

Determining the effects of forest harvesting on habitat nutritional quality for koalas

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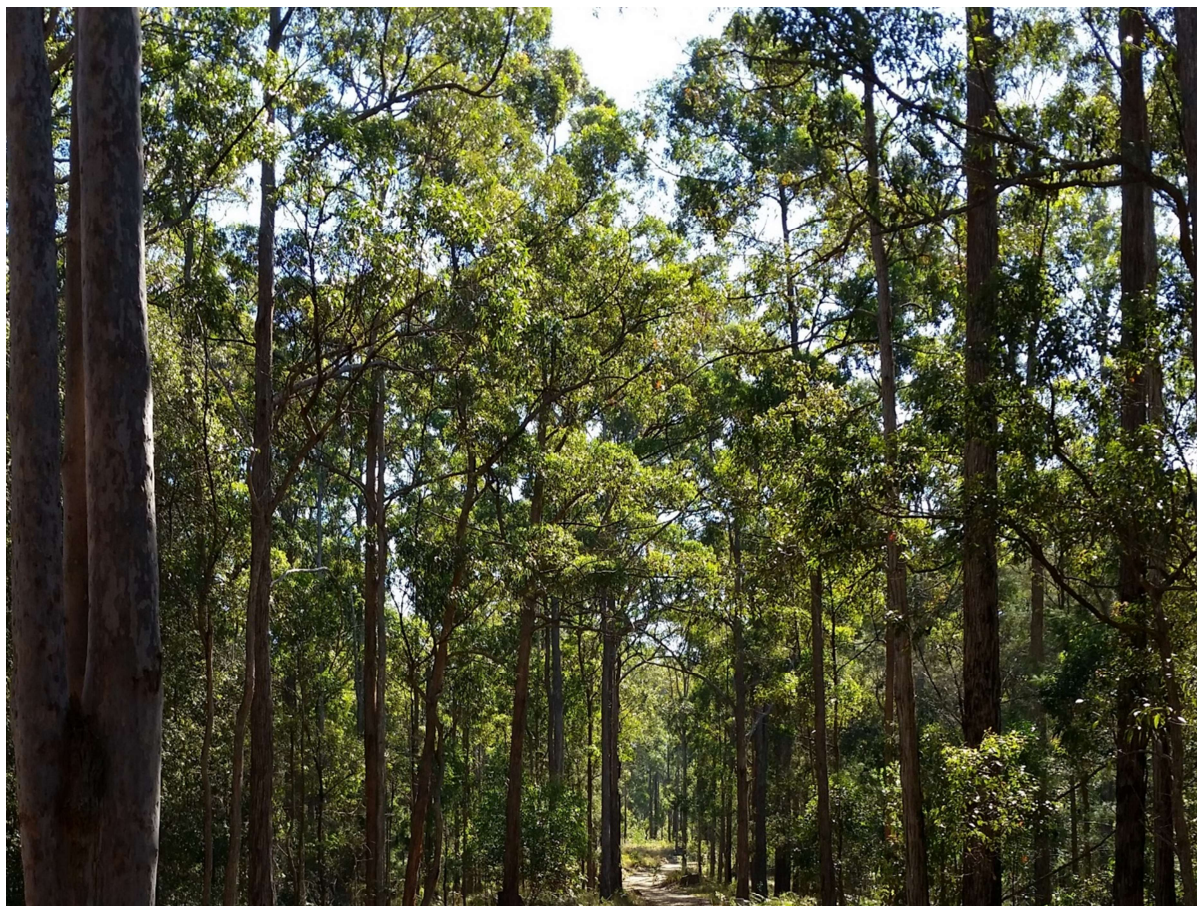


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Abbreviations

BIC	Bayesian information criterion
DBH	Diameter at breast height
DM	Dry matter
FPCs	Formylated phloroglucinol compounds
GPS	Global positioning system
N	Nitrogen
NIRS	Near-infrared reflectance spectroscopy
NSW	New South Wales
PSMs	Plant secondary metabolites
RN17	Research Note 17 forest types
UBFs	Unsubstituted B-ring flavanones

Summary

The project aimed to determine the effects of regeneration harvesting on koala habitat nutritional quality on the NSW North Coast. Nutrients (total and digestible nitrogen (N)) and plant secondary metabolites (formylated phloroglucinol compounds (FPCs) and unsubstituted B-ring flavanones (UBFs)) known to be important to koalas were measured in fully expanded leaves from more than 900 trees of 19 *Eucalyptus* and 3 *Corymbia* species across three tree size classes from the study region. There were substantial differences in concentrations of key nutritional constituents between eucalypt species, but there were no differences between tree size classes. This latter finding suggests that, if the mixture of available species is suitable, koalas should be able to find food of adequate nutritional quality in a regrowing forest dominated by trees as small as 10 cm diameter at breast height. However, it is not known from this study whether forests dominated by trees as small as 10 cm would also provide sufficient shelter for koalas.

Species regarded as being koala browse trees under Coastal Integrated Forestry Operations Approval protocols generally contained the highest average concentrations of digestible N, confirming their potential nutritional value to koalas. However, koalas may be unable to eat a small proportion of the trees within each browse species because some individual trees contained high concentrations of deterrent FPCs or UBFs. *Eucalyptus pilularis* (blackbutt), an important timber species, was one of the poorest quality available eucalypt species for koalas due to low average concentrations of digestible N and reasonably high concentrations of UBFs. In addition, blackbutt and *Eucalyptus microcorys* (tallowwood, a primary browse species), the two most widespread species, were generally poorer quality food at higher elevations.

The average nutritional quality of sites sampled in NSW North Coast state forests was relatively low compared to koala habitat sampled at other locations around Australia. However, sites with higher proportions of koala browse species, lower proportions of blackbutt, and/or lower proportions of other non-preferred eucalypt species tended to have the highest digestible N concentrations. Sites were predicted to retain their nutritional quality after harvesting and regeneration if they regrew with a similar species composition to their pre-harvest state. However, if the proportion of koala browse trees increased, or blackbutt or other non-preferred trees decreased, site nutritional quality was predicted to improve, while it was predicted to decline if the proportion of blackbutt or other non-preferred trees increased and/or browse trees decreased.

Forests with lower proportions of blackbutt relative to other species, particularly if those species are koala browse, are likely to support higher koala densities. However, koalas may be able to persist at low densities even in forests of low average nutritional quality on the NSW North Coast. Harvesting and regeneration practices that alter the proportions of tree species in the landscape are also likely to affect the density of koalas that a site can support. Specifically, the predicted koala density index increases with reduced proportions of blackbutt, but decreases under the reverse scenario.

Background

The nutritional quality of eucalypt forests can influence koala habitat quality and population densities (Moore et al. 2010; Au 2018). Forests with higher average foliar concentrations of critical nutrients and lower concentrations of herbivore deterrent plant secondary metabolites (PSMs) support more koalas (Au 2018; Au et al. 2019). The nutritional quality of eucalypts varies within and between tree species because the chemical determinants of browse quality are genetically and environmentally determined (Moore et al. 2004; Andrew et al. 2005; Marsh et al. 2020). The nutritional composition of eucalypt species from one region may therefore differ from the nutritional composition of the same tree species from another region. Even within the same forest, trees of the same species can show substantial differences in concentrations of key nutrients and herbivore deterrent PSMs (Wallis et al. 2002).

There are three main drivers of eucalypt browse nutritional quality for koalas: 1) digestible nitrogen (digestible N), 2) formylated phloroglucinol compounds (FPCs), and 3) unsubstituted B-ring flavanones (UBFs). Protein (usually measured as total N) can be a limiting nutrient for herbivores because it is less available in plant-based diets. Digestible N is the relative amount of protein available for digestion (DeGabriel et al. 2008), and it is influenced by the concentration of total N in leaves and the types and concentrations of tannins that bind to proteins (Marsh et al. 2020). In general, eucalypts are considered to be a poor source of digestible N (Wallis et al. 2010), so trees containing high digestible N concentrations may be particularly valuable to eucalypt folivores. For example, diets higher in digestible N can increase reproductive fitness (DeGabriel et al. 2009) and improve tolerance to some herbivore-deterrent PSMs (Au et al. 2013). At a landscape scale, koala densities have a strong positive correlation with the average concentration of digestible N at a site (Au 2018; Figure 1).

In contrast to digestible N, concentrations of FPCs and UBFs negatively influence koala densities (Au 2018; Figure 1). FPCs and UBFs are specific classes of eucalypt secondary metabolites that are known to deter koala browsing (Marsh et al. 2007; Marsh et al. 2021). FPCs occur in *Eucalyptus* species belonging to the *Symphyomyrtus* and *Alveolata* subgenera (common name symphyomyrtle), whereas UBFs occur in species belonging to the *Eucalyptus* subgenus (common name monocalypt) (Tucker et al. 2010). Variation in FPC and UBF concentrations within and between eucalypt species create chemically complex landscapes with differing levels of palatability (Moore et al. 2010; Marsh et al. 2014; Au et al. 2019).

Landscape disturbance from fire and/or logging can naturally or artificially favour the proliferation of some species over others, altering the eucalypt species composition of a forest over time (King 1985; Nicholson 1999; Au et al. 2019). This, in turn, can affect overall nutritional quality, and the value of habitat to koalas (Au et al. 2019). These effects should be considered in the context of mitigation strategies where the intent is to maintain viable koala populations in native timber production forests in the longer term. The overall aim of this project was to determine how harvesting and regeneration on the NSW North Coast affect the nutritional quality of habitat for koalas, and, as such, the expected effects on koala population densities. We used a combination of foliar nutritional quality data from eucalypt trees sampled in the field and statistical modelling to assess the nutritional quality of a range of eucalypt species and forest sites from the NSW North Coast. We also determined how different proportions of 1) koala browse species, 2) *E. pilularis* (blackbutt, an important timber species; Horne 1994), and 3) other eucalypt species influenced koala habitat nutritional quality and the predicted koala density index. Finally, we investigated whether selectively retaining koala browse trees in scenarios in which blackbutt was preferentially allowed to

regenerate improved habitat nutritional quality relative to replacement scenarios in which browse trees were not prioritised.

