Relationship between Leaf Traits, Insect Communities and Resource Availability

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Thesis submitted for the degree of Doctor of Philosophy

November 2005

The work described in this thesis is original and has not been submitted in any form for a higher degree at any other university or institution. All of the work presented in this thesis is my own and was undertaken during my PhD candidature: February 2002 to November 2005.

November 2005

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Abstract

This project used the resource availability hypothesis (Coley *et al*., 1985) as a framework for investigating the relationship between resource availability (as defined by soil nutrients), leaf traits, insect herbivore damage and insect community structure. According to the hypothesis, plants from low resource environments should be better-defended, have longer leaf lifespans and slower growth rates than plants from higher resource environments. Higher resource plant species are expected to suffer higher levels of herbivory and recover faster from herbivory than low resource plant species (Coley *et al*. 1985). A corollary to this hypothesis is that plants from higher resource sites should support greater densities of insect herbivores than low resource species.

The study was performed in Sydney, Australia, providing a temperate, southern hemisphere complement to most previous studies on herbivory conducted in the tropics and the northern hemisphere. The project had five components. Comparisons between high and low resource sites were made in terms of: (i) leaf traits of mature and immature leaves; (ii) phenology of leaf maturation; (iii) herbivore damage in the field and laboratory; (iv) diversity and abundance of herbivorous insect fauna; and (v) ability to recover from herbivory.

It was found that leaves from low resource environments were better defended by phenols, but not by physical defences such as leaf toughness. Species from low resource areas did not have longer leaf lifespans or slower leaf expansion rates than species from higher resource areas. In addition, plants from higher resource sites did not suffer greater levels of herbivory or support greater densities of insect herbivores, and they did not recover faster from artificial defoliation compared to plants from low resource environments.

Several expectations of the resource availability hypothesis were supported by these data, whilst others were not. Leaves from low resource environments appeared less palatable and better defended chemically, at least in terms of

carbon-based defences, than leaves from higher resource environments. However, the expectations that low resource plants would have better physical defences and longer leaf lifespans were not met, and the expectations that mesic species would suffer greater herbivore damage and support higher densities of herbivores than dry sclerophyll species were not supported. There was also no evidence that soil nutrients assisted plants in recovering from artificial defoliation.

It is likely that plants from different resource environments are employing different strategies to defend their tissues from herbivores rather than one vegetation type being quantitatively better defended than the other. Leaf characteristics traditionally perceived as providing defence against herbivory, such as phenols, may in fact be contributing to plant resilience to environmental stress.

Acknowledgments

I thank Dr Lesley Hughes for supervision.

I thank Dr Robert Gittins for introducing me to biplots. Thank you for your time, advice and training. You bring energy and excitement to statistics.

I thank my parents Dr John Laxton and Mrs Elizabeth Laxton for providing access to laboratory facilities and the dining room table. I thank my father for his assistance with photography and general advice.

I thank Dr Joanne Jamie and Ms Nynke Brouwer for their advice and access to laboratory facilities; and Mr James Hooker for running my C:N samples. I thank Mrs Tracey Adams for assistance in identifying Coleoptera to family level.

I also thank Dr Marie Elizabeth Herberstein for the loan of 30 leaf stick insects and numerous crickets for the cafeteria experiments, and Ms Jessica Santos for helping me process the photographs taken during the cafeteria experiments.

I thank the Chemical Safety Officer for ELS, Mrs Jenny Minard for her kindness and efficiency in tracking down, ordering and having delivered the many chemicals I have needed throughout this project. I thank Wendy Southwell and Laura McMillan, financial administrative staff in the Environmental Life Sciences division, for their efficiency and advice.

I thank Mr Ken Blade, Mr Peter Hay, Mrs Jacqueline Sedgewicke and Ms Patsy Ross from NSW National Parks and Wildlife Service for providing keys to gates, making collections so much easier.

This research was funded by an Australian Postgraduate Award and the Department of Biological Sciences at Macquarie University.

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Chapter 1
Introduction

The degree of herbivory and the effectiveness of plant defences vary widely among plant species (Aide, 1993; Bolser and Hay, 1996; Coley and Aide, 1991; Coley, 1983a; Coley, 1983b; Lowman, 1992a; Lowman, 1992b, Lowman and Heatwole, 1992). Past studies on plant-herbivore interactions have attempted to understand differences in the susceptibility of plant species to herbivory and variation in defences against herbivory by:

(i) examining the nutritional quality of plant tissues that influence herbivore food choice (eg. Feeny, 1970; Lightfoot et al. 1987; Marquis, 1996; Mattson, 1980; Neuvonen et al. 1984);

(ii) analysing chemical and structural defences of plants (e.g. Bryant and Kuropat, 1980; Campbell, 1985; Coley, 1986; Coley et al., 1996; Cronin and Hay, 1996; Feeny, 1970; Hay et al. 1987; Hay et al. 1994; Lucas et al. 2000; Milton, 1979; Robbins et al., 1987; Iddles et al., 2003);

(iii) monitoring the phenological characteristics of plants (e.g Aide, 1988; Aide et al., 1989; Aide, 1993; Kursar et al., 1991; Kursar and Coley, 1992; Read et al., 2003; Gras et al., 2005);

(iv) measuring rates of herbivory in the field and/or laboratory and correlating these to leaf characteristics (e.g Grime et al., 1968; Cates and Orians, 1975; MacLean and Jensen, 1985; Lowman, 1985; Landsberg, 1988; Lowman, 1992b; Lowman and Heatwole, 1992; Perez-Harguindeguy et al., 2003); and

(v) surveying arboreal arthropod communities and relating insect herbivore abundance and species richness to differences in leaf characteristics or biotic factors such as climate (e.g. McWilliam and Death, 1998; Basset, 2001; Peeters, 2001; Peeters, 2002; Peeters, 2002b).

To understand the general patterns that have been observed in these and other studies, a number of conceptual frameworks have been developed (reviewed in Hartley and Jones, 1997). These include:

I. **Raison d'être theory** (Fraenkel, 1959) which states that plants evolved secondary metabolites to defend themselves against insect herbivores and that insects were important selective agents on plants.

II. **Coevolution theory** (Ehrlich and Raven, 1964) that interprets plant-insect interactions in terms of reciprocal, step-wise coevolution. Ehrlich and Raven suggested that plants and animals are engaged in a continuous evolutionary 'arms race', with plants evolving new secondary metabolites and herbivores evolving adaptations to the compounds.

III. **Sequential evolutionary theory** (Jermy, 1976) which argues that the evolution of flowering plants has been propelled by selection forces that are much more potent than insect attacks (e.g. climate and plant-plant interactions). Jermy asserted that it is the evolution of plants that influences the evolution of phytophagous insects, rather than the other way round.

IV. **Productivity theory** (Janzen, 1974) that proposes a habitat with extremely low primary productivity should select strongly for plants that are rich in chemical defences because of the high cost of replacing damaged tissues.

V. Concept of '**apparency'** (Feeny, 1976 and Rhoades and Cates, 1976) that suggests long-lived plants are more 'apparent' and therefore require higher concentrations of quantitative digestibility-reducing defences, while herbaceous 'unapparent' plants are better defended by qualitative or toxic defensive compounds.

VI. **Carbon/ nutrient balance hypothesis** (Bryant, Chapin and Klein, 1983) that states the availability of carbon and nitrogen in the environment determines the amount and kind of chemicals that a plant allocates to defence versus growth.

VII. **Resource availability hypothesis** (Coley, Bryant and Chapin III, 1985) evolved from the earlier work of Janzen (1974). The hypothesis

proposes that the variation in herbivore pressure between plant species can be largely understood in terms of the resources available to plants.

The ‘raison d’être’ theory interpreted the presence/absence of insects on plant species solely in terms of secondary substances (metabolites) (Fraenkel, 1959), and was therefore regarded as too narrow in its focus for this current study. Similarly, the ‘sequential evolution’ theory was not used as a framework because its primary emphasis is on the evolution and adaptation of insect herbivores (Jermy, 1976).

Coevolution theory has driven much of the research in plant-herbivore interactions (Hartley and Jones, 1997). Studies have demonstrated that secondary chemicals can affect herbivores, that herbivores can adapt to plant chemical defences and that herbivores can inherit the ability to deal with plant defences (Dawkins, and Krebs, 1979; Cornell and Hawkins, 2003; Archetti and Brown, 2004). However, little evidence exists to support the notion that insect herbivores affect the evolution of plant chemical composition (Jermy, 1976; Bernays and Graham, 1988; Crawley, 1989; Berenbaum and Zangerl, 1992). Much of the evidence for coevolution is case-specific and subject to alternative explanations (Hartley and Jones, 1997).

The carbon/ nutrient balance hypothesis has had a direct bearing on more than 200 studies (Hamilton et al., 2001). Despite examples of studies supporting the hypothesis (Waring et al. 1985; Bryant et al. 1987), an increasing number of studies and reviews have identified conceptual limitations (Gershenzon 1994, Berenbaum 1995) and numerous empirical studies have failed to find support for the predictions (reviewed in Herms and Mattson, 1992 Koricheva et al. 1998; Koricheva 2002). Recent reviews have suggested the hypothesis be dismissed as a useful predictive tool (Hamilton et al. 2001; Nitao et al., 2002) and there is currently a heated debate on this subject (Lerdau and Coley, 2002; Koricheva, 2002).

1.1 The resource availability hypothesis

The resource availability hypothesis was selected as a framework for this study because it offers a broad model for understanding the relationship between environmental variables, plant traits and herbivore communities. This hypothesis evolved from the work of Janzen (1974). Janzen (1974) proposed that a habitat with extremely low primary productivity should select for plants that are rich in chemical defences. Plants growing in nutrient-poor environments would lose fitness proportionally more when consumed than plants in more fertile habitats because the cost of replacing damaged tissues and nutrients would be greater in infertile habitats (Janzen, 1974). As a corollary, there is a strong expectation that plant species from low resource environments should invest heavily in defences to protect their photosynthetic tissues (Janzen, 1974). Over a decade later, these ideas were further developed by Coley et al. (1985) and came to be known as the resource availability hypothesis.

Coley et al. (1985) hypothesized that when resources are limited, plants with inherently slow growth are favoured over those with fast growth rates, and that slow growth rates in turn favour large investments in anti-herbivore defences. Plants growing in resource-limited environments are expected to have long-lived, more heavily-defended leaves that consequently suffer less intense herbivore pressure. By contrast, plants growing in resource-rich environments are predicted to have faster growing short-lived leaves with fewer defences and as a consequence, experience higher levels of herbivory. Plants with inherently slow growth are expected to recover more slowly from herbivory than plants with faster growth rates.

Since 1985, the resource availability hypothesis has been cited widely in the literature (eg. Bazzaz et al. 1987; Blossey and Notzold, 1995; Lincoln and Couvet, 1989; Martin et al. 2002). Surveys of herbivore damage in different ecosystems have generally supported the idea that fast-growing plants in more productive environments are subject to more intense herbivory (Cebrian et al. 1994; Jing et al. 1990; Bolser & Hay, 1996). This has led to the generalization that plants in tropical ecosystems are subject to more intense herbivore pressure than those in

temperate regions (Coley & Aide, 1991; Coley & Barone, 1996). However, when studies from tropical regions are compared to those of temperate regions (Coley and Aide, 1991; Coley and Barone, 1996), differences in resource availability (be they soil nutrients, light or water) are confounded not only by major climatic differences but also by differences in the pool of available herbivores. Any apparent increase in herbivore pressure in the tropics may be due simply to the increased abundance and species richness of available herbivores, rather than to resource availability per se.

1.2 Project components and associated aims

This project used the resource availability hypothesis (Coley et al. 1985) as a framework for investigating the relationship between resource availability, leaf traits, insect herbivore damage and insect community structure on a wide variety of plant species of varying growth forms from paired low and higher resource sites within the same region. The study was performed in Sydney, Australia, thus providing a temperate, southern hemisphere complement to most previous studies on herbivory conducted in the tropics and the northern hemisphere (Coley, 1988; Aide and Londono, 1989; Bryant et al. 1989; Jing and Coley, 1990; Coley and Aide, 1991; Bolser and Hay, 1996).

The paired sites were within 5 km of each other, had similar climate and geology, and were therefore likely to be subject to similar regional insect herbivore fauna. Resources were defined in terms of soil characteristics: - soil moisture, percent organic matter, nitrogen and phosphorus concentrations.

The project had five components. Comparisons between high and low resource sites were made in terms of:

1. Leaf traits of mature and immature leaves
2. Phenology of leaf maturation
3. Herbivore damage in the field and laboratory
4. Diversity and abundance of herbivorous insect fauna
5. Ability to recover from herbivory

The first and second components tested the hypothesis that leaves of plants from low resource environments would be well defended, long-lived, and have slow growth rates, compared to leaves of plants from higher resource environments.

Mature, immature and expanding leaves were analysed for a number of chemical and physical characteristics in order to answer three major questions:

1. Do the leaves of plants from resource-poor environments have higher concentrations of chemical and physical defences than plants from resource-rich environments?

2. Do leaves from low resource environments live longer and have slower expansion rates than leaves from higher resource environments?

3. Do leaves of plant species from resource-poor environments become better defended at a faster rate than plant species from environments that are richer in resources?

Components 3 and 4 tested the hypothesis that plants in low resource environments suffer less insect herbivore damage and have fewer insect herbivores compared to plants in more fertile areas. Herbivore damage was monitored monthly for three years at the field sites. Cafeteria laboratory experiments were also conducted to investigate leaf palatability.

Component 4 examined the diversity and abundance of insect herbivores at the field sites using pyrethrum spraying and branch clipping. The orders Coleoptera (beetles), Hemiptera (bugs) and Lepidoptera (butterflies and moths) were the focus of this study. The specific questions addressed in components 3 and 4 were:

4. (i) Do plants from low resource environments suffer less herbivory than plants from more fertile habitats? (ii) How much plant tissue is lost to herbivores in the field and laboratory?

5. Is herbivore damage correlated with (i) nutritional value (percent nitrogen, fiber content) of the foliage, (ii) concentration of chemical defences (total phenols), (iii) degree of physical defence (toughness, force of fracture, lamina thickness), (iv) other leaf traits (specific leaf area, percent water)?

6. How is the herbivore damage distributed amongst insect feeding guilds (chewing, sucking, etc), and is this distribution different at the sites with differing resources?

7. Does the structure of insect herbivore communities differ: (i) between plant species within a site (ii) between sites of different resource availability at a location (iii) between locations?

8. Are specific leaf traits correlated with particular insect orders/ families/ guilds?

The fifth component tested the hypothesis that plant species from low resource environments are less able to recover from herbivore damage than species from higher resource environments. This component was tested with a glasshouse experiment designed to monitor recovery rates of dry sclerophyll and mesic plant species following artificial herbivory. The questions investigated were:

9. Do plants species from vegetation communities growing in more fertile environments recover faster from artificial herbivory than plant species from communities in less fertile environments?

10. Do plant species from infertile environments recover faster from defoliation when they have access to greater concentrations of soil nutrients?

1.3 Thesis structure

Chapter 2 describes the study sites.

Chapter 3 tests the first of three major hypotheses: that leaves from low resource environments will be better defended (both physically and chemically), live longer and have slower expansion rates. The characteristics of mature and immature leaves from low and higher resource sites, and the phenology of leaf maturation for plants growing in nutrient poor and richer environments are presented.

Chapter 4 tests the hypothesis that plants from low resource environments experience less herbivore damage than plants from higher resource environments. Results of field studies and laboratory experiments monitoring herbivore damage and selection are presented.

Chapter 5 continues to test the second major expectation of the resource availability hypothesis by presenting data on the insect herbivore communities found in the low and high resource sites.

Chapter 6 describes the results of a glasshouse experiment designed to monitor recovery rates of plants from artificial herbivory. This chapter tests the hypothesis that plants from low resource environments will have less ability to recover from herbivore damage compared to plants from resource rich areas.

Chapter 7 presents conclusions.

Chapter 2
Study sites

2.1 Site locations

The paired sites on infertile and more fertile soils were situated in two localities in the Sydney Basin , New South Wales: Ku-ring-gai Chase National Park and Royal National Park (Fig. 2.1). The localities were 30 km north and south of Sydney respectively.

In Ku-ring-gai Chase National Park, the infertile site was located along Challenger Track (151°16'35.6"E 33°35'47.6"S) and the more fertile site 2 km away near the Resolute Picnic Area (151°17'37.8"E 33°34'44.6"S) (Fig. 2.2). In Royal National Park, the infertile site was located off Bundeena Road (151°05'09.8"E, 34°07'32.9"S) and the fertile site approximately 4 km away, along Bola Creek opposite Walumarra track (151°01'56.1"E, 34°08'51.6"S) (Fig. 2.3). Photographs of the sites are presented in Appendix 1.

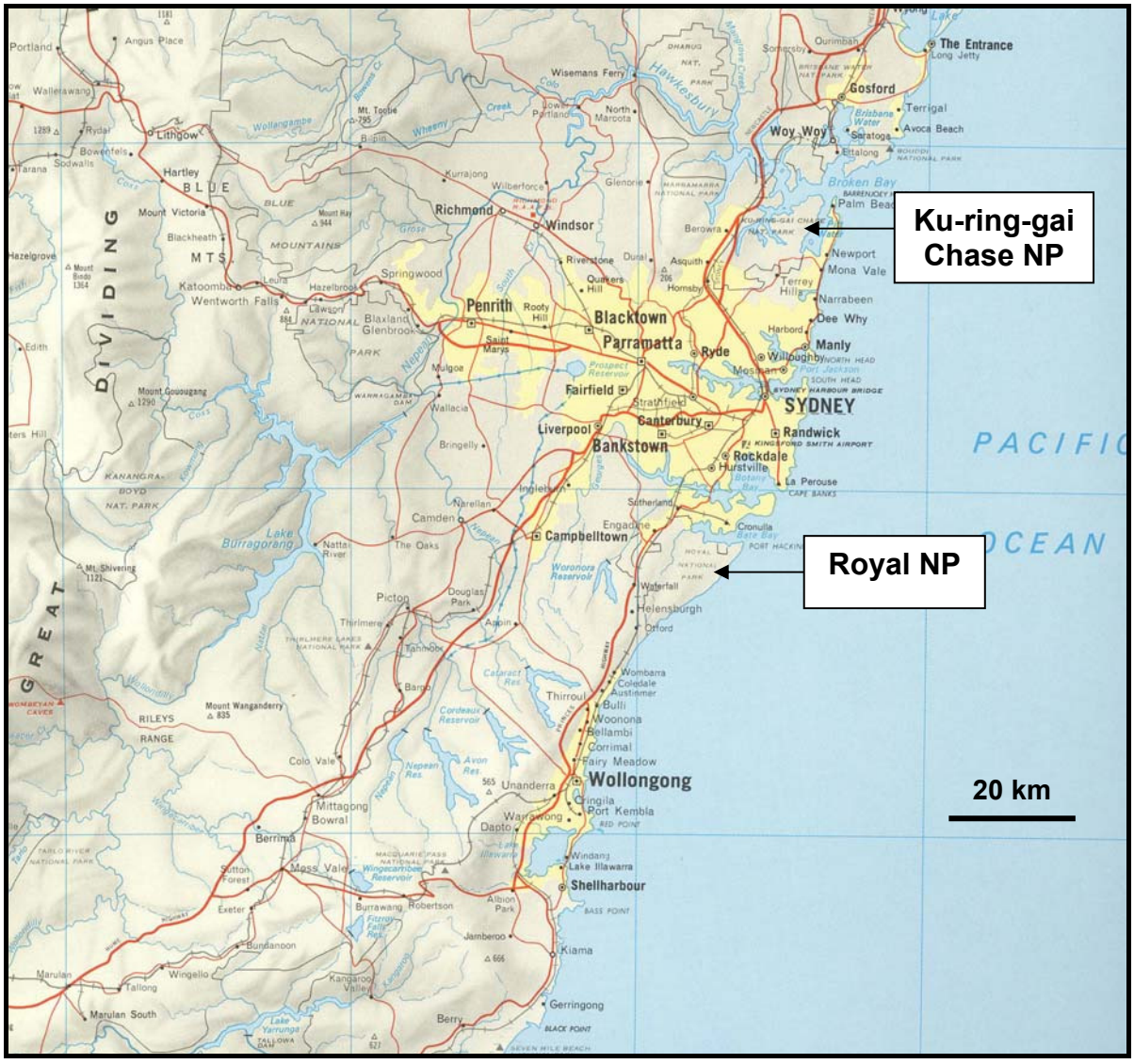
Fieldwork occurred seasonally between 2002 and 2004.

2.2 Climate

Between the years 2002 to 2004, the maximum and minimum air temperatures for the Sydney region ranged from 3.6°C to 41.8°C, with the highest monthly maximum temperatures occurring between November to December, and the lowest monthly minimum temperatures between May and July (Table 2.1). The annual mean daily maximum was 22.1°C and the annual mean daily minimum was 13.2°C (www.bom.gov.au/climate/averages/).

The nearest rainfall data for Ku-ring-gai Chase National Park is recorded daily at St Ives, approximately 10 km south-west of the Park. Rainfall data for Royal

National Park is collected at Audley in the Park itself, about 8 km from the sites (Fig. 2.3). The two recording stations showed similar patterns of rainfall (paired t-test comparing differences of each month's rainfall: t = 1.60, p = 0.12; Pearson Chi-Square comparing days of rain with days without rain: value of 3.75, p > 0.05) (Fig. 2.7). The annual average rainfall for St Ives was 84 mm in 2002, 123 mm in 2003 and 83 mm in 2004. Audley had an average annual rainfall of 72 mm in 2002, 118 mm in 2003 and 79 mm in 2004.



(from *The Macquarie Atlas*, 1995: p146.)

