conductance under the same conditions. Stomatal density was increased as well under the same conditions resulting in higher transpiration rate as compared to 4 and 12mM [N]. A positive correlation between foliar [N] and transpiration rate was obtained under 6mM [N] and elevated [CO_2], suggesting the involvement of transpiration in nutrient uptake. Water use efficiency was not affected by an increase in nitrogen supplies in this study though it was higher under elevated [CO_2] as expected for a C_3 plant.

As mentioned earlier, nitrogen is the most important nutrient element for the synthesis of proteins, enzymes and amino acids in plants. As a result, the decline in foliar nitrogen under elevated [CO_2] grown plants was seen to affect NAR adversely during this study. This scenario was exacerbated under the 6mM [N]. The cause of this could be that nitrogen derived from Rubisco, the largest source of N among the photosynthetic components (Wojcieska, 1994) could be reallocated. The re-allocation could be into the electron transport chain or Pi regeneration enzymes, which have been observed to limit NAR during long term especially to high CO_2 (see Mjwara, 1996 and references cited therein).

Another cause of NAR down-regulation, may be correlated to the observed larger leaf areas, as a result affecting NAR per unit of leaf area (Mjwara, 1996). Analysis of foliar non structural carbohydrates (NSC) must provide the answers as to whether excessive NSC particularly starch resulted in the dilution effects as was suggested by Akey and Kimball (1989).

The *in vivo* changes in carboxylation efficiency (CE) were analysed from the A/C_i curves. Carboxylation efficiency was seen to decline with age, in high CO_2 grown plants. This clearly indicates that the activity of Rubisco is affected by age more especially as high CO_2 accelerates ageing of plants (see Mjwara, 1996 and references cited therein). Finally, NAR appears to have been affected by a combination of factors viz. foliar [N], decline in chlorophyll content, stomatal density and conductance.

Chloroplasts are the important sites for photosynthesis and amino acid synthesis (Wallsgrove *et al*., 1983). In the present study photosynthetic pigments were observed to decline concomitantly with NAR and in addition NAR was correlated to foliar [N], confirming the results of Sage (1987) who reported a significant positive correlation between total chlorophyll content and foliar [N]. A decline in the photosynthetic pigments and foliar [N] decreases the quantities of proteins and amino acids involved in photosynthesis resulting in acclimation of NAR.

6.4 Elevated CO_2 and N interaction on growth of plants

There is a large body of evidence dealing with the effects of elevated [CO_2] on plants growth. For an example see Kimball, (1983); Enoch and Zieslin, (1988); Mjwara *et al*., (1996); Stitt and Krapp, (1999) and references therein). There is consensus that elevated [CO_2] results in enhanced plant growth but this response is species-dependent.

In the present study, elevated [CO_2] and increase in nitrogen supply increased TPH, TPB, LA, SDW and RDW. The effects were more pronounced under the 6mM [N] regime for all growth parameters including foliar [N] and NUE. Increased growth occurred at the expense of foliar [N] as a result attributing enhanced growth to higher NUE (Hocking and Meyer, 1991). Nitrogen may have been reallocated away from Rubisco to other processes (growth) resulting in enhanced growth. However, leaf senescence is not ruled out as having reduced leaf area under 4 and 6mM [N] at 28 DAG onwards. The significant increase in RDW and R: S under 6mM [N] and elevated [CO_2] is attributed to additional roots as a result of more tillering under elevated [CO_2].

Grain yield increased with the increase in nitrogen supply under elevated [CO_2]. At 35 DAG onwards most of the foliar [N] is diverted to grain filling. In the present study, at 35 DAG foliar [N] was very low under the 4 and 6mM [N] compared to the 12mM [N] at the same age. This suggests reduced grain yield as a result of nitrogen stress under 4 and 6mM [N].

Mesophyll chloroplast ultrastructure of the leaves harvested at 25 DAG, did not show any damaged thylakoids as a result of larger starch grains. This shows that acclimation was not as result of damaged photosynthetic machinery. The reduction in starch grain size with an increase in nitrogen supply under elevated [CO_2] appears to be due to the shift in source and sink capacities.