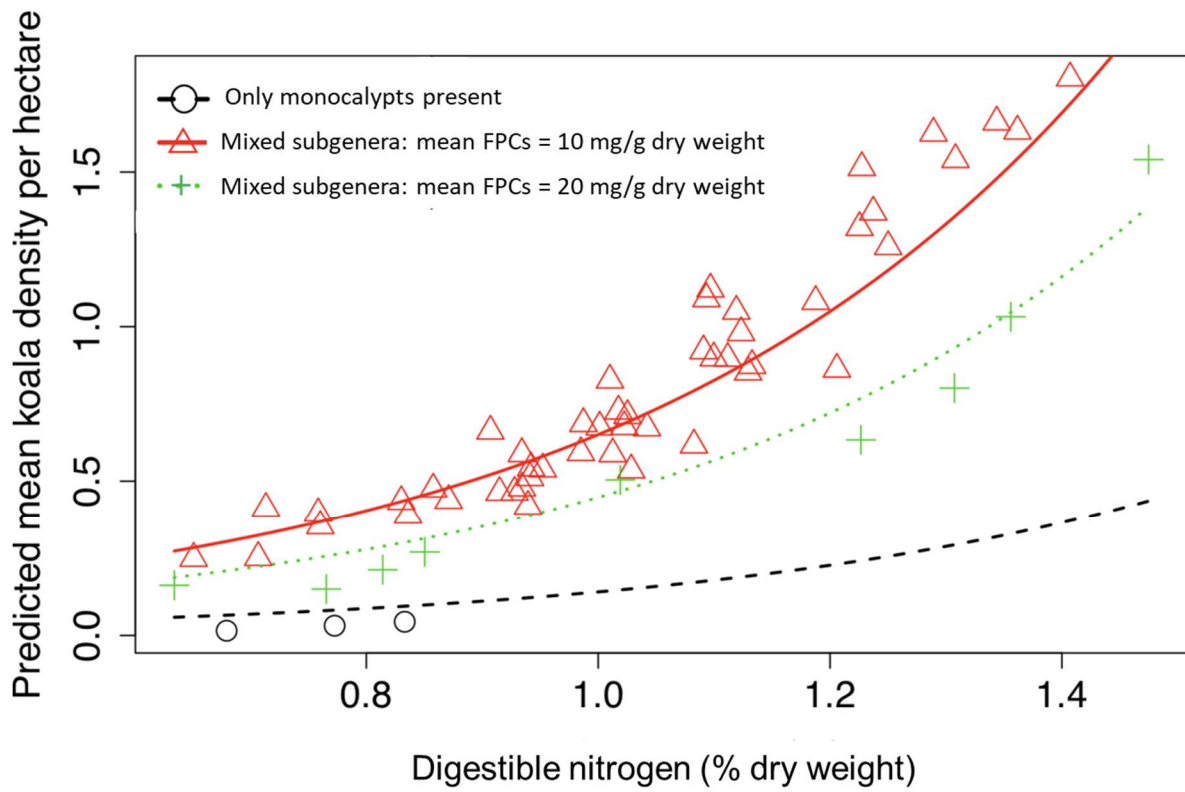


Figure 1. The relationship between average site nutritional quality and koala density at 75 sites across eastern Australia. Reproduced from Au (2018).

Objectives

The project had four related objectives:

1) Determine the current nutritional composition of forests within the regeneration forestry zone on the North Coast of NSW.

We investigated variation in nutritional composition between and within the eucalypt species and communities commonly available to koalas within the regeneration forestry zone on the NSW North Coast. We also compared the nutritional composition of trees of different sizes to determine whether forest and tree age influences food quality for koalas. The data set also gave us the necessary baseline information to address later objectives.

2) Model the way in which habitat nutritional quality is affected by harvesting and regeneration scenarios.

Blackbutt is one of the most valuable timber species in NSW North Coast forests (Horne 1994), but the harvesting strategy (e.g. selective or intensive) can influence whether blackbutt regrowth is suppressed or favoured relative to other species (Florence 1996). We conducted a series of statistical simulations to explore how different proportions of koala browse species, blackbutt and other eucalypts influenced site nutritional quality. We also investigated whether the nutritional composition of forest plots differed between scenarios that randomly removed and replaced trees with blackbutt, relative to those in which koala browse trees were preferentially retained.

3) Predict changes in koala densities under harvesting and regeneration scenarios.

We used the relationship between nutritional quality and koala population densities developed by Au (2018) to determine how different proportions of koala browse species, blackbutt and other eucalypts are likely to influence koala densities. We also investigated the expected direction of any potential changes in koala densities that may result directly from a shift in species composition towards different proportions of eucalypt species. This exercise utilised current measured habitat nutritional values together with those generated through simulations in Objective 2.

4) Identify strategies that minimise long-term impacts of forestry on koala populations.

We utilised the results from Objective 3 to inform potential strategies to minimise long-term impacts of forestry on koala populations.

Methods

Selection of sites

Site selection was designed to ensure that a range of tree species were encountered across different age classes throughout the designated regeneration forestry zone. To achieve this, we generated a dataset for every logging compartment within the regeneration harvesting zone that included RN17 forest types (classification of tree communities; Forestry Commission of New South Wales 1989) and harvest event history (the date of last harvest) based on a geodatabase provided by the NSW Forestry Corporation. We further stratified compartments into three geographic regions (north, mid and south). We then randomly selected one compartment in each geographic region for each of the eight most common forest types (Table A 1) in three harvest history categories (pre 2000, 2000-2009, 2010-2019). Ten forest type/harvest history combinations were absent from some geographic regions, and thus our final selection consisted of 62 sites. Seven of these sites were inaccessible due to logging activities or active bushfires, but three additional sites were included to overlap with koala acoustic survey work, bringing the final number of sites to 58 (Figure 2; Table A 2).

Survey of eucalypt species composition

Sites were visited between May and September 2019. At each selected site, we conducted a survey of eucalypt species composition along a 420m transect. Every 60 m we recorded specific details (GPS location, elevation, species, diameter at breast height (DBH), surrounding topography) of the closest four *Eucalyptus* trees (>10 cm DBH) to the transect point. Thus, we collected data on 32 trees per transect.

Collection of samples for nutritional analysis

At each 60 m point along transects, we collected mature leaves from one tree of every *Eucalyptus* species present, unless another tree of that species had been collected within the previous 80 m. This spacing reduced the chance of collecting closely related individuals, which are more likely to be similar in nutritional composition (Andrew et al. 2005). Thus, we collected a maximum of four samples per species per transect. We preferentially collected samples from trees that had been included in the survey of eucalypt species composition. In addition to *Eucalyptus*, we collected leaves from 11-16 individuals from three species of the closely related genus, *Corymbia*, which koalas occasionally eat (NSW Office of Environment and Heritage 2018).

We used a throw line launcher to pull down a small branch from each tree (Youngentob et al. 2016), and removed approximately 80 g leaves from their stems. For each tree, we also recorded the following information; GPS coordinates, elevation, topography, species, DBH, harvest context (e.g. retained or regrowth), and density of surrounding understorey. Leaves were placed into paper bags, weighed, and then immediately frozen on dry ice. They were later transferred to a freezer at -20 °C for storage until preparation for analysis.

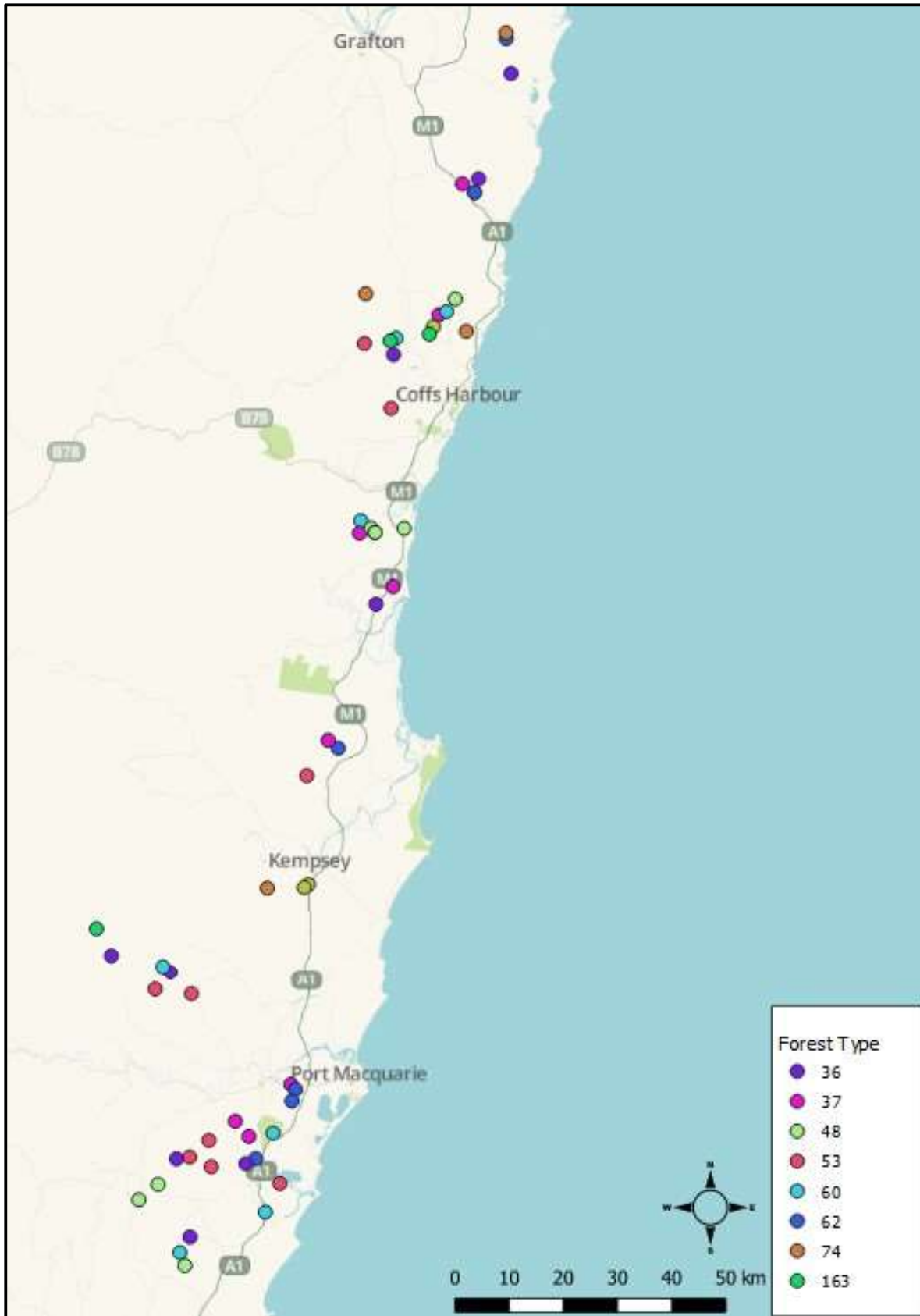


Figure 2. Sites selected for sampling. The different colours represent different forest types (see key), which are described in Table A 1.

