THE UNIVERSITY OF QUEENSLAND
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Influence of leaf chemistry on dietary choice and habitat quality of koala (*Phascolarctos cinereus*) populations in southwest Queensland

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Abstract

Protecting high quality habitat is an important wildlife conservation action. Spatial and temporal variation in habitat quality in heterogeneous landscapes influences habitat use and population persistence. Populations living at the margins of species' geographic ranges are particularly sensitive to fluctuations in habitat quality, especially if species occupy narrow ecological niches. For arboreal folivores, foliar chemical composition is a key factor influencing habitat quality. To understand the spatial and temporal dynamics of foliar chemistry and hence the habitat quality for an arboreal folivore species, I applied theories and methods from chemical ecology, nutritional ecology and landscape ecology to understand foliar chemical/folivore interactions in a seasonally changing environment.

I used populations of koalas (*Phascolarctos cinereus*) in two semi-arid regions of Queensland, Australia, as a case study. Koalas are specialist folivores with complex feeding behaviour from *Eucalyptus* species. My aim was to identify the influence of foliar chemicals (moisture content, digestible nitrogen (DigN) and a toxin formylated phloroglucinol compounds (FPC) concentrations) and associated environmental factors on koala habitat use and diet across three rainfall seasons. I addressed three specific questions: 1) How do tree characteristics and environmental factors influence on spatial and temporal variation in leaf chemistry composition of koala food tree species? 2) What are relative influences of leaf chemistry, tree characteristics and environmental factors on koala habitat use and diet? and 3) Can we use the WorldView-3 satellite imagery to accurately map foliar nutrition at high resolution in koala habitats in semi-arid regions?

The research was conducted in the Mulga Lands and Brigalow Belt South bioregions of southwest Queensland. *Eucalyptus* leaf chemicals, koala habitat use and diet were examined. A hierarchical sampling design was applied to select 34 sites (6-10 trees in each) from ten landscapes (each 10×10 km) across the 62,500 km^2 area. Leaf samples were collected from 261 trees and repeated over three seasons with contrasting rainfall. Leaf moisture content, DigN and FPC concentrations were analysed in the laboratory. Koala habitat use was indicated by the presence of fresh faecal pellets at the tree scale. Koala diet composition was assessed by histological analysis of leaf cuticles from fresh faecal pellets collected from sites and the adjacent transects along creeks. Generalised mixed effects modelling was applied to analyse the influence of tree characteristics and environmental factors on foliar chemistry and the presence/absence of koala pellets. The relationship between foliar chemical composition and temporal variation in koala diet composition was also investigated. Two satellite images captured by WorldView-3 were used to extract tree spectral reflectance for eight sites from two landscapes. Spectral indices were calculated from the tree spectra. The correlations between spectral indices and foliar DigN concentrations were examined.

Rainfall within the previous six-months and surface water availability were the primary determinants of leaf moisture and secondary determinants of foliar DigN and FPC. All foliar chemicals varied among the four eucalypt species sampled. The riparian species *E. camaldulensis* had higher leaf moisture content, DigN and FPC concentrations than the floodplain species *E. populnea*. Koala presence was positively influenced by foliar DigN concentration, tree size and long-term (three years) soil moisture. Koalas used taller *E. camaldulensis* in riparian areas and the long-term soil moisture in the Brigalow Belt South was positively associated with koala presence. More than 50% of the koala diet was from *E. camaldulensis*. *E. coolabah* was eaten more than *E. populnea* and *E. melanophloia* where it occurred. Koalas increased consumption of *E. melanophloia* in the dry season, probably to increase moisture intake but ate more *E. populnea* in wet seasons in response to higher DigN levels. Leaf moisture was lower in the dry season whereas DigN and FPC were more stable across seasons. The normalised difference index using bands ‘Coastal’ and ‘NIR1’ extracted from WorldView-3 satellite images was best correlated with DigN concentrations. The index was used to map foliar DigN at landscape scale.

The significance of this research lies in demonstrating the importance of leaf moisture and DigN for semi-arid koala populations, especially in *E. camaldulensis* as the koala primary food tree species. Leaf moisture decreased in dry seasons and was a key factor limiting foliar nutrition for koalas. The high variation and concentration of foliar FPC in *E. camaldulensis* indicated koalas could cope with FPC through physiological tolerance and using trees with comparatively lower FPC. The temporal variation in diet of *E. populnea* and *E. melanophloia* revealed the supplementary function of secondary koala habitats. Therefore, preserving riparian habitats and surface water bodies is essential for the survival of western koala populations under a hotter and drier climate. Protecting secondary floodplain habitats is important for their long-term persistence. Because foliar DigN did not show strong and unique absorption features, the relationship between the best spectral index and DigN is indirect. The applicability of this index for mapping DigN in other areas needs to be verified.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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Publications during candidature

No publications.

Publications included in this thesis

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Contributions by others to the thesis

Dr Bill Venables helped with statistical modelling in R.

Professor Noam Levin helped with analysis and interpretation of satellite images.

Statement of parts of the thesis submitted to qualify for the award of another degree

None.

Research Involving Human or Animal Subjects

Protocol for this study was approved by the Animal Ethics Committee at The University of Queensland (GPEM/394/14). The animal ethics approval certificate is in Appendix A.

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List of Abbreviations Used in The Thesis

ADF	Acid Detergent Fibre
AIC	Akaike Information Criterion
ASD	Analytical Spectral Devices
BIC	Bayesian Information Criterion
DBH	Diameter at Breast Height
DM	Dry Matter
DigN	Digestible Nitrogen
FPC	Formylated Phloroglucinol Compounds
HPLC	High-Performance Liquid Chromatography
IBRA	the Interim Biogeographic Regionalisation for Australia
N	Total Nitrogen
NDF	Neutral Detergent Fibre
NDI	Normalized Difference Indices
NDVI	Normalized Difference Vegetation Index
NIR	Near-infrared
NIRS	Near-infrared Spectroscopy
PEG	Polyethylene Glycol
PSMs	Plant Secondary Metabolites
RMSE	Root Mean Square Error
SWIR	Shortwave Infrared
UBF	Unsubstituted B-ring Flavanones
VNIR	Visible and Near-infrared
WV2	WorldView-2
WV3	WorldView-3

Chapter 1 General Introduction

1.1 Introduction

Increasingly, human activities are dramatically changing the global environment and biodiversity (Sterling et al. 2013). The main threats to biodiversity are habitat loss and climate change and these are leading to rapid declines in the population size of many species (Jantz et al. 2015). These threats make it vital that habitat be retained of sufficient area, connectivity and quality to sustain viable local populations within a species' range (Hodgson et al. 2011). Environmentally sustainable development requires that decisions to conserve habitat should be based on ecological scientific evidence. To better understand and protect species from rapid population declines, knowledge of the spatial and temporal dynamics of habitat quality for the species is required in their changing environments (Van Teeffelen et al. 2012).

For wildlife species, habitats provide biotic and abiotic conditions that are essential for their survival and reproduction (Hall et al. 1997). Habitat quality, a fundamental concept in ecology, is determined by the availability of resources required by a given species for its survival including water, food, shelter and suitable climate conditions (Hall et al. 1997, Doligez and Boulinier 2008, Krebs 2009, Mayor et al. 2009). These resources are distributed heterogeneously in space and time, resulting in patchy species distributions (Mayor et al. 2009). Although species can be widely distributed across their geographic range (Whittingham et al. 2007, McAlpine et al. 2008), changes in habitat quality, quantity and configuration at the landscape scale (1000s ha) can have direct influences on the survival of populations (McAlpine et al. 2006). In addition, habitat quality tends to decrease closer to the edge of the geographic range of a species (Seabrook et al. 2014). Populations living at the margins of their geographic ranges can be more sensitive to fluctuations in resource availability, especially for species occupying narrow ecological niches. Therefore, studying the dynamics of habitat resources and their quality are critically important to understanding habitat requirements for population persistence across a spatially and temporally heterogeneous landscape.

Arboreal folivores are a group of species that have a narrow ecological niche, living in trees and almost exclusively eating leaves (Seabrook et al. 2014). For many folivores, trees provide food and shelter and are the primary resource unit of species management (Matthews et al. 2007). The quantity and quality of food tree resources have been used to determine habitat quality and to predict the occurrence of arboreal mammalian folivores within heterogeneous landscapes (Chapman et al. 2004, Moore et al. 2010, Youngentob et al. 2015). Spatial and temporal variation in food quality for folivores is often influenced by the chemical composition of leaves (Hume 1999). Many plants have evolved a chemical defence system against browsing and it is challenging for folivores to balance the benefits and costs of diet in using habitats (DeGabriel et al. 2010, Castillo et al. 2013). Populations of folivores which live at the edge of their distribution are close to their physiological and environmental limits and extreme weather events can directly cause population crashes within months (Gordon et al. 1988). To understand the spatial and temporal dynamics of food resources and hence the habitat quality for folivorous species, we need to understand plant-animal interactions in a changing environment using theories and methods of chemical ecology, nutritional ecology and landscape ecology (Moore et al. 2004a).

1.2 Problem Statement

The koala (*Phascolarctos cinereus*) provides an ideal example of arboreal folivore species which is widely distributed but which has a narrow ecological niche. The koala is endemic to Australia. As shown in Figure 1.1, the distribution of koala is from tropical Queensland to temperate Victoria and South Australia (Rhodes et al. 2015). The habitat types within the koala's distribution are highly variable at the regional, landscape and tree scale (McAlpine et al. 2008). Koala habitats have been highly modified by human land use, resulting in extensive habitat loss and fragmentation (McAlpine et al. 2015). In addition, because Australia has a highly variable climate, the food, water and other resources available to koalas are also highly variable, especially in semi-arid regions where koalas can occur (Gordon et al. 1988, Ellis et al. 1995). This variation in resources is driven by inter-seasonal and inter-annual variation in rainfall (Seabrook et al. 2014). Rainfall is becoming more variable due to climate change (Adams-Hosking et al. 2012). The long-term survival of koala populations requires knowledge of habitat composition and quality, how this varies in space and time, and how koalas respond to resource variability.

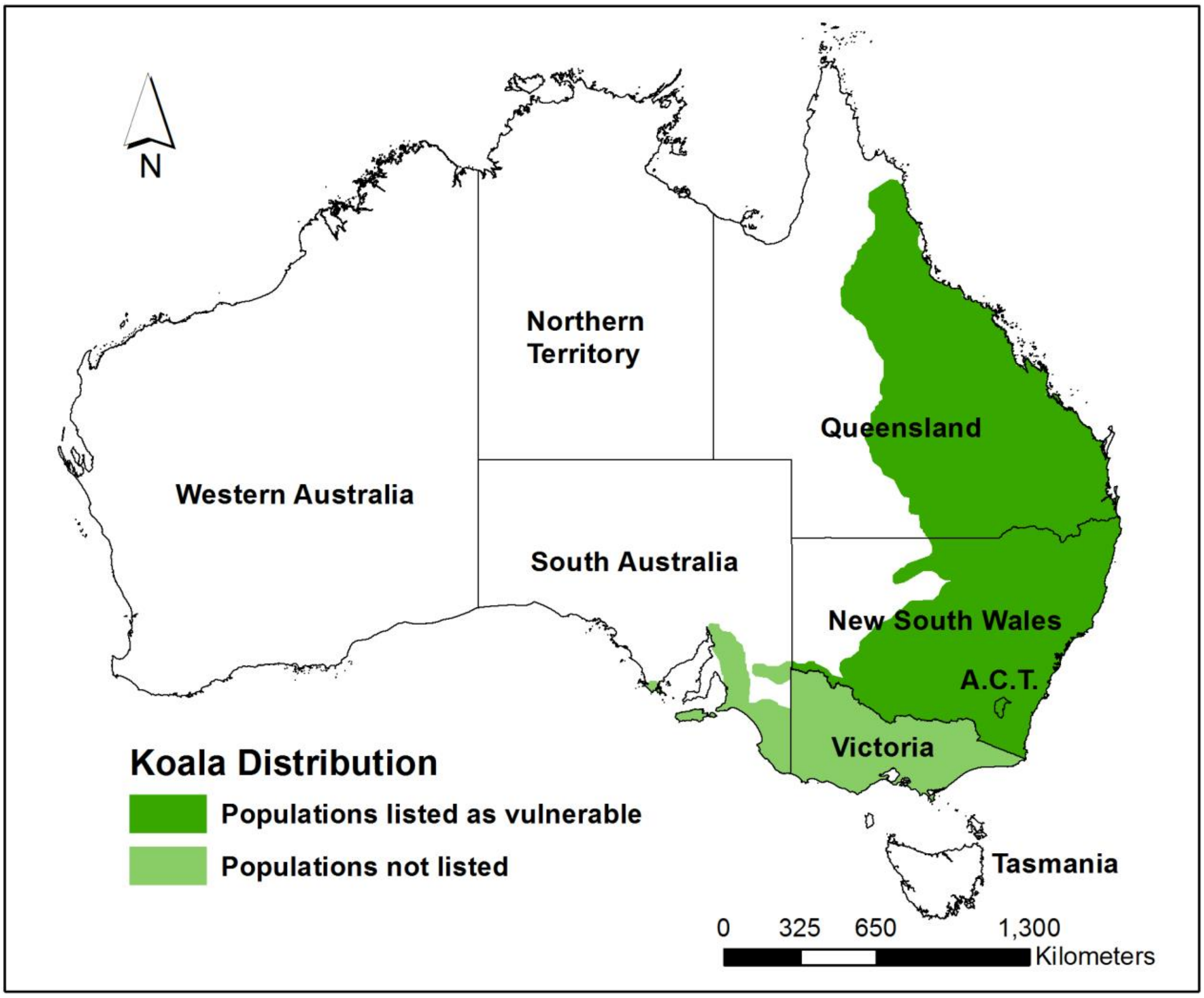

Figure 1.1 Map of koala distribution and listed populations in Australia. (Source of data: Rhodes et al. 2015)

Koala populations occupying the hot semi-arid regions of southwest Queensland are on the front line of koala range shifts due to climate change (Adams-Hosking et al. 2011). Land clearing and climate change are directly affecting the habitats and dynamics of these western populations (Sullivan et al. 2004, Seabrook et al. 2011). Bioclimatic models predict that western koala habitats may become uninhabitable in the next 50 years under the more extreme climate change scenarios (Adams-Hosking et al. 2011). The koala populations in southwest Queensland, therefore, provide a suitable case study of the spatial and temporal dynamics of their habitat, in particular food quality, under changing climatic conditions.

The koala populations of southwest Queensland form a significant regional population (Sullivan et al. 2004, Seabrook et al. 2011). The nutritional quality of their habitat has at times been degraded by severe droughts and heatwaves which are increasing in frequency and severity with climate change (NRMMC 2009). In 1980, a heatwave and drought in southwest Queensland caused food tree defoliation/dieback and a dramatic decline in koala populations from malnutrition and dehydration (Gordon et al. 1988). More recent research found that drought and heat-waves resulted in a decline in the koala population in southwest Queensland of 80% from 1995 to 2009 (Seabrook et al. 2011, Davies et al. 2014). There were also significant relationships between spatial and temporal patterns of tree use, distribution and habitat use by koalas and rainfall variability and surface water availability (Davies et al. 2013a, Smith et al. 2013b, Smith et al. 2013c).

As specialist folivores, koalas are browsers showing complex variations in using trees of eucalypt species ("eucalypt" encompasses the genera *Eucalyptus, Corymbia*, *Angophora*, *Arillastrum*, *Allosyncarpia*, *Stockwellia* and *Eucalyptopsis*), and leaf chemistry of which plays an important role in tree and leaf use (Moore et al. 2004a). In plant-folivore interactions, leaf chemicals play a role in nutrition and deterrence which influence food quality for, and diet variation by, folivores. *Eucalyptus* leaves are known to have low nutritional quality and high toxicity (Hume 1999). This presents a challenge for koalas to choose leaves with sufficient nutritional values and tolerable levels of toxins. Previous studies found evidence that koalas showed complex food tree use decisions, which was influenced by concentrations of leaf chemicals including toxins (Moore et al. 2004a).

Most previous studies of the influence of leaf chemistry on koala diet have focused on the temperate forests of southeast Australia (Lawler et al. 1998, Moore and Foley 2005, Marsh et al. 2014). In contrast, there is little research on the leaf chemistry of trees used by semi-arid koala populations which are vulnerable to seasonal and inter-annual rainfall variations (Gordon et al. 1988). My previous study (Wu et al. 2012) was the first leaf chemistry study for the semi-arid koala populations in southwest Queensland but it was limited to one season. Furthermore, there is an absence of studies of how the leaf chemistry of the same tree species varies at the landscape-regional scale with environmental variability, especially climate variability and surface water availability. This question is critical for understanding how regional climatic change is likely to influence the leaf chemistry of koala food trees and koala population persistence in semi-arid regions (Smith et al. 2013b, Seabrook et al. 2014). Hence further investigation is required to understand how spatial and temporal variation in leaf chemical constituents such as digestible nitrogen and plant secondary metabolites influence koala diet. This knowledge can then be applied to assess and map koala habitat quality for regional conservation planning (McAlpine et al. 2008). It would also be useful for koala conservation planning if foliar nutrition could be estimated via remote sensing, which may be a cost-effective way to identify high quality habitat and monitor habitat change.

1.3 Aims and Objectives

This thesis investigates the spatial and temporal effects of ecological factors on koala diets, mediated by foliar chemistry in the Queensland section of the Mulga Lands and Brigalow Belt South biogeographic regions. It aims to identify the leaf chemical attributes that determine koala habitat quality in these semi-arid regions and the environmental and climatic factors that influence habitat quality.

The project addresses the following objectives for western koala populations:

1. Identify the spatial and temporal influence of tree characteristics (species, size and condition) and environmental factors (proximity to surface water, short-term rainfall and annual rainfall) on leaf chemistry composition of koala food tree species.
2. Determine the spatial and temporal influence of leaf chemistry, tree characteristics and environmental factors on koala habitat use and diet.
3. Explore the use of WorldView-3 satellite imagery to accurately map foliar nutrition at high resolution in koala habitats in semi-arid regions.

1.4 Significance

This project represents the first study of spatial and temporal variation in the foliar chemistry of koala food trees at multiple scales of a western koala population inhabiting a semi-arid environment. The significant contributions of this project are to:

1. Identify the key leaf chemistry characteristics of koala food trees and how they vary spatially and temporally. This information will provide new scientific evidence to help prioritise areas for koala habitat protection, management and restoration.
2. Assess the predictive capacity of satellite spectra in foliar chemistry mapping. If the spectral indices derived from space-borne multispectral data are proved to be able to predict the foliar nutrition of eucalypt leaves used by koalas, it will improve the efficiency and accuracy of mapping koala habitat quality at a regional scale (1000s km^2).
3. Provide new scientific evidence for developing koala climate change adaptation strategies for the koala's long-term survival. Bioclimatic models show that the western koala populations and their food sources are more vulnerable than the coastal populations to the threats from increasing droughts and heatwaves with habitat loss (Adams-Hosking et al. 2011). Studying the ecological response of western koalas and their key food trees can provide better understanding of the vulnerabilities of this species to future climatic threats.

1.5 Disciplinary Basis

This study is based on the theories and methods of chemical ecology, nutritional ecology and landscape ecology. One of the major objectives of chemical ecology is to understand the variation in plant chemicals and how they mediate the interactions between plant and plant-eaters (Harborne 2001). Previous studies of *Eucalyptus*-folivore interactions provide knowledge of the leaf chemicals that can influence koala foraging decisions and will help us to identify the leaf chemicals to target in this study. Nutritional ecology focuses on understanding how animals regulate the intake of multiple nutrients simultaneously in a changing ecological environment (including food quality and quantity, and abiotic components), and how nutrition effects the survival and persistence of animal populations (Raubenheimer et al. 2009). Previous studies provide the basis of my conceptual model (Chapter 2) which guides the following chapters to understand the influence of nutrition (leaf chemistry) on patterns of koala habitat use. The core of landscape ecology is to understand the causes and ecological consequences of spatial heterogeneity on a factor of interest, and how they vary at multiple scales in space and time (Turner 2005). Scaling is one of the most important themes of landscape ecology and it is associated with variation in space and time (Weins 2002). This provides the conceptual basis for determining the sampling design of field data collection, and the analysis of leaf chemistry, koala diet and habitat use in space and time.

1.6 Study Area

The study focused on a 62,500 km^2 area of southwest Queensland, Australia (Figure 1.2a). Australia's landscapes are classified into 89 large geographically distinct bioregions by the Interim Biogeographic Regionalisation for Australia (IBRA version 7), based on common climate, geology, landform, native vegetation and species information (Department of Environment 2012). The study area of this study covers two biogeographic regions: the Mulga Lands bioregion in the west and the Brigalow Belt South bioregion in the east. The average daily temperature range is 3-19 °C in winter (May-August) and 19-35 °C in summer (December-February). The average annual rainfall of the range in this study is 440-500 mm in the Mulga Lands and 535-593 mm in the Brigalow Belt South. However, monthly and annual rainfall is highly variable (Figure 1.2b, c). The whole study area experienced droughts, below-average rainfall (265-460 mm), from 2012 until late 2015, which corresponded with the field data collection (Bureau of Meteorology 2016). The average annual relative humidity is 62% at 9 am and 47% at 3 pm (Bureau of Meteorology 2016).

The tree communities are dominated by *Eucalyptus camaldulensis* and *Eucalyptus coolabah* along the riparian areas, whereas *Eucalyptus populnea* and *Eucalyptus melanophloia* dominate the surrounding floodplains and low hills. The watercourses in the study area are ephemeral and the overall drainage patterns are from north to south. The 2012-2015 droughts caused extensive tree die-back or leaf-fall especially for the *E. camaldulensis* along stretches of dry creek beds. The main land use in both bioregions is cattle grazing, with mixed grazing-cropping also common in the eastern Brigalow Belt South.

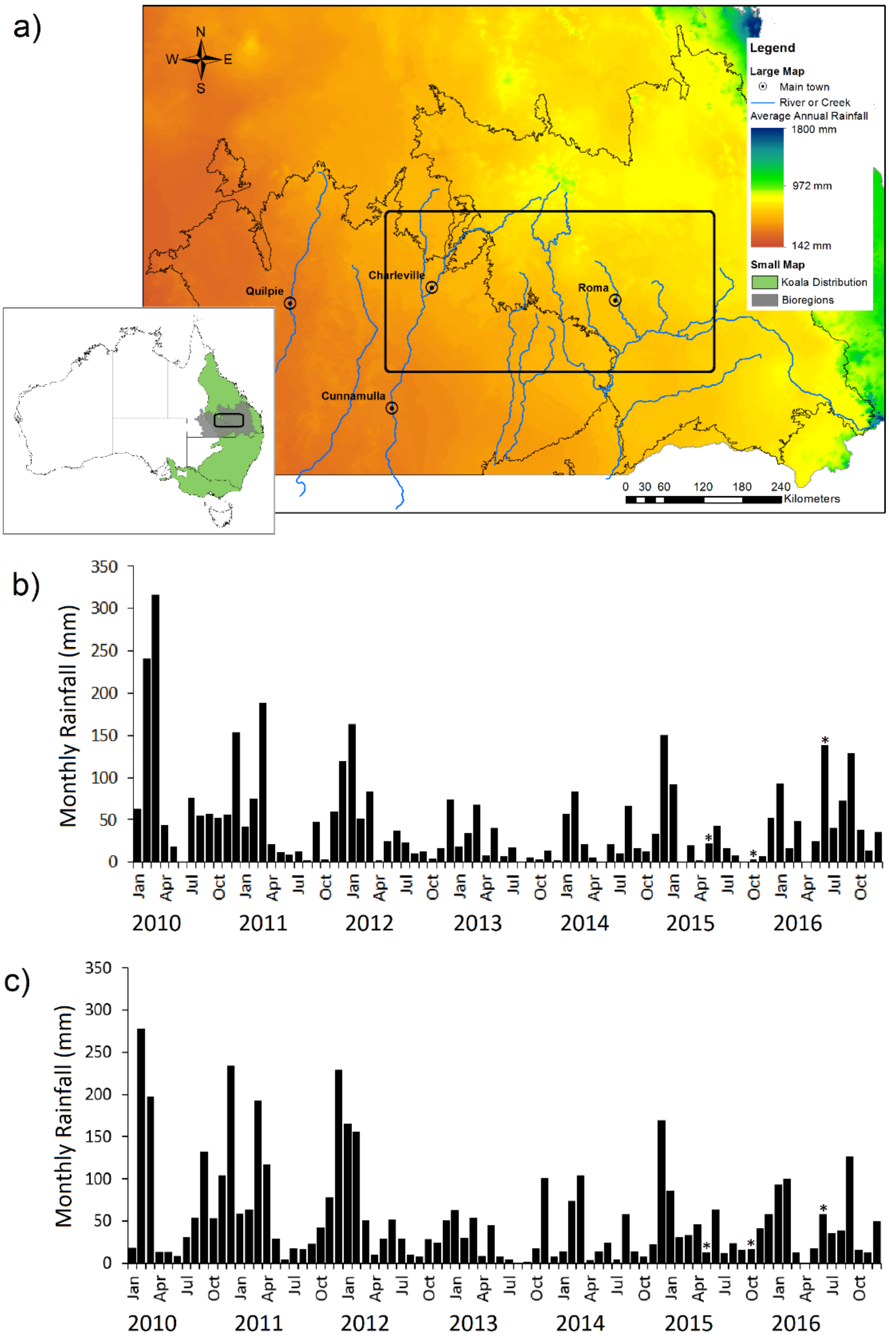

Figure 1.2 Study area of the project: (a) Location of study area (black rectangle) within Mulga Lands and Brigalow Belt South bioregions with average annual rainfall (source: Bureau of Meteorology). Insert map shows koala distribution (green), Mulga Lands and Brigalow Belt South bioregions (grey) and location of study area (black rectangle). Monthly rainfall of (b) Charleville weather station (26.41 °S, 146.26 °E) and (c) Roma weather station (26.55 °S, 148.77 °E) in 2010-2016 with asterisks indicating the dates of field sampling.

1.7 Thesis Structure

This thesis consists of six chapters (Figure 1.3). This introduction (Chapter 1) sets out the background, aims and objectives. Chapter 2 provides a literature review of the background concepts, methods and key findings of relevant previous studies. This is followed by three core data chapters (Chapters 3-5) that address the aims and objectives. Chapter 3 presents variation in leaf chemistry and its drivers. Chapter 4 links koala diet and habitat use to leaf chemical variation. Chapter 5 explores the use of satellite images to map leaf chemical for koala habitat. A final discussion and conclusion chapter (Chapter 6) synthesises the key findings of the research, discusses future research needs and provides recommendations for koala habitat management and conservation.

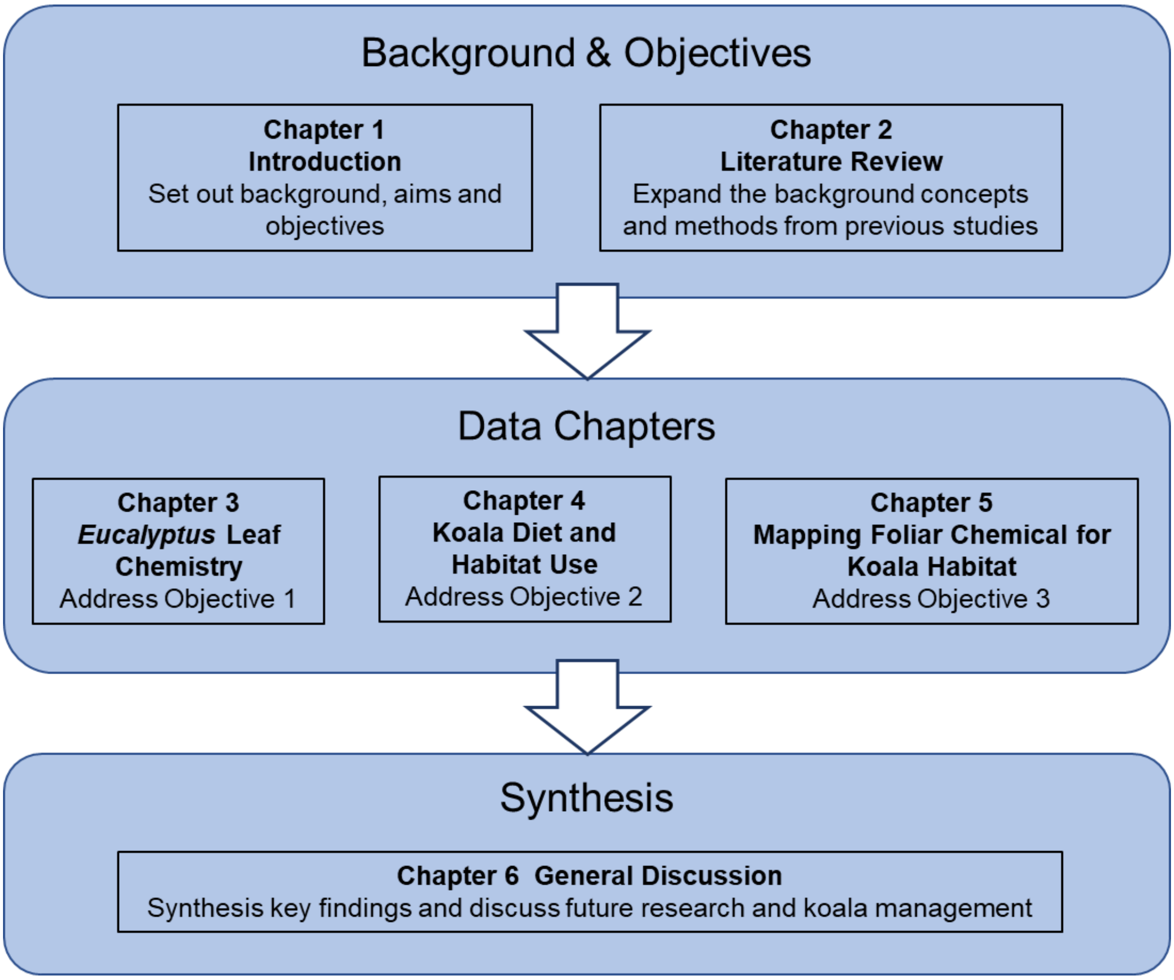

Figure 1.3 Thesis structure showing the core components of the thesis and the related chapter(s).

Chapter 2 Literature Review

2.1 *Introduction*

The aim of this literature review is to 1) identify the fundamental concepts integral to this project, 2) review previous studies as background to this study, 3) introduce methods that I will use to measure leaf chemistry, koala diet and habitat quality evaluation, 4) identify knowledge gaps in ecological science and practical conservation in this field, and 5) develop hypotheses in the form of a conceptual model. This chapter is divided into four sections, with sections 2-4 covering the three main aspects of the study and section 5 providing the conceptual model.

In this chapter, I summarise the background knowledge and research progress of studies into foliar chemistry, the causes of variation in leaf chemistry and the range of analysis methods available, with a focus on *Eucalyptus* species (Section 2.2). I then examine the needs of arboreal folivores in terms of diet as influenced by leaf chemistry, with a focus on the koala (Section 2.3). Finally, I review methods for applying leaf chemical analysis at greater spatial scales to map habitat quality for wildlife conservation, with a focus on koala habitat in southwest Queensland (Section 2.4).

2.2 *Foliar Chemistry*

Plants contain water and minerals, produce primary metabolites related to plant growth and development (e.g. protein and carbohydrate), and evolve toxic/anti-nutritional plant secondary metabolites (PSMs) to defence against herbivores as a result from the evolutionary competition between plant species and foraging animals (Moore et al. 2004a, Youngentob et al. 2011, Castillo et al. 2013). To understand the chemical mediated plant-herbivore interactions, identifying the functions of foliar chemicals and their distributions are among the highest priorities in chemical ecology (Harborne 2001). Driven by genetics and environmental conditions, leaf chemicals can vary across individual trees and taxa, and across space and time (Shipley et al. 2009, Bustos-Segura et al. 2017). These variations in leaf chemicals create patchy resource availability to animals and influence the foraging behaviour and population dynamics of herbivores (DeGabriel et al. 2014). For example, high foliar nutritional quality is related to important food trees and habitats of the ring-tailed lemur (*Lemur catta*) and high biomass of leaf-specialist lemurs (*Lepilemur leucopus* and *Propithecus verreauxi*) in Madagascar (Mertl-Millhollen et al. 2003, Simmen et al. 2012).

This section reviews the commonly studied foliar chemicals (including water) that represent both nutritional value and potentially toxic or anti-nutritional constituents affecting arboreal folivores. It will focus on the leaf chemicals of *Eucalyptus*, including the variations of chemical compositions and concentrations among individual trees, sub-species, species and between subgenera, and the influences of genes and environments on these variations.

2.2.1 Leaf Nutrients and Deterrents

To foraging animals, leaf chemicals can be divided into nutrients and deterrents. The fundamental and essential nutrients for animals, including folivores, are carbohydrates, protein, lipids, minerals (e.g. potassium, calcium and phosphorus), vitamin and water (Barboza et al. 2009). The most well studied nutrient is protein, which is usually estimated from total nitrogen (N) content, which has been suggested to be the most important element for folivores (Mattson 1980, Kavanagh and Lambert 1990, White 1993). Because leaves contain little protein in comparison to animal tissue, folivores have evolved strategies to maximize nitrogen absorption and to recycle nitrogen (Barboza et al. 2009). For folivores, relative water content is not examined as widely as protein in studies of food quality because leaves are relatively rich in water and most animals can acquire additional water from drinking, including from dew (Barboza et al. 2009). However, water may become limiting in hot and dry areas as leaf water content decreases and water requirements increase for cooling via evapotranspiration and because of greater rates of water loss in dry atmospheres (Munks et al. 1996, Barboza et al. 2009).

The main foliar chemicals against folivores include PSMs and cell walls, which either decrease digestibility or are toxic to animals (Hume 1999). PSMs are groups of different chemicals not essential to the survival of plants compared to the primary metabolites, and which commonly play roles as chemical defences against herbivores or pathogens (Barboza et al. 2009). The commonly known PSMs acting as deterrents to folivores are phenolics, alkaloids and terpenoids (Robbins 1993). Cell walls are composed of largely indigestible fibre including lignin, cellulose and hemicellulose (Hume 1999). Tannins (groups of polyphenolics that occur widely in browse plants) and cell wall components have been well studied for their negative effects on leaf digestibility and protein absorption in folivores. In low-pH guts, insoluble complexes form between tannins and protein which are indigestible and eventually lost in the faeces (Jones and Mangan 1977). Because tannins can bind the plant protein, microbial protein and microbial polysaccharide-degrading enzymes in the hindgut, tannins not only reduce the digestibility of foliar protein but also reduce the hindgut fermentation efficiency which is an important digestive process for many monogastric herbivores (Jones and Mangan 1977, Hume 1999).

Food quality for folivores is usually represented using one nutrient value or a ratio of key foliar chemicals. For years, it has been common to use N to represent crude protein, and standardised measures of fibre fractions, such as neutral detergent fibre and acid detergent fibre (NDF and ADF), and N:ADF ratio to indicate overall food quality (Mattson 1980, Van Soest et al. 1991, Chapman et al. 2004, Simmen et al. 2012). DeGabriel et al. (2008) introduced the use of digestible nitrogen (DigN), which incorporates nitrogen concentration with the biological effects of tannins and fibre. It is thus closer to the protein value that folivores can use than N (Wallis et al. 2012).

2.2.2 Foliar Chemistry of Eucalyptus

About 40 million years ago, Australia separated from other continents and began moving northward, leading to drier weather and consequently altering its fauna and flora profoundly (Tyndale-Biscoe 2005). Australian sclerophyll flora has adapted to the dry environments and the highly weathered and infertile soil (Cox 2000). Up to half the Australian sclerophyll genera are endemic to Australia and the eucalypts (Myrtaceae) are perhaps the most familiar and typical, making this continent the "gum tree" country (Cox 2000). The eucalypts comprise seven genera. The largest genus, *Eucalyptus*, is divided into a number of subgenera, the largest of which are *Symphyomyrtus* (~500 species) and *Eucalyptus* (~130 species) and these include the tree species that are most favoured by marsupial folivores (Moore et al. 2004a).

Previous studies have found that the water content of eucalypt leaves across Queensland ranged between 39-65% wet weight (Munks et al. 1996, Ellis et al. 2002, Clifton 2010, Wu et al. 2012). Foliar moisture content of eucalypt trees is similar to the leaf moisture 48-66% found in tamarind (*Tamarindus indica*) in Africa (Mertl-Millhollen et al. 2003) and is lower than the leaf moisture for 162 tropical forest species (43-79%) in north Queensland, Australia (Asner and Martin 2008). Young leaves in eucalypts had higher water content than old leaves (O'Reilly-Wapstra et al. 2007) and so the presence of new growth, which can be stimulated by rainfall (Gherlenda et al. 2016), strongly influenced the water content of available foliage.

