Ecophysiological responses of *Themeda triandra* Forsk. and other southern African C_4 grass species to increases in atmospheric CO_2 concentrations.

Stephanie Juliane Editha Wand

Thesis Presented for the Degree of
DOCTOR OF PHILOSOPHY
in the Department of Botany
UNIVERSITY OF CAPE TOWN

March 1999

University of Cape Town

The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.

University of Cape Town

Abstract

Ecophysiological responses of *Themeda triandra* Forsk. and other southern African C_4 grass species to increases in atmospheric CO_2 concentrations.

This study was undertaken to investigate the potential effects of rising atmospheric carbon dioxide (CO_2) concentrations on southern African C_4 grass species, with respect to carbon assimilation and allocation, growth, and morphological development. Altered CO_2-responsiveness with simultaneous exposure to other environmental influences was also assessed in order to estimate the impacts of elevated CO_2 on the future distribution and productivity of grasslands in southern Africa. The initial hypothesis was based on established theory and current perceptions, which predict no significant photosynthetic or growth responses to elevated CO_2 in C_4 grasses.

Three different approaches were employed to test a range of questions:

- A quantitative literature review (meta-analysis) was undertaken of physiological and growth responses to elevated CO_2 of wild C_3 and C_4 grass species worldwide, in order to test the current perception of non-responsiveness in C_4 species.

- Selected representative southern African C_4 grass species were grown from seed or from small tufts for periods of at least four months in open-top chambers in a greenhouse under either ambient (360 µmol mol^{-1}) or elevated (650-700 µmol mol^{-1}) CO_2 concentration. Both inter- and intra-specific variability in the CO_2-response were studied, under different seasonal temperature regimes. The CO_2 treatments were also combined with defoliation and increased ultraviolet-B (UV-B) radiation treatments, and comparisons were made with the CO_2-responsiveness of woody grassland invaders, in order to examine the potentially moderating influence of other climatic and biological factors on C_4 responses.

- Photosynthetic responses to elevated CO_2 of selected C_4 and C_3 grass species were determined under natural conditions in a field experiment situated in a C_4-dominated grassland in Kwazulu/Natal. Potential seasonal shifts in CO_2-responsiveness were investigated over the course of a year, to determine possible linkages with plant phenology.

This study shows that some dominant southern African C_4 grass species are capable of responding positively to increasing atmospheric CO_2 concentrations. The meta-analysis revealed that wild C_4 grasses are not intrinsically less responsive to elevated CO_2 than C_3

University of Cape Town

grasses with respect to gas exchange and plant growth. Seedlings of the C_3 woody invasive *Acacia karoo* are also not more responsive than C_4 grasses. At the leaf level, C_4 grasses do not show any sink limitation, but some are capable of downregulating their photosynthetic capacity after prolonged exposure to high CO_2. However, growth responses are manifested early in plant development and persist into maturity. The main responses are increases in leaf length and canopy leaf biomass and area. *Themeda triandra*, a widespread palatable species over southern Africa, showed consistent positive growth responses across three different ecotypes, particularly when plants developed over a longer growth period under moderate temperatures.

Not all C_4 grass species studied responded favourably to elevated CO_2. Species belonging to the NADP-ME photosynthetic sub-type showed significant leaf biomass increases in elevated CO_2, whereas positive responses in species belonging to the NAD-ME and PCK sub-types were due to increases in non-leaf shoot fractions. CO_2-responsiveness under field conditions was strongly dependent on phenological development of individual species. Defoliation did not influence the response to elevated CO_2 in comparison to undefoliated plants (no interaction between treatments), but total shoot productivity was maximal under high CO_2 and defoliation. Moderate defoliation could mitigate the accelerated self-shading of mature stands in elevated CO_2. However, repeated defoliation at short intervals was accompanied by reduced plant vigour and nutrient uptake capacity. Forage quality (leaf nitrogen concentration) was also reduced in elevated CO_2. Longer-term reductions in both forage quality and quantity could be detrimental to grazers and other herbivores.

Expected increases in global temperature may favour the expansion of C_4 grasses into cooler regions of the grassland biome, aided by CO_2-induced growth enhancements. Improved plant and soil water relations due to reductions in transpiration in high CO_2 will also reduce evapotranspiration. Increasing UV-B radiation may reduce the relative growth enhancement in C_4 grasses, but is unlikely to alter the response to elevated CO_2 significantly.

In conclusion, this study demonstrates that C_4-dominated grasslands in southern Africa may constitute significant carbon sinks for CO_2 emissions and are unlikely to give way to C_3 grasses in this region over the next century.

University of Cape Town

Acknowledgements

This study was conducted while in the employ of the National Botanical Institute (NBI), who funded the project. I am grateful to Prof. B.J. Huntley (Chief Director), Dr. M.C. Rutherford (Deputy Director for Research: Ecology and Conservation), Prof. G. Smith (Director: Research), and Dr. G.W. Davis (former Programme Leader: Stress Ecology), for their generous and committed support. Greenhouse facilities were constructed with the financial support of the Roland and Leta Hill Trust, administered by the World Wildlife Fund (Southern Africa).

I thank my supervisor, Prof. William Stock, for his interest and involvement in the project, and for the instrumental role he played in locating and setting up the field experiment. Prof. William Stock, Prof. William Bond (University of Cape Town) and Dr. Guy Midgley (NBI) promoted knowledgeable, challenging and stimulating discussions. I am deeply grateful to Guy for sharing his hard-earned theoretical and technical expertise. Without his successful design and development of the open-top chamber fumigation system, I would not have been able to embark on this study. Dr. Bert Drake of the Smithsonian Environmental Research Centre, USA, provided a most stimulating opportunity for me to work on his elevated CO_2 field experiments in Maryland and Florida, which further kindled my interest in this field.

The continued interest and support of Mr. Guy Clegg of Pleasant View Farm, on whose land the field experiment is located, is gratefully acknowledged. Mr. Nick Bennett, Mr. Deryck de Witt, Mr. Stanley Snyders, Mr. Barry Jagger and Mrs. Judy Arnolds helped with the construction of the open-top chamber experiments, the maintenance of experiments, as well as harvesting and leaf carbohydrate analyses. Dr. Charles Musil is thanked for his assistance with the ultraviolet-B treatment, including dose calculations, and for providing UV-B lamps and filters.

Dr. Peter Curtis and Dr. Michael Jones of The Ohio State University (USA), and their assistants, are thankfully acknowledged for their role in running the meta-analysis of grass CO_2-responses. I am also grateful to Dr. Oula Ghannoum (Australia) and Dr. Jack Morgan (USA) for suggestions and valuable discussion.

University of Cape Town

Themeda triandra seed was supplied by Dr. Timm O'Connor (formerly of the Döhne Agricultural Development Institute) and the Gene Bank (Agricultural Research Council, Pretoria). Dr. John Randall of the University of Stellenbosch provided guidance for the statistical design and analysis of some of the experiments.

Most of all I want to thank Guy for his constant support, and for his infectious love of science and quest for understanding of our physical and natural world, which were a never-ending source of inspiration and motivation.

University of Cape Town

Table of Contents

		Page
Abstract		i
Acknowledgements		iii
Table of Contents		v
List of abbreviations		ix
Chapter 1	**Introduction**	1
1.1	Global climate change and rising atmospheric CO_2 concentrations	1
1.2	African grasslands and climate change	2
1.3	C_4 grasses: biochemistry and ecophysiology	4
1.4	C_4 grasses: distribution and evolution	7
1.5	C_4 grasses and elevated atmospheric CO_2	8
1.6	Response mechanisms and influences on CO_2-responsiveness	10
1.7	Study objectives and key questions	11
1.7.1	Overall objectives	11
1.7.2	Key questions	13
Chapter 2	**Responses of wild C_4 and C_3 grass (Poaceae) species to elevated atmospheric CO_2 concentration: a meta-analytic test of current theories and perceptions**	15
2.1	Introduction	15
2.2	Materials and Methods	17
2.2.1	Database compilation	17
2.2.2	Meta-analyses	20
2.3	Results	20
2.3.1	Relative CO_2-responses of C_3 and C_4 species	20
2.3.2	Influence of environmental stress	23
2.3.3	Exposure methods and growth conditions	25
2.4	Discussion	28
2.4.1	Relative CO_2-responses of C_3 and C_4 species	28
2.4.2	Influence of environmental stress and growth methodology	33
Chapter 3	**Materials and Methods: Greenhouse Elevated CO_2 Experiments**	36
3.1	Introduction	36
3.2	Open-top chambers	36
3.3	CO_2 fertilization and calibration	39
3.4	Environmental monitoring of chambers	40
3.5	Pots and irrigation	43

University of Cape Town

Chapter 4	**Physiological and growth responses of *Themeda triandra* (C_4 grass) and *Acacia karroo* (C_3 shrub/tree) to combined increases in CO_2 and UV-B radiation**	45
4.1	Introduction	45
4.2	Materials and methods	47
4.2.1	Plant establishment	47
4.2.2	Design and treatments	47
4.2.3	Photosynthetic gas exchange	50
4.2.4	Harvest	50
4.2.5	Leaf chemical analyses	51
4.2.6	Statistical analysis	52
4.3	Results	52
4.3.1	Photosynthetic gas exchange	52
4.3.2	Leaf chemistry	54
4.3.3	Biomass	54
4.4	Discussion	60
4.4.1	*Themeda triandra*	60
4.4.2	*Acacia karroo*	62
4.4.3	Conclusion	65
Chapter 5	**Physiological and growth responses of *Themeda triandra* to elevated CO_2: interaction with defoliation**	66
5.1	Introduction	66
5.2	Materials and methods	69
5.2.1	Plant establishment	69
5.2.2	Design and treatments	70
5.2.3	Photosynthetic gas exchange	71
5.2.4	Defoliation and harvest	72
5.2.5	Statistical analysis	73
5.3	Results	73
5.3.1	Regrowth following defoliation	73
5.3.2	Canopy gas exchange	77
5.3.3	Final harvest - interaction with defoliation	79
5.3.4	Final harvest - interaction with CO_2 switch-over (last three weeks)	81
5.4	Discussion	81
5.4.1	Hypothesis 1: CO_2-response in defoliated vs. undefoliated plants	81
5.4.2	Hypothesis 2: Regrowth in elevated vs. ambient CO_2	84
5.4.3	Hypothesis 3: Long-term indirect stimulation vs. short-term direct stimulation of regrowth in elevated CO_2	86
5.4.4	Growth form and productivity responses under different treatment combinations	88
Chapter 6	**The developmental sequence of morphological and photosynthetic CO_2-responses of *Themeda triandra***	90
6.1	Introduction	90
6.2	Materials and methods	91
6.2.1	Morphological measurements	91
6.2.2	Leaf-level gas exchange measurements	91
6.2.3	Statistical analysis	92

University of Cape Town

6.3	Results	92
6.4	Discussion	94
Chapter 7	**Gas exchange and above-ground growth responses to elevated CO_2 in C_4 NADP-ME, C_4 NAD-ME and C_4 PCK and C_3 grasses**	99
7.1	Introduction	99
7.2	Materials and methods	101
7.2.1	Plant establishment	101
7.2.2	Design and treatments	102
7.2.3	Photosynthetic gas exchange	103
7.2.4	Harvest	105
7.2.5	Statistical analysis	105
7.3	Results	106
7.3.1	Biomass, morphology and leaf chemistry	106
7.3.2	Gas exchange	115
7.4	Discussion	133
7.4.1	Gas exchange in elevated CO_2	133
7.4.2	Growth in elevated CO_2	135
7.4.3	Photosynthetic acclimation in elevated CO_2	138
7.4.4	Conclusion	139
Chapter 8	**Seasonal gas exchange responses in C_4 and C_3 grass species grown in elevated CO_2 in the field**	145
8.1	Introduction	145
8.2	Materials and methods	146
8.2.1	Site description	146
8.2.2	Controlled CO_2 fumigation	147
8.2.3	Photosynthetic gas exchange	149
8.2.4	Statistical analysis	150
8.3	Results	150
8.4	Discussion	161
Chapter 9	**Photosynthetic temperature responses in C_4 and C_3 grass species grown in elevated CO_2**	167
9.1	Introduction	167
9.2	Materials and methods	170
9.2.1	Experiment 1	170
9.2.2	Experiment 2	171
9.2.3	Experiment 4	171
9.2.4	Curve analysis	172
9.2.5	Chamber air temperatures	172
9.3	Results	172
9.4	Discussion	184
9.4.1	Photosynthetic temperature optima vs. growth temperature	184
9.4.2	Photosynthetic temperature optima in elevated CO_2	186
9.4.3	Influence of LAVPD on the temperature response	190
9.4.4	Conclusion	192

University of Cape Town

Chapter 10	**Summary discussion**	193
10.1	CO_2-responsiveness of C_3 and C_4 species	193
10.2	Genetic (intrinsic) determinants of CO_2-responsiveness in C_4 grasses	195
10.2.1	*Themeda triandra* (C_4): intra-specific variation in CO_2-responsiveness	195
10.2.2	Inter-specific and between-sub-type variation in CO_2-responsiveness	197
10.3	Mechanisms of the CO_2-response in C_4 grasses	199
10.4	Implications for productivity with and without defoliation	199
10.5	CO_2-responses and other concomitant climate changes	200
10.6	Conclusion	201
References		202
Appendix 1	References included in database for meta-analysis	220
Appendix 2	References, C_3 species, exposure and growth conditions, and interaction with other environmental stresses, for studies used in the analysis	224
Appendix 3	References, C_4 species, exposure and growth conditions, and interaction with other environmental stresses, for studies used in the meta-analysis	226
Appendix 4	CO_2-responses of *Themeda triandra* (ecotype 2) in summer	227
A1	Materials and methods	227
A1.1	Plant establishment	227
A1.2	Design and treatments	227
A1.3	Harvest and statistical analysis	228
A2	Results	228

University of Cape Town

List of Abbreviations

α	Apparent quantum efficiency (μmol CO_2 μmol^{-1} PPFD)
Γ_c	Photosynthetic CO_2 compensation point (μmol mol^{-1} CO_2)
Γ_l	Photosynthetic light compensation point (μmol m^{-2} s^{-1} PPFD)
k	Apparent carboxylation efficiency (μmol CO_2 μmol^{-1} c_i)
A	Net CO_2 assimilation rate (μmol m^{-2} s^{-1})
A_{max}	Light-saturated rate of net CO_2 assimilation (μmol m^{-2} s^{-1})
c_a	Atmospheric CO_2 concentration (μmol mol^{-1})
c_i	Intercellular CO_2 concentration (μmol mol^{-1})
$[CO_2]ms$	Cuvette CO_2 concentration during measurements (μmol mol^{-1})
$[CO_2]trt$	CO_2 concentration during growth (CO_2 treatment) (μmol mol^{-1})
E	Transpiration rate (mmol m^{-2} s^{-1})
g_s	Stomatal conductance (mmol m^{-2} s^{-1})
IRGA	Infra-red gas analyser
J_e	Light- and CO_2-saturated rate of net CO_2 assimilation (μmol m^{-2} s^{-1})
N	Leaf nitrogen concentration (mg g^{-1})
NAD-ME	Nicotinamide adenine dinucleotide malic enzyme
NADP-ME	Nicotinamide adenine dinucleotide phosphate malic enzyme
NUE	Photosynthetic nitrogen use efficiency
PEP	Phosphoenolpyruvate
PCK	Phosphoenolpyruvate carboxykinase
PPFD	Photosynthetic photon flux density (μmol m^{-2} s^{-1})
R_d	Dark respiration rate (μmol m^{-2} s^{-1})
Rubisco	Ribulose-1,5-bisphosphate carboxylase/oxygenase
RuBP	Ribulose-1,5-bisphosphate
TNC	Leaf total non-structural carbohydrate concentration (mg g^{-1})
T_{opt}	Photosynthetic temperature optimum (°C)
UV-B	Ultraviolet-B radiation
WUE	Leaf instantaneous water use efficiency (μmol CO_2 $mmol^{-1}$ H_2O)

University of Cape Town

Chapter 1

Introduction

1.1 Global climate change and rising atmospheric CO_2 concentrations

The potential effect of changes in global climatic patterns has been recognised as one of the most serious threats and challenges facing the biosphere during the next century (IPCC 1995). The United Nations Framework Convention on Climate Change (FCCC 1992) is one of four major international conventions drawn up in recent years, which aims to achieve "stabilization of greenhouse gas concentrations in the atmosphere at a level that would prevent dangerous anthropogenic interference with the climate system. Such a level should be achieved within a time frame sufficient to allow ecosystems to adapt naturally to climate change, to ensure that food production is not threatened and to enable economic development to proceed in a sustainable manner." Subsequent protocols, such as the Kyoto Protocol of 1997, and the agreement reached in Buenos Aires in 1998, have suggested guidelines as to the implementation of greenhouse gas emission controls.

The best documented and undisputed aspect of climate change is the fact that concentrations of carbon dioxide (CO_2) in the atmosphere are increasing at a rate unprecedented in geological history (Keeling 1986, Vitousek 1994). Atmospheric CO_2 concentrations have risen from pre-industrial levels of about 280 μmol mol^{-1} to more than 360 μmol mol^{-1} (Schimel 1995, IPCC 1995), an increase of about 30%, or 1.5 μmol mol^{-1} per year (Houghton 1997). By the middle of the next century, this concentration is expected to double from pre-industrial levels and will likely reach 600-700 μmol mol^{-1} by the end of the 21st century (Trabalka *et al.* 1985). The cause of this rapid rise is the release of CO_2 into the atmosphere due to the burning of fossil fuels, as well as the effects of deforestation on a large scale (Gates 1993). Because CO_2 effectively absorbs long-wave radiation, making it a major greenhouse gas, an increase in CO_2 concentration is expected to drive changes in other climatic patterns, such as increases in global temperatures and altered rainfall regimes (IPCC 1995, Houghton 1997). The potential impact of these perturbations on the biosphere and human well-being has highlighted the need for an improved understanding of the role and

University of Cape Town

importance of terrestrial ecosystems as sinks of greenhouse gases such as CO_2, and has prompted a call for relevant scientific research which will help us to understand and address global change effectively (FCCC 1992).

It is also recognized that countries with arid and semi-arid areas, prone to drought and desertification, would be particularly vulnerable to the adverse effects of climate change. Southern Africa is not only highly vulnerable to aridification, but is also a developing region with rising energy requirements and a significant per capita CO_2 emission rate (Scholes & van der Merwe 1995). South Africa ratified the FCCC in 1997 and is now required to develop and implement policies in this regard (Climate Change: a South African Policy Discussion Document 1998). Trade-offs between development, conservation and food security are required. Both conservation and food security are strongly dependent on the diversity, survival and productivity of plants under fluctuating climatic conditions. Since plants are intimately dependent on, and adapted to, prevailing levels of CO_2, temperature and rainfall, perturbations of these factors may have serious direct and indirect impacts on natural vegetation. Global terrestrial photosynthesis represents a major sink for CO_2 second only to the oceans (Schimel 1995). Consequently, a considerable amount of research has been focussed on the potential response of natural vegetation to elevated CO_2, including predictions of future carbon balances (Bazzaz 1990, Körner & Bazzaz 1996, Koch & Mooney 1996, Walker & Steffen 1996).

1.2 African grasslands and climate change

Grasslands cover at least 20% of the earth's land surface (Hall *et al.* 1995), and can be classified as either temperate or tropical. Temperate grasslands occur in the middle latitudes, and include the central plains of North America and temperate regions of Eurasia and Australia. They primarily contain grasses having the C_3 photosynthetic mechanism, grading to more C_4 grasses in the warmer regions. Tropical grasslands occur in tropical and subtropical regions of Africa, Central and South America, the Indian subcontinent, and Australia, and are dominated by C_4 grass species (Long *et al.* 1992). They occupy about 15 million km^2 and are nearly equal to tropical forests in terms of both land area and productivity (Hall & Scurlock 1991). In addition, the herbaceous component of savannas contains a high proportion of C_4 graminoids. Most research on climate change responses has been conducted in the Northern Hemisphere, which differs from the Southern Hemisphere in

University of Cape Town

key respects, both in terms of hemispheric differences in the climate systems, as well as physical, biological, economic and cultural contrasts that affect vulnerability to climate change (Giambelluca & Henderson-Sellers 1996). Much of sub-equatorial Africa is dominated by tropical savanna and grassland vegetation, but very few empirical studies on species from tropical C_4-dominated grasslands and savannas have been conducted. The emphasis thus far has been on modelling the effects of climate change and rising CO_2 on grassland ecosystems, mainly using the CENTURY model. CENTURY simulations which included the direct effects of elevated CO_2 on plant water use efficiency, nitrogen use efficiency and productivity (via a 20% decrease in transpiration) for both C_3 and C_4 grasslands predicted an increased net primary productivity for all temperate and tropical grasslands (Hall *et al.* 1995, Parton *et al.* 1995). These models suggest that grasslands could well provide a substantial sink for anthropogenic CO_2 emissions (Hall & Scurlock 1991). Since these models do not allow for any direct stimulation of carbon assimilation in C_4 grasslands, and simulate increased productivity only in response to improved water use, these results could underestimate the sink capacity of grasslands for CO_2 if indeed C_4 grasses are capable of increasing their photosynthetic rates. On a more cautious note, Long & Hutchin (1991) reviewed the responses of different physiological processes underlying grassland production to climate change, and concluded that there was insufficient information to predict accurately the response of primary production to climate change.

For southern Africa, Midgley & O'Callaghan (1993) made an initial assessment of likely impacts of climate change on the natural vegetation. They predicted increased aridification and water stress and possible shifts in species composition and vegetation structure for the savanna and grassland biomes. The sensitivity of the grassland biome of South Africa to predicted climate change (2°C increase in temperature, 15% decrease in rainfall) was modelled by Ellery *et al.* (1991) and yielded a significant decrease in the biome area. They predicted large-scale invasion of the grassland biome by savanna, both east and west of the escarpment, as well as invasion of the savanna in the eastern Cape by nama-karoo. Hulme (1996) recently developed models of southern African vegetation responses to climate change, and emphasized the importance of including the direct impacts of elevated CO_2 on photosynthesis and water use in these models. He predicted a reduction in the grassland biome in South Africa of 30-55% when only changes in temperature and rainfall were incorporated. However, when the increase in plant water use efficiency in elevated CO_2 was

University of Cape Town

included, the biome was predicted to decrease by only 10-20%, or even increase by up to 20% under a "wet" climate scenario. Clearly, a good understanding of the direct effects of CO_2 on grass species is required in order to improve the predictive value of such models.

Empirical studies of the responses of South African plant species to rising atmospheric CO_2 concentrations were initiated in 1992 by the National Botanical Institute (NBI), starting on a group of sclerophyllous woody fynbos species (Midgley 1996). Continued work on fynbos is underway at the University of Cape Town. However, up until 1994, when this study was initiated at the NBI in Cape Town, and the South African Greenhouse Experiment on Savannas (SAGES) project (effects of elevated CO_2 on model savannas) began in Pretoria (Scholes & Bailey 1996), no research had been conducted on the effects of elevated CO_2 on southern African savannas or grasslands. In particular, the CO_2-responses of tropical and sub-tropical C_4 grasses had received no attention.

1.3 C_4 grasses: biochemistry and ecophysiology

The C_4 photosynthetic pathway was initially discovered in the 1960's (Kortschak *et al.* 1965, Hatch & Slack 1966) and has since received much attention (Sage & Monson 1998), particularly with respect to its specialized biochemical mechanisms, and how these may be of adaptive significance under specific environmental conditions experienced over the distributional ranges of plants possessing this pathway. The main feature of C_4 photosynthesis is the CO_2 concentrating mechanism that effectively pumps CO_2 from the mesophyll cells to the vascular bundle sheath cells (Hatch & Osmond 1976, Ehleringer & Monson 1993). The initial carboxylation reaction occurring in the mesophyll cells involves phosphoenolpyruvate (PEP) and atmospheric CO_2 as substrates, and is catalysed by PEP carboxylase. The product of this reaction, oxaloacetate, is a four-carbon acid (hence the term C_4 photosynthesis). This acid is converted to either malate or aspartate (depending on the C_4 sub-type), which diffuse from the mesophyll through plasmodesmata to the bundle sheath cells, where the C_4 acid is decarboxylated. The released CO_2 is then used to carboxylate ribulose-1,5-bisphosphate (RuBP), a reaction catalysed by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), thereby starting the C_3 photosynthetic cycle ("photosynthetic carbon reduction [PCR] cycle"). The C_3 cycle is therefore limited to the bundle sheath cells. Since PEP carboxylase has a higher affinity for CO_2 and a greater maximum velocity than Rubisco, the CO_2 concentration in the bundle sheath cells is significantly higher than in the mesophyll. The difference can be

University of Cape Town

an order of magnitude (~200 μmol mol^{-1} in the mesophyll chloroplasts of C_3 plants compared to ~2000 μmol mol^{-1} in the bundle sheath chloroplasts of C_4 plants). This effectively eliminates or masks photorespiration in C_4 plants, the term used to describe the oxygenation of RuBP, also catalysed by Rubisco, which can account for losses of 25% in C_3 species at present atmospheric CO_2:O_2 ratios, thereby reducing the efficiency of photosynthesis (Bowes 1993). These differences between C_3 and C_4 photosynthesis can be seen when comparing their respective A/c_i responses (photosynthetic response to intercellular CO_2 concentration): at sub-ambient atmospheric CO_2 concentration, photosynthesis of C_4 plants is considerably more efficient, and compensates for any respiratory losses at lower c_i (<10 μmol mol^{-1}) than in C_3 plants (30-70 μmol mol^{-1}) (Pearcy & Ehleringer 1984). On the other hand, net CO_2 assimilation is saturated at relatively low c_i in C_4 plants (100-150 μmol mol^{-1}) compared to C_3 plants (>300 μmol mol^{-1}).

Three variants (sub-types) of the C_4 acid cycle have been identified, based on different biochemical enzymatic mechanisms to achieve C_4 acid decarboxylation in the bundle sheath cells. The three enzymes in question are NADP-ME (NADP-malic enzyme), NAD-ME (NAD-malic enzyme) and PCK (phosphoenolpyruvate carboxykinase) (Hattersley & Watson 1992). The sub-types can also be grouped into aspartate formers (NAD-ME and PCK) and malate formers (NADP-ME), based on the type of acid that is transported from the mesophyll to the bundle sheath cells. The utilisation of the different decarboxylases is generally expressed in conservative patterns across phylogenies, with the supertribe Andropogonodae, for example, possessing only NADP-ME, and most genera being consistently of one sub-type (Hattersley 1992, Hattersley & Watson 1992). The three C_4 sub-types exhibit unique stable carbon isotope discrimination ($\delta^{13}C$) values, apparently related to the degree of "leakiness" to CO_2 in the bundle sheath cells (Hattersley 1982).

Owing to the greater efficiency of carboxylation in C_4 plants and the fact that photosynthesis is largely independent of leaf internal CO_2 concentration, C_4 plants generally operate under lower stomatal conductances than C_3 plants, thus reducing transpirational water loss without compromising carbon assimilation (Pearcy & Ehleringer 1984, Fitter & Hay 1987). Consequently, instantaneous leaf water use efficiency, the ratio of photosynthesis to transpiration, is significantly higher in C_4 than in C_3 plants (Osmond *et al.* 1982, Ehleringer & Monson 1993). This alone does not necessarily confer greater tolerance to drought or low

University of Cape Town

leaf water potentials on C_4 plants, since both groups exhibit wide variation in tolerance, depending on many other factors such as leaf form, growth form, and life history (Pearcy & Ehleringer 1984, Fitter & Hay 1987). However, they may have a better chance of survival under conditions of both water stress and high temperatures, as suggested by their geographic distributions (Hattersley & Watson 1992).

The C_4 photosynthetic mechanism also affects the response of photosynthesis to temperature. Photosynthetic rates in C_4 plants generally peak between 30-48°C under high light conditions (Long & Hutchin 1991), in contrast to C_3 plants, which generally show a photosynthetic temperature optimum below 30°C. This is due to the absence of photorespiration in C_4 plants, which increases relatively more at higher temperatures than carboxylation. This effect can be seen when C_3 photosynthesis is measured at non-limiting CO_2 concentrations, resulting in an upward shift in the temperature optimum (Osmond *et al.* 1980).

Theoretically, C_4 photosynthesis carries with it a higher energetic cost: five ATP (adenosine triphosphate) molecules are required for CO_2 fixation and RuBP regeneration, as opposed to the three ATP and two NADPH required by C_3 plants. Nevertheless, owing to their lack of photorespiration and the loss of energy associated with it, C_4 plants generally have a higher quantum yield (ratio of CO_2 assimilation to absorbed photons) than C_3 plants under current atmospheric conditions (Ehleringer & Björkman 1977, Ehleringer & Pearcy 1983, Ehleringer *et al.* 1997). The quantum yield remains constant as temperatures increase, unlike C_3 plants which show significant reductions in quantum yield with increasing temperature (Ehleringer & Björkman 1977, Ehleringer *et al.* 1997). There are subtle differences in quantum yield among C_4 photosynthetic sub-types, with NAD-ME species having the lowest, and NADP-ME species having the highest quantum yields. This has implications for the differential geographical distributions of these sub-types and of C_3 species (Ehleringer *et al.* 1997).

Nitrogen-use efficiency also differs between C_3 and C_4 plants. C_4 plants contain three to six times less Rubisco than C_3 plants, owing to the fact that photosynthetic rates can be maintained with less Rubisco, as this enzyme operates close to CO_2 saturation in C_4 plants. As a result, leaf nitrogen concentrations are significantly lower, and the photosynthetic rate per unit of leaf nitrogen, or nitrogen-use efficiency (NUE) is higher in C_4 than C_3 plants (Brown 1978). Whether this could provide an advantage to C_4 species in nitrogen-poor habitats is not

University of Cape Town

yet clear. Tilman (1990) showed that C_4 grass species persist under lower soil nitrogen concentrations than C_3 grass species (although he did not explicitly categorize the species used as C_3 or C_4).

1.4 C_4 grasses: distribution and evolution

C_4 species comprise about half of the approximately 10 000 known grass species (Hattersley 1992) and account for approximately 18-21% of the total global productivity (Lloyd & Farquhar 1994, Ehleringer *et al.* 1997). C_4 grasses are particularly important in Africa and other developing regions both as staple food (cereals such as maize) and for grazing of wild animals (tourism) and domesticated stock. In southern Africa they serve the additional very important function of cladding a large proportion of the country's water catchments.

The C_4 photosynthetic pathway evolved in recent geological times, probably primarily in response to declining atmospheric CO_2 concentrations (Ehleringer & Monson 1993; Cole & Monger 1994, Cerling *et al.* 1998), and in association with high irradiance and temperature (in particular with high minimum temperatures during the growing season), and marked rainfall seasonality. C_4 photosynthesis is particularly successful in regions with high temperatures (>25-28°C) during the rainy season, usually in summer. This is borne out by present-day geographical distributions of C_4 grasses in North America (Teeri & Stowe 1976), South Africa (Vogel *et al.* 1978), Namibia (Ellis *et al.* 1980), Australia (Hattersley 1983) and Argentina (Cavagnaro 1988). Vogel *et al.* (1978) showed that, even though only a small portion of South Africa has a tropical or sub-tropical climate, most of the country has almost exclusively C_4 grasses in the vegetational graminoid cover. While temperature of the growing season is the key variable which determines the proportion of C_3 to C_4 grasses, the biochemical diversity within C_4 grasses is best correlated with rainfall parameters. In Namibia (Ellis *et al.* 1980) and Australia (Hattersley 1992), increasing annual rainfall is associated with an increasing contribution of the NADP-ME type of C_4 photosynthesis, while the contribution of the NAD-ME type decreases with increasing rainfall and is greatest in the more arid regions. The PCK type shows a maximum contribution to the grass flora at intermediate rainfall levels. This pattern is also reflected in the differential carbon isotope ratios ($\delta^{13}C$) of the different C_4 photosynthetic types across rainfall gradients (Schulze *et al.* 1996).

University of Cape Town

Phylogenetic studies have shown that the C_4 pathway occurs only in the more advanced angiosperm families (17 known families), and has probably evolved independently a number of times, even among genera (Hattersley & Watson 1992, Ehleringer *et al.* 1997). It occurs most frequently in herbaceous species, and is especially prevalent in the grass (Poaceae) and the sedge (Cyperaceae) families. Two of the five recognised subfamilies within the Poaceae (the Bambusoideae and Pooideae) contain only C_3 species, whereas the Chloridoideae is almost entirely C_4, the Panicoideae has both C_3 and C_4 species, and the Arundinoideae is predominantly C_3 but has five C_4 genera (Gibbs Russel *et al.* 1990, Hattersley & Watson 1992, Ehleringer & Monson 1993). Lineages originating throughout tropical and subtropical regions tend to have C_4 photosynthesis, whereas those originating from the temperate or arctic regions tend to have C_3 photosynthesis. Although most grass genera are consistently either C_3 or C_4, there are exceptions, one of which shows variation in pathway at the sub-specific level (*Alloteropsis semialata* has both C_3 and C_4 sub-species, Gibbs Russel *et al.* 1990).

One of the strong drivers of C_4 evolution appears to be the atmospheric CO_2 concentration: this has been generally decreasing over the last 65 million years, from a level of more than 1000 μmol mol^{-1} at the end of the Cretaceous period, to glacial and interglacial levels fluctuating between about 200-300 μmol mol^{-1}, respectively, in the last million years or so. Recent studies (Cole & Monger 1994, Ehleringer *et al.* 1997, Cerling *et al.* 1998) have put forward a strong argument that the increasing dominance of C_4 plants in the tropics and sub-tropics from the end of the Miocene (about 7 million years ago) was linked to the reduction in atmospheric CO_2 levels below about 500 μmol mol^{-1}.

University of Cape Town

1.5 C_4 grasses and elevated atmospheric CO_2

It has been widely predicted that C_3 species will gain a competitive advantage over C_4 species as global CO_2 concentrations rise (Bazzaz 1990; Bowes 1993; Ehleringer & Monson 1993). Collatz *et al.* (1998), using a modelling approach, suggest that extensive regions that were previously C_3/C_4 mixed grassland (pre-industrial) are now dominated by C_3 grasses. The question therefore arises as to whether C_4 plants will be threatened by global climate change. Will the advantages in productivity, water use efficiency, and temperature tolerance that the CO_2 concentrating mechanism confers on C_4 plants over C_3 plants be diminished under rising CO_2 concentrations (Henderson *et al.* 1994)? Early experiments on agricultural species did indeed show that biomass and yield in C_3 crops increased in elevated CO_2 by about 30-40%,

whereas C_4 crops showed a smaller growth response (Enoch & Kimball 1986; Cure & Acock 1986). A number of studies using both C_3 and C_4 plants, grown either individually or in competition, reported that C_3 species had greater growth responses and became more competitive with C_4 species under elevated CO_2 (Patterson & Flint 1980, Carter & Peterson 1983; Zangerl & Bazzaz 1984, Wray & Strain 1987). However, this outcome was often not conclusive and confounded by other environmental variables such as irradiance, temperature or water stress, or by inconsistencies in the expected C_3/C_4 dichotomy (Bazzaz & Carlson 1984; Bazzaz *et al.* 1989; Garbutt *et al.* 1990; Imai & Okamoto-Sato 1991). For example, high ambient temperatures as they are generally experienced over tropical and sub-tropical grasslands generally favour a greater positive CO_2-response (Long 1991, Idso & Idso 1994), but may do so differentially in C_3 and C_4 species.

Variable results have also been obtained when intact graminoid-dominated C_3/C_4 ecosystems were exposed to elevated CO_2. In the estuarine marsh of Chesapeake Bay, USA, elevated CO_2 increased shoot density and biomass in *Scirpus*, the C_3 species, but there was no effect on *Spartina*, the C_4 species. Mixed C_3/C_4 communities had intermediate responses (Drake *et al.* 1996). In a study using intact soil cores of the dominant grass species of the short-grass steppe in the USA (Morgan *et al.* 1994), all plants had higher CO_2 assimilation rates after one year of exposure to elevated CO_2, with a relatively greater response in the cool-season C_3 species. After two growing seasons, biomass was 19% greater under elevated CO_2, with no significant difference between the C_3 and C_4 grass (Hunt *et al.* 1996). In contrast, Newton *et al.* (1995) found that the only C_4 grass in an intact C_3-dominated turf community from a cool temperate environment showed increased CO_2 assimilation rates but decreased harvestable yield, and its proportion in the total biomass declined. The response of grasses to elevated CO_2 has also been studied in the tallgrass prairie of the USA (Owensby *et al.* 1996b, 1997). Elevated CO_2 significantly increased production of C_4 grasses but not of C_3 grasses, and the relative abundance of C_3 grasses declined (Owensby *et al.* 1993). The authors suggested that in natural ecosystems with periodic water stress, increased water use efficiency under elevated CO_2 could have a greater impact on productivity irrespective of photosynthetic pathway.

Rather than support expectations based on theory and clarify our understanding of C_4 responses to elevated CO_2, the diversity of responses reported in recent experiments under

University of Cape Town

more natural environmental conditions has thrown this question wide open again. It has also become clear that more experiments must be performed on natural C_4 grasses, both from temperate and tropical regions, since much of the older studies reported in the literature used crop species, weedy species, and species from salt marshes, which may not be representative of the vast majority of natural C_4 grass species.

1.6 Response mechanisms and influences on CO_2-responsiveness

The mechanisms for C_4 responses to elevated CO_2 are not as well understood as those for C_3 species (Stitt 1991), primarily because photosynthetic understanding does not make provision for enhanced carbon assimilation in C_4 species. However, increased photosynthesis in high CO_2 has been reported for a number of C_4 species (e.g. Ziska & Bunce 1997, LeCain & Morgan 1998). In C_3 species, sink strength limitations resulting in foliar carbohydrate accumulation, and photosynthetic downregulation after prolonged exposure to elevated CO_2 have emerged as important factors in plant CO_2-responsiveness (Wullschleger 1993, Midgley *et al.*, in press). Whether or not this also plays a role in C_4 responses is not clear, as some researchers refute its importance in C_4 species (Sage 1994), but others have shown evidence of photosynthetic and stomatal downregulation in elevated CO_2 (LeCain & Morgan 1998). Whether or not carbon assimilation rates are increased in elevated CO_2, C_4 species may still exhibit positive growth responses due to improved leaf water relations (Knapp *et al.* 1993) and increased leaf temperatures (Kirkham *et al.* 1993) linked to reduced transpirational water loss, although there is not much direct evidence for this in the literature. Aboveground growth enhancement in C_4 grasses could develop either via stimulation of tiller numbers and therefore leaf numbers, or via stimulation of leaf area development. Although some evidence for both exists in the literature, it has not been critically assessed, although there appears to be more evidence for leaf area stimulation (Coleman & Bazzaz 1992, Ackerly *et al.* 1992).

Plant CO_2-responsiveness is determined both by genetic (intrinsic) and environmental (extrinsic) factors (Midgley 1996). Genetic controls over CO_2-responsiveness can be found at both inter-specific (Poorter 1993, Poorter *et al.* 1996) and intra-specific levels. The latter can occur both within populations, as reported for reproductive responses in elevated CO_2 in *Phlox drummondii* (Garbutt & Bazzaz 1984) and *Raphanus raphanistrum* (Curtis *et al.* 1994), and between ecotypes. Ecotypic variation in CO_2-responsiveness reported between three North American ecotypes of the C_4 grass species *Echinochloa crus-galli* (Potvin & Strain

University of Cape Town

1985a, b) suggested that, in terms of productivity, southern ecotypes (from Mississippi and North Carolina) would benefit more from CO_2 enrichment than the northern one (from Québec). Changes in spatial distribution were also predicted under a global warming scenario, based on the differential interaction of the CO_2-response with growth temperature in different ecotypes.

At the inter-specific level, different types of plant life history strategy, which are largely determined by genome, have been linked to CO_2-responsiveness, with "competitive" species showing greater responsiveness than "stress tolerant" species (Hunt *et al.* 1991, 1993). Another genetically-determined influence on CO_2-responsiveness in C_3 species is the differential phloem loading and transport capacity and sink limitation between species (Körner *et al.* 1995). On the other hand, environmental factors which influence critical resource levels such as rooting volume and nutrient supply (Arp 1991, Midgley *et al.*, in press), as well as temperature, light and water supply during growth (Idso & Idso 1994) can alter the response to high CO_2. Most of these studies have been conducted on C_3 species, and it is unclear what the determinants of CO_2-responsiveness are in C_4 species. C_4 grasses, in particular, are morphologically and functionally relatively similar, but they do show some genetic and ecological divergence (e.g. C_4 sub-types or ecotypes) which, together with environmental factors, could influence the relative response to elevated CO_2 .

1.7 Study objectives and key questions

This study was initiated in order to gain an ecophysiological understanding of the potential responses of southern African C_4 grasslands to climate change, and in particular the rise in atmospheric CO_2 concentrations. The main overall objectives were as follows:

1.7.1 Overall objectives

- To experimentally determine the effects of elevated atmospheric CO_2 concentrations on representative southern African C_4 grass species, with respect to carbon assimilation and allocation, growth, and morphological development, and to assess potential changes in this responsiveness when exposed simultaneously to other environmental and biological influences.

University of Cape Town

- To formulate the potential direct and indirect impacts of rising atmospheric CO_2 on the future distribution and productivity of grasslands in southern Africa.

The initial working hypothesis, based on widespread current perceptions, predicted no photosynthetic or growth responses to elevated CO_2 in C_4 grasses as the null hypothesis. Following the results of the meta-analytic literature review (Chapter 2) and the first empirical greenhouse experiment (Chapter 4), it became clear that this was a simplistic assumption which did not hold for the C_4 grass group. Consequently, new experiments were devised based on a revised hypothesis which allowed for positive photosynthetic and growth responses to elevated CO_2 in C_4 grass species. Furthermore, these experiments broadened the focus by aiming to elucidate potential response mechanisms, to examine intra- and interspecific variability in CO_2-responsiveness, and to study interactions with other climatic and biological factors which could potentially alter plant CO_2-responsiveness.

Themeda triandra Forsk. was chosen as the first and primary study species, owing to its widespread dominance over South Africa and much of the eastern sub-continent, and its preferred status as a grazing species (van Oudtshoorn 1992). Its grazing and reproductive ecology have been well studied (e.g. O'Connor 1994), and this served as a useful background to this study. It is also a highly ecologically variable species, with a number of recognised ecotypes of divergent growth form, chemistry and ecology in the semi-arid grasslands of southern Africa (Theunissen 1992, S. van Rensburg, unpublished data). Potential intra-specific variability in CO_2-responsiveness could, therefore, be assessed using different ecotypes. This species also lent itself to a study on the interaction between defoliation and elevated CO_2. In order to provide a broader assessment of C_4 grass CO_2-responsiveness (inter-specific variability), and to study the potential differential responsiveness between C_4 photosynthetic sub-types, a range of C_4 grass species which dominate a site in southern Kwazulu/Natal was subsequently studied both in the greenhouse and the field.

In order to address the above objectives, literature studies and empirical experiments were devised with the following key questions in mind, presented here on a scale from the leaf to the landscape level (i.e. not necessarily in chronological order). Although the main focus of measurements lay at the leaf level and the whole plant level, even for the field experiment, an attempt was made to interpret results at a larger scale with respect to productivity and land management, but within reasonable limits of extrapolation. In spite of

University of Cape Town

the fact that individual species responses are not always sustained in a multi-species competitive environment over the longer-term, they do give a useful indication of potential responsiveness. The field experiments that are required to make such predictions with greater certainty are not within the logistical and financial ambit of researchers in South Africa at present, but the rapidity of atmospheric CO_2 increases and the urgency to formulate policy in this regard, demands some initial forecasts with which scientists and managers can work.

1.7.2 Key questions

- Do C_3 and C_4 grasses worldwide respond differentially to elevated atmospheric CO_2? (literature review). Are there pronounced trends in the published literature which can be applied to most grasses, or to C_4 grasses specifically?

- What are the photosynthetic responses of selected representative southern African C_4 grass species to elevated CO_2? Does photosynthetic capacity acclimate after prolonged exposure to high CO_2? Do the three C_4 photosynthetic sub-types display differential photosynthetic responses to elevated CO_2?

- Is the photosynthetic temperature optimum of C_4 grasses affected by growth in elevated CO_2? Is this response dependent on seasonal temperature shifts or on genetic acclimation potential?

- Does leaf water use efficiency change when C_4 grasses are grown in elevated CO_2? Does this response differ between C_4 sub-types?

- What are the growth and morphological responses of C_4 grasses to elevated CO_2? What is the developmental sequence of such responses? Does the growth response differ between seasons? Is this response consistent at a C_4 sub-type level, or is it species-specific, or does it vary even at the sub-species level (ecotypes)?

- Does defoliation alter the response of C_4 grasses to elevated CO_2? Is this response dependent on short-term photosynthetic responses, or to longer-term carbon accumulation patterns? What are the effects of repeated defoliation on resource use, and how is this altered in elevated CO_2?

University of Cape Town

- Will concomitant increases in ultraviolet-B (UV-B) radiation alter the response of C_4 grasses to elevated CO_2?

- Will C_3 invasive trees and shrubs, such as *Acacia karroo*, respond more favourably to elevated CO_2 than C_4 grasses? How could this affect bush encroachment of southern African grasslands?

- How will these responses to elevated CO_2 alter C_4 grassland distribution and productivity in southern Africa? Is there a need to re-assess management practices in the light of future changes in grassland function in elevated CO_2?

University of Cape Town

Chapter 2

Responses of wild C_4 and C_3 grass (Poaceae) species to elevated atmospheric CO_2 concentration: a meta-analytic test of current theories and perceptions

Note: This study was carried out in collaboration with Drs M.H. Jones and P.S. Curtis of The Ohio State University, USA, who provided data from additional references, ran the meta-analysis program and commented on data interpretation and presentation. This chapter is currently in press in slightly altered form. I hereby state that my contribution was at least 80%, including most of the conceptualization and writing.

2.1 Introduction

The effects of atmospheric CO_2 enrichment have been studied in great detail for agricultural crops (Cure & Acock 1986), trees (Ceulemans & Mousseau 1994), and other plant types (Bazzaz 1990, Poorter 1993, Idso & Idso 1994). The great majority of these studies have been carried out on C_3 species. Much of the early research into CO_2-responses of C_4 species focused on weedy and old-field dicotyledonous (dicot) species (e.g. *Amaranthus* sp., Bazzaz & Carlson 1984), or important planted C_4 crop species (e.g. *Zea mays* and *Sorghum* sp., Morison & Gifford 1984). Despite the fact that about half of the world's grass species possess the C_4 photosynthetic pathway, and they account for approximately 18% of the total global productivity (Lloyd & Farquhar 1994, Ehleringer *et al.* 1997), fewer studies have tested the responses of wild temperate C_4 grasses to elevated CO_2, and only a handful have used tropical C_4 grasses. Any changes in C_4 productivity driven by CO_2 and other climatic perturbations will, consequently, have a substantial impact on global gross primary productivity.

By far the greatest proportion of C_4 species are monocotyledonous (monocot), whereas C_4 dicots are relatively uncommon, both in terms of species representation and abundance (Ehleringer *et al.* 1997). Many C_4 dicots are noxious weeds and old-field invaders, and gain importance only in disturbed sites. Thus, studies using C_4 dicots and bred crops may not represent the potential CO_2-responsiveness of natural, relatively undisturbed ecosystems with a significant C_4 monocot component, such as prairies, tropical grasslands and savannas. Nevertheless, results obtained from many of these studies, and from the first field-based study

University of Cape Town

of a C_4-containing ecosystem, a salt marsh on Chesapeake Bay (Curtis *et al.* 1989, Drake *et al.* 1996), appeared to confirm the theory that C_4 plants should not show significant growth responses to elevated CO_2, due to their CO_2-concentrating mechanism in the bundle sheath cells (Osmond *et al.* 1982, Pearcy & Ehleringer 1984, Bowes 1993, see Chapter 1). It follows, in theory, that C_4 plants should not benefit from increased atmospheric CO_2 availability, and may suffer reduced competitive advantage over C_3 species (Bazzaz 1990, Bowes 1993, Ehleringer & Monson 1993, Polley *et al.* 1996b). As a result of this common perception, the potential contribution of C_4-dominated ecosystems to the global carbon budget in a future high-CO_2 environment, especially in the highly productive tropics, has been largely discounted or ignored.

It is now becoming increasingly clear that the response of C_4 species to elevated CO_2 is not as clearcut as previously thought (Henderson *et al.* 1994), and that many C_4 plants show significant photosynthetic and growth responses to CO_2. In a review of the literature, Poorter (1993) found an average growth enhancement of 22% for C_4 species, although this was reduced to 10% in an updated review (Poorter *et al.* 1996). Owensby *et al.* (1993) reported significant above-ground biomass increases in the C_4 component of a tall-grass prairie site exposed to elevated CO_2. This was explained by the reduced water loss under high CO_2 of C_4 species relative to competing C_3 species, especially during a dry year. However, there also appears to be a primary direct enhancement of photosynthetic activity in elevated CO_2 in a number of C_4 species (Sionit & Patterson 1984, Knapp *et al.* 1993), suggesting that the assumption of photosynthesis saturation at current CO_2 concentration may need to be re-evaluated.

Interacting environmental stresses can influence the response to elevated CO_2 in plants (Idso & Idso 1994, Lloyd & Farquhar 1996, Curtis 1996, Curtis & Wang 1998), and may do so differentially for different functional types. The literature of C_4 responses to elevated CO_2 shows that environmental factors, especially those known to be of importance to C_4 productivity and biogeographic distributions (high minimum temperatures and high light levels), may influence the relative CO_2-response. Responses of C_4 species under stressful conditions may not emerge clearly from experiments employing growth conditions optimal for C_3 plants. These factors could account for some of the poor responses to high CO_2 previously reported for environmentally controlled experiments, as opposed to significant

University of Cape Town

responses measured more recently under natural field conditions (Owensby *et al.* 1993).

The purpose of this review is to assess critically from the literature, using meta-analytic methods (e.g. Curtis 1996, Curtis & Wang 1998), the physiological and growth responses of wild C_4 grass species (family: Poaceae) to elevated atmospheric CO_2. To enable a critical test of current theories and perceptions, a similar literature review was carried out for the CO_2-responses of wild C_3 grass species (Poaceae). This provides a clear comparison, controlled for genotypic and morphological variability. The influences of exposure and growth conditions were also analysed, in order to determine whether current understanding of the relative responses of C_3 and C_4 species, and resulting uncritical extrapolation to natural environments, may be biased by experimental conditions very different from natural conditions.

2.2 Materials and Methods

2.2.1 Database compilation

The data analysed in this study were taken from published sources, and combined with a similar database compiled for the CO_2 Meta-Analysis Project, Ohio State University, USA. In cases where the two individual databases overlapped, data were used from the CO_2 Meta-Analysis Project only. Non-overlapping data were checked for consistency. The studies addressing C_4 pathway grass species that were included in this analysis were as comprehensive as possible for all years (1980-1997), while the studies addressing C_3 pathway grass species were as comprehensive as possible for 1991-1997, with most studies from 1980-1990 also included. The final database used for the meta-analysis comprised 64 papers (Appendix 1).

The following criteria were used for incorporation of studies in the database:

a) The species was wild or semi-wild, a member of the family Poaceae, and the photosynthetic pathway (C_3 or C_4) was either clearly stated or otherwise unambiguous.

b) Only data which included response means, sample sizes (N), and either standard deviation (SD) or standard error (SE) were used, since a weighted meta-analysis gives a more robust analysis than if resampling tests must be used to estimate variances or if

University of Cape Town

an unweighted analysis is used (Rosenberg *et al.* 1997).

c) The paper was published between 1980 and 1997.

d) The ambient CO_2 treatment concentration was between 300 and 400 μmol mol^{-1}, and the elevated CO_2 treatment was between 550 and 750 μmol mol^{-1}.

e) Data were presented for individual plants, or for individual species where plants were grown in stands or in mixture with other species.

f) Only absolute data were used, not relative data such as relative growth rates.

g) At least one of the following parameters was measured:

- A: Leaf-based light-saturated net CO_2 assimilation rates measured at the growth CO_2 concentration
- g_s: Leaf-based stomatal conductance measured at the growth CO_2 concentration
- WUE: Instantaneous leaf water use efficiency at the growth CO_2 concentration, either published as such or calculated from net CO_2 assimilation rates and transpiration rates
- TOTWT: Total plant biomass, either presented as such or calculated as the sum of above- and belowground biomass.
- AGWT: Aboveground biomass
- BGWT: Belowground biomass
- INDLA: Individual leaf area
- TILLERS: Number of tillers
- SLA: Specific leaf area either presented as SLA, or calculated as the inverse of specific leaf mass
- TNC: Concentration of total non-structural carbohydrates in leaves, either presented as such or calculated as the sum of total sugar and starch concentrations, and expressed on a dry mass basis
- N: Leaf total nitrogen concentration expressed on a dry mass basis

The responses at elevated and ambient CO_2 were extracted either from tables, or manually digitised from figures. Where the interaction between CO_2 treatments and deliberately imposed light, temperature, water, or nutrient stress treatments was reported, the

University of Cape Town

CO_2-response was entered separately under both levels of the stressful environmental factor. For those analyses testing a response to stress, all possible data in which plants were not stressed were included as controls for the meta-analysis ("no stress"), rather than only the data for non-stressed plants ("controls" within those studies) in studies reporting the response under intentional factorial stress treatments. This necessitated careful decisions about how to code some treatment responses. For example, "high nutrient levels" or normal nutrient levels (comparable to the field situation) were equivalent to "no nutrient stress", and "high light levels" or light levels which were deemed normal or sufficiently high, were similarly equivalent to "no light stress". In these instances, the level of stress was recorded as "none". Furthermore, only the extreme levels of any given stress were utilized. That is, only "low nutrients" (nutrient stress) and "high nutrients" (no nutrient stress, or normal) were included in the analyses, but not intermediate levels (e.g. "medium nutrient levels"). Studies which provided data on interactions with environmental stresses are identified in Appendix 2 (C_3) and Appendix 3 (C_4).

Where additional environmental stresses (such as salinity or ozone treatments) were imposed factorially, only the CO_2-response at the ambient, non-stressful level of this other factor was used. Unintentional stresses were not taken into account, except in the case of separately reported data for wet and dry years in some prairie studies. Where competition treatments were intentionally and differentially imposed, only the CO_2 response at the lowest level of competition was used.

Response parameters were combined whenever appropriate in order to overcome the problem of low sample sizes. For example, rather than differentiate between what some authors termed root biomass and others termed belowground biomass, these data were pooled and reported as belowground biomass. Thus, while some potential detail in the analysis is lost, the ability to generalize and distinguish among effects is generally improved (Gurevitch & Hedges 1993).

In order to test for potential influences of exposure methodology on the responses to elevated CO_2, the following categorical variables were assigned to each data entry:

(i) Pot size: ≤10 L, >10 L, or in-ground. These size classes have been previously used in a similar meta-analytic review (e.g. Curtis 1996).

University of Cape Town

(ii) Duration of exposure (from treatment initiation until measurement): ≤60 days, 61-120 days, >120 days. Where repeated measurements were taken, only the last measurement was used (usually at harvest). However, in some field studies showing marked seasonal responses, declining towards the end of the growth season, a single date at or just after the mid-season peak was chosen.

(iii) Exposure method: GC = indoor controlled-environment growth chamber, GH = outdoor enclosed mini-greenhouse or enclosed portion of greenhouse, OTC = open-top chamber in the field or greenhouse, FACE = free-air CO_2 enrichment.

2.2.2 Meta-analyses

Meta-analyses were conducted with MetaWin (Rosenberg *et al.* 1997), using the natural log of the response ratio (response in elevated CO_2/response in ambient CO_2) as the metric (Hedges *et al.*, in press). The mixed-effects model was used in this analysis, because of the large number of diverse studies examined and the assumption that there is random variation among studies in the effects of interest. Consequently, the confidence intervals generated are larger than those of a fixed-effects model, and as such represent potentially more conservative interpretation. In general, means of single response variables were considered significantly different from zero (significant response to elevated CO_2) if their 95% confidence intervals did not overlap zero. Similarly, means of two different response variables (e.g. stress treatment classes) were considered significantly different from each other if their 95% confidence intervals did not overlap. Some results are also discussed in terms of trends and tendencies in order to highlight interesting comparisons, even if they did not satisfy this statistical guideline. For a more detailed description of the statistical approach see Curtis & Wang (1998) and Hedges *et al.* (in press).

University of Cape Town

2.3 Results

Sample sizes for all variables presented in the Figures are given in Table 2.1.

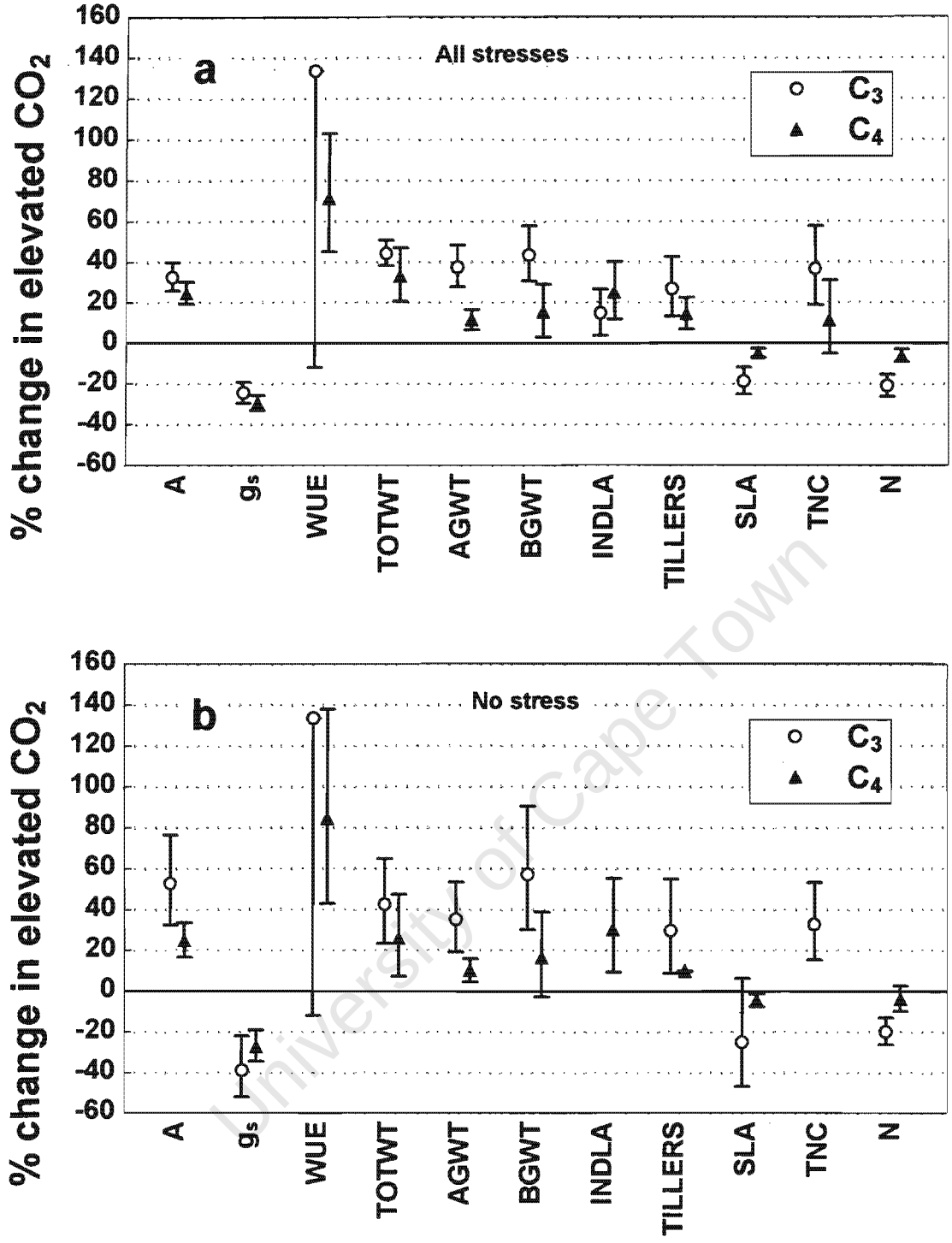
2.3.1 Relative CO_2-responses of C_3 and C_4 species

CO_2 responses of the full data set, including responses under interacting stress variables, are presented as the mean percentage change in elevated CO_2 (Fig. 2.1a). Net CO_2 assimilation rates (A) increased significantly in both C_3 and C_4 species, by 33% and 25%, respectively.

Table 2.1 Number of data entries (N) used for analyses of the CO_2-responses of C_3 and C_4 grasses. Abbreviations of variables as for Fig. 2.1. "None" refers to no stress; "low" or "high" refers to stress. For pot size, category numbers represent (1) <10 L (2) >10 L (3) in-ground. For duration, category numbers represent (1) <60 days (2) 61-120 days (3) >120 days. For method, category numbers represent (1) GC (2) GH (3) OTC (4) FACE.

Variable	Photo. pathway	CO_2-responses (Fig. 1)		Environmental stress (Fig. 2)				Exposure methods / Growth conditions (Figs 3-5)		
		All stresses	No stress	Light none;low	Temp. none; low/high	Water none;low	Nutrients none;low	Pot size cat.1;2;3	Duration cat. 1;2;3	Method cat. 1;2;3;4
A	C_4	48	15		38 ; 8	42 ; 6		34 ; - ; 12	29 ; 7 ; 12	29 ; 6 ; 13 ; -
	C_3	68	8				41 ; 19	27 ; 32 ; 9	55 ; 8 ; 5	50 ; 11 ; 3 ; 4
g_s	C_4	47	16		40 ; 5	41 ; 6	44 ; 3	35 ; - ; 10	28 ; 7 ; 12	27 ; 7 ; 13 ; -
	C_3	7	3					4 ; - ; -	4 ; - ; 3	4 ; - ; 3 ; -
WUE	C_4	13	7							
	C_3	2	2							
TOTWT	C_4	25	6	22 ; 3				20 ; - ; 2	18 ; 2 ; 5	
	C_3	71	9				54 ; 13	49 ; 17 ; 5	49 ; 13 ; 9	
AGWT	C_4	19	10			16 ; 3		19 ; - ; -	19 ; - ; -	18 ; - ; - ; -
	C_3	40	23		36 ; 3		37 ; 3	18 ; 13 ; 9	7 ; 15 ; 18	11 ; 10 ; 17 ; 2
BGWT	C_4	9	4					9 ; - ; -		
	C_3	30	8				21 ; 9	19 ; 10 ; -		
INDLA	C_4	14	5			10 ; 4		9 ; - ; 2	9 ; - ; 5	10 ; 2 ; 2 ; -
	C_3	8	0					6 ; - ; -	2 ; 2 ; 4	7 ; - ; - ; -
TILLERS	C_4	9	5						4 ; - ; 5	5 ; 2 ; 2 ; -
	C_3	12	6						2 ; 7 ; 3	8 ; - ; 4 ; -
SLA	C_4	19	6							
	C_3	21	5							
TNC	C_4	4	0							
	C_3	12	11							
N	C_4	15	5							
	C_3	37	10				22 ; 11			

University of Cape Town



University of Cape Town

Fig. 2.1 Comparative photosynthetic, growth, morphological and chemical responses of wild C_3 and C_4 grass species to elevated atmospheric CO_2 concentrations. **a:** full data set including responses under all levels of environmental interactions other than CO_2. **b:** selected data set of CO_2-responses under non-stressful environmental conditions. Abbreviations: A = net CO_2 assimilation rate; g_s = stomatal conductance; WUE = instantaneous leaf water use efficiency; TOTWT = total plant dry weight; AGWT = aboveground dry weight; BGWT = belowground dry weight; INDLA = individual leaf area; TILLERS = tiller number; SLA = specific leaf area; TNC = leaf total non-structural carbohydrate concentration; N = leaf total nitrogen concentration. No data were available for INDLA (C_3 species) and TNC (C_4 species) for non-stressful environmental conditions. Data represent percentage change in elevated CO_2 with 95% confidence intervals.

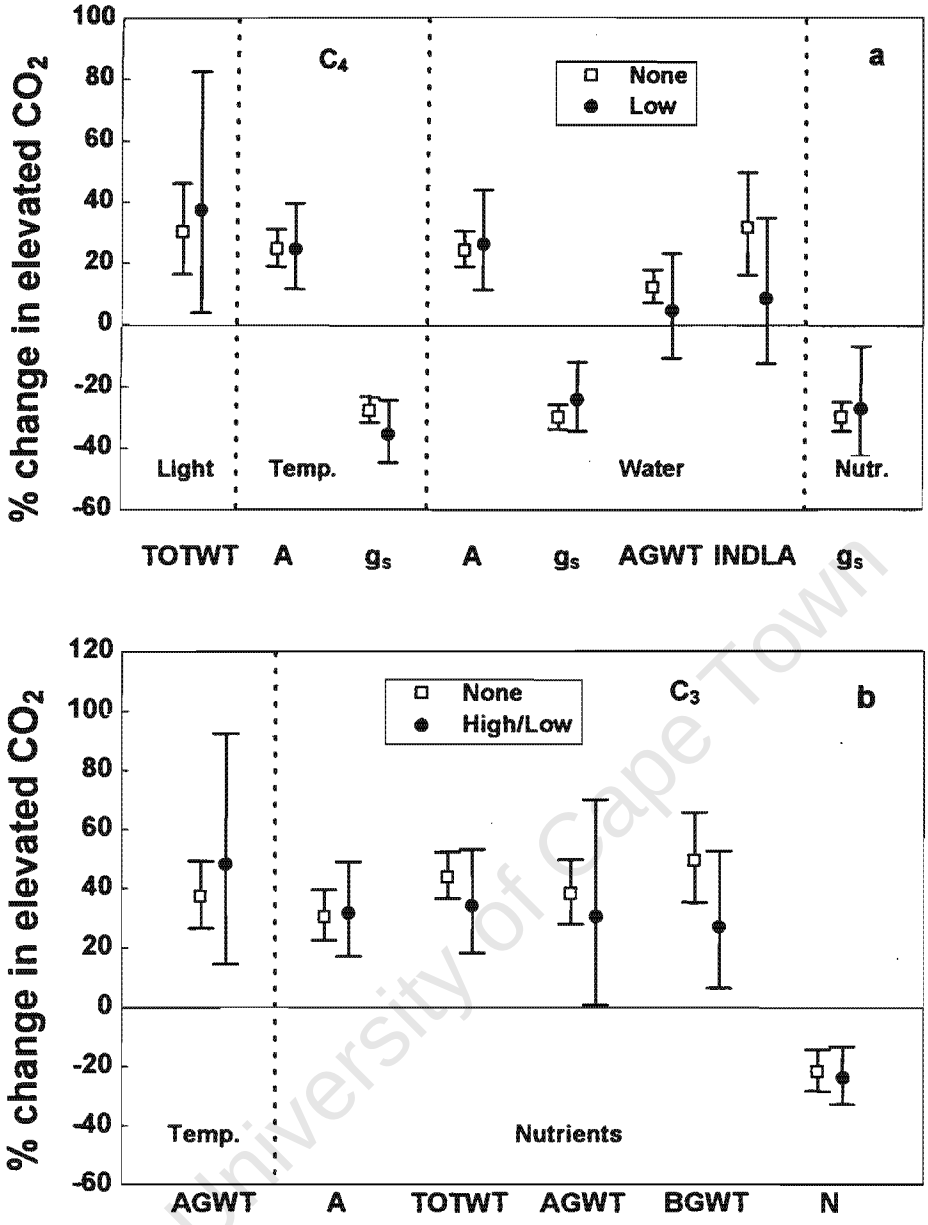
Stomatal conductances (g_s) decreased significantly by 24% and 29% for C_3 and C_4, respectively. Increases in instantaneous leaf water use efficiency (WUE) were significant only in C_4 species (72%); the sample size for C_3 was small and variability high. Total plant biomass (TOTWT) was enhanced in both C_3 (44%) and C_4 species (33%). C_3 species showed greater CO_2-induced increases in aboveground biomass (AGWT, 38%) and belowground biomass (BGWT, 44%), where these were reported individually, than C_4 species. This suggests a deficiency in data for C_4 biomass partitioning into above- and belowground components, as the smaller effect here does not concur with the larger positive result for TOTWT. Due to reporting shortcomings, the data set for above- and belowground biomass was often drawn from a different set of publications than that for total plant biomass, likely contributing to the lack of correspondence between the results for the three variables. Individual leaf area (INDLA) increased by 15% and 25% and tiller numbers increased by 27% and 14% in C_3 and C_4 species, respectively. C_3 species showed greater decreases in specific leaf area (SLA, 19%) and foliar total nitrogen (N) concentrations (21%) than C_4 species. Only C_3 species showed significantly increased foliar total non-structural carbohydrate (TNC) concentrations (37%) in elevated CO_2.

2.3.2 Influence of environmental stress

Under non-stressful growth conditions (Fig. 2.1b), the relative mean stimulation of photosynthetic rate in C_3 species increased from 33% to 53%, and belowground biomass enhancement rose from 44% to 57%. However, both changes were not significant according to the 95% confidence interval (CI) overlap test. The CO_2-responses of all other variables remained similar compared to the "all stresses" analysis (Fig. 2.1a). By contrast, when interacting stresses were removed from the database for C_4 species, the response of total biomass to elevated CO_2 decreased from 33% to 26%, the tillering response was reduced (from a 14% increase to a 10% increase), but stimulation of individual leaf area rose from 25% to 30%. Again, these responses were not significant according to the CI overlap test.

The influence of environmental stresses was further explored by comparing the CO_2-responses of deliberately stressed plants with the responses of all other plants (not deliberately stressed) for each stress variable individually (Fig. 2.2). Only results which can be interpreted with reasonable confidence, taking into account the sample size (>2), the confidence interval, and the power to draw robust statistical conclusions, are presented. For example, no studies

University of Cape Town



University of Cape Town

Fig. 2.2 CO_2-responses of wild C_4 (**a**) and C_3 (**b**) grass species as influenced by interaction with low light, low or high temperature, low water, or low nutrient stress. Abbreviations as for Fig. 2.1. Data represent percentage change in elevated CO_2 with 95% confidence intervals.

addressing the interactions between elevated CO_2 and light or water stress in C_3 grass species existed or were suitable for use in the meta-analysis, and in many other cases the number of studies for a particular measurement category and stress factor were too small, or did not exist.

In C_4 species (Fig. 2.2a), low light, low temperature, or low nutrient supply levels did not alter the mean responses to elevated CO_2. Droughting treatments (low water supply) similarly did not alter the CO_2-responses of gas exchange in C_4 species, but resulted in the loss of a significant CO_2-response for aboveground biomass and individual leaf area (95% confidence intervals overlap zero), compared to plants that were not water stressed. There were no data for C_4 plants under high temperature stress, probably because high temperatures are not regarded as being potentially harmful to C_4 plants compared to C_3 plants. In C_3 species (Fig. 2.2b), on the other hand, abnormally high temperatures increased the mean aboveground biomass response to elevated CO_2, but this tendency was not significant. Low nutrient stress did not alter the mean CO_2-response of photosynthesis and leaf nitrogen concentration in C_3 species. By contrast, mean CO_2-induced increases in total plant, above- and belowground biomass tended to diminish under low nutrient supply levels, although these changes were not significant.

2.3.3 Exposure methods and growth conditions

The effects of exposure methods and growth conditions on the relative responses to elevated CO_2 are presented in Figs 2.3-2.5. Those variables for which data for at least two categories were available for either the C_3 or C_4 data set, and which had reasonable sample sizes and the potential for meaningful statistical inferences, are presented. These are matched with the results for the corresponding variable in the other (C_3 or C_4) data set, even if the sample sizes are small and categories missing. This was done in order to allow at least a rudimentary comparison between C_3 and C_4 species. Even though this comparison is largely fragmentary, it exposes gaps in the knowledge base, particularly with regard to the lack of information from long-term studies in the field, and could provide a guideline for future studies (and publication of existing data).

An increasing volume of available rooting space ("pot size", Fig. 2.3) allowed for slightly greater CO_2-induced increases in photosynthetic rate in C_3 species, although not

University of Cape Town

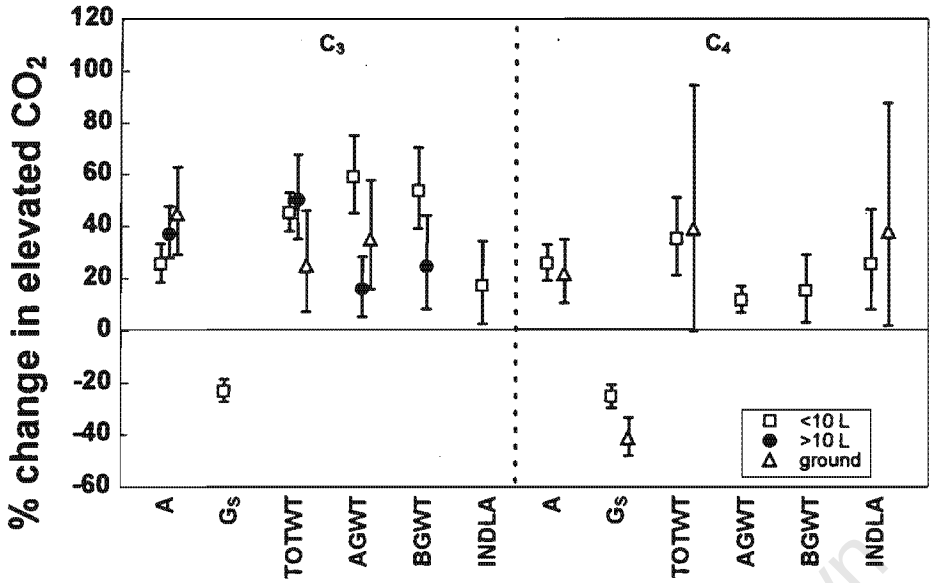


Fig. 2.3 Effect of pot size on CO_2-response of C_3 and C_4 grass species. Legend refers to pot size in L, or plants grown in-ground. Abbreviations as for Fig. 2.1. Data represent percentage change in elevated CO_2 with 95% confidence intervals.

University of Cape Town

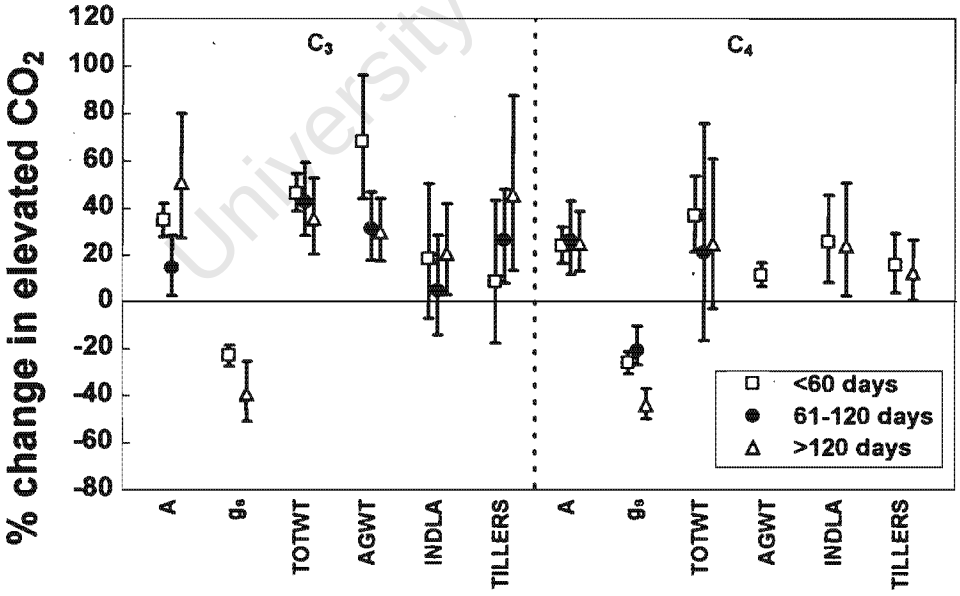


Fig. 2.4 Effect of duration of exposure on the CO_2-response of C_3 and C_4 grass species. Abbreviations as for Fig. 2.1. Data represent percentage change in elevated CO_2 with 95% confidence intervals.

significantly according to the CI overlap test. The mean photosynthetic CO_2-response in C_4 species was not altered. Stomatal conductances were reduced more strongly in C_4 species growing in open ground than in small pots. Large positive responses in biomass in C_3 species were favoured by growth in smaller rooting volumes, as supported by no or only minimal overlap in the confidence intervals between pot size classes. This effect on biomass was not discernible in C_4 species, although interpretation here is strongly limited by insufficient sample sizes. Significant increases in individual leaf area in C_4 species in high CO_2 were measured in plants growing both in small pots and in the ground.