Preparation and analysis of leaf samples

Frozen leaves were freeze dried and then reweighed to determine the mass lost during the drying process. This value was used to calculate the dry matter (DM) content, or conversely the amount of water, in leaves as a percentage of the total wet mass of leaves collected. Dried leaf samples were ground using a Cyclotec mill (Foss Tecator, Hillerod, Denmark) until they passed through a 1 mm sieve. The spectra of ground samples were collected between 400 nm and 2,498 nm using a Foss XDS near-infrared reflectance (NIR) spectrometer (Foss, Hillerod, Denmark). We used NIRS calibration models developed from wet chemical assays on a subset of 300 samples to measure total N, and digestible N using the laboratory methods described in Degabriel et al. (2008). We used a calibration developed by Marsh et al. (2019) to measure UBFs and a calibration from Au et al. (2020) to measure FPCs.

Comparison between species and tree size classes

We calculated the range, mean and median concentrations of total N, digestible N, UBFs and FPCs for leaves collected from different species and tree size classes. We investigated the effect of topography, elevation and tree size on the nutritional composition and moisture content of the two most widely sampled species, *E. pilularis* (blackbutt) and *E. microcorys* (tallowwood). We used linear models for each of these measures with the following explanatory variables: species, topography (upper slope, mid-slope, lower slope, ridge line, flat), elevation, tree size (three categories) and all of the two-way interactions between them. Because UBFs only occur in blackbutt, while FPCs only occur in tallowwood, models for these constituents were confined to the relevant species and did not include the species and interaction terms. The final models for all constituents were determined using stepwise selection based on the Bayesian information criterion (BIC) model fit. The topography variable, which was retained only in the model for moisture content, was then further simplified by pairwise testing of the 5 different topographies. The models were also assessed by residual plots; the results were satisfactory after excluding 3 outliers from analysis.

Nutritional composition of sites

The nutritional composition of sites was determined using a combination of the data sets on site species composition and leaf nutritional quality. Specifically, we used the survey of eucalypt species composition to determine the relative availability of each species at a site. Where we had sampled leaves from a surveyed tree, we used the specific nutritional data for that tree. For trees that we did not specifically sample, we substituted the nutritional data from other nearby trees of the same species. If there were no nearby trees of the same species, we used a randomly selected tree of the same species from any site. The final measures for each nutritional constituent at a site therefore included 32 trees of the specific species mix recorded during the survey of eucalypt species composition. The nutritional composition of tree species from genera other than *Eucalyptus* (i.e. *Corymbia*) were not included in site quality measures.

Effect of tree species composition on site nutritional value

We used random selection from the full pool of analysed trees to simulate sites containing varying proportions of three categories of trees: 1) blackbutt (*E. pilularis*), 2) koala browse trees (combined primary and secondary browse trees as defined in the Coastal Integrated Forestry Operations Approval (IFOA) protocols: *E. microcorys*, *E. tereticornis*, *E. saligna*, *E. propinqua* and *E. andrewsii*; NSW Environment Protection Authority 2020), and 3) other eucalypt species (all other species) at 20 % intervals for each category from 0-100 %. These were plotted to allow visualisation of 1) the approximate current average nutritional composition of any site in NSW North Coast forests based

on the proportions of trees in each category, and 2) how changing the proportions of trees in different ways are likely to influence site nutritional composition.

We also conducted a series of statistical simulations to determine how replacing trees with blackbutt affected predicted post-harvest and post-regeneration nutritional quality. The purpose of this exercise was to explore whether the preferential regrowth of blackbutt affected site nutritional quality at different replacement levels and in different forest types. In these simulations, we used the mean nutritional values for each site as the baseline nutritional composition. First, we sequentially removed randomly selected trees from each site and replaced them with an individual blackbutt randomly drawn from the full data set. We recalculated the average nutritional quality of a site using the new simulated tree composition at 10 % intervals of proportional replacement by blackbutt. This process was repeated 50 times for each site. The range and median value for each 10% replacement interval derived from this modelling are reported.

Second, we repeated the above process using a non-random selection of trees to explore the potential effect of selective retention of koala browse trees on site nutritional quality. Specifically, we divided trees into two categories: 1) koala browse trees (as defined above), and 2) all other species including blackbutt. Trees in the second category were removed and replaced with blackbutt before trees in the first category.

Predicted effects on koala densities

We used the established relationship between average site nutritional quality and koala density (Au 2018; Figure 1; Model A1) to determine 1) a koala density index for sites containing different simulated proportions of koala browse trees, blackbutt and other eucalypt species, and 2) the direction and degree of change in koala density index with simulated replacement of trees by blackbutt in different forest types with random or selective retention. Specifically, we applied the mean site nutritional values from the simulations in the previous section to koala density models (Model A2 and A3) to generate a series of predicted koala density indexes for each site, forest type, replacement scenario and proportion of different tree categories.

A koala density index was used rather than absolute density, because of the likelihood that an absolute density estimate would be misleading. This is because 1) there are slight differences in the way in which nutritional constituents were measured between this and the original model development study, 2) the techniques used to estimate koala densities at sites in the study by Au (2018) differ from those currently being used to assess koala densities in NSW North Coast forests, and 3) the initial model did not incorporate the effects of UBFs on koala densities, even though they may have a substantial negative impact (Au 2018; Figure 3). Despite these considerations, the density index can be used to determine relative differences in predicted densities between sites (i.e. where one site is expected to support more koalas than another), and the expected direction of any change in the capacity to support koalas with changing forest species composition.

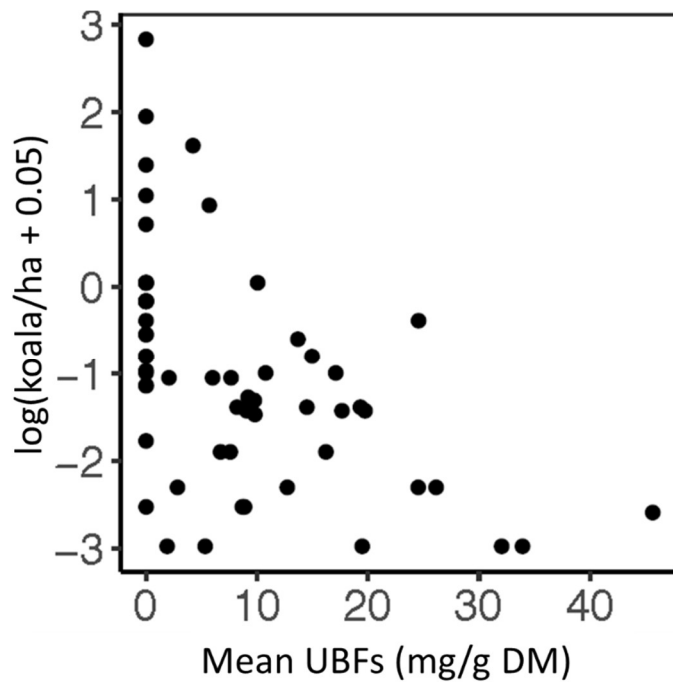


Figure 3. The raw relationship between koala densities and mean UBF concentrations of eucalypts sampled at the 75 sites from which koala density nutritional models were originally developed. This relationship has not yet been incorporated into koala density nutritional models. Reproduced from Au (2018).

Results

Samples collected

Leaf samples were collected from 921 trees representing 19 *Eucalyptus* and 3 *Corymbia* species (Table 1). Widespread and common species were encountered, and therefore sampled, more often than rarer species. The tree species composition of sites was highly variable, ranging from 0-100 % koala browse trees, 0-88 % blackbutt, and 0-100 % other eucalypts (Table A 2). Sampled trees ranged in size from 5 cm to 166 cm DBH (mean = 30 cm).

Table 1. Summary of leaf samples collected from NSW North Coast state forests.

Species	Total samples	DBH 5-15cm	DBH 15-25cm	DBH >25cm
All	921	218	230	473
<i>E. pilularis</i>	177	44	51	82
<i>E. microcorys</i>	181	39	45	97
<i>E. propinqua</i>	99	17	28	54
<i>E. siderophloia</i>	88	25	20	43
<i>E. resinifera</i>	78	26	16	36
<i>E. carnea</i>	68	16	16	36
<i>E. acmenoides</i>	44	12	4	28
<i>E. saligna</i>	40	5	13	22
<i>E. grandis</i>	30	8	7	15
<i>C. gummifera</i>	16	7	3	6
<i>C. intermedia</i>	16	5	5	6
<i>E. paniculata</i>	16	4	3	9
<i>E. robusta</i>	12	4	3	5
<i>C. maculata</i>	11	2	4	5
<i>E. globoidea</i>	10	1	5	4
<i>E. pyrocarpa</i>	8	0	3	5
<i>E. laevopinea</i>	7	1	0	6
<i>E. umbra</i>	7	1	3	3
<i>E. tereticornis</i>	5	0	0	5
<i>E. andrewsii</i>	4	0	1	3
<i>E. planchoniana</i>	2	0	0	2
<i>E. agglomerata</i>	1	0	0	1
<i>E. racemosa</i>	1	1	0	0

Nutritional composition of eucalypt leaves

The concentrations of total N, digestible N, FPCs and UBFs varied between species (Figure 4; Table 2). Species with the highest digestible N concentrations were all classed as “high use” or “significant use” in a review of koala tree use by the NSW Office of Environment and Heritage (2018); namely, *E. grandis*, *E. tereticornis*, *E. microcorys*, *E. propinqua* and *E. robusta* (Figure 4b). All but *E. grandis* are also listed as koala browse trees for retention in NSW Coastal IFOA protocols (Table 2; NSW Environment Protection Authority 2020). The highest concentrations of UBFs occurred in *E. andrewsii*, with some *E. pilularis* individuals also containing high UBF concentrations (Figure 4c). FPC concentrations were highly variable both within and between species. For example, three species known to be eaten by koalas (*E. microcorys*, *E. propinqua* and *E. resinifera*) contained individual trees that ranged from less than 5 to more than 40 mg g⁻¹ dry matter (DM; Figure 4d). The moisture content of leaves ranged from 37 % to 60 % of wet mass, with species means being 43 % to 52 %.

