The effect of elevated atmospheric CO_2 on the growth and physiology of *Chromolaena odorata*

By

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Submitted in fulfilment of the academic requirements
for the degree of Masters of Science in the
School of Biological and Conservation Sciences,
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March 2008

ABSTRACT

Rising atmospheric CO_2 (C_a) concentrations have generated concern among scientists, mainly because of CO_2's role as a greenhouse gas and its influence on plant growth and development. Previous research has suggested that future CO_2 enriched atmospheres may enhance the success of invasive aliens. *Chromolaena odorata* is an example of an invasive alien proving to be a serious threat to indigenous vegetation in South Africa, and effective control measures are desperately needed to curb infestations in the future. The current study aimed at assessing the response of *C. odorata* to elevated C_a and interactive factors, and was divided into two trials. During PART A, *C. odorata* was grown in competition with 2 grass species: *Eragrostis curvula* and *Themeda triandra* (selected for their differential preferences to nutrient availability). All three species were potted in a greenhouse at the University of KwaZulu-Natal (Howard College). There were 16 pots in total, and each pot contained four *C. odorata* plants, four *T. triandra* seedlings, and four *E. curvula* seedlings. Eight pots were exposed to elevated C_a (~700ppm), and eight pots were exposed to ambient C_a (~370ppm). The pots at each C_a treatment were further divided: four received high nutrient treatments (3L per addition), while the other four received low nutrient treatments (300 ml per addition). Studies on growth (e.g. plant height, dry weight, etc.), as well as physiology (e.g. J_{max}), were undertaken. Results showed that generally, plants responded positively to high nutrient treatments. In contrast, elevated C_a did not affect growth or any of photosynthetic parameters of *C. odorata* significantly, but did reduce stomatal limitations. During PART B, *C. odorata* plants were grown monospecifically to assess whether there was a "chamber effect" associated with planting density. Pots at both C_a treatments contained either four *C. odorata* or two *C. odorata* seedlings. Growth and physiology were assessed. The fact that elevated C_a did not affect any of the photosynthetic parameters studied, suggests that photosynthetic down-regulation did not occur. This, together with the fact that no increase in stomatal limitations were observed in elevated C_a, implies that enhancement of photosynthetic assimilation could have occurred in *C. odorata* plants exposed to CO_2 enrichment. Results from this study (PART A and PART B), when compared to previous

research on this species, suggests that CO_2 enrichment may enhance the success of monoculture populations of *C. odorata*. However, other species may gain competitive advantages over *C. odorata* occurring in mixed communites, under CO_2 enriched environments. In addition, results of this study support the prediction that increasing C_a will reduce the importance of carbon as an external limiting resource, and that the extent of a plant's response to C_a enrichment will depend on resources other than CO_2. If increases in temperature caused by elevated C_a increases nutrient availability in the soil, then C_a could *indirectly* enhance the success of *C. odorata* occurring in mixed communities.

PREFACE

The experimental work described in this dissertation was carried out in the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Howard College, from January 2004 to January 2007, under the supervision of Professor Norman Pammenter.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. The work of others, where used, has been duly acknowledged in the text.

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Reshnee Lalla (Miss)

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ACKNOWLEDGEMENTS

I wish to express my gratitude to a few people, who have contributed (in various ways) to the successful undertaking of this study. Firstly, my heartfelt appreciation goes out to my family, who have supported and encouraged me every step of the way. To Professor Norman Pammenter: Thank you for being a great supervisor, and for all your help and guidance during my research. My sincere thanks also goes out to Vishal Bharuth, for his motivation, assistance, and patience. To Alana Patton, co-researcher and fellow colleague, thank you for your inspiring company and dedication to the study. I would also like to acknowledge Mr. Kay Govindsamy and Mr. Sagren Govender, for their technical assistance, as well as Dr. Robin Mackay for her statistical advice. Last but certainly not least, I wish to acknowledge the National Research Foundation (NRF), for providing funding for the undertaking of this study.

ABBREVIATIONS

ºC	degrees Celsius
%	percentage
CO_2	Carbon dioxide
N_2O	nitrous oxide
CH_4	methane
CFCs	chlorofluorocarbons
C_a	atmospheric carbon dioxide concentration
C_i	intercellular CO_2 concentration
ppm	parts per million
PCR cycle	photosynthetic carbon reduction cycle
rubisco	ribulose-1.5-bisphosphate carboxylase/oxygenase
RuBP	ribulose-1.5-bisphosphate
$V_{c,max}$	maximum carboxylation velocity of rubisco
J_{max}	maximum electron transport rate
A_{max}	maximum net CO_2 assimilation rate
$H_2O_{(g)}$	water vapour
g_c	stomatal conductance to CO_2
g_w	stomatal conductance to water vapour
FACE	Free Air CO_2 enrichment
WUE	water-use efficiency
LAR	leaf area ratio
SLA	specific leaf area

SD	stomatal density
P	phosphorus
Pi	inorganic phosphate
N	nitrogen
K	potassium
C/N ratio	carbon/nitrogen ratio
CPR	crop performance ratio
RGR	relative growth rate
OTCs	open-top chambers
L	litre
ml	milliliters
K-S test	Kolmogorov-Smirnof test
ANOVA	Analysis of variance
Mean±SD	mean ± standard deviation
A&LN treatment	Ambient CO_2 and x low nutrient treatment
A&HN treatment	Ambient CO_2 and x high nutrient treatment
E&LN treatment	Elevated CO_2 and x low nutrient treatment
E&HN treatment	Elevated CO_2 and x high nutrient treatment
A&LD treatment	Ambient CO_2 and x low density treatment
A&HD treatment	Ambient CO_2 and x high density treatment
E&LD treatment	Elevated CO_2 and x low density treatment
E&HD treatment	Elevated CO_2 and x high density treatment

PP	per plant
PC	per community
TNCs	total non-structural carbohydrates
R^2 value	coefficient of determination value

CHAPTER 1. INTRODUCTION

1.1 Global climate change: A serious threat to ecosystems

According to Ehrlich (1991), climates have always changed in response to changes in solar output, the Earth's orbit, variations in the tilt of its axis, volcanic activity, the drifting of continents, and so forth. An example of climate change is the temperature difference of 5-8 °C between an Ice age and an interglacial warm period (King, 2005). However, it is not climate change itself that threatens the world today, but it is the potential *rate* of the change which is cause for great concern (Ehrlich, 1991).

Woodwell (1995) suggested that global climates are moving from a period of slow change into a period of accelerating change. The earth's climate includes many linkages and feedbacks between atmospheric temperature, oceanic heat storage, clouds, humidity, ice cover, energy budgets, heat transport, i.a. (Houghton, 1991). It would be expected that even a small change in any one of these links would have an impact on overall climate (Morel, 1989). Therefore, world climate should not be seen as a constant. The difficulty in accurately predicting the impacts of climatic changes has lead to a substantial increase in the magnitude of literature on global climate change, and its potential impacts.

In an attempt to highlight the severity of the issue, Kingslover (1996) proposed that biological consequences of the rapid rates of climate change would preclude evolutionary responses: species will either adjust ecologically, or become extinct. Over a decade later, his prediction is proving true, as climate change is now recognized as a major threat to the survival of species, and integrated ecosystems worldwide (Thomas et al., 2004; Hulme, 2005).

1.2 Elevated Atmospheric CO_2 and the greenhouse effect

The accelerating changes in global climate are probably linked to the accumulation of heat-trapping gases in the atmosphere (Woodwell, 1995; Hulme, 2005). These gases are aptly termed "greenhouse gases," and it was the French mathematician Fourier in 1827 who first put forward the greenhouse gas concept: our atmosphere absorbs heat

that would otherwise radiate out into space (King, 2005). The Earth's average surface temperature is kept about 15ºC by this blanket effect of the atmosphere that surrounds it. Without the warming effect of greenhouse gases, the average Earth surface temperature would be a mere -18°C (King, 2005). Carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4), are examples of greenhouse gases, which enable the atmosphere to act as a "heat sink" (Houghton, 1991; Wallace et al., 1996). However, it is the 'greenhouse effect" of CO_2 in particular, that is the reason for major concern regarding climatic effects (Bolin et al., 1987). Although methane (produced in copious quantities by cows) has recently been identified as a more efficient greenhouse gas than CO_2, the effects of methane on climate/ecosystems will not be discussed, as it does not form any part of the current study. Instead, CO_2 effects will be discussed in some detail.

While CO_2 is transparent to incoming short wave radiation from the sun, it absorbs outgoing long wave radiation and re-emits this energy in all directions. Therefore, an increase in atmospheric CO_2 concentration (C_a), will lead to a warming of the earth's surface and lower atmosphere (Bolin et al., 1987; Houghton, 1991; Wallace et al., 1996).

Analysis of air trapped in glacial ice from Greenland and Antarctica has indicated that the average partial pressure of C_a during the last 220 000 years has been considerably lower than it is currently (Tissue et al., 1995). More specifically, there has been a 15% increase in C_a in the past 80 years alone (Wallace et al., 1996). C_a increased from 315 parts per million (ppm) in 1958, to 343 ppm in 1984 (Bolin et al., 1987). Current literature shows that C_a levels are now rising at a rate of approximately 2 ppm per annum. C_a was measured to be 379 ppm in 2004, 40 % higher than pre-industrial levels (King, 2005). Figure 1.1 clearly illustrates the steep rise in C_a from 1958-2004, and future predictions of C_a concentrations range between about 450 and 600 ppm by the year 2050 (Woodward, 2002).

Fig. 1.1 The relentless rise of atmospheric CO_2 (According to readings taken at Mauna Loa in Hawaai, C_a concentrations are 35% higher than pre-industrial levels; Adapted from http://www.newscientist.com/data/images/archive/2486/24861401.jpg)

Although fires and respiration are sources of C_a, the observable changes in C_a and climate are a direct consequence of human activities that have occurred during the past century (Kingslover, 1996). In particular, combustion of fossil-fuels – primarily oil, gas and coal, has been shown to be the major source of increasing C_a (Woodwell, 1995). Human activities could *double* C_a over the next 40 years (Wallace et al., 1996).

Not surprisingly, global warming is probably linked to increasing C_a (Weltzin et al., 2003), and from data of the Vostok core, it was previously estimated that an approximate 1ºC increase in temperature is associated with every 7-10 ppm increase in C_a (Woodwell, 1995). However, an increase in C_a from 315 ppm in 1958 to 379 ppm in 2004 (Bolin et al., 1987; King, 2005), should have resulted in a global temperature increase of 6ºC during that period. This is clearly not the case. This example points out that many inaccuracies and uncertainties regarding climate change predictions in the literature do exist. Nevertheless, the fact that global temperatures have in fact increased by 0.6 °C over the past century (King, 2005), highlights the strong correlation between increasing C_a and climate change.

As the subject of C_a enrichment reaches maturity, much attention now focuses on the fate of the "extra" CO_2 in the atmosphere.

1.3 Plant response to elevated C_a

The terrestrial biosphere is the major sink for increasing concentrations of C_a (Fujita et al., 2003). More than one eighth of C_a is exchanged with terrestrial ecosystems each year, through the biological processes of photosynthesis and respiration (Lashof et al., 1997). As a result, concern regarding the continuing increase in C_a has prompted a great deal of research on responses of plants to CO_2 enrichment (Bazzaz, 1990).

According to Warrick et al. (1987), there are two ways in which plants/ecosystems can respond to rising levels of C_a. The first is through changes in climate i.e. *indirectly.* There is evidence that the direct correlation between global temperatures and increasing C_a is part of a positive-feedback system: warming produces more C_a, which will in turn favour a further warming (Woodwell, 1995). Extreme temperatures caused by increasing C_a may make more resources available to plants, by thawing frozen soil, or by changing rates of nutrient cycling, and allowing plants to grow faster than they would otherwise (Weltzin et al., 2003). Kimball et al. (1993) suggested that the average growth response of plants to doubled C_a, could be significantly higher in warmer climates. Therefore, elevated C_a could affect plants *indirectly.*

In addition to being an environmental factor, CO_2 is also the carbon source that supports the growth of plants via photosynthesis (Murray, 1995). Therefore, the second way in which ecosystems can be affected by increasing C_a is through *direct* effects on plant growth and development (Conroy et al., 1986; Warrick et al., 1987; Bazzaz, 1990). An increase in the CO_2 concentration in the atmosphere in contact with vegetation acts to increase the CO_2 gradient between the atmosphere and the air spaces within leaves (Jarvis et al., 1999). Given no adjustment to this change, the rate of CO_2 diffusion through the stomatal pores could rise in proportion to the increase in ambient CO_2. Therefore the rise in CO_2 availability directly impacts photosynthetic processes (Hulme, 2005). However, this evokes a wide range of physiological and morphological responses in plants. These responses vary among species: differences in photosynthetic pathways, intrinsic growth rates (Dukes, 2000) as well as growth conditions, differences in the degree of enrichment, and the duration of CO_2

enrichment (Mott, 1990), all influence the response. However, despite these complex interactive factors, certain trends can be identified.

C_3 species are plants which fix carbon by the photosynthetic carbon reduction (PCR) cycle (Wallace et al., 1996). These plants show the greatest potential for responding to elevated C_a (Garbutt et al., 1990), and a number of studies confirm this is indeed the case (Mott, 1990; Poorter et al., 1996; Dukes, 2000; Lloyd and Farquhar, 2000; Ainsworth et al., 2002). C_4 plants, on the other hand, have an anatomy and biochemistry, that in effect, concentrate CO_2 into the bundle sheath cells of leaves for subsequent assimilation of carbon by the C_3 pathway. That is, the CO_2 concentration is already "elevated" in C_4 species. Therefore, C_4 species are not expected to respond as strongly as C_3 species to increasing C_a, and many studies confirm this prediction (Johnson et al., 1993; Poorter, 1993; Polley et al., 1994; Dippery et al., 1995; Tissue et al., 1995). However, results on a study on three C_4 species, showed a stimulation of leaf photosynthesis in elevated C_a of all three species (Ziska et al., 1999). Results from this study suggest that certain C_4 species may respond directly to increasing C_a. Wand et al. (1999) conducted a literature review and meta-analysis of data sets on responses of C_4 vs C_3 grasses to CO_2 enrichment, and observed a significant positive response of C_4 grasses. These authors maintain that the previous prediction of C_4 grasses losing their competitive ability over C_3 grasses, is premature. Despite results from these two studies, the general pattern is that plants with the C_3 metabolism are more sensitive to CO_2 than plants with C_4 metabolism (Garbutt et al., 1990).

In light of the above, the remainder of this chapter will focus only on C_3 plants, and their responses to elevated C_a. According to Mott (1990), plants demonstrate a number of physiological and morphological responses to changes in C_a. For the purpose of this thesis, plant responses to elevated C_a will be divided into two categories: 1) physiological responses, and 2) growth and morphological responses.

1.3.1 Physiological responses

According to Long et al. (2004), plants can perceive a change in C_a only through tissues that are exposed to the open air. With the exception of some reproductive organs, only the photosynthetic organs of plants have direct contact with the atmosphere. The protective cuticle of higher-plant leaves and other photosynthetic organs means that only the inner surfaces of stomatal guard cells and the mesophyll can directly sense a change in C_a (Long et al., 2004). Not surprisingly, respiration, transpiration and photosynthesis appear to be the only three physiological processes by which plants and ecosystems can sense and respond directly to rising C_a (Drake and Gonzàlez-Meler, 1997). For the purpose of this study, only photosynthesis, stomata and transpiration responses to elevated C_a will be discussed.

Photosynthesis:

Photosynthesis plays a central role in the physiology of plants (Mott, 1990). According to Bazzaz (1990), CO_2 would enhance photosynthesis of C_3 plants over a wide range of concentrations, provided that other environmental resources and factors are present at adequate levels. Several lines of evidence do in fact illustrate a stimulation of photosynthesis in elevated C_a (Ackerson et al., 1984; Garbutt et al., 1990). Photosynthesis of *Plantago major* was enhanced during the first 2 weeks of exposure to elevated C_a (den Hertog et al., 1993). A study conducted on *Pinus taeda* trees showed similar results: carbon assimilation (photosynthesis) was twice as high in elevated C_a than ambient C_a (Teskey, 1995). Drake and Gonzàlez-Meler (1997) conducted a survey of 60 experiments, and found that growth of plants in elevated C_a increased photosynthesis 58% compared with the rate for plants grown at ambient C_a.

Recent literature maintains that the observed stimulation in photosynthesis in elevated C_a, results from properties of the enzyme, rubisco (Long et al., 2004), an enzyme which has been shown to deactivate under moderate heat stress (Salvucci and Crafts-Brandner, 2004). At this point, the theory behind the function of rubisco, deserves a little attention.

Rubisco (ribulose-1.5-bisphosphate carboxylase/oxygenase), catalyses the carboxylation reaction of the PCR cycle (Wallace et al., 1996). Its main function is to

add CO_2 to the 5-carbon molecule, ribulose-1.5-bisphosphate (RuBP). However, rubisco has a very low catalytic rate, despite being one of the largest enzymes in nature (Salvucci and Spreitzer, 2002). It also has a low affinity for CO_2 on carboxylation, a reaction which is not saturated at current C_a (Drake and Gonzàlez-Meler, 1997; Long et al., 2006). To make matters worse, rubisco also has an affinity to bind to O_2. This oxygenation reaction leads to photorespiration, a process which decreases the net effect of photosynthesis by 20-30 % (Drake and Gonzàlez-Meler, 1997). In light of the above, it is not difficult to see how increasing C_a would competitively inhibit the oxygenation reaction of rubisco, thereby decreasing photorespiratory CO_2 loss and diverting ATP and NADPH (generated by the light reactions) away from photorespiratory metabolism to photosynthetic assimilation (Usuda and Shimogawara, 1998; Long et al., 2004).

The above explanation may be adequate in describing the short-term photosynthetic responses of plants to elevated C_a. But what happens when plants are exposed to CO_2 enrichment for prolonged periods of time?

According to Drake and Gonzàlez-Meler (1997), if plants are unable to use all the additional carbohydrate that photosynthesis in elevated C_a can provide, then a decrease in source activity must result. There is in fact, abundant evidence that in the long-term, and in some species, photosynthesis acclimates to elevated C_a, i.e. the photosynthetic properties of leaves developed at elevated C_a differ from those developed at current C_a (Drake and Gonzàlez-Meler, 1997; Bazzaz, 1990; Kauder et al., 2000; Vu, 2005). Plants grown at elevated C_a often fail to sustain the initial stimulation of net assimilation (Rogers and Humphries, 2000). For example, the observed initial increase in photosynthesis of *Plantago major* plants transferred to elevated C_a, decreased after 2 weeks and nearly reached the level of control plants (den Hertog et al., 1993). Acclimation of leaf net assimilation of *Ginkgo biloba*, occurred after two years of growth in elevated C_a, and maximum net CO_2 assimilation was 56% higher at ambient C_a than at 700 ppm C_a (Overdieck and Strassemeyer, 2005). Many researchers have attempted to explain the phenomenon of photosynthetic acclimation: why and how it occurs. This has often resulted in contradicting views, especially along the time line.

Earlier studies on the photosynthetic acclimation to elevated C_a in five C_3 species, indicated that the rubisco content of leaves in elevated C_a remained in excess of that required (Sage et al., 1989). In contrast, more recent studies have shown that acclimation of photosynthesis is accompanied by lower concentrations of leaf rubisco content, because less rubisco is needed in elevated C_a (Drake and Gonzàlez-Meler, 1997; Adam et al., 2004). Research which supports this more recent point of view was conducted on *Nardus stricta* (Cook et al., 1998). Plants were growing near a natural CO_2 spring in Iceland where they were exposed to more than 100 years of elevated C_a (~ 790 ppm). These plants showed reductions in photosynthetic capacity (~ 25%), rubisco content (~ 26%), and rubisco activity (~ 40 %), when compared to plants growing away from the spring (~ 360 ppm C_a). More specifically, Rogers and Humphries (2000) showed that the failure of plants grown in elevated C_a to maintain the initial stimulation of photosynthesis, can be attributed almost entirely to the decrease in the maximum carboxylation velocity of rubisco ($V_{c,max}$).

This has been shown to be the case for *Polygonum cuspidatum,* a species which showed a decline in $V_{c,max}$ and J_{max} (maximum electron transport rate), when exposed to elevated C_a for six months (Onoda et al., 2005). Similarly, a study conducted on *Larrea tridentate,* an evergreen Mojave Desert shrub, showed that well-watered plants significantly down-regulated photosynthesis at elevated C_a, reducing maximum photosynthetic rates (A_{max}), and carboxylation efficiencies of rubisco (Huxman et al., 1998). Photosynthetic down-regulation observed in nodulated alfalfa plants exposed to elevated C_a, was also a direct consequence of reduced carboxylation efficiency, as a result of reduced rubisco content and activity (Aranjuelo et al., 2005).

According to two earlier studies on cotton, photosynthetic acclimation to elevated C_a is mediated by shifts in allocation between leaves and the rest of the plant, such that carbohydrate supply remains in balance with the utilization capacity of sink tissue (Thomas and Strain, 1991; Barrett and Gifford, 1995). Results from these two studies suggested that reductions in $V_{c,max}$ (and hence rubisco activity), may be responsive to plant source-sink balance, rather than C_a as a single factor. This idea is supported by recent research. According Long et al. (2004), at a whole plant level, restricted capacity to utilize photosynthate drives a loss of photosynthetic capacity. Ainsworth

et al. (2002) also maintain that a decrease in photosynthetic capacity would not occur if plants have adequate sink strength. Futher support for this idea came from a study conducted on three component species of chalk grassland swards (Bryant et al., 1998). After 14 months of exposure to elevated C_a, $V_{c,max}$ and J_{max} were reduced in two of the three species. However, after a change in source-sink balance brought about by defoliation, photosynthetic capacity was fully restored. Similarly, Usuda and Shimogawara (1998) concluded that the 105% increase in root production of radish in elevated C_a, occurred to enhance sink capacity. This enhanced capacity seemed to be responsible for the absorption of elevated levels of photosynthate, and resulted in the absence of over-accumulation of carbohydrates in source leaves, and the absence of photosynthetic acclimation at elevated C_a.

Therefore the correlation between a decrease in $V_{c,max}$ and photosynthetic acclimation (and photosynthetic down-regulation), should not be seen as a hard and fast rule. Different responses have been reported in the literature, and these cannot be ignored. For example, a step increase in C_a on *Plantago* plants, resulted in a 50% increase in photosynthesis, which lead to a 20-24% decrease in leaf nitrogen (N) content. The offset of the C:N ratio in leaves, induced nitrogen stress. This, and not reduced $V_{c,max}$ resulted in photosynthetic down-regulation (Hui et al., 2002).

In addition to reduced $V_{c,max}$, Drake and Gonzàlez-Meler (1997) also proposed that photosynthetic acclimation is accompanied by high carbohydrate concentrations in the leaf. Bazzaz (1997) also maintained that accumulation of starch in plant cells may cause down-regulation of photosynthesis. However a study on the acclimation of potato plants to elevated C_a, has shown that carbohydrates formed by CO_2 - assimilation in leaves during the rapid growth of plants in elevated C_a, were exported to sink tissues and used for accelerated shoot growth and tuber induction. These results indicate that carbohydrate accumulation could not have resulted in down-regulation of photosynthesis in these plants (Kauder et al., 2000).

To further complicate matters, there are some cases in which no photosynthetic acclimation to elevated C_a occurs. Lifelong exposure of *Quercus pubescens* to CO_2 enrichment, resulted in no photosynthetic down-regulation of these plants, as net photosynthesis was enhanced by 36 to 77% (Stylinski, et al., 2000). Similarly, no

photosynthetic down-regulation was observed in sweetgum trees (*Liquidambar styraciflua*) after 3 years of exposure to elevated C_a (Herrick and Thomas, 2001). There were non-significant differences in A_{max}, rubisco content, activity and carboxylation capacity between 'controls' and plants under chronic C_a enrichment in both studies. A more recent study on sweetgum trees (Sholtis et al., 2004), also showed no decrease in photosynthetic capacity, as net photosynthetic rates were 44% higher in trees grown in elevated C_a, than ambient C_a over the 3-year period. Calfapietra et al. (2005) also showed no clear signs of photosynthetic acclimation of a poplar plantation, after five years of elevated C_a exposure. A study was conducted on perennial ryegrass (*Lolium perenne*), in which plants were exposed to 10 years of CO_2 enrichment (Ainsworth et al., 2003). Results showed that although daily carbon assimilation was significantly increased in plants grown at elevated C_a, there was no significant change in photosynthetic stimulation across the 10-year period, and no greater acclimation in $V_{c,max}$ and J_{max} in the later years.

The aim of this reviewing of contradicting results and contrasting research, is to demonstrate the difficulty of predicting how a single aspect of terrestrial vegetation (i.e. photosynthesis), could respond to increasing C_a, and hence global climate change.

In order to increase our understanding of the differential photosynthetic responses reviewed above, closer attention needs to be drawn to stomata, pore-like structures that permit the exchange of gases between photosynthetic cells and the atmosphere (Wallace et al., 1996).

Stomata:

Stomata permit inward diffusion of CO_2 for photosynthesis, and outward diffusion of water ($H_2O_{(g)}$) during transpiration (Wallace et al., 1996). For the purpose of this thesis, stomatal conductance to CO_2 is abbreviated as g_c, while g_w symbolizes stomatal conductance to $H_2O_{(g)}$).

Stomata of most species close as C_a increases, i.e. g_c is reduced in elevated C_a (Garbutt et al., 1990; Mott 1990; Drake and Gonzàlez-Meler, 1997; Medlyn et al., 2001). Studies on *Lolium perenne* (Ainsworth et al., 2003), and *Ginkgo biloba* (Overdieck and Strassemeyer, 2005), have shown a 30% decrease in g_c, under CO_2

enrichment. Long et al. (2004) conducted a meta-analysis of results of 200 independent FACE (Free Air CO_2 enrichment) studies on C_3 plants, and found an average 20% decrease in g_c in elevated C_a. Vu (2005) also demonstrated a decrease in g_c of *Arachis hypogaea* (peanuts) in elevated C_a.

However the correlation between reduced g_c and increasing C_a is not universal. No significant effect of elevated C_a on g_c was noted for *Pinus taeda* trees (Teskey, 1995) and Douglas fir trees (Apple et al., 2000). Some species have even shown an increase in g_c in elevated C_a. Wheeler et al. (1999) conducted a study to determine if stomata open at very high C_a concentrations. Results showed that in three of the four species investigated, g_c increased when plants were exposed to super-high (1000 and 10 000 ppm) C_a concentrations.

An early study (Mott, 1988) showed that g_c actually responds to intercellular CO_2 concentrations within the leaf (C_i), and not C_a *per se*. Nevertheless, Murray (1995) attempted to explain how elevated C_a was sensed by stomata. He questioned the possibility of CO_2 sensors being located in the plasmalemma or other membranes. However, years later, the mechanism by which stomata sense CO_2 concentrations, and where in the leaf CO_2 is sensed, is still unclear (Long et al., 2004).

Because assimilation of carbon is strongly dependent on the availability of CO_2 and the gradient of CO_2 between the atmosphere and the chloroplast (Kauder et al., 2000), g_c could also affect photosynthesis. Since photosynthesis has been shown to acclimate to elevated C_a, it is natural to wonder if stomata behave in the same manner. In a meta-analysis of data from 13 long-term (>1 year) studies on the effects of elevated C_a on forest trees, no evidence for acclimation of g_c to elevated C_a was found (Medlyn et al., 2001). However, results from a study on a C_3 perennial forb, *Solanum dimidiatum*, in which g_c-C_i response curves differed significantly across the range of growth CO_2 treatments (200-500 $\mu mol.mol^{-1}$), suggests that stomata of C_3 herbaceous species, *could* acclimate to elevated C_a.

Transpiration:

Literature shows that in elevated C_a, reduced g_c leads to a decrease in transpiration (Bazzaz, 1990; Drake and Gonzàlez-Meler, 1997; Jarvis et al., 1999). For example, reduced g_c of poplar trees (*Populus* x *euramericana)* grown in elevated C_a, was shown to decrease tranpirational rates (Calfapietra et al., 2005).

Because loss of water vapour ($H_2O_{(g)}$) via transpiration has a cooling effect on leaves, reductions of transpirational rates in elevated C_a also decreases evaporative cooling of leaves, which leads to increases in leaf temperatures (Jarvis et al., 1999). This could have an effect on many physiological processes that occur in plants since most biological enzymes are temperature sensitive (Wallace et al., 1996). For example, the O_2/CO_2 specificity of rubisco, is affected by temperature.

Although photosynthesis, g_c and transpiration have been discussed as separate categories under the discussion of physiological responses of plants to elevated C_a, the links between these processes, and subsequently generated feedbacks of these links, should not be forgotten. For example, an earlier study on cotton and maize plants, clearly showed that increased water-use efficiency (WUE) in elevated C_a, was due to reduced transpiration in some plants, and to increased assimilation in others (Wong, 1979). The complexity of the various relationships and feedback loops present in leaf cells, such as the relationships between the three parameters described above, makes the topic of 'plant responses to elevated C_a' such a dynamic and interesting one.

However, the picture is still incomplete at this stage. For example, what do plants do with the 'extra' assimilated carbon in elevated C_a? Is all of it invested directly into biomass, and if so, is biomass allocation uniform throughout the entire plant? Does reduced g_c also mean that plants in elevated C_a will produce fewer stomata?

In order to investigate these questions, growth and morphological responses of plants to elevated C_a will now be discussed.

1.3.2 Growth and morphological responses

Plant primary production is ultimately dependent on photosynthetic CO_2 uptake (Körner, 1991). Therefore, under CO_2 enrichment, increased photosynthesis should also lead to increased growth of plants. Most literature indicates an increase in plant dry weights in elevated C_a (Thomas and Strain, 1991; Hand et al.,1993; Kimball et al., 1993; Drake and González-Meler, 1997; Hui et al., 2002). An early study demonstrated an increase in total plant growth of six early successional plant species in elevated C_a (Carlson and Bazzaz, 1982). Elevated C_a also caused an increase in total plant biomass of C_3 grasses (Wilsey et al., 1997), and biomass of *Lotus corniculatus* (Bazin et al., 2002). In a study conducted on radish, *Raphanus sativus,* elevated C_a (700 ppm) increased dry matter production by 111% (Usuda and Shimogawara, 1998). Growth rates of wheat, *Triticum aestivum,* increased with elevated C_a (Polley et al., 1993a). Poorter (1993) compiled the results of growth responses of 156 species to elevated C_a. Averaged over all species, a doubling of ambient C_a resulted in a stimulation of plant growth of 37%. Similarly, a 37% increase in total dry weight of soybean (*Glycine max*) in elevated C_a was also observed, when a meta-analysis of data from 111 studies on soybean, was performed (Ainsworth et al., 2002).

Enhancement of growth, according to Mott (1990), is associated with increased photosynthetic assimilation under elevated C_a. However, plant growth is affected by a multitude of attributes other than photosynthesis (Körner, 1991), and increases in plant growth in elevated C_a, are actually a result of interactions between many factors (Mott, 1990).

Time, for example is one of the factors that can influence a plant's growth response to elevated C_a (Eamus, 1991; Poorter, 1993). Most studies on the effects of elevated C_a show an initial enhancement in growth, and like photosynthesis, this enhancement is especially large when resources are plentiful (Bazzaz, 1990). Centritto et al. (1999) conducted a study to determine if the increases in total biomass brought about by enhanced C_a, was a result of a transient or persistent effect. In this study, four clones of Sitka spruce and cherry, were grown for three and two seasons, respectively, at two CO_2 concentrations (350 $\mu mol.mol^{-1}$). Sitka spruce and cherry seedlings showed a

positive growth response to elevated C_a, and at the end of the experiments, both species were ~ 40% larger in elevated C_a. However, the differences in plant dry mass at the end of the experiments, were a consequence of the more rapid growth in the *early* phase of exposure to elevated C_a. A similar result was found by den Hertog et al. (1993) who showed that the initial 30% increase in total dry weight of *Plantago major* was due to a stimulation of relative growth rates (RGR) during the first ten days of exposure to elevated C_a. These observations seem to suggest that growth may acclimate to elevated C_a, in a similar way that photosynthesis and stomatal conductance (g_s) have been shown to acclimate to elevated C_a (Drake and González-Meler, 1997).

Another point to ponder, regarding growth, is whether increases in plant growth in elevated C_a occurs uniformly throughout the plant. An early study on *Plantago major* (den Hertog et al., 1993), showed although total dry matter was increased by 30% in elevated C_a, there were no effects on partitioning ratios of dry matter. Similarly, biomass allocation of soybean (*Glycine max)* was unaffected by growth in elevated C_a, as there was no effect on root:shoot ratios (Ainsworth et al., 2002).

However, according to Taylor et al. (1994), additional amounts of carbon could be allocated to below-ground material, i.e. roots, under CO_2 enrichment. Root growth, according to these authors, could be a direct result of increased cell production, cell expansion, or both, due to the fact that root cell turgor pressure and root cell wall extensibility are promoted by exposure of shoots to elevated C_a. He and Bazzaz (2003) also maintain that an increase in carbon acquisition in elevated C_a, should result in a shift in allocation toward roots, until root activity is proportionally enhanced. Wechsung et al. (1999) showed a 37% increase in root dry weight of wheat, under elevated C_a. Increases in growth of a C_3 grass under elevated C_a, occurred primarily in the roots (Wilsey et al., 1997). Elevated C_a also increased dry weights of storage roots of radish, *Raphanus sativus* by 105%, only 46 days after planting (Usuda and Shimogawara, 1998). These authors maintain that plants exposed to elevated C_a, accumulate more biomass as roots to enhance sink capacity.

Farrar (1999) proposed that roots of plants grown in elevated C_a are heavier because of the earlier and greater production of nodal roots and the greater rate of production

of laterals on seminal roots. In contrast, Pritchard et al. (1999) charged that root *length* increases in elevated C_a. However, if plants are grown in pots, root growth restrictions may occur. Thomas and Strain (1991) demonstrated root restriction as a factor in photosynthetic acclimation of cotton seedlings grown in elevated C_a. In this study, plants were grown in two pot sizes (0.38 and 1.75 litres), and exposed to three C_a concentrations. Reduced photosynthetic capacity observed for plants grown at elevated C_a, was clearly associated with inadequate rooting volume (small pot size).

Nevertheless, Rogers et al. (1999) maintain that increased rooting generally observed under elevated Ca, has the potential to significantly alter the edaphic environment through increased carbon deposition and /or nutrient uptake by plants.

In contrast to the literature reviewed above, many studies show an increase in above-ground biomass (leaves and stems), in elevated C_a. A study on *Lotus corniculatus* showed an increase in total biomass under elevated C_a (Bazin et al., 2002), However, shoot dry mass was 2.3 times greater, while root dry mass was 1.8 times greater under elevated C_a conditions, indicating a greater biomass allocation to shoots, compared with roots. These results are consistent with Hunt et al. (1991), who showed an increase in shoot:root ratio in 14 C_3 species. Polley et al. (1993b) showed that *only* above-ground biomass (leaves and stems) of three C_3 annuals (oats, wild mustard and wheat), increased linearly and nearly proportionately with increasing C_a concentrations. Results from these studies support the prediction that in general, elevated C_a would alter the balance between growth and availability of resources, thus leading to reduced water stress (because of increased water use efficiency) and consequently, reduced root:shoot ratios, as plants increase their above-ground biomass allocation (Friedlingstein et al., 1999). In contrast to all of the above studies, Ainsworth et al. (2002) showed that root:shoot ratios of soybean did not differ between ambient- and elevated C_a-grown plants.

As can be seen, results in the literature concerning the root:shoot ratios under conditions of CO_2 enrichment are mixed (Hunt et al., 1991). This, together with the fact that even coexisting species may widely differ in root:shoot ratios even though receiving the same severe environmental constraints (Körner, 1991), indicates a high degree of variability of root: shoot ratios in elevated C_a.

Total leaf area, according to Taylor et al. (1994), may be stimulated following exposure to elevated C_a. Wong (1979) and O'Leary and Knecht (1981) showed that elevated C_a increased leaf area in cotton and *Phaseolus vulgaris*, respectively. Vu (2005) also reported an enhancement of leaf area of peanut in elevated C_a. Carlson and Bazzaz (1982) conducted a study on six early successional plant species, and found that leaf area increased with increasing C_a. A more recent study has shown an increase in leaf area of three cultivars of a C_3 wheat species (Polley et al., 1993a). Pritchard et al. (1999) reviewed available literature, and found that 66% of studies reported an increase in leaf area per plant.

These results could be due to the fact that both the production and expansion of leaf cells may be stimulated by exposure to elevated C_a (Taylor et al., 1994). However, Usuda and Shimogawara (1998) maintain that it is the leaf area *ratio* (LAR; amount of leaf area per unit of plant dry mass), that provides an indication of the proportion of a plant that is active in photosynthesis. Results from this study showed that elevated C_a increased the rate of expansion of radish leaves, but decreased LAR possibly because of the production of large storage roots. Leaf area ratios of Sitka spruce and cherry seedlings also decreased in response to elevated C_a (Centritto et al. (1999). This indicates that these plants allocated more carbon to compartments such as stems or roots, instead of producing more leaves to acquire resources. An increase in leaf thickness of soybean, loblolly pine and sweet gum, stimulated by elevated C_a (Thomas and Harvey, 1983), could also contribute to the observed decreases in LAR. Harmens et al. (2000) conducted a study on *Dactylis glomerata*, and showed that the reduction of LAR in CO_2 enrichment, was due solely to a decrease in SLA (leaf area/leaf dry weight). Similarly, Hui et al. (2002) and Overdieck and Strassemeyer (2005) showed a significant decrease in SLA with increasing C_a. Bazzaz (1990) maintains that reduced SLA in elevated C_a is often associated with increased starch levels and reduced nitrogen (N) concentrations.

Stomatal density (SD), is the number of stomata per unit area (Woodward, 1987). There is a lot of evidence that indicates a reduction of SD decreases as C_a increases (O'Leary and Knecht, 1981; Woodward, 1987; Beerling and Chaloner, 1993a; Beerling and Chaloner, 1993b). A survey conducted by Woodward and Kelly (1995) on 100 species and 122 observations, showed that 74% of the cases exhibited a

decrease in SD. In contrast, SD may also increase at higher Ca (Knapp et al., 1994; Maherali et al., 2002). Apple et al. (2000) showed that elevated C_a had no effect on SD of Douglas fir needles. A study conducted on four chalk grassland herbs, showed contrasting effects of elevated C_a on SD of these species (Ferris and Taylor, 1994).

The contrasting results in the literature reviewed above, are not surprising since many factors could influence the effect of elevated C_a on SD. Altitude (Woodward and Bazzaz, 1988), temperature change (Beerling and Chaloner, 1993b), LAR (O'Leary and Knecht, 1981), species specificity (Knapp et al., 1994), and canopy development (Tricker et al., 2005) have been documented as factors that increase the complexity of C_a interactions with SD, and subsequently the whole plant.

1.4 Elevated C_a and interactive factors

In the real word, rising C_a is always interacting with other environmental and biological parameters in determining actual changes in material and energy fluxes in ecosystems (Luo et al., 1999). On this basis, it would be more beneficial to conduct studies on plant responses to *interactive* effects of elevated C_a with other environmental factors, rather than elevated C_a alone. Significant CO_2 times family interactions, were noted for photosynthetic rates of *Plantago lanceolata*, when 18 families from two populations of *P. lanceolata* were studied under CO_2 enrichment (Klus et al., 2001). Sallas et al. (2003) conducted a study on differences of the response of two conifer seedlings (Norway spruce and Scots pine), when exposed to elevated C_a and elevated temperature. Results showed that in general, effects of elevated C_a on the studied parameters were small, compared with the effects of elevated temperature.

For the purpose of the study undertaken, interactive effects of elevated C_a with the following three factors, will be reviewed: 1) nutrient availability, 2) interspecific competition and 3) planting density.

1.4.1 Plants, elevated C_a and nutrients

CO_2 is just one of the many inorganic resources that are required by plants (Stitt and Krapp, 1999). Increasing C_a reduces the importance of carbon as a limiting external resource (Lynch and St. Clair, 2004). Therefore the extent of plant responses to C_a enrichment will depend on the availability of resources other than CO_2 (Zanetti et al., 1997). Nutrient availability, according to Poorter et al. (1996), is an important factor that influences C_a responses of plants. These sentiments are echoed by Körner (2003), who maintains that plant nutrition is a key issue in CO_2 response research, as CO_2 enrichment *per se*, is rarely the major driver of responses.

Many studies have been undertaken in an attempt to investigate the interactions between increasing C_a, and nutrient supply or availability, on plants. Bezemer et al. (2000) showed no significant CO_2 times nutrient interactions on an annual grass species (*Poa annua*). Similarly, Pi (inorganic phosphate) limitations reduced dry matter formation of barley roots, at ambient *and* elevated C_a (Sicher, 2005).

In contrast, Midgley (1996) showed that in three of the four *Leucadendron* species investigated, the response of plant biomasss to elevated C_a, was reduced at high nutrient supply. Similarly, the response of total biomass of an annual plant community to elevated C_a was found to be dependent on nutrient levels (He et al., 2002). In a study conducted by Stöcklin and Körner (1999), the biomass of legumes increased significantly by 29% when treated with phosphorus (P). In addition, the above-ground biomass response to elevated C_a, was much larger with P fertilization. Similar results emerged from a study on *Pinus radiata* seedlings. Elevated C_a increased total dry weight of *P. radiata* by an average of 30% (Conroy et al., 1986). However, in P-deficient seedlings, the increase was only 13%. A similar trend was observed for other parameters: assimilation and the number, length, weight and diameter of needles increased with elevated C_a, but the effect was decreased by P-deficiency.

The effect of P observed in this study was similar to the effect of another macronutrient, potassium (K), in a recent study on cotton. Reddy and Zhao (2005) found that stimulation of physiological and growth parameters (e.g. photosynthesis,

leaf area and biomass production) of cotton observed under elevated C_a, was lost under severe K deficiency. In addition, plants grown under elevated C_a were more sensitive to K deficiency.

Because ~50% of leaf N is used for photosynthetic activities (Onoda et al., 2004), potential interactions of N with CO_2 and other nutrients, could play a vital role in plant responses to simultaneous increases in C_a and nutrient supply.

Nitrogen-nutrient interactions (at ambient C_a):

An experiment was carried out on boreal rich-fen vegetation, in which plants were fertilized with N, P and K at three different sites (Øien, 2004). Results showed that in two of the three sites, above-ground biomass increased when plants received N and P together, indicating a strong relationship between these two nutrients. The addition of K to the nutrient treatment did not increase biomass more than when N and P were added. Similarly, addition of N alone significantly increased above-ground biomass of two Austrialian grasslands (Bennet and Adams, 2001). However, combined addition of N and P had similar effects to addition of N alone. These two studies reiterate the point that the relationship between nutrient supply and growth is not always linear. In a study on *Sphagnum* spp., the lack of significant increases in growth under elevated C_a and N deposition, was attributed to low supplies of K and P (Hoosbeek et al., 2002). Results from these studies highlight an important point: nutrient-nutrient interactions need to be taken into consideration in studies on plant responses to increasing C_a and nutrient supply, especially when nutrients are supplied as a nutrient solution/treatment (comprising various combinations of different nutrients).

Nitrogen-C_a interactions:

Harmens et al. (2000) showed that both CO_2 and N enrichment stimulated net dry matter production of *Dactylis glomerata*. Responses of two semiarid shrubs (*Prosopis glandulosa* and *Prosopis flexuosa),* to elevated C_a were found to be dependent on N, with the largest effects evident at high N supply (Causin et al., 2004). Similarly, Deng and Woodward (1998) showed that elevated C_a increased fruit yield of strawberry plants, but the effect was more profound at high N supply, than at low N supply.

On the other side of the coin, responses of *Hippeastrum* spp. to N were also affected by C_a enrichment (Silberbush et al., 2003). In this study, bulbs of an initial diameter were grown in a greenhouse, on dune sand either enriched with 1000 ppm CO_2, or with ambient concentration, and exposed to different combinations of nitrogen and phosphorus. Both nutrients significantly increased bulb growth, but the optimal response of larger bulbs (greatest bulb growth) was at the high C_a concentration, for both nutrients. Results from these four studies suggest a strong interaction between N and CO_2.

Onoda et al. (2004) has shown that plants can alter their nitrogen allocation to increase the rate or duration of carbon assimilation. Therefore, in addition to nutrient availability, the way in which nutrients are utilized by plants also influences the long-term response of photosynthesis and growth to elevated C_a (Stitt and Krapp, 1999).

1.4.2 Plants, elevated C_a and competition

Plants respond less predictably to C_a enrichment when they are grown in mixed communities (Dukes and Mooney, 1999). Component species of plant communities may differ in their responses to elevated C_a (Ferris and Taylor, 1994), and interspecific variation in plant responses may lead to community-level changes in species dominance, composition and diversity (Weltzin et al., 2003).

According to Johnson et al. (1993), the effects of increasing C_a concentrations on plant-plant interactions in communities, are not mediated through competition for CO_2 *per se*, but depend primarily on how the rate of carbon supply influences individual growth rates and alters the acquisition and utilization of other resources. This idea is supported by Dukes (2000), who stated that whereas the growth of a solitary plant might be limited by the availability of CO_2, plants in communities are likely to be limited by the availability of light, space, water, and nutrients, for which they perceptibly compete with other plants. Differences in species ability to compete for potentially *limiting* resources could influence species responses to elevated C_a (Reynolds, 1996).

Navas (1998) conducted a literature review, and found that competition largely altered the response of plant species to CO_2 enrichment. The observed responses of plants grown in mixtures to elevated C_a, were much lower than the estimated responses calculated from plants grown individually, indicating a depressing effect of inter-specific competition on the response of plant mixtures to elevated C_a. Experimental conditions of the 20 studies selected for the literature review, differed, and plant densities varied according to the growth form of a species. Observations from this literature review highlight the fact that species responses to CO_2 enrichment when grown in mixed communities, cannot be extrapolated from its response in monoculture, and this could be attributed to the fact that when species are grown in mixture, competitive interaction changes the *amount* they require of an available resource, relative to their acquisition in monoculture (He et al., 2002). Bazzaz and Garbutt (1988) demonstrated strong interactions between different species in mixtures, and in some cases, interactions cancelled out the effects of CO_2 enhancement. Therefore, interactions between plants in communities could be positive, negative or even neutral.

Physiological responses of different plants to elevated C_a, might affect growth and competition by causing limitation of other resources to abate (or intensify). The species that best responds to the full suite of CO_2-driven changes in resource availability are most likely to benefit from the C_a increase (Dukes, 2000). According to Hunt et al. (1991), increasing C_a clearly has the potential to induce shifts in species composition toward a *competitive strategy*. One of the general patterns that has emerged, is that plants with C_3 metabolism are more sensitive to increasing C_a than plants with C_4 metabolism (Garbutt et al., 1990; Johnson et al., 1993; Poorter, 1993; Tissue et al., 1995). The striking differences observed in photosynthesis and growth between C_3 and C_4 species are directly connected to the presence of the process of photorespiration in the former but not in the latter (Johnson et al., 1993).

Gavazzi et al. (2000) demonstrated a favouring of community development of C_3 weeds over C_4 weeds, in elevated C_a. An early study on *Abutilon theophrasti* (C_3 weed) and *Amaranthus retroflexus* (C_4 weed), in which both species were grown individually, and in competition with each other, showed elevated C_a had a positive effect on the biomass of *A. retroflexus*, and to a lesser extent, *A. theophrasti* (Bazzaz

et al., 1989). However, these effects were limited to the early parts of the experiment, in the case of individually grown plants. These results are in direct contrast to two later studies on the same two species. Dippery et al. (1995) and Tissue et al. (1995) conducted studies which compared the effects of elevated C_a on *Abutilon theophrasti,* and *Amaranthus retroflexus,* grown in competition with each other. Dippery et al. (1995) showed that after 35 days of exposure to CO_2 enrichment, there were no effects on relative growth rate (RGR), total biomass, or partitioning of biomass of the C_4 species. On the contrary, C_3 plants grown under 700 ppm C_a, had greater root mass and root:shoot ratios than C_3 plants grown at lower C_a partial pressures. Tissue et al. (1995) demonstrated similar results: net photosynthesis at growth CO_2 concentration increased with increasing C_a for *A. theophrasti* (C_3 weed), but not for *A. retroflexus* (C_4 weed). Contrary to the three studies on *A. theophrasti* and *A. retroflexus* discussed above, Coleman and Bazzaz (1992) showed that when both species were grown individually, elevated C_a had *no* effect of the final biomass of the C_3 species, A. *theophrasti*.

Further evidence, which supports the idea that the response of C_3 - C_4 mixed communities may not be as straightforward as expected, comes from a study conducted on the Poaceae family (Wand et al., 1999). In this study, both C_3 and C_4 grass species increased total biomass significantly, in response to elevated $C_{a.}$ However, the overall stimulation of C_3 carbon assimilation rates was reduced by stress. Environmental stresses did not alter the C_4 response to elevated $C_{a.}$ These results suggest that CO_2-enriched environments characterized by several stresses may favor C_4 grasses over C_3 grasses.

The focus of the literature reviewed thus far, has been on interactions between plants of the same life form, for e.g. weed-weed, or grass-grass interactions. However, one needs to consider that in reality, plant communities are usually comprised of plants of different life forms. Annuals, perennials, grasses, crops, and even weeds co-occur in nature. Therefore, in order to get a more accurate representation of the role of competition in plant responses to elevated C_a, it would perhaps be more beneficial to conduct studies on different life forms grown in competition with each other.

According to Polley et al. (1994), increases in global C_a since the beginning of the 19th century, potentially favour a greater increase in growth of a C_3 weed species, *Prosopis glandulosa*, than C_4 grasses. Patterson and Flint (1980) also investigated the competitiveness of C_3 and C_4 crops and weeds under elevated C_a conditions. Results from this study suggested that C_a enrichment would make C_3 weeds more competitive than C_4 crops. This was shown to be the case in a more recent study: sorghum (C_4 crop) was grown in competitive mixtures with common cocklebur (C_3 weed), in elevated C_a (Ziska, 2001). Aboveground biomass and leaf area increased significantly for cocklebur, but decreased significantly for sorghum. Results from this study indicate that vegetative growth, competition and potential yield of C_4 crops, could be reduced by co-occurring C_3 weeds as C_a increases. On the other hand, Patterson (1980) predicted that C_4 weeds might become less competitive when grown with C_3 crops.

Despite the many conflicting reports on whether increasing C_a would generally favor C_3 or C_4 species, it is clear that global CO_2-enrichment will affect the competitive interactions between C_3 and C_4 species in some way, and this may even affect seasonal niche separation and species distribution (Carter and Peterson, 1983).

1.4.3 Plants, elevated C_a and planting density

Many lines of evidence illustrate a response of plants to different planting density. Gan et al. (2002) conducted research on the responses of three genotypes of soybean (*Glycine max)*, to planting density. Results from the field showed that total biomass and seed yield per unit area, for all three genotypes responded positively to increased plant density. The effect of planting density on individual plant characteristics varied across genotypes in this study. In contrast, seed yield per plant, plant width and number of branches of *Lesquerella fendleri*, was significantly reduced with increasing planting density (Brahim et al., 1998). Minor decreases in plant height observed in this study, was attributed to competition for available space at greater planting densities.

A study on loblolly pine stands reported a significant increase in stem growth rates with increasing planting density (Will et al., 2005). However, increases were not

proportional, indicating the presence of competition-induced limitations to growth. In contrast, no adverse effects of increasing planting density on growth /yield of rubber/banana crops was observed, when these two species were grown together (Rodrigo et al., 1997). In this study, rubber crops were grown in a single row, and increasing planting density was achieved by increasing the number of rows of *banana* crops, from one to three. The relative performance of component crops in different cropping systems were analysed in terms of the crop performance ratio (CPR), which refers to the production of an intercrop per unit area of ground, compared with that expected from sole crops sown in the same proportions (Rodrigo et al., 1997). Interestingly, CPR and dry matter productivity of rubber increased with increasing banana planting density. Leaf area and total dry matter of stands, as well as total yield per hectare, were significantly increased with increasing planting density.

Effects of spacing on planting density were demonstrated by Proe et al. (2002), who conducted research on red alder and balsam spire poplar. In this study, both species were grown in rows, that were spaced at either 1m or 1.5m apart. Root:shoot ratios and leaf weight ratios of both species increased when planting occurred at wider spacing. This was attributed to the fact that with planting density at wider spacing, leaf and root biomass increased for longer, before competition for above- and below-ground resources commenced. However, planting at wider spacing reduced stocking density by 56%, and led to a 35% decrease in total community biomass (dry matter/hectare). Results from this study shows that in addition to the number of plants/area (plant density), the *distance* between plants could also influence plant behaviour, and should be taken into consideration in plant studies.

It should be noted that in the above-mentioned studies, plant species were exposed only to planting density, and *not* to elevated C_a. Since plants in nature occur in populations of variable planting density, it would be beneficial to conduct studies on the influence of planting density on the response of plants to elevated C_a. Literature on plant responses to a combination of planting density *and* elevated C_a was scarce, and proved difficult to find. However, three such studies are discussed below.

He and Bazzaz (2003) investigated population- and individual-level responses of reproductive allocations of *Phytolacca americana* to elevated C_a. At the population-

level, the interaction between C_a and density was insignificant, but at the individual-level, the effects of elevated C_a on reproductive allocation was density-dependent. For example, elevated C_a decreased the reproductive mass per vegetative mass at low density, but increased it at high density. In addition, this study showed that net photosynthesis of *P. americana* increased under elevated C_a, but decreased with density, indicating a C_a times density interaction. Rutuerto et al. (1996) conducted a study to investigate the influence of plant density on the response of *Sinapis alba* to increasing atmospheric CO_2. A strong density effect on individual plant biomass was observed for both CO_2 levels (350 and 700 $\mu L.L^{-1}$). Similarly, total individual plant biomass values were significantly enhanced with elevated C_a, at all plant densities. Interestingly, when measured as a population/stand response, there was no effect of density on CO_2 responses. Wayne et al. (1999) also conducted a similar study, in which stands of *Brassica kaber* were grown at a range of six densities in both ambient and elevated CO_2 environments. Results showed that early in stand development, CO_2 enhancement of above-ground biomass was highly density-dependent. With regard to above-ground biomass, the density-dependence of the response of *B. kaber* to elevated CO_2 was reduced, as stands matured. At the final harvest, no apparent density-dependence of the response of stand communities of *B. kaber* to elevated CO_2, were observed.

Results from these three studies highlight the importance of considering plant density when assessing the potential impacts of CO_2 enrichment on plants.

1.5 Invasive aliens and elevated C_a

Biological invasions, the human-mediated breakdown of biogeographical barriers to species dispersal, could be a consequence of global environmental change (Witkowski, 2002). For several years, biological invasions have threatened to degrade the natural biological diversity of many nature reserves (Loope et al.1988). Patterson (1995) predicted that global warming and other climatic changes would affect the growth, phenology, and geographical distribution of weeds. More specifically, Dukes and Mooney, (1999) and Weltzin et al. (2003) proposed that most aspects of global climate change, especially the interactive effects between increasing C_a and global warming, would favour invasive alien species.

This idea is supported by other studies. For example, Poorter (1993) and Dukes (2000) suggested that many C_3 plants that are fast growing tend to respond more strongly to elevated C_a. Since vigorous growth rates are characteristic of many invasive species (Willis and Blossey, 1999; Simons, 2003), a positive response of invasive plant species to elevated C_a would be expected. However, Lloyd and Farquhar (2000) proposed that ranking of plants with different inherent growth abilities should *not* be independent of growth conditions. When grown individually or in monoculture, several lines of evidence have demonstrated a positive response of invasive plants to elevated C_a (Sasek and Strain, 1988; Dukes and Mooney, 1999; Dukes, 2000; Ziska, 2001). Ziska (2003) conducted a study on the response of six invasive species to different CO_2 concentrations: 284, 380 or 719 ppm CO_2. These averages corresponded roughly to the CO_2 concentrations which existed in the beginning of the 20th century, the current CO_2 concentration, and the future CO_2 concentration predicted for the end of the 21st century, respectively (Ziska, 2003). On average, the stimulation of plant biomass among the invasive species from current to future CO_2 concentrations, was 46%. Studies on invasive weed, *Pueraria lobata* (kudzu), showed a 51% increase in biomass, and 58% increase in stem height, when exposed to elevated C_a (1000 ppm) (Sasek and Strain, 1988).

However, research also suggests that invasive plants in mixed communities may respond differently to elevated C_a, compared with plants grown monospecifically. When *Centaurea solstitalis* (an invasive species), was grown in monoculture, it responded strongly to CO_2 enrichment by increasing above-ground biomass production by 70% (Dukes, 2002). However, when grown in competition with common serpentine grassland species, *C. solstitalis* responded to CO_2 enrichment with similar but *non-significant* increases (69% increase in above-ground biomass production). In contrast, above-ground production of an invasive annual C_3 grass grown in a mixed community, was shown to increase more at elevated C_a, than several of the native annuals (Smith et al., 2000), suggesting that not all plants behave differently in communities, regarding their response to CO_2 enrichment.

Because invasive aliens are major agents of land transformation, disruptors of ecosystem functioning and a threat to biodiversity (Richardson et al., 1997), the

predicted increase in the success of invasive alien plants in CO_2-enriched environments, is cause for major concern.

1.5.1 *Chromolaena odorata*

Background:

Chromolaena odorata (Linn.) King and Robinson (Asteraceae), formerly known as *Eupatorium odoratum*, is a common pan-tropical weed found in waste places, roadsides, and farmlands (Apori et al., 2000; Taiwo et al., 2000). It is commonly called 'Siam weed,' but was previously also known as Kingsweed, triffid weed and Christmas bush (Liggit, 1983; Udosen and Udodiong, 1999; Apori et al., 2000).

C. odorata is a perennial, semi-lignified herbaceous shrub that can form dense tangled bushes up to 1.5-5m in height (McFadyen and Skarret, 1996; Udosen and Udodiong, 1999; Apori et al., 2000; Witkowski and Wilson, 2001). Growth is optimal in the open or in partial shade, and pale-bluish-lilac or white flowers are produced in winter (McFadyen and Skarret, 1996).

Uses:

Many beneficial aspects of this weed have been identified. It has been widely used with considerable success as a fertilizer (Liggit, 1983). According to Udosen and Udodiong (1999), a decoction of the leaf is used in combination with lemon grass and guava leaves for treatment of malaria. In Vietnam, Eupolin ointment (made from leaves of *C. odorata*) has been licensed for the treatment of soft tissue wounds and burns (Phan et al., 2001). Apori et al. (2000) conducted a study which revealed that *C. odorata* leaves are of high nutritive value, and might have the potential to be used as a protein supplement for ruminants.

C. odorata poses a problem:

However, despite its many uses *C. odorata* has become a serious pest in the humid tropics of South East Asia, Africa and Pacific Islands (Ye et al., 2004). In Nigeria for example, it constitutes an aggressive weed that is very difficult to control in young single and mixed crop plantations of oil palm, rubber and cocoa (Ikuenobe and Anoliefo, 2003).

Studies have showed that *C. odorata* can be killed physically or chemically without too much difficulty (Liggit, 1983; Goodall and Erasmus, 1996; Zachariades and Goodall, 2002). The problem lies in keeping an area free from the weed once it has been cleared. The success of *C. odorata* as an alien invader, could therefore be attributed to the fact that it possesses many characteristics necessary for rapid spread and establishment.

Factors promoting spread of C. odorata:

C. odorata is fast growing and rapidly multiplying. High competitive ability, production of phytotoxins (allelopathy), and general hardiness and wide environmental tolerance, further render it a problem plant (Liggit, 1983). Plants attain reproductive maturity relatively early (Witkowski, 2002), and efficient short- and long-distance dispersal abilities ensure easy dispersal of the large quantity of seeds produced by this species (Ye et al., 2004). The fact that a small but significant proportion of seeds persist in the soil for more than a year (Witkowski, 2002), further enhances this species' exceptional reproductive ability. These properties enable *C. odorata* to invade disturbed areas and recolonize cleared land very rapidly (Liggit, 1983). Another particularly dangerous attribute of *C. odorata*, is its high flammability: it burns even when green in midsummer, due to the presence of essential oils in its stems and leaves. Nevertheless, it is rarely killed by fire and the ashbed that results from fires provides a good germination site for *C. odorata* seeds (Liggit, 1983).

An early study demonstrated soil moisture as an important criterion for the survival of *C. odorata* (Kushwaha et al., 1981). Support for this idea comes from a recent study, which showed that *C. odorata* grows mainly in areas with a rainfall of > 800 mm per annum (Zachariades and Goodall, 2002). Bright sunlight and high relative humidity have also been documented as environmental factors which promote vigorous growth of this species (Ambika, 2002). Therefore environmental factors, in combination with the properties of *C. odorata* described above, facilitate the spread of this weed throughout the tropical world, making this species extremely difficult to control.

C. odorata in South Africa:

C. odorata was first recorded in South Africa in 1947 near Ndwedwe, KwaZulu-Natal (Retief, 2002). During the next 30 years, *C. odorata* spread southwards and northwards throughout the coastal, subtropical region of KwaZulu-Natal (Liggit, 1983; Goodall and Erasmus, 1996). By 1986, *C. odorata* was identified as one of 47 alien invasive plant species, capable of transforming habitats and landscapes in South Africa (Wells et al., 1986).

Other studies conducted in South Africa, have revealed that *C. odorata* suppresses indigenous vegetation through physical smothering and allelopathy, and the impenetrable tangles resulting from its growth form has the potential of shading out all natural vegetation (Macdonald, 1983; Zachariades and Goodall, 2002). *C. odorata* also forms dense thickets on the edges of forest and riverine forest, ecotones which are normally fire-excluding (Macdonald, 1983). Following invasion by *C. odorata*, fires burning in adjacent grassland and woodland areas cross the ecotone with ease and burn right into the canopies of fire sensitive forest trees. This could explain the current severe threat that *C. odorata* poses to forest biodiversity in South Africa (Zachariades and Goodall, 2000). A recent study conducted near St. Lucia in Kwa-Zulu Natal, confirmed that *C. odorata* was most abundant in indigenous forest reserves, compared to other land regimes (van Gils et al., 2006). Therefore, in South Africa, this species of weed is *primarily* viewed as a threat to biodiversity conservation, and *secondarily* to commercial agriculture and forestry (Liggit, 1983; Goodall and Erasmus, 1996).

C. odorata was first observed in the Hluhluwe Game Reserve (north-eastern KwaZulu-Natal) in the early 1970's (Howison and Balfour, 2002), and was classified as the most widespread alien invader in the reserve by the 1980's (Macdonald, 1983). By 1998, approximately 2100 hectares of Hluhluwe Game Reserve were densely infested by *C. odorata* (Howison and Balfour, 2002).

Although there are large tropical and subtropical areas in eastern and central Africa, where *C. odorata* is absent or limited to small infestations (McFadyen and Skarret, 1996), figures regarding the Hluhluwe Game Reserve in South Africa suggest that the potential threat of *C. odorata* should not be underestimated.

Control:

Biological control of a weed involves the introduction of host-specific natural enemies of a weed from its original native range (Fowler et al., 2000). In South Africa, a review reported extensive use of biological control of 38 alien plant species (Richardson et al., 1997). More recently, the success of biological control of environmental weeds was reported in New Zealand (Fowler et al., 2000). Goodall and Erasmus (1996) proposed that *biological control* is the only viable solution for reducing the current and potential impacts of *C. odorata*.

However, a recent study conducted near Scottburgh on the KwaZulu-Natal coast, showed that sugar-cane farmers *could* resort to a very costly option of tractor-mounted herbicide sprayers to curb *C. odorata* infestations on their farms (Goodall, 1997). According to Richardson et al. (1997), satisfactory control of weeds is usually achieved only when several complementary methods, including biological control, improved land management practices, herbicides and mechanical methods are carefully intergrated. Therefore, an effective *intergrated* control programme needs to be developed, to reduce *C. odorata* infestations in South Africa (Witkowski and Wilson, 2001; Witkowski, 2002).

1.6 Experimental approaches to plant studies on responses to elevated C_a

According to Long et al. (2004), most information about plant responses to elevated C_a has been derived from experimental studies that use greenhouses, artificially illuminated controlled environmental chambers, and in the field, transparent enclosures or open-top chambers (OTCs). Many of these studies, including some of the field studies, have used plants grown in pots.

Thomas and Strain (1991) showed that inadequate rooting volume (observed for plants grown in small pots), reduced the photosynthetic capacity of cotton seedlings, clearly demonstrating that the loss of response to increased C_a through acclimation, was an artifact of pot size. Even large pots have been shown to restrict the response of plants to elevated C_a. Ainsworth et al. (2002) conducted a survey on studies of soybeans grown at elevated C_a, and found that plants grown in the field without

restrictions on rooting volume showed a significant three fold increase in yield, compared to plants grown in large pots (> 9 litres volume).

Most field studies have been based on the use of OTCs (Long et al., 2004). According to these authors, there are important differences between the environment within the best-engineered OTCs and the environment surrounding the chamber, despite the fact that the tops, or large portions of the tops, of OTCs are open to the atmosphere. One obvious effect of an OTC is that wind is removed, preventing wind damage and dispersal of pathogens, rainfall interception is dramatically reduced, and plant-atmosphere coupling is altered (Long et al., 2004).

Some field studies have even been conducted with the aid of branch chambers, where separate branches of trees are isolated and enclosed in a chamber (Teskey, 1995). According to this study, the relatively small size of these chambers helps provide a similar microclimate to that outside the chamber. However, a limitation of this technique is that only a small portion of the crown is exposed to elevated C_a, and so phenomena e.g. photosynthetic acclimation, would not be observed, unless it is localized.

Free Air CO_2 enrichment (FACE) experiments have also been reported in many studies on plant responses to CO_2 enrichment (Bryant et al., 1998; Wechsung et al., 1999; Herrick and Thomas, 2001; Bernacchi et al., 2005). A typical FACE apparatus consists of a 20-m-diameter plot within the cropfield, in which CO_2 is released just above the crop surface on the upwind side of the plot (Long et al., 2006). The greater size of FACE plots by comparison to OTCs not only reduces edge effects but also allows simultaneous studies of many plant processes Within chamber systems, such holistic approaches are precluded by the damaging effect that would result from destructive sampling of soil and leaves (Long et al., 2004). Woodward (2002) and Ainsworth and Long (2005) also maintain that FACE methods minimize experimental artifacts by allowing researchers to work under natural field conditions, without enclosures.

However, FACE is not without limitations. Long et al. (2004) maintain that one potential disadvantage of FACE is that it depends on continuous air movement. In

addition, wind generates a dilution gradient across treatment plots, a gradient which becomes more pronounced in larger plots. Further evidence, which supports the fact that FACE may not always be the better option, comes from a review of data obtained from 12 large-scale FACE experiments, which showed that trees were more responsive to FACE than herbaceous species (Ainsworth and Long, 2005)

Although a study on *Larrea tridentate* showed that FACE *and* glasshouse well-watered plants displayed similar behaviour when exposed to elevated C_a (Huxman et al., 1998), there are important differences between experimental approaches, which cannot be ignored. For example, when Long et al. (2006) assessed model projections of global crop yield in CO_2 enrichment, elevated C_a in FACE studies enhanced yield of crops by ~50% less than in enclosure studies.

Although chamber studies have used a wide range of elevated C_a, averaging 700 $\mu mol.mol^{-1}$, FACE studies have used an elevation of 550-600 $\mu mol.mol^{-1}$ (Long et al., 2004). The high cost of FACE experiments (Conroy et al., 1986), is probably the reason for this difference. However, this may have serious consequences, in that responses to CO_2 enrichment which are small in magnitude, may not be detected at the low C_a elevations used in FACE studies.

From the literature reviewed above, it is clear that there advantages and disadvantages associated with both experimental approaches (OTCs and FACE). The use of OTCs was considered the best option in the current study, particularly with respect to cost.

1.7 This study

Background:

The severity of the problem of *Chromolaena odo*rata as an alien invader in South Africa, has been outlined in Section 1.5.1. CO_2-enriched atmospheres, as well as a potential increase in the success of invasive aliens in elevated C_a, have been predicted for the future (See Sections 1.2 and 1.5., respectively). Therefore, a study on the response of *C. odorata* to elevated C_a, is required to assist future control measures of this particular weed in the South Africa.

Naidoo, personal communication, conducted a study in which *C. odorata* was grown in competition with another C_3 species, *Chrysanthemoides monilifera*. Results showed that *C. monilifera* out-competed *C. odorata* in CO_2 enriched atmospheres. Based on the fact that *C. odorata* is a serious invader of grasslands, the present author (Lalla, unpublished) investigated the effects of elevated C_a on *C. odorata*, when grown in competition with a C_4 grass, *Hyparhenia dregeana*. Results from this study showed no enhancement of growth (e.g. total leaf area, plant height etc.) of *C. odorata* when grown in elevated C_a. However, the lack of photosynthetic data in that study should be noted.

Experimental approach:

It is agreed that the most realistic picture of plant responses to elevated C_a will be found *in situ*, but a disadvantage of this approach is that the mechanisms of CO_2 effects will be obscured by a multitude of unknown interactions (Stöcklin and Körner, 1999). The current project attempts to bypass this problem by studying artificial plant communities growing in pots in a greenhouse.

Treatments:

Nutrient availability, according to Poorter et al. (1996) could affect a plant's response to elevated C_a. In the current study, *Eragrostis curvula* and *Themeda triandra* were chosen as competitors for *C.odorata*, because of their preference for different nutrient levels. *E. curvula* is a perennial C_4 grass native of South Africa that grows naturally in many semi-arid regions (Colom and Vazzana, 2003). Also known as weeping lovegrass, *E. curvula* is able to adapt to climatic and edaphic factors. This species is well adapted to high fertility, and a strong reaction to nitrogenous fertilization has been observed (Rethman, 1973). Therefore, growth of this species is favoured by high nutrient supply. *T. triandra* on the other hand, is a perennial warm-season C_4 grass which performs better in poor nutrient soils (Groves et al., 2003).

According to Weltzin et al. (2003), plant responses to elevated C_a are often restricted by biotic (e.g. competition), and abiotic (e.g. soil nutrients) factors, that interact with each other and with changing C_a concentrations. On this basis, one of the main aims of the current study, was to explore the relationship between competition and nutrient availability, and its potential impacts on the response of *C. odorata* to elevated C_a.

In previous studies on *C. odorata* (Naidoo, personal communication; Lalla, unpublished), this species was grown in competition with other species. In addition, the density of the experimental pots in these two studies, were kept constant. It would be interesting to investigate the *C. odorata* responses to elevated C_a, when grown monospecifically, and subjected to different planting densities.

Experimental trials:

This study was divided into two experimental trials. The first of these (referred to as PART A), considers the effect of elevated C_a on interspecific competition. *C. odorata* was grown in competition with *T. triandra* and *E. curvula*, and planting density was kept constant. Plants were subjected to two factors: C_a and nutrient availability.

The second trial (PART B) explores the effect of planting density on responses of *C. odorata* to elevated C_a. In this trial, *C. odorata* was grown monospecifically, at two different planting densities. Therefore, C_a and planting density were the two factors, to which plants from PART B were exposed.

In both experimental trials, the effect of elevated C_a on the growth and physiology of *C. odorata*, was assessed.

CHAPTER 2. MATERIALS AND METHOD

2.1 Collection of Plant Material

PART A: During April 2004, sixty-four intact young, single-stemmed, plants with roots, of *C. odorata* were collected from Manor Gardens, Durban, KwaZulu-Natal in South Africa (29º 51.781'S, 30º 58.286'E). A single collection site was opted for, to minimize variation. It was also for this reason that plants of similar height and stem diameter were chosen. Once collected, the plants were completely defoliated and cut back to a single node, before planting. During this growth trial, two grass species (*Eragrostis curvula* and *Themeda triandra)*, were selected as potential competitors with *C. odorata*. These grasses were obtained from the Grassland Science Department, University of KwaZulu-Natal, Pietermaritzburg. Grass plants were growing as plugs in "seedling trays," before they were potted.

PART B: During February 2005, sixty-four young plants of *C .odorata* (of similar height and stem diameter) were collected from the grounds of the University of KwaZulu-Natal, Durban (29º 52.238'S, 30º 58.513'E). Once again, these plants were defoliated and cut back to a single node, before planting. During this experimental trial, *C. odorata* was grown in the absence of competitors.

2.2 Experimental Set-up

During PART A and PART B, plants were potted in open-top chambers (OTCs) in a greenhouse at the University of KwaZulu-Natal, Durban. Sixteen large round pots (50cm in diameter and 26cm in depth) were used in the study. Several holes were drilled into the bottom of each pot to provide drainage. Pots were then filled with 3cm of coarse gravel, to serve as a water filter. After the gravel was treated with a fungicide (Dursban 2 E, AgrEvo, chloropyriphos (organophosphate), pots were filled to the brim with river sand. The combined effect of the drainage holes and gravel was to provide drainage and prevent excess water retention in the soil. To avoid soil overheating, pots were painted white. All 16 pots were then placed on stands in the greenhouse.

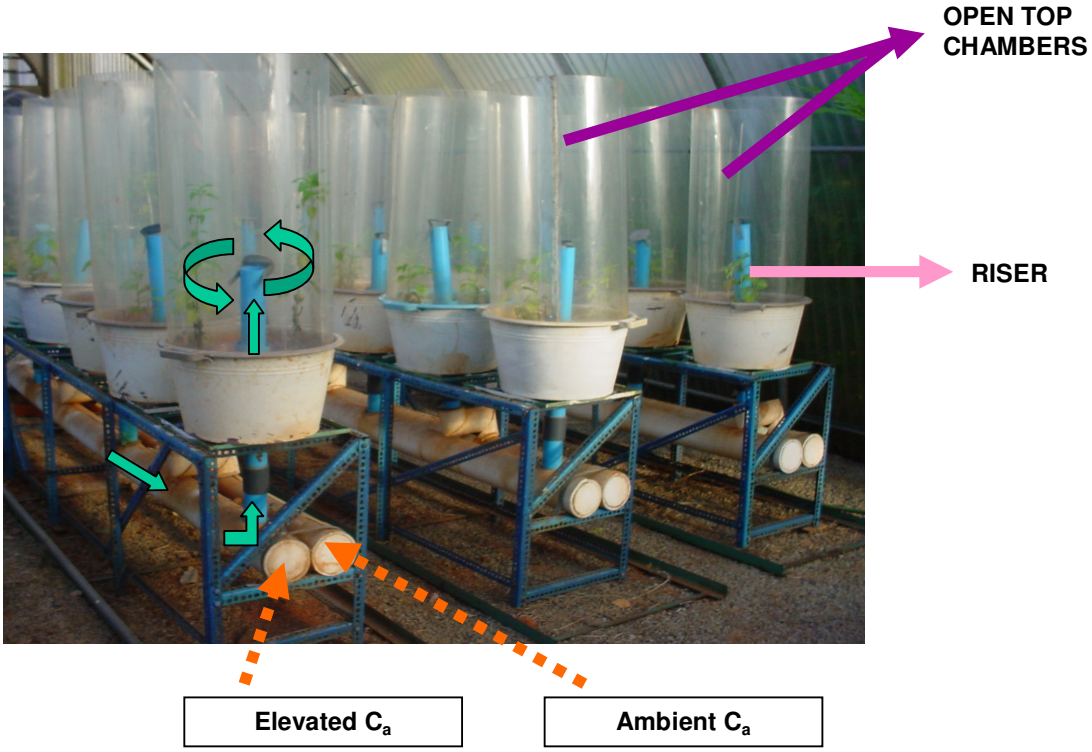


Fig. 2.1 Arrangement of 16 pots in the greenhouse. Green arrows indicate direction of airflow through each pot. Broken arrows illustrate which pipe transports elevated or ambient C_a, in each row

Air (from the outside) entered the greenhouse through an inlet, and was supplied to each of the 16 pots, via white insulated pipes (Fig. 2.1). Air flowed through the pipes, up the riser pipe (63 mm in diameter), and into the open top chamber. The risers were fitted with valves, which were manipulated to ensure that all pots received air at the same flow rate. In addition, risers were fitted with baffles to distribute the air in the chamber. The OTCs were 0.82 m in height and 0.44 m in diameter, giving a volume of 0.125 m^3. The flow rate was set in each pot, with the help of an air velocity meter. The 'valves' in the risers were adjusted manually, until the air-velocity meter gave a reading of ~2.45 ms^{-1} for each pot. This supplied air sufficiently rapidly to provide three changes of air per minute in the OTCs.

2.3 Experimental design

The experimental factor common to both experimental trials, was C_a. During the study, eight of the sixteen pots were exposed to ambient C_a (~370 ppm), and the other 8 pots were exposed to elevated C_a (~720 ppm). The source of elevated C_a (a CO_2 cylinder), was connected to only three of the six white pipes (Fig. 2.1). This kind of experimental design made it possible for plants to be exposed to different C_a treatments, even though all 16 pots were situated adjacent to each other.

2.3.1 Treatments

PART A: Each of the 16 pots contained four *C. odorata*, four *E. curvula* and four *T. triandra* seedlings (Fig. 2.2).

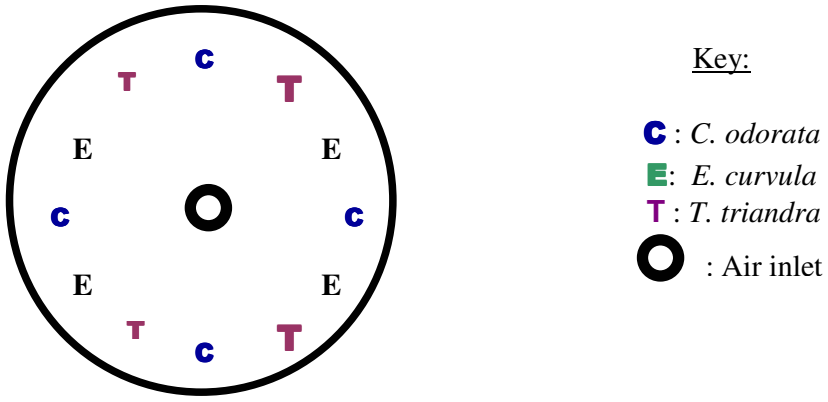


Fig. 2.2 Illustration showing species arrangement in each pot, for PART A (Aerial view)

Consequently, the density of each pot was kept constant (12 plants in total). In addition to C_a, another influencing factor was introduced: nutrient treatments. Stock solutions of Hoagland's nutrient solution (Hoagland and Arnon, 1950 in Salisbury and Ross, 1992), were prepared, and supplied fortnightly to the pots. The various nutrients used to make up the nutrient solution supplied to the pots, are shown in Tables 2.1 and 2.2.

Table 2.1 Macronutrient concentration of final solution

Nutrient	Concentration ($mol.L^{-1}$)
K	0.006
P	0.001
N	0.01
Ca	0.005
Mg	0.002
S	0.002
Fe	9×10^{-5}

Table 2.2 Micronutrient concentration of final solution

Nutrient	Concentration ($mol.L^{-1}$)
B	4.69×10^{-5}
Cu	9.19×10^{-6}
Zn	7.67×10^{-7}
Mo	4.49×10^{-7}
Mn	1.11×10^{-7}

This solution was prepared by diluting the following stock solution (Table 2.3):

Table 2.3 Stock solution for Hoagland's nutrient solution

Salt	Concentration (g/L)	Dilution factor
KH_2PO_4	136	1:1000
KNO_3	101	5:1000
$Ca(NO_3)_2$	164	5:1000
$MgSO_4$	120	2:1000
H_3BO_3	2.86	1:10000
$MnCl_2.4H_2O$	1.81	
$ZnSO_4.7H_2O$	0.22	
$CuSO_4.H_2O$	0.08	
$H_2MoO_4.H_2O$	0.02	
Fe-EDTA	33	

At each C_a treatment, four pots received high nutrient treatments (3 L per addition), and the other four pots received low nutrient treatments (300 ml per addition), every two weeks. All plants were watered on a regular basis. A diagrammatic representation of treatments for PART A, can be seen in Fig. 2.3.

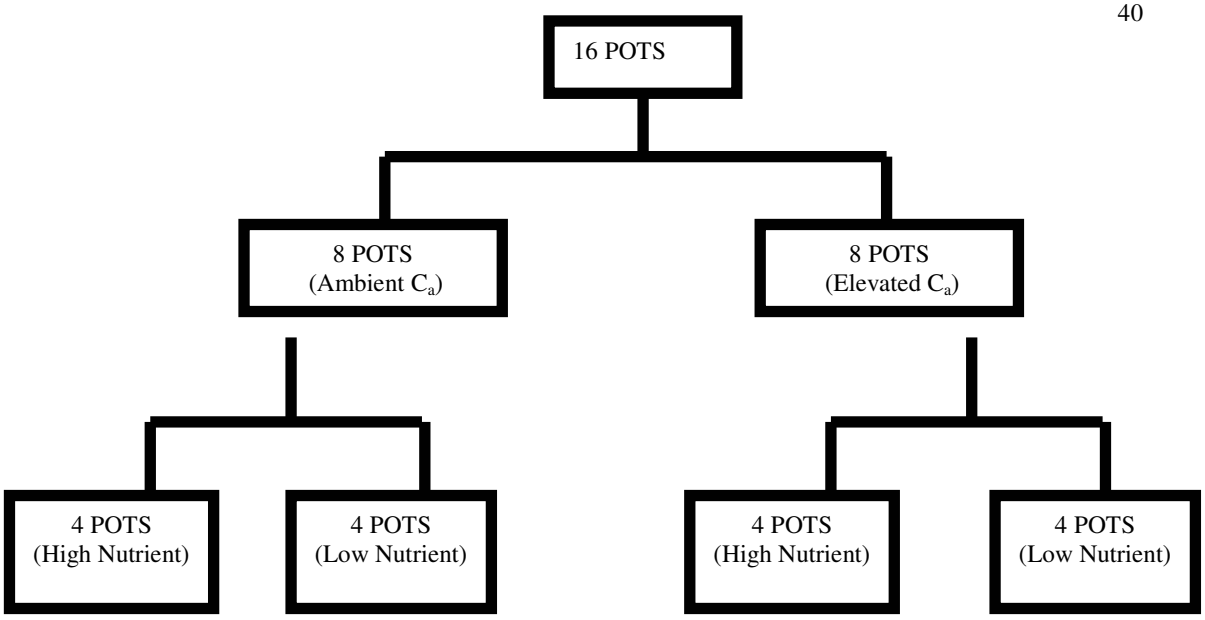


Fig. 2.3 Illustration showing experimental design of PART A

Treatments, and the respective abbreviations for PART A, are summarized in Table 2.4 below.

Table 2.4 The four treatments applied in PART A

C_a	Nutrient	Treatment Abbreviation
Ambient	Low	A & LN
Ambient	High	A & HN
Elevated	Low	E & LN
Elevated	High	E & HN

PART B: For this experiment, *C. odorata* was grown monospecifically. At each C_a treatment, 4 pots contained four plants each, and the remaining 4 pots contained two plants each. Figure 2.4 shows a simple diagrammatic representation of the treatments in PART B of this study.

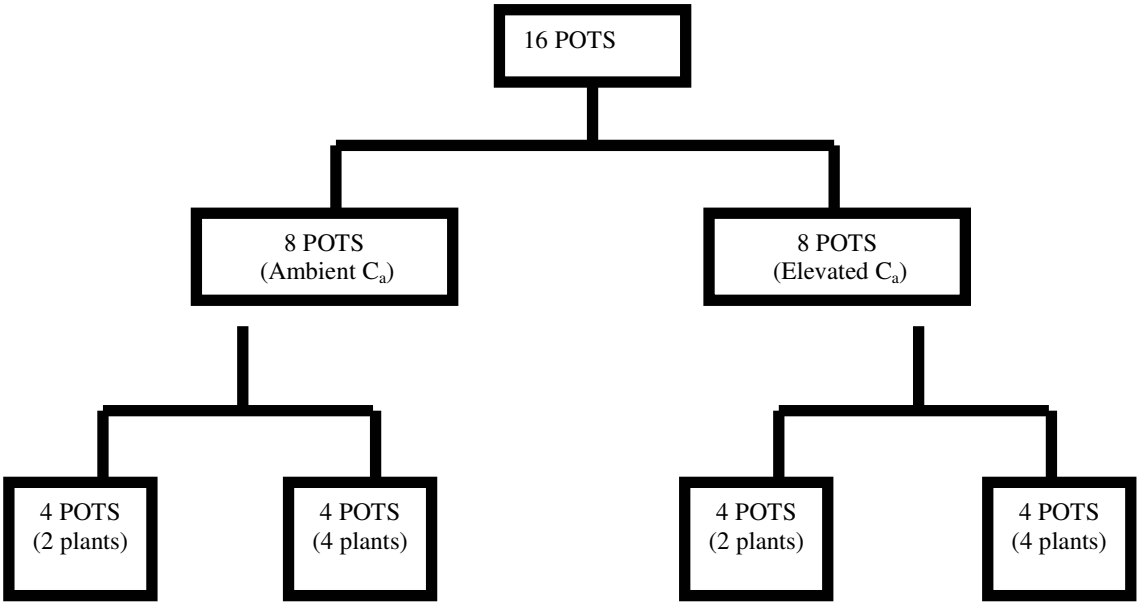


Fig. 2.4 Illustration showing experimental design of PART B

Plants grown in this experimental trial were also exposed to two influencing factors (C_a and density), and four treatments (summarized in Table 2.5).

Table 2.5 The four treatments applied in PART B

C_a	Density (no.of seedlings)	Treatment Abbreviation
Ambient	2 plants	A & LD
Ambient	4 plants	A & HD
Elevated	2 plants	E & LD
Elevated	4 plants	E & HD

2.4 Data collection

Plants were watered regularly and allowed to grow for reasonable periods of time (6 months for PART A and 8 months for PART B), before harvesting. Occasionally plants were treated with insecticides (e.g. Kelthane and Ludwigs insect spray), to remove ants and aphids. The data collected have been separated into 2 categories: physiological data and growth data.

2.4.1 Physiological data

Photosynthetic measurements:

These were essentially measurements of the response of net CO_2 assimilation (A) to intercellular CO_2 concentrations (C_i), and were obtained using the Li-Cor 6400 portable infrared gas analyzer (IRGA) (LiCor, Lincoln, Nebraska, U.S.A). For PART A, two mature leaves from each pot were selected for measurements, and one leaf per plant was sampled in PART B. Once a leaf was enclosed in the leaf chamber of the IRGA, a C_a value of either 400 or 700 ppm was selected, depending on which C_a treatment the leaf had been exposed to during the growth trial. The flow rate was set to 500 $\mu mol.s^{-1}$, and a block temperature of 25 °C was selected. To eliminate light as a compounding factor, a constant light intensity was used. This value was selected as 1000 $\mu mol.m^{-2}s^{-1}$ photosynthetic photon flux density (PPFD), since preliminary studies showed that photosynthesis of *C. odorata* occurs maximally at this light intensity. Figure 2.5 shows the use of the IRGA, in obtaining A:C_i data in the study.

Fig. 2.5 Picture showing IRGA-use in the greenhouse

To allow for acclimation to chamber conditions, the leaf remained in the chamber for ~10 minutes before any readings were taken. An A:C_i autoprogram was then selected from the menu. In this autoprogram, the user is required to enter desired C_a values, to which the leaf would be exposed. The initial value was selected as either 400 or 700 ppm (depending on the C_a treatment). This initial value was decreased stepwise to 50 ppm, and then increased stepwise to 1000 ppm. During the increase, the leaf was exposed to the original C_a value again. It should be noted that stepwise increasing and decreasing of C_a values was done at regular intervals (200 ppm at a time), to avoid unnecessary stress for the leaf. However, smaller intervals (50 ppm) were used at lower C_a concentrations to improve the accuracy of the initial slopes of A:C_i curves. Data captured by the IRGA over the range of C_a values selected, included air temperature (T_a), stomatal conductance to CO_2 (g_c), intercellular CO_2 concentration (C_i) and photosynthetic net CO_2 assimilation rate (A).

The response of A to C_a itself cannot be interpreted easily since it is affected by boundary layer, stomatal, and mesophyll processes; the response of A to C_i however, eliminates the effect of the boundary layer and stomata, depending solely on the mesophyll processes. Hence it is not surprising that one of the most commonly reported responses of CO_2 uptake is the response of A to C_i (Long and Bernarcchi, 2003).

A plot of assimilation versus C_i, is called an A:C_i curve.

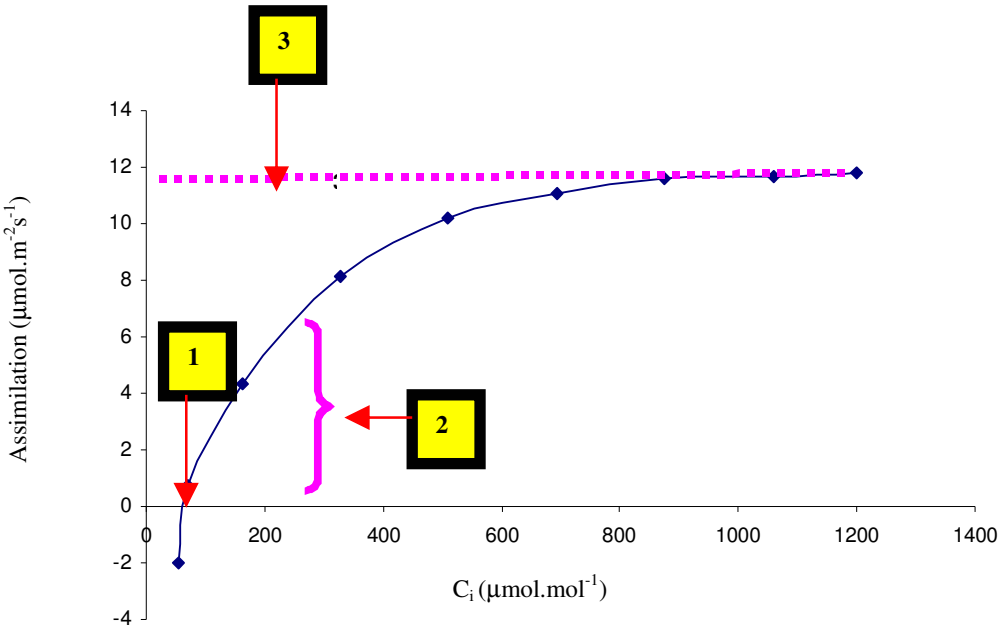


Fig. 2.6 Idealized A:C_i curve (1: CO_2 compensation point, 2: initial slope and 3: J_{max})

Although A:C_i curves show general trend patterns, Long and Bernarcchi (2003) state that one of the most common purposes of these curves is to extract other information, such as the maximum rate of electron transport used in the regeneration of RuBP (J_{max}). For this reason, the model curve in Fig. 2.6 can be analysed to obtain the values of three parameters: 1) The CO_2 compensation point, 2) the initial slope of the curve, and 3) J_{max}. The CO_2 compensation point is the C_i concentration at which the rates of gross assimilation and respiration are equal. The initial slope of the curve gives the carboxylation efficiency, and is a measure of the activity of the enzyme rubisco. J_{max} is the maximum assimilation rate, and is a measure of the maximum rate of flow of electrons down the photosynthetic electron transport chain (Farquhar and Sharkey, 1982).

To investigate how these three parameters of photosynthesis responded to the treatments, if at all, data from the $A:C_i$ curves were fitted to the monomolecular function $A=a*(1-(\exp(b-c*C_i))$, (Causston and Dale, 1990), to yield values of a, b and c for each curve. These values were used to estimate the values of the parameters, in the following way:

$a = J_{max}$

$b/c = CO_2$ compensation point

and $a*c*e^b$ = initial slope of the curve. This is derived from differentiating the curve and solving for $C_i=0$

Results from these calculations were used to construct an "average" $A:C_i$ curve, for each treatment.

Stomatal measurements:

In order to investigate the stomatal response of *C. odorata* to elevated C_a, stomatal conductance to CO_2 (g_c), and stomatal limitations to CO_2, were assessed. Unfortunately, no direct measurements of g_c under CO_2 enrichment, were taken. To compensate for this, values of g_c which corresponded with C_a values closest to current C_a (~370 ppm) and elevated C_a (~720 ppm) were extrapolated from $A:C_i$ data, and averaged per treatment.

Stomatal limitations were calculated for each $A:C_i$ curve (see Fig. 2.7), using the equation (Farquhar and Sharkey, 1982):

$$\text{Stomatal limitation} = (A_o-A)/A_o$$

where, A_o= Assimilation when stomatal conductance to CO_2 is infinite ($g_{c=\infty}$).

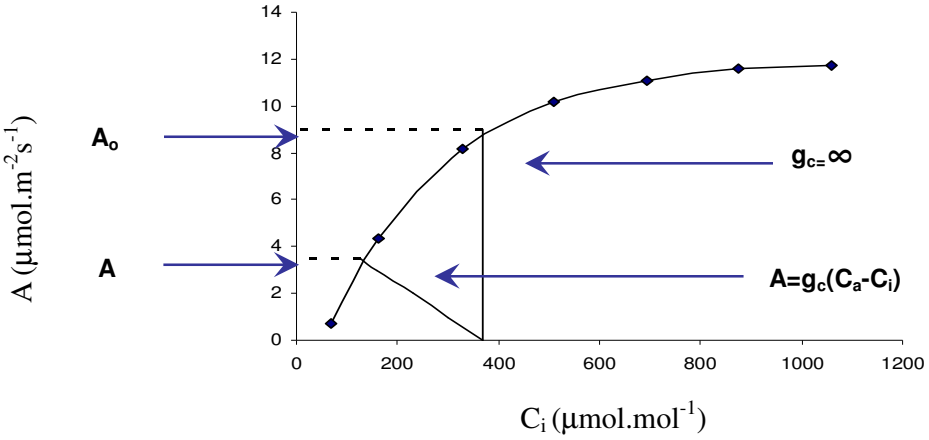


Fig. 2.7 Idealized A:C_i curve, showing variables used in the formula to calculate stomatal limitations

A was calculated using the equation: $A=g_c(C_a-C_i)$. For this equation, g_c was selected at C_i =~370 ppm (close to atmospheric CO_2) and C_a was entered as 370 ppm. C_i was initially entered as 370 ppm, but was then decreased at regular intervals to yield arbituary values of A. This equation describes the supply function of photosynthesis: as A increases, C_i decreases linearly and in inverse proportion to g_c. The point of interception between the supply function and A:C_i curve (demand function), gives the operating C_i of the plant (Long et al., 2004). If there was no diffusion barrier, (i.e. $g_{c=\infty}$), C_i would equal to C_a, as indicated by the vertical line originating from ~370 ppm on the x axis. In many plants, the value of A at operational C_i is commonly about 90% of what it would be without the epidermis as a barrier to water loss and CO_2 diffusion into intercellular spaces (Drake and Gonzàlez-Meler, 1997). Since A is the actual rate of assimilation at actual C_i, the limitation imposed by stomata is given by $(A_o-A)/A_o$ (in this example (Fig. 2.7), stomatal limitation=(9-3.8)/9=0.58).

2.4.2 Morphological and Growth data

It is appropriate at this point, to introduce the terminology of "per plant" (PP) and "per community" (PC), which will be used frequently from hereon, with respect to growth data. These terms will be explained with the aid of the hypothetical example: root dry weight. Root dry weight PP will refer to the effect of a stimulus on the root dry weight of an individual plant. Root dry weight PC refers to the response of a community (all plants in a given treatment) to an influencing factor, and will be calculated by adding together the root dry weights of all plants belonging to the community (a specific treatment).

It should be noted that PC data were only analyzed for PART B of the experiment, in which planting density was one of the influencing factors. In PART A, planting density was kept constant, and so it was thought that PC calculations were irrelevant. Morphological and growth data have been separated into data collected prior to harvesting, and data collected after harvesting.

2.4.2.1 Pre-harvest data

Stomatal density (SD):

This parameter was calculated only for PART A. Two *C. odorata* leaves from each plant were randomly selected. Because data were eventually pooled together, this meant that 32 leaves were sampled for each of the 4 treatments.

Accoding to Wallace et al. (1996), stomata are usually concentrated in the abaxial epidermis of leaves. In addition, an earlier study on *Phaseolus vulgaris*, showed a significant C_a effect on SD of abaxial, and *not* adaxial leaf surfaces (O'Leary and Knecht, 1981). In light of the above, only abaxial surfaces of *C. odorata* were sampled. With the aid of a medicine dropper, acetone was applied to a section of leaf. This section was randomly chosen, since a previous study (Lalla, unpublished) showed no spatial heterogeneity in stomatal density across the leaf surface of *C. odorata*. A small strip of cellulose acetate was gently pressed onto the moist surface to obtain a "surface print" of the leaf. After the strip was dry, it was peeled off the leaf and placed on a microscope slide. At this point (after drying), the strip

had hardened and curled around the edges. So, to solve the problem, a small amount of acetone was placed on the slide before placing the strip. This "flattened" out the strip, and facilitated its adherence to the slide.

Slides were viewed under a light microscope, at a magnification of 25X. When an image (with clear, visible stomata) was in focus, it was captured. Three images per leaf were captured for replication purposes. This resulted in the capturing of 96 images for each of the 4 treatments. All images were then imported into the Adobe Photoshop software program. For each image, as all visible stomata were manually highlighted, the program counted the number of stomata in the field of view, and values of stomatal density per mm^2 of leaf surface were calculated. No obvious visual differences among treatments, in the lengths of pores and guard cells, were observed.

Plant height and Stem cross-sectional area:

Plant height (PP) was measured using a measuring tape. Each plant in each pot was sampled, resulting in 16 replicates per treatment. Measurements were taken from the point of new growth to the tallest part of the plant. Thereafter, total stem length (PC) was calculated by summing up the individual plant heights of all plants belonging to a treatment. Stem diameter measurements were obtained with the use of vernier calipers. Because plants of similar stem diameter were initially collected (See Section 2.1.), averages of stem diameter were calculated for each plant, at the point of new growth. Stem cross-sectional areas PP, and stem cross-sectional area PC were calculated.

2.4.2.2 Post-harvest data

Total leaf area, specific leaf area and leaf dry weight:

All the leaves of each plant were plucked by hand, and the total area per plant measured using a portable leaf area meter. All the leaves (except one average-sized leaf) were placed in a marked brown paper bag, and dried in the oven at 80 °C for 2 days, after which they were weighed. The area of the individual selected leaf was measured with the leaf area meter, the leaf was dried and weighed, and the specific leaf area (SLA) was calculated. SLA measurements were taken only for PART B. Total leaf area (PC) and leaf dry weight (PC) were also calculated. For PART A of

the study, total leaf area of the experimental grasses (*Eragrostis curvula* and *Themeda triandra*, were also measured.

Stem dry weight, root dry weight and total plant biomass and ratios:

Stems of each plant were cut off with a pair of secateurs, and placed in brown paper bags. To obtain stem dry weight (PP), the paper bags were placed in an oven (80 °C) for 2 days. Root data were not captured for PART A, since it was impossible to separate the below-ground material of the individual plants in each pot. However, root dry weight (PP) was measured for PART B, since *C. odorata* was grown monospecifically. Roots of individual plants were collected by physically pulling the plants out of the soil, placed in a brown paper bag, and then left in an 80ºC oven for 2days. Unfortunately, one of the disadvantages of this technique is that some root material (especially the finer roots), could have escaped detection and remained in the soil. However, effort was made to ensure that most of the root material was removed when the plants were uprooted. Roots were not washed after they were pulled out of the soil, which resulted in soil particles being collected as well. However, this did not pose a problem, since oven-drying caused the soil to separate from the roots and accumulate at the bottom of the paper bags. This, together with the fact that roots were physically shaken prior to being weighed, suggests that soil particles could not have affected root dry weight values. Values of leaf, stem and root dry weight of each plant were then added together to produce a value for total plant biomass (PP).

Biomass partitioning to leaves, stems and roots was then calculated as percentages of total plant biomass, for each plant. Averages for leaf dry weight (PC), stem dry weight (PC) and root dry weight (PC) of each treatment, were added together to calculate total plant biomass (PC). Community allocation to leaves, stems and roots were then calculated as percentages of total plant biomass (PC), for each treatment. Leaf area ratios (LAR; total leaf area per total plant biomass), were calculated at a PP and PC level. For PART A, total above-ground biomass of the two grasses (*Eragrostis curvula* and *Themeda triandra*), were assessed, in addition to measurements for *C. odorata.*

2.5 Controls for experimental trials

During PART A, eight additional pots, containing only grass species, were exposed only to nutrient treatments, as they were grown at ambient (~370 ppm) C_a. This was done to investigate if there was a major difference in the behaviour of grasses grown without *C. odorata (*non-experimental grasses) compared to grasses grown together with *C. odorata* (experimental grasses). Only leaf area and total above-ground biomass were calculated for the non-experimental grasses in PART A.

For PART B, four additional pots were not subjected to density treatments, as they each contained 2 *C. odorata* seedlings. These plants (termed "non-treatment plants") were grown at ambient C_a concentrations for seven months, after which they were exposed to elevated C_a concentrations. Measurements of J_{max} were taken 2days after exposure to C_a enrichment, and thereafter at regular intervals until 22 days. This was done to futher investigate the possibility of photosynthetic down-regulation in *C. odorata* exposed to elevated C_a.

2.6 Statistical analysis

Physiological data (J_{max}, initial slope and CO_2 compensation points) as well as stomatal limitations for each plant, were also subjected to K-S tests and analysis of variances across the treatments. Differences between g_c values at C_a=370 ppm and C_a=720 ppm, were assessed using a t-test. Analysis of variance (ANOVA) across all four treatments was performed for g_c values at C_a=370 ppm, and C_a=720 ppm, to investigate treatment effects.

Growth data were subjected to the Kolmogorov-Smirnof test (K-S test) to test for normality of the distribution of the data. As the data were normally distributed, a two-way Analysis of variance (ANOVA) across all 4 treatments was performed, for each parameter. Since leaf, stem and root weight ratios PP and PC were subjected to the ANOVA as percentages, the residuals of the analyses for these three parameters had to be tested for normality. Results from K-S tests showed that they were normal.

CHAPTER 3. RESULTS (PART A)

EXPERIMENTAL POTS: Influence of nutrients on the response to C_a

3.1 Physiology data

3.1.1 Photosynthesis

Data from A:C_i curves for each individual leaf were fitted to the monomolecular function y=a*(1-exp(b-c*C_i)), to yield values for CO_2 compensation point, initial slope and J_{max}. Data for these three parameters were subjected to a 2-way ANOVA, to investigate differences amongst the four treatments. Mean ± SD values and p-values are presented in Table 3.1.

Table 3.1 Mean±SD values for 3 photosynthetic parameters of *C. odorata*, grown at four treatments, as well as P-values for C_a, nutrient and C_a*nutrient effects

Parameter	Mean ± SD				P-values		
	A&LN	**A&HN**	**E&LN**	**E&HN**	C_a	nutrient	C_a*nutrient
J_{max} ($\mu mol.m^{-2}s^{-1}$)	10.6 ± 1.9	13.5 ± 2.3	11.7 ± 1.9	15.8 ± 3.0	0.075	0.001	0.559
Initial slope ($mol.m^{-2}s^{-1}$)	0.07 ± 0.05	0.08 ± 0.04	0.08 ± 0.04	0.05 ± 0.02	0.513	0.667	0.239
CO_2 compensation point (($\mu mol.mol^{-1}$)	74 ± 10	62 ± 8	70 ± 13	66 ± 8	0.980	0.067	0.368

Statistical analysis revealed significant nutrient effects on J_{max}, and means show that the highest, and second highest J_{max} values can be observed for the two high nutrient treatments (Table 3.1). C_a increased J_{max}, but this was a non-significant effect (p=0.075; Table 3.1). High nutrient supply decreased CO_2 compensation points, but with a non-significant p-value of 0.067 (Table 3.1).

An "average" A:C_i curve was constructed from the monomolecular function y=a*(1-(exp(b-c*C_i)), and the average values of the constants a, b and c for each of the four treatments. Figure 3.1 below, is a plot of "average" assimilation versus C_i, for *C. odorata* plants grown at elevated or ambient C_a, and at low or high nutrient supply.

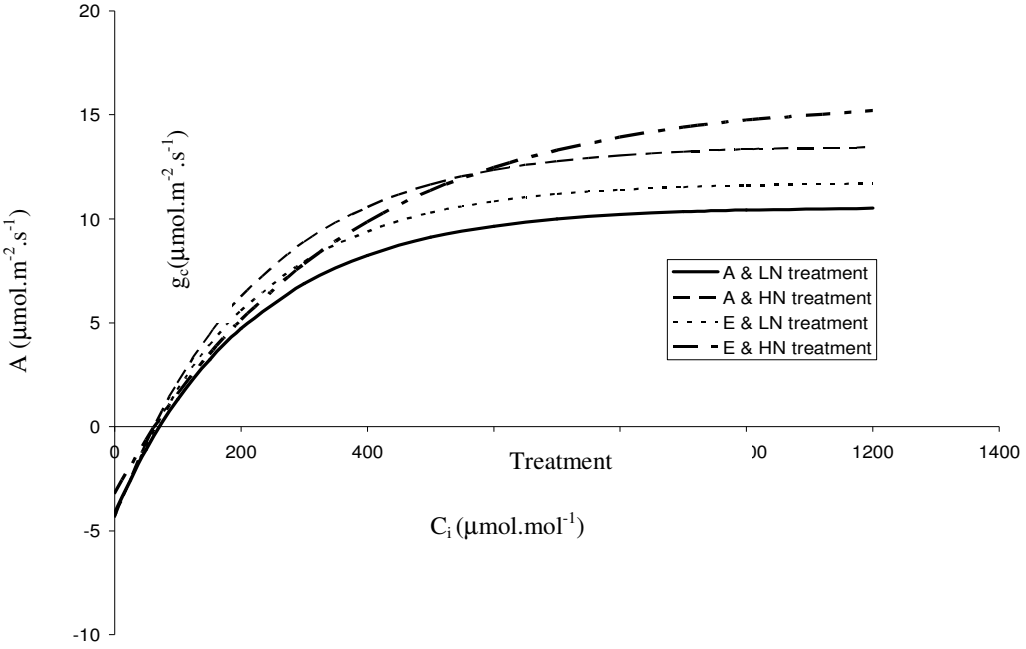


Fig. 3.1 "Average" A:C_i curves for all four treatments

Fig. 3.1 shows a general trend of the response of photosynthetic assimilation to C_i. Although lines for all four treatments are similar, statistical analysis of photosynthetic parameters has shown a significant effect of nutrients on J_{max} (Table 3.1). Since elevated C_a did not result in a decrease in any of the photosynthetic parameters, this suggests that photosynthetic down-regulation did not occur in *C. odorata* treatment plants.

3.1.2 Stomatal conductance

Unfortunately, no instantaneous g_c measurements were taken. To compensate for this, values for g_c at C_a=370 ppm, and C_a=720 ppm, were extrapolated from A:C_i data. These g_c values were averaged per treatment, after which, *within* treatment and *across* treatment comparisons were assessed. A graphical representation of the results (Fig. 3.2), precedes a summary of results of statistical analyses (Table 3.2).

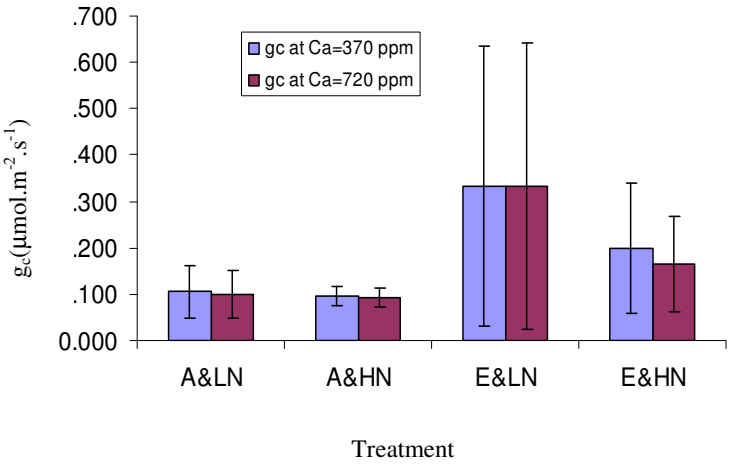


Fig. 3.2 Bar graph showing comparisons of g_c at C_a=370 ppm, and C_a=720ppm, of *C. odorata* grown under the four treatments. Error bars refer to standard deviations

Table 3.2 Within, and across treatment comparisons of mean ± SD g_c values, at $C_{a=}$370 ppm and 720 ppm

Row	Parameter	Mean ± SD				P-values		
		A&LN	**A&HN**	**E&LN**	**E&HN**	C_a	nutrient	C_a*nutrient
1	**g_c (µmol.m^{-2}.s^{-1}), at C_a=370 ppm**	0.10 ± 0.06	0.10 ± 0.02	0.33 ± 0.30	0.20 ± 0.14	0.031	0.329	0.398
2	**g_c (µmol.m^{-2}.s^{-1}), at C_a=720 ppm**	0.10 ± 0.05	0.09 ± 0.02	0.33 ± 0.31	0.17 ± 0.10	0.037	0.226	0.265
3	**P-value**	0.089	0.000	0.941	0.136			

P-values for C_a, nutrient, and C_a*nutrient effects, on g_c at C_a=370 ppm and C_a=720 ppm, *across* treatments, are shown in Rows 1 and 2, respectively (Table 3.2). A significant C_a effect can be observed for g_c at C_a=370 ppm (p=0.031; Table 3.2), and for g_c at C_a=720 ppm (p=0.037; Table 3.2). Figure 3.2 shows that g_c values at both C_a concentrations, were higher for *C. odorata* grown in elevated C_a, irrespective of

nutrient treatment. *Within* treatment comparisons of g_c at C_a=370 ppm and C_a=720 ppm, showed that the A&LN treatment was the only treatment in which a significant difference can be noted (Table 3.2; Row 3). However, this is most likely due to a small sample size, as data from only four plants were assessed for the A&HN treatment.

Stomatal limitations of plants in each treatment, were calculated and subjected to statistical analysis. Results are presented in Table 3.3.

Table 3.3 Mean ± SD values for stomatal limitations for *C. odorata*, grown with high or low nutrients, at elevated or ambient C_a, as well as P-values for C_a, nutrient and C_a*nutrient effects

Parameter	Mean ± SD				P values		
	A&LN	**A&HN**	**E&LN**	**E&HN**	C_a	Nutrient	C_a*nutrient
STOMATAL LIMITATIONS	0.14 ± 0.06	0.17 ± 0.05	0.08 ± 0.06	0.14 ± 0.08	0.059	0.077	0.596

Results show that nutrients, and the interaction between C_a and nutrients, had no significant effect on stomatal limitations of *C. odorata* exposed to the treatments. In contrast, a significant C_a effect on stomatal limitations can be observed (p=0.059; Table 3.3), as elevated C_a reduced the limitations that stomata could have imposed on CO_2 assimilation.

3.2 Morphological and growth data

3.2.1 *Chromolaena odorata*

Growth and biomass partitioning characteristics of *C. odorata* under the four treatments are shown in Table 3.4. Since planting density of all experimental pots was kept constant, all growth measurements were obtained, and will be presented and discussed, on a per plant basis. Results from K-S tests showed that the data for all parameters were normally distributed, and so they were subjected to a 2-way ANOVA.

Table 3.4 Summary of differences in mean ± SD values of nine structural parameters for *C. odorata* plants, across all four treatments. P-values describe C_a, nutrient and C_a*nutrient effects

Parameter	Mean ± SD				P values		
	A&LN	**A&HN**	**E&LN**	**E&HN**	C_a	nutrients	C_a*nutrients
Stomatal density (mm^2)	224 ± 51	177 ± 30	201 ± 29	190 ± 44	0.596	0.005	0.073
Total leaf area (cm^2	165 ± 126	1394 ± 342	196 ± 122	820 ± 476	0.008	<0.001	0.003
Plant height (cm)	15 ± 4	43 ± 11	20 ± 6	39 ± 14	0.716	<0.001	0.077
Stem cross sectional area (cm^2)	0.06 ± 0.03	0.13 ± 0.05	0.08 ± 0.03	0.15 ± 0.07	0.097	<0.001	0.896
Leaf dry weight (g)	2.0 ± 1.5	15.3 ± 6.5	3.1 ± 2.3	14.1 ± 9.3	0.645	<0.001	0.675
Stem dry weight (g)	1.1 ± 0.9	9.5 ± 4.5	1.7 ± 1.3	8.3 ± 6.6	0.762	<0.001	0.377
Total above-ground biomass (g)	3.1 ± 2.4	24.8 ± 11.0	4.7 ± 3.6	22.4 ± 15.9	0.958	<0.001	0.449

From Table 3.4, it can be noted that nutrients had a significant effect on all seven biomass parameters measured for *C. odorata*. Only one parameter (total leaf area), was significantly affected by C_a. Significant C_a*nutrient effects were observed for only one parameter.

Figs. 3.3-3.7 below, illustrate error-bar graphs for each of the nine biomass parameters described in Table 3.4, above. In the following figures, error bars refer to standard deviations.

Stomatal density:

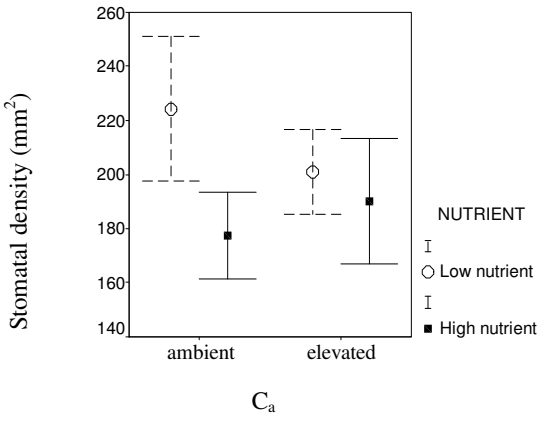


Fig. 3.3 Clustered error-bar graph illustrating stomatal density of *C. odorata* plants, grown under four treatments

No C_a effects were noted for this parameter (p=0.596; Table 3.4). At each C_a treatment, plants grown in low nutrients, had higher stomatal density than plants grown in high nutrients (Fig. 3.3). This is indicative of a strong nutrient effect (p=0.005; Table 3.4). The A&LN treatment showed the highest stomatal density (224 mm^{-2}), while the lowest value was observed for the A&HN treatment (177 mm^{-2}). There was no interaction between C_a and nutrients, on stomatal density (p=0.073; Table 3.4).

Leaf Area:

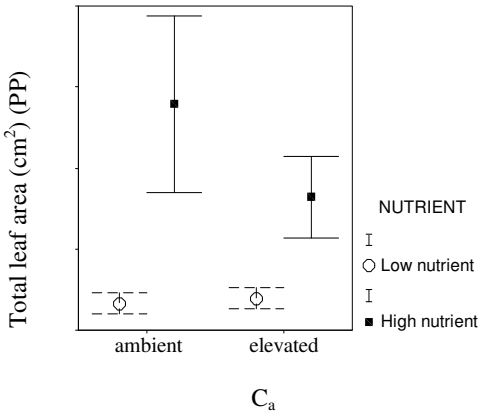


Fig. 3.4 Clustered error-bar graph illustrating total leaf area of *C. odorata* plants, grown at elevated or elevated C_a, and high or low nutrients

Figure 3.4 shows that the A&HN treatment generated the highest total leaf area (1394 cm^2), followed by the E&HN treatment (820 cm^2). Although C_a significantly influenced total leaf area (p=0.008; Table 3.4), the *direction* of this response could not be determined because of the significant interactive effect of C_a times nutrient (p=0.003; Table 3.4). Figure 3.4 clearly shows that high nutrients significantly increased total leaf area of *C. odorata*, irrespective of C_a treatment (p<0.001; Table 3.4).

Plant height and stem cross sectional area:

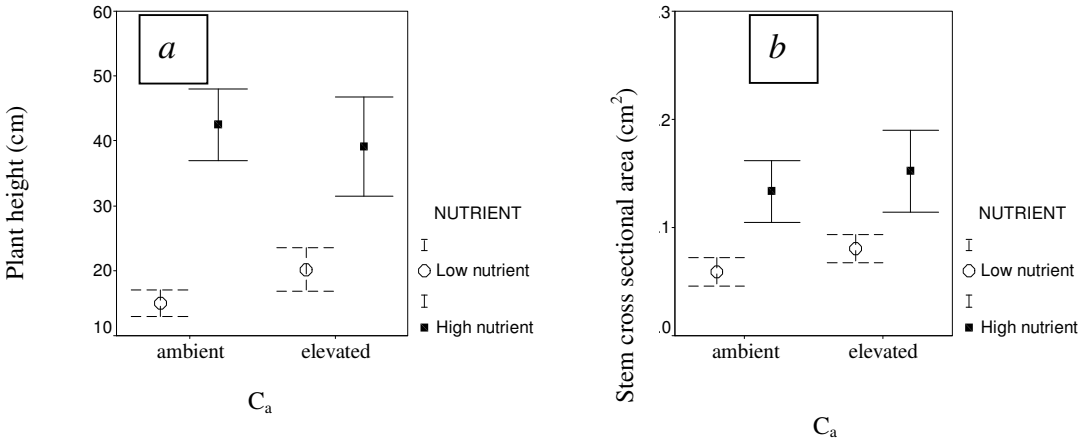


Fig. 3.5 Clustered error-bar graphs illustrating plant height (a) and stem cross-sectional areas (b), respectively for *C. odorata* plants grown under four treatments

Although plant height was not influenced by C_a, stem cross sectional area was increased by elevated C_a, but with a non-significant p-value of 0.097 (Table 3.4). There was no significant effect of C_a times nutrient on plant height (p=0.077; Table 3.4), and stem cross sectional area (p=0.896; Table 3.4).

A similar nutrient effect can be observed for plant height (Fig. 3.5a) and stem cross sectional area (Fig. 3.5b): At both C_a treatments, plants supplied with high nutrients displayed greater values than plants supplied with low nutrients. Not surprisingly, a very significant nutrient effect (p=0.000) can be observed for both parameters (Table 3.4). The tallest *C. odorata* plants were observed for the A&HN treatment (43 cm; Table 3.4), while plants with the thickest stems were characteristic of the E&HN treatment (0.15 cm^2; Table 3.4).

Biomass and biomass partitioning; per plant:

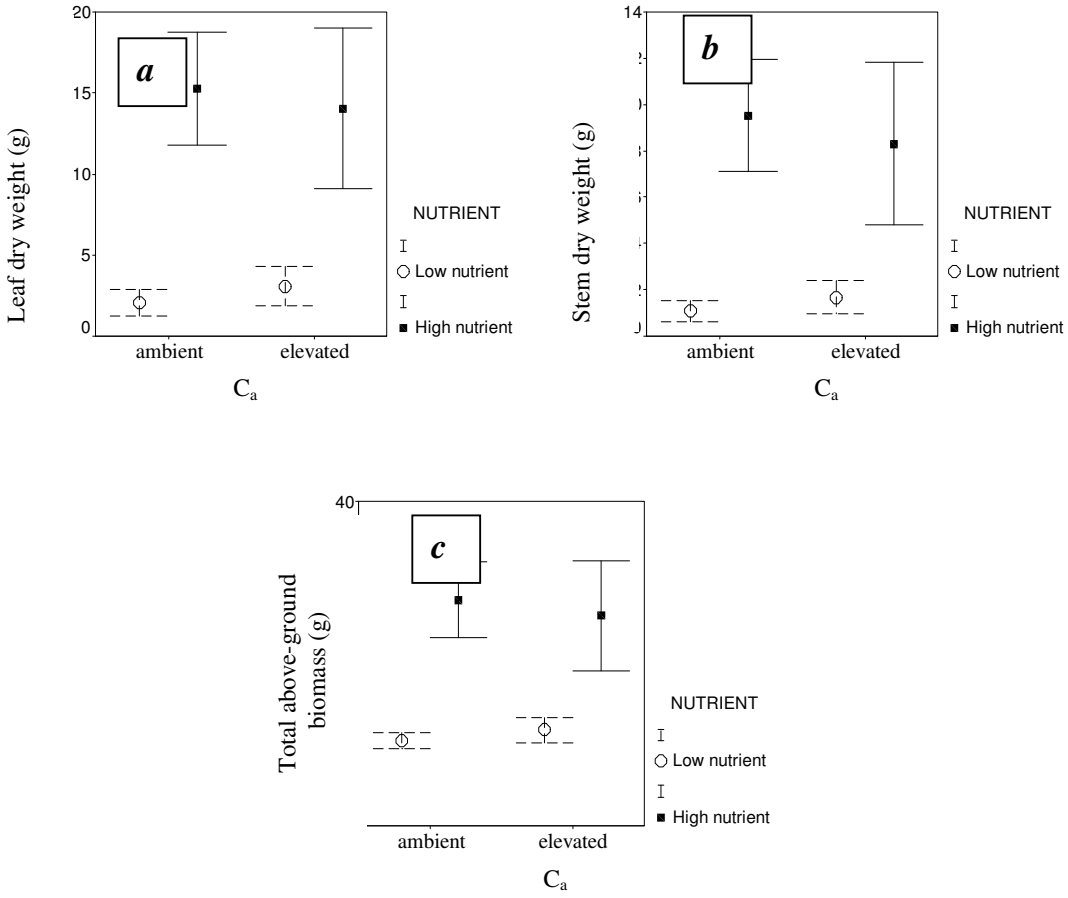


Fig. 3.6 shows clustered error-bar graphs for leaf dry weight (*a*), stem dry weight (*b)* and total above-ground biomass (*c*) of *C. odorata* plants, grown under high or low nutrients, and elevated or ambient C_a

C_a, and C_a times nutrient effects on biomass partitioning were non-significant (Table 3.4), while nutrients significantly affected all three parameters ($p<0.001$; Table 3.4). Figures. 3.6a-c show similar nutrient effects, as has been observed for plant height (Fig. 3.5a) and stem cross sectional area (Fig. 3.5b) above. Greater leaf dry weight (Fig. 3.6a), stem dry weight (Fig. 3.6b) and total above-ground biomass (Fig. 3.6c), can be noted for plants grown in high nutrients, compared to plants in lower nutrients, irrespective of C_a treatment.

3.2.2 Grasses

Only above-ground biomass and total leaf area for *E. curvula* and *T. triandra*, were assessed. Results from K-S tests showed that data were normally distributed, and so they were subjected to a 2-way ANOVA.

Table 3.5 Summary of differences in mean values for total above-ground biomass and total leaf area of *E.curvula* and *T. triandra* grasses, grown at ambient or elevated C_a, and high or low nutrient supply. P-values describe C_a, nutrients, and C_a*nutrient effects

Parameter	Mean ± SD				P values		
	A&LN	**A&HN**	**E&LN**	**E&HN**	C_a	nutrients	C_a*nutrients
***E. curvula* Total above-ground biomass (g)**	3.0 ± 1.6	9.4 ± 9.9	3.0 ± 1.4	9.6 ± 4.5	0.941	<0.001	0.948
***E. curvula* Leaf area (cm^2)**	188 ± 129	728 ± 520	210 ± 079	714 ± 390	0.965	<0.001	0.828
***T. triandra* Total above-ground biomass (g**	1.6 ± 0.9	15.8 ± 8.6	1.0 ± 0.6	17.1 ± 12.7	0.849	<0.001	0.624
***T. triandra* Leaf area (cm^2)**	85 ± 86	1167 ± 692	45 ± 29	1205 ± 953	0.997	<0.001	0.795

C_a, and the interaction between C_a and nutrients, had no significant influence on biomass parameters for both grasses (Table 3.5). In direct contrast, a very significant response to nutrients (p=<0.001) can be observed for both grass species: High nutrient supply increased total above-ground biomass and total leaf area, for both grasses, and from a comparison of the averages amongst the treatments, it can be observed that the magnitude of this effect was higher for *T. triandra* (Table 3.5).

Since such a strong response of the experimental grasses to high nutrient supply was noted (see above), "control" grasses (grown without C_a treatments, and without *C. odorata*), were assessed for major differences in responses to high nutrient supply.

Table 3.6 P-values for "Control" grasses, subjected only to nutrient treatment

E. curvula* (*Control)	**P-value for nutrient treatment**
Above-ground biomass (g)	0.000
Total leaf area (cm^2)	0.010
T. triandra* (*Control)	
Above-ground biomass (g)	0.000
Total leaf area (cm^2)	0.000

From Table 3.6, it can be seen that the "control' grasses behaved similarly to the experimental grasses, with regard to the response to high nutrient supply, even in the absence of C_a effects and competition with *C. odorata*.

CHAPTER 4. DISCUSSION (PART A)

There has been a considerable amount of research on global climate change and increasing C_a in recent decades. Given that the terrestrial biosphere is the major sink for increasing concentrations of C_a (Fujita et al., 2003), and the fact that CO_2 is a substrate for photosynthesis, much focus has been placed on plant responses to CO_2 enrichment. Almost two decades of research on this topic (Woodward, 2002), has provided a rich suite of data that has enabled the identification of certain trends.

For example, many invasive plants have shown a positive response to elevated C_a, when grown individually or in monoculture (Dukes, 2000). C_3 plants have also been shown to respond more strongly to CO_2 enrichment, than C_4 species (Garbutt et al., 1990; Poorter, 1993). In addition, several studies have demonstrated a dependence of plant responses to elevated C_a, on nutrient levels (Stöcklin and Körner, 1999; He et al., 2002). PART A of this study, assessed the *interactive* effects of competition between C_3 and C_4 species, and different nutrient concentrations, on the response of *C. odorata* to elevated C_a. Discussions of results from this experimental trial will now follow.

4.1 Physiology

Enhancement of photosynthesis under CO_2 enrichment, has been demonstrated in various studies (den Hertog et al., 1993; Teskey, 1995). Increasing CO_2 competitively inhibits the oxygenation reaction of the enzyme, rubisco, thus leading to a stimulation of photosynthesis. (Long et al., 2004). A previous study showed that growth of a C_3 weed, *Abutilon theophrasti*, in elevated (700 ppm) C_a increased net photosynthesis 27% relative to growth in ambient (350 ppm) C_a, when the species was grown in competition with C_4 weed, *Amaranthus retroflexus* (Tissue et al., 1995). However, a recent study showed no change in instantaneous CO_2 assimilation rates of *C. odorata* under CO_2 enrichment (Naidoo, personal communication). Unfortunately, no measurements of instantaneous rates of photosynthetic assimilation in elevated C_a, were taken in the current study. The "average" $A:C_i$ curve constructed for the treatments, showed that lines for all four treatments were similar (Fig. 3.1; Chapter 3). J_{max} was the only photosynthetic parameter to display a significant response: High

nutrient supply significantly increased J_{max} of *C. odorata* (p=0.001; Table 3.1 in Chapter 3), and this contradicts results of a previous study on *Populus euamericana*, which showed no response of photosynthetic parameters to N fertilization (Calfapietra et al., 2005). The current study also showed that elevated C_a increased J_{max}, but with a non-significant p-value of 0.075 (Table 3.1 in Chapter 3). Perhaps this could have been a significant response, if the duration of the growth period had been longer, or if measurements had been taken earlier, before full acclimation. High nutrient supply decreased CO_2 compensation points, but this was also a marginal effect (p=0.067; Table 3.1 in Chapter 3). CO_2 compensation points of *C. odorata* under elevated C_a (70 and 66 $\mu mol.mol^{-1}$; Table 3.1 in Chapter 3), were generally lower than that reported for *Ginkgo biloba* in elevated C_a (75-84 $\mu mol.mol^{-1}$) (Overdieck and Strassemeyer, 2005), and this may simply be due to the different species studied.

Since elevated C_a did not decrease any of the photosynthetic parameters studied, this *could* suggest that CO_2 enrichment did not result in photosynthetic down-regulation in *C. odorata*. However, the lack of *direct* photosynthetic data in elevated C_a, makes it impossible to determine if this were the case.

Stomata are the entry site for CO_2 used in photosyntheis (Wallace et al., 1996). During this trial, stomatal conductance (g_c) and stomatal limitations of *C. odorata*, were assessed. Stomatal acclimation to elevated C_a, is a process that would be demonstrated if stomatal behaviour of plants *grown* at contrasting CO_2 concentrations differed when *measured* at the same CO_2 concentration (Maherali et al., 2002). Although *direct* g_c data in elevated C_a were not analysed, results from analysis of g_c values extrapolated from $A:C_i$ data, showed that elevated C_a significantly increased g_c values at C_a=370 ppm and C_a=720 ppm (Table 3.2; Chapter 3). This suggests that stomatal acclimation of *C. odorata* in elevated C_a could have occurred, but conclusive evidence in the form of direct g_c data in elevated C_a, is lacking.

It should be noted that for the A&HN treatment, data from only one pot was assessed for photosynthetic responses, compared with four pots for each of the other three treatments. This difference was due to the fact that *C. odorata* plants from three out of the four pots belonging to the A&HN treatment, suffered an inadvertent water stress prior to photosynthetic measurements. Therefore, a small sample size is most

likely the reason for the significant *within* treatment comparison of g_c at C_a=370 ppm, and g_c C_a=720 ppm, in the A&HN treatment (Table 3.2; Chapter 3).

According to Drake and Gonzàlez-Meler (1997), it is not g_c *per se*, which limits photosynthesis. Stomatal limitations to photosynthesis, according to Farquhar and Sharkey (1982), could be quantified in a simple, practical way from A:C_i curves. Table 3.3 shows that elevated C_a significantly reduced stomatal limitations to photosynthesis, of *C. odorata* grown under the four treatments (p=0.059; Table 3.3 in Chapter 3). This is not really surprising, since results of assessments of g_c values extrapolated from A:C_i data, showed that significantly higher g_c values were observed for the elevated C_a treatments (Fig. 3.2 and Table 3.2; Chapter 3).

Different photosynthetic pathways is the major reason that C_3 plants are expected to respond better to elevated C_a, than C_4 plants (Johnson et al., 1993; Reynolds, 1996). Based on the fact that *C. odorata* and both grasses were grown *together* in the current trial, photosynthetic measurements for grasses could have aided in providing insight into the poor photosynthetic response of *C. odorata*. It is unfortunate that photosynthesis of both grass species was not assessed, during PART A.

4.2 Growth and Morphology

C. odorata:

Although a *significant* C_a effect on stem cross sectional area was not observed, Fig. 3.5b (Chapter 3), suggests that CO_2 enriched atmospheres are likely to result in *C. odorata* plants with thicker stems, even in poor nutrient (mineral stressed) soils. Due to a dieback of many plants belonging to the A&HN treatment, total leaf area for this treatment was assessed from only four plants, instead of 16. Incidentally, this was the only morphological parameter to be significantly affected by C_a (p=0.008; Table 3.4 in Chapter 3). However, the *direction* of this response could not be determined, due to a significant interaction between C_a and nutrients for this parameter (p=0.003; Table 3.4 in Chapter 3). For plants treated with low nutrient supply, growth in elevated C_a resulted in higher total leaf area, than growth in ambient C_a (Fig. 3.4; Chapter 3). In direct contrast to results of a study on spring wheat (Li et al., 2004), elevated C_a *reduced* total leaf area of *C. odorata* plants supplied with high nutrients.

Higher rates of growth in elevated C_a, according to Stitt and Krapp (1999), will lead to an increased demand for nutrients. These authors maintain that this demand could be met by using nutrients more efficiently, and/or by increasing the rate of nutrient uptake. Therefore, the fact that elevated C_a decreased total leaf area of high-nutrient grown plants, could have been a result of increased allocation to roots for increasing nutrient uptake, to keep up with the demand for more nutrients in elevated C_a (Stitt and Krapp, 1999), and/or to restore C:N balances which may have been offset by elevated C_a (Hartwig et al., 1996). Unfortunately, with the lack of root data, and leaf concentrations of total non-structural carbohydrates and nitrogen, it is impossible to determine if the above statements hold true in the current trial. At this stage, it is sufficient to say that nutrient supply modified the response of total leaf area of *C. odorata* to elevated C_a.

Co-incidentally, elevated C_a also reduced plant height, leaf dry weight, and total above-ground biomass of high-nutrient treatment *C. odorata* plants (Figs. 3.5a; 3.6a; 3.6c; respectively, in Chapter 3), but with non-significant differences (Table 3.4; Chapter 3). These results are in contrast with previous studies which have shown that elevated C_a enhanced biomass parameters of high nutrient treatment plants (Conroy et al., 1986; Stöcklin and Körner, 1999; Causin et al., 2004; Reddy and Zhao, 2005). These contrasting results are understandable; if one considers that *C. odorata* in the current trial was subjected to competition with C_4 species, *in addition* to competition for nutrients.

When the grassland invader, *Prosopis glandulosa* was grown in competition with a C_4 grass, *Schizachyrium scoparium*, results showed that biomass of *P. glandulosa* was not affected by CO_2 concentrations (Polley et al., 1994). This is in accord with the current study. In contrast, Ziska (2001) demonstrated a significant increase in total above-ground biomass and leaf area of C_3 weed, common cocklebur, when grown in competition with a C_4 crop (sorgum). It should be mentioned that there were no nutrient treatments in these two studies. In the current study, interactions between competition for nutrients, and competition between C_3-C_4 species, had come into play.

According to Petersen (2005), differences in root length of plants in nutrient-rich zones, may be related to characteristics of the species involved. If this is true, then

interactions between competition for nutrients, *and* competition between C_3-C_4 species in PART A, is more than likely, and it is difficult, if not impossible, to predict the *exact* effect of either type of competition on the response of *C. odorata* to elevated C_a.

However, because total leaf area was the *only* parameter to be significantly influenced by elevated C_a, this suggests that CO_2 enrichment did not result in a stimulation of growth of *C. odorata*, when grown in competition with C_4 grasses, and exposed to different nutrient treatments. Due to the lack of photosynthetic down-regulation of *C. odorata* in the current trial (see Section 4.1; this study), one would have expected an enhancement of biomass of *C. odorata* in elevated CO_2, most likely due to an increase in net assimilation rates. However, the relatively low CO_2 compensation points observed for *C. odorata* under elevated C_a (see Section 4.1; this study), are more characteristic of C_4 species. Therefore, it is possible that *C. odorata* has evolved a more efficient photosynthetic physiology than most other C_3 species. This would explain the lack of stimulation of biomass under CO_2 enrichment, even in the absence of photosynthetic down-regulation.

Alternatively, the response of *C. odorata* to high nutrient supply could have been so strong, that any potential C_a response could have easily been overshadowed, and thus remained undetected. Midgley (1996) showed that increasing nutrient supply reduced the biomass response of three *Leucadendron* species to elevated C_a. This could have occurred in the current trial, if one considers the significant effect of nutrient treatment on all seven morphological parameters measured for *C. odorata*: High nutrient supply resulted in taller *C. odorata* plants with thicker stems, with greater total leaf area and stomatal density, and greater biomass (leaf dry weight, stem dry weight and total above-ground biomass), irrespective of C_a treatment (Figs. 3.3-3.6; Chapter 3). In addition, high nutrient supply also increased J_{max} (Table 3.1), highlighting the fact that the response to nutrients was strong enough to be carried through structurally *and* physiologically.

The fact that plants from only one of the four pots belonging to the A&HN treatment, survived growth, also re-iterates the strength of the nutrient effect on *C. odorata* plants. When nutrient availability is high, excessive and potentially detrimental levels

of accumulation of nutrients in plants leaves could occur (Baker, 1983). High nutrient concentrations in the soil proved detrimental to plants grown at ambient C_a (A&HN treatment), but not for plants grown at elevated C_a (E&HN treatment), possibility as a result of restoration of C:N balances in the latter, but not in the former treatment. Alternatively, the fact that only a few plants from the A&HN treatment survived growth, could merely be a result of water stress.

Results from this experimental trial yield no conclusive evidence which supports the prediction that increasing C_a would reduce the importance of CO_2 as an external limiting resource (Lynch and St. Clair, 2004), and cause limitations of other external resources to intensify (Dukes, 2000), since a strong nutrient response of *C. odorata* was observed for both C_a treatments. For this reason, PART A also does not support the notion that higher C_a would benefit plant growth by enhancing resource-use efficiencies (Polley et al., 1993a). However, more CO_2-enrichment studies should be conducted on *C. odorata* and other resources, (e.g. water, light, temperature, space, etc.), which could provide evidence which may support these two hypotheses.

Based on the fact that altered root:shoot ratios (often noted in elevated C_a), are indicative of shifts in functional relationships between these organs (Pritchard et al., 1999), and that the capture of applied nutrients depends on roots (Petersen, 2005), the lack of below-ground data (and subsequent LAR, total plant biomass, and biomass allocation ratio data), in the current experimental trial, cannot be overstated. In addition, relative growth rate data (RGR), should be incorporated into future studies, since research has shown that increases in plant growth in elevated C_a results mainly from initial stimulation, which may decline or even disappear over time (Centritto et al., 1999).

Grasses:

C_a and C_a times nutrient treatments, had no effect on the two structural parameters measured for the experimental grasses (Table 3.5; Chapter 3). However, a strong response of both experimental grass species to nutrient treatments can be observed: High nutrient supply significantly increased above-ground biomass and total leaf area of *E. curvula* and *T. triandra*, when grown in competition with *C. odorata*, and exposed to different C_a and nutrient concentrations (Table 3.5; Chapter 3). The fact

that the "control" grasses (grown in absence of *C. odorata*, and not exposed to C_a treatments), also displayed strong positive responses to high nutrient treatment (Table 3.6; Chapter 3), makes the observed response of the experimental grasses to high nutrient supply, all the more robust.

E. curvula and *T. triandra* were chosen as competitors for *C. odorata* in this experimental trial (PART A), because of their preference for different nutrient levels. *E. curvula* has previously been shown to respond well to high nutrient level soils (Rethman, 1973), wheras *T. triandra* performs better in poor nutrient soils (Groves et al., 2003). However, from comparisons of means for above-ground biomass and leaf area of both grasses, amongst the four treatments (Table 3.5; Chapter 3), it can be concluded that although both grasses responded positively to high nutrient levels, the effect was larger for *T. triandra*. This contradicts a recent study on the responses of different grass species to nutrient gradients, which showed that at the *lowest* nutrient level, *T. triandra* was the most productive species in monoculture (Groves et al., 2003).

These contrasting results could be due to C_a effects and/or competition with a C_3 species, and highlights the fact that the opposite scenario could have also occurred: competition with C_4 grasses *and* nutrient treatments, could have, and most probably did, alter the CO_2 effects on *C. odorata*.

CHAPTER 5. RESULTS (PART B)

EXPERIMENTAL POTS: Influence of planting density on the response to C_a

5.1 Physiology data

5.1.1 Photosynthesis

Data from A:C_i curves for each individual leaf were fitted to the monomolecular function A=a*(1-exp(b-c*C_i)), to yield values for CO_2 compensation point, initial slope and J_{max}. Data for these three parameters were subjected to a 2-way ANOVA, to investigate differences amongst the four treatments. Mean ± SD values and p-values are presented in Table 5.1.

Table 5.1 Mean ± SD values for 3 photosynthetic parameters of *C. odorata*, grown at four treatments, as well as P-values for C_a, density and C_a*density effects

Parameter	Mean ± SD				P-values		
	A&LD	**A&HD**	**E&LD**	**E&HD**	C_a	density	C_a*density
J_{max} (μmol.m^{-2}s^{-1})	12.5 ± 0.8	11.9 ± 1.2	11.8 ± 1.6	11.2 ± 1.3	0.133	0.191	0.931
Initial slope (mol.m^{-2}s^{-1})	0.15 ± 0.08	0.10 ± 0.02	0.08 ±0.07	0.10 ± 0.04	0.165	0.534	0.152
CO_2 compensation point ((μmol.mol^{-1})	65 ± 22	57 ± 4	59 ± 7	61 ± 4	0.821	0.479	0.251

Statistical analysis revealed no significant effects of C_a, density or C_a*density on any of the photosynthetic parameters (Table 5.1). The A&LD treatment showed the highest average for all three parameters.

An "average' A:C_i curve was constructed from the monomolecular function A=a*(1-(exp(b-c*C_i)), and the average values of the constants a, b and c for each of the four treatments. Figure 5.1 is a plot of "average" assimilation versus C_i, for *C. odorata* plants grown at elevated or ambient C_a, and at low or high planting densities.

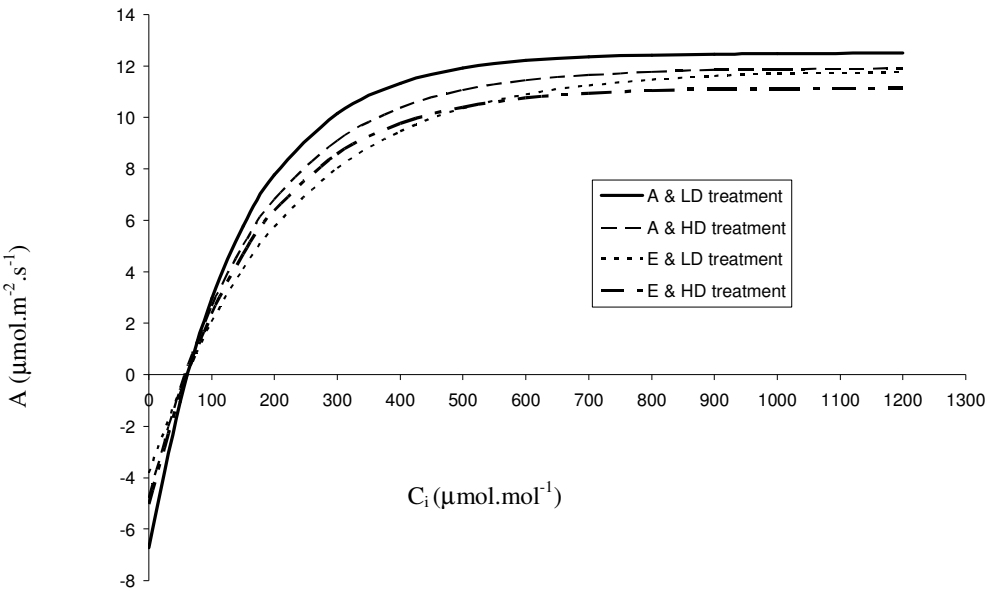


Fig. 5.1 "Average" A:C_i curve for all four treatments

The general trend of responses of photosynthetic assimilation to C_i, can be seen from Fig. 5.1. Lines for all four treatments are similar, and statistical analysis of photosynthetic parameters (Table 5.1) showed no significant differences among the treatments. This implies that growth of *C. odorata* in elevated C_a resulted in no photosynthetic down-regulation of these plants.

No significant effect of elevated C_a on J_{max}, was also reported in two separate studies on *Liquidambar styraciflua* (Herrick and Thomas, 2001; Sholtis et al., 2004). CO_2 compensation points of *C. odorata* in the elevated C_a treatments (59-61 μmol.mol^{-1}; Table 5.1 in Chapter 5), were generally lower than that reported for this species in elevated C_a, when grown in competition with C_4 grasses and subjected to different nutrient concentrations (66-70 μmol.mol^{-1}) (Table 3.1 in Chapter 3; this study).

5.1.2 Stomatal conductance

In an attempt to investigate the response of stomata to elevated C_a, stomatal conductance to CO_2 (g_c), as well as stomatal limitations of the treatment plants, were assessed. Unfortunately, no instantaneous g_c measurements were taken. To compensate for this, values for g_c at C_a=370 ppm, and C_a=720 ppm, were extrapolated from A:C_i data obtained from the IRGA. These values were averaged, and compared within treatments, and across treatments. Figure 5.2 is a graphical representation of the results, and results of statistical analyses are presented in Table 5.2.

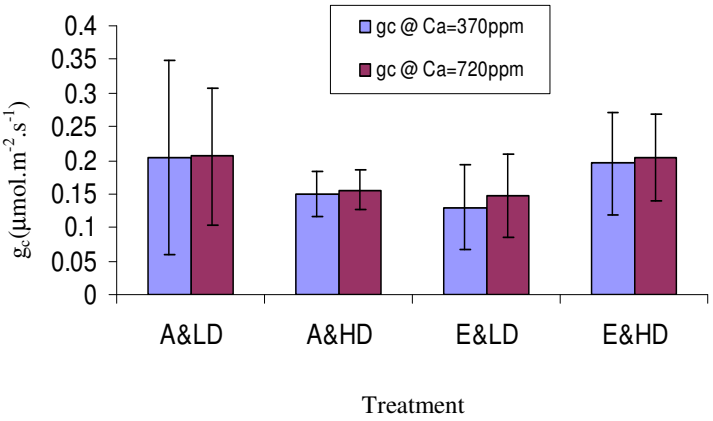


Fig. 5.2 Bar graph showing comparisons of g_c at C_a=370 ppm, and C_a=720ppm, of *C. odorata* grown under the four treatments. Error bars refer to standard deviations

Table 5.2 Within, and across treatment comparisons of mean ± SD g_c values, at C_a=370 ppm and 720 ppm

Row	Parameter	Mean ± SD				P-values		
		A&LD	**A&HD**	**E&LD**	**E&HD**	C_a	density	C_a*density
1	**g_c (µmol.m^{-2}.s^{-1}), at C_a=370 ppm**	0.20 ± 0.15	0.15 ± 0.03	0.13 ± 0.06	0.20 ± 0.08	0.730	0.916	0.089
2	**g_c (µmol.m^{-2}.s^{-1}), at C_a=720 ppm**	0.21 ± 0.10	0.16 ± 0.03	0.15 ± 0.07	0.20 ± 0.07	0.862	0.885	0.042
3	**P-value**	0.913	0.506	0.026	0.119			

P-values for C_a, density, and C_a*density effects, on g_c at C_a=370 ppm and C_a=720 ppm, *across* treatments are shown in Rows 1 and 2, respectively (Table 5.2). A significant interactive effect of C_a times density can be observed for g_c at C_a=720 ppm, of plants belonging to the E&LD treatment (Table 5.2; Row 2). Figure 5.2 shows that for *C. odorata* grown at ambient C_a, g_c at C_a=720 ppm was higher for plants grown at low planting density, compared with plants grown at high density. However, for elevated C_a-grown plants, growth at low planting density showed lower g_c averages at C_a=720 ppm, than plants grown at high density (Fig. 5.2). *Within* treatment comparisons of g_c at C_a=370 ppm and C_a=720ppm, showed that the E&LD treatment was the only treatment in which a significant difference can be noted (Table 5.2; Row 3). It can be observed from Fig. 5.2, that mean g_c values at C_a=720 ppm were higher than at C_a=370 ppm, for the E&LD treatment.

Stomatal limitations of plants in each treatment, were calculated, and subjected to statistical analysis. Results are presented in Table 5.3.

Table 5.3 Mean±SD values for stomatal limitations for *C. odorata*, grown with high or low density, at elevated or ambient C_a, as well as P-values for C_a, density and C_a*density effects

Parameter	Mean ± SD				P values		
	A&LD	**A&HD**	**E&LD**	**E&HD**	C_a	density	C_a*density
STOMATAL LIMITATIONS	0.10 ± 0.12	0.09 ± 0.03	0.09 ± 0.05	0.06 ± 0.02	0.501	0.384	0.670

Decreases in stomatal limitations are generally expected in plants that are grown under CO_2 enrichment. However, results of the current study, show no significant effects of C_a, density or C_a times density on stomatal limitations of *C. odorata* subjected to the treatments.

5.1.3 Non-treatment pots

In a further attempt to investigate the physiological response of *C. odorata* to elevated C_a, four additional "non-treatment pots" were obtained. These pots contained two *C. odorata* plants each, thus eliminating density as an influencing factor. The "non-treatment" plants were grown for seven months in ambient C_a, without OTCs, after which they were exposed to elevated C_a in OTCs. Maximum assimilation rates (J_{max}) were measured two days after exposure to elevated C_a (700 ppm), and thereafter at regular intervals. For non-treatment *C. odorata* plants, J_{max} was plotted against time (days after exposure to elevated C_a) (Fig. 5.3). It should be noted that an initial reading of J_{max} for "non- treatment" plants, was not taken (0 days after exposure to elevated C_a). In an attempt to compensate for this, the average J_{max} value of treatment *C. odorata* plants from the A&LD treatment, was substituted for an initial J_{max} value of "non-treament" plants.

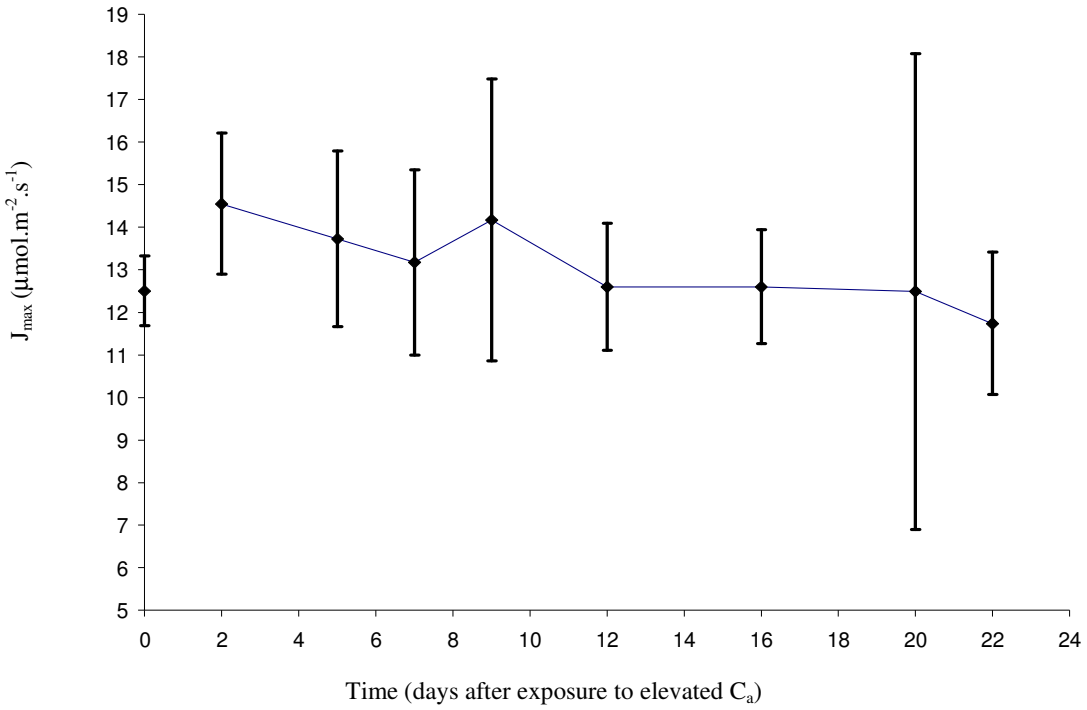


Fig. 5.3 Change in J_{max} with time after transfer from ambient to elevated C_a

On the second day, J_{max} had a value of ~14.5 μmol.$m^{-2}s^{-1}$, greater than that of ~12.5 μmol.$m^{-2}s^{-1}$ (day 0). Despite some variation in J_{max} (Fig. 5.3), a general decrease in J_{max} (14.55 μmol.$m^{-2}s^{-1}$ at 2 days, and 11.74 μmol.$m^{-2}s^{-1}$ after 22 days), can be observed. Data from day 2 to day 20 were subjected to a linear regression analysis, to ascertain whether the observed decrease in J_{max} was significant or not. Results of the analyses showed a significant decrease in J_{max}, as the number of days after exposure to C_a increased ($p=0.03$). Although these results suggest that photosynthetic down-regulation of "non-treatment" *C. odorata* plants in elevated C_a could have occurred, the possibility of a "chamber effect" cannot be ruled out. These non-treatment pots were exposed to elevated C_a the same time they were enclosed in chambers. Distinguishing between potential chamber effects and photosynthetic down-regulation on the significant decreases in J_{max} observed for these "non-treatment" plants, is beyond the scope of this study. However, earlier physiology results of the treatment *C. odorata* plants, in which no photosynthetic down-regulation occurred, suggests that the observed significant decrease in J_{max} of the "non-treatment" *C. odorata* plants, is most likely a result of a "chamber effect".

5.2 Morphological and growth data

Growth and biomass allocation characteristics per plant (PP), of *C. odorata* under the four treatments are shown in Table 5.4. Table 5.5 shows the same characteristics, per community (PC), of *C. odorata* under the four treatments. Results from K-S tests showed that the data for all parameters (PP and PC) were normally distributed, and so they were subjected to a 2-way ANOVA.

Table 5.4 Summary of differences in mean ± SD values of 12 structural parameters per plant (PP) for *C. odorata plants*, across all four treatments. P-values describe C_a, density and C_a*density effects

Parameter	Mean ± SD				P values		
	A&LD	**A&HD**	**E&LD**	**E&HD**	C_a	density	C_a*density
Total leaf area (cm^2)	504 ± 158	342 ± 334	557 ± 220	287 ± 155	0.987	0.006	0.469
Specific leaf area (cm^2)	212 ± 28	279 ± 82	193 ± 34	183 ± 56	0.003	0.133	0.041
Leaf area ratio ($cm^2.g^{-1}$)	65 ± 25	97 ± 39	60 ± 20	51 ± 28	0.009	0.228	0.035
Plant height (cm)	63 ± 15	35 ± 17	75 ± 35	49 ± 22	0.065	<0.001	0.917
Stem cross sectional area (cm^2)	0.15 ± 0.06	0.06 ± 0.03	0.18 ± 0.09	0.11 ± 0.04	0.350	0.014	0.603
Leaf dry weight (g)	2.9 ± 1.0	1.5 ± 1.0	4.2 ± 1.7	1.8 ± 0.8	0.038	<0.001	0.155
Stem dry weight (g)	3.8 ± 2.1	0.9 ± 1.2	4.9 ± 4.6	2.3 ± 1.4	0.086	<0.001	0.866
Root dry weight (g)	1.9 ± 1.0	0.5 ± 0.5	1.7 ± 1.4	2.2 ± 1.1	0.018	0.096	0.003
Total plant biomass (g)	8.7 ± 3.8	2.9 ± 1.9	10.8 ± 6.6	6.2 ± 2.9	0.021	<0.001	0.607
Leaf weight ratio (%)	36.9 ± 11.7	52.3 ± 18.9	46.0 ± 19.5	29.7 ± 8.5	0.155	0.924	0.001
Stem weight ratio (%)	41.6 ± 10.6	31 ± 19.8	38.1 ± 16.2	35.2 ± 7.6	0.959	0.152	0.411
Root weight ratio (%)	21.5 ± 4.1	16.8 ± 14.2	15.9 ± 9.7	35.1 ± 9.7	0.063	0.036	0.001

Table 5.5 Summary of differences in mean ± SD values of 10 structural parameters per community (PC) for *C. odorata plants*, across all four treatments. P-values describe C_a, density and C_a*density effects

Parameter	Mean ± SD				P values		
	A&LD	**A&HD**	**E&LD**	**E&HD**	C_a	density	C_a*density
Total leaf area (cm^2)	1008 ± 81	1370 ± 1069	1115 ± 334	1076 ± 379	0.757	0.597	0.512
Leaf area ratio ($cm^2.g^{-1}$)	64 ± 24	107 ± 28	60 ± 19	48 ± 13	0.012	0.182	0.026
Stem cross sectional area (cm^2)	0.29 ± 0.11	0.23 ± 0.1	0.36 ± 0.17	0.40 ± 0.13	0.079	0.826	0.442
Leaf dry weight (g)	5.9 ± 0.9	6.1 ± 0.7	8.3 ± 1.6	6.6 ± 0.8	0.018	0.187	0.093
Stem dry weight (g)	7.6 ± 3.4	3.7 ± 3.9	9.8 ± 8.5	8.6 ± 3.6	0.204	0.357	0.629
Root dry weight (g)	3.8 ± 1.6	1.9 ± 1.4	3.4 ± 2.5	8.1 ± 3.5	0.034	0.292	0.017
Total plant biomass (g)	17.3 ± 5.8	11.6 ± 5.9	21.5 ± 12.3	23.2 ± 7.3	0.080	0.641	0.388
Leaf weight ratio (%)	36.6 ± 10.6	58.4 ± 16.6	46.6 ± 20.2	30.1 ± 7.3	0.232	0.727	0.022
Stem weight ratio (%)	41.8 ± 7.7	27 ± 14.1	38.9 ± 17.3	36.3 ± 4.7	0.601	0.173	0.331
Root weight ratio (%)	21.6 ± 3.8	14.6 ± 7.2	14.4 ± 4.1	33.6 ± 5.0	0.043	0.038	<0.001

From Table 5.4, it can be seen that as a main factor, density significantly affected seven of the 12 morphological parameters (PP) measured for *C. odorata* ($p<0.05$). In addition, density influenced five parameters, through an interaction with C_a. Similarly, five parameters were significantly affected by C_a, however, seven parameters were influenced by C_a if one includes interactive effects between C_a and density. C_a times density effects were observed for five parameters.

When assessed on a community basis, only four out of 10 parameters (PC) were significantly affected by C_a (Table 5.5). Density influenced only one parameter significantly. Significant interactive effects between C_a and density were noted for four parameters.

For a clear illustration, results have been presented per plant (PP), and then per community (PC). In the following graphs, error bars refer to standard deviations.

Leaf Area:

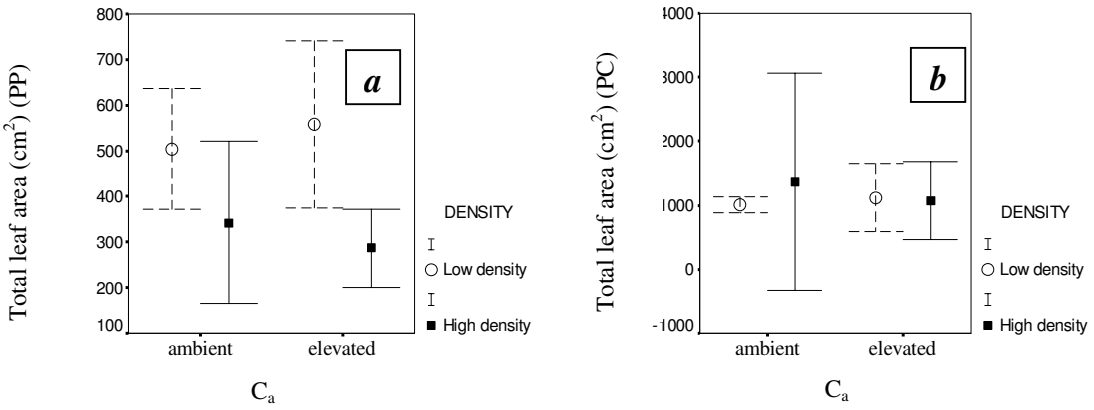


Fig. 5.4 Clustered error-bar graphs showing total leaf area per plant (***a***) and per community (***b***) of *C. odorata* grown at elevated or ambient C_a and high or low planting density

PP: Table 5.4 shows a variation of total leaf area per plant from a low value of 287 cm^2 (E&HD treatment), to a high value of 557 cm^2 (E&LD treatment). C_a did not significantly affect total leaf area PP (p=0.987; Table 5.4). P-values from Table 5.4 indicate a highly significant density effect on total leaf area PP of *C. odorata* (p=0.006; Table 5.4). At each C_a treatment, plants grown at lower density had greater total leaf areas PP, than plants grown at high density (Fig. 5.4a). There was no significant interaction between C_a and density (p=0.469; Table 5.4).

PC: Total leaf area per plant community, ranged from a low value of 1008 cm^2 (A&LD treatment) to a high value of 1370 cm^2 (A&HD treatment) (Fig. 5.4b). No significant C_a, density or C_a*density effects were observed (p=0.757; p=0.597; p=0.512, respectively; Table 5.5), for total leaf area PC.

Specific leaf area:

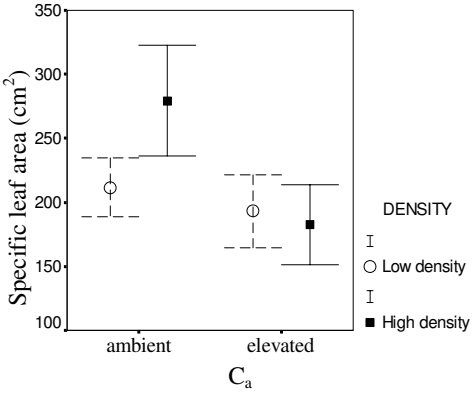


Fig. 5.5 Clustered error-bar graph of specific leaf area (PP) of *C. odorata* grown at elevated or ambient C_a and high or low planting density

Specific leaf area was measured per plant only, and not per community. Fig. 5.5 shows that plants belonging to the E&HD treatment had the lowest SLA values (183 $cm^{2;}$ Table 5.4), while the A&HD treatment showed the highest SLA average (279 cm^2; Table 5.4). C_a had a significant effect on SLA (p=0.003), with lower SLA's at elevated C_a, while density did not (p=0.133). At ambient C_a, plants grown with higher densities had greater SLA values than plants from the lower density treatments. This pattern is reversed at elevated C_a (although the effect is small), clearly indicating a significant interactive effect of C_a and density on SLA of *C. odorata* plants (p=0.041; Table 5.4).

Leaf area ratio:

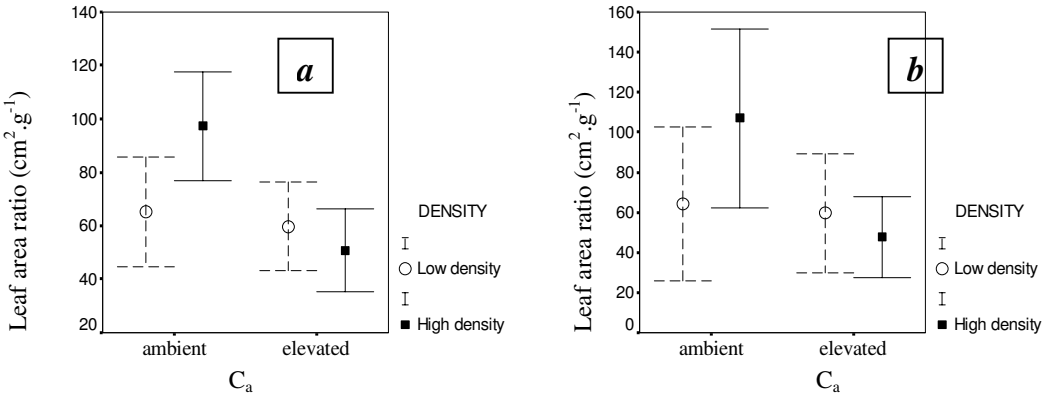


Fig. 5.6 Clustered error-bar graphs showing Leaf area ratio per plant (***a***) and per community (***b***) of *C. odorata* grown at elevated or ambient C_a and high or low planting density.

PP: Elevated C_a significantly reduced LAR, of both density classes (p=0.009; Table 5.4). Although density did not significantly influence LAR, a significant interaction between C_a and density, can be noted (p=0.035; Table 5.4). At ambient C_a, plants in the low density treatment had lower LARs than plants from the high density treatment. This pattern is reversed at elevated C_a (Fig. 5.6a). Plants from the A&HD treatment showed the highest LAR values.

PC: The response of LAR PC to the treatments (Fig. 5.6b), was exactly the same as that of LAR PP (Fig. 5.5a). Significant responses to elevated C_a (p=0.012; Table 5.5) and to the interaction between C_a and density (p=0.026; Table 5.5), can be noted for this parameter at a PC level.

Plant height:

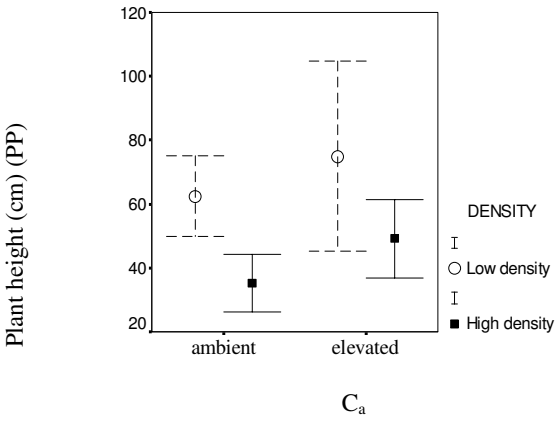


Fig. 5.7 Clustered error-bar graphs illustrating plant height of *C. odorata* grown under four treatments (ambient or elevated C_a, and low or high density)

PP: Plant height (PP) ranged from 35 cm (A&HD treatment), to 75 cm (E&LD treatment) (Fig. 5.7a). Elevated C_a increased plant height of both density classes, but with a non-significant p-value of 0.065. However, density had a very significant effect on plant height (PC) ($p<0.001$). At both C_a treatments, plants grown at lower densities were taller than plants grown at high densities. No interactive effects of C_a and density were noted ($p=0.917$; Table 5.4).

PC: With the lack of measurements for lengths of side branches, plant height PC (total stem lengths) would have merely been a sum of plant heights of individual plants for each treatment. High density treatment communities would obviously have greater total stem lengths than communities grown with low density, simply because there were more plants. Plant height PC is actually meaningless and has not been calculated.

Stem cross sectional area:

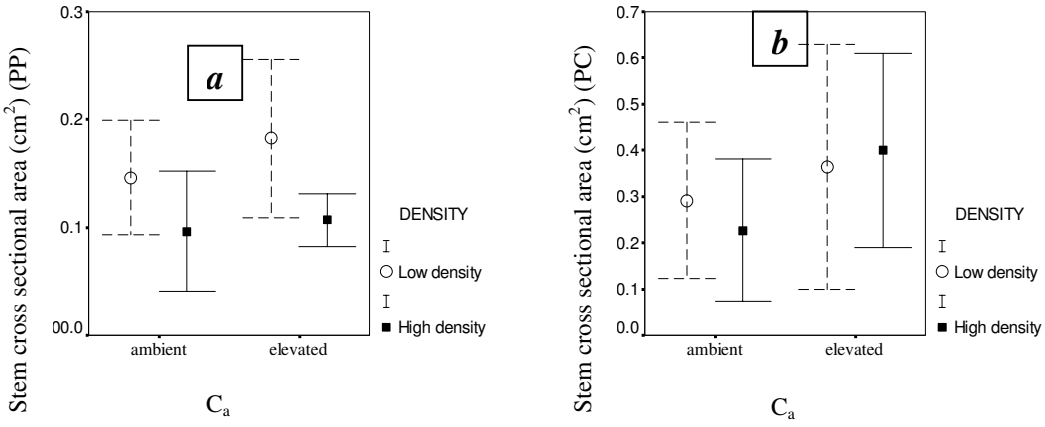


Fig. 5.8 Clustered error-bar graphs showing stem cross sectional area per plant (***a***) and per community (***b***) of *C. odorata* grown under the four treatments

PP: Averages for stem cross-sectional area were lowest (0.057 cm^2) for the A&HD treatment, and highest (0.182 cm^2) for the E&LD treatment (Fig. 5.8a). Although C_a had no influence (p=0.350; Table 5.4), stem cross-sectional area PP of *C. odorata* was significantly influenced by planting density (p=0.014). At both C_a treatments, *C. odorata* plants grown at lower densities had thicker stems than plants grown at higher densities. No C_a*density effects were observed (p=0.603; Table 5.4).

PC: Lowest stem total basal area (PC) was observed for communities belonging to the A&HD treatment (0.226 cm^2), while the community growing in the E&HD treatment showed the highest stem basal area (PC) values (0.4 cm^2) (Table 5.5). Elevated C_a increased basal area (PC) of the communities with both density classes (Fig. 4b), but with a non-significant P-value of 0.079 (Table 5.5). Although density had no *significant* effect on total basal area (PC), it can be seen that for the ambient C_a treatments, communities grown at low density had greater total basal area than the community of higher density. This pattern is reversed for the high C_a treatments (Fig. 5.8b). Surprisingly, there was no significant interactive effect between C_a and density, on total basal area (PC) (p=0.442; Table 5.5).

Biomass and biomass partitioning; per plant:

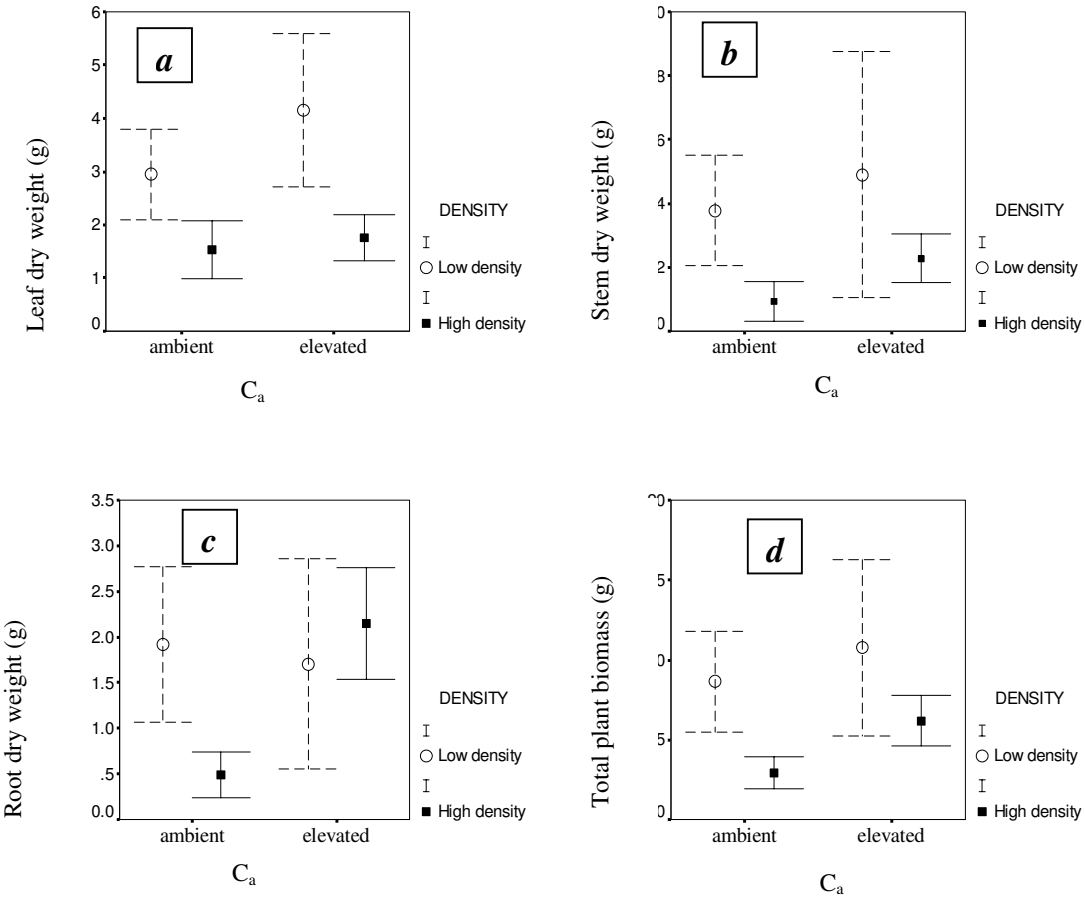


Fig. 5.9 Clustered error-bar graphs showing leaf dry weight per plant (a), stem dry weight per plant (b), root dry weight per plant (c) and total biomass per plant (d) of *C. odorata* grown at elevated or ambient C_a, and high or low planting density

PP: Leaf dry weight (PP) averaged between 1.5 g (A&HD treatment) and 4.2 g (E&LD treatment) (Fig. 5.9a), stem dry weight averages ranged from 0.9 g (A&HD treatment) to 4.9 g (E&LD treatment) (Fig. 5.9b), and root dry weights were from 0.5 g (A&HD treatment) to 2.2g (E&HD treatment) (Fig. 5.9c). Elevated C_a increased leaf (Fig. 5.9a) and root (Fig. 5.9c) dry weights (PP) significantly ($p=0.038$ and $p=0.018$, respectively), but had no significant effect on stem dry weight ($p=0.086$) (Fig. 5.9b). However, from Fig. 5.9b, it can be seen that stem dry weights at elevated C_a are generally greater than those at ambient C_a, for both density classes. Table 5.4

shows a significant effect of planting density on leaf and stem dry weights (PP) ($p<0.001$ for both parameters). From Fig. 5.9a and 5.9b, it can be seen that at both C_a treatments, plants grown at lower densities had greater leaf, and stem dry weights, than plants grown at higher densities. This pattern is observed only at ambient C_a, for root dry weight (PP). Fig. 5.9c shows that at elevated C_a, the pattern was reversed: plants grown at high density had heavier root mass than plants grown at low density. Therefore, root dry weight showed an interactive effect of C_a*density ($p=0.003$), although it was not significantly affected by planting density ($p=0.096$).

Fig. 5.9d shows the response of total biomass (PP) of *C. odorata* to the four treatments. Total biomass (PP) was lowest (2.9 g) for the A&HD treatment, and highest (10.8 g) for the E&LD treatment (Table 5.4). Total plant biomass (PP) was significantly affected by C_a ($p=0.021$), and density ($p<0.001$) (Table 5.4). Elevated C_a increased total biomass PP in both density classes. At ambient and elevated C_a, plants grown at lower densities had greater total biomass, compared with plants grown at higher densities (Fig. 5.9d).

PP weight ratios:

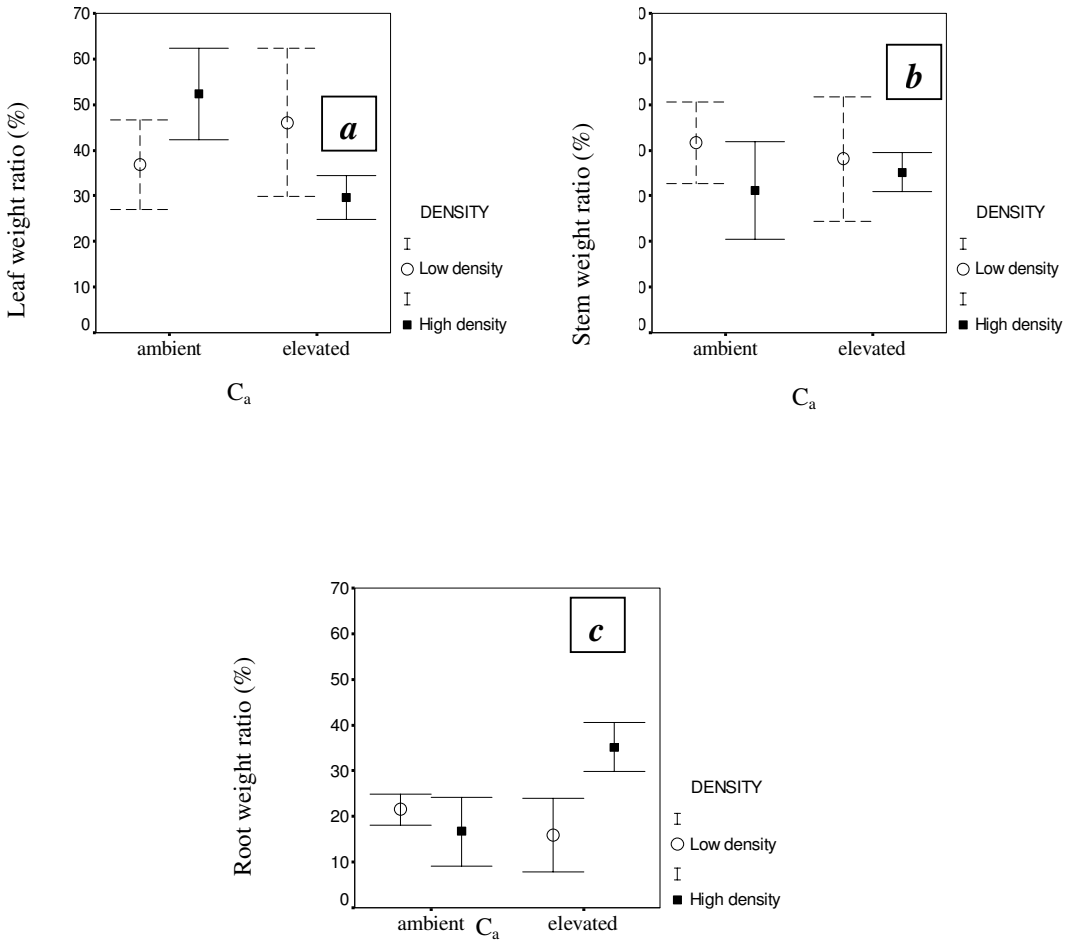


Fig. 5.10 shows error-bar graphs for leaf weight ratio per plant (a), stem weight ratio per plant (b) and root weight ratio per plant (c) of *C. odorata* grown at ambient or elevated C_a, and high or low density

PP: Biomass partitioning to leaves, stems and roots were calculated as ratios to total plant biomass, for each plant. Assumptions of parametric ANOVA were tested, and found to be satisfied. From Fig. 5.10a, it can be seen that leaf weight ratio (PP) was not significantly affected by Ca (p=0.155), or density (p=0.924). However, a

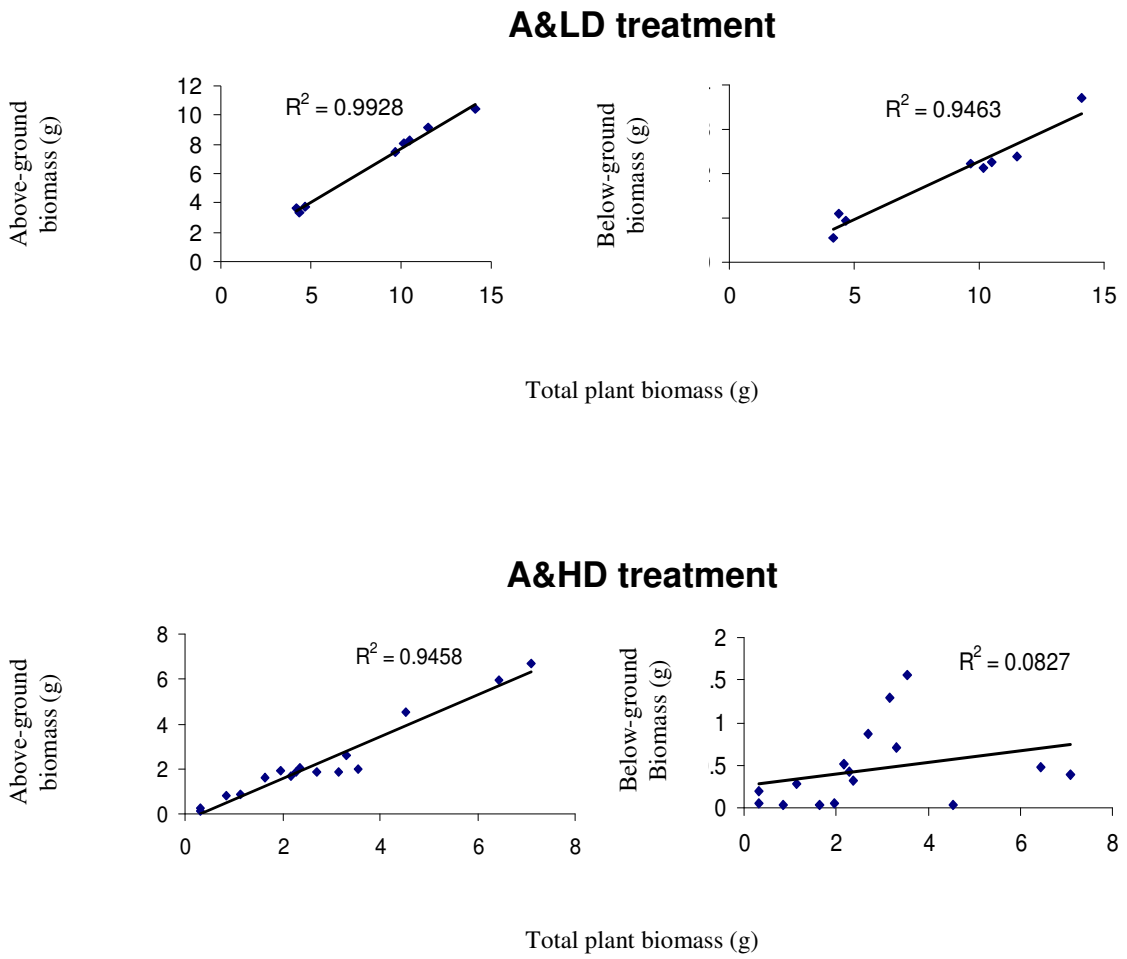
significant interactive effect of C_a*density was noted (p=0.001). Plants grown at high density allocated more biomass to leaves (PP) at ambient C_a, with the reverse for plants grown at elevated C_a.

There were no significant effects of C_a (p=0.959), density (p=0.512), or C_a*density (p=0.411), on stem weight ratio (PP) of *C. odorata*. The highest stem weight ratio (PP) was observed for the A&LD treatment (41.6 %) and the lowest average was observed for the A&HD treatment (31%) (Fig. 5.10b).

Proportional biomass partitioning to roots (PP) (Fig. 5.10c) was affected by C_a, but with a non-significant p-value of 0.063 (Table 5.4), and significantly affected by density (p=0.036; Table 5.4). There was a considerable allocation of biomass to roots in the elevated C_a, high planting density treatment, giving rise to a significant C_a*density interaction (p=0.001).

Potential pot size biomass restrictions:

To investigate potential physical limitations that pot size or OTCs could have had on biomass PP responses to the treatments, above- and below-ground biomass were plotted as functions of total plant biomass for each treatment (Figs. 5.11a and 5.11b below).



Figs. 5.11a Changes in above- and below-ground biomass, in relation to total plant biomass, of the A&LD and A&HD treatment

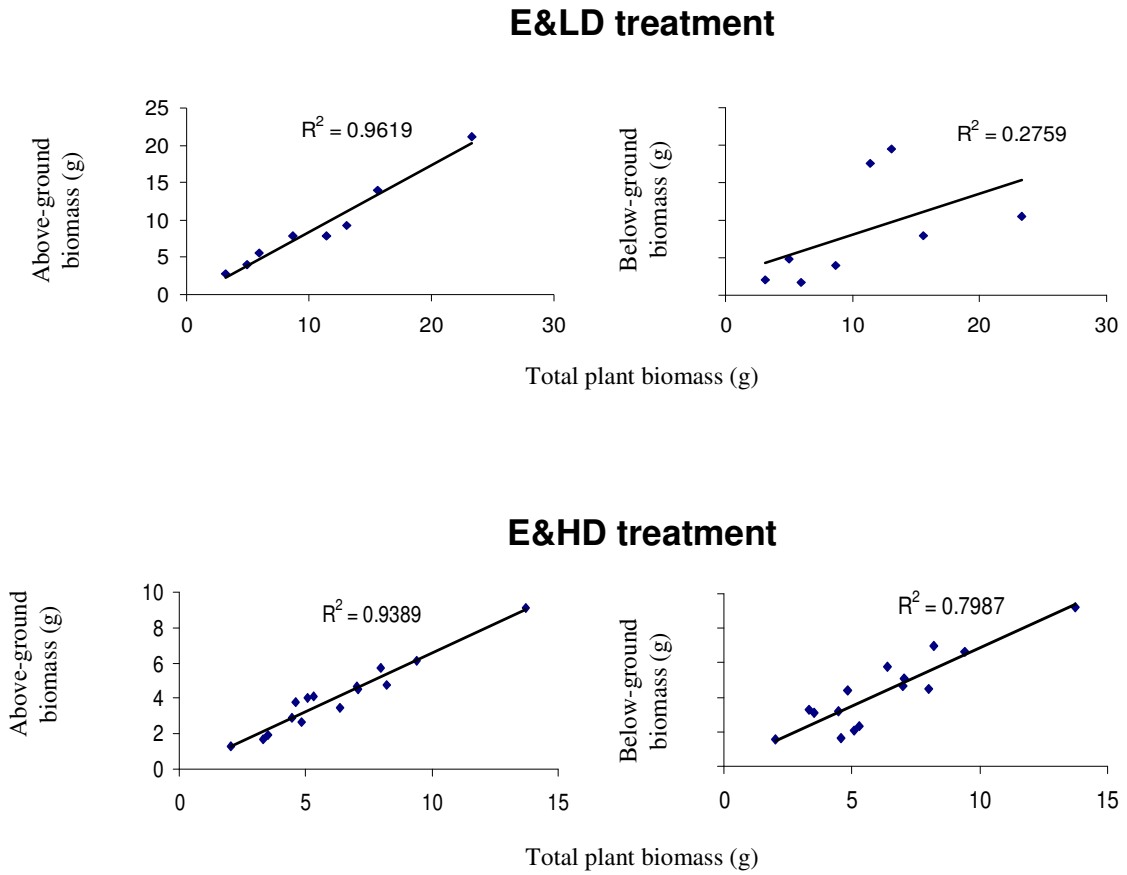


Fig. 5.11b Changes in above- and below-ground biomass, in relation to total plant biomass, of the E&LD and E&HD treatment

From the linear relationships between above-ground biomass and total plant biomass of plants from all four treatments (Figs. 5.11a and 5.11b), it can be concluded that pot size or OTCs did not limit growth of above-ground biomass (stems and leaves) of *C. odorata*, when exposed to elevated or ambient, and high or low planting densities. In contrast, growth of below-ground biomass of *C. odorata* from three treatments (A&HD; E&LD and E&HD treatments), could have been restricted by pot size. R-squared values (coefficients of determinations), are indicators that reveal how closely the estimated values for the trendline correspond to the actual data. Since trendlines are most reliable when its R-squared value is at or near 1, the highest degree of pot-size limitation on root growth of *C. odorata*, can be observed for plants in the A&HD treatment (Fig. 5.11a).

Biomass and biomass partitioning; community level:

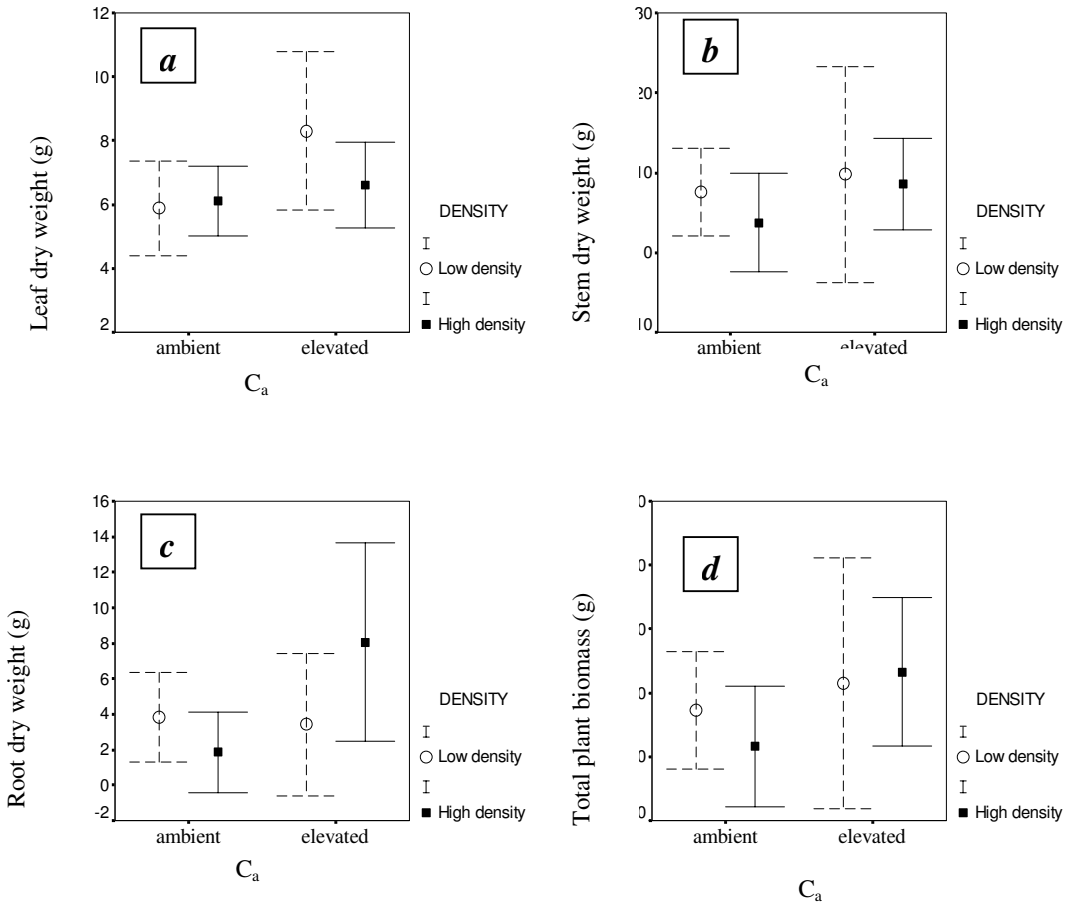


Fig. 5.12 Clustered error-bar graphs showing leaf dry weight per community (a), stem dry weight per community (b), root dry weight per community (c) and total biomass per community (d) of *C. odorata* grown under the four treatments

PC: Leaf dry weight (PC) averaged between 5.9 g (A&LD treatment) and 8.3 g (E&LD treatment) (Fig. 5.12a), stem dry weight means ranged from 3.7 g (A&HD treatment) to 9.8 g (E&LD treatment) (Fig. 5.12b), and root dry weights were from 1.9 g (A&HD treatment) to 8.1 g (E&HD treatment) (Fig. 5.12c).

The pattern that can be observed from Fig. 5.12a is that communities exposed to elevated C_a had greater total leaf dry weight (PC), than communities exposed to

ambient C_a, for both density classes. The effect of C_a on communitiy total leaf weight was significant (p=0.018; Table 5.5). No significant density, or C_a*density effects were noted for community leaf dry weight (PC) (Table 5.5).

Although C_a and density did not affect community stem dry weight significantly, the pattern that emerges from Fig. 5.12b shows a decrease in stem dry weight (PC), with increasing planting density, for both C_a treatments. The E&LD treatment once again showed the greatest variation in the data (SD=8.5; Table 5.5). No significant interactive effects between C_a and density were observed for stem dry weight (PC).

Significant C_a and C_a*density effects were observed for community root dry weight (p=0.034 and p=0.017, respectively; Table 5.5). For the ambient C_a treatment, communities with lower planting densities had more root than communities with at higher planting densities (Fig. 5.12c). As can be seen, this pattern is reversed for the elevated C_a treatment: There was more root in communities of high density than the low density communities. The highest root dry weight was observed for the community under the E&HD treatment (Fig. 5.12c; Table 5.5).

Elevated C_a increased total community biomass of both density classes, but with a non-significant p-value of 0.08 (Fig. 5.12d). The lowest biomass (11.6 g) was observed for the A&HD treatment, and the highest value (23.2 g) belonged to the E&HD treatment (Table 5.5). There were no significant density or C_a*density effects on total community biomass (Table 5.5).

PC weight ratios:

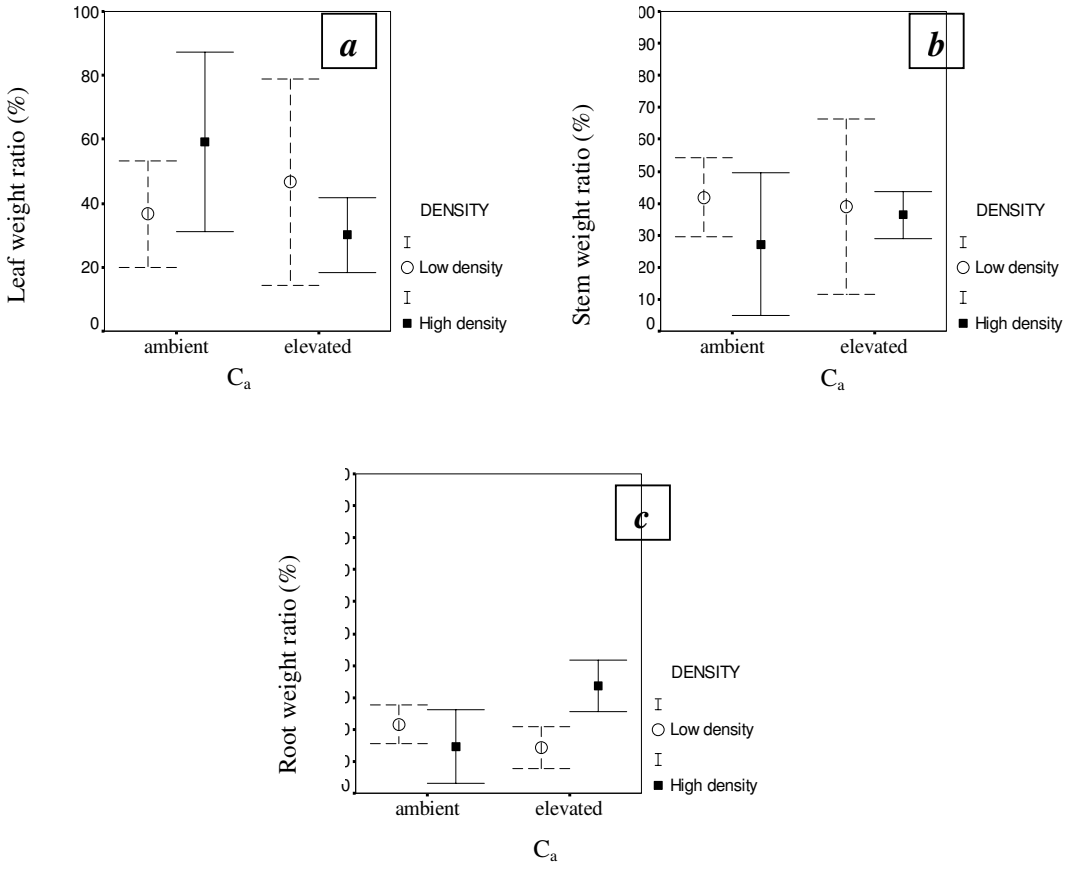


Fig. 5.13 shows error-bar graphs for leaf weight ratio per community (a), stem weight ratio per community (b) and root weight ratio per community (c) of *C. odorata* grown under the four treatments

PC: Biomass weight ratios PC were similar to that of PP, for leaf, stem and root weight ratios. The highest leaf weight ratio PC was observed for the A&HD treatment (58.4 %) and the lowest value was noted for the E&HD treatment (30.1 %; Table 5.5, Fig. 5.13a). This pattern was also noted for leaf weight ratio (PP) (Fig. 5.10a). C_a and density did not influence leaf weight ratio PC significantly (p=0.232 and p=0.727, respectively, Table 5.5). Fig. 5.13a shows that for the ambient C_a treatment, communities grown at higher density, had a greater biomass allocation to leaves, compared to communities grown at lower density. For the elevated C_a

treatment, this pattern is reversed (Fig. 5.13a). Not surprisingly, a significant interactive effect between C_a and density was noted for community leaf weight ratio ($p=0.022$).

Communities of plants grown under the A&LD treatment, had the highest allocation to stems (41.8 %) while the lowest community stem weight ratio was observed for the A&HD treatment (27 %) (Table 5.5). However, there were no significant C_a, density or interactive C_a and density effects on community stem weight ratio, Fig. 5.13b shows that for each C_a treatment, communities with lower densities allocated more biomass to stems than plants grown at higher densities. Incidentally, this pattern was also observed for stem weight ratio (PP) (Fig. 5.10b).

Community root weight ratios were significantly influenced by C_a ($p=0.043$), and by density ($p=0.038$) (Table 5.5). For the ambient C_a treatment, overall community allocation to roots was higher at low planting density, than at higher planting density (Fig. 5.13c). The opposite pattern can be observed for elevated C_a-grown communities. Therefore, a significant C_a*density interaction on community root weight ratio can be noted ($p<0.001$) (Table 5.5).

CHAPTER 6. DISCUSSION (PART B)

Although much is known about the individual effects of CO_2 enrichment and planting density on plant growth, very little is known about the *interactive* effects of these two factors on plants. PART B is the first study thus far, in which *C. odorata* was grown in monoculture. Plants were grown at ambient or elevated C_a, and with high or low planting density. Growth analyses were done at an individual plant (PP) level, as well as at a per community (PC) level. Physiology results were based on single-leaf photosynthetic data.

6.1. Physiology

Stimulation of photosynthesis in elevated C_a has been demonstrated in various studies (Poorer et al, 1996; Lloyd and Farquhar, 2000; Ainsworth et al., 2002), and has been attributed to the properties of the enzyme rubisco. Increasing C_a competitively inhibits the oxygenation reaction of rubisco, thus increasing photosynthetic assimilation (Johnson et al., 1993). However, a recent study showed no change in instantaneous CO_2 assimilation rates of *C. odorata* under CO_2 enrichment (Naidoo, personal communication). It is unfortunate that measurements of instantaneous rates of photosynthetic assimilation under elevated C_a, were not taken in the current study. Nevertheless, the "average" A:C_i curve constructed (Fig. 5.1; Chapter 5), showed that lines for all four treatments were similar, and statistical analysis revealed no significant effects of C_a, density or C_a times density on J_{max}, initial slopes and CO_2 compensation points, of *C. odorata* grown in elevated or ambient C_a, and high or low density. Therefore, in terms of changes to A:C_i curves, no noticeable photosynthetic down-regulation can be noted. Although this *indirectly* suggests that enhancement of photosynthesis of *C. odorata* could have occurred in elevated C_a, the lack of *direct* data for assimilation rates under CO_2 enrichment, makes it impossible to determine if this was indeed the case.

Stomatal responses of *C. odorata* to elevated C_a, was investigated by assessing stomatal conductance, and stomatal limitation measurements.

Previous studies have shown that in elevated C_a, g_c could increase (Wheeler et al., 1999), decrease (Calfapietra et al., 2005; Overdieck and Strassmeyer, 2005), or remain unaffected (Teskey, 1995). In a recent study, the significant decrease in g_c of *C. odorata* in elevated C_a (when grown in mixed arrays with another C_3 species), was attributed to resource-sink limitations, rather than elevated C_a *per se* (Naidoo, personal communication). Unfortunately, data for instantaneous g_c values in elevated C_a, were not analysed in the current study. To compensate for this, mean g_c values at C_a= 370 ppm and C_a=720 ppm, were extrapolated from A:C_i data for each treatment, and analyzed statistically.

According to Maherali et al. (2002), a major factor that could alter stomatal responses to CO_2 is the degree to which stomata acclimate to growth CO_2. These authors maintain that physiological stomatal acclimation would be demonstrated if the stomatal behaviour of plants grown at contrasting CO_2 concentrations differ when measured at the same CO_2 concentration. Since there were no significant effects of C_a on g_c at C_a=370 ppm, and g_c at C_a=720 ppm between ambient- and elevated-grown plants (Table 5.2; Chapter 5), it can be concluded that stomatal acclimation to elevated C_a did not occur in the current study. Nevertheless, g_c values at C_a=720 ppm, were significantly higher than g_c at C_a=370 ppm, for the E&LD treatment (Fig. 5.2; Chapter 5). This could have resulted in, or have been a result of, the significant *interactive* effect of C_a and density on g_c at C_a=720 ppm, across treatments (Table 5.2; Chapter 5).

According to Drake and Gonzàlez-Meler (1997), the observed general decreases in g_c in elevated C_a, by itself, does not limit photosynthesis. Limitations that stomata could impose on CO_2 assimilation, can be quantified in a simple, practical way from A:C_i responses (Long and Bernacchi, 2003), and have been calculated in the current study. Results showed no significant influence of C_a, density or C_a times density on stomatal limitations of *C. odorata* (Table 5.3; Chapter 5). These results are not really surprising, if one considers the "slope" of the 'average' A:C_i curve constructed (Fig. 5.1; Chapter 5). A comparison of "average" A:C_i curves for both experimental trials of the current study (Fig. 3.1; Chapter 3, and Fig. 5.1; Chapter 5), as well as initial slope values (Table 3.1; Chapter 3, and Table 5.1; Chapter 5), showed that relatively steep inclines for all four treatments can be observed in the "average" A:C_i curve

constructed for PART B (Fig. 5.1; Chapter 5). This implies that photosynthesis was saturated at generally low C_i concentrations, and for a plant operating at 700 ppm (elevated C_a treatments), reduced g_c would not decrease assimilation. In this regard, *C. odorata* is effectively acting as a C_4 plant, and the fact that elevated C_a had no effect on stomatal limitations, is not unexpected. However, these results are in contrast to a study on sweetgum (*Liquidambar styraciflua*), which demostrated a 26% decrease in stomatal limitations under long-term CO_2 enrichment (Herrick et al., 2004).

Research on physiological responses of plants to elevated C_a, has shown that after prolonged exposure to CO_2 enrichment (as is the case when plants are *grown* in elevated C_a), photosynthetic down-regulation may occur as plants fail to sustain the initial stimulation of photosynthesis (Rogers and Humphries, 2000; Overdieck and Strassemeyer, 2005). In the current study, results from A:C_i curves (Table 5.1; Chapter 5), indicated that photosynthetic down-regulation did not occur in *C. odorata* grown at elevated C_a, at high or low planting density. However, to investigate the possibility of photosynthetic down-regulation even further, four "non-treatment" pots containing two *C. odorata* each, were obtained. Significant decreases in J_{max} with time (days after exposure to elevated C_a) of the "non-treatment" plants, provides evidence that photosynthetic downregulation could have occurred in these plants, when exposed to elevated C_a (Fig. 5.3; Chapter 5). However, it should be noted that the "non-treatment" plants were exposed to elevated C_a by being enclosed in OTCs, and the response observed could in fact, be due to a chamber effect. Distinguishing between possible chamber effects and photosynthetic down-regulation, on the significant decrease in J_{max} of "non-treatment" *C.odorata* plants, is beyond the scope of this study.

Instead of resorting to this "by process of elimination" approach, photosynthetic down-regulation, or the lack of it, needs to be thoroughly investigated in future research, as it is an important phenomenon clearly demonstrated in various studies. According to Aranjuelo et al. (2005), photosynthetic down-regulation may be caused by stomatal- and non-stomatal limitations. Since substantial stomatal limitations to photosynthesis did not occur in the current study, it can be concluded that *if* photosynthetic down-regulation had occured in leaves of *C. odorata* when exposed to

elevated C_a and different planting densities, it would have been attributed to non-stomatal limitations, and this could range from low leaf rubisco (Cook et al., 1998) and offset in leaf C:N balances (Hui et al., 2002), to decreased carboxylation efficiencies (Long et al., 2004) and nutrient deficiencies (Barret and Gifford, 1995), as well as changes in source-sink balance (Ainsworth et al., 2002), or even a reduction in light capture (PS11 activity) (Aranjuelo et al., 2005). Hui et al., 2002). These options need to be investigated in future, to allow for proper understanding of photosynthetic down-regulation in *C. odorata* in elevated C_a, if the process occurs at all. In addition, photosynthetic measurements should be taken at various intervals during growth, instead of only at the end of the growth period. This would also help investigate the potential for photosynthetic down-regulation.

Pritchard et al. (1999) mentioned the possibility of plant limitations to carbon assimilation e.g. inefficiencies of assimilate transport from sources to sinks. These potential limitations of plant physiology and structure to photosynthesis, should be included in future research on *C. odorata* responses to CO_2 enrichment.

6.2 Growth and Morphology

Growth and Morphology PP:

The fact that density significantly influenced seven of the 12 biomass parameters PP, while C_a affected only five parameters significantly, suggests that density effects could have "overridden" the effects of C_a on *C. odorata* plants. This means that in cases where a lack of C_a effects on a biomass parameter is observed, it does not necessarily mean that C_a did not affect the parameter at all; it could just mean that the response of the plant to density could have been stronger, and thus overshadowed the plant's response to C_a. He and Bazzaz (2003) showed that density modified the response of reproductive allocations of *Phytolacca americana* to elevated C_a. Therefore, density reducing the response of a plant to elevated C_a could have occurred in the current study. Significant interactive effects between C_a and density were observed for five biomass parameters, and this re-iterates the possibility of overshadowing occurring between density and C_a effects, on biomass parameters (PP) of *C. odorata*.

Although many studies show an increase in total leaf area of plants when exposed to CO_2 enrichment (Wong, 1979; O'Leary and Knecht, 1981; Polley et al., 1993a; Pritchard et al., 1999), total leaf area PP of *C. odorata* in the current study was not significantly affected by C_a (p=0.987; Table 5.4 in Chapter 5). This is in direct contrast to results of two similar studies: Firstly, Wayne et al. (1999) studied density-dependent CO_2 responses of *Brassica kaber*, and showed a stimulation of leaf area in elevated C_a. Secondly, leaf area of six invasive species was also enhanced in elevated C_a (Ziska, 2003). However, recent studies on *C. odorata* have shown results similar to the current study: Total leaf area PP of *C. odorata* was not significantly influenced by C_a, when this species was grown in elevated C_a, and subjected to interspecific competition with another C_3 plant (Naidoo, personal communication), grown in competition with a C_4 grass (Lalla, unpublished), or subjected to different nutrient treatments (Patton, personal commnication). In the current study, planting density had a significant effect on total leaf area PP of *C. odorata* (p=0.006; Table 5.4 in Chapter 5). For each C_a treatment, plants grown at lower densities had greater total leaf area than plants grown at higher densities (Fig. 5.4a; Chapter 5). This result could simply be attributed to space: fewer plants in a pot have more space in to which to expand their leaves, and therefore an increase in leaf area per plant is not unexpected. Although no significant *interactive* effect between C_a and density was noted, Fig. 3a shows that elevated C_a increased total leaf area of the low-density class only, and this trend was observed by Rutuerto et al. (1996). This observation suggests that once a certain threshold of self-shading occurs, the CO_2 response of total leaf area is no longer density-dependent.

Values for SLA were obtained by dividing the area of a single, average sized leaf by its weight. Thus, high SLA values are usually characteristic of thin leaves. In direct contrast to total leaf area, SLA was found to be significantly affected by C_a, and not by density (Table 5.4; Chapter 5). Irrespective of planting density at which they were grown, *C. odorata* grown in elevated C_a had lower SLA values (thicker leaves) than plants grown in ambient C_a (Fig. 5.5; Chapter 5). This is in accord with Thomas and Harvey (1983), Usuda and Shimogawara (1998) and Hui et al. (2002), who demonstrated a stimulation of leaf thickness (decrease in SLA) in elevated C_a. Bazzaz (1990) suggested that plants grown in elevated C_a reduce their SLA (have thicker leaves) because they become photosynthetically more efficient. However, it is

impossible to determine if this were the case in the current trial, due to the lack of direct data for CO_2 assimilation rates in elevated C_a. At this stage, it is sufficient to say that investing in thicker leaves, could simply have been a way for elevated C_a-grown plants of dealing with extra carbon.

Although density did not significantly affect SLA, significant C_a times density effects were noted for SLA of *C. odorata* ($p=0.041$; Table 5.4 in Chapter 5). Since the same C_a effect can be observed for both density classes, it can be concluded that C_a modified the response of SLA to density. At ambient C_a, plants grown at high density had greater SLA values (thin leaves) than plants grown at lower density. In fact, the A&HD treatment showed the highest SLA values (Fig. 5.5a; Chapter 5). The pattern is reversed at elevated C_a, and the E&HD treatment showed the lowest SLA values (thickest leaves). Previous studies on *C. odorata* have shown a variation in the responses of SLA to elevated C_a. Patton (personal communication), however showed a significant decrease in SLA of *C. odorata* in elevated C_a (similar to the current study), while Lalla (unpublished) showed no difference between the SLA of ambient vs elevated C_a-grown *C. odorata* plants. The variation in the results of how SLA of a single species responds to elevated C_a, highlights the need for future studies to be conducted to eliminate these discrepancies. A starting point would be the fact that decreased SLA is often associated with increased starch levels in leaves (Bazzz, 1990). Therefore, future research on *C. odorata* should include measurements of total non-structural carbohydrates like starch (TNCs) in order to get a better understanding of how elevated C_a affects SLA.

According to Usuda and Shimogawara (1998), leaf area ratio (LAR; leaf area per unit of plant dry mass), provides an indication of the proportion of a plant that is active in photosynthesis. Decreasing LAR with increasing C_a concentrations, were reported for five out of six invasive species studied (Ziska, 2003). Ishizaki et al. (2003) also showed a significant reduction in LAR of *Polygonum cuspidatum* in elevated C_a. Similarly, in the current trial, elevated C_a significantly reduced LAR PP of *C. odorata*, independently of which density group they were grown at ($p=0.009$; Table 5.4 in Chapter 5). However, a possible link between the reductions of LAR in elevated C_a, and the proportion of *C. odorata* plants that were active in photosynthesis, cannot be

established in the current trial, since instantaneous rates of photosynthetic assimilation in elevated C_a, were not obtained.

LAR PP was unaffected by planting density (p=0.228; Table 5.4 in Chapter 5), and this is in accord with a previous study on *Sinapis alba* (Rutuerto et al., 1996). In the current trial, a significant interactive effect of C_a and density on LAR can also be noted (p=0.035; Table 5.4 in Chapter 5). Since the trend of the response of LAR to C_a, is exactly the same as that observed for SLA (discussed above), it can be concluded that elevated C_a reduced LAR, solely due to a decrease in SLA; an idea which has been previously demonstrated by Harmens et al. (2000), in a study on *Dactylis glomerata*.

An earlier study on an invasive alien, kudzu, showed a 58% increase in stem height, when exposed to elevated C_a (1000 ppm) in chambers (Sasek and Strain, 1988). Similarly, elevated C_a increased plant heights of both density classes of *C. odorata*, but with a *non*-significant p-value of 0.065 (Table 5.4; Chapter 5). If the growth period of the current study was increased, this *may* have developed into a significant C_a effect on plant height. However, this is speculation since previous studies have shown no significant influence of elevated C_a on plant height of this species (Lalla, unpublished; Patton, personal communication). Athough one might expect a positive relationship between planting density and plant height, because of competition for light, the current study demonstrated a significant decrease in plant height with increasing density, of both C_a treatments. Brahim et al. (1998) attributed minor decreases in plant height of *Lesquerella fendleri* with increasing planting density, to competition for available space, and this could explain the plant height responses of *C. odorata* to density in the current study.

Pritchard et al. (1999) conducted a review of studies on elevated C_a and plant structure, and reported that in many of the studies, stem diameter increased under CO_2 enrichment. However, previous studies on *C. odorata* showed no significant effect of C_a on stem diameter (Lalla, unpublished; Patton, personal communication). Similarly, C_a had no significant effect on stem cross sectional areas of *C. odorata* in the current study (p=0.350; Table 5.4 in Chapter 5). However, in direct contrast to a study on loblolly pines (Will et al., 2005), increasing planting density was

significantly associated with reduced stem cross sectional areas of *C. odorata* ($p=0.014$; Table 5.4 in Chapter 5). This pattern was also observed for plant height, and the correlation is not surprising: thicker stems would be needed to support taller plants.

Elevated C_a significantly increased leaf dry weight of *C. odorata*, irrespective of planting density ($p=0.038$; Table 5.4 in Chapter 5). Although stem dry weights were not *significantly* affected by C_a ($p=0.086$; Table 5.4 in Chapter 5), the pattern that is obvious from Fig. 5.9b (Chapter 5) is similar to that of leaf dry weight: elevated C_a increased stem dry weights of both density classes. These results indicate an increase in above-ground biomass of *C. odorata* in elevated C_a, a relationship which has been demonstrated in previous studies on different species (Polley et al., 1993b; Hunt et al., 1991; Bazin et al., 2002). Results from those studies support the prediction that plants would alter the balance between growth and availability of resources in elevated C_a, such that water stress is reduced (because of increased water-use efficiency due to decreased g_c) (Friedingstein et al., 1999). A consequence of this, according to these authors, is a reduction in the root:shoot ratio as plants increase their above-ground biomass. (At this stage, it is impossible to assess if this prediction holds true in the current study, due to lack of direct g_c data). The fact that increasing planting density significantly decreased leaf and stem dry weights, irrespective of C_a exposure, is not surprising ($p<0.001$ for both parameters; Table 5.4 in Chapter 5). With less competition for space, plants in the low density classes generally grew bigger. It should be mentioned that this observation, thus far applies to *above-ground* growth (taller plants, thicker and heavier stems and leaves).

Research similar to the current study was conducted on *Brassica kaber*, and results showed that root dry weight was significantly increased by density, but unaffected by C_a, or the interaction between C_a and density (Wayne et al., 1999). In direct contrast, below-ground biomass of *C. odorata* in the current study was significantly influenced by C_a, and by the interaction between C_a and density. At ambient C_a, plants grown with low density had heavier roots than plants grown with high density. However, at elevated C_a, this pattern was reversed, and the significant interactive effect between C_a and density, rendered it impossible to determine the *direction* of the root dry

weight response to elevated C_a (i.e. whether elevated C_a had a positive or negative response on root dry weight).

The significant increase in total plant biomass in elevated C_a (Fig. 5.9d; Chapter 5), can be attributed mainly to the increase in above-ground biomass (leaf- and stem dry weights) under CO_2 enrichment (Fig. 5.9a and Fig. 5.9b; Chapter 5). This positive response of total plant biomass to elevated C_a, has been observed in other studies (Carlson and Bazzaz, 1982; Wilsey et al., 1997; Ainsworth et al., 2002; Bazin et al., 2002). Ruetuerto et al. (1996) showed an increase in total plant biomass of *Sinapis alba* in elevated C_a, irrespective of planting density; results identical to the current study (Fig. 5.9d; Chapter 5).

The idea of a positive correlation between the enhancement of growth, and an increase in photosynthetic assimilation rates, under CO_2 enrichment, cannot be proven in the current trial due to a lack of direct data for CO_2 assimilation rates in elevated C_a. However, the fact that elevated C_a did not result in photosynthetic down-regulation, nor an increase in stomatal limitations, does suggest that rates of photosynthesis could have been increased in elevated C_a. However, a study similar to the current trial, showed no enhancement of instantaneous CO_2 assimilation rates under CO_2 enrichment (Naidoo, personal communication).

In addition to photosynthesis, plant growth is affected by a multitude of other factors (Körner, 1991). Time, according to Easmus (1991) and Poorter (1993), is one of the factors that could influence plant growth responses to elevated C_a. Many studies have shown that initial increases in plant biomass are not maintained throughout growth in elevated C_a (Bazzaz, 1990; den Hertog et al., 1993; Centritto et al., 1999). This suggests that growth, may in fact acclimate to elevated C_a, and in a similar way to photosynthesis and g_c (Drake and Gonzàlez-Meler, 1997). To re-iterate this point, a study on *Dactylis glomerata* showed that elevated C_a changed biomass allocation patterns only transiently, during early stages of growth, if at all (Harmens et al., 2000). Previous RGR (relative growth rate) data on *C. odorata* seedlings has shown that growth rates are quite high during the first 30 days, decreases considerably during the next 2 months, and the decline is greater in the subsequent period (after 2 months) (Ambika, 2002). In addition, that study showed that the decline in growth rate of

C. odorata is slower in the roots. Therefore, future research on this species, should incorporate regular biomass measurements (initial, during growth and prior to harvesting measurements), to generate RGR data. This would help establish whether the observed increase in total plant biomass of *C. odorata* in elevated C_a, is a transient or persistent effect, and to determine if the RGR results for *C. odorata* seedlings holds true for cuttings from intact plants. Density significantly decreased total plant biomass (Fig. 5.9d; Chapter 5), in direct contrast to a study on rubber/banana plantations (Rodrigo et al., 1997).

From Figs. 5.10a-c (Chapter 5), it can be concluded that in general, there was greater proportional allocation of dry matter of *C. odorata* to above-ground biomass (leaves and stems), rather than to below-ground biomass. One of the major reasons behind the high rate of invasiveness of *C. odorata*, is the fact that this weed suppresses natural vegetation through physical smothering and by forming impenetrable tangles which shade out indigenous vegetation (Macdonald, 1983; Goodall, 2002). This, together with the fact that *C. odorata* is not a woody species with a long lifespan, but rather a rapid-growing shrub with a high reproductive rate, suggests that the general biomass partitioning pattern observed above, permit the observed growth patterns in the field.

Altered root:shoot ratios are often noted in elevated C_a, and this suggests a shift in the functional relationship between these organs (Pritchard et al., 1999). A closer look at the response of biomass partitioning patterns of *C. odorata* to the treatments, will now follow. Stem weight ratios were not siginificantly affected by any of the treatments, and so will not be discussed. Although leaf weight ratios were not significantly influenced by C_a or density, a significant *interactive* effect between the two factors can be observed ($p=0.001$; Table 5.4 in Chapter 5). This implies that density could have modified the CO_2 response of leaf weight ratios, or vice-versa, and distinguishing between the two is impossible at this stage: suffice it to say that there was some kind of significant interaction between C_a and density, on the response of leaf weight ratios to the treatments.

At ambient C_a, high density grown plants allocated more carbon to leaves, than low density grown plants. With a larger number of plants per pot, competition for

increasing surface areas for the absorption of CO_2 and light must have arisen. However, with the abundant availability of CO_2 in the elevated C_a treatments, competition of high density grown plants for CO_2 was not that strong, and so these plants allocated more biomass to roots. Significant C_a times density interactions were noted for root weight ratios. C_a had a marginal effect on this parameter ($p=0.063$; Table 5.4). Although this may have transpired into a significant effect, if time had permitted, it could also have been lost over a longer growth period. Nevertheless, the extremely high root weight ratios for the E&HD treatment, suggests that results from this study may support the prediction that elevated C_a will promote root growth (Taylor et al., 1994). On the other hand, because of reduced g_c (in many cases), elevated C_a could reduce proportional allocation to roots.

Usuda and Shimogawara (1998), attributed the extreme 105% increase in dry weight of storage roots of radish observed in their study, to increasing sink capacity. These sentiments were echoed by Rogers et al. (1999), who maintained that increased rooting observed under CO_2-enrichment occurs to increase carbon deposition and or nutrient uptake. These predictions seem logical if the general expectation of plants enhancing photosynthesis and growth in elevated C_a, is met. More sinks would be needed for the "extra" photoassimilate produced in the leaves (source), and the greater demands of the plant in elevated C_a, could result in the production of more roots to increase nutrient/water uptake to become more efficient. However, without reference to direct data of photosynthetic rates in elevated C_a, it is impossible to determine if the above explanation could account for the response of root weight ratio or root dry weight, to elevated C_a. However, it is possible that the changes in dry matter allocation patterns of *C. odorata* in elevated C_a, could be a secondary response through changes in C/N uptake, rather than a direct response to elevated C_a, as has been demonstrated by Ishizaki et al. (2003). Ratios of C/N content in *C. odorata*, should therefore be included in future studies on the species.

Thomas and Strain (1991) attributed photosynthetic acclimation of cotton seedlings, to inadequate rooting volume, as root restrictions were evident for plants grown in small pots. To investigate potential pot size limitations in the current study, above- and below-ground biomass were plotted as functions of total plant biomass. Linear relationships between total biomass and either plant component, according to

Centritto et al. (1999), indicate an absence of pot size limitations. From Figs 5.11a and Fig. 5.11b (Chapter 5), and from the coefficient of determination (R^2) value, it is evident that root restrictions due to pot size limitations, could have occurred in the A&HD, E&LD and E&HD treatments, in the current study. Further evidence for this is provided by the fact that when collected, roots had formed masses which in many cases, had reached the sides of pots. If photosynthetic down-regulation had occurred in the current study (see Section 6.1; this study), this could have been attributed to restricted root growth due to pot size limitations, as has been demonstrated by Thomas and Strain (1991). Larger pots should be used in future CO_2 enrichment studies on *C. odorata*. On the other side of the coin, linear relationships between above-ground biomass and total plant biomass, provide evidence that neither pot size, nor OTC dimensions restricted above-ground growth, and therefore any observed responses of above-ground biomass to the treatments, could not have been an artifact of experimental technique.

Results of growth analysis (PP) in this trial, suggests that elevated C_a would result in individual *C. odorata* plants, with thick leaves, and high leaf-, and total plant biomass, irrespective of the number of *C. odorata* plants growing together.

Growth and morphology PC:

All three studies reviewed on the responses of plants to elevated C_a *and* density in Section 1.4.3 in Chapter 1, have shown profound density-dependent responses of plants to elevated C_a at an individual level, but a "withering away" effect of this response at population level. To ascertain if this were the case in the current study, per community (PC) biomass measurements were taken to investigate whether differences, if any, in the biomass responses of individual *C. odorata* plants to the treatments, scaled up to whole communities of *C. odorata*,. A distinct decline in the responses of *C. odorata* PC, compared to that observed PP, to the treatments, is obvious from the comparisons of Table 5.4 and Table 5.5, in Chapter 5. Total leaf area, stem cross sectional area and stem dry weight were significantly affected by density at an individual plant level, but not on a community level. The significant interactive effect of C_a and density on total plant biomass PP, was lost at PC level. Leaf dry weight was significantly affected by C_a *and* density at PP level, but only by C_a at PC level. These observations suggest that to some extent, a decline in the effects

of the treatments on plants occurred at community level, and this has been reported in previous studies (He and Bazzaz, 1990; Rutuerto et al., 1996; Wayne et al., 1999).

When grown at different planting densities, competition for resources was indirectly incorporated into the study, as a factor which could have influenced the response of *C. odorata* to elevated C_a. According to Dukes (2000), growth of a single plant might be limited by the availability of CO_2, but plants in communities are likely to be limited by the availability of light, nutrients, water and space, for which they have to compete with one another. This could explain the decline in effects of five out of nine biomass parameters from a PP to a PC level, observed in the study. In plant communities, the greater number of plants increases competition for the same resources, and any one (or combinations) of the resources mentioned above, could have become limiting, and it is difficult to narrow down the possibilities.

Despite this potential limitation of resources at community level, five parameters PC were still significantly influenced by the treatments (C_a, density or C_a times density), and these will now be discussed in detail.

Effects of the treatments on root dry weight PP and PC, were identical (Fig. 5.9c and Fig. 5.12c; Chapter 5). The fact that the response of this particular parameter of individual plants, was carried through to the whole community, highlights the importance of the effect of the treatments on root dry weights. In this particular case, this refers to the significant C_a, and C_a times density effects on root dry weight PC and PP, and the latter has already been discussed.

Leaf dry weight PP was significantly increased by increasing C_a, and decreased with increasing density (Fig. 5.9a; Chapter 5). When assessed on a PC level, leaf dry weight was still significantly increased by elevated C_a ($p=0.018$; Table 5.5 in Chapter 5). Once again, this observation of "following through" of the response of leaf dry weight from individual, to community level, serves to make the significant C_a effect more profound. Comparisons of the responses of leaf weight ratio PC and PP, to the treatments, showed a significant interactive effect between C_a and density, at both levels. At ambient C_a, individual plants and communities of *C. odorata* grown with high density allocated more biomass to leaves, compared with plants from the low

density treatment (Fig. 5.12a; Chapter 5). This pattern was reversed at elevated C_a, suggesting that competition among the high-density grown plants in elevated C_a, was transferred to below-ground biomass, as more roots were produced. This is confirmed by the response of root weight ratio PC to the treatments (Fig. 5.12c; Chapter 5). Root weight ratio PP and PC were significantly influenced by density, and there was a significant C_a times density effect. Elevated C_a significantly affected root weight ratio PC ($p=0.043$; Table 5.5 in Chapter 5). C_a did affect root weight ratio PP, but with a non-significant p-value of 0.063. Perhaps, this would have transpired into a significant effect, if time had permitted. Nevertheless, the significant C_a times density on root weight ratio and leaf weight ratio, at PP and PC level, renders it impossible to determine the direction of the response of either parameter, to C_a or density as individual factors.

From these results, it can be concluded that in *C. odorata* populations comprising a small number of plants, growing under CO_2 enrichment, a greater allocation of community biomass to leaves would result. But as the number of plants in the community increases, dry matter partitioning of the community would change in favour of the production of roots. This suggests that the "withering away" of biomass responses to the treatments discussed earlier, could have been due to a limitation of nutrients and water, rather than resources such as light and above-ground space.

CHAPTER 7. CONCLUDING REMARKS

7.1 Conclusion: PART A and PART B

Invasive aliens are major agents of land transformation, disruptors of ecosystem functioning, and a threat to biodiversity (Richardson et al., 1997). Future CO_2-enriched atmospheres (Wallace et al., 1996; King, 2005), are likely to enhance the success of invasive aliens (Patterson, 1995; Dukes and Mooney, 1999; Weltzin et al., 2003). Therefore, the emphasis is on *current* studies, which in most cases, are conducted under artificial controlled conditions, in order to predict, and curb rates of infestations of invasive plants in the future. *C. odorata* is an invasive alien posing a serious threat to biodiversity in Africa (Ye et al., 2004), and more specifically, South Africa (Zachariades and Goodall, 2000; Howison and Balfour, 2002).

The current study, undertaken on the basis of providing insight into *C. odorata* responses to *interactive* effects of C_a and other factors, was divided into two experimental trials, namely PART A and PART B. Thus far, the two trials have been discussed separately to avoid confusion. However, several similarities between the two experiments, in addition to being part of the overall study, demands concluding comments be brought together in a single chapter.

During PART A, *C. odorata* was grown in competition with two C_4 grasses, and exposed to two levels of C_a and nutrients. It should be noted that although linked, there were two major types of competition that had come into play during this trial: competition for nutrients, and competition between C_3 and C_4 species for CO_2.

Numerous studies on increasing C_a effects on C_3-C_4 communities have given rise to the idea that CO_2-enriched atmospheres would favour C_3 plants over C_4 species (Dippery et al., 1995; Gavazzi et al., 2000), even more so, if C_3 plants are invasive species (Dukes and Mooney, 1999; Weltzin et al., 2003). Assessment of photosynthetic parameters of *C. odorata* in PART A, showed no *significant* effect of CO_2 enrichment on any of the parameters, although a marginal increase in J_{max} was noted. Results from analysis of indirect g_c data obtained from A:C_i data, showed an enhancement of g_c values at C_a=370 ppm and C_a=720 ppm, in elevated C_a. Not

surprisingly, elevated C_a reduced stomatal limitations during this trial. As total leaf area was the only structural parameter to be significantly influenced by C_a, it was concluded that generally, growth of *C. odorata* during PART A, was not enhanced by elevated C_a. Both grass species also showed no growth stimulation under CO_2 enrichment.

When grown individually or in monoculture, several studies have demonstrated a positive response of invasive species to elevated C_a (Dukes and Mooney, 1999; Dukes, 2000; Ziska, 2001). However, responses of invasive species in mixtures, to CO_2 enrichment, has been shown to be influenced by competition ((Bazzaz et al., 1989; Dukes, 2002; Ziska, 2002). Unfortunately, no prior studies have been conducted in which *C. odorata*, *T. triandra* and *E. curvula* were grown in monoculture in elevated C_a. This is indeed a pity, since data from polyculture growth of *C. odorata* and both grasses (PART A), cannot be compared with data from monoculture CO_2-enrichment studies on these three species, to help understand the role that competition between C_3 and C_4 species, could have had on the poor response of *C. odorata* to elevated C_a, in PART A.

In addition, the lack of photosynthetic assessments of grasses, and the small number of structural parameters studied (seven for *C. odorata*; two for the grasses), suggests that results of PART A of this study, are inadequate in aiding recent research on predicting the success of C_3 species (especially invasive aliens) in mixed communities, growing under CO_2-enrichment (Smith et al., 2000; Dukes, 2002; Naidoo, personal communication).

The second type of co-occurring competition during PART A (competition for nutrients), proved to more dominant, as growth of *C. odorata* and the grasses, and to some extent, physiology of *C. odorata*, was enhanced by high nutrient supply. Mineral stress, according to Lynch and St. Clair (2004), is a primary constraint of plant growth over the majority of the earth's land surface. In nutrient-poor systems, nutrient fertilization would spur the success of faster-growing species (Dukes, 2000). Therefore, the strong response of *C. odorata* to high nutrient supply is not really surprising.

In this trial, it is possible that any C_a effect could have been reduced, or even completely overshadowed, by the extremely strong nutrient effect, a concept which has been demonstrated by numerous studies (Conroy et al., 1986; Reddy and Zhao, 2005; Midgley, 1996).

According to Weltzin et al. (2003), increasing C_a may lead to an increase in other resources, e.g. nutrients, through changes in rates of nutrient cycling or by thawing frozen soil. Up until the previous decade, no plant invaders likely to benefit from changes in nutrient availability associated with rising C_a, had been identified (Dukes, 2000). PART A of this study, identifies *C. odorata* as an invasive alien likely to respond positively to high nutrient availability, which may be induced by future CO_2-enriched atmospheres.

Sage and Coleman (2001) put forward an interesting concept: low C_a concentrations in previous years may have acted as an evolutionary agent in selecting plants that are adapted to CO_2 deficiency, and this might constrain the responses of these plants to rising C_a. Research on *C. odorata* thus far (including PART A of this study), suggests that *C. odorata* may be a species that has adapted to previous low C_a concentrations, and this could explain the observed low responsiveness of this species to elevated C_a (Naidoo, personal communication; Lalla; unpublished). However, from PART B of this study, it is clear that the above explanation does not apply to *C. odorata*, as the species did, to some extent, respond to elevated C_a, during this trial.

During PART B, *C. odorata* was grown in monoculture, and exposed to different C_a concentrations, and different planting densities. Although *direct* photosynthetic assimilation data in elevated C_a were not analysed, results from *indirect* data (no photosynthetic down-regulation, and no increase in stomatal limitions in elevated C_a, suggest that photosynthetic assimilation rates of *C. odorata* could have been enhanced under CO_2 enrichment. On a per plant basis, elevated C_a significantly increased leaf thickness, dry weight of leaves, and total plant biomass of *C. odorata*. Plant height, stem dry weight and stem cross sectional areas of individual *C. odorata* plants, were also increased in elevated C_a, but these were non-significant effects. Results from this trial suggests that the idea of low-responsiveness of *C. odorata* to elevated C_a, generated by previous studies on this species (Naidoo, personal communication;

Lalla, unpublished; Patton, personal communication), should not be taken as the norm, as growth conditions clearly play a vital role in the response of *C. odorata* to elevated C_a.

From this experimental trial (PART B), it can be predicted that CO_2 enriched atmospheres, as have predicted by global climate change studies (King, 2005)**,** could enhance growth of individual *C. odorata* plants to some extent, irrespective of the number of co-occurring plants in monoculture populations. Results from growth analysis at a community level, showed that in CO_2-enriched environments with monoculture *C. odorata* communities comprising a small number of plants, community biomass allocation would be directed towards leaves (above-ground growth). However, as the number of *C. odorata* plants in the community increases, community biomass would change in favour of roots (below-ground growth), most probably as a response to a depletion of soil nutrients. This observation, supports previous research (Körner, 2003; Sicher, 2005), in highlighting the importance of avoiding nutrient stress during CO_2 enrichment studies.

Taken as a whole, this study (PART A and PART B), together with previous studies on this species (Naidoo, personal communication; Lalla, unpublished), suggests that CO_2 enriched environments may enhance the success of monoculture populations of *C. odorata*, to some extent. However, when occurring in mixtures comprising other plants (e.g. C_4 grasses, C_3 species), *C. odorata* may fall short in competitive strategy, as other species gain in competitive advantage over *C. odorata*, in CO_2 enriched atmospheres. This study also supports previous research, which has shown a "depressing" effect of invasive plants in mixtures, compared to monoculture populations (Bazzaz et al., 1989; Dukes, 2002; Ziska, 2002). The extremely strong response of ambient- *and* elevated C_a-grown *C. odorata* plants to high nutrient levels during PART A, provides no conclusive evidence to support the ideas that elevated C_a reduces carbon as a limiting external resource (Lynch and St. Clair, 2004), and that the extent of plant responses to CO_2 enrichment will depend of the availability of resources other than CO_2 (Zanetti et al., 1997). However, if extreme temperatures caused by increasing C_a increases resource (nutrient) availability in the soil by altering rates of nutrient cycling (Weltzin et al., 2003), then it is likely that elevated C_a would *indirectly* enhance the success of *C. odorata* occurring in mixed populations.

7.2 Experimental technique

After conducting a study of this nature, it is only natural to wonder about the experimental approach used in the research (OTC study, instead of FACE study), and whether it would be beneficial to continue with this experimental approach in future research on *C. odorata* responses to CO_2 enrichment. When reviewing the literature on plant responses to elevated C_a (Section 1.3 and 1.4; Chapter 1), distinguishing between OTC and FACE studies would have been tedious, and was thus avoided. Instead, a short review of the two techniques was provided in Section 1.6 (Chapter 1).

Despite some parallel trends observed between FACE and OTC studies, there are important differences which cannot be ignored (Long et al., 2004). For example, trees have been shown to be more responsive than herbaceous species, to FACE studies (Ainsworth and Long, 2005). Although this provides a good reason for the choice of experimental approach in the current study, OTCs were selected mainly due to the high cost associated with FACE studies. Long et al. (2006) assessed models of CO_2 responses on crop yield, and concluded that FACE studies projected approximately 50% less increase in yield, when compared to enclosure studies. Ths reiterates the point that plant responses to elevated C_a are not independent of growth conditions. Differences in species studied, and duration of CO_2 enrichment have been documented as factors that have resulted in inconsistent and often conflicting reports, concerning plant responses to elevated C_a (Mott, 1990). Therefore it would be beneficial to compare results of *similar* studies (e.g. similar experimental approach, similar species, similar light conditions etc.

The foundation of the current study was placed a few years ago, when Naidoo (personal communication) generated first results on the CO_2 response of *C. odorata*. This topic was further investigated in two consecutive studies (Lalla, unpublished and Patton, personal communication), and the current study is the fourth of its kind. Since all four studies on *C. odorata* responses to CO_2 enrichment, were OTC studies, and were conducted under similar conditions (e.g. same greenhouse with similar experimental setup), it seems logical to continue use of OTCs in future research. At some stage in the future, once data from a number of studies on *C. odorata* responses to elevated C_a have accumulated, a meta-analysis of data sets can be performed,

which has already shown to provide invaluable insight into plant responses to elevated C_a (Ainsworth et al., 2002; Ainsworth et al., 2005). Meta-analyses demands effective communication amongst researchers in a particular field, and this more "coherent" approach to understanding plant responses to CO_2 enrichment, is no doubt more valuable than isolated research.

Ultimately, data from accumulative studies on *C. odorata* in elevated C_a, could be compared with CO_2-enrichment studies on other invasive aliens which are seriously threatening natural vegetation in South Africa, to develop management strategies which will aid existing control measures for these invasive species in the future. In particular, the apparent lack of photosynthetic down-regulation, but no enhancement of growth, needs to be investigated further.

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