The mean CO_2-responses for photosynthetic rate and conductance in C_3 species were greater in the longer term (>120 days, Fig. 2.4) than in the medium term (61-120 days). The mean aboveground biomass response, by contrast, was greatest in the short term (<60 days). The positive tillering response to elevated CO_2 achieved significance (95% confidence interval not overlapping zero) only after 60 days of exposure to high CO_2, and continued to increase with experimental duration, although not significantly. In C_4 species, relative decreases in conductance were significantly greater in the longer term (>120 days). The biomass response to elevated CO_2 appeared to decrease somewhat after 60 days (although small sample sizes and large confidence limits preclude a clear interpretation), so that mean biomass increases were no longer significant after 60 days (confidence intervals overlap zero). Similar increases in leaf area in elevated CO_2 were found both in the short and longer term.

Increases in photosynthetic rates in elevated CO_2 appeared to be greatest when C_3 species were grown in open-top chambers (73%), and smallest in FACE systems (12%, Fig. 2.5), although the latter is probably attributable to the generally lower CO_2 concentrations used in these systems than in the other types of growth facilities. In both C_3 and C_4 species, mean decreases in conductances were greatest in OTCs. Aboveground biomass increases were found for all exposure methods in C_3 species, but responses were particularly high in growth chambers (95% increase in GC compared to 23-32% increases in other facilities). The mean tillering response was greater in OTCs (45% increase) than in growth chambers (18%). In C_4 species, CO_2-induced increases in photosynthetic rates were similar in all exposure facilities. Leaf area increases were found for most exposure methods (with the possible exception of greenhouses, where confidence intervals overlapped zero), and the tillering response to

University of Cape Town

elevated CO_2 was small for all methods and not significant in GHs and OTCs (CI overlapped zero) , but in both cases interpretation was hampered by insufficient sample sizes.

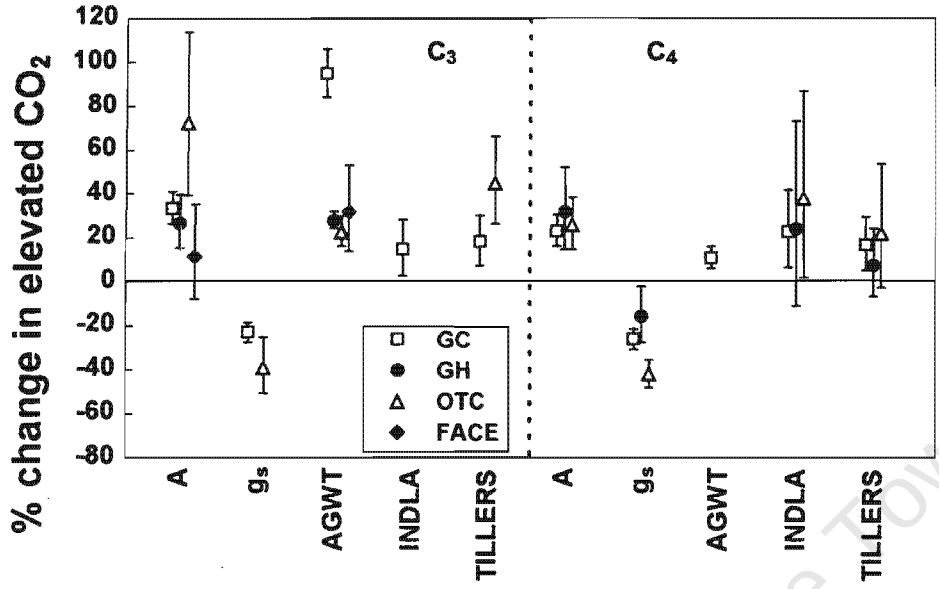


Fig. 2.5 Effect of exposure method on the CO_2-response of C_3 and C_4 grass species. Abbreviations as for Fig. 2.1. In the legend, GC = growth chamber; GH = greenhouse; OTC = open-top chamber; FACE = free-air CO_2 enrichment. Data represent percentage change in elevated CO_2 with 95% confidence intervals. Note that GC, GH and OTC treatments typically have 2x ambient [CO_2] (650-700 µmol mol^{-1}), and that FACE is typically about 550 µmol mol^{-1} [CO_2].

University of Cape Town

2.4 Discussion

2.4.1 Relative CO_2-responses of C_3 and C_4 species

The results of this meta-analysis confirm the widely-held view that the relative responses of C_4 species to elevated CO_2 are usually smaller than those for C_3 species, especially for growth under non-stressful environmental conditions. Nevertheless, differences in CO_2 response between C_3 and C_4 grass species are not as large as current perceptions have it. A similar conclusion was drawn from a previous semi-quantitative minireview of C_3 versus C_4 responses (Poorter 1993). The present analysis shows that C_4 grasses are certainly responsive to elevated CO_2 particularly with regard to gas exchange and leaf area development.

Photosynthetic stimulation of C_4 species is, surprisingly, comparable to that of C_3 species. This contradicts the general view that C_4 photosynthesis does not increase in elevated CO_2, due to the specialized CO_2 concentrating mechanism in C_4 leaves (Bowes 1993). Many of the definitive early studies of C_4 photosynthesis were performed on crop species, such as maize, which appears to be CO_2-saturated at ambient CO_2 levels and shows very low responsiveness to higher CO_2 concentrations, compared to wild C_4 species (Ziska & Bunce 1997). A closer examination of gas exchange in other C_4 grasses reveals that photosynthesis is not necessarily saturated at current CO_2 levels and can increase at higher c_i (Sionit & Patterson 1984, Imai & Okamoto-Sato 1991). This simple explanation may account for the majority of cases of photosynthetic stimulation in C_4 species. For example, LeCain & Morgan (1998) showed that photosynthesis was not saturated at ambient CO_2 concentration in any of the six wild C_4 grass species studied. Ziska & Bunce (1997) similarly measured higher photosynthetic rates at elevated CO_2 in 8 out of 10 C_4 species studied, due to the effect of increased c_i. Another study has shown that under favourable growth conditions (high light), the operational c_i of a tropical C_4 grass was close to the inflection point of the A/c_i response, so that increasing CO_2 supply caused slight enhancements in the CO_2 assimilation rates, and improved growth (Ghannoum *et al.* 1997). On the other hand, under growth-limiting conditions (low light), the operational c_i was well above the CO_2 saturation level for photosynthesis, and no photosynthetic or growth response to elevated CO_2 was measured. The relative "saturation level" may, therefore, change with changing conditions, and this may play a role in photosynthetic responses to elevated CO_2 in wild C_4 grasses.

Stimulation of photosynthetic rates due to increases in c_i represents a simple short-term effect. In addition, longer term biochemical changes, such as altered enzyme efficiencies, or altered regeneration rates of PEP or RuBP, may develop in elevated CO_2. These are termed "regulatory" or "acclimatory" responses and change the shape of the A/c_i response. Unlike for many C_3 species (Wullschleger 1993), instances of up- or downregulation of photosynthetic capacity in C_4 species have not been given much attention in the literature as they have appeared to be rare. Sage (1994) concluded that little adjustment is found in the A/c_i response in C_4 species under elevated CO_2 (see also Ziska & Bunce 1997), except possibly downregulation under conditions of nutrient deficiency (Wong 1979, Morgan *et al.* 1994, Ghannoum & Conroy, 1998). Nevertheless, photosynthetic downregulation has also been measured under conditions not apparently stressful (Read *et al.*

University of Cape Town

1997, LeCain & Morgan 1998). Many earlier reports of unchanged or reduced photosynthetic rates in C_4 species in elevated CO_2 may have been due to the acclimation phenomenon, but are not identifiable without full A/c_i measurements (e.g. Wray & Strain 1986). The mechanisms of photosynthetic acclimation in C_4 species are apparently not related to feedback inhibition resulting from carbohydrate (TNC) accumulation, or to reductions in leaf nitrogen (N) concentrations (LeCain & Morgan 1998, Read *et al.* 1997), as they are in C_3 species (Stitt 1991, Sage 1994, Cotrufo *et al.* 1998). The meta-analysis confirmed that accumulation of TNC and reductions in leaf N in elevated CO_2 are insignificantly low in C_4 species. There is currently no available information on the possible mechanisms of photosynthetic acclimation in C_4 species, and this warrants further attention.

Natural seasonal dynamics of photosynthetic capacity in C_4 species may also influence the response to elevated CO_2. Significant increases in CO_2 assimilation rates in the salt marsh C_4 species *Spartina patens* in high CO_2 were confined to the early season, when absolute rates of assimilation were highest (Ziska *et al.* 1990). Knapp *et al.* (1993) have also reported upregulation in *Andropogon gerardii* (big bluestem), and Chen *et al.* (1994) modelled this response. Recent reports indicate that C_4 photosynthetic physiology may change with progressive developmental stages, showing more similarities with C_3 physiology (lower CO_2-concentrating ability) when leaves are young or senescent, compared to mature leaves (Dai *et al.* 1995, He & Edwards 1996). This was tentatively proposed as an explanation for ontogenetic shifts in CO_2-responsiveness (Ghannoum *et al.* 1997), but subsequent work on C_4 grass species does not support this explanation, as C_4 photosynthetic characteristics were already fully developed in young leaves (Ghannoum *et al.* 1998). In conclusion, this aspect of C_4 response to elevated CO_2 requires more attention, as conflicting evidence also exists. For example, photosynthetic downregulation was found in both young and older *Bouteloua gracilis* (blue grama) plants (Read *et al.* 1997). Furthermore, seasonal dependencies of C_4 growth responses, in contrast with responses of carbon assimilation, are not evident in many field-based elevated CO_2 experiments (Curtis *et al.* 1989, Kirkham *et al.* 1991, Hamerlynck *et al.* 1997).

The issue of whether biochemical differences between C_4 photosynthetic subtypes may shed light on the reasons for interspecific differences in CO_2 responsiveness (Henderson *et al.* 1994) is beyond the scope of the present review, but some brief comments can be made.

University of Cape Town

The three C_4 subtypes (NADP-ME (NADP-malic enzyme), PCK (phosphoenolpyruvate carboxykinase), and NAD-ME (NAD-malic enzyme)) exhibit increasing levels of "leakiness" to CO_2 from the bundle sheath to the mesophyll, in the above order (Hattersley 1982, Furbank & Hatch 1987, Jenkins *et al.* 1989, Brown & Byrd 1993, but see Hatch *et al.* 1995). This amounts to a loss of between 10 and 40% of carbon fixed by PEP carboxylase, which could, conceivably, be counteracted by increased CO_2 supply from the atmosphere. A recent study investigating the relative responsiveness of the subtypes to elevated CO_2 yielded counter-intuitive results, with the least "leaky" NADP-ME showing the largest responses (LeCain & Morgan 1998). Nevertheless, this line of investigation may well contribute to an improved understanding of photosynthetic responses of C_4 plants to elevated CO_2.

Elevated CO_2 has significant positive effects on plant water relations in both C_3 and C_4 grass species, via reductions in stomatal conductance (g_s). In fact, this response, coupled with reduced transpirational water loss and the corresponding increases in WUE, are probably the most ubiquitous responses to elevated CO_2 for almost all plant functional types (Gifford & Morison 1985, Chaves & Pereira 1992, Tyree & Alexander 1993). C_4 and C_3 responses in g_s to elevated CO_2 were similar (Fig. 2.1a), but clear interpretation is hampered by the fact that fewer data are available for C_3 Poaceae. The decrease in g_s in C_4 species is consistent across a range of environmental stresses, but greatest in plants grown in the ground and exposed to elevated CO_2 for more than 120 days. This suggests that developmental changes in g_s, possibly related to altered stomatal sizes or densities, may occur as leaves mature in high CO_2. Information on long-term changes in g_s (e.g. changing stomatal densities) is limited (Ghannoum *et al.* 1997). It is generally thought that CO_2-induced reductions in g_s are primarily short-term effects, but some researchers have measured acclimatory responses in stomatal physiology in C_4 species, as shown by the responses of conductance to increasing CO_2 (g_s/c_i curves) (Morgan *et al.* 1994, Read *et al.* 1997, LeCain & Morgan 1998). This can take the form of either up-regulation (higher g_s at equivalent c_i for leaves grown in elevated CO_2), or downregulation (reduced g_s at low c_i in elevated CO_2). Reduced transpirational water loss in elevated CO_2 and the resulting improvement in soil water content over the course of the growing season, as reported for the tall-grass prairie (Kirkham *et al.* 1993), are likely to be reflected in longer term changes in stomatal conductances.

The stimulation of C_4 whole plant growth under elevated CO_2 (mean of 33%, 95%

University of Cape Town

confidence interval 21%-47%) is slightly higher than the 22% reported by Poorter (1993), and considerably higher than Poorter's updated estimate of 10% (Poorter *et al.* 1996). Growth stimulation could be either a direct effect of greater carbon assimilation rates (discussed above), or an indirect effect of improved soil and leaf water relations resulting from reduced stomatal conductances and transpirational water loss (Knapp *et al.* 1993, Owensby *et al.* 1993). Cell elongation and blade extension rates in developing grass leaves are positively correlated with leaf water potentials (Boyer 1970, Toft *et al.* 1987). Although not included in the meta-analysis, the C_4 literature database clearly showed a consistent and significant positive increase in shoot water potentials in grasses exposed to elevated CO_2 (e.g. Kirkham *et al.* 1993, Hamerlynck *et al.* 1997).

The growth response of C_3 species in this review is a little larger than that for C_4 species (44%) and comparable to the C_3 herbaceous monocot component of Poorter's 1993 database (42%). This supports Poorter's original conclusion that differences in growth stimulation between C_3 and C_4 plants are probably not as large as suggested by current perceptions. Unfortunately, the responses for above- and belowground biomass in C_4 species in this meta-analysis do not match those for whole-plant biomass, and care must be taken in interpretation. Also, many field studies using C_4 species have not reported biomass responses adequately, probably due to logistical difficulties and an unwillingness to disturb the ecosystem in longer term experiments. More information is needed on whether increased carbon assimilation rates will lead to sustained enhanced biomass production in C_4-grass-dominated ecosystems such as prairies and savannas, which comprise a large percentage of productive land surface (Hall *et al.* 1995). This would help to improve our models of global carbon dynamics and clarify the potential of C_4-grass-dominated ecosystems as significant carbon sinks.

An interesting contrast emerged regarding the morphological development of C_3 and C_4 species under elevated CO_2. C_3 species generally develop more tillers, with only small increases in leaf area, but decreased specific leaf areas (increased leaf density or thickness). C_4 species, on the other hand, appeared to respond mainly with increased leaf areas, and smaller increases in tiller numbers. This contrast may indicate a greater sensitivity in C_4 species to self-shading of the basal nodes from which tillers are initiated (Deregibus *et al.* 1985, Everson *et al.* 1988), and may provide the mechanism for growth stimulation even

University of Cape Town

under moderate photosynthetic enhancement. Gradually increasing canopy leaf areas, leading to a progressive increase in whole-canopy carbon assimilation rates, would result in a continuously greater supply of carbon products to support enhanced growth. Early increases in leaf area, leaf area duration (the cumulative leaf area over the growth period), leaf area ratio (the proportion of leaf area to plant biomass), plant height and total plant biomass of C_4 species in elevated CO_2 have been reported by Patterson & Flint (1980), Riechers & Strain (1988) and Ackerly *et al.* (1992). Early responses in biomass and leaf area, which persist for the whole growth period, have also been found for some C_3 species (Bowler & Press 1993), but the stimulation of leaf area, in particular, appears to be characteristic of the CO_2-response of C_4 species. Coleman & Bazzaz (1992) and Ackerly *et al.* (1992) came to the conclusion that standing photosynthetically active leaf area (net leaf area production and loss) in a C_4 species was the primary influence on growth responses in elevated CO_2.

2.4.2 Influence of environmental stress and growth methodology

Environmental stresses tend to reduce (although not significantly) the potential CO_2-response in C_3 species, as evidenced by the suppression of mean photosynthetic and belowground biomass responses when all stresses are included in the analysis (Figs 2.1a, b), as well as the reductions in mean growth responses when nutrients are limiting (Fig. 2.2b). By contrast, C_4 species were generally not negatively impacted by environmental stresses, although leaf area stimulation was sensitive to water stress (Fig. 2.2a). The current view that CO_2-responses in C_4 graminoids are particularly marked under conditions of water stress (Nie *et al.* 1992, Knapp *et al.* 1993, Owensby *et al.* 1993, 1996b, Ham *et al.* 1995) was not borne out by the meta-analysis, possibly due to the lack of data suitable for inclusion in the database. Many of these studies do not present plant- and leaf-level responses, concentrating on canopy-level gas fluxes instead.

C_4 graminoids may well benefit from increased CO_2 supply under some stressful environmental conditions, such as low soil fertility. However, there is a lack of information about the changes in plant nitrogen use efficiency in C_3 and C_4 graminoids in elevated CO_2. This understanding may be critical in predicting changes in their relative competitive abilities, as it has been suggested that competitive advantage, especially in grasslands, may be due to a greater ability to extract nitrogen from the soil (Tilman 1990). This in turn may be associated with greater plant and photosynthetic nitrogen use efficiency (Richardson *et al.*, in press).

University of Cape Town

Contrary to strong evidence for non-graminoid C_3 species showing reduced CO_2-responsiveness in small rooting volumes (Arp 1991, McConnaughay *et al.* 1993, 1996), C_3 grass species were more responsive in small pots, with respect to above- and belowground growth. In C_4 species, rooting volume did not appear to play a role in the biomass response to CO_2, but more data are needed to confirm this as the sample size was too small for a confident interpretation. Nevertheless, field studies with C_3 species have not supported the expected significant productivity increases based on earlier pot studies, and predictions of competitive advantages over C_4 species should be cautiously reviewed. In addition, aboveground biomass enhancement of C_3 species diminished with increasing duration of exposure to elevated CO_2, even though tiller number appeared to be stimulated, which suggests that individual tiller size is progressively reduced in this group.

In conclusion, responses to elevated CO_2 in wild C_4 and C_3 Poaceae at the leaf and whole plant levels are summarised in Fig. 2.6, and demonstrate many trends common to both photosynthetic types. At the leaf level, the greater carbohydrate accumulation and greater reductions in leaf nitrogen concentration in the C_3 type alone differentiated the types, and constituted the only evidence for so-called "sink limitation" which is often invoked in elevated CO_2 studies on C_3 dicots. Average photosynthetic responsiveness did not concur with predictions based solely on photosynthetic theory. However, at the shoot level, there were clearcut differences between types resulting from disparate effects on aboveground morphologies. These, rather than photosynthetic differences between the types, might be of greater importance when evaluating responses to elevated CO_2.

The combined shoot structural and water use efficiency responses of these functional types are likely to have consequential implications for the water balance of important catchments and rangelands throughout the world, especially in semi-arid subtropical and temperate regions. Improved water relations would be highly beneficial to C_4 grasses growing in marginal semi-arid sites where growing season may be limited by soil water availability, such as over much of southern Africa and parts of North America. The results of this meta-analysis suggest that it may be premature to predict that the C_4 type will lose its competitive advantage in certain regions as CO_2 levels rise, based solely on differential photosynthetic mechanisms (Collatz *et al.* 1998).

University of Cape Town

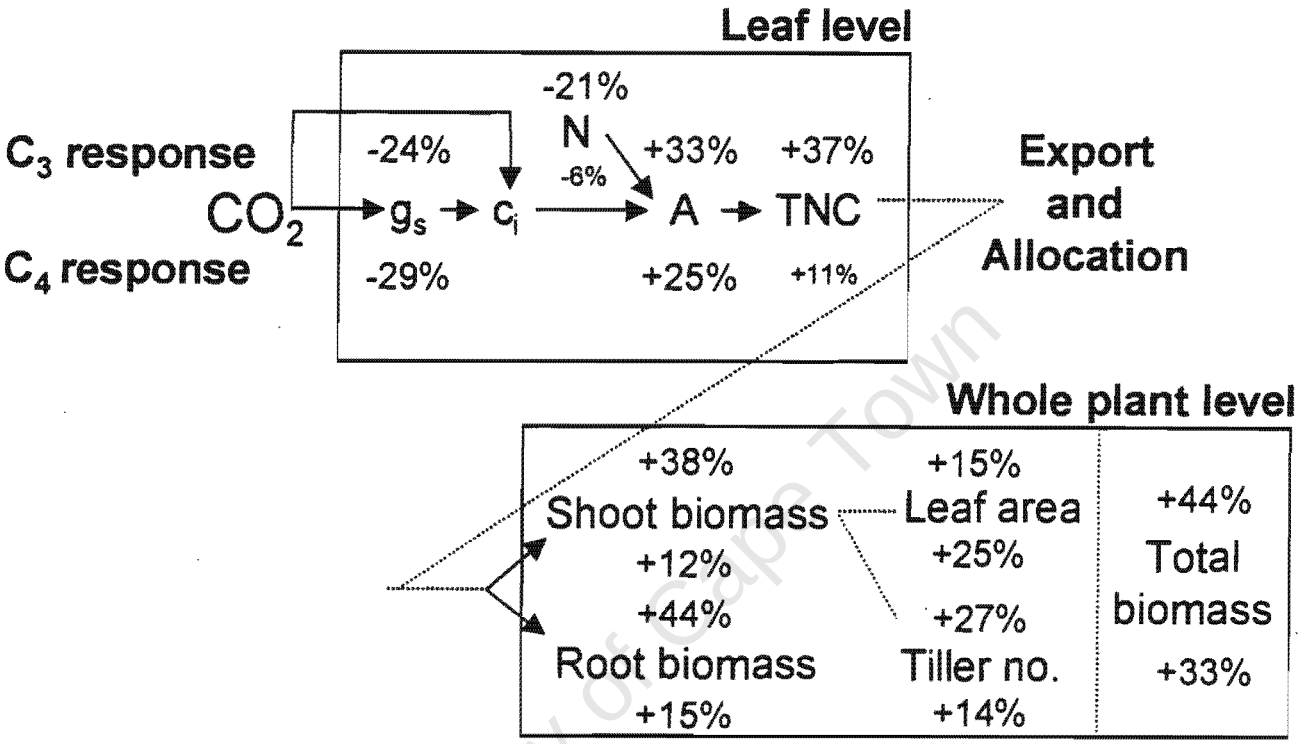


Fig. 2.6 Summary scheme of the CO_2-response levels (% change) and their relationships under all conditions (full data set). C_3 responses are given above and C_4 responses given below the stated parameter. Response levels printed in small font are not significantly different from zero. Abbreviations as for Fig. 1, and c_i = intercellular CO_2 concentration.

University of Cape Town

Chapter 3

Materials and Methods: Greenhouse Elevated CO_2 Experiments

3.1 Introduction

Most of the experimental work presented hereafter was performed in the Hill Greenhouse, part of the research greenhouse complex of the National Botanical Institute, Kirstenbosch, Cape Town. This greenhouse is covered with translucent polycarbonate which allows transmittance of ca. 85% of incident visible radiation (>400 nm), and greenhouse temperature is controlled at below about 28°C by means of a fan and a wetwall.

The methods described in this chapter cover the general experimental setup, including open-top chamber design and calibration, and the environmental conditions created inside the open-top chambers housed in the greenhouse. More detailed descriptions of experiment-specific methods of plant establishment and fertilisation, experimental design and treatments other than elevated CO_2, average temperatures and irradiance, measurement techniques, and statistical analyses are described in individual chapters.

Four experiments were carried out in the greenhouse during the course of four years. They are summarised in Table 3.1.

University of Cape Town

3.2 Open-top chambers

The open-top chambers were designed by Midgley (1996), based on the design of Rogers *et al.* (1983). Eight hexagonal open-top chambers were constructed of polycarbonate (1.8 mm thick), measuring 0.58 m in diameter from point to point, and 0.50 m in height, and which could cover 12 pots (0.1 m diameter, 0.5 m long). Each chamber was topped with a removable polycarbonate frustum of 20 mm width. The eight open-top chambers were placed in pairs on four tables along the length of the east-facing greenhouse wall (Fig. 3.1). Air was drawn into each chamber from outside the greenhouse by means of a 12V D.C. brushless fan (model FP-108, Commonwealth, Taiwan) positioned inside a 0.5 m-long square polycarbonate box, which in turn was connected to flexible piping inserted through holes in the greenhouse wall. The air was then circulated in a plenum surrounding the base of the

Table 3.1 Summary of elevated CO_2 experiments carried out in the greenhouse. Ecotypes refer to origin of plant material.

Exp.	Species	Treatments	Propagation and origin	Growth medium	Duration	Chapters in which detailed methods and data are presented
1	*Themeda triandra* (ecotype 1) *Acacia karroo*	elevated CO_2/ increased UV-B radiation interaction	from seed - Eastern Cape (Döhne)	hydroponic sand culture	13 April - 10 November 1994 (*Themeda*) 29 April - 28 November 1994 (*Acacia*)	4, 9
2	*Themeda triandra* (ecotype 2)	elevated CO_2	from seed - Transvaal Lowveld (Kruger Park)	hydroponic sand culture	23 October 1995 - 9 February 1996	9, Appendix 4
3	*Themeda triandra* (ecotype 2)	elevated CO_2/ defoliation interaction	from seed - Transvaal Lowveld (Kruger Park)	hydroponic sand culture	28 May - 23 December 1996	5, 6
4	*Alloteropsis semialata* subsp. *eckloniana* (C_3) *Themeda triandra* (C_4 NADP-ME) *Andropogon appendiculatus* (C_4 NADP-ME) *Digitaria natalensis* (C_4 NADP-ME) *Eragrostis curvula* (C_4 NAD-ME) *Eragrostis racemosa* (C_4 NAD-ME) *Sporobolus pyramidalis* (C_4 PCK) *Melinis repens* subsp. *repens* (C_4 PCK)	elevated CO_2 - differing photosynthetic pathways	tufts excavated from the field - southern Kwazulu/ Natal (Paddock area)	mix of field-excavated soil and Kirstenbosch potting soil	3 October 1997 - 30 March 1998	7, 9

University of Cape Town



Fig. 3.1 View of experimental setup in the greenhouse.

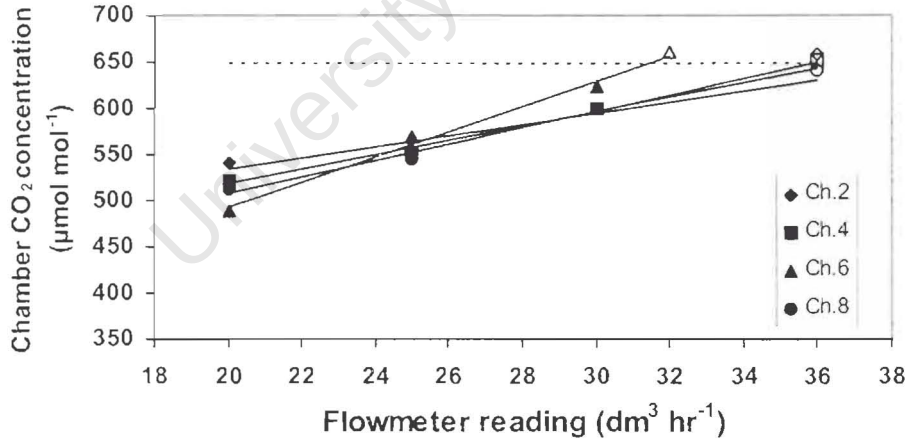


Fig. 3.2 CO_2 calibration of four elevated CO_2 chambers (Chambers 2, 4, 6 and 8) on 16 May 1994. Chamber CO_2 concentration is plotted as a function of the flow rate of CO_2, and extrapolation is used to achieve the target concentration of 650 µmol mol^{-1} (dotted line). Open markers indicate the final CO_2 concentration at the flowmeter set point.

University of Cape Town

chamber. The inner wall of the plenum was perforated by holes which allowed air to enter the chamber at equal rate from all directions.

3.3 CO_2 fertilization and calibration

In each pair of adjacent chambers, the air entering one chamber was enriched with CO_2 gas 70 mm upstream of the fan to give the desired average CO_2 concentration of between 650 and 700 μmol mol^{-1} throughout the chamber. The CO_2 (industrial grade, Afrox Pty Ltd., Cape Town and Fedgas Pty Ltd., Cape Town) was supplied by two banks of four 37 kg cylinders, connected to a manifold. The CO_2 gas contained low concentrations of impurities, with ethylene estimated to account for less than 0.001% v/v (personal communication from gas company). The CO_2 supply rate was controlled by means of needle valve flow controllers (model DK800, Krohne, Germany), calibrated at the start of each experiment, and re-adjusted to set point when necessary. Calibrations were checked once or twice during the course of the experiment but did not require significant adjustment. A float metering valve with pressure regulator (model DK800RA, Krohne, Germany) minimised fluctuations in flow rates due to diurnal temperature changes. The air in the other chamber of each pair remained at ambient CO_2 concentration (about 350-360 μmol mol^{-1}). The choice of using a double-ambient CO_2 concentration for the high CO_2 treatment conforms with the majority of similar studies worldwide (Cure & Acock 1986, Curtis & Wang 1998) and allows comparisons to be drawn with the international literature (see literature review and meta-analysis in Chapter 2).

The four elevated CO_2 chambers were calibrated using a portable infra-red gas analyzer (IRGA, LI-6200, Li-Cor, Lincoln, NE, USA). The IRGA had been calibrated previously with a certified gas standard which had been independently checked using the LI-6000-01 gas calibration cylinder (Li-Cor, Lincoln NE, USA). Measurements (average concentration during a 30 s period) were made at plant height at either six or twelve positions throughout the chamber in order to characterize the spatial variability. Using the calibration for Experiment 1 (16 May 1994) as an example (Fig. 3.2), average chamber CO_2 concentrations at six positions were plotted against flowmeter readings at full fan speed (12 V) ranging from 20 to 30 dm^3 hr^{-1}. Extrapolation to the target CO_2 concentration (650 μmol mol^{-1}) gave the required flow rates for each chamber. After flow rates were set to this value (flowmeter readings of about 32 dm^3 hr^{-1} for chamber 6, and 36 dm^3 hr^{-1} for chambers 2,4 and 8), measured CO_2 concentrations were 659 ± 34 (chamber 2), 653 ± 66 (chamber 4), 661 ± 55 (chamber 6) and 641 ± 47 (chamber 8) μmol mol^{-1} (open markers in Fig. 3.2). The errors around these means represent the spatial variability within chambers, which was

University of Cape Town

considered acceptable. Calibration of subsequent experiments yielded reduced spatial variability (see sections 5.2.2 and 7.2.2), possibly due to improved control of gas supply and more intensive sampling methods. Nevertheless, the potential effect of this variability was minimised by randomisation of pots within chambers on a monthly basis.

3.4 Environmental monitoring of chambers

During the course of most experiments, solar radiation flux density measurements were collected by means of a tube solarimeter (Delta-T Devices Ltd., Cambridge, UK) placed on the table surface (soil level) in one of the chambers and connected to a data logger (MC Systems, Cape Town). Although the solarimeter was positioned perpendicularly to the sun, most radiation was diffuse due to scattering by the greenhouse roof and chamber walls. Air temperatures were monitored using thermistors, connected to the same data logger. Six thermistors were calibrated together before the start of each experiment, so that their readings did not deviate more than 0.6°C from each other. Four thermistors were installed below white aluminium radiation shields at mid-chamber height in a subset of chambers, one thermistor was installed beneath the tables supporting the chambers, and one outside the greenhouse at the height of the chamber air inlet pipes (about 70 cm above the ground, also below a white radiation shield). Air temperatures were measured every minute, and mean, minimum and maximum temperatures recorded every three hours.

The temperature data of Experiment 2 (Fig. 3.3) and Experiment 3 (Fig. 3.4) illustrate the general seasonal temperature trends and chamber effects during summer and winter/spring, respectively. During early summer, midday differences in air temperature between the warmest and coolest of the four measured chambers averaged 1.8°C, increasing gradually to about 3.8°C during mid-summer (Fig. 3.3a). This may have been caused by a slight temperature gradient from the northern to the southern end of the greenhouse wall, but any potential effect on treatments was probably overcome by regular randomisation of tables, and by the fact that CO_2 treatments were stratified from one end of the array to the other. Also, this represents the worst-case scenario, as it is based on the warmest three-hour period of the day. Average midday chamber air temperatures in summer were generally 7-11°C higher than average midday ambient air temperatures outside the greenhouse (Fig. 3.3b). Plants therefore experienced midday temperatures of about 30-36°C, which compares well with daily maximum summer temperatures experienced in the field in South African grasslands and savannas (Schulze 1997), and are highly favourable for C_4 grass growth (Ehleringer *et al.* 1997). During winter and spring (1996), midday temperature differences

University of Cape Town

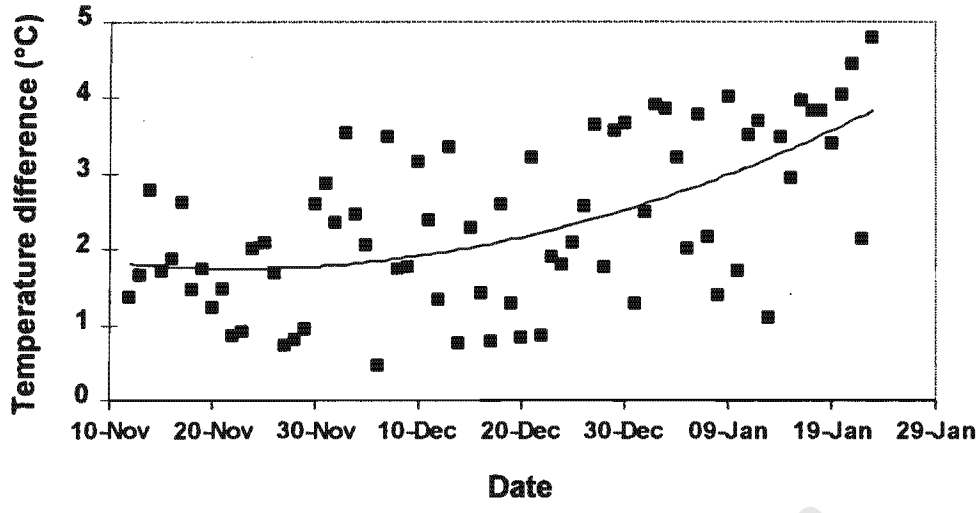


Fig. 3.3a Daily midday (12:00 to 15:00) air temperature difference between warmest and coolest chambers. Data from Experiment 2, 12 November 1995 to 23 January 1996. Polynomial illustrates seasonal trend.

University of Cape Town

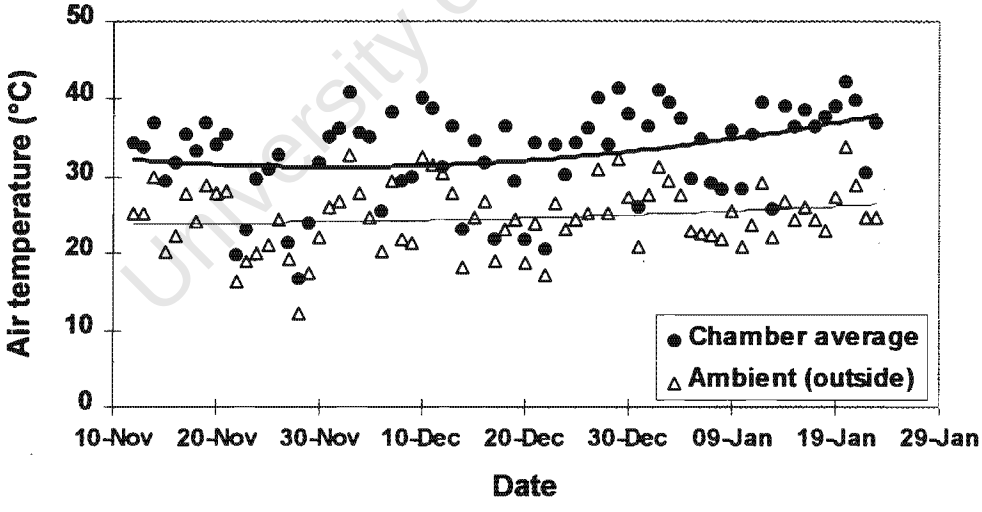


Fig. 3.3b Average daily midday (12:00 to 15:00) chamber and ambient air temperatures. Data from Experiment 2, 12 November 1995 to 23 January 1996. Thick- and thin-lined polynomials illustrate seasonal trends for chambers and ambient air, respectively.

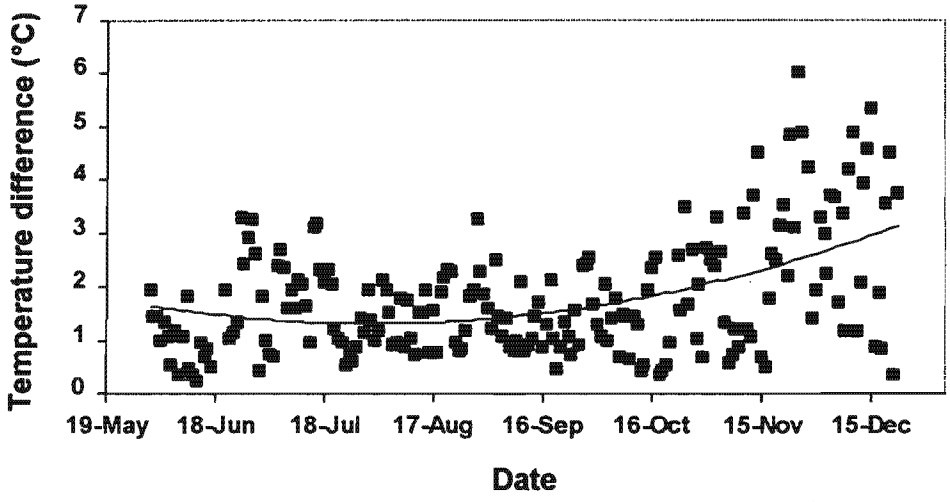


Fig. 3.4a Daily midday (12:00 to 15:00) air temperature difference between warmest and coolest chambers. Data from Experiment 3, 28 May 1996 to 23 December 1996. Polynomial illustrates seasonal trend.

University of Cape Town

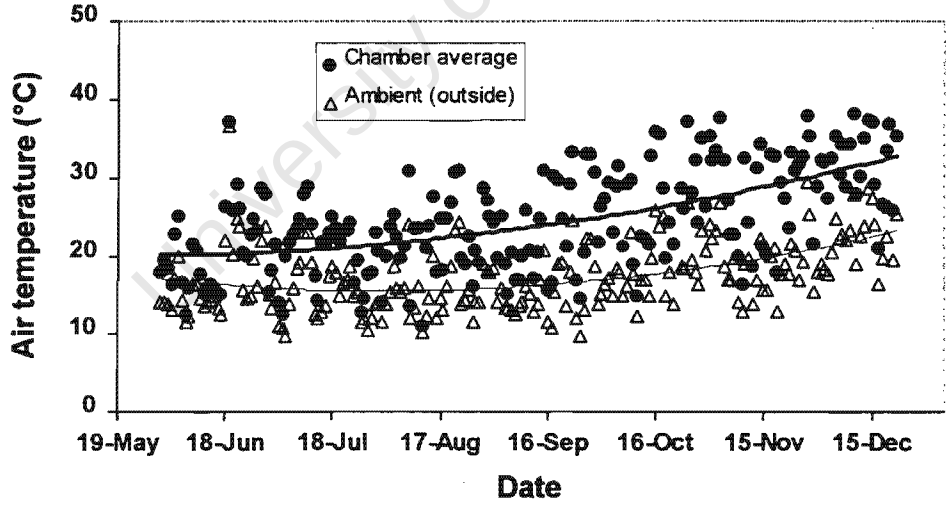


Fig. 3.4b Average daily midday (12:00 to 15:00) chamber and ambient air temperatures. Data from Experiment 3, 28 May 1996 to 23 December 1996. Thick- and thin-lined polynomials illustrate seasonal trends for chambers and ambient air, respectively.

between the warmest and coolest chambers were about 1.7°C, increasing to on average 3.1°C in early summer (Fig. 3.4a). Average midday chamber air temperatures were 4-10°C higher than outside the greenhouse (Fig. 3.4b), so that plants experienced winter (June-September) midday air temperatures of about 20-23°C. This compares well with field environmental conditions for the grassland biome, which has warmer winter daytime temperatures than those typical for the cool winters around Cape Town. Therefore, the temperature regime in winter allowed for reasonable C_4 grass growth, as C_4 physiology is generally disadvantaged below about 18°C (Ehleringer & Pearcy 1983).

Visible radiation levels (photosynthetic photon flux density, PPFD) are given for each experiment in the respective chapters (Table 4.1 for Experiment 1; Table A1 in Appendix 4 for Experiment 2; Table 5.1 for Experiment 3). Given that photosynthesis of leaves exposed to full sunlight in most C_4 species saturates at ≥1000 μmol m^{-2} s^{-1} (Nobel 1991, however see Chapter 7 for examples of light saturation in C_4 grasses at <1000 μmol m^{-2} s^{-1}), irradiance levels were probably mostly non-saturating for photosynthesis during the winter and spring months, but saturating during the peak summer months of November, December and January. Self-shading of developing grass canopies and eventual canopy closure can substantially alter the responses of grass growth to experimentally imposed treatments, compared to unshaded isolated plants (McNaughton 1992). In my experiments, artificial collars to simulate this effect were not used, but the attenuation of incoming radiation through the greenhouse roof and chamber walls created a light environment comparable to that experienced by a grass canopy under intermittently cloudy conditions or with gradual canopy closure (McNaughton 1992).

University of Cape Town

3.5 Pots and irrigation

Plants were grown in 3.9 L plastic pots (0.1 m diameter, 0.5 m long), suspended beneath the tables through holes cut in the surface, and held by means of adjustable plastic collars (Fig. 3.5). This prevented excessive pot and soil heating, and ensured that only above-ground plant parts were fumigated with CO_2. Water was supplied automatically once or twice daily, depending on the season, through microtubes inserted in the growth medium and connected to a supply pipe beneath the table. Pots received similar amounts of water, as confirmed by an initial test where the volume of water pumped through each microtube during one watering cycle was measured.

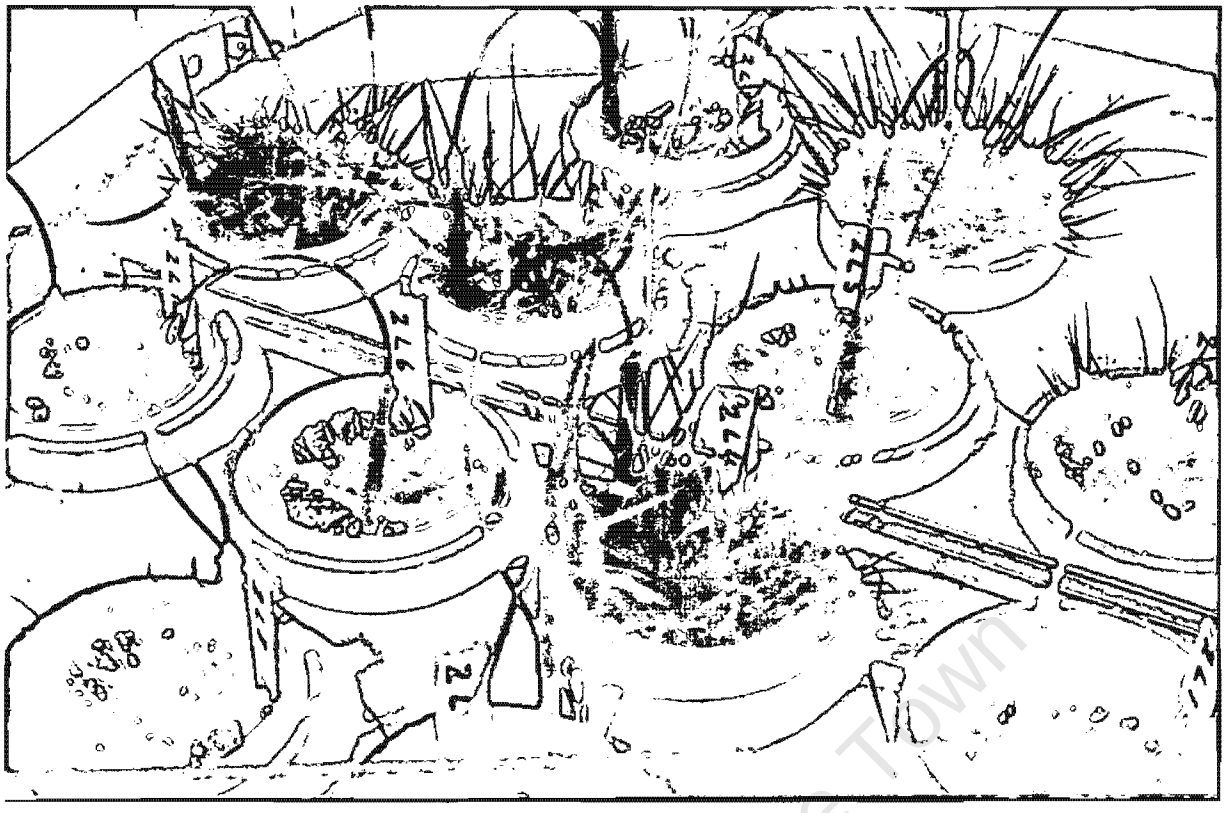


Fig. 3.5 View of long pots suspended from plastic collars beneath tables. Microtubes supply daily water to individual plants. Photograph taken during Exp. 3 after the first defoliation (see Chapter 5), showing defoliated and undefoliated *Themeda* plants.

University of Cape Town



Fig. 3.5 View of long pots suspended from plastic collars beneath tables. Microtubes supply daily water to individual plants. Photograph taken during Exp. 3 after the first defoliation (see Chapter 5), showing defoliated and undefoliated *Themeda* plants.

University of Cape Town

Chapter 4

Physiological and growth responses of *Themeda triandra* (C_4 grass) and *Acacia karroo* (C_3 shrub/tree) to combined increases in CO_2 and UV-B radiation

4.1 Introduction

The potential effects of rising CO_2 on C_4 grasslands will depend not only on the direct responses of individual grass plants and communities, but will also be influenced by interaction with other biotic and abiotic factors, especially those which are currently changing concomitantly with atmospheric CO_2. One such biotic interaction of critical importance to semi-arid grasslands worldwide and in southern Africa, is the expanding distributional range and increasing density of woody species, commonly termed bush encroachment. This is causing rising concern, since it increasingly threatens pasture resources and human livelihoods (Acocks 1988, Archer *et al.* 1988). Bush encroachment has been attributed to both climatic variability and anthropogenic interference, such as altered grazing and fire regimes. Recently, it was proposed that global increases in atmospheric CO_2 concentrations may be contributing to, or even driving bush encroachment (Johnson *et al.* 1993, Polley *et al.* 1994, 1996a, b, Archer *et al.* 1995). Most affected grasslands are dominated by species having the C_4 photosynthetic pathway, whereas woody encroaching species are C_3. Owing to their specialised photosynthetic mechanism, it has generally been held that C_4 species will exhibit a smaller photosynthetic and growth stimulation in elevated CO_2 than C_3 species (Arp *et al.* 1993, Chapter 1). Current perceptions are that increasing CO_2 levels will shift the competitive balance in favour of C_3 species (Johnson *et al.* 1993, Polley *et al.* 1996b).

Interactions between elevated CO_2 and other environmental factors may, however, alter the CO_2 response. Owensby *et al.* (1993) found substantially greater increases in growth in C_4 than in C_3 grass species in a tallgrass prairie under enriched CO_2 during a year with water stress, resulting in improved competitive ability of the C_4 species in that community. Also, nutrient limitations may mitigate the potential CO_2 response over the medium to long term (Wong 1979, Midgley *et al.* 1995), since nutrient demand increases to support sustained growth enhancement. Nitrogen-fixing species exhibit a greater growth stimulation in elevated

University of Cape Town

CO_2 than non-N_2-fixing species, due to the greater availability of carbohydrates to sustain the high energy demand of N_2-fixation (Norby 1987, Arnone & Gordon 1990). This may provide encroaching woody species, many of which are N_2-fixing legumes, with an added competitive advantage.

Rising CO_2 concentrations are currently going hand in hand with another important atmospheric change, the equally imperceptible but potentially serious increase in ultraviolet-B (UV-B, 280-320 nm) radiation resulting from stratospheric ozone depletion (Kerr & McElroy 1993). Plant growth stimulation in elevated CO_2 may be limited under high levels of UV-B radiation (Ziska & Teramura 1992). Increased UV-B applied independently can reduce net assimilation rates and growth in many species (Caldwell & Flint 1994, Teramura & Sullivan 1994), but there is substantial inter-specific variability in UV-B sensitivity. The development of protective mechanisms against harmful UV-B radiation, such as foliar accumulation of UV-B absorbing compounds which limit UV-B transmittance to sensitive targets, may explain some of the differences in UV-B tolerance between species (Teramura & Sullivan 1994). There have been reports of low sensitivity of monocotyledonous species, such as wheat (Teramura 1980) and annual herbs (Musil 1995), to increased UV-B.

In most crop and some natural species investigated to date, increased UV-B radiation levels reduced or negated the CO_2 growth enhancement effect when both factors were applied simultaneously (Teramura *et al.* 1990, Ziska & Teramura 1992), with little interaction between UV-B and CO_2 responses (Stewart & Hoddinott 1993, van de Staaij *et al.* 1993, Sullivan & Teramura 1994, Yakimchuk & Hoddinott 1994). Differential shoot morphological responses to increased CO_2 and/or UV-B, such as changes in branching, tillering, and leaf size, may lead to changes in canopy architectures, which could alter the species competitive balance by modifying foliar light interception patterns (Bazzaz & McConnaughay 1992, Ryel *et al.* 1990). This may be particularly important during seedling establishment.

The possible contribution of increasing CO_2 and UV-B to the problem of bush encroachment in southern African grasslands has not yet received attention. Few data are available on CO_2 responses of species from arid or semi-arid grasslands or savannas (Chapter 1), whereas studies of the combined effects of CO_2 and UV-B have been restricted to crop and conifer species. For this study, both the individual and combined effects of CO_2 and UV-B enhancement on two dominant species from the semi-arid Eastern Cape were

University of Cape Town

investigated, where serious bush encroachment has already occurred. *Acacia karroo* Hayne is a C_3 nitrogen-fixing invasive tree, and *Themeda triandra* Forsk. is a dominant C_4 grass in the region (these species will be called only by their generic names hereafter). I tested the hypothesis that increased atmospheric CO_2 would enhance photosynthetic CO_2 uptake and growth to a greater extent in *Acacia* than *Themeda*, but that interaction with increased UV-B radiation could mitigate this response and possibly reduce the advantage of *Acacia* in elevated CO_2.

4.2 Materials and Methods

4.2.1 Plant establishment

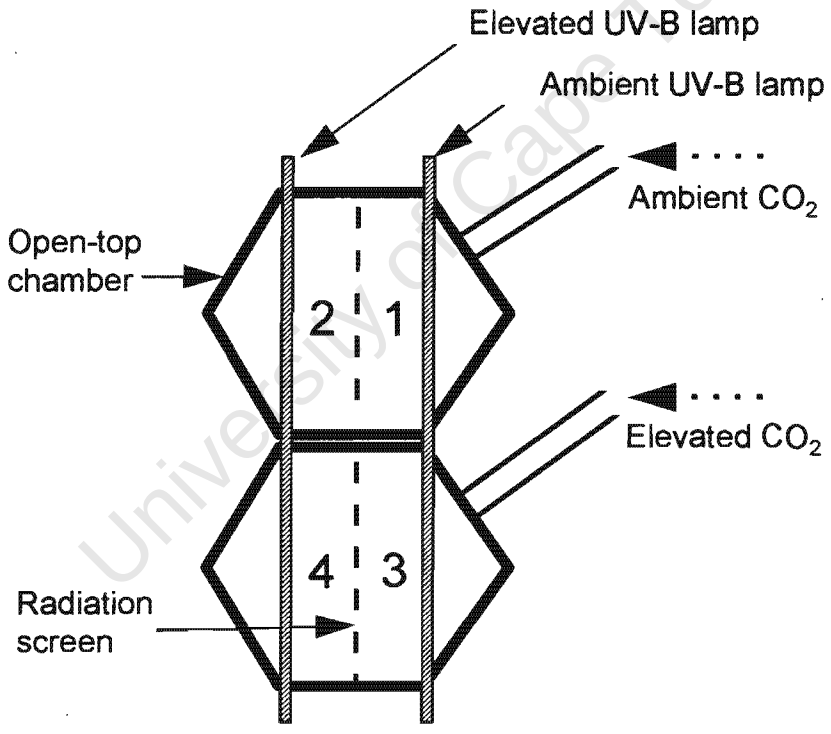
Themeda and *Acacia* plants were grown from seeds in 3.9 L pots filled with a sand/Perlite mixture. *Themeda* seeds (designated "ecotype 1") were supplied by the Döhne Agricultural Development Institute, Department of Agriculture, Stutterheim. *Acacia* seeds were supplied by Kirstenbosch National Botanical Garden, Cape Town, and had been collected in the northern Cape. *Acacia* seedlings were inoculated with *Rhizobium* obtained from root nodules of young plants excavated in the field. All plants were fertilized weekly (150 ml) with a complete Rorison nutrient solution (Booth *et al.* 1993). Inoculated *Acacia* seedlings were also fertilized with the complete solution as they showed symptoms of nitrogen deficiency when initially given the solution without nitrogen. The nutrient concentrations in the solution were as follows: 56 mg L^{-1} N (= 4 mM NO_3^-), 31 mg L^{-1} P, 78 mg L^{-1} K, 80 mg L^{-1} Ca, 24 mg L^{-1} Mg, 3 mg L^{-1} Fe, 0.5 mg L^{-1} Mn, 0.5 mg L^{-1} B, 0.1 mg L^{-1} Mo, 0.1 mg L^{-1} Zn, 0.1 mg L^{-1} Cu. Weekly nutrient supply rates were therefore: 8.4 mg N, 4.7 mg P, 11.7 mg K, 12.0 mg Ca, 3.6 mg Mg, 450 µg Fe, 75 µg Mn, 75 µg B, 15 µg Mo, 15 µg Zn, and 15 µg Cu. Nutrient availabilities in the field vary widely across the grassland biome, but final leaf N concentrations suggest that N uptake in the pots was comparable to most field situations.

4.2.2 Design and treatments

The experimental design was a split-block (Fig. 4.1), with four main plots (=blocks; CO_2 treatments) each split into sub-plots (UV-B treatments), giving four different treatment combinations: 1) ambient CO_2, ambient UV-B (control); 2) ambient CO_2, increased UV-B (+UV-B); 3) elevated CO_2, ambient UV-B (+CO_2); elevated CO_2, increased UV-B (+CO_2 +UV-B). Each block comprised two adjacent hexagonal open-top chambers. The CO_2 ranges after calibration in the four amended chambers were 552-734, 442-855, 490-857, and 475-779 µmol mol^{-1}, with 50% of measurements falling within 10% of the target (650 µmol mol^{-1}).

University of Cape Town

Artificial UV-B radiation was supplied by two fluorescent sun lamps (Phillips TL/12 40 W UV-B, The Netherlands) suspended above each chamber pair, with each lamp spanning both chambers. Half the length of each lamp irradiated the ambient CO_2 chamber, and the other half irradiated the elevated CO_2 chamber. Each chamber was subdivided between the two lamps into two equal halves by a clear sheet of polycarbonate (2 mm thick, no transmission below 400 nm) that extended 1.5 m above the top of each chamber to prevent radiation contamination between UV-B treatments. The radiation screen was perforated at its base to allow sufficient CO_2 mixing in the chamber, which, in combination with the air distribution through the plenum, ensured minimal variability of CO_2 concentration within the chamber.



University of Cape Town

Fig. 4.1 Schematic representation of one block of the experimental design, which was replicated three times. Two UV-B lamps were suspended above two adjacent open-top chambers, with each lamp supplying either ambient or increased (20% ozone depletion) levels of UV-B radiation. This matrix resulted in four treatments, with 3 plants per treatment (12 plants per block): 1) ambient CO_2, ambient UV-B (control); 2) ambient CO_2, increased UV-B (+UV-B); 3) elevated CO_2, ambient UV-B (+CO_2); 4) elevated CO_2, increased UV-B (+CO_2, +UV-B).

Absolute spectral irradiances of filtered lamps (0.075 mm thick cellulose acetate film, no transmission below 290 nm) were measured after sunset with a monochromator spectroradiometer (IL-1700, International Light Inc., Newburyport, USA), calibrated for absolute responsivity and checked for wavelength alignment, which was interfaced with a personal computer. Measured spectral irradiances were weighted with the generalized plant response action spectrum (Caldwell 1971), normalized at 300 nm and integrated over wavelength to obtain biologically effective UV-B irradiances as a function of distance from the lamp (Musil & Bhagwandin 1992). Lamp heights were set individually to provide the two UV-B treatments in each block. These simulated the daily biologically effective UV-B irradiances received at ground level under clear sky conditions at 1094 m elevation at Queenstown in the eastern Cape (31°54'S, 26°52'E) at ambient (1979) stratospheric ozone concentrations (UV-B range of 1.56 to 8.66 kJ m^{-2} d^{-1}) and at anticipated 20% ozone depletion (UV-B range of 2.22 to 11.93 kJ m^{-2} d^{-1}) over the 30- week growth period, calculated according to an empirical model (Green 1983). UV-B radiation was supplied over an 8-h period centred on solar noon. Lamp height adjustments were made monthly to simulate seasonal changes in UV-B irradiances and to accomodate plant height increases. Plastic collars fitted with rubber O-rings facilitated precise vertical pot adjustments. All lamps were wrapped in 0.075 mm thick cellulose acetate film which was replaced weekly. A UV-A (320-380 nm) control treatment was not used due to design and cost limitations. Therefore, UV-A was supplied only by the lamps, as was UV-B (due to screening of UV radiation by the polycarbonate greenhouse covering), and its flux would have increased with increased UV-B treatment. This may have had a small influence on plant responses, possibly of photomorphogenetic nature, but this was not quantified in this study.

Six potted 4-week-old plants of each species were randomised within each open-top chamber, with 3 plants of each species on either side of the radiation screen. Pots within each sub-block were randomised monthly to minimise spatial variations in CO_2 and UV-B. Block locations were also randomised monthly. The experiment was carried out from 13 April 1994 to 10 November 1994 (*Themeda*) and from 29 April 1994 to 28 November 1994 (*Acacia*), for 211 and 213 days, respectively.

University of Cape Town

Table 4.1 Average monthly midday chamber air temperatures and irradiance (PPFD) from 19 July to 28 November 1994.

Month	Avg midday temperature (°C) (12:00 - 15:00)	Avg midday irradiance (PPFD, µmol m^{-2} s^{-1}) (12:00 - 15:00)
July	20.4	445
August	21.2	513
September	26.0	687
October	32.7	950
November	33.3	958

4.2.3 Photosynthetic gas exchange

Gas exchange measurements were performed after 26 to 29 weeks, using an IRGA (LI-6200, Li-Cor, Lincoln, NE, USA) under a bank of 50 W tungsten-halogen incandescent lamps (Decostar 51, Osram, Germany). The response of net CO_2 assimilation rate to PPFD (the photosynthetic light response) was determined in 3 plants from each treatment selected randomly from different blocks. A range of 6-7 PPFD values (60 to 1930 µmol m^{-2} s^{-1}), measured with a quantum sensor (LI-189, Li-Cor, Lincoln, NE, USA) at leaf level, was obtained through combinations of lamps controlled by individual switches. Leaf temperatures were maintained at 28.4 ± 1.4 °C using external cooling. Light response curves were determined at cuvette CO_2 concentrations of both 360 and 650 µmol mol^{-1} to distinguish between short-term and long-term responses to CO_2. Response curves were fitted individually using non-linear regression (SAS 1989), and the monomolecular function $y=a(1-e^{b-cx})$ given by Causton & Dale (1990). The fitted curve coefficient "a" gave the light-saturated rate of net CO_2 assimilation (A). Transpiration rates and A were used to calculate instantaneous leaf water use efficiency (WUE).

4.2.4 Harvest

Plants were harvested after 30 weeks and sub-divided into leaves, stems and roots in *Acacia*, and senesced tillers, green leaf blades, leaf sheaths, reproductive tillers and roots in *Themeda*. Leaf and/or tiller numbers were recorded. Root nodules in *Acacia* were removed, counted and

University of Cape Town

the total dry mass determined. In *Themeda*, the length and breadth of 10 randomly sampled fully developed leaf blades were measured. All material was oven-dried to constant mass at 65°C, and milled.

4.2.5 Leaf chemical analyses

Readily oxidizable organic leaf carbon concentration

This was determined by potassium dichromate in a sulphuric acid medium, and was performed by Matrolab (Pty) Ltd., Cape Town. The excess $Cr_20_7^{2-}$ was determined by titration with a standard $FeSO_4$ solution and the concentration of oxidised substances calculated from the quantity of reduced $Cr_20_7^{2-}$ (modification of the Walkley-Black method, original reference unknown). This method analyses the active forms of carbon (ca. 80% of total organic carbon).

Total leaf nitrogen concentration

This was measured by Matrolab (Pty) Ltd., Cape Town, using the micro-Kjeldahl method. Dried leaf material was digested in concentrated H_2SO_4 the nitrogen converted quantitatively to $(NH_4)_2SO_4$, and the solution made alkaline. The released ammonia was steam-distilled into a standard H_2SO_4 solution and titrated against a standard NaOH solution. Total foliar nitrogen content (total pool) was calculated as the product of the leaf nitrogen concentration (expressed on a leaf dry mass basis) and total plant foliar biomass. For Experiment 1, both C and N concentrations were expressed on a starch-free leaf dry mass basis, to eliminate possible bias introduced by CO_2-induced starch accumulation.

Leaf UV-B absorbing compound concentration

This was determined by extracting 5 mg of dry leaf material in 10 ml acidified methanol (methanol:water:HCl, 79:20:1, [v/v]) overnight, then centrifuging for 10 min at 12100 g. Absorbance of the supernatant at 300 nm, appropriately diluted if necessary, was measured using a Beckman (DU640) spectrophotometer (Beckman Instruments Inc., Fullerton, USA).

Leaf total non-structural carbohydrate (TNC) concentration

The modified phenol-sulphuric acid method (Buysse & Merckx 1993) was used to determine TNC concentrations, and samples were extracted in duplicate. Dry leaf material (50 mg) was

University of Cape Town

extracted overnight in 20 ml of 80% ethanol (v/v) and centrifuged for 10 min at 12100 g. The supernatant was quantitatively transferred to volumetric flasks and made up to 25 ml with 80% ethanol. The pellet was hydrolysed in 3% HCl for three hours, then centrifuged for 10 min at 12100 g. The supernatant was transferred into 25 ml volumetric flasks, the pellet washed with 12 ml 80% ethanol and re-centrifuged, and the combined supernatant made up to 25 ml with 80% ethanol. Total sugar (sucrose, glucose and fructose) and starch concentrations were analyzed spectrophotometrically (Beckman DU640, Beckman Instruments Inc., Fullerton, USA) using 28% w/w phenol and concentrated H_2SO_4 (Buysse & Merckx 1993) against glucose and starch standards.

4.2.6 Statistical analysis

A split-block analysis of variance (ANOVA) with 4 blocks was used to test for significant differences in biomass data between treatments. This is a special case of the split-plot ANOVA, in which the error term for main effect of CO_2 is Blocks x CO_2, that for main effect of UV-B is Blocks x UV-B, and that for their interaction is the residual error (df=3). Means of three plants per treatment and block were entered into the ANOVA. These analyses were performed using SAS (1989) and the level of confidence was set at $P \leq 0.10$. Gas exchange parameters were analysed using a two-way ANOVA, with CO_2 and UV-B treatments as factors.

University of Cape Town

4.3 Results

4.3.1 Photosynthetic gas exchange

Net CO_2 assimilation rates (A) of *Acacia* plants grown and measured at elevated CO_2 were significantly greater (53%) than those grown and measured at ambient CO_2 (Table 4.2). Instantaneous leaf water use efficiency (WUE) increased by 47% in elevated CO_2 due to the increase in A. In *Themeda* there was no significant change in A in response to elevated CO_2 or UV-B, but CO_2 enrichment increased WUE significantly by 35%. Stomatal conductance (g_s) of *Acacia* (Fig. 4.2, statistical analysis in Table 4.3) showed interaction between CO_2 and UV-B, with g_s decreasing where elevated CO_2 and UV-B were applied separately, but not

Table 4.2. Gas exchange parameters of *Acacia* and *Themeda* grown under elevated CO_2 and/or UV-B. A = net light-saturated CO_2 assimilation rate measured at the growth CO_2 concentration; WUE = instantaneous leaf water use efficiency at the growth CO_2 concentration. Values are means ± SE (n=3). * P≤0.05; ** P≤0.01; NS not significant according to a split-block ANOVA.

Treatment	*Acacia karroo*		*Themeda triandra*	
	A (μmol m^{-2} s^{-1})	WUE (μmol CO_2 $mmol^{-1}$ H_20)	A (μmol m^{-2} s^{-1})	WUE (μmol CO_2 $mmol^{-1}$ H_20)
Control	21.0 ± 1.0	3.72 ± 0.40	20.1 ± 3.5	6.62 ± 0.36
+UV-B	17.8 ± 2.5	4.89 ± 1.10	14.9 ± 2.5	8.36 ± 0.59
+CO_2	27.5 ± 1.8	7.23 ± 1.12	15.5 ± 2.6	10.53 ± 1.96
+UV-B +CO_2	32.0 ± 3.2	5.41 ± 0.44	12.4 ± 1.7	9.67 ± 0.43
UV-B	NS	NS	NS	NS
CO_2	**	*	NS	*
UV-B x CO_2	NS	NS	NS	NS

University of Cape Town

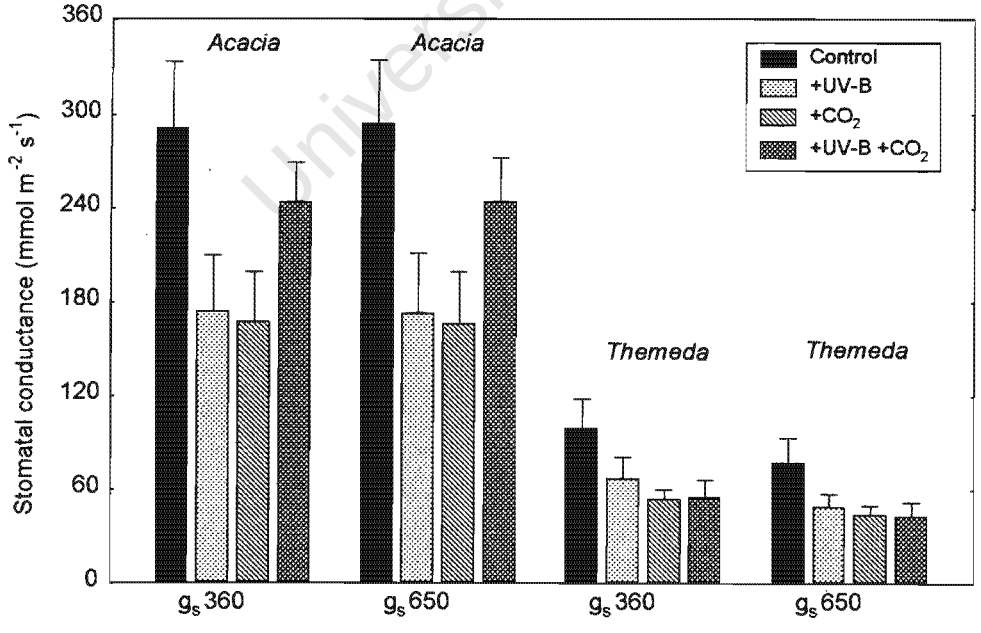


Fig. 4.2 Leaf stomatal conductance of *Acacia* and *Themeda* grown under elevated CO_2 and/or UV-B, and measured at cuvette CO_2 concentrations of 360 (g_s360) and 650 (g_s650) μmol m^{-2} s^{-1}. Values are means (n=3) ± SE. Statistical analysis in Table 4.3.

where these treatments were combined. Conductances did not respond to short-term changes in cuvette CO_2 concentration. In *Themeda*, elevated CO_2 decreased g_s by 48% when treatments were compared at growth CO_2 concentration (long-term response), although there was also a weaker short-term decrease in g_s of ambient CO_2-grown plants measured at elevated cuvette CO_2 concentrations.

4.3.2 Leaf chemistry

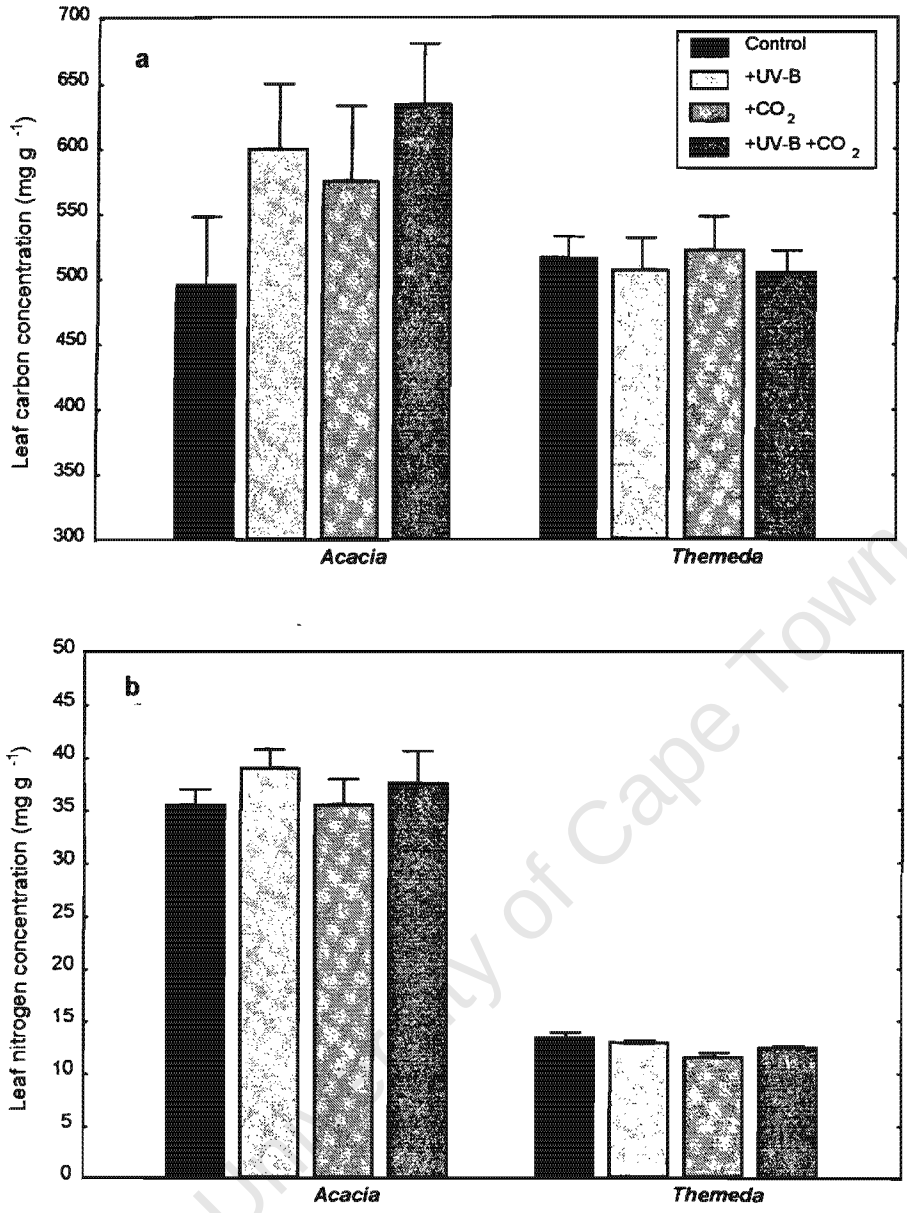
In *Acacia*, leaf starch-free carbon concentrations increased significantly under enhanced UV-B (on average 15%) at both ambient and elevated CO_2 (Fig. 4.3a, statistical analysis in Table 4.3). Leaf nitrogen concentrations decreased significantly in elevated CO_2 ($P=0.02$) when expressed on a total leaf dry mass basis (data not shown), but not when expressed on a starch-free basis (Fig. 4.3b, Table 4.3). By contrast, leaf nitrogen concentrations (starch-free basis) in *Themeda* (Fig. 4.3b, Table 4.3) did decrease significantly (9%) in elevated CO_2, especially under ambient UV-B. Total foliar nitrogen content did not differ between treatments (data not shown). Neither increased CO_2 nor UV-B affected leaf carbon concentrations in *Themeda* (Fig. 4.3a, Table 4.3).

Leaf soluble sugar concentrations were not significantly affected by elevated CO_2 and/or UV-B in either *Acacia* or *Themeda* (Fig. 4.4a, statistical analysis in Table 4.3). Leaf starch concentrations increased in *Acacia* in elevated CO_2, but relatively more so under ambient UV-B (23%) than under enhanced UV-B (14%) (Fig. 4.4b, Table 4.3). Leaf starch concentrations were similar in all treatments in *Themeda*. Absorbances of leaf extracts in the mid-UV-B range (300 nm) did not differ significantly between treatments in both species (data not shown).

4.3.3 Biomass

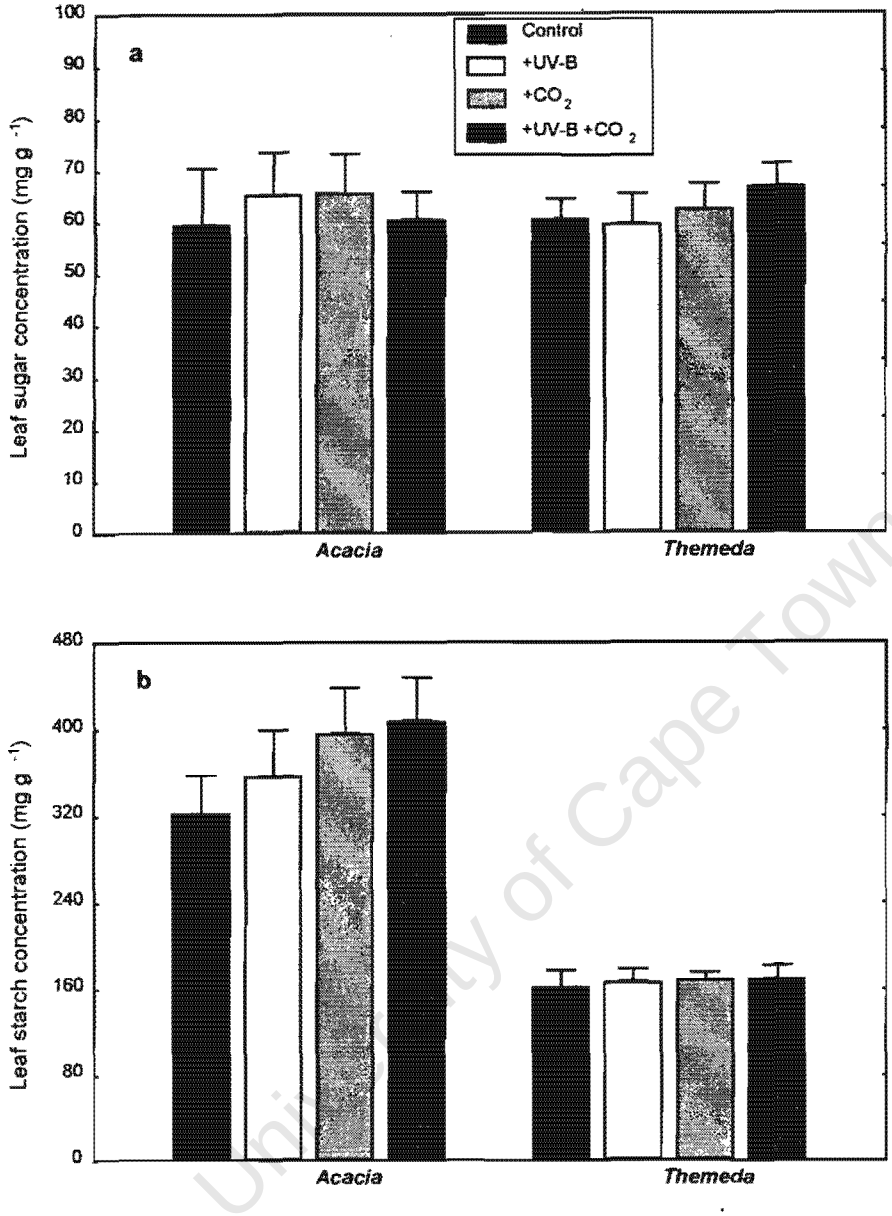
In *Acacia*, a greater relative increase in stem mass in elevated CO_2 was found under ambient (34%) than enhanced UV-B (16%) (Fig. 4.5a, statistical analysis in Table 4.4). A total plant biomass increase of 16% in elevated CO_2 was not statistically significant at $P \leq 0.10$. Plants grown in elevated CO_2 produced more root nodules, resulting in an increased total dry nodule mass per plant, particularly under enhanced UV-B (Table 4.5). However, individual nodule dry mass, and total nodule dry mass relative to total plant dry mass ratios (the nodule weight ratio), were not affected by elevated CO_2 and UV-B.