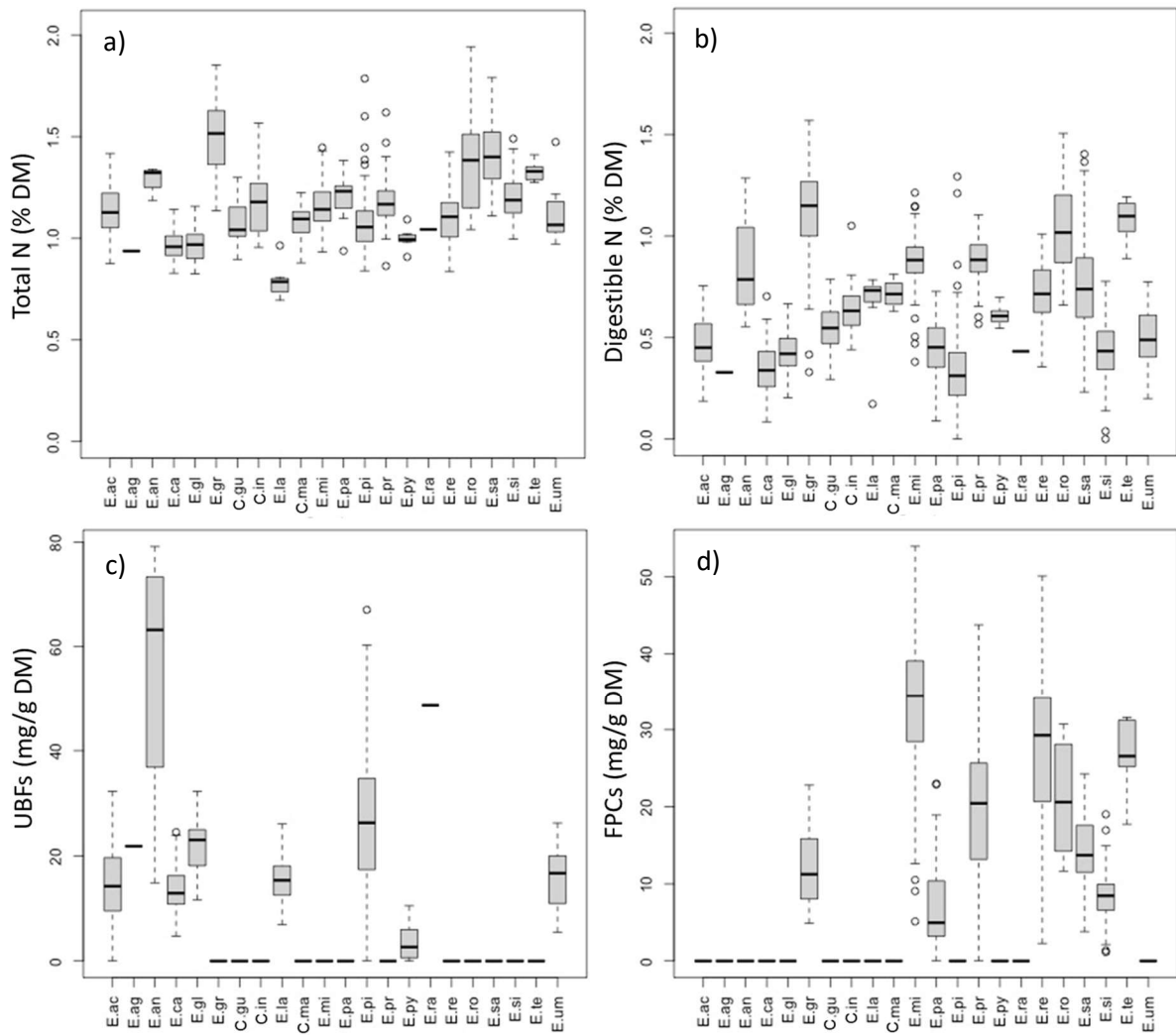


Figure 4. Concentrations of a) total N, b) digestible N, c) UBFs, and d) FPCs in the species collected from NSW North Coast State Forests. Upper and lower bars show the maximum and minimum values respectively for each species, with outliers shown as circles. Boxes encompass values between the first and third quartiles, with the mid-line indicating the median. Species names are abbreviated to the first two letters to reduce crowding. Full species names and the number of individuals measured within each species are given in Table 1.

Table 2. The relative mean nutritional value of eucalypt species sampled in NSW North Coast forests. Species can be considered to be of highest nutritional quality for koalas when they contain high concentrations of digestible N and low concentrations of FPCs or UBFs. Species classified as primary or secondary koala browse trees in NSW Coastal IFOA protocols are highlighted in grey. The mean concentration of each constituent within each species was assigned to a category according to the following criteria; Digestible N: low <0.38, medium = 0.39-0.77, high >0.78 % dry matter (DM). Formylated phloroglucinol compounds (FPCs): low <19, medium = 20-34, high >35 mg.g⁻¹ DM. Unsubstituted B-ring flavanones (UBFs): low <10, medium = 11-20, high >20 mg.g⁻¹ DM.

Species (number of trees sampled)	Digestible N	FPCs	UBFs
<i>E. pilularis</i> (177)	Low		High
<i>E. microcorys</i> (181)	High	Medium	
<i>E. propinqua</i> (99)	High	Low	
<i>E. siderophloia</i> (88)	Medium	Low	
<i>E. resinifera</i> (78)	Medium	Medium	
<i>E. carnea</i> (68)	Low		Medium
<i>E. acmenoides</i> (44)	Medium		Medium
<i>E. saligna</i> (40)	High	Low	
<i>E. grandis</i> (30)	High	Low	
<i>C. gummifera</i> (16)	Medium		
<i>C. intermedia</i> (16)	Medium		
<i>E. paniculata</i> (16)	Medium	Low	
<i>E. robusta</i> (12)	High	Medium	
<i>C. maculata</i> (11)	Medium		
<i>E. globoidea</i> (10)	Medium		High
<i>E. pyrocarpa</i> (8)	Medium		Low
<i>E. laevopinea</i> (7)	Medium		Medium
<i>E. umbra</i> (7)	Medium		Medium
<i>E. tereticornis</i> (5)	High	Medium	
<i>E. andrewsii</i> (4)	High		High
<i>E. planchoniana</i> (2)	Low		Low
<i>E. agglomerata</i> (1)	Low		High
<i>E. racemosa</i> (1)	Medium		High

Topography had no effect on any of the measured nutritional constituents in the two most widely sampled eucalypt species, blackbutt and tallowwood ($p > 0.05$ for all). However, the moisture content of leaves from these species varied with topography, with trees on mid-slopes having a higher water content (lower % DM) than trees in other topographic positions at sea level equivalent ($t(231) = -3.30$, $p < 0.001$; Table 3). There was also a significant interaction between elevation and mid-slope for moisture content; % DM increased with elevation for mid-slope topographies, but decreased for other topography categories. This complex relationship is unlikely to be meaningful to koalas. Elevation was also correlated with several nutritional variables (Table 3). At higher elevations blackbutt (but not tallowwood) had higher concentrations of digestible N ($t(238) = 3.073$, $p < 0.01$) and higher concentrations of UBFs ($t(116) = 37.24$, $p < 0.001$). Concentrations of FPCs in tallowwood were also positively correlated with elevation ($t(122) = 13.65$, $p < 0.001$). Concentrations of total N were unaffected by elevation in either species ($p > 0.05$), but total N was lower in the largest tree size class ($t(238) = -0.07$, $p < 0.001$; Table 3). There were no differences in the measured concentrations of other nutritional constituents or moisture content between tree size classes for all species (Figure 5). Figure A 1 and Figure A 2 show these relationships in more detail for individual species.

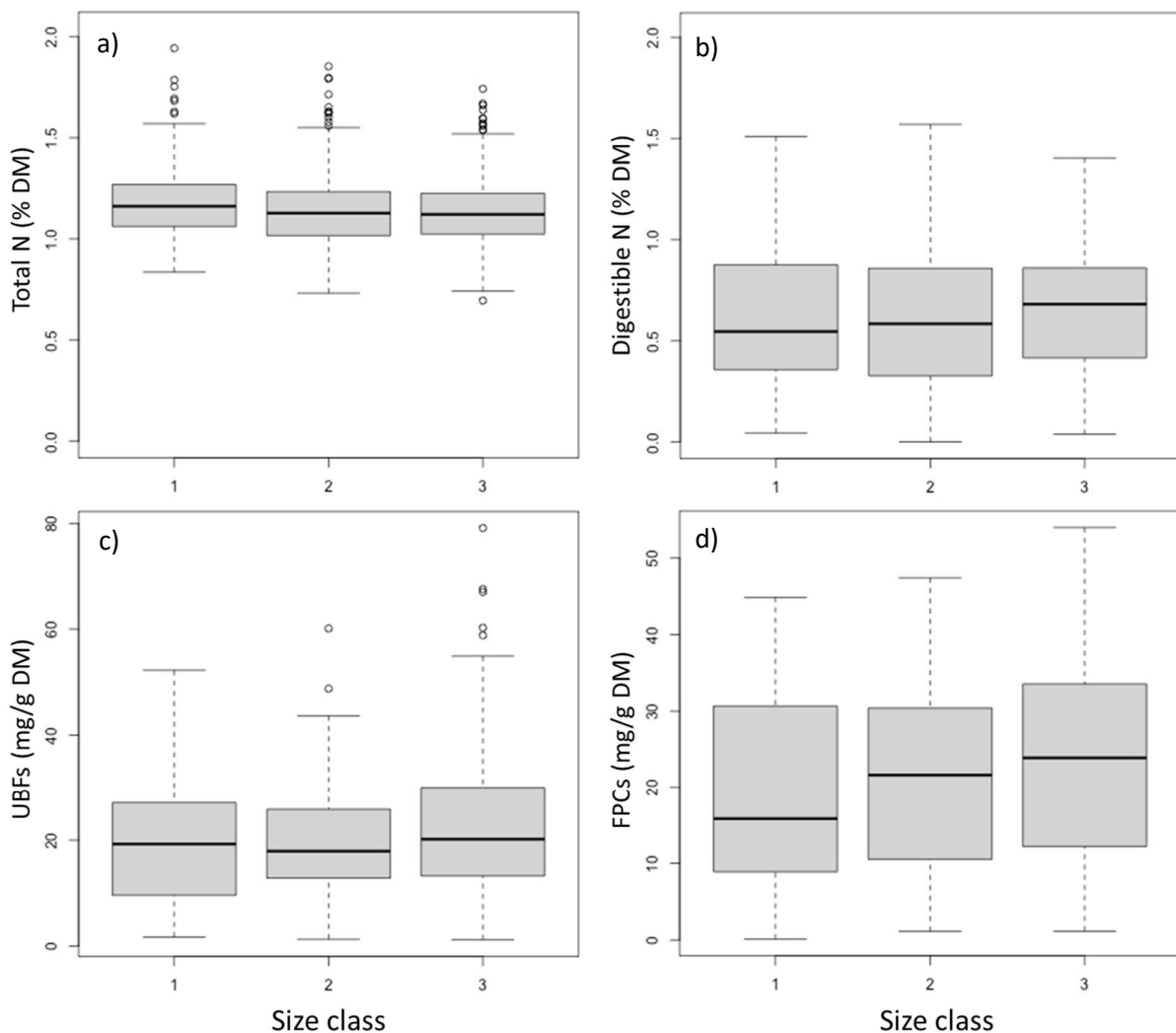


Figure 5. Nutritional composition relative to the size class of trees collected from NSW North Coast State Forests. Size class 1 = 5-15 cm DBH ($n = 228$), 2 = 15.1-25 cm DBH ($n = 240$), and 3 = >25 cm DBH ($n = 478$). Boxes encompass values between the first and third quartiles, with the mid-line indicating the median. Bars show the maximum and minimum values, with outliers shown as circles.

