

Effects of elevated CO₂ on the reproductive performance and early life fitness of long-lived woody plant species

By

Corey O'Brien (BSc)

A thesis submitted in fulfilment for the degree of Master of Research

Hawkesbury Institute for the Environment
Western Sydney University
December 2019

Acknowledgements

I would like to thank my wife and family for all of their support while undertaking my studies over the past 5 years and my Master of Research supervisors Dr Paul Rymer and Distinguished Professor David Tissue, for their guidance in undertaking this project; particularly Paul who managed to talk me through any doubts I had and helped guide me through the project. Critical feedback from members of the Rymer lab group has been greatly appreciated throughout the project; in no particular order, Katherine Rolls, Colin Ahrens, John Whale, Osazee Oyanoghafo, Victoria Perez Martinez, Manuel Esperon-Rodriguez, Desi Quintans, Vaibhav Dagg, and Anthea Challis. This project could not have happened without all of the extra help in conducting experiments, so I'd also like to thank all of the following for their respective contributions to the studies within this thesis:

Collecting Plant Material: Paul Rymer, Amy-Marie Gilpin, Katherine Rolls

Processing Plant Material: Vaibhav Dagg, Emily O'Brien

Assistance with Experiments: Katherine Rolls, John Whale, Colin Ahrens

EucFACE Technical Staff: Vinod Kumar, Craig McNamara

HIE Technical Staff: Andrew Gherlanda (Growth Chambers), Pushpinder Matta (Chemical Analysis), Serina Majewski (Materials), Chelsea Maier (Calorimetry), Renee Smith (Li-Cor)

Statement of Authentication

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

..... 

Corey O'Brien

22 December 2019

Table of Contents

LIST OF TABLES	2
LIST OF FIGURES	3
ABBREVIATIONS	5
ABSTRACT	6
1 INTRODUCTION	8
1.1 CO ₂ PAST, PRESENT AND FUTURE.....	8
1.2 LAND PLANTS AND CO ₂	9
1.3 PLANT CO ₂ EXPERIMENTS	10
1.4 OVERVIEW OF THESIS.....	13
2 EFFECTS OF ELEVATED CO₂ ON THE REPRODUCTIVE PERFORMANCE OF <i>EUCALYPTUS TERETICORNIS</i> AND <i>HAKEA SERICEA</i>	15
2.1 INTRODUCTION.....	15
2.2 METHODS	17
2.3 RESULTS	21
2.4 DISCUSSION.....	26
3 DIRECT AND MATERNAL EFFECTS OF CO₂ ON THE EARLY LIFE FITNESS OF <i>HAKEA SERICEA</i>	30
3.1 INTRODUCTION.....	30
3.2 METHODS	32
3.3 RESULTS	38
3.4 DISCUSSION	46
4 DISCUSSION	51
4.1 THESIS SUMMARY	51
4.2 REPRODUCTIVE EFFORT	51
4.3 GERMINATION	52
4.4 EARLY-LIFE FITNESS.....	54
4.5 ACCLIMATION OF PHOTOSYNTHETIC CAPACITY	55
4.6 LIMITATIONS OF STUDY	56
4.7 CONCLUSION AND FUTURE WORK	56
5 REFERENCES	58
6 APPENDIX	64

List of Tables

TABLE 1. SUMMARY OF SAMPLE MEANS +/- STANDARD ERROR AND ONE-WAY ANOVA FOR REPRODUCTIVE EFFORT, REPRODUCTIVE OUTPUT AND SEED GERMINATION TRAITS OF EUCALYPTUS TERETICORNIS AND HAKEA SERICEA GROWN IN AMBIENT AND ELEVATED CO₂ FACE SITES. SIGNIFICANCE CODES 'NS' = NOT SIGNIFICANT; '.' = < 0.1; '*' = < 0.05; '**' = < 0.01; '***' = < 0.001. NOTE: '-' SIGNIFIES NO ANALYSIS WAS CONDUCTED25

TABLE 2. SUMMARY OF TWO-WAY ANOVA (MATERNAL CO₂ CONCENTRATION X GROWING CO₂ CONCENTRATION) FOR EMERGENCE, GROWTH, FUNCTIONAL TRAITS, AND GAS EXCHANGE MEASUREMENTS OF H. SERICEA. SIGNIFICANCE CODES; . = < 0.1; * = < 0.05; ** = < 0.01; *** = < 0.001; NS = NOT SIGNIFICANT; - = NOT TESTED.44

TABLE 3. SUMMARY OF EMERGENCE, GROWTH, FUNCTIONAL TRAITS AND GAS EXCHANGE MEASUREMENTS OF H. SERICEA FOR WHICH SEED WERE SOURCED FROM MATERNAL AMBIENT AND ELEVATED CO₂ ENVIRONMENTS AND SUBSEQUENTLY GROWN IN PRE-INDUSTRIAL (280PPM), AMBIENT (410PPM), AND ELEVATED (650PPM) GROWING CO₂ ENVIRONMENTS.45

List of Figures

FIGURE 1. ATMOSPHERIC CO₂ CONCENTRATION FOR THE PAST 800,000 YEARS AS DETERMINED FROM ICE CORE DATA. MEASUREMENTS AFTER 1958 ARE DERIVED FROM DIRECT MEASUREMENTS AT MAUNA LOA, HAWAII. ACCESSED 6 DECEMBER 2019. FIGURE SOURCED FROM [HTTPS://SCRIPPS.UCSD.EDU/PROGRAMS/KEELINGCURVE/WP-CONTENT/PLUGINS/SIO-BLUEMOON/GRAPHS/CO₂_800K.PDF](https://scripps.ucsd.edu/programs/keelingcurve/wp-content/plugins/sio-bluemoon/graphs/co2_800k.pdf).....9

FIGURE 2. CONCEPTUAL OVERVIEW OF PREDICTED OUTCOMES FROM THIS STUDY GIVEN THE FINDINGS FROM PREVIOUS STUDIES 14

FIGURE 3. PERCENTAGE CHANGE WITH 95% CONFIDENCE INTERVAL OF REPRODUCTIVE EFFORT TRAITS MEASURED FROM *E. TERETICORNIS* BRANCHES HARVESTED FROM ELEVATED CO₂ FACE SITES IN RELATION TO BRANCHES HARVESTED FROM AMBIENT CO₂ FACE SITES. SIGNIFICANCE CODES ‘.’ = < 0.1; ‘*’ = < 0.05; ‘**’ = < 0.01; ‘***’ = < 0.001.22

FIGURE 4. REPRODUCTIVE ALLOCATION +/- STANDARD ERROR FOR BIOMASS OF FLORAL BUDS OF *EUCALYPTUS TERETICORNIS* GROWN IN FACE SITES FOR CROPS IN 2018 AND 2019. COLUMNS WITH THE SAME LETTER ABOVE THEM DO NOT SIGNIFICANTLY DIFFER FROM ONE ANOTHER.22

FIGURE 5. PERCENTAGE CHANGE WITH 95% CONFIDENCE INTERVAL OF REPRODUCTIVE OUTPUT TRAITS MEASURED FROM *E. TERETICORNIS* BRANCHES AND ABOVEGROUND BIOMASS OF *H. SERICEA* HARVESTED FROM ELEVATED CO₂ FACE SITES IN RELATION TO BRANCHES/ABOVEGROUND BIOMASS HARVESTED FROM AMBIENT CO₂ FACE SITES. SIGNIFICANCE CODES ‘.’ = < 0.1; ‘*’ = < 0.05; ‘**’ = < 0.01; ‘***’ = < 0.001. .24

FIGURE 6. CUMULATIVE GERMINATION OF *EUCALYPTUS TERETICORNIS* AND *HAKEA SERICEA*. SOLID LINES INDICATE SEED SOURCED FROM MATERNAL PLANTS GROWN IN AMBIENT FACE SITES, AND DASHED LINES INDICATE SEED SOURCED FROM MATERNAL PLANTS GROWN IN ELEVATED FACE SITES. VERTICAL LINES SIGNIFY T₅₀ FOR CORRESPONDING CURVE. G_{MAX} DERIVED FROM FINAL % GERMINATION. INSET TABLE WITH SUMMARY OF TWO WAY ANOVA OF T₅₀ AND G_{MAX} WITH CO₂ TREATMENT AND SPECIES AS FIXED EFFECTS.24

FIGURE 7. CONCEPTUAL DIAGRAM FOR EXPERIMENTAL DESIGN. SEEDS SOURCED FROM MATERNAL PLANTS GROWN IN FACE SITES WITH AMBIENT (410PPM) AND ELEVATED (560PPM) CO₂ CONCENTRATIONS AND SUBSEQUENTLY SOWN IN CLIMATE-CONTROLLED GROWTH CHAMBERS IMITATING PRE-INDUSTRIAL (280PPM), AMBIENT (410PPM), AND ELEVATED (650PPM) CO₂ CONCENTRATIONS. DIAMOND SHAPES INDICATE MATERNAL CO₂ TREATMENTS AND CIRCLES INDICATE GROWING CO₂ TREATMENTS.....33

FIGURE 8. EXPERIMENTAL DESIGN TO COMPARE THE LONG- AND SHORT-TERM EFFECTS OF CO₂ CONCENTRATION ON THE PHOTOSYNTHETIC CAPACITY OF *H. SERICEA*. SOLID LINES INDICATE HOW LONG-TERM RESPONSE WAS MEASURED WITH SEEDS SOURCED FROM THE FACE SITES AT AMBIENT (410 PPM) AND ELEVATED (560 PPM) MATERNAL CO₂ ENVIRONMENTS AND GROWN AT PRE-INDUSTRIAL (280 PPM), AMBIENT (410 PPM) AND ELEVATED (650 PPM); DASHED LINES INDICATE SHORT-TERM RESPONSE TO CO₂ BY MEASURING A_{SAT} AT DIFFERENT CO₂ CONCENTRATIONS FOR PLANTS GROWN UNDER THE SAME CONDITIONS. DIAMOND SHAPES INDICATE MATERNAL CO₂ ENVIRONMENT, CIRCLES INDICATE THE CO₂ TREATMENTS, AND TRIANGLES INDICATE THE CO₂ CONCENTRATION OF CUVETTE FOR GAS EXCHANGE MEASUREMENTS.36

FIGURE 9. CUMULATIVE EMERGENCE OF *HAKEA SERICEA* SEEDS EXPOSED TO PRE-INDUSTRIAL, AMBIENT AND ELEVATED CO₂ CONCENTRATIONS AND SOURCED FROM MATERNAL PLANTS GROWN IN (A) AMBIENT AND (B) ELEVATED ATMOSPHERIC CO₂ CONCENTRATIONS. VERTICAL DASHED LINES INDICATE TIME TO REACH 50% OF TOTAL SEEDS TO SUCCESSFULLY EMERGE (T₅₀).38

FIGURE 10. MEASUREMENTS OF (A) BASAL DIAMETER AND (B) HEIGHT OF *H. SERICEA* THROUGHOUT THE EXPERIMENTAL PERIOD FOR WHICH SEEDS WERE SOURCED FROM MATERNAL PLANTS SUBJECT TO AMBIENT AND ELEVATED CO₂ IN FACE AND SUBSEQUENTLY GROWN IN GROWTH CHAMBERS IN PRE-INDUSTRIAL, AMBIENT AND ELEVATED CO₂ ENVIRONMENTS.39

FIGURE 11. PLANT GROWTH MEASUREMENTS AT THE TIME OF HARVEST OF *H. SERICEA* FOR WHICH SEEDS WERE SOURCED FROM MATERNAL PLANTS EXPOSED TO AMBIENT (410PPM) AND ELEVATED (560PPM) ATMOSPHERIC CO₂ IN FACE AND SUBSEQUENTLY PROPAGATED AND GROWN IN GROWTH CHAMBERS IN PRE-INDUSTRIAL (280 PPM), AMBIENT (410 PPM) AND ELEVATED (650 PPM) CO₂ CONCENTRATIONS FOR 5 MONTHS. MEASUREMENTS FOR (A) HEIGHT, (B) EFFECTIVE BASAL DIAMETER, AND (C) DRY BIOMASS ARE ALL STANDARDISED FOR AGE AT TIME OF HARVEST. BARS WITH THE SAME LETTERS ABOVE THEM DO NOT SIGNIFICANTLY DIFFER FROM ONE ANOTHER.40

FIGURE 12. FUNCTIONAL TRAITS OF *H. SERICEA* FOR WHICH SEEDS WERE SOURCED FROM MATERNAL PLANTS GROWN IN AMBIENT AND ELEVATED CO₂ ENVIRONMENTS IN FACE SITES AND SUBSEQUENTLY GROWN IN GROWTH CHAMBERS IN PRE-INDUSTRIAL (280 PPM), AMBIENT (410 PPM) AND ELEVATED (650 PPM) CO₂ ENVIRONMENTS. (A) LEAF FRACTION, (B) SPECIFIC LEAF AREA, AND (C) NITROGEN CONTENT ARE AS MEASURED AT TIME OF HARVEST 5 MONTHS AFTER PROPAGATING. BARS WITH THE SAME LETTERS ABOVE THEM DO NOT SIGNIFICANTLY DIFFER FROM ONE ANOTHER.....41

FIGURE 13. GAS EXCHANGE MEASUREMENTS OF *H. SERICEA* FOR WHICH SEEDS WERE SOURCED FROM MATERNAL PLANTS GROWN IN AMBIENT AND ELEVATED CO₂ ENVIRONMENTS AND SUBSEQUENTLY PROPAGATED AND GROWN IN PRE-INDUSTRIAL, AMBIENT, AND ELEVATED CO₂ ENVIRONMENTS. MEASUREMENTS OF (A) PHOTOSYNTHESIS IN SATURATING LIGHT AND CO₂ CONDITIONS (A_{MAX}), (B) PHOTOSYNTHESIS IN SATURATING LIGHT CONDITIONS (A_{SAT}), (C) STOMATAL CONDUCTANCE AT SATURATING LIGHT CONDITIONS (G_s) DETERMINED FROM SPOT MEASUREMENTS. MEASUREMENTS FOR (D) APPARENT MAXIMAL ELECTRON TRANSPORT RATE (J_{MAX}), (E) APPARENT MAXIMAL CARBOXYLATION RATE (V_{CMAX}), AND (F) THE RATIO OF THESE PARAMETERS WERE DETERMINED FROM FITTING MODELS TO A/Ci CURVES. BARS WITH THE SAME LETTERS ABOVE THEM DO NOT SIGNIFICANTLY DIFFER FROM ONE ANOTHER.43

FIGURE 14. LONG- AND SHORT-TERM RESPONSE TO CO₂ FOR *H. SERICEA*. SOLID LINES INDICATE LONG-TERM RESPONSE; PLANTS FOR WHICH SEED WERE SOURCED FROM AMBIENT AND ELEVATED CO₂ MATERNAL ENVIRONMENTS AND GROWN IN PRE-INDUSTRIAL (280PPM), AMBIENT (410PPM), AND ELEVATED (650PPM) CO₂ ENVIRONMENTS. DASHED LINES INDICATE SHORT-TERM RESPONSE; PLANTS FOR WHICH SEED WERE SOURCED FROM AMBIENT AND ELEVATED MATERNAL CO₂ ENVIRONMENTS AND GROWN IN AMBIENT (410PPM) CO₂ CONDITIONS. PHOTOSYNTHESIS AT SATURATING LIGHT (A_{SAT}) MEASURED AT CO₂ CONCENTRATIONS CORRESPONDING WITH THE X-AXIS.44

Abbreviations

A/C_i	Relationship between photosynthesis and intercellular CO_2 concentration
AIC	Akaike's Information Criterion
A_{max}	Net photosynthesis at saturating light and carbon dioxide
ANOVA	Analysis of Variance
A_{sat}	Net photosynthesis at saturating light
C	Carbon
C_i	Intercellular concentration of carbon dioxide
cm	Centimetres
CO_2	Carbon dioxide
FACE	Free Air Carbon-dioxide Enrichment
g	Gram
GLMM	Generalised Linear Mixed Model
G_{max}	Maximum number of germinants
g_s	Stomatal conductance
H_C	Heat of Combustion
J	Joules
J_{max}	Maximum rate of electron transport
L	Litre
LMM	Linear Mixed Model
m	Metre
mg	Milligram
mL	Millilitre
mm	Millimetre
N	Nitrogen
NPP	Net Primary Productivity
ppm	Parts per million
psi	Pounds per Square Inch
Rubisco	Ribulose-1,5-bisphosphate carboxylase/oxygenase
s	Second
t	Tonne
T_{50}	Time to reach 50% emergence/germination
V_{Cmax}	Maximum rate of carboxylation
μmol	Micromole

Abstract

Atmospheric concentrations of CO₂ have risen considerably since the industrial revolution due to human activity. Without extreme intervention, climate forecasts indicate that atmospheric CO₂ concentrations will continue to rise. Plant growth and productivity in response to elevated CO₂ has been studied extensively, however, few studies explore how reproduction will be affected under future CO₂ concentration; particularly for long-lived woody plant species. This study is undertaken within the EucFACE facility in Western Sydney (Australia), with naturally growing populations, on nutrient limited soils, which form part of the remnant Cumberland Plains Woodland. The effects of elevated CO₂ on vegetative growth in this ecosystem have previously been demonstrated to be absent due to phosphorous limitation despite maintaining higher photosynthetic rates. Little is known how reproductive performance of long-lived woody plants species will respond to increased CO₂ concentrations in this environment. The main objective of this thesis is to explore direct effects of elevated CO₂ on the reproductive performance of a dominant tree, *Eucalyptus tereticornis*, and a perennial shrub, *Hakea sericea*, in addition to the subsequent maternal effects on early-life fitness of *H. sericea*. It is the first study to assess the reproductive performance of mature dominant evergreen angiosperm (*E. tereticornis*) in response to rising CO₂ concentration. Several experiments were undertaken to determine how reproductive performance and subsequent early-life fitness is affected by elevated CO₂; (i) The impacts on reproductive effort and output through quantifying the proportion of biomass invested into floral buds, fruit and seeds for *E. tereticornis* and *H. sericea*. (ii) Seed quality was assessed through conducting germination experiments for *E. tereticornis* and *H. sericea*. (iii) Seed sourced from maternal *H. sericea* plants grown in ambient and elevated CO₂ conditions were propagated into growth chambers simulating pre-industrial, ambient, and future CO₂ concentrations to assess early-life fitness through measurements of biomass, photosynthesis, and functional traits. Key findings from these experiments were; (i) Reproductive effort for *E. tereticornis* is increased for plants growing in elevated CO₂, however, there is great inter-annual variability which will require further exploration, (ii) Germination success is differentially affected by elevated CO₂, with *H. sericea* (large seeded species) seeing greater benefits than *E. tereticornis* (small seeded species), (iii) Despite having lower biomass, seedlings with maternal plants grown in ambient CO₂ conditions had greater photosynthetic capacity than seedlings with maternal plants grown in elevated CO₂, and (iv) Photosynthesis was down-regulated in response to long-term exposure of elevated CO₂ only for seedlings

with maternal plants grown in elevated CO₂ indicating acclimation may occur across generations but not necessarily within a generation. Differential responses between species may have profound impacts on vegetation community composition, thereby altering ecosystem structure and function.

1 Introduction

1.1 CO₂ Past, Present and Future

Atmospheric concentration of Carbon-dioxide (CO₂) has been demonstrated to be increasing in recent history, with the longest record of direct measurements commencing in 1958 from Mauna Loa Observatory, Hawaii (Keeling et al. 1976). In 2016, for the first time since direct measurements began, atmospheric CO₂ concentration remained consistently above 400 ppm and latest measurements indicate an annual average of 411 ppm for 2019; a stark contrast to glacial (~150 ppm), pre-industrial (~ 280 ppm) and the 1959 annual average of 316 ppm (NOAA 2019). Assessment of atmospheric CO₂ concentrations earlier than records derived from direct measurements is made possible through the analysis of air bubbles trapped within ice (Friedli et al. 1986). Air bubbles form in ice during the transformation of snow into ice (firnification), preserving a sample of the atmosphere in an air-tight ice matrix (Raynaud & Barnola 1985). Information provided from ice core records are currently limited to the previous 800,000 years (Figure 1), however, there are plans to extend this record to over 1 million years in the Antarctic ice sheet (Schiermeier 2019). To date, ice core records have shown that atmospheric CO₂ concentration prior to the industrial revolution fluctuated between approximately 180 ppm and 270 ppm throughout 8 glacial cycles (Figure 1). With the onset of the industrial revolution in the mid-18th century, burning of fossil fuels has resulted in unmatched atmospheric CO₂ concentration and rate of increase in at least the past 800,000 years (IPCC 2014). Atmospheric composition is estimated for time periods earlier than what is provided from ice core records from geochemical models such as GEOCARB and GEOCARBSULF which indicate highly variable CO₂ concentrations within the last 500 million years, with concentrations exceeding 20 times the mean concentration of the past 1 million years (Bernier 2006). Such models indicate that current atmospheric CO₂ concentration has not been matched for approximately 3 million years (Martínez-Botí et al. 2015). Looking to the future, without extreme intervention, atmospheric CO₂ concentration is expected to continue increasing (IPCC 2014). A series of Representative Concentration Pathways (RCPs) based on emission, land-use, and concentration trajectories outline future scenarios up to the year 2100 estimating atmospheric CO₂ concentration to range from 410 ppm (mitigation scenario) to over 900 ppm (high baseline scenario; van Vuuren et al. 2011).

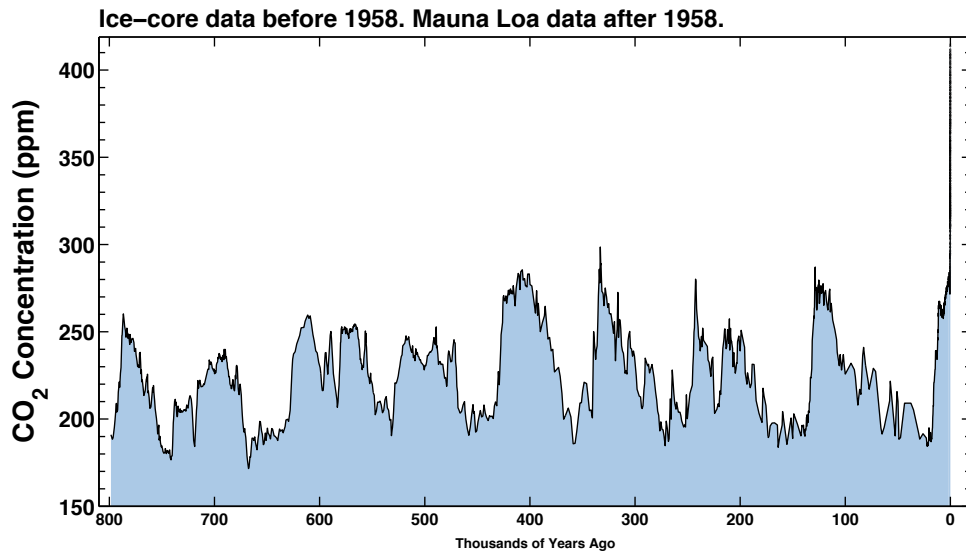


Figure 1. Atmospheric CO₂ concentration for the past 800,000 years as determined from ice core data. Measurements after 1958 are derived from direct measurements at Mauna Loa, Hawaii. Accessed 6 December 2019. Figure sourced from [https://scripps.ucsd.edu/programs/keelingcurve/wp-content/plugins/sio-blumoon/graphs/CO₂_800k.pdf](https://scripps.ucsd.edu/programs/keelingcurve/wp-content/plugins/sio-blumoon/graphs/CO2_800k.pdf)

1.2 Land Plants and CO₂

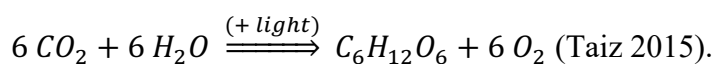
Photosynthesis is the process of synthesising organic carbon compounds from inorganic substrates and solar energy and is thought to have first evolved 3.2 - 3.5 billion years ago, shortly after the origin of life on Earth (Blankenship 2010). It is estimated oxygenic photosynthesis evolved from this earliest form 2.4 billion years ago, producing oxygen as a waste product, which forever changed the atmosphere of the Earth (Blankenship 1992). Photosynthetic ability in multicellular life forms (Eukarya) is derived from an endosymbiotic event with photosynthetic cyanobacteria approximately 1.5 billion years ago (McFadden 2001; Yoon et al. 2004). Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is the key enzyme involved in carbon fixation for phototrophic organisms and is the most abundant protein on Earth (Andersson & Backlund 2008). Rubisco performs the first step of photosynthetic carbon fixation, known as the Calvin-Benson cycle, facilitating the carboxylation of an acceptor molecule, Ribulose-1,5-bisphosphate (RuBP) with CO₂ and H₂O to generate two molecules of 3-phosphoglycerate used in the remaining reactions of the Calvin-Benson cycle (Taiz 2015).

Fossil evidence estimates the evolution of land plants occurred approximately 480 million years ago (Kenrick & Crane 1997) from simple liverwort-like plants which have since diversified to form the major clades of plants today; liverworts, hornworts, mosses, and tracheophytes (vascular plants) (Fiz-Palacios et al. 2011). Vascular plants are comprised of

ferns, lycophytes and seed plants, the last of which is further divided into gymnosperms and angiosperms (Fiz-Palacios et al. 2011). Interactions and feedbacks between plants and the abiotic environment have had great impact on climatic factors such as temperature and rainfall over geological timescales, which in turn create selective pressures driving the evolution and diversification of plant life (Boyce & Lee 2017). The great success of early land plants in the late Devonian period (~380 million years ago) resulted in a large scale decreasing of atmospheric CO₂ over 40 million years which is thought to have acted as one of the greatest selective pressures driving the diversification of plants (Beerling & Berner 2005; Franks & Beerling 2009). As atmospheric CO₂ concentration declines, the efficiency of Rubisco is decreased due to low specificity for CO₂, which can alternatively catalyse O₂ with RuBP driving the process known as photorespiration and resulting in a loss of carbon from the plant (Taiz 2015). In more recent plant evolutionary history, CO₂ concentrating photosynthetic pathways such as Crassulacean Acid Metabolism (CAM; 20-30 million years ago) and C₄ (4-7 million years ago) have evolved in response to low atmospheric CO₂ concentrations in tandem with high light and temperatures inhibiting the common photosynthetic pathway (C₃) and increasing photorespiration (Keeley & Rundel 2003; Taiz 2015). These pressures are thought to be highly selective as is evident from the independent evolution at least 30 times each for C₄ and CAM photosynthetic pathways (Keeley & Rundel 2003).

1.3 Plant CO₂ Experiments

Elements of the photosynthesis reaction were discovered throughout the 18th century, with experiments of Charles Bonnet demonstrating that illuminated leaves under water produce bubbles (1754); Joseph Priestly discovering that plants can ‘purify’ air to prolong the life of a mouse and burning candle in an enclosed space (1772); Jan Ingen-Housz demonstrating that the experiments by Priestly were dependant on sunlight touching green tissues of a plant (1779); Jean Senebier discovering that CO₂ was essential for photosynthesis and oxygen was released (1782); and finally Nicolas Theodore de Saussure identifying the incorporation of water as part of photosynthesis (1804; Krogmann 2005). These experiments together form the basic understanding of the photosynthesis reaction, when balanced;



Contemporary plants have evolved under considerably lower atmospheric CO₂ concentrations for at least the past 800,000 years as determined from the ice core records (Lüthi et al. 2008), but likely the past 3 million years (Martínez-Botí et al. 2015). It was calculated by Svante Arrhenius in 1896 that anthropogenic CO₂ emissions could cause global warming (Anderson, Hawkins & Jones 2016), however, since direct evidence of rapidly increasing CO₂ concentration has been demonstrated (Keeling et al. 1976), there has been extensive research on the effects elevated concentrations of CO₂ will have on plants (Parmesan & Hanley 2015; Ward & Strain 1999). The earliest studies were undertaken in the context of agriculture and food production (Pritchard et al. 1999), with a more recent focus shifting toward broader ecological impacts and climate regulation (Parmesan & Hanley 2015). Plant responses to elevated CO₂ have been studied since late in the 19th century (Kreusler 1885; Parmesan & Hanley 2015), however, the earliest of these studies produced highly variable results due to harmful impurities in the CO₂ supply from different sources, and difficulties in maintaining CO₂ concentrations, humidity, light, and temperature conditions (Bolas & Henderson 1928). Most studies consist of individual species grown in controlled environments, considerably altering the natural conditions that would normally be experienced by the plants which may result in chamber effects that may be greater than the effect of CO₂ (Ainsworth & Long 2005; Arp 1991); nevertheless, these studies have provided the foundations for our mechanistic understanding of plant-atmosphere interactions. Latest technologies have helped to overcome these limitations to better understand the long-term impacts of elevated CO₂ on plant growth and development. Free Air Carbon-Dioxide Enrichment (FACE) technology has made experimental CO₂ studies on long-lived plants and/or plants in natural environments feasible. Globally, FACE sites have been established within numerous ecosystems since its development such as grasslands, bogs, and forests (Ainsworth & Long 2005; Hendrey et al. 1999; Norby & Zak 2011).

In similar fashion, studies of plant responses to pre-industrial (~280 ppm) and glacial (~150 ppm) CO₂ concentrations have been undertaken to determine impacts on the relatively recent anthropogenic increase of atmospheric CO₂ and improve our understanding of how plants will respond to future change (Gerhart & Ward 2010). These studies have also had to overcome technological hurdles in order to maintain desired CO₂ concentrations with early studies as reviewed by Gerhart and Ward (2010). Such studies included surrounding C₃ plants with more efficient C₄ plants to draw down the CO₂ concentration in the vicinity of the subject plant (Sharma, Griffing & Scholl 1979); growing plants in an outdoor tunnel with air

pumped from one end where CO₂ concentration is relatively high and is drawn down by plants as it passes through and is decreased as it reaches the far end thereby creating a CO₂ gradient (Mayeux et al. 1993); and more recently, climate controlled growth chambers with the ability of scrubbing CO₂ from the atmosphere by passing air through soda lime scrub boxes (Ward et al. 2000).

Many studies have led to the broad conclusions that elevated CO₂ concentration results in increased photosynthesis (Galmes et al. 2013), increased biomass (Curtis & Wang 1998; Ghannoum et al. 2010a), reduced stomatal conductance (Ainsworth & Rogers 2007; Becklin et al. 2017; Drake, González-Meler & Long 1997), and reduced leaf nitrogen (Curtis & Wang 1998; Taub & Wang 2008) in C₃ plants but minimal or no effects on C₄ plants (Dippert et al. 1995; Tissue et al. 1995). Increased photosynthesis occurs at elevated CO₂ by increasing the efficiency of Rubisco by facilitating higher carboxylation rates due to less competition with oxygen (Galmes et al. 2013). When CO₂ concentration is high, stomatal conductance is adjusted to optimise the trade-off in water loss through transpiration and the intake of CO₂ into the substomatal cavity for photosynthesis, thereby making plants more water use efficient (Drake, González-Meler & Long 1997). After long-term exposure to elevated CO₂, downregulation of photosynthesis resulting in reduced Rubisco content, and therefore leaf nitrogen content, is observed as plants adjust to new conditions while maintaining growth thereby making plants more nitrogen use efficient (Drake, González-Meler & Long 1997; Taub & Wang 2008), however, it is widely debated if plants acclimate to elevated CO₂ concentrations after long-term exposure, or if apparent acclimation is due to limitations on sink capacity (Arp 1991). Inversely, long-term exposure to pre-industrial or glacial CO₂ concentrations results in upregulation of photosynthesis to maintain growth with higher Rubisco content in leaves, and therefore nitrogen content (Gerhart & Ward 2010). Where plants have been grown in conditions without limitation of nutrients or root growth, the effects of elevated CO₂ seem to persist after long-term exposure (Curtis & Wang 1998). In numerous studies, it has been demonstrated that when one or more nutrients are limiting, as is usually the case in natural ecosystems, the CO₂ fertilisation effect is absent or diminished (Ellsworth et al. 2017; Parmesan & Hanley 2015; Tissue & Lewis 2010).

The broader ecological impacts of elevated CO₂ either directly through impacts on growth and reproduction of plants, or indirectly through its contribution to climate change, has been the subject of many studies (Bazzaz 1990; Becklin et al. 2017; Parmesan & Hanley 2015; Parmesan & Yohe 2003; Pritchard et al. 1999; Ward & Strain 1999). Further to observing

greater photosynthetic capacity and growth in response to increased CO₂ concentration, biomass allocation has been demonstrated to be altered by increasing the ratio of root-shoot biomass, especially when nutrients and/or water is limited, thereby altering water and nutrient uptake (Bazzaz 1990; Rogers et al. 1992).

A less studied area is how reproductive performance is affected by increased CO₂ concentration, particularly for long-lived species (Parmesan & Hanley 2015). Highly variable results have been obtained on how increased CO₂ affects reproductive effort (LaDeau & Clark 2001; Reekie & Bazzaz 1987), allocation (Bazzaz, Ackerly & Reekie 2005; Wang, Taub & Jablonski 2015), and output (Way et al. 2010). Differential impacts of increased CO₂ concentration on the reproductive performance of different plant functional types or species may alter ecosystem structure and function over time (Garbutt, Williams & Bazzaz 1990). Much of the work in this area is conducted on crop or annual species with little known on the responses in natural systems, however, general impacts on reproductive performance in response to increased CO₂ concentration include increased flowering, fruiting, seed production, individual seed weight, and germination success (Jablonski, Wang & Curtis 2002; Marty & BassiriRad 2014). The few studies conducted on long-lived species indicate increased reproductive performance through increased fecundity and/or increased seed quality (Darbah et al. 2007; Hussain, Kubiske & Connor 2001; Ladeau & Clark 2006; Way et al. 2010). These findings are derived from only two species; the coniferous *Pinus taeda* and deciduous angiosperm *Betula papyrifera*, and therefore the study of a broader range of plant functional types is required before being able to infer findings to other ecosystems.

1.4 Overview of Thesis

This thesis explores how the reproductive allocation and initial fitness in response to increased atmospheric CO₂ concentration for two co-occurring evergreen, perennial, woody angiosperms with contrasting life-history strategies. An obligate seeding shrub (*Hakea sericea*) producing a small number of large seeds, and a tall, resprouting, tree (*Eucalyptus tereticornis*) producing numerous tiny seeds. The first data chapter is designed to test if the reproductive allocation is affected by atmospheric CO₂ concentration for the two species growing within FACE sites in a mature natural open woodland environment and initial fitness is tested via germination experiments for seeds sourced from ambient and elevated CO₂ conditions. The second data chapter explores effects of maternal and growth CO₂ environment on seedling emergence, plant growth and photosynthesis of *H. sericea*. Long

term (growth CO₂) and short-term (measurement CO₂) effects on photosynthesis were assessed under pre-industrial (280ppm), ambient (410ppm), and future (650ppm) CO₂ concentrations. The expected outcomes from both data chapters are outlined in Figure 2. Key findings are synthesized and discussed in relation to the ability for plants to respond to climate change with suggestions for future research.

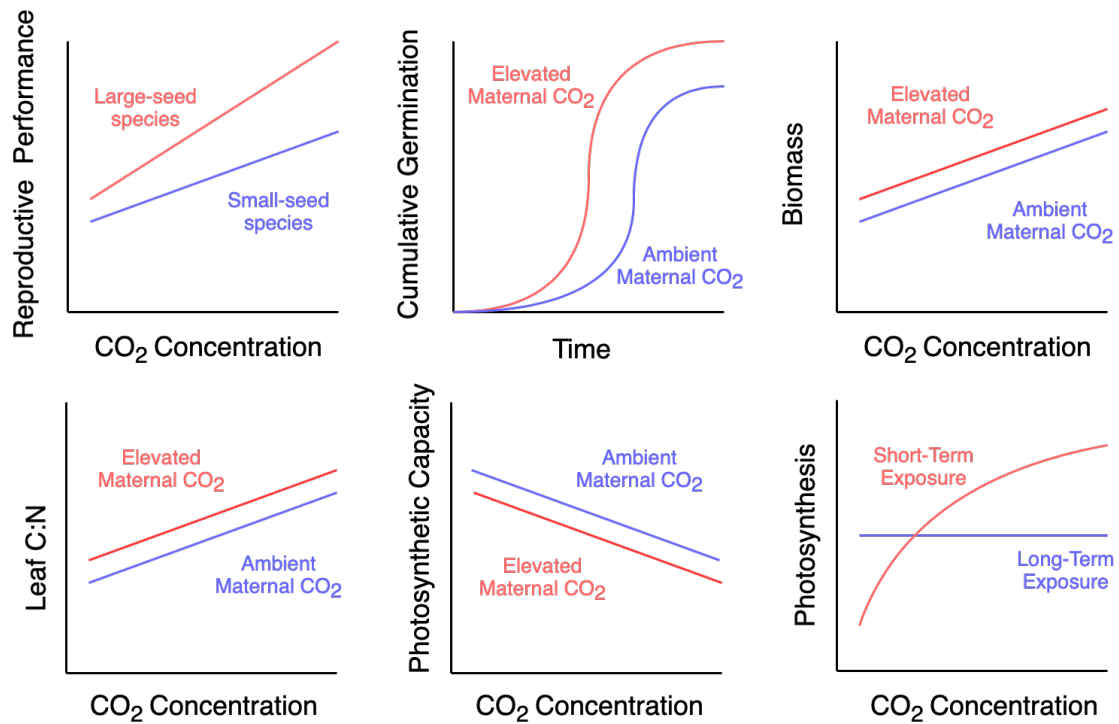


Figure 2. Conceptual overview of predicted outcomes from this study given the findings from previous studies

2 Effects of Elevated CO₂ on the Reproductive Performance of *Eucalyptus tereticornis* and *Hakea sericea*

2.1 Introduction

Atmospheric CO₂ concentration has risen rapidly since the industrial revolution due to human activity (IPCC 2014). Present day concentrations (*ca.* 410 ppm) are greater now than anytime in at least the past 800,000 years (*ca.* 180-270 ppm) as determined through the analysis of ice cores (Lüthi et al. 2008), and it is likely that current atmospheric CO₂ concentration has not been matched in the past 3 million years (Martínez-Botí et al. 2015). Climate models indicate that without extreme intervention, atmospheric CO₂ concentration will reach values of 540-940 ppm by the end of the 21st century (van Vuuren et al. 2011). Increasing atmospheric CO₂ concentration has been demonstrated to increase temperature by keeping radiant energy within the earth's atmosphere resulting in global warming, altered rainfall, and extreme events including heat waves and droughts (Edwards, Clark & Newton 2001; Hussain, Kubiske & Connor 2001; Jablonski, Wang & Curtis 2002; Way et al. 2010). Plants response to climate change is complex and mediated through the direct and/or indirect effects of increased atmospheric CO₂ concentrations.

The development and timing of flowering, resources to attract pollinators, seed and fruit development are all integral processes of angiosperm sexual reproduction. External factors that may interfere with any of these processes can influence the overall reproductive performance of plants. Mixed results have been observed for the impacts of elevated CO₂ concentrations on plant reproduction. A meta-analysis conducted by Jablonski et al. (2002) observed a general increase in the number of flowers, fruit, and seed across many crop and wild species. This is observed alongside increased individual seed mass and reproductive allocation; the relative proportion of biomass invested in reproductive tissues (Bazzaz, Ackerly & Reekie 2005), however, other studies have observed a decrease in seed mass (Andalo et al. 1998; Parmesan & Hanley 2015). Many of the findings are derived from studies on crop, annual and grass species with little information on how the reproduction of long-lived woody plants may be impacted by increased atmospheric CO₂ concentration (Jablonski, Wang & Curtis 2002; Marty & BassiriRad 2014). Differential effects to increased atmospheric CO₂ concentration may alter community structure and function as conditions become increasingly favourable for some species and having little or negative effect on other

species (Norby & Zak 2011). It is therefore essential to further our understanding of impacts to reproductive performance for a broader range of plant functional types.

The introduction of Free Air Carbon-dioxide Enrichment (FACE) technology has made it possible to study long-term impacts of elevated CO₂ on long-lived and large plant species with no restriction on root growth and exposed to nutrient limitations and biotic interactions in natural environments. For long-lived species, this has proven to be more feasible than past technologies such as growing plants inside climate-controlled chambers (Ainsworth & Long 2005). Since its development, FACE facilities have been established globally and within numerous ecosystems including bogs, grasslands and forests (Hendrey et al. 1999; Norby & Zak 2011). The effects of elevated CO₂ have been explored in a range of forest biomes with FACE technology including mixed-deciduous forest (WebFACE; Switzerland), coniferous forest (DukeFACE; USA), temperate deciduous woodland (BiFoR-FACE; England), broadleaf evergreen tropical rainforest (AmazonFACE; Brazil), sub-tropical evergreen forest (EucFACE; Australia) and hemiboreal forest (SwedFACE; Sweden) (Hendrey et al. 1999; Lapola & Norby 2014; Norby et al. 2016; Pepin & Körner 2002; WSU 2019). To date, much of the work in these facilities has revolved around the effects of elevated CO₂ on vegetative growth with little emphasis on the impacts on reproductive performance.

The few studies that have explored the effects of elevated CO₂ on the reproductive performance of long-lived woody plant species are currently centred around two species; the coniferous *Pinus taeda* (Hussain, Kubiske & Connor 2001; Ladeau & Clark 2006; Way et al. 2010), and a deciduous angiosperm *Betula papyrifera* (Darbah et al. 2008). All of these studies report increased reproductive performance through an increase in fecundity and/or seed quality, however, these studies are both northern hemisphere, temperate climate, plantations. As such, it remains uncertain if other systems would respond in a similar way, and it is of fundamental importance to determine the response of broad natural ecosystems including the sub-tropical forests of Australia where 74% of dominant canopy species are *Eucalyptus sp.*; broad leaf evergreen angiosperms (ABARES 2017).

This study is undertaken within the EucFACE facility in Western Sydney (Australia), with naturally growing populations, on nutrient limited soils, which form part of the remnant Cumberland Plains Woodland ecological community. The effects on vegetative growth have been demonstrated to be absent or diminished when one or more nutrients are limiting (Crous, Ósváldsson & Ellsworth 2015; Ellsworth et al. 2017; Parmesan & Hanley 2015), leaving much unknown on how reproduction may be affected. The main objective of this

study is to investigate the effect of elevated atmospheric CO₂ (150 ppm greater than ambient) on the reproductive performance of two long-lived woody plant functional types; a woody perennial shrub *Hakea sericea* and dominant evergreen tree *Eucalyptus tereticornis*.

Components of reproductive performance explored here are: reproductive effort (investment of reproductive tissues), and reproductive output (fruit and seed quality/quantity). Within this study, the following hypotheses are tested:

H₁: Reproductive effort in terms of investment to floral buds of *E. tereticornis* will increase for plants within elevated CO₂ sites

H₂: Due to increased reproductive effort, the reproductive output will be greater for *E. tereticornis* and *H. sericea* in terms of the number and mass of seed and fruit

H₃: Germination success and germination rate will increase for seeds produced in CO₂ enriched sites with effects more pronounced in the large seeded species *H. sericea*

2.2 Methods

2.2.1 Study Site

Plant material for this study was grown within the Eucalyptus Free Air Carbon-Dioxide Enrichment (EucFACE) facility at ambient (*ca.* 410ppm) and elevated (ambient + 150ppm) atmospheric CO₂ conditions. The EucFACE facility was established in 2012 at Western Sydney University's Hawkesbury campus (Richmond, NSW, Australia), approximately 50km northwest of Sydney, Australia (33°36'58"S 150°44'22"E; (WSU 2019) on a relatively flat site ranging from 22-25 m above sea level. The EucFACE facility consists of 6 carbon-fibre ring structures (hereafter rings) 25m in diameter (~ 0.05 ha) and 28m in height surrounding remnant Cumberland Plain Woodland within a 35 ha patch of vegetation. The vegetation community in this area has been identified as Cumberland Shale Plains Woodland (Tozer et al. 2010), dominated by *E. tereticornis*, with the understorey consisting of grasses and forbes with occasional shrub species (Crous, Ósvaldsson & Ellsworth 2015). The EucFACE facility is located on stagnant alluvial soil described as being slightly acidic (pH = 4.5 - 6.5) loamy sand at the surface with 75% sand content, and sandy clay loam with > 30% clay and silt at depths ranging 50 - 300cm (Gimeno et al. 2018).

Long-term climate data for 1960-1990 in Richmond, NSW, indicate a mean annual rainfall of *ca.* 900 mm and mean annual temperature of 17.1 °C (Hijmans et al. 2005). Since fumigation

at EucFACE began in 2012, Mean Annual Precipitation (MAP) and Mean Annual Temperature (MAT) as recorded by the Bureau of Meteorology (BOM) as 730 mm yr⁻¹ and 18.2 °C respectively (BOM station 67021). Potential Evapotranspiration (PET) for Richmond, NSW, is approximately 1209 mm yr⁻¹ based on the 1960-1990 average (Appendix 2), resulting in an Aridity Index (AI) of *ca.* 0.74; indicating the EucFACE site is on the borderline of humid and dry sub-humid climatic zones (UNEP, Middleton & Thomas 1992).

Three rings at the EucFACE site serve as controls, and the other three as treatment to fumigate the surrounded vegetation with CO₂ 150 ppm greater than ambient conditions. Fumigation began in 2012 with gradual steps of 30 ppm increased over 6 months until reaching the targeted 150 ppm above ambient concentration (Ellsworth et al. 2017). Fumigation is computer controlled, releasing CO₂ into the surrounded area during daylight hours and when wind velocity threshold is not exceeded (5.5 m s⁻¹ for 5 minutes).

2.2.2 Study Species

Eucalyptus tereticornis SM. (Myrtaceae) is a dominant, evergreen, angiosperm tree native to the east coast of Australia. It is widely distributed on well-drained alluvial soils from northern Queensland to south-eastern Victoria (Fairley & Moore 2010), and a dominant tree in the Cumberland Plain Woodland. With fast growth and a maximum height of over 40 m *E. tereticornis* is a valuable forestry species, especially in India and Africa. *Eucalyptus tereticornis* is a facultative resprouting species that produces numerous small seeds (*c.* 1 mm) within a small woody fruit. *Eucalyptus tereticornis* is Spring flowering, with clusters of 7-11 flowers blooming between August and November (Fairley & Moore 2010). Periods between flowering events for the *Eucalyptus* genus can range from 1-7 years, however, a large number *Eucalyptus* species flower every 2-4 years (Birtchnell & Gibson 2006). *Hakea sericea* Schrad. & J.C.Wendl. (Proteaceae) is an Australian native perennial woody shrub growing up to 3 m tall in open forest, woodland and heath vegetation. It is widely distributed along the eastern and south-eastern coast of Australia, ranging from southern Queensland to south-western Victoria (Fairley & Moore 2010), and a common component of the Cumberland Plain Woodland. Outside of its natural range, *H. sericea* is known to be an invasive, particularly in South Africa and New Zealand (Brunel et al. 2010). *Hakea sericea* is a Late-Winter/Early-Spring flowering plant, with flowering occurring from June-September. *Hakea sericea* is an obligate re-seeder species with large woody fruits that encapsulate two large (*c.*

7mm) winged seeds that are released following the death of the maternal plant, usually by fire.

2.2.3 Plant Material

To estimate the reproductive allocation for *E. tereticornis* and *H. sericea* plant material was destructively harvested from trees and shrubs growing under the treatment conditions within the EucFACE facilities in August to September 2018 and 2019. Two different sampling approaches were employed: (i) All *E. tereticornis* trees had a subsample of upper canopy (10-20m) branches harvested and (ii) *H. sericea* shrubs had all above ground material sampled. Standardised length branches, 45 cm in length, were harvested from dominant *E. tereticornis* trees, accessing the tree canopy by crane. Initial surveys of all dominant trees within the experimental rings (~6 trees / ring) was conducted to determine which trees were producing floral buds and fruits. In 2018, reproductive plant material was harvested for randomly selected two trees from two rings for each CO₂ treatment. Six branches were harvested for each tree; three of which contained floral buds as the predominant reproductive structures, and the remaining three where fruits were the predominant reproductive structure. In 2019, three trees from all six rings (3 ambient and 3 elevated CO₂ rings) were randomly selected to harvest three branches with floral buds (fruits were too limited to sample).

Following the harvest, all tissues of branches (bud, fruit, leaf, stem) were separated and stored in paper bags or envelopes. Fruits within envelopes were kept in a sealed plastic box with a calcium chloride desiccant (Sunfresh Moisture Absorber) to prevent mould outbreak. The fruits ripened within 2 weeks, opening valves to release seed and chaff held within. Where necessary, fruits were shaken in sealed plastic container and cleaned out with pointed tweezers to release seed and chaff that remained within. Floral bud, leaf, stem and fruit material was dried in ovens set at 65 °C for a minimum of 72 hours prior to recording the count and/or biomass of all tissues. Mass of separated seed and chaff were recorded separately, and seed counts were made per fruit.

Hakea sericea plants were grown within each of the six rings at EucFACE established in four plots (with four plants / plot) in November 2014 (Collins et al. 2018). The entire above-ground mass of *H. sericea* was harvested in 2017 and dry vegetative (leaf, stem) mass recorded. Fruits for each plot within a ring were combined into a single collection and kept in paper bags at room temperature for 6 months. Seeds were then removed from large woody

fruits and counted, after which fruits were oven-dried at 65 °C for 72 hours prior to recording dry mass. Methods for measuring energy content by calorimetry are outlined in Appendix 11.

2.2.4 Germination Experiment

Seeds were surface sterilised following the protocols of Davies et al. (2015), with seeds of *H. sericea* and *E. tereticornis* rinsed in 70% ethanol for 30 seconds and soaked in 0.5% Sodium Hypochlorite (NaOCl; White King Bleach) for 10 minutes, rinsed with distilled water after each step. Agar solution (Sigma Aldrich Noble Agar; 1%) was prepared in sterilised 90 mm petri dishes as the medium for seeds to germinate on. For *E. tereticornis*, 10 seeds were each placed in 5 replicate petri dishes for 2 trees, 2 rings, and 2 CO₂ treatments (40 dishes of 10 seeds). Similarly, for *H. sericea*, 10 seeds were each placed in 5 replicate petri dishes for 4 plots, 3 rings, and 2 CO₂ treatments (40 dishes of 10 seeds). Petri dishes were covered with parafilm to prevent desiccation and contamination, and then placed in an incubator set at 20°C, 16 hour day / 8 hour night and 70% humidity. Seeds were monitored every 2 days over two-weeks, at which point new germinants were recorded and removed. Seeds were determined to have germinated when radicle emergence was at least 2 mm long.

2.2.5 Data Handling and Analysis

All data were collated in Microsoft Excel and analyses conducted with R v3.6.1 (R Core Team 2019). Linear Mixed Models (LMM) and Generalised Linear Mixed Models (GLMM) were produced using the *lme4* R package (Bates et al. 2015) with CO₂ treatment as a fixed effect and EucFACE ring, tree ID, and/or plot ID as random effects. Extreme outliers were removed from data, and where necessary, data were arcsine square-root or log transformed to meet the assumption of normality and homoscedasticity. Where appropriate, Shapiro-Wilk tests of normality were conducted to ensure residuals were normally distributed. Models were selected such that Akaike's Information Criterion (AIC) was lowest. Analysis of Variance (ANOVA) was conducted using the *car* R package (Fox & Weisberg 2019) with degrees of freedom calculated by the Kenward-Roger approximation. *Post hoc* Tukey tests were undertaken using the *emmeans* R package (Lenth 2019) to identify where results significantly differed. For response variables with count data, GLMM's were produced with a Poisson distribution, and G_{\max} was tested by logistic regression using *germinated/not germinated* as the response variable with a logit link function and binomial errors. Goodness of fit for

GLMM's was tested using the *DHARMA* R package (Hartig 2019). With the *germinationmetrics* R package (Aravind et al. 2019), time to reach 50% germination (T_{50}) was calculated using the 'Farooq' method, and a logistic regression fitted for cumulative germination with a Four Parameter Hill Function. Differential responses of G_{max} and T_{50} between *E. tereticornis* and *H. sericea* were tested with species and CO_2 as fixed effects and tree/plot as a random effect for GLMM and LMM.

Traits were grouped as reproductive effort, reproductive output or seed traits depending on which phase of reproduction they represented. Reproductive effort traits (only measured for *E. tereticornis*) include those involved in the initial investment of resources for reproduction and included individual bud weight, total bud weight, bud count, reproductive allocation (proportion of branch biomass invested in buds), bud energy content, and bud N content. Reproductive output refers to traits that describe the payoff of the initial investment. These traits include individual fruit mass, total fruit mass, fruit count, reproductive allocation (proportion of total branch/aboveground biomass for *E. tereticornis*/*H. sericea* in fruits and seed), number of seed per fruit, individual seed weight, total seed weight, and seed count. Seed traits included G_{max} and T_{50} .

2.3 Results

2.3.1 Reproductive Effort

At elevated CO_2 , individual bud weight and the number of buds per branch of *E. tereticornis* increased by 12% and 41%, respectively (Figure 3). It follows that total bud weight per branch also increased (49%) and with marginal significance ($P < 0.1$ LMM; Table 1). Due to increased individual bud weight and a greater number of buds per branch, reproductive allocation was greater (not significant; Table 1) with negligible impacts on tissue quality in terms of energy and N content (not significant; Table 1; Figure 3). Although the 95% confidence interval does not intersect with 0% (indicative of significance) for individual bud weight or reproductive allocation; the transformation of data to conform to the assumptions of normality and homoscedasticity in addition to tree and ring included as random effects yielded non-significant results from ANOVA (Appendix 5).

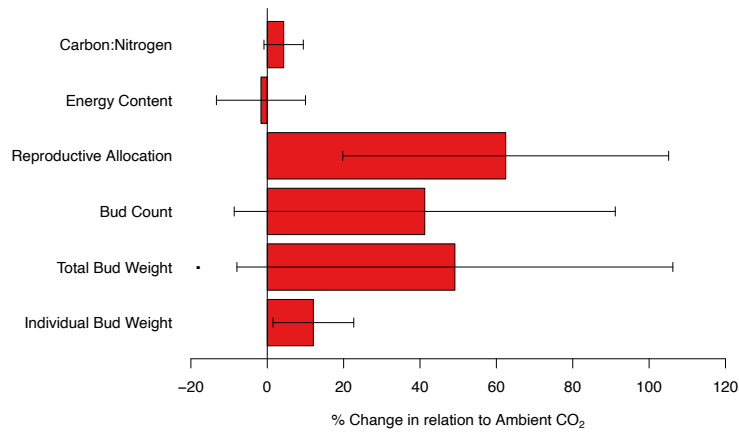


Figure 3. Percentage change with 95% confidence interval of reproductive effort traits measured from *E. tereticornis* branches harvested from elevated CO₂ FACE SITES in relation to branches harvested from ambient CO₂ FACE sites. Significance codes ‘.’ = < 0.1; ‘*’ = < 0.05; ‘**’ = < 0.01; ‘***’ = < 0.001.

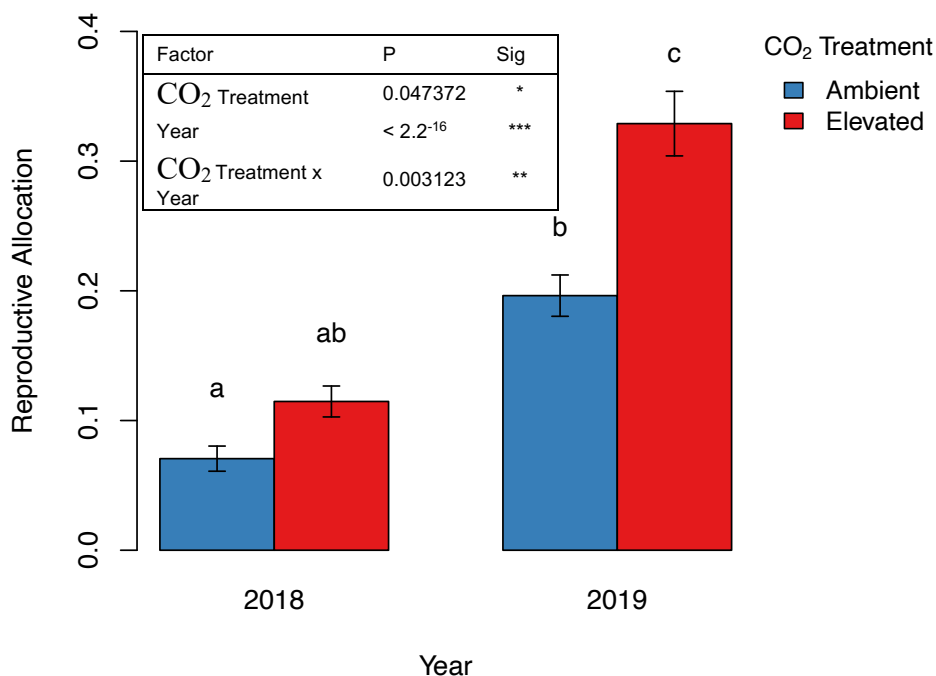


Figure 4. Reproductive allocation +/- standard error for biomass of floral buds of *Eucalyptus tereticornis* grown in FACE sites for crops in 2018 and 2019. Columns with the same letter above them do not significantly differ from one another.

Relative allocation of biomass to floral buds of *E. tereticornis* varied widely across CO₂ treatments (ambient, elevated) and years (2018, 2019) from 0.07-0.33 (Figure 4). Flowering was observed to be much more intense across all trees, treatments and the surrounding area in 2019 than in 2018 (Amy Gilpin; unpublished data). Reproductive allocation for buds of *E. tereticornis* was observed to be greater for branches harvested from elevated CO₂ for both years combined, and significantly within 2019 but not 2018 (Figure 4). Significant differences for the reproductive allocation of biomass of floral buds of *E. tereticornis* were

observed for CO₂ treatment, year, and the interaction of CO₂ treatment and year (Inset table of Figure 4).

2.3.2 Reproductive Output

Individual fruit weight increased for *E. tereticornis* (~ 40%) and decreased for *H. sericea* (~ 16%) under elevated CO₂ (Figure 5). This was accompanied with a decrease in the number of *E. tereticornis* fruits (~ 19%) and increase of *H. sericea* fruits (~ 38%). The net result was a gain of total fruit mass for both species (6.6% *E. tereticornis* branch; 32% *H. sericea* plot). Individual seed weight marginally decreased under elevated CO₂ for *E. tereticornis* (~ 5%) and *H. sericea* (~ 6%), but compensated with greater seed number (~ 29 % and ~ 35% for *E. tereticornis* and *H. sericea* respectively) resulting in a net gain of total seed weight (~ 13 % *E. tereticornis*; ~ 34% *H. sericea*; Figure 5). For *E. tereticornis*, this was accompanied with greater number of seed per fruit (~ 64%), but *H. sericea* rather consistently produces 2 seed per fruit resulting in essentially no difference in seed per fruit (~ 2%). Altogether, the relative proportion of biomass for reproductive output (combined fruit and seed mass) compared with vegetative biomass was greater for *E. tereticornis* and *H. sericea* (~ 14% and ~ 54% respectively; Figure 5). No significant differences were observed for reproductive output traits from ANOVA on LMM's (Table 1).

2.3.3 Seed Germination Traits

Time to reach 50% germination (T₅₀) was not affected in *E. tereticornis*, however it was increased for *H. sericea* seeds sourced from elevated CO₂ maternal FACE sites (Figure 6). There was no significant difference within species between CO₂ treatments for T₅₀ (Table 1), however, T₅₀ significantly differed between species (P < 0.01; Inset table of Figure 6) with *E. tereticornis* germinating more quickly than *H. sericea*. Germination success (G_{max}) was observed to increase for *E. tereticornis* and *H. sericea* for seeds sourced from maternal plants growing in elevated CO₂ FACE sites (Figure 6). Difference in germination success between CO₂ treatments was significantly greater for *H. sericea* (elevated ~ 99%; ambient ~ 83%) than for *E. tereticornis* (elevated ~ 90%; ambient ~ 83%) as signified by the interaction of CO₂ treatment and species (P < 0.05; Inset table of Figure 6).

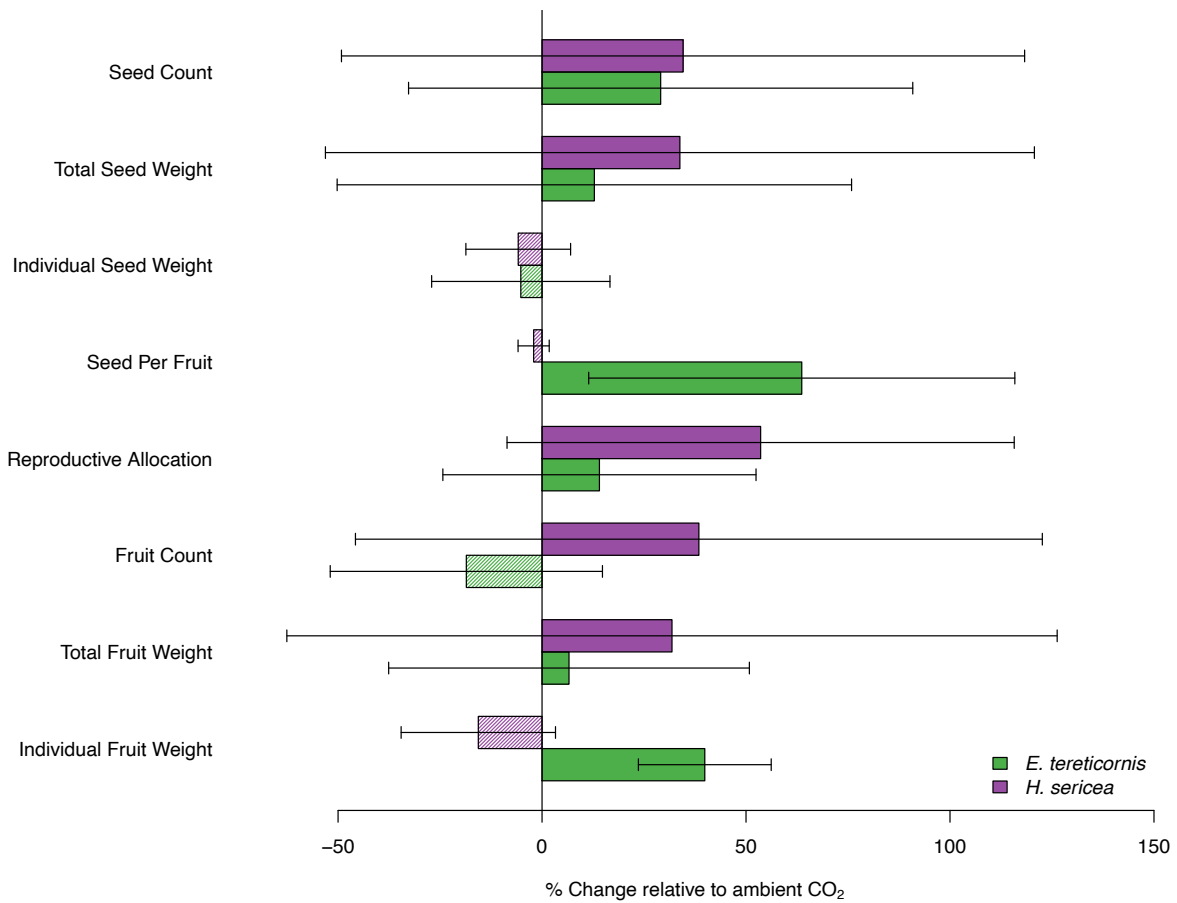


Figure 5. Percentage change with 95% confidence interval of reproductive output traits measured from *E. tereticornis* branches and aboveground biomass of *H. sericea* harvested from elevated CO₂ FACE SITES in relation to branches/aboveground biomass harvested from ambient CO₂ FACE sites. Significance codes ‘.’ = < 0.1; ‘*’ = < 0.05; ‘***’ = < 0.01; ‘****’ = < 0.001.

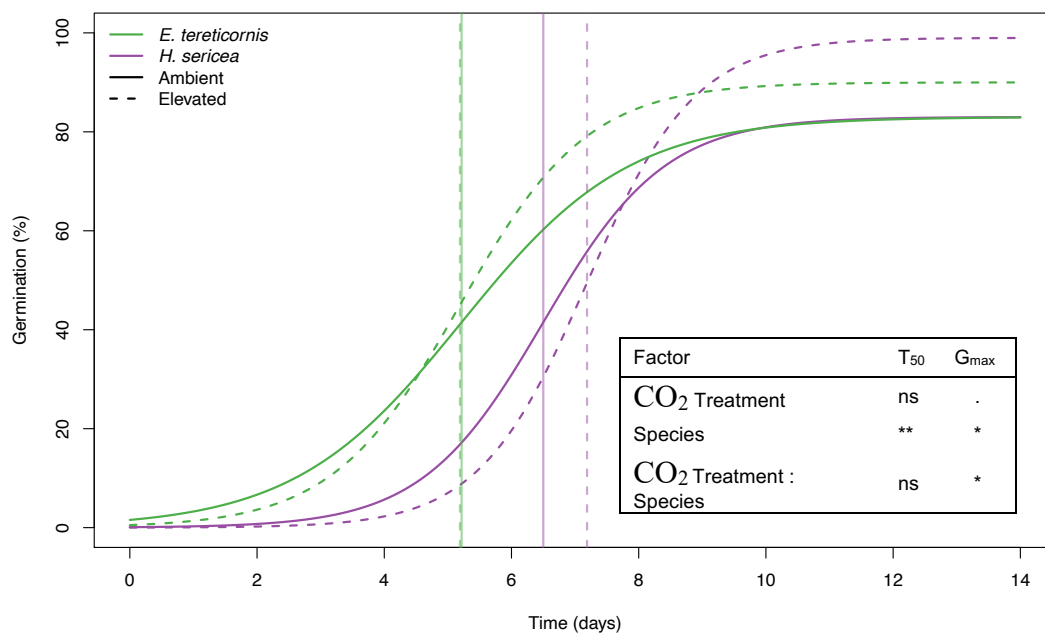


Figure 6. Cumulative germination of *Eucalyptus tereticornis* and *Hakea sericea*. Solid lines indicate seed sourced from maternal plants grown in ambient FACE sites, and dashed lines indicate seed sourced from maternal plants grown in elevated FACE sites. Vertical lines signify T₅₀ for corresponding curve. G_{max} derived from final % germination. Inset table with summary of Two way ANOVA of T₅₀ and G_{max} with CO₂ treatment and species as fixed effects.

Table 1. Summary of sample means +/- standard error and one-way ANOVA for reproductive effort, reproductive output and seed germination traits of *Eucalyptus tereticornis* and *Hakea sericea* grown in ambient and elevated CO₂ FACE sites. Significance codes 'ns' = not significant; '.' = < 0.1; '*' = < 0.05; '**' = < 0.01; '***' = < 0.001. Note: '-' signifies no analysis was conducted

Response Variable	Ambient	Elevated	P	Sig
<i>Eucalyptus tereticornis</i>				
Individual Bud Weight (g)	0.052 +/- 0.002	0.058 +/- 0.002	0.245	ns
Total Bud Weight (g)	2.23 +/- 0.45	3.35 +/- 0.49	0.074	.
Bud Count (branch ⁻¹)	40.38 +/- 6.43	57.03 +/- 8.02	0.199	ns
Reproductive Allocation (Bud)	0.071 +/- 0.010	0.115 +/- 0.012	0.171	ns
Bud Energy Content (J)	321.13 +/- 17.64	326.96 +/- 12.76	0.296	ns
Bud C:N	52.28 +/- 0.99	54.52 +/- 0.95	0.569	ns
Individual Fruit Weight (g)	0.059 +/- 0.004	0.079 +/- 0.002	0.146	ns
Total Fruit Weight (g)	0.486 +/- 0.095	0.518 +/- 0.054	0.903	ns
Fruit Count (branch ⁻¹)	7.68 +/- 1.15	6.26 +/- 0.62	0.203	ns
Reproductive Allocation (Fruit)	0.046 +/- 0.006	0.053 +/- 0.007	0.676	ns
Seed Count (fruit ⁻¹)	10.9 +/- 2.04	17.86 +/- 2.08	0.258	ns
Individual Seed Weight (mg)	0.156 +/- 0.014	0.148 +/- 0.011	0.811	ns
Total Seed Weight (mg)	12.50 +/- 3.63	14.10 +/- 1.72	0.901	ns
Seed Count (branch ⁻¹)	82.42 +/- 22.45	106.39 +/- 13.09	0.125	ns
G _{max} (%)	83 +/- 3.8	90 +/- 2.1	0.156	ns
T ₅₀ (days)	5.0 +/- 0.14	5.2 +/- 0.15	0.644	ns
<i>Hakea sericea</i>				
Individual Fruit Weight (g)	1.88 +/- 0.11	1.58 +/- 0.14	0.307	ns
Total Fruit Weight (g)	45.47 +/- 14.79	59.96 +/- 16.16	0.488	ns
Fruit Count (plot ⁻¹)	25.10 +/- 8.15	34.75 +/- 7.06	0.740	ns
Reproductive Allocation (Fruit)	0.103 +/- 0.023	0.159 +/- 0.016	0.322	ns
Seed Count (fruit ⁻¹)	1.96 +/- 0.03	1.93 +/- 0.03	-	-
Individual Seed Weight (mg)	30.02 +/- 0.89	28.27 +/- 1.75	0.636	ns
Total Seed Weight (g)	1.455 +/- 0.471	1.947 +/- 0.440	0.393	ns
Seed Count (plot ⁻¹)	50.10 +/- 16.33	67.42 +/- 13.83	0.125	ns
G _{max} (%)	83 +/- 4.7	99 +/- 0.7	0.014	*
T ₅₀ (days)	7.0 +/- 0.25	6.5 +/- 0.28	0.347	ns

2.4 Discussion

This study has explored the effects of elevated CO₂ (150 ppm above ambient) on the reproductive performance of two long-lived woody plant species; a dominant evergreen tree *Eucalyptus tereticornis*, and an understory perennial shrub *Hakea sericea*. I have found trends indicating that reproductive effort and reproductive output is increased for long-lived woody plant species, however, no conclusive evidence to support my first two hypotheses. There is mixed support for my third hypothesis; that germination success and germination rate would increase for seeds produced in elevated CO₂ conditions. There is no evidence for the effect of elevated CO₂ on the germination rate of either *E. tereticornis* or *H. sericea*, however, the germination success of both species was differentially affected, with *H. sericea* seeing greater benefits than *E. tereticornis*. Altogether, these results indicate reproductive performance may increase under future CO₂ conditions with differential impacts between species.

2.4.1 Reproductive Effort

Previous studies conducted within the EucFACE facilities have determined that there is no increase in vegetative growth of *E. tereticornis* due to phosphorous limitation, despite observing increased photosynthesis (Crous, Ósváldsson & Ellsworth 2015; Ellsworth et al. 2017). It would then be expected then that any increase in reproductive effort results in greater reproductive allocation. While an increase in reproductive allocation of floral buds was observed, there were large amounts of variation within treatments yielding non-significant results for the branches harvested in 2018. Reproductive allocation significantly increased in both CO₂ treatments for branches harvested in 2019 ($P < 0.001$), with significantly greater increase observed in the elevated CO₂ treatment ($P < 0.01$; Figure 4). This counters the observation that reproductive allocation remains a constant proportion of the total biomass of many plant functional types (Thürig, Körner & Jürg 2003; Way et al. 2010), however, this study has been conducted at the branch level and Net Primary Productivity (NPP) has not been estimated for the entire tree (Way et al. 2010). Furthermore, the effect of other environmental variables has not been fully explored (e.g. nutrients, water, temperature). The observed increase of investment in floral buds for *E. tereticornis* was not accompanied with a comparable decrease in nitrogen or energy content suggesting there may be negligible impact on floral resources to attract pollinators and/or successfully reproduce. This would support the findings of Way et al. (2010) who found that increased seed

production did not decrease seed quality. Further study on pollen, nectar and seed quality of *E. tereticornis* would be required to substantiate these observations.

2.4.2 Reproductive Output

Although no significant differences were observed, there was a strong trend for increased reproductive output in *E. tereticornis* and *H. sericea* grown in elevated CO₂. Reproductive allocation was increased via different methods for the two species; *E. tereticornis* producing fewer but larger fruits, and *H. sericea* producing a greater number of smaller fruits. There was no information obtained for the reproductive effort of *E. tereticornis* in 2017 from which the reproductive output of 2018 is derived. This study sampled *E. tereticornis* branches once fruits were mature so information regarding the payoff of initial investment from reproductive effort in 2017 is not obtained due to not being able to account for loss of developing fruits to wind and/or insect damage before maturation. Research is ongoing where fruit set is being estimated from tagged branches to track flowers to mature fruits

Contrary to studies on *Pinus taeda* and *Betula papyrifera* (Darbah et al. 2008; Hussain, Kubiske & Connor 2001), this study finds no evidence of CO₂ effect on individual seed mass. It was hypothesised that *H. sericea*, being a comparatively large seeded species, would devote greater resources provided under elevated CO₂ conditions to developing seed as observed in *Pinus taeda* (Hussain, Kubiske & Connor 2001), while small seeded species *E. tereticornis* would have limited capacity to enhance seed mass. Although not significant, overall seed output was greater in elevated CO₂ for *E. tereticornis* through increasing the number of seed per fruit, and *H. sericea* through producing more fruits which consistently contain two seeds. Multi-year studies would be required to conclusively determine the impacts of CO₂ on reproductive performance due to inter-annual variation (Ladeau & Clark 2006).

2.4.3 Seed Germination Traits

Seed germination represents the first important step in the establishment of a new plant, and therefore the success and rate of germination can provide an estimate of initial fitness. In this study, germination characteristics were measured under optimal conditions (moisture, temperature, sterile) and therefore may not accurately represent recruitment in natural conditions where competition for establishment may be enhanced (e.g. sub-optimal

conditions, resources limiting). Germination success (G_{\max}) is a hard fitness trait where seed fail to germinate they have a fitness of zero, when measured under optimal conditions with fresh seed, G_{\max} should reflect the innate ability to germinate. The results of this study show significant differences in G_{\max} between maternal CO_2 treatment, species, and the $\text{CO}_2 \times$ species interaction. While the CO_2 treatment was significant overall, G_{\max} was only significantly greater under elevated CO_2 than ambient CO_2 for *H. sericea* but the difference between the CO_2 treatments was not significant for *E. tereticornis*. This highlights potential differential effects between plant functional types (Marty & BassiriRad 2014). Large seed species are recognised to have greater germination success than small seed species due to investing more resources to produce fewer seeds (*H. sericea* in this study), rather than produce larger numbers of small seeds (*E. tereticornis* in this study; Moles & Westoby 2006). Furthermore, *H. sericea* is an obligate re-seeding species and relies on high quality seed to persist in fire-prone landscapes, whereas *E. tereticornis* is a facultative resprouting species, and has to provide resources for two competing functions (production of seed and epicormic buds) to persist in fire-prone landscapes (Bazzaz, Ackerly & Reekie 2005; Lawes & Clarke 2011). Increased G_{\max} was found to be positively correlated with an increase in biomass in a meta-analysis conducted by Marty & BassiriRad (2014), however, this study observed increased G_{\max} with no change in seed mass. A potential hypothesis is that seed grown under the CO_2 treatments may have a different composition, perhaps additional carbohydrates, amino acids or lipids may be found in seed produced under elevated CO_2 conditions. Germination rate (T_{50}) provides a complimentary measure that may enhance fitness, where seed that germinate rapidly may be able to access resources (e.g. water, nutrient, light) before seed that are slow to germinate. T_{50} in this study was only found to significantly differ between species and was not affected by maternal CO_2 conditions. It is likely that the faster germination rate observed for *E. tereticornis* compared with *H. sericea* is due to physical difference in size; with smaller seeds imbibing more quickly (Shipley & Parent 1991). Increased germination rate has been shown to be positively correlated with increased plant fitness (growth, fecundity) in controlled environments, however, it remains to be tested if this occurs in natural conditions where resources may be limited (Verdú & Traveset 2005).

2.4.4 Conclusion and Future Work

While many of the results presented in this study were non-significant, taken altogether, the increase in seed production and the increase in germination success is likely to result in

overall reproductive performance. Increased reproductive performance of *H. sericea* may contribute to the woody plant encroachment observed in Africa (Venter, Cramer & Hawkins 2018), however, it is not likely to be the sole cause. Further study would be required to completely understand the ecological implications and investigate the effects of CO₂ on seed quality and seed longevity as *H. sericea* may hold onto seeds for numerous years. This study is limited to two species; however, it demonstrates that plant functional types may respond differentially to future atmospheric CO₂ concentrations, highlighting the need to represent a broader range of plant functional types. These findings improve our understanding on how future CO₂ concentration may impact ecosystem dynamics and future studies should explore how other aspects of the plant reproductive cycle may be impacted by increased CO₂ concentration including plant-pollinator interactions, phenology, seed quality, and transgenerational effects.

3 Direct and Maternal Effects of CO₂ on the Early Life Fitness of *Hakea sericea*

3.1 Introduction

The concentration of atmospheric Carbon dioxide (CO₂) has risen considerably since the industrial revolution from *c.* 280 ppm (IPCC 2014) to an annual concentration of *c.* 407 ppm in 2018 (Blunden & Arndt 2019). Prior to the industrial revolution, ice core records indicate atmospheric CO₂ concentration has fluctuated between 180 and 280 ppm for the past 800,000 years with the occurrence of 8 glacial periods; the last of which occurred 18,000 - 20,000 years ago (Lüthi et al. 2008). It has been approximately 3 million years since atmospheric CO₂ concentrations matched current conditions (Martínez-Botí et al. 2015), and forecasts indicate that without intervention we will experience CO₂ concentrations greater than any time in the last 20 million years by the end of the 21st century (Foster, Royer & Lunt 2017; van Vuuren et al. 2011). Most species on earth have evolved under relatively stable atmospheric CO₂ concentration of around 240 ppm (Körner 2006), and the rapid increase of atmospheric CO₂ and the associated effects on the global climate give cause for alarm.

Increased atmospheric CO₂ has been demonstrated to affect plant growth and reproduction directly through impacts on physiology and indirectly through its contribution to climate change (Bazzaz 1990; Parmesan & Hanley 2015). Although the indirect effects of CO₂ on plants may be avoided through migration, assisted or otherwise, exposure to increased global atmospheric CO₂ concentration will continue to have impact on plant growth and reproduction. Differential responses between plant species may over time result in altered ecosystem structure and function (Garbutt, Williams & Bazzaz 1990), highlighting the need to undertake studies on a broad range of plant functional types. Many studies have explored the effects of elevated CO₂ on plant growth and development but have largely focused on the vegetative growth of crop and annual species (Ainsworth & Long 2005; Norby & Zak 2011). Few studies explore the effects of elevated CO₂ on seed quality and subsequent early life fitness of progeny, particularly for long-lived species (Parmesan & Hanley 2015). This is concerning given the lower adaptive potential due to longer generation times, and the importance of the ecological services provided by forests and woodlands.

It has been extensively demonstrated that short-term increase of CO₂ concentration can enhance photosynthesis of C₃ plants through improving the efficiency of carboxylation reactions of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco; Galmes et al. 2013).

These observations are usually associated with a long-term increase in growth and productivity (Drake, González-Meler & Long 1997; Körner 2006), however, little or no gain is found when one or more nutrients and/or resources are limiting (Ellsworth et al. 2017; Parmesan & Hanley 2015; Tissue, Thomas & Strain 1993). The initial benefits of increased CO₂ have been demonstrated to diminish over time due to photosynthetic downregulation and acclimation of new environmental conditions (Drake, González-Meler & Long 1997; Galmes et al. 2013; Körner 2006; Parmesan & Hanley 2015). The long-term downregulation of photosynthesis has been observed via two mechanisms; the production of Rubisco binding inhibitors, and/or reduced Rubisco content (Galmes et al. 2013). The latter is thought to occur to maintain balance between carbon source and sink (Arp 1991), thereby reducing Rubisco content resulting in lower nitrogen concentration of photosynthetic tissues (Drake, González-Meler & Long 1997). Several hypotheses have been put forward to explain the reduction in nitrogen following long-term exposure to increased atmospheric CO₂ concentration. Among these include the dilution of nitrogen from the accumulation of non-structural carbohydrates or secondary compounds; decreased uptake due to lower stomatal conductance and therefore transpiration; or decreased production of Rubisco enzymes due to improved photosynthetic efficiency from higher carboxylation rates (Taub & Wang 2008).

Regeneration by seed is a crucial stage in the life history of plants but still remains understudied in response to climate change (Parmesan & Hanley 2015). Reports of seed quality in response to elevated CO₂ have yielded highly variable results, however, a correlation between the increase in seed size due to elevated CO₂ and germination success has been identified (Jablonski, Wang & Curtis 2002; Marty & BassiriRad 2014). The study of a broader range of plant functional types is required as many studies revolve around crops, grasses, and forbs and more recently with trees grown under Free Air Carbon-Dioxide Enrichment (FACE) technologies (Andalo et al. 1998; Hussain, Kubiske & Connor 2001; Steinger, Gall & Schmid 2000, 2000; Way et al. 2010). Seed traits in response to elevated concentrations of many plant functional types have remained largely unexplored. There is substantial variation within the plant functional types already studied indicating that responses may be species specific (Jablonski, Wang & Curtis 2002; Marty & BassiriRad 2014).

The main objective of this study is to address this knowledge gap through experiments designed to isolate the short-term, long-term, and maternal effects of atmospheric CO₂ on plant growth, functional traits and physiology. Following the findings presented in chapter

two of my thesis where enhanced seed germination was observed in the perennial woody shrub species *Hakea sericea* (Proteaceae), this experiment sources seed from maternal plants grown in ambient (410 ppm) and elevated (560 ppm) atmospheric CO₂ to propagating and growing seedlings in pre-industrial (280 ppm), present (410 ppm) and future (650 ppm) CO₂ concentrations to identify long-term and maternal effects of elevated CO₂ on progeny. Within this study, the following hypotheses are tested:

H₁: Germination success and rate will increase with increasing CO₂ concentration (Direct effect) with seed sourced from an elevated CO₂ maternal environment performing better than those sourced from an ambient CO₂ maternal environment (Maternal effect)

H₂: Plant growth in terms of height, basal diameter and biomass will increase with increasing concentrations of CO₂ (Direct effect) and seed sourced from elevated CO₂ will outperform seed from ambient CO₂ (Maternal effect)

H₃: Nitrogen concentration of *H. sericea* leaves will decrease with increasing CO₂ concentration (Direct effect) with plants sourced from elevated maternal CO₂ environments having lower Nitrogen concentration than those from ambient maternal CO₂ environment (Maternal Effect)

H₄: Photosynthetic capacity of plants will decrease with increasing CO₂ concentration (Direct effect) with photosynthetic capacity of plants sourced from elevated CO₂ maternal environments having lower photosynthetic capacity than plants sourced from ambient CO₂ maternal environments (Maternal Effect)

H₅: Photosynthesis will increase with increasing CO₂ concentration when exposure is short term. Plants will acclimate to new conditions with long-term exposure and benefits of increased CO₂ will be diminished, and inversely, long-term exposure to decreased CO₂ will result in higher photosynthetic capacity than the short-term response.

3.2 Methods

3.2.1 Plant Material and Experimental Design

Hakea sericea Schrad. & J.C.Wendl. is a perennial woody shrub of the Proteaceae family native to Australia with a wide distribution occurring in dry sclerophyll forests in coastal regions of eastern and south eastern Australia (Fairley & Moore, 2010). Maternal plants were grown in ambient (410 ppm) and elevated (560 ppm) atmospheric CO₂ conditions within the

EucFACE facility at Western Sydney University’s Hawkesbury Campus (33°36'57"S, 150°44'22"E) (WSU 2019). The EucFACE facility was established in 2012, consisting of six carbon-fibre ring structures 25 m in diameter encompassing natural forest, three of which serve as control replicates circulating ambient air, and three that increase the atmospheric concentration of CO₂ to levels 150 ppm greater than ambient conditions. Fumigation is computer controlled, releasing CO₂ only in daylight hours and when wind velocity is sufficiently low. A detailed description of the EucFACE facility is outlined by Crous et al. (2015).

In the context of previous research undertaken at EucFACE by Collins et al. (2018), four *H. sericea* plants were transplanted and grown in four replicate plots within each of the six rings at EucFACE in November 2014. Half of the plants were grown in the presence of competitive grass species and half with no competition. The entire above-ground mass was harvested in November 2017 and the dry vegetative (leaf, stem) mass recorded. Fruits from each plot were pooled into a single collection and kept in paper bags at ambient conditions for 12 months while fruits opened to release winged seeds. Fruits were then dried at 70°C for 72 hours prior to recording the dry mass. Seed collections for this experiment were chosen such that they were sourced from maternal plants grown in the absence of competitive grass species. Six healthy looking seeds were chosen from 4 seed collections for each maternal CO₂ environment (ambient 410ppm; elevated 560ppm) to be propagated into pre-industrial (280ppm), ambient (410ppm), and elevated (650ppm) CO₂ environments (Figure 7).

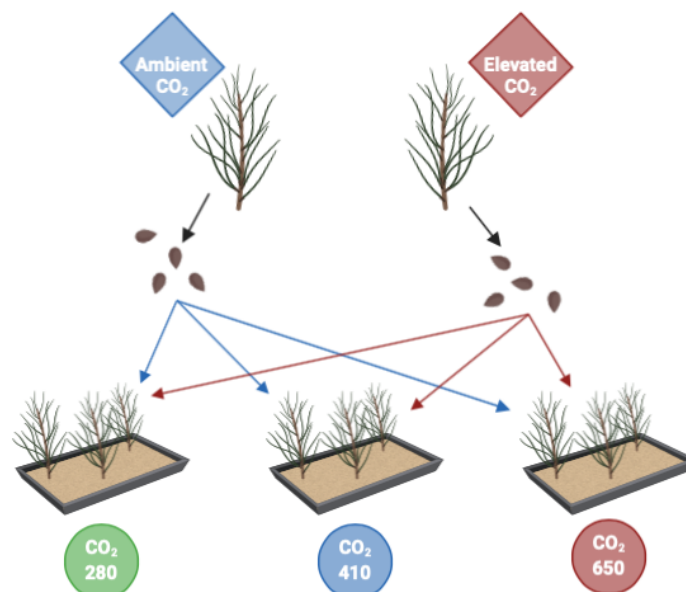


Figure 7. Conceptual diagram for experimental design. Seeds sourced from maternal plants grown in FACE sites with ambient (410ppm) and elevated (560ppm) CO₂ concentrations and subsequently sown in climate-controlled growth chambers imitating pre-industrial (280ppm), ambient (410ppm), and elevated (650ppm) CO₂ concentrations. Diamond shapes indicate maternal CO₂ treatments and circles indicate growing CO₂ treatments.

3.2.2 Growing Conditions

Seeds were sown into Osmocote Native Potting and Planting Mix within 70 mm² (0.55L) tube pots and covered with a 1 cm layer of Perlite (Bunnings Pty Ltd) used to retain moisture in soil. Trays of pots were placed within climate-controlled chambers to assess germination characteristics and subsequent early-life fitness under various atmospheric CO₂ regimes. Three CO₂ treatments were set to pre-industrial (280ppm), ambient (410ppm) and elevated (650ppm) atmospheric CO₂ concentrations and imposed on seed sourced from maternal plants grown at FACE sites in ambient (~410 ppm) and elevated (~560 ppm) CO₂ environments for 5 months. Elevated CO₂ concentration was selected from a previous study by Ghannoum et al. (2010b) to make results comparable. In order to minimise chamber effects, plants and treatments were rotated between chambers mid-way through the experiment. Mean CO₂ concentration throughout the experimental period remained within 5 ppm for the ambient and elevated CO₂ treatments; however, for the pre-industrial treatment, the mean concentration of CO₂ was approximately 30 ppm higher than the set-point (309 ppm; Appendix 7). All other growing conditions were matched across the climate-controlled chambers and were programmed to simulate summer growing conditions in Richmond (NSW, Australia) with relative humidity set at 70% and a 12 hour day/8 hour night, with a 2 hour ramp at the beginning and ending of each day for Photosynthetic Active Radiation (PAR; 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and temperature (26/18°C). Mean temperature and relative humidity throughout the experimental period closely followed the desired set-points; however, lighting conditions deviated substantially from the desired setpoint for the pre-industrial (~20% below setpoint) and ambient (~5% below setpoint) treatments for the final 2 months of the experiment (Appendix 7).

For the initial phase of the experiment, pots were passively watered by placing pots in trays of water and supplemented with fine misting every 3 days. Following the end of germination experiment period (*ca.* 8 weeks), germinants were transplanted into 20 cm round (5 L) pots with Debco Native Potting Mix, watered in with a low concentration of Yates Dynamic Lifter Organic Plant Food Liquid Concentrate (2.2 mL L⁻¹), and had 30 g of Osmocote Native Gardens Controlled Release Fertiliser applied. All plants were watered every 2-3 days and liquid fertiliser was reapplied once every two weeks for 8 weeks to ensure plants were not nutrient or water limited prior to gas exchange measurements.

3.2.3 Emergence and Growth Measurements

Emergence of *H. sericea* germinants was assessed and recorded every 3 days for the first 7 weeks of the experiment. Plant height and basal diameter were measured every 2-3 weeks for the remainder of the experiment. Plant height was determined as the length of the longest stem from the soil level, and basal diameter as the diameter of the main stem 1 cm above the dicotyledons. Where plants had two or more stems, the largest was chosen for measurement. All plants were harvested over 3 weeks starting from 154 days since sowing. The harvest was undertaken such that one individual from each of the 6 treatments was randomly selected at a time to avoid confounding treatment with time of harvest.

At the time of harvest, a final height, basal diameter (all stems for multi-stemmed plants), and perpendicular widths of canopy cover were recorded prior to partitioning the aboveground biomass into stem and leaf tissues and subsequently dried at 70°C for a minimum of 72 hours prior to recording the dry. All growth measurements at the time of harvest were standardised for the time from emergence to time of harvest. From these measurements, canopy volume was calculated as the product of perpendicular canopy widths and plant height, and effective basal diameter as the square root of the sum of basal diameters for up to 3 stems of multi-stemmed plants (Eq. 1). Where b = the basal diameter of a single stem;

$$\text{Effective Basal Diameter} = \sqrt{\sum_{i=1}^n b_i^2} \quad \text{Eq. 1}$$

3.2.4 Gas Exchange Measurements

Gas exchange measurements were undertaken using the Li-Cor LI-6400XT portable photosynthesis system on *H. sericea* plants 20 weeks after sowing. All measurements were conducted at saturating light supplied by a red/blue light-emitting diode source at 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ as determined by light response curves (Appendix 8). To test the procedure was suitable, oven-dried needle leaves were measured to assess potential leaks (Long 2003). Any gaps in the seal where leaves were positioned were filled with blu-tak (Bostik) to minimise leaks. Minor diffuse leakage was detected but deemed to be negligible (*ca.* 2 ppm; Appendix 9).

Gas exchange measurements were conducted for nine randomly selected plants for each treatment combination (Maternal CO₂ x Growing CO₂). To distribute variation between Li-Cor units, A/C_i curves were produced such that after a measurement of one plant sourced from ambient and one from elevated CO₂ environments within each CO₂ treatment chamber

then the Li-Cor systems were rotated between chambers. For each measurement, 5 healthy needle leaves were selected and positioned across the longest side of the 3 cm x 2 cm chamber and care was taken to ensure that leaves did not cross over each other. A/C_i curves were measured with external CO_2 concentration starting at the growing conditions, gradually stepping down to 0 ppm then stepping up to 1750 ppm before finally settling back on the growing CO_2 concentration with a total of 19 measurements of 15 unique concentrations. Measurements were automatically recorded and given 1-3 minutes to equilibrate between each point. Leaves used for measuring A/C_i curves were harvested immediately after measurements, and width of needles recorded to correct photosynthesis measurements for leaf area post processing. Leaf area for needle leaves were estimated as $length \times width \times 2$ as suggested by Pérez-Harguindeguy et al. (2013). Long-term response was assessed through measuring photosynthesis at CO_2 concentrations matching the treatment CO_2 concentration (280 ppm, 410 ppm, 650 ppm) of *H. sericea* for which seeds were sourced from ambient and elevated CO_2 maternal environments; and short term response was assessed through measuring photosynthesis at the corresponding CO_2 treatments (280 ppm, 410 ppm, 650 ppm) for plants grown in ambient CO_2 conditions (Figure 8).

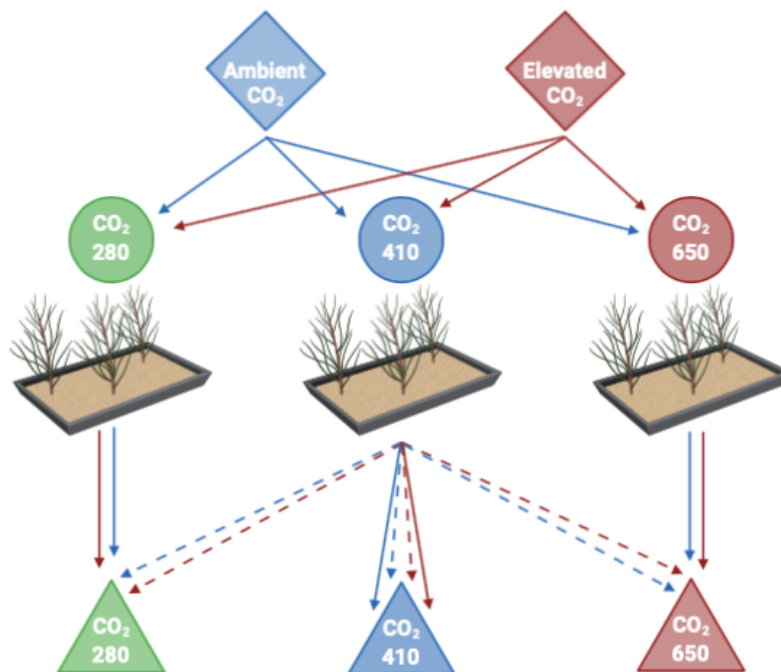


Figure 8. Experimental design to compare the long- and short-term effects of CO_2 concentration on the photosynthetic capacity of *H. sericea*. Solid lines indicate how long-term response was measured with seeds sourced from the FACE sites at ambient (410 ppm) and elevated (560 ppm) maternal CO_2 environments and grown at pre-industrial (280 ppm), ambient (410 ppm) and elevated (650 ppm); dashed lines indicate short-term response to CO_2 by measuring A_{sat} at different CO_2 concentrations for plants grown under the same conditions. Diamond shapes indicate maternal CO_2 environment, circles indicate the CO_2 treatments, and triangles indicate the CO_2 concentration of cuvette for gas exchange measurements.

3.2.5 Data Handling and Analysis

All data were collected and collated in Microsoft Excel and analyses undertaken with R v3.6.1 (R Core Team 2019). Emergence rate of *H. sericea* seed was assessed using the *germinationmetrics* R package (Aravind et al. 2019). Time to reach 50% emergence (T_{50}) was calculated as the number of days until half of the total number of emergents had emerged using the method described by Farooq et al. (2005). A/C_i curves were fitted using the *plantecophys* R package (Duursma 2015) from which measurements of photosynthetic capacity were derived: light saturated net photosynthetic rate (A_{sat}), light and CO₂ saturated photosynthetic rate (A_{max}), apparent maximal electron transport limited photosynthetic rate (J_{max}), and apparent maximal Rubisco limited photosynthetic rate (V_{Cmax}). All data collected from one Li-Cor unit was excluded from analyses due to recording values much lower than the two other Li-Cor units used throughout the experiment. Further filtering and refining of the data was undertaken by removing outliers to improve fitting models to A/C_i curves, or removing entire curves where models could not be fitted.

Linear mixed effects models used the *lme4* R package (Bates et al. 2015) with maternal and growing CO₂ environments as categorical fixed effects and seed collection or EucFACE ring as a random effect. Data that did not conform to the assumptions of normality were log transformed or arcsine square root transformed and subsequently tested for normality with a Shapiro-Wilk test. A Generalised Linear Mixed Model (GLMM) with a logit link function and binomial errors was produced with *emerged/not emerged* as the response variable; maternal and growing CO₂ environments as fixed effects; and seed collection as a random variable. The interaction term of fixed effects for all models was included to test for maternal effects. All models were selected such that Akaike's Information Criteria (AIC) was lowest. Analysis of Variance (ANOVA) was conducted using the *car* R package (Fox & Weisberg 2019) with degrees of freedom calculated by the Kenward-Roger approximation. *Post Hoc* Tukey tests were conducted using the *emmeans* R package to identify where results significantly differed (Lenth 2019). Two sample t-tests of *H. sericea* sourced from ambient and elevated CO₂ environments were conducted for each trait and each CO₂ treatment to test for differences due to maternal effects alone.

3.3 Results

3.3.1 Seedling Emergence

Across all treatments, 79-87 % of *H. sericea* seeds emerged (Figure 9). There were no significant differences in emergence success between growing CO₂ treatment, maternal CO₂ treatment or the interaction of treatments (analysis not shown). Time to reach 50% emergence (T₅₀) differed marginally between the treatments, ranging from 26-29 days from sowing seeds (Figure 9). No statistical analyses were conducted for emergence rate as the cumulative emergence was calculated for all seeds in each of the six treatment combinations (2 x Maternal CO₂; 3 x Growing CO₂) and therefore no replication was attained (however, see germination experiment in chapter 2). Seed weight between maternal treatments did not significantly differ (analysis not shown; see reproductive allocation in chapter 2).

3.3.2 Plant Growth

Throughout the experiment, basal diameter of *H. sericea* seedlings sourced from ambient maternal CO₂ environment (FACE) remained greater than those sourced from elevated CO₂ maternal environment (FACE) within each growing CO₂ treatment (Figure 10A). Plant height throughout the experimental period was comparable across all treatments for the first 114 days since sowing, again with the exception of plants grown in elevated CO₂ sourced from ambient maternal CO₂ environment that appear to have a delayed growth (Figure 10B). At 114 days following sowing, plant height between treatments began to diverge with the final mean height measurements at 154 days ranging from 256-330 mm.

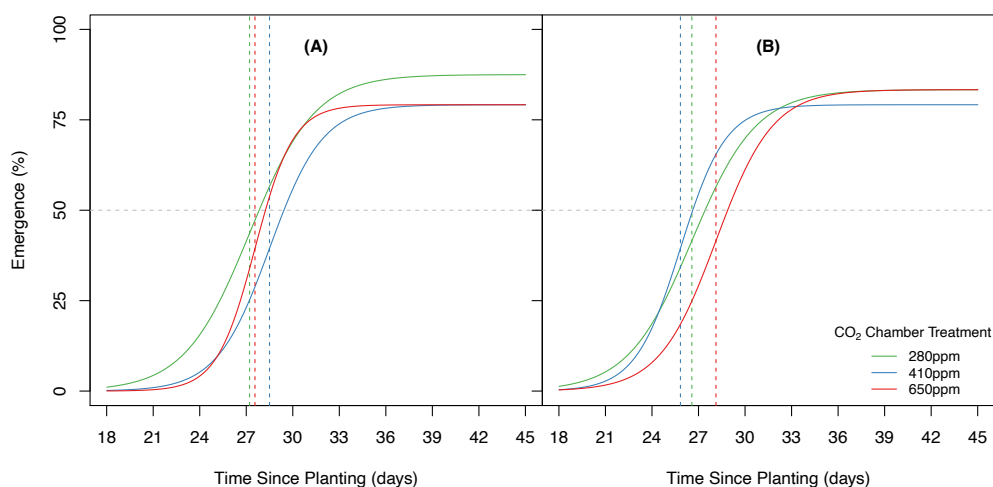


Figure 9. Cumulative emergence of *Hakea sericea* seeds exposed to pre-industrial, ambient and elevated CO₂ concentrations and sourced from maternal plants grown in (A) ambient and (B) elevated atmospheric CO₂ concentrations. Vertical dashed lines indicate time to reach 50% of total seeds to successfully emerge (T₅₀).

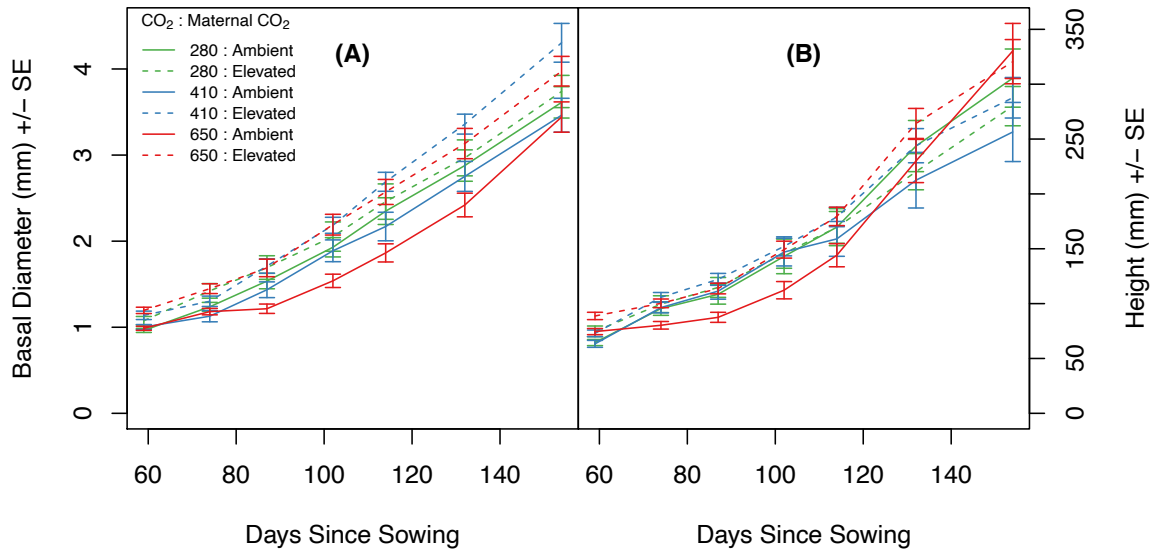


Figure 10. Measurements of (A) basal diameter and (B) height of *H. sericea* throughout the experimental period for which seeds were sourced from maternal plants subject to ambient and elevated CO₂ in FACE and subsequently grown in growth chambers in pre-industrial, ambient and elevated CO₂ environments.

At the time of harvest, height of *H. sericea* was significantly affected by the long-term CO₂ growing conditions ($P \approx 0.003$; Figure 11A). In linear mixed models with maternal environment as a random effect, plant height is significantly greater for *H. sericea* grown at 650 ppm than 410 ppm and 280 ppm CO₂ environments (analysis not shown). *Post-Hoc* Tukey test indicates that this was driven by the difference in plants sourced from ambient maternal environments grown in 650 ppm ($\bar{x} \approx 363$ mm) and 410 ppm ($\bar{x} \approx 259$ mm) environments. However, no significant differences in height were observed between plants for which seeds were sourced from ambient and elevated CO₂ maternal environments.

No significant difference for effective basal diameter was observed between growing CO₂ treatments; however, there is weak evidence ($P \approx 0.07$) to indicate differences in maternal source of seed for *H. sericea* grown at 410 ppm (Figure 11B). At each growing CO₂ treatment, *H. sericea* sourced from elevated CO₂ maternal environments had greater aboveground biomass than ambient sourced *H. sericea*, significantly so at 410 ppm and 650 ppm (Figure 11C). No significant results were obtained from linear mixed effects model when seedlot was included as a random effect, due to a reduction in the power of the analysis.

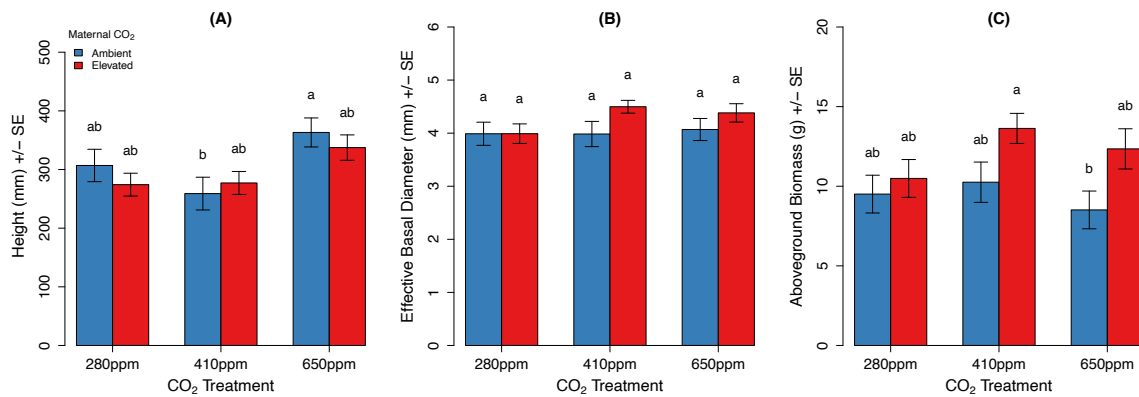


Figure 11. Plant growth measurements at the time of harvest of *H. sericea* for which seeds were sourced from maternal plants exposed to ambient (410ppm) and elevated (560ppm) atmospheric CO₂ in FACE and subsequently propagated and grown in growth chambers in pre-industrial (280 ppm), ambient (410 ppm) and elevated (650 ppm) CO₂ concentrations for 5 months. Measurements for (A) height, (B) effective basal diameter, and (C) dry biomass are all standardised for age at time of harvest. Bars with the same letters above them do not significantly differ from one another.

Leaf fraction of *H. sericea* was 3.5% greater for plants sourced from an ambient maternal CO₂ environment and grown in 410 ppm environment than for plants grown in 280 ppm and 650 ppm CO₂ environments (Figure 12 A). Mean leaf fraction between all treatments ranged from ~ 82.5% to ~ 85.3%. No difference was observed at any growth CO₂ treatment for *H. sericea* sourced from elevated maternal CO₂ environments, and similarly, no difference within CO₂ treatments between plants sourced from ambient and elevated maternal environments was observed. Specific Leaf Area (SLA) of *H. sericea* plants sourced from ambient maternal environments was observed to be relatively constant across the three growing CO₂ treatments, and also for *H. sericea* sourced from elevated maternal CO₂ environments with marginally lower SLA with the exception of plants grown in a 280 ppm CO₂ environment (Figure 12 B). Carbon-Nitrogen ratio was highest for plants from both maternal sources (ambient, elevated) grown in 410 ppm growth chambers, with elevated sourced plants with significantly higher than ambient sourced plants grown in 280 ppm and 650 ppm growth chambers (Figure 12 C).

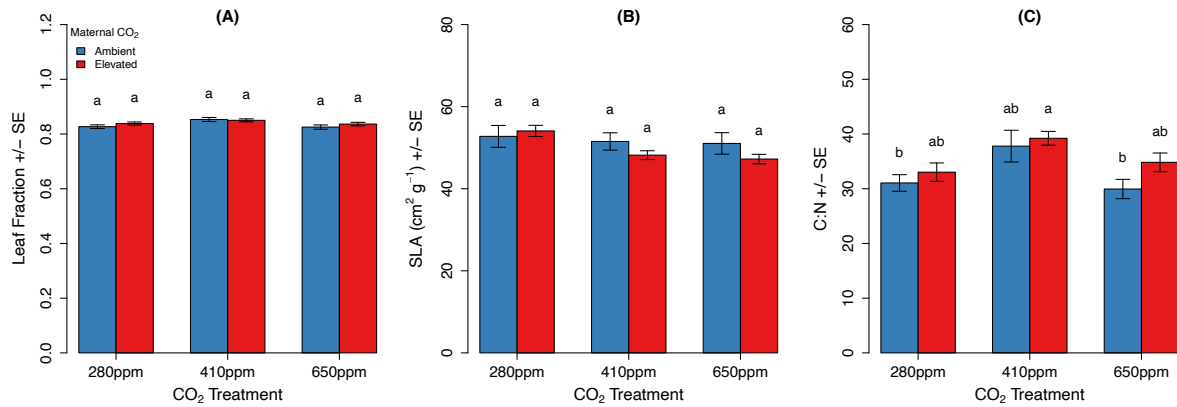


Figure 12. Functional traits of *H. sericea* for which seeds were sourced from maternal plants grown in ambient and elevated CO₂ environments in FACE sites and subsequently grown in growth chambers in pre-industrial (280 ppm), ambient (410 ppm) and elevated (650 ppm) CO₂ environments. (A) Leaf fraction, (B) Specific Leaf Area, and (C) Nitrogen Content are as measured at time of harvest 5 months after propagating. Bars with the same letters above them do not significantly differ from one another.

3.3.3 Photosynthesis

It should be noted that much of the data acquired from gas-exchange measurements was discarded due to large variations between photosynthesis systems. For the remaining data, photosynthetic capacity at saturating light (1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and CO₂ concentration (1750 ppm; A_{max}) did not significantly differ between growing CO₂ treatments, however, there is weak evidence ($P < 0.1$) to indicate *H. sericea* with maternal plants grown in ambient CO₂ concentrations had higher photosynthetic capacity than elevated sourced counterparts (Figure 13 A). A_{max} generally decreased with increasing growing CO₂ concentration as would be expected due to increased Rubisco content in lower CO₂ concentrations, however, there is no apparent correlation of A_{max} with N content (representative of Rubisco content) suggesting that down-regulation of photosynthesis may not be due to reduced Rubisco content. It was thought that due to A_{max} results only presented for a subset of experimental plants ($n = 4-6$) after filtering of poor-quality data that N content of leaves were not representative of the plants for which A_{max} measurements were taken. Accounting for these differences, there was still no correlation between A_{max} and N observed (analysis not shown). Data were further refined in producing A/C_i curves ($n = 3-4$ each treatment) from which J_{max} and V_{Cmax} were derived. In align with A_{max} , there is evidence for greater J_{max} ($P < 0.1$) and V_{Cmax} ($P < 0.05$) for *H. sericea* sourced from ambient CO₂ maternal plants.

Photosynthetic capacity under saturating light (1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$) with CO₂ concentration matching the growing CO₂ environment (A_{sat}) significantly differed between growing CO₂ concentration ($P < 0.001$), maternal CO₂ treatment ($P < 0.05$), and there was marginal

significance for the interaction of maternal and growing CO₂ treatments ($P < 0.1$; Figure 13 B; Table 2). Pairwise comparisons from a *Post-Hoc* Tukey test indicate results significantly differed between ambient sourced *H. sericea* grown at 650 ppm to ambient sourced *H. sericea* in all other growing CO₂ environments, and elevated sourced *H. sericea* grown in 280 ppm CO₂ environment. Furthermore, at 650 ppm growing conditions, ambient sourced *H. sericea* had significantly greater photosynthetic capacity than elevated sourced counterparts. No discernible pattern was observed for stomatal conductance (g_s), with contrasting results for the two maternal sources of plants (Figure 13 C). No significant differences were observed across growing treatments or maternal treatments, but plants sourced from ambient maternal environments tended to have lower g_s when grown in ambient CO₂ than in pre-industrial or elevated conditions, and inversely, plants sourced from elevated maternal environments tended to have higher g_s in ambient conditions, and lower g_s in pre-industrial and elevated CO₂ conditions. No significant differences between growing treatments were observed for the apparent maximal electron transport rate (J_{max}), apparent maximal carboxylation rate (V_{Cmax}), or the ratio of these two parameters, likely due to the small sample size after filtering poor quality data, however, V_{Cmax} was significantly higher on average for plants sourced from ambient maternal treatment for which J_{max} was marginally significant (Figure 13 E-G; Table 2). Nevertheless, J_{max} and V_{Cmax} generally decreased with increasing CO₂ concentration, with the exception of elevated sourced plants grown at 280 ppm that was observed to have lower rates.

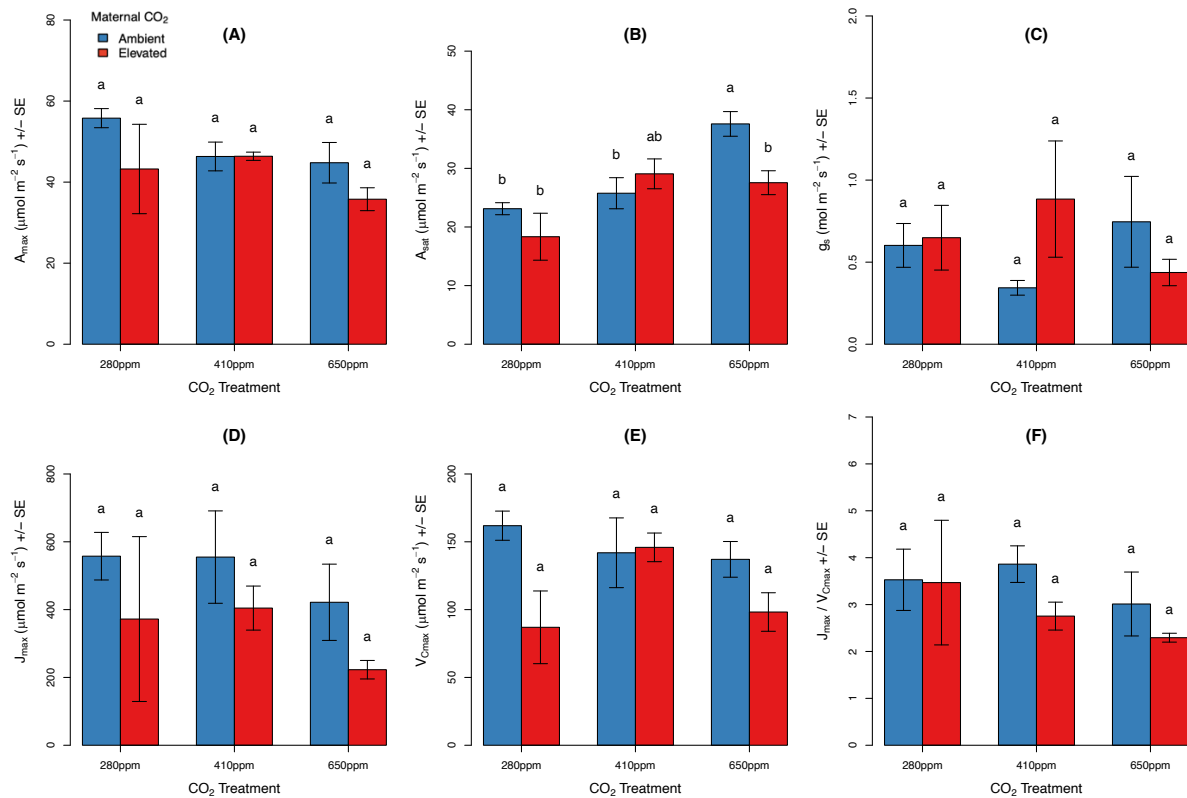


Figure 13. Gas exchange measurements of *H. sericea* for which seeds were sourced from maternal plants grown in ambient and elevated CO₂ environments and subsequently propagated and grown in pre-industrial, ambient, and elevated CO₂ environments. Measurements of (A) Photosynthesis in saturating light and CO₂ conditions (A_{max}), (B) Photosynthesis in saturating light conditions (A_{sat}), (C) Stomatal conductance at saturating light conditions (g_s) determined from spot measurements. Measurements for (D) Apparent maximal electron transport rate (J_{max}), (E) Apparent maximal carboxylation rate (V_{Cmax}), and (F) the ratio of these parameters were determined from fitting models to A/Ci curves. Bars with the same letters above them do not significantly differ from one another.

Short-term response to CO₂ concentration for plants sourced from ambient and elevated maternal CO₂ conditions and grown in 410 ppm CO₂ environment showed a positive correlation of photosynthesis at saturating light (A_{sat}) with cuvette CO₂ concentration of Li-Cor portable photosynthesis system. While the pattern is consistent for maternal treatments, plants sourced from elevated maternal CO₂ environment having consistently higher photosynthetic capacity (Figure 14). Plants sourced from elevated maternal CO₂ environments and grown at 410 ppm CO₂ had higher photosynthetic capacity than ambient CO₂ sourced counterparts; however, photosynthetic capacity of elevated CO₂ sourced plants was considerably lower than ambient CO₂ sourced counterparts when grown in 280 ppm and 650 ppm CO₂ environments. Significant differences were observed for cuvette CO₂ concentration (280 ppm, 410 ppm, 650 ppm; $P < 0.001$) and the interaction of maternal treatment (ambient, elevated) and response trend (long-term, short-term; $P < 0.05$).

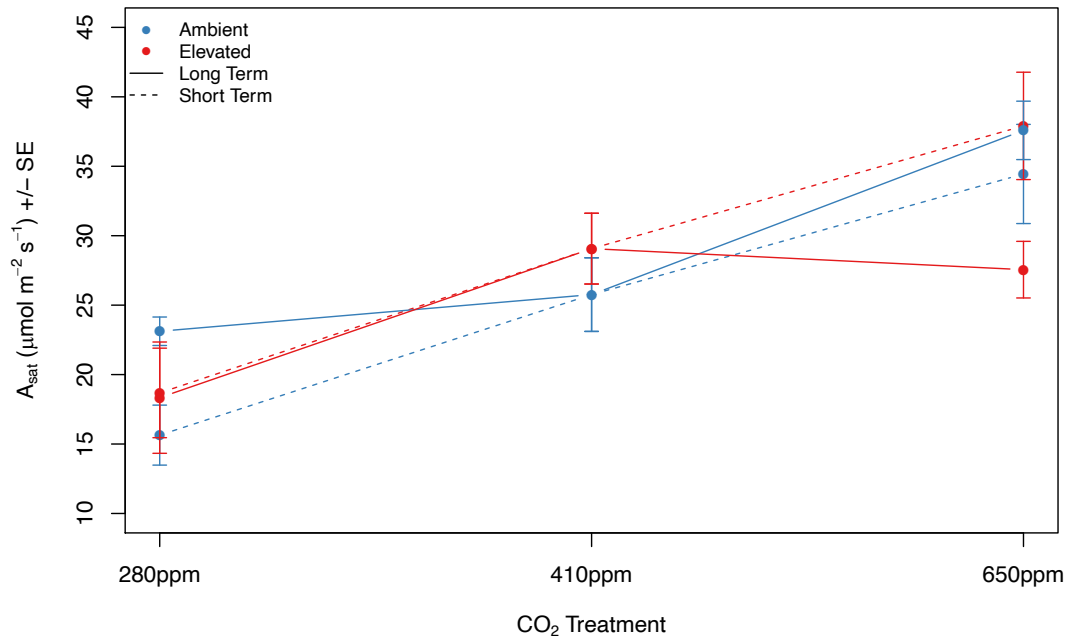


Figure 14. Long- and short-term response to CO₂ for *H. sericea*. Solid lines indicate long-term response; plants for which seed were sourced from ambient and elevated CO₂ maternal environments and grown in pre-industrial (280ppm), ambient (410ppm), and elevated (650ppm) CO₂ environments. Dashed lines indicate short-term response; plants for which seed were sourced from ambient and elevated maternal CO₂ environments and grown in ambient (410ppm) CO₂ conditions. Photosynthesis at saturating light (A_{sat}) measured at CO₂ concentrations corresponding with the x-axis.

Table 2. Summary of Two-way ANOVA (maternal CO₂ concentration x Growing CO₂ concentration) for emergence, growth, functional traits, and gas exchange measurements of *H. sericea*. Significance codes; . = < 0.1; * = < 0.05; ** = < 0.01; *** = < 0.001; ns = not significant; - = not tested.

Variable Type	Variable	Treatment	Maternal Treatment	Interaction
Emergence	Seed Weight	-	ns	-
	Maximum Emergence	ns	ns	ns
	Emergence Rate	-	-	-
Growth	Aboveground Biomass	ns	**	ns
	Height	**	ns	ns
	Basal Diameter	ns	.	ns
Functional Traits	Leaf Fraction	**	ns	ns
	SLA	.	ns	ns
	C:N	***	ns	ns
Gas Exchange	A _{max}	ns	.	ns
	A _{sat}	***	*	.
	g _s	ns	ns	ns
	J _{max}	ns	.	ns
	V _{cmax}	ns	*	ns
	J _{max} /V _{cmax}	ns	ns	ns

Table 3. Summary of emergence, growth, functional traits and gas exchange measurements of *H. sericea* for which seed were sourced from maternal ambient and elevated CO₂ environments and subsequently grown in pre-industrial (280ppm), ambient (410ppm), and elevated (650ppm) growing CO₂ environments.

Variable Type	Variable	Maternal CO ₂ Treatment	280ppm	410ppm	650ppm	
Emergence	G _{max} (%)	Ambient	87.5 +/- 12.5	79.2 +/- 15.8	83.3 +/- 11.8	
		Elevated	83.3 +/- 16.7	79.2 +/- 4.2	83.3 +/- 16.7	
	T ₅₀ (days)	Ambient	27.2	28.5	27.6	
		Elevated	26.6	25.8	28.1	
Growth	Height (mm)	Ambient	306.9 +/- 27.6	259.0 +/- 27.9	363.2 +/- 24.7	
		Elevated	274.2 +/- 19.5	277.1 +/- 19.6	337.4 +/- 21.7	
	Effective Basal Diameter (mm)	Ambient	3.99 +/- 0.22	3.98 +/- 0.24	4.07 +/- 0.21	
		Elevated	3.99 +/- 0.18	4.50 +/- 0.12	4.38 +/- 0.17	
	Aboveground Biomass (g)	Ambient	9.51 +/- 1.19	10.25 +/- 1.27	8.51 +/- 1.19	
		Elevated	10.49 +/- 1.19	13.63 +/- 0.95	12.35 +/- 1.27	
	Functional Traits	Specific Leaf Area (cm ² g ⁻¹)	Ambient	26.4 +/- 1.33	27.8 +/- 2.21	25.5 +/- 1.31
			Elevated	27.0 +/- 0.68	24.1 +/- 0.54	24.7 +/- 1.22
Leaf Fraction		Ambient	0.83 +/- 0.007	0.85 +/- 0.007	0.82 +/- 0.008	
		Elevated	0.84 +/- 0.006	0.85 +/- 0.005	0.84 +/- 0.006	
C:N		Ambient	31.06 +/- 1.51	37.79 +/- 2.90	29.95 +/- 1.76	
		Elevated	33.02 +/- 1.69	39.22 +/- 1.26	34.83 +/- 1.71	
Gas Exchange		A _{max} (μmol m ⁻² s ⁻¹)	Ambient	55.7 +/- 1.93	46.3 +/- 3.55	44.8 +/- 5.00
			Elevated	38.4 +/- 9.82	42.6 +/- 3.56	35.8 +/- 2.82
	A _{sat} (μmol m ⁻² s ⁻¹)	Ambient	21.4 +/- 0.86	21.9 +/- 1.70	34.8 +/- 1.80	
		Elevated	15.4 +/- 2.92	22.6 +/- 1.89	26.9 +/- 1.84	
	g _s (mol m ⁻² s ⁻¹)	Ambient	0.49 +/- 0.08	0.30 +/- 0.03	0.66 +/- 0.18	
		Elevated	0.55 +/- 0.13	0.45 +/- 0.14	0.40 +/- 0.06	
	J _{max} (μmol m ⁻² s ⁻¹)	Ambient	558 +/- 70	555 +/- 136	421 +/- 112	
		Elevated	372 +/- 243	405 +/- 65	223 +/- 27	
	V _{Cmax} (μmol m ⁻² s ⁻¹)	Ambient	162 +/- 11	142 +/- 26	137 +/- 13	
		Elevated	87 +/- 27	146 +/- 11	98 +/- 14	
	J _{max} /V _{Cmax}	Ambient	3.53 +/- 0.65	3.86 +/- 0.39	3.01 +/- 0.68	
		Elevated	3.47 +/- 1.33	2.75 +/- 0.30	2.29 +/- 0.09	

3.4 Discussion

This study has assessed the direct and maternal effects of elevated atmospheric CO₂ on the emergence and early life fitness of *H. sericea*, a perennial woody shrub. Seeds were sourced from maternal plants grown in ambient (~ 410 ppm) and elevated (~ 560 ppm) CO₂ environments at EucFACE, and subsequently propagated and grown for 5 months in growth chamber environments simulating pre-industrial (280 ppm), ambient (410 ppm) and future (650 ppm) conditions. Key findings from this study were (i) elevated CO₂ directly increased plant height of *H. sericea* plants, and aboveground biomass was influenced by maternal CO₂ environment, and (ii) maternal CO₂ environment influenced the ability of progeny to acclimate to pre-industrial or elevated CO₂ concentrations with plants from ambient CO₂ maternal environments capable of up-regulating photosynthesis, and plants from elevated CO₂ maternal environments capable of down-regulating photosynthesis.

3.4.1 Emergence Rate

Direct and maternal effects of elevated CO₂ on seed quality and germination traits have been demonstrated to vary widely between species and plant functional groupings (Jablonski, Wang & Curtis 2002; Marty & BassiriRad 2014). Many studies in this area have largely revolved around crop, annual, and grass species. More recently with the utilisation of Free Air Carbon-dioxide Enrichment (FACE) technologies, it has become feasible to study the long-term and legacy effects of elevated CO₂ on the growth and reproduction of large and long-lived species in natural environments (Hendrey et al. 1999). Large gaps still remain in the knowledge of how other plant functional types will respond to future atmospheric CO₂ concentrations. Here, we try to address this gap with insight regarding the direct and maternal effects of CO₂ on a perennial woody shrub *H. sericea*.

Given the reported increase of seed mass of seeds developed in CO₂ enriched environments, particularly for trees (Hussain M., Kubiske M. E. & Connor K. F. 2001; Jablonski, Wang & Curtis 2002; Steinger, Gall & Schmid 2000; Thürig, Körner & Jürg 2003), it was expected that individual seed mass of *H. sericea* would be higher in elevated CO₂, but I found no evidence to support this hypothesis (also see chapter 2). Seed mass may not have increased due to nutrient limitation restricting the use of extra CO₂ or acclimation of maternal plants to the elevated CO₂ environment (Parmesan & Hanley 2015). While the soils at the EucFACE facilities, where the maternal plants were grown, are nutrient limited, Ochoa-Hueso et al.

(2017) demonstrated that rhizosphere Nitrogen (N) and Phosphorous (P) concentrations increased in elevated CO₂ sites. This offers the potential that plants may have acclimated to these environmental conditions, rather than experiencing nutrient limitations. The dominant canopy tree species has not increased growth under elevated CO₂ (Ellsworth et al. 2017). The growth of *H. sericea* was not affected by CO₂ concentration, but was altered by competition from herbaceous neighbours (Collins et al. 2018; see chapter 2 for analysis on maternal plants). Thus, the lack of vegetative response to elevated CO₂ would not be expected to generate differences in seed mass in elevated CO₂.

In their meta-analysis on parental and direct effects of rising CO₂ on seed germination, Marty & BassisiRad (2014) identified a correlation between germination success and increased seed mass for seed developed in CO₂ enriched environments. There was no significant difference in individual seed mass of *H. sericea* produced in ambient and elevated CO₂ FACE sites, and no difference in emergence success or rate indicating that seed composition (lipids, proteins) was comparable between the two seed sources.

3.4.2 Growth and Physiology

As in a study undertaken of *H. sericea* grown in FACE sites by Collins et al. (2018), total biomass was not affected by CO₂ concentration, although interestingly in the present study, *H. sericea* with maternal plants grown in elevated CO₂ conditions had significantly greater basal diameter and biomass except when grown in 280 ppm CO₂ growth chambers. The non-responsiveness of biomass accumulation in response to CO₂ concentration within seed source (maternal ambient/elevated) contrasts with the findings of Huang et al. (2015) for their study also conducted at Western Sydney University on another member of the Proteaceae family, *Telopea speciosissima*, which responded positively to elevated CO₂ concentration; highlighting that responses may be species specific. Aboveground dry biomass was negatively associated with Specific Leaf Area (SLA) but not clearly linked with leaf nitrogen content or shifts in allocation between leaf and stem of aboveground biomass. Leaf fraction remained relatively constant across all treatments (0.83 - 0.86). The small differences observed in leaf fraction are in agreement with the determination of no impact of elevated CO₂ on biomass allocation for woody plants (Curtis & Wang 1998), however, belowground biomass was not assessed in the present study so increased allocation to roots cannot be ruled out. Final height measurements were found not to differ between the two maternal sources of seed (ambient/elevated), but growing CO₂ chamber concentration had a significant effect

with the tallest plants found in 650 ppm. Plant biomass has been demonstrated to increase in a multitude of species exposed to increased concentrations of CO₂, however, the response is known to be diminished for plants grown in pots and growth chambers (Curtis & Wang 1998; Jablonski, Wang & Curtis 2002). This is thought to occur due to sink limitations, with plants unable to utilise the extra carbon because of restrictions on root growth (Kirschbaum 2011), however, some studies have observed no growth response to elevated CO₂ when grown in the ground (Collins et al. 2018). Plant growth has shown to be diminished in CO₂ limiting environments (Dillery et al. 1995; Gerhart & Ward 2010; Ghannoum et al. 2010a), however, this was not observed in the present study with comparable results of plant height between 280 ppm and 410 ppm CO₂ concentrations. This could be in part due to the effect of high air circulation within the growth chambers required to closely monitor CO₂ concentration that has been shown to affect stem growth and root:shoot ratios of woody plants (Gardiner, Berry & Moulia 2016). Furthermore, there was difficulty in maintaining pre-industrial CO₂ conditions in growth with concentrations periodically reaching ambient concentrations and averaging 310 ppm throughout the experimental period that could influence the results (Appendix 6).

The increase of leaf C:N in response to elevated CO₂ concentration has been extensively reported (Bazzaz 1990; Ceulemans & Mousseau 1994; Curtis & Wang 1998; Drake, González-Meler & Long 1997; Taub & Wang 2008; Temme et al. 2017), however, it was not observed in the present study. Rather, leaf C:N was significantly higher for plants grown in 410 ppm CO₂ conditions than both 280 ppm and 650 ppm concentrations. While increased nitrogen content (decreased C:N) is generally observed in lower CO₂ concentrations (Gerhart & Ward 2010), it is unusual for leaf C:N to decrease in elevated CO₂. This may be possible with increased allocation or production of finer roots, allowing for greater N acquisition (Finzi et al. 2007), particularly in this system where nutrients and water were not limiting factors, however, without information regarding belowground biomass this remains speculation.

3.4.3 *Photosynthesis*

Maternal treatments were shown to have an effect on photosynthetic capacity. For the two maternal sources of *H. sericea* grown in 280 ppm, A_{\max} was higher for ambient sourced plants despite having comparable total N content and g_s suggesting a maternal effect of

elevated CO₂ shifting N allocation to non-photosynthetic features within leaves as observed by Tissue & Lewis (2010) or inhibition of Rubisco (Galmes et al. 2013). Furthermore, apparent maximal rate of carboxylation (V_{Cmax}) and electron transport (J_{max}) were observed to be lower for plants from elevated maternal environments grown in 280 ppm chambers, however the balance between the two functions (J_{max}/V_{Cmax}) is the same (Chen et al. 1993). Despite large differences in g_s (however non-significant) between maternal treatments of *H. sericea* grown in 410 ppm, A_{max} , A_{sat} , V_{Cmax} , and N content were all comparable. This might indicate a similar response to what was observed from elevated sourced *H. sericea* grown in 280 ppm, with a shift of N allocation to non-photosynthetic features within the leaves but compensating for reduced Rubisco content with higher g_s . Additionally, it appears g_s is driving the photosynthetic responses observed in *H. sericea* grown in 650 ppm as N content is comparable, however, A_{sat} (significant) and A_{max} (not significant) were increased with higher g_s . From this study, it is not known if differences in g_s are due to changes in stomatal size, density or aperture however previous studies have observed that stomatal density decreases in response to increased atmospheric CO₂ concentration (Saban, Chapman & Taylor 2019; Woodward 1987).

Direct effects of CO₂ concentration on photosynthetic capacity were also observed within each maternal treatment. For *H. sericea* sourced from ambient maternal environment, despite having comparable N and g_s when grown in 280 ppm and 650 ppm, A_{max} was higher for plants grown in 280 ppm indicating increased N allocation to photosynthetic factors. This upregulation of photosynthetic capacity when grown in 280 ppm was not observed for *H. sericea* sourced from elevated CO₂ maternal environments. Inversely *H. sericea* sourced from ambient CO₂ maternal environments did not acclimate or downregulate photosynthesis when grown at 650 ppm, suggesting the ability to acclimate may be passed onto progeny via maternal exposure to increased CO₂ concentration. Here the possible mechanisms may be through an epigenetic mechanism (Kinoshita & Seki 2014; Singroha & Sharma 2019), or enhanced maternal provisioning of seed (Way et al. 2010). Alternatively, increased growth of *H. sericea* from elevated CO₂ maternal environments may have made these plants more sink-limited with greater root mass in pots resulting in apparent acclimation (Arp 1991). Further studies would, however, be required to resolve the process.

3.4.4 Conclusion and Future Work

From this study, I have found (H₁) No evidence of direct or maternal effects on emergence success or rate; (H₂) Plant height was directly affected by CO₂ concentration with plants grown in elevated CO₂ growing taller, in addition to maternal effects on total above ground biomass and basal diameter for plants sourced from elevated CO₂ maternal environments; (H₃) Direct effects of N concentration, however, in the opposite direction from what was expected with lowest N observed for plants grown in 410 ppm; (H₄) Weak evidence for diminished photosynthetic capacity of plants from elevated CO₂ maternal source; and (H₅) Partial support that plants will acclimate to long-term exposure to pre-industrial and elevated CO₂, with plants from ambient CO₂ maternal sources capable of upregulating photosynthesis, and inversely, plants from elevated CO₂ maternal sources capable of downregulating photosynthesis.

Together, these findings indicate that early-life fitness as measured by height and/or biomass (Younginger et al. 2017) is increased directly influenced by growing in elevated CO₂, and indirectly through maternal plants grown in elevated CO₂. I have demonstrated remarkably different capabilities of photosynthetic acclimation within a single generation where plants grown under elevated CO₂ produce seed that down-regulate photosynthesis at 650 ppm, while there is up-regulation of photosynthesis at 280 ppm for seed produced in ambient CO₂. Teng et al. (2009) failed to detect maternal effects of elevated CO₂ over 15 generations of *Arabidopsis thaliana*, however, plant response to increased CO₂ concentration is known to be extremely variable between species (Curtis & Wang 1998). Future work should include multiple generations in FACE facilities with no restriction on root growth and for a broader range of plant functional types as undertaken by Collins et al. (2018) to identify differential responses within plant communities to future conditions. Although not addressed in this study, increased global concentration of CO₂ imposes other pressures through its contribution to climate change, altering temperature, water, and nutrient availability. Although a number of studies are addressing the interactions of CO₂ concentration with additional abiotic pressures for long-lived plants (Ellsworth et al. 2017; Ghannoum et al. 2010a; Souza et al. 2019; Tissue & Lewis 2010; Tissue, Thomas & Strain 1993), more work is required in this area.

4 Discussion

4.1 Thesis Summary

The experiments undertaken for this thesis were designed to identify if and how increased CO₂ concentrations impact reproduction and subsequent early life fitness of long-lived woody plant species. Two widespread angiosperm species (both evergreen, woody plants with C₃ photosystems) with constraining growth form and reproductive strategy were chosen as representatives of different plant functional types. *Hakea sericea* is a perennial obligate re-seeding woody shrub that produces two seeds in large woody fruits released after the death of the plant, usually by fire. *Eucalyptus tereticornis* is a dominant evergreen facultative resprouting tree that produces many small seeds within comparatively small woody fruits. Quantitative assessment of the reproductive effort (Chapter 2, *E. tereticornis*), reproductive output (Chapter 2, *E. tereticornis* and *H. sericea*), and subsequent early-life fitness (Chapter 3, *H. sericea*) was undertaken throughout a series of growth and germination experiments. Key findings derived from these studies include:

1. Reproductive effort is increased for *E. tereticornis* grown in elevated CO₂, however, there is great inter-annual variation that requires further exploration.
2. Germination success was increased differentially between the study species, with benefits more pronounced in *H. sericea* (a large-seeded species) than *E. tereticornis*, (a small-seeded species).
3. Early-life fitness of *H. sericea* was increased for progeny of plants subject to elevated CO₂ and/or grown in elevated CO₂ despite having lower photosynthetic capacity
4. Acclimation of photosynthesis under pre-industrial and future atmospheric CO₂ concentrations was differentially up/down regulated depending on the maternal CO₂ environment.

4.2 Reproductive Effort

Reproductive effort is commonly assessed through flowering intensity and is generally shown to increase in response to increased CO₂ concentration (Jablonski, Wang & Curtis 2002), however, some studies indicate that Aboveground Net Primary Productivity (ANPP) is increased by the same magnitude, thereby keeping the biomass allocation to reproduction constant (Thürig, Körner & Jürg 2003; Way et al. 2010). Other climatic variables are shown

to interact with increased elevated CO₂ to affect reproductive allocation such as water stress (increased reproductive allocation), ozone (decreased reproductive allocation) and nutrient limitation (no change in reproductive allocation) (Wang, Taub & Jablonski 2015). A disproportionate number of studies on the effects of elevated CO₂ on reproductive allocation are conducted on crop species which have been artificially selected to increase reproductive allocation. The effects of elevated CO₂ on reproductive allocation is shown to be greater for crop species than wild species (Jablonski, Wang & Curtis 2002), however, wild species remain understudied with limited knowledge for many plant functional types.

In this study (Chapter 2), it was hypothesised that reproductive allocation would be increased at the branch level for *E. tereticornis*, with greater relative biomass allocation to floral buds for trees subject to elevated CO₂. It was found that reproductive effort was significantly greater for CO₂ enriched *E. tereticornis* in 2019, but not in 2018. This was observed alongside no significant difference in nitrogen or energy content in 2018 (no energy or nitrogen data are currently available for the 2019 flowering). In the first 3 years of fumigation at EucFACE, Ellsworth et al. (2017) found that light saturated photosynthesis for *E. tereticornis* was increased, however, no increase in ANPP was observed. The absent response of CO₂ fertilisation was attributed to phosphorous limitation, however, it may be that reproductive tissues are an alternative C-sink for extra C fixed by photosynthesis (Arp 1991). From the findings of Wang et al. (2015), it would be expected that phosphorous limitation would result in no change in reproductive allocation, however, it could be that water stress in association with elevated CO₂ is resulting in greater reproductive effort as there has been four consecutive years of rainfall well below the long-term average (Appendix 1). It may be that trees exposed to elevated CO₂ are more water use efficient (Drake, González-Meler & Long 1997) and as water becomes limiting, the difference between CO₂ enriched and ambient treatments is exacerbated. Environmental stressors do not occur in isolation, and it is likely that complex interactions between increased CO₂ concentration in tandem with altered water and/or nutrient availability may result in varied responses between and within species.

4.3 Germination

The germination response to elevated CO₂ is known to be highly variable between and within plant functional groupings, however, germination success is generally shown to increase (Jablonski, Wang & Curtis 2002; Marty & BassiriRad 2014). This is thought to be in part due

to increased seed size (Marty & BassiriRad 2014) which has been observed in numerous studies for a wide range of plant functional types (Darbah et al. 2008; Hussain, Kubiske & Connor 2001; Steinger, Gall & Schmid 2000; Thürig, Körner & Jürg 2003). Larger seed mass between species, and within species, has been associated with increased germination success (Jones & Reekie 2007; Steinger, Gall & Schmid 2000). Increased seed mass from elevated CO₂ has been observed to decrease relative seed N concentration of C₃ species (Grünzweig & Dumbur 2012; Hovenden et al. 2008) except for legumes due to increased uptake of N from symbiosis with N-fixing bacteria (Jablonski, Wang & Curtis 2002). In some cases, seed C:N was observed to increase by the same magnitude as seed mass, thereby resulting in same total N content in seeds (Steinger, Gall & Schmid 2000), or seed mass and N remaining unchanged in response to C enrichment (Way et al. 2010), both of which resulted in no difference in germination success.

From these observations, it was hypothesised that seed produced in elevated CO₂ environments would have greater germination success and germination rate, with greater benefits observed in large-seeded species *H. sericea* than small-seeded species *E. tereticornis*. Mixed support for this hypothesis was found with differentially increased germination success between *H. sericea* and *E. tereticornis* in response to CO₂ concentration of the maternal environment (Chapter 2), however, no difference in seed weight or germination rate was observed (Chapter 2) and no difference in emergence success was observed due to maternal or growing CO₂ environment when propagated in soil (Chapter 3). It may be that radicle emergence (Chapter 2) does not reflect seedling survival and cotyledon emergence (Chapter 3), however, the sterile media and highly controlled conditions are likely to have less variation than propagation in soil with potential differences in microclimate (moisture, light, air circulation). The impacts on seed quality are likely to go beyond the influence of elevated CO₂ on seed size as the driver of increased germination success (Jones & Reekie 2007; Marty & BassiriRad 2014). An alternative mechanism, given the differential response between large-seed species *H. sericea* and small-seed species *E. tereticornis*, could be improved germination success is a result from a shift in resource allocation and/or nutrient stoichiometry with the large-seeded species seeing greater benefits. Further study would be required to substantiate this proposed mechanism.

4.4 Early-Life Fitness

Plant height and biomass is generally accepted as a proxy for fitness as it is positively correlated with fecundity (Younginger et al. 2017). Elevated CO₂ has been shown to increase the initial Relative Growth Rate (RGR), and inversely, lower CO₂ concentrations reduce RGR (Bazzaz 1990; Temme et al. 2015). Initial benefits of elevated CO₂ appear to be diminished in longer term experiments, particularly in one or more nutrients are limiting (Parmesan & Hanley 2015). Furthermore, other environmental stressors associated with climate change (temperature, water availability) are shown to have greater impact on growth than CO₂ concentration (Curtis & Wang 1998). Less studied is how elevated CO₂ affects growth performance of progeny. In their study on *Arabidopsis thaliana*, Teng et al. (2009) did not observe any lasting maternal effects over 15 generations in ambient or elevated CO₂, but consistent differences within each generation. Due to inherent difficulties of the task, few studies explore the maternal effects of elevated CO₂ on long-lived woody plant species. No difference in biomass was observed in the coniferous *Pinus taeda* with maternal plants grown in ambient and elevated CO₂ in a study by Hussain et al. (2001), however morphology was observed to differ with greater root length and more needles in plants sourced from elevated CO₂ maternal environments.

In this thesis, it was hypothesised that growth in terms of height and biomass would be greater for plants grown in elevated CO₂, and, despite past studies not observing maternal effects, greater growth for plants sourced from elevated CO₂ maternal environments than ambient CO₂ maternal environments due to greater provisioning of resources to seed. There was mixed support for this hypothesis, with *H. sericea* growing taller in elevated CO₂, and increased basal diameter and aboveground dry mass for plants sourced from elevated CO₂ maternal environments (Chapter 3). These results were striking, given there was no difference in biomass between plants from ambient CO₂ maternal environments grown in ambient and elevated CO₂ concentrations (as was seen for *H. sericea* planted into the EucFACE site; Collins et al. 2018), indicating growth performance of *H. sericea* may be more responsive to maternal effects of increased CO₂ than direct effects. It is unknown if, or how, the reproductive performance of progeny is affected by increased CO₂, as shifts in reproductive allocation may result in overall increase or decrease of reproductive output; the true measure of fitness. Given the observed increase in reproductive allocation (Chapter 2; not significant), there is potential for positive feedback to increase plant size and relative reproductive performance over successive generations resulting in overall increased fitness;

however, multi-generation studies on long-lived woody plants are required to test for positive feedback.

4.5 Acclimation of Photosynthetic Capacity

Short-term exposure to increased CO₂ concentrations has been extensively demonstrated to increase the productivity of the C₃ photosynthetic pathway through favouring the carboxylation reactions of Rubisco (Bazzaz 1990; Parmesan & Hanley 2015), however, when exposure is extended, plants may acclimate and photosynthetic capacity is down-regulated (Drake, González-Meler & Long 1997; Leakey et al. 2012). It is thought that acclimation of photosynthesis is an artefact of source-sink relations, with limitations on sink capacity when plants are grown in pots and apparent acclimation observed as photosynthesis is down-regulated (Arp 1991). Subsequent studies have shown that increased photosynthetic rates can be maintained when root capacity and/or nutrients are not limiting (Ellsworth et al. 2017; Ghannoum et al. 2010a; Norby et al. 1999). Photosynthetic capacity can be down-regulated via reduced Rubisco content and/or the production of Rubisco binding inhibitors (Galmes et al. 2013).

In Chapter 3 of this thesis, it was hypothesised that photosynthesis will increase with increasing CO₂ concentration when exposure is short-term; and that plants will acclimate to new conditions with long-term exposure and benefits of increased CO₂ will be diminished, and inversely, long-term exposure to lower CO₂ concentration will up-regulate photosynthetic capacity. Mixed support for this hypothesis was obtained, with photosynthesis of *H. sericea* sourced from elevated CO₂ maternal environments and grown in elevated CO₂ growth chamber acclimated to long-term exposure of elevated CO₂ by down-regulating photosynthesis. There was no decrease in leaf nitrogen content observed alongside this down-regulation of photosynthesis, so it is thought that either there is a shift in N allocation within leaves from Rubisco to non-photosynthetic components (Tissue, Thomas & Strain 1993), or the production of Rubisco binding inhibitors (Galmes et al. 2013). Interestingly, the same group of *H. sericea* (elevated CO₂ maternal environment) did not up-regulate photosynthesis in pre-industrial CO₂ concentrations; however, *H. sericea* sourced from ambient CO₂ maternal environments did acclimate to pre-industrial CO₂ concentrations by up-regulating photosynthesis but did not acclimate to elevated CO₂. This strong maternal effect in the ability to acclimate to CO₂ environments suggests an epigenetic mechanism may be involved.

4.6 Limitations of Study

This study has been limited to assessing the effects of elevated CO₂ on the reproductive performance of *E. tereticornis* and *H. sericea*, and subsequent early-life fitness of *H. sericea*. It is recognised that the increase of global atmospheric CO₂ does not occur in isolation and the abiotic environment is altered through changes in temperature and precipitation regimes. Many studies are exploring the interactions of temperature, water, and nutrient availability alongside elevated CO₂; however, the impacts on reproduction of long-lived woody plant species are largely understudied and differential impacts between plant functional types are poorly understood so more studies in this area are required to improve understanding of underlying mechanisms.

The studies undertaken for this thesis were not without challenges. Lower flowering intensity of *E. tereticornis* in 2018 resulted in fewer sampled branches than the desired replication. Additionally, initial experimental designs included *E. tereticornis* for assessment of early-life fitness and maternal effects in response to elevated CO₂, however, they had to be discarded from the experiment due to such low emergence in the growth chambers (showing sensitivity to desiccation with the high air flow in the growth chambers). It was thought that this might be due to limitations on Photosynthetically Active Radiation (PAR; 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) or no Ultraviolet (UV) Radiation. Gas exchange measurements on the needle leaves of *H. sericea* proved to be difficult, with much of the collected data having to be discarded. Conifer chambers for the Li-Cor photosynthesis systems were considered, however, due to the introduction of more variables (no light control, self-shading) the conifer chamber was ultimately decided against. This study has shown that plants which may generally be classed as the same plant functional type (evergreen woody perennial) differentially respond to future CO₂ concentrations. However, in order to make broader predictions on ecosystem responses, finer resolution of plant functional types need to be defined based on ecological principles (persistent, recolonisers). Species reliant on seed for regeneration are more likely to provision them with the resources and information required for survival.

4.7 Conclusion and Future Work

Findings from this study indicate reproductive performance of long-lived woody plant species will be increased under future atmospheric CO₂ concentrations through increases in reproductive effort, reproductive output and increased germinability of seed; however, the

magnitude of these responses are likely to differ between species with different life-histories. Differential responses between plant functional types to future CO₂ concentration over time may have profound implications on ecosystem structure and function (Garbutt, Williams & Bazzaz 1990). It is known that the maternal environment can influence the fitness of progeny (Vivas et al. 2019), however, for long-lived woody plants, maternal effects of elevated CO₂ has been overlooked. In this study, it is demonstrated that maternal effects may have greater impact on early-life fitness (as determined by biomass and photosynthetic capacity) than what is observed for a single generation in elevated CO₂. As such, future research should include multiple generations of exposure to elevated CO₂ to accurately determine responses to future climate conditions. Given that Teng et al. (2009) did not see any maternal effects over 15 generations of *Arabidopsis thaliana*, responses are likely to differ between plants with different life-histories. A finer resolution of plant functional types based on ecological principles is required to make broader predictions on ecosystem responses to future climate conditions. Although early-life fitness was increased for plants sourced from maternal plants grown in elevated CO₂, it remains unknown how subsequent reproductive performance may be affected. Further work in this area is required, and future studies could include impacts on phenology, pollen and nectar quality, and provisioning of seed resources.

5 References

- ABARES 2017, *Australia's forests at a glance*, Australian Government, Department of Agriculture and Water Resources, Canberra, Australia
- Ainsworth, E & Long, S 2005, 'What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂', *New Phytologist*, vol. 165, no. 2, pp. 351-372.
- Ainsworth, E & Rogers, A 2007, 'The response of photosynthesis and stomatal conductance to rising [CO₂]: mechanisms and environmental interactions', *Plant, Cell & Environment*, vol. 30, no. 3, pp. 258-270.
- Andalo, C, Raquin, C, Machon, N, Godelle, B & Mousseau, M 1998, 'Direct and Maternal Effects of Elevated CO₂ on Early Root Growth of Germinating *Arabidopsis thaliana* Seedlings', *Annals of Botany*, vol. 81, no. 3, pp. 405-411.
- Anderson, T, Hawkins, E & Jones, P 2016, 'CO₂, the greenhouse effect and global warming: from the pioneering work of Arrhenius and Callendar to today's Earth System Models', *Endeavour*, vol. 40, no. 3, pp. 178-187.
- Andersson, I & Backlund, A 2008, 'Structure and function of Rubisco', *Plant Physiology and Biochemistry*, vol. 46, Plant structural biology, no. 3, pp. 275-291.
- Aravind, J, Devi, S, Radhamani, J, Jacob, S & Kalyani Srinivasan 2019, 'germinationmetrics: Seed Germination Indices and Curve Fitting'. R package version 0.1.3
- Arp, W 1991, 'Effects of source-sink relations on photosynthetic acclimation to elevated CO₂', *Plant, Cell & Environment*, vol. 14, no. 8, pp. 869-875.
- Bates, D, Mächler, M, Bolker, B & Walker, S 2015, 'Fitting Linear Mixed-Effects Models Using lme4', *Journal of Statistical Software*, vol. 67, no. 1, pp. 1-48.
- Bazzaz, F 1990, 'The Response of Natural Ecosystems to the Rising Global CO₂ Levels', *Annual Review of Ecology and Systematics*, vol. 21, no. 1, pp. 167-196.
- Bazzaz, F, Ackerly, D & Reekie, E 2005, 'Reproductive Allocation in Plants', p. 31.
- Becklin, K, Walker, S, Way, D & Ward, J 2017, 'CO₂ studies remain key to understanding a future world', *New Phytologist*, vol. 214, no. 1, pp. 34-40.
- Beerling, D & Berner, R 2005, 'Feedbacks and the Coevolution of Plants and Atmospheric CO₂', *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 5, pp. 1302-1305.
- Berner, R 2006, 'GEOCARBSULF: A combined model for Phanerozoic atmospheric O₂ and CO₂', *Geochimica et Cosmochimica Acta*, vol. 70, no. 23, pp. 5653-5664.
- Birtchnell, M & Gibson, M 2006, 'Long-term flowering patterns of melliferous *Eucalyptus* (Myrtaceae) species', *Australian Journal of Botany*, vol. 54, no. 8, p. 745.
- Blankenship, R 1992, 'Origin and early evolution of photosynthesis', *Photosynthesis Research*, vol. 33, no. 2, pp. 91-111.
- Blankenship, R 2010, 'Early Evolution of Photosynthesis', *Plant Physiology*, vol. 154, no. 2, pp. 434-438.
- Blunden, J & Arndt, D 2019, 'State of the Climate in 2018', *Bulletin of the American Meteorological Society*, vol. 100, no. 9, p. 325.
- Bolas, B & Henderson, F 1928, 'The Effect of Increased Atmospheric Carbon Dioxide on the Growth of Plants', *Annals of Botany*, vol. 42, no. 2, pp. 509-523.
- Boyce, C & Lee, J 2017, 'Plant Evolution and Climate Over Geological Timescales', *Annual Review of Earth and Planetary Sciences*, vol. 45, no. 1, pp. 61-87.
- Brunel, S, Schrader, G, Brundu, G & Fried, G 2010, 'Emerging invasive alien plants for the Mediterranean Basin: Emerging invasive alien plants for the Mediterranean Basin', *EPPO Bulletin*, vol. 40, no. 2, pp. 219-238.

- Ceulemans, R & Mousseau, M 1994, 'Tansley Review No. 71 Effects of elevated atmospheric CO₂ on woody plants', *New Phytologist*, vol. 127, no. 3, pp. 425-446.
- Chen, J, Reynolds, J, Harley, P & Tenhunen, J 1993, 'Coordination theory of leaf nitrogen distribution in a canopy', *Oecologia*, vol. 93, no. 1, pp. 63-69.
- Collins, L, Boer, M, de Dios, V, Power, S, Bendall, E, Hasegawa, S, Hueso, R, Nevado, J & Bradstock, R 2018, 'Effects of competition and herbivory over woody seedling growth in a temperate woodland trump the effects of elevated CO₂', *Oecologia*, vol. 187, no. 3, pp. 811-823.
- Crous, K, Ósváldsson, A & Ellsworth, D 2015, 'Is phosphorus limiting in a mature Eucalyptus woodland? Phosphorus fertilisation stimulates stem growth', *Plant and Soil*, vol. 391, no. 1-2, pp. 293-305.
- Curtis, P & Wang, X 1998, 'A meta-analysis of elevated CO₂ effects on woody plant mass, form, and physiology', *Oecologia*, vol. 113, no. 3, pp. 299-313.
- Darbah, J, Kubiske, M, Nelson, N, Oksanen, E, Vaapavuori, E & Karnosky, D 2007, 'Impacts of Elevated Atmospheric CO₂ and O₃ on Paper Birch (*Betula papyrifera*): Reproductive Fitness', *The Scientific World Journal*, vol. 7, pp. 240-246.
- Darbah, J, Kubiske, M, Nelson, N, Oksanen, E, Vaapavuori, E & Karnosky, D 2008, 'Effects of decadal exposure to interacting elevated CO₂ and/or O₃ on paper birch (*Betula papyrifera*) reproduction', *Environmental Pollution*, vol. 155, no. 3, pp. 446-452.
- Davies, R, Di Saco, A & Newton, R 2015, 'Germination testing: procedures and evaluation', *Technical Information Sheet 13a*, Royal Botanic Gardens, Kew.
- Dippery, J, Tissue, D, Thomas, R & Strain, B 1995, 'Effects of low and elevated CO₂ on C₃ and C₄ annuals', *Oecologia*, vol 101, no. 1, pp. 13-20.
- Drake, B, González-Meler, M & Long, S 1997, 'More Efficient Plants: A Consequence of Rising Atmospheric CO₂?', *Annual Review of Plant Physiology and Plant Molecular Biology*, vol. 48, no. 1, pp. 609-639.
- Duursma, R 2015, 'Plantecophys - An R Package for Analysing and Modelling Leaf Gas Exchange Data', *PLoS ONE*, vol. 10, no. 11, p. e0143346.
- Edwards, G, Clark, H & Newton, P 2001, 'The effects of elevated CO₂ on seed production and seedling recruitment in a sheep-grazed pasture', *Oecologia*, vol. 127, no. 3, pp. 383-394.
- Ellsworth, D, Anderson, I, Crous, K, Cooke, J, Drake, J, Gherlenda, A, Gimeno et al. 2017, 'Elevated CO₂ does not increase eucalypt forest productivity on a low-phosphorus soil', *Nature Climate Change*, vol. 7, no. 4, pp. 279-282.
- Fairley, A & Moore, P 2010, *Native plants of the Sydney region: From Newcastle to Nowra and west to the Dividing Range*, 3rd edn, Allen & Unwin.
- Farooq, M, Basra, S, Ahmad, N & Hafeez, K 2005, 'Thermal Hardening: A New Seed Vigor Enhancement Tool in Rice', *Journal of Integrative Plant Biology*, vol. 47, no. 2, pp. 187-193.
- Finzi, A, Norby, R, Calfapietra, C, Gallet-Budynek, A, Gielen, B, Holmes, W, Hoosbeek, M et al. 2007, 'Increases in nitrogen uptake rather than nitrogen-use efficiency support higher rates of temperate forest productivity under elevated CO₂', *Proceedings of the National Academy of Sciences*, vol. 104, no. 35, pp. 14014-14019.
- Fiz-Palacios, O, Schneider, H, Heinrichs, J & Savolainen, V 2011, 'Diversification of land plants: insights from a family-level phylogenetic analysis', *BMC Evolutionary Biology*, vol. 11, no. 1, p. 341.
- Foster, G, Royer, D & Lunt, D 2017, 'Future climate forcing potentially without precedent in the last 420 million years', *Nature Communications*, vol. 8, p. 14845.
- Fox, J & Weisberg, S 2019, *An R Companion to Applied Regression*, Third, Sage, Thousand Oaks CA.
- Franks, P & Beerling, D 2009, 'CO₂-forced evolution of plant gas exchange capacity and water-use efficiency over the Phanerozoic', *Geobiology*, vol. 7, no. 2, pp. 227-236.
- Friedli, H, Löttscher, H, Oeschger, H, Siegenthaler, U & Stauffer, B 1986, 'Ice core record of the 13 C/ 12 C ratio of atmospheric CO₂ in the past two centuries', *Nature*, vol. 324, no. 6094, pp. 237-238.

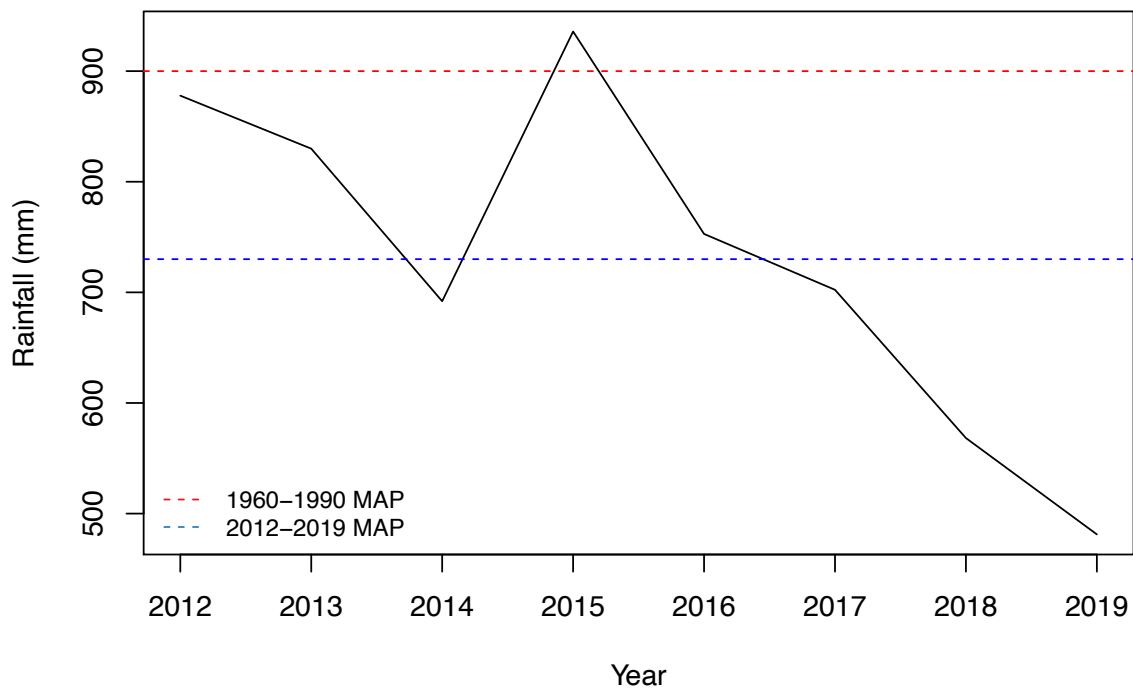
- Galmes, J, Aranjuelo, I, Medrano, H & Flexas, J 2013, 'Variation in Rubisco content and activity under variable climatic factors', *Photosynthesis research*, vol. 117, no. 1-3, pp. 73-90.
- Garbutt, K, Williams, W & Bazzaz, F 1990, 'Analysis of the Differential Response of Five Annuals to Elevated CO₂ during Growth', *Ecology*, vol. 71, no. 3, pp. 1185-1194.
- Gardiner, B, Berry, P & Moulia, B 2016, 'Review: Wind impacts on plant growth, mechanics and damage', *Plant Science*, vol. 245, pp. 94-118.
- Gerhart, L & Ward, J 2010, 'Plant responses to low [CO₂] of the past', *New Phytologist*, vol. 188, no. 3, pp. 674-695.
- Ghannoum, O, Phillips, N, Conroy, J, Smith, R, Attard, R, Woodfield, R, Logan, B et al. 2010a, 'Exposure to preindustrial, current and future atmospheric CO₂ and temperature differentially affects growth and photosynthesis in Eucalyptus', *Global Change Biology*, vol. 16, no. 1, pp. 303-319.
- Ghannoum, O, Phillips, N, Sears, M, Logan, B, Lewis, J, Conroy, J & Tissue, D 2010b, 'Photosynthetic responses of two eucalypts to industrial-age changes in atmospheric [CO₂] and temperature', *Plant, Cell & Environment*, vol. 33, no. 10, pp. 1671-1681.
- Gimeno, T, McVicar, T, O'Grady, A, Tissue, D & Ellsworth, D 2018, 'Elevated CO₂ did not affect the hydrological balance of a mature native *Eucalyptus* woodland', *Global Change Biology*, vol. 24, no. 7, pp. 3010-3024.
- Grünzweig, J & Dumbur, R 2012, 'Seed traits, seed-reserve utilization and offspring performance across pre-industrial to future CO₂ concentrations in a Mediterranean community', *Oikos*, vol. 121, no. 4, pp. 579-588.
- Hartig, F 2019, *DHARMA: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models*, R package version 0.2.4.
- Hendrey, G, Ellsworth, D, Lewin, K & Nagy, J 1999, 'A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO₂', *Global Change Biology*, vol. 5, no. 3, pp. 293-309.
- Hijmans, R, Cameron, S, Parra, J, Jones, P & Jarvis, A 2005, 'Very high resolution interpolated climate surfaces for global land areas', *International Journal of Climatology*, vol. 25, no. 15, pp. 1965-1978.
- Hovenden, M, Wills, K, Chaplin, R, Vander Schoor, J, Williams, A, Osanai, Y & Newton, P 2008, 'Warming and elevated CO₂ affect the relationship between seed mass, germinability and seedling growth in *Austroanthonia caespitosa*, a dominant Australian grass', *Global Change Biology*, vol. 14, no. 7, pp. 1633-1641.
- Huang, G, Rymer, P, Duan, H, Smith, R & Tissue, D 2015, 'Elevated temperature is more effective than elevated [CO₂] in exposing genotypic variation in *Telopea speciosissima* growth plasticity: implications for woody plant populations under climate change', *Global Change Biology*, vol. 21, no. 10, pp. 3800-3813.
- Hussain M, Kubiske M & Connor K 2001, 'Germination of CO₂-enriched *Pinus taeda* L. seeds and subsequent seedling growth responses to CO₂ enrichment', *Functional Ecology*, vol. 15, no. 3, pp. 344-350.
- IPCC 2014, *Climate change 2014: synthesis report*, Intergovernmental Panel on Climate Change, Geneva, Switzerland.
- Jablonski, L, Wang, X & Curtis, P 2002, 'Plant reproduction under elevated CO₂ conditions: a meta-analysis of reports on 79 crop and wild species', *New Phytologist*, vol. 156, no. 1, pp. 9-26.
- Jones, T & Reekie, E 2007, 'Effect of seed size on seedling growth response to elevated CO₂ in *Picea abies* and *Picea rubens*', *Plant Biology*, vol. 9, no. 6, pp. 766-775.
- Keeley, J & Rundel, P 2003, 'Evolution of CAM and C₄ Carbon-Concentrating Mechanisms', *International Journal of Plant Sciences*, vol. 164, no. S3, pp. S55-S77.
- Keeling, C, Bacastow, R, Bainbridge, A, Ekdahl, C, Guenther, P, Waterman, L & Chin, J 1976, 'Atmospheric carbon dioxide variations at Mauna Loa Observatory, Hawaii', *Tellus*, vol. 28, no. 6, pp. 538-551.
- Kenrick, P & Crane, P 1997, 'The origin and early evolution of plants on land', *Nature*, vol. 389, no. 6646, pp. 33-39.

- Kinoshita, T & Seki, M 2014, 'Epigenetic Memory for Stress Response and Adaptation in Plants', *Plant and Cell Physiology*, vol. 55, no. 11, pp. 1859-1863.
- Kirschbaum, M 2011, 'Does Enhanced Photosynthesis Enhance Growth? Lessons Learned from CO₂ Enrichment Studies', *Plant Physiology*, vol. 155, no. 1, pp. 117-124.
- Körner, C 2006, 'Plant CO₂ responses: an issue of definition, time and resource supply', *New Phytologist*, vol. 172, no. 3, pp. 393-411.
- Kreusler, U 1885, 'Über eine methode zur beobachtung der assimilation und atmung der pflanzen und über einige diese vorgänge beeinflussende momente', *Landwirtschaftliche Jahrbücher*, vol. 14, pp. 913-965.
- Krogmann, D 2005, 'Discoveries in oxygenic photosynthesis (1727-2003): a perspective', *Photosynthesis Research*, vol. 80, no. 1-3 pp. 63-105.
- LaDeau, S & Clark, J 2001, 'Rising CO₂ Levels and the Fecundity of Forest Trees', *Science*, vol. 292, no. 5514, pp. 95-98.
- Ladeau, S & Clark, J 2006, 'Elevated CO₂ and tree fecundity: the role of tree size, interannual variability, and population heterogeneity', *Global Change Biology*, vol. 12, no. 5, pp. 822-833.
- Lapola, D & Norby, R 2014, 'AmazonFACE: Assessing the effects of increased atmospheric CO₂ on the ecology and resilience of the Amazon forest science plan and implementation strategy', Brasília: Ministério de Ciência, Tecnologia e Inovação, p. 56.
- Lawes, M & Clarke, P 2011, 'Ecology of plant resprouting: populations to community responses in fire-prone ecosystems', *Plant Ecology*, vol. 212, no. 12, pp. 1937-1943.
- Leakey, A, Ainsworth, E, Bernacchi, C, Zhu, X, Long, S & Ort, D 2012, 'Photosynthesis in a CO₂-rich atmosphere', *Photosynthesis*, vol. 34, pp. 733-768.
- Leakey, A, Ainsworth, E, Bernacchi, C, Zhu, X, Long, S & Ort, D 2012, 'Photosynthesis in a CO₂-Rich Atmosphere', *Photosynthesis: Advances in Photosynthesis and Respiration*, vol 34. Springer, Dordrecht.
- Lenth, R 2019, *emmeans: Estimated Marginal Means, aka Least-Squares Means*. R package version 1.4
- Long, S 2003, 'Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error', *Journal of Experimental Botany*, vol. 54, no. 392, pp. 2393-2401.
- Lüthi, D, Le Floch, M, Bereiter, B, Blunier, T, Barnola, J, Siegenthaler, U, Raynaud, D et al. 2008, 'High-resolution carbon dioxide concentration record 650,000-800,000 years before present', *Nature*, vol. 453, no. 7193, pp. 379-382.
- Martínez-Botí, M, Foster, G, Chalk, T, Rohling, E, Sexton, P, Lunt, D, Pancost, R et al. 2015, 'Plio-Pleistocene climate sensitivity evaluated using high-resolution CO₂ records', *Nature*, vol. 518, no. 7537, pp. 49-54.
- Marty, C & BassiriRad, H 2014, 'Seed germination and rising atmospheric CO₂ concentration: A meta-analysis of parental and direct effects', *New Phytologist*, vol. 202, no. 2, pp. 401-414.
- Mayeux, H, Johnson, H, Polley, H, Dumesnil, M & Spanel, G 1993, 'A Controlled Environment Chamber for Growing Plants Across a Subambient CO₂ Gradient', *Functional Ecology*, vol. 7, no. 1, pp. 125-133.
- McFadden, G 2001, 'Primary and Secondary Endosymbiosis and the Origin of Plastids', *Journal of Phycology*, vol. 37, no. 6, pp. 951-959.
- Moles, A & Westoby, M 2006, 'Seed size and plant strategy across the whole life cycle', *Oikos*, vol. 113, no. 1, pp. 91-105.
- NOAA 2019, *ESRL Global Monitoring Division - Global Greenhouse Gas Reference Network*, US Department of Commerce, viewed 18 December 2019 <<https://www.esrl.noaa.gov/gmd/ccgg/trends/global.html#global>>.
- Norby, R, De Kauwe, M, Domingues, T, Duursma, R, Ellsworth, D, Goll, D, Lapola, D et al. 2016, 'Model-data synthesis for the next generation of forest free-air CO₂ enrichment (FACE) experiments', *New Phytologist*, vol. 209, no. 1, pp. 17-28.
- Norby, R, Wullschleger, S, Gunderson, C, Johnson, D & Ceulemans, R 1999, 'Tree responses to rising CO₂ in field experiments: implications for the future forest', *Plant, Cell & Environment*, vol. 22, no. 6, pp. 683-714.

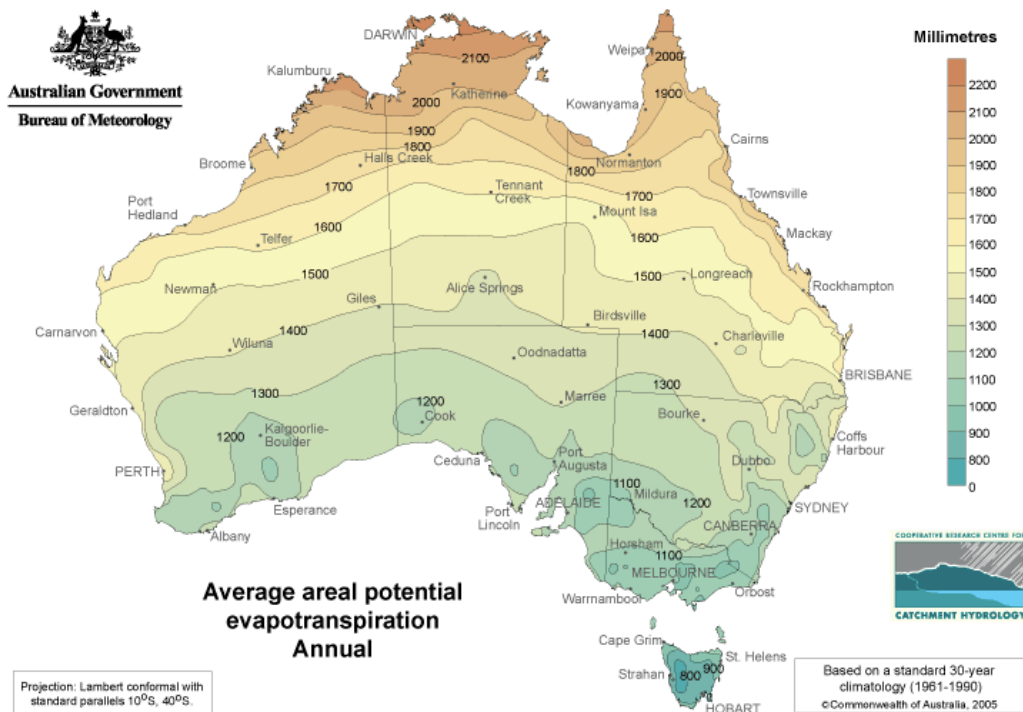
- Norby, R & Zak, D 2011, 'Ecological Lessons from Free-Air CO₂ Enrichment (FACE) Experiments', *Annual Review of Ecology, Evolution, and Systematics*, vol. 42, pp. 181-203.
- Ochoa-Hueso, R, Hughes, J, Delgado-Baquerizo, M, Drake, J, Tjoelker, M, Piñeiro, J & Power, S 2017, 'Rhizosphere-driven increase in nitrogen and phosphorus availability under elevated atmospheric CO₂ in a mature Eucalyptus woodland', *Plant and Soil*, vol. 416, no. 1-2, pp. 283-295.
- Parnesan, C & Hanley, M 2015, 'Plants and climate change: complexities and surprises', *Annals of Botany*, vol. 116, no. 6, pp. 849-864.
- Parnesan, C & Yohe, G 2003, 'A globally coherent fingerprint of climate change impacts across natural systems', *Nature*, vol. 421, no. 6918, pp. 37-42.
- Pepin, S & Körner, C 2002, 'Web-FACE: A new canopy free-air CO₂ enrichment system for tall trees in mature forests', *Oecologia*, vol. 133, no. 1, pp. 1-9.
- Pérez-Harguindeguy, N, Díaz, S, Garnier, E, Lavorel, S, Poorter, H, Jaureguiberry, P, Bret-Harte, M et al. 2013, 'New handbook for standardised measurement of plant functional traits worldwide', *Australian Journal of Botany*, vol. 61, no. 3, p. 167.
- Pritchard, S, Rogers, H, Prior, S & Peterson, C 1999, 'Elevated CO₂ and plant structure: A review', *Global Change Biology*, vol. 5, no. 7, pp. 807-837.
- R Core Team 2019, *R: A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing, Vienna, Austria.
- Raynaud, D & Barnola, J 1985, 'An Antarctic ice core reveals atmospheric CO₂ variations over the past few centuries', *Nature*, vol. 315, no. 6017, pp. 309-311.
- Reekie, E & Bazzaz, F 1987, 'Reproductive Effort in Plants. 1. Carbon Allocation to Reproduction', *The American Naturalist*, vol. 129, no. 6, pp. 876-896.
- Rogers, H, Peterson, C, McCrimmon, J & Cure, J 1992, 'Response of plant roots to elevated atmospheric carbon dioxide', *Plant, Cell & Environment*, vol. 15, no. 6, pp. 749-752.
- Saban, J, Chapman, M & Taylor, G 2019, 'FACE facts hold for multiple generations; Evidence from natural CO₂ springs', *Global Change Biology*, vol. 25, no. 1, pp. 1-11.
- Schiermeier, Q 2019, 'Antarctic project to drill for oldest-ever ice core', *Nature*, vol. 567, pp. 442-443.
- Sharma, R, Griffing, B & Scholl, R 1979, 'Variations among races of *Arabidopsis thaliana* (L.) heynh for survival in limited carbon dioxide', *Theoretical and Applied Genetics*, vol. 54, no. 1, pp. 11-15.
- Shiple, B & Parent, M 1991, 'Germination Responses of 64 Wetland Species in Relation to Seed Size, Minimum Time to Reproduction and Seedling Relative Growth Rate', *Functional Ecology*, vol. 5, no. 1, pp. 111-118.
- Singroha, G & Sharma, P 2019, 'Epigenetic Modifications in Plants under Abiotic Stress', *Epigenetics*, IntechOpen
- Souza, J, Jesus Melo, N, Halfeld, A, Vieira, K & Rosa, B 2019, 'Elevated atmospheric CO₂ concentration improves water use efficiency and growth of a widespread Cerrado tree species even under soil water deficit', *Acta Botanica Brasilica*, vol. 33, no. 3, pp. 425-436.
- Steinger, T, Gall, R & Schmid, B 2000, 'Maternal and direct effects of elevated CO₂ on seed provisioning, germination and seedling growth in *Bromus erectus*', *Oecologia*, vol. 123, no. 4, pp. 475-480.
- Taiz, L 2015, *Plant Physiology and Development*, Sinauer Associates.
- Taub, D & Wang, X 2008a, 'Why are Nitrogen Concentrations in Plant Tissues Lower under Elevated CO₂? A Critical Examination of the Hypotheses', *Journal of Integrative Plant Biology*, vol. 50, no. 11, pp. 1365-1374.
- Temme, A, Liu, J, Cornwell, W, Cornelissen, J & Aerts, R 2015, 'Winners always win: growth of a wide range of plant species from low to future high CO₂', *Ecology and Evolution*, vol. 5, no. 21, pp. 4949-4961.
- Temme, A, Liu, J, van Hal, J, Cornwell, W, Cornelissen, J & Aerts, R 2017, 'Increases in CO₂ from past low to future high levels result in "slower" strategies on the leaf economic spectrum', *Perspectives in Plant Ecology, Evolution and Systematics*, vol. 29, pp. 41-50.

- Teng, N, Jin, B, Wang, Q, Hao, H, Ceulemans, R, Kuang, T & Lin, J 2009, 'No detectable maternal effects of elevated CO₂ on *Arabidopsis thaliana* over 15 generations', *PLoS ONE*, vol. 4, no. 6, p. e6035.
- Thürig, B, Körner, C & Jürg, S 2003, 'Seed production and seed quality in a calcareous grassland in elevated CO₂', *Global Change Biology*, vol. 9, no. 6, pp. 873-884.
- Tissue, D, Griffin, K, Thomas, R & Strain, B 1995, 'Effects of low and elevated CO₂ on C₃ and C₄ annuals', *Oecologia*, vol. 101, no. 1, pp. 21-28.
- Tissue, D & Lewis, J 2010, 'Photosynthetic responses of cottonwood seedlings grown in glacial through future atmospheric [CO₂] vary with phosphorus supply', *Tree Physiology*, vol. 30, no. 11, pp. 1361-1372.
- Tissue, D, Thomas, R & Strain, B 1993, 'Long-term effects of elevated CO₂ and nutrients on photosynthesis and rubisco in loblolly pine seedlings', *Plant, Cell & Environment*, vol. 16, no. 7, pp. 859-865.
- Tozer, M, Turner, K, Keith, D, Tindall, D, Pennay, C, Simpson, C, MacKenzie, B et al. 2010, 'Native vegetation of southeast NSW: a revised classification and map for the coast and eastern tablelands', *Cunninghamia*, vol. 11, no. 3, pp. 359-406.
- UNEP, Middleton, N & Thomas, D 1992, *World atlas of desertification*, Edward Arnold, London.
- Venter, Z, Cramer, M & Hawkins, H 2018, 'Drivers of woody plant encroachment over Africa', *Nature Communications*, vol. 9, no. 1, pp. 1-7.
- Verdú, M & Traveset, A 2005, 'Early Emergence Enhances Plant Fitness: A Phylogenetically Controlled Meta-Analysis', *Ecology*, vol. 86, no. 6, pp. 1385-1394.
- Vivas, M, Rolo, V, Wingfield, M & Slippers, B 2019, 'Maternal environment regulates morphological and physiological traits in *Eucalyptus grandis*', *Forest Ecology and Management*, vol. 432, pp. 631-636.
- van Vuuren, D, Edmonds, J, Kainuma, M, Riahi, K, Thomson, A, Hibbard, K, Hurtt, G et al. 2011, 'The representative concentration pathways: An overview', *Climatic Change*, vol. 109, no. 1-2, pp. 5-31.
- Wang, X, Taub, D & Jablonski, L 2015, 'Reproductive allocation in plants as affected by elevated carbon dioxide and other environmental changes: A synthesis using meta-analysis and graphical vector analysis', *Oecologia*, vol. 177, no. 4, pp. 1075-1087.
- Ward, J, Antonovics, J, Thomas, R & Strain, B 2000, 'Is atmospheric CO₂ a selective agent on model C₃ annuals?', *Oecologia*, vol. 123, no. 3, pp. 330-341.
- Ward, J & Strain, B 1999, 'Elevated CO₂ studies: past, present and future', *Tree Physiology*, vol. 19, no. 4-5, pp. 211-220.
- Way, D, Ladeau, S, McCarthy, H, Clark, J, Oren, R, Finzi, A & Jackson, R 2010, 'Greater seed production in elevated CO₂ is not accompanied by reduced seed quality in *Pinus taeda* L.', *Global Change Biology*, vol. 16, no. 3, pp. 1046-1056.
- Western Sydney University (WSU) 2019, *EucFACE*, viewed 25 November 2019, <<https://www.westernsydney.edu.au/hie/EucFACE>>.
- Woodward, F 1987, 'Stomatal numbers are sensitive to increases in CO₂ from pre-industrial levels', *Nature*, vol. 327, no. 6123, pp. 617-618.
- Yoon, H, Hackett, J, Ciniglia, C, Pinto, G & Bhattacharya, D 2004, 'A Molecular Timeline for the Origin of Photosynthetic Eukaryotes', *Molecular Biology and Evolution*, vol. 21, no. 5, pp. 809-818.
- Younginger, B, Sirová, D, Cruzan, M & Ballhorn, D 2017, 'Is biomass a reliable estimate of plant fitness?', *Applications in Plant Sciences*, vol. 5, no. 2, p. 1600094.

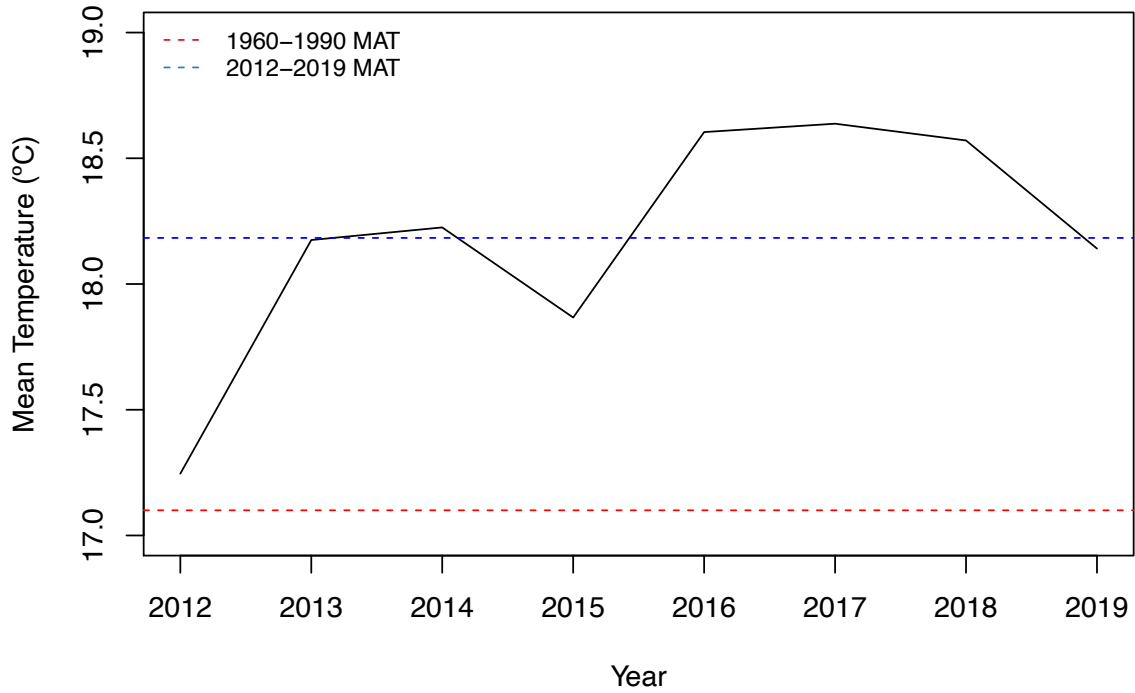
6 Appendix



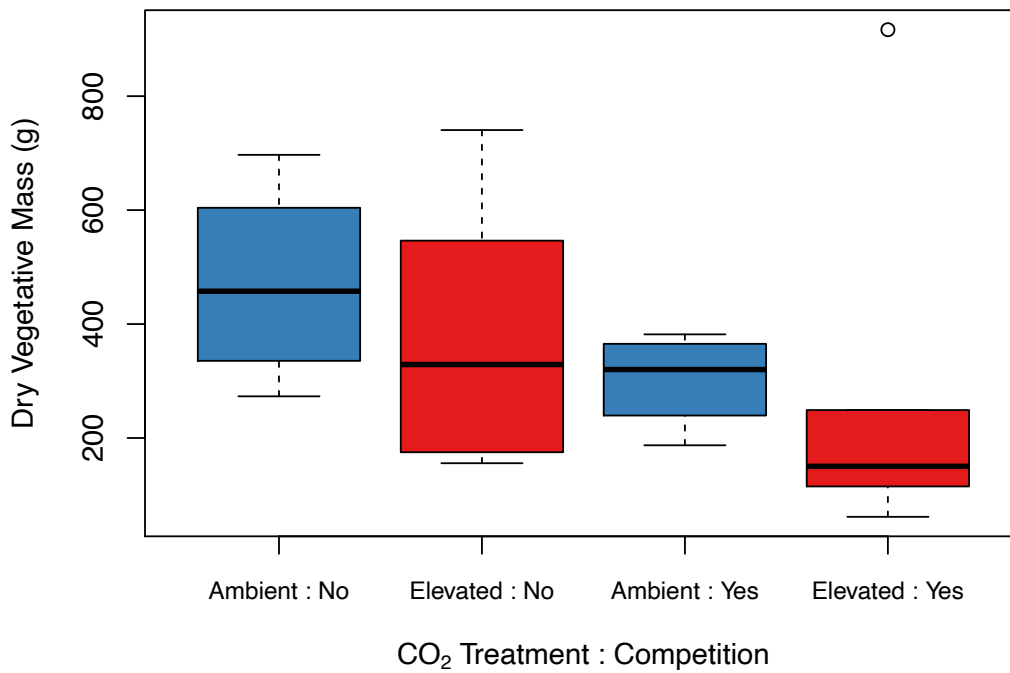
Appendix 1. Annual rainfall for Richmond, NSW, recorded by Bureau of Meteorology (BOM Station 67021) since fumigation began at EucFACE. Long-term Mean Annual Precipitation (MAP) derived from WorldClim (Hijmans et al. 2005)



Appendix 2. Potential evapotranspiration in Australia (Richmond PET $\approx 1209 \text{ mm yr}^{-1}$). Graph sourced from BOM



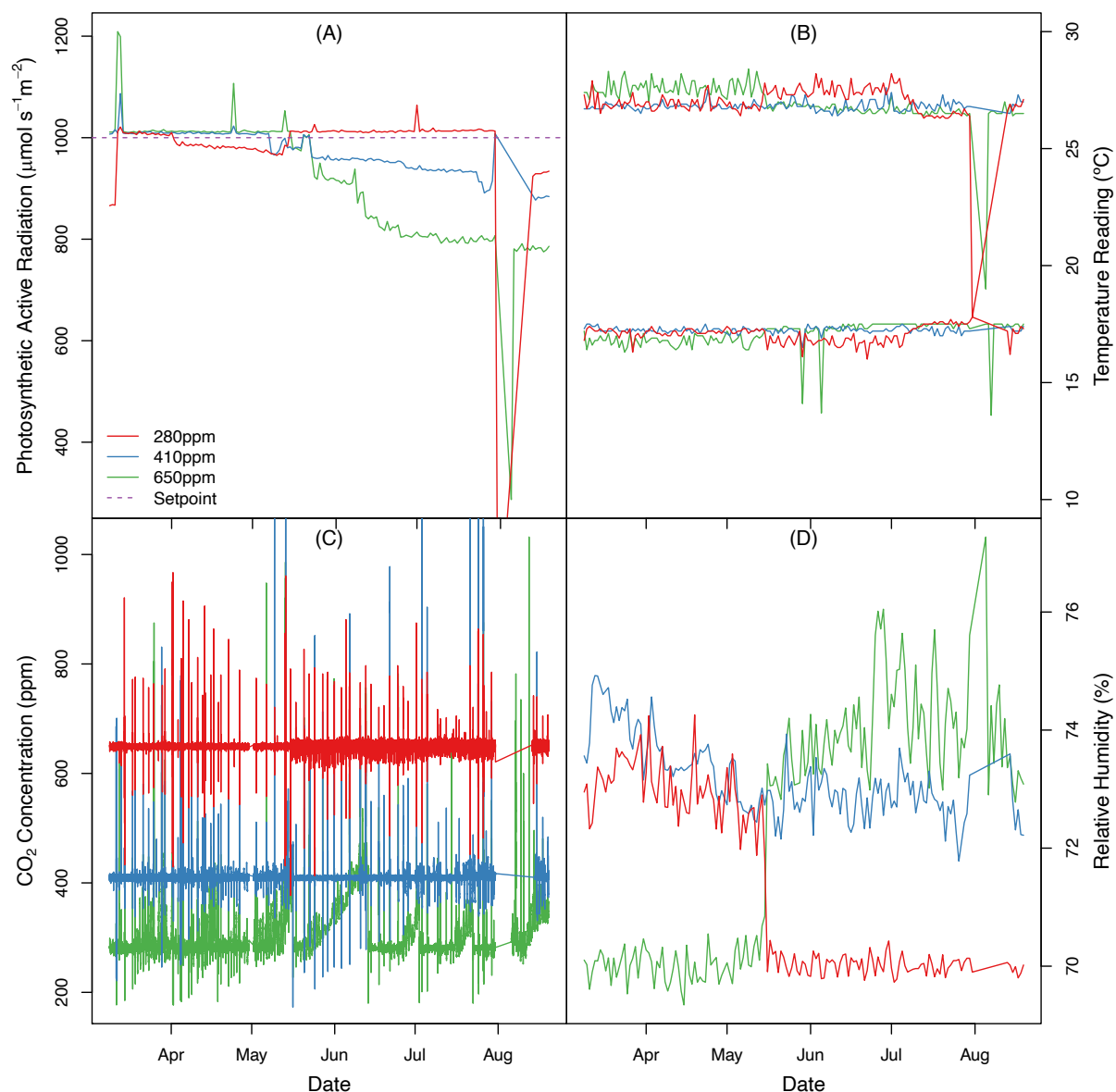
Appendix 3. Mean annual temperature in Richmond, NSW, recorded by Bureau of Meteorology (BOM Station 67021) since fumigation began at EucFACE. Mean temperature calculated as average of daily minimum and maximum temperatures. Long-term Mean Annual Temperature (MAT) derived from WorldClim (Hijmans et al. 2005).



Appendix 4. Dry vegetative aboveground mass of *H. sericea* grown in ambient and elevated CO₂ with and without the presence of competitive herbaceous species in the context of Collins et al. (2018).

Appendix 5. Summary of models used to test for the effect of CO₂ on reproductive performance of *E. tereticornis* and *H. sericea*. All models were Linear Mixed Models (LMM) or Generalised Linear Mixed Models (GLMM). * = Reproductive allocation for buds of *E. tereticornis*; # = Reproductive allocation of fruits for *E. tereticornis* and *H. sericea*

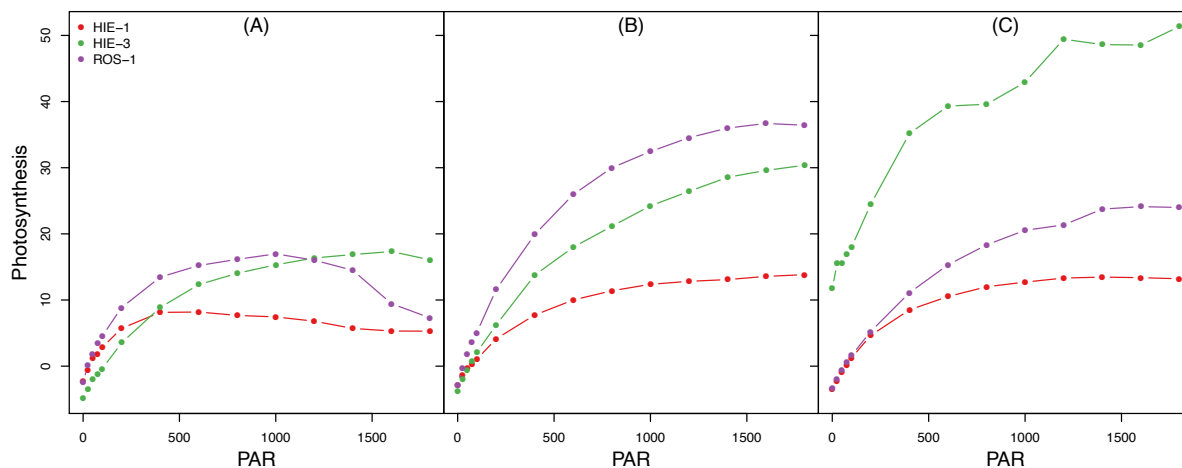
Response Variable	Model	Random Effects	Distribution	Transformation	P
<i>Eucalyptus tereticornis</i>					
Individual Bud Weight	LMM	Tree	Normal	None	0.2449
Total Bud Weight	LMM	Tree	Normal	Log _e	0.07352
Bud Count	GLMM	Tree	Poisson	None	0.1989
Reproductive Allocation *	LMM	Ring	Normal	Arcsine Square-root	0.1706
Energy Content	LMM	Ring	Normal	None	0.296
C:N	LMM	Ring/Tree	Normal	None	0.5693
Individual Fruit Weight	LMM	Tree	Normal	None	0.1463
Total Fruit Weight	LMM	Tree	Normal	Log _e	0.9027
Fruit Count	GLMM	Tree	Poisson	None	0.2028
Reproductive Allocation #	LMM	Tree	Normal	Log _e	0.6758
Seed per Fruit	LMM	Tree	Normal	None	0.2583
Individual Seed Weight	LMM	Tree	Normal	None	0.811
Total Seed Weight	LMM	Tree	Normal	None	0.9008
Seed Count	GLMM	Tree	Poisson	None	0.909
germinated/not germinated	GLMM	Tree	Binomial	None	0.1559
T ₅₀	LMM	Tree	Normal	None	0.644
<i>Hakea sericea</i>					
Individual Fruit Weight	LMM	Ring	Normal	None	0.3066
Total Fruit Weight	LMM	Ring	Normal	Log _e	0.4882
Fruit Count	GLMM	Ring	Poisson	None	0.7402
Reproductive Allocation #	LMM	Ring	Normal	None	0.3215
Seed per Fruit	-	-	-	-	-
Individual Seed Weight	LMM	Ring	Normal	None	0.6359
Total Seed Weight	LMM	Ring	Normal	Log _e	0.3934
Seed Count	GLMM	Ring	Poisson	None	0.125
germinated/not germinated	GLMM	Plot	Binomial	None	0.01363
T ₅₀	LMM	Plot	Normal	None	0.3472



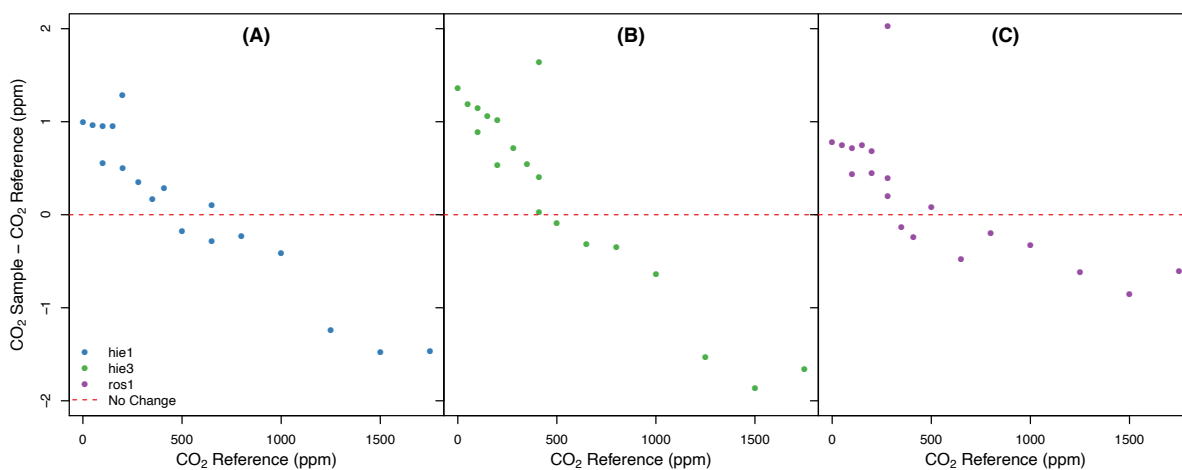
Appendix 6. Climate-controlled growth chamber conditions throughout the experimental period for (A) Photosynthetically Active Radiation, (B) day and night temperature, (C) chamber CO₂ concentration, and (D) relative humidity. Data for early August are missing.

Appendix 7. Summary of chamber conditions throughout experimental period. Values are calculated means +/- standard error.

Parameter	Pre-Industrial	Ambient	Elevated
CO ₂ (ppm)	309.41 +/- 0.25	413.36 +/- 0.20	649.97 +/- 0.07
Relative Humidity (%)	72.30 +/- 0.02	73.17 +/- 0.02	71.34 +/- 0.02
Temperature Day (°C)	25.97 +/- 0.003	25.97 +/- 0.002	25.97 +/- 0.003
Temperature Night (°C)	17.93 +/- 0.003	17.94 +/- 0.002	17.95 +/- 0.003
PAR (μmol m ⁻² s ⁻¹)	897.89 +/- 0.69	960.56 +/- 0.31	983.83 +/- 0.27



Appendix 8. Light response curves of *Hakea sericea* with the Li-Cor units used for gas exchange measurements at (A) pre-industrial, (B) ambient, and (C) elevated CO_2 concentrations.



Appendix 9. Diffuse leakage of Li-Cor LI-6400 portable photosynthesis systems taking measurements on oven-dried needle leaves of *H. sericea* with increasing CO_2 concentration.

Appendix 10. Summary of parameter settings used in measuring A/C_i curves for *H. sericea*

Parameter	Setpoint
Flow Rate ($\mu\text{mol s}^{-1}$)	300
Block Temperature ($^{\circ}\text{C}$)	25
PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	1200
Stability (min)	1 - 3 (min/max)
CO_2 (ppm)	{Growing CO_2 } 200, 150, 100, 50, 0, 100, 200, 280, 350, 410, 500, 650, 800, 1000, 1250, 1500, 1750, {Growing CO_2 }

Appendix 11. Calorimetry methods

Dried bud tissues were finely ground using Foss Cyclotec Sample Mill with a 0.5 mm screen and stored in Microcentrifuge Eppendorf Tubes. Grinding equipment was cleaned with ethanol between samples to prevent cross contamination from sticky residue. Ground bud tissues were used to determine energy and nitrogen content. To determine the energy content of *E. tereticornis* floral buds, heat of combustion (H_C) was measured for ground plant material. To measure H_C , 14 mg of ground material was weighed out with a 4-decimal balance prior to being pressed into a pellet with Specac 5 mm Evacuatable Pellet Die with 1.25 t of pressure using a hydraulic press, cleaning all press and die equipment with 70% methanol between each sample. The final weight of each pellet was recorded prior to combustion with pressure at 400 psi within a microbomb calorimeter and temperature difference recorded with CR800 data logger. A standard curve with benzoic acid of known calorific value was produced to determine energy content within bud tissues.