University of Cape Town



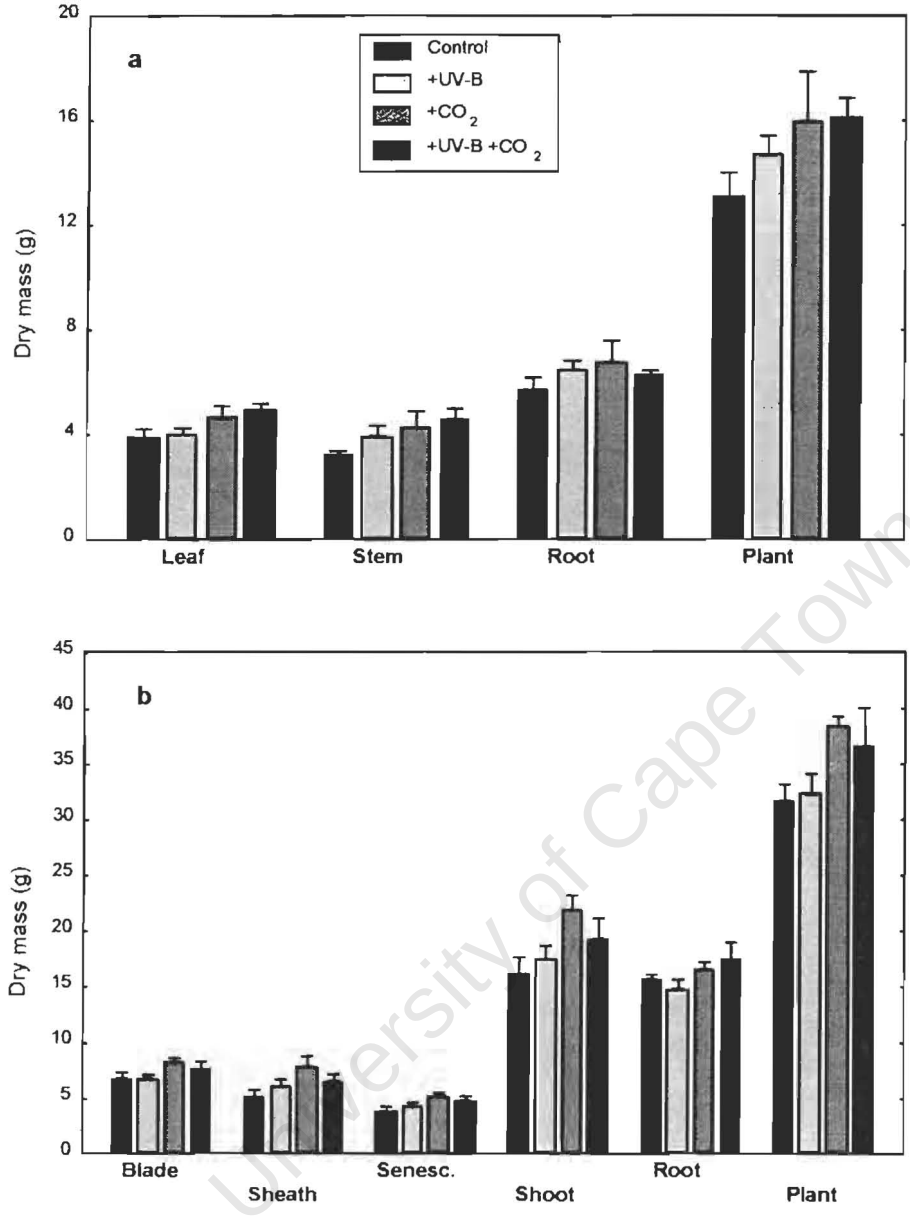
University of Cape Town

Fig. 4.3 Leaf carbon (a) and nitrogen (b) concentrations, expressed on a starch-free basis, of *Acacia* and *Themeda* grown under elevated CO_2 and/or UV-B. Values are means (n=4) ± SE. Statistical analysis in Table 4.3.



University of Cape Town

Fig. 4.4 Leaf soluble sugar (a) and starch (b) concentrations of *Acacia* and *Themeda* grown under elevated CO_2 and/or UV-B. Values are means (n=4) ± SE. Statistical analysis in Table 4.3.



University of Cape Town

Fig. 4.5 Biomass of *Acacia* (a) and *Themeda* (b) grown under elevated CO_2 and/or UV-B. Values are means (n=4) ± SE. Statistical analysis in Table 4.4.

Table 4.5. Effect of elevated CO_2 and/or UV-B on root nodulation of *Acacia karroo*. Nodule weight ratio calculated as total nodule dry weight/total plant dry weight. Values are means ± SE (n=4). + P≤0.10; * P≤0.05; NS not significant according to a split-block ANOVA.

Treatment	Nodule no.	Total nodule DW (mg)	DW per nodule (mg)	Weight ratio (mg g^{-1})
Control	84 ± 14	319 ± 19	4.40 ± 0.60	24.6 ± 0.4
+UV-B	100 ± 15	309 ± 23	3.71 ± 0.50	22.6 ± 1.9
+CO_2	122 ± 13	348 ± 24	3.71 ± 0.39	22.5 ± 1.7
+CO_2 +UV-B	139 ± 25	369 ± 31	3.51 ± 0.20	23.1 ± 2.1
UV-B	NS	NS	NS	NS
CO_2	+	*	NS	NS
UV-B x CO_2	NS	+	NS	NS

Table 4.6. Effect of elevated CO_2 and/or UV-B on plant and leaf morphological characteristics of *Themeda triandra*. Values are means ± SE (n=4). + P≤0.10; * P≤0.05; NS not significant according to a split-block ANOVA.

Treatment	Tiller no.	Leaf no.	Leaf length (mm)	DW per leaf (mg)	DW per leaf sheath (mg)
Control	135 ± 4	510 ± 20	205 ± 7	13.7 ± 0.5	40.7 ± 6.4
+UV-B	121 ± 12	446 ± 41	211 ± 6	15.4 ± 0.8	50.0 ± 1.5
+CO_2	103 ± 8	416 ± 32	247 ± 17	20.1 ± 2.0	80.9 ± 16.2
+CO_2 +UV-B	102 ± 7	399 ± 23	248 ± 6	19.3 ± 1.0	65.8 ± 2.0
UV-B	NS	NS	NS	NS	NS
CO_2	*	NS	*	*	*
UV-B x CO_2	NS	NS	NS	*	+

University of Cape Town

Biomass of all vegetative fractions in *Themeda* was significantly increased in elevated CO_2 (Fig. 4.5b, Fig. 4.6, statistical analysis in Table 4.4). The greatest increases, averaged over both UV-B treatments, occurred in senesced tiller mass (24%) and leaf sheath mass (29%). Total shoot mass increased by 23% and root mass by 11%. Enhanced UV-B moderated the CO_2 enhancement effect on leaf sheath mass: a 54% increase under ambient UV-B was reduced to 8% under enhanced UV-B. This trend was also reflected at the whole shoot level. Green leaf blade mass decreased under enhanced UV-B when this treatment was combined with elevated CO_2.

Morphological responses of *Themeda* to elevated CO_2 (Table 4.6) included decreased tiller numbers (20% reduction), but leaf numbers were not significantly reduced at $P \leq 0.10$. Leaf blade lengths increased, accompanied by greater individual leaf blade mass, particularly under ambient UV-B (47%) compared to enhanced UV-B (25%). CO_2-induced increases in individual leaf sheath mass (99% under ambient UV-B) were reduced to 32% under enhanced UV-B.

4.4 Discussion

4.4.1 *Themeda triandra*

Significant growth enhancement (21% for whole plant biomass) of *Themeda* was measured in elevated CO_2, in contrast to widespread perception that C_4 species exhibit no or only very low stimulation under elevated CO_2 (Chapter 1). However, similar rates of net CO_2 assimilation were measured just before harvest in all treatments. This level of growth enhancement compares well with a synthesis by Poorter (1993), who calculated an average biomass increase of 22% in 15 C_4 species, and the meta-analysis of CO_2-responses of C_4 grasses (Wand *et al.* in press), which revealed an average total plant growth stimulation of 26%. It contrasts, however, with an updated analysis by Poorter *et al.* (1996), which showed only a 10% increase in biomass in 24 C_4 species. In *Themeda*, assimilation rates may have been higher during an earlier developmental stage, resulting in early growth stimulation. If this was the case, there was apparently a subsequent negative acclimation of CO_2 uptake (also reported by Wong 1979 and Morgan *et al.* 1994) as evidenced by substantial decreases in stomatal conductance. The latter was predominantly a long-term response, although a smaller short-term decline in conductances was also found. High CO_2-grown plants may also have matured more quickly following initial stimulation of CO_2 assimilation and growth, with later decreases in assimilation rate merely reflecting an age-related decline in relative growth rate.

University of Cape Town



Fig. 4.6 Biomass response of *Themeda triandra* (ecotype 1), showing the growth enhancement and change in plant morphology in plants from adjacent chambers grown under elevated CO_2 (three plants on the left) compared to those grown under ambient CO_2 (three plants on the right). The depicted plants received ambient UV-B radiation levels.

University of Cape Town

Alternatively, the growth stimulation in elevated CO_2 may be explained by the greater canopy leaf area, resulting in greater canopy net assimilation rates, or by more efficient utilization of resources. Increases in leaf water use efficiencies may have facilitated greater biomass production (Wong 1979, Morison & Gifford 1984). Enhanced photosynthetic rates and growth of a C_4 tall-grass prairie species in elevated CO_2 during periods of water stress have been linked to improved leaf water potentials (Knapp *et al.* 1993, Owensby *et al.* 1993). *Themeda* plants were not water stressed but may still have benefitted from an improved leaf water status. In addition to improved water use, altered mineral use efficiencies may have contributed to improved growth. Leaf nitrogen concentrations in *Themeda* were reduced in elevated CO_2, but foliar nitrogen pools were unchanged due to foliar biomass increases. This suggests that nitrogen was re-allocated within the shoot to support increases in leaf and sheath biomass. Leaf sheaths, in particular, may have developed more strongly to provide carbohydrate sinks, as stored sheath carbohydrates are mobilized during the initial stages of regrowth of *Themeda* following defoliation (Danckwerts & Gordon 1990).

Elevated CO_2 also elicited distinct morphological responses, such as longer leaf blades but reduced tiller numbers. It is likely that the greater canopy leaf area led to increased self-shading of the basal nodes from which tillers are initiated, with consequent reductions in tiller initiation. This has been observed in *Themeda* following a range of shade treatments (Everson *et al.* 1988). In contrast, enhanced UV-B had no effect on leaf and tiller numbers or leaf length, suggesting that there was no interference with meristematic activity or cell elongation. This does not agree with some UV-B studies which have reported predominantly morphological changes in leaf and canopy characteristics (Barnes *et al.* 1990, Ryel *et al.* 1990). Significantly moderating effects of UV-B were apparent only on individual leaf blade and leaf sheath masses, and on total sheath and shoot biomass. In previous studies of the UV-B/CO_2 interaction, increased UV-B generally depressed biomass production under both ambient and elevated CO_2, with no interaction between the treatments (Ziska & Teramura 1992, van de Staaij *et al.* 1993, Stewart & Hoddinott, Yakimchuk & Hoddinott 1994). However, in concurrence with our study, UV-B-induced reductions in pine seedling biomass were found only at elevated CO_2 (Sullivan & Teramura 1994). Under predicted future climatic conditions, biomass enhancements in elevated CO_2 may be mitigated by concomitant UV-B increases, but will not be eliminated.

4.4.2 *Acacia karroo*

If the increase in global atmospheric CO_2 concentration is accelerating the encroachment of *Acacia* into semi-arid C_4 grasslands in southern Africa, one would expect this to be attributable to, amongst other possibilities, improved seedling growth or improved seedling establishment due to more efficient resource capture or resource use. The data gathered in this experiment do not support the improved-growth hypothesis since there was only insignificant biomass enhancement in *Acacia* after 30 weeks in elevated CO_2. In a compilation of data from 106 C_3 plant species, Poorter (1993) reported an average CO_2-induced biomass increase of 41%, which was increased to 50% in the case of N_2-fixing species. A subsequent analysis confirmed this small relative increase, but not significantly (Poorter *et al.* 1996). Two N_2-fixing woody species with similar invasive potential as *Acacia karroo*, *Acacia melanoxylon* and *Prosopis glandulosa*, show either a small biomass enhancement of 21% or no effect on

University of Cape Town

above-ground biomass, respectively (P.J. Mathias, J.E. Conroy & S.E.W.R. Barlow, unpublished, reported in Poorter 1993, Polley *et al.* 1994).

The absence of a CO_2-induced growth response in *Acacia* may be due to some sort of sink limitation. Positive growth responses are largely influenced by the presence of active growth sites or other strong sinks (Stitt 1991, Loehle 1995), as well as efficient partitioning and transport of carbohydrates to these sinks (Körner *et al.* 1995). There appears to be a positive correlation between potential growth rate and CO_2-induced growth enhancement (Hunt *et al.* 1991, 1993, Poorter 1993, Poorter *et al.* 1996). Shoot growth rates in *Acacia* were slow during initial plant establishment, but increased towards the middle of the experiment. Most biomass accumulation may initially have been belowground, but there were no signs of root restriction at harvest since the tap root had only just reached the bottom of the long pot, if at all, and lateral root growth was healthy but not visibly restricted. It is unlikely that limiting pot size or poor nutritional conditions were responsible for the lack of a growth response (Arp 1991). High leaf nitrogen concentrations and healthy levels of root nodulation indicated that nutrient limitation was also unlikely. Total root nodule mass increased only in proportion to small biomass increases, supporting previous studies showing that total nitrogenase activity is increased owing to greater nodule mass in elevated CO_2 (Norby 1987, Arnone & Gordon 1990). However, total foliar nitrogen pools did not differ in ambient or elevated CO_2, indicating that nitrogen accretion, at least for leaves, was not altered. This agrees with results obtained by Polley *et al.* (1994) with *Prosopis glandulosa*. It is now thought that N_2-fixation is tuned in response to nitrogen demand (N sink strength) as determined (amongst others) by changes in plant growth, and may involve some kind of nitrogen feedback regulation. This means that, under elevated CO_2, nitrogen fixation increases in concert with increased biomass production (Hartwig *et al.* 1996).

Net CO_2 assimilation rates of *Acacia* seedlings were substantially higher in elevated CO_2, and increases in foliar starch concentrations suggest that additional carbohydrates could not be completely exported out of source leaves to sink tissue. This in turn may have led to feedback inhibition and negative acclimation of CO_2 uptake (Stitt 1991, Loehle 1995), and was accompanied by reduced stomatal conductances in the long term. Foliar starch concentrations were particularly high in *Acacia*, indicating that this species may be a natural starch accumulator with perhaps restricted capacity for carbohydrate export (Körner *et al.*

University of Cape Town

1995). Nevertheless, the possibility that extra carbon products were exported to roots for longer-term reserve accumulation (Midgley *et al.* 1998), signifying a more conservative allocation (Loehle 1995) rather than immediate utilisation for above-ground growth, cannot be discounted.

Prolonged exposure of *Acacia* to increased UV-B radiation had no effect on seedling growth, but interaction between CO_2 and UV-B treatments was found at a physiological level. The decline in stomatal conductances under elevated CO_2 and UV-B supplied separately, but not in combination, suggests that a demand for additional carbon substrate was developed under the combination of treatments, which may have prevented the reduction in stomatal conductance. Curtis (1996), in a meta-analysis of tree stomatal responses to elevated CO_2, found that moderate reductions in conductance in unstressed trees were not evident in stressed trees. Enhanced UV-B also resulted in increased foliar carbon concentrations at both CO_2 levels. Concentrations of non-structural carbohydrates and UV-B-absorbing compounds were not affected by elevated UV-B, so that an accumulation of structural carbohydrates may have taken place. Thickened epidermal cell walls can provide improved protection of sensitive targets against UV-B (Bornman & Vogelmann 1991). The development of such UV-B screening mechanisms would have created a demand for carbon which could be better met in elevated CO_2. Sullivan & Teramura (1994) reported that CO_2 enrichment reduced the inhibitory effects of UV-B on photosynthesis in loblolly pine seedlings, possibly by reducing UV-B penetration into leaves as a result of changes in leaf anatomy and chemistry in elevated CO_2, such as increases in specific leaf mass and UV-B-absorbing compounds. In jack pine seedlings, enhanced UV-B stimulated photosynthesis at a CO_2 concentration of 400 $\mu mol\ mol^{-1}$ or higher, but below this concentration UV-B was inhibitory (Stewart & Hoddinott 1993). This was attributed to increased Rubisco activation or concentration in high UV-B, but relatively greater UV-B induced photorespiratory losses in ambient than in elevated CO_2. It appears that UV-B-induced reductions in CO_2 assimilation at ambient CO_2 may be moderated by elevated CO_2 in woody species, although conclusive evidence for the mechanism is lacking.

University of Cape Town

4.4.3 Conclusion

The direct effects of elevated CO_2 and UV-B on young *Themeda* and *Acacia* plants did not follow expected patterns, and do not offer a satisfactory explanation of bush encroachment (Polley *et al.* 1994, 1996b, Archer *et al.* 1995). Nevertheless, leaf and canopy morphological changes could alter the mosaic of resource availability in *Themeda* canopies, and may facilitate *Acacia* seedling establishment even in the absence of direct growth enhancements. To investigate such effects would require different and more long-term approaches, including studies of patch dynamics and population biology in the field (O'Connor 1995, Polley *et al.* 1996a), and these fall outside the possibilities of open-top chamber studies as employed here. However, this experiment has shown that simultaneous UV-B enhancement is unlikely to substantially alter the CO_2 response of these two species.

University of Cape Town

Chapter 5

Physiological and growth responses of *Themeda triandra* to elevated CO_2: interaction with defoliation

5.1 Introduction

In Chapter 4, the responses of *Themeda* and *Acacia* to elevated atmospheric CO_2, and how they may affect bush encroachment, were investigated interactively with another current atmospheric change, the increase in UV-B radiation. Defoliation is another key factor in grassland functioning which may also change the way these systems respond to elevated CO_2 in future. African C_4-dominated grassland and grass co-dominated (savanna) ecosystems have evolved over many millenia, probably since the late Miocene, in close conjunction with the evolution of grazing mammals (Cerling *et al.* 1998, McNaughton *et al.* 1982). The change from more forested conditions to the more open habitat characteristic of grasslands and savannas went hand in hand with increases in fire, both natural and anthropogenic in origin, which have played an important role in the evolution of these ecosystems (Tainton & Mentis 1984). Both these defoliating agents continue to exert the main environmental influences, together with water availability, on grassland ecosystem function, in particular on productivity and nutrient dynamics (McNaughton *et al.* 1982), and have become important tools for land management both for commercial gain and conservation purposes (Tainton & Walker 1993).

Themeda triandra is one of the most common, widely distributed and utilized grass species in Africa. In South Africa it occurs in almost all grassland communities, dominating most of the grassland biome (Tainton & Walker 1993, O'Connor & Bredenkamp 1997). This species is regarded as one of the best grazing grasses in the region, as leaf production is high and it is very palatable in the young stage during the active growing season (van Oudtshoorn 1992). Due to its sensitivity to overgrazing, it is classed as a "decreaser" species (Hurt *et al.* 1993), dominating in relatively undisturbed veld (fire-climax grasslands), but decreasing under heavy grazing pressure (Coughenour *et al.* 1985, Danckwerts & Nel 1989, Hodgkinson *et al.* 1989). It is especially vulnerable under conditions of combined heavy grazing and drought (O'Connor 1994). *Themeda* does, however, thrive under moderate fire or grazing

University of Cape Town

regimes, and requires periodic defoliation to ensure high productivity and persistence (Mentis & Tainton 1984), given enough time between defoliations to recover and build up new reserves (Danckwerts & Nel 1989, Hodgkinson *et al.* 1989).

The ability of grasses to recover quickly from defoliation has long been thought to be influenced primarily by the long-term accumulation of labile carbon reserves in roots, crowns and sheaths, and their re-mobilization following defoliation to stimulate leaf regrowth (Weinmann 1948, Trlica 1977, Menke & Trlica 1981). Despite evidence to support this, some researchers have questioned the idea that long-term storage is the primary carbon source for regrowth, and have proposed a greater role for carbon assimilated by concurrent photosynthesis, especially after the first 2-5 days following defoliation (White 1973, Caldwell *et al.* 1981, Richards & Caldwell 1985). For *Themeda*, Danckwerts & Gordon (1990) showed, using ^{14}C-labelling techniques, that carbon reserves in roots and stem bases were re-mobilized after defoliation and incorporated into new shoot growth. These reserves probably comprise mainly non-reducing sugars in roots and starch in leaf sheath bases (Weinmann & Reinhold 1946). Subsequently, Danckwerts (1993) studied the relative contribution of reserve carbon and concurrent photosynthesis to regrowth of *Themeda* after defoliation, and found that reserve carbon played an important but transient role in regrowth, by facilitating the rapid emergence of new photosynthetically active leaf tissue, which became the main carbon source three days after clipping.

In Experiment 1 (Chapter 4), it was found that leaf sheath biomass of *Themeda* was higher in elevated CO_2, and root mass was also increased. It was suggested that sheaths may have developed more strongly in high CO_2 to provide sinks for excess carbohydrates. Carbohydrate accumulation in plants exposed to elevated CO_2 has been widely reported, especially when strong carbon sinks are available (Stitt 1991, Loehle 1995). Larger carbohydrate reserves in sheaths and roots after CO_2 enrichment would conceivably be available for regrowth following defoliation (Fajer *et al.* 1991), thereby stimulating regrowth over and above that induced by defoliation *per se*. Although carbohydrate dynamics are central to the defoliation responses of grasses, this response is also influenced by the complex interactions (McNaughton *et al.* 1982) of altered water relations (McNaughton *et al.* 1983, Coughenour *et al.* 1985), nutrient dynamics (Ruess 1988, Hiernaux & Turner 1996), meristematic potential (Richards & Caldwell 1985), and light availability (McNaughton

University of Cape Town

1992). On the other hand, responses to elevated CO_2 are also finely tuned to resource availabilities and changing resource use efficiencies. For example, carbohydrate accumulation (Stitt 1991) and foliar nitrogen reductions (Cotrufo *et al.* 1998, Chapters 2 and 4) after exposure to high CO_2 have been strongly implicated in long-term downregulation of photosynthetic capacity and muted growth responses (Sage 1994, Midgley 1996). However, increased foliar nitrogen uptake (under moderate defoliation intensity) and improved light availability following defoliation (McNaughton 1992) may counteract these effects and allow for sustained growth stimulation in elevated CO_2 of defoliated grasslands.

So far, very little information is available on the responses of grasses to combined treatments of elevated CO_2 and defoliation. Studies on the effects of elevated CO_2 and plant herbivore activity have focused mainly on insects (Lincoln *et al.* 1993). In general, insects feeding on high CO_2-grown plant material show retarded growth and development, despite increased consumption rates linked to decreased leaf nitrogen concentrations (Fajer *et al.* 1989, Lincoln 1993), although more complex responses have been reported in a whole ecosystem context (Arnone *et al.* 1995). However, simulated insect defoliation did not affect the regrowth response of *Plantago lanceolata* in elevated CO_2 (Fajer *et al.* 1991).

Recent studies on the responses of C_3-dominated temperate grassland swards to elevated CO_2 have included regular defoliation (simulating grazing or mowing), but not as a factorial treatment, so it is not possible to establish potential interactions between high CO_2 and defoliation (e.g. Newton *et al.* 1995, Schapendonk *et al.* 1997). Where defoliation treatments have been explicitly included (e.g. Fischer *et al.* 1997, Zanetti *et al.* 1997), the responses to elevated CO_2 have been closely associated with altered nitrogen availabilities. Only one researcher and his colleagues have addressed the question of plant-mammal herbivore interactions and CO_2 concentration in the context of tropical C_4 grasslands (Wilsey 1995, in Wilsey 1996). Wilsey *et al.* (1994) reported that simulated grazing had no effect on the muted CO_2-response of an African C_4 grass. A subsequent study compared the effects of elevated CO_2 and defoliation on grasses from different C_3, C_4 and mixed grassland ecosystems worldwide, and confirmed this lack of interaction, but again under conditions of low CO_2-responsiveness in the C_4 species used (Wilsey *et al.* 1997). Only the C_3 species from a northern temperate grassland showed biomass responses in high CO_2, due mainly to growth increases of crowns and roots, the storage organs. Compensatory shifts in allocation following

University of Cape Town

defoliation were ecosystem-specific and not altered by elevated CO_2. However, in species such as *Themeda triandra*, which shows significant growth increases in elevated CO_2 and favours moderate defoliation for sustained productivity, we may find that defoliation boosts the potential for productivity increases in high CO_2, provided that carbon is readily available either in the form of reserves or concurrent assimilation products, and other nutrients such as nitrogen do not become limiting. Unfortunately, this beneficial effect of elevated CO_2 on productivity could be moderated by reductions in forage quality due to increases in the C:N ratio (Owensby *et al.* 1996a).

The purpose of this experiment was to test the the following main hypotheses:

1. Elevated CO_2 will stimulate growth of both undefoliated and defoliated *Themeda*, but the relative stimulation of productivity will be greater in defoliated plants. Possible mechanisms could include (i) triggered re-mobilization of carbon reserves following defoliation, (ii) higher foliar nitrogen concentrations and photosynthetic capacity of regrowth, and (iii) increased light availability in the canopy.

2. Elevated CO_2 will allow for more sustained regrowth following repeated defoliations, compared to regrowth in ambient CO_2. This could be due to the interaction between improved foliar water status in elevated CO_2 and increased carbon assimilation rates of regrowth, leading to greater carbon availability to buffer diminishing carbon reserves.

3. Increased regrowth in elevated CO_2 will draw mainly on greater carbon reserves, and will continue following short-term reversion to ambient CO_2. Similarly, short-term increases in carbohydrates in elevated CO_2 in plants grown previously under ambient CO_2 will become available for shoot growth in the longer term following sufficient carbon accumulation. This hypothesis aims to tease apart the "long-term indirect stimulation" versus the "short-term direct stimulation" possibilities.

5.2 Materials and Methods

5.2.1 Plant establishment

Themeda plants were grown individually from seed in 3.9 L pots filled with a sand/Perlite mixture. *Themeda* seeds (designated "ecotype 2") were supplied by the Gene Bank of the

University of Cape Town

Range and Forage Institute, Agricultural Research Council, Pretoria, and originated from the Transvaal Lowveld region (Kruger National Park). Plants were fertilized weekly (150 ml) with a complete Rorison nutrient solution (Booth *et al.* 1993). The nutrient concentrations were as described for Experiment 1 (section 4.2.1).

5.2.2 Design and treatments

The experimental design was a split-plot, with CO_2 concentration as the main plot, and defoliation treatment as the sub-plot. The CO_2 ranges after calibration in the four amended chambers were 648-746, 661-732, 655-726, and 598-777 μmol mol^{-1}, with 75-100% of measurements falling within 10% of the target (680 μmol mol^{-1}). At the start of the experiment (28 May 1996), twelve 46-day-old seedlings were placed randomly within each chamber. Plants in each chamber were evenly divided, at random, between two sub-treatments: no defoliation, or three defoliations to 4 cm height, on 23 October, 13 November and 4 December. This defoliation regime provided for a short recovery period, and probably some stress towards the end of the regime due to potential carbon and nitrogen draw-down. Recommended grazing regimes for *Themeda* veld allow for a maximum of two to three grazing cycles per annum under optimal conditions, with resting periods considerably longer than three weeks (Danckwerts & Nel 1989). During the last three weeks before harvest (4-23 December), after the third defoliation, three of the six defoliated plants in each chamber were switched ("CO_2 switch-over group") to the adjacent chamber on the same table, with a different (higher or lower) CO_2 concentration. The remaining three were left at their original growth CO_2 concentration. The experiment was carried out from 28 May to 23 December 1996, for 209 days.

University of Cape Town

Table 5.1 Average monthly midday chamber air temperatures and irradiance (PPFD) from 1 June to 23 December 1996.

Month	Avg midday temperature (°C) (12:00 - 15:00)	Avg midday irradiance (PPFD, µmol m^{-2} s^{-1}) (12:00 - 15:00)
June	21.0	296
July	20.8	403
August	22.4	539
September	23.0	544
October	27.3	804
November	28.2	968
December	32.0	1325

University of Cape Town

5.2.3 Photosynthetic gas exchange

Canopy gas exchange measurements were performed 25-26 weeks after the start of CO_2 fumigation (20-26 November, one to two weeks after the second defoliation) on two plants per treatment (CO_2 x defoliation) per chamber, giving 8 replicates per treatment combination. A square cuvette (28.8 cm width, 33.5 cm height) was constructed of polycarbonate, with a hole at the bottom through which a long pot could be suspended in an air-tight manner, and with an air-tight lid to facilitate pot changes. Ventilation within the chamber was provided by a 12 V fan. A closed-system gas exchange system was established by linking to an LI-6200 IRGA. The rate of leakage between the chamber atmosphere and air outside the chamber was tested using a long pot filled with sand/perlite mix but no plant, and was found to be minimal (-0.075 mmol s^{-1} for the chamber volume) at ambient CO_2 levels (360 µmol mol^{-1}). When the chamber atmosphere was enriched to 670 µmol mol^{-1} CO_2, an equilibrated exchange rate of 0.479 mmol s^{-1} was measured for the chamber volume, possibly owing to leaks associated with the high CO_2 gradient, or CO_2 uptake from the sand/Perlite growth medium. These values were subtracted from all measurements at these respective CO_2 concentrations. Each plant was measured at both ambient and elevated cuvette CO_2 concentration. Measurements were recorded over a one-minute period, during which the chamber temperature did not increase due to the large air volume and adequate mixing. Irradiance (PPFD > 1000 µmol m^{-2} s^{-1}) was measured at plant level, and was supplied by a light bank as described above (section 4.2.3).

Net canopy CO_2 assimilation rates (whole chamber volume) were measured, and converted to average plant leaf-area-based rates. For undefoliated plants, the total estimated canopy leaf area (obtained at harvest on 27 November immediately following gas exchange measurements) was used to calculate rates expressed on a leaf area basis. For defoliated plants, which were measured one to two weeks after the second defoliation, and one to two weeks before the third defoliation, the leaf area of the third clipping was used. This was regarded as the best possible estimate of total leaf area at measurement, given the available data. Even if not entirely accurate, this calculation represents the most conservative estimate of area-based assimilation rates in defoliated plants.

5.2.4 Defoliation and harvest

For each defoliation (23 October, 13 November and 4 December), freshly clipped material (mainly leaves) was weighed, oven-dried at 65°C, re-weighed, and milled. The fresh mass, dry mass and leaf area (all three defoliations) and leaf length (only on 4 December) of five randomly selected fully developed leaf blades per clipped plant were determined. Specific leaf mass (SLM, g m^{-2}) was calculated on the subsample and used together with total leaf dry mass to calculate estimated canopy leaf area. Leaf water content was calculated for the bulk sample. Carbohydrate determinations were immediately performed on fresh clippings, using the method described in section 4.2.5.

Undefoliated plants were harvested on 27 November 1996, after 183 days of fumigation, as they were showing early signs of senescence. Final harvest of defoliated plants was performed on 23 December, after 209 days. Plant material was sub-divided into senesced tillers, green leaf blades, leaf sheaths, reproductive tillers and roots. Leaf and tiller numbers were recorded, and material was oven-dried to constant mass at 65°C, and milled. The length, area and dry mass of 10 randomly sampled fully developed leaf blades were measured. SLM was calculated on the subsample and used together with total leaf dry mass to calculate estimated canopy leaf area.

Leaf total nitrogen concentrations were analysed on dried milled material of clipped and harvested plants, using the micro-Kjeldahl method. For each of the first two defoliations, leaf clippings of the six replicate plants per chamber were pooled (n=4). Due to lack of sufficient material at the third defoliation, clippings of all plants per CO_2 treatment were

University of Cape Town

pooled, giving only one measurement per treatment. These data were not included in the statistical analysis. The total nitrogen pool in offtake material per combined group of six replicate plants was calculated from leaf N concentration and the total dry mass of combined clippings (n=4). After harvesting of undefoliated plants, leaf material of the six replicate plants per chamber was pooled into two samples of three plants each (selected at random) (n=8). Leaf material of harvested defoliated plants was pooled according to the switch-over sub-treatment i.e. the three replicates for each combination of CO_2 long-term treatment/CO_2 short-term treatment (switch-over) were pooled (n=4).

5.2.5 Statistical analysis

Three-way ANOVA was used to analyse gas exchange data, with CO_2 concentration (treatment), CO_2 concentration (measurement) and defoliation treatment as factors. Clippings data were analysed using a repeated measures two-way ANOVA with CO_2 treatment and clipping date as factors. Harvest data were subjected to split-plot ANOVA with CO_2 treatment as the main plot, and defoliation treatment as the sub-plot. Only data from plants grown in the same chamber for the full duration of the experiment (not switched) were used in the case of the defoliation treatment (giving 24 undefoliated plants and 12 defoliated plants for each CO_2 treatment). For leaf N concentrations and N pools, n=8 for undefoliated and n=4 for defoliated plants. In addition, harvest data were analysed separately for defoliated plants only, using a split-plot ANOVA with long-term CO_2 treatment as the main plot, and the CO_2 concentration for the last three weeks (CO_2 switch-over) as the sub-plot (giving 12 plants per treatment combination, except for leaf N concentration where n=4).

University of Cape Town

5.3 Results

5.3.1 Regrowth following defoliation

On the first defoliation date (23 October), shoot offtake (clippings) biomass was already significantly increased in elevated CO_2 (Fig. 5.1a, Fig. 5.2, Table 5.2). Strong shoot regrowth after the first defoliation was followed by a reduction in regrowth after the second defoliation. The effects of the third defoliation are shown in the harvest data (Table 5.4) but are addressed below due to differing methods of measurement and analysis, making a direct comparison unjustifiable. Offtake biomass was consistently greater in elevated CO_2 for the period from

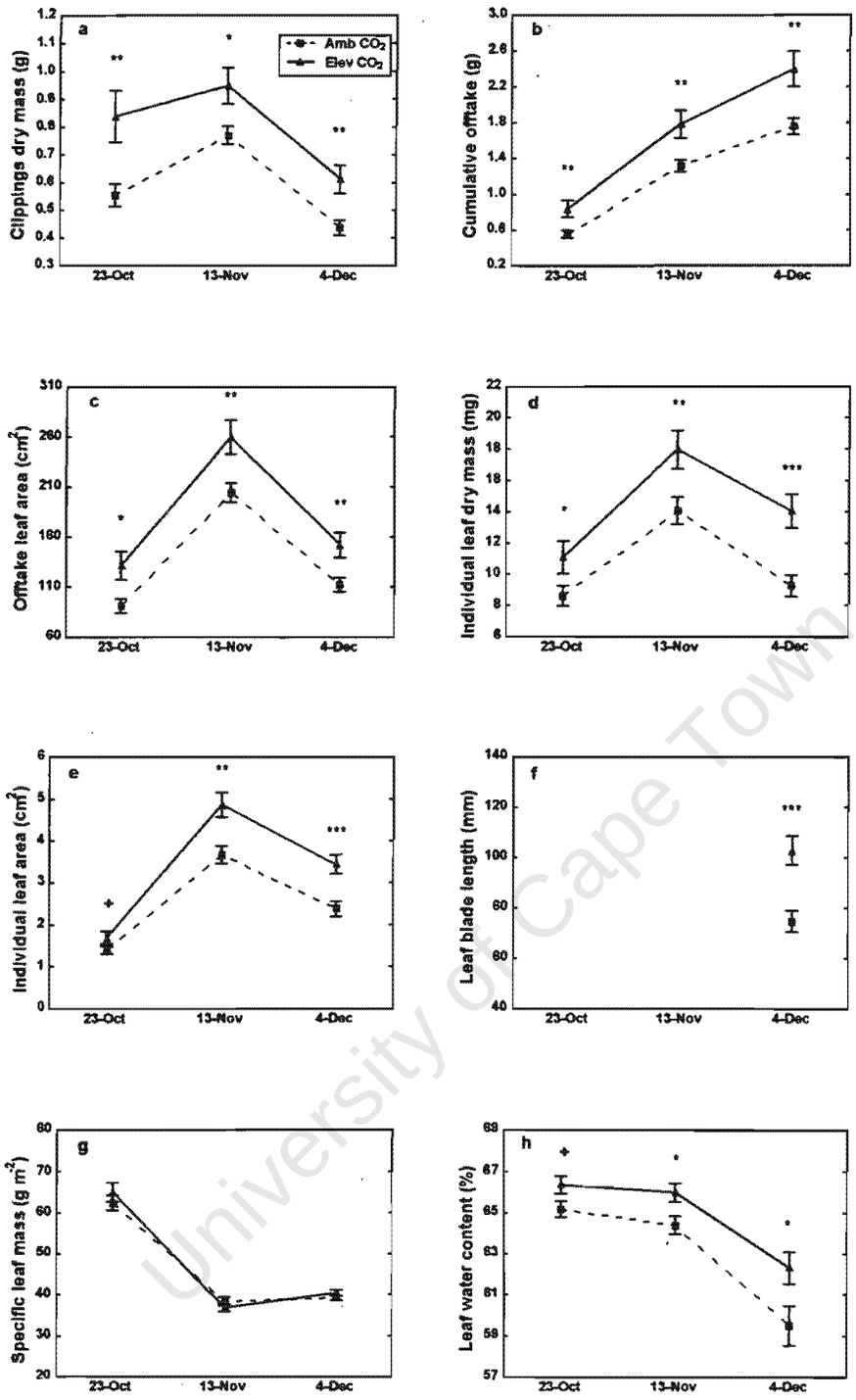


Fig. 5.1 Shoot offtake and leaf characteristics at three defoliation times. Values are means per treatments (n=24) ± SE. + P≤0.10, * P≤0.05, ** P≤0.01, *** P≤0.001 according to one-way ANOVA for each date of defoliation. Results of repeated measures two-way ANOVA presented in Table 5.2.

University of Cape Town

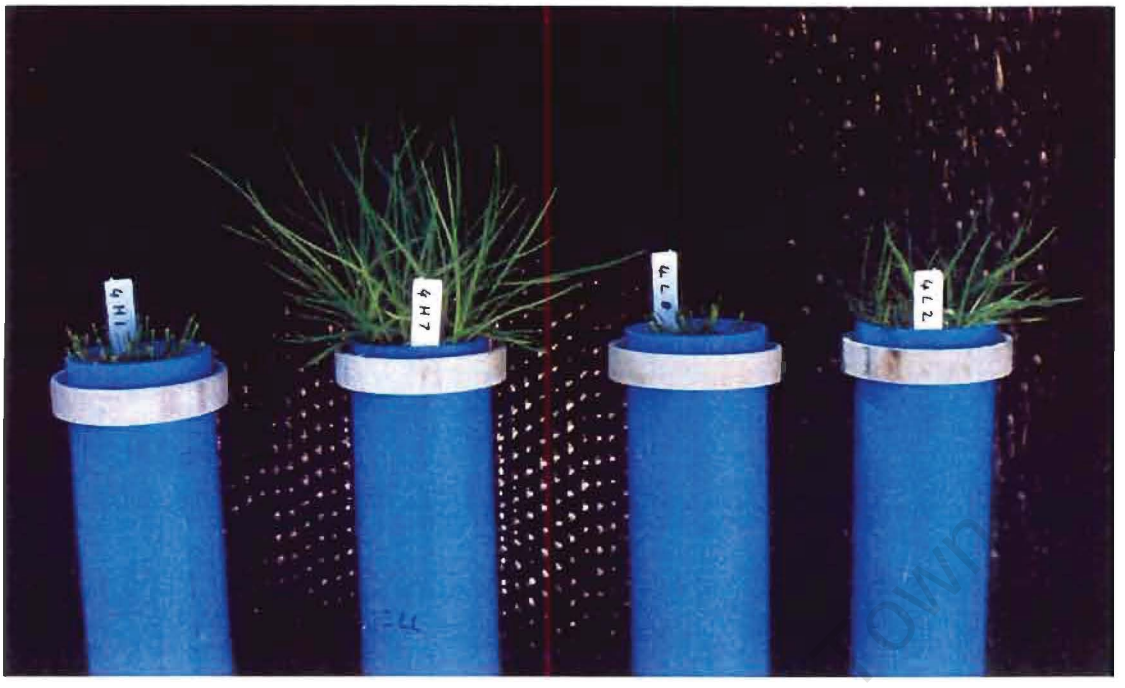


Fig. 5.2 *Themeda* plants from adjacent chambers after the first defoliation. Treatments are f.l.t.r. elevated CO_2, defoliated; elevated CO_2, undefoliated; ambient CO_2, defoliated; ambient CO_2, undefoliated.

University of Cape Town

the first to the third defoliation, and this response was similar for all three clippings (no CO_2/date interaction). Cumulative offtake was consistently greater under elevated CO_2, with the relative increase rising after each defoliation (Fig. 5.1b). Estimated offtake leaf area in elevated CO_2 (Fig. 5.1c) paralleled the response for offtake biomass, as did individual leaf dry mass (Fig. 5.1d) and individual leaf area (Fig. 5.1e). The latter was due mainly to longer leaf blades (Fig. 5.1f) in elevated CO_2, as shown at the third defoliation. At the individual leaf level, however, significant interaction was found between CO_2 treatment and defoliation date, due to the progressive increase in the relative CO_2-response from the first to the third defoliation. Leaf regrowth had reduced specific leaf mass (SLM) compared to pre-defoliation levels, and SLM did not differ between CO_2 treatments (Fig. 5.1g). Leaf percentage water content progressively declined following successive defoliations, particularly after the second defoliation, possibly due to increasing ambient temperatures and increased transpirational water loss of softer leaves. However, leaf water content was consistently higher in plants grown in elevated CO_2 (Fig. 5.1h).

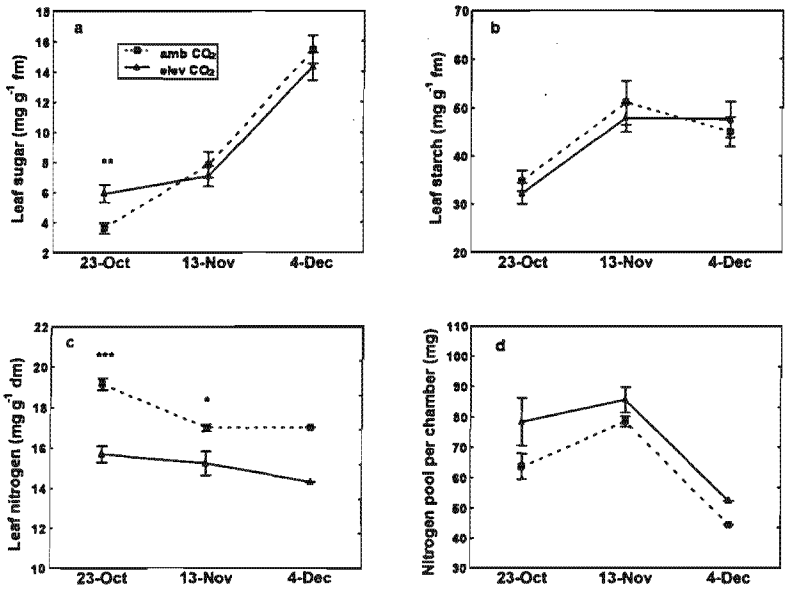


Fig. 5.3 Leaf sugar, starch and nitrogen concentrations, and offtake nitrogen pools per chamber (6 plants) at three defoliation times. Values are means per treatment (n=24) ± SE. * P≤0.05; ** P≤0.01; *** P≤0.001 according to one-way ANOVA for each date of defoliation. Results of repeated measures two-way ANOVA presented in Table 5.2.

Table 5.2 Statistical significance (repeated measures two-way ANOVA) of the effects of elevated CO_2 concentration and date of defoliation, and their interaction, on shoot offtake and leaf characteristics at three defoliation times. + P≤0.10; * P≤0.05; ** P≤0.01; *** P≤0.001; NS not significant.

Parameter	Main effects		Interaction
	$[CO_2]$	Date	$[CO_2]$ x Date
Clippings dry mass	**	***	NS
Cumulative offtake	**	***	***
Offtake leaf area	**	***	NS
Individual leaf dry mass	**	***	+
Individual leaf area	**	***	***
Individual leaf length	***	-	-
SLM	NS	***	NS
Water content	*	***	NS
Sugar concentration	NS	***	*
Starch concentration	NS	***	NS
Nitrogen concentration	**	**	*
Nitrogen pool	NS	*	NS

University of Cape Town

Total sugar concentrations of freshly clipped leaf material (Fig. 5.3a, Table 5.2) were low, but increased progressively after successive defoliations. Sugar levels were only higher in elevated CO_2 at the first defoliation, but not thereafter. Foliar starch concentrations (Fig. 5.3b) increased after the first defoliation but stabilized after the second defoliation, and were not significantly altered by elevated CO_2. Leaf total nitrogen concentrations (Fig. 5.3c) decreased gradually after successive defoliations, but more so in ambient CO_2 (significant interaction). [N] was consistently lower in elevated CO_2, particularly at the first defoliation. The nitrogen content (total pool) of clippings rose after the first defoliation (Fig. 5.3d) but decreased after the second defoliation. Elevated CO_2 did not alter the total N uptake into shoots i.e. similar N pools were allocated to growing shoots in ambient and elevated CO_2.

5.3.2 Canopy gas exchange

Net CO_2 assimilation rates measured one week after the second defoliation, and expressed on an average leaf area basis, were significantly greater in defoliated than in undefoliated plants (Fig. 5.4a, Table 5.3). Short-term CO_2 enrichment in the IRGA cuvette during measurement ($[CO_2]$ms) consistently led to increased assimilation rates compared to ambient cuvette CO_2 concentrations. However, long-term elevated CO_2 treatment ($[CO_2]$trt) resulted in decreased assimilation rates probably associated with photosynthetic downregulation, particularly in defoliated plants. This led to similar area-based assimilation rates being measured between plants grown and measured under ambient CO_2, and plants grown and measured under elevated CO_2 concentration, for both defoliated and undefoliated plants.

When expressed on a whole canopy basis (Fig. 5.4b, Table 5.3), defoliated plants had smaller net CO_2 assimilation rates due to the smaller canopy leaf area (Table 5.4). Short-term CO_2 enrichment boosted assimilation rates in both undefoliated and defoliated plants, irrespective of CO_2 treatment. However, there were no significant long-term differences between plants grown under ambient or elevated CO_2 when measured at the same cuvette CO_2 concentration. Nevertheless, plants grown and measured under elevated CO_2 had greater canopy assimilation rates than plants grown and measured under ambient CO_2 concentration due to the short-term CO_2 effect.

University of Cape Town

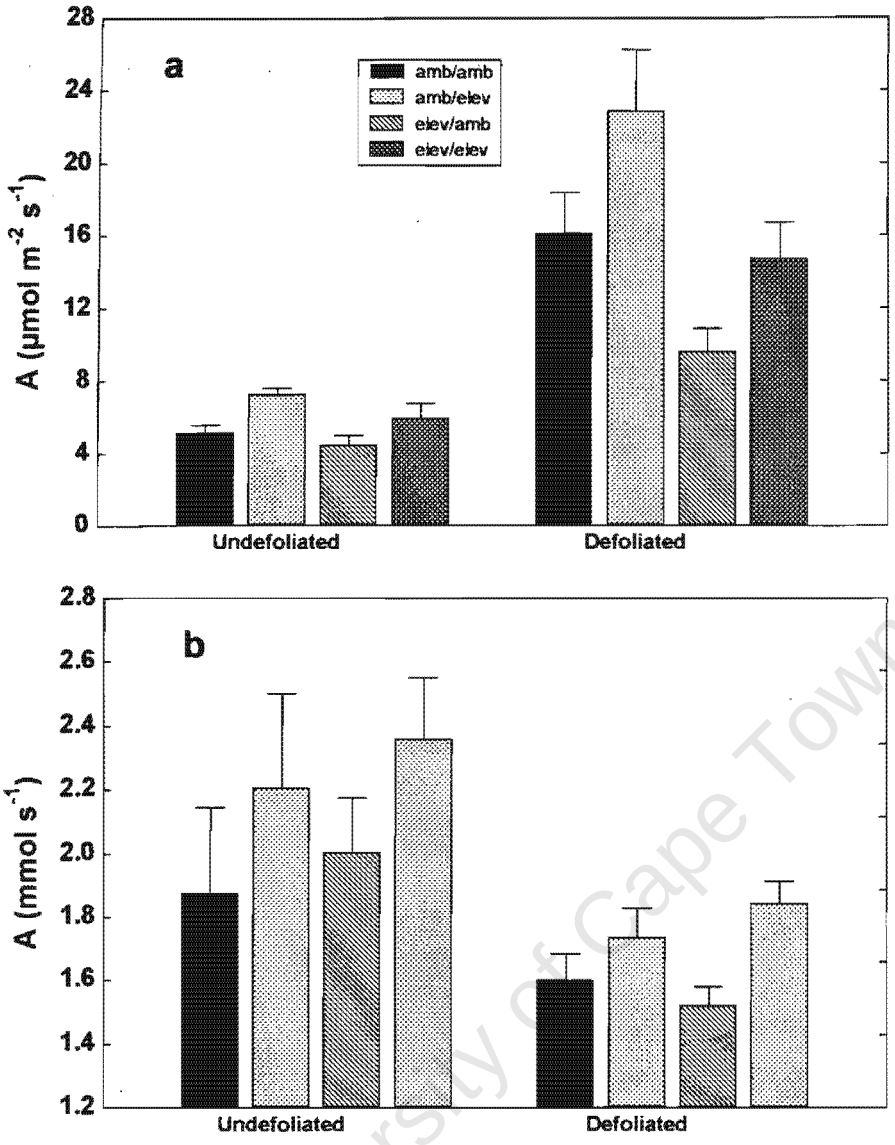


Fig. 5.4 Net CO_2 assimilation rates (A) (**a**, area-based; **b**, canopy-based) in ambient or elevated CO_2, with or without defoliation. Gas exchange measured at both ambient (360 μmol m^{-2} s^{-1}) and elevated (680 μmol m^{-2} s^{-1}) CO_2 concentration. In the legend, the first abbreviation before the slash (amb or elev) refers to the treatment CO_2 concentration, and the second abbreviation after the slash refers to the cuvette (measurement) CO_2 concentration. Values are means per treatment (n=8) ± SE.

University of Cape Town

Table 5.3 Statistical significance of the effects of CO_2 concentration during growth ($[CO_2]$trt), CO_2 concentration during measurement ($[CO_2]$ms), defoliation, and their interaction on net CO_2 assimilation rates (A) at leaf and canopy level. * P≤0.05; ** P≤0.01; *** P≤0.001; NS not significant according to three-way ANOVA.

Parameter	Main effects			Interactions			
	$[CO_2]$trt	$[CO_2]$ms	Defol	$[CO_2]$trt x $[CO_2]$ms	$[CO_2]$trt x defol	$[CO_2]$ms x defol	$[CO_2]$trt x $[CO_2]$ms x defol
A (area-based)	**	**	***	NS	*	NS	NS
A (canopy-based)	NS	*	***	NS	NS	NS	NS

5.3.3 Final harvest - interaction with defoliation

Defoliation resulted in increased numbers of tillers and leaves (Table 5.4), but elevated CO_2 reduced leaf numbers in both undefoliated and defoliated plants. Total leaf biomass at harvest, representing residual material and three weeks of regrowth, was less in defoliated compared to undefoliated plants. However, when total leaf offtake from the previous three defoliations was added to the harvest leaf fraction (giving total plant leaf production), defoliated plants showed significantly higher leaf production under both CO_2 concentrations than undefoliated plants, as well as greater senesced tiller dry mass and total plant production. Sheath, shoot, root and total residual plant biomass at harvest were not altered by defoliation. Root:shoot ratios were higher in defoliated than undefoliated plants, due to slightly greater root biomass at ambient CO_2 levels, and slightly lower shoot biomass at elevated CO_2. Elevated CO_2 stimulated leaf production, shoot, root and total dry mass at harvest, as well as total plant production in both undefoliated and defoliated plants (no interaction).

Individual leaf lengths were reduced in defoliated plants (Table 5.4), but CO_2 enrichment increased leaf lengths. Similar patterns were observed for individual leaf area, dry mass per leaf and dry mass per sheath. Individual leaf area was particularly stimulated by elevated CO_2 in undefoliated plants, and this was the only significant (though small) interaction between the treatments for any parameter. Canopy leaf area was increased in elevated CO_2, but reduced by defoliation. There were no effects of CO_2 or defoliation treatment on specific leaf mass at harvest. Final total nitrogen concentrations in leaves were reduced by both elevated CO_2 and defoliation treatments, with no interaction. Total leaf nitrogen pools were also reduced by defoliation, but were not altered in elevated CO_2.

Although undefoliated plants were harvested earlier than defoliated plants, I regard the measured effects of defoliation as "real", as they mainly reflect the residual plant appearance, except possibly tiller number. At harvest, undefoliated plants were large, self-shading and showing signs of senescence, compared to the healthy appearance of defoliated plants. The "extra" regrowth period between 4 and 23 December (to facilitate the "switch-over" experiment) may have resulted in a relatively greater increase in total leaf production relative to undefoliated plants. Subtraction of this contribution from total leaf production (as an estimate of productivity at the time of harvest of undefoliated plants) does not, however,

University of Cape Town

Table 5.4 Final harvest biomass responses of *Themeda* grown under elevated CO_2 with/without defoliation. amb/undef = ambient CO_2 undefoliated; amb/def = ambient CO_2 defoliated; elev/undef = elevated CO_2 undefoliated; elev/def = elevated CO_2 defoliated. Values are means ± SE (n=24 for undefoliated, n=12 for defoliated, except for leaf [N] where n=8 for undefoliated and n=4 for defoliated). + P≤0.10; * P≤0.05; ** P≤0.01; *** ≤0.001; NS not significant according to split-plot ANOVA.

Parameter	Treatment				Statistical significance		
	amb/undef	amb/def	elev/undef	elev/def	[CO_2]	Def	[CO_2] x Def
Residual:							
Tiller number	39 ± 4	59 ± 4	34 ± 2	56 ± 4	NS	***	NS
Leaf number	161 ± 12	203 ± 13	141 ± 8	188 ± 10	+	***	NS
Leaf dry mass (g)	1.66± 0.14	1.35 ± 0.07	2.04 ± 0.17	1.42 ± 0.05	+	**	NS
Sheath dry mass (g)	0.69 ± 0.06	0.59 ± 0.05	0.71 ± 0.05	0.80 ± 0.08	NS	NS	NS
Senesced dry mass (g)	0.30 ± 0.03	0.44 ± 0.05	0.39 ± 0.05	0.47 ± 0.04	NS	*	NS
Shoot dry mass (g)	2.70 ± 0.21	2.59 ± 0.10	3.47 ± 0.33	2.70 ± 0.13	*	NS	NS
Root dry mass (g)	4.22 ± 0.32	4.59 ± 0.18	4.99 ± 0.33	4.94 ± 0.20	+	NS	NS
Total dry mass (g)	6.92 ± 0.51	7.18 ± 0.21	8.47 ± 0.60	7.64 ± 0.30	*	NS	NS
Root:shoot ratio	1.60 ± 0.07	1.80 ± 0.11	1.55 ± 0.07	1.86 ± 0.07	NS	**	NS
Leaf length (mm)	112 ± 6	92 ± 3	147 ± 6	109 ± 5	***	***	NS
Leaf area (cm^2)	3.79 ± 0.25	3.01 ± 0.16	5.19 ± 0.21	3.51 ± 0.25	***	***	+
Dry mass per leaf (mg)	18.2 ± 1.4	13.7 ± 0.7	24.7 ± 1.2	16.7 ± 1.3	***	***	NS
Dry mass per sheath (mg)	19.1 ± 1.5	10.4 ± 1.0	21.1 ± 1.1	15.6 ± 2.5	+	***	NS
Canopy leaf area (cm^2)	345 ± 24	295 ± 13	429 ± 33	299 ± 9	*	**	NS
Residual + offtake:							
Total leaf production (g)	1.66 ± 0.14	3.10 ± 0.18	2.04 ± 0.17	3.91 ± 0.29	**	***	NS
Total plant production (g)	6.92 ± 0.51	8.93 ± 0.33	8.47 ± 0.60	10.12 ± 0.53	*	**	NS
Chemistry:							
SLM ($g\ m^{-2}$)	47.3 ± 1.4	45.8 ± 0.6	47.3 ± 0.7	47.5 ± 1.0	NS	NS	NS
Leaf [N] ($mg\ g^{-1}$)	11.3 ± 0.2	10.1 ± 0.1	10.1 ± 0.3	9.2 ± 0.2	**	**	NS
N pool (mg)	55.7 ± 2.5	40.8 ± 1.7	60.6 ± 4.2	38.9 ± 0.5	NS	***	NS

University of Cape Town

alter the result as leaf production remains significantly greater in defoliated plants even after only two defoliations.

5.3.4 Final harvest - interaction with CO_2 switch-over (last three weeks)

Defoliated plants that were switched from elevated to ambient CO_2 for the last three weeks of regrowth had slightly reduced leaf numbers compared to those remaining at elevated CO_2, whereas those switched from ambient to elevated CO_2 also showed leaf number reductions relative to those remaining at ambient CO_2 (marginally significant interaction, Table 5.5). There were no other interactions between short-term and long-term responses to high CO_2. Leaf, sheath and shoot biomass were smaller in plants grown under ambient CO_2 for the last three weeks, when plants having the same long-term exposure history are compared. The same response was found for individual leaf length, area, dry mass and individual sheath dry mass, as well as estimated canopy leaf area. On the other hand, long-term exposure to elevated CO_2 prior to the switch led to reduced leaf and shoot biomass and canopy leaf area, when plants with the same final CO_2 treatment are compared. This resulted in greater root:shoot ratios.

Leaf nitrogen concentrations (Table 5.5) were reduced primarily over the short-term i.e. plants grown in elevated CO_2 for the last three weeks had lower leaf [N], irrespective of their previous long-term CO_2 treatment. However, total leaf nitrogen pools were decreased in all plants initially exposed to elevated CO_2, and this was not altered by the CO_2 concentration provided for the last three weeks.

University of Cape Town

5.4 Discussion

5.4.1 Hypothesis 1: CO_2-response in defoliated vs. undefoliated plants

The results of this experiment support the first part of the first hypothesis, namely that elevated CO_2 stimulates growth of both undefoliated and defoliated *Themeda* plants. However, there was no evidence that the relative stimulation of productivity was greater in defoliated plants, as shown by the lack of interaction between defoliation and CO_2 treatments. In fact, the responses were largely additive. Defoliation stimulated leaf productivity by 87% and 92% in ambient and elevated CO_2, respectively, whereas elevated CO_2 increased leaf productivity by 23% and 26% in undefoliated and defoliated plants, respectively. This

Table 5.5 Final harvest biomass responses of defoliated *Themeda* grown under ambient or elevated CO_2 with/without switching to the other CO_2 concentration for the last three weeks. amb/amb = ambient CO_2 initially, ambient CO_2 after switch; amb/elev = ambient CO_2 initially, elevated CO_2 after switch; elev/amb = elevated CO_2 initially, ambient CO_2 after switch; elev/elev = elevated CO_2 initially, elevated CO_2 after switch. $[CO_2]$trt, initial CO_2 treatment; $[CO_2]$sw, CO_2 treatment after switch. Values are means ± SE (n=12, except for leaf [N] where n=4). + P≤0.10; * P≤0.05; ** P≤0.01; NS not significant according to split-plot ANOVA.

Parameter	Treatment				Statistical significance		
	amb/amb	amb/elev	elev/amb	elev/elev	$[CO_2]$trt	$[CO_2]$sw	$[CO_2]$trt x $[CO_2]$sw
Tiller number	59 ± 4	54 ± 5	47 ± 4	56 ± 4	NS	NS	NS
Leaf number	203 ± 13	184 ± 14	162 ± 10	188 ± 10	NS	NS	+
Leaf dry mass (g)	1.35 ± 0.07	1.49 ± 0.09	1.18 ± 0.05	1.42 ± 0.05	+	**	NS
Sheath dry mass (g)	0.59 ± 0.05	0.74 ± 0.05	0.60 ± 0.05	0.80 ± 0.08	NS	**	NS
Senesced dry mass (g)	0.44 ± 0.05	0.48 ± 0.04	0.38 ± 0.04	0.47 ± 0.04	NS	NS	NS
Shoot dry mass (g)	2.59 ± 0.10	2.81 ± 0.14	2.23 ± 0.09	2.70 ± 0.13	*	**	NS
Root dry mass (g)	4.59 ± 0.18	4.51 ± 0.34	4.44 ± 0.33	4.94 ± 0.20	NS	NS	NS
Total dry mass (g)	7.18 ± 0.21	7.32 ± 0.43	6.67 ± 0.38	7.64 ± 0.30	NS	NS	NS
Root:shoot ratio	1.80 ± 0.11	1.60 ± 0.10	1.99 ± 0.13	1.86 ± 0.07	*	NS	NS
Leaf length (mm)	92 ± 3	108 ± 5	102 ± 5	109 ± 5	NS	*	NS
Leaf area (cm^2)	3.01 ± 0.16	3.57 ± 0.21	3.38 ± 0.18	3.51 ± 0.25	NS	+	NS
Dry mass per leaf (mg)	13.7 ± 0.7	16.8 ± 1.0	15.6 ± 1.1	16.7 ± 1.3	NS	+	NS
Dry mass per sheath (mg)	10.4 ± 1.0	14.3 ± 1.3	13.4 ± 1.5	15.6 ± 2.5	NS	+	NS
Canopy leaf area (cm^2)	295 ± 13	320 ± 22	257 ± 12	299 ± 9	*	*	NS
SLM ($g\ m^{-2}$)	45.8 ± 0.6	47.0 ± 1.1	46.0 ± 0.8	47.5 ± 1.0	NS	NS	NS
Leaf [N] ($mg\ g^{-1}$)	10.1 ± 0.1	9.3 ± 0.1	10.0 ± 0.2	9.2 ± 0.2	NS	***	NS
N pool (mg)	40.8 ± 1.7	41.7 ± 2.8	35.2 ± 1.3	38.9 ± 0.5	*	NS	NS

University of Cape Town

suggests that a history of exposure to high CO_2 and the development of some residual effect such as greater carbon reserves in roots and sheath bases, did not influence the regrowth response following defoliation, at least not within a single growing season. This is supported indirectly by the lack of interaction between defoliation and CO_2 treatments with respect to root and sheath biomass at harvest. Root biomass was slightly increased (13%) in elevated CO_2, but did not respond to defoliation. This contrasts with Wilsey *et al.* (1997) who found that defoliation of African C_4 grasses stimulated root and crown growth, but this was likewise not influenced by CO_2 concentration. Sheath biomass was not altered by any treatment in this experiment. Although not directly measured, it seems unlikely that roots and sheaths contributed significant carbon to leaf extension and new tiller and leaf growth, in neither ambient nor elevated CO_2. On the other hand, senesced aboveground biomass increased in response to defoliation (by 34%), possibly signifiying the re-mobilization of carbon from portions of residual shoot material to help fund regrowth (McNaughton *et al.* 1982, Welker *et al.* 1985). Again, this response was not significantly different in ambient or elevated CO_2. Therefore, intra-plant carbon allocation patterns in response to defoliation were apparently not altered by CO_2 concentrations, or conversely, elevated CO_2 elicited the same responses in undefoliated and defoliated plants. Danckwerts (1993) showed that reserve carbon re-mobilization to new growth in *Themeda* played a transient role for the first few days after defoliation. In this study, utilization of reserve carbon may have occurred initially after each defoliation, especially from residual above-ground material (Welker *et al.* 1985), but to no greater extent in high CO_2.

Although foliar nitrogen concentrations ([N]) were slightly reduced in defoliated plants at harvest (Table 5.4), this result is misleading as it does not reflect the differential rates of decreasing leaf [N] with time in undefoliated and defoliated plants. Fig. 5.3c shows that, on the first defoliation date, nitrogen concentrations in leaf offtake were 19.2 mg g^{-1} and 15.7 mg g^{-1} in ambient and elevated CO_2, respectively. On the third defoliation date (4 December), nitrogen concentrations in leaf offtake had dropped slightly to 17.0 mg g^{-1} and 14.3 mg g^{-1}, whereas in undefoliated plants (harvested one week earlier) leaf N concentrations were considerably lower at 11.3 mg g^{-1} and 10.1 mg g^{-1} in ambient and elevated CO_2, respectively. As shown before for grasses (Ruess 1988, Hiernaux & Turner 1996), defoliation retarded the senescence- and shading-related reduction in leaf [N]. At about the same time, carbon assimilation rates (leaf area basis, Fig. 5.4a) were significantly higher in defoliated

University of Cape Town

than undefoliated plants. This was probably attributable to a combination of photosynthetic up-regulation (enhanced Rubisco activity linked to higher [N]) and increased light penetration into the defoliated canopy (McNaughton *et al.* 1982). This stimulation of photosynthetic capacity following defoliation was, however, accompanied by photosynthetic down-regulation in elevated CO_2. All in all, when measured at growth CO_2 concentrations, defoliated plants had greater carbon assimilation rates (area basis) than undefoliated plants, but not more so in elevated CO_2. At a whole-canopy level (Fig. 5.4b), total carbon uptake rates were lower and relatively less stimulated in elevated CO_2 in defoliated (15%) than in undefoliated plants (26%). It seems that defoliation of *Themeda* did not confer any advantages which favoured a relatively greater CO_2-response during the first growth season, at least not for the imposed defoliation regime.

In the longer term (e.g. the next growth season), this lack of interaction could change, since the absence of any defoliation would result in canopy closure, which could limit the CO_2-induced growth stimulation of young growth due to reduced light use efficiency. It is interesting to note that Wilsey *et al.* (1994, 1997) found that a range of tropical and sub-tropical C_4 grass species, including *Themeda triandra* from the African Serengeti, were relatively unresponsive to elevated CO_2, with or without defoliation. However, they employed collars around individual plants simulating canopy closure (following the advice of McNaughton 1992), and this level of shading may have precluded a significant CO_2-response at the young growth stages. Also, their plants were sourced from the field in Africa in the early 1970's, and have been kept in a greenhouse at Syracuse University ever since. They would be fully acclimated to the cooler climate in the north-eastern USA, possibly altering the potential CO_2-responsiveness.

University of Cape Town

5.4.2 Hypothesis 2: Regrowth in elevated vs. ambient CO_2

The prediction that elevated CO_2 would allow for relatively greater and more sustained regrowth following repeated defoliation, was not supported by the evidence. Although CO_2-induced stimulation of offtake dry mass and leaf area was sustained during three-weekly regrowth periods following the first two defoliations (Fig. 5.1a,c), the relative stimulation was not significantly different between the first growth period (pre-defoliation) and after the first two defoliations. This was shown by the lack of interaction between CO_2 treatment effect and defoliation date. If greater carbon availability from, for example, roots

and shoot bases following long-term exposure to high CO_2 had played an important role in shoot regrowth, one would have expected a relatively larger regrowth response in elevated than in ambient CO_2 after the first defoliation, and a greater buffering against the reduction in regrowth following the second defoliation. Instead, the relative stimulation appeared to be constant, or even slightly decreased after successive defoliations.

In fact, the positive effect of elevated CO_2 on whole shoot regrowth all but disappeared after the third defoliation, as measured at harvest. Residual leaf biomass (+5%) and canopy leaf area (+1%) were only marginally increased in elevated CO_2. This may have been attributable to reductions in tiller numbers (-5%, just not significant) and leaf numbers (-7%) in elevated CO_2. By contrast, the CO_2-induced enhancement of individual leaf growth persisted and was even increased, in relative terms, after each defoliation. It is likely that this could be attributable to improved leaf water relations in elevated CO_2 (Fig.5.1h). What could account for the apparent down-regulation of whole shoot growth responses in elevated CO_2 following repeated defoliations? Leaf nitrogen concentrations emerge as a likely explanation. At the third defoliation, [N] was still relatively high (14-17 mg g^{-1}, Fig. 5.1), though reduced in elevated CO_2, and the shoot nitrogen pool had declined since the second defoliation. Three weeks later, [N] had dropped to 9-10 mg g^{-1} (Table 5.4) and the nitrogen pool had further decreased. This indicates a significant and rapid decline in plant vigour and nutrient uptake capacity, or nitrogen remobilization (Fig. 5.3d, Thornton & Millard 1997) to help replenish lost shoot material, which may already have been evident after the second defoliation. The weakened regrowth potential then also weakened the potential CO_2-response, similarly to the apparent correlation between CO_2-responsiveness and relative growth rate (or vigour) in a wide range of plant species (Poorter 1993, Poorter *et al.* 1996). Gifford & Morison (1985) showed that in a C_4 grass sward exposed to elevated CO_2, the growth enhancement initially measured under a regime of low water supply became attenuated with successive regrowth following repeated defoliation. This was linked to the response of leaf area to soil water deficit in the long term. It seems that any condition leading to a gradual decrease in plant vigour as measured by shoot regrowth, such as water stress, nutrient draw-down or possibly carbon reserve depletion under high grazing intensity, would in the longer term depress the potential regrowth response in elevated CO_2. The CO_2 exposure history appears to have little influence over, or buffering capacity against these effects of defoliation pressure.

University of Cape Town

5.4.3 Hypothesis 3: Long-term indirect stimulation vs. short-term direct stimulation of regrowth in elevated CO_2

The third hypothesis provided the opportunity for a novel approach to help tease apart the relative importance of long-term versus short-term resource utilization and allocation for regrowth following defoliation, and how this may be affected by elevated CO_2. Plants were grown in ambient or elevated CO_2 for about five months, and then subjected to three consecutive defoliations. However, after the third and last defoliation, half the plants in each CO_2 treatment were switched to the opposite CO_2 treatment for the last three weeks of regrowth. The final harvest data (Table 5.5) shows long-term CO_2 responses ([CO_2]trt) for certain parameters when compared at the same final CO_2 treatment level: decreased residual leaf and shoot biomass, increased root:shoot ratios, decreased canopy leaf areas, and reduced shoot N pools. When one compares this "anomalous" response to the data in Table 5.4, it becomes clear that short-term stimulation of leaf and shoot growth in elevated CO_2 masks a longer-term suppression of above-ground growth, which only emerges when the short-term stimulus is removed. This is also reflected in the disappearance of the large CO_2-induced increases in individual leaf length, area, mass and individual sheath mass when the CO_2 stimulus is removed. On the other hand, these responses emerged when plants previously grown in ambient CO_2 became acclimated to elevated CO_2 for the last three weeks. The statistical analysis shows clearly that above-ground growth increases of individual leaves and sheaths, as well as of the whole canopy, are predominantly short-term effects which are easily lost when the atmospheric CO_2 concentration is reduced.

If CO_2 exposure history had no apparent influence on CO_2-induced growth stimulation, and in fact appeared to reduce the potential for positive growth responses, what were the long-term mechanisms of diminishing responsiveness, and the short-term mechanisms of increased responsiveness? One possible explanation for the former is the downregulation of shoot nitrogen uptake capacity after prolonged exposure to high CO_2, as shown by the reduced leaf offtake nitrogen pools (Table 5.5). Nitrogen supply was shown by McNaughton *et al.* (1983) to be the primary determinant of regrowth biomass following clipping of African C_4 species. In this study, the mechanism was possibly related to long-term changes in belowground biochemistry of defoliated plants, as no such effect was apparent in undefoliated plants (Table 5.4), and the effect was not inducible by short-term exposure of

University of Cape Town

shoots to elevated CO_2 or reversible by short-term absence of high CO_2 (Table 5.5). A limitation would, therefore, be placed on the regrowth response when the short-term CO_2-stimulus of leaf and shoot growth was removed. This stimulus was apparently not sensitive to lower N uptake, as available nitrogen was simply "diluted" to fund the regrowth response in elevated CO_2.

Growth stimulation in elevated CO_2 in the short term is likely associated with changes in photosynthetic capacity, canopy area development, improved water relations, and possibly increased leaf temperature (see Chapter 9). Gas exchange measurements of both undefoliated and defoliated plants after the second defoliation treatment revealed almost complete homeostasis in photosynthetic carbon uptake (on a leaf area basis) due to photosynthetic down-regulation. This means that, on an area basis, similar amounts of carbon were fixed by plants grown and measured at ambient or at elevated CO_2. Nevertheless, both undefoliated and defoliated plants growing in elevated CO_2 had increased total canopy carbon assimilation rates owing to their larger canopy leaf areas. This may have provided most if not all of the additional carbon required for increased growth in elevated CO_2. Defoliated plants did not accumulate carbohydrates in their leaves, so that any extra carbohydrates produced in elevated CO_2 would have been used for leaf growth, or allocated below-ground (root growth or exudation). This contrasts with the responses measured in C_3 grasses, where conditions leading to a carbon-sink limitation, such as low N supply, lead to increases in foliar carbohydrate concentrations in elevated CO_2 in defoliated and undefoliated plants (Bowler & Press 1996, Fischer *et al.* 1997, Zanetti *et al.* 1997, Chapter 2), and restrict the growth response to elevated CO_2. Carbohydrate accumulation does not usually occur in C_4 grasses (Ghannoum *et al.* 1997, Read & Morgan 1996, Wilson 1975, Chapters 2 and 4) and may reflect higher efficiencies of phloem loading (Körner *et al.* 1995) and higher nutrient use efficiencies (Brown 1978) than in C_3 grasses.

The stimulus (trigger) for increased leaf growth was possibly related to the improved leaf water content in elevated CO_2 (Patterson 1986, Knapp *et al.* 1993, Ghannoum & von Caemmerer, pers. communication). A positive relationship exists between leaf water potential and cell elongation and leaf extension in cereal crops (Boyer 1970) and grasses (Toft *et al.* 1987).

University of Cape Town

5.4.4 Growth form and productivity responses under different treatment combinations

When one examines the effects of defoliation *per se*, it becomes clear that, apart from obvious responses such as reduced residual leaf biomass and smaller individual leaves and sheaths at harvest, the most striking changes are the greatly increased leaf and plant productivity, and altered shoot morphology. *Themeda* has previously been shown to compensate for defoliation i.e. total production of defoliated plants equals or exceeds that of undefoliated plants (Coughenour *et al.* 1985, Oesterheld & McNaughton 1988). Compensatory responses measured in this study have been commonly reported in previous studies of grasses, and include increased allocation to leaf area production, increased photosynthetic rates related to higher leaf nitrogen concentrations and improved light and water use efficiencies, higher relative growth rates, higher number but smaller size of tillers, and lower specific leaf mass (McNaughton *et al.* 1982, Oesterheld & McNaughton 1988). With respect to shoot morphology, increases in basal tiller initiation after defoliation are common where meristems are damaged or removed (Caldwell *et al.* 1981, McNaughton *et al.* 1982). The elevated meristems of *Themeda* are particularly susceptible even at the clipping height employed in this experiment (4 cm), this being one of the reasons this species is sensitive to frequent defoliation, as new tillers must be initiated and established. In addition, tiller development in *Themeda* is stimulated by increased light penetration to basal meristems (Everson *et al.* 1988) and the altered quality of light in an open canopy (Deregibus *et al.* 1985).