Figure 2.1 Locality map of Ku-ring-gai Chase National Park and Royal National Park, NSW Australia

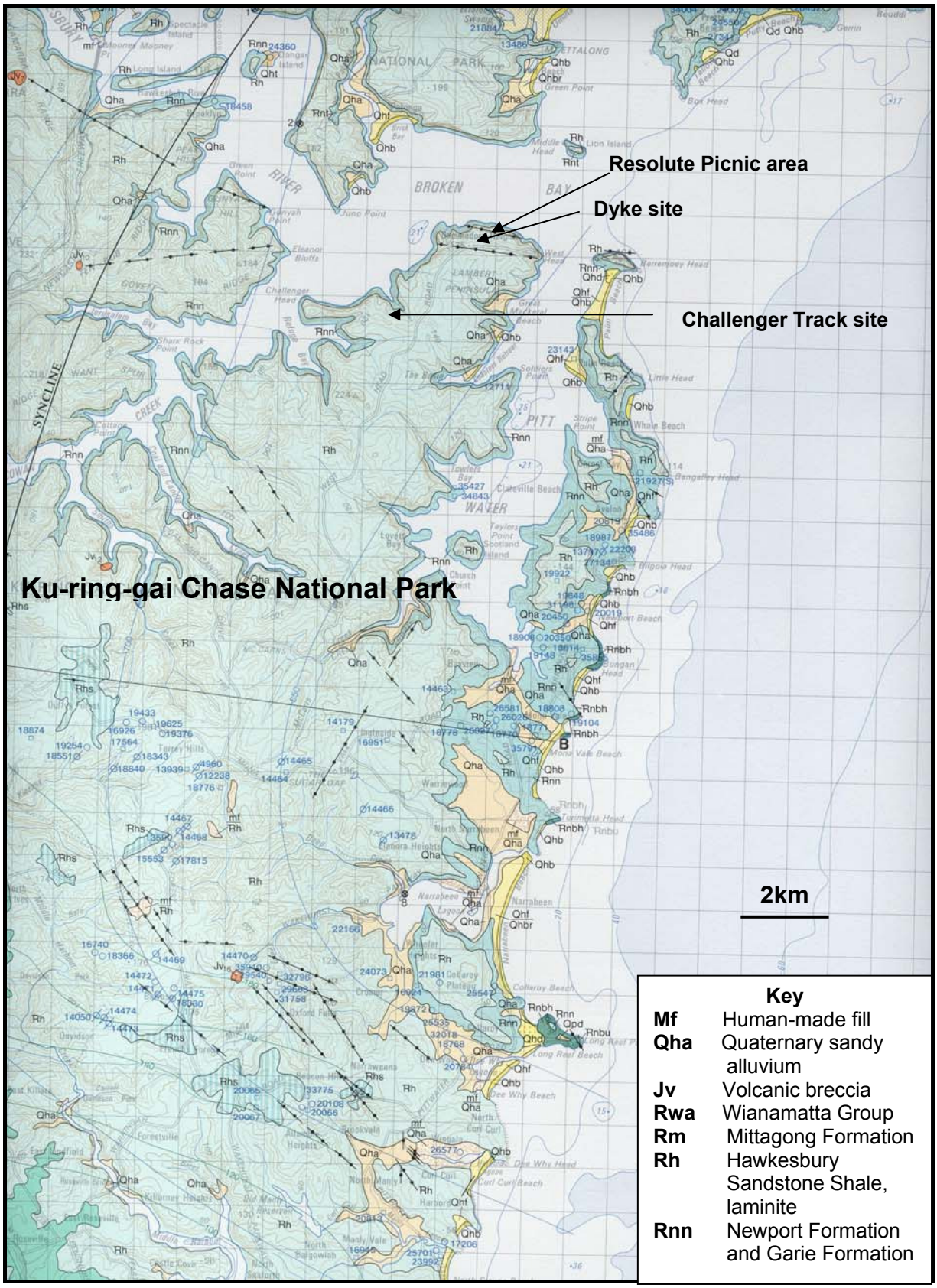


Figure 2.2 **Geology of West Head Peninsula, Ku-ring-gai Chase National Park** (from Sydney 1:100000 Geological Series map, NSW Department of Mineral Resources)

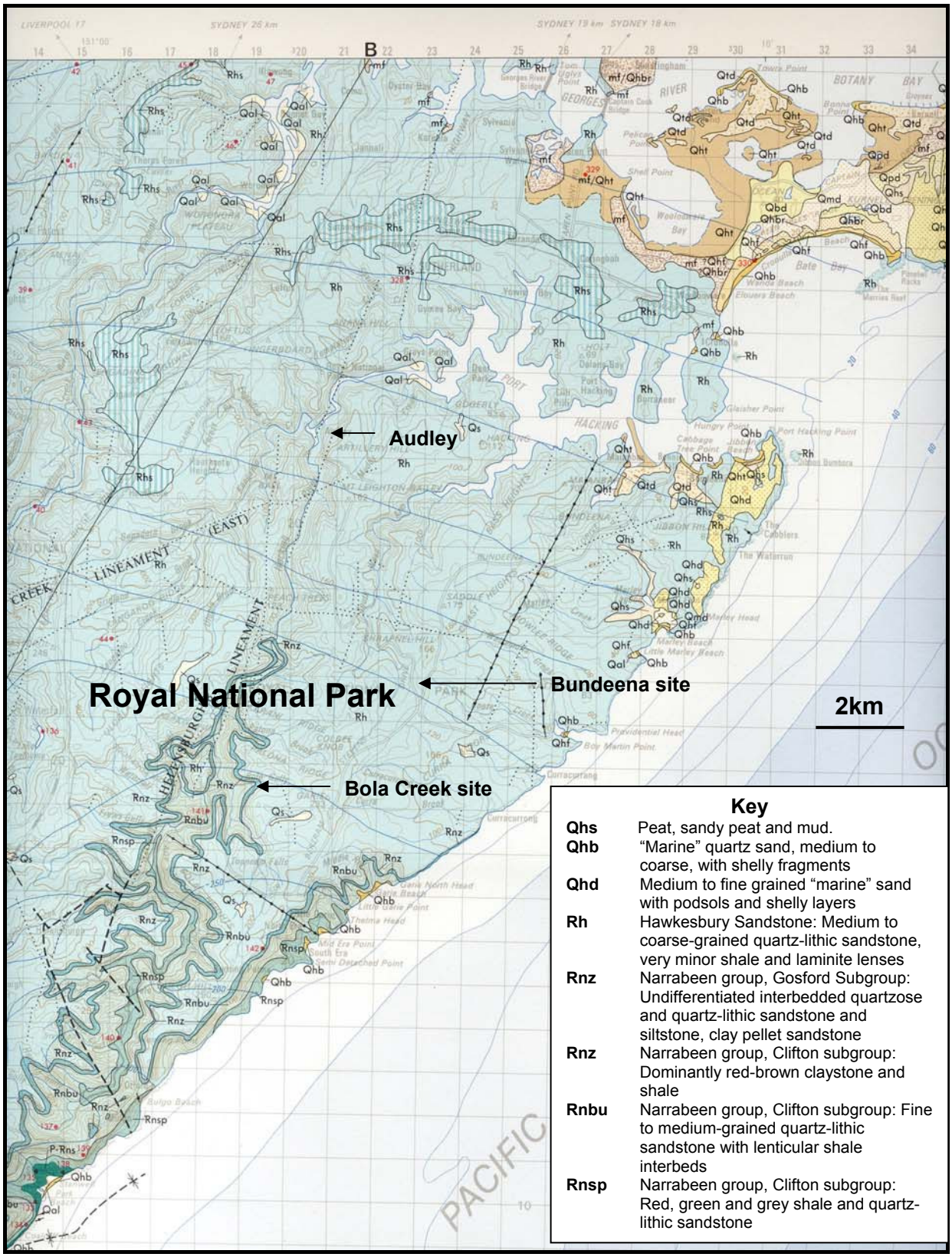


Figure 2.3 **Geology of Royal National Park** (from Wollongong-Port Hacking 1:100000 Geological Series map, NSW Department of Mineral Resources)

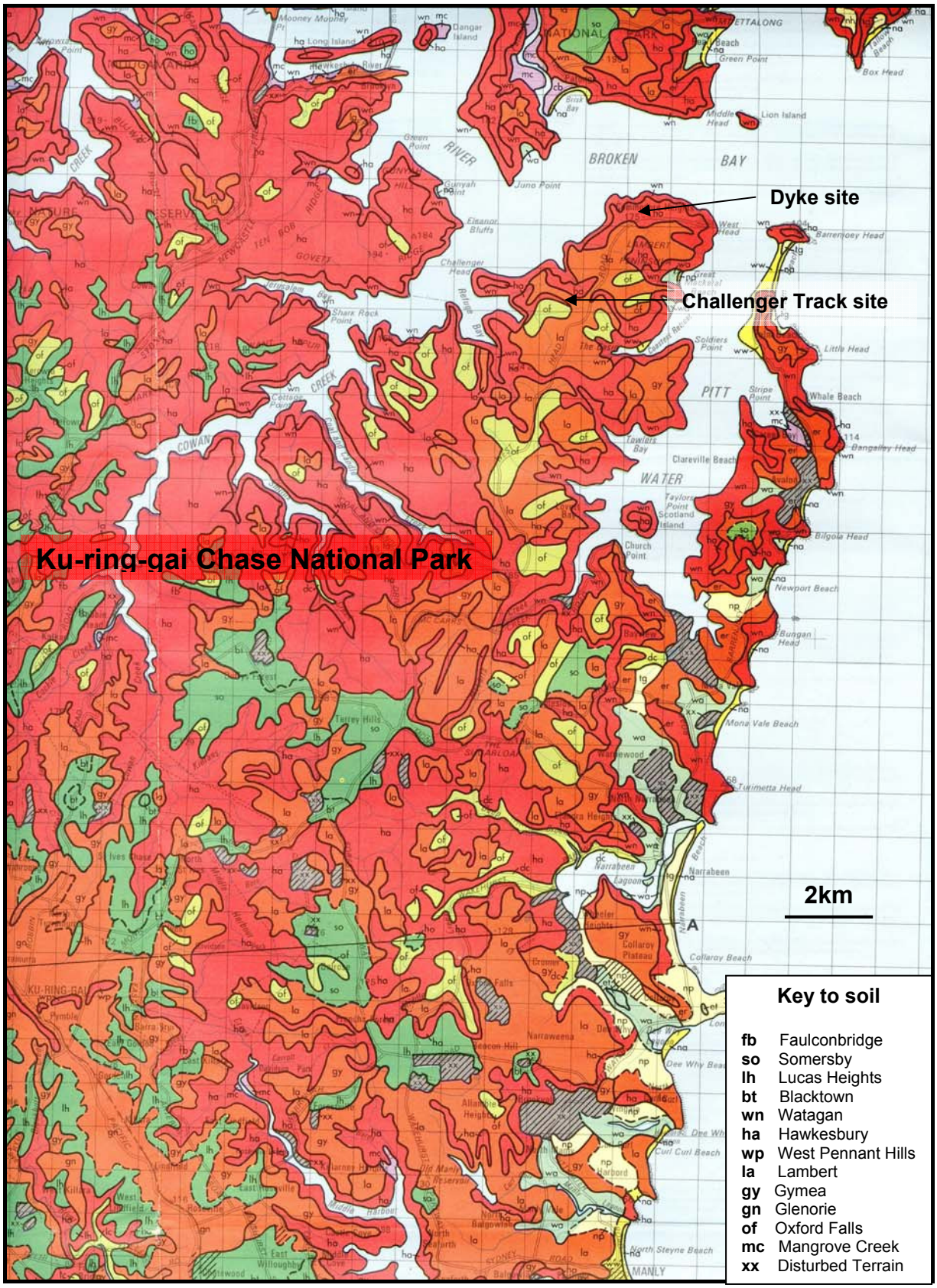


Figure 2.4 **Soil landscape of West Head Peninsula, Ku-ring-gai Chase National Park** (from Soil landscapes of the Sydney 1:100000 sheet compiled by Chapman, G.A. and Murphy, C.L.)

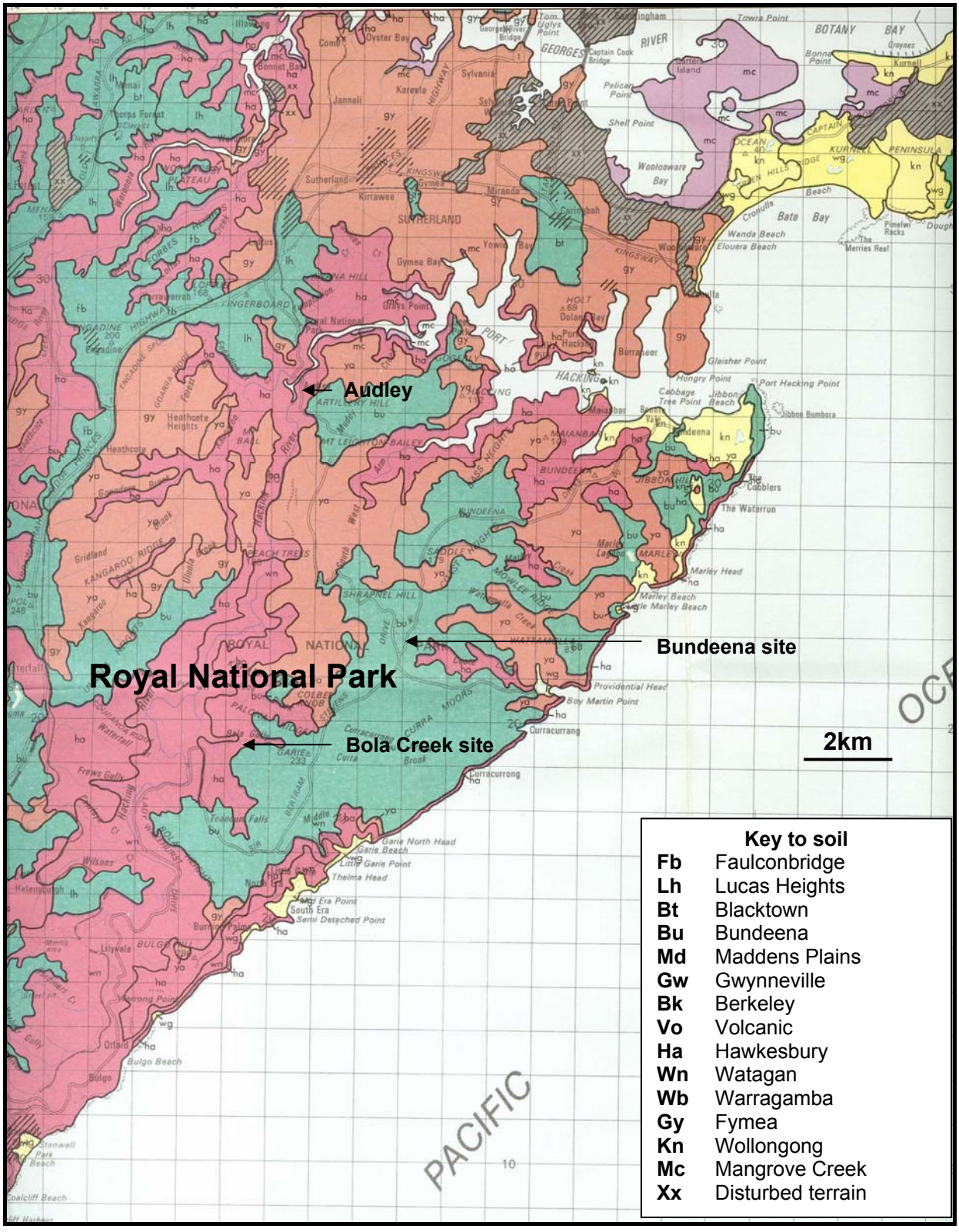


Figure 2.5 Soil landscape of Royal National Park (from Soil landscapes of the Wollongong-Port Hacking 1:100 000 sheet compiled by Hazelton, P.A., Bannerman, S.M. and Tillie, P.J)

There was below average rainfall (the average being 1322 mm) during the period of study (Fig. 2.6). From the St. Ives data, there were three months in 2002, six months in 2003, and three months in 2004 that received over 100 mm of rain (Fig. 2.7), and 13 months out of 36 with greater than 10 raindays (Fig. 2.7). From the incomplete Audley dataset, the number of months that received over 100 mm of rainfall were two months in 2002, about two months in 2003 and at least one month in 2004 (Fig. 2.7). Six months out of 28 received over 10 days of rainfall during 2002 to 2004.

Table 2.1 Monthly maximum and minimum air temperatures (^{o}C) for Sydney Airport 2002-2004 (elevation 6m)

	2002		2003		2004	
Month	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**
January	37.4	16.1	38.3	16.7	35.0	15.8
February	32.1	15.3	33.8	16.9	36.5	16.1
March	36.0	17.1	31.5	13.5	40.3	15.9
April	27.5	13.0	27.1	11.8	31.5	11.2
May	25.5	8.2	26.4	9.2	27.0	6.9
June	24.8	5.4	24.4	6.8	25.4	6.0
July	22.3	3.6	23.8	4.4	25.0	6.2
August	27.3	6.1	25.8	5.8	25.6	6.9
September	30.8	6.9	34.2	7.0	30.8	6.3
October	34.4	8.9	30.1	7.2	39.1	11.5
November	37.4	11.8	29.3	10.4	40.0	13.2
December	36.9	13.6	39.0	16.5	41.8	13.2

Australian Government Bureau of Meteorology data sent on CD March 2005

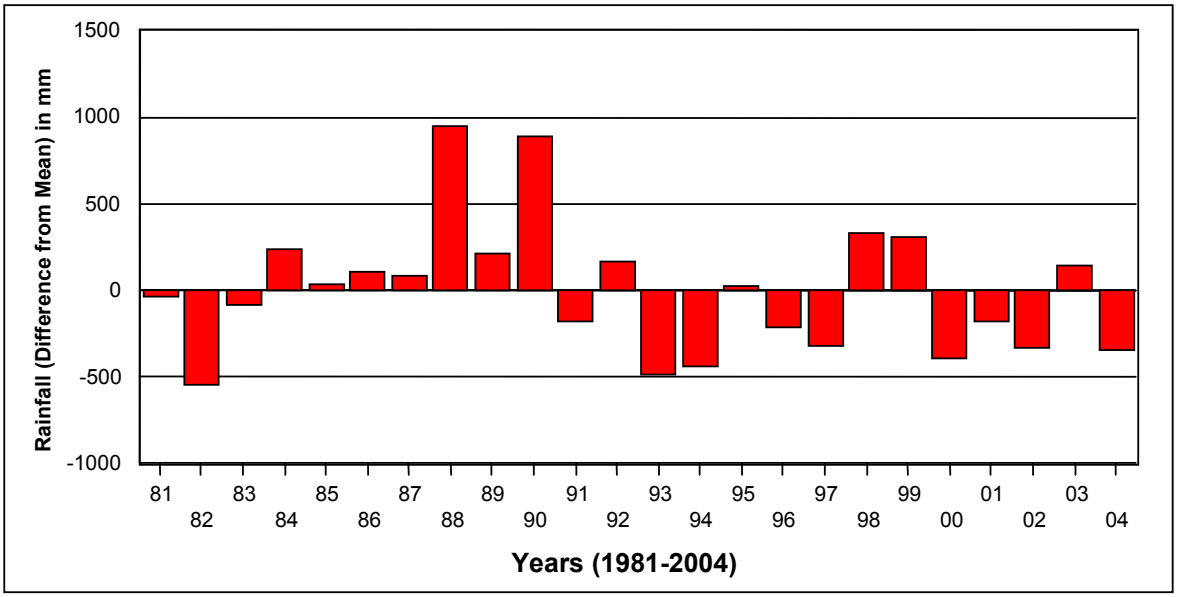
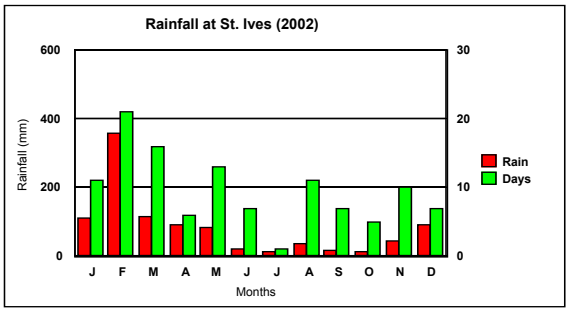
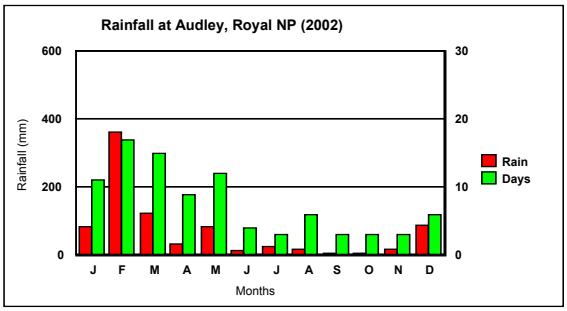


Figure 2.6 Difference between total rainfall of year and mean rainfall of period (1981 to 2004) at St Ives in millimetres



(A)



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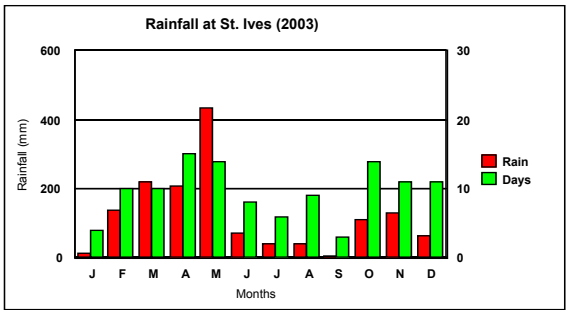
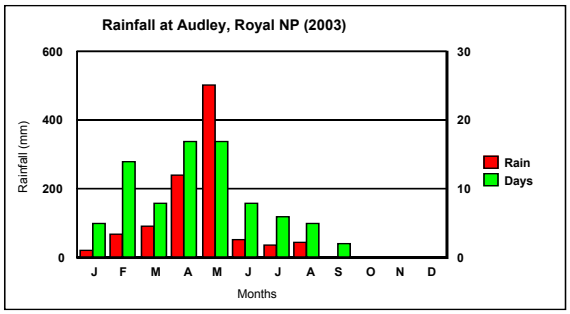
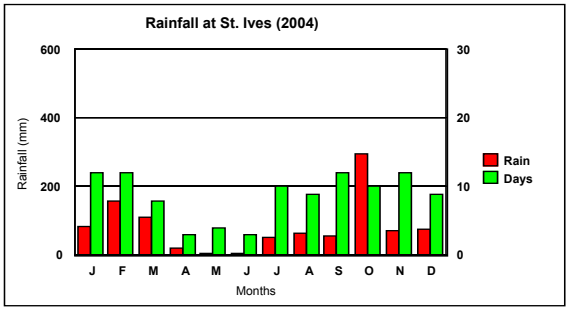


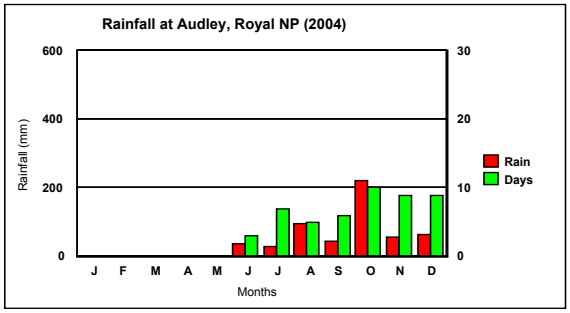
(B)



(E)



(C)



(F)

Figure 2.7 **Rainfall at St Ives and Audley (2002-2004).** St Ives data courtesy of J.H & E.S. Laxton Environmental Consultants P/L. Audley data courtesy of the Australian Government Bureau of Meteorology. Bargraphs show the two recording stations had similar patterns of rainfall.