In general, compared to the dry matter composition of grasses, legumes and vegetables (Figure 2.1), *Eucalyptus* foliage is low in nutrients (minerals, protein and digestible carbohydrate) but high in indigestible and toxic chemicals (lignin and PSMs) reflecting the infertile soils of Australia (Hume 1999). The highly-lignified fibre causes low digestibility of eucalypt leaves by diluting the content of digestible constituents and by impending access to cell contents (Hume 1999). The main PSM groups in eucalypts are terpenoids (e.g. 1,8 cineole), phenolics including the polymeric condensed and hydrolysable tannins, flavanones, simple phenolics, and formylated phloroglucinol compounds (FPC, e.g. jensenal, grandinal, Macrocarpals G and Sideroxylonal), the last of which are known to deter folivores including the koala (Moore and Foley 2005, Jensen et al. 2014). Twenty-three eucalypt species are also known to produce cyanogenic glucosides (Gleadow et al. 2008). FPC were first isolated from *Eucalyptus* in a marsupial folivore nutrition study and proved to be a strong deterrent of marsupial browsing (Pass et al. 1998). Studies have provided evidence supporting FPC as the most promising foliage compounds to explain food quality for marsupial folivores feeding from eucalypts from the subgenus *Symphyomytus* (Moore et al. 2004a, DeGabriel et al. 2010, Youngentob et al. 2011). More recently, another class of compounds, the unsubstituted B-ring flavanones (UBF) have been discovered from the subgenus *Eucalyptus* (Tucker et al. 2010). These are absent from the subgenus *Symphyomyrtus*, but appear to be effective deterrents against the common brushtail possum (*Trichosurus vulpecula*), a marsupial folivore which eat more *Symphyomyrtus* species than *Eucalyptus* species (Marsh et al. 2015).

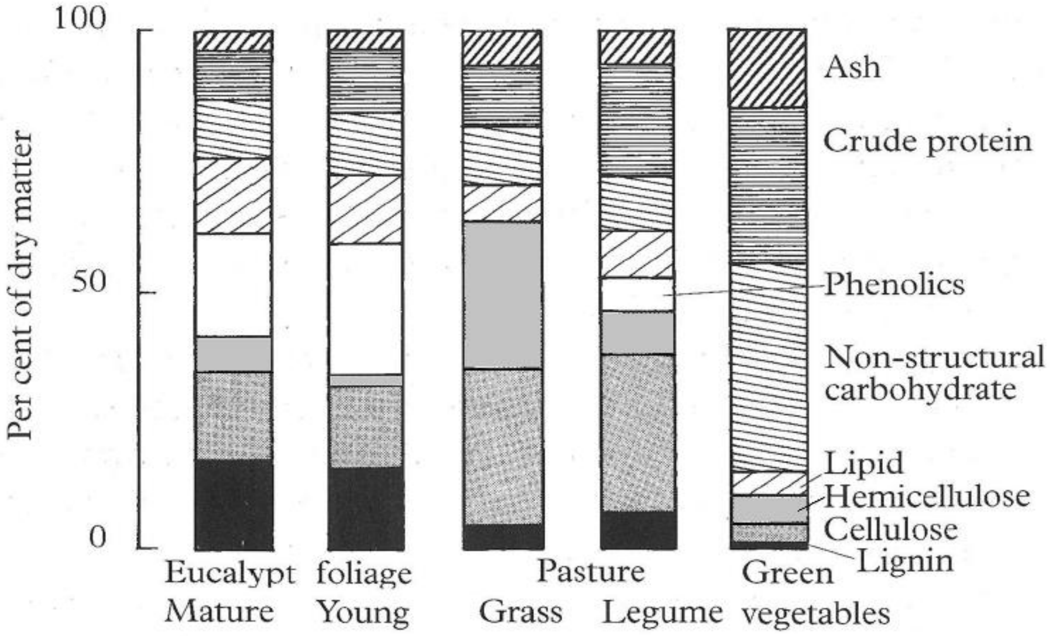

Figure 2.1 Dry matter composition of *Eucalyptus punctata* and other plant groups subject to browsing (figure from Hume 1999).

2.2.3 Variation of Genus Eucalyptus Foliar Chemistry due to Genes

Foliar water content is controlled through plant physiological adjustments, whereas most leaf chemicals are synthesised in plant tissues, which are both, to some extent, related to gene expressions (e.g. synthesis of enzyme, structural protein or PSMs) (Yan et al. 2000, Freeman et al. 2008, Külheim et al. 2011). For example, FPC and terpenes have a strong genetic basis which results in significant heritability, and hence the spatial genetic variation is correlated with the spatial structure of chemical variation (Andrew et al. 2005, Külheim et al. 2011). Condensed tannins have a weaker genetic basis and are more likely to be influenced by environmental variation (Andrew et al. 2005, O'Reilly-Wapstra et al. 2005).

Different genotypes, which are the genetic constitutions of organisms (Allaby 2012), can lead to variations of foliar chemistry along taxonomy, among individuals and even within one plant (Eschler et al. 2000, Youngentob et al. 2011, Padovan et al. 2012, Moore et al. 2014). The variation of leaf moisture between tree species has been studied in semi-arid and sub-humid areas, and showed interspecific variation (Munks et al. 1996, Wu et al. 2012, Melzer 1995). There are more studies of the variation of nitrogen and PSMs within *Eucalyptus* taxonomic divisions. For example, results from the subgenera *Symphyomyrtus* and *Eucalyptus* species indicated that nitrogen concentrations can vary between subgenera and among species (Cork et al. 1984, Dearing and Cork 1999, O'Reilly-Wapstra et al. 2007, DeGabriel et al. 2008), but this is not always the case (Noble 1989). For PSMs, studies show significant variation of foliage concentrations of cyanogenic glycosides, phenolics and FPC from the subgenus level down to the individual species and even trees of the same species. At the subgenus level, species of *Eucalyptus* are recognized as UBF and tannin-rich species whereas those from *Symphyomyrtus* are FPC-rich species (Wallis et al. 2010, Marsh et al. 2015, Eschler et al. 2000). Folivore species show different diet composition at the subgenera level due to the differing tolerances they have evolved (Jensen et al. 2014). A comparison between 31 species of subgenus *Eucalyptus* and 83 *Symphyomyrtus* species across the continent showed significantly higher condensed tannins in the subgenus *Eucalyptus* foliage (resulting in lower mean digestible nitrogen) and considerable variation of tannins within each subgenus (Wallis et al. 2010). Because of the strong genetic basis, FPC variation is widely studied and its genetic control has been explored down to molecular level (Freeman et al. 2008, Külheim et al. 2011). Interspecific variation of FPC compounds and concentrations were found in *Symphyomyrtus* species whereas *Eucalyptus* species had no FPC concentrations (Dearing and Cork 1999, Eschler et al. 2000). At individual tree level, evidence showed a significant difference of FPC concentration among trees of the same species growing side by side (Lawler et al. 2000, Scrivener et al. 2004) and among branches in a single tree (mosaic tree) (Padovan et al. 2012), indicating a genetic control of these chemicals., These variations in leaf chemistry can have a spatial effect on the tree use and the associated distribution of arboreal folivores (Moore et al. 2010, Youngentob et al. 2011).

2.2.4 Environmental Variation and Genus Eucalyptus Foliar Chemistry

A variety of external environmental conditions were found to influence genus *Eucalyptus* foliar chemistry across different tree species and study areas (Noble 1989, O'Reilly-Wapstra et al. 2005). These environmental factors include water availability, nutrient availability, temperature and atmospheric CO_2 concentration (Noble 1989, Moore et al. 2004a). Different leaf chemicals can have different responses to the same environmental variability (O'Reilly-Wapstra et al. 2005). One difficulty in studying the environmental effects on foliar chemistry in natural forests is avoiding the effects of genotype (e.g. the spatial pattern of species distribution along environmental gradients) (Braithwaite 1983). Hence it is common to study a single species of one or more populations through glasshouse experiments with seedlings (Gleadow et al. 1998, O'Reilly-Wapstra et al. 2005) or field trials with plantation in common gardens (O'Reilly-Wapstra et al. 2007).

Water availability to trees varies both spatially and temporally which influences leaf chemistry in different ways. In semi-arid areas of Queensland, *Eucalyptus* trees occurring along creek-lines, where more folivores also occur, had higher leaf moisture than in trees growing far from creek-lines (Munks et al. 1996, Wu et al. 2012). In addition, presence of surface water was positively associated with leaf moisture (Wu et al. 2012) and surface water can make up to 50% of the water source to trees when it is available in long-term (Thorburn and Walker 1994). In sub-humid central Queensland, seasonal leaf monitoring in *Eucalyptus* showed higher moisture contents during summer, which is the rainy season (Ellis et al. 1995, Ellis et al. 2010). Water availability can also change the concentrations of foliar PSMs by influencing the growth rate of trees (Cork et al. 1990, Munks et al. 1996). For example, *Eucalyptus viminalis* only produces cyanide during the wet seasons in Western Australia, causing variation in the foraging response of captive koalas to this species (Congreve and Betts 1978). Prolonged extreme dry conditions during severe droughts can even cause extensive defoliate which profoundly affect leaf chemicals and availability (Gordon et al. 1988).

Nutrient availability to plants can change nutritional and anti-nutritional components in *Eucalyptus* foliage. A classic example of the influence of nutrient availability on *Eucalyptus* foliar chemicals is the discovery of positive relationships between foliar nutrient concentrations and soil fertility near Eden, New South Wales (Braithwaite et al. 1984). The patchy occurrence of the highly fertile soils from Devonian intrusions creates a patchy distribution of *Eucalyptus* communities with highly nutritional foliage, leading 63% of the arboreal herbivores to inhabit just 9% of the forest area (Braithwaite 1983). When fertiliser was applied to *Eucalyptus blakelyi* and *Eucalyptus globulus* seedlings, they had significantly higher leaf moisture, nitrogen, soluble sugars but lower condensed tannins and terpenes, whereas FPC did not change (Landsberg 1987, O'Reilly-Wapstra et al. 2005).

The leaf chemistry of *Eucalyptus* may be influenced by climate variables, such as temperature and the concentration of atmospheric CO_2 (Moore et al. 2004a). A significant increase of FPC and tannin concentrations in *Eucalyptus microcorys* was found at higher elevation sites where temperatures were 7 °C lower than in low-elevation sites (Moore et al. 2004b). But this study was not able to separate the effects of genotype and temperature. The elevation of atmospheric CO_2 may decrease food quality for marsupial folivores (Lawler et al. 1997). For example, glasshouse trials on

Eucalyptus tereticornis and *Eucalyptus cladocalyx* seedlings (food tree species for marsupial folivores) found that doubling ambient CO_2 concentration caused significant decrease in foliar nutrients such as moisture, nitrogen concentration (by 18-36%) or protein but increase in total phenolics and condensed tannins (Lawler et al. 1997, Gleadow et al. 1998). The influence of elevated CO_2 is mostly studied in the glasshouse, and looking at only seedlings, but a recent study on mature trees showed different results. In a mature *Eucalyptus* woodland, a free-air CO_2 enrichment experiment found that foliar nitrogen concentration was not influenced by elevated CO_2 (Gherlenda 2016). Atmospheric CO_2 concentrations have increased by about 36.7% since industrialization, and average warming for the next two decades is projected to be 0.4 °C (UNFCCC 2009). Therefore, the effects of increasing temperature and atmospheric CO_2 on foliar chemistry of eucalypts forests and woodlands can be potentially wide but complex, and requires further studies measuring these environmental effects in field-grown trees.

2.2.5 Measuring Foliar Chemistry in Genus Eucalyptus

The assessment of foliar chemistry has depended on general laboratory assays of leaf samples, which requires a large amount of samples, time and money. Scientists have been exploring alternative approaches to reduce costs. For example, near-infrared spectroscopy (NIRS) was introduced as a rapid and cost-effective auxiliary approach to reduce the sample size for laboratory assays (Foley et al. 1998). Laboratory assays in conjunction with NIRS have been well developed and now commonly used in ecological studies (McIlwee et al. 2001, Ebbers et al. 2002, Moore et al. 2010). Before analysing different leaf chemicals in laboratory and NIRS, leaf sample preparation should not be overlooked. The leaf chemicals commonly studied in chemical ecology, especially PSMs, can in some cases degrade once leaves are removed from trees (Foley and Hume 1987, Landsberg 1987). Hence, rigorous sample collection and preparation are essential for the accuracy for both laboratory assays and NIRS. Freeze-dried ground leaf is mostly used and proved to be better than oven-dried samples because the low temperature and water deprivation inactivates enzymatic and oxidative degradation of targeted chemicals (Foley and Hume 1987, Wallis et al. 2003).

2.2.5.1 Laboratory assays

In this subsection, frequently used techniques for foliar chemistry analysis are introduced and discussed.

Relative water content is determined by the weight loss from fresh weight to dry weight, and is recorded as % wet weight (Ellis et al. 1995, Wu et al. 2012). Leaves are oven dried, but the drying temperature varies from 45 °C to 80 °C (Ellis et al. 1995, Krockenberger et al. 1998, Ellis et al.

2002, Clifton 2010). The accuracy of leaf water content measurement will decrease if there is loss of essential oils during oven drying. For example, Ellis et al. (2002) decreased the drying temperature from 80 °C to less than 60 °C because they found most eucalypt essential oils in their study area volatilises at 70 °C. Yet there remains no agreement on the best drying temperature or drying method to prevent the loss of essential oils. Thus, it is important that studies report the drying temperature used.

The Kjeldahl method is the classic way to measure N in foliar chemistry (Ma and Zuazaga 1942). The analysis involves the following steps: the sample is dissolved by sulfuric acid which converts nitrogen into ammonium sulphate; then ammonium sulphate is converted to ammonium gas and quantified through neutralization and titration processes; finally, nitrogen concentration is estimated based on the amount of ammonium gas (Ma and Zuazaga 1942). However, in the tannin-rich eucalypt leaves, condensed tannins bind to protein and reduce nitrogen digestibility to folivores. Existing methods for analysing phenolics cannot completely extract tannin or phenolics from samples with fibre and phenolics concentration is not a measurement of their nitrogen binding effects (DeGabriel et al. 2009). Therefore, N cannot accurately reflect the nitrogen value in *Eucalyptus* for folivores and DigN should be measured instead (DeGabriel et al. 2009, Wallis et al. 2010).

The measurement of DigN extends the Kjeldahl method by using an *in vitro* enzymatic digestion to simulate an animal digestive system. The leaf sample is sequentially digested by pepsin and cellulase at 37 °C and then the nitrogen loss from the leaf sample to the digestion residue (measured by elemental analysis) is used to calculate the amount of DigN (% dry matter (DM)) (DeGabriel et al. 2008). Because leaf nitrogen is digested with interactions among foliar chemicals, DigN reflects the nitrogen value available to animals along with the negative effects of tannins and fibre (Wallis et al. 2010). Therefore, DigN was used in this research project.

In contrast to the complex methods measuring nitrogen, there is one commonly used method to estimate FPC concentrations. FPC are extracted in an organic solvent (acetonitrile), then separated and quantified by high performance liquid chromatography (HPLC) using UV detection and authentic standards as reference (Eschler et al. 2000, Moore et al. 2005, Youngentob et al. 2011).

2.2.5.2 Near-infrared spectroscopy (NIRS)

The principle of NIRS is that different organic chemicals absorb incident radiation at different frequencies, because the composition of atoms and chemical bonds in molecular structure are diverse (Burns and Ciurczak 2001). Molecular bonds of organic chemicals of *Eucalyptus* foliage mainly absorb near infrared radiation and hence the composition of chemicals can be represented in the NIR spectrum (Foley et al. 1998).

The technique of spectral data acquisition in *Eucalyptus* foliar chemistry analysis has been developed from standard methods used in agriculture developed by the American Society for Testing and Materials (Anon 1995). The most frequently used procedure is summarized in Foley et al. (1998) and McIlwee et al. (2001). It should be noted that NIRS is an indirect method that estimates chemical concentrations by comparing spectra with samples of known composition, which means it cannot entirely replace wet chemical assays (Shenk et al. 1992). Wet chemical assays are still needed for calibration and validation of NIRS predictive models but NIRS can reduce the number of samples assayed by the time-consuming and often costly traditional assays (Shenk et al. 1992).

The spectra of freeze-dried ground leaf samples are recorded by a NIR spectrometer between 400-2500 nm at 0.5 nm intervals in a controlled room at 22 °C and 55% relative humidity with an appropriate light source (e.g. tungsten-halogen). A predictive model is then developed to predict chemicals concentrations from leaf spectral data. The procedure to develop a calibration equation for predictive models in *Eucalyptus* foliage chemicals applications includes applying pre-processing techniques to spectral data, using algorithms to identify the calibration population, completing wet chemical assays on leaf samples from the calibration population, establishing a regression equation based upon calibration population data, and equation validation.

The development of NIRS in estimating chemical composition of *Eucalyptus* foliage is suitable for the development of methods to be used in the field for landscape-scale studies. Landscape-scale studies have proved the feasibility of using fresh whole leaves as NIRS samples to analyse well-defined compounds such as sideroxylonal and cineole, however, spectral quality is lower than that from dried, powdered samples (Dury et al. 2000, Ebbers et al. 2002). Pioneering studies have demonstrated the possibility of assessing the variation of foliar chemicals from airborne high-resolution spectral data (Youngentob et al. 2012, Cheng et al. 2014, De Jong et al. 2014).

2.3 Diet of Arboreal Folivores

Arboreal mammalian folivores are tree-living animals that specialise in eating leaves from trees. Leaf chemistry has been found to influence their diet (Hume 1999). For example, ring-tailed lemurs in Madagascar frequently used tamarind leaves with higher water and protein (Vaughan et al. 2007) and the common langurs (*Presbytis entellus*) in India feed on leaves with higher protein and lower fibre (Kar-Gupta and Kumar 1994).

In Australia, common ringtail possums (*Pseudocheirus peregrinus*), common brushtail possums, greater gliders (*Petauroides volans*) and koalas have been classified and studied as marsupial folivores of *Eucalyptus* because they are the only marsupial species that can use *Eucalyptus* foliage as their primary food source (Jensen et al. 2014). Marsupial folivores have developed a complicated relationship with *Eucalyptus* vegetation communities. Although these four Australian marsupial species share *Eucalyptus* as the main proportion of their diets, they appear to have different characteristics in terms of their dependence on *Eucalyptus* foliage. It is extensively agreed that koalas and greater gliders rely the most on *Eucalyptus* foliage, ringtail possums are less reliant on *Eucalyptus* foliage whereas brushtail possums are the least reliant (Moore et al. 2004a, Hume 2005). Hence, brushtail possums are known as generalists and ringtail possums, greater gliders and koalas are defined as specialists. However, both possum species can include eucalypt foliage as the major component of their diet under some circumstances (DeGabriel et al. 2009).

2.3.1 Koalas as Specialist Arboreal Folivores

Koalas are one of the iconic fauna endemic to Australia, living along the eastern part of the Australian mainland (Jackson 2008). As specialist arboreal folivores, they have developed a distinct digestive system and behaviour to survive on this low nutrition food source (Hume 2005). Their gut capacity is limited by their body size (6-15 kg) (Moore et al. 2004a, Tyndale-Biscoe 2005). Their leaf-eating characteristics include high crown molar teeth for cutting and breaking the cell walls, an enormous caecum containing bacteria for hindgut fermentation of polysaccharides to volatile fatty acids, 79% resting time to decrease daily metabolic consumption and a long lactation to avoid the peak nutrition demands of reproduction (Smith 1979, Hume 1999, Tyndale-Biscoe 2005). Koalas are known for their highly complex food tree use and feed from about 35 eucalypt species (Hume 1999). The variation in food tree use has been found at subgenus, species, populations and even individual tree levels. From the aspect of chemical ecology, they are using food trees based on foliar chemistry.

2.3.2 Diet of Koalas

Koalas have a highly specialized diet which has been relatively well studied. They mainly feed on a number of *Eucalyptus* species (Moore and Foley 2000, Tyndale-Biscoe 2005), although other eucalypt and non-eucalypt genera are also included occasionally to a smaller extent (Melzer et al. 2014). Among the subgenus of eucalypts, *Symphyomyrtus* makes up the biggest proportion of koala diet, followed by *Eucalyptus* and *Corymbia* (Moore et al. 2004a, Melzer et al. 2014).

At the species level, the variation of koala diet is even higher. It is broadly agreed that only a few species form the majority food source of koala at any locality and the diet composition varies from place to place. In koala studies, the tree species form the largest proportion of koala diet at a location are usually referred as primary food tree species and the tree species have a smaller proportion of koala diet are called secondary food tree species (Seabrook et al. 2014, Callaghan et al. 2011, Law et al. 2017, Phillips and Callaghan 2000). In south Victoria, all-day observations found that koalas mainly eat *E. viminalis, Eucalyptus obliqua,* and *Eucalyptus ovata* (Marsh et al. 2014, Nagy and Martin 1985). In eastern New South Wales, *Eucalyptus punctata* and *Eucalyptus robusta* are reported as primary food tree species for koalas based on all-day observations or faecal cuticle analysis (Matthews et al. 2007, Sluiter et al. 2002). In Queensland, the primary food tree species indicated by faecal cuticle analysis are *E. tereticornis*, *E. robusta*, *E. camaldulensis*, *E. coolabah* and *E. populnea* (Ellis et al. 2002, Tucker et al. 2008, Melzer et al. 2014).

Koalas also show fine scale food use variation amongst individual trees within one *Eucalyptus* species. This can cause severe defoliation of favoured trees but leaving other neighbouring trees untouched (Moore et al. 2004a). The defoliation can cause tree death and the decline of these local folivore populations (Whisson et al. 2016). The variation in food tree use at the individual tree level has been reported for marsupial folivores and its important role in the diets of marsupial folivores is recognized (Lawler et al. 1998, Moore et al. 2004a). For example, a previous study suggested that the significant variation of food intake of the common ringtail possum at tree level, rather than species level, may reflects fine-scale heterogeneity in habitat quality (Lawler et al. 1998).

Seasonal changes of koala diet have been found in Queensland which was suggested to be influenced by nutritional or moisture availability in leaves or requirements of koalas (Davies et al. 2014, Melzer et al. 2014). In southwest Queensland, studies reveal seasonal changes in the proportion in koala diet, with a significant increase in the use of *E. populnea* and decrease in the use of *E. coolabah* under wet conditions (Davies et al. 2014). The development of the *Eucalyptus* diet of koalas involves multiple aspects of folivore-foliage relationships with spatial and temporal variations along sites. Such complicated flora-fauna relationships are affected by foliage chemistry which is challenging to study and not fully understood (Wu et al. 2012, Melzer et al. 2014).

2.3.3 Leaf Chemistry Influencing Food Quality for Koalas

Existing observations on the relationships between koalas and *Eucalyptus* foliage repeatedly confirm that foliar chemistry is a critical factor (Moore et al. 2004a, Ellis et al. 2009, DeGabriel et al. 2010). Koalas appear to have evolved to have a number of strict sequential criteria for foliar chemistry when choosing food and have become highly adapted to eat a narrow range of food sources. Occupying such a narrow dietary niche makes koalas vulnerable to the changes of food quality and availability due to landscape and climatic changes (Youngentob et al. 2011).

The importance of water has been emphasised for marsupial folivores in semi-arid areas, especially in harsh dry conditions (Gordon et al. 1988, Munks et al. 1996). Consuming PSMs increases the water requirements of animals to excrete urinary metabolites of PSMs (Beale et al. 2017). In hot weather, koalas have higher rates of water influx for cooling via evapotranspiration (Ellis et al. 1995). It is likely that foliar moisture fulfils most of koala's water requirement and becomes the first feeding criterion for koalas in the region (Munks et al. 1996). Permanent waterholes have an important role in supporting healthy food trees with a higher leaf water content were important for koala's survival during severe droughts and heatwaves in western Queensland (Gordon et al. 1988, Munks et al. 1996, Wu et al. 2012). In the Mulga Lands of southwest Queensland, foliar moisture varies in eucalypt species and was observed to be positively related to food tree species use of koalas (Wu et al. 2012). However, if leaf moisture cannot meet water requirements, free water including creek/dam water and dew on leaf surfaces overnight may also be significant (Ellis et al. 1995).

Foliar nitrogen has been well studied as a protein indicator because it is especially low in *Eucalyptus* foliage compared to many non-*Eucalyptus* foliage (Moore and Foley 2000). However, most results indicate the relationships between foliar nitrogen and foraging by koalas are either mixed or non-significant. Wallis et al. (2012) showed that total nitrogen failed to explain koala diet because it is the DigN that is nutritionally important. In a recent study, DigN did not influence decision making of tree visits and food tree use of koalas, but was positively related to time spent browsing in a tree when foliar FPC were low (Marsh et al. 2014). In related studies, total nitrogen has not been shown to be as important as other foliar chemicals (Marsh et al. 2014, Stalenberg et al. 2014, Lawler et al. 1998). This indicates that koalas have adapted to a low nitrogen diet but does not exclude the likelihood that low foliar N prevents koalas from using many eucalypt species as food staples. The minimum nitrogen demand of koalas has been calculated to be 1% of *Eucalyptus* foliage dry matter (Cork 1986), which is extremely close to the lower end of the nitrogen range (0.8-2% dry matter) of eucalypt leaves (Moore et al. 2004a).

In contrast, studies have found significant and coherent responses of koalas to foliar FPC concentrations, making FPC the most important foliage chemical in explaining koala dietary variations from subgenus levels to individual tree levels (Marsh et al. 2014, Stalenberg et al. 2014, Lawler et al. 1998). Such dietary variation is related to whether the type and concentration of FPC in leaves fall in the toleration range of koalas. Koalas not only have high tolerance to FPC (50 mg g^{-1} DM) but also accept foliage with moderate FPC (21 mg g^{-1} DM) instead of choosing only the lowest concentrations (Moore et al. 2005, Moore and Foley 2005, Marsh et al. 2007). This explains why they frequently use species of the FPC-rich subgenus *Symphomyrtus* over *Eucalyptus*, despite the latter lacking foliar FPC (Moore et al. 2004a). At individual tree level, the latest evidence suggests that FPC are negatively related to koala tree visit and foraging strategy, leading to intraspecific dietary variations of koala (Marsh et al. 2014).

2.3.4 Measuring Koala Diet

Behavioural observations show that koalas feed at all times of day but predominately at night (Hindell et al. 1985, Tyndale-Biscoe 2005, Marsh et al. 2007), so researchers often use indirect methods for determining koala diet to avoid 24-hour or over-night observations. Hindell et al. (1985) concluded that daytime occupancy of trees was reliable in predicting the food tree use of wild koalas in Victoria. However, day-time use of tree species is usually different from night-time use tree species which are important food tree species for koalas (Marsh et al. 2014, Matthews et al. 2007). The study of Tun (1993) showed that to be unreliable in Queensland and adopted an alternative approach, cuticle analysis, which has been used widely on other herbivores (Pahl 1987, Stewart 1967). This method was latter validated by Ellis et al. (1999) in captive koalas and has been commonly used by subsequent studies (Phillips and Callaghan 2000, Sullivan et al. 2003b, Davies et al. 2014, Melzer et al. 2014).

Cuticle analysis determines the proportion of eucalypt species from koala faecal pellets based on the principle that the appearances of the cuticle of eucalypt species are different enough to be distinguish under the microscope (Tun 1993, Ellis et al. 1999). The cuticle remains of a feeding event are defecated between 1.5-6.5 days after ingestion (Sullivan et al. 2003b). By collecting fresh faecal pellets aged less than one week from the field (bright green shiny with exterior smell present), cuticle analysis can show what the koala ate 1.5-14 days ago from the date of sample collection, including which species were eaten and the proportion of each species. This analysis is not necessarily related to the tree under which the faecal pellets are found (Melzer et al. 2011). A limitation of this method is that it can only reveal koala diet down to tree species level. Within species food tree use of koala still requires tracking and observing individual koalas, which is resource intensive and time consuming (Marsh et al. 2014). Another limitation of this method is that it only works when the cuticle patterns of all eucalypts in the study area are distinctive and can be recognised using a microscope (Ellis et al. 1999). Although there are drawbacks to cuticle analysis, it remains the preferred method to study the diet of low density koala populations, such as the southwest Queensland (Sullivan et al. 2004), because it samples faecal pellets instead of the animal, which is much harder to find.

Current studies attempt to develop a genetic analysis to identify tree species from koala faecal samples, which would be a faster, more accurate and widely applicable method for koala diet analysis (Schultz et al. 2018). A recent study first reported that sequences of *Eucalyptus grandis* can be identified from the koala faecal DNA sequencing products, which was a known food tree species for the sampled koalas (Schultz et al. 2018). This study indicates the possibility of genetically measuring koala diet in the near future.

2.4 Habitat for Arboreal Folivores

Habitat is defined as an area within a larger landscape which can support the survival and reproduction of individual species (Hall et al. 1997. To further categorize habitat at a species or an individual, habitat quality is used as a criterion, which is defined as the fitness of individual living in this type of habitat (Hall et al. 1997). Given the unevenness of habitat quality within a landscape, animals tend to choose and occupy habitats with higher quality to access sufficient resources for survival and reproduction (Hall et al. 1997). Habitat quality can be estimated by measuring the fitness components of species, such as food, water, shelter from predators, resting-sites and venues for social activities. Different species living in different areas within their geographical range can weight these fitness components in different ways. Hence, choosing key fitness components to accurately estimate habitat quality is a major concern for understanding population dynamics and identifying optimal conservation management strategies (Hall et al. 1997, Callaghan et al. 2011).

For arboreal folivores, food resources can outweigh other factors in determining habitat quality which is crucial for species survival (Callaghan et al. 2011). In addition, for many species, primary food tree species provide not only high nutrient food, but also sleeping sites and refuges, such as for the three-toed sloth (*Bradypus variegatus*) and the two-toed sloth (*Choloepus hoffmanni*) (Vaughan et al. 2007). Hence, quality and availability of food trees can be a key indicator of habitat quality for arboreal folivores, such as Mexican mantled howler monkeys (*Alouatta palliata mexicana*), maned sloths (*Bradypus torquatus*) and koalas (Arroyo-Rodriguez et al. 2007, McAlpine et al. 2008, Moore et al. 2010, Callaghan et al. 2011, Cassano et al. 2011).

2.4.1 Koala Habitat and Conservation Status

The current habitat of koala ranges from the *Eucalyptus* forests and woodlands along the eastern seaboard to the semi-arid woodlands of inland regions (Hume 1999). Despite inhabiting multiple biogeographic regions with different vegetation and landforms, koala occurrence are always positively associated with koala’s food tree species, and this relationship is always stronger with primary food tree species than secondary food tree species (Adams-Hosking et al. 2016). Previous studies have shown that the abundance and nutritional value of primary food tree species are essential resources of high quality habitats for koalas (Braithwaite et al. 1984, Kavanagh and Lambert 1990, Lawler et al. 2000, McAlpine et al. 2008). Hence, knowing the nutritional ecology of the koala provides guidelines to assess habitat quality and to develop habitat management strategy for conservation purposes (Moore et al. 2010, Callaghan et al. 2011).

In comparison to historical records in the early 1800's, koala habitat is now shrinking towards the eastern coast and is increasingly fragmented (Tyndale-Biscoe 2005, Gordon et al. 2006, Seabrook et al. 2014). The national population was estimated to have decreased by 33-43% from 1990 to 2010, and the populations of QLD, NSW and the ACT were listed as "Vulnerable" in 2012 (Department of Environment 2014). Koalas are listed as 'Vulnerable' by the IUCN red list due to series of threats including habitat loss and fragmentation, bushfires, disease and drought (Woinarski and Burbidge 2016). Other threats to koalas include road mortality, dog attack and climate change (Lawler et al. 1997, McAlpine et al. 2006, Adams-Hosking et al. 2011). An assessment on the decline in the distribution of koalas in Queensland showed that the contraction of the area of koala occupancy has occurred on the western and northern margins (Gordon et al. 2006).

A study of McAlpine et al. (2008) concluded that habitat factors (e.g. primary food tree species, patch size, soil, road density, etc.) are weighted differently by the models of koala distribution of different regions (south-east Queensland, central coast of New South Wales, and central western Victoria). Hence, it is necessary to develop region-specific conservation management strategies based on scientific knowledge of the key regional factors to support sustainable koala populations. Further, bioclimatic models show that the populations living at the current western margins are likely to be more vulnerable under increasing droughts and heatwaves than the coastal populations (Adams-Hosking et al. 2011). However, the western populations have had less attention than the eastern populations in terms of scientific research and conservation effort (Sullivan et al. 2002, Sullivan et al. 2004, Shumway et al. 2015).

2.4.2 Koala Habitat and Food Tree Species in Southwest Queensland

For western koala populations, the average home range size is 170 ha (± 85 ha) and increases as annual rainfall declines (Davies et al. 2013a). The regional-scale distribution of koalas in southwest Queensland has been found to be positively related to annual rainfall, with the higher densities in the wetter northern and eastern areas (Sullivan et al. 2003b, Seabrook et al. 2011). At the landscape scale, Seabrook et al. (2011) categorized koala habitats according to four types of landform and dominant species:

- riverine *Eucalyptus* woodland,
- floodplain *Eucalyptus* woodland,
- plains *Eucalyptus* and *Acacia* woodland, and
- residual plains *Eucalyptus* woodland.

Riverine habitat supported 45% of the western koala population but covered only 0.9% of the area of the region, whereas the other habitat types were occupied at much lower densities (Sullivan et al. 2004). Hence, the riverine habitat was defined as primary habitat and the other three habitat types (also described as poplar box and mulga woodland communities) were recognized as secondary habitat (Smith et al. 2013c).

Primary and secondary habitats are dominated by primary and secondary *Eucalyptus* food tree species respectively (Sullivan et al. 2003b). Primary riverine habitat is dominated by *E. camaldulensis*, which can make up to 58.6% of koala diet in drier periods (Sullivan et al. 2003b, Davies et al. 2014). This is a tree species that mainly grows alongside drainage lines with surface water and rarely occurs in plains or residual habitats (Sullivan et al. 2003b). Compared to the secondary koala food tree species (*E. coolabah, E. populnea and Eucalyptus thozetiana*), *E. camaldulensis* has significantly higher foliar moisture, which was the foliar component found to be positively related to koala food tree species utilisation (Wu et al. 2012). The importance of watercourses in semi-arid areas for the long-term survival of koalas has also been widely recognised because they form valuable refuge habitat during droughts by providing higher food water and drinking water which are unavailable in the drier floodplain (Gordon et al. 1988, Barboza et al. 2009).

Secondary habitat is dominated by secondary and supplementary koala food tree species including *E. coolabah, E. populnea, E. thozetiana, Eucalyptus ochrophloia, Eucalyptus largiflorens, E. melanophloia, Eucalyptus intertexta* and *Eucalyptus microcarpa* (Seabrook et al. 2011). Among these species, *E. coolabah, E. populnea* and *E. thozetiana* each form more than 10% of koala diet, and they contain less foliar moisture but lower phenolics concentrations than *E. camaldulensis* (Sullivan et al. 2003b, Wu et al. 2012, Davies et al. 2014). Koalas only spend about 20% of time in secondary habitat (Davies et al. 2013a). However, these habitats are still of high value to the animal. For example, the spatial interaction between the amount of primary and secondary habitat in the landscape was far more important than the amount of either individually to the persistence of koalas in this region (Smith et al. 2013c).

2.4.3 Mapping Koala Habitat

Although some koala habitats are protected, areas where koalas occur largely overlap human settlements and they are inevitably being, or will be, exploited and modified by humans (McAlpine et al. 2015). The high variation in food tree use by koalas means that in a landscape not all trees will be of equal value for koalas (Marsh et al. 2014, Youngentob et al. 2012). There is a danger of losing important koala habitat when there are clashes between development and koala conservation

(McAlpine et al. 2015). For instance, the policy of offsetting allows alternative areas to replace the proposed cleared areas and it is likely to replace primary koala habitat with marginal habitat, which will harm koala conservation in the future (McAlpine et al. 2015). If cost-effective methods can be developed that can map koala habitat quality in terms of the chemical landscape that is important for the species, this will be of great interest to conservationists because such maps highlight areas to conserve koalas effectively (Youngentob et al. 2012). Accurate and appropriate mapping is a useful scientific output to be applied to decision making in wildlife management and development planning (Law et al. 2017).