Following from these responses, the different combinations of CO_2 and defoliation treatments used in this experiment resulted in characteristic growth forms and productivity levels. Defoliation in ambient CO_2 produced plants with a greater density but with a shorter canopy, whereas defoliation in elevated CO_2 produced plants with a similarly dense canopy but a canopy height almost equal to that of undefoliated plants in ambient CO_2. This treatment combination also resulted in the highest plant and leaf productivity levels. In contrast, elevated CO_2 in the absence of defoliation produced a shoot structure that was open (fewer tillers) combined with increased height, similar to that described in Experiment 1 (Chapter 4). This growth form was associated with a productivity lower than that for both defoliated treatments, and bears a striking resemblance to that described by McNaughton (1992) for grasses grown with collars simulating gradual canopy closure. The results suggest

University of Cape Town

that elevated CO_2 may have an initial positive effect on *Themeda* growth mediated mainly via increased leaf extension, but this would accelerate self-shading and canopy closure as the growth season progresses. As a result, further potential growth enhancement in elevated CO_2 would be limited unless the canopy was rejuvenated through defoliation of one sort or another. However, if sustained high productivity of *Themeda* veld is the objective, increasing defoliation frequencies would have to be carefully balanced against the potential loss of plant vigour associated with reduced nutrient uptake capacity and reserve depletion. In addition, the reduction in forage quality due to reductions in leaf [N] in elevated CO_2 (Owensby *et al.* 1996a, Fig. 5.3c) could play an important moderating role, as nutrient draw-down linked to defoliation pressure will be exacerbated in elevated CO_2.

University of Cape Town

Chapter 6

The developmental sequence of morphological and photosynthetic CO_2-responses of *Themeda triandra*

6.1 Introduction

Elevated CO_2 studies in the past have commonly reported the responses of plants only at the end of experiments, usually at final harvest. More recently, new approaches incorporating sequential harvest over the whole growth period, or measurements of plant performance at more than one time, have been developed. This follows on the recognition that the relative response to elevated CO_2 may depend on the developmental or phenological stage of the plant, and this history can mediate effects on plant growth at later stages (Ackerly *et al.* 1992, Reekie 1996). Quite often, the CO_2 response for photosynthesis and growth will be most pronounced during periods of active carbon assimilation and high relative growth rates (see Chapter 8), whereas responses such as CO_2-induced carbohydrate accumulation will be more dominant during seasons when carbon reserve accumulation is naturally high (Loehle 1995). These developmentally-linked responses to elevated CO_2 are not identifiable or are masked when plants are measured on one occasion only.

In Experiments 1 (Chapter 4) and 3 (Chapter 5), certain CO_2 effects of *Themeda* were measured at harvest, the interpretation of which required an understanding of the relative timing of their emergence during the course of plant development. For example, photosynthetic rates expressed on a leaf area basis were similar or slightly lower in elevated than ambient CO_2 in mature plants in both experiments, owing to downregulation of photosynthetic capacity. However, significant growth stimulation was also found, which would require increased carbon availability. This could have been achieved either through (1) increased area-based photosynthetic rates, possibly at an earlier developmental stage, (2) increased canopy-level carbon assimilation, which depends on changes in canopy leaf area development, (3) utilization of reserve carbon (not significant, see Chapter 5), or (4) decreased respiration rates (not measured in these studies, but see Chapter 8). Prolonged growth in elevated CO_2 also gave rise to pronounced shoot morphological changes. Plants developed a taller, more open canopy, due to longer leaf blades and leaf sheaths, but reduced

University of Cape Town

tiller numbers. It was suggested that the greater canopy leaf area led to increased self-shading of the basal nodes from which tillers are initiated (Everson *et al.* 1988). This explanation was based on the assumption that early increases in sheath and leaf blade elongation preceded the reduction in tiller initiation, so that tiller development was only secondarily affected by CO_2 concentration.

In this chapter, these questions will be addressed by investigating the developmental sequence of CO_2-induced morphological responses, and the differences in leaf- and canopy-level carbon assimilation rates between young and mature *Themeda* plants in Experiment 3. Some of the data have been taken from Chapter 5 (gas exchange of mature plants and morphological data at harvest) for comparative and interpretive purposes.

6.2 Materials and Methods

See Chapter 5 for plant establishment, experimental design and treatments, climatic conditions, gas exchange measurements of mature plants, and final harvest. Data for undefoliated plants only are used in this chapter.

6.2.1 Morphological measurements

Measurements of morphological development were performed on 3 June, 10 July, 6 August and 5 September. Sheath height, tiller number, numbers of emerging, extended and senesced leaves, and the leaf blade length of the two youngest extended leaves, were recorded for each plant. On the last measurement date, numbers of emerging and extended leaves and leaf lengths were not recorded due to increasing plant size. Morphological data (tiller number and leaf length) for undefoliated plants at harvest were taken from Chapter 5.

6.2.2 Leaf-level gas exchange measurements

Photosynthetic CO_2-response curves (A/c_i) were determined 10 weeks after the start of CO_2 fumigation, using five plants per CO_2 treatment. These measurements were performed at the Botany Department, University of Cape Town by Mr. F. Ludwig, and the data originally presented in his undergraduate project report (Ludwig 1996). An ADC LCA-3 IRGA was used, and cuvette CO_2 concentrations were varied between 0 and 1100 µmol mol^{-1}, and assimilation rates measured at 100 µmol mol^{-1} intervals. Light intensity was kept constant at

University of Cape Town

about 1000 µmol m^{-2} s^{-1} with a halogen lamp suspended above a water bath to prevent excessive heating of the cuvette. Cuvette temperature was constant at about 28°C. Humidity of incoming air was controlled by first saturating the air with water in a "water bubbler", and thereafter the air was passed through a tube filled with ferrous oxide to obtain a constant humidity of about 72% at 28°C. Response curves were fitted individually using non-linear regression and the monomolecular function $y=a(1-e^{b-cx})$ (Causton & Dale 1990), which provided a very good fit to data in this and other sets of measurements (Chapters 4, 7, 8). The strong feature of this model lies in its more realistic estimate of the light- and CO_2-saturated maximum rate of net CO_2 assimilation, compared to Michaelis-Menton-type functions which tend to overestimate this value (Causton & Dale 1990). Current C_3 photosynthesis models (e.g. Harley *et al.* 1992, Wullschleger 1993) cannot be applied to C_4 species due to their specialised photosynthetic biochemistry and physiology e.g. they lack photorespiration and possess different kinetics of the A/c_i response (Collatz *et al.* 1992). Although I do not use the Collatz model in the interests of simplicity (the purpose here was a simple comparison of maximum photosynthetic rate in ambient and elevated CO_2), their terminology is applied, so that "J_e" represents the light- and CO_2-saturated rate of net CO_2 assimilation, as given by the fitted curve coefficient "a" in the monomolecular function.

6.2.3 Statistical analysis

Data for morphological development were analysed using repeated measures two-way ANOVA, with CO_2 concentration and date as factors, but excluding harvest date measurements. Data were also subjected to one-way ANOVA for individual measurement dates. Early gas exchange measurements were tested for CO_2 effects using one-way ANOVA.

6.3 Results

Significant CO_2 effects on morphological characteristics (sheath height, senesced leaf number and leaf length) were found during early development (Fig. 6.1, Table 6.1). One week after the start of CO_2 fumigation (3 June), no morphological differences were measured in seedlings growing under elevated CO_2 (Fig. 6.1a-d). However, five weeks later (10 July) and from then onwards, sheath heights and leaf blade lengths were significantly greater in elevated CO_2 (Fig. 6.1a and Fig. 6.1b, respectively). This response became more pronounced with time, as shown by the significant interactions between CO_2 treatment and date of

University of Cape Town

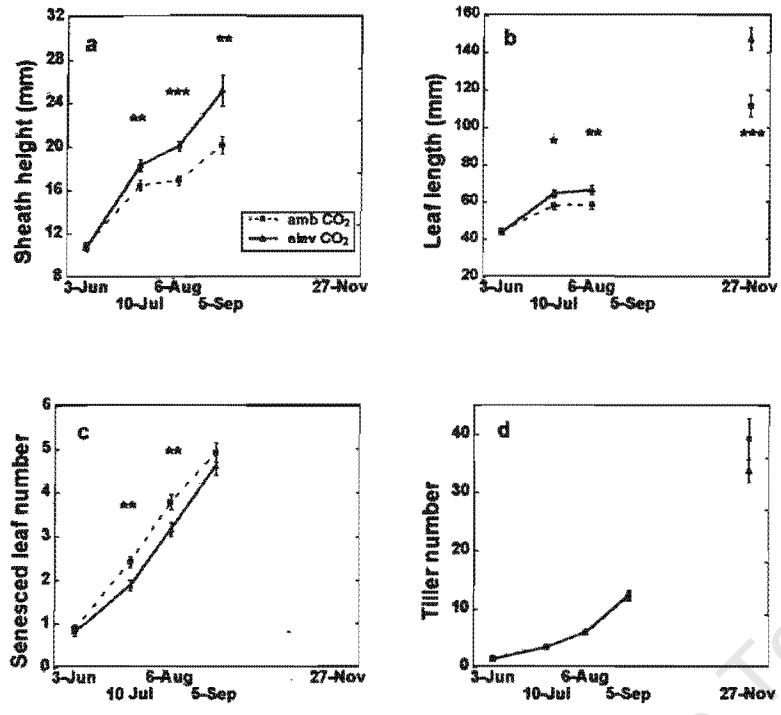


Fig. 6.1 Developmental sequence of the morphological responses of *Themeda* to elevated CO_2. CO_2 fumigation began on 28 May 1996, when seedlings were 46 days old. Values are means per treatment (n=48 for the first three dates, n=24 for the last date (harvest)). * P≤0.05; ** P≤0.01; *** ≤0.001 according to repeated measures 2-way ANOVA.

University of Cape Town

Table 6.1 Statistical significance (repeated measures two-way ANOVA) of the effects of elevated CO_2 on morphological development of *Themeda*. + P≤0.10; * P≤0.05; ** P≤0.01; *** P≤0.001; NS not significant.

Parameter	Main effects		Interaction
	$[CO_2]$	Date	$[CO_2]$ x Date
Sheath height	*	***	***
No. senesced leaves	*	***	NS
No. extended leaves	NS	***	NS
No. emerging leaves	NS	***	NS
Total leaf number	NS	***	NS
Leaf length	+	***	***
Tiller number	NS	***	NS

measurement for sheath height and leaf length. On 10 July and 6 August, fewer senesced leaves were counted per plant in elevated CO_2, but this response was reduced by 5 September (Fig. 6.1c). Elevated CO_2 did not affect numbers of extended or emerging leaves, or total leaf number per plant (data not shown), but a slight decrease in total leaf numbers was found at harvest (see Table 5.4). Tiller numbers per plant did not differ between CO_2 treatments during the period of early morphological measurements (Fig. 6.1d), but were reduced (just not significantly, P=0.153) at harvest (27 November).

Early-season gas exchange measurements showed that maximum net CO_2 assimilation rates (J_e) under saturating light and CO_2 concentration (800 μmol mol^{-1}) were significantly higher (P=0.014, one-way ANOVA) for plants growing in elevated CO_2 (Fig. 6.2). These plants had an average J_e of 24.3 μmol m^{-2} s^{-1}, whereas J_e for those grown under ambient CO_2 was 18.9 μmol m^{-2} s^{-1}. This indicates photosynthetic up-regulation under CO_2 enrichment. It follows that photosynthetic rates of plants grown and measured at elevated CO_2 (650 μmol mol^{-1}) were significantly higher than for those grown and measured at ambient CO_2 (360 μmol mol^{-1}).

In mature plants, CO_2 assimilation rates expressed on a leaf area basis did not differ significantly between plants grown in ambient or elevated CO_2 (Table 6.2). This was due to photosynthetic downregulation in elevated CO_2 (see Fig. 5.3 and Table 5.3 for full data set). However, canopy leaf area of plants grown in elevated CO_2 was 24.1% greater than that for plants grown in ambient CO_2 (Table 6.2). Consequently, canopy net CO_2 assimilation rates were 25.8% higher in elevated than ambient CO_2.

University of Cape Town

6.4 Discussion

CO_2-induced growth enhancement of young *Themeda* plants, particularly increased leaf and sheath extension, was already visible six weeks after the start of the experiment. Although sheath heights were not measured at harvest, increased leaf extension persisted throughout the remaining growth period until harvest. This early growth stimulation was evidently funded by increased leaf-level carbon assimilation rates in elevated CO_2, and the data even suggest an upregulation of photosynthetic capacity in young plants (higher J_e). During maturation, however, reductions in the relative effect of elevated CO_2 on photosynthesis eventually resulted in photosynthetic downregulation in elevated CO_2, so that net CO_2 assimilation rates were similar in elevated or ambient CO_2 in mature plants. Simultaneously, canopy leaf area

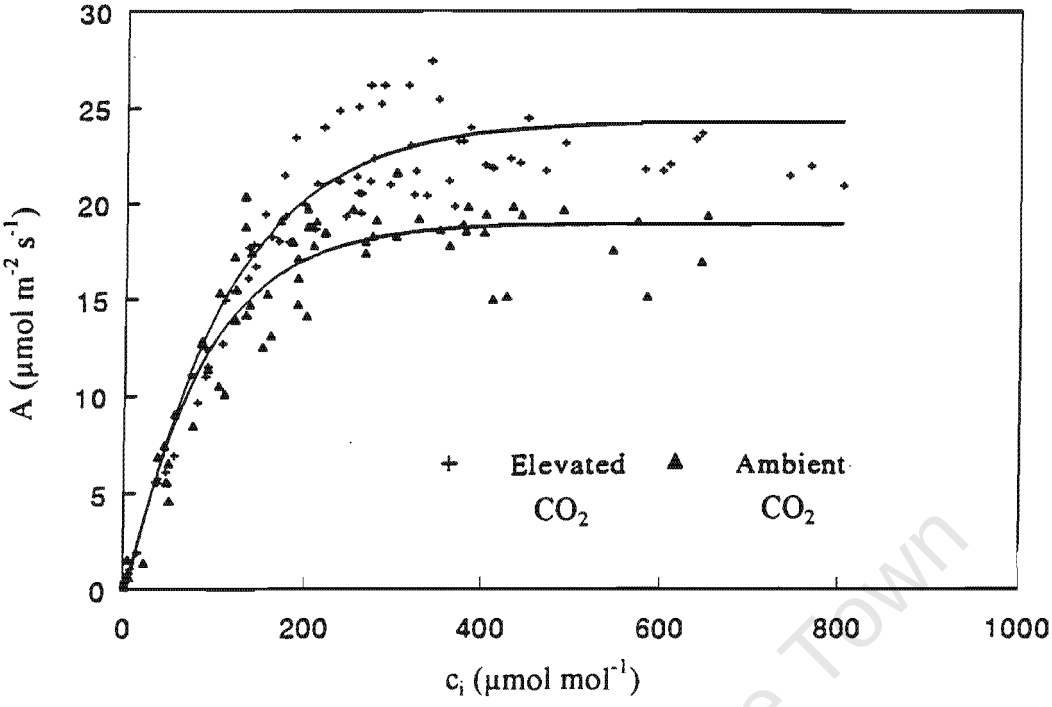


Fig. 6.2 Photosynthetic response to intercellular CO_2 concentration (A/c_i) for leaves of *Themeda* grown in ambient or elevated CO_2 for 10 weeks. Data points of five different plants of each treatment are plotted together and a monomolecular function fitted for each treatment. From Ludwig (1996).

University of Cape Town

Table 6.2 Net CO_2 assimilation rates (A) of mature undefoliated plants, expressed on both a leaf area basis and a whole canopy basis, and estimated canopy leaf areas, after exposure to elevated CO_2 for 25 weeks. Data taken from Chapter 5 using only measurements performed at the growth CO_2 concentration. Values are means ± SE (n=8).

CO_2 treatment	A (area-based) ($\mu mol\ m^{-2}\ s^{-1}$)	A (canopy-based) ($mmol\ s^{-1}$)	Canopy leaf area (cm^2)
ambient	5.11 ± 0.46	1.87 ± 0.27	345 ± 24
elevated	5.88 ± 0.86	2.36 ± 0.19	429 ± 33

gradually increased in elevated CO_2, leading to a progressive increase in whole-canopy carbon assimilation rate, which compensated for photosynthetic downregulation and resulted in a continuously greater supply of carbon products to support enhanced growth at the whole plant level. Early increases in leaf area, leaf area duration (the cumulative leaf area over the growth period), leaf area ratio (the proportion of leaf area to plant biomass), plant height and total plant biomass of C_4 species in elevated CO_2 have been reported by Patterson & Flint (1980), Riechers & Strain (1988) and Ackerly *et al.* (1992). Early responses in biomass and leaf area, which persist for the whole growth period, have also been found for some C_3 grasses (Bowler & Press 1993), but the stimulation of leaf area, in particular, appears to be characteristic of the CO_2-response of C_4 species (Chapter 2). Coleman & Bazzaz (1992) and Ackerly *et al.* (1992) came to the conclusion that standing photosynthetically active leaf area (net leaf area production and loss) in a C_4 dicot was the primary influence on growth responses in elevated CO_2. In *Themeda*, the transiently reduced rate of leaf senescence in elevated CO_2 would also have contributed to a greater active leaf area during early development, and so add to increasing canopy CO_2 uptake rates.

Downregulation of photosynthetic capacity in C_4 species under elevated CO_2 (Wong 1979; Morgan *et al.* 1994; Ghannoum *et al.* 1997; LeCain & Morgan 1998) has not yet been widely acknowledged, possibly because this response may sometimes be out of phase with growth responses. Smith *et al.* (1987) showed that photosynthetic rates of *Eragrostis orcuttiana* were reduced by 44% in elevated CO_2 after 90 days, but shoot biomass was already 51% greater after 70 days, increasing to 150% after 120 days. Similarly, Polley *et al.* (1996a) reported a 61% increase in biomass in *Schizachyrium scoparium* despite a 30% decrease in photosynthetic rate. The mechanisms of downregulation in C_4 plants are not yet well elucidated but appear not to be related to alterations in leaf carbohydrate or nitrogen levels, as they are in C_3 plants (Sage 1994; Stitt 1991; Conroy 1992; Wullschleger 1993). Even in C_3 grasses, CO_2-induced photosynthetic downregulation has not been commonly reported (Baxter *et al.* 1995; Ryle *et al.* 1992). In two temperate species, *Poa alpina* and *Festuca vivipara*, it appeared to go hand in hand with successively decreasing absolute rates of photosynthesis during the course of plant development and gradual senescence (Baxter *et al.* 1995). Coleman & Bazzaz (1992), studying a C_4 dicotyledonous annual, reported slightly higher rates of photosynthesis and greater photosynthetically active leaf area during early growth in elevated CO_2, but not in older plants. Nevertheless, plants attained a larger final

University of Cape Town

biomass. Early CO_2-induced photosynthetic increases in C_4 species may be related to developmental changes in biochemistry and bundle sheath cell membrane properties from young to mature plants (Dai *et al.* 1995). It seems that photosynthetic and growth responses of grasses in elevated CO_2 are temporally uncoupled to a certain extent, and that early and transient changes in physiological activity can affect growth patterns right up to the end of the growth season.

Mature *Themeda* plants had longer leaves and fewer tillers in elevated CO_2, giving rise to a taller and more open canopy structure. This growth form bears a striking resemblance to that described by McNaughton (1992) for *Themeda* grown with "collars" simulating the decreasing light environment of a closing canopy. *Themeda* is classified as a "decreaser" species owing to its intolerance of over- and under-utilization (Hurt *et al.* 1993). This group of grass species is more sensitive to low light intensities than "increaser" species (Everson *et al.* 1988), due to the fact that the nodes from which tillers develop are borne above the soil surface, and new tillers will not develop from heavily shaded basal nodes (Tainton & Mentis 1984). *Themeda* develops significantly fewer tillers with 60% or greater shading (Everson *et al.* 1988), but tillering is stimulated by regular defoliation, either by fire (Tainton & Mentis 1984) or moderate grazing (McNaughton *et al.* 1982), which increases light penetration to the basal nodes and stimulates compensatory growth through activation of new meristems (see Chapter 5). Deregibus *et al.* (1985) attributed these shading responses to decreases in the red:far red light ratio at the base of the plant, and showed that greater tillering could be induced when additional red light was supplied to the crown of C_4 grasses. Such photomorphogenic regulation of tillering and senescence, mediated through the phytochrome system, has been known for some time (Schopfer 1984).

Inhibition of tiller initiation in *Themeda* grown under elevated CO_2 was only introduced sometime between the last measurement date and final harvest, well after the early leaf growth responses, but during the period of greatest tiller development (Fig. 5.1d). This indicates an indirect, secondary response to elevated CO_2, mediated through early and sustained increases in canopy leaf area, and the resulting acceleration of self-shading of the basal nodes. In a review of the literature (Chapter 2), only one other C_4 grass (*Eleusine indica*) was found to display a similar decrease in tiller number (Patterson 1986). Increases in tiller number were reported only under constant high light levels in one study (Sionit &

University of Cape Town

Patterson 1984), but all other studies showed no significant differences between ambient and elevated CO_2. However, all except one of these studies were conducted in growth chambers employing constant daytime irradiation levels and long photoperiods. In this study, on the other hand, plants received fluctuating levels of ambient irradiation, depending on the natural photoperiod and subject to periods of cloudiness, which better simulated the field light environment. This, in conjunction with the morphological characteristics of *Themeda*, could explain the tillering response in elevated CO_2.

University of Cape Town

Chapter 7

Gas exchange and above-ground growth responses to elevated CO_2 in C_4 NADP-ME, C_4 NAD-ME and C_4 PCK and C_3 grasses

7.1 Introduction

In this thesis so far, the focus of experimental work has been on the CO_2-responses of one grass species only, namely *Themeda triandra*. Although this species can justifiably be regarded as one of the most important grass species in southern Africa due to its widespread distribution and dominance in productive rangelands, it can by no means represent the diversity of grasses in the region. Valid predictions of grassland responses to rising atmospheric CO_2 must take into account the potential range of CO_2-responses within the Poaceae, as discussed in Chapter 2. Screening of every grass species in southern Africa is obviously an impossible task, and alternative methods of selecting and assessing the responses of representative species must be found. In recent years, there has been increasing international interest in the concept of plant functional types (PFT), particularly with respect to issues surrounding global change (Box 1996, Poorter *et al.* 1996, Smith *et al.* 1996, Diaz & Cabido 1997). This approach is based on the view that species can be grouped variously on the basis of shared physiological or other functional responses to environmental perturbations e.g. photosynthetic pathway, morphogenetic and architectural traits, reproductive and phenological traits (Diaz 1995, Diaz & Cabido 1997). This provides a framework for predicting plant and ecosystem responses at larger spatial scales (Box 1996), in the absence of direct experimentation. This approach lends itself to spatial modelling at landscape and regional scales, provided one can define the vegetational composition on the basis of relative PFT representation.

Much of the diversity within the grass family (Poaceae) can be attributed to divergence in photosynthetic pathways in response to changing CO_2 concentrations and associated changes in temperature and rainfall since the end of the Cretaceous (Hattersley & Watson 1992). Diversity levels with respect to growth form, morphology, life history and reproductive systems are particularly low in this family, so functional type categorizations do

University of Cape Town

not emerge naturally for these types of traits. However, categorizations based on photosynthetic mechanisms are fairly robust, well researched and documented, and have obvious application in the search for common responses to elevated CO_2. The differential CO_2-responses of C_3 versus C_4 grass species have been discussed in Chapter 2. In South Africa, C_3 and C_4 species are strongly separated geographically, with C_3 grasses predominating only in the winter rainfall region of the Western Cape and the higher altitudes of the Drakensberg and Eastern Cape mountain ranges (Vogel *et al.* 1978). However, over most of the summer rainfall region of South Africa, more than 75% of grass species are C_4, and in large areas more than 95% are C_4 (Vogel *et al.* 1978). Therefore, a sub-division within the C_4 group is required to achieve a finer resolution and enhanced predictive power of potential vegetation change in grasslands and grass co-dominated savannas.

Within the C_4 pathway, three photosynthetic sub-types have been identified based on the C_4 acid decarboxylation enzyme, namely NADP-ME (NADP-malic enzyme), NAD-ME (NAD-malic enzyme) and PCK (phosphoenolpyruvate carboxykinase) (Hattersley & Watson 1992), which have differing photosynthetic biochemistry and potentially different responses to resources required for photosynthesis and growth. Ellis *et al.* (1980, for Namibia) and Hattersley (1992, for Australia) showed that the abundance of these sub-types is correlated with mean annual rainfall, with NADP-ME species becoming more abundant with increasing rainfall, whereas NAD-ME species dominate in areas of low rainfall and decrease in abundance as rainfall increases. PCK species are intermediate between NADP-ME and NAD-ME species. This close relationship has yet to be explained physiologically (Hattersley & Watson 1992, Ellery *et al.* 1995), but suggests that C_4 sub-types may be strong potential candidates for PFTs that are likely to change with changing rainfall and temperature regimes, and which lend themselves to spatial modelling. Will they also show differential responses to rising CO_2?

It has been shown that the three C_4 sub-types exhibit unique stable carbon isotope discrimination ($\delta^{13}C$) values, apparently related to the degree of "leakiness" to CO_2 in the bundle sheath cells (Hattersley 1982, Farquhar 1983). "Leakiness" can be defined as the ratio of leakage of CO_2 from the bundle sheath to the rate of PEP carboxylase (Henderson *et al.* 1994), and is attributable to both the physical conductance of bundle sheath cell walls to CO_2 (Brown & Byrd 1993) and the relative biochemical capacities of the C_3 and C_4 cycles.

University of Cape Town

Leakiness is lowest in the NADP-ME sub-type (highest $\delta^{13}C$ values), intermediate in PCK (intermediate $\delta^{13}C$), and highest in NAD-ME (lowest $\delta^{13}C$) (Hattersley 1982, Farquhar 1983, Furbank & Hatch 1987, Jenkins *et al.* 1989, Brown & Byrd 1993, but see Hatch *et al.* 1995 for contradictory results). Passive leakage of CO_2 out of the bundle sheath cells amounts to a loss of between 10 and 40% of carbon fixed by PEP carboxylase (the exact levels are still uncertain due to differences between studies). Henderson *et al.* (1994) proposed that leakiness levels could provide an indication of the responses of C_4 species to elevated CO_2. Those species most affected by leakage (NAD-ME) could, conceivably, benefit most from elevated atmospheric CO_2. LeCain & Morgan (1998) tested this hypothesis in the first study thus far: contrary to predictions, growth of NADP-ME grasses was generally greater under elevated CO_2, and none of the NAD-ME grasses studied had a significant growth response. Due to the fact that one of the NAD-ME species used had a positive growth response in previous experiments (Read *et al.* 1997), LeCain & Morgan (1998) concluded that no generalisations could be made about the CO_2-response of C_4 species based on C_4 sub-type.

Since my study was initiated and completed prior to publication of LeCain and Morgan's study, I present here my original hypothesis, namely that elevated CO_2 will enhance photosynthesis and growth to a larger degree in NAD-ME species than in NADP-ME species, with PCK species showing an intermediate response. I also investigated the relative ability of the C_4 sub-types to acclimate to elevated CO_2 in the longer term, with regard to photosynthesis and stomatal conductance, as this has not been widely reported and is assumed to be rare and of lesser importance for C_4 species (Sage 1994). This may be a premature assumption (LeCain & Morgan 1998). For general comparisons between grass photosynthetic types, and to allow cross-comparisons with the field CO_2 experiment (Chapter 8), one C_3 grass species was also included in this experiment.

7.2 Materials and methods

7.2.1 Plant establishment

Eight locally dominant grass species representing either the C_3 , NADP-ME C_4, NAD-ME C_4, or PCK C_4 photosynthetic pathways, were selected from the Bongwan field CO_2 experimental site in Kwazulu/Natal (see Chapter 8 for experimental description). These species had been previously identified by staff at the Natal Herbarium (National Botanical Institute, Durban)

University of Cape Town

from dried and pressed specimens. The selected species were (to be named only by their generic names hereafter, except for the two *Eragrostis* species): *Andropogon appendiculatus* Nees (NADP-ME), *Digitaria natalensis* Stent (NADP-ME), *Themeda triandra* Forssk. (NADP-ME), *Eragrostis curvula* (Schrad.) Nees (NAD-ME), *Eragrostis racemosa* (Thunb.) Stend. (NAD-ME), *Sporobolus pyramidalis* Beauv. (PCK), *Melinis repens* (Willd.) Zizka subsp. *repens* FP 23:914 (PCK), and *Alloteropsis semialata* (R.Br.) Hitchc. subsp. *eckloniana* (Nees) Gibbs Russel (C_3). The identity and photosynthetic pathway of the last sub-species (as opposed to the closely related C_4 *Alloteropsis semialata* subsp. *semialata*), was confirmed using ^{13}C isotope determinations (W.D. Stock, UCT, personal communication). Photosynthetic sub-types for C_4 species were determined from the literature (Ellis *et al.* 1980, Schulze *et al.* 1996), and confirmed by R.P. Ellis (ARC, Pretoria, pers. comm.). Clumps of each species were excavated from the field site, well away from the CO_2 vent, during April 1997, transferred to Cape Town and potted into 0.2 m diameter plastic pots using a standard Kirstenbosch potting mix (coarse sand, leaf mould and loam, 2:1:1 v/v) packed around the clumps of field soil. Plants were left in the open, although partially sheltered from rain, to acclimatise.

On 3 September 1997, clumps were separated into smaller tufts of similar size and potted into 3.9 L long pots using the standard Kirstenbosch potting mix with some remaining field soil around the roots, to give 16 individual plants per species. Care was taken to minimise variation in initial tuft size, as no baseline biomass data could be obtained due to constraints on available material. Only *Melinis* yielded fewer than 16 plants (8 plants). Pots were moved into the greenhouse to acclimatise. All plants were fed with an organic liquid fertilizer (Seagro (Pty) Ltd., Cape Town, 5 ml diluted to 2 L, 120 ml each) on two occasions before the start of the experiment, and again 39 days after the start. Thereafter, no fertilization took place.

7.2.2 Design and treatments

This experiment was conducted in a second set of eight chambers, identical in design to the chambers described in Chapter 3, except that they were slightly larger and could hold 16 instead of 12 pots. This chamber set was placed at the southern end of the same side of the greenhouse as the smaller set. Mechanisms of CO_2 fumigation were identical to those previously described (Chapter 3). The experimental design was a split-plot design, with CO_2

University of Cape Town

treatment as the main plot and photosynthetic pathway as the sub-plot. The CO_2 ranges after calibration in the four amended chambers were 611-704, 624-687, 608-737, and 601-706 μmol mol^{-1}, with 90-100% of measurements falling within 10% of the target (660 μmol mol^{-1}).

At the start of the experiment, all plants were defoliated to 5 cm height. Two plants of each species were placed in each chamber, except *Melinis*, for which only one plant per chamber was available. Pot positions were randomised at the start of the experiment, but not thereafter. The experiment was carried out from 3 October 1997 to 30 March 1998, for 178 days. Greenhouse air temperature was measured every minute at table height adjacent to the chamber array, and average, minimum and maximum temperatures recorded every three hours. Radiation levels (PPFD) were not recorded in this experiment. Between 3 October 1997 and 11 March 1998, the following average monthly greenhouse temperatures were recorded:

Table 7.1 Average monthly midday greenhouse air temperatures from 3 October to 11 March 1998.

Month	Avg midday temperature (°C) (12:00 - 15:00)
October	29.7
November	28.8
December	31.4
January	32.3
February	34.1
March	30.8

University of Cape Town

7.2.3 Photosynthetic gas exchange

Gas exchange measurements (Light and A/c_i response curves) were performed after 6-12 weeks of CO_2 fumigation, using a LI-6400 IRGA (Li-Cor, Lincoln, Nebraska, USA). For each species, 4-5 plants from each CO_2 treatment were selected at random from different chambers. A/c_i responses were determined at 6-7 different cuvette CO_2 concentrations ranging between 50-1000 μmol mol^{-1} (C_4 species) and between 100-1000 μmol mol^{-1} (C_3 species). Cuvette CO_2 concentrations were regulated using pressurised CO_2 canisters (soda

chargers) controlled electronically by the IRGA. Leaf temperatures were regulated at 30°C, and light level kept constant at 1500 μmol m^{-2} s^{-1} (C_4 species) or 800 μmol m^{-2} s^{-1} (C_3 species), since C_4 photosynthesis can potentially saturate at higher light levels than most C_3 plants (Fitter & Hay 1987). The data subsequently showed that both C_3 and C_4 grasses were light-saturated for photosynthesis at about 800-1000 μmol m^{-2} s^{-1}, with the exception of *Andropogon* and *Sporobolus*, which saturated at about 1500 μmol m^{-2} s^{-1} (Fig. 7.6). Light was provided by an LED light source inside the IRGA cuvette. Leaf-to-air vapour pressure difference (LAVPD) ranged between 1.7 and 3.2 kPa but never changed by more than 0.6 kPa for any individual A/c_i response curve. Between one and four enclosed mid-leaf sections (number depending on leaf width) were allowed to equilibrate at 500 μmol mol^{-1} CO_2 until photosynthetic rate and stomatal conductance were constant (at least 15 minutes). Thereafter, CO_2 concentrations were progressively decreased to the lowest level, then increased to 1000 μmol mol^{-1}, allowed to stabilise, and then decreased to 650 μmol mol^{-1} for the last measured point. Care was taken that the beginning and end points lay on a smooth curve, signifiying sufficient acclimation time and lack of cuvette-induced changes in photosynthetic capacity. After the A/c_i response measurement, leaves were allowed to equilibrate at the treatment CO_2 concentration (360 or 650 μmol mol^{-1}) and light levels of 2000 μmol m^{-2} s^{-1} for C_4 species and 1250 μmol m^{-2} s^{-1} for C_3 species. Different light levels were used for C_3 and C_4 species for reasons stated above, and proved to be saturating for all species under their respective light levels (Fig. 7.6). Gas exchange measurements were then taken at 6-7 progressively lower light levels up to just above light compensation point (about 70 μmol m^{-2} s^{-1} PPFD). Leaf temperatures were regulated at 30°C. LAVPD ranged between 1.7 and 3.3 kPa but did not change by more than 0.7 kPa for any individual light response curve.

After gas exchange, projected leaf areas were calculated using the lengths and breadths of the enclosed leaf sections. Response curves were fitted individually using non-linear regression (UNISTAT 3.0) and the monomolecular function $y=a(1-e^{b-cx})$ given by Causton & Dale (1990). The reasoning and justification for using this model have been discussed in section 6.2.2 of Chapter 6. For the A/c_i response curves, the fitted curve coefficient "a" gave the light- and CO_2-saturated rate of net CO_2 assimilation (termed J_e), "b/c" gave the CO_2 compensation point (termed Γ_c), and "ace^b" gave the apparent carboxylation efficiency (termed *k*, the slope of the A/c_i response at x=0). The equivalent constants and formulae for the light response curves gave the light-saturated rate of net CO_2 assimilation (termed A_{max}), the light compensation point (termed Γ_l), and the apparent quantum efficiency (termed α). In

University of Cape Town

addition, the predicted dark respiration rate (termed R_d) was calculated from the light response using "$a(1-e^b)$" (Causton & Dale 1990).

In addition to curve analysis, gas exchange values were extracted from the A/c_i responses at cuvette CO_2 concentrations of 360 and 650 μmol m^{-2} s^{-1} for further analysis. The photosynthesis/transpiration ratio (A/E) at these CO_2 levels was used as a measure of instantaneous leaf water use efficiency (WUE). The ratio of intercellular to atmospheric CO_2 concentration (c_i/c_a) was calculated.

7.2.4 Harvest

All plants were defoliated to 5 cm height during the second week of January (14 weeks after the start of CO_2 fumigation) and again at the end of the experiment (end March). For each plant, green leaves were separated from other shoot material (senesced, sheaths, reproductive) and the fresh mass of the green leaf component determined. Blade lengths of ten fully developed leaves were measured. Leaf areas were determined using a digital image analysis system (Delta-T Devices, Cambridge, England). For the first harvest, total leaf area was measured where possible, but in some species the leaf area and dry mass of a subsample of 20 leaves was measured. Total leaf area was then calculated using specific leaf mass of the subsample and total leaf dry mass. For the second harvest, subsamples of 10 leaves were measured for each species and total leaf area and dry mass calculated. All material was oven-dried at 65°C, weighed and leaf material milled. Leaf total nitrogen concentrations were determined on pooled dry leaf material from both harvests, using the micro-Kjeldahl method (section 4.2.5).

University of Cape Town

7.2.5 Statistical analysis

Biomass data for individual species for each harvest were subjected to one-way ANOVA. For each harvest, biomass data were then combined according to photosynthetic pathway and subjected to a split-plot ANOVA, with CO_2 treatment as the main plot and photosynthetic pathway as the sub-plot. Each photosynthetic pathway was also individually subjected to one-way ANOVA. In order to test for possible changes in the CO_2-response of photosynthetic types between the harvests (interaction), and, in the potential absence of such interaction, to provide a more powerful statistical test for CO_2 main effects (increased replication), a repeated measures two-way ANOVA was performed on each species, with CO_2 treatment and harvest date as factors.

With respect to gas exchange data extracted from A/c_i response curves, data for individual species were analysed using two-way ANOVA with CO_2 treatment concentration and CO_2 measurement concentration as factors. The species-based comparison between measurements performed at the respective growth CO_2 concentrations was tested using one-way ANOVA. Gas exchange responses for different photosynthetic types were analysed using three-way ANOVA, with CO_2 treatment concentration, CO_2 measurement concentration and photosynthetic pathway as factors. Measurements performed at the respective growth CO_2 concentrations were analysed by two-way ANOVA, with photosynthetic pathway and CO_2 concentration as factors. Curve parameters derived from both A/c_i and light response curves were analysed by one-way ANOVA for individual species, and by two-way ANOVA to test for differences between photosynthetic pathways.

7.3 Results

7.3.1 Biomass, morphology and leaf chemistry

Data for both aboveground harvests for individual species are presented in Table 7.2, with the statistical analysis for individual species and harvests in Table 7.3. Photographs of individual species at harvest are presented in Figs 7.8-7.11 (pages 141-144). At the first harvest, *Andropogon* (NADP-ME) had significantly increased leaf biomass, canopy leaf area, and leaf length in elevated CO_2, with total shoot biomass increased by 72%. Leaf moisture content was significantly increased by 5%. Leaf moisture also increased slightly in *Digitaria* (NADP-ME), but no other significant CO_2-responses were measured in this species. *Themeda* (NADP-ME) showed increases of around 118% in leaf biomass, other shoot biomass (senesced and sheath material, but no reproductive tillers), total shoot biomass and canopy leaf area in elevated CO_2. Of the two *Eragrostis* species (NAD-ME), only *Eragrostis curvula* responded significantly to elevated CO_2, with increased non-leaf shoot biomass (senesced, sheath and reproductive tillers) and total shoot biomass, but slightly reduced leaf moisture content. *Sporobolus* (PCK) also responded to elevated CO_2 with increased non-leaf shoot biomass (senesced, sheath and reproductive tillers) and total shoot biomass. *Melinis* (PCK) and *Alloteropsis* (C_3) showed no growth responses to elevated CO_2.

University of Cape Town

Table 7.2 Harvest data for eight grass species grown under elevated CO_2 for 96 days (first harvest January 1998) and 178 days (second harvest March 1998). January and March material pooled for leaf [N] determinations. Values are means ± SE (n=7-8 except n=4 for *Melinis*, and n=4 for leaf [N]).

Photosynthetic pathways:
C_4 NADP-ME: *Andropogon appendiculatus*, *Digitaria natalensis*, *Themeda triandra*
C_4 NAD-ME: *Eragrostis curvula*, *Eragrostis racemosa*
C_4 PCK: *Sporobolus pyramidalis*, *Melinis repens* subsp *repens*
C_3: *Alloteropsis semialata* subsp *eckloniana*

Parameter / Species	January harvest		March harvest	
	Amb CO_2	Elev CO_2	Amb CO_2	Elev CO_2
Leaf dry mass (g):				
Andropogon appendiculatus	0.81 ± 0.14	1.41± 0.19	1.72 ± 0.19	2.33 ± 0.31
Digitaria natalensis	1.38 ± 0.22	1.59 ± 0.26	1.53 ± 0.15	1.81 ± 0.31
Themeda triandra	0.34 ± 0.07	0.75 ± 0.08	1.17 ± 0.08	1.41 ± 0.18
Eragrostis curvula	0.64 ± 0.05	0.85 ± 0.12	0.46 ± 0.04	0.58 ± 0.07
Eragrostis racemosa	0.94 ± 0.14	1.10 ± 0.23	2.05 ± 0.22	2.27 ± 0.21
Sporobolus pyramidalis	1.99 ± 0.17	2.38 ± 0.22	3.14 ± 0.33	3.43 ± 0.39
Melinis repens subsp *repens*	0.54 ± 0.11	0.55 ± 0.04	0.63 ± 0.12	0.65 ± 0.10
Alloteropsis semialata subsp *eckloniana*	0.50 ± 0.09	0.67 ± 0.12	0.72 ± 0.12	0.91 ± 0.12
Other shoot dry mass (g):				
Andropogon appendiculatus	0.07 ± 0.01	0.10 ± 0.05	0.96 ± 0.14	1.29 ± 0.42
Digitaria natalensis	0.81 ± 0.18	0.78 ± 0.25	3.79 ± 0.49	4.51 ± 0.66
Themeda triandra	0.76 ± 0.16	1.65 ± 0.15	3.24 ± 0.32	4.89 ± 0.54
Eragrostis curvula	1.85 ± 0.35	3.37 ± 0.63	3.32 ± 0.63	4.07 ± 0.55
Eragrostis racemosa	0.29 ± 0.04	0.32 ± 0.04	0.98 ± 0.09	1.04 ± 0.13
Sporobolus pyramidalis	0.64 ± 0.19	1.56 ± 0.44	4.63 ± 0.34	3.93 ± 0.71
Melinis repens subsp *repens*	0.71 ± 0.20	1.05 ± 0.05	1.19 ± 0.31	1.70 ± 0.43
Alloteropsis semialata subsp *eckloniana*	0.41 ± 0.08	0.62 ± 0.15	1.11 ± 0.20	1.68 ± 0.26
Total shoot dry mass (g):				
Andropogon appendiculatus	0.88 ± 0.14	1.51 ± 0.19	2.68 ± 0.25	3.62 ± 0.66
Digitaria natalensis	2.18 ± 0.34	2.38 ± 0.49	5.33 ± 0.53	6.33 ± 0.78
Themeda triandra	1.10 ± 0.22	2.41 ± 0.21	4.41 ± 0.39	6.31 ± 0.69
Eragrostis curvula	2.50 ± 0.35	4.22 ± 0.67	3.79 ± 0.64	4.65 ± 0.60
Eragrostis racemosa	1.22 ± 0.17	1.42 ± 0.27	3.03 ± 0.24	3.32 ± 0.26
Sporobolus pyramidalis	2.63 ± 0.32	3.94 ± 0.62	7.77 ± 0.45	7.36 ± 0.64
Melinis repens subsp *repens*	1.25 ± 0.31	1.60 ± 0.08	1.82 ± 0.43	2.36 ± 0.51
Alloteropsis semialata subsp *eckloniana*	0.91 ± 0.16	1.30 ± 0.25	1.83 ± 0.31	2.59 ± 0.36
Canopy leaf area (cm^2):				
Andropogon appendiculatus	108 ± 16	192 ± 26	233 ± 23	307 ± 36
Digitaria natalensis	122 ± 18	144 ± 19	301 ± 26	348 ± 57
Themeda triandra	93 ± 17	204 ± 20	283 ± 34	366 ± 49
Eragrostis curvula	84 ± 5	105 ± 16	-	-
Eragrostis racemosa	140 ± 23	172 ± 26	352 ± 36	384 ± 32

University of Cape Town

Sporobolus pyramidalis	116 ± 16	147 ± 20	348 ± 33	349 ± 31
Melinis repens subsp *repens*	89 ± 21	94 ± 10	119 ± 24	122 ± 20
Alloteropsis semialata subsp *eckloniana*	63 ± 11	84 ± 16	117 ± 18	141 ± 18
Individual leaf length (mm):				
Andropogon appendiculatus	188 ± 13	253 ± 12	272 ± 11	295 ± 11
Digitaria natalensis	224 ± 20	261 ± 19	273 ± 11	298 ± 16
Themeda triandra	144 ± 7	157 ± 5	182 ± 5	175 ± 9
Eragrostis curvula	180 ± 9	186 ± 6	174 ± 9	154 ± 4
Eragrostis racemosa	140 ± 7	135 ± 9	147 ± 3	150 ± 5
Sporobolus pyramidalis	356 ± 23	393 ± 14	451 ± 25	467 ± 28
Melinis repens subsp *repens*	72 ± 7	82 ± 4	75 ± 4	83 ± 6
Alloteropsis semialata subsp *eckloniana*	115 ± 13	143 ± 10	200 ± 11	234 ± 11
Leaf moisture (%):				
Andropogon appendiculatus	56.0 ± 0.8	58.8 ± 0.6	56.7 ± 0.4	57.1 ± 0.5
Digitaria natalensis	68.7 ± 0.6	70.7 ± 0.7	69.8 ± 0.6	71.2 ± 0.5
Themeda triandra	52.5 ± 1.5	53.6 ± 0.8	52.7 ± 1.0	50.0 ± 1.2
Eragrostis curvula	55.2 ± 1.2	52.2 ± 0.9	48.7 ± 2.5	49.3 ± 2.6
Eragrostis racemosa	56.8 ± 1.0	55.4 ± 1.6	58.6 ± 1.0	60.3 ± 0.9
Sporobolus pyramidalis	58.6 ± 0.8	58.2 ± 2.4	58.6 ± 0.1	58.6 ± 0.8
Melinis repens subsp *repens*	63.3 ± 0.9	63.8 ± 1.3	59.3 ± 1.9	58.3 ± 1.5
Alloteropsis semialata subsp *eckloniana*	61.9 ± 1.0	62.5 ± 1.2	62.7 ± 1.8	64.6 ± 1.3
Specific leaf mass (g m^{-2}):				
Andropogon appendiculatus	73.1 ± 2.5	73.2 ± 2.4	73.3 ± 1.7	75.1 ± 1.6
Digitaria natalensis	111 ± 5.4	106 ± 6.8	50.9 ± 2.1	52.1 ± 2.1
Themeda triandra	35.9 ± 1.6	36.9 ± 1.1	41.3 ± 1.6	40.1 ± 1.2
Eragrostis curvula	76.9 ± 4.3	81.3 ± 2.9	-	-
Eragrostis racemosa	69.5 ± 3.5	73.9 ± 5.0	58.0 ± 2.7	58.1 ± 1.7
Sporobolus pyramidalis	189 ± 21	180 ± 25	89.6 ± 1.5	97.5 ± 5.5
Melinis repens subsp *repens*	61.7 ± 3.1	59.7 ± 3.0	53.0 ± 1.5	54.3 ± 1.1
Alloteropsis semialata subsp *eckloniana*	79.3 ± 1.3	80.8 ± 2.6	66.5 ± 1.9	68.9 ± 1.8
Leaf [N] (mg g^{-1})			January+March	
Andropogon appendiculatus			9.0 ± 0.3	8.1 ± 0.3
Digitaria natalensis			7.2 ± 0.3	7.2 ± 0.3
Themeda triandra			12.4 ± 0.1	11.3 ± 0.1
Eragrostis curvula			9.5 ± 0.6	9.0 ± 0.3
Eragrostis racemosa			10.2 ± 0.9	9.3 ± 0.6
Sporobolus pyramidalis			7.6 ± 0.1	7.3 ± 0.4
Melinis repens subsp *repens*			11.0 ± 0.4	10.6 ± 0.5
Alloteropsis semialata subsp *eckloniana*			19.6 ± 0.4	17.5 ± 0.5

University of Cape Town

Table 7.3 Statistical significance of the effects of elevated CO_2 on harvest parameters in grass species of differing photosynthetic pathway, as calculated individually for species and harvest date by one-way ANOVA. Percentage changes in elevated CO_2 are given where differences are significant. +, P≤0.10; *, P≤0.05; **, P≤0.01; ***, P≤0.001; NS, not significant, according to one-way ANOVA.

Species	Parameter	Harvest date	
		January	March
Andropogon appendiculatus (NADP-ME)	Leaf dry mass	+74% *	NS
	Other shoot dry mass	NS	NS
	Total shoot dry mass	+72% *	NS
	Canopy leaf area	+77% *	NS
	Individual leaf length	+35% **	NS
	% leaf moisture	+5% *	NS
	SLM	NS	NS
	Leaf [N]	-10% +	
Digitaria natalensis (NADP-ME)	Leaf dry mass	NS	NS
	Other shoot dry mass	NS	NS
	Total shoot dry mass	NS	NS
	Canopy leaf area	NS	NS
	Individual leaf length	NS	NS
	% leaf moisture	+3% +	NS
	SLM	NS	NS
	Leaf [N]	NS	
Themeda triandra (NADP-ME)	Leaf dry mass	+119% **	NS
	Other shoot dry mass	+118% **	+51% *
	Total shoot dry mass	+118% ***	+43% *
	Canopy leaf area	+118% ***	NS
	Individual leaf length	NS	NS
	% leaf moisture	NS	NS
	SLM	NS	NS
	Leaf [N]	-9% ***	
Eragrostis curvula (NAD-ME)	Leaf dry mass	NS	NS
	Other shoot dry mass	+82% +	NS
	Total shoot dry mass	+69% +	NS
	Canopy leaf area	NS	-
	Individual leaf length	NS	-12% *

University of Cape Town

	% leaf moisture	-5% +		NS
	SLM	NS		-
	Leaf [N]		NS	
Eragrostis racemosa (NAD-ME)	Leaf dry mass	NS		NS
	Other shoot dry mass	NS		NS
	Total shoot dry mass	NS		NS
	Canopy leaf area	NS		NS
	Individual leaf length	NS		NS
	% leaf moisture	NS		NS
	SLM	NS		NS
	Leaf [N]		NS	
Sporobolus pyramidalis (PCK)	Leaf dry mass	NS		NS
	Other shoot dry mass	+143% +		NS
	Total shoot dry mass	+50% +		NS
	Canopy leaf area	NS		NS
	Individual leaf length	NS		NS
	% leaf moisture	NS		NS
	SLM	NS		NS
	Leaf [N]		NS	
Melinis repens subsp *repens* (PCK)	Leaf dry mass	NS		NS
	Other shoot dry mass	NS		NS
	Total shoot dry mass	NS		NS
	Canopy leaf area	NS		NS
	Individual leaf length	NS		NS
	% leaf moisture	NS		NS
	SLM	NS		NS
	Leaf [N]		NS	
Alloteropsis semialata subsp *eckloniana* (C_3)	Leaf dry mass	NS		NS
	Other shoot dry mass	NS		NS
	Total shoot dry mass	NS		NS
	Canopy leaf area	NS		NS
	Individual leaf length	NS		+17% +
	% leaf moisture	NS		NS
	SLM	NS		NS
	Leaf [N]		-11% *	

University of Cape Town

At the second harvest, which involved removal of aboveground regrowth following the first defoliation, CO_2-responses for biomass and morphology were evident in most species, but were relatively smaller and not statistically significant in most cases (Tables 7.1 and 7.2). Only *Themeda* showed a significant increase in total shoot biomass, due to increases in non-leaf shoot biomass (senesced and sheath material). *Eragrostis curvula* had slightly shorter leaf blades in elevated CO_2, whereas *Alloteropsis* had slightly longer leaf blades.

For both harvests combined, leaf total nitrogen concentrations were significantly reduced in elevated CO_2 in *Andropogon* (10%), *Themeda* (9%) and *Alloteropsis* (11%). Comparison of absolute leaf [N] with that of plants growing in the field (see Chapter 8) shows that leaf [N] was twice as high in this experiment than in the field (19.6 mg g^{-1} versus ca. 9.5 mg g^{-1} for *Alloteropsis*, 12.4 mg g^{-1} versus ca. 6.0 mg g^{-1} for *Themeda*, data for the field obtained from Ludwig 1996). However, the field data were obtained in May, at the end of the growth season when grasses are senescing and [N] is gradually reduced.

When data from species belonging to the same photosynthetic pathway were combined, significant CO_2-induced increases in leaf biomass, other shoot (non-leaf) biomass and total shoot biomass, as well as increases in canopy leaf area and leaf length were found for the first harvest (Fig. 7.1, Table 7.4). At the second harvest, only total shoot biomass and canopy leaf area were significantly increased. No significant interactions were found between photosynthetic pathway and CO_2-response for either harvest (Table 7.4), even though one-way ANOVAs for individual photosynthetic types showed statistically significant CO_2-responses mostly in the NADP-ME pathway (Fig. 7.1).

A repeated-measures ANOVA for the two harvests showed no significant interactions (except for leaf length in NADP-ME) between harvest time and CO_2-response (Table 7.5), signifying that CO_2-response trends were similar for both harvests. This allows for valid interpretation based on the CO_2 main effect over both harvests, which shows statistically significant increases in leaf biomass, other shoot biomass (marginal), total shoot biomass and canopy leaf area only in the NADP-ME pathway, with the exception of marginal increases in total shoot biomass in NAD-ME, and an increase in leaf length in the C_3 pathway.

Leaf moisture content remained relatively constant in all species over both harvests (Table 7.2), with no overall response to elevated CO_2 in any photosynthetic type (Fig. 7.1,

University of Cape Town

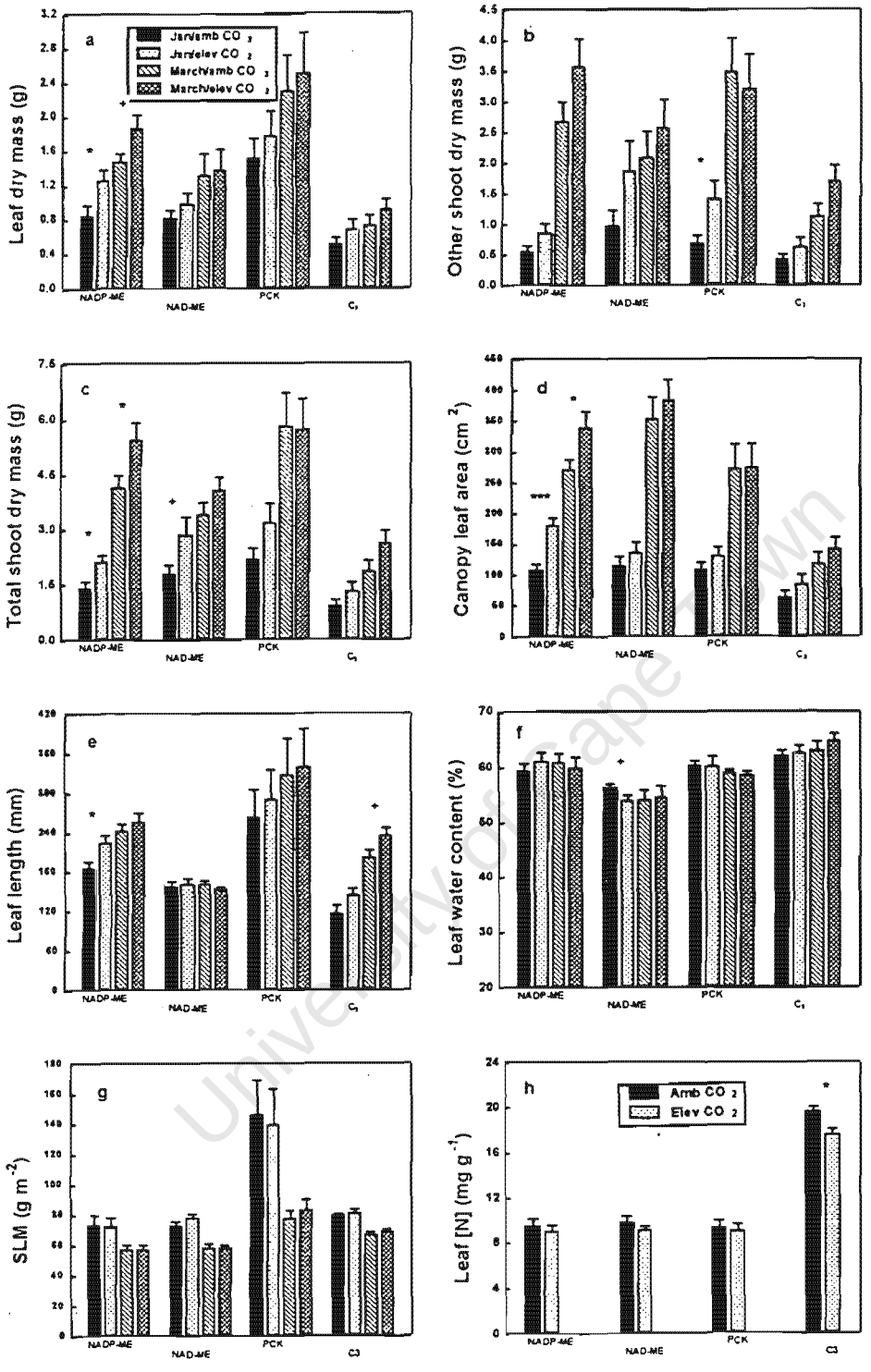


Fig. 7.1 Effects of growth in elevated CO_2 on harvest parameters (two harvests in January and March 1998) in grasses representing four different photosynthetic pathways (C_4 NADP-ME, C_4 NAD-ME, C_4 PCK and C_3). Values are means ± SE bars. +, $P \leq 0.10$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$, according to one-way ANOVA.

Table 7.4 Statistical significance of the effects of elevated CO_2 on grass species of differing photosynthetic pathway (PP), as calculated by split-plot ANOVA. +, P≤0.10; *, P≤0.05; **, P≤0.01; ***, P≤0.001; NS, not significant, according to two-way ANOVA.

Parameter:	Main effects				Interaction	
	PP		$[CO_2]$		PP x $[CO_2]$	
	Jan 98	Mar 98	Jan 98	Mar 98	Jan 98	Mar 98
Leaf dry mass	***	***	*	NS	NS	NS
Other shoot dry mass	**	**	**	NS	NS	NS
Total shoot dry mass	***	***	***	*	NS	NS
Canopy leaf area	***	***	***	+	NS	NS
Individual leaf length	***	***	+	NS	NS	NS
% leaf moisture	***	***	NS	NS	NS	NS
SLM	***	***	NS	NS	NS	NS
Leaf [N]	***		NS		NS	

University of Cape Town

Table 7.5 Statistical significance of the effects of elevated CO_2 on harvest parameters (two harvest times) of differing photosynthetic pathways, as calculated by repeated measures two-way ANOVA. Data for species belonging to the same photosynthetic pathway are pooled. Percentage changes in elevated CO_2 are given where differences are significant. +, $P \leq 0.10$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; NS, not significant, according to two-way ANOVA (repeated measures).

Species	Parameter	Main effects		Interaction
		$[CO_2]$	Harvest time	$[CO_2]$ x Harv
C_4 NADP-ME				
	Leaf dry mass	+34% *	***	NS
	Other shoot dry mass	+37% +	***	NS
	Total shoot dry mass	+36% *	***	NS
	Canopy leaf area	+39% ***	***	NS
	Individual leaf length	NS	***	*
	% leaf moisture	NS	NS	**
	SLM	NS	**	NS
C_4 NAD-ME				
	Leaf dry mass	NS	*	NS
	Other shoot dry mass	NS	**	NS
	Total shoot dry mass	+31% +	***	NS
	Canopy leaf area	NS	***	NS
	Individual leaf length	NS	NS	NS
	% leaf moisture	NS	NS	NS
	SLM	NS	***	NS
C_4 PCK				
	Leaf dry mass	NS	***	NS
	Other shoot dry mass	NS	***	NS
	Total shoot dry mass	NS	***	NS
	Canopy leaf area	NS	***	NS
	Individual leaf length	NS	***	NS
	% leaf moisture	NS	NS	NS
	SLM	NS	***	NS
C_3				
	Leaf dry mass	NS	*	NS
	Other shoot dry mass	NS	***	NS
	Total shoot dry mass	NS	***	NS
	Canopy leaf area	NS	***	NS
	Individual leaf length	+20% *	***	NS
	% leaf moisture	NS	NS	NS
	SLM	NS	***	NS

University of Cape Town

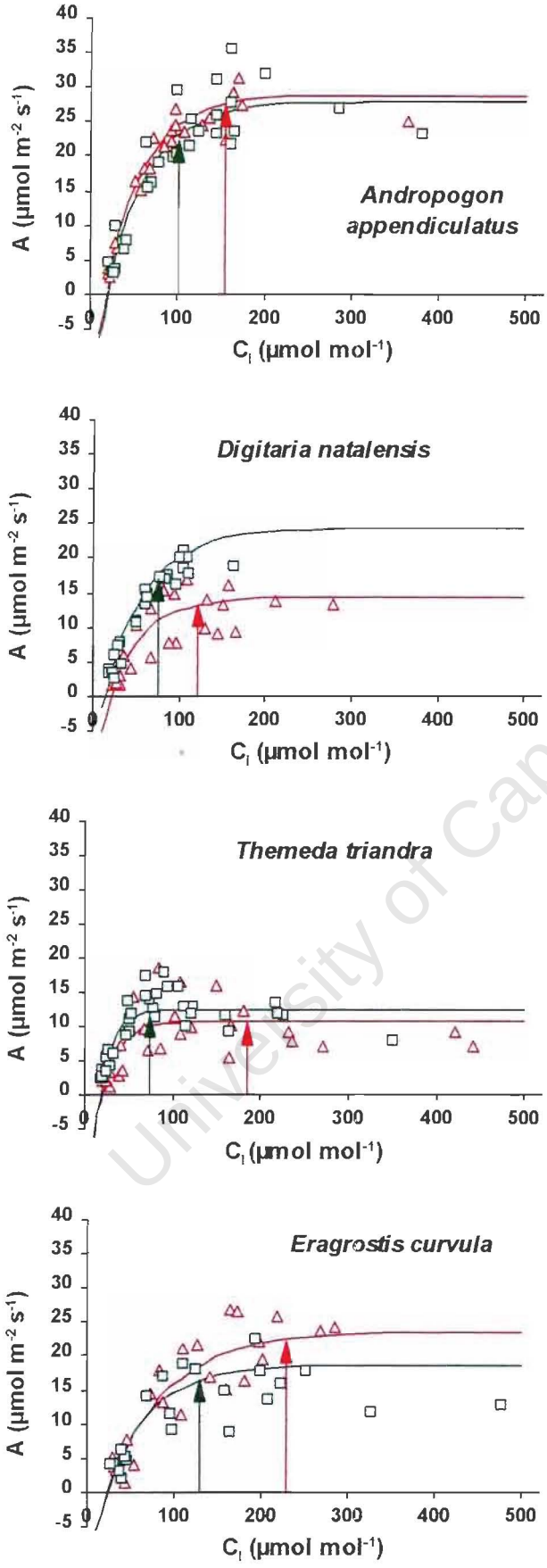
Table 7.5), although there were some species-specific responses (Table 7.3). Significant differences in leaf moisture content were found between pathways (Table 7.4), with the C_3 pathway having the highest moisture content, the NADP-ME and PCK pathways having intermediate moisture content, and the NAD-ME pathway having the lowest leaf moisture content (according to a Student-Newman-Keuls Multiple Range Test $P \leq 0.05$). SLM was reduced between the first and second harvests in all four pathways (Table 7.5), but this was mainly attributable to defoliation responses of some species having high initial SLM (*Digitaria* and *Sporobolus*, Table 7.2). Elevated CO_2 did not alter SLM in any photosynthetic type (Table 7.5). Leaf [N] was similar between the three C_4 sub-types, but was twice as high in the C_3 than the C_4 pathway (Fig. 7.1).

7.3.2 Gas exchange

The responses of net CO_2 assimilation rate (A) to intercellular CO_2 concentration (c_i) in the eight species studied are presented in Fig. 7.2. Measured data points for all replicate curves are plotted, and the average of individually modelled response curves for each CO_2 treatment overlaid. Changes in stomatal conductance (g_s) with changing c_i are presented in Fig. 7.3, which shows all data points and one polynomial (logarithmic function) to fit all points. Gas exchange data for both ambient (360 μmol m^{-2} s^{-1}) and elevated (650 μmol m^{-2} s^{-1}) cuvette CO_2 concentrations, as extracted from the A/c_i response, are given for each species in Table 7.6 (statistical analysis in Table 7.7). The statistical analysis of differences between plants grown and measured at ambient CO_2 and plants grown and measured at elevated CO_2 is given in Table 7.8.

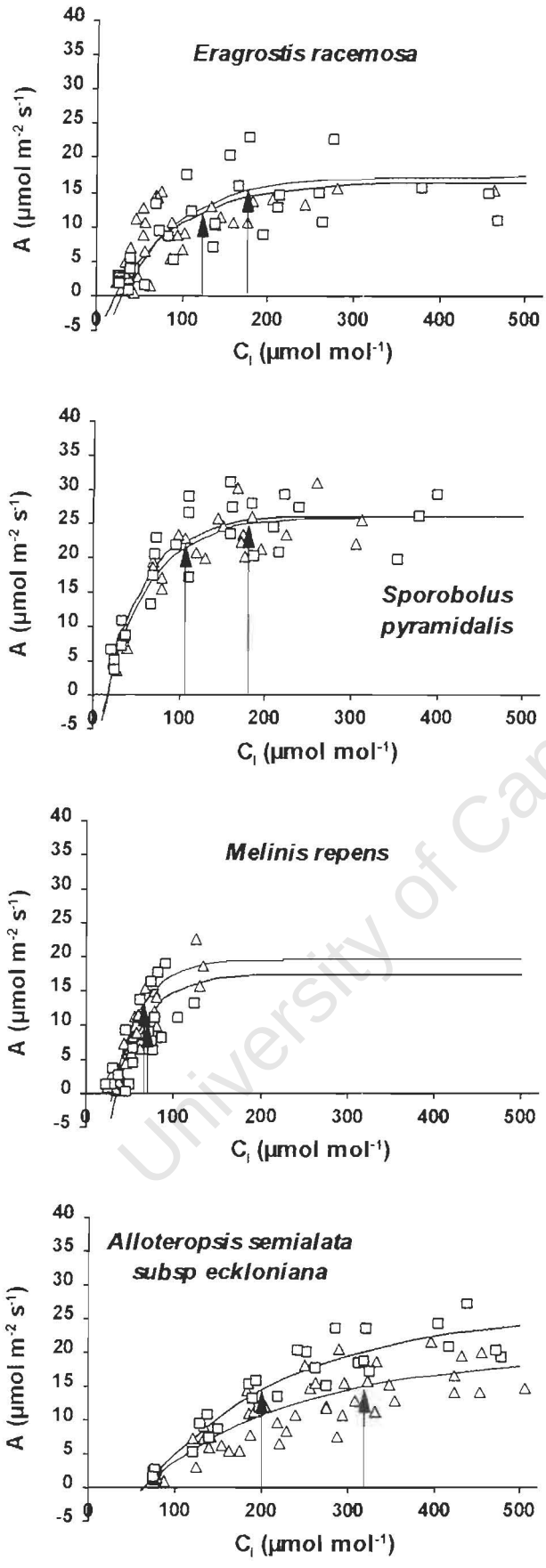
Only three species showed responses of net photosynthetic rate to elevated CO_2 in the short- ($[CO_2]$ms) or long-term ($[CO_2]$trt; Table 7.7). *Digitaria* grown under elevated CO_2 had significantly lower rates when measured at the same cuvette CO_2 level as plants grown under ambient CO_2, but short-term increases in cuvette CO_2 level resulted in higher photosynthetic rates in all plants. Consequently, there were no net differences in photosynthetic rates when plants were measured at their growth CO_2 concentration (Table 7.8). A similar trend was found in *Alloteropsis*. *Eragrostis curvula* showed significant long-term increases in photosynthetic rates when grown under elevated CO_2 (Table 7.7), but there were no short-term responses to increasing cuvette CO_2 concentration. Rates measured at growth CO_2 concentration were, therefore, higher in plants grown under elevated CO_2 (Table 7.8).

University of Cape Town



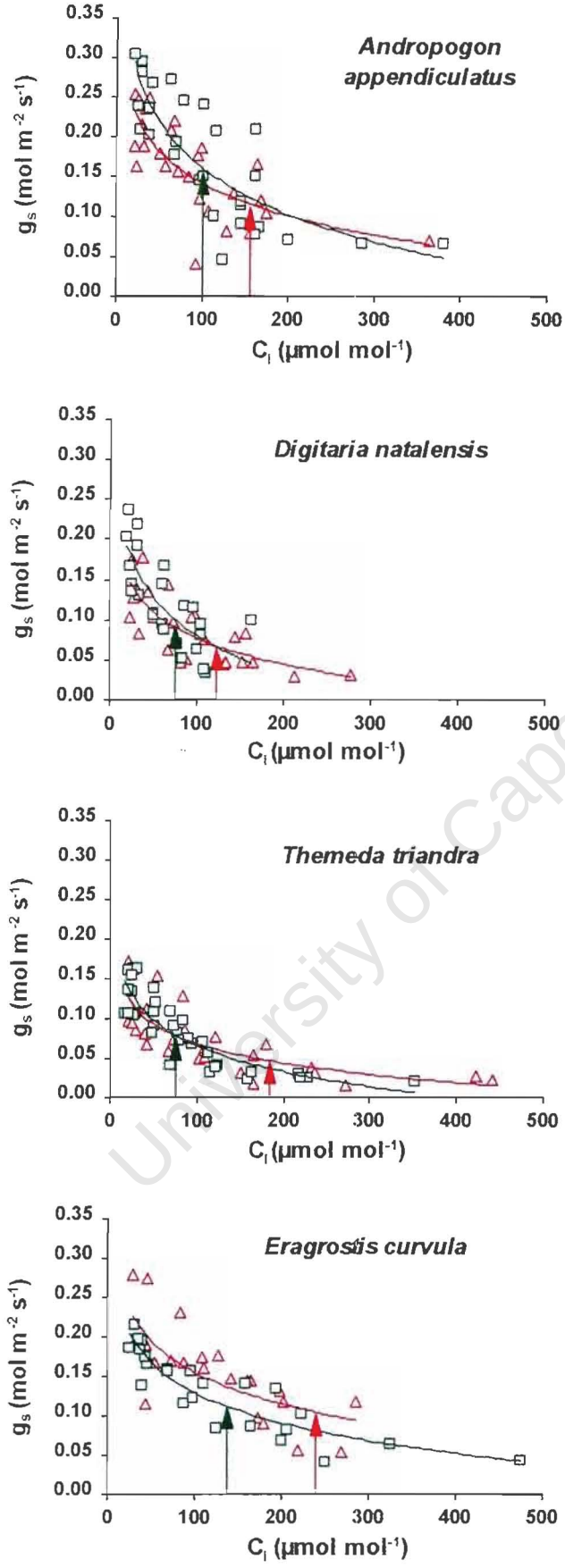
University of Cape Town

Fig. 7.2 Photosynthetic CO_2-response curves (A/c_i) of grass species grown under ambient (green squares and polynomials) or elevated (red triangles and polynomials) CO_2. Symbols represent all data points and polynomials represent averages for individually modelled curves. Arrows indicate A values at operational c_i. Cont.



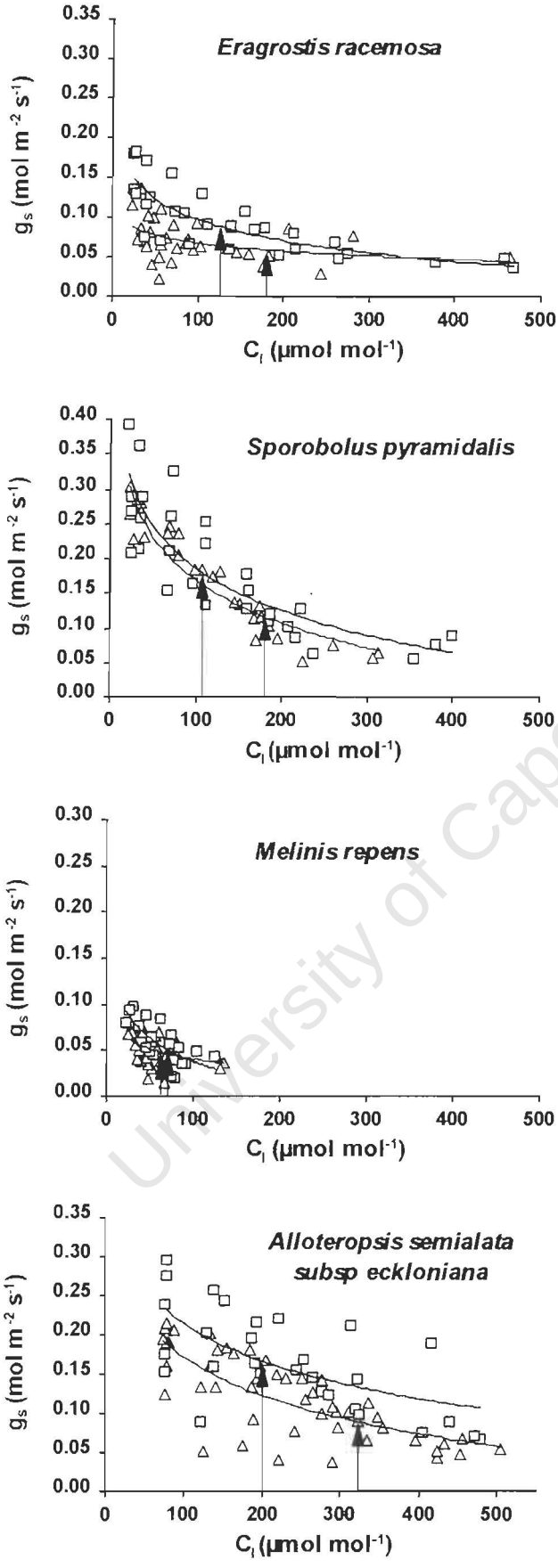
University of Cape Town

Fig. 7.2 (cont.) Photosynthetic CO_2-response curves (A/c_i) of grass species grown under ambient (green squares and polynomials) or elevated (red triangles and polynomials) CO_2. Symbols represent all data points and polynomials represent averages for individually modelled curves. Arrows indicate A values at operational c_i.



University of Cape Town

Fig. 7.3 CO_2-response of stomatal conductance (g_s/c_i) in grass species grown under ambient (green squares and polynomials) or elevated (red triangles and polynomials) CO_2. Symbols represent all data points and polynomials (logarithmic function) are fitted to all data points. Arrows indicate g_s values at operational c_i. Cont.



University of Cape Town

Fig. 7.3 (cont.) CO_2-response of stomatal conductance (g_s/c_i) in grass species grown under ambient (green squares and polynomials) or elevated (red triangles and polynomials) CO_2. Symbols represent all data points and polynomials (logarithmic function) are fitted to all data points. Arrows indicate g_s values at operational c_i.

Table 7.6 Gas exchange data for eight grass species grown under elevated CO_2 for 6-12 weeks. Data taken from A/c_i response curves at cuvette CO_2 concentrations of 360 µmol mol^{-1} and 650 µmol mol^{-1}. A = net CO_2 assimilation rate; g_s = stomatal conductance; E = transpiration rate; WUE = leaf instantaneous water use efficiency; c_i = intercellular CO_2 concentration; c_a = atmospheric CO_2 concentration. Values are means ± SE (usually n=4 but ranges between 2-5, except n=8 (elevated CO_2) for *Alloteropsis*).