Table 3. Final models describing regressions of nutritional constituents (total and digestible nitrogen (N), formylated phloroglucinol compounds (FPCs), and unsubstituted B-ring flavanones (UBFs)) and dry matter content (% DM) on species, elevation, topography and tree size class for blackbutt and tallowwood. Standard errors of coefficients are shown in brackets.

Model term	Total N	Digestible N	FPCs	UBFs	% DM
Blackbutt	-0.11 *** (0.01)	-0.61 *** (0.03)			-1.79 *** (0.34)
Elevation (km)		0.05 (0.06)	13.65 *** (3.10)	37.24 *** (7.18)	-2.49* (1.06)
Tree size class 2	-0.03 (0.02)				-0.46 (0.53)
Tree size class 3	-0.07*** (0.02)				0.89 (0.48)
Mid-slope					-3.30 *** (0.68)
Blackbutt:elevation		0.26 ** (0.10)			
Mid-slope:elevation					13.57 *** (2.58)
n	238	238	122	116	231
R ²	0.26	0.83	0.14	0.19	0.25

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

Nutritional value of sites

The trees at each site spanned a range of values for each nutritional constituent, although the median concentrations clearly differed between some sites (Figure 6-Figure 9).

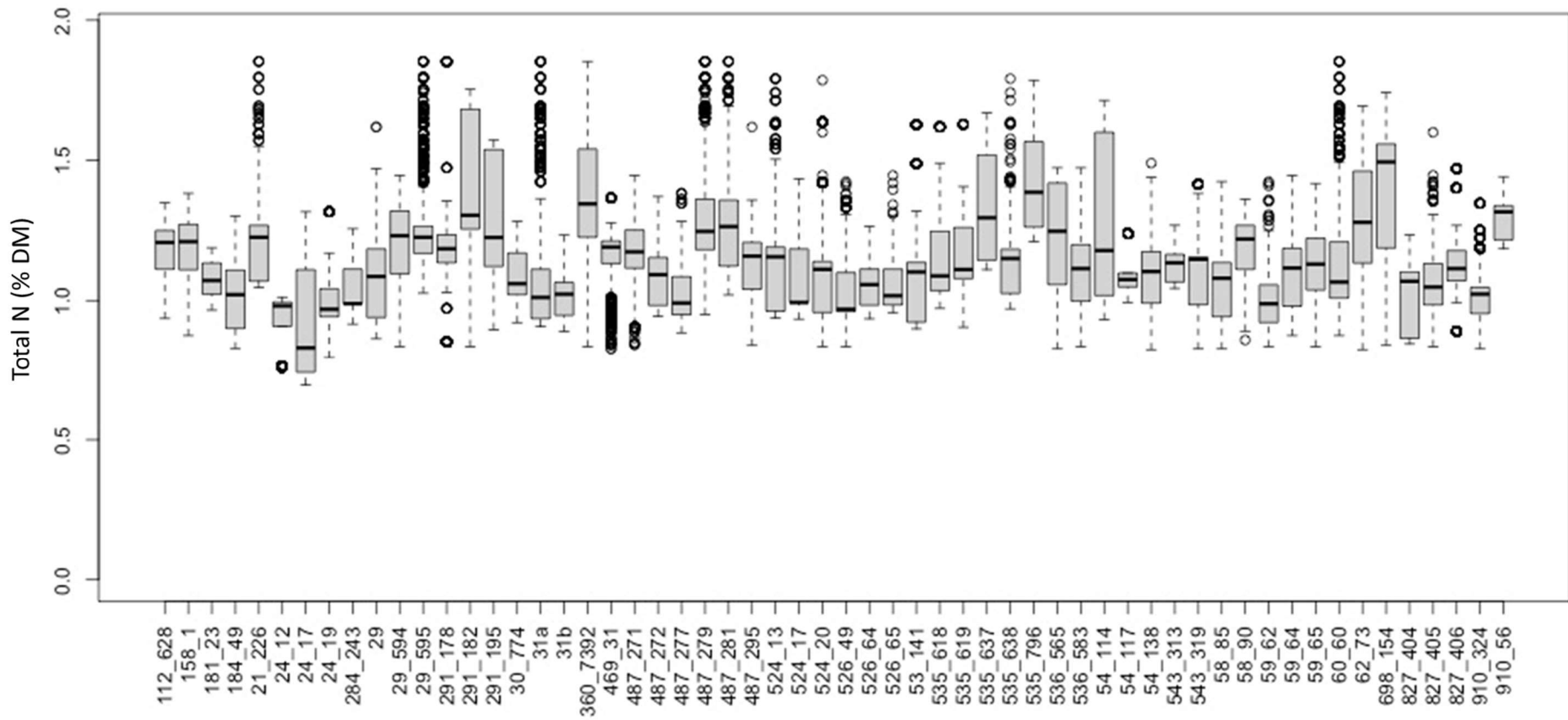


Figure 6. The range and median concentration of total N in Eucalyptus trees (n = 32) from each surveyed site. Details of each site are given in Table A 2.

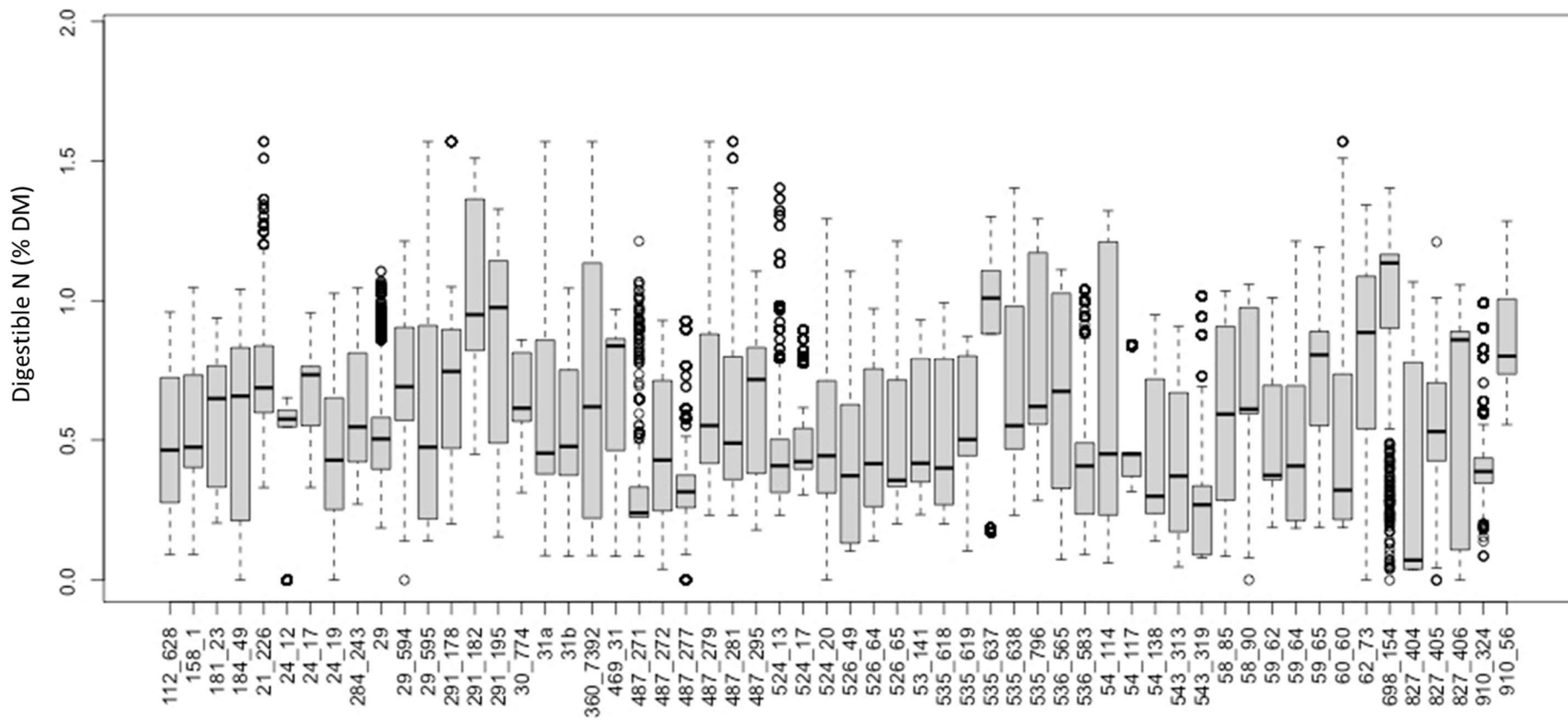


Figure 7. The range and median concentration digestible N in Eucalyptus trees (n = 32) from each surveyed site. Details of each site are given in Table A 2.