Koala habitat mapping has tended to focus on the eastern koala habitats and the common approach is to rank koala habitat by integrating koala occurrence and vegetation types (Lunney et al. 2000, Scarth et al. 2001, Callaghan et al. 2011, GHD 2009). Although koala sighting data or a landcover map can be accessed from existed database, government authorities or local organisations to determine koala occurrence or habitat type, accurate koala habitat mapping still requires field surveys for ground-truth koala utilisation and vegetation features (GHD 2009). Because *Eucalyptus* tree species varies among regions, it is almost impossible to generalise the current field survey results to a new region. Each mapping study need to conduct field surveys included koala absence/presence and vegetation surveys (Lunney et al. 2000, Callaghan et al. 2011). Koala habitats are ranked by koala densities in addition with vegetation features (e.g. main tree species of patches, patch size, patch fragmentation) (Lunney et al. 1998, GHD 2009). Koala survey methods are mainly koala sighting or faecal pellet searching, with other approaches (e.g. radio tracking, using koala detection dogs) in recent mapping (GHD 2009, Callahan et al. 2011, Environment Protection Authority 2016). The size of area mapped can vary from site scale to regional scale, and so does the mapping methods and source of data. In studies of Lunney et al. (2000) and Callaghan et al. (2011), the ranking of vegetation types was applied on the existing vegetation map to produce a koala habitat map, which covered an area around 900 km^2 with coarse spatial resolution. However, Scarth et al. (2001) used an airborne image to map the individual trees of primary koala food tree species (*E. microcorys* and *Lophostemon conferta*) in an area about 100 km^2. Despite these koala habitat maps being used for local planning (Lunney et al. 2000), it is recognized that incorporating foliar nutritional quality into mapping methods can improve mapping accuracy (Callaghan et al. 2011). Airborne spectra have been shown to be useful for mapping foliar DigN in *Eucalyptus* (Youngentob et al. 2012). However, airborne images are expensive and cover small areas so they are not cost-effective for mapping koala habitat quality at local and regional scales (Zengeya et al. 2013). Hence, there is a need for methods using high spatial resolution satellite images to map *Eucalyptus* foliar chemistry over large areas.

2.5 Conceptual Model

The preceding literature review summarises the background knowledge and research progress on the distribution and determinants of foliar chemistry, diet and habitat of specialist arboreal folivores, and the relationships among them. Using these concepts, I developed a conceptual model (Figure 2.2) to illustrate how leaf chemicals mediate the influence of ecological factors to the diet and habitat occupancy of western koala populations in Queensland.

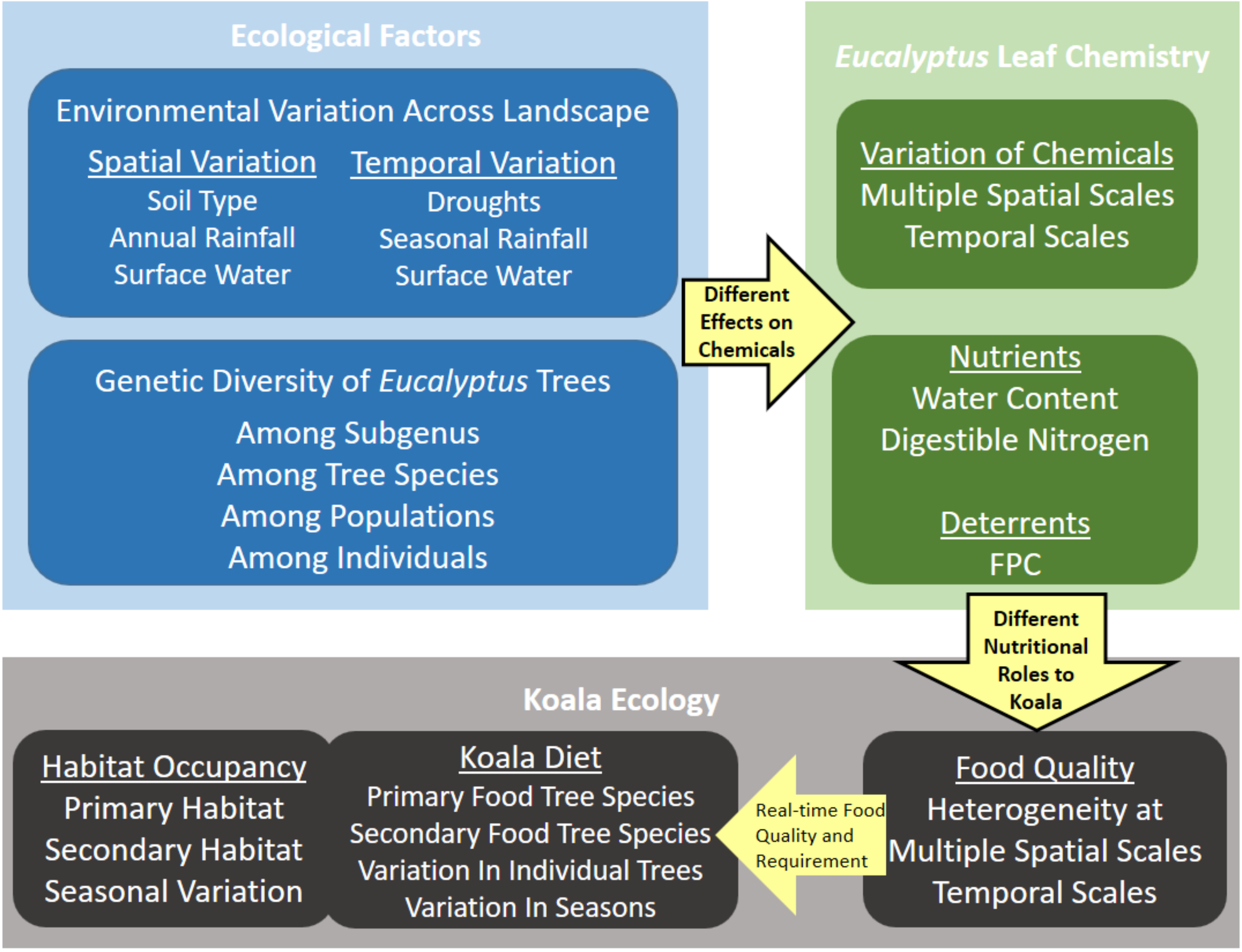

Figure 2.2 Conceptual model illustrating how leaf chemicals mediate the influence of ecological factors to the diet and habitat occupancy of western koala populations in Queensland.

The variation of a foliar chemical can result from the effects of variability in environmental conditions and in tree genotypes (O'Reilly-Wapstra et al. 2005). Different leaf chemicals respond differently to environmental variations and genetic diversity. The variations of different environmental variables can be spatial and temporal, creating spatial patterns of leaf chemical distribution across landscapes and some may change seasonally or annually (Braithwaite et al. 1984, Ellis et al. 1995, Gleadow and Woodrow 2000). The effects of genetic diversity of *Eucalyptus* trees on foliar chemicals can occur at multiple scales, i.e. among subgenus, among tree species, between and within intraspecific populations (Eschler et al. 2000, Moore et al. 2014, Marsh et al. 2017a).

Because different leaf chemicals play a certain role in influencing the nutritional value of tree for koala, the chemical profile of leaves determines the food quality of a tree (Moore et al. 2004a). The spatial and temporal variations of foliar chemistry and consequently the food quality of trees cause heterogeneity in habitat food quality for koalas at temporal scales and at multiple spatial scales (i.e. tree scale, site scale, landscape scale and regional scale) (Lawler et al. 2000, Smith et al. 2013c). Therefore, koalas show temporal food tree use variation from individual trees, tree species and habitat types with higher food quality based on their metabolic requirements and detoxifying ability (Ellis et al. 1995, Krockenberger 2003, Jensen et al. 2014). The occurrence and diet of koalas in a habitat at a time of year is related to a higher food quality and quantity of the area at that time (Smith et al. 2013c, Davies et al. 2014).

Based on the conceptual model, four *a priori* predictions were developed from the existed knowledge of koala-eucalypt interactions. The objectives of this project (see section 1.2) have been designed to test these postulated predictions:

Postulate 1: Foliar moisture and DigN will have positive relationship with water availability in the environment. DigN will also have positive relationship with more fertile soil type. FPC will be largely related to tree species.

Postulate 2: Among koala food tree species, *E. camaldulensis* will be primary food tree species, having highest proportion in koala diet. Secondary food tree species will be *E. coolabah*, *E. populnea* and *E. melanophloia*. In wetter season, the proportion of secondary food tree species will increase due to the change of leaf chemistry.

Postulate 3: Koalas will show more frequent use of trees with higher foliar moisture, moderate FPC, and higher DigN.

Postulate 4: Habitat with higher koala occupancy will be dominated by *E. camaldulensis* and have *Eucalyptus* trees with higher foliar moisture and DigN, moderate FPC, bigger tree size and higher surface water availability.

Chapter 3 Spatial and Temporal Effects of Environmental Factors on Subgenus *Eucalyptus* Leaf Chemistry

3.1 Introduction

In chemical ecology, plant nutrients and PSMs have been widely studied because of their effects on the interactions between herbivores and plant (Bryant et al. 1992, Ali and Agrawal 2012, Rodríguez et al. 2017). The type and amount of plant chemicals affects the foraging behaviour and food quality for herbivores (Kimball et al. 1998, Mertl-Millhollen et al. 2003). Because human-induced environmental modifications can cause spatial and temporal changes in food resources, the dynamics of plant chemicals should be a consideration in the conservation of wildlife species (Adams-Hosking et al. 2012, Forbey et al. 2013). This can be important to specialist species that depend on a narrow range of food sources, such as the koala, giant panda (Nie et al. 2015) and monarch caterpillars *Danaus plexippus* (Zalucki et al. 2001). These species have low dietary plasticity (Williams et al. 2008) and are likely to be threatened when changes of chemical properties in food sources exceed their tolerance to plant chemical defences or reduce nutritional quality (Forbey et al. 2013). Understanding the spatial and temporal variations of foliar nutrition and chemical defences is important for studying the ecological response of specialist species to external change, especially in a seasonally dynamic environment (Forbey et al. 2013, Nie et al. 2015).

The koala is a typical example of a specialist species because it feeds almost exclusively on the leaves of a few *Eucalyptus* species (Shipley et al. 2009). Details of the koala's tolerance of foliar toxins and their food tree use variation driven by foliar nutritional quality was discussed in Chapter 2. The semi-arid regions in the koalas' distribution are often seen to be marginal distribution range because of the effect of climate variability on the quality and availability of forage resources (Lunney et al. 2012, Butt et al. 2013, Duan et al. 2014). Yet to date there is little direct evidence of how leaf chemistry varies temporally among tree species and between seasons in semi-arid environments. The eucalypt communities in semi-arid southwest Queensland provide an ideal case study for understanding the temporal variation in foliar chemical properties that are critical for the fitness and survival of the koalas in this region.

This chapter focuses on Objective 1 of this thesis, which is to identify the spatial and temporal influence of tree characteristics (species, size and condition) and environmental factors (proximity to surface water, short-term rainfall and annual rainfall) on leaf chemistry composition of koala food tree species. I addressed two research questions for Objective 1: 1) how does the leaf chemistry of *Eucalyptus* vary temporally and spatially, and 2) which environmental factors are correlated with these changes? Leaf chemistry in my study included leaf moisture content, DigN and FPC concentrations, because they influence the occurrence and feeding strategy of koalas (Munks et al. 1996, Marsh et al. 2014). I applied a hierarchical sampling design to investigate the effect of spatial and temporal variability in these leaf chemicals for the main koala food tree species in largely semi-arid southwest Queensland. I focused on the influence of temporal variation in rainfall and water availability on leaf chemistry because droughts are one of main threats to koalas in the region (Seabrook et al. 2014).

3.2 Methods

3.2.1 Conceptual Model

I developed a conceptual model to explain how explanatory variables might influence selected leaf chemicals at three spatial scales (tree, site and landscape scales) and across time (sampling interval of six months) (Figure 3.1). In this model, leaf chemicals vary at tree scale in part according to tree species, height, DBH and tree condition. Leaf chemicals varies along tree species (Eschler et al. 2000, Wallis et al. 2010, Munks et al. 1996). The primary food tree species *E. camaldulensis* in the study area (Sullivan et al. 2003) is expected to have higher leaf moisture content (Wu et al. 2012), and higher DigN concentration or lower FPC concentration. Larger tree size (height and DBH) may have higher foliar FPC (Moore and Foley 2005). Better tree condition may have more young or fresh leaves which contain higher DigN but higher FPC (O'Reilly-Wapstra et al. 2007, Marsh et al. 2018).

At the site scale (stands of 6-10 trees), soil fertility and surface water availability potentially influence the chemical concentration of eucalypt foliage. Higher soil fertility (N and K) may lead to higher leaf moisture and DigN, but it may not influence FPC (O'Reilly-Wapstra et al. 2005). Soil pH is important because it can affect the form of nutrients and hence their availability to plants (Binkley and Vitousek 1989). Presence of surface water is expected to provide additional water source for tree surface roots acquiring water and nutrition hence higher foliar moisture and DigN (Mensforth et al. 1994).

At the landscape scale, short-term and inter-annual variations in rainfall can influence the foliar chemicals directly or indirectly via changing surface water availability and underlying soil moisture (root-zone soil moisture, deep soil moisture and deep drainage). Higher rainfall is expected to be associate with presence of surface water and higher soil moisture, and they may cause higher leaf moisture (Mensforth et al. 1994, Merchant et al. 2010).

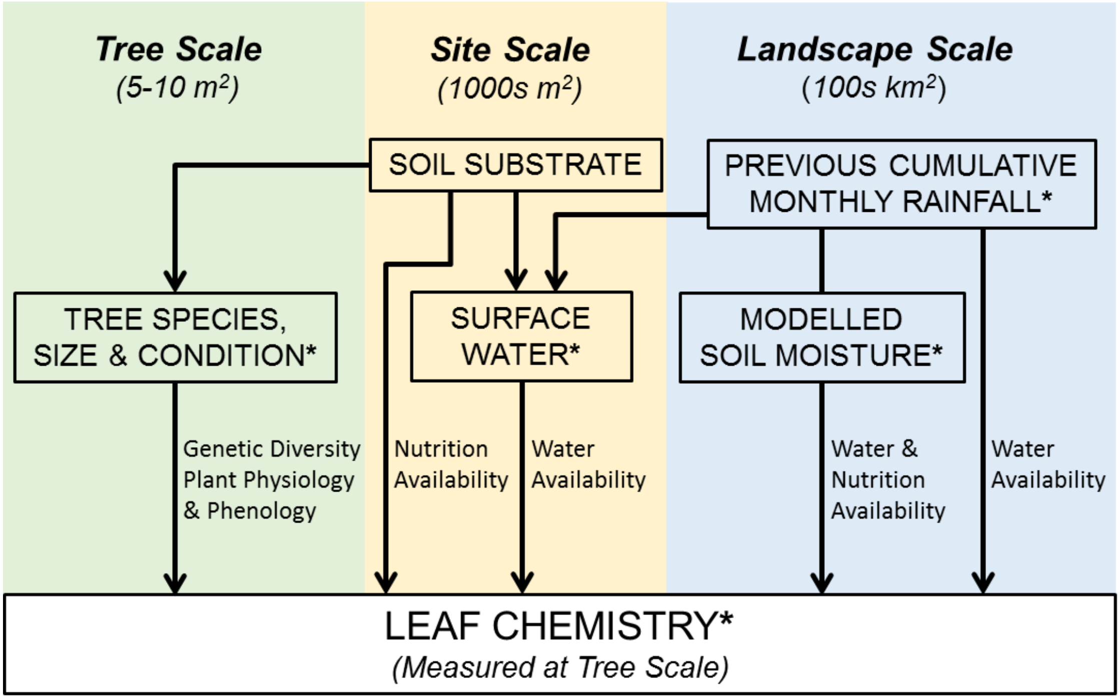

Figure 3.1 Conceptual model of variables influencing eucalypt leaf chemistry (including nutritional components and plant secondary metabolites) at three spatial scales (tree, site and landscape scales). '*' indicates temporal variation.

3.2.2 Survey Design

Ten non-contiguous landscapes (each 10×10 km) were selected along creeks within the study area in different rainfall zones according to the studies of Seabrook et al. (2014) and Sullivan et al. (2003b) (Figure 3.2a). All landscapes were on cattle grazing properties and all surveys were conducted with the property owners' permission.

For each landscape, two on-creek sites (2-7 km apart) were selected (Figure 3.2b). Two corresponding off-creek sites were located between 200 m and 300 m from the creek. Ten trees from each on-creek site and six trees from each off-creek site were marked for measuring leaf chemistry. There are less trees selected at off-creek sites is because the low tree density at off-creek sites caused a lower number of trees available at the site scale (1000s m^2). This caused a bigger sample size of *Eucalyptus camaldulensis* than other *Eucalyptus* tree species. All selected trees had diameters at breast height (DBH) >10 cm following the tree sampling methods in previous koala studies (Phillips et al. 2000, Seabrook et al. 2011, Smith et al. 2013c). On-creek sites were in riparian vegetation dominated by *E. camaldulensis*, whereas off-creek sites were located in woodlands dominated by *Eucalyptus populnea*, *Eucalyptus coolabah* and *Eucalyptus melanophloia*. In total 261 trees were marked and sampled. Selected trees had no mistletoe. Based on the buds of the sampled *E. camaldulensis*, they are of the subspecies *E.* var. *acuminata* at all landscapes. Leaf samples were collected from the lower canopy of the selected trees between 7:00–10:30 am using an extendable pruner or a sling shot. All leaf sample were collected under weather of no rain. Because koalas eat both young and mature foliage (Moore and Foley 2000), leaf samples are expanding and expanded adult leaves (young soft leaves and mature leaves mixed), or epicormic growth when adult leaf absented, to represent the leaves available to koalas. Leaves were collected from healthy stems and cocoons or major galls were excluded. Because rainfall is dominant from November to March (the extended summer), each site was visited before and after this period (Figure 3.2c).

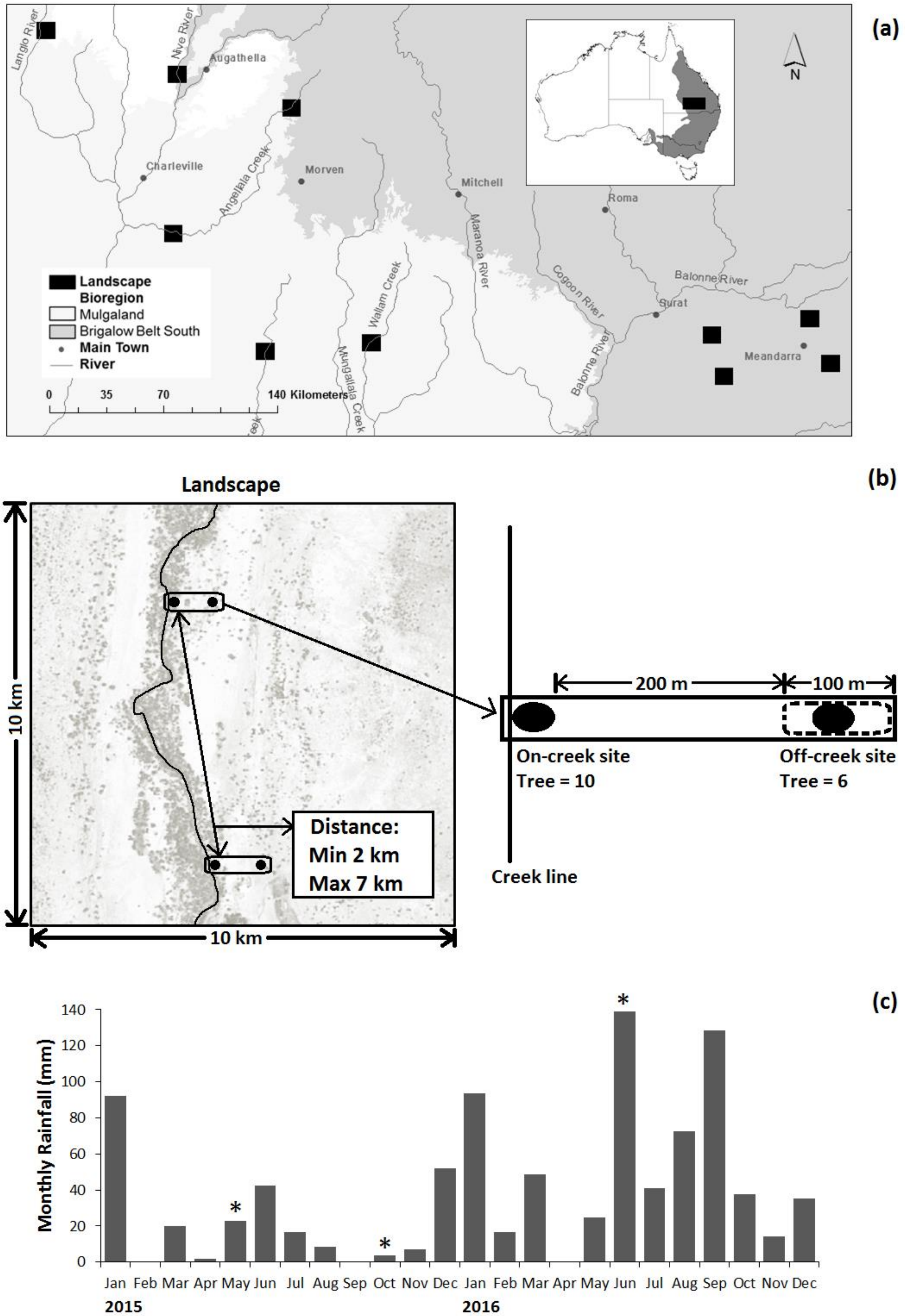

Figure 3.2 Hierarchical sampling design of the project: (a) Distribution of ten landscapes (dark squares) chosen within the Mulga Lands and Brigalow Belt South bioregions. The inserted map shows approximate koala distribution (grey) and location of study area (black). (b) The location of two on-creek sites and two off-creek sites in each landscape. (c) Monthly rainfall at the Charleville weather station for 2015-2016 with asterisk indicating the dates of field sampling.

Based on the conceptual model, a summary of all explanatory variables used for modelling is provided in Table 3.1. Leaf samples and associated explanatory data were collected in May 2015, October 2015 and June 2016. Tree species was recorded. The classes of tree condition was modified from the study of Seabrook et al. (2014) with class 4 (nearly dead tree) removed because there was no sample tree belongs to this class. Tree height was measured by a laser range finder to 0.1 m. Tree DBH of all tree trunk(s) was measured by a DBH tape and the maximum DBH measurement was recorded for a tree.

Surface water includes in-stream water holes and dams. Distance from creek is the distance from the edge of the lower terrace of the 1 or 2 order streams (depends on different landscapes). Old channels were not included. There was no anastomosing system in any landscapes of this study. Surface soil samples (top 0.1 m) were collected with a hand auger (0.1 m) from each site in June 2016 and stored at -20 °C (CF-110ACVERB 106L portable fridge/freezer, WAECO). Roots, leaf litter and stones were removed before laboratory analysis done by the Analytical Services Unit of the School of Agriculture and Food Sciences at the University of Queensland.

For each landscape, cumulative rainfall at different time-lags prior to sampling were calculated from daily rainfall records provided by either property owners or the Bureau of Meteorology (2016). Daily rainfall records were measured to 0.2 mm with a manual rain gauge by property owners or a tipping bucket rain gauge of a weather station. The locations and methods of rainfall measurement are in Appendix 3.1. The averaged soil moisture for different soil layers (root-zone, deep soil and deep drainage) and for different time-lags prior to sampling were calculated from the data extracted from the Australian Landscape Water Balance dataset (Bureau of Meteorology 2017).

Table 3.1 Summary of explanatory variables shown in the conceptual model and subsequently used in the analysis. Variables that vary temporally are indicated by '*'.

Scale	Variable	Description
Tree	Tree species	*Eucalyptus* species for all study sites, 1 = *E. camaldulensis*, 2 = *E. populnea*, 3 = *E. coolabah*, 4 = *E. melanophloia*
	Tree condition*	Ordinal ranking 1-3 classes, where 1 = healthy crown, 2 = minor dead branches, 3 = major dead branch or epicormics growth
	Tree height	Height of tree (m)
	Tree DBH	Diameter (cm) of trunk at about 1.3 m above ground
Site	Surface water*	Visible water, 0 = absence, 1 = presence
	Distance from creek	Distance of a site to nearest creek (m)
	Soil N	Surface soil total nitrogen (% dry matter)
	Soil K	Surface soil total potassium (% dry matter)
	Soil pH	Surface soil pH
Landscape	Previous Rainfall*	Cumulative rainfall at different time-lags pre-sampling (mm). Time-lags include 1-6 months (monthly) and 1-3 years (annually).
	Root-zone soil moisture*	Averaged root-zone soil moisture (0-1 m) at different time-lags pre-sampling (%). Time-lags include 1-6 months (monthly) and 1-3 years (annually).
	Deep soil moisture*	Averaged deep soil moisture (1-6 m) at different time-lags pre-sampling (%). Time-lags include 1-6 months (monthly) and 1-3 years (annually).
	Deep drainage*	Averaged deep drainage (below 6 m) at different time-lags pre-sampling (mm). Time-lags include 1-6 months (monthly) and 1-3 years (annually).
	Bioregion	1 = Mulga Lands, 2 = Brigalow Belt South
Temporal	Season*	0 = Wet season, 1 = Dry season

3.2.3 Leaf Chemistry

Moisture Assays

The measurement of leaf moisture was adjusted based on the method of Ellis et al. (2002). Twenty grams of leaves were collected from each tree and placed in zip lock bags. The wet weight was measured immediately after collection to the nearest 0.01g using an electronic scale (EJ-610, A & D Mercury). The leaf samples were stored in paper bags, kept cool and allowed to air dry in the field.

In the laboratory, samples were oven dried at 43 °C for 5–7 days to a consistent weight. Samples were allowed to equilibrate to room temperature in a desiccator before weighing. Moisture content is represented as (weight loss/wet weight) *100%.

Near-infrared Spectroscopy (NIRS)

NIRS was applied to analyse the DigN and FPC concentrations of the leaf samples in the laboratory. The principle of NIRS was introduced in Section 2.2.5.2 of this thesis. I used this method to reduce the number of samples in the laboratory analysis.

A sample of approximately 50g of leaves were collected from each selected tree and stored in paper bags at -20 °C (CF-110ACVERB 106L portable fridge/freezer, WAECO). Sample preparation followed the method of Wallis and Foley (2003). Samples were freeze dried and ground to pass through a 1-mm screen (ZM200 Retsch®). Leaf samples were then scanned using an NIR spectrophotometer (XDS^{TM} Rapid Content Analyser, FOSS). Based on NIR absorbance data, a subset of 200 samples was selected using the "Select" procedure from the 783 samples as a reference population in WinISI^{TM}. This procedure progressively eliminates spectra with the highest similarity (in a principal component analysis) to other spectra. The spectra were transformed by principal components, then a distance matrix was formed using Mahalanobis distances between all pairs of spectra, and the spectrum with the most neighbours were retained then its neighbours discarded (Shenk and Westerhaus 1991). To obtain the reference values of DigN and total FPC, laboratory analysis was applied to this subset of samples. Calibration equations were derived from the spectra and reference values using modified partial least-squares regression with cross-validation (Shenk and Westerhaus 1991). Equations were developed with a number of different mathematical treatments on raw spectra. Linear regression analysis was performed to test the relationship between predicted concentration and lab chemical concentration for choosing an optimal equation for prediction (Appendix 3.2). Chemical concentrations of the rest of the sample population (n = 583) were estimated (predicted) by the optimal calibration equation (Shenk and Westerhaus 1991).

Digestible Nitrogen

The DigN concentration of the subset 200 leaf samples was calculated using an *in vitro* analysis which is a two-stage *in vitro* digestion with pepsin and cellulase, following the procedure of DeGabriel et al. (2008). For each sample, 800 ± 10 mg of dry leaf were weighed into each of three ANKOM F57 filter bags. Digestions were performed at 37 °C in a shaking incubator. The bags were washed thoroughly then oven dried to constant mass at 70 °C. The nitrogen concentration (% DM) in residue and original leaf samples were quantified using a combustion method with Leco TruMac

CN analyser (Leco Corporation, USA). The concentration of DigN calculated according to the formula:

Digestible Nitrogen (% DM) = $(N_{origin} – N_{residue} / W_{origin}) * 100\%$

where N_{origin} and $N_{residue}$ are nitrogen content (mg of DM) in original leaf and residue. W_{origin} is the dry weight of original leaf sample (mg).

Formylated Phloroglucinol Compounds

The FPC concentration of the subset of 200 leaf samples was determined using high performance liquid chromatography (HPLC) (Wallis et al. 2003). FPC were extracted from 10 mg of leaf sample in 1ml of solvent (7% water in acetonitrile containing 0.1% trifluoroacetic acid and 0.3 g L^{-1} of the internal standard 2-ethylphenol). The diluted extraction was loaded in the Agilent 1290 Infinity Quaternary LC System for HPLC. I measured the peak response at 275 nm. The FPC detected in the samples were quantified by standard curves prepared from purified standard at known amounts.

3.2.4 Statistical Analysis

All data analysis was carried out in R version 3.4.1 (R Project for Statistical Computing, http://www.r-project.org). Continuous explanatory variables were standardized to a mean of 0 and a standard deviation of 1 to enable comparison of effect size. Preliminary univariate modelling was applied to estimate the contribution of each explanatory variable. Spearman's rank correlation coefficient was used to test cross-correlations between explanatory variables. There were strong correlations between surface water and distance from creek ($r = 0.72$), bioregion and 2-year rainfall ($r = 0.69$), bioregion and 3-year root-zone soil moisture ($r = 0.67$), 6-month rainfall and 5-month root-zone soil moisture ($r = 0.72$), 6-month rainfall and season ($r = - 0.82$). To avoid collinearity between explanatory variables in regression, I compared the contribution of the correlated variables by their Akaike Information Criteria (AIC) in univariate modelling and kept the one with lower AIC (Dormann et al. 2013). The explanatory variables retained were surface water, bioregion and 6-month rainfall.

I applied generalized linear mixed modelling to identify the best-fitting models for each leaf chemical response using the 'lme4' package with maximum likelihood estimation (Bates et al. 2014). I used random intercepts on grouping factors 'Site' and 'Tree' with all model combinations. The random intercept models account for the correlation between neighbouring trees within a site and between repeat measurements of a tree (Zuur et al. 2009). The FPC data were zero-inflated, and were modelled using a two-stage hurdle model (Chipeta et al. 2013): the presence-absence of FPC was first modelled then FPC concentration values were modelled as a Gamma distribution with log link. Due to the large number of explanatory variables, fixed effects of full models had six to seven explanatory variables with lower AIC from univariate modelling as well as likely interactions as indicated by prior graphical exploration (Buckley et al. 2003). To select the best fixed effect structure, the likelihood ratio test was applied to determine if a certain fixed effect should be dropped from the full model. The best-fitting model was selected by keeping the fixed effects having a significant influence on the model indicated by likelihood ratio test. Model averaging was applied to the best-fitting model for each leaf chemical to estimate the relative importance of explanatory variables using Bayesian information criterion (BIC) in the 'MuMIn' package (Barton 2017).

I assessed model adequacy using normalized quantile-quantile plots, with a linear relationship between the predicted and fitted residuals of the best-fitting model indicating a good model fit (Landwehr et al. 1984). To test for spatial autocorrelation of the residuals of the most parsimonious models, I created spline correlograms using the 'ncf' package in R (Bjørnstad 2016). Spline correlograms display the spatial correlation using a smoothed spline with 95% confidence intervals calculated by bootstrapping (Bjørnstad and Falck 2001). Splines that are flat and centred on zero demonstrate spatial randomness, (i.e. the data are spatially independent), and splines that are not flat with 95% confidence intervals that do not encapsulate zero show spatial autocorrelation (Bjørnstad and Falck 2001).

3.3 Results

3.3.1 Summary

A total of 261 trees were selected and sampled repeatedly over three field seasons. There were 132 *E. camaldulensis*, 88 *E. populnea*, 28 *E. coolabah* and 13 *E. melanophloia* trees. The mean of three leaf chemicals from the four tree species over three field seasons is summarised in Figure 3.3. One-way ANOVA showed that the difference among the three field seasons was strongly significant for leaf moisture ($P < 0.0001$), not significant for DigN ($P = 0.890$), and significant for FPC ($P = 0.049$). Overall, the mean and standard error of leaf chemicals were 48.8 ± 4.6 % for moisture, 0.98 ± 0.36 % for DigN and 9.95 ± 8.32 mg g^{-1} for FPC.

Raw data and the modelling analysis are available from an online data archive folder of figshare via the link: https://figshare.com/s/b97276822cd7901b9a2e

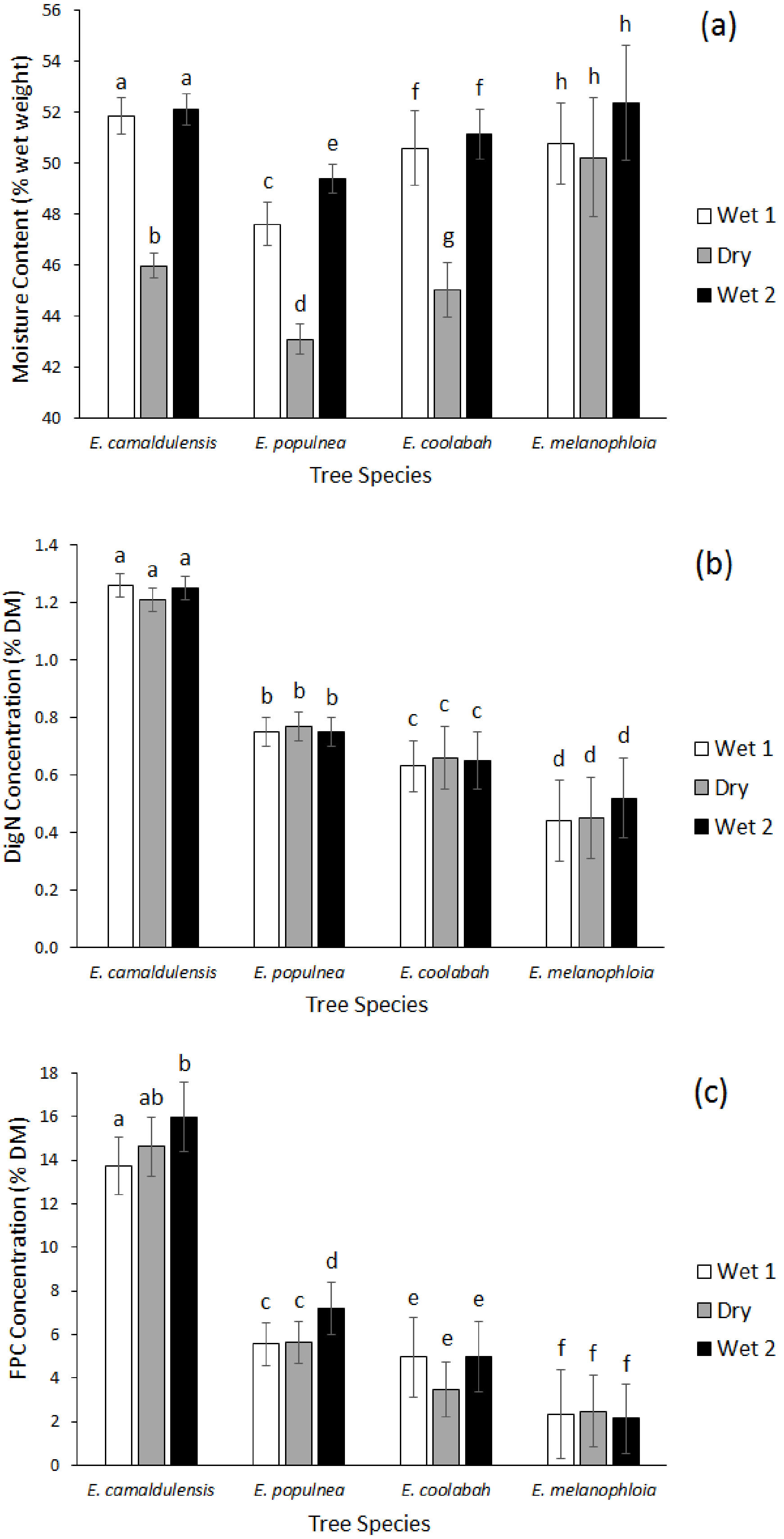

Figure 3.3 Mean with 95% confidence interval of: a) leaf moisture content, b) digestible nitrogen (DigN) concentration, and c) formylated phloroglucinol compounds (FPC) concentration of four *Eucalyptus* species over three field seasons (Wet 1 = May-2015, Dry = October-2015, Wet 2 = June-2016). Different letters within one tree species indicate significant differences between seasons ($P < 0.05$).

3.3.2 Influence of Explanatory Variables

Leaf Moisture

The effect size of the best-fitting generalized linear mixed models for leaf moisture are shown in Figure 3.4a ($R^2 = 0.84$). At the tree scale, *E. camaldulensis* had higher leaf moisture than *E. populnea* throughout the sampling period. Tree height positively influenced leaf moisture for three species, especially for *E. melanophloia*, but not for *E. camaldulensis*. Leaf moisture was higher in trees of poor condition. At the site scale, leaf moisture was significantly higher when surface water was present. The previous 6-month rainfall showed a strong positive effect. Because 6-month rainfall varied among field seasons (t-test, $P < 0.0001$; Appendix 3.3) but not between bioregions (t-test, $P = 0.615$), the effect of 6-month rainfall is temporal. Similar effects were found for 5-month rainfall, 5-month root-zone moisture and dry/wet season (Figure 3.3a) although they were weaker (results not shown). The ranking of the variables according to the Akaike weight showed that tree species, surface water and 6-month rainfall were the most important variables for leaf moisture (Figure 3.4b). Tree condition and the interaction between tree species and tree height were less important.

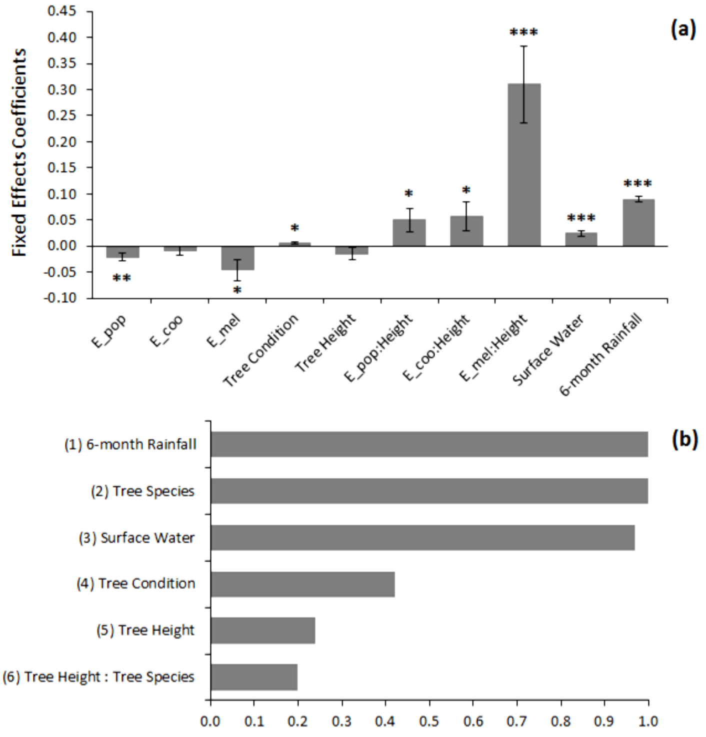

Figure 3.4 Bar charts showing a) the fixed effect coefficient with standard error of explanatory variables and b) relative importance of explanatory variables ranked by the sum of the Akaike weights derived from model averaging for leaf moisture content (%) measured at the tree scale. Note: tree species and surface water are treated as factors, and their coefficient values are relative to the coefficient for the reference species (*E. camaldulensis* and surface water absence). Tree species abbreviations are E_pop = *E. populnea*, E_coo = *E. coolabah* and E_mel = *E. melanophloia*. The asterisks indicate the levels of significance: (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.001$.

Foliar DigN

The effect size of the best-fitting generalized linear mixed models for leaf DigN concentration are shown in Figure 3.5a ($R^2 = 0.91$). At the tree scale, tree species strongly influenced DigN with the highest concentration in *E. camaldulensis*, and lower concentrations in *E. populnea* and *E. coolabah* and *E. melanophloia* in that order. At the site scale, DigN in *E. coolabah* slightly increased as soil nitrogen increased. At the landscape scale, DigN concentrations in *E. camaldulensis*, *E. populnea* and *E. melanophloia* were higher in the Mulga Lands than in Brigalow Belt South, even though the Mulga Lands has a lower mean annual rainfall. The interaction between tree species and 5-month rainfall showed that DigN concentration for *E. camaldulensis* and *E. melanophloia* was positively related to the previous 5-month rainfall. For DigN, tree species was the most important variable followed by 5-month rainfall and bioregion (Figure 3.5b). Soil nitrogen and its interaction with tree species were relatively less important.

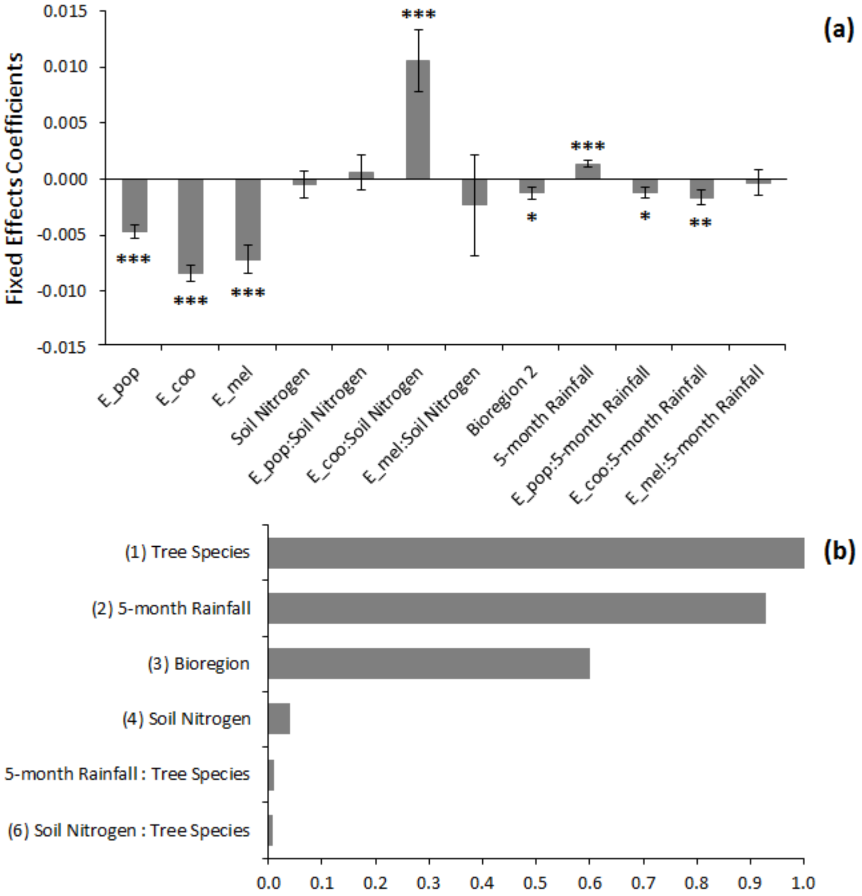

Figure 3.5 Bar charts showing a) the fixed effect coefficient with standard error of explanatory variables and b) relative importance of explanatory variables ranked by the sum of the Akaike weights derived from model averaging for foliar digestible nitrogen concentration (% dry matter) measured at the tree scale. Note: tree species and bioregion are treated as factors, and their coefficient values are relative to the coefficient for the reference species (*E. camaldulensis* and bioregion 'Mulga Lands'). Tree species abbreviations are E_pop = *E. populnea*, E_coo = *E. coolabah* and E_mel = *E. melanophloia*. The asterisks indicate the levels of significance: (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.001$.

Foliar FPC

The effect size of the best-fitting generalized linear mixed models for leaf FPC are shown in Figure 3.6a for FPC presence ($R^2 = 0.35$) and Figure 3.6b for FPC concentrations ($R^2 = 0.86$). Tree species strongly influenced the presence and concentrations of leaf FPC (Figure 3.7). Compared to the other species, *E. camaldulensis* had a higher proportion of trees containing FPC and those trees displayed a high mean and wider range of FPC concentrations (10-30 mg g^{-1}). In contrast, *E. populnea* and *E. coolabah* had fewer trees containing FPC. FPC concentrations for these two tree species were lower and less variable (range 2-13mg g^{-1}) and a greater proportion of *E. coolabah* contained lower FPC. Nearly 40% of *E. melanophloia* had no FPC and the remainder had FPC concentrations below 10 mg g^{-1}. The previous one-month rainfall had a weak positive influence on the non-zero FPC concentrations. The previous one-month rainfall was higher in June-2016 compared to other field seasons (ANOVA, $P < 0.0001$). The variation of previous one-month rainfall was temporal instead of spatial. For FPC, tree species was the only important variable to explain the presence-absence of FPC, whereas tree species and the previous one-month rainfall were important explaining the variation in positive FPC concentrations (Figure 3.6c).

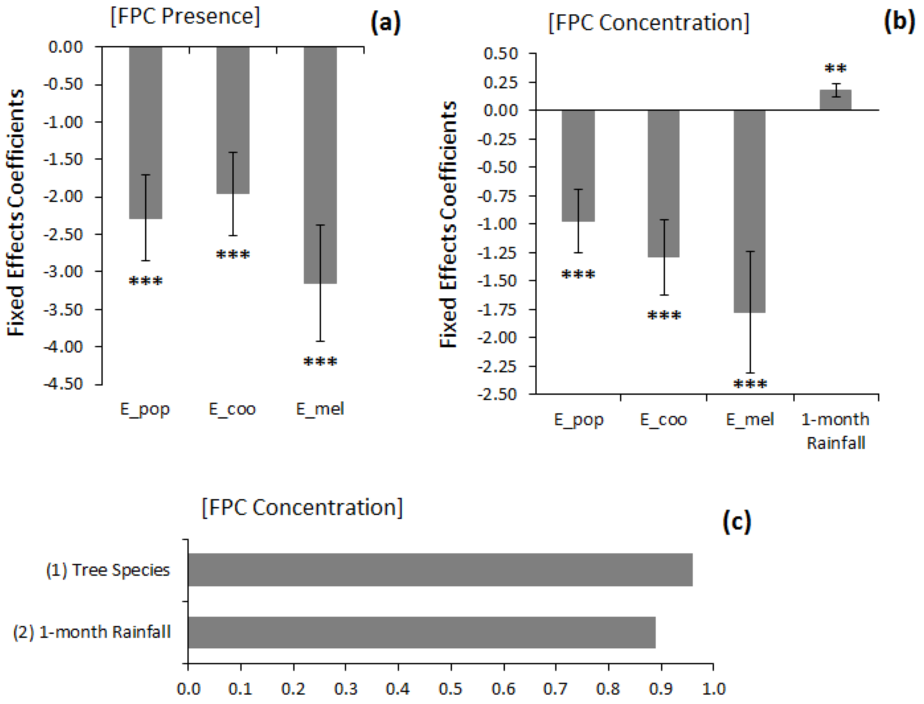

Figure 3.6 Bar charts showing the fixed effect coefficient with standard error of the explanatory variables for foliar formylated phloroglucinol compounds (FPC) measured at the tree scale: (a) presence of FPC and (b) FPC concentration (mg g^{-1} dry matter); and (c) relative importance of explanatory variables ranked by the sum of the Akaike weights derived from model averaging for FPC concentration. Note: tree species is treated as factor, and its coefficient values are relative to the coefficient for the reference species (*E. camaldulensis*). Tree species abbreviations are E_pop = *E. populnea*, E_coo = *E. coolabah* and E_mel = *E. melanophloia*. The asterisks indicate the levels of significance: (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.001$.

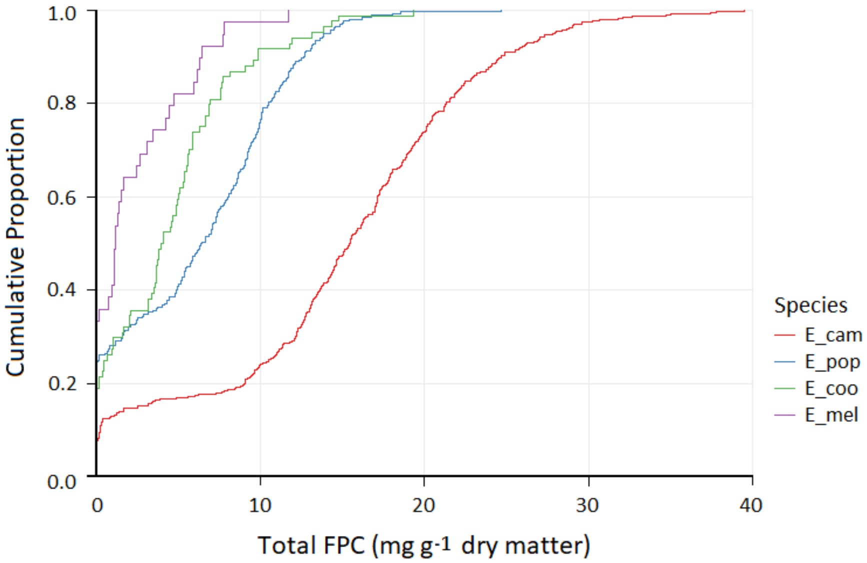

Figure 3.7 The cumulative proportion of leaf formylated phloroglucinol compounds (total FPC) concentration (mg g^{-1} dry matter) in the four target tree species E_cam = *E. camaldulensis*, E_pop = *E. populnea*, E_coo = *E. coolabah* and E_mel = *E. melanophloia*.

3.3.3 Model Fit

The normalized quantile–quantile plots of the best-fitting models for the leaf chemical response variables all showed linear relationships between the predicted residuals and fitted residuals, indicating good model fits (Appendix 3.4). Similarly, the spline correlograms produced for the fitted residuals of the best-fitting models for the leaf chemical response variables respectively showed no significant spatial autocorrelation, indicating the final models adequately explained the spatial variance in leaf chemicals (Appendix 3.5).

3.4 Discussion

Understanding the spatial and temporal variability of nutrients and PSMs in plant communities is one of the most important and challenging steps to link food quality and spatial population dynamics in wild mammals (DeGabriel et al. 2014). Currently, there are very few studies investigating the effect of tree characteristics and environmental factors on the distribution of leaf chemicals at both the spatial and the temporal scale. In this study, I addressed the problem by asking two questions: 1) how does leaf chemistry change temporally? and 2) which environmental factors cause these changes? I addressed these questions for *Eucalyptus* species growing in two semi-arid regions of eastern Australia and over three contrasting seasons. I found that the rainfall within the previous six-months and surface water availability were the primary determinant of leaf moisture and a secondary determinant of leaf DigN and FPC. Tree species strongly influenced all leaf chemicals and it was the key variable explaining the spatial pattern of food quality in a landscape.

3.4.1 Foliar Moisture Content

In my study, the temporally variation of leaf moisture is consistent with the temporally leaf moisture variation of eucalypts in the sub-humid inland and on a wet tropical island in Queensland (Ellis et al. 2002, Clifton et al. 2007). Because of the high correlation between short-term rainfall and root-zone soil moisture, it is likely that short-term rainfall influenced leaf moisture by influencing root-zone soil moisture. However, the effect of short-term root-zone soil moisture was weaker than short-term rainfall in this study. This may be due to the lower data accuracy because soil moisture data was not field measured but modelled by the Australian Water Resource Assessment Landscape (AWRA-L) Model at 5 km spatial resolution using national rainfall and solar radiation data (Bureau of Meteorology 2017). Other reasons for the weaker effect from soil moisture may be the factors influencing soil moisture but were not measured in this study, such as evaporation and evapotranspiration (Dermody et al. 2007), or the presence of palaeochannels in sub-surface (Colloff 2014).

The spatial distribution of leaf moisture was primarily influenced by tree species at the tree scale, surface water at the site scale and rainfall at the landscape scale. The leaf moisture in *E. camaldulensis* was constantly higher than *E. populnea* during the drought and in a wet winter for the same study area (Wu et al. 2012). These main tree species differ in their association with creek lines, and it is uncertain whether the difference was due to tree species or the accessibility to stream water (Mensforth et al. 1994). However, when compared to *E. populnea*, the significantly higher leaf moisture of other floodplain tree species *E. melanophloia* and *E. coolabah* is indicative of the effect of tree species regardless of stream water availability. It may be explained by the finding of the study of Melzer (1995) which found the absolute water content differed among species which affect tree species resilience to dry condition. In addition, the best-fitting model also showed taller trees tend to have higher leaf moisture especially in *E. melanophloia*. Within a landscape, leaf moisture content was higher next to creeks with permanent surface water. Thorburn and Walker (1994) found that *E. camaldulensis* acquired about 50% water from permanent surface water where it was available. Trees that do not have this permanent water source need to rely on groundwater, rain-derived soil moisture and floods. Permanent stream water is an advantage for trees especially during droughts because groundwater alone may not be sufficient for tree use (Catelotti et al. 2015). The spatial distribution of short-term rainfall was highly variable among landscapes but this variation was not related to bioregion, unlike the long-term rainfall. Therefore, leaf moisture is highly variable at the landscape scale regardless of bioregion and is higher in riparian areas especially when surface water is present.

3.4.2 Foliar Digestible Nitrogen

DigN is an integrated measurement of the concentrations and interactions among total nitrogen, tannins and plant cell wall constituents (fibre) and it reflects the leaf nitrogen concentration that can be digested and used by folivores (DeGabriel et al. 2008). DigN differs between subgenera and species of *Eucalyptus*. In my study, tree species was the most important and strongest effect influencing leaf DigN concentration at the tree scale, however DigN did not show significant temporal variation. A previous study compared DigN across 138 *Eucalyptus* species and found higher average DigN in the subgenus *Symphyomyrtus* (0.59% DM) than in other subgenera *Eucalyptus*, *Corymbia* and *Eudesmia* (0.26-0.29% DM) (Wallis et al. 2010). All tree species in my study were from the relatively DigN-rich subgenus *Symphyomyrtus*. In this study, riparian-dominant *E. camaldulensis* had higher DigN than other floodplain species and most other tree species in the studies discussed above. In addition, the spatial distribution of tree species contributed to a higher foliar DigN concentration in riparian habitats than floodplain habitats.

DigN concentration for different tree species also responded in different ways to soil nitrogen, five-month rainfall and bioregion which had weaker effects on DigN in my study. Only DigN in *E. coolabah* showed a positive relationship with soil nitrogen at the site scale. At the landscape scale, DigN in *E. camaldulensis* responded positively to five-month rainfall. Of the two bioregions, DigN concentration of *E. camaldulensis* and *E. populnea* was higher in Mulga Lands. From previous studies, I found four possible reasons for the different DigN patterns within tree species. First, leaf nitrogen increased and tannins decreased in the foliage of *Eucalyptus globulus* seedlings from more fertile soil (O'Reilly-Wapstra et al. 2005). Second, both foliar nitrogen and tannin was higher in young leaves than adult leaves of *E. globulus* and *Eucalyptus viminalis* (McKiernan et al. 2014). Third, epicormic growth of *E. globulus* contained no tannins but higher nitrogen (O'Reilly-Wapstra et al. 2007). Finally, leaf tannin and total nitrogen concentrations are inheritable and can show geographic structure within species (Moore et al. 2004b, Andrew et al. 2005, O'Reilly-Wapstra et al. 2007). In *E. coolabah*, the higher DigN in higher soil nitrogen and Brigalow Belt South is explained primarily by the first reason. The second reason may support the positive response of *E. camaldulensis* to the previous five-month rainfall because the previous two-month cumulative rainfall strongly affects vegetation growth (Malo and Nicholson 1990). However, further studies are required to test if young leaves contain higher DigN than old leaves of *E. camaldulensis*.

3.4.3 Foliar Formylated Phloroglucinol Compounds

FPC include a number of structural groupings such as macrocarpals and sideroxylonals which are highly heritable (Andrew et al. 2005, Freeman et al. 2008). They are common in *Eucalyptus* especially the subgenus *Symphyomyrtus* and have genetic variations among species, among populations and within populations (Eschler et al. 2000). In this study, tree species was the only measured factor determining whether or not a tree had FPC and what concentrations were observed in leaves. Compared to the FPC concentrations in *Eucalyptus* species previously reported (Eschler et al. 2000, Moore and Foley 2005, Youngentob et al. 2011, Mann et al. 2012), *E. camaldulensis* had moderate levels of FPC, whereas *E. populnea* and *E. coolabah* showed low FPC concentrations and *E. melanophloia* contained very low FPC. Similar to DigN, the higher foliar FPC in riparian habitats were explained by the distribution of tree species. In regard to intraspecific variation, trees of all four species could be divided into FPC-present and FPC-absent types. In *E. camaldulensis*, *E. populnea* and *E. coolabah*, these two types co-occurred in most landscapes. This provides further evidence of genetic variation within population which was reported in other *Symphyomyrtus* species (Youngentob et al. 2011, O'Reilly-Wapstra et al. 2013).

Within populations or genotypes, there are several studies investigating the response of leaf FPC to different leaf forms and environmental factors for *E. globulus* and *E. viminalis* (O'Reilly-Wapstra et al. 2007, McKiernan et al. 2012). In my study, FPC showed a positive relationship with recent rainfall (one-month pre-sampling) which increased in the third field season. This variation reflects the significantly higher concentration of foliar FPC in *E. camaldulensis* and *E. populnea* in the third field season in the Mulga Lands. Concentration of FPC was found higher in young leaves than old leaves of *E. globulus* and *E. viminalis* and was not influenced by low water availability (McKiernan et al. 2014). Hence the likely explanation for my finding is that trees may have more new growth in the third field season as the study area experienced heavy rainfall after a four-year drought.

3.5 Summary

This chapter reported the major influences of tree species, short-term rainfall and surface water on the spatial and temporal distribution of leaf moisture, DigN and FPC of the marginal koala distribution range in southwest Queensland. Leaf moisture varied between seasons following short-term rainfall. The riparian habitat dominated by *E. camaldulensis* contained trees with higher moisture content, higher DigN and FPC concentrations. By integrating chemical ecology with landscape ecology, this chapter provided new evidence of the variations of biotic and abiotic factors in the semi-arid habitats which may influence the ecological response of the koala. The strong influence of short-term rainfall on leaf moisture indicates that leaf moisture content can be monitored indirectly via rainfall records to evaluate the influence of drought events on koala habitats. The results of this chapter underpin the next objective of this thesis which links the spatially-complex and varying foliar chemistry with the diet and habitat use of koalas in the same time and areas.

Chapter 4 Influence of Leaf Chemistry and Environmental Factors on Koala Habitat Use and Diet

4.1 Introduction

The koala has a patchy but widespread distribution across its geographic range and most populations are of low density (McAlpine et al. 2015). The presence of koalas is associated with the distribution of frequently use particular eucalypt species of higher nutritional quality, often growing on more fertile soils (Crowther et al. 2009), putting them in conflict with agricultural and urban development in eastern Australia (Woinarski and Burbidge 2016). Most current protected areas are unsuitable for koalas as they occur on infertile soils, and are usually dominated by less frequently used food tree species (McAlpine et al. 2015). Hence identifying the characteristics of suitable habitat is one of the koala conservation priorities for more efficient conservation actions (Lunney et al. 2000, Adams-Hosking et al. 2011).

Vegetation communities, primary food trees species and rainfall are commonly used to predict koala occurrence in both coastal and semi-arid regions (McAlpine et al. 2008, Smith et al. 2013d, Seabrook et al. 2014). Current methods of koala habitat mapping use the distribution of vegetation types and primary food tree species to categorise areas into koala habitat-quality classes (Lunney et al. 2000, Callaghan et al. 2011). However, food nutritional quality is determined primarily by leaf chemistry which reflects the integrative effects of food tree species and genotype, and environmental factors on the nutritional value of individual trees for koalas. Food nutritional quality can be highly variable at a small spatial scale and result in habitat quality variation within areas of the same vegetation type or within a food tree species (Moore et al. 2010). Atmospheric and climatic change are significant new threats to koalas. Climate change is altering rainfall regimes, temperatures, which together with increased atmospheric CO_2 levels, may lower the nutritional quality of foliage over time (Munks et al. 1996, Lawler et al. 1997, Lunney et al. 2012). Therefore, foliar chemistry is an important ecological component, and understanding of foliar chemistry can enhance understanding of koala habitat use across landscapes and may improve mapping of koala habitat quality in the landscapes modified by human and climate change (Moore et al. 2010, Callaghan et al. 2011). However, there are few studies on the response of koalas to spatial and temporal variations in the nutritional and foraging environment at the landscape scale (Moore et al. 2010).

The aim of this chapter is to address Objective 2 of this thesis: Determine the spatial and temporal influence of leaf chemistry, tree characteristics and environmental factors on koala habitat use and diet. Leaf chemistry and environmental data from Chapter 3 were integrated and analysed with data on koala habitat use and diet in this chapter to achieve Objective 2. I use presence/absence of koala faecal pellets to indicate koala habitat use, and the proportional dietary inclusion of tree species derived from koala faecal pellets to represent koala diet composition. The koala population in southwest Queensland is of low density and hence it is practical to identify koala presence and thus habitat use through indirect signs, such as faecal pellets (Sullivan et al. 2002, Rhodes et al. 2011). The presence of koala faecal pellets is not sufficient to separate foraging trees from resting trees, but the analysis of cuticle fragments within faecal pellets is a more effective technique to understand the proportions of food tree species in the koala diet (Melzer et al. 2011).

4.2 Methods

4.2.1 Sampling Design

Sampling consisted of two related activities which were conducted in three seasons (i.e. May and October 2015, June 2016).

First, surveys of koala faecal pellets indicating indirect signs of the presence/absence of koalas at the tree scale were conducted at each study site. I acknowledge the false-negative errors of assigning absence of pellets to absence of koalas (Woosnam-Merchez et al. 2012). However, in a study of Sullivan (2000) for the Mulga Lands, the estimated koala abundance from a faecal pellet survey was only slightly lower than that from direct observation. During my study, pellets were not likely to be affected by running water because creeks were not running or had low stream flow during the sampling seasons. In addition, the faecal pellet survey method is more resource and time-efficient than direct observation for the low-density and scattered koala population of southwest Queensland (Seabrook et al. 2011). Therefore, although faecal pellet survey is not absolutely accurate, it has low false negative errors (Rhodes et al. 2011) and is a practical approach for this study (Munks et al. 1996, Sullivan 2000). In total, there were 261 trees selected from the 34 study sites within the ten landscapes as per Figure 3.2, Chapter 3. The number of trees sampled at each site was 10 for on-creek sites and six for off-creek sites. The purpose of these surveys was to identify which trees koalas were using, and whether tree use was related to leaf chemistry, tree species and size, and environmental factors such as previous rainfall.

Second, searches for koala faecal pellets were conducted along17 transects (10 in Mulga Lands and 7 in Brigalow Belt South) following creek lines (Figure 4.1). The purpose of the transect surveys was to collect fresh faecal pellets for koala diet composition analysis. Transects started from an on-creek site, extended approximately 500 m along the creek and were about 5 m wide. Transects were searched by two persons (one on each side of a creek) for two hours. I only surveyed transects starting at the on-creek sites to maximize the chance of finding fresh koala faecal pellets, given time constraints on searching. Koalas more frequently use riparian habitats rather than the adjacent floodplain habitats, especially during drought conditions (Gordon et al. 1988, Seabrook et al. 2011).

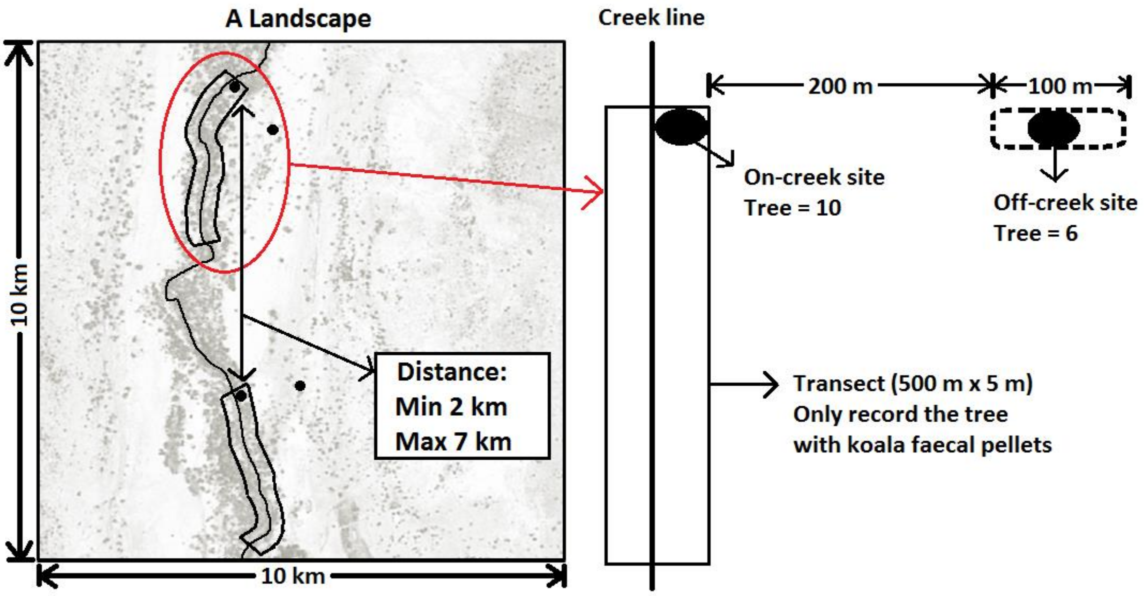

Figure 4.1 Schematic of koala faecal pellet survey conducted for the 34 study sites and 17 transects adjacent to a sub-set of on-creek sites. Information on faecal pellets (absence/presence, age) and tree species were recorded for the 10 trees at each on-creek site, the 6 trees at each off-creek site. Similar information was recorded for any trees with faecal pellets present along transects.

4.2.2 Koala Faecal Pellet Surveys

A koala produces approximately 150 faecal pellets per day, mostly between 6 pm and midnight (Ellis et al. 1998, Sullivan et al. 2004). The presence of koala faecal pellets can indicate the presence of koalas at a location, however its association with a particular tree is not sufficient to represent koala diet (Rhodes et al. 2011, Woosnam-Merchez et al. 2012). At each site, koala faecal pellets were searched for under the whole canopy of each tree (Woosnam-Merchez et al. 2012). Koala faecal pellets were distinguished from pellets of the common brushtail possum, which commonly co-occur with koalas in this region, by their size, shape, smell and colour of the exterior and interior (Triggs 2004). Koala pellets generally have rounded or pointed ends and thick round in the middle while pellets of the brushtail possum are usually uniform along the length (Triggs 2004). Pellets of koala have strong eucalypt odour and finer interior particles, but pellets of brushtail possum have a stinky smell and coarser interior particles (Triggs 2004). If two or more pellets were found closer than 10 cm, they were assigned to a pellet group (Munks et al. 1996). The usual pellet group size was about 15-30. The presence/ absence and group age of faecal pellets were recorded for each tree. The group age of koala faecal pellets was estimated according to the criteria in Table 4.1.

Table 4.1 Criteria for the identification of group age of koala faecal pellets (data derived from Witt and Pahl 1995).

Age	Description
I	Fresh samples, exterior smell present, colour bright green and generally shiny. Age less than 1 week.
II	Lack exterior odour but retained an interior smell when crushed, dark green or brown in colour. Age less than 1 month but older than 1 week.
III	No smell when crushed but intact and greyish green/brown. Age greater than 1 month.

4.2.3 Faecal Sampling for Koala Diet Analysis

For each 500 m transect, only fresh koala faecal pellets (age I-II) were searched for under the whole canopy of trees. Where a fresh pellet or a group of pellets were found, group size, group age, date of collection, tree position and tree species were recorded. Fresh pellet groups were collected and stored at -20 °C (CF-110ACVERB 106L portable fridge/freezer, WAECO) in brown paper bags (for old and dry pellets) or in zip-lock plastic bags with 75% ethanol (for fresh or wet pellets).

In the laboratory, koala diet was determined using cuticle analysis (Ellis et al. 1999) from the samples collected in the field. Cuticle analysis compares cuticle reference slides from fresh leaves of specific tree species with slides of the leaf fragments obtained from koala faecal pellets (Wu et al. 2012).

The preparation of cuticle reference slides was conducted following Tun (1993). Three young and three mature expanded adult leaves of common tree species and shrubs were collected from each landscape, cut into small pieces (1.5cm x1.0cm) and fixed in 100% ethanol at room temperature. Leaf segments were heated gently (heater or water bath) in hydrogen peroxide: acetic acid (6:1, v:v) solution until the cuticle separated from the mesophyll. Both the upper (adaxial) and lower (abaxial) cuticle layers of leaf segments were cleaned, stained with aqueous gentian violet and mounted on slides with glycerine. Reference slides were examined under a light microscope at x10, x20 and x40 magnifications and photos were taken for each tree species as a reference.

Up to ten pellets from each group were used to make faecal fragment slides following the protocol of Witt and Pahl (1995) and Tun (1993). Pellets were soaked and washed in water to obtain a suspension containing leaf fragments from the faecal pellets. Leaf fragments were then bleached in 4% sodium hypochlorite until white, stained with aqueous gentian violet and mounted on slides with glycerine. Three replicate slides were made for each pellet group (Sullivan et al. 2003b). Each slide contained approximately 150 leaf fragments. From each slide, the tree species of the first 100 fragments (with appropriate size for identifiable cuticle pattern and guard cells of stomata) counted were identified under the microscope at x20 magnification and cross-referenced with the reference slides (Sullivan et al. 2003a). To avoid double counting, each slide was analysed using a systematic traverse (Ellis et al. 1999). The averaged frequency of a tree species from the three replicate slides was recorded as its respective proportion in a pellet group.

4.2.4 Statistical Analysis of Koala Faecal Presence

The presence/absence of koala faecal pellets for the 261 trees surveyed was modelled using binomial generalised linear models. Pellet presence/absence was modelled as a function of foliar moisture content, DigN and FPC concentrations, and the explanatory variables listed in Chapter 3 (Table 3.1). All data analyses were conducted in R version 3.4.1 (R Project for Statistical Computing, http://www.r-project.org).

Continuous explanatory variables were standardized to a mean of 0 and a standard deviation of 1 to enable comparison of the effect size. Exploratory univariate modelling was applied to estimate the contribution of each explanatory variable. Spearman's rank correlation was used to test for pairwise correlations between explanatory variables. There were strong correlations between tree species and foliar DigN ($r = -0.72$), and between 6-month rainfall and foliar moisture ($r = -0.61$). After comparing the contribution of the correlated variables in the univariate modelling, foliar DigN and moisture were retained because they had lower univariate AIC values (Dormann et al. 2013).

I applied generalized linear mixed modelling to select the best-fitting models for koala faecal presence using the 'lme4' package with maximum likelihood estimation (Bates et al. 2014). Random intercepts of the grouping factors 'Site' and 'Tree' were used for all model combinations. The random intercept models account for the correlation between neighbouring trees within a site and between repeat measurements of a tree (Zuur et al. 2009). Fixed effect combinations of the full models included six or seven explanatory variables and potential interactions. These explanatory variables were selected from variables with lower AICs in the univariate modelling. To select the best fixed effect structure, the likelihood ratio test was applied to determine if a certain fixed effect should be dropped from the full model. The best-fitting model was selected by keeping the fixed effects having a significant effect on the model fit as indicated by the likelihood ratio test. Model averaging was then applied to the best-fitting model for each leaf chemical to estimate the relative importance of explanatory variables using BIC in the ‘MuMIn’ package (Barton 2017). To test for spatial autocorrelation of the residuals of the most parsimonious model, I created spline correlograms using the ‘ncf’ package in R (Bjørnstad 2016).

4.3 Results

4.3.1 Summary

In total, 261 trees were searched for koala faecal pellets in three field seasons. There were 109 records of koala faecal pellet groups (age I-III) which were analysed in the mixed effect model. A total of 25 fresh koala faecal pellet groups (age I-II) were collected for koala diet analysis across the three field seasons (Figure 4.2). Only four on-creek sites from three landscapes had koala faecal pellets present in all three field seasons. More faecal pellet groups were found in landscapes in the Brigalow Belt South compared to the Mulga Lands.

Raw data are accessible online via this link: https://figshare.com/s/b97276822cd7901b9a2e

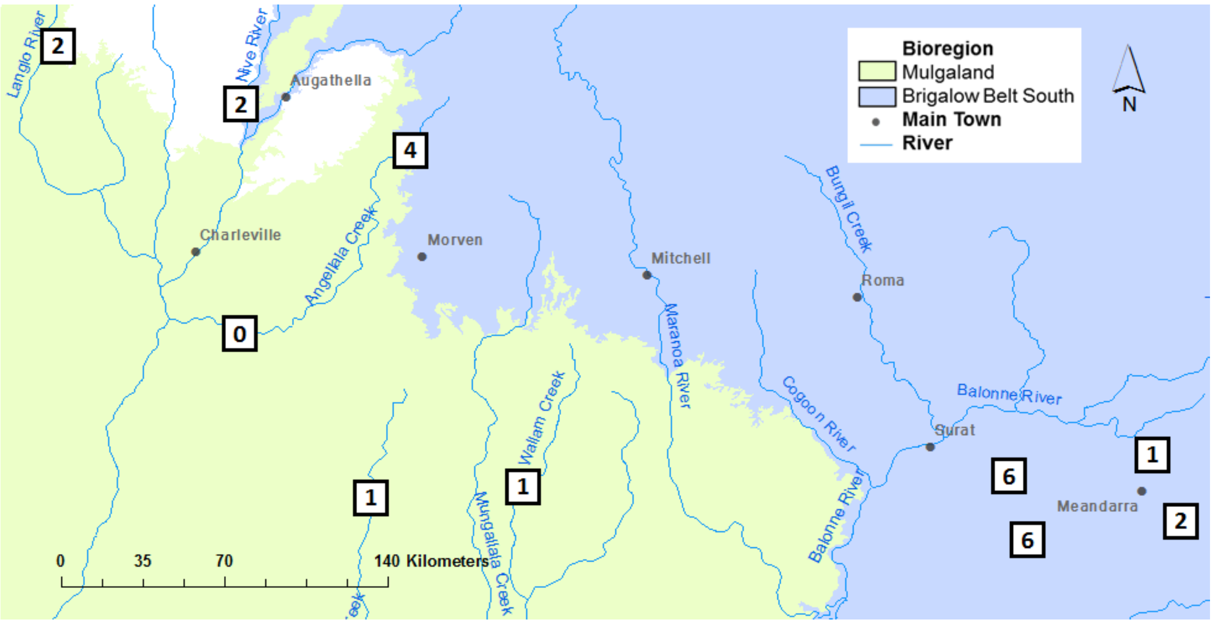

Figure 4.2 The total number of fresh koala faecal pellet groups collected from each landscape over three field seasons.

4.3.2 Average Foliar Chemicals in the Sites Used by Koalas

All sites surveyed over three field seasons were categorised into three koala use types: no koala use (faecal pellet absence, n = 70), previous koala use (faecal age II or III, n = 11) and current koala use (faecal age I, n = 21). Figure 4.3 shows the average foliar chemistry for sites with each use type in the three field seasons. The foliar moisture content of the current koala use sites was higher than sites without koala use in the wet season but not in the dry season (Figure 4.3a). Only in wet seasons, foliar DigN concentrations of the sites with current and previous koala use were higher than the sites without koala use (Figure 4.3b). Sites with current and previous koala use always had higher foliar FPC concentrations than the sites without koala use (Figure 4.3c).

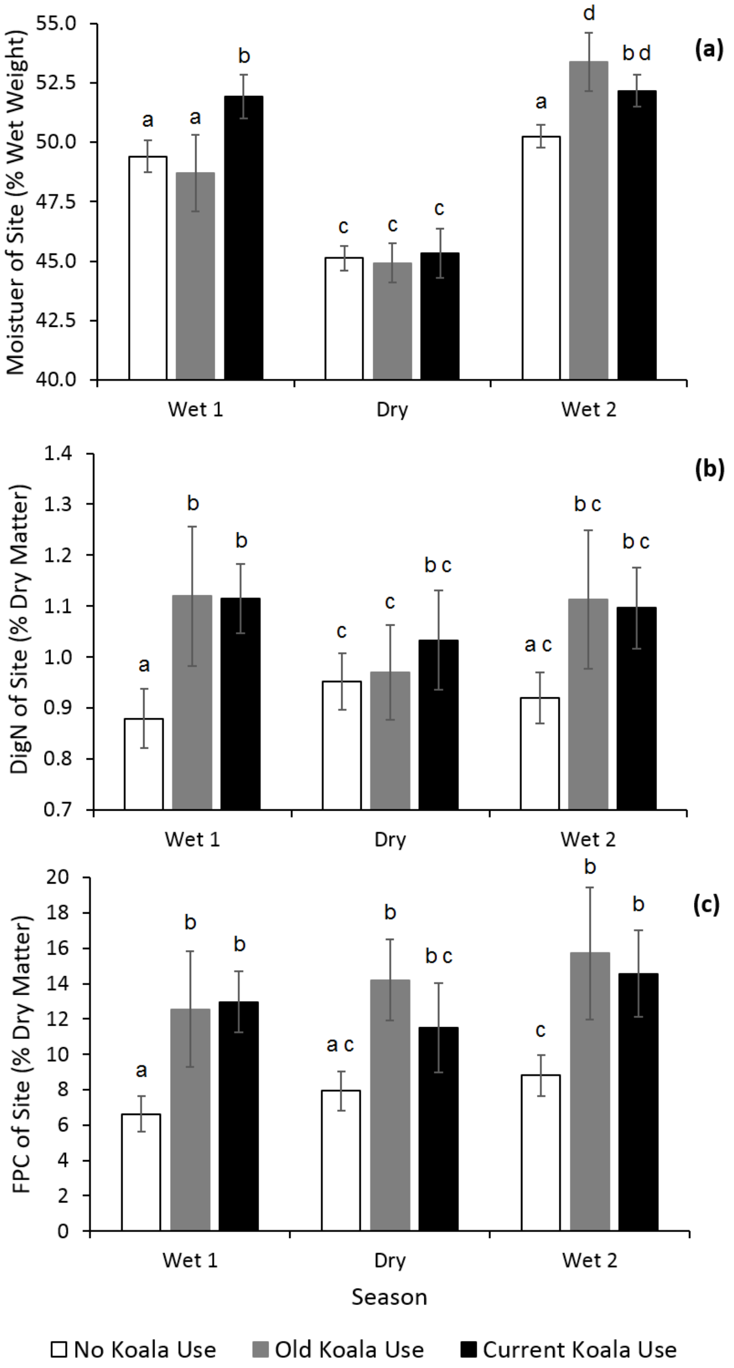

Figure 4.3 Mean with 95% confidence interval of: a) leaf moisture content, b) digestible nitrogen (DigN) concentration, and c) formylated phloroglucinol compounds (FPC) concentration of three koala use types over three field seasons (Wet 1 = May-2015, Dry = October-2015, Wet 2 = June-2016). Different letters indicate significant differences (*P* < 0.05) among letter groups and same letters indicate values that are not significantly different from each other.

4.3.3 Probability of Koala Faecal Presence

The effect sizes of the variables in the best-fitting generalized linear mixed model ($R^2 = 0.44$) for koala faecal presence at the tree scale are shown in Figure 4.4a. Trees with larger DBH and higher foliar DigN concentration had a higher probability of the presence of koala faecal pellets. The average of the previous 3-year root-zone soil moisture measured at the landscape scale had a positive effect. The 3-year root-zone soil moisture varied between bioregions (Brigalow Belt South > Mulga Lands; t-test, $P < 0.0001$) and among field seasons (May-2015 > Jun-2016 > Oct-2015; ANOVA, $P < 0.0001$). The ranking of the variables according to their Akaike weight showed that tree DBH, was the most important variable for koala faecal presence (Figure 4.4b). Foliar DigN concentration and 3-year root-zone soil moisture were less important.

The spline correlograms produced for the fitted residuals of the best-fitting model for koala faecal presence showed no significant spatial autocorrelation, indicating the model adequately explained the spatial variance in the response variable (Appendix 4.1).

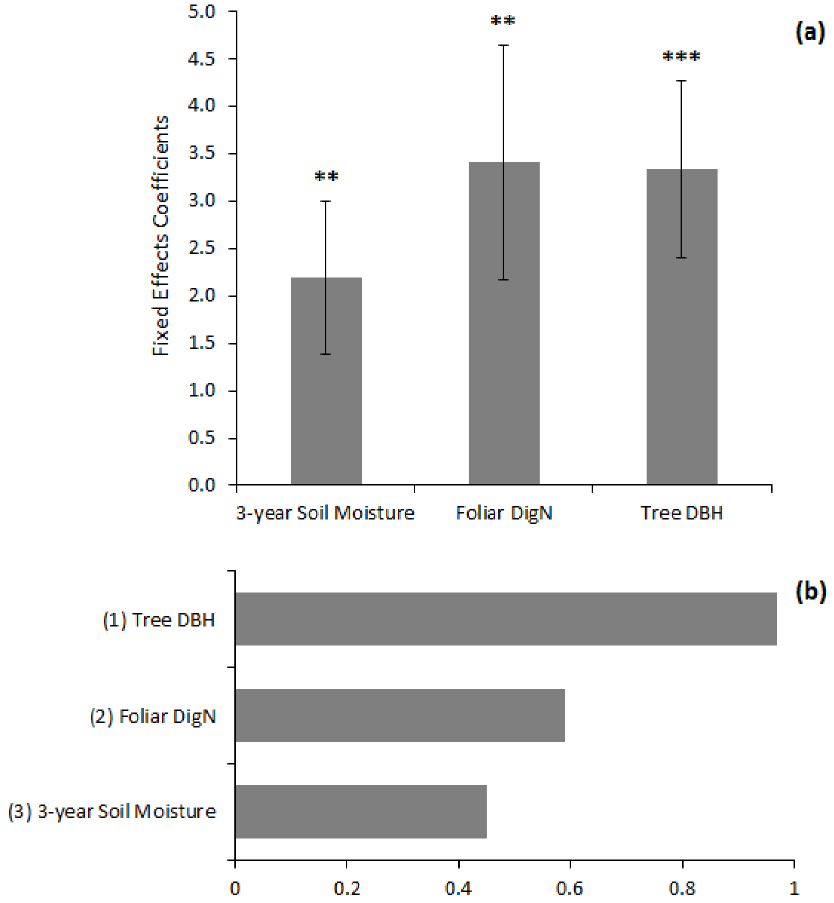

Figure 4.4 Bar charts showing a) the fixed effect coefficient with standard error of standardised explanatory variables and b) relative importance of explanatory variables ranked by the sum of the Akaike weights derived from model averaging for the koala faecal pellet presence measured at the tree scale. Three-year soil moisture is the averaged root-zone soil moisture of three years pre-sampling. The asterisks indicate the levels of significance: (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.001$.

4.3.4 Koala Diet

Koala diet composition data were collected at the transect scale across three field seasons. Unfortunately, the small sample size of fresh koala faecal pellet groups (n = 25) did not allow us to model the relationship between koala diet composition and the variables of foliar chemistry and the environmental factors. As an alternative, the variation of koala diet compositions was explored using t-test (two tails) and the correlation between koala diet compositions and the three foliar chemical variables.

The proportion of *Eucalyptus camaldulensis* in koala diets was higher than other tree species in all landscapes for all field seasons (t (48) = 8.68, $P < 0.0001$). When *Eucalyptus coolabah* was absent from the landscape, the proportion of *Eucalyptus populnea* and *Eucalyptus melanophloia* were not significantly different (t (38) = 1.60, $P = 0.118$) but they were both higher (t (22) = 5.85, $P < 0.0001$ for *E. populnea*; t (25) = 5.36, $P < 0.0001$ for *E. coolabah*) than other tree species in the koala diets (Figure 4.5a, b). The average proportions of *E. camaldulensis* were similar between the two bioregions (t (17) = -1.13, $P = 0.275$). In wet seasons (May 2015 and June 2016), *E. populnea* was more frequently used over *E. melanophloia* (t (15) = 2.25, $P = 0.040$) whereas the consumption of *E. melanophloia* increased in the dry season (t (23) = -2.10, $P = 0.047$). When *E. coolabah* was present in the landscape (Figure 4.5c), this species was eaten a bigger amount by koalas than *E. populnea* (t (5) = 8.24, $P < 0.001$) and *E. melanophloia* (t (8) = 7.14, $P < 0.0001$). The proportion of *E. coolabah* in koala diets was higher in the dry season than in the two wet seasons (t (2) = 10.98, $P = 0.008$). Apart from these four main tree species, I also identified three other eucalypt species (*Eucalyptus thozetiana*, *Eucalyptus ochrophloia* and *Eucalyptus crebra*) from koala faecal pellets which were also reported in the study of Sullivan et al. (2003b).

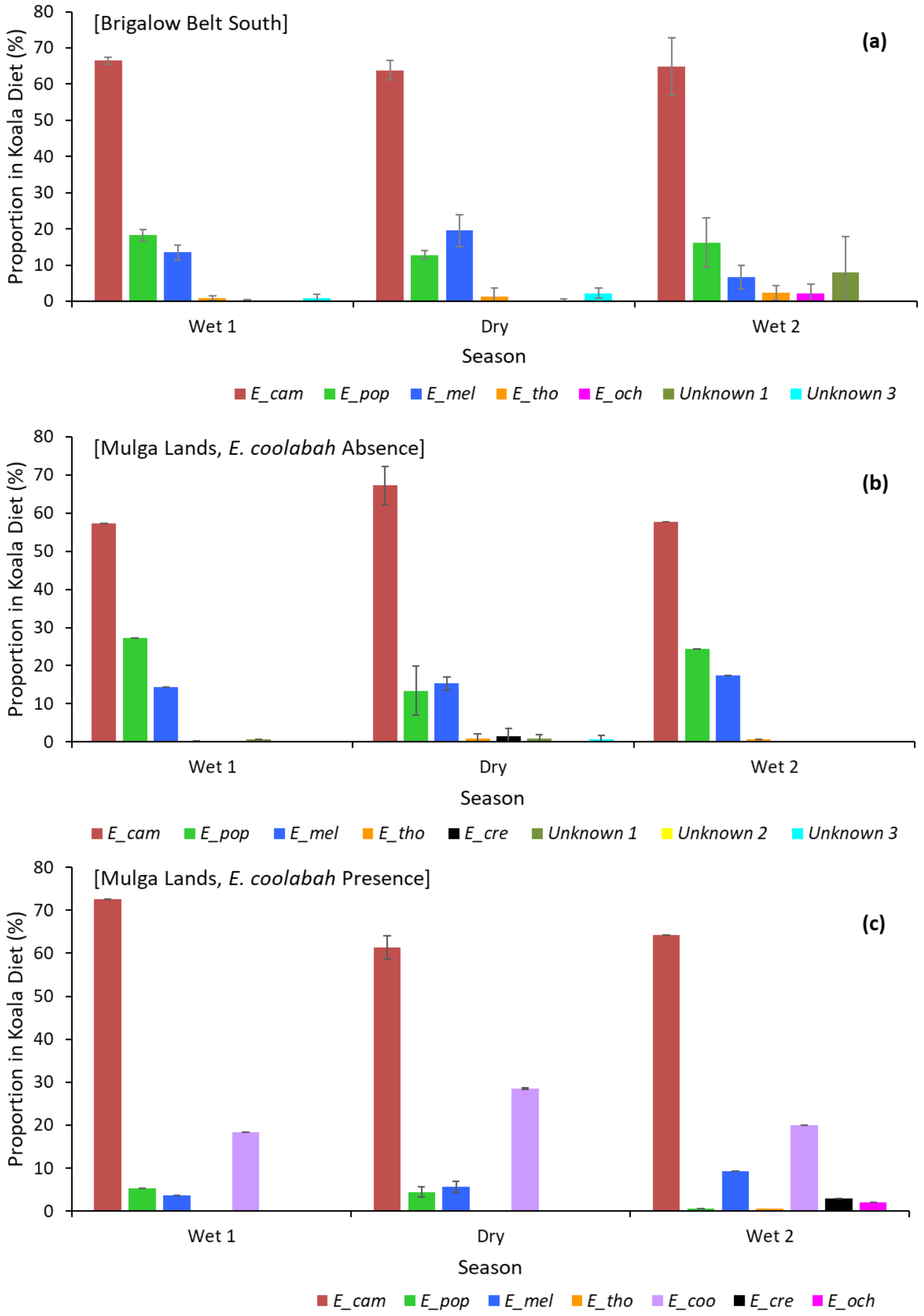

Figure 4.5 Mean with 95% confidence interval of the proportion of tree species in koala diet over three field seasons (Wet 1 = May-2015, Dry = October-2015, Wet 2 = June-2016) for the landscapes of a) Brigalow Belt South, b) Mulga Lands with *Eucalyptus coolabah* and c) Mulga Lands without *E. coolabah*. Tree species abbreviations are *E_cam* = *E. camaldulensis*, *E_pop* = *E. populnea*, *E_mel* = *E. melanophloia*, *E_tho* = *E. thozetiana*, *E_cre* = *E. crebra*, *E_och* = *E. ochrophloia*, *E_coo* = *E. coolabah* and three unknown species which could not be identified.

For each study site, the values of leaf moisture, DigN and FPC of all trees were averaged respectively to represent the overall leaf chemical concentrations of site. The correlations between the average foliar chemical concentrations and the tree species' proportions in koala diets at the same study site were evaluated. Average foliar moisture content had a weak negative correlation with the proportion of *E. melanophloia* (Figure 4.6a). Average foliar DigN concentration had a weak positive correlation with the proportion of *E. populnea* (Figure 4.6b).

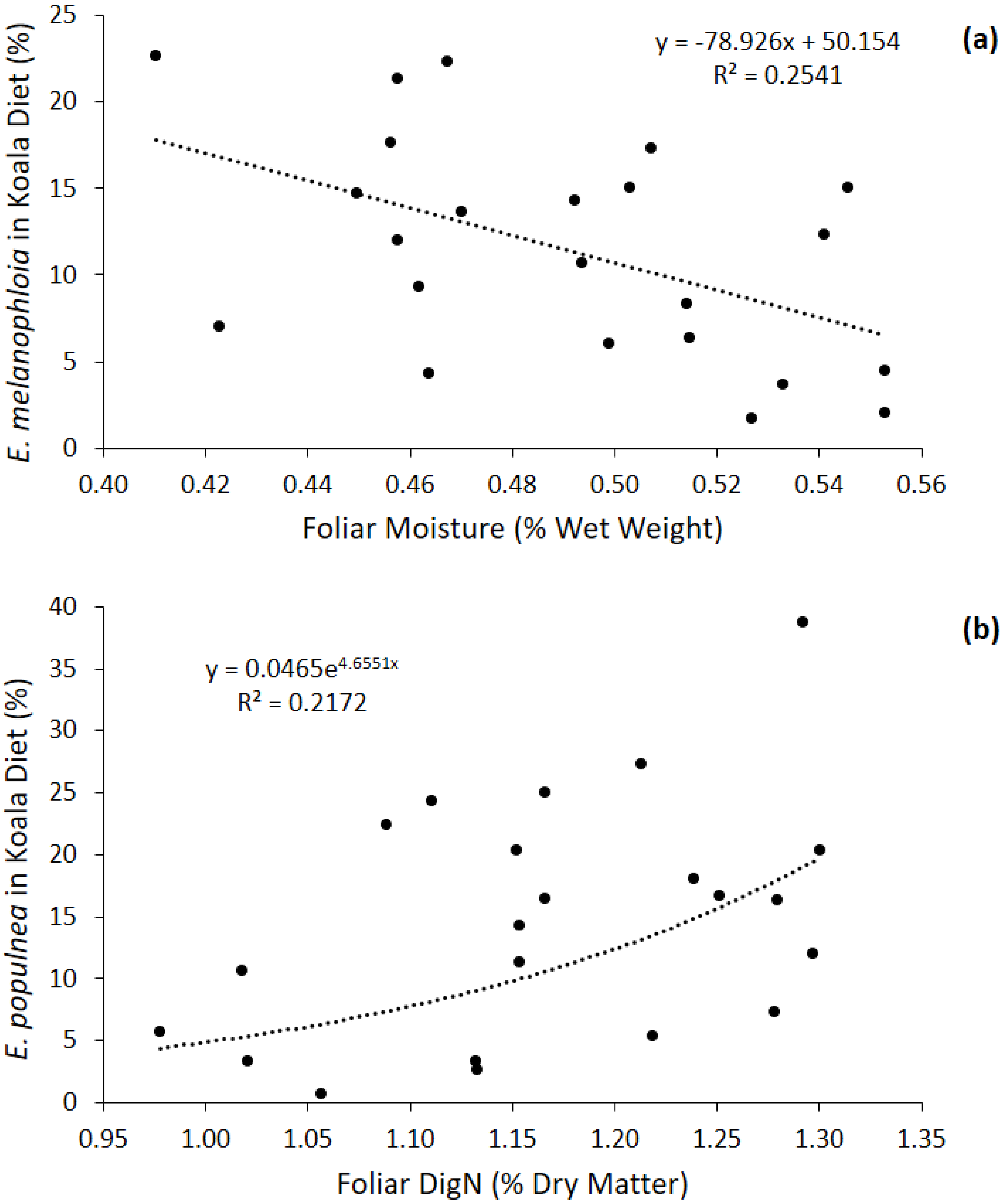

Figure 4.6 Regression analysis showing the relationships between a) foliar moisture content (averaged at the site scale) and the proportion of *E. melanophloia* and b) between foliar digestible nitrogen (DigN, averaged at the site scale) concentration and the proportion of *E. populnea.*

4.4 Discussion

Understanding the response of koalas in semi-arid habitats to variations in foliar chemistry of different *Eucalyptus* trees, and to abiotic factors such as soil moisture in semi-arid habitats is a key focus of this thesis. This chapter contributes to previous studies of this koala population (Sullivan et al. 2003b, Smith et al. 2013c, Davies et al. 2014, Seabrook et al. 2014) by providing insights into the nutritional ecology of koalas in semi-arid regions.

For Objective 2 of this thesis, the analysis presented in this chapter identified the relative influence of leaf chemistry, tree characteristics and environmental factors on koala habitat use and diet. Koala tree use was approximately presented as the presence of koala faecal pellets at the tree scale and it was positively related to tree DBH, foliar DigN concentration and long-term soil moisture level. Among the foliar chemical properties of trees used by koalas, it was leaf moisture content that varied most between seasons. Koala diet was determined from the tree species composition of faecal pellets. *E. camaldulensis* was the primary food tree species for this koala population across all landscapes and seasons. *E. coolabah* was eaten more over *E. populnea* and *E. melanophloia*. Temporal variation in the use of *E. populnea* and *E. melanophloia* was related to foliar chemicals.

Variation in foliar chemistry was related to the occurrence of koalas and the patterns of koala diet composition, including primary food tree species use and temporal variation in secondary food tree species identity.

4.4.1 Presence of Koalas

A number of studies of the koala population in southwest Queensland have investigated the spatial pattern of koala distribution, and estimated the influence of tree characteristics and environmental factors on koala habitat use (Sullivan et al. 2003a, Smith et al. 2013b, Seabrook et al. 2014). In my study, foliar chemistry was added to explain variation in the presence/absence of koalas. Tree DBH, foliar DigN concentration and long-term soil moisture each had a positive influence on koala presence in this study. These environmental factors are consistent with those identified by previous studies: tree species, tree height, tree DBH and rainfall (Sullivan et al. 2003a, Smith et al. 2013b, Seabrook et al. 2014).

Chapter 3 demonstrated that tree species is the most important factor influencing foliar DigN concentration. In the models of koala faecal presence, foliar DigN showed better performance than tree species in explaining the variation in the presence of koala faeces at the tree scale. Hence a potential reason for koalas frequently use *E. camaldulensis* is that this tree species has a higher foliar DigN concentration. In addition, *E. camaldulensis* had higher leaf moisture than the floodplain species *E. populnea*. This is consistent with the inference by Smith et al. (2013c) that koalas frequently use *E. camaldulensis* in the Mulga Lands due to its higher foliar moisture and nutrients. The foliar chemistry of *E. camaldulensis* explains why it has been recognized as koalas' primary food tree species and riparian habitat as primary habitat by previous studies (Sullivan et al. 2003a, Smith et al. 2013c, Seabrook et al. 2014).

Tree DBH and foliar DigN explain koala habitat use within a landscape. The more frequently use of greater DBH trees was found in previous studies and in my study. The same finding was reported for the coastal koala populations in southeast Australia by koala faecal survey and radio-tracking (Phillips and Callaghan 2000, Matthews et al. 2007). Trees of bigger size (larger DBH) were considered to be more associated with shelter from predators and shade from the summer heat rather than to foraging (Moore and Foley 2005, Matthews et al. 2007). The importance of large trees highlights the need to preserve mature and old-growth trees for koala habitat management (Matthews et al. 2007).

Long-term soil moisture explains possibility of koala occurrence within a region. The effect of long-term soil moisture found in this study is consistent with the positive influence of average rainfall reported by Seabrook et al. (2014) and Sullivan et al. (2003a) because long-term soil moisture is associated with long-term rainfall. Another factor, bioregion, had high correlation with long-term soil moisture and showed similar effect on koala faecal presence. Brigalow Belt South had a higher chance of having koalas than the Mulga Lands which is consistent with the study of Davies et al. (2013a). One possible reason is that Brigalow Belt South has higher long-term winter rainfall and hence a smaller fluctuation of water availability between dry/wet seasons (Appendix 3.2). Another potential reason is the lower summer mean temperature (1961-1990) in Brigalow Belt South (24-27 °C) than in Mulga Lands (27-30 °C) (Bureau of Meteorology 2016), which may cause lower heat stress for koalas.

Foliar FPC concentration was not included in the best-fitting model for koala faecal presence. The average FPC levels in the sites used by koalas, which were dominated by primary food tree species *E. camaldulensis*, were higher than those without koala use across seasons. This may associate with koala's tolerance to FPC as discussed in Section 2.3.3 and the limitations of the koala survey in this study. The study of Marsh et al. (2014) found koalas eat more from trees with higher foliar DigN and lower foliar FPC. However, neither the presence of koala faecal pellets nor koala diet composition can determine how much koala ate from a particular tree. Although foliar FPC had high intraspecific variation at the tree scale, my study methods were not able to measure the influence of foliar FPC on koala food tree use. This requires methods that can estimate koala food intake at the tree scale such as high-temporal frequency radio and GPS tracking, direct observation through 24 hours, or acoustic telemetry (Marsh et al. 2014).

In wet seasons, the average foliar moisture content, DigN and FPC concentrations of sites used by koalas were higher than those of sites not used by koalas. However, in the dry season, the average leaf moisture content did not differ between sites with or without koala use. This potentially explains why leaf moisture content was not important in the model of koala faecal presence at the tree scale. In the sites with fresh koala faecal pellets, average leaf moisture content decreased in the dry season whereas the average foliar DigN and FPC concentrations were more stable between dry/wet seasons. Higher physiological stress in koalas represented by faecal cortisol metabolite levels was associated with lower short-term rainfall in southwest Queensland (Davies et al. 2013b). The influence of variability in short-term rainfall on leaf moisture supports the inference of Davies et al. (2013b) that the influence of rainfall on koala stress level is mediated by leaf moisture in semi-arid regions. It is possible that leaf moisture content is a limiting factor for koalas in winter. However, the water demand of koalas in dry and wet seasons maybe different due to various requirements of thermoregulation, energy production and metabolism of koalas. In central Queensland, the water influx rate, indicating water requirement, of free-ranging male koalas was higher in summer (Ellis et al. 1995). A number of studies provided evidence that free-ranging male and female koalas had higher water requirements in summer but higher energy requirements in winter (Ellis et al. 1995, Krockenberger 2003, Clifton et al. 2007, Ellis et al. 2010). However, free water appeared to supplement water intake for koalas in winter (Ellis et al. 1995) and the water influx rates of captive koalas were higher in winter when drinking water was available (Degabriele et al. 1978). Although leaf moisture is an important source of water for koalas, they may also obtain water from dew on leaves, creeks or dams, and metabolic water as supplement. To test if leaf moisture is limiting for koalas in semi-arid regions, further research is required to assess the adequacy of leaf moisture for koalas under different weather conditions, and the dependency of koalas on free water in various combinations of leaf moisture levels and weather conditions.

4.4.2 Temporal Variation in Koala Diet

Studies of the tree species composition in koala diet of the riparian communities in these semi-arid regions confirms that *E. camaldulensis* is the main food species and can make up of above 50% koala diet regardless of rainfall conditions (Sullivan et al. 2003b, Wu et al. 2012, Davies et al. 2014). Although the sample size of my koala diet analysis was small, the composition of *E. camaldulensis* (63.9% ± 6.0%) indicates its role as dominant food tree species across landscapes and seasons. The dietary proportion of this species was 58.6% ± 8.1% from the study of Davies et al. (2014) on the same koala population. The annual rainfall of 2009 (345 mm, Charleville weather station) as the "Drought" condition in the study of Davies et al. (2014) was higher than the annual rainfall in 2015 (267 mm) in this study (Bureau of Meteorology 2016). In a koala population crash during droughts and heatwaves recorded in southwest Queensland, koalas survived well in the healthy *E. camaldulensis* woodlands adjacent to permanent waterholes (Gordon et al. 1988). Hence the drier the conditions, the more koalas rely on habitats with water and healthy *E. camaldulensis* to survive the droughts and heatwaves.

Apart from *E. camaldulensis*, *E. coolabah* form bigger diet proportion than *E. populnea* and *E. melanophloia* when it was available in the landscape. In Brigalow Belt South and Mulga Lands, *E. coolabah* can co-occur with *E. camaldulensis* in riparian woodlands and on the adjacent floodplains (Sullivan et al. 2003b). As seen in Chapter 3, *E. coolabah* has a higher leaf moisture than *E. populnea* and higher DigN than *E. melanophloia*. The higher diet proportion of *E. coolabah* may also reflect its proximity to riparian habitats and more suitable foliar chemistry for koalas.

There were temporal variations in the consumption of *E. populnea* and *E. melanophloia*. In wet seasons, koalas ate more *E. populnea* than *E. melanophloia*. Similarly, an increased proportion of *E. populnea* in koala diets under wetter conditions was found in the study of Davies et al. (2014). However, the proportion of *E. melanophloia* in koala diets increased in the dry season. The correlations between leaf chemistry and the two tree species indicated that the proportion of *E. populnea* increased with higher average foliar DigN concentration of sites, and the proportion of *E. melanophloia* increased when site average leaf moisture decreased. This has not been reported in other studies. Due to the small sample size of koala diet in this study, future research is required to investigate these relationship with a larger sample size and expanded to seasons with above-average rainfall.

Davies et al. (2014) suggested that the higher proportion of *E. populnea* in wetter conditions was due to the higher nutritional value (lower phenolics and higher total nitrogen) in this species. However, this study revealed a medium nutritional value for *E. populnea* and it was significantly lower than *E. camaldulensis* regardless of previous rainfall conditions. Koalas' increased use of *E. populnea* in wetter conditions may be driven by different reasons such as breeding and dispersal. In dry areas, higher short-term rainfall causes the increases of mobility and home range size of only male koalas, which reflects breeding or dispersal movements (Davies et al. 2013a). This emphasises the importance of using DigN to measure nitrogen value in *Eucalyptus* and it can be misleading to estimate and compare leaf nitrogen value using total nitrogen and phenolics. Phenolics concentration is not a measure of their nitrogen binding effects because many existing methods treat the extraction containing different phenolics as the same although they have different reaction with the reagent and diverse biological effects (DeGabriel et al. 2009).

4.5 Summary

This chapter identified the important leaf chemicals and environmental factors that associate to the diet and habitat use of koalas living in southwest Queensland. Variations in foliar chemistry were related to the occurrence of koalas and the patterns of koala diet compositions including the significant higher use of primary food tree species and the temporal variations in secondary food tree species. A higher DigN of foliage is associated with the presence of koalas in a landscape based on pellet presence. The outstanding nutritional value but high leaf toxin concentration of *E. camaldulensis* as primary food tree species provided important insights into the ecological significance of riparian and non-riparian habitats to koalas found in previous studies. That is, riparian habitats function as primary habitat and as refugia in droughts, and non-riparian habitats serve as secondary and supplementary habitats in wet seasons. This study highlights the importance of protecting riparian habitats and the adjacent floodplains from the aspect of leaf chemicals and food quality dynamics. Leaf moisture contents of koala food trees fluctuated significantly between seasons whereas the values of foliar DigN and FPC were relatively stable across seasons. This indicates a need to verify the limiting effect of leaf moisture for koalas in semi-arid areas in future research. An important implication from this chapter is that high foliar DigN can indicate important koala habitats in a landscape. This finding at the tree scale is then used for the third objective to try to map foliar DigN at the landscape scale using multispectral remote sensing from a new satellite, which can potentially be applied to koala conservation.

Chapter 5 Estimating Foliar Digestible Nitrogen Using WorldView-3 Multispectral Data

5.1 Introduction

Mapping and monitoring the habitat quality of threatened species at the landscape and regional scale ($\geq$100s km^2) is crucial for developing effective conservation management (Callaghan et al. 2011, Lunney et al. 1998, GHD 2009, Environment Protection Authority 2016). This is especially important for koalas because their distribution at the landscape and regional scale is determined largely by the occurrence of nutrient-rich foliage of food tree species (Moore et al. 2004a, McAlpine et al. 2006). Current published mapping of koala habitat quality relies mainly on vegetation community mapping, especially the relative proportion of primary food tree species (Callaghan et al. 2011). Incorporating leaf nutritional information is one of the suggested enhancements to this method (Callaghan et al. 2011) because of a patchy distribution of forage nutrients across large areas found in this study and previous studies (Munks et al. 1996, Moore et al. 2010). For most folivores species, studies use total nitrogen (N) or protein-to-fibre ratio to estimate plant nutritional value (Mertl-Millhollen et al. 2006, Simmen et al. 2012). However, foliar DigN better reflects the nutritional value than N in tannin-rich food (Wallis et al. 2010) and it was found strongly influences koala feeding decision (Marsh et al. 2014). In addition, higher foliar DigN was found to associate with high quality koala habitats in Chapter 4. Mapping DigN, therefore, would be suitable for the study of koala habitat distribution because tannins are widely distributed in eucalypt species as chemical defences (DeGabriel et al. 2008).

However, assessing foliar nutrients over large areas using field-sampling and chemical analysis requires a very large sample size and is expensive (Govender et al. 2008, Youngentob et al. 2012, Eitel et al. 2007). Remote sensing applications offer new opportunities to address this problem by providing proximal data for estimating plant chemicals (Adjorlolo et al. 2014). An ideal remote sensing data source for mapping foliar DigN would have adequate spatial resolution to provide individual tree spectra, an appropriate spectral response to provide sensitive indices and a relatively low cost. It is encouraging that foliar DigN was accurately modelled by the airborne hyperspectral data in the Australian temperate eucalypt woodlands in a recent study (Youngentob et al. 2012). However, mapping large areas using airborne remote sensing is generally more expensive compared to satellite remote sensing (Zengeya et al. 2013). There is a need to find satellite-derived data that have high spatial resolution and can provide sensitive spectral indices of foliar DigN.

No studies mapping DigN with satellite remote sensing were found during a literature search, but a wide range of studies have used satellite-derived data (from WorldView-2, SPOT, Hyperion, Aster and IKONOS) to map N in crops and trees (Reyniers and Vrindts 2006, Bagheri et al. 2013, Boegh et al. 2013, Adjorlolo et al. 2014, Zandler et al. 2015). These studies tested the correlation between N and the potential spectral indices, which spectral transformation of two or more spectral bands contain absorption features of N (e.g. 850 nm) or chlorophyll (550 nm, 670 nm, 700 nm) (Herrmann et al. 2010). Several suitable spectral indices have been found for mapping N using satellite multispectral data, such as the modified triangle vegetation index 2 (Bagheri et al. 2013, Adjorlolo et al. 2014). Testing potential spectral indices may be suitable to identify appropriate spectral indices for estimating and mapping DigN. Yet the requirement of high spatial resolution of mapping foliar DigN narrows the range of suitable sensors. For example, the spatial resolution of Hyperion (30 m), Aster (15 m) and SPOT (10 m) are not adequate to provide pure tree pixel for individual trees with crown diameter around 5-10 m in this study area. In terms of spectral response, most satellite sensors have only visible and near-infrared bands which may restrict their mapping performance on the target in interest having absorption features beyond these bands. Foliar N is highly correlated with spectral indices in the shortwave infrared region using field hyperspectral data (Martin et al. 2008, Herrmann et al. 2010). DigN is affected by the compositions of N, tannins and fiber in leaves which have important absorbtion features in the shortwave infrared region (Youngentob et al. 2012). A new multispectral sensor WorldView-3 (WV3) was launched in 2014 to collect eight shortwave infrared (SWIR) bands at 3.2 m spatial resolution, in addition to the eight multispectral bands in the visible and near-infrared (VNIR) bands (at 1.2 m spatial resolution). The higher spatial resolution compared to other space-borne sensors and the eight SWIR bands of WV3 offers an opportunity to map foliar DigN, in eucalypt trees used by koalas. For example, the SWIR bands of WV3 contain nitrogen absorption features at 1645, 2180, 2240, 2300 and 2350 nm (Curran 1989, Youngentob et al. 2012), tannins and lignin absorption features (1658,1675 and 1668 nm), absorption features of cellulose (2280 nm) and lignin (2272 nm) (Curran 1989, Soukupova et al. 2002).

This chapter is focused on Objective 3 of this thesis: Explore the use of WorldView-3 satellite imagery to accurately map foliar nutrition at high resolution in koala habitats in semi-arid regions. The study area is suitable but also challenging for testing spectral mapping methods because it has varied tree species and soil types. This chapter addressed two research questions: 1) does the WV3 sensor provide better spectral indices to estimate plant N when including SWIR bands? and 2) is the WV3 data able to accurately estimate and map plant DigN? This study attempted to identify an appropriate DigN mapping index following the existing methods of mapping N using satellite multispectral data. Hence WV3 data were used to map both foliar N and DigN to compare the index performance with that in previous studies to test consistency. Mapping foliar DigN in koala habitat was the primary focus and goal of this chapter.

5.2 Methods

5.2.1 Study Areas and Survey Design

Two landscapes (Landscape A and Landscape B) located ~200 km apart were chosen from the ten landscapes in this thesis (Figure 5.1). The main landforms are riparian strips dominated by *Eucalyptus camaldulensis*, *Eucalyptus populnea* dominated floodplains and *Acacia aneura* or *Acacia harpophylla* dominated plains (Figure 5.2). The riparian areas and floodplains in Landscape B also have *Eucalyptus coolabah* and some *Eucalyptus melanophloia*.

Each landscape had four sample sites and 32 eucalypt trees that were selected in Chapter 3 (see Figure 3.2b). There are 64 trees in total, including 35 *E. camaldulensis*, seven *E. coolabah* and 21 *E. populnea* and one *E. melanophloia*. Both field sampling and WV3 image acquisition occurred in October and November 2015, which is the late dry season. From October 2012 to November 2015, average annual rainfall was 342 mm in Landscape A and 317 mm in Landscape B. That was much lower than the average annual rainfall (400-600 mm) and a severe rainfall deficit was reported for this period (Bureau of Meteorology 2016). Ground cover was senescent in the dry season which made it easier to distinguish tree canopy from ground cover such as grass. There also was a higher chance of having cloud-free satellite images.

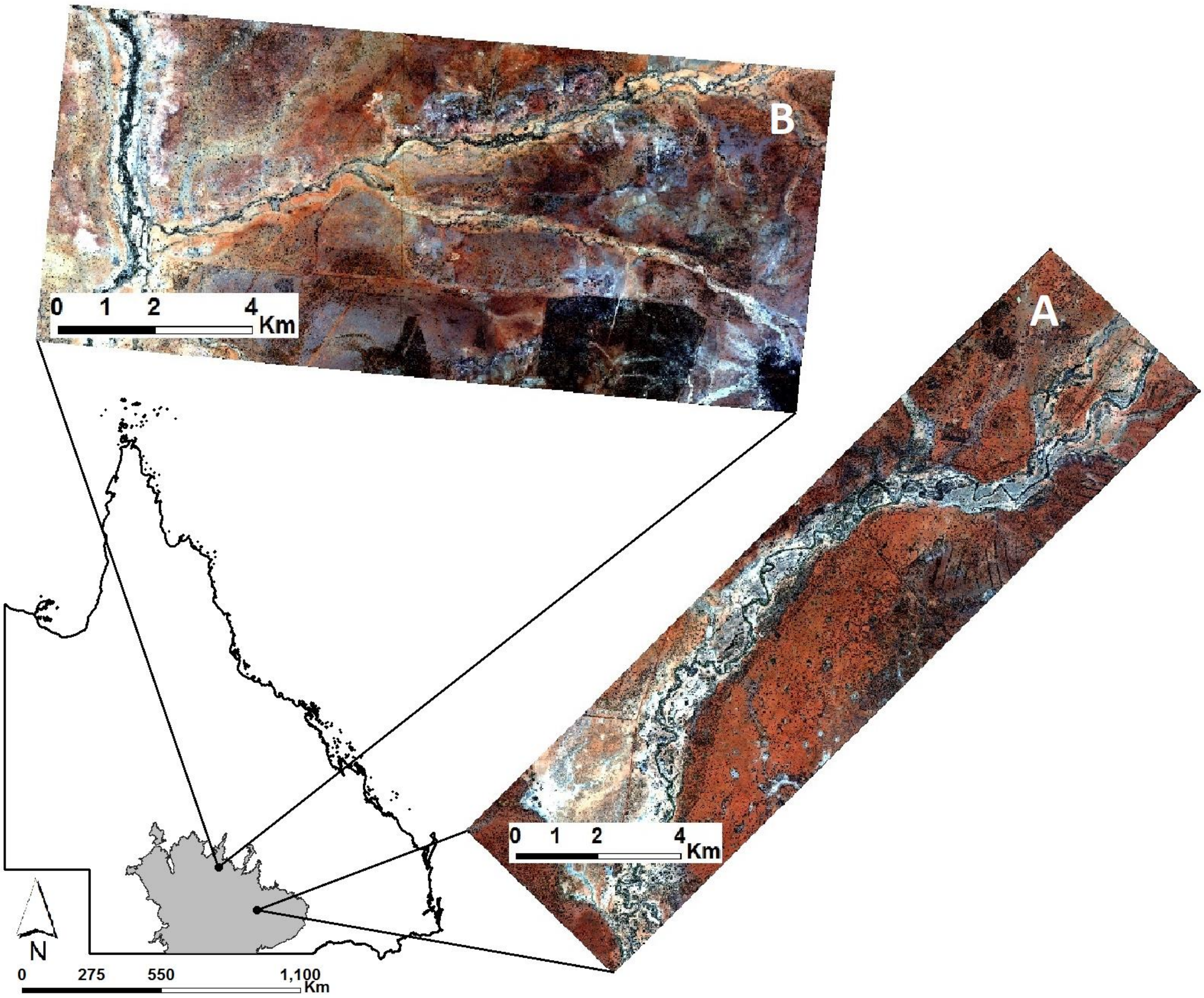

Figure 5.1 WorldView-3 satellite images in approximate true colour at Landscape A (right) and Landscape B (top) captured on 21 November 2015. The map of Queensland at the bottom left corner shows the Mulga Lands bioregion as a grey polygon and the locations of two landscapes as black dots.

Figure 5.2 Field photos of Landscape A showing a riparian habitat dominated by *Eucalyptus camaldulensis* on the left and a floodplain habitat dominated by *E. populnea* on the right in October 2015.

5.2.2 Field Spectral Sampling and Foliar Chemical Analysis

From 19-24 October 2015, the tree species and the location of each selected tree were recorded using a handheld GPS (Garmin eTrex® 30). To check and correct the tree GPS location when overlaying on the image, five distinct and widely dispersed locations in each landscape were selected as GPS control points for image geo-referencing. GPS coordinates and a description of each GPS control point were recorded by the same GPS device. Four tree species were sampled: *E. camaldulensis*, *E. populnea*, *E. coolabah* and *E. melanophloia*. Leaf samples were collected as described in Chapter 3 for field spectral sampling and chemical analysis.

Fresh leaf spectra were acquired with an ASD FieldSpec® high-resolution spectrometer between 350 to 2500 nm with 1.1-1.4 nm bandwidth. A white reference panel was used to standardise and convert relative radiance to reflectance. A leaf clip and a plant probe with halogen bulb as light source were used to collect leaf surface reflectance. For each tree, three leaves were randomly selected and ten spectra were collected from both sides. A total of 60 spectra were collected for each tree and the average spectra were calculated as the ASD leaf spectra. The ASD leaf spectra were resampled to simulate WV3 spectra based on the spectral response of WV3 bands following the methods in Eitel et al. (2007).

After spectral sampling, leaf samples were processed for foliar N and DigN analysis as described in Chapter 3. Foliar N was measured in the process of DigN analysis as the nitrogen concentration (% DM) in original leaf samples.

5.2.3 WV3 Image Acquisition and Pre-processing

The WV3 multispectral images (DigitalGlobe, USA) used in this study comprised eight multispectral and eight SWIR bands (Table 5.1). SWIR bands were collected at 3.7 m spatial resolution by WV3, but distributed at 7.5 m due to restrictions imposed by the US Government. It caused a lower spatial resolution for SWIR bands which increases the chance of mixed pixels when identifying tree pixels. One image was captured for each landscape during 10:39-10:40 AM AEST, 21 November 2015. It was at nadir so that the image was captured at the downward-facing view without geometric distortion. Each image covered an area of 100 km^2 and cost AU$109 per km^2. The georeferencing quality was assessed by overlaying the satellite images with the field-measured GPS control points in ArcMap (Version 10.4). Minor deviations were corrected to the nearest 0.3 m (the spatial resolution of WV3 panchromatic band). Satellite images were atmospherically corrected using Quick Atmospheric Correction in ENVI (Version 5.2) to remove and atmospheric effects and retrieve the inherent spectral reflectance of the surface materials (Bernstein et al. 2012). The eight VIS-NIR multispectral bands were resampled to 7.5 m spatial resolution to calculate the indices involved SWIR bands.

Table 5.1 Details of the sensor bands of WorldView-3 images acquired for this study.

Band Name	**Wavelength (nm)**	**Spatial Resolution**	**Band Name**	**Wavelength (nm)**	**Spatial Resolution**
MUL1: Coastal	400-450	1.2 m	SWIR1	1195-1225	7.5 m
MUL2: Blue	450-510		SWIR2	1550-1590	
MUL3: Green	510-580		SWIR3	1640-1680	
MUL4: Yellow	585-625		SWIR4	1710-1750	
MUL5: Red	630-690		SWIR5	2145-2185	
MUL6: Red Edge	705-745		SWIR6	2185-2225	
MUL7: NIR1	770-895		SWIR7	2235-2285	
MUL8: NIR2	860-1040		SWIR8	2295-2365	

5.2.4 Collecting Tree Image Spectra

For each tree, the pixel corresponding to the tree's GPS position was used to extract image spectra (Figure 5.3). The normalized difference vegetation index (NDVI) was calculated using Red and NIR1 bands for both ASD leaf spectra and WV3 image spectra (Rouse et al. 1974). By comparing the ASD and WV3 NDVI, trees with WV3 NDVI values below 0.6 were lower than the average ASD NDVI and the spectra were mixed with other features such as soil, tree trunks or big stems.

These trees were not used for this study as the image spectra of these trees were not pure tree reflection but mixed spectra with other features.

In the 1.2 m WV3 images, small trees or those with epicormic growth were not used if their canopies were covered by or mixed with neighbouring canopies. In the 7.5 m WV3 images, most pixels covered more than one tree so the average N or DigN values were used. As a result, trees used for 1.2 m WV3 images were 49 (29 *E. camaldulensis*, 5 *E. coolabah* and 14 *E. populnea* and one *E. melanophloia*) and trees used for 7.5 m WV3 images were 27 (19 *E. camaldulensis*, 2 *E. coolabah* and five *E. populnea* and one *E. melanophloia*), out of the total 64 trees.

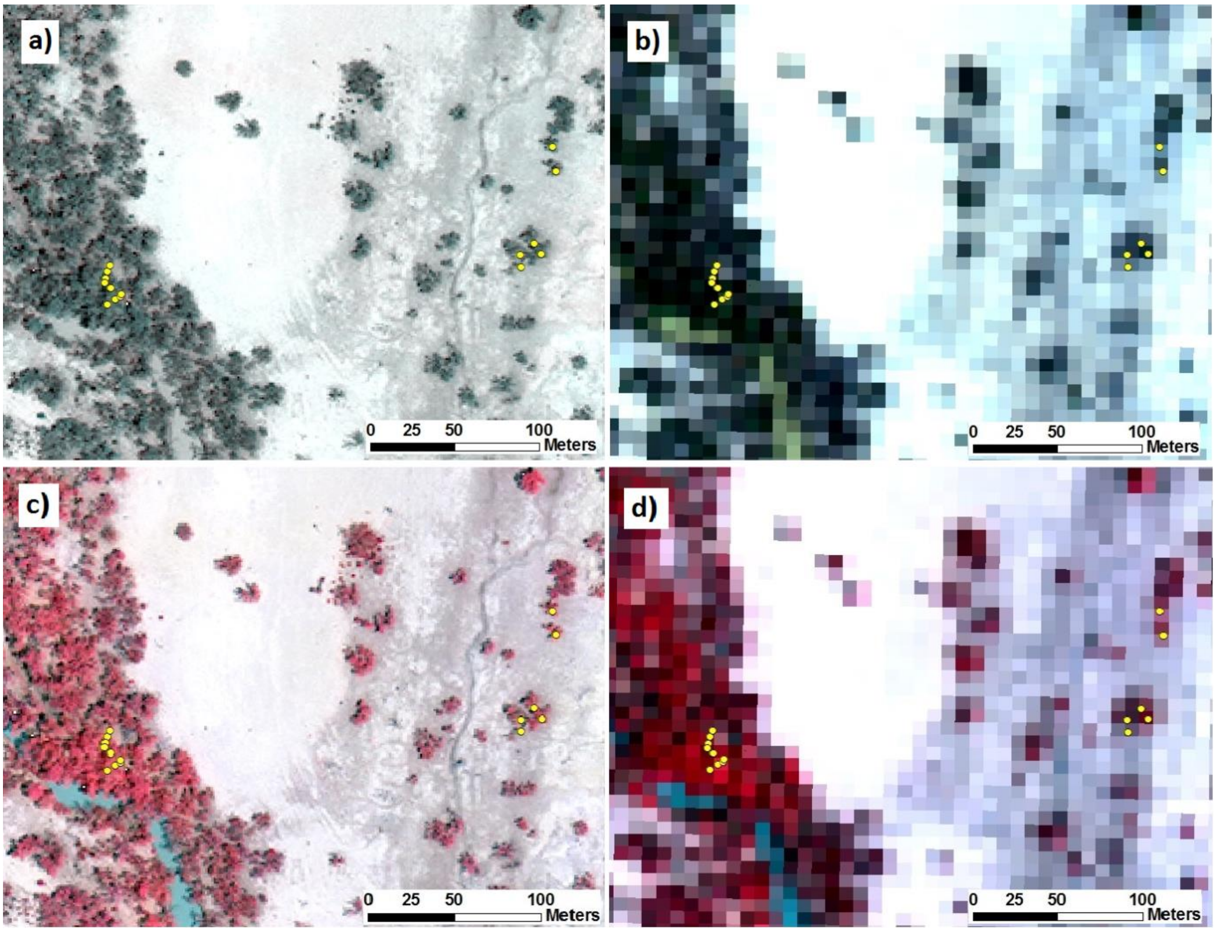

Figure 5.3 WorldView-3 image displayed in RGB using bands Blue, Green, Red (a, b) and bands NIR1, Red and Blue (c, d) showing the same sample sites in Landscape B at 1.2 m (a, c) and 7.5 m (b, d) spatial resolutions. Yellow dots denote pixels correspond to tree GPS locations.

5.2.5 Spectral Indices

Single bands, 1st derivative spectra and spectral indices selected from previous studies of N estimation were calculated (Table 5.2). All indices were calculated from ASD resampled spectra, WV3 1.2 m spectra and WV3 7.5 m spectra. I aimed to compare the indices' performance in leaf-acquired hyperspectral spectra and satellite-derived multispectral data. Some spectral indices were developed from narrow band data so they were calculated from ASD leaf spectra (narrow band) to compare with indices of ASD resampled spectra.

A number of spectral indices were selected from the recent studies estimating plant N using remotely sensed spectra. Normalized Difference Indices (NDI) from WorldView-2 (WV2) spectra were used to estimate grass N concentration (Adjorlolo et al. 2014). The ratio of Transformed Chlorophyll Absorption in Reflectance Index and Optimized Soil Adjusted Vegetation Index (TCARI/OSAVI) was used to measure chlorophyll content which is related to nitrogen content (Haboudane et al. 2002). The TCARI/ OSAVI was then modified by Herrmann et al. (2010) into $TCARI_{1510}/OSAVI_{1510}$ because the 1510 nm band is directly related to N content. Four SWIR-based indices including $TCARI_{1510}/OSAVI_{1510}$ from ASD field spectra showed better estimation for N content of potato fields (Herrmann et al. 2010). Another ratio of Modified Chlorophyll Absorption Ratio Index and the second Modified Triangular Vegetation Index (MCARI/MTVI2) showed better performance than TCARI/OSAVI in estimating wheat N content using multispectral data (Eitel et al. 2007).

Table 5.2 Spectral indices used for estimation of foliar N and DigN in this study. R_a represents the reflectance of a certain band. λ_a represents the average wavelength of a certain band.

Spectral Index	Formula	N in 1.2 m	N in 7.5 m	Reference
Single band	R_a	8	16	
1st Derivative	$D_{a\text{-}b} = (R_a - R_b)/(\lambda_a - \lambda_b)$	7	15	
Normalized difference indices (NDI)	$NDI_{a\text{-}b} = (R_a - R_b)/(R_a + R_b)$	28	120	Adjorlolo et al. (2014)
Transformed chlorophyll absorption in reflectance index (TCARI)	TCARI = 3[(Red edge-Red)-0.2(Red edge-Green)(Red edge/Red)]	1	1	Haboudane et al. (2002)
Optimized soil adjusted vegetation index (OSAVI)	OSAVI = 1.16 [(NIR1 - Red)/(NIR1 + Red + 0.16)]	1	1	Rondeaux et al. (1996)
Transformed chlorophyll absorption in reflectance index 1510 ($TCARI_{1510}$)	$TCARI_{1510}$ = 3[(Red edge-SWIR2)-0.2(Red edge-Green)(Red edge/SWIR2)]	0	1	Adjusted from Herrmann et al. (2010)
Optimized soil adjusted vegetation index 1510 ($OSAVI_{1510}$)	$OSAVI_{1510}$ = 1.16 [(NIR1 - SWIR2)/(NIR1 + SWIR2+ 0.16)]	0	1	
Modified chlorophyll absorption in reflectance index (MCARI)	MCARI = [(Red edge-Red)-0.2(Red edge-Green)](Red edge/Red)	1	1	Daughtry et al. (2000)
Modified triangle vegetation index 2 (MTVI2)	MTVI2 = [1.8(NIR1-Green)-3.75(Red-Green)]/sqrt[(2NIR1+1)^2-6NIR1+5sqrt(Red)-0.5]	1	1	Haboudane et al. (2004)
Combined index	TCARI/OSAVI	1	1	Eitel et al. (2007)
	MCARI/MTVI2	1	1	
	$TCARI_{1510}/OSAVI_{1510}$	0	1	Herrmann et al. (2010)

5.2.6 Data Analysis

The correlation coefficients between the spectral indices and N or DigN concentrations were used to evaluate the performance of indices. For the index with best correlation coefficients, linear and second-order regression equations were used to determine the best estimation of N or DigN. The coefficient of determination (R^2), *p*-value and root mean square error (RMSE) were used to compare the performance of the spectral indices. All data analysis was performed in Excel.

5.2.7 Mapping N and DigN with Selected Indices

A mask of NDVI > 0.6 was applied to the WV3 images to subset the areas of vegetation. Using the region of interest (ROI) tool in ENVI, a plot of average spectra of eucalypt trees, non-eucalypt trees (*Acacia aneura* and *Geijera parviflora*) and dense grass patches (mixed species) was created to find ways to mask out grass and non-eucalypt trees (Figure 5.4, detail statistics are provided in Appendix 5.1). Vegetation areas with $R_{NIR1} > 0.455$ were masked out to exclude grass, whereas the remaining tree areas with $(R_{NIR1}–R_{Red}) < 0.16$ were masked out to exclude non-eucalypt trees. The equation of the selected index for N or DigN was applied to the masked image using band math in ENVI. The foliar N or DigN concentration of eucalypt tree areas were mapped and displayed in colour.

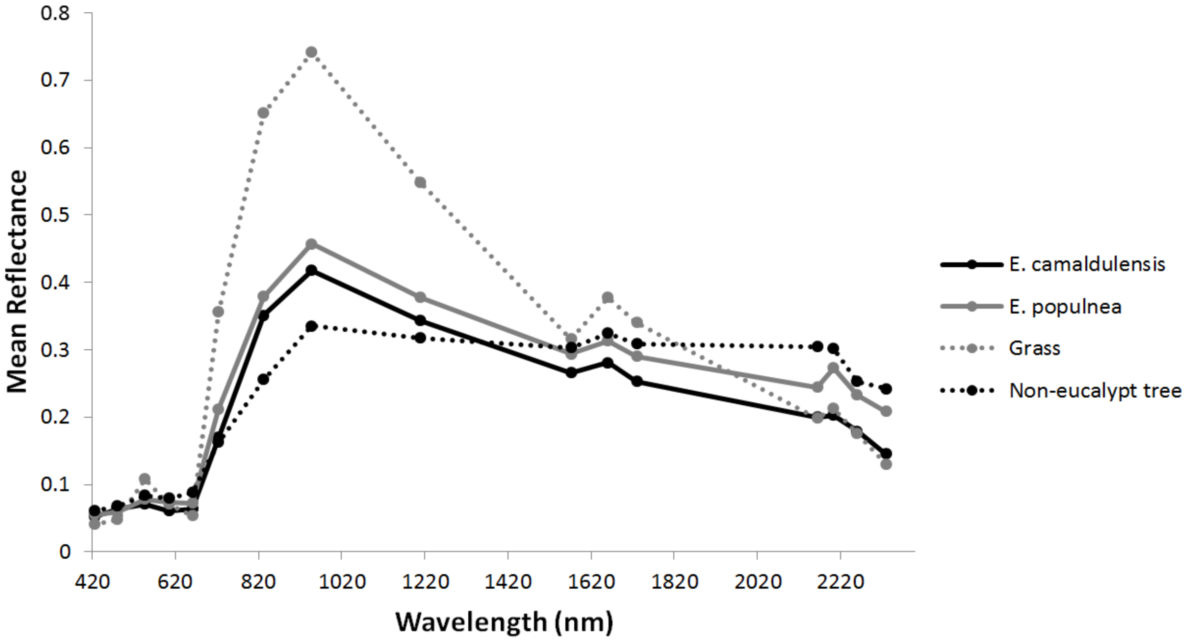

Figure 5.4 Average reflectance of two eucalypt species (solid lines), non-eucalypt trees and grass at different wavelengths from WorldView-3 images at 7.5 m spatial resolution.

5.3 Results

5.3.1 Laboratory Measured Foliar N and DigN Concentrations

The results of foliar N and DigN concentrations of 64 trees out of the 261 trees analysed in Chapter 3 were relevant to this chapter. The Pearson correlation coefficient between DigN concentration and dry matter digestibility ($r = 0.75$, $P < 0.001$) was higher than that between DigN and N ($r = 0.46$, $P < 0.001$). No significant correlation was found between dry matter digestibility and N ($r = 0.05$, $P = 0.71$). Table 5.3 shows the descriptive statistics for the measured N and DigN concentrations of all samples (n = 64), samples for 1.2 m WV3 images (n = 49) and 7.5 m WV3 images (n = 27).

Table 5.3 Foliar N and DigN concentrations (% dry matter) of all samples, sample subsets for 1.2 m and 7.5 m WorldView-3 images.

Response Variable	Samples Size	Mean	Min	Max	SD	CV (%)
N	64	1.65	1.09	2.20	0.22	13.12
N 1.2 m	49	1.65	1.09	2.20	0.22	13.62
N 7.5 m	27	1.66	1.20	2.20	0.23	13.56
DigN	64	1.12	0.35	1.86	0.33	29.12
DigN 1.2 m	49	1.14	0.35	1.86	0.34	29.86
DigN 7.5 m	27	1.20	0.35	1.86	0.35	29.46

5.3.2 Estimation of N Concentration with ASD Resampled Spectra and WV3 Spectra

The best index to estimate foliar N concentration was $NDI_{Coastal\text{-}SWIR3}$ using WV3 7.5 m spectra. Its regression relationship with N concentration ($R^2 = 0.60$, $P < 0.001$, RMSE = 0.140% DM) is shown in Figure 5.5a.

From the ASD leaf spectra (before resampled), the performance of $TCARI_{1510}$ ($R^2 = 0.48$, $P < 0.001$, RMSE = 0.155% DM) and $TCARI_{1510}/OSAVI_{1510}$ ($R^2 = 0.47$, $P < 0.001$, RMSE = 0.156% DM) were better than the VNIR based indices (either lower R square or not significant). This was consistent with the results of Herrmann et al. (2010). The performance of these indices from ASD resampled spectra were similar (Table 5.4), with $TCARI_{1510}$ and $TCARI_{1510}/OSAVI_{1510}$ doing better than other indices. However, these two indices had inferior performance in WV3 7.5 m spectra.

The performance of other indices in ASD resampled spectra and WV3 spectra were different for N concentration (Table 5.4). The 1st Derivative between Red-edge and NIR1 ($D_{6\text{-}7}$) showed good regression fit in ASD resampled spectra but not WV3 spectra. In contrast, two single bands (Coastal and Blue) and a number of NDIs performed better in WV3 spectra, especially at the 7.5 m resolution. The NDIs containing a SWIR band performed better than the NDIs used only VNIR bands.

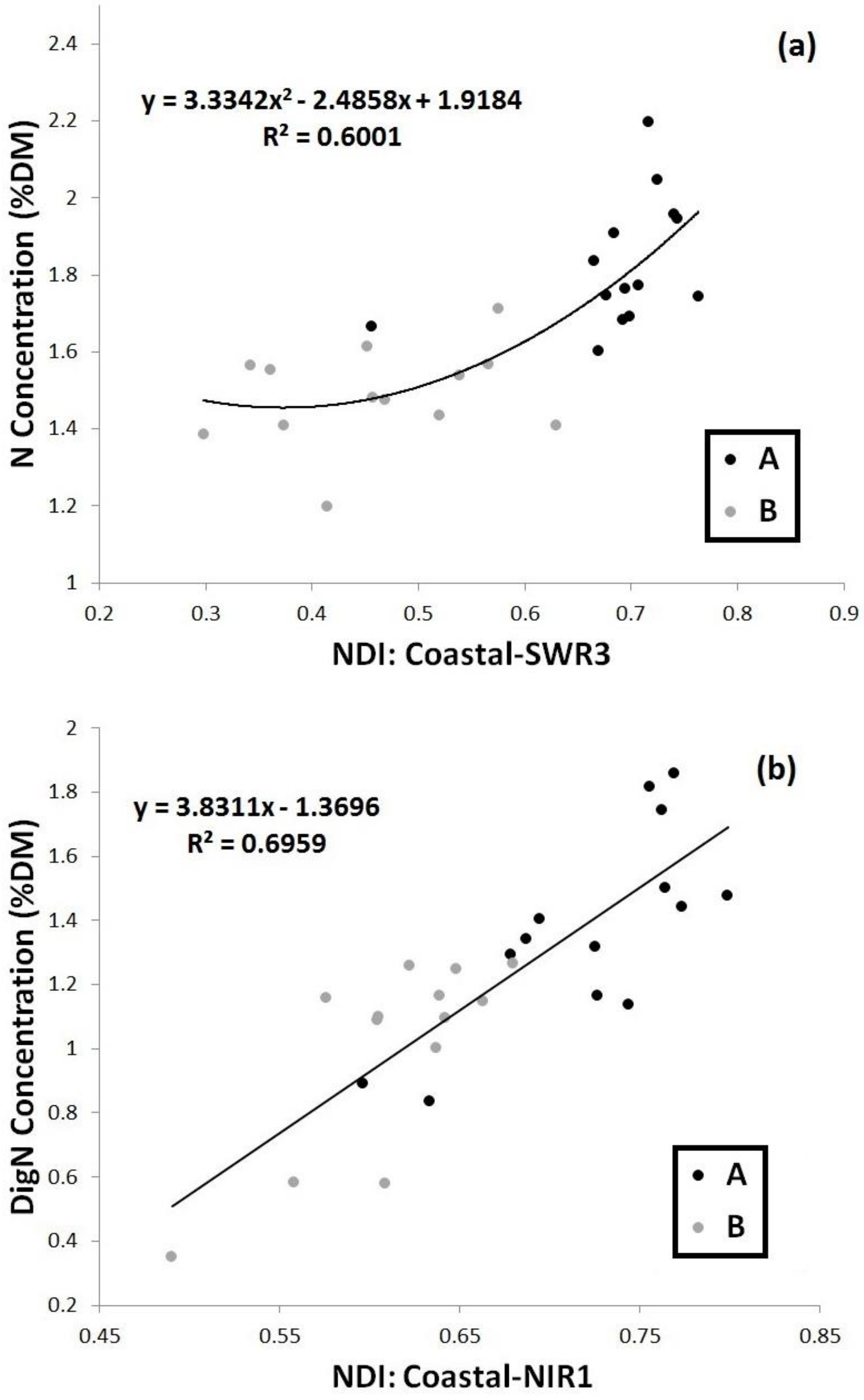

Figure 5.5 Regression of the relationship between (a) foliar total nitrogen (N; % dry matter (DM)) and normalised difference index (NDI) of MUL1 and SWIR3 bands, and (b) foliar digestible nitrogen (DigN; % DM) and NDI of MUL1 and MUL7 bands. NDIs were calculated from WorldView-3 images (Landscapes A and B) at 7.5 m spatial resolution.

Table 5.4 Coefficient of determination (R^2), p-value and RMSE (% dry matter) for various spectral indices used as regression estimators of foliar nitrogen concentration (N; % dry matter) in eucalypts. Spectral indices were calculated from three datasets including ASD resampled spectra, WorldView-3 (WV3) 1.2 m spectra and WV3 7.5 m spectra. R^2 over 0.40 are shown in bold text.

Spectral Index		**ASD resampled**			**WV3 1.2 m**			**WV3 7.5 m**		
		R^2	p	**RMSE**	R^2	p	**RMSE**	R^2	p	**RMSE**
Previous Indices	MCARI	0.14	0.002	0.199	0.02	0.358	0.220	0.01	0.574	0.220
	MTVI2	0.32	<0.001	0.176	0.00	0.707	0.222	0.29	0.004	0.186
	MCARI /MTVI 2	0.06	0.048	0.208	0.06	0.099	0.216	0.02	0.447	0.219
	TCARI	0.17	<0.001	0.196	0.28	<0.001	0.188	0.20	0.018	0.197
	$TCARI_{1510}$	**0.50**	<0.001	0.151	-	-	-	0.26	0.006	0.190
	TCARI/OSAVI	0.24	<0.001	0.187	0.25	<0.001	0.192	0.31	0.003	0.184
	$TCARI_{1510}$ $/OSAVI_{1510}$	**0.50**	<0.001	0.151	-	-	-	0.00	0.836	0.221
Single band	Coastal	0.11	0.008	0.203	**0.43**	<0.001	0.168	**0.58**	<0.001	0.144
	Blue	0.15	0.002	0.198	**0.45**	<0.001	0.165	**0.56**	<0.001	0.146
	SWIR3	0.00	0.827	0.215	-	-	-	0.10	0.116	0.210
1st D	D_{6-7}	**0.47**	<0.001	0.157	0.00	0.765	0.222	0.06	0.219	0.215
	D_{14-15}	0.02	0.274	0.213	-	-	-	0.01	0.561	0.220
	D_{15-16}	0.01	0.503	0.214	-	-	-	0.02	0.437	0.219
NDI	Coastal-NIR1	0.12	0.005	0.201	0.32	<0.001	0.183	**0.42**	0.001	0.168
	Coastal-NIR2	0.12	0.005	0.201	0.38	<0.001	0.174	**0.47**	<0.001	0.161
	Coastal-SWIR1	0.13	0.004	0.200	-	-	-	**0.59**	<0.001	0.141
	Coastal-SWIR3	0.16	<0.001	0.196	-	-	-	**0.60**	<0.001	0.140
	Blue-NIR1	0.17	<0.001	0.195	0.35	<0.001	0.179	**0.47**	<0.001	0.161
	Blue-NIR2	0.17	<0.001	0.195	**0.40**	<0.001	0.172	**0.52**	<0.001	0.153
	Blue-SWIR1	0.18	<0.001	0.194	-	-	-	**0.59**	<0.001	0.141
	Green-Yellow	0.13	0.004	0.200	0.03	0.263	0.219	**0.45**	<0.001	0.164
	Red Edge-NIR1	0.36	<0.001	0.172	0.14	0.009	0.206	0.32	0.002	0.183
	Red Edge-NIR2	0.34	<0.001	0.174	0.29	<0.001	0.188	**0.42**	<0.001	0.169

5.3.3 Estimation of DigN Concentration with ASD Resampled Spectra and WV3 Spectra

Indices with higher R square (> 0.4, in bold text) for DigN concentration (Table 5.4) differed from those for N concentration (Table 5.5). Only four bands (Coastal, Blue, NIR1 and NIR2) were able to estimate both DigN and N in the WV3 data. The best index to estimate foliar DigN concentration was $\mathrm{NDI}_{\mathrm{Coastal\text{-}NIR1}}$ using WV3 7.5 m spectra. Its regression relationship with DigN concentration ($R^2 = 0.70$, $P < 0.001$, RMSE = 0.191% DM) is shown in Figure 5.5b.

From ASD leaf spectra (before resampled), the best indices were TCARI_{1510} ($R^2 = 0.14$, $P = 0.003$, RMSE = 0.301% DM) and $\mathrm{TCARI}_{1510}/\mathrm{OSAVI}_{1510}$ ($R^2 = 0.17$, $P = 0.001$, RMSE = 0.296% DM) compared to MCARI, MTVI2, MCARI/MTVI2, TCARI and TCARI/OSAVI (either lower R square or not significant), but they all performed poorly in ASD resampled spectra and WV3 spectra.

The performance of indices in ASD resampled spectra and WV3 spectra were different for DigN concentration (Table 5.5). The 1st Derivative including SWIR bands ($D_{14\text{-}15}$ and $D_{15\text{-}16}$) were better than $D_{6\text{-}7}$ in ASD resampled spectra but this was reversed in WV3 spectra. Similarly, single band SWIR3 did well in ASD resampled spectra whereas Coastal was better in WV3 7.5 m spectra. NDIs were poor at estimating DigN from ASD resampled spectra but had good performance from the WV3 7.5 m data. The NDIs using only VNIR bands performed better than those containing the SWIR band in WV3 7.5 m spectra.

Table 5.5 Coefficient of determination (R^2), p-value and RMSE (% dry matter) for various spectral indices used as regression estimators of foliar digestible nitrogen concentration (DigN; % dry matter) in eucalypts. Spectral indices were calculated from three datasets including ASD resampled spectra, WorldView-3 (WV3) 1.2 m spectra and WV3 7.5 m spectra. R^2 over 0.40 are shown in bold text.

Spectral Index		**ASD resampled**			**WV3 1.2 m**			**WV3 7.5 m**		
		R^2	p	**RMSE**	R^2	p	**RMSE**	R^2	p	**RMSE**
Previous Indices	MCARI	0.00	0.674	0.324	0.03	0.242	0.332	0.18	0.026	0.312
	MTVI2	0.02	0.254	0.324	0.13	0.011	0.315	**0.42**	<0.001	0.264
	MCARI /MTVI 2	0.02	0.214	0.320	0.00	0.692	0.336	0.04	0.327	0.339
	TCARI	0.12	0.005	0.304	0.14	0.009	0.313	0.08	0.147	0.331
	$TCARI_{1510}$	0.05	0.069	0.316	-	-	-	0.01	0.612	0.344
	TCARI/OSAVI	0.07	0.031	0.312	0.20	0.001	0.302	0.23	0.012	0.304
	$TCARI_{1510}$ /$OSAVI_{1510}$	0.02	0.304	0.322	-	-	-	0.01	0.694	0.345
Single band	Coastal	0.14	0.002	0.300	0.34	<0.001	0.274	**0.45**	<0.001	0.257
	Blue	0.11	0.007	0.305	0.29	<0.001	0.284	0.36	<0.001	0.277
	SWIR3	**0.48**	<0.001	0.235	-	-	-	0.06	0.222	0.335
1st D	D_{6-7}	0.04	0.136	0.319	0.05	0.137	0.329	0.36	<0.001	0.276
	D_{14-15}	**0.52**	<0.001	0.225	-	-	-	0.15	0.049	0.319
	D_{15-16}	**0.51**	<0.001	0.226	-	-	-	0.00	0.937	0.346
NDI	Coastal-NIR1	0.13	0.004	0.303	**0.40**	<0.001	0.262	**0.70**	<0.001	0.191
	Coastal-NIR2	0.13	0.004	0.303	**0.45**	<0.001	0.250	**0.66**	<0.001	0.202
	Coastal-SWIR1	0.12	0.005	0.305	-	-	-	0.32	0.002	0.285
	Coastal-SWIR3	0.08	0.027	0.312	-	-	-	0.12	0.074	0.324
	Blue-NIR1	0.09	0.018	0.301	0.32	<0.001	0.277	**0.60**	<0.001	0.218
	Blue-NIR2	0.09	0.018	0.310	0.36	<0.001	0.269	**0.55**	<0.001	0.232
	Blue-SWIR1	0.08	0.025	0.311	-	-	-	0.27	0.006	0.296
	Green-Yellow	0.03	0.143	0.319	0.19	0.002	0.303	0.34	0.001	0.281
	Red Edge-NIR1	0.02	0.294	0.321	0.19	0.002	0.302	**0.46**	<0.001	0.255
	Red Edge-NIR2	0.02	0.298	0.321	0.34	<0.001	0.274	0.34	0.001	0.281

5.3.4 Mapping N and DigN with Selected Indices

The regression equations from $NDI_{Coastal\text{-}NIR1}$ and $NDI_{Coastal\text{-}SWIR3}$ were applied to the WV3 7.5 m images to map foliar DigN concentration (Figure 5.6) and N concentration (Figure 5.7) across landscapes of both Landscape A and Landscape B. In both landscapes, the foliar DigN or N along creek lines was outstanding on the maps where the tree density was relatively higher. Overall there was lower foliar DigN and N concentration in Landscape B. The trees mapped on the floodplain (around 300 m from the creek) in Landscape A showed a high DigN concentration. However, these trees were not surveyed in the field and it was not possible to determine their species. GPS collar data showed koalas in the Mulga Land spent more than 80% time on average within riverine habitat (Davies et al. 2013) and koalas occur within 300 m from creek lines (Davies, N. personal communication, 24 April 2014). Therefore, the trees 300 m away from creek were of less importance in this study and were not observed in the field. The cluster of higher DigN in the zoomed in area of Landscape B was the main house garden with frequent irrigation, which contained mature *E. camaldulensis* (grown from local seedlings), non-eucalypt trees, bushes and grass.

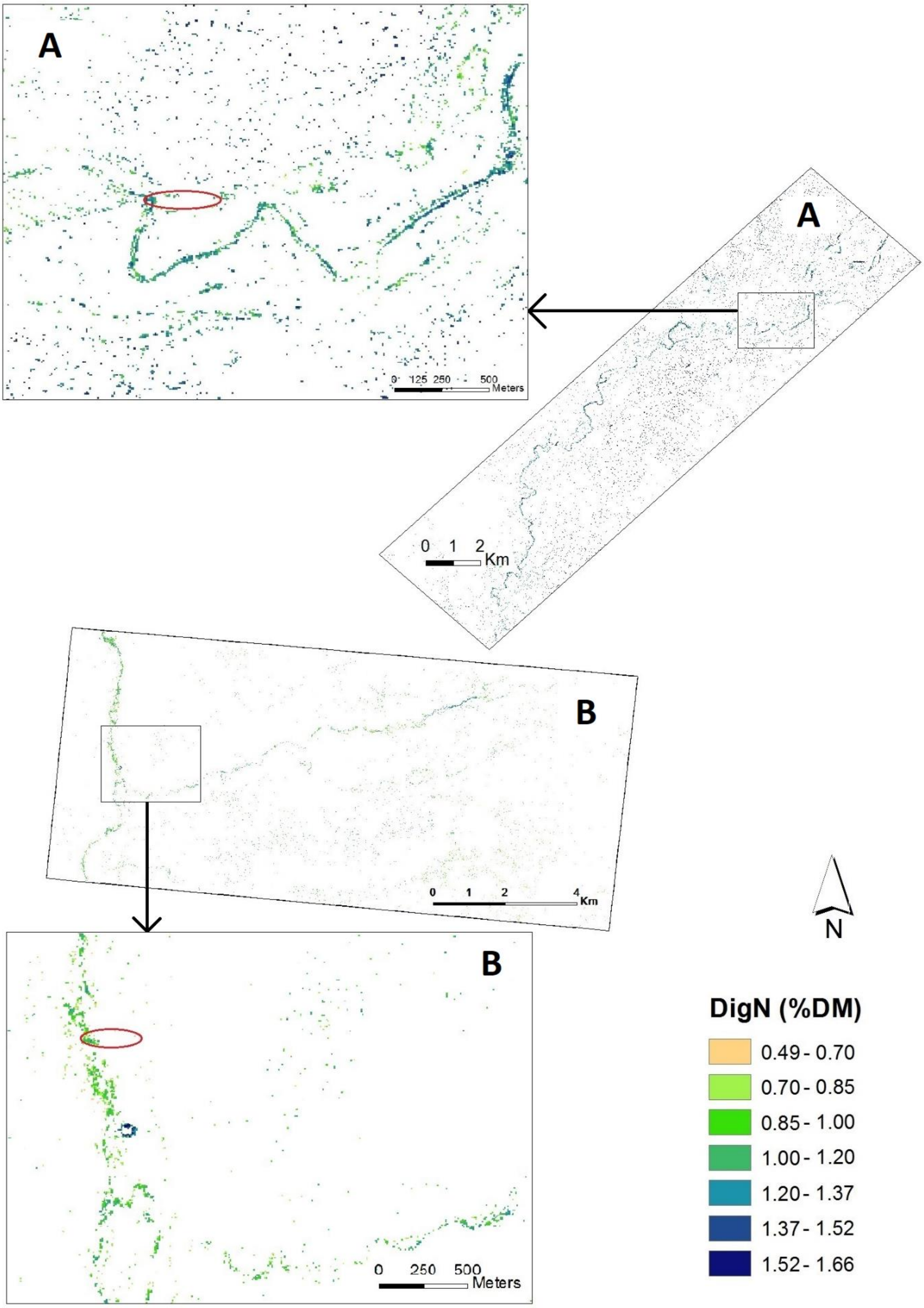

Figure 5.6 Maps of foliar digestible nitrogen (DigN) at Landscape A and Landscape B in colour. Black boxes in full maps indicate location of zoomed in areas. Red circles in zoomed in areas indicate locations of sample sites. Features in non-tree areas (soil, water and grass) are white.

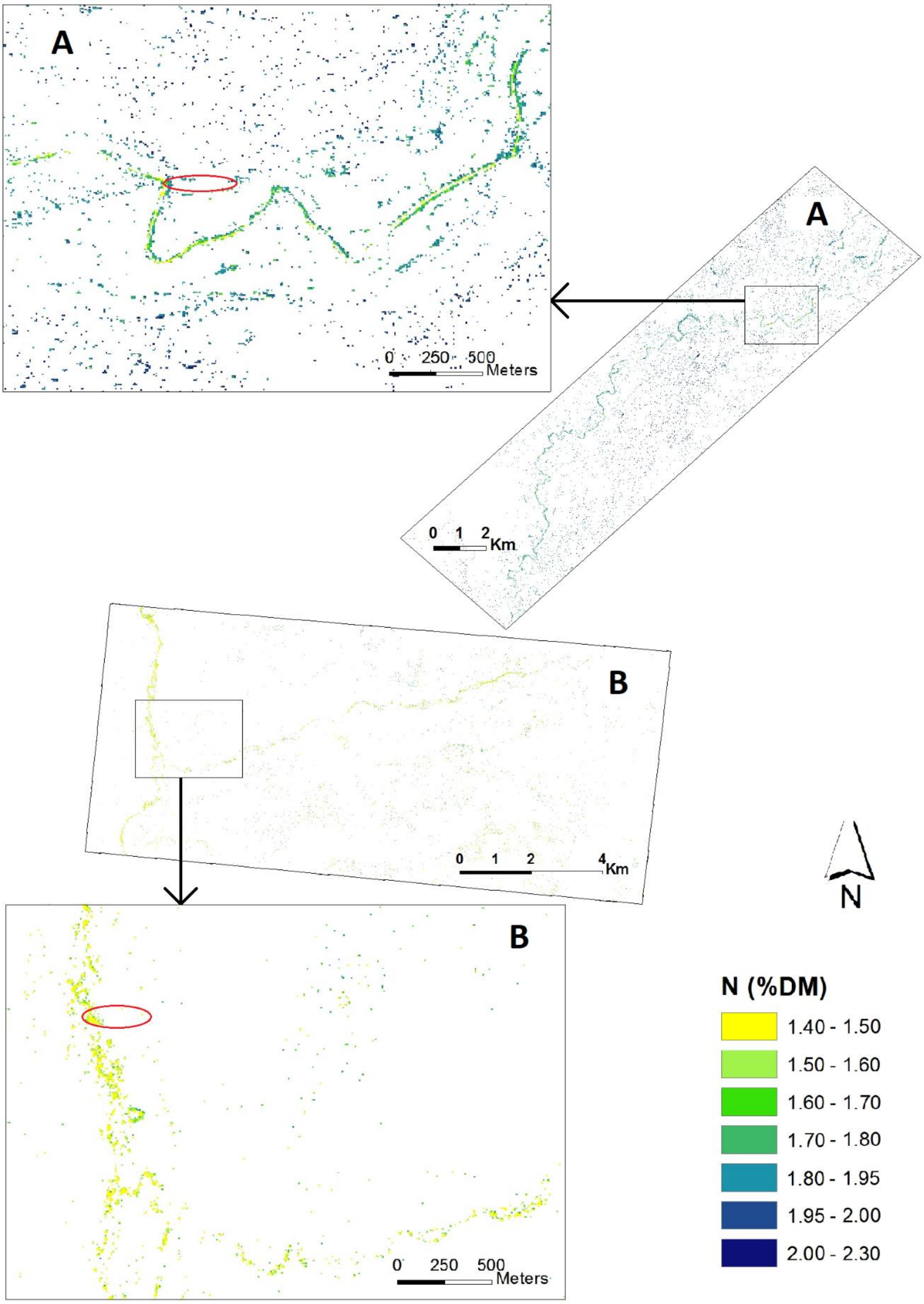

Figure 5.7 Maps of foliar total nitrogen (N) at Landscape A and Landscape B in colour. Black boxes in full maps indicate location of zoomed-in areas. Red circles in zoomed-in areas indicate the locations of sample sites. Features of non-tree areas (soil, water and grass) are white.

5.4 Discussion

This study assessed the potential of using WV3 multispectral data to map foliar N and DigN concentrations in eucalypt woodlands. I examined the relationships between WV3-derived spectral indices and foliar N or DigN concentrations of eucalypt trees from two landscapes in the semi-arid Mulga Lands bioregion, Australia. Two indices were identified to estimate foliar N ($NDI_{Coastal\text{-}SWIR3}$, $R^2 = 0.60$) and DigN concentrations ($NDI_{Coastal\text{-}NIR1}$, $R^2 = 0.70$). Index performance is within the range of R^2 of the best spectral index (0.56-0.77) in previous studies for N estimation (Eitel et al. 2007, Herrmann et al. 2010, Adjorlolo et al. 2014). Therefore, it is possible to estimate and map the variation in foliar N and DigN over large areas using WV3-derived multispectral data.

5.4.1 Spectral Indices to Estimate Foliar N Concentration

Regarding the first research question, the results indicated a limited improvement in estimating foliar N concentration using the SWIR-based spectral indices over the VNIR-based spectral indices from WV3-derived data. It is uncertain if WV3 gives better spectral indices for mapping N than other satellite-derived multispectral data such as WV2, despite the additional SWIR bands.

Within the ASD leaf spectra and ASD resampled spectra, $TCARI_{1510}$ and $TCARI_{1510}/OSAVI_{1510}$ were highly correlated with foliar N concentration, which had similar R^2 to the study of Herrmann et al. (2010) using narrow band canopy spectra in potato fields. Spectral bands used in these indices include one nitrogen absorption band at 1510 nm and two bands sensitive to chlorophyll at 550 and 700 nm (Herrmann et al. 2010). However, the indices did not perform well in WV3 spectra. This suggests that indices developed using narrow band field-measured spectra may not be transferable to broad band satellite-derived spectra for estimating plant N concentration. As the relationships between satellite-derived spectral indices and plant N are indirect (Herrmann et al. 2010), any subtle variation in spectral reflectance can influence the estimation capacity of a spectral index. One possible reason may be the different sources of reflectance spectra (Daughtry et al. 2000, Huber et al. 2008), as the ASD spectra were pure leaf spectra whereas the WV3 spectra include the reflectance of an entire tree canopy and possibly background soil.

Spectral indices from WV3 spectra showed high correlations with foliar N concentration in this study. This indicated that WV3 spectra can be used to map the foliar N concentration over large areas. These spectral indices were single band or NDIs involving Coastal, Blue, NIR1, NIR2, SWIR1 and SWIR3. Although the relationship is indirect, several nitrogen absorption features can be found in these bands. Coastal and Blue have chlorophyll absorption features at 430 and 460nm, whereas NIR1, NIR2, SWIR1 and SWIR3 have nitrogen or protein absorption features at 808, 868, 910, 1020, 1195 and 1645 nm (Curran 1989, William 2001, Coops et al. 2003, Youngentob et al.

2012). In the recent studies using WV2 spectra, NDIs involve Coastal, Blue or NIR2 were also important in estimating grass N concentration (Zengeya et al. 2013, Adjorlolo et al. 2014). NDIs with SWIR bands had slightly higher performance (higher R^2 and lower RMSE) than those using only VNIR bands in this study, but they were not superior in compared to the best index of the study using WV2 spectra (Zengeya et al. 2013). Therefore, the additional SWIR bands of WV3 are not likely to provide better spectral indices for estimating plant N.

5.4.2 Spectral Indices to Estimate and Map Foliar DigN

This is the first attempt I know to estimate foliar DigN using satellite-derived spectra since the study of Youngentob et al. (2012) successfully estimated foliar DigN using airborne hyperspectral spectra. DigN is an integrated foliar nutrition measurement influenced by nitrogen, tannins, cellulose and lignin (DeGabriel et al. 2008) which means it is related to more absorption features than N alone. Consequently, the relationship between spectral indices and foliar DigN concentration would be indirect. It can be harder to estimate DigN using multispectral data which may explain the higher RMSE for DigN compared with N. However, the best index from WV3 spectra, $\text{NDI}_{\text{Coastal-Blue}}$, showed good estimation capability. For the second research question, this study suggested that WV3 data can be potentially used to estimate and map foliar DigN in eucalypt across a landscape, but further study is needed to confirm it with ground truthing.

The ASD and WV3 spectra gave good spectral indices from distinct regions of the spectrum. In the ASD resampled spectra, good spectral indices were from the SWIR region (R^2=0.48-0.52). SWIR3, 6 and 8 contain nitrogen absorption features at 1645, 2180, 2240, 2300 and 2350 nm (Curran 1989, Youngentob et al. 2012). SWIR3 also contains tannins and lignin absorption features (1658,1675 and 1668 nm), whereas SWIR7 has absorption features of cellulose (2280 nm) and lignin (2272 nm) which influence dry matter digestibility (Curran 1989, Soukupova et al. 2002). In contrast, good spectral indices in WV3 spectra were from the VNIR region, involving four bands of Coastal, Blue, NIR1 and NIR2. These VNIR bands were also important for estimating N whereas SWIR bands in WV3 did not provide good indices for DigN. Apart from the absorption features of chlorophyll or nitrogen mentioned above, spectral indices using these four bands contain absorption features of tannins (803 nm in NIR1, 948 and 993 nm in NIR2) and lignin (478 nm in Blue) (Curran et al. 2001, Ferwerda et al. 2006).

5.4.3 Better Performance of WV3 7.5 m Spectra than 1.2 m Spectra

When estimating either foliar N or DigN, most spectral indices showed higher performance in WV3 7.5 m spectra than in WV3 1.2 m spectra. The first reason may be a statistical artefact, due to the smaller sample size of foliar N or DigN for 7.5 m than for 1.2 m. The second reason is the difficulty of identifying pure pixels for a single tree canopy from WV3 1.2 m images. Most trees sampled in this study were *E. camaldulensis* which had overlapping canopies and one pixel may contain the leaf reflection of more than one tree. Therefore, defining tree spectra of one tree from WV3 1.2 m images based on tree trunk GPS has a risk to include the reflection of its neighbours and may not precisely represent the pure spectra of a specific tree. Given the intraspecific variations of foliar N and DigN in *Eucalyptus* trees (Moore et al. 2004a), the impure tree spectra can have a lower correlation with foliar N or DigN for a specific tree. In contrast, at 7.5 m spatial resolution, when one pixel covered more than one tree GPS, its spectra were defined as the combined tree spectra of the trees within this pixel. The combined tree spectra were correlated with the combined foliar N or DigN of these trees. However, it does not mean WV3 1.2 m data is less useful. Sampling big and isolated trees may improve spectral indices performance in WV3 1.2 m data.

5.5 Summary

This chapter reported the first attempt in correlating satellite-derived multispectral data with foliar DigN, which is an important measure of food quality for koalas. Because the best indices identified by this study were developed across two study areas and four eucalypt species, they can be used for new areas with similar eucalypt species. In summary, WV3 data can estimate and map foliar DigN in open eucalypt woodlands in semi-arid Queensland. Foliar DigN map derived from WV3 data would improve the mapping of high quality habitat at the landscape scale for effective koala conservation and habitat management. This technique, once tested to assess its reliability with different tree species, could be used to validate the accuracy of coarse koala habitat mapping based on vegetation communities and improve koala habitat classification by adding finer foliar nutritional information.

Chapter 6 General Discussion

In this chapter, I synthesise the key research findings, highlight the important implications for koala habitat management and conservation in semi-arid regions, discuss the limitations and propose future research questions.

6.1 Synthesis

It is essential to understand how food quality influences foraging decisions and constrains population persistence of wildlife for effective conservation management (Rothman 2015). Where possible, the spatial and temporal heterogeneity in plant nutritional quality for mammalian herbivores needs to be considered with other biotic and abiotic factors in the evaluation of habitat quality (DeGabriel et al. 2014). This study was undertaken with the aim of identifying the leaf chemical attributes, and the environmental and climatic factors that determine koala habitat quality in the semi-arid environment of southwest Queensland, Australia. It extends on previous studies of the ecology of koalas in the region (Sullivan et al. 2003b, Wu et al. 2012, Smith et al. 2013c, Davies et al. 2014, Seabrook et al. 2014). The novel contribution of this study is the inclusion of nutritionally important foliar chemistry (Moore et al. 2004a) in my analysis of koala habitat use and diet under different rainfall seasons. This study integrated the theory and methods of chemical ecology, nutritional ecology and landscape ecology to identify how leaf chemistry influences koala's utilisation for food tree species and habitats. It is consistent with previous studies which demonstrate the influence of plant chemistry on diet and patterns of habitat use of other herbivores (Snyder 1992, Diner et al. 2009, Nie et al. 2015) and omnivores (Kimball et al. 1998) in different ecosystems. A major outcome of this study is an insight into how and why koalas use habitats and food tree species under different rainfall conditions in semi-arid regions. This is important for informing management decisions to conserve the koala from increasingly severe droughts and heatwaves driven by global climate change (Seabrook et al. 2014). The study also identified a potential method for mapping foliar chemical and habitat quality for koala conservation using high-spatial resolution multispectral satellite imagery.

To achieve my first objective (Chapter 3), I identified the spatial and temporal influence of tree characteristics and environmental factors on leaf chemistry (moisture content, DigN and FPC concentrations) of koala key food tree species in the semi-arid study area. Tree species was found to strongly influence all three leaf chemical attributes. Higher leaf moisture contents, foliar DigN and

FPC concentrations all occurred in *Eucalyptus camaldulensis*-dominated riparian habitats than in the adjacent floodplain habitats across three field seasons. Moisture content and FPC concentration also showed temporal variation related to previous rainfall. Leaf moisture content was strongly related to water availability including 6-month rainfall and the presence of surface water, whereas 1-month rainfall had a weak influence on FPC concentration which was higher in the third field season. These findings fill important gaps in the knowledge base of the spatially- and temporally-variable complex nutritional and foraging environment encountered by the koala in semi-arid regions, which has been identified as a priority in nutritional ecology (Rothman 2015). In addition, the foliar DigN concentration of the four main tree species produced an interpretation of nutritional values of the tree species studied that differed from the interpretation based on foliar total nitrogen and tannins by Davies et al. (2014). Previous work (DeGabriel et al. 2009) and the improved agreement with observed patterns of koala tree use suggest that DigN is a more accurate measurement than total nitrogen and tannins to estimate protein value in tannin-rich foods.

In the second objective (Chapter 4), I determined the spatial and temporal influence of leaf chemistry, tree characteristics and environmental factors on koala habitat use and diet. Results from objective 1 formed the knowledge base for the investigation in objective 2. Koalas were more likely to be found in taller and bigger trees, in sites with higher foliar DigN concentrations, and in landscapes with higher long-term (three years) soil moisture. Previous studies found *E. camaldulensis* was one of the main factors determining koala occurrence (Smith et al. 2013c, Seabrook et al. 2014), and in this study, the variation in foliar chemistry between tree species showed the subtlety of why koalas feed on this food tree species more than others. The findings of my study support the argument of Ellis et al. (1995) that food quality of an individual tree at a certain time is more important than its species in determining koala use. It has been previously demonstrated, using faecal diet analysis, that *E. camaldulensis* is the primary food tree species in the region (Sullivan et al. 2003b, Davies et al. 2014). However, although this species has higher foliar DigN concentration, it also has higher FPC levels which is a deterrent to koalas (Moore et al. 2005). Mammalian browsers, especially those with narrow diet range, often confront food with higher nutrition but also higher cost of PSMs (Shipley et al. 2009). For example, the bamboo lemurs (genera *Hapalemur* and *Prolemur*) consume bamboos rich in nitrogen and cyanogenic glucosides (Eppley et al. 2017), and blue duiker (*Cephalophus monticola*) depend on wild figs that are high in protein and tannins (Kendrick et al. 2009). In contrast to the consistent high use of *E. camaldulensis* in the koala diets, the dietary inclusion of *Eucalyptus populnea* and *Eucalyptus melanophloia* showed temporal variation that was likely due to the differences of leaf nutrient levels of these species. The temporal diet variations found in Davies et al. (2014) and this study indicate koalas may adjust their diet composition to meet different temporal requirements. Temporal diet variations were found in koala populations in other regions driven by the temporal requirements and concentrations of some foliar chemicals (Moore and Foley 2000). Temporal diet variation was also observed in the giant panda to regulate nutrient intake to meet different nutritional requirements of reproduction stages (Nie et al. 2015).

For the third objective (Chapter 5), I explored the use of WorldView-3 satellite imagery to accurately map foliar nutrition at high resolution in koala habitats in semi-arid regions. Foliar DigN was targeted because it was found to strongly influence koala occurrence (Chapter 4). Two WV3 satellite images, obtained from two landscapes in Mulga Lands, were used to map DigN with the field measurements of foliar DigN (Chapter 3). The normalised difference index using bands 'Coastal' and 'NIR1' extracted from WV3 satellite images was best correlated with the foliar DigN concentrations. Foliar DigN integrates the influence of nitrogen, phenolics and fibre, and these chemicals have multiple absorption features co-occur in several spectral bands. I acknowledge that the relationship between the spectral index and DigN may not be causal and the applicability of the index to the mapping of DigN needs to be validated on an independent data set. Only one study of estimating DigN with remotely sensed data was found (Youngentob et al. 2012). That study used data from the airborne hyperspectral sensor HyMap with a similar estimation accuracy. However, airborne hyperspectral imagery is relatively more expensive per unit area compared to the satellite-based WV3 imagery (Adjorlolo et al. 2014). Therefore, the method developed in Chapter 5 is potentially a cost-effective approach to mapping koala habitat quality at the landscape and regional scale. The map of DigN would be useful to be integrated with the data of koala occurrence and tree species distribution for koala habitat mapping to improve habitat management.

Based on the findings of the three objectives discussed above, I now address the postulated predictions stated in the literature review (Chapter 2):

Postulate 1: Foliar moisture and DigN will have positive relationship with water availability in the environment. DigN will also have positive relationship with more fertile soil type. FPC will be largely related to tree species.

Key finding: This study confirmed that foliar moisture and DigN have a positive relationship with water availability in semi-arid environments. I identified an effect of soil fertility on DigN but only in *Eucalyptus coolabah*. The results confirmed that foliar FPC is mainly related to tree species.

Postulate 2: Among koala food tree species, *E. camaldulensis* will be primary food tree species, having highest proportion in koala diet. Secondary food tree species will be *E. coolabah*, *E.*

populnea and *E. melanophloia*. In wetter season, the proportion of secondary food tree species will increase due to the change of leaf chemistry.

Key finding: This study confirmed that *E. camaldulensis* is the primary food tree species with the highest proportion in koala diets, and secondary food tree species are *E. coolabah*, *E. populnea* and *E. melanophloia*. However, the postulated higher proportion of secondary food tree species in wetter seasons was rejected.

Postulate 3: Koalas will show more frequent use of trees with higher foliar moisture, moderate FPC, and higher DigN.

Key finding: The results confirmed that koalas showed more frequent use of trees with higher foliar moisture and DigN except for moderate FPC. Primary food tree species contain higher foliar FPC than other tree species. Perhaps intake of this deterrent is regulated by koalas through using trees with lower FPC and toleration.

Postulate 4: Habitat with higher koala occupancy will be dominated by *E. camaldulensis* and have *Eucalyptus* trees with higher foliar moisture and DigN, moderate FPC, bigger tree size and higher surface water availability.

Key finding: This study confirmed that habitats with higher koala occupancy have *Eucalyptus* trees with higher foliar DigN and bigger tree size. Tree species is highly correlated to foliar DigN and also related to koala occurrence. In addition, this study identified that higher long-term soil moisture level is related to koala occurrence. The influence of surface water availability, foliar moisture and FPC on koala habitat use is relatively less important.

6.2 Implications for Conservation and Management

Across much of its geographic range, koala populations are continuing an overall decline in numbers, and habitat loss and hotter droughts are among the major threats identified (McAlpine et al. 2015). Compared to the koala populations occupying wetter, more moderate areas, the koalas living in drier and more variable environments have different characteristics in terms of home range size and spatial pattern, foraging behaviour, and body size (Melzer 1995, Davies et al. 2013a). Western koalas appear to be a regionally adapted population that has evolved the ability to live in hot and dry conditions (Melzer 1995). Koalas in southwest Queensland form a large regional population occupying in marginal distribution range (Sullivan et al. 2004). They are under increasing pressure from heatwaves and prolonged droughts such as the Millennium drought (2001-2009) and more recent droughts (Seabrook et al. 2011, Davies et al. 2013b). The koala population of southwest Queensland has significant conservation value because their adaptation to a semi-arid hot climate is a reference population for understanding how the resilience of other koala populations can be improved under predicted climate change. Based on the insights into the foliar chemistry and nutritional dynamics of the koala habitats in this region, I make the following recommendations for conserving and managing the koala population in semi-arid regions:

1. Maintain and restore riparian habitats and the adjacent floodplains.

Maintaining the habitat quality of riparian habitats has been recommended by previous studies (Sullivan et al. 2004, Seabrook et al. 2011, Smith et al. 2013c, Davies et al. 2014). The study of Smith et al. (2013a) suggests a 1 km buffer of riparian habitats is required to provide supplementary habitat for koalas. The findings of this thesis highlight the importance of these habitats because of their high leaf moisture content and DigN concentration even during drought. I recommend that protecting riparian habitats and the adjacent floodplains should be a priority for conserving koala populations in southwest Queensland. Conserving these high-quality habitats require maintenance of the riparian woodlands dominated by healthy *E. camaldulensis* and the woodlands in the adjacent floodplains dominated by *E. populnea*, *E. coolabah* and *E. melanophloia*.

Maintaining healthy riparian and floodplain habitats under climate change requires improving the drought resilience of these areas. Riparian and floodplain vegetation rely on ground water to survive through droughts, but ground water availability is being reduced by over-extraction and pollution (Kath et al. 2015). Reduced water sources, including groundwater depth and quality, have caused widespread *E. camaldulensis* dieback across the extensive Murray-Darling Basin (including southwest Queensland) which support a large number of koala populations in Australia (Cunningham et al. 2011, Mac Nally et al. 2011). Re-vegetation is a recommended solution because one of the ecological functions of riparian vegetation is to improve water quality and reduce groundwater salinity (Kath et al. 2015). This action may increase the ground water availability to riparian and floodplain communities during droughts. However, revegetation can also increase evapotranspiration and consequently decrease streamflow (Scott and Prinsloo 2008). Hence, caution is required in planning vegetation restoration to balance the cost of water balance change and the benefit of water quality improvement.

Partly funded by the government, some landholders have started to fence riparian sections of their property to prevent erosion of river banks by livestock. Although this action is intended to maintain the condition of creek banks and the depth of creek beds, it also benefit the riparian habitats by reducing water pollution from erosion and the disturbance of livestock to vegetation (Belsky et al. 1999). Monitoring programs are required to evaluate the effectiveness of these actions and the potential effects on wildlife movements. Maintaining healthy and intact riparian ecosystems appears to increase the drought and heatwave resilience of local people, koalas and other mammals, reptiles or insects that reply on *E. camaldulensis* and riparian habitats.

On the floodplains, bigger and taller trees of eucalyptus, mulga and brigalow provide koalas with supplementary food trees or shelter from hot weather and predators (Davies et al. 2013a). Landholders usually clear regrowth vegetation to reduce the density of woody vegetation, thereby promoting the growth of pasture for livestock. This management practice prevents dense woodlands with small trees from developing into open woodlands with large, mature trees on the floodplain which provide important supplementary habitats for the koalas. Keeping a reasonable number of mature and replacement trees on floodplains and plains is likely to benefit koalas by providing food and habitat, and the livestock by providing shades and shelter. It also helps to decrease the dependence of livestock on riparian habitats for shelter in hot sunny days. Therefore, maintaining a 1 km buffer of floodplain vegetation (Smith et al. 2013a) and apply thinning to regrowth instead of clearing needs to be considered for conserving high quality koala habitats.

2. Evaluate and monitor leaf chemicals of koala habitats across landscapes.

Mapping and monitoring habitat quality for the koala at the landscape scale (100s km^2) is crucial for developing effective conservation management (Callaghan et al. 2011). Current koala habitat mapping identifies high quality habitat for conservation based on the distribution of vegetation types and primary food tree species (Lunney et al. 2000, Callaghan et al. 2011). This approach provides a coarse koala habitat map and it is not sufficient to show the variation in nutritional quality for koalas. For effective and specific conservation actions, the approach of mapping koala habitat quality needs to be improved to narrow down areas with high food quality. A new method of evaluating foliar DigN at the landscape scale was explored in this study. With further development, it should be possible to map habitat nutritional quality for koalas over large areas. This could complement the existing habitat maps by looking at variations in nutritional quality within broad vegetation communities and also check if habitat classed as low quality does indeed have low nutritional value. This will be a useful tool to identify the networks of suitable habitats and refugia as the prioritised protected areas and to evaluate the influence of mining or urban development projects on the persistence of local koala populations.

Drought is a major threat to koala populations in semi-arid regions of Queensland and New South Wales (Seabrook et al. 2011, Lunney et al. 2012, Davies et al. 2013b). Monitoring the change in leaf moisture can help to evaluate the influence of drought events on koala habitat quality. The strong effect of short-term rainfall on leaf moisture indicates that leaf moisture content can be monitored indirectly via rainfall records. The open access database from Bureau of Meteorology provides rainfall data to estimate leaf moisture conditions when field sampling is not possible. For example, the daily rainfall data are measured and recorded in a large number of weather stations in Australia and can be easily accessed from the government website. The Bureau can provide data on any areas or time periods in interest, and be more time and cost-effective than field sampling.

6.3 Limitations

There are a number of limitations in the sampling design of this study. A key limitation was the unbalanced sample size of the trees in different landforms and tree species. Because of the spatial distribution patterns of *Eucalyptus* tree species, sampling focused on riparian habitats resulted in an unbalanced sample size of the different eucalypt tree species. This caused difficulty in estimating and modelling the leaf chemistry of *E. coolabah* and *E. melanophloia* with the same confidence as for *E. camaldulensis* and *E. populnea*. Non-riparian areas act as supplementary habitats (Smith et al. 2013c) and there is a risk that they have been overlooked. In a koala pellet survey in 1995, more than 40% koala pellets were recorded in plain and residual areas (Sullivan et al. 2003b). There may be a koala community reliant on residual and plain habitats, or koalas may rely on these areas to disperse across different creeks which maintains sustainable gene flow and allows re-establishment of locally extinct populations throughout broader metapopulations. It should be noted that these habitats are often of greater spatial extent than riparian woodlands, and so might support significant numbers of koalas, even if those populations are of low density. Yet most studies on the southwest Queensland koalas, including mine, have focused on riparian habitats and the dominant species due to the restricted research funding and time (Seabrook et al. 2011, Smith et al. 2013c, Davies et al. 2014). Foliar chemistry of *E. coolabah* and *E. melanophloia* in this study was not sufficiently investigated due to small sample sizes. There is a lack of study of the foliar chemistry of *Eucalyptus thozetiana* which is dominant in potentially important residual habitats. Further study is required to confidently draw conclusions about the leaf chemical dynamics in secondary food tree species including *E. coolabah*, *E. melanophloia* and *E. thozetiana*.

For mapping DigN using WV3 satellite images, the first limitation is the sampling design. It was difficult to extract individual tree spectra from WV3 images at both 1.2 m and 7.5 m spatial resolutions. One of the reasons was that tree sampling in this study was designed primarily to select koala food trees for foliar chemical analysis, rather than to be compared with satellite sensors. The canopies of dense vegetation along creeks overlapped which caused mixed tree pixels. A better sampling design would choose isolated trees with denser and bigger canopies that are identifiable in the image (Youngentob et al. 2012). The second limitation was the sparse canopy in the dry season when the images were obtained. However, work by Ream (2013) and this study showed that image acquisition in both wet and dry seasons face different limitations. In the wet season, tree canopies were less distinct from grass, shrubs and the creek bed (Ream 2013) but isolated tree canopies can be sparse in dry seasons. This region experienced a severe rainfall deficiency in the study period (Bureau of Meteorology 2016). This caused many mixed pixels with the reflectance from trees and soil. In addition, for improving the mapping method, field reference information should not just focus on eucalypt species. The distribution of non-eucalypt species should be recorded which helps to exclude non-eucalypt trees from images when mapping food quality for specialist folivores like koalas. The last limitation is the lack of a validation sample subset to test the accuracy of the mapping results because of limited time and resource of this study.

In terms of the scope of foliar chemistry in this study, there is a limitation of the number of sampling seasons under different rainfall conditions. This research was conducted during a drought with below-average rainfall. Although the rainfall differed among three field seasons, I was not able to measure leaf chemistry under above-average rainfall conditions. Rainfall is closely correlated with the production of new foliage, and the differences in foliar chemistry between new and old leaves can produce different foraging environments for koalas under various rainfall conditions (Gherlenda et al. 2016, Marsh et al. 2017b). The leaf chemistry of food tree species in wet conditions, especially the foliar FPC, is likely to provide further understanding of the value of supplementary habitats to the reproduction and dispersal of koalas (Davies et al. 2013a, Davies et al. 2014). Future research should measure leaf chemicals in wetter condition (e.g. after flood) for a better knowledge of spatial and temporal variation of leaf chemicals.

6.4 Future Research

It is a priority to conserve western koala populations because of the extent and rate of population decrease (McAlpine et al. 2015). Hence a priority of future research is to understand the mechanisms underlying the main threats to koalas and identify the ecological effects that are directly causing population decline. This study focused on the variations of foliar chemistry that mediate the ecological influence of increasing droughts on koalas. I highlight future studies that may help to identify effective actions to conserve koalas against droughts in semi-arid regions.

1. Further understand the response of koalas to foliar chemistry.

Studying the response of wild koalas to foliar chemistry in the study area requires methods that directly record koala tree use with more details. This is because koalas show variation in feeding decision at the tree scale and adjust feeding rates in different trees based on the foliar chemistry (Marsh et al. 2007, Marsh et al. 2014). This study found an outstanding nutritional value but high leaf toxin concentration in *E. camaldulensis* and there was a moderate positive correlation between foliar DigN and FPC. This indicated a challenge for koalas to balance the cost (FPC) and benefit (DigN and moisture) when choosing food at the tree scale. However, koala faecal pellet surveys and diet compositional analysis cannot provide details of which individual trees koalas fed from nor for how long they fed. Radio tracking combined with acoustic telemetry provides a potential solution which has been used to record the tree use and foraging behaviour for wild koalas (Marsh et al. 2014). This method may be time consuming, but it provides valuable details of koala foraging behaviours. Integrating the results of field observations and leaf chemical analysis can inform the influence of foliar chemicals on the feeding strategy of koalas at the tree scale. Conducting foraging behaviours observations temporally and long-term GPS-tracking over the same year may allow us to study the temporal variations of koala foraging strategy and habitat use.

Because koala stress level is influenced by short-term rainfall (Davies et al. 2013b) and short-term rainfall influences leaf moisture contents, future research should test if the change of koala stress level is driven by the variation of leaf moisture content in different rainfall conditions. Excessive stress levels may indicate reduced individual health condition and population sustainability caused by ecological stressors (Davies et al. 2013b). When food quantity and quality are influenced by the increasing variability of climate conditions, understanding nutritional challenges becomes urgent (Rothman 2015) and is an essential component of approaches to identify conservation solutions. If koala stress levels increase with low leaf moisture and even exceed the normal physiological range of koalas, it is necessary to test if conservation actions (e.g. protecting permanent water holes and providing drinking water) helps to reduce koala stress level in dry seasons and if they are viable at the landscape scale (Gordon et al. 1988, Mella 2017).

2. Further understanding the population dynamic of koalas in southwest Queensland.

Although the sampling design and sample size of this study did not allow us to estimate the trend of koala population size, four landscapes in the Mulga Lands in my study were also sampled in the study of Davies et al. (2014). In these landscapes, I not only conducted the survey in the sites and transects but also visited additional areas where koalas were found in the study of Davies et al. (2014) in 2010 (after the Millennium Drought). I found almost no koala faecal pellets from two of these landscapes, and the other two landscapes had fewer locations with koala faecal pellets. A lower success rate in finding koalas and koala faecal pellets was observed. Consequently, the sample size of koala faecal pellets in this study was small and it restricted data analysis to address research questions. Unfortunately, I could not provide comparative data to show a population decline as the survey methods were different. The increased difficulty of finding koala faecal pellets should sound an alert that the koala population in the Mulga Lands may be continuing to decrease.

A new population size survey following the methods of Seabrook et al. (2011) and Sullivan et al. (2004) is required to further understand the koala population trends in semi-arid regions. However, the low density of this koala population presents a challenge in population size estimation with limited research funding and time. A recent study training dogs to search for koala faecal pellets (Cristescu et al. 2015). Law et al. (2017) successfully estimated koala occupancy from the acoustic data of koala bellows during a breeding season in the north coast of New South Wales. These new techniques may provide solutions to improve the efficiency of population surveys for low density koala populations.

In addition to trends in koala population size, understanding koala dispersal is important to ensure the long-term persistence of koala metapopulations in semi-arid regions. Future research should study the koala metapopulation dynamics among riparian, plain and residual habitats to investigate whether there are source and sink habitats at the landscape scale. This is important for habitat management in conserving the number and genetic diversity of koala populations at the regional scale. Tracking the movement of the dispersing sub-adult koalas and testing the genetic parentage and kinship analyses may provide better understanding of population dynamics in these fragmented habitats.

6.5 Conclusion

This study investigated the spatial and temporal heterogeneity of nutritional quality, and the habitat use and diet of the specialist folivore, the koala, in marginal semi-arid distribution range in southwest Queensland, Australia. It fills an important knowledge gap in koala ecology by identifying the spatial distribution of three nutritionally important aspects of foliar chemistry at the tree, site and landscape scales across three different rainfall seasons. It demonstrated a higher koala habitat use for mature *E. camaldulensis*-dominated riparian habitats with permanent surface water especially during droughts and heatwaves. It is important to conserve and ensure the persistence of these high-quality habitats to support the koala populations in this region. The temporal variations in eucalypt species composition of koala diets emphasised that it is essential to preserve the adjacent floodplains as secondary habitats for the long-term persistence of these koala populations. The lower koala occurrence in the landscapes with lower long-term water availability at the south-western margin of study area raised an alarm about the decline and imminent local extinction of these koala populations under predicted climate change. I recommend further research on the western koalas to study the spatial and temporal variations of the water requirements of koalas and the availability of leaf moisture contents and drinking water, and immediate actions to conserve koala habitats and monitor habitat quality in the semi-arid regions.

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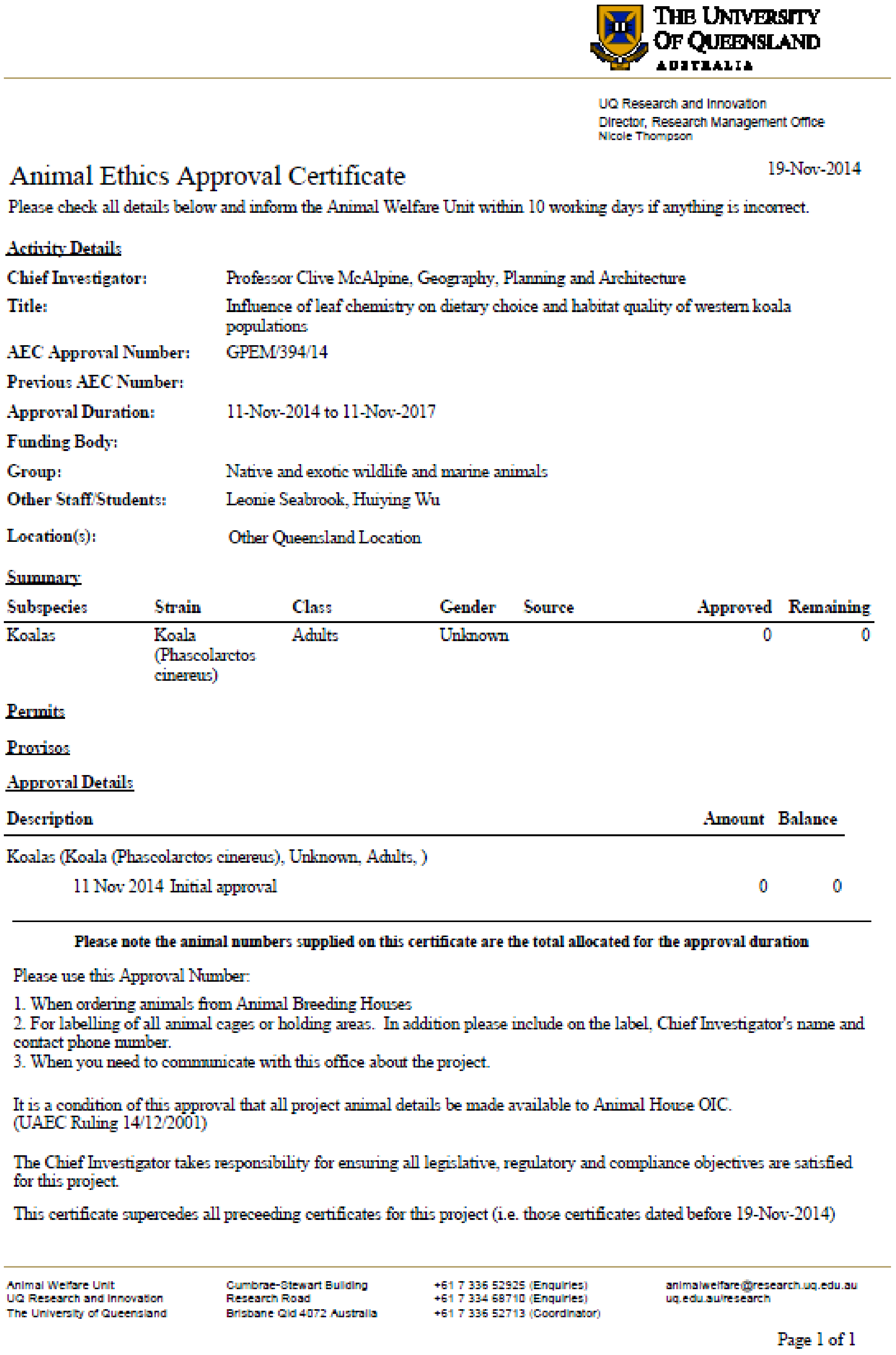
Appendices

Appendix A. Animal Ethics Approval Certificate.



THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

UQ Research and Innovation
Director, Research Management Office
Nicole Thompson

Animal Ethics Approval Certificate

19-Nov-2014

Please check all details below and inform the Animal Welfare Unit within 10 working days if anything is incorrect.

Activity Details

Chief Investigator:	Professor Clive McAlpine, Geography, Planning and Architecture
Title:	Influence of leaf chemistry on dietary choice and habitat quality of western koala populations
AEC Approval Number:	GPEM/394/14
Previous AEC Number:	
Approval Duration:	11-Nov-2014 to 11-Nov-2017
Funding Body:	
Group:	Native and exotic wildlife and marine animals
Other Staff/Students:	Leonie Seabrook, Huiying Wu
Location(s):	Other Queensland Location

Summary

Subspecies	Strain	Class	Gender	Source	Approved	Remaining
Koalas	Koala (Phascolarctos cinereus)	Adults	Unknown		0	0

Permits

Provisos

Approval Details

Description	Amount	Balance
Koalas (Koala (Phascolarctos cinereus), Unknown, Adults,)		
11 Nov 2014 Initial approval	0	0

Please note the animal numbers supplied on this certificate are the total allocated for the approval duration

Please use this Approval Number:

1. When ordering animals from Animal Breeding Houses
2. For labelling of all animal cages or holding areas. In addition please include on the label, Chief Investigator's name and contact phone number.
3. When you need to communicate with this office about the project.

It is a condition of this approval that all project animal details be made available to Animal House OIC. (UAEC Ruling 14/12/2001)

The Chief Investigator takes responsibility for ensuring all legislative, regulatory and compliance objectives are satisfied for this project.

This certificate supercedes all preceeding certificates for this project (i.e. those certificates dated before 19-Nov-2014)

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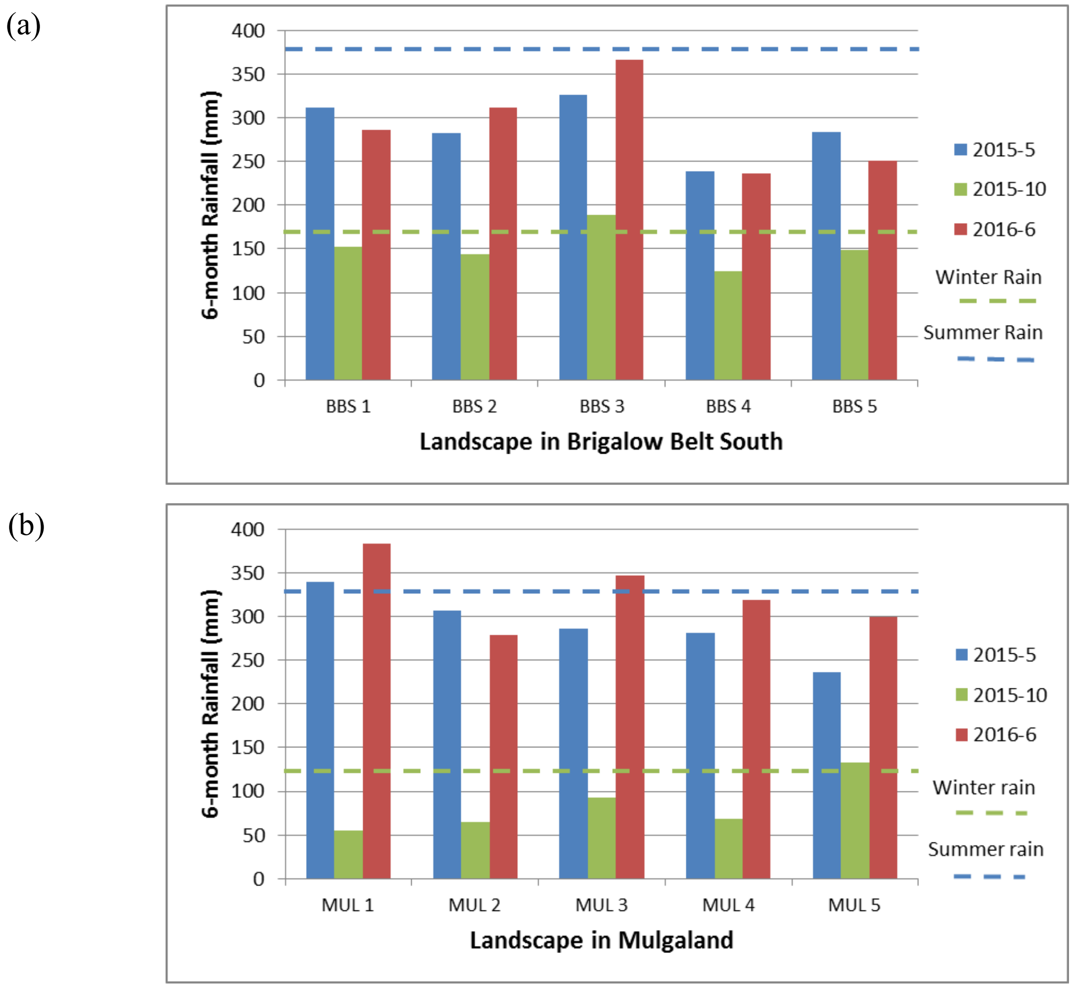
Appendix 3.1 Locations and methods of rainfall measuring. For measuring methods, 'Manual' is measured with manual rain gauge by property owners. 'Automatic' is measured by tipping bucket rain gauge and the data were automatically sent to the Bureau of Meteorology and made accessible online. All measurement methods were following the instruction from the Bureau of Meteorology (http://www.bom.gov.au/climate/cdo/about/rain-measure.shtml).

Landscape	Methods	Longitude	Latitude
Bullecourt	Manual	25.59°S	145.67°E
Biddenham	Automatic	25.80°S	146.42°E
Angellala Downs	Automatic	26.02°S	147.03°E
Prairie Flats	Manual	26.39°S	146.21°E
Abbieglassie	Manual	27.25°S	147.58°E
Boatman	Manual	27.26°S	146.91°E
Telgazlie	Manual	27.29°S	147.34°E
Gaybriel Downs	Manual	27.48°S	149.46°E
Burigal	Manual	27.25°S	150.01°E
Undulla	Manual	27.17°S	149.92°E

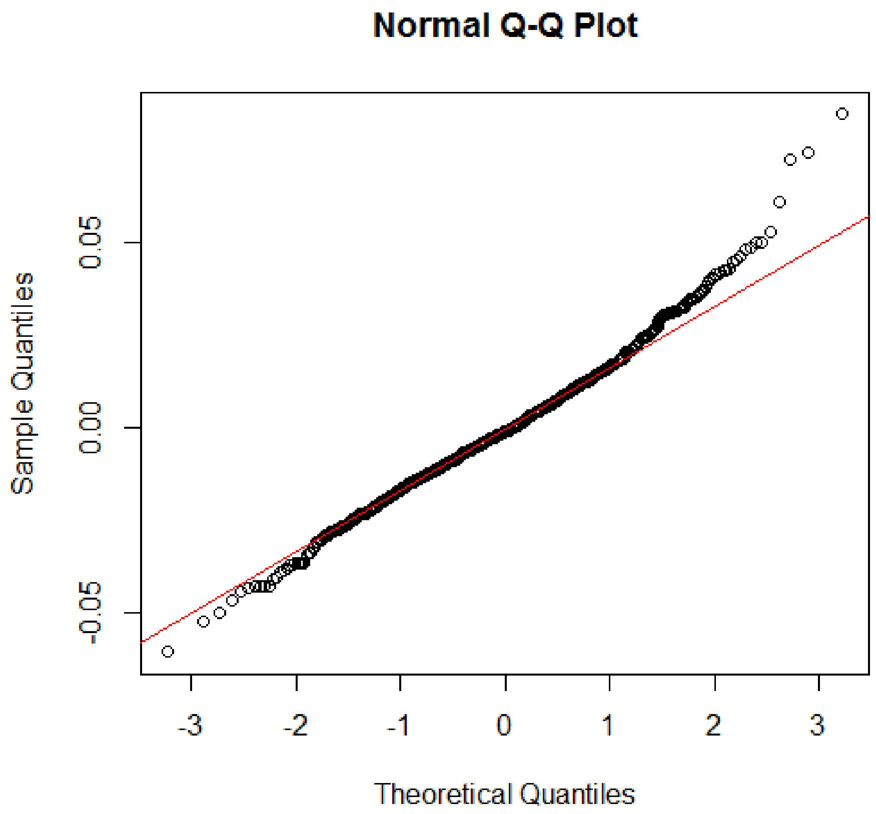
Appendix 3.2 NIRS modified partial least-squares regression models predicting foliar concentrations of digestible nitrogen (DigN) and formylated phloroglucinol compounds (FPC). SECV is standard error of cross validation. Math describes the mathematical treatment applied to raw spectra (stored as log[1/reflectance]). The first two numbers describe the derivative used; the third and fourth numbers indicate the degrees of primary and secondary smoothing performed on the derivative. SNV transformation is described by Boegh et al. (2013).

Constituent	r^2	SECV	Wavelengths (nm)	Math	Transformation	Factor
DigN	0.97	0.09	1100-2492	2, 8, 6, 1	None	8
FPC	0.94	2.68	1100-2492	1, 4, 4, 1	SNV	10



Appendix 3.3 Plots of 6-month rainfall of three seasons in ten landscapes in (a) Brigalow Belt South (BBS) and (b) Mulga Lands (MUL). Dashed lines indicate average winter (May-August) or summer (December-February) rainfall since 1881 for Brigalow Belt South (Surat weather station, 26.41 °S 146.26 °E) and since 1942 for Mulga Lands (Charleville weather station). Average rainfall data were obtained from the Bureau of Meteorology (2016).

(a)


Normal Q-Q Plot
Sample Quantiles
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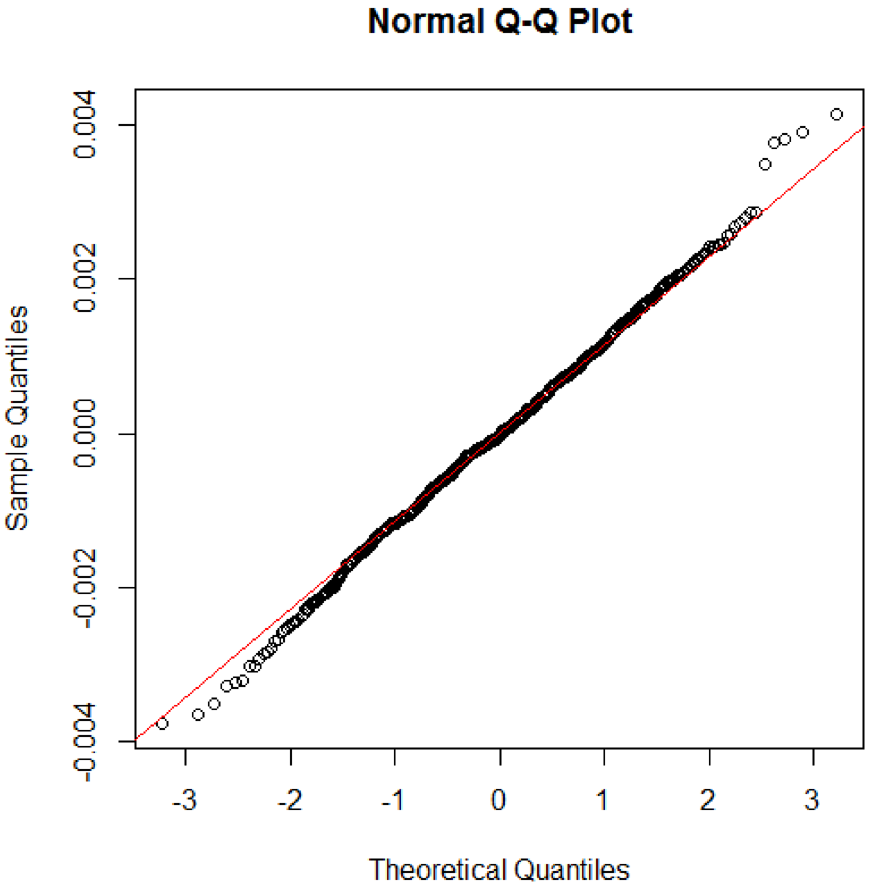
0.00
0.05
-3
-2
-1
0
1
2
3
Theoretical Quantiles

(b)


Normal Q-Q Plot
Sample Quantiles
-0.004
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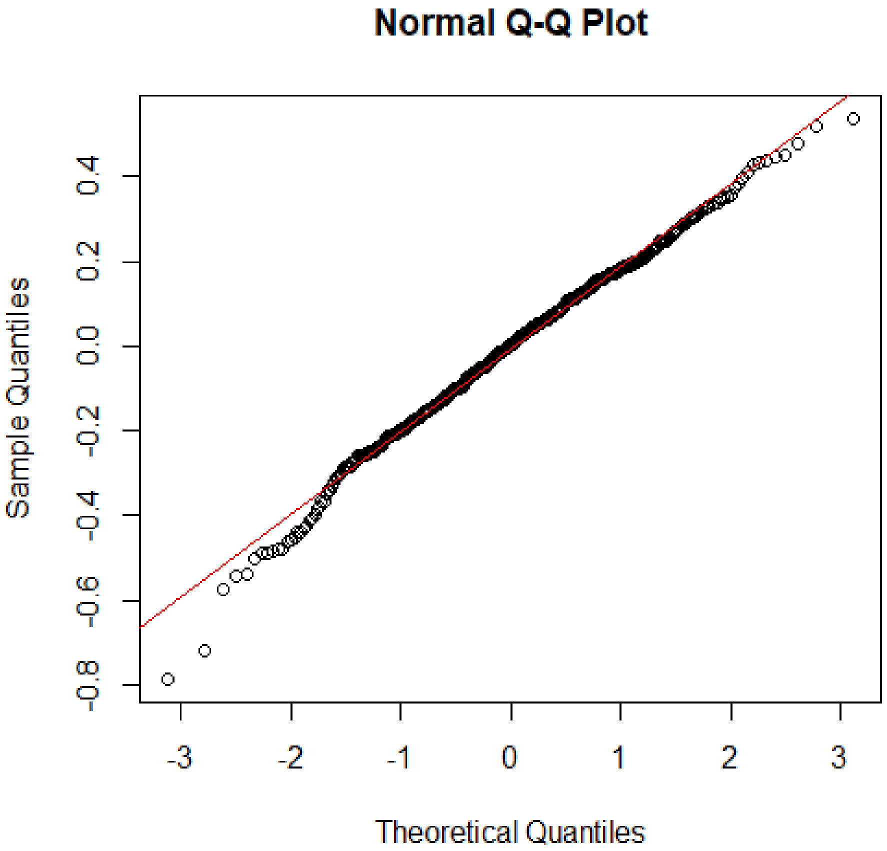
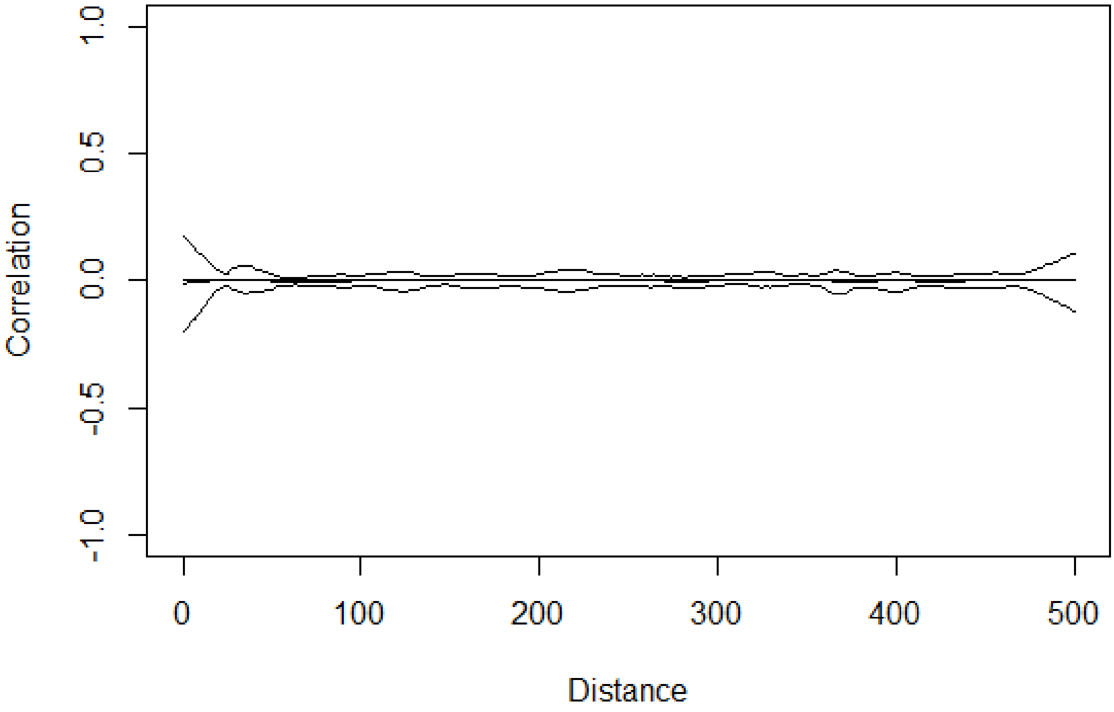
0.000
0.002
0.004
-3
-2
-1
0
1
2
3
Theoretical Quantiles

(c)



Appendix 3.4 The normalized quantile-quantile plots of the optimal models of leaf moisture content (a), digestible nitrogen concentration (b) and positive formylated phloroglucinol compounds concentration (c, samples with value < 4 were excluded).

(a)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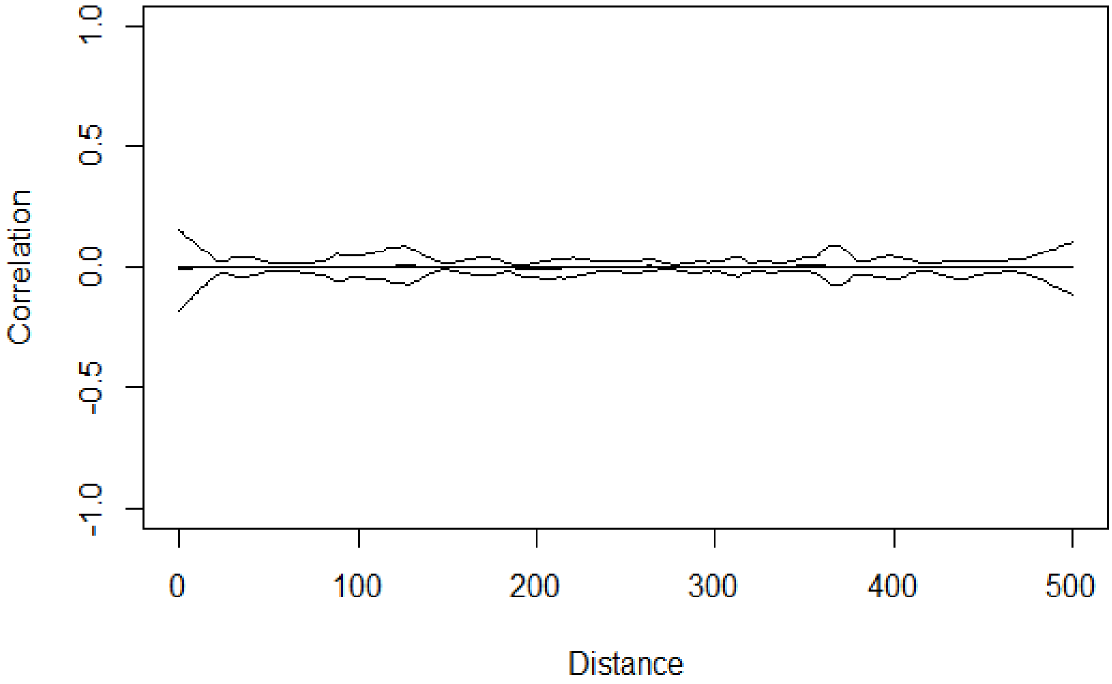
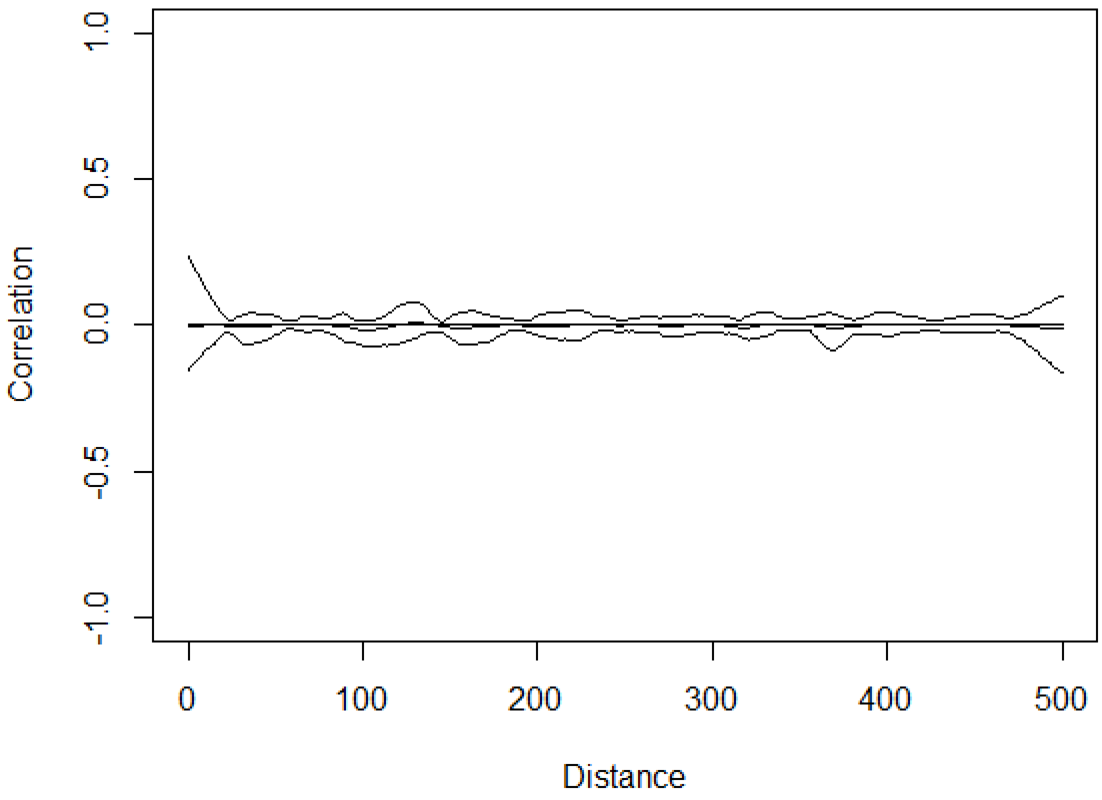
1.0
0.5
0.0
-0.5
-1.0
0
100
200
300
400
500
Distance

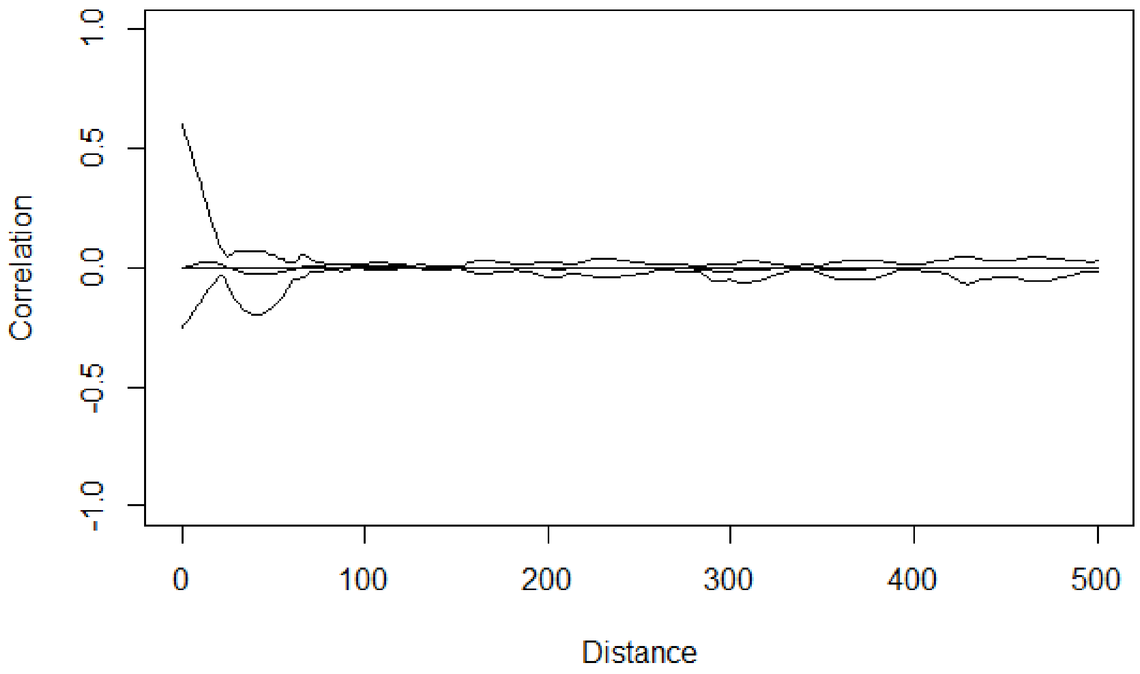
(b)


Correlation
1.0
0.5
0.0
-0.5
-1.0
0
100
200
300
400
500
Distance

(c)



Appendix 3.5 The fitted residuals of the optimal models using the spline correlograms of leaf moisture content (a), digestible nitrogen (b) and formylated phloroglucinol compounds (c). Distance is in kilometres.



Appendix 4.1 The fitted residuals of the best-fitting model using the spline correlograms of koala faecal pellet presence. Distance is in kilometres.

Appendix 5.1 The summary statistics of region of interest (ROI) of *Eucalyptus camaldulensis*, *Eucalyptus populnea*, non-eucalypt trees (*Acacia aneura* and *Geijera parviflora* mixed) and dense grass patches (mixed species). Data collected from WorldView-3 images at 7.5 m spatial resolution.

ROI	Band	Min	Max	Mean	St Dev
E. camaldulensis	Band 1	394	518	437.6	48.0
	Band 2	461	631	518.8	69.3
	Band 3	504	702	574.4	75.8
	Band 4	435	606	498.4	64.9
	Band 5	435	636	503.0	80.7
	Band 6	1338	1691	1465.6	133.9
	Band 7	2923	3508	3130.4	225.1
	Band 8	3499	4171	3700.4	277.5
	Band 9	2919	3431	3185.4	189.1
	Band 10	1981	2661	2380.8	267.6
	Band 11	2167	2806	2574.6	265.4
	Band 12	2028	2526	2248.8	202.5
	Band 13	1422	2002	1682.6	225.9
	Band 14	1461	2026	1807.8	231.5
	Band 15	1073	1789	1462.6	258.1
	Band 16	827	1454	1166.4	226.8
E. populnea	Band 1	477	555	513.7	39.2
	Band 2	499	592	544.3	46.5
	Band 3	616	778	712.0	85.1
	Band 4	558	731	659.3	90.2
	Band 5	632	720	686.3	47.5
	Band 6	1581	2120	1895.0	280.3
	Band 7	2932	3787	3484.3	479.1
	Band 8	3625	4569	4201.0	505.2
	Band 9	2892	3778	3464.0	496.2
	Band 10	2215	2935	2593.0	361.3
	Band 11	2407	3133	2830.0	377.6
	Band 12	2209	2899	2586.3	349.5
	Band 13	1806	2445	2204.0	347.2
	Band 14	2084	2730	2384.3	325.4
	Band 15	1535	2329	1923.0	397.3
	Band 16	1196	2084	1701.0	456.4
Non-eucalypt	Band 1	514	607	569.0	48.8
	Band 2	524	676	604.7	76.4
	Band 3	660	834	729.7	92.0
	Band 4	609	788	680.7	94.7
	Band 5	626	881	752.3	127.5
	Band 6	1137	1622	1363.7	244.0
	Band 7	1816	2562	2185.0	373.1
	Band 8	2504	3351	2906.3	425.1
	Band 9	2499	3178	2814.0	342.1

	Band 10	2800	3032	2933.7	120.0
	Band 11	2952	3240	3131.7	156.7
	Band 12	2542	3087	2826.3	273.3
	Band 13	2495	3046	2798.0	279.6
	Band 14	2744	3013	2844.7	146.7
	Band 15	2044	2526	2313.0	245.8
	Band 16	1809	2420	2132.7	307.1
Grass	Band 1	375	404	389.5	20.5
	Band 2	468	474	471.0	4.2
	Band 3	1026	1084	1055.0	41.0
	Band 4	683	707	695.0	17.0
	Band 5	517	534	525.5	12.0
	Band 6	3336	3561	3448.5	159.1
	Band 7	6108	6509	6308.5	283.5
	Band 8	7162	7408	7285.0	173.9
	Band 9	4495	5482	4988.5	697.9
	Band 10	2511	3154	2832.5	454.7
	Band 11	2907	3780	3343.5	617.3
	Band 12	2512	3407	2959.5	632.9
	Band 13	1692	1984	1838.0	206.5
	Band 14	1794	2125	1959.5	234.1
	Band 15	1484	1752	1618.0	189.5
	Band 16	1032	1303	1167.5	191.6