2.3 Geology and soils

Both localities are situated in the Sydney Basin, a major depositional basin on the east coast of Australia (Bembrick et al., 1980, Ray et al., 1996).

The infertile sites in Ku-ring-gai Chase National Park and Royal National Park are on the Tertiary geological deposits of Hawkesbury Sandstone (Sydney and Wollongong Port Hacking 1:100 000 geological map sheets, NSW Department of Mineral Resources. Fig. 2.2, 2.3). Hawkesbury Sandstone-derived soils are

described as loose, coarse quartz sand that has high permeability, low available water holding capacity, low fertility, very high aluminium concentrations and pH ranges from 4 to 6 (Chapman & Murphy, 1989).

The more fertile site in Ku-ring-gai Chase National Park was located on an approximately 20 m wide igneous dyke (Fig. 2.4), which has weathered to form deep red soils with a higher nutrient content than the surrounding areas.

The more fertile site in Royal National Park was located on Narrabeen Shales (Fig. 2.5). Soils belong to the Watangan soil group and are described as shallow to deep (30-200 cm) loose, stony, brownish black fine sandy or clay loam that has strong acidity and high potential aluminium concentrations (Hazelton & Tille, 1990).

The higher nutrient sites in Ku-ring-gai Chase and Royal National Parks are surrounded by infertile siliceous sandy soils derived from Hawkesbury Sandstone. They are, in essence, biogeographic islands.

2.4 Soil chemistry

Four soil samples from each site were collected from randomly chosen locations and analysed for pH, percent water content, percent organic matter, total Kjeldahl Nitrogen (free ammonia and organic nitrogen compounds), Nitrite-N $(NO_2)^-$, Nitrate-N $(NO_3)^-$, total Nitrogen, Orthophosphate-P, and total Phosphorus concentrations (Table 2.2).

Samples were collected with a 10 cm x 10 cm core. Surface leaf litter was removed, and soil was sieved through a 2 mm mesh. Samples were analysed by J.H. & E.S. Laxton Environmental Consultants P/L. The pH was measured with a Yeo-Kal 611 SDL. Water content was measured by placing a weighed sample of fresh soil into an incubator (45^{o}C for 48 hours) and drying to a constant weight. Percent organic matter was determined by combustion. The dried sample was placed into a muffle furnace at 550^{o}C for 90 minutes and then reweighed. Nitrites and nitrates were determined by the sulphanilamide/n(-1 naphthyl) ethylene

diaminodihydrochloride method (based on Greenberg et al., 1985). Orthophosphate determinations were done by the single solution method, Acidic molybdate/ ascorbic acid/ antimonyl tartrate (based on Greenberg et al., 1985). Total Kjeldahl nitrogen and total phosphorus analyses were determined using the Kjeldahl digestion method (based on Greenberg et al., 1985).

Soils from Bola Creek, RNP and the Dyke, KCNP had higher concentrations of total nitrogen, percent organics and total phosphorus concentrations than the soils collected from the dry sclerophyll sites of Challenger, KCNP and Bundeena, RNP (Table 2.2). Bola Creek soils were the most fertile, followed by the Dyke soils and then the two dry sclerophyll sites. The more fertile soils also had higher percent water content than the dry sclerophyll sites. The highest concentrations of oxidized nitrogen and orthophosphate phosphorus occurred at the Bola Creek site in Royal National Park (Table 2.2).

Table 2.2 Mean pH, water content and concentrations of Nitrogen (mg-N/kg) and total phosphorus (mg-P/kg) for soils collected from study sites in Ku-ring-gai Chase and Royal National Parks (n = 4 for each site). Standard deviations and maximum and minimum values shown.

Organics – organic matter; Ox N – oxidised nitrogen; TKN – total Kjeldahl nitrogen; Total N – total nitrogen; Ortho P – orthophosphate phosphorus; Total P – total phosphorus

Site		pH	Water %	Organics %	Ox N Mg-N/kg	TKN mg-N/kg	Total N mg-N/kg	Ortho P mg-P/kg	Total P mg-P/kg
Challenger Tk, KCNP	Mean	5.6	2.0	4.7	0.1	405	**405**	0.08	**180**
	Std	0.1	0.6	1.6	0.03	153	**153**	0.03	**39**
	Max	5.8	2.5	6.8	0.2	577	**577**	0.09	**220**
	Min	5.5	1.2	3.0	0.1	257	**257**	0.04	**140**
Bundeena Rd, RNP	Mean	5.8	1.4	2.7	0.2	377	**377**	0.03	**109**
	Std	0.2	0.9	1.0	0.2	81	**81**	0.01	**41**
	Max	6.0	2.3	3.9	0.4	479	**479**	0.05	**156**
	Min	5.5	0.5	1.7	0.1	288	**288**	0.02	**64**
Dyke, KCNP	Mean	5.7	8.5	11.1	0.09	405	**626**	0.07	**227**
	Std	0.2	0.3	4.4	0.02	79	**79**	0.02	**15**
	Max	6.0	12.4	14.6	0.1	728	**728**	0.09	**249**
	Min	5.5	5.6	5.1	0.1	549	**549**	0.05	**216**
Bola Ck, RNP	Mean	6.1	27.1	26.8	1.5	664	**666**	0.2	**291**
	Std	0.2	9.0	9.7	0.02	202	**202**	0.1	**46**
	Max	6.4	36.3	39.3	3.0	893	**894**	0.33	**337**
	Min	6.0	18.1	18.8	0.4	409	**410**	0.07	**237**

Two-way ANOVAs are shown in Table 2.3. Locality (KCNP and RNP) and Soil ("fertile"/ "infertile") were the factors. Prior to analysis, the variables water, organic

nitrogen, orthophosphate phosphorus and oxidised nitrogen were logged to normalise the distribution.

Table 2.3 Results of Two-way ANOVAs for pH, water content, organic matter (Organics %), oxidised nitrogen (Ox N), total Kjeldahl nitrogen (TKN), total nitrogen (Total N), orthophosphate-P (Ortho P) and total phosphorus (Total P). Factors were Locality (KCNP and RNP) and Soil ("fertile" and "infertile").

Response Variable	Indicator variables	DF	F	P
pH	Locality	1	8.0	0.01
	Soil	1	6.2	0.01
	Locality:Soil	1	1.5	0.25
Water %	Locality	1	1.8	0.21
	Soil	1	94.2	<0.001
	Locality:Soil	1	12.9	0.001
Organics %	Locality	1	0.9	0.37
	Soil	1	63.4	<0.001
	Locality:Soil	1	14.2	0.001
Ox N	Locality	1	18.4	0.001
	Soil	1	11.4	0.001
	Locality:Soil	1	18.2	0.001
TKN	Locality	1	0.01	0.94
	Soil	1	13.4	0.001
	Locality:Soil	1	0.2	0.64
Total N	Locality	1	47.7	0.001
	Soil	1	19.4	0.001
	Locality:Soil	1	0.2	0.68
Ortho P	Locality	1	0.08	0.78
	Soil	1	12.5	0.001
	Locality:Soil	1	13.6	0.001
Total P	Locality	1	2.4	0.84
	Soil	1	7.8	<0.001
	Locality:Soil	1	8.7	0.003

2.5 Vegetation of the sites

The resource-poor sites were characterised by sclerophyll heath vegetation. The higher resource sites were characterised by wet sclerophyll forest (dyke, KCNP) or temperate rainforest (Bola Creek, RNP). There was no plant species overlap between the soils of contrasting fertility, and overlap between the sites of similar fertility.

The sites supported relatively intact vegetation with no exotic plant species recorded in a fixed 20 m x 20 m sampling area. The more fertile sites at the Dyke, KCNP and Bola Creek, RNP were characterised by a greater diversity of tree species and vines, but lower shrub and ground layer (herbs, graminoids and ferns) species diversity than the infertile sites (Table 2.5).

Table 2.5 Vegetation structure for paired sites in Ku-ring-gai Chase (KCNP) and Royal National (RNP) Parks

Sites	Total plant species	Canopy species	Shrub species	Ground layer	Vines
Challenger Tk KCNP	59	4	39	15	1
Bundeena Rd RNP	71	4	46	19	2
Dyke KCNP	50	10	14	19	7
Bola Ck RNP	38	19	5	8	6

Table 2.6 Tree species found within a 20 m x 20 m sampling area at four sites

Species	Challenger KCNP	Bundeena RNP	Dyke KCNP	Bola Ck RNP
Acacia floribunda			X	
Acmena smithii				X
Allocasuarina distyla	X	X		
Allocasuarina torulosa			X	
Angophora floribunda			X	
Backhousia myrtifolia				X
Banksia serrata	X	X		
Ceratopetalum apetalum				X
Claoxylon australe				X
Corymbia gummifera	X	X		
Cryptocarya microneura				X
Cryptocarya sp.				X
Diospyros australis				X
Diploglottis australis				X
Doryphora sassafras				X
Eucalyptus haemastoma	X	X		
Eucalyptus paniculata			X	
Eucalyptus piperita			X	
Eucalyptus scias subsp. scias			X	
Gmelina leichardtii				X
Livistona australis			X	X
Notelaea venosa				X
Pittosporum undulatum				X
Rapanea variabilis			X	X
Schizomeria ovata				X
Sloanea australis				X
Stenocarpus salignus				X
Syncarpia glomulifera			X	X
Synoum glandulosum			X	
Trochocarpa laurina				X

Four canopy species (*Allocasuarina distyla, Banksia serrata, Corymbia gummifera* and *Eucalyptus haemastoma*) were recorded at both of the infertile sites, but were not recorded at either of the more fertile sites (Table 2.6). There was a higher diversity of canopy species unique to the more fertile sites than recorded at the infertile sites. There were only three canopy species in common between the two fertile sites (Table 2.6).

There were 19 shrub species recorded in common at the two infertile sites (out of 39 shrub species at the Challenger track site, KCNP and the 46 shrub species at the Bundeena road site, RNP). At the more fertile sites, there was one shrub species (*Citriobatus pauciflorus*) in common out of 14 shrub species recorded at the dyke (KCNP) and the 5 shrub species recorded at Bola Creek (RNP). None of the shrub species at the infertile sites were recorded at the more fertile sites. Similarly, shrub species from the more fertile sites were not recorded at the infertile sites (Table 2.7).

Table 2.7 Shrub species found within a 20 m x 20 m sampling area at four sites

Species	Challenger Tk KCNP	Bundeena Rd RNP	Dyke KCNP	Bola Ck RNP
Acacia echinula		X		
Acacia linifolia			X	
Acacia myrtifolia		X		
Acacia suaveolens		X		
Angophora hispida	X	X		
Astrotricha floccosa			X	
Baeckea diosmifolia	X			
Baeckea sp.		X		
Banksia ericifolia	X	X		
Banksia oblongifolia	X			
Boronia ledifolia	X	X		
Boronia pinnata	X			
Boronia serrulata	X			
Bossiaea heterophylla		X		
Bossiaea scolopendria	X	X		
Brachyloma daphnoides		X		
Breynia oblongifolia			X	
Bursaria spinosa var. spinosa			X	
Calytrix tetragona	X	X		
Cephalaralia cephalobotrys				X
Citriobatus pauciflorus			X	X
Conospermum ellipticum		X		
Conospermum ericifolium	X			
Cryptandra amara var. amara		X		

Species	Challenger Tk KCNP	Bundeena Rd RNP	Dyke KCNP	Bola Ck RNP
Dampiera stricta	X	X		
Darwinia fascicularis subsp. Fascicularis	X	X		
Dillwynia floribunda	X			
Dillwynia retorta	X	X		
Dillwynia sp.		X		
Epacris microphylla	X			
Epacris pulchella	X			
Euryomyrtus ramosissima		X		
Gompholobium grandiflorum		X		
Gompholobium sp.		X		
Grevillea buxifolia	X			
Grevillea oleoides		X		
Grevillea sericea	X			
Grevillea speciosa	X			
Grevillea sphacelata		X		
Hakea dactyloides	X			
Hakea gibbosa	X	X		
Hakea laevipes subsp.		X		
Hakea teretifolia	X			
Hemigenia purpurea	X	X		
Hibbertia monogyna	X			
Hibbertia sp. (unidentified)		X		
Isopogon anethifolius	X	X		
Kunzea capitata	X	X		
Lambertia formosa	X	X		
Lasiopetalum sp.		X		
Leptospermum arachnoides		X		
Leptospermum trinervium	X	X		
Leucopogon esquamatus	X			
Leucopogon microphyllus	X	X		
Lomatia silaifolia		X		
Macrozamia communis			X	
Maytenus silvestris				
Micrantheum ericoides		X		
Micromyrtus ciliata	X			
Mirbelia rubiifolia	X			
Notelaea longifolia			X	
Persoonia lanceolata	X	X		
Persoonia linearis			X	
Persoonia pinifolia	X			
Petrophile pulchella	X	X		
Philotheca buxifolia		X		
Phyllota phylicoides	X			
Pimelea linifolia	X			
Platysace linearifolia		X		
Pomaderris ferruginea			X	
Prostanthera howelliae			X	
Psychotria loniceroides				X
Pultenaea daphnoides			X	

Species	Challenger Tk KCNP	Bundeena Rd RNP	Dyke KCNP	Bola Ck RNP
Pultenaea elliptica	X	X		
Pultenaea flexilis			X	
Pultenaea sp. (unidentified)		X		
Tasmannia insipida				X
Telopea speciosissima		X		
Tetratheca juncea			X	
Wilkiea huegeliana				X
Woollsia pungens		X		
Xanthorrhoea arborea			X	
Xanthorrhoea media	X	X		
Xanthorrhoea resinifera		X		
Xanthorrhoea sp.		X		

The site with the lowest diversity of ground layer plants was Bola Creek, RNP (Table 2.5).

The architectural characteristics of plant species studied at each site, along with the known habitats and flowering times are summarized in Appendix 2.

2.6 Fire history

Fire is one of the physical factors influencing the Australian environment to which native plants and animals have become adapted (NSW NPWS, 2002a). Available records show that over the last 50 years, most of Ku-ring-gai Chase National Park has been subject to a fire frequency of 10 -15 years, particularly the ridges and upper slopes (NSW NPWS, 2002a). There has been an average of 10 small (usually less than 5 hectares) wildfires each year, with extensive wildfires (over 500 hectares) occurring in 1943, 1946, 1958, 1965, 1968, 1971, 1979, 1980, 1983, 1990, 1994 and 2004 (NSW NPWS, 2002a). The January 1994 fire burnt 7110 hectares or almost half the park. After the 1994 fires, it was estimated that only about 1% of the park was unburnt for more than 21 years (Conroy, 1996). Approximately 1400 hectares of bushland in Ku-ring-gai Chase National Park was burnt in January 2004 (Cowper, 2005). The lack of charcoal on trees along Challenger track suggests the study site has not been burnt recently. However, charcoal scarring on trees in the Dyke indicate fire has impacted the site.

Extensive fires have also occurred in Royal National Park. In 1965 and again in October 1988, over 50 percent of Royal National Park was burnt. In January 1994 over 90% of the Park was burnt. In 2001/02, extensive fires occurred again with approximately 60% of Royal National Park being burnt. The majority of these fires were considered to have been deliberately lit (NSW NPWS 2000, 2002b).

The infertile site along Bundeena Road (RNP) has been burnt four times since 1978/79, and once in 2001/2002. The more fertile site along Bola Creek has also experienced fire in recent years.

2.7 Conclusions

According to the resource availability hypothesis (Coley et al., 1985), resources such as soil nutrients, water and light influence the characteristics of leaves which subsequently effect herbivores.

The paired sites in the two localities were appropriate sites for the current study. Climatic conditions (rainfall and air temperatures) were similar for both localities, and the sites designated as lower resource sites had similar soil nutrient levels (percent organic matter, total nitrogen and total phosphorus), but lower nutrient levels than sites designated as higher resource sites.

There was a similarity in plant species composition in the infertile sites and substantial differences between the infertile and more fertile paired sites.

Chapter 3
Leaf characteristics and resource availability

3.1 Introduction

Herbivory can affect individual plants by reducing growth (Meyer et al., 1993), fitness (Marquis, 1984) and reproductive capacity (Belskey, 1986; Crawley, 1987), and can affect plant communities by influencing competitive outcomes and community composition (Harper, 1969; Kulmon, 1971; Chew, 1974; Morrow et al., 1978; Prins et al., 1990). To inhibit or prevent consumption by animals, plants have evolved an extensive array of chemical, physical and phenological characteristics.

Plant traits that contribute to plant palatability include water and nitrogen content (McNeil et al., 1979; Lightfoot et al., 1987). Young leaves (Kursar et al., 1991), and leaves from fast-growing or successional plant species (Mattson, 1980) are considered vulnerable to herbivores because they are succulent and frequently contain higher concentrations of nitrogen. Low nitrogen and water content in leaves may be a defensive strategy by plants to reduce herbivory (Neuvonen and Haukioja, 1984).

Secondary metabolites reduce digestibility and plant nutritional quality by binding to digestive enzymes and dietary proteins (Robbins et al., 1987). These chemicals can affect the nervous systems, and cardiac functions of herbivores (van Alstyne, 1988), and often make plants toxic or bitter tasting (Moles & Westoby, 2000). In terrestrial plants, secondary metabolites such as condensed tannins (Coley, 1986; Feeny, 1976; Rosenthal et al., 1979), alkaloids (McKey, 1974; Levin, 1976; Levin et al., 1978) and cyanogenic glycosides (Jones et al., 1978) may be associated with reduced herbivory in field and laboratory experiments (Coley, 1986; Shure and Wilson, 1993; Behmer et al, 2002). Terpenoids may also play an important

role in reducing the impact of grazers on populations of marine plants (van Alstyne, 1988; Hay et al., 1987; Hay et al., 1994; Hay, 1991).

Secondary metabolites are not always effective defences against herbivores. Many insect species consume plants containing highly toxic chemicals without ill effect (Brattsten, 1979). Some insects are also able to sequester toxins to use against predators (Brower, 1969; Rothschild, 1973; Nishida, 2002). Small marine sedentary herbivores such as amphipods, small crabs and gastropods are frequently resistant to chemicals found in seaweeds (Duffy et al., 1994). Some plants therefore require additional strategies to defend photosynthetic tissues.

Structural characteristics such as toughness (Coley, 1983a; Raupp, 1985; Iddles et al., 2003; Lucas et al., 2000; Read et al., 2003) and fibre content (Coley, 1983a; Coley and Aide, 1991) play a significant role in inhibiting herbivory. Plants with high force of fracture and toughness may deter herbivores by wearing out contact parts such as mandibles and teeth, or preventing animals from shearing leaves (Lucas et al., 2000; Sanson et al., 2001). Other structures that can reduce herbivore consumption are trichomes such as spines and hairs (Campbell, 1968; Grubb, 1992). Whilst not always effective (Pollard, 1986; Hulley, 1988; Potter et al., 1988; van Dam and Hare, 1998), trichomes have been observed to prevent some sucking (Hoffman and McEvoy, 1985) and chewing (Ramalho et al., 1984; Oghiakhe et al., 1992) insects from feeding and moving about on leaves. Surface waxes may also protect leaves from insect herbivory (Edwards, 1982; Peeters, 2002a).

Herbivores prefer generally young leaves to mature leaves (Coley, 1980; Basset, 1991). Leaf toughness is not an effective defence for immature leaves because fibre, lignin and cuticular thickening constrain leaf expansion (Aide, 1993). Other strategies must therefore exist to reduce losses to herbivores. It has been suggested that phenological characteristics of young leaves such as delayed greening (Kursar & Coley, 1992), fast expansion rates (Aide et al., 1989; Kursar et al., 1991; Moles & Westoby, 2000; Moles & Westoby, 2003), and timing of leaf production (Aide, 1988) may influence the degree to which leaves are vulnerable to herbivores. Delayed greening in leaves involves a delay in the input of

chlorophyll, rubisco, nitrogen and energy. This mechanism may have evolved to minimise losses to herbivores by delaying input of valuable resources until after the leaf is fully expanded and better protected by toughness (Kursar & Coley, 1992). Fast expansion rates in maturing leaves have the potential to reduce herbivore damage by shortening the developmental period and by employing mechanical defences like toughness more rapidly (Hay et al., 1988; Aide et al., 1989; Aide, 1993). Synchronous leaf production may also reduce damage to young leaves by satiating herbivores (Aide, 1993), and timing of leaf production could result in plant species avoiding peaks in herbivore emergence (Aide, 1988).

The general aim of this chapter is to compare leaf traits of plants growing in nutrient-poor, dry sclerophyll vegetation to those of nutrient-richer wet sclerophyll/ temperate rainforest environments. The chemical and physical characteristics of mature, immature and maturing leaves were the focus of the study. The specific aim was to test one expectation of the resource availabiltiy hypothesis, that leaves of plants from low resource environments will be better defended both physically and chemically, live longer and have slower growth rates (Coley et al. 1985). While numerous studies have investigated the relationship between soil nutrients and relative growth rate (Bradshaw et al., 1960; Clarkson, 1966; Rorison, 1968; Christie, 1975; Grime et al., 1975; Chapin III, 1980), few have explored the relationship between physical and chemical leaf characteristics and soil nutrients.

3.2 Plant species

Plant species were chosen for the study in such a way as to construct a set of phylogenetically independent contrasts (Felsenstein, 1985; Burt, 1989; Armstrong & Westoby, 1993). This method uses phylogenetic relationships to establish independent cases of evolutionary divergence (Armstrong & Westoby, 1993). Each independent contrast serves as a statistical replicate for testing whether the presence of an evolved trait is associated with (1) the existence of another trait, or (2) species' environment (eg. soil nutrients).

Thirteen phylogenetically independent contrasts (PICs) were selected (Figure 3.10). Each PIC consisted of one plant species found at one of the nutrient-rich

sites, paired with one found at the nutrient-poor sites, within the same locality (Fig. 3.1). For each pair to be a phylogenetically independent contrast, the phylogenetic path connecting two species must be independent of the path connecting any other two species. Graphically, this means that the path connecting a pair cannot meet or share a vertical line with the path connecting another pair. The phylogenetic tree was based on within-family taxonomies from the *Flora of Australia* (1982, 1984, 1988).

Plant species were also selected to represent a wide range of plant families, and different growth forms (trees, shrubs, herbs and vines) at each site (Appendix 2).

3.3 Methods

Traits of mature leaves were assessed for forty-six common plant species (at least fifteen species per site), representing twenty-eight families (Fig. 3.1). Traits of immature leaves were assessed on a subset of thirty-nine plant species from twenty-four plant families. The phenology of leaf maturation was assessed for nine plant species from five families (Fig. 3.1).

In the mature leaf trait study, all leaf variables were measured on fully-matured expanded leaves. Young expanding leaves less than a third the size of an average mature leaf were measured for each species in the immature leaf trait study. Phenology of leaf maturation was monitored from bud to full expansion. Where possible, immature leaves were collected from the same plants as mature leaves.

Leaf traits measured on mature, immature and expanding leaves were:

- Lamina thickness,
- Force of fracture and toughness,
- Leaf area and specific leaf area,
- Percent water content,
- Total nitrogen and total carbon concentrations, and
- Total phenol concentrations

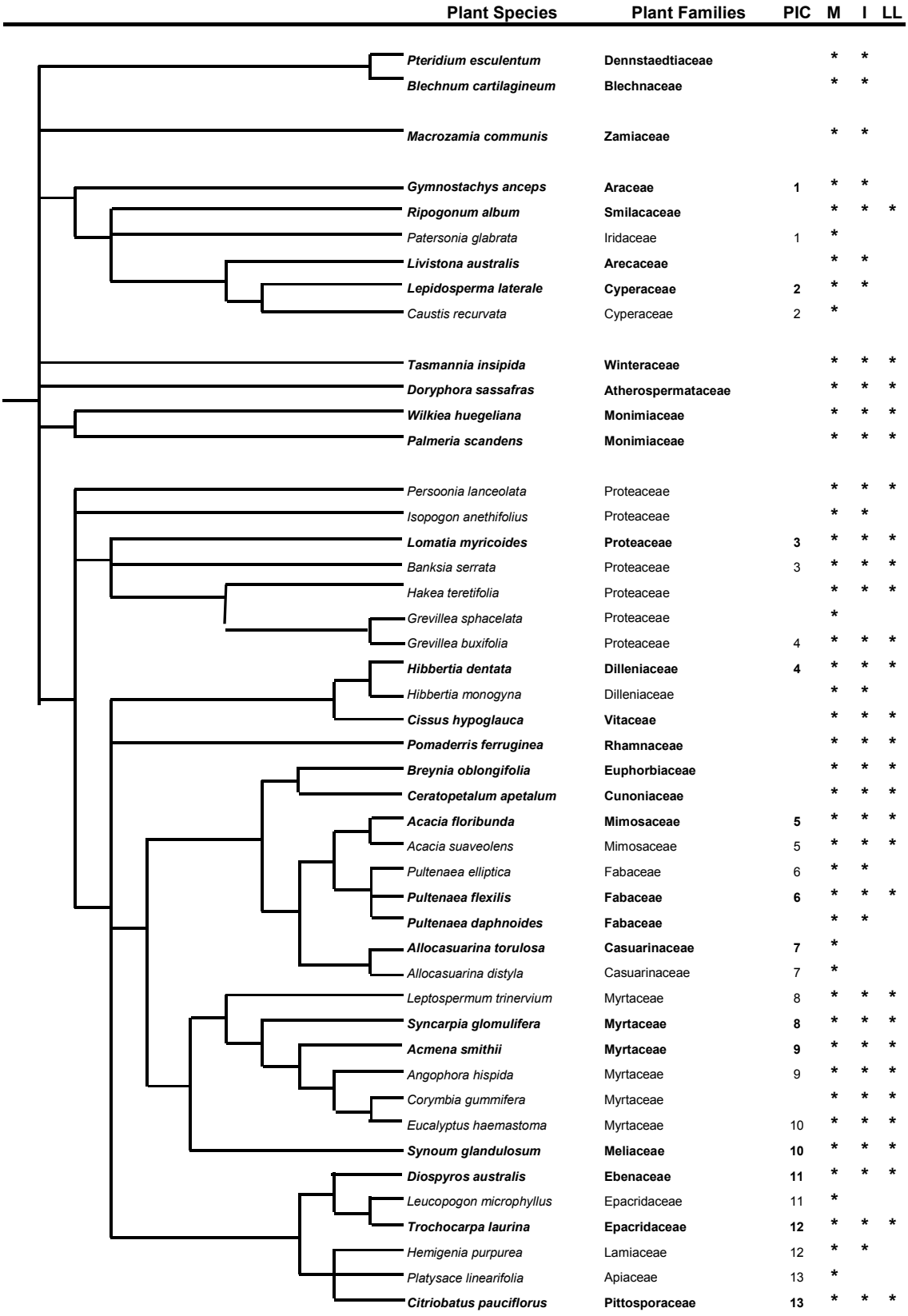


Figure 3.1 Phylogenetic relationships of plant species from paired low and higher resource sites analysed for chemical and physical characteristics. Branch lengths are not proportional to time since evolutionary divergence. (* indicate species used in mature (M) and immature (I) leaf trait studies and leaf lifespan (LL) study. Species in bold are from higher resource sites.)

Due to a limited supply of plant material, only mature leaves were tested for:

- Condensed tannin concentrations,
- Presence/ absence of alkaloids,
- Presence/ absence of cyanogenic glycosides,
- Neutral Detergent Fibre (hemicellulose, cellulose, lignin, cutin, silica and some CW-protein)
- Acid Detergent Fibre (cellulose, lignin, cutin and silica), and
- Acid Detergent Lignin (lignin, cutin and some silica)

At each site (Fig.2.2; Fig.2.3), during the early mornings, up to five leaves were clipped randomly from five individual plants representing each plant species (i.e. sample size approximately 25 leaves per species at each site). Where possible, sunlit leaves were collected. Leaves were then wrapped separately in paper towelling, placed in plastic bags and moistened with water. They were refrigerated at 4^{o}C and processed within 72 hours of collection. No individual plant was sampled more than once.

Fresh leaves were weighed and were either photocopied or scanned directly to obtain leaf area. Leaf area was determined with a flat-bed scanner and DELTA-T SCAN software (Delta-T, Cambridge, UK).

Leaf thickness was measured at three to four points per leaf with a dial gauge micrometer. Major veins and the midrib were avoided on all but the smallest leaves.

Force of fracture, or the amount of energy (work) required to fracture a leaf (Lucas et al., 1990), was determined using a purpose-built leaf-cutter designed to provide a generalised measure of physical defence (Wright & Cannon, 2001). The midrib was dissected from all leaves, which were cut at the widest point along the lamina or halfway between the leaf base and tip. The leaf-cutter measures the force required to cut a leaf at a constant cutting angle (20^{o}) and speed. The cutting blade is supported by a cantilevered arm, which rises and falls according to the direction of rotation of a lead screw driven by a computer-controlled stepper motor.

The leaf is placed on an anvil, providing a reference face against which sample shearing occurs. The force of fracture is concentrated in the centre of a double concave, thin section of the cantilevered arm, and measured via paired strain gauges mounted on either side of the arm at this point. Output from the strain gauges is in the form of a series of force measurements taken at regular intervals as the blade traverses the sample (9.1 Hz, equivalent to every 0.03 mm along the edge of the anvil), giving a force x displacement graph. The mean force of fracture for a sample was calculated as the average force registered across the cutting trajectory divided by the gain and multiplied by a conversion constant (Wright & Cannon, 2001). Toughness (N m^{-1}= J m^{-2}) is the mean force of fracture (N) divided by lamina thickness (mm).

Fresh leaves collected in the early morning, transported from the field between wet sheets of towelling and stored in a refrigerator for a few hours, were patted dry and weighed (fw). Leaves were then placed in paper envelopes, oven dried (60 ^{o}C for at least 72 hours) and weighed again for dry weight (dw). Specific leaf area (SLA in cm^2/g) and percent water content (fw – dw/ fw) were then calculated. Leaves were ground using a mill grinder, sieved through a 1mm mesh and stored in 30 mL vials ready for total nitrogen, total carbon, condensed tannin and total phenol analyses.

Total nitrogen and total carbon was analysed by a Leco CN analyzer at Macquarie University. Acetanilide was used as the reference material.

An estimate of condensed tannin concentration (expressed as absorbance) was determined using the Proanthocyanidin or Butanol-HCl method (Waterman & Mole, 1994). The process involves the hydrochloric acid catalysed depolymerization of condensed tannin in butanol to yield a red anthocyanin product that can be detected spectrophotometrically.

Total phenol concentration was measured on fresh and dried mature leaves, and on dried ground leaves for the immature leaf trait study. When leaves are dried, volatile phenolic compounds can be lost, reducing the overall concentration of phenols (Waterman & Mole, 1994). Tests on fresh leaves are thus the preferred

method for analysis of these compounds. Fresh leaves for the mature leaf trait study were collected and processed within 24 hours. Small branches or sections of plant were cut from trees, shrubs, herbs and vines and placed immediately into large sealed buckets of cool water. Leaves were transported from the field to the laboratory within 20 minutes – 1.5 hours, and finely ground using a pestle and mortar with acid-washed sand and some extractant. Total soluble phenols were determined using the Folin-Ciocalteu method (basic outline in Waterman & Mole, 1994), with 70% analytical grade ethanol as the extractant and tannic acid as the reference material (supplied by Sigma). The reaction is an oxidation-reduction reaction in which the phenolate ion is oxidized under alkaline conditions while reducing the phosphotungstic-phosphomolybdic complex in the reagent to a blue-coloured solution (Waterman & Mole, 1994).

Alkaloids are often referred to as nitrogen-based chemical compounds. In acid solution, alkaloids give precipitates with heavy-metal reagents. The presence of alkaloids in leaves was detected using the Mayer's and Dragendorff's reagents (Houghton & Raman, 1998). Prior to the main study, reagents were tested for reliability using a standard.

Cyanogenic glycosides, which are also nitrogen-based chemical compounds, were detected using the Feigl-Anger method (Brinker *et al.* 1989). For samples that gave a negative response after 24 hours, a fresh test was set up and a few drops of beta-glucosidase added (Brinker *et al.* 1989). Tannins can inhibit the hydrolysis of cyanogenic glycosides and thereby be responsible for negative tests (Brinker *et al.* 1989). Beta-glucosidase (MP Biochemicals Cat. No. 100348) prevents this from occurring. These tests were carried out in November 2004 and again in December 2004.

Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) tests were conducted by Diagnostic and Analytical Services, NSW Department of Primary Industries Industries using an Ankom Fibre Analyser (AFIA 2005). Percentages of hemicellulose, cellulose and lignin were calculated as follows:

$$\% \text{ Hemicellulose} = \text{NDF} - \text{ADF}$$

% Hemicellulose and cellulose = NDL – ADL

% Cellulose = ADF – ADL

% Lignin = ADL

3.4 Statistics

Mature and immature leaf characteristics were analysed with principal component biplots produced by the R-statistical program (http://www.r-project.org/) based on the work of Gabriel (1971) and Gabriel and Odoroff (1990). Though the principal components underlying each biplot are not themselves displayed, the length of one unit in the X-axis direction (1st principal component) is identical to the length of one unit in the Y-axis direction (2nd principal component). Points in the matrix were obtained by standardising the mean of each species for each variable. This was done by subtracting the variable (column) mean from the species (cell) mean and dividing the subsequent value by the variable or column mean. The variables leaf area, specific leaf area, leaf thickness, toughness and condensed tannins were logged prior to standardisation to make values commensurate.

To accompany the PC biplots, PAST was used to perform an analysis of similarity (ANOSIM) comparing dry sclerophyll and mesic species (Hammer et al. 2001). The ANOSIM used an Euclidean distance with 5000 permutations and was performed on the transformed dataset to reduce the colinearity and multidimensionality of the original data (Quinn & Keough 2002).

In the biplots, the numbered points in yellow represent the means of individual rainforest and wet sclerophyll leaves collected from the higher resource sites at Bola Creek, RNP and the dyke, KCNP (Figs. 3.3, 3.4, 3.6, 3.7). Numbers in orange are the means of individual dry sclerophyll leaves collected from the lower resource sites at Bundeena Rd, RNP and Challenger track, KCNP (Figs. 3.3, 3.4, 3.6, 3.7). Arrows represent the leaf trait variables. The longer the arrow the more important the leaf trait variable is in differentiating the plant communities or plant species. Arrows or leaf trait variables that subtend angles less than 90^{o} are positively correlated ($0^{o} \leq r_{xy} \leq 1$). The smaller the angle the stronger the correlation. Variables that subtend an angle of 180 ^{o} are negatively correlated ($r_{xy} \approx$

-1). Arrows or variables that subtend an angle of 90^o are uncorrelated (r_{xy}=0). The goodness-of-fit indicates the percentage of variation in the data that has been presented in the biplot.

The Welch Two Sample t-test and ANOVAs were used to compare particular traits of leaves from low nutrient environments to those of higher nutrient environments. ANOVAs were of a block design with region designated as the block and resource level as the factor. Prior to analysis, checks were made to ensure assumptions on which the tests were based were satisfied. Quantile-quantile plots (Hamilton, 1992) were used to identify any departures from normality, and *F*-tests were used to ensure population variances were equal (Everitt, 1994).

3.5 Mature leaf characteristics

Mesic leaves from sites with higher soil nutrients were characterised by greater leaf areas and specific leaf areas, and higher concentrations of nitrogen and water content compared to mature dry sclerophyll leaves from nutrient-poor sites (Fig. 3.2; Fig. 3.3). Mesic plants defined by their particularly large leaf areas were *Livistona australis* and the fern *Pteridium esculentum*. The plant species with the toughest leaves and highest cellulose concentrations were the wet sclerophyll and temperate rainforest plant species *Gymnostachys anceps*, *Lepidosperma laterale* (both monocotyledons), and *Macrozamia communis* (Fig. 3.3). The dry sclerophyll plant species *Hakea teretifolia* and *Allocasuarina distyla* had the greatest lamina thickness due to their cylindrical leaves (Fig. 3.3; Appendix 2).

(Henceforth, for convenience, leaves from wet sclerophyll and temperate rainforest environments are referred to as ‘mesic’).

Mature dry sclerophyll leaves have greater variability in their characteristics compared to mesic leaves, as evidenced by the broader cloud of points (Fig. 3.3). Mature dry sclerophyll leaves were characterised by higher concentrations of total phenols and condensed tannins, higher carbon:nitrogen ratios and thicker leaves compared to the wet sclerophyll and rainforest species (Fig. 3.3). With outliers (ie points on the periphery of the biplot, in this case points 7, 8, 22, 23, 30, 39 and 54)

removed, the dry sclerophyll species with the toughest mature leaves were shown to be *Patersonia glabrata*, *Angophora hispida* and *Banksia serrata* (Fig. 3.4). Seven dry sclerophyll plant species were also characterised by small leaf areas, while temperate rainforest plants such as *Ripogonum album*, *Wilkiea huegelianna* and *Ceratopetalum apetalum* had relatively large leaf areas (Fig. 3.4).

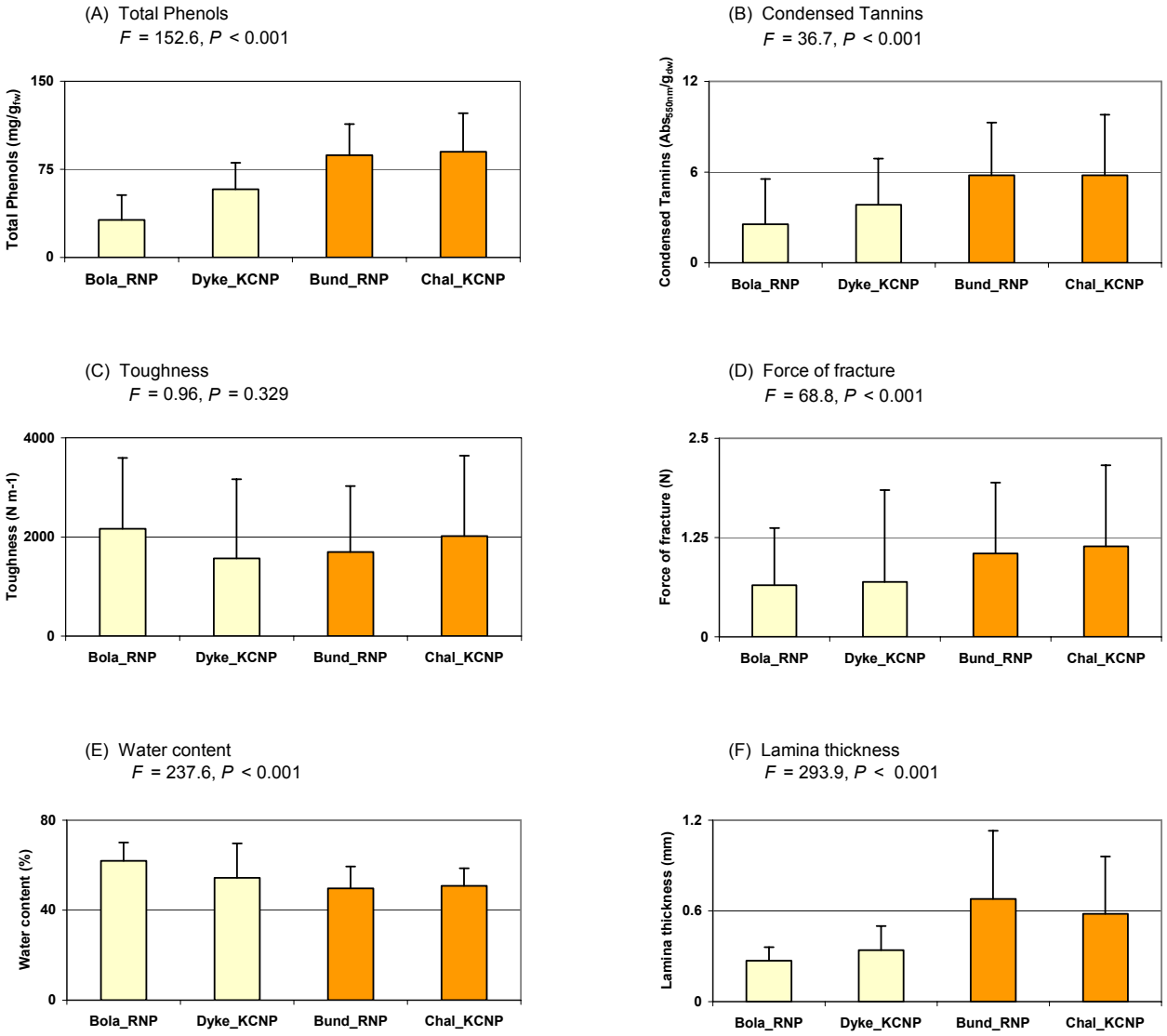


Figure 3.2(a) Overall mean mature leaf characteristics obtained for each site in Royal National Park (RNP) and Ku-ring-gai Chase National Park (KCNP), NSW Australia. One-way ANOVA testing the means of the paired sites and standard deviations are given. Yellow bars represent the more fertile sites and the orange bars the less fertile sites.