6.5 Elevated CO_2 and mineral nutrition in plants

Foliar [N] and [P] appear to have received much attention in most crop species (Conroy and Hocking, 1993). On the other hand a wide spectrum of both macro- and micronutrients have received little attention, especially their foliar concentration partitioning in plants (Newton, 1991; Stulen and Den Hertog, 1993; Overdieck, 1993) and yet these nutrient elements play a crucial role in CO_2 fixation in plants. For an example down regulation of NAR may be as a result of deficiencies in manganese (Mn), zinc (Zn) and magnesium (Mg) as these play a crucial role as functional or structural regulatory cofactors including enzyme activation in photosynthesis (Hipkins, 1983; Davis, 1994).

Magnesium is a central atom in the chlorophyll molecule, and serves as a pH regulator within the cell and also plays a vital role in modulation of Rubisco as its binding with Rubisco increases its affinity for the substrate CO_2 and its turnover rate (Marchner, 1986; Dreyer *et al*., 1994; Hewitt, 1983). On the other hand, Zn and Mn are associated with electron transport and photophosphorylation at PSII (Raven, 1990; Geider and La Roche, 1994).

Iron (Fe) is involved in chlorophyll synthesis, since it is an essential element in coproporphyrinogen oxidase, an enzyme involved in catalysing chlorophyll synthesis (Mjwara *et al*., 1996 and references therein). In the present study the decline in the total chlorophyll content may have been as a result of the interaction and the uptake of nutrient elements under high [CO_2] (Mjwara *et al*., 1996).

Mjwara *et al*., (1996) reported down regulation of bean leaves as having resulted from deficiencies in Mg and Mn. In addition, Mjwara *et al*., (1996) reported a correlation between Fe deficiency and total chlorophyll content. The authors further pointed out that although Mn uptake was significantly increased its role might have been reduced by reduction of other elements in that study. This confirms the hypothesis that, the presence or the levels of one element may affect the uptake of others in the soil solution (Chapin, 1980).

In the present study, P was supplied at high levels (12mM [N]) and nitrogen was varied whilst the rest of the elements were kept constant. As a result this may have resulted in down regulation of NAR, by affecting uptake of other crucial elements involved in CO_2 fixation as described elsewhere.

6.6 Future research

A change in the [CO_2] around the photosynthetic organisms is guaranteed to alter many physiological activities in these organisms. Regulation can be mediated indirectly through anatomical, morphological and physiological

changes or more directly through biochemical effects on photosynthesis (Bowes, 1991).

Carefull attention needs to be devoted to nitrogen metabolism in parallel with investigations of photosynthesis carbon allocation (using carbon isotopes) and growth under elevated [CO_2]. In future studies researchers should examine changes in foliar nutrient concentration of both micro and macronutrients under elevated [CO_2] in order to help elucidate interactions with other parameters.

Analysis of amino acids and protein levels in both leaves and grain of elevated [CO_2] grown plants is essential as this may assist in identifying partitioning of nutrient elements like nitrogen as well as providing solutions to the problems relating to fertiliser management in the growth medium. In addition, amino acid composition will assist plant breeders in their quest develop new crop varieties, that will not only cope with the changing climatic conditions, but also meet the demands of industry.

Another avenue that needs to be examined is a comparative assessment and the involvement of mycorrhiza under elevated [CO_2] and ambient [CO_2] conditions, as it is well established that these organisms significantly improve nutrient uptake by plants.

Finally, a more holistic approach is required in order to better understand the interaction of elevated [CO_2] and plant nutrition. Progress in this regard is

dependent on the costs involved in purchasing facilities that will closely resemble the natural environment. For an example, CO_2 enrichment facilities such as free-air CO_2 enrichment (FACE), open top chambers (OTC) and sunlit controlled environment chambers (Soil, Plant, Atmospheric Research chambers, SPAR). However as more mechanistic surveys need to be done, high CO_2 controlled environment (CE) chambers seem to be the best option for this purpose as stated elsewhere in this report, in so far as all the basic physiological inputs ([CO_2], light intensity and daylength, temperature and saturated vapour pressure deficit) can strictly be controlled. Clearly, elevated CO_2 and plant nutrition still remain a topic of great interest as we enter the new millennium.

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Appendix 1

The full Long-Ashton nutrient solution used in this study as taken from Hewitt (1966).

