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## Ecotypic variation and plasticity of morphological and physiological traits of *Eucalyptus loxophleba* ssp. *lissophloia* along a climate gradient in south-west Australia

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**Ecotypic variation and plasticity of  
morphological and physiological traits of  
*Eucalyptus loxophleba* ssp. *lissophloia* along a  
climate gradient in south-west Australia**

By  
Vanessa Stylianou

A Thesis submitted in partial fulfilment of the requirements for the award of  
Bachelor of Science (Environmental Management) Honours

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## USE OF THESIS

The Use of Thesis statement is not included in this version of the thesis.

# ABSTRACT

The adaptive capacity of plant species will be important for increasing their resilience in a changing climate. By 2070, a change in rainfall of +10 to -40 % of current mean annual rainfall and warming of between +2 to +5 °C in south-west Western Australia, is predicted. Plant species may cope with changing climatic characteristics through natural selection or phenotypic plasticity responses. Greater ecotypic variation in traits between stands of a single species may reduce its vulnerability in a changing climate as there is a greater selection of traits to increase fitness. Phenotypic plasticity has been highlighted as a mechanism to potentially enhance resistance and resilience in a changing climate in the short-term. Given that the Mediterranean region of the south-west of Western Australia is considered to be the most vulnerable to contraction of all Mediterranean systems under predicted climate change scenarios, the region is a priority area for research into climate resilience and adaptive ecotypic variation in plant species.

This project examined differences in morphological and physiological traits between nine stands of the widespread species *E. loxophleba* ssp. *lissophloia* L.A.S. Johnson & K.D. Hill (Smooth-barked York Gum) across a climate gradient in south-western Australia. Morphological and physiological traits known to promote efficient water use and drought tolerance were compared across its natural range (450 km from W to E), with samples of the same provenances also grown in a common garden (plantation) which receives higher long-term average annual rainfall. Ten trees per natural stand and per provenance were selected for sampling. Traits measured were total leaf length, maximum leaf width, the ratio of leaf length to leaf width, area per leaf, dry mass, specific leaf area, leaf nitrogen, carbon and nitrogen isotope ratios, instantaneous water use efficiency, maximum photosynthetic and transpiration rate and wood density.

The eastern end of the gradient tended to have lower long-term average rainfall and higher long-term average temperatures than the sites further west. However, in the twelve months prior to sampling (01/06/2010 to 31/05/2011) rainfall patterns were anomalous and sites to the west of the gradient received below average rainfall. This meant that the long-term rainfall gradient was actually inverted when considering annual rainfall in the twelve months prior to sampling. The natural stands along the climate gradient and the plantation were similar in their soil characteristics and biotic structure.

Results showed significant differences (one-way ANOVA) between the nine natural stands along the climate gradient for all of the traits measured, with the exception of wood density. This showed the high ecotypic variation present within the widespread species in both foliar morphological and physiological traits measured. However, there was no single, major climatic variable which was the primary cause of variation of all the traits, as determined by linear regression analysis.

Comparisons of the natural versus planted stands showed high phenotypic plasticity responses in the physiological traits to prevailing climatic conditions, which should allow the species to cope with climate changes of a limited scale over the short-term. Morphological leaf traits showed a more conservative response, although the high ecotypic variation between stands suggests there is variation to facilitate change if given sufficient time. In conclusion, some adaptive capacity of traits relating to drought tolerance and water conservation has been shown in a single species. Despite the ability to cope with a changing climate afforded by phenotypic responses and ecotypic variation within a species, the necessity of mitigating climate change remains paramount.

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# LIST OF ABBREVIATIONS

$A_{\max}$	Maximum Photosynthetic Rate	An instantaneous physiological trait measured under controlled conditions by a LiCor
DW	Deionised Water	Purified water that contains only hydrogen, oxygen and hydroxyl ions
$\delta^{15}\text{N}$	Nitrogen Natural Abundance	The natural abundance of stable nitrogen isotopes ( $^{14}\text{N}$ , $^{15}\text{N}$ )
EC	Electrical Conductivity	Reflects the ability of a material to conduct electrical currents. Can reflect salinity or total dissolved salts
Evap	Evaporation	A measure of average daily evaporation ( $\text{mm d}^{-1}$ )
LOI	Loss on Ignition	Measure of soil organic carbon
PI	Plasticity Index	The plasticity index was a measure of plasticity calculated using the formula in Godoy et al. (2011).
Radn	Radiation	Average daily solar radiation measured in megajoules per square metre ( $\text{MJ m}^{-2} \text{d}^{-1}$ )
RH	Relative Humidity	Measured at either maximum temperature (RHmaxT) or minimum temperature (RHminT)
SLA	Specific Leaf Area	The ratio of leaf area (petiole inclusive) to dry mass expressed in $\text{mm}^2 \text{mg}^{-1}$
SE	Standard Error	Determined by the standard deviation divided by the square root of N
Temp	Temperature	Measured in degrees Celsius ( $^{\circ}\text{C}$ )
TN	Total Nitrogen	
TP	Total Phosphorus	
VP	Vapour Pressure	The pressure exerted by a vapour measured in hPa
WUEi	Instantaneous Water-use Efficiency	An instantaneous measure of the carbon fixed per unit of water used (photosynthetic rate / transpiration rate)
WUEs	Seasonal Water-use Efficiency	An integrated, season-length measure of water-use efficiency as determined using carbon isotopes

# CHAPTER 1: INTRODUCTION

## 1.1.1 A Changing Climate

Global climatic changes have been recorded over the last century and there is evidence of the effects this has already had on species and ecosystems (Hughes 2003, IPCC 2007). Precipitation is one of several climatic variables in which changes have been noted, as the amount, frequency, duration, intensity and type of precipitation have changed (IPCC 2007). In Australia, areas such as the northwest have experienced increased annual precipitation, whereas the south-west had a 25 % decline in winter rainfall over the last century (Hennessy et al. 1999, IPCC 2007). By 2070, rainfall is projected to increase by up to 40 % of current levels in the northern, summer-rainfall dominated parts of Australia whilst it could change by +10 to -40 % of current mean annual rainfall in the south-western, winter-rainfall dominated area (CSIRO 2007).

Temperature is another climatic variable predicted to change as a result of greenhouse gas emissions (CSIRO 2007). Congruent with trends observed globally (IPCC 2007), average temperatures in Australia have already increased by approximately 0.8 °C from 1910 to 2000 (Collins 2000). It has been estimated that temperatures across Australia will increase by approximately 1 °C by 2030 (relative to 1990) (CSIRO 2007). Best estimates of a 1.8 °C or 3.4 °C increase under a low or high greenhouse gas emission scenario, respectively, have been predicted by 2070 (CSIRO 2007). In the south-west Western Australia, warming of between +2 to +5 °C in all seasons by 2070 is predicted (CSIRO 2007). Furthermore, changes in temperature extremes will be experienced, with more frequent heat waves expected (CSIRO 2007).

It is difficult to predict the impact of altered precipitation regimes and temperature patterns on vegetation using modelling approaches, due to simultaneous changes in other climatic variables and elevated carbon dioxide levels (Hughes 2003). However, projections suggest that altered rainfall and temperature may cause considerable changes to species composition, abundance and productivity (Howden et al. 1999, Hughes 2003, Prober et al. 2011). Modelling of species ranges under various climate change scenarios predict a reduced or more fragmented distribution for many Australian plant species (Witkowski & Lamont 2006, Yates et al. 2010a). Such reductions in

species abundance and ranges would be undesirable, given that the South-West Botanical Province is a globally renowned 'hotspot' for plant diversity and that the rate at which plants are becoming endangered or extinct in Western Australia exceeds the rate exhibited in any other Mediterranean region (Greuter 1994, Hobbs 2003, Witkowski & Lamont 2006).

Outcomes for plant species abundance, range and distribution not only depend on the climatic changes and how environmental processes are affected, but also how individuals, populations, species and ecosystems respond to these changes (Prober et al. 2011). Responses to climatic changes are species specific, with some plant taxa having a higher sensitivity and a lower adaptive capacity to maintain their fitness in a changing environment. For instance, it is predicted that 23 % of *Eucalyptus* species cannot tolerate more than 20 % variation in mean annual rainfall received (Hughes et al. 1996). *Eucalyptus* forest types dominate approximately 79 % of Australia's native forest area (MIG 2008), yet many eucalypts are currently located in areas where temperature is expected to increase and rainfall is expected to decrease beyond levels in which populations of these species are currently found (Hughes et al. 1996). Thus, it is vital to understand whether there are severe population loss or extinction risks posed by predicted climatic changes or whether these species (and other plant species) have the capacity to tolerate and respond to a changing climate.

### **1.1.2 Coping in a Changing Climate**

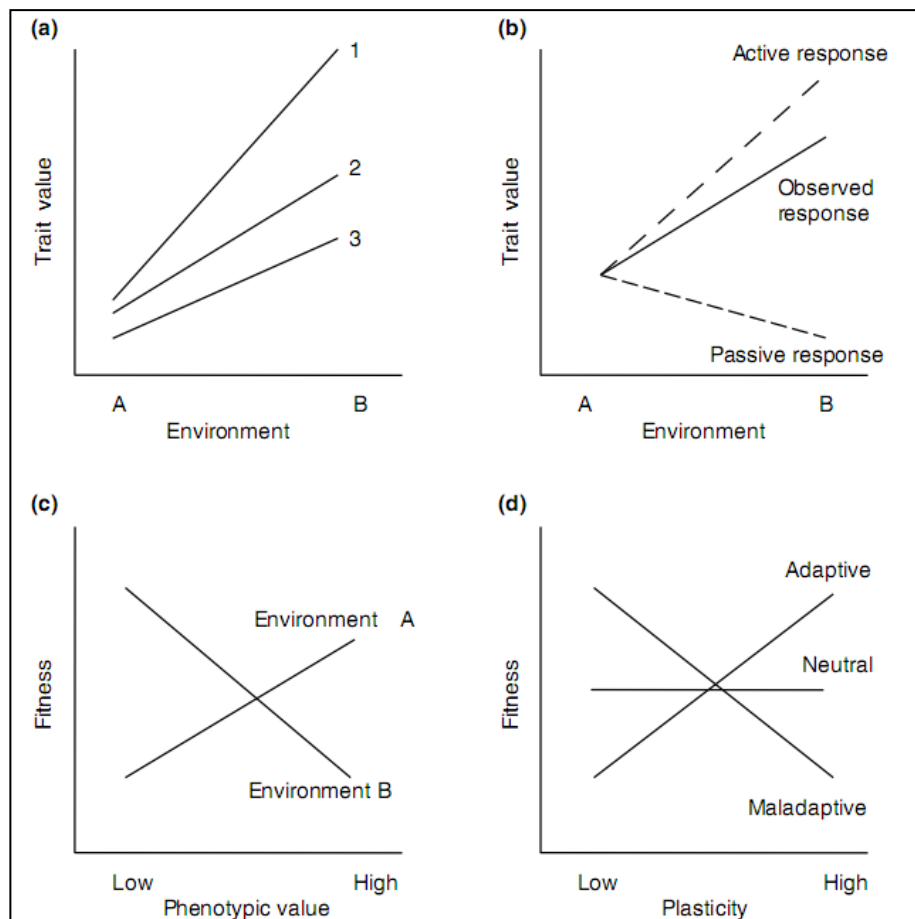
Plant species may cope with changing climatic characteristics through migration, natural selection or phenotypic plasticity (Helmuth et al. 2005, Nicotra et al. 2010). Migration is restricted by factors such as dispersal, establishment and/or reproductive capacity, degree of landscape fragmentation, soil type and site nutrient levels (Hughes et al. 1996, Hughes 2003, Witkowski & Lamont 2006). Natural selection may provide an adaptive response to changing conditions but only in the long-term. Ecotypic and within-stand variation and diversity in morphological and physiological traits are assumed to make species more resistant and less vulnerable to externalities such as a changing climate as there is a greater selection of traits to increase fitness in varying conditions (Whisenant 1999, Lindenmayer & Burgman 2005). In this way, the ability of species to adapt to climate change in the long-term may depend on the amount of variation present within the species (Lindenmayer & Burgman 2005). It is expected that

the ecotypic variation in a widespread species is generally greater than in a narrowly distributed species (Whisenant 1999, Lindenmayer & Burgman 2005).

Genetic changes through natural selection only occur over several generations (Helmuth et al. 2005). Given the rapid rate at which climate changes are predicted, it may prove problematic or impossible for some plant species to evolve or migrate quickly enough to remain in conditions within their tolerance ranges (Witkowski & Lamont 2006). The importance of phenotypic plasticity to tolerate increasingly arid conditions is therefore increased (Jump & Peñuelas 2005).

Phenotypic plasticity refers to alternative expressions of a single genotype induced by environmental factors (Nicotra et al. 2010). Where this increases a genotype's global fitness, the change is said to be an adaptive phenotypic response (Nicotra et al. 2010). Different genotypes, populations or species have varying plasticity levels of traits (Figure 1a) and the combination of both active and passive plasticity responses in a given environment will determine the phenotype observed (Figure 1b). It is important to recognise that a high phenotypic value of a certain trait may increase fitness in one environment, but could convey low fitness in a different environment (Figure 1c). In addition, not all plastic responses will be adaptive and increase fitness (Figure 1d), they may simply be a response to resource limitation or ecological or physical processes (van Kleunen & Fischer 2005, Nicotra et al. 2010). However, even passive plastic responses may be advantageous in offering new phenotypes for which natural selection may act on to facilitate adaptive plasticity in the future (Ghalambor et al. 2007).

Phenotypic plasticity has been highlighted as a mechanism to potentially enhance resistance and resilience in a changing climate (Prober et al. 2011). In this study, any changes in the morphological or physiological traits in the individuals studied as a response to altered environmental conditions within the lifetime of the plant will be considered a phenotypic plasticity response (Bradshaw 1965, Jump & Peñuelas 2005). Alternatively, changes which occurred over several generations in response to climatic conditions could be considered the result of genetic changes (Bradshaw 1965) which result in ecotypic differentiation.



Source: Nicotra et al. 2010.

**Figure 1:** a.) Trait values of different genotypes, populations or species whereby line 1 exhibits the greatest variation between environment A and B.

b.) The observed phenotype of a trait is a combination of both active and passive responses to a given environment.

c.) Phenotypes convey varying levels of fitness depending on the environment. In environment A, the phenotype conveys high fitness whereas it conveys low fitness in environment B.

d.) Adaptive traits tend to increase fitness whereas high plasticity of maladaptive traits will decrease fitness.

Although requiring further investigation, phenotypic responses in a changing climate may be observed in numerous plant morphological and physiological traits (Nicotra et al. 2010). Of course, all plasticity originally arises from physiological changes, however, those resulting in primarily a morphological effect may be known as morphological plasticity (Bradshaw 1965). Changes in physiology only are often less permanent than morphological changes (Bradshaw 1965) and thus may be expected to show higher plasticity. Studies have shown that morphological traits such as leaf length, leaf width, the ratio of length to width, leaf area, specific leaf area (SLA) (the ratio of leaf area to dry mass) and wood density, as well as the physiological traits of

transpiration and photosynthetic rate ( $A_{\max}$ ), instantaneous water-use efficiency, seasonal water-use efficiency as determined by carbon isotopes ( $\delta^{13}\text{C}$ ), foliar nitrogen content (%N) and nitrogen natural abundance ( $\delta^{15}\text{N}$ ), may all exhibit plastic responses to water deficits and arid conditions.

For instance, *Eucalyptus globulus* had smaller leaves in water-stressed environments than when it was well-watered (Osório et al. 1998). Likewise, smaller leaf area is a documented response to water deficit in other genera such as in *Quercus ilex* (Castro-Díez et al. 1997). This species also increased specific leaf mass to cope with water deficits, whereas *Q. faginea* increased leaf nitrogen content and decreased specific leaf mass in response to drought conditions. Reduced SLA to tolerate aridity is another phenotypic response that has been shown in studies of *Eucalyptus* and other Australian plant species (Schulze et al. 1998, Warren et al. 2006, Merchant et al. 2007). Greater wood density may also enhance drought tolerance through providing greater resistance to xylem cavitations under highly negative water pressures (Hacke et al. 2001).

Carbon isotope discrimination is related to season-length water use efficiency (WUE) (Farquhar et al. 1982) and thus, stands in more water stressed environments may exhibit greater WUE as illustrated by more positive  $\delta^{13}\text{C}$  values (Stewart et al. 1995, Schulze et al. 2006). Corcuera et al. (2010) found a high level of plasticity of  $\delta^{13}\text{C}$  in *Pinus pinaster* when two provenance progeny trials, one in a mesic location and one in a xeric location, were examined. Seedlings in the drier site increased WUE regardless of their origin. Instantaneous measures of WUE may complement season-length WUE. Ponton et al. (2002) found a strong correlation between  $\delta^{13}\text{C}$  and instantaneous WUE in two *Quercus* spp. Nitrogen isotopes are also useful for studying differences between climatic environments, with higher  $\delta^{15}\text{N}$  values generally found in drier, warmer conditions (Amundson et al. 2003, Craine et al. 2009). Furthermore, Merchant et al. (2007) also noted a decrease in photosynthetic rate, another physiological trait, associated with water stress. It may be assumed that species which have a high variability in these traits in response to increasing water and heat stress would be less vulnerable in a drying, warming climate, and may be able to occupy a broader range of climatic conditions than their current distributions may suggest (Pohlman et al. 2005).

### 1.2.1 Significance of the Study

Most studies of relationships between environment and plant traits have focused on variation between rather than within species, and many only compare growth traits, rather than physiological traits (*see* Thuiller et al. 2004, Wright et al. 2004, Schulze et al. 2006, Witkowski & Lamont 2006, Merchant et al. 2007, Yates et al. 2010b). In comparison, this study sought to determine both the ecotypic variation (between stands) of the morphological and physiological traits studied within a species and the relative plasticity of these traits. Greater variation between stands of a single species may reflect higher adaptive capacity to respond to a changing climate, which would improve resilience. Given the predictions for continuing rainfall decline and increases in temperature in Australia's south-west (Hughes 2003), such knowledge of adaptive potential is crucial to inform climatic modelling of species distribution changes (Helmuth et al. 2005).

Climate change models use the present environmental conditions and current distribution of a species to generate the 'climatic envelope' it may occupy (Jeschke & Strayer 2008). From this, the models predict where environmental conditions tolerated by the species will likely shift to, and consequently, where the species may be found under future climate scenarios (Loehle & LeBlanc 1996, Jeschke & Strayer 2008). However, this could exaggerate the effects of a changing climate, as phenotypic plasticity may allow species to occupy areas they currently do not. Knowledge of ecotypic variation in species morphology and physiology, as well as their ability to phenotypically adjust to water deficit and higher temperatures, is therefore essential for climate change models to improve predictions of range, distribution and composition changes in a changing climate (Loehle & LeBlanc 1996, Helmuth et al. 2005, Nicotra et al. 2010). Indeed, the level of phenotypic plasticity remains unknown for most plant species in south-west Western Australia (Prober et al. 2011). Knowledge gained will also help inform and improve effective conservation and management plans (Witkowski & Lamont 2006). High ecotypic variation may focus restoration efforts so that genetic material is sourced from stands which may be more adapted to coping with water deficits (thereby enhancing success of restoration efforts in a changing climate).

Nicotra et al. (2010) reviewed current knowledge relating to phenotypic plasticity in a changing climate and identified several unresolved questions. My study may contribute to three of these key, outstanding questions, specifically:

- I. "How much variation is there for plasticity...?"
- II. "Will the incidence of adaptive plasticity vary among types of traits (e.g. those related to anatomy... versus physiology?)"
- III. "How will plasticity contribute to rapid evolution in response to climate change?" (Nicotra et al. 2010, p. 686).

Provenance trials are commonly used to test the ability of a species and its ecotypes to cope with varying climatic conditions. However, comparisons between the natural and plantation stands are rare in the literature with the emphasis being placed on performance in common gardens across climate gradients rather than at all the collection locations. Common garden experiments are nonetheless useful to ascertain whether intra-specific phenotypic differences between stands is the result of ecotypic adaptation or phenotypic plasticity (Prat 2003). Such knowledge is crucial when aiming to conserve genetic diversity, since preserving ecotypes of a species would require each stand from each environment to be protected, whereas, if the differences were a plasticity response, only a sub-sample of the stands would require conservation to maintain the diversity of taxa (Prat 2003).

The maintenance of genetic diversity is critical if we are to preserve the ability of a species to adapt to, and cope with, future conditions such as global climate changes (Prat 2003, Lindenmayer & Burgman 2005). Currently, simultaneous studies using molecular markers for adaptive traits in *E. loxophleba* ssp. *lissophloia* are being undertaken to ascertain genetic diversity within the taxa (Byrne M., *pers. comm.*). Recently, studies have shown that genetic markers can identify loci in a plant related to climatic conditions and thus adaptive alleles may be detected (Hancock et al. 2011). Therefore my study will contribute to knowledge of *E. loxophleba* ssp. *lissophloia* eco-physiology which can be compared to genetic analyses to determine whether in-depth genetic studies can be undertaken directly.



### 1.2.2 Study Area and Species Description

The Mediterranean region of the south-west of Western Australia is considered to be the most vulnerable to contraction of all Mediterranean systems worldwide under predicted climate change scenarios (Klausemeyer & Shaw 2009). The region is therefore a priority area for research into the climate resilience and adaptive variation present in the plant species. Within the south-western Australian region, native vegetation of the Western Australian wheatbelt has become highly fragmented and degraded, and is thus the focus of significant restoration investment (Prober & Smith 2009). By contrast, vegetation of the adjacent, mostly drier region known as the Great Western Woodlands, has to date remained relatively intact. These two areas share many vegetation types, with many species spanning the climatic gradient from the wheatbelt to the Great Western Woodlands further east. Approximately 30 % of Australia's eucalypt species are found in the Great Western Woodlands of south-west Western Australia alone (Watson et al. 2008). They thus offer an ideal scenario for examining adaptive variation across environmental gradients, which in turn can inform climate adaptation strategies in degraded and intact landscapes (Prober & Smith 2009, Prober et al. 2011).

This project examined differences in morphological and physiological traits between stands of a eucalypt species across this climate gradient. This approach was taken to observe changes in the traits in stands located in increasingly warmer and drier conditions similar to those predicted in a changing climate. Significant ecotypic variation or differences in the traits depending on the site may increase adaptive potential and improve species resilience to climatic changes. Comparisons were also made regarding the differences (or plasticity) in traits observed between natural stands and the stands grown in an established plantation, to determine the short-term responses of a species to altered climatic conditions. This helped indicate whether the underlying basis for observed ecotypic variation was genetic or plastic.

*Eucalyptus loxophleba* Benth. (York Gum) has four subspecies, the *lissophloia* and *gratae* subspecies of mallee habit and the *loxophleba* and *supralaevis* subspecies of tree habit (Hines & Byrne 2001). *E. loxophleba* ssp. *lissophloia* L.A.S. Johnson & K.D. Hill (Smooth-barked York Gum) was studied (Fig. 2). This subspecies occurs as a mallee in woodlands and tall shrublands generally on shallow sand over clays, has a C<sub>3</sub> photosynthetic pathway and is lignotuberous (Hines & Byrne 2001, Boland et al. 2006).

*E. loxophleba* ssp. *lissophloia* was suitable given that it is geographically widespread in south-western Australia across a climate gradient, meaning that differences in traits relating to progressively more arid climatic conditions could be observed. Likewise, *E. loxophleba* is of commercial interest for oil production, owing to the (R)-4-methyl-2-pentyl acetate in the oil of its leaves and terminal branchlets, a component not found in the oil of other eucalypts (Grayling & Knox 1991, Davis 2002). Therefore, the subspecies has been grown in oil mallee plantations in more mesic environments, thus allowing a comparison between the natural stands and those sourced from the same provenance but grown in a plantation. Genetic analyses on this subspecies have revealed no strong genetic differentiation in neutral markers between *E. loxophleba* ssp. *lissophloia* populations (Hines & Byrne 2001).



**Figure 2:** *Eucalyptus loxophleba* ssp. *lissophloia* L.A.S. Johnson & K.D. Hill (Smooth barked York Gum) was the sub-species examined in this study. This subspecies is geographically widespread in south-western Australia.

### 1.2.3 Aims and Objectives

In light of the warming and drying trends predicted for the south-west of Western Australia, this study aimed to examine the amount of variation present between stands of a single subspecies in progressively warmer and drier conditions. If stands located in the cooler, wetter end of its range were significantly different to those currently found toward the warmer, drier end of the gradient then the potential exists for these stands in more mesic conditions to exhibit traits similar to those in the arid extreme of the gradient. This would therefore improve species resilience to projected climatic changes. The study also aimed to examine which traits showed the greatest phenotypic plasticity which would increase resistance and resilience to climatic changes in the short-term. Specific objectives were to:

1. Characterise the ecotypic variation in morphological and physiological traits between naturally occurring stands of the species, *Eucalyptus loxophleba* ssp. *lissophloia*, across a climate gradient in the south-west of Western Australia.
2. Determine the relative plasticity of morphological and physiological traits between natural stands of *Eucalyptus loxophleba* ssp. *lissophloia* and a provenance plantation trial located to the western end of the climate gradient in the south-west of Western Australia.

### 1.2.4 Thesis Outline

Chapter 1 has provided a background of climate changes which have occurred and are predicted to continue. It outlined the major mechanisms plants have for coping with these climatic changes and identified traits which are likely to respond to changing climatic conditions. The significance of the research was explained and the implications the findings may have for species resilience in a changing climate and improving bioclimatic modelling and restoration success were described. The study area and species of interest were described and the aims and the objectives of the project outlined.

Chapter 2 provides background information on the south-west Australian study area and outlines the sampling design and site selection criteria used to select which natural stands to sample. A description of the plantation to the western end of the climate

gradient used to assess plasticity between the natural stands exposed to modified climatic conditions is then provided. This is followed by the field and laboratory methods and statistical analyses used in this research.

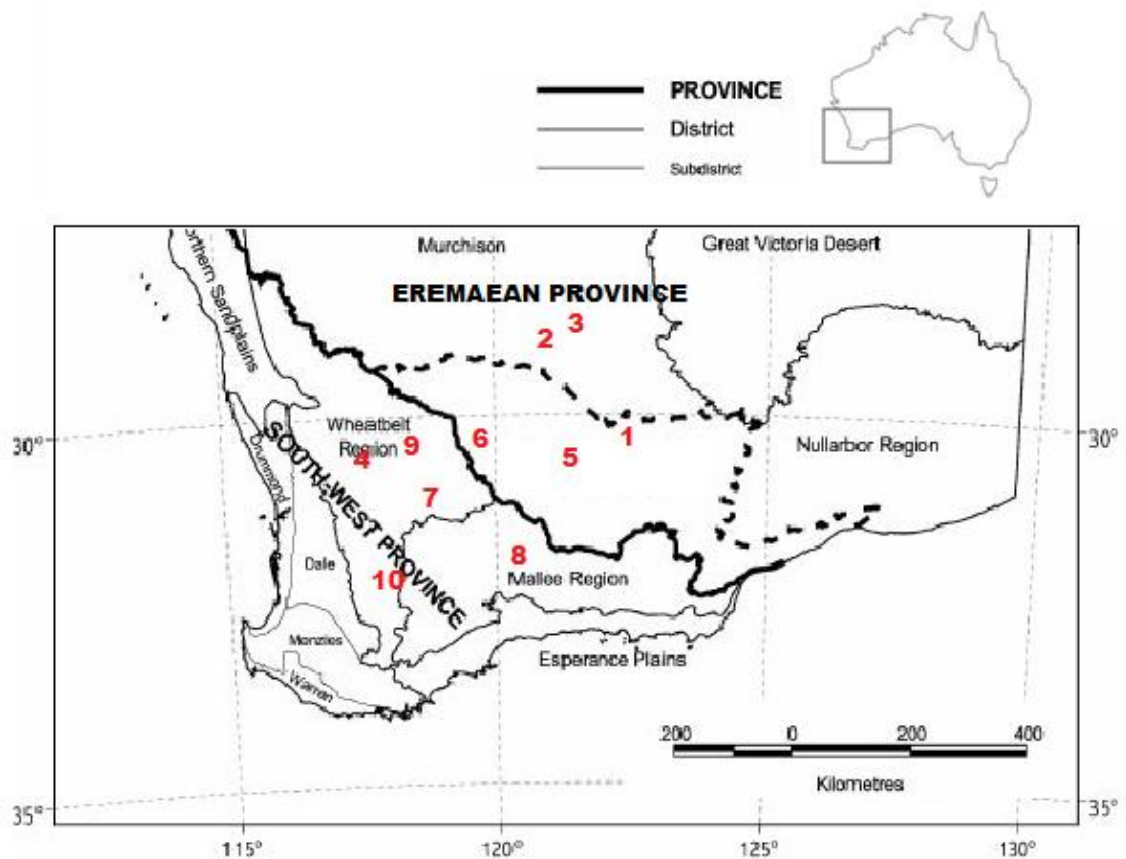
Chapter 3 describes and illustrates the major results found and is divided into three main sections. The first section summarises the characteristics of the natural sites along the gradient, as well as the plantation, with regard to long-term and recent climatic conditions experienced at the site and their soil characteristics and biotic structure. The second section describes the variation and trends in morphological and physiological traits between the natural stands along the gradient as well as the relationships between these traits and the climatic variables of the site. The third section examines the traits within the plantation site depending on their source of origin, and the relative plasticity of the traits studied compared to their respective natural source stand.

Chapter 4 discusses the results from Chapter 3 with regards to the ecotypic variation in, and plasticity of, the traits studied. It highlights the differences in ecotypic variation and plasticity between morphological and physiological traits. Important climatic determinants of the traits are explained. A description of the application of the results to resilience in a changing climate and how such knowledge can be used to improve restoration efforts and genetic analyses is provided.

# CHAPTER 2: METHODS

## 2.1 Study Area Description

The study was located in the south-west of Western Australia across a climate gradient with a range in long-term average annual rainfall from 230 to 350 mm. Sites were situated in the South-West Botanical Province and extended into the Eremaean Botanical Province (Murchison district) (Fig. 3). These provinces are distinguished by differences in climate, geology, flora, fauna and topography. However, granite geology underlies much of the south-west study area (Withers 2000).

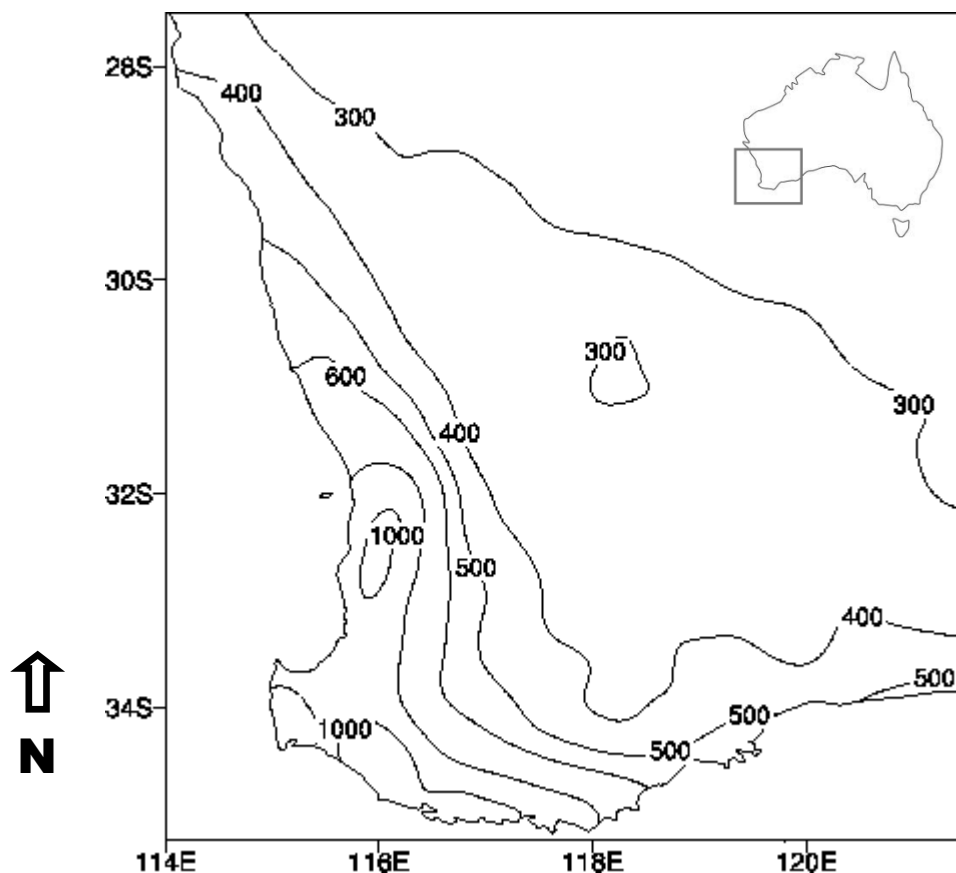


**Figure 3:** The location of the nine natural stands and the plantation of *E. loxophleba* ssp. *lissophloia* sampled in the South-West Botanical Province and extending into the Eremaean Botanical Province, in south-west Western Australia. Map reproduced from Beard et al. (2000).

The South-West Botanical Province is largely winter-rainfall dominated and annually receives between 1200 mm of rainfall in the extreme south-west and this decreases further inland to 300 mm (Beard et al. 2000). The climate is warm Mediterranean, where hot, dry summers and cool wet winters are experienced (Bamford 1995, Hobbs

2003, Prober & Smith 2009). The area is characterised by generally poor, sandy soils dominated by eucalypt woodland and mixed shrubland (Beard et al. 2000).

The research was also conducted within the south-western interzone and Murchison region in the Eremaean Botanical Province (Fig. 3). This region is distinctly drier (250-300 mm) and consists of uplands dominated by mallees and scrub-heaths as well as acacia woodlands and broad valleys with eucalypt woodlands (Hopper et al. 1997, Beard et al. 2000, Cowan et al. 2001). The seasonality of rainfall is more constant throughout the year and is summer-dominated in some areas (Beard et al. 2000). Rainfall contours for south-west Western Australia therefore reveal a general decrease in mean annual rainfall proceeding inland from the south-west to the north-east (Fig. 4).



**Figure 4:** Long term annual rainfall (mm) isohyets as averaged from 1925 to 2003 in south-west Western Australia. Proceeding inland from the south-west to the north-east, there is a general decrease in rainfall. Map reproduced from Jones & Weymouth (1997).

In addition to these rainfall trends, there is also a general increase in average temperature and evaporation rates and a decrease in humidity from the south-west to the

north-east. In the more northern areas of the south-west of Western Australia, maximum temperature in January is between 33-36 °C and annual average 9AM relative humidity ranges from 50-60 %, whereas the more southern areas in the South-West Botanical Province have maximum January temperatures of between 30-33°C and annual average 9AM relative humidity values ranging between 60-70 % (BOM 2011b, BOM 2011c).

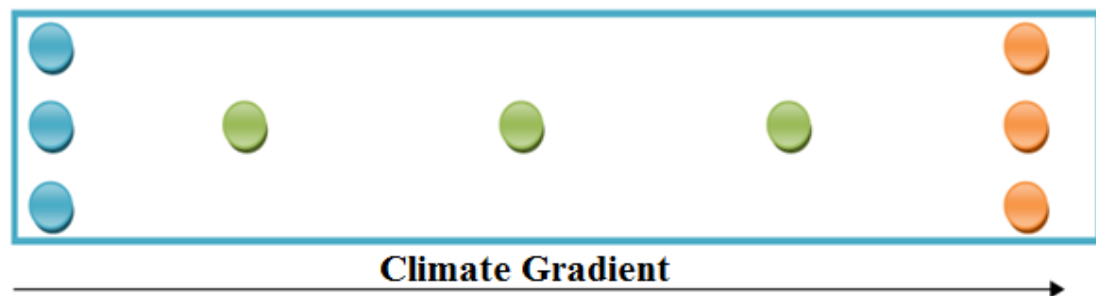
## 2.2 Sampling Design

In the south-west of Western Australia, rainfall decreases and temperature increases with increasing distance from the coast and in the higher latitudes of more northern locations. Sites were selected across this climate gradient from SW to NE.

Nine natural stands of *E. loxophleba* ssp. *lissophloia* were studied, as well as six provenance sources within one plantation trial. For the purpose of this study, a stand herein is defined as a collection of individual plants of the same species occurring continuously and within a small enough area as to be examined as a single unit, with no substantial differences in site conditions such as soil type or average rainfall.

- *Objective 1: Characterise the ecotypic variation in morphological and physiological traits between naturally occurring stands of the species, Eucalyptus loxophleba ssp. lissophloia, across a climate gradient in the south-west of Western Australia.*

Nine natural stands of *E. loxophleba* ssp. *lissophloia* were studied along the climate gradient from the cooler and wetter stands in the west to warmer and drier stands in the east. The nine stands included three stands at both extremes of the climate gradient, as well as three additional stands at intermediate locations along the gradient (Fig. 5). This allowed the extreme ends of the species range to be more thoroughly examined.



**Figure 5:** Conceptual diagram representing the location of nine sampling locations of *E. loxophleba* ssp. *lissophloia* across a climate gradient in south-west Western Australia. Three stands were sampled at the both the cooler and wetter end to the warmer and drier extreme of the gradient, as well as three stands at intermediate locations along the transect.

### 2.2.1 Site Selection and Location

Potential sampling sites along the entire transect were examined prior to any sampling being undertaken. This allowed careful and informed site selection and sampling only once all possible sites had been considered.

The natural stands sampled were required to meet several criteria. Importantly, they had to satisfy the sampling design and cover the entire rainfall range that the sub species is known to occupy. Stands which may have been influenced by nearby constructs such as roads, tracks, highways and other hard surfaces which could potentially alter natural drainage distribution patterns, surface runoff flows and volumes to the site, were avoided (Montgomery 1994, Forman & Alexander 1998). Furthermore, the tree subspecies *Eucalyptus loxophleba* ssp. *loxophleba* has been found growing in association with granite rocks (Mullan 2000). Thus, the potential exists for the *lissophloia* subspecies to exhibit the same association with a water collecting environment. As a precaution therefore, no stands within 50 m of exposed granite outcrops were studied. It is acknowledged however, that influence of potential underlying granites could not be accounted for.

It is common for *E. loxophleba* ssp. *lissophloia* to co-occur with other plant species including *Acacia acuminata* (jam), *Santalum spicatum* (sandalwood), *Eucalyptus salmonophloia* (salmon gum), *E. salubris* (gimlet) and *E. wandoo* (wandoo) (Boland et al. 2006). Thus, the stands sampled were dominated by the species of interest where minimal co-occurrence of other plant species occurred (Fig. 6). In addition, stands in



recently burnt areas (time since fire  $\leq 3$  years) were not selected for sampling as the disturbance from fire could be a potentially complicating factor. However, this was merely a precaution, as another study of Australian plant species in sites with time since fire  $\leq 3$  years showed no significant difference in the leaf traits examined, including specific leaf area, carbon and nitrogen isotope ratios and leaf nitrogen concentration, to those which had less recent fire activity (Schulze et al. 1998). Grazing has been shown to produce a considerable increase in nitrogen isotope values and a decrease in leaf nitrogen concentration (Schulze et al. 1998). As such, sites with a low to medium grazing level were sampled where possible. All stands were free of obvious disease or pathogens and were situated on low to gently undulating land.



**Figure 6:** Natural stands selected for sampling were dominated by *E. loxophleba* ssp. *lissophloia* where minimal co-occurrence of other plant species occurred.

Stands that seed had been sourced from for the Toolibin plantation were sampled where these criteria were met. The nine natural stands covered both a broad geographic range (approximately 450 km in length) across the south-west of Western Australia as well as the climate gradient (Fig. 3). Outcrossing between the natural stands studied could

potentially influence the relationship with the average climatic variables of the site. However, this is unlikely to have occurred as outcrossing between populations of *E. loxophleba* is limited to a maximum of around 2 km (Sampson & Byrne 2008).

Sites ranged from 235 mm long term average annual rainfall at site 1 at the drier end, to 335 mm long term average rainfall at site 9 at the wetter end (Table 1, Fig. 3). This allowed the subspecies to be sampled across the entire rainfall range that it is known to occupy (250-350 mm) (Florabank 2011).

**Table 1:** The site name, number (No.), code, type and coordinates of the sample sites of *E. loxophleba* ssp. *lissophloia* in south-west Western Australia

Site Name	Site No.	Site Code	Stand Type	Coordinates
Karonie	1	KAR	Natural	31°01' 09.8" S; 123°03' 57.2" E
Goongarrie	2	GOO	Natural	29°58' 21.3" S; 121°03' 22.0" E
Pianto Road	3	PIA	Natural	29°54' 59.4" S; 121°40' 21.4" E
Hines Hill	4	HIN	Natural	31°32' 20.0" S; 118°03' 40.2" E
Quairnie Rock	5	QUR	Natural	31°15' 54.9" S; 121°05' 13.1" E
Yellowdine	6	YEL	Natural	31°17' 33.7" S; 119°40' 28.9" E
Narembeen	7	NAR	Natural	32°01' 06.4" S; 118°32' 32.3" E
Graham Rock	8	GRA	Natural	32°27' 52.0" S; 119°03' 24.5" E
Burracoppin	9	BUR	Natural	31°23' 02.7" S; 118°27' 21.1" E
Toolibin	10	TBN	Plantation	32°53' 28.3" S; 117°37' 09.2" E

*E. loxophleba* ssp. *lissophloia* and other mallee eucalypts can potentially acquire soil water as deep as 8-10 m (Robinson et al. 2006). However, given the often saline and acidic quality of groundwater in the region (Farrington et al. 1994), it is unlikely that heavy use of groundwater was occurring, which may have otherwise significantly impacted on relationship with the climate gradient. This is further exacerbated by the study taking place during winter, when reduced groundwater use may be required, due to generally lower transpiration rates and higher rainfall (Farrington et al. 1994, Wildly et al. 2004).

- *Objective 2: Determine the relative plasticity of morphological and physiological traits between natural stands of Eucalyptus loxophleba ssp. lissophloia and a provenance plantation trial located to the western end of the climate gradient in the south-west of Western Australia.*

In 1999, the Department of Environment and Conservation established an oil mallee plantation trial in Toolibin, approximately 40 km east of Narrogin, Western Australia (Table 1, Fig. 3). With a long term average rainfall of almost 355 mm, this site is narrowly outside the rainfall range the sub-species is known to naturally inhabit (Florabank 2011), and was therefore wetter than the natural stands sampled. Seed for the plantation was sourced from six of the natural stands I sampled. This allowed a comparison between genetically similar individuals in differing climatic conditions to determine how plastic the traits measured may be as a consequence of external environmental influences as opposed to genetic factors determined by the seeds source of origin (provenance).

The plantation was structured in a grid, with 30 rows by 4 columns for a total of 120 plots (Fig. 7). Each plot contained 60 individuals in a 10 x 6 layout. In 2005, the orchard underwent thinning of individuals with low leaf cineole levels and/or biomass production, to 10 individuals per plot. I selected ten individuals from each of the six difference provenances across the plantation and carried out leaf trait sampling and instantaneous measurements of physiological traits. Individuals that had primarily regrowth shoots due to being coppiced during thinning in 2005 were not sampled. This is because coppicing has been shown to alter water-use relations and leaf area indices in eucalypts in the Western Australian wheatbelt (Wildly et al. 2004).

Whilst selection for high leaf cineole concentration occurred during the thinning of the plantation, this was not expected to have biased or significantly influenced water use characteristics or traits. Leaf oil production and cineole concentrations in mature leaves of *Eucalyptus camaldulensis* were not found to be notably influenced by water deficit (Doran & Bell 1994, Stone & Bacon 1994). In this way, it was unlikely that selection favouring aspects of oil characteristics in the eucalypt species grown would have any correlation to water use conservation or drought tolerance traits. The composition of leaf oil is thought to be chiefly controlled by inheritance, although it is recognised that



other environmental factors may influence the yield and concentration of oil (Barton et al. 1989, Doran & Bell 1994, Wildly et al. 2000b).



**Figure 7:** Toolibin plantation site of *E. loxophleba* ssp. *lissophloia* established by the Department of Environment and Conservation and planted in a grid structure.

The thinning undertaken in 2005 also removed individuals with low biomass production. However, in a study involving *E. loxophleba* ssp. *lissophloia* and other eucalypts at twelve oil mallee plantation sites in the Western Australian wheatbelt, the total biomass produced by the first harvest was influenced by the site's access to water (Wildly et al. 2000a). In this way, the thinning in 2005, which removed individuals with low biomass production, could have increased traits relevant to water conservation or retrieval which caused those which obtained a greater biomass to be retained.

## 2.3 Field and Laboratory Methods

Field sampling was undertaken in May, June and July 2011. At each site details recorded included: site name, GPS location, name of the nearest town, recent rainfall events, a grazing index value (very low, low, medium) and general site observations. The GPS location of each individual tree and soil sample was also recorded. I sampled ten individuals at each site which were selected for maturity, health and average height, with a minimum distance of 10 m between the trees sampled. The subjective criteria used to estimate health were minimal leaf discolouration or insect-damage, intact mallee stems and located away from the exposed outside edge of the stand. The height of each mallee was measured and a stem sample to measure wood density was taken. Twenty healthy, mature, sun-exposed leaves were picked to analyse average leaf length, width and dry mass, area per leaf, specific leaf area, leaf nitrogen concentration, carbon ( $\delta^{13}\text{C}$ ) and nitrogen isotope ( $\delta^{15}\text{N}$ ) ratios. In addition, transpiration rates and photosynthetic rate ( $A_{\text{max}}$ ) were measured. Details of method are given below.

For each natural stand and the plantation, long term, 5 year, 2 year, 18, 12, 6 and 3 month averages of rainfall, maximum and minimum temperature, evaporation, radiation, vapour pressure, relative humidity at maximum and minimum temperature and evapo-transpiration were interpolated using SILO climatic data from the Bureau of Meteorology.

### 2.3.1 Morphological traits

Height was estimated using a calibrated tool whereby one length of the tool represented approximately 3 m in height when held at arm's length at a distance of ten metres from an individual. Estimates of the number of lengths of the tool from the base to the tip of the tallest mallee stem (excluding dead protruding branches) were made. Average height was ascertained on ten individuals at each site, which is the recommended minimum sample size for this trait (Cornelissen et al. 2003).

At each site twenty leaves were collected from the sun-exposed crown (Schulze et al. 1998) of each of the ten individual mallees. Only mature, fully expanded leaves in the healthiest condition were selected. Juvenile or insect-damaged leaves were avoided. I measured the maximum length along the leaves mid-vein to obtain total leaf length and measured the greatest width across the leaf blade to give maximum leaf width. Dividing the former by the latter provided a ratio of total length to maximum width.

I then measured the projected area of the leaf blade with the petiole and without the petiole for calculation of specific leaf area (SLA) and measurement of area per leaf respectively. A Leaf Area Meter and the program WinDias 2.0 was used. Twenty leaves from each of the ten individuals per site exceeded the minimum recommended sampling size for these traits (of two leaves on five individuals) (Cornelissen et al. 2003). An analysis of variance was calculated on three leaves after measuring them 10 separate times each. The variance was less than 1% for all three leaves and thus measuring area per leaf only once per leaf was sufficient. Where leaves were not completely flat I placed a plastic screen on top of the leaf before the projected area of the leaf was measured to ensure that area per leaf was not underestimated. Leaves were dried at 60°C until a constant mass was attained (>72h) before I weighed them using a calibrated scale to get dry mass (g). SLA was the projected leaf area with petiole divided by the oven-dried mass.

Five individuals is the minimum sample size advocated for measuring wood density, however, wood from ten individuals per site was assessed, which is preferred for this trait (Cornelissen et al. 2003). One main stem segment from each individual mallee was taken. I cut each sample down to at least 150 mm in length and then measured the average diameter of both ends of the wood sample. The volume was then calculated as the volume of a cylinder where the diameter at both ends was equal or as per a frustum of a cone when the diameters of the ends were not equal. Wood samples were then dried at 60 °C until a constant mass was achieved (>120 hours). Their dry weight was measured and divided by their fresh volume weight to obtain the wood density of each stem (Hacke et al. 2000).

I was not able to measure wood density at the Toolibin plantation due to the single-stemmed nature of all the individuals there, which would have necessitated the destruction of the trees to obtain a wood sample. However, previous analysis by the Department of Environment and Conservation had destructively measured wood density through taking a disk from 29 individuals at ground level, 10 %, 20 %, 30 %, 40 %, 50 %, 60% and 70 % of the total top height, and averaging the basic density of each disc to give an average tree basic density.

### 2.3.2 Physiological traits

Five leaves from each of the ten individuals per site were bulked and analysed for foliar nitrogen content (%N), nitrogen natural abundance ( $\delta^{15}\text{N}$ ) and carbon isotope values ( $\delta^{13}\text{C}$ ). I took a sub-section of the leaves across their mid-section (including the mid-vein) and excluded the tip and base of the leaf. I rough chopped the leaf material and placed it in eppendorf tubes. These were oven dried for 12 hours at 57 °C before I ground these samples at 30 hertz for four minutes using a Retsch MM200 ball mill and two stainless steel ball-bearings (6.3 mm).

For analysis of %N and  $\delta^{15}\text{N}$ , I weighed out 5.00-5.50 mg of sample in to 8x5 mm tin capsules. These were combusted (ANCA-GSL, Europa, Crewe, United Kingdom) and then analysed using a continuous flow isotope mass spectrometer (20-20 IRMS, Europa, Crewe, United Kingdom) by the Edith Cowan University, School of Natural Sciences Analytical Services (Joondalup, WA). Reference standards were used for calibration and were placed amongst the samples. Nitrogen isotopic values are measured in parts per thousand relative to the  $\text{N}_2$  in the atmosphere.

As an indicator of seasonal water use efficiency, carbon isotopes ( $\delta^{13}\text{C}$ ) were measured from the cellulose component of the leaves. Cellulose was selected to analyse  $\delta^{13}\text{C}$  as it is less variable and representative of the whole plant, and is also fairly immobile (Wilson & Grinstead 1977, Deines 1980, Leavitt & Danzer 1993, Macfarlane et al. 1999). Indeed,  $\delta^{13}\text{C}$  measured from whole wood was found to be highly variable between heartwood and sapwood whilst the lignin-cellulose ratio is also inconsistent within an individual tree (Wilson & Grinstead 1977). To validate this, I initially analysed the  $\delta^{13}\text{C}$  of the whole leaf when analysing %N and  $\delta^{15}\text{N}$ . I compared the results to the  $\delta^{13}\text{C}$  of just the cellulose portion. The results were highly variable and were between 2 and 19 % higher when measured in the cellulose portion. Therefore,  $\delta^{13}\text{C}$  of the bulked leaf would not have provided an accurate representation of the entire plant owing to its apparent variability.

Cellulose was extracted using the modified diglyme-HCl method (Macfarlane et al. 1999). This extraction method is a rapid process that does not require highly specific glassware (Macfarlane et al. 1999). I weighed out 100-250 mg of sample from the remainder of the ground samples of the five bulked leaves from all the individuals per

site used for %N and  $\delta^{15}\text{N}$  analysis in to 15 ml vials. 2 ml of diglyme and 0.5 ml of 10 M HCl were added and then sealed with an aluminium lid with teflon-butyl liners. Vials were then placed in a shaking water bath heated to 90 °C for one hour. I then removed the seals and filtered the residue of each sample using a vacuum through a 90 mm cellulose hardened ashless filter paper, which was folded into a cone and placed in a funnel in a 100 ml Erlenmeyer flask. Samples were rinsed with approximately 20 ml of methanol followed by 40-60 ml of boiling water. I placed the filter papers in a 57 °C oven overnight before 2 mg (+/-0.50) of dried cellulose was carefully removed from the paper and weighed into tin capsules for  $\delta^{13}\text{C}$  analysis.

I trialled this cellulose extraction method and  $\delta^{13}\text{C}$  analysis using five duplicates of four trees and analysed them for  $\delta^{13}\text{C}$ . The average coefficient of variation was less than 0.8% between the trial samples and thus duplicates were not necessary. Foliar  $\delta^{13}\text{C}$  were measured by combusting samples to  $\text{CO}_2$  (ANCA-GSL, Europa, Crewe, United Kingdom) and then analysed using a continuous flow isotope ratio mass spectrometer (20-20 IRMS, Europa, Crewe, United Kingdom). Carbon isotopic values (parts per thousand) relative to V-PDB, as well as nitrogen isotope values, were determined using the general formula:

$$\delta^{\text{NE}} (\text{‰}) = (\text{R}_{\text{sample}} / \text{R}_{\text{Standard}} - 1)$$

Instantaneous physiological traits were measured in the field using an infra red gas analyser and LiCor 6400. The LiCor is a portable photosynthesis system which can measure the maximum photosynthetic and transpiration rate of each individual. These measurements were recorded between the hours of 1100 and 1400 h as the sampling was undertaken in late autumn to winter, meaning that the leaves were not stressed at this time and that any dew had evaporated. Conditions within the infra-red gas analyser chambers were controlled with  $\text{CO}_2$  levels set at 390 ppm, temperature at 25°C, light intensity (provided using the red-blue LED light source) at  $1500 \text{ mol m}^{-2} \text{ s}^{-1}$ . Leaves on the sun-exposed crown were measured whilst still attached to the stem. The transpiration ratio (net photosynthesis per unit of transpiration) was calculated from these measurements to provide a measure of instantaneous water use efficiency (WUEi).



### 2.3.3 Soil characteristics

As a widespread species, York Gum has been documented growing on a variety of soil textures including loams, clay loams, sandy loams and loamy sands (McArthur 1991). pH in 0.01M CaCl<sub>2</sub> in the top 10-20 cm of the soil is generally slightly acidic (4.7 to 7.6) but it can tolerate slightly alkaline soils with a pH in water of at least 8.3 (McArthur 1991). York Gum is also versatile in the range of soil electrical conductivities it can withstand. McArthur (1991) observed York Gum in soils with EC ranging from 1- 46 mS/cm in the top soil horizon. Organic carbon is commonly between <1 % to 10 % whilst clay content is approximately 5 % to almost 30 %. York Gum may also tolerate soils with total N from 0.02 to 0.16 % and total P of 57 mg kg<sup>-1</sup> up to at least 340 mg kg<sup>-1</sup> in the top 10-20 cm of the soil (McArthur 1991). Due to the versatility of the species, I analysed the soil at each site.

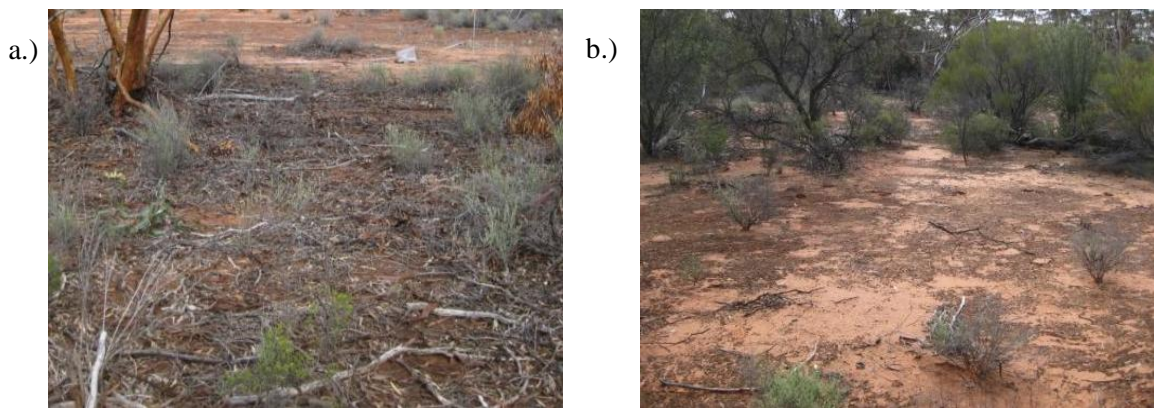
I took samples to a depth of 150 mm and a diameter of 300 mm at three locations at each of the sites at a distance of 2 m from one of the trees sampled. Soil was placed in plastic zip-lock bags for further analysis (Rowell 1994). At the Toolibin plantation six samples from across the entire plot to include the entire length and width of the area were taken. I measured soil field texture on unsieved soil using the techniques and field texture grade outlined in McDonald & Isbell (2009). Soil was moistened and kneaded with the degree to which it can be manipulated providing an approximate measure of texture.

Soil was oven dried at 105 °C for 24 hours and sieved (2 mm). I then measured the carbon loss on ignition as a measure of organic carbon by placing pre-weighed oven-dried soil in a furnace set at 500 °C for three hours. Samples were cooled in a desiccator and re-weighed. The difference in weight was used to ascertain loss on ignition. I also analysed soil for electrical conductivity and pH in water and pH in CaCl<sub>2</sub>. A 10 g soil sample and 50 mL of distilled water were placed in 75 mL plastic vials and shaken for ten minutes. Two replicates of each soil sample were measured. pH was measured using a calibrated WTW pH/mV 330 Meter. Once samples had settled for fifteen minutes conductivity was measured using a calibrated WTW LF Conductivity 330 Meter. 1 mL of 0.01M CaCl<sub>2</sub> was then added to each soil sample and shaken for another ten minutes. pH in CaCl<sub>2</sub> was then measured using the calibrated WTW pH/mV 330 Meter.

I used a Munsell colour chart to provide an indication of soil colour. Repellency was measured by placing two to three drops of liquid (starting at deionised water and proceeding up to 4 M ethanol) onto a soil sample and determining when the water or ethanol could not penetrate the soil within a ten second time frame. Samples were sent to CSBP Limited analytical laboratories (Bibra Lake, WA) for analysis of soil total nitrogen and total phosphorus.

#### 2.3.4 Site characteristics

Stand density was measured to reflect differences in intra-specific competition and ecology between stands. In the natural stands, a 20 m x 20 m quadrat was randomly located in each stand and the percent cover of trees, dead trees, shrubs, understorey, leaf litter and bare ground (Fig. 8) was estimated. Maximum tree height, number of stems and number of trees per quadrat were recorded.



**Figure 8:** Depiction of what was considered a.) leaf litter and, b.) bare ground within quadrats when estimating their percent cover in natural stands of *E. loxophleba* ssp. *lissophloia* sampled across the south-west of Western Australia.

#### 2.4 Statistical Analysis

Means and standard deviations were calculated for each individual tree and also for all the trees at a site. I performed one-way analysis of variance (ANOVA) on the means of the traits measured on the ten trees per site ( $n=10$ ) where the assumptions of an ANOVA were met. Homogeneity of data was tested by a Levene's test (significance  $>0.05$ ) and normality of data distribution was determined by the Kolmogorov-Smirnov and Shapiro-Wilk tests ( $n=1$ ) (Sig. 0.05). The data was transformed when necessary to meet these assumptions. I used log transformation of  $A_{\max}$  and  $WUE_i$  and natural log transformation of  $\delta^{15}\text{N}$  and transpiration rate. When the ANOVA was significant I performed a *post hoc* Tukey's test to determine which groups were different. In addition, biotic structure and soil data from the sites were also compared using one-way ANOVA

once I had shown that the assumptions had been met. Cosine transformation for EC, square root transformation for TN and sin transformation for TP was used.

Given the atypical rainfall patterns across the gradient preceding leaf growth, traits were related to climatic conditions averaged over the long term, 5 and 2 years, and 18, 12, 6 and 3 months prior to sampling (from 01/06/2011). Pearson correlation coefficients showed which climatic variables were correlated (5% significance). Several indices, such as long term, 5 year, 2 year, 18 and 12 month minimum temperatures, were highly correlated, and thus only the longest average data were included and used as a proxy for the more recent climatic variables in these cases to avoid exaggerating the influence of one variable. Graphing the climatic data against the traits showed linear relationships. Therefore, stepwise linear regressions were performed on each of the morphological and physiological plant traits (with stepping method criteria: probability of F to enter < 0.05 and to remove > 0.10). This determined which environmental variables significantly contributed to the traits and the adjusted  $r^2$  values. Where two of the same climatic variables (different time frames that were not significantly correlated, for example, long-term and twelve month average rainfall) were significant in the regression, the less significant variable was removed and the regression recalculated. This was to ensure that one variable did not bias or be over-accounted for in the analysis.

A plasticity index (PI) was calculated using the formula outlined in Godoy et al. (2011) (adapted from Valladares et al. 2000), to compare the means of traits in the natural environment (env 1) to the plantation environment (env 2). The formula,

$$PI = (\text{Mean}[\text{env1}] - \text{Mean}[\text{env2}] / (\text{MAXMean}[\text{env1}], \text{Mean}[\text{env2}])$$

ranged from zero to (+/-) one whereby zero was no plasticity and (+/-) one was maximum plasticity. MAXMean was the higher of the two means (from either environment 1 or 2) for each trait. A negative PI indicated that the mean value of a given trait was higher in environment two. Absolute average plasticity was calculated on both a per-site and trait basis. Despite the array of plasticity estimators available (*see* Valladares et al. 2006), this index was chosen as it is simple, easy to calculate, appropriate along gradients where the level of plasticity is expected to differ and only has statistical limitations when comparing species (Valladares et al. 2006).

# CHAPTER 3: RESULTS

## 3.1 Climatic and soil conditions and the biotic structure of the study sites

### 3.1.1 Long-term climate characteristics of the study sites

The long-term average rainfall of the natural stands studied ranged from approximately 235 to 335 mm per annum. The Toolibin plantation site had a long term average of 355 mm (Table 2). Sites situated further east tended to be drier, with approximately 40-50% of the average 5 year rainfall received in the summer months. The three driest sites, all receiving less than 245 mm annually, were Karonie, Goongarrie and Pianto Road. These sites were the most inland and were located in the arid to semi-arid warm Mediterranean Eremaean Botanical Province.

The sites along the gradient with intermediate long-term average rainfall levels were situated in the middle of the transect. Hines Hill was the fourth driest site despite its relatively western position, followed by Quairnie Rock and Yellowdine (averaging 275, 280 and 320 mm per annum, respectively) (Table 2). The sites further west were typically wetter and experienced winter-dominated rainfall. Indeed, 35-40 % of the 5 year average rainfall was received during the winter months. The three wettest sites, Narembeen, Graham Rock and Burracoppin, were all located in the South-West Botanical Province. The long term average rainfall was at least 325 mm per annum (Table 2). The Toolibin plantation site had the highest average long-term rainfall of 354 mm (Table 2).

There was also a temperature gradient across the study sites. There was a difference in long-term maximum temperature of 2 °C from Goongarrie in the northeast of the gradient, with an average maximum temperature of 26.4 °C, to the cooler Graham Rock (the most southern site) with an average maximum temperature of 24.4 °C (Table 2). The three driest sites to the east had the highest average long-term maximum temperature (Table 2). Two of the intermediate sites, Hines Hill and Yellowdine, again exhibited a midway maximum temperature of 25.1 °C. Quairnie Rock and the three wettest sites located furthest west along the gradient had the lowest average long-term maximum temperature, all under 25 °C. Toolibin was cooler than the natural sites, with the lowest maximum temperature of 22.7 °C (Table 2).

**Table 2:** Summary of the long-term (01/06/1940 to 31/05/2011) climatic variable conditions of the ten sites studied across the south-west Western Australia, based on SILO interpolated data from the Bureau of Meteorology.

Variable	KAR	GOO	PIA	HIN	QUR	YEL	NAR	GRA	BUR	TBN
Annual Rainfall (mm)	234.6	241.0	243.3	275.9	278.8	320.1	323.0	329.7	332.7	354.0
Temperature Max (°C)	25.2	26.4	26.2	25.1	24.5	25.1	24.5	24.4	24.8	22.7
Radiation (MJ m <sup>-2</sup> d <sup>-1</sup> )	18.8	19.4	19.4	18.9	18.8	18.8	18.6	18.2	19.0	17.8
Daily Evaporation (mm d <sup>-1</sup> )	6.6	7.3	7.4	6.0	6.4	6.2	5.6	5.4	6.0	4.6
Relative Humidity (max temp) (%)	36.5	33.6	33.5	40.9	37.6	37.6	41.4	41.4	40.2	45.2

There was a small variation in long-term average radiation along the gradient, although it was generally higher towards the eastern end of the gradient (especially in Goongarrie and Pianto, the most northern sites at the extreme east end of the gradient) (Table 2). Likewise, long-term radiation levels were lower towards the western end of the gradient and, of the natural stands, were lowest in the most southern site, Graham Rock. The Toolibin plantation had the lowest long-term average radiation of all the sites at 17.8 MJ m<sup>-2</sup> d<sup>-1</sup> (Table 2).

Evaporation rates were highest towards the eastern end of the gradient. Long-term evaporation rates were greatest in the two sites with the highest average long-term maximum temperature: Pianto Road and Goongarrie. Both of these sites had an average evaporation rate of over 7 mm d<sup>-1</sup>. Karonie, also at the dry end of the gradient had the

next highest evaporation rate, averaging  $6.6 \text{ mm d}^{-1}$  (Table 2). Following the same general trends as rainfall and maximum temperature, sites further west along the gradient, which typically experienced lower average temperatures and higher long-term rainfall, had slightly lower rates of evaporation. Graham Rock had the lowest average rate of evaporation of the natural stands at  $5.4 \text{ mm d}^{-1}$  whereas Toolibin was again an outlier with a much lower average evaporation rate of  $4.6 \text{ d}^{-1} \text{ mm}$  (Table 2).

The more inland, easterly sites with lower average rainfall and higher maximum temperatures recorded the lowest relative humidity levels. Pianto Road had the lowest relative humidity at maximum temperature at 33.5 % and was the most inland of all the sites. This was followed by Goongarrie and Karonie, also at the dry end of the gradient (Table 2). Of the intermediate sites, Quairnie Rock and Yellowdine had a lower relative humidity than Hines Hill, despite their higher long-term average rainfall and similar average maximum temperature. Hines Hill, which occupied a more coastal location, had a relative humidity at maximum temperature averaging almost 41 % whereas Quairnie Rock and Yellowdine were both 37.6 % (Table 2).

The three wettest sites all had an average relative humidity at maximum temperature above 40 % and it was equally highest in Narembreen and Graham Rock at 41.4 %. These sites were the furthest south of all the natural stands studied. Toolibin was again the outlier with a substantially greater relative humidity averaging 45.2 % (Table 2).

In summary, there were clear climate gradients between the study sites sampled. The driest sites were found at the eastern extreme of the gradient and tended to have higher maximum temperatures, higher evaporation rates and lower relative humidity (at maximum temperature). The inverse of this was shown in the wetter sites, typically found towards the west of the gradient. These had lower maximum temperatures and evaporation rates and higher average relative humidity (at maximum temperature) based on long-term data. There were apparent differences between the Toolibin plantation and the natural stands, as the plantation had the highest rainfall and humidity levels as well as the lowest maximum temperature and evaporation rates of all the sites sampled.

### **3.1.2 Recent trends in climate characteristics of the study sites**

In the twelve months prior to sampling (01/06/2010 to 31/05/2011) SILO interpretations (Bureau of Meteorology) indicated that rainfall patterns were anomalous and sites to the west of the gradient received below average rainfall. Indeed, south-west Western Australia had its driest year on record in 2010 (BOM 2011a) whereas the sites further east received above average rainfall. This meant that the wetter sites in terms of their long-term rainfall, including Yellowdine, Burracoppin, Narembeen and Graham Rock which all generally have over 320 mm per annum, had only received approximately 222, 272, 206 and 193 mm respectively (Table 3). Therefore, the long-term rainfall gradient studied was inverted when considering annual rainfall in the twelve months prior to sampling.

In this way, the 'wetter' end of the gradient when considering long-term trends had significantly lower rainfall in the last twelve months prior to sampling. As mentioned above, Graham Rock received well below the average annual rainfall, receiving 40 % less in the period from 01/06/2010 to 31/05/2011 than the long-term average of the site. Toolibin also received over 100 mm less than the long-term average whilst Hines Hill and Narembeen received barely 200 mm (Table 3).

The greatest variation from the long-term rainfall average in the twelve months prior to sampling was in Graham Rock, where rainfall received was 137 mm below the long-term average. Narembeen and Yellowdine followed, with an average twelve month rainfall 117 and 98 mm below their respective long-term average rainfall. Karonie had the lowest rainfall variability between these two time frames, recording less than a 3 mm difference between long-term and recent twelve month rainfall averages (Tables 2 and 3).

In the eastern end of the gradient, average maximum temperatures over the last twelve months have been lower than the long-term average (Tables 2 and 3). However, the most western sites typically experienced higher average maximum temperature over the last twelve months than the long-term average. Narembeen, Graham Rock and Burracoppin went from an average maximum temperature under 25°C to almost 26 °C (Tables 2 and 3). The average maximum temperatures over the last twelve months recorded at Toolibin also increased by over 1 °C to an average of 23.8 °C.

**Table 3:** Summary of the recent (01/06/2010 to 31/05/2011) climatic variable conditions of the ten sites studied across the south-west Western Australia, based on SILO interpolated data from the Bureau of Meteorology.

Variable	KAR	GOO	PIA	HIN	QUR	YEL	NAR	GRA	BUR	TBN
Annual Rainfall (mm)	237.0	317.2	317.3	204.0	213.7	221.7	206.2	193.0	271.6	221.5
Temperature Max (°C)	25.2	26.4	26.2	25.1	24.5	25.1	24.5	24.4	24.8	23.8
Radiation (MJ m <sup>-2</sup> d <sup>-1</sup> )	17.7	18.7	18.5	19.5	18.1	18.9	19.1	18.8	19.4	18.7
Daily Evaporation (mm d <sup>-1</sup> )	6.6	7.3	7.4	6.0	6.4	6.2	5.6	5.4	6.0	4.6
Relative Humidity (max temp) (%)	36.5	33.6	33.5	40.9	37.6	37.6	41.4	41.4	40.2	40.5

In the twelve months prior to sampling, average radiation was also inverted. The lowest levels of radiation were recorded in the more eastern sites, including Karonie, Quairnie Rock and Pianto Road whilst higher average radiation levels were recorded to the west, especially in Hines Hill, Burracoppin and Narembeen (Table 3). The mean radiation level in Toolibin was intermediate compared to the natural stands (Table 3).

Recent rates of evaporation revealed similar trends to the long-term patterns whereby sites at the eastern end of the gradient exhibited higher rates of evaporation than those at the more western end. The same held for relative humidity at maximum temperature. Goongarrie and Pianto Road still had the lowest average relative humidity whilst Narembeen in the west had the highest level of humidity at almost 40 % (Table 3).



However, the three driest sites along the gradient, Karonie, Goongarrie and Pianto Road, had higher average relative humidity at maximum temperature in the last twelve months than the long-term average. In contrast, the other seven sites further west, including the Toolibin plantation, all had drier, lower relative humidity levels at maximum temperature. This meant that the gradient in relative humidity across the sites also decreased from a range of almost 12 % between the most and least humid sites when based on long-term data, to less than half of this range at a 5.7 % difference between the most and least humid sites based on recent humidity levels (Tables 2 and 3).

The long-term, five year, two year, 18 month, 12 month, 6 month and 3 month average climatic conditions of the sites, based on SILO interpolated data from the Bureau of Meteorology, is provided in Appendix I.

### **3.1.3 Soil characteristics and biotic structure of the study sites**

The study sites along the climate gradient were similar in their soil characteristics and biotic structure. Soil electrical conductivity (EC) was fairly uniform, ranging only slightly from approximately  $166 \mu\text{S cm}^{-1}$  at Goongarrie to  $240 \mu\text{S cm}^{-1}$  at Narembeen. Soil pH was also consistent across the gradient varying from 6.5 to 7.5 in water and 6.2 to 7.2 in  $\text{CaCl}_2$ . Yellowdine had the most acidic pH of the sites and Pianto Road had the most alkaline soils. Water repellency of the soil at all sites was low with deionised water (DW) penetrating within 10 seconds and soil TP was within a  $100 \text{ mg kg}^{-1}$  range from 35 to  $135 \text{ mg kg}^{-1}$  (Table 4). One way ANOVAS showed no significant differences between the plantation or the natural sites in any of these soil characteristics (Table 4). In addition, the level of soil water repellency was the same at each site.

In contrast, one-way ANOVA showed significant differences in soil total nitrogen (TN) content and loss on ignition (LOI) between sites ( $P < 0.005$  and  $0.001$  respectively). TN was highest in Pianto Road at 0.14 % and lowest in Hines Hill and Toolibin, both averaging 0.03 %. LOI ranged from just below 2 % at Hines Hill to a maximum of around 6.5 % at Narembeen (Table 4). However, these differences were not seen as biologically significant as the reference soil sites from McArthur (1991) where York Gum is located suggests that the species is commonly found in soils with TN from 0.02-0.16 % and organic carbon (measured by LOI) between  $<1$  % to 10 %. As the values for

all the study sites fell within these ranges it was apparent that both the natural sites and the plantation were located on soils typical of the species.

The structure of the sites was very similar across the gradient with no significant differences ( $p \leq 0.05$ ) in the percent cover of trees, dead trees, shrubs, ground layer, bare ground or leaf litter, or in the maximum tree height (Table 4). However, there was generally a higher mean cover of trees and shrubs in the more eastern sites with decreasing cover in the sites located in the extensively cleared wheatbelt area. Karonie and Goongarrie, for example, found further east, had approximately 48 and 37 % cover of trees respectively, whilst Toolibin and Graham Rock only had 5 % and 10 % tree cover respectively. Nevertheless, all sites had  $<50$  % coverage of trees and  $<5$  % dead trees (Table 4). There was a significant correlation between the percent cover of trees and shrubs, with a Pearson's correlation of 0.675 ( $P < 0.001$ ). Ground layer was variable between the sites, with Hines Hill, Burracoppin and Toolibin having around one third to almost one half covered by a ground layer (mosses, grasses and *Borya*; a resurrection plant). Comparatively, the other sites had less than a 5 % ground layer coverage. Goongarrie, Narembreen and Yellowdine had the greatest amount of bare ground (averaging between 55 % and 65%) whilst leaf litter was found in the highest average abundance ( $\geq 60$  %) in Karonie, Quairnie Rock and Toolibin (Table 4).

The numbers of *E. loxophleba* ssp. *lissophloia* within the sites were significantly different ( $P < 0.001$ ) as the Toolibin plantation had the greatest number whilst Pianto Road had the lowest total number of the species. Yellowdine and Burracoppin had the greatest number of stems of *E. loxophleba* ssp. *lissophloia*, averaging almost 40 stems in a 20 m by 20 m quadrat whilst Pianto Road and Graham Rock had the least (less than ten stems on average) (Table 4). The greatest total number of trees in each stand were found at the wetter end of the gradient, and fewest at the drier end. Goongarrie in particular had a higher percentage cover of trees, implying that although the trees were fewer in number, they occupied a greater area. There were no significant differences in the maximum tree height achieved (which ranged from 6.1 m to 11.2 m) (Table 4). However, estimates of the average height of the trees sampled were significantly different with the drier sites tending to have slightly taller individuals on average, generally over 6 m, whilst Toolibin and the three natural sites located towards the west had the shortest trees, with a mean of less than 6 m (Table 4).

**Table 4:** Soil and biotic characteristics of the ten sites studied across the south-west Western Australia, averaged across three 20 m x 20 m quadrats in each site. Bold font denotes a significant difference as determined by one-way ANOVA. Means followed by the same letter are not significantly different (Tukey's HSD post-hoc output at  $P \leq 0.05$ ). The subscript NS denotes a non-significant result of one-way ANOVA.

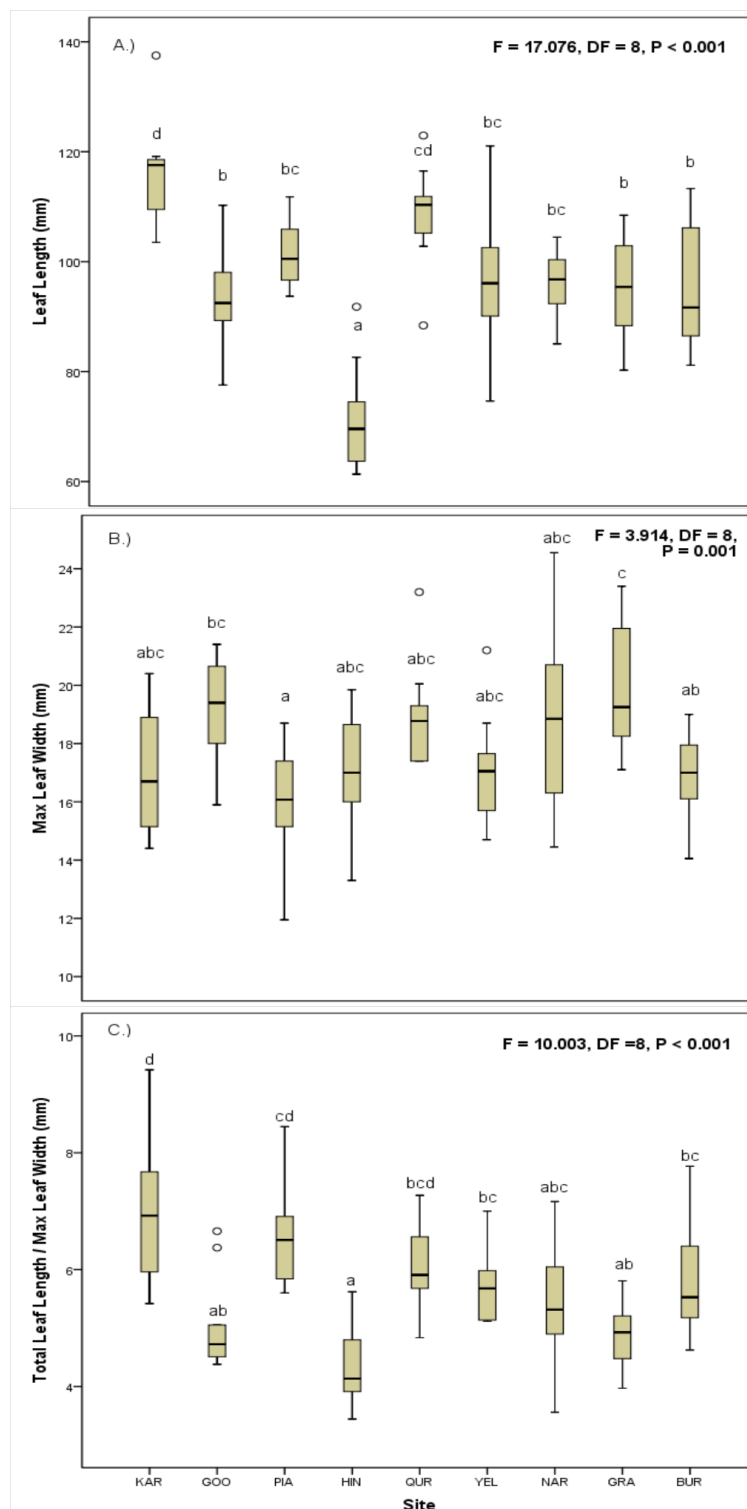
Site	KAR	GOO	PIA	HIN	QUR	YEL	NAR	GRA	BUR	TBN	ANOVA 'P'
Type	Natural	Natural	Natural	Natural	Natural	Natural	Natural	Natural	Natural	Plantation	
pH water	6.94	7.06	7.46	7.11	7.07	6.51	6.97	6.68	6.65	6.77	0.466 <sup>NS</sup>
pH CaCl <sub>2</sub>	6.67	6.86	7.17	7.05	6.68	6.22	6.81	6.38	6.26	6.52	0.354 <sup>NS</sup>
EC (μS/cm)	199.6	165.9	224.4	199.5	226.3	186.8	260.5	205.6	186.8	239.3	0.971 <sup>NS</sup>
TN (%)	0.13 <sup>bc</sup>	0.04 <sup>abc</sup>	0.14 <sup>c</sup>	0.03 <sup>a</sup>	0.07 <sup>abc</sup>	0.09 <sup>abc</sup>	0.05 <sup>abc</sup>	0.06 <sup>abc</sup>	0.05 <sup>abc</sup>	0.03 <sup>ab</sup>	<b>0.005</b>
TP (mg/Kg)	115.5	132.4	128.1	36.3	61.7	83.0	44.8	102.0	45.5	103.7	0.675 <sup>NS</sup>
LOI (%)	5.20 <sup>bc</sup>	4.53 <sup>bc</sup>	6.39 <sup>c</sup>	1.91 <sup>a</sup>	2.97 <sup>ab</sup>	4.42 <sup>bc</sup>	6.66 <sup>c</sup>	3.27 <sup>ab</sup>	3.88 <sup>ab</sup>	3.59 <sup>ab</sup>	<b>0.001</b>
Water Repellency	DW	DW	DW	DW	DW	DW	DW	DW	DW	DW	
% Cover Trees	48	37	15	13	28	23	15	10	17	5	0.078 <sup>NS</sup>
% Cover Dead Trees	0	0	1	0	1	0	0	2	2	0	0.374 <sup>NS</sup>
% Cover Shrubs	40	30	28	4	25	13	25	18	3	0	0.154 <sup>NS</sup>
% Cover Ground Layer	1	0	1	35	3	3	3	1	43	35	0.740 <sup>NS</sup>
% Cover Bare Ground	22	65	7	32	40	58	60	43	32	15	0.054 <sup>NS</sup>
% Cover Leaf Litter	62	30	27	35	60	42	43	57	28	60	0.057 <sup>NS</sup>
Stems of LoxLiss	13.0 <sup>ab</sup>	15.0 <sup>ab</sup>	7.7 <sup>a</sup>	20.3 <sup>ab</sup>	24.7 <sup>ab</sup>	38.7 <sup>b</sup>	23.7 <sup>ab</sup>	8.0 <sup>a</sup>	37.0 <sup>ab</sup>	20.0 <sup>ab</sup>	<b>0.009</b>
Trees of LoxLiss	3.7 <sup>ab</sup>	3.3 <sup>ab</sup>	1.7 <sup>a</sup>	8.0 <sup>abc</sup>	9.0 <sup>bc</sup>	15.7 <sup>bc</sup>	5.7 <sup>abc</sup>	3.0 <sup>ab</sup>	14.7 <sup>bc</sup>	20.0 <sup>c</sup>	<b>0.001</b>
Total Trees	7.3 <sup>abc</sup>	3.3 <sup>ab</sup>	1.7 <sup>a</sup>	8.0 <sup>abcd</sup>	9.0 <sup>bcde</sup>	17.3 <sup>cde</sup>	5.7 <sup>ab</sup>	9.7 <sup>bcde</sup>	18.7 <sup>de</sup>	20.0 <sup>e</sup>	<b>0.001</b>
Max Tree Height (m)	6.1	11.2	7.0	6.7	7.9	7.2	6.2	7.4	6.7	6.2	0.651 <sup>NS</sup>
Mean Tree Height(m)	6.1 <sup>b</sup>	8.6 <sup>c</sup>	6.5 <sup>b</sup>	6.0 <sup>ab</sup>	6.6 <sup>b</sup>	6.8 <sup>bc</sup>	5.1 <sup>ab</sup>	4.9 <sup>ab</sup>	5.9 <sup>ab</sup>	4.1 <sup>a</sup>	<b>0.001</b>

### **3.2 Trends in morphological and physiological traits between sites along the gradient**

There were significant differences (one-way ANOVA) between the nine natural stands along the climate gradient for all of the morphological and physiological traits measured, with the exception of wood density. Karonie had the greatest average leaf length of all the natural stands studied, at 116 mm. Hines Hill was significantly different to the other sites along the gradient, having the lowest average leaf length of 71 mm. Yellowdine exhibited the greatest range in average leaf length per tree whilst Pianto Road, closely followed by Narembeen, had the least variation (Fig. 9A). Although significantly different across the gradient ( $p < 0.001$ ), a Tukey's HSD test revealed that Goongarrie, Pianto Road, Yellowdine, Narembeen, Graham Rock and Burracoppin were not significantly different from each other in their leaf length.

Average maximum leaf width tended to increase in sites with higher long-term average rainfall. The narrowest leaves were located in Pianto Road to the east of the gradient, which had a mean width of 16 mm and one individual with an average width of just below 12 mm. Graham Rock, a site in a higher rainfall, western location, had the broadest leaves averaging just under 20 mm. Narembeen had the greatest range of leaf widths whilst Burracoppin had the smallest range (Fig. 9B).

In contrast, the leaf length to width ratio tended to decrease in the sites further west along the gradient, which had lower average rainfall in the twelve months prior to sampling (Fig. 9C). Similar to leaf length, Karonie had the highest median length to width ratio (that is, longer, narrower leaves), which were significantly higher than all other sites with the exception of Pianto Road and Quairnie Rock, also located to the east of the gradient. Hines Hill, the site furthest west, had the lowest median and average length to width ratio (reflecting generally shorter, broader leaves) (Fig. 9C).

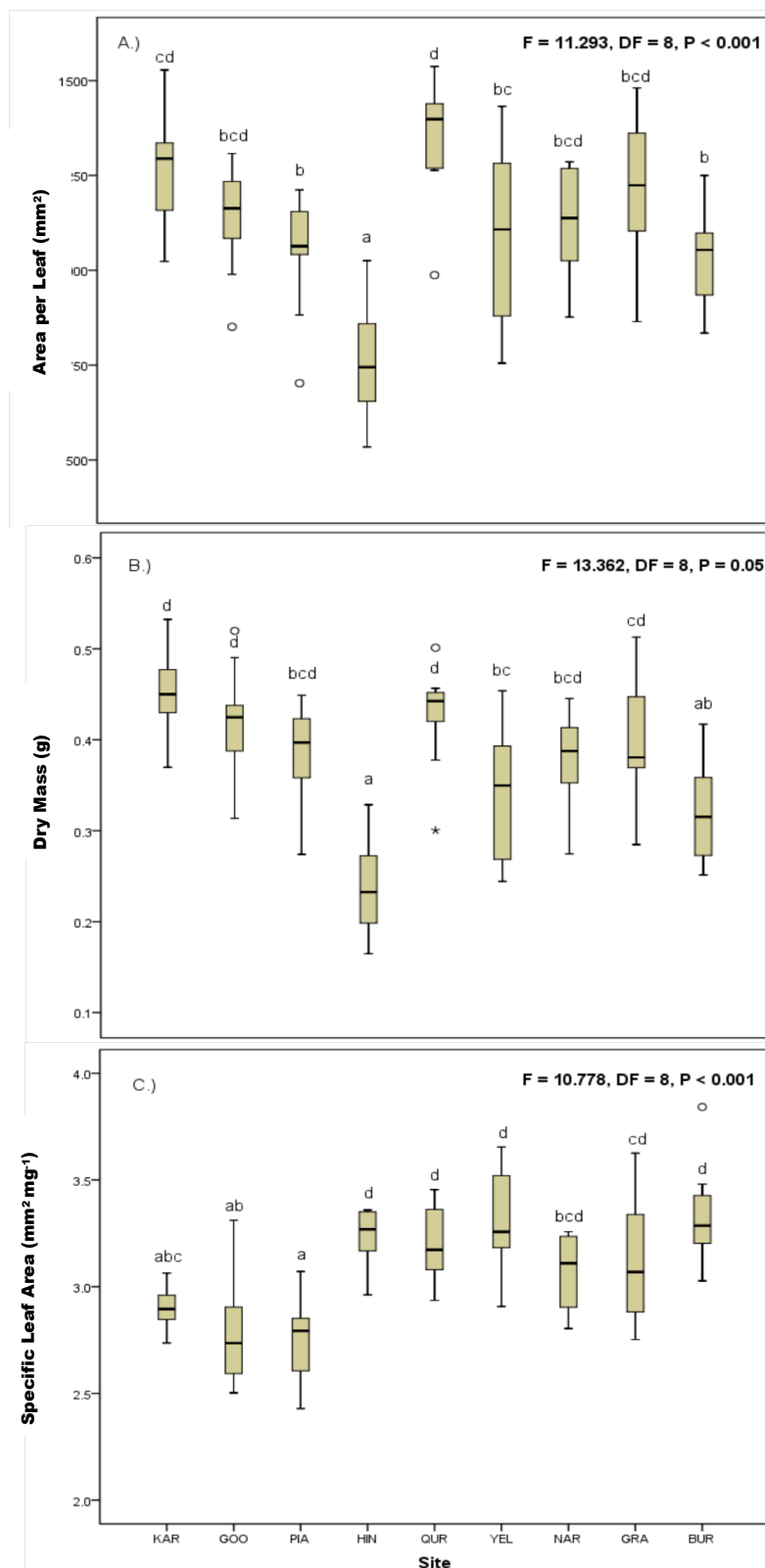


**Figure 9:** The values of A.) Leaf length, B.) Maximum leaf width and, C.) Total leaf length divided by maximum leaf width, (mm) as averaged for each individual mallee at the nine stands of *Eucalyptus loxophleba* spp. *lissophloia* studied along a climate gradient in south-west Australia are shown. Sites are arranged from lowest to highest long-term average annual rainfall. Box and whisker plots show the median value as the horizontal line in the box and the 25<sup>th</sup> and 75<sup>th</sup> percentile marking the ends of the 'box'. The whiskers represent the maximum and minimum values up to 1.5 times the inter-quartile range (the difference between the 75<sup>th</sup> and 25<sup>th</sup> percentile). Outliers outside this range are represented as circles and a star showed an extreme outlier over 3 times the inter-quartile range above the 75<sup>th</sup> or below the 25<sup>th</sup> percentile for all consequent box and whisker plots. Results of one-way ANOVA analysis are shown ( $P \leq 0.05$ , degrees of freedom (DF) and the F statistic). Letters above the box and whisker plot show the result of Tukey's HSD post-hoc test where the same letter indicates no significant difference at  $P \leq 0.05$ .

As expected, Hines Hill, which had the lowest average values of both leaf length and leaf length to width ratio, had the smallest average area per leaf and was significantly different to the rest. Indeed, it had an average area per leaf of around  $750 \text{ mm}^2$  whilst the next lowest mean area per leaf was in Burracoppin, with an average just over  $1000 \text{ mm}^2$ . The greatest average area per leaf were measured in sites located further east (Quairnie Rock followed by Karonie with average leaf areas of  $1350 \text{ mm}^2$  and almost  $1300 \text{ mm}^2$  respectively) (Fig. 10A). The most southern site, Graham Rock, had the greatest range in area per leaf with more than  $600 \text{ mm}$  between the largest and smallest average width. Located to the extreme north-east of the gradient, Goongarrie had the smallest range in area per leaf of just over  $300 \text{ mm}$  difference between the largest and smallest average area per leaf (Fig 10A).

The trends for dry mass appeared very similar to those of area per leaf with the lowest average dry mass occurring in Hines Hill at  $0.24 \text{ g}$  whilst the leaves at Karonie were the heaviest, with a dry mass averaging  $0.45 \text{ g}$  (Fig. 10B). Indeed, dry mass and area per leaf were strongly correlated, with a Pearson's correlation coefficient of  $0.898$  ( $P < 0.001$ ).

Specific leaf area was significantly different across the gradient ( $p < 0.001$ ) and the drier and warmer sites (based on long-term data) at the eastern end of the gradient displayed lower values of SLA. Goongarrie had the smallest median ( $2.74 \text{ mm}^2 \text{ mg}^{-1}$ ) and Pianto Road had the lowest average SLA both per site and per tree ( $2.76$  and  $2.43 \text{ mm}^2 \text{ mg}^{-1}$  respectively). The sites at the more western end of the gradient, including Hines Hill, Quairnie Rock, Yellowdine, Graham Rock and Burracoppin, were significantly different from the drier end yet had no significant differences between them. Graham Rock had the greatest range in SLA values across the site, from  $2.75$  to  $3.63 \text{ mm}^2 \text{ mg}^{-1}$  and Burracoppin had the highest average SLA at  $3.32 \text{ mm}^2 \text{ mg}^{-1}$  (Fig. 10C).



**Figure 10:** The values of A.) Area per leaf (mm<sup>2</sup>), B.) Dry Mass (g), and C.) Specific Leaf Area (mm<sup>2</sup> mg<sup>-1</sup>), as averaged for each individual mallee at the nine stands of *Eucalyptus loxophleba* spp. *lissophloia* studied along a climate gradient in south-west Australia. Results of one-way ANOVA analysis are shown (P ≤ 0.05, degrees of freedom (DF) and the F statistic). Letters above the box and whisker plot indicate the result of Tukey's HSD post-hoc test where the same letter indicates no significant difference at P ≤ 0.05. See Fig. 9 for a description of the box and whisker plot.

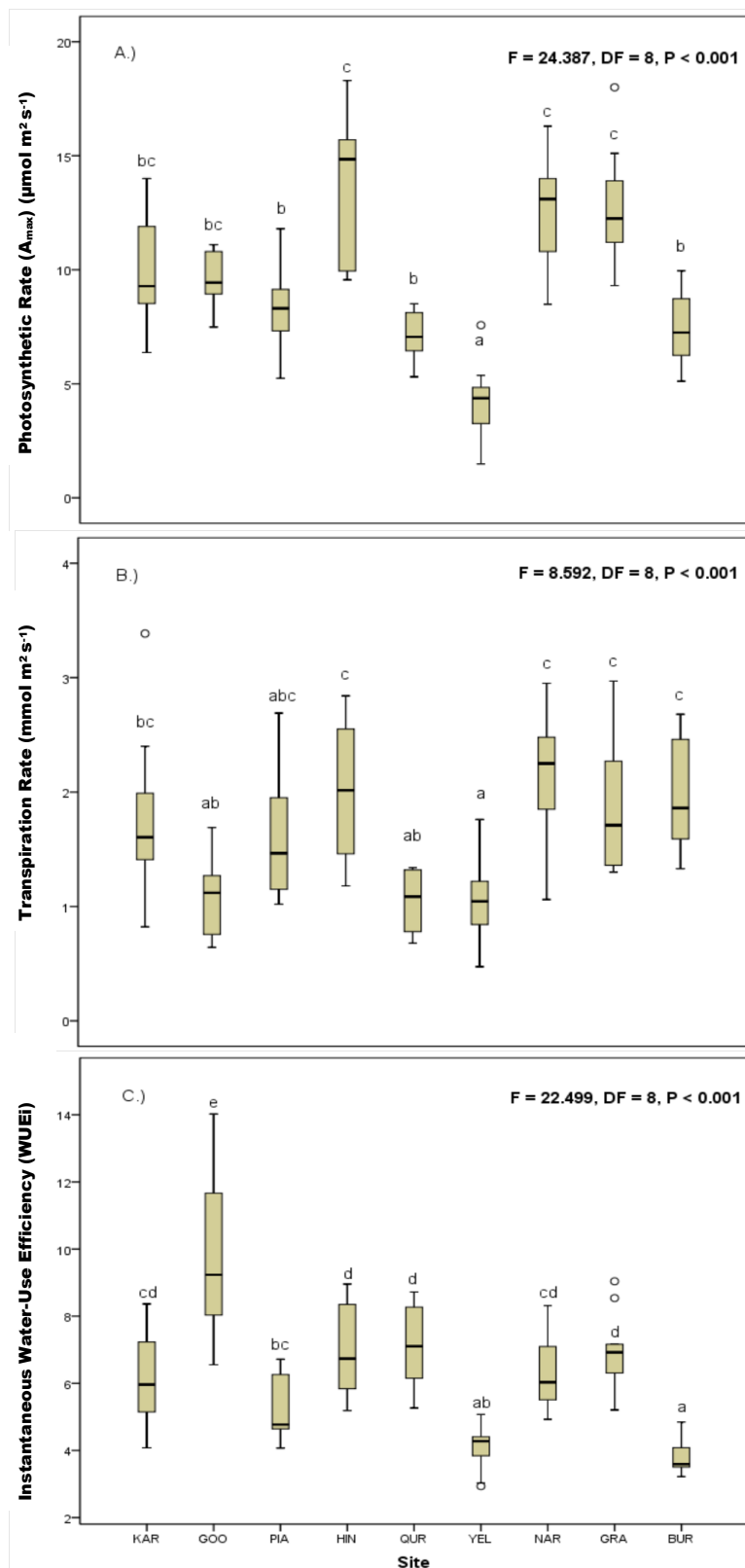
Photosynthetic rate ( $A_{\max}$ ) varied between stands across the climate gradient with Yellowdine in particular exhibiting a significantly lower average  $A_{\max}$  of  $4.3 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ . Average  $A_{\max}$  was higher in the sites further west along the gradient, being greatest at Hines Hill at  $13.5 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ , followed by Graham Rock and Burracoppin, which both averaged around  $12.6 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  (Fig. 11A). Furthermore, Hines Hill had the greatest within-stand range (as determined by the difference between maximum and minimum values) in  $A_{\max}$  whilst Quairnie Rock had the lowest within-stand range.

Results for transpiration rates were similar, again exhibiting a slight increasing trend towards the western side of the gradient. Yellowdine and Quairnie Rock had the lowest rates of transpiration, both averaging  $1.04 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ . Narembeen had the greatest average transpiration rate of just over  $2 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$  whilst Hines Hill and Burracoppin had similarly high average rates of  $1.99$  and  $1.96 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$  respectively (Fig. 11B). Similar to  $A_{\max}$ , Quairnie Rock also had the lowest within-stand range in transpiration rate between individuals within the site.

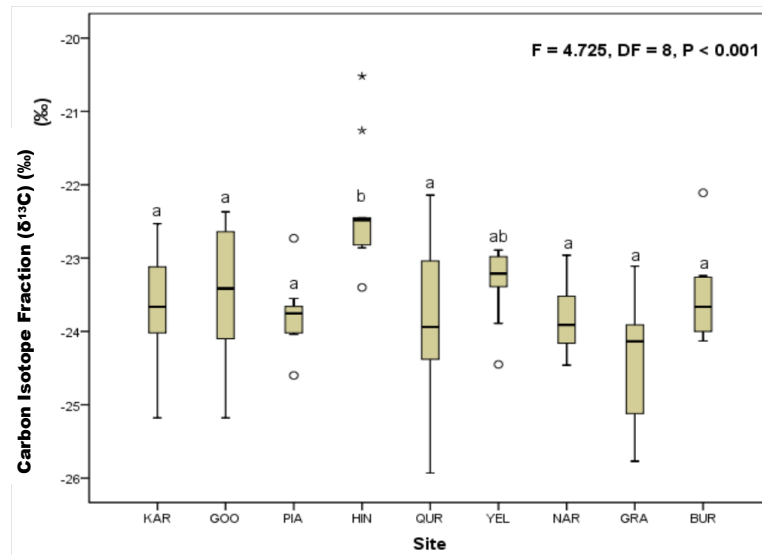
Instantaneous water use efficiency (WUEi) was significantly different across the sites and showed a slight decreasing trend with increasing long-term rainfall (Fig. 11C). Indeed, Goongarrie, the second driest site, had the highest average WUEi of  $9.57 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} / \text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$  with one tree obtaining a WUEi of  $14.02 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} / \text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ . Burracoppin, which had the highest long-term average rainfall, had the lowest average WUEi of  $3.80 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} / \text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ . Moreover, Goongarrie had the greatest within-stand range in WUEi, whilst Burracoppin had the lowest within-stand range ( $7.5$  and  $1.6 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} / \text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$  respectively).

Carbon isotope fractionation ( $\delta^{13}\text{C}$ ) of foliar cellulose varied significantly across the sites, with Hines Hill in particular exhibiting significantly higher levels of discrimination (suggesting greater season-length WUE) than the other sites. Indeed, the average  $\delta^{13}\text{C}$  in Hines Hill was  $-22.14 \text{ ‰}$  and the maximum was  $-20.46 \text{ ‰}$ . The average of all the other natural stands along the gradient was at least  $-23.3 \text{ ‰}$  in Yellowdine to  $-24.4 \text{ ‰}$  in Graham Rock (Fig. 12). There were no significant differences in  $\delta^{13}\text{C}$  in eight of the nine sites along the gradient. Quairnie Rock exhibited the greatest range in  $\delta^{13}\text{C}$  between individuals in the stand whereas Narembeen had the lowest range.





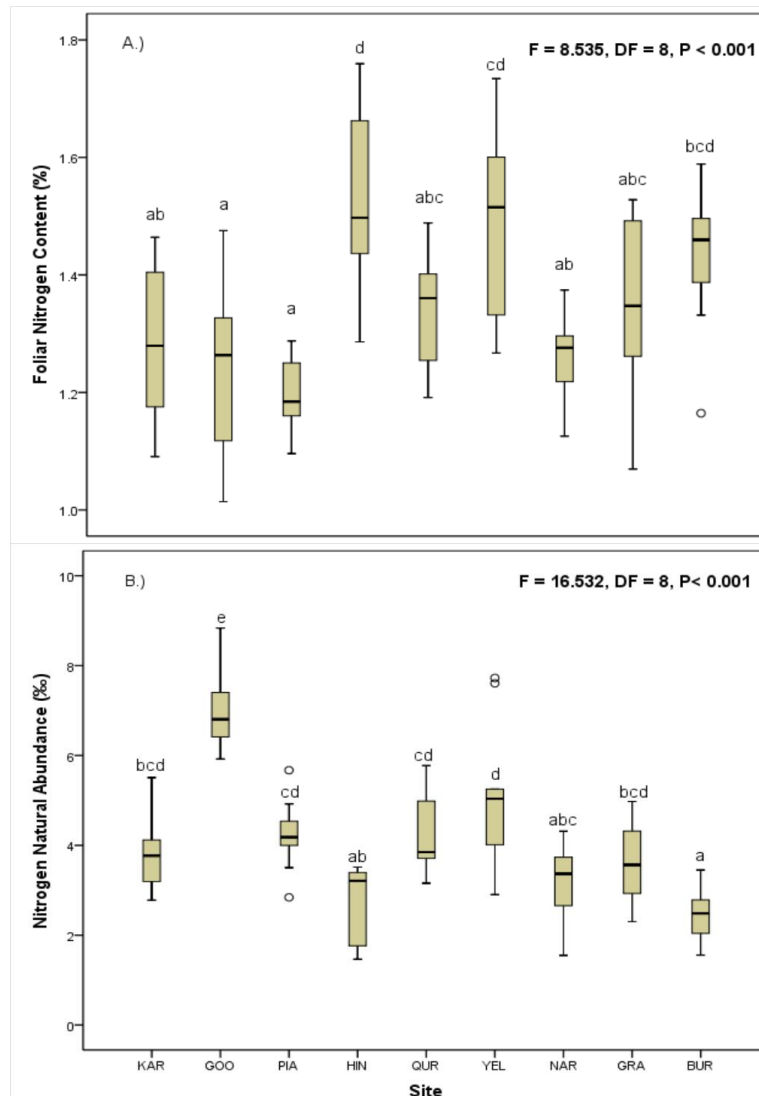
**Figure 11:** The values of A.) Photosynthetic Rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), B.) Transpiration Rate ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), and, C.) Instantaneous Water Use Efficiency ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} / \text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), for each individual mallee at the nine stands of *Eucalyptus loxophleba* spp. *lissophloia* studied along a climate gradient in south-west Australia. Results of one-way ANOVA analysis are shown ( $P \leq 0.05$ , degrees of freedom (DF) and the F statistic). Letters above the box and whisker plot indicate the result of Tukey's HSD post-hoc test where the same letter indicates no significant difference at  $P \leq 0.05$ . See Fig. 9 for a description of the box and whisker plot.



**Figure 12:** The values of carbon isotope fraction ( $\delta^{13}\text{C}$ ) (‰) of foliar cellulose, for each individual mallee at the nine stands of *Eucalyptus loxophleba* spp. *lissophloia* studied along a climate gradient in south-west Australia. Results of one-way ANOVA analysis are shown ( $P \leq 0.05$ , degrees of freedom (DF) and the F statistic). Letters above the box and whisker plot indicate the result of Tukey's HSD post-hoc test where the same letter indicates no significant difference at  $P \leq 0.05$ . See Fig. 9 for a description of the box and whisker plot.

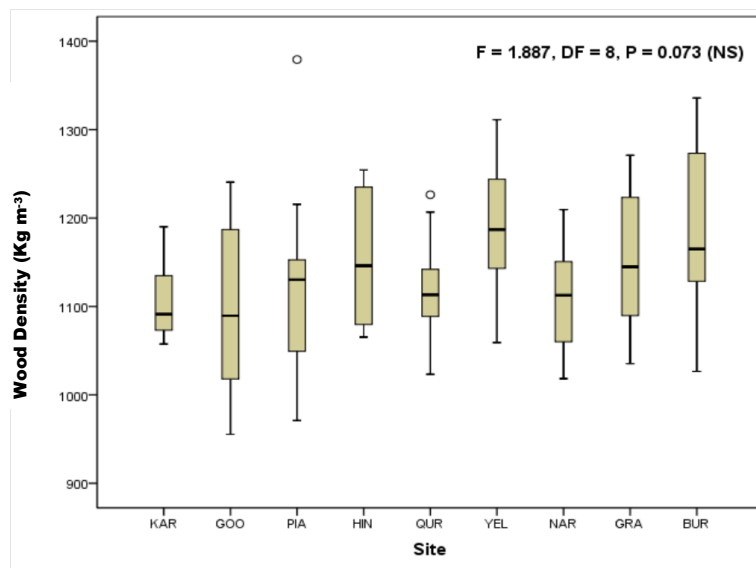
The natural stands located further east generally had lower foliar nitrogen content (%N) than the sites located further west. %N varied from a low of 1.19 % in Pianto Road to 1.53 % in Yellowdine (Fig. 13A).  $A_{\text{max}}$  and %N were not significantly correlated, with a Pearson's coefficient of -0.099 ( $P = 0.352$ ).

The opposite trends were evident when examining nitrogen natural abundance ( $\delta^{15}\text{N}$ ). There were higher  $\delta^{15}\text{N}$  levels at the drier end of the gradient, and this decreased with increasing long-term rainfall and decreasing temperature in the more western sites. Differences between the natural stands were again significant. Goongarrie in the east had significantly higher values of this trait with an average of 7 ‰ whilst Burracoppin, located further west, had the lowest average  $\delta^{15}\text{N}$  of 2.44 ‰ (Fig. 13B). Burracoppin also had the lowest within-stand range in  $\delta^{15}\text{N}$  between individuals whereas Yellowdine had the greatest within-stand range in values. A Pearson's correlation revealed that there was a significant relationship between soil TN and foliar nitrogen content ( $P=0.022$ ), however, the low coefficient of just -0.241 portrayed this as only a weak correlation. The two foliar nitrogen traits (%N and  $\delta^{15}\text{N}$ ) were also weakly correlated at the 5% significance level ( $P=0.025$ ) although this was again a weak correlation with a Pearson's coefficient of -0.237.



**Figure 13:** The values of A.) Foliar nitrogen content (%) and, B.) Nitrogen natural abundance (%), for each individual mallee at the nine stands of *Eucalyptus loxophleba* spp. *lissophloia* studied along a climate gradient in south-west Australia. Results of one-way ANOVA analysis are shown ( $P \leq 0.05$ , degrees of freedom (DF) and the F statistic). Letters above the box and whisker plot indicate the result of Tukey's HSD post-hoc test where the same letter indicates no significant difference at  $P \leq 0.05$ . See Fig. 9 for a description of the box and whisker plot.

Wood density was similar across the gradient, with less than a 10 % difference between the natural sites studied. The average wood density per site varied from almost 1100 kg m<sup>-3</sup> at Goongarrie to just below 1200 kg m<sup>-3</sup> at Yellowdine. ANOVA analysis showed that these differences were not significant (Fig. 14).



**Figure 14:** The values of Wood Density ( $\text{kg m}^{-3}$ ), for each individual mallee at the nine stands of *Eucalyptus loxophleba* spp. *lissophloia* studied along a climate gradient in south-west Australia. Results of one-way ANOVA analysis are shown ( $P \leq 0.05$ , degrees of freedom (DF) and the F statistic). NS denotes a non-significant difference at  $P \leq 0.05$  as determined by one-way ANOVA. See Fig. 9 for a description of the box and whisker plot.

### 3.2.1 Linear regression analysis of traits against climatic variables

A stepwise linear regression analysis performed on the morphological and physiological traits measured against the climatic variables over different time frames revealed that the differences that were conveyed between the sites were, at least in part, related to the varying climatic conditions experienced along the gradient. A Pearson's correlation was used to determine which of the climatic variables were significantly correlated with each other. This allowed proxies to be used for one or more other climatic variables (Table 5). Owing to the similarities in both the soil characteristics and biotic structure of the sites, they were excluded from the analysis.

**Table 5:** List of climatic variables and the climatic variables they are a proxy for in the Linear Regression Analysis. Variables were said to be correlated and useable as proxies when the Pearson's Correlation coefficient  $\geq 0.80$  ( $P < 0.05$ ).

<b>Climatic Variable</b>	<b>Useable as a Proxy for:</b>	<b>Pearson's Correlation</b>	<b>Sig (2-tailed)</b>
Long-term rainfall average	5yr rainfall average	0.869	0.002
12 month rainfall average	6 month rainfall average	0.981	0.001
5 yr summer (Dec-Feb) rainfall ave.	12 month summer rainfall average	0.857	0.003
5 yr winter (June-Aug) rainfall ave.	12 month winter rainfall average	0.831	0.005
Long-term Temp Max	5yr Temp max	0.944	0.001
18month Temp Max	12 month Temp Max	0.983	0.001
	6 month Temp Max	0.871	0.002
	3 month Temp Max	0.851	0.004
Long-term Temp Min	5yr month Temp Min	0.983	0.001
	2yr month Temp Min	0.987	0.001
	18 month Temp Min	0.992	0.001
	12 month Temp Min	0.979	0.001
6 month Temp Min	3 month Temp Min	0.914	0.001
Long-term Evaporation	5 yr Evaporation	0.990	0.001
	2 yr Evaporation	0.998	0.001
	18 month Evaporation	0.983	0.001
	12 month Evaporation	0.983	0.001
	3 month Evaporation	0.868	0.002
2yr Radiation	18 month Radiation	0.975	0.001
	12 month Radiation	0.960	0.001
	6 month Radiation	0.874	0.002
	3 month Radiation	0.958	0.001
Long-term Vapour Pressure	5 yr Vapour Pressure	0.993	0.001
	2 yr Vapour Pressure	0.981	0.001
	18 month Vapour Pressure	0.966	0.001
	12 month Vapour Pressure	0.972	0.001
	6 month Vapour Pressure	0.901	0.001
	3 month Vapour Pressure	0.940	0.001

Long-term Relative Humidity (Max temp)	5 yr Relative Humidity (maxT)	0.987	0.001
	2 yr Relative Humidity (maxT)	0.968	0.001
	18mth Relative Humidity (maxT)	0.849	0.004
	12 mth Relative Humidity (maxT)	0.834	0.005
6 Month Relative Humidity (Max temp)	3 mth Relative Humidity (maxT)	0.843	0.004
Long-term Relative Humidity (Min temp)	5 yr Relative Humidity (minT)	0.992	0.001
	2 yr Relative Humidity (minT)	0.998	0.001
18 Month Relative Humidity (Min temp)	12 mth Relative Humidity (minT)	0.998	0.001
	6 mth Relative Humidity (minT)	0.957	0.001
	3 mth Relative Humidity (minT)	0.913	0.001
Long-term ETo	5yr ETo	0.988	0.001
	2 yr ETo	0.935	0.001
18 month ETo	12 month ETo	0.991	0.001
6 month ETo	3 month ETo	0.947	0.001

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Of the climatic variables entered into the linear regression, 58% of the variation in average leaf length was explained by three climatic factors, primarily maximum temperature averaged over the previous 18 months (and also the previous 12, 6 and 3 months of which this variable is a proxy for), 12 month annual rainfall (and therefore 6 month rainfall) and long-term temperature minimum (5 and 2 year, and 18 and 12 month temperature minimum) (Table 6). Average leaf length was greatest in Karonie and smallest in Hines Hill. In accord with these results, the average maximum temperature in the 18 months prior to the study was lowest at 26.0 °C at Karonie and highest in Hines Hill at 27.7 °C. Indeed, average maximum temperature in the previous 18 months explained almost half of the variation in leaf length.

Maximum leaf width was less influenced by climatic variables with long-term radiation, 18 month rainfall and 2 year maximum temperature accounting for just over 15 % of the variation in the data (Table 6). Pianto Road had the narrowest leaves on average of the sites and also the highest long-term radiation levels whilst Graham Rock had the broadest leaves and also, the lowest rates of long-term radiation of the sites along the gradient. Both leaf length and leaf width responded to the most significant climatic variables in a similar time frame of around 18 months to 2 years. The leaf length to width ratio showed a strong relationship to the more recent climatic conditions of 12

month average rainfall and minimum temperatures of the last 6 months. These two variables alone accounted for almost 40 % of the variation in the data (Table 6).

Area per leaf differed in its response to climatic conditions along the gradient. The most influential climatic variables, accounting for almost 48 % of the data were more short-term ones, including 2 year radiation, 2 year average rainfall and 6 month evapotranspiration. The smallest average values of area per leaf were documented in the sites with the greatest recent levels of radiation. Indeed, Karonie had the lowest levels of 2 year radiation at  $18.1 \text{ MJ m}^{-2}$ , and Hines Hill the most at  $19.4 \text{ MJ m}^{-2}$ . Correspondingly, Karonie had the greatest average area per leaf and Hines Hill had the smallest average. However, in terms of long-term average radiation, Karonie and Hines Hill received  $18.8$  and  $18.9 \text{ MJ m}^{-2}$  respectively, indicating that the trait is exhibiting a plastic response to recent conditions rather than displaying traits more attuned to learned, long-term trends. The same pattern was evident in average foliar dry mass. Two year radiation again had a considerable influence on the foliar dry mass measured (with dry mass tending to increase as radiation decreased), as well as 18 month rainfall, 6 month evapotranspiration and relative humidity (at maximum temperature) and 18 month maximum temperature, together explaining over 50 % of the data (Table 6).

The climatic variables examined explained almost half of the variation in specific leaf areas between the sites (Table 6). Long-term maximum temperature, relative humidity (min temp) and summer rainfall levels were noted as significant contributors. Goongarrie and Pianto Road had the lowest median and mean SLA respectively and they also had the highest long-term maximum temperature of all the sites along the gradient. Likewise, they both also had the lowest long-term, 18 and 12 month relative humidity (max and min temp) and were summer rainfall dominated. In comparison, sites further west had higher SLA values and these sites had lower maximum temperatures and higher humidity levels, as well as lower summer rainfall.

Average photosynthetic rate was highly variable across the gradient, as almost 70 % of the data were explained by environmental factors, particularly long-term vapour pressure (Table 6). Hines Hill had the greatest average vapour pressure and also the highest photosynthetic rate. Transpiration rate also responded primarily to long-term vapour pressure (which alone explained almost one quarter of the data) (Table 6).

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**Table 6:** Formulas from stepwise linear regression analysis (with stepping method criteria: probability of F to enter < 0.05 and to remove > 0.10) on traits at the nine natural stands of *E. loxophleba* ssp. *lissophloia* against the climatic variables averaged over the long-term (LT), five years (5Y), two years (2Y), 18, 12, 6 and 3 months (mth) prior to sampling. Adjusted  $r^2$  values are in bold font.

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Leaf Length (mm) = 618.493 - (17.778 x 18mth TMax) + (0.236 x 12mth rainfall ave) - (9.299 x LT TMin)	<b>Adjusted <math>r^2</math> = 0.580</b>
Max Leaf Width (mm) = 85.644 - (6.287 x LT Radn) + (0.036 x 18mth rainfall ave) + (1.669 x 2Y TMax)	<b>Adjusted <math>r^2</math> = 0.155</b>
Leaf Length / Width (mm) = 25.983 + (0.025 x 12mth rainfall ave) - (1.732 x 6mth TMin)	<b>Adjusted <math>r^2</math> = 0.396</b>
Area per leaf (mm <sup>2</sup> ) = 13250.592 - (943.306 x 2Y Radn) + (4.704 x 2Y rainfall ave) + (977.817 x 6mth ETo)	<b>Adjusted <math>r^2</math> = 0.475</b>
Dry Mass (g) = 3.530 - (0.843 x 2Y Radn) + (0.003 x 18mth rainfall ave) + (1.613 x 6mth ETo) + (0.082 x 6mth RHmaxT) + (0.061 x 18mth TMax)	<b>Adjusted <math>r^2</math> = 0.532</b>
Specific Leaf Area (mm <sup>2</sup> mg <sup>-1</sup> ) = 22.204 - (0.434 x LT TMax) - (0.088 x 18mth RHminT) - (0.016 5Y Summer rainfall)	<b>Adjusted <math>r^2</math> = 0.470</b>
Photosynthetic Rate (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> ) = -13.976 + (0.529 x LT VP) + (0.016 x 5YSummer rainfall) - (0.006 x 5YWinter rainfall) + (0.063 x 18mth RHminT) (0.206 x 6mth TMin)	<b>Adjusted <math>r^2</math> = 0.676</b>

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Table 6 continued...

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Transpiration Rate ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) = $-10.929 + (0.597 \times \text{LT VP}) + (0.094 \times \text{6mth RHmaxT}) + (0.003 \times \text{12mth rainfall ave})$	<b>Adjusted <math>r^2</math> = 0.342</b>
WUEi ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} / \text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) = $5.199 + (5.115 \times \text{LT Evap}) + (0.255 \times \text{LT RHminT}) - (0.264 \times \text{6mth RHmaxT}) - (4.406 \times \text{LT ETo}) - (1.627 \times \text{LT Radn}) + (0.018 \times \text{3mth rainfall total}) + (0.008 \times \text{5YWinter rainfall})$	<b>Adjusted <math>r^2</math> = 0.685</b>
Carbon Isotope Fraction of Foliar Cellulose ( $\delta^{13}\text{C}$ ) (‰) = $-82.795 + (0.564 \times \text{6mth RHmaxT}) - (0.022 \times \text{12mth rainfall ave}) + (9.630 \times \text{18mth ETo})$	<b>Adjusted <math>r^2</math> = 0.283</b>
Foliar nitrogen content (%) = $16.448 - (0.013 \times \text{5YSummer rainfall}) + (0.560 \times \text{LT VP}) - (0.126 \times \text{18mth RHminT}) - (0.328 \times \text{6mth TMin}) - (0.287 \times \text{LT Radn})$	<b>Adjusted <math>r^2</math> = 0.427</b>
Nitrogen natural abundance (‰) = $35.388 - (0.589 \times \text{LT RHmaxT}) - (2.001 \times \text{LT Evap}) + (0.027 \times \text{3mth rainfall total})$	<b>Adjusted <math>r^2</math> = 0.524</b>
Wood Density ( $\text{kg m}^{-3}$ ) = $252.827 - (0.112 \times \text{LT rainfall ave})$	<b>Adjusted <math>r^2</math> = 0.060</b>

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Of all the traits measured, WUEi demonstrated the strongest relationship with the climatic variables used, with an adjusted  $r^2$  of 0.685 (Table 6). However, this involved a high number of explanatory variables. The main variables WUEi was influenced by were evaporation, relative humidity and evapo-transpiration, which alone gave an adjusted  $r^2$  value of 0.520. Goongarrie had the highest average WUEi and also had some of the highest average evaporation and evapo-transpiration levels and the lowest humidity levels, whilst Burracoppin had much lower WUEi and had much less evaporation and higher humidity relative to the other sites.

Carbon isotope fractionation of foliar cellulose ( $\delta^{13}\text{C}$ ) showed a weak relationship (adjusted  $r^2$  of 0.283) with more recent climatic variables of 6 month relative humidity (maximum temperature), 12 month rainfall and 18 month evapo-transpiration (Table 6) as opposed to more long-term climatic conditions.

Of the climatic variables examined, foliar nitrogen content (%N) was primarily influenced by summer rainfall (Table 6). The sites further west, which are more winter rainfall dominated, had higher levels of %N than sites located further east, which had more summer rainfall. In fact, the three driest sites all located to the east of the gradient had the lowest levels of %N, yet the fourth driest site (based on long-term data), Hines Hill, which receives predominately winter rainfall had the second highest %N levels of the sites along the gradient, indicating that seasonality could be important. Relative humidity at maximum temperature, evaporation and 3 month total rainfall all significantly influenced nitrogen natural abundance, explaining over 50 % of the data (Table 6). Sites with lower relative humidity and higher evaporation rates tended to have significantly higher values of nitrogen natural abundance.

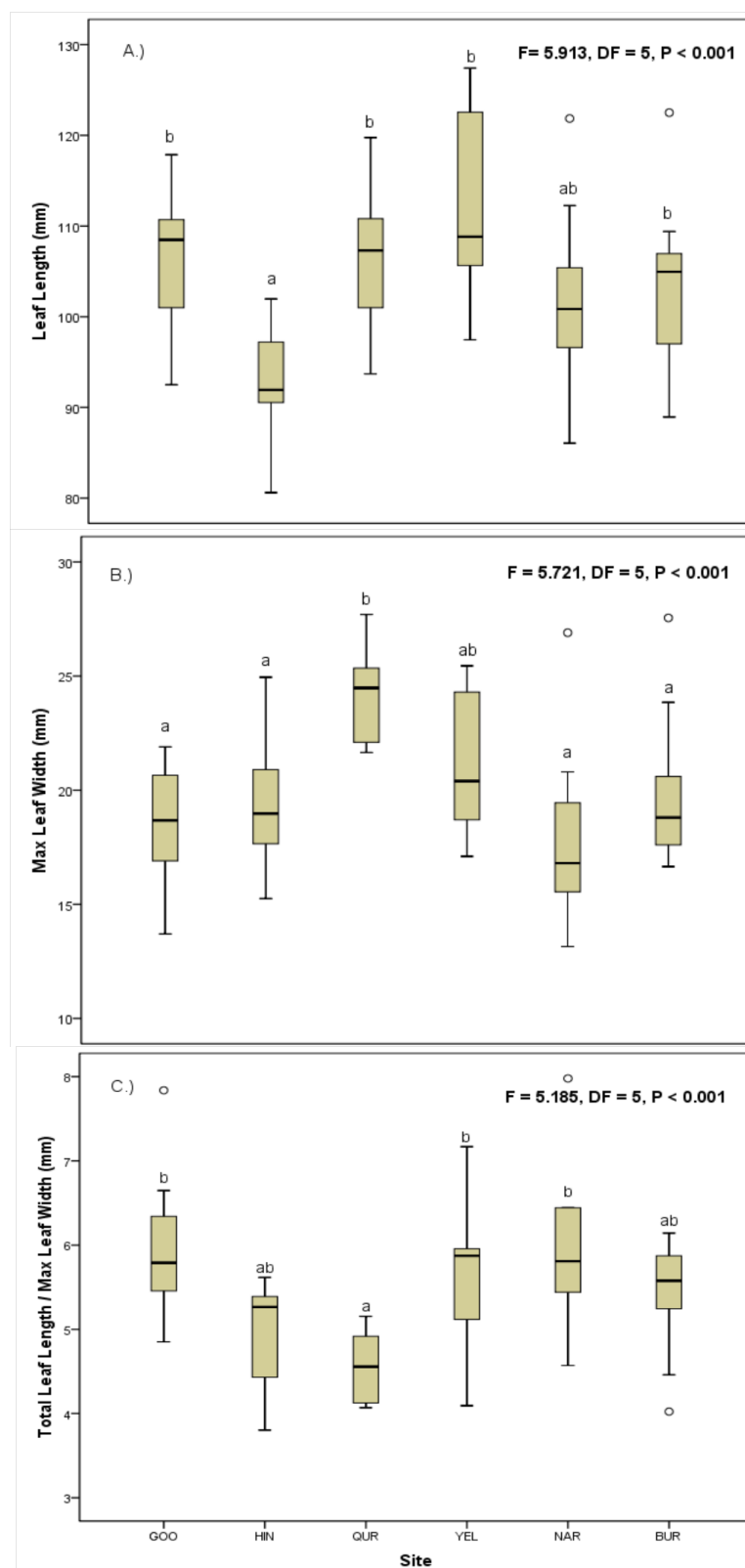
There were no significant differences in wood density across the sites, and as expected there was an even weaker relationship between this trait and the climatic variables, with an adjusted  $r^2$  of 0.060 (Table 6). This suggests that wood density was less influenced by differences in climatic conditions across the gradients than the leaf traits measured.

### **3.3 Trends in morphological and physiological traits within the Toolibin plantation**

Morphological leaf traits within the Toolibin plantation differed depending on the site the seed was sourced from, suggesting that the differences in morphological traits between natural stands along the gradient were the result of ecotypic variation. The patterns evident in leaf length between provenances in the Toolibin plantation mimicked those of the natural populations. As in the natural stands, the leaves sourced from Hines Hill had the smallest average leaf length when grown in the Toolibin plantation, suggesting a stronger correlation with the provenance than climatic variables. Leaves sourced from Yellowdine had the greater leaf lengths (Fig. 15A). The greatest range in average leaf lengths were in the individuals sourced from wetter provenances whereas the smallest range in this trait was measured in the drier Hines Hill. Indeed, the significant differences in leaf length within the plantation based on provenance indicated that the immediate environmental conditions were not the main variable influencing leaf length.

There were also significant differences in maximum leaf width between individuals from contrasting provenances. The differences relating to the provenance the individual was selected from, despite their growth in the same site, illustrates that genetics are an important influence and the individuals may have limited plasticity to alter this trait to suit the prevailing climatic conditions. The largest maximum leaf widths were measured in the individuals sourced from stands in an intermediate position along the gradient and the narrowest leaves on average were in individuals sourced from the western Narembeen stand (Fig. 15B).

As in the natural stands, total leaf length to width ratios were also significantly different between provenances within the plantings at Toolibin. However the pattern of decreasing length to width ratio with increasing long-term rainfall and decreasing average temperature evident between the natural stands was not shown. Within the plantation, the greatest average leaf length to width ratios were in individuals sourced from both a relatively wet, western (Narembeen) and dry, eastern (Goongarrie) site, averaging 5.94 and 5.93 mm respectively. An intermediate site along the gradient (Quairnie Rock) was the source of the individuals with the lowest leaf length to width ratio, with an average of 4.55 mm (Fig. 15C).



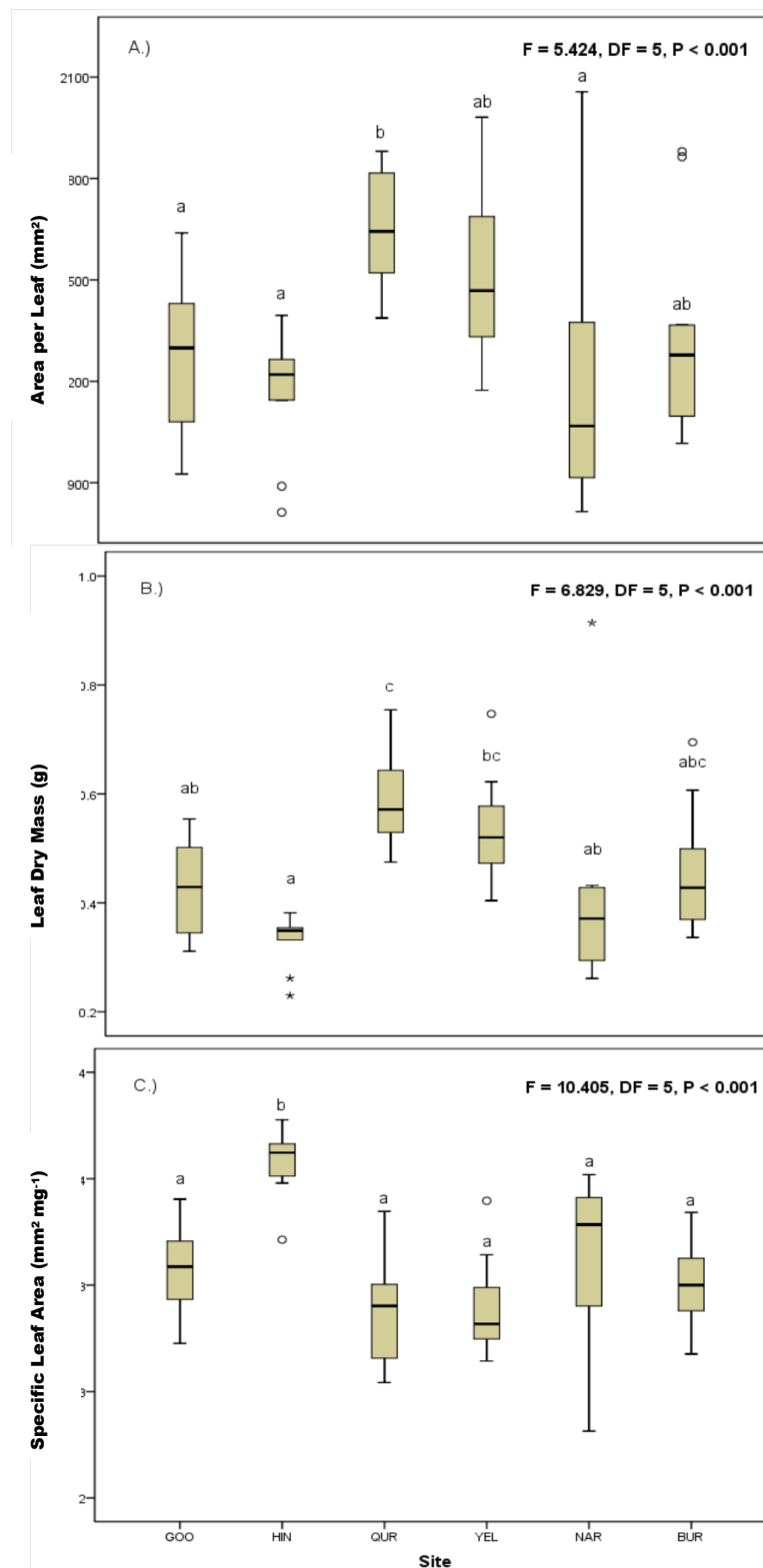
**Figure 15:** The values of A.) Leaf length, B.) Maximum leaf width and, C.) Total leaf length divided by maximum leaf width, (mm) as averaged for each individual mallee of *Eucalyptus loxophleba* spp. *lissophloia* at the Toolibin plantation from six different seed source stands along a climate gradient in south-west Australia. Results of one-way ANOVA analysis are shown ( $P \leq 0.05$ , degrees of freedom (DF) and the F statistic). Letters above the box and whisker plot indicate the result of Tukey's HSD post-hoc test where the same letter indicates no significant difference at  $P \leq 0.05$ . See Fig. 9 for a description of the box and whisker plot.

There were significant differences in area per leaf between individuals sourced from different provenances but grown together in the plantation (Fig. 16A). Individuals originating from the western sites had the lowest area per leaf when grown in the Toolibin site (Fig. 16A). Indeed, both Hines Hill and Narembeen provenances had less than 1200 mm<sup>2</sup> average area per leaf. Individuals sourced from Quairnie Rock had the greatest average area per leaf of 1650 mm<sup>2</sup>. This mimicked the patterns evident between the individuals in the natural stands, since Quairnie Rock had the highest average area per leaf and Hines Hill had the lowest in their original locations. However, all individuals grown in the Toolibin plantation had a greater average area per leaf than the individuals measured in the respective natural stand they were sourced from.

The heaviest leaves within the Toolibin plantation were from the individuals sourced from Quairnie Rock, with an average dry mass of 0.60 g. In contrast, Hines Hill had the lightest leaves, with an average dry mass of 0.33 g (Fig. 16B). The same pattern was evident in the natural stands, where Quairnie Rock had the heaviest leaves (after Karonie and Graham Rock which were not grown in the Toolibin plantation) and Hines Hill had the lightest weight leaves. As with the other leaf morphological traits examined above, there were again significant differences within the plantation between individuals sourced from varying parent stands in their dry mass. All individuals grown in the Toolibin plantation obtained a greater average dry mass than when grown in their respective source stands.

SLA was fairly uniform in the Toolibin plantation for the individuals sourced from five of the six provenances. Only those sourced from Hines Hill had a significantly higher SLA (average of 3.58 mm<sup>2</sup> mg<sup>-1</sup>) than those sourced from the other provenances. Individuals sourced from an intermediate provenance along the transect had the lowest average SLA (Fig. 16C). Goongarrie, Hines Hill and Narembeen had a higher average SLA in Toolibin than in respective natural stand whereas Quairnie Rock, Yellowdine and Burracoppin had a greater mean SLA in the natural stand than in the plantation.

Individuals sourced from Narembeen and grown in Toolibin had the greatest range in average SLA, area per leaf and dry mass. Those sourced from Hines Hill had the smallest range in average SLA and dry mass, and those sourced from Quairnie Rock had the smallest range in average area per leaf.

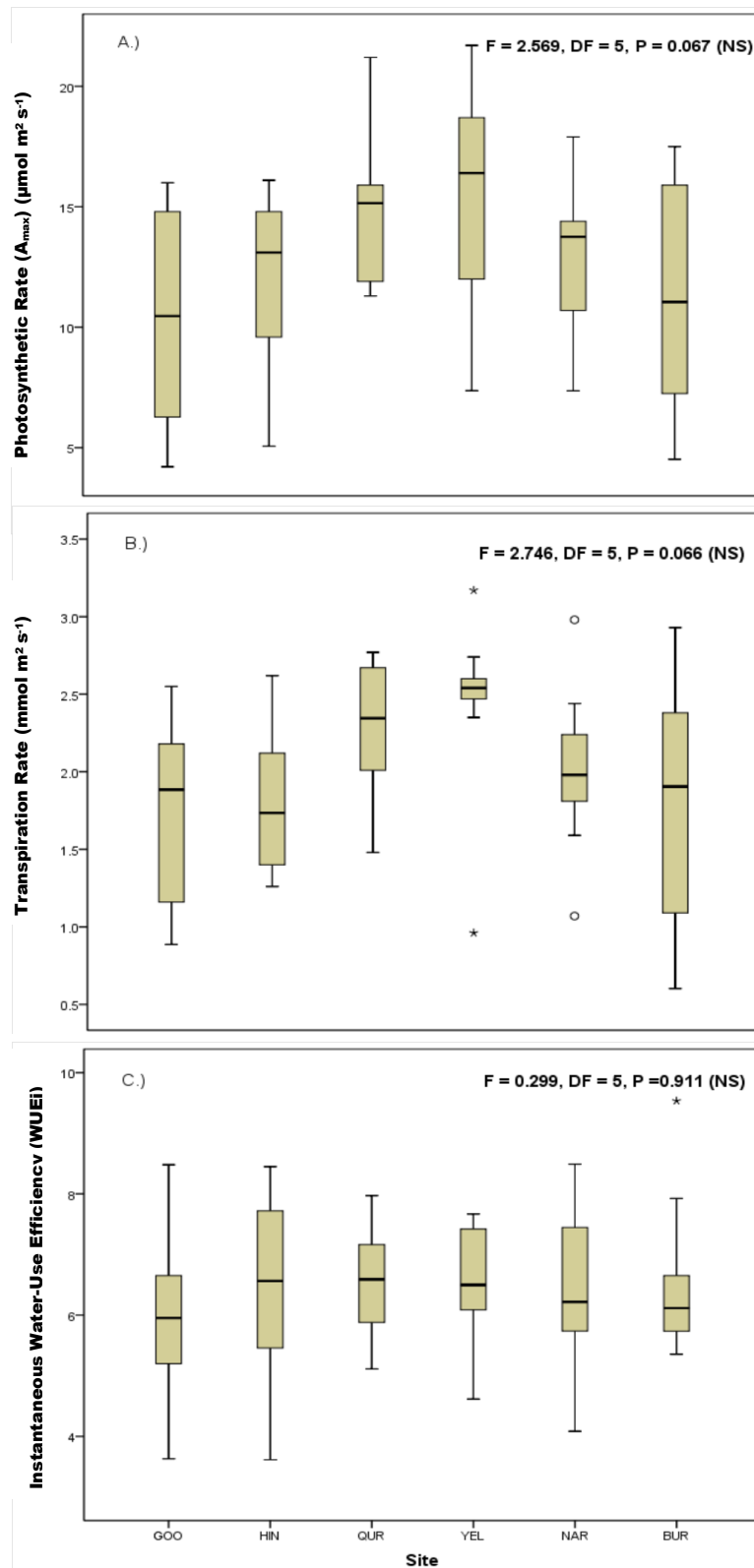


**Figure 16:** The values of A.) Area per leaf ( $\text{mm}^2$ ) B.) Dry Mass (g), and, C.) Specific Leaf Area ( $\text{mm}^2 \text{mg}^{-1}$ ), as averaged for each individual mallee of *Eucalyptus loxophleba* spp. *lissophloia* at the Toolibin plantation from six different seed source stands along a climate gradient in south-west Australia. Results of one-way ANOVA analysis are shown ( $P \leq 0.05$ , degrees of freedom (DF) and the F statistic). Letters above the box and whisker plot indicate the result of Tukey's HSD post-hoc test where the same letter indicates no significant difference at  $P \leq 0.05$ . See Fig. 9 for a description of the box and whisker plot.

Physiological traits were more similar for the individuals within the plantation, regardless of the provenance, than the morphological traits. Indeed, one-way ANOVA showed no significant differences in photosynthetic rate ( $A_{\max}$ ), transpiration rate or instantaneous water-use efficiency (WUEi) between the varying seed sites of origin when all grown under the same climatic conditions. This suggests that the differences observed in these traits between different natural stands along the gradient were perhaps responding to immediate climatic conditions, rather than ecotypic variation.

Although Yellowdine had the lowest average  $A_{\max}$  in the natural stands studied, it had the greatest average  $A_{\max}$  ( $15.6 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) of all the provenances grown in Toolibin. However, this was not significantly higher than the other provenances in the plantation (Fig. 17A). The lowest average  $A_{\max}$  was  $10.5 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ , in the individuals sourced from Goongarrie. With the exception of Hines Hill, all individuals attained a higher  $A_{\max}$  within the Toolibin plantation, than when measured in their natural source stand. Indeed, maximum photosynthetic rates were almost four times higher in mallees from Yellowdine that were grown in Toolibin, than those located in the natural Yellowdine stand. There was a similar range (within-stand variation) in the  $A_{\max}$  between individuals sourced from the six different, natural stands (Fig. 17A). Indeed, there was only a 4 % difference between the average range of the individuals in the driest provenances versus the three wettest provenances (based on long term data).

Like photosynthetic rate, there were no significant differences ( $p > 0.05$ ) in the transpiration rate achieved with respect to the provenance. Individuals sourced from Yellowdine had the greatest average transpiration rate at almost  $2.5 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ , whilst those which originated in Goongarrie had the lowest average transpiration rate, averaging  $1.7 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$  (Fig. 17B). Individuals from a provenance located further east exhibited a greater average transpiration rate in the Toolibin plantation than in their respective source stands. In contrast, mallees originating from a more western location all had a greater average transpiration rate in their natural source stand than the individuals grown in the Toolibin plantation, although the difference was extremely marginal between Narembeen individuals ( $0.06 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$  difference). Again, the within-stand variation in this trait was similar in the six provenances examined (Fig. 17B) with a 5 % difference between the average range of the three driest provenances versus the three wettest provenances.

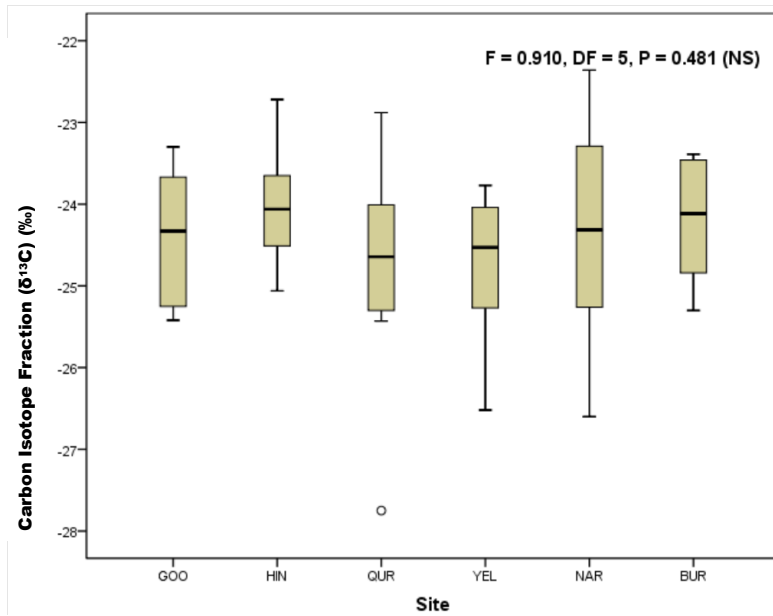


**Figure 17:** The values of A.) Photosynthetic Rate ( $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ ), B.) Transpiration Rate ( $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ ), and, C.) Instantaneous Water Use Efficiency ( $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1} / \text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ ), for each individual mallee of *Eucalyptus loxophleba* spp. *lissophloia* at the Toolibin plantation from six different seed source stands along a climate gradient in south-west Australia. Results of one-way ANOVA analysis are shown ( $P \leq 0.05$ , degrees of freedom (DF) and the F statistic). NS denotes a non-significant difference as determined by one-way ANOVA. See Fig. 9 for a description of the box and whisker plot.



Instantaneous water-use efficiency (WUEi) was consistent across the individuals originating from varying provenances but grown in the Toolibin plantation (Fig. 17C). The range in WUEi ranged from  $6.0 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} / \text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$  in those sourced from Goongarrie to  $6.6 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} / \text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$  in those sourced in Quairnie Rock. One-way ANOVA showed a non-significant difference in WUEi based on the source provenance ( $P = 0.911$ ). In contrast, there were significant differences ( $P < 0.001$ ) in WUEi between the natural stands across the climate gradient. Individuals originally found in the drier sites (Goongarrie, Quairnie Rock and Hines Hill) had a greater WUEi in the natural stands than when grown in the Toolibin plantation. In contrast, individuals sourced from the more mesic natural stands (Yellowdine, Narembreen and Burracoppin) had a greater average WUEi in the individuals grown in the Toolibin plantation than their respective natural source sites.

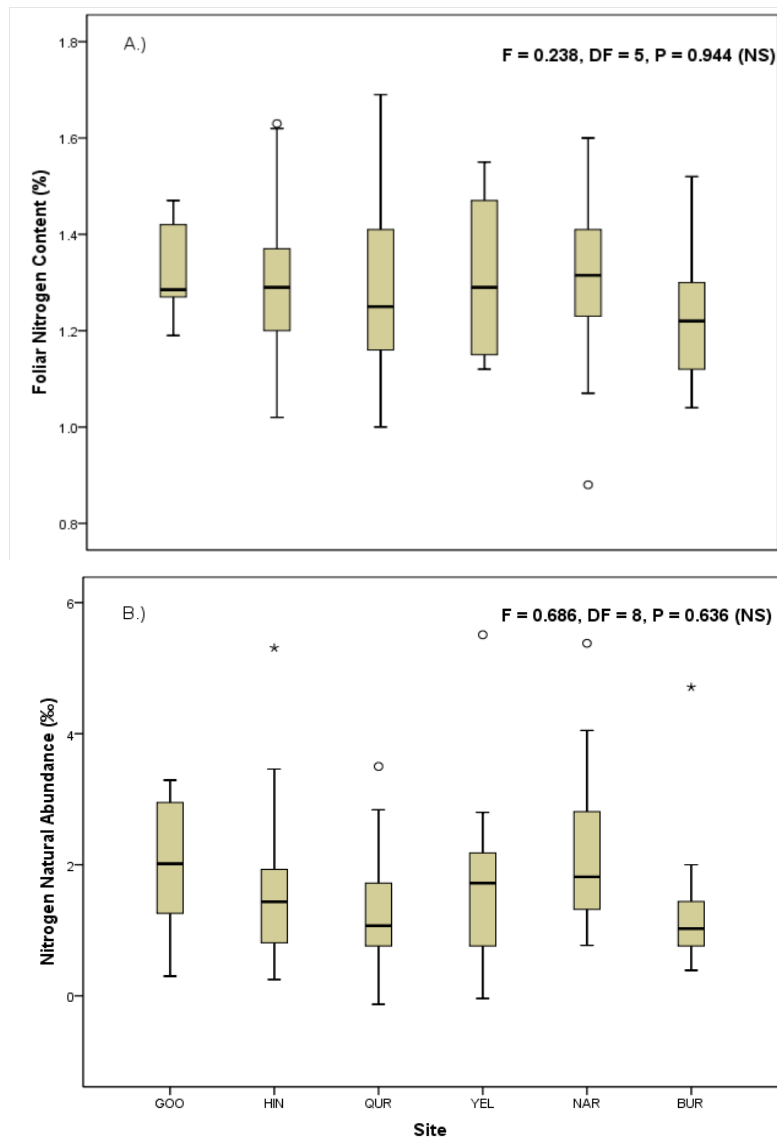
The carbon isotope fraction of foliar cellulose ( $\delta^{13}\text{C}$ ) is a season-length, integrated measure of water-use efficiency (WUE). As with WUEi, WUE as determined by carbon isotopes did not differ between individuals sourced from different sites along the gradient when grown together in the Toolibin plantation.  $\delta^{13}\text{C}$  ranged only marginally, from the highest (most positive) average of  $-24.0 \text{ ‰}$  in the individuals sourced from the western Hines Hill site, to  $-24.8 \text{ ‰}$  in the mallees originating from the more easterly located Quairnie Rock (Fig. 18). With the exception of Yellowdine, all sites had a higher (more positive) average  $\delta^{13}\text{C}$  in the Toolibin site than their respective source stands.



**Figure 18:** The values of Carbon isotope fractionation ( $\delta^{13}\text{C}$ ) (‰) of foliar cellulose, for each individual mallee of *Eucalyptus loxophleba* spp. *lissophloia* at the Toolibin plantation from six different seed source stands along a climate gradient in south-west Australia. Results of one-way ANOVA analysis are shown ( $P \leq 0.05$ , degrees of freedom (DF) and the F statistic). NS denotes a non-significant difference as determined by one-way ANOVA. See Fig. 9 for a description of the box and whisker plot.

Foliar nitrogen content was fairly uniform across the Toolibin plantation, irrespective of the provenance it was sourced from (Fig. 19A). There were no significant differences between the percent foliar nitrogen when grown in the same plantation ( $P = 0.944$ ), despite the differences observed in the natural stands along the gradient.

There were also no significant differences in the nitrogen natural abundance ( $\delta^{15}\text{N}$ ) measured between different provenances. Individuals sourced from Goongarrie (an eastern, low long-term average rainfall site) had the greatest median value of  $\delta^{15}\text{N}$  whilst those from Narembeen (a more westerly, higher long-term average rainfall site) had the highest average  $\delta^{15}\text{N}$ , at 2.33 ‰ (Fig. 19B). Individuals sourced from Quairnie Rock had the lowest average value of this trait, at 1.35 ‰, although this was not significantly lower than the individuals sourced from the other sites along the gradient.



**Figure 19:** The values of A.) Foliar nitrogen content (%) and, B.) Nitrogen natural abundance (%), for each individual mallee of *Eucalyptus loxophleba* spp. *lissophloia* at the Toolibin plantation from six different seed source stands along a climate gradient in south-west Australia. Results of one-way ANOVA analysis are shown ( $P \leq 0.05$ , degrees of freedom (DF) and the F statistic). NS denotes a non-significant difference as determined by one-way ANOVA. See Fig. 9 for a description of the box and whisker plot.

### **3.4 Relative plasticity of morphological and physiological traits between natural stands of the *E. loxophleba* ssp. *lissophloia* and a provenance plantation trial exposed to modified climatic conditions.**

The plasticity exhibited within a single subspecies in response to environmental conditions was evident when comparing morphological and physiological traits in the wetter plantation compared to the progressively drier natural sites along the climate gradient the seed was sourced from. The plasticity index (PI) used by Godoy et al. (2011) (adapted from Valladares et al. 2000), ranges from zero to (+/-) one, whereby zero is no plasticity and (+/-) one is maximum plasticity. A negative PI signifies that the mean value of a trait is higher in environment two, that is, the wetter plantation site in Toolibin, than in environment one (the respective natural stand located along the climate gradient).

There was significant variation between stands along the climate gradient in their foliar morphological traits. Likewise, when individuals from different provenance locations were grown in the plantation under the same climatic conditions, there were still significant differences between them based on provenance. This would imply that the traits are highly correlated to the environmental conditions at the seed place of origin and are less responsive to the immediate climatic conditions. The plasticity index supported this, as all of the foliar morphological traits had a low PI, especially relative to the physiological traits (Table 7).

Leaf length, leaf width and the ratio of leaf length to leaf width were similar in their low plasticity as reflected by the index, with an average trait PI of 0.11, 0.12 and 0.12 respectively (Table 7). Therefore provenance appeared to affect these traits more than the environment they were grown in. Seed sourced from drier sites (based on long-term data) demonstrated higher plasticity in leaf length. This was particularly the case for individuals from Hines Hill, which had a PI of -0.23. Individuals with a higher rainfall at the provenance location generally had slightly lower PI values recorded (Table 7). The same pattern was also noted for the ratio of leaf length to leaf width, as the three driest sites exhibited relatively higher plasticity than the individuals sourced from the three wettest sites (Table 7).

**Table 7:** Plasticity index (PI) values per site and per trait. The PI ranges from zero to (+/-) one (which represents minimal to maximum plasticity). A negative PI shows a higher mean in environment two (the plantation), than in environment one (the respective natural stand). Results in bold are absolute averages per site and trait.

	Leaf Length	Leaf Width	Length/ Width	Area per leaf	Dry Mass	SLA	Wood Density	A <sub>max</sub>	Trans.	WUEi	$\delta^{13}\text{C}$	%N	$\delta^{15}\text{N}$	<b>Ave. Site Plasticity</b>	Std error (s.e.)
GOO	-0.12	0.03	-0.15	-0.10	-0.01	-0.10	0.33	-0.09	-0.38	0.37	-0.04	-0.09	0.72	<b>0.18</b>	0.073
HIN	-0.23	-0.12	-0.13	-0.35	-0.28	-0.10	0.36	0.14	0.08	0.07	-0.08	0.14	0.34	<b>0.18</b>	0.057
QUR	0.02	-0.22	0.24	-0.18	-0.28	0.10	0.34	-0.52	-0.54	0.07	-0.04	0.03	0.68	<b>0.23</b>	0.087
YEL	-0.14	-0.18	0.02	-0.27	-0.37	0.12	0.38	-0.72	-0.57	-0.37	-0.06	0.15	0.65	<b>0.29</b>	0.097
NAR	-0.05	0.04	-0.09	-0.05	-0.07	-0.02	0.34	-0.02	0.03	-0.01	-0.02	-0.03	0.27	<b>0.08</b>	0.034
BUR	-0.09	-0.16	0.07	-0.22	-0.31	0.10	0.38	-0.35	0.10	-0.42	-0.03	0.13	0.43	<b>0.20</b>	0.068
<b>Ave. Trait Plasticity</b>	<b>0.11</b>	<b>0.12</b>	<b>0.12</b>	<b>0.20</b>	<b>0.22</b>	<b>0.09</b>	<b>0.36</b>	<b>0.31</b>	<b>0.29</b>	<b>0.22</b>	<b>0.05</b>	<b>0.09</b>	<b>0.52</b>		
Std error (s.e.)	0.034	0.046	0.061	0.044	0.010	0.042	0.134	0.131	0.123	0.059	0.007	0.041	0.079		

Of the leaf morphological traits, dry mass and area per leaf were the most plastic with an absolute average PI of 0.22 and 0.20 respectively. There was a general trend again of decreasing plasticity in these traits as the long-term average rainfall at the provenance location increased. Indeed, the PI values of area per leaf ranged from a maximum of -0.35 in Hines Hill at the drier end of the gradient (based on long-term data) to a minimum PI of -0.05 in the mallees from Narembreen, which has a much higher long-term average rainfall.

Specific Leaf Area showed low plasticity of the foliar morphological traits relating to leaf size, with an absolute mean PI of 0.09 and a minimum PI of only -0.02 at Narembreen. The remaining five sites were fairly uniform, with an absolute PI around 0.10 (Table 7). In contrast, however, wood density was relatively plastic with an average PI of 0.36. The wood density of the natural stands was consistently and fairly evenly higher than in the Toolibin plantation site, giving rise to a relatively unvarying PI across the sites for this morphological trait (s.e. 0.134).

Several of the physiological traits were more plastic than the foliar morphological traits measured. The absolute average PI was 0.31 for maximum photosynthetic rate ( $A_{\max}$ ), 0.29 for transpiration rate and 0.22 for WUEi. Again, plasticity was variable amongst the different provenances when related to the Toolibin plantation (Table 7).

Individuals sourced from the relatively higher rainfall Narembreen provenance displayed a very small plasticity response in its photosynthetic and transpiration rate (-0.02 and 0.03 respectively). In comparison, there were greater plasticity responses in these traits in the intermediate sites (Table 7). Indeed, Quairnie Rock had a PI of -0.52 and -0.54 whilst Yellowdine had a PI of -0.72 and -0.57 for  $A_{\max}$  and transpiration respectively. Hines Hill and Burracoppin were both more plastic in their photosynthetic rate than their transpiration, whilst the opposite was shown in Goongarrie (Table 7).

The transpiration rate was higher in the natural stands furthest west along gradient than when grown in the Toolibin site, whereas transpiration was higher in the plantation for the individuals sourced from the eastern side of the gradient (Table 7).

The level of plasticity of WUEi was variable between the different provenances. There was almost no difference in average WUEi between the Narembeen site and the trees sourced from Narembeen grown in the Toolibin plantation, as the PI was a mere -0.01. There was also low plasticity of WUEi in Hines Hill and Quairnie Rock (Table 7). In contrast, WUEi was much more plastic in Goongarrie, Yellowdine and Burracoppin, all which had an absolute PI of at least 0.37 (Table 7). Despite the relatively high average plasticity of WUEi, carbon isotope fractionation was the least plastic of the traits, with an absolute average PI of 0.05 (Table 7).

Foliar nitrogen content (%N) also had a very low average PI calculated of 0.09 with a maximum PI of 0.15 in Yellowdine. This was despite the linear regression analysis revealing that the trait responded to varying climatic conditions along the gradient quite strongly (adjusted  $r^2$  of 0.427). Of the traits examined, nitrogen natural abundance ( $\delta^{15}\text{N}$ ) was the most plastic on average with a PI of 0.52 (s.e. 0.079). The highest PI values of this trait were recorded in the driest sites (based on long-term data); Goongarrie, Quairnie Rock and Yellowdine, which had a PI of 0.72, 0.68 and 0.65 respectively for  $\delta^{15}\text{N}$ . The lowest average PI of  $\delta^{15}\text{N}$  was in Narembeen, with a PI of 0.27, yet this was still relatively plastic compared to the other traits (Table 7).

On a site basis, individuals sourced from intermediate locations along the gradient were the most plastic. Yellowdine had an average site PI of 0.29, followed by Quairnie Rock which had an average site PI of 0.23. There was less plasticity exhibited at the higher rainfall, western site, Narembeen, with an average site PI of 0.08 (Table 7).

## CHAPTER 4: DISCUSSION

There was high ecotypic variation in both morphological and physiological traits between the natural stands along the climate gradient as shown by the significant differences (one-way ANOVA) depending on site. However, there was no single, major climatic variable which was the primary cause of variation of all the traits. Foliar morphological traits tended to be more conservative whereas the physiological traits showed higher plasticity and were more responsive to prevailing climatic conditions.

### 4.1 Leaf size across environments: A conservative trait?

Ecotypic variation in leaf morphology and size in *E. loxophleba* ssp. *lissophloia* was measured as differences in total leaf length, maximum leaf width, length to width ratio, area per leaf, dry mass and specific leaf area (SLA) across the climate gradient. There was high ecotypic variation in these traits with a general reduction in leaf size across the gradient with warmer, drier conditions. There were significant differences in leaf morphological traits relating to provenance when grown in the Toolibin plantation, which suggested that they were conservative traits. The low PI values for leaf morphological traits (Table 7) verified the limited plasticity of these traits to adapt to local environmental conditions. This was consistent with a reciprocal transplant study of *Acer negundo*, which also found that leaf traits were more affected by the origin of the individual than the surrounding environment (Erfmeier et al. 2011). In addition, Warren et al. (2006) concluded that smaller, narrower leaves with reduced water and nutrient availability were primarily the result of long-term genotypic adaptation.

It has been well-documented that leaf size tends to decrease with water deficit in plant species (Dudley 1996, Castro- Díez et al. 1997, Osório et al. 1998, Thuiller et al. 2004, Warren et al. 2006). Osório et al. (1998) found that water-stressed *Eucalyptus globulus* had smaller leaves than when they were grown in well-watered environments, since the leaves ceased to grow when under mild water deficits. Thus, in this study it was expected that ecotypic variation in leaf size would be primarily influenced by rainfall. However, linear regression analysis showed either maximum temperature or radiation levels to be the primary influence on leaf size for five of the six foliar morphological traits in this study (Table 6). Only one foliar morphological trait (leaf length to width



ratio) was principally influenced by rainfall. This was consistent with Yates et al. (2010b) who noted that reduced leaf size in the 97 species of *Protea*, *Leucadendron* and *Leucospermum* had little significance for water conservation, as the reduction in transpirational cooling of the leaves was minimal as leaf size decreased.

Rather, smaller leaf size in warmer climates may be an adaptive strategy to promote a greater thermoregulation benefit because it provides an increased rate of conductive/convective heat loss (Yates et al. 2010b). Potvin and Tousignant (1996) similarly found that *Brassica juncea* reduced its leaf area in environmental conditions predicted in the future (higher temperatures and elevated CO<sub>2</sub> levels) than under control conditions. In this study, lower temperatures gave rise to significant ecotypic variation along the gradient whereby leaf length and SLA were higher in the cooler natural stands along the gradient. Indeed, maximum temperature in the 18 months prior to sampling explained almost half of the variation in leaf length. The longest leaves on average were recorded in Karonie, despite it being the driest site based on long-term rainfall data, yet it had the second lowest maximum temperature in the 18 months prior to the study. This supports the significance of temperature in affecting leaf length. Furthermore, long-term maximum temperature correlated more strongly with the general decrease in SLA from the eastern to the western edge of the gradient than rainfall did (Table 6).

Plants with a low SLA thrive in resource-poor environments due to their efficient conservation of resources whereas a high SLA is more suited to relatively rich resource conditions (Li et al. 2005). Several studies have examined SLA response to water stress in particular and have noted that reductions in this trait is a common phenotypic response to lower water conditions (Cunningham et al. 1999, Wright et al. 2004, Warren et al. 2006, Merchant et al. 2007, Austin et al. 2009). In addition, Schulze et al. (1998) measured a decrease in SLA with increasing aridity in 50 tree species (15 of which were eucalypts) in northern Australia. However, there have been comparatively fewer studies examining SLA along a temperature gradient, although long-term rainfall and long-term maximum temperature were moderately correlated ( $r^2 = 0.526$ ) in this study. Leaf length to width ratio was one trait that responded primarily to recent 12 month rainfall. In addition, four of the six leaf size traits had a measure of rainfall as the second most influential variable as shown in linear regression analysis (Table 6). This demonstrates that rainfall is still an important determinant of leaf size across a climate gradient.

Differences in radiation levels also significantly affected leaf size and shape (Table 6). Smaller leaf sizes with increasing radiation may reflect increased heat stress and evapotranspiration rates in sites with higher radiation. A narrowing of leaves was evident in the sites with higher radiation levels. Generally the more eastern sites had higher long-term radiation levels and correspondingly lower maximum leaf widths. In contrast, area per leaf and dry mass also responded to radiation levels, but to the more recent 2 year average radiation. The smallest leaf areas and dry mass were measured in more western sites which had higher levels of recent ( $> 3$  months to  $< 2$  year) radiation. As area per leaf and dry mass responded to the more recent climatic variables, it would suggest that they were more plastic than measurements of leaf width. Comparisons with the Toolibin plantation confirmed this, as leaf width had an absolute average PI of 0.12 whilst area per leaf and dry mass had the highest PI values (0.20 and 0.22 respectively) of the foliar morphological traits (Table 7). Cernusak et al. (2011) also found that leaf mass per area was the most plastic trait of eucalypts studied in northern Australia across a 1500 km rainfall gradient. The average area per leaf of individuals in the Toolibin plantation was larger than when they were grown in their respective natural stand. The response of a smaller leaf area measured in the natural stands is a positive indication of resilience in a changing climate (Dudley 1996, Potvin & Tousignant 1996). The inverse relationship between leaf area and recent radiation were consistent with the findings of James & Bell (2000) on *Eucalyptus globulus* ssp. *globulus*. As sunlight decreased from 100 % full sunlight to 50 %, leaf area increased. Austin et al. (2009) also noted a marked increase in leaf area of two *Eucalyptus* species with increasing light availability.

Greater within-stand variation, or range (as determined by the difference between maximum and minimum values) in a site may reflect higher adaptive capacity to respond to a drying climate, which would improve species resilience (Whisenant 1999, Lindenmayer & Burgman 2005). However, there was not a distinctly greater range in the morphological traits in any one of the natural stands, or those in more stressful (drier, warmer) conditions. In this way, there was no singular stand, or general trend from east to west across the climate gradient, for the amount of within-stand variation present in the morphological traits within a site.

Within the Toolibin site, there were significant differences based on provenance for all of the foliar morphological traits studied. This was supported by Warren et al. (2006) who also showed that leaf area and leaf length/width were correlated with the rainfall at the place seed was collected from, rather than the environmental conditions of the common garden it was grown in. In addition, the plasticity index in this study showed a low PI for leaf morphological traits compared to physiological traits, showing them to be conservative in their plasticity (Table 7). These findings were contradictory to what was recently noted in a study on eucalypts in northern Australia savannas. Higher plasticity responses were noted in foliar morphological traits (leaf area index) than gas exchange measurements (Cernusak et al. 2011). This highlights that different areas may require specific management to account for varying trends in plasticity responses between different regions and/or climates.

Similar to the results of my study, Godoy et al. (2011) found SLA to show low plasticity (PI from 0.02 to 0.10) when they used the same plasticity index for plants in response to low-med and med-high nutrient environments in a Mediterranean system. However, a study by Warren et al. (2006) between 29 eucalypt species conflicted with the results of my study. They found SLA to be a relatively plastic trait as there was no relationship between SLA and rainfall at the place where seed was collected for the species studied when grown in two common garden sites in south-eastern Australia. Likewise, Li et al. (2005) found that SLA varied in their study on 20 sand-dune species in northern China, where species in the more resource-rich areas had significantly higher SLA. In my study, the low plasticity response of SLA, as well as the other leaf morphological traits, may be due in part to the high inter-annual variability of rainfall and water-availability in the south-west of Western Australia. The unpredictable and highly changeable nature of environmental conditions in Mediterranean ecosystems can mean that any plastic responses can actually reduce fitness if other variables are also erratic and stressful (Valladares et al. 2007, Godoy et al. 2011). Therefore, the average trait values, rather than the plasticity of the trait, may be more important for survival in a highly seasonal climate (Godoy et al. 2011).

Overall, it was evident that leaf morphological traits exhibited high ecotypic variation and long-term adaptation to climatic conditions of the site. Although there was no clear distinction between sites located east or west of the gradient for all the foliar

morphological traits measured, there was a general decrease in leaf size across the gradient with warmer, drier conditions which would likely provide an adaptive response that would increase the individual's fitness in a more arid environment. However, given the conservative nature of the morphological leaf size traits examined, it is unlikely that a rapid plasticity response would be observed in a changing climate. These findings can be applied to revegetation efforts, as resilience of planted species to climate change may be increased through sourcing genetic material from stands with morphological traits which exhibit long-term adaptive changes to suit drier conditions, rather than selecting stands based on their physiological traits which may be more plastic. However, it would also be useful for future studies to focus on demonstrating that these leaf traits affect fitness and capacity to persist in warmer environments.

Given the high ecotypic variation of foliar morphological traits and the relatively conservative nature of the traits, they would be considered an effective indicator of long-term environmental conditions and for research of genetic markers that can be used to identify adaptive alleles (*see* Hancock et al. 2011). The additional leaf trait of leaf dry matter content, a measure of the leaf oven-dry mass to water-saturated fresh mass (Cornelissen et al. 2003), may also be considered. This trait is generally similar to  $1/\text{SLA}$  and has been viewed as preferable to SLA by some, owing to its lower sensitivity to differences in soil nutrient supply (Li et al. 2005).

#### **4.2 Ecotypic variability and plasticity of water-use efficiency measures**

Greater average instantaneous water-use efficiency ( $\text{WUE}_i$ ) was measured at the sites with lower long-term average rainfall and higher long-term average temperature. There was significant ecotypic variation in this trait, yet when individuals sourced from these sites were grown in the plantation, there were no significant differences between them based on provenance. This has implications in a changing climate since it suggests that there are potentially high levels of variation and short-term adaptability within the genome of a single subspecies in each local stand or individual, which allows them to occupy a range of variable sites along the environmental gradient. The relatively high PI value for this physiological trait further indicates that the sub-species has the capacity to respond to a range of conditions that change in the short- (< 3months) to long-term (> annual) conditions. However, these findings were inconsistent with a study by Heschel et al. (2002) who found that  $\text{WUE}_i$  was markedly higher in individuals sourced from a

dry environment (driest soil water potential of -0.05 mPa), than the individuals sourced from a wet environment (driest soil water potential of -0.02 mPa), when they were grown together under water-stressed conditions in a common garden experiment in Rhode Island, USA. In contrast with Heschel et al. (2002), my results imply plasticity in this trait which may reflect capacity for adaptation in a changing climate.

My results showing a decreasing trend in WUEi with decreasing long-term water stress across the gradient from east to west were consistent with studies showing that plants in lower rainfall environments generally have higher water-use efficiency (Sun et al. 1996, Nativ et al. 1999, Cregg & Zhang 2001). That is, they are able to assimilate more carbon per unit of water used. Not only did climatic variables explain almost 70 % of the variation in this trait across the natural stands, but the trait also showed a relatively high plasticity response which will provide a fitness benefit in a future climate predicted to be increasingly warmer and drier. However, given the strong influence of weather conditions on the day of sampling it would perhaps be a less useful trait in future studies concerning long-term adaptation or genetic changes in a changing climate.

A measure of season-length water-use efficiency (WUEs) was investigated using carbon isotopes. Carbon isotope values ( $\delta^{13}\text{C}$ ) are related to water-use efficiency, and species which are more enriched with  $^{13}\text{C}$ , that is, have more positive  $\delta^{13}\text{C}$  values, will exhibit higher water-use efficiency (Farquhar et al. 1982, Bond & Stock 1990, Sun et al. 1996). In southern Queensland, Australia, Stewart et al. (1995) documented a strong relationship between  $^{13}\text{C}$  natural abundance and rainfall across a 1700-350 mm per annum rainfall gradient, covering mainly *Acacia* to rainforest species, where  $^{13}\text{C}$  natural abundance was higher (more positive) in lower rainfall areas, reflecting increased water-use efficiency. However, Le Roux et al. (1996) noted that the relationship between carbon isotope discrimination and WUEi was evident in eucalypts grown under high soil water treatments but there were limitations of the use of  $\delta^{13}\text{C}$  as an indicator of WUE under low rainfall conditions and water-stress. This was because stomatal closure constrains photosynthesis for most of the growing season (Le Roux et al. 1996). Further examination of  $\delta^{13}\text{C}$  in plants in the low rainfall (200 to 450 mm) range was advocated by Schulze et al. (1998) who observed highly variable responses in  $\delta^{13}\text{C}$  of eucalypts in low rainfall environments (Schulze et al. 1998, Schulze et al. 2006).

There was little ecotypic variation in WUE as determined by  $\delta^{13}\text{C}$  values across the climate gradient as the significant differences across the natural stands were because the individuals in Hines Hill had significantly higher  $\delta^{13}\text{C}$  values than the other eight sites along the gradient. Indeed, climatic variables only predicted 28% of the variability in  $\delta^{13}\text{C}$  values in the linear regression analysis. Therefore, my results supported the findings of Le Roux et al. (1996) who noted that the relationship between  $\delta^{13}\text{C}$  in plants is limited in environments with low-water availability. Previous studies have demonstrated even lower correlations between carbon isotope values and mean annual precipitation. For example, Cernusak et al. (2011) found little variation in foliar carbon isotope discrimination in eucalypts across an annual rainfall gradient from 1700 mm to 300 mm in northern Australia. Foliar  $\delta^{13}\text{C}$  showed inconsistent responses in a study conducted by Miller et al. (2001), where five eucalypts exhibited decreased  $\delta^{13}\text{C}$  with decreasing rainfall, one species increased and seven species had no apparent relationship between  $\delta^{13}\text{C}$  and the rainfall gradient in north-western Australia. Further weak relationships between rainfall and eucalyptus have been shown in Australian studies by Schulze et al. (2006) and Warren et al. (2006), however, access to groundwater was not considered in either of these studies. Indeed, Turner et al. (2008) suggested that rainfall may only indirectly impact  $\delta^{13}\text{C}$  values of *Eucalyptus* species through its direct effects on SLA and leaf nitrogen content. However, in my study,  $\delta^{13}\text{C}$  values were not significantly correlated with SLA or leaf nitrogen content. It may be in this study that the opposing short- and long-term rainfall trends cancelled out the effect on the carbon isotopes (if there was both a substantial plastic and genetic component).

The lack of relationship between the climate gradient and  $\delta^{13}\text{C}$  values may be because there is no direct relationship between water-use efficiency as determined by carbon isotopes and mean annual rainfall in *Eucalyptus* found in low-rainfall environments. Studies have shown a relationship between rainfall and carbon isotope discrimination exists in many areas of the world. Diefendorf et al. (2010) undertook a global between-species analysis and found a strong, positive correlation between carbon isotope fractionation and mean annual precipitation at 105 locations. Midgley et al. (2004) noted higher water-use efficiency as determined by carbon isotopes in areas of southern Africa with lower mean annual rainfall and the same was noted by Sun et al. (1996) in white spruce. However, the results of my study and other Australian studies on eucalypts (*see* Le Roux et al. 1996, Miller et al. 2001, Schulze et al. 2006, Warren et al.

2006, Cernusak et al. 2011) do not support this relationship (with notable exceptions, *see* Stewart et al. 1995). It is therefore questionable whether such approaches are of use when studying water-use efficiency in this genus in low rainfall (200 to 450 mm) environments (Schulze et al. 1998). Given the less predictable response of  $\delta^{13}\text{C}$  values in low water environments, the use of this trait would be more appropriate in higher rainfall sites or studies spanning larger environmental gradients.

The PI measured for  $\delta^{13}\text{C}$  was also very low (0.05), suggesting that because this is a more season-length, long-term variable than WUEi, it has less adaptive capacity to rapidly change in different climatic conditions. This contradicts the findings of Warren et al. (2006) who found no relationship between  $\delta^{13}\text{C}$  and the rainfall at the place seed was collected from when 29 eucalypt species from a range of xeric and mesic habitats were grown in two common gardens (1220 mm and 600 mm rainfall per annum). The low plasticity in  $\delta^{13}\text{C}$  may be due to the high heritability of the trait, regardless of the site the individual is grown in. Carbon isotope discrimination has been shown to have a heritability as high as 92 % in Asteraceae (Schuster et al. 1992). The relatively high plasticity of photosynthetic rate ( $A_{\text{max}}$ ) (Table 7) is another possible reason accounting for the subsequently low plasticity of carbon isotope discrimination required by the plant (Lauteri et al. 2004). Stands further east had lowered their photosynthetic rates (Fig. 11A) meaning the time stomata were open taking in carbon dioxide was reduced so that less water was lost through transpiration, therefore decreasing the need to improve water-use efficiency.

In this study, WUEs did not vary greatly along the gradient and exhibited a low plasticity response to climatic conditions. Season-length measurements of water-use efficiency were intended to complement measures of WUEi and provide an indication of the accuracy of such instantaneous measures under low rainfall conditions. However, they were not statistically correlated, as they had a Pearson's coefficient of 0.088 (sig. 0.412). Therefore, although instantaneous measures of WUE are quick they are not a reliable or accurate surrogate for WUEs as determined by carbon isotope analysis in *E. loxophleba* ssp. *lissophloia* as the two traits revealed very different trends in both variation and plasticity in this study. However, given the poor relationship evident between  $\delta^{13}\text{C}$  and the climate gradient, WUEi may be a more valuable measure when studying *Eucalyptus* provided that it is undertaken across all seasons.

### 4.3 Ecotypic variation and plasticity in maximum photosynthetic and transpiration rates

There was a high degree of ecotypic variation in the physiological processes of maximum photosynthetic rate ( $A_{\max}$ ) and transpiration rate in *E. loxophleba* ssp. *lissophloia* as shown by the significant differences between natural stands across the climate gradient. Natural sites located in the western extreme of the gradient had greater average rates of these physiological traits in comparison to the eastern stands. However, there were no significant differences ( $p < 0.05$ ) in the traits when grown in the Toolibin site, and in addition, there was a high PI value calculated for both  $A_{\max}$  and transpiration rate (0.31 and 0.29 respectively). In addition, over two-thirds of the variation in  $A_{\max}$  between the natural stands was explained by climatic variables. Thus it was likely that these physiological traits were highly responsive to the prevailing climatic conditions across the gradient.

The highest plasticity in physiological processes was measured in the intermediate sites along the climate gradient.  $A_{\max}$  had a Pi of -0.52 and -0.72 for Quairnie Rock and Yellowdine respectively. This was consistent with a greenhouse study on *Holcus lanatus* which found that photosynthetic rates were higher in plants with intermediate, 50-34% water availability, than those in well-watered conditions or severe water deficit (Pedrol et al. 2000). This was attributed to the investment of photosynthate for growth, and therefore the photosynthetic rate was correlated with leaf size index and aerial biomass (Pedrol et al. 2000). However, in my study above-ground biomass was not measured, although  $A_{\max}$  was correlated with leaf length, leaf length to width ratio and SLA ( $P < 0.05$ ).

Transpiration was almost as plastic as  $A_{\max}$  and again, was the most plastic in the intermediate sites along the climate gradient. Transpiration rates were higher in the Toolibin plantation for the individuals sourced from the eastern side of the gradient than their respective source stands. This is because in the drier natural stands, the individuals may have reduced their transpiration rates through reduced stomatal apertures so that water loss is minimised (Gratani et al. 2003, Franks et al. 2009). Given that these physiological processes were quite plastic in this study and showed a short-term response to current conditions at the time of sampling, rather than a long-term adaptive



response, they would be less useful in studies of ecotypic variation and adaptation in a changing climate.

Differences in long-term (< 40 year mean) vapour pressure were the most significant influence on  $A_{\max}$  and transpiration rate between the natural stands as determined by regression analysis. Long term vapour pressure was strongly correlated with all the more recent averages of this trait and therefore it is difficult to determine how quickly the plants could adjust their physiological processes to varying vapour pressure. Higher vapour pressure may reduce water stress in plants, as it promotes cooler leaf surface temperatures and less water is lost to the atmosphere (Schulze 1986). Indeed, it was apparent that the sites located further west (including Hines Hill and Narembeen) with higher vapour pressure, had higher rates of  $A_{\max}$  and transpiration. Transpiration may have been relatively less influenced by average rainfall along the gradient owing to the decreased dependence of trees in low rainfall areas (< 300 mm) on this water source, as other available supplies such as stored soil water could potentially be used (Wildy et al. 2004). Net photosynthesis has also been shown to decrease as differences in vapour pressure between the leaf and air increase (Khairi & Hall 1976, Bunce 1984, Merchant et al. 2007). Merchant et al. (2007) found a decrease in photosynthesis and stomatal conductance during a ten week experiment in six eucalypt species exposed to water deficit, as opposed to well-watered conditions. This relationship between photosynthesis and vapour pressure might explain why the more mesic western sites had a higher average  $A_{\max}$ .

Five year summer and winter rainfall averages may also have affected  $A_{\max}$ . Carbon assimilation is known to decrease in the dry season (Eamus et al. 1998) and since this study was conducted in winter, the sites at the eastern end of the gradient, all of which are summer rainfall dominated, were in their dry season. This may explain why the sites located further west along the gradient, all winter dominated sites, had higher relative rates of photosynthesis.

Photosynthesis and transpiration may be affected by differences in the leaf age and position on the tree (Xie & Luo 2003). Net photosynthesis has been shown to increase with leaf age in the early stage of its development before reaching a plateau once it is fully expanded (Xie & Luo 2003). Likewise, transpiration rate may also be higher in

older leaves at the base of the branch (Xie & Luo 2003). Although these were controlled for as far as possible, through selecting only fully expanded, mature leaves and avoiding the apex of the branch, there might be different aged leaves along the gradient because of prevailing climatic conditions.

Greater within-stand variation as shown by the range in physiological traits may reflect higher adaptive capacity, which could improve species ability to cope in a changing climate (Whisenant 1999, Lindenmayer & Burgman 2005). The range in physiological traits within a stand was generally higher in the sites in more stressful (higher long-term average temperature and lower long-term average rainfall) conditions. For instance, Goongarrie the greatest within-stand range in  $WUE_i$  between individuals, Hines Hill had the greatest range in  $A_{max}$  and Karonie had the greatest range in transpiration. Therefore, as there was higher within-stand variation (range) in the drier, hotter sites in physiological traits, it suggests they would have a greater source of within-stand variation to respond to different, uncertain, changing climatic conditions.

#### **4.4 Relationship between $\delta^{15}N$ and %N with environmental conditions consistent with global trends**

There was clear ecotypic variation in  $\delta^{15}N$  across the gradient with higher  $\delta^{15}N$  values in the sites located to the east of the gradient in drier, warmer conditions, consistent with trends recognised worldwide (Amundson et al. 2003, Craine et al. 2009). These warmer, drier environments tend to have the greatest availability of soil nitrogen (consistent with this study, Table 4) which leads to increased foliar  $\delta^{15}N$  and %N (Craine et al. 2009). Higher  $\delta^{15}N$  in the eastern stands may make them less vulnerable to water deficit as they utilise more reliable nitrogen sources and are not reliant on precipitation to meet nitrogen requirements (Evans & Ehleringer 1994, Nadelhoffer & Fry 1994). The lower  $\delta^{15}N$  values to the western side of the gradient are consistent with global trends for lower average  $\delta^{15}N$  in sites dominated by winter-rainfall (Craine et al. 2009). Lower  $\delta^{15}N$  values suggest that the western sites had lower nitrogen losses and thus were more efficient in their nitrogen use than sites further east (Nadelhoffer & Fry 1994). Indeed, it has been suggested that, as rainfall increases and temperature decreases, conservation and cycling of plant-available mineral nitrogen in plants becomes more efficient (Amundson et al. 2003). In this way, there was a significant progression from water-use efficient at the eastern end of the gradient, to nutrient-use efficient at the western end of

the gradient, where efficiency in one meant less efficiency in the other, a trend shown in other studies (Austin & Vitousek 1998).

Regression analysis explained over half of the variation in  $\delta^{15}\text{N}$ . In addition, despite the ecotypic variation in  $\delta^{15}\text{N}$  values, there were no significant differences depending on provenance when grown in the Toolibin site. This indicates that the trait was highly variable and responsive to the conditions it was found in, and indeed, was the most plastic, on average, of all the traits.  $\delta^{15}\text{N}$  was higher in all the natural stands than when grown in the wetter, cooler Toolibin site, again consistent with global trends (Amundson et al. 2003, Craine et al. 2009). In addition, the Toolibin plantation was fenced and had lower grazing pressure than the natural stands studied. Increases in  $\delta^{15}\text{N}$  by more than 5 ‰ have been demonstrated under grazed conditions (Schulze et al. 1998), therefore further accounting for the higher  $\delta^{15}\text{N}$  in the grazed, natural sites.

An increase in foliar nitrogen (%N) is a common response to drying conditions because %N can improve photosynthetic efficiency and thereby limit the time stomata must remain open (causing elevated water loss) to take up carbon dioxide (Wright et al. 2001, Wright et al. 2004, Schulze et al. 2006). However, the western sites recorded higher %N than the stands located further east. This was perhaps due to the rainfall anomaly in the south-west causing below-average rainfall in the western sites. Regression analysis showed that 5 year summer-rainfall was the most influential factor on %N. Seasonality may have been important as sampling was undertaken at the start of winter. Therefore, the eastern stands, dominated by summer-rainfall would perhaps be less water-stressed than the western winter-rainfall dominated stands which had only just received their start-of-season rainfall and had not yet received the majority of their annual rainfall. In addition, microbial biomass nitrogen and nitrogen mineralisation also increase with rainfall marking the start of the wet season (Mazzarino et al. 1991, Davidson et al. 1993). Therefore, the western, winter-rainfall dominated sites may have had higher %N due to the greater amount of plant-available inorganic nitrogen from the mineralisation process.

The lower %N in the drier sites may also be partly attributable to the differences in plant height between the sites. It has been demonstrated that, with increasing tree size and stem volume, the allocation of nutrients, including nitrogen, in *E. loxophleba* ssp.

*lissophloia* will increase to the stems and decrease in the canopy (Grove et al. 2007). This may explain why the natural stands to the western side of the gradient, which had smaller average plant heights than the eastern sites, had higher foliar nitrogen content. Despite the high variation across the natural stands and the uniformity of %N measured in the Toolibin site, the trait actually had a very low PI (0.09). This indicates that, although there is a high level of ecotypic variation in the trait, it does not rapidly respond to environmental conditions.

There are opposing findings regarding the relationship between  $A_{\max}$  and foliar nitrogen content. Wright et al. (2001), for example, noted an increase in photosynthetic capacity with %N whereas Daas-Ghrib et al. (2011) and Cernusak et al. (2011) found no effect of nutrient or leaf nitrogen availability, respectively, on  $A_{\max}$ . My study supported the latter, as  $A_{\max}$  was weakly, negatively correlated with %N. The very small range in %N in the individuals studied is a possible contributor to the lack of distinct correlation shown (Cernusak et al. 2011).

#### **4.5 Low ecotypic variation in wood density**

There were no significant differences in wood density between the natural stands reflecting very low ecotypic variation. In addition, linear regression analysis revealed a lack of relationships with climatic variables (adjusted  $r^2$  of 0.060). The low ecotypic variation was perhaps due to the relatively small climate gradient relative to other studies. Thomas et al. (2004) for instance, measured wood density across a temperature range from 20 °C to 37 °C in a controlled experiment, whereas the temperature range in the natural stands in my study was only 2 °C difference. Indeed, Searson et al. (2004) assessed wood density in three eucalypt species in both well-watered and water-limited environments. Similarly to my study, wood density increased minimally (between 0-9 % overall) in the water-limited environment, and the difference was only significant for one of the eucalypts studied. This indicates that much larger differences in climatic conditions may be required to facilitate significant changes in wood density across the gradient (Searson et al. 2004) and thus the trait may only be useful for studies of ecotypic variation over very large climatic gradients.

Despite the low ecotypic variation, the plasticity index showed wood density to be quite plastic. This was not consistent with other studies which have shown that wood density

has limited plasticity. Wang et al. (1984) and Greaves et al. (1997) showed that differences in wood density were due to genetic differences in *Eucalyptus grandis* and *E. nitens*. In addition, studies on *Eucalyptus urophylla* concluded that basic density is a highly heritable trait (0.71) (Wei & Borralho 1997). The primary reason for the differences observed between the natural stands and the Toolibin site (and the consequently high plasticity) was perhaps due to the different methods for calculating wood density. Wood density was measured at Toolibin by the Department of Environment and Conservation, where a mean tree basic density was obtained by averaging the basic wood density of eight discs taken at various heights of the tree, whereas I measured only a single stem for wood density in the natural stands. Wood density varies both progressively up the stem and horizontally from corewood to outerwood (Burdon et al. 2004) and therefore the inconsistency in the position that wood was sampled from in the plantation is likely to have also contributed to the large differences measured. Furthermore, wood density has been shown to be positively associated with stem biomass (Iida et al. 2011) and thus the selection for high biomass trees in the thinning of the Toolibin plantation in 2005 may have affected the results obtained. Likewise, given how close the seedlings were planted in the Toolibin plantation, the greater competition for light resources may have promoted low-density wood for vertical stem expansion than higher-density wood for lateral branch expansion (Iida et al. 2011). Therefore the trait is not very useful, as it was very dependent on where the wood was sampled and so is less comparable to other studies. Measuring wood density is also quite destructive, particularly if single-stemmed species were to be studied.

#### **4.6 Resilience in a changing climate**

My study measured differences in phenotypic expression as an indication of ecotypic variation. However, given the low plasticity shown when grown in a common garden, it was likely that the phenotypic variation in morphological traits was the result of some genotypic differentiation along the climate gradient. Recent studies have shown a link between the phenotypic differentiation in an Alpine species in a common garden in Switzerland to the genetic differences in the widespread species adapted to different climatic conditions (Frei et al. 2011). Furthermore, Hancock et al. (2011) have recently shown that genetic markers on *Arabidopsis thaliana* can accurately identify loci in the plant that are related to climatic conditions. This implies that genome-wide scans can

detect alleles adapted to a changing climate (Hancock et al. 2011). Thus, it will be useful to compare eco-physiological studies, such as this, to the genetic analyses being undertaken by Byrne et al. to determine whether such eco-physiological studies are even necessary or if in-depth genetic studies can be undertaken directly to gauge the adaptive potential of a species in a changing climate.

Given that leaf morphological traits were conserved in the common garden, it appears that some differentiation had a genetic basis. The significant ecotypic variation in morphological traits between natural stands along the gradient suggests that there is variation to facilitate change to warming and drying conditions. However, species with distinct ectopic adaptations may be restricted in their ability to change and evolve under a rapidly changing climate due to the time delay in new adaptive mutations (Jump & Peñuelas 2005, Aitken et al. 2008, Hancock et al. 2011). Should these traits be shown to directly affect fitness (for instance, through improved temperature regulation), this implies that the resilience of plants may be enhanced in a changing climate by sourcing genetic material from the drier, hotter end of the gradient. Currently, restoration efforts source genetic material from local populations or areas, assuming them to be the most successful as they are adapted to the local conditions. However, in a changing climate, it is advisable to instead source genetic material from stands displaying traits with a higher fitness advantage given predicted climatic changes. In this way, resilience of plants in a may be enhanced changing climate by sourcing genetic material from the drier end of the gradient. Intermediate locations along the gradient, such as Yellowdine and Quairnie Rock, could also be selected, as they were the most plastic and thus may have a higher adaptive capacity to respond to uncertain climatic conditions in the short-term.

In contrast, physiological traits reflecting water use characteristics appeared highly plastic. This implies that *E. loxophleba* ssp. *lissophloia* individuals already have some capacity to adjust to variable moisture environments which should allow it to cope with climate changes of a limited scale over the short-term. However, it is noted that the common garden environment represented a lower stress environment than the natural populations, and thus it would be valuable to test whether responses are similar in common gardens in higher stress environments. The high plasticity shown in the physiological traits in the species is a positive indication of resilience to climate change

in the short-term. A study on 40 plant species demonstrated that native and non-native species vary in their mean trait values, but not the plasticity of these traits (Godoy et al. 2011). This implies that the results in this study, illustrating the high level of plasticity in the physiological traits, might be applicable to other plant species.

It should be recognised however, that despite the findings of Godoy et al. (2011) suggesting that plasticity of traits between native and non-native species does not vary, geographically restricted species may have lower phenotypic plasticity than more geographically widespread species, such as *E. loxophleba* (Hughes et al. 1996, Pohlman et al. 2005, Godoy et al. 2011). Therefore, complementary studies on variation in geographically restricted species would be valuable. Indeed, current climatic modelling typically uses the environmental conditions of the past and current distribution of a species to generate the 'climatic envelope' it may occupy (Jeschke & Strayer 2008). However, restricted species may actually have a higher variability in traits and potential plasticity to respond to water deficit than their present distribution would suggest, but they are restricted in their geographic range owing to other factors, such as landscape clearing, dispersal abilities or biotic interactions (Loehle & LeBlanc 1996, Witkowski & Lamont 2006). Thus, it would be important to measure the relative plasticity of geographically restricted species to determine if the effects of climate change on their distribution are being over-estimated.

Some adaptive capacity of traits relating to drought tolerance and water conservation has been demonstrated in a single species. Even greater differences between the natural and plantation sites may have been observed had sampling been undertaken at the end of winter, when the eastern sites may have been more stressed and the winter sites less stressed, leading to more pronounced ecotypic variation and phenotypic plasticity responses (Cernusak et al. 2011). However, the Mediterranean region of the south-west of Western Australia is regarded as one of the most vulnerable to suffering contractions under predicted climate change scenarios (Klausemeyer & Shaw 2009). Therefore, despite the ability to cope with a changing climate afforded by phenotypic responses, the necessity of mitigating climate change and associated increased temperatures and altered rainfall distribution, remains paramount (Prober et al. 2011).

#### **4.7 Conclusions**

In summary, there was a high degree of ecotypic variation within a single subspecies along a climate gradient in the majority of morphological and physiological traits studied. These findings may be used to enhance restoration success in a changing climate as resilience of plant species can be increased through sourcing genetic material from stands which have traits suited to drier, warmer conditions. However, there was no single climatic variable that was principally responsible for the progressive changes measured in both morphological and physiological traits along the gradient as determined by linear regression analysis. A multitude of climatic variables affected the traits differently, and with varying levels of influence. Thus, the consequences for plant species in a changing climate where a multitude of interrelated climatic variables are predicted to change, remains uncertain.

Despite the ecotypic variation in foliar morphological traits, they showed conservative plasticity responses in this study. They were significantly different in the Toolibin plantation depending on the provenance, which suggests that some differentiation in morphological traits had a genetic basis. Physiological traits were more plastic on average than foliar morphological traits. In this way, plasticity in physiological traits to prevailing climatic conditions may provide short-term resilience in a changing climate.



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# APPENDIX I

*The long-term (01/06/1940 to 31/05/2011), five year (01/06/2006 to 31/05/2011), two year (01/06/2009 to 31/05/2011), 18 month (01/01/2010 to /31/05/2011), 12 month (01/06/2010 to 31/05/2011), 6 month (01/12/2010 to 31/05/2011) and 3 month (01/03/2011 to 31/05/2011) average climatic conditions of the ten sites studied across the south-west Western Australia, based on SILO interpolated data from the Bureau of Meteorology.*

Variable	Time Period	KAR	GOO	PIA	HIN	QUR	YEL	NAR	GRA	BUR	TBN
Rainfall (mm)	Long term average	234.6	241.0	243.3	275.9	278.8	320.1	323.0	329.7	332.7	354.0
	5 yr average	196.7	231.1	225.8	218.8	239.1	243.0	259.1	269.3	284.0	282.8
	2 yr average	222.3	265.7	258.2	233.4	269.0	236.7	239.8	220.0	286.8	255.2
	18 month average	199.1	274.3	263.3	163.3	247.4	189.1	181.7	159.3	229.7	210.9
	12 month average	237.0	317.2	317.3	204.0	213.7	221.7	206.2	193.0	271.6	221.5
	6 month total	134.4	198.2	200.0	111.5	129.8	126.5	108.5	101.7	146.8	117.0
	3 month total	25.7	31.5	25.0	58.6	36.0	30.5	52.4	34.7	46.5	49.6
Maximum Temperature (°C)	Long term average	25.2	26.4	26.2	25.1	24.5	25.1	24.5	24.4	24.8	22.7
	5 yr average	25.8	27.1	26.8	26.2	25.3	25.9	25.7	25.6	25.9	23.6
	2 yr average	25.4	26.7	26.4	26.3	25.1	25.8	25.8	25.8	26.0	23.7
	18 month average	26.0	27.4	27.0	27.7	25.9	26.9	27.1	27.0	27.3	25.3
	12 month average	24.4	25.8	25.4	26.3	24.3	25.4	25.8	25.6	25.9	23.8
	6 month average	26.9	28.5	28.0	30.1	27.2	28.7	29.6	29.1	29.5	27.7
	3 month average	24.2	25.6	25.1	27.2	24.5	25.8	26.7	26.4	26.7	25.0

Variable	Time Period	KAR	GOO	PIA	HIN	QUR	YEL	NAR	GRA	BUR	TBN
Minimum	Long term average	10.9	12.6	12.5	11.1	10.7	10.7	10.3	9.9	10.9	9.5
Temperature (°C)	5 yr average	11.4	13.0	13.1	11.1	11.1	10.6	10.6	9.9	11.2	9.8
	2 yr average	11.4	13.1	13.1	11.3	11.1	10.7	10.7	10.2	11.3	9.9
	18 month average	12.2	14.0	14.0	12.3	11.9	11.7	11.6	11.1	12.3	10.8
	12 month average	11.0	12.7	12.7	11.3	10.8	10.6	10.7	10.3	11.3	9.8
	6 month average	14.4	16.2	16.0	15.5	14.4	14.9	15.0	14.7	15.4	13.5
	3 month average	11.9	13.6	13.4	13.0	12.1	12.3	12.9	12.9	13.1	11.8
Mean Daily	Long term average	6.6	7.3	7.4	6.0	6.4	6.2	5.6	5.4	6.0	4.6
Evaporation (mm d <sup>-1</sup> )	5 yr average	6.6	7.3	7.3	5.6	6.4	6.0	5.3	5.2	5.6	4.5
	2 yr average	6.1	6.7	6.7	5.6	5.9	5.8	5.3	5.1	5.6	4.6
	18 month average	6.2	7.0	6.9	6.0	6.1	6.1	5.6	5.4	5.9	5.1
	12 month average	5.7	6.4	6.4	5.5	5.6	5.6	5.1	5.0	5.4	4.6
	6 month average	6.0	6.7	6.6	6.4	6.0	6.2	6.0	5.7	6.2	5.8
	3 month average	4.8	5.4	5.4	5.0	4.8	4.9	4.7	4.5	4.9	4.3

Variable	Time Period	KAR	GOO	PIA	HIN	QUR	YEL	NAR	GRA	BUR	TBN
Radiation (MJ m <sup>-2</sup> d <sup>-1</sup> )	Long term average	18.8	19.4	19.4	18.9	18.8	18.8	18.6	18.2	19.0	17.8
	5 yr average	18.5	19.4	19.2	19.4	18.7	19.1	19.0	18.6	19.4	18.4
	2 yr average	18.1	19.0	18.9	19.4	18.4	18.9	19.0	18.7	19.3	18.4
	18 month average	18.1	19.2	18.9	19.8	18.5	19.2	19.4	19.0	19.7	19.4
	12 month average	17.7	18.7	18.5	19.5	18.1	18.9	19.1	18.8	19.4	18.7
	6 month average	17.3	18.6	18.3	20.4	18.1	19.2	19.8	19.3	20.2	20.4
	3 month average	15.3	16.3	16.1	17.4	15.7	16.6	16.8	16.3	17.4	16.4
Vapour Pressure (hPa)	Long term average	11.1	10.8	10.7	12.3	10.9	11.3	12.1	12.0	11.8	11.9
	5 yr average	11.1	10.8	10.6	12.8	10.7	11.4	12.5	12.2	12.1	11.7
	2 yr average	11.2	10.9	10.8	12.9	10.7	11.3	12.8	12.3	12.2	11.6
	18 month average	11.9	11.5	11.4	13.4	11.2	11.7	13.2	12.7	12.7	12.0
	12 month average	11.5	11.1	11.0	13.0	10.9	11.4	12.8	12.3	12.3	11.6
	6 month average	14.4	14.2	14.1	16.1	13.7	14.2	15.4	14.8	15.4	13.9
	3 month average	13.0	12.3	12.2	14.7	12.2	12.6	14.0	13.5	14.1	12.9

Variable	Time Period	KAR	GOO	PIA	HIN	QUR	YEL	NAR	GRA	BUR	TBN
Relative Humidity	Long term average	36.5	33.6	33.5	40.9	37.6	37.6	41.4	41.4	40.2	45.2
At Maximum	5 yr average	35.4	31.9	31.9	39.4	35.1	36.1	39.7	38.7	38.1	42.2
Temperature	2 yr average	36.8	33.4	33.5	39.7	35.9	36.5	40.4	38.7	38.5	41.7
(%)	18 month average	37.3	33.3	33.8	37.6	35.5	34.9	38.2	37.0	36.5	38.7
	12 month average	39.1	34.8	35.4	39.2	37.1	36.6	39.7	38.3	38.3	40.5
	6 month average	42.2	37.8	38.7	38.6	39.3	37.0	38.1	37.6	38.3	38.4
	3 month average	44.2	38.5	39.4	41.8	40.9	38.9	41.1	40.2	41.3	42.1
Relative Humidity	Long term average	82.3	73.9	73.3	87.1	81.8	83.8	89.1	90.5	85.5	91.6
At Minimum	5 yr average	80.1	71.7	70.5	88.6	79.0	83.5	89.8	90.7	85.2	89.4
Temperature	2 yr average	81.0	72.5	71.5	88.2	78.8	82.0	90.2	90.0	84.8	88.6
(%)	18 month average	81.4	71.7	71.1	86.5	78.0	80.4	88.8	88.8	83.0	86.8
	12 month average	83.8	74.0	73.6	88.1	80.3	82.3	89.9	89.3	84.9	88.4
	6 month average	85.3	75.8	75.9	86.9	81.3	81.4	87.5	85.4	84.1	86.7
	3 month average	89.4	78.1	78.3	91.0	83.7	84.6	90.3	87.3	87.7	89.7



Variable	Time Period	KAR	GOO	PIA	HIN	QUR	YEL	NAR	GRA	BUR	TBN
Mean Daily	Long term average	4.2	4.5	4.5	4.1	4.1	4.2	3.9	3.8	4.1	3.6
Evapo-transpiration (mm d <sup>-1</sup> )	5 yr average	4.2	4.7	4.6	4.2	4.2	4.3	4.1	4.1	4.2	3.8
	2 yr average	4.1	4.6	4.5	4.2	4.2	4.3	4.1	4.1	4.3	3.8
	18 month average	4.2	4.7	4.6	4.5	4.3	4.5	4.4	4.3	4.5	4.2
	12 month average	3.9	4.4	4.3	4.2	4.0	4.2	4.1	4.1	4.2	3.9
	6 month average	4.1	4.6	4.5	4.8	4.3	4.7	4.7	4.6	4.8	4.6
	3 month average	3.2	3.7	3.7	3.7	3.4	3.7	3.7	3.6	3.7	3.4