Parameter	Growth [CO_2]: 360		650	
	Measurement [CO_2]: 360	650	360	650
A (µmol m^{-2} s^{-1}):				
Andropogon appendiculatus	23.8 ± 2.3	25.6 ± 2.1	23.2 ± 0.7	26.4 ± 1.9
Digitaria natalensis	15.8 ± 0.7	18.6 ± 1.6	11.7 ± 1.8	14.3 ± 0.6
Themeda triandra	14.0 ± 1.1	11.7 ± 0.9	11.9 ± 2.3	9.8 ± 2.4
Eragrostis curvula	14.9 ± 2.1	14.7 ± 3.0	18.7 ± 1.6	25.5 ± 1.2
Eragrostis racemosa	11.8 ± 2.2	15.8 ± 2.6	11.2 ± 1.1	13.8 ± 1.1
Sporobolus pyramidalis	23.6 ± 2.7	25.6 ± 1.9	21.8 ± 0.8	25.0 ± 2.0
Melinis repens subsp *repens*	9.6 ± 2.1	12.9 ± 2.9	9.5 ± 1.3	12.1 ± 2.7
Alloteropsis semialata subsp *eckloniana*	13.9 ± 0.7	20.7 ± 1.3	10.1 ± 1.0	14.7 ± 1.4
g_s (mmol m^{-2} s^{-1}):				
Andropogon appendiculatus	186 ± 23	92 ± 8	167 ± 9	97 ± 10
Digitaria natalensis	104 ± 8	57 ± 6	83 ± 12	47 ± 0
Themeda triandra	90 ± 7	37 ± 2	83 ± 16	36 ± 7
Eragrostis curvula	121 ± 13	66 ± 3	158 ± 7	109 ± 10
Eragrostis racemosa	92 ± 14	65 ± 8	79 ± 6	53 ± 9
Sporobolus pyramidalis	193 ± 27	106 ± 9	181 ± 3	97 ± 8
Melinis repens subsp *repens*	60 ± 13	40 ± 9	56 ± 8	36 ± 9
Alloteropsis semialata subsp *eckloniana*	190 ± 14	132 ± 16	135 ± 14	85 ± 9
WUE (µmol CO_2 $mmol^{-1}$ H_20):				
Andropogon appendiculatus	5.1 ± 0.1	10.1 ± 0.3	5.3 ± 0.1	10.0 ± 0.2
Digitaria natalensis	5.7 ± 0.3	11.6 ± 0.1	5.2 ± 0.2	10.5 ± 0.7
Themeda triandra	6.1 ± 0.3	11.6 ± 0.6	5.8 ± 0.4	10.2 ± 0.7
Eragrostis curvula	5.0 ± 0.5	8.6 ± 1.2	4.7 ± 0.3	9.3 ± 0.4
Eragrostis racemosa	4.9 ± 0.4	8.9 ± 0.7	5.6 ± 0.8	10.6 ± 1.8
Sporobolus pyramidalis	5.3 ± 0.3	9.5 ± 0.5	5.1 ± 0.1	10.0 ± 0.3
Melinis repens subsp *repens*	5.5 ± 0.4	10.9 ± 0.8	5.8 ± 0.1	11.5 ± 0.2
Alloteropsis semialata subsp *eckloniana*	3.5 ± 0.3	7.1 ± 0.6	3.6 ± 0.1	7.6 ± 0.3
c_i/c_a:				
Andropogon appendiculatus	0.29 ± 0.01	0.24 ± 0.01	0.25 ± 0.02	0.24 ± 0.02
Digitaria natalensis	0.22 ± 0.03	0.14 ± 0.01	0.29 ± 0.04	0.19 ± 0.03
Themeda triandra	0.22 ± 0.01	0.18 ± 0.02	0.27 ± 0.03	0.29 ± 0.05
Eragrostis curvula	0.37 ± 0.05	0.40 ± 0.10	0.39 ± 0.03	0.35 ± 0.09
Eragrostis racemosa	0.35 ± 0.03	0.35 ± 0.03	0.29 ± 0.04	0.27 ± 0.07
Sporobolus pyramidalis	0.31 ± 0.01	0.32 ± 0.01	0.33 ± 0.02	0.28 ± 0.01
Melinis repens subsp *repens*	0.20 ± 0.01	0.14 ± 0.02	0.16 ± 0.01	0.10 ± 0.01
Alloteropsis semialata subsp *eckloniana*	0.57 ± 0.02	0.51 ± 0.03	0.56 ± 0.02	0.49 ± 0.01

University of Cape Town

Table 7.7 Statistical significance of the effects of growth in elevated CO_2 ($[CO_2]$trt) on gas exchange of individual grass species, as calculated by two-way ANOVA. Abbreviations as for Table 7.6. Data taken from A/c_i response curves at cuvette CO_2 concentrations ($[CO_2]$ms) of 360 µmol mol^{-1} and 650 µmol mol^{-1}. Percentage changes in elevated CO_2 are given where differences are significant. +, P≤0.10; *, P≤0.05; **, P≤0.01; ***, P≤0.001; NS, not significant, according to two-way ANOVA.

Species	Parameter	Main effects		Interaction
		$[CO_2]$trt	$[CO_2]$ms	$[CO_2]$trt x $[CO_2]$ms
Andropogon appendiculatus (NADP-ME)	A	NS	NS	NS
	g_s	NS	-46% ***	NS
	WUE	NS	+92% ***	NS
	c_i/c_a	NS	-12% *	+
Digitaria natalensis (NADP-ME)	A	-23% *	+16% +	NS
	g_s	-23% +	-45% **	NS
	WUE	NS	+101% ***	NS
	c_i/c_a	NS	-33% *	NS
Themeda triandra (NADP-ME)	A	NS	NS	NS
	g_s	NS	-58% ***	NS
	WUE	NS	+84% ***	NS
	c_i/c_a	+39% *	NS	NS
Eragrostis curvula (NAD-ME)	A	+41% *	NS	NS
	g_s	+37% **	-37% **	NS
	WUE	NS	+84% ***	NS
	c_i/c_a	NS	NS	NS
Eragrostis racemosa (NAD-ME)	A	NS	NS	NS
	g_s	NS	-31% *	NS
	WUE	NS	+86% **	NS
	c_i/c_a	NS	NS	NS
Sporobolus pyramidalis (PCK)	A	NS	NS	NS
	g_s	NS	-46%***	NS
	WUE	NS	+87% ***	NS
	c_i/c_a	NS	NS	+
Melinis repens subsp *repens* (PCK)	A	NS	NS	NS
	g_s	NS	-35%+	NS
	WUE	NS	+96% ***	NS
	c_i/c_a	-23% *	-31% **	NS
Alloteropsis semialata subsp *eckloniana* (C_3)	A	-28% ***	+47% ***	NS
	g_s	-32% ***	-34% ***	NS
	WUE	NS	+108% ***	NS
	c_i/c_a	NS	-11% **	NS

University of Cape Town

Table 7.8 Statistical significance of the effects of elevated CO_2 on gas exchange of individual grass species, as calculated by one-way ANOVA. Abbreviations as for Table 7.6. Data taken from A/c_i response curves at cuvette CO_2 concentrations equivalent to growth CO_2 concentrations. +, $P \leq 0.10$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; NS, not significant.

Species	Parameter	$[CO_2]$
Andropogon appendiculatus (NADP-ME)	A	NS
	g_s	-48% *
	WUE	+95% ***
	c_i/c_a	-18% *
Digitaria natalensis (NADP-ME)	A	NS
	g_s	-55% **
	WUE	+85% ***
	c_i/c_a	NS
Themeda triandra (NADP-ME)	A	NS
	g_s	-61% ***
	WUE	+68% ***
	c_i/c_a	NS
Eragrostis curvula (NAD-ME)	A	+71% *
	g_s	NS
	WUE	+86% **
	c_i/c_a	NS
Eragrostis racemosa (NAD-ME)	A	NS
	g_s	-43% +
	WUE	+118% *
	c_i/c_a	NS
Sporobolus pyramidalis (PCK)	A	NS
	g_s	-50% *
	WUE	+88% ***
	c_i/c_a	-10% +
Melinis repens subsp *repens* (PCK)	A	NS
	g_s	NS
	WUE	+107% ***
	c_i/c_a	-50% **
Alloteropsis semialata subsp *eckloniana* (C_3)	A	NS
	g_s	-55% ***
	WUE	+115% ***
	c_i/c_a	-13% **

University of Cape Town

Responses of stomatal conductances followed the same long-term patterns as described for photosynthetic rates, (Tables 7.6, 7.7). Growth under elevated CO_2 resulted in decreased conductances in *Digitaria* and *Alloteropsis*, but increased conductances in *Eragrostis curvula*, when compared to ambient CO_2-grown plants at the same cuvette CO_2 level. However, all species had strongly reduced conductances in the short term, ranging between 31-58%, when measured at elevated CO_2, irrespective of long-term CO_2 treatment (Table 7.7). When compared at growth CO_2 concentration, all species except *Eragrostis curvula* and *Melinis* had significantly reduced conductances (43-61%; Table 7.8). The resulting highly significant increases in WUE in all species ranged between 68-118% (Table 7.8).

The ratio of intercellular to atmospheric CO_2 concentration (c_i/c_a) was increased under the elevated CO_2 treatment (long-term) in *Themeda*, but decreased in *Melinis* (Table 7.7). Under high cuvette CO_2 concentrations (short-term effect), four species (*Andropogon*, *Digitaria*, *Melinis* and *Alloteropsis*) showed significantly reduced c_i/c_a. When plants were compared at their growth CO_2 concentrations (Table 7.8), *Andropogon*, *Sporobolus*, *Melinis* and *Alloteropsis* plants grown and measured under elevated CO_2 operated at a lower c_i/c_a than those grown and measured under ambient CO_2.

Data for species belonging to the same photosynthetic pathway were combined, and the differences in short- and long-term photosynthetic CO_2-responses between them tested (Fig. 7.4, Table 7.9). There was no interaction between the factors with respect to photosynthetic rate, and interactions for other parameters were marginally significant ($P \leq 0.10$). Photosynthetic rates were significantly increased by elevated CO_2 in the short-term by 18%, on average, across all four photosynthetic pathways (Table 7.9). Long-term decreases in stomatal conductance were more pronounced in the C_3 pathway (Table 7.9, Fig. 7.4). All pathways showed highly significant short-term decreases in conductance in elevated cuvette CO_2 concentrations, by on average 41%. This resulted in increases of about 92% in WUE in all pathways, but particularly in the C_3 pathway (108%). Short-term decreases (9%) in c_i/c_a were measured across all pathways under elevated cuvette CO_2 concentrations. Photosynthetic pathways differed significantly from one another with respect to their photosynthetic characteristics: a Student-Newman-Keuls Multiple Range Test ($P \leq 0.05$) showed that the C_3 pathway had higher g_s, lower WUE, and higher c_i/c_a ratios than all three

University of Cape Town

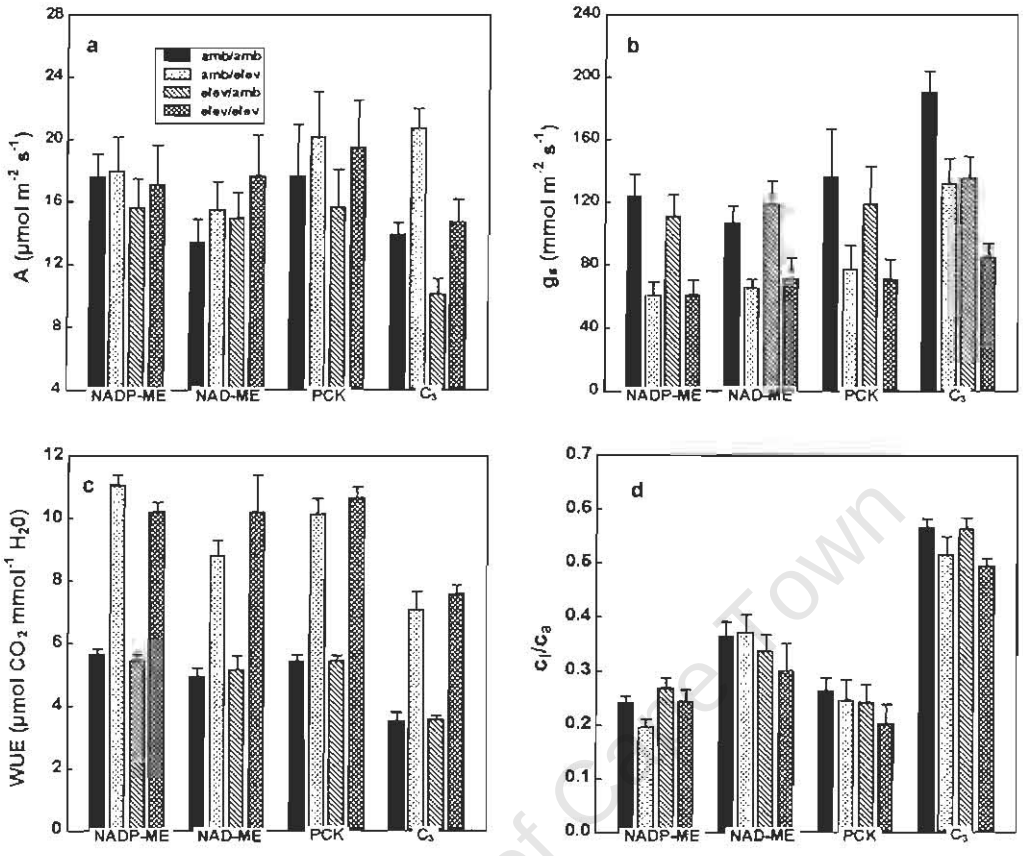


Fig. 7.4 Effects of growth in elevated CO_2 on gas exchange of grasses representing four different photosynthetic types (C_4 NADP-ME; C_4 NAD-ME; C_4 PCK; C_3). Data taken from A/c_i responses at cuvette CO_2 concentrations of 350 and 650 µmol m^{-2} s^{-1}. In the legend, the first abbreviation (amb or elev) refers to the treatments CO_2 concentration, and the second abbreviation refers to the cuvette (measurement) concentration. Values are means ± SE bars. n=11-13 for NADP-ME; n=6-8 for NAD-ME; n=7-8 for PCK; n=5-8 for C_3.

University of Cape Town

Table 7.9 Statistical significance of the effects of growth in elevated CO_2 ($[CO_2]$trt) on gas exchange of grass species of differing photosynthetic pathway (PP), as calculated by three-way ANOVA. Abbreviations as for Table 7.6. Data taken from A/c_i response curves at cuvette CO_2 concentrations ($[CO_2]$ms) of 360 μmol mol^{-1} and 650 μmol mol^{-1}. +, P≤0.10; *, P≤0.05; **, P≤0.01; ***, P≤0.001; NS, not significant.

Parameter	Main effects			Interactions			
	PP	$[CO_2]$trt	$[CO_2]$ms	PP x $[CO_2]$trt	PP x $[CO_2]$ms	$[CO_2]$trt x $[CO_2]$ms	PP x $[CO_2]$trt x $[CO_2]$ms
A	+	NS	+18% *	NS	NS	NS	NS
g_s	***	-10% +	-41% ***	+	NS	NS	NS
WUE	***	NS	+92%***	+	+	NS	NS
c_i/c_a	***	NS	-9% **	+	NS	NS	NS

Table 7.10 Statistical significance of the effects of elevated CO_2 on gas exchange of grass species of differing photosynthetic pathway (PP), as calculated by two-way ANOVA. Abbreviations as for Table 7.6. Data taken from A/c_i response curves at cuvette CO_2 concentrations equivalent to growth CO_2 concentrations. *, P≤0.05; ***, P≤0.001; NS, not significant.

Parameter:	Main effects		Interaction
	PP	$[CO_2]$	PP x $[CO_2]$
A	NS	NS	NS
g_s	*	-46% ***	NS
WUE	***	+89% ***	NS
c_i/c_a	***	-5% *	NS

University of Cape Town

C_4 pathways. The NAD-ME pathway had higher c_i/c_a ratios than the NADP-ME or PCK pathways.

When measurements were compared at growth CO_2 concentrations, no interactions were found between pathway and CO_2-response (Table 7.10). There were no significant effects of elevated CO_2 on photosynthetic rate. Conductances decreased (46%), WUE increased (89%) and c_i/c_a decreased (5%) across all pathways, owing to the short-term effect described above (Table 7.9).

A/c_i curve parameters are given for individual species (with statistical analysis) in Table 7.11. The only significant responses were decreases in J_e in *Digitaria* and *Alloteropsis*. When data were combined for different photosynthetic pathways, no significant CO_2-responses were found, with no interaction between CO_2 treatment and pathway (Fig. 7.5, Table 7.12). However, there were significant differences between pathways: a Student-Newman-Keuls Multiple Range Test ($P \leq 0.05$) showed that the CO_2 compensation point (Γ_c) was higher in the NAD-ME pathway than in the NADP-ME pathway, with PCK being intermediate, but Γ_c was considerably higher in the C_3 pathway than in all three C_4 pathways. The apparent carboxylation efficiency (k) was lower in the C_3 and NAD-ME pathways than in the NADP-ME and PCK pathways (Fig. 7.5).

The responses of net CO_2 assimilation rate (A) to light levels (photosynthetic photon flux density, PPFD) in the eight species studied are presented in Fig. 7.6. Measured data points for all replicate curves are plotted, and the average of individually modelled response curves for each CO_2 treatment overlaid. Light response curve parameters are given for individual species (with statistical analysis) in Table 7.13. Light-saturated photosynthetic rates measured at growth CO_2 concentration (A_{max}) were significantly higher under elevated CO_2 in *Andropogon* and *Eragrostis curvula*, but lower in *Digitaria*. When compared to Table 7.8, the responses for *Andropogon* and *Digitaria* are similar but slightly greater than found from A/c_i measurements, although only marginally significant. This could be attributable to slight lag effects of high cuvette CO_2 concentration prior to measurement in the latter. Dark respiration rates (R_d) were significantly lower in elevated CO_2 in *Andropogon*, *Eragrostis curvula* and *Alloteropsis*. This had the effect of decreasing the light compensation point (Γ_l) in these species. The apparent quantum use efficiency (α) was significantly reduced in elevated CO_2 in *Andropogon*, *Digitaria* and *Alloteropsis*.

University of Cape Town

Table 7.11 Photosynthetic CO_2-response curve (A/c_i) parameters for eight grass species grown under elevated CO_2 for 6-12 weeks. Abbreviations: J_e, light- and CO_2-saturated net CO_2 assimilation rate; k, apparent carboxylation efficiency; Γ_c, CO_2 compensation point. Values are means ± SE (usually n=4 but ranges between 3-5, except n=8 (elevated CO_2) for *Alloteropsis*). Statistical significances calculated by one-way ANOVA. *, $P \leq 0.05$; NS, not significant, according to one-way ANOVA.

Parameter Species	Ambient CO_2	Elevated CO_2	Significance
J_e (μmol m^{-2} s^{-1}):			
Andropogon appendiculatus	27.9 ± 2.3	28.9 ± 1.6	NS
Digitaria natalensis	24.4 ± 3.2	14.4 ± 1.8	*
Themeda triandra	12.5 ± 0.9	10.9 ± 2.1	NS
Eragrostis curvula	18.6 ± 1.9	23.6 ± 2.1	NS
Eragrostis racemosa	16.6 ± 2.6	17.4 ± 2.6	NS
Sporobolus pyramidalis	26.1 ± 2.2	26.0 ± 2.0	NS
Melinis repens subsp *repens*	17.5 ± 4.0	19.6 ± 3.8	NS
Alloteropsis semialata subsp *eckloniana*	25.8 ± 1.8	19.4 ± 1.9	*
Γ_c (μmol mol^{-1}):			
Andropogon appendiculatus	19.8 ± 2.2	17.9 ± 1.0	NS
Digitaria natalensis	13.7 ± 2.1	19.7 ± 2.7	NS
Themeda triandra	17.1 ± 1.4	18.5 ± 2.5	NS
Eragrostis curvula	20.5 ± 4.7	24.6 ± 4.0	NS
Eragrostis racemosa	22.0 ± 4.6	31.5 ± 5.4	NS
Sporobolus pyramidalis	15.9 ± 1.5	15.1 ± 1.4	NS
Melinis repens subsp *repens*	29.8 ± 7.8	29.6 ± 2.5	NS
Alloteropsis semialata subsp *eckloniana*	63.4 ± 1.8	62.4 ± 2.6	NS
k (μmol CO_2 μmol^{-1} c_i):			
Andropogon appendiculatus	0.93 ± 0.06	0.99 ± 0.10	NS
Digitaria natalensis	0.61 ± 0.07	0.79 ± 0.29	NS
Themeda triandra	3.11 ± 0.87	1.90 ± 1.06	NS
Eragrostis curvula	0.72 ± 0.32	0.50 ± 0.07	NS
Eragrostis racemosa	0.31 ± 0.09	0.39 ± 0.08	NS
Sporobolus pyramidalis	0.86 ± 0.12	0.68 ± 0.10	NS
Melinis repens subsp *repens*	2.30 ± 1.67	2.14 ± 1.20	NS
Alloteropsis semialata subsp *eckloniana*	0.23 ± 0.03	0.16 ± 0.03	NS

University of Cape Town

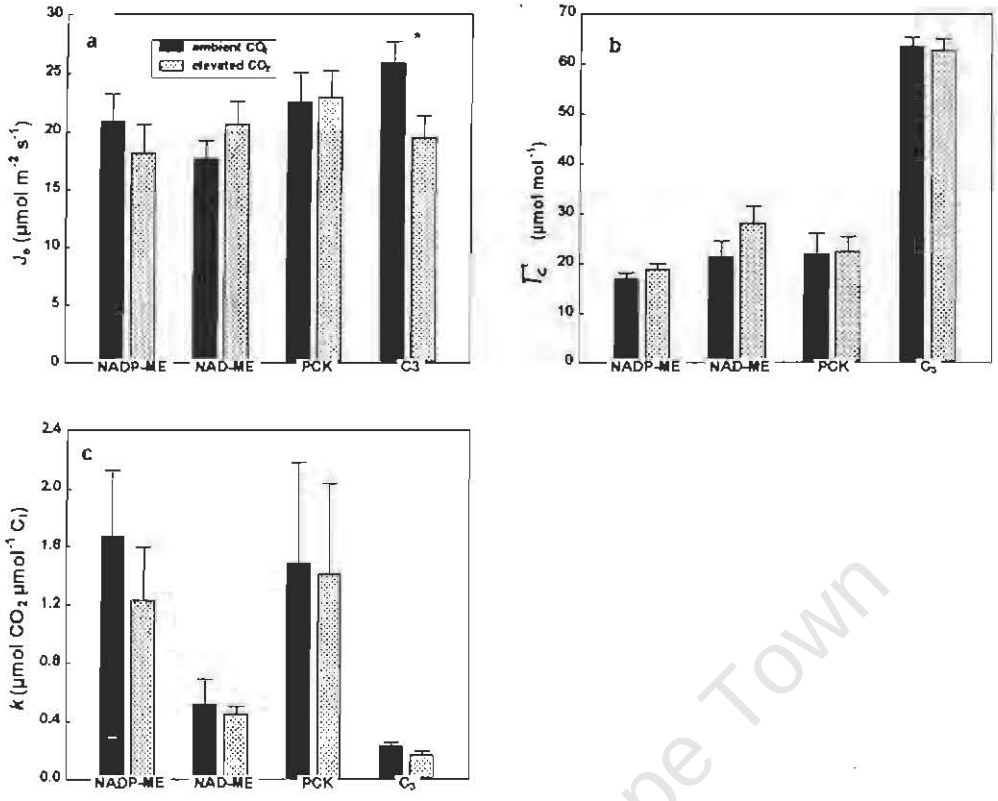
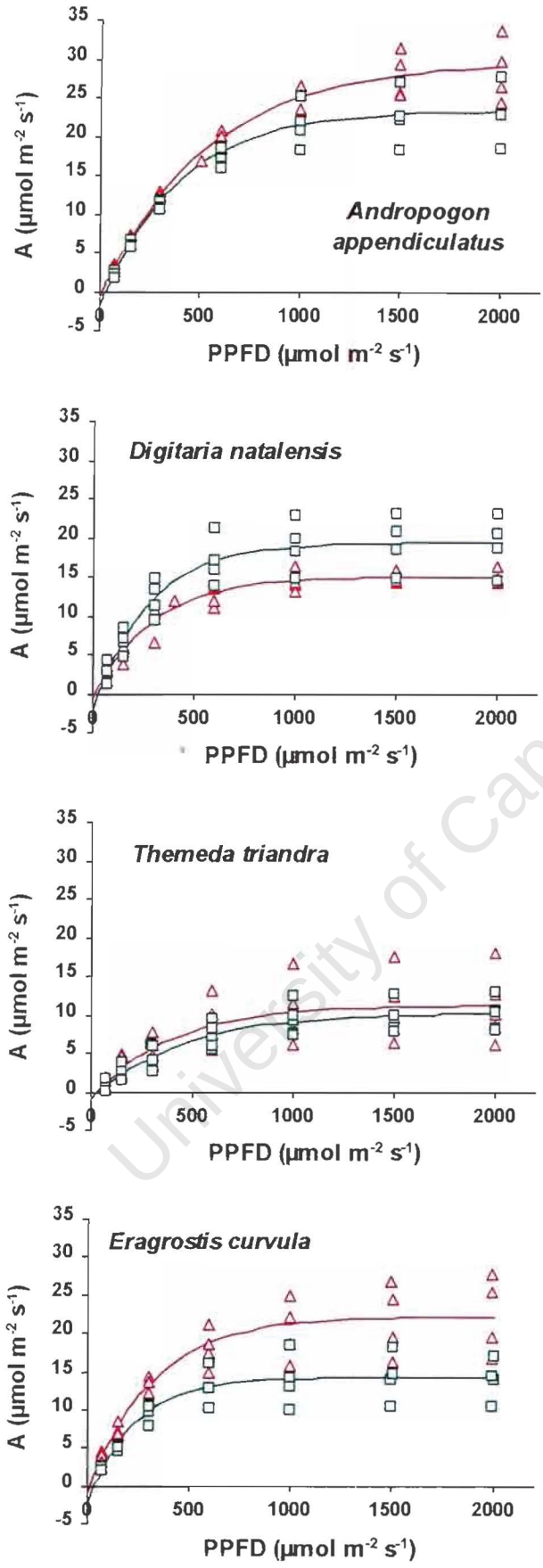


Fig. 7.5 Effects of growth in elevated CO_2 on A/C_i response curve parameters of grasses representing four different photosynthetic types (C_4 NADP-ME; C_4 NAD-ME; C_4 PCK; C_3). Values are means ± SE bars. n=12-13 for NADP-ME; n=8 for NAD-ME; n=7-8 for PCK; n=5-8 for C_3. * $P \leq 0.05$ according to one-way ANOVA.

University of Cape Town

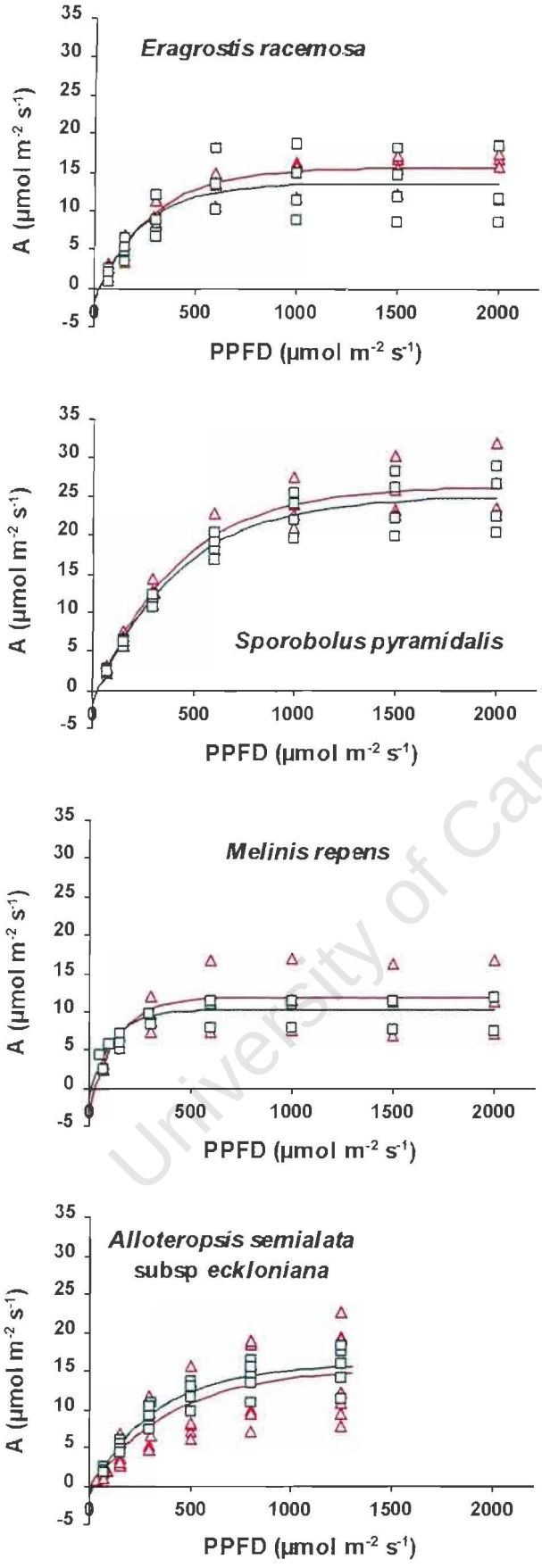
Table 7.12 Statistical significance of the effects of elevated CO_2 on photosynthetic CO_2-response curve (A/c_i) parameters of grass species of differing photosynthetic pathway (PP), as calculated by two-way ANOVA. Abbreviations as for Table 7.11. **, $P \leq 0.01$; ***, $P \leq 0.001$; NS, not significant.

Parameter:	Main effects		Interaction
	PP	$[CO_2]$	PP x $[CO_2]$
J_c	NS	NS	NS
Γ_c	***	NS	NS
k	**	NS	NS



University of Cape Town

Fig. 7.6 Photosynthetic light response curves of grass species grown under ambient (green squares and polynomials) or elevated (red triangles and polynomials) CO_2. Measurements made at growth CO_2 concentration. Symbols represent all data points and polynomials represent averages for individually modelled curves. Cont.



University of Cape Town

Fig. 7.6 (cont.) Photosynthetic light response curves of grass species grown under ambient (green squares and polynomials) or elevated (red triangles and polynomials) CO_2. Measurements made at growth CO_2 concentration. Symbols represent all data points and polynomials represent averages for individually modelled curves.

Table 7.13 Photosynthetic light-response curve parameters for eight grass species grown under elevated CO_2 for 6-12 weeks. A_{max}, maximum light-saturated net CO_2 assimilation rate at growth CO_2 concentration; Γ_i, light compensation point; R_d, predicted dark respiration rate; α, apparent quantum use efficiency. Values are means ± SE (usually n=4 but ranges between 3-5, except n=7 (elevated CO_2) for *Alloteropsis*). Statistical significances calculated by one-way ANOVA. +, P≤0.10; *, P≤0.05; **, P≤0.01; NS, not significant.

Parameter Species	Ambient CO_2	Elevated CO_2	Significance
A_{max} (μmol m^{-2} s^{-1}):			
Andropogon appendiculatus	23.4 ± 2.1	29.9 ± 2.2	+
Digitaria natalensis	21.2 ± 2.8	15.1 ± 0.5	+
Themeda triandra	10.2 ± 0.9	11.3 ± 2.1	NS
Eragrostis curvula	14.2 ± 1.6	22.2 ± 2.6	*
Eragrostis racemosa	13.6 ± 2.2	15.6 ± 1.3	NS
Sporobolus pyramidalis	25.0 ± 2.1	26.4 ± 2.1	NS
Melinis repens subsp *repens*	10.2 ± 1.2	12.0 ± 2.8	NS
Alloteropsis semialata subsp *eckloniana*	15.7 ± 1.3	15.3 ± 2.3	NS
Γ_i (μmol m^{-2} s^{-1}):			
Andropogon appendiculatus	31.2 ± 6.1	13.0 ± 5.9	+
Digitaria natalensis	25.7 ± 8.8	12.9 ± 5.1	NS
Themeda triandra	42.2 ± 10.4	28.7 ± 7.0	NS
Eragrostis curvula	35.8 ± 1.6	12.9 ± 4.3	**
Eragrostis racemosa	34.3 ± 6.9	31.2 ± 9.9	NS
Sporobolus pyramidalis	30.2 ± 2.2	27.2 ± 3.3	NS
Melinis repens subsp *repens*	21.7 ± 11.0	31.4 ± 2.2	NS
Alloteropsis semialata subsp *eckloniana*	25.0 ± 2.1	14.3 ± 4.3	+
R_d (μmol m^{-2} s^{-1}):			
Andropogon appendiculatus	1.95 ± 0.44	0.73 ± 0.35	+
Digitaria natalensis	1.85 ± 0.62	0.68 ± 0.30	NS
Themeda triandra	0.97 ± 0.27	0.78 ± 0.14	NS
Eragrostis curvula	2.36 ± 0.17	0.88 ± 0.30	**
Eragrostis racemosa	2.12 ± 0.57	1.70 ± 0.48	NS
Sporobolus pyramidalis	1.87 ± 0.15	1.80 ± 0.18	NS
Melinis repens subsp *repens*	1.62 ± 0.88	2.87 ± 0.63	NS
Alloteropsis semialata subsp *eckloniana*	1.28 ± 0.11	0.61 ± 0.22	*
α (μmol CO_2 μmol^{-1} PPFD):			
Andropogon appendiculatus	0.064 ± 0.003	0.056 ± 0.002	+
Digitaria natalensis	0.075 ± 0.007	0.053 ± 0.008	+
Themeda triandra	0.026 ± 0.004	0.030 ± 0.004	NS
Eragrostis curvula	0.071 ± 0.002	0.070 ± 0.004	NS
Eragrostis racemosa	0.066 ± 0.010	0.061 ± 0.005	NS
Sporobolus pyramidalis	0.064 ± 0.002	0.069 ± 0.002	NS
Melinis repens subsp *repens*	0.094 ± 0.015	0.102 ± 0.020	NS
Alloteropsis semialata subsp *eckloniana*	0.053 ± 0.003	0.039 ± 0.006	+

University of Cape Town

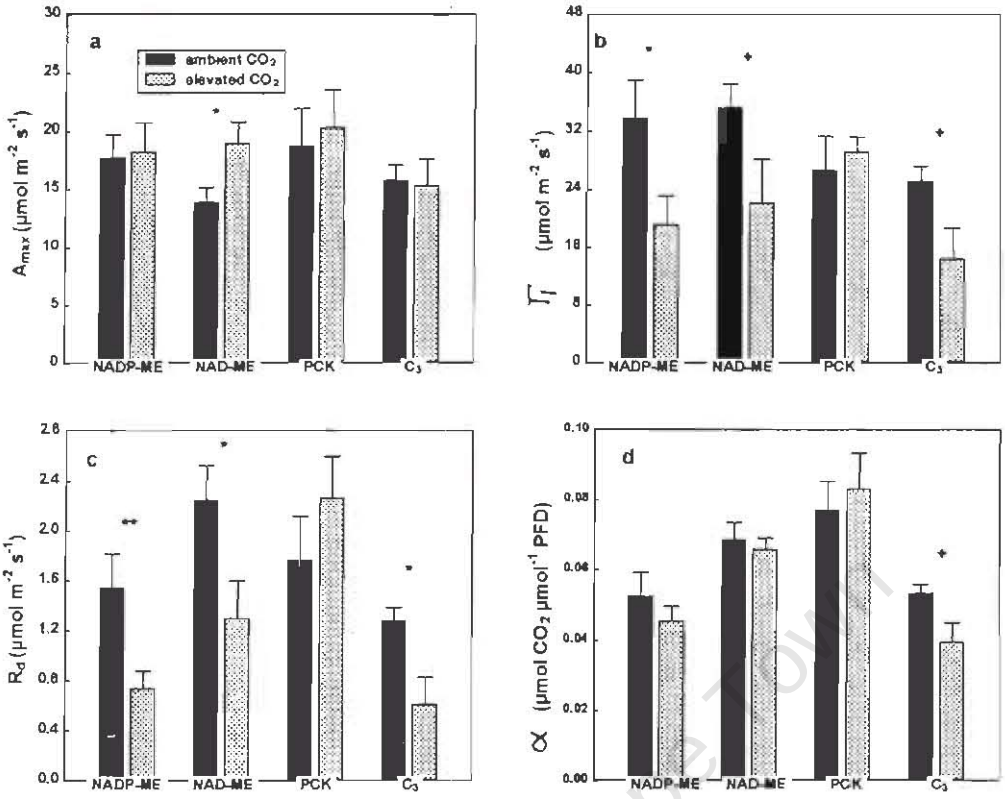


Fig. 7.7 Effects of growth in elevated CO_2 on photosynthetic light response curve parameters of grasses representing four different photosynthetic types (C_4 NADP-ME; C_4 NAD-ME; C_4 PCK; C_3). Values are means ± SE bars. n=13 for NADP-ME; n=8 for NAD-ME; n=7 for PCK; n=5-7 for C_3. + P≤0.10; * P≤0.05; ** P≤0.01 according to one-way ANOVA.

University of Cape Town

Table 7.14 Statistical significance of the effects of elevated CO_2 on photosynthetic light-response curve parameters of grass species of differing photosynthetic pathway (PP), as calculated by two-way ANOVA. Abbreviations as for Table 7.13. +, P≤0.10; **, P≤0.01; ***, P≤0.001; NS, not significant.

Parameter:	Main effects		Interaction
	PP	$[CO_2]$	PP x $[CO_2]$
A_{max}	NS	NS	NS
Γ_l	NS	**	NS
R_d	***	**	+
α	***	NS	NS

Light response curve characteristics for different photosynthetic pathways are presented in Fig. 7.7, with the statistical analysis in Table 7.14. There were no overall effects of elevated CO_2 on A_{max} (Table 7.14), despite an increase in the NAD-ME pathway when analysed individually (Fig. 7.7). There were significant overall decreases in R_d and Γ_l under elevated CO_2 in the C_3, NADP-ME and NAD-ME, but not in the PCK pathway (Fig. 7.7). No significant CO_2-responses emerged overall for α, although the C_3 pathway had reduced α when pathways were analysed individually. Photosynthetic pathways differed from one another with respect to R_d and α (Student-Newman-Keuls Multiple Range Test $P \leq 0.05$): the C_3 and NADP-ME pathways had lower R_d and α than the NAD-ME and PCK pathways.

7.4 Discussion

7.4.1 Gas exchange in elevated CO_2

The hypothesis that NAD-ME pathway grass species will show greater positive photosynthetic and growth responses to elevated CO_2 than NADP-ME pathway grasses was rejected in this study. Only one species (*Eragrostis curvula*, NAD-ME) showed increased carbon assimilation rates at elevated compared to ambient CO_2 concentrations owing to longer-term enhanced photosynthetic capacity (Tables 7.6 and 7.7). While two other species (*Digitaria*, NADP-ME, and *Alloteropsis*, C_3) showed short-term increases in carbon assimilation rates at higher CO_2 concentration owing to non-saturation of photosynthesis at ambient CO_2, this was countered by longer-term photosynthetic downregulation (Table 7.7, J_e in Table 7.11). Non-saturation of photosynthesis is to be expected in C_3 species, but has only recently been shown to occur widely in C_4 species as well (Sionit & Patterson 1984, LeCain & Morgan 1998, Ziska & Bunce 1997). Although *Eragrostis curvula* belongs to the NAD-ME C_4 sub-type, which was expected to show the greatest photosynthetic response, the lack of CO_2-response in the other NAD-ME species, *Eragrostis racemosa*, suggests that this was a species-specific response rather than a sub-type-specific response (Tables 7.8 and 7.9). The C_4 sub-types did differ amongst each other with respect to photosynthetic characteristics: apparent carboxylation efficiencies were significantly lower, c_i/c_a ratios were higher, and CO_2 compensation points were higher (due to greater respiration rates) in NAD-ME than in NADP-ME or PCK species. In these respects, NAD-ME species showed intermediate gas exchange characteristics between NADP-ME/PCK and C_3 species. Nevertheless, these differences did not influence the relative CO_2-responsiveness between C_4 sub-types, except in

University of Cape Town

the case of R_d, which was not reduced in elevated CO_2 in the PCK sub-type, as it was in the NADP-ME or NAD-ME sub-types.

In most species, photosynthetic response to elevated CO_2 was poorly linked to growth response, with only *Eragrostis curvula* showing a positive relationship between enhanced carbon assimilation and enhanced non-leaf shoot growth. The large positive above-ground (particularly leaf) growth responses to elevated CO_2 in *Andropogon* (NADP-ME) and *Themeda* (NADP-ME), and in *Sporobolus* (PCK) for non-leaf growth, were not related to carbon assimilation responses and could not have been predicted from photosynthetic measurements alone. These results confirm those of LeCain & Morgan (1998), who found growth increases in only two of the six species studied, although photosynthetic rates in all six species were not saturated at ambient CO_2 concentrations and were increased under elevated CO_2. They also concluded that bundle sheath leakiness in C_4 grasses is not simply associated with plant growth responses to elevated CO_2.

The lack of a photosynthetic response to elevated CO_2 in *Themeda triandra* contrasts with the positive response measured in a previous experiment (Fig. 6.2) after a similar exposure period. However, the latter measurements were performed on seedlings (ecotype 2) with high relative growth rates and high J_e (19-24 $\mu mol\ m^{-2}\ s^{-1}$), whereas the data presented in this chapter were from mature plants excavated from the field (ecotype 3) and showing low relative growth rates and J_e (11-12.5 $\mu mol\ m^{-2}\ s^{-1}$). Significantly, the same plants of ecotype 2, when mature, showed photosynthetic downregulation (Fig. 5.3, Table 5.3). One could thus conclude that both results are valid, and that young vigorous plants show positive responses, which disappear in mature plants with lower photosynthetic capacity and vigour.

Stomatal conductances (g_s) were significantly reduced in all species due to short-term responses to elevated cuvette CO_2 concentration (Table 7.7). When measured at the growth CO_2 concentration, however, *Eragrostis curvula* and *Melinis* did not show significant reductions in conductance (Table 7.8), owing to longer-term upregulation of conductance in elevated CO_2 in the former and high variability in the latter (Table 7.7). The short-term stomatal response to CO_2 did not differ between photosynthetic pathways, and neither did the longer-term response differ between C_4 sub-types. At the respective growth CO_2 concentration, conductance was reduced overall by 46%, with no response differences between photosynthetic pathways. Therefore, changes in stomatal function in elevated CO_2 were fairly consistent across species and photosynthetic pathways, and can confidently be predicted for the majority of grass species (see also Chapter 2). However, these changes bore no direct relationship to growth responses.

University of Cape Town

Similarly, substantial increases in leaf instantaneous water use efficiency (WUE) in high CO_2, when compared at growth CO_2 levels, were measured in all species (on average 89%). The level of responsiveness in C_4 species could not, however, be differentiated on the basis of photosynthetic sub-type, and there was no link with the growth response.

7.4.2 Growth in elevated CO_2

Having established that the gas exchange response to elevated CO_2 is either species-specific (A) or near-ubiquitous (g_s and WUE), and not directly related to the growth response, the next question is whether the growth response can be linked to any functional groupings. Two of the three NADP-ME species (*Andropogon* and *Themeda*) showed significant aboveground growth enhancement in elevated CO_2, mainly due to the stimulation of leaf growth, although *Themeda* also showed enhanced non-leaf shoot growth. None of the NAD-ME or PCK species showed leaf growth enhancement, but *Eragrostis curvula* and *Sporobolus* had greater non-leaf biomass in high CO_2. This is contrary to the expectation that NAD-ME would show the greatest and NADP-ME the lowest CO_2-responses, with PCK intermediate. These results do, however, confirm the results of the only other similar study carried out thus far, using species from the North American short-grass prairie (LeCain & Morgan 1998). The latter study also found growth enhancement in two out of three NADP-ME grass species, and no response in three NAD-ME species. The statistical analysis for the present study does not reveal any significant differences in the CO_2-response of different photosynthetic pathways when analysed separately for each harvest (no interaction between pathway and CO_2-response, Table 7.4). However, a combined analysis for both harvests confirmed that significant leaf growth enhancement was strongly linked to the NADP-ME pathway, with NAD-ME species showing only a marginal increase in total shoot biomass, and PCK species showing no consistent CO_2-responses (Table 7.5).

Two possible explanations for the enhancement of leaf growth in NADP-ME sub-type grass species could be put forward. The first is based on the finding that wild C_4 NADP-ME monocots have a 12% higher quantum yield for CO_2 uptake, on average, than NAD-ME monocots (Ehleringer *et al.* 1997). The greater quantum yield of NADP-ME grasses provides them with a competitive advantage over NAD-ME grasses in ecosystems with conditions conducive to higher productivity, and this is borne out by their differential distribution patterns (Ehleringer *et al.* 1997). Under variable and often non-saturating light conditions experienced in this experiment, and which are usual in the field, the higher quantum yield may also allow for a greater relative response to elevated CO_2 (a strong "growth factor") in NADP-ME than in NAD-ME grasses under well-watered conditions.

University of Cape Town

The second explanation is based on possible differences in water relations between the C_4 sub-types. Although biogeographic distribution patterns have shown strong correlations between sub-type abundance and rainfall gradients (Ellis *et al.* 1980, Hattersley 1992), we have little ecophysiological knowledge about potential differences in leaf and plant water relations between C_4 sub-types. NADP-ME species tend to dominate in the mesic eastern part of South Africa (the "sourveld", Ellery *et al.* 1995). This veld type responds well to high rainfall during the growing season (summer), has a long growth season (>120 days), and is generally favoured by conditions conducive to carbon assimilation and growth (Ellery *et al.* 1995), but is generally drought-sensitive. On the other hand, NAD-ME species dominate the semi-arid "sweetveld" towards the west of the country. This veld type has a shorter growth season and is less productive, but is more drought-resistant, and offers nutritious grazing throughout most of the year. Improved leaf water relations in elevated CO_2, resulting from stomatal closure and reduced transpirational water loss, may well benefit NADP-ME grasses more than NAD-ME or PCK grasses, considering their likely strategy of maximal leaf growth during favourable conditions. NAD-ME grasses may have a more conservative leaf growth response to improved water relations, since the risks associated with high leaf and canopy area are higher (a "feedback control" situation in an arid environment). Hattersley & Watson (1992) suggested that the NAD-ME sub-type evolved in the tropical and sub-tropical zones primarily in response to aridization of the climate, whereas the NADP-ME sub-type evolved in the productive wet-dry "monsoon" (summer rainfall) tropics primarily in response to decreasing CO_2. If this is so, then NADP-ME taxa may be inherently more flexible and responsive to changing CO_2 than NAD-ME taxa.

Partial evidence for this "CO_2 vs arid climate" explanation may be seen in the leaf moisture data: two species (*Andropogon* and *Digitaria*), both NADP-ME types, showed increased percentage leaf moisture content in elevated CO_2 at the first harvest. The third NADP-ME species (*Themeda*) also had greater leaf moisture, although this was not significant. On the other hand, *Eragrostis curvula* (NAD-ME) showed reduced leaf moisture (Table 7.3). NAD-ME species also had lower absolute values of leaf moisture content than the other photosynthetic pathways, even though all plants were watered daily and were never water-stressed. In addition, leaf lengths remained remarkably constant in the NAD-ME sub-type over both harvests and in ambient and elevated CO_2 (Fig. 7.1), in contrast with the other pathways, supporting the idea that genetic control over leaf extension overrides

University of Cape Town

environmental stimuli. *Eragrostis curvula* even showed a significant reduction in leaf length in high CO_2 at the second harvest. Before any conclusions can be drawn, more data must be obtained on the water relations and growth patterns of the C_4 sub-types and how flexible these are under changing environmental conditions.

Leaf total nitrogen concentration did not differ between C_4 sub-types, but was twice as high in C_3 than C_4 leaves, as expected. Although there were no overall CO_2-induced reductions in [N], the two species showing leaf growth enhancement (*Andropogon* and *Themeda*) also had reduced [N], suggesting that this was a dilutory effect (in the absence of any changes in SLM).

An analysis of the literature on C_4 grass responses to elevated CO_2 reveals that only a few species belonging to the NAD-ME or PCK pathways have been studied. *Bouteloua gracilis* (NAD-ME) has shown both significantly positive shoot biomass responses (Hunt *et al.* 1996, Read & Morgan 1996) and no response (LeCain & Morgan 1998). A related species, *Bouteloua curtipendula* (NAD-ME) does not respond (Volin & Reich 1996). The only other NAD-ME species to show shoot enhancement was *Eragrostis orcuttiana*, a noxious weed of the Great Basin Desert of North America (Smith *et al.* 1987). Another weedy annual of this sub-type, *Eleusine indica*, develops greater whole plant biomass in elevated CO_2 (Sionit & Patterson 1984, Patterson 1986). All other wild grass species showing significantly positive shoot growth responses belong to the NADP-ME sub-type, notably *Andropogon gerardii* (Knapp *et al.* 1993, Owensby *et al.* 1993, LeCain & Morgan 1998), *Themeda triandra* (Chapters 4 and 5), *Panicum antidotale* (Ghannoum *et al.* 1997) and *Sorghastrum nutans* (LeCain & Morgan 1998). Whole plant biomass enhancement has also been measured in *Schizachyrium scoparium* (Polley *et al.* 1996a, but not significant in LeCain & Morgan 1998), and a number of weedy NADP-ME grass species. This literature survey, together with the results presented here, would appear to indicate a greater effect of elevated CO_2 on shoot growth in NADP-ME species. However, possible bias may be introduced by the skewed representation of sub-types in past studies, and a larger sample of NAD-ME and PCK species needs to be tested. Also, the positive effect of elevated CO_2 on non-leaf growth in *Eragrostis curvula* and *Sporobolus* should not be overlooked, and the potential link with reproductive stage investigated. These two species flowered profusely at the time of the first

University of Cape Town

harvest, and since they are relatively tall species with long reproductive culms, much of the biomass enhancement may have occurred in the reproductive stalks.

An interesting link between responsive species of the NADP-ME sub-type appears to be their taxonomic relationship. As the NADP-ME sub-type probably arose three times in different ancestral lines, it is quite possible that there is considerable genetic diversity even within this sub-type (Hattersley & Watson 1992), which may account for differential responsiveness to environmental factors such as CO_2 concentration. Most of the CO_2-responsive genera (e.g. *Andropogon*, *Themeda*, *Sorghastrum* and *Schizachyrium*) belong to the supertribe Andropogonodae (Gibbs Russel *et al.* 1990, Hattersley & Watson 1992), or to a branch of the Paniceae (e.g. *Panicum antidotale*), which, together with the Arundinelleae (which to my knowledge have not been tested for CO_2-responsiveness), share the common and exclusive feature of having lost their outer bundle sheaths as they evolved (Hattersley & Watson 1992). By contrast, other NADP-ME genera such as *Digitaria*, *Paspalum*, *Echinochloa* and *Setaria* (Paniceae) seem to be responsive to elevated CO_2 only if they are early successional, weedy species growing under favourable conditions e.g. *Echinochloa crus-galli* (Sionit & Patterson 1985) and *Digitaria sanguinalis* (Sionit & Patterson 1984). These ideas of an evolutionary link to potential CO_2-responsiveness, at least in non-weedy wild grass species, are circumstantial and speculative at this stage, and the potential mechanism a complete mystery, but the apparent pattern does merit further investigation.

7.4.3 Photosynthetic acclimation in elevated CO_2

Long-term acclimatory responses of gas exchange capacity to elevated CO_2 in C_4 grasses occurred in only two out of seven species: one case of photosynthetic downregulation was found (*Digitaria*) and one of upregulation (*Eragrostis curvula*). Such acclimation has been well documented for many C_3 species (Wullschleger 1993), but has been considered rare or even absent in C_4 species (Sage 1994, Ziska & Bunce 1997), except for instances of downregulation under conditions of nutrient deficiency (Wong 1979, Morgan *et al.* 1994, Ghannoum & Conroy, 1998). However, photosynthetic downregulation has recently been measured in wild C_4 grasses under non-stressful conditions (Read *et al.* 1997, LeCain & Morgan 1998). The mechanisms were apparently not related to carbohydrate accumulation or reductions in leaf nitrogen concentrations, as they are in C_3 species (Stitt 1991, Sage 1994, Cotrufo *et al.* 1998). In this study, leaf nitrogen concentrations were not altered in elevated

University of Cape Town

CO_2 in the two species concerned (Table 7.3), suggesting that photosynthetic acclimation was not attributable to changes in photosynthetic enzyme content. Ghannoum *et al.* (1997) measured no significant reductions in Rubisco content in a C_4 grass, *Panicum antidotale*, under elevated CO_2. In C_4 leaves, the activity of PEP carboxylase is represented by the initial slope of the A/c_i curve (apparent carboxylation efficiency, *k*), which was similar in this study in ambient and elevated CO_2 (Table 7.11). However, Rubisco activity is represented by the plateau of the curve (J_e), which was reduced in elevated CO_2 in *Digitaria* and in *Alloteropsis* (C_3). Similar findings have been reported for *Bouteloua gracilis* (LeCain & Morgan 1998). On the other hand, J_e was increased in *Eragrostis curvula*, although not significantly. The available evidence suggests that Rubisco acitivation rather than content may be altered in elevated CO_2 in C_4 grass species, or that reductions in Rubisco content are more difficult to detect than in C_3 species due to the lower proportional allocation of nitrogen to photosynthetic enzymes (LeCain & Morgan 1998).

The more obvious and likely explanation for photosynthetic acclimation was the strong link with long-term changes in stomatal conductance in both C_4 and C_3 species (Table 7.7). Acclimatory responses in stomatal physiology in C_4 species, as shown by g_s/c_i curves, have been reported by Morgan *et al.* (1994), Read *et al.* (1997) and LeCain & Morgan (1998), and took the form of either up- or downregulation. In the meta-analysis (Chapter 2) it was shown that decreases in g_s in C_4 species are greatest in plants exposed to elevated CO_2 for more than 120 days, suggesting that developmental changes in g_s may occur as leaves mature in high CO_2. This could be achieved through altered stomatal sizes or densities. Although Ghannoum *et al.* (1997) found no changes in stomatal densities in elevated CO_2 in *Panicum antidotale*, more data is needed on leaf anatomical responses in C_4 grasses to explain stomatal acclimation.

7.4.4 Conclusion

In conclusion, the grouping of C_4 grass species into functional types based on C_4 photosynthetic sub-type, in order to ascertain potential CO_2-responsiveness, was not successful in predicting gas exchange responses either in the short- or long-term, but yielded mixed success in predicting above-ground growth responses. The evidence suggests a strong tendency for greater leaf growth enhancement in NADP-ME species, although not all species of this sub-type responded significantly. Total shoot growth enhancement was not limited to

University of Cape Town

any sub-type/s but appeared to be more species-specific and possibly linked to reproductive phenology. Larger-scale modelling of grassland and savanna responses to elevated CO_2 on the basis of photosynthetic pathway is not yet possible, but there are promising leads which merit further study.

University of Cape Town



Fig. 7.8 *Alloteropsis semialata* subsp. *eckloniana* (top) and *Themeda triandra* (bottom) grown under ambient and elevated CO_2.

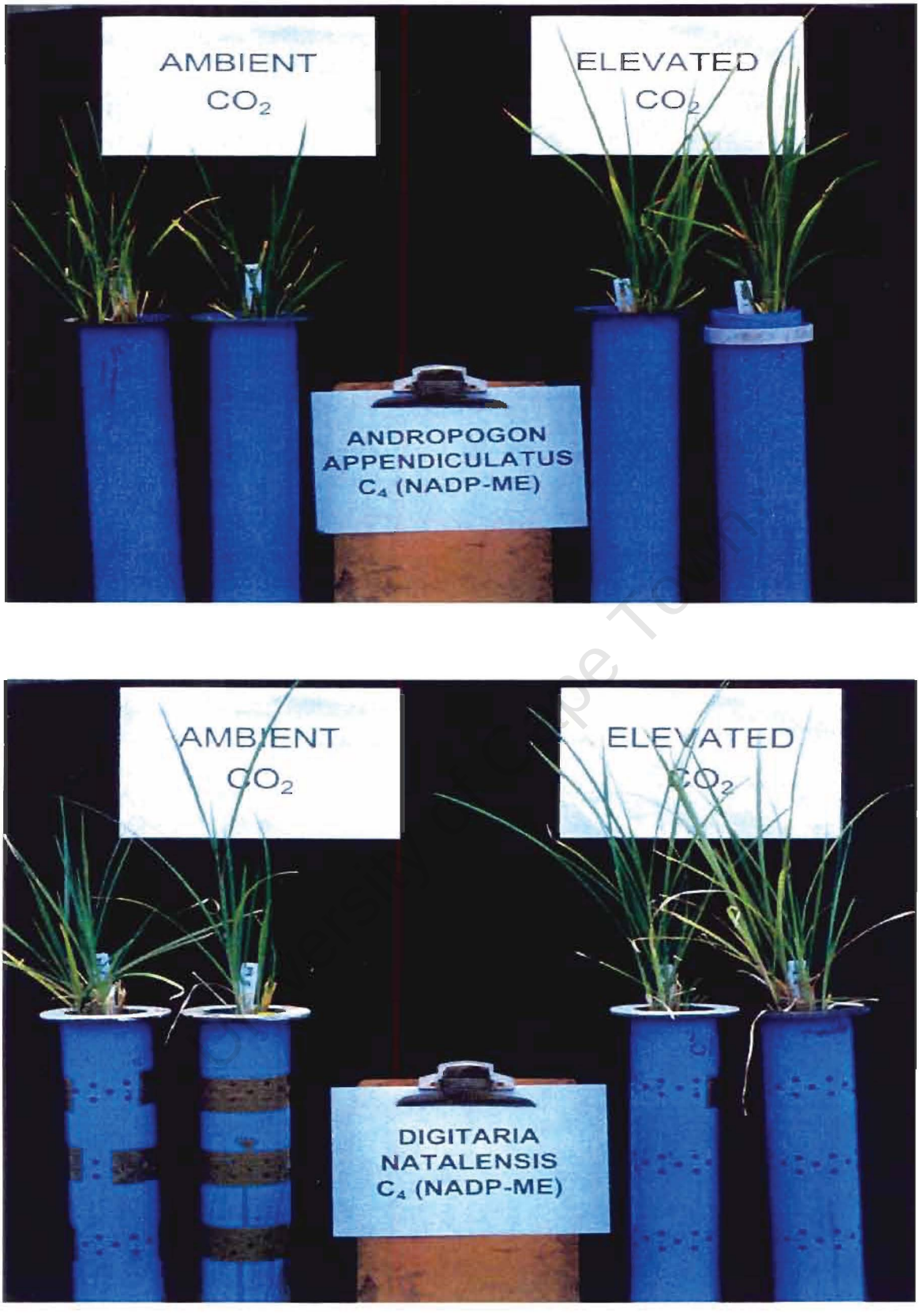


Fig. 7.9 *Andropogon appendiculatus* (top) and *Digitaria natalensis* (bottom) grown under ambient and elevated CO_2.

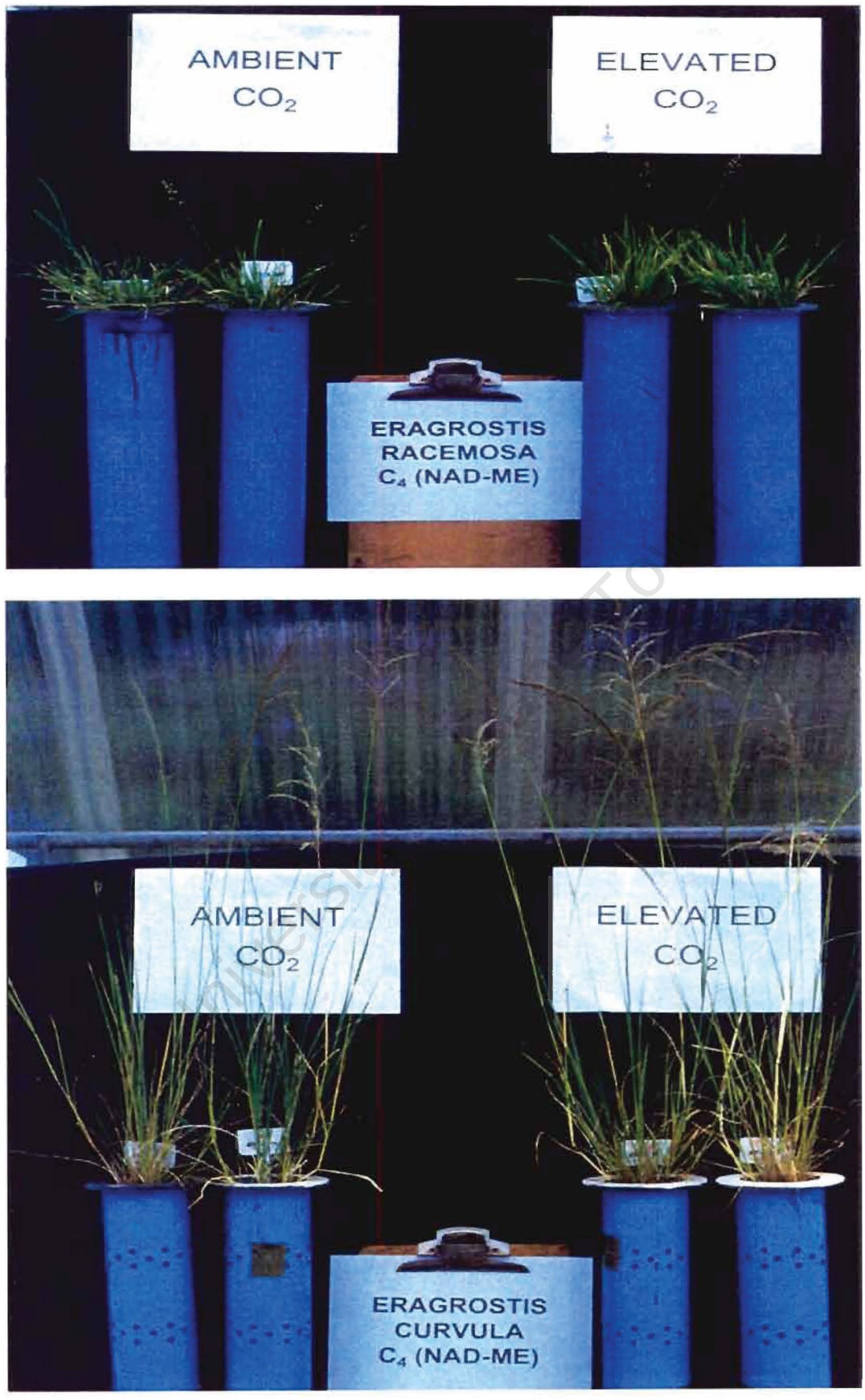


Fig. 7.10 *Eragrostis racemosa* (top) and *Eragrostis curvula* (bottom) grown under ambient and elevated CO_2.

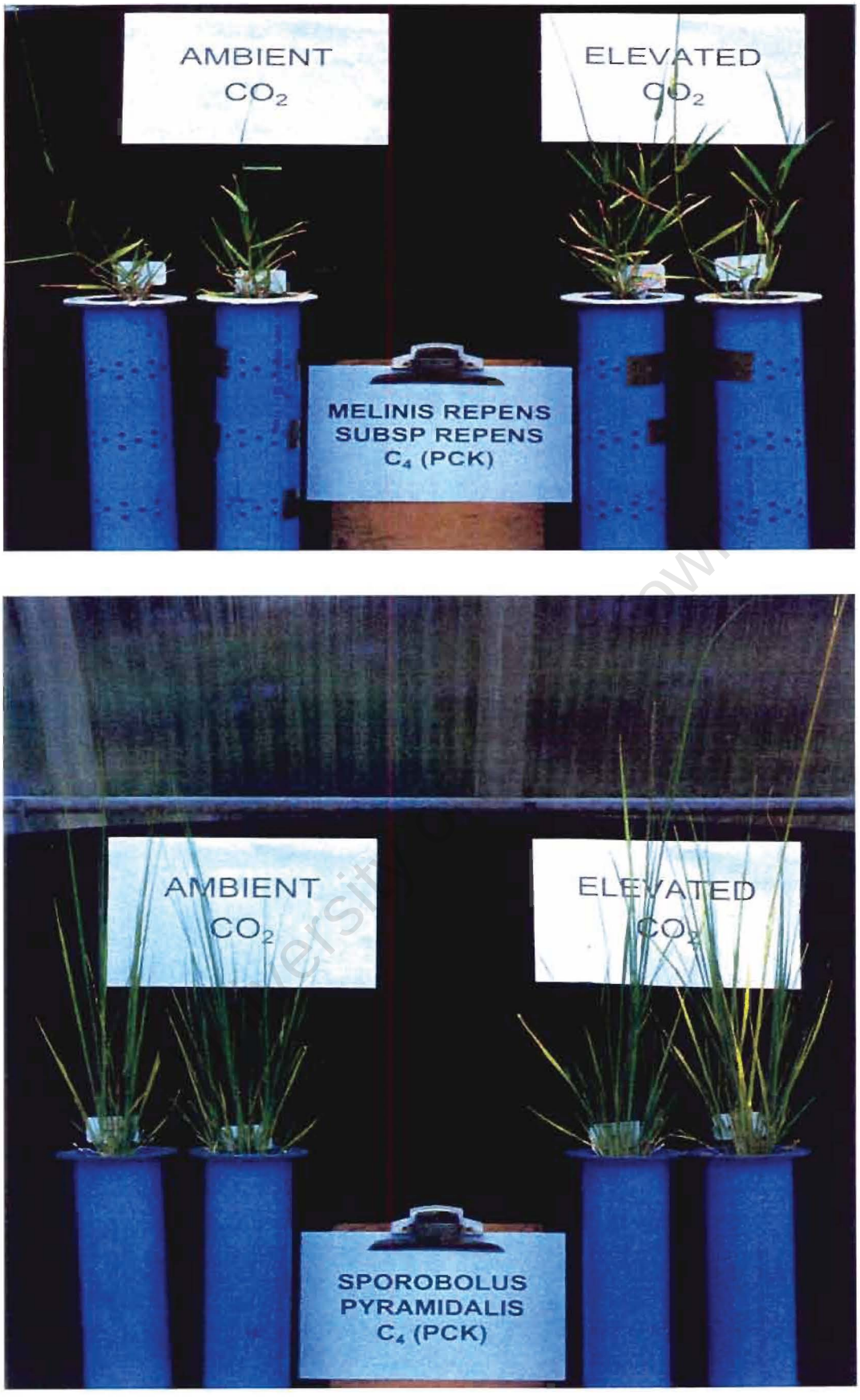


Fig. 7.11 *Melinis repens* subsp. *repens* (top) and *Sporobolus pyramidalis* (bottom) grown under ambient and elevated CO_2

Chapter 8

Seasonal gas exchange responses in C_4 and C_3 grass species grown in elevated CO_2 in the field

8.1 Introduction

Studies of the effects of elevated CO_2 on plants grown under controlled conditions e.g. in a greenhouse, are useful in gaining an initial understanding of species' potential responses to high CO_2. They also allow the study of interactive responses with other environmental conditions, such as increased UV-B radiation and defoliation, under otherwise constant environmental conditions of water, temperature etc. However, direct extrapolation of greenhouse-based results to the field is always tenuous owing to the confounding effects which may be introduced by factors such as sub-optimal nutrient and water supply, seasonal changes in climate and vegetation dynamics, and intra- and interspecific competition. Short-term measurements of gas exchange responses to elevated CO_2 may not reflect potential biochemical acclimation in the longer term in a natural, sub-optimal environment. These considerations have led to a concerted international effort over the last decade or so to establish longer term elevated CO_2 experiments in the field (Koch & Mooney 1996).

The prohibitive cost of establishing and running elevated CO_2 experiments in the field, using open-top chamber or FACE (Free Air CO_2 Enrichment) technology, has raised interest in the potential for studying vegetation around natural CO_2 springs (Raschi *et al.* 1997). These sites have the added advantage of having been exposed to high levels of CO_2 for long periods, often for decades or centuries. One such CO_2 spring in Italy has been extensively studied, and has shown that certain responses measured in greenhouses or growth chambers also occur in the field, although by no means all (Körner & Miglietta 1994; Miglietta *et al.* 1995). In the Arctic region, however, striking consistencies were found between data obtained for sub-Arctic graminaceous species at a long-term CO_2 spring and shorter-term elevated CO_2 experiments (Cook *et al.* 1998). Field CO_2 experiments have also highlighted the potential dependence of CO_2-responsiveness on seasonal changes in vegetation dynamics and soil properties, which could ultimately alter phenological patterns and species composition (Field *et al.* 1996). This may be particularly important in vegetation

University of Cape Town

types which exhibits strong seasonality of growth, such as sub-tropical grasslands with their typical wet-dry climates. South African grasses, particularly in the sourveld of the eastern regions, exhibit distinctive growth cycles, peaking in mid-summer (wet season) and senescing during late summer and autumn, when rainfall decreases. This annual cycle may alter the CO_2-responsiveness during the course of the growth season, and elevated CO_2 may even change the timing and duration of the growth season itself via its influence on water relations, for example (Field *et al.* 1997).

The existence of a strong and relatively pure CO_2 spring in Kwazulu/Natal, South Africa, gave South African botanists (W.D. Stock, University of Cape Town and scientists from the National Botanical Institute, Kirstenbosch) the opportunity to study the grassland vegetation nearby to ascertain whether any long-term effects of exposure to high CO_2 levels could be found. In addition, the gas could be harnessed to fumigate a plot of grassland at controlled double-ambient CO_2 concentrations. This experiment has allowed me to expand my study of grass responses to elevated CO_2 to a wider selection of species, and to test whether gas exchange responses obtained in previous greenhouse experiments could be corroborated in the field. Measurements were performed at three times during the course of a growth season in order to determine potential shifts in CO_2-responsiveness.

8.2 Materials and Methods

8.2.1 Site description

The field site is situated in southern Kwazulu-Natal, South Africa, between the towns of Port Shepstone and Harding (about 30°40'S 30°00'E). Natural CO_2 springs in the area around Bongwan were first described by geologists in the 1920's and by Gevers (1941). The CO_2 gas is probably formed by the reaction of acidified water from the upper soil layers draining onto underlying carbonate rocks at depth (Harris *et al.* 1997). The gas is emitted through a gas fault which extends for about 80 km, and is more than 97% pure CO_2, with trace amounts of other gases (Gevers 1941).

The field experiment was established around one such CO_2 vent on the farm Pleasant View, making use of an existing pipe sunk into the spring during the 1940's. This pipe emits about one ton of gas daily (Harris *et al.* 1997), but fluctuates somewhat depending on the

University of Cape Town

season. It is situated on a grassed hillside surrounded by sugar cane plantations. The grassland was used for cattle grazing prior to 1992 but has not been utilized since. It is burnt annually in winter as a fire precaution. The soil is of the Kroonstad formation, with a depth of about 20-50 cm, underlain by a seasonally waterlogged gley layer on bedrock. The loamy sand is acidic (pH 4.3) and leached, and of relatively low fertility (total N ~0.15% of soil dry mass). Soil water content varies seasonally between about 10-19%. The site has an altitude of about 650 m and the climate is sub-tropical, with an average annual rainfall of about 800 mm, most of which falls in summer. Although temperatures are only moderately warm (see climate data in Table 8.1), the grassland vegetation of the region is dominated by the C_4 photosynthetic type. Vegetative re-growth commences in spring after the annual burn, and reaches a peak during late summer, after which senescence sets in. There are marked differences in phenology between species, with *Alloteropsis semialata* subsp. *eckloniana* (C_3 grass), *Andropogon appendiculatus* (C_4 grass) and the geophytes and herbs showing strong early season growth following the burn, with the other C_4 grasses, including *Themeda triandra* and *Eragrostis racemosa*, showing maximal growth during the mid- to late-season (personal observation). These species were chosen for gas exchange measurements, and will hereafter be called by their generic names.

University of Cape Town

8.2.2 Controlled CO_2 fumigation

In April 1996 an initial survey of the site was conducted, including a characterisation of species composition, soil properties, plant and soil nutrient concentrations and plant carbon stable isotope (^{13}C) composition, along transects radiating out from the CO_2 source (Ludwig 1996, Stock *et al.* unpublished data). Three experimental plots (7 m x 7 m) were laid out on similar vegetation at distances of about 20 m (elevated CO_2 treatment plot), about 40 m (first control plot) and about 50 m (second control plot) from the source pipe. The CO_2 gas was harnessed from the source pipe using 10 cm diameter plastic irrigation piping which was buried around the perimeter of the treatment plot as well as in a 1.2 m x 1.2 m square in the middle of the plot. Copper rizers were inserted at regular intervals in the irrigation pipe and were positioned in such a way that the gas blows gently over the vegetation from all sides at a height of about 15 cm. The natural pressure of the spring gas was sufficient to achieve fumigation at the desired CO_2 concentration (650 μmol mol^{-1}), and any excess pressure was vented by means of a T-piece in the irrigation pipe regulated by a tap. This design ensured relatively constant fumigation over the entire plot, taking into account the prevailing winds at

the site (generally upslope during the day and downslope at night). Measurements of CO_2 concentration were carried out over the elevated CO_2 plot in a grid-wise fashion during the course of three days, using a LI-6200 IRGA (Li-Cor, Lincoln, Nebraska, USA) in combination with an anemometer to measure wind direction and speed. With a typical windspeed of 1 m s^{-1}, a CO_2 concentration of 600-800 µmol mol^{-1} was measured over 80% of the plot, with 4% of the area (in the center) having [CO_2] greater that 800 µmol mol^{-1} and 16% of the area (on the downwind edge of the plot) less than 600 µmol mol^{-1}. Similar measurements over the first control plot (closest to the treatment plot) revealed only minimal contamination of CO_2 due to rapid diffusion of CO_2 in air. The concentration over this plot was on average no more than 400 µmol mol^{-1}. The second control plot has been maintained but was not used in this study.

Owing to the difficulties of measuring CO_2 concentrations accurately in the field under highly variable conditions, and the need for a more integrated seasonal trace, the carbon stable isotope composition ($^{13}\delta C$) of the vegetation was determined after 12 months of fumigation. Using a formula which incorporates this value, as well as the CO_2 concentration and $^{13}\delta C$ value (-8.0 ‰) of the atmosphere, the $^{13}\delta C$ value of the spring gas (-0.8 ‰, Harris *et al.* 1997), and the carbon isotope discrimination of C_4 plants, the average CO_2 concentration over the plot during this period was calculated as 680 µmol mol^{-1} (W.D. Stock, personal communication). This agreed very well with the IRGA measurements. Nevertheless, it is recognised that further characterisation of the elevated CO_2 environment is required in order to understand the spatial and temporal variability of CO_2 over the fumigated plot, since the finer patterns of enrichment could influence relative plant responses.

Meteorological measurements were obtained from the South African Weather Bureau. Mean monthly minimum, maximum and highest temperature were obtained from the Paddock meteorological station (30°49'S 30°13'E, about 35 km east of the field site) for the period 1961-1990 (Table 8.1). Mean monthly rainfall was obtained from the Eureka meteorological station (which monitors only rainfall) (30°43'S 30°01'E, about 5 km from the field site) for the period 1936-1998.

University of Cape Town

Table 8.1 Mean monthly minimum, maximum and highest temperature at Paddock (1961-1990) and mean monthly rainfall at Eureka (1936-1998).

Month	Avg daily min. temp. (°C)	Avg daily max. temp. (°C)	Avg highest temp. (°C)	Rainfall (mm)
January	17.1	24.8	32.2	100.8
February	17.2	25.0	31.6	100.8
March	16.6	24.8	31.8	97.0
April	14.4	23.4	30.5	53.2
May	12.4	22.0	29.4	33.6
June	10.2	20.4	27.3	22.0
July	10.2	20.4	28.1	18.7
August	10.7	21.0	30.7	28.6
September	12.0	21.4	32.4	59.9
October	13.0	21.4	31.3	87.1
November	14.3	22.3	31.3	96.4
December	16.0	24.1	31.6	102.8
Whole year:	13.7	22.6		801.1

University of Cape Town

8.2.3 Photosynthetic gas exchange

Photosynthetic CO_2 response curves (A/c_i) were measured on individuals of one C_3 (*Alloteropsis*) and three C_4 grass species (*Themeda*, *Andropogon*, *Eragrostis*) using an LI-6400 IRGA (Li-Cor, Lincoln, Nebraska, USA). Measurements were made during the early season (August 1997, five weeks after burning), mid-season (December 1996) and late season (April 1997). For each species, 4-5 plants from the treatment plot (elevated CO_2) and first control plot (ambient CO_2) were measured, selected at various positions within the plots. A/c_i responses were determined at 6-9 different cuvette CO_2 concentrations ranging between 50-1000 µmol mol^{-1} (C_4 species) or between 100-1000 µmol mol^{-1} (C_3 species). Cuvette CO_2 concentrations were regulated using pressurised CO_2 canisters (soda chargers). Leaf temperatures were regulated at either 25°C (August) or 30°C (December and April), and light levels were kept constant at 1200 µmol m^{-2} s^{-1}, provided by an LED light source in the IRGA cuvette. Leaf-to-air vapour pressure difference (LAVPD) ranged between 0.8 and 2.2 kPa (August) and between 1.9 and 3.0 kPa (April), but never changed by more than 0.6 kPa for any individual A/c_i response curve.

After gas exchange, projected leaf areas were calculated using the lengths and breadths of the enclosed mid-leaf leaf sections (1-6 leaves depending on species). Response curves were fitted individually using non-linear regression (UNISTAT 3.0) and the monomolecular function $y=a(1-e^{b-cx})$ given by Causton and Dale (1990) (see Chapter 6, section 6.2.2). Transpiration and net CO_2 assimilation rates (A/E) were used to calculate instantaneous leaf water use efficiency (WUE).

Due to time constraints, only *Themeda* and *Alloteropsis* were measured in December (mid-season). In August (early season), *Eragrostis* could not be measured because the plants were still too small. All four species were measured in April (late season).

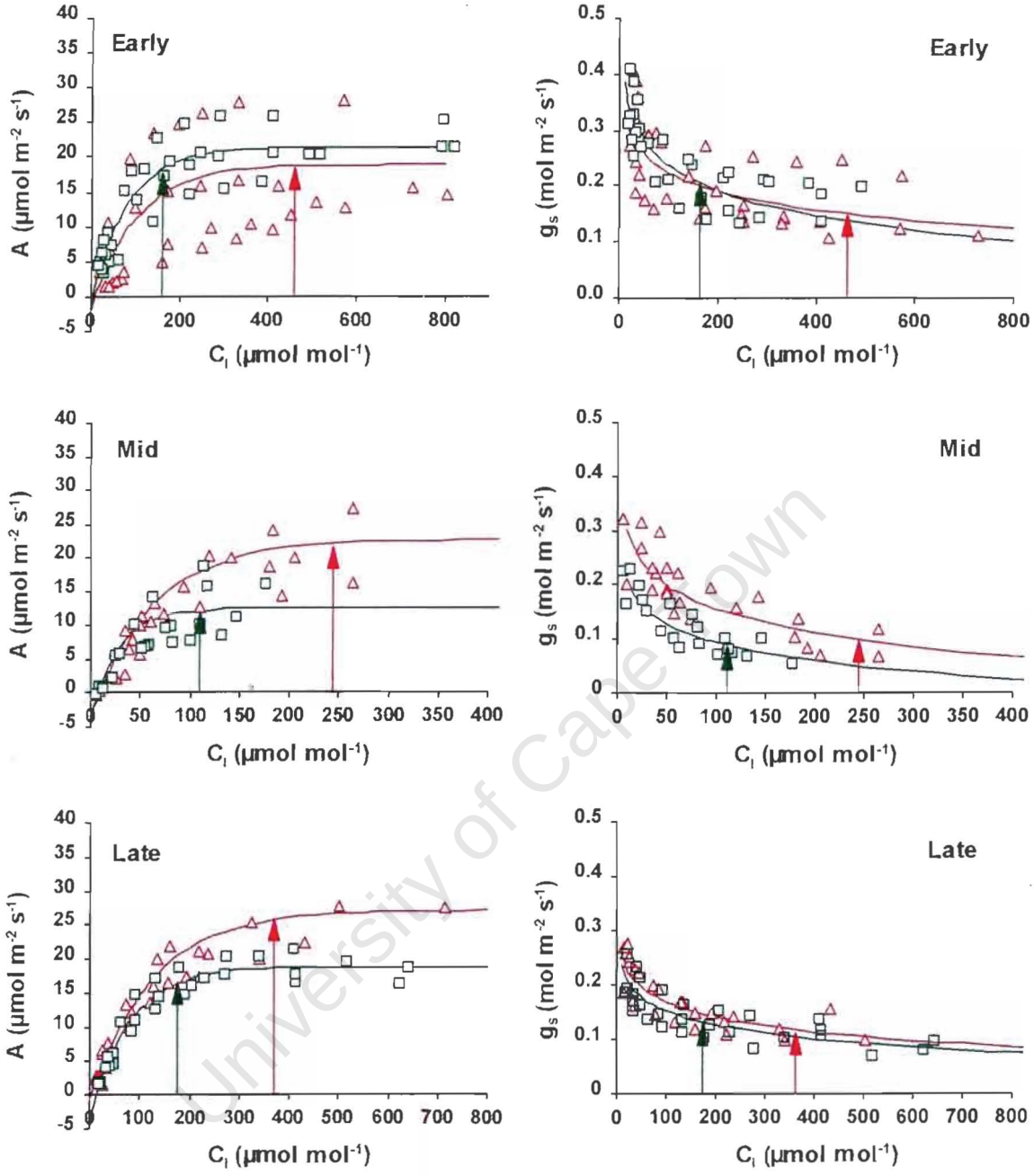
8.2.4 Statistical analysis

Gas exchange data were extracted from A/c_i response curves at both ambient (350 µmol mol^{-1}) and elevated (700 µmol mol^{-1}) cuvette CO_2 concentrations. These were analysed for each species at each measurement time (season) using two-way ANOVA with CO_2 treatment ($[CO_2]$trt) and cuvette CO_2 concentration ($[CO_2]$ms) as factors, in order to test both long- and short-term effects of elevated CO_2. Subsequently, only data measured at the plant's growth CO_2 concentration were used and the effects of season and $[CO_2]$ tested for each species using two-way ANOVA. Due to significant interaction between $[CO_2]$ and season, a one-way ANOVA was performed for seasons individually to ascertain the timing of the significant CO_2 response. A/c_i curve parameters were also subjected to two-way ANOVA with $[CO_2]$ and season as factors, as well as one-way ANOVA for each season.

University of Cape Town

8.3 Results

Seasonal responses of net CO_2 assimilation rate (A) and stomatal conductance (g_s) to intercellular CO_2 concentration (c_i) in the four species studied are presented in Fig. 8.1 (*Themeda*), Fig. 8.2 (*Alloteropsis*), Fig. 8.3 (*Andropogon*) and Fig. 8.4 (*Eragrostis*). Measured data points for all replicate curves are plotted, and the average of individually modelled response curves for each CO_2 treatment overlaid on the points. Gas exchange data for both ambient and elevated cuvette CO_2 concentrations are given in Table 8.2 and the corresponding statistical analysis is in Table 8.3. Comparisons of plants grown and measured at ambient CO_2 versus plants grown and measured at elevated CO_2 are illustrated in Fig. 8.5



University of Cape Town

Fig. 8.1 CO_2-responses of photosynthesis (A/c_i) and stomatal conductance (g_s/c_i) of *Themeda triandra* grown under ambient (green squares and polynomials) or elevated (red triangles and polynomials) CO_2 in the field, and measured during three seasons (early, mid- and late season). Symbols represent all data points and polynomials represent averages for individually modelled curves (A/c_i) or a logarithmic function (g_s/c_i). Arrows indicate A and g_s values at operational c_i.

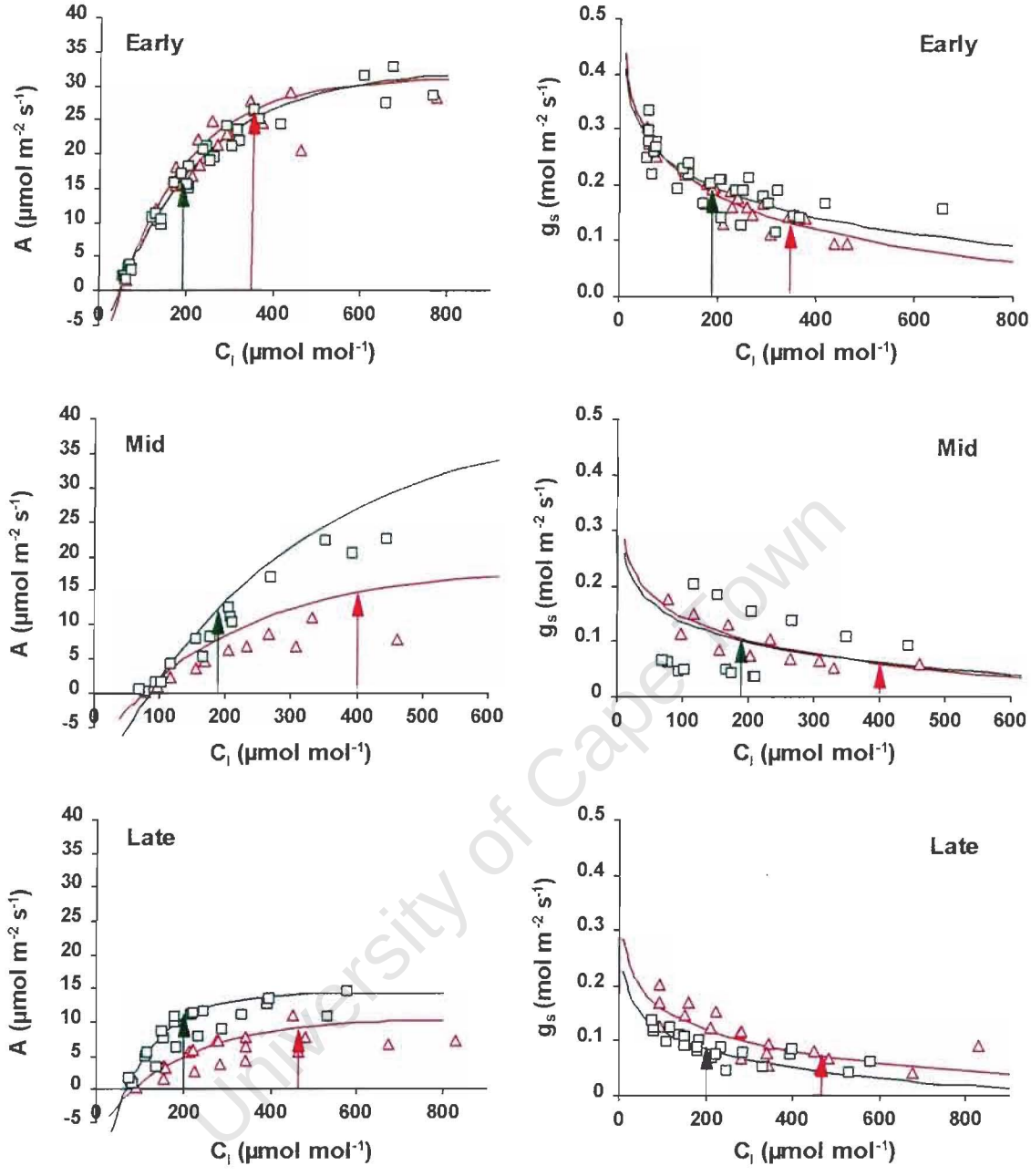


Fig. 8.2 CO_2-responses of photosynthesis (A/c_i) and stomatal conductance (g_s/c_i) of *Alloteropsis semialata* subsp *eckloniana* grown under ambient (green squares and polynomials) or elevated (red triangles and polynomials) CO_2 in the field, and measured during three seasons (early, mid- and late season). Symbols represent all data points and polynomials represent averages for individually modelled curves (A/c_i) or a logarithmic function (g_s/c_i). Arrows indicate A and g_s values at operational c_i.

University of Cape Town

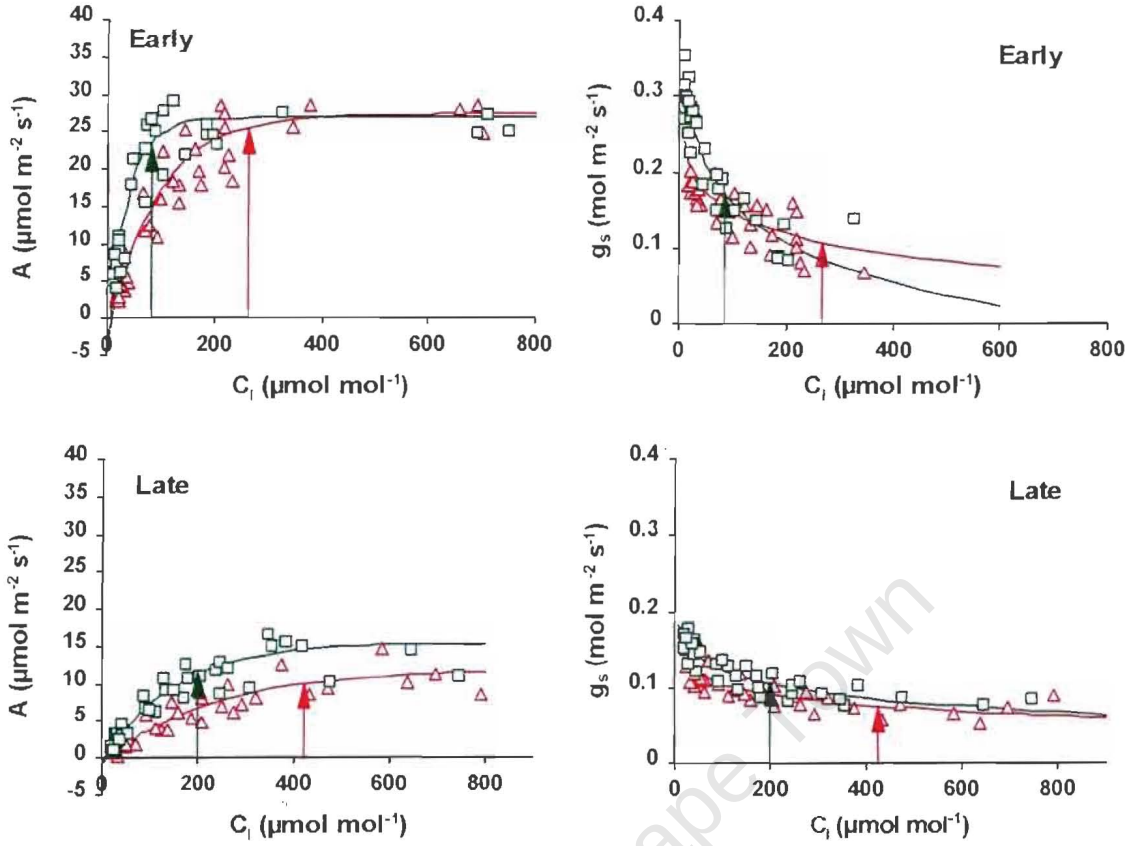


Fig. 8.3 CO_2-responses of photosynthesis (A/c_i) and stomatal conductance (g_s/c_i) of *Andropogon appendiculatus* grown under ambient (green squares and polynomials) or elevated (red triangles and polynomials) CO_2 in the field, and measured during two seasons (early and late season). Symbols represent all data points and polynomials represent averages for individually modelled curves (A/c_i) or a logarithmic function (g_s/c_i). Arrows indicate A and g_s values at operational c_i.

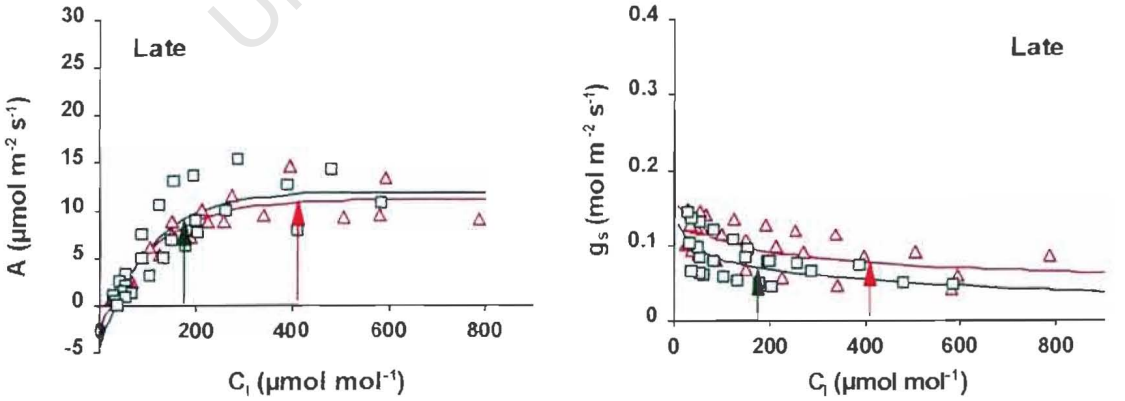


Fig. 8.4 CO_2-responses of photosynthesis (A/c_i) and stomatal conductance (g_s/c_i) of *Eragrostis racemosa* grown under ambient (green squares and polynomials) or elevated (red triangles and polynomials) CO_2 in the field, and measured during one season (late season). Symbols represent all data points and polynomials represent averages for individually modelled curves (A/c_i) or a logarithmic function (g_s/c_i). Arrows indicate A and g_s values at operational c_i.

University of Cape Town

Table 8.2 Gas exchange data for four grass species grown under elevated CO_2 in the field and measured during early season (August), mid-season (December) and late season (April). Abbreviations: A = net CO_2 assimilation rate; g_s = stomatal conductance; WUE = instantaneous water use efficiency. Data taken from A/c_i response curves at cuvette CO_2 concentrations of 350 µmol mol^{-1} and 700 µmol mol^{-1}. Values are means ± SE (n=4 for early season; n=1-3 for mid-season; n=2-4 for late season).

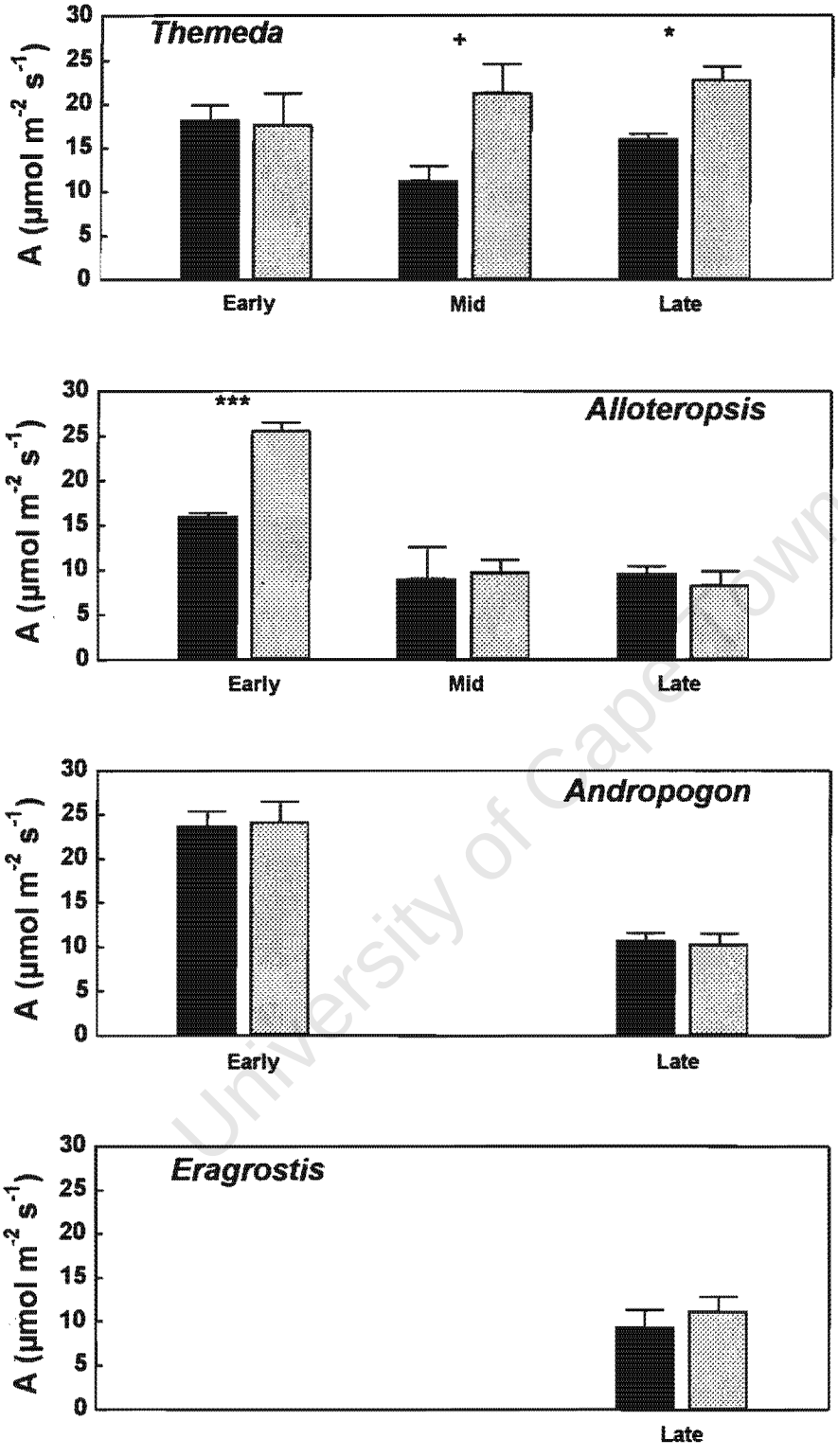
Parameter	Season	Growth $[CO_2]$: 360		650	
		Measurement $[CO_2]$: 360	650	360	650
A (µmol m^{-2} s^{-1}):					
Andropogon appendiculatus	Early	23.7 ± 1.7	25.3 ± 1.0	18.2 ± 1.6	24.1 ± 2.4
	Late	10.7 ± 0.9	14.4 ± 1.5	7.5 ± 0.5	10.2 ± 1.3
Themeda triandra	Early	18.1 ± 1.7	21.8 ± 1.4	13.9 ± 3.7	17.6 ± 3.5
	Mid	11.2 ± 1.7	16.0 ± 0	17.6 ± 2.5	21.2 ± 3.3
	Late	15.9 ± 0.6	19.5 ± 1.4	17.9 ± 1.0	22.6 ± 1.5
Eragrostis racemosa	Late	9.3 ± 2.0	13.9 ± 1.3	8.8 ± 0.8	11.1 ± 1.8
Alloteropsis semialata	Early	15.9 ± 0.5	24.8 ± 0.6	16.3 ± 0.6	25.5 ± 1.0
	Mid	9.0 ± 3.5	16.7 ± 5.6	6.7 ± 0.2	9.6 ± 1.5
	Late	9.5 ± 0.9	12.1 ± 0.8	6.1 ± 1.2	8.3 ± 1.6
g_s (mmol m^{-2} s^{-1}):					
Andropogon appendiculatus	Early	167 ± 11	99 ± 13	144 ± 12	109 ± 25
	Late	101 ± 7	92 ± 4	86 ± 4	70 ± 6
Themeda triandra	Early	200 ± 18	193 ± 25	193 ± 27	152 ± 24
	Mid	84 ± 10	53 ± 0	143 ± 26	84 ± 16
	Late	131 ± 12	116 ± 11	147 ± 14	126 ± 17
Eragrostis racemosa	Late	74 ± 13	69 ± 5	95 ± 16	76 ± 14
Alloteropsis semialata	Early	198 ± 10	141 ± 11	190 ± 8	136 ± 9
	Mid	101 ± 52	73 ± 36	95 ± 10	55 ± 3
	Late	87 ± 2	61 ± 12	99 ± 16	66 ± 10
WUE (µmol CO_2 $mmol^{-1}$ H_20):					
Andropogon appendiculatus	Early	7.6 ± 0.7	12.9 ± 2.0	7.5 ± 0.3	12.5 ± 1.1
	Late	4.1 ± 0.4	5.8 ± 0.5	3.1 ± 0.3	5.1 ± 0.3
Themeda triandra	Early	7.2 ± 1.6	8.7 ± 1.0	4.5 ± 0.8	6.8 ± 1.3
	Mid	4.7 ± 0.7	10.3 ± 0	4.7 ± 0.4	9.7 ± 0.7
	Late	4.7 ± 0.5	6.4 ± 0.5	5.6 ± 0.2	8.3 ± 0.9
Eragrostis racemosa	Late	4.6 ± 0.7	8.0 ± 2.1	3.8 ± 0.4	6.1 ± 1.0
Alloteropsis semialata	Early	5.1 ± 0.3	10.2 ± 0.8	5.1 ± 0.2	10.6 ± 0.5
	Mid	3.7 ± 0.3	11.1 ± 2.3	4.1 ± 0.4	7.8 ± 0.6
	Late	4.2 ± 0.5	7.4 ± 1.1	2.3 ± 0.2	4.6 ± 0.6

University of Cape Town

Table 8.3 Statistical significance of the effects of growth in elevated CO_2 ($[CO_2]$trt) in the field on gas exchange of individual grass species at different seasons, as calculated by two-way ANOVA. Abbreviations as for Table 8.2. Data taken from A/c_i response curves at cuvette CO_2 concentrations ($[CO_2]$ms) of 350 μmol mol^{-1} and 700 μmol mol^{-1}. Percentage changes in elevated CO_2 are given where differences are significant. +, $P \leq 0.10$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; NS, not significant.

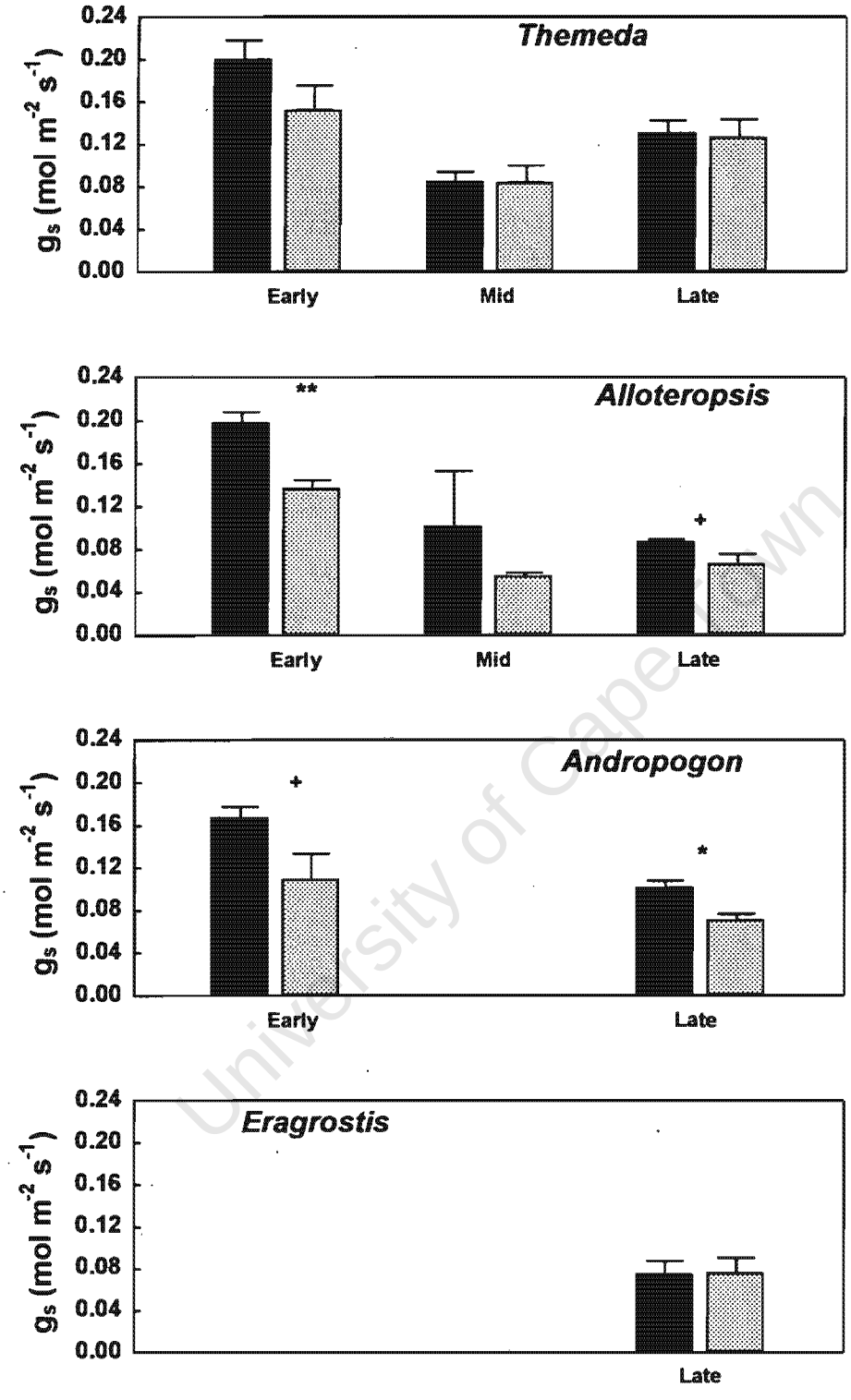
Species	Parameter	Season	Main effects		Interaction
			$[CO_2]$trt	$[CO_2]$ms	$[CO_2]$trt x $[CO_2]$ms
Andropogon appendiculatus (C_4)	A	Early	-14% +	+18% *	NS
		Late	-31% **	+38% *	NS
	g_s	Early	NS	-33% **	NS
		Late	-18% **	-12% *	NS
	WUE	Early	NS	+69% **	NS
		Late	-20% +	+51%***	NS
Themeda triandra (C_4)	A	Early	NS	NS	NS
		Mid	+56% +	NS	NS
		Late	+15% +	+24% **	NS
	g_s	Early	NS	NS	NS
		Mid	+49% +	-33% +	NS
		Late	NS	NS	NS
	WUE	Early	-29% +	NS	NS
		Mid	NS	+109% ***	NS
		Late	+25% *	+42% **	NS
Eragrostis racemosa (C_4)	A	Late	NS	+34% +	NS
	g_s	Late	NS	NS	NS
	WUE	Late	NS	+61% *	NS
Alloteropsis semialata subsp *eckloniana* (C_3)	A	Early	NS	+56%***	NS
		Mid	NS	NS	NS
		Late	-34% *	+30% +	NS
	g_s	Early	NS	-28% ***	NS
		Mid	NS	NS	NS
		Late	NS	-32% *	NS
	WUE	Early	NS	+105% ***	NS
		Mid	NS	+140%**	NS
		Late	-40% *	+84% **	NS

University of Cape Town



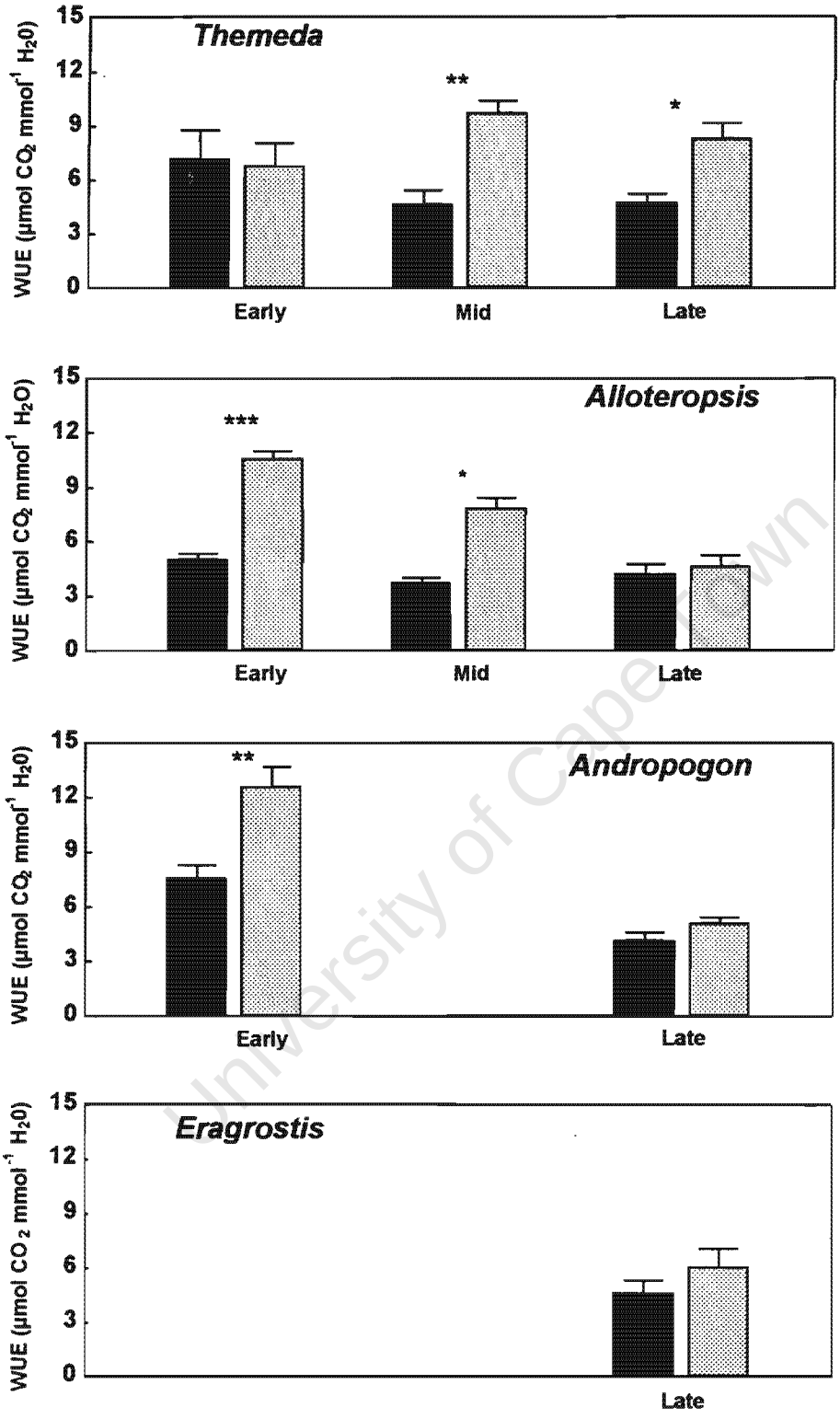
University of Cape Town

Fig. 8.5 Responses of net CO_2 assimilation rate (A) to elevated CO_2 in the field, as measured at growth CO_2 concentration, at three different seasons. +, $P \leq 0.10$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$, according to one-way ANOVA. Black bars = ambient CO_2, stippled bars = elevated CO_2.



University of Cape Town

Fig. 8.6 Responses of stomatal conductance (g_s) to elevated CO_2 in the field, as measured at growth CO_2 concentration, at three different seasons. +, P≤0.10; *, P≤0.05; **, P≤0.01; ***, P≤0.001, according to one-way ANOVA.



University of Cape Town

Fig. 8.7 Responses of instantaneous water use efficiency (WUE) to elevated CO_2 in the field, as measured at growth CO_2 concentration, at three different seasons. +, $P \leq 0.10$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$, according to one-way ANOVA.

(photosynthetic rate), Fig. 8.6 (stomatal conductance) and Fig. 8.7 (WUE), and the statistical analysis of $[CO_2]$ by seasonal effects is presented in Table 8.4.

Table 8.4 Statistical significance of the effects of elevated CO_2 on gas exchange of individual grass species in the field at different seasons, as calculated by two-way ANOVA. Abbreviations as for Table 8.2. Data taken from A/c_i response curves at cuvette CO_2 concentrations equivalent to growth CO_2 concentrations. Percentage changes in elevated CO_2 are given where differences are significant. +, P≤0.10; *, P≤0.05; **, P≤0.01; ***, P≤0.001; NS, not significant.

Species	Parameter	Main effects		Interaction
		$[CO_2]$	Season	$[CO_2]$ x Season
Andropogon appendiculatus (C_4)	A	NS	***	NS
	g_s	-31% *	**	NS
	WUE	+59% **	***	*
Themeda triandra (C_4)	A	+31% *	NS	NS
	g_s	NS	***	NS
	WUE	+42% *	NS	+
Alloteropsis semialata subsp *eckloniana* (C_3)	A	+33% **	***	**
	g_s	-32% **	***	NS
	WUE	+77% ***	***	***

University of Cape Town

In *Andropogon*, significant long-term reductions in photosynthetic capacity occurred when plants were grown in elevated CO_2, although elevated cuvette CO_2 concentrations increased photosynthetic rates in the short-term (Table 8.3). This resulted in similar carbon uptake rates when plants were compared at their respective growth CO_2 concentrations (Fig. 8.5, Table 8.4). Photosynthetic rates in *Themeda* were not affected by CO_2 during the early season, but significant long- and short-term stimulation occurred during the mid- to late season (Table 8.3), leading to higher carbon uptake rates when compared at the growth CO_2 concentration (Fig. 8.5, Table 8.4). *Alloteropsis* showed strong positive photosynthetic responses to elevated cuvette CO_2 concentration during the early season, and a smaller response during the late season (Table 8.3). However, the latter was accompanied by long-term decreases in photosynthetic capacity. There was no net carbon gain during the mid- and late-season when comparisons were made at the growth CO_2 concentration in contrast with the early season (Fig. 8.5, Table 8.4). Photosynthetic rates increased at high cuvette CO_2 levels in *Eragrostis* (Table 8.3), but no significant differences were found between ambient-

and elevated CO_2-grown plants when compared at growth CO_2 concentrations (Fig. 8.5, Table 8.4).

Stomatal conductances in *Andropogon* always decreased significantly in response to short-term CO_2 elevation, and there was added downregulation of conductance in the long-term towards the end of the season (Table 8.3). At growth [CO_2], conductances were, therefore, always significantly lower in elevated CO_2 (Fig. 8.6, Table 8.4). Apart from some variable long-term increases and short-term decreases in conductance in *Themeda* during the mid-season, this species showed no significant responses to elevated CO_2 (Table 8.3), so that no differences in conductance were found at growth [CO_2] (Fig. 8.6, Table 8.4). Conductances in *Alloteropsis* decreased in response to elevated [CO_2] throughout the season (Fig. 8.6, Table 8.4), but only significantly so during early and late season measurements. This was purely a short-term response (Table 8.3). *Eragrostis* showed no responses in conductance to elevated CO_2.

Decreased transpiration rates (data not shown) in elevated CO_2 led to increased instantaneous leaf water use efficiency (WUE) in *Andropogon* only during the early season (Fig. 8.7, Table 8.4), owing to long-term down-regulation in elevated CO_2 towards the end of the season (Table 8.3). WUE in *Themeda* was increased significantly in elevated CO_2 during the mid- to end-season as a result of increased carbon uptake rates (Tables 8.3 and 8.4; Fig. 8.7), but not during the early season. In *Alloteropsis*, WUE was strongly increased in elevated CO_2 throughout the season, as a result of both increases in carbon uptake rate and decreases in transpiration rate (Fig. 8.7, Table 8.4). This was more pronounced during the early season than later, due to long-term photosynthetic downregulation in elevated CO_2 towards the end (Table 8.3). *Eragrostis* showed no increases in WUE during the late season when compared at the growth [CO_2] (Fig. 8.7, Table 8.4).

Gas exchange capabilities changed significantly through the season in all species (Figs 8.5-8.7, Table 8.4). *Themeda* was the least variable and showed no significant changes in photosynthetic rates or WUE between seasons, but lower conductances during mid-season than during early- or late-season, and highest conductances during early season (according to a Student-Newman-Keuls Multiple Range Test $P \leq 0.05$). Both *Andropogon* and *Alloteropsis* had significantly lower photosynthetic rates and conductances during mid- and late-season, compared to early-season.

University of Cape Town

A/c_i curve parameters also showed seasonal changes both in absolute terms and in their responses to elevated CO_2 (Tables 8.5 and 8.6). CO_2- and light-saturated net CO_2 assimilation rate (J_e) decreased in *Andropogon* and *Alloteropsis* towards the end of the season. The CO_2 compensation point (Γ_c) was lower in *Alloteropsis* during early-season than later in the season. In *Andropogon*, the apparent carboxylation efficiency (k) decreased substantially from early- to late-season. The effects of elevated CO_2 during growth on A/c_i curve parameters were species-specific and dependent on season (Tables 8.5 and 8.6). J_e was significantly increased in *Themeda* during late-season, but also (non-significantly) during mid-season. In *Alloteropsis*, J_e was significantly decreased in elevated CO_2 when analysed over the whole season, but particularly from mid- to late-season, although significance was not achieved for individual seasons (Table 8.5). In *Themeda*, Γ_c decreased significantly in elevated CO_2 during late-season. *Andropogon* showed large reductions in k in elevated CO_2, particularly during the early season. This had the effect of increasing Γ_c slightly.

8.4 Discussion

These data clearly show the important influence of season on gas exchange characteristics in grasses, and how this interacts with altered CO_2 concentrations. The observed annual succession of grass re-growth following the burn (personal observation) went hand in hand with seasonally changing photosynthetic capacities. The early-season dominants *Alloteropsis* and *Andropogon* had highest CO_2 assimilation rates, stomatal conductances and leaf water use efficiencies during the early season. By contrast, *Themeda*, which starts off more slowly and achieves greater dominance during the mid- to late-season, had similar assimilation rates throughout the season, although conductances were lower during the mid-season, probably owing to drought stress (G.F. Midgley, unpublished soil moisture data). The level of CO_2-responsiveness was closely related to these seasonal changes in activity, and was generally highest during each species' period of greatest assimilation and growth, at least with respect to photosynthesis and leaf water use efficiency. Although there was no interaction between CO_2 treatment and season in *Themeda* (Table 8.4), there was a strong trend towards enhanced CO_2 assimilation under elevated CO_2 during the mid- to late-season (Fig. 8.5). Conversely, *Alloteropsis* had enhanced assimilation rates in elevated CO_2 only during the early season. Similar patterns were observed for leaf water use efficiency, with *Andropogon* also showing a significant CO_2 effect only during the early season. Poorter (1993) and Poorter *et al.* (1996)

University of Cape Town

Table 8.5 Photosynthetic CO_2-response curve (A/c_i) parameters for four grass species grown under elevated CO_2 in the field and measured during early season (August), mid-season (December) and late season (April). Abbreviations: J_e, light- and CO_2-saturated net CO_2 assimilation rate; k, apparent carboxylation efficiency; Γ_c, CO_2 compensation point. Values are means ± SE (n=4 for early season; n=1-3 for mid-season; n=2-4 for late season). Statistical significances calculated by one-way ANOVA. +, P≤0.10; *, P≤0.05; **, P≤0.01; NS, not significant.

Parameter	Season	Ambient CO_2	Elevated CO_2	Sign.
J_e (μmol m^{-2} s^{-1}):				
Andropogon appendiculatus	Early	26.8 ± 0.8	27.4 ± 1.1	NS
	Late	15.4 ± 1.8	11.8 ± 1.5	NS
Themeda triandra	Early	21.4 ± 1.7	19.0 ± 3.1	NS
	Mid	12.4 ± 2.8	22.7 ± 4.7	NS
	Late	18.8 ± 0.9	27.1 ± 1.3	**
Eragrostis racemosa	Late	11.9 ± 1.9	11.2 ± 1.6	NS
Alloteropsis semialata	Early	32.4 ± 1.8	31.3 ± 3.5	NS
	Mid	40.2 ± 13.0	18.3 ± 7.2	NS
	Late	14.3 ± 1.9	10.6 ± 2.6	NS
Γ_c (μmol mol^{-1}):				
Andropogon appendiculatus	Early	5.34 ± 0.46	8.79 ± 1.08	*
	Late	7.22 ± 2.91	9.70 ± 5.43	NS
Themeda triandra	Early	4.57 ± 1.14	9.09 ± 3.65	NS
	Mid	9.0 ± 1.6	11.2 ± 3.5	NS
	Late	14.2 ± 0.8	1.83 ± 1.83	**
Eragrostis racemosa	Late	33.5 ± 4.9	24.9 ± 2.5	NS
Alloteropsis semialata	Early	46.7 ± 2.0	49.3 ± 1.1	NS
	Mid	80.6 ± 8.0	75.2 ± 1.0	NS
	Late	70.1 ± 3.2	82.4 ± 3.9	+
k (μmol CO_2 μmol^{-1} c_i):				
Andropogon appendiculatus	Early	0.885 ± 0.194	0.329 ± 0.082	*
	Late	0.113 ± 0.021	0.057 ± 0.011	+
Themeda triandra	Early	0.297 ± 0.072	0.238 ± 0.113	NS
	Mid	0.591 ± 0.160	0.371 ± 0.033	NS
	Late	0.294 ± 0.061	0.220 ± 0.047	NS
Eragrostis racemosa	Late	0.171 ± 0.047	0.125 ± 0.010	NS
Alloteropsis semialata	Early	0.196 ± 0.024	0.252 ± 0.039	NS
	Mid	0.169 ± 0.079	0.099 ± 0.029	NS
	Late	0.338 ± 0.157	0.103 ± 0.057	NS

University of Cape Town

showed that fast-growing species have a greater CO_2-response than slow-growing species in short-term experiments, and this may also be applicable to seasonal changes in growth rate within the same plant/species. However, responses of stomatal conductance to elevated CO_2 were more consistent throughout the growth season, with *Themeda* showing no significant responses, but *Andropogon* and *Alloteropsis* having reduced conductances of, on average, 31-32%, throughout the season. *Eragrostis*, which develops most strongly during the mid- to late-season, did show short-term increases in assimilation rates and WUE in elevated CO_2 during the late season, but these were not significant when plants were compared at their respective growth CO_2 concentrations. These results support the plea made by Körner (1995) and Loehle (1995) for a recognition that timing of measurements is important due to temporal changes in the vigour of growth and the developmental speed of the system under investigation, which can affect the relative CO_2-responsiveness. At any one time, faster-growing species will out-top the slower-growing ones and their CO_2-response will be larger, as growth and development is not synchronized among species. These differences can ultimately lead to changes in population dynamics (Reekie & Bazzaz 1991; Morse & Bazzaz 1994).

Table 8.6 Statistical significance of the effects of growth in elevated CO_2 in the field on photosynthetic CO_2-response (A/c_i) parameters of individual grass species at different seasons, as calculated by two-way ANOVA. Percentage changes in elevated CO_2 are given where differences are significant. Abbreviations as for Table 8.5. +, $P \leq 0.10$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; NS, not significant.

Species	Parameter	Main effects		Interaction
		$[CO_2]$	Season	$[CO_2]$ x Season
Andropogon appendiculatus (C_4)	J_e	NS	***	NS
	Γ_c	NS	NS	NS
	k	-61% *	***	*
Themeda triandra (C_4)	J_e	+26% +	NS	+
	Γ_c	NS	NS	**
	k	NS	+	NS
Alloteropsis semialata subsp *eckloniana* (C_3)	J_e	-27% +	*	NS
	Γ_c	+8% +	***	*
	k	NS	NS	NS

University of Cape Town

Longer-term photosynthetic acclimation to elevated CO_2 differed between species. *Andropogon* exhibited early downregulation of photosynthetic capacity in elevated CO_2, which persisted and became greater as the season progressed. This was associated with reduced apparent carboxylation capacity, an indicator of the capacity of PEP-carboxylase in the initial photosynthetic reaction. Stomatal conductance was also significantly downregulated in high CO_2 towards the end of the season. *Alloteropsis* also downregulated its photosynthetic capacity towards the end of the growth season, owing to a similar reduction in apparent carboxylation capacity, although not statistically significant. In a study of plant responses to long-term elevated CO_2 near a natural spring in Iceland, the dominant C_3 grass species *Nardus stricta* also exhibited strong downregulation of the photosynthetic apparatus, including reductions in Rubisco content and Rubisco activity and activation state (Cook *et al.* 1998). At Bongwan, *Eragrostis* showed no long-term acclimatory effects of elevated CO_2, but photosynthetic capacity in *Themeda* was strongly upregulated, a rather unusual phenomenon (Wullschleger 1993). This effect was measured from the mid- to late-season and led to a significant increase in J_e. Stomatal upregulation was measured during the mid-season, and may have been in response to the drought stress experienced during this time. Bazzaz & Miao (1993) found that growth enhancement of early-successional tree seedlings in elevated CO_2 was more sensitive to nutrient levels, while in the late-successional species the enhancement was more sensitive to light levels. Differing nutrient (especially nitrogen) availabilities or competition between plants for nutrients, may have played a role in photosynthetic downregulation in the early-season *Andropogon* and *Alloteropsis*. The burst of nitrogen availability following the fire would allow for a positive CO_2-response only for a short period, until competition from other species would reduce the availability of nitrogen, resulting in the downregulation of photosynthetic capacity.

A comparison between gas exchange responses measured in the field experiment and those measured in the greenhouse experiment (Chapter 7) provides interesting contrasts (Table 8.7).

University of Cape Town

Table 8.7 Comparison of net CO_2 assimilation rates ($\mu mol\ m^{-2}\ s^{-1}$) measured at growth CO_2 concentration (taken from Table 7.6 and Table 8.2). * $P \leq 0.05$, *** $P \leq 0.001$, NS not significant according to one-way ANOVA.

	Greenhouse			Field			
Species	Amb. CO_2	Elev. CO_2	% Change	Season	Amb. CO_2	Elev. CO_2	% Change
Andropogon	23.8	26.4	+11% NS	early	23.7	24.1	+2% NS
				late	10.7	10.2	-5% NS
Themeda	14.0	9.8	-30% NS	early	18.1	17.6	-3% NS
				late	15.9	22.6	+42% *
Eragrostis racemosa	11.8	13.8	+17% NS	late	9.3	11.1	+19% NS
Alloteropsis	13.9	14.7	+6% NS	early	15.9	25.5	+60% ***
				late	9.5	8.3	-13% NS

Absolute rates of CO_2 assimilation were relatively similar between the greenhouse and field. In general, the assimilation rates measured in the greenhouse on actively growing plants during mid-summer reflected those measured in the field during the earlier part of the growth period. However, responses to elevated CO_2 differed between the greenhouse and field studies. *Andropogon* showed no photosynthetic or stomatal downregulation, and *Themeda* showed no photosynthetic or stomatal upregulation in the greenhouse, in contrast with the field. Only the responses to elevated CO_2 of *Eragrostis* (no response) and *Alloteropsis* (photosynthetic downregulation) were similar in the greenhouse and field. It appears that *Andropogon* was sensitive to limitations experienced in the field which were not evident in the greenhouse, and this species may be particularly sensitive to competition for nutrients from an early developmental stage.

Photosynthetic upregulation in *Themeda* was also measured in the greenhouse in Experiment 3 (Chapter 6) in young, active plants, but this changed to downregulation in mature plants, possibly due to canopy closure and light limitation. In the field, *Themeda* plants were perhaps not yet of a similar seasonal maturity as in the greenhouse, or not subject to any limitations experienced in the greenhouse, such as reduced light levels, and therefore did not lose the higher photosynthetic capacity in elevated CO_2 as the season progressed. Alternatively, the slightly lower nitrogen availabilities in the field may have resulted in a

University of Cape Town

greater relative CO_2-induced improvement in nitrogen use efficiency, which could account for photosynthetic upregulation.

It could be expected that the reduced conductances and transpiration rates measured in elevated CO_2 in the field could lead to improved soil water relations, as has been measured in a number of field experiments worldwide (Bremer *et al.* 1996; Owensby *et al.* 1997; Field *et al.* 1997). Field and his co-workers (Field *et al.* 1996 and Field *et al.* 1997) showed that increased soil water content towards the end of the growth season under elevated CO_2 stimulated continued growth of plants, when plants growing in the control plots were already senescing. A similar effect in the Bongwan field site under elevated CO_2 may also have benefited some of the late-season grasses, possibly contributing to the sustained photosynthetic upregulation measured in *Themeda*. This would have been particularly evident during the mid-season drought stress, when plants growing on the elevated CO_2 plot showed greater inherent stomatal conductances than those on the drier ambient CO_2 plot.

This study has confirmed that enhanced CO_2 assimilation occurs in some species, and reduced conductances and transpirational water loss occurs in most measured species when grown in elevated CO_2 in the field, and corroborates the results obtained for these species in the greenhouse experiment. In addition, it has highlighted the close dependence of CO_2-responsiveness on season and changing resources, a result which should be taken into account when attempting to predict vegetation dynamics and longer term effects of elevated CO_2 on natural plant communities (Körner 1995).

University of Cape Town

Chapter 9

Photosynthetic temperature responses in C_4 and C_3 grass species grown in elevated CO_2

9.1 Introduction

In previous chapters it was shown that one of the most consistent and characteristic responses of some C_4 grasses to elevated atmospheric CO_2 is the stimulation of leaf length and individual leaf area, leading to an increase in canopy leaf area and biomass. Some of the possible physiological mechanisms that could account for this stimulation, such as increased carbon assimilation rates in young leaves and improved leaf water potentials, have been discussed. Another possible mechanism, which has received little attention thus far in the literature, is the stimulation of leaf extension by the increased leaf temperatures brought about by stomatal closure under elevated CO_2. Increases in leaf and canopy temperature resulting from reduced transpiration are an established physiological phenomenon based on physical laws of heat exchange, and are routinely incorporated into leaf energy balance calculations (Nobel 1991). The suggestion that leaf temperature increases occur under elevated CO_2 has been sporadically brought up (e.g. Idso *et al.* 1987, Bowes 1993), but has received little attention in the literature. Where interaction between elevated CO_2 and increasing temperature has been addressed, this has usually focussed on controlled leaf temperature elevation treatments, applied factorially with CO_2 treatments (e.g. Potvin & Strain 1985a, b, Read *et al.* 1997, Nijs *et al.* 1997). In general, the relative growth enhancement in elevated CO_2 increases with increasing air temperature during growth (Drake & Leadley 1991, McMurtrie & Wang 1993, Idso & Idso 1994). However, the indirect increase in leaf temperature could also affect the response to elevated CO_2 (Long 1991).

Idso *et al.* (1993) showed that, for sour orange trees, water hyacinth and cotton, leaf temperature responses to elevated CO_2 were directly proportional to the degree of stomatal closure i.e. greater leaf warming was found where stomatal closure was greatest. From first principles, Long (1991) calculated that a 30% decrease in stomatal conductance under elevated CO_2 would translate to an increase in leaf temperature of ca. 1°C (in full sunlight, 25°C, LAVPD=0.5 kPa). A similar calculation by Hogan *et al.* (1991) yielded a leaf

University of Cape Town

temperature increase of 1-2°C. In Chapter 2 it was shown that C_4 grasses generally experience substantial stomatal closure (29% reduction) in high CO_2. This was subsequently confirmed for a number of southern African C_4 grass species (Chapters 4, 7, 8). Grassland communities exposed to elevated CO_2 experience significant reductions in canopy evapotranspiration (Kirkham *et al.* 1993, Bremer *et al.* 1996), sometimes more so in C_4- than in C_3-dominated communities (Drake *et al.* 1997). In the tallgrass prairie of North America, this resulted in increased canopy temperatures of 0.8-1.7°C (Kirkham *et al.* 1993). Idso *et al.* (1987) showed that midday leaf temperatures in cotton plants increased by 1.1°C in elevated CO_2. Leaf growth in grasses is strongly related to temperature, so that total growth depends linearly on the number of degree-days, a cumulative measure of thermal time (Gallagher 1979). Clifton-Brown & Jones (1997) reported that in different genotypes of the perennial C_4 grass, *Miscanthus*, plant extension rate (accounted for mainly by leaf extension) was highly significantly related to plant temperature. It is thus possible that elevated CO_2 could stimulate leaf extension in grasses indirectly via increases in leaf temperature.

The most direct method of recording such an effect is the instantaneous measurement of leaf and canopy temperature under ambient and elevated CO_2. Alternatively, the photosynthetic response to increasing leaf temperature can provide an indirect indication of longer-term average leaf and canopy temperatures. Many plant species have the capacity to acclimate to changing air and leaf temperatures (Berry & Björkman 1980, Fitter & Hay 1987), so that their photosynthetic capacities at different temperatures reflect the thermal environment experienced during growth. The most commonly used index of thermal acclimation is the leaf temperature at which optimal (maximal) net CO_2 assimilation takes place, the so-called photosynthetic temperature optimum, or T_{opt}. This optimal temperature is influenced by both genetic and environmental factors. C_4 species generally possess higher T_{opt} at normal concentrations of CO_2 than C_3 species (Berry & Björkman 1980, Monson *et al.* 1983), due to the effect of increasing photorespiration with increasing temperature in C_3 plants (see Chapter 1). These differential temperature responses of photosynthesis are partly the reason for the temporal displacement of growth in mixed C_3/C_4 grasslands, with C_3 grasses dominating during the cooler seasons and C_4 grasses dominating during the warmer seasons (Williams 1974, Kemp & Williams 1980). However, within individual plants, there is often a tendency for T_{opt} to "track" changes in the plant's thermal environment, often reflecting the mean maximum temperature (Slatyer & Morrow 1977) or the mean daily

University of Cape Town

temperature (Battaglia *et al.* 1996) of the week or two prior to the date of measurement. Plants from habitats with large temperature variations during the growth season tend to possess a greater potential for this phenological type of acclimation over a wider temperature range than do plants with relatively stable temperatures during the growth season. For four C_3 and C_4 grass and sedge species from the temperate short-grass prairie in Colorado, Monson *et al.* (1983) measured upward adjustment in T_{opt} in all species when plants were grown at 30°C compared to 25°C. The two C_4 grasses showed T_{opt} of 42°C and 40°C at the warm growth temperature, respectively, compared to 40°C and 35°C at the cooler growth temperature.

Elevated CO_2 also exerts a direct effect on the photosynthetic temperature response of plants, particularly those with the C_3 photosynthetic mechanism (Berry & Björkman 1980). The temperature response is influenced by the ratio of CO_2 to O_2 concentrations during measurement. Oxygenation of RuBP (photorespiration) is more sensitive to leaf temperature increases than the carboxylation reaction i.e. the rate of oxygenation increases relatively faster at higher temperature. However, at elevated CO_2 concentrations, photorespiration is repressed, and the photosynthetic temperature optimum shifts to a higher temperature (Long 1991). For C_3 plants, this increase in T_{opt} relative to that measured under ambient CO_2 can amount to about 3°C (at 500 μmol mol^{-1} CO_2) and 5°C (at 650 μmol mol^{-1} CO_2) (Long 1991). It has generally been thought that this effect is restricted to C_3 plants. However, Chen *et al.* (1994, 1996), using a combination of modelling and empirical measurement, found an upward shift in T_{opt} in elevated CO_2 in both a C_3 (3-5°C) and a C_4 grass (1-2°C).

In this chapter, the photosynthetic temperature responses of various C_3 and C_4 grass and shrub species of differing origin (differing climatic regimes) and grown in elevated CO_2 during the course of three different experiments, are presented. Measurements of leaf and canopy temperature were also measured *in situ* during one experiment. The objectives were two-fold: firstly, to determine how well these species adjust to their growth environment i.e. whether T_{opt} reflects the temperature regimes experienced during the experiments; secondly, to determine whether growth in elevated CO_2 alters the leaf thermal environment, and whether this potential indirect effect of high CO_2, or the possible direct effect of high CO_2 on photosynthetic temperature responses leads to any acclimatory shifts in T_{opt}.

Stomatal responses to leaf temperature increases (externally imposed) in plants grown and measured under either ambient or elevated CO_2, and the resulting effects on transpiration,

University of Cape Town

are also presented. Increases in leaf-to-air vapour pressure difference (LAVPD) which accompany leaf temperature increases under constant air vapour pressure, can result in stomatal closure (Farquhar & Sharkey 1982, Schulze 1986), and could interfere with any responses to elevated CO_2 or temperature *per se* (Bunce 1993, 1998, Will & Teskey 1997). Due to technical constraints, particularly at the high temperatures (>35°C) frequently required for the C_4 grasses, it was not possible to keep LAVPD constant during the temperature response measurements. In order to characterize the potential influence of increasing LAVPD on the temperature response, particularly with respect to stomatal conductance, gas exchange responses to increasing LAVPD at constant leaf temperature were determined during the last experiment (Exp. 4, using the technically advanced LI-6400 Photosynthesis System).

9.2 Materials and Methods

Photosynthetic temperature response curves were determined during the course of three experiments: **Experiment 1** (*Acacia karroo* and *Themeda triandra* ecotype 1 [Döhne], description of experimental methods in Chapter 4), **Experiment 2** (*Themeda triandra* ecotype 2 [Kruger National Park], description of experimental methods in Appendix 4), and **Experiment 4** (*Themeda triandra* ecotype 3, *Andropogon appendiculatus* and *Alloteropsis semialata* subsp. *eckloniana* [all from Bongwan, Kwazulu/Natal], description of experimental methods in Chapter 7). Methods used in each experiment to determine temperature responses were as follows:

University of Cape Town

9.2.1 Experiment 1

Photosynthetic temperature response curves were determined 24-25 weeks after the start of CO_2 fumigation (27 September - 5 October 1994), using an LI-6200 IRGA (Li-Cor, Lincoln, NE, USA) under a bank of 50 W tungsten-halogen incandescent lamps (Decostar 51, Osram, Germany). Measurements were performed on three plants per CO_2 treatment, selected from different chambers, but only from the ambient UV-B treatment. Rate-saturating light intensities (PPFD = 1500 to 2000 µmol m^{-2} s^{-1}) were provided, as determined from prior light response measurements. PPFD was measured with a quantum sensor (LI-189, Li-Cor, Lincoln, NE, USA) at leaf level. A polycarbonate cuvette was developed for measuring temperature responses. The temperature of the leaves enclosed within the cuvette was controlled by either heating or cooling an aluminium heat sink built into the base of the

cuvette, using a Peltier module. Relatively rapid air temperature changes were made possible by ventilating the heat sink with a small fan which also stirred the air within the cuvette. Photosynthetic temperature responses were performed starting at the lowest attainable leaf temperature (about 22°C), and then increasing the temperature in steps of approximately 5°C until the net CO_2 assimilation rate was decreasing steadily (maximum leaf temperatures used were about 38°C for *Acacia* and 45°C for *Themeda*). Measurements were carried out at a cuvette CO_2 concentration of both 360 μmol mol^{-1} and 650 μmol mol^{-1} to distinguish between short- and long-term responses to CO_2. Air vapour pressure increased from about 1.7 to 2.7 kPa during the course of the temperature response; LAVPD also increased with leaf temperature. Sampled leaves were harvested and their areas measured with a leaf area meter (LI-3000, Li-Cor, Lincoln, NE, USA) in the case of *Themeda*, or calculated from a regression of measured leaf area versus pinnae length and breadth (*Acacia*).

9.2.2 Experiment 2

Photosynthetic temperature response curves were determined 13-14 weeks after the start of CO_2 fumigation (23 January 1995 - 5 February 1996), using the same method as described for Experiment 1. Measurements were performed on four plants per CO_2 treatment, one from each chamber, under high light intensities (PPFD > 1000 μmol m^{-2} s^{-1}).

Immediately following the last temperature response measurements (6 February), gas exchange measurements were performed in the greenhouse (*in situ*) under high ambient light intensities (PPFD ~1000 μmol m^{-2} s^{-1}), using the standard cuvette of the LI-6200 IRGA. Measurements were performed only at growth CO_2 concentrations on 13-16 plants per treatment, from different chambers. Leaf temperatures were measured using the thermocouple within the cuvette, immediately after leaf insertion. On the same day, canopy temperatures were measured using an infra-red thermometer (Instatherm, Barnes Engineering Co., Stamford, Conn., USA), on 15-25 plants per treatment, from different chambers.

9.2.3 Experiment 4

Photosynthetic temperature response curves were determined 18-20 weeks after the start of CO_2 fumigation (10-20 February 1998) using a LI-6400 IRGA (Li-Cor, Lincoln, NE, USA). Measurements were performed on four plants per species per CO_2 treatment, one from each

University of Cape Town

chamber. Three species grown during the experiment were measured: *Andropogon*, *Themeda* and *Alloteropsis*. Light was provided inside the cuvette by an LED light source and light levels were controlled at 1500 µmol m^{-2} s^{-1}. Cuvette CO_2 concentrations were controlled at the growth [CO_2] using pressurised CO_2 canisters attached to the IRGA. Leaf temperatures were increased stepwise from about 25°C to 44°C (C_4 species) or from 25°C to 38°C (C_3 species), with sufficient acclimation time between temperature changes as determined by monitoring of ΔCO_2 and ΔH_20. LAVPD was not controlled and increased with increasing leaf temperatures in the range 1.5-6.5 kPa. In order to test how increasing LAVPD *per se* affected the photosynthetic response, LAVPD responses (in the range 2-5 kPa) were measured on one or two plants per species (plants grown in elevated CO_2), during which leaf temperature was kept constant at 32°C.

9.2.4 Curve analysis

Photosynthetic temperature response curves were fitted individually using non-linear regression and a 3rd-order polynomial (UNISTAT 3.0). The fitted curve coefficients (a, b, c and d) were then used to calculate the temperature (T_{opt}) at which the maximum rate of net CO_2 assimilation was achieved, using differentiation (ie. x, where $b+2cx+3dx^2 = 0$). Average temperature response curves for each treatment were plotted using the averages of the coefficients. T_{opt} values were subjected to either a two-way ANOVA, with growth [CO_2] and measurement [CO_2] as factors (Experiments 1 and 2), or one-way ANOVA (Experiment 4). *In situ* stomatal conductance and leaf and canopy temperatures (Experiment 2) were analysed using one-way ANOVA.

University of Cape Town

9.2.5 Chamber air temperatures

Average open-top chamber air temperatures for the midday period (12:00 - 15:00) were determined from daily logged records, for the 14-day period preceding temperature response measurements as well as the measurement period itself.

9.3 Results

Temperature responses of photosynthesis, stomatal conductance and transpiration for *Acacia karroo* are presented in Fig. 9.1, and the photosynthetic temperature optima (T_{opt}) are given in Table 9.1, with the statistical analysis in Table 9.2. Plants grown in ambient and elevated CO_2

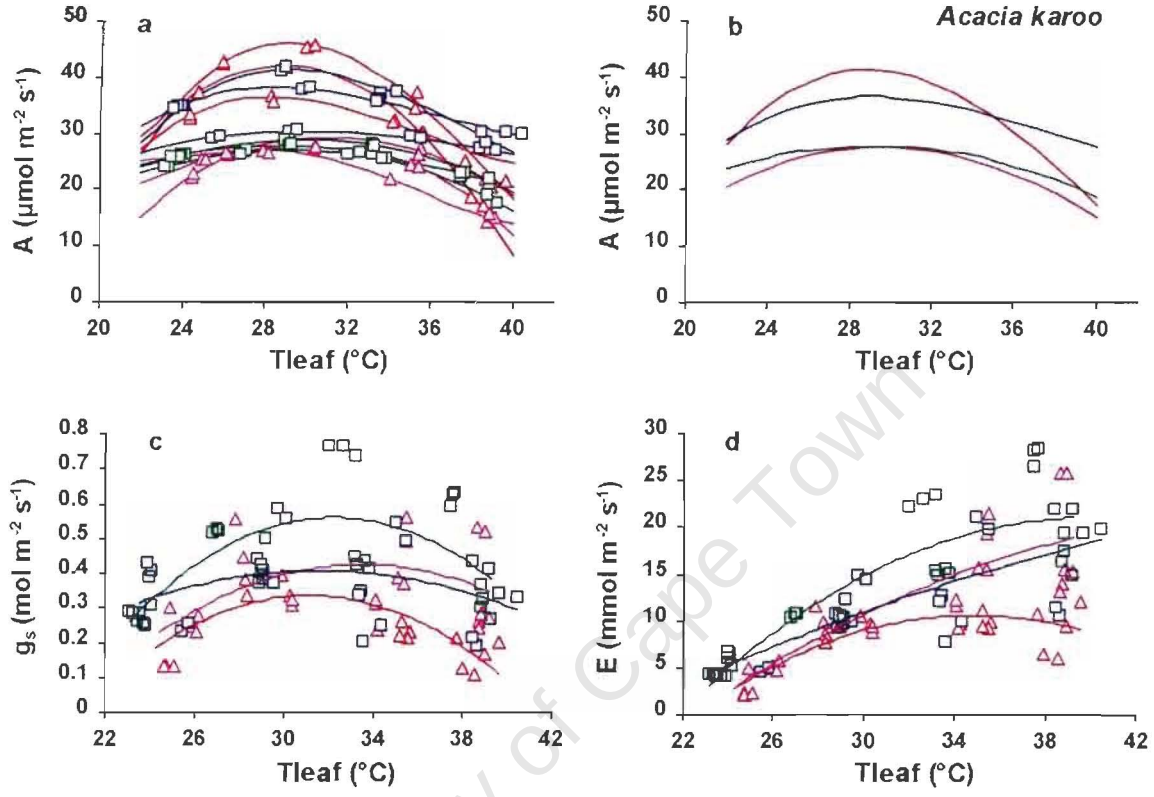
had the same T_{opt} (about 29°C) irrespective of the CO_2 concentration they were measured under. However, in the same experiment, *Themeda triandra* showed a large significant increase in T_{opt} when grown under elevated CO_2 (Fig. 9.2, Tables 9.1 and 9.2). Ambient CO_2-grown plants had a T_{opt} of about 28-30°C, whereas those grown in elevated CO_2 had a T_{opt} of about 37-38°C, irrespective of cuvette CO_2 concentrations during measurement. During this experiment conducted in winter/spring, average open-top chamber air temperatures before and during measurements were around 26°C (Table 9.1). When a shorter ecotype of *Themeda triandra* (ecotype 2) was grown in elevated CO_2 during summer (average chamber air temperatures of 36°C before and during measurements), absolute photosynthetic rates were considerably lower than in Exp. 1, but the temperature optima were again significantly higher (Fig. 9.3, Tables 9.1 and 9.2). In ambient CO_2, T_{opt} was about 36°C, whereas in elevated CO_2 T_{opt} was 38-39°C. Cuvette [CO_2] had no effect on T_{opt}.

During the summer experiment (Exp. 2), stomatal conductances measured *in situ* were generally low, but were further decreased in elevated CO_2 (Table 9.3). Leaf temperatures measured *in situ* were about 1.6°C higher in elevated compared to ambient CO_2. Both responses were marginally significant ($P \leq 0.10$). Canopy temperatures were significantly higher (by about 0.8°C) in elevated CO_2.

In Experiment 4, all three species showed no change in T_{opt} under elevated CO_2 (Table 9.2). Chamber air temperatures during this experiment were about 34°C (Table 9.1). *Themeda triandra* had a T_{opt} of about 34-35°C (Fig. 9.4, Table 9.1), T_{opt} for *Andropogon appendiculatus* was about 38°C (Fig. 9.5, Table 9.1), and T_{opt} for *Alloteropsis semialata* was about 29-30°C (Fig. 9.6, Table 9.1).

Responses of stomatal conductance (g_s) to increasing leaf temperatures differed depending on whether the species was a grass or shrub, and whether it was C_3 or C_4. Only *Acacia karroo* (C_3 shrub) showed a parabolic response (Fig. 9.1), with g_s peaking at just over T_{opt} and then decreasing with further temperature increases. The other C_3 species, *Alloteropsis semialata*, a grass, showed a slight linear decrease in g_s with increasing temperature when grown and measured under ambient CO_2, but g_s remained constant under elevated CO_2 (Fig. 9.6). In all other cases for C_4 grasses (Figs 9.2 to 9.5), g_s either remained constant or increased with increasing leaf temperature. *Andropogon appendiculatus* showed a particularly strong increase in g_s as leaf temperature rose. In all species, g_s was reduced when measured

University of Cape Town



University of Cape Town

Fig. 9.1 Temperature responses of photosynthesis (A, graphs **a** and **b**), stomatal conductance (g_s, graph **c**), and transpiration (E, graph **d**) in *Acacia karoo* during Experiment 1. Plants were either grown and measured under ambient CO_2 (green symbols and lines), grown under ambient CO_2 and measured at elevated CO_2 (dark blue symbols and lines), grown and measured under elevated CO_2 (red symbols and lines), or grown under elevated CO_2 and measured at ambient CO_2 (pink symbols and lines). **a**: all data points of replicate curves with individually modelled 3rd-order polynomial functions; **b**: average 3rd-order polynomial function for each treatment. For **c** and **d**, 2nd-order polynomials are fitted to all data points per treatment.

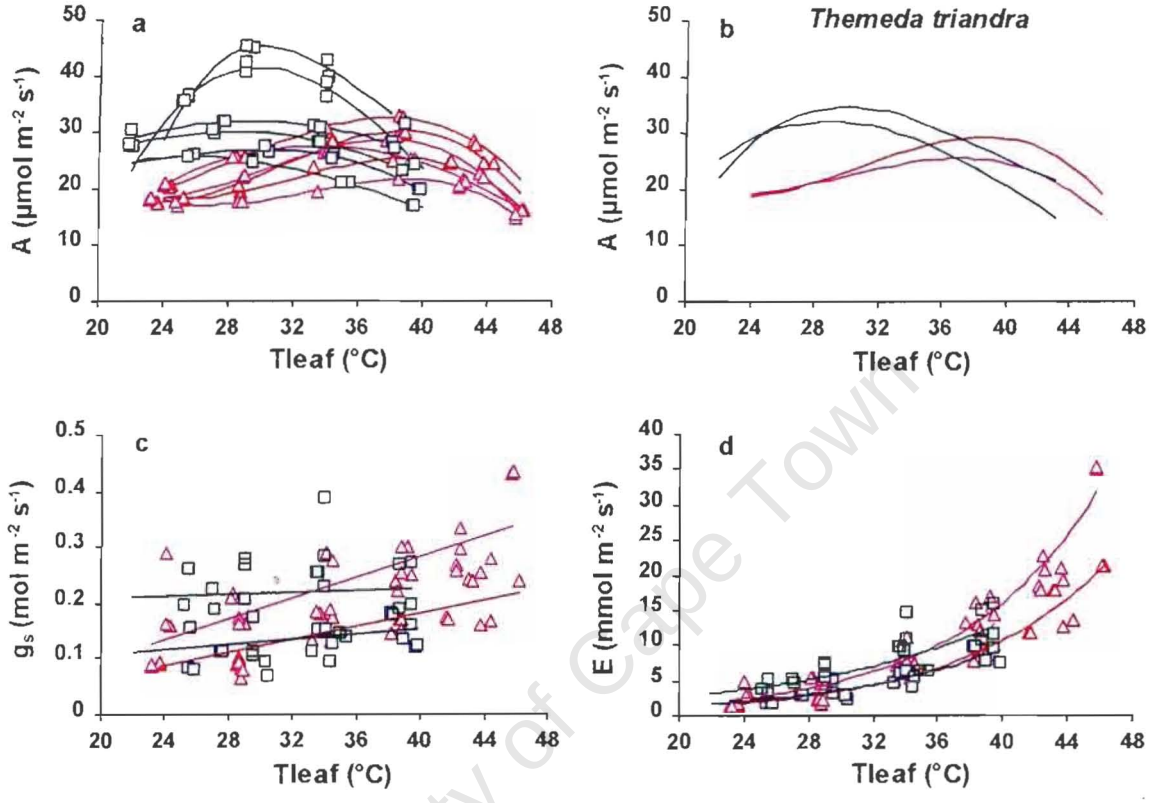


Fig. 9.2 Temperature responses of photosynthesis (A), stomatal conductance (g_s), and transpiration (E) in *Themeda triandra* during Experiment 1 (ecotype 1, winer/spring). For legend and description of graphs see Fig. 9.1. For **c** and **d**, exponential functions are fitted to all data points per treatment.

University of Cape Town

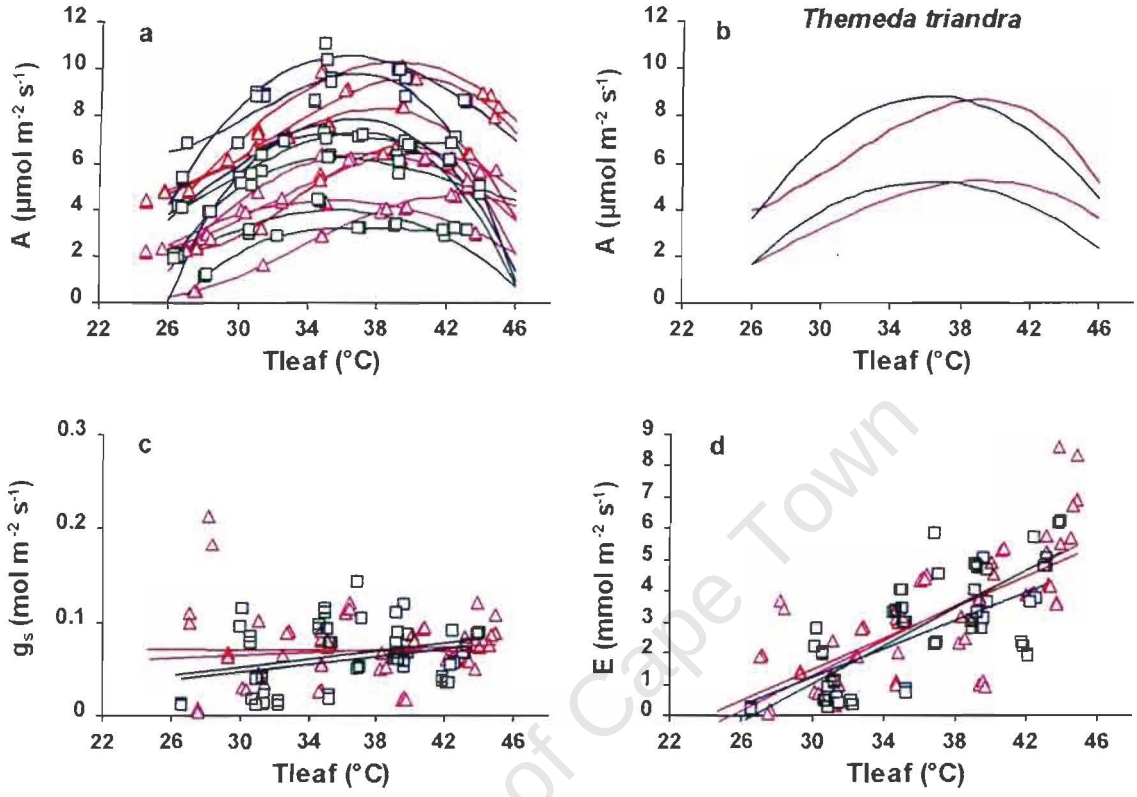


Fig. 9.3 Temperature responses of photosynthesis (A), stomatal conductance (g_s), and transpiration (E) in *Themeda triandra* during Experiment 2 (ecotype 2, summer). For legend and description of graphs see Fig. 9.1. For **c** and **d**, linear functions are fitted to all data points per treatment.