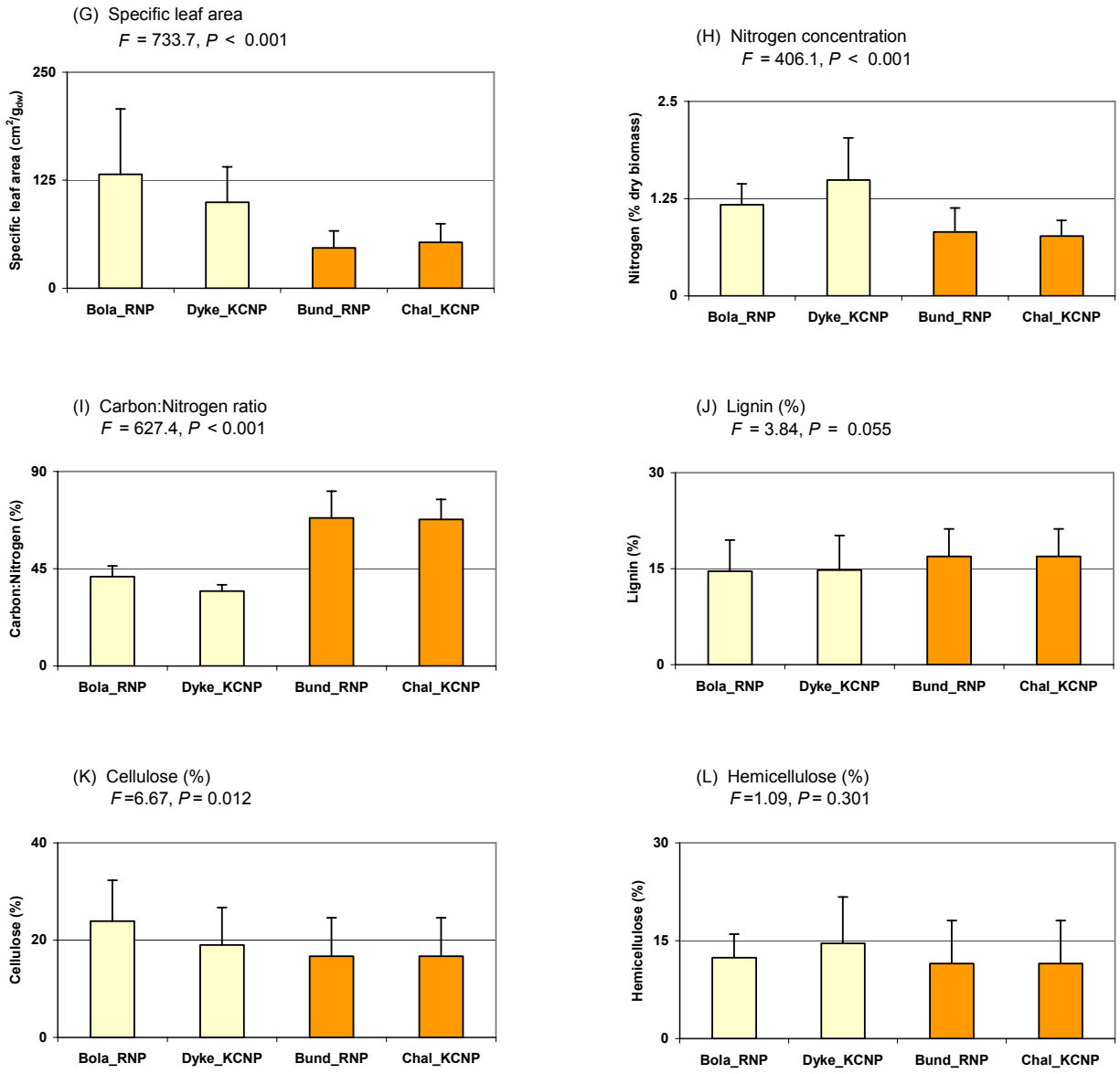


Figure 3.2(b) Overall mean mature leaf characteristics obtained for each site in Royal National Park (RNP) and Ku-ring-gai Chase National Park (KCNP), NSW Australia. One-way ANOVA testing the means of the paired sites and standard deviations are given. Yellow bars represent the more fertile sites and the orange bars the less fertile sites.

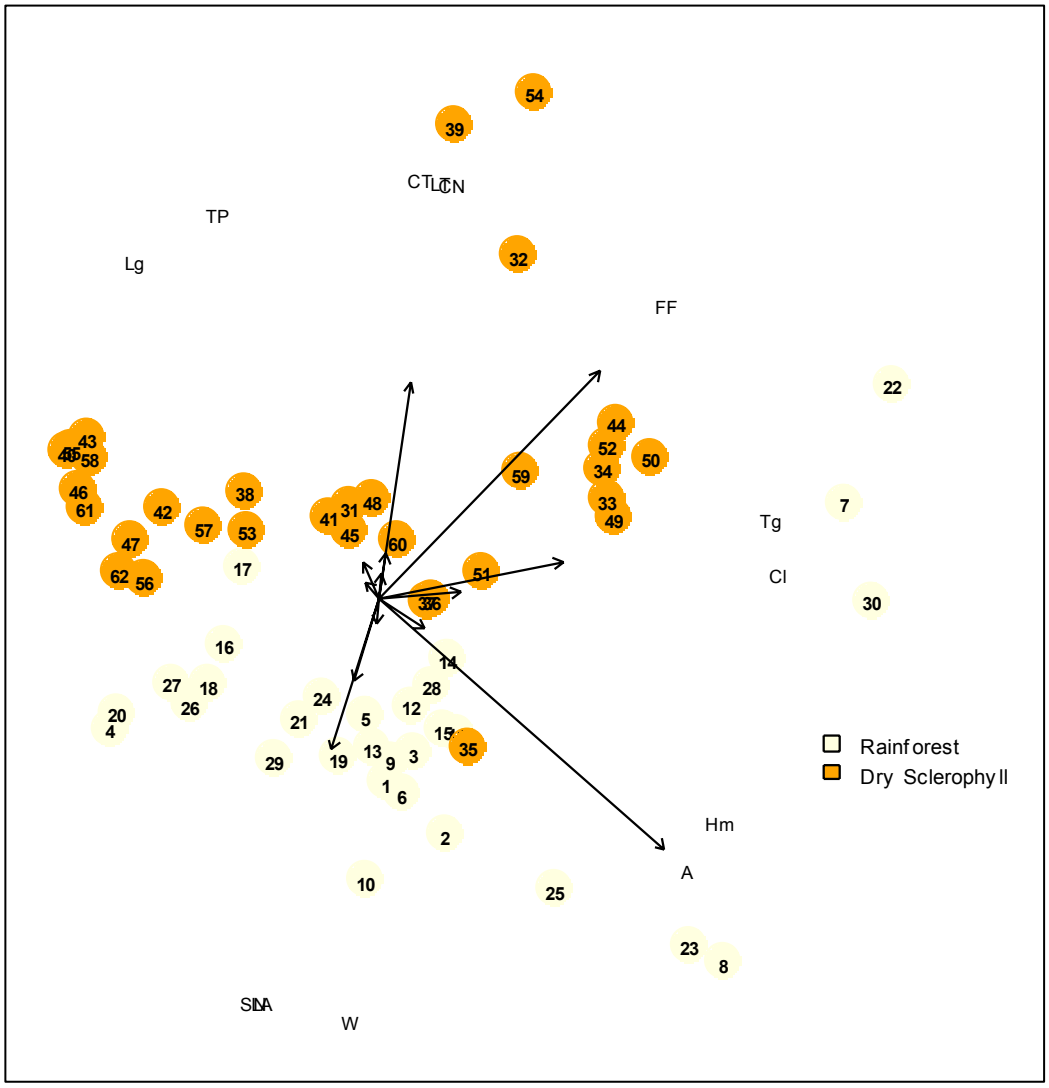


Fig. 3.3 Mature leaf characteristics of common rainforest and dry sclerophyll plant species from four sites in NSW (PC biplot goodness-of- fit: 70.76%; ANOSIM: R = 0.27, p = 0.0001; all species included)

Temperate rainforest Bola Ck, Royal NP	**Wet Sclerophyll forest Dyke, Ku-ring-gai NP**	**Dry sclerophyll heathland Bundeena, Royal NP**	**Dry sclerophyll heathland Challenger, Ku-ring-gai NP**
1 *Acmena smithii*	16 *Acacia floribunda*	31 *Acacia suaveolens*	48 *Acacia suaveolens*
2 *Blechnum cartilagineum*	17 *Allocasuarina torulosa*	32 *Allocasuarina distyla*	49 *Angophora hispida*
3 *Ceratopetalum apetalum*	18 *Breynia oblongifolia*	33 *Angophora hispida*	50 *Banksia serrata*
4 *Citriobatus pauciflorus*	19 *Cissus hypoglauca*	34 *Banksia serrata*	51 *Corymbia gummifera*
5 *Diospyros australis*	20 *Citriobatus pauciflorus*	35 *Caustis recurvata*	52 *Eucalyptus haemastoma*
6 *Doryphora sassafras*	21 *Hibbertia dentata*	36 *Corymbia gummifera*	53 *Grevillea buxifolia*
7 *Gymnostachys anceps*	22 *Lepidosperma laterale*	37 *Eucalyptus haemastoma*	54 *Hakea teretifolia*
8 *Livistona australis*	23 *Livistona australis*	38 *Grevillea sphacelata*	55 *Hemigenia purpurea*
9 *Lomatia myricoides*	24 *Pomaderris ferruginea*	39 *Hakea teretifolia*	56 *Hibbertia monogyna*
10 *Palmeria scandens*	25 *Pteridium esculentum*	40 *Hemigenia purpurea*	57 *Leptospermum trinervium*
11 *Ripogonum album*	26 *Pultenaea daphnoides*	41*Isopogon anethifolius*	58 *Leucopogon microphyllus*
12 *Syncarpia glomulifera*	27 *Pultenaea flexilis*	42*Leptospermum trinervium*	59 *Patersonia glabrata*
13 *Tasmannia insipida*	28 *Syncarpia glomulifera*	43*Leucopogon microphyllus*	60 *Persoonia lanceolata*
14 *Trochocarpa laurina*	29 *Synoum glandulosum*	44*Patersonia glabrata*	61 *Platysace linearifolia*
15 *Wilkiea huegelianna*	30 *Macrozamia communis*	45*Persoonia lanceolata*	62 *Pultenaea elliptica*
		46*Platysace linearifolia*	
		47 *Pultenaea elliptica*	

A Leaf Area
SLA Specific Leaf Area
W %Water content
N Nitrogen

FF Force of Fracture
Tg Toughness
Lg Lignin
Hm Hemicellulose

Cl Celulose
CN Carbon: Nitrogen ratio

LT Lamina thiickness
CT Condensed tannins
TP Total Phenols

Toughness and to a lesser extent, force of fracture, played little to no part in differentiating rainforest and sclerophyll plant populations in Royal National Park and Ku-ring-gai Chase National Park. There were just as many wet sclerophyll and rainforest plant species with tough leaves as there were dry sclerophyll plants with soft leaves (Fig. 3.4; see Fig. 3.5 for examples).

Leaf traits with high positive correlations were specific leaf area, nitrogen concentration, and water content; leaf area and concentrations of cellulose and hemicellulose; and leaf toughness and force of fracture. Lamina thickness, total phenol concentrations, carbon:nitrogen ratios, lignin, and condensed tannin concentrations also had very high positive correlations (Fig. 3.4).

The variables characterising mesic plant species (i.e. high specific leaf area, water content and nitrogen concentration) correlated negatively with those characterising dry sclerophyll leaves (i.e. greater lamina thickness, lignin concentration and C:N ratios). Leaf trait variables with no or very little correlation included condensed tannins against area and cellulose, and force of fracture against hemicellulose (Fig. 3.4).

Of the 45 plant species tested for alkaloids, only two mesic plant species, *Doryphora sassafras* and *Acacia floribunda*, produced precipitates indicating a positive presence (Table A3.10). As a considerable amount of work has been done on the presence of alkaloids in Australian plants, it was possible to confirm the positive results with the literature (CSIRO, 1990).

Four plant species periodically contained cyanogenic glycosides. These were the rainforest plant species *Tasmannia insipida, Palmeria scandens* and *Gymnostachys anceps*, and the dry sclerophyll shrub *Hemigenia purpurea* (Table A3.10). The periodic positive results suggest cyanogenic glycosides may be inducible chemical compounds. Further study is required to identify mechanisms instigating cyanogenic glycoside production.

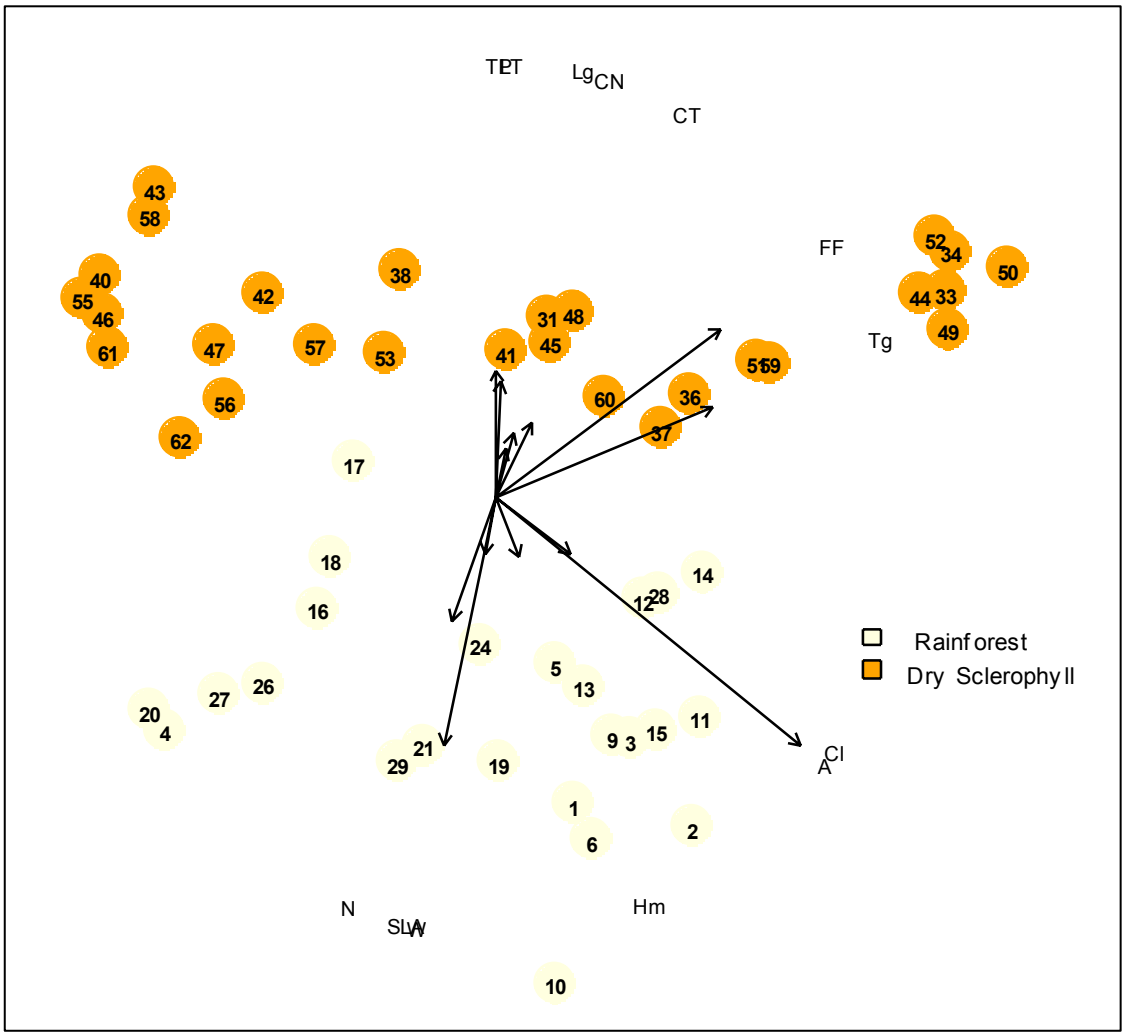


Fig. 3.4 Mature leaf characteristics of common rainforest and dry sclerophyll plant species from four sites in NSW (PC biplot goodness-of- fit: 67.53%; ANOSIM: R = 0.38, p = 0.0001; outliers excluded)

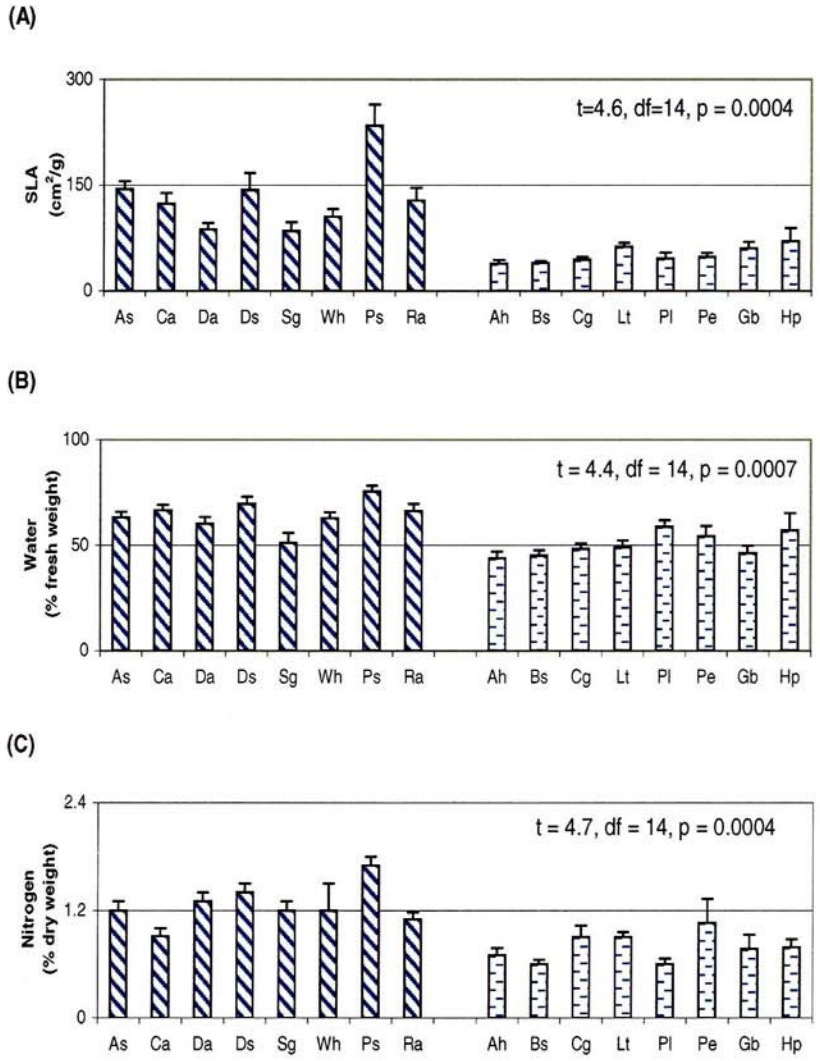
Temperate rainforest Bola Ck, Royal NP	**Wet Sclerophyll forest Dyke, Ku-ring-gai NP**	**Dry sclerophyll heathland Bundeena, Royal NP**	**Dry sclerophyll heathland Challenger, Ku-ring-gai NP**
1 *Acmena smithii*	16 *Acacia floribunda*	31 *Acacia suaveolens*	48 *Acacia suaveolens*
2 *Blechnum cartilagineum*	17 *Allocasuarina torulosa*	33 *Angophora hispida*	49 *Angophora hispida*
3 *Ceratopetalum apetalum*	18 *Breynia oblongifolia*	34 *Banksia serrata*	50 *Banksia serrata*
4 *Citriobatus pauciflorus*	19 *Cissus hypoglauca*	36 *Corymbia gummifera*	51 *Corymbia gummifera*
5 *Diospyros australis*	20 *Citriobatus pauciflorus*	37 *Eucalyptus haemastoma*	52 *Eucalyptus haemastoma*
6 *Doryphora sassafras*	21 *Hibbertia dentata*	38 *Grevillea sphacelata*	53 *Grevillea buxifolia*
9 *Lomatia myricoides*	24 *Pomaderris ferruginea*	40 *Hemigenia purpurea*	55 *Hemigenia purpurea*
10 *Palmeria scandens*	26 *Pultenaea daphnoides*	41 *Isopogon anethifolius*	56 *Hibbertia monogyna*
11 *Ripogonum album*	27 *Pultenaea flexilis*	42*Leptospermum trinervium*	57 *Leptospermum trinervium*
12 *Syncarpia glomulifera*	28 *Syncarpia glomulifera*	43*Leucopogon microphyllus*	58 *Leucopogon microphyllus*
13 *Tasmannia insipida*	29 *Synoum glandulosum*	44 *Patersonia glabrate*	59 *Patersonia glabrata*
14 *Trochocarpa laurina*		45 *Persoonia lanceolata*	60 *Persoonia lanceolata*
15 *Wilkiea huegelianna*		46 *Platysace linearifolia*	61 *Platysace linearifolia*
		47 *Pultenaea elliptica*	62 *Pultenaea elliptica*

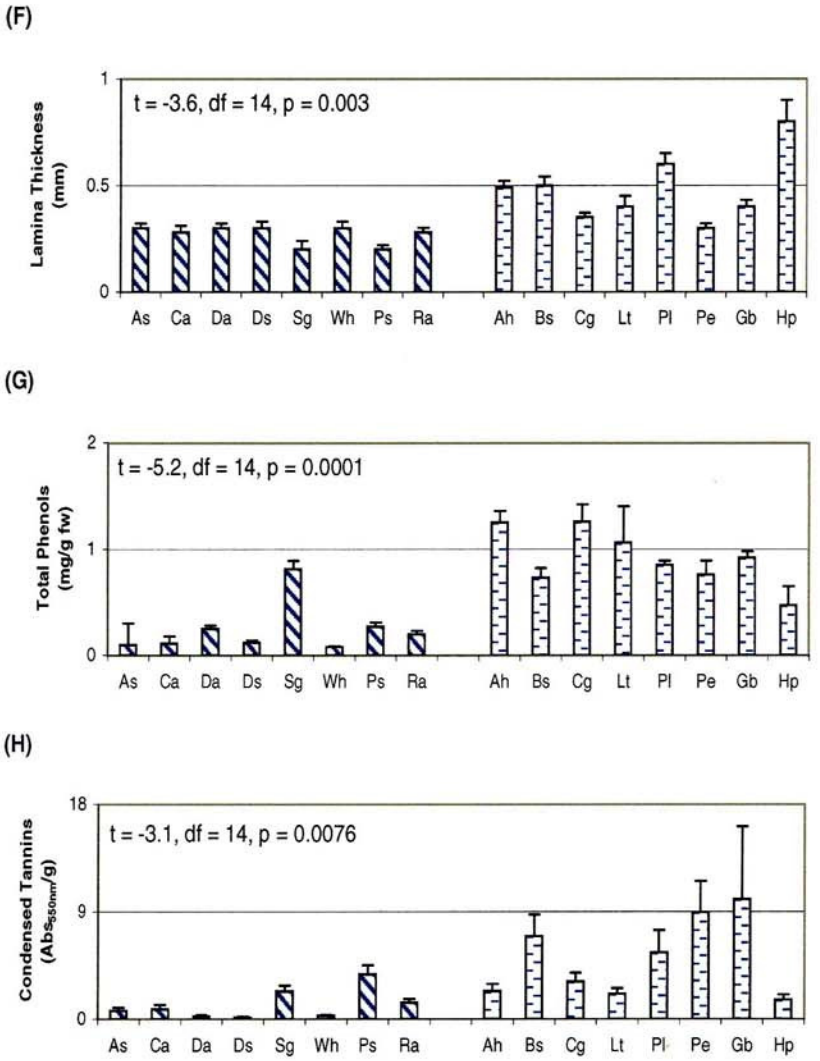
A Leaf Area
SLA Specific Leaf Area
W %Water content
N Nitrogen

FF Force of Fracture
Tg Toughness
Lig Lignin
Hem Hemicellulose

Cel Celulose
CN Carbon: Nitrogen ratio
LT Lamina thickness

CT Condensed tannins
TP Total Phenols


(A)
SLA (cm²/g)
300
150
0
t=4.6, df=14, p = 0.0004
As Ca Da Ds Sg Wh Ps Ra Ah Bs Cg Lt Pl Pe Gb Hp
(B)
Water (% fresh weight)
100
50
0
t = 4.4, df = 14, p = 0.0007
As Ca Da Ds Sg Wh Ps Ra Ah Bs Cg Lt Pl Pe Gb Hp
(C)
Nitrogen (% dry weight)
2.4
1.2
0
t = 4.7, df = 14, p = 0.0004
As Ca Da Ds Sg Wh Ps Ra Ah Bs Cg Lt Pl Pe Gb Hp


(F)
Lamina Thickness (mm)
1
0.5
0
t = -3.6, df = 14, p = 0.003
As Ca Da Ds Sg Wh Ps Ra Ah Bs Cg Lt Pl Pe Gb Hp
(G)
Total Phenols (mg/g fw)
2
1
0
t = -5.2, df = 14, p = 0.0001
As Ca Da Ds Sg Wh Ps Ra Ah Bs Cg Lt Pl Pe Gb Hp
(H)
Condensed Tannins (Abs550nm/g)
18
9
0
t = -3.1, df = 14, p = 0.0076
As Ca Da Ds Sg Wh Ps Ra Ah Bs Cg Lt Pl Pe Gb Hp

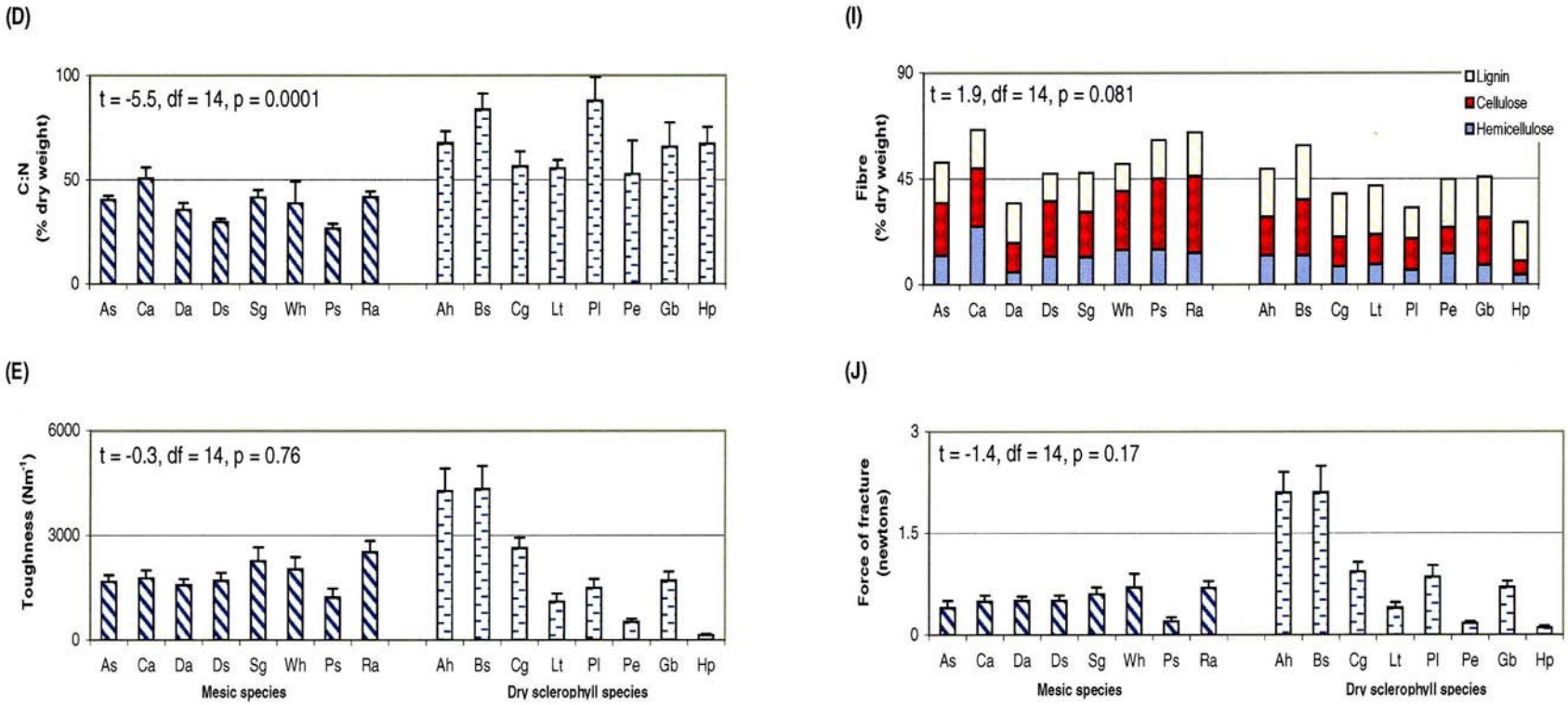


Figure 3.5 Leaf characteristics of 8 mature rainforest and 8 mature dry sclerophyll plant species found in Ku-ring-gai Chase and Royal National Parks.

Mesic species (As - *Acmena smithii*; Ca - *Ceratopetalum apetalum*; Da - *Diospyros australis*; Ds - *Doryphora sassafras*; Sg - *Syncarpia glomulifera*; Wh - *Wilkiea huegelianna*; Ps - *Palmeria scandens*; Ra - *Ripogonum album*.
Dry sclerophyll species (Ah - *Angophora hispida*; Bs - *Banksia serrata*; Cg - *Corymbia gummifera*; Lt - *Leptospermum trinervium*; Pl - *Persoonia lanceolata*; Pe - *Pultenaea elliptica*; Gb - *Grevillea buxifolia*; Hp - *Hemigenia purpurea*. Welch t-tests show significant differences between mesic and dry sclerophyll plant species.

3.6 Phylogenetically Independent Contrasts (PICs)

In this study, phylogenetically independent contrasts were used to detect the effects of soil nutrients on leaf characteristics by removing the potentially confounding effects caused by evolutionary divergence. Consistent slope directions indicate a consistency in responses by plants to resource availability.

The slope directions of the phylogenetically independent contrasts were consistent for five out of the eight mature leaf trait variables and inconsistent for three mature leaf trait variables (Fig. 3.6). Variables that had PICs with consistent responses to soil nutrients were total phenols, percent nitrogen, specific leaf area, lamina thickness and water content. Variables that had PICs with inconsistent directions were leaf toughness, force of fracture and leaf area (Fig. 3.6).

For the variables that had consistent responses to soil nutrients, it was found that out of thirteen PICs:

- eleven dry sclerophyll species had higher total phenol concentrations than their mesic pair, and two had similar total phenol concentrations (Fig. 3.6A);
- twelve mesic species had higher nitrogen concentrations than their dry sclerophyll partner, whilst one had a lower concentration (Fig. 3.6B);
- nine mesic species had greater specific leaf areas than their PIC partner, and four had similar SLAs (Fig. 3.6F);
- ten dry sclerophyll plants had higher mean lamina thickness than their mesic PIC partner, whilst two had lower and one had similar mean lamina thickness (Fig. 3.6G); and
- ten mesic species had higher percent water content than their dry sclerophyll pair, whilst two had lower and one PIC pair similar percent water content (Fig. 3.6H).

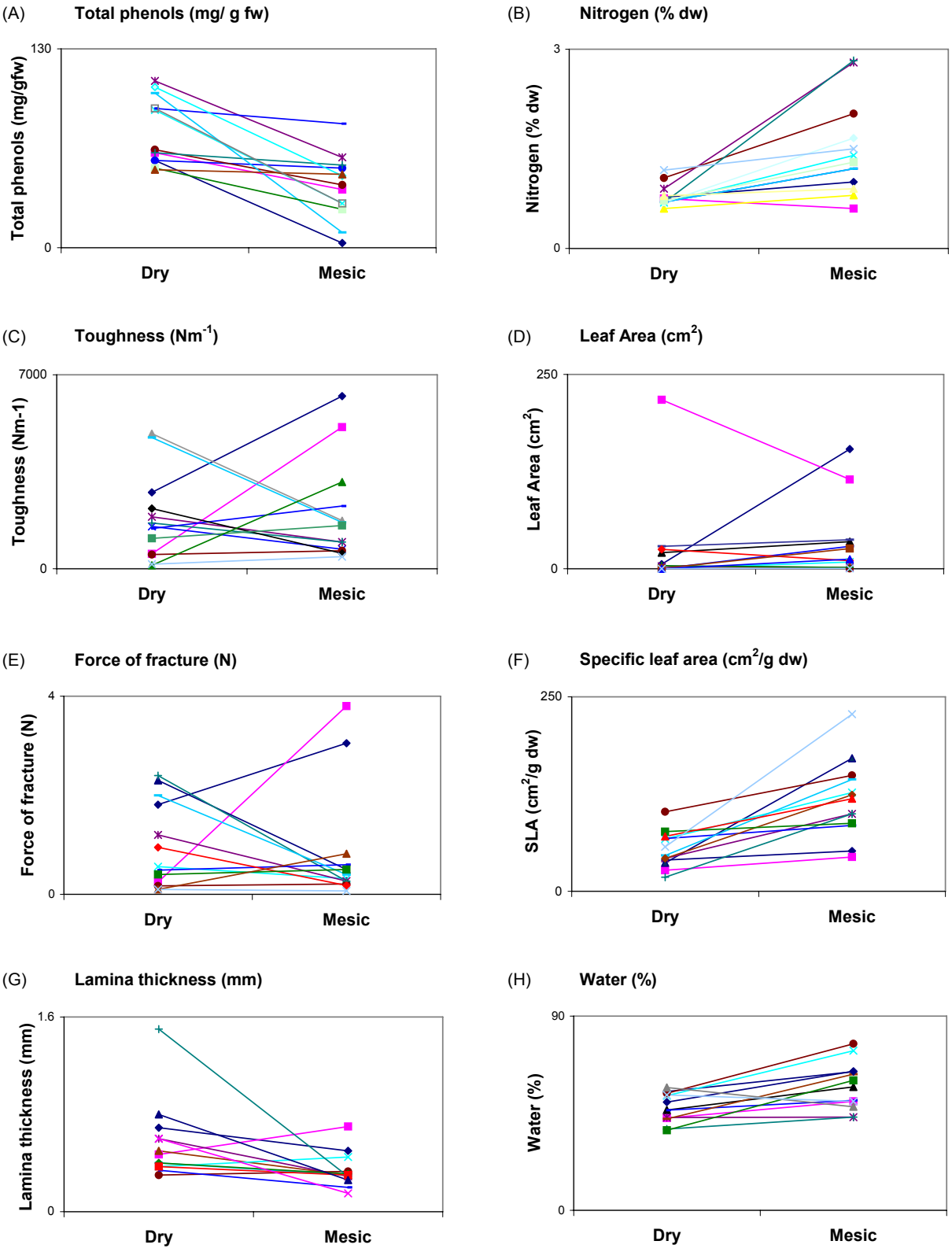


Figure 3.6 Comparisons of mature leaf traits within each of 13 phylogenetically independent contrasts. Values shown are means. Lines connect the dry sclerophyll (Dry) and mesic species within each PIC.

For variables that had inconsistent responses to soil nutrients, it was found that out of thirteen PICs:

- six dry sclerophyll plants had higher leaf toughness than their mesic PIC, and three had lower and four had similar leaf toughness (Fig. 3.6C);
- five dry sclerophyll plants had higher forces of fracture than their mesic PIC, and three had lower and five had similar forces of fracture (Fig. 3.6E); and
- eight mesic species had larger leaf areas than their dry sclerophyll PIC, and four had smaller and one had similar sized leaf areas. (Fig. 3.6)

3.7 Immature leaf characteristics

The leaf trait results for immature leaves reflected those found for mature leaves. Immature mesic leaves from the higher resource sites had very high specific leaf areas, water content and nitrogen concentrations (Fig. 3.7). In comparison, the dry sclerophyll immature leaves from the low resource sites were characterised by very high concentrations of total phenols, higher carbon:nitrogen ratios and greater lamina thickness (Fig. 3.7). The plant species with the toughest juvenile leaves were *Gymnostachys anceps*, *Macrozamia communis*, and *Lepidosperma laterale*. *Livistona australis*, *Pteridium esculentum* and *Blechnum cartilagineum* had the greatest immature leaf areas compared to the other plant species studied (Fig. 3.7).

To observe the variation within the majority of the plant species, the dominating outliers (points 18, 19, 21, 27, 32 and 33) were removed and the data reexamined (Fig. 3.8). Total phenol concentration became the most important variable defining immature dry sclerophyll leaves, followed by specific leaf area, nitrogen concentration and carbon:nitrogen ratios. Leaf area and leaf toughness did not contribute to the differentiation of the plant communities (Fig. 3.8).

High total phenol concentration, lamina thickness and carbon:nitrogen ratios were highly positively correlated with each other, as were high specific leaf area, nitrogen concentration and water content (Fig. 3.8). Total phenols, lamina thickness and carbon:nitrogen ratios were negatively correlated with SLA, nitrogen

concentration and water content. Carbon: nitrogen ratios were not correlated with leaf area (Fig. 3.8).

In contrast to the results for mature leaves, young dry sclerophyll leaves did not exhibit the greatest variation in leaf trait characteristics. There was just as much variation in the overall mean characteristics of immature dry sclerophyll leaves from resource-poor environments, as there was in the immature mesic leaves from the higher resource sites (Fig. 3.8).

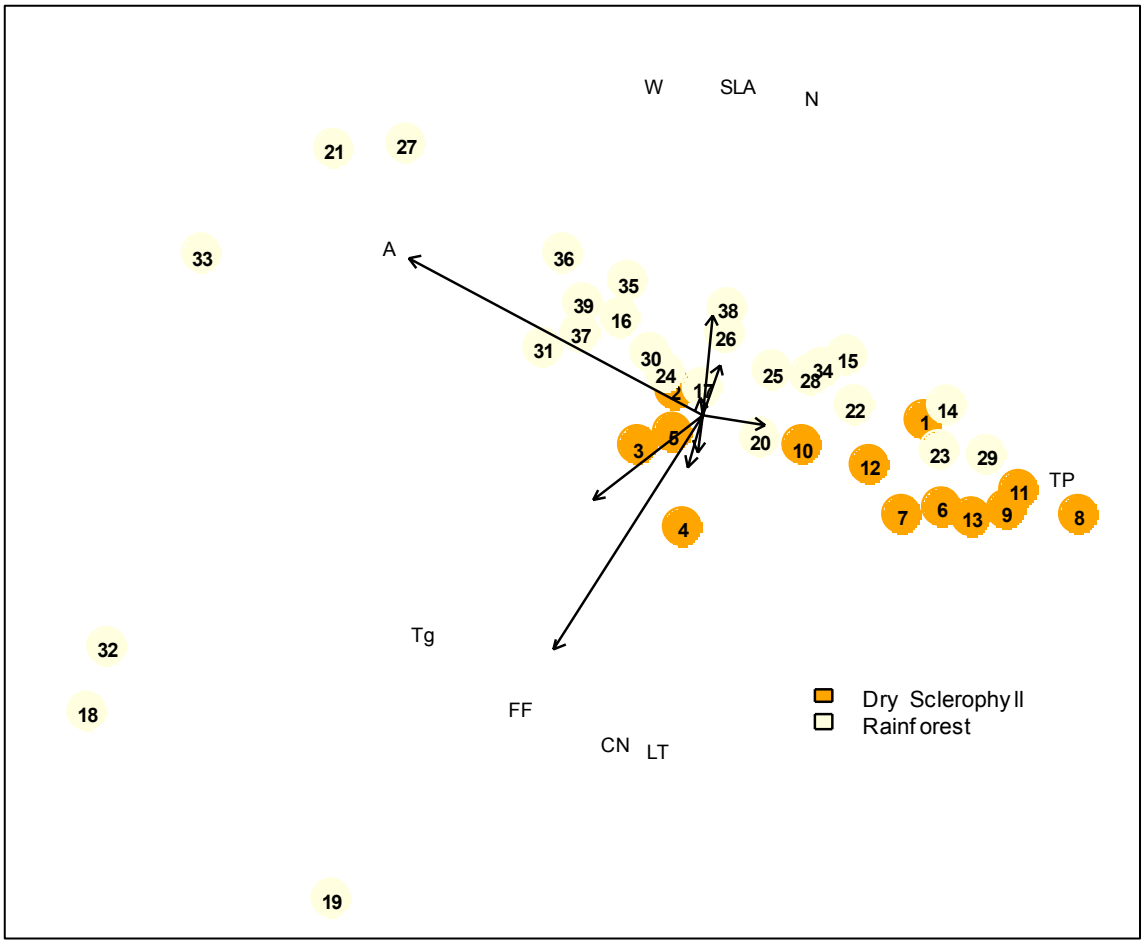


Fig. 3.7 Immature leaf characteristics of common rainforest and dry sclerophyll plant species in NSW (PC biplot goodness-of- fit: 81.64%; ANOSIM: R = 0.17, p=0.019; all species included).

Dry sclerophyll plant species	Wet sclerophyll plant species	Rainforest plant species	Variables
1. Acacia suaveolens	*14 Acacia floribunda*	*26. Acmena smithii*	**A** Area
2. Angophora hispida	*15 Breynia oblongifolia*	*27. Blechnum cartilagineum*	**SLA** Specific Leaf Area
3. Banksia serrata	*16 Cissus hypoglauca*	*28. Ceratopetalum apetalum*	**W** %Water content
4. Corymbia gummifera	*17 Hibbertia dentata*	*29. Citriobatus pauciflorus*	**N** Nitrogen
5. Eucalyptus haemastoma	*18 Lepidosperma laterale*	*30. Diospyros australis*	**FF** Force of Fracture
6. Grevillea buxifolia	*19 Macrozamia communis*	*31. Doryphora sassafras*	**Tg** Toughness
7. Hakea teretifolia	*20 Pomaderris ferruginaea*	*32. Gymnostachys anceps*	**LT** Leaf thickness
8. Hemigenia purpurea	*21 Pteridium esculentum*	*33. Livistona australis*	**TP** Total Phenols
9. Hibbertia monogyna	*22 Pultenaea daphnoides*	*34. Lomatia myricoides*	**CN** Carbon:Nitrogen ratio
10. Isopogon anethiofolius	*23 Pultenaea flexilis*	*35. Palmeria scandens*	
11. Leptospermum trinervium	*24. Syncarpia glomulifera*	*36. Ripogonum album*	
12. Persoonia lanceolata	*25. Synoum glandulosum*	*37. Tasmannia insipida*	
13. Pultenaea elliptica		*38. Trochocarpa laurina*	
		39. Wilkiea huegelianna	

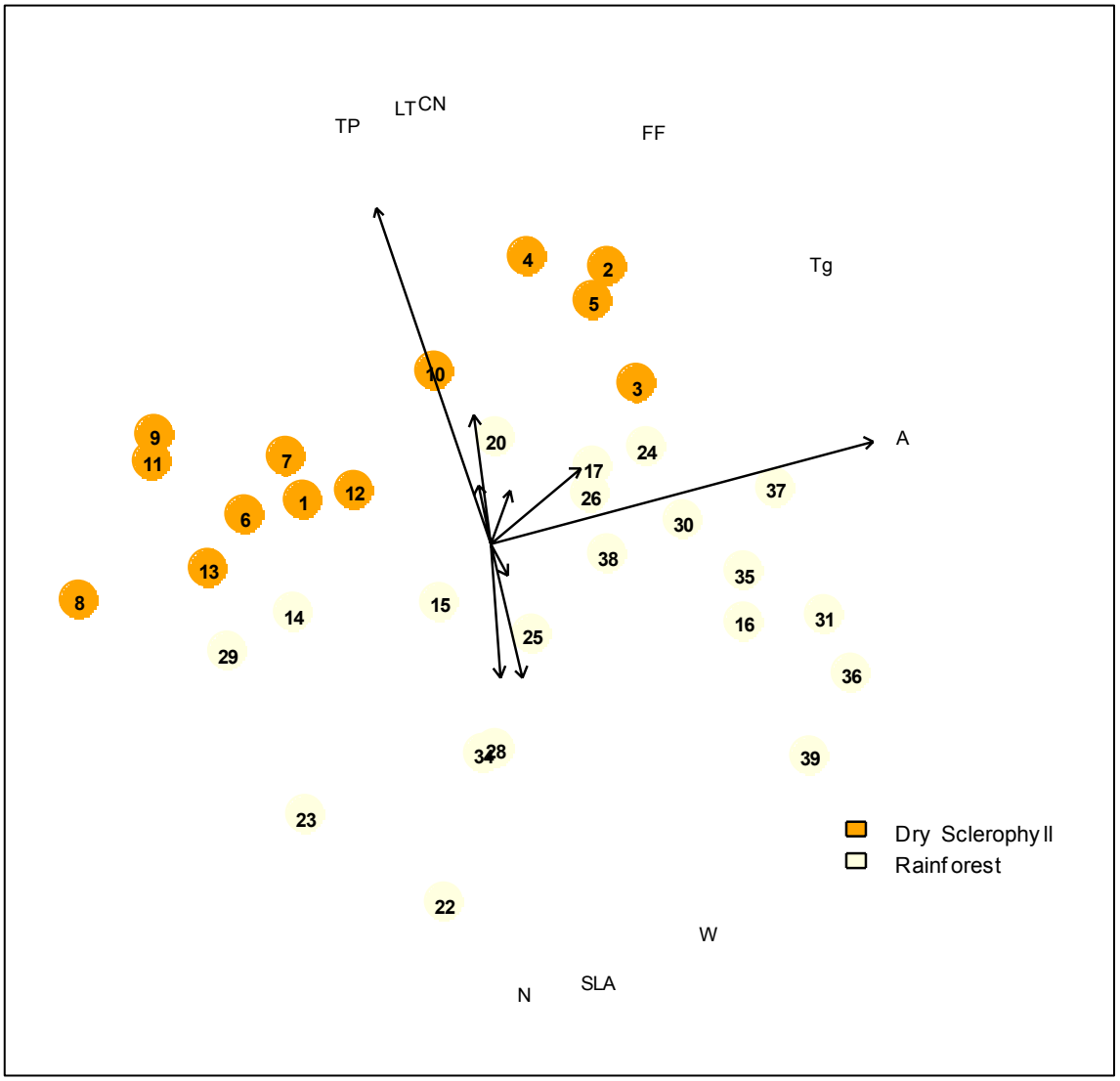


Fig. 3.8 Immature leaf characteristics of common rainforest and dry sclerophyll plant species in NSW (PC biplot goodness-of- fit: 82.3%; ANOSIM: R = 0.41, p = 0.0001; outliers excluded)

Dry sclerophyll species	Wet sclerophyll species	Rainforest plants	Variables
1. Acacia suaveolens	*14 Acacia floribunda*	*26. Acmena smithii*	**A** Area
2. Angophora hispida	*15 Breynia oblongifolia*	*28. Ceratopetalum apetalum*	**SLA** Specific Leaf Area
3. Banksia serrata	*16 Cissus hypoglauca*	*29. Citriobatus pauciflorus*	**W** %Water content
4. Corymbia gummifera	*17 Hibbertia dentata*	*30. Diospyros australis*	**N** Nitrogen
5. Eucalyptus haemastoma	*20 Pomaderris ferruginaea*	*31. Doryphora sassafras*	**FF** Force of Fracture
6. Grevillea buxifolia	*22 Pultenaea daphnoides*	*34. Lomatia myricoides*	**Tg** Toughness
7. Hakea teretifolia	*23 Pultenaea flexilis*	*35. Palmeria scandens*	**LT** Leaf thickness
8. Hemigenia purpurea	*24. Syncarpia glomulifera*	*36. Ripogonum album*	**TP** Total Phenols
9. Hibbertia monogyna	*25. Synoum glandulosum*	*37. Tasmannia insipida*	**CN** Carbon:Nitrogen ratio
10. Isopogon anethiofolius		*38. Trochocarpa laurina*	
11. Leptospermum trinervium		*39. Wilkiea huegelianna*	
12. Persoonia lanceolata			
13. Pultenaea elliptica			

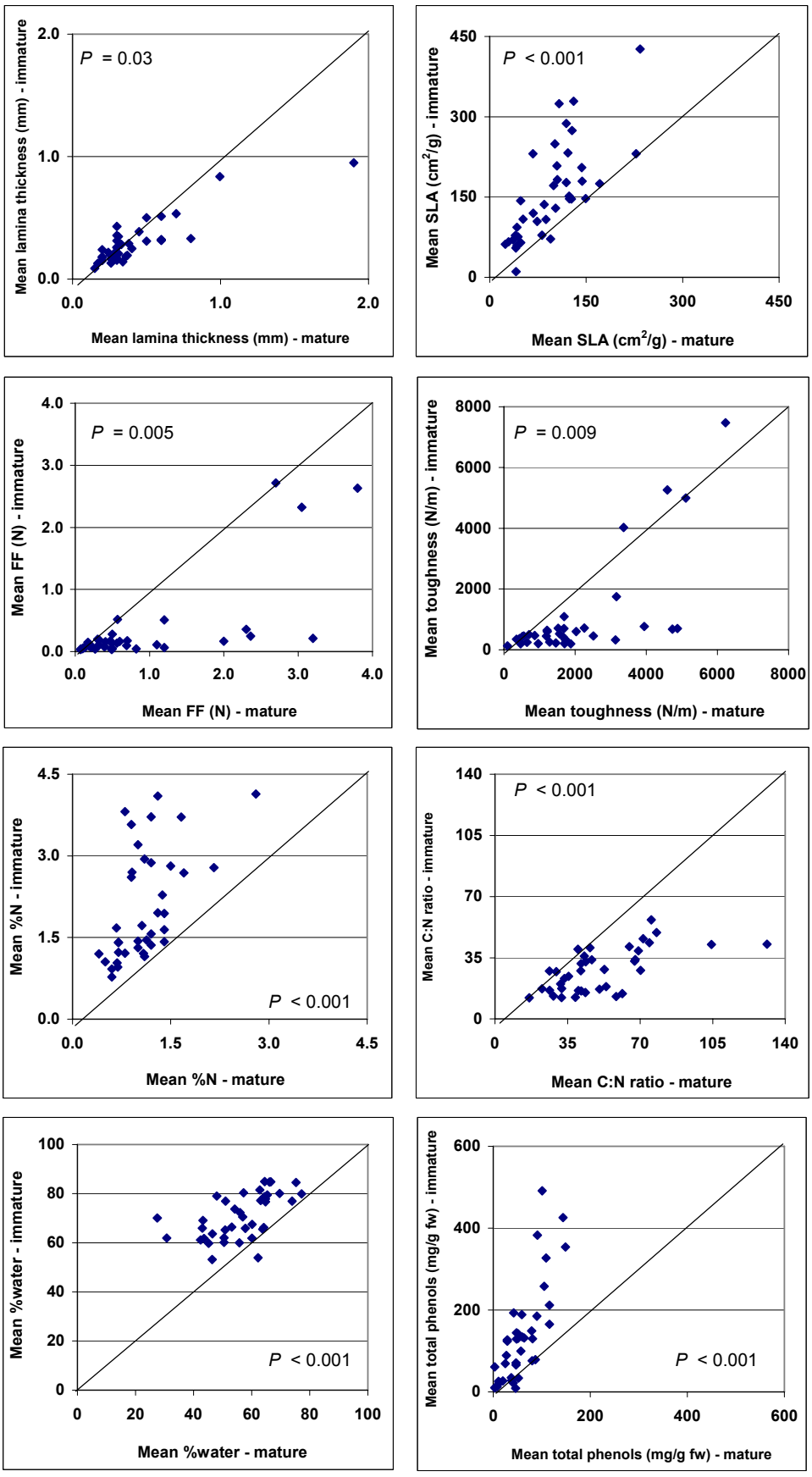


Figure 3.9 Comparison of mature and immature leaves for 39 plant species. Scatterplots include dry and wet sclerophyll, and temperate rainforest plant species. *P* - values from Welch Two Sample t-tests presented.

A comparison between mature and immature leaf trait characteristics for 39 dry sclerophyll and mesic plant species from low and higher resource sites is presented in Figure 3.9. Regardless of vegetation type and resource availability, mature leaves tended to have thicker lamina, higher carbon:nitrogen values, and greater force of fracture and toughness compared to younger leaves. Young leaves were characterised by much higher specific leaf areas, water content, and nitrogen and total phenol concentrations than mature leaves.

3.8 Phenology of leaf maturation

Expanding leaves are at greater risk of herbivory (Ernest, 1989) and environmental damage (eg. wind, drought) than mature leaves. Phenology of leaf maturation was monitored from leaf formation to full maturity for nine plant species located in Royal National Park and Ku-ring-gai Chase National Park.

3.8.1 Methods

The majority of plants were selected for study because they began synchronously producing new leaves in the winter of June 2003, and were a subset of the 46 plant species analysed in the mature and immature leaf trait studies. The plant species and collection sites are shown in Table 3.1.

Leaf buds from four to five individual plants of each species were tagged with wiremarker tape. Between two and five leaves from each plant were randomly sampled in the early mornings on a weekly (first three weeks), fortnightly (next month and a half) and later monthly basis (following four months) for approximately 192 days. As leaves were collected, they were wrapped separately in paper towelling, placed in plastic bags and moistened. The leaves were refrigerated at 4^{o}C and processed within 72 hours of collection.

Leaves were analysed for water content, area, specific leaf area, lamina thickness, toughness, force of fracture, carbon:nitrogen ratio and total phenol concentrations.

Table 3.1 Collection sites for plant species used in the phenology study

Plant species	Family	Description	Collection Site
Acmena smithii	Myrtaceae	Rainforest sp.	Bola ck, Royal NP
Eucalyptus haemastoma	Myrtaceae	Dry sclerophyll sp.	Bundeena rd, Royal NP
Syncarpia glomulifera	Myrtaceae	Wet sclerophyll sp.	Bola ck, Royal NP
Corymbia gummifera	Myrtaceae	Dry sclerophyll sp.	Bundeena rd, Royal N
Trochocarpa laurina	Epacridaceae	Rainforest sp.	Bola ck, Royal NP
Angophora hispida	Myrtaceae	Dry sclerophyll sp.	Challenger tk, KCNP
Ceratopetalum apetalum	Cunoniaceae	Rainforest sp.	Bola ck, Royal NP
Hakea teretifolia	Proteaceae	Dry sclerophyll sp.	Challenger tk, KCNP
Diospyros australis	Ebenaceae	Rainforest sp.	Bola ck, Royal NP

3.8.2 Statistical analysis

The phenological patterns for each variable were analysed by repeated measures ANOVA performed using DataDesk. The analysis was designed as a nested model, with the factor species nested within the factor communities (dry sclerophyll and mesic).

3.8.3 Results

Expanding dry sclerophyll leaves from nutrient poor sites did not have significantly higher force of fracture (P = 0.56) and toughness (P = 0.31) than expanding mesic leaves from higher nutrient sites (Fig. 3.10, Table 3.2), and there was no significant difference between the vegetation communities over time (P_{FF} = 0.49, P_{Tg} = 0.53). Force of fracture and toughness differed significantly between species (P < 0.0001). Expanding leaves with the greatest toughness included the dry sclerophyll leaves of *E. haemastoma* (averages from 382 Nm^{-1} to 2705 Nm^{-1}) and *C. gummifera* (averages from 431 Nm^{-1} to 2812 Nm^{-1}) and the mesic leaves of *T. laurina* (means from 322 Nm^{-1} to 4150 Nm^{-1}) and *S. glomulifera* (averages from 573 Nm^{-1} to 2154 Nm^{-1}) (Fig. 3.10).

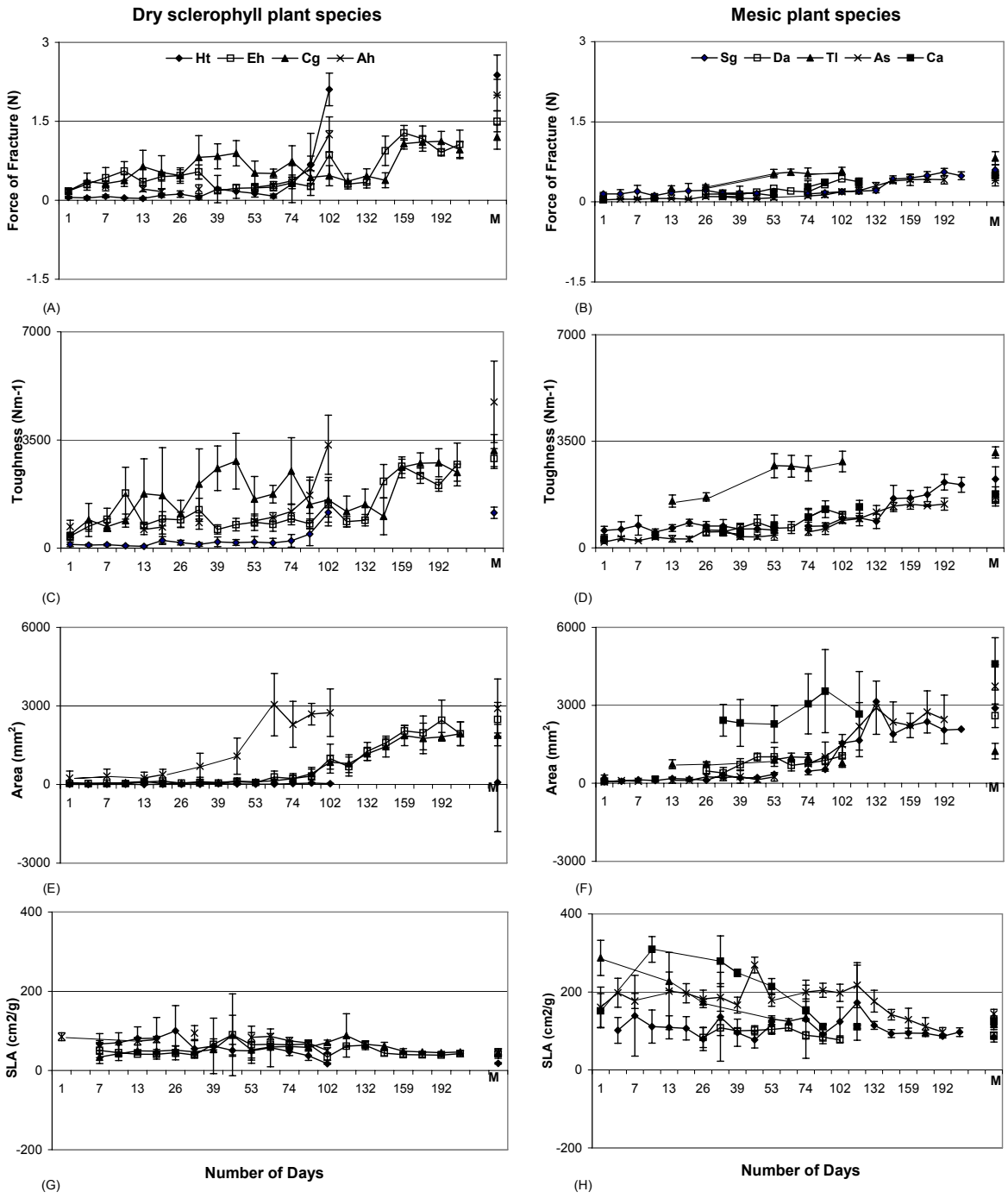


Figure 3.10 Phenology of force of fracture, toughness, area and specific leaf area (SLA) for four dry sclerophyll and five mesic plant species from infertile and more fertile environments respectively (mean ± s.d.). Ht – *Hakea teretifolia*, Eh – *Eucalyptus haemastoma*, Cg – *Corymbia gummifera*, Ah – *Angophora hispida*, Sg – *Syncarpia glomulifera*, Da – *Diospyros australis*, As – *Acmena smithii* and Cs – *Ceratopetalum apetalum*. M – mature leaves.

Table 3.2 Summary of repeated measures ANOVA statistics for phenology of leaf maturation.

Variable	Source	df	F-ratio	P
Toughness	Comm	1	0.4	0.56
	Species	3	81.3	< 0.001
	Comm*Rpt	7	0.9	0.53
	Species*Rpt	21	3.8	< 0.001
Force of fracture	Comm	1	1.5	0.31
	Species	3	102.2	< 0.001
	Comm*Rpt	7	1.0	0.49
	Species*Rpt	21	3.1	< 0.001
Total phenols	Comm	1	7.9	0.04
	Comm*Rpt	4	0.6	0.67
Water content	Comm	1	1.3	0.34
	Species	3	24.7	< 0.001
	Comm*Rpt	6	1.1	0.38
	Species*Rpt	18	1.8	0.03
Nitrogen	Comm	1	1.7	0.51
	Comm*Rpt	9	5.7	0.003
C:N ratio	Comm	1	27.8	0.03
	Comm*Rpt	9	3.3	0.02
Lamina thickness	Comm	1	14.3	0.03
	Species	3	6.8	0.002
	Comm*Rpt	7	1.3	0.29
	Species*Rpt	21	1.0	0.52
Area	Comm	1	7.4	0.11
	Species	2	7.5	0.005
	Comm*Rpt	7	3.5	0.02
	Species*Rpt	14	0.9	0.55
Specific leaf area (SLA)	Comm	1	11.1	0.04
	Species	3	19.1	< 0.001
	Comm*Rpt	6	2.5	0.06
	Species*Rpt	18	0.9	0.61

Expanding dry sclerophyll leaves toughened at a faster rate initially than expanding mesic leaves (Table 3.3). *Eucalyptus haemastoma*, *C. gummifera* and *H. teretifolia* reached 50 percent mature toughness within 13 to 19 days. In comparison, *A. smithii* took between 57 and 71 days, *S. glomulifera* between 85 to

99 days, *T. laurina* 54 days, *C. apetalum* approximately 30 days and *D. australis* between 42 to 70 days to reach 50 % mature leaf force of fracture and toughness. However, expanding dry sclerophyll and mesic leaves reached their 90th percentile toughness and force of fracture at approximately the same time, ie in 150 days (Table 3.3).

Table 3.3 Summary of days to maturity (10, 50 and 90th percentiles) for each plant species for each variable measured. (FF – force of fracture, Tg – toughness, %W – percent water, SLA – specific leaf area, LT – lamina thickness, TP – total phenols, N – nitrogen. Blanks indicate consistent values over time)

Dry sclerophyll species		FF	Tg	%W	Area	SLA	LT	TP	N
Eucalyptus haemastoma	**10%** **50%** **90%**	1-5 13-19 146-159	5-7 13-19 146-159	~7 53-60 192	5-10 74 146-159	-	-	32-53 117 192-206	-
Corymbia gummifera	**10%** **50%** **90%**	5-10 13-19 159-173	5-10 13-19 173-192	~13 60 206	7-10 60 159-192	-	-	10-19 102-117 173-206	~13 ~74 173-192
Angophora hispida	**10%** **50%** **90%**	1-7 50-63 107	1-14 50 107	~7-14 21 107	14 50 107	1-7 78 107	~1 ~63 93 –107	~21 50 - 63 93 - 107	1 – 14 50 – 63 93 - 107
Hakea teretifolia	**10%** **50%** **90%**	1-5 19 74-89	1-5 19 74-89	~7-10 ~39 ~102	5-7 26-39 74-89	19-26 32-39 74-89	1-5 19-32 74-89	53-60 60-74 102	8-13 53-60 102+
Mesic plant species		**FF**	**Tg**	**%W**	**Area**	**SLA**	**LT**	**TP**	**N**
Trochocarpa laurina	**10%** **50%** **90%**	1 - 15 54 98	1 – 15 54 98- 118	1 – 15 80 118+	1 – 15 54 118+	1 – 15 25 – 54 118+	1 – 15 54 118+	1 – 15 25 98-118	1 – 15 54 ~98
Ceratopetalum apetalum	**10%** **50%** **90%**	1 – 10 30 ~100	1 –10 55 ~93	1 –10 55 ~93	1 -10 ~30 ~93	10 – 30 ~69 ~69 – 93	1 – 10 ~41 69 – 93	1 – 10 41 ~93	1 – 10 41 – 55 ~93
Diospyros australis	**10%** **50%** **90%**	1 – 15 42 -70 108-122	1 – 15 42 - 70 108-122	1 – 15 42 – 70 108-122	1 – 15 28 - 42 122+	1 – 15 70 – 80 108 – 122	1 – 28 28 – 42 108	1 – 15 56 – 70 122+	1 – 15 70 - 80 122+
Acmena smithii	**10%** **50%** **90%**	3 - 9 57 - 71 155	3 - 9 71 - 85 155	3 – 6 57 – 71 169	3 - 6 57 - 71 169	~15 113-128 155-169	6 71 142	6-15 ~71 142-155	28-35 85 155
Syncarpia glomulifera	**10%** **50%** **90%**	3 85-99 169	1-3 85 169-202	6-9 ~57 202	3-9 57-71 169	35 49 – 57 ~ 188	-	28-38 71-85 169	6-15 49 155-188

In general, expanding dry sclerophyll leaves in low nutrient environments contained higher concentrations of phenols compared to maturing mesic leaves in higher nutrient sites (Fig. 3.11, P = 0.04). The dry sclerophyll species *E. haemastoma*, *C. gummifera* and *A. hispida* had ranges between 522 to 175 mg/g dry weight, compared to the mesic species *A. smithii, S. glomulifera*, *D. australis*

and *T. laurina* that had ranges between 215 and 31 mg/g dry weight. The exception to the trend was the dry sclerophyll species *H. teretifolia* that had concentrations from 185 to 48 mg/g dry weight (Fig. 3.11). The concentration of phenols declined for all species, indicating a dilution effect as maturing leaves expanded (Fig. 3.11).

Mesic expanding leaves had greater ranges and higher concentrations of nitrogen than expanding dry sclerophyll leaves (Fig. 3.11). Within the mesic species, *A. smithii* (3.8% to 1.3% N) and *C. apetalum* (3.2% to 0.9% N) had the highest concentrations of nitrogen in their leaves, whilst *S. glomulifera* (2.3% to 0.9% N) and *D. australis* (2.4% to 1.4% N) had the lowest concentrations of nitrogen. In comparison, dry sclerophyll leaves had concentrations from 1.7% to 0.7% for *H. teretifolia*; 1.3% to 0.8% for *E. haemastoma*; 1.0% to 0.7% for *C. gummifera* and 1.4% to 1.0% for *A. hispida.* Percent nitrogen therefore remained relatively constant throughout the development of the majority of dry sclerophyll leaves. On the other hand, the phenology of nitrogen in mesic species was characterised by very high concentrations followed by a steady decline in total nitrogen (Fig. 3.11). As mesic leaves expanded, the concentration of nitrogen was diluted with addition of carbon, as shown in the carbon:nitrogen ratios (Fig. 3.11).

There was no significant difference in water content between expanding mesic and dry sclerophyll leaves either in general ($P = 0.34$) or over time (Fig. 3.11, $P = 0.38$), but there was a significant difference between species ($P < 0.0001$, Table 3.2). The species that had the highest water content in expanding leaves were *H. teretifolia* (91.2% - 50.1%, dry sclerophyll), *C. apetalum* (85.2% - 69.0%, mesic sp.), *A. smithii* (79.7% - 59.8%, mesic sp.) and *T. laurina* (79.0% - 54.2%, mesic sp.). The expanding leaves with the lowest water content were from the dry sclerophyll species *E. haemastoma* (65.1% - 42.7%), *C. gummifera* (69.9% - 43.7%) and *A. hispida* (65.9% - 47.2%). Percent water content was highest when leaves were young, and declined as leaves matured (Fig. 3.11).

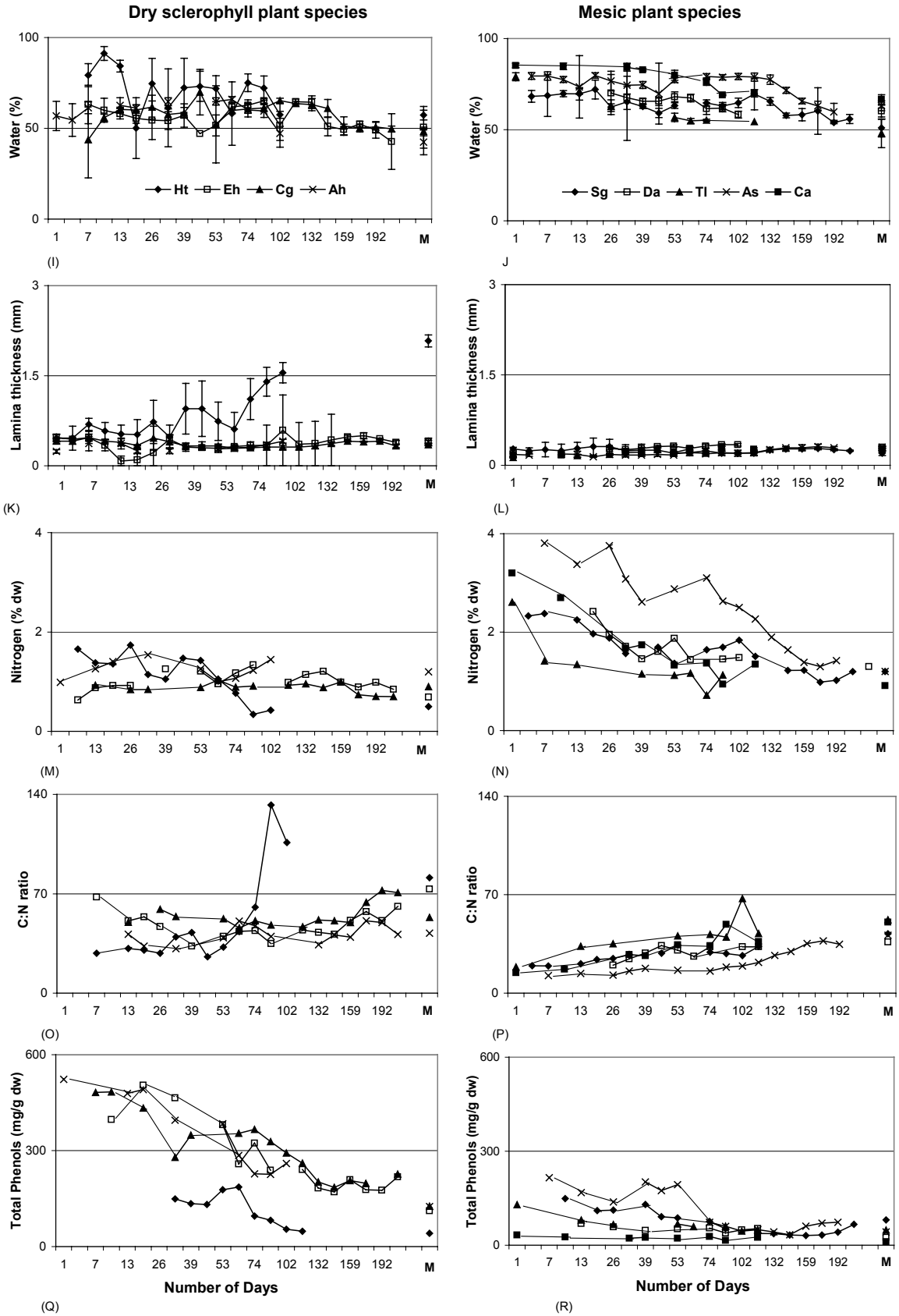


Figure 3.11 Phenology of water, lamina width (mean ± s.d.), nitrogen, carbon:nitrogen and phenols (pooled samples) for four dry sclerophyll and five mesic plant species from infertile and more fertile environments respectively. Ht – *Hakea teretifolia*, Eh – *Eucalyptus haemastoma*, Cg – *Corymbia gummifera*, Ah – *Angophora hispida*, Sg – *Syncarpia glomulifera*, Da – *Diospyros australis*, As – *Acmena smithii* and Cs – *Ceratopetalum apetalum*. M – mature leaves.

Leaf area differed significantly between the vegetation communities over time (P = 0.02) and between species (P = 0.005), but there was no significant difference between the communities in general (Table 3.2, P = 0.11) or between the species over time (P = 0.55). The plants with the greatest leaf areas were the dry sclerophyll species *A. hispida* (247 mm^2 – 2745 mm^2) and *E. haemastoma* (37 mm^2 – 2453 mm^2) and the mesic species *C. apetalum* (69 mm^2 – 3547 mm^2), *A. smithii* (59 mm^2 – 2910 mm^2) and *S. glomulifera* (56 mm^2 – 2368 mm^2). The dry sclerophyll species *H. teretifolia* (3.0 mm^2 – 51 mm^2) and *C. gummifera* (38 mm^2 – 1936 mm^2), and the mesic species *D. australis* (396 mm^2 – 1053 mm^2) and *T. laurina* (259 mm^2 – 1009 mm^2) had the smallest leaf areas (Fig. 3.10).

Expansion rates of dry sclerophyll leaves from low nutrient environments did not differ greatly from mesic leaves from higher nutrient sites. On average dry sclerophyll plant species took about 54 days to reach 50% of mature leaf area, and approximately 132 days to attain 90% of mature leaf area. In comparison, mesic plant species reached 50% mature leaf area in about 44 days and reached 90% mature leaf area in 128 days (Table 3.3).

Specific leaf areas were lowest for maturing dry sclerophyll leaves, compared to expanding mesic leaves (Fig. 3.10, P = 0.04). The dry sclerophyll species *E. haemastoma* and *C. gummifera* had means that ranged from 90 cm^2/g to 34 cm^2/g. However, averages for mesic leaves ranged from 269 cm^2/g to 97 cm^2/g for *A. smithii*, 173 cm^2/g to 78 cm^2/g for *S. glomulifera* and 108 cm^2/g to 77 cm^2/g for *D. australis* (Fig. 3.10). All plant species used in this study had leaves that demonstrated the same trend of having relatively constant SLAs for at least 130 days of development (or a few weeks prior to full expansion) followed by a slight decline until full expansion was reached (Fig. 3.10).

3.9 Leaf Lifespan

Individual leaves die for one of four reasons: (a) They are consumed by an herbivore or infected by a pathogen; (b) they are mechanically removed by wind, hail, or sand abrasion; (c) a physical stress disrupts cellular activity, inducing

senescence; or (d) a genetically determined lifespan is terminated by an hormonally-mediated senescence process that may itself be triggered by an external or internal cue (Chabot & Hicks, 1982).

A major expectation of the resource availability hypothesis is that leaves from high resource environments will have short leaf lifespans and that leaves from low resource habitats will have long leaf lifespans (Coley et al., 1985).

3.9.1 Methods

Eight wet sclerophyll, 11 rainforest and 9 dry sclerophyll plant species were monitored from August 2002 to August 2005 to determine leaf lifespan. Whilst leaf lifespan is defined here as the age of the oldest surviving leaf (leaf lifespan actually achieved) for a plant species, it should be noted that 9 temperate rainforest species and 1 dry sclerophyll species had leaves that survived longer than the 3 year monitoring period.

Maximum leaf lifespan for the 28 plant species was determined by tagging at least 5 leaves on four individual plants per species with wiremarker tape and monitoring survivorship monthly using digital photography. All tagged dry sclerophyll and wet sclerophyll leaves were in direct sunlight. However a number of the temperate rainforest plants were found in shade only. Therefore for this component, resources were defined in terms of soil and light. Dry sclerophyll leaves were exposed to full sun (high resource), but lower soil nutrients (low resource); wet sclerophyll leaves were exposed to full sun and higher soil nutrients; and temperate rainforest leaves were exposed to dappled light and even higher soil nutrients.

3.9.2 Results

Temperate rainforest plants at the highest nutrient site (Bola Creek, Royal National Park) had leaf lifespans that were significantly longer than dry sclerophyll (Welch t-test: t = 3.4, df = 19, P = 0.003) and wet sclerophyll leaves (Welch t-test: t = 3.3, df = 24, P = 0.003) (Fig. 3.12). Average maximum leaf lifespan for the studied

temperate rainforest plant species was over 2.9 years, compared to 2.1 years for dry sclerophyll species. Wet sclerophyll leaves had an average leaf lifespan of 1.9 years. Dry sclerophyll and wet sclerophyll leaf ages did not differ significantly (Welch t-test: t = -0.35, df = 21, P = 0.73).

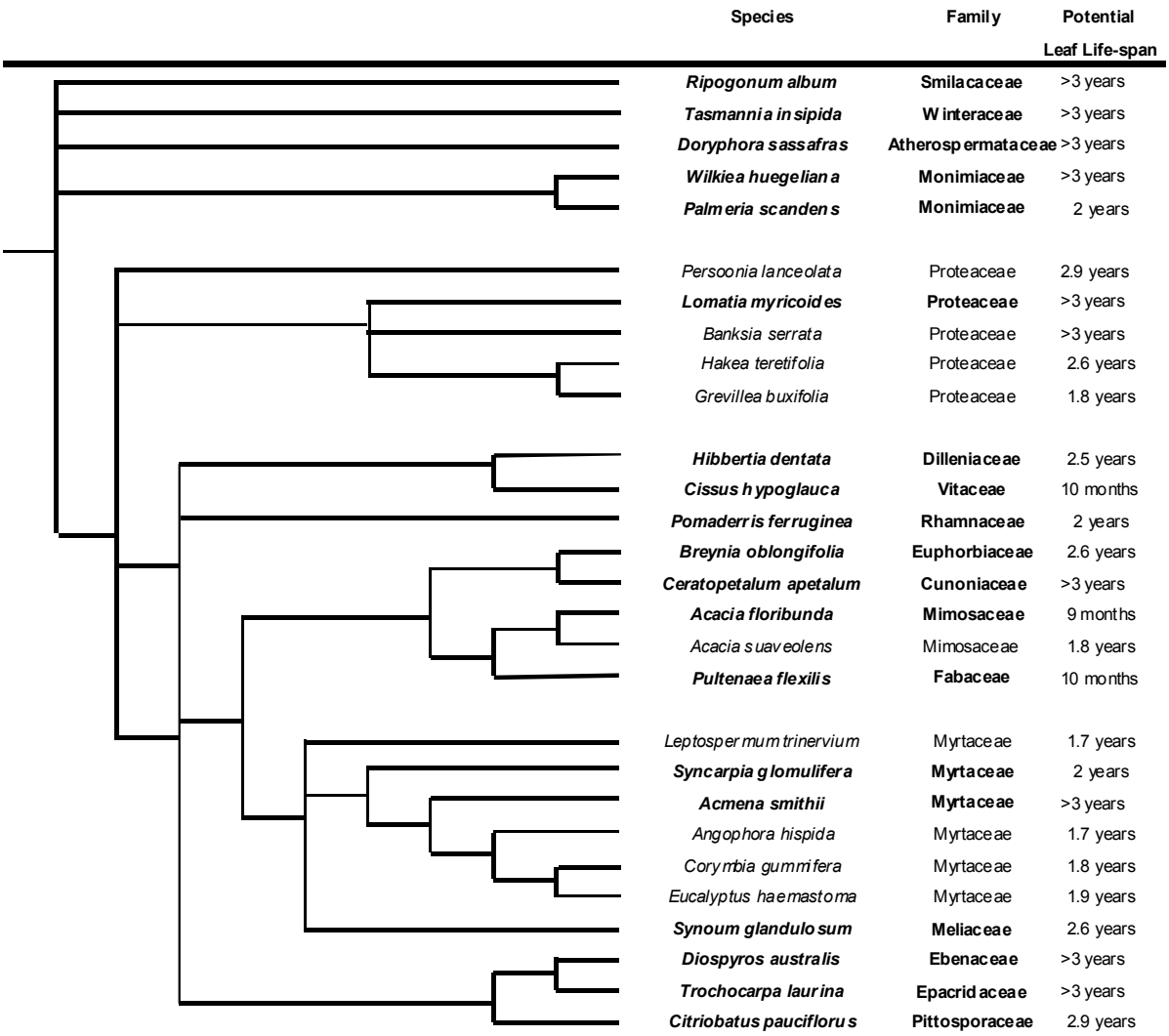


Figure 3.12 Phylogeny and leaf lifespan of 28 plant species from eastern New South Wales. Mesic species are in bold.

3.10 Discussion

The resource availability hypothesis predicts leaves from resource-poor environments will be better defended, have slower intrinsic growth rates, and

longer leaf lifespans than leaves from higher resource sites (Coley et al., 1985). In this study, several of these expectations were supported whilst others were not.

It was found that plants from nutrient-poor soils generally had higher concentrations of carbon-based chemical compounds than plants from higher resource habitats. Leaves from dry sclerophyll plant species contained higher concentrations of total phenols and condensed tannins than rainforest and wet sclerophyll leaves. Condensed tannins act as antiherbivore compounds forming complexes with plant proteins and carbohydrates. They bind to digestive enzymes to disrupt digestive processes and reduce nutrient availability (Rhoades, 1979). If phenolic compounds are an important plant defence, these results support the resource availability hypothesis.

However, five out of 27 rainforest plant species tested positive for either alkaloids or cyanogenic glycosides. As thousands of secondary metabolites have been found in leaves (Waterman & Mole, 1994), it is possible that mesic leaves are being defended by alternative chemically-derived or inducible secondary metabolites. The presence of protein-derived molecules such as lectins, chitinases, proteinase inhibitors and α–amylase inhibitors (Mello et al., 2002) have also not been examined in this study. As these molecules have the ability to interfere in the absorption of nutrients, increase absorption of toxic substances (Falco et al., 2001), damage insect midgut (Falco et al., 2001), and inhibit digestive enzymes (Silva et al., 2001; Pompermayer et al., 2001), further investigations are needed.

Dry sclerophyll leaves from low nutrient soils had slightly higher concentrations of lignin, higher carbon:nitrogen ratios, and lower nitrogen and water content than leaves of plants from higher resource sites. The mechanism of producing relatively unpalatable leaves may have evolved as a potential defensive strategy against herbivory by plants growing in low resource environments. Lignin reduces the digestibility of plants by forming hydrogen-bonded complexes with plant carbohydrates and proteins (Rhoades & Cates, 1976). Assuming these characteristics do indeed make leaves less palatable to herbivores and reduce herbivory, these findings support the resource availability hypothesis.

Leaf force of fracture and toughness are probably the most important physical defences against herbivory. Whilst several dry sclerophyll leaves from low nutrient sites were found to have particularly high forces of fracture (eg. *Banksia serrata*, *Angophora hispida* and *Eucalyptus haemastoma*), the majority of leaves from low and higher resource sites were not differentiated by force of fracture and toughness, ie there were just as many higher resource plants with tough leaves as there were low resource plants with soft leaves. These findings consequently do not support the expectations of the resource availability hypothesis.

Expanding dry sclerophyll leaves from nutrient poor environments reached their 50th-percentile toughness faster than immature mesic leaves from higher resource sites. This ability to toughen faster may contribute to the foliar defence of immature dry sclerophyll leaves, and would therefore provide support for the expectations of the resource availability hypothesis. However, because fibre, lignin and cuticular thickening constrain leaf expansion (Aide, 1993), it is unlikely leaf toughness is an important physical anti-herbivore defence for young expanding leaves. Instead, the higher initial rate of leaf toughness of dry sclerophyll leaves may be part of a mechanism designed to defend delicate tissues from high sunlight levels and desiccating winds.

Some studies have suggested that phenological characteristics may contribute to foliar defence (Aide et al., 1989; Kursar et al., 1991). In the present study, young leaves were found to contain much higher concentrations of phenols than mature leaves. As leaves expanded, toughness and force of fracture increased steadily and the concentration of total phenols declined as phenols were diluted with addition of carbon. This trend may be an example of maturing leaves investing fewer resources in chemical defences as they invest more resources into physical defences. However, there is an alternative explanation. Total phenols have functions other than defence such as reducing the photodestruction of exposed tissues (Waterman & Mole, 1994; Close & McArthur, 2002). Phenols achieve this by acting as antioxidants, varying concentrations with environmental conditions in order to counteract potential photodamage (Close & McArthur, 2002). Close and McArthur (2002) suggest that phenol levels are low under some environmental conditions not because resources to produce them are limited, but simply because

the risk of photodamage is low and they are not required. Therefore, a decline in total phenols with expansion may indicate a leaf's increased ability to withstand harsh sunlight. In addition, low phenol concentrations in rainforest and wet sclerophyll plants may be indicative of lower photodamage risk for plants growing beneath partially closed canopies. This could explain the lack of correlation between phenols and herbivory in several studies (eg. Coley 1983a, Hatcher & Paul 1994, Read et al. 2003, also see chapter 4), and the tendency of phenol concentrations to increase as light levels rise (Shure & Wilson, 1993, Dudt & Shure 1994, Hatcher & Paul 1994, Weinig et al. 2004).

Recent research has found that small leaves have relatively short leaf expansion rates compared to plants with large leaves (Moles & Westoby, 2000, 2003). Rapid leaf expansion may be a phenological characteristic that contributes to plant defence (Moles & Westoby, 2000), imposing severe constraints on host-finding by specialist herbivores and shortening the period of exposure to generalists (Coley & Barone, 1996). In the current study, leaves from low and higher nutrient environments with similar mature leaf areas (1886 mm^2 to 4597 mm^2) were monitored from leaf formation to maturity. It was found that leaves from higher resource environments did not have faster expansion rates than leaves from resource poor habitats. The expectation of the resource availability hypothesis that plants from resource rich environments would have fast expansion rates was therefore not supported.

The resource availability hypothesis suggests plants from resource-poor environments will have longer leaf lifespans than those from resource-richer habitats (Coley et al., 1985). Whilst many of the temperate rainforest leaves in the current study had lifespans longer than 3 years, it is clear they have significantly longer leaf lifespans than either wet or dry sclerophyll leaves (means of 1.9 and 2.1 years respectively). Though it should be acknowledged that many rainforest leaves were forming in dappled light (low resource), leaves monitored at the wet sclerophyll dyke, KCNP were generally in full sunlight. In addition, the leaf lifespans of dry and wet sclerophyll leaves from low and higher soil nutrient sites did not differ significantly. The leaf lifespan study therefore did not support the expectations of the resource availability hypothesis.

Recent Australian studies have demonstrated that plant species receiving higher rainfall often have greater concentrations of foliar nitrogen, higher specific leaf areas and longer leaf lifespans than plants from environments with lower rainfall (Wright *et al.* 2001; Wright *et al*. 2002). In addition, species receiving higher light levels can have smaller leaf areas than species growing in habitats with moderate shade (Bragg & Westoby 2002). There is no doubt that resources have an effect on leaf traits. What is less clear is whether resources influence the type, quantity and quality of plant defences, and whether those defences are actually effective in reducing herbivore consumption. Some of these issues are addressed in chapters four and five.

In conclusion, several expectations of the resource availability hypothesis were supported by these data, whilst other expectations were not. Leaves from low resource environments contained higher concentrations of phenols and may therefore be better defended chemically than mesic leaves from higher resource environments. They may also be less palatable than higher resource leaves as they have high carbon:nitrogen ratios, higher lignin content and contain less water. However: (1) leaf toughness, one of the most important physical defences, was not a variable that differentiated leaves from low and higher resource sites;

(2) there is evidence that phenols act primarily as photoinhibitors rather than anti-herbivore chemical compounds;

(3) there are thousands of alkaloids, cyanogenic glycosides and other secondary compounds, and protein inhibitors that have not been tested in this project that may be defending mesic leaves in higher resource environments; and

(4) leaves from low resource areas did not have longer leaf lifespans than leaves from higher resource sites.

Despite the exceptions, the two resource environments had distinct suites of leaf characteristics. It is possible that plants from different resource environments are simply employing *different* strategies to defend their tissues from herbivores rather than one vegetation type being quantitatively better defended than the other.