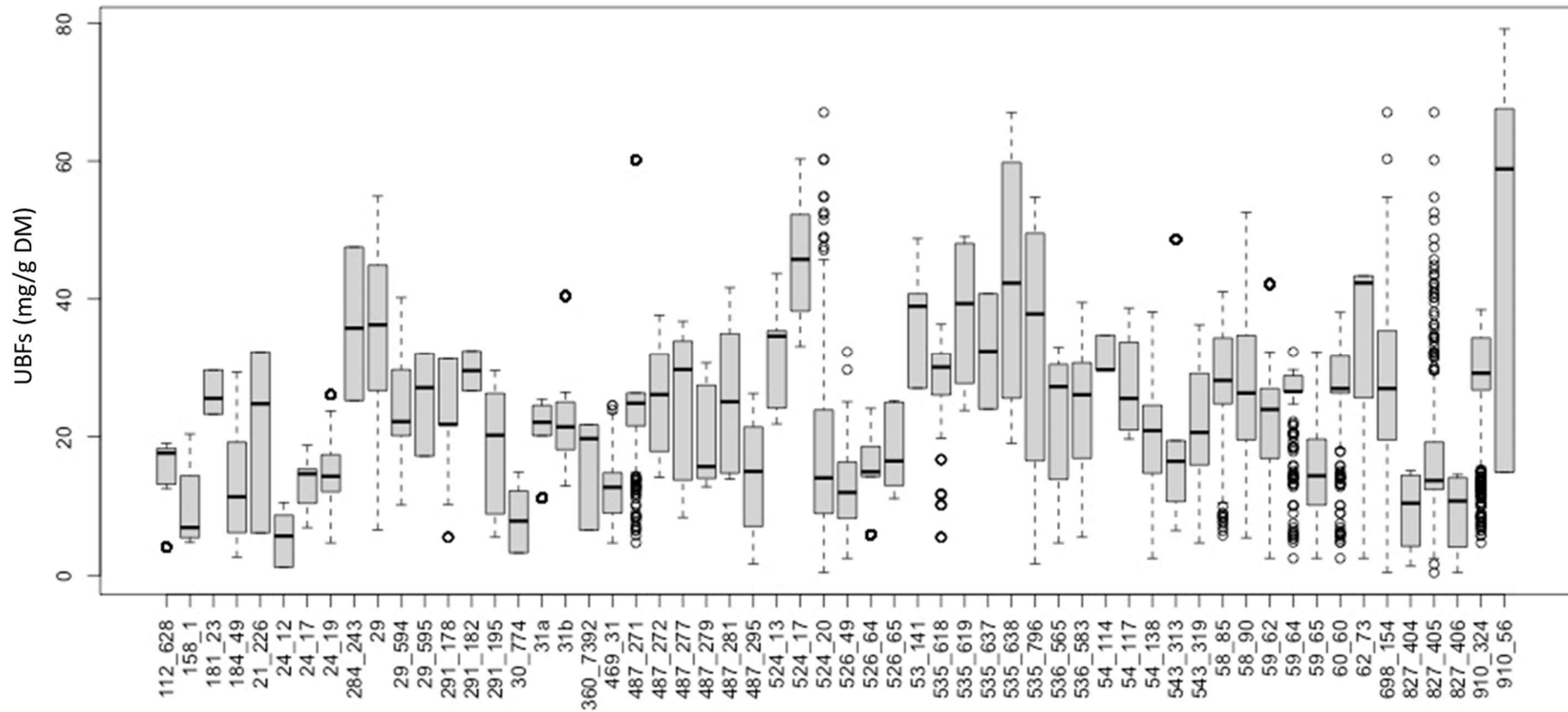


Figure 8. The range and median concentration of UBFs in Eucalyptus trees of the Monocalyptus subgenus from each surveyed site. Details of each site are given in Table A 2.

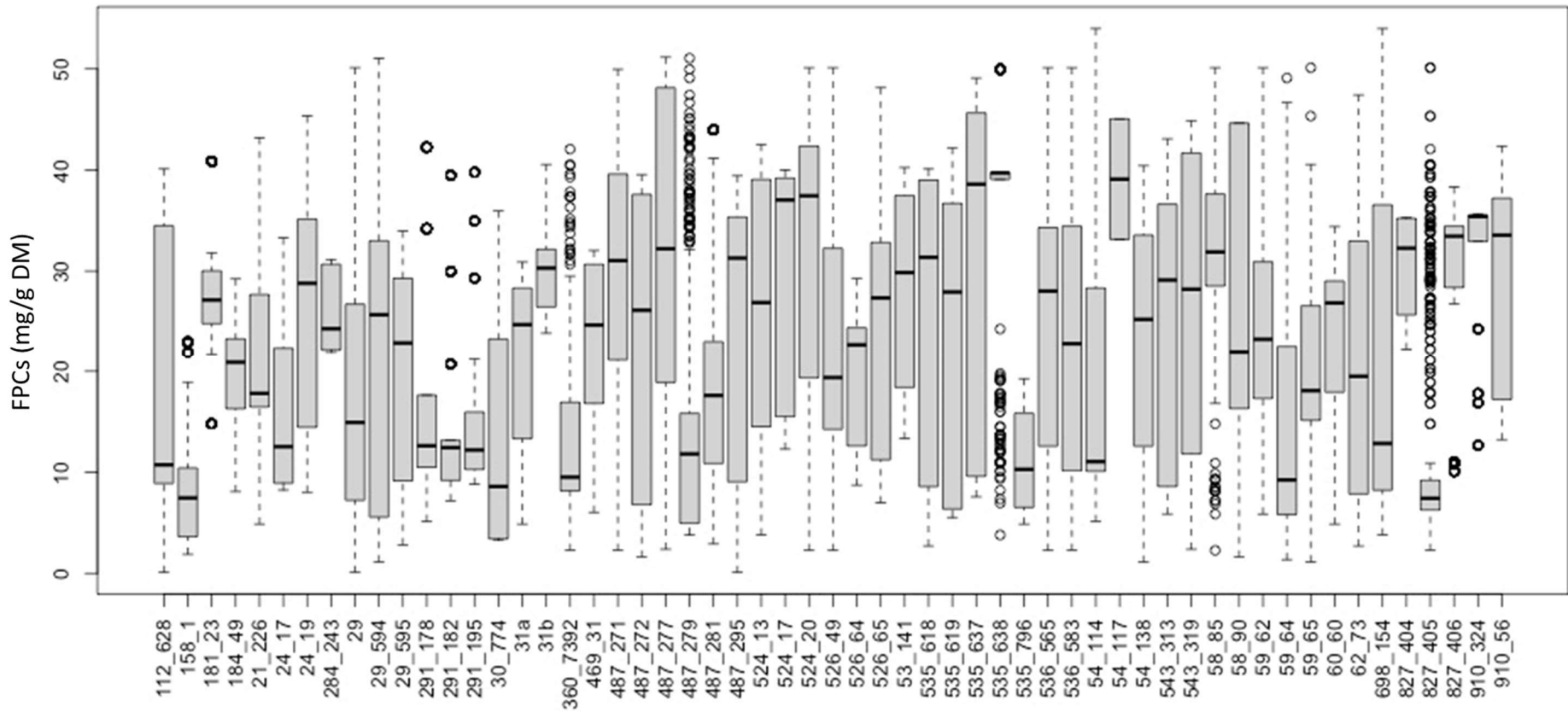


Figure 9. The range and median concentrations of FPCs in Eucalyptus trees of the Symphyomyrtus and Alveolata subgenera from each surveyed site. Details of each site are given in Table A 2.

Effect of tree species composition on site nutritional quality

The proportions of koala browse species, blackbutt, and other eucalypts influenced the average digestible N, FPC, and UBF concentrations at surveyed and simulated sites. Sites containing high proportions of koala browse species were generally the highest in average digestible N concentrations, although this effect was also moderated by the proportion of blackbutt in the landscape (Figure 10). For example, the average proportion of koala browse trees in sites sampled within forest types 53 and 74 (E and F, respectively, on Figure 10) were similar (just over 30%), but there was a substantial difference in the proportion of blackbutt relative to other eucalypt species. This led to sites in forest type 53, which had more blackbutt on average, having lower mean digestible N concentrations.

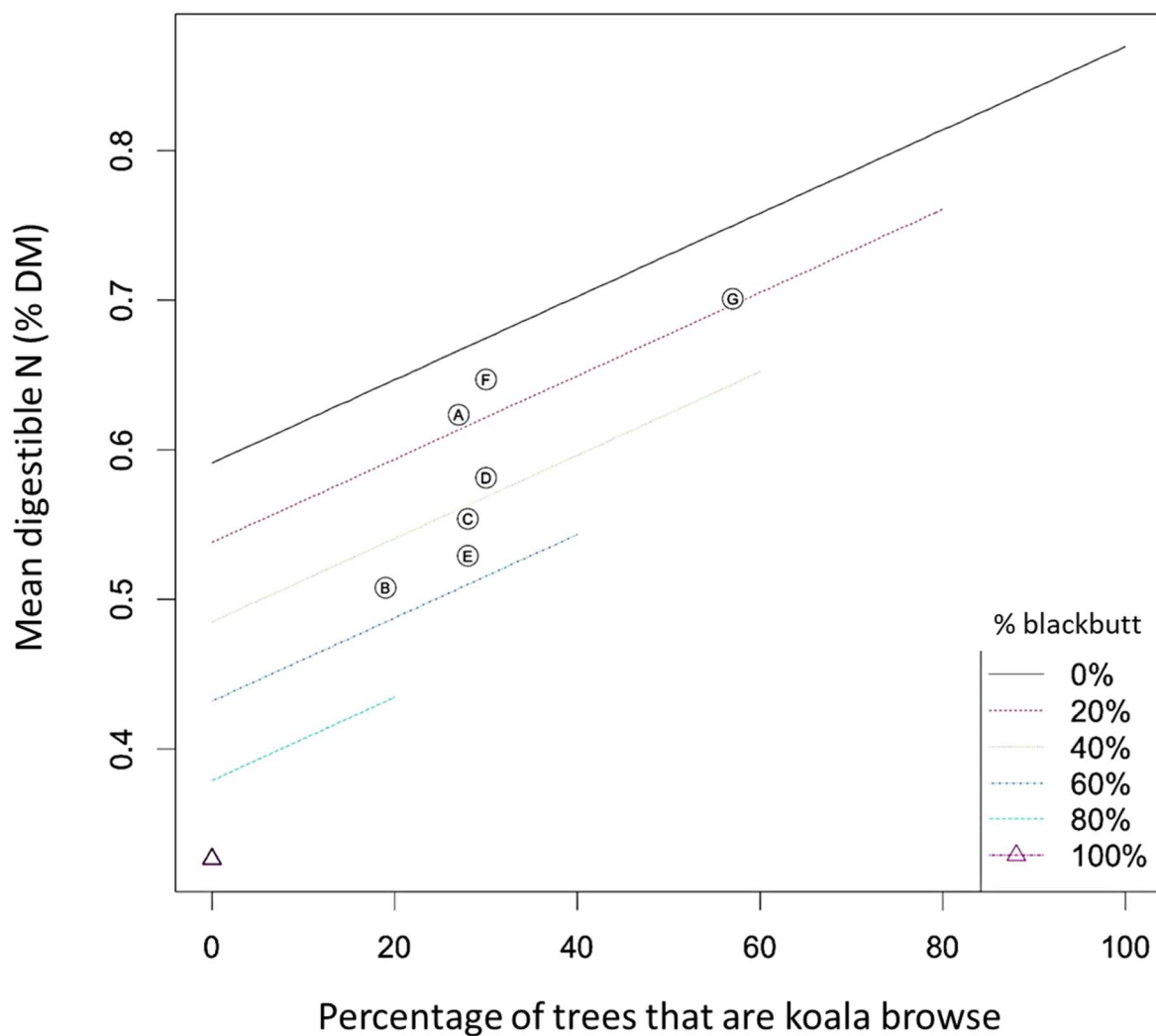


Figure 10. The effect of the percentage of koala browse trees, blackbutt, and other eucalypts on mean site digestible N concentration. Letters in circles indicate the average tree species proportions for sampled sites within selected RN17 forest types (Forestry Commission of New South Wales 1989); A = 62, B = 36, C = 37, D = 48 and 60, E = 53, F = 74, G = 163.

