Faculty of Science and Engineering
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Evolutionary Adaptations to Climate Change in Australian Flora

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made.

This thesis contains no material that has been accepted for the award of any other degree or diploma in any university.

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Abstract

Climate changes have been impacting ecosystems worldwide, causing shifts in the habitats suitable for plant species and threatening their continuing persistence. Mediterranean-type Ecosystems contain extraordinarily high levels of plant diversity and endemism, yet are vulnerable to climate and environmental changes. Southwest Western Australia is recognised as a global biodiversity hotspot, though has been experiencing decreased rainfall and increased drought over the past 40 years, causing great concerns for the long-term survival of its extraordinary plant diversity. Plants may respond to changes in climate by migrating to new habitats, persisting in their current habitat, or by adapting to changes through evolution. In this thesis, I investigated the potential of Southwest Western Australian plants to rapidly evolve to tolerate changes in climate, particularly decreased rainfall and increased temperature. I first assessed the floristic composition of a nature reserve in speciose kwongan heathland following four decades of decreased rainfall and increased temperature through comparison of a present day vegetation survey to historic surveys. In Chapter 3, I further investigated the potential of seed banks to mitigate the effects of decreased rainfall through comparison of the growth of seedlings descended from parents established in years of either average or below-average rainfall when subjected to drought and control treatments. In Chapter 4, I explored the potential of *Banksia hookeriana* (Proteaceae) to accumulate adaptive genetic variation in order to resist the effects of a drying climate through the assessment of the fitness of seedlings established from seed produced pre- and post- drought to determine whether post-drought seed displayed increased drought tolerance. Finally, in Chapter 5, I investigated whether adaptive genetic diversity in *B. attenuata* was affected by rainfall and temperature gradients, or altered fire regime through genomic scanning and environmental association analysis on plants distributed across its range to determine candidate genes associated with these environmental variables. Overall, results from my study suggest that the flora of Southwest Western Australia has been able to tolerate the changes in climate that have occurred so far, and that there may be potential for tolerance of further changes in climate. However changes in fire regime in the region may have a detrimental effect on the ability of some species to adapt and survive.

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List of Abbreviations

AMPK	Adenosine monophosphate-activated protein kinase gene
ANOSIM	Analysis of Similarity
ANOVA	Analysis of Variance
AR	Arthur River
ATP	Adenosine Triphosphate
BAM	Binary version of SAM file of extension .bam
BLASTN	Basic Local Alignment Search Tool for Nucleotides
bp	Base pairs
BW	Brunswick
cDNA	Complementary DNA
CSB	Canopy Seed Bank
DEGs	Differentially Expressed Genes
DEPC Water	Diethylpyrocarbonate Water
DNA	Deoxyribonucleic acid
EN	Eneabba
E-value	Expected value
FR	Fitzgerald River
Fst	Fixation Index; measure of population differentiation
GB	Gigabytes
GC content	Guanine-cytosine base content
GEF	Guanine nucleotide exchange factor gene
GM	Goomalling
GO term	Gene Ontology term
He	Expected Heterozygosity
HiR	High Rainfall
IBRA	Interim Biogeographic Regionalisation for Australia
KB	Kalbarri
KEGG	Kyoto Encyclopedia of Genes and Genomes
LD	Leda
LMA	Leaf Mass per Area
LoR	Low Rainfall
LU	Cape Leeuwin
Ma	Million years
Mb	Million base pairs

MDH	Malate Dehydrogenase
MID barcode	Molecular Identifier barcode
MTE	Mediterranean Type Ecosystem
NCBI	National Center for Biotechnology Information
nMDs	Non-metric Multi-dimensional scaling
Nr	Non redundant (protein database)
NRS	Non Resprouter
mRNA	Messenger RNA
N	Ambiguous nucleotide bases
nt	Nucleotide
PPL	Percentage Polymorphic Loci
Q	Quality score
RAD-Seq	Restriction-site Associated DNA Sequencing
RNA	Ribonucleic Acid
RS	Resprouter
S1P	Sphingosine-1-phosphate phosphatase gene
SAM	Tab delimited file of extension .sam
SE genome	Single End
SE	Standard Error
SENR	South Eneabba Nature Reserve
SLA	Specific Leaf Area
SNPs	Single Nucleotide Polymorphisms
SSB	Soil Seed Bank
SSR	Simple Sequence Repeats
SWA	Southwest Australia
SWAFR	Southwest Australian Floristic Region
WA	Western Australia
YC	Yanchep

Chapter 1: An introduction to the kwongan vegetation of Southwest Australia and potential threats from climate change.

1.1 Climate change and plant response

1.1.1 Climate change over the past half century

Climate is the most dominant factor influencing the broad scale distribution of plant species (Sykes 2009). Global changes in climate are altering the suitable habitats for plant species worldwide, causing concern for the continued persistence of numerous species (Sykes 2009; Urban 2015). The Intergovernmental Panel on Climate Change (IPCC 2013) reports that many changes in climate and weather events have occurred across the globe over the period from the 1950s until now and are forecast to continue into the future. Days and nights have become warmer, the frequency of hot days and nights has increased and the number of cold days and nights has decreased. The frequency and intensity of heavy precipitation events, drought events, and heat waves have all increased, as well as the frequency of intense cyclones. Additionally, polar ice sheets have been losing mass, glaciers have been shrinking, and Northern Hemisphere snow cover has been decreasing. There has also been a warming of ocean surface temperatures by 0.11˚C per decade since 1971, an increase in the rate of sea-level rise by 0.19 m over the past century, and an increase in atmospheric concentrations of greenhouse gases by 40% since pre-industrial times. These changes in climate are already influencing vegetation patterns and will continue to do so into the future, shifting the spatial ranges in which species can survive (Sykes 2009).

1.1.2 Plant responses to climate change

Plants respond to climate change in one of four ways; they persist within their current distribution, they migrate to more suitable areas, they face local extinction, or they adapt through evolution (Aitken *et al*. 2008; Corlett & Westcott 2013). Species may persist within their current habitat and range if the changes in climate that occur are insufficient in eliciting a stronger response, or if changes in climate are within the range of tolerance for the species. For example; some *Eucalyptus* species have been found to exhibit tolerance to a wider range of climatic conditions than their natural distribution

would suggest (Booth *et al*. 1988; Butt *et al.* 2013; Booth *et al.* 2015). Booth *et al*. demonstrated that *Eucalyptus regnans* was able to grow at sites 5˚C warmer than the hottest location in its natural habitat (Booth *et al*. 1988; Booth *et al*. 2013). Migration, a geographic shift in the distribution of a species, may occur when species move to areas adjacent to their current range of distribution when these areas become suitable habitat as a result of climate change (Christmas *et al.* 2016). Plants achieve migration through seed dispersal into areas with a suitable climate, and rare instances of long-distance dispersal of seeds are beneficial in hastening the process of migration (Aitken *et al*. 2008; Corlett & Westcott 2013; Christmas *et al*. 2016). For example; Kelly and Goulden (2008) demonstrated range shifts in dominant mountain flora in California over 40 years of climate warming, with species ranges shifting up an elevation gradient into areas of cooler temperatures. Plant species may become extinct at a local level or as a whole as a result of climate change; this can be due to a species not being able to physiologically tolerate changes such as higher temperatures or decreased rainfall, or due to indirect effects of climate change. For example, changes in climate may lead to changes in the distribution of pollinators essential for the continued existence of plant species, or increased distribution of pathogens and competitor species, all of which could lead to the eventual loss of a species from an area (Cahill *et al.* 2012). Alternatively, plants may respond to changes in climate through evolution. Rapid evolution, the process of evolutionary changes occurring at the timescale of decades, has in the past been a rarely considered cause for changes observed in populations. However, an increasing number of studies have observed rapid evolutionary changes over recent decades (Thompson 1998; Hendry *et al*. 2010; Sgrò *et al.* 2010). Changing climates may result in strong environmental selection pressure, inducing evolutionary adaptations in species, and increasing a species' tolerance to new climatic conditions (Bijlsma & Loeschcke 2005; Hoffmann & Willi 2008; Williams *et al.* 2008).

1.1.3 Evolutionary adaptations as a result of climate change

Changes in climate may impose strong selection pressure on species, which may result in the selection of more stress-tolerant genotypes. In theory, all plants have some innate capacity to adapt to changing conditions through either phenotypic plasticity or evolutionary adaptation (Williams *et al.* 2008). Phenotypic plasticity is the process of a single genotype producing a variety of phenotypes depending on the environment to which it is exposed (Bijlsma & Loeschcke 2005). Phenotypic plasticity can result in organisms bearing traits that are better adapted to their environment, and if phenotypic plasticity occurs as a result of epigenetic processes, these adaptations may

be passed on to subsequent generations (Herrera & Bazaga 2010; Zhang *et al*. 2013). Alternatively, evolutionary adaptation is the natural selection of traits caused by changes in allele frequency within the gene pool of a population, with these traits being passed on to subsequent generations (Bijlsma & Loeschcke 2005; Williams *et al.* 2008). A number of studies report evolutionary adaptation in a range of species as a result of climate change over short periods of time (Bradshaw & Holzapfel 2006; Nevo *et al*. 2012; Scheffers *et al*. 2016). These adaptations are usually related to the timing of seasons or seasonal events (Bradshaw & Holzapfel 2006; Nevo *et al*. 2012).

For example; Nevo *et al*. (2012) examined populations of wild wheat (*Triticum dicoccoides*) and barley (*Hordeum spontaneum*) in Israel in 1980 and then again in 2008 after 28 years of climate change. Significant changes in flowering time were observed, with plants sampled in 2008 displaying earlier flowering times than plants sampled in 1998, and with barley flowering significantly earlier than wheat (Nevo *et al.* 2012). Similarly, Vigouroux *et al.* (2011) examined adaptive traits in pearl millet (*Pennisetum glaucum*) in Niger for plants grown from seed collected in 1976 and 2003. Significantly shorter life cycles, smaller plants and flowering spikes and earlier flowering times were observed for plants grown from seed collected in 2003, and this correlated with a decrease in rainfall that had occurred in the area over the prior 50 years (Vigouroux *et al*. 2011). Guerin *et al*. (2012) examined herbarium specimens and contemporary field samples of *Dodonaea viscosa* collected in South Australia. A significant difference in leaf morphology was observed, with leaves having become narrower over the past 127 years, this correlated with an increase in temperature that had occurred in the region (Guerin *et al.* 2012).

The level of existing genetic variation present within populations of a species may be an important factor in the species' ability to evolve at a fast enough rate to keep pace with climate change, with high levels of genetic variation within a population presenting a greater amount of material for future adaptations (Bürger & Lynch 1995; Gomulkiewicz & Holt 1995; Barrett & Schluter 2008; Williams *et al*. 2008). Populations with low genetic diversity will be at the greatest risk of extinction, and as the rate of environmental change increases so too does the risk of extinction (Bürger & Lynch 1995). Gomulkiewicz and Holt (1995) suggest that population size, as well as the initial level of adaptation to environmental stress, are deciding factors in whether a species will be able to evolve rapidly to keep up with climate change. Large populations that are not too poorly adapted to conditions will have the greatest chance of survival, while

small populations and those that are poorly adapted will have the lowest chance of survival, reaching critically low population levels (Gomulkiewicz & Holt 1995).

1.2 Vegetation, ecology and adaptations in the Southwest Australian Floristic Region, a global biodiversity hotspot

1.2.1 The Southwest Australian Floristic Region

The Southwest Australian Floristic Region (SWAFR) is a Mediterranean-type Ecosystem (MTE) occupying 302,627 km^2 of Southwest Australia (SWA), and is bounded by the Indian Ocean to the west and south, and by the 300 mm rainfall isohyet to the north and east that separates it from arid areas (Hopper & Gioia 2004; Figure 1.1). The SWAFR is geologically stable, located on a flat, highly weathered plateau with some emergent granite and few mountains, and consists of ancient, nutrient deficient soils (Hopper & Gioia 2004). The flora is well adapted to the nutrient-poor soils, dry conditions and frequent fire and is dominated by sclerophyllous species with tough, often prickly leaves and species with persistent seed banks (Hopper & Gioia 2004). The SWAFR has been designated as one of the world's 25 biodiversity hotspots; containing high levels of endemic species under threat of habitat loss (Myers *et al.* 2000), and is home to 7,380 native plant species, mainly from the families Myrtaceae, Proteaceae, Fabaceae, Orchidaceae and Ericaceae, with 49% of taxa being endemic to the region (Hopper & Gioia 2004). The SWAFR is of high conservation significance, with 2,500 plant species listed as threatened, with the main threat to the flora being further habitat loss and fragmentation (Hopper & Gioia 2004).

1.2.2 Mediterranean Type Ecosystems and climate change

The five Mediterranean-type climate regions of the world (the Mediterranean Basin, central Chile, the Cape Region of South Africa, California, and Southern and Southwest Australia) are expected to be some of the areas most significantly affected by climate change (Klausmeyer & Shaw 2009; Yates *et al.* 2010; Urban 2015). All five Mediterranean-type ecosystems are dominated by highly biodiverse evergreen sclerophyll shrublands, known as kwongan in SWA, chaparral in California, fynbos in the Cape Region of South Africa, matorral in Chile, and maquis in the Mediterranean Basin (Rundel *et al*. 2016). These regions are some of the most biodiverse places on earth, covering less than 5% of global land area, but being home to 20% of the Earth's

vascular plant diversity (Cowling *et al.* 1996), and all are classified as global biodiversity hotspots; regions that contain high numbers of endemic species but that are under threat of habitat loss (Myers *et al.* 2000).

The Mediterranean-type climate is characterised by cool wet winters and hot dry summers, with SWA and the Cape Region of South Africa experiencing small amounts of summer rainfall and displaying low interannual variability (Rundel *et al*. 2016). Climate models project that rainfall is likely to decrease across Mediterranean climate regions and temperature is projected to increase (IPCC 2013), leading to concerns about the potential loss of biodiversity from these regions (Klausmeyer & Shaw 2009; Yates *et al*. 2010). Klausmeyer and Shaw (2009) determined the projected extent of the Mediterranean climate areas of the world under future climate scenarios and concluded that Australia's Mediterranean climate zones are at the greatest risk of loss of biodiversity. The extent of the Mediterranean climate zones in Australia (Southern and Southwest Australia) are projected to contract by 49-77%, with only 50-60% of current reserves and protected areas expected to lie within this contracted area, and about a third of this area already having land uses other than conservation (Klausmeyer & Shaw 2009). The high level of Mediterranean climate zone contraction and the low level of protected areas creates a high-risk situation; there is reduced potential for species to move to colonise new areas and for adaptation to occur (Klausmeyer & Shaw 2009; Urban 2015).

1.2.3 Southwest Australia and climate change

Over the past 40 years, the climate of SWA has become warmer and drier causing concern for the continued existence of many species of flora. SWA has experienced a greater than 30% decrease in annual rainfall since the mid-1970s, with a marked reduction in early winter rainfall (IOCI 2013), and mean annual temperatures have increased at a rate of 0.15˚C per decade since the 1970s (Bates *et al*. 2008). The consensus of nine climate models is that decreasing annual rainfall and increasing temperatures will be a trend that continues into the future for SWA (Bates *et al.* 2008). Species distribution modelling for SWA has forecast that climate change will result in substantial declines in suitable habitats for most species which will present as range contractions and loss, resulting in changes in the composition of the flora (Fitzpatrick *et al*. 2008; Yates *et al*. 2010; Urban 2015).

1.2.4 Future species distributions – current projections and caveats

Modelling of species distributions under future climate scenarios has indicated that as the severity of climate change increases, the loss of species habitat and risk of species extinction will also increase and accelerate (Fitzpatrick *et al*. 2008; Yates *et al.* 2010; Urban 2015). Consideration of factors other than climate that influence species distribution (such as dispersal ability, habitat available for colonisation, and species interactions) greatly increase the forecast risk of species extinction or range loss (Fitzpatrick *et al*. 2008; Yates *et al.* 2010; Urban 2015). The iconic *Banksia* species of SWA are projected to suffer significant declines in suitable habitat under future climate scenarios, resulting in range contraction for most species and total range loss and extinction for some others (Fitzpatrick *et al.* 2008; Yates *et al*. 2010). Species distribution modelling has projected that the predominant response of SWA *Banksia* species to climate change will be range contraction, with the degree of contraction increasing with the severity of climate change (Fitzpatrick *et al.* 2008; Yates *et al*. 2010).

For example; Yates *et al.* (2010) investigated the effects of climate change and land transformation on range size of 18 *Banksia* species from SWA. The distributions of *Banksia* species were modelled for 'no migration' and 'full migration' (migration of species to any area suitable) across low-, medium-, and high- severity future climate scenarios, taking into account land use (Yates *et al.* 2010). Yates *et al*. (2010) report that the dominant response was range contraction, with increasing climate change severity resulting in greater contraction for all species. It was also found that the addition of land use data into climate change models resulted in even greater range contractions, with species migrations having little ability to counter the loss of range; as 52% of areas modelled as future species ranges are unavailable as potential habitat, having been cleared of native vegetation and being in use for agriculture or urban settlements (Yates *et al.* 2010). Similarly, Fitzpatrick *et al*. (2008) combined different climate change scenarios and migration rates to determine the impact of these factors on the distribution of 100 wind dispersed *Banksia* species endemic to SWA. The distribution of *Banksia* species was modelled for 'no migration', 5 km per decade migration, and 'full migration' across low-, mid-, and high- severity future climate scenarios. It was found that 66% of *Banksia* species are projected to decline in range across all climate change severity scenarios, while 6% of species are projected to expand in range or remain stable (Fitzpatrick *et al*. 2008). Depending on the severity of changes to climate between 5-25% of *Banksia* species are projected to suffer total

range loss, and thus extinction, by 2080 (Fitzpatrick *et al*. 2008). Species migration rates were found to have little impact on the projected species distributions, as ranges are projected to contract rather than shift, meaning there would be no new areas to colonise, though migration was found to become more important as climate change severity increased (Fitzpatrick *et al*. 2008). Thus Fitzpatrick *et al*. (2008) and Yates *et al.* (2010) concur that climate change is the most influential factor in determining which species will be at risk, and that migration will not serve as a viable mechanism through which species can avoid range contraction or extinction, with areas of potential range expansion being mostly unavailable for colonisation due to existing land uses.

As previously discussed, migration is not the only mechanism through which plants may be able to respond to climate change; they may be able to persist in their current habitat if changes in climate are tolerable, or adapt to the changes in climate through evolution (Aitken *et al.* 2008; Hoffmann & Sgrò 2011). Although the aforementioned models account for species migration and dispersal ability under future climate scenarios, they do not take into consideration the ability of species to adapt *in situ* to changes in climate. Changes in climate may impose strong selection pressure on species, which may result in selection of more stress-tolerant genotypes (Hoffmann & Willi 2008; Hoffmann & Sgrò 2011). Thus projections of great losses of habitat for species may be overestimated, as there has been little consideration of the ability of species to rapidly adapt to changes in climate.

1.2.5 Fire and climate as drivers of ecological adaptation in SWA

Fire has been a force on the evolution of terrestrial plants for over 100 million years (He *et al*. 2011; Keeley *et al*. 2011; Lamont & He 2012), and humans have been influencing the occurrence of fire in SWA for tens of thousands of years (Burrows 2008). Before human arrival in SWA, fire existed as infrequent but widespread events ignited by lightning during the hot, dry, summer and autumn months (McCaw & Hanstrum 2003; Bowman *et al.* 2012). When the Aboriginal people arrived in SWA over 40,000 years ago, they altered the incidence of fire in the landscape, using fire as a tool for hunting animals and for managing the vegetation (McCaw & Hanstrum 2003; Bowman *et al.* 2012). In areas of high resource availability, such as woodlands and savannas, the Aboriginal people set small but frequent fires, creating a mosaic of different vegetation structures and fuel ages across the landscape (McCaw & Hanstrum 2003; Bowman *et al.* 2012). Other areas of the landscape, however, such as the kwongan vegetation of the northern sandplains of SWA were not as high in resource

availability, and would not have been occupied or managed as frequently by the Aboriginal people (Enright & Thomas 2008).

The European settlement of SWA in the 1800s brought substantial changes to the natural landscape with high levels of clearing and ecosystem fragmentation; this has resulted in further changes to fire in the landscape, with fire in SWA now highly influenced by land use (Bowman *et al.* 2012; Burrows & McCaw 2013). There has been a recent increase in the occurrence of fire in natural areas due to increased instances of wild fires, as well as due to the implementation of prescribed burning which has been used since the 1960s to manage fuel loads in areas surrounding urban settlements and farms in order to reduce the risk of larger, out of control fires (Burrows & McCaw 2013). In other areas there has been a reduction in fire or total fire suppression; in highly fragmented areas isolated remnants are often unburned for many decades, and in areas adjacent to urban settlements fire is controlled and suppressed to protect the lives and property of people (Hobbs 2003; Harris *et al*. 2010).

Climate change is expected to alter fire regimes across SWA. The intensity, frequency, spread and timing of fire is influenced by the growth rates of plants, which are in turn influenced by climate (Huston 2003; Cary *et al.* 2012; Enright *et al*. 2012). In SWA the majority of rainfall occurs during the cool, wet winters, while the hot, dry summers often bring drought as well as lightning storms – conditions that promote the ignition and spread of fire (McCaw & Hanstrum 2003). SWA is already experiencing drier summers, and winter rainfall has been decreasing since the 1970s; during which time the fire season has lengthened and the number of days of fire risk have increased (McCaw & Hanstrum 2003). Decreased rainfall will slow down plant growth rates, but will also reduce plant moisture content and the rate of decomposition, which in turn could promote the spread of fire (Enright *et al*. 2012). Regions such as SWA that are projected to become both warmer and drier under future climate scenarios are expected to experience significantly altered fire regimes, leading to increased fire risk and longer fire seasons resulting in high risk of species loss (Enright *et al.* 2012; Enright *et al*. 2015). Warmer, drier climates will result in increased seedling mortality, and decreased plant growth and reproductive ability (Enright *et al*. 2015). This in turn will mean that plants will require longer to reach reproductive age, requiring longer fire-free periods, the opposite to which is forecast (Enright *et al*. 2015).

The flora of SWA possesses a number of traits that allow coexistence with fire, with the products of fire- such as heat, ash and smoke- being beneficial to many species (Dixon

& Barrett 2003). Two fire response syndromes exist within the SWA flora; resprouting and re-seeding. Resprouting species survive fire (if it is not too intense) and resprout from epicormic buds stored beneath the bark or from lignotubers stored below the ground (Bell *et al*. 1984; Bell 2001). Re-seeding species are killed by fire and re-establish from seed stored within canopy or soil seed banks (Bell *et al*. 1984; Bell 2001).

The fire survival strategies of SWA species range from protection from fire to encouraging fire, and even the requirement of fire to trigger biological processes (Bowman *et al.* 2012). Species that employ protection strategies may do so by shielding their regenerative tissue or seeds. Resprouting species insulate their regenerative tissue from the heat of fire either beneath thick bark in the case of epicormic buds (e.g. *Eucalyptus* and *Banksia* species) or below ground in the case of lignotubers (e.g. many Proteaceae species; Bowman *et al*. 2012). Species may store seed in seed banks insulated from the heat of fire either within woody capsules in the canopy (e.g. Proteaceae species) or within the soil (e.g. Fabaceae species; Bowman *et al*. 2012). Species that employ seed banks benefit two-fold from fire; fire first cues seed release from canopy seed banks or cracks open seed stored in the soil seed bank, and then the conditions created post fire are ideal for seedling growth (Lamont *et al.* 1991; Causley *et al.* 2016). Some species possess characteristics that make them highly flammable, encouraging combustion of the plant, and promoting the spread of fire. For example; the hard, dry leaves displayed by sclerophyllous families such as Proteaceae and Myrtaceae, the high oil content of foliage in families such as Myrtaceae, and the retention of dead foliage on the plant such as in the family Xanthorrhoeaceae all increase the flammability of the plant and encourage the spread of fire (Bowman *et al*. 2012).

Fire can stimulate reproductive cycles and growth in some species (Bell *et al.* 1993; Dixon & Barrett 2003; Bowman *et al.* 2013). For example; fire promotes flowering in species such as those from the genera *Haemodorum* and *Xanthorrhoea,* it is the main cue for seed release for many canopy seed bank species (and accelerates the process in others) such as those from the family Proteaceae, it cues germination in fire ephemerals and soil seed bank species by cracking the hard seed coat such as for those from the family Fabaceae, and breaks seed dormancy through the chemicals found in smoke in other species (Bell *et al.* 1984; Bell *et al.* 1993; Dixon & Barrett 2003; Bowman *et al.* 2013). The environment that is left post-fire is conducive to vigorous plant growth (Bell *et al*. 1993; Dixon & Barrett 2003). Fire removes biomass from

ecosystems, resulting in increased space, light, water, and nutrient availability and also produces a layer of nutrient rich ash on top of the soil, all factors that promote germination and growth (Bell *et al.* 1993; Dixon & Barrett 2003).

Climate change in SWA is expected to result in altered fire regimes, which may intensify selection pressure on plant species and have a detrimental effect on the flora (Keeley *et al*. 2011; Enright et al 2012). Altered fire regimes could lead to changes in community structure and may cause range contractions, range shifts, and even extinctions in some species (Enright *et al.* 2012; Pekin *et al.* 2012). The plants of SWA are adapted to a variety of fire regimes, with no single regime being suitable for all plants (Burrows 2008). Increased fire and shortened fire intervals are beneficial for re-sprouting species that can regenerate quickly, but can be detrimental for non-resprouting species (Bell 2001; Burrows 2008; Enright *et al.* 2012). Shortened fire intervals may result in the time between fires being less than the time required for some non-resprouting species to mature, flower, and set seed, resulting in the exhaustion of the seed banks of these species, and possible loss of these species from ecosystems, while promoting regeneration in species that quickly accumulate biomass such as grasses and resprouting species (Bell 2001; Burrows 2008; Cary *et al*. 2012; Enright *et al.* 2012). Increased biomass of grasses and other fast growing resprouting species may in turn further increase fire risk due to increased fuel loads (Cary *et al* 2012). On the other hand, some areas are expected to experience a decrease in fire under climate change; longer fire intervals are beneficial for non-resprouting species (re-seeders) but can be detrimental for resprouting species (Bell 2001; Burrows 2008; Enright *et al.* 2012). Longer fire intervals would mean a lack of the stimulation required for resprouting species to regenerate, as well as a greater risk of whole-plant death due to larger, more intense fires fed by increased fuel loads, making non-resprouting species more competitive in these areas (Bell 2001; Burrows 2008; Enright *et al.* 2012).

1.3 Eneabba Sandplain: Kwongan vegetation and current climate change

1.3.1 Kwongan vegetation of the Eneabba sandplain

Located within the SWAFR, the kwongan vegetation of the Eneabba sandplain was selected as the study system for this research. The Eneabba sandplain occurs within the Geraldton Sandplains IBRA (Interim Biogeographic Regionalisation of Australia)

Region and the Irwin District of the Southwest Botanical Province. *Banksia* woodland is the dominant form of vegetation in the area, with taxa from the families Proteaceae and Myrtaceae particularly prominent. Species from the family Proteaceae were chosen as the focus species for this study. Proteaceae is a dominant family within the Mediterranean climate areas of SWA (Hopper & Gioia 2004; Groom & Lamont 2015), and species within this family display various adaptations to the infertile soils, hot temperatures, low rainfall and recurrent fires to which they are exposed (Groom & Lamont 2015). These adaptations include canopy-stored seed banks, with seeds protected in woody fruits which crack open with the heat of fire, resulting in seed release on to a nutrient-enriched soil surface where they germinate with the onset of winter rains.

Banksia and *Hakea* are two genera of the Proteaceae family that display canopy seed banks, both belonging to the subfamily Grevilleoideae (Lamont *et al.* 2016). These genera occur across Australia, with their main centre of diversity in SWA, where they are thought to have originated (Lamont *et al*. 2016). *Hakea* consists of over 150 species, with 65% of species occurring in SWA, of which 95% are endemic (Lamont *et al*. 2007). Populations of *Hakea* are concentrated on the northern and southern sandplains of SWA within sclerophyll shrublands and low woodlands where they occur as shrubs or small trees and are pollinated by birds or insects (Lamont *et al.* 2007). *Banksia* consists of 84 species, 80% of which occur in SWA, of which 75% are endemic (Lamont *et al*. 2007). Populations of *Banksia* occur on the northern and southern sandplains of SWA, where they vary from rhizomatous creepers to large, single stemmed trees up to 10 m in height and are pollinated mainly by birds (Lamont *et al.* 2007). Some of the main threats to the conservation of these two genera are loss of habitat which has occurred as a result of clearing land for agriculture and urban settlements, susceptibility to the root pathogen *Phytophthora cinnamomi*, and altered fire regimes (Lamont *et al.* 2007). Shortened fire intervals can have a negative effect on fire killed (non resprouting) species including the 60% of *Banksia* and *Hakea* species that display this type of fire response; if fire intervals are shorter than the juvenile period of these species their seed bank may become depleted and they may be lost from ecosystems (Lamont *et al.* 2007; Enright *et al.* 2012). Longer fire intervals can have a negative effect on resprouting species, including the 40% of *Banksia* and *Hakea* species that display this type of fire response; these species require regular fire to promote resprouting, which results in vigorous growth of the plant (Enright *et al.* 2012).

1.3.2 Eneabba study site and climate

The study area on the Eneabba Sandplain is located 275 - 300 km north of Perth, Western Australia, centred on the town of Eneabba (Figure 1.1), and has a Mediterranean-type climate characterised by cool, wet winters and hot, dry summers (Schimper *et al.* 1903). Mean annual rainfall at Eneabba is 491 mm, with 80% of rainfall occurring in the winter months, between May and September (Eneabba Weather Station number 008225; Bureau of Meteorology 2017a). The mean maximum temperature is 27.9˚C, and the mean minimum temperature is 13.6 ˚C while the mean maximum temperature of the hottest month (February) is 36.4˚C (Bureau of Meteorology 2017b*;* Figure 1.2). Historically, SWA receives relatively consistent rainfall, the majority of which falls in the winter months. However, there has been a recent decrease in the amount of rain falling in late autumn and early winter, with winter rainfall having steadily decreased by 30% since the 1970s (Hope *et al.* 2006; IOCI 2013; Figure 1.3). The mean annual rainfall over the past 30 years was significantly lower than for the previous 20 years (t=32.39, p<0.001), as was the mean winter rainfall (t=25.98, p<0.001; Figure 1.3). The study system experienced severe drought in 2006 and 2010, with annual rainfall during these years being around 30% lower than the long-term average (He & Lamont 2010; Bureau of Meteorology 2017).

The Eneabba sandplain is located within the Geraldton Coastal Soil Landscape Zone, characterised by ancient dunes with alluvial plains and sand sheets, low limestone hills, and calcareous and siliceous dunes (Stuart-Street 2007). Soils are mostly well drained, shallow yellow-brown sands, deep yellow sands, deep and shallow calcareous sands, and deep pale sands, and are nutrient deficient (Stuart-Street 2007). The study system possesses low baseline soil-water availability, and plants rely on winter rainfall to survive the dry summer and autumn months (He & Lamont 2010). As such, a further decrease in rainfall is of particular concern, as drought can cause sudden and extreme vegetation change (He & Lamont 2010, Yates *et al.* 2010).

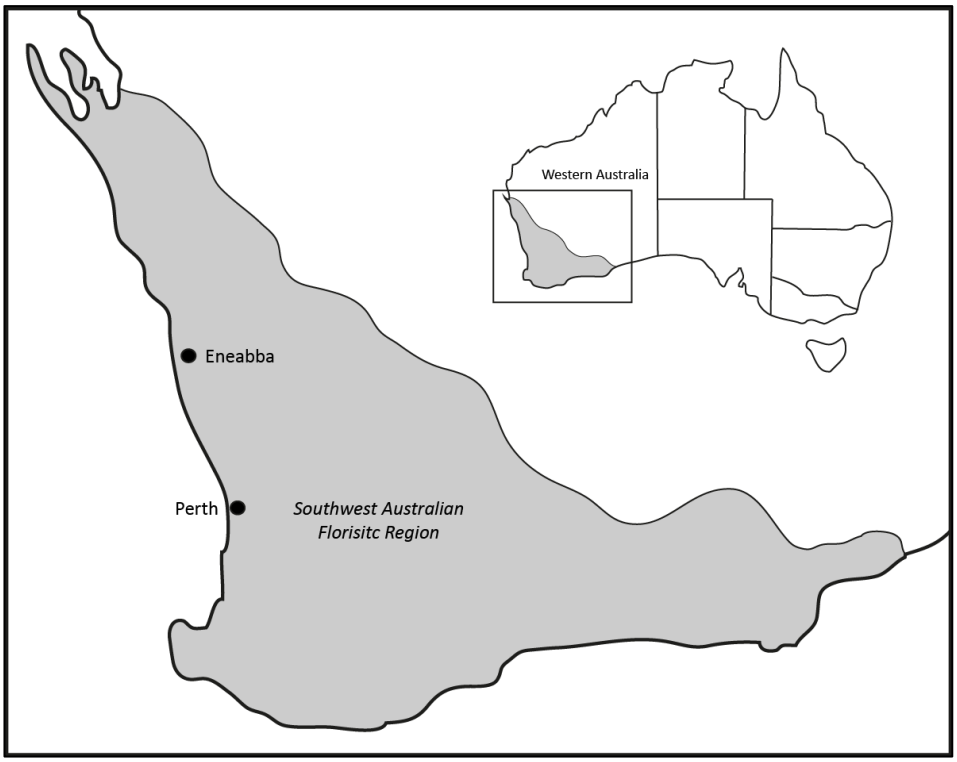

Figure 1.1: Inset shows the location of the South West Australian Floristic Region within Western Australia. The location of Eneabba in relation to Perth, the capital of Western Australia is shown on the main map.

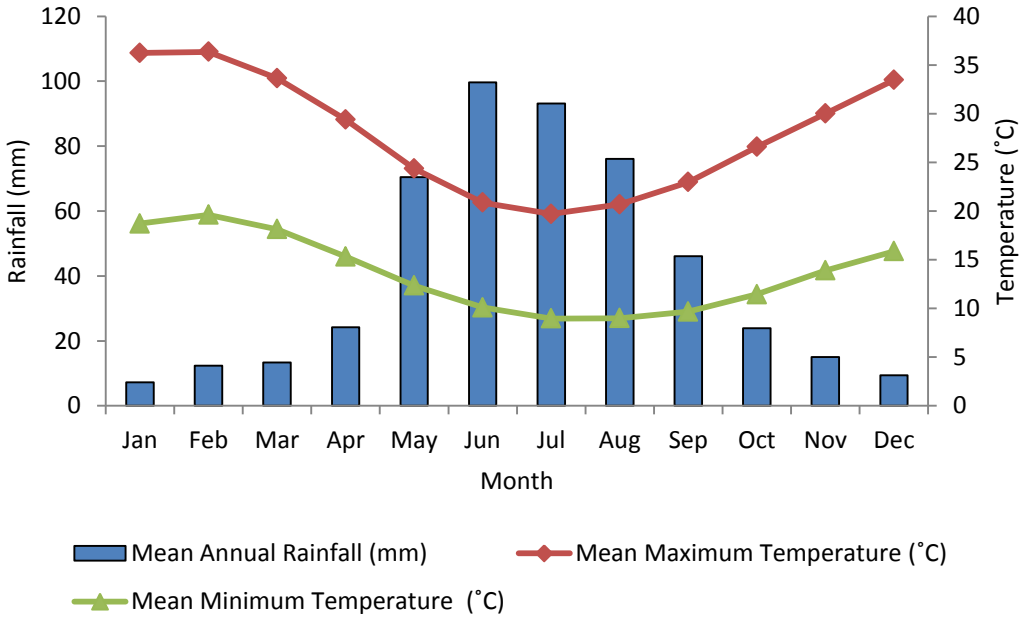

Figure 1.2: Long-term average climate data for Eneabba. Mean annual rainfall (since 1964), mean maximum, and mean minimum temperatures (since 1972; Bureau of Meteorology 2017a and 2017b) are displayed.

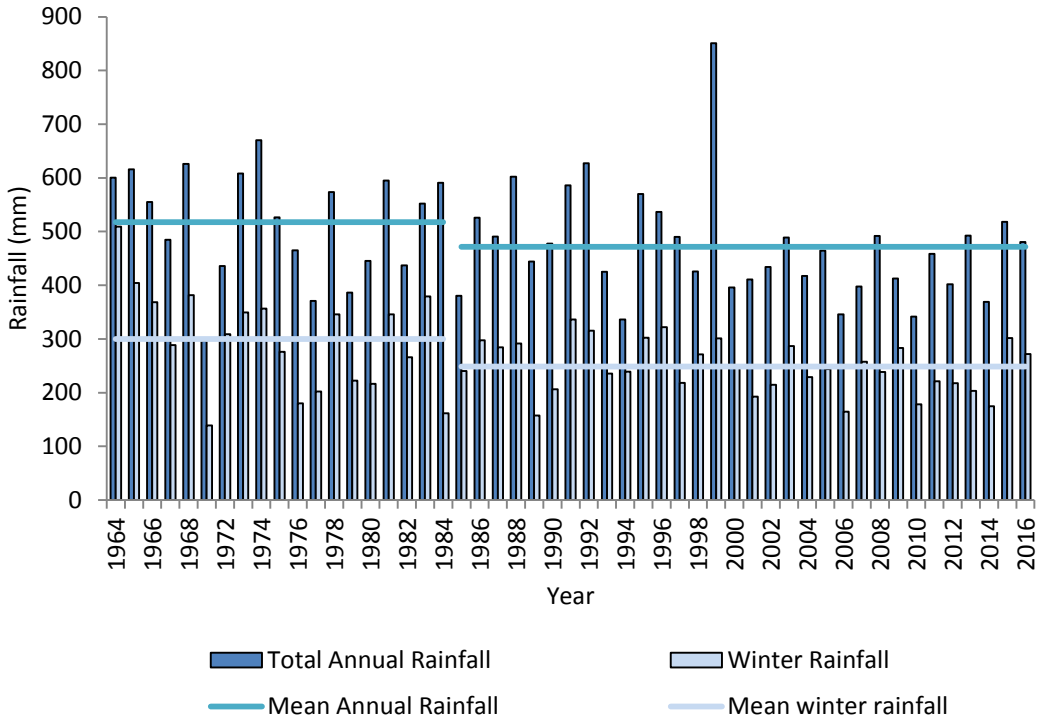

Figure 1.3: Total annual rainfall at Eneabbba since 1964, with rain falling in winter also indicated. Mean annual and mean winter rainfall is presented for the years 1964-1984 and 1985-2016 (Bureau of Meteorology 2016a).

1.4 Objectives and structure of this thesis

This research aimed to determine whether the flora of Southwest Australia can persist within current distributions as changes in climate occur, in particular declining rainfall. This was investigated by assessing how plants and plant communities respond to changes in climate through the use of community composition surveys, greenhouse experiments, and genetic analyses. There were four main objectives of this research:

1. To determine whether the steady decrease in rainfall and increase in temperature over the past 40 years at Eneabba, Western Australia has led to a change in the composition of the vegetation.
2. To determine whether seed banks of four fire-killed, serotinous species, *Banksia hookeriana*, *B. leptophylla*, *Hakea costata* and *H. polyanthema* have the potential to mitigate the effects of a drying climate through the rapid expression of drought-tolerant genotypes.
3. To determine whether *B. hookeriana* is capable of accumulating adaptive genetic variation fast enough to resist the effects of drought.

4. To determine adaptive genetic diversity in *B. attenuata* populations spanning across its range.

The following hypotheses were tested for the above objectives:

Hypothesis 1: Lower rainfall, higher temperatures, and more frequent fires will have resulted in changes in the composition of the flora at Eneabba, with the proportion of resprouting species to non-resprouting species having increased as well as the proportion of soil seed bank species to canopy seed bank species.

Hypothesis 2: Species will respond evolutionarily to drought through the use of genetic material stored in the seed bank.

Hypothesis 3: Increased resistance to drought will have built up in the seed bank following recent periods of prolonged drought due to mortality of plants bearing susceptible genotypes.

Hypothesis 4: Adaptive genetic variation of populations of *B. attenuata* will vary based on rainfall, temperature and fire frequency.

To achieve the above objectives, the potential of SWA species to tolerate changes in climate through evolutionary adaptation were investigated on different levels, as described in Chapters Two to Five. Chapter 2 focuses on the first objective, examining changes in a community over a ~30 year period of time, by surveying South Eneabba Nature Reserve, and comparing the composition of the flora recorded in this survey to that of surveys conducted in the same area in the 1970s and 1980s to assess whether a change has occurred. Chapter 3 focuses on the second objective, assessing the accumulation of drought tolerance over one generation, which was investigated through growing seedlings of four species descended from seed produced in either high or low winter rainfall years, subjecting these seedlings to drought and control treatments, and determining differences in gene expression between control and drought species from high and low rainfall origins. Chapter 4 focuses on the third objective, assessing the possibility of accumulation of drought tolerance within a generation, which was investigated through growing seedlings of *B. hookeriana* from seed produced pre-drought, post one drought, and post two droughts, subjecting these seedlings to drought and control treatments, and determining differences in drought tolerance across the year cohorts. Chapter 5 focuses on the fourth objective, the consequence of multiple generations of adaptation and evolution in *B. attenuata*, which was examined through the sequencing of DNA extracted from leaves of plants that had

been sampled from across the species' range, and determining which genes were associated with different rainfall and temperature gradients and fire intervals. The findings of this research could be used to better inform models of the future, which should take the capacity of the flora to adapt to changes in climate into consideration to provide a more accurate assessment of the effects of future climate change on species distributions.

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Chapter 2: Effect of decreasing rainfall on floristic composition of South Eneabba Nature Reserve, Eneabba, Western Australia.

2.1 Abstract

Climate is the main factor influencing the broad scale distribution of plant species worldwide. Since the 1950s, changes in climate and the frequency of extreme weather events have been occurring across the globe, raising concern for the persistence of many species. Southwestern Australia (SWA), a global biodiversity hotspot, has experienced a steady decrease in winter rainfall since the 1970s, and is one of the regions of the world projected to be at greatest risk of biodiversity loss under future climate change scenarios. This study aimed to determine whether the biodiverse kwongan vegetation of South Eneabba Nature Reserve (SENR), SWA, has experienced changes in floristic composition as rainfall has decreased and temperature increased in the area. Sixty 10 m x 10 m plots were surveyed across the reserve in 2015, and vegetation composition was compared to the composition recorded in earlier surveys of the reserve (dating from 1975-1985). Results indicate that there has been no measurable change in the florisitic composition of SENR over the past 40 years. Nor have there been any changes in the distribution of readily quantifiable plant functional traits. The proportion of resprouting to non-resprouting species has remained the same, as has the proportion of soil seed bank species to canopy seed bank species. There were a number of species identified in the historic surveys that were not present in the 2015 survey; however the absence of some of these species may be explained by the great richness of the flora in the area, the specific areas of the reserve surveyed, taxonomic errors, incomplete identifications, and misidentifications. It is unlikely that there has been a significant change in the floristic composition of SENR over the past 40 years, however monitoring should be continued in order to detect any changes that may occur in the future, and the plots established in this survey allow for future detailed comparisons.

2.2 Introduction

The broad scale distribution of plant species is influenced by a number of factors including soil type, geography, available nutrients, and fire regime; however no factor is as dominant in determining species distributions as climate. Global changes in climate are leading to variations in the suitable habitats for plant species world-wide, causing concern for the continued persistence of many species (Sykes 2009; IPCC 2013; Urban 2015). As stated in the report by the Intergovernmental Panel on Climate Change (2015) changes in climate and the frequency of extreme weather events ascribed to anthropogenic impacts have been occurring across the globe at least since the 1950s and are forecast to continue into the future. Overall temperature has increased, with the frequency of hot days and nights having increased while the frequency of cold days and nights has decreased. The frequency and intensity of heavy precipitation events, drought events, heat waves and cyclones have all increased. Polar ice sheets have been losing mass, glaciers have been shrinking, and Northern Hemisphere snow cover has been decreasing. There has also been a warming of ocean surface temperatures by 0.11˚C per decade since 1971 and mean sea level has risen by 0.19 m over the past century. Atmospheric concentrations of greenhouse gases have also increased by 40% since pre-industrial times. These changes in climate are already influencing vegetation patterns and will continue to do so into the future, shifting the spatial ranges in which species can survive (Sykes 2009).

Some of the regions expected to be most significantly affected by climate change are global biodiversity hotspots; regions that contain both high numbers of endemic species and that are under threat of habitat loss (Myers *et al.* 2000; Klausmeyer & Shaw 2009, Yates *et al.* 2010). These regions cover less than 5% of global land area, but are home to 20% of the Earth's vascular plant diversity (Rundel *et al.* 2016). The five Mediterranean climate regions of the world (the Mediterranean Basin, Chile, South Africa, California, and Southern and Southwest Australia) are all classified as biodiversity hotspots (Myers *et al.* 2000). The Mediterranean climate is characterised by cool, wet winters and hot, dry summers. Climate models project that rainfall is likely to decrease across Mediterranean regions while temperature is projected to increase (IPCC 2013). This leads to concerns for potential loss of biodiversity from these regions (Klausmeyer & Shaw 2009; Yates *et al*. 2010). Klausmeyer and Shaw (2009) determined the projected extent of the Mediterranean climate areas of the world in future climate change scenarios, and concluded that Australia's Mediterranean climate zones are at the greatest risk of loss of biodiversity. The extent of the Mediterranean

climate zones in Australia (located in Southern and Southwest Australia) are projected to contract by 49-77%, with only 50-60% of current reserves and protected areas expected to lie within this contracted area, and about a third of this area already having land uses other than conservation (Klausmeyer & Shaw 2009). The high level of Mediterranean climate zone contraction and the low level of protected areas create a high risk situation; there is reduced potential for species to migrate and colonise new areas and for adaptation to occur (Klausmeyer & Shaw 2009).

2.2.1 Climate change and the flora of Southwest Australia

Southwest Australia (SWA) is already experiencing a change in climate, and this change is projected to have significant impacts on the composition of the flora (Malcolm *et al.* 2006; Fitzpatrick *et al*. 2008; Yates *et al*. 2010; Urban 2015). For more information, see Chapter 1.

In situ rapid adaptation may be one of the most important mechanisms through which species can survive climate change (Hoffmann & Willi 2008), and species with different plant functional traits will respond to climate change in different ways (Debouk *et al*. 2015). Species with certain functional traits are expected to be able to better tolerate increased temperatures and drought and the associated increase in fire frequency than others (Enright *et al*. 2014). For example; it is expected that the impacts of increased drought will be less severe on resprouting species than non-resprouting species (Bell 2001; Enright *et al*. 2014; Zeppel *et al*. 2015). Resprouting species have the ability to regenerate from underground lignotubers and rhizomes or epicormic buds when above ground biomass is damaged, while non-resprouting species must regenerate from seed, allowing resprouting species to tolerate stresses and recover from disturbances faster than non-resprouting species (Zeppel *et al.* 2015). There is evidence that as fire intervals become shorter, as is expected under climate change scenarios, the proportion of resprouting species to fire killed species present in a community increases (Bell 2001; Auld & Denham 2006; Enright *et al.* 2014). This is because resprouting species are adapted to survive fire, while non-resprouting species are dependent on seed banks for regeneration which become depleted as fire intervals shorten (Bell 2001; Auld & Denham 2006; Enright *et al.* 2014).

It is also expected that the effect of increased drought will be less detrimental for soil seed bank species than for canopy seed bank species. Soil seed bank species are reported to be more tolerant to frequent fire than canopy seed bank species (Auld & Denham 2006; Buma *et al*. 2013; Enright *et al*. 2014). The soil seed bank acts as a fire

resistant genetic reserve with seeds from a range of years being stored in the soil (Auld & Denham 2006; Enright *et al*. 2014). After a fire, seeds are stimulated to germinate, though some seeds remain in the soil seed bank and are carried over to the next recruitment opportunity (Auld & Denham 2006; Enright *et al*. 2014). In contrast, canopy stored seed is released post-fire, and either germinates in the first winter after fire or perishes (Enright *et al.* 2014). Thus soil seed bank species may have greater resilience to a drier climate than canopy seed bank species (Auld & Denham 2006; Enright *et al*. 2014).

2.2.2 Value of historic data in climate change studies

Changes in vegetation as a consequence of climate change can be monitored with the use of historic data, which can deliver insight into patterns and processes of change in biodiversity over time (Stockli *et al*. 2010; Vellend *et al.* 2013). Historic surveys documenting the vegetation at a certain point in time with a certain climate can be compared to subsequent surveys of the same site to reveal changes in community composition, such as an increase or decrease in the abundance of certain species or traits (Stockli *et al*. 2010; Vellend *et al.* 2013). For example; Kelly and Goulden (2008) used historic data and contemporary data to detect range shifts in plant species of Southern California's Santa Rosa Mountains. The average elevation of species was found to have risen by about 65 m in the 30 years between an initial survey in 1977 and a re-survey in 2007, and this was attributed to changes in the local climate (Kelly & Goulden 2008). Harrison *et al.* (2010) used historic data to detect changes in the understorey herb community structure of Oregon's Siskiyou Mountains. Cold-adapted species were found to have decreased in relative abundance at low elevations and mean specific leaf area had decreased over the 60 year period between a historic survey in 1949 and a re-survey in 2009, during which time temperatures had increased by 2˚C (Harrison *et al.* 2010). Historical collections, such as those archived in museums or herbariums may also be used to draw comparisons between past and present vegetation composition and characteristics (Suarez & Tsutsui 2004; Guerin *et al.* 2012). For example, Guerin *et al.* (2012) were able to determine that *Dodonaea viscosa* had developed narrower leaves over 127 years of climate change in South Australia through comparison of leaf morphology of herbarium specimens and field surveys.

This study uses contemporary and historic data to investigate whether the steady decrease in rainfall and increase in temperature in SWA over the past 40 years have resulted in a significant change in the composition of the flora of South Eneabba Nature

Reserve- a conservation reserve located in the diverse Eneabba Sandplains of SWA, and provides a baseline of data for use in future monitoring. The aim was to determine whether changes in plant community composition have occurred over the period of time between the historic surveys and the present survey. It was hypothesised that lower rainfall and higher temperatures will have resulted in changes in the composition of the flora, with the proportion of resprouting species to non-resprouting species having increased, as well as the proportion of soil seed bank species to canopy seed bank species. Data recorded in the historic surveys are at the coarse level of species lists and cover abundance of non-fixed plots; this study aimed to collect data on species numbers in fixed plots to provide a baseline for comparison in the future.

2.3 Methods

2.3.1 Study system

See Chapter 1, Section 1.3 for details on the study system.

2.3.2 Site selection

South Eneabba Nature Reserve (SENR) is representative of the kwongan vegetation of the Eneabba Sandplain, containing a high level of floristic diversity, with plants displaying varied functional traits. Previous surveys have been conducted in the area between 1974 and 1984 (Lamont 1976; Hnatiuk & Hopkins 1981; Griffin *et al*. 1983; Elkington & Griffin 1985) and serve as a baseline for comparisons with this study.

Banksia woodland is the main vegetation community in the area, with taxa from the families Proteaceae, Myrtaceae and Fabaceae dominating. The study area is approximately 4,946 ha and is located 8 km south of the town of Eneabba (S 29° 58’ 25.82” E 115° 17’ 25.02”). The reserve is of relatively even fuel age, with three major bushfires having burned large areas of the reserve in 2002, 2003, and 2005. Twenty locations within SENR were selected so that they were accessible by 4WD vehicle along tracks within or along the edges of the reserve and with the aim of having sites evenly spread across the reserve (Figure 2.1). The north-eastern part of SENR is an active mine, so this area was excluded from the surveys.

2.3.3 Vegetation surveys

Three 10 m x 10 m vegetation plots (approximately 100 m apart) were established along each of twenty transects (Table S2.1). Plot size was selected based on the size of plots used in previous studies in the area (Griffin *et al*. 1983; Elkington & Griffin 1985). These studies in turn based plot size on an earlier study (George *et al.* 1979) that indicated that 100 m^2 plots of kwongan vegetation can be expected to include 80% of the flora present in a 1000 m^2 plot of kwongan vegetation. Plots were located 50-200 m apart, and situated along an environmental gradient if one was present in the area (for example; slope, soil type, fire age), with the aim of maximising sampling diversity. The location of the north-west corner of each plot was recorded with a GPS, and notes were made on the dominant vegetation, soil type, aspect, and fire history, based on visual inspection. For each species present within the plot, abundance was determined and given a rank on the Braun-Blanquet cover abundance scale: "r"= solitary individual, "+"= few individuals, "1"= 1-5% cover, "2"= 5-25% cover, "3"= 25-50% cover, "4"= 50-75% cover, and "5"=75-100% cover (Wikum & Shanholtzer 1978). Each species was subsequently categorised by seed bank type (canopy seed bank, soil seed bank or no seed bank), and fire response (resprouter or non-resprouter). A voucher specimen of each species was collected and pressed for identification. Plant specimens were identified to species level with the use of resources at the Western Australian Herbarium, Kensington, or by herbarium taxonomists. Voucher specimens were archived within the Department of Environment and Agriculture, Curtin University, Bentley.

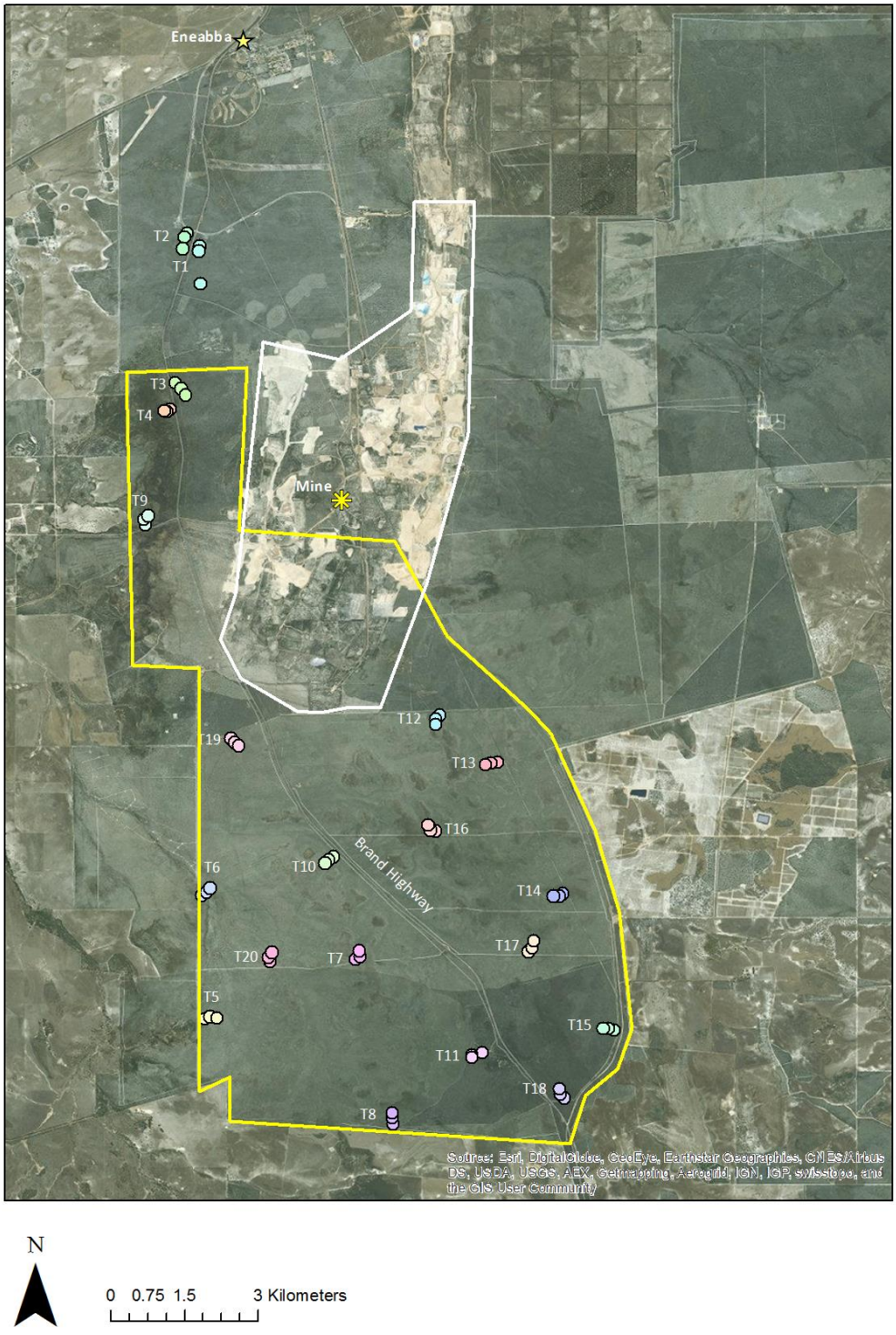

Figure 2.1: Locations of the 20 transects of three plots within SENR. The boundary of SENR is outlined in yellow; the extent of the mine is outlined in white. The location of the town of Eneabba is also indicated.

2.3.4 Historical surveys in the area

Four surveys were previously conducted in the area (Lamont 1976, Hnatiuk & Hopkins 1981, Griffin *et al*. 1983, Elkington & Griffin 1985) that are within or overlap with SENR (Figure 2.2). These surveys had similar intermediate post-fire ages at the time of surveying (Table 2.1).

In 1975 Lamont surveyed the vegetation in a 600 ha area from mid-August to late-September. Presence/absence of species in the area was recorded, with a total of 239 species found across the study area, with 40 families represented by 109 genera (Lamont 1976).

In 1977 Hnatiuk and Hopkins surveyed the vegetation in a 2,000 ha area in September and October. Their plots were laid out in a grid pattern, with 400 m between plots, and a total of 87 plots. Plots were 4 m x 4 m in size and species presence was recorded. A total of 429 species were recorded, with 50 families represented by 162 genera (Hnatiuk & Hopkins 1981). Voucher specimens were collected for most species and lodged with the Western Australian Herbarium, Perth, marked 'Eneabba Survey 1977' (Hnatiuk & Hopkins 1981).

In 1978 and 1979 Griffin *et al*. surveyed the vegetation in a 500, 000 ha area centred on Eneabba, extending from Jurien in the south-west to Three Springs in the north-east, plots were selected to give a representative sample of kwongan vegetation on laterite (Griffin *et al.* 1983). A total of 31 plots were sampled, each 10 m x 10 m in size, and each for which species present, their height, and estimated cover abundance (Braun-Blanquet scale) were recorded. A total of 348 species were recorded at the 31 sites, with 38 families represented by 125 genera. Of the 31 plots sampled six occurred in the vicinity of SENR and only these plots were used for comparison in this study. A total of 177 species were recorded in the six plots. Voucher specimens were collected and lodged with the Western Australian Herbarium, Perth, marked 'EAG Laterite Survey 1978/79' (Griffin *et al*. 1983).

In 1984 (31st of May and the 29th of June) Elkington and Griffin (1985) surveyed the vegetation in a 1,600 ha area. Their plots were laid out along east-west drill lines. A total of 135 10 m x 10 m plots were sampled and species presence was recorded. Cover (modified Domin-Krajina scale) was recorded for only 40 common taxa. A total of 295 species were found in the 135 plots, with 36 families represented by 114 genera (Elkington & Griffin 1985).

The data recorded by the Lamont (1976), Hnatiuk & Hopkins (1981), Elkington & Griffin (1985) and Griffin *et al*. (1983) surveys were sufficient for comparison of species presence/absence between the historic surveys and the 2015 survey. However the data recorded by the Elkington and Griffin (1985) survey presented plot level abundance data for 40 species, while the Griffin *et al*. (1983) study presented plot level abundance data for all species recorded, allowing comparisons of species abundance between these two historic surveys and the 2015 survey (comparisons were limited to 40 species for the Elkington & Griffin (1985) data set).

In order to compare the four historic surveys to one another and to the 2015 survey the data sets had to be made comparable and oddities dealt with. The first step in making surveys comparable was to ensure that taxonomy was current and consistent across surveys. Surveys had been conducted at different times, and taxonomy had changed between surveys, for example; the genus *Dryandra* has been merged with the genus *Banksia*. Each taxon recorded was checked against FloraBase (Western Australian Herbarium 2016) to determine the current taxonomic classification. Once taxonomy was current, a matrix detailing the presence/absence of each taxon in each survey was created. In order to make comparisons of abundance between surveys that had recorded abundance data (Griffin *et al*. (1983) survey; Elkington and Griffin (1985) survey, and 2015 survey) data had to be converted to the same cover scale. The Griffin *et al.* (1983) survey had recorded cover of taxa using the Braun Blanquet Scale, the Elkington and Griffin (1985) survey had recorded cover of taxa using a modified Domin-Krajina Scale, while counts of each species had been recorded as well as cover for the 2015 survey. In order to make the three surveys comparable, the cover data from the 2015 and Elkington and Griffin (1985) surveys was converted into the Braun-Blanquet Scale. However, this was at the expense of the resolution of data from the 2015 and Elkington and Griffin (1985) surveys, due to the wide cover classes of the Braun-Blanquet Scale.

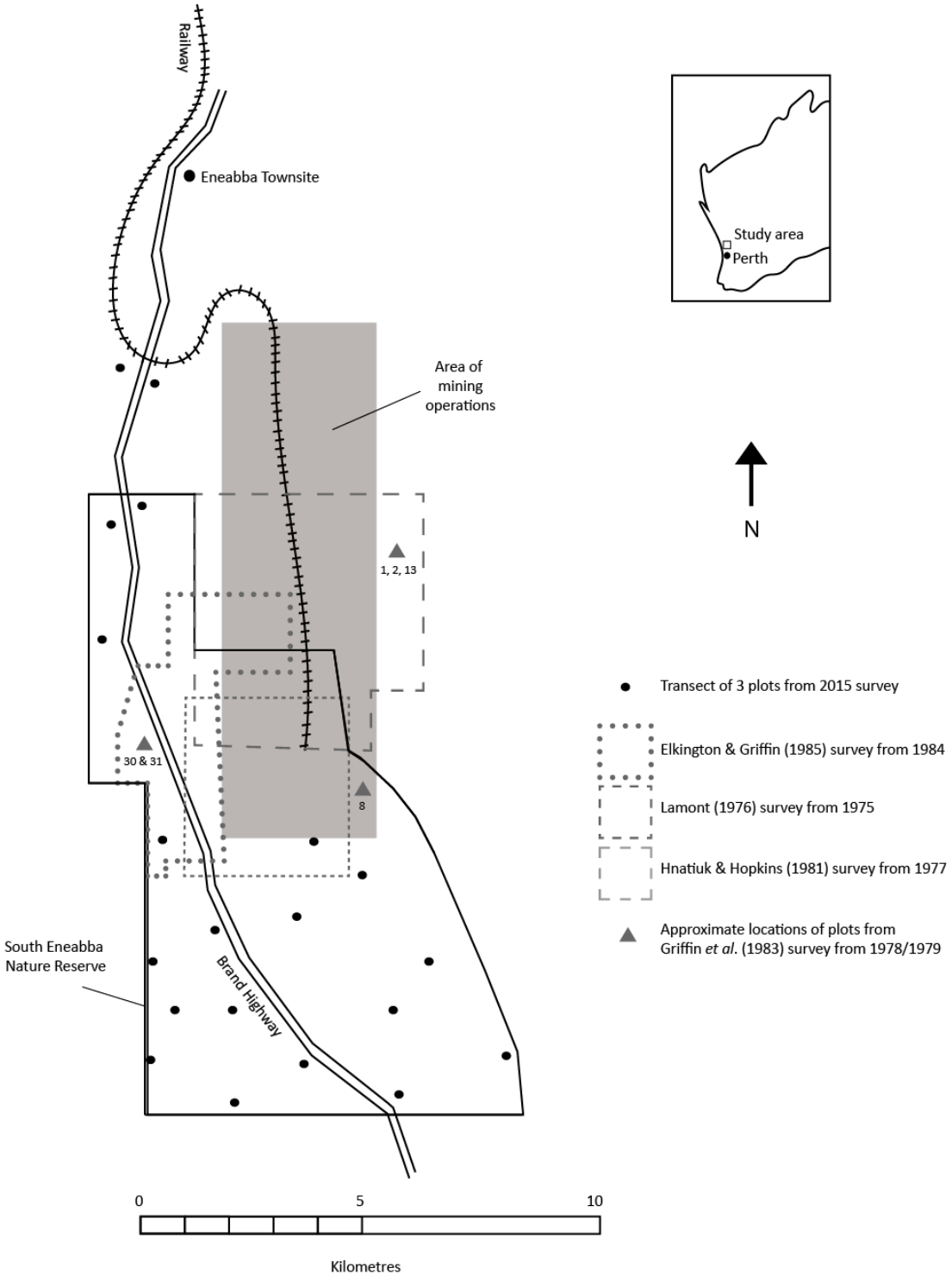

Figure 2.2: Location of the 2015 survey transects and historic surveys in relation to SENR and the town of Eneabba (adapted from Hopkins & Hnatiuk 1981 and Elkington & Griffin 1985).

Table 2.1: Summary of the 2015 survey and the four historic surveys conducted within or overlapping with SENR. Note that only a portion of the Griffin *et al*. (1983) survey is within the current study area.

	2015	Elkington & Griffin 1985	Griffin *et al*. 1983	Hnatiuk & Hopkins 1981	Lamont 1976
Year surveyed	2015	1984	1978/1979	1977	1975
Location	SENR	SENR	From Jurien to Three Springs	Mine site north of SENR	SENR
Time since fire	~10 years	17 years	~8 years	10 years	7 years
Study area	~8,200 ha	~1,600 ha	500,000 ha	2,000 ha	600 ha
Plot size	10 m x 10 m	10 m x 10 m	10 m x 10 m	4 m x 4 m	-
Number of plots	60	135	31 (6 in study area)	87	-
Total plot area surveyed	6,000 m^2	13,500 m^2	3,100 m^2 (600 m^2 in study area)	1,392 m^2	-
Species present	316	295	348 (177 in study area)	429	239

2.3.5 Statistical analysis

Chi-squared analyses were performed in SPSS Statistics (Version 21; IBM Corp. 2012) to determine the expected proportion of resprouting to non-resprouting species and soil seed bank to canopy seed bank species, with the mean number of species representing these traits from the historic surveys being used as the expected values.

Two dimensional non-metric multidimensional scaling (nMDS) was used to observe the relative groupings of the plots from the 2015, Elkington and Griffin (1985) and Griffin *et al*. (1983) surveys based on species composition (both presence/absence and Braun-Blanquet cover-abundance) and to identify outliers in the data. Two dimensional nMDS was performed in the statistical program PAST (Version 3; Hammer *et al*. 2001) using the Bray-Curtis Similarity Index. Sample rarefaction (Mao's tau) of the 40 species surveyed in the Elkington and Griffin (1985) survey and of the species found in the

2015 survey was also performed in PAST, providing an estimate of expected species richness as a function of the number of plots sampled.

Diversity indices (Number of taxa, Dominance, Shannon Diversity, Evenness) were calculated for each plot from the Griffin *et al*. (1983), Elkington and Griffin (1985) and 2015 surveys in PAST from transformed Braun-Blanquet cover-abundance values. The Braun-Blanquet scale is an ordinal scale, thus values were turned into cardinal numbers by using the mid-point values of each cover class (Table 2.2). Mann-Whitney U tests were used to determine whether there was a significant difference in the mean diversity (Number of taxa, Dominance, Shannon Diversity, Evenness) of the plots from Griffin *et al*. (1983) and 2015. Kruskal-Wallis tests were used to determine whether there was a significant difference in the mean diversity (Number of taxa, Dominance, Shannon Diversity, Evenness) of 40 species (those targeted in the Elkington and Griffin (1985) survey) between the plots from the Griffin *et al*. (1983), Elkington and Griffin (1985) and 2015 surveys.

Table 2.2: Classification of vegetation percentage cover on the Braun-Blanquet scale, with midpoint of each cover class indicated.

Braun Blanquet Scale	Cover Class (%)	Cover Class Midpoint (%)
5	75-100	87.5
4	50-75	62.5
3	25-50	37.5
2	5-25	15
1	1-5	3
+	<5, few individuals	0.5

2.4 Results

2.4.1 Floristic analysis of 2015 survey

A total of 316 species were found in the 60 plots surveyed within SENR from September to November 2015 (Table S2.2; Table S2.3). The 316 species represented 107 genera from 36 families. Seven families (19.5% of the total) accounted for over 70% of species: Proteaceae, with 71 species present from 13 genera; Myrtaceae, with 61 species present from 19 genera; Fabaceae, with 37 species present from eight

genera; Cyperaceae and Restionaceae, both with 15 species present from six genera, Ericaceae, with 14 species present from five genera, and Haemodoraceae, with 10 species present from five genera. Eleven families (30.5% of the total) were represented by a single taxon; Asteraceae, Cupressaceae, Euphorbiaceae, Frankeniaceae, Haloragaceae, Hemerocallidaceae, Iridaceae, Lauraceae, Polygalaceae, Sapindaceae, and Zamiaceae. A total of 53 genera (50% of the total) were represented by one species. Eight genera were represented by eight or more species, which accounted for 30% of the total number of species*: Banksia* with 23 species; *Hakea* with 15 species; *Daviesia* with 12 species; *Petrophile* with 10 species; *Acacia* with 10 species; and *Melaleuca, Verticordia,* and *Stylidium* all with eight species. Twelve taxa were represented by material inadequate for identification. In four instances, species were too similar to provide a confident identification; in this case two species were considered a single taxonomic unit, as per the following species groupings: *Gastrolobium polystachyum*/*Cristonia biloba*; *Daviesia podophylla/Daviesia quadrilatera*; *Conostylis micrantha/Conostylis teretifolia*; and *Eucalyptus pleurocarpa/Eucalyptus xtetragona*.

A total of 17 species of conservation significance were recorded during the survey; one Priority One species, three Priority Two species, seven Priority Three species, and five Priority Four species (Table 2.3).

Table 2.3: List of species of conservation significance identified in the 2015 survey of SENR, with Conservation Code indicated.

Family	Species	Conservation Code
Myrtaceae	*Chamelaucium* sp. Bunjil	Priority 1
Myrtaceae	*Homalocalyx chapmanii*	
Myrtaceae	*Verticordia argentea*	Priority 2
Proteaceae	*Persoonia filiformis*	
Cyperaceae	*Mesomelaena stygia* subsp. *deflexa*	
Lamiaceae	*Hemiandra* sp. Eneabba	
Myrtaceae	*Hypocalymma gardneri*	
Proteaceae	*Banksia cypholoba*	Priority 3
Proteaceae	*Grevillea uniformis*	
Restionaceae	*Desmocladus biformis*	
Restionaceae	*Lepidobolus quadratus*	
Myrtaceae	*Calytrix chrysantha*	
Myrtaceae	*Calytrix superba*	
Proteaceae	*Banksia chamaephyton*	Priority 4
Proteaceae	*Grevillea rudis*	
Restionaceae	*Desmocladus elongatus*	

A total of 208 species (66% of the total) were identified as Resprouters (RS; resprout after fire), while 102 species (32% of the total) were identified as Non-resprouters (NRS; killed by fire and population regenerates from seed). The fire response strategy of six species (2% of the total) could not be determined. A total of 227 species (71.8% of the total) were identified as storing seed in a Soil Seed Bank (SSB), while 88 species (27.8% of the total) were identified as storing seed in a Canopy Seed Bank (CSB), with one species (0.32% of the total) being an annual and hence not having a long term seed storage strategy (Table S2.2).

The number of species in the 10 m x 10 m plots averaged 45.5 (± 1.41 SE) species per plot, ranging from 16 species (Transect 9, Plot 1) to 62 species (Transect 13, Plot 2 and Transect 16, Plot 1). The composition of each of the 60 plots was compared to determine whether there were distinct vegetation communities present across SENR (Figure 2.3). The vegetation community present within the three plots from Transect 9 was distinct from all other plots (Group A, Figure 2.3). These plots were dominated by *Eucalyptus-Casuarina* over-storey and *Casuarina campestris* understorey and had few

species in the understorey and thus low species richness (16-23 species per plot). The three plots from Transect 4 (Group B, Figure 2.3) also displayed a vegetation community distinct from all other plots. These plots were dominated by *Acacia acuminata*-Myrtaceae thicket and also had few species in the understorey and low overall species richness (29-41 species per plot). Both plots One and Three from Transect 6 displayed vegetation communities distinct from all other plots (Groups C and E, Figure 2.3). Plot One (Group C, Figure 2.3) was dominated by low shrubs including *Calytrix chrysantha*, *Hakea gilbertii*, and *Mesomelaena stygia*, was at the top of a slight ridge, and displayed low species richness (26 species total). Plot Three (Group E, Figure 2.3) was dominated by large *Banksia* shrubs, with little understorey and thus low species richness (21 species total). All other plots were similar in composition (Group D, Figure 2.3), dominated by low shrubs such as *Banksia attenuata*, *Xanthorrhoea drummondii*, *Allocasuarina humilis*, and *Beaufortia elegans*, with high diversity, ranging in species richness from 31 to 62 species per plot.

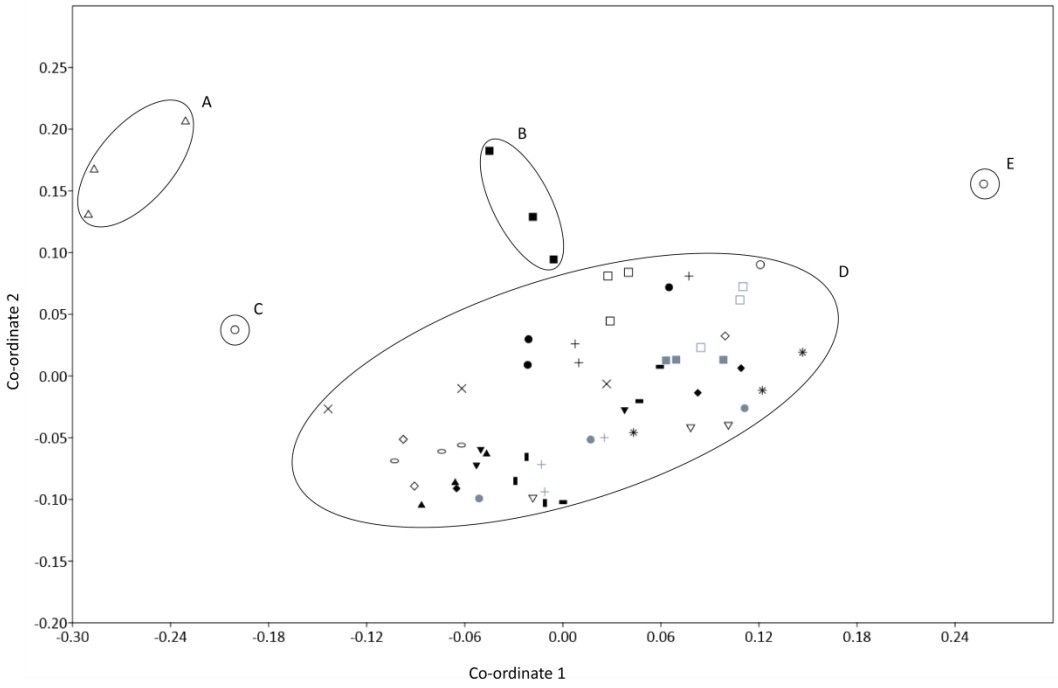

Figure 2.3: Two dimensional non-metric multidimensional scaling (Bray-Curtis Similarity) of Braun-Blanquet cover of species in each of the 60 plots from the 2015 survey, where different symbols represent the 20 transects. Groupings represent different vegetation communities. Stress= 0.1543.

2.4.2 Comparison with previous surveys

2.4.2.1 Comparison with Griffin et al*. (1983) survey*

A total of 316 taxa were found across the 60 plots surveyed in 2015 with an average of 45.5 (± 1.41 SE) taxa per plot; while 177 taxa were found across the six plots sampled by Griffin *et al*. (1983) in 1978/1979 with an average of 59 (± 7.15 SE) taxa per plot. A total of 13% of taxa recorded in the Griffin *et al*. (1983) survey were annuals. After removal of annual species from the data set to aid comparison between the two surveys the number of taxa in the 2015 survey data set was reduced to 315 with an average of 45.5 (± 1.41 SE) taxa per plot, while the Griffin *et al*. (1983) dataset was reduced to 156 taxa with an average of 53 (± 10.7 SE) taxa per plot.

Sample rarefaction was performed on the 2015 plot data. The 177 species found in six plots from the Griffin *et al*. (1983) survey is within the expected number of species to be found in six plots based on the 2015 data (Figure 2.4).

Prior to analysis of plot diversity between the two surveys, taxa that were deemed non-comparable were removed from the data sets- this saw the removal of 19 taxa from the 2015 data set (0.6% of all taxa), and the removal of 49 taxa from the Griffin *et al.* (1983) data set (27% of all taxa). These taxa were deemed non-comparable between surveys due to the lack of complete identifications, suspected misidentifications, or because they were annual species (the 2015 survey did not sample across seasons for annual species). A Mann-Whitney U test (α=0.05) indicated that the Griffin *et al*. (1983) and 2015 surveys did not differ in three measures of diversity; Number of taxa (U=0.137.5, p=0.352), Dominance (U=254.0, p=0.102) and Shannon Diversity (U=105.0; p=0.097), however they did differ in Evenness (U=1.0, p<0.001), with the 2015 survey having lower species Evenness (Table 2.4).

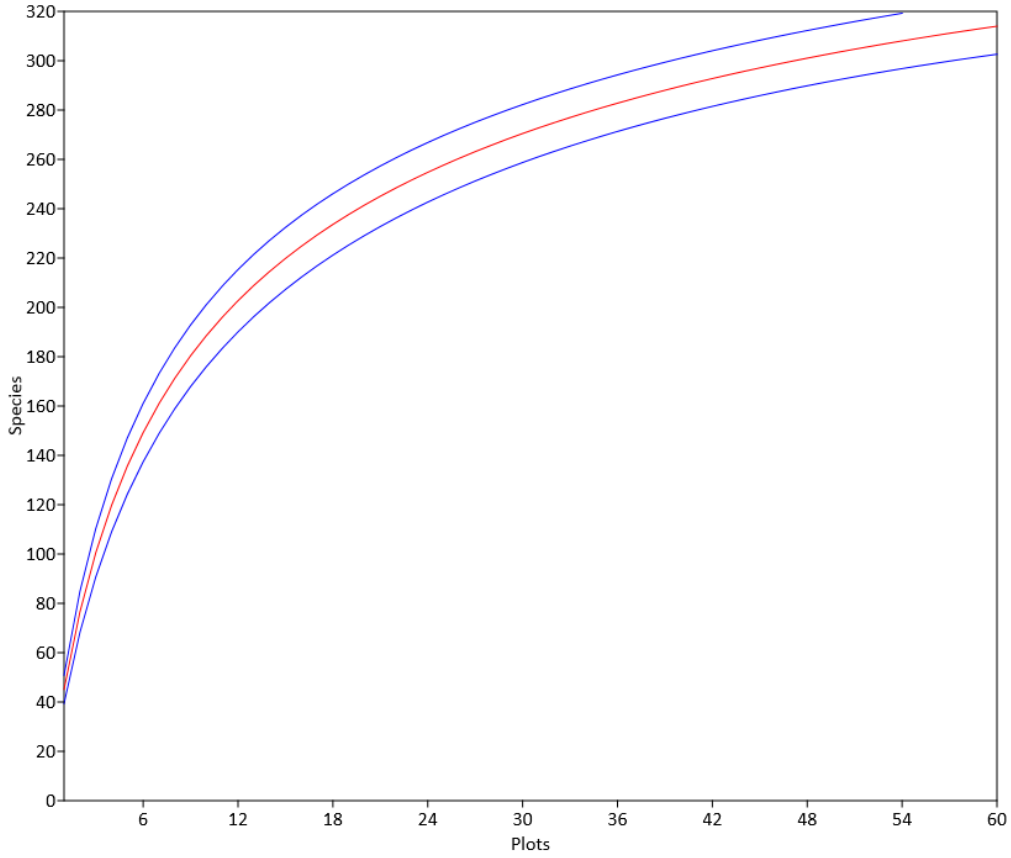

Figure 2.4: Sample rarefaction of species in the sixty plots surveyed in 2015. 95% confidence intervals are indicated.

Table 2.4: Measures of diversity for plots from the Griffin *et al*. (1983) and 2015 surveys, displayed as mean and standard error.

	Griffin *et al*. (1983) survey	2015 survey
Number of taxa	45.17 ± 9.48	44.38 ± 1.37
Dominance	0.05 ± 0.02	0.06 ± 0.01
Shannon Diversity	3.37 ± 0.32	2.91 ± 0.03
Evenness	0.77 ± 0.03	0.44 ± 0.01

The abundance (Braun-Blanquet cover) of species identified in the 2015 survey and the Griffin *et al*. (1983) survey (with non-comparable data removed, i.e. annual species, incomplete identifications, and misidentifications) were compared (Figure 2.5; note that the comparison of presence-absence data was very similar, Figure S2.1). A One-way ANOSIM indicated that the community composition of the surveys was not the same (p<0.001, R=0.6359). The six Griffin *et al*. (1983) plots were distinct from the 2015 plots in the ordination, with two of the Griffin *et al*. (1983) plots being grouped

together (Group A, Figure 2.5), and the four others being grouped together (Group E, Figure 2.5). Three of the 2015 plots (from one transect) were grouped separately from the rest of the 2015 plots in the ordination (Group B, Figure 2.5), lying between the bulk of the 2015 plots (Group C, Figure 2.5) and two of the Griffin *et al*. (1983) plots (Group A, Figure 2.5), while one other plot from the 2015 survey was grouped separately from all others (Group D, Figure 2.5).

The four outlier plots from the 2015 survey (Groups B and D, Figure 2.5) were the westernmost plots of the SENR survey. The plots in Group B were dominated by *Allocasuarina-Eucalyptus* woodland with little understorey, and thus had low plot diversity (Table 2.5). The plot in Group D was dominated by large *Banksia* and *Adenanthos cygnorum* shrubs, also with little understorey, and thus low plot diversity (Table 2.5). All six plots from the Griffin *et al*. (1983) survey were outliers (Groups A and E, Figure 2.5). The plots in Group A were dominated by *Allocasuarina campestris*, *Calothamnus quadrifidus*, and *Melaleuca* sp. and had few species in the understorey (Griffin *et al*. 1983; Table 2.5). The plots in Group A were also located on red-brown sandy loams, whereas the majority of other plots surveyed were on white-grey sands (Griffin *et al*. 1983). The plots from Group E were dominated by low shrubs such as *Allocasuarina humilis*, *A. microstachya*, *Hibbertia crassifolia*, and *Banksia shuttleworthiana* (Table 2.5). Three of these plots are spatially separate from all other plots within SENR, being located in the north-eastern corner of the reserve, within the area now occupied by the mine (Plots 1, 2, and 13, Figure 2.2).

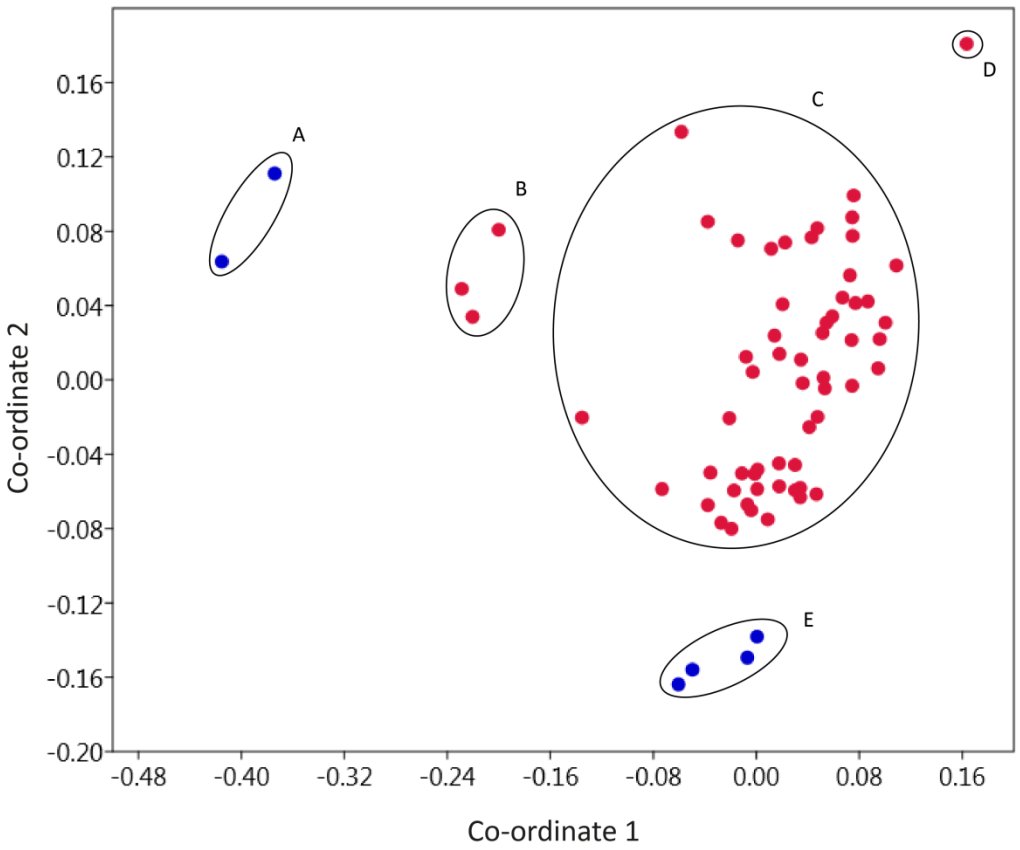

Figure 2.5: Two dimensional non-metric multidimensional scaling (Bray-Curtis similarity) of Braun-Blanquet cover of species in plots from the Griffin *et al*. (1983) survey (blue triangles), and the 2015 survey (red dots). Stress=0.1405.

Table 2.5: Vegetation communities present within SENR as determined from the 2015 and Griffin *et al.* (1983) surveys.

	Vegetation Community				
	A	**B**	**C**	**D**	**E**
Number of plots	2	3	56	1	4
Dominant species	*Allocasuarina campestris, Calothamnus quadrifidus, Melaleuca* sp., *Jacksonia hakeoides, Petrophile media, Stylobasium australe*	*Allocasuarina campestris, Eucalyptus gittinsii, E. leptopoda, Melaleuca tinkeri*	*Xanthorrhoea drummondii, Allocasuarina humilis*, *A. microstachya, Mesomelaena pseudostygia, Schoenus subflavus, Hibbertia hypericoides, Beaufortia elegans*	*Adenanthos cygnorum, Astroloma xerophyllum, Banksia attenuata, B. hookeriana, B. menziesii, Jacksonia furcellata, Lechenaultia biloba*	*Allocasuarina humilis, A. microstachya, Banksia shuttleworthiana, Gastrolobium plicatum, Hakea auriculata, Hibbertia crassifolia, Melaleuca trichophylla, Mesomelaena stygia*
Indicator species	*Petrophile media, Stylobasium australe, Trachymene pilosa, Schoenus nanus, Podotheca gnaphalioides, Pterochaeta paniculata, Thelymitra villosa*	*Allocasuarina campestris, Melaleuca tinkeri, Scholtzia* sp. Eneabba*, Verticordia eriocephala*.	*Schoenus subflavus, Hibbertia hypericoides, Beaufortia elegans, Allocasuarina humilis, A. microstachya*	*Astroloma xerophyllum, Banksia menziesii, Lechenaultia biloba, Verticordia argentea, Jacksonia furcellata*	*Babingtonia grandiflora, Conostylis androstemma, Gastrolobium plicatum, Goodenia coerulea, Petrophile megalostegia, Tetraria octandra.*
Mean plot diversity (Shannon)	2.40 ± 0.37	2.38 ± 0.20	2.95 ± 0.05	2.38	3.86 ± 0.06

2.4.2.2 Comparison of presence of 40 species across three surveys

Elkington and Griffin (1985) identified 40 species as being dominant in their 1984 survey of SENR (Table 2.6). The presence of these species in the Griffin *et al*. (1983) survey was investigated as well as their presence in the 2015 survey, in order to determine whether they were still present within SENR. A total of 34 of these 40 species occurred within the 2015 survey plots, indicating little change in the presence of these species across SENR. The six species that were not recorded in the 2015 survey were *Jacksonia hakeoides, Hibbertia glaberrima, Lysinema ciliatum, Scaevola canescens, Verticordia nitens,* and *Verticordia muelleriana.* The distribution range of three of these species does not cover the Eneabba area: *Lysinema ciliatum* occurs from Perth to Esperance; *Verticordia nitens* occurs from Gingin to Harvey; and *Hibbertia glaberrima* is limited to an area within the Pilbara region (Western Australian Herbarium 2016). Therefore it was concluded that these three taxa were likely misidentified in the Elkington and Griffin (1985) survey, and were removed from the data set.

Table 2.6: List of 40 species identified as dominant in Elkington and Griffin (1985). Note that species marked with an asterisk were removed from the data set prior to analysis.

Family	Species
Casuarinaceae	*Allocasuarina humilis*
Cupressaceae	*Callitris acuminata*
Cyperaceae	*Mesomelaena stygia*
Dasypogonaceae	*Calectasia cyanea*
Ericaceae	*Andersonia heterophylla*
Fabaceae	*Acacia auronitens*
Fabaceae	*Acacia latipes*
Fabaceae	*Jacksonia floribunda*
Fabaceae	*Jacksonia hakeoides*
Goodeniaceae	*Scaevola canescens*
Myrtaceae	*Beaufortia elegans*
Myrtaceae	*Calothamnus quadrifidus*
Myrtaceae	*Calothamnus sanguineus*
Myrtaceae	*Darwinia neildiana*
Myrtaceae	*Eucalyptus todtiana*
Myrtaceae	*Leptospermum erubescens*

Myrtaceae	*Melaleuca systena*
Myrtaceae	*Phymatocarpus porphyrocephalus*
Myrtaceae	*Scholtzia involucrata*
Myrtaceae	*Verticordia densiflora*
Myrtaceae	*Verticordia muelleriana*
Proteaceae	*Adenanthos cygnorum*
Proteaceae	*Banksia attenuata*
Proteaceae	*Banksia candolleana*
Proteaceae	*Banksia hookeriana*
Proteaceae	*Banksia menziesii*
Proteaceae	*Banksia shuttleworthiana*
Proteaceae	*Banksia tridentata*
Proteaceae	*Grevillea shuttleworthiana*
Proteaceae	*Hakea auriculata*
Proteaceae	*Hakea candolleana*
Proteaceae	*Hakea incrassata*
Proteaceae	*Hakea obliqua*
Proteaceae	*Petrophile macrostachya*
Proteaceae	*Stirlingia latifolia*
Proteaceae	*Xylomelum angustifolium*
Xanthorrhoeaceae	*Xanthorrhoea drummondii*
Ericaceae	*Lysinema ciliatum**
Myrtaceae	*Verticordia nitens**
Dilleniaceae	*Hibbertia glaberrima**

Sample rarefaction was performed on the data from the Elkington and Griffin (1985) survey, and indicated that fifty 10 m x 10 m plots should be sufficient in collecting all 37 species (Figure 2.6). The 2015 survey collected 34 species from 60 plots, while the Griffin *et al*. (1983) survey collected 17 species from six plots.

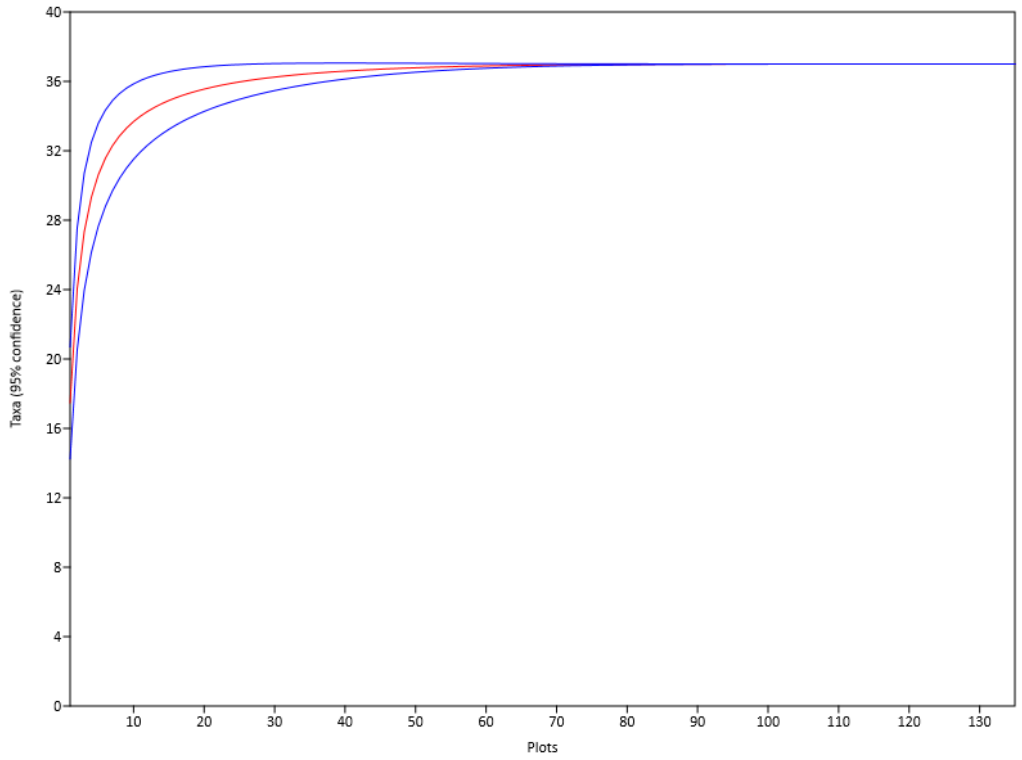

Figure 2.6: Sample rarefaction of 37 species in the Elkington and Griffin (1985) survey plots. 95% confidence intervals are indicated.

A Kruskal-Wallis test (α=0.05) indicated that the Griffin *et al*. (1983), Elkington and Griffin (1985) and 2015 survey plots differed in four measures of diversity; Number of taxa (χ^2=109.952, p<0.001), Dominance (χ^2=116.737, p<0.001), Shannon Diversity (χ^2=116.245; p<0.001), and Evenness (χ^2=90.053, p<0.001). Pairwise comparisons between the surveys indicated that for three measures of diversity (Number of taxa, Dominance, and Shannon Diversity) the 2015 survey did not differ from the Griffin *et al*. (1983) survey (p=1.00 in all cases). However both the 2015 survey and the Griffin *et al*. (1983) survey had a lower Number of taxa, higher Dominance, and lower Shannon Diversity than the Elkington and Griffin (1985) survey (p<0.002 in all cases). Pairwise comparisons indicated that the 2015 survey had significantly lower species Evenness than both the Griffin *et al*. (1983) and Elkington and Griffin (1985) surveys (p<0.001 in

both cases), while Evenness did not differ between the Griffin *et al*. (1983) and Elkington and Griffin (1985) surveys (p=0.764; Table 2.7).

Table 2.7: Measures of diversity for 37 species from plots from the Griffin *et al*. (1983), Elkington and Griffin (1985) and 2015 surveys, displayed as mean and standard error. Like letters indicate values that are not significantly different (Kruskal- Wallis test, α=0.05).

	Griffin *et al*. (1983)	**Elkington & Griffin (1985)**	**2015**
Number of taxa	6.667 ± 1.585[b]	17.482 ± 0.324[a]	8.850 ± 0.368[b]
Dominance	0.350 ± 0.139[b]	0.095 ± 0.01[a]	0.311 ± 0.025[b]
Shannon Diversity	1.489 ± 0.363[b]	2.543 ± 0.036[a]	1.396 ± 0.060[b]
Evenness	0.833 ± 0.059[a]	0.767 ± 0.010[a]	0.514 ± 0.019[b]

The abundance (Braun-Blanquet cover) of the 37 species identified in the Elkington and Griffin (1985) survey plots was compared with the abundance of these species found in the Griffin *et al*. (1983) and 2015 surveys (Figure 2.7; note that two dimensional nMDS of presence-absence data for these surveys presented near identical results; Figure S2.2). A One-way ANOSIM indicated that composition of plots between the surveys was not the same (p<0.001, R=0.449). Pairwise ANOSIMs indicated that the composition of plots in the Elkington and Griffin (1985) survey was different from the Griffin *et al*. (1983) survey (p<0.001, R=0.6874) and the 2015 survey (p<0.001, R=0.4488), while the abundance of species between the 2015 survey and the Griffin *et al*. (1983) survey was similar (p=0.0628, R=0.1553). The majority of the Elkington and Griffin (1985) plots are clustered closely together in the ordination, while the 2015 plots are spread. Two of the Griffin *et al*. (1983) plots (those indicated in Figure 2.7) are grouped separately from the rest of the plots in the ordination while the other four plots are grouped with the 2015 plots.

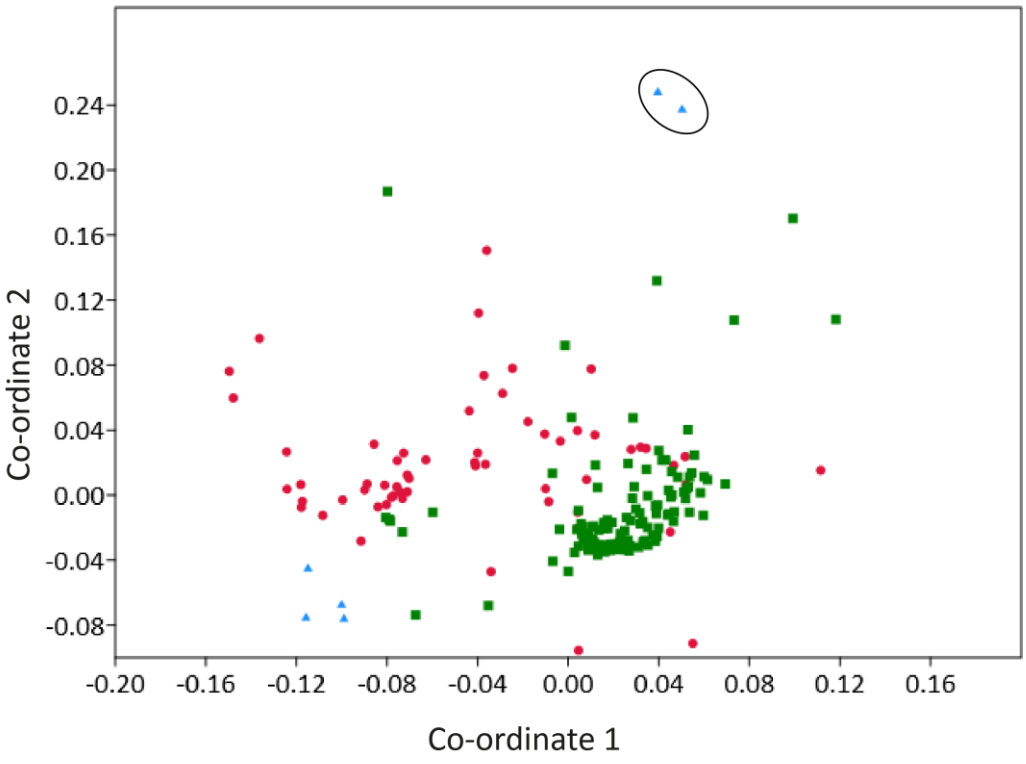

Figure 2.7: Two dimensional non-metric multidimensional scaling (Bray-Curtis similarity) for Braun-Blanquet cover of 37 species in plots from the Elkington and Griffin (1985) survey (green squares), Griffin *et al*. (1983) survey (blue triangles), and 2015 survey (red dots). Stress=0.2402.

2.4.2.3 Plant functional trait analysis

The observed proportion of RS to NRS species in the 2015 survey was not significantly different from the expected ratio based on the four historic surveys (χ^2=0.16, p= 0.899, α=0.05; Figure 2.8). Note that 165 species from the historic surveys were excluded from this analysis as their capability to resprout after fire is not known.

The observed proportion of SSB species to CSB species in the 2015 survey was not significantly different from the expected ratio based on the four historic surveys (χ^2=0.548, p= 0.459, α=0.05; Figure 2.9). However, the ratio of seed bank species to annual species was different (χ^2=4.329, p= 0.037, α=0.05). Note that nine species were excluded from this analysis as their categorisation as an annual, SSB, or CSB species is not known.

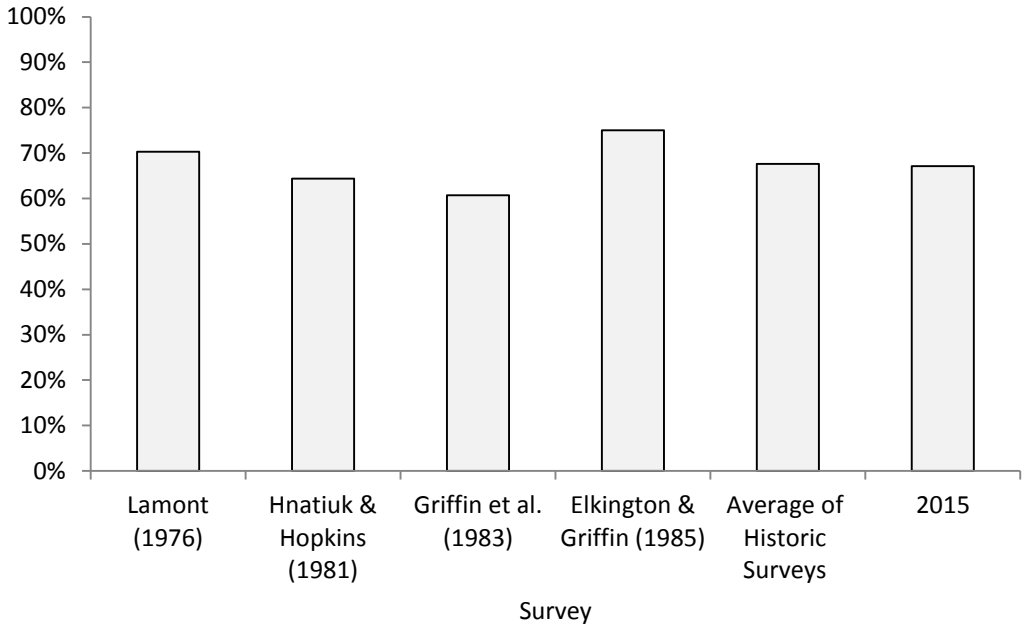

Figure 2.8: Proportion of resprouting species in the four historic surveys, the average of the four historic surveys, and the 2015 survey.

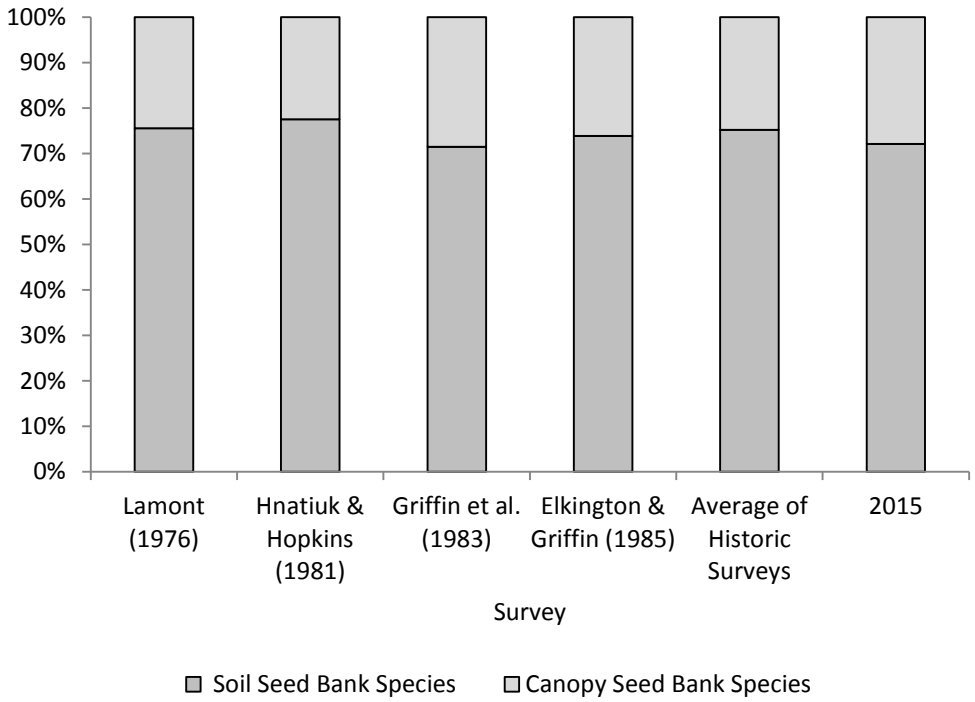

Figure 2.9: Proportion of soil seed bank species to canopy seed bank species and annual species in the four historic surveys, the average of the four historic surveys, and the 2015 survey. Note that annual species were not included in the analysis.

2.4.2.4 Species composition comparison

A total of 316 taxa were found in the 2015 survey and 610 taxa across all historic surveys (235 taxa in the Lamont (1976) survey, 418 taxa in the Hnatiuk and Hopkins (1981) survey, 177 taxa in the Griffin *et al*. (1983) survey, and 265 taxa in the Elkington and Griffin (1985) survey). Of the species found in the 2015 survey, 58% also occurred within at least one of the historic surveys, while 42% only occurred in the 2015 survey (Table 2.8). There were 427 species found only in the historic surveys (Table 2.8).

Species that did not occur in the 2015 survey but did occur in at least one of the historic surveys were further investigated. In order to make the historic data comparable with the 2015 survey, species were removed from the data set if they met certain criteria (outlined in Table 2.9), which resulted in a total of 285 taxa being removed from the historic survey data set (Table 2.8). A total of 19 taxa with only partial identifications were also removed from the 2015 data set.

The remaining 142 species that were only found in the historic surveys were further investigated to determine why they did not occur in the 2015 survey (Table 2.10). Two of these species were parasitic, nine species could be explained by incomplete identifications in 2015, 21 species only occurred in one historic survey with the survey area being at the edge of the species' range, 24 species occurred in more than one historic survey with the survey area being at the edge of the species' range, 42 species occurred in only one historic survey but have a wide distribution, while 39 species occurred in more than one historic survey and have a wide distribution (Table 2.10).

Table 2.8: Number of species shared between the 2015 survey and 1, 2, 3, and 4 reference surveys, and species present only in the 2015 or reference surveys, before and after removal of non-comparable taxa.

		2015 survey and;						
		4 ref. surveys	3 ref. surveys	2 ref. surveys	1 ref. survey	2015 survey only	Ref. surveys only	Total
Original data set	Number of species	46	61	45	31	133	427	743
	Percentage of species	6.19	8.21	6.06	4.17	17.9	57.47	100
Data set after removals	Number of species	46	61	45	31	114	142	439
	Percentage of species	10.48	13.9	10.25	7.06	25.97	32.35	100

Table 2.9: Number of taxa removed from data set and criteria used to determine which taxa should be removed.

Number of taxa removed	Reason for removal from data set
129	Only partial identifications are provided for these taxa.
59	Species are not known to occur within the Irwin District of the Southwest Botanical Province (as per distributions recorded in FloraBase; Western Australian Herbarium 2016), and are likely a misidentification.
9	Species occur in swampy, marshy or wet habitat, area containing these habitat types is located near the mine site north of the area of SENR surveyed in 2015 (Hnatiuk & Hopkins 1981).
16	Species are restricted to soil types that do not occur within the 2015 survey area.
13	Species are from the family Orchidaceae, occur seasonally. No species from the family Orchidaceae identified in the 2015 survey.
11	Species are exotic weeds. No weed species identified in the 2015 survey.
48	Species are annuals; only occur at certain times of the year. Only one annual species was identified in the 2015 survey.

Table 2.10: Species absent from the 2015 survey of SENR but recorded in at least one historic survey. Species are classified into groups that may help explain their absence from the 2015 survey.

Family	Genus and Species	Lamont 1976	Hnatiuk & Hopkins 1981	Griffin *et al.* 1983	Elkington & Griffin 1985
Parasitic species					
Loranthaceae	*Amyema miquelii*		✔		
Apodanthaceae	*Pilostyles hamiltonii*	✔			
Inaccurate identification in 2015 survey					
Droseraceae	*Drosera macrantha*	✔	✔	✔	
Droseraceae	*Drosera pallida*	✔	✔		✔
Lauraceae	*Cassytha flava*				✔
Lauraceae	*Cassytha pomiformis*			✔	✔
Lauraceae	*Cassytha pubescens*		✔		
Lauraceae	*Cassytha racemosa*			✔	
Proteaceae	*Banksia lanata*			✔	✔
Proteaceae	*Banksia vestita*	✔	✔		✔
Proteaceae	*Hakea lissocarpha*		✔	✔	
Recorded in one historic survey, occurring at edge of species' range					
Asparagaceae	*Laxmannia grandiflora*	✔			
Asparagaceae	*Laxmannia ramosa*	✔			
Asparagaceae	*Laxmannia squarrosa*				✔
Asparagaceae	*Lomandra sericea*			✔	
Asparagaceae	*Thysanotus ?tenellus*		✔		
Casuarinaceae	*Allocasuarina ramosissima*			✔	
Fabaceae	*Daviesia dielsii*		✔		
Fabaceae	*Gastrolobium capitatum*		✔		
Fabaceae	*Gompholobium aristatum*		✔		
Goodeniaceae	*Dampiera alata*	✔			
Haemodoraceae	*Conostylis setigera*	✔			
Myrtaceae	*Babingtonia camphorosmae*			✔	
Myrtaceae	*Ericomyrtus serpyllifolia*	✔			
Myrtaceae	*Eucalyptus drummondii*		✔		
Myrtaceae	*Eucalyptus wandoo*	✔			
Myrtaceae	*Regelia ciliata*	✔			

Polygalaceae	*Comesperma confertum*	✔			
Proteaceae	*Banksia sphaerocarpa*	✔			
Proteaceae	*Persoonia rudis*	✔			
Rutaceae	*Philotheca pinoides*		✔		
Santalaceae	*Leptomeria empetriformis*		✔		
Recorded in more than one historic survey, occurring at edge of species' range					
Asparagaceae	*Thysanotus spiniger*	✔			✔
Myrtaceae	*Eucalyptus rhodantha*	✔	✔		
Ericaceae	*Leucopogon obtectus*		✔		✔
Asparagaceae	*Lomandra caespitosa*		✔	✔	
Cyperaceae	*Tetraria octandra*		✔	✔	✔
Dasypogonaceae	*Dasypogon bromeliifolius*		✔		✔
Dilleniaceae	*Hibbertia huegelii*		✔	✔	
Droseraceae	*Drosera leucoblasta*	✔	✔		
Droseraceae	*Drosera paleacea*		✔		✔
Euphorbiaceae	*Monotaxis grandiflora*	✔	✔		✔
Fabaceae	*Gastrolobium obovatum*		✔		✔
Fabaceae	*Gastrolobium plicatum*	✔	✔	✔	
Fabaceae	*Hovea stricta*	✔	✔	✔	✔
Fabaceae	*Sphaerolobium macranthum*	✔		✔	
Goodeniaceae	*Dampiera carinata*	✔	✔		✔
Haemodoraceae	*Conostylis crassinervia*		✔	✔	
Lamiaceae	*Hemiandra pungens*	✔	✔		✔
Polygalaceae	*Comesperma drummondii*	✔	✔		✔
Proteaceae	*Adenanthos drummondii*		✔		✔
Proteaceae	*Conospermum incurvum*	✔	✔		✔
Proteaceae	*Hakea ruscifolia*	✔	✔		✔
Proteaceae	*Persoonia angustiflora*	✔	✔		
Myrtaceae	*Eucalyptus jucunda*		✔		✔
Celastraceae	*Stackhousia dielsii*	✔	✔		✔
Occurs in one historic survey, wide distribution.					
Asparagaceae	*Chamaescilla corymbosa*			✔	
Asparagaceae	*Laxmannia sessiliflora*		✔		
Asparagaceae	*Thysanotus teretifolius*		✔		
Celastraceae	*Stackhousia pubescens*	✔			
Colchicaceae	*Wurmbea dioica*		✔		
Cyperaceae	*Schoenus unispiculatus*				✔
Dilleniaceae	*Hibbertia acerosa*		✔		

Droseraceae	*Drosera glanduligera*			✔	
Euphorbiaceae	*Monotaxis bracteata*	✔			
Fabaceae	*Acacia alata*			✔	
Fabaceae	*Kennedia prostrata*	✔			
Fabaceae	*Labichea lanceolata*			✔	
Goodeniaceae	*Dampiera linearis*				✔
Goodeniaceae	*Dampiera oligophylla*		✔		
Goodeniaceae	*Dampiera teres*		✔		
Goodeniaceae	*Scaevola humifusa*		✔		
Goodeniaceae	*Scaevola phlebopetala*		✔		
Gyrostemonaceae	*Gyrostemon ramulosus*	✔			
Haemodoraceae	*Anigozanthos manglesii*		✔		
Haemodoraceae	*Haemodorum spicatum*		✔		
Hemerocallidaceae	*Corynotheca micrantha*		✔		
Lamiaceae	*Quoya verbascina*		✔		
Myrtaceae	*Calytrix oldfieldii*				✔
Myrtaceae	*Eucalyptus macrocarpa*				✔
Myrtaceae	*Eucalyptus rudis*		✔		
Myrtaceae	*Leptospermum oligandrum*	✔			
Myrtaceae	*Scholtzia capitata*				✔
Myrtaceae	*Scholtzia umbellifera*		✔		
Myrtaceae	*Thryptomene mucronulata*		✔		
Myrtaceae	*Verticordia chrysantha*		✔		
Poaceae	*Austrostipa variabilis*		✔		
Proteaceae	*Banksia fraseri*			✔	
Proteaceae	*Banksia prionotes*				✔
Proteaceae	*Grevillea brachystachya*				✔
Proteaceae	*Grevillea leucopteris*		✔		
Proteaceae	*Grevillea pinaster*			✔	
Proteaceae	*Persoonia rufiflora*				✔
Proteaceae	*Petrophile megalostegia*			✔	
Rhamnaceae	*Cryptandra pungens*				✔
Rhamnaceae	*Polianthion wichurae*			✔	
Santalaceae	*Exocarpos sparteus*		✔		
Surianaceae	*Stylobasium australe*			✔	
	Occurs in more than one historic survey, wide distribution.				
Apiaceae	*Platysace xerophila*		✔		✔
Asparagaceae	*Laxmannia omnifertilis*		✔		✔

Celastraceae	*Tripterococcus brunonis*	✔	✔		✔
Colchicaceae	*Burchardia congesta*	✔	✔	✔	✔
Cyperaceae	*Lepidosperma scabrum*		✔	✔	
Cyperaceae	*Lepidosperma tenue*			✔	✔
Elaeocarpaceae	*Tetratheca confertifolia*	✔	✔	✔	
Ericaceae	*Leucopogon conostephioides*		✔		✔
Ericaceae	*Leucopogon hispidus*		✔		✔
Fabaceae	*Acacia blakelyi*		✔	✔	✔
Fabaceae	*Acacia saligna*		✔	✔	
Fabaceae	*Gompholobium shuttleworthii*		✔	✔	
Fabaceae	*Jacksonia hakeoides*		✔	✔	✔
Goodeniaceae	*Goodenia coerulea*		✔	✔	✔
Goodeniaceae	*Scaevola canescens*	✔	✔	✔	✔
Goodeniaceae	*Verreauxia reinwardtii*	✔	✔		✔
Haemodoraceae	*Conostylis aculeata*	✔	✔		✔
Haemodoraceae	*Conostylis androstemma*		✔	✔	✔
Haemodoraceae	*Conostylis dielsii*	✔			✔
Hemerocallidaceae	*Arnocrinum preissii*		✔		✔
Hemerocallidaceae	*Tricoryne elatior*	✔	✔		✔
Loganiaceae	*Orianthera spermacocea*		✔		✔
Loranthaceae	*Nuytsia floribunda*	✔	✔		✔
Molluginaceae	*Macarthuria australis*	✔	✔		
Myrtaceae	*Babingtonia grandiflora*	✔	✔	✔	✔
Myrtaceae	*Calytrix flavescens*		✔	✔	✔
Myrtaceae	*Calytrix strigosa*	✔	✔		
Myrtaceae	*Eucalyptus johnsoniana*				✔
Myrtaceae	*Hypocalymma xanthopetalum*	✔	✔	✔	✔
Polygalaceae	*Comesperma calymega*	✔	✔		✔
Proteaceae	*Conospermum acerosum*	✔	✔		✔
Proteaceae	*Conospermum triplinervium*	✔	✔		✔
Proteaceae	*Grevillea polybotrya*		✔		✔
Proteaceae	*Hakea prostrata*	✔	✔		
Rubiaceae	*Opercularia spermacocea*			✔	✔
Rutaceae	*Boronia coerulescens*	✔	✔		✔
Rutaceae	*Diplolaena ferruginea*		✔	✔	✔
Stylidiaceae	*Stylidium rigidulum*	✔	✔		
Violaceae	*Hybanthus floribundus*		✔		✔

The 115 species (the 19 taxa with incomplete identifications were removed from the data set) that were only found in the 2015 survey were further investigated to determine whether their presence may be explained by migration southwards into SENR over the past thirty years. The range of each species was analysed, and it was found that only one species, *Acacia megacephala*, may have migrated southwards, as the ranges of all other species either cover areas much further south of the Eneabba region, or are restricted to the Eneabba region itself (Table 2.11).

Table 2.11: Species present in only the 2015 survey of SENR and their distributions.

Family	Species	Distribution
Anarthriaceae	*Lyginia imberbis*	Widespread
Asparagaceae	*Lomandra micrantha* subsp. *micrantha*	Widespread
Asteraceae	*Cephalipterum drummondii*	Widespread
Cyperaceae	*Baumea juncea*	Widespread
Cyperaceae	*Cyathochaeta avenacea*	Widespread
Cyperaceae	*Lepidosperma apricola*	Widespread
Cyperaceae	*Lepidosperma costale*	Widespread
Cyperaceae	*Lepidosperma squamatum*	Widespread
Cyperaceae	*Schoenus grandiflorus*	Widespread
Dasypogonaceae	*Dasypogon obliquifolius*	Local area
Dilleniaceae	*Hibbertia leucocrossa*	Local area
Dilleniaceae	*Hibbertia rostellata*	Widespread
Droseraceae	*Drosera eneabba*	Widespread
Droseraceae	*Drosera porrecta*	Widespread
Ecdeiocoleaceae	*Georgeantha hexandra*	Local area
Ericaceae	*Astroloma glaucescens*	Widespread
Ericaceae	*Astroloma oblongifolium*	Local area
Ericaceae	*Astroloma* sp. *Eneabba*	Local area
Ericaceae	*Conostephium pendulum*	Widespread
Ericaceae	*Leucopogon crassiflorus*	Local area
Ericaceae	*Leucopogon* sp. *Carnamah*	Local area
Ericaceae	*Lysinema pentapetalum*	Widespread
Fabaceae	*Acacia acuminata*	Widespread
Fabaceae	*Acacia andrewsii*	Widespread
Fabaceae	*Acacia megacephala*	North, Priority 3
Fabaceae	*Acacia stenoptera*	Widespread
Fabaceae	*Bossiaea eriocarpa*	Widespread

Fabaceae	*Daviesia benthamii*	Widespread
Fabaceae	*Daviesia debilior*	Local area
Fabaceae	*Daviesia physodes*	Widespread
Fabaceae	*Daviesia triflora*	Widespread
Fabaceae	*Gastrolobium axillare*	Local area
Fabaceae	*Gastrolobium oxylobioides*	Widespread
Fabaceae	*Gompholobium preissii*	Widespread
Fabaceae	*Hardenbergia comptoniana*	Widespread
Fabaceae	*Jacksonia condensata*	Widespread
Fabaceae	*Jacksonia furcellata*	Widespread
Fabaceae	*Jacksonia lehmannii*	Widespread
Frankeniaceae	*Frankenia pauciflora*	Widespread
Goodeniaceae	*Scaevola repens* subsp. Northern Sandplains	Local area
Haemodoraceae	*Conostylis hiemalis*	Local area
Haemodoraceae	*Conostylis latens*	Local area
Lamiaceae	*Hemiandra* sp. Eneabba	Local area
Lamiaceae	*Microcorys* sp. Coomallo	Local area
Malvaceae	*Guichenotia micrantha*	Widespread
Malvaceae	*Thomasia grandiflora*	Widespread
Myrtaceae	*Baeckea grandis*	Local area
Myrtaceae	*Calothamnus glaber*	Widespread
Myrtaceae	*Calytrix chrysantha*	Local area
Myrtaceae	*Calytrix drummondii*	Local area
Myrtaceae	*Calytrix glutinosa*	Widespread
Myrtaceae	*Chamelaucium* sp. Bunjil	Local area
Myrtaceae	*Darwinia capitellata*	Widespread
Myrtaceae	*Eremaea ectadioclada*	Local area
Myrtaceae	*Eremaea hadra*	Local area
Myrtaceae	*Eucalyptus celastroides*	Widespread
Myrtaceae	*Eucalyptus gittinsii*	Widespread
Myrtaceae	*Eucalyptus leptopoda*	Widespread
Myrtaceae	*Homalocalyx chapmanii*	Local area
Myrtaceae	*Hypocalymma gardneri*	Local area
Myrtaceae	*Malleostemon roseus*	Widespread
Myrtaceae	*Melaleuca ciliosa*	Local area
Myrtaceae	*Melaleuca leuropoma*	Widespread
Myrtaceae	*Melaleuca orbicularis*	Local area
Myrtaceae	*Melaleuca tinkeri*	Widespread

Myrtaceae	*Melaleuca urceolaris*	Local area
Myrtaceae	*Melaleuca zonalis*	Local area
Myrtaceae	*Scholtzia leptantha*	Widespread
Myrtaceae	*Scholtzia* sp. Eneabba	Widespread
Myrtaceae	*Scholtzia* sp. Wongonderrah	Local area
Myrtaceae	*Thryptomene cuspidata*	Widespread
Myrtaceae	*Verticordia argentea*	Local area
Myrtaceae	*Verticordia eriocephala*	Widespread
Myrtaceae	*Verticordia huegelii*	Widespread
Myrtaceae	*Verticordia nobilis*	Widespread
Olacaceae	*Olax scalariformis*	Widespread
Proteaceae	*Banksia armata* var. *armata*	Widespread
Proteaceae	*Banksia cypholoba*	Local area
Proteaceae	*Banksia densa*	Widespread
Proteaceae	*Banksia glaucifolia*	Local area
Proteaceae	*Banksia micrantha*	Local area
Proteaceae	*Banksia nana*	Local area
Proteaceae	*Banksia sclerophylla*	Local area
Proteaceae	*Banksia sessilis*	Widespread
Proteaceae	*Conospermum unilaterale*	Local area
Proteaceae	*Conospermum wycherleyi* subsp. *wycherleyi*	Local area
Proteaceae	*Grevillea uniformis*	Local area
Proteaceae	*Hakea eneabba*	Widespread
Proteaceae	*Hakea neospathulata*	Widespread
Proteaceae	*Hakea polyanthema*	Widespread
Proteaceae	*Hakea psilorrhyncha*	Widespread
Proteaceae	*Persoonia comata*	Widespread
Proteaceae	*Persoonia filiformis*	Local area
Proteaceae	*Petrophile aculeata*	Local area
Proteaceae	*Petrophile brevifolia*	Widespread
Proteaceae	*Petrophile pilostyla*	Widespread
Proteaceae	*Petrophile rigida*	Widespread
Proteaceae	*Petrophile seminuda*	Widespread
Proteaceae	*Synaphea aephynsa*	Local area
Restionaceae	*Alexgeorgea subterranea*	Local area
Restionaceae	*Desmocladus biformis*	Local area
Restionaceae	*Desmocladus elongatus*	Local area
Restionaceae	*Desmocladus myriocladus*	Widespread

Restionaceae	*Desmocladus parthenicus*	Widespread
Restionaceae	*Desmocladus semiplanus*	Local area
Restionaceae	*Desmocladus virgatus*	Local area
Restionaceae	*Hypolaena exsulca*	Widespread
Restionaceae	*Lepidobolus quadratus*	Local area
Rhamnaceae	*Stenanthemum humile*	Local area
Rhamnaceae	*Stenanthemum notiale*	Widespread
Stylidiaceae	*Stylidium cygnorum*	Widespread
Stylidiaceae	*Stylidium stenosepalum*	Local area
Thymelaeaceae	*Pimelea ferruginea*	Widespread
Xanthorrhoeaceae	*Xanthorrhoea brunonis*	Local area
Zamiaceae	*Macrozamia fraseri*	Widespread

The number of species found per square metre of land surveyed was determined in order to compare species richness between surveys (data set with non-comparable species removed was used). It was found that the 2015 survey and the Elkington and Griffin (1985) survey recorded fewer species per 100 m^2 sampled than the Hnatiuk and Hopkins (1981) and Griffin *et al*. (1983) surveys (Table 2.12).

Table 2.12: Number of taxa and taxa per square metre found in the 2015 survey and the historic surveys. Note that there was no survey area provided for the Lamont (1976) survey.

		Survey			
	2015	**Lamont (1976)**	**Hnatiuk & Hopkins (1981)**	**Griffin *et al.* (1983)**	**Elkington & Griffin (1985)**
Taxa found	297	176	245	121	195
Area surveyed m^2	6,000	N/A	1,392	600	13,500
Taxa found per 100 m^2	4.95	N/A	17.60	20.17	1.44

2.5 Discussion

This study indicates that the recent changes in climate, mainly decreased rainfall and increased temperature that have occurred over the past 40 years have not had a measurable impact on the composition and diversity of the flora within South Eneabba Nature Reserve (SENR). The proportion of two key functional traits present in the flora

has not changed significantly; the proportion of resprouting (RS) to non-resprouting (NRS) species has remained the same across the past 40 years, as has the proportion of canopy seed bank (CSB) to soil seed bank (SSB) species.

The diversity of plants within SENR has not changed significantly over time but there were some differences with the historic surveys. The 2015 survey did not differ from the Griffin *et al*. (1983) survey on three levels of diversity; Number of taxa, Dominance, and Shannon diversity, although it did record lower species Evenness. The 2015 survey contained the majority of the 37 common species identified in the Elkington and Griffin (1985) survey; although it recorded lower Dominance, Shannon diversity, and species Evenness for these species than the Elkington and Griffin (1985) survey. The 2015 survey had a similar Number of taxa, Dominance, and Shannon diversity to the Griffin *et al*. (1983) survey for these 37 species.

There were a large number of species found to be unique to the historic surveys or to the 2015 survey, though most are unlikely to be due to species migrations or extinctions but likely explained by the high diversity of the study area, and spatial and habitat variation between some of the plots.

2.5.1 Plant functional traits

There has been no significant change in plant functional traits within SENR over the past 40 years, which reflects the absence of significant change in species composition in the Reserve. The proportion of RS to NRS species has not significantly changed; this does not support the hypothesis that the proportion of RS species should have risen with increased drought. It is thought that the high level of fire stress in SWA resulted in the high proportion of RS species found in SWA compared to other Mediterranean regions (Bell 2001), and future increased drought is expected to result in more frequent fire. RS species are generally able to recover from fire and other stresses faster than NRS species due to the large amounts of resources allocated to the root system (Bell 2001; Enright *et al.* 2014; Zeppel *et al*. 2015). This large root system allows RS species to accumulate reserves and provides access to deep water sources, assisting the plant during stressful conditions by allowing the plant to accumulate biomass and set seed faster than NRS (re-seeding) species and thus giving RS species a competitive advantage (Bell 2001; Enright *et al*. 2014; Zeppel *et al*. 2015; Pausas *et al.* 2016). RS species are also able to lower drought stress by avoiding tissue dehydration through stomatal closure, and tissue water storage (Pausas *et al*. 2016). The changes in climate that have occurred at Eneabba over the past 40 years may not have induced

sufficient stress to affect the proportion of RS to NRS species, though it is possible that greater climate induced stress may affect this in the future.

The proportion of CSB species to SSB species has not significantly changed in SENR over the past 40 years. This does not support the hypothesis that the proportion of SSB species would have risen with increased drought. SSB species are able to store viable seed in a below ground seed bank until conditions are suitable for germination, some of this seed carries over to the following season, while CSB species release all seed post-fire (Auld & Denham 2006; Ooi 2012; Buma *et al*. 2013; Enright *et al*. 2014). In instances where fire is frequent, as is forecast for future climate scenarios, the CSB can become depleted, giving SSB species a competitive advantage (Ooi 2012; Enright *et al*. 2014). There is no evidence that fire frequency has changed at Eneabba over the past 40 years, and so there has been no driver for change in the proportion of SSB to CSB species. However, if the climate continues to dry, the frequency of fire is expected to increase, which could result in loss of CSB species and NRS species from the community.

2.5.2 Comparison between the 2015 survey and Griffin et al. *(1983) survey*

The species composition of SENR has not changed significantly over the past 40 years. The 2015 survey and the Griffin *et al*. (1983) survey of SENR did not significantly differ in three measures of diversity; Number of taxa, Dominance, and Shannon diversity; though species Evenness was significantly lower in the 2015 survey. Although diversity measures were similar, assemblages of plots surveyed by Griffin *et al.* (1983) were distinct from the 2015 plots.

It is unlikely that these differences in species assemblages are a result of changed overall composition of SENR, but are likely to be a reflection of specific site characteristics; five distinct vegetation communities were present within the 2015 and Griffin *et al*. (1983) plots (Figure 2.5). The Griffin *et al*. (1983) Plots 1, 2, 13, and 8 (Group E in Figure 2.5) are located to the north-east of SENR, in the area currently occupied by the mine and thus are spatially separate from the 2015 plots, none of which were located within the mine area. The Griffin *et al*. (1983) Plots 30 and 31 that were also a distinct grouping (Group A in Figure 2.5) were in an area of red and brown sandy loam soils, as opposed to the sandy soils present at all other sites (Griffin *et al*. 1983), and also had the lowest species richness of 10 and 19 species respectively. The 2015 plots 9.1, 9.2, and 9.3 that were a distinct grouping (Group B in Figure 2.5) are geographically close, all occurring around Rocky Springs Road on brown soils, and had

low species richness (17, 23, and 16 species respectively). The 2015 site 6.3 was an outlier (Group D in Figure 2.5), vegetation here appeared unburned for many years, with large *Banksia* shrubs and little understorey and thus low species richness (26 species). The remainder of the plots were grouped together (Group C in Figure 2.5), species richness was high in these plots (~35-55 species) and soil and vegetation composition was similar.

2.5.3 Comparison of abundance of 37 species across three surveys

Comparison of the abundance of 37 species sampled in the Elkington and Griffin (1985) survey to the abundance of these species in the Griffin *et al.* (1983) survey and the 2015 survey indicates that there has likely been little change in composition of these species across SENR. The difference in diversity between the Elkington and Griffin (1985) survey and both the 2015 and Griffin *et al*. (1983) survey is likely due to the fact that the 37 species for which Elkington and Griffin (1985) report abundance and distribution are not representative of the flora of SENR as a whole. Instead, these 37 species were chosen due to their representation of the different floristic groups present in the survey area (for example; wetland vegetation in depressions, *Banksia-Xylomelum* heath on dunes, low mixed heath on swales, low open woodland) and thus some of these species such as *Calothamnus quadrifidus*, *Hakea auriculata*, and *Jacksonia hakeoides* have very limited abundance and distribution in the overall Reserve which is dominated by low *Banksia* heathland (Community C, Table 2.5), and thus these species cannot be used as a surrogate for total biodiversity of SENR.

Aside from the three species that were removed from the data due to likely misidentifications (*Lysinema ciliatum*, *Hibbertia glaberrima* and *Verticordia nitens*), an additional three species were identified in the Elkington and Griffin (1985) survey that were not present in the 2015 survey; *Scaevola canescens*, *Jacksonia hakeoides* and *Verticordia muelleriana*. However, it is unlikely that the absence of these species from the 2015 survey represents a change in species composition but is more likely a reflection of the great floristic diversity of SENR. *Scaevola canescens* was found in 28 of the original 135 Elkington and Griffin (1985) plots, however all of these were within the area currently occupied by the mine and were not sampled in the 2015 survey. *Jacksonia hakeoides* was not widespread in the original Elkington and Griffin (1985) survey, occurring in only five plots, three of which are now within the area occupied by the mine, thus it is likely that the 2015 survey did not cover the species' distribution area. *Verticordia muelleriana* was relatively widespread in the original Elkington and

Griffin (1985) survey, occurring in 35 plots, the reason for the absence of this species from the 2015 survey is unknown- although it is possible that this species did occur in the 2015 survey, but may have been misidentified as a similar species (eight species of *Verticordia* were present in the 2015 survey, including the widespread *V. grandis* that is similar in appearance and habitat) or vice versa. Herbarium records show that all three of these species have been collected from the region in recent years; *Scaevola canescens* in 2011 (Western Australian Herbarium collection number PERTH 8293902), *Jacksonia hakeoides* in 2004 (Western Australian Herbarium collection number PERTH 6987079) and *Verticordia muelleriana* (Western Australian Herbarium collection number PERTH 8136319) and thus are still present within the region (Atlas of Living Australia 2017).

The groupings of plots displayed in the ordination of species abundance appear to be mainly explained by the species richness of the plots. The Griffin *et al*. (1983) Plots 30 and 31 were grouped separately from all others (highlighted in Figure 2.7); these plots contained very few of the 37 species (two in each plot) and occur in different soil types to all other plots, having red and brown loam soils as opposed to sand (Griffin *et al*. 1983). Sample rarefaction of the Elkington and Griffin (1985) survey data indicated that 50 plots should be required to sample the 37 species identified in the survey. The 2015 survey sampled 34 species from sixty plots; this variance is likely explained by the differences in plot locations, restricted distributions of some species, and the targeted choice of species to sample in the Elkington and Griffin (1985) survey, as mentioned above.

2.5.4 Species presence-absence across all surveys

There was a high level of floristic variability between the surveys. A number of species that were found in the historic surveys were not found in the 2015 survey, and vice versa. There are a number of possible explanations for this variability. Firstly, kwongan vegetation is an extremely biodiverse ecosystem, consisting of over 2,540 species from 433 genera and 91 families (Lamont *et al*. 1984; Hopper & Gioia 2004). The study region contains high species richness within sample plots (alpha diversity) as well as high species turnover or variation between sample plots (beta diversity) and it is likely that this diversity contributed to the high species variability encountered (Lamont *et al*. 1984). There is also a large number of infrequently occurring species in the Eneabba region; these species may have a wide distribution but not be locally abundant, they may be rare, or they may have restricted distributions (Hopkins & Griffin 1984). Hopkins and Griffin (1984) found that 100 m^2 plots in the Eneabba

region ranging from 0 to 80 km apart rarely exceeded 45% similarity (Jaccard similarity index), and even large plots rarely contained all species in the area. The sample rarefaction of the 2015 survey data supports this idea; the majority of, but not all species were sampled across the 60 plots. Thus a greater number of plots would aid in higher species detection and should be used in future surveys. Five different vegetation communities were sampled in the 2015 survey; future monitoring should consider these communities separately when assessing whether a change in composition has occurred.

Secondly, mineral sands mining commenced in Eneabba in 1975. The scale of mining operations has increased since Lamont's original 1975 survey of the area, with approximately 2,300 ha of vegetation having since been disturbed by mining operations (Iluka 2013). The expansion of the mine has meant that some habitats, vegetation communities and the species contained within them that were sampled in the historic surveys, especially those localised to the area within the mine site, were absent from the 2015 study. It is possible that these species were absent from the 2015 study due to lack of sampling in the mine site area, although it is unknown whether they still exist within this area or not. Future studies could gain access to the mine site in order to survey the area to determine if these species are still present.

Thirdly, seasonality can account for some of the variability in the surveys. Some species not detected in the 2015 survey were annuals, seasonal or exotic weeds. The time of year that the 2015 survey was performed meant that there was a reduced chance of detecting annual and seasonal species, such as orchids. The 2015 survey area was largely free of weeds; most weed species in the area are annuals, and the location of plots away from the edges of the Nature Reserve and access tracks, where weeds are abundant, reduced the chances of detection. Future surveys should be repeated across the seasons to ensure greater representation of seasonal species, in order to determine whether these species are in decline.

Fourthly, some species identified in the historic surveys but not detected in the 2015 survey occur in habitats or soil types that were not covered by the 2015 survey area. The historic surveys included areas of wetlands, winter wet depressions, marshes and rocky outcrops, and soils of clay and peat, all of which were not represented in the 2015 survey area, or in the case of the winter wet depressions, were not present during the sampling season. The wetland and marsh habitats present in the historic surveys are located north of the 2015 survey area within the mine area (Hnatiuk & Hopkins

1981; Landgate 2015), thus it is not known whether climate change has had an effect on these habitats, potentially reducing their size or eliminating them entirely. Future surveys could aim to establish plots that include these habitats and soil types in order to represent a greater number of species and determine whether certain habitat types are being lost; surveying across the seasons would also assist with this. Data collected from these plots could be used in future comparisons of diversity.

Finally, there was a high level of uncertainty surrounding taxonomy and species identifications recorded in the historic surveys (outlined in the following section), which lead to many taxa not being comparable between the historic and 2015 surveys, and may have inflated or understated certain differences between the surveys.

2.5.5 Potential species for use as indicators of change

Although no change in floristic composition was observed in this study, several international studies have found evidence of change in species distributions in response to climate change (Parmesan & Yohe 2003; Chen *et al*. 2012). For example; Foden *et al*. (2007) reported range contraction in the Namib Desert species *Aloe dichotoma,* likely as a result of a regional increase in temperature and decrease in rainfall. It was found that although population declines and mortalities were observed at the equatorial end of the species' distribution no range expansion was observed in newly climatically suitable habitat to the south of the species' range, indicating a lag between contraction away from the equator and expansion southwards (Foden *et al*. 2007).

Range shifts to higher elevations are analogous to range shifts to higher latitudes, both comprising species moving into cooler areas. For several northern hemisphere species, tree lines (which are thought to be limited by temperature) have been found to be advancing to areas of higher elevation or latitude, where temperatures are lower (Davis *et al*. 2001; Harsch *et al*. 2009). The majority of studies regarding the effect of climate change on species distributions have been conducted on alpine ecosystems and have reported shifts or contractions of species ranges to areas of higher elevation (Davis *et al*. 2001; Kelly & Goulden 2008; Parolo & Rossi 2008; Harsch *et al*. 2009). For example; Parolo and Rossi (2008) reported a change in floristic composition of the Rhaetian Alps in Italy over 46 years, which coincided with warming of the climate. There had been an increase in species richness along a 700 m elevation gradient, with 28 species present that had not been previously recorded in the study area, and 52 species recorded at elevations at least 50 m higher than their 1950s limit, indicating a shift in these species'

ranges up the elevation gradient (Parolo & Rossi 2008). The shifts in species ranges to areas of higher latitudes or elevations that have been reported by these studies indicate the need to focus efforts on species that are at their northern (for Southern hemisphere species) limits.

Particular groups of species could be used as indicators of change in the floristic composition of SENR occurring as a result of climate change, and these species should be monitored closely in future surveys. The species listed in Table 2.13 that were identified as being absent from the 2015 survey but present within the historic surveys, and that are near the edge of their range or have a small distribution should be monitored. The species listed in Table 2.14 that occur in the 2015 survey but that are non-resprouters and/or have canopy seed banks, particularly those with SENR at the northern edge of their range should also be monitored. All species of conservation significance should also continue to be monitored (Table 2.3, Table 2.14). The aforementioned groups of species are predicted to be at greatest risk of range shift or contraction occurring, a shift away from the warmer, drier edges of their range, or could even be totally lost from the community. Seasonal species are also of interest, with changing climate may come changes in the suitability of habitats for these species and they may disappear from the community. The proportion of RS to NRS and SSB to CSB species should continue to be monitored in the fixed plots from this survey in order to detect any shift towards or away from certain plant functional traits. Although there was no evidence of change in the 2015 survey, the proportion of SSB species to CSB species is expected to increase as rainfall decreases, and so too is the proportion of RS species to NRS species (Bell 2001; Auld & Denham 2006; Enright *et al.* 2014; Zeppel *et al.* 2015).

Table 2.13: List of species occurring in the historic surveys but absent from the 2015 survey that are at the edge of their range and of potential interest for monitoring in future surveys. Species with conservation significance are indicated with an asterisk.

Family	Species	Family	Species
	Laxmannia grandiflora	Haemodoraceae	*Conostylis setigera*
	Laxmannia ramosa		*Conostylis crassinervia*
	Laxmannia squarrosa	Lamiaceae	*Hemiandra pungens*
Asparagaceae	*Lomandra sericea*		*Babingtonia camphorosmae*
	Thysanotus ?tenellus		*Ericomyrtus serpyllifolia*
	Thysanotus spiniger		*Eucalyptus drummondii*
	Lomandra caespitosa	Myrtaceae	*Eucalyptus johnsoniana*Threatened*
Casuarinaceae	*Allocasuarina ramosissima*Priority 3*		*Eucalyptus wandoo*
Celastraceae	*Stackhousia dielsii*		*Regelia ciliata*
Cyperaceae	*Tetraria octandra*		*Eucalyptus rhodantha*
Dasypogonaceae	*Dasypogon bromeliifolius*		*Eucalyptus jucunda*
Dilleniaceae	*Hibbertia huegelii*	Polygalaceae	*Comesperma confertum*
Droseraceae	*Drosera leucoblasta*		*Comesperma drummondii*
	Drosera paleacea		*Banksia sphaerocarpa*
Ericaceae	*Leucopogon obtectus*Threatened*		*Persoonia rudis*
Euphorbiaceae	*Monotaxis grandiflora*		*Adenanthos drummondii*
	*Daviesia dielsii*Threatened*	Proteaceae	*Conospermum incurvum*
	Gastrolobium capitatum		*Hakea ruscifolia*
	Gompholobium aristatum		*Persoonia angustiflora*
Fabaceae	*Gastrolobium obovatum*		*Persoonia rudis* Priority 3*
	Gastrolobium plicatum	Rutaceae	*Philotheca pinoides*
	Hovea stricta	Santalaceae	*Leptomeria empetriformis*
	Sphaerolobium macranthum		
Goodeniaceae	*Dampiera alata*		
	Dampiera carinata		

Table 2.14: List of species occurring in the 2015 survey that may be at risk of range loss under future climate change scenarios. Resprouting ability; resprouter (RS) or non resprouter (NRS) and seed bank type; soil seed-bank (SSB) or canopy seed-bank (CSB), are indicated. Species for which SENR is located at the northern edge of their distribution are indicated, as well as species that are restricted to the Eneabba region.

Family	Species	RS / NRS	CSB / SSB	Distribution
Apiaceae	*Platysace juncea*	NRS	SSB	-
Casuarinaceae	*Allocasuarina humilis*	RS	CSB	-
Casuarinaceae	*Allocasuarina campestris*	NRS	CSB	-
Cupressaceae	*Callitris acuminata*	RS	CSB	Northern edge
Dilleniaceae	*Hibbertia rostellata*	NRS	SSB	-
Dilleniaceae	*Hibbertia subvaginata*	NRS	SSB	-
Droseraceae	*Drosera eneabba*	NRS	SSB	Restricted
Ericaceae	*Andersonia heterophylla*	NRS	SSB	Northern edge
Ericaceae	*Astroloma serratifolium*	NRS	SSB	-
Ericaceae	*Astroloma* sp. Eneabba	NRS	SSB	Northern edge
Ericaceae	*Astroloma xerophyllum*	NRS	SSB	Northern edge
Ericaceae	*Leucopogon crassiflorus*	NRS	SSB	Northern edge
Ericaceae	*Leucopogon leptanthus*	NRS	SSB	Northern edge
Ericaceae	*Lysinema pentapetalum*	NRS	SSB	Northern edge
Euphorbiaceae	*Stachystemon axillaris*	NRS	SSB	-
Fabaceae	*Acacia acuminata*	NRS	SSB	-
Fabaceae	*Acacia andrewsii*	NRS	SSB	-
Fabaceae	*Acacia lasiocarpa*	NRS	SSB	-
Fabaceae	*Acacia megacephala** *P3 species*	NRS	SSB	-
Fabaceae	*Acacia pulchella*	NRS	SSB	-
Fabaceae	*Bossiaea eriocarpa*	NRS	SSB	-
Fabaceae	*Daviesia benthamii*	NRS	SSB	-
Fabaceae	*Daviesia daphnoides*	NRS	SSB	-
Fabaceae	*Daviesia podophylla/quadrilatera*	NRS	SSB	-
Fabaceae	*Gastrolobium spinosum*	NRS	SSB	-
Fabaceae	*Gompholobium confertum*	NRS	SSB	Northern edge
Fabaceae	*Gompholobium preissii*	NRS	SSB	Northern edge
Fabaceae	*Gompholobium tomentosum*	NRS	SSB	-
Fabaceae	*Hardenbergia comptoniana*	NRS	SSB	Northern edge

Fabaceae	*Jacksonia condensata*	NRS	SSB	Northern edge
Frankeniaceae	*Frankenia pauciflora*	NRS	SSB	-
Goodeniaceae	*Lechenaultia stenosepala*	NRS	SSB	Northern edge
Goodeniaceae	*Scaevola repens* subsp. Northern Sandplains	NRS	SSB	Northern edge
Haloragaceae	*Glischrocaryon aureum*	NRS	SSB	-
Lauraceae	*Cassytha* sp.	NRS	SSB	-
Malvaceae	*Guichenotia micrantha*	NRS	SSB	-
Myrtaceae	*Baeckea grandis*	NRS	SSB	-
Myrtaceae	*Beaufortia bracteosa*	NRS	CSB	Northern edge
Myrtaceae	*Beaufortia elegans*	NRS	CSB	-
Myrtaceae	*Calothamnus glaber*	RS	CSB	-
Myrtaceae	*Calothamnus quadrifidus*	RS	CSB	-
Myrtaceae	*Calothamnus sanguineus*	RS	CSB	-
Myrtaceae	*Calothamnus torulosus*	RS	CSB	Northern edge
Myrtaceae	*Calytrix aurea*	NRS	SSB	Northern edge
Myrtaceae	*Calytrix chrysantha** [P4 species]	NRS	SSB	Northern edge
Myrtaceae	*Calytrix drummondii*	NRS	SSB	-
Myrtaceae	*Calytrix glutinosa*	NRS	SSB	-
Myrtaceae	*Calytrix sapphirina*	NRS	SSB	-
Myrtaceae	*Conothamnus trinervis*	RS	CSB	-
Myrtaceae	*Darwinia capitellata*	NRS	SSB	-
Myrtaceae	*Darwinia sanguinea*	NRS	SSB	Restricted
Myrtaceae	*Eremaea beaufortioides*	RS	CSB	-
Myrtaceae	*Eremaea ectadioclada*	RS	CSB	Northern edge
Myrtaceae	*Eremaea hadra*	RS	CSB	Restricted
Myrtaceae	*Eremaea pauciflora*	RS	CSB	-
Myrtaceae	*Eremaea violacea*	RS	CSB	-
Myrtaceae	*Eucalyptus celastroides*	RS	CSB	-
Myrtaceae	*Eucalyptus pleurocarpa/xtetragona*	RS	CSB	Northern edge
Myrtaceae	*Eucalyptus gittinsii*	RS	CSB	-
Myrtaceae	*Eucalyptus leptopoda*	RS	CSB	-
Myrtaceae	*Eucalyptus todtiana*	RS	CSB	Northern edge
Myrtaceae	*Leptospermum erubescens*	RS	CSB	-
Myrtaceae	*Leptospermum spinescens*	RS	CSB	-
Myrtaceae	*Malleostemon roseus*	NRS	SSB	-
Myrtaceae	*Melaleuca ciliosa*	NRS	CSB	-

Myrtaceae	*Melaleuca leuropoma*	RS	CSB	-
Myrtaceae	*Melaleuca orbicularis*	RS	CSB	Northern edge
Myrtaceae	*Melaleuca systena*	RS	CSB	-
Myrtaceae	*Melaleuca tinkeri*	RS	CSB	Northern edge
Myrtaceae	*Melaleuca trichophylla*	RS	CSB	-
Myrtaceae	*Melaleuca urceolaris*	NRS	CSB	Northern edge
Myrtaceae	*Melaleuca zonalis*	RS	CSB	Restricted
Myrtaceae	*Phymatocarpus porphyrocephalus*	RS	CSB	-
Myrtaceae	*Scholtzia* sp. Eneabba	NRS	SSB	-
Myrtaceae	*Scholtzia involucrata*	NRS	SSB	Northern edge
Myrtaceae	*Scholtzia* sp. Wongonderrah	NRS	SSB	Northern edge
Myrtaceae	*Thryptomene cuspidata*	NRS	SSB	-
Myrtaceae	*Verticordia argentea*	NRS	SSB	Restricted
Myrtaceae	*Verticordia eriocephala*	NRS	SSB	-
Myrtaceae	*Verticordia huegelii*	NRS	SSB	-
Myrtaceae	*Verticordia nobilis*	NRS	SSB	-
Olacaceae	*Olax benthamiana*	NRS	SSB	Northern edge
Olacaceae	*Olax scalariformis*	NRS	SSB	Northern edge
Proteaceae	*Adenanthos cygnorum* subsp. *cygnorum*	NRS	SSB	-
Proteaceae	*Banksia armata* var. *armata*	RS	CSB	Northern edge
Proteaceae	*Banksia attenuata*	RS	CSB	-
Proteaceae	*Banksia bipinnatifida* subsp. *multifida*	RS	CSB	Northern edge
Proteaceae	*Banksia candolleana*	RS	CSB	Northern edge
Proteaceae	*Banksia carlinoides*	NRS	CSB	-
Proteaceae	*Banksia chamaephyton** [P4 species]	RS	CSB	Northern edge
Proteaceae	*Banksia cypholoba** [P3 species]	RS	CSB	Restricted
Proteaceae	*Banksia densa*	NRS	CSB	Northern edge
Proteaceae	*Banksia glaucifolia*	NRS	CSB	Northern edge
Proteaceae	*Banksia grossa*	RS	CSB	Restricted
Proteaceae	*Banksia hookeriana*	NRS	CSB	Restricted
Proteaceae	*Banksia incana*	RS	CSB	Northern edge
Proteaceae	*Banksia kippistiana* var. *kippistiana*	NRS	CSB	Northern edge
Proteaceae	*Banksia leptophylla*	NRS	CSB	-
Proteaceae	*Banksia menziesii*	RS	CSB	-
Proteaceae	*Banksia micrantha*	RS	CSB	Northern edge
Proteaceae	*Banksia nana*	RS	CSB	Restricted
Proteaceae	*Banksia nivea*	NRS	CSB	Northern edge
Proteaceae	*Banksia sclerophylla*	RS	CSB	Restricted

Proteaceae	*Banksia sessilis*	NRS	CSB	-
Proteaceae	*Banksia shuttleworthiana*	RS	CSB	-
Proteaceae	*Banksia tortifolia*	RS	CSB	Restricted
Proteaceae	*Banksia tridentata*	RS	CSB	Northern edge
Proteaceae	*Conospermum nervosum*	NRS	SSB	Northern edge
Proteaceae	*Conospermum unilaterale*	NRS	SSB	Northern edge
Proteaceae	*Grevillea uniformis*[P3 species]*	NRS	SSB	Restricted
Proteaceae	*Hakea auriculata*	RS	CSB	-
Proteaceae	*Hakea candolleana*	RS	CSB	-
Proteaceae	*Hakea conchifolia*	RS	CSB	Northern edge
Proteaceae	*Hakea costata*	NRS	CSB	-
Proteaceae	*Hakea eneabba*	RS	CSB	-
Proteaceae	*Hakea flabellifolia*	RS	CSB	Northern edge
Proteaceae	*Hakea gilbertii*	NRS	CSB	Northern edge
Proteaceae	*Hakea incrassata*	RS	CSB	-
Proteaceae	*Hakea neospathulata*	RS	CSB	Northern edge
Proteaceae	*Hakea obliqua*	NRS	CSB	Northern edge
Proteaceae	*Hakea polyanthema*	NRS	CSB	-
Proteaceae	*Hakea psilorrhyncha*	NRS	CSB	-
Proteaceae	*Hakea smilacifolia*	NRS	CSB	-
Proteaceae	*Hakea stenocarpa*	RS	CSB	-
Proteaceae	*Hakea trifurcata*	NRS	CSB	-
Proteaceae	*Isopogon adenanthoides*	NRS	CSB	Northern edge
Proteaceae	*Isopogon inconspicuus*	NRS	CSB	Restricted
Proteaceae	*Isopogon linearis*	RS	CSB	-
Proteaceae	*Isopogon tridens*	RS	CSB	-
Proteaceae	*Lambertia multiflora*	RS	CSB	Northern edge
Proteaceae	*Petrophile aculeata*	NRS	CSB	Restricted
Proteaceae	*Petrophile brevifolia*	RS	CSB	-
Proteaceae	*Petrophile drummondii*	NRS	CSB	Northern edge
Proteaceae	*Petrophile linearis*	RS	CSB	Northern edge
Proteaceae	*Petrophile macrostachya*	RS	CSB	-
Proteaceae	*Petrophile pilostyla*	RS	CSB	-
Proteaceae	*Petrophile rigida*	RS	CSB	Northern edge
Proteaceae	*Petrophile seminuda*	RS	CSB	-
Proteaceae	*Petrophile serruriae*	NRS	CSB	Northern edge
Proteaceae	*Petrophile shuttleworthiana*	RS	CSB	-
Proteaceae	*Strangea cynanchicarpa*	RS	CSB	Restricted

Proteaceae	*Xylomelum angustifolium*	RS	CSB	-
Rhamnaceae	*Stenanthemum notiale*	NRS	SSB	-
Rutaceae	*Geleznowia verrucosa*	NRS	SSB	-
Stylidiaceae	*Stylidium adpressum*	NRS	SSB	-
Stylidiaceae	*Stylidium carnosum*	NRS	SSB	Northern edge
Stylidiaceae	*Stylidium crossocephalum*	NRS	SSB	-
Stylidiaceae	*Stylidium cygnorum*	NRS	SSB	Northern edge
Stylidiaceae	*Stylidium diuroides*	NRS	SSB	-
Stylidiaceae	*Stylidium maitlandianum*	NRS	SSB	-
Stylidiaceae	*Stylidium repens*	NRS	SSB	-
Stylidiaceae	*Stylidium stenosepalum*	NRS	SSB	Northern edge
Thymelaeaceae	*Pimelea ferruginea*	NRS	SSB	Northern edge
Thymelaeaceae	*Pimelea sulphurea*	NRS	SSB	Northern edge
Zamiaceae	*Macrozamia fraseri*	RS	CSB	Northern edge

2.5.6 The value and challenges of using historic data

Historic data that is accessible, well mapped or georeferenced and that has reproducible methods is useful for accurately tracking changes in species composition at sites over time (Stöckli *et al.* 2012, Vellend *et al*. 2013). If used correctly, historic data can provide a baseline to which contemporary surveys can be compared, contributing to the understanding of long term changes in plant composition in an area (Vellend *et al*. 2013). However, often surveys are not designed with future re-surveying in mind, leading to problems with data comparisons (Stöckli *et al.* 2012, Vellend *et al*. 2013).

Studies conducted by Kelly and Goulden (2008) and Harrison *et al.* (2010) that detected changes in vegetation composition in California's Santa Rosa Mountains and Oregon's Siskiyou Mountains respectively had the advantage of the availability of detailed, high resolution historic data. This allowed these researchers to re-survey the exact locations of the plots from the historic surveys, enabling quality comparisons to be made and allowing strong conclusions to be drawn from the data. Unfortunately, the 2015 study of South Eneabba Nature Reserve was restricted by the limited information from the available historic data.

Challenges were faced when comparing the present composition of the vegetation of SENR to the historic composition. One issue was the relative scarcity of historic plot

data; there have been numerous studies conducted in and around SENR, though few present data on the species found, and fewer yet present data on the abundance of these species. The historic surveys used here (Lamont 1976, Hnatiuk & Hopkins 1981, Griffin *et al*. 1983, Elkington & Griffin 1985) all included species lists for the total area surveyed, allowing for an overall comparison of species richness in the area.

Abundance data however, allows for greater comparison between surveys. Abundance data enables increases or decreases in species numbers to be determined and assists with detection of common and rare species (Stöckli *et al*. 2010). The Elkington and Griffin (1985) survey presented plot level abundance data for only 40 species, while the Griffin *et al*. (1983) study presented plot level abundance data for all species recorded. Thus it was only possible to compare all three surveys based on the plot level abundance data for the 40 species sampled by Elkington and Griffin (1985). It was possible though to compare the Griffin *et al*. (1983) survey and the 2015 survey based on plot level abundance data for all species recorded.

The exact plot locations were not provided for any of the historic surveys used in this study. This meant that replication of exact plot locations was not possible. Insufficient records of plot locations or the lack of permanent plot markers makes re-surveying an area difficult, and is a common problem faced when using historic data (Vellend *et al*. 2013). Contemporary studies can overcome problems with plot locations by surveying plots in the vicinity of the historic plots, as was done with the 2015 survey (Vellend *et al*. 2013).

Outdated and incorrect taxonomy had been used in the historic surveys to which the 2015 survey was compared. This is a common occurrence when using historic data (Stöckli *et al.* 2012, Vellend *et al*. 2013). Compiling contemporary species lists independent of the historic species lists, as was done here, can increase the reliability of contemporary surveys (Stöckli *et al.* 2012). By tracking changes in taxonomy and if necessary using coarse taxonomic groupings, historic data can be compared to contemporary data with more confidence (Stöckli *et al.* 2012, Vellend *et al*. 2013). Considerable effort was made to update the taxonomy of the historic surveys to allow for greater comparison ability with the 2015 survey, though there were many taxa that were suspected to have been misidentified in the historic surveys. Reference collections were only available for two of the historic surveys, the Griffin *et al*. (1983) and Hnatiuk and Hopkins (1981) surveys.

A large proportion of taxa found in the historic surveys were absent from the 2015 survey, with many of these taxa being non-comparable between surveys. The decision was made to remove non-comparable taxa from the data sets. Incomplete identifications could have artificially inflated the species richness counts for the historic surveys- unidentified taxa may have been duplicates of taxa already identified in the surveys that were too difficult to identify to species level. Though alternatively, removal of these taxa from the data set could have artificially decreased species richness counts if taxa were genuinely unique to all other taxa surveyed.

Around 10% of taxa recorded in the historic surveys have no record of occurrence in the Eneabba area, and so it is suspected that they were possibly misidentified at the time of surveying. Many taxa that were suspected to have been misidentified were very similar to species found to occur in the 2015 survey. For example; it is suspected that the species that was recorded as *Desmocladus fasciculatus* in all four historic surveys was recorded as *Desmocladus parthenicus* in the 2015 survey- the two species look very similar, though *D. fasciculatus* is recorded as having a distribution located south of Eneabba (FloraBase; Western Australian Herbarium 2016). Another example is the recording of *Macrozamia riedlei* in two of the historic surveys and *M. fraseri* in the 2015 survey- *M. riedlei* is recorded as occurring much further south of Eneabba, in the south-western most corner of SWA, while *M. fraseri* is recorded in the Eneabba area (FloraBase; Western Australian Herbarium 2016). These possible misidentifications may have artificially inflated the differences between the historic and 2015 surveys, although some may have been genuine records, and thus removal of these taxa from analyses may have incorrectly reduced the difference between the historic and 2015 surveys.

2.6 Conclusions and implications of results

Use of historic datasets indicates that over the past 40 years, there has been little change in the vegetation of SENR in terms of species diversity, abundance, composition, and functional traits. The results do not support the hypothesis that changes would have occurred in floristic composition and in the proportion of certain functional traits present. Decreased rainfall and increased temperatures have not resulted in an increase in the proportion of RS species to NRS species, nor SSB species to CSB species.

This study provides an indication of the condition of the vegetation contained within 60 plots across SENR in 2015, as well as a summary of data collected from four historic surveys of the reserve. This data can be used as a baseline for further monitoring of SENR to detect changes in the composition of the flora. Future surveys of SENR could use the GPS coordinates provided here to relocate the plots in order to perform repeat surveys, specimens collected for this study could be used to cross-check taxonomy, and the plot data recorded in Table A3 could be used to draw comparisons with any new data collected.

This study investigated changes in the floristic composition of SENR over the past 40 years through analysis of species cover and proportion of two plant functional traits; however these are coarse measures of change. For finer scale analysis of changes in floristic composition more detailed data are required. For example; although this study assessed whether the proportion (abundance) of RS to NRS and CSB to SSB species has changed over the past 40 years, it provides no indication of whether the proportion (size) of plants displaying these traits has changed; measurements of plant height, size and biomass are required to make these comparisons. Additional plant functional traits could also be monitored in the future, such as leaf size. Leaf size has been found to decrease with increased temperature and drought, as small leaved species are less susceptible to water loss (Ackerly *et al.* 2012; Guerin *et al.* 2012). Leaf size could be monitored by measuring leaf properties (i.e. shape, thickness, biomass, leaf mass per area) of plants within fixed plots to determine whether a shift is occurring toward smaller leaves, either within a species or through loss of larger leaved species. Future studies could also delve into the historical collections stored at museums and herbariums to investigate changes that may have occurred at a location over a temporal scale (Suarez & Tsutsui 2004). These changes could include differences in the species recorded at a location over time, or changes in their characteristics (Guerin *et al.* 2012). The growth rate and reproductive ability of species could also be assessed and compared over time to determine whether changes have occurred. Measurements of the number of flowers and fruit per plant, follicles per cone (in *Banksia* species), and proportion of viable seeds could all give indication of changes occurring. For example; Enright *et al*. (2015) found evidence of a shift in the endemic shrub *Banksia hookeriana* towards production of fewer cones and seed over 40 years at Eneabba. Data pertaining to the location of species within the landscape would also be useful for analysis of whether species are retreating to certain habitats, for example; moving to deeper soils, or up slope. An overall analysis of plant biomass in an area could be used to determine whether changes have occurred over time as the climate changes. Total plot cover data

or remotely sensed vegetation cover data could be used to determine this. Thus is it recommended that more data is collected at each of the permanent plots in the future; additional functional traits could be recorded, vegetation cover could be assessed, and for select species location in the landscape, height, number of flowers and fruit and proportion of viable seeds could be recorded.

Although an increase in the proportion of RS species to NRS species was not observed in this study, nor an increase in SSB species to CSB species, it is possible that these proportions may change in the future with forecast drier conditions, and so proportions of these traits should continue to be monitored. The species outlined in Table 2.13 and 2.14; CSB and NRS species, species at the edge of their range that were absent from the 2015 survey, and species of conservation significance, should be of particular interest for future monitoring. Continued monitoring could determine if the species recorded in the historic surveys but not in the 2015 survey (Table 2.13) are actually missing from the area or just not sampled in the 2015 survey, and if species of conservation significance recorded here are changing in distribution.

Overall, this study suggests that the plant communities within SENR have coped with the changes in climate that have occurred over the past 40 years; however unidirectional climate change is continuing and change in the vegetation is expected. The plots established in 2015 provide an ideal dataset to test theories of community responses into the future.

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Chapter 3: Phenotypic variation and differentiated gene expression of Australian plants in response to declining rainfall*

3.1 Abstract

Declining rainfall is projected to have negative impacts on the demographic performance of plant species. Little is known about the adaptive capacity of species to respond to drying climates, and whether adaptation can keep pace with climate change. In fire-prone ecosystems, episodic recruitment of perennial plant species in the first year post-fire imposes a specific selection environment, offering a unique opportunity to quantify the scope for adaptive response to climate change. We examined the growth of seedlings of four fire-killed species under control and drought conditions for seeds from populations established in years following fire receiving average-to-above-average winter rainfall, or well-below-average winter rainfall. We show that offspring of plants that had established under drought had more efficient water-uptake, and/or stored more water per unit biomass, or developed denser leaves, and all maintained higher survival in simulated drought than did offspring of plants established in average annual rainfall years. Adaptive phenotypic responses were not consistent across all traits and species, while plants that had established under severe drought or established in years with average-to-above-average rainfall had an overall different physiological response when growing either with or without water constraints. Seedlings descended from plants established under severe drought also had elevated gene expression in key pathways relating to stress response. Our results demonstrate the capacity for rapid adaptation to climate change through phenotypic variation and regulation of gene expression. However, effective and rapid adaptation to climate change may vary among species depending on their capacity to maintain robust populations under multiple stresses.

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Authors' contributions: The supervisory team (Tianhua He and Neal Enright) conceived the idea. Haylee D'Agui designed the study in consultation with Tianhua He and Neal Enright. Haylee D'Agui collected the data and performed the study with assistance from William Fowler (Research Assistant). Haylee D'Agui analysed the data with assistance from Tianhua He and Sim Lin Lim for transcriptome data. Haylee D'Agui drafted the manuscript. Haylee D'Agui coordinated the publication process, with assistance with revisions from Tianhua He, Neal Enright, Sim Lin Lim and William Fowler.

3.2 Introduction

Bioclimatic modelling of species distributions suggests that extinction rates may increase dramatically in response to future climate change, with potentially large losses in biodiversity (Parmesan & Yohe 2003; Thuiller *et al*. 2005; Fitzpatrick *et al.* 2008; Thuiller *et al.* 2011; Urban 2015). These projections raise great concerns about the deleterious consequences globally for biodiverse floras (Enright *et al*. 2015). However, the validity of these extinction predictions is uncertain as critical gaps remain in our knowledge of the intrinsic capacity of species to respond to climate changes through rapid phenotypic and physiological change for better survival (i.e. adaptation). Species may have the potential to mitigate the effects of a changing climate through rapid selection and adaptation that lead to 'effect dampening' within a short time frame (Hoffmann & Sgrò 2011; Leuzinger *et al.* 2011).

Mediterranean-type ecosystems (MTEs) are among the most biologically diverse terrestrial ecosystems globally (Cowling *et al.* 1996; Myers *et al.* 2000), and are highly vulnerable to species extinction under global change (Sala *et al.* 2000; Malcolm *et al.* 2006). Both drought and fire play an important role in shaping the structure and composition of MTE vegetation, as the distribution and abundance of plant species is determined primarily by their ability to tolerate water stress and extreme temperatures in the summer, and to re-establish themselves after fire. In Southwestern Australia (SWA), a MTE global biodiversity hotspot, the climate has undergone a dramatic drop in annual rainfall (>30%) since the 1970s, with decreases in rainfall most apparent in late autumn and early winter (Hope *et al*. 2006; Bates *et al*. 2008). Significant decrease in rainfall is coupled with an increased frequency of extreme drought events (Bates *et al.* 2008; He & Lamont 2010; Dai 2012). Increases in drought are of particular concern because drought has the capacity to cause sudden and extreme vegetation change, especially when combined with fire in such Mediterranean-type shrublands which already have low baseline water levels (He & Lamont 2010; Yates *et al.* 2010).

Plants may respond to climate change by migrating or shifting their geographic range if possible (Corlett & Westcott 2013). Recent discoveries have shown that Australian plant species have the capacity to disperse their seeds to distant habitats up to 3 km away in a single dispersal event (He *et al.* 2004; He *et al.* 2009; He *et al.* 2010). However, most Australian species seem to have persisted through major climatic changes over the past few million years, rather than moving long distances to track

changing climates (Dodson & Macphail 2004; Byrne 2007). This supports the idea that plant species may be able to adapt *in situ* to new climatic conditions, to some extent at least, through rapid evolutionary adaptation. Evolutionary adaptation can be rapid (Hoffmann & Sgrò 2011) and can help species to counter environmental stresses arising from climate change (Hoffmann & Willi 2008). It is important to understand the capacity of species to tolerate climate change and the mechanisms that might buffer them against the consequences of such changes in environmental conditions.

Plants in SWA offer a unique opportunity to quantify the pace of selection and adaptation to climate change. In fire-prone ecosystems of SWA many plant species are characterised by cohort regeneration after fire, so that populations are largely single-aged, with stands of fire-killed species being replaced after each fire (for more details see Chapter 1). This means that all individuals in a stand are established in the same year, and have experienced the same environmental circumstances (the same selection filter), while individuals in stands established in other years will have experienced different environmental circumstances, representing different strengths of selection pressure from climate conditions. Fire is patchy every year, and rainfall also varies between years, creating populations established under different strengths of selection pressure. For example, in a year with low rainfall following fire, the populations established in that year will have been filtered by strong selection from drought; conversely, in a wet year following fire, selection pressure from drought would be relaxed. Climate extremes in the year of regeneration may therefore impose a 'hard' selection upon those species, and select for stress-tolerant genotypes within a single generation. Many shrub and tree species in SWA are serotinous, with seeds stored in woody fruits in the living canopy for several to many years and released *en masse* following fire. Individuals subjected to abiotic stress, such as drought, may retain an imprint of this stress that facilitates higher protection from stress in future generations (For example; Kou *et al.* 2011; Walter *et al.* 2011), and such trans-generational response may be a potential mechanism of rapid adaptation to environmental and climate change. Episodic (cohort) recruitment of perennial plant species in the first year after fire, imposing a specific selection of abiotic stress, offers a unique opportunity to quantify the scope for rapid adaptive response to climate change. Here we aimed to determine whether seed banks of four fire-killed, serotinous species, *Banksia hookeriana*, *B. leptophylla*, *Hakea costata* and *H. polyanthema*, have the potential to mitigate the effects of a drying climate through rapid expression of drought-tolerant genotypes.

3.3 Materials and Methods

3.3.1 Glasshouse experiment

Four serotinous species from the family Proteaceae, *Banksia hookeriana* Meisn., *Banksia leptophylla* A.S. George, *Hakea costata* Meisn., and *Hakea polyanthema* Diels., from the biodiverse SWA kwongan were investigated (for details see Appendix; Supplementary Information: Chapter 3). For each species, canopy-stored seeds set one to two years prior to the investigation were collected from five sites (three for *Hakea costata*) of different post-fire age at eight locations near Eneabba, Western Australia (Table S3.1). The sites are geographically proximate (2-60 km) and with similar species composition (typical kwongan vegetation dominated by species from the families Proteaceae and Myrtaceae), and soils (low nutrient, acidic white sands). All sites have experienced the same long-term climate while fire history may vary, as fires are patchy in size and location (Miller *et al.* 2007). Distances between sites are sufficiently large such that genes are not immediately mixed through pollen and seed dispersal after fire (He *et al.* 2004; He *et al.* 2009). All sites were last burnt at least eight years ago, and so supported mature stands of the selected species. Sites were classified either as average-to-wet winter (HiR; high rainfall populations) or dry winter (LoR; low rainfall populations) based on total rainfall in the first winter/spring following the last fire, with 'dry' defined as >20% below the long term average mean winter rainfall at Eneabba (Table S3.2).

Seeds were extracted from woody fruits and geminated at 15°C before being transferred to custom-made pots (15 cm diameter, 100 cm deep - to facilitate the natural pattern of early tap root growth). Seedlings were grown in a temperature-controlled glasshouse (air temperature ranged from 12.9˚C to 36.1˚C, and soil temperature ranged from 12.4˚C to 31.2˚C). Seedlings were watered every second day with 200 mL water for four weeks to allow seedlings to establish. Once established, seedlings from HiR and LoR sites for each species were divided into two treatment groups, with up to 60 replicates per species per group and an equal number of seedlings from each location in each group. Seedlings were subjected to either a control (mean winter rainfall at Eneabba over the past 30 years equivalent; approximately 200 mL per plant every second day) or drought regime (equivalent to a 50% decrease in mean winter rainfall at Eneabba; 100 mL per plant every second day) for two weeks and then no water over the next three months simulating severe drought. After three months of growth, half of the seedlings (up to 24, Table S3.4) of

each species were harvested for measurement of growth and phenotypic variation in drought resistance traits. The remaining half of the seedlings were grown on without water supply in the greenhouse for a further 12 weeks with mortality recorded each week. For more details see Appendix; Supplementary Information: Chapter 3.

3.3.2 Trait measurements and statistical analysis

After three months, up to twenty-four plants from each of the treatment groups and sites (HiR and LoR) were harvested for each species. Growth (total dry biomass) and five traits that are related to drought resistance were measured: Root length, Leaf mass per area (LMA), Water content per unit biomass; Water content per unit root length, and Water content per leaf area. Relative fitness for each trait was represented as standardised trait values, with data standardised as (v-Min)/(Max-Min), where v is trait value, and Min and Max are the minimum and maximum values in each trait. We first used Canonical Discriminant Function Analysis to summarise drought and growth traits for the overall physiological response of seedlings derived from HiR and LoR sites under average versus droughted growing conditions. We used Wilks' lambda and associated chi-square statistic as a measure of the difference in overall physiological response between HiR and LoR seedlings of each species. Variation in each growth and drought parameter, and survival between sources of seeds (HiR or LoR) were compared using one-way ANOVAs. Variations between treatments with different sources of seeds (fixed variates), and different watering regimes (covariates) were compared using two-way ANOVAs. In the case of unequal variance, Welch F-tests were used. Median values of trait measurements between seed sources (HiR or LoR) were compared using Kruskal-Wallis tests. Statistical analyses were performed in PAST V3 (Hammer *et al*. 2001) and SPSS 22 (IBM Corp. 2012). Significance level was set at $p< 0.05$ for all statistical tests.

3.3.3 Differentially expressed genes in relation to drought treatment

Banksia hookeriana was investigated further using transcriptome analyses, due to its known susceptibility to drought (He & Lamont 2010). Samples of *Banksia hookeriana* for genetic analyses were harvested ten weeks into the drought treatment (as species in SWA generally have three months growth after germination before the onset of the dry season). We collected and pooled five seedlings from each of the two source population groups (HiR and LoR) from both control and drought treatment regimes. Total RNA was extracted, and cDNA libraries were constructed and then sequenced using the Illumina HiSeq 2000 Sequencing System (Illumina Inc. San Diego, CA, USA),

yielding ~50 million 100-bp reads per sample. Sequence data were processed for length and quality, and aligned to protein databases before functional analysis and pathway enrichment analysis (see Appendix; Supplementary Information: Chapter 3 for more details on pipeline of all data processing steps and parameters). The assembled *Banksia hookeriana* transcriptome sequences have been deposited in the NCBI database (accession number: GBXB00000000).

3.4 Results

3.4.1 Growth and drought resistance of seedlings under control and drought conditions

We first examined the physiological response in growth and phenotypic traits in simulated drought for the four species. Multivariate analysis through Canonical Discriminant Function suggested significant overall difference in physiological response for six measured growth and drought related traits between seedlings derived from populations established in years with average-to-above-average winter rainfall (HiR) and those derived from populations established in years with at least 20% below-average winter rainfall (LoR) in *B. hookeriana* and *H. costata,* when growing in conditions with full water supply (control). Overall differences in physiological response between HiR and LoR populations were revealed for *B. leptophylla*, *Hakea costata* and *H. polyanthema* when growing under drought conditions (Table 3.1).

Table 3.1: Canonical Discriminant Function analysis of overall difference of growth and physiological traits between HiR (high rainfall) and LoR (low rainfall) seedlings of the four species growing in conditions either with full water supply or with simulated drought. Significant *p*- values are indicated with an asterisk (α=0.05).

Species	Watering regime	Wilks' Lambda	Probability
Banksia hookeriana	Full water	0.684	0.038*
	Simulated drought	0.860	0.680
Banksia leptophylla	Full water	0.850	0.476
	Simulated drought	0.690	0.048*
Hakea polyanthema	Full water	0.760	0.860
	Simulated drought	0.528	0.049*
Hakea costata	Full water	0.341	0.006*
	Simulated drought	0.519	0.024*

When growing in conditions with average water availability, seedlings in two species, derived from LoR populations, *Banksia hookeriana* and *Hakea costata,* showed higher relative fitness in four traits than seedlings derived from HiR populations (Figure3.1). Seedlings of *B. hookeriana* from LoR sites had higher water content per unit root length and higher water content per leaf area than seedlings from HiR sites, indicating higher efficiency in water uptake and water use in those seedlings whose parents were established in drought years compared with those whose parents were established in average-to-wet years. Apart from storing more water per unit biomass, seedlings of *H. costata* from LoR sites developed higher leaf mass per area (LMA) than HiR seedlings (Figure 3.1) when grown in conditions with average water availability.

LoR seedlings of *B. leptophylla* and *H. polyanthema* showed no difference in growth and drought resistance traits compared with HiR seedlings when grown in conditions with average water availability (Figure 3.1). When grown under drought conditions, LoR seedlings of *B. leptophylla* had longer roots, higher LMA and higher water content per leaf area than HiR seedlings (Figure 3.2); LoR seedlings of *H. polyanthema* had higher water content per unit biomass and higher water content per unit leaf area than HiR seedlings, and LoR seedlings of *H. costata* had higher LMA than HiR seedlings when grown in drought conditions (Figure 3.2).

Despite the results that improved fitness in relation to drought tolerance was not consistent across all traits and species in relation to their origin, two-way ANOVAs

indicated that seedlings from LoR populations generally had higher LMA, higher water content per unit root length, higher water content per unit leaf area, and higher water content per unit biomass than seedlings from HiR populations (Table 3.2). Since climate extremes in the year of regeneration impose a 'hard' selection upon those species, such selection would then remove, or decrease the frequency of, less stress-tolerant genotypes. Indeed, LoR populations generally had a smaller proportion of individuals having lower relative fitness in relation to drought resistance than HiR populations (p= 0.016, one sample test of Chi-square goodness of fit).

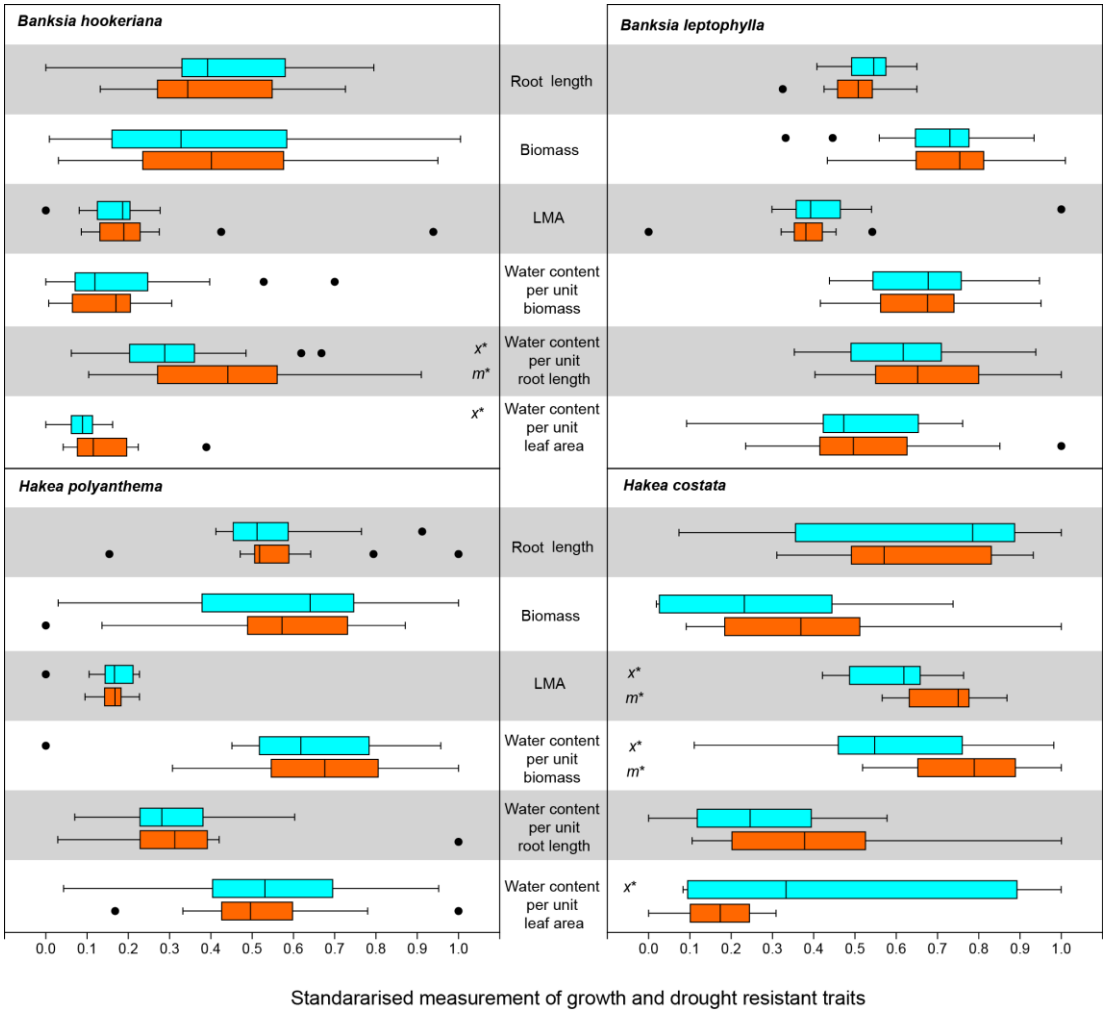

Figure 3.1: Standardised measurement of growth and drought traits of seedlings derived from HiR (high rainfall; blue) and LoR (low rainfall; orange) populations in the glasshouse experiment with full water supply. Box indicates 25th to 75th percentile, black dots represent outliers. An "x" with an asterisk indicates a significant difference in mean values, and an "m" with an asterisk indicates a significant difference in median values between HiR and LoR populations

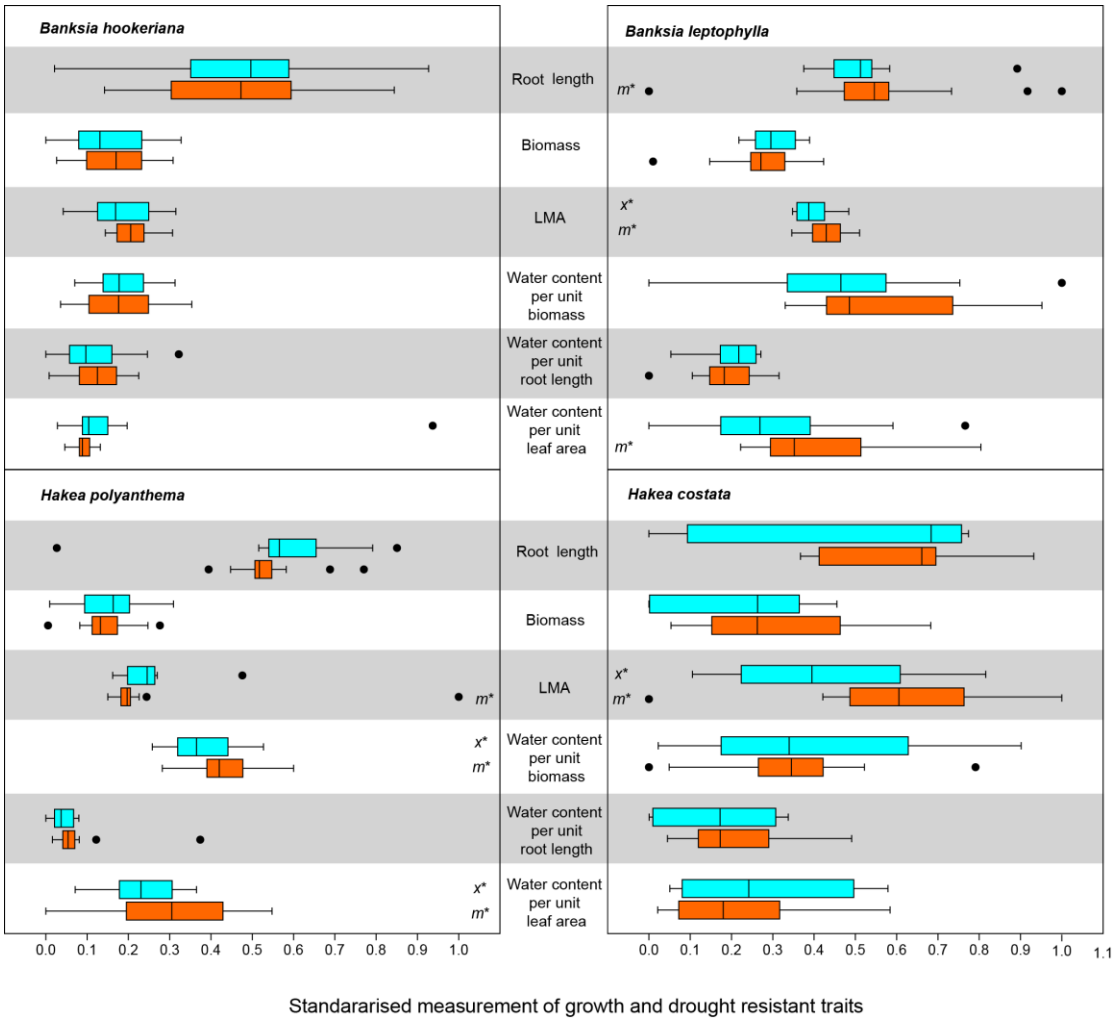

Figure 3.2: Standardised measurement of growth and physiological traits of seedlings derived from HiR (high rainfall; blue) and LoR (low rainfall; orange) populations in the glasshouse experiment under conditions of water deficit. Box indicates 25th to 75th percentile, black dots represent outliers. An "x" with an asterisk indicates a significant difference in mean values and an "m" with an asterisk indicates a significant difference in median values between HiR and LoR populations.

Table 3.2: Two-way ANOVA of each trait with source of seedlings (high rainfall or low rainfall) as fixed variants, and different watering regime as covariant. Probabilities (< 0.10) of overall difference between sources of seedlings are shown. Significant *p*-values are marked with an asterisk (α=0.05).

Species	Root length	Biomass	LMA	Water content/ Biomass	Water content/ Root length	Water content/ Leaf area
Banksia hookeriana	-	-	0.073	-	0.095	-
Banksia leptophylla	-	-	-	-	0.093	-
Hakea polyanthema	0.040*	-	-	-	0.045*	-
Hakea costata	-	-	0.015*	0.045*	-	0.014*

3.4.2 Mortality under severe drought

We further assessed survival under simulated severe drought for three of the four species (there were insufficient *Hakea costata* seedlings after harvesting for trait measurements) by terminating the water supply after five and a half months and monitoring continued growth and survival for three months (Figure 3.3A). At the end of the experiment (i.e. after 8.5 months), seedlings from LoR populations had lower mortality than those from HiR populations (Figures. 3.3B, 3.3C). For all three species, seedlings that had been treated with drought at the start of the experiment had significantly lower mortality than seedlings from the control group (Figure 3.3D).

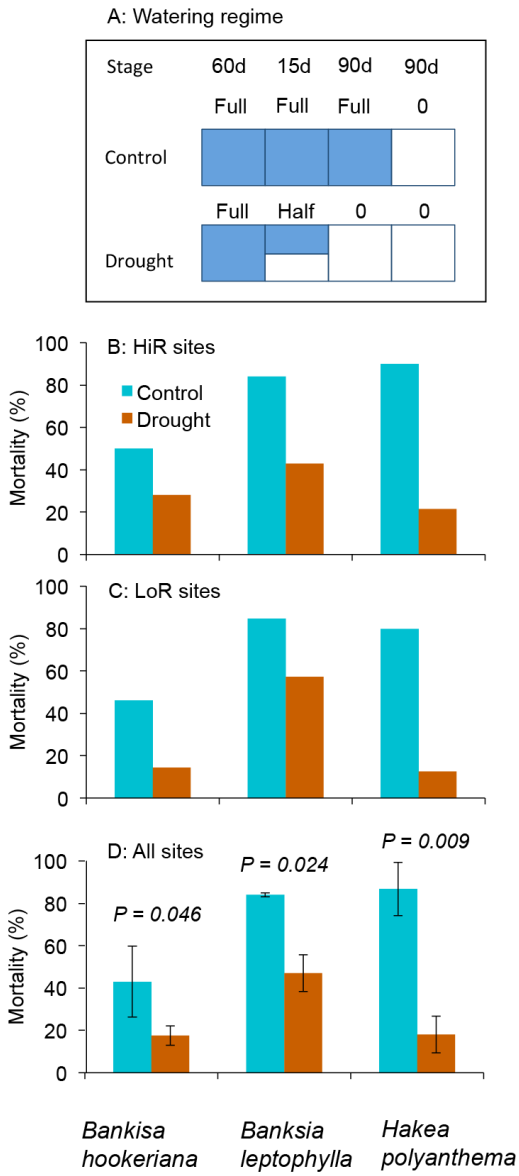

Figure 3.3: Watering regime and mortality (%) of seedlings derived from HiR (high rainfall) and LoR (low rainfall) sites of three species in the glasshouse experiment under simulated and prolonged drought (*Hakea costata* was not included due to low survivorship, which left insufficient samples to be monitored further for mortality under drought). A) Watering regime; four stages were arranged, ranging from 15 days to 90 days over a period of 8.5 months. "Full" indicating full water supply (200 mL per plant, every second day) over the stage; "Half" indicating half water supply (100 mL per plant, every second day); "0" indicating no water supply. B) Mortality of seedlings from HiR sites; C) Mortality of seedlings from LoR sites; D) Mortality of seedlings over all sites in the two treatments. *P*-values are probabilities of equal mortalities of seedlings from HiR and LoR sites (one-way ANOVA).

3.4.3 Differentiated gene expression and regulation networks in Banksia hookeriana

Finally, we assessed differentiated gene expression and regulation networks in seedlings of *B. hookeriana* derived from HiR and LoR populations and grown under simulated drought, versus average water availability (control). Among the 59,064 transcripts assembled from all samples, 8.2% had differentiated expression between samples derived from HiR populations and LoR populations (Natural logarithm transformed fold change $t > 2.5$, False Discovery Rate < 0.05). Gene Ontology (GO term) comparison revealed that differentially expressed genes (DEGs) were mostly involved in the oxidation-reduction process, protein phosphorylation, metabolic process, response to stress, and regulation of transcripts, regardless of the source of seedlings, when grown in conditions with simulated drought.

Gene regulation networks created through Pathway Enrichment Analysis revealed gene function with significant expression change ($t > 2.5$ or $t < -2.5$) in *B. hookeriana* seedlings derived from HiR and LoR populations and grown in simulated drought conditions when compared to seedlings grown in control conditions. Cellular pathways related to growth, development and metabolism were down-regulated in all seedlings, irrespective of the source of populations (Table 3.3), which is consistent with reduced growth observed in seedlings in terms of biomass accumulation when growing in drought conditions. Defense response and response to stress pathways were up-regulated in seedlings from both sources when grown in drought conditions, however, HiR and LoR seedlings responded to drought via regulation with different plant hormones. In HiR seedlings, jasmonic acid biosynthesis and metabolism were more active under drought conditions, while in LoR seedlings, salicylic acid and brassinosteroid biosynthesis and metabolism were stronger (Table 3.3). Pathways of programmed cell death were also up-regulated in LoR seedlings when under drought conditions.

Table 3.3: Biological pathways with significant expression change in seedlings of *Banksia hookeriana* from HiR (high rainfall) and LoR (low rainfall) populations growing in drought conditions. Differentially expressed genes were identified by a False Discovery Rate of ≤0.001 and fold change value of > 2. Differentially expressed genes clusters were identified by an adjusted *p*-value < 0.05.

Seedlings with HiR origin	Seedlings with LoR origin
Down-regulated pathways	
Developmental growth	Cell proliferation
Meristem development	Meristem development
Root morphogenesis	Xylem development
Stomatal complex morphogenesis	Stomatal complex morphogenesis
Cell-wall biogenesis	Cell wall biogenesis
Peptide transport	
Protein phosphorylation	Protein phosphorylation
Response to gibberellin stimulus	
Up-regulated pathways	
Programmed cell death	Programmed cell death
Jasmonic acid biosynthesis and metabolism	Salicylic acid biosynthesis and metabolism
Response to salicylic acid stimulus	Response to biotic stimulus
Response to stress	Response to stress
Heat acclimation	Defense response
	Cellular response to nutrient deficiency

3.4.4 Genetic diversity in Banksia hookeriana

Intensified selection under droughting may have reduced genetic diversity in *B. hookeriana*. We detected an average of 66,377 SNPs in seedlings derived from LoR populations, with an average of 50.6% heterozygous SNPs, compared to 62.9% heterozygous SNPs in seedlings from HiR populations (with an average of 65,799 SNPs detected). Among the transcripts that belonged to genes in salicylic acid biosynthesis and metabolism, 48.1% SNPs were heterozygous in LoR seedlings, while 62.3% were

heterozygous in HiR seedlings. Note that these results need to be interpreted with caution as they are indicative of the overall presence of heterozygotes and homozygotes in the sample and cannot be attributed to individual plants due to the bulking of mRNA for sequencing.

3.5 Discussion

Both phenotypic plasticity and adaptive evolution may contribute to population persistence in a changing environment (Nicotra *et al.* 2010; Vedder *et al.* 2013). Although it is difficult to parse out the relative contributions of adaptive evolution and phenotypic plasticity in our study, we suggest that rapid evolutionary adaptation might be the more significant in our study system. Our LoR and HiR sites were geographically proximate with similar climates and environments (but different fire histories). Significant differentiation in drought-related traits of seedlings from LoR and HiR sites was revealed in a common garden experiment using average water supply versus water deficient growing conditions. Moreover, our transcriptome analysis of *Banksia hookeriana* revealed that differentiated phenotype was related to differentiated expression of genes, indicating that the adaptive mechanism is heritable through natural selection or epigenetic processes (For example; Zhang *et al*. 2013). Climate, acting as an environmental filter (i.e. rainfall change) at the time of population establishment may select for drought-resistant alleles (Hoffmann & Willi 2008), resulting in a more drought-tolerant population compared to its parent population. On the other hand, phenotypic plasticity may also determine the immediate response of a natural population to changing climate (Richards *et al.* 2010), since phenotypic plasticity may be adaptive and can evolve rapidly in response to selection if it has a heritable genetic basis, for example; through epigenetic processes such as DNA methylation in generating plasticity (Herrera & Bazaga 2010; Zhang *et al.* 2013).

Our glasshouse experiment revealed that climate changes (declining rainfall in this case) can drive adaptive morphological change in a single generation. Despite such morphological changes not being consistent across all studied species, our results provide evidence of general presence of positive adaptation to drought. Plants with higher LMA are more water-use efficient in terms of assimilation to transpiration rate (Groom & Lamont 1997), and in our glasshouse experiment, seedlings of *B. leptophylla* and *H. costata* from populations that had been filtered by drought (i.e. LoR) developed higher LMA when growing under water deficit conditions but not when growing under

control conditions. In water-limited habitats, such as those here, the rapid elongation of the root increases the chances of first year seedlings maintaining contact with receding soil water over the first summer (Enright & Lamont 1992, Milberg & Lamont 1997), which is the key to successful seedling recruitment following fire. Seedlings of *B. leptophylla* from populations that experienced the drought filter grew deeper roots than seedlings from populations that did not experience the drought filter. Seedlings from populations that had been filtered by drought might also be more efficient in water uptake, as measured by water content per root length in *B. hookeriana*, and may have more water for transpiration per unit leaf area.

Water deficit led to significant changes in gene expression in seedlings derived from both HiR and LoR populations of *B. hookeriana*, with seedlings actively reprogramming their metabolism, growth and response to stress. In response to water deficit, cellular pathways of stress avoidance and tolerance were up-regulated to promote survival; pathways related to growth were generally down-regulated. Therefore, plants were able to redirect resources from growth to stress-resistance functions for increased chance of survival (Roelofs *et al.* 2010). The most significant results from our transcriptome analysis are that LoR derived seedlings up-regulated cellular pathways of salicylic acid biosynthesis and metabolism (as distinct from jasmonic acid biosynthesis and metabolism in HiR derived seedlings), and programmed cell death. Salicylic acid is involved in a range of cell activities as a response to stress, enhancing tolerance to heat, cold, and drought stress, regulating cell growth, regulating stomatal movement and photosynthetic activity in guard cells of stomata, and initiating flowering and reproduction under stress conditions and cell starvation (Senaratna *et al*. 2000; Stevens *et al.* 2006). Salicylic acid has antagonistic effects on jasmonic acid signaling downstream (Van der Does *et al.* 2013), and modifies transcriptional regulators that are involved in suppression of jasmonic acid-dependent genes (Caarls *et al*. 2015), suggesting a deep layer mechanism of stress adaptation of activating salicylic acid biosynthesis and metabolism.

Programmed cell death is thought to be a mechanism of adaptive response to stress, maintaining cell survival under stress conditions by allowing the degradation and recycling of non-essential components of the cell (Elmore 2007, Mizushima 2007). The adaptive advantages of activating these two cellular pathways (i.e. degradation and recycling of non-essential components) are apparent, as we recorded much lower mortality under drought than control conditions in our glasshouse experiment. The ability to activate these two biological processes after a selection filter in a single

generation suggests that there may be an intrinsic capacity for rapid adaptation to stress, likely from standing genetic variation within the population, rather than from new mutations (Barrett & Schluter 2008), or through an epigenetic process such as DNA methylation resulting in phenotypic plasticity (Richards *et al*. 2010).

Directional selection resulting in phenotypic change may increase the fitness of an organism; it also could decrease genetic variability in adaptive evolution (Hoffmann & Sgrò 2011). Our results suggest that drought may have selected for homozygotes associated with salicylic acid biosynthesis and metabolism, and reduced genome wide heterozygosity. It is likely that the homozygous state of those drought related genes could contribute to up-regulated expression, and therefore confer higher fitness when under stress conditions. Consistent with observed lower mortality rate in the glasshouse under simulated drought, the lower mortality of natural *B. hookeriana* populations with lower microsatellite DNA heterozygosity was also observed after the severe 2006 drought in SWA (He & Lamont 2010). The temporal and spatial heterogeneity of selection suggested by this study, together with high gene flow via pollen (Barrett *et al.* 2005) and seed dispersal (He *et al.* 2004) might lead to a reservoir of adaptive genetic variation in *B. hookeriana* and other co-occurring species that facilitates rapid adaptation to a changing climate. We observed a considerable number of genes with significant expression change when under drought, suggesting that the genetic basis of adaptation to a drier climate is strongly multigenic.

Our results suggest that some species and ecosystems might be more resilient to climate change than we currently believe, with adaptive evolution through natural selection and/or heritable phenotypic plasticity as results of epigenetic processes occurring within a relatively short time frame (Leuzinger *et al.* 2011), in our case, a single generation. Plant communities in biodiverse SWA may be able to tolerate further changes in rainfall through rapid adaptive evolution. Our results suggest drought experienced by a population results in reduced growth, but natural selection across this fitness differential results in a population that is better adapted to water deficit conditions, which represents potential for adapting to a drying climate. For this process to occur, two conditions must be met. First, there must be genetic variation within the population that allows a physiologically beneficial response to low water availability. A study on adaptive genetic variation in response to rainfall and temperature in *Banksia attenuata* (usually co-occurring with the species studied here) indicated that even populations occurring in wet habitats have genetic variation favouring survival under dry conditions (He *et al.* 2016). However, directional

selection could deplete population genetic variation, which limits the species' adaptive potential for other stresses that require different suites of genes and regulation networks. Indeed, we observed that LoR populations had fewer individuals with lower fitness specific to drought resistance than HiR populations, indicating lower genetic variation within LoR populations than HiR populations. Second, populations must be robust, with high reproductive capacity. This second condition is important because the capacity to respond favourably to stress requires a balance of growth and survival (Claeys & Inzé 2013; Pespeni *et al.* 2013). Populations experiencing selection may pay a selective cost in terms of reduced growth, as we observed in our glasshouse experiment, which could lead to lowered reproductive potential (Lamont *et al*. 2003; Groom & Lamont 2011). Populations impacted by climate change, or by other stresses, such as frequent fire in SWA (Enright *et al*. 2015), may have low population growth and reduced capacity to cope with selective impacts of a drying climate. Our glasshouse experiment revealed poorer early growth of seedlings from populations that experienced drought selection than seedlings from populations that did not experience drought selection, even under favourable growing conditions. In conclusion, although plant species in SWA may possess the capacity for rapid adaptation to a drying climate, the extent of rapid adaptation is finite, and maintenance of robust populations in the future is an important part of any climate response strategy. Future studies are needed to empirically test the effect of loss of genetic diversity through rapid evolution in response to a drying climate, or other stressors, on overall population variability.

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Chapter 4: Does drought tolerance increase in the canopy seed bank of *Banksia hookeriana* under rising water stress?

4.1 Abstract

Climate change is having documented effects on ecosystems globally, potentially threatening their persistence. Mediterranean Type Ecosystems are experiencing decreased rainfall and increased drought; and this is evident in Southwestern Australia, a globally significant biodiversity hotspot. Climate change may act as a selection pressure for stress-tolerant genotypes, inducing adaptations that increase a species' tolerance to drought. The accumulation of stress-tolerant genotypes can be analysed through resurrection studies; studies that use stored genetic material to compare the response of ancestral and descendent genotypes to stressors. Genera such as *Banksia* (Proteaceae) that possess persistent canopy seed banks of dormant, viable seed traceable to each year of seed production are ideal candidates for resurrection studies. This study aimed to determine whether *Banksia hookeriana* is capable of accumulating adaptive genetic variation within the life-time of individual plants. Resistance to drought may build up in the seed bank over periods of drought, which has occurred at Eneabba over the past 40 years as rainfall has decreased. Seed of *B. hookeriana* produced each year from 2003 to 2013 was collected from Eneabba, Western Australia, germinated, and seedlings grown in a greenhouse over 11 months where they were divided among control and drought treatments. No significant difference in growth was observed between seedlings established from seed produced in years prior to drought, post one drought, or post two droughts, indicating that accumulation of drought tolerant genes has not occurred in *B. hookeriana* as parents experienced drought. Climate change at Eneabba may not have been severe enough to induce selection pressure for drought tolerant genes in this species, implying that *B. hookeriana* may be capable of withstanding additional changes in climate. Further investigation could confirm whether the responses observed in this study are consistent across a variety of taxa.

4.2 Introduction

Changes in climate due to anthropogenic influences are now becoming apparent across the globe (IPCC 2013). In Mediterranean Type Ecosystems (MTEs) these effects are presenting as decreased winter rainfall (increased drought) and increased temperature in all seasons (IPCC 2013). In Southwest Australia (SWA), a biodiversity hotspot, rainfall has been steadily decreasing since the 1970s, and temperature has been increasing (Hope *et al*. 2006; Bates *et al*. 2008), causing concern for the persistence of the flora.

Plants may respond to changes in climate through evolutionary adaptation, a process that may occur as a result of environmentally induced selection pressure (Bijlsma & Loeschcke 2005; Williams *et al*. 2008). Changing climates may therefore induce evolutionary adaptations in species and increase species' tolerance to new climatic conditions (Hoffmann & Willi 2008). For further information regarding climate change in MTEs, the study system, and plant responses to climate change see Chapter 1.

The study outlined here seeks evidence of accumulation of drought tolerant genes within a generation (i.e. between sibling seeds produced by the same maternal plant but with different pollen donors over different years). This is made possible through the use of the unique study system available within fire killed serotinous species of the SWA flora; viable seed stored in the canopy that is identifiable to the year of production.

4.2.1 Use of resurrection studies to identify evolutionary changes

The pattern and speed of accumulation of stress tolerant genotypes can be determined through experiments using genetic material such as seeds or eggs that have been stored across years when climate change is occurring (Kerfoot *et al.* 1999; Franks *et al*. 2007; Franks *et al*. 2008; Hoffman & Sgrò 2011; Thomann *et al*. 2015). The method of raising ancestral genotypes alongside descendant genotypes from the same location and comparing the response of the two genotypes to a stressor is known as 'resurrection ecology' and can indicate evolutionary changes (Kerfoot *et al*. 1999; Franks *et al*. 2007; Franks *et al*. 2008). For example; Kerfoot *et al*. (1999) used resting eggs of the small, aquatic crustacean *Daphnia* to analyse the effects of copper mining on the biota of the Keweenaw Waterway in Michigan, USA. Dormant *Daphnia* eggs were extracted from sediment cores dating up to 70 years old and the species of *Daphnia* that had been present in the waterway across time were determined. *Daphnia* hatched

from eggs collected from different sediment layers were exposed to toxicity tests and the differences in *Daphnia* tolerance to dissolved copper concentrations occurring over the years were determined (Kerfoot *et al.* 1999). Similarly, Thomann *et al*. (2015) examined cornflower (*Centaurea cyanus*) in a common garden experiment in northern France using ancestral seed collected in 1992 and descendent seed collected in 2010 following 18 years of climate change. Significant changes in reproductive traits were observed, with plants grown from seed collected in 2010 flowering earlier and having larger floral displays than plants grown from seed collected in 1992 (Thomann *et al*. 2015).

4.2.2 The seed bank as a source of stored genetic material

Plants may store seed in persistent soil or canopy seed banks where it will remain until germination is triggered (Lamont *et al*. 1991; Crawford *et al.* 2011). Species that store overlapping years of seed production in woody fruits in the canopy are said to possess canopy seed banks (CSBs), and are termed 'serotinous', meaning that seed is stored on the plant for extended periods of time (Lamont *et al*. 1991; Crawford *et al.* 2011). Seed within the CSB must remain viable until conditions for release are met, though seeds gradually lose viability with age, and the proportion of seed released spontaneously increases with time (Lamont *et al*. 1991; Crawford *et al.* 2011). Seed stored within the CSB lies dormant until follicles open and seed is released (Lamont *et al*. 1991; Barrett *et al*. 2005). Thus CSB species possess a reserve of dormant, viable seed traceable to each year of production, provided that the individual age of the cones can be determined, making CSB species ideal candidates for use in resurrection studies.

CSBs promote fitness in a fire prone environment by ensuring seed is released at a time conducive to establishment (Lamont *et al.* 1991; Causley *et al.* 2016). CSB species display varying levels of serotiny, ranging from weakly serotinous to highly serotinous (Lamont *et al.* 1991). Weakly serotinous species may release seed from their woody fruits at maturity, over time with the ageing of the fruit, after the death of branches, or due to fire (Lamont *et al.* 1991). Highly serotinous species however, generally require fire to crack open the woody fruit to release seed (Lamont *et al*. 1991; Enright *et al.* 2008). The post-fire environment is more conducive to seedling establishment than the inter-fire period; it is free of leaf litter which allows seeds to reach the soil, there is decreased competition and thus increased space, light, water and nutrient availability, and the ash produced by the fire is nutrient rich (Lamont *et al*. 1991; Causley *et al*. 2016). Thus fire stimulates seed release in CSB species, and ensures that seeds are

released into an environment favourable to germination and growth. The accumulation of seed crops from a number of years also raises the genetic diversity of the offspring population and gives greater capacity for regeneration under a range of conditions (Lamont *et al*. 1991).

CSB species occur in abundance in the sclerophyll vegetation of Australia, South Africa, and North America- areas that are fire prone, highly seasonal and generally possess Mediterranean type climates (Lamont *et al.* 1991). Serotiny has been recorded in 40 genera worldwide but is most developed in the sclerophyll vegetation of Australia where it is displayed by species within the families Proteaceae, Myrtaceae, Ericaceae, Cupressaceae, and Asteraceae (Lamont *et al.* 1991). Therefore the fire prone flora of SWA provides the opportunity to investigate possible timelines of adaptation of plants to stress, with many species of SWA's kwongan vegetation retaining seeds from sequences of years in persistent CSBs (Groom & Lamont 2015).

Proteaceae is an important family in the kwongan vegetation of SWA. It is high in species richness and abundance, and often dominates the local flora (George 1998). A number of genera within the Proteaceae including *Banksia, Grevillea, Hakea, Isopogon, Petrophile*, and *Xylomelum* display CSBs. The genus *Banksia,* with 80% of species endemic to SWA, is perhaps the most well-known of SWA's serotinous plant groups. Banksias produce large, woody cones that bear follicles encasing the seeds (George 1998). The degree of serotiny varies between species, with seed typically stored on the plant for 3 - 10 years (Barrett *et al*. 2005; Lamont *et al*. 2007). Follicles of weakly serotinous species open to release seed in the first autumn after production, while follicles of strongly serotinous species remain closed until the heat of fire cracks them open, which may take several years (Barrett *et al*. 2005; Lamont *et al*. 2007).

4.2.3 Banksia hookeriana

An ideal species for use in investigating the influence of drought on the flora of SWA is *Banksia hookeriana* Meisn. A number of factors make *B. hookeriana* a suitable study species; firstly it is strongly serotinous (Crawford *et al*. 2011), storing seed on the plant for up to 15 years (Groom & Lamont 2015). Secondly, it is killed by fire, stimulating follicles to release seeds that generate a new, single aged population cohort; instances of inter-fire recruitment are very rare (Cowling *et al.* 1987; Lamont *et al.* 2007). Thirdly, the age of cones and the seed contained within them can be reliably determined using stem node counts (Lamont 1985). Finally, *B. hookeriana* is known to be susceptible to drought (He & Lamont 2010). Mortality in *B. hookeriana* is directly

related to the rainfall of the previous winter/spring, with low rainfall resulting in increased plant mortality the following summer at a rate of a 6% increase in mortality per 100 mm of rainfall below the long term average (Keith *et al*. 2014). The species relies on accessing groundwater with its deep tap root over the summer and becomes vulnerable to drought when water is not recharged annually (Keith *et al*. 2014). Mortality rates are highest for seedlings, declining with plant age as deeper tap roots develop (Keith *et al*. 2014). He & Lamont (2010) reported 25-50% mortality of adult *B. hookeriana* plants in 15 individual populations at Eneabba, Western Australia following prolonged drought in 2006.

Banksia hookeriana is considered at risk under future climate change scenarios due to its restricted distribution on the sand dunes around Eneabba (Fitzpatrick *et al*. 2008; He & Lamont 2010; Yates *et al*. 2010). In addition, the species' range has contracted by 40% in area since the 1960s due to clearing for farming and mining, frequent fire, and commercial wildflower picking, hence further range contraction, clearing or plant death could have dire consequences for *B. hookeriana* (He & Lamont 2010).

This study aimed to determine whether *B. hookeriana* is capable of accumulating adaptive genetic variation rapidly enough to resist the effects of climate change, in particular drought. It was hypothesised that increased drought resistance would have built up in the seed bank during recent years of drought (2006 and 2010). The populations of *B. hookeriana* at Eneabba that were sampled in this experiment are of a similar age, thus all individuals in the populations have experienced the same selection pressure from drought. It was hypothesised that the populations present prior to the drought of 2006 would have been made up of a combination of comparatively 'drought tolerant' and 'drought susceptible' genotypes (Figure 4.1A). However, after the drought of 2006 a number of drought susceptible individuals would have perished decreasing the proportion of this genotype within the populations (Figure 4.1B). Thus it was hypothesised that seed produced from the years 2006 onwards would produce a higher proportion of drought tolerant seedlings, as the proportion of pollen donors for this seed that were drought susceptible had decreased due to the selection pressure exhibited by the drought (note that *B. hookeriana* is an out-crossing species; Barrett *et al.* 2005). After the drought of 2010 further drought susceptible individuals would have perished, further decreasing the proportion of this genotype within the populations (Figure 4.1C). Thus it was hypothesised that seed produced from 2010 onwards would produce the highest proportion of drought tolerant seedlings, as the

proportion of pollen donors for this seed that were drought susceptible was low due to the selection pressure exhibited by both the 2006 and 2010 droughts.

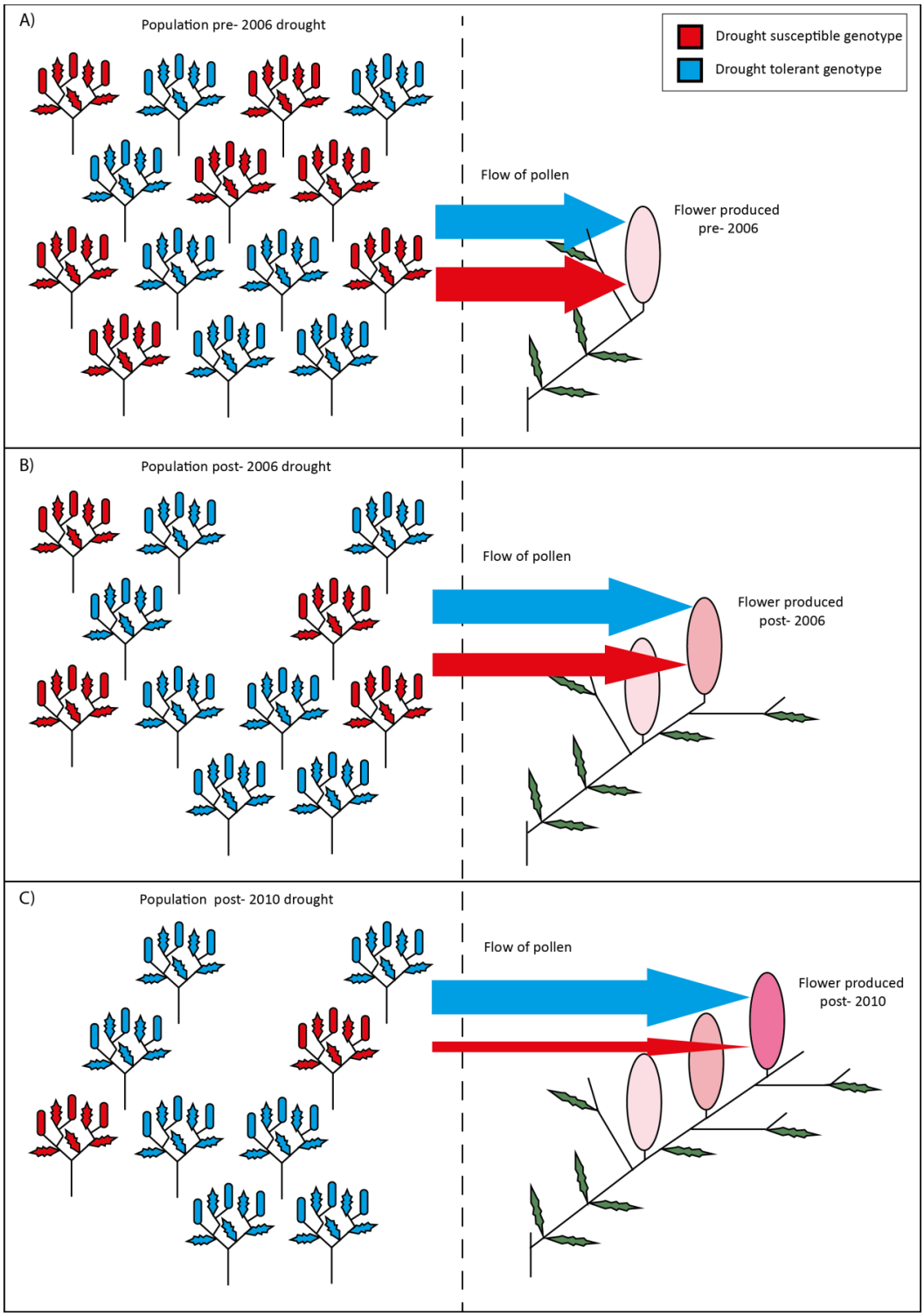

Figure 4.1: Representation of the hypothetical genotypic composition of *B. hookeriana* plants at Eneabba A) before drought, B) after the 2006 drought, and C) after the 2010 drought, with hypothetical proportion of pollen flow from each genotype indicated.

4.3 Materials and Methods

4.3.1 Population selection and seed collection

Details on the study system within Southwestern Australia and Eneabba are given in Chapter 1. Populations of *B. hookeriana* were sampled from stands of Banksia woodland at three locations at Eneabba, Western Australia. Locations used were determined by the presence of *B. hookeriana* plants of post-fire age of at least 15 years, with 10-11 years of cone production (*B. hookeriana* begins to flower at age 3-4 years; Barrett *et al*. 2005). Time since fire for each site was provided by the Department of Parks and Wildlife (Western Australia), the government agency responsible for managing and monitoring fire across Western Australia. Study sites were located at Lake Logue Nature Reserve (S 29˚45'21.1" E 115°11'22.0"), Leda Nature Reserve (S 29˚43'53.7" E 115˚14'38.8") and by the side of the train tracks 9 km north of Eneabba (S 29˚44'09.0" E 115˚13'34.7"; Figure 4.2).

In October 2014, cones produced each year from 2003 up until 2013 were collected from *B. hookeriana* plants at each site. Cones were aged using stem node counts as described in Lamont (1985); starting from the tip of a stem the years of growth were counted by identifying the number of annual growth nodes along the stem (present as branching or as a nodule/scar), this was repeated for multiple stems per plant, and for multiple plants per stand to achieve an accurate assessment of the age of the plants. Figure 4.3 displays a *B. hookeriana* plant with multiple nodes of growth indicated along the stem.

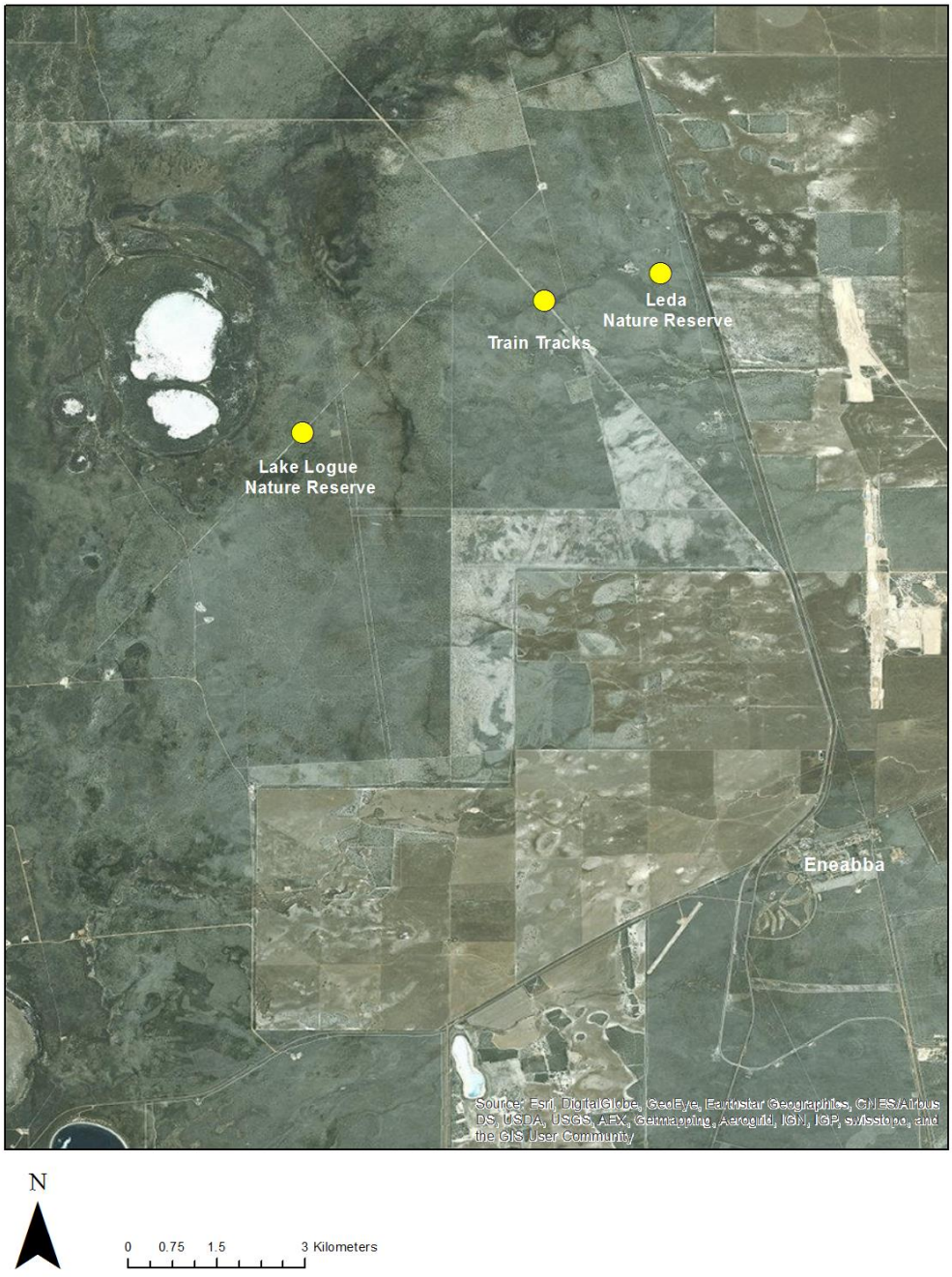

Figure 4.2: Location of *B. hookeriana* collection sites at Lake Logue Nature Reserve, Leda Nature Reserve, and along the train tracks. The location of the town of Eneabba is also indicated.

Figure 4.3: *Banksia hookeriana* plant with six years of growth indicated along the stem**.** Photograph by Tianhua He, 2013.

4.3.2 Seed preparation and germination

Seed was extracted from fruits of *B. hookeriana* by scorching cones with a gas torch until follicles split open and then soaking cones in cold water over night. Cones were then dried in an oven at 60°C until follicles were open wide enough for seed to be removed with a pair of forceps.

In order to assess whether the recent droughts experienced by plants in Eneabba had had an impact on the drought tolerance of their offspring, seed was grouped under one of three categories relating to the drought experience of parent plants in Eneabba: 1) Pre-drought, meaning seed was produced by parent plants prior to the recent droughts, 2) Post one drought, meaning seed was produced by parent plants after they had experienced the drought of 2006, or 3) Post two droughts, meaning seed was produced by parent plants after they had experienced the droughts of 2006 and 2010 (Table 4.1). Seed that had been collected across the three sites but produced in the same year cohort was combined in order to maximise genetic diversity within the samples.

Table 4.1: Categorisation of *B. hookeriana* seed by drought exposure of parent plants.

	Drought exposure		
	Pre-drought	**Post one drought**	**Post two droughts**
Year seed produced	2003-05	2006-09	2010-13
Recent drought experience	No recent drought experienced	Experienced 2006 drought	Experienced 2006 and 2010 drought

Seed was surface sterilised in a solution of 20% sodium hypochlorite with a drop of Tween 80 surfactant (polyoxyethylene sorbitan mono-oleate) for 15 minutes, then rinsed with sterile deionised water (Downes *et al.* 2010). Sterilised seed was placed into 150 mm Petri dishes containing two sheets of Whatman No. 1 filter paper, five pieces of 2 cm^2 portions of Wettex sponge and 16 mL of water. A total of eighty seeds were used from each individual year of seed production with the exception of the years 2003 (11 seeds), 2004 (25 seeds), 2005 (27 seeds) and 2006 (72 seeds) for which less seed was available. Petri dishes were sealed with Parafilm wax then placed in a germination cupboard at 15°C with a 12 hr light/dark cycle for four weeks. The number of seeds to germinate each day (germination rate) was recorded.

4.3.3 Greenhouse experiment

Germinants were sown into tube pots (PVC pipe with capping; 15 cm diameter, 100 cm height) containing washed white sand on the 16th June 2015. A total of 24 seeds were grown for the 2003-05 cohort, 94 seeds were grown for the 2006-09 cohort, and 136 seeds were grown for the 2010-13 cohort (Table 4.2). An even number of seeds were sourced from each individual year group within cohorts where possible. Where there was spare seed (for seed collected in the years 2006 to 2013) two seeds were planted per pot, with one serving as a spare to be removed once established. Seedlings were grown in a greenhouse at Curtin University, Bentley, Western Australia. All seedlings were frequently watered for the first six days and then three times a week for five weeks to allow seedlings to establish. Once seedlings were established, extra seedlings were removed so that only one seedling remained per pot. Seedlings were then divided between a control and a drought treatment (Table 4.2).

Table 4.2: Number of *B. hookeriana* seedlings from each year cohort allocated to the control and drought treatment, where one seedling is one replicate.

	Year seed produced		
	2003-05	**2006-09**	**2010-13**
Control treatment	12	47	66
Drought treatment	12	47	66

Once established, watering of seedlings in the drought treatment was ceased, while watering of seedlings in the control treatment was continued three times a week for six weeks (Table 4.3). Soil moisture was monitored throughout the experiment with Odyssey Multi-profile soil moisture loggers (Dataflow Systems Ltd, Christchurch, New Zealand). Monitoring allowed watering volumes to be altered if required, to prevent soil moisture levels from ranging too far from desired thresholds: no less than 10% soil moisture content for the control treatment, and no greater than 5% soil moisture content for the drought treatment (Dodd *et al.* 1984).

After the six week treatment period, some seedlings were showing signs of nutrient deficiency (yellowing of leaves) so one tablespoon of fertiliser (Scotts Osmocote Plus Trace Elements: Native Gardens) was added to each pot, and all plants were watered for three days to activate the fertiliser. Plants in the control treatment were then watered once a week for nine weeks, while plants in the drought treatment were not

watered at all. During December 2015, extreme summer heat resulted in the death of 60 plants. Starting on the 23rd of December 2015 all plants were watered once a week for three weeks to avoid further mortality, after which watering returned to once a week for plants in the control treatment and once a month for plants in the drought treatment for a further three months.

Table 4.3: Watering regime for *B. hookeriana* seedlings subjected to control and drought treatments over an 11 month period.

	Period of time						
	6 days	**5 weeks**	**6 weeks**	**3 days**	**9 weeks**	**3 weeks**	**3 months**
Control Treatment	Every day	3 times a week	3 times a week	Every day	Once a week	Once a week	Once a week
Drought Treatment	Every day	3 times a week	0 times a week	Every day	0 times a week	Once a week	Once a month

Plants were harvested between the 19th and 22nd of April 2016, and measurements were taken of the Number of leaves, Shoot length, Root length, Biomass, Leaf area (of five leaves per plant, using the freeware program ImageJ; Rasband 1997) and Leaf thickness (of five leaves per plant). Seedlings were dried in an oven at 80˚C after which Total dry biomass and Biomass of individual leaves (five per plant) were recorded. Water content (fresh mass – dry mass), Specific leaf area (SLA; leaf dry mass/leaf area), Leaf mass per area (LMA; 1/SLA), and Root to shoot ratio (dry root mass/dry shoot mass) were also calculated.

4.3.4 Statistical analysis

All statistical analyses were performed in SPSS Statistics (Version 21; IBM Corp. 2012). A Kruskal-Wallis test (α=0.05) was used to determine whether the mean mass of seed produced in each year cohort was similar.

Tests for homogeneity of variance (Levene's test; α=0.05) and normality (Shapiro-Wilk test; α=0.05) indicated that data for the following growth variables were non parametric; Number of leaves, Root length, Root fresh biomass, Total fresh biomass, Root dry biomass, Root-to-shoot ratio. Log (x+1) transformations were performed on these data in order to reduce variation, and transformed data were used in further analyses.

Two-way ANOVAs (α=0.05) were performed on the following measurements: Number of leaves, Root length, Shoot length, Leaf thickness, Root fresh biomass, Shoot fresh biomass, Total fresh biomass, Leaf dry biomass, Root dry biomass, Shoot dry biomass, Total dry biomass, Leaf area, Water content, Root to shoot ratio, SLA and LMA. Tests were performed in order to determine 1) whether there was a significant difference in means between control and drought treated plants, 2) whether there was a significant difference in means between plants established from parents with different recent drought exposure, or 3) whether there was a significant interaction between the two factors (treatment and recent drought exposure).

4.4 Results

4.4.1 Seed collection and germination

Cones were collected from each year of production from 2003 to 2013. Cones and seed from earlier years were few, with many cones having been predated by birds or insects, opened with time, or decayed, leaving no seeds inside of the follicles. A summary of the cones collected from the three sites at Eneabba and the seed extracted is displayed in Table 4.4.

Table 4.4: Number of *B. hookeriana* cones collected from each of the three sites at Eneabba for each year of production and number of seeds extracted from cones.

	Number of cones collected				Number of seeds collected			
	Lake Logue	Train Tracks	Leda Reserve	Total	Lake Logue	Train Tracks	Leda Reserve	Total
2003-05	16	17	6	39	32	28	3	63
2006-09	44	43	40	127	303	159	170	632
2010-13	27	27	32	86	404	340	451	1,195

Seed mass differed across year cohorts (One-way ANOVA; p<0.001), ranging from an average of 43.35 mg (± 0.71 SE) for seed produced in years post two droughts (2010-13) to 49.84 mg (± 0.39 SE) for seed produced in years post one drought (2006-09; Figure 4.4). Time until germination increased with the age of the seed, while the percentage of seed germinating decreased with the age of the seed (Figure 4.5).

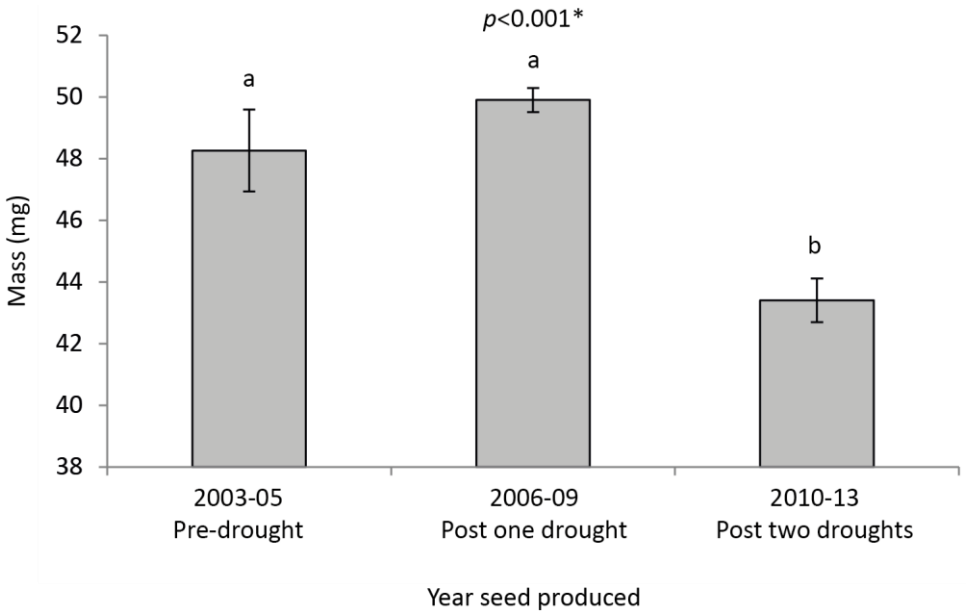

Figure 4.4: Mass (mean ± SE) of *B. hookeriana* seed produced across three seed age cohorts. Measure of significance between treatments is from a One-way ANOVA (α=0.05), asterisk indicates significance.

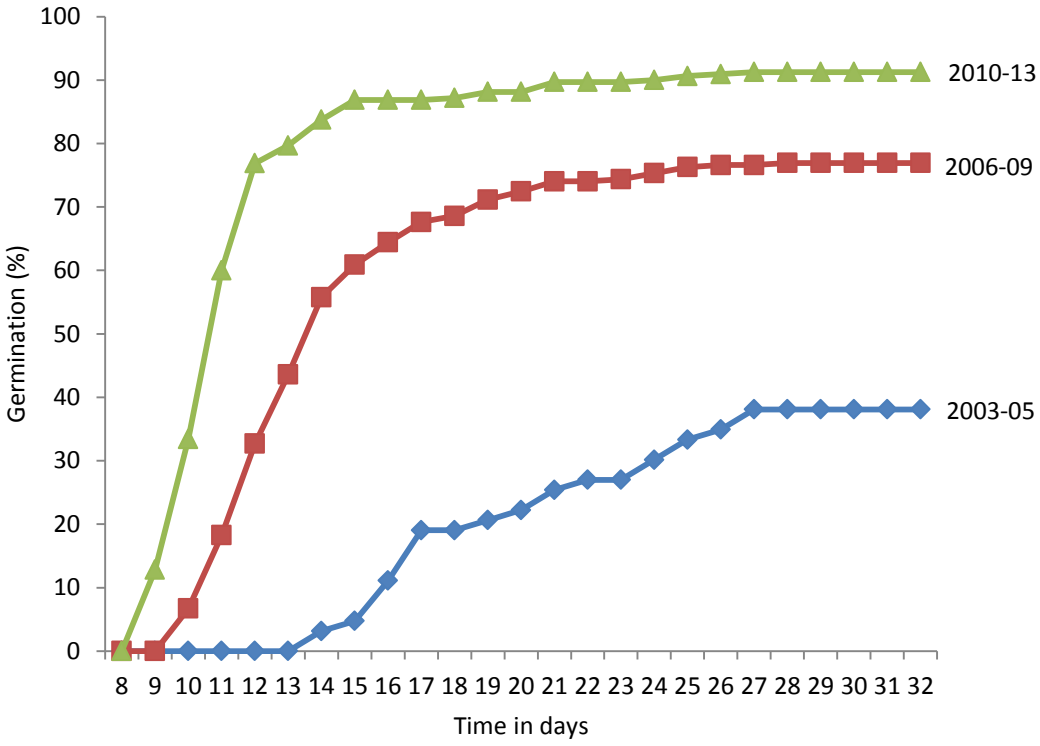

Figure 4.5: Germination rate (cumulative percentage) of *B. hookeriana* seed produced from 2003-05, 2006-09, and 2010-13. Note that a total of 63 seeds were used from 2003-2005, 312 seeds were used from 2006-09, and 320 seeds were used from 2010-13.

4.4.2 Growth measurements

There was no significant interaction between age cohort (Pre-drought, 2003-05; Post one drought, 2006-09; and Post two droughts, 2010-13) and treatment (control or drought) on growth of *B. hookeriana* seedlings (p>0.05 in all cases; Two-way ANOVAs; α=0.05). Age cohort had no significant effect on the growth of seedlings when they were subjected to the control and drought treatments (Figure 4.6 a-j, Figure S4.1 a-f; p>0.05 in all cases).

The treatment to which *B. hookeriana* seedlings were exposed had an effect on their growth, with seedlings subjected to the drought treatment displaying reduced growth compared to seedlings subjected to the control treatment for the following variables: Shoot height, Root length, Total fresh biomass, Total dry biomass, Number of leaves, Leaf thickness, Leaf area (Figure 4.6 a-d, g-i), Shoot fresh biomass, Root fresh biomass, Shoot dry biomass, Root dry biomass, and Leaf dry biomass (Figure S4.1 a-e; p<0.001 in all cases), with seedlings in the control treatment displaying higher shoots, longer roots, greater fresh and dry biomasses of roots, shoots and total plant, a greater number of leaves, thicker leaves, and greater leaf area. *Banksia hookeriana* seedlings subjected to the control and drought treatments displayed similar Root to shoot ratios (p=0.054; Figure 4.6 e), Water content (p=0.238; Figure 4.6 f), LMA (p=0.514; Figure 4.6 j) and SLA (p=0.528; Figure S4.1 f).

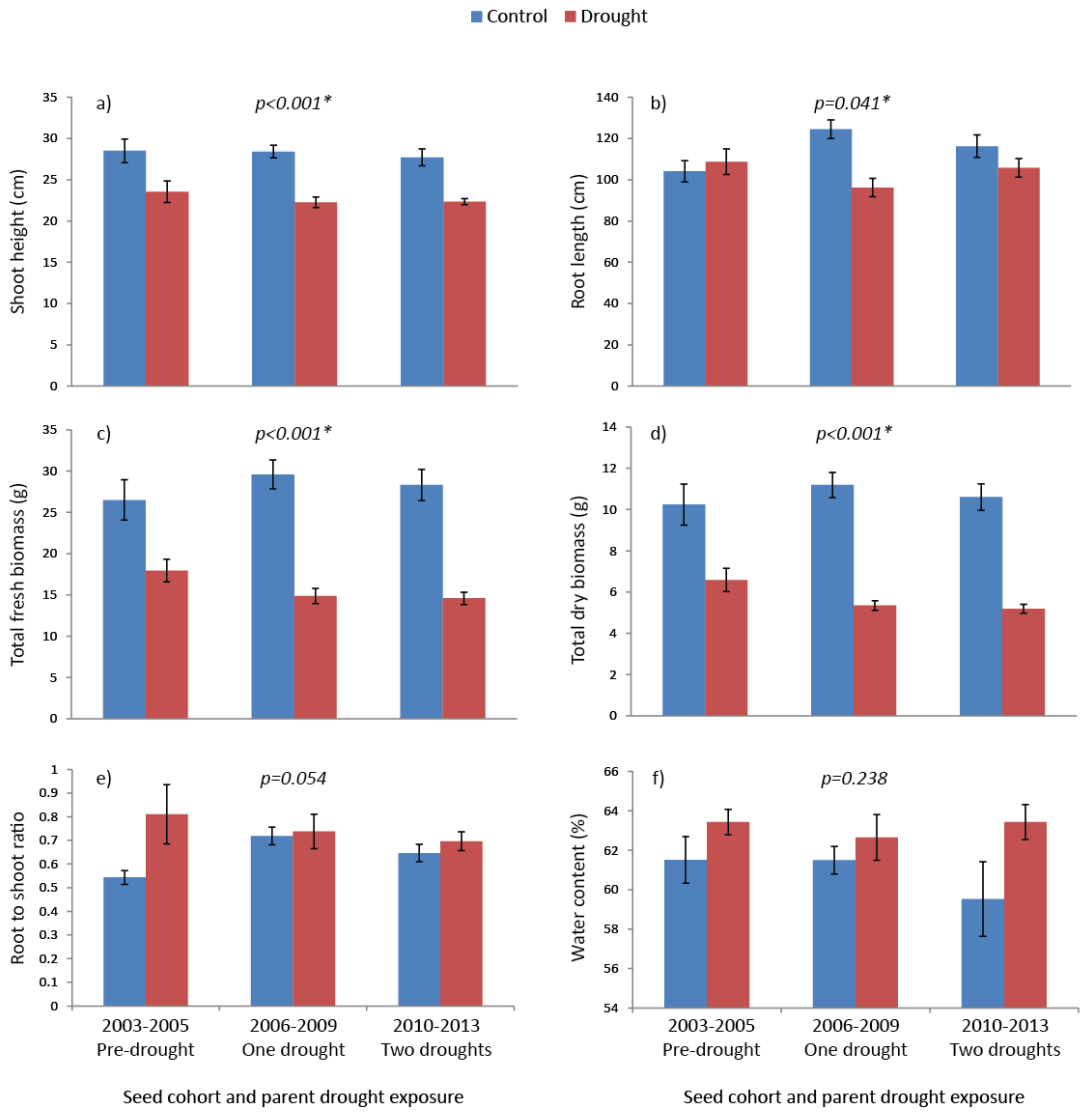

Figure 4.6 a-f: Shoot height, Root length, Total fresh biomass, Total dry biomass, Root to shoot ratio, and Water content of *B. hookeriana* seedlings grown in the control and drought treatments. Data presented as means ± SE. Measures of significance between treatments are from Two-way ANOVAs (α=0.05), significant values are marked with an asterisk.

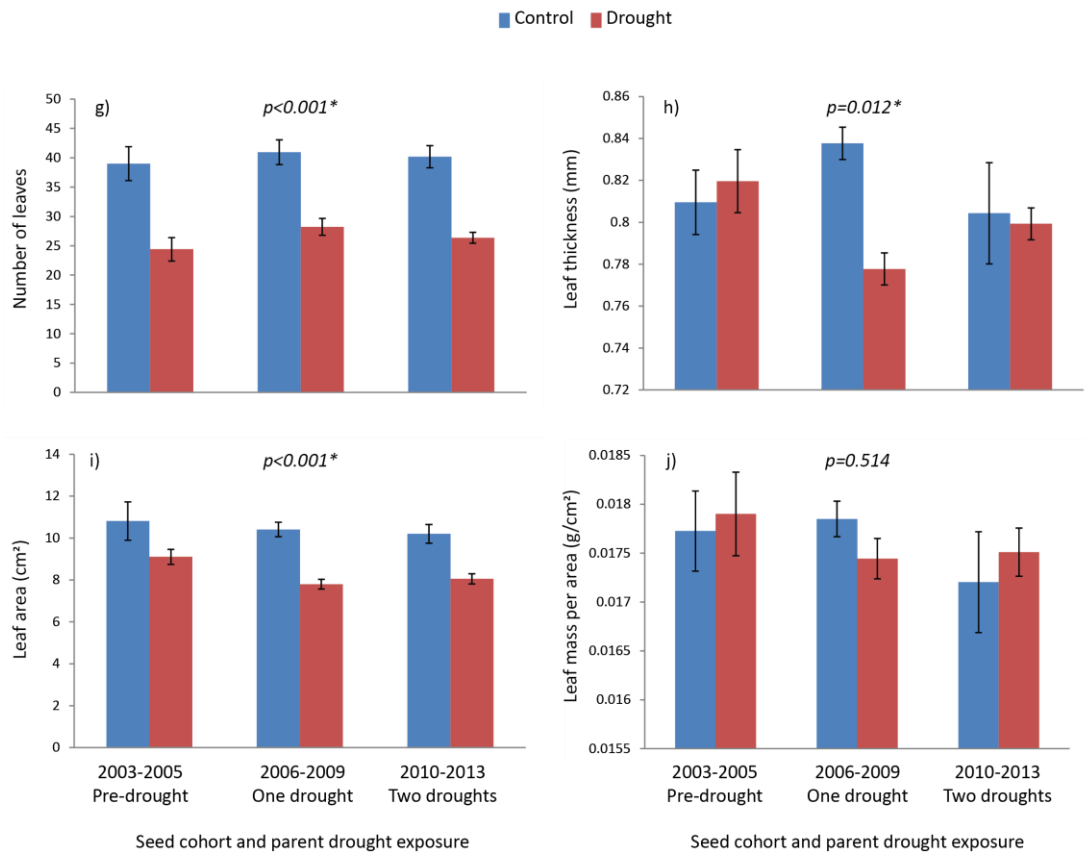

Figure 4.6 g-j: Number of leaves, Leaf thickness, Leaf area, and Leaf mass per area of *B. hookeriana* seedlings grown in the control and drought treatments. Data are presented as means ± SE. Measures of significance between treatments are from Two-way ANOVAs (α=0.05), significant values are marked with an asterisk.

4.4.3 Mortality

Before treatments had ceased 40% of the seedlings planted had perished (114 seedlings out of 284). The majority of seedlings that perished were from the drought treatment, with 53% of drought treated seedlings perishing (76 out of 142 seedlings; Figure 4.7), while 27% of seedlings allocated to the control treatment perished (38 out of 142 seedlings; Figure 4.7). Drought treated seedlings established from Pre-drought and Post one drought (2003-05 and 2006-09) seed had the highest mortality rates of 58.3% and 57.8% respectively (7 out of 12 seedlings and 37 out of 64 seedlings perished respectively; Figure 4.7), while seedlings established from Post one drought (2006-09) seed in the control treatment had the lowest mortality rate of 18.75% (12 out of 64 seedlings perished; Figure 4.7). Overall, seedlings established from Pre-drought (2003-05) seed had the highest mortality rate of 45.8% (11 out of 24 seedlings

perished), while seedlings from the Post one drought (2006-09) seed had the lowest mortality rate of 38.3% (49 out of 128 seedlings perished).

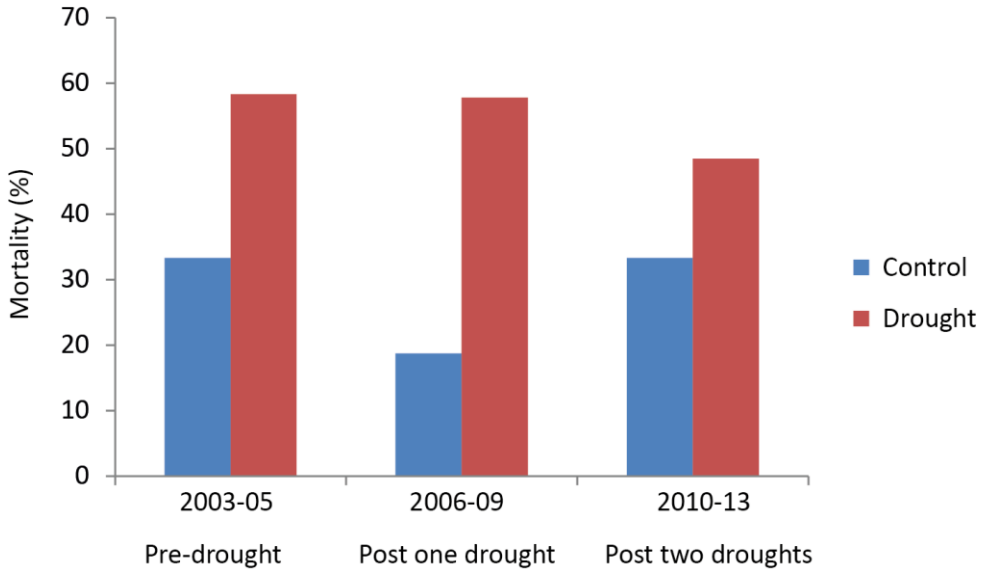

Figure 4.7: Proportion of seedlings from each year cohort of seed production subjected to the drought and control treatments that did not survive the experimental period.

4.5 Discussion

4.5.1 Overall findings

The mass plant deaths that occurred in Eneabba following the droughts of 2006 and 2010 are expected to have resulted in the loss of many drought susceptible plants from the community. It was hypothesised that the death of drought susceptible pollen donors would have reduced the proportion of drought susceptible genotypes in the community, and would have led to a higher proportion of drought tolerant offspring (Figure 4.1). Thus cohorts of seed produced post-drought years were expected to produce seedlings displaying greater drought tolerance than cohorts of seed produced pre-drought. The results of this study however do not support the hypothesis, instead indicating that there is no difference in drought tolerance between seedlings established from seed produced pre-drought and those established from seed produced post drought, with differences observed likely accounted for by general interannual variation.

For all but four measures of growth, seedlings grown under the control treatment fared significantly better than those subjected to the drought treatment, displaying higher

shoots, longer roots, greater fresh and dry biomasses of roots, shoots and total plant, a greater number of leaves, thicker leaves, and greater leaf area. However, for four traits directly related to drought tolerance- water content, root to shoot ratio, specific leaf area, and leaf mass per area- there was no significant difference between seedlings that survived under the drought or control treatments.

4.5.2 Explanations for similar growth rates

No year cohort of seedlings displayed a better or worse response than any other with regards to the treatments. Seedlings from all year cohorts however coped poorly with the drought treatment. This may indicate that the selection pressure from climate change in Eneabba was not significant enough to elicit a change in the genetic composition of the population; the decline in rainfall may not have been severe enough to remove the majority of drought-susceptible genes, and therefore increase the proportion of drought-tolerant genes. Although the mortality of *B. hookeriana* seedlings after the drought of 2006 was high, recorded to be on average 40-47% (He & Lamont 2010; He & Lamont 2014), the selection pressure imposed on *B. hookeriana* seedlings while establishing during the first summer post-fire can be high; Lamont *et al*. (1993) recorded 67% mortality of *B. hookeriana* seedlings at the end of their first summer in 1989, though this was following a year that displayed below-average rainfall (444.5 mm recorded in 1989, 502.8 mm long term average).

Detectable evolutionary adaptation occurs at different rates for different species and circumstances, and has been reported to occur within five years for some species and over thirty years for others (Bradshaw & Holzapfel 2006). For example; Nevo *et al.* (2012) recorded significant changes in the flowering time of barley (*Hordeum spontaneum*) and wheat (*Triticum dicoccoides*) after 28 years of climate change, Vigouroux *et al*. (2011) recorded significantly shorter life cycles and smaller plants in pearl millet (*Pennisetum glaucum*) after 27 years of decreased rainfall, and Guerin *et al.* (2012) recorded significantly narrower leaves in *Dodonaea viscosa* after 127 years of rising temperatures. Thus *B. hookeriana* may be evolving, but at a rate that is too slow to be detected by the methods used here.

The droughts of 2006 and 2010 may not have resulted in a sufficient enough change in the gene pool of *B. hookeriana* to induce evolutionary adaptation. After the 2006 drought He and Lamont (2010) reported an average of 40% mortality for *B. hookeriana* plants across 15 populations at Eneabba, with mortality ranging from 25-50% within individual populations. However, *B. hookeriana* is abundant in the local area, and the

death of some plants through drought stress may not have been enough to have long-term effects on the genetic make-up of the larger population.

The similar response to drought displayed by seedlings established from different seed year cohorts reported here is consistent with the findings of Barrett *et al.* (2005) who investigated the genetic structure of a nine year old seed bank of *B. hookeriana* plants near Eneabba. Barrett *et al*. (2005) found that for 304 seeds produced by five plants across nine years 83.43% of molecular variance could be accounted for by among individual differences, 12.73% of variance could be accounted for by among maternal plant differences, while only 3.83% of variance could be accounted for by among year of seed production differences. Thus the level of genetic variation between seeds produced by the same plant but in different years is very low when compared to between plant genetic variation for *B. hookeriana*. Barrett *et al.* (2005) suggest that this is due to high levels of genetic outcrossing in the population that leads to high levels of genetic diversity within the seedbank and decreases the likelihood of detecting large differences between years of seed production.

Banksia hookeriana also displays a high level of genetic variation between populations (Enright *et al*. 2003; Krauss *et al*. 2006). Krauss *et al.* (2006) sampled 15 populations of *B. hookeriana* from across the species' range and found that 30% of genetic variation occurred between populations. Similarly, Enright *et al.* (2003) sampled six populations of *B. hookeriana* from across its range and found that 32% of genetic variation occurred between populations. *B. hookeriana* is pollinated by insects and small mammals but nectar-feeding birds are the main pollinator of the species (Krauss *et al.* 2009). Birds are highly mobile, and can fly long distances to spread genes between populations of *B. hookeriana* (Lamont *et al*. 2003). This high level of genetic diversity present between populations of *B. hookeriana* paired with highly mobile bird pollinators results in a high level of gene flow between populations of *B. hookeriana* and high levels of genetic diversity that may buffer against evolutionary change.

The maternal plants from which the seed for this experiment was collected may themselves already have been relatively drought tolerant. These maternal plants have already passed drought selection with survival of the 2006 and 2010 droughts, while the more drought susceptible plants of the population would have perished. Thus it is possible that all of the seed used in this experiment already possessed a level of drought tolerance as inherited from hardy maternal plants.

4.5.3 Mortality rates

There was a high level of plant mortality recorded overall. Seedlings subjected to the drought treatment had a mortality rate twice that of the control, though mortality rates did not differ greatly between seedlings established from seed produced in different year cohorts, and there was no observable pattern to mortality. Mortality rates ranged from 18.75% to 58.3%, which is in line with results reported by Enright and Lamont (1989), who found that mortality of *B. hookeriana* seedlings at the end of their first summer ranged from 35% in areas burned in spring to 68% in areas burned in autumn and Lamont *et al*. (1993) who recorded 67% mortality of *B. hookeriana* seedlings at the end of the first summer post-fire.

4.5.4 Limitations of the study and directions for future study

It is likely that the sample size used in this study was a limiting factor, and perhaps not large enough to detect a treatment effect. A limitation on the initial sample size was the lack of seed available from cones produced in earlier years. As seed ages in the CSB it reduces in viability, is predated upon by birds and insects, or decays (Crawford *et al*. 2011). In their 2011 study of *B. hookeriana*, Crawford *et al*. (2011) found that 10% of seeds aged ten years or older were decayed, and up to 30% of seeds aged six years or older were damaged by insects. They also found that only 50% of *Banksia* seeds remaining on a plant after 13 years could be expected to be viable. In this study, the time *B. hookeriana* seeds took to germinate increased with the age of the seed and the percentage of seeds to germinate decreased with the age of the seed. Seed from the years 2006-09 and 2010-13 started to germinate from eight or nine days after placement within the germination cupboard, while seed that was over ten years old, from the years 2003-2005 started to germinate five days later, at day 14. However, seed that was over 10 years old still reached 38% germination. This is in line with a study by Barrett *et al*. (2005) on *B. hookeriana* that reported that viability of *B. hookeriana* seeds decreased with age, while time taken for seeds to germinate increased. Thus older seed can be hard to come by, and more of it is required to gain viable seeds for use in experiments.

Seed size was not consistent across year cohorts; with seed produced after the 2006 and 2010 droughts being significantly smaller than seed produced before these droughts. Water stress has been reported to produce smaller seeds in some agricultural species (Viera *et al.* 1991; Alqudah *et al.* 2010), though seed size is influenced by a range of factors, including soil type, nutrient availability, and pollinator

presence, and is inversely related to the number of seeds produced by a plant (Baker 1972; Esler *et al.* 1989). Larger seeds generally produce larger seedlings, due to their higher stores of nutrients (Baker 1972; Leishman & Westoby 1994); this had the potential to affect the final growth measurements of seedlings in this experiment, however, no relationship between larger seeds and larger seedlings was observed.

A limitation on the final sample size was the high proportion of seedlings that perished during the study. A total of 53% of drought treated seedlings and 27% of control seedlings perished, resulting in a lower than planned sample size with which to test treatment effects. Most seedlings perished during December 2015, which was 1.1˚C above the mean maximum December temperature for Perth, at 30.1˚C, with seven days of December 2015 reaching temperatures greater than 35˚C (Bureau of Meteorology 2016a). A larger sample size is recommended for future studies to provide greater confidence in determining whether a difference exists between cohorts of seed.

It is possible that some of the pollen that fertilised the seed used in this study was donated by plants that had not experienced drought filtering (i.e. plants established post 2010). However, the likelihood of this having occurred is low, as instances of inter-fire recruitment are very rare (Cowling *et al.* 1987), and the majority of mating occurs within populations of *B. hookeriana*; Krauss *et al*. (2009) reported that 84% of pollen fertilising flowers originated from within the same population, with the average distance between mates being 29.9 m.

The methods used here were only able to detect potential differences in seedling growth; however it is possible that differing levels of drought stress tolerance may become evident at later stages of development, such as influencing the age and size that plants are at the onset of flowering or fruiting. For example; evolutionary adaptations can occur as changes in phenology such as timing of flowering, as has been found for other plant species (Franks *et al.* 2007; Vigouroux *et al*. 2011; Nevo *et al.* 2012). A study by Franks *et al.* (2007) with similar underlying methods demonstrated a rapid evolutionary response of flowering time in an annual plant (*Brassica rapa*) in response to decades of drought; when descendent seed and ancestral seed were exposed to extreme drought, it was found that descendent seedlings flowered sooner – a shift to earlier flowering time had occurred with drought. Future studies could investigate whether *B. hookeriana* seedlings from seed produced pre-drought or post-drought display any differences in later stages of development, such as reproductive capacity and output.

It is possible that differences in gene expression may exist between seedlings from different seed-age cohorts when subjected to drought, and this could be tested through molecular analysis. As discussed in Chapter 3, seedlings of four Proteaceae species from Eneabba (*Banksia hookeriana*, *B. leptophylla*, *Hakea costata* and *H. polyanthema*) descended from plants established in low or average winter rainfall displayed few differences in growth response to drought treatments. However, when gene expression of *B. hookeriana* was analysed, it was found that seedlings descended from plants established under drought conditions had greater gene expression in pathways relating to stress response than seedlings descended from plants established under average rainfall conditions (D'Agui *et al*. 2016). For example; pathways relating to stress avoidance and tolerance were upregulated while pathways related to growth were down-regulated, allowing plants to redirect resources from growth to stress-resistance, enabling them to better tolerate the drought treatment (D'Agui *et al*. 2016). Future studies could analyse the gene expression of seedlings from different seed-age cohorts when subjected to drought treatments to determine whether there is a difference in expression between years of seed production.

4.6 Conclusion

The results indicate no difference in drought tolerance between *B. hookeriana* seedlings established from a range of seed-age cohorts. This implies that seeds of *B. hookeriana* have not accumulated more drought tolerant genes as parent plants have passed through drought events. The changes in climate experienced by *B. hookeriana* at Eneabba so far, mainly decreased rainfall, may not have affected population genetic variation, nor produced severe enough selection pressure to result in accumulation of drought tolerant genes. This may indicate that *B. hookeriana* could potentially withstand further changes to climate, and may accumulate stress tolerant genes under future, more severe conditions. Given its capacity to disperse seeds to distant habitats (with the potential to colonise habitat where *B. hookeriana* has become locally extinct as a result of drought), near complete outcrossing, and effective pollination system, drought tolerant genes could rapidly spread and be maintained in *B. hookeriana* populations. However, the results of this study should be interpreted with caution due to low sample sizes surviving to assessment, and further investigation is warranted.

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Chapter 5: Evolutionary potential and adaptation of *Banksia attenuata* (Proteaceae) to climate and fire regime in southwestern Australia, a global biodiversity hotspot*

5.1 Abstract

Substantial climate changes are evident across Australia, with declining rainfall and rising temperature in conjunction with frequent fires. Considerable species loss and range contractions have been predicted; however, our understanding of how genetic variation may promote adaptation in response to climate change remains uncertain. Here we characterised candidate genes associated with rainfall gradients, temperatures, and fire intervals through environmental association analysis. We found that overall population adaptive genetic variation was significantly affected by shortened fire intervals, whereas declining rainfall and rising temperature did not have a detectable influence. Candidate SNPs associated with rainfall and high temperature were diverse, whereas SNPs associated with specific fire intervals were mainly fixed in one allele. Gene annotation further revealed four genes with functions in stress tolerance, the regulation of stomatal opening and closure, energy use, and morphogenesis with adaptation to climate and fire intervals. *B. attenuata* may tolerate further changes in rainfall and temperature through evolutionary adaptations based on its adaptive genetic variation. However, the capacity to survive future climate change may be compromised by changes in the fire regime.

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Authors' contributions: The supervisory team (Tianhua He and Neal Enright) and Yiqi Luo conceived the idea. Haylee D'Agui designed the study alongside Tianhua He. Haylee D'Agui collected and prepared experimental materials with assistance from Tianhua He. Haylee D'Agui, Sim Lin Lim and Tianhua He analysed the data. Haylee D'Agui, Tianhua He and Neal Enright drafted the manuscript. Tianhua He coordinated the publication process, with assistance with revisions from Haylee D'Agui, Neal Enright, Sim Lin Lim and Yiqi Luo.

5.2 Introduction

Mediterranean type ecosystems (MTEs) are among the most biologically diverse terrestrial ecosystems globally and are thought to be highly vulnerable to species loss under global change (Cowling *et al.* 1996; Myers *et al.* 2000; Sala *et al.* 2000). Both drought and fire play an important role in shaping the structure and composition of MTE vegetation, as the distribution and abundance of plant species is determined primarily by their ability to tolerate water stress and extreme temperatures in the summer, and to re-establish themselves after disturbance, usually from fire. Significant climate trends of warming and drying are already evident across the world's MTEs, raising concerns about the consequences for their diverse floras (Enright *et al.* 2015). Moreover, climate change is redefining management strategies and conservation goals and concepts (McDonald-Madden *et al.* 2011).

Malcolm *et al.* (2006) identify South-west Australia (SWA) and the Cape Region of South Africa as two of the most vulnerable MTE regions globally, potentially losing more than 2,000 plant species each over the next 100 years in the face of climate change. The climate of SWA has undergone dramatic change since the mid-1970s, with annual rainfall decreasing by 30% and mean maximum temperature increasing by 0.15–0.20 °C per decade (Hope *et al*. 2006; Bates *et al*. 2008). Global climate models project a further temperature increase of 1–3 °C across all seasons of the year, a further 10–20% reduction in rainfall (largely in winter), and a higher frequency of extreme events such as droughts. In a bioclimatic envelope modeling analysis, Fitzpatrick *et al*. estimated that up to 25% of *Banksia* species (Proteaceae) were projected to become extinct by 2080 (Fitzpatrick *et al*. 2008). Similarly, Yates *et al*. simulated the impacts of climate-change scenarios on *Banksia* species distributions and reported an increased risk of decline for all species (Yates *et al.* 2010). More recently, Urban has predicted that 14% of native species in Australia and New Zealand will become extinct by 2100 if the current trend of climate change continues (Urban 2015).

However, the validity of these sobering extinction predictions is uncertain as critical gaps remain in our knowledge of the intrinsic capacity of species to respond to climate and other environmental changes. For instance, species may be able to adapt *in situ* to new climatic conditions based on genetic variation within populations and plasticity, for example; most species can persist outside their natural range (Butt *et al*. 2013), albeit under altered competition contexts. Recently, attempts to predict the impacts of climate change on biodiversity have moved beyond species-level models and toward a

greater consideration of intraspecific variations in tolerances and adaptation (Jump & Peñuelas 2005; Jay *et al*. 2012). Broad niche breadth and higher adaptive genetic variation could buffer genotypes from the immediate effects of climate and environmental change (Lee & Mitchell-Olds 2012). To effectively assess the responses of a species to climate change, we need to understand both the current levels of adaptation within a species and its future adaptive potential (Cochrane *et al.* 2015). We therefore need to know the level of adaptive genetic variation in extant populations and the variation associated with adaptation to components of climate and other environmental conditions.

SWA is also one of the most fire-prone regions in the world (Abbott & Burrows 2003), and plants here display remarkable adaptations to recurrent fire (Keeley *et al*. 2011). Although the expected interaction with climate change is complex, a projected hotter and drier climate with more high fire danger days will likely lead to more fire, and so, shorter fire intervals (Enright *et al.* 2012). However, other human activities also play an important role in determining fire regimes. Both historical accounts and evidence from current land-use practices support the argument that Aboriginal peoples used fire as a land management tool over the past 50,000 years before the European settlement of Australia, with increased occurrence of fire under their land management in areas with high resource availability (Enright & Thomas 2008). Since the 1950s, the managed use of fire to reduce fuel loads in public estate vegetation types has been the major strategy employed by government agencies in Australia to mitigate the risk of fire spreading into private lands (Esplin *et al.* 2003; Burrows & McCaw 2013). Such altered fire frequency (shortened fire intervals) is an important component of environmental change and has been implicated in shifts in community structure (Pekin *et al.* 2009), species loss, and invasions. Experimental studies show mixed results, with overall species richness adversely affected in shrublands (Enright *et al.* 1998) but not in wetter forests burned at 3–5-year intervals (Burrows & Wardell-Johnson 2003), whereas the abundance of specific plant functional types (for example; obligate seeding shrubs) was significantly reduced in both.

Plants may respond to climate and environmental changes either by persisting *in situ* through tolerating and/or adapting to the changes, or by migrating to suitable habitats if possible. Recent research has shown that many Australian plant species have the capacity to disperse their seeds over long distances, especially after fire (He *et al*. 2009a; He *et al*. 2009b; He *et al.* 2010; Merwin *et al.* 2012). However, in at least the past 700,000 years (Byrne 2007), and possibly since the mid-Pliocene (Dodson &

Macphail 2004), most Australian species seem to have persisted through major climatic changes in localised habitats rather than by moving long distances. Species may be able to retreat to nearby refugia in the face of climatic and other types of environmental change, thereby allowing them to persist locally (Tapper *et al.* 2014; Keppel *et al*. 2015). This pattern emphasises the importance of maintaining an adaptive life-history trait set with adequate genetic variation in populations so that species can persist through changing conditions (Blows & Hoffmann 2005).

Investigating adaptive genetic variation may reveal the role of genetic diversity in buffering species and communities against the effects of changing climate (Reush & Wood 2007; Ouburg *et al*. 2010). Variations in neutral genetic markers (for example; microsatellite DNAs) have traditionally been used as indicators of the evolutionary potential of wild populations. However, recent studies have questioned the usefulness of molecular indices of neutral genetic variability as surrogates of the evolutionary potential of natural populations, as these markers are generally not under selection (He & Lamont 2010), though such a view overlooks that presently neutral variation may become adaptive if new selection pressures emerge (Barrett & Schluter 2008). Nevertheless, the challenge now is to identify whether species harbor sufficient adaptive genetic capacity (Jump & Peñuelas 2005; Hoffmann & Sgrò 2011). In plants, the functional traits linked to phenology, growth and stress resistance are shaped by selection along environmental gradients (in space and time). Those functional traits exhibiting sufficient genetic variation are expected to facilitate rapid evolutionary adaptation to climate change (Savolainen *et al.* 2007). Given rapid climate change, the immediate adaptation of populations to recruitment and growing conditions must rely on this existing genetic variation, as these variations were selected over many generations and are capable of providing immediate adaptive value to the population when facing rapid environmental changes (Barrett & Schluter 2008). Research is now emerging that takes genetic adaptation and evolutionary capacity into account in predictions of species or ecosystem responses to climate change (Savolainen *et al.* 2007; Steane *et al.* 2014; Kovach *et al*. 2015).

The rapidly falling costs of next-generation sequencing are now enabling the genome-wide characterisation of adaptive genetic variation, which offers unprecedented power to identify the loci that mediate local adaptation (Bragg *et al.* 2015). Recent research has demonstrated the value of single-nucleotide polymorphisms (SNPs) in detecting selection -and adaptation-related candidate genes (Steane *et al*. 2014; Yoder *et al.* 2014; Bragg *et al.* 2015; Kovach *et al*. 2015). SNPs have clear advantages for

accommodating models of evolutionary change and their potential roles in functional evolution. By screening large numbers of SNPs, genome-scale studies open the possibility of identifying loci that mediate fitness in different environments and contribute to local adaptation (Vitti *et al.* 2013). Restriction-site-associated DNA sequencing (RAD-seq) combines enzymatic fragmentation of the genome with high-throughput sequencing to generate large numbers of SNP markers (Baird *et al.* 2008). This process enables large-scale studies of genomic variation in species lacking a reference (Reitzel *et al*. 2013).

Banksia attenuata (Proteaceae) is one of the most prominent and widespread woody plants in SWA. It occurs in semi-arid shrubland to mesic forest and is highly resilient to fire, recovering by resprouting from its trunk or base. Here, we utilize RAD-seq to screen large numbers of SNPs and characterise adaptive genetic diversity in *B. attenuata* populations spanning a broad range of precipitation, temperature, and fire regimes (mean fire intervals) in SWA. Our approach detects SNPs that show concordant differences in allele frequencies across populations with respect to specific local climates and fire regimes. We screened those genetic variations that are putatively associated with genes under directional selection and then used environmental association analysis to identify putative genes associated with adaptation to specific precipitation levels, temperatures, and fire intervals. Finally, we annotated those genes for their potential biological function. Our study attempts to answer the critical question of whether those alleles that confer adaptation to local climate factors and fire regime occur globally with varying frequencies or whether they are highly localised in specific populations. Our research quantifies genetic variations associated with adaptation to climate and fire regimes, identifies geographic regions that are predicted to be most sensitive to the disruption of current patterns of local adaptation under climate change, and provides critical insight into the evolutionary potential of further climate change for an iconic species in SWA.

5.3 Materials and Methods

5.3.1 Species and sampling

Banksia attenuata (Proteaceae) is a member of the iconic Australian genus *Banksia*, an important element of the flora, with over 180 species in SWA. This species forms an important component of open *Eucalyptus* and *Banksia* woodlands and shrublands as a dominant or understory tree or tall shrub. The distribution of *B. attenuata* spans a wide climate and environmental range, and it is the most widely distributed of all western banksias. *Banksia attenuata* is found across much of SWA west of the 400 mm isohyet, with a few populations penetrating slightly east into areas with less than 400 mm annual rainfall, through to the west coast of WA, north to Kalbarri National Park (with an annual rainfall less than 400 mm), south to Cape Leeuwin (>1000 mm annual rainfall) and across to the Fitzgerald River region (Figure 5.1). This species has an evolutionary history of ~19 million years and is one of the oldest members of the extant *Banksia* (He *et al.* 2011), implying a historically strong capacity to adapt to climatic and environmental changes. Individuals of *B. attenuata* are estimated to live for 300 years or more (Enright & Lamont 1992) and can disperse seeds up to at least 2.6 km in a single dispersal event (He *et al*. 2009a).

Up to ten individuals of *Banksia attenuata* were randomly sampled from each of nine locations across its range in SWA (Table 5.1; Figure 5.1). These nine locations span a rainfall gradient from 330 mm to over 1000 mm and cover three major vegetation types (shrubland, woodland, and forest). Long-term weather observations (since the 1930s) show a marked decline (15%–30% reduction) in annual rainfall since 1975 in seven of the nine locations (Figure 5.2a), and a 0.5–1 °C increase in mean temperature in the hottest month (February) in all nine locations compared with pre-1975 (Figure 5.2b; Bureau of Meteorology 2016). Historical fire intervals range from ~10 years in the north to 140 years in the south (Figure 5.3). Data for contemporary fire intervals indicate major changes in the southern (shortened fire intervals) but little change in the northern parts of the species' geographic range (Figure 5.3; data for fire intervals from Burrows *et al*. 1995; Hobbs 2002; Hassell & Dodson 2003; Enright & Thomas 2008; Parsons & Gosper 2011; Enright *et al.* 2012).

Table 5.1: Locations and long-term climate of the sampled *Banksia attenuata* populations.

Location	Habitat	Annual Rainfall (mm)	Fire interval (years)		T-max[a] °C	T-ext[b] °C
			Historical	Contemporary		
Kalbarri (KB)	shrubland	345	15	15-22	34.3	47.2
Leda (LD)	shrubland	491	13-21	13-21	36.4	48.7
Eneabba (EN)	shrubland	510	13-21	13-21	34.8	46.9
Yanchep (YC)	woodland	739	40	12	33.3	46.3
Goomalling (GM)	woodland	366	63	44-66	34.9	46.9
Brunswick (BW)	forest	987	>100	15-20	33.1	43.6
Arthur River (AR)	forest	431	>65	15-20	31.1	44.0
Cape Leeuwin (LU)	forest	1001	>81	15-20	27.2	40.6
Fitzgerald River (FR)	shrubland	385	50-140	22	28.7	45.0

a: mean maximum temperature of the hottest month (February); b: hottest temperature recorded.

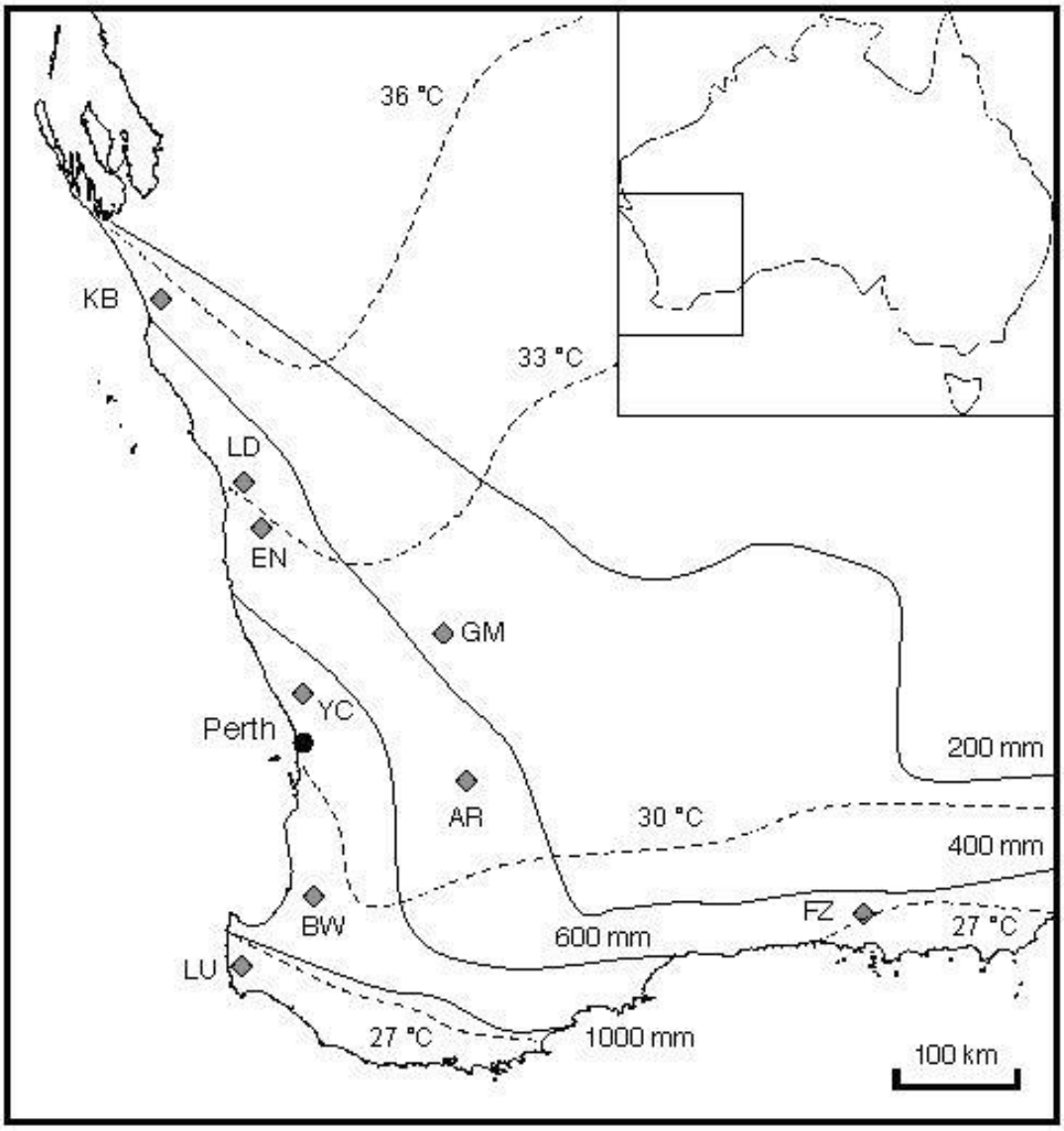

Figure 5.1: Locations of the sampled populations of *Banksia attenuata* in Western Australia. KB-Kalbarri National Park, LD-Leda Nature Reserve/Beekeepers Nature Reserve, EN-South Eneabba Nature Reserve, YC-Yanchep National Park, GM-Goomalling road reserve, BW- Brunswick Junction road reserve, AR- Arthur River road reserve, LU- Leeuwin Naturaliste National Park, FR- Fitzgerald River National Park. Continuous lines indicate annual rainfall isohyets, and broken lines indicate isotherms of average temperatures for February. Annual rainfall and temperature data represent a 30-year average (1980–2010) and are from the Australian Bureau of Meteorology. Map is adapted from an outline map from The University of Melbourne Library Map Collection (http://www.lib.unimelb.edu.au/collections/maps).

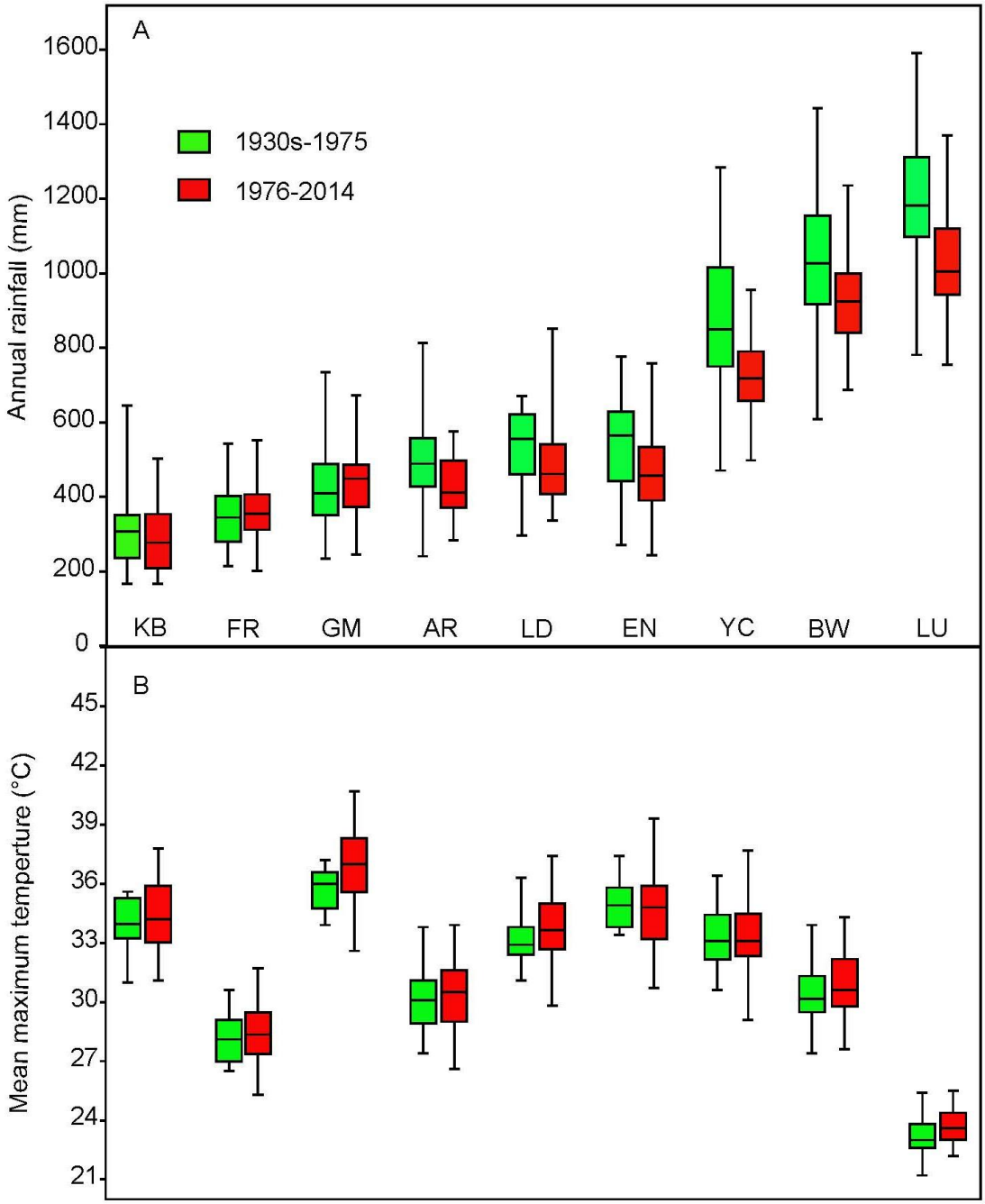

Figure 5.2: Change in annual rainfall and mean maximum summer temperature (February) since 1975 at nine sampling locations. Data are from the Australian Bureau of Meteorology.

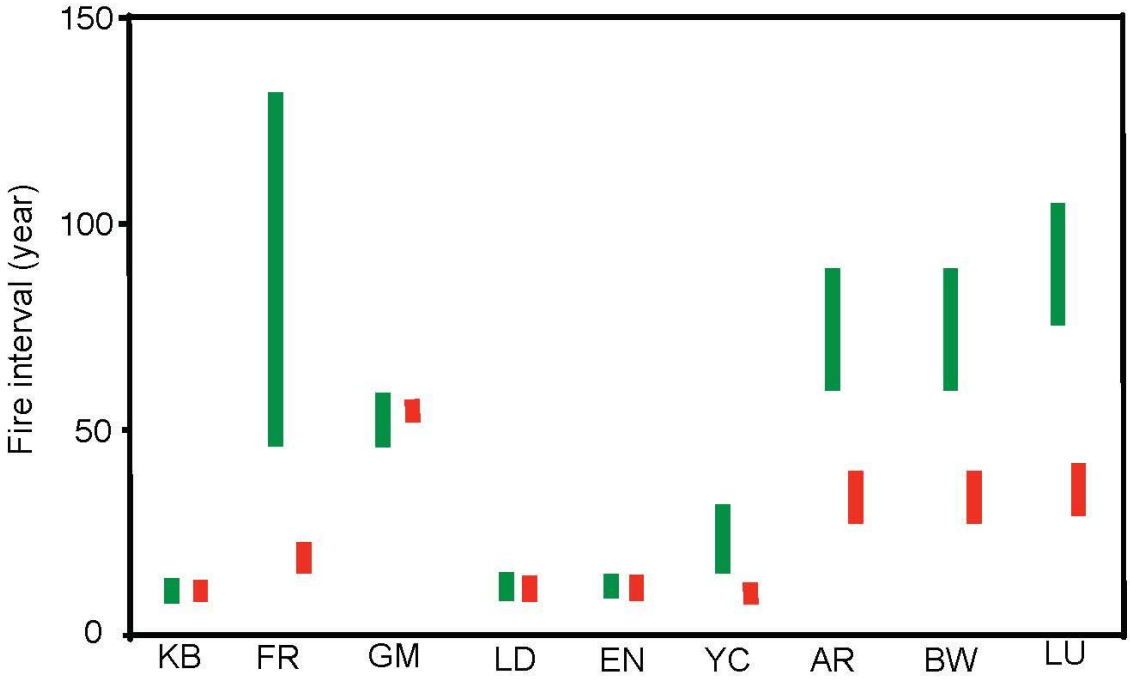

Figure 5.3: Changes in fire regime at nine sampling locations. Green bars indicate variations in historical fire intervals; red bars indicate the contemporary interval. Data compiled from Burrows *et al*. 1995, Hobbs 2002, Hassell & Dodson 2003, Enright & Thomas 2008, Parsons & Gosper 2011, and Enright *et al.* 2012.

5.3.2 RAD-seq and SNP discovery

Genomic DNA from each individual was extracted and then fragmented by the corresponding enzyme (EcoRI, recognition site: 5'-G/AATTC-3'). EcoRI is a frequent cutter, resulting in the detection of more markers in RAD sequencing. For library construction, two 100 bp single-end sequencing libraries were constructed using the eight-nucleotide multiplex identifiers. Each library contained five individual samples. Each sample was assigned to a unique molecular identifier (MID) barcode. The RAD products from the 80 plants were processed on an Illumina HiSeq2000 platform (Illumina Inc., San Diego, CA, USA) at Beijing Genomics Institute (Shenzhen, China). Sequencing data were segregated by individual specific MID. Reads from each plant were clustered into tag reads by sequence similarity (allowing two mismatches at most between any two reads within each tag reads cluster). To ensure quality, the raw data were modified by the following two steps: first, the adapter pollutions and index sequence in the reads were deleted, and then the reads which contained more than 50% low-quality bases (quality value ≤ 5) were discarded.

Because there is no reference genome available for *Banksia* or closely related taxa, a *de novo* RAD reference genome was constructed. Reitzel *et al*. demonstrated that the results from analyses with and without a reference genome detect similar sets of SNPs (Reitzel *et al*. 2013), highlighting that RAD-seq can be efficiently applied to species lacking existing genomic resources. RAD-seq reads from 20 randomly chosen samples were used for *de novo* assembly. At the initial step, pair-reads were collapsed into RAD sequence clusters if the SE (Single End; first RAD tag) shared 100% sequence identity across the Illumina reads. The RAD sequence cluster was set at a range of 50–750× to maximise the efficient assembly of sequences. The paired-end sequences corresponding to the selected SE were extracted for further assembly. The selected pair-end reads were input into the Velvet sequence assembler, and k-mer 40 was used for *B. attenuata* contig assembly (Hassell & Dodson 2003). Assembled contigs less than 200 bp were excluded from further analysis.

The final filtered RAD reference genome assemblies should thus represent single-copy genomic sequences. Reads for each individual were then aligned to the RAD reference using BOWTIE (Zerbino *et al.* 2008; Langmead *et al.* 2009), again using sequence quality information and allowing a two mismatch maximum as well as permitting alignment to no more than one reference region per read. SAM Tools were used to convert Bowtie alignments into BAM and pileup files for SNP identification (Li *et al.* 2009). Sequence variants from the pileups were then condensed into a variant call format (VCF) file. For an SNP to be recognised, it had to appear in all sequenced samples.

5.3.3 Analysis of adaptive genetic variation

To assess the presence and extent of adaptive genetic variation in populations, we began by asking how many SNP loci have diverged under selection in *B. attenuata*. A locus under balancing selection should show uniform allele frequencies across populations, whereas loci under local directional selection should show large differences among populations. We used the F_{st}-outlier approach to detect SNP loci that are putatively under selection, and these markers were expected to reveal a signal of adaptive variation related to local climate and environment (Eveno *et al*. 2008; Dillon *et al.* 2014). F_{st}-outlier identification followed the approach of hierarchy modeling and coalescent simulation (Eveno *et al*. 2008) and was implemented in Arlequin v3.5 (Excoffier *et al.* 2009; Excoffier *et al* 2010). Briefly, coalescent simulations were used to obtain a null distribution and confidence intervals around the

observed values and to determine whether observed locus-specific F_{st} values could be considered as F_{st} outliers conditioned on the globally observed F_{st} value. The populations in our samples were separated by large geographic distances and could be considered as independent units, which minimises false positives in hierarchy modeling and coalescent simulation (Excoffier *et al.* 2010). Using a stringency of p= 0.01, we categorized SNPs into three groups: SNPs under directional selection, those under balanced selection, and neutral SNPs. For each subset of SNPs, population genetic variation (percentage of polymorphic loci, PPL; expected heterozygosity H_e) and pairwise population differentiation (F_{st}) were estimated in Arlequin v3.5 (Excoffier *et al.* 2010). To test isolation by distance, a correlation analysis was performed between pairwise population differentiation F_{st}, as estimated from SNPs that were detected under directional selection, F_{st} estimated from those SNPs other than under directional selection, and geographical distance (transformed using the logarithm function).

To investigate whether climate change (i.e., declining rainfall and rising temperature) since ~1970 and changes in fire interval have impacted the level of adaptive genetic diversity in each population, we used multiple linear regression with adaptive genetic diversity (expected heterozygosity, H_e) as the dependent variable, and change in climate (annual rainfall and average temperature in the hottest month, usually February) and fire interval as independent variables. Climate change was quantified as [Mean (post-1975) − Mean (pre-1975)] / [Mean (post-1975) + Mean (pre-1975)]. Change in fire interval was based on the historical versus contemporary intervals (Table 5.1) and used the same formula. Multiple Linear Regression was performed in PAST (Hammer *et al.* 2001), with a Bonferroni correction employed for multiple comparisons. A multidimensional scaling ordination, drawn using PAST (Hammer *et al.* 2001), was used to illustrate associations among variables.

The subset of SNPs putatively under directional selection was further used to detect specific candidate genes associated with adaptation to annual precipitation, temperature (mean maximum temperature of hottest month, and hottest temperature), solar exposure, and historical fire intervals using environmental associations (Hancock *et al*. 2008; Coop *et al.* 2010). Association with contemporary fire intervals was not examined because *B. attenuata* is a long-lived plant with a long generation time (the sampled individuals were adult trees and likely more than 100 years old). We employed a Bayesian method that estimates the empirical pattern of covariance in allele frequencies between populations from a set of markers and then uses this as a

null model for a test of individual SNPs (Coop *et al.* 2010). The Bayesian method uses environmental correlations to identify underlying local adaptation of loci and largely overcomes problems of differences in sample sizes and the neutral correlation of allele frequencies across populations due to shared history and gene flow (Coop *et al.* 2010). Both simulation and empirical datasets suggest this approach is very useful for identifying selected loci via their correlation with environmental variables and can be applied to continuous or discrete environmental variables (Coop *et al*. 2010). Analysis of environmental association was implemented in *Bayenv* (http://gcbias.org/bayenv/). Only one SNP locus from the same contig was used for environmental association.

5.3.4 Annotation of genes putatively associated with rainfall and fire regime

We further investigated the current functional annotation and classification of the candidate genes for local adaptation as revealed in the above environmental associations. The *de novo* assembled contigs of *B. attenuata* were used for BLASTN searches and annotation against an annotated *B. hookeriana* transcriptome assembly (obtained from http://www.ncbi.nlm.nih.gov) using an E-value cut-off of 10^{-10} (Expect Value; E-value < 10^{-10}; Altschul *et al*. 1990). *B. hookeriana*-annotated transcripts' information (Nr protein database similarity, GO annotation and KEGG pathway annotations) was applied to *B. attenuata* contigs if they were matched against each other.

5.4 Results

RAD sequencing coverage averaged 16 million reads and 1.53 GB of data per individual for each of the 80 individuals of *B. attenuata* sampled across the nine locations. One sample with 2.1 million reads was discarded due to its low coverage. The average quality score (Q20) was 99.4%, with the lowest being 99.1%, suggesting very high quality for the obtained sequences. The final RAD reference genomes generated from the RAD sequences of twenty individuals using *de novo* assembly contained 241,259 contigs, with N_{50} = 264 and a total length of 63.6 million base pairs. The RAD reference genome had GC contents of 38.0%.

A total of 9,887 SNPs passed initial filters in our variant calling approach. We identified 5,701 SNPs that existed in all 80 sampled individuals. Overall, 71.9% of the SNPs were bi-allelic, 27.5% were tri-allelic, and 0.5% contained more than three alleles. Applying a stringency of p = 0.01, the F_{st}-outlier approach using a hierarchical population model

and coalescent simulations identified 560 SNPs (9.8%) putatively under directional selection (Figure 5.4).

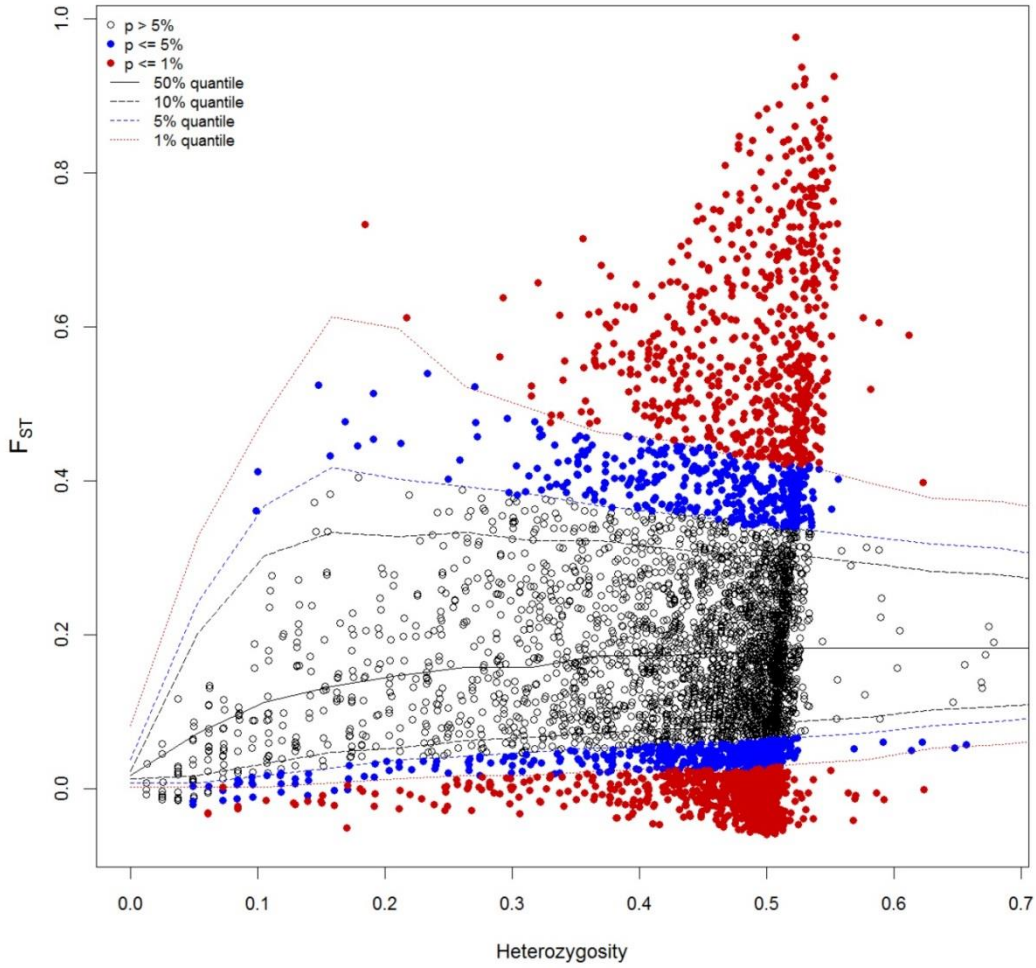

Figure 5.4: Detection of loci (SNPs) under selection from genome scans using the F_{st}-outliers approach.

Adaptive genetic diversity, as measured by the 560 SNPs putatively under directional selection, showed considerable differences among populations. PPL varied from 28% in the population at FR to 81% at GM, with an average of 59%, and expected heterozygosity H_e ranged from 0.12 at FR to 0.36 at GM and averaged 0.21 (Figure 5.5). Population genetic diversity measured by the rest of the SNPs (neutral or possibly under balanced selection) was uniform across all populations other than population FR, which showed lower PPL and H_e (Figure 5.5). Multiple Linear Regression analysis suggested that the level of adaptive genetic diversity (H_e) in each population was largely determined by the change in fire interval (R^2 = 0.734, p = 0.003) and not by change in local climate (R^2 = 0.004, p= 0.982 for rainfall; R^2 = 0.063, p = 0.264 for high temperature; Figure 5.6). The five populations with lower H_e (YC, BW, AR, LU, FR) have

much more frequent contemporary fires compared with historical fires, whereas fire intervals in the other four populations have not changed significantly (Figure 5.3).

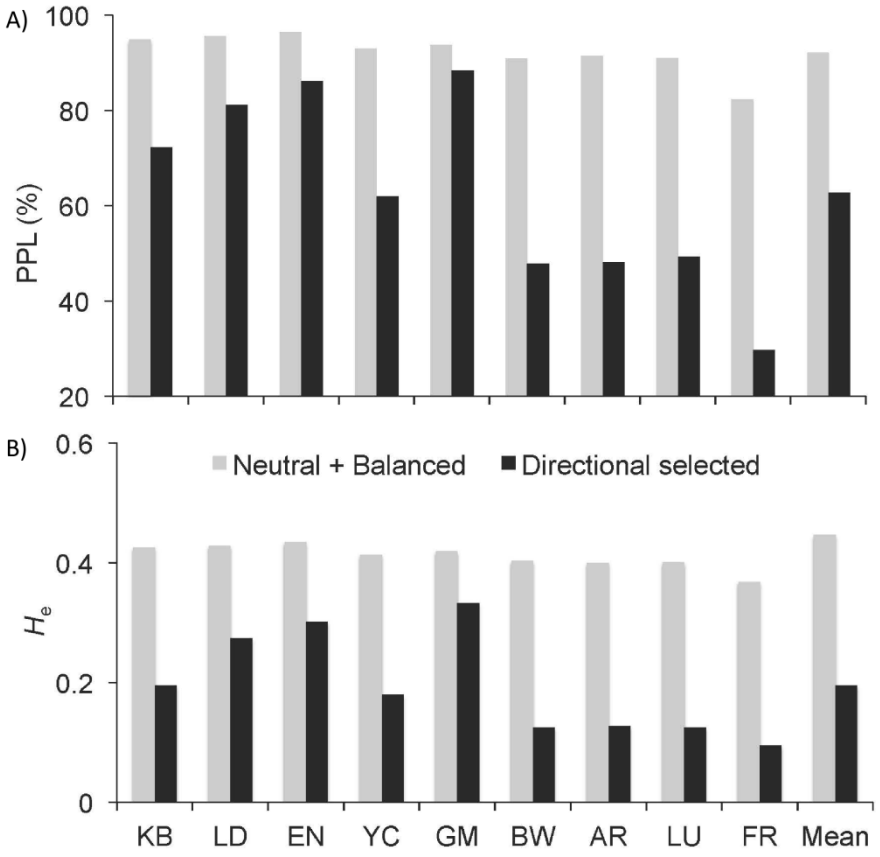

Figure 5.5: Genetic variation in nine populations of *Banksia attenuata* putatively under balanced or neutral selection, or directional selection, as measured by SNPs: A) percentage of polymorphic SNPs; B) expected heterozygosity.

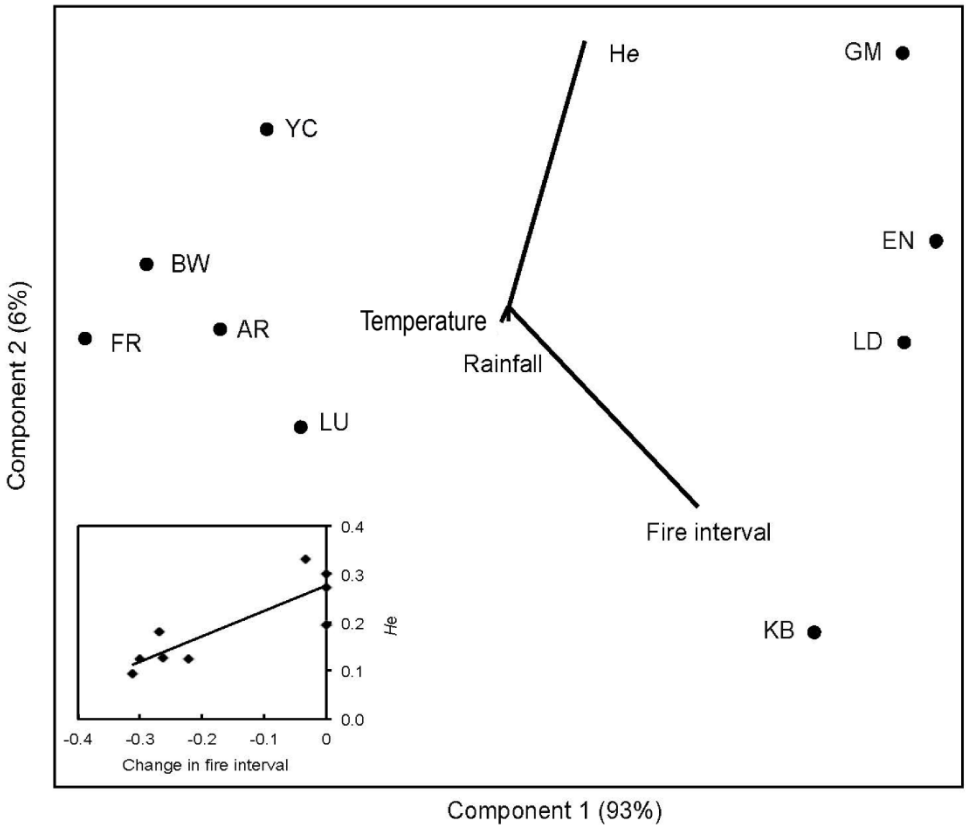

Figure 5.6: A multidimensional scaling ordination of adaptive genetic variation in nine populations of *Banksia attenuata*, with effects of changes in local climate and fire interval overlaid as correlated environmental vectors.

For the 5,141 neutral loci, population pairwise F_{st} values ranged from 0.003 to 0.342 and averaged 0.113. Populations are significantly differentiated, with all pairwise F_{st} values statistically greater than zero. SNPs putatively under directional selection revealed much greater genetic differentiation among populations, with pairwise F_{st} values ranging from 0.086 to 0.809 and averaging 0.438. For both measures, the geographic distance between populations has contributed to genetic differentiation, with differentiation increasing with increasing geographic distance between populations (Figure 5.7).

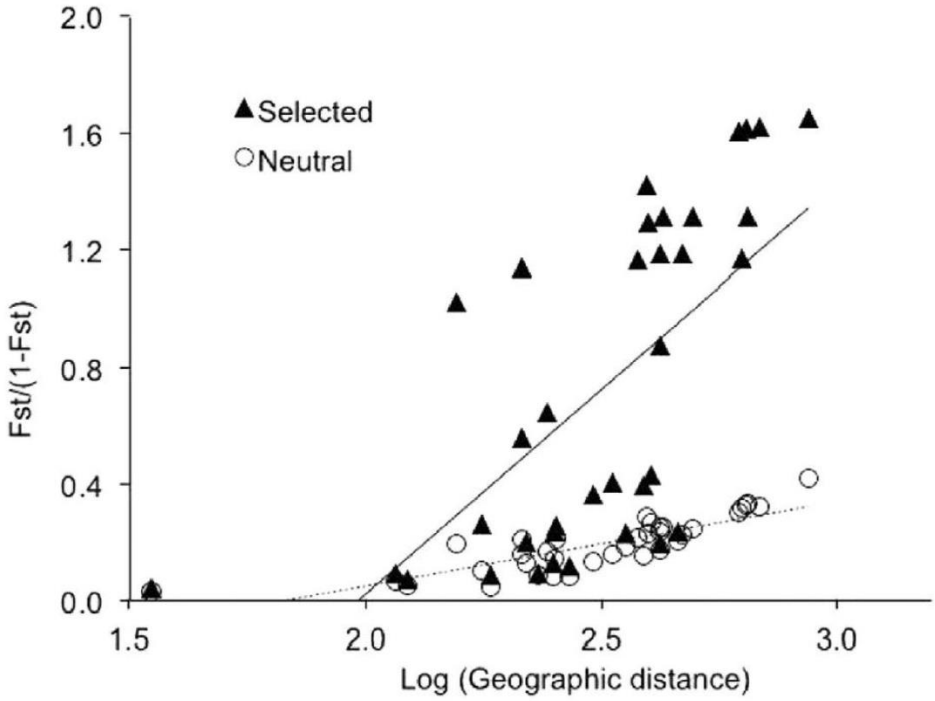

Figure 5.7: Correlation of pairwise population genetic distance and geographic distance among nine *Banksia attenuata* populations in SW Australia.

Environmental correlation using *Bayevn* identified 25 SNPs as significantly associated with rainfall gradients, 18 with maximum temperature, 37 with extreme high temperature, and 11 with historical fire intervals. A total of 14 SNPs are associated with at least two climate factors associated with temperature and solar exposure, whereas only one SNP was associated with rainfall and temperature. SNPs associated with historical fire regime were also specific, with only one of 25 associated with high temperature and one with rainfall.

The majority of the SNPs associated with rainfall, maximum temperature, and extreme temperature were not fixed in one allele in a specific population, whereas SNPs associated with specific fire intervals were mainly fixed in one allele (Figure 5.8). A total of 20% of SNPs for rainfall and 17% for maximum temperature and extreme temperature together were fixed at one allele in a specific population, whereas overall, 34% of the SNPs that were associated with specific fire intervals were fixed in specific populations. In the population at FR, 10 out of the 11 SNPs were fixed in one allele, with the remaining SNP severely skewed toward one allele (with a frequency of 0.83).

Aligning the results here with the annotated *Banksia hookeriana* leaf transcriptome revealed a total of 16 candidate genes with biological functions, including four

candidate genes with functions for adaptation to rainfall, 16 to high temperature and solar exposure, and two to fire intervals (Table 5.2). Among these, the malate dehydrogenase (MDH) gene was identified as important in adaptation to both rainfall and high temperature. The sphingosine-1-phosphate phosphatase (S1P) gene was linked to adaptation to rainfall, and the 5' AMP activated protein kinase (AMPK) gene was identified as important in adaptation to high temperature. For fire intervals, the multi-functional guanine nucleotide exchange factor (GEF) gene was one of the most important candidate genes.

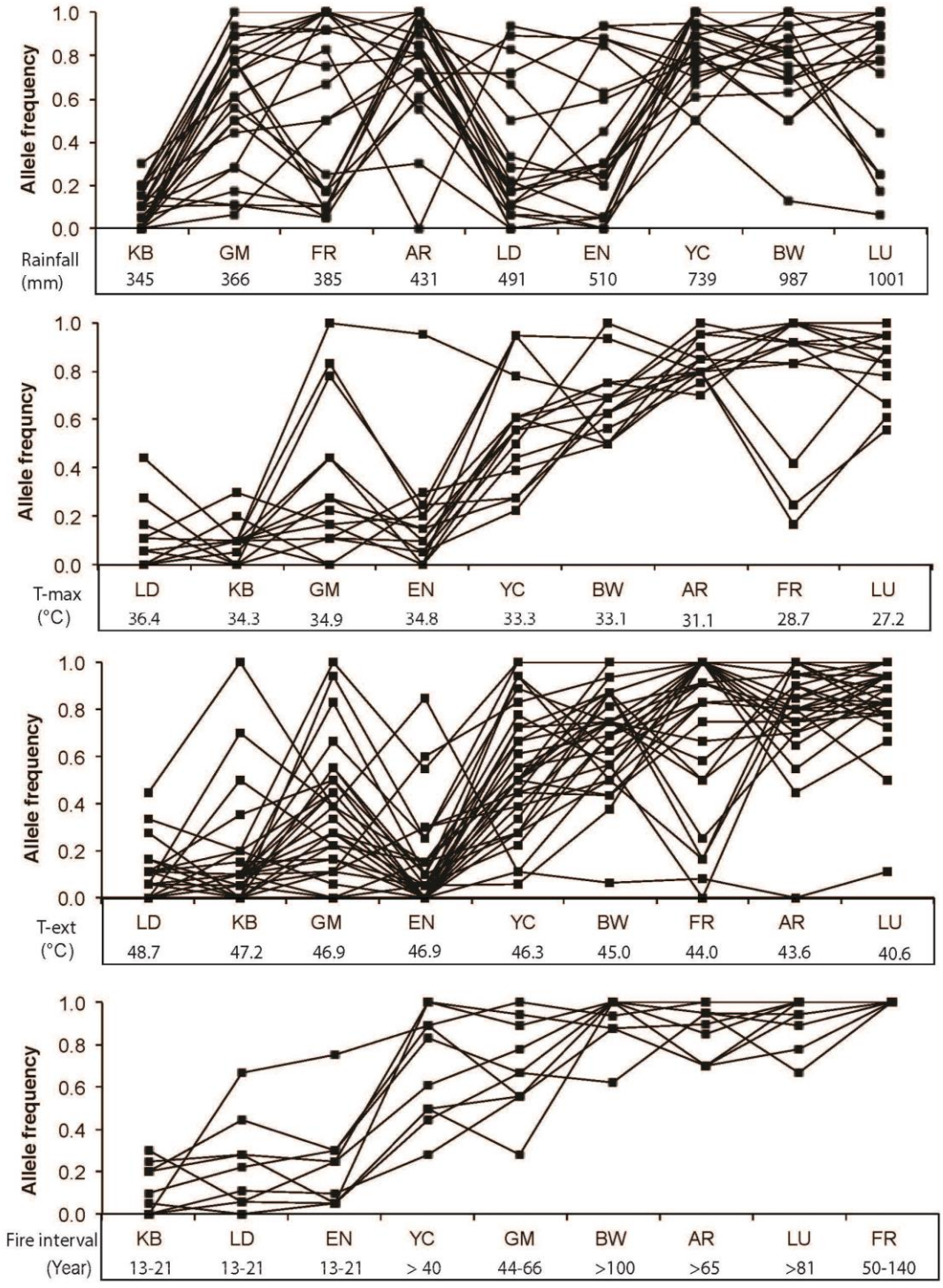

Figure 5.8: Distribution of allele frequencies for SNPs associated with climate and fire intervals in nine populations of *Banksia attenuata*. Note: only one allele per SNP loci is plotted.

Table 5.2: SNPs and corresponding candidate genes for *Banksia attenuata* from 9 populations in SWA that have been annotated and whose molecular function and biological processes have been identified.

A. Related to rainfall

Name	Bayes Factor	KEGG-related Pathway	Biological Process	Molecular Function
SNP066	3.6			ATP-dependent DNA helicase HFM1/MER3 [EC:3.6.4.12]
SNP086	3.7		Methionine biosynthetic process Proteolysis RNA splicing	Lysosomal ProX carboxypeptidase [EC:3.4.16.2] Carboxypeptidase activity Erine-type peptidase activity
SNP120	7.8	Metabolic pathways Biosynthesis of secondary metabolites Pyruvate metabolism Carbon fixation in photosynthetic organisms Glyoxylate and dicarboxylate metabolism Citrate cycle (TCA cycle)	Response to salt stress Defense response to bacterium Response to cold Malate metabolic process Tricarboxylic acid cycle Cellular carbohydrate metabolic process Response to cadmium ion	Malate dehydrogenase [EC:1.1.1.37] Copper ion binding L-malate dehydrogenase activity Nucleotide binding
SNP246	3.0		Sphingolipid metabolic process Stomatal closure Response to abscisic acid stimulus	Sphingosine 1-phosphate phosphatase activity

B. Related to adaptation to temperature

Name	T-max	T-ext	KEGG-related Pathway	Biological Process	Molecular Function
	Bayes Factor				
SNP036	3.0	3.1			Proteasome component ECM29
SNP108	1.9	3.8	RNA transport	Negative regulation of biological process; Protein import into nucleus; Organ morphogenesis; Xylem and phloem pattern formation; Flower morphogenesis; Determination of bilateral symmetry; Protein targeting to chloroplast.	Nuclear pore complex protein Nup62; Transmembrane signaling receptor activity; Hydrolase activity; Acting on acid anhydrides.
SNP120	0.8	3.1	Metabolic pathways; Biosynthesis of secondary metabolites; Pyruvate metabolism; Carbon fixation; Glyoxylate and dicarboxylate metabolism; Citrate cycle (TCA cycle).	Response to salt stress; Defense response to bacterium; Response to cold; Malate metabolic process; Tricarboxylic acid cycle; Cellular carbohydrate metabolic process; Response to cadmium ion.	Malate dehydrogenase [EC:1.1.1.37]; Copper ion binding; L-malate dehydrogenase activity; Nucleotide binding.
SNP214	1.3	4.3		Protein ubiquitination; Protein phosphorylation;	Interleukin 1 receptor-associated kinase 4 [EC:2.7.11.1]; ATP binding;

					Ubiquitin protein ligase activity; Protein serine/threonine kinase activity.
SNP230	2.8	3.1			Structure specific endonuclease subunit SLX1 [EC:3.6.1.].
SNP238	1.0	8.4			tRNA (cytosine 38C5) methyltransferase [EC:2.1.1.204].
SNP303	5.2	1.7		Cellular response to glucose starvation; Protein autophosphorylation.	5'AMP activated protein kinase; Regulatory gamma subunit; Protein binding; Protein kinase activator activity; Protein serine/threonine kinase activity.
SNP306	0.7	5.4		Borate transmembrane transport; Response to boron-containing substance.	Inorganic anion exchanger activity; Borate efflux transmembrane transporter activity.
SNP311	5.3	12.0	Metabolic pathways; Pyrimidine metabolism.	DNA dependent transcription; Nucleotide phosphorylation.	UMPCMP kinase [EC:2.7.4. 2.7.4.14]; ATP binding; Nucleoside triphosphate adenylate kinase activity; Nucleotide kinase activity.
SNP348	3.2	6.3		Embryo development ending in seed dormancy; Microtubule cytoskeleton organization.	Transferase activity.

SNP355	3.7	1.0	Metabolic pathways; Biosynthesis of secondary metabolites; Stilbenoid; Diarylheptanoid and gingerol biosynthesis; Limonene and pinene degradation.		
SNP412	2.3	7.7	Circadian rhythm plant.	Blue light signaling pathway; Proteasomal protein catabolic process; Response to red light; Regulation of transcription; Flower development.	Clock associated PAS protein ZTL; Blue light photoreceptor activity.

C. Related to (historical) fire interval

Name	Bayes Factor	Function-Description	Biological Process	Molecular Function
SNP004	4.81	SEC7 domain proteins.	Seed maturation; Endosome transport via multi-vesicular body sorting pathway; Actin nucleation; Lateral root formation; Regulation of chromosome organization; Proteasomal protein catabolic process; Regulation of vesicle targeting to, from or within Golgi; Floral organ formation; Basipetal auxin transport; Unidimensional cell growth.	Guanine nucleotide-exchange factor; GTP: GDP antiporter activity; ARF guanyl-nucleotide exchange factor activity; Protein homodimerization activity.
SNP361	3.06	Cytochrome P450.	Oxidation-reduction process.	Heme binding; Iron ion binding; Electron carrier activity; Aromatase activity.

5.5 Discussion

5.5.1 Frequent fire depletes adaptive genetic diversity in Banksia attenuata

Our results suggest that altered fire regime, and particularly shortened fire intervals in some parts of SWA, has had a significant impact on the level of adaptive genetic variation in populations of the widespread and abundant *Banksia attenuata*. The five populations in the southern forests of SWA all show a much lower adaptive genetic variation (H_e) as measured by 560 SNPs putatively under directional selection than those of populations from the northern part of the species' geographic range. Principal components analysis suggests that the change in fire interval was the main driver of decline of adaptive genetic variation in *B. attenuata* populations. Recurrent fire has been a major evolutionary force in the evolution of terrestrial plants for at least 100 million years (He *et al*. 2011; Keeley *et al*. 2011; Lamont & He 2012). However, plants are not adapted to fire *per se* but, rather, to specific fire regimes that include fire frequency, fire intensity, and patterns of fuel consumption (Keeley *et al.* 2011). It has been argued that increasing fire frequency intensifies the selection on plant species in these environments (Keeley *et al.* 2011). This effect has likely led to depletion of adaptive genetic variation in some *B. attenuata* populations in SWA, where fire occurrence has increased as a result of human influence, but not in others where historical fire regimes remain largely intact.

Multiple lines of evidence support the idea of dramatic changes in fire interval in the southern forests of SWA. Burrows *et al*. reported a mean fire interval of approximately 80 years for tree-scarring fires in jarrah (*Eucalyptus marginata*) forests of SWA in the pre-European period (Miller *et al.* 2007). More recently, the fire interval has decreased due to a combination of more frequent wild fires and regular prescribed burning, which is implemented in this region for a range of land management objectives (Burrows & McCaw 2013). Hobbs also posits a change in fire regime from infrequent (~50 years) to frequent (6–8 years) following the European settlement in temperate banksia woodlands of SWA (Dodson *et al.* 2005). Aboriginals most likely did not permanently occupy the semi-arid shrublands of Fitzgerald River National Park in the pre-European period due to food resource limitations, and analyses of charcoal from sediment cores indicate intervals between major fires of 50 to 140 years (Miller *et al.* 2007). Similarly, kwongan vegetation in northern SWA is low in resource availability from the perspective of Aboriginals and would not have warranted regular occupation and "management" using fire (Enright & Thomas 2008). Field observations and analysis of

satellite imagery over the past 40 years suggest that fire intervals in kwongan vegetation average approximately 13 years (Miller *et al*. 2007).

Palynological evidence suggests that the Proteaceae-Myrtaceae kwongan scrub vegetation (community dominants include *Acacia, Banksia, Casuarina, Eucalyptus, Grevillea, Melaleuca* and *Xylomelum angustifolium*) has changed little since 2.9 Ma in Yallalie, SWA, a location close to the populations at Eneabba (Beekeepers Nature Reserve and South Eneabba Nature Reserve) used in our study. The mid-Pliocene fire interval at Yallalie was proposed to be slightly longer than 10 years (Dodson *et al.* 2005), suggesting an incredibly reliable fire return time in this region over evolutionary timescales.

The juvenile period in woody species is generally correlated with longevity, such that longer-lived species have longer juvenile stages (Morrison *et al*. 1996). Thus, if the fire interval between successive fires is shorter than the time required for long-lived woody species (recovering from fire either by resprouting or from seed) to mature and set seed, the abundance of these species will decline (Burrows & Wardell-Johnson 2003; Pekin *et al.* 2012). Enright *et al.* estimated that individuals of *Banksia attenuata* near Eneabba, SWA, may live for up to 300 years (Enright & Lamont 1992). Field observations show that the secondary juvenile stage (resprouting of existing individuals after fire) lasts 2–3 years, with little or no viable seed available until at least four years following a fire. Thus, few seedlings will be recruited for fires at 3–5 year intervals. Frequent fires would further impact long-term population size, as seedlings are more vulnerable to fire than are resprouts. Frequent fires can also deplete carbohydrate stores in resprouting species (Pate *et al*.1990), resulting in reduced survivorship and vigour. With the increasing selective pressure from shortened fire intervals, only individuals with beneficial alleles survive, leading to a *de facto* selective sweep.

Genome-wide analysis of adaptive genetic variation in *Banksia attenuata* revealed a clear signature of increased fire frequency as a consequence of fire management, first by Aboriginal people, then European settlers, to the current government fire management agency programs. We identify the presence of higher adaptive genetic variation in populations where fire frequency has been relatively stable over long periods of time but much lower genetic variation in populations where fire has become more frequent as a result of changes to the mean fire interval. Because changes in fire regime in certain parts of SWA have been relatively recent, new adaptive mutations are

not likely to have appeared, leaving the species reliant on existing genetic diversity to facilitate persistence. High adaptive genetic variation must have existed in those populations (as it continues to do in some others reported here). This contemporary evolutionary response to frequent fire has reduced variability at the selected loci. Such selective sweeps may reduce the population's ability to respond genetically to future fluctuations in fire regime, leading to unpredictable effects on the species' presence and abundance at the level of the plant community. Given the current very low level of adaptive genetic variation in populations in southern SWA, the capacity to survive more frequent fires is highly uncertain.

5.5.2 Adaptive variation to rainfall and high temperature

Rapid environmental changes have long been recognised as powerful driving forces for positive directional selection (Anderson *et al.* 2012). Directional selection is reliant on the availability of genetic diversity upon which selection can act. Our genome-wide study has revealed high levels of adaptive genetic variation in populations of *Banksia attenuata* across its range. Most of the candidate genes (~80%) associated with rainfall and high temperature have multiple alleles, and the results of our principal components analysis suggest that recent changes in rainfall and temperature have had little impact so far on within-population adaptive genetic variation. These results are perhaps not surprising because inter-annual fluctuations in rainfall and temperature are normal occurrences and different genotypes of a species may be favored in different years. Inter-annual environmental fluctuations may be one driver by which functional genetic variation is maintained in natural populations (Jump *et al.* 2008). The regions inhabited by *B. attenuata* range from semi-arid with an annual rainfall of 360 mm to the high rainfall zone with over 1000 mm annually, supporting the interpretation that species spanning a wide climate range may have greater intrinsic adaptability due to high adaptive genetic variation. In *Arabidopsis thaliana*, Lee and Mitchell-Olds similarly demonstrated that environmental adaptation contributes to gene polymorphism across the genome (Lee & Mitchell-Olds 2012). Given the existence of such diverse climate-related genetic variability within natural populations of *B. attenuata*, its capacity to adapt to changes in climate (declines in rainfall and rising temperatures) may be large.

The impacts of declining rainfall on plant species may be greater in combination with increasing temperatures, prompting the hypothesis that genes that confer fitness under drought stress may overlap with those associated with tolerance to high temperatures.

However, our results show that the opposite is true for *B. attenuata*. Although there is considerable overlap of the SNPs associated with the mean temperature of the hottest month, with 14 SNPs (out of 45) associated with at least two of these climate factors, of the 25 SNPs associated with annual rainfall, only one was associated with temperature. Such weak genetic correlations may allow traits to respond to selection independently (Etterson & Shaw 2001).

Over the last four decades, an unplanned experiment has shown the impact that increasing temperatures, declining rainfall and retreating groundwater levels may have had on *Banksia* species in woodlands near Perth, SWA. Extensive deaths of mature individuals, including *B. attenuata*, have been recorded since the 1970s (Groom *et al.* 2000). However, no *Banksia* species have become locally extinct during the more than 40 years of continuous decline in the water table. Furthermore, new individuals that became established under the changed ecohydrological state of lower groundwater availability have been less stressed by drought compared with their parent populations (Froend *et al*. 2013). In *Eucalyptus*, experimental trials indicate that climatic tolerances of the species may be greater than suggested by their natural distributions (Butt *et al*. 2013). For example; Booth *et al.* showed that *Eucalyptus regnans* was able to grow well at trial sites where the annual mean temperature was 5 °C warmer than the hottest location in its natural distribution (Booth *et al.* 2015). Taken together, our results showing a high level of adaptive genetic variation and an abundance of alleles in those candidate genes associated with adaptation to rainfall and high temperature suggest an intrinsic adaptability in populations of *B. attenuata* to tolerate further changes in rainfall and temperature.

5.5.3 Ecologically important genes in adaptation to climate and fire regimes

Among the candidate genes that have been identified with close associations to both climate (rainfall and high temperature) and fire intervals in *B. attenuata*, four candidate genes have clear implications for molecular functions in the adaptation to stress as revealed by experimental studies in many other plants. The malate dehydrogenase (MDH) gene was identified as closely related to rainfall variation and high temperature in our study. Previous studies have shown that MDH is sensitive to abiotic stresses and that the expression of MDH is positively correlated with the growth vigour of plants and cells under stress (Baisakh *et al.* 2008; Evers *et al*. 2012). It is likely that the enrichment of the MDH gene in *B. attenuata* populations confers a substantial capacity for the species to adapt to a broad spectrum of rainfall and high temperatures,

including adaptation to dry and hot environments such as in the northern sandplain of SWA. The sphingosine 1-phosphate phosphatase (S1P) gene has been identified as specifically linked to adaptation to rainfall variation in *B. attenuata*. The S1P gene has important functions in controlling stomatal opening and closure (Coursol *et al.* 2003; Chalfant & Spiege 2005). Stomatal closure has negative effects on CO_2 uptake, photosynthesis, and transpirational cooling as well as on water and nutrient uptake. The ability to close the stomata during unfavourable conditions (usually drought stress) represents an important intrinsic adaptation to repeated drought in *B. attenuata.*

High temperature causes a negative carbon balance, which increases the risk of carbon starvation (Zhao *et al.* 2013). It is not surprising that 5' AMP-activated protein kinase (AMPK) has been identified as closely associated with adaptation to high temperature (the temperature in the hottest month) in *B. attenuata*. High temperatures promote stomatal closure, which leads to decreased CO_2 uptake and subsequently lowers net photosynthesis. AMPK is a sensor of energy status and switches on catabolic pathways that generate ATP, which maintains cell survival during energy starvation (Hardie 2011). The guanine nucleotide-exchange factor gene (GEF) has been found to be important in morphogenesis, including the regulation of root growth, lateral root formation, root hair differentiation and floral organ formation, and regulation of the formation of plant vascular networks (Sieburth *et al.* 2006). The GEF gene has been identified as closely associated with fire interval, reflecting its critical role in promoting and regulating post-fire growth and survival in *B. attenuata*.

Eckert *et al*. investigated the genetic basis of climatic adaptation in loblolly pine (*Pinus taeda*) by evaluating the associations between environmental clines and allelic variation using genome-wide markers; they revealed five loci that were significantly associated with aridity gradients (Eckert *et al.* 2010). These genes were putatively orthologous to the *Arabidopsis* (*Arabidopsis thaliana*) genes that confer stress tolerance. Our genome-wide scans identified candidate genes that are related to stress tolerance, the regulation of stomatal closure, energy use, and morphogenesis in the adaptation to climate and fire regime in *B. attenuata*. Further research points to experimentally investigating and validating the functional and physiological pathways of these candidate genes.

5.5.4 Methodological considerations

The present effort of searching candidate genes involved in the adaptation to climate

and fire regimes in SWA must be considered as preliminary, and the list of candidate genes is far from complete. Although our RAD-seq approach has generated data equivalent to a 2–3× coverage of the genome, the reference genome obtained *de novo* from RAD sequences was only 65 MB, equivalent to approximately one-tenth of the whole genome (assuming a genome size of 650–850 MB for *Banksia*). The resulting 5,701 SNPs scattered across the genome may be sufficient for providing an overall evaluation of the level of adaptive genetic variation within populations of *B. attenuata* but may not be sufficient to cover all the genes that confer adaptive fitness to climate and fire regime. Furthermore, to facilitate the comparison of the genetic variation among populations and across the range of *B. attenuata*, only those SNPs that were present in all sampled individuals and populations were investigated, which may omit population- or individual-specific genetic variations. Indeed, over 61,000 SNPs (61,000–74,000) were discovered in individual samples, as estimated from the raw RAD sequences. Future advances in analytic methodology may make full use of all the data generated from high-throughput sequencing. Finally, many of the candidate genes in this study are still not well characterised at the functional or transcriptional level. Indeed, among the 18 candidate genes identified by aligning to the *Banksia hookeriana* leaf transcriptome, only the function for four genes can be connected to adaptation to local climate and fire regime. Future research on a fully annotated *Banksia* genome is anticipated to provide a critical platform for the ecology, evolution and adaptation of this iconic genus.

5.6 Conclusion

Genetic variability has the capacity to buffer species against specific environmental changes. Our study was able to detect a high level of adaptive genetic variation and candidate genes with a clear ecological function associated with adaptation to local climate and fire regimes in natural plant populations. Our results suggest that *B. attenuata*, and most likely other species with a similar life history and distribution, may be able to tolerate further changes in rainfall and temperature based on adaptive genetic variation within populations. This is corroborated with the results from studies of the impacts of declining water availability on banksias (Groom *et al*. 2000; Froend *et al*. 2013) and paleo-evidence of climate change and the persistence of banksias *in situ* for almost 3 million years (Dodson & Macphail 2004). Our results contribute to the recently proposed notion that some species and ecosystems might be more resilient to climate change than we currently believe, with genetic adaptation leading to “effect

dampening" within a relatively short time frame (Leuzinger *et al*. 2011).

Our results reveal that shortened fire intervals, predominantly a consequence of recent human activities, imposed the strongest selection pressure on *B. attenuata* populations in southern SWA. Frequent fires have been driving changes in gene frequency within natural plant populations and have led to selective sweep. Given the current very low level of adaptive genetic variation in those populations of southern SWA, the capacity to survive more frequent fires and further environmental fluctuations in their habitat is substantially reduced. Even if adaptive genetic variations exist in *B. attenuata* populations and an evolutionary response to further climatic changes can occur, this may not be sufficient to ensure the survival of the population. The projected decline in rainfall in SWA, in conjunction with the continuing rise of summer temperature, may result in longer fire seasons and increased fire likelihood, thus further shortening fire intervals (Burrows & McCaw 2013).

Finally, despite the presence of positive correlations in heterozygosity as measured by neutral SNPs and by SNPs putatively under directional selection, the pattern of neutral genetic variation in populations of *B. attenuata* was not representative of adaptive genetic variation, particularly in those populations experiencing shortened fire intervals in the southern part of SWA. Our results highlight the long-held concerns regarding the use of neutral genetic variation as a surrogate for adaptive genetic variation.

5.7 References

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Chapter 6: Summary, conclusions, significance and recommendations

6.1 Summary of major results and conclusions from this thesis

The changes in climate that are occurring across the globe are resulting in shifts in the habitats suitable for occupation by plant species, putting the future persistence of many species at risk (Sykes 2009; IPCC 2013; Urban 2015). Plants will respond to changes in climate by persisting within their current distribution, migrating to suitable habitats, or by adapting through evolution (Aitken *et al.* 2008). Southwest Australia (SWA), a global biodiversity hotspot, has experienced a 30% decrease in annual rainfall since the mid-1970s, and mean annual temperatures have increased by 0.15˚C per decade over the same time period, with these trends forecast to continue into the future (Bates *et al*. 2008; IOCI 2012). Species distribution modelling indicates that substantial declines in suitable habitat will occur for SWA species under future climate change scenarios, resulting in range shifts, contractions or even total range losses (Fitzpatrick *et al.* 2008; Yates *et al.* 2010; Urban 2015).

This thesis provides a comprehensive analysis of evolutionary adaptations of the flora of SWA in response to decreased rainfall and increased drought on a number of levels; from one species to multiple species to the community level, and from within a single generation to over multiple generations, as well as over a long-term time frame. I draw the overall conclusion that the flora of SWA is tolerating the changes in climate that have occurred so far, and have the potential to cope with further changes in climate, and that the capacity to tolerate climate may be compromised by altered fire regimes. Specifically:

1. The flora of South Eneabba Nature Reserve has been able to tolerate the changes in climate that have occurred in the area over the past 40 years, and has the potential to tolerate further changes in climate; however should extreme change in climate take place in the future, it is likely that the persistence of many species will be at risk.

2. Species and ecosystems may be more resilient to climate change than is currently believed; with adaptive evolution through natural selection or heritable phenotypic plasticity occurring over a short time frame in this study. Plants in Southwest Australia may possess the capacity for rapid adaptation, but this is

dependent on robust, genetically diverse populations and the rate at which climate change occurs.

3. There is no evidence of gradual accumulation of drought tolerant genotypes in the seed bank of *Banksia hookeriana* when seedlings established from different seed age cohorts (pre-drought and post- drought) from a parent plant are raised alongside one another. However, results need to be interpreted with caution due to a low sample size, and the possibility of changes in phenology cannot be ruled out.

4. Declining rainfall and rising temperatures have no detectable influence on overall population genetic variation in *B. attenuata*; however shortened fire intervals do significantly affect population genetic variation. *Banksia attenuata* may be able to tolerate further changes in rainfall and temperature through evolutionary adaptation; however the associated changes in fire regime that are expected with a drier, warmer climate may be detrimental for the species.

6.2 Significance and implications of this research

The research presented in this thesis indicates that plant species of Southwest Australia (SWA) have been able to tolerate the changes in climate that have occurred in the region over the past 40 years, specifically decreased rainfall and increased temperature, and that they may possess the capacity to rapidly adapt to the continued changes in climate that are forecast under future climate change scenarios. Recent research predicts high extinction rates, range contractions and migrations for species under future climate scenarios (Thomas *et al*. 2004; Thuiller *et al*. 2005, Fitzpatrick *et al*. 2008; Urban 2015). My study suggests that plants, and indeed all organisms, have the potential to adapt and evolve in a changing environment. Future bioclimatic modelling should take the intrinsic capacity of adaptation into consideration.

However, there are limitations to the ability of species to rapidly adapt to changes in climate, and species persistence will depend on a variety of factors. The rate at which climate change occurs will play a substantial role in determining whether species will be able to adapt, with the risk of extinction increasing along with the rate of environmental change (Bürger & Lynch 1995). The level of genetic variation existing within a population will also be a significant factor in determining the ability of a species to rapidly evolve to tolerate climate changes (Bürger & Lynch 1995;

Gomulkiewicz & Holt 1995; Williams *et al*. 2008). As is reported in Chapter 5 for *Banksia attenuata*, populations with low genetic diversity are at the greatest risk of extinction, having a smaller gene pool from which to source tolerant genotypes. Changes in fire regime will also influence the ability of species to adapt (Huston 2003; Cary *et al.* 2012; Enright *et al*. 2012); the shortened fire intervals that are expected to occur in SWA as a result of the warmer, drier conditions forecast under future climate scenarios could be detrimental to some species, but beneficial to others. Fire-killed species, particularly those with long juvenile periods are expected to be at greatest risk, while resprouting species that regenerate quickly after fire will benefit from reduced competition but will suffer depleted energy stores (Bell 2001; Burrows 2008; Enright *et al.* 2012).

The research presented here aids progress towards improved bioclimatic modelling of species distributions under future climate change scenarios. Although current bioclimatic modelling does consider species migrations, it fails to take the ability of species to rapidly adapt to changes in climate into account when making predictions for the future (Fitzpatrick *et al.* 2008; Yates *et al*. 2010). Further investigation into the ability of species from a range of genera and families to rapidly adapt under pressure from climate change is required in order to provide sufficient data for incorporation into future bioclimatic models.

The research presented here has implications for biodiversity management and can assist in informing approaches to adaptive conservation management. Conservation management does not currently take the ability of species to adapt to changes in climate into account, although it has been advocated for in the past (Pressey *et al*. 2007). Little information is available regarding the ability of species to rapidly adapt to changes in climate and so further research will be required to provide conservation managers with reliable information for use in development and implementation of strategies for best conserving biodiversity.

6.3 Recommendations for future study

The results from my study offer a few recommendations for future research:

1. Further research into the floristic composition of South Eneabba Nature Reserve should focus on continued monitoring of the fixed plots established in this study. Data should be collected in greater detail (recording of plant size, assessment of reproductive ability, recording of additional plant functional traits) to assist in

detecting fine-scale changes in floristic composition that may be occurring. Particular attention should be paid to the species listed in Chapter 2 as possible indicators of floristic change as a result of climate change (species absent from the 2015 survey but present in historic surveys, species at the northern edge of their range or restricted to the Eneabba region, resprouting species, species with a canopy seed bank, seasonal species, and species of conservation significance).

2. Further research into the rapid evolutionary adaptation of Southwest Australian plants in response to declining rainfall should focus on testing the effect of rapid evolution and other factors on the loss of genetic diversity and the effect that this has on overall population variability. Plants from a variety of families should be studied (the species studied in Chapter 3 are all from the family Proteaceae) as well as a range of species to determine whether responses observed here are consistent across families and genera.

3. Further research into the ability of plants to accumulate drought tolerance in the seed bank should focus on using a greater sample size and a range of different species to determine whether the responses observed here are representative. The possibility of changes having occurred that effect later stages of development despite no measured change in growth should also be investigated in the future.

4. Further research into the evolutionary potential of *Banksia attenuata* should focus on examining and validating the pathways of the candidate genes that were identified in the study as being associated with stress tolerance, regulation of stomatal closure, energy use and morphogenesis in the adaptation of the species to climate and fire regimes.

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Appendices

Supplementary Information: Chapter 2

Table S2.1: GPS coordinates of the 60 plots surveyed within South Eneabba Nature Reserve in 2015. GPS locations were recorded in the north-west corner of each plot.

Plot name	Latitude	Longitude
1.1	S 29˚ 51' 41.8"	E 115˚ 15'23.8"
1.2	S 29˚ 51' 17.0"	E 115˚ 15'23.5"
1.3	S 29˚ 51' 20.2"	E 115˚ 15'22.9"
2.1	S 29˚ 51' 08.7"	E 115˚ 15'15.5"
2.2	S 29˚ 51' 11.4"	E 115˚ 15'13.5"
2.3	S 29˚ 51' 18.7"	E 115˚ 15'12.3"
3.1	S 29˚ 52' 47.4"	E 115˚ 15'07.7"
3.2	S 29˚ 52' 50.6"	E 115˚ 15'11.4"
3.3	S 29˚ 52' 55.2"	E 115˚ 15'14.7"
4.1	S 29˚ 53' 04.8"	E 115˚ 15'03.6"
4.2	S 29˚ 53' 06.4"	E 115˚ 15' 01.9"
4.3	S 29˚ 53' 05.6"	E 115˚ 14' 59.9"
5.1	S 29˚ 59' 46.9"	E 115˚ 15'27.1"
5.2	S 29˚ 59' 45.2"	E 115˚ 15'30.9"
5.3	S 29˚ 59' 46.3"	E 115˚ 15'34.9"
6.1	S 29˚ 58' 25.1"	E 115˚ 15'25.2"
6.2	S 29˚ 58' 22.7"	E 115˚ 15'28.0"
6.3	S 29˚ 58' 20.2"	E 115˚ 15'30.4"
7.1	S 29˚ 59' 07.3"	E 115˚ 17' 06.6"
7.2	S 29˚ 59' 05.3"	E 115˚ 17' 09.6"
7.3	S 29˚ 59' 01.8"	E 115˚ 17' 09.1"
8.1	S 30˚ 00' 55.5"	E 115˚ 17' 31.4"
8.2	S 30˚ 00' 52.0"	E 115˚ 17' 30.9"
8.3	S 30˚00' 48.9"	E 115˚ 17' 30.7"
9.1	S 29˚54' 20.8"	E 115˚14' 47.8"
9.2	S 29˚ 54' 17.1"	E 115˚ 14' 46.8"
9.3	S 29˚ 54' 14.5"	E 115˚ 14' 49.6"
10.1	S 29˚ 57' 59.3"	E 115˚ 16' 52.1"
10.2	S 29˚ 58' 01.4"	E 115˚ 16' 48.9"

10.3	S 29˚ 58' 04.0"	E 115˚ 16' 46.4"
11.1	S 30˚ 00' 09.0"	E 115˚ 18' 30.2"
11.2	S 30˚ 00' 10.7"	E 115˚ 18' 23.3"
11.3	S 30˚ 00' 12.3"	E 115˚ 18' 23.3"
12.1	S 29˚56'26.1"	E 115˚ 18' 01.8"
12.2	S 29˚56'28.8"	E 115˚17'59.6"
12.3	S 29˚56'32.3"	E 115˚ 17'59.4"
13.1	S 29˚ 56' 57.3 "	E 115˚ 18' 40.4"
13.2	S 29˚56' 57.8"	E 115˚ 18' 35.7"
13.3	S 29˚ 56' 59.3"	E 115˚ 18' 32.4"
14.1	S 29˚ 58' 24.0"	E 115˚ 19' 23.4"
14.2	S 29˚ 58' 25.6"	E 115˚ 19' 20.4"
14.3	S 29˚ 58' 25.4"	E 115˚ 19' 16.6"
15.1	S 29˚59'54.2"	E 115˚ 19' 57.3"
15.2	S 29˚59'53.0"	E 115˚ 19' 53.3"
15.3	S 29˚59'53.2"	E 115˚ 19' 49.6"
16.1	S 29˚57' 42.8"	E 115˚ 17' 59.2"
16.2	S 29˚57' 42.1"	E 115˚ 17' 55.8"
16.3	S 29˚57' 39.0"	E 115˚ 17' 53.9"
17.1	S 29˚59'02.2"	E 115˚ 19' 00.6"
17.2	S 29˚58'59.3"	E 115˚ 19' 03.0"
17.3	S 29˚58'55.4"	E 115˚ 19' 04.0"
18.1	S 30˚00'38.5"	E 115˚ 19' 24.4"
18.2	S 30˚00'36.2"	E 115˚ 19' 21.6"
18.3	S 30˚00'33.0"	E 115˚ 19' 20.9"
19.1	S 29˚56'41.5"	E 115˚ 15' 44.0"
19.2	S 29˚56'44.4"	E 115˚ 15' 46.8"
19.3	S 29˚56'46.5"	E 115˚ 15' 49.4"
20.1	S 29˚59'09.0"	E 115˚ 16' 10.2"
20.2	S 29˚59'05.6"	E 115˚ 16' 09.0"
20.3	S 29˚59'02.4"	E 115˚ 16' 11.2"

Table S2.2: Species identified from 60 plots during the 2015 survey of South Eneabba Nature Reserve. Response after fire- Resprouter (RS) or Non-resprouter (NRS) and seed storage syndrome- Canopy Seed Bank (CSB), Soil Seed Bank (SSB) or Annual/No Seed Bank (ANN) are indicated for each species.

Family	Genus and Species	RS/NRS	CSB/SSB/ANN
Anarthriaceae	*Lyginia barbata*	RS	SSB
Anarthriaceae	*Lyginia imberbis*	RS	SSB
Apiaceae	*Platysace juncea*	NRS	SSB
Apiaceae	*Xanthosia huegelii*	RS	SSB
Asparagaceae	*Acanthocarpus preissii*	RS	SSB
Asparagaceae	*Lomandra hastilis*	RS	SSB
Asparagaceae	*Lomandra micrantha* subsp. *micrantha*	RS	SSB
Asparagaceae	*Lomandra preissii*	RS	SSB
Asparagaceae	*Thysanotus dichotomus*	RS	SSB
Asparagaceae	*Thysanotus patersonii*	RS	SSB
Asparagaceae	*Thysanotus sparteus*	RS	SSB
Asparagaceae	*Thysanotus triandrus*	RS	SSB
Asparagaceae	*Thysanotus* sp.	?	SSB
Asteraceae	*Cephalipterum drummondii*	NRS	ANN
Casuarinaceae	*Allocasuarina humilis*	RS	CSB
Casuarinaceae	*Allocasuarina microstachya*	RS	CSB
Casuarinaceae	*Allocasuarina campestris*	NRS	CSB
Cupressaceae	*Callitris acuminata*	RS	CSB
Cyperaceae	*Baumea juncea*	RS	SSB
Cyperaceae	*Caustis dioica*	RS	SSB
Cyperaceae	*Cyathochaeta avenacea*	RS	SSB
Cyperaceae	*Lepidosperma apricola*	RS	SSB
Cyperaceae	*Lepidosperma costale*	RS	SSB
Cyperaceae	*Lepidosperma squamatum*	RS	SSB
Cyperaceae	*Mesomelaena pseudostygia*	RS	SSB
Cyperaceae	*Mesomelaena tetragona*	RS	SSB
Cyperaceae	*Mesomelaena stygia*	RS	SSB
Cyperaceae	*Schoenus brevisetis*	RS	SSB
Cyperaceae	*Schoenus curvifolius*	RS	SSB
Cyperaceae	*Schoenus grandiflorus*	RS	SSB
Cyperaceae	*Schoenus pedicellatus*	RS	SSB

Cyperaceae	*Schoenus pleiostemoneus*	RS	SSB
Cyperaceae	*Schoenus subflavus*	RS	SSB
Dasypogonaceae	*Calectasia cyanea*	RS	SSB
Dasypogonaceae	*Dasypogon obliquifolius*	RS	SSB
Dilleniaceae	*Hibbertia crassifolia*	RS	SSB
Dilleniaceae	*Hibbertia glomerosa*	RS	SSB
Dilleniaceae	*Hibbertia hypericoides*	RS	SSB
Dilleniaceae	*Hibbertia leucocrossa*	RS	SSB
Dilleniaceae	*Hibbertia rostellata*	NRS	SSB
Dilleniaceae	*Hibbertia spicata*	RS	SSB
Dilleniaceae	*Hibbertia subvaginata*	NRS	SSB
Droseraceae	*Drosera eneabba*	NRS	SSB
Droseraceae	*Drosera erythrorhiza*	RS	SSB
Droseraceae	*Drosera humilis*	RS	SSB
Droseraceae	*Drosera menziesii*	RS	SSB
Droseraceae	*Drosera porrecta*	RS	SSB
Ecdeiocoleaceae	*Ecdeiocolea monostachya*	RS	SSB
Ecdeiocoleaceae	*Georgeantha hexandra*	RS	SSB
Ericaceae	*Andersonia heterophylla*	NRS	SSB
Ericaceae	*Astroloma glaucescens*	RS	SSB
Ericaceae	*Astroloma oblongifolium*	RS	SSB
Ericaceae	*Astroloma serratifolium*	NRS	SSB
Ericaceae	*Astroloma* sp. Eneabba	NRS	SSB
Ericaceae	*Astroloma stomarrhena*	RS	SSB
Ericaceae	*Astroloma xerophyllum*	NRS	SSB
Ericaceae	*Conostephium pendulum*	RS	SSB
Ericaceae	*Conostephium preissii*	RS	SSB
Ericaceae	*Leucopogon crassiflorus*	NRS	SSB
Ericaceae	*Leucopogon leptanthus*	NRS	SSB
Ericaceae	*Leucopogon striatus*	RS	SSB
Ericaceae	*Leucopogon* sp. Carnamah	RS	SSB
Ericaceae	*Lysinema pentapetalum*	NRS	SSB
Euphorbiaceae	*Stachystemon axillaris*	NRS	SSB
Fabaceae	*Acacia acuminata*	NRS	SSB
Fabaceae	*Acacia andrewsii*	NRS	SSB
Fabaceae	*Acacia auronitens*	RS	SSB
Fabaceae	*Acacia barbinervis*	RS	SSB
Fabaceae	*Acacia fagonioides*	RS	SSB

Fabaceae	*Acacia lasiocarpa*	NRS	SSB
Fabaceae	*Acacia latipes*	RS	SSB
Fabaceae	*Acacia megacephala*	NRS	SSB
Fabaceae	*Acacia pulchella*	NRS	SSB
Fabaceae	*Acacia stenoptera*	RS	SSB
Fabaceae	*Bossiaea eriocarpa*	NRS	SSB
Fabaceae	*Daviesia benthamii*	NRS	SSB
Fabaceae	*Daviesia chapmanii*	RS	SSB
Fabaceae	*Daviesia daphnoides*	NRS	SSB
Fabaceae	*Daviesia debilior*	RS	SSB
Fabaceae	*Daviesia decurrens*	RS	SSB
Fabaceae	*Daviesia divaricata*	RS	SSB
Fabaceae	*Daviesia epiphyllum*	RS	SSB
Fabaceae	*Daviesia nudiflora*	RS	SSB
Fabaceae	*Daviesia pedunculata*	RS	SSB
Fabaceae	*Daviesia physodes*	RS	SSB
Fabaceae	*Daviesia podophylla/quadrilatera*	NRS	SSB
Fabaceae	*Daviesia triflora*	RS	SSB
Fabaceae	Fabaceae sp. 01	RS	SSB
Fabaceae	*Gastrolobium axillare*	RS	SSB
Fabaceae	*Gastrolobium oxylobioides*	RS	SSB
Fabaceae	*Gastrolobium polystachyum/ Cristonia biloba*	RS	SSB
Fabaceae	*Gastrolobium spinosum*	NRS	SSB
Fabaceae	*Gompholobium confertum*	NRS	SSB
Fabaceae	*Gompholobium preissii*	NRS	SSB
Fabaceae	*Gompholobium tomentosum*	NRS	SSB
Fabaceae	*Hardenbergia comptoniana*	NRS	SSB
Fabaceae	*Jacksonia condensata*	NRS	SSB
Fabaceae	*Jacksonia floribunda*	RS	SSB
Fabaceae	*Jacksonia furcellata*	RS	SSB
Fabaceae	*Jacksonia lehmannii*	RS	SSB
Fabaceae	*Mirbelia spinosa*	RS	SSB
Frankeniaceae	*Frankenia pauciflora*	NRS	SSB
Goodeniaceae	*Dampiera juncea*	RS	SSB
Goodeniaceae	*Dampiera lindleyi*	RS	SSB
Goodeniaceae	*Dampiera spicigera*	?	SSB
Goodeniaceae	*Lechenaultia biloba*	RS	SSB
Goodeniaceae	*Lechenaultia hirsuta*	?	SSB

Goodeniaceae	*Lechenaultia stenosepala*	NRS	SSB
Goodeniaceae	*Scaevola repens* subsp. Northern Sandplains	NRS	SSB
Goodeniaceae	*Scaevola* sp. 01	RS	SSB
Haemodoraceae	*Anigozanthos humilis*	RS	SSB
Haemodoraceae	*Anigozanthos* sp. 01	RS	SSB
Haemodoraceae	*Blancoa canescens*	RS	SSB
Haemodoraceae	*Conostylis aurea*	RS	SSB
Haemodoraceae	*Conostylis hiemalis*	RS	SSB
Haemodoraceae	*Conostylis latens*	RS	SSB
Haemodoraceae	*Conostylis micrantha/teretifolia*	RS	SSB
Haemodoraceae	*Conostylis neocymosa*	RS	SSB
Haemodoraceae	*Haemodorum* sp.	RS	SSB
Haemodoraceae	*Macropidia fuliginosa*	RS	SSB
Haloragaceae	*Glischrocaryon aureum*	NRS	SSB
Hemerocallidaceae	*Johnsonia pubescens*	RS	SSB
Iridaceae	*Patersonia juncea*	RS	SSB
Lamiaceae	*Lachnostachys eriobotrya*	RS	SSB
Lamiaceae	*Hemiphora bartlingii*	RS	SSB
Lamiaceae	*Hemiandra* sp. Eneabba	RS	SSB
Lamiaceae	*Microcorys* sp. Coomallo	RS	SSB
Lamiaceae	*Pityrodia hemigenioides*	RS	SSB
Lauraceae	*Cassytha* sp.	NRS	SSB
Malvaceae	*Guichenotia micrantha*	NRS	SSB
Malvaceae	*Lasiopetalum drummondii*	RS	SSB
Malvaceae	*Thomasia grandiflora*	RS	SSB
Myrtaceae	*Baeckea grandis*	NRS	SSB
Myrtaceae	*Beaufortia bracteosa*	NRS	CSB
Myrtaceae	*Beaufortia elegans*	NRS	CSB
Myrtaceae	*Calothamnus glaber*	RS	CSB
Myrtaceae	*Calothamnus quadrifidus*	RS	CSB
Myrtaceae	*Calothamnus sanguineus*	RS	CSB
Myrtaceae	*Calothamnus torulosus*	RS	CSB
Myrtaceae	*Calytrix aurea*	NRS	SSB
Myrtaceae	*Calytrix chrysantha*	NRS	SSB
Myrtaceae	*Calytrix depressa*	RS	SSB
Myrtaceae	*Calytrix drummondii*	NRS	SSB
Myrtaceae	*Calytrix glutinosa*	NRS	SSB
Myrtaceae	*Calytrix sapphirina*	NRS	SSB

Myrtaceae	*Calytrix superba*	RS	SSB
Myrtaceae	*Chamelaucium* sp. Bunjil	RS	SSB
Myrtaceae	*Conothamnus trinervis*	RS	CSB
Myrtaceae	*Darwinia capitellata*	NRS	SSB
Myrtaceae	*Darwinia neildiana*	RS	SSB
Myrtaceae	*Darwinia sanguinea*	NRS	SSB
Myrtaceae	*Darwinia speciosa*	RS	SSB
Myrtaceae	*Eremaea beaufortioides*	RS	CSB
Myrtaceae	*Eremaea ectadioclada*	RS	CSB
Myrtaceae	*Eremaea hadra*	RS	CSB
Myrtaceae	*Eremaea pauciflora*	RS	CSB
Myrtaceae	*Eremaea violacea*	RS	CSB
Myrtaceae	*Eucalyptus celastroides*	RS	CSB
Myrtaceae	*Eucalyptus pleurocarpa/xtetragona*	RS	CSB
Myrtaceae	*Eucalyptus gittinsii*	RS	CSB
Myrtaceae	*Eucalyptus leptopoda*	RS	CSB
Myrtaceae	*Eucalyptus todtiana*	RS	CSB
Myrtaceae	*Homalocalyx chapmanii*	RS	SSB
Myrtaceae	*Hypocalymma gardneri*	RS	SSB
Myrtaceae	*Leptospermum erubescens*	RS	CSB
Myrtaceae	*Leptospermum spinescens*	RS	CSB
Myrtaceae	*Malleostemon roseus*	NRS	SSB
Myrtaceae	*Melaleuca ciliosa*	NRS	CSB
Myrtaceae	*Melaleuca leuropoma*	RS	CSB
Myrtaceae	*Melaleuca orbicularis*	RS	CSB
Myrtaceae	*Melaleuca systena*	RS	CSB
Myrtaceae	*Melaleuca tinkeri*	RS	CSB
Myrtaceae	*Melaleuca trichophylla*	RS	CSB
Myrtaceae	*Melaleuca urceolaris*	NRS	CSB
Myrtaceae	*Melaleuca zonalis*	RS	CSB
Myrtaceae	Myrtaceae sp. 01	NRS	SSB
Myrtaceae	Myrtaceae sp. 02	NRS	SSB
Myrtaceae	*Phymatocarpus porphyrocephalus*	RS	CSB
Myrtaceae	*Pileanthus filifolius*	RS	SSB
Myrtaceae	*Scholtzia* sp. Eneabba	NRS	SSB
Myrtaceae	*Scholtzia involucrata*	NRS	SSB
Myrtaceae	*Scholtzia laxiflora*	RS	SSB
Myrtaceae	*Scholtzia leptantha*	RS	SSB

Myrtaceae	*Scholtzia* sp. Wongonderrah	NRS	SSB
Myrtaceae	*Thryptomene cuspidata*	NRS	SSB
Myrtaceae	*Verticordia argentea*	NRS	SSB
Myrtaceae	*Verticordia densiflora*	RS	SSB
Myrtaceae	*Verticordia eriocephala*	NRS	SSB
Myrtaceae	*Verticordia grandis*	RS	SSB
Myrtaceae	*Verticordia huegelii*	NRS	SSB
Myrtaceae	*Verticordia nobilis*	NRS	SSB
Myrtaceae	*Verticordia ovalifolia*	RS	SSB
Myrtaceae	*Verticordia pennigera*	RS	SSB
Olacaceae	*Olax benthamiana*	NRS	SSB
Olacaceae	*Olax scalariformis*	NRS	SSB
Poaceae	*Amphipogon turbinatus*	RS	SSB
Poaceae	*Neurachne alopecuroidea*	RS	SSB
Polygalaceae	*Comesperma acerosum*	RS	SSB
Proteaceae	*Adenanthos cygnorum* subsp. *cygnorum*	NRS	SSB
Proteaceae	*Banksia armata* var. *armata*	RS	CSB
Proteaceae	*Banksia attenuata*	RS	CSB
Proteaceae	*Banksia bipinnatifida* subsp. *multifida*	RS	CSB
Proteaceae	*Banksia candolleana*	RS	CSB
Proteaceae	*Banksia carlinoides*	NRS	CSB
Proteaceae	*Banksia chamaephyton*	RS	CSB
Proteaceae	*Banksia cypholoba*	RS	CSB
Proteaceae	*Banksia densa*	NRS	CSB
Proteaceae	*Banksia glaucifolia*	NRS	CSB
Proteaceae	*Banksia grossa*	RS	CSB
Proteaceae	*Banksia hookeriana*	NRS	CSB
Proteaceae	*Banksia incana*	RS	CSB
Proteaceae	*Banksia kippistiana* var. *kippistiana*	NRS	CSB
Proteaceae	*Banksia leptophylla*	NRS	CSB
Proteaceae	*Banksia menziesii*	RS	CSB
Proteaceae	*Banksia micrantha*	RS	CSB
Proteaceae	*Banksia nana*	RS	CSB
Proteaceae	*Banksia nivea*	NRS	CSB
Proteaceae	*Banksia sclerophylla*	RS	CSB
Proteaceae	*Banksia sessilis*	NRS	CSB
Proteaceae	*Banksia shuttleworthiana*	RS	CSB
Proteaceae	*Banksia tortifolia*	RS	CSB

Proteaceae	*Banksia tridentata*	RS	CSB
Proteaceae	*Conospermum crassinervium*	RS	SSB
Proteaceae	*Conospermum nervosum*	NRS	SSB
Proteaceae	*Conospermum unilaterale*	NRS	SSB
Proteaceae	*Conospermum wycherleyi* subsp. *wycherleyi*	RS	SSB
Proteaceae	*Grevillea eriostachya*	RS	SSB
Proteaceae	*Grevillea rudis*	RS	SSB
Proteaceae	*Grevillea shuttleworthiana*	?	SSB
Proteaceae	*Grevillea synapheae subsp. pachyphylla*	RS	SSB
Proteaceae	*Grevillea uniformis*	NRS	SSB
Proteaceae	*Hakea auriculata*	RS	CSB
Proteaceae	*Hakea candolleana*	RS	CSB
Proteaceae	*Hakea conchifolia*	RS	CSB
Proteaceae	*Hakea costata*	NRS	CSB
Proteaceae	*Hakea eneabba*	RS	CSB
Proteaceae	*Hakea flabellifolia*	RS	CSB
Proteaceae	*Hakea gilbertii*	NRS	CSB
Proteaceae	*Hakea incrassata*	RS	CSB
Proteaceae	*Hakea neospathulata*	RS	CSB
Proteaceae	*Hakea obliqua*	NRS	CSB
Proteaceae	*Hakea polyanthema*	NRS	CSB
Proteaceae	*Hakea psilorrhyncha*	NRS	CSB
Proteaceae	*Hakea smilacifolia*	NRS	CSB
Proteaceae	*Hakea stenocarpa*	RS	CSB
Proteaceae	*Hakea trifurcata*	NRS	CSB
Proteaceae	*Isopogon adenanthoides*	NRS	CSB
Proteaceae	*Isopogon inconspicuus*	NRS	CSB
Proteaceae	*Isopogon linearis*	RS	CSB
Proteaceae	*Isopogon tridens*	RS	CSB
Proteaceae	*Lambertia multiflora*	RS	CSB
Proteaceae	*Persoonia acicularis*	RS	SSB
Proteaceae	*Persoonia comata*	RS	SSB
Proteaceae	*Persoonia filiformis*	RS	SSB
Proteaceae	*Petrophile aculeata*	NRS	CSB
Proteaceae	*Petrophile brevifolia*	RS	CSB
Proteaceae	*Petrophile drummondii*	NRS	CSB
Proteaceae	*Petrophile linearis*	RS	CSB
Proteaceae	*Petrophile macrostachya*	RS	CSB

Proteaceae	*Petrophile pilostyla*	RS	CSB
Proteaceae	*Petrophile rigida*	RS	CSB
Proteaceae	*Petrophile seminuda*	RS	CSB
Proteaceae	*Petrophile serruriae*	NRS	CSB
Proteaceae	*Petrophile shuttleworthiana*	RS	CSB
Proteaceae	*Stirlingia latifolia*	RS	SSB
Proteaceae	*Strangea cynanchicarpa*	RS	CSB
Proteaceae	*Synaphea aephynsa*	RS	SSB
Proteaceae	*Synaphea spinulosa*	RS	SSB
Proteaceae	*Xylomelum angustifolium*	RS	CSB
Restionaceae	*Alexgeorgea nitens*	RS	SSB
Restionaceae	*Alexgeorgea subterranea*	RS	SSB
Restionaceae	*Chordifex sinuosus/sphacelatus*	RS	SSB
Restionaceae	*Desmocladus biformis*	RS	SSB
Restionaceae	*Desmocladus elongatus*	RS	SSB
Restionaceae	*Desmocladus myriocladus*	RS	SSB
Restionaceae	*Desmocladus parthenicus*	RS	SSB
Restionaceae	*Desmocladus semiplanus*	RS	SSB
Restionaceae	*Desmocladus virgatus*	RS	SSB
Restionaceae	*Hypolaena exsulca*	RS	SSB
Restionaceae	*Lepidobolus chaetocephalus*	RS	SSB
Restionaceae	*Lepidobolus preissianus*	RS	SSB
Restionaceae	*Lepidobolus quadratus*	RS	SSB
Restionaceae	*Loxocarya striata*	RS	SSB
Restionaceae	Unidentified species 10	RS	SSB
Rhamnaceae	*Stenanthemum humile*	RS	SSB
Rhamnaceae	*Stenanthemum notiale*	NRS	SSB
Rutaceae	*Boronia purdieana*	?	SSB
Rutaceae	*Geleznowia verrucosa*	NRS	SSB
Rutaceae	*Philotheca spicata*	RS	SSB
Sapindaceae	*Dodonaea ericoides*	RS	SSB
Stylidiaceae	*Stylidium adpressum*	NRS	SSB
Stylidiaceae	*Stylidium carnosum*	NRS	SSB
Stylidiaceae	*Stylidium crossocephalum*	NRS	SSB
Stylidiaceae	*Stylidium cygnorum*	NRS	SSB
Stylidiaceae	*Stylidium diuroides*	NRS	SSB
Stylidiaceae	*Stylidium maitlandianum*	NRS	SSB
Stylidiaceae	*Stylidium repens*	NRS	SSB

Stylidiaceae	*Stylidium stenosepalum*	NRS	SSB
Thymelaeaceae	*Pimelea angustifolia*	RS	SSB
Thymelaeaceae	*Pimelea ferruginea*	NRS	SSB
Thymelaeaceae	*Pimelea leucantha*	?	SSB
Thymelaeaceae	*Pimelea sulphurea*	NRS	SSB
Xanthorrhoeaceae	*Xanthorrhoea drummondii*	RS	SSB
Xanthorrhoeaceae	*Xanthorrhoea brunonis*	RS	SSB
Zamiaceae	*Macrozamia fraseri*	RS	CSB
Unknown	Unidentified species 01	RS	SSB
Unknown	Unidentified species 02	NRS	SSB
Unknown	Unidentified species 03	NRS	SSB
Unknown	Unidentified species 04	NRS	SSB
Unknown	Unidentified species 05	NRS	SSB
Unknown	Unidentified species 06	RS	SSB
Unknown	Unidentified species 07	RS	SSB
Unknown	Unidentified species 08	NRS	SSB
Unknown	Unidentified species 09	NRS	SSB
Unknown	Unidentified species 11	RS	SSB
Unknown	Unidentified species 12	RS	SSB

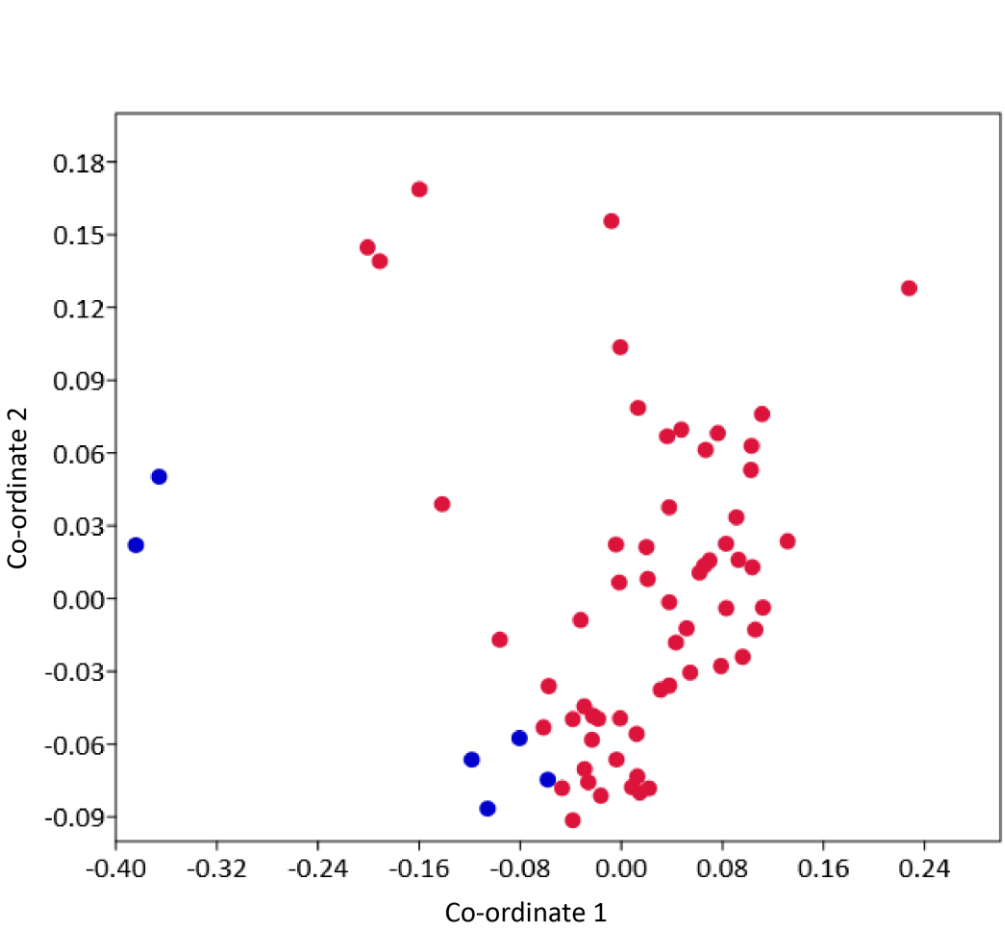

Figure S2.1: Two dimensional non-metric multidimensional scaling (Bray-Curtis similarity) of presence-absence of species in plots from the Griffin *et al*. (1983) survey blue) and the 2015 survey (red). Stress= 0.1477.

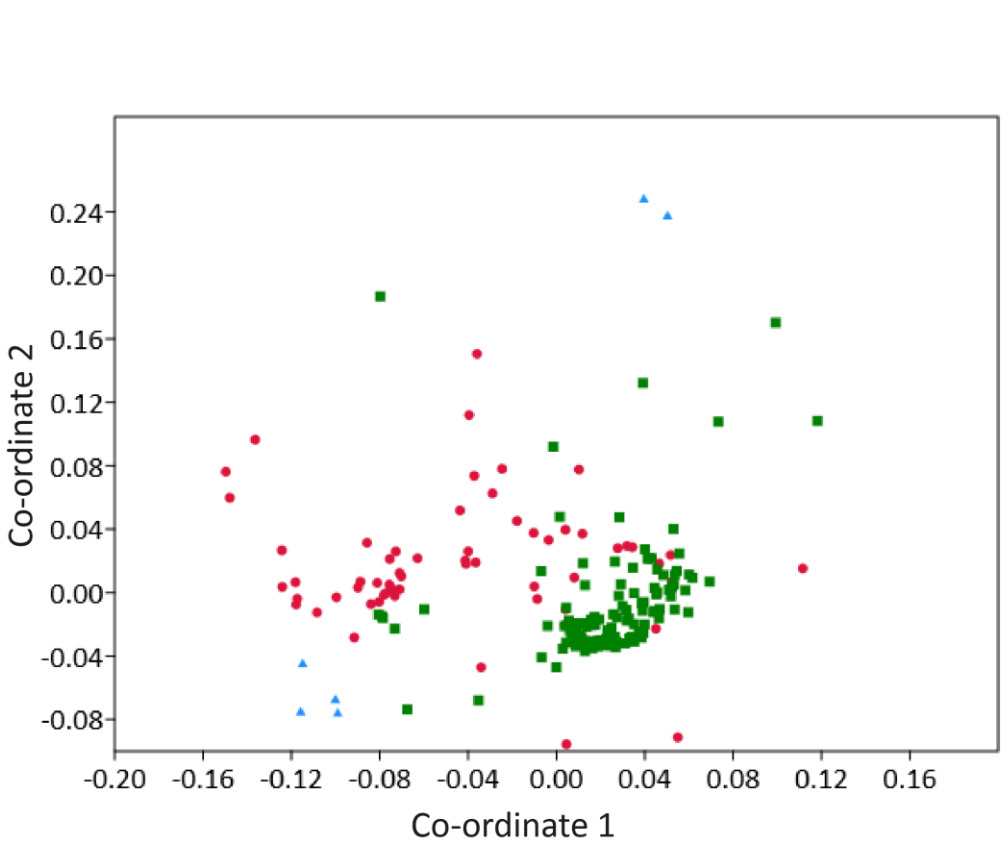

Figure S2.2: Two dimensional non-metric multidimensional scaling (Bray-Curtis similarity) of presence-absence of 37 species in plots from the Elkington and Griffin (1985) survey (green squares), the Griffin *et al*. (1983) survey (blue triangles) and the 2015 survey (red dots). Stress= 0.2431.

Table S2.3: Taxa recorded in the 60 plots surveyed in South Eneabba Nature Reserve in 2015 with cover of each taxon recorded for each plot (Braun-Blanquet Scale). Resprouting ability; resprouter (RS) or non-resprouter (NRS) and seed bank type; soil seed bank (SSB) or canopy seed bank (CSB) indicated for each taxon.

Family	Species	RS/NRS	SSB/CSB	1.1	1.2	1.3	2.1	2.2	2.3	3.1	3.2	3.3	4.1	4.2	4.3	5.1	5.2	5.3	6.1	6.2
Anarthriaceae	*Lyginia barbata*	RS	SSB																	
Anarthriaceae	*Lyginia imberbis*	NRS	SSB			1														
Apiaceae	*Platysace juncea*	NRS	SSB																	
Apiaceae	*Xanthosia huegelii*	RS	SSB						+											
Asparagaceae	*Acanthocarpus preissii*	RS	SSB																	
Asparagaceae	*Lomandra hastilis*	RS	SSB								+									
Asparagaceae	*Lomandra micrantha* subspecies *micrantha*	RS	SSB					+										1		
Asparagaceae	*Lomandra preissii*	RS	SSB	+																
Asparagaceae	*Thysanotus dichotomus*	RS	SSB	+	+	+	+	+			+	+					+			+
Asparagaceae	*Thysanotus patersonii*	NRS	SSB	+	+		+									+			+	
Asparagaceae	*Thysanotus* sp. 1	NRS	SSB															+		
Asparagaceae	*Thysanotus* sp. 2	NRS	SSB			+														
Asparagaceae	*Thysanotus triandrus*	RS	SSB																	
Asteraceae	*Cephalipterum drummondii*	NRS	SSB														+			
Casuarinaceae	*Allocasuarina campestris*	NRS	CSB																+	
Casuarinaceae	*Allocasuarina humilis*	RS	CSB	+	+		+	1		1	1	+	+	+		+	1	+		+
Casuarinaceae	*Allocasuarina microstachya*	RS	CSB	1	2			+								+	+		+	
Cupressaceae	*Callitris acuminata*	RS	CSB			1	+	+	1		+							1		+
Cyperaceae	*Baumea juncea*	RS	SSB																	
Cyperaceae	*Caustis dioica*	NRS	SSB	1	+		+						+			1	+			
Cyperaceae	*Cyathochaeta avenacea*	RS	SSB																	
Cyperaceae	*Lepidosperma apricola*	RS	SSB		+			1	+	+										
Cyperaceae	*Lepidosperma costale*	RS	SSB																	
Cyperaceae	*Lepidospermum squamatum*	RS	SSB																	
Cyperaceae	*Mesomelaena pseudostygia*	RS	SSB	2	1	1	1	1	1	1	+	1	+			2	1	1	1	+
Cyperaceae	*Mesomelaena stygia* subspecies *deflexa*	RS	SSB					+												
Cyperaceae	*Mesomelaena tetragona*	RS	SSB					+												
Cyperaceae	*Schoenus brevisetis*	RS	SSB																	
Cyperaceae	*Schoenus curvifolius*	RS	SSB			1	+		+		+									
Cyperaceae	*Schoenus grandiflorus*	RS	SSB																	
Cyperaceae	*Schoenus pleiostemoneus*	RS	SSB		+															
Cyperaceae	*Schoenus pedicellatus*	RS	SSB															+		
Cyperaceae	*Schoenus subflavus*	RS	SSB	1			1		1	1	1	1	1				1	1	1	
Dasypogonaceae	*Calectasia cyanea*	RS	SSB	+	+			+	+	+										
Dasypogonaceae	*Dasypogon obliquifolius*	RS	SSB															+		
Dilleniaceae	*Hibbertia crassifolia*	RS	SSB																	
Dilleniaceae	*Hibbertia glomerosa*	RS	SSB							+				+						
Dilleniaceae	*Hibbertia hypericoides*	RS	SSB	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Dilleniaceae	*Hibbertia leucocrossa*	RS	SSB																	
Dilleniaceae	*Hibbertia rostellata*	NRS	SSB	1		+	+	1									+	+		
Dilleniaceae	*Hibbertia spicata*	RS	SSB													1	+		1	
Dilleniaceae	*Hibbertia subvaginata*	RS	SSB																	
Droseraceae	*Drosera eneabba*	NRS	SSB		1	1	1	1	1	1		1						1		
Droseraceae	*Drosera erythrorhiza*	NRS	SSB		1											+				
Droseraceae	*Drosera humilis*	NRS	SSB									1	+							

Family	Species	RS/NRS	SSB/CSB	1.1	1.2	1.3	2.1	2.2	2.3	3.1	3.2	3.3	4.1	4.2	4.3	5.1	5.2	5.3	6.1	6.2
Droseraceae	*Drosera menziesii*	NRS	SSB	1	1	+	+	1	+	1	+									
Droseraceae	*Drosera porrecta*	NRS	SSB	+	+	+	1	+	+	1	+									
Ecdeiocoleaceae	*Ecdeiocolea monostachya*	NRS	SSB	+	1		1	+	+					+			1		1	
Ecdeiocoleaceae	*Georgeantha hexandra*	RS	SSB																	
Ericaceae	*Andersonia heterophylla*	NRS	SSB			+	+	+	+									1		
Ericaceae	*Astroloma glaucescens*	NRS	SSB														1			
Ericaceae	*Astroloma oblongifolium*	RS	SSB																	
Ericaceae	*Astroloma serratifolium*	RS	SSB	+		+	+	+			+						+			
Ericaceae	*Astroloma* sp. Eneabba	NRS	SSB																	
Ericaceae	*Astroloma stomarrhena*	RS	SSB																	
Ericaceae	*Astroloma xerophyllum*	NRS	SSB		+						+						1	1		
Ericaceae	*Conostephium pendulum*	RS	SSB																	
Ericaceae	*Leucopogon striatus*	NRS	SSB					+				+					+			
Ericaceae	*Leucopogon crassiflorus*	NRS	SSB																	
Ericaceae	*Leucopogon leptanthus*	NRS	SSB																	
Ericaceae	*Leucopogon* sp. Carnamah	RS	SSB																	
Ericaceae	*Lysinema pentapetalum*	NRS	SSB													+	1	+		
Euphorbiaceae	*Stachystemon axillaris*	NRS	SSB											2						
Fabaceae	*Acacia acuminata*	NRS	SSB										3	3	3					
Fabaceae	*Acacia andrewsii*	NRS	SSB																	
Fabaceae	*Acacia auronitens*	NRS	SSB																	
Fabaceae	*Acacia barbinervis* subspecies *borealis*	RS	SSB				+	+		+										
Fabaceae	*Acacia fagonioides*	RS	SSB															1		
Fabaceae	*Acacia lasiocarpa*	NRS	SSB										+	+						
Fabaceae	*Acacia latipes* subspecies *latipes*	RS	SSB									+		1		+				
Fabaceae	*Acacia megacephala*	NRS	SSB																	
Fabaceae	*Acacia pulchella*	RS	SSB							1										
Fabaceae	*Acacia stenoptera*	RS	SSB																	
Fabaceae	*Bossiaea eriocarpa*	NRS	SSB																	
Fabaceae	*Daviesia benthamii*	NRS	SSB																	
Fabaceae	*Daviesia chapmanii*	RS	SSB																	
Fabaceae	*Daviesia daphnoides*	NRS	SSB																	
Fabaceae	*Daviesia debilior*	RS	SSB													+	1			
Fabaceae	*Daviesia decurrens*	RS	SSB																	
Fabaceae	*Daviesia divaricata*	RS	SSB			+			+	+										+
Fabaceae	*Daviesia epiphyllum*	RS	SSB																	
Fabaceae	*Daviesia nudiflora*	RS	SSB	1			1	1										+		
Fabaceae	*Daviesia pedunculata*	RS	SSB	1	1		1	2												
Fabaceae	*Daviesia physodes*	NRS	SSB																	
Fabaceae	*Daviesia podophylla/Daviesia quadrilatera*	NRS	SSB															+		
Fabaceae	*Daviesia triflora*	RS	SSB					2	1		+			+						
Fabaceae	Fabaceae species 01	RS	SSB																	
Fabaceae	*Gastrolobium axillare*	RS	SSB													+				
Fabaceae	*Gastrolobium oxylobioides*	RS	SSB													+	+			
Fabaceae	*Gastrolobium polystachyum/Cristonia biloba*	NRS	SSB																	
Fabaceae	*Gastrolobium spinosum*	NRS	SSB																1	
Fabaceae	*Gompholobium confertum*	RS	SSB																	
Fabaceae	*Gompholobium preissii*	NRS	SSB																	
Fabaceae	*Gompholobium tomentosum*	RS	SSB										1	+		+				
Fabaceae	*Hardenbergia comptoniana*	NRS	SSB																	

Family	Species	RS/NRS	SSB/CSB	1.1	1.2	1.3	2.1	2.2	2.3	3.1	3.2	3.3	4.1	4.2	4.3	5.1	5.2	5.3	6.1	6.2
Fabaceae	*Jacksonia condensata*	RS	SSB													+				
Fabaceae	*Jacksonia floribunda*	RS	SSB		1	2	1	+	1									+		+
Fabaceae	*Jacksonia furcellata*	RS	SSB											+	+					
Fabaceae	*Jacksonia lehmannii*	RS	SSB				+								1	+	1			
Fabaceae	*Mirbelia spinosa*	RS	SSB									+			+				1	
Frankeniaceae	*Frankenia pauciflora*	NRS	SSB													+				
Goodeniaceae	*Dampiera juncea*	RS	SSB																	
Goodeniaceae	*Dampiera lindleyi*	RS	SSB			1					1							+		
Goodeniaceae	*Dampiera spicigera*	NRS	SSB		1	1	+	+	+	+						+	+			1
Goodeniaceae	*Lechenaultia biloba*	RS	SSB																	
Goodeniaceae	*Lechenaultia hirsuta*	?	SSB										+							
Goodeniaceae	*Lechenaultia stenosepala*	NRS	SSB																	
Goodeniaceae	*Scaevola repens* subspecies Northern Sandplains	RS	SSB																	
Goodeniaceae	*Scaevola species 01*	RS	SSB																	
Haemodoraceae	*Anigozanthos humilis*	RS	SSB							+				+			+			
Haemodoraceae	*Anigozanthos* species 01	RS	SSB															+		
Haemodoraceae	*Blancoa canescens*	RS	SSB																	
Haemodoraceae	*Conostylis aurea*	RS	SSB	+	1	1	1	1	1	+	1	+	+	1	+			1		
Haemodoraceae	*Conostylis hiemalis*	RS	SSB	+	+		+													1
Haemodoraceae	*Conostylis latens*	RS	SSB	+	+		1	1	+	+	+		+					+		
Haemodoraceae	*Conostylis micrantha*	RS	SSB			+	+		+							1	+			
Haemodoraceae	*Conostylis neocymosa*	RS	SSB																	1
Haemodoraceae	Haemodorum species 01	RS	SSB				+	+								+				
Haemodoraceae	*Macropidia fuliginosa*	RS	SSB																	
Haloragaceae	*Glischrocaryon aureum*	RS	SSB																	
Hemerocallidaceae	*Johnsonia pubescens*	RS	SSB						+			+								
Iridiaceae	*Patersonia juncea*	RS	SSB																	
Lamiaceae	*Hemiandra* sp Eneabba	RS	SSB																	
Lamiaceae	*Hemiphora bartlingii*	RS	SSB																	+
Lamiaceae	*Lachnostachys eriobotrya*	RS	SSB																	
Lamiaceae	*Microcorys* sp. Coomallo	RS	SSB																	
Lamiaceae	*Pityrodia hemigenioides*	RS	SSB																	+
Lauraceae	*Cassytha* sp.	NRS	SSB			1	1	1	1	1	1	1		+		+	+	1		
Malvaceae	*Guichenotia micrantha*	RS	SSB																	
Malvaceae	*Lasiopetalum drummondii*	RS	SSB	1				1												
Malvaceae	*Thomasia grandiflora*	RS	SSB													+	+			
Myrtaceae	*Baeckea grandis*	RS	SSB	+	+											+	1			
Myrtaceae	*Beaufortia bracteosa*	NRS	CSB																	
Myrtaceae	*Beaufortia elegans*	NRS	CSB	1	1	2	1	1		+	1	+	+	1	+		+	1		2
Myrtaceae	*Calothamnus glaber*	RS	CSB																	
Myrtaceae	*Calothamnus quadrifidus* subspecies *angustifolius*	RS	CSB																1	
Myrtaceae	*Calothamnus sanguineus*	RS	CSB	+							1	+	+	+			+			
Myrtaceae	*Calothamnus torulosus*	RS	CSB	+				+								1				
Myrtaceae	*Calytrix aurea*	NRS	SSB					1												
Myrtaceae	*Calytrix chrysantha*	NRS	SSB									1	1	+	1			+	1	
Myrtaceae	*Calytrix depressa*	RS	SSB																	
Myrtaceae	*Calytrix drummondii*	NRS	SSB	1		+					1					+			+	
Myrtaceae	*Calytrix glutinosa*	NRS	SSB																	
Myrtaceae	*Calytrix sapphirina*	NRS	SSB				1			1			1	1	+					1
Myrtaceae	*Calytrix superba*	RS	SSB				+													

Family	Species	RS/NRS	SSB/CSB	1.1	1.2	1.3	2.1	2.2	2.3	3.1	3.2	3.3	4.1	4.2	4.3	5.1	5.2	5.3	6.1	6.2
Myrtaceae	*Chamelaucium* sp. Bunjil	RS	SSB					1										1		
Myrtaceae	*Conothamnus trinervis*	RS	CSB	+			+											+		
Myrtaceae	*Darwinia capitellata*	NRS	SSB													+	+		+	
Myrtaceae	*Darwinia neildiana*	NRS	SSB	+	1	+		+			+			+		+	1			
Myrtaceae	*Darwinia sanguinea*	NRS	SSB															1		+
Myrtaceae	*Darwinia speciosa*	RS	SSB	+	1		+	+										+		
Myrtaceae	*Eremaea beaufortioides*	RS	CSB						+	1	+	1		+				1		+
Myrtaceae	*Eremaea ectadioclada*	NRS	CSB				+			+	+	+	+					+		+
Myrtaceae	*Eremaea hadra*	RS	CSB																	
Myrtaceae	*Eremaea pauciflora*	RS	CSB																	
Myrtaceae	*Eremaea violacea*	RS	CSB	1	2		+	+												
Myrtaceae	*Eucalyptus celastroides*	RS	CSB																	
Myrtaceae	*Eucalyptus pleurocarpa/xtetragona*	RS	CSB		1		1									+	1	1		
Myrtaceae	*Eucalyptus gittinsii*	RS	CSB																	
Myrtaceae	*Eucalyptus leptopoda*	RS	CSB																	
Myrtaceae	*Eucalyptus todtiana*	RS	CSB								+			1	+					
Myrtaceae	*Homalocalyx chapmanii*	RS	SSB																	
Myrtaceae	*Hypocalymma gardneri*	NRS	SSB															+		
Myrtaceae	*Leptospermum erubescens*	RS	CSB										+		+					
Myrtaceae	*Leptospermum spinescens*	NRS	CSB		+			+			+	+	+	+						
Myrtaceae	*Malleostemon roseus*	NRS	SSB																	
Myrtaceae	*Melaleuca ciliosa*	NRS	CSB							+	+									
Myrtaceae	*Melaleuca systena*	RS	CSB									+	+	+	+					
Myrtaceae	*Melaleuca leuropoma*	RS	CSB	1	1	1			+		+	+								+
Myrtaceae	*Melaleuca orbicularis*	RS	CSB																	
Myrtaceae	*Melaleuca tinkeri*	RS	CSB										1	2	1					
Myrtaceae	*Melaleuca trichophylla*	RS	CSB	1	1		1	1								2	1	1	+	
Myrtaceae	*Melaleuca urceolaris*	NRS	CSB																	
Myrtaceae	*Melaleuca zonalis*	RS	CSB																	
Myrtaceae	Myrtaceae species 01	NRS	SSB				+	+			+									
Myrtaceae	Myrtaceae species 02	NRS	SSB																	
Myrtaceae	*Phymatocarpus porphyrocephalus*	NRS	SSB						1											
Myrtaceae	*Pileanthus filifolius*	RS	SSB																	
Myrtaceae	*Scholtzia involucrata*	RS	SSB							1			2	1	2					
Myrtaceae	*Scholtzia laxiflora*	NRS	SSB		+		1	+	1		+	+			+					+
Myrtaceae	*Scholtzia leptantha*	NRS	SSB																	
Myrtaceae	*Scholtzia* sp. Eneabba	NRS	SSB																	
Myrtaceae	*Scholtzia* sp. Wongonderrah	NRS	SSB							+			+	+	1					
Myrtaceae	*Thryptomene cuspidata*	NRS	SSB									+								
Myrtaceae	*Verticordia argentea*	NRS	SSB																	+
Myrtaceae	*Verticordia densiflora*	RS	SSB																	
Myrtaceae	*Verticordia eriocephala*	NRS	SSB																	
Myrtaceae	*Verticordia grandis*	NRS	SSB			+		+	+	+		+	+					+		+
Myrtaceae	*Verticordia huegelii*	NRS	SSB																	
Myrtaceae	*Verticordia nobilis*	NRS	SSB							+										
Myrtaceae	*Verticordia ovalifolia*	NRS	SSB						+											
Myrtaceae	*Verticordia pennigera*	RS	SSB													+	+			
Olacaceae	*Olax benthamiana*	NRS	SSB																+	
Olacaceae	*Olax scalariformis*	NRS	SSB																	
Poaceae	*Amphipogon turbinatus*	RS	SSB	+			+	+	+	+	+	+					+		+	

Family	Species	RS/NRS	SSB/CSB	1.1	1.2	1.3	2.1	2.2	2.3	3.1	3.2	3.3	4.1	4.2	4.3	5.1	5.2	5.3	6.1	6.2
Poaceae	*Neurachne alopecuroidea*	RS	SSB									+								
Polygalaceae	*Comesperma acerosum*	NRS	SSB															+		
Proteaceae	*Adenanthos cygnorum* subspecies *cygnorum*	NRS	SSB																	
Proteaceae	*Banksia armata* var. *armata*	RS	CSB																+	
Proteaceae	*Banksia attenuata*	NRS	CSB						+	+				+						1
Proteaceae	*Banksia bipinnatifida*	RS	CSB	+		+		+									+			
Proteaceae	*Banksia candolleana*	RS	CSB			+			+											
Proteaceae	*Banksia carlinoides*	RS	CSB													2	1			
Proteaceae	*Banksia chamaephyton*	RS	CSB																	
Proteaceae	*Banksia cypholoba*	RS	CSB														+			
Proteaceae	*Banksia densa*	RS	CSB															+		
Proteaceae	*Banksia glaucifolia*	NRS	CSB																	
Proteaceae	*Banksia grossa*	RS	CSB																	
Proteaceae	*Banksia hookeriana*	NRS	CSB																	1
Proteaceae	*Banksia incana*	RS	CSB																	
Proteaceae	*Banksia kippistiana* var. *kippistiana*	RS	CSB																	
Proteaceae	*Banksia leptophylla*	NRS	CSB							+	2	2	2	2	2			+		+
Proteaceae	*Banksia menziesii*	RS	CSB																	1
Proteaceae	*Banksia micrantha*	RS	CSB																	
Proteaceae	*Banksia nana*	RS	CSB																	
Proteaceae	*Banksia nivea*	RS	CSB	+	+	+	+	+	+	+	+	1	+	+						
Proteaceae	*Banksia sclerophylla*	RS	CSB																	
Proteaceae	*Banksia sessilis*	NRS	CSB								+		+		+					
Proteaceae	*Banksia shuttleworthiana*	RS	CSB	1	1		1	1		+	+					+	1	1		
Proteaceae	*Banksia tortifolia*	RS	CSB								+									+
Proteaceae	*Banksia tridentata*	RS	CSB	+	1	2	1	1									+	+		
Proteaceae	*Conospermum crassinervium*	NRS	SSB							+										
Proteaceae	*Conospermum nervosum*	NRS	SSB																	
Proteaceae	*Conospermum unilaterale*	RS	SSB																	
Proteaceae	*Conospermum wycherleyi* subspecies *wycherleyi*	RS	SSB	+	+	1		+	1				+	+	+		+	1		1
Proteaceae	*Grevillea eriostachya*	RS	SSB						+											
Proteaceae	*Grevillea rudis*	RS	SSB													+	+			
Proteaceae	*Grevillea shuttleworthiana*	RS	SSB																	
Proteaceae	*Grevillea synapheae* subspecies *pachyphylla*	RS	SSB																	
Proteaceae	*Grevillea uniformis*	NRS	SSB																	
Proteaceae	*Hakea auriculata*	RS	CSB													+				
Proteaceae	*Hakea candolleana*	RS	CSB				+													
Proteaceae	*Hakea conchifolia*	RS	CSB														+	1		
Proteaceae	*Hakea costata*	NRS	CSB					+			+		+	+						
Proteaceae	*Hakea eneabba*	RS	CSB					+												+
Proteaceae	*Hakea flabellifolia*	RS	CSB									+								
Proteaceae	*Hakea gilbertii*	NRS	CSB																2	
Proteaceae	*Hakea incrassata*	RS	CSB		+			+								1	1	+	+	
Proteaceae	*Hakea neospathulata*	RS	CSB																+	
Proteaceae	*Hakea obliqua* subspecies *parviflora*	RS	CSB													+				
Proteaceae	*Hakea polyanthema*	NRS	CSB										+							
Proteaceae	*Hakea psilorrhyncha*	NRS	CSB																	
Proteaceae	*Hakea smilacifolia*	NRS	CSB																	
Proteaceae	*Hakea stenocarpa*	RS	CSB																	
Proteaceae	*Hakea trifurcata*	NRS	CSB										+	+						

Family	Species	RS/NRS	SSB/CSB	1.1	1.2	1.3	2.1	2.2	2.3	3.1	3.2	3.3	4.1	4.2	4.3	5.1	5.2	5.3	6.1	6.2
Proteaceae	*Isopogon adenanthoides*	NRS	CSB																	
Proteaceae	*Isopogon inconspicuus*	RS	CSB													+				
Proteaceae	*Isopogon linearis*	RS	CSB											+						
Proteaceae	*Isopogon tridens*	RS	CSB		+			+				+								
Proteaceae	*Lambertia multiflora*	RS	CSB														+	1		
Proteaceae	*Persoonia acicularis*	RS	SSB			+			+									+		1
Proteaceae	*Persoonia comata*	RS	SSB																	
Proteaceae	*Persoonia filiformis*	RS	SSB																	
Proteaceae	*Petrophile aculeata*	NRS	CSB																	
Proteaceae	*Petrophile brevifolia*	RS	CSB	+	+		+	+		+	+		+		+	+	1	1		+
Proteaceae	*Petrophile drummondii*	NRS	CSB						1	1	+	1	+							1
Proteaceae	*Petrophile linearis*	NRS	CSB								+									
Proteaceae	*Petrophile macrostachya*	RS	CSB		+	+	+	+				+	+					+		
Proteaceae	*Petrophile pilostyla*	RS	CSB																	
Proteaceae	*Petrophile rigida*	RS	CSB																	
Proteaceae	*Petrophile seminuda*	RS	CSB											+	+					
Proteaceae	*Petrophile serruriae*	NRS	CSB																	
Proteaceae	*Petrophile shuttleworthiana*	RS	CSB																	
Proteaceae	*Stirlingia latifolia*	RS	SSB							1	+									+
Proteaceae	*Strangea cynanchicarpa*	RS	CSB																	
Proteaceae	*Synaphea aephynsa*	RS	SSB																	
Proteaceae	*Synaphea spinulosa* subspecies *spinulosa*	RS	SSB												+	+	+		+	
Proteaceae	*Xylomelum angustifolium*	RS	CSB							+	1			+						
Restionaceae	*Alexgeorgea nitens*	NRS	SSB											+	+			+		
Restionaceae	*Alexgeorgea subterranea*	NRS	SSB																	
Restionaceae	*Chordifex sinuosus/sphacelatus*	RS	SSB			1	1	1	1	1	1	1	+	+	+			+		
Restionaceae	*Desmocladus biformis*	RS	SSB										1							
Restionaceae	*Desmocladus elongatus*	RS	SSB																	
Restionaceae	*Desmocladus myriocladus*	RS	SSB																	
Restionaceae	*Desmocladus parthenicus*	RS	SSB											1						
Restionaceae	*Desmocladus semiplanus*	RS	SSB																	
Restionaceae	*Desmocladus virgatus*	RS	SSB																	
Restionaceae	*Hypolaena exsulca*	RS	SSB																	
Restionaceae	*Lepidobolus chaetocephalus*	RS	SSB																	+
Restionaceae	*Lepidobolus preissianus*	RS	SSB								1	+								
Restionaceae	*Lepidobolus quadratus*	NRS	SSB				+													
Restionaceae	*Loxocarya striata*	RS	SSB			+		+	+	+				+				+		
Rhamnaceae	*Stenanthemum humilis*	RS	SSB																	
Rhamnaceae	*Stenanthemum notiale*	NRS	SSB												+					
Rutaceae	*Boronia crassifolia*	RS	SSB																	
Rutaceae	*Geleznowia verrucosa*	NRS	SSB																1	
Rutaceae	*Philotheca spicata*	RS	SSB																	
Sapindaceae	*Dodonaea ericoides*	RS	SSB																	
Stylidiaceae	*Stylidium adpressum*	NRS	SSB	+	+		+	+								+	+			
Stylidiaceae	*Stylidium carnosum*	NRS	SSB	+																
Stylidiaceae	*Stylidium crossocephalum*	NRS	SSB	1	+	1	1	1	1	1	1	+	+				+	1		
Stylidiaceae	*Stylidium cygnorum*	NRS	SSB																	
Stylidiaceae	*Stylidium diuroides* subspecies *paucifoliatum*	NRS	SSB													+	+		1	
Stylidiaceae	*Stylidium maitlandianum*	NRS	SSB			+		+			+									
Stylidiaceae	*Stylidium repens*	NRS	SSB																	

Family	Species	RS/NRS	SSB/CSB	1.1	1.2	1.3	2.1	2.2	2.3	3.1	3.2	3.3	4.1	4.2	4.3	5.1	5.2	5.3	6.1	6.2
Stylidiaceae	*Stylidium stenosepalum*	NRS	SSB													+				
Thymelaeaceae	*Pimelea angustifolia*	NRS	SSB													+				
Thymelaeaceae	*Pimelea leucantha*	NRS	SSB				+											+		
Thymelaeaceae	*Pimelea ferruginea*	NRS	SSB		+		+										+			
Thymelaeaceae	*Pimelea sulphurea*	NRS	SSB		+		+	+												
Xanthorrhoeaceae	*Xanthorrhoea brunonis*	RS	SSB																	
Xanthorrhoeaceae	*Xanthorrhoea drummondii*	RS	SSB	1	2						2		+	2	1	2	2	1	1	
Zamiaceae	*Macrozamia fraseri*	RS	CSB											+						
Unknown	Unidentified species 01	RS	SSB								+									
Unknown	Unidentified species 02	NRS	SSB																	
Unknown	Unidentified species 03	NRS	SSB																	
Unknown	Unidentified species 04	NRS	SSB																	
Unknown	Unidentified species 05	NRS	SSB																	
Unknown	Unidentified species 06	RS	SSB																	+
Restionaceae	Unidentified species 07	RS	SSB			+	+													
Unknown	Unidentified species 09	NRS	SSB																+	
Restionaceae	Unidentified species 10	RS	SSB																	
Unknown	Unidentified species 11	RS	SSB																	
Unknown	Unidentified species 12	RS	SSB				1							+	1					

Family	Species	RS/NRS	SSB/CSB	6.3	7.1	7.2	7.3	8.1	8.2	8.3	9.1	9.2	9.3	10.1	10.2	10.3	11.1	11.2
Anarthriaceae	*Lyginia barbata*	RS	SSB															
Anarthriaceae	*Lyginia imberbis*	NRS	SSB													+		
Apiaceae	*Platysace juncea*	NRS	SSB															
Apiaceae	*Xanthosia huegelii*	RS	SSB															
Asparagaceae	*Acanthocarpus preissii*	RS	SSB															
Asparagaceae	*Lomandra hastilis*	RS	SSB															
Asparagaceae	*Lomandra micrantha* subspecies *micrantha*	RS	SSB															
Asparagaceae	*Lomandra preissii*	RS	SSB															
Asparagaceae	*Thysanotus dichotomus*	RS	SSB			+								+	+	+		
Asparagaceae	*Thysanotus patersonii*	NRS	SSB												+			
Asparagaceae	*Thysanotus* sp. 1	NRS	SSB															
Asparagaceae	*Thysanotus* sp. 2	NRS	SSB															
Asparagaceae	*Thysanotus triandrus*	RS	SSB															
Asteraceae	*Cephalipterum drummondii*	NRS	SSB			+												
Casuarinaceae	*Allocasuarina campestris*	NRS	CSB							+	3	3	2					
Casuarinaceae	*Allocasuarina humilis*	RS	CSB			1			1	1				+	+	1	+	1
Casuarinaceae	*Allocasuarina microstachya*	RS	CSB		1	1										+		
Cupressaceae	*Callitris acuminata*	RS	CSB		+		2	2	+					+	+		+	+
Cyperaceae	*Baumea juncea*	RS	SSB									+						
Cyperaceae	*Caustis dioica*	NRS	SSB		+	+				+							+	+
Cyperaceae	*Cyathochaeta avenacea*	RS	SSB															1
Cyperaceae	*Lepidosperma apricola*	RS	SSB													+	+	+
Cyperaceae	*Lepidosperma costale*	RS	SSB									1						
Cyperaceae	*Lepidospermum squamatum*	RS	SSB															
Cyperaceae	*Mesomelaena pseudostygia*	RS	SSB		1	1		+		+		1	+	1			1	1
Cyperaceae	*Mesomelaena stygia* subspecies *deflexa*	RS	SSB		+	+									+	+	+	+
Cyperaceae	*Mesomelaena tetragona*	RS	SSB		+	+									+	+	+	+
Cyperaceae	*Schoenus brevisetis*	RS	SSB															
Cyperaceae	*Schoenus curvifolius*	RS	SSB					+	+									
Cyperaceae	*Schoenus grandiflorus*	RS	SSB															
Cyperaceae	*Schoenus pleiostemoneus*	RS	SSB														+	+
Cyperaceae	*Schoenus pedicellatus*	RS	SSB												1	+		
Cyperaceae	*Schoenus subflavus*	RS	SSB		1	1	1	1	1						1	1		
Dasypogonaceae	*Calectasia cyanea*	RS	SSB													+		
Dasypogonaceae	*Dasypogon obliquifolius*	RS	SSB					1	1	1								
Dilleniaceae	*Hibbertia crassifolia*	RS	SSB		+										+	+		
Dilleniaceae	*Hibbertia glomerosa*	RS	SSB															
Dilleniaceae	*Hibbertia hypericoides*	RS	SSB		1	1		1	1	1				1	1	1	2	1
Dilleniaceae	*Hibbertia leucocrossa*	RS	SSB							+								
Dilleniaceae	*Hibbertia rostellata*	NRS	SSB			+				+				+				
Dilleniaceae	*Hibbertia spicata*	RS	SSB		+								+				1	1
Dilleniaceae	*Hibbertia subvaginata*	RS	SSB	+						+								
Droseraceae	*Drosera eneabba*	NRS	SSB					+		+				+				
Droseraceae	*Drosera erythrorhiza*	NRS	SSB								+							
Droseraceae	*Drosera humilis*	NRS	SSB															

Family	Species	RS/NRS	SSB/CSB	6.3	7.1	7.2	7.3	8.1	8.2	8.3	9.1	9.2	9.3	10.1	10.2	10.3	11.1	11.2
Droseraceae	*Drosera menziesii*	NRS	SSB															
Droseraceae	*Drosera porrecta*	NRS	SSB															
Ecdeiocoleaceae	*Ecdeiocolea monostachya*	NRS	SSB								1	1						
Ecdeiocoleaceae	*Georgeantha hexandra*	RS	SSB		1	1						+				+		
Ericaceae	*Andersonia heterophylla*	NRS	SSB	+			1	1	1	1				1	1			
Ericaceae	*Astroloma glaucescens*	NRS	SSB		1													1
Ericaceae	*Astroloma oblongifolium*	RS	SSB															
Ericaceae	*Astroloma serratifolium*	RS	SSB															
Ericaceae	*Astroloma* sp. Eneabba	NRS	SSB										+					
Ericaceae	*Astroloma stomarrhena*	RS	SSB															
Ericaceae	*Astroloma xerophyllum*	NRS	SSB	1				1	1	1				1				
Ericaceae	*Conostephium pendulum*	RS	SSB					+	+									
Ericaceae	*Leucopogon striatus*	NRS	SSB					+	+								+	+
Ericaceae	*Leucopogon crassiflorus*	NRS	SSB															
Ericaceae	*Leucopogon leptanthus*	NRS	SSB															
Ericaceae	*Leucopogon* sp. Carnamah	RS	SSB						1						1	1		
Ericaceae	*Lysinema pentapetalum*	NRS	SSB				1							1	2	1	1	1
Euphorbiaceae	*Stachystemon axillaris*	NRS	SSB															
Fabaceae	*Acacia acuminata*	NRS	SSB															
Fabaceae	*Acacia andrewsii*	NRS	SSB															
Fabaceae	*Acacia auronitens*	NRS	SSB														+	+
Fabaceae	*Acacia barbinervis* subspecies *borealis*	RS	SSB													+		+
Fabaceae	*Acacia fagonioides*	RS	SSB															
Fabaceae	*Acacia lasiocarpa*	NRS	SSB															
Fabaceae	*Acacia latipes* subspecies *latipes*	RS	SSB															
Fabaceae	*Acacia megacephala*	NRS	SSB				+								+			
Fabaceae	*Acacia pulchella*	RS	SSB		+													
Fabaceae	*Acacia stenoptera*	RS	SSB														+	
Fabaceae	*Bossiaea eriocarpa*	NRS	SSB			+			+									
Fabaceae	*Daviesia benthamii*	NRS	SSB						+									
Fabaceae	*Daviesia chapmanii*	RS	SSB															
Fabaceae	*Daviesia daphnoides*	NRS	SSB		2	+												
Fabaceae	*Daviesia debilior*	RS	SSB															
Fabaceae	*Daviesia decurrens*	RS	SSB															
Fabaceae	*Daviesia divaricata*	RS	SSB				+	+						+	+			
Fabaceae	*Daviesia epiphyllum*	RS	SSB		+													
Fabaceae	*Daviesia nudiflora*	RS	SSB							+				1	2		1	
Fabaceae	*Daviesia pedunculata*	RS	SSB											+				
Fabaceae	*Daviesia physodes*	NRS	SSB								1		+					
Fabaceae	*Daviesia podophylla/Daviesia quadrilatera*	NRS	SSB				1		+							+		
Fabaceae	*Daviesia triflora*	RS	SSB															
Fabaceae	Fabaceae species 01	RS	SSB												1		+	
Fabaceae	*Gastrolobium axillare*	RS	SSB								1	1						+
Fabaceae	*Gastrolobium oxylobioides*	RS	SSB													1		+
Fabaceae	*Gastrolobium polystachyum/Cristonia biloba*	NRS	SSB		+	+											+	+
Fabaceae	*Gastrolobium spinosum*	NRS	SSB															
Fabaceae	*Gompholobium confertum*	RS	SSB															
Fabaceae	*Gompholobium preissii*	NRS	SSB															
Fabaceae	*Gompholobium tomentosum*	RS	SSB	1		1	1				1	1						
Fabaceae	*Hardenbergia comptoniana*	NRS	SSB															

Family	Species	RS/NRS	SSB/CSB	6.3	7.1	7.2	7.3	8.1	8.2	8.3	9.1	9.2	9.3	10.1	10.2	10.3	11.1	11.2
Fabaceae	*Jacksonia condensata*	RS	SSB															
Fabaceae	*Jacksonia floribunda*	RS	SSB				+		+					1	1			
Fabaceae	*Jacksonia furcellata*	RS	SSB	1														
Fabaceae	*Jacksonia lehmannii*	RS	SSB		+					+					1			
Fabaceae	*Mirbelia spinosa*	RS	SSB															
Frankeniaceae	*Frankenia pauciflora*	NRS	SSB			1												
Goodeniaceae	*Dampiera juncea*	RS	SSB											1			+	
Goodeniaceae	*Dampiera lindleyi*	RS	SSB											+				
Goodeniaceae	*Dampiera spicigera*	NRS	SSB				+											
Goodeniaceae	*Lechenaultia biloba*	RS	SSB	1														
Goodeniaceae	*Lechenaultia hirsuta*	?	SSB															
Goodeniaceae	*Lechenaultia stenosepala*	NRS	SSB															
Goodeniaceae	*Scaevola repens* subspecies Northern Sandplains	RS	SSB															
Goodeniaceae	*Scaevola species 01*	RS	SSB															
Haemodoraceae	*Anigozanthos humilis*	RS	SSB				+			+								
Haemodoraceae	*Anigozanthos* species 01	RS	SSB		+													+
Haemodoraceae	*Blancoa canescens*	RS	SSB	+														
Haemodoraceae	*Conostylis aurea*	RS	SSB				+			1								
Haemodoraceae	*Conostylis hiemalis*	RS	SSB				1							1				
Haemodoraceae	*Conostylis latens*	RS	SSB					1	+	1					1			
Haemodoraceae	*Conostylis micrantha*	RS	SSB													1	1	+
Haemodoraceae	*Conostylis neocymosa*	RS	SSB	+														
Haemodoraceae	Haemodorum species 01	RS	SSB		1					+					1	+		+
Haemodoraceae	*Macropidia fuliginosa*	RS	SSB															
Haloragaceae	*Glischrocaryon aureum*	RS	SSB			1												
Hemerocallidaceae	*Johnsonia pubescens*	RS	SSB						+									
Iridiaceae	*Patersonia juncea*	RS	SSB						+									
Lamiaceae	*Hemiandra* sp Eneabba	RS	SSB				+	+						+	+			
Lamiaceae	*Hemiphora bartlingii*	RS	SSB							+								
Lamiaceae	*Lachnostachys eriobotrya*	RS	SSB															
Lamiaceae	*Microcorys* sp. Coomallo	RS	SSB														1	+
Lamiaceae	*Pityrodia hemigenioides*	RS	SSB															
Lauraceae	*Cassytha* sp.	NRS	SSB			+	1	1	1	1	+		1	1	1		+	+
Malvaceae	*Guichenotia micrantha*	RS	SSB								1							
Malvaceae	*Lasiopetalum drummondii*	RS	SSB							+					+			+
Malvaceae	*Thomasia grandiflora*	RS	SSB															
Myrtaceae	*Baeckea grandis*	RS	SSB		+					+					+	+		+
Myrtaceae	*Beaufortia bracteosa*	NRS	CSB															
Myrtaceae	*Beaufortia elegans*	NRS	CSB				1		1	1			+	2	1	2	+	+
Myrtaceae	*Calothamnus glaber*	RS	CSB									+	+					
Myrtaceae	*Calothamnus quadrifidus* subspecies *angustifolius*	RS	CSB															
Myrtaceae	*Calothamnus sanguineus*	RS	CSB						+					+	+	+	+	1
Myrtaceae	*Calothamnus torulosus*	RS	CSB		1					+						+	1	1
Myrtaceae	*Calytrix aurea*	NRS	SSB															+
Myrtaceae	*Calytrix chrysantha*	NRS	SSB								1	+		1				
Myrtaceae	*Calytrix depressa*	RS	SSB															
Myrtaceae	*Calytrix drummondii*	NRS	SSB															
Myrtaceae	*Calytrix glutinosa*	NRS	SSB								+	1						
Myrtaceae	*Calytrix sapphirina*	NRS	SSB	+														
Myrtaceae	*Calytrix superba*	RS	SSB															

Family	Species	RS/NRS	SSB/CSB	6.3	7.1	7.2	7.3	8.1	8.2	8.3	9.1	9.2	9.3	10.1	10.2	10.3	11.1	11.2
Myrtaceae	*Chamelaucium* sp. Bunjil	RS	SSB															
Myrtaceae	*Conothamnus trinervis*	RS	CSB		+					+				+	+	+		+
Myrtaceae	*Darwinia capitellata*	NRS	SSB															
Myrtaceae	*Darwinia neildiana*	NRS	SSB		1	+											+	1
Myrtaceae	*Darwinia sanguinea*	NRS	SSB		+		1		+	+				1	1		+	1
Myrtaceae	*Darwinia speciosa*	RS	SSB				+	1	+					1				
Myrtaceae	*Eremaea beaufortioides*	RS	CSB	+		+	1			+				1				
Myrtaceae	*Eremaea ectadioclada*	NRS	CSB	+			+	+	+							+		
Myrtaceae	*Eremaea hadra*	RS	CSB															
Myrtaceae	*Eremaea pauciflora*	RS	CSB					1	+									
Myrtaceae	*Eremaea violacea*	RS	CSB		+	+				+						+	+	1
Myrtaceae	*Eucalyptus celastroides*	RS	CSB															
Myrtaceae	*Eucalyptus pleurocarpa/xtetragona*	RS	CSB													2		
Myrtaceae	*Eucalyptus gittinsii*	RS	CSB								1							
Myrtaceae	*Eucalyptus leptopoda*	RS	CSB								1	+						
Myrtaceae	*Eucalyptus todtiana*	RS	CSB															
Myrtaceae	*Homalocalyx chapmanii*	RS	SSB															
Myrtaceae	*Hypocalymma gardneri*	NRS	SSB		1													+
Myrtaceae	*Leptospermum erubescens*	RS	CSB				+											
Myrtaceae	*Leptospermum spinescens*	NRS	CSB			+			+					+	+		+	+
Myrtaceae	*Malleostemon roseus*	NRS	SSB					+										
Myrtaceae	*Melaleuca ciliosa*	NRS	CSB	1														
Myrtaceae	*Melaleuca systena*	RS	CSB		+									+				
Myrtaceae	*Melaleuca leuropoma*	RS	CSB				1		+						1		+	
Myrtaceae	*Melaleuca orbicularis*	RS	CSB															
Myrtaceae	*Melaleuca tinkeri*	RS	CSB								1	2	2					
Myrtaceae	*Melaleuca trichophylla*	RS	CSB		1	1			+	1							1	+
Myrtaceae	*Melaleuca urceolaris*	NRS	CSB										+					
Myrtaceae	*Melaleuca zonalis*	RS	CSB									+						
Myrtaceae	Myrtaceae species 01	NRS	SSB															
Myrtaceae	Myrtaceae species 02	NRS	SSB															
Myrtaceae	*Phymatocarpus porphyrocephalus*	NRS	SSB		+													
Myrtaceae	*Pileanthus filifolius*	RS	SSB											+	+		+	
Myrtaceae	*Scholtzia involucrata*	RS	SSB															
Myrtaceae	*Scholtzia laxiflora*	NRS	SSB															
Myrtaceae	*Scholtzia leptantha*	NRS	SSB															
Myrtaceae	*Scholtzia* sp. Eneabba	NRS	SSB								2	2	+					
Myrtaceae	*Scholtzia* sp. Wongonderrah	NRS	SSB															
Myrtaceae	*Thryptomene cuspidata*	NRS	SSB															
Myrtaceae	*Verticordia argentea*	NRS	SSB	1														
Myrtaceae	*Verticordia densiflora*	RS	SSB													+		
Myrtaceae	*Verticordia eriocephala*	NRS	SSB									1						
Myrtaceae	*Verticordia grandis*	NRS	SSB				+	+		+				+	+			
Myrtaceae	*Verticordia huegelii*	NRS	SSB									+						
Myrtaceae	*Verticordia nobilis*	NRS	SSB															
Myrtaceae	*Verticordia ovalifolia*	NRS	SSB						+									
Myrtaceae	*Verticordia pennigera*	RS	SSB			+												
Olacaceae	*Olax benthamiana*	NRS	SSB				+											
Olacaceae	*Olax scalariformis*	NRS	SSB															
Poaceae	*Amphipogon turbinatus*	RS	SSB		+		+	+	+					+	+		+	+

Family	Species	RS/NRS	SSB/CSB	6.3	7.1	7.2	7.3	8.1	8.2	8.3	9.1	9.2	9.3	10.1	10.2	10.3	11.1	11.2
Poaceae	*Neurachne alopecuroidea*	RS	SSB															
Polygalaceae	*Comesperma acerosum*	NRS	SSB							+								
Proteaceae	*Adenanthos cygnorum* subspecies *cygnorum*	NRS	SSB	1				1	1									
Proteaceae	*Banksia armata* var. *armata*	RS	CSB															
Proteaceae	*Banksia attenuata*	NRS	CSB	1			+	1	1	+								
Proteaceae	*Banksia bipinnatifida*	RS	CSB													+	+	
Proteaceae	*Banksia candolleana*	RS	CSB					+	1	+				1				
Proteaceae	*Banksia carlinoides*	RS	CSB		2	1							2					
Proteaceae	*Banksia chamaephyton*	RS	CSB			+												
Proteaceae	*Banksia cypholoba*	RS	CSB				+									+	+	
Proteaceae	*Banksia densa*	RS	CSB			+												
Proteaceae	*Banksia glaucifolia*	NRS	CSB		1											2	1	1
Proteaceae	*Banksia grossa*	RS	CSB				+		+	1						1		1
Proteaceae	*Banksia hookeriana*	NRS	CSB	+														
Proteaceae	*Banksia incana*	RS	CSB						+					1	1			
Proteaceae	*Banksia kippistiana* var. *kippistiana*	RS	CSB										1			+		
Proteaceae	*Banksia leptophylla*	NRS	CSB			+	1	1								1	1	
Proteaceae	*Banksia menziesii*	RS	CSB	1				+										
Proteaceae	*Banksia micrantha*	RS	CSB		+	+									1	+		
Proteaceae	*Banksia nana*	RS	CSB													+		
Proteaceae	*Banksia nivea*	RS	CSB						+									
Proteaceae	*Banksia sclerophylla*	RS	CSB															
Proteaceae	*Banksia sessilis*	NRS	CSB										+					
Proteaceae	*Banksia shuttleworthiana*	RS	CSB		+		+				+			+	+		1	1
Proteaceae	*Banksia tortifolia*	RS	CSB					1	+					+	+			
Proteaceae	*Banksia tridentata*	RS	CSB													+		
Proteaceae	*Conospermum crassinervium*	NRS	SSB	1				1	1									
Proteaceae	*Conospermum nervosum*	NRS	SSB															
Proteaceae	*Conospermum unilaterale*	RS	SSB											+				
Proteaceae	*Conospermum wycherleyi* subspecies *wycherleyi*	RS	SSB				+			+				2	+			
Proteaceae	*Grevillea eriostachya*	RS	SSB												+			
Proteaceae	*Grevillea rudis*	RS	SSB		1	1												
Proteaceae	*Grevillea shuttleworthiana*	RS	SSB															
Proteaceae	*Grevillea synapheae* subspecies *pachyphylla*	RS	SSB													+	+	+
Proteaceae	*Grevillea uniformis*	NRS	SSB		+	+												
Proteaceae	*Hakea auriculata*	RS	CSB		+	+										+		1
Proteaceae	*Hakea candolleana*	RS	CSB												+			
Proteaceae	*Hakea conchifolia*	RS	CSB		+	+				1						1	+	1
Proteaceae	*Hakea costata*	NRS	CSB													+		
Proteaceae	*Hakea eneabba*	RS	CSB				+	1						+		+		
Proteaceae	*Hakea flabellifolia*	RS	CSB				+		+	+						+		
Proteaceae	*Hakea gilbertii*	NRS	CSB								1	1	1					
Proteaceae	*Hakea incrassata*	RS	CSB		+		+					+				+	1	1
Proteaceae	*Hakea neospathulata*	RS	CSB															
Proteaceae	*Hakea obliqua* subspecies *parviflora*	RS	CSB															
Proteaceae	*Hakea polyanthema*	NRS	CSB															
Proteaceae	*Hakea psilorrhyncha*	NRS	CSB											+				
Proteaceae	*Hakea smilacifolia*	NRS	CSB															+
Proteaceae	*Hakea stenocarpa*	RS	CSB															
Proteaceae	*Hakea trifurcata*	NRS	CSB															

Family	Species	RS/NRS	SSB/CSB	6.3	7.1	7.2	7.3	8.1	8.2	8.3	9.1	9.2	9.3	10.1	10.2	10.3	11.1	11.2
Proteaceae	*Isopogon adenanthoides*	NRS	CSB													+	+	+
Proteaceae	*Isopogon inconspicuus*	RS	CSB		+													
Proteaceae	*Isopogon linearis*	RS	CSB															+
Proteaceae	*Isopogon tridens*	RS	CSB															
Proteaceae	*Lambertia multiflora*	RS	CSB		+					2				+	+	1	1	1
Proteaceae	*Persoonia acicularis*	RS	SSB											+				+
Proteaceae	*Persoonia comata*	RS	SSB									+						
Proteaceae	*Persoonia filiformis*	RS	SSB															
Proteaceae	*Petrophile aculeata*	NRS	CSB															+
Proteaceae	*Petrophile brevifolia*	RS	CSB		+	+	1							+	1	+		
Proteaceae	*Petrophile drummondii*	NRS	CSB															
Proteaceae	*Petrophile linearis*	NRS	CSB				+	1	+					+		+		
Proteaceae	*Petrophile macrostachya*	RS	CSB				+	+	+					+	+	+		
Proteaceae	*Petrophile pilostyla*	RS	CSB															
Proteaceae	*Petrophile rigida*	RS	CSB		+													1
Proteaceae	*Petrophile seminuda*	RS	CSB									1	1					
Proteaceae	*Petrophile serruriae*	NRS	CSB															
Proteaceae	*Petrophile shuttleworthiana*	RS	CSB		+	+												1
Proteaceae	*Stirlingia latifolia*	RS	SSB	+			+	1	+									
Proteaceae	*Strangea cynanchicarpa*	RS	CSB															
Proteaceae	*Synaphea aephynsa*	RS	SSB															
Proteaceae	*Synaphea spinulosa* subspecies *spinulosa*	RS	SSB														+	
Proteaceae	*Xylomelum angustifolium*	RS	CSB				1							+				
Restionaceae	*Alexgeorgea nitens*	NRS	SSB	+			+		1									
Restionaceae	*Alexgeorgea subterranea*	NRS	SSB					1	1	1		+						
Restionaceae	*Chordifex sinuosus/sphacelatus*	RS	SSB	+			+	1	1	1				1	1	+		+
Restionaceae	*Desmocladus biformis*	RS	SSB															
Restionaceae	*Desmocladus elongatus*	RS	SSB														+	1
Restionaceae	*Desmocladus myriocladus*	RS	SSB															
Restionaceae	*Desmocladus parthenicus*	RS	SSB															
Restionaceae	*Desmocladus semiplanus*	RS	SSB															
Restionaceae	*Desmocladus virgatus*	RS	SSB															
Restionaceae	*Hypolaena exsulca*	RS	SSB						1					+				
Restionaceae	*Lepidobolus chaetocephalus*	RS	SSB															
Restionaceae	*Lepidobolus preissianus*	RS	SSB															
Restionaceae	*Lepidobolus quadratus*	NRS	SSB															
Restionaceae	*Loxocarya striata*	RS	SSB				+		+					+				
Rhamnaceae	*Stenanthemum humilis*	RS	SSB															
Rhamnaceae	*Stenanthemum notiale*	NRS	SSB															
Rutaceae	*Boronia crassifolia*	RS	SSB															
Rutaceae	*Geleznowia verrucosa*	NRS	SSB															
Rutaceae	*Philotheca spicata*	RS	SSB							+								
Sapindaceae	*Dodonaea ericoides*	RS	SSB															
Stylidiaceae	*Stylidium adpressum*	NRS	SSB						1									
Stylidiaceae	*Stylidium carnosum*	NRS	SSB															
Stylidiaceae	*Stylidium crossocephalum*	NRS	SSB						1	1				1	+			
Stylidiaceae	*Stylidium cygnorum*	NRS	SSB		+													
Stylidiaceae	*Stylidium diuroides* subspecies *paucifoliatum*	NRS	SSB		+	1												
Stylidiaceae	*Stylidium maitlandianum*	NRS	SSB															
Stylidiaceae	*Stylidium repens*	NRS	SSB											+				

Family	Species	RS/NRS	SSB/CSB	6.3	7.1	7.2	7.3	8.1	8.2	8.3	9.1	9.2	9.3	10.1	10.2	10.3	11.1	11.2
Stylidiaceae	*Stylidium stenosepalum*	NRS	SSB															
Thymelaeaceae	*Pimelea angustifolia*	NRS	SSB															
Thymelaeaceae	*Pimelea leucantha*	NRS	SSB							+								
Thymelaeaceae	*Pimelea ferruginea*	NRS	SSB		+													
Thymelaeaceae	*Pimelea sulphurea*	NRS	SSB							+				+	+		+	+
Xanthorrhoeaceae	*Xanthorrhoea brunonis*	RS	SSB				+											
Xanthorrhoeaceae	*Xanthorrhoea drummondii*	RS	SSB		2	2	1	+		2	1	1		2	2	2	2	2
Zamiaceae	*Macrozamia fraseri*	RS	CSB															
Unknown	Unidentified species 01	RS	SSB															
Unknown	Unidentified species 02	NRS	SSB		+													
Unknown	Unidentified species 03	NRS	SSB															
Unknown	Unidentified species 04	NRS	SSB															
Unknown	Unidentified species 05	NRS	SSB															
Unknown	Unidentified species 06	RS	SSB															
Restionaceae	Unidentified species 07	RS	SSB		+													
Unknown	Unidentified species 09	NRS	SSB															
Restionaceae	Unidentified species 10	RS	SSB															+
Unknown	Unidentified species 11	RS	SSB															
Unknown	Unidentified species 12	RS	SSB															

Family	Species	RS/NRS	SSB/CSB	11.3	12.1	12.2	12.3	13.1	13.2	13.3	14.1	14.2	14.3	15.1	15.2	15.3	16.1
Anarthriaceae	*Lyginia barbata*	RS	SSB														1
Anarthriaceae	*Lyginia imberbis*	NRS	SSB								+	+		+			
Apiaceae	*Platysace juncea*	NRS	SSB										1				+
Apiaceae	*Xanthosia huegelii*	RS	SSB														
Asparagaceae	*Acanthocarpus preissii*	RS	SSB														
Asparagaceae	*Lomandra hastilis*	RS	SSB														
Asparagaceae	*Lomandra micrantha* subspecies *micrantha*	RS	SSB									+					
Asparagaceae	*Lomandra preissii*	RS	SSB														
Asparagaceae	*Thysanotus dichotomus*	RS	SSB														
Asparagaceae	*Thysanotus patersonii*	NRS	SSB														
Asparagaceae	*Thysanotus* sp. 1	NRS	SSB														
Asparagaceae	*Thysanotus* sp. 2	NRS	SSB														
Asparagaceae	*Thysanotus triandrus*	RS	SSB														
Asteraceae	*Cephalipterum drummondii*	NRS	SSB														
Casuarinaceae	*Allocasuarina campestris*	NRS	CSB														
Casuarinaceae	*Allocasuarina humilis*	RS	CSB	+	+	+	+	1	+	+	+	1	+	2	+	1	1
Casuarinaceae	*Allocasuarina microstachya*	RS	CSB		+	1	1	1	1	+						+	+
Cupressaceae	*Callitris acuminata*	RS	CSB	+	+	+			+			1		+	1		+
Cyperaceae	*Baumea juncea*	RS	SSB		+												
Cyperaceae	*Caustis dioica*	NRS	SSB			+	1		1	+	+			1			
Cyperaceae	*Cyathochaeta avenacea*	RS	SSB			1		1	+	+	1				+	+	
Cyperaceae	*Lepidosperma apricola*	RS	SSB	+					+					+		+	
Cyperaceae	*Lepidosperma costale*	RS	SSB														
Cyperaceae	*Lepidospermum squamatum*	RS	SSB														
Cyperaceae	*Mesomelaena pseudostygia*	RS	SSB	1	1	+	1	3	1	+	+	1		1	+	1	1
Cyperaceae	*Mesomelaena stygia* subspecies *deflexa*	RS	SSB	+					+							+	+
Cyperaceae	*Mesomelaena tetragona*	RS	SSB	+					+							+	+
Cyperaceae	*Schoenus brevisetis*	RS	SSB														
Cyperaceae	*Schoenus curvifolius*	RS	SSB														
Cyperaceae	*Schoenus grandiflorus*	RS	SSB														
Cyperaceae	*Schoenus pleiostemoneus*	RS	SSB						1					+		1	+
Cyperaceae	*Schoenus pedicellatus*	RS	SSB					+								+	
Cyperaceae	*Schoenus subflavus*	RS	SSB		1	1	1	1	1	1	1	1	1	1	1	1	1
Dasypogonaceae	*Calectasia cyanea*	RS	SSB				+				+					+	
Dasypogonaceae	*Dasypogon obliquifolius*	RS	SSB													+	
Dilleniaceae	*Hibbertia crassifolia*	RS	SSB	+						+					+	1	+
Dilleniaceae	*Hibbertia glomerosa*	RS	SSB														
Dilleniaceae	*Hibbertia hypericoides*	RS	SSB	1	1	1	1	1	1	1	1	1	1	1	2	2	1
Dilleniaceae	*Hibbertia leucocrossa*	RS	SSB								1	1	1		1		+
Dilleniaceae	*Hibbertia rostellata*	NRS	SSB					+								+	
Dilleniaceae	*Hibbertia spicata*	RS	SSB	1				+		+							
Dilleniaceae	*Hibbertia subvaginata*	RS	SSB	1				+	+		+					1	
Droseraceae	*Drosera eneabba*	NRS	SSB														
Droseraceae	*Drosera erythrorhiza*	NRS	SSB														
Droseraceae	*Drosera humilis*	NRS	SSB														

Family	Species	RS/NRS	SSB/CSB	11.3	12.1	12.2	12.3	13.1	13.2	13.3	14.1	14.2	14.3	15.1	15.2	15.3	16.1
Droseraceae	*Drosera menziesii*	NRS	SSB														
Droseraceae	*Drosera porrecta*	NRS	SSB														
Ecdeiocoleaceae	*Ecdeiocolea monostachya*	NRS	SSB			1			1								+
Ecdeiocoleaceae	*Georgeantha hexandra*	RS	SSB		1	1	+	1	+	2							
Ericaceae	*Andersonia heterophylla*	NRS	SSB						+			1	1		+		1
Ericaceae	*Astroloma glaucescens*	NRS	SSB		1	1	1	1		1	1	+				+	
Ericaceae	*Astroloma oblongifolium*	RS	SSB														
Ericaceae	*Astroloma serratifolium*	RS	SSB		+											+	
Ericaceae	*Astroloma* sp. Eneabba	NRS	SSB														
Ericaceae	*Astroloma stomarrhena*	RS	SSB												+		
Ericaceae	*Astroloma xerophyllum*	NRS	SSB												+		
Ericaceae	*Conostephium pendulum*	RS	SSB												+		+
Ericaceae	*Leucopogon striatus*	NRS	SSB	+	+		1		1		2						
Ericaceae	*Leucopogon crassiflorus*	NRS	SSB													1	
Ericaceae	*Leucopogon leptanthus*	NRS	SSB									1	1				
Ericaceae	*Leucopogon* sp. Carnamah	RS	SSB									1	1		+		1
Ericaceae	*Lysinema pentapetalum*	NRS	SSB	1	+				1			1	+				1
Euphorbiaceae	*Stachystemon axillaris*	NRS	SSB											1		1	
Fabaceae	*Acacia acuminata*	NRS	SSB														
Fabaceae	*Acacia andrewsii*	NRS	SSB						+	+							
Fabaceae	*Acacia auronitens*	NRS	SSB						+								
Fabaceae	*Acacia barbinervis* subspecies *borealis*	RS	SSB														
Fabaceae	*Acacia fagonioides*	RS	SSB												+		
Fabaceae	*Acacia lasiocarpa*	NRS	SSB														
Fabaceae	*Acacia latipes* subspecies *latipes*	RS	SSB														
Fabaceae	*Acacia megacephala*	NRS	SSB										+				
Fabaceae	*Acacia pulchella*	RS	SSB														+
Fabaceae	*Acacia stenoptera*	RS	SSB	+													
Fabaceae	*Bossiaea eriocarpa*	NRS	SSB												+		
Fabaceae	*Daviesia benthamii*	NRS	SSB														
Fabaceae	*Daviesia chapmanii*	RS	SSB		+						+						
Fabaceae	*Daviesia daphnoides*	NRS	SSB			2	+		1	2	2						
Fabaceae	*Daviesia debilior*	RS	SSB					+	1								
Fabaceae	*Daviesia decurrens*	RS	SSB			1											
Fabaceae	*Daviesia divaricata*	RS	SSB					+				1	+	+			
Fabaceae	*Daviesia epiphyllum*	RS	SSB			+	+	+	1	1							
Fabaceae	*Daviesia nudiflora*	RS	SSB			1				1	1	1	+	+	+		1
Fabaceae	*Daviesia pedunculata*	RS	SSB						+						1		
Fabaceae	*Daviesia physodes*	NRS	SSB														
Fabaceae	*Daviesia podophylla/Daviesia quadrilatera*	NRS	SSB												+		+
Fabaceae	*Daviesia triflora*	RS	SSB														
Fabaceae	Fabaceae species 01	RS	SSB														
Fabaceae	*Gastrolobium axillare*	RS	SSB							+							
Fabaceae	*Gastrolobium oxylobioides*	RS	SSB	+													
Fabaceae	*Gastrolobium polystachyum/Cristonia biloba*	NRS	SSB	+					+	+	+						
Fabaceae	*Gastrolobium spinosum*	NRS	SSB														
Fabaceae	*Gompholobium confertum*	RS	SSB					+	+		+						
Fabaceae	*Gompholobium preissii*	NRS	SSB														
Fabaceae	*Gompholobium tomentosum*	RS	SSB											+			
Fabaceae	*Hardenbergia comptoniana*	NRS	SSB	1							+						

Family	Species	RS/NRS	SSB/CSB	11.3	12.1	12.2	12.3	13.1	13.2	13.3	14.1	14.2	14.3	15.1	15.2	15.3	16.1
Fabaceae	*Jacksonia condensata*	RS	SSB		1												
Fabaceae	*Jacksonia floribunda*	RS	SSB										+		+		
Fabaceae	*Jacksonia furcellata*	RS	SSB														
Fabaceae	*Jacksonia lehmannii*	RS	SSB		+		+				1					+	
Fabaceae	*Mirbelia spinosa*	RS	SSB														
Frankeniaceae	*Frankenia pauciflora*	NRS	SSB														
Goodeniaceae	*Dampiera juncea*	RS	SSB	+													
Goodeniaceae	*Dampiera lindleyi*	RS	SSB					+			+			+			
Goodeniaceae	*Dampiera spicigera*	NRS	SSB														
Goodeniaceae	*Lechenaultia biloba*	RS	SSB														
Goodeniaceae	*Lechenaultia hirsuta*	?	SSB									+					
Goodeniaceae	*Lechenaultia stenosepala*	NRS	SSB									+	+				
Goodeniaceae	*Scaevola repens* subspecies Northern Sandplains	RS	SSB														+
Goodeniaceae	*Scaevola species 01*	RS	SSB														+
Haemodoraceae	*Anigozanthos humilis*	RS	SSB														
Haemodoraceae	*Anigozanthos* species 01	RS	SSB						+								
Haemodoraceae	*Blancoa canescens*	RS	SSB									+			1		
Haemodoraceae	*Conostylis aurea*	RS	SSB						+	+	+	1	+			+	
Haemodoraceae	*Conostylis hiemalis*	RS	SSB							+			+				
Haemodoraceae	*Conostylis latens*	RS	SSB						+			+	+		+		1
Haemodoraceae	*Conostylis micrantha*	RS	SSB			+										+	
Haemodoraceae	*Conostylis neocymosa*	RS	SSB														
Haemodoraceae	*Haemodorum* species 01	RS	SSB	+							+						
Haemodoraceae	*Macropidia fuliginosa*	RS	SSB														
Haloragaceae	*Glischrocaryon aureum*	RS	SSB														
Hemerocallidaceae	*Johnsonia pubescens*	RS	SSB		+												
Iridiaceae	*Patersonia juncea*	RS	SSB														
Lamiaceae	*Hemiandra* sp Eneabba	RS	SSB									+					+
Lamiaceae	*Hemiphora bartlingii*	RS	SSB									+					
Lamiaceae	*Lachnostachys eriobotrya*	RS	SSB														
Lamiaceae	*Microcorys* sp. Coomallo	RS	SSB	+				+								1	
Lamiaceae	*Pityrodia hemigenioides*	RS	SSB														
Lauraceae	*Cassytha* sp.	NRS	SSB	1	+		+		+	+		1	1	1	1		+
Malvaceae	*Guichenotia micrantha*	RS	SSB														
Malvaceae	*Lasiopetalum drummondii*	RS	SSB					1	+		+				+	1	
Malvaceae	*Thomasia grandiflora*	RS	SSB														
Myrtaceae	*Baeckea grandis*	RS	SSB														
Myrtaceae	*Beaufortia bracteosa*	NRS	CSB		1	1	1	1	1	9							
Myrtaceae	*Beaufortia elegans*	NRS	CSB		1	1	+		+		+	1	1	1	1	1	2
Myrtaceae	*Calothamnus glaber*	RS	CSB														
Myrtaceae	*Calothamnus quadrifidus* subspecies *angustifolius*	RS	CSB														
Myrtaceae	*Calothamnus sanguineus*	RS	CSB	+			+	+	+	+		+		1	+		+
Myrtaceae	*Calothamnus torulosus*	RS	CSB	1	1		+	+		1	1					1	
Myrtaceae	*Calytrix aurea*	NRS	SSB														
Myrtaceae	*Calytrix chrysantha*	NRS	SSB														
Myrtaceae	*Calytrix depressa*	RS	SSB													+	
Myrtaceae	*Calytrix drummondii*	NRS	SSB														
Myrtaceae	*Calytrix glutinosa*	NRS	SSB														
Myrtaceae	*Calytrix sapphirina*	NRS	SSB														
Myrtaceae	*Calytrix superba*	RS	SSB														

Family	Species	RS/NRS	SSB/CSB	11.3	12.1	12.2	12.3	13.1	13.2	13.3	14.1	14.2	14.3	15.1	15.2	15.3	16.1
Myrtaceae	*Chamelaucium* sp. Bunjil	RS	SSB									+				+	
Myrtaceae	*Conothamnus trinervis*	RS	CSB	+		1			+	+					+	+	+
Myrtaceae	*Darwinia capitellata*	NRS	SSB				1									+	
Myrtaceae	*Darwinia neildiana*	NRS	SSB	1	1	+	+	+	1		+	+					+
Myrtaceae	*Darwinia sanguinea*	NRS	SSB	1											+	+	+
Myrtaceae	*Darwinia speciosa*	RS	SSB									+					
Myrtaceae	*Eremaea beaufortioides*	RS	CSB		+			1	1		+					+	
Myrtaceae	*Eremaea ectadioclada*	NRS	CSB										+		+		+
Myrtaceae	*Eremaea hadra*	RS	CSB														
Myrtaceae	*Eremaea pauciflora*	RS	CSB														
Myrtaceae	*Eremaea violacea*	RS	CSB	+							+	+	+	+	+		+
Myrtaceae	*Eucalyptus celastroides*	RS	CSB														2
Myrtaceae	*Eucalyptus pleurocarpa/xtetragona*	RS	CSB			+	+	1	+	1							
Myrtaceae	*Eucalyptus gittinsii*	RS	CSB														
Myrtaceae	*Eucalyptus leptopoda*	RS	CSB														
Myrtaceae	*Eucalyptus todtiana*	RS	CSB									1			1		
Myrtaceae	*Homalocalyx chapmanii*	RS	SSB														
Myrtaceae	*Hypocalymma gardneri*	NRS	SSB	+	1		+				1				+		
Myrtaceae	*Leptospermum erubescens*	RS	CSB														
Myrtaceae	*Leptospermum spinescens*	NRS	CSB	+	+			+	+			+	+	+	+		+
Myrtaceae	*Malleostemon roseus*	NRS	SSB														
Myrtaceae	*Melaleuca ciliosa*	NRS	CSB					+									
Myrtaceae	*Melaleuca systena*	RS	CSB														
Myrtaceae	*Melaleuca leuropoma*	RS	CSB									1	1	1			+
Myrtaceae	*Melaleuca orbicularis*	RS	CSB														
Myrtaceae	*Melaleuca tinkeri*	RS	CSB														
Myrtaceae	*Melaleuca trichophylla*	RS	CSB	1	2	2	1	1			1				1	+	
Myrtaceae	*Melaleuca urceolaris*	NRS	CSB														
Myrtaceae	*Melaleuca zonalis*	RS	CSB					+	1	1						+	
Myrtaceae	Myrtaceae species 01	NRS	SSB														
Myrtaceae	Myrtaceae species 02	NRS	SSB														
Myrtaceae	*Phymatocarpus porphyrocephalus*	NRS	SSB														
Myrtaceae	*Pileanthus filifolius*	RS	SSB									1	+	+	1	+	
Myrtaceae	*Scholtzia involucrata*	RS	SSB														
Myrtaceae	*Scholtzia laxiflora*	NRS	SSB			1											
Myrtaceae	*Scholtzia leptantha*	NRS	SSB											1			
Myrtaceae	*Scholtzia* sp. Eneabba	NRS	SSB														
Myrtaceae	*Scholtzia* sp. Wongonderrah	NRS	SSB												+		
Myrtaceae	*Thryptomene cuspidata*	NRS	SSB														
Myrtaceae	*Verticordia argentea*	NRS	SSB														1
Myrtaceae	*Verticordia densiflora*	RS	SSB						1						+		
Myrtaceae	*Verticordia eriocephala*	NRS	SSB														
Myrtaceae	*Verticordia grandis*	NRS	SSB									+	+	+			
Myrtaceae	*Verticordia huegelii*	NRS	SSB														
Myrtaceae	*Verticordia nobilis*	NRS	SSB														
Myrtaceae	*Verticordia ovalifolia*	NRS	SSB												+		
Myrtaceae	*Verticordia pennigera*	RS	SSB														
Olacaceae	*Olax benthamiana*	NRS	SSB														
Olacaceae	*Olax scalariformis*	NRS	SSB												+		+
Poaceae	*Amphipogon turbinatus*	RS	SSB										+	+	+		+

Family	Species	RS/NRS	SSB/CSB	11.3	12.1	12.2	12.3	13.1	13.2	13.3	14.1	14.2	14.3	15.1	15.2	15.3	16.1
Poaceae	*Neurachne alopecuroidea*	RS	SSB														
Polygalaceae	*Comesperma acerosum*	NRS	SSB									+					
Proteaceae	*Adenanthos cygnorum* subspecies *cygnorum*	NRS	SSB										1				1
Proteaceae	*Banksia armata* var. *armata*	RS	CSB														
Proteaceae	*Banksia attenuata*	NRS	CSB										1		+		
Proteaceae	*Banksia bipinnatifida*	RS	CSB	+	+				+		+						
Proteaceae	*Banksia candolleana*	RS	CSB										1	+	1		
Proteaceae	*Banksia carlinoides*	RS	CSB		1	+	2	1	+	1	1						
Proteaceae	*Banksia chamaephyton*	RS	CSB			+		+	+							+	
Proteaceae	*Banksia cypholoba*	RS	CSB									+					
Proteaceae	*Banksia densa*	RS	CSB			+		+	+	+							
Proteaceae	*Banksia glaucifolia*	NRS	CSB	1	+	1	1	1		1	+						
Proteaceae	*Banksia grossa*	RS	CSB	1								+	1	1	+	1	1
Proteaceae	*Banksia hookeriana*	NRS	CSB														
Proteaceae	*Banksia incana*	RS	CSB											+	+	1	
Proteaceae	*Banksia kippistiana* var. *kippistiana*	RS	CSB		+	1	2				+						
Proteaceae	*Banksia leptophylla*	NRS	CSB		1	+	+	+	1	+						1	
Proteaceae	*Banksia menziesii*	RS	CSB														
Proteaceae	*Banksia micrantha*	RS	CSB		1	+	+	+	+		+					+	
Proteaceae	*Banksia nana*	RS	CSB														
Proteaceae	*Banksia nivea*	RS	CSB			+				+					+	+	+
Proteaceae	*Banksia sclerophylla*	RS	CSB													1	
Proteaceae	*Banksia sessilis*	NRS	CSB														
Proteaceae	*Banksia shuttleworthiana*	RS	CSB		+	1		1	1		1					1	1
Proteaceae	*Banksia tortifolia*	RS	CSB									+	+				
Proteaceae	*Banksia tridentata*	RS	CSB									+		+	+		+
Proteaceae	*Conospermum crassinervium*	NRS	SSB														+
Proteaceae	*Conospermum nervosum*	NRS	SSB						+								
Proteaceae	*Conospermum unilaterale*	RS	SSB														
Proteaceae	*Conospermum wycherleyi* subspecies *wycherleyi*	RS	SSB									+	+		+		
Proteaceae	*Grevillea eriostachya*	RS	SSB														
Proteaceae	*Grevillea rudis*	RS	SSB														
Proteaceae	*Grevillea shuttleworthiana*	RS	SSB														
Proteaceae	*Grevillea synapheae* subspecies *pachyphylla*	RS	SSB	+				+	+	+						+	
Proteaceae	*Grevillea uniformis*	NRS	SSB								1						
Proteaceae	*Hakea auriculata*	RS	CSB		+	1	1	+	+	1	+						
Proteaceae	*Hakea candolleana*	RS	CSB														
Proteaceae	*Hakea conchifolia*	RS	CSB	1	+	+		+	1	+	+					1	
Proteaceae	*Hakea costata*	NRS	CSB									+					
Proteaceae	*Hakea eneabba*	RS	CSB										+		+		+
Proteaceae	*Hakea flabellifolia*	RS	CSB						+						+		+
Proteaceae	*Hakea gilbertii*	NRS	CSB														
Proteaceae	*Hakea incrassata*	RS	CSB	1	+	1	+	1	1	+	1						+
Proteaceae	*Hakea neospathulata*	RS	CSB						1		+						
Proteaceae	*Hakea obliqua* subspecies *parviflora*	RS	CSB			+	+	1	+	1							
Proteaceae	*Hakea polyanthema*	NRS	CSB														
Proteaceae	*Hakea psilorrhyncha*	NRS	CSB											+			
Proteaceae	*Hakea smilacifolia*	NRS	CSB														
Proteaceae	*Hakea stenocarpa*	RS	CSB				+										
Proteaceae	*Hakea trifurcata*	NRS	CSB														

Family	Species	RS/NRS	SSB/CSB	11.3	12.1	12.2	12.3	13.1	13.2	13.3	14.1	14.2	14.3	15.1	15.2	15.3	16.1
Proteaceae	*Isopogon adenanthoides*	NRS	CSB	1	+	+		+		+	1					+	
Proteaceae	*Isopogon inconspicuus*	RS	CSB		+		+										
Proteaceae	*Isopogon linearis*	RS	CSB													+	
Proteaceae	*Isopogon tridens*	RS	CSB														
Proteaceae	*Lambertia multiflora*	RS	CSB	1	1	2		+							+	1	+
Proteaceae	*Persoonia acicularis*	RS	SSB	+													
Proteaceae	*Persoonia comata*	RS	SSB														
Proteaceae	*Persoonia filiformis*	RS	SSB	+				+									
Proteaceae	*Petrophile aculeata*	NRS	CSB	+													
Proteaceae	*Petrophile brevifolia*	RS	CSB	+	+			+									+
Proteaceae	*Petrophile drummondii*	NRS	CSB														
Proteaceae	*Petrophile linearis*	NRS	CSB	+								+	+		+		1
Proteaceae	*Petrophile macrostachya*	RS	CSB									+		1			1
Proteaceae	*Petrophile pilostyla*	RS	CSB												+		
Proteaceae	*Petrophile rigida*	RS	CSB													+	
Proteaceae	*Petrophile seminuda*	RS	CSB														
Proteaceae	*Petrophile serruriae*	NRS	CSB						+								
Proteaceae	*Petrophile shuttleworthiana*	RS	CSB		1	1	1	1	+	1	1						
Proteaceae	*Stirlingia latifolia*	RS	SSB										+		1		1
Proteaceae	*Strangea cynanchicarpa*	RS	CSB												+		
Proteaceae	*Synaphea aephynsa*	RS	SSB														
Proteaceae	*Synaphea spinulosa* subspecies *spinulosa*	RS	SSB			+						+	+				+
Proteaceae	*Xylomelum angustifolium*	RS	CSB									+	1				
Restionaceae	*Alexgeorgea nitens*	NRS	SSB									1	1	+	+		
Restionaceae	*Alexgeorgea subterranea*	NRS	SSB									+					
Restionaceae	*Chordifex sinuosus/sphacelatus*	RS	SSB	+		+		+	+	+			+			1	+
Restionaceae	*Desmocladus biformis*	RS	SSB														
Restionaceae	*Desmocladus elongatus*	RS	SSB					+	+	+							
Restionaceae	*Desmocladus myriocladus*	RS	SSB														
Restionaceae	*Desmocladus parthenicus*	RS	SSB														
Restionaceae	*Desmocladus semiplanus*	RS	SSB									1	1				
Restionaceae	*Desmocladus virgatus*	RS	SSB									+					
Restionaceae	*Hypolaena exsulca*	RS	SSB										+		1		
Restionaceae	*Lepidobolus chaetocephalus*	RS	SSB														
Restionaceae	*Lepidobolus preissianus*	RS	SSB														
Restionaceae	*Lepidobolus quadratus*	NRS	SSB														
Restionaceae	*Loxocarya striata*	RS	SSB										+				+
Rhamnaceae	*Stenanthemum humilis*	RS	SSB										+				
Rhamnaceae	*Stenanthemum notiale*	NRS	SSB														
Rutaceae	*Boronia crassifolia*	RS	SSB														
Rutaceae	*Geleznowia verrucosa*	NRS	SSB														
Rutaceae	*Philotheca spicata*	RS	SSB														
Sapindaceae	*Dodonaea ericoides*	RS	SSB				+										
Stylidiaceae	*Stylidium adpressum*	NRS	SSB														
Stylidiaceae	*Stylidium carnosum*	NRS	SSB														
Stylidiaceae	*Stylidium crossocephalum*	NRS	SSB									+					+
Stylidiaceae	*Stylidium cygnorum*	NRS	SSB														
Stylidiaceae	*Stylidium diuroides* subspecies *paucifoliatum*	NRS	SSB														
Stylidiaceae	*Stylidium maitlandianum*	NRS	SSB														
Stylidiaceae	*Stylidium repens*	NRS	SSB														

Family	Species	RS/NRS	SSB/CSB	11.3	12.1	12.2	12.3	13.1	13.2	13.3	14.1	14.2	14.3	15.1	15.2	15.3	16.1
Stylidiaceae	*Stylidium stenosepalum*	NRS	SSB														
Thymelaeaceae	*Pimelea angustifolia*	NRS	SSB														
Thymelaeaceae	*Pimelea leucantha*	NRS	SSB									1					+
Thymelaeaceae	*Pimelea ferruginea*	NRS	SSB														
Thymelaeaceae	*Pimelea sulphurea*	NRS	SSB	+	+			+			+						+
Xanthorrhoeaceae	*Xanthorrhoea brunonis*	RS	SSB														+
Xanthorrhoeaceae	*Xanthorrhoea drummondii*	RS	SSB	2	3	2	2	2	2	1	2	1	1		2	2	1
Zamiaceae	*Macrozamia fraseri*	RS	CSB														
Unknown	Unidentified species 01	RS	SSB														
Unknown	Unidentified species 02	NRS	SSB														
Unknown	Unidentified species 03	NRS	SSB							+							
Unknown	Unidentified species 04	NRS	SSB														
Unknown	Unidentified species 05	NRS	SSB						+	1							
Unknown	Unidentified species 06	RS	SSB														
Restionaceae	Unidentified species 07	RS	SSB														
Unknown	Unidentified species 09	NRS	SSB														+
Restionaceae	Unidentified species 10	RS	SSB														
Unknown	Unidentified species 11	RS	SSB													+	
Unknown	Unidentified species 12	RS	SSB								1		1				

Family	Species	RS/NRS	SSB/CSB	16.2	16.3	17.1	17.2	17.3	18.1	18.2	18.3	19.1	19.2	19.3	20.1	20.2	20.3
Anarthriaceae	*Lyginia barbata*	RS	SSB														
Anarthriaceae	*Lyginia imberbis*	NRS	SSB									+					+
Apiaceae	*Platysace juncea*	NRS	SSB														
Apiaceae	*Xanthosia huegelii*	RS	SSB														
Asparagaceae	*Acanthocarpus preissii*	RS	SSB										+	+			
Asparagaceae	*Lomandra hastilis*	RS	SSB														
Asparagaceae	*Lomandra micrantha* subspecies *micrantha*	RS	SSB												+		
Asparagaceae	*Lomandra preissii*	RS	SSB														
Asparagaceae	*Thysanotus dichotomus*	RS	SSB										+		1		+
Asparagaceae	*Thysanotus patersonii*	NRS	SSB														
Asparagaceae	*Thysanotus* sp. 1	NRS	SSB														
Asparagaceae	*Thysanotus* sp. 2	NRS	SSB														
Asparagaceae	*Thysanotus triandrus*	RS	SSB				+								+		
Asteraceae	*Cephalipterum drummondii*	NRS	SSB														
Casuarinaceae	*Allocasuarina campestris*	NRS	CSB														
Casuarinaceae	*Allocasuarina humilis*	RS	CSB	1	1	1	+	1	1	2	2			+	+	+	+
Casuarinaceae	*Allocasuarina microstachya*	RS	CSB	1	1		1			+							
Cupressaceae	*Callitris acuminata*	RS	CSB	+	+	1			+	+	+	+	1	1	+	+	
Cyperaceae	*Baumea juncea*	RS	SSB			+		+							+		
Cyperaceae	*Caustis dioica*	NRS	SSB	1	1					+	1				+		
Cyperaceae	*Cyathochaeta avenacea*	RS	SSB		1		1	1									
Cyperaceae	*Lepidosperma apricola*	RS	SSB											+			
Cyperaceae	*Lepidosperma costale*	RS	SSB														
Cyperaceae	*Lepidospermum squamatum*	RS	SSB							+	+						
Cyperaceae	*Mesomelaena pseudostygia*	RS	SSB	+	+	1	1	1	+	+		+	1	+	1	+	1
Cyperaceae	*Mesomelaena stygia* subspecies *deflexa*	RS	SSB	+			+	+	+	+	+						
Cyperaceae	*Mesomelaena tetragona*	RS	SSB	+			+	+	+	+	+						
Cyperaceae	*Schoenus brevisetis*	RS	SSB					+			1						
Cyperaceae	*Schoenus curvifolius*	RS	SSB					+							+	1	
Cyperaceae	*Schoenus grandiflorus*	RS	SSB							+							
Cyperaceae	*Schoenus pleiostemoneus*	RS	SSB				1		+								
Cyperaceae	*Schoenus pedicellatus*	RS	SSB					+								+	
Cyperaceae	*Schoenus subflavus*	RS	SSB		1	1	1	1	1	1	1	1			1	1	1
Dasypogonaceae	*Calectasia cyanea*	RS	SSB	+								+					
Dasypogonaceae	*Dasypogon obliquifolius*	RS	SSB			+											
Dilleniaceae	*Hibbertia crassifolia*	RS	SSB											+	+	+	+
Dilleniaceae	*Hibbertia glomerosa*	RS	SSB														
Dilleniaceae	*Hibbertia hypericoides*	RS	SSB	1	1	1	1	1	1	1	1	1	1		1	1	1
Dilleniaceae	*Hibbertia leucocrossa*	RS	SSB		1		+	+							+	+	1
Dilleniaceae	*Hibbertia rostellata*	NRS	SSB		+		+		+	+	1	1	+		+	+	
Dilleniaceae	*Hibbertia spicata*	RS	SSB	+													
Dilleniaceae	*Hibbertia subvaginata*	RS	SSB	+	+		+		+	1	1						
Droseraceae	*Drosera eneabba*	NRS	SSB												1		
Droseraceae	*Drosera erythrorhiza*	NRS	SSB														
Droseraceae	*Drosera humilis*	NRS	SSB														

Family	Species	RS/NRS	SSB/CSB	16.2	16.3	17.1	17.2	17.3	18.1	18.2	18.3	19.1	19.2	19.3	20.1	20.2	20.3
Droseraceae	*Drosera menziesii*	NRS	SSB														
Droseraceae	*Drosera porrecta*	NRS	SSB														
Ecdeiocoleaceae	*Ecdeiocolea monostachya*	NRS	SSB						1								
Ecdeiocoleaceae	*Georgeantha hexandra*	RS	SSB	1	1		1					+		+			1
Ericaceae	*Andersonia heterophylla*	NRS	SSB					1									
Ericaceae	*Astroloma glaucescens*	NRS	SSB				1		+	+	+						
Ericaceae	*Astroloma oblongifolium*	RS	SSB	+													
Ericaceae	*Astroloma serratifolium*	RS	SSB	+									+		+		+
Ericaceae	*Astroloma* sp. Eneabba	NRS	SSB														
Ericaceae	*Astroloma stomarrhena*	RS	SSB				+	+	+		+						
Ericaceae	*Astroloma xerophyllum*	NRS	SSB					+									
Ericaceae	*Conostephium pendulum*	RS	SSB					+	1								+
Ericaceae	*Leucopogon striatus*	NRS	SSB								+	+					
Ericaceae	*Leucopogon crassiflorus*	NRS	SSB						+								
Ericaceae	*Leucopogon leptanthus*	NRS	SSB					1									
Ericaceae	*Leucopogon* sp. Carnamah	RS	SSB			1										1	1
Ericaceae	*Lysinema pentapetalum*	NRS	SSB	1				1				+	+		1	+	+
Euphorbiaceae	*Stachystemon axillaris*	NRS	SSB														
Fabaceae	*Acacia acuminata*	NRS	SSB														
Fabaceae	*Acacia andrewsii*	NRS	SSB														
Fabaceae	*Acacia auronitens*	NRS	SSB	+	1												
Fabaceae	*Acacia barbinervis* subspecies *borealis*	RS	SSB														
Fabaceae	*Acacia fagonioides*	RS	SSB														
Fabaceae	*Acacia lasiocarpa*	NRS	SSB														
Fabaceae	*Acacia latipes* subspecies *latipes*	RS	SSB														
Fabaceae	*Acacia megacephala*	NRS	SSB			1											
Fabaceae	*Acacia pulchella*	RS	SSB														
Fabaceae	*Acacia stenoptera*	RS	SSB		+		+				1						
Fabaceae	*Bossiaea eriocarpa*	NRS	SSB														
Fabaceae	*Daviesia benthamii*	NRS	SSB														
Fabaceae	*Daviesia chapmanii*	RS	SSB		+												
Fabaceae	*Daviesia daphnoides*	NRS	SSB				2										
Fabaceae	*Daviesia debilior*	RS	SSB														
Fabaceae	*Daviesia decurrens*	RS	SSB		+						+						
Fabaceae	*Daviesia divaricata*	RS	SSB			+						+		+	1	1	+
Fabaceae	*Daviesia epiphyllum*	RS	SSB	1	+												
Fabaceae	*Daviesia nudiflora*	RS	SSB	+	+	+	+		+	+	+	1		+	+		1
Fabaceae	*Daviesia pedunculata*	RS	SSB	+	+		+										
Fabaceae	*Daviesia physodes*	NRS	SSB														
Fabaceae	*Daviesia podophylla/Daviesia quadrilatera*	NRS	SSB			+											
Fabaceae	*Daviesia triflora*	RS	SSB														
Fabaceae	Fabaceae species 01	RS	SSB	+													
Fabaceae	*Gastrolobium axillare*	RS	SSB					+	1								
Fabaceae	*Gastrolobium oxylobioides*	RS	SSB		+												
Fabaceae	*Gastrolobium polystachyum/Cristonia biloba*	NRS	SSB	+	+		+										
Fabaceae	*Gastrolobium spinosum*	NRS	SSB														
Fabaceae	*Gompholobium confertum*	RS	SSB	+	+						+			+			
Fabaceae	*Gompholobium preissii*	NRS	SSB									+					
Fabaceae	*Gompholobium tomentosum*	RS	SSB			+										+	
Fabaceae	*Hardenbergia comptoniana*	NRS	SSB				+			1	+						

Family	Species	RS/NRS	SSB/CSB	16.2	16.3	17.1	17.2	17.3	18.1	18.2	18.3	19.1	19.2	19.3	20.1	20.2	20.3
Fabaceae	*Jacksonia condensata*	RS	SSB														
Fabaceae	*Jacksonia floribunda*	RS	SSB	+		1		1				1	1	1	1	1	1
Fabaceae	*Jacksonia furcellata*	RS	SSB														
Fabaceae	*Jacksonia lehmannii*	RS	SSB				+										
Fabaceae	*Mirbelia spinosa*	RS	SSB														
Frankeniaceae	*Frankenia pauciflora*	NRS	SSB														
Goodeniaceae	*Dampiera juncea*	RS	SSB									+	3				
Goodeniaceae	*Dampiera lindleyi*	RS	SSB			+						+					
Goodeniaceae	*Dampiera spicigera*	NRS	SSB														
Goodeniaceae	*Lechenaultia biloba*	RS	SSB														
Goodeniaceae	*Lechenaultia hirsuta*	?	SSB			+											
Goodeniaceae	*Lechenaultia stenosepala*	NRS	SSB														
Goodeniaceae	*Scaevola repens* subspecies Northern Sandplains	RS	SSB		+	+											
Goodeniaceae	*Scaevola* species 01	RS	SSB										+				
Haemodoraceae	*Anigozanthos humilis*	RS	SSB			1											
Haemodoraceae	*Anigozanthos* species 01	RS	SSB						+								
Haemodoraceae	*Blancoa canescens*	RS	SSB										1	+	1	+	
Haemodoraceae	*Conostylis aurea*	RS	SSB					+	+	1	1	1	+	+	+	+	1
Haemodoraceae	*Conostylis hiemalis*	RS	SSB			1											
Haemodoraceae	*Conostylis latens*	RS	SSB				+	1	1	1	+			+	1		
Haemodoraceae	*Conostylis micrantha*	RS	SSB	+			+		1		+						+
Haemodoraceae	*Conostylis neocymosa*	RS	SSB									1	1	+			
Haemodoraceae	*Haemodorum* species 01	RS	SSB														
Haemodoraceae	*Macropidia fuliginosa*	RS	SSB								+						
Haloragaceae	*Glischrocaryon aureum*	RS	SSB														
Hemerocallidaceae	*Johnsonia pubescens*	RS	SSB													+	
Iridiaceae	*Patersonia juncea*	RS	SSB														
Lamiaceae	*Hemiandra* sp Eneabba	RS	SSB									+	+	+		+	+
Lamiaceae	*Hemiphora bartlingii*	RS	SSB													+	+
Lamiaceae	*Lachnostachys eriobotrya*	RS	SSB										+	+			
Lamiaceae	*Microcorys* sp. Coomallo	RS	SSB	1	1		+			+	+				1		
Lamiaceae	*Pityrodia hemigenioides*	RS	SSB									1	1	1			
Lauraceae	*Cassytha* sp.	NRS	SSB	+	+			+	1	+	1	1	1		1	1	1
Malvaceae	*Guichenotia micrantha*	RS	SSB														
Malvaceae	*Lasiopetalum drummondii*	RS	SSB	+	+	+	+	+	+								
Malvaceae	*Thomasia grandiflora*	RS	SSB														
Myrtaceae	*Baeckea grandis*	RS	SSB												1		
Myrtaceae	*Beaufortia bracteosa*	NRS	CSB	1	+		1	+									
Myrtaceae	*Beaufortia elegans*	NRS	CSB	1	1	1		1	1	+		2	2	2	1	1	1
Myrtaceae	*Calothamnus glaber*	RS	CSB														
Myrtaceae	*Calothamnus quadrifidus* subspecies *angustifolius*	RS	CSB														
Myrtaceae	*Calothamnus sanguineus*	RS	CSB	+	1	1		1	1	1	1						
Myrtaceae	*Calothamnus torulosus*	RS	CSB	+			+	+		1	1						
Myrtaceae	*Calytrix aurea*	NRS	SSB														
Myrtaceae	*Calytrix chrysantha*	NRS	SSB														
Myrtaceae	*Calytrix depressa*	RS	SSB														
Myrtaceae	*Calytrix drummondii*	NRS	SSB														
Myrtaceae	*Calytrix glutinosa*	NRS	SSB														
Myrtaceae	*Calytrix sapphirina*	NRS	SSB			+											
Myrtaceae	*Calytrix superba*	RS	SSB														+

Family	Species	RS/NRS	SSB/CSB	16.2	16.3	17.1	17.2	17.3	18.1	18.2	18.3	19.1	19.2	19.3	20.1	20.2	20.3
Myrtaceae	*Chamelaucium* sp. Bunjil	RS	SSB						+							+	+
Myrtaceae	*Conothamnus trinervis*	RS	CSB				+	+		+	+						
Myrtaceae	*Darwinia capitellata*	NRS	SSB														
Myrtaceae	*Darwinia neildiana*	NRS	SSB		+		+	+							+		1
Myrtaceae	*Darwinia sanguinea*	NRS	SSB		+				+						+		
Myrtaceae	*Darwinia speciosa*	RS	SSB					+				+				+	
Myrtaceae	*Eremaea beaufortioides*	RS	CSB		+	1		+			1	1	1	+	1	1	
Myrtaceae	*Eremaea ectadioclada*	NRS	CSB					+							+	+	
Myrtaceae	*Eremaea hadra*	RS	CSB			+											
Myrtaceae	*Eremaea pauciflora*	RS	CSB														
Myrtaceae	*Eremaea violacea*	RS	CSB			+	+		+	1					+		+
Myrtaceae	*Eucalyptus celastroides*	RS	CSB					2									
Myrtaceae	*Eucalyptus pleurocarpa/xtetragona*	RS	CSB	1	2		+									+	
Myrtaceae	*Eucalyptus gittinsii*	RS	CSB														
Myrtaceae	*Eucalyptus leptopoda*	RS	CSB														
Myrtaceae	*Eucalyptus todtiana*	RS	CSB			+						+					
Myrtaceae	*Homalocalyx chapmanii*	RS	SSB									+			+	+	
Myrtaceae	*Hypocalymma gardneri*	NRS	SSB			+	+	+	1	+	+	+	+				
Myrtaceae	*Leptospermum erubescens*	RS	CSB														
Myrtaceae	*Leptospermum spinescens*	NRS	CSB	+	+	+	+	+	+		+	+	+	+	+	+	+
Myrtaceae	*Malleostemon roseus*	NRS	SSB														
Myrtaceae	*Melaleuca ciliosa*	NRS	CSB									+					
Myrtaceae	*Melaleuca systena*	RS	CSB														
Myrtaceae	*Melaleuca leuropoma*	RS	CSB			1			2	+		+	1	2	1	1	1
Myrtaceae	*Melaleuca orbicularis*	RS	CSB							+							
Myrtaceae	*Melaleuca tinkeri*	RS	CSB														
Myrtaceae	*Melaleuca trichophylla*	RS	CSB	1	+		2	1	1	1	+						
Myrtaceae	*Melaleuca urceolaris*	NRS	CSB								+						
Myrtaceae	*Melaleuca zonalis*	RS	CSB				1										
Myrtaceae	Myrtaceae species 01	NRS	SSB														
Myrtaceae	Myrtaceae species 02	NRS	SSB		+												
Myrtaceae	*Phymatocarpus porphyrocephalus*	NRS	SSB									1		+		+	
Myrtaceae	*Pileanthus filifolius*	RS	SSB					+	1	+		+	+	+	1	1	+
Myrtaceae	*Scholtzia involucrata*	RS	SSB														
Myrtaceae	*Scholtzia laxiflora*	NRS	SSB	+									+	+			
Myrtaceae	*Scholtzia leptantha*	NRS	SSB														
Myrtaceae	*Scholtzia* sp. Eneabba	NRS	SSB														
Myrtaceae	*Scholtzia* sp. Wongonderrah	NRS	SSB					+									
Myrtaceae	*Thryptomene cuspidata*	NRS	SSB														
Myrtaceae	*Verticordia argentea*	NRS	SSB														
Myrtaceae	*Verticordia densiflora*	RS	SSB										+				
Myrtaceae	*Verticordia eriocephala*	NRS	SSB														
Myrtaceae	*Verticordia grandis*	NRS	SSB									+	+	1	+	+	1
Myrtaceae	*Verticordia huegelii*	NRS	SSB														
Myrtaceae	*Verticordia nobilis*	NRS	SSB														
Myrtaceae	*Verticordia ovalifolia*	NRS	SSB														
Myrtaceae	*Verticordia pennigera*	RS	SSB														
Olacaceae	*Olax benthamiana*	NRS	SSB														
Olacaceae	*Olax scalariformis*	NRS	SSB														
Poaceae	*Amphipogon turbinatus*	RS	SSB			+						+			+	+	+

Family	Species	RS/NRS	SSB/CSB	16.2	16.3	17.1	17.2	17.3	18.1	18.2	18.3	19.1	19.2	19.3	20.1	20.2	20.3
Poaceae	*Neurachne alopecuroidea*	RS	SSB														
Polygalaceae	*Comesperma acerosum*	NRS	SSB			+			+		+					+	
Proteaceae	*Adenanthos cygnorum* subspecies *cygnorum*	NRS	SSB													1	
Proteaceae	*Banksia armata* var. *armata*	RS	CSB														
Proteaceae	*Banksia attenuata*	NRS	CSB									1	+	1		1	+
Proteaceae	*Banksia bipinnatifida*	RS	CSB					+									
Proteaceae	*Banksia candolleana*	RS	CSB			1			1					+	1	1	1
Proteaceae	*Banksia carlinoides*	RS	CSB	1	+												
Proteaceae	*Banksia chamaephyton*	RS	CSB	+	+		+		+	+	+						
Proteaceae	*Banksia cypholoba*	RS	CSB		+		+			1		+					
Proteaceae	*Banksia densa*	RS	CSB	+	+		+		+	+							
Proteaceae	*Banksia glaucifolia*	NRS	CSB	+	1		1	1		+	1						
Proteaceae	*Banksia grossa*	RS	CSB			1		1	1	1	1	+				+	+
Proteaceae	*Banksia hookeriana*	NRS	CSB									1	1	1		1	1
Proteaceae	*Banksia incana*	RS	CSB						+	1	1				1		
Proteaceae	*Banksia kippistiana* var. *kippistiana*	RS	CSB														
Proteaceae	*Banksia leptophylla*	NRS	CSB		1						+						
Proteaceae	*Banksia menziesii*	RS	CSB														
Proteaceae	*Banksia micrantha*	RS	CSB		+		+			+							
Proteaceae	*Banksia nana*	RS	CSB							+	1						
Proteaceae	*Banksia nivea*	RS	CSB	+				+	1		1		+	+			
Proteaceae	*Banksia sclerophylla*	RS	CSB		1				+	1	+						
Proteaceae	*Banksia sessilis*	NRS	CSB														
Proteaceae	*Banksia shuttleworthiana*	RS	CSB	1	1		1	+	+	+	1	+					
Proteaceae	*Banksia tortifolia*	RS	CSB			+						+			+		+
Proteaceae	*Banksia tridentata*	RS	CSB			+		1		+		+	+	+	+		+
Proteaceae	*Conospermum crassinervium*	NRS	SSB									+					
Proteaceae	*Conospermum nervosum*	NRS	SSB	+													
Proteaceae	*Conospermum unilaterale*	RS	SSB														
Proteaceae	*Conospermum wycherleyi* subspecies *wycherleyi*	RS	SSB			1			+			1	1	1	1	+	1
Proteaceae	*Grevillea eriostachya*	RS	SSB											+			+
Proteaceae	*Grevillea rudis*	RS	SSB														
Proteaceae	*Grevillea shuttleworthiana*	RS	SSB									+					
Proteaceae	*Grevillea synapheae* subspecies *pachyphylla*	RS	SSB		+		+	+	+	+	+						
Proteaceae	*Grevillea uniformis*	NRS	SSB														
Proteaceae	*Hakea auriculata*	RS	CSB	+	1		+			+							
Proteaceae	*Hakea candolleana*	RS	CSB												+		
Proteaceae	*Hakea conchifolia*	RS	CSB	+			1	+	1	1	1						
Proteaceae	*Hakea costata*	NRS	CSB										+	+			
Proteaceae	*Hakea eneabba*	RS	CSB										+				
Proteaceae	*Hakea flabellifolia*	RS	CSB					+									
Proteaceae	*Hakea gilbertii*	NRS	CSB														
Proteaceae	*Hakea incrassata*	RS	CSB	+	+		1	+			+						
Proteaceae	*Hakea neospathulata*	RS	CSB														
Proteaceae	*Hakea obliqua* subspecies *parviflora*	RS	CSB														
Proteaceae	*Hakea polyanthema*	NRS	CSB														
Proteaceae	*Hakea psilorrhyncha*	NRS	CSB											+	+	+	+
Proteaceae	*Hakea smilacifolia*	NRS	CSB			+											
Proteaceae	*Hakea stenocarpa*	RS	CSB					+									
Proteaceae	*Hakea trifurcata*	NRS	CSB														

Family	Species	RS/NRS	SSB/CSB	16.2	16.3	17.1	17.2	17.3	18.1	18.2	18.3	19.1	19.2	19.3	20.1	20.2	20.3
Proteaceae	*Isopogon adenanthoides*	NRS	CSB	1			1			+	+						
Proteaceae	*Isopogon inconspicuus*	RS	CSB														
Proteaceae	*Isopogon linearis*	RS	CSB					+	+		+						
Proteaceae	*Isopogon tridens*	RS	CSB										+	+			
Proteaceae	*Lambertia multiflora*	RS	CSB	+	+		+	2	1	2	1					+	
Proteaceae	*Persoonia acicularis*	RS	SSB			+							1		1		
Proteaceae	*Persoonia comata*	RS	SSB														
Proteaceae	*Persoonia filiformis*	RS	SSB									+	+		+		
Proteaceae	*Petrophile aculeata*	NRS	CSB				+										
Proteaceae	*Petrophile brevifolia*	RS	CSB	+	+	+	+			+							+
Proteaceae	*Petrophile drummondii*	NRS	CSB									+	1	1	1	1	2
Proteaceae	*Petrophile linearis*	NRS	CSB					+	1		+						
Proteaceae	*Petrophile macrostachya*	RS	CSB					1	+			+	+		+		
Proteaceae	*Petrophile pilostyla*	RS	CSB									+	+	+	+	+	
Proteaceae	*Petrophile rigida*	RS	CSB				+		+	+	+						
Proteaceae	*Petrophile seminuda*	RS	CSB														
Proteaceae	*Petrophile serruriae*	NRS	CSB														
Proteaceae	*Petrophile shuttleworthiana*	RS	CSB	+	+		1			+	+						
Proteaceae	*Stirlingia latifolia*	RS	SSB			+		+	+		+	1	+	+	+	+	
Proteaceae	*Strangea cynanchicarpa*	RS	CSB														
Proteaceae	*Synaphea aephynsa*	RS	SSB		+					+	+			+			
Proteaceae	*Synaphea spinulosa subspecies spinulosa*	RS	SSB	+									1				
Proteaceae	*Xylomelum angustifolium*	RS	CSB														
Restionaceae	*Alexgeorgea nitens*	NRS	SSB										+				
Restionaceae	*Alexgeorgea subterranea*	NRS	SSB				+										
Restionaceae	*Chordifex sinuosus/sphacelatus*	RS	SSB	+			+	+	1	1	+	1		1	+		+
Restionaceae	*Desmocladus biformis*	RS	SSB														
Restionaceae	*Desmocladus elongatus*	RS	SSB				+				+				+		
Restionaceae	*Desmocladus myriocladus*	RS	SSB	1	1												
Restionaceae	*Desmocladus parthenicus*	RS	SSB														
Restionaceae	*Desmocladus semiplanus*	RS	SSB						+								
Restionaceae	*Desmocladus virgatus*	RS	SSB					1									
Restionaceae	*Hypolaena exsulca*	RS	SSB														
Restionaceae	*Lepidobolus chaetocephalus*	RS	SSB									+	1	+	+	+	1
Restionaceae	*Lepidobolus preissianus*	RS	SSB			+									+		1
Restionaceae	*Lepidobolus quadratus*	NRS	SSB														
Restionaceae	*Loxocarya striata*	RS	SSB							+				+	+		
Rhamnaceae	*Stenanthemum humilis*	RS	SSB					+									
Rhamnaceae	*Stenanthemum notiale*	NRS	SSB														
Rutaceae	*Boronia crassifolia*	RS	SSB							1							
Rutaceae	*Geleznowia verrucosa*	NRS	SSB														
Rutaceae	*Philotheca spicata*	RS	SSB														
Sapindaceae	*Dodonaea ericoides*	RS	SSB														
Stylidiaceae	*Stylidium adpressum*	NRS	SSB														
Stylidiaceae	*Stylidium carnosum*	NRS	SSB														
Stylidiaceae	*Stylidium crossocephalum*	NRS	SSB			+						+			+		+
Stylidiaceae	*Stylidium cygnorum*	NRS	SSB														
Stylidiaceae	*Stylidium diuroides* subspecies *paucifoliatum*	NRS	SSB	+													
Stylidiaceae	*Stylidium maitlandianum*	NRS	SSB														
Stylidiaceae	*Stylidium repens*	NRS	SSB														

Family	Species	RS/NRS	SSB/CSB	16.2	16.3	17.1	17.2	17.3	18.1	18.2	18.3	19.1	19.2	19.3	20.1	20.2	20.3
Stylidiaceae	*Stylidium stenosepalum*	NRS	SSB														
Thymelaeaceae	*Pimelea angustifolia*	NRS	SSB									+			+		
Thymelaeaceae	*Pimelea leucantha*	NRS	SSB	+		+						+					
Thymelaeaceae	*Pimelea ferruginea*	NRS	SSB														
Thymelaeaceae	*Pimelea sulphurea*	NRS	SSB	+							+						
Xanthorrhoeaceae	*Xanthorrhoea brunonis*	RS	SSB														
Xanthorrhoeaceae	*Xanthorrhoea drummondii*	RS	SSB	1	2		1	2	1	1	2	+		+	+	+	1
Zamiaceae	*Macrozamia fraseri*	RS	CSB														
Unknown	Unidentified species 01	RS	SSB														
Unknown	Unidentified species 02	NRS	SSB														
Unknown	Unidentified species 03	NRS	SSB	+													
Unknown	Unidentified species 04	NRS	SSB	+													
Unknown	Unidentified species 05	NRS	SSB														
Unknown	Unidentified species 06	RS	SSB														
Restionaceae	Unidentified species 07	RS	SSB														
Unknown	Unidentified species 09	NRS	SSB														
Restionaceae	Unidentified species 10	RS	SSB			+											
Unknown	Unidentified species 11	RS	SSB														
Unknown	Unidentified species 12	RS	SSB														

Supplementary Information: Chapter 3

S3.1 Study species, population selection and seed collection

Four species characterised by canopy seed storage and cohort recruitment after fire were selected; *Banksia hookeriana* Meisn., *Banksia leptophylla* A.S. George, *Hakea costata* Meisn., and *Hakea polyanthema* Diels, all belonging to the family Proteaceae. *Banksia hookeriana* was chosen for further genetic studies due to its greater susceptibility to drought (He & Lamont 2010).

Populations of the four species were sampled from stands with different post-fire ages at eight locations near Eneabba, Western Australia (Table S3.1). Seed source locations used were classified as high rainfall (HiR) if they received an average to above-average rainfall in the first winter after fire, or low rainfall (LoR) if they experienced drought (less than 80% of average rainfall) in the first winter following fire (Table S3.2; Bureau of Meteorology 2015a). The time elapsed since last fire for each site was provided by the Department of Parks and Wildlife (Western Australia), the government agency responsible for managing and monitoring fire across the state, and was also verified by the age of plants at each site using annual stem node counts for *Banksia hookeriana* (following Lamont 1985).

Each of the four species were sampled from five of eight locations (except for *H. costata*, which was only present at three locations), from June-August 2013, with fruits harvested from 15 individuals per species, with the aim of collecting 150 viable seeds per species per site. The seeds collected were set over the two years prior to collection to maximise seed viability and to minimise environmental variation in any factors affecting seed set across years. The seed collection site locations and details are shown in Table S3.1.

Table S3.1: Site categorisation, location and species collected from locations at Eneabba. LoR (Low Rainfall) refers to low rainfall years and HiR (High Rainfall) to average/above average rainfall years.

Site	Year last burned/ plants established	Winter/ spring rainfall (mm) and category	GPS coordinates	Species collected			
				Banksia hookeriana	*Banksia leptophylla*	*Hakea polyanthema*	*Hakea costata*
1	2006	260 (LoR)	S 29°38'32" E 115°12'43"		✔	✔	
2	2002	320 (LoR)	S 29°37'03" E 115°12'00"		✔	✔	✔
3	2000	311 (LoR)	S29°45'26" E115°11'11"	✔		✔	✔
4	2002	320 (LoR)	S 29°52'15" E 115°14'54"	✔	✔		
5	2005	429 (HiR)	S 29°56'34" E 115°16'00"	✔			
6	2005	429 (HiR)	S 29°45'27" E 115°11'14"	✔			
7	1998	401 (HiR)	S 29°35'50" E 115°10'12"		✔	✔	✔
8	1991	512 (HiR)	S 29°37'03" E 115°12'02"	✔	✔	✔	

Table S3.2: Criteria for classifying rainfall at Eneabba. Long term average was calculated using data from 1965 to 2012.

	Annual rainfall (mm)	Winter/spring rainfall (mm)
Long term average (Average rainfall)	492	426
Long term average -20% (Low rainfall-LoR)	394	341

S3.2 Seed preparation and germination

Seed was extracted from fruits of *Hakea* species by allowing fruits to air dry and open naturally. Seed was extracted from fruits of *Banksia* species by first scorching cones with a gas torch until follicles split open, then soaking cones in cold water over night, and drying cones in an oven at 60°C until follicles were open wide enough for seed to be extracted with a pair of forceps.

Seed was surface sterilised in a solution of 20% sodium hypochlorite with a drop of Tween 80 surfactant (polyoxyethylene sorbitan mono-oleate) then placed under pressure in a vacuum chamber for five minutes, removed from the vacuum for five minutes, and returned to the vacuum chamber for another five minutes, then rinsed three times in sterile deionised water (Downes *et al.* 2010). Sterilised seed was placed into Petri dishes of varying size (120 mm for *H. costata* seed, and 150 mm for large *H. polyanthema*, *B. hookeriana* and *B. leptophylla* seeds) containing two sheets of Whatman No. 1 filter paper, 2 cm^2 pieces of Wettex sponge (4 pieces for 120 mm Petri dishes, and 5 pieces for 150 mm Petri dishes), and water (13 mL for 120 mm Petri dishes, 16 mL for 150 mm Petri dishes). Petri dishes were then placed in a germination cupboard at 15°C for one month, until all viable seeds had germinated.

S3.3 Greenhouse experiment

Germinants were removed from the germination cupboard and sown into 100 cm x 15 cm diameter PVC tube pots containing low nutrient, acid sand (Bassendean sand; McArthur & Bettenay 1960) similar in type to sands at Eneabba, with three germinants of the same species from the same site sown per tube, and thirty replicates made of each. After a month of growth, ash (produced by burning local native plant species) was added to the surface of the soil at a rate of 6.68 grams (one tablespoon) per tube or 0.03789 grams of ash per cm^2 (Enright *et al.* 1997). Seedlings were grown in a greenhouse at Murdoch University, Perth, Western Australia, where they were watered every second day with 200 mL of water (simulating Eneabba mean winter rainfall

equivalent) for one month to allow seedlings to establish, after which seedlings were harvested down to one seedling per tube. Once established, seedlings were divided into two treatment groups (15 replicates per treatment) and subjected to either a control (mean winter rainfall at Eneabba over the past 30 years equivalent; 200 mL per plant every second day) or drought regime (a 50% decrease in mean winter rainfall at Eneabba; 100 mL per plant every second day) over a period of three months (Table S3.3).

Table S3.3: Watering regime in simulated drought and control treatments. 100% water refers to 200 mL of water per plant, every second day, 50% water refers to 100 mL of water per plant every second day, and 0% water refers to no water.

Treatment	Time			
	2 months	**2 weeks**	**3 months**	**3 months**
Control	100% water	100% water	100% water	0% water
Drought	100% water	50% water	0% water	0% water

Samples of *Banksia hookeriana* to be used for genetic analyses were harvested after two months (see below). After three months up to 24 plants from each of the treatment groups and sites were harvested for each species (Table S3.4), and measurements were taken of the number of leaves, leaf area (of 5 leaves per plant, using the freeware program ImageJ; Rasband 1997), leaf thickness and total fresh biomass. Seedlings were dried in an oven at 80˚C after which total dry biomass and biomass of individual leaves (5 per plant) were recorded. Leaf mass per area was calculated as leaf dry weight/leaf area. Water content was obtained as total fresh biomass – total dry biomass. Remaining seedlings were kept growing in the greenhouse for 12 weeks, with mortality recorded each week.

Table S3.4: Allocation of seedlings to experiments. Number of seedlings used for physical measurements and mortality data are listed. Eight seedlings were used from each site and data for seedlings from sites of the same type were pooled for analysis.

Species	Treatment	Type	Sites	Seedlings used for measurements	Seedlings used in mortality experiment
Banksia hookeriana	100%	Wet	3	24	16
		Dry	2	16	24
	50%	Wet	3	24	18
		Dry	2	16	14
Banksia leptophylla	100%	Wet	2	16	13
		Dry	3	24	19
	50%	Wet	2	16	14
		Dry	3	24	21
Hakea polyanthema	100%	Wet	2	16	10
		Dry	3	24	20
	50%	Wet	2	16	14
		Dry	3	24	16
Hakea costata	100%	Wet	1	7	0
		Dry	2	16	4
	50%	Wet	1	8	0
		Dry	2	16	5

S3.4 RNA sequencing and differential expression of genes

S3.4.1 Experimental material

Differential gene expression analyses were performed using seedlings of *B. hookeriana*, as it is the most drought-susceptible of the four study species (He & Lamont 2010). Leaf material was collected after two months of growth, with five seedlings harvested from each of the four treatment groups of seedlings: 1) those descended from plants recruited in a HiR year treated with 50% mean annual winter rainfall equivalent; 2) those descended from plants recruited in a HiR year treated with 100% mean annual

winter rainfall equivalent; 3) those descended from plants recruited in a LoR year, treated with 50% mean annual winter rainfall equivalent; and 4) those descended from plants recruited in a LoR year, treated with 100% mean annual winter rainfall equivalent. Leaf material was cleaned with DEPC water (Diethylpyrocarbonate water, an RNase enzyme inhibitor), and stored in RNAlater (Life Technologies Australia Pty Ltd.).

S3.4.2 RNA Sequencing

For each treatment, leaf samples were collected from four seedlings and pooled. Samples were stored in RNAlater RNA Stabilization Reagent (Thermo Fisher Scientific Inc. Perth) before being processed. RNA extraction, cDNA library preparation, and sequencing was carried out by Beijing Genomics Institute BGI (Shenzhen, China). Briefly, samples were homogenised, total RNA was isolated from leaf samples using Total RNA Purification System (Invitrogen), RNA quality and concentration were checked with Nanodrop and Agilent 2100 Bioanaylzer. Poly (A) mRNAs were isolated using beads with oligo(dT) (Qiagen GmbH, Hilden, Germany). The mRNA was fragmented and used as a template. cDNAs were obtained using a random hexamer to synthesize first-strand. The short cDNA fragments were then connected using sequencing adapters. The paired-end library (100 base pair insertion) was prepared following the protocol of the Illumina TruSeq RNA Sample Preparation Kit (Illumina), and the library was sequenced using Illumina HiSeq 2000 (Illumina Inc., San Diego, CA, USA). Raw reads were quality controlled by removing adaptor sequences, empty reads and low quality reads.

S3.4.3 Transcriptome De Novo Assembly

Transcriptome *de novo* assembly was carried out using a de Bruijn graph and the short read assembling program Trinity that consists of 3 independent programs; Inchworm, Chrysalis, and Butterfly (Grabherr *et al.* 2011, Haas *et al*. 2013). Inchworm first assembled the RNA-seq data into the unique sequences of transcripts (contigs) with a certain overlap length (k-mer = 25) and minimum overlap coverage of 3 reads. The resulting contigs were then clustered by Chrysalis into clusters and complete de Bruijn graphs were constructed for each cluster. In the final step, Butterfly processed the individual graphs in parallel, tracing the paths that reads and pairs of reads take within the graph, ultimately reporting full- length transcripts for alternatively spliced isoforms (unigenes).

S3.4.4 Transcriptome De Novo assembly of sequence read of Banksia hookeriana

An average of 47,287,067 clean reads with total average of 4,728,706,650 nucleotides (nt) and an average guanine-cytosine (GC) content of 45.73% were generated from four samples of *Banksia hookeriana*. We defined the reads with Q > 20 (99% probability of detecting correct base) and no ambiguous sequences (N) as high-quality reads, which resulted in 46,289,310 (97.89%) clean reads. An average of 99,439 contigs were assembled from those clean reads. The length of contigs ranged from 100 nt to 12,556 nt with an average length of 402 nt. Trinity's Butterfly software was used to connect the contigs to form a unigene. This method assembled 59,063 unigenes, where 25, 912 unigenes were distinct clusters, and 33, 151 unigenes were distinct singletons. The mean size of unigenes was 1098 bp with lengths ranging from 300 bp to > 3,000 bp, and an N50 of 1813 nt. The assembled sequences have been deposited in the NCBI database (accession number: GBXB00000000).

S3.4.5 Differential Expression of Genes and Pathway Enrichment Analysis

A rigorous algorithm has been developed to identify differentially expressed genes between two samples by BGI based on 'The significance of digital gene expression profiles' (Audic & Claverie 1997). We used a very stringent cutoff, "FDR" (False Discovery Rate) of ≤0.001 and fold change value of 2 for identifying differentially expressed genes. We further studied the biological significance of differentially expressed genes by an enrichment analysis of gene ontology (GO) terms using Blast2GO (version 2.3.5; http://www.blast2go.org/). GO functional enrichment analysis was carried out, and KEGG pathway analyses were performed using Path_finder software against the KEGG databases (Kanehisa *et al.* 2008). Statistically significant over-representation of GO categories in response to the drought treatment was determined. A list of differentially expressed genes clusters was reported after correcting for multiple comparisons (adjusted p-value < 0.05).

S3.5 References

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Supplementary Information: Chapter 4

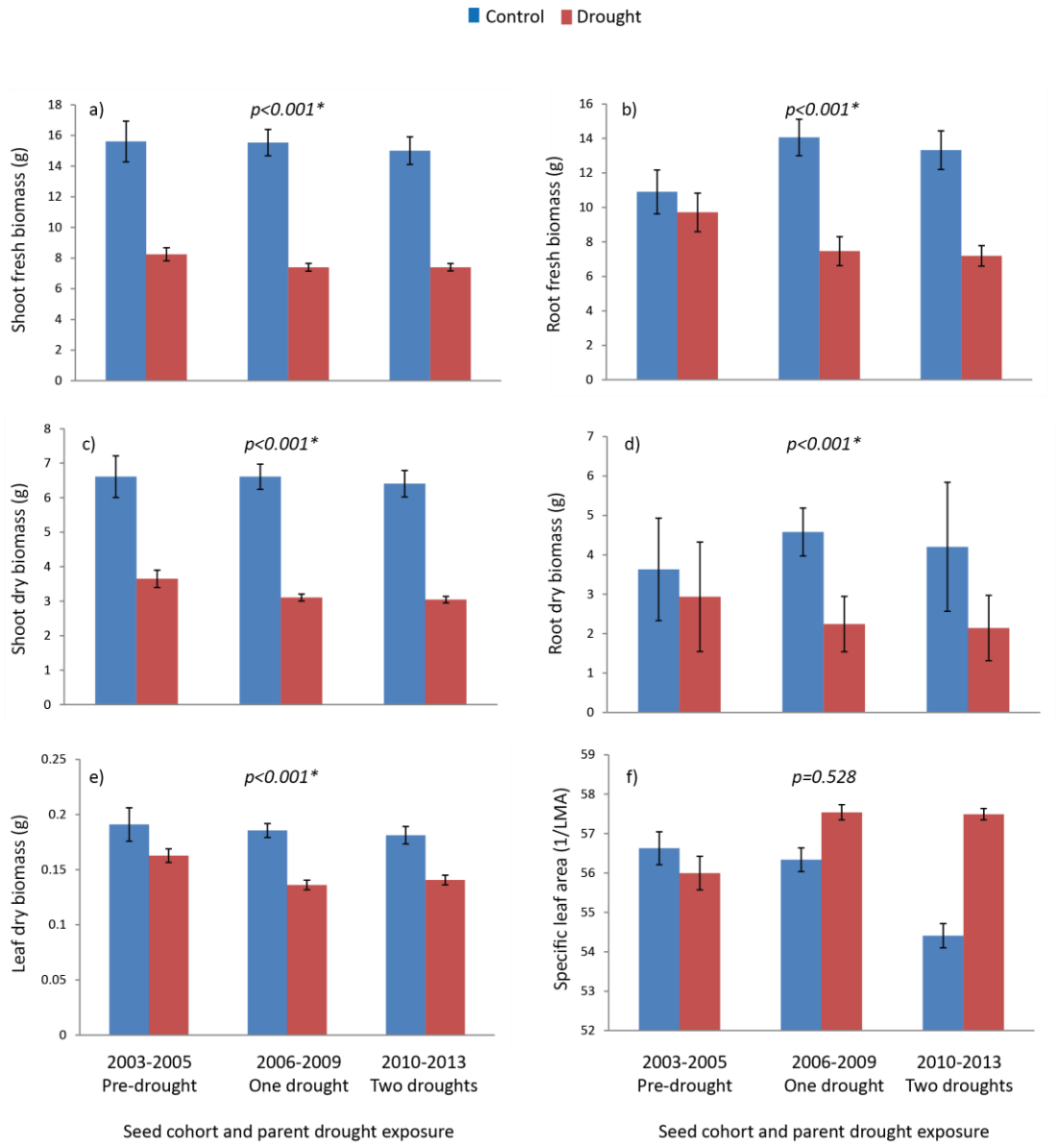

Figure S4.1 a-f: Shoot fresh biomass, Root fresh biomass, Shoot dry biomass, Root dry biomass, Leaf dry biomass, and Specific leaf area of *B. hookeriana* seedlings grown under the control and drought treatments. Data presented as mean ± SE. Measures of significance between treatments are from Two-way ANOVAs (α=0.05), significant values are marked with an asterisk.

Other publications related to this thesis

The following publications have been produced from work related to this thesis.

Characterization of Leaf Transcriptome in *Banksia hookeriana*

Sim Lin Lim, Haylee M. D'Agui, Neal J. Enright, Tianhua He

Abstract

Banksia is a significant element in vegetation of southwestern Australia, a biodiversity hotspot with global significance. In particular, *B. hookeriana* represents a species with significant economic and ecological importance in the region. For better conservation and management, we reported an overview of transcriptome of *B. hookeriana* using RNA-seq and *de novo* assembly. We have generated a total of 202.7 million reads (18.91 billion of nucleotides) from four leaf samples in four plants of *B. hookeriana*, and assembled 59,063 unigenes (average size = 1098 bp) through *de novo* transcriptome assembly. Among them, 39,686 unigenes were annotated against the Swiss-Prot, Clusters of Orthologous Groups (COG), and NCBI non-redundant (NR) protein databases. We showed that there was approximately one single nucleotide polymorphism (SNP) per 5.6–7.1 kb in the transcriptome, and the ratio of transitional to transversional polymorphisms was approximately 1.82. We compared unigenes of *B. hookeriana* to those of *Arabidopsis thaliana* and *Nelumbo nucifera* through sequence homology, Gene Ontology (GO) annotation, and KEGG pathway analyses. The comparative analysis revealed that unigenes of *B. hookeriana* were closely related to those of *N. nucifera*. *B. hookeriana*, *N. nucifera*, and *A. thaliana* shared similar GO annotations but different distributions in KEGG pathways, indicating that *B. hookeriana* has adapted to dry-Mediterranean type shrublands via regulating expression of specific genes. In total 1927 potential simple sequence repeat (SSR) markers were discovered, which could be used in the genotype and genetic diversity studies of the *Banksia* genus. Our results provide valuable sequence resource for further study in *Banksia*.

Reference

Lim SL, D'Agui HM, Enright NJ & He T 2017. Characterization of leaf transcriptome in *Banksia hookeriana*. Genomics, Proteomics , Bioinformatics 15: 49-56.

Environmental drivers and genomic architecture of trait differentiation in fire-adapted *Banksia attenuata* ecotypes

Tianhua He, Byron B. Lamont, Neal J. Enright, Haylee M. D'Agui, William Stock

Abstract

Phenotypic trait divergence between populations is considered an adaptive response to different environments, but to what extent this response is accompanied by genetic differentiation is less clear. We analysed phenotypic variation between two *Banksia attenuata* growth forms, lignotuberous (shrub) and epicormic resprouting (tree), in fire-prone environments to identify the environmental factors that might have driven this phenotypic divergence. We linked genotype with phenotype and traced candidate genes using differential gene expression analysis. Fire intervals determined the phenotypic divergence related to growth forms in *B. attenuata*. A genome-wide association study identified 66 single nucleotide polymorphisms putatively associated with growth form, and differential gene expression analysis identified 37 genes/transcripts that were differentially expressed in the two growth forms. A small heat-shock protein gene was associated with lignotuber presence and thus was differentially expressed in the shrub and tree forms. Phenotypic plasticity of resprouting is a general strategy for surviving variable fire regimes in fire-prone environments. Different fire regimes likely induce phenotypic polymorphism in *B. attenuata*, whereas phenotypic trait divergence reflects allelic frequency variation and the differential expression of a small fraction of genes that strongly interact with the disturbance regime.

Reference

Molecular Ecology (under review).