Salt	**Wt used (g)**	**V. stock Sol. (ml)**	**V. stock sol. dil. In 25 L (ml)**	**Conc. In final V. of 25 L (mM0**
Macronutrient				
KNO_3	101	500	25	2
K_2SO_4	43	500	25	1
$Ca(NO_3)_2$	164	500	25	4
$CaCl_2$	111	500	25	4
$MgSO_47H_20$	92	500	25	1.5
$NaH_2PO_4.2H_2O$	104	500	25	4
Micronutrient				
$MnSO_4.4H_2O$	11.20	500	2.5	0.02

$CuSO_4.5H_2O$	1.25	500	2.5	0.002
$ZnSO_4.7H_2O$	1.45	500	2.5	0.002
H_3BO_3	15.50	500	2.5	0.05
$Na_2Mo_4.2H_2O$	0.605	500	2.5	0.0005
NaCl	29.30	500	2.5	0.1
Fe-Citrate (3H2O)	29.90	500	2.5	0.6

Appendix 2

Parameters calculations and equations

(Adopted from Mjwara, 1991)

The basic equations (Farquhar *et al*., 1980; von Caemmerer and Farquhar, 1981; Ziegler-Jöns and Selinger, 1987; Field *et al*., 1989) which were also incorporated in the IRCAL programme are presented here without details of the theory. Photosynthetic CO_2 assimilation (A, µmol m^{-2} s^{-1}) was calculated from the depletion of CO_2 in the gas stream. According to the above mentioned authors, A depends on the velocity of carboxylation used Γ^* is the CO^2 compensation point of photosynthesis in the absence of dark respiration and C_i is the internal carbon dioxide concentration. Thus the net rate of CO_2 assimilation in the absence of day respiration R_d is: V_{cmax}, Rubisco which is

an unstable enzyme capable of carboxylationor oxygenation, hence K_c and K_o which are the Michaelis-Menten constant for CO_2 and O_2 are used. Thus the net rate of CO_2 assimilation in the absence of day respiration (R_d) is:

$$A = V_{c\max}\left[\frac{C_i - \Gamma^*}{C_i + K_c\left(\frac{1+0}{K_o}\right)}\right] - R_d \qquad \text{Equation 7.1}$$

Farquhar *et al*., (1980) established the dependence of A to intercellular CO_2 using equation 7.1. The resultant equation (Equation 7.3) is directly related to the equation proposed by Ku and Edwards (1977):

$$CE = \frac{APS}{CO_2 - \Gamma^*} \qquad \text{Equation 7.2}$$

which estimated carboxylation efficiency (*CE*) from the initial slope of *A* versus C_i, where *APS* is the apparent rate of photosynthesis.

The dependence of *A* on the intercellular CO_2 is then:

$$\frac{dA}{dC} = V_{c\max}\left[\frac{\Gamma^* + K_c\left(\frac{1+0}{K_o}\right)}{\left[C + K_c\left(\frac{1+0}{K_o}\right)\right]^2}\right] \qquad \text{Equation 7.3}$$

Carbon dioxide compensation point (Γ*, μmol m^{-2} s^{-1}) has bee used for the calculation of *ACE* and its calculation is based on the following equation:

$$\Gamma^* = \frac{\Gamma + K_c\left(\frac{1+0}{K_o}\right)\frac{R_d}{V_{c\max}}}{1 - \frac{R_d}{V_{c\max}}} \qquad \text{Equation 7.4}$$

Farquhar and Sharkey (1982) and later Field *et al*. (1989) pointed out that the power of photosynthetic measurement is greatly increased by simultaneous measurement of transpiration (*E*, mmol m^{-2} s^{-1}) as illustrated in equation 7.5. Once *E* has been calculated, it is possible to calculate leaf conductance to water vapour, which is the critical parameter for the determination of internal CO_2 concentration (C_i). By rearranging equation 7.5

$$A = g_c\left(C_a - C_i\right) - \left(\frac{C_i + C_a}{2}\right)E \qquad \text{Equation 7.5}$$

The resultant equation (7.6, below) is incorporated in the IRCAL software package that was used, adequately estimates C_i as outlined by Field *et al*. (1989).

$$C_i = \frac{\left(g_{tc} - \frac{E}{2} C_a\right) - A_n}{\left(g_{tc} + \frac{E}{2}\right)}$$ Equation 7.6

where g_{tc} is the total conductance to CO_2 (mol m^{-2} s^{-1}), C_a is the mole fraction of CO_2 in the ambient air (μmol mol^{-1}) and A_n is the net assimilation rate (μmol m^{-2} s^{-1}).