Average FPC concentrations were generally lowest in sampled forest types with high proportions of blackbutt and low proportions of koala browse species. However, these results should be interpreted with caution, because most koala browse species contain FPCs, whereas blackbutt contains UBFs. Thus, when UBFs are considered, average UBF concentrations are lowest at sites with low proportions of blackbutt and high proportions of koala browse species.

Changes in species composition after harvesting and regeneration could alter the average nutritional quality of koala habitat in either a positive or negative way depending on which species are affected. For example, an increase in the proportion of koala browse species or a decrease in blackbutt would improve mean digestible N concentrations at a given site, while a decrease in koala browse or an increase in blackbutt would reduce it (Figure 11). It is more difficult to interpret the effects on FPCs and UBFs when they are only present in a portion of the trees at a site. For example, an increase in the proportion of koala browse species or a decrease in blackbutt would result in higher mean site FPC concentrations, suggesting the potential for poorer quality food. However, the concomitant decrease in mean site UBF concentrations under the same treatment would result in an improvement in average site quality.

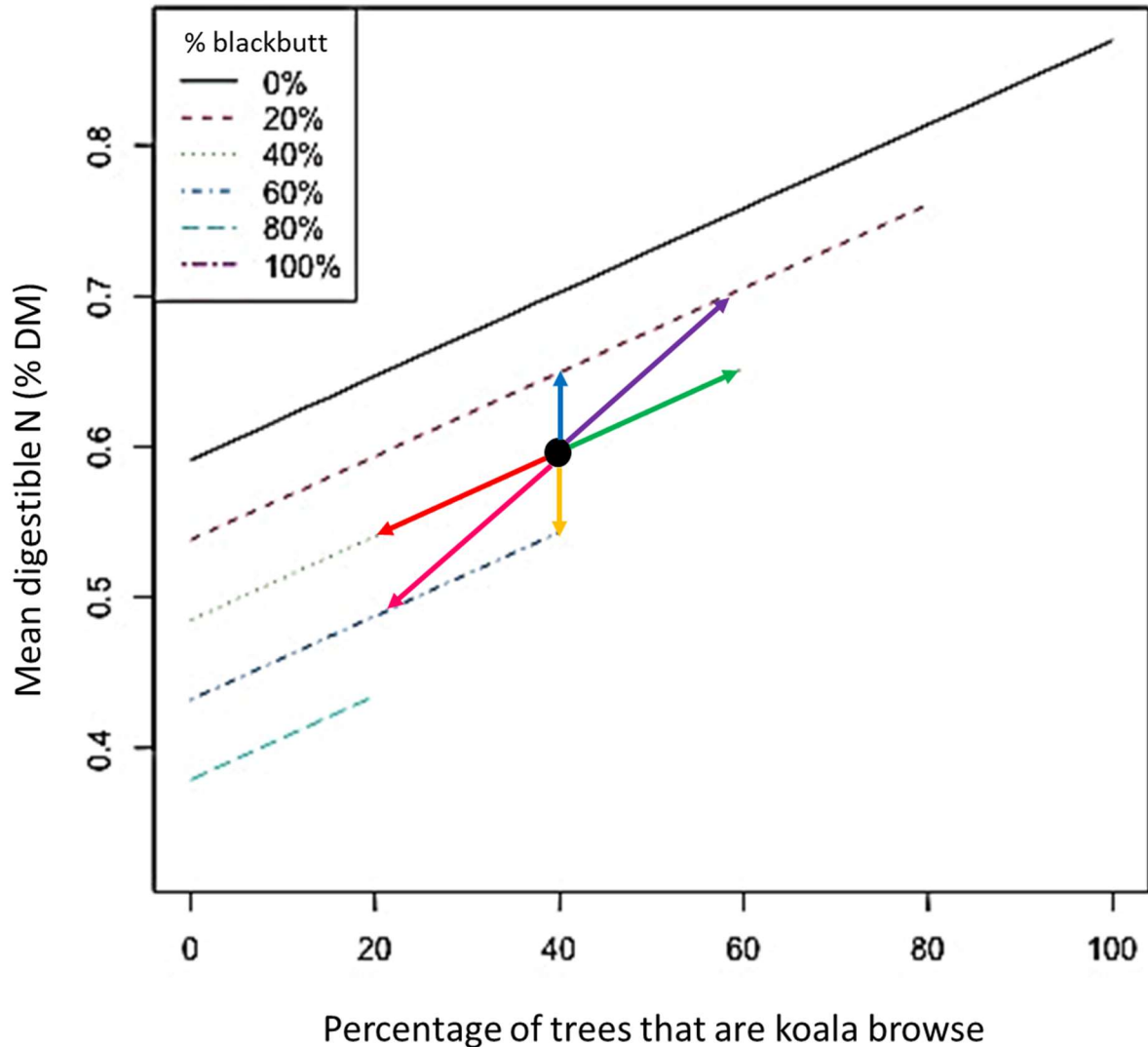


Figure 11. Expected effects on mean site digestible N concentration if there was a maximum 20 % change in species composition within different tree categories relative to a pre-harvest composition of 40 % browse trees, 40 % blackbutt and 20 % other species (black circle). The green arrow shows a 20 % increase in browse species, no change in blackbutt, and a 20 % decrease in other eucalypts. The purple arrow shows a 20 % increase in browse, 20 % decrease in blackbutt, and no change in other eucalypts. The blue arrow shows no change in browse, a 20 % decrease in blackbutt, and a 20 % increase in other eucalypts. The red arrow shows a 20 % decrease in browse, no change in blackbutt, and a 20 % increase in other eucalypts. The pink arrow shows a 20 % decrease in browse, 20 % increase in blackbutt, and no change in other eucalypts. The orange arrow shows no change in browse, a 20 % increase in blackbutt, and a 20 % decrease in other eucalypts.

Simulated replacement of trees with blackbutt had little effect on median site digestible N concentrations at surveyed sites that already had high proportions of blackbutt, regardless of whether koala browse trees were selectively retained or not (e.g. RN17 forest type 37; Figure 12a, b). In contrast, there was a noticeable reduction in median site digestible N with increasing replacement by blackbutt in sites that naturally contained higher proportions of koala browse trees and lower proportions of blackbutt (e.g. RN17 forest type 74; Figure 12c, d). In these forest types,

the selective retention scenario allowed the median digestible N concentration and the upper range of digestible N in available trees to remain higher at a greater proportion of replacement with blackbutt (Figure 12d). Thus, selective retention of preferred koala food trees allowed small increases in the proportion of blackbutt in the landscape with minimal impact on site nutritional value, at least in terms of digestible N.

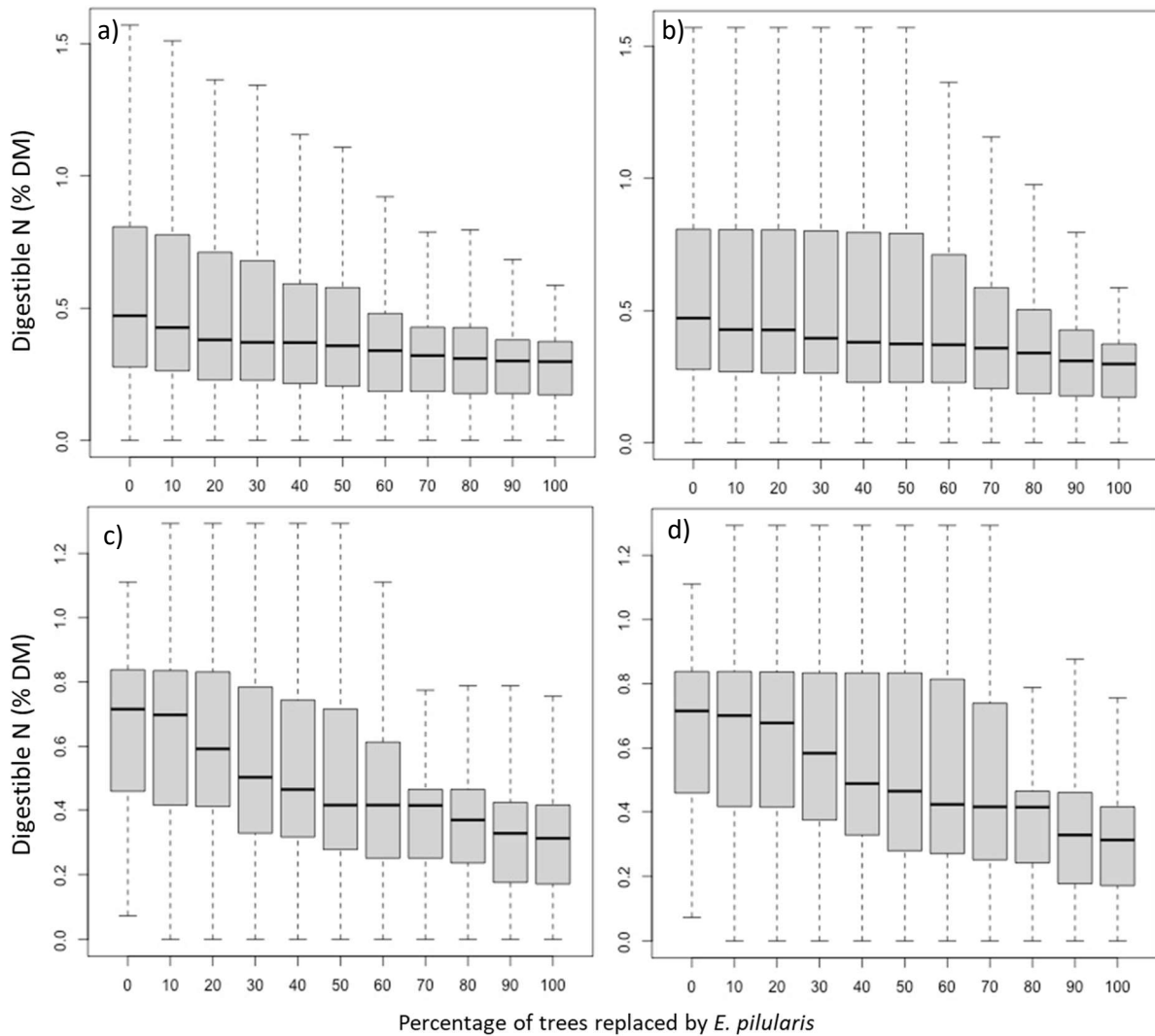


Figure 12. The digestible N concentration of sites comprising RN17 forest types 37 (a, b) and 74 (c, d) relative to random (a, c) or selective (b, d) replacement with blackbutt.

Predicted effects on koala densities

The average nutritional quality of sites sampled in NSW North Coast state forests was low compared to many of the sites sampled across the range of the koala in which the relationship between nutritional quality and koala densities has previously been established (i.e. Figure 1). Thus, the predicted koala density index was also relatively low at each site for current forest species compositions (Figure 13). Without considering the effect of UBFs on koala densities, the mean predicted koala density index was highest at surveyed and simulated sites within forest types with low proportions of blackbutt. There was little effect of the proportion of koala browse species or

other eucalypt species on predicted koala densities, likely because FPCs were considered but UBFs were not. As a consequence, the model predictions likely overestimate the koala density index at sites with high proportions of blackbutt, and underestimate the potential for higher proportions of koala browse species to improve the koala density index.

The applied model predicts that the capacity of a site to support koalas (i.e. the koala density index) is maintained when blackbutt regenerates in similar proportions after harvesting, and increases if the proportion of blackbutt decreases (Figure 13). Increasing the proportion of koala browse species at a site has little effect on the koala density index under the applied model, unless the proportion of blackbutt is very high (e.g. > 80 %), or the blackbutt proportion is simultaneously reduced (Figure 13). These preliminary predictions should be used with caution until UBF effects have been incorporated into the model.

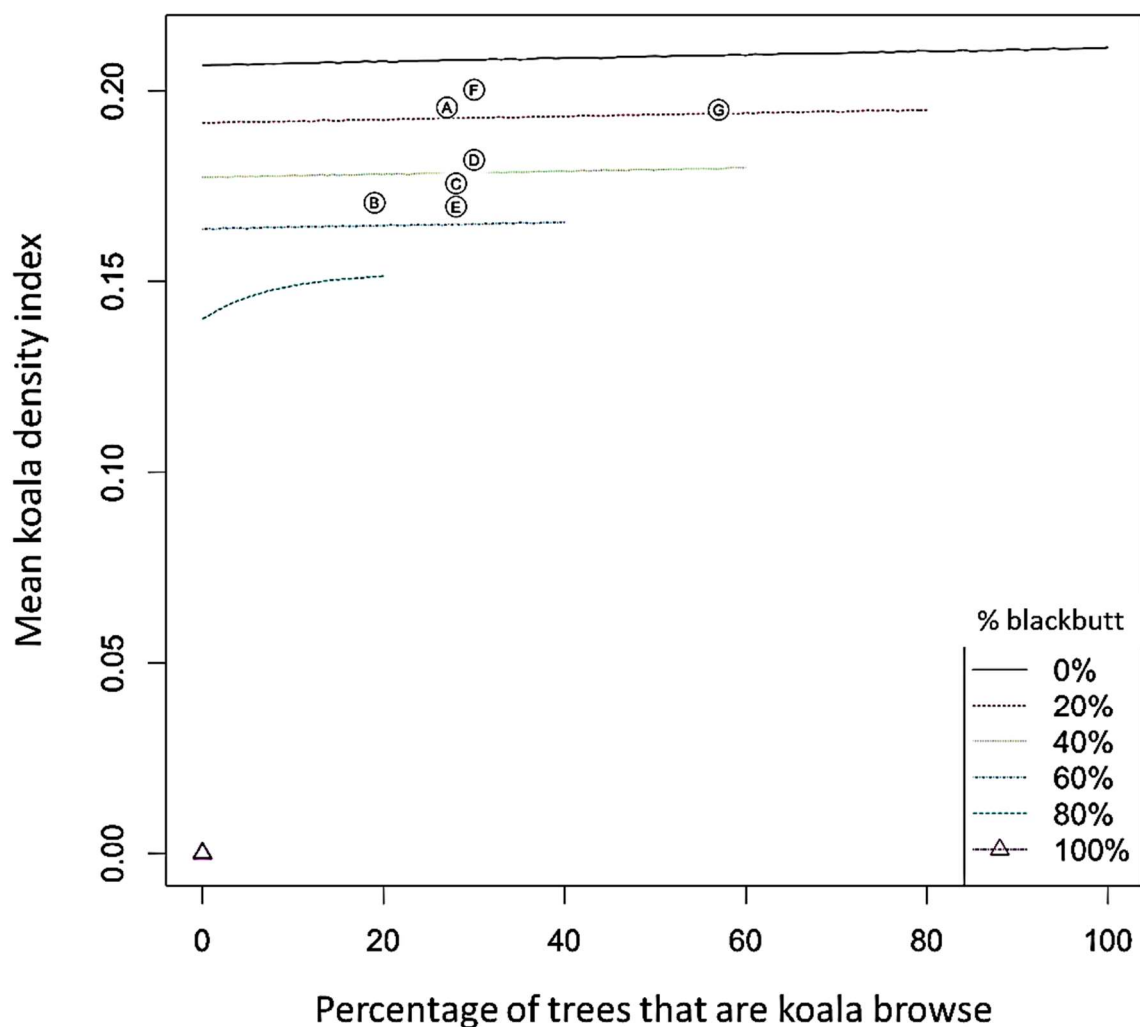


Figure 13. The effect of the percentage of koala browse trees, blackbutt, and other eucalypts at a site on the mean site koala density index. UBF concentrations are assumed to have no impact on koala density index in the applied model, so high proportions of *Monocalyptus* species (including blackbutt) are likely to reduce the koala density index to a greater extent than is shown on the figure. Letters in circles indicate the average tree species proportions for sampled sites within different RN17 forest types; A = 62, B = 36, C = 37, D = 48 and 60, E = 53, F = 74, G = 163.

Without considering UBFs, the effect of removing and replacing trees with blackbutt differed between forest types. In forest types currently dominated by blackbutt, the replacement of trees with blackbutt had little effect on the koala density index until greater than 60 % replacement during random replacement scenarios (Figure 14a), or until greater than 80 % replacement in selective retention scenarios (Figure 14b). In forest types with lower starting proportions of blackbutt, koala densities declined more steadily with increasing replacement by blackbutt (Figure 14c). However, the rate of decline was initially slower during selective retention of koala browse species (Figure 14d), suggesting that this scenario can assist with maintaining koala densities in the landscape if blackbutt proportions were to increase slightly at sites where this species is not currently dominant.

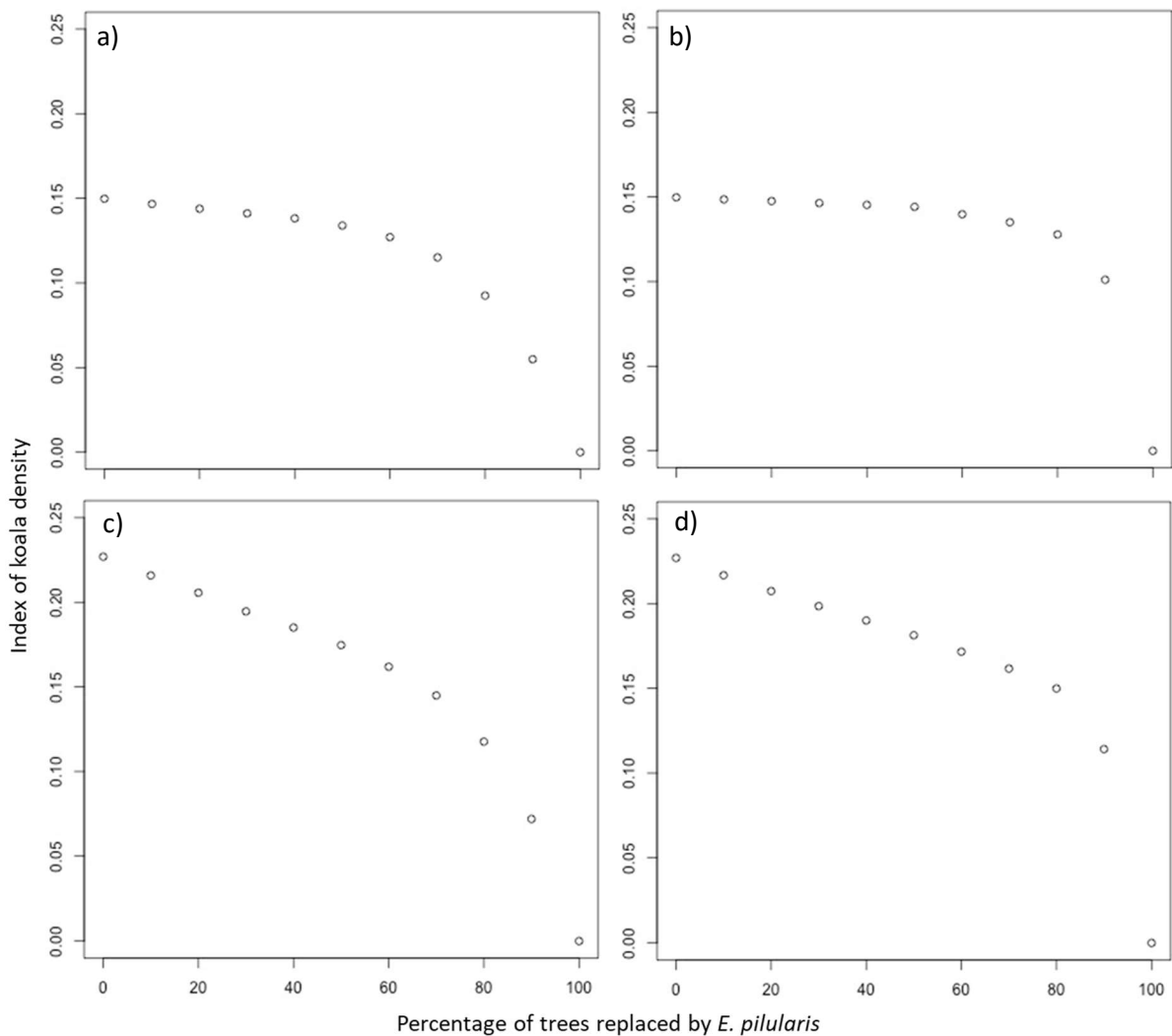


Figure 14. Index of koala density for RN17 forest types 37 (a, b) and 74 (c, d) relative to random (a, c) or selective (b, d) replacement with *E. pilularis*.

Discussion

Koala presence and abundance are strongly influenced by the underlying nutritional quality of the eucalypt trees in their habitat (Au 2018; Au et al. 2019). Thus, an understanding of the nutritional value of different eucalypt species for koalas and how the species composition of forests influence habitat nutritional quality within a management region can be used to inform management decisions. Mean concentrations of three important nutritional constituents, digestible N, FPCs and UBFs, differed between eucalypt species and forest sites sampled on the NSW North Coast. Furthermore, statistical simulations showed that the average nutritional value of sites and the predicted koala density index were influenced by the relative proportions of trees within three categories: koala browse species, blackbutt, and other eucalypts. The results suggest that habitat nutritional value and the predicted koala density index may increase, decrease, or stay the same after harvesting and regeneration depending on whether and how the proportions of eucalypt species differ from pre-harvest values. In particular, the retention and regrowth of eucalypt species that are currently classified as primary and secondