Impacts of elevated CO_2 on the growth, production and water use of a South African C_4-dominated grassland community.

Nthabiseng Motete

Submitted in fulfilment of the academic requirements of the degree of Doctor of Philosophy in the School of Life and Environmental Sciences, University of Kwazulu-Natal, Durban.

June 2004

All work for this thesis was completed at the former University of Natal.

PREFACE

The experimental work described in this thesis was carried out in the School of Life and Environmental Sciences, University of Kwa Zulu-Natal, Durban Campus, under the supervision of Professor Norman W. Pammenter, with Dr. Guy F. Midgley of the National Botanical Institute (NBI) as co-supervisor.

These studies represent the original work by the author and where use was made of the work of others, it has been duly acknowledged in the text.

NTHABISENG MOTETE

14/04/2005

ABSTRACT

A three year microcosm experiment consisting of four C_4 grass species, one C_3 grass, and a C_3 geophyte was set up to investigate production and water use efficiency of a grassland community (coastal Ngongoni veld) in response to increasing concentrations of atmospheric CO_2 and different levels of simulated rainfall. The Ngongoni grassland community is dominated by species that possess a C_4 photosynthetic pathway, predominantly of the NADP-me. Dominant C_4 grass species irrespective of photosynthetic pathway include *Andropogon appendiculatus*, *Eragrostis racemosa*, *Sprobolus pyramidalis*, and *Themeda triandra*. Only one C_3 grass species, *Alloteropsis semialata* sub-species *eckloniana*, is common in this grassland community. There are also a few forbs.

The experimental system was assembled in a greenhouse, where microcosms were arranged in three rows representing four randomly arranged treatment groups with four replicates per treatment. Community canopy development and phenology were studied qualitatively from the beginning to the end of each growing season. Community above-ground production was determined at end-of-year harvests in a manner that differentiated contributions of different species. Above-ground biomass of grass species was further sorted by components in order to illustrate how these influenced canopy structure and possibly competitive interactions. Changes in above-ground biomass production of the grass species in the three years were used to infer species dominance changes in response to a factorial combination of CO_2 and water treatments. Assessment of community water use was done by measurements of evapotranspiration using a weighing lysimeter, and by measurements of soil water content using a moisture probe. Fluxes of carbon and water vapour were also determined by canopy gas exchange in the second and third years of study. Leaf gas exchange measurements were performed at three intervals (beginning, middle and end) during the third year of study in order to investigate a correlation between photosynthesis and biomass production. Measurements done at the final harvest included total below ground biomass, distribution of roots with depth, and crown biomass (below-ground biomass could not be split into species-specific components).

In the first year, watering simulated a stochastic rainfall distribution typical of the site. Results after the first year showed a significant positive response of above ground production to elevated CO_2, but only at rainfall values typical of the field site from which the community was derived (mean annual rainfall, MAR, 730 mm). There was no CO_2 effect on above-ground production at a rainfall treatment 20% lower than MAR (CO_2 x water treatment interaction $p<0.01$). In the first year elevated CO_2 reduced community water use more at MAR than under dry conditions. A reduction in cumulative water use led to an increase in pot mass as a consequence of soil water accumulation in all water treatments. In the second year rainfall treatments were adjusted to MAR and MAR + 20% (wet), using regular application as opposed to stochastic application. Results of the second year showed that the CO_2 effect on community production was identical to that of the previous year under the MAR treatment. In the third year, a reduction in biomass production occurred in all treatments, and the main effects of CO_2 and water treatments were not statistically significant.

Responses of canopy structure to elevated CO_2 treatment were characterised by higher production of community leaf biomass in upper canopy layers (height of about 40 cm and above) due to significant treatment effects. The taller grass species influenced responses of canopy structure the most. Among taller grasses, *Sporobolus pyramidalis* and *Themeda triandra*, were responsive to elevated CO_2 + MAR, and their leaf biomass in the 40-60 cm layer was equivalent to 50% of each of their leaf biomass in the dense basal layers (5-20 cm or 20-40 cm); while contributions of *Alloteropsis semialata* and *Andropogon appendiculatus* in the 40-60 cm layer were each no more than 10-15% of their respective contributions in the dense basal layers (5-20 cm or 20-40 cm). There was a dense presence of leaf biomass in the bottom part of the canopy below 40 cm, and treatment effects in that part of the canopy were not statistically significant. Lack of statistical significance of treatment effects on the amount of leaf biomass in the basal layer of the canopy suggests that important functional processes that are successfully maintained by dense lower canopy may not be altered by elevated CO_2.

Responses of community phenology show that elevated CO_2 caused early sprouting, early flowering and delayed senescence, even though the responses were species

specific, and sometimes dependent on water supply. Early sprouting occurred under elevated CO_2 in all three years, and was further enhanced by a higher water supply (MAR and 120%MAR). Sprouting responses of the species was characterised by three groups, which categorise *Themeda triandra* as an early sprouter, *Eragrostis racemosa* and *Sporobolus pyramidalis* as intermediate, and *Sporobolus pyramidalis*, *Andropogon appendiculatus* and *Alloteropsis semialata* as late sprouters. The observed trends in sprouting are in contradiction with sprouting phenology of mixed grasslands, where cool-season C_3 grass species sprout earlier than warm-season C_4 grass species. This may suggest a response to greenhouse conditions, especially less extreme night time temperatures.

Elevated CO_2 reduced community evapotranspiration, and increased community water use efficiency. The highest recorded reduction in evapotranspiration was 10%. Reduction in evapotranspiration resulted in a significant increase in soil water in the rooting zone and underlying clay layer under elevated CO_2 in both wet and MAR conditions. Soil water content was found to increase with soil depth. A reduction in community water use under elevated CO_2 was consistently measured in all three years by all methods of assessment used.

Canopy gas exchange data were in agreement with community production and water use data in the sense that carbon gain was 20-30% higher under elevated CO_2, and water vapour flux was reduced under elevated CO_2. Results of leaf gas exchange measurements in the third year showed higher rates photosynthesis in the C_4 grass species than the C_3 grass. A reduction in stomatal conductance was observed both in the C_3 and C_4 grass species.

The geophyte (*Eriospermum mackenii*) did not show a response to treatments in the above-ground organs in the first year. In the second and third years, above-ground biomass increased under both treatments, but the increase in the second year was higher than the increase in the third year, possibly indicating an acclimation response. Elevated CO_2 caused a 6-11% increase in the dry mass of below-ground organs of the geophyte from the time of planting to final harvest.

Generally, grass species most responsive to the elevated CO_2 treatment possessed the C_4 photosynthetic pathway. The C_3 grass – *Alloteropsis semialata*, showed non-responsiveness to elevated CO_2 relative to the C_4 grasses, as indicated by delayed sprouting at beginning of growing season, an earlier onset of senescence, and lower above-ground biomass at harvest. The results suggest that elevated CO_2 may cause changes in community composition of warm-season vs. cool season grasses where the two types co-occur. These results will be useful in predictive modeling of future impacts of elevated CO_2 on C_4 grassland composition and catchment yield, particularly because South African C_4 grasslands cover major catchments and occur in areas otherwise suitable for C_3 vegetation.

ACKNOWLEDGEMENTS

This study is a product of a collaboration between the School of Life and Environmental Sciences at the University of Natal, Durban, and the National Botanical Institute (NBI) where I was subsequently employed. I would like to thank the management of the NBI, especially the Research Directorate for making this opportunity possible. I am grateful to my two supervisors Professor Norman W. Pammenter (University of Natal) and Dr. Guy F. Midgley (NBI), who have been extensively involved in all aspects of this study. Professor Norman W. Pammenter has supervised most of my post-graduate studies at the University of Natal and I could not have asked for a better mentor. Dr. Guy F. Midgley facilitated an enjoyable professional development for me at the NBI.

During the planning stages of this study Dr. SJE Wand made a significant contribution with regards to selection and identification of plant species. The plant material and soil used in the study were obtained from Mr. Guy Clegg's farm, near Oribi Gorge, southern KwaZulu Natal. Construction of the experimental set-up was skilfully undertaken by Mr. Kay Govindsamy and Mr. Sagie Govender of the University of Natal. My colleagues at the NBI, Mr. Deryck de Witt and Mr. Stanley Snyders assisted with the final harvest of below-ground biomass.

I am also very thankful to Professor Patricia Berjak for the many ways in which she has contributed to my professional development in all the years I spend in the School of Life and Environmental Sciences.

And to my family, for always being there for me.

TABLE OF CONTENTS

List of abbreviations

k	Apparent carboxylation efficiency (μmol CO_2 μmol^{-1} c_i)
α	Apparent quantum efficiency (μmol CO_2 μmol^{-1} PPFD)
Γc	Photosynthetic CO_2 compensation point (μmol μmol^{-1} CO_2)
Γ_l	Photosynthetic light compensation point (μmol m^{-2} s^{-1} CO_2)
A	Net CO_2 assimilation rate (μmol m^{-2} s^{-1})
A_{max}	Light saturated rate of net CO_2 assimilation rate (μmol m^{-2} s^{-1})
B_o	Community total above-ground biomass of starting plant material (g)
B_1	Community total above-ground biomass at the end of the first year (g)
B_2	Community total above-ground biomass at the end of the second year (g)
B_3	Community total above-ground biomass at the of the third year (g)
g_s	Stomatal conductance (mmol m^{-2} s^{-1})
c_a	Intercellular CO_2 concentration (μmol mol^{-1})
c_i	Atmospheric CO_2 concentration (μmol mol^{-1}) IRGA Infra red gas analyser
J_{max}	Light and CO_2 saturated rate of net CO_2 assimilation (μmol m^{-2} s^{-1})
LUE	Light use efficiency
MAR	Mean annual rainfall
NAD-me	Nicotinamide adenine dinucleotide malic enzyme
NADP-me	Nicotinamide adenine dinucleotide phosphate malic enzyme
PEP	Phosphoenolpyruvate
PCK	Phosphoenolpyruvate carboxykinase
PPFD	Photosynthetic photon flux density
Rubisco	Ribulose-1,5-bisphosphate carboxylase/oxygenase
RuBP	Ribulose-1,5-bisphosphate
WUE	Leaf instantaneous water use efficiency (μmol CO_2 $mmol^{-1}$ H_2O)
	Canopy level water use efficiency (mmol CO_2 mol^{-1} H_2O)

List of Figures

List of Tables

CHAPTER 1

INTRODUCTION

1.1. Increasing atmospheric CO_2 concentrations and global climate change

The turn of the twenty first century is experiencing exceptionally high increases in the concentration of atmospheric carbon dioxide. Data from polar ice cores show that in the middle of the nineteenth century, before the industrial revolution, the concentration of carbon dioxide in the atmosphere was 275 ± 10 ppm (Neftel et al. 1985). Additional data collected at Mauna Loa Observatory show an indisputable increase from 315 ppm in 1958 to 350 ppm in 1988 (Keeling et al. 1989) and a continued rise since then. Although CO_2 concentrations have changed over geological time scales, present changes are occurring at a rate higher than at any time over the last 160 000 years BP (Barnola et al. 1987), and even more rapidly than the changes that occurred 3 million years ago (Houghton et al. 2001). The principal sources of increasing levels of atmospheric CO_2 are anthropogenic activities that release carbon from major reservoirs (Keepin et al. 1986). The increases in CO_2 and other greenhouse gases are expected to cause global warming by increasing the absorbance of long-wave radiation by the lower atmosphere (IPCC, 1990). Even though some suggestions indicated that global warming could be negated by planetary cooling forces that are intensified by warmer temperatures and by biological processes that are enhanced by rising levels of atmospheric CO_2 (Idso 1998), it has become clear that the planet has warmed by 0.5 ^{o}C in the past century (IPCC, 1990), and models (IPCC, 1995) suggest that it will continue to warm well beyond the year 2100. CO_2 on the other hand is a substrate for photosynthesis, and elevated CO_2 will affect natural ecosystems by its direct impact on vegetation.

This introductory chapter will discuss some of the commonly reported impacts of elevated CO_2 on grassland ecosystems, particularly C_4-dominated grasslands. A brief outline of major studies on grassland ecosystems will be given in section 1.2., and further reference will be made to the content of section 1.2 in greater detail under section 1.5 where a specific account will tease out differences in response between C_3 and C_4 grassland vegetation. There will be a section on advantages conferred by the C_4 photosynthetic pathway on C_4 species, and the impacts of elevated CO_2 on those

benefits, and whether responses to elevated CO_2 will be different for the C_4 functional subtypes. Lastly, responses of C_4-dominated grassland communities to elevated CO_2 will be discussed in the South African context.

1.2. Increasing atmospheric CO_2 concentrations and grasslands

Responses of grassland communities and ecosystems to elevated CO_2 have been studied for a variety of climates and habitats, but the majority of studies have been conducted on northern hemisphere temperate grasslands, with very few studies done on African tropical and sub-tropical grasslands. Several ecosystem characteristics such as water flux, light regime, nutrients, temperature, the predominant mode of photosynthesis in dominant plant species (C_3, C_4, or CAM) vary among different grassland types, and all of these factors are important in how grassland ecosystems will respond to elevated CO_2 (Wilsey et al. 1997).

The first running grassland ecosystem experiment looking at the effects of elevated CO_2 on key processes that regulate ecosystem carbon metabolism, and also measuring the response of these effects on ecosystem carbon accumulation, was set up in a salt marsh in Chesapeake Bay, Maryland (Drake et al. 1989). The study site consisted of two monospecific stands (a C_3 sedge community of *Scirpus olneyi* and a C_4 grass community of *Spartina patens*), and a mixed community of *Scirpus olneyi*, *Spartina patens* and another C_4 grass *Distichlis spicata*, all exposed to elevated CO_2 since 1987 (Drake et al. 1989). Following one year of exposure to elevated CO_2, there was a significant increase in above-ground biomass in the C_3 sedge, and no significant treatment effects on above-ground biomass in the C_4 grass community (Curtis et al. 1989a). The C_4 component of the mixed community also showed no measurable response of above-ground biomass to elevated CO_2 (Curtis et al. 1989a). Production of the C_3 sedge community was further enhanced by delayed senescence. That pattern of response in primary production was confirmed through seven years of CO_2 exposure (Drake et al. 1996). Elevated CO_2 also increased annual net ecosystem CO_2 uptake throughout the first year in all three communities (Drake and Leadley 1991). Net ecosystem CO_2 uptake was continually enhanced for seven years in the C_3 sedge community under elevated CO_2, but only for the first four years in the C_4 grass community and in the mixed community only during the first, third, sixth and seventh years (Drake et al. 1996).

The second longest running grassland ecosystem study was initiated in a pristine tallgrass prairie in Manhattan, Kansas in 1989, on vegetation consisting of a mixture of C_3 and C_4 perennial species, with C_4 grasses *Andropogon geradii* and *Sorghastrum nutans* as dominant species. The tallgrass prairie experiment involved a combination of experimental approaches, from measuring leaf water potential to whole-ecosystem gas exchange. The results show that a C_4 tallgrass prairie exposed to elevated CO_2 can sustain reduced water use, which in turn is sufficient to increase above- and below-ground biomass production in years when water stress is frequent (Knapp et al. 1993a, Ham et al. 1995, Owensby et al. 1997). Improved water use efficiency conferred by elevated CO_2 on the tallgrass prairie is a result with profound implications, considering that production of that ecosystem is commonly limited by water availability (Owensby et al. 1969). Furthermore, improved water use efficiency in the tallgrass prairie under elevated CO_2 is in agreement with one of the most purported effects of elevated CO_2, which is enhanced water use efficiency as a consequence of reduced stomatal conductance (e.g. Chaves and Pereira, 1992; Morrison 1993; Wand et al. 1999). Elevated CO_2 apparently had a greater impact on the production of C_4 grass species and C_3 forbs than of the C_3 grass species, and Owensby and co-workers (1993) partly attribute the non-responsiveness of C_3 grasses to lack of grazing, which they suggest may have allowed taller C_4 grasses to overtop the shorter C_3 grass species. The results are exemplary in demonstrating that competitive advantages conferred upon C_3 species (under elevated CO_2) by the C_3 photosynthetic pathway, do not override other environmental factors that govern plant competitive interactions.

Another major ecosystem-level study is the Jasper Ridge annual grassland experiment undertaken since 1992 in California, to quantify the roles of ecosystem characteristics such as species composition, soil moisture, and nutrients, as well as ecosystem processes such as photosynthesis and evapotranspiration, in controlling ecosystem responses to elevated CO_2 (Field et al. 1995). The study communities consist of single species field microcosms, and mixed species field microcosms, in addition to chambered and unchambered field plots of C_3 grasses growing on serpentine soil and sandstone soil (Field et al. 1996). Elevated CO_2 significantly enhanced productivity in both sandstone and serpentine communities, and

consequently enhanced ecosystem water use efficiency (WUE) of communities, even though there were no statistically significant CO_2 effects on evapotranspiration (Fredeen et al. 1995). Effect of CO_2 on ecosystem gas exchange was statistically significant only in the serpentine community, but higher rates of ecosystem gas exchange were measured in the sandstone grassland compared to the serpentine grassland, suggesting that the response of above-ground production to elevated CO_2 may be dependent on grassland type in that ecosystem (Fredeen et al. 1995). Despite lack of statistically significant differences in evapotranspiration measured under ambient and elevated CO_2 treatments in the sandstone grassland, slightly lower rates were measured under elevated CO_2, while evapotranspiration in the serpentine grassland showed no sensitivity to elevated CO_2 (Fredeen et al. 1995). Elevated CO_2 had a strong effect on leaf level processes such as net CO_2 assimilation, transpiration, stomatal conductance, instantaneous water use efficiency, and mid-day leaf water potential of the dominant species *Avena barbata* in sandstone grassland (Jackson et al. 1994). Higher mid-day leaf water potential and lower stomatal conductance under elevated CO_2 of the dominant species in the sandstone community (Jackson et al. 1994), resulted in increased soil water availability at the ecosystem level (Fredeen et al. 1996). Jackson et al. (1995) show that other notable effects of elevated CO_2 are increased density of a late-season species such as the C_3 grass *Hemizonia congesta*, and enhanced litter production.

Other research groups around the world are pursuing ecosystem level studies to investigate impacts of elevated CO_2 on other types of grasslands. Wilsey and co-workers (1997) studied the response of grassland communities from three different ecosystems exposed to similar treatments of elevated CO_2 with or without defoliation. The three ecosystems represented the African tropical grassland of Serengeti dominated by C_4 species, a South American temperate grassland of Flooding Pampa dominated by a mixture of C_3 and C_4 species, and a North American temperate grassland at Yellowstone National Park dominated by C_3 species. In the North American temperate grassland, elevated CO_2 caused an increase in total biomass of crowns and roots (storage organs), and no effect on above-ground biomass. In the South American temperate grassland and East African tropical grasslands, there were no significant CO_2 effects on either storage-organs or above-ground biomass. Lack of significant CO_2 effects on above-ground biomass in species from any of the three

ecosystems were interpreted to imply no effect of CO_2 on the quality of forage. Wilsey et al. (1997) deduced that lack of interactive effect of CO_2 and defoliation suggested that herbivores will not affect the way grasses respond to elevated CO_2 under average nutrient conditions.

A pioneer experiment investigating potential impacts of elevated CO_2 on southern African C_4 grass species started in 1994 at the National Botanical Institute in Kirstenbosch, Cape Town. The objective of that study was to investigate how carbon assimilation and allocation, growth, and morphological development of the representative southern African C_4 grass species will be affected by elevated CO_2 (Wand, 1999). A further objective was to formulate the potential direct and indirect impacts of rising atmospheric CO_2 on the future distribution and production of grasslands in southern Africa. At the time when that study was initiated, it was common perception that C_4 grass species would not be responsive to elevated CO_2, and that under elevated CO_2, C_4 grasses would be out-competed by their C_3 counterparts in communities where both co-occur. Wand and co-workers (1999) performed a critical assessment, using meta-analysis methods, of published literature on the physiological and growth responses of wild C_4 vs. C_3 species to elevated CO_2. The analysis showed that elevated CO_2 has a significant positive effect on plant water relations in both C_3 and C_4 grass species, as a consequence of reduced stomatal conductance (g_s). These authors (Wand et al. 1999) also indicated that at the leaf level, greater carbohydrate accumulation and greater reductions in leaf nitrogen concentration in C_3 species were the only patterns which significantly differentiated C_3 from C_4 responses, and constituted the only evidence for sink limitation. However, there were substantial differences between C_3 and C_4 species at the shoot level, which resulted from shoot allocation differences and effects on above-ground morphologies. Those differences, and other differences in photosynthetic pathway, might explain the tendency towards biomass response differences.

The study described in this thesis forms part of the Climate Change Research Programme at the National Botanical Institute in Kirstenbosch, Cape Town, and was initiated in April 1998 to further understand community level responses to impacts of elevated CO_2 on South African C_4-dominated subtropical grasslands, with specific emphasis on community production and water use.

1.3. C_3 and C_4 photosynthetic pathways and increasing atmospheric CO_2

Plants that possess the C_4 photosynthetic pathway evolved as a result of reductions in atmospheric CO_2 concentrations (Ehleringer et al. 1991), and are at present abundant in tropical, sub-tropical and temperate regions with warm-season rainfall (Ehleringer et al. 1997). It is interesting that the climatic variables that were important in the evolution of the C_4 pathway, are the ones being altered anthropogenically (Henderson et al 1995), and even more intriguing are the ecological implications of these climatic changes on the future of C_4 grassland ecosystems.

The C_4 photosynthetic pathway serves to concentrate CO_2 in the bundle sheath cells, where the carbon fixing enzyme, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco) and the photosynthetic carbon reduction cycle are specifically located (Pearcy and Ehleringer 1984). The CO_2 concentrating mechanism in C_4 species enables rubisco to function at CO_2 concentrations near saturation ($\approx$2000 $\mu l\ l^{-1}$), which is about ten times greater than those experienced by rubisco in C_3 species. Carboxylation in C_3 plants is limited furthermore by photorespiration, such that at current ambient CO_2 concentrations the maximum rate of CO_2 fixation in leaves of C_3 species is about 20% of the maximum capacity of rubisco (Collatz 1977). Photorespiration consumes extra ATP and NADPH derived from the light reactions of photosynthesis, thus lowering the effective quantum yield of CO_2 fixation, that effect becoming more pronounced at higher temperatures (Ehleringer and Björkman, 1977). Although the quantum yield of C_4 plants is independent of temperature, the CO_2 concentrating mechanism requires extra ATP derived from the light reactions. The extra energy required is associated with the regeneration of phosphoenolpyruvate by the C_4 cycle in the mesophyll cells, thus reducing the potential quantum efficiency (Ehleringer and Björkman, 1977). These authors argue that under high CO_2, the quantum efficiency of CO_2 fixation in C_3 plants will be superior to that of C_4 plants, and by implication, C_4 species may not benefit from elevated CO_2 as much C_3 species under high irradiances. In an experiment undertaken to study the interactive growth effects between different levels of irradiance and elevated CO_2 on C_4 and C_3 grasses, Ghannoum et al. (1997) show that elevated CO_2 enhances plant dry weight by 1.41 and 1.71 times at both high and low light respectively in the C_3 grass, and only by 1.28 times at high light in the C_4 grass.

The mechanisms of C_4 plant responses to elevated CO_2 are not as well understood as those of C_3 plants, especially at the leaf and single plant levels (Stitt, 1991). Elevated CO_2 increases photosynthetic assimilation in the C_3 pathway (i) by decreasing photorespiration because of increased intercellular CO_2 concentration (c_i), and (ii) by increasing the rate of CO_2 fixation by rubisco (Stitt, 1991). A consequence of increased c_i is a reduction of stomatal conductance and concomitant water use efficiency. Another likely response mechanism of C_3 species is reduced mitochondrial respiration under elevated CO_2 (Drake et. al. 1999), although this response is not unequivocal. There are suggestions that C_4 species may be out-competed by C_3 species under elevated CO_2, even in regions otherwise favourable for C_4 species (Collatz et. al. 1998). But then again, it would seem that the magnitude of C_4 responsiveness, especially in mixed C_3/C_4 communities, could depend on whether C_3 species do take advantage of elevated CO_2 (Henderson et al. 1995).

The nature of the C_4 pathway confers physiological flexibility that is well suited to the ecological advantages associated with elevated CO_2 (Henderson et. al. 1995), which suggests that C_4 species will do well under elevated CO_2. The purported physiological flexibility of the C_4 pathway is attributed to, among other factors, co-ordinated compartmentalisation of metabolism (Henderson et. al. 1995). These authors argue that the elaborate specialisation of photosynthetic functions between the mesophyll and bundle sheath cells in C_4 plants permits good regulations of metabolite transport and pool sizes both within and between cells. The close proximity of bundle sheath cells to the vascular system may support a higher capacity for sucrose translocation in C_4 plants (Henderson et al. 1995), lack of which limits the capability of C_3 plants to take advantage of elevated CO_2 (Stitt, 1991). Secondly, the C_4 pathway confers water use efficiency under ambient atmospheric CO_2 concentrations because of a reduction in stomatal conductance (Henderson et al. 1995), and reports in the literature indicate that this benefit is further enhanced under elevated CO_2 concentrations (Knapp et al. 1993b; Wand et al. 1999). Thirdly, C_4 species use less rubisco to sustain high photosynthetic rates under ambient CO_2 concentrations, and therefore the nitrogen use efficiency potential of the C_4 pathway places C_4 species at an advantage over C_3 species under elevated CO_2, and arguments on this suggestion are discussed below.

One of the critical arguments about whether C_4 species will respond positively to elevated CO_2 on the basis of nitrogen use efficiency is that if nitrogen is a limiting factor to plant growth under elevated CO_2, the nitrogen use potential of the C_4 pathway could be lost because nitrogen deficiency reduces rubisco activity more than the activity of PEP carboxylase in C_4 species, hence the rate of delivery of CO_2 to the bundle sheath becomes faster than its fixation (Ghannoum and Conroy, 1998). C_3 species would be at an advantage if nitrogen is limiting to plant growth because the C_3 pathway uses less rubisco in elevated CO_2 due to the elimination of photorespiration (Stitt, 1991). The advantage of nitrogen use efficiency may fail to be sustained in either C_3 or C_4 species due to lack of sink strength, which in turn leads to accumulation of total non-structural carbohydrates and photosynthetic acclimation (Stitt, 1991). As a result, the capability of either C_3 or C_4 plants to take advantage of elevated CO_2 would be limited. Response mechanisms of C_4 and C_3 grasses are less clearly understood at canopy and ecosystem levels, and that is a major drawback in assessing whether grasslands have the potential to sequester carbon in the long-term.

1.4. C_4 subtypes and elevated CO_2

C_4 photosynthetic subtypes are named according to the principal four-carbon acid (malate or aspartate) decarboxylating enzyme, and they are NAD-dependent malic enzyme (NAD-me), NADP-dependent malic enzyme (NADP-me), and phosphoenolpyruvate carboxykinase (PCK) (Hattersly and Watson 1992). Grass species that possess the NAD-me pathway are dominant in drier regions, while those that possess the NADP-me pathway are dominant in regions of higher precipitation (Ehleringer et al. 1997). The PCK photosynthetic sub-type is dominant in more arid regions than the NAD-me variant (Hattersly, 1983). Generally, there are slight differences in the quantum yields of the C_4 grass subtypes that are often associated with the leakiness of CO_2 in the bundle sheaths or lack of it. CO_2 leakiness is considered an energy cost that is manifested in the quantum yield (Pearcy and Ehleringer, 1984). The NADP-me subtype is purported to have the tightest bundle sheath cells, NAD-me the most leaky, and the PCK group is intermediate (Pearcy and Ehleringer, 1984). Those authors suggested that differences in leakiness between C_4 subtypes might be related to the conductance of the bundle sheath cells.

Subsequently, Henderson et al. (1995) hypothesised that levels of CO_2 leakiness could serve as predictors of the responses of C_4 species to elevated CO_2. This interesting hypothesis was by coincidence tested almost simultaneously by two research groups (LeCain and Morgan 1998, and Wand 1999), who further proposed that if higher bundle sheath cell wall conductance in the NAD-me subtype implies lower CO_2 concentration in the bundle sheath cell, then the photosynthesis of species belonging to that subtype would be more responsive to elevated CO_2 than those belonging to NADP-me and PCK subtypes. Results of both studies did not support generalisations about gas exchange response of C_4 grasses to elevated CO_2 based on subtype, but they show that growth response of well watered NADP-me grasses to elevated CO_2 tends to be larger than of NAD-me subtypes, although not all species respond the same. In another study, Seneweera et al. (1998) found that elevated CO_2 ameliorates the effect of soil water deficit on the growth of a C_4 NAD-me wild grass. At present, no conclusive generalisations can be made about the responsiveness of the different C_4 subtypes to elevated CO_2.

1.5. C_4 grassland communities and their responses to elevated CO_2

Rigorous ecosystem-level studies on the responses of C_4 vs. C_3 grassland communities to elevated CO_2 have been conducted on a mixed C_4 and C_3 salt marsh at Chesapeake Bay in Maryland (Curtis et. al. 1989a,b); a predominantly C_4 tallgrass prairie at Kansas, Manhattan (Owensby et. al. 1993); an annual C_3 grassland at Jasper Ridge, California (Field et al. 1996); and a shortgrass steppe in Colorado (Morgan et al. 2001). Other studies have been conducted in controlled environments using soil cores (Morgan et. al. 1994), or grasses planted from seed (Morgan et al. 1998; Le Cain and Morgan, 1998; Ghannoum et. al. 1997; 1998; Wand 1999). The experiment on the salt marsh is the longest running, and its initial findings indicated that elevated CO_2 has a relatively larger effect on C_3 species than C_4 species (Curtis et. al. 1989a,b). Those results are in agreement with predictions based on differences in photosynthetic pathways, that C_4 species will not respond due to their photosynthetic pathway – and this places them at a competitive disadvantage. The greatest response in C_3 species was above-ground biomass production, which was stimulated only after mid-season, increasing thereafter (Curtis et. al. 1989a,b). However, as the absolute values of above-ground biomass increased, the relative stimulation by elevated CO_2 decreased (Arp et. al. 1993), indicating a short-term effect of elevated CO_2 on above-

ground biomass in C_3 species which according to those authors coincided with a period of high temperature and drought. An important observation by Drake et al. (1996) is that generally, C_3 species in the salt marsh responded better to elevated CO_2 during times of greatest stress in which C_4 species were least productive.

Findings of the tallgrass prairie experiment on the other hand, indicated that responses of C_4 grass species to elevated CO_2 did not conform to predictions based on differences in photosynthetic pathways. Compared with ambient CO_2 levels, elevated CO_2 increased total biomass and leaf area of C_4 grass species, but not of C_3 grass species, although the relative increase in biomass was greater below-ground than above-ground (Owensby et. al. 1993). The authors argued that the reduction in C_3 grasses can be attributed to lack of grazing, which allowed taller C_4 grasses to quickly overtop the shorter C_3 species. The taller C_3 forbs in that study increased in basal cover under elevated CO_2, supporting a suggestion that canopy responses to competition for light associated with CO_2 enrichment may affect interspecific competition. Furthermore, a positive effect of elevated CO_2 on biomass production of a dominant C_4 grass species, *Andropogon gerardii*, was substantially greater in dry years than in wet years during the growing season (Owensby et. al. 1993). Such a response may be indicative of an increased competitive edge of C_4 species over C_3 under elevated CO_2.

The positive response of C_4 grass biomass production of the tallgrass prairie under elevated CO_2 was accompanied by an improvement in plant water relations measured as increased leaf xylem pressure potentials (Knapp et. al. 1993a), associated with a reduction in stomatal conductance (Knapp et. al. 1993b). Maintenance of high leaf water potentials during periods of low water availability by plants growing in elevated CO_2 improves water use efficiency, while high precipitation during growth in elevated CO_2 has been shown to moderate the effect of water use efficiency on biomass production (Ham et. al. 1995). Those observations have led to speculation that any increases in production of C_4 grasslands under elevated CO_2 would be most apparent during dry periods (Hamerlynck et al. 1997). Data from another experiment (Hunt et. al. 1996), showed that elevated CO_2-induced increase in biomass was greatest at an intermediate water level.

Increased water use efficiency in elevated CO_2 is often attributed to either greater photosynthetic assimilation associated with high CO_2 availability or lower rates of transpiration resulting from decreased stomatal conductance, or a combination of the two. In their review, Tyree and Alexander (1993) proposed that a combination of the two factors, rather than each one singly produces increased water use efficiency, especially because lower stomatal conductance is more limiting to evapotranspiration than to assimilation. In the tallgrass prairie experiment, elevated CO_2 does not seem to have a direct effect on photosynthetic assimilation, but seems to influence production by altering the water relations of the ecosystem (Ham et. al. 1995). During periods of either extended drought or extended rain, no differences in biomass production may occur under ambient or elevated CO_2 either because of equally limiting water stress or high photosynthetic rates (Ham et. al. 1995), hence a suggestion by the authors that if repeated wetting and drying cycles occur during the growing season, elevated CO_2 will induce more production because reduced evapotranspiration will delay the onset of water stress during each drying cycle.

Differences in the growth strategies of C_3 and C_4 grasses may explain the differences in their responses to elevated CO_2. Generally, root:shoot ratio determines the patterns of water supply and demand within the plant, and in some instances early rapid development of leaves and leaf area under elevated CO_2 has been linked to enhanced rates of assimilation and a decrease in transpiration. The work of Morgan et al. (1998) shows that root:shoot ratio in the C_3 grass *Pascopyrum smithii* increased in response to elevated CO_2 while the root:shoot ratio of the C_4 *Bouteloua gracilis* remained unaltered. Their explanation of the results is that the cool-season C_3 grass sequestered total non-structural carbohydrate, storage carbohydrates and biomass below-ground in preparation for summer dormancy while resource allocation remained unaltered in the warm-season C_4 grass.

1.6. Stomatal responses to elevated CO_2 and their implications for evapotranspiration and community water use

The interactions of a plant with its environment under elevated CO_2 can be described by several key processes relating to the role of stomata (Eamus, 1991; Jarvis et al. 1999). Increased CO_2 concentration around the leaf surface has a powerful effect on stomatal aperture and conductance (g_s), which regulates the fluxes of CO_2 and water

vapour into and out of the leaf and thus c_i (Mott, 1988). The extent of regulation of CO_2 and water vapour fluxes is dependent on the rate of diffusion, the demand for these fluxes, and the stomatal control of the fluxes (Jarvis et al. 1999). Mechanisms that control or regulate g_s include among others (i) malate pools, (ii) ABA, (iii) pH, and (iv) ion channels, even though specific roles are not well understood (Jarvis et al. 1999). The direct overall effect of reduced leaf level conductances is reduced leaf transpiration rates and a concomitant improvement in water use efficiency irrespective of whether or not assimilation (A) is increased by elevated CO_2 (Eamus, 1991). Therefore, elevated CO_2 enhances the ratio of leaf net CO_2 assimilation (A_{net}) to evapotranspiration (E), a relationship termed instantaneous water use efficiency. Oftentimes, reduced leaf level conductances are accompanied by increased rates of CO_2 assimilation, although the extent of increase in rate of CO_2 assimilation differs somewhat for different species, photosynthetic pathways (C_3 vs. C_4), for crop and natural vegetation, and also whether that effect is sustained in the long-term.

The potential for elevated CO_2 to reduce leaf transpiration is reported to be effective in the long-term (Morgan et al. 1994; Radoglou et al. 1992). Although an increase in leaf area tends to offset the effect of reduced transpiration, the benefit of enhanced water use efficiency often remains at the canopy level (Morrison and Gifford, 1994; Nijs et al. 1989); hence, elevated CO_2 can ameliorate the negative effects of drought in many species (Morrison 1993). Moreover, reductions in water use as a result of partial stomatal closure could indirectly affect other important ecosystem processes and delay the onset of stress during drying cycles (Field et al. 1995; Hungate et al. 1997). The stimulation of A_{net}/E by CO_2 enrichment, along with responses such as changes in leaf area, root water access, and hydraulic conductivity will determine species performance with rising CO_2, particularly in water limited situations. This suggests that enhancement of ecosystem production by elevated CO_2 would be greater under drought than well-watered conditions. However, not all species and ecosystems would respond similarly.

The production of many ecosystems together with seasonal dynamics of production, are coupled with the surface water balance; hence many ecological and biophysical processes could be altered by CO_2-induced changes in plant-water relations (Bremer et al. 1996). For water-limited systems, elevated CO_2 can result in greater water

availability for longer in the growing season, especially if there is not an increase in leaf transpiration surface per unit of ground area (Campbell et al. 1997; Field et al. 1997, Owensby et al. 1997, and Volk et al. 2000). By implication, the hydrological consequences of elevated CO_2 in water-limited systems can be as significant as the direct CO_2 fertilisation effect on photosynthesis. Such speculation however, could become uncertain if current experimental designs fail to mimic the actual coupling between atmosphere and vegetation (McLeod and Long 1999).

1.7. Long term implications of elevated CO_2 on South African grasslands

The South African grassland biome is climatically hospitable and agriculturally the most productive ecosystem, contributing greatly to the country's gross annual production of maize, beef, and fresh milk and other dairy products. The economic potential is further enhanced by the discovery of large coal deposits and the world's richest gold mines. This coincidence of agricultural, fossil fuel and mineral wealth and the accompanying economic growth is not without serious environmental repercussions and potential for pressure on resources (Mentis and Huntley 1982), thus compromising food security, especially in the face of changing local land use practices and global climatic patterns. Water resource is another prominent area of concern because of the spatial and temporal scarcity of rainfall in the country. The area weighted annual average rainfall in South Africa is below the world average, although some parts of the country such as the eastern seaboard get rainfall higher than the world annual average. A reduction in rainfall reliability as predicted by Ellery and co-workers (1991) would make this situation much worse.

Global change research aims to reduce levels of uncertainties among decision makers and policy makers seeking to develop an appropriate and evidence-based legislative and regulatory environment (Campbell and Smith 2000). Often some of the important decisions need to be made whether pertinent research results are available or not. On the other hand, researchers who focus on the detailed aspects of climate change caution against extrapolation before the understanding of the changes is robust (Huebert, 1999). It is hoped that the results of this study will contribute towards increased confidence in formulating policy on some aspects of South African grasslands.

South African grasslands are dominated by C_4 grass species, for which some of the characteristic determinants of their distribution are high temperature during the growing season and a frequent occurrence of dry spells (Vogel et al. 1978). The C_4 photosynthetic pathway confers a competitive advantage on C_4 grasses in water limited environments (Pearcy and Ehleringer 1984), and elevated CO_2 coincidentally brings about increased water use efficiency by vegetation. If the positive effect of elevated CO_2 on water use is maintained together with the advantages of the C_4 pathway, then there is a potential for a "water saving" effect on South African C_4-dominated grasslands.

C_4 grassland communities in some parts of the world have undergone drastic encroachment by C_3 shrubs in the last 125 years, possibly as a consequence of increased competitive abilities of C_3 species as a consequence of rising atmospheric CO_2 concentrations (Polley et al. 1996, 2002). Bush encroachment is considered by some global change scientists (Pacala et al. 2001) to be a substantially stable carbon sink, estimated to have sequestered 18 to 34 percent of total North American carbon stocks over the 1980-1990 period. However, an important aspect of carbon sequestration is not only the potential of woody vegetation to bind more carbon, but how long it will be before the sequestered carbon is released back to the atmosphere through economic use or natural breakdown, a phenomenon that Körner (2001) terms "buying time with respect to atmospheric CO_2 enrichment". As far as mitigation of CO_2 enrichment is concerned, the size of the carbon pools is more important than the rate at which carbon cycles through the pool (Steffen et al. 1998). Hence, preservation of old forests may represent a larger carbon pool than a rapidly expanding young forest. But for purposes of CO_2 mitigation, if old forests are not dynamic enough to fix more CO_2, perhaps rapidly expanding young forests remain an alternative mitigation tool. Other scientists (Gill et al. 2002; Jackson et al. 2002) suggest that the potential of woody vegetation to sequester carbon at the expense of natural grassland ecosystems is not as extensive as Pacala et al. (2001) suggest. Jackson et al. (2002) illustrate that encroachment can in fact reduce the carbon sequestration potential in high precipitation grassland ecosystems in the northern hemisphere. The work of Gill et al. (2002) further illustrate that the capacity of vegetation (grasslands in particular) to moderate impacts of elevated CO_2 by storing additional carbon may be limited. Such confounding reports (Pacala et al. 2001; Gill et al. 2002; and Jackson et al.

2002) have major implications for policy formulation on Global Climatic Change, particularly ratification of the Kyoto Protocol and issues of trading in carbon stocks.

Predictions for South Africa are that increasing levels of atmospheric CO_2 and global climate change will accelerate invasion of both C_4 and C_3 grasslands by perennial savannah and nama-karoo elements (grassy dwarf shrubland) at rates and magnitudes that exceed traditional explanations of bush encroachment (Ellery et al. 1991). If those predictions hold, long-term impacts will threaten the sustainability of major services yielded by the grassland biome, carbon sequestration notwithstanding. South African grasslands are unique in that they are dominated by C_4 grass species (Vogel et al. 1978), and serve as major water catchments for a large proportion of the country's population. Another major impact of global climate change driven changes on vegetation as a consequence of increasing atmospheric CO_2, would therefore be an alteration in the hydrology of C_4-grassland ecosystems (Joffre and Rambal, 1993). However, predictions of vegetation changes on South African grasslands are based on a southern African climate change scenario of mean annual temperature increase of 2°C and the mean annual precipitation decrease by 15% (Ellery et al. 1991). That study did not incorporate the positive effect of elevated CO_2 on plant water use efficiency, which if included in climate change models (Hulme et al. 1996), could predict a different scenario for the grassland biome, but nonetheless, it represents earlier significant attempts to understand climate change impacts on southern African grasslands.

The ability to predict the impact of elevated CO_2 on grassland ecosystems is complicated by perceptions that C_4 grasses will suffer a competitive disadvantage relative to C_3 species. Mechanisms by which C_4 grass species respond to elevated CO_2 may not be based as strongly on predictions based on differences in photosynthetic pathways as is the case for C_3 species. Furthermore, it has been illustrated that C_4 species have the physiological flexibility necessary to realise the ecological advantage and growth potential of elevated CO_2 (Henderson et. al. 1995). A recent review by Wand et al. (1999) on the responses of wild C_4 and C_3 grass species to elevated CO_2 concentrations also indicates that there is a significant positive response of C_4 grasses at both leaf and whole plant levels. It is necessary to further investigate responses of South African C_4 grasslands at levels of mixed community or even higher levels in

order to facilitate scaling-up. Reviews by Navas et al. (1999) and Poorter and Navas (2003) of past studies indicate that predictions of vegetation responses to elevated CO_2 become more powerful when growth analyses are done at the mixed stand level than at the level of individual plants. This is because in mixed stands, responses to elevated CO_2 do not only depend on individual species physiological and morphological characteristics, but also on interactions that arise with other species competing for the same resources (Firbank and Watkinson, 1990). Furthermore, community level studies can be designed to allow for interaction of elevated CO_2 with other environmental parameters such as rainfall or nutrients.

1.8. Objectives

(i) To investigate interactive effects of elevated CO_2 and different amounts of rainfall on ecosystem production and water use of a C_4–dominated grassland.

(ii) To determine treatment effects on component species representing key functional types.

1.9. Experimental approach

The study was undertaken in greenhouse based microcosms using a re-constituted grassland community, sampled from Ngongoni field site in southern Kwazulu-Natal, South Africa (30°22'S 30°00'E, altitude 650 m). The Ngongoni grassland community is dominated by species that possess a C_4 photosynthetic pathway, predominantly of the NADP-me. Dominant C_4 grass species irrespective of photosynthetic pathway include *Andropogon appendiculatus*, *Eragrostis racemosa*, *Sprobolus pyramidalis*, and *Themeda triandra*. Only one C_3 grass species, *Alloteropsis semialata* sub-species *eckloniana*, is common in this grassland community. There are also a few forbs.

The following plant species were used: a C_3 grass – *Alloteropsis semialata* (R. Br.) Hitchc. sub-species *eckloniana*, a C_3 tuberous geophyte – *Eriospermum mackenii* (Hook. f.) Baker, subsp. m*ackenni*; four C_4 grasses representative of three C_4 photosynthetic pathways: *Andropogon appendiculatus* Nees and *Themeda triandra* Forssk. both NADP-me; *Eragrostis racemosa* (Thumb.) Stued., NAD-me; *Sporobulus pyramidalis* Beauv., PCK. The species composition and the soil of the established microcosm communities resembled those of the Ngongoni grassland community. An experimental approach of a greenhouse based microcosm enabled increased control

and precision on treatment inputs of CO_2 and water, and measurement of outputs such as community water loss (evapotranspiration) using weighing lysimetry, while at the same time offering the benefit of working on a mixed community that is representative of the field site. Furthermore, data from the greenhouse microcosm experiment would augment findings from other investigations related to the field site that had either been conducted prior to the study described in this thesis (Wand 1999, Morrow 2002) or during the same period (Hattas 2002).

1.10. Key questions

Experiments in this study were designed to address the following key questions, in a manner that is not mutually exclusive.

(i) What effects will elevated CO_2 have on canopy development and structure of microcosm communities, and will responses be dependent on water supply?

(ii) Will elevated CO_2 change above-ground biomass production at species and community levels?

(iii) To what extent will above-ground biomass production be influenced by a combined effect of elevated CO_2 and different watering treatments?

(iv) Will community-level water use be changed by long-tem exposure to elevated CO_2?

(v) Will the responsiveness and proportional representation of C_4 functional types be altered by a combined effect of elevated CO_2 and different watering treatments?

(vi) What are the long-term implications of elevated CO_2 on South African grasslands as water catchments?

CHAPTER 2

MATERIALS AND METHODS

2.1. Microcosm design and set-up

The Ngongoni field site has previously been used for *in situ* measurements of leaf level photosynthesis of C_3 and C_4 grass species in response to a natural source of elevated CO_2 coming out of a natural CO_2 spring (Wand, 1999; Wand et al. 2002). Stock et al. (2004) undertook community level measurements at the Ngongoni field site to characterise species composition, plant growth, leaf properties and soil nutrient, carbon and water dynamics in response to long-term exposure to a natural source of elevated CO_2. Morrow (2002) studied aspects of below-ground responses at the field site. The study reported in this thesis aimed to achieve a high level of control on environmental parameters, and replication that was not feasible in the field. The advantage of using microcosms is that they enable thorough manipulation of specific environmental parameters and facilitate understanding of the role such parameters play in an ecological community (Fraser and Keddy, 1997).

Mixed C_4-grass microcosm communities were constructed and installed in a greenhouse at the University of Natal in order to address the objectives and key questions of this study. Each microcosm contained a species assemblage sampled from a coastal Ngongoni C_4-grassland community (30°22'S 30°00'E, altitude 650 m), about 30 km west of Paddock (30°49' S:30°13'E: 514m) and 15 km south east of Harding (30°34S:29°53'E: 820m) in southern Kwazulu-Natal, South Africa.

The set up consisted of three rows of painted steel framework supporting 16 microcosms, representing four randomly arranged treatment groups with four replicates per treatment. Two peripheral rows carried five microcosms each and a central row carried six microcosms (Figure 2.1.a and b). Beneath the microcosms on each row were two air supply pipes running horizontally along the length of the framework. One pipe was for elevated CO_2 and the other for ambient CO_2. The supply of air was driven by a large fan which blew ambient outside air, through a pipe of 0.2 m internal diameter, through the wall of the greenhouse. The air source pipe was 5 m tall to avoid extreme fluctuations in ambient CO_2 that would otherwise occur if the

source was at ground level. The top end of the 5 m tall pipe was fitted with a filter and an elbow joint in order to exclude unwanted objects in the air stream. Downstream from the fan the air supply was split, to provide air streams of ambient or elevated CO_2. A bank of four cylinders of industrial grade CO_2 (Air Products, Pinetown, South Africa) connected to a manifold, served as a CO_2 source for the elevated CO_2 air stream. Pure CO_2 was injected into the elevated CO_2 supply pipe at a controlled pressure 2 m downstream from the fan. Injected CO_2 mixed with ambient air along the length of the pipe-layout which subsequently delivered air and CO_2 mixture to the array of 16 microcosms.

The rate of gas delivery in each microcosm was maintained at 0.38 m^3min^{-1} to facilitate three volume changes of air per minute which was sufficient to reduce over-heating to acceptable levels. The fan and all pipe-layout downstream from the fan were insulated with a white 10 mm thick "33 closed cell density" polyethylene foam (Sandor Industries, Pinetown, South Africa) to minimise heating problems in the air stream, and plant pots were painted white to minimise a temperature build-up in the soil (Figure 2.1.b).

A diagram of a single microcosm is illustrated in Figure 2.1.c depicting a 37 litres PVC plant pot of top radius 0.225 m and 0.165 m bottom radius, by 0.3 m deep, fitted with a polycarbonate open-top chamber of 0.8 m height and radius 0.225 m. Also shown in the diagram is a movable supporting metal frame of 0.4 m x 0.4 m that was laid over the main framework at a height of 0.7 m from the floor of the greenhouse. The movable supporting frame enabled microcosms to be lifted independently for weighing without interfering with the rest of the set-up using a cantilevered balance. A sleeve was attached to the centre of each microcosm in order to accommodate a CO_2 delivery pipe rising from the main supply pipe. Risers were fitted with adjustable butterfly valves in order to maintain air velocity close to 2.1 ms^{-1}, which was adequate to supply 0.38 m^3min^{-1} (to permit three changes of air per minute in the open top chambers). The risers were designed to be detachable from the main supply pipes to prevent contact between the riser and the central sleeve when plant pots were lifted during weighing.

Figure 2.1.a: General view of the experimental set-up during construction, showing three rows of painted steel framework supporting 16 microcosms. Air and CO_2 delivery pipes rising from the two supply pipes (one for ambient air and the other for elevated CO_2) can be seen beneath each row of microcosms. The cantilevered balance can be seen in the middle row.

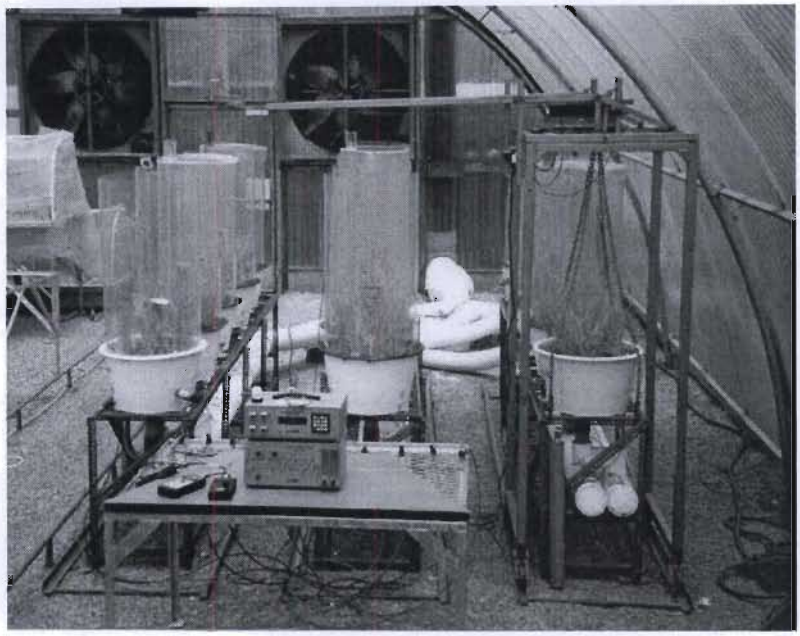


Figure 2.1.b: General view of the experiment during a growing season, showing microcosms fitted with open-top chambers. Operation of the cantilevered balance is illustrated on the right hand row. The microcosms have been painted white to minimise temperature build up in the soil. The fan and all pipe-layout downstream from the fan were insulated with white polythene foam to minimise heating problems in the air stream.

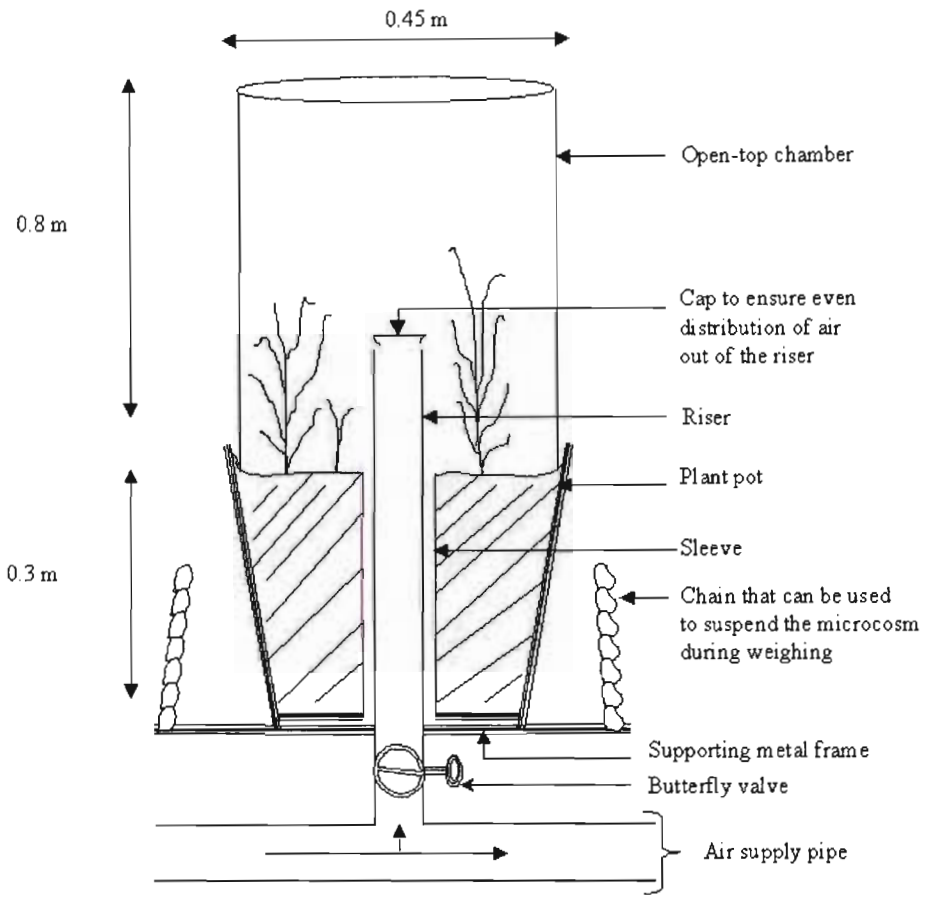


Figure 2.1.c: Schematic diagram of a cross-section of a microcosm with an open-top chamber and other accessories.

The soil placed in the microcosms attempted to reproduce field conditions as closely as possible. All soil was sourced from the field site. A 5 cm layer of clay was placed into the bottom of the pots followed by a 20 cm deep silty loam characterised by a fine texture and low gravel content. The bottom of plant pots was fitted with two 1cm wide by 0.5 m long drainage tubes, sealed at one end with detachable plugs for quantifying drainage output. Drainage tubes were equidistant from the central sleeve attached to the CO_2 supply pipe. The design allowed for measuring ecosystem water output in addition to evapotranspiration. An additional opening was made on the side of each pot at 10 cm depth from the soil surface, and was fitted with a perforated PVC tube that extended 1 cm outside the pot. The perforated tube was used as a portal for measurement of soil temperature. Two sets of small openings that could fit the probes of a Delta-T ThetaProbe (Delta-T Devices Ltd., Cambridge, UK) soil moisture sensor were made at 12 cm and 22 cm depths on each side of a plant pot. The soil moisture sensor was used to monitor changes in soil water at 6 cm below the soil surface, at a 12 cm depth in the rooting layer, and at 22 cm depth in the clay layer. The average mass of a microcosm including soil, plants, and associated accessories excluding the chamber was 31.6 kg before watering, and about 41.3 kg after watering to field capacity.

2.2. Plant collection and establishment in the greenhouse

Experimental plants and soil were collected from the field site on 16th April 1998, and the C_4 grass communities were established in microcosms within 72 hours of collection. Heavy rain had fallen on the field site a few days prior to collection of plants and soil, and water content of the soil and underlying clay was 19 ± 1 % and 20 ± 1 % respectively, hence plants were not subjected to any sort of water stress during transplanting. It was ensured that the species composition and the soil of the established communities resembled those of the Ngongoni grassland community. Resemblance of the microcosms to the field site was critical because factors such as species density (Wayne and Bazzaz 1995, 1997; Wayne et al. 1999) and composition (Chiariello and Field, 1996) have an influence on responsiveness of mixed communities to elevated CO_2. Plant communities were allowed four months to re-establish in the greenhouse. No plant mortality was observed after planting, and there were no major differences in the basal cover and height of plant communities in all 16 microcosm after four months of establishment, and this was a satisfactory state of

affairs prior to application of treatments. At the end of the establishment phase, and before the treatments were applied, the above-ground biomass of established plants was harvested and quantified by species, and then burned in a muffle furnace at 500 °C. The plant ash was sprinkled on to the soil surface to simulate effects of burning on nutrient turnover.

Composition of the established communities included a C_3 grass – *Alloteropsis semialata* (R. Br.) Hitchc. sub-species *eckloniana*, a C_3 tuberous geophyte – *Eriospermum mackenii* (Hook. f.) Baker, subsp. m*ackenni*; four C_4 grasses representative of three C_4 photosynthetic pathways: *Andropogon appendiculatus* Nees and *Themeda triandra* Forssk., both NADP-me; *Eragrostis racemosa* (Thumb.) Stued., NAD-me; *Sporobulus pyramidalis* Beauv., PCK. Each plant was replicated twice per pot to make a total of 12 plants equally distributed on a total surface area of 0.125 m^2 per pot. Earthworms collected from the same site as the plants were included in the microcosm to facilitate nutrient turn-over. In most grasslands, earthworms make up a dominant fraction of the biomass of soil animals and have important effects on the structure and function of the ecosystems (Zallar and Arnone III, 1997). Principal activities of earthworms include soil turnover, incorporation of organic matter, improvement of soil aeration, and preservation of soil structure through humification.

2.3. Weed and pest control

Microcosms were hand weeded when weeds appeared. On two occasions in October 1999 at the beginning of the second year, the microcosms were sprayed for mites when required, using a domestic insecticide; Baysol contact spray manufactured by Bayer Chemicals (South Africa) active ingredient Cyfluthrin (Pyethroid), was used.

2.4. The simplified weighing lysimeter

Weighing lysimetres are well recognised as the best technique available for measuring evapotranspiration of grasses (Howell et a., 1991). The technique is convenient for confined soil systems without the spatial and temporal variations characteristic of field measurements. The cost involved in construction of the lysimeter was minimised by use of locally available materials and labour. Control of water inputs is easy and outputs can be measured by incorporating evapotranspiration, drainage loss and a

change in the amount of soil water in the lysimeter over a known period of time, provided surface run-off is prevented, otherwise the volume of net surface run-off should be included in the final equation. The lysimeter used in this study was designed and constructed in the workshop of the School of Life and Environmental Sciences at Natal University. Each plant pot and associated drainage accessories formed a confined soil column of known surface area and soil volume as described in section 2.1 above.

The weighing apparatus consisted of a rail-guided and hand-operated mobile crane to which a load cell with a millivolt meter was attached (Figure 2.1.b). The load cell and reading meter were supplied and calibrated by Scales for Africa (Johannesburg, South Africa). The balance was placed on top of the crane, and the read-out LCD screen was attached at breast height on the side of the crane facing the operator. The weighing capacity of the load cell was 60 kg, which was sufficient for the average pot mass of 37–39 kg in the low water treatments and 41–43 kg in the high water treatments. Weighing was done by lifting the pots with the hand-operated crane such that they were suspended from the load cell. The crane was fitted with castors that enable it to move along the rails in either direction. Metal rails were fitted on the floor of the greenhouse along rows of the main framework to ensure that the lysimeter remained stable and sturdy during measurements.

2.5. Treatments

The treatments consisted of two by two factorial combinations of CO_2 and watering, each replicated four times. A considerable degree of control over CO_2 concentrations and watering within set ranges was achieved. As a result, some of the inherent complexities of doing this kind of work in the field were eliminated, although the limitations were restricted space and over-simplified ecosystem interactions.

2.5.1. CO_2 treatments

Ambient CO_2 treatment fluctuated around 380 μmol mol^{-1}, while the elevated CO_2 treatment was ambient plus 370 μmol mol^{-1}. Temporal fluctuations in CO_2 concentration around 10 μmol mol^{-1} in all chambers were associated with fluctuations in CO_2 concentrations of ambient air. Calibration of CO_2 treatments was checked weekly using a portable infrared gas analyser (IRGA). An Analytical Development

Company LCA-2 was used in the first year of the experiment and a Li-Cor 6262 was used in the subsequent two years. A Li-Cor 6400 was also used to check CO_2 concentrations in the third year. For some reason the CO_2 concentration in the elevated CO_2 treatment in the third row of microcosms was about 50 μmol mol^{-1} lower than the set point. Despite several attempts this problem could not be solved and the two elevated CO_2 microcosm in this row remained at CO_2 concentrations slightly lower than the other six microcosms throughout the duration of the study.

2.4.2. Watering treatments

Watering treatments were based on monthly average rainfall of a 50-year data set falling in the period 1936 to 1990 measured at Eureka weather station (30°43'S 30°01'E), 5 km from Ngongoni field site (Figure 2.2). In the first year a high watering treatment was equivalent to a mean annual rainfall (MAR) of 736 mm, and the low watering treatment was set at 80% of MAR, a figure close to the average rainfall of dry years. The volume of water required for watering events was determined from rainfall in mm, and the soil surface area of plant pots (an average of the top and bottom radius of the plant pot, 0.225 m and 0.165 m respectively, was used to calculate the soil surface area). It was also taken into consideration that the central part of plant pots was fitted with a riser for air delivery (ambient and CO_2-enriched), and that the riser was surrounded by a sleeve of 0.045 m radius (Figure 2.1c).

In the first year, water volumes per month were according to annual rainfall patterns (Table 2.1), but within each month water was supplied in a stochastic manner, to simulate natural rainfall. During the course of the first year, it became apparent that the stochastic pattern at 80%MAR watering treatment was sometimes stressful for the plants during prolonged periods of no watering. Hence, water treatments were changed to MAR and 120%MAR in the second year starting August 1999, and application was changed from stochastic to regular (Table 2.2) at intervals of every three and four days to avoid prolonged dry periods. In the third year, water treatments were swapped as shown by the change in the direction of arrows from second to third season in Table 2.2, to assess the effects of changes in rainfall pattern.

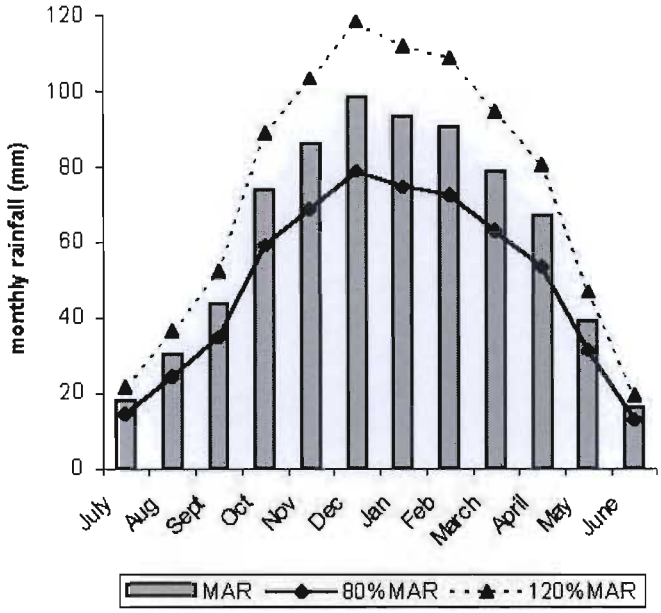


Figure 2.2: Mean seasonal rainfall data for the Ngongoni field site represented as MAR (mean annual rainfall), shown with two other experimental treatments of 80% MAR and 120% MAR.

Table 2.1: Monthly watering amounts in mm per treatment.

Month	Monthly watering at MAR (mm)	Monthly watering at 80%MAR (mm)	Monthly watering at 120%MAR (mm)
July	18	14	22
August	30	24	37
September	44	35	52
October	74	59	89
November	86	69	103
December	99	79	118
January	93	75	112
February	91	72	109
March	79	63	95
April	67	54	80
May	39	31	47
June	16	13	19
Total	**736**	**588**	**883**

Table 2.2: Changes in annual rainfall treatment and manner of application.

Year	Rainfall amount (mm)		
CO_2 and water treatments 1998/1999	Stochastic 80%MAR (low water)	Stochastic MAR (high water)	
1999/2000		Regular MAR (low water)	Regular 120%MAR (high water)
2000/2001		Regular MAR (low water)	Regular 120%MAR (high water)
Effective water treatment at end of year 2		**110%MAR** (high water)	**90%MAR** (low water)
Effective water treatment at end of year 3		**107%MAR** (high water)	**MAR**

2.6. Monitoring of experimental micro-climatic conditions

A major advantage of using experimental microcosms is the potential for good control of environmental variables. Attempts to attain such control on the microcosm set-up used in this study include reduction of direct radiation on plant pots by painting all pots with white paint, and use of 40% density shade cloth skirts hung to mid-canopy height. Air flow through the open-top chambers was controlled with butterfly valves at the bottom of the risers, and flow rate out of the risers was monitored at the top of the riser to ensure that it was maintained at 2.1 ms^{-1}. Monitoring of CO_2 concentrations was manual, using an LCA2 in the first year of the experiment, and a Li-Cor 6262 in the second and third years of the experiment. Control of CO_2 treatments (ambient and elevated) was satisfactory, and periodically fluctuated around 10 $\mu mol\ mol^{-1}$ of set point due to fluctuations in ambient air. A minor concern as mentioned in section 2.4.1, was that two elevated CO_2 microcosms in the third row of microcosms measured about 50 $\mu mol\ mol^{-1}$ lower than set point, despite numerous attempts to solve the problem. As a result the two elevated CO_2 microcosms remained at CO_2 concentrations slightly lower than the other six microcosms. However, several studies have used elevated CO_2 concentrations of 680 $\mu mol\ mol^{-1}$ with satisfactory results.

Air temperature inside and outside the chambers as well as soil temperature were monitored in a subset of microcosms at hourly intervals from September to May each year, using thermistors attached to a data logger (MCS 120-EX, MC Systems, Cape Town). Thermistors were calibrated at the start of the experiment so that their readings did not deviate by 0.6°C from each other. Air temperature inside the chambers was monitored at the top of the canopy (80 cm) and at mid-canopy height (40 cm). Thermistors measuring air temperature were covered with shields made of white polystyrene to avoid effects of direct radiation. Generally, there was no difference in air temperature at the top of the canopy (80 cm) and at mid-canopy height (40 cm). During the cooler months of the year, mid-day air temperature inside the chambers ranged between 25 °C – 32 °C. Air temperature inside the chambers was 3 °C warmer than outside the chambers during the cooler months of the year, and during the warmer months mid-day temperatures inside the chambers ranged between 30 °C – 41 °C. Air inside the chambers at mid-day was 6 °C warmer than air outside the chambers during the hottest months of the year. Soil temperature was monitored at

a depth of 10 cm with a thermistor inserted in the soil through a horizontal perforated PVC sleeve. Mean, minimum and maximum readings were recorded hourly. Day time soil temperature at the 10 cm depth was usually 4 °C lower than air temperature, with a characteristic time lag as air cooled down and warmed up before the soil.

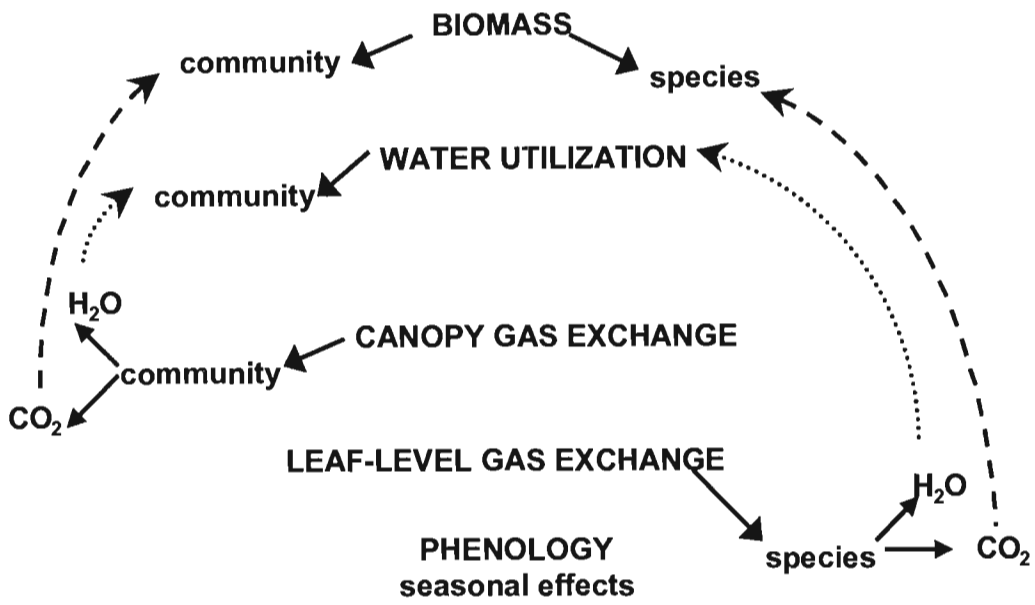
Net incoming radiation was monitored at the top of the open top-chamber using an MCS 155-1 radiation sensor attached to the same data logger as the thermistors. Calibration of the radiation sensor was done at MC Systems in Cape Town. Recorded values of net radiation at mid-day during clear and cloudless summer days of the year measured 670 Wm^{-2}, and mid-day values recorded during clear, cloudless winter days of the year were around 450 Wm^{-2}. The distribution of photosynthetically active radiation within plant canopies was also measured at full canopy once each year.

2.7. Experimental design and statistical analysis

The set-up consisted of four open-top chambers per treatment by four treatments. There were five grass species and one geophyte per treatment, and the plants were replicated twice in each chamber. A classical two-way analysis of variance was performed on most of the results, giving fifteen degrees of freedom per species and thirty-one degrees of freedom in total. Analyses were performed using Unistat for Windows version 4.53.

2.8. Work plan

Experiments were planned to generate data that would be consolidated into major themes as illustrated in the concept map below.



Year 1: (September 1998 – May 1999)

Resprouting plants were exposed to ambient and elevated CO_2 and watering treatments (Tables 2.1 and 2.2) for an entire growing season. Measurements of community water use were taken with the weighing lysimeter, from which annual rate of evapotranspiration could be calculated. Phenological observations were made, and canopy development assessed by measurements of leaf growth rate on two leaves per plant per pot in October, December, February and April. The set-up was assessed for performance of CO_2 delivery, spatial and temporal distribution of CO_2 within the open top chambers, and temperature fluctuations. End of year above-ground biomass was harvested, and quantified by species into two components *viz.* leaf and stem-plus-floral parts.

Year 2: (September 1999 – May 2000)

A revised watering schedule as shown in Table 2.2 was applied, and the following measurements commenced once plants had re-sprouted.

1. Weekly measurements of water use with the weighing lysimeter, which were indicators of annual rates of evapotranspiration.
3. Canopy level gas exchange using the Li-6262 IRGA once a month for all pots/treatments.
4. A data logger was used to collect concurrent measurements of total incoming radiation at the top of selected and representative open-top chambers, and canopy and soil temperatures.
5. Visual phenological observations at three monthly intervals to estimate the proportion standing dead. This permits assessment of species composition in terms of live biomass.
6. Harvesting at the end of the year to quantify total above ground biomass.

Year 3: (August 2000 – May 2001)
Watering treatments were changed again as shown in Table 2.2 such that pots that were subjected to MAR in the previous year received a watering treatment that was increased to 120%MAR and vice versa, while the frequency of application remained unchanged (twice weekly application). The reason for swapping water treatments was to investigate whether there was a carry over-effect of previous water treatments on responses to elevated CO_2 after two years of exposure. Measurements of leaf level gas exchange were done using the Li-Cor 6400, in order to evaluate individual species contribution to above-ground productivity, evapotranspiration, and total community water use. Other physiological measurements were similar to those taken in the previous year.

In order for community and ecosystem level studies to make a meaningful contribution towards understanding potential impacts of climate change on present and future vegetation distribution pattern, and to have a predictive capacity, they have to continue for a number of years. Mooney et al. (1991) suggest that at least a decade is required to allow a response trajectory to be established.

CHAPTER 3

CANOPY STRUCTURE AND PHENOLOGY

3.1. Introduction

Canopy structure describes the spatial and temporal organisation of leaf area with respect to the positional distributions of stems and branches in a stand of vegetation (Norman and Campbell, 1989). Phenology, on the other hand, describes the timing and progress of plants through identifiable stages of development in response to changes in climate or photoperiod. Effects of elevated CO_2 on plant development (Bazzaz 1990), including seedling establishment, growth, biomass allocation, time of flowering and senescence, have a remarkable influence on plant competitive outcomes, because even small differences in time of emergence among seedlings have significant consequences for the ability of plants to compete with their neighbours (Ross and Harper 1972). CO_2-induced changes in the rate of plant development are usually borne through leaf and stem morphology (Reekie 1996), and consequently modification of canopy structure, which in turn has an effect on species' competitive interactions (Gaudet and Keddy 1988; Beyschlag et al. 1990; Anten and Hirose, 2001), particularly with regards to above-ground production.

There is a very good correlation between parameters of plant form (canopy structure) and functional processes of net primary production (Lambers and Poorter 1992) nutrient cycling (Eviner and Chapin 2003), hydrology (LeMaitre et al. 1999), and plant water use (Passioura 1984). Thus, it is suggested that the influence of canopy structure on net primary production is sometimes greater than that of photosynthetic CO_2 uptake. This is a contention that is supported by the experimental work of Beyschlag and co-workers (1990), which illustrates the significance of plant structural features beyond photosynthetic characteristics (carboxylation efficiency, maximum photosynthetic capacity, and quantum efficiency). Lambers and Poorter (1992) estimate that in herbaceous C_3 species, an average of 10% increase in relative growth rate is associated with a 7.5% increase in leaf area ratio and a 2.4% increase in net assimilation rate. CO_2-induced changes in structural parameters such as canopy height (Hartz-Rubin and DeLucia, 2001) and leaf area distribution (Ellsworth and Reich, 1992, Hirose and Werger 1995) influence primary production in a manner

proportional to the vertical gradients of the light regime. In this regard, Barnes et al. (1990) demonstrated that modifications of canopy structural parameters in the upper canopy layers have more profound consequences for net photosynthesis, relative to structural modifications in the lower layers of the canopy.

The importance of phenology in plant functional processes is especially highlighted in models of primary production that predict potential consequences of global climate change on the terrestrial carbon cycle (Jackson et al. 2001; Kramer et al. 1996). As far as nutrient cycling is concerned, senescence constitutes a major trigger for translocation (Son and Gower, 1991; Jach and Ceulemans 1999), with important implications for timing of senescence under elevated CO_2. Phenological changes of leaf production and loss of green leaf area due to senescence are long-term mechanisms that affect plant water use (Passioura, 1984). Effects of elevated CO_2 on phenology can influence mechanisms by which phenology itself affects plant water use. Importantly, elevated CO_2 affects plant water use in the short-term via reduction in stomatal conductance (Knapp et al. 1993a; Wand et al. 1999), which in the long-term results in significant reductions in canopy transpiration (Knapp et al. 1993a, Harmelynck et al. 1997) and in some instances significant increases in soil water (Owensby et al. 1999; Grünzweig and Körner, 2001; Morgan et al. 2001). On the other hand, stimulation of leaf growth under elevated CO_2 (Taylor et al. 1994) is a developmental response that can result in larger plants and earlier canopy closure, whose consequence on plant water use is to negate the water saving effect due to reduced stomatal conductance (Field et al. 1995). However, in ecosystems with low leaf areas such as grasslands, a stimulation of leaf growth that is accompanied by an increase in leaf area index can lead to a reduction in evaporation from the soil surface (Field et al. 1995), and consequently an improvement in ecosystem water use efficiency.

The current chapter will characterise treatment effects on phenology and canopy structure of the model C_4-dominated grassland community by assessing phenology of sprouting, flowering and senescence; and how responses of leaf area distribution influence canopy structure. Results obtained will be used to answer the first key question of the study which states: (i) what effects will elevated CO_2 have on canopy development and structure of microcosm communities, and will responses be

dependent on water supply? The data will also contribute to the broader objective of the study by enabling an assessment of whether treatment effects on community structure and phenology have a bearing on community production and water use (Chapter 8 – General Discussion).

In this introductory section of the chapter, reference is made to other studies on C_4-dominated grasslands and to communities and grasslands with C_3 grasses for comparison. Previous studies generally indicate that elevated CO_2 influences plant phenology through changes in timing of leaf emergence, the time it takes for developing leaves to reach maximum leaf area, longevity of leaf area, senescence, and the rate of leaf turnover (Reekie and Bazzaz, 1991; Knapp et al. 1999; and Reich et al. 2001).

In C_4-dominated tallgrass prairie elevated CO_2 was reported to enhance the rate of leaf expansion, and to reduce the time required for leaves to reach maximum leaf area, by 29% (Knapp et al. 1999), while leaf senescence was delayed at the end of the growing season (Ham et al. 1995, Knapp et al. 1999). Positive effects of elevated CO_2 on growth in tallgrass prairie were particularly enhanced under low precipitation (Owensby et al. 1993; Knapp et al. 1999), and the mechanism was through enhanced soil water at the end of the growing season. In an estuarine marsh ecosystem on the other hand (Curtis et al. 1989a), elevated CO_2 delayed senescence only in C_3 species with no effect on growth in C_4 species. In a study that measured leaf area longevity under elevated CO_2 with no restriction on water supply (Craine and Reich 2001), increased longevity was measured in C_3 species only, with no effect on C_4 species. From the above studies, it seems that effect of elevated CO_2 on vegetative phenology of grasslands is strongly mediated by water availability, which differentiates between C_3 and C_4 responsiveness. It is also clear that the appropriate level at which to study this phenomenon is the community level, and not at individual species level.

A recent meta-analysis of responses of plant reproduction to elevated CO_2 (Jablonski et al. 2002) suggests that wild species are less responsive compared to crop species. Furthermore, studies on wild perennial grasses are scarce. The few studies that have investigated responses of plant reproduction to elevated CO_2 in wild annual grasses show highly variable results. For instance, Grünzweig and Körner (2000) reported no

change in reproductive output of two C_3 grass species (*Aegilops kitschyi* and *Hordeum spontaneum*), and a decline in reproductive output of a third C_3 grass species (*Aegilops peregrina*). On the contrary, Jackson and co-workers (1994) observed a 30% increase in seed production of a C_3 annual grass *Avena barbata* Brot. under elevated CO_2. With regards to C_4 annual grasses, Alberto et al. (1996) reported no consistent effect of elevated CO_2 on grain yield in *Echinochloa glabrescens*, suggesting a non-consistent effect of elevated CO_2 on reproduction responses. A study by Potvin and Strain (1985) showed that flowering timing of a C_4 annual grass (*Echinochloa crus-galli*) was advanced by elevated CO_2 only in populations derived from localities with shorter growing seasons, suggesting that positive effect of elevated CO_2 on floral initiation may be dependent on temperature.

Responses of plant reproduction to elevated CO_2 appear to be influenced by abiotic environmental parameters such as water availability. For instance in a study on pepper plants – *Caspium annum* (Penuelas et al. 1995), flower and fruit production increased when there was sufficient water. Several studies in the USA have shown that elevated CO_2 has the potential to increase soil water availability in grasslands (Ham et al. 1995; Owensby et al. 1997; Morgan et al. 2001). However, it is not clear how reproduction responses will benefit from increased soil water availability. Perhaps the benefit of reproduction response to elevated CO_2 and enhanced soil water availability will be realised mostly in plant species that are not dependent on specific pollinator association and/or species whose flowering is not dependent on photoperiod or degree days.

Effects of elevated CO_2 on canopy structure are manifested through increases in leaf area and canopy height (Taylor et al. 1994, Hartz-Rubin and DeLucia, 2001), even though responses are species specific and/or dependent on other environmental factors such as variation in amount and timing of rainfall (Jackson et al. 1998, Knapp et al. 1993a, Owensby et al. 1996, 1999). Precipitation indirectly influences canopy structure of subhumid grasslands (Knapp 1984) through competition for light (Lane et al. 2000), by either increasing canopy height without any effects on basal cover or by increasing basal cover without an effect on canopy height (Lane et al. 2000). As above-ground biomass and leaf area increase with increasing precipitation, new individuals become established only when above-ground gaps make light available

(Lauenroth and Coffin, 1992; Jurik and Pleasants, 1990). In the absence of elevated CO_2, there is a positive correlation between precipitation and above-ground net primary production of grasslands (Sala et al. 1988; Lane et al. 1998; Titlyanova et al. 1999). However, this positive effect is reported to diminish under elevated CO_2 (Owensby et al. 1993), and production is enhanced under elevated CO_2 preferentially when precipitation is low or variable.

3.2. Materials and Methods and statistical analysis

3.2.1. Materials and Methods

Experimental plants were allowed to establish for four months in a greenhouse (from April to July 1998), after which they were clipped to a 5 cm stubble. Application of CO_2 and water treatments commenced on the 1st August of each year of study, continuasly for a complete growing season, until time of biomass harvest (June/July). Canopy development and seasonal phenology were monitored as outlined below, at specific times from beginning of the year until the end, when above-ground foliage was harvested.

Time of sprouting was recorded bi-weekly at intervals of three and four days. Plants were considered to have sprouted on observation of three or more leaves emerging from the first tiller, when the height of emerging leaves measured 5 cm or more. Time was recorded as days elapsed since the application of treatments, so generating a continuous variable. Day of year (DOY) was not used because the growing season incorporates the end and beginning of two consecutive calendar years (August to May), and so would generate a discontinuous variable.

Time of appearance of first floral parts was assessed by external evidence of rapid elongation of the upper internodes of the flower stalk plus attached leaf sheaths, which was shortly followed by emergence of the inflorescence; and quantified in days elapsed since application of treatment.

Time of loss of greenness (beginning of senescence) was assessed qualitatively by visual observation of 50% senesced above-ground plant tissue for each species in different treatments, and quantified in number of days elapsed since application of

treatments. The **extent of senescence at harvest** was estimated qualitatively as proportion of dead (brown leaves) to live (green leaves) plant tissue.

Estimation of leaf distribution within a canopy was done by a stratified harvesting procedure at the end of each year. Plants were clipped at intervals of 20 cm from top to bottom of the canopy, leaving a 5 cm stubble to ensure that meristematic tissues were not destroyed during clipping. Plant biomass from each layer was sorted into leaves, stems and flowers where present, and placed in labelled brown paper bags and oven-dried at 60°C for 5 days. Dry leaves were weighed separately from dry stems and flowers. Only leaf biomass data by layer was used to estimate leaf distribution within the canopy.

3.2.2. Statistical analysis

Statistical analysis was performed on quantitative data only, and qualitative data such as loss of greenness (senescence) and its extent are referred to as personal observations. Data analysis was aimed at elucidating main and interactive effects of CO_2 and water treatments on time to sprouting, leaf growth, time to first appearance of floral parts, and canopy structure, by performing a two-way ANOVA (Unistat version 4.53) at the $\alpha = 0.05$ level of significance, using the Classical Experimental Approach. Where possible, data were analysed separately for species level and community level treatment effects. Assessment of treatment effects on species responses to time to sprouting and time to of flowering, as well as species contributions to community structure were done by use of "species" as a third factor in addition to CO_2 and water in a three-way ANOVA. If the ANOVA indicated significant treatment effects on a factor with more than two levels, Tukey-HSD (Highly Significant Differences) Multiple Comparison test was performed to find out which of the levels were significantly different. Missing values due to lack of sprouting for instance, created a situation of an unbalanced design in the data set. Nonetheless, the Unistat's ANOVA procedure of Classical Experimental Approach takes unbalanced designs into consideration so that the sum of squares computed for two or three factors and their interaction, are calculated after making adjustments for main effects.

3.3. Results

3.3.1. Time to sprouting

3.3.1.1. First year (CO_2 350, 700 ppm and water MAR, 80%MAR)

Sprouting took place 17 days after application of treatments. *Themeda* was the first species to sprout, followed by *Eragrostis*, then *Sporobolus*, *Alloteropsis* and *Andropogon*. Trends in the response are illustrated in Figure 3.1.a., and statistical significance of treatments is presented in Table 3.1. Early sprouting in *Themeda* and *Eragrostis* occurred under elevated CO_2 + MAR treatment, and the effect of CO_2 on the response was statistically significant for both species, but effect of water treatments was not statistically significant on those species as shown in Table 3.1. CO_2 treatments did not have a significant effect on sprouting in *Alloteropsis* and *Sporobolus*, but effect of water treatments was significant. In *Andropogon* which sprouted last, both CO_2 and water treatments (without interaction) influenced time to sprouting.

To present the results in a community context, data were pooled to generate three factors, namely; CO_2 treatment, water treatment, and species. A three-way ANOVA was performed to test if differences in the mean values of time of sprouting among CO_2 treatments, water treatments, and species are greater than would be expected by chance after allowing for the effects of differences in other factors. Results of the three-way ANOVA (Table 3.2) showed a statistically significant effect ($P < 0.001$) of CO_2, water and presence of different species, and there was also a significant interaction between CO_2 and water treatments ($P = 0.0370$). There was no apparent interaction of CO_2 and water treatments when a two-way ANOVA (Table 3.1) was performed on results of individual species. The significant main effect of species, and an interaction of CO_2 and water treatments in a three way ANOVA highlight treatment effects on competitive outcome of different sprouting capacities at a community level. To isolate which species differ from the others in their sprouting capacity, Tukey-HSD multiple comparison procedure was performed (Table 3.3), and results suggest that the sprouting response of the grasses can be grouped in to three, with *Themeda* as an early sprouter, then *Eragrostis* and *Sporobolus* as intermediate, and lastly *Sporobolus*, *Andropogon*, *Alloteropsis* as late sprouters.

Table 3.1: Statistical significance of treatment effects on time to sprouting in the first year.

Species	CO2	Water	Interaction
Themeda	P = 0.0294	NS	NS
Eragrostis	P = 0.0263	NS	NS
Sporobolus	NS	P = 0.0290	NS
Alloteropsis	NS	P = 0.0256	NS
Andropogon	P = 0.0321	P = 0.0417	NS

Table 3.2: Results of a three-way ANOVA for CO_2 x water x species at the $\alpha = 0.05$ level on time to sprouting.

Due To	Sum of Squares	DoF	Mean Square	F-Stat	Signif
Main Effects	1056.475	6	176.079	18.134	0.0000
CO2	166.056	1	166.056	17.102	0.0001
Water	162.006	1	162.006	16.685	0.0001
Species	728.413	4	182.103	18.755	0.0000
2 Way Interactions	82.431	9	9.159	0.943	0.4899
CO2 × Water	43.056	1	43.056	4.434	0.0370
CO2 × Species	24.537	4	6.134	0.632	0.6406
Water × Species	14.838	4	3.709	0.382	0.8212
3 Way Interactions	1.662	4	0.416	0.043	0.9965
CO2 × Water × Species	1.662	4	0.416	0.043	0.9965
Explained	1140.569	19	60.030	6.182	0.0000
Error	1359.375	140	9.710		
Total	2499.944	159	15.723		

Table 3.3: Tukey-HSD multiple comparisons test for time to sprouting (days) in the first year for all treatments combined, and data classified by species at the α = 0.05 level.
* denotes significantly different pairs and vertical bars show homogeneous subsets.

Group	Mean	*Themeda*	*Eragrostis*	*Sporobolus*	*Alloteropsis*	*Andropogon*	Homogenous subsets
Themeda	17.7188		*	*	*	*	|
Eragrostis	20.6250	*			*	*	|
Sporobolus	22.8125	*					| |
Alloteropsis	23.0313	*	*				|
Andropogon	23.4063	*	*				|

3.3.1.2. Second year (CO_2 350, 700 ppm and water MAR, 120%MAR)

Sprouting was delayed by a few days in the second year compared to the first year, probably due to a mild drought brought about by long periods of no watering during a stochastic application of watering treatment in the first year. *Themeda* was once again the first species to sprout after 18 and 20 days in elevated CO_2 + 120%MAR and elevated CO_2 + MAR treatments respectively. The pattern of sprouting is represented graphically in Figure 3.1.b., and the statistical significance of the response is presented in Table 3.4. CO_2 treatments had a significant effect on sprouting in *Themeda* and *Andropogon*, such that plants growing under elevated CO_2 flowered earlier than plants growing under ambient CO_2 in these two species, even though sprouting in *Andropogon* occurred much later than in *Themeda*. Water treatments had a significant effect on sprouting in *Sporobolus*, and plants growing under the 120%MAR treatments sprouted earlier than their counterparts in the MAR treatments irrespective of CO_2. In *Eragrostis*, the earliest date of sprouting was recorded under elevated CO_2 + 120%MAR, and the ANOVA result was significant for both CO_2 and water without interaction. There were no treatment effects on the sprouting pattern of *Alloteropsis* in the second year.

Incidents of lack of sprouting were recorded in *Alloteropsis* under ambient CO_2 + MAR and in *Eragrostis* under elevated CO_2 + MAR. The frequency of lack of sprouting was one plant in each of the two species.

Table 3.4: Statistical significance of treatment effects on time to sprouting in the second year.

Species	CO2	Water	Interaction
Themeda	P = 0.0213	NS	NS
Eragrostis	P = 0.0211	P = 0.0354	NS
Sporobolus	NS	P = 0.0178	NS
Andropogon	P = 0.0248	NS	NS
Alloteropsis	NS	NS	NS

All data for the second year were combined to generate factors CO_2, water and species, in order to assess the significance of treatment effects in a community context using a three-way ANOVA. Results of the three-way ANOVA (Table 3.5) were very similar to results of the first year because effects of all three factors: CO_2, water, and species were significant (P = <0.001), with a significant interaction between CO_2 and water treatments (P = 0.0198). A multiple comparison test performed on species revealed three categories of sprouting response, where *Themeda* is an early sprouter, *Eragrostis*, *Sporobolus* and *Andropogon* are intermediate, and *Andropogon* and *Alloteropsis* are late sprouters (Table 3.6).

Table 3.5: Results of a three-way ANOVA for CO_2 x water x species at the $\alpha = 0.05$ level.

Due To	Sum of Squares	DoF	Mean Square	F-Stat	Signif
Main Effects	1673.202	6	278.867	32.287	0.0000
CO2	155.942	1	155.942	18.055	0.0000
Water	134.311	1	134.311	15.551	0.0001
Species	1394.183	4	348.546	40.355	0.0000
2 Way Interactions	83.554	9	9.284	1.075	0.3852
CO2 × Water	47.968	1	47.968	5.554	0.0198
CO2 × Species	28.392	4	7.098	0.822	0.5134
Water × Species	7.141	4	1.785	0.207	0.9343
3 Way Interactions	5.485	4	1.371	0.159	0.9587
CO2 × Water × Species	5.485	4	1.371	0.159	0.9587
Explained	1762.241	19	92.750	10.739	0.0000
Error	1191.911	138	8.637		
Total	2954.152	157	18.816		

Table 3.6: Tukey-HSD multiple comparisons test for time to sprouting (days) in the second year for all treatments combined, and data classified by species at the $\alpha = 0.05$ level.

* denotes significantly different pairs and vertical bars show homogeneous subsets.

Group	Mean	*Themeda*	*Eragrostis*	*Sporobolus*	*Andropogon*	*Alloteropsis*	Homogenous subsets
Themeda	20.84		*	*	*	*	|
Eragrostis	26.35	*				*	|
Sporobolus	27.00	*				*	|
Andropogon	28.37	*					||
Alloteropsis	29.29	*	*	*			|

3.3.1.3. Time to sprouting in the third year

A notable trend of the third year is that responses were influenced by CO_2 treatments in all species except in *Sporobolus*, while water treatments had no significant effect on response of any of the species (Figure 3.1.c. and Table 3.7.). The earliest time of sprouting recorded was 18 days in *Themeda* under elevated CO_2 + 120%MAR and 20 days in the same species under elevated CO_2 + MAR. The response time is almost a

replica of the previous year, only with a higher level of statistical significance for the CO_2 treatment (P = 0.0068). When a three-way ANOVA was performed on combined data (Table 3.8), the effect of CO_2 treatments and species were statistically significant (P < 0.001) but effects of water treatments were not significant. Interaction of CO_2 and water treatments was highly significant (P = 0.0099). Results of a multiple comparison test categorised by species revealed two classes of sprouting capacity, one with *Themeda* and another with the other four species (Table 3.9).

Table 3.7: Statistical significance of treatment effects on time to sprouting in the third year.

Species	CO2	Water	Interaction
Themeda	P = 0.0068	NS	NS
Sporobolus	NS	NS	NS
Eragrostis	P = 0.0271	NS	P = 0.0395
Andropogon	P = 0.0346	NS	NS
Alloteropsis	P = 0.0453	NS	NS

Table 3.8: Results of a three-way ANOVA for CO_2 x water x species at the $\alpha = 0.05$ level.

Due To	Sum of Squares	DoF	Mean Square	F-Stat	Signif
Main Effects	1356.759	6	226.126	20.805	0.0000
CO2	252.366	1	252.366	23.219	0.0000
Water	17.051	1	17.051	1.569	0.2128
Species	1090.623	4	272.656	25.086	0.0000
2 Way Interactions	96.685	9	10.743	0.988	0.4531
CO2 × Water	74.616	1	74.616	6.865	0.0099
CO2 × Species	10.699	4	2.675	0.246	0.9116
Water × Species	21.332	4	5.333	0.491	0.7426
3 Way Interactions	24.901	4	6.225	0.573	0.6829
CO2 × Water × Species	24.901	4	6.225	0.573	0.6829
Explained	1478.344	19	77.808	7.159	0.0000
Error	1336.858	123	10.869		
Total	2815.203	142	19.825		

Table 3.9: Tukey-HSD multiple comparisons test for time to sprouting (days) in the third year for all treatments combined, and data classified by species at the α = 0.05 level.
* denotes significantly different pairs and vertical bars show homogeneous subsets.

Group	Mean	*Themeda*	*Sporobolus*	*Eragrostis*	*Andropogon*	*Alloteropsis*	Homogenous subsets
Themeda	21.32		*	*	*	*	|
Sporobolus	26.89	*					|
Eragrostis	26.90	*					|
Andropogon	28.12	*					|
Alloteropsis	29.22	*					|

After analysing for treatment effects on annual patterns of sprouting per species, it is necessary to do further analysis to determine if similarities and/or differences in time of sprouting per species among years are statistically significant. Data of the three years were combined for each species to generate the factors: CO_2, water and year, and a three-way ANOVA was performed to determine the statistical significance of year of study (Table 3.10), CO_2 and water treatments. Difference in the mean values of time of sprouting among the different years of study are greater than would be expected by chance after allowing for the effects of differences in CO_2 and water ($P = <0.001$). A multiple comparison test was done to determine which year(s) differ from others. In all species, sprouting happened sooner in year one than in years two and three. There was no statistically significant difference in the time of sprouting in years two and three. In *Themeda*, sprouting occurred approximately three days earlier in year one compared to years two and three. In the other four grass species, sprouting in year one occurred approximately five to six days earlier compared to years two and three.

Table 3.10: Results of a three-way ANOVA for CO_2 x water x year at the $\alpha = 0.05$ level.

Due To	Sum of Squares	DoF	Mean Square	F-Stat	Signif
Main Effects	921.812	5	184.362	22.333	0.0000
CO2	78.248	1	78.248	9.479	0.0029
Water	70.255	2	35.128	4.255	0.0177
Season	661.566	2	330.783	40.070	0.0000
2 Way Interactions	22.438	3	7.479	0.906	0.4422
CO2 × Water	9.017	2	4.508	0.546	0.5814
CO2 × Season	4.121	2	2.061	0.250	0.7797
Water × Season	6.889	1	6.889	0.834	0.3638
3 Way Interactions	0.000	0			
CO2 × Water × Season	34.533	1	34.533	4.183	0.0442
Explained	978.783	7	139.826	16.938	0.0000
Error	635.640	77	8.255		
Total	1614.424	84	19.219		

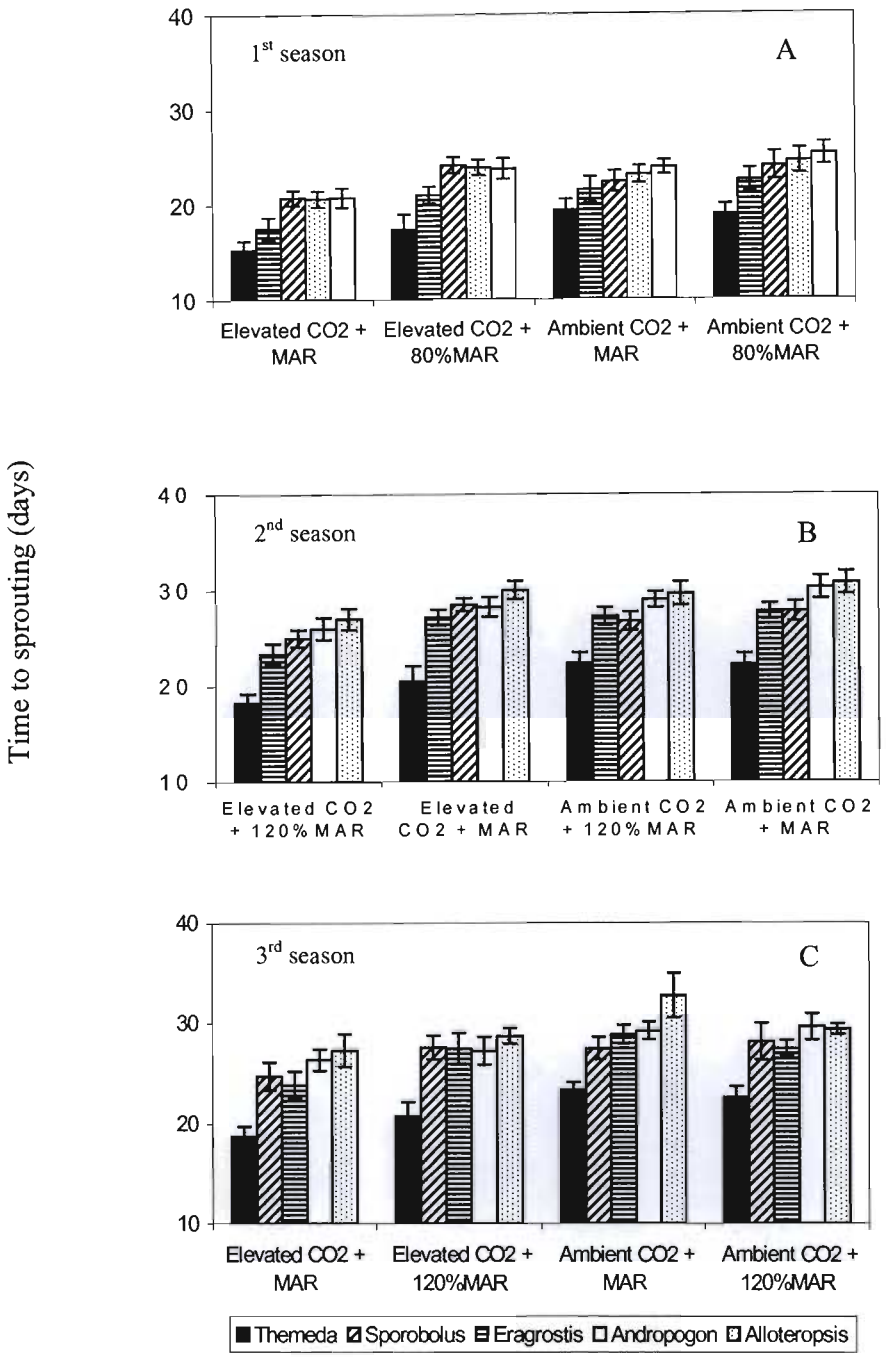


Figure 3.1 (a-c): Treatment effect on time to sprouting of the grass species over three years. Error bars indicate standard error on this figure, and in subsequent figures throughout this Chapter.

3.3.2. Time to flowering

Data are presented for only three of the five grass species used in the study (*Eragrostis*, *Sporobolus* and *Themeda* in Figure 3.2.a,b,c), because of either poor flowering or no flowering in *Alloteropsis* and *Andropogon*. *Andropogon* flowered rather poorly with a frequency of one or two plants out of eight plants per treatment in the first and second years. No flowering was recorded in that species in the third year. *Alloteropsis* and the geophyte did not flower in any of the three years. *Eragrostis* was the first species to flower about 75 days after application of treatments. Plants exposed to ambient CO_2 flowered earlier than plants exposed to elevated CO_2 ($P = 0.0002$) (Table 3.11). Effect of watering treatments were not statistically significant in *Eragrostis*. A treatment combination of elevated CO_2 + MAR induced early flowering in *Themeda* after 82 days, and effects of both CO_2 and water were statistically significant ($P = 0.0093$ and $P = 0.0018$ respectively) but there was no interaction. The flowering response of *Sporobolus* was similar to that of *Themeda*, but in *Sporobolus* the interaction of CO_2 and water was significant ($P = 0.0089$).

In the second and third years, flowering responses of *Eragrostis* and *Themeda* were very similar to the first year. In *Sporobolus*, effect of water treatments was not significant in the second year, and effect of CO_2 treatments was not significant in the third year (Table 3.11). Data from the three years was combined for each species in order to perform a three-way ANOVA with factors CO_2, water and year, to determine differences and/or similarities between years. Annual trends of flowering were similar in *Eragrostis* and *Themeda*. In *Sporobolus*, the flowering pattern of the first year was significantly different from the flowering pattern of the second and third year.

Table 3.11: Statistical significance of treatment effects on time to flowering

Year	Species	CO2	Water	Interaction
1	*Eragrostis*	P = 0.0002	NS	NS
1	*Sporobolus*	P = 0.0173	P = 0.013	P = 0.0089
1	*Themeda*	P = 0.0093	P = 0.0018	NS
2	*Eragrostis*	P < 0.0001	NS	NS
2	*Sporobolus*	P = 0.0120	NS	P = 0.037
2	*Themeda*	P = 0.0018	P = 0.0006	NS
3	*Eragrostis*	P = 0.0011	NS	NS
3	*Sporobolus*	NS	P = 0.0408	P = 0.0323
3	*Themeda*	P = 0.0013	P = 0.0001	NS

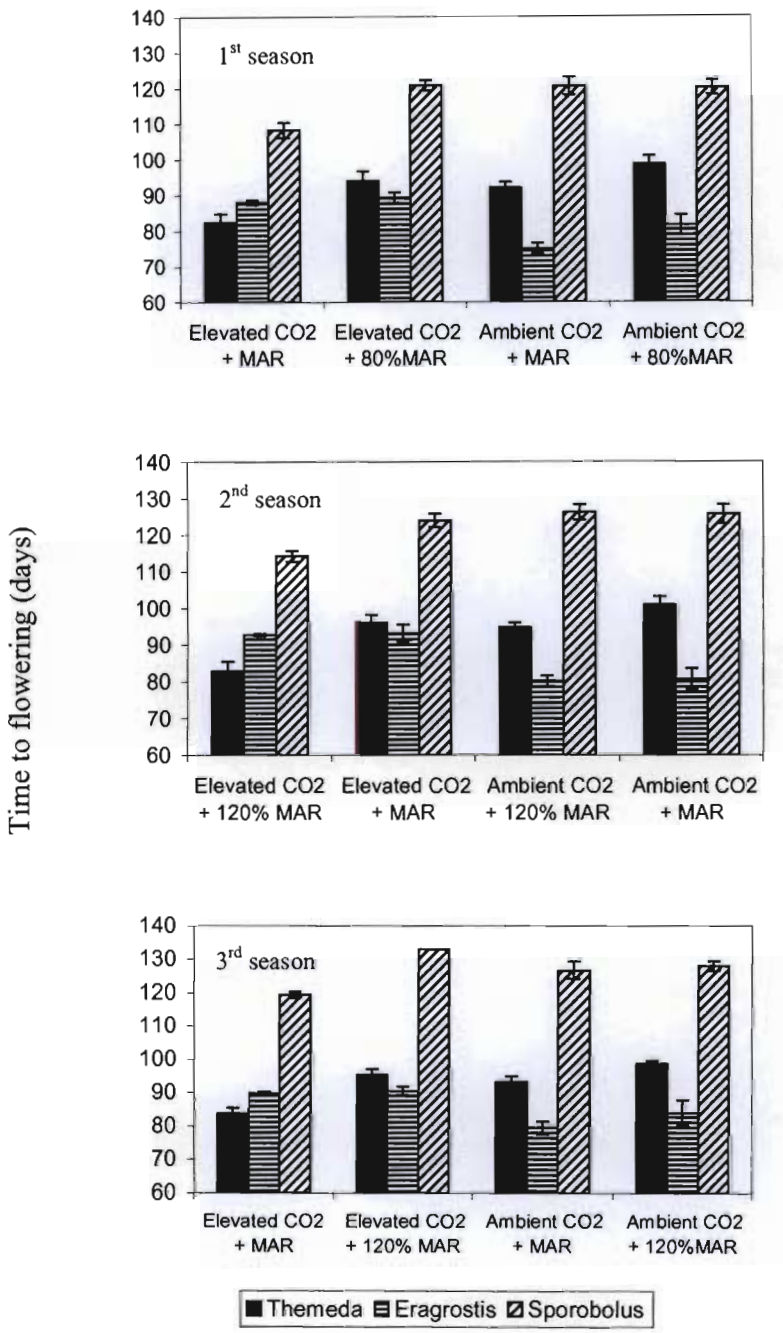


Figure3.2: Treatment effect on time to flowering of the grass species over three years.

3.3.3. Canopy structure

3.3.3.1 Community contribution to canopy structure in the first year

Analysis of treatment effects on canopy structure was firstly done on total leaf biomass per treatment replicate without dividing the canopy into layers, and further analysis was done after dividing the canopy into layers. In the first analysis, leaf biomass per treatment replicate was summed across species prior to a two-way ANOVA. Results of the ANOVA on the combined community leaf biomass (Table 3.12) show a highly significant effect of water treatment ($P = 0.0044$) and a significant interaction of CO_2 and water treatments ($P=0.0431$), while main effect of CO_2 treatment was not statistically significant.

Analysis of distribution of leaf biomass per layer per treatment shows a gradual decrease with canopy height (Figure 3.3), resulting in a canopy that is sparse at the top in the height ranges of 40-60 cm and >60 cm, becoming denser below 40 cm. Such a structure allows a degree of light penetration and interception by leaves in the lower part of the canopy. The most dense layer of leaf biomass is between 20 cm and the root crown. Data were also analysed to find out at which layers in the canopy the treatments had a significant effect on leaf biomass. It emerged that CO_2 and water treatments, either singly or interactively, had no significant effect on leaf biomass in the dense layers of the canopy, 5-20 cm and 20-40 cm (Table 3.12). However, higher up in the 40-60 cm and >60 cm layers, both main effects of CO_2 and water were highly significant (Table 3.12), resulting in more leaf biomass under the elevated CO_2 + MAR treatment in these layers (Figure 3.3). Thus, a treatment combination of CO_2 + MAR affects canopy structure by increasing leaf biomass in the top part of the canopy.

Table 3.12: Statistical significance of treatment effects on canopy structure in the first year, expressed by layers and as total leaf biomass.

Layer	CO2	Water	Interaction
Combined leaf biomass of all layers	NS	P = 0.0044	P = 0.0431
5-20 cm	NS	NS	NS
20-40 cm	NS	NS	NS
40-60 cm	P = 0.0327	P = 0.0162	NS
>60 cm	P = 0.0326	P = 0.0469	NS

3.3.3.2. Species contribution to canopy structure in the first year

Species contributions to leaf biomass in each layer were analysed and results are presented in Figures 3.4. a-d. Contributions per layer in each treatment are presented as absolute amounts in grams. Different species contributed different amounts of leaf biomass, and so it is important to assess the statistical significance of treatment effect on differences in species contributions within respective layers. That analysis was done by a three-way ANOVA, whereby data within a specific layer were combined to generate three factors *viz.*, CO_2 treatments, water treatments and species. Whenever the differences in leaf biomass of the species were statistically significant, Tukey-HSD multiple comparisons test was performed to show which species contributed different amounts of leaf biomass, and which species contributed similar amounts. Indeed, the differences in species were significant in each layer, and groupings of similar and different species will be discussed by layer.

Starting at the bottom 5-20 cm layer, *Sporobolus* had the highest absolute leaf biomass of all the species under elevated CO_2. A further slight enhancement of leaf biomass is observed in that species at MAR relative to 80%MAR, although the difference is not statistically significant. The order of proportional contribution in the bottom 5-20 cm layer under elevated CO_2 treatments is as follows: *Sporobolus* > *Andropogon* > *Eragrostis* > *Alloteropsis* > *Themeda* (Figure 3.4.a). Under ambient CO_2 treatments however, all five grass species contributed similar amounts of leaf biomass. Effects of CO_2 and water treatments on species contributions to leaf biomass

in this dense layer are not statistically significant (Table 3.13). Results of a three-way ANOVA on combined data in this layer however, show highly significant differences in species ($P < 0.0001$), and a multiple comparison test grouped contributions of *Sporobolus* as significantly different from contributions of *Themeda*, *Eragrostis* and *Alloteropsis*.

Higher up in the canopy within the 20-40 cm layer, a positive effect of elevated CO_2 on the contribution of *Sporobolus* was statistically significant ($P=0.0233$). Leaf biomass of *Sporobolus* was enhanced under elevated CO_2 + MAR compared with the other three treatments (Figure 3.4.b). Relative contributions of each species were more ordered in this layer than the previous one, possibly in a manner that correlates leaf biomass with species height. A multiple comparisons test in this layers classified proportional contributions of *Sporobolus* and *Themeda* as similar, and that pair was different from *Alloteropsis and Eragrostis*, and *Eragrostis* was in turn was different form *Andropogon*. *Eragrostis* did not grow beyond a 40 cm height, and it was the shortest of the five grasses at the field site where the plant material was collected.

Themeda and *Sporobolus* were the dominants in the 40-60 cm layer across all treatments (Figure 3.4. c), and the contribution of each of the two species was highest under elevated CO_2 + MAR. A multiple comparison test classified contributions of *Sporobolus* and *Themeda* as similar, but different from contributions of *Alloteropsis* and *Andropogon*. It was difficult to do meaningful analysis of data in the >60 cm layer because of a high incidence of missing values since most of the plants did not grow to that height (Figure 3.4.d). *Andropogon*, *Sporobolus* and *Themeda* are the only species that grew that tall, and of the three, *Sporobolus* made the highest contribution to leaf biomass.

Table 3.13: Statistical significance of differences in leaf biomass at different layers of the canopy in the first year due to (i) presence of different species (ii) CO_2, and (iii) water treatments analysed by a three-way ANOVA.

Layer	Presence of different Species	CO_2	Water	Interaction
5-20 cm	P <0.001	NS	NS	NS
20-40 cm	P <0.001	NS	NS	NS
40-60 cm	P <0.001	P = 0.0120	P = 0.0046	NS
>60 cm	P = 0.0224	NS	NS	NS

3.3.3.3. Community contribution to canopy structure in the second year

General community response pattern was analysed first as combined canopy leaf biomass and subsequently as layers of leaf biomass. Treatment effects on combined canopy leaf biomass were not statistically significant (Table 3.14). Results of a three-way ANOVA on the other hand showed highly significant effects of CO_2 treatment ($P = 0.0208$) and canopy layers ($P < 0.001$), but no significant effect of water treatment ($P = 0.99$). On the other hand, analysis of response of canopy layers can be summarised as an apparent slight increase in leaf biomass in the 5-20 cm and 20-40 cm layers, which was accompanied by slightly reduced leaf biomass in the upper layers of 40-60 cm and >60 cm, relative to the previous year (Figure 3.5). Either of two factors *viz.* watering amounts increased by 20% across the board in the second year (Table 1, Chapter 2) or elevated CO_2, or a combination of the two could have contributed to the observed changes in the pattern of distribution of leaf biomass within the canopy. Whatever the cause, it was certainly not carry-over effects from the previous year because all above-ground material was harvested at the end of the first year (unless the water saving benefit of elevated CO_2 had come into effect, which would then qualify as a carry-over effect). However, the observed responses to the treatments of the two bottom layers were not statistically significant (Table 3.14).

The pattern of response among treatments in the 5-20 cm layer indicated the lowest leaf biomass value for the ambient CO_2 + MAR treatment. Elevated CO_2 enhanced

leaf biomass by small amounts in the 20-40 cm layer compared to the leaf biomass under ambient CO_2 treatments, but the differences were too small to be of any statistical significance (Table 3.14). Effects of watering treatments became apparent once more further up in the canopy within the 40-60 cm layer (P = 0.0379), as was the case in the first year. The amount of leaf biomass under elevated CO_2 + MAR was greater than amount of leaf biomass under all other treatments in the 40-60 cm layer. Interestingly, the amount of leaf biomass was more under ambient CO_2 + MAR than under elevated CO_2 + 120%MAR in this layer. That result may be indicative of the fact that elevated CO_2 enhances leaf biomass only at rainfall values typical of the field site from which the grass community was derived. There were no significant treatment effects in the >60 cm layer although Figure 3.5 depicts greater amount of leaf biomass under elevated CO_2 + MAR.

Table 3.14: Statistical significance of treatment effects on canopy structure in the second year, expressed by layers and as total leaf biomass.

Layer	CO2	Water	Interaction
Combined leaf biomass of all layers	NS	NS	NS
5-20 cm	NS	NS	NS
20-40 cm	NS	NS	NS
40-60 cm	NS	P = 0.0379	NS
>60 cm	NS	NS	NS

3.3.3.4. Species contribution to canopy structure in the second year

The bulk of leaf biomass was in the bottom 5-20 cm layer for all grass species (Figure 3.6.a). *Sporobolus* contributed the largest proportion of leaf biomass under elevated CO_2 in both watering treatments, but there was slightly more leaf biomass under MAR than under 120%MAR. The positive effect of elevated CO_2 on leaf biomass of *Sporobolus* was statistically significant (P = 0.0468). The response of *Themeda* on the other hand was not influenced by either CO_2 or water treatments, and even though leaf biomass of *Themeda* under ambient CO_2 + MAR seems higher than in other

treatments (Figure 3.6.a), the difference is not statistically significant. The other two C_4 species *Eragrostis* and *Andropogon* showed a response that was marginally statistically significant with respect to the high water treatment under ambient CO_2 ($P = 0.0686$ and 0.0542 respectively). CO_2 and water treatments had a weak interactive effect on the amount of leaf biomass in *Alloteropsis* ($P = 0.0773$).

Response pattern to CO_2 and water treatments in the 20-40 cm layer (Figure 3.6.b) depicts an increase in leaf biomass of *Themeda* across all treatments relative to the first year, but a very high level of variability meant that there were no statistically significant differences between treatments. The response of *Sporobolus* CO_2 and water treatments is similar to the trend observed in the 5-20 cm layer (Figure 3.6.a), but treatment effects were not significant. Responses of other species were also not influenced by treatments. Leaf biomass in the 40-60 cm layer was harvested from *Sporobolus*, *Themeda* and *Andropogon* in order of their proportional contributions (Figures 3.6.c and 3.6.d). There were no statistically significant treatment effects on leaf biomass above 40 cm, although the data indicate greater leaf biomass for *Sporobolus* under elevated CO_2.

3.3.3.5. Community contribution to canopy structure in the third year

The most marked response in the third year was reduced canopy leaf biomass in all species, treatments, and layers relative to the previous two years. Most reduction in canopy leaf biomass was observed in the part of the canopy above 40 cm (Figure 3.7). A recurrent observation on canopy structure, that it is denser in the bottom 5-20 cm layer, becoming more sparse with increasing height, was noted. Effect of CO_2 and water treatments on combined canopy leaf biomass was not statistically significant, (Table 3.15). Generally, the watering treatment of MAR resulted in slightly higher leaf biomass irrespective of CO_2 treatment in both the 5-20 cm and 20-40 cm layers. That response was most pronounced in the 20-40 cm layer, especially under elevated CO_2 than under ambient CO_2 (Figure 3.7). The observation is supported by a statistically significant effect of CO_2 and water treatments, as well as their interaction in the 20-40 cm layer (Table 3.15). CO_2 treatments did not have any effect on the amount of leaf biomass in the 40-60 cm layer, but a statistically significant effect of the watering treatment was apparent (Table 3.15), and there was no interactive effect

of CO_2 and watering treatments. There was very little leaf biomass above 60 cm and there were also no apparent treatment effects.

Table 3.15: Statistical significance of treatment effects on canopy structure in the third year, expressed by layers and as total leaf biomass.

Layer	CO2	Water	Interaction
Combined leaf biomass of all layers	NS	NS	NS
5-20 cm	NS	NS	NS
20-40 cm	P = 0.0140	P = 0.0018	P = 0.0144
40-60 cm	NS	P = 0.0316	NS
>60 cm	NS	NS	NS

3.3.3.6. Species contribution to canopy structure in the third year

An interesting shift in proportional contributions of species occurred such that the normal dominance of *Sporobolus* under elevated CO_2 + MAR was lacking in the 5-20 cm layer (Figure 3.6.a). However, leaf biomass of *Sporobolus* was highest under elevated CO_2 + 120%MAR. Leaf biomass in *Themeda* within the 5-20 cm layer was higher under elevated CO_2, with a slightly larger contribution in 120%MAR than MAR. Nonetheless, observed treatment effects on *Themeda* lacked statistical significance. Responses of other species also lacked statistical significance, including a particularly high leaf biomass response of *Eragrostis* under ambient CO_2.

Leaf biomass in the 20-40 cm layer and other layers above (Figures 3.6.b,c, and d) was characterised by the dominance of *Themeda* in most treatments. In the 20-40 cm and 40-60 cm layers, both *Themeda* and *Sporobolus* responded best under elevated CO_2 + 120%MAR, but *Sporobolus* was subordinate to *Themeda*. The 120%MAR treatment also enhanced leaf biomass, but only in the two upper layers of the canopy (Figures 3.6.c and d), and this result could imply that ample supply of water influences canopy structure by enhancing plant height.

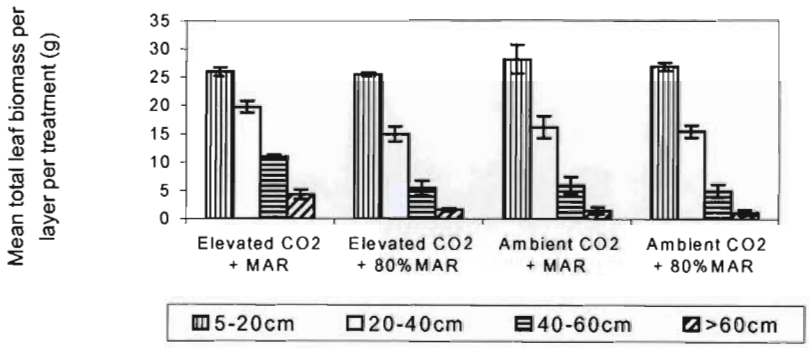


Figure 3.3: Treatment effect on placement of leaf biomass in the first year.

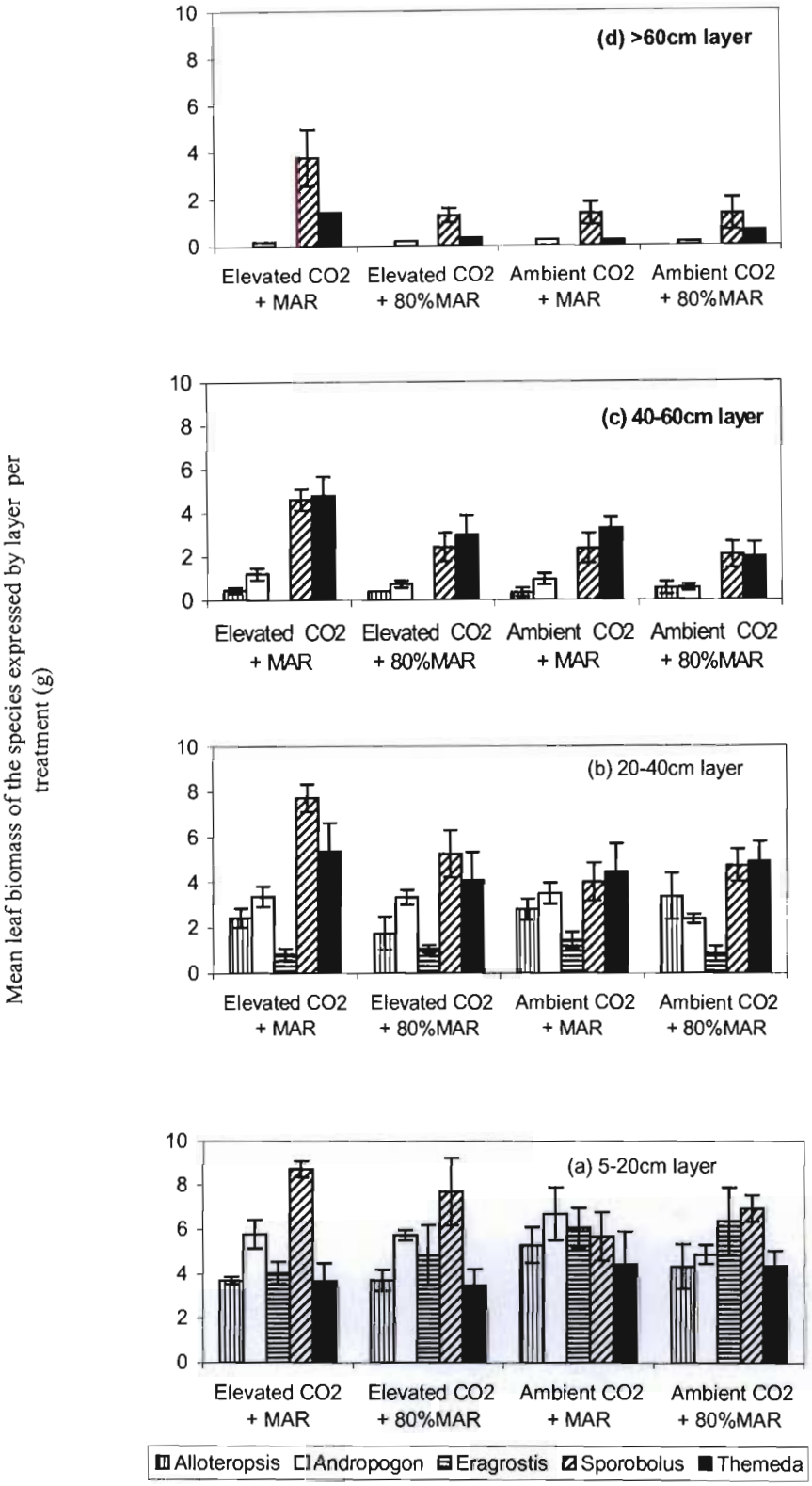


Figure 3.4. (a-d): Treatment effect on placement of leaf biomass of each species in the first year.

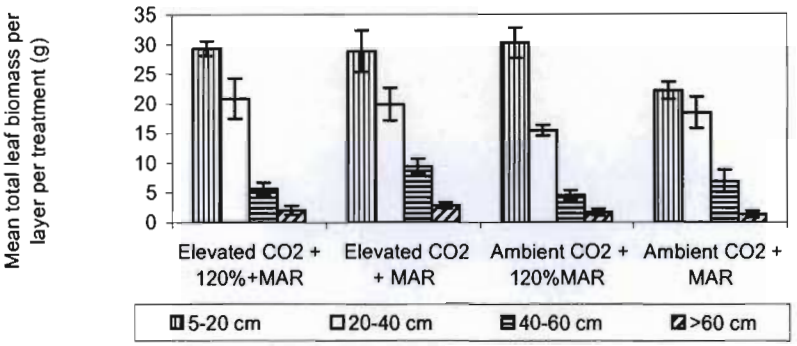


Figure 3.5: Treatment effect on placement of leaf biomass in the second year.

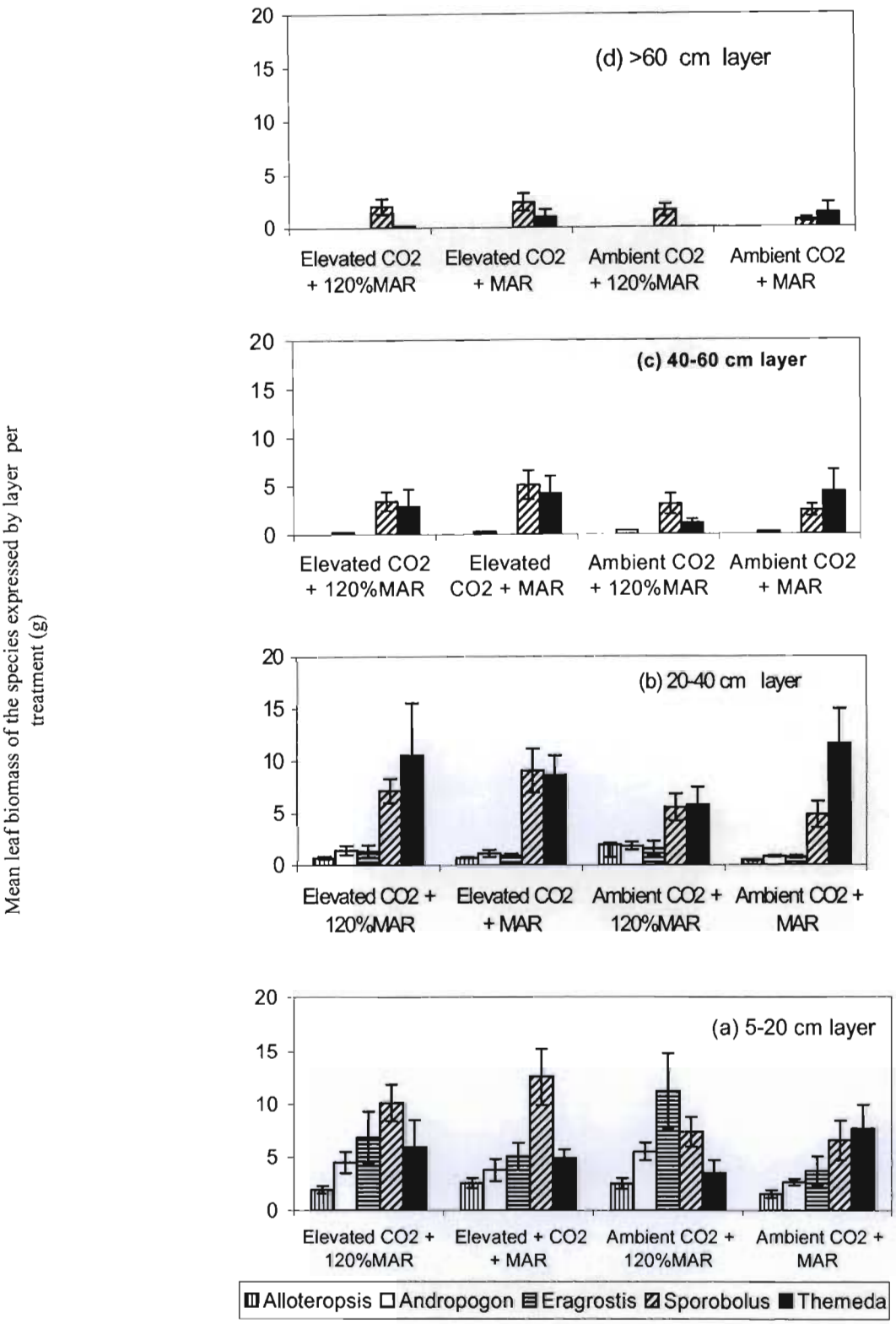


Figure 3.6 (a-d): Treatment effect on placement of leaf biomass of each species in the second year.

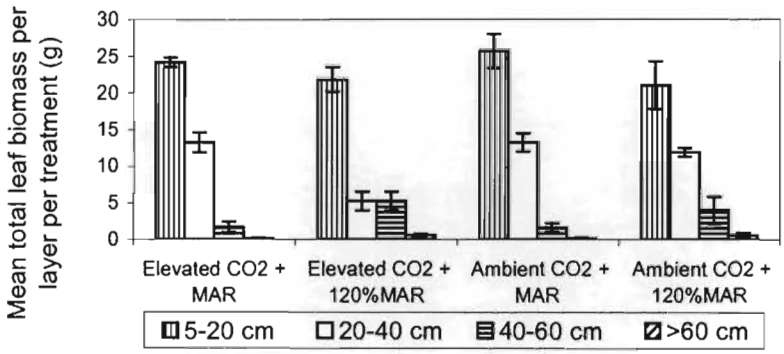


Figure 3.7: Treatment effect on placement of leaf biomass in the third year.

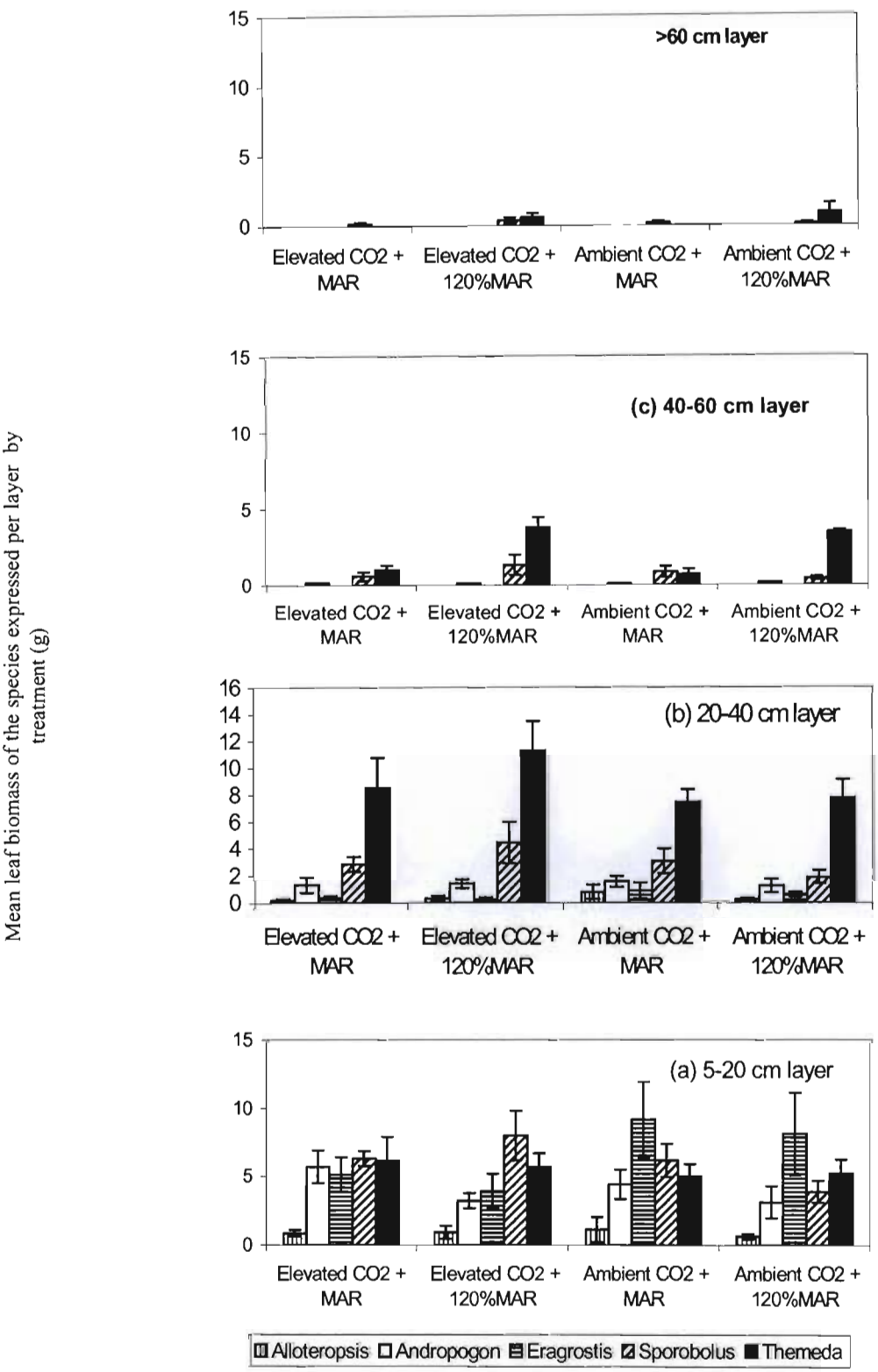


Figure 3.8.a-d: Treatment effect on placement of leaf biomass of each species in the third seas

3.4 Discussion

The data have successfully fulfilled the main objective of this chapter by enabling characterisation of effect of elevated CO_2 on canopy development (sprouting, flowering and senescence) and canopy structure (leaf distribution) of the C_4-dominated microcosm community. They also show which parameters of canopy development and canopy structure responses to elevated CO_2 are dependent on water supply. Generally, elevated CO_2 treatment caused early sprouting, early flowering, and delayed senescence, even though the responses were species specific, and sometimes dependent on water supply. Effect of elevated CO_2 on canopy structure was most apparent in upper canopy layers between 20-40 and 40-60 cm, because greater leaf biomass was produced in elevated CO_2 relative to ambient CO_2 in these respective canopy layers. Response of community canopy structure (combined leaf biomass of all canopy layers) was not dependent on water supply at watering treatments higher than MAR.

Responses of community sprouting show that early regrowth (sprouting) occurred under elevated CO_2 in all three years, and was further enhanced by a higher water supply (MAR and 120%MAR), as indicated by a statistical test (Tables 3.2; 3.5; 3.8), while water treatment on its own had an effect only during first and second years. At the species level on the other hand, effect of CO_2 treatment on sprouting was not dependent on water supply (Tables 3.1; 3.4; 3.7), except in *Eragrostis* in the third year. Elevated CO_2 caused earlier sprouting in all grass species, but statistically the effect was significant for *Themeda*, *Eragrostis*, and *Andropogon* in all three years, and in *Alloteropsis* only in the third year, while effect of water treatment was statistically significant in *Sporobolus* in the first and second years. Sprouting response of the species was explicitly characterised by three groups, that categorise *Themeda* as an early sprouter, *Eragrostis* and *Sporobolus* as intermediate, and *Sporobolus*, *Andropogon* and *Alloteropsis* as late sprouters.

Extrapolation of the sprouting data to field conditions suggests that future scenarios of high atmospheric CO_2 will cause a big change in sprouting phenology at the Ngongoni grassland community, because at the field site, the C_4 grass species *Themeda* and *Eragrostis* show mid- to late-season post-burn growth, while the *Alloteropsis* (C_3) and *Andropogon* (C_4) show early post-burn growth (Wand et al.

2002). Even though observations of Wand and co-workers (2002) on sprouting trends at the field site were made after an annual burn, while sprouting responses reported in this study occurred subsequent to end of year clipping and removal of litter, experimental evidence (Hulbert 1988) suggests that clipping and removal of litter can result in responses similar to those following fire. Therefore the observed results on sprouting are of interest because they are in contradiction with sprouting phenology of mixed grasslands, where cool-season C_3 grass species sprout earlier than warm-season C_4 grass species. This may suggest a response to greenhouse conditions, especially less extreme night time temperature causing C_4 species to sprout earlier.

Annual differences in time of sprouting were statistically significant (three-way ANOVA Table 3.10) and a multiple comparison test suggests that sprouting responses in year one differed from those of years two and three (both of which were similar). From an experimental design point of view, a major difference between year one and the subsequent two years was that water treatments in year one were 20% lower. But a lack of interaction between year and water treatment (Table 3.10) may suggest that annual differences in sprouting intervals were not dependent on water supply. On the other hand it may suggest acclimation to greenhouse conditions of less extreme night temperatures.

It is noteworthy in the first year there was a difference of approximately five days between the earliest (*Themeda*) and last (*Andropogon*) recorded sprouting event (Table 3.3). In the second and third years (Tables 3.3 and 3.9), there was a difference of approximately nine days between the earliest and last recorded dates of sprouting. From the sprouting data alone, it is not easy to say if the observed intervals between dates of sprouting have any likely consequence for length of growing season. Length of growing season was assessed by time of loss of greenness. Qualitative observations made during each growing season allude to a two to three weeks delay in senescence in microcosms exposed to elevated CO_2, but still there is insufficient evidence to attribute the delay in senescence (longer growing season) to time of sprouting. Delayed senescence in response to elevated CO_2 has been reported in other grassland systems (Ham et al. 1995; Drake et al. 1996; Knapp et al. 1999) with important implication for increased production, but with potentially negative impacts on diversity if a subset of species benefit from delayed senescence.

Grass species maintain their populations by both vegetative and sexual reproduction, and vegetative reproduction is the dominant form of growth in semiarid and mesic African grasslands (Belsky 1992). The benefit of sexual reproduction is maintenance of genetic diversity, therefore, it is likely that experimental manipulations which alter flowering pattern can pose a threat to population recovery after large-scale abiotic stress or disturbance. On the other hand, if experimental manipulations induce repetitive flowering events during a growing season in some species, such a response may result in increased population diversity with a higher potential for recovery or resilience to large scale abiotic stress and disturbance. In this study, the objective of assessing flowering response to treatments was to understand phenological development, and possibly its implications for ecological and rapid evolutionary changes in the community. Results of this study suggest that grass species that tend not to produce reproductive shoots under elevated CO_2 such as *Alloteropsis* and *Andropogon* may be under the risk of not maintaining genetic diversity. In South Africa, *Themeda* is already over-utilised, and consistent production of reproductive shoots under future scenarios of elevated CO_2 may help to achieve its regeneration potential. The other two grasses *Eragrostis* and *Sporobolus*, which showed significant flowering response under elevated CO_2 also stand a good chance of regenerating their populations as atmospheric CO_2 increases. Seeds from different generations were not collected, hence inter-generational effects of elevated CO_2 were not studied. Some of the reported inter-generation effects of elevated CO_2 in grasses include increased tillering and biomass production from first to second generation (Bezemer et al. 1998).

Treatment effect on reproduction phenology was assessed by noting time of flowering. Flowering is mainly controlled by photoperiod, but it can also be altered by other environmental variables such as precipitation and temperature. Anthesis occurred at mid-canopy development, and *Eragrostis* was the first species to produce open flowers, about 75 days after application of treatment. Because *Eragrostis* is a species of short stature, early flowering might be an important mechanism for achieving reproductive development prior to canopy closure and a consequent reduction in availability of light. *Themeda* started flowering at mid-canopy also, at 82 days after application of treatments under elevated CO_2 + MAR, and flowering

occurred about 10 days later in other treatments. *Sporobolus* flowered towards the end of mid-canopy at 108 days under elevated CO_2 + MAR, and about 12 days later in other treatments. The above trends in response to treatments were similar for all three years, but there was a general delay in years two and three.

Responses of canopy structure to elevated CO_2 treatment was characterised by a higher production of community leaf biomass in upper canopy layers (height of about 40 cm and above). Among the taller grasses, *Sporobolus* and *Themeda* were most responsive to elevated CO_2 + MAR, and their respective leaf biomass in the 40-60 cm layer was equivalent to 50% of each of their leaf biomass in the dense basal layers (5-20 cm or 20-40 cm); while contributions from *Alloteropsis and Andropogon* in the 40-60 cm layer were each no more than 10-15% of their respective contributions in the dense basal layers (5-20 cm or 20-40 cm).

The observed high contribution of leaf biomass production within upper canopy layers (above 40 cm height) by *Sporobolus* and *Themeda* demonstrates that a tall stature is an important adaptation for persistence in competitive and productive grasslands, owing to reductions in light with canopy depth. But on the other hand, grass species that respond to elevated CO_2 by increase in height may be more susceptible to defoliation than species that retain a short stature such as *Eragrostis*. Response of the vertical structure of *Alloteropsis and Andropogon* showed no tendency towards tall stature, unlike their natural appearance in the field. Elevated CO_2 generally caused an increase in canopy height in *Sporobolus* and *Themeda* during the first two years, but subsequently leaf biomass was reduced throughout the canopy in the third year, thus benefiting short stature *Eragrotis* whose leaf biomass increased. Also, a change in dominance of contribution to leaf biomass was observed in the third year relative to previous years. The most notable change occurred between *Sporobolus* and *Themeda*, whereby the latter species was a dominant contributor to leaf biomass in the part of the canopy above 20 cm. The highest contribution of *Themeda* occurred in all treatments in the 20-40 cm layer, and only under watering treatment of 120%MAR in the layers above 40 cm. Gain in dominance of *Themeda* over *Sporobolus* however, did not make up for reduction in canopy leaf biomass that accompanied loss in dominance of *Sporobolus*.

Structural dynamics in the bottom layers of the canopy included a dense presence of leaf biomass in the bottom layers below 40 cm in the first and second years, and in the third year the most dense part of the canopy was at 20 cm and below. Lack of statistical significance of treatment effects (CO_2 and water treatments or their interaction) on the amount of leaf biomass in the basal layer of the canopy suggests that important functional processes that are successfully maintained by dense lower canopy may not be altered by elevated CO_2. Ecosystem benefits of a dense basal layer of a grass canopy include reduced evaporation loss and increased infiltration, thus improving soil water status. However, a drawback is that if a dense basal cover persists unmanaged by defoliation or fire, it may impede tiller initiation of grass species that cannot tolerate shading (Everson et al. 1988), and perhaps subsequently induce conditions that are suitable for invasion by woody elements.

From the collated data, it appears that canopy development (sprouting, flowering, and senescence) may be advanced by about one to one-and-a-half weeks in under future scenarios of high atmospheric CO_2. Secondly, responses to elevated CO_2 may be dependent on water supply at the community level but may not always be dependent on water supply at species level. Thirdly, trends in species competitive interactions may influence annual response at community level.

CHAPTER 4

COMMUNITY PRODUCTION

4.1. Introduction

Net primary production is an important functional attribute of plant communities because it represents energy available within a system, and it sets a potential upper limit for all other processes. Increasing atmospheric CO_2 enhances net production significantly in the absence of competitive interactions (Poorter 1993; Wand et al. 1999), hence consequences of production on carbon sequestration may also be affected by competitive interactions. Earlier speculation on responses of natural ecosystems to increasing atmospheric CO_2 was that competition would favour C_3 over C_4 species as a result of increased photosynthetic ability and reduced photorespiration in C_3 species (Bazzaz 1990). However, field experiments have generally failed to confirm large increases in production (e.g. forest systems), and it therefore appears that the response of ecosystem production to elevated CO_2 would be overridden by the most limiting ecosystem resources (Field et al. 1992), which in most ecosystems are nitrogen or water.

Data from the first ecosystem level study on a mixed C_3 and C_4 community (Curtis et al. 1989a) showed that elevated CO_2 enhanced above-ground production of a C_3 sedge but not of C_4 grass species in a salt marsh ecosystem. Besides the fact that C_4 species are saturated at an ambient CO_2 concentration of 350 ppm, Curtis and co-workers (1989b) associated the non-responsiveness of C_4 grasses with a limited ability to mobilise nitrogen resources within the plant compared to C_3 sedge used in that study. The initial response pattern in primary production of the salt marsh ecosystem, in which C_4 grasses were non-responsive, was confirmed through seven years of exposure to elevated CO_2 (Drake et al. 1996). Modelling analyses (Rasse et al. 2003) confirm that the high responsiveness of the C_3 sedge to elevated CO_2 in the salt marsh, is attributed mainly to high foliage nitrogen concentrations. Meanwhile, a study on the tallgrass prairie ecosystem (Owensby et al. 1993, 1996, 1999) reported enhanced primary production of a dominant C_4 grass species and a C_3 forb, but no measurable increment in production of a C_3 grass species after eight years of exposure

to elevated CO_2, contrary to predictions based on differences in photosynthetic pathway.

Collective data on response patterns of natural ecosystems to elevated CO_2 challenged the notion of C_4 non-responsiveness. Consequently, it has been established that outcomes of species competitive interactions cannot be generalised along biochemical and photosynthetic differences in C_3 and C_4 functional types (Wand et al. 1999). The meta-analysis by Wand et al. (1999) illustrated that both C_3 and C_4 functional types are susceptible to reduced production under conditions of limited resources. Water stress causes a reduction in the stimulation of leaf area by CO_2 in C_4 species, while overall stress reduces rate of carbon assimilation in C_3 species and nutrient stress particularly reduces biomass response in C_3 species. Water is a limiting resource in many grassland ecosystems, and predictions that climatic change associated with increased atmospheric CO_2 may be accompanied by variable rainfall in South Africa (Ellery et al. 1991) necessitates an understanding of how production of the grassland ecosystem will respond to the predicted changes in atmospheric CO_2 and water availability.

In Chapter 3, an outline of treatment effects on canopy structure and phenology was given. In the present chapter, the analysis is taken further to determine treatment effect on community production, thereby integrating all components representative of the potential energy available within communities under different treatments. The objective will be achieved by addressing two key questions, namely:-

(i) Will elevated CO_2 change above-ground biomass production at community and species levels, and below-ground biomass production at community level?

(ii) To what extent will biomass production (community above- and below-ground and species above-ground) be dependent on watering treatment?

(iii) Will CO_2 response depend on watering treatment?

4.2. Materials and Methods

The methods used in this study placed emphasis on end of year above-ground production in three consecutive years, and total below-ground production at the end of a three-year period. Harvest procedures are outlined separately for biomass of above-ground parts of the grass species, litter, crown material, roots, biomass of the

geophyte, and the amount of soil organic matter. Results for different biomass units have been presented in separate sections.

4.2.1. Above-ground production and litter

Above-ground biomass was quantified by a stratified harvest method per plant per species. Biomass layers were harvested starting at a plant height-range of 60 cm and above followed by 40 – 60 cm, then 20 – 40 cm, ending at 5 – 20 cm. Harvest dates for the first, second and third years were 8th – 14th June 1999, 15th – 21st July 2000 and 23rd – 28th July 2001 respectively. Harvesting commenced at pot 1 and continued progressively through to pot 16. Plant material from each layer of each plant was bagged separately, labeled and oven-dried at 70°C for two days to reach a constant mass. Dried plant material was separated into a leaf component and a stem plus floral parts component, and each component was weighed separately. Community above-ground production and species contributions to above-ground production were quantified as averages of four treatment replicates.

Care was taken when handling plant pots and chambers to minimise incidents of breaking plant parts, at the same time avoiding affecting the natural process of litter accumulation. Litter that accumulated on the soil surface of each pot was hand-picked, bagged and labeled by pot number without separating by species or plant part. Litter that fell to the floor of the greenhouse during the course of the growing season was hand picked and bagged by pot number as it fell, to be combined with other litter at final harvest. Drying of litter was done as for above-ground biomass, followed by weighing.

4.2.2. Crown and below-ground production

Crown and root biomass were quantified at final harvest from 2nd – 8th August 2001. Harvest of root biomass was performed in a manner that allowed for determination of root density with depth, and an estimate of total community root biomass. Harvest of below-ground parts enabled observations on the degree of soil compaction, and activity and survival of earthworms that were added to the soil at the beginning of the experiment. Each plant pot was demarcated into halves, ensuring equal representation of plant species. One half of a pot was used to determine root density at three depths by extracting horizontal soil cores. Additional vertical soil cores were obtained from

the same half of plant pot for determining soil organic matter content (section 4.2.3). It was important to remove soil cores from intact pots to ensure minimal disturbance to the soil structure. To enable removal of horizontal soil cores, a key-hole saw was used to make 3 cm diameter holes at three depths on the outer side of the first half of each pot at marked positions directly below each grass crown. Two hundred and forty horizontal soil cores were collected, representing four replicates of four treatment groups for each of the five grass species at three depths. Root material in the soil cores was separated from soil by sieve-washing. Washed roots were oven-dried to a constant mass at 70 °C and weighed to determine treatment effect on root density with depth.

Plant pots were split into halves, and the 5 cm stubble of grass crown that remained subsequent to harvest of above ground biomass were separated from root material on the two halves of each pot. Identification of crown material by species was aided by attached plant labels. Excess soil was shaken off the crowns, which were individually placed in labeled bags, oven-dried to a constant mass at 70 °C, and weighed. Differences in total crown biomass per treatment would be taken as potential indicators of treatment effect on reserve accumulation at the end of the year.

Sampling for community below-ground production was done on the second half of each pot. All roots were separated from the soil by sieve-washing and allowed to drain sufficiently before placing them in bags labeled by treatment and pot number. The roots were oven-dried to a constant mass at 70 °C and weighed. The recorded root mass of each half pot was multiplied by two to estimate pot totals. Community below-ground production was estimated as an average of the four replicates per treatment.

4.2.3. The geophyte - *Eriospermum mackenii* (Hook. f.) Baker, subsp. m*ackenni*

Above-ground biomass of the geophyte was harvested at end of each year, at the same time as above-ground biomass of the grasses (section 4.2.1.). Leaf material was oven dried to a constant mass at 70 °C, and weighed. The species of geophyte used in the experiment (*Eriospermum mackenii* Hook. f.) Baker, accumulates a small above-ground biomass, which comprises no more than two to five leaves (Perry 1994). Starting fresh mass of the main organ, the bulb, was measured at the beginning of the experiment. Treatment effects on growth of the bulb were assessed at final harvest.

Bulbs were separated from soil, washed, and their fresh mass recorded for comparison with starting fresh mass to determine treatment effects on the capacity for reserve storage. Bulbs were subsequently oven-dried and weighed to compare water content at the end of experiment with samples dried and weighed at the start.

4.2.4. Soil organic matter content

Soil organic matter was quantified at final harvest at the end of the third year. Pots were divided into two, one half for estimating total community measurements, and the other half for species-specific measurements. Sampling was performed on the half of plant pot designated for determining root density with depth (section 4.2.3). Five vertical soil cores of 0.14 dm^3 (3 cm diameter by 20 cm length) were collected randomly, and placed in brown paper bags labeled by pot number. Large roots were removed from samples by passing the soil through a 2 mm sieve, prior to determining soil organic matter content by loss of mass on ignition (Allen 1989). A small sample of preweighed oven-dried soil was ignited in a muffle furnace at 850 °C for 30 minutes, cooled and weighed (McRae 1988). The amount of organic matter was determined as the difference in mass of soil before and after ignition. The procedure was repeated about two to three times to ensure that combustion was complete.

4.2.5. Data analysis

Biomass produced during the year will depend on the initial biomass of the crown, as well as on the treatment. Biomass of the crown could not be determined at the beginning of the each year because it is a destructive measure. However, if it is assumed that the amount of above-ground biomass produced is related to the amount of crown material, then the above-ground biomass at the end of the previous year can be taken as an indicator of crown mass at the beginning of the next year. Thus expressing above-ground biomass accumulated during the year relative to the above-ground biomass at the end of the previous year should overcome the problem of differing crown biomass among replicates within a treatment. The above-ground biomass of each plant removed after initial planting was labeled B_0, and that at the end of the first, second and third years B_1, B_2 and B_3, respectively. Growth over a year, accounting for initial crown biomass was then expressed as B_1/B_0, B_2/B_1, and B_3/B_2 for the first, second and third years. A ratio greater or less than 1 would serve as

an indicator of whether treatment effects caused an enhancement or reduction in production over the study period.

Data analysis was aimed at elucidating main and interactive effects of CO_2 and water on community production and on annual changes in production by performing two-way ANOVA tests. In instances where variables were ratios, data were transformed prior to the ANOVA (Zar 1984). Assessment of treatment effects on species contributions to production involved use of "species" as a third factor in addition to CO_2 and water. Five grass species were used in the microcosms, hence the factor "species" had five levels. In the case of treatment effects on root density with depth, the factor "depth" had three levels. If an ANOVA indicated significant effects of a factor with more than two levels, Tukey-HSD (Highly Significant Difference) Multiple Comparison test was performed to find out which of the levels were significantly different.

Cumulative above-ground production at the end of years two ($B_1 + B_2$) and three ($B_1 + B_2 + B_3$) was computed, and statistically analysed by two-way ANOVA. However, water treatments changed in each of the three year, and therefore, the effective accumulated water treatment on cumulative above-ground production at the end of years two and three was an average of water treatments of the respective years as illustrated in Table 4.1. Water treatments lower than MAR are referred to as low water, and water treatments higher than MAR are referred to as high water.

It is important to note that water treatments will be expressed slightly differently for biomass units that were quantified only at the end of the third year, such as below-ground biomass and crown biomass, but which had been exposed to water treatments of all three years. The effective water treatments at harvest will be as shown in Table 4.1.

Table 4.1: Effective water treatment resulting from changes in annual rainfall treatment and manner of application.

Year	Rainfall amount (mm)		
CO_2 and water treatments 1998/1999	Stochastic 80%MAR (low water)	Stochastic MAR (high water)	
1999/2000		Regular MAR (low water)	Regular 120%MAR (high water)
2000/2001		Regular MAR (low water)	Regular 120%MAR (high water)
Effective water treatment at end of year 2		**110%MAR** (high water)	**90%MAR** (low water)
Effective water treatment at end of year 3		**107%MAR** (high water)	**MAR**

4.3. Results

4.3.1. Response of community above-ground production

4.3.1.1. 1st Year

The highest community average above-ground production was recorded for the high CO_2 + MAR treatment at 74.5 g, which is equivalent to 467 g m^2 (Figure 4.1.a), and values of production in the other three treatments ranged between 54 g to 60 g (equivalent to 340-377 g m^2). The main effects of CO_2 concentration and water treatment as well as their interactive effect were highly significant (P = 0.040, 0.0024 and 0.018 respectively). Leaf and stem production was affected by treatments in different ways (Figs 4.1.b and c). The main effect of CO_2 was significant on community leaf biomass (P = 0.0008) but effect of water treatment was not significant (P = 0.2170). There was a significant interactive effect of CO_2 and water (P = 0.0003) on community leaf biomass. The fraction of stem biomass was highest under ambient CO_2 and MAR (Fig 4.1.c), and an ANOVA test on that result showed a highly significant effect of water (P < 0.001) and CO_2 (P = 0.0283) but no interaction (P = 0.0632).

A comparison of above-ground production at the end of year one with above-ground biomass at the start of the experiment (B_1/B_0) showed biomass accumulation above that at start of the experiment under all treatments (Fig 4.1.d), and relative increase in production under elevated CO_2 + MAR was significantly higher than the increase under other treatments (P = 0.049, 0.005 and 0.024 for CO_2, water, and their interaction respectively). One would have expected no change in biomass production under the control treatment (ambient CO_2 + MAR) at end of year one compared to the starting biomass. Except for the disturbance of transplanting, perhaps the warmer microclimate in the chambers relative to field conditions, caused the observed difference in values of control biomass between the field and microcosms.

4.3.1.2. 2nd Year

Community above-ground production was highest under elevated CO_2 + MAR (for a second consecutive year), possibly indicating a requirement for an optimum amount of water for the effect of elevated CO_2 on production to become apparent. The recorded value of 79.9 g (503 g m^2) under elevated CO_2 + MAR in the second year (Figure 4.2.a) was slightly higher than the value recorded in the first year under the same treatment (Figure 4.1.a). However, communities that were exposed to elevated CO_2 + MAR in the second year had been exposed to elevated CO_2 + 80%MAR in the first year. Results of an ANOVA nonetheless showed no significant main effects of either CO_2 and water, or their interactive effect in the second year. Dividing production into leaves and stems indicated stimulatory effects of elevated CO_2 on leaf biomass (Figure 4.2.b) and MAR on stem biomass (Figure 4.2.c), even though not in a statistically significant manner.

A comparison of community above-ground production at end of the second year relative to above-ground biomass at the end of the first year (B_2/B_1) showed an increment under three treatments, while a slight reduction in biomass occurred under one treatment (Figure 4.2.d). Changes in water treatment from MAR to 120%MAR effected a small increment of 5% in production under ambient CO_2 and a 7% reduction in production under elevated CO_2, while a change from 80%MAR to MAR effected a 15% increment in production under ambient CO_2 and 47% under elevated CO_2. A 7% decrease in production when water treatments changed from MAR to 120%MAR under elevated CO_2 does not necessarily imply that excessive water

availability under elevated CO_2 inhibits growth. A two-way ANOVA on transformed B_2/B_1 ratios showed a highly significant effect of water ($P = 0.007$) but not of CO_2 ($P = 0.340$), and significant interactive effects of CO_2 and water ($P = 0.047$).

Cumulative above-ground production of years one and two; $B_1 + B_2$ (Fig 4.2.e) was marginally influenced by CO_2 treatment ($P = 0.054$). Cumulative biomass was higher under elevated CO_2 irrespective of effective average water treatment, but there was not effect of water treatment. Similarly, $(B_1 + B_2)/B_0$ was marginally influenced by CO_2 treatment ($P = 0.0590$), and there was no effect of water.

4.3.1.3. 3rd Year

Production was substantially lower at the end of the third year than it had been in years one and two (Figure 4.3.a), and treatment effects were not statistically significant. The highest production was recorded under elevated CO_2 + MAR treatment as was the case in the first and second years (Figures 4.1.a and 4.2.a). Figure 4.3.b shows treatment effects on leaf production, and that parameter was affected by a weak interaction of CO_2 and water ($P = 0.0593$). Stem biomass was also not affected by treatments in a statistically significant manner.

Cumulative above-ground production of the three years; $B_1 + B_2 + B_3$ (Figure 4.3.c) was significantly influenced by CO_2 treatments ($P = 0.0249$), but effect of water treatment was not significant ($P = 0.4$). As was the case at the end of the second year, cumulative biomass production at the end of the third year was higher under elevated CO_2 than under ambient CO_2 irrespective of effective average water treatment. Production at the end of year three relative to year two (B_3/B_2 ratio), was not statistically different among treatments, and in fact was characterised by an 11% reduction. Even though community biomass at end of the third year was lower than in the two previous years, still there was a degree of stimulation on biomass production ($B_3/B_0 \approx 1.5$) compared to biomass at start of the experiment. However, treatment effects on (B_3/B_0) were not statistically significant.

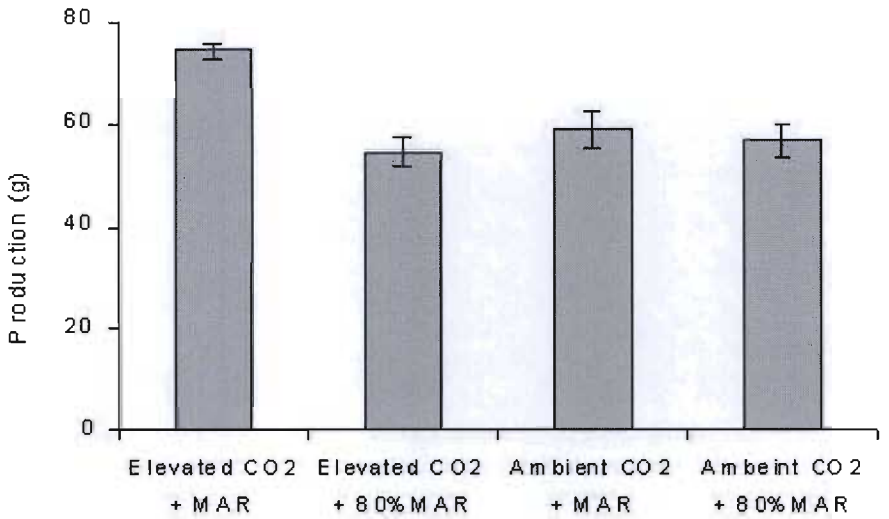


Figure 4.1.a: Treatment effect on community above-ground production at the end of season one.

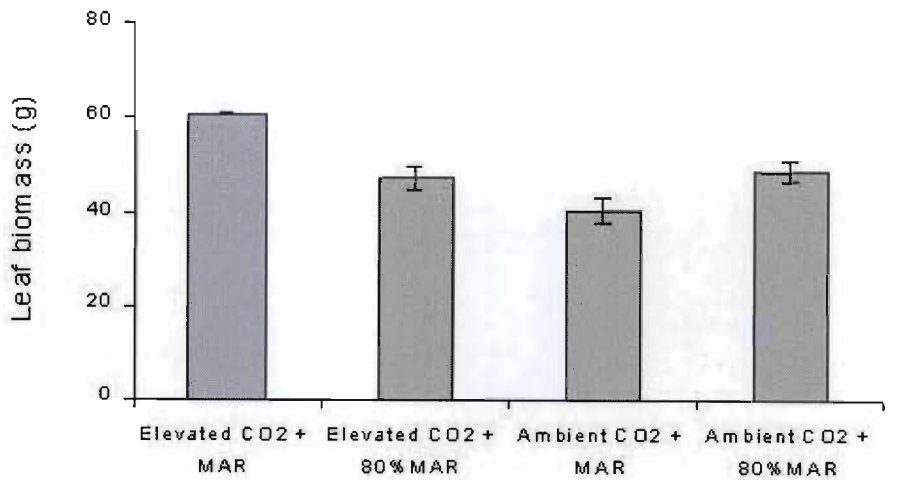


Figure 4.1.b: Treatment effect on community leaf biomass at the end of season one.

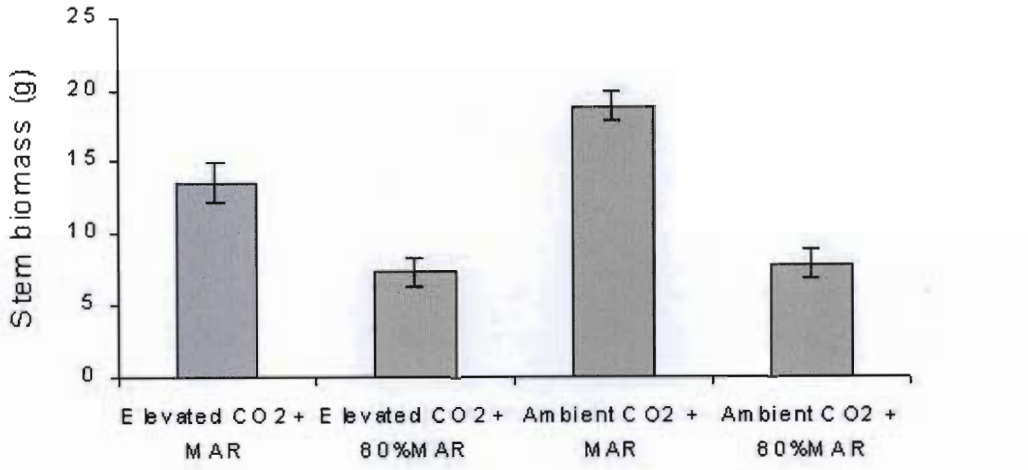


Figure 4.1.c: Treatment effect on community stem biomass at the end of season one.

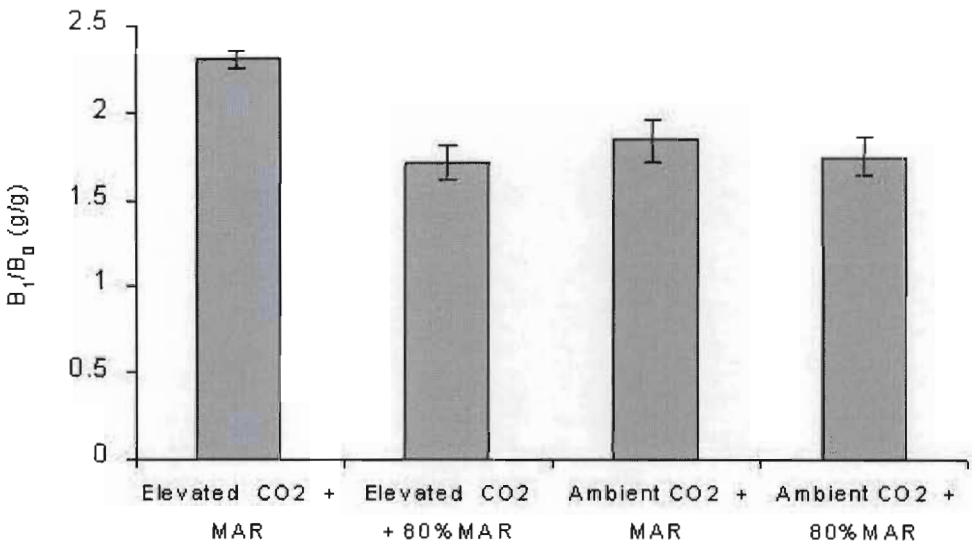


Figure 4.1.d: Treatment effect on above-ground production in season one relative to above-ground biomass at the time of planting.

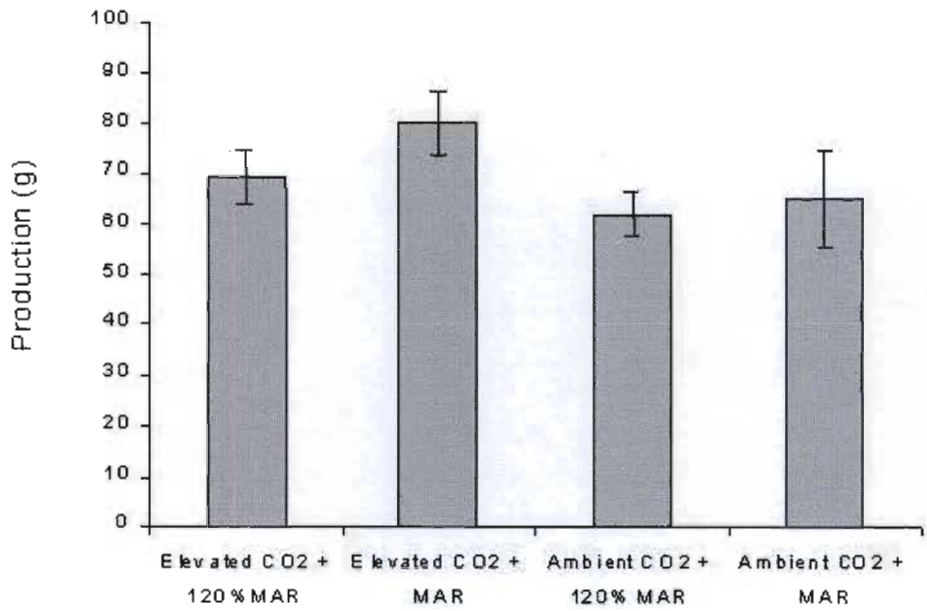


Figure 4.2.a: Treatment effect on community above-ground production at the end of season two.

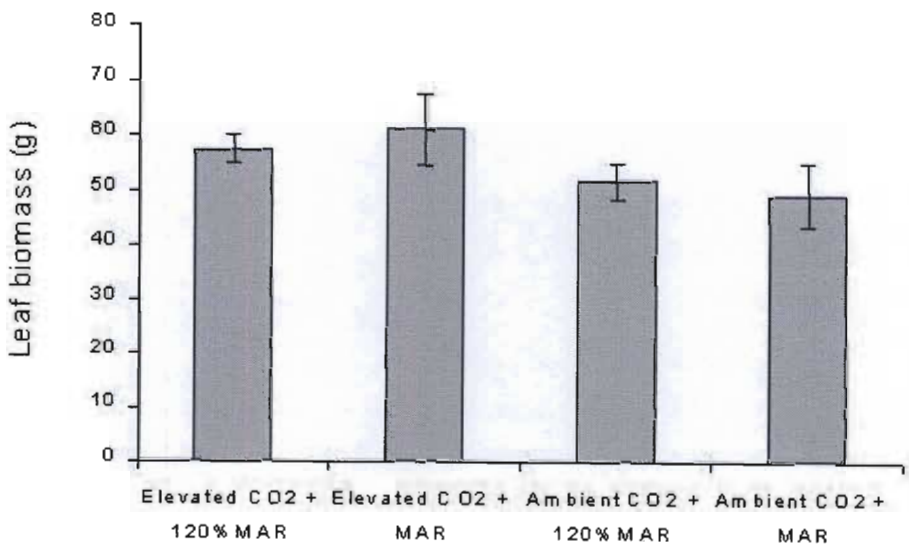


Figure 4.2.b: Treatment effect on community leaf biomass at the end of season two.

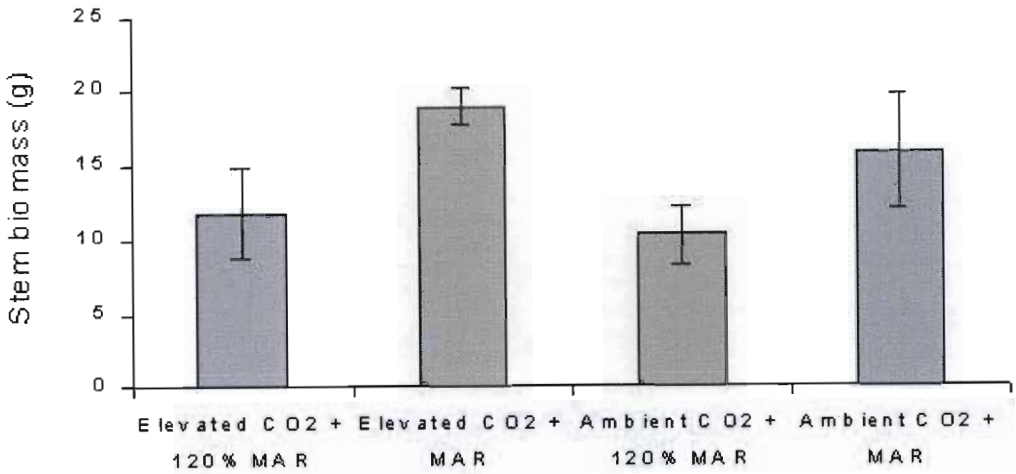


Figure 4.2.c: Treatment effect on community stem biomass at the end of season two.

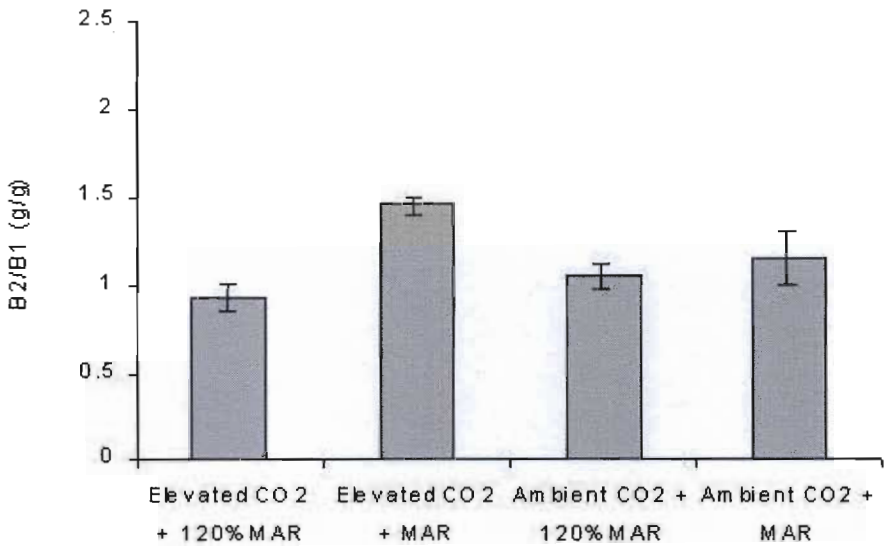


Figure 4.2.d: Treatment effect on above-ground production in season two relative to above-ground biomass at the end of season one.

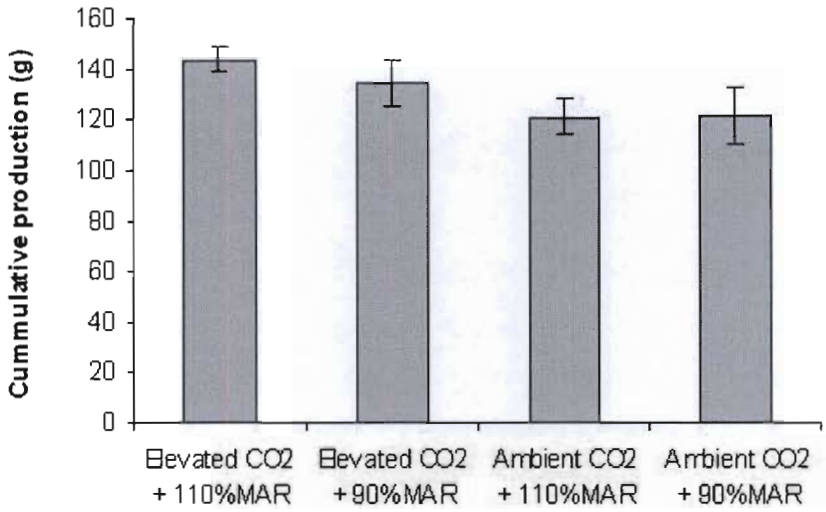


Figure 4.2.e: Treatment effect on community cumulative above-ground Production of the first tow seasons

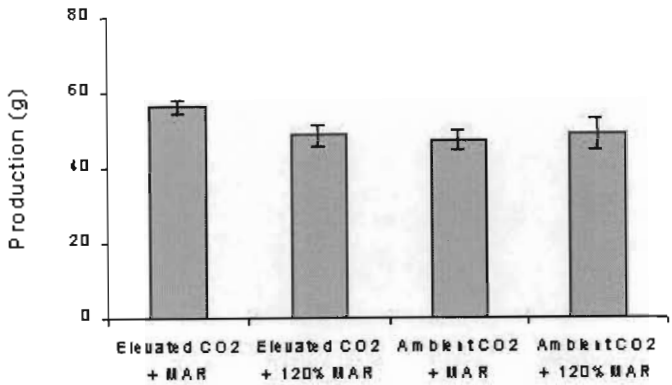


Figure 4.3.a: Treatment effect on community above-ground production at end of season three

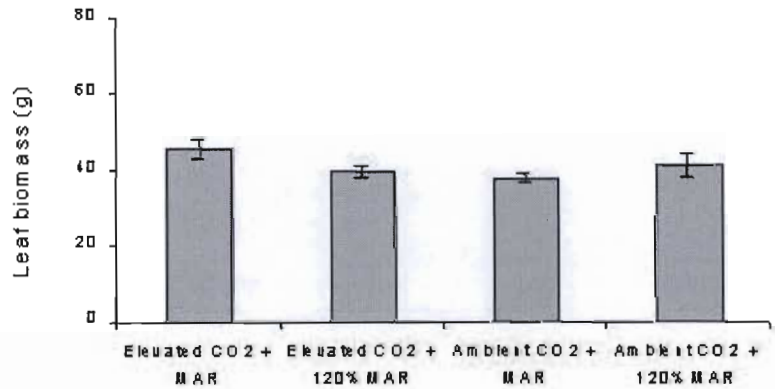


Figure 4.3.b: Treatment effect on community leaf biomass at the end of season three

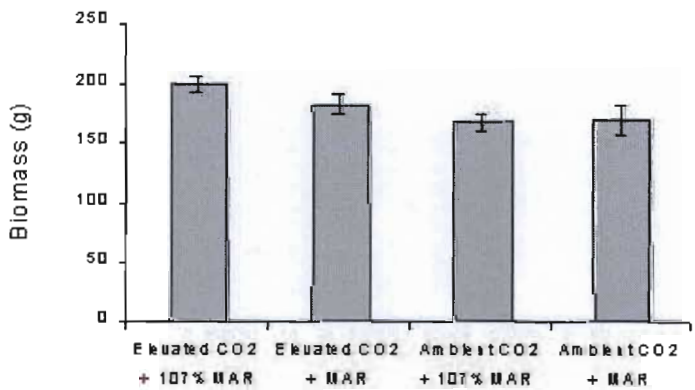


Figure 4.3.c: Community cumulative above-ground production of three seasons

4.3.2. Community litter

Average annual litter production in the microcosms was 6 g per unit ground area of 0.159 m^2, which is equivalent to 38 g m^{-2}. In all three years, no significant main effects of CO_2 and water treatments on litter production, or their interaction were noted (Figures 4.4.a-c). A higher amount of litter was collected in the second year compared to first and third years, and similarly, higher community production was recorded for the second year in all treatments compared to the first and third years. There were no significant differences in cumulative litter among the treatment groups as shown in (Figure 4.4.d.). Data on litter production was not included in the analysis of community above-ground production (Section 4.3.1) in order to avoid inherent difficulties of precisely sorting litter derived from leaves and parts of the crown. However, litter production has been included in the analysis of community cumulative biomass production (Section 4.3.6), where all biomass components have been combined.

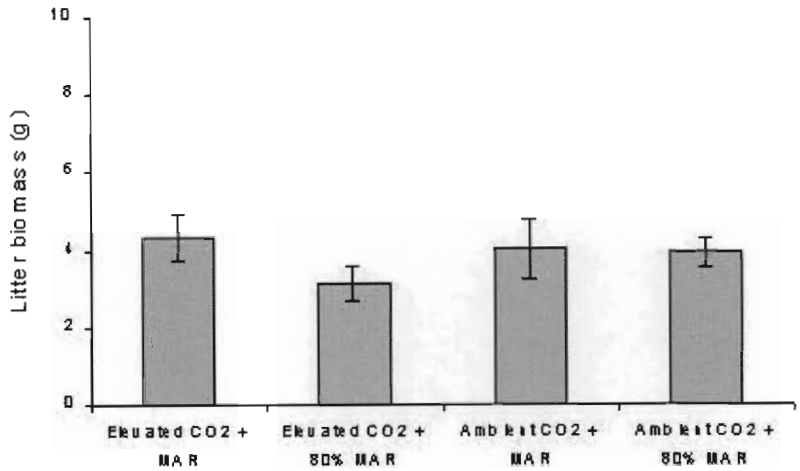


Figure 4.4.a: Treatment effect on litter biomass of season one

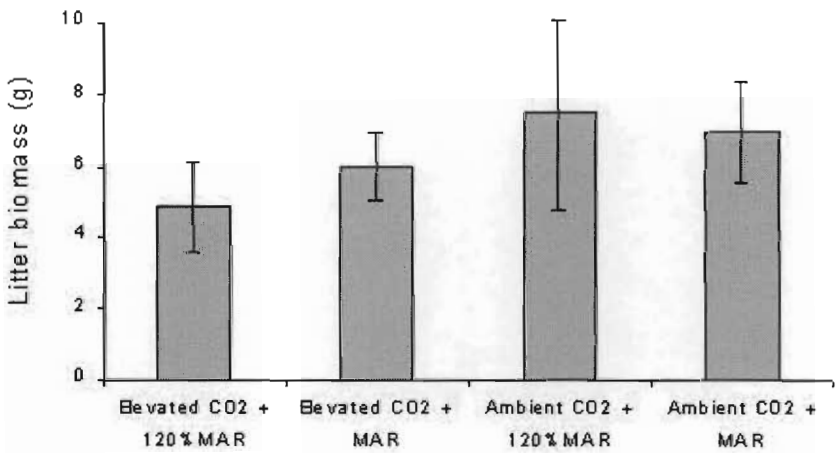


Figure 4.4.b: Treatment effect on litter biomass of season two

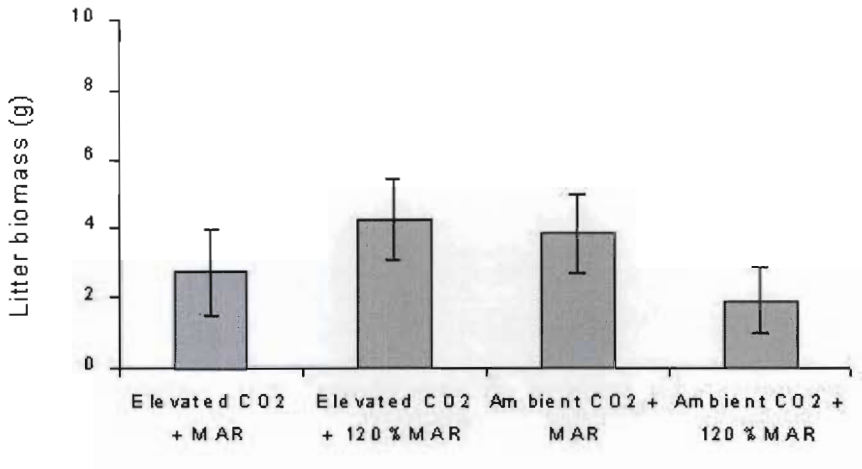


Figure 4.4.c: Treatment effect on litter biomass of season three

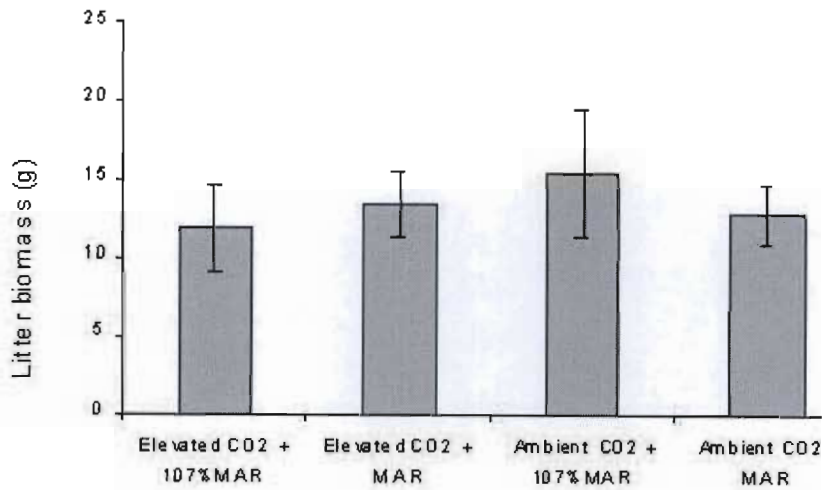


Figure 4.4.d: Cumulative litter over three seasons

4.3.3. Species contribution to above-ground production

Analysis of above-ground production by species serves to identify key species contributing towards ecosystem production, to assess treatment effects on species competitive interactions, and to highlight potential trends in population dynamics as a consequence of elevated CO_2. The corollary of species responsiveness to elevated CO_2 is enhanced production, which for some species might be accompanied by changes in carbon allocation that influence competitive interactions, whereby aggressive competitors become dominant in the community. Following a quantitative analysis, a qualitative description of species contributions to production will be given as either high, intermediate, or low for grasses.

4.3.3.1. 1st Year

Biomass contributions of *Sporobolus* and *Themeda* to community production were the highest, and biomass contributions of *Alloteropsis*, *Andropogon* and *Eragrostis* were low (Fig 4.5.a). Results of a three-way ANOVA showed that CO_2 treatment did not have a significant effect ($P = 0.186$) on species contributions, but effects of water ($P = 0.030$) and species ($P = <0.001$) were statistically significant. There was no interaction between CO_2 and water ($P = 0.090$) and between water and species ($P = 0.446$), but there was a statistically significant interaction between CO_2 and species ($P = 0.020$). Results of a multiple comparison test presented in Table 4.2 confirmed two categories of species, with *Sporobolus* and *Themeda* in a category of high contributions and *Alloteropsis*, *Andropogon* and *Eragrostis* in a category of low contributions. Data on leaf and stem biomass fractions showed that *Themeda* allocated more biomass to stems and reproductive parts than *Sporobolus*. On the basis of total species biomass, *Sporobolus* was more competitive than *Themeda*.

4.3.3.2. 2nd Year

Sporobolus and *Themeda* were again the dominant contributors to above-ground production, and contributions of the other three species; *Alloteropsis*, *Andropogon* and *Eragrostis*, were lower (Fig 4.5.b). Treatment main effects were not statistically significant for CO_2 ($P = 0.368$) or water ($P = 0.710$), but contributions of different species were significantly different ($P < 0.0001$). A multiple comparison test placed contributions of five grass species into two categories (Table 4.3). Among the species

making lower contributions to production, *Eragrostis* gained a slight competitive edge over *Alloteropsis* and *Andropogon*, despite a general trend of decline in biomass in that category.

Themeda was a dominant competitor under ambient CO_2 and MAR with a biomass increment of 54.4% compared to the first year. A slightly higher proportion of the increment in biomass was allocated to the stem fraction including floral parts. *Themeda* responded better at MAR irrespective of CO_2. *Sporobolus* on the other hand had a preference for a higher watering treatment of 120%MAR, interacting with elevated CO_2 to enhance biomass.

4.3.3.3. 3rd Year

There was a general decline in biomass of all species compared to the first and second years (Fig 4.5.c). Main effects of CO_2 and water treatments were not statistically significant ($P = 0.5286$ and 0.6522 respectively), but contributions of different species were significantly different ($P < 0.0001$). A multiple comparison test on species showed that *Themeda* was the most competitive species, and biomass contribution of *Sporobolus* was reduced from high to intermediate as was *Andropogon* and *Eragrostis*, while contribution of *Alloteropsis* was reduced to low (Table 4.4).

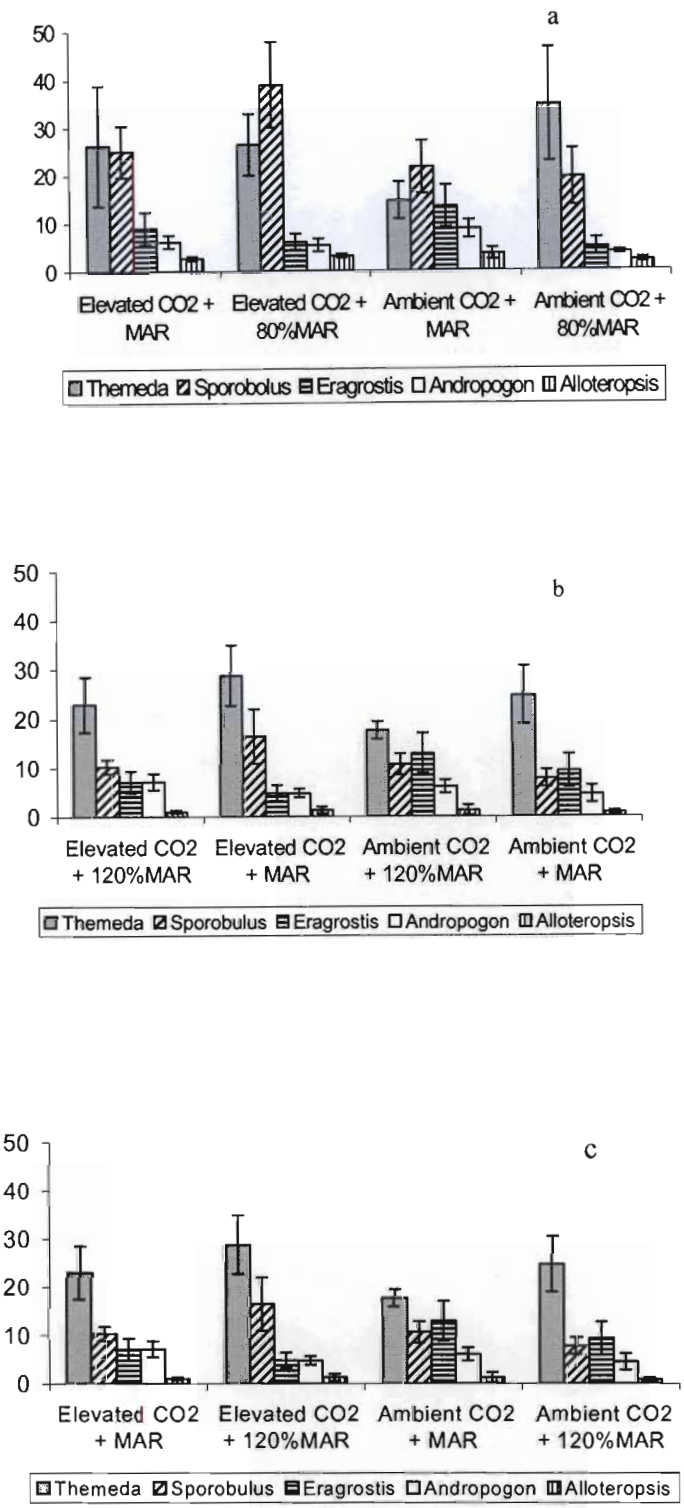


Figure 4.5. a-c: Species contribution to above-ground production in first, second and third years respectively.

Table 4.2: Multiple Comparisons for community above-ground production of the first year classified by species using 95% Tukey-HSD interval (* denotes significantly different pairs and vertical bars show homogeneous subsets).

Group	Cases	Mean	*Eragrostis*	*Alloteropsis*	*Andropogon*	*Themeda*	*Sporobolus*	Homogenous subsets
Eragrostis	16	6.7108				*	*	|
Alloteropsis	16	6.8966				*	*	|
Andropogon	16	10.3845				*	*	|
Themeda	16	16.8938	*	*	*			|
Sporobolus	16	20.2030	*	*	*			|

Table 4.3: Multiple Comparisons for community above-ground production of the second year classified by species using 95% Tukey-HSD interval (* denotes significantly different pairs, and vertical bars show homogeneous subsets).

Group	Cases	Mean	*Alloteropsis*	*Andropogon*	*Eragrostis*	*Themeda*	*Sporobolus*	Homogenous subsets
Alloteropsis	16	6.0108				*	*	|
Andropogon	16	6.0108				*	*	|
Eragrostis	16	8.3607				*	*	|
Themeda	16	25.4517	*	*	*			|
Sporobolus	16	26.2202	*	*	*			|

Table 4.4: Multiple Comparisons for community above-ground production of the third year classified by species using 95% Tukey-HSD interval (* denotes significantly different pairs, and vertical bars show homogeneous subsets).

Group	Cases	Mean	*Alloteropsis*	*Andropogon*	*Eragrostis*	*Sporobolus*	*Themeda*	Homogenous subsets
Alloteropsis	16	1.1053			*	*	*	|
Andropogon	16	5.6175					*	| |
Eragrostis	16	8.5190	*				*	|
Sporobolus	16	11.2816	*				*	|
Themeda	16	23.5031	*	*	*	*		|

4.3.4. Response of the crown material

A two-way ANOVA of treatment effects on community crown biomass showed a high statistical significance of treatment main effects (P = 0.0125 for CO_2, and P = 0.0086 for water treatment), but no interaction (P = 0.4178) as shown in Figure 4.6.a. The result implies that differences in mean crown biomass values among ambient and elevated CO_2 groups is greater than would be expected by chance after allowing for effects of differences in water, and that differences in mean crown biomass values among high water (107%MAR) and MAR is greater than would be expected by chance after allowing for effects of differences in CO_2. Lack of a statistically significant interaction between CO_2 and water (P = 0.418) means the effect of different levels of CO_2 does not depend on what level of water is present. The data suggest that communities under elevated CO_2 and high water allocate reserves to the crown. A benefit of such mobilisation would be enhanced recovery of C_4 grassland communities following a disturbance.

Further analysis was done by including species as a third factor, and the results showed statistically significant main effects of CO_2 (P = 0.048), water (P = 0.025) and species (P = <0.001). However, there was not a statistically significant interaction between (i) CO_2 and water (P = 0.600), (ii) CO_2 and species (P = 0.133) and (iii) water and species (P = 0.546). Analysis on species basis showed a significant CO_2 effect only on *Sporobolus* (P = 0.018) and a marginally significant effect of water on *Andropogon* (P = 0.056). Species averages are plotted in Figure 4.6.b. A rank of grass species by crown biomass was as follows: *Eragrostis* > *Sporobolus* > *Themeda* > *Andropogon* >*Alloteropsis*. A consideration of these results raises the question of whether grass species that respond to elevated CO_2 by development of new tillers would have larger crowns than grass species that respond through development of leaf area, and what the long-term benefits of either mode of response would be with regards to competition.

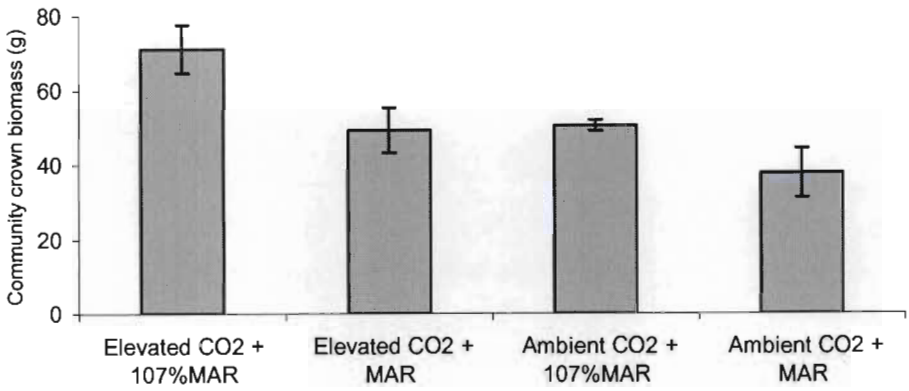


Figure 4.6.a: Treatment effect on community crown biomass

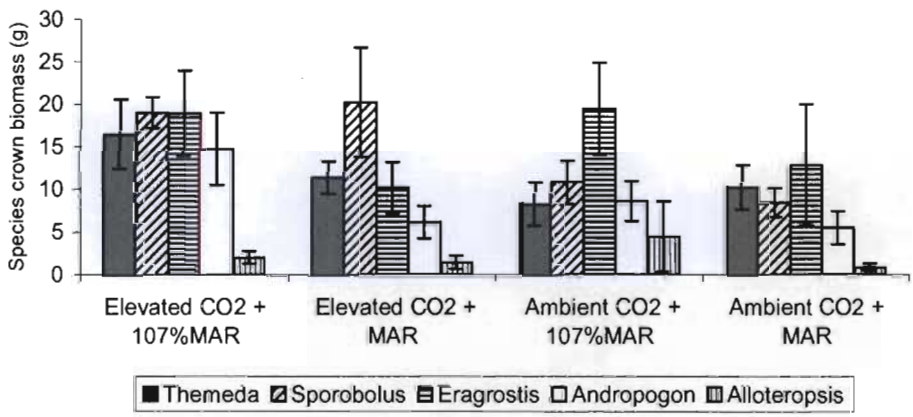


Figure 4.6.b: Treatment effect on mean species crown biomass

4.3.5. Below-ground responses

4.3.5.1. Community total below-ground biomass

The amount of below-ground biomass accumulated at the end of the experiment was highest in the high CO_2 + 107%MAR treatment (Figure 4.7), although the main effects of CO_2 and water were not significant (P = 0.2656 and P = 0.7330 respectively), neither was their interaction (P = 0.3000).

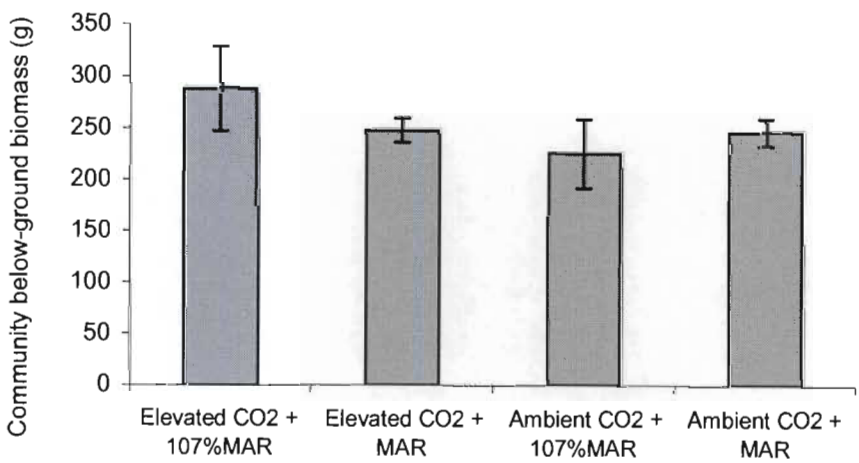


Figure 4.7: Treatment effect on community below-ground biomass

4.3.5.2. Response of root density with depth

Root distribution was significantly influenced by depth ($P < 0.0001$) and watering treatments ($P = 0.032$), but the effect of CO_2 was not significant ($P = 0.66$), neither were interactive effects of CO_2, water and depth. Communities that were exposed to average MAR invested more carbon below-ground, particularly in the top layer (Figure 4.8.a), thereby enabling effective water acquisition during and following a watering event. Almost 50% of the roots were in the top layer under all treatments, and there were no significant differences in root density between the middle and bottom layers. A low rooting density in the bottom layer was indicative of grass roots avoiding the clay layer despite increased water availability there. Data obtained from soil moisture probe measurements showed increased accumulation of soil water in the bottom and clay layers of the soil (section 5.2).

When all root density data were pooled irrespective of depth and plotted against treatment (Figure 4.8.b), the trend of high root density in response to MAR which was observed only within the top layer in Figure 4.8.a, was observed in Figure 4.8.b, although the statistical significance of the response was not maintained ($P = 0.09$). Differences between response of root density to treatment (Fig 4.8.b) and response of community below-ground biomass to treatment (Fig 4.7.) could be explained by differences in the sampling method. Sampling for community root biomass required use of the entire plant pot, while sampling for root density was done form a soil core below the crown. Perhaps root densities differ below the plants and between plants.

Analysis of top layer root density data using species as a third factor in addition to CO_2 and water treatments, showed a significant effect of water and species ($P = 0.0008$ and 0.0098 respectively) and no effect of CO_2 ($P = 0.62$). Differences in response among species in the top layer were tested using Tukey-HSD multiple comparisons (Table 4.4) which showed that *Andropogon* responded differently from either *Themeda* or *Eragrostis*. The three factors did not have interactive effects on root density in all three layers. In the middle layer, differences in response to CO_2 treatment were significant ($P = 0.0236$), but effects due to water treatment or presence of different species were not significant ($P = 0.1987$ and 0.8940 respectively). There were no significant differences in the response in the bottom layer.

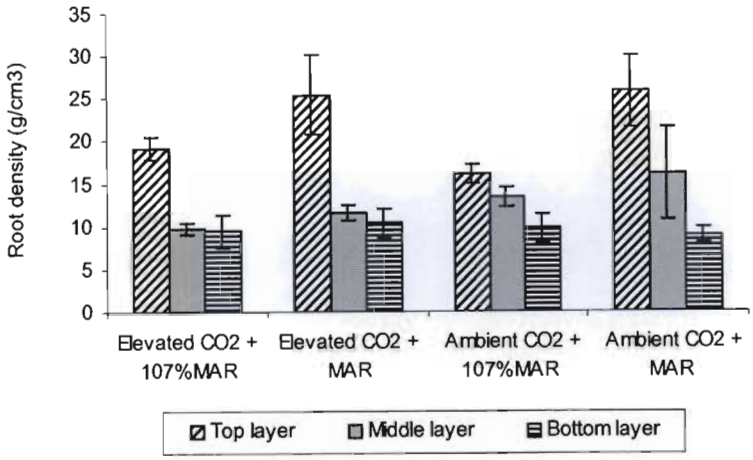


Figure 4.8.a: Treatment effect on root density at three depths

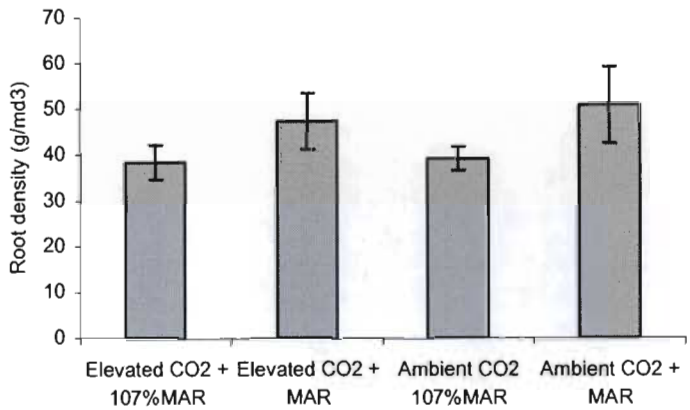


Figure 4.8.b: Treatment effect on pooled root density

Table 4.4: Multiple comparisons for root density in the top layer classified by species using 95% Tukey-HSD interval (* denotes significantly different pairs, vertical bars show homogeneous subsets).

Group	Cases	Mean	*Themeda*	*Eragrostis*	*Sporobolus*	*Alloteropsis*	*Andropogon*	Homogenous subsets
Themeda	16	2.7358					*	|
Eragrostis	16	3.0166					*	|
Sporobolus	16	4.1004						||
Alloteropsis	16	5.3042						||
Andropogon	16	6.4377	*	*				|

4.3.6. Community total above- and below-ground production of the grass species

Having analysed treatment effects on separate biomass fractions, further analysis of treatment effect on total production over the entire period of study was done by adding accumulated three years above-ground biomass, below-ground biomass, and biomass of the crown. It is important though to note that the data presented in this analysis will be influenced by the initial size of the crown and below-ground biomass at the start of the experiment in the following manner. Part of the crown and below-ground biomass that was measured at final harvest was present at the start of the experiment, hence it is not really part of the production over the three year period. Nonetheless, results of a two-way ANOVA performed on the accumulated community production show a statistically significant effect of CO_2 treatments ($P = 0.0407$). There was no significant effect of water treatments, and there was no interaction of CO_2 and water treatments. Accumulated community production under elevated CO_2 + 107%MAR treatment was 19% and 21% higher than accumulated community production under ambient CO_2 + MAR and ambient CO_2 + 107%MAR respectively (Figure 4.9). Enhancement of accumulated community production under elevated CO_2 + 107%MAR was 14% higher than under elevated CO_2 + MAR.

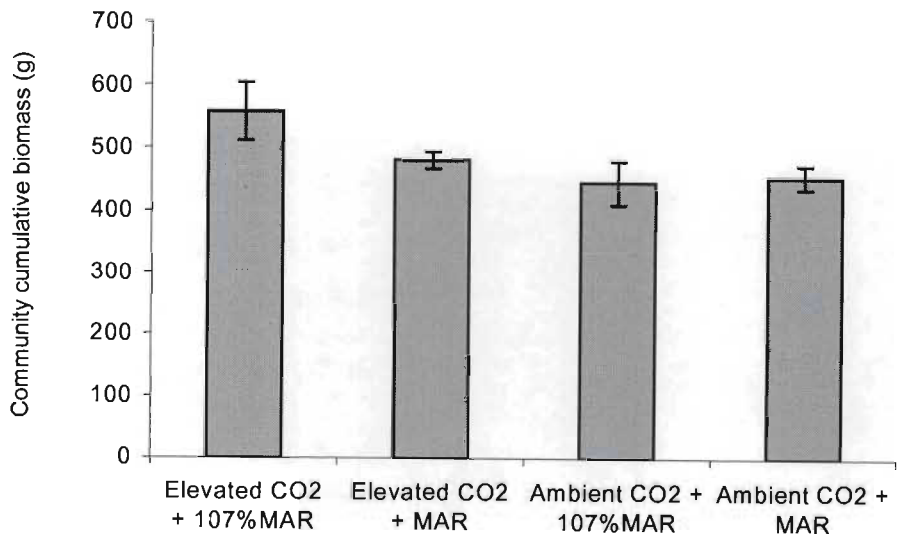


Figure 4.9: Treatment effect on community cumulative biomass

4.3.7. Response of the geophyte - *Eriospermum mackenii* (Hook. f.) Baker, subsp. *mackenni*

Data on absolute above-ground biomass of *E. mackenii* are presented as mean ± standard error per treatment in Table 4.5. The above-ground biomass of *E. mackenii* is notably small at no more than 1g dry mass, compared to approximately 15g dry mass of the below-ground tuber. In the first year, above-ground biomass was similar among all treatments (Table 4.5). Speculation on lack of a growth response in above-ground plant parts is that the geophyte prioritises carbon allocation to the tuber. In the second and third years, there was an increase in above-ground biomass compared to the first year. Even though CO_2 and water main effects were not statistically significant on above-ground biomass production in the second year ($P = 0.4263$ and 0.1483 respectively), there was a statistically significant interaction ($P = 0.0183$). In the third year however, neither the main effects of CO_2 or water treatment ($P = 0.7843$ and 0.0738 respectively) nor interactions ($P = 0.0963$) were significant on above-ground biomass production. Furthermore, the increase in above-ground biomass was not similar for all treatments in the second and third years. For instance, the lowest production of biomass in the second year was recorded under elevated CO_2 + 120%MAR, while slightly higher production was recorded in the other three treatments (Table 4.5). The pattern of response was reversed in the third year, whereby a higher increment was observed under elevated CO_2 + 120%MAR, than the other three treatments. It is tempting to speculate that differences in patterns of biomass accumulation above-ground may be a mimetic expression of development in the bulbs. Lack of treatment effects on above-ground biomass was apparent even on the basis of three year accumulation.

Below-ground biomass at final harvest had increased by different amounts under different treatments ranging between 6-11% (Figure 4.10.). The highest increase in mass of the bulb was under elevated CO_2 + MAR average water treatment. Water treatments seemed to influence responses under both ambient and elevated CO_2, because biomass increase under MAR was higher than under 107%MAR under both ambient and elevated CO_2. Water content of bulbs at harvest was similar for all treatment, at a value of 65-68%. Those values were not different from a mean value of 69% recorded at the start of the experiment.

Table 4.5: Annual above-ground production of the geophyte under different treatments. No treatments had a statistically significant effect on above-ground biomass when tested at $\alpha = 0.05$. Mean value are recorded with standard errors.

	Treatment		
Year	**CO_2**	**Water**	**Biomass (g)**
Year 1	Elevated	MAR	0.18 ± 0.04
	Elevated	80%MAR	0.20 ± 0.07
	Ambient	MAR	0.19 ± 0.09
	Ambient	80%MAR	0.19 ± 0.04
Year 2	Elevated	120%MAR	0.37 ± 0.11
	Elevated	MAR	0.87 ± 0.13
	Ambient	120%MAR	0.78 ± 0.11
	Ambient	MAR	0.64 ± 0.11
Year 3	Elevated	MAR	0.20 ± 0.08
	Elevated	120%MAR	0.54 ± 0.012
	Ambient	MAR	0.32 ± 0.03
	Ambient	120%MAR	0.34 ± 0.09

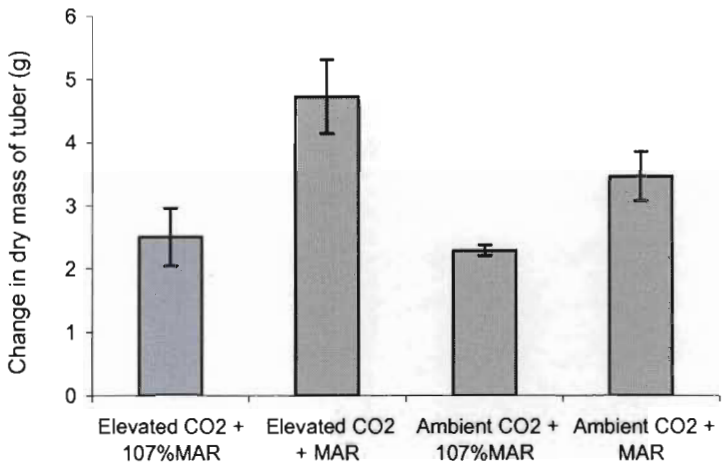


Figure 4.10: Treatment effect on growth of the bulb - *Eriospermum mackenii* (Hook. f.) Baker, subsp. m*ackenni* after three years of exposure

4.3.8. Soil organic matter content

After three years of treatment application, there were no differences in soil organic matter content of all four treatment groups, with mean values ranging between 7.5 and 7.7 percent (Figure 4.11.).

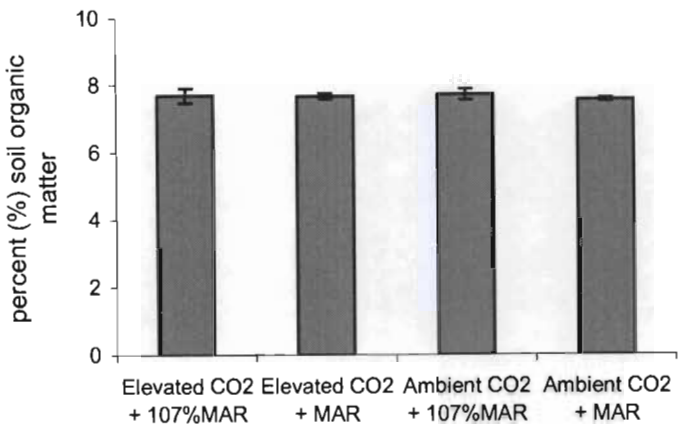


Figure 4.11: Mean percent soil organic matter content per treatment

4.4. Discussion

The experiment set out to investigate what effect elevated CO_2 will have on above-ground biomass production at community and species levels, and on below-ground biomass production at the community level. A second objective was to investigate whether CO_2 response of biomass production (community above- and below-ground, and species above-ground) is dependent on watering treatment.

Community above-ground biomass was greater under elevated CO_2 + MAR than any other treatment combination in the first, second and third years of the experiment. However, when the responses are considered at the 95% significance level, main and interactive effects of CO_2 and water were highly significant only in the first year. To illustrate the purported optimum biomass response under elevated CO_2 + MAR observed in all the three years of the study, average biomass data of the three years were plotted against water treatment (Figure 4.12). A two-way ANOVA was performed on the data pooled over three years, and the effect of water treatment on

community biomass was not statistically significant (P = 0.13), while the effect of CO_2 treatment was significant (P = 0.036). Mean values for pooled community above-ground biomass at ambient and elevated CO_2 treatments were 56.23 g ± 2.28 and 63.76 g ± 2.78 respectively. Figure 4.12 shows that community responses to water treatments were more apparent at elevated CO_2 than ambient CO_2. A one-way ANOVA was then performed to test the statistical significance of water treatment on biomass data of communities treated with elevated CO_2 only (excluding biomass of communities exposed to ambient CO_2), and a significance level of P = 0.056 was observed. This suggests that above-ground biomass production of South African C_4-grasslands may not respond to CO_2 under unfavourably low rainfall scenarios, or acclimate in years of rainfall scenarios higher than MAR.

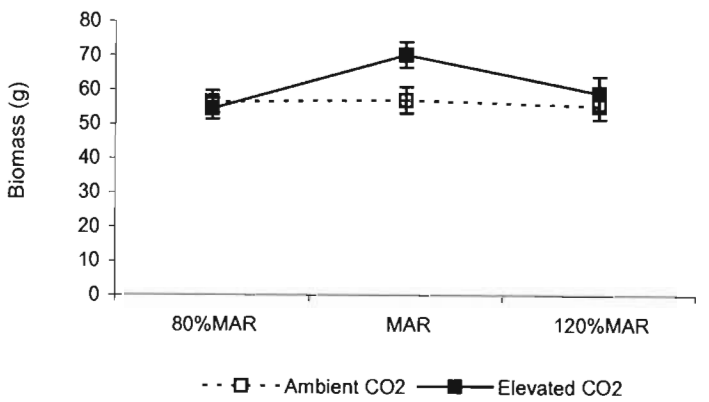


Figure 4.12: A comparison of community above-ground response to water treatment at ambient and elevated CO_2.

Studies on grassland ecosystems in other parts of the world have also reported that effect of elevated CO_2 on biomass production varies with levels of water availability, whereas data in this thesis indicates an optimum response at MAR. In the mesic tallgrass prairie, biomass production under elevated CO_2 was higher in relatively dry years (Owensby et al. 1993, 1999). In the water-limited shortgrass steppe, above-ground biomass production was greatly enhanced by elevated CO_2 in years of greater

than average precipitation (Morgan et al. 2001). The contrasting influence of water availability on responses of biomass production to elevated CO_2 that is observed in the various grassland ecosystems highlights the importance of basing regional predictions of future climate change scenarios on experimental data derived under regional conditions.

Cumulative community biomass production (above- and below-ground) was higher under elevated CO_2 than ambient CO_2 treatments (Figure 4.9). In order to assess cumulative effect of water treatment on cumulative biomass, results were pooled over time. Thus two categories of water treatment applied over three years are defined: (MAR, 120%MAR, MAR) and (80%MAR, MAR, 120%MAR). Mean values of the two categories are 107%MAR and MAR respectively. Effect of CO_2 treatment on community cumulative production was significant at 95% level, but effect of water treatment was not. Among the elevated CO_2 treatments, an average water treatment of 107%MAR resulted in a higher cumulative biomass production than an average water treatment of MAR (Figure 4.9). Trends in annual biomass production over the three years suggest that highest biomass production is attained at MAR, but the cumulative data suggest that highest biomass production is attained at a cumulative water supply higher than MAR (107%MAR). The difference between 107%MAR and MAR categories of water treatments is that the former represents both a narrower fluctuation of rainfall treatments and more water input. So the result suggests that both quantity and level of variability of water supply may be critical for response to elevated CO_2. Even though this is a short-term experiment, the results suggest a potential for positive long-term effects of elevated CO_2 on production of South African C_4-grasslands.

Analysis of biomass production by species serves as an indicator of competitive interactions, and consequently population dynamics of a community. The key species that contributed to above-ground production in all three years are *Sporobolus* and *Themeda*, which are both C_4 grasses although of different photosynthetic sub-types namely PCK and NADP-me respectively. Low biomass contributions were recorded for the other two C_4 grasses; *Andropogon* and *Eragrostis* of photosynthetic sub-types NADP-me and NAD-me respectively. Biomass production of the C_3 grass *Alloteropsis*, was also low under elevated CO_2. Greatest biomass response to elevated

CO_2 occurred in warm season C_4 grasses, possibly as a result of less extreme night time temperatures in the greenhouse. The results suggest that not all C_4 grasses can respond positively to elevated CO_2, and that responses may vary with location. Also, elevated CO_2 may cause changes in community composition of warm-season vs. cool-season grasses where the two types co-occur. Therefore, phenological attributes such as sprouting and senescence may play an important role in vegetation composition and competitive interactions of communities.

Owensby and co-workers (1999) evaluated effect of elevated CO_2 on cool-season vs. warm-season grasses in a mixed community, and reported greater responses in warm-season (C_4 photosynthetic pathway) than in cool-season (C_3 photosynthetic pathway). Comparison of species biomass responses in the field were also made by Morgan and co-workers (2001), who reported biomass enhancement in one C_3 species and not in the other co-dominant C_3 and C_4 species.

Response of the crown biomass was highly influenced by CO_2 and water treatments, but not their interaction. Communities that were exposed to elevated CO_2 and a higher water treatment allocated more biomass to the crown, implying a higher rate of reserve deposition in those communities and possibly a potential for enhanced recovery of C_4-grasslands following disturbance. There was a definite species effect on crown biomass and the order of species contribution starting with the highest was: *Eragrostis* > *Sporobolus* > *Themeda* > *Andropogon* > *Alloteropsis*. Questions that arise from these data are (i) whether grass species that respond to elevated CO_2 by development of new tillers would have larger crowns than species that respond through development of leaf area and (ii) does the size of the crown have an influence on the competitive ability of a species? All grasses used in this study reproduce by tillers. It is not clear whether grass species which respond to elevated CO_2 through leaf biomass would allocate more carbon to the crown than species which respond through development of stems. Perhaps an increase in stem mass could reflect an increase in tiller numbers.

Below-ground production did not respond to CO_2 and water treatments or their interaction. Assessment of treatment effect on below-ground growth may have been complicated by the fact that new root growth was not separated from part of the

biomass that was present at the beginning of the experiment, and the two components were measured together at the final harvest. Root restriction may have also contributed to non-responsiveness of below-ground production. Root density however, was higher in communities exposed to low water irrespective of CO_2 treatment. Almost 50% of root biomass was found in the top 12 cm of the soil in all treatments, thus enabling efficient water acquisition following a watering event. The shallow rooted nature of the grassland community could confer instantaneous benefit to growth-stimulating processes that occur predominantly in the upper layer of the soil such as nutrient mineralisation (Hungate et al. 1997; Arnone and Hohlen 1998), microbial activity (Rice et al. 1994) and earthworm activity (Zaller and Arnone, 1997). On the other hand, shallow rootedness could easily dispose the grassland community to bush encroachment because woody shrubs would have prior access to soil water conserved under elevated CO_2 by virtue of spatial separation of their root systems.

The geophyte (*Eriospermum mackenii* (Hook.f.) Baker, subsp. m*ackenni*) did not show a response to treatments in the above-ground organs in the first year. In the second and third years, above-ground biomass increased, but the increase in the second year was higher than the increase in the third year, possibly indicating an acclimation response. Similarly, the above-ground biomass production of the grasses was lower in the third year compared to the second year. There was a 6-11% increase in the dry mass of tubers over the three years of the study, and the highest increase was recorded under elevated CO_2 + MAR.

Amount of surface litter accumulated at the end of the year comprised about 5-10% of community above-ground production. An average value was about 6 g per unit ground area of 0.159 m^2. Contribution of the two dominant grass species (*Sporobolus* and *Themeda*) to surface litter was proportionally higher than the contribution of other species. Senesced plant material started falling from the canopy after full canopy development. There were no significant differences in treatment effect on amount of surface litter accumulation in each of the three years, and even when considered as a three year cumulative effect. Lack of treatment effect on surface litter could also imply that the physical attributes such as insulation of soil surface that limits evaporation of soil water, and promotion of water infiltration were not differentially

influenced by the presence of different amounts of litter. It is also speculated that the negative effects usually associated with presence of plant litter in communities (Xiong and Nilsson 1999) could not have influenced response of microcosm communities to treatments because a meta-analysis by Xiong and Nilsson (1999) suggested that litter quantities of less than 200 g m^{-2} are commonly associated with positive effects on plant communities. Soil organic matter content of the microcosms was also not significantly different among treatments after three years of the experiment, and it measured an average of just under 8% across treatments. Most of the soil organic matter input comes from root litter, even though some of the surface litter may eventually form soil organic matter after decomposition (though grass litter is known to have very low decomposition rates (Cornelissen and Thompson 1997)). Lack of treatment effect on soil organic matter content of the microcosms may be indicative of none-responsiveness of root growth to treatment or a physical restriction on root growth by pot size.

The amount of soil organic matter at the end of the three year study was similar under all treatments, and had not changed from the beginning of the experiment. Soil organic matter correlates with ecosystem biogeochemical pools and processes, and litter is a major component of biogeochemical processes such as decomposition. Furthermore, rate of decomposition can be affected by soil water. Treatments in this study did not have any effects on the amount of litter accumulated, and that may have influenced lack of changes in soil organic matter. It is also difficult to speculate if lack of treatment effects on litter and soil organic matter content was influenced by phenology or a high dominance of C_4 grasses as opposed to C_3 grasses in the microcosms. Some workers (Epstein et al. 1999) have shown that composition of C_3 and C_4 functional types in a grassland can have important influences on biogeochemical pools and processes. Their results showed that soil organic matter was relatively stable in C_4 dominated communities with respect to changes in precipitation seasonality, whereas soil organic matter in the C_3 community was sensitive to seasonality of precipitation changes.

CHAPTER 5

COMMUNITY WATER USE

5.1. Introduction

Water is a primary factor limiting growth and production in grasslands (Schulze et al. 1987), and small changes in soil water balance are known to cause large changes in composition and function of grassland ecosystems (Epstein et al. 1999; Sala et al. 1992). Overall composition of South African grasslands is in part determined by the seasonality of rainfall and associated occurrence of dry spells, and is to a large extent characterised by a regional distribution pattern whereby C_4 grasses grade into regions that are suitable for C_3 grasses (Vogel et al. 1978), as summer rainfall grades into winter rainfall. At a global scale however, an analysis by Ehleringer and co-workers (1997) suggests that the correlation between total precipitation and distribution of C_4 grasslands is less critical relative to the stronger correlation between distribution of C_4 grasslands and minimum growing-season temperature.

Competitive interactions among C_3 and C_4 grass species under elevated CO_2 are likely to influence compositional balance of South African grassland communities where both C_3 and C_4 types co-occur. Furthermore, yield of grassland catchments could also be influenced by elevated CO_2 through effects on plant water use and soil water balance. At the time when the current study was undertaken, there were no experimental investigations on the effect of increasing atmospheric CO_2 on water use of South African natural grassland communities. However, there are several reports in the literature on effects of elevated CO_2 on water use of natural grasslands from other parts of the world, and a clear message that comes out of some of the reports (Owensby et al. 1997) is that there is an observed sustenance of plants in drier environments due to enhanced availability of soil water under increasing levels of atmospheric CO_2.

Paleoclimate studies and recent evidence (Williams and Balling 1996) support the fact that areas that are now drylands of the central and western United States, southern South America and Western Australia, were much more vegetated in past epochs with high atmospheric CO_2 concentrations. For southern Africa, on the contrary, Williams

and Balling (1996) reported a trend of increasing aridity concurrent with increasing levels of atmospheric CO_2. Recent predictions for South Africa based on a climate change scenario assuming increase in atmospheric CO_2 to 550 ppm, modelled by Midgley, Rutherford and Bond, and reported by Ashwell (2001) suggest that the Succulent Karoo Biome will become more arid, particularly in the west. Cognisance of different climate change predictions for southern Africa relative to other parts of the world strongly warrants more climate change research in the southern African region. It is apparent that climate change mitigation for southern African cannot be based on extrapolation of predictions that are specific for other parts of the world. Within southern Africa, there may be predicted climate change scenarios of enhanced soil water availability for some ecosystems, co-occurring with increasing aridity for other ecosystems. Increases or reductions in aridity of terrestrial ecosystems under elevated CO_2 has a potential to alter geographical range of species. More arid regions will offer fewer restricted refuge sites for plants, while less aridity prone regions will offer broader refuge sites. The scenarios highlight the dire need for in-depth understanding of the unique southern African regional impacts of climate change on biodiversity and other ecosystem-based resources.

The first study to report an increase in ecosystem water use efficiency under elevated CO_2 was conducted on a mixed C_3/ C_4 mesic tallgrass prairie in Kansas (Knapp et al. 1993a), a result that has been confirmed through several years in that community (Ham et al. 1995, Bremer et al. 1996, Owensby et al. 1999). The authors attributed the observed responses to reduced g_s. Other studies in which a similar trend of increased water use efficiency was reported include a C_3 semi-arid annual grassland in California (Freeden et al. 1996), and C_3 mesic perennial grasslands in Switzerland (Niklaus et al. 1998) and Sweden (Sindhøj et al. 2000). Positive effects of elevated CO_2 on water use have also been reported in reconstituted grassland communities (Grünzweig and Körner, 2001, Volk et al. 2000).

In the study by Grünzweig and Körner (2001), more than two CO_2 concentration treatments were applied, *viz.*, 280, 440, and 660 μmol mol^{-1}; and community water use efficiency was increased more at a higher CO_2 concentration of 660 μmol mol^{-1} than at an intermediate CO_2 concentration of 440 μmol mol^{-1}, due to a greater

reduction in evapotranspiration at the higher CO_2; i.e. evapotranspiration was 2% and 11% lower at 440 and 600 μmol mol^{-1} respectively relative to 280 μmol mol^{-1}. The response of increased water use efficiency in communities and microcosms exposed to elevated CO_2 is unequivocally attributed to reduced stomatal conductance as a consequence of increased c_i (Jackson et al. 1994; Ham et al. 1995; Wand et al. 1999), which results in improved water status at the leaf and whole plant level (Tyree and Alexander, 1993).

Ecophysiological benefits of improved water use efficiency under elevated CO_2 include (i) extended periods of photosynthetic activity in ecosystems that are otherwise water-limited, and (ii) increased carbon allocation to root biomass to enhance the capacity for extraction of soil water and better exploitation of water limited environments (Owensby et al. 1997). Additionally, improved water use efficiency could potentially result in decreased allocation to root development in ecosystems where water is not a limiting factor for growth. However, Wullschleger et al. (2002) argue that the capacity of the root system for the uptake of water does not depend only on root biomass, but on rooting volume, rooting depth, root density, and fine root surface area activity. These authors (Wullschleger et al. 2002) further argue that effects of elevated CO_2 on rooting volume become less significant when soil water is adequate to meet transpirational losses. However, Hungate and co-workers (1997) presented a more comprehensive proposal that if elevated CO_2 could reduce canopy transpiration and root uptake of water regardless of any effect on root volume, that would also result in increased soil water.

The current chapter investigates changes in evapotranspiration (ET) and soil water status of microcosm communities under ambient and elevated CO_2 coupled with different water treatments. The key question is whether community-level water use will be changed by long-term exposure to elevated CO_2. The working hypotheses for the study are that: 1) elevated CO_2 will reduce ET and improve soil water status, 2) effects of elevated CO_2 will depend on the amount and frequency of application of water treatments, and 3) responses of ET will be related to canopy developmental stages and phenology. Potential consequences of a change in community evapotranspiration under elevated CO_2 include (i) changes in soil water status at the

end of a growing season, (ii) changes in growing season length, or (iii) changes in leaf area index, and the study will also investigate which of the three potential consequences will occur.

The hypotheses were tested by measurement of evapotranspiration using lysimetry, and volumetric soil water content was measured with a soil moisture probe in order to determine soil water status. The experimental set-up was designed to eliminate runoff so that output was measured as evapotranspiration plus drainage loss during the first year, and as evapotranspiration only for second and third years. Water output by drainage was measured only in the first year, and was subsequently ignored in the second and third years without any major consequences for computation of water use in the microcosms.

5.2 Materials and Methods

5.2.1 Community evapotranspiration by lysimetry

Ideally, the most direct method of measuring evapotranspiration is eddy covariance methodology. However the technology is not appropriate at small scales, and therefore other direct methods may be considered. A weighing lysimeter qualifies as a direct method for measurement of evapotranspiration under conditions where rain or irrigation is controlled, provided deep drainage is accounted for. Weighing lysimetry was used in the current study as a direct method of measuring community evapotranspiration because it fulfilled requirements of permitting control of water input together with precision and replication, while being easily available and cost effective. The weighing lysimeter consisted of a rail-guided and hand-operated mobile crane to which a cantilevered balance consisting of a load cell and a millivolt meter, were attached (Chapter 2 section 2.2).

The capacity of the load cell on the lysimeter was 60 kg with an error margin of 10 g. Evapotranspiration in the study comprised of transpirational water loss, foliar interception, and evaporation from the soil surface. Foliar interception was however kept to a complete minimum by applying watering treatments very close to the soil surface. A potential source of error in the measurement of evapotranspiration that could occur due to incremental changes in plant biomass was expected to be insignificant, because common estimates of such errors are said to be in the vicinity of

less than 1% of change in water storage (Dunin et al. 1983). Biomass data obtained in the current study was not used to estimate the magnitude of error arising from incremental changes in plant mass because water content of the foliage had not been determined during the course of the growing season.

Measurements were performed by weighing individual microcosms (plant pot) at weekly intervals, before application of watering treatment. The "after watering" mass of microcosms was determined by adding to the "before watering" value of pot mass, the mass of water supplied. However, at the beginning of the first year, measurements were performed more frequently, and some occasions twice or three times a week. Precision of frequent measurements in the first year was usually satisfactory, except on occasions when recorded values of water loss were very low as a consequence of a stochastic pattern of watering. Thus to minimise concerns about measurement precision, a stochastic pattern of treatment application was replaced by a regular pattern of twice weekly watering in the second and third years. From then on, measurements were done weekly until end of the study. The best time of day for weighing was either in the morning or late in the afternoon, during periods of low evaporative demand.

Evapotranspiration was calculated as the difference in pot mass between two consecutive weighing events, taking into account the mass of water applied between the two weighing events. Evapotranspiration was assessed from the beginning to the end of a growing season in the three years of the experiment, and the beginning of a growing season was taken as the time of increase in watering, which according to the rainfall data (Figure 2.4.2) was beginning of September each year.

The lysimetry data can provide an indication of treatment effect on weekly, monthly and annual cumulative evapotranspiration loss. On the other hand, when values of water loss are considered against total amount of water supplied, the data could also allow for estimation of the amount of water accumulated in the soil. A slight limitation of determining soil water status as a difference between water added and water evapotranspired would be lack of an indication of spatial distribution of accumulated soil water in the soil profile. Further measurements were therefore

undertaken using a Delta-T Theta Probe soil sensor (section 5.2.3) to assess patterns of water distribution within the soil profile in the second and third years.

5.2.2 Drainage loss in the first year

Microcosms were fitted with 50 cm drainage tubing at the bottom of plant pots. The hanging ends of drainage tubes were plugged to ensure control over collection and quantifying of drainage loss. Volume of drainage liquid was collected and quantified whenever drainage tubes were full, and if present, subsequently collections were added up to make monthly totals, which were in turn added up to calculate a cumulative annual value. An ANOVA was performed to assess the statistical significance of treatment effect on the cumulative annual value.

5.2.3 Volumetric soil water content

Volumetric soil water content was measured *in situ* at soil depths of 6 cm, 12 cm and 20 cm on each plant pot using an ML2x Delta-T Theta Probe (Delta-T Devices Ltd., Cambridge, UK). The instrument consisted of a sensor head adjacent to a PVC case enclosing power transmission and measuring circuitry. A PVC case of 112 mm was connected to a hand-held voltage output device by an input/output cable of 5 m. The sensor head was made of four sharp-ended 60 mm stainless steel rods. A template of the sensor rods was made on a PVC sheet and used to drill permanent access holes at demarcated positions on plant pots to enable entry of rods when taking measurements. Short wooden plugs of similar thickness to rods were used as plugs in access holes in order to prevent soil from drying when measurement was not in progress.

Principle of operation of the Theta Probe is based on a relationship between the dielectric constant of soil (ε) and voltage output signal (V) explained by a third order polynomial (Theta Probe User Manual 1997):

Equation 1: $\sqrt{\varepsilon} = \mathbf{1.07 + 6.4V - 6.4V^2 + 4.7V^3} \qquad \mathbf{(R^2 = 0.998)}$

Volumetric soil water content, θ, was determined by a linear relationship with dielectric constant (Whalley, 1993; White et al. 1994):

Equation 2: $\sqrt{\varepsilon} = \mathbf{a0 + a1 \,.\, \theta}$

The instrument was operated within a range of 0 to 1 V, which corresponded to a volumetric soil water content of 0 to 0.55 m^3m^{-3} (Theta Probe User Manual, 1997). Soil specific calibrations were performed for each of two soil types used in the experiment *viz.*, silty loam for potting and silty clay lining the bottom 5 cm of plant pots; in order to determine coefficients a_1 and a_0 in equation 2. Measurements for instrument calibration were taken on soil at various levels of wetness, from saturation (drainage upper limit) through to oven-dryness. Three replicate calibrations were done for each soil type on one-litre samples of known mass.

Assessment of treatment effects on volumetric soil water content was done during the second and third years at intervals of once a week. Two sets of readings were taken on each sampling event, one set just before watering and the other an hour after watering. The trend in "before-" and "after watering" readings could give an indication of change in volumetric soil water content, even though the differences would not necessarily have a linear relationship. Taking measurements an hour after a watering event allowed sufficient time for even distribution of water in the soil. Measurements at 6 cm depth were taken at five random positions in each pot by vertically inserting sensor rods at the soil surface. Measurements at 12 and 20 cm depths were each taken at two demarcated horizontally opposite replicate positions on either side of a plant pot. Sampling was done either in the morning or late in the afternoon, when evaporative demand was low.

5.3 Results

5.3.1 Evapotranspiration in the first year

5.3.1.1 Annual cumulative evapotranspiration and water use efficiency (WUE)

Cumulative evapotranspiration at end of the first year indicated higher water loss under ambient CO_2 relative to elevated CO_2 (Figure 5.1). The results also suggest a strong effect of water treatment within ambient and elevated CO_2 treatments. Evapotranspiration was reduced by 12% under elevated CO_2 + MAR relative to ambient CO_2 + MAR treatment (Figure 5.1). The observed responses were demonstrated by statistically significant treatment main effects of both CO_2 and water ($P < 0.001$ for both) and their interaction ($P = 0.0203$). However, a more meaningful

comparison of treatment effect on community water use strategies under different treatments would be based on the relationship between total evapotranspirational water loss and the amount of biomass produced, WUE (Table 5.1). Community WUE at the end of the first year was calculated as total above-ground biomass (g) produced (Chapter 4, Figure 4.4.1.a) per kg of water lost by evapotranspiration. A short-coming of the analysis was that the WUE ratio obtained did not include below-ground production because root biomass was harvested only at the end of the third year. Nonetheless, effect of CO_2 treatment on WUE was statistically significant ($P = 0.0016$), but effect of water treatment was not ($P = 0.45$) and treatment interactions were statistically significant ($P = 0.0095$) indicating that responses to CO_2 treatment were dependent on water supply.

Table 5.1: WUE as a ratio of the above-ground biomass produced to the total evapotranspiration in the first year.

Treatment	WUE (g/kg)
Elevated CO_2 + MAR	1.24 ± 0.04
Elevated CO_2 + 80%MAR	1.11 ± 0.08
Ambient CO_2 + MAR	0.84 ± 0.05
Ambient CO_2 + 80%MAR	1.058 ± 0.05

5.3.1.2 Annual cumulative evapotranspiration and soil water status

A comparison of cumulative water loss by evapotranspiration against total amount of water added during the first year (from beginning of September 1998 to the end of May 1999) gives an indication of the amount of water remaining in the soil, taking into consideration amount of water lost as drainage (section 5.3.2). That comparison indicates that 79% of added water was lost as evapotranspiration under the elevated CO_2 + MAR even though leaf biomass production increased, and that 91% was lost under ambient CO_2 + MAR. Under the treatments of lower water supply namely, elevated CO_2 + 80%MAR and ambient CO_2 + 80%MAR, cumulative evapotranspiration accounted for 81% and 88% of applied watering treatment

respectively. It follows that soil water accumulation would therefore be highest under elevated CO_2 + MAR (44%), followed by elevated CO_2 + 80%MAR (42%), then ambient CO_2 + 80%MAR (39%), and least under ambient CO_2 + MAR (34%).

Assessment of treatment effect on soil water accumulation was examined by considering change in pot mass throughout the year. Increase in values of pot mass recorded before watering serve as better indicators of soil water status than values of pot mass recorded after watering, because the latter would be influenced by addition of water. Taking into consideration the fact that pot mass was different for all microcosms at the beginning of the experiment, the best way to compare the trend of changes in pot mass would be a comparison of the slope of the graphs representing the data. Figure 5.2 illustrates a consistent increase in values of pot mass recorded before watering during the period between day 53 and day 151 since application in watering treatment. Values of pot mass recorded between days 0-39 since application of watering, which represents a period from beginning of September to beginning of October, show a decline of about 1.6-2 kg despite a 59% increase in amount of watering for the same period. During the period before application of CO_2 and watering treatments, plant pots were watered to field capacity to ensure efficient establishment. The origin of the water lost during days 0-39 since application of watering which resulted in the observed initial decline in pot mass at that period, is speculated to have come from the saturated condition that was maintained during establishment.

Comparison of slopes for increase in pot mass (Figure 5.3) was performed in order to assess treatment effect on soil water accumulation, by calculating the regression coefficients and their respective 95% confidence intervals (upper and lower limits) using the "studentized range" method (Sokal and Rohlf, 1995). The 95% intervals of the regression coefficients are represented in Figure 5.3. A similar analysis was performed for second and third year data on change in pot mass. The analysis illustrated in Figure 5.3 was performed on data points representing the period from days 44-151 on Figure 5.2, during which a similar response pattern was observed in all four treatments. The regression coefficients whose intervals do not overlap show statistically significant differences in treatment effect. The analysis suggests that the

greatest increase in pot mass occurred under elevated CO_2 + MAR., but that increase in pot mass was not significantly different from ambient CO_2 + MAR.

Data points falling beyond day 151 were excluded from the analysis of regression coefficients because opposite response patterns in soil water status were observed in ambient and elevated CO_2 treatments (Figure 5.2). Pot mass declined progressively in the ambient CO_2 microcosms, and by end of year values were similar to values recorded at the beginning of year. Among the ambient CO_2-treated microcosms, pots receiving 80%MAR underwent a quicker rate of reduction in mass due to water loss than pots receiving MAR, nonetheless remaining at a similar value by end of year. Pot mass of microcosms exposed to elevated CO_2 on the other hand remained high after day 151, declining only slightly by end of year. Among the elevated CO_2 microcosms, pots receiving 80%MAR continued to accumulate soil water, reaching a similar level of mass as pots receiving MAR by end of year. Differences in response under ambient and elevated CO_2 could be attributed to two different phenomena. Pot mass of microcosms exposed to ambient CO_2 increased with increasing water supply, and declined when a reduction in water supply occurred after day 151. While increase in water supply may have also contributed to the observed increment in pot mass under elevated CO_2, maintenance of high pot mass through the period of reduced water supply after day 151 under elevated CO_2 strongly suggests a CO_2 treatment effect.

The data on cumulative evapotranspiration and soil water accumulation (increase in pot mass with time) were used to determine coarse estimates of soil water balance in the microcosms under different treatments. That procedure was performed for the period representing beginning to end of year (days 44-273 Figure 5.2). In that way, the analysis would differentiate actual treatment effects on soil water balance from consequences of increase in water supply, because any increase in soil water accumulation that occurred due to increase in water supply during the course of year would have ceased by end of year. Results of the analysis show an accumulation of 2% soil water under elevated CO_2 + MAR, 6% soil water accumulation in elevated CO_2 + 80%MAR, no soil water accumulated in ambient CO_2 + MAR, and 1.9% soil water accumulation in ambient CO_2 + 80%MAR.

5.3.1.3 Monthly cumulative evapotranspiration

The pattern of monthly evapotranspiration (Figure 5.4) could be described as dependent on water supply and somewhat related to canopy development. At the beginning of growing season during September, evapotranspiration accounted for 50-60% of the water applied. A large component of evapotranspiration was surface evaporation because there was little foliage present. The rate of water loss was similar among all four treatments, particularly during September. Statistical significance of treatment effects on monthly evapotranspiration was assessed separately for each month as shown in Table 5.2. Treatment main effects and their interaction did not have statistically significant effects on water loss during September. In October, the effect of water treatment was statistically significant, while CO_2 effect was marginally significant, and CO_2 interaction with water treatment was not significant. Microcosms receiving MAR lost more water than microcosms receiving 80%MAR under both CO_2 treatments from October until at the end of the growing season (Figure 5.4). Among microcosms receiving similar water treatments, the group of microcosms under elevated CO_2 lost less water than those under ambient CO_2 between October and March. Thereafter, a transition occurred whereby water loss decreased under ambient CO_2 at both MAR and 80%MAR, notably at the time when senescence started. The canopy developmental phase of senescence was noted to occur earlier under ambient CO_2 treatments, thus indicating longer growing season for microcosms exposed to elevated CO_2. The apparent higher rate of water loss observed in elevated CO_2 microcosms during April and May was a consequence of delayed senescence resulting in prolonged physiological activity. Initial rate of water loss during the first half of the growing season (September to January), and the difference in water loss among and between groups was more moderate. Subsequently, a high increase in water loss was noted under MAR treatments during February and March, a time corresponding with full canopy. A more steady rate of water loss was observed under elevated CO_2 + 80%MAR throughout the year. Treatment main effects on the observed responses were significant during most months except in September and December.

Data on weekly rate of water loss was characterised by a high degree of variability because of the direct effects of weather fluctuations, hence data are not shown. Nonetheless, typical values for weekly rate of water loss ranged between just over 0.5

kg at beginning of growing season in September, to just over 1.5 kg by end of growing season in May. The maximum rate of weekly water loss occurred in the middle of the growing season and typical values ranged between 1.7 to 3.0 kg.

Table 5.2: Statistical significance of treatment effects on monthly total evapotranspiration in the first year. NS notes lack of statistical significance of treatment effect in parenthesis.

Month	**Statistical significance of treatment effect**		
	CO_2	**Water**	**Interaction**
September	P = 0.863 (NS)	P = 0.0566 (NS)	P = 0.744 (NS)
October	P = 0.0562 (NS)	P = 0.026	P = 0.914 (NS)
November	P = 0.0490	P < 0.001	P = 0.5905 (NS)
December	P = 0.914 (NS)	P = 0.1 (NS)	P = 0.892 (NS)
January	P < 0.001	P < 0.001	P = 0.2499 (NS)
February	P < 0.001	P < 0.001	P = 0.1392 (NS)
March	P < 0.001	P < 0.001	P = 0.0019
April	P = 0.0791 (NS)	P < 0.001	P < 0.001
May	P = 0.001	P = 0.1391 (NS)	P = 0.0083

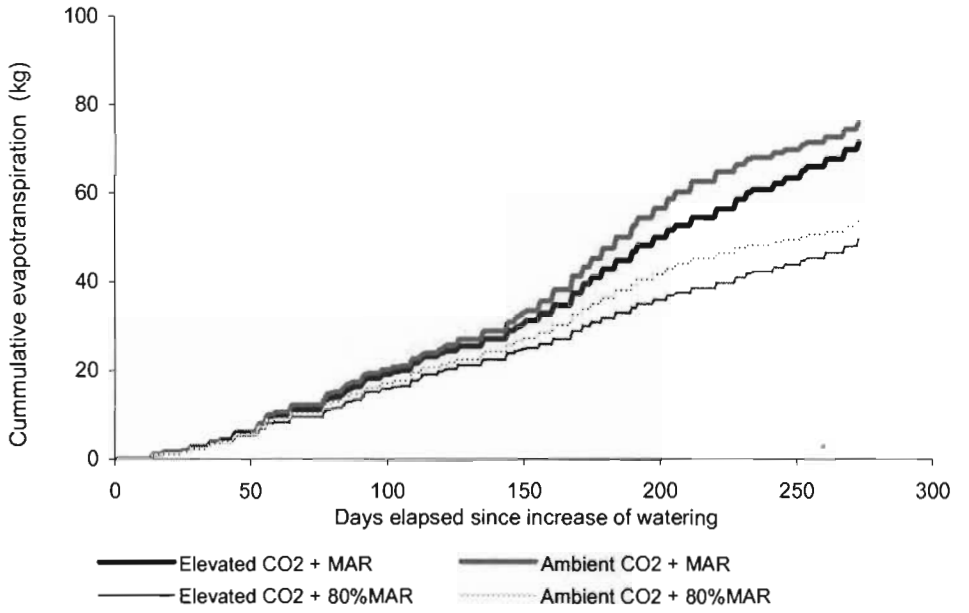


Figure 5.1: Treatment effect on cumulative evapotranspiration in the first year.

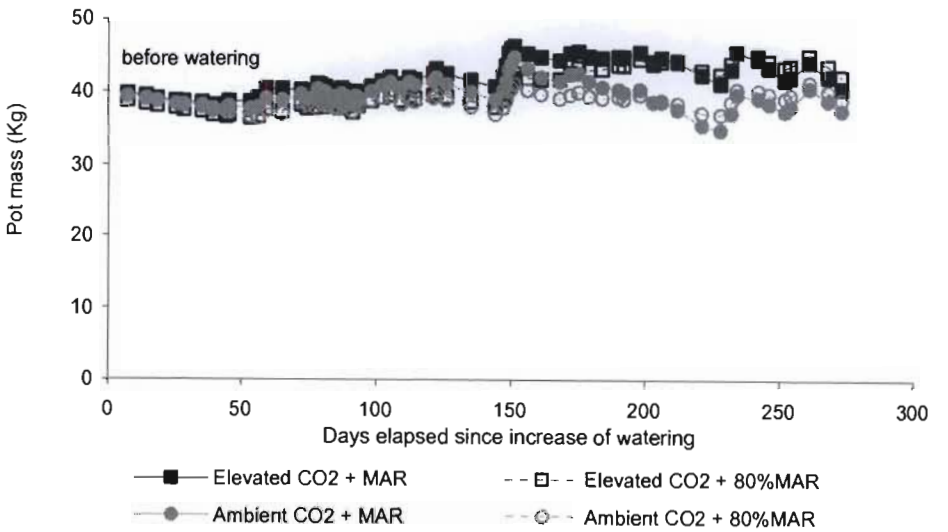


Figure 5.2: Treatment effect on change in pot mass reflecting soil water status in the first year.

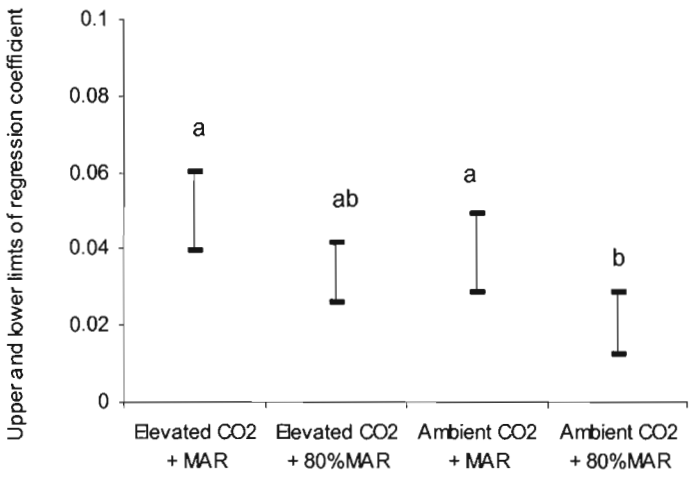


Figure 5.3: Studentized range showing 95% confidence intervals for the regression coefficients of change in pot mass for the period between days 44-151 in the first year. Similar letters assigned different treatments indicate lack of significant statistical differences and vice versa.

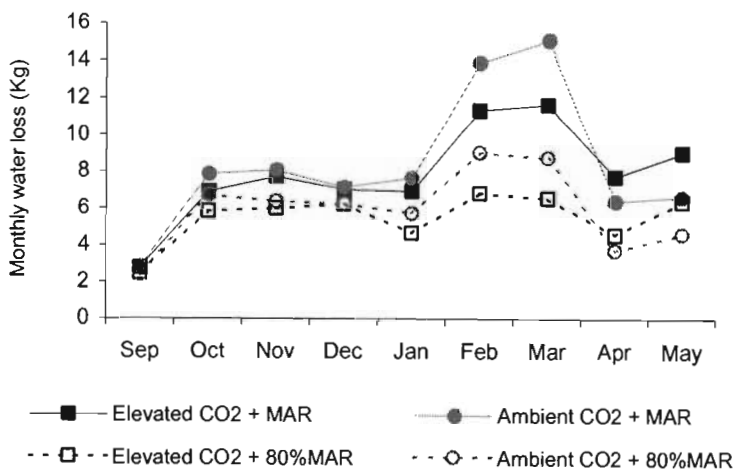


Figure 5.4: Treatment effect on monthly cumulative evapotranspiration in the first year.

5.3.1.4 Drainage loss in the first year

Drainage loss occurred when watering in excess of the equivalent of 25 mm of rainfall was applied within a period of two days. Drainage volume collected from microcosms receiving a water treatment of MAR under both ambient and elevated CO_2 was higher than drainage volume collected from microcosms receiving 80%MAR under both ambient and elevated CO_2. The first incident of drainage was recorded towards the end of November 1998, and it was concurrent with the largest watering event since beginning of the growing season. Subsequent collections of drainage were made during December 1998, January 1999 and February 1999. During December 1998 and February 1999, drainage was also collected from microcosms receiving 80%MAR. Cumulative drainage output was estimated at 3mm relative to annual rainfall of 734 mm. Treatment effects on drainage output were not statistically significant with the following P values: $P = 0.28$ for CO_2 treatment, $P = 0.93$ for water treatment, and $P = 0.47$ interactions. No drainage was collected during years two and three because when the stochastic watering programme was changed to regular watering, thus eliminating application of large quantities of water which would result in drainage.

5.3.2 Evapotranspiration in the second year

5.3.2.1 Annual cumulative evapotranspiration and water use efficiency (WUE)

Treatment effect on cumulative evapotranspiration at the end of year two is illustrated in Figure 5.5. Statistical analysis of the data showed a highly significant effect ($P < 0.0001$) of the main treatments and their interaction. Lower cumulative evapotranspiration was observed in microcosms exposed to elevated CO_2 compared to microcosms exposed to ambient CO_2 at similar water treatments. Expressing cumulative evapotranspiration data as a proportion of total amount of water added in the second year (from beginning of September 1999 to May 2000) illustrated that the difference in response among CO_2 treatments was greater at 120%MAR than at MAR. For instance, microcosms exposed to elevated CO_2 + 120%MAR lost 12% less water than microcosms exposed to ambient CO_2 + 120%MAR, while microcosms exposed to elevated CO_2 + MAR lost 3% less water compared to microcosms exposed to

ambient CO_2 + MAR. Within elevated CO_2 treatments, cumulative evapotranspiration was 7% lower at 120%MAR compared to MAR, but within the ambient CO_2 treatments cumulative evapotranspiration was 2% higher at 120%MAR compared to MAR.

An annual comparison for first and second years showed a slight reduction in evapotranspiration relative to water supply during the second year despite two modifications in watering treatment that increased the quantity and frequency of water supply, *viz.*, 20% increment in water treatment and regular application (Chapter 2 Table 2.2). It would be expected that a 20% increment in water supply would render the soil surface wetter on a regular basis compared to the first year, possibly increasing chances of surface evaporation prior to canopy closure. Indeed, the absolute values of water evapotranspired in the second year had increased compared to the absolute values of the first year (Figures 5.1 and 5.5), but the response pattern changed when evapotranspiration values were expressed as a proportion of total water supply. An exception to this pattern was noted under elevated CO_2 + MAR in terms of both absolute amount of water evapotranspired, but proportionally 56% of added water was lost under that treatment in the first and second years. The difference however, is that more above-ground biomass was produced in the second year than in the first year under elevated CO_2 + MAR, resulting in a WUE of 1.31 g above-ground biomass kg^{-1} water lost (Table 5.3) compared to 1.24 g kg^{-1} (Table 5.1) observed in second and first years respectively. Comparison of WUE in the other three treatments during the first and second years respectively showed either no change or slight reduction in WUE (Tables 5.1 and 5.3). Treatment main effects of CO_2 and water treatments on WUE were statistically significant ($P = 0.026$ and 0.03 respectively), and treatment interactions were not statistically significant ($P = 0.82$) in the second year.

Table 5.3: WUE as a ratio of the above-ground biomass produced to the total evapotranspiration in the second year.

Treatment	WUE (g/kg)
Elevated CO_2 + 120%MAR	1.04 ± 0.08
Elevated CO_2 + MAR	1.31 ± 0.12
Ambient CO_2 + 120%MAR	0.8 ± 0.06
Ambient CO_2 + MAR	1.04 ± 0.15

5.3.2.2 Annual cumulative evapotranspiration and soil water status

Assessment of treatment effect on soil water accumulation was done by comparing change in pot mass for measurements taken "before watering" throughout the second year (Figure 5.6). Pot mass generally increased in all treatments indicating accumulation of soil water from beginning of growing season until day 213. The highest increment in pot mass occurred under elevated CO_2 + 120%MAR, and the lowest increment occurred under ambient CO_2 + MAR (Figure 5.6). Over the last part of the growing season little change was observed in pot mass of microcosms exposed to elevated CO_2, whereas a decline in pot mass was observed in the ambient CO_2 treatments and also elevated CO_2 + MAR. Soil water retained in elevated CO_2-treated microcosms towards end of growing season sustained further physiological activity and a delay in senescence.

Evaluation of treatment effect on increase in pot mass as an indicator of soil water accumulation was done by regression analysis of the slopes of graphs in Figure 5.6, for a period from 62-189 days. The 95% confidence intervals of the regression coefficients are shown in Figure 5.7. Regression coefficients whose intervals do not overlap are indicative of statistically significant differences due to treatment effect. There was some overlap in coefficient intervals within either ambient CO_2 treatments and elevated CO_2 treatments, suggesting a lack of statistically significant effect of water treatment within each CO_2 treatment group. The overlap was bigger for ambient CO_2 treatments than for elevated CO_2 treatments.

Estimates of soil water balance were done for the period between days 62-273 by subtracting cumulative water loss for that period from the amount of water added over the same period. Results of that analysis show an accumulation of 8.75% soil water under elevated CO_2 + 120%MAR, 4% soil water accumulation in elevated CO_2 + MAR, no soil water accumulated in ambient CO_2 + 120%MAR, and 2.3% soil water accumulation in ambient CO_2 + MAR.

5.3.2.3 Monthly cumulative evapotranspiration

Total monthly evapotranspiration increased continuously from September to February, and then declined during March until May (Figure 5.8). A peak in evapotranspiration occurred during February at the time of full canopy, which was a two month lag behind a peak in total monthly rainfall. Increases in evapotranspiration during September to October, and during January to February were steeper than the increases that occurred during November to January. It seemed that substantially higher rates of evapotranspiration coincided with periods of initial growth (September to October) and full canopy (January and February). Total monthly evapotranspiration during each of the nine months of measurement were separately subjected to a two-way ANOVA (Table 5.4). Results of the ANOVA show that effects of CO_2 and water treatments were significant during most part of the growing season, except in October when only effects of water treatments were significant, but effects of CO_2 and its interaction with water treatment were not significant. Effect of CO_2 treatment was again non-significant during February.

Weekly rates of evapotranspiration in the second year were less variable (data not shown) than weekly rates of evapotranspiration in the first year, probably because of a change from stochastic to regular watering. The pattern of weekly water loss was generally similar to a pattern of water supply, but also with a strong influence of short fluctuations in weather conditions. Initial weekly water loss ranged around just over 0.5 kg and it increased to about 2.2 kg by end of growing season.

Table 5.4: Statistical significance of treatment effects on total monthly evapotranspiration in the second year.

Month	Statistical significance of treatment effect		
	CO_2	Water	Interaction
September	P = 0.0047	P = 0.0004	P = 0.0006
October	P = 0.765 (NS)	P = 0.0073	P = 0.913 (NS)
November	P = 0.0026	P = 0.0033	P = 0.956 (NS)
December	P < 0.001	P < 0.001	P = 0.0758 (NS)
January	P < 0.0001	P < 0.001	P = 0.0042
February	P = 0.129 (NS)	P < 0.0001	P < 0.0001
March	P = 0.0003	P < 0.0035	P = 0.483 (NS)
April	P < 0.0001	P < 0.0001	P < 0.0001
May	P < 0.0001	P < 0.0001	P < 0.0001

5.3.2.4 Volumetric soil water content

Assessment of treatment effect on soil water content was done by measurements recorded just before a watering event, instead of on measurements recorded after a watering event. Figures 5.9: (a-c) show that soil water content generally increased with soil depth in all treatments. The highest soil water content was measured at the bottom clay layer, and the second highest measured in the rooting layer, while the lowest soil water content was measured at the soil surface. Dynamics of soil water content at the soil surface are under the control of both direct evaporation from the soil and utilisation by plant below-ground organs. However, logic dictates that evaporative demand on the soil would be highest in the first few millimetres of soil layer after a watering event, and thereafter movement of water vapour molecules across the soil surface would be constrained by tortuousity of the diffusion pathway. Differences in soil water content observed at the soil surface would hence be to a large extent a consequence of treatment effects on plant water utilisation, as would be the case in the deeper layers of the soil profile.

The highest soil water content at the soil surface was measured in microcosms exposed to elevated CO_2 + 120%MAR, and the lowest surface soil water content was measured in microcosms exposed to ambient CO_2 + MAR (Figure 5.9a). There were however, a lot of similarities in water content values recorded in microcosms treated with elevated CO_2 + MAR and ambient CO_2 + 120%MAR. Compared to Figures 5.9 b and c Figure 5.9a shows less variability among treatments throughout the year.

A different pattern of treatment effect on soil water content was observed in the rooting layer Figures 5.9b, in that microcosms treated with elevated CO_2 clearly retained more water in the soil at both 120%MAR and MAR than microcosms treated with ambient CO_2 at both watering treatments. At either CO_2 treatment however, higher volumetric soil water was consistently recorded under 120%MAR than MAR, but the difference in soil water content due to water treatment was biggest under elevated CO_2 than under ambient CO_2. Dynamics of soil water content in the clay layer (Figure 5.9c) were more influenced by water treatment than CO_2 treatment, but within each water treatment, higher volumetric soil water content was measured under elevated CO_2.

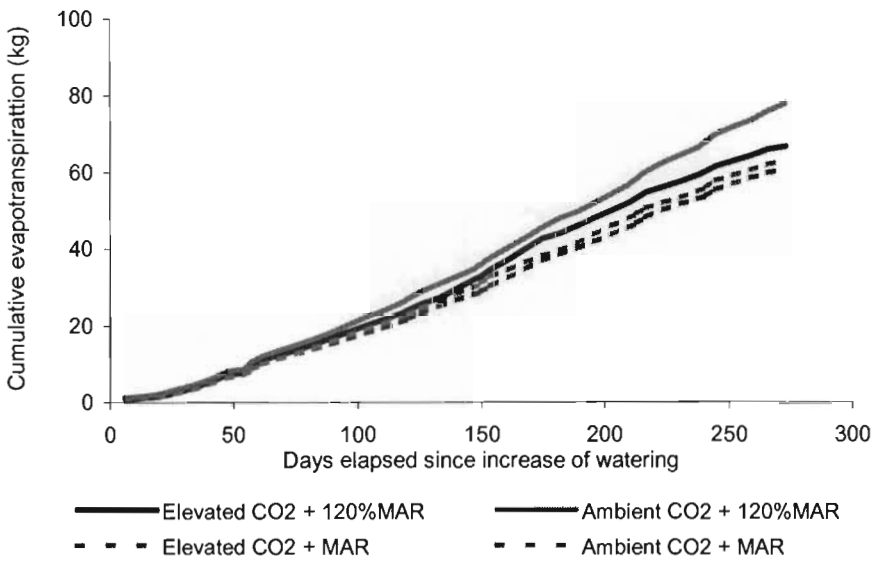


Figure 5.5: Treatment effect on cumulative evapotranspiration in the second year.

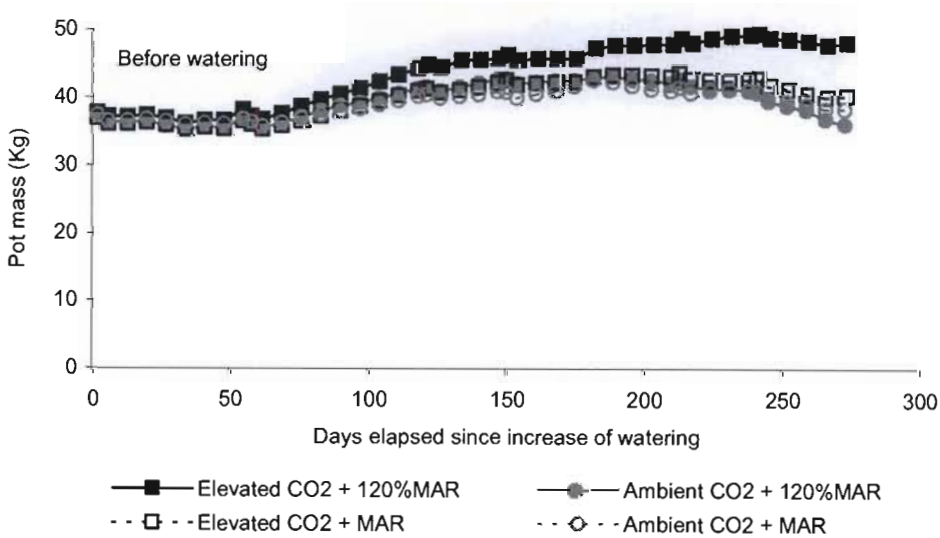


Figure 5.6: Treatment effect on change in pot mass as a consequence of soil water status in the second year.

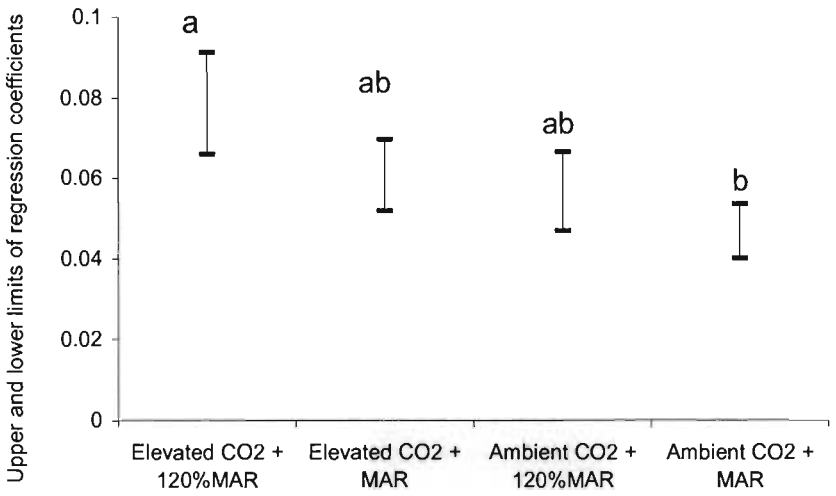


Figure 5.7: Studentized range showing 95% confidence intervals for the regression coefficients of change in pot mass for the period between days 44-151 in the second year. Similar letters assigned different treatments indicate lack of significant statistical differences and vice versa.

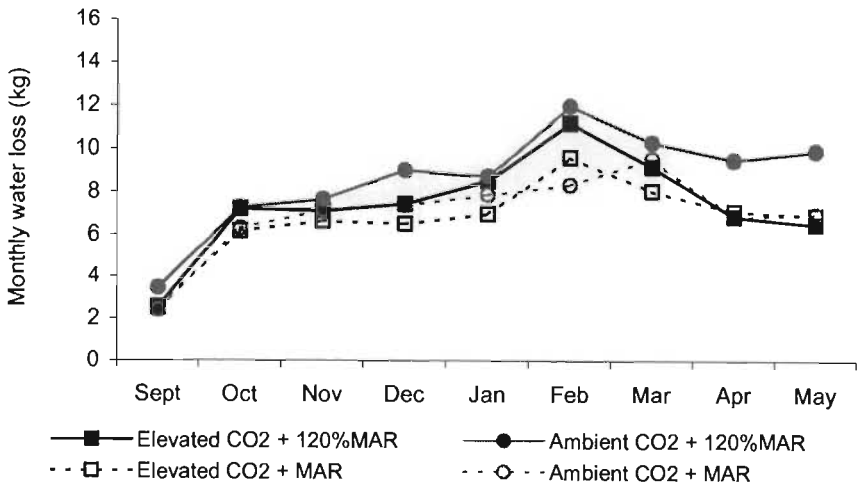
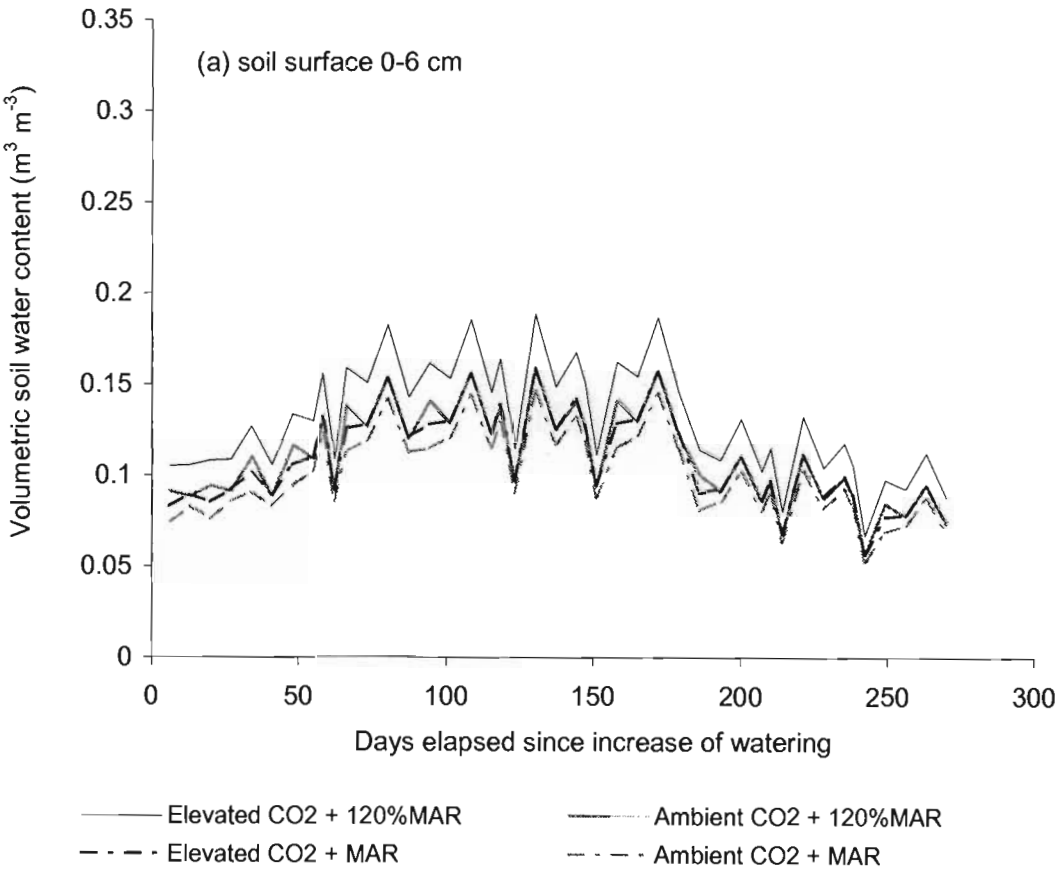
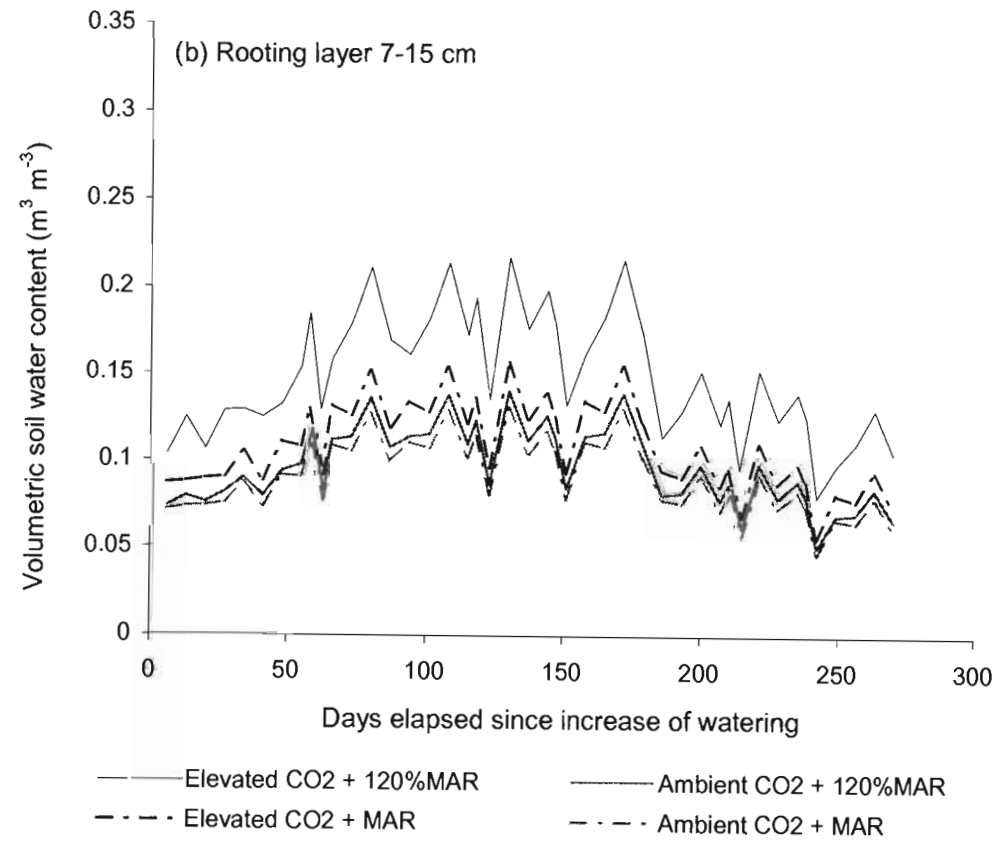


Figure 5.8: Treatment effect on monthly cumulative evapotranspiration in the second year.


(a) soil surface 0-6 cm
Volumetric soil water content (m^3 m^{-3})
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
50
100
150
200
250
300
Days elapsed since increase of watering
Elevated CO2 + 120%MAR
Ambient CO2 + 120%MAR
Elevated CO2 + MAR
Ambient CO2 + MAR


(b) Rooting layer 7-15 cm
Volumetric soil water content (m^3 m^{-3})
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
50
100
150
200
250
300
Days elapsed since increase of watering
Elevated CO2 + 120%MAR
Ambient CO2 + 120%MAR
Elevated CO2 + MAR
Ambient CO2 + MAR

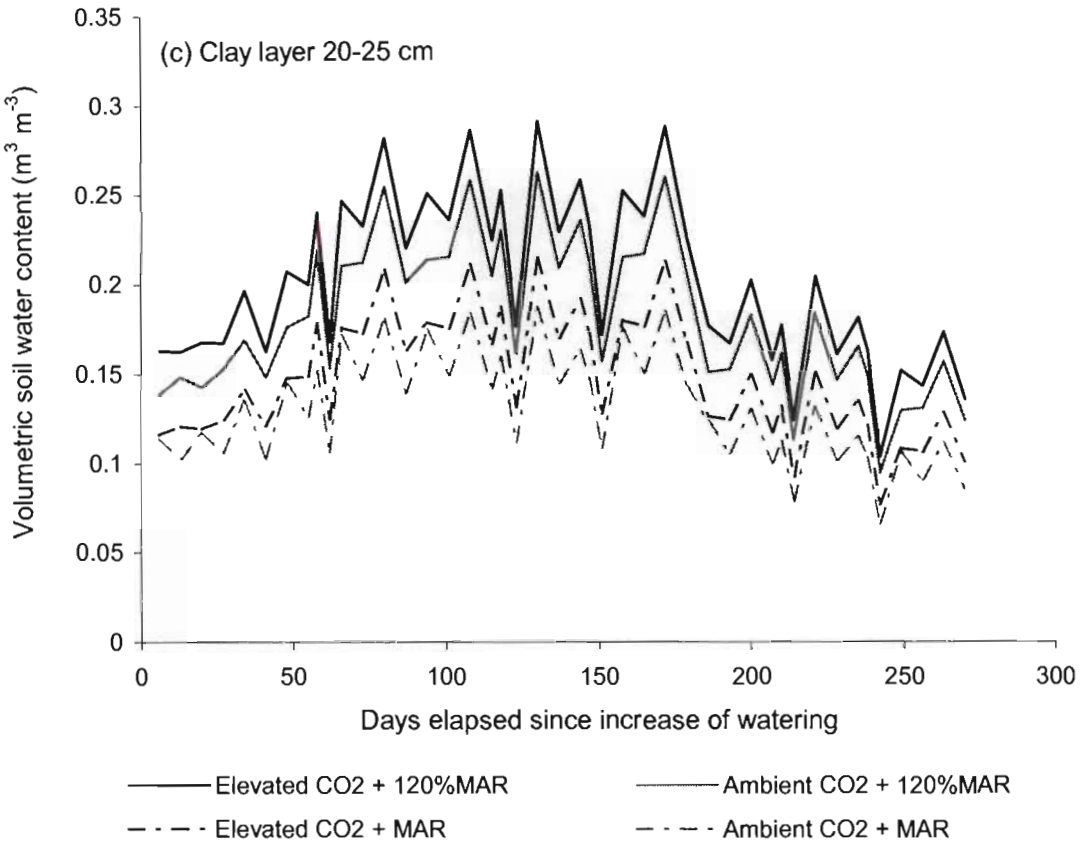


Figure 5.9 (a-c): Treatment effect on volumetric soil water content in the second year measured at different depths before a watering event.

5.3.3. Evapotranspiration in the third year

5.3.3.1 Annual cumulative evapotranspiration and water use efficiency (WUE)

The pattern of water loss that was observed in the third year was consistently similar to the pattern observed in the first and second years, which was characterised by higher cumulative evapotranspiration under ambient CO_2 compared to elevated CO_2 at similar water treatments (Figure 5.10). Elements of contrast however, were that in the third year the highest annual cumulative water loss was lower than that recorded in either the first or second years. Furthermore, differences in water loss between treatments were smaller in the third year, but nonetheless of high statistical significance with regards to main effect of CO_2 and water ($P < 0.001$), even though their interaction was not statistically significant ($P = 0.18$). Microcosms supplied with 120%MAR lost more water (in absolute units) than those supplied with MAR. However, when annual cumulative evapotranspiration was expressed relative to the total amount of water supplied, microcosms supplied with MAR lost a higher proportion of the water available to them than those receiving 120%MAR. In a further analysis annual cumulative evapotranspiration was expressed relative to total biomass produced, in order to determine WUE. MAR treatments yielded higher WUE than 120%MAR in both ambient and elevated CO_2 treatments (Table 5.5). Highest WUE among the four treatments occurred under elevated CO_2 + MAR.

Table 5.5: WUE as a ratio of the above-ground biomass produced to the total evapotranspiration.

Treatment	WUE
Elevated CO_2 + MAR	0.94 ± 0.02
Elevated CO_2 + 120%MAR	0.71 ± 0.05
Ambient CO_2 + MAR	0.73 ± 0.04
Ambient CO_2 + 120%MAR	0.68 ± 0.05

5.3.4.2 Annual cumulative evapotranspiration and soil water status

The extent of soil water accumulation was determined from changes in pot mass prior to each watering event throughout the year (Figure 5.11). The data show higher pot mass for microcosms receiving 120%MAR than microcosms receiving MAR regardless of CO_2 treatment in the earlier part of the year. The trend in the data could be attributed to both (i) annual increase in water supply and (ii) the physiological consequences of increase in water supply. An increase in pot mass that occurred due to a physiological consequence of treatment effect would be expected to be prolonged subsequent to a reduction in water supply, while an increase in pot mass that occurred due only to increased water supply would cease subsequent to a reduction in water supply. Plant pots generally showed increase in mass from day 33 since the increase in watering treatment, and remained at high mass throughout the period of high water supply. Reduction in pot mass that occurred subsequent to a reduction in water supply was first observed at day 166 in the ambient CO_2 + MAR treatment, followed by the treatment receiving ambient CO_2 + 120%MAR at day 194. Elevated CO_2-treated microcosms on the other hand retained high pot mass due to accumulation of soil water until after day 250, implying that accumulated soil water was available for a longer period to sustain physiological activity and delay senescence.

Pot mass data were subjected to a regression analysis for comparison of upper and lower limits of regression coefficients of the slopes of the graphs shown in Figure 5.11. The regression analysis was done for data points representing days 40-187 from the increase in watering treatment, and the results are shown in Figure 5.12. Statistically significant differences in the effect of treatments on pot mass were denoted by non-overlap of the regression coefficients. The effects of CO_2 treatment on soil water accumulation were significantly different at MAR but were not significantly different at 120%MAR. The results indicate that elevated CO_2 can reduce cumulative evapotranspiration of grassland microcosm communities at MAR, but the effect does not become obvious at the excess water supply of 120%MAR.

5.3.4.3 Monthly cumulative evapotranspiration

Total monthly evapotranspiration is illustrated in Figure 5.13. Overall response pattern in the first four months of the growing season depicted a higher rate of water loss under elevated CO_2 treatments relative to ambient CO_2 at similar water treatments. Thereafter, water loss under ambient CO_2 treatments increased to values higher than observed in elevated CO_2. A high initial rate of water loss under elevated CO_2 could have been due to a head start in canopy development, while a lag in canopy development was observed in microcosms exposed to ambient CO_2 at beginning of growing season. By the middle of the growing season, canopy development under ambient CO_2 was profuse, hence the observed high rates of evapotranspiration in ambient CO_2 between January and March. Generally, a steadier rate of evapotranspiration occurred in elevated CO_2 throughout the growing season, and even the start of a reduction in water loss after February was moderate, as opposed to abrupt changes in response patterns in ambient CO2-treated microcosms. For instance in ambient CO_2, there were three clearly distinct phases of evapotranspiration that could be related to stages of canopy development. The third phase of evapotranspiration in ambient CO_2 was a reduction in water loss, which occurred after March and was physiologically associated with beginning of senescence. Beginning of senescence in ambient CO_2-treated microcosms was also associated with a reduction in water supply. Senescence was delayed in elevated CO_2-treated microcosms (Chapter 3).

The statistical significance of treatment effects on monthly rates of evapotranspiration is presented in Table 5.6. Main effects of CO_2 and water treatments were highly significant in September, but their interactive effect was not significant. In the subsequent three months (October to December), effect of CO_2 treatment and its interaction with water treatment were statistically significant while effect of water treatment was statistically significant only in October. The period between January and March marked a second phase of evapotranspiration, and the main effects of CO_2 and water treatments were statistically significant, while their interaction was significant only in February. In the third phase of response during April and May, treatment main effects and their interactions were also significant, except for the CO_2 treatment in May. Recorded weekly

rates of evapotranspiration ranged from just over 0.5 kg at beginning of growing season to just under 2.5 kg by end of growing season.

Table 5.6: Statistical significance of treatment effects on total monthly evapotranspiration in the third year.

Month	Statistical significance of treatment effect		
	CO_2	Water	Interaction
September	P = 0.0013	P < 0.0001	P = 0.287 (NS)
October	P < 0.0001	P = 0.0148	P = 0.0012
November	P = 0.0005	P = 0.084 (NS)	P = 0.0419
December	P = 0.0157	P = 0.54 (NS)	P = 0.0311
January	P = 0.001	P < 0.0001	P = 0.348 (NS)
February	P < 0.0001	P < 0.0001	P = 0.019
March	P < 0.0001	P < 0.0001	P = 0.111 (NS)
April	P = 0.0021	P < 0.0001	P = 0.0461
May	P = 0.203 (NS)	P < 0.0001	P = 0.0012

5.3.4.4 Volumetric soil water content

Response patterns observed in the third year were very similar to those observed in the second year. Soil water content generally increased with soil depth in all treatments Figures 5.14: (a-c). The clay layer at the bottom of plant pots retained higher soil water than the rooting and surface layers. Treatment effects on soil water content could be described as follows: elevated CO_2 + 120%MAR induced the highest retention of soil water, while ambient CO_2 + MAR induced the lowest retention of soil water at all soil depths. Further still, a lot of similarities were observed in values of water content recorded for microcosms treated with elevated CO_2 + MAR and ambient CO_2 + 120%MAR.

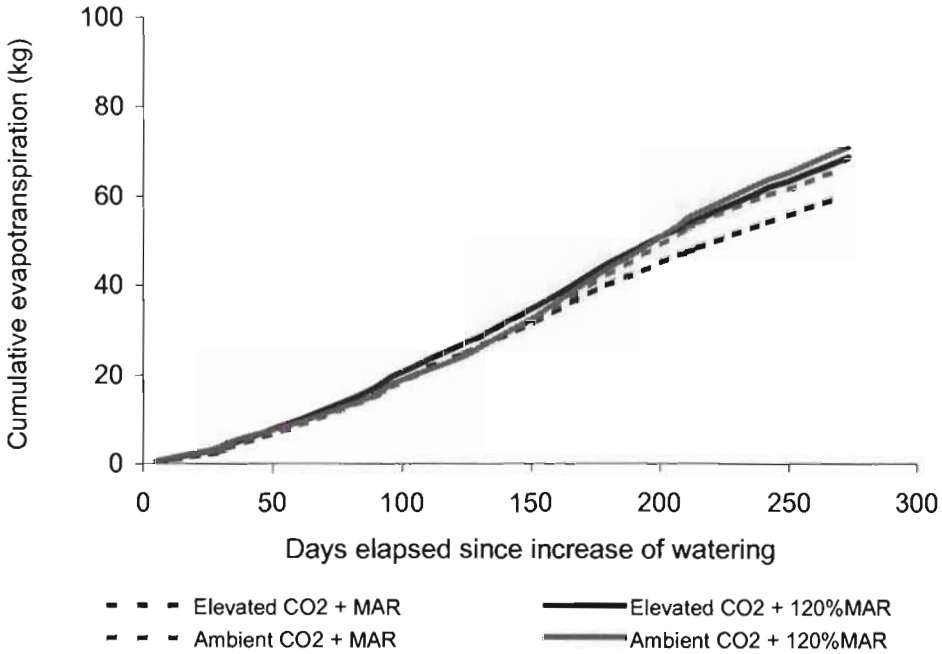


Figure 5.10 Treatment effect on cumulative evapotranspiration in the third year.

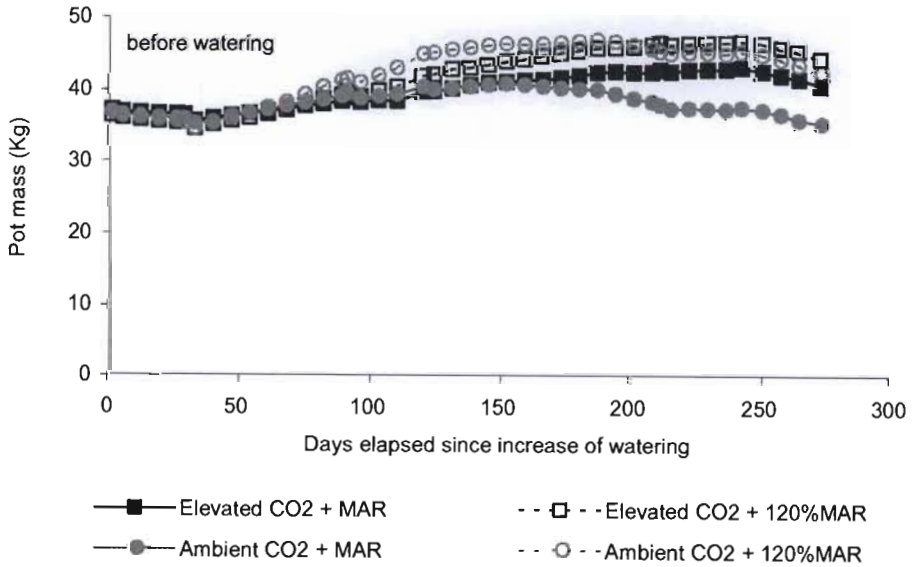


Figure 5.11: Treatment effect on change in pot mass as a consequence of soil water status in the third year.

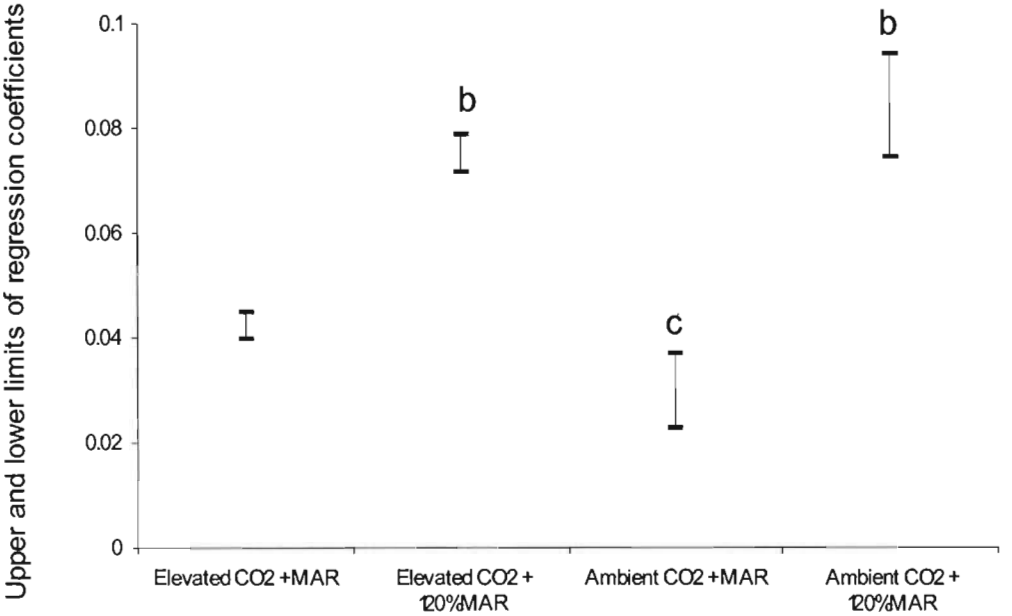


Figure 5.12: Studentized range showing 95% confidence intervals for the regression coefficients of change in pot mass for the period between days 44-151 in the third year.

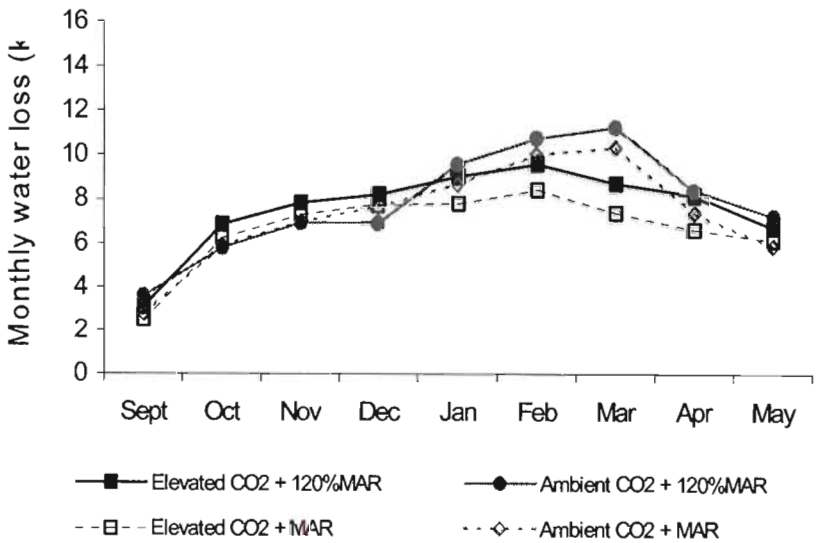
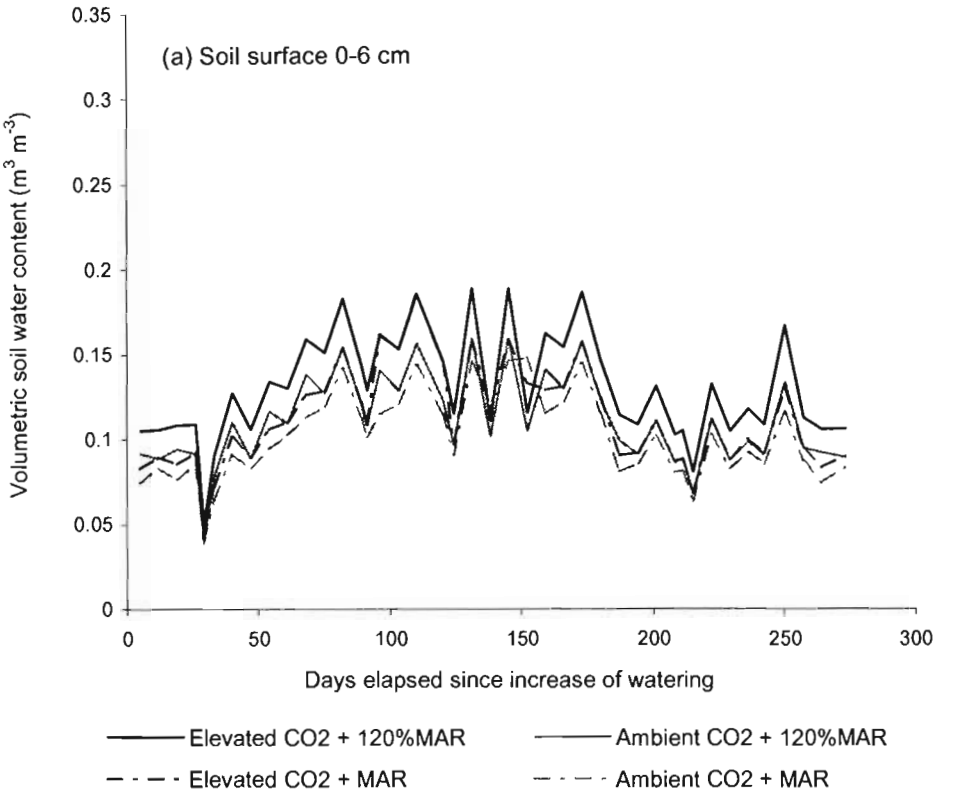
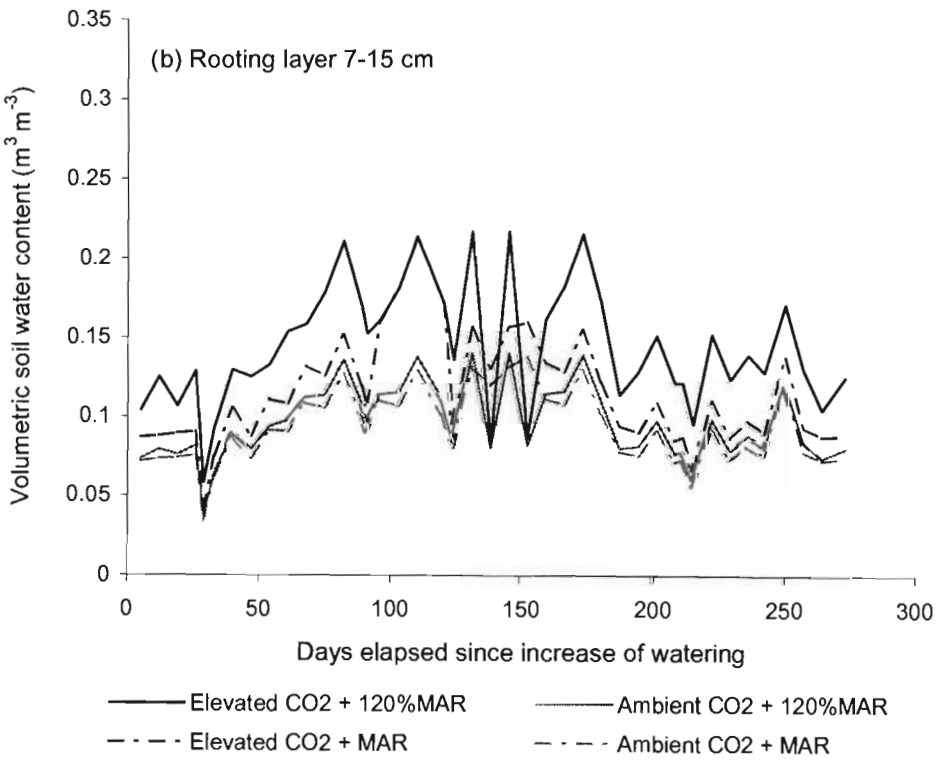


Figure 5.13: Treatment effect on monthly cumulative evapotranspiration in the third year.


(a) Soil surface 0-6 cm
Volumetric soil water content (m3 m-3)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
50
100
150
200
250
300
Days elapsed since increase of watering
Elevated CO2 + 120%MAR
Ambient CO2 + 120%MAR
Elevated CO2 + MAR
Ambient CO2 + MAR


(b) Rooting layer 7-15 cm
Volumetric soil water content (m3 m-3)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
50
100
150
200
250
300
Days elapsed since increase of watering
Elevated CO2 + 120%MAR
Ambient CO2 + 120%MAR
Elevated CO2 + MAR
Ambient CO2 + MAR

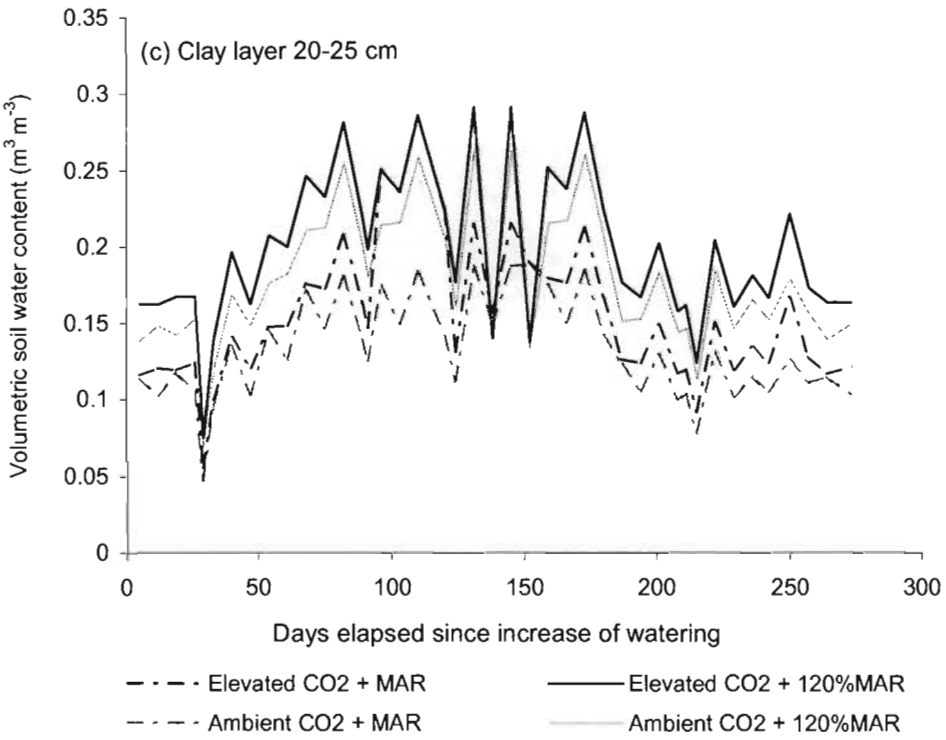


Figure 5.14 (a-c): Treatment effect on volumetric soil water content in the third year measured at different depths before a watering event.

5.4 Discussion

Three direct methods of measurement *viz.*, weekly evapotranspiration by lysimetry, long-term change in pot mass, and soil water content, were used to assess treatment effect on community water use, in an attempt to answer the key question of whether community-level water use will be changed by long-term exposure to elevated CO_2. The data obtained by all three methods support hypothesis 1 which postulated that long-term exposure to elevated CO_2 will change community-level water use. There was also some evidence that evapotranspiration responses are dependent on water supply, thus supporting hypothesis 2. When community water use data were interpreted in the light of results of the previous two chapters (Chapters 3 and 4), it became apparent that evapotranspiration responses were related to stages of canopy development, as postulated by hypothesis 3. It is important to mention that an indirect assessment of treatment effect on community water use will also be considered from gas exchange measurements of canopy water vapour fluxes in Chapter 6.

To illustrate support for hypothesis 1, it appears that long-term exposure to elevated CO_2 does change community-level water use, elevated CO_2 reduced community evapotranspiration where the highest recorded cumulative reduction was 12% under elevated CO_2 + MAR relative to a 7% reduction under elevated CO_2 + 80%MAR in the first year. In the second year, an 8% reduction in cumulative evapotranspiration was recorded under elevated CO_2 + MAR. Even though cumulative evapotranspiration was higher in the second year relative to the first year, there was more biomass produced per kg of water lost under elevated CO_2 + MAR in the second year, hence WUE was higher in that treatment. This (WUE) remained relatively unchanged in other treatments. The lowest reduction in evapotranspiration under elevated CO_2 was recorded in the third year.

Two-way ANOVA interactions serve as a powerful tool for assessing if responses to one experimental factor are dependent on another experimental factor, and in this study the interactions are particularly important for assessing if responses to CO_2 treatment are dependent on water supply because of the anticipated reduction in rainfall reliability predicted for South Africa (Ellery et al. 1991). The second hypothesis at the beginning of

this chapter states that responses of community water use to CO_2 treatment will be dependent on water supply. Analysis of evapotranspiration data showed significant treatment interactions in the first and second years but not in the third, suggesting that responses to CO_2 treatment were dependent on water supply only in the first and second year. It is speculated that the relatively lower annual cumulative water loss observed in the third year relative to first and second years, together with small differences in evapotranspiration that were observed between treatment in the third year could have resulted in lack of interaction between CO_2 and water treatments. Dependence of CO_2 responses on water supply has been reported in other studies on grasslands, with a major trend being greater effect of CO_2 response in years of low rainfall or soil water availability. In this study however, greater effect of CO_2 response was observed at MAR.

Hypothesis 3 stated at the beginning of this chapter postulates that evapotranspiration responses will be related to canopy development, and the trends in monthly cumulative evapotranspiration fully support the hypothesis. Differences in water loss were not apparent at the beginning of the growing season because surface evaporation was the predominant process of community water loss due to lack of foliage. At the beginning of each growing season microcosm communities receiving higher water supply lost relatively more water irrespective of CO_2 treatment, and in some instances effect of CO_2 treatment was not statistically significant during the first two months of application of treatment. As the growing season progressed, the rate of water loss was higher in communities with greater leaf area development, and highest levels of monthly evapotranspiration were recorded at the time of full canopy in all three years. A striking difference in monthly water loss was observed towards end of the growing season as a result of interactions of CO_2 and water treatment whereby a delay in senescence was induced.

Reduction of community evapotranspiration under elevated CO_2 is a culmination of several phenomena operating at different scales of community organisation (stomatal conductance, leaf transpiration, sap flow, energy balance etc) and sometimes logistics do not permit assessment of all of these parameters in a single study. But, analysis of data in

the literature shows trends of positive effects of elevated CO_2 on these various parameters that serve as indicators of community water use. The tallgrass prairie has been extensively studied in this regard, and reductions in stomatal conductance, canopy conductance, sap flow and evapotranspiration have been measured (Ham et al. 1995) as well as reductions in transpiration (Bremer et al. 1996). A 22% reduction in ET was measured in the tallgrass prairie relative to the 10% measured in the current study. In a model grassland community derived from the Negev in Israel, Grünzweig and Körner (2001) measured 2% reduction in ET under an elevated CO_2 treatment of 400 ppm and 11% reduction under 600 ppm.

Consequences of reduction in ET for biomass production under different treatments could be summarised as follows: firstly WUE was higher under elevated CO_2 + MAR relative to other treatments in all three years. Secondly, increases in the amount and frequency of water supply from one year to another did not instantaneously enhance WUE in all treatments; WUE was enhanced only at elevated CO_2 provided the increase in water supply did not exceed MAR. Thirdly, continued ample supply of watering as characterised by treatments in years two and three, enhanced soil water accumulation (section 5.3.3.2) without a corresponding enhancement in production.

Reduction in ET resulted in higher volumetric soil water content measured under elevated CO_2 in the current study, and the trend was further confirmed by a measurable increase in mass of plant pots due to water accumulation in the soil. Soil water content was found to increase with soil depth, hence the soil in the rooting layer was found to be on average 20% wetter than soil on the surface under elevated CO_2. Improved soil water status of 10-28% was measured in a study using grassland assemblages (Volk et al. 2000). Deep drainage has also been observed to increase under elevated CO_2 in some grassland studies as a consequence of soil water accumulation under elevated CO_2 (Jackson et al. 1998; Grünzweig and Körner 2001), especially during the wetter part of the growing season and not during the drier part of the growing season (Grünzweig and Körner 2001). In the current study, drainage loss was measured only when single water applications were in excess of the equivalent of 25mm rainfall event during the first year. Treatment effects on

drainage loss were not significant, and that parameter was subsequently not measured in the second and third years.

Effect of swapping water treatments between MAR and 120%MAR in the third year were not profound, and even a reduction in biomass production that occurred in the last year could not be attributed to such an experimental manipulation.

In conclusion, the data presented in this chapter have unequivocally shown through application of three methods of assessment, namely evapotranspiration, change in pot mass as a consequence of soil water accumulation, and measurements of soil water content, that elevated CO_2 reduces community water use. Consistent observations in that regard were made throughout three years, and evapotranspiration was reduced by approximately 12% under elevated CO_2. The balance between evaporation and transpiration seems to regulated by leaf area index to a certain extent, because under 80%MAR, there was low leaf area index hence most water was lost by evaporation through the soil surface. The data also showed that community responses of water use were dependent on water supply. Microcosms that received high water supply under both CO_2 treatments invariably underwent higher rates of evapotranspiration than microcosms receiving lower water supply. The highest response of community water use were recorded at elevated CO_2 + MAR. These results are in agreement with findings of similar studies on grassland communities in other parts of the world.

CHAPTER 6

COMMUNITY CARBON AND WATER VAPOUR EXCHANGE

6.1 Introduction

Measurement of carbon and water vapour fluxes is a requisite for quantifying community/ecosystem carbon and water balance, which in turn illustrate energy and material flow across spatial and temporal scales. The significance placed on flux measurements has increased substantially with the trend of continuous increase in concentrations of atmospheric CO_2, because of the application of these measurements to predictions of ecosystem responses and their feedbacks to global climate change (Mooney 1991; Mooney et al. 1991; Pitelka 1994).

Carbon balance of a community/ecosystem integrates all aspects of carbon metabolism, including photosynthesis, plant respiration and soil respiration. The total carbon fixed in gross photosynthesis is referred to as gross primary production (GPP), whereas GPP minus total plant respiration is net primary production (NPP). An alternative definition of NPP is the total organic matter produced over a given time interval, usually annual (Chapter 4). Descriptively, NPP constitutes the total annual above- and below-ground growth increment, together with the amount of growth lost in decomposition, herbivory, reproduction, plant death, root exudation, senescence, and volatilisation (Long et al. 1989, 1992; Roberts 1993).

Measurement of net carbon exchange (NCE) or net ecosystem production (NEP) is a non-destructive technique for estimating production, and can complement destructive methods (such as biomass harvesting) to determine production (Chapter 4). In some treatments, NCE and NEP are used interchangeably, but more often than not, NCE is used to refer to measurements of gas exchange rates over time scales of hours. NEP on the other hand is used to refer to processes, if measurements are based on ecosystem carbon exchanges measured over a minimum period of one year. Net carbon exchange of a community/ecosystem can be positive, negative or zero depending on the dynamics of carbon balance within a system. In grasslands for example, a large component of annually produced biomass tends to turn over, with a result that there are no large pools of accumulating biomass, hence NCE may be

somewhat balanced. However, if carbon sequestration occurs, the build up in soil carbon can turn the system into a carbon sink. On the other hand, occurrence of disturbance such as fire lead to loss of accumulated carbon sinks, thus causing the carbon balance of the system to become negative, which makes the system a carbon source. Systems that undergo recurring disturbance would therefore have a carbon balance characterised by a series of peaks and troughs as the dynamics change from positive to negative or sink to source.

Early experimental investigations on response of C_3 vs. C_4 communities to CO_2 enrichment in natural grass vegetation have been undertaken in the C_3 tussock tundra (Grulke et al. 1990), in C_3 and C_4 monospecific stands in the salt marsh (Drake and Leadley, 1991), and in the C_4 tallgrass prairie (Ham et al. 1993). Expectations were that communities would respond along predictions based on differences in photosynthetic pathways, whereby C_3 species would constitute a stronger sink in their respective communities compared to C_4 species. Initial findings from C_3 tussock tundra studies indicated that elevated CO_2 induced a negative annual carbon balance. However, recent findings from the tussock tundra indicated a previously undemonstrated capacity for that ecosystem to adjust to decade long changes in climate by acting as a net sink for atmospheric CO_2 during the summer growing season, yet remaining a source on an annual basis (Oechel et al. 2000). The response mechanism was attributed to adjustment at different levels (plant, soil, microbial, and whole-ecosystem) including nutrient cycling, physiological acclimation, and population and community reorganisation. In the wild C_3/C_4 salt marsh ecosystem, elevated CO_2 significantly increased net carbon exchange of the C_3 community components, but had much less effect in the C_4 community components (Drake and Leadley, 1991). The positive response of the C_3 community was further supported by a modelling simulation (Rasse et al. 2003). In the C_4-dominated tallgrass prairie, elevated CO_2 positively enhanced net carbon exchange only when water was limiting (Ham et al. 1993). In a C_3 annual grassland, Freeden and co-workers (1995) reported increased net ecosystem CO_2 uptake under elevated CO_2, but the capacity of the response was reduced by acclimation due to a decrease in rubisco activity.

Most of these early studies on community fluxes were carried out in open-top chambers. Subsequent research in other ecosystems has predominantly employed the

eddy correlation technique as part of the long term ecological monitoring of climate change impacts.

Measurement of community water vapour fluxes is important for determination of community/ecosystem water balance. In Chapter 5, community ET was measured by lysimetry, and the data were interrelated with measurements of soil water status (change in pot mass as a consequence of soil water accumulation) to estimate water balance of the microcosm communities. In the work reported in the current Chapter ET was measured by flux exchange of water vapour from the canopy, the advantage being that shorter time scale mechanism of water savings is revealed. The use of several methods for assessment of community water use in this study was found necessary to gain confidence in the results, taking into consideration the importance attached to understanding effects of elevated CO_2 on community water use and its implications for community production. Effect of elevated CO_2 on ET of grasslands is attributed to reduction in stomatal conductance (g_s), and effect of g_s on ET under elevated CO_2 is recognised as the second most responsive parameter after photosynthesis (Field et al. 1995). Exhaustive studies of community water vapour flux that have influenced the current scientific dogma on effects of elevated CO_2 on ecosystem ET were undertaken in the C_4-dominated tallgrass prairie. Results of these studies showed a 22% reduction in daily ET under elevated CO_2 (Ham et al. 1995) and a 50% reduction in stomatal conductance (Owensby et al. 1997), and therefore tying in with other work (Wand et al. 2001) that shows a reduction in g_s at the leaf level.

Carbon and water vapour flux responses to elevated CO_2 are more readily comprehended at the leaf level than they are at the canopy level, because of the complexities of the canopy boundary layer and light regime. Such complexities occur because each leaf in a canopy modifies the environment of adjacent leaves through reduced irradiances, wind speed, and vapour pressure deficit. Furthermore, canopy fluxes are generally greater than the sum of fluxes of individual leaves due to contributions of the rhizosphere. As a result, carbon and water vapour fluxes of vegetation canopies cannot be adequately predicted from the study of individual leaves. The open-top chamber technique is widely used for canopy flux studies (Drake and Leadley, 1991, Grulke et al. 1990, Ham et al. 1995). Nonetheless,

influence of chamber microclimate conditions cannot be overlooked. Measurement of chamber microclimate conditions performed during experimental set-up indicated an increase of 3-5 °C in air temperature within the canopy, more than 5% reduction in PAR compared with conditions outside the greenhouse (Section 2.5). Nonetheless, Jones et al. (1985) previously suggested that physiological responses to elevated CO_2 are often not sensitive to temperature changes less than 5 °C (Jones et al. 1985). Besides, a similar effect of chamber microclimate was prevalent in all microcosms in this study.

The main objectives of undertaking measurements of canopy carbon and water vapour exchange are to investigate whether (i) South African C_4-dominated grassland communities can increase CO_2 uptake under elevated CO_2 (ii) whether their water use will be reduced, and WUE will change under elevated CO_2 and (iii) whether the response patterns in (i) and (ii) above relate to water input and subsequent canopy development/LAI.

6.2 Materials and methods and data analysis

6.2.1 Materials and methods

Monthly measurements of day-long (diel) community gas exchange were performed on four pots of each of the four treatments, at intervals of one hour beginning at about 7:00 a.m. and ending at about 5:00 p.m. A Li-Cor 6262 CO_2/H_2O IRGA was used in differential mode to record CO_2 and water vapour fluxes. The polycarbonate chambers on the plant pots were fitted with detachable polycarbonate chimneys during measurement. Four chimneys at a time were fitted to four open-top chambers attached to four replicate microcosms. Two manifolds made of tubing and four plastic taps (Festo (Pty) Ltd. Durban, South Africa) were connected each to the reference and sample air inlets of the IRGA. Each manifold opened into four long pieces of tubing. The tubing coming out of the "reference air" manifold were connected through small ports at the base of four risers (Figures 2.1.b and c) supplying ambient or elevated CO_2 air to each of four replicate microcosms. The tips of tubing connected to the "sample air" manifold sampled air from 5 cm below the top of exit chimneys on the open-top chambers. Gas exchange of four replicate microcosms was measured within 15 minutes by manually recording ten intermittently random readings of differentials of CO_2 and water vapour exchange. Thereafter, the chimneys and manifolds were

cycled to another set of treatment replicates to take measurements. It took approximately 15 minutes to perform measurements on four replicates per treatment, which was sufficient to complete 16 hourly measurements for all treatments. Hourly readings of PAR inside the greenhouse were also recorded along with flux measurements.

6.2.2 Data analysis

Fluxes of carbon and water vapour per unit ground area of microcosms were calculated from recorded differential values of CO_2 and H_2O:-

$$\text{Carbon flux} = (\text{flow rate} \times \Delta CO_2)/\text{microcosm ground area}$$

$$\text{ET} = (\text{flow rate} \times \Delta H_2O)/\text{microcosm ground area}$$

Flow rate in the chambers was maintained at about $0.381 m^3 min^{-1}$ in order to enable three changes of air per minute ($381 l min^{-1}$ which was equivalent to $0.283 mol sec^{-1}$). The ground area of the microcosms was $0.159 m^2$. ΔCO_2 values were recorded in umol/mol, and ΔH_2O values were recorded in $mmol mol^{-1}$. Daily time course response curves of carbon and water vapour fluxes were determined for each of the four replicate treatments. Subsequently, the area under each replicate response curve per treatment was calculated by integration to produce four replicate daily estimates of carbon and water vapour fluxes per treatment. Daily estimates were considered as representative of monthly estimates, and the monthly estimates were plotted to produce an annual time course of community fluxes. Statistical significance of treatment effects on the monthly and annual estimates of canopy fluxes was tested by two-way ANOVA. Also, an annual time course of photosynthetic efficiency was determined for each treatment as the quotient of total CO_2 assimilated during the measurement period in each month and total incident PAR for that period.

Dark respiration rate on any measurement day was determined as the mean value of respiratory fluxes indicated as negative assimilation on the diel response curve. Total daily respiratory flux was determined by integrating the mean value of dark respiration rate over the total number of hours of "no light". The analysis does not take into consideration the differences in dark respiration rate during the day and

night that arise as a result of differences in temperature. Nonetheless, extreme fluctuations in temperature were minimised through-out the experiment because the microcosm set-up was housed under controlled greenhouse conditions. Ultimately, total annual respiratory fluxes were compared with total carbon assimilation, in order to estimate the carbon balance of the microcosms for the duration of each year.

6.3 Results

6.3.1 Community fluxes in the second year

6.3.1.1 Carbon flux

The diel response of community carbon exchange was characterised by low rates of carbon fixation in the first three hours of measurement (7:00-9:00 a.m.), followed by a steady increase in carbon fixation as PAR increased (Figure 6.1). A daily maximum rate of carbon fixation was observed to occur between 12:00 noon and 14:00 p.m., followed by a reduction in assimilation as PAR decreased. Differences in community responses due to treatment effects were least apparent during the time of the day when PAR was low, and were highest around mid-day when PAR was high. Therefore a comparison of A_{max} was one of the rational ways of assessing treatment effect on net carbon exchange. Figure 6.2 shows that low values of maximum net carbon exchange were recorded at the beginning of the growing season and towards the end of the growing season. The reason for low values of A_{max} at the beginning of the growing season was that there was low LAI, and senescence at the end of the growing season led to a reduction in carbon fixation. Typical values of A_{max} at the beginning of the growing season ranged between 1.8 and 3.4 μmol $m^{-2}s^{-1}$. There was a distinct difference in A_{max} due to CO_2 and water treatment as well as their interaction at 95% level (Table 6.1). Treatment interactions were not statistically significant at the beginning of full canopy development during December and January, even though differences in A_{max} during that period of rapid growth were even greater in absolute values. Peak photosynthetic activity of the communities was measured in February, and high rates of net carbon exchange were measured in all treatments. Rates of carbon fixation at peak season ranged between four to seven-fold relative to the beginning of the growing season, and the highest relative increase occurred under ambient CO_2 treatments. That observation implies that microcosms exposed to ambient CO_2 fixed a large amount of carbon only for a limited period of about four

weeks at peak season, while more steady rates of carbon fixation occurred under elevated CO_2 consistently throughout peak season. Differences in response to treatments were not so pronounced during the peak season, relative to the preceding stage of canopy development. In the later phase of growth, lower rates of net carbon exchange were measured in all treatments, but still the rates of carbon exchange measured at the end of the growing season were still approximately twice as high as the rates measured at the start of the growing season.

Table 6.1: Statistical significance of treatment effect on maximum rate of community carbon exchange (A_{max}) in the second year.

Month	Statistical significance of treatment effect		
	CO_2	Water	Interaction
October	$P < 0.001$	$P < 0.001$	$P < 0.001$
November	$P < 0.001$	$P < 0.001$	$P = 0.0019$
December	$P < 0.001$	$P < 0.001$	$P = 0.36$ (NS)
January	$P < 0.001$	$P < 0.001$	$P = 0.0057$
February	$P < 0.001$	$P = 0.0005$	$P < 0.001$
March	$P < 0.001$	$P < 0.001$	$P < 0.001$
April	$P < 0.001$	$P = 0.207$	$P < 0.001$
May	$P < 0.001$	$P < 0.001$	$P < 0.001$

Effect of time of year on A_{max} was analysed by a three-way ANOVA, where all data were pooled to three factors namely, month, CO_2 treatment, and water treatment. Results of the three-way ANOVA showed a significant effect of "month" at 95% level (Table 6.2). A multiple comparison test was performed to assess differences in A_{max} recorded in different months. Table 6.3 shows results of the multiple comparison test, and it emerges that the biggest differences in A_{max} became apparent during the months of full canopy development, *viz.*, December, January, February, and March.

Table 6.2: Results of a three-way ANOVA for CO_2 x water x month on A_{max} at the $\alpha = 0.05$ level, in the second year.

Due To	Sum of Squares	DoF	Mean Square	F-Stat	Signif
Main Effects	969610.150	9	107734.461	22830.136	0.0000
Month	898850.542	7	128407.220	27210.925	0.0000
CO2	67629.225	1	67629.225	14331.389	0.0000
Water	3130.383	1	3130.383	663.363	0.0000
2 Way Interactions	20141.722	15	1342.781	284.550	0.0000
Month × CO2	13366.210	7	1909.459	404.636	0.0000
Month × Water	4697.000	7	671.000	142.192	0.0000
CO2 × Water	2078.513	1	2078.513	440.460	0.0000
3 Way Interactions	3795.777	7	542.254	114.910	0.0000
Month × CO2 × Water	3795.777	7	542.254	114.910	0.0000
Explained	993547.650	31	32049.924	6791.737	0.0000
Error	453.020	96	4.719		
Total	994000.670	127	7826.777		

Data from the diel responses was integrated to yield estimates of daily net carbon exchange in units of mmol $m^{-2}d^{-1}$ on a annual time course (Figure 6.3). The trend in daily carbon flux of the different months was very similar to the pattern observed for the trend in A_{max} during the course of the growing season.

Annual photosynthetic light use efficiency (LUE) (Figure 6.4) reached a peak between January and March. The duration of peak phase lasted longer in elevated CO_2 treatments relative to a more brief peak phase in ambient CO_2. Differences in LUE between treatments were less pronounced in the first two months of measurement, possibly as a result of a slow rate of leaf biomass development rather than limiting PAR. Differences between treatments were not statistically significant ($P > 0.05$) during the first two months of measurement, as well as during February.

Differences in dark respiration rate were observed during December to April (Figure 6.5). Respiratory fluxes were particularly high under elevated CO_2 + 120%MAR, relative to other treatments. The integrated data on CO_2 assimilation rate and dark respiration of different months was ultimately summed to yield estimates of annual net carbon exchange of microcosms under different treatments (Figure 6.6), for the period of 273 days that the experiments were conducted. Overall, respiratory loss accounted for 30% of assimilated CO_2 during the period of active canopy

development. Hence, microcosm communities in both ambient and elevated CO_2 served as sinks of for atmospheric CO_2 during all phases of active canopy development. Gas exchange measurements were not undertaken during the dormant phase. Even though respiration would continue to occur after senescence, a limitation would ensue as a result of reduced water supply during the dormant phase of the year. The data also showed a marked difference of about 20% in annual net carbon exchange due to CO_2 treatment, but the differences due to water treatment within ambient and elevated CO_2 groups were not significant.

Table 6.3: Tukey-HSD multiple comparison test for integrated daily values of rate of community carbon exchange in different months of the growing season, measured in the second season for all treatments combined, and data classified by month at the 95% significance level.
* denotes significantly different pairs. Vertical bars show homogeneous subsets.

Month	Cases	Mean	October	May	April	November	December	March	January	February
October	16	48.5250		*	*	*	*	*	*	*
May	16	89.1375	*				*	*	*	*
April	16	107.0625	*				*	*	*	*
November	16	116.4063	*				*	*	*	*
December	16	171.1563	*	*	*	*		*	*	*
March	16	227.3063	*	*	*	*	*			*
January	16	245.7188	*	*	*	*	*			*
February	16	308.9000	*	*	*	*	*	*	*	

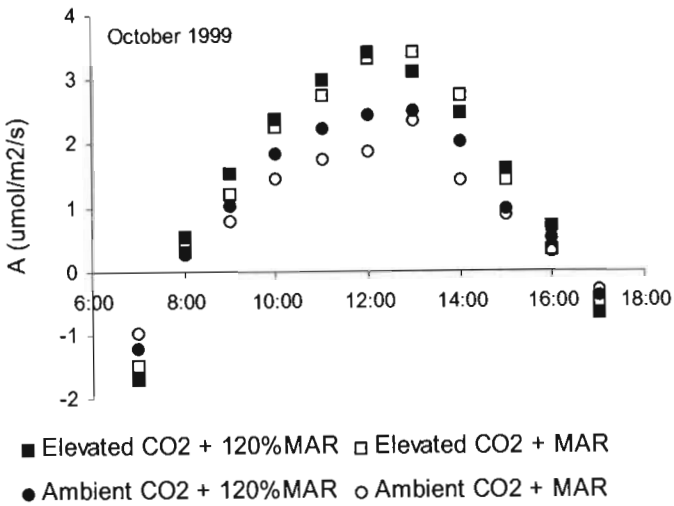


Figure 6.1: Typical diel response of community net carbon exchange per unit ground area of microcosm, shown for the first month of measurement in the second year.

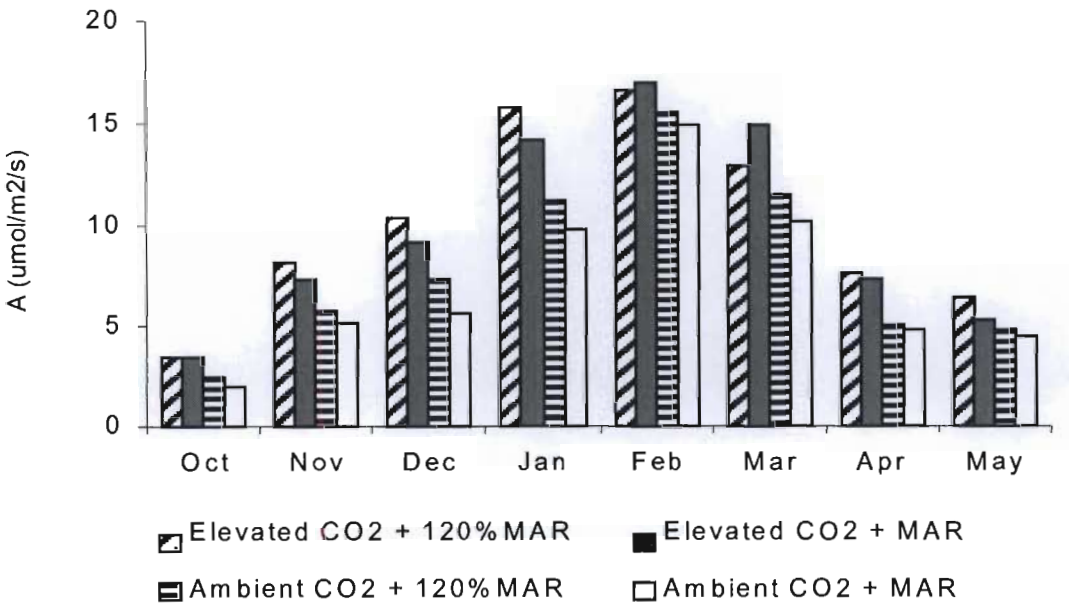


Figure 6.2: Treatment effect on values of maximum rate of net community carbon exchange (A_{max}) per unit ground area of microcosm, in the second year.

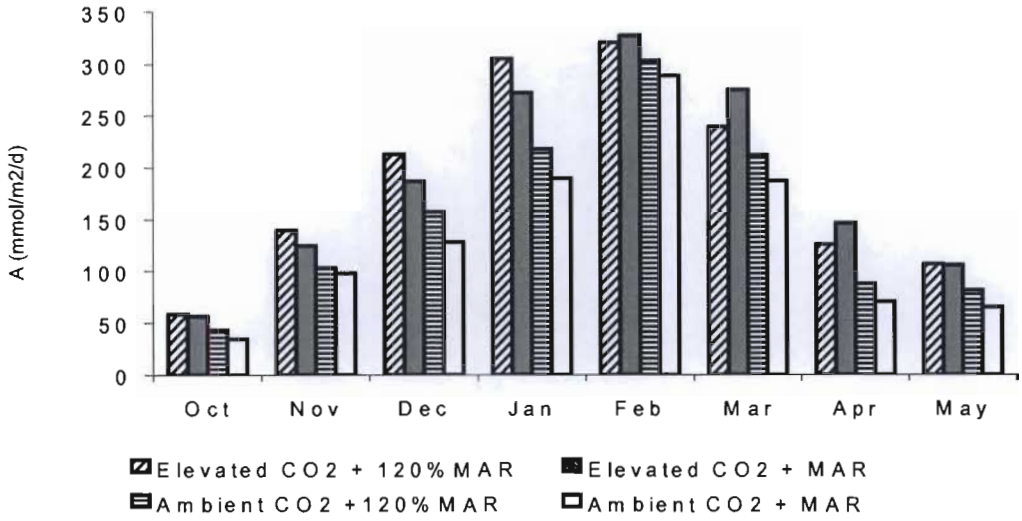


Figure 6.3: Integrated daily net carbon exchange (mmol $m^{-2}d^{-1}$) per unit ground area of microcosm, on an annual time course in the second year.

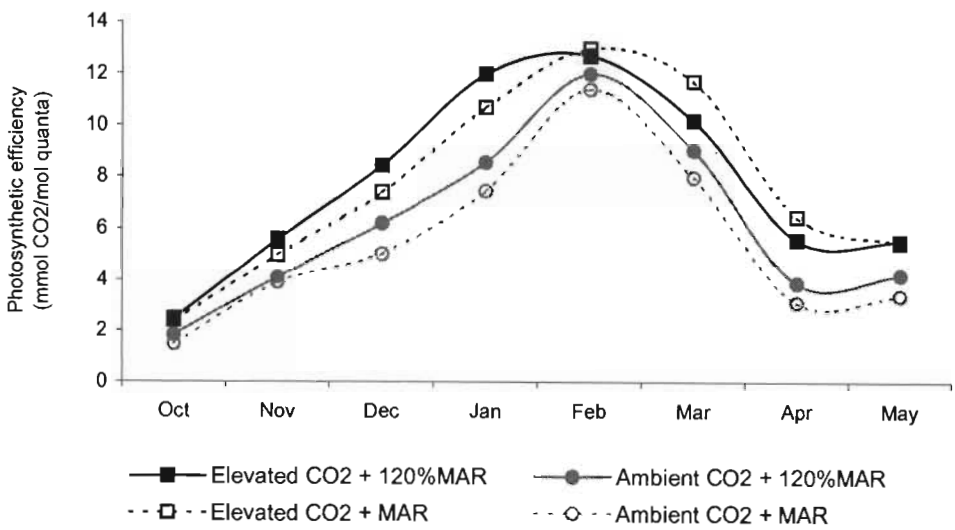


Figure 6.4: Treatment effect on photosynthetic efficiency (mmol CO_2 mol^{-1} quanta) in the second year.

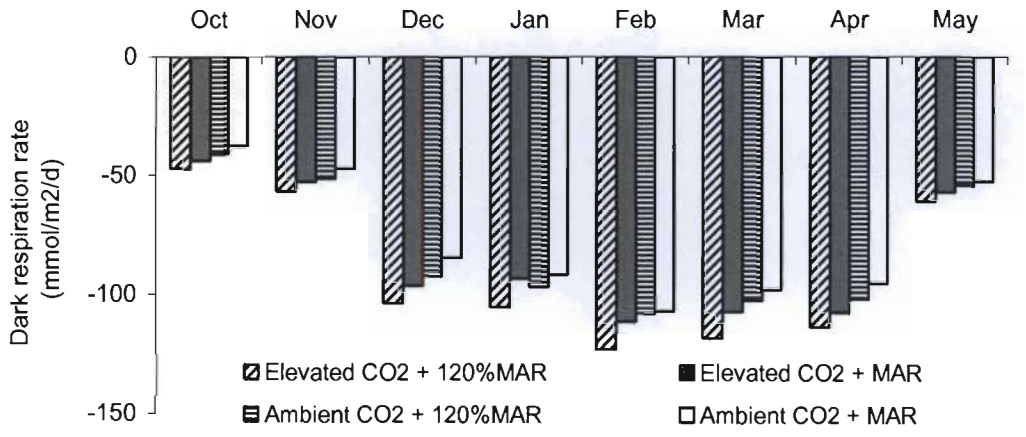


Figure 6.5: Treatment effect on annual course of respiratory flux (mmol $m^{-2}d^{-1}$) per unit ground area of microcosm, in the second year.

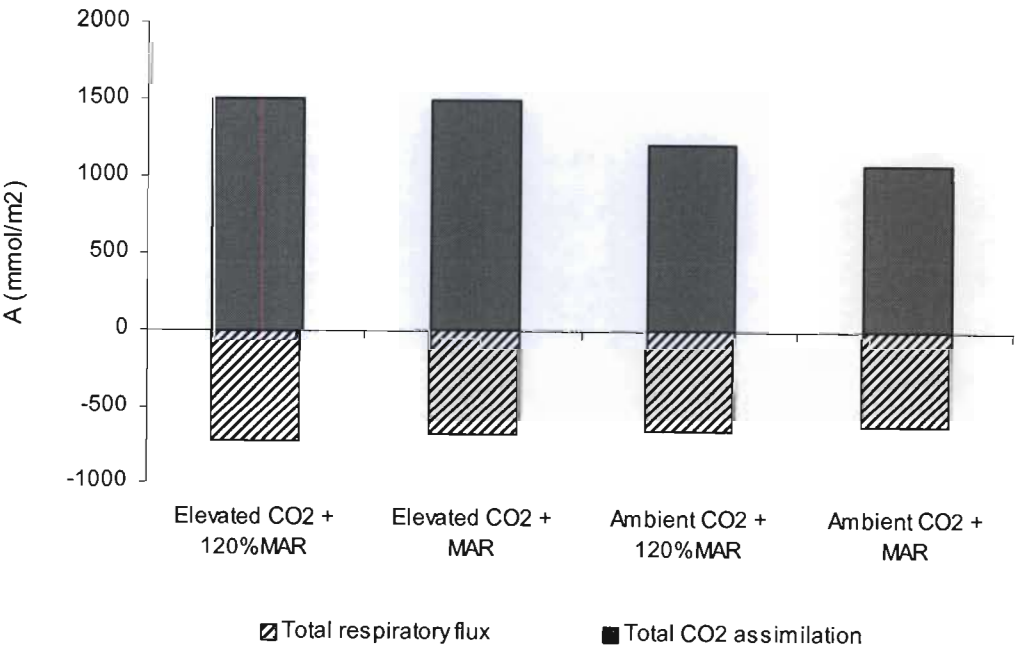


Figure 6.6: Treatment effect on integrated annual carbon exchange (mmol m^{-2}) per unit ground area of microcosm, in the second year.

6.3.1.2 Water vapour flux

Trends in diel water vapour flux depicted low rates of ET in the morning and in the afternoon, and the highest rate of water loss was reached by mid-day for the most part of the growing season (e.g. Figure 6.7.). Rate of ET before noon seemed to be slightly higher than rate of ET in the afternoon. The biggest differences in water loss between treatments occurred during the time of day when ET was highest.

An annual time course of integrated daily rates of ET showed relatively low rates of water loss in the first two months of the growing season (Figure 6.8), characterised by very small differences, that were nonetheless statistically significant at 95% level (Table 6.4), except for the effect of water treatment during November. The low rate of ET at the beginning of the growing season (October and November) was about 50% less than rate of water loss at the end of the growing season (April and May). However, the amount of watering applied in the first two months of the growing season (October and November) was about one and a half times higher than amount of watering applied in the last two months of annual measurement (April and May). It is postulated that the high rate of ET observed at end of the growing season despite low amount of watering applied occurred because there was a substantial amount of water in the soil at end of the growing season, as well as greater canopy area at the end of the growing season. Even though senescence was starting to take place by April, hence reducing the proportion of leaf area actively contributing to transpirational water loss, a fair proportion of the water that was conserved during the course of the growing season could still be lost by evaporation from the soil surface. The highest rates of daily integrated ET were recorded in January, during the time of peak net carbon exchange. Effect of treatments on daily annual trend in ET was summarised as higher ET under ambient CO_2 treatments relative to elevated CO_2, and also as higher in microcosms receiving 120%MAR than MAR.

Table 6.4: Statistical significance of treatment effect on once a month measurements of water vapour flux integrated over a day.

Month	Statistical significance of treatment effect		
	CO_2	Water	Interaction
October	$P < 0.001$	$P < 0.001$	$P < 0.001$
November	$P < 0.001$	$P = 0.52$	$P < 0.001$
December	$P < 0.001$	$P < 0.001$	$P < 0.001$
January	$P < 0.001$	$P < 0.001$	$P = 0.0057$
February	$P < 0.001$	$P < 0.001$	$P < 0.001$
March	$P < 0.001$	$P < 0.001$	$P < 0.001$
April	$P < 0.001$	$P < 0.001$	$P < 0.001$
May	$P < 0.001$	$P < 0.001$	$P < 0.001$

Data on ET was pooled for all treatments and all months of the growing season to generate three factors, *viz.*, CO_2, water and month. A three-way ANOVA was applied to pooled data to assess whether differences in ET that were observed in different months were statistically significant. Results of the three-way ANOVA showed a significant effect of "month", CO_2 and water at 95% level (Table 6.5).

Table 6.5: Results of a three-way ANOVA for CO_2 x water x month on ET at the $\alpha = 0.05$ level, in the second year.

Due To	Sum of Squares	DoF	Mean Square	F-Stat	Signif
Main Effects	46684.110	9	5187.123	70309.049	0.0000
Month	41121.966	7	5874.567	79627.022	0.0000
CO2	3694.776	1	3694.776	50080.970	0.0000
Water	1867.369	1	1867.369	25311.318	0.0000
2 Way Interactions	2154.456	15	143.630	1946.844	0.0000
Month × CO2	1186.912	7	169.559	2298.292	0.0000
Month × Water	830.799	7	118.686	1608.728	0.0000
CO2 × Water	136.744	1	136.744	1853.508	0.0000
3 Way Interactions	251.456	7	35.922	486.910	0.0000
Month × CO2 × Water	251.456	7	35.922	486.910	0.0000
Explained	49090.022	31	1583.549	21464.273	0.0000
Error	7.083	96	0.074		
Total	49097.105	127	386.591		

A multiple comparison test was performed to find out how ET differed among months of the growing season. Table 6.6 shows results of the multiple comparison test, which categorises responses of ET into three groups. The three categories can be conveniently described as low, intermediate, and high. Low rates of ET occurred at the beginning (October and November) and end of the growing season (May) while high rates of ET occurred at peak season (January and February). Intermediate rates of ET were observed at transitional stages of canopy development, from beginning of the growing season to peak season (December) and from peak season to end of the growing season (March and April).

Annually integrated ET was highest under ambient CO_2 + 120%MAR, followed by ambient CO_2 + MAR, then elevated CO_2 + 120%MAR, and was least under elevated CO_2 + MAR (Figure 6.9).

Community flux data were further analysed by calculating the ratio of community carbon assimilation to community water loss of the annual integration and annual totals, in order to estimate seasonal and annual WUE. Overall, annual WUE was highest at the beginning and at the end of growing season, and was lowest in the middle of the growing season when the canopy was undergoing high rates of physiological activity (Figure 6.10). The between treatment analysis showed significantly higher WUE in elevated CO_2 relative to ambient CO_2. The among treatment analysis indicated smaller differences due to water treatment under elevated CO_2 at the beginning of the growing season, and the differences became larger towards the end of the growing season, with higher WUE observed at MAR than 120%MAR towards the end of the growing season in elevated CO_2. With regard to microcosms exposed to ambient CO_2, there were also no differences between watering treatments in WUE at the beginning of the growing season and further still no differences in WUE at the end of the growing season. The only differences in WUE observed in ambient CO_2 occurred at full canopy development stage.

As expected, annual WUE was characterised by a higher trend in elevated CO_2 than ambient CO_2 (Figure 6.11). The interesting aspect of the trend was that difference due to water treatment among CO_2 levels was greater in elevated CO_2 than in ambient CO_2 (significant interaction). Furthermore, communities receiving MAR in elevated

CO_2 had a higher WUE than communities exposed to 120%MAR in elevated CO_2. Also, communities exposed to elevated CO_2 + 120%MAR had a higher WUE than those exposed to ambient CO_2 + MAR.

Table 6.6: Tukey-HSD multiple comparison test for for integrated daily values of rate of community water vapour flux in different months of the growing season, measured in the second season for all treatments combined, and data classified by month at the 95% significance level.
* denotes significantly different pairs. Vertical bars show homogeneous subsets.

Group	Cases	Mean	October	November	May	April	December	March	February	January
October	16	12.7188				*	*	*	*	*
November	16	13.2688				*	*	*	*	*
May	16	20.5250				*	*	*	*	*
April	16	37.3000	*	*	*				*	*
December	16	45.3375	*	*	*				*	*
March	16	45.5313	*	*	*				*	*
February	16	57.8063	*	*	*	*	*	*		
January	16	60.9063	*	*	*	*	*	*		

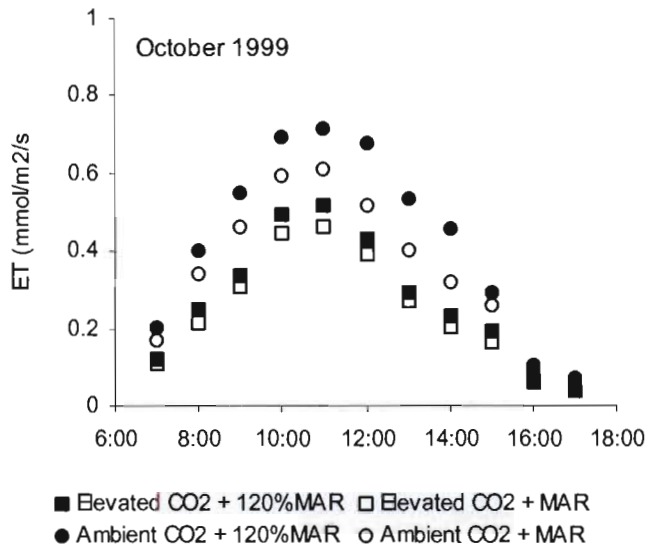


Figure 6.7: Typical diel response of community ET per unit ground area of microcosm, shown for the first month of measurement in the second year.

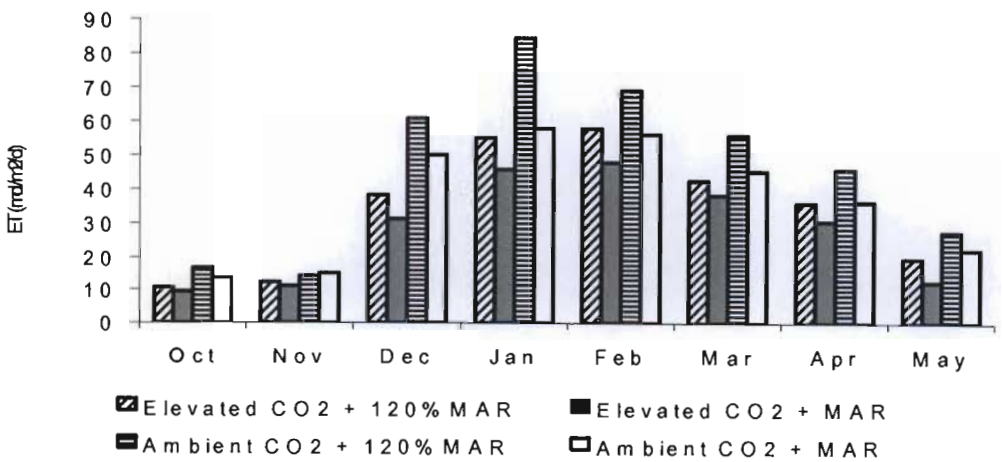


Figure 6.8: Integrated daily community ET (mol m^{-2} d^{-1}) per unit ground area of microcosm, on an annual time course in the second year.

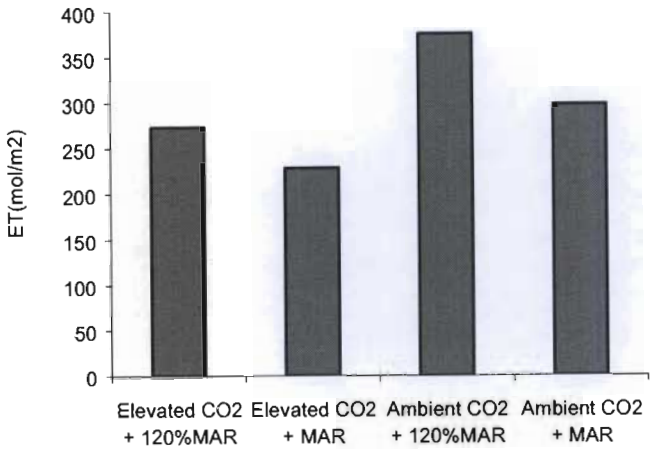


Figure 6.9: Treatment effect on integrated annual community ET (mol m^{-2}) per unit ground area of microcosm, in the second year.

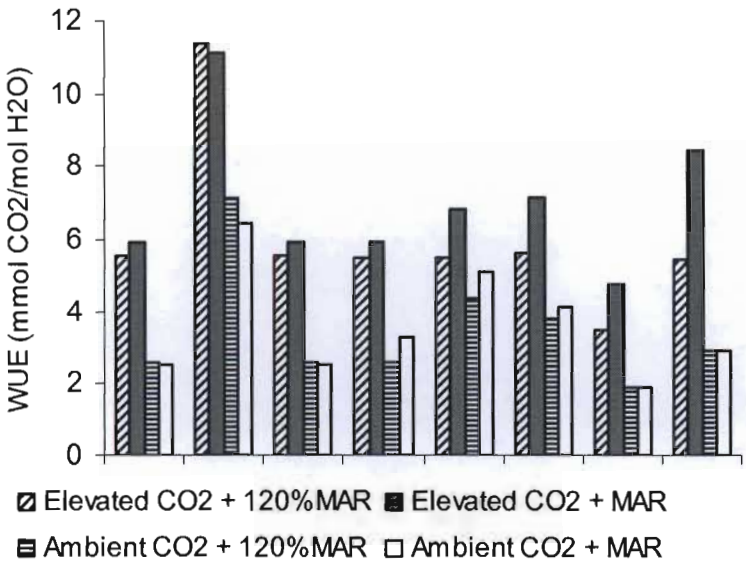


Figure 6.10: Treatment effect on community WUE on an annual time course in the second year.

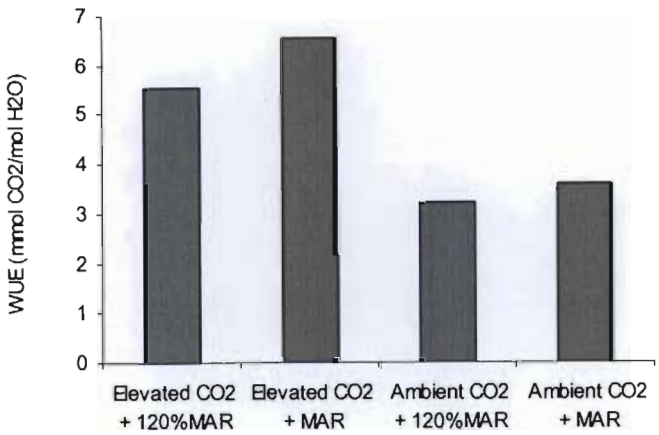


Figure 6.11: Treatment effect on an annual estimate of community WUE in the second year.

6.3.2 Community fluxes in the third year

The diel and monthly integrated trends of community carbon and water vapour flux of the third year were found to be very similar to the trends depicted in the figures presented in sections 6.3.1.1 and 6.3.1.2. This is simply because the same treatments were applied during the second and third years, with a slight modification of swapping the water treatments. To avoid redundancy, data that are very similar to that which has been presented in the previous sections will not be shown. However, interesting differences in response were noted with regards to annually integrated fluxes of carbon and water vapour, despite the similarity in treatments.

A reduction of about 15-19% in annually integrated net carbon exchange occurred during the third year relative to the second year in microcosms subjected to elevated CO_2 treatment, but no reduction in net carbon exchange was observed in ambient CO_2 (compare Figure 6.12 and Figure 6.6). Lower rates of CO_2 assimilation in the third year suggest a less efficient use of PAR per unit ground area in the third year relative to the second year. Rates of dark respiration in the third year were similar to respiratory fluxes of the second year, and as a result the sink potential of the microcosms was reduced (Figure 6.12). Furthermore, the observed reduction in net

carbon exchange under elevated CO_2 may explain some of the changes in above-ground biomass production that were described for third year production in Chapter 4. There was however, no major difference in net carbon exchange data of the second and third years under ambient CO_2, and that result does not help to explain a reduction in above-ground biomass that occurred across treatments in the third year.

The response pattern of ET in the third year was generally similar to the response pattern of the second year. The difference though, was that absolute quantities of ET were lower by about 8% in the third year (Figure 6.13). Incidentally, the lysimetry data of the third year also indicated lower rates of ET (Chapter 5). When a ratio of annual carbon flux to annual water vapour flux of the third year was calculated, the values indicated a reduction in WUE of microcosms subjected to elevated CO_2 treatment, and a slight improvement in the WUE of microcosms subjected to ambient CO_2 treatments (Figure 6.14).

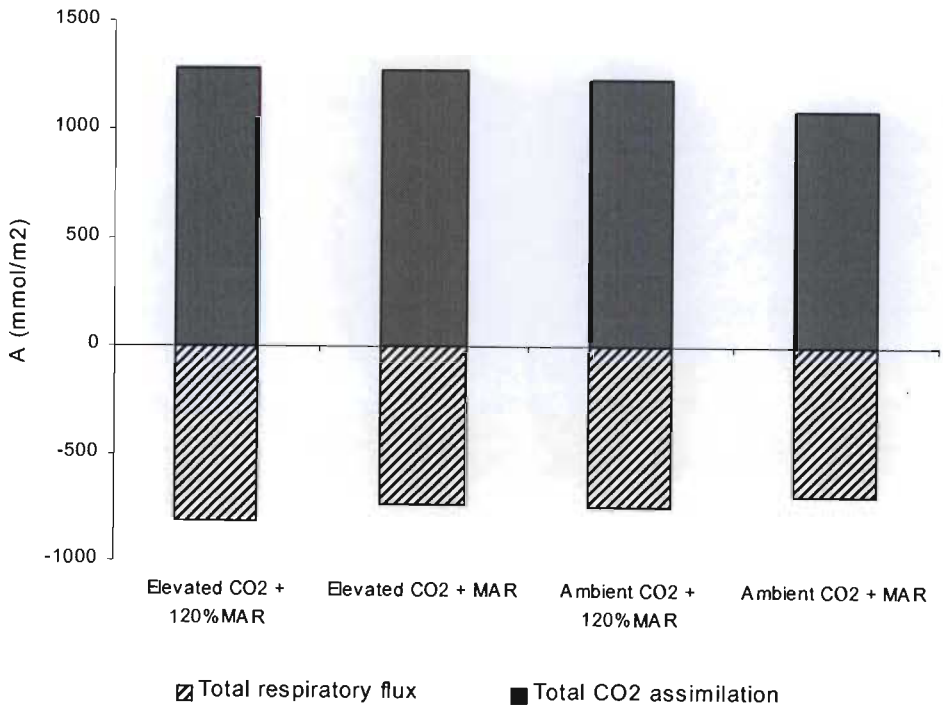


Figure 6.12: Treatment effect on integrated annual carbon exchange (mmol m^{-2}) per unit ground area of microcosm, in the third year.

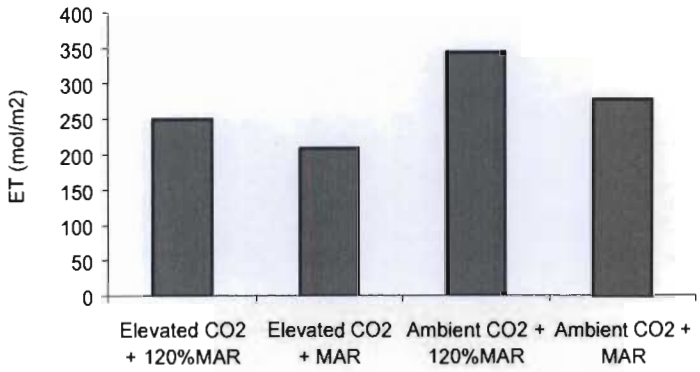


Figure 6.13: Treatment effect on integrated annual ET (mol m^{-2}) per unit ground area of microcosm, in the third year.

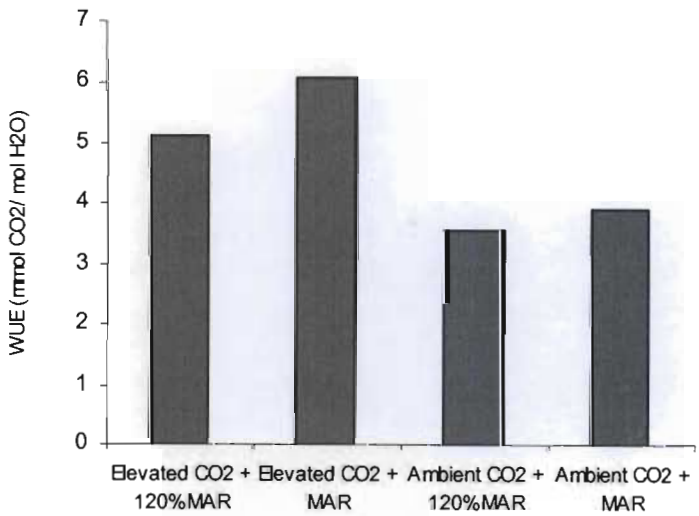


Figure 6.14: Treatment effect on an annual estimate of WUE in the third year.

6.4 Discussion

Measurements of community carbon and water vapour fluxes were undertaken as a non-destructive technique for estimating annual time course of community production and water use in the microcosms. Monthly measurements of community carbon exchange relate community production to seasonal canopy development stages, particularly leaf biomass development (Chapter 3). Integration of monthly measurements of community carbon exchange over a growing season complement end of growing season harvest data as indicators of community production (Chapter 4). Measurements of community vapour flux were undertaken to study the annual time course of community water use in relation to stages of canopy development, and to complement water use data presented in Chapter 5. So, the objectives of measuring fluxes of community carbon and water vapour exchange were to investigate whether (i) South African C_4-dominated grassland communities can increase CO_2 uptake under elevated CO_2 (ii) whether their water use will be reduced, and WUE will change under elevated CO_2 and (iii) whether the response patterns in (i) and (ii) above relate to water input and subsequent canopy development/LAI.

The data show that microcosm communities under elevated CO_2 acquired an annual carbon gain of about 1500 mmol m^{-2} over approximately 273 days in the second year irrespective of whether the system received 120%MAR or MAR. That value was 20-30% higher than the carbon gain of microcosms treated with ambient CO_2. In the third year, the carbon gain of microcosms treated with elevated CO_2 decreased by about 15-19%, while no reduction was observed under ambient CO_2 over a similar interval of running the experiment. Proportional differences in community carbon exchange seem to compare well with proportional difference in pooled values of community above-ground biomass at ambient and elevated CO_2, which were 56.2 g ± 2.3 and 63.7 g ± 2.8 respectively (Figure 4.12). Community carbon gain remained higher later in the growing season under elevated CO_2 in both second and third year because of a delay in senescence, which came about as a consequence of conserved soil water. Effect of water treatment on carbon exchange was highly significant during most periods of monthly measurement (Table 6.1).

Dark respiration accounted for 25-30% of CO_2 assimilation in the second year (Figure 6.6). The magnitude of respiratory fluxes in the third year was 5-10% higher relative to the second year, and that observation was attributed to a continual accumulation of soil water. The proportion of dark respiration relative to CO_2 assimilation was higher in the third year compared with the second year, partly because lower rates of CO_2 assimilation were measured in the third year. The relatively lower rates of CO_2 assimilation measured in the third year resulted in less efficient use of PAR per ground area.

The annual time course of community carbon exchange suggest that stimulation by elevated CO_2 was highest earlier in the growing season compared to later in the growing season (Figure 6.3). Furthermore, annual trend in response followed a similar pattern as canopy light use efficiency (Figure 6.4), suggesting that changes in canopy structure may enhance light use efficiency. A significant effect of treatment on canopy leaf biomass placement was apparent in the top canopy layers between 40-60 cm than in the lower canopy layers below 40 cm. The results suggest that a canopy structural change of increase in height due to treatment, may lead to improved canopy light use efficiency, and consequently greater carbon accumulation. It becomes apparent therefore that differences in height of plants in a community can cause variability in carbon exchange, with important consequences for species' competitive interactions.

Effects of elevated CO_2 on water vapour flux of the community integrated over the growing season show a very high reduction in ET. When the reduction in ET is coupled with an increase in rate of community carbon exchange, a huge increase in WUE (Figure 6.11) becomes apparent. The trend was observed in the second and third years, and the observation serves as corroboration of the data presented in Chapter 5. Annual trends in monthly ET show low rates of water loss in the beginning and at end of growing season, and higher rates of water loss at peak season. Treatment effects were statistically significant during most months of the growing season. Higher rates of ET observed at peak season are a consequence of increase in leaf area as the canopy closes, and probably increases in VPD with increasing air temperature.

Results of community carbon and water vapour fluxes link coherently to data presented in Chapters 3, 4, and 5, particularly with respect to community level responses of carbon exchange and water use. The chapter that follows will discuss leaf level gas exchange responses in order to relate contributions of individual species to the bigger picture.

CHAPTER 7

LEAF LEVEL GAS EXCHANGE

7.1 Introduction

Measurement of leaf-level gas exchange of different species in a plant community permits deductions to be made about relative species contributions to canopy carbon gain and water fluxes. Furthermore, leaf gas exchange responses of plants grown in a community tend to differ from responses of individually grown plants because of the consequences of competition for resources. Wand and co-workers (1999) showed that leaf level gas exchange of C_4 grasses grown without competition does not show any sink limitation, but that some species are capable of down regulating their photosynthetic capacity after prolonged exposure to high CO_2. The rate of CO_2 assimilation (A) that is measured at or near light saturation determines intercellular CO_2 concentration (c_i) and influences stomatal conductance (g_s). The A/c_i relationship is important for understanding mechanisms that underlie photosynthetic responses by showing the limitations to photosynthesis due to carboxylation efficiency (V_{cmax}) versus light saturated rate of potential electron transport (J_{max}). A measure of g_s serves as an indication of rate of plant water use. Thus, differences in g_s of different species in a plant community in response to elevated CO_2 can help to attribute species contributions to community water use. Regarding light response characteristics, some leaves in a plant canopy continuously experience sub-optimal light conditions due to shading by other leaves, and during cloudy days the entire canopy experiences low light, as a result the capacity for photosynthetic carbon gain would be dependent on light-limited rate of carbon fixation; quantum yield (ϕ), rather than light-saturated rate of carbon fixation (A_{max}) of a light response curve.

A systematic approach in studying responses to elevated CO_2 of mixed communities is one that allows for response patterns to be categorised by plant functional groups (Poorter 1993; Box 1996, Diaz and Cabido 1997, Ghanoum et al. 2001; Wand et al. 1999, 2001; Ni, 2003; Poorter and Navas 2003). The importance of plant functional groups is based on the premise that species with common functional traits show similar responses to change in environmental factors (Smith et al. 1997; Lavorel,

2002). This approach makes studies of leaf level gas exchange amenable to extrapolation to higher levels of plant organisation (community, ecosystem and landscape), in order to facilitate understanding of biosphere responses (Körner, 2000). Grass species used in the current study represent C_3 and C_4 functional groups, and within the C_4 photosynthetic pathway, choice of species was representative of all three sub-types *viz.*, NAD-ME, NADP-ME and PCK as described in Chapter 2.

7.2 Materials and methods and data analysis

7.2.1 Photosynthetic gas exchange

Measurement of A/c_i and light responses were performed using a Li-6400 portable photosynthesis system (Li-Cor, Lincoln, Nebraska, USA), in the middle of the third growing season during January. Measurements were generally performed on one individual plant per species in each chamber, thus four individuals per species per treatment, but in some instances, three plants per treatment were measured. Because of the narrow shape of plant leaves, three or four leaves arranged side-by-side without over-lapping were placed in the cuvette. The projected area of leaves within the cuvette was recorded and incorporated in the gas exchange calculations. A/c_i response measurements were performed by varying cuvette CO_2 concentrations between 50-1000 μmol mol^{-1} for C_4 species, and between 100-1000 μmol mol^{-1} for the C_3 species. CO_2 supply in the cuvette was by means of pressurised canisters, and was regulated electronically by the instrument. Light was provided by a blue/red LED light source inside the cuvette. Light intensity was set at 1000 μmol mol^{-1} s^{-1} during measurement, and leaf temperature was maintained at 28 °C. Leaves that were selected for measurement were allowed 15 minutes to reach steady state in the cuvette at a CO_2 concentration of 380 μmol mol^{-1} before measurement commenced. During measurement, cuvette CO_2 concentrations were reduced from ambient (380 μmol mol^{-1}) to 100 μmol mol^{-1} or 50 μmol mol^{-1} for C_3 and C_4 species respectively, and then increased back to ambient, followed by a step-wise increase to 1000 μmol mol^{-1}. Sufficient time was allowed for a measurement at any CO_2 concentration to reach steady state before proceeding to the next CO_2 concentration. Upon completion of an A/c_i response measurement, leaves enclosed in the cuvette were allowed about 5 minutes to stabilise at growth or treatment CO_2 concentration.

Light response measurements were performed at growth or treatment CO_2 concentration, starting at a light intensity of 1000 μmol mol^{-1} s^{-1} reducing to 50 μmol mol^{-1} s^{-1}. Leaf temperature was regulated to 28 °C as for A/c_i response measurements. Once again, the leaves enclosed in the cuvette were allowed about 5 minutes to stabilise at saturated light before being taken out of the chamber.

7.2.2 Data analysis

Analysis of the A/c_i and light response data was done using the mathematical function $y = a(1-e^{b-cx})$, described by Causton and Dale (1990), because it provided a better fit to the data than Michaelis-Menton functions. The latter functions tend to over-estimate the light- and CO_2-saturated maximum rate of CO_2 assimilation (Causton and Dale, 1990). In the case of A/c_i response curves, "y" is the dependent variable, rate of CO_2 exchange and "x" is the independent variable "c_i", "a" is the light- and CO_2-saturated rate of CO_2 exchange (J_{max}) which is equivalent to the maximum rate of RuBP regeneration, "b/c" is the CO_2 compensation point (Γ_c), and "ace^b" is the carboxylation efficiency (V_{cmax} or k which is the slope or derivative of the curve at the CO_2 compensation point). In the case of a light response curves, "y" is the dependent variable, rate of CO_2 exchange (A) and "x" is the independent variable PFD, "a" is the light saturated rate of CO_2 exchange (A_{max}), "b/c" gives the light compensation point (Γ_l), "$a(1-e^b)$" gives the dark respiration rate (R_d), and "ace^b" is used to derive the apparent quantum yield α, which is the slope or derivative of the curve at the light compensation point. Individual response curves were modeled independently, and the output presented as treatment averages.

7.3. Results

Results of the A/c_i and light response measurements for each species are presented in Figures 7.1 and 7.2 respectively, and the photosynthetic characteristics of stomatal conductance (g_s) and instantaneous water use efficiency (WUE) for each species at growth CO_2 concentration are presented in Figures 7.3 and 7.4 respectively. The response curves in Figures 7.1 and 7.2 were drawn using mean values of the parameters (Section 7.2.2) for each treatments.

The rates of carbon dioxide assimilation shown in the A/c_i responses in Figure 7.1 are low for C_4 plants, particularly in relation to greenhouse and field measurements of Wand et al. (2001, 2002). The A/c_i response of the C_3 species *Alloteropsis* grown under elevated CO_2 showed a lower rate of CO_2 assimilation at higher cuvette CO_2 concentrations compared with response of plants grown under ambient CO_2 (Figure 7.1). Analysis of light response measurements for that species showed lower rates of light-saturated CO_2 assimilation (A_{max}) under elevated CO_2 relative to ambient CO_2 (Figure 7.2). A/c_i responses of *Andropogon* showed slightly higher rates of CO_2 assimilation in microcosms treated with ambient CO_2 compared with microcosms treated with elevated CO_2, although differences in J_{max} between CO_2 treatments were not statistically significant (Table 7.2). On the other hand, A_{max} measured in light responses of *Andropogon* was higher under elevated CO_2 + 120%MAR compared to ambient CO_2 treatments, but the response observed in ambient CO_2 treatments was similar to the response in elevated CO_2 + MAR.

Results on *Eragrostis*, *Sporobolus* and *Themeda* showed a stimulation of CO_2 assimilation to high cuvette CO_2 concentrations in A/c_i response measurements (Figure 7.1), because plants grown under elevated CO_2 had higher J_{max}. Data for those three species, *Eragrostis*, *Sporobolus* and *Themeda* suggest a long-term stimulation of photosynthetic response. Light response data of *Eragrostis*, *Sporobolus* and *Themeda* at growth CO_2 concentration showed a higher A_{max} under elevated CO_2 (Figure 7.2), and differences between treatments were more marked in *Eragrostis*.

Stomatal conductance (g_s) determined from $A:c_i$ responses at growth CO_2 concentration was generally lower under elevated CO_2 relative to ambient CO_2 in all five species at mid-season (Figure 7.3). A consequence of a reduction in g_s was a reduction in transpiration, which resulted in higher instantaneous water use efficiency (WUE) under elevated CO_2 (Figure 7.4).

Data showing treatment effects on modeled photosynthetic parameters of A/c_i and light response measurements are summarised in Tables 7.1-7.10. Notable effects include a reduction in light- and CO_2-saturated rate of net CO_2 assimilation (J_{max}) in *Alloteropsis* and *Andropogon* under elevated CO_2, but the reduction was not statistically significant. Carboxylation efficiency was lower in *Alloteropsis,*

Andropogon and *Egarostis*, and higher in *Sporobolus* and *Themeda*. Surprisingly, CO_2 compensation point in the C_3 species *Alloteropsis* was much lower than values recorded for the C_4 species. Furthermore, lower dark respiration (R_d) in *Alloteropsis* was apparent and consequently a lower light compensation point in the C_3 species under elevated CO_2. Quantum use efficiency was reduced in *Alloteropsis* under elevated CO_2.

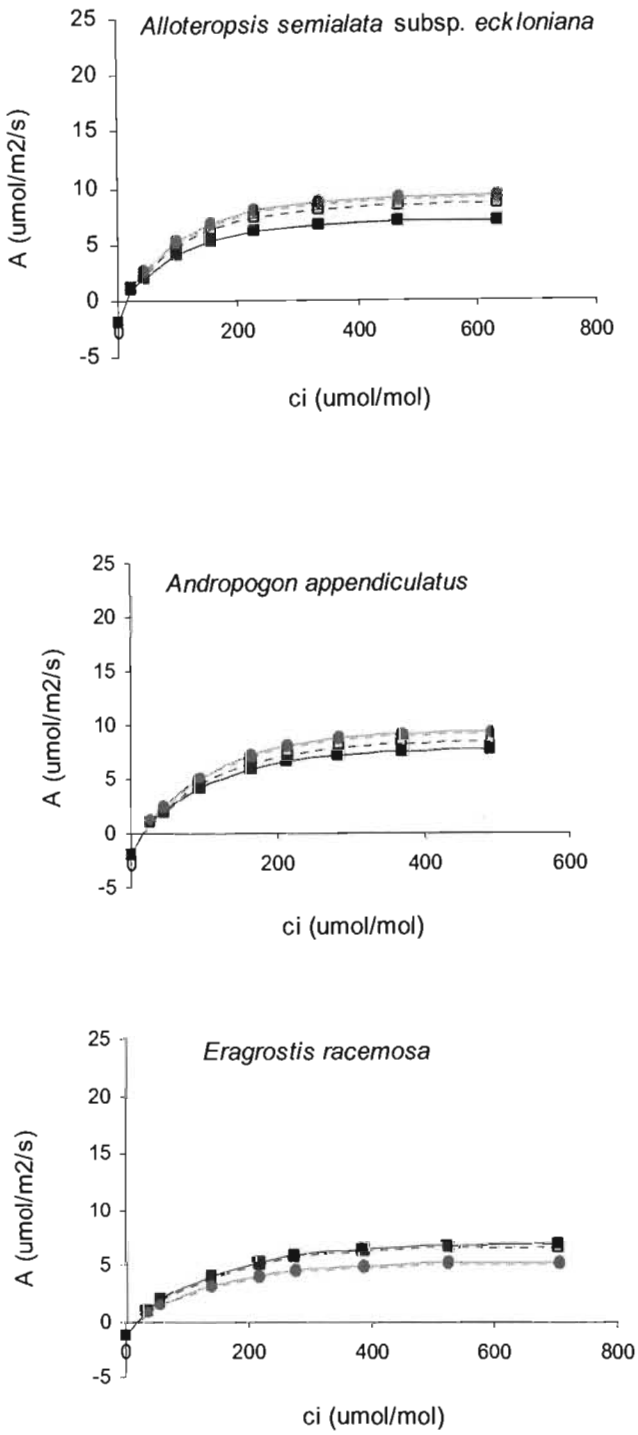


Figure 7.1: CO_2 response of photosynthesis (A/c_i) of the grass species measured at mid-season. Square symbols represent elevated CO_2 treatments, and circles represent ambient CO_2 treatments. Dashed lines among the circles and squares represent mean annual rainfall (MAR) and solid lines among circles and squares represent 120%MAR.

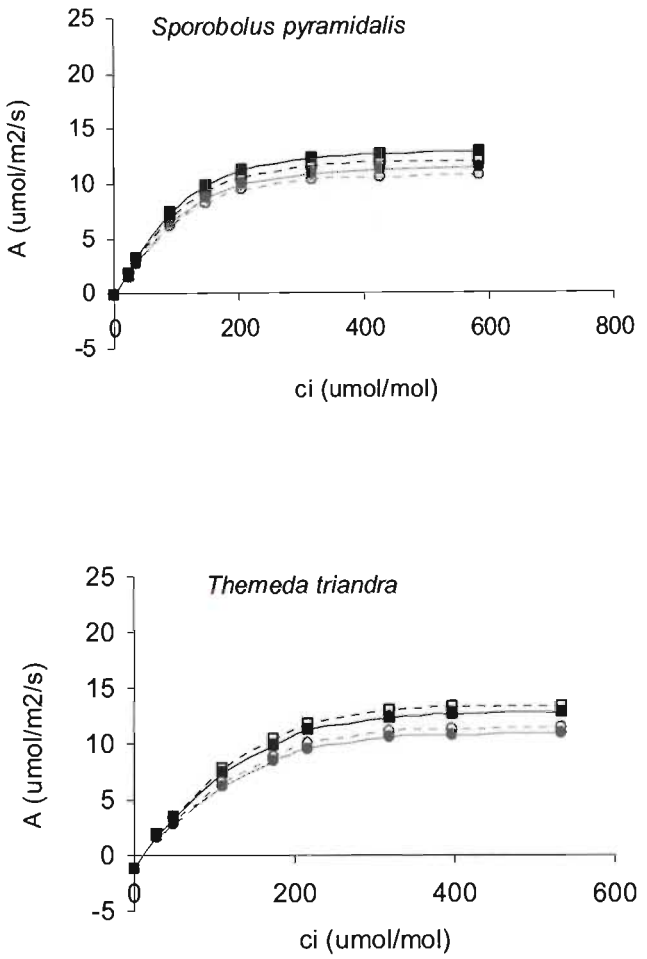


Figure 7.1 (continued) CO_2 response of photosynthesis (A/c_i) of the grass species measured at mid-season. Square symbols represent elevated CO_2 treatments, and circles represent ambient CO_2 treatments. Dashed lines among the circles and squares represent mean annual rainfall (MAR) and solid lines among circles and squares represent 120%MAR.

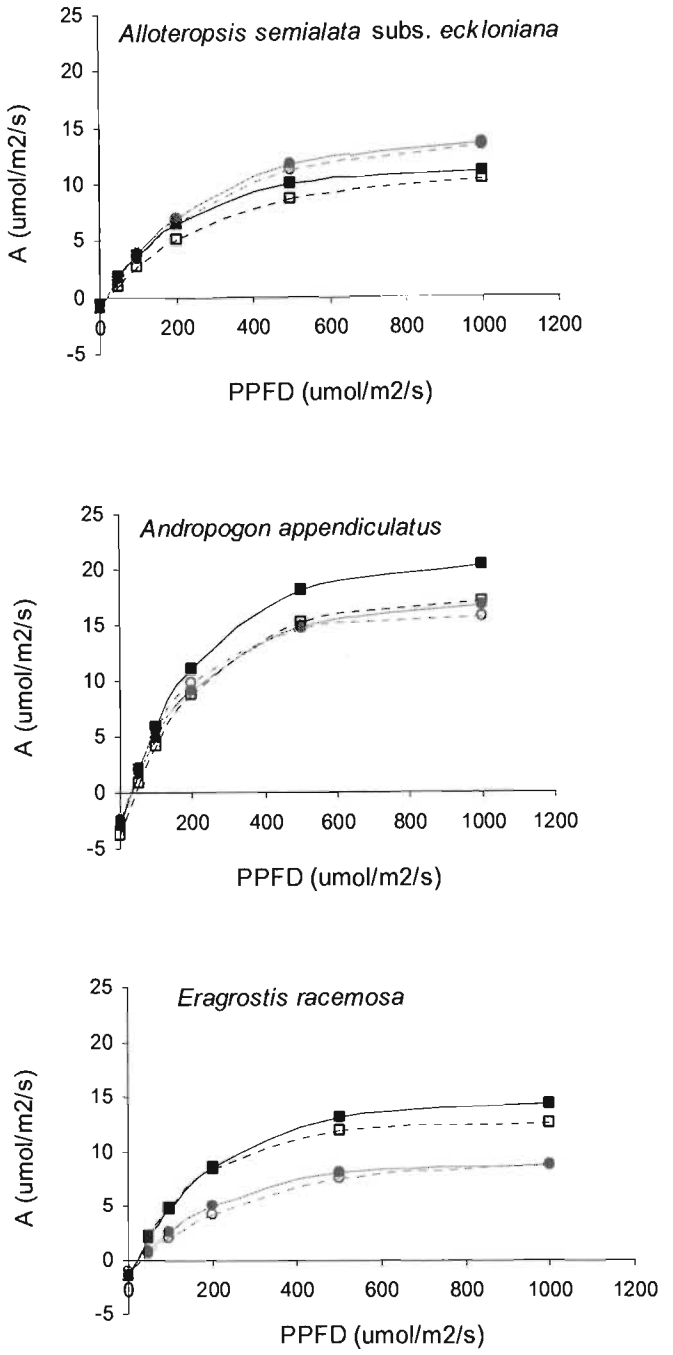


Figure 7.2: Light response of photosynthesis of the grass species measured at mid-season. Square symbols represent elevated CO_2 treatments, and circles represent ambient CO_2 treatments. Dashed lines among the circles and squares represent mean annual rainfall (MAR) and solid lines among circles and squares represent 120%MAR. Measurements were made under growth CO_2 concentrations.

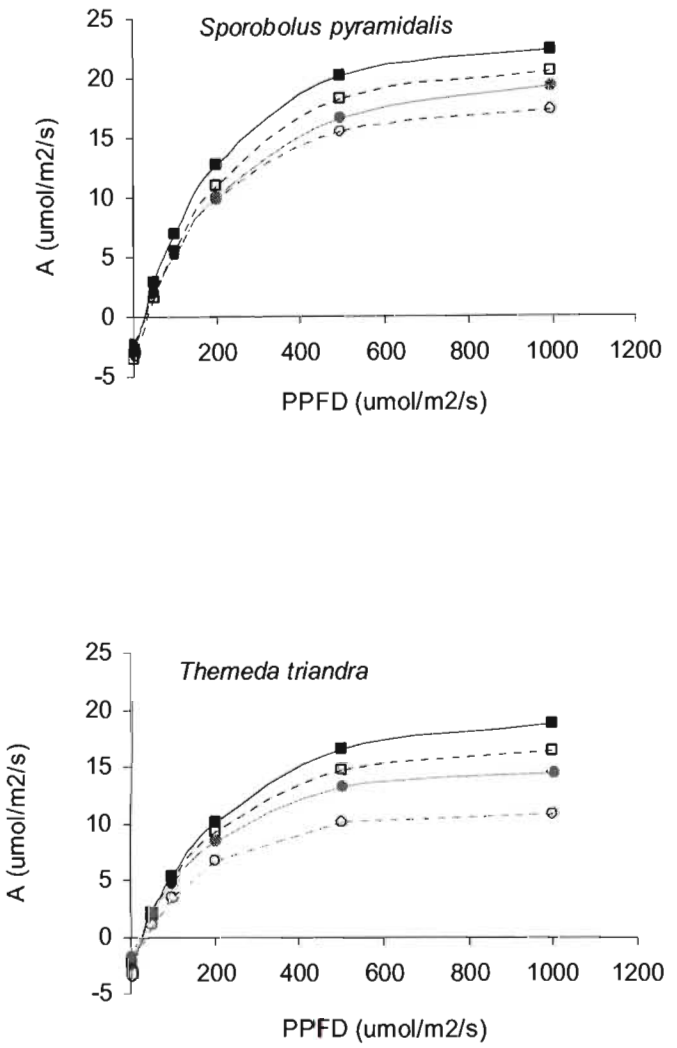


Figure 7.2 (continued) Light response of photosynthesis of the grass species measured at mid-season. Square symbols represent elevated CO_2 treatments, and circles represent ambient CO_2 treatments. Dashed lines among the circles and squares represent mean annual rainfall (MAR) and solid lines among circles and squares represent 120%MAR. Measurements were made under growth CO_2 concentrations.

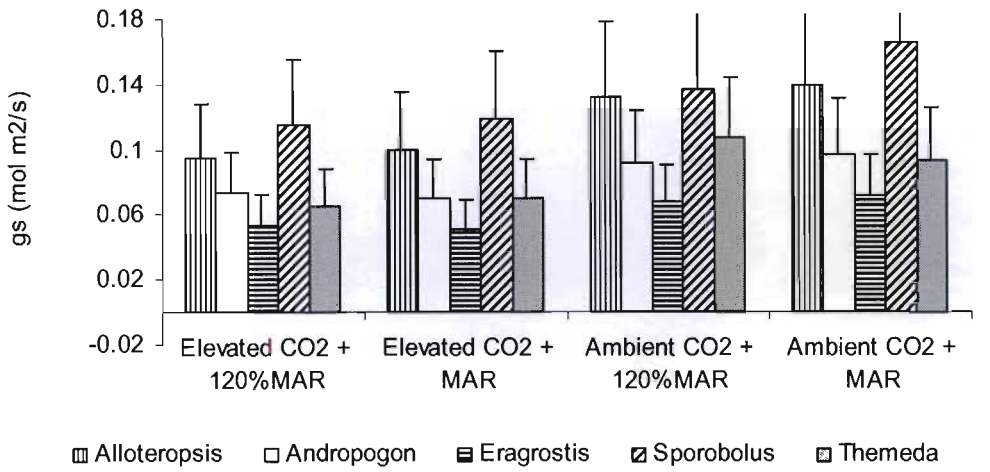


Figure 7.3: The response of stomatal conductance (g_s) to treatment at mid-season.

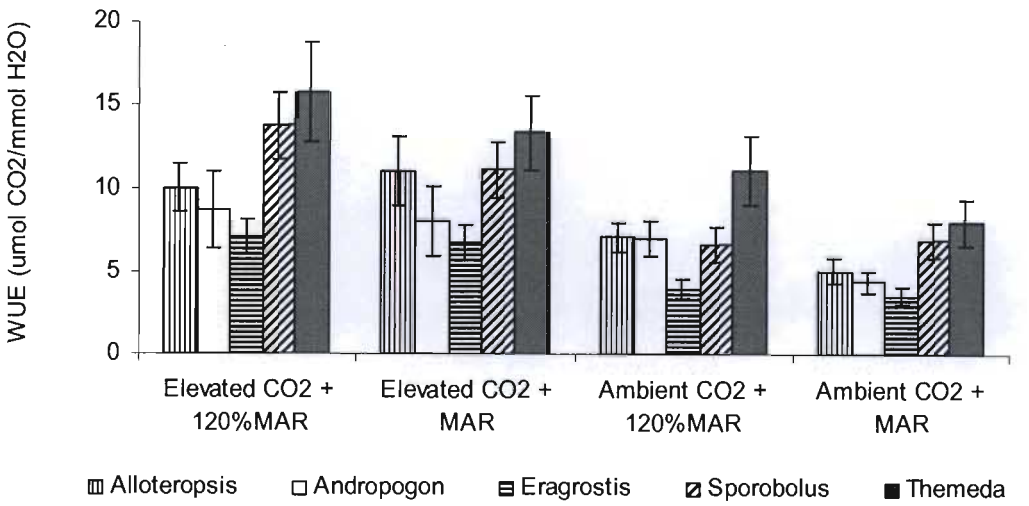


Figure 7.4: The response of WUE to treatment at mid-season.

Table 7.1. Response of A/c_i parameters of *Alloteropsis* to treatment, presented together with the results of a two-way ANOVA.

Species	Parameter	Treatment	Value of parameter	Statistical significance (ANOVA)		
				CO_2	H_2O	Inter-action
Alloteropsis	J_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	Elevated CO_2 + 120%MAR	7.08	NS	NS	NS
		Elevated CO_2 + MAR	8.50			
		Ambient CO_2 + 120%MAR	9.21			
		Ambient CO_2 + MAR	9.00			
	Γ_c ($\mu mol\ mol^{-1}$)	Elevated CO_2 + 120%MAR	6.62	NS	NS	NS
		Elevated CO_2 + MAR	6.6			
		Ambient CO_2 + 120%MAR	7.62			
		Ambient CO_2 + MAR	9.3			
	k ($\mu mol\ CO_2\ \mu mol^{-1}\ c_i$)	Elevated CO_2 + 120%MAR	0.069	NS	NS	NS
		Elevated CO_2 + MAR	0.083			
		Ambient CO_2 + 120%MAR	0.09			
		Ambient CO_2 + MAR	0.088			

Table 7.2. Response of A/c_i parameters of *Andropogon* to treatment presented together with the results of a two-way ANOVA.

Species	Parameter	Treatment	Value of parameter	Statistical significance (ANOVA)		
				CO_2	H_2O	Inter-action
Andropogon	J_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	Elevated CO_2 + 120%MAR	7.81	NS	NS	NS
		Elevated CO_2 + MAR	8.51			
		Ambient CO_2 + 120%MAR	9.46			
		Ambient CO_2 + MAR	9.3			
	Γ_c ($\mu mol\ mol^{-1}$)	Elevated CO_2 + 120%MAR	12.33	NS	NS	NS
		Elevated CO_2 + MAR	12.2			
		Ambient CO_2 + 120%MAR	12.43			
		Ambient CO_2 + MAR	12.18			
	k ($\mu mol\ CO_2\ \mu mol^{-1}\ c_i$)	Elevated CO_2 + 120%MAR	0.082	NS	NS	NS
		Elevated CO_2 + MAR	0.089			
		Ambient CO_2 + 120%MAR	0.098			
		Ambient CO_2 + MAR	0.097			

Table 7.3. Response of A/c_i parameters of *Eragrostis* to treatment, presented together with the results of a two-way ANOVA.

Species	Parameter	Treatment	Value of parameter	Statistical significance (ANOVA)		
				CO_2	H_2O	Inter-action
Eragrostis	J_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	Elevated CO_2 + 120%MAR	6.86	NS	NS	NS
		Elevated CO_2 + MAR	6.64			
		Ambient CO_2 + 120%MAR	5.28			
		Ambient CO_2 + MAR	5.11			
	Γ_c ($\mu mol\ mol^{-1}$)	Elevated CO_2 + 120%MAR	10.22	NS	NS	NS
		Elevated CO_2 + MAR	10.88			
		Ambient CO_2 + 120%MAR	10.17			
		Ambient CO_2 + MAR	10.98			
	k ($\mu mol\ CO_2\ \mu mol^{-1}\ c_i$)	Elevated CO_2 + 120%MAR	0.051	NS	NS	NS
		Elevated CO_2 + MAR	0.049			
		Ambient CO_2 + 120%MAR	0.039			
		Ambient CO_2 + MAR	0.059			

Table 7.4. Responses of A/c_i parameters of *Sporobolus* to treatment, presented together with the results of a two-way ANOVA.

Species	Parameter	Treatment	Value of parameter	Statistical significance (ANOVA)		
				CO_2	H_2O	Inter-action
Sporobolus	J_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	Elevated CO_2 + 120%MAR	12.74	NS	NS	NS
		Elevated CO_2 + MAR	12.02			
		Ambient CO_2 + 120%MAR	11.34			
		Ambient CO_2 + MAR	10.69			
	Γ_c ($\mu mol\ mol^{-1}$)	Elevated CO_2 + 120%MAR	10.52	NS	NS	NS
		Elevated CO_2 + MAR	10.78			
		Ambient CO_2 + 120%MAR	10.39			
		Ambient CO_2 + MAR	10.47			
	k ($\mu mol\ CO_2\ \mu mol^{-1}\ c_i$)	Elevated CO_2 + 120%MAR	0.15	NS	NS	NS
		Elevated CO_2 + MAR	0.14			
		Ambient CO_2 + 120%MAR	0.13			
		Ambient CO_2 + MAR	0.12			

Table 7.5. Response of A/c_i parameters of *Themeda* to treatment, presented together with the results of a two-way ANOVA.

Species	Parameter	Treatment	Value of parameter	Statistical significance (ANOVA)		
				CO_2	H_2O	Inter-action
Themeda	J_{max} (μmol m^{-2} s^{-1})	Elevated CO_2 + 120%MAR	13.12	NS	NS	NS
		Elevated CO_2 + MAR	13.70			
		Ambient CO_2 + 120%MAR	11.09			
		Ambient CO_2 + MAR	11.64			
	Γ_c (μmol mol^{-1})	Elevated CO_2 + 120%MAR	11.66	NS	NS	NS
		Elevated CO_2 + MAR	13.73			
		Ambient CO_2 + 120%MAR	13.7			
		Ambient CO_2 + MAR	13.7			
	k (μmol CO_2 μmol $^{-1}$ c_i)	Elevated CO_2 + 120%MAR	0.12	NS	NS	NS
		Elevated CO_2 + MAR	0.13			
		Ambient CO_2 + 120%MAR	.011			
		Ambient CO_2 + MAR	0.12			

Table 7.6. Treatment effect on light response parameters of *Alloteropsis*, presented together with the results of a two-way ANOVA.

Species	Parameter	Treatment	Value of parameter	Statistical significance (ANOVA)		
				CO_2	H_2O	Inter-action
Alloteropsis	A_{max} (μmol m^{-2} s^{-1})	Elevated CO_2 + 120%MAR	11.08	NS	NS	NS
		Elevated CO_2 + MAR	10.61			
		Ambient CO_2 + 120%MAR	13.91			
		Ambient CO_2 + MAR	13.88			
	Γ_l (μmol m^{-2} s^{-1})	Elevated CO_2 + 120%MAR	12.09	NS	NS	NS
		Elevated CO_2 + MAR	20.84			
		Ambient CO_2 + 120%MAR	15.57			
		Ambient CO_2 + MAR	19.36			
	α (μmol CO_2 μmol $^{-1}$ PPFD)	Elevated CO_2 + 120%MAR	0.05	NS	NS	NS
		Elevated CO_2 + MAR	0.04			
		Ambient CO_2 + 120%MAR	0.05			
		Ambient CO_2 + MAR	0.05			
	R_d (μmol m^{-2} s^{-1})	Elevated CO_2 + 120%MAR	-0.7	NS	NS	NS
		Elevated CO_2 + MAR	-1.61			
		Ambient CO_2 + 120%MAR	-0.93			
		Ambient CO_2 + MAR	-1.31			

Table 7.7. Treatment effect on light response parameters of *Andropogon*, presented together with the results of a two-way ANOVA.

Species	Parameter	Treatment	Value of parameter	Statistical significance (ANOVA)		
				CO_2	H_2O	Inter-action
Andropogon	A_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	Elevated CO_2 + 120%MAR	20.53	NS	NS	NS
		Elevated CO_2 + MAR	17.39			
		Ambient CO_2 + 120%MAR	16.86			
		Ambient CO_2 + MAR	15.79			
	Γ_1 ($\mu mol\ m^{-2}\ s^{-1}$)	Elevated CO_2 + 120%MAR	25.13	NS	NS	NS
		Elevated CO_2 + MAR	39.1			
		Ambient CO_2 + 120%MAR	26.46			
		Ambient CO_2 + MAR	21.37			
	α ($\mu mol\ CO_2\ \mu mol^{-1}$ PPFD)	Elevated CO_2 + 120%MAR	0.10	NS	NS	NS
		Elevated CO_2 + MAR	0.09			
		Ambient CO_2 + 120%MAR	0.085			
		Ambient CO_2 + MAR	0.098			
	R_d ($\mu mol\ m^{-2}\ s^{-1}$)	Elevated CO_2 + 120%MAR	-2.43	NS	NS	NS
		Elevated CO_2 + MAR	-3.27			
		Ambient CO_2 + 120%MAR	-2.13			
		Ambient CO_2 + MAR	-1.98			

Table 7.8. Treatment effect on light response parameters of for *Eragrostis*, presented together with the results of a two-way ANOVA.

Species	Parameter	Treatment	Value of parameter	Statistical significance (ANOVA)		
				CO_2	H_2O	Inter-action
Eragrostis	A_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	Elevated CO_2 + 120%MAR	14.51	P = 0.02	P = 0.04	P = 0.03
		Elevated CO_2 + MAR	12.54			
		Ambient CO_2 + 120%MAR	8.78			
		Ambient CO_2 + MAR	8.96			
	Γ_1 ($\mu mol\ m^{-2}\ s^{-1}$)	Elevated CO_2 + 120%MAR	19.37	P = 0.01	P = 0.02	P = 0.046
		Elevated CO_2 + MAR	17.75			
		Ambient CO_2 + 120%MAR	30.46			
		Ambient CO_2 + MAR	33.73			
	α ($\mu mol\ CO_2\ \mu mol^{-1}$ PPFD)	Elevated CO_2 + 120%MAR	0.077	NS	NS	NS
		Elevated CO_2 + MAR	0.084			
		Ambient CO_2 + 120%MAR	0.052			
		Ambient CO_2 + MAR	0.038			
	R_d ($\mu mol\ m^{-2}\ s^{-1}$)	Elevated CO_2 + 120%MAR	-5.05	P = 0.001	P = 0.003	P = 0.0001
		Elevated CO_2 + MAR	-4.54			
		Ambient CO_2 + 120%MAR	-1.89			
		Ambient CO_2 + MAR	-1.99			

Table 7.9. Treatment effect on light response parameters of *Sporobolus*, presented together with the results of a two-way ANOVA.

Species	Parameter	Treatment	Value of parameter	Statistical significance (ANOVA)		
				CO_2	H_2O	Inter-action
Sporobolus	A_{max} (μmol m^{-2} s^{-1})	Elevated CO_2 + 120%MAR	22.48	NS	NS	NS
		Elevated CO_2 + MAR	20.82			
		Ambient CO_2 + 120%MAR	19.57			
		Ambient CO_2 + MAR	17.39			
	Γ_l (μmol m^{-2} s^{-1})	Elevated CO_2 + 120%MAR	21.24	NS	NS	NS
		Elevated CO_2 + MAR	31.17			
		Ambient CO_2 + 120%MAR	28.81			
		Ambient CO_2 + MAR	21.10			
	α (μmol CO_2 μmol $^{-1}$ PPFD)	Elevavted CO_2 + 120%MAR	0.12	P = 0.002	P = 0.004	P = 0.001
		Elevated CO_2 + MAR	0.11			
		Ambient CO_2 + 120%MAR	0.87			
		Ambient CO_2 + MAR	0.88			
	R_d (μmol m^{-2} s^{-1})	Elevated CO_2 + 120%MAR	-2.23	NS	NS	NS
		Elevated CO_2 + MAR	-4.62			
		Ambient CO_2 + 120%MAR	-2.39			
		Ambient CO_2 + MAR	-2.19			

Table 7.10. Treatment effect on light response parameters of *Themeda*, presented together with the results of a two-way ANOVA.

Species	Parameter	Treatment	Value of parameter	Statistical significance (ANOVA)		
				CO_2	H_2O	Inter-action
Themeda	A_{max} (μmol m^{-2} s^{-1})	Elevated CO_2 + 120%MAR	19.0	P = 0.007	P = 0.01	NS
		Elevated CO_2 + MAR	16.3			
		Ambient CO_2 + 120%MAR	14.35			
		Ambient CO_2 + MAR	10.86			
	Γ_l (μmol m^{-2} s^{-1})	Elevated CO_2 + 120%MAR	23.9	NS	P = 0.013	
		Elevated CO_2 + MAR	37.3			
		Ambient CO_2 + 120%MAR	30.9			
		Ambient CO_2 + MAR	46.5			
	α (μmol CO_2 μmol $^{-1}$ PPFD)	Elevated CO_2 + 120%MAR	0.074	NS	NS	NS
		Elevated CO_2 + MAR	0.072			
		Ambient CO_2 + 120%MAR	0.059			
		Ambient CO_2 + MAR	0.054			
	R_d (μmol m^{-2} s^{-1})	Elevated CO_2 + 120%MAR	-0.84	NS	P = 0.0056	NS
		Elevated CO_2 + MAR	-1.28			
		Ambient CO_2 + 120%MAR	-1.00			
		Ambient CO_2 + MAR	-1.82			

7.4. Discussion

Leaf level gas exchange measurements were undertaken in order to relate responses of individual species to responses of biomass and water use at the community level. All five grass species were tested for short-term stimulation of CO_2 assimilation in response to high cuvette CO_2 concentrations by doing A/c_i response measurements. Down regulation occurred in two species in elevated CO_2 treatments, one a C_3 (*Alloteropsis*) and another a C_4 (*Andropogon*) The other three species (*Eragrostis*, *Sporobolus*, and *Themeda*), all possessing a C_4 photosynthetic pathway, showed an up regulation under elevated CO_2. Differences among treatments were however not statistically significant, except for light response of *Eragrostis* and *Themeda*. Values of A_{max} measured in this study were low relative to many C_4 systems, but a pattern in response for *Themeda* was similar to findings of Wand et al. (2002) in the field and Wand (1999) in the laboratory. A contention for lack of statistical significance on differences in treatment effect could be optimum growth conditions that prevailed at the time of measurement at mid-season. While it would have been interesting to study annual trends in gas exchange of individual species at various phases of development during the growing season, greater emphasis in this study was placed on community responses because earlier findings of Wand and co-workers (2001, 2002) extensively characterised leaf level responses of all species used in this study in the greenhouse and in the field respectively. Nonetheless, it was pertinent to relate biomass production, canopy gas exchange and community water use of the microcosm communities in this study to the leaf level gas exchange response of individual species within the microcosms.

The two species with a response of photosynthetic down regulation (*Alloteropsis* and *Andropogon*), contributed less to community biomass production (Chapter 4) compared with *Sporobolus* and *Themeda*, which did not undergo measurable photosynthetic down regulation at the time of leaf gas exchange measurement. In fact, *Sporobolus* and *Themeda* were dominant species in the community in terms of biomass production (Chapter 4). Photosynthetic up-regulation under elevated CO_2 was also measured in *Eragrostis*, as well as high rates of dark respiration. But biomass production in *Eragrostis* was higher under ambient CO_2 compared with elevated CO_2, suggesting a disproportional response of leaf level photosynthesis and leaf biomass production in elevated CO_2. A higher rate of carbon loss by respiratory

flux as observed for *Eragrostis* is one of the factors attributed to disproportional response of photosynthesis and biomass production (Luo et al. 1997).

Reduction in stomatal conductance due to elevated CO_2 was observed in all grass species, and instantaneous water use efficiency remained higher under elevated CO_2 in C_4 species, as well as in the C_3 species. The response of stomatal conductance and leaf level water use efficiency tie in very well with community water use data (Chapter 5). Improved water use under elevated CO_2 in grassland ecosystems (Ham et al. 1995, Freeden et al. 1996, Owensby et al. 1997 and Morgan et al. 2001) is a major driver of positive response in biomass production.

CHAPTER 8

GENERAL DISCUSSION

Despite substantial research effort over the past decade and more, significant uncertainty remains about the response of C_4 grass species to rising atmospheric CO_2, and how these will be reflected in multi-species assemblages and grassland ecosystems. The combined impact of rising atmospheric CO_2 and variable rainfall is an especially critical concern under future climate scenarios. This topic is of great importance to South Africa, both because major river catchments are found in landscapes characterised by C_4 dominated grasslands, and because extensive rangeland farming activities depend on these ecosystems. Several studies in the USA (Ham et al. 1995, Field et al. 1997, Freeden et al. 1996, Owensby et al. 1997 and Morgan et al. 2001) have shown that CO_2 has the potential to ameliorate the effects of reduced rainfall.

Several methods were used in this study to investigate the potential impacts of increasing atmospheric CO_2 and simulated rainfall amount on the production and water use of a South African C_4-dominated grassland microcosm community. The research aimed to address the following five key questions:

(i) Will elevated CO_2 change above-ground community biomass production, biomass allocation, and leaf-area indices in the long-term?

(ii) To what extent will above-ground biomass development be influenced by a combined effect of elevated CO_2 and different watering treatments?

(iii) Will the responsiveness and proportional representation of C_4 functional types be altered by a combined effect of elevated CO_2 and different watering treatments?

(iv) Will community-level water use be changed by long-term exposure to elevated CO_2?

(v) What are the long-term implications of elevated CO_2 on South African grasslands as water catchments?

The results presented in the thesis strongly illustrate that elevated atmospheric CO_2 significantly enhanced above-ground community production and water use efficiency.

Increased production was largely a consequence of CO_2 stimulation of only two grass species, *Sporobolus* and *Themeda*, out of the five grass species forming the microcosm communities. Species competitive interactions influenced community responses through treatment effects on vertical placement of leaf biomass in the canopy, and as a result treatment effects on canopy structure also had major consequences for above-ground production. Effects of elevated CO_2 occurred mostly as a result of interactions with rainfall amount when the applied water treatment was lower than or equal to MAR, but independently of watering treatment when rainfall amount was higher than MAR.

8.1 Community above-ground production

End of growing season above-ground production was enhanced for three consecutive years under a treatment combination of elevated CO_2 + MAR relative to other treatment combinations (Chapter 4). In the first year, above-ground production under elevated CO_2 + MAR was 74.5 g ± 3.1 per unit ground area of 0.16 m^2, which is equivalent to 465.6 g m^{-2} $year^{-1}$. That value was 26-36% higher than values recorded under other treatments in the first year. At the end of the second year, a 15-29% enhancement, relative to other treatments, was recorded under elevated CO_2 + MAR. At the end of the third year the enhancement by this treatment, and other treatments was 15-18%. This reduction in the enhancement by elevated CO_2 + MAR in the third year was accompanied by a general decrease in biomass production across all treatments (Figures 4.1a, 4.2a and 4.3a). This suggests that the reduction in enhancement by elevated CO_2 + MAR was not simply an acclimation to elevated CO_2. The reported production values in the current microcosm experiment are comparable to production values recorded at the field site (Stock et al. 2004). Those authors measured 300-400 g m^{-2} annual peak standing biomass on control plots at the field site, relative to approximately 500 g m^{-2} in plots fumigated with a natural source of elevated CO_2. However, Stock et al. (2004) indicated that differences in annual peak standing biomass of control and CO_2-fumigated plots could not be attributed to CO_2 fumigation alone.

Annual comparisons for treatment effects involving elevated CO_2 + the other two watering amounts (80%MAR and 120%MAR) on above-ground production could not be done because of changes in amount of water supplied. However, the objective of

deducing overall effects of elevated CO_2 + watering treatment from results obtained under elevated CO_2 + MAR is not far-fetched considering that, of the three watering treatments applied in the study (80%MAR, MAR, and 120%MAR), the highest enhancement in above-ground production in all three years, was observed under elevated CO_2 + MAR as illustrated in Figure 4.11 in Chapter 4. The annual trend in above-ground production of communities exposed to elevated CO_2 + MAR, suggested a 50% reduction in growth stimulation by elevated CO_2 in the long-term when results of the first year were compared to results of the third year. Despite this decline, biomass production in elevated CO_2 remained higher than in ambient CO_2. Furthermore, a three-year cumulative above-ground biomass production under elevated CO_2 was significantly higher than under ambient CO_2 ($P = 0.0249$), despite the observed reduction in enhancement of production in year one relative to year three.

Acclimation to the CO_2 effect on biomass production is commonly attributed to restriction of the rooting volume for experiments conducted within containers (Arp 1991; Thomas and Strain 1991, Barrett and Gifford 1995, Drake et al. 1997). Additionally, nutrient limitation may ensue following unreplenishable uptake of nitrogen in container studies (Petterson and McDonald 1994), a situation that may lead to a phenomenon of sink strength limitation (Midgley et al. 1995). This study was not designed to test for the effect of root restriction; nonetheless, observations made at the final harvest showed that a large volume of the soil contained root material, which was indicative of some degree of root restriction. The experimental set-up was designed to simulate root space as it was in the field from which the microcosm communities were derived, hence it is speculated that some degree of sink limitation would occur in the field as well.

At the beginning of the experiment, initial leaf samples were set aside for analysis of nitrogen and phosphorus content, for comparison with nutrient content of leaf material from the final harvest at the end of the third year. Results of that analysis showed no differences in nutrient status of leaf material at the beginning and at the end of the experiment in all treatments, although the data have not been included in the thesis because the samples were not sufficient for a statistical analysis to be performed. It is also reasonable to assume that there was little loss of nutrients from the microcosms

during the course of the study, because there was not drainage, and litter and harvested material was ashed and returned to the pots. At this point, root restriction remains a strong contender as cause for the observed trend in biomass production from first to third year. The speculation is further supported by the fact that a reduction in biomass production was also observed under ambient CO_2 treatments in the third year relative years one and two.

Field studies overcome a problem of restriction on rooting volume. However, field studies that are conducted in chambers can also induce microclimate conditions that could influence responses. Under ideal circumstances, studies that utilise FACE technique may be pertinent for testing the hypothesis of nutrient limitation versus sink strength as causes of acclimation to elevated CO_2, but the biggest draw-back is the associated exorbitant cost of such research in developing countries. Nonetheless, inferences can be drawn from FACE studies in the literature. For example, Rogers and others (1998) studied nitrogen limitation versus sink strength limitation in ryegrass, and concluded that acclimation was caused by limitation of sink development rather than it being a direct effect of nitrogen supply on photosynthesis.

Another important highlight with regards to above-ground biomass production was an apparent requirement for a critical amount of water above or below which effect of elevated CO_2 on production became less marked in this particular grassland community. To reiterate, the highest enhancement in production under elevated CO_2 occurred consistently at MAR during all three years, given a range of water treatments (80%MAR, MAR and 120%MAR) applied in the study. The observation is certainly unique to the grassland community used in this study. Reports on past studies on *in situ* grassland communities in other parts of the world have reported greater enhancement of above-ground production only during years when water availability was lower than mean annual rainfall (Owensby et al. 1993, 1997). Amelioration of water stress remains an adequate mechanism that influences biomass responses to low water availability under elevated CO_2, but consensus does not exist on the type of mechanisms that cause waning biomass responses in surplus water under elevated CO_2. Huxman and co-workers (1998) cited photosynthetic down-regulation as the cause of reduced biomass enhancement in well-watered desert plants under elevated CO_2, suggesting that elevated CO_2 ameliorates drought-induced stress to the

photosynthetic apparatus in the arid ecosystem. An observed 50% stimulation of production in anomalously wet years in the same arid ecosystem was reported by Smith et al. (2000) to favour growth of invasive grasses.

A conclusion that can be drawn from the data on annual and cumulative above-ground biomass production is that a transient effect of CO_2 may occur in South African C_4-dominated grasslands, and also the degree of stimulation may vary from year to year depending on rainfall conditions.

8.1.1. Influence of canopy structure, phenology and species contributions on above-ground production

Species that contribute most biomass above-ground usually have the most influence on above-ground processes, but the degree of influence may also depend on whether allocation prioritises leaf biomass or stem biomass. Production of high leaf biomass correlates positively with high rates of canopy photosynthesis, while biomass allocation to reproductive stalks may depend on factors conducive to reproductive success such as nitrogen availability (even though this parameter was not tested in this study). The data showed a stem:leaf biomass allocation ratio of 1:4 in the first and second years, and a slight increase in allocation to leaf biomass in the third year (1:4.5). Leaf biomass decreased with canopy height, but the significance of treatment effect on leaf biomass was realised in the upper, less dense, layers of the canopy in the height ranges of 40-60 cm and >60 cm, possibly because there was no limitation of light at the top of the canopy. During the first two years, CO_2 treatment had a statistically significant effect on community leaf biomass while water treatment had statistically significant effect on community stem biomass, but, treatment effects on leaf and stem biomass fractions were not significant in the third year.

The end-of-year harvestable biomass of each species was a culmination of treatment effect on developmental stages from time of sprouting, including time of flowering and annual changes in rate of leaf gas exchange (carbon fixation and water use). In terms of absolute values of biomass harvest at end of year, *Sporobolus* and *Themeda* were the dominant species in the first two years, contributing more than 50% of the community above-ground production. Among the dominant species, biomass of *Sporobolus* was 25% higher than biomass of *Themeda* at the end of the first year. By

the end of the second year, an increase in harvestable biomass of both *Sporobolus* and *Themeda* was noted while biomass of the other three grass species (*Alloteropsis, Andropogon,* and *Eragrostis*) had decreased. A higher biomass increment occurred in *Themeda* than *Sporobolus*, resulting in both species contributing almost equal amounts to production at the end of the second year. At the end of the third year, an 8% reduction in biomass production was noted in *Themeda*, relative to a 50% reduction in *Sporobolus*, suggesting a greater degree of resilience in the dominance of *Themeda*.

Sporobolus and *Themeda* also had a significantly greater proportion of leaf placement in the upper layers of the canopy in the height range of 20 cm and above, while the other three species had a greater proportion of biomass than *Sporobolus* and *Themeda* in the bottom 5-20 cm layer. Part of the competitive edge in *Sporobolus* and *Themeda* was attributed to their intrinsically tall stature, even though the same attribute was not advantageous to the competitive capacity of *Alloteropsis* and *Andropogon*. A further attribute for a positive response of *Themeda* was early sprouting in all three years, on which the CO_2 treatment had a highly significant effect, even though there was no significant effect of water treatment nor significant interactive effect of CO_2 and water treatments.

Flowering occurred only in *Eragrostis*, *Sporobolus*, and *Themeda* in all three years. The effects of CO_2 and water treatments and their interaction were significant on the flowering of *Sporobolus* in all three years. In *Eragrostis*, time of flowering was influenced by CO_2 treatment alone and not water treatment nor its interaction with CO_2.

Gas exchange responses at the species and community levels in this study largely corroborate the biomass production data. Canopy CO_2 assimilation was 20% higher under elevated CO_2 compared to ambient CO_2. Respiratory carbon was proportionally higher in elevated CO_2 relative to ambient CO_2, nonetheless a positive carbon balance was realised during the growing season. Gas exchange at the species level showed a photosynthetic up-regulation in response to high cuvette CO_2 concentrations during measurements of A/ci responses of *Themeda* and *Sporobolus* at mid-season. Those two species were dominant in the community on the basis of biomass production.

Photosynthetic down regulation occurred in *Alloteropsis* and *Andropogon* at mid-season. Phenological influences must be considered in the interpretation of leaf gas exchange data of one-time measurement at mid-season in this study.

8.2. Below-ground responses

Results of this study suggest that total below-ground production of the grassland community did not respond to CO_2 and water treatments or their interaction. However, assessment of treatment effect on below-ground growth may have been complicated by the fact that new root growth was not separated from the part of the biomass that was present at the beginning of the experiment, and the two components were measured together at the final harvest. Root restriction may also have contributed to non-responsiveness of below-ground production. However, there was a very distinct distribution of fine root biomass with depth as determined from root cores, with almost 50% of root biomass present in the upper 12 cm of the soil in all treatments. Microcosm communities that were exposed to a lower water treatment overall had a higher density of root biomass in the upper soil layers. The shallow rooted nature of the grassland community could confer instantaneous benefit to growth-stimulating processes that occur predominantly in the upper layer of the soil such as nutrient mineralisation (Hungate et al. 1997; Arnone and Bohlen 1998), microbial activity (Rice et al. 1994) and earthworm activity (Zaller and Arnone, 1997). On the other hand, shallow-rootedness could easily dispose the grassland community to bush encroachment because woody shrubs would have prior access to soil water conserved under elevated CO_2 by virtue of spatial separation of their root systems (Bond and Midgley 2000).

Response of the crown biomass was highly influenced by CO_2 and water treatments, but interactively. Communities that were exposed to elevated CO_2 and a higher water treatment allocated more biomass to the crown, implying a higher rate of reserve deposition for future mobilisation in those communities. There was a definite species effect on crown biomass and the order of species contribution starting with the highest was: *Eragrostis* > *Sporobolus* > *Themeda* > *Andropogon* > *Alloteropsis*. Two questions that arise from these data are (i) whether grass species that respond to elevated CO_2 by development of new tillers would have larger crowns than species

that respond through development of leaf area? (ii) what the long-term benefits of either mode of response would be with regards to competition?

The amount of surface litter accumulated at the end of the growing season comprised about 5-10% of community above-ground production. An average value was about 6 g per unit ground area of 0.159 m^2. Contribution of the two dominant grass species (*Sporobolus* and *Themeda*) to surface litter was proportionally higher than the contribution of other species. Senesced plant material started falling from the canopy after full canopy development. There were no significant differences in treatment effect on amount of surface litter accumulation in each of the three years, and even when considered as a three year cumulative. Lack of treatment effect on surface litter could also imply that the physical attributes such as insulation of soil surface that limits evaporation of soil water, and promotion of water infiltration were not influenced by the presence of different amounts of litter. It is also considered that the negative effects usually associated with presence of plant litter in communities (Xiong and Nilsson 1999) is not likely to have influenced the response of the microcosm communities to treatments, because a meta-analysis by Xiong and Nilsson (1999) suggested that litter quantities of less than 200 g m^{-2} are commonly associated with positive effects on plant communities. Soil organic matter content of the microcosms was also not significantly different among treatments after three years of the experiment, and it measured an average of just under 8% across treatments. Most of the soil organic matter input comes from root litter, even though some of the surface litter may eventually form soil organic matter after decomposition (though grass litter is known to have very low decomposition rates (Cornelissen and Thompson 1997)). Lack of treatment effect on soil organic matter content of the microcosms may be indicative of non-responsiveness of root growth to treatment, or a physical restriction on root growth by pot size.

8.3. Community water use

Three direct methods of measurement *viz.*, evapotranspiration by lysimetry, change in pot mass, and soil water content, were used to assess treatment effect on community water use, as discussed in Chapter 5. Canopy water vapour exchange (Chapter 6) was also measured to assess community evapotranspiration. A further indirect assessment of treatment effect was inferred from gas exchange measurements of canopy water

vapour fluxes. Overall, elevated CO_2 reduced community evapotranspiration, and the highest recorded cumulative reduction was 12% under elevated CO_2 + MAR relative to a 10.2% reduction under elevated CO_2 + 80%MAR in the first year. It is noteworthy that maximum reduction in evapotranspiration occurred at elevated CO_2 + MAR in all three years of study, as did maximum enhancement of biomass. Even though cumulative evapotranspiration seemed higher in the second year relative to the first year, WUE was higher. The lowest reduction in evapotranspiration was recorded in the third year.

Reduction of community evapotranspiration under elevated CO_2 is a culmination of several phenomena operating at different scales of community organisation (leaf stomatal conductance, leaf transpiration, sap flow, energy balance etc) and sometimes logistics do not permit assessment of all of these parameters in a single study. But, analysis of data in the literature shows trends of positive effects of elevated CO_2 on these various parameters that serve as indicators of community water use. The tallgrass prairie has been extensively studied in this regard, and reductions in stomatal conductance, canopy conductance, sap flow and evapotranspiration have been measured (Ham et al. 1995) as well as reductions in transpiration (Bremer et al. 1996). A 22% reduction in ET was measured in the tallgrass prairie relative to the 10% measured in the current study. In a model grassland community derived from the Negev in Israel, Grünzweig and Körner (2001) measured 2% reduction in ET under an elevated CO_2 treatment of 400 ppm and 11% reduction under 600 ppm.

Reduction in ET resulted in higher volumetric soil water content measured under elevated CO_2 in the current study, and the trend was further confirmed by a measurable increase in mass of plant pots due to water accumulation in the soil. Soil water content was found to increase with soil depth, hence the soil in the rooting layer was found to be on average 20% wetter than soil on the surface under elevated CO_2. Improved soil water status of 10-28% was measured in a study using grassland assemblages (Volk et al. 2000). Deep drainage has also been observed to increase under elevated CO_2 in some grassland studies as a consequence of soil water accumulation under elevated CO_2 (Jackson et al. 1998; Grünzweig and Körner 2001), especially during the wetter part of the growing season and not during the drier part of the growing season (Grünzweig and Körner 2001). In the current study, drainage loss

was measured only when water application was in excess of the equivalent of 25mm rainfall event during the first year. Treatment effects on drainage loss were not significant. Drainage was subsequently not measured in the second and third growing seasons.

The effect of swapping water treatments between MAR and 120%MAR and vice versa in the third year were not profound, and even a reduction in biomass production that occurred in the last year could not be attributed to such an experimental manipulation.

8.5. Concluding remarks

Five key questions of the study are reiterated at the beginning of this Chapter, followed by an account of how the results interrelate. This section of the thesis uses the synthesised data to provide answers to the key questions. The data provides satisfactory answers to some questions, while other questions cannot be sufficiently answered.

The first two questions address impacts of elevated CO_2 on above-ground production and canopy structure, and answering them requires an integration of leaf-level gas exchange and whole-plant characteristics (phenology, plant structure and biomass allocation patterns). Measurements were done predominantly at the community level relative to the leaf-level because earlier greenhouse and field studies by Wand et al. (2001, 2002) extensively characterised leaf-level responses of species used in this study. Results of leaf gas exchange (Chapter 7) show a down regulation of photosynthesis in the C_3 grass *Alloteropsis*, and in a C_4 grass *Andropogon* under elevated CO_2. Photosynthetic up regulation under elevated CO_2 was measured in the C_4 grasses *Eragrostis*, *Sporobolus*, and *Themeda*. In turn, the species that underwent photosynthetic down regulation (*Alloteropsis* and *Andropogon*), contributed less to community biomass production under elevated CO_2, while two of the species that underwent photosynthetic up regulation (*Sporobolus* and *Themeda*) contributed more than 50% of community above-ground production. Biomass production of *Eragrostis* was intermediate. Canopy structure was mostly influenced by species that contributed higher biomass in the higher layers of the canopy *viz. Sporobolus* and *Themeda*. Placement of leaf biomass in upper canopy layers enabled better light harvesting. The

competitive edge of *Sporobolus* and *Themeda* was further attributed to early sprouting under elevated CO_2, and all of these factors together culminated in a 12% increase in community above-ground production under elevated CO_2.

The response to elevated CO_2 of above-ground production was higher under MAR than other watering treatments (80% MAR and 120%MAR). This point answers the third question of whether responses to elevated CO_2 would be influenced by an interaction with variable watering treatment.

Data on species-level responses does not sufficiently answer the third question on effects of elevated CO_2 on proportional representation of photosynthetic functional types (C_3 and the three variants of the C_4). Clearly, the C_3 species (*Alloteropsis*) did not respond positively to elevated CO_2, but one of the C_4 species (*Andropogon*) did not respond positively to elevated CO_2. More importantly, *Andropogon* and *Themeda* both belong to the NADP-me C_4 subtype, yet their biomass production, leaf gas exchange, sprouting and flowering were different.

The fourth question addresses long-term impacts of elevated CO_2 on community water use. Evidently, the data suggest that community level water use of South African C_4-dominated grasslands will be improved under elevated CO_2 as consequences of improved leaf-level water use efficiency and 12% reduction in community evapotranspiration. As a result, 20% higher soil water content was measured in microcosm communities exposed to elevated CO_2, even at the end of the growing season. A study conducted by Stock et al. (2004) at a South African natural CO_2 spring (occurring at the field site from which experimental material for the current study was derived) measured higher soil water content at end of growing season at the sites closest to the CO_2 source, for three consecutive years. Whether similar responses of improved community water use will be realised at a landscape level depends on a number of other interacting environmental parameters. It would also be interesting to do an analysis of catchment run-off data of the past 50 years to see if any trends emerge that could perhaps be associated with increases in concentrations of atmospheric CO_2.

In conclusion, the data suggest that the long-term implications (key question 5) for elevated CO_2 on South African grasslands will be characterised by enhanced water use efficiency and biomass production. However, a response of increased biomass production may be transient while water use efficiency may be longer lasting. Implications of effects of elevated CO_2 on C_4-dominated grasslands at a landscape scale, particularly in their role as water catchments, may be greatly influenced by catchment management styles. This study represents the first investigation on combined effects of elevated CO_2 and controlled water treatment on a South African natural grassland community.

References

Alberto AMP, Ziska LH, Cervancia CR and Manalo PA (1996) The influence of increasing carbon dioxide and temperature on competitive interactions between a C_3 crop, rice (*Oryza sativa*) and a C_4 weed (*Echinochloa glabrescens*). *Australian Journal of Plant Physiology* **23**, 795-802.

Allen SE (1989) *Chemica Analysis of Ecological Material*. 2nd Edition. Blackwell Scientific Publication, Oxford, UK.

Anten NPR and Hirose T (2001) Limitations on photosynthesis of competing individuals in stands and the consequences for canopy structure. *Oecologia* **129**, 186-196.

Arnone JA III and Bohlen PJ (1998) Stimulated N_2O flux from intact grassland monoliths after two growing seasons under elevated atmospheric CO_2. *Oecologia* **116**,331-335.

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

Arp WJ, Drake BG, Pockman Wt, Curtis PS and Whigham DF (1993) Interactions between C_3 and C_4 salt marsh plant species during fours of exposure to elevated CO_2. *Vegetatio* **104/105**, 133-143.

Ashwell A (ed.) (2001) *The heat is on: Impacts of climate change on plant diversity in South Africa*. pp 4-5. National Botanical Institute, Cape Town, South Africa.

Barnes PW, Beyschlag W, Ryel R, Flint SD, and Caldwell MM (1990) Plant competition for light analysed with a multispecies canopy model III. Influence of canopy structure in mixtures and monocultures of wheat and wild oat. *Oecologia* **82**, 560-566.

Barrett DJ and Gifford RM (1995) Photosynthetic acclimation to elevated CO_2 in relation to biomass allocation in cotton. *Journal of Biogeography* **22**, 331-339.

Barnola JM, Raynaud D, Korotkevitch YS and Lorius C (1987) Vostok ice core: A 160 000-year record of atmospheric CO_2 concentration. *Nature* **329**, 408-411.

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

Belsky AJ (1992) Effects of grazing competition, disturbance and fire on species composition and diversity in grassland communities. *Journal of Vegetation Science* **3**, 187-200.

Beyschlag W, Barnes PW, Ryel R, Caldwell MM and Flint SD (1990) Plant competition for light analysed with a multispecies canopy model. II. Influence of photosynthetic characteristics on mixtures of wheat and wild oat. *Oecologia* **82,** 374-380.

Bezemer TM, Thompson LJ and Jones TH (1998) *Poa annua* shows inter-generational differences in response to elevated CO_2. *Global ChangeBiology* **4**, 687-691.

Bond WJ and Midgley GF (2000) A proposed CO_2-controlled mechanism of wood plant invasion in grasslands and savannas. *Global Change Biology* **6**, 1-5.

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

Bowes G (1996) Elevated carbon dioxide. In: *Advances in Photosynthesis, Vol. 3. Environmental Stress and Photosynthesis* (ed. Baker N), pp 387-407. Academic Press, Dordrecht.

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

Bremmer DJ, Ham JM and Owensby CE (1996) Effect of elevated atmospheric carbon dioxide and open-top chambers on transpiration in a tallgrass prairie. *Journal of Environmental Quality* **25**, 691-701.

Campbell BD, Stafford Smith DM and McKeon GM (1997) Elevated CO_2 and water supply interactions in grasslands: a pastures and rangelands management perspective. *Global Change Biology* **3**, 177-187.

Campbell BD and Stafford Smith DM (2000) A synthesis of recent global change research on pasture and rangeland production: reduced uncertainties and their management implications. *Agriculture, Ecosystems and Environment* **82**, 39-55.

Cao M and Woodward I (1998) Net primary and ecosystem production and carbon stocks of terrestrial ecosystems and their responses to climate change. *Global Change Biology* **4**, 185-198.

Causton DR and Dale MP (1990) The monomolecular and rectangular hyperbola as empirical models of the response of photosynthetic rate to photon flux density, with application to three *Veronica* species. *Annals of Botany* **65**,389-394.

Chaves MM and Pereira JS (1992) Water stress, CO_2 and climate change. *Journal of Experimental Botany* **43**, 1131-1139.

Chiariello NR and Field CB (1996) Annual grassland responses to elevated CO_2 in multi-year community microcosms. In: *Carbon Dioxide, Populations, and Communities* (eds. C. Körner and FA Bazzaz), pp 139-157. Academic Press, Inc., San Diego, CA.

Collatz GJ (1977) Influence of certain environmental factors on photosynthesis and photorespiration in *Simmondsia chinensis*. *Planta* **134**, 127-132.

Collatz GJ, Berry JA and Clark JS (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.

Cornelissen JHC and K Thompson (1997) Functional leaf attributes predict litter decomposition rate in herbaceous plants. *New Phytologist* **135**,109-114.

Craine JM and Reich PB (2001) Grassland species effects on soil CO_2 flux track the effects of elevated CO_2 and nitrogen. *New Phytologist* **150**, 425-434.

Curtis PS, Drake BG, Leadley PW, Arp WJ and Whigham DF (1989a) Growth and senescence in plant communities exposed to elevated CO_2 concentrations on estuarine marsh. *Oecologia* **78**, 20-29.

Curtis PS, Drake BG and Whigham DF (1989b) Nitrogen and carbon dynamics in C_3 and C_4 estuarine marsh plants grown under elevated CO_2 in situ. *Oecologia* **78**, 297-301.

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

Dore S, Hymus GJ, Johnson DP, Hinkle CR, Valentini R and Drake BG (2003) Cross validation of open-top chamber and eddy covariance measurements of ecosystem CO_2 exchange in a Florida scrub-oak ecosystem. *Global Change Biology* **9**, 84-95.

Drake BG Leadley PW, Arp WJ, Nassiry D and Curtis PS (1989) An open top Chamber for field studies of elevated atmospheric CO_2 concentration on saltmarsh vegetation. *Functional Ecology* **3**, 363-371.

Drake BG and Leadley PW (1991) Canopy photosynthesis of crops and native plants communities exposed to long-term elevated CO_2 treatment. *Plant, Cell and Environment* **14**, 853-860.

Drake BG, Peresta G, Beugeling E and 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. GW Koch and HA Mooney), pp197-214. Academic Press, San Diego.

Drake BG, Gonzàlez-Meler MA and Long SP (1997) More efficient plants: a consequence of rising atmospheric CO_2? *Annual Review of Plant Physiology and Plant Molecular Biology* **48**, 609-638.

Drake BG, Azcon-Bieto J, Berry J, Bunce J, Dijkstra P, Farrar J, Gifford RM, Gonzalez-Meler, MA, Kock G, Lambers H, Siedow J and Wullschleger S

(1999) Does elevated atmospheric CO_2 concentration inhibit mitochondrial respiration in green plants? *Plant Cell and Environment* **22**, 649-657.

Dunin FX, McIlroy IC and O'Loughlin EM (1983) A lysimeter characterization of evaporation by eucalypt forest and its representativeness for the local environment. The Forest-Atmosphere Interaction; *Proceedings of the Forest Environmental Measurements Conference*, Oak Ridge, Tenn., D. Reidel Publ., 271-291.

Eamus D (1991) The interaction of rising CO_2 and temperature with water use efficiency. *Plant, Cell and Environment* **14**, 843-852.

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

Ehleringer JR, Sage RF, Flanagan LB and Pearcy RW (1991) Climate change and the evolution of C_4 photosynthesis *Trends in Ecology and Evolution* **6**, 95-99.

Ehleringer JR, Cerling TE and Helliker BR (1997) C_4 photosynthesis, atmospheric CO_2, and climate. *Oecologia* **112**, 285-299.

Ellery WN, Scholes RJ and Mentis MT (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.

Ellsworth DS and Reich PB (1992) Leaf mass per area, nitrogen content and photosynthetic carbon gain in *Acer Saccharum* seedlings in contrasting light environments. *Functional Ecology* **6**, 423-435.

Epstein HE, Burke IC and Lauenroth WK (1999) Responses of the shortgrass steppe to changes in rainfall seasonality. *Ecosystems* **2**, 139-150.

Everson CS, Everson TM and Tainton NM (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.

Eviner VT and Chapin III FS (2003) Functional matrix: a conceptual framework for predicting multiple plant effects on ecosystem processes. *Annual Review of Ecology, Evolution, and Systematics* **34**, 455-485.

Farnsworth EJ and Bazzaz FA (1995) Inter- and intra generic differences in growth, reproduction and fitness of nine herbaceous annual species grown in elevated CO_2. *Oecologia* **104**, 139-146.

Field CB, Chapin III FS, Matson PA and Mooney HA (1992) Response of terrestrial ecosystems to the changing atmosphere: A resource based approach. *Annual Review of Ecology and Systematics* **23**, 210-235.

Field CB, Jackson RB and Mooney HA (1995) Stomatal responses to increased CO_2: implications from the plant to the global scale. *Plant, Cell and Environment* **18**, 1214-1225.

Field CB, Chapin III FS, Chiariello NR, Holland EA and Mooney HA (1996) The Jasper Ridge CO_2 experiment: Design and motivation . In: *Carbon Dioxide and Terrestrial Ecosystems* (eds. GW Koch and HA Mooney), pp121-145. Academic Press, San Diego.

Field CB, Lund CP, Chiariello NR and Mortimer BE (1997) CO_2 effects on the water budget of grassland microcosm communities. *Global Change Biology* **3,** 197-206.

Firbank LG and Watkinson AR (1990) On effects of competition: from monocultures to mixtures. In: *Perspectives on plant competition.* (eds. JB Grace and D Tilman), pp 165-192. Academic Press, London, UK.

Fraser LH and Keddy P (1997) The role of experimental microcosms in ecological research *Trends in Ecology and Evolution* **12**, 478-481.

Fredeen AL, Koch GW and Field CB (1995) Effects of atmospheric CO_2 enrichment on ecosystem CO_2 exchange in a nutrient and water limited grassland. *Journal of Biogeography* **22**, 215-219

Fredeen AL, Randerson JT, Holbrook NM and Field CB (1996) Elevated atmospheric CO_2 increases water availability in a water-limited grassland ecosystem. *Journal of American Water Resource Association* **33**, 1033-1039.

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

Gaudet CL and Keddy PA (1988) A comparative approach to predicting competitive ability from plant traits. *Nature* 334, 242-243.

Ghannoum O, von Caemmerer S, and Conroy JP (2001) Carbon and water economy of Australian NAD-ME and NADP-ME C_4 grasses. *Australian Journal of Plant Physiology* **28**, 213-223.

Ghannoum O, Siebke K, von Caemmerer S and Conroy JP (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 EWR and Conroy JP (1997) The effect of CO_2 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.

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

Gill R, Polley HW, Johnson HB, Anderson LJ, Maherali H and Jackson RB (2002) Non-linear grassland responses to past and future atmospheric CO_2. *Nature* **417**, 279-282.

Grulke NE, Riechers GH, Oechel WC, Hjelm U and Jaeger C (1990) Carbon balance of tussock tundra under ambient and elevated atmospheric CO_2. *Oecologia* **83**, 485-494.

Grünzweig JM and Körner C (2001) Growth, water and nitrogen relations in grassland model ecosystems of the semi-arid Negev of Israel exposed to elevated CO_2. *Oecologia* **128**, 251-262.

Grünzweig JM and Körner C (2000) Growth and reproductive responses to elevated CO_2 in wild cereals of the northern Negev of Israel. *Global Change Biology* **6**, 631-638.

Ham JM, CE Owensby and PI Coyne (1993) Technique for measuring air flow and CO_2 flux in large open-top chambers. *Journal of Environmental Quality* **22**, 759-766.

Ham JM, Owensby CE, Coyne PI and Bremer DJ (1995) Fluxes of CO_2 and water vapour form a prairie ecosystem exposed to ambient and elevated atmospheric CO_2. *Agricultural and Forest Meteorology* **77**, 73-93.

Hamerlynck EP, McAllister CA, Knapp AK and Owensby CE (1997) Photosynthetic gas exchange and water relation responses of three tallgrass prairie species to elevated carbon dioxide and moderate drought. *International Journal of Plant Science* **158**, 608-616.

Hartz-Rubin JS and DeLucia EH (2001) Canopy development of a model herbaceous community exposed to elevated atmospheric CO_2 and soil nutrients. *Physiologia Plantarum* **113**, 258-266.

Hattas D (2002) *Phytochemical changes in leaves of sub-tropical grasses and Fynbos shrubs at elevated atmospheric CO_2 concentrations*. MSc Thesis, University of Cape Town.

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

Hattersly PW and Watson L (1992) Diversification of photosynthesis. In: *Grass Evolution and Domestification* (ed. GP Chapman), pp. 38-116. Cambridge University Press, Cambridge.

Henderson S, Hattersly P, von Caemmerer S and Osmond CB (1995) Are C_4 Pathway Plants Threatened by Global Climatic Change? In: *Ecophysiology of photosynthesis* (eds. ED Schulze and MM Caldwell), pp. 529-549. Heidelberg: Springer-Verlag, Berlin.

Hirose T and Werger (1995) Canopy structure and photon flux partitioning among species in a herbaceous plant community. *Ecology* **76**, 466-474.

Howell TA, Schneider AD and Jensen ME (1991) History of lysimeter design and use for evapotranspiration measurements. In: *Lysimeters for Evapotranspiration and Environmental Measurements* (eds. Allen RG et al.), pp 1-9. American Society of Civil Engineers. Reston, Va.

Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linden PJ, Dai X, Maskell K and Johnson CA (eds.) (2001) *Climate Change 2001: The Scientific Basis. Contributions of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press, Cambridge, UK.

Huebert B (1999) IGBP's vision scares me. *IGBP News Letter* **No. 38**, 10-11.

Hulbert LC (1988) Causes of fire effects in tallgrass prairie. *Ecology* **69**, 46-58.

Hulme M, Arntzen J, Downing T, Leemans R, Malcolm J, Reynard N, Ringrose S, Rogers D, Chiziya E, Conway D, Joyce A, Jain P, Magadza C, Markham A, and Mulenga H (1996) *Climate Change and southern Africa: an extrapolation of some potential impacts and implications in the SADC region.* Report to WWF International, University East Anglia, Climate Research Unit and WWF International, Gland Switzerland, 104pp.

Hungate BA, Holland EA, Jackson RB, Chapin III FS, Mooney HA and Field CB (1997) On the fate of carbon in grasslands under carbon dioxide enrichment *Nature* **388**, 576-579.

Hunt HW, Elliott ET, Detling JK, Morgan JA and Cheng D-X (1996) Responses of a C_3 and C_4 perennial grass to elevated CO_2 and temperature under different water regimes. *Global Change Biology* **2**, 35-47.

Huxman TE, Hamerlynck EP, Moore BD, Smith SD, Jordan DN, Zitzser SF, Nowak RS, Coleman JS and Seemann JR (1998) Photosynthetic down-regulation in *Larrea tridentata* exposed to elevated atmospheric CO_2: interaction with drought under glasshouse and field (FACE) exposure. *Plant, Cell and Environment* **21**, 1153-1161.

Idso KE (1998) CO_2-induced global warming: a sceptic's view of potential climate change. *Climate Research* **10**, 69-82.

Intergovernmental Panel on Climate Change (IPCC) 1990. *Climate change - The IPCC scientific assessment.* Houghton JT, Jenkins GJ and Ephraums JJ, (eds.) The Cambridge University Press, Cambridge, p365.

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

Jablonski LM, Wang X and Curtis PS (2002) Plant reproduction under elevated CO2 conditions: a meta-analysis of reports on 79 crop and wild species. *New Phytologist* **156**, 9-26.

Jach ME and Ceulemans R (1999) Effects of elevated atmospheric CO_2 on phenology, growth and crown structure of Scots pine (*P. sylvestris*) seedlings after two years of exposure in the field. T*ree Physiology* 19, 289-300.

Jackson RB, Sala OE, Field CB and Mooney HA (1994) CO_2 alters water use, carbon gain, and yield for the dominant species in a natural grassland. *Oecologia* **98**, 257-262.

Jackson RB, Lou Y, Gardon ZG, Sala OE, Field CB and Mooney HA (1995) Photosynthesis, growth and density for the dominant species in a CO_2-enriched grassland. *Journal of Biogeography* **22**, 221-225.

Jackson RB, Sala OE, Paruelo JM and Mooney HA (1998) Ecosystem water fluxes for two grasslands in elevated CO2: a modelling analysis. *Oecologia* **113**, 537-546.

Jackson RB, Lechowicz MJ, Li X and Mooney HA (2001) Phenology, growth, and allocation in global terrestrial productivity. In: *Terrestrial Global Productivity: Past, Present, and Future.* (eds. B Saugier, J Roy, HA Mooney), pp 61-82. Academic Press, San Diego.

Jackson RB, Banner JL, Jobbágy EG, Pockman WT and Wall DH (2002) Ecosystem carbon loss with woody plant invasions of grasslands *Nature* **418**, 623-626.

Jarvis AJ, Mansfield TA and Davies WJ (1999) Stomatal behaviour, photosynthesis and transpiration under rising CO_2. *Plant Cell and Environment* **22**, 639-648.

Joffre R and Rambal S (1993) How tree cover influences the water balance of Mediterranean rangelands. *Ecology* **74**, 570-582.

Jones P, Allen, LH Jr. and Jones JW (1985) Responses of soybean canopy photosynthesis and transpiration to whole-day temperature changes in different CO2 environments. *Agronomy Journal* 77, 242-249.

Jurik TW and Pleasants JM (1990) The effects of light and plant size on fate of seedlings of the prairie compass plant (*Silphium laciniatum* L.). *Functional Ecology* **4**, 661-665.

Keeling C, Bacastow R, Carter A, Piper S, Whorf T, Heimann M, Mook W and Roeloffzen H. (1989) A three-dimensional model of atmospheric CO_2 transport based on observed winds 1. Analysis of observational data. In: *Aspects Of Climate Variability In The Pacific And The Western Americas* (ed. DH Peterson), pp 165-236. Washington DC.

Keepin W, Mintzer I and Kristoreson L (1986) Emission of CO_2 into the atmosphere: The rate of release of CO_2 as a function of future energy developments. In: *The greenhouse effect, climatic change and ecosystems.* (eds. Bolin B, Döös BR, Jager J, and Warrick RA), pp 35-91 Scope 29. John Wiley, Chichester.

Knapp AK (1984) Water relations and growth of three grasses during wet and drought years in a tallgrass prairie. *Oecologia* **65**, 35-43.

Knapp AK, Hamerlynck EP and Owensby CE (1993a) Photosynthetic and water relations responses to elevated CO_2 in the C_4 grass *Andropogon geradii. International Journal of Plant Science* **154**, 459-466.

Knapp AK, Fahnstock JT and Owensby CE (1993b) Elevated atmospheric CO_2 alters stomatal responses to sunlight in a C_4 grass. *Plant, Cell and Environment* **17**, 189-195.

Knapp AK, Bargmann N, Maragni LA, McAllister CA, Bremmer DJ, Ham JM and Owensby CE (1999) Elevated CO_2 and leaf longevity in the C_4-grassland dominant *Andropogon geradii. International Journal of Plant Sciences* **160**, 1057-1061.

Körner C (2000) Biosphere responses to CO_2 enrichment. *Ecological Applications* **10**, 1590-1619.

Körner C (2001) Experimental plant ecology: some lessons from global change research. In: *Ecology: Achievement and Challenge BES 41*(eds. Press MC, Huntly NJ and Simon Levin), pp 227-247. Blackwell Publishing.

Kramer K, Friend A and Leinonen I (1996) Modelling comparison to evaluate the importance of phenology and spring frost damage for the effects of climate change on growth of mixed temperate-zone deciduous forest. *Climate Research* **7**, 31-41.

Lambers H and Poorter H (1992) Inherent variation in growth rate between higher plants: A search for physiological causes and ecological consequences. *Advances in Ecological Research* **23**, 187-261.

Lane DR, Coffin DP and Lauenroth WK (1998) Effects of soil texture ad precipitation on above-ground net primary productivity and vegetation structure across the Central Grassland region of the United States. *Journal of Vegetation Science* **9**, 239-250.

Lane DR, Coffin DP and Lauenroth WK (2000) Changes in grassland canopy structure across a precipitation gradient. *Journal of Vegetation Science* **11**, 359-368.

Lauenroth WK and Coffin DP (1992) Below-ground processes and the recovery of semiarid grasslands from disturbance. In: *Ecosystem rehabilitation: preamble*

to sustainable development. Volume 2: Ecosystem analysis and synthesis (ed. MK Wali), pp131-150. SPB Academic Publishing, The Netherlands.

Lavorel S (2002) Plant functional types. In *The Earth System: Biological and Ecological Dimensions of Global Environmental Change, Vol. 2* (eds. Mooney HA and Candell J). pp 481-489. John Wiley & Sons. Chichester. UK.

Le Cain DR and Morgan JA (1998) Growth, grass 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.

Le Maitre D, Scott DF and Colving C (1999) A review of information on interactions between vegetation and ground water. *Water South Africa* **25**, 137-152.

Long SP, Garcia Moya E, Imbamba SK, Kamnalrut A, Piedade MTF, Scurlock JMO, Shen YK and Hall DO (1989) Primary productivity of natural grass ecosystems of the tropics: a reappraisal. *Plant and Soil* **115**, 155-166.

Long SP, Jones MB and Roberts MJ eds. (1992) *Primary Productivity of Grass Ecosystems of the Tropics and Sub-tropics*. Chapman and Hall, London. 267 pp.

Luo Y, Chen J-L, Reynolds JF, Field CB and Mooney HA (1997) Disproportional increases in photosynthesis and plant biomass in a Californian grassland exposed to elevated CO_2: a simulation analysis. *Functional Ecology*, **11**:696-704.

McLeod AR and Long SP (1999). Free-air carbon dioxide enrichment (FACE) in global change research: a review. *Advances in Ecological Research* **28**, 1-56.

McRae SG (1988) *Practical Pedology: Studying Soils in the Field.* Ellis Horwood Limited, Chichester, England. Pp 227.

Mentis MT and Huntley BJ (1982) *A description of the Grassland Biome Project. South African National Scientific Programmes report no: 62.* CSIR, Pretoria.

Midgley GF, Stock WD and Juritz JM (1995) Effects of elevated CO_2 on Cape Fynbos species adapted to different nutrient status: nutrient and CO_2-responsiveness. *Journal of Biogeography* **22**, 185-191.

Mooney HA (1991) Biological response to climate change: an agenda for research. *Ecological Applications* **1**, 112-117.

Mooney HA, Drake BG, Luxmoore RJ, Oechel WC and Pitelka LF (1991) Predicting ecosystem responses to elevated CO_2. *Bioscience* **41**, 96-104.

Morgan JA, Hunt HW, Monz CA and Le Cain DR (1994) Consequences of long term growth at various [CO_2] and temperatures for leaf gas exchange of

Pascopyrum smithii (C_3) and *Bouteloua gracilis* (C_4). *Plant Cell and Environment* **17**, 1023-1033.

Morgan JA, LeCain DR and Read JJ (1998) Photosynthetic pathway and ontogeny affect water relations and the impact of CO_2 on *Bouteloa gracilis* (C_4) and *Pascopyrum smithii* (C_3). *Oecologia* **114**, 483-493.

Morgan JA, LeCain DR, Mosier AR and Milchunas DG (2001) Elevated CO_2 enhances water relations and productivity and affects gas exchange in C_3 and C_4 grasses of the Colorado shortgrass steppe. *Global Change Biology* **7**: 451-466.

Morrison JIL (1993) Response of plants to CO_2 under water limited conditions. *Vegetatio* **104/105**, 193-209.

Morison JIL and Gifford RM (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.

Morrow CD (2002) *The effects of elevated atmospheric CO_2 on below-ground processes in Fynbos and C_4 ecosystems*. PhD thesis. University of Cape Town.

Mott KA (1988) Do stomata respond to CO_2 concentrations other than intercellular? *Plant Physiology* **86**, 200-203 .

Navas M-L, Garnier E, Austin MP and Gifford RM (1999) Effect of competition on the response of grasses and legumes to elevated atmospheric CO_2 along a nitrogen gradient: differences between isolated plants and multi-species mixtures. *New Phytologist* **143**, 323-331.

Neftel A, Moor E, Oeschger H and Stauffer B (1985) Evidence from polar ice cores for the increase in atmospheric CO_2 in the past two centuries. *Nature* **315**, 45-47.

Ni J (2003) Plant functional types and climate change along a precipitation gradient in temperate grasslands, north-east China and south-east Mongolia. *Journal of Arid Environments* **53**, 501-516.

Nijs I, Impens I and Behaeghe T (1989) Effects of long-term elevated atmospheric CO_2 concentration on *Lolium perenne* and *Trifolium repens* canopies in the course of a terminal drought stress period. *Canadian Journal of Botany* **67**, 2720-2725.

Niklaus PA, Leadley PW, Stocklin J and Korner C (1998) Nutrient relations in calcareous grassland under elevated CO_2. *Oecologia* **116**, 67-75.

Norman JM and Campbell GS (1989) Canopy structure. In: *Plant Physiological Ecology; Field methods and instrumentation* (eds. Pearcy RW, Ehleringer J R, Mooney HA and Rundel PW), pp 301-325. Chapman and Hall, New-York.

Oechel WC, Vourlitis GL, Hastings SJ, Zulueta RC, Hinzman L and Kane D (2000) Acclimation of ecosystem CO_2 exchange in the Alaskan Arctic in response to decadal climate warming. *Nature* **406**, 978-981.

Owensby CE, Hyde RM and Anderson KL (1969) Effects of clipping and supplemental nitrogen and water on loamy upland bluestem range. *Journal of Range Management* **23**, 341-346.

Owensby CE, Coyne PI, Ham JM, Auen LM and Knapp AK (1993) Biomass production in a tallgrass prairie ecosystem exposed to ambient and elevated CO_2. *Ecological Applications* **3**, 644-653.

Owensby CE, Ham JM, Knapp AK, Rice CW, Coyne PI and Auen LM (1996) Ecosystem-level Responses of Tallgrass Prairie to Elevated CO_2. In: *Carbon Dioxide and Terrestrial Ecosystems* (eds. Koch GW and Mooney HA), pp. 147-162. Academic Press, San Diego.

Owensby CE, Ham JM, Bremer D and Auen LM (1997) Water vapour fluxes and their impact under elevated CO_2 in a C_4-tallgrass prairie. *Global Change Biology* 3, 189-195.

Owensby CE, Ham JM, Knapp AK and Auen LM (1999) Biomass production and species composition change in a tallgrass prairie ecosystem after long-term exposure to elevated atmospheric carbon dioxide. *Global Change Biology* **5**, 497-506.

Pacala SW, Hurtt GC, Baker D, Peylin P, Houghton RA, Birdsey RA, Heath L, Sundquist ET, Stallard RF, Ciais P, Moorcroft P, Caspersen JP, Shevliakova E, Moore B, Kohlmaier G, Holland E, Gloor M, Harmon ME, Fan S-M, Sarmiento JL, Goodale CL, Schimel D and Field CB (2001) Consistent land and atmosphere-based U.S. carbon sink estimates. *Science* **292**, 2316-2320.

Passioura JB (1984) Hydraulic resistance of plants. I. Constant or variable? *Australian Journal of Plant Physiology* **11**, 333-339.

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

Penuelas J, Biel C and Estiarte M (1995) Growth, biomass allocation, and phenology response of pepper to elevated CO_2 concentrations and different nitrogen and water supply. *Photosynthetica* **3**, 91-99.

Perry PL (1994) A revision of the genus *Eriospermum* (Eriospermaceae). *Bolus Herbarium Contributions* **17**, 1-320.

Pitelka LF (1994) Ecosystem response to elevated CO_2. *Trends in Ecology and Evolution* **9**, 204-206.

Pettersson R and McDonald JS (1994) Effects of nitrogen supply on the acclimation of photosynthesis to elevated CO_2. *Photosynthesis Research* **39**, 389-400.

Polley HW (1997) Implications of rising atmospheric carbon dioxide for rangelands. *Journal of Rangeland Management* **50**, 562-577.

Polley HW, Johnson HB, Mayeux HS and Tischler CR (1996) Are Some of the Recent Changes in Grassland Communities a Response to Rising CO_2 Concentrations? In: *Carbon Dioxide, Populations, and Communities* (eds. Körner C and Bazzaz FA), pp 177-195. Academic Press, San Diego.

Polley HW, Johnson HB and Tischler CR (2002). Woody invasion of grasslands: evidence that CO_2 enrichment indirectly promotes establishment of *Prosopis glandulosa*. *Plant Ecology* **164**, 85-94.

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 and Navas M-L (2003) Plant growth and competition at elevated CO_2: on winners, losers and functional groups. *New Phytologist* **157**, 175-198.

Potvin C and Strain BR (1985) Effects of CO_2 enrichment and temperature on growth in two C_4 weeds, *Echinochloa crus-galli* and *Eleusineindica*. *Canadian Journal of Botany* **63**, 1495.

Radoglou KM, Aphalo P and Jarvis PG (1992) Response of photosynthesis, stomatal conductance and water use efficiency to elevated CO_2 and nutrient supply in acclimated seedlings of *Phaseolus vulgaris* L. *Annals of Botany* **70**, 257-264.

Rasse DP, Li J-H and Drake BG (2003) Carbon dioxide assimilation by a wetland sedge canopy exposed to ambient and elevated CO_2: measurements and model analysis. *Functional Ecology* **17**, 222-230.

Reekie EG (1996) The effect of elevated CO_2 on developmental processes and its implications for plant-plant interactions. In *Carbon dioxide, Populations and Communities*. (eds. Körner C and Bazzaz FA), pp. 333-346. Academic Press, San Diego.

Reekie EG and Bazzaz FA (1991) Phenology and growth in four annual species grown in ambient and elevated CO_2. *Canadian Journal of Botany* **69**, 2475-2481.

Reich PB, Tilman D, Craine J, Ellsworth D, Tjoelker MG, Knops J, Wedin D, Naeem S, Bahauddin D, Goth J, Bengtson W, and Lee TA (2001) Do species and functional groups differ in acquisition and use of C, N and water under varying atmospheric CO_2 and N availability regimes? A field test with 16 grassland species. *New Phytologist* **150**: 435-448.

Rice CW, Garcia FO, Hampton CO and OwensbyCE (1994) Soil microbial response in tallgrass prairie to elevated CO_2. *Plant Soil* **165**, 67–74.

Roberts MJ, Long SP, Tieszen LL and Beadle CL (1993) Measurement of plant biomass and net primary production, In: *Photosynthesis and Production in a Changing Environment: a Field and Laboratory Manual* (eds. Hall DO et al.), pp 1-21. Chapman and Hall, London.

Rogers A, Fischer BU, Bryant J, Frehner M, Blum H, Raines CA and Long SP (1998) Acclimation of photosynthesis to elevated CO_2 under low-nitrogen nutrition is affected by the capacity for assimilate utilisation. Perennial ryegrass under free-air CO_2 enrichment. *Plant Physiology* **118**, 683-689.

Ross MA and Harper JL (1972) Occupation of biological space during seedling establishment. *Journal of Ecology* **60**, 77-88.

Sala OE, Parton WJ, Joyce LA and Lauenroth WK (1988) Primary production of the central grassland region of the United States. *Ecology* **69**, 40-45.

Sala OE, Lauenroth WK and Parton WJ (1992) Long-term soil water dynamics in the shortgrass steppe. *Ecology* **73**, 1175-1181.

Scholes RJ and Archer S (1997) Tree grass interactions in savannas. *Annual Review of Ecology and Systematics* **28**, 517-544.

Schulze E-D, Bobichaux RH, Grace J, Rundel PW and Ehleringer JR (1987) Plant water balance. *Bioscience* **37**, 30-37.

Seneweera SP, Ghannoum O and Conroy J (1998) High vapour pressure deficit and low soil water availability enhance shoot growth responses of a C_4 grass (*Panicum coloratum* cv. Bambatsi) to CO_2 enrichment. *Australian Journal of Plant Physiology* **25**, 287-292.

Sindhøj E, Hansson A-C, Andren O, Katterer T, Marissink M and Petterson R (2000) Root dynamics in a semi- arid grassland in relation to atmospheric carbon dioxide enrichment, soil water, and shoot biomass. *Plant and Soil* **223**, 253-263.

Smith SD, Huxman TE, Zitzer SF, Charlet , Houseman DC, Coleman JS, Fenstermaker LK, Seemann JR and Nowak RS. (2000) Elevated CO_2 increases productivity and invasive species success in an arid ecosystem. *Nature* **408**, 79-82.

Smith TM, Shugart HH and Woodward FI (1997) *Plant Functional Types: Their Relevance to Ecosystem Properties and Global Change.* Cambridge. Cambridge University Press. 369 pp.

Sokal RR and Rohlf J (1995) *Biometry: Principles and Practice of Statistics in Biological Research.* 3rd ed. Page 507. Freeman. New York.

Son Y and Gower ST (1991) Above-ground nitrogen and phosphorus use by five plantation-grown trees with different leaf longevities. *Biogeochemistry* **14**, 167-191.

Steffen W, Noble I, Canadell J, Apps M, Schulze ED, Jarvis PG, Baldocchi D, Ciais P, Cramer W, Ehleringer J, Farquhar G, Field CB, Ghazi A, Gifford R, Heimann M, Houghton R, Kabat P, Korner C, Lambin E, Linder S, Mooney HA, Murdiyarso D, Post WM, Prentice IC, Raupach MR, Schimel DS, Shvidenko A, Valentini R (1998) The terrestrial carbon cycle: Implications for the Kyoto Protocol. *Science* **280**, 1393-1394.

Stitt M (1991) Rising CO_2 levels and their potential significance for carbon flow in photosynthetic cells. *Plant Cell and Environment* **14**, 741-762.

Stock WD, Ludwig F, Morrow C, Midgley GF, Wand SJE, Allsopp N and Bell T (2004) Long-term effects of elevated atmospheric CO_2 on species composition and productivity of a southern African C_4 dominated grassland in the vicinity of a CO_2 exhalation. *Journal of Ecology* (In press).

Taylor G, Ranasinghe S, Bosac C, Gardner SDI and Ferris R (1994) Elevated CO_2 and plant growth: cellular mechanisms and responses of whole plants. *Journal of Experimental Botany* **45**, 1761-1774.

Theta Probe User Manual (1999) ML2x-UM-1.21. Cambridge. UK.

Thomas RB and Strain BR (1991) Root restriction as a factor in photosynthetic acclimation of cotton seedlings grown in elevated carbon dioxide. *Plant Physiology* **96**, 627-634.

Titlyanova AA, Romanova IP, Kosykh NP and Mironycheva-Tokareva NP (1999) Pattern and process in above-ground and below-ground components of grassland ecosystems. *Journal of Vegetation Science* **10**, 307-320.

Tyree MT and Alexander JD (1993) Plant water relations and the effect of elevated CO_2: a review and suggestions for future research. *Vegetatio* **104/105**, 47-62.

Vogel JC, Fuls A, and Ellis RP (1978) The geographical distribution of Kranz grasses in South Africa. *South African Journal of Science* **74**, 209-219.

Volk M, Niklaus PA and Körner Ch (2000) Soil moisture effects determine CO_2 responses of grassland species. *Oecologia* **125**, 380-388.

Wand SJE (1999) *Ecophysiological responses of Themeda triandra Forsk. and other southern African C_4 grass species to increases in atmospheric CO_2 concentrations*. PhD thesis, University of Cape Town.

Wand SJE, Midgley GF, Jones MH and Curtis PS (1999) Responses of wild C_4 and C_3 grass (Poaceae) species to elevated atmospheric CO_2 concentration: a meta-analytical test of current theories and perceptions. *Global Change Biology* **5**, 723-741.

Wand SJE, Midgley GF and Stock WD (2001) Growth responses to elevated CO_2 in NADP-ME, NAD-ME and PCK C_4 grasses and a C_3 grass from South Africa. *Australian Journal of Plant Physiology* **28**, 13-25.

Wand SJE, Midgley GF and Stock WD (2002) Response to elevated CO_2 from a natural spring in a C_4-dominated grassland depends on seasonal phenology. *African Journal of Range and Forage Science* **19(2)**: 81-91.

Wayne P, Bazzaz FA (1995) Seedling density modifies the growth responses of yellow birch maternal families to elevated carbon dioxide. *Global Change Biology* **1**, 315-324.

Wayne PM, Carnelli AL, Connolly J and Bazzaz FA (1999) The density dependence of plant responses to elevated CO_2. *Journal of Ecology* **87**, 183-192.

Whalley WR (1993) Considerations on the use of time-domain reflectometry (TDR) for measuring soil moisture content. *Journal of Soil Science* **44**, 1-9.

White I, Knight JH, Zegelin SJ and Topp GC (1994) Comments of "Considerations on the use of time-domain reflectometry (TDR) for measuring soil moisture content" by WR Whalley. *Journal of soil Science* **45**, 503-508.

Williams MAJ and Balling RC Jr. eds. (1996) *Interactions of Desertification and Climate*. pp 190. Edward Arnold Press, London, England.

Wilsey BJ, Coleman JS and McNaughton SJ (1997) Effects of elevated CO_2 and defoliation on grasses: a comparative ecosystem approach. *Ecological Applications* **7**, 844-853.

Wullscgleger SD, Tschaplinski TJ and Norby RJ (2002) Plant water relations at elevated CO_2 – implications for water-limited environments. *Plant, Cell and Environment* **25**, 319-331.

Xiong S and C Nilsson (1999) The effects of plant litter on vegetation: a meta-analysis. *Journal of Ecology* **87**, 984-994.

Zaller JG and Arnone III JA (1999) Interaction between plant species and earthworm casts in a calcareous grassland under elevated CO_2. **80**, 873-881.

Zaller JG and Arnone III JA (1997) Activity of surface casting-casting earthworms in a calcareous grassland under elevated atmospheric CO_2. *Oecologia* **111**, 249-254.

Zar JH (1984) *Biostatistical Analysis*. Second Edition. Englewood Cliffs, Prentice Hall.