University of Cape Town

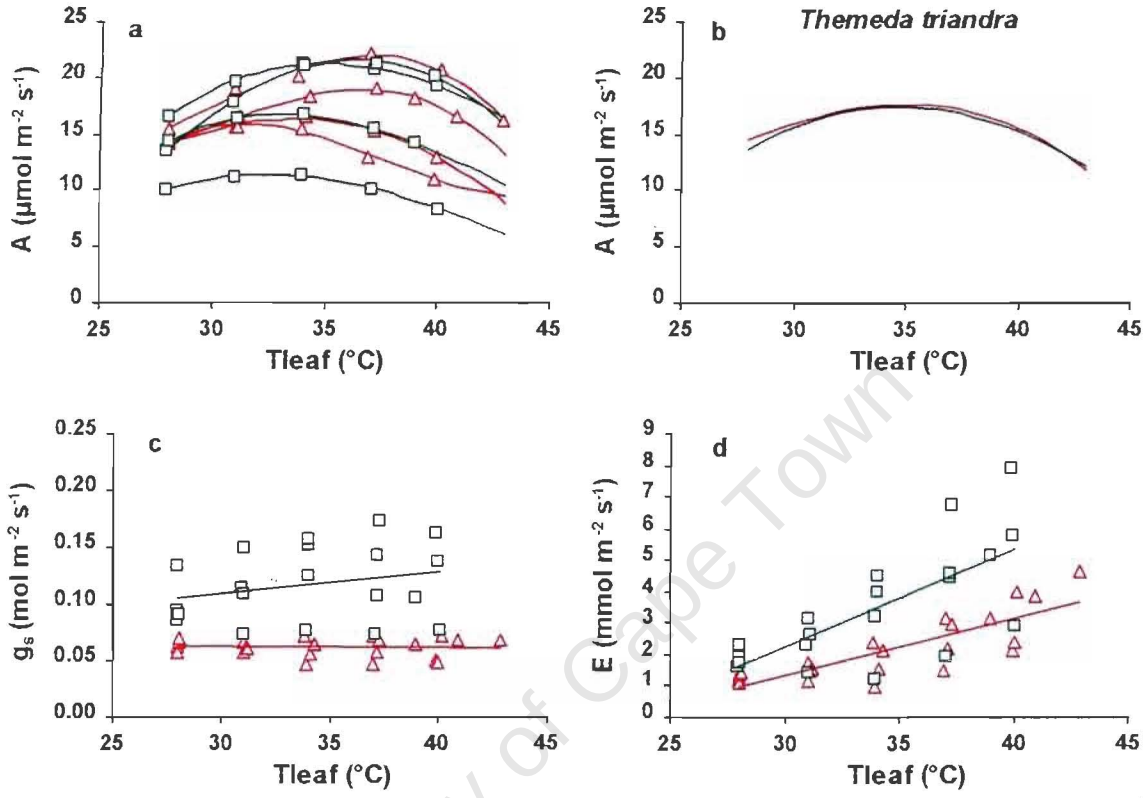


Fig. 9.4 Temperature responses of photosynthesis (A), stomatal conductance (g_s), and transpiration (E) in *Themeda triandra* during Experiment 4 (ecotype 3 excavated in field, summer). For legend and description of graphs see Fig. 9.1. For **c** and **d**, linear functions are fitted to all data points per treatment.

University of Cape Town

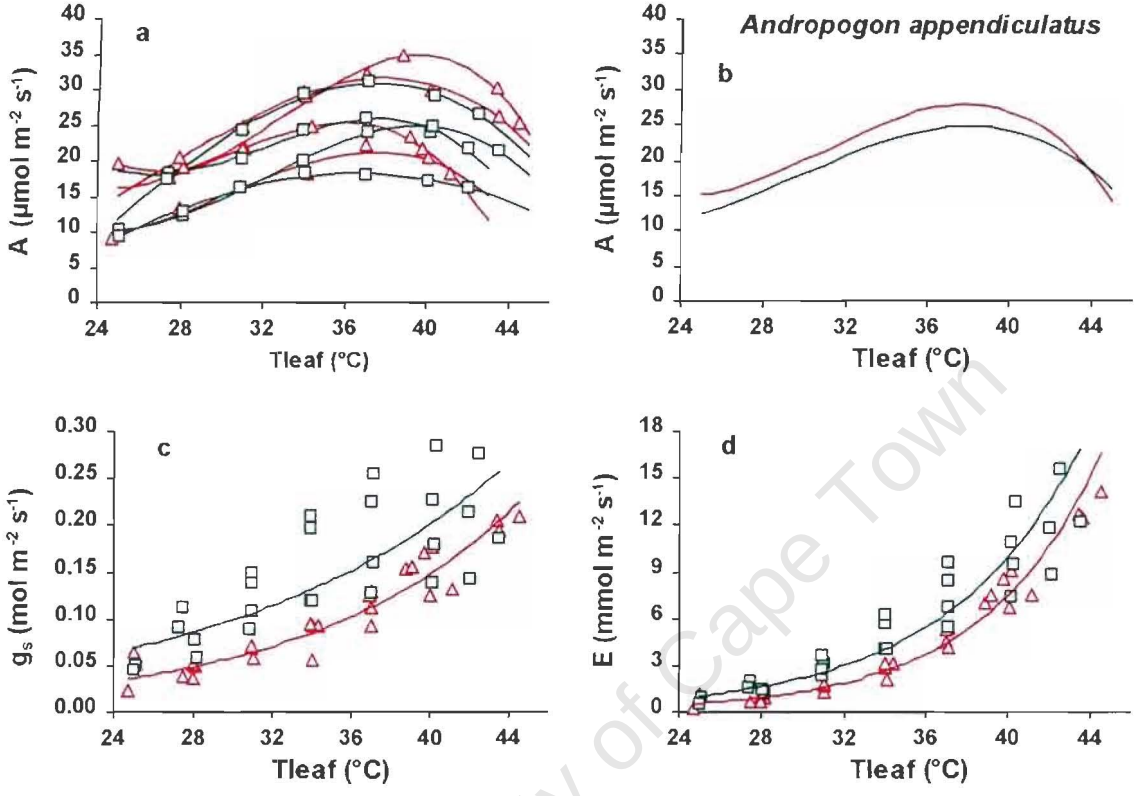


Fig. 9.5 Temperature responses of photosynthesis (A), stomatal conductance (g_s), and transpiration (E) in *Andropogon appendiculatus* during Experiment 4 (excavated in field, summer). For legend and description of graphs see Fig. 9.1. For **c** and **d**, exponential functions are fitted to all data points per treatment.

University of Cape Town

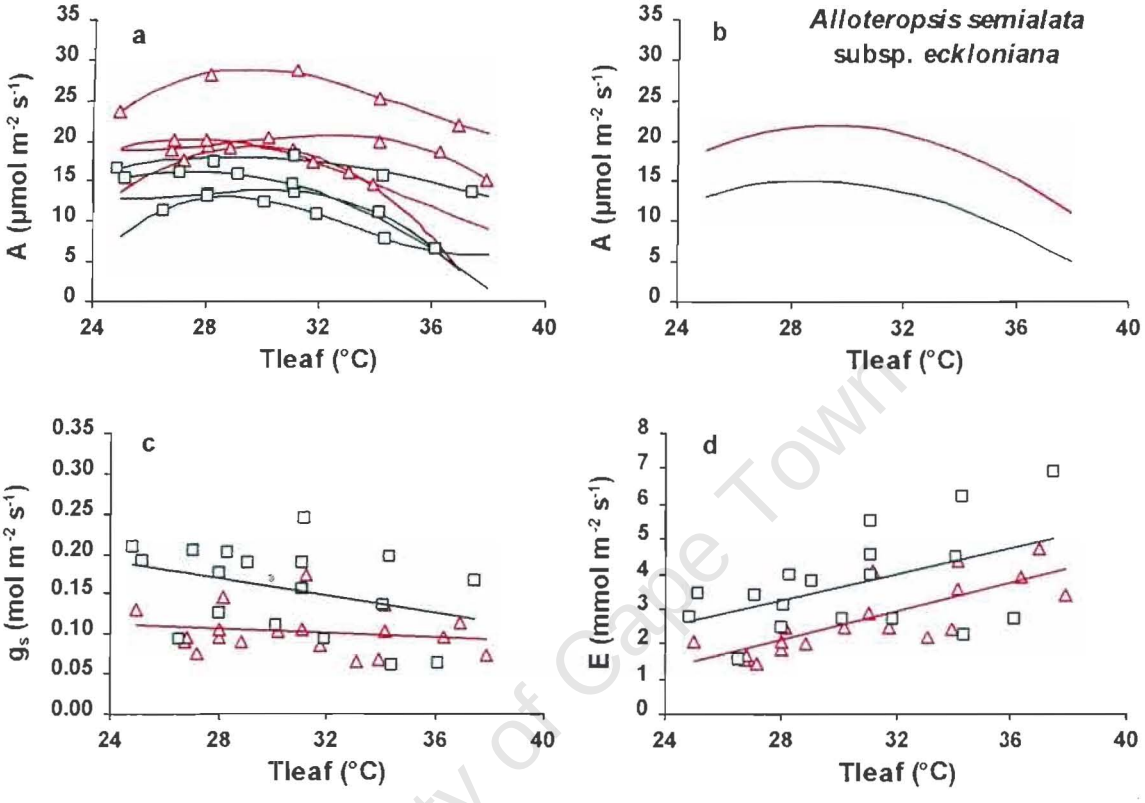


Fig. 9.6 Temperature responses of photosynthesis (A), stomatal conductance (g_s), and transpiration (E) in *Alloteropsis semialata* subsp. *eckloniana* during Experiment 4 (excavated in field, summer). For legend and description of graphs see Fig. 9.1. For **c** and **d**, linear functions are fitted to all data points per treatment.

University of Cape Town

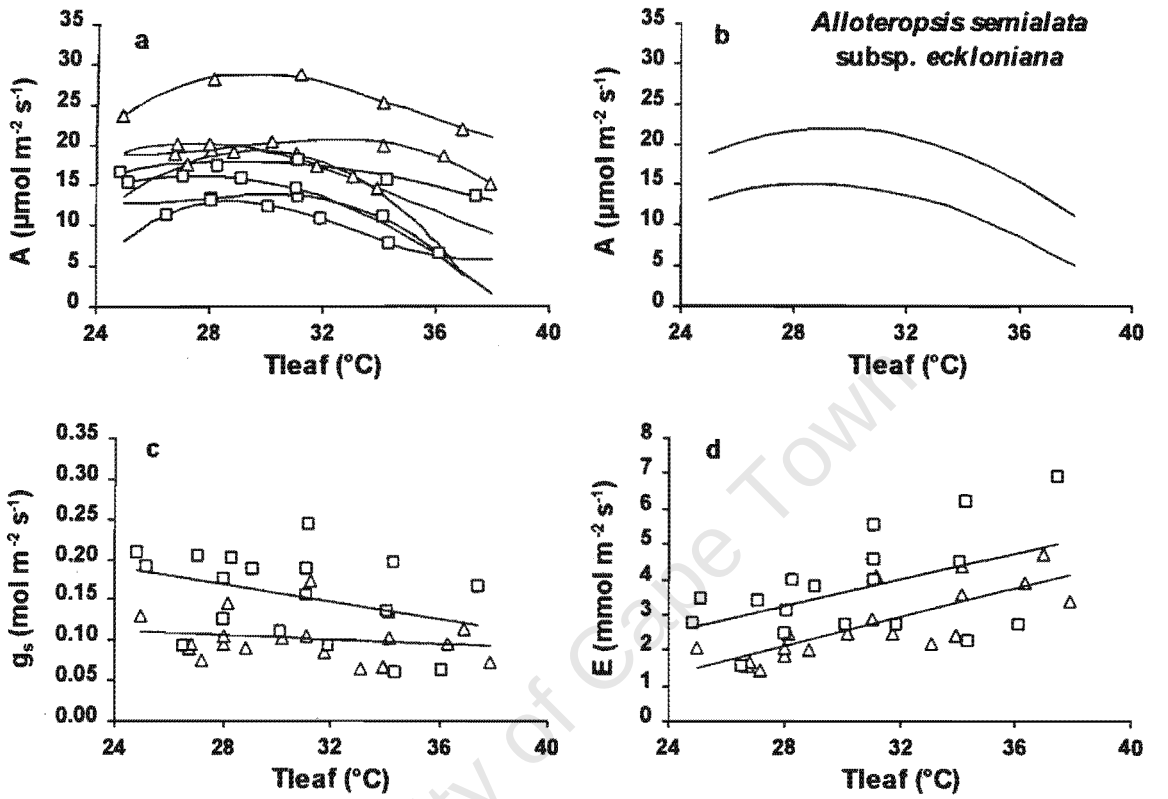


Fig. 9.6 Temperature responses of photosynthesis (A), stomatal conductance (g_s), and transpiration (E) in *Alloteropsis semialata* subsp. *eckloniana* during Experiment 4 (excavated in field, summer). For legend and description of graphs see Fig. 9.1. For **c** and **d**, linear functions are fitted to all data points per treatment.

University of Cape Town

Table 9.1 The temperature optimum (T_{opt}, °C) of net CO_2 assimilation of *Acacia karroo* (C_3) and *Themeda triandra* (C_4) (Exp. 1), *T. triandra* (Exp. 2), and *T. triandra*, *Andropogon appendiculatus* (C_4) and *Alloteropsis semialata* subsp. *eckloniana* (C_3) (Exp. 4) grown under either ambient or elevated CO_2. For Experiments 1 and 2, measurements were performed at both ambient and elevated cuvette CO_2 concentrations. Values are means ±SE of temperature optima obtained from individually fitted temperature response curves (n=3-4). Mean (±SE) ambient midday (12:00-15:00) chamber air temperatures for the 14-day period preceding temperature response measurements and the period of measurement, are also given.

	Acacia karroo (Exp. 1)		*Themeda triandra* (ecotype 1, Exp. 1)		*Themeda triandra* (ecotype 2, Exp. 2)		*Themeda triandra* (ecotype 3, Exp. 4)		*Andropogon appendiculatus* (Exp. 4)		*Alloteropsis semialata* subsp. *eckloniana* (Exp. 4)	
Growth $[CO_2]$	Cuvette $[CO_2]$		Cuvette $[CO_2]$		Cuvette $[CO_2]$		Cuvette $[CO_2]$		Cuvette $[CO_2]$		Cuvette $[CO_2]$	
	ambient	elevated	ambient	elevated	ambient	elevated	ambient	elevated	ambient	elevated	ambient	elevated
ambient	29.2 ± 0.4	29.2 ± 0.5	27.9 ± 1.2	29.8 ± 0.3	36.1 ± 0.3	36.0 ± 0.3	34.3 ± 0.9		37.9 ± 0.6		28.8 ± 0.5	
elevated	29.9 ± 0.8	28.9 ± 0.2	36.7 ± 1.7	38.4 ± 0.3	37.7 ± 1.4	39.2 ± 0.4		34.7 ± 1.3		37.8 ± 0.7		29.7 ± 1.0
Air temp (°C)	25.8 ± 6.2				35.6 ± 4.8		33.7 ± 4.9					

University of Cape Town

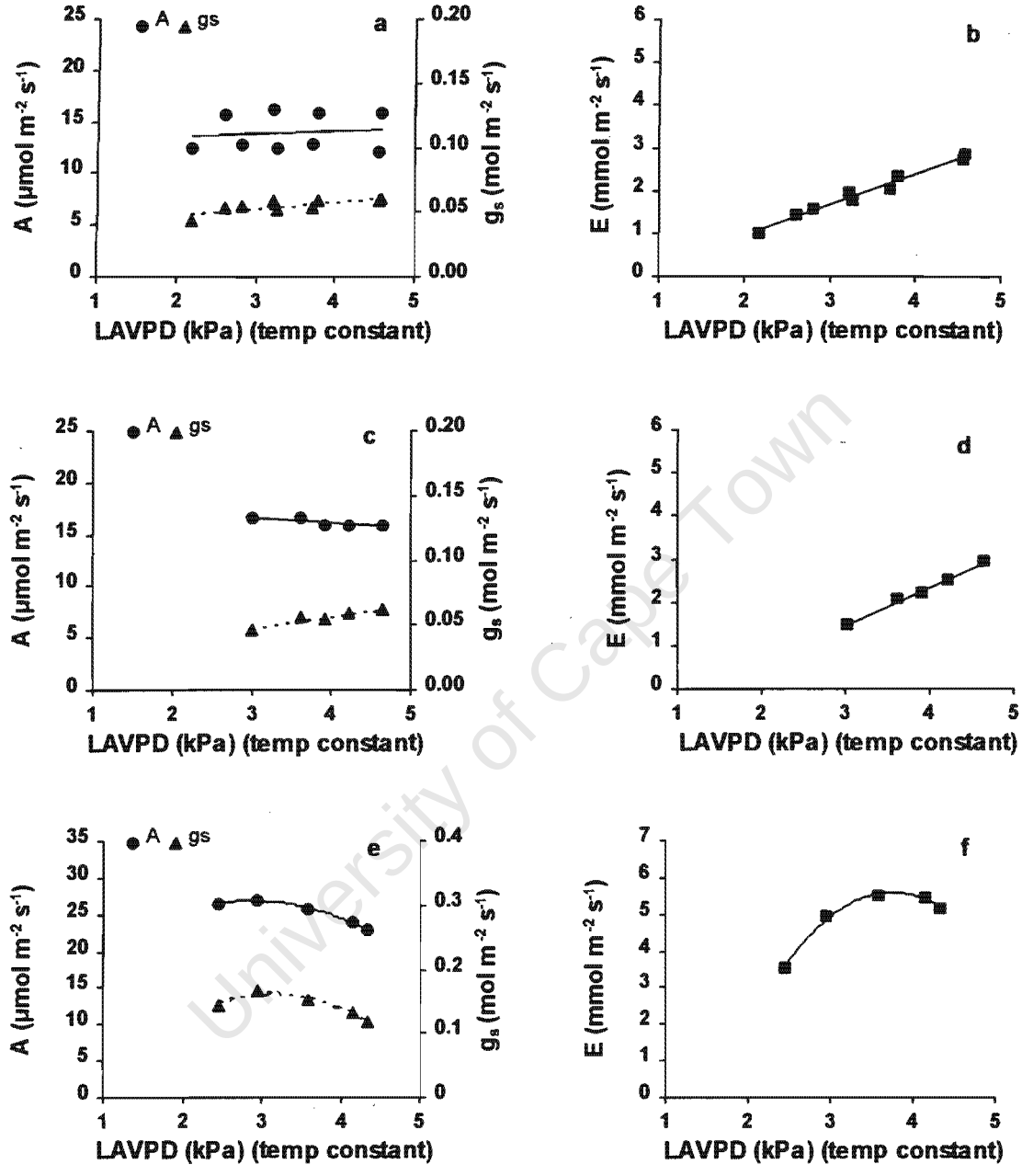
Table 9.2 Statistical significance of the effects of elevated CO_2 during growth ($[CO_2]$trt) or during measurement ($[CO_2]$ms) on the temperature optimum (T_{opt}, °C) of net CO_2 assimilation. ** P≤0.01; *** P≤0.001; NS not significant, according to either two-way ANOVA (Experiments 1 and 2) or one-way ANOVA (Experiment 4).

	Acacia karroo (Exp. 1)	*Themeda triandra* (ecotype 1, Exp. 1)	*Themeda triandra* (ecotype 2, Exp. 2)	*Themeda triandra* (ecotype 3, Exp. 4)	*Andropogon appendiculatus* (Exp. 4)	*Alloteropsis semialata* subsp. *eckloniana* (Exp. 4)
$[CO_2]$trt	NS	***	**	NS	NS	NS
$[CO_2]$ms	NS	NS	NS			
$[CO_2]$trt x $[CO_2]$ms	NS	NS	NS			

Table 9.3 *In situ* stomatal conductance and leaf and canopy temperatures of *Themeda triandra* grown under ambient or elevated CO_2 concentration during summer (ecotype 2, Exp. 2). Values are means ± SE (n=sample size). + P≤0.10; * P≤0.05, according to one-way ANOVA.

$[CO_2]$ treatment	n	Stomatal conductance (mmol m^{-2} s^{-1})	n	Leaf temperature (°C)	n	Canopy temperature (°C)
ambient	14	83 ± 6.6	13	37.3 ± 0.7	15	29.9 ± 0.3
elevated	16	67 ± 4.9	16	38.9 ± 0.6	25	30.7 ± 0.2
$[CO_2]_{trt}$		+		+		*

University of Cape Town



University of Cape Town

Fig. 9.7 LAVPD-responses of photosynthesis (A), stomatal conductance (g_s), and transpiration (E) in *Themeda triandra* (**a** and **b**), *Andropogon appendiculatus* (**c** and **d**) and *Alloteropsis semialata* subsp. *eckloniana* (**e** and **f**) during Experiment 4. Leaf temperatures were held constant at 32°C. All plants had been grown under elevated CO_2, with 2 plants measured for *Themeda*, and one each for *Andropogon* and *Alloteropsis*.

under elevated cuvette [CO_2], except in *Themeda* in Experiment 2 (which did, however, show reduced g_s when measured *in situ*, Table 9.3). Transpiration rates (E) generally increased with increasing leaf temperatures, either linearly or exponentially (the latter response was most pronounced in *Andropogon*). In *Acacia* (Fig. 9.1) the relative increase in rates was reduced at higher temperatures, reflecting the reductions in g_s at these temperatures. Transpiration rates were consistently lower in elevated than in ambient CO_2, except in *Themeda* in Experiment 2.

When A, g_s and E were plotted against LAVPD rather than T_{leaf} in *Themeda*, *Andropogon* and *Alloteropsis*, the resulting curves (data not shown) were very similar to the temperature response curves, reflecting the correlation between leaf temperature and LAVPD. When LAVPD was increased at constant temperature in *Themeda* and *Andropogon*, g_s remained constant and E increased linearly (Fig. 9.7a-d). Therefore, stomatal function was not sensitive to LAVPD in the C_4 grasses, but transpirational water loss was strongly influenced by changes in LAVPD. In *Andropogon* the exponential increase in g_s with concurrently rising leaf temperature and LAVPD (Fig. 9.5) was not found when LAVPD was increased at constant temperature (Fig. 9.7c), suggesting that stomatal function is sensitive to changes in leaf temperature *per se*. In *Alloteropsis*, g_s decreased at LAVPD levels higher than 3 kPa when leaf temperature was held constant (Fig. 9.7e), resulting in a drop-off in transpiration rate (Fig. 9.7f).

For interpretive purposes, the relationships between T_{opt} and T_{air} (mean midday chamber air temperature of the preceding two weeks) were plotted for C_3 and C_4 species individually (Fig. 9.8). Because only two C_3 species were studied, the linear function for C_4 species only is presented, although this regression should be treated with caution as it is based on an insufficient number of data points particularly at lower temperatures. Nevertheless, the regression for the C_4 species suggests that plants were capable of acclimating to growth temperature. In C_3 species, however, a T_{opt} of about 29°C was measured at both moderate (26°C) and high (34°C) growth temperatures.

University of Cape Town

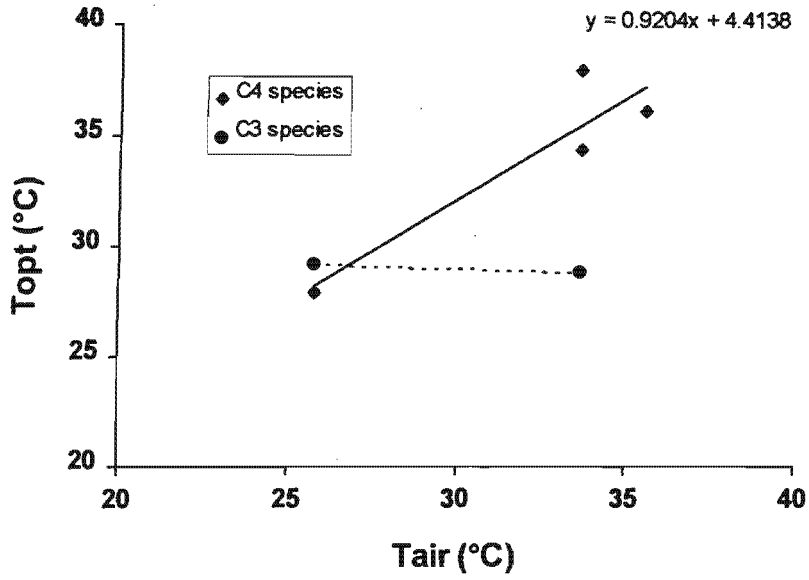


Fig. 9.8 Relationship between T_{opt} (photosynthetic temperature optimum) and T_{air} (mean midday chamber air temperature of the preceding two weeks) for C_3 and C_4 species grown under ambient CO_2 concentration. The equation represents the linear relationship for C_4 species.

University of Cape Town

9.4 Discussion

9.4.1 Photosynthetic temperature optima vs. growth temperature

Photosynthetic temperature responses were characteristic for C_3 and C_4 species. In both C_3 species (*Acacia* and *Alloteropsis*), photosynthetic temperature optima were about 29°C, which is comparable to many other C_3 species from warm environments (Anderson 1982, Seemann *et al.* 1984, Sage *et al.* 1995). This T_{opt} is also similar to that previously reported for *Alloteropsis semialata* subsp. *eckloniana* from the eastern Cape (25°C, Botha & Russel 1988) at similar photosynthetic rates. By contrast, C_4 grass species had characteristically higher T_{opt} of between 28 and 38°C under ambient atmospheric conditions. Botha & Russel (1988) also found that the temperature optima for a range of C_4 grasses from the eastern Cape lay between 30 and 35°C at comparable photosynthetic rates.

T_{opt} in C_4 species closely reflected the ambient temperature regime within open-top chambers for the two weeks preceding measurements (Fig. 9.8). C_4 grasses were capable of acclimating to high ambient growth temperatures. This type of acclimation usually occurs relatively rapidly, within a matter of one or two weeks (Battaglia *et al.* 1996, Slatyer &

Morrow 1977). Plants originating from regions with large seasonal fluctuations in air temperature often display better acclimation capacities than those from regions with less variable seasonal temperatures (Berry & Björkman 1980). In this study, growth temperatures for C_4 grasses compared as follows with temperature regimes in their regions of origin (Table 9.4).

Table 9.4 Annual temperature regimes for Döhne, Skukuza (Kruger National Park) and Paddock, Kazulu/Natal (SA Climate Statistics 1997), and chamber temperatures prior to measurements.

Origin	Mean daily maximum temperature of the warmest month	Mean daily temperature from the coldest to the warmest month	Range in mean daily temperature per month	Mean chamber air temperature two weeks preceding measurements
Döhne (Exp. 1)	25.2°C	7.1 - 14.7°C	7.6°C	25.8°C
Skukuza (Exp. 2)	32.6°C	5.6 - 20.6°C	15.0°C	35.6°C
Paddock (Exp. 4)	25.0°C	10.2 - 17.2°C	7.0°C	33.7°C

University of Cape Town

Themeda plants grown from seed sourced from Döhne (Exp. 1) or the Kruger National Park (Exp. 2) were grown at temperatures well within the summer ranges experienced at these places, so that their acclimation capacity was not tested beyond familiar conditions. However, the two C_4 grass species sourced from southern Kwazulu/Natal (Bongwan, near Paddock), an area with moderately high summer temperatures and low annual temperature variability, also acclimated well at 34°C in the greenhouse (Exp. 4), whilst maintaining high photosynthetic rates. In fact, both *Themeda* and *Andropogon* were apparently not stressed by high temperatures in this greenhouse experiment, as their maximum photosynthetic rates were similar to those measured in the field under more moderate temperatures (compare Figures 9.4 and 9.5 with Fig. 8.5 in Chapter 8). C_3 and C_4 shrub species native to cooler regions (Berry & Björkman 1980), and a C_3 grass species from temperate grasslands (Williams 1974) have been shown to acclimate to higher growth temperature (shift in T_{opt}), but suffered damage and reduced productivity due to reductions in photosynthetic rates. By contrast, *Bouteloua gracilis*, a C_4 grass, maintained significantly

higher photosynthetic rates at high temperature (Williams 1974). This would suggest that C_4 grasses are capable of full thermal acclimation while resisting damage to, or inhibition of the photosynthetic system at high temperatures. Downing & Groves (1985) demonstrated how ecotypes of *Themeda* originating from cool alpine to hot sub-tropical habitats in southern Africa, were all able to grow and reproduce equally well at temperatures up to 36/31°C.

Alloteropsis (C_3), on the other hand, was unable to acclimate to high growth temperatures, and had a T_{opt} 4.9°C lower than mean chamber air temperature (Table 9.1, Fig. 9.8). This was not surprising, since net CO_2 assimilation in C_3 plants is more sensitive to increasing temperatures as a result of the relatively greater increase in oxygenation than carboxylation of RuBP. In addition, quantum yield for CO_2 uptake in C_3 species is highly sensitive (negatively) to increasing temperature, unlike C_4 species (Ehleringer *et al.* 1997). This restricts the capacity of C_3 species to acclimate to higher temperatures, particularly above an apparent "ceiling" of about 28-30°C, which may represent the temperature above which thermal instability or damage to photosynthetic enzymes occurs. In *Chenopodium album*, temperature acclimation occured when plants were grown at 25°C relative to 17°C, but not at 34°C relative to 25°C (Pearcy *et al.* 1981, Sage *et al.* 1995), with a maximum T_{opt} of about 30°C. Anderson (1982) found that T_{opt} (23-28°C) for *Tamarix chinensis*, a C_3 desert shrub, was well below the typical midday temperatures observed in the field (32-38°C). This upper limit for T_{opt} may have negative implications for productivity: in both *Tamarix* (Anderson 1982) and *Alloteropsis* (Fig. 9.6), photosynthetic rates at high ambient temperatures were effectively reduced by 20% below maximum photosynthetic rates at T_{opt}.

University of Cape Town

9.4.2 Photosynthetic temperature optima in elevated CO_2

When *Acacia* and *Alloteropsis* were grown and measured under elevated CO_2, photosynthetic rates were higher than in ambient CO_2, even at temperatures 5°C higher than the optimum (Table 9.5). This apparently compensated for the lack of upward adjustment of T_{opt}, suggesting that maintenance of carbon assimilation and avoidance of damage were of primary importance. Elevated CO_2 also did not affect T_{opt} in *Chenopodium album* when grown at 34°C, but photosynthetic rates were higher than in ambient CO_2 (Sage *et al.* 1995). These authors suggested that thermal acclimation may not be important when warming occurs at or above the maximum T_{opt} of about 30°C. Further support for the "ceiling" idea can be found in the fact that no upward shift in T_{opt} was measured in response to short-term (cuvette)

Table 9.5 Comparison between maximum net CO_2 assimilation rate (A) at optimum temperature ($T_{opt\ ambient}$) in plants grown and measured under ambient CO_2, and A at the same temperature ($T_{opt\ ambient}$) and at 5°C and 10°C higher than $T_{opt\ ambient}$ in plants grown and measured under elevated CO_2. The last column depicts the shift in T_{opt} induced by growth under elevated CO_2. ** P≤0.01; *** P≤0.001; NS not significant, according to either two-way ANOVA (Experiments 1 and 2) or one-way ANOVA (Experiment 4).

Species	A at $T_{opt\ ambient}$ in ambient CO_2 ($\mu mol\ m^{-2}\ s^{-1}$)	A at $T_{opt\ ambient}$ in elevated CO_2 ($\mu mol\ m^{-2}\ s^{-1}$)	A at $T_{opt\ ambient}$ +5°C in elevated CO_2 ($\mu mol\ m^{-2}\ s^{-1}$)	A at $T_{opt\ ambient}$ +10°C in elevated CO_2 ($\mu mol\ m^{-2}\ s^{-1}$)	ΔT_{opt} ($T_{opt\ elevated}$ - $T_{opt\ ambient}$) (°C)
Acacia karroo (Exp. 1)	27.9	41.4	35.3		-0.3 NS
Themeda triandra (ecotype 1, Exp. 1)	32.1	21.3	26.3	29.4	10.5 ***
Themeda triandra (ecotype 2, Exp. 2)	5.2	8.1	8.5		3.1 **
Themeda triandra (ecotype 3, Exp. 4)	17.5	17.6	16.3		0.4 NS
Andropogon appendiculatus (Exp. 4)	24.8	27.9	21.3		-0.1 NS
Alloteropsis semialata subsp. *eckloniana* (Exp. 4)	15.1	21.9	18.7		0.9 NS

University of Cape Town

increases in CO_2 in the two C_3 species, unlike most C_3 plants measured under more moderate temperatures.

Themeda (ecotype 2, Exp. 2) displayed low stomatal conductances and photosynthetic rates. As in the two C_3 species, higher photosynthetic rates were also measured in this ecotype when grown in elevated compared to ambient CO_2 at optimum and above-optimum leaf temperatures (Table 9.5). After only 13 weeks under elevated CO_2, photosynthetic downregulation had not yet set in (see Chapter 6). With the 1-2°C increase in leaf temperature in elevated CO_2 (Table 9.3), the photosynthetic temperature optimum shifted upwards by 3.1°C, thereby increasing assimilation rates over and above the direct increase experienced under high CO_2. This effectively resulted in a 62% increase in photosynthetic rate in elevated compared to ambient CO_2 at leaf temperatures 1-2°C higher than $T_{opt\ ambient}$. This ecotype originated from the Kruger National Park, a region experiencing high summer temperatures and high annual temperature variability (see Table 9.4). This history may have conferred on it a greater inherent tolerance of high temperatures, and possibly a greater acclimation capability at or above such high temperatures (Hikosaka 1997). Seemann *et al.* (1984) showed that desert plants are capable of acclimation to above-ambient increases in temperature, as their threshold for high temperature damage is shifted upwards. This may be attributable to the high optimum temperature for electron transport rate, and therefore RuBP regeneration, in hot desert species compared to temperate species, and increased thermal stability of the photosynthetic machinery at higher temperatures (Armond *et al.* 1978, Badger *et al.* 1982). Nevertheless, the shift in T_{opt} in this *Themeda* ecotype grown at high temperature was not nearly as large as that measured in ecotype 1 grown under more moderate temperatures, which may indicate a "ceiling" to heat tolerance and thermal stability of enzymes in *Themeda* at about 39°C.

The most productive ecotype of *Themeda* studied in these experiments was that sourced from the eastern Cape (ecotype 1, Döhne). This ecotype was grown under moderately high temperature conditions, similar to those experienced in Döhne, for 24 weeks before measurements were started. Although photosynthetic rates were high under ambient CO_2, substantial photosynthetic downregulation had taken place in plants grown under elevated CO_2, as seen from Fig. 9.2 (comparison at leaf temperatures <34°C) and Table 9.5. However, when the 10.5°C increase in the temperature optimum under elevated CO_2 is taken into

University of Cape Town

consideration, photosynthetic rates at this optimum (38°C) were only slightly lower than the maximum in ambient CO_2 (29.4 and 32.1 μmol m^{-2} s^{-1}, respectively). This large shift in T_{opt} was substantially greater than the potential increase in leaf temperature attributable to stomatal closure and reduced transpiration (not more than 2-3°C according to leaf energy balance calculations). Seasonal increases in T_{opt} of more than 10°C have been measured in desert shrubs (Berry & Björkman 1980), and are physiologically possible in some species. Whatever the stimulus for this response in *Themeda*, the potential benefit for carbon assimilation was nevertheless significant, since even only a 3°C increase in leaf temperature under elevated CO_2 yielded a 14% higher photosynthetic rate than that measured under elevated CO_2 in the absence of leaf warming.

It is debatable whether this response can be regarded as compensatory in the face of long-term photosynthetic downregulation, as the mechanisms are not sufficiently well studied. Altered partitioning of nitrogen between photosynthetic components has been shown to result in changes in the photosynthetic temperature response (Hikosaka 1997). Leaf nitrogen concentration decreased significantly under elevated CO_2 in this *Themeda* ecotype (see Table 4.3 in Chapter 4), a characteristic of photosynthetic downregulation in many species (Sage 1994), and the organization of the photosynthetic apparatus may have undergone a change. Using a modelling approach, Hikosaka (1997) demonstrated how T_{opt} shifts from lower to higher temperatures if investment of nitrogen into the photosynthetic process having a rate-limiting lower optimal temperature is increased. This argument is only valid where changes in stomatal conductance are not responsible for the shift in T_{opt}. An upward shift in T_{opt} could also take place if stomatal conductance increases with increasing temperature, as shown in Fig. 9.2 for this *Themeda* ecotype grown under elevated CO_2. This divergent temperature response of stomatal conductance under elevated and ambient CO_2 could partially explain the upward shift in T_{opt}. It is not clear whether this stomatal response is causal i.e. a direct response to high temperature, or whether it is a secondary response following stimulation of the photosynthetic mechanism.

Both C_4 grass species studied in Exp. 4 (from Bongwan) showed no significant shifts in T_{opt} in response to elevated CO_2. Although leaf temperatures were not determined, any potential increase in leaf temperature under elevated CO_2 due to stomatal closure (which was measured in both species) did not appear to elicit any changes in the photosynthetic

University of Cape Town

temperature response. Temperature responses were broad (i.e. near-optimal photosynthetic rates were maintained over a wide temperature range) and there was no short- or long-term effect of elevated CO_2 on carbon assimilation. Consequently, photosynthetic rates were similar at optimum temperature under ambient or elevated CO_2, and were only slightly reduced under elevated CO_2 and a 5°C leaf warming scenario compared to ambient CO_2-grown plants at optimum temperature (Table 9.5). These species were, therefore, capable of acclimating to relatively high growth temperatures, compared to field conditions at Bongwana, but did not acclimate to potential smaller increases in leaf temperature under the elevated CO_2 treatment. Knapp *et al.* (1993) also found no change in T_{opt} in *Andropogon gerardii* (C_4 prairie grass) grown under elevated CO_2.

9.4.3 Influence of LAVPD on the temperature response

Photosynthetic temperature responses are potentially altered under conditions of decreasing relative humidity and the related rise in LAVPD (Bunce 1993, Will & Teskey 1997) due to reductions in stomatal conductance (Schulze 1986). It is generally thought that stomata respond to the rate of transpiration rather than to humidity *per se* (Mott & Parkhurst 1991, Monteith 1995), although the exact nature of the sensing mechanism is still debated (Bunce 1996, Meinzer *et al.* 1997). The most commonly measured response is a linear decrease in stomatal conductance with increasing rate of transpiration (Anderson 1982, Bunce 1993, Monteith 1995).

In this study, stomatal conductances of both C_4 grass species studied in Exp. 4 did not respond to increasing LAVPD at 32°C leaf temperature, remaining constant between a range of 2-5 kPa (Fig. 9.7a and c), while transpiration rates rose linearly (Fig. 9.7b and d). However, conductances were low (~0.05 mol m^{-2} s^{-1}) resulting in relatively low rates of transpiration (1-3 mmol m^{-2} s^{-1}). Under conditions of regular water supply, as in this study, the maintenance of high photosynthetic rates was probably of greater importance to plant productivity than the restriction of water loss to the atmosphere. Maroco *et al.* (1997) recently reported that two out of three C_4 semi-arid Sahelian grass species displayed flat responses of g_s and A to increasing LAVPD at constant leaf temperature. These were regarded as "drought-escaping" species, which complete their life cycle within a limited period before the onset of the dry season. Three "drought-resistant" species (two C_3 dicots and one C_4 grass) displayed the more usual and conservative negative response of g_s and A to increasing

University of Cape Town

LAVPD. They argued that in semi-arid climates, where dry air is not necessarily accompanied by soil water shortage, stomatal closure at high LAVPD might be disadvantageous, as this would reduce the opportunity for carbon assimilation when there is still adequate soil water available. Morison & Gifford (1983) demonstrated that the sensitivity of stomatal conductance to LAVPD is linearly proportional to the magnitude of stomatal conductance. By extrapolation, plants with very low conductances should show almost no response of conductance to changing LAVPD.

In *Andropogon*, increasing leaf temperature *per se* increased conductances and transpiration rates (Fig. 9.5c and d). This allowed sustained high photosynthetic rates at very high leaf temperatures, and a high T_{opt}, and the resulting large transpirational water loss was apparently not detrimental. Two C_4 millet cultivars with similarly low stomatal conductances have also shown increases in stomatal conductance with increasing leaf temperature in the range 23-33°C (Imai & Okamoto-Sato 1991). Increases in g_s with increasing leaf temperature have been found in other species either where LAVPD is kept constant, or where species are relatively insensitive to LAVPD (Šantrucek & Sage 1996). The C_3 grass *Alloteropsis*, on the other hand, showed slight reductions in g_s and A with increasing LAVPD (>3 kPa) (Fig. 9.7e). Transpiration was maximal at a LAVPD of about 3.8 kPa, before decreasing again (Fig. 9.7f). Conductances and transpiration rates were comparatively high, and would have led to more rapid reductions in leaf water potential than in the C_4 grasses.

In general, the response of A to increasing leaf temperature in the C_4 grasses was not confounded by any effects of increasing LAVPD on g_s and A, either in ambient or in elevated CO_2. This supports the finding by Bunce (1993) that there was no significant effect of LAVPD on assimilation rate in *Amaranthus* (C_4) for either increased growth or measurement CO_2 concentration. It is, therefore, justifiable to say that the responses of T_{opt} in C_4 grasses in this study are "real" despite the variability in LAVPD. Even in the two C_3 species, there was no change in T_{opt} which could have been attributable to LAVPD effects. The only experiment which may represent the exception was Exp. 1 (*Themeda* ecotype 1), but in the absence of the appropriate measurements it is not clear whether increasing leaf temperature or LAVPD induced the increased stomatal conductance under long-term elevated CO_2 which may have contributed to the upward shift in T_{opt}.

University of Cape Town

9.4.4 Conclusion

Some direct evidence was found for an increase in leaf temperature due to stomatal closure in elevated CO_2. The absence of upward shifts in T_{opt} in some species does not necessarily imply that leaf temperatures were not increased, and it cannot be ruled out that higher leaf temperatures contribute to the significant leaf growth response in elevated CO_2 in some C_4 species (Gallagher 1979, Clifton-Brown & Jones 1997). Growth responses are notoriously difficult to correlate with CO_2 assimilation rate and improved growth may occur even where this rate is not optimally adjusted. Future studies should test these hypotheses by measuring leaf temperatures and leaf extension directly under differing concentrations of CO_2.

University of Cape Town

Chapter 10

Summary discussion

In this study, I have shown that wild C_4 grass species from southern Africa and elsewhere in the world are capable of responding favourably to expected increases in atmospheric CO_2 concentrations during the next few decades. Whether or not they will do so depends on a number of factors, which can be grouped into those which are under genetic control (e.g. intra- and inter-specific differences in CO_2-responsiveness), and those which can modify the potential CO_2-response in the shorter-term (e.g. interaction with other climatic and biological factors). Both will need to be taken into consideration when predictions of grassland responses to elevated CO_2 are attempted.

10.1 CO_2-responsiveness of C_3 and C_4 species

Contrary to current international perceptions based on theory and some earlier experiments, I found no evidence to suggest that wild C_3 grass species are intrinsically more responsive to elevated CO_2 than wild C_4 grass species. A meta-analysis of the available literature (Chapter 2) revealed similar responses in photosynthetic rate, stomatal conductance and total plant biomass between C_3 and C_4 grasses in elevated CO_2. However, C_4 species showed a greater increase in leaf area in elevated CO_2, whereas C_3 species showed a greater increase in tillering. Although the importance of leaf area responses in C_4 species have been emphasized by a few researchers previously, the meta-analysis highlights this response as one of the most important and distinguishing characteristics of C_4 grass growth in elevated CO_2.

Both C_3 experimental study species displayed signs of sink limitation. This effect apparently prevented *Acacia karroo* (C_3 shrub, Chapter 4) from accumulating significantly more biomass in elevated CO_2 in spite of increased photosynthetic rates. With the exception of a small increase in leaf length, no growth responses were measured in elevated CO_2 for *Alloteropsis semialata* subsp. *eckloniana*, a C_3 grass (Chapter 7). This species showed significant photosynthetic downregulation in high CO_2 in the greenhouse (Chapter 7) and in the field after prolonged exposure (Chapter 8). Both *Acacia* and *Alloteropsis* also showed no thermal acclimation in elevated CO_2 which may have been of benefit to plant productivity (Chapter 9). By contrast, significant growth responses in elevated CO_2 were measured in

University of Cape Town

some C_4 grass species during this study. Although only a small C_3 sample size was tested, the results emphasize that care must be taken when making assumptions of greater CO_2-responsiveness in C_3 than in C_4 species. It is apparently not the possession of one or the other photosynthetic pathway which will determine how species will respond, but rather their ability to convert greater carbon assimilation into greater biomass, and to sustain this response in the longer-term. This supports the suggestion made by Henderson *et al.* (1994), that any potential threat posed to the C_4 pathway will not be determined by advantages lost in high CO_2, but by whether C_3 plants capitalize on the potential advantage of elevated CO_2 and thereby improve their competitive abilities.

It is possible that *Acacia* and other woody C_3 species may accumulate carbon belowground and only show significant shoot responses to elevated CO_2 in older plants with sufficient reserve carbon (Midgley *et al.* 1998), particularly if soil moisture levels are improved. Such potential phenological determinants of CO_2-responsiveness in C_3 shrubs and trees operate over years rather than one growing season as in grasses, and are difficult to demonstrate in short-term experiments. Despite the absence of a direct growth enhancement in *Acacia* seedlings, the role of rising CO_2 in the problem of widespread bush encroachment of C_4 grasslands cannot be discounted and requires further study. However, besides the potential threat from invading woody C_3 species, C_4 grasses will probably continue to thrive under increasing concentrations of CO_2 wherever they now occur in mixed C_3/C_4 or C_4-dominated tropical and sub-tropical grasslands.

My study has emphasized the importance of conducting empirical experiments which test and/or validate (or invalidate) efforts to model vegetation responses to changing atmospheric CO_2 at larger spatial scales. For example, a recent model (Collatz *et al.* 1998) which has attempted this for C_4-containing grasslands worldwide, although based on sound theoretical principles of C_3 and C_4 photosynthetic physiology and climatic preference, has failed to take into consideration the potential direct effects of high CO_2 on C_4 grasses. This model has, consequently, yielded predictions of past, current and future distributions of C_4 grasses which are inaccurate for southern Africa. It is to be hoped that emerging empirical evidence, such as presented in this thesis, will be incorporated into existing and new models in order to more accurately predict future vegetation changes.

University of Cape Town

10.2 Genetic (intrinsic) determinants of CO_2-responsiveness in C_4 grasses

10.2.1 *Themeda triandra* (C_4): intra-specific variation in CO_2-responsiveness

Curtis *et al.* (1996) stated that "the first step in understanding how communities and ecosystems evolve under elevated CO_2 requires characterization of the variation within populations that constitute the community". In other words, the intra-specific variability in the CO_2-response is a key determinant of the long-term response of that species to rising CO_2. This is a relatively new focus of interest within the elevated CO_2 debate, and although some excellent work has been done at the population level with annuals (e.g. by F.A. Bazzaz and colleagues), I am aware of only one other study investigating the CO_2-response of different ecotypes of the same grass species but differing geographical origin (Potvin & Strain 1985a, b). For *Themeda triandra* growing in southern Africa, enormous morphological and ecological differences between many different ecotypes have perplexed scientists and land managers for some time. Any predictions with regard to future performance of this species must take this into account. Potential functional differences between these ecotypes have not been studied, so the work presented here gives a first indication of the potential range of present and future functional characteristics of ecotypes of *Themeda*.

In this study, three different ecotypes of *Themeda triandra* were investigated (see Chapter 4, Chapter 5, Chapter 7, and Appendix 4). The climatic regimes of the respective regions of origin are given in Chapter 9, Table 9.4. Ecotype 1, originating from a region of moderate summer temperatures (eastern Cape), attained about three times more total plant biomass when grown in winter/spring (Fig. 4.6) as ecotype 2 (Fig. 5.2), grown in both summer and winter/spring. The former ecotype was characterised by large numbers of tillers and leaves, and a higher proportion of sheath biomass and a lower proportion of root biomass than in ecotype 2. Ecotype 3 also had a low proportion of sheath biomass, but differed from ecotype 2 in having thin, spindly and slightly taller culms and thinner leaves (Fig. 7.8). Significant increases in aboveground biomass components in response to elevated CO_2 were found during growth in winter/spring for both ecotypes 1 and 2, but not significantly for ecotype 2 during summer. In winter/spring, leaf biomass was increased for both ecotypes in elevated CO_2, but sheath biomass increased only in ecotype 1. In ecotype 3 grown in summer, leaf and total shoot growth were strongly stimulated in elevated CO_2. Root biomass was not

University of Cape Town

significantly enhanced in elevated CO_2 for ecotypes 1 or 2, although such a trend was visible for ecotype 2. Leaf length was increased in elevated CO_2 in all experiments, although not significantly in ecotype 3.

A number of generalisations could be made regarding the CO_2-responsiveness of *Themeda*. Firstly, stimulation of leaf length was a near-ubiquitous response, regardless of ecotype or growth season. Total leaf biomass enhancement was also found in all ecotypes studied, but the relative response appeared to be more dependent on growth season and developmental time (see discussion below). Secondly, biomass stimulation of each ecotype was more marked in the plant fraction that constituted a larger proportion of the total biomass in comparison with other ecotypes. So, in ecotype 1, sheath biomass was highly responsive to elevated CO_2, whereas root biomass was more responsive in ecotype 2 (although just not significant). Thirdly, relative differences in aboveground growth rate between ecotypes influenced the relative CO_2-response, but this was moderated by seasonal temperature regimes. Aboveground biomass stimulation was large in ecotype 1, the most productive ecotype, under moderately high temperatures (<30°C). This supports Hunt *et al.* (1991, 1993), Poorter *et al.* (1996), and Loehle (1995) who predicted that fast-growing competitive species will show the greatest growth responses to elevated CO_2. I propose that this may also be true for ecotypes of the same species. The shorter and less productive ecotype 2, when grown under high summer temperatures (>30°C) and greater nutrient supply rate, did not respond significantly, possibly due to a shortage of time required to develop significant effects. Plants had higher growth rates, matured more quickly, and developed more tillers with larger leaves and shorter sheaths when grown in summer and greater nutrient supply than in winter/spring with lower nutrient supply. Accelerated maturation and flowering with increasing temperature has been shown for different ecotypes of *Themeda* sourced from a range of thermal environments (Downing & Groves 1985). However, during the winter/spring season (<30°C), when plants grew more slowly and matured later, growth was stimulated in elevated CO_2 almost to the same degree as in ecotype 1. This indicates a possible cumulative effect ("compound interest") of elevated CO_2 on grass growth, which could be related to the time-dependency of morphological CO_2-induced responses such as increases in individual leaf biomass (which were measured in winter/spring in both ecotypes 1 and 2 but not in summer in ecotype 2). It appears that the generally reported increase in CO_2-responsiveness with increasing temperature (Drake & Leadley 1991, Idso & Idso 1994) is dependent on developmental and growth rates in highly seasonal plants such as tropical and

University of Cape Town

sub-tropical grasses, and CO_2-responsiveness will be lower than expected if plants grow and mature quickly under favourable conditions of high temperature and high nutrient supply. Increased nutrient supply, in spite of increasing plant growth, does not necessarily increase CO_2-responsiveness, and the CO_2-response may even be higher under lower nutrient supply (Idso & Idso 1994, McConnaughay *et al.* 1996). The productivity of ecotype 3 was more difficult to assess and compare with the other ecotypes because it was grown from excavated tillers rather than from seed, but the growth rate appeared to be moderately high when grown at high temperatures (>30°C), and shoot growth was significantly enhanced in elevated CO_2.

The responses of the three *Themeda* ecotypes to elevated CO_2 suggest the following future scenario for this species over southern Africa: All ecotypes, when growing under moderate temperatures (<30°), will show increased productivity. Ecotypes from cooler regions will also respond favourably to CO_2 enrichment when growing at higher temperatures (>30°C) e.g. ecotype 3 from southern Kwazulu/Natal. This may represent the scenario over much of the southern grassland biome if predictions of global warming are realized. However, the CO_2-response will be smaller in ecotypes from warm regions, when growing at high temperatures (>30°C) e.g. ecotype 2 from the Kruger National Park. Under these conditions, photosynthetic rates are low but growth and maturation occur quickly, and elevated CO_2 has a reduced effect possibly due to the limited time over which the effects can emerge.

10.2.2 Inter-specific and between-sub-type variation in CO_2-responsiveness

Inter-specific variation in CO_2-responsiveness has been studied to some extent mainly in C_3 species and various reasons for this variability put forward which include both genetic determinants and environmental influences (see Chapter 1, section 1.6). In my experiments which used more than one species (Chapters 7, 8), experimental growth conditions for all species were similar (Arp 1991, Idso & Idso 1994), functional aspects relating to metabolite transport and sink capacity (Körner *et al.* 1995) were unlikely to be significantly divergent for the C_4 grasses studied, and plant life history strategy (Hunt *et al.* 1991, 1993) was probably similar between a group of relatively dominant C_4 species from the same site. Therefore, inter-specific variability in CO_2-responsiveness in this study could be ascribed mainly to an inherent genetic predisposition. Some C_4 grasses responded positively to elevated CO_2 with both leaf and non-leaf growth enhancement, while others showed only non-leaf enhancement

University of Cape Town

or no response at all (Chapter 7). It was argued that the level of responsiveness may be determined by the C_4 photosynthetic sub-type, with only species belonging to the NADP-ME sub-type showing significant leaf biomass increases in elevated CO_2. This is only the second report of differential CO_2-responses in C_4 sub-types, but the first to report detailed gas exchange and growth analyses at the specific and sub-type level for all three sub-types.

An evolutionary explanation for CO_2-responsiveness in C_4 grasses was suggested as a potentially fruitful line of investigation, and is appealing in the light of strong evidence that C_4 species evolved in response to decreasing atmospheric CO_2 levels (Cerling *et al.* 1998), but that some sub-types (notably NAD-ME) appear to have evolved more specifically in response to reduced water availability (Hattersley & Watson 1992). Nevertheless, variability at the species level may not be discounted, since not all NADP-ME species showed increased growth under elevated CO_2, and non-leaf growth enhancement in the other two sub-types was also species-specific. This will complicate any attempt at making predictions regarding the future productivity of C_4 grasslands in southern Africa. At most, it could be predicted that the eastern mesic regions of South Africa having a high proportion of NADP-ME species, particularly those belonging to the supertribe Andropogonodae such as *Themeda* and *Andropogon*, will be more likely to show significantly increased leaf productivity at a community level under rising CO_2 concentrations. On the other hand, the more semi-arid grasslands to the west of the country may also benefit from elevated CO_2, depending on individual species' responsiveness, and particularly if the positive effects on plant and soil water relations are incorporated.

Results obtained from the field experiment for three C_4 grass species (Chapter 8) supported the findings of differential CO_2-responsiveness measured in the greenhouse. More importantly, this experiment demonstrated the phenological dependence of the CO_2-response in different species, and underscored the need to monitor responses throughout the growth period before arriving at conclusions regarding CO_2-responsiveness. Grassland responses to high CO_2 could fluctuate seasonally, depending on which species are most active at any given time and their proportional representation in the community. Such dynamics could be of benefit to managers with regard to palatable species, such as *Themeda*, but could increase the problems associated with unpalatable and/or invasive grass species at certain times in the seasonal or successional cycles.

University of Cape Town

10.3 Mechanisms of the CO_2-response in C_4 grasses

Positive responses of C_4 grass species to elevated CO_2 were generally found at the leaf level (increased photosynthetic rates, decreased stomatal conductance, reduced transpiration, and increased instantaneous leaf water use efficiency), but positive responses also emerged at the whole plant level (increased biomass and increased leaf area). One of the most consistent and potentially highly ecologically significant findings was the large improvement in water use efficiency (Chapters 4,7,8), which could be of particular importance for southern African semi-arid grasslands. Although this response is well-established for many plant growth forms worldwide, including C_4 grasses (Owensby *et al.* 1997), this is the first confirmation of large improvements in WUE for a dominant group of southern African species. This group (C_4 grasses) clads our major catchments and plays an important role in determining water yield for the countries in the region.

The improvement in plant and leaf water relations which must result from stomatal closure and its effect on transpiration, is likely one mechanism by which leaf growth is stimulated in C_4 grasses in elevated CO_2. Another stimulus may be the increased leaf temperatures which result from the reductions in transpiration (Chapter 9). Although some researchers have previously shown increases in leaf temperature in elevated CO_2, the link with improved leaf growth, particularly in graminoid leaves, has to my knowledge not been put forward previously. I have also demonstrated the direct enhancement of growth via increased carbon assimilation in some southern African C_4 grasses. Growth enhancement in *Themeda* was maintained according to the rule of "compound interest" even after photosynthetic capacity was reduced after prolonged exposure to elevated CO_2 (Chapter 6), possibly owing to more rapid growth, maturation and/or self-shading. Increasing leaf length and leaf area were early responses which in turn allowed for greater canopy carbon assimilation as plants matured.

10.4 Implications for productivity with and without defoliation

The results and arguments presented in Chapter 5 apply only to *Themeda*, and need to be tested using other palatable C_4 grass species. Nevertheless, *Themeda* is dominant or at least widespread over many productive rangelands throughout southern Africa, and its future in a high CO_2 world will be of importance to many managers. This study has shown with some certainty that, even in the absence of defoliation, *Themeda* will display increased productivity, at least during the early growth stages following the last defoliation, or in

University of Cape Town

seedlings. However, increased leaf area and taller plants in high CO_2 will eventually lead to more rapid self-shading and self-inhibition and moribund stands, which could dampen any further responses to elevated CO_2. With regular moderate defoliation, on the other hand, increased productivity will be sustained for longer, and grazing frequency may even be increased. If grazing pressure is increased too much, this benefit will gradually be lost and result in reduced productivity and reduced CO_2-responsiveness, due to inadequate supply of nitrogen and possibly other nutrients to fund this growth. In fact, the potential benefits of elevated CO_2 for productivity must be weighed up against the likely reductions of forage quality and palatability because of increasing carbon:nitrogen ratios, since leaf nitrogen concentrations are reduced (Owensby *et al.* 1996a).

10.5 CO_2-responses and other concomitant climate changes

C_4 grass species, and *Themeda* in particular, appear to be highly adaptable to changes in temperature (Chapter 9, Downing & Groves 1985). With the predicted 1-2°C increase in mean temperatures over southern Africa, this could be of critical importance to the sustained productivity of grasslands. An expansion of the growth season for grasses is possible in the cooler regions (Long & Hutchin 1991), provided that soil water and nutrient supply are not limiting. We may even see a southerly and altitudinal expansion of C_4 grass species into cooler regions, aided by higher CO_2-responsiveness at moderate temperatures (similar to the northerly expansion of *Echinochloa* in North America, Potvin & Strain 1985a, b). Although increased temperatures and altered rainfall regimes could lead to aridification, this will very likely be countered by the positive effects of elevated CO_2 on plant and soil water relations (as modelled by Hall *et al.* 1995, Parton *et al.* 1995 and Hulme 1996). Increased soil water availability, particularly towards the end of the rainy season when soils are slowly drying out, could extend grass growth for days or even weeks. In this way, the benefits of growth elevated CO_2 can be enjoyed for a longer period. At the very least, the direct and indirect effects of elevated CO_2 will help to sustain grassland productivity at current levels, rather than decrease under conditions of aridification (Ellery *et al.* 1991, Hulme 1996).

Increasing global temperatures and altered rainfall patterns are expected to be accompanied by increases in ultraviolet-B radiation reaching the earth, due to the gradual thinning of the ozone layer. My study of the interactive effects of elevated CO_2 and UV-B on *Themeda* (Chapter 4) showed that high UV-B levels may slightly reduce the CO_2-induced

University of Cape Town

increases in leaf and shoot growth, but this is unlikely to alter significantly the positive responses of *Themeda* to elevated CO_2.

10.6 Conclusion

The widely-held belief that C_4 species will not show the same positive responses to rising atmospheric CO_2 concentrations as C_3 species, must be re-assessed in the context of mixed C_3/C_4 or C_4-dominated grassland responses for southern Africa. In this thesis, I have presented evidence that some dominant southern African C_4 grasses can respond significantly to rising atmospheric CO_2 concentrations predicted for the next century. A quantitative literature survey highlighted the finding that wild C_3 grasses are not inherently more responsive to elevated CO_2 than wild C_4 grasses. Controlled greenhouse experiments showed that various ecotypes of the widespread and economically important African C_4 grass *Themeda triandra* showed significant and relatively consistent growth enhancement under elevated CO_2, mainly as a result of increased carbon assimilation at a canopy level and improved leaf area development. Other species which responded positively to elevated CO_2 were *Andropogon appendiculatus*, *Eragrostis curvula* and *Sporobolus pyramidalis*. However, not all C_4 grass species responded to elevated CO_2. Inter-specific variability and the phenological dependence of CO_2-responsiveness largely preclude the formulation of confident predictions for grassland responses to rising CO_2 at smaller spatial scales, as this will depend on the species mix. Nevertheless, the findings presented in this thesis provide evidence that tropical and sub-tropical African C_4-dominated grasslands could constitute significant sinks for anthropogenically emitted CO_2 due to the direct stimulatory effects of elevated CO_2 on photosynthesis and growth in some species, as well as the indirect stimulation of productivity owing to the ameliorating effects of elevated CO_2 on water use of grasslands.

University of Cape Town

References

Ackerly, D.D., Coleman, J.S., Morse, S.R. & Bazzaz, F.A. (1992) CO_2 and temperature effects on leaf area production in two annual plant species. *Ecology* **73**, 1260-1269.

Acocks, J.P.H. (1988) Veld types of South Africa. *Memoirs of the Botanical Society of South Africa* **57**.

Anderson, J.E. (1982) Factors controlling transpiration and photosynthesis in *Tamarix chinensis* Lour. *Ecology* **63**, 48-56.

Archer, S., Schimel, D.S. & Holland, E.A. (1995) Mechanisms of shrubland expansion: land use, climate or CO_2? *Climatic Change* **29**, 91-99.

Archer, S., Scifres, C., Bassham, C.R. & Maggio, R. (1988) Autogenic succession in a subtropical savanna: conversion of grassland to thorn woodland. *Ecological Monographs* **58**, 111-127.

Armond, P.A., Schreiber, U. & Björkman, O. (1978) Photosynthetic acclimation to temperature in the desert shrub, *Larrea divaricata*. II. Light-harvesting efficiency and electron transport. *Plant Physiology* **61**, 411-415.

Arnone III, J.A. & Gordon, J.C. (1990) Effect of nodulation, nitrogen fixation and CO_2 enrichment on the physiology, growth and dry mass allocation of seedlings of *Alnus rubra* Bong. *New Phytologist* **116**, 55-66.

Arnone III, J.A., Zaller, J.G., Ziegler, C., Zandt, H. & Körner, Ch. (1995) Leaf quality and insect herbivory in model tropical plant communities after long-term exposure to elevated atmospheric CO_2. *Oecologia* **104**, 72-78.

Arp, W.J. (1991) Effects of source-sink relations on photosynthetic acclimation to elevated CO_2. *Plant, Cell and Environment* **14**, 869-875.

Arp, W.J., Drake, B.G., Pockman, W.T., Curtis, P.S. & Whigman, D.F. (1993) Interactions between C_3 and C_4 salt marsh plant species during four years of exposure to elevated atmospheric CO_2. *Vegetatio* **104/105**, 133-143.

Badger, M.R., Björkman, O. & Armond, P.A. (1982) An analysis of photosynthetic response and adaptation to temperature in higher plants: temperature acclimation in the desert evergreen *Nerium oleander* L. *Plant, Cell and Environment* **5**, 85-99.

Barnes, P.W., Flint, S.D. & Caldwell, M.M. (1990) Morphological responses of crop and weed species of different growth forms to ultraviolet-B radiation. *American Journal of Botany* 77, 1354-1360.

Battaglia, M., Beadle, C. & Loughhead, S. (1996) Photosynthetic temperature responses of *Eucalyptus globulus* and *Eucalyptus nitens*. *Tree Physiology* **16**, 81-89.

Baxter, R., Bell, S.A., Sparks, T.H., Ashenden, T.W. & Farrar, J.F. (1995) Effects of elevated CO_2 concentrations on three montane grass species. III. Source leaf metabolism and whole plant carbon partitioning. *Journal of Experimental Botany* **46**, 917-929.

Bazzaz, F.A. (1990) The response of natural ecosystems to the rising global CO_2 levels. *Annual Review of Ecology and Systematics* **21**, 167-196.

Bazzaz, F.A. & Carlson, R.W. (1984) The response of plants to elevated CO_2. I. Competition among an assemblage of annuals at two levels of soil moisture. *Oecologia* **62**, 196-198.

University of Cape Town

Bazzaz, F.A. & McConnaughay, K.D.M. (1992) Plant-plant interactions in elevated CO_2 environments. *Australian Journal of Botany* **40**, 547-563.

Bazzaz, F.A. & Miao, S.L. (1993) Successional status, seed size, and responses of tree seedlings to CO_2, light, and nutrients. *Ecology* **74**, 104-112.

Bazzaz, F.A., Garbutt, K., Reekie, E.G. & Williams, W.E. (1989) Using growth analysis to interpret competition between a C_3 and a C_4 annual under ambient and elevated CO_2. *Oecologia* **79**, 223-235.

Berry, J. & Björkman, O. (1980) Photosynthetic response and adaptation to temperature in higher plants. *Annual Review of Plant Physiology* **31**, 491-543.

Booth, R.E., Mackey, J.M.L., Rorison, I.H., Spencer, R.E., Gupta, P.L. & Hunt, R. (1993) ISP germination and rooting environments: sand, compost and solution culture. In *Methods in Comparative Plant Ecology* (eds G.A.F. Hendry & J.P. Grime), pp. 19-24. Chapman & Hall, London.

Bornman, J.F. & Vogelmann, T.C. (1991) The effect of UV-B radiation on leaf optical properties measured with fiber optics. *Journal of Experimental Botany* **42**, 547-554.

Botha, C.E.J. & Russel, S. (1988) Comparison of photosynthetic temperature response and light optima in selected C_3 and C_4 grasses from the E. Cape/Ciskei region. *South African Journal of Botany* **54**, 133-136.

Bowes, G. (1993) Facing the inevitable: plants and increasing atmospheric CO_2. *Annual Review of Plant Physiology and Plant Molecular Biology* **44**, 309-332.

Bowler, J.M. & Press, M.C. (1993) Growth responses of two contrasting upland grass species to elevated CO_2 and nitrogen concentration. *New Phytologist* **124**, 515-522.

Bowler, J.M. & Press, M.C. (1996) Effects of elevated CO_2, nitrogen form and concentration on growth and photosynthesis of a fast- and slow-growing grass. *New Phytologist* **132**, 391-401.

Box, E.O. (1996) Plant functional types and climate at the global scale. *Journal of Vegetation Science* **7**, 309-320.

Boyer, J.S. (1970) Leaf enlargement and metabolic rates in corn, soybean, and sunflower at various leaf water potentials. *Plant Physiology* **46**, 233-235.

Bremer, D.J., Ham, J.M. & Owensby, C.E. (1996) Effect of elevated atmospheric carbon dioxide and open-top chambers on transpiration in a tallgrass prairie. *Journal of Environmental Quality* **25**, 691-701.

Brown, R.H. (1978) A difference in N use efficiency in C_3 and C_4 plants and its implications in adaptation and evolution. *Crop Science* **18**, 93-98.

Brown, R.H. & Byrd, G.T. (1993) Estimation of bundle sheath cell conductance in C_4 species and O_2 insensitivity of photosynthesis. *Plant Physiology* **103**, 1183-1188.

Bunce, J.A. (1993) Effects of doubled atmospheric carbon dioxide concentration on the responses of assimilation and conductance to humidity. *Plant, Cell and Environment* **16**, 189-197.

Bunce, J.A. (1996) Does transpiration control stomatal responses to water vapour pressure deficit? *Plant, Cell and Environment* **19**, 131-135.

Bunce, J.A. (1998) Effects of environment during growth on the sensitivity of leaf conductance to changes in humidity. *Global Change Biology* **4**, 269-274.

University of Cape Town

Buysse, J. & Merckx, R. (1993) An improved colorimetric method to quantify sugar content of plant tissue. *Journal of Experimental Botany* **44**, 1627-1629.

Caldwell, M.M. (1971) Solar ultraviolet radiation and the growth and development of higher plants. In *Photophysiology*, Vol. 6 (ed. A.C. Giese), pp. 131-177. Academic Press, New York.

Caldwell, M.M. & Flint, S.D. (1994) Stratospheric ozone reduction, solar UV-B radiation and terrestrial ecosystems. *Climatic Change* **28**, 375-394.

Caldwell, M.M., Richards, J.H., Johnson, D.A., Nowak, R.S. & Dzurec, R.S. (1981) Coping with herbivory: photosynthetic capacity and resource allocation in two semiarid *Agropyron* bunchgrasses. *Oecologia* **50**, 14-24.

Carter, D.R. & Peterson, K.M. (1983) Effects of a CO_2-enriched atmosphere on the growth and competitive interaction of a C_3 and a C_4 grass. *Oecologia* **58,** 188-193.

Causton, D.R. & Dale, M.P. (1990) The monomolecular and rectangular hyperbola as empirical models of the response of photosynthetic rate to photon flux density, with applications to three *Veronica* species. *Annals of Botany* **65**, 389-394.

Cavagnaro, J.B. (1988) Distribution of C_3 and C_4 grasses at different altitudes in a temperate arid region of Argentina. *Oecologia* **76,** 273-277.

Cerling, T.E., Ehleringer, J.R. & Harris, J.M. (1998) Carbon dioxide starvation, the development of C_4 ecosystems, and mammalian evolution. *Philosophical Transactions of the Royal Society London Series B* **353**, 159-171.

Ceulemans, R. & Mousseau, M. (1994) Effects of elevated atmospheric CO_2 on woody plants. *New Phytologist* **127**, 425-446.

Chaves, M.M. & Pereira, J.S. (1992) Water stress, CO_2 and climate change. *Journal of Experimental Botany* **43**, 1131-1139.

Chen, D-X., Coughenour, M.B., Knapp, A.K. & Owensby, C.E. (1994) Mathematical simulation of C_4 grass photosynthesis in ambient and elevated CO_2. *Ecological Modelling* **73**, 63-80.

Chen, D.-X., Hunt, H.W. & Morgan, J.A. (1996) Responses of a C_3 and C_4 perennial grass to CO_2 enrichment and climate change: Comparison between model predictions and experimental data. *Ecological Modelling* **87**, 11-27.

Clifton-Brown, J.C. & Jones, M.B. (1997) The thermal response of leaf extension rate in genotypes of the C_4-grass *Miscanthus*: an important factor in determining the potential productivity of different genotypes. *Journal of Experimental Botany* **48**, 1573-1581.

Climate Change: a South African Policy Discussion Document (1998) Department of Environmental Affairs and Tourism, South Africa.

Cole, D.R. & Monger, H.C. (1994) Influence of atmospheric CO_2 on the decline of C_4 plants during the last deglaciation. *Nature* **368,** 533-536.

Coleman, J.S. & Bazzaz, F.A. (1992) Effects of CO_2 and temperature on growth and resource use of co-occurring C_3 and C_4 annuals. *Ecology* **73**, 1244-1259.

Collatz, G.J., Berry, J.A. & Clark, J.S. (1998) Effects of climate and atmospheric CO_2 partial pressure on the global distribution of C_4 grasses: present, past, and future. *Oecologia* **114**, 441-454.

University of Cape Town

Collatz, G.J., Ribas-Carbo, M. & Berry, J.A. (1992) Coupled photosynthesis-stomatal conductance model for leaves of C_4 plants. *Australian Journal of Plant Physiology* **19**, 519-538.

Conroy, J.P. (1992) Influence of elevated atmospheric CO_2 concentrations on plant nutrition. *Australian Journal of Botany* **40**, 445-456.

Cook, A.C., Tissue, D.T., Roberts, S.W. & Oechel, W.C. (1998) Effects of long-term elevated [CO_2] from natural CO_2 springs on *Nardus stricta*: photosynthesis, biochemistry, growth and phenology. *Plant, Cell and Environment* **21**, 417-425.

Cotrufo, M.F., Ineson, P. & Scott, A. (1998) Elevated CO_2 reduces the nitrogen concentration of plant tissues. *Global Change Biology* **4**, 43-54.

Coughenour, M.B., McNaughton, S.J. & Wallace, L.L. (1985) Responses of an African graminoid (*Themeda triandra* Forsk.) to frequent defoliation, nitrogen, and water: a limit of adaptation to herbivory. *Oecologia* **68**, 105-110.

Cure, J.D. & Acock, B. (1986) Crop response to carbon dioxide doubling: a literature survey. *Agricultural and Forest Meteorology* **38**, 127-145.

Curtis, P.S. (1996) A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. *Plant, Cell and Environment* **19**, 127-137.

Curtis, P.S. & Wang, X. (1998) A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. *Oecologia* **113**, 299-313.

Curtis, P.S., Drake, B.G., Leadley, P.W., Arp, W.J. & Whigham, D.F. (1989) Growth and senescence in plant communities exposed to elevated CO_2 concentrations on an estuarine marsh. *Oecologia* **78**, 20-26.

Curtis, P.S., Klus, D.J., Kalisz, S. & Tonsor, S.J. (1996) Intraspecific variation in CO_2 responses in *Raphanus raphanistrum* and *Plantago lanceolata*: assessing the potential for evolutionary change with rising atmospheric CO_2. In *Carbon Dioxide, Populations, and Communities* (eds Ch. Körner & F.A. Bazzaz), pp. 13-22. Academic Press, San Diego.

Curtis, P.S., Snow, A.A. & Miller, A.S. (1994) Genotype-specific effects of elevated CO_2 on fecundity in wild radish (*Raphanus raphanistrum*). *Oecologia* **97**, 100-105.

Dai, Z., Ku, M.S.B. & Edwards, G.E. (1995) C_4 photosynthesis: The effects of leaf development on the CO_2-concentrating mechanism and photorespiration in maize. *Plant Physiology* **107**, 815-825.

Danckwerts, J.E. (1993) Reserve carbon and photosynthesis: their role in regrowth of *Themeda triandra*, a widely distributed subtropical graminaceous species. *Functional Ecology* 7, 634-641.

Danckwerts, J.E. & Gordon, A.J. (1990) Partitioning, storage and remobilization of ^{14}C assimilated by *Themeda triandra* Forskk. *Journal of the Grassland Society of Southern Africa* 7, 97-105.

Danckwerts, J.E. & Nel, L.O. (1989) The effect of frequency of defoliation on *Themeda triandra* in the False Thornveld of the eastern Cape. *Journal of the Grassland Society of Southern Africa* **6**, 32-36.

Deregibus, V.A., Sanchez, R.A., Casal, J.J. & Trlica, M.J. (1985) Tillering responses to enrichment of red light beneath the canopy in a humid natural grassland. *Journal of Applied Ecology* **22**, 199-206.

Diaz, S. (1995) Elevated CO_2 responsiveness, interactions at the community level and plant functional types. *Journal of Biogeography* **22**, 289-295.

University of Cape Town

Diaz, S. & Cabido, M. (1997) Plant functional types and ecosystem function in relation to global change. *Journal of Vegetation Science* **8**, 463-474.

Downing, B.H. & Groves, R.H. (1985) Growth and development of four *Themeda triandra* populations from southern Africa in response to temperature. *South African Journal of Botany* **51**, 350-354.

Drake, B.G. & Leadley, P.W. (1991) Canopy photosynthesis of crops and native plant communities exposed to long-term elevated CO_2. *Plant, Cell and Environment* **14**, 853-860.

Drake, B.G., Gonzàlez-Meler, M.A. & Long, S.P. (1997) More efficient plants: a consequence of rising atmospheric CO_2? *Annual Review of Plant Physiology and Plant Molecular Biology* **48**, 609-639.

Drake, B.G., Peresta, G., Beugeling, E. & Matamala, R. (1996) Long-term elevated CO_2 exposure in a Chesapeake Bay wetland: ecosystem gas exchange, primary production, and tissue nitrogen. In *Carbon Dioxide and Terrestrial Ecosystems* (eds G.W. Koch & H.A. Mooney.), pp. 197-214. Academic Press, San Diego.

Ehleringer, J.R. & Björkman, O. (1977) Quantum yields for CO_2 uptake in C_3 and C_4 plants: dependence on temperature, CO_2, and O_2 concentration. *Plant Physiology* **59**, 86-90.

Ehleringer, J.R. & Monson, R.K. (1993) Evolutionary and ecological aspects of photosynthetic pathway variation. *Annual Review of Ecology and Systematics* **24**, 411-439.

Ehleringer, J.R. & Pearcy, R.W. (1983) Variation in quantum yields for CO_2 uptake among C_3 and C_4 plants. *Plant Physiology* **73**, 555-559.

Ehleringer, J.R., Cerling, T.E. & Helliker, B.R. (1997) C_4 photosynthesis, atmospheric CO_2, and climate. *Oecologia* **112**, 285-299.

Ellery, W. N., Scholes, R. J. & Mentis, M. T. (1991) An initial approach to predicting the sensitivity of the South African grassland biome to climate change. *South African Journal of Science* **87**, 499-503.

Ellery, W.N., Scholes, R.J. & Scholes, M.C. (1995) The distribution of sweetveld and sourveld in South Africa's grassland biome in relation to environmental factors. *African Journal of Range and Forage Science* **12**, 38-45.

Ellis, R.P., Vogel, J.C. & Fuls, A. (1980) Photosynthetic pathways and the geographical distribution of grasses in South West Africa/Namibia. *South African Journal of Science* **76**, 307-314.

Enoch, H.Z. & Kimball, B.A. (1986) *Carbon dioxide enrichment of greenhouse crops. Volume II: Physiology, yield and economics.* CRC Press Inc., Boca Raton. Florida.

Everson, C.S., Everson, T.M. & Tainton, N.M. (1988) Effects of intensity and height of shading on the tiller initiation of six grass species from the Highland sourveld of Natal. *South African Journal of Botany* **54**, 315-318.

Fajer, E.D., Bowers, M.D. & Bazzaz, F.A. (1989) The effects of enriched carbon dioxide atmospheres on plant-insect herbivore interactions. *Science* **243**, 1198-1200.

Fajer, E.D., Bowers M.D. & Bazzaz, F.A. (1991) Performance and allocation patterns of the perennial herb, *Plantago lanceolata*, in response to simulated herbivory and elevated CO_2 environments. *Oecologia* **87**, 37-42.

Farquhar, G.D. (1983) On the nature of carbon isotope discrimination in C_4 species. *Australian Journal of Plant Physiology* **10**, 205-226.

University of Cape Town

Farquhar, G.D. & Sharkey, T.D. (1982) Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology* **33**, 317-345.

FCCC (1992) *Framework Convention on Climate Change*. United Nations Conference on Environment and Development, Rio de Janeiro, Brazil.

Field, C.B., Chapin III, F.S., Chiariello, N.R., Holland, E.A. & Mooney, H.A. (1996) The Jasper Ridge CO_2 experiment: design and motivation. In *Carbon Dioxide and Terrestrial Ecosystems* (eds G.W. Koch & H.A. Mooney), pp. 121-145, Academic Press, San Diego.

Field, C.B., Lund, C.P., Chiariello, N.R. & Mortimer, B.E. (1997) CO_2 effects on the water budget of grassland microcosm communities. *Global Change Biology* **3**, 197-206.

Fischer, B.U., Frehner, M., Hebeisen, T., Zanetti, S., Stadelmann, F., Lüscher, A., Hartwig, U.A., Hendrey, G.R., Blum, H. & Nösberger, J. (1997) Source-sink relations in *Lolium perenne* L. as reflected by carbohydrate concentrations in leaves and pseudo-stems during regrowth in a free air carbon dioxide enrichment (FACE) experiment. *Plant, Cell and Environment* **20**, 945-952.

Fitter, A.H. & Hay, R.K.M. (1987) *Environmental Physiology of Plants*. Second Edition. Academic Press, London, San Diego.

Furbank, R.T. & Hatch, M.D. (1987) Mechanism of C_4 photosynthesis. The size and composition of the inorganic carbon pool in bundle-sheath cells. *Plant Physiology* **85**, 958-964.

Gallagher, J.N. (1979) Field studies of cereal leaf growth. I. Initiation and expansion in relation to temperature and ontogeny. *Journal of Experimental Botany* **30**, 625-636.

Garbutt, K. & Bazzaz, F.A. (1984) The effects of elevated CO_2 on plants. III. Flower, fruit and seed production and abortion. *New Phytologist* **98**, 433-446.

Garbutt, K., Williams, W.E. & Bazzaz, F.A. (1990) Analysis of the differential response of five annuals to elevated CO_2 during growth. *Ecology* **71**, 1185-1194.

Gates, D.M. (1993) *Climate Change and its Biological Consequences*. Sinauer Associates, Inc., Sunderland, Massachusetts, USA.

Gevers, T.W. (1941) Carbon dioxide springs and exhalations in northern Pondoland and Alfred County, Natal. *Transactions of the Geological Society of South Africa* **44**, 233-301.

Ghannoum, O. & Conroy, J.P. (1998) Nitrogen deficiency precludes a growth response to CO_2 enrichment in one C_3 and two C_4 *Panicum* grasses. *Australian Journal of Plant Physiology* **25**, 627-636.

Ghannoum, O., Siebke, K., von Caemmerer, S. & Conroy, J.P. (1998) The photosynthesis of young *Panicum* C_4 leaves is not C_3-like. *Plant, Cell and Environment* **21**, 1123-1131.

Ghannoum, O., von Caemmerer, S., Barlow, E.W.R. & Conroy, J.P. (1997) The effect of CO_2 enrichment and irradiance on the growth, morphology and gas exchange of a C_3 (*Panicum laxum*) and a C_4 (*Panicum antidotale*) grass. *Australian Journal of Plant Physiology* **24**, 227-237.

Giambelluca, T.W. & Henderson-Sellers, A. (eds) (1996) *Climate Change. Developing Southern Hemisphere Perspectives*. John Wiley & Sons, Chichester, U.K.

Gibbs Russel, G.E., Watson, L., Koekemoer, M., Smook, L., Barker, N.P., Anderson, H.M. & Dallwitz, M.J. (1990) *Grasses of Southern Africa*. Memoirs of the Botanical Survey of South Africa No. 58, National Botanic Gardens/Botanical Research Institute, South Africa.

University of Cape Town

Gifford, R.M. & Morison, J.I.L. (1985) Photosynthesis, water use and growth of a C_4 grass stand at high CO_2 concentration. *Photosynthesis Research* 7, 77-90.

Green, A.E.S. (1983) The penetration of ultraviolet radiation to the ground. *Physiologia Plantarum* **58**, 351-359.

Gurevitch, J. & Hedges, L.V. (1993) Meta-analysis: combining the results of independent experiments. In: *Design and Analysis of Ecological Experiments* (eds S.M. Scheiner & J. Gurevitch), pp 378-398. Chapman and Hall, New York.

Hall, D.O. & Scurlock, J.M.O. (1991) Climate change and productivity of natural grasslands. *Annals of Botany* **67** (Supplement 1), 49-55.

Hall, D.O., Ojima, D.S., Parton, W.J. & Scurlock, J.M.O. (1995) Response of temperate and tropical grasslands to CO_2 and climate change. *Journal of Biogeography* **22**, 537-547.

Ham, J.M., Owensby, C.E., Coyne, P.I. & Bremer, D.J. (1995) Fluxes of CO_2 and water vapour from a prairie ecosystem exposed to ambient and elevated atmospheric CO_2. *Agricultural and Forest Meteorology* 77, 73-93.

Hamerlynck, E.P., McAllister, C.A., Knapp, A.K., Ham, J.M. & Owensby, C.E. (1997) Photosynthetic gas exchange and water relation responses of three tallgrass prairie species to elevated carbon dioxide and moderate drought. *International Journal of Plant Sciences* **158**, 608-616.

Harley, P.C., Thomas, R.B., Reynolds, J.F. & Strain, B.R. (1992) Modelling photosynthesis of cotton grown in elevated CO_2. *Plant, Cell and Environment* **15**, 271-282.

Harris, C., Stock, W.D. & Lanham, J. (1997) Stable isotope constraints on the origin of CO_2 gas exhalations at Bongwan, Natal. *South African Journal of Geology* **100**, 261-266.

Hartwig, U.A., Zanetti, S., Hebeisen, T., Lüscher, A., Frehner, M., Fischer, B., van Kessel, C., Hendrey, G.R., Blum, H. & Nösberger, J. (1996) Symbiotic nitrogen fixation: one key to understand the response of temperate grassland ecosystems to elevated CO_2? In *Carbon Dioxide, Populations, and Communities* (eds Ch. Körner & F.A. Bazzaz), pp. 253-264. Academic Press, San Diego.

Hatch, M.D. & Osmond, C.B. (1976) Compartmentation and transport in C_4 photosynthesis. In *Encyclopedia of Plant Physiology, New Series,* Vol. 3 (ed. C.R. Stocking & U. Heber), pp. 144-184. Springer-Verlag, Berlin, Heidelberg, New York.

Hatch, M.D., Agostino, A. & Jenkins, C.L.D. (1995) Measurement of the leakage of CO_2 from bundle-sheath cells of leaves during C_4 photosynthesis. *Plant Physiology* **108**, 173-181.

Hatch, M.D. & Slack, C.R. (1966) Photosynthesis by sugar-cane leaves. A new carboxylation reaction and the pathway of sugar formation. *Biochemical Journal* **101**, 103-111.

Hattersley, P.W. (1982) $\delta^{13}C$ values of C_4 types in grasses. *Australian Journal of Plant Physiology* **9**, 139-154.

Hattersley, P.W. (1983) The distribution of C_3 and C_4 grasses in Australia in relation to climate. *Oecologia* **57**, 113-128.

Hattersley, P.W. (1992) C_4 photosynthetic pathway variation in grasses (Poaceae): its significance for arid and semi-arid lands. In *Desertified Grasslands: their Biology and Management* (ed. G.P. Chapman), pp. 181-212. Linnean Society Symposium Series 13, Academic Press, London.

University of Cape Town

Hattersley, P.W. & Watson, L. (1992) Diversification of photosynthesis. In *Grass Evolution and Domestication* (ed. G.P. Chapman), pp. 38-116, Cambridge University Press, Cambridge.

He, D. & Edwards, G.E. (1996) Estimation of diffusive resistance of bundle sheath cells to CO_2 from modeling of C_4 photosynthesis. *Photosynthesis Research* **49**, 195-208.

Hedges, L.V., Gurevitch, J. & Curtis, P.S. (in press) The meta-analysis of response ratios in experimental ecology. *Ecology*.

Henderson, S., Hattersley, P., von Caemmerer, S. & Osmond, C.B. (1994) Are C_4 pathway plants threatened by global climatic change? In *Ecophysiology of Photosynthesis* (eds E.-D. Schulze & M.M. Caldwell), pp. 529-549. Springer-Verlag, Heidelberg.

Hiernaux, P. & Turner, M.D. (1996) The effect of clipping on growth and nutrient uptake of Sahelian annual rangelands. *Journal of Applied Ecology* **33**, 387-399.

Hikosaka, K. (1997) Modelling optimal temperature acclimation of the photosynthetic apparatus in C_3 plants with respect to nitrogen use. *Annals of Botany* **80**, 721-730.

Hodgkinson, K.C., Ludlow, M.M., Mott, J.J. & Baruch,Z. (1989) Comparative responses of the Savanna grasses *Cenchrus ciliaris* and *Themeda triandra* to defoliation. *Oecologia* **79**, 45-52.

Hogan, K.P., Smith, A.P. & Ziska, L.H. (1991) Potential effects of elevated CO_2 and changes in temperature on tropical plants. *Plant, Cell and Environment* **14**, 763-778.

Houghton, J. (1997) *Global Warming: the Complete Briefing*. Cambridge University Press, Cambridge.

Hulme, M. (ed.) (1996) *Climate change and southern Africa: an exploration of some potential impacts and implications in the SADC region.* Climatic Research Unit, UEA, Norwich, UK, and WWF International, Switzerland.

Hunt, H.W., Elliott, E.T., Detling, J.K., Morgan, J.A. & Chen, D.-X. (1996) Responses of a C_3 and a C_4 perennial grass to elevated CO_2 and temperature under different water regimes. *Global Change Biology* **2**, 35-47.

Hunt, R., Hand, D.W., Hannah, M.A. & Neal, A.M. (1991) Response to CO_2 enrichment in 27 herbaceous species. *Functional Ecology* **5**, 410-421.

Hunt, R., Hand, D.W., Hannah, M.A. & Neal, A.M. (1993) Further responses to CO_2 enrichment in British herbaceous species. *Functional Ecology* **7**, 661-668.

Hurt, C.R., Hardy, M.B. & Tainton, N.M. (1993) Identification of key grass species under grazing in the Highland Sourveld of Natal. *African Journal of Range and Forage Science* **10**, 96-102.

Idso, K.E. & Idso, S.B. (1994) Plant responses to atmospheric CO_2 enrichment in the face of environmental constraints: a review of the past 10 years' research. *Agricultural and Forest Meteorology* **69**, 153-203.

Idso, S.B., Kimball, B.A. & Mauney, J.R. (1987) Atmospheric carbon dioxide enrichment effects on cotton midday foliage temperature: Implications for plant water use and crop yield. *Agronomy Journal* **79**, 667-672.

Idso, S.B., Kimball, B.A., Akin, D.E. & Kridler, J. (1993) A general relationship between CO_2-induced reductions in stomatal conductance and concomitant increases in foliage temperature. *Environmental and Experimental Botany* **33**, 443-446.

University of Cape Town

Imai, K. & Okamoto-Sato, M. (1991) Effects of temperature and CO_2 dependence of gas exchanges in C_3 and C_4 crop plants. *Japanese Journal of Crop Science* **60**, 139-145.

IPCC (1995) *Climate Change 1995. Impacts, adaptations and mitigation of climate change: scientific-technical analyses.* Intergovernmental Panel on Climate Change (IPCC), Cambridge University Press, Cambridge.

Jenkins, C.L.D., Furbank, R.T. & Hatch, M.D. (1989) Mechanism of C_4 photosynthesis.. A model describing the inorganic carbon pool in bundle sheath cells. *Plant Physiology* **91**, 1372-1381.

Johnson, H.B., Polley, H.W. & Mayeux, H.S. (1993) Increasing CO_2 and plant-plant interactions: effects on natural vegetation. *Vegetatio* **104/105**, 157-170.

Keeling, C.D. (1986) *Atmospheric CO_2 Concentrations.* Mauna Loa Observatory, Hawaii 1958-1986. NDP-001/R1. Carbon dioxide information analysis center. Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Kemp, P.R. & Williams III, G.J. (1980) A physiological basis for niche separation between *Agropyron smithii* (C_3) and *Bouteloua gracilis* (C_4). *Ecology* **61**, 846-858.

Kerr, J.B. & McElroy, C.T. (1993) Evidence for large upward trends of ultraviolet-B radiation linked to ozone depletion. *Science* **262**, 1032-1034.

Kirkham, M.B., He, H., Bolger, T.P., Lawlor, D.J. & Kanemasu, E.T. (1991) Leaf photosynthesis and water use of big bluestem under elevated carbon dioxide. *Crop Science* **31**, 1589-1594.

Kirkham, M.B., Nie, D., He, H. & Kanemasu, E.T. (1993) Responses of plants to elevated levels of carbon dioxide. *Proceedings of the Symposium on Plant Growth and Environment, Suwon, Korea*, pp. 130-161.

Knapp, A.K., Hamerlynck, E.P. & Owensby, C.E. (1993) Photosynthetic and water relations responses to elevated CO_2 in the C_4 grass *Andropogon gerardii*. *International Journal of Plant Science* **154**, 459-466.

Koch, G.W. & Mooney, H.A. (eds) (1996) *Carbon Dioxide and Terrestrial Ecosystems.* Academic Press, San Diego.

Körner, Ch. (1995) Towards a better experimental basis for upscaling plant responses to elevated CO_2 and climate warming. *Plant, Cell and Environment* **18**, 1101-1110.

Körner, Ch. & Bazzaz, F.A. (eds) (1996) *Carbon Dioxide, Populations, and Communities.* Academic Press, San Diego.

Körner, Ch. & Miglietta, F. (1994) Long term effects of naturally elevated CO_2 on mediterranean grassland and forest trees. *Oecologia* **99**, 343-351.

Körner, Ch., Pelaez-Riedl, S. & van Bel, A.J.E. (1995) CO_2 responsiveness of plants: a possible link to phloem loading. *Plant, Cell and Environment* **18**, 595-600.

Kortschak, H.P., Hartt, C.E. & Burr, G.O. (1965) Carbon dioxide fixation in sugarcane leaves. *Plant Physiology* **40**, 209-213.

LeCain, D.R. & Morgan, J.A. (1998) Growth, gas exchange, leaf nitrogen and carbohydrate concentrations in NAD-ME and NADP-ME C_4 grasses grown in elevated CO_2. *Physiologia Plantarum* **102**, 297-306.

University of Cape Town

Lincoln, D.E. (1993) The influence of plant carbon dioxide and nutrient supply on susceptibility to insect herbivores. *Vegetatio* **104/105**, 273-280.

Lincoln, D.E., Fajer, E.D. & Johnson, R.H. (1993) Plant-insect herbivore interactions in elevated CO_2 environments. *Trends in Ecology and Evolution* **8**, 64-68.

Lloyd, J. & Farquhar, G.D. (1994) ^{13}C discrimination during CO_2 assimilation by the terrestrial biosphere. *Oecologia* **99**, 201-215.

Lloyd, J. & Farquhar, G.D. (1996) The CO_2 dependence of photosynthesis, plant growth responses to elevated atmospheric CO_2 concentrations and their interaction with soil nutrient status. I. General principles and forest ecosystems. *Functional Ecology* **10**, 4-32.

Loehle, C. (1995) Anomalous responses of plants to CO_2 enrichment. *Oikos* **73**, 181-187.

Long, S.P. (1991) Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO_2 concentrations: Has its importance been underestimated? *Plant, Cell and Environment* **14**, 729-739.

Long, S.P. & Hutchin, P.R. (1991) Primary production in grasslands and coniferous forests with climate change: an overview. *Ecological Applications* **1**, 139-156.

Long, S.P., Jones, M.B. & Roberts, M.J. (eds) (1992) *Primary Productivity of Grass Ecosystems of the Tropics and Sub-tropics*. Chapman & Hall, London.

Ludwig, F. (1996) Long-term effects of elevated atmospheric CO_2 concentrations on a South African grassland. Research report submitted in fulfilment of the requirements for the undergraduate degree in Environmental Biology, Utrecht University, The Netherlands.

Maroco, J.P., Pereira, J.S. & Chaves, M.M. (1997) Stomatal responses to leaf-to-air vapour pressure deficit in Sahelian species. *Australian Journal of Plant Physiology* **24**, 381-387.

McConnaughay, K.D.M., Berntson, G.M. & Bazzaz, F.A. (1993) Limitations to CO_2-induced growth enhancement in pot studies. *Oecologia* **94**, 550-557.

McConnaughay, K.D.M., Nicotra, A.B. & Bazzaz, F.A. (1996) Rooting volume, nutrient availability, and CO_2-induced growth enhancements in temperate forest tree seedlings. *Ecological Applications* **6**, 619-627.

McMurtrie, R.E. & Wang, Y.-P. (1993) Mathematical models of the photosynthetic response of tree stands to rising CO_2 concentrations and temperatures. *Plant, Cell and Environment* **16**, 1-13.

McNaughton, S.J. (1992) Laboratory-simulated grazing: interactive effects of defoliation and canopy closure on Serengeti grasses. *Ecology* **73**, 170-182.

McNaughton, S.J., Coughenour, M.B. & Wallace, L.L. (1982) Interactive processes in grassland ecosystems. In *Grasses and Grasslands Systematics and Ecology* (eds J.R. Estes, R.J. Tyrl & J.N. Brunken), pp. 167-193. University of Oklahoma Press, Norman.

McNaughton, S.J., Wallace, L.L. & Coughenour, M.B. (1983) Plant adaptation in an ecosystem context: effects of defoliation, nitrogen, and water on growth of an African C_4 sedge. *Ecology* **64**, 307-318.

Meinzer, F.C., Hinckley, T.M. & Ceulemans, R. (1997) Apparent responses of stomata to transpiration and humidity in a hybrid poplar canopy. *Plant, Cell and Environment* **20**, 1301-1308.

University of Cape Town

Menke, J.W. & Trlica, M.J. (1981) Carbohydrate reserve, phenology, and growth cycles of nine Colorado range species. *Journal of Range Management* **34**, 269-277.

Mentis, M.T. & Tainton, N.M. (1984) The effect of fire on forage production and quality. In *Ecological Effects of Fire in South African Ecosystems* (eds P.de V. Booysen & N.M. Tainton), pp. 245-254. Springer Verlag, Berlin, Heidelberg.

Midgley, G.F. (1996) *Influence of genotype and nutrients on the response to elevated CO_2 of plants of a nutrient limited ecosystem*. Ph.D. thesis, University of Natal.

Midgley, G.F. & O'Callaghan, M. (1993) *Review of likely impacts of climate change on South African flora and vegetation.* A report prepared for the Southern African Nature Foundation. National Botanical Institute, Cape Town.

Midgley, G.F., Bond, W.J., Roberts, R. & Wand, S.J.E. (1998) Will Gullivers travel? Potential causes of changes in savanna tree success due to rising atmospheric CO_2. In: *Towards sustainable natural resource management in the Kalahari Region: Abstracts and report from the Kalahari Transect Regional Scientific Workshop, Gaborone, Botswana.* START Report No. 2 (eds R. Chanda, S. Ringrose & L. Magole), pp. 21-22.

Midgley, G.F., Stock, W.D. & Juritz, J.M. (1995) Effects of elevated CO_2 on Cape Fynbos species adapted to soils of different nutrient status: nutrient- and CO_2-responsiveness. *Journal of Biogeography* **22**, 185-191.

Midgley, G.F., Wand, S.J.E. & Pammenter, N. (in press) Nutrient and genotypic constraints on CO_2-responsiveness: Photosynthetic regulation in congenerics of a nutrient-poor environment. *Journal of Experimental Botany.*

Miglietta, F., Badiani, M., Bettarini, I., van Gardingen, P., Selvi, F. & Raschi, A. (1995) Preliminary studies of the long-term CO_2 response of Mediterranean vegetation around natural CO_2 vents. In *Global Change and Mediterranean-type Ecosystems* (eds J.M. Moreno & W.C. Oechel), pp. 102-120. Springer Verlag, New York.

Monson, R.K., Littlejohn, R.O. & Williams III, G.J. (1983) Photosynthetic adaptation to temperature in four species from the Colorado shortgrass steppe: a physiological model for coexistence. *Oecologia* **58**, 43-51.

Monteith, J.L. (1995) A reinterpretation of stomatal responses to humidity. *Plant, Cell and Environment* **18**, 357-364.

Morgan, J.A., Hunt, H.W., Monz, C.A. & Lecain, D.R. (1994) Consequences of growth at two carbon dioxide concentrations and two temperatures for leaf gas exchange in *Pascopyrum smithii* (C_3) and *Bouteloua gracilis* (C_4). *Plant, Cell and Environment* **17**, 1023-1033.

Morison, J.I.L. & Gifford, R.M. (1983) Stomatal sensitivity to carbon dioxide and humidity. A comparison of two C_3 and two C_4 grass species. *Plant Physiology* **71**, 789-796.

Morison, J.I.L. & Gifford, R.M. (1984) Plant growth and water use with limited water supply in high CO_2 concentrations. I. Leaf area, water use and transpiration. *Australian Journal of Plant Physiology* **11**, 361-374.

University of Cape Town

Morse, S.R. & Bazzaz, F.A. (1994) Elevated CO_2 and temperature alter recruitment and size hierarchies in C_3 and C_4 annuals. *Ecology* **75**, 966-975.

Mott, K.A. & Parkhurst, D.F. (1991) Stomatal responses to humidity in air and helox. *Plant, Cell and Environment* **14**, 509-515.

Musil, C.F. (1995) Differential effects of elevated ultraviolet-B radiation on the photochemical and reproductive performances of dicotyledonous and monocotyledonous arid-environment ephemerals. *Plant, Cell and Environment* **18**, 844-854.

Musil, C.F. & Bhagwandin, N. (1992) The SUN program for computation of solar ultraviolet spectral irradiances: solar exposure limits in South Africa. *South African Journal of Science* **88**, 406-410.

Newton, P.C.D., Clark, H., Bell, C.C., Glasgow, E.M., Tate, K.R., Ross, D.J., Yeates, G.W. & Saggar, S. (1995) Plant growth and soil processes in temperate grassland communities at elevated CO_2. *Journal of Biogeography* **22**, 235-240.

Nie, D., Kirkham, M.B., Ballou, L.K., Lawlor, D.J. & Kanemasu, E.T. (1992) Changes in prairie vegetation under elevated carbon dioxide levels and two soil moisture regimes. *Journal of Vegetation Science* **3**, 673-678.

Nijs, I., Ferris, R., Blum, H., Hendrey, G. & Impens, I. (1997) Stomatal regulation in a changing climate: a field study using Free Air Temperature Increase (FATI) and Free Air CO_2 enrichment (FACE). *Plant, Cell and Environment* **20**, 1041-1050.

Nobel, P.S. (1991) *Physicochemical and Environmental Plant Physiology*. Academic Press, San Diego.

Norby, R.J. (1987) Nodulation and nitrogenase activity in nitrogen-fixing woody plants stimulated by CO_2 enrichment of the atmosphere. *Physiologia Plantarum* **71**, 77-82.

O'Connor, T.G. (1994) Composition and population responses of an African savanna grassland to rainfall and grazing. *Journal of Applied Ecology* **31**, 155-171.

O'Connor, T.G. (1995) *Acacia karroo* invasion of grassland: environmental and biotic effects influencing seedling emergence and establishment. *Oecologia* **103**, 214-223.

O'Connor, T.G. & Bredenkamp, G.J. (1997) Grassland. In *Vegetation of Southern Africa* (eds R.M. Cowling, D.M. Richardson & S.M. Pierce), pp. 215-257. Cambridge University Press, Cambridge.

Oesterheld, M. & McNaughton, S.J. (1988) Intraspecific variation in the response of *Themeda triandra* to defoliation: the effect of time of recovery and growth rates on compensatory growth. *Oecologia* **77**, 181-186.

Osmond, C.B., Björkman, O. & Anderson, D.J. (1980) *Physiological Processes in Plant Ecology: Toward a Synthesis with* Atriplex. Ecological Studies, Vol. 36, Springer Verlag, Berlin, Heidelberg, New York, pp. 291-377.

Osmond, C.B., Winter, K. & Ziegler, H. (1982) Functional significance of different pathways of CO_2 fixation in photosynthesis. In *Encyclopedia of Plant Physiology, New Series,* Vol. 12B (eds O.L. Lange, P.S. Nobel, C.B. Osmond & H. Ziegler), pp. 480-547. Springer-Verlag, Berlin, Heidelberg, New York.

Owensby, C.E., Cochran, R.C. & Auen, L.M. (1996a) Effects of elevated carbon dioxide on forage quality for ruminants. In *Carbon Dioxide, Populations, and Communities* (eds Ch. Körner & F.A. Bazzaz), pp. 363-371. Academic Press, San Diego.

Owensby, C.E., Coyne, P.I., Ham, J.M., Auen, L.M. & Knapp, A.K. (1993) Biomass production in a tallgrass prairie ecosystem exposed to ambient and elevated CO_2. *Ecological Applications* **3**, 644-653.

University of Cape Town

Owensby, C.E., Ham, J.M., Knapp, A.K., Bremer, D. & Auen, L.M. (1997) Water vapour fluxes and their impact under elevated CO_2 in a C_4-tallgrass prairie. *Global Change Biology* **3**, 189-195.

Owensby, C.E., Ham, J.M., Knapp, A., Rice, C.W., Coyne, P.I. & Auen, L.M. (1996b) Ecosystem-level responses of tallgrass prairie to elevated CO_2. In *Carbon Dioxide and Terrestrial Ecosystems* (eds G.W. Koch & H.A. Mooney), pp. 147-162. Academic Press, San Diego.

Parton, W.J., Scurlock, J.M.O., Ojima, D.S., Schimel, D.S., Hall, D.O. & Scopegram group members (1995) Impact of climate change on grassland production and soil carbon worldwide. *Global Change Biology* **1**, 13-22.

Patterson, D.T. (1986) Responses of soybean (*Glycine max*) and three C_4 grass weeds to CO_2 enrichment during drought. *Weed Science* **34**, 203-210.

Patterson, D.T. & Flint, E.P. (1980) Potential effects of global atmospheric CO_2 enrichment on the growth and competitiveness of C_3 and C_4 weed and crop plants. *Weed Science* **28**, 71-75.

Pearcy, R.W. & Ehleringer, J. (1984) Comparative ecophysiology of C_3 and C_4 plants. *Plant, Cell and Environment* 7, 1-13.

Pearcy, R.W., Tumosa, N. & Williams, K. (1981) Relationships between growth, photosynthesis and competitive interactions for a C_3 and a C_4 plant. *Oecologia* **48**, 371-376.

Polley, H.W., Johnson, H.B. & Mayeux, H.S. (1994) Increasing CO_2: comparative responses of the C_4 grass *Schizachyrium* and grassland invader *Prosopis*. *Ecology* **75**, 976-988.

Polley, H.W., Johnson, H.B., Mayeux, H.S., Brown, D.A. & White, J.W.C. (1996a) Leaf and plant water use efficiency of C_4 species grown at glacial to elevated CO_2 concentrations. *International Journal of Plant Science* **157**, 164-170.

Polley, H.W., Johnson, H.B., Mayeux, H.S. & Tischler, C.R. (1996b) Are some of the recent changes in grassland communities a response to rising CO_2 concentrations? In *Carbon Dioxide, Populations, and Communities* (eds Ch. Körner & F.A. Bazzaz), pp. 177-195. Academic Press, San Diego.

Poorter, H (1993) Interspecific variation in the growth response of plants to an elevated ambient CO_2 concentration. *Vegetatio* **104/105**, 77-97.

Poorter, H., Roumet, C. & Campbell, B.D. (1996) Interspecific variation in the growth response of plants to elevated CO_2: a search for functional types. In *Carbon Dioxide, Populations, and Communities* (eds Ch. Körner & F.A. Bazzaz), pp. 375-412. Academic Press, San Diego.

Potvin, C. & Strain, B.R. (1985a) Photosynthetic response to growth temperature and CO_2 enrichment in two species of C_4 grasses. *Canadian Journal of Botany* **63**, 483-487.

Potvin, C. & Strain, B.R. (1985b) Effects of CO_2 enrichment and temperatureon growth in two C_4 weeds, *Echinochloa crus-galli* and *Eleusine indica*. *Canadian Journal of Botany* **63**, 1495-1499.

Raschi, A., Miglietta, F., Tognetti, R. & van Gardingen, P. (eds) (1997) *Plant Responses to Elevated CO_2: Evidence from Natural Springs*. Cambridge University Press, New York.

Read, J.J. & Morgan, J.A. (1996) Growth and partitioning in *Pascopyrum smithii* (C_3) and *Bouteloua gracilis* (C_4) as influenced by carbon dioxide and temperature. *Annals of Botany* 77, 487-496.

University of Cape Town

Read, J.J., Morgan, J.A., Chatterton, N.J. & Harrison, P.A. (1997) Gas exchange and carbohydrate and nitrogen concentrations in leaves of *Pascopyrum smithii* (C_3) and *Bouteloua gracilis* (C_4) at different carbon dioxide concentrations and temperatures. *Annals of Botany* **79**, 197-206.

Reekie, E.G. (1996) The effect of elevated CO_2 on developmental processes and its implications for plant-plant interactions. In *Carbon Dioxide, Populations, and Communities* (eds Ch. Körner & F.A. Bazzaz), pp. 333-346. Academic Press, San Diego.

Reekie, E.G. & Bazzaz, F.A. (1991) Phenology and growth in four annual species grown in ambient and elevated CO_2. *Canadian Journal of Botany* **69**, 2475-2481.

Richards, J.H. & Caldwell, M.M. (1985) Soluble carbohydrates, concurrent photosynthesis and efficiency in regrowth following defoliation: a field study with *Agropyron* species. *Journal of Applied Ecology* **22**, 907-920.

Richardson, D.M., Bond, W.J., Dean, W.R.J., Higgins, S.I., Midgley, G.F., Milton, S.J., Powrie, L.W., Rutherford, M.C., Samways, M.J. & Schulze, R.E. (in press) Invasive alien species and global change: a South African perspective. In: *The Impact of Global Change on Invasive Species* (eds H.A. Mooney & R. Hobbs). Island Press.

Riechers, G.H. & Strain, B.R. (1988) Growth of blue grama (*Bouteloua gracilis*) in response to atmospheric CO_2 enrichment. *Canadian Journal of Botany* **66**, 1570-1573.

Rogers, H.H., Heck, W.W. & Heagle, A.S. (1983) A field technique for the study of plant responses to elevated carbon dioxide concentration. *Journal of the Air Pollution Control Association* **33**, 42-44.

Rosenberg, M.S., Adams, D.C. & Gurevitch, J. (1997) MetaWin: statistical software for meta-analysis with resampling tests. Version 1.0. Sinauer Associates, Sunderland, Massachusetts.

Ruess, R.W. (1988) The interaction of defoliation and nutrient uptake in *Sporobolus kentrophyllus*, a short-grass species from the Serengeti Plains. *Oecologia* 77, 550-556.

Ryel, R.J., Barnes, P.W., Beyschlag, W., Caldwell, M.M. & Flint, S.D. (1990) Plant competition for light analyzed with a multispecies canopy model. I. Model development and influence of enhanced UV-B conditions on photosynthesis in mixed wheat and wild oat canopies. *Oecologia* **82**, 304-310.

Ryle, G.J.A., Powell, C.E. & Tewson, V. (1992) Effect of elevated CO_2 on the photosynthesis, respiration and growth of perennial ryegrass. *Journal of Experimental Botany* **43**, 811-818.

SA Climate Statistics up to 1990 (1997) South African Department of Environmental Affairs and Tourism: Weather Bureau.

Sage, R.F. (1994) Acclimation of photosynthesis to increasing atmospheric CO_2: The gas exchange perspective. *Photosynthesis Research* **39**, 351-368.

Sage, R.F. & Monson, R.K. (1998) *C_4 Plant Biology*. Academic Press, San Diego.

Sage, R.F., Šantrucek, J. & Grise, D.J. (1995) Temperature effects on the photosynthetic response of C_3 plants to long-term CO_2 enrichment. *Vegetatio* **121**, 67-77.

Šantrucek, J. & Sage, R.F. (1996) Acclimation of stomatal conductance to a CO_2-enriched atmosphere and elevated temperature in *Chenopodium album*. *Australian Journal of Plant Physiology* **23**, 467-478.

SAS (1989) SAS Users Guide: Statistics. SAS Institute Inc, Cary, NC, USA.

University of Cape Town

Schapendonk, A.H.C.M., Dijkstra, P., Groenwold, J., Pot, C.S. & van de Geijn, S.C. (1997) Carbon balance and water use efficiency of frequently cut *Lolium perenne* L. swards at elevated carbon dioxide. *Global Change Biology* **3**, 207-216.

Schimel, D.S. (1995) Terrestrial ecosystems and the carbon cycle. *Global Change Biology* **1**, 77-91.

Scholes, R.J. & Bailey, C.L. (1996) Can savannas help balance the South African greenhouse gas budget? *South African Journal of Science* **92,** 60-61.

Scholes, R.J. & van der Merwe, M.R. (1995) *South Africa greenhouse gas inventory.* FORDEA 918. Forestek, CSIR.

Schopfer, P. (1984) Photomorphogenesis. In *Advanced Plant Physiology* (ed. M.B. Wilkins), pp. 380-407. Pitman Publishing Ltd., London.

Schulze, E.-D. (1986) Carbon dioxide and water vapor exchange in response to drought in the atmosphere and in the soil. *Annual Review of Plant Physiology* **37**, 247-274.

Schulze, E.-D., Ellis, R., Schulze, W., Trimborn, P. & Ziegler, H. (1996) Diversity, metabolic types and $\delta^{13}C$ carbon isotope ratios in the grass flora of Namibia in relation to growth form, precipitation and habitat conditions. *Oecologia* **106,** 352-369.

Schulze, R.E. (1997) Climate. In *Vegetation of Southern Africa* (eds R.M. Cowling, D.M. Richardson & S.M. Pierce), pp. 21-42. Cambridge University Press, Cambridge.

Seemann, J.R., Berry, J.A. & Downton, W.J.S. (1984) Photosynthetic response and adaptation to high temperature in desert plants. A comparison of gas exchange and fluorescence methods for studies of thermal tolerance. *Plant Physiology* **75**, 364-368.

Sionit, N. & Patterson, D.T. (1984) Responses of C_4 grasses to atmospheric CO_2 enrichment. I. Effect of irradiance. *Oecologia* **65**, 30-34.

Sionit, N. & Patterson, D.T. (1985) Responses of C_4 grasses to atmospheric CO_2 enrichment. II. Effect of water stress. *Crop Science* **25**, 533-537.

Slatyer, R.O. & Morrow, P.A. (1977) Altitudinal variation in the photosynthetic characteristics of snow gum, *Eucalyptus pauciflora* Sieb. ex Spreng. I. Seasonal changes under field conditions in the Snowy Mountains area of south-eastern Australia. *Australian Journal of Botany* **25**, 1-20.

Smith, S.D., Strain, B.R. & Sharkey, T.D. (1987) Effects of CO_2 enrichment on four Great Basin grasses. *Functional Ecology* **1**, 139-143.

Smith, T.M., Shugart, H.H. & Woodward, F.I. (1996) *Plant Functional Types.* Cambridge University Press, Cambridge.

Stewart, J.D. & Hoddinott, J. (1993) Photosynthetic acclimation to elevated atmospheric carbon dioxide and UV irradiation in *Pinus banksiana*. *Physiologia Plantarum* **88**, 493-500.

Stitt, M. (1991) Rising CO_2 levels and their potential significance for carbon flow in photosynthetic cells. *Plant, Cell and Environment* **14**, 741-762.

Sullivan, J.H. & Teramura, A.H. (1994) The effects of UV-B radiation on loblolly pine. 3. Interaction with CO_2 enhancement. *Plant, Cell and Environment* **17**, 311-317.

Tainton, N.M. & Mentis, M.T. (1984) Fire in Grassland. In *Ecological Effects of Fire in South African Ecosystems* (eds P.de V. Booysen & N.M. Tainton), pp. 115-147. Springer Verlag, Berlin, Heidelberg.

University of Cape Town

Tainton, N.M. & Walker, B.H. (1993) Grasslands of Southern Africa. In *Ecosystems of the World*, Vol. 8B, *Natural Grasslands, Eastern Hemisphere and Résumé* (ed. R.T. Coupland), pp. 265-290. Elsevier, Amsterdam.

Teeri, J.A. & Stowe, L.G. (1976) Climatic patterns and the distribution of C_4 grasses in North America. *Oecologia* **23**, 1-12.

Teramura, A.H. (1980) Effects of ultraviolet-B irradiances on soybean. I. Importance of photosynthetically active radiation in evaluating ultraviolet-B irradiance effects on soybean and wheat growth. *Physiologia Plantarum* **48**, 333-339.

Teramura, A.H. & Sullivan, J.H. (1994) Effects of UV-B radiation on photosynthesis and growth of terrestrial plants. *Photosynthesis Research* **39**, 463-473.

Teramura, A.H., Sullivan, J.H. & Ziska, L.H. (1990) Interaction of elevated ultraviolet-B radiation and CO_2 on productivity and photosynthetic characteristics in wheat, rice, and soybean. *Plant Physiology* **94**, 470-475.

Theunissen, J.D. (1992) An ecosystematic investigation of *Themeda triandra* (Poaceae: Andropogoneae) in the semi-arid grasslands of southern Africa. *Journal of Arid Environments* **23**, 35-44.

Thornton, B. & Millard, P. (1997) Increased defoliation frequency depletes remobilization of nitrogen for leaf growth in grasses. *Annals of Botany* **80**, 89-95.

Tilman, D. (1990) Constraints and tradeoffs: toward a predictive theory of competition and succession. *Oikos* **58**, 3-15.

Toft, N.L., McNaughton, S.J. & Georgiadis, N.J. (1987) Effects of water stress and simulated grazing on leaf elongation and water relations of an east African grass, *Eustachys paspaloides*. *Australian Journal of Plant Physiology* **14**, 211-226.

Trabalka, J.R., Edmonds, J.A., Reilly, J.M., Gardner, R.H. & Voorhees, L.D. (1985) Human alterations of the global carbon cycle and the projected future. In *Atmospheric Carbon Dioxide and the Global Carbon Cycle* (ed. J.R. Trabalka), pp. 247-287. DOE/ER-0239, US Department of Energy, Washington DC, USA.

Trlica, M.J. (1977) Distribution and utilization of carbohydrate reserves in range plants. In *Rangeland Plant Physiology* (ed. R.E. Sosebee), pp. 73-96. Society for Range Management, Denver.

Tyree, M.T. & Alexander, J.D. (1993) Plant water relations and the effects of elevated CO_2: a review and suggestions for future research. *Vegetatio* **104/105**, 47-62.

van de Staaij, J.W.M., Lenssen, G.M., Stroetenga, M. & Rozema, J. (1993) The combined effects of elevated CO_2 levels and UV-B radiation on growth characteristics of *Elymus athericus* (= *E. pycnanathus*). *Vegetatio* **104/105**, 433-439.

van Oudtshoorn, F.P. (1992) *Guide to Grasses of South Africa*. Briza Publikasies Cc, Cape Town.

Vitousek, P.M. (1994) Beyong global warming: ecology and global change. *Ecology* 75, 1861-1876.

Vogel, J.C., Fuls, A. & Ellis, R.P. (1978) The geographical distribution of Kranz grasses in South Africa. *South African Journal of Science* **74**, 209-215.

Volin, J.C. & Reich, P.B. (1996) Interaction of elevated CO_2 and O_3 on growth, photosynthesis and respiration of three perennial species grown in low and high nitrogen. *Physiologia Plantarum* 97, 674-684.

University of Cape Town

Walker, B. & Steffen, W. (eds) (1996) *Global Change and Terrestrial Ecosystems*. International Geosphere-Biosphere Programme Book Series No. 2. Cambridge University Press, Cambridge.

Wand, S.J.E., Midgley, G.F., Jones, M.H. & Curtis, P.S. (in press) Responses of wild C_4 and C_3 grass (Poaceae) species to elevated atmospheric CO_2 concentration: a meta-analytic test of current theories and perceptions. *Global Change Biology*.

Weinmann, H. (1948) Underground development and reserves of grasses. A review. *Journal of the British Grassland Society* **3**, 115-140.

Weinmann, H. & Reinhold, L. (1946) Reserve carbohydrates in South African grasses. *Journal of South African Botany* **12**, 57-73.

Welker, J.M., Rykiel, Jr., E.J., Briske, D.D. & Goeschl, J.D. (1985) Carbon import among vegetative tillers within two bunchgrasses: assessment with carbon-11 labelling. *Oecologia* **67**, 209-212.

White, L.M. (1973) Carbohydrate reserves of grasses: a review. *Journal of Range Management* **26**, 13-18.

Will, R.E. & Teskey, R.O. (1997) Effect of irradiance and vapour pressure deficit on stomatal response to CO_2 enrichment of four tree species. *Journal of Experimental Botany* **48**, 2095-2102.

Williams III, G.J. (1974) Photosynthetic adaptation to temperature in C_3 and C_4 grasses. A possible ecological role in the shortgrass prairie. *Plant Physiology* **54**, 709-711.

Wilsey, B.J. (1995) *Effects of elevated CO_2 on plant-grazer interactions*. Ph.D. dissertation, Syracuse University.

Wilsey, B.J. (1996) Urea additions and defoliation affect plant responses to elevated CO_2 in a C_3 grass from Yellowstone National Park. *Oecologia* **108**, 321-327.

Wilsey, B.J., Coleman, J.S. & McNaughton, S.J. (1997) Effects of elevated CO_2 and defoliation on grasses: a comparative ecosystem approach. *Ecological Applications* 7, 844-853.

Wilsey, B.J., McNaughton, S.J. & Coleman, J.S. (1994) Will increases in atmospheric CO_2 affect regrowth following grazing in C_4 grasses from tropical grasslands? A test with *Sporobolus kentrophyllus*. *Oecologia* **99**, 141-144.

Wilson, J.R. (1975) Comparative response to nitrogen deficiency of a tropical and temperate grass in the interrelation between photosynthesis, growth, and the accumulation of non-stuctural carbohydrate. *Netherlands Journal of Agricultural Science* **23**, 104-112.

Wong, S.C. (1979) Elevated atmospheric partial pressure of CO_2 and plant growth. 1. Interactions of nitrogen nutrition and photosynthetic capacity in C_3 and C_4 plants. *Oecologia* **44**, 68-74.

Wray, S.M. & Strain, B.R. (1986) Response of two old field perennials to interactions of CO_2 enrichment and drought stress. *American Journal of Botany* **73**, 1486-1491.

Wray, S.M. & Strain, B.R. (1987) Competition in old field perennials under CO_2 enrichment. *Ecology* **68**, 1116-1120.

Wullschleger, S.D. (1993) Biochemical limitation to carbon assimilation in C_3 plants - a retrospective analysis of the A/C_i curves from 109 species. *Journal of Experimental Botany* **44**, 907-920.

Yakimchuk, R. & Hoddinott, J. (1994) The influence of ultraviolet-B light and carbon dioxide enrichment on the growth and physiology of seedlings of three conifer species. *Canadian Journal of Forestry Research* **24**, 1-8.

University of Cape Town

Zanetti, S., Hartwig, U.A., van Kessel, C., Lüscher, A., Hebeisen, T., Frehner, M., Fischer, B.U., Hendrey, G.R., Blum, H. & Nösberger, J. (1997) Does nitrogen nutrition restrict the CO_2 response of fertile grassland lacking legumes? *Oecologia* **112**, 17-25.

Zangerl, A.R. & Bazzaz, F.A. (1984) The response of plants to elevated CO_2. II. Competitive interactions among annual plants under varying light and nutrients. *Oecologia* **62,** 412-417.

Ziska, L.H. & Bunce, J.A. (1997) Influence of increasing carbon dioxide concentration on the photosynthetic and growth stimulation of selected C_4 crops and weeds. *Photosynthesis Research* **54**, 199-208.

Ziska, L.H. & Teramura, A.H. (1992) CO_2 enhancement of growth and photosynthesis in rice (*Oryza sativa*). Modification by increased ultraviolet-B radiation. *Plant Physiology* **99**, 473-481.

Ziska, L.H., Drake, B.G. & Chamberlain, S. (1990) Long-term photosynthetic response in single leaves of a C_3 and C_4 salt marsh species grown at elevated atmospheric CO_2 in situ. *Oecologia* **83**, 469-472.

University of Cape Town

Appendix 1 References included in database for meta-analysis

Baxter, R., Ashenden, T.W., Sparks, T.H. & Farrar, J.F. (1994a) Effects of elevated carbon dioxide on three montane grass species. 1. Growth and dry matter partitioning. *Journal of Experimental Botany* **45**, 305-315.

Baxter, R., Gantley, M., Ashenden, T.W. & Farrar, J.F. (1994b) Effects of elevated carbon dioxide on three grass species from montane pasture. *Journal of Experimental Botany* **45**, 1267-1287.

Baxter, R., Bell, S.A., Sparks, T.H., Ashenden, T.W. & Farrar, J.F. (1995) Effects of elevated CO_2 concentrations on three montane grass species. III. Source leaf metabolism and whole plant carbon partitioning. *Journal of Experimental Botany* **46**, 917-929.

Baxter, R., Ashenden, T.W. & Farrar, J.F. (1997) Effect of elevated CO_2 and nutrient status on growth, dry matter partitioning and nutrient content of *Poa alpina* var *vivipara* L. *Journal of Experimental Botany* **48**, 1477-1486.

Bowler, J.M. & Press, M.C. (1993) Growth responses of two contrasting upland grass species to elevated CO_2 and nitrogen concentration. *New Phytologist* **124**, 515-522.

Bowler, J.M. & Press, M.C. (1996) Effects of elevated CO_2, nitrogen form and concentration on growth and photosynthesis of a fast- and slow-growing grass. *New Phytologist* **132**, 391-401.

Bowman, W.D. & Strain, B.R. (1987) Interaction between CO_2 enrichment and salinity stress in the C_4 non-halophyte *Andropogon glomeratus* (Walter) BSP. *Plant, Cell and Environment* **10**, 267-270.

Campbell, B.D., Laing, W.A., Greer, D.H., Crush, J.R., Clark, H., Williamson, D.Y. & Given, M.D.J. (1995) Variation in grassland populations and species and the implications for community responses to elevated CO_2. *Journal of Biogeography* **22**, 315-322.

Carlson, R.W. & Bazzaz, F.A. (1982) Photosynthetic and growth response to fumigation with SO_2 at elevated CO_2 for C_3 and C_4 plants. *Oecologia* **54**, 50-54.

Casella, E., Soussana, J.F. & Loiseau, P. (1996) Long-term effects of CO_2 enrichment and temperature increase on a temperate grass sward. 1. Productivity and water use. *Plant and Soil* **182**, 83-99.

Curtis, P.S., Drake, B.G., Leadley, P.W., Arp, W.J. & Whigham, D.F. (1989) Growth and senescence in plant communities exposed to elevated CO_2 concentrations on an estuarine marsh. *Oecologia* **78**, 20-26.

Curtis, P.S., Balduman, L.M., Drake, B.G. & Whigham, D.F. (1990) Elevated atmospheric CO_2 effects on belowground processes in C_3 and C_4 estuarine marsh communities. *Ecology* **71**, 2001-2006.

Ferris, R., Nijs, I., Behaeghe, T. & Impens, I. (1996) Contrasting CO_2 and temperature effects on leaf growth of perennial rye grass in spring and summer. *Journal of Experimental Botany* **47**, 1033-1043.

Fischer, B.U., Frehner, M., Hebeisen, T., Zanetti, S., Stadelmann, F., Lüscher, A., Hartwig, U.A., Hendrey, G.R., Blum, H. & Nösberger, J. (1997) Source-sink relations in *Lolium perenne* L. as reflected by carbohydrate concentrations in leaves and pseudo-stems during regrowth in a free air carbon dioxide enrichment (FACE) experiment. *Plant, Cell and Environment* **20**, 945-952.

Fitter, A.H., Self, G.K., Wolfenden, J., vanVuuren, M.M.I., Brown, T.K., Williamson, L., Graves, J.D. & Robinson, D. (1996) Root production and mortality under elevated atmospheric carbon dioxide. *Plant and Soil* **187**, 299-306.

University of Cape Town

Garbutt, K., Williams, W.E. & Bazzaz, F.A. (1990) Analysis of the differential response of five annuals to elevated CO_2 during growth. *Ecology* **71**, 1185-1194.

Ghannoum, O., von Caemmerer, S., Barlow, E.W.R. & Conroy, J. (1997) The effect of CO_2 enrichment and irradiance on the growth, morphology and gas exchange of a C_3 (*Panicum laxum*) and a C_4 (*Panicum antidotale*) grass. *Australian Journal of Plant Physiology* **24**, 227-237.

Gifford, R.M. & Morison, J.I.L. (1985) Photosynthesis, water use and growth of a C_4 grass stand at high CO_2 concentration. *Photosynthesis Research* 7, 77-90.

Gloser, J. & Bartak, M. (1994) Net photosynthesis, growth rate and biomass allocation in a rhizomatous grass *Calamagrostis epigejos* grown at elevated CO_2 concentration. *Photosynthetica* **30**, 143-150.

Greer, D.H., Laing, W.A. & Campbell, B.D. (1995) Photosynthetic responses of thirteen pasture species to elevated CO_2 and temperature. *Australian Journal of Plant Physiology* **22**, 713-722.

Hakala, K. & Mela, T. (1996) The effects of prolonged exposure to elevated temperatures and elevated CO_2 levels on the growth, yield and dry matter partitioning of field-sown meadow fescue. *Agricultural and Food Science in Finland* **5**, 285-298.

Hamerlynck, E.P., McAllister, C.A., Knapp, A.K., Ham, J.M. & Owensby, C.E. (1997) Photosynthetic gas exchange and water relation responses of three tallgrass prairie species to elevated carbon dioxide and moderate drought. *International Journal of Plant Sciences* **158**, 608-616.

Jackson, R.B. & Reynolds, A.L. (1996) Nitrate and ammonium uptake for single- and mixed species communitites grown at elevated CO_2. *Oecologia* **105**, 74-80.

Jackson, R.B., Sala, O.E., Field, C.B. & Mooney, H.A. (1994) CO_2 alters water use, carbon gain, and yield for the dominant species in a natural grassland. *Oecologia* **98**, 257-262.

Jackson, R.B., Luo, Y., Cardon, Z.G., Sala, O.E., Field, C.B. & Mooney, H.A. (1995) Photosynthesis, growth and density for the dominant species in a CO_2-enriched grassland. *Journal of Biogeography* **22**, 221-225.

Jones, M.B., Jongen, M. & Doyle, T. (1996) Effects of elevated carbon dioxide concentrations on agricultural grassland production. *Agricultural and Forest Meteorology* **79**, 243-252.

Kirkham, M.B., He, H., Bolger, T.P., Lawlor, D.J. & Kanemasu, E.T. (1991) Leaf photosynthesis and water use of big bluestem under elevated carbon dioxide. *Crop Science* **31**, 1589-1594.

Knapp, A.K., Hamerlynck, E.P. & Owensby, C.E. (1993) Photosynthetic and water relations responses to elevated CO_2 in the C_4 grass *Andropogon gerardii*. *International Journal of Plant Sciences* **154**, 459-466.

Larigauderie, A., Hilbert, D.W. & Oechel, W.C. (1988) Effects of CO_2 enrichment and nitrogen availability on resource acquisition and resource allocation in a grass, *Bromus mollis*. *Oecologia* 77, 544-549.

Leadley, P.W. & Stoecklin, J. (1996) Effects of elevated CO_2 on model calcareous grasslands: community, species, and genotype level responses. *Global Change Biology* **2**, 389-397.

Lenssen, G.M., Vandium, W.E., Jak, P. & Rozema, J. (1995) The response of *Aster tripolium* and *Puccinellia maritima* to atmospheric carbon dioxide enrichment and their interaction with flooding and salinity. *Aquatic Botany* **50**, 181-192.

University of Cape Town

Marks, S. & Clay, K. (1990) Effects of CO_2 enrichment, nutrient addition, and fungal endophyte-infection on the growth of two grasses. *Oecologia* **84**, 207-214.

Marks, S. & Strain, B.R. (1989) Effects of drought and CO_2 enrichment on competition between two old-field perennials. *New Phytologist* **111**, 181-186.

Morgan, J.A., Hunt, H.W., Monz, C.A. & LeCain, D.R. (1994a) Consequences of growth at two carbon dioxide concentrations and two temperatures for leaf gas exchange in *Pascopyrum smithii* (C_3) and *Bouteloua gracilis* (C_4). *Plant, Cell and Environment* **17**, 1023-1033.

Morgan, J.A., Knight, W.G., Dudley, L.M. & Hunt, H.W. (1994b) Enhanced root system C-sink activity, water relations and aspects of nutrient acquisition in mycotrophic *Bouteloua gracilis* subjected to CO_2 enrichment. *Plant and Soil* **165**, 139-146.

Newton, P.C.D., Clark, H., Bell, C.C., Glasgow, E.M., Tate, K.R., Ross, D.J., Yeates, G.W. & Saggar, S. (1995) Plant growth and soil processes in temperate grassland communities at elevated CO_2. *Journal of Biogeography* **22**, 235-240.

Nie, D., He, H., Kirkham, M.B. & Kanemasu, E.T. (1992) Photosynthesis of a C_3 grass and a C_4 grass under elevated CO_2. *Photosynthetica* **26**, 189-198.

Nijs, I., Teughels, H., Blum, H., Hendrey, G. & Impens, I. (1996) Simulation of climate change with infra-red heaters reduces the productivity of *Lolium perenne* L. in summer. *Environmental and Experimental Botany* **36**, 271-280.

Nijs, I., Ferris, R., Blum, H., Hendrey, G. & Impens, I. (1997) Stomatal regulation in a changing climate: a field study using Free Air Temperature Increase (FATI) and Free Air CO_2 enrichment (FACE). *Plant, Cell and Environment* **20**, 1041-1050.

Polley, H.W., Johnson, H.B., Mayeux, H.S., Brown, D.A. & White, J.W.C. (1996) Leaf and plant water use efficiency of C_4 species grown at glacial to elevated CO_2 concentrations. *International Journal of Plant Sciences* **157**, 164-170.

Potvin, C. & Strain, B.R. (1985) Photosynthetic response to growth temperature and CO_2 enrichment in two species of C_4 grasses. *Canadian Journal of Botany* **63**, 483-487.

Read, J.J., Morgan, J.A., Chatterton, N.J. & Harrison, P.A. (1997) Gas exchange and carbohydrate and nitrogen concentrations in leaves of *Pascopyrum smithii* (C_3) and *Bouteloua gracilis* (C_4) at different carbon dioxide concentrations and temperatures. *Annals of Botany* **79**, 197-206.

Ryle, G.J.A., Powell, C.E. & Tewson, V. (1992) Effects of elevated CO_2 on the photosynthesis, respiration and growth of perennial ryegrass. *Journal of Experimental Botany* **43**, 811-818.

Saebo, A. & Mortensen, L.M. (1995) Growth and regrowth of *Phleum pratense*, *Lolium perenne*, *Trifolium repens* and *Trifolium pratense* at normal and elevated CO_2 concentration. *Agriculture, Ecosystems & Environment* **55**, 29-35.

Saebo, A. & Mortensen, L.M. (1996) The influence of elevated CO_2 concentration on growth of seven grasses and one clover species in a cool maritime climate. *Acta Agriculturae Scandinavica Section B, Soil and Plant Sciences* **46**, 49-54.

Schäppi, B. & Körner, Ch. (1996) Growth responses of an alpine grassland to elevated CO_2. *Oecologia* **105**, 43-52.

University of Cape Town

Sionit, N. & Patterson, D.T. (1984) Responses of C_4 grasses to atmospheric CO_2 enrichment. I. Effect of irradiance. *Oecologia* **65**, 30-34.

Sionit, N. & Patterson, D.T. (1985) Responses of C_4 grasses to atmospheric CO_2 enrichment. II. Effect of water stress. *Crop Science* **25**, 533-537.

Stewart, J. & Potvin, C. (1996) Effects of elevated CO_2 on an artificial grassland community: competition, invasion and neighbourhood growth. *Functional Ecology* **10**, 157-166.

Stirling, C.M., Davey, P.A., Williams, T.G. & Long, S.P. (1997) Acclimation of photosynthesis to elevated CO_2 and temperature in five British native species of contrasting functional type. *Global Change Biology* **3**, 237-246.

Teughels, H., Nijs, I., van Hecke, P. & Impens, I. (1995) Competition in a global change environment: The importance of different plant traits for competitive success. *Journal of Biogeography* **22**, 297-305.

Thompson, G.B. & Drake, B.G. (1994) Insects and fungi on a C_3 sedge and a C_4 grass exposed to elevated atmospheric CO_2 concentrations in open-top chambers in the field. *Plant, Cell and Environment* **17**, 1161-1167.

van de Staaij, J.W.M., Lenssen, G.M., Stroetenga, M. & Rozema, J. (1993) The combined effects of elevated CO_2 levels and UV-B radiation on growth characteristics of *Elymus athericus* (= *E. pycnanathus*). *Vegetatio* **104/105**, 433-439.

Volin, J.C. & Reich, P.B. (1996) Interaction of elevated CO_2 and O_3 on growth, photosynthesis and respiration of three perennial species grown in low and high nitrogen. *Physiologia Plantarum* **97**, 674-684.

Wand, S.J.E., Midgley, G.F. & Musil, C.F. (1996) Physiological and growth responses of two African species, *Acacia karroo* and *Themeda triandra*, to combined increases in CO_2 and UV-B radiation. *Physiologia Plantarum* **98**, 882-890.

Wilsey, B.J. (1996) Urea additions and defoliation affect plant responses to elevated CO_2 in a C_3 grass from Yellowstone National Park. *Oecologia* **108**, 321-327.

Wilsey, B.J., McNaughton, S.J. & Coleman, J.S. (1994) Will increases in atmospheric CO_2 affect regrowth following grazing in C_4 grasses from tropical grasslands? A test with *Sporobolus kentrophyllus*. *Oecologia* **99**, 141-144.

Wilsey, B.J., Coleman, J.S. & McNaughton, S.J. (1997) Effects of elevated CO_2 and defoliation of grasses: A comparative ecosystem approach. *Ecological Applications* **7**, 844-853.

Wray, S.M. & Strain, B.R. (1986) Response of two old field perennials to interactions of CO_2 enrichment and drought stress. *American Journal of Botany* **73**, 1486-1491.

Wray, S.M. & Strain, B.R. (1987) Competition in old-field perennials under CO_2 enrichment. *Ecology* **68**, 1116-1120.

Zanetti, S., Hartwig, U.A., van Kessel, C., Lüscher, A., Hebeisen, T., Frehner, M., Fischer, B.U., Hendrey, G.R., Blum, H. & Nösberger, J. (1997) Does nitrogen nutrition restrict the CO_2 response of fertile grassland lacking legumes? *Oecologia* **112**, 17-25.

Ziska, L.H., Hogan, K.P., Smith, A.P. & Drake, B.G. (1991) Growth and photosynthetic response of nine tropical species with long-term exposure to elevated carbon dioxide. *Oecologia* **86**, 383-389.

University of Cape Town

Appendix 2 References, C_3 species, exposure and growth conditions, and interaction with other environmental stresses, for studies used in the meta-analysis.

(GC = growth chamber; GH = greenhouse; OTC = open-top chamber; FACE = free-air CO_2 enrichment)

Reference	C_3 Species	Exposure facility	Pot size (liter)	Duration (days)	Interacting stresses Nutr.	Temp.	Water
Baxter *et al.* 1994a	*Agrostis capillaris*	OTC	0.7	79			
	Festuca vivipara	OTC	0.7	189			
	Poa alpina	OTC	0.7	105			
Baxter *et al.* 1994b	*Agrostis capillaris*	OTC	0.4	43			
	Festuca vivipara	OTC	0.4	189			
	Poa alpina	OTC	0.4	105			
Baxter *et al.* 1995	*Agrostis capillaris*	OTC	0.7	79			
	Festuca vivipara	OTC	0.7	189			
	Poa alpina	OTC	0.7	105			
Baxter *et al.* 1997	*Poa alpina*	GC	2.5	50			
Bowler & Press 1993	*Agrostis capillaris*	GC	3.8	58	*		
	Nardus stricta	GC	3.8	63	*		
Bowler & Press 1996	*Agrostis capillaris*	GC	25	42	*		
	Nardus stricta	GC	25	49	*		
Campbell *et al.* 1995	*Agrostis capillaris*	GC	0.8	28		*	
	Bromus willdenowii	GC	0.8	42		*	
	Dactylis glomerata	GC	0.8	28		*	
	Festuca arundinacea	GC	0.8	42		*	
	Lolium multiflorum	GC	0.8	28		*	
	Lolium perenne	GC	0.8	42		*	
	Phalaris aquatica	GC	0.8	28		*	
Casella *et al.* 1996	*Lolium perenne*	GH	220	720	*		
Ferris *et al.* 1996	*Lolium perenne*	GH	3.7	133		*	
Fischer *et al.* 1997	*Lolium perenne*	FACE	G	426	*		
Fitter *et al.* 1996	*Festuca ovina*	OTC	G	730			
Ghannoum *et al.* 1997	*Panicum laxum*	GH	7.0	49			
Gloser & Bartak 1994	*Calamagrostis epigejos*	GC	0.5	21			
Greer *et al.* 1995	*Lolium perenne*	GC	1.2	28/56		*	
	Agrostis capillaris	GC	1.2	28/56		*	
Hakala & Mela 1996	*Festuca pratensis*	OTC	G	510		*	
Jackson & Reynolds 1996	*Avena fatua*	OTC	30	135	*		
	Bromus hordeaceus	OTC	30	135	*		
	Lolium multiflorum	OTC	30	135	*		
	Vulpia microstachys	OTC	30	135	*		
Jackson *et al.* 1994	*Avena barbata*	OTC	G	430			
Jackson *et al.* 1995	*Avena barbata*	OTC	G	790			
	Avena sativa	GH	3.1	30			
Jones *et al.* 1996	*Lolium perenne*	OTC	G	735			
Larigauderie *et al.* 1988	*Bromus mollis*	GC	3	129	*		
Leadley & Stöcklin 1996	*Bromus erectus*	GC	24.3	126			
	Festuca ovina	GC	24.3	126			
Lenssen *et al.* 1995	*Puccinellia maritima*	GH	1.8	28			
Marks & Clay 1990	*Lolium perenne*	GC	0.5	70	*		
Morgan *et al.* 1994a	*Pascopyrum smithii*	GC	20.4	460		*	
Newton *et al.* 1995	*Lolium perenne*	GC	150	340			
Nie *et al.* 1992	*Poa pratensis*	GH	G	61			*
Nijs *et al.* 1996	*Lolium perenne*	FACE	G	23		*	
Nijs *et al.* 1997	*Lolium perenne*	FACE	G	143		*	
Read *et al.* 1997	*Pascopyrum smithii*	GC	6	49		*	
Ryle *et al.* 1992	*Lolium perenne*	GC	2	49			
Saebo & Mortensen 1995	*Lolium perenne*	OTC	48	147			

University of Cape Town

	Phleum pratense	OTC	48	147		
Saebo & Mortensen 1996	*Agrostis capillaris*	OTC	48	60		
	Dactylis glomerata	OTC	48	64		
	Festuca arundinaceae	OTC	48	64		
	Festuca duruiscula	OTC	48	72		
	Festuca pratensis	OTC	48	72		
	Festuca rubra	OTC	48	71		
	Poa pratensis	OTC	48	72		
Schäppi & Körner 1996	*Poa alpina*	OTC	G	310	*	
Stewart & Potvin 1996	*Poa pratensis*	GC	27.4	61		
	Poa pratensis	OTC	G	61		
	Elymus athericus	GH	1.8	65		
Stirling *et al.* 1997	*Poa alpina*	GH	4.7	75		*
	Poa annua	GH	4.7	75		*
Teughels *et al.* 1995	*Lolium perenne*	GH	6.2	60		
	Festuca arundinaceae	GH	6.2	30		
van de Staaij *et al.* 1993	*Elymus athericus*	GH	1.8	65		
Volin & Reich 1996	*Agropyron smithii*	GC	2.5	58	*	
Wilsey 1996	*Stipa occidentalis*	GC	2	86	*	
Wilsey *et al.* 1997	*Agropyron caninum*	GC	2.0	75		
	Festuca idahoensis	GC	2.0	75		
	Briza subaristata	GC	2.0	75		
	Stipa occidentalis	GC	2.0	75		
Zanetti *et al.* 1997	*Lolium perenne*	FACE	G	913		
Ziska *et al.* 1991	*Pharus latifolius*	OTC	12.5	100		

University of Cape Town

Appendix 3 References, C_4 species, exposure and growth conditions, and interaction with other environmental stresses, for studies used in the meta-analysis

(GC = growth chamber; GH = greenhouse; OTC = open-top chamber)

Reference	C_4 Species	Exposure facility	Pot size (liter)	Duration (days)	Interacting stresses: Temp.	Water	Light
Bowman & Strain 1987	*Andropogon glomeratus*	GC	1	56			
Campbell *et al.* 1995	*Digitaria sanguinalis*	GC	0.8	42	*		
	Paspalum dilatatum	GC	0.8	42	*		
Carlson & Bazzaz 1982	*Setaria faberii*	GH	1	32			
	Setaria lutescens	GH	1	32			
Curtis *et al.* 1989	*Spartina patens*	OTC	G	124			
Curtis *et al.* 1990	*Spartina patens*	OTC	G	580			
Garbutt *et al.* 1990	*Setaria faberii*	GH	1	78			
Ghannoum *et al.* 1997	*Panicum antidotale*	GH	7	49			*
Gifford & Morison 1985	*Paspalum plicatulum*	GC	3.2	131		*	
Hamerlynck *et al.* 1997	*Andropogon gerardii*	OTC	G	1255		*	
Kirkham *et al.* 1991	*Andropogon gerardii*	OTC	G	214		*	
Knapp *et al.* 1993	*Andropogon gerardii*	OTC	G	480		*	
Marks & Clay 1990	*Tridens flavus*	GC	0.5	70			
Marks & Strain 1989	*Andropogon virginicus*	GC	0.5	16		*	
Morgan *et al.* 1994a	*Bouteloua gracilis*	GC	8	76		*	
Morgan *et al.* 1994b	*Bouteloua gracilis*	GC	20	190			
Newton *et al.* 1995	*Paspalum dilatatum*	GC	150	340			
Nie *et al.* 1992	*Andropogon gerardii*	GH	G	61		*	
Polley *et al.* 1996a	*Schizachyrium scoparium*	GH	30	480			
Potvin & Strain 1985a	*Echinochloa crus-galli*	GC	1	48	*		
	Eleusine indica	GC	1	48	*		
Read *et al.* 1997	*Bouteloua gracilis*	GC	6	49	*		
Sionit & Patterson 1984	*Digitaria sanguinalis*	GC	1	22			*
	Echinochloa crus-galli	GC	1	22			*
	Eleusine indica	GC	1	22			*
	Setaria faberii	GC	1	22			*
Sionit & Patterson 1985	*Digitaria sanguinalis*	GC	2	43		*	
	Echinochloa crus-galli	GC	2	43		*	
	Eleusine indica	GC	2	43		*	
	Setaria faberii	GC	2	43		*	
Thompson & Drake 1994	*Spartina patens*	OTC	G	1600			
Volin & Reich 1996	*Bouteloua curtipendula*	GC	2.5	58			
Wand *et al.* 1996	*Themeda triandra*	OTC	3.9	210			
Wilsey *et al.* 1994	*Sporobolus kentrophyllus*	GC	4.2	42			
Wilsey *et al.* 1997	*Sporobolus kentrophyllus*	GC	2	75			
	Paspalum dilatum	GC	2	75			
	Digitaria macroblephara	GC	2	75			
	Themeda triandra	GC	2	75			
Wray & Strain 1986	*Andropogon virginicus*	GC	0.5	56		*	
Wray & Strain 1987	*Andropogon virginicus*	GC	0.5	63			
Ziska *et al.* 1990	*Spartina patens*	OTC	G	500			

University of Cape Town

Appendix 4

CO_2-responses of *Themeda triandra* (ecotype 2) in summer.

A1 Materials and Methods

A1.1 Plant establishment

Themeda triandra plants were grown from seed in 3.9 L pots filled with a sand/Perlite mixture. *Themeda* seeds (designated "ecotype 2") were supplied by the Gene Bank of the Range and Forage Institute, Agricultural Research Council, Pretoria, and originated from the Transvaal Lowveld region (Kruger National Park). Plants were fertilized twice weekly (100 ml each) with an organic liquid fertilizer ("Seagro", Seagro (Pty) Limited, Cape Town, 40 ml dissolved in 20 L water), and once a week (100 ml each) with a water soluble fertilizer containing all micronutrients (Multifeed $P_{(43)}$ "10 in 1", Plaaskem., Houghton, 25 g dissolved in 20 L water). The weekly nutrient supply rate was: 49 mg N, 14 mg P, 27 mg K, 0.11 mg Mg, 94 µg Fe, 38 µg Mn, 125 µg B, 88 µg Mo, 44 µg Zn, and 94 µg Cu. These were calculated for N, P and K for both "Seagro" and "10 in 1", but for micronutrients only for "10 in 1", since the concentration of micronutrients was not given on the "Seagro" bottle.

A1.2 Design and treatments

The experimental design was a randomized block design, with four blocks of two chambers each (one at ambient and one at elevated CO_2 concentration). The CO_2 ranges after calibration in the four amended chambers were 588-810, 583-803, 550-751, and 568-719 µmol mol^{-1}, with 70-90% of measurements falling within 10% of the target (660 µmol mol^{-1}). Twelve 32-day-old plants were placed randomly within each chamber. Pots within each chamber were randomised monthly to minimise spatial variations in CO_2. Block locations were also randomised monthly. Due to intermittent problems with the automatic irrigation system during the early stages of the experiment, seedling mortality was experienced in all chambers, so that final replication per treatment and block varied between 3 and 7 (total of 17 plants for ambient and 22 plants for elevated CO_2 treatments). The experiment was carried out from 23 October 1995 to 9 February 1996, for 109 days.

University of Cape Town

Table A1 Average monthly midday chamber air temperatures and irradiance (PPFD) from 13 November 1995 to 9 February 1996.

Month	Avg midday temperature (°C) (12:00 - 15:00)	Avg midday irradiance (PPFD, μmol m^{-2} s^{-1}) (12:00 - 15:00)
November	30.0	1133
December	33.1	1081
January	35.2	1028
February	33.4	467

University of Cape Town

A1.3 Harvest and statistical analysis

Plants were harvested on 9 February 1996, and sub-divided into senesced tillers, green leaf blades, leaf sheaths, reproductive tillers and roots. Leaf and tiller numbers were recorded. The length and breadth of 10 randomly sampled fully developed leaf blades were measured. All material was oven-dried at 65°C, and milled. Two-way ANOVA, with [CO_2] and blocks as factors, was used to analyse all data.

A2 Results

Plant growth rates were greater in this ecotype in summer than in winter/spring (see Chapter 5), probably due to higher temperatures and greater nutrient supply rate. A final plant biomass of 10.9 g (ambient CO_2) was achieved in 109 days (Fig. A1), as compared to 6.9 g (ambient CO_2) in 183 days during winter/spring. By contrast, stimulatory effects of elevated CO_2 on growth were present but relatively smaller than in winter/spring, and could not be statistically distinguished in this summer experiment. Only individual leaf length was marginally increased in elevated CO_2 (Table A2).

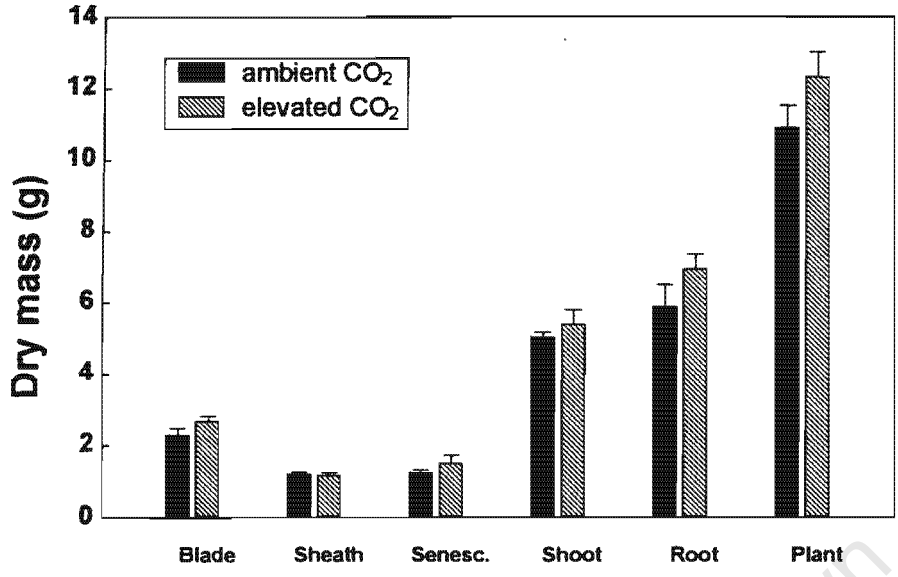


Fig. A1 Biomass of *Themeda triandra* (ecotype 2) grown under elevated CO_2 in summer. Values are block means (n=4) ± SE. There were no statistically significant differences between treatments.

University of Cape Town

Table A2. Effects of elevated CO_2 on plant and leaf morphological characteristics of *Themeda triandra* (ecotype 2) grown in summer. Values are block means ± SE (n=4). + $P \leq 0.10$; NS not significant, according to two-way ANOVA with [CO_2] and blocks as factors.

CO_2 treatment	Tiller no.	Leaf no.	Leaf length (mm)	DW per leaf (mg)	DW per leaf sheath (mg)
ambient	47 ± 4	165 ± 15	147 ± 6	14.2 ± 1.1	7.9 ± 1.0
elevated	44 ± 1	161 ± 8	176 ± 11	17.0 ± 1.0	7.6 ± 0.5
	NS	NS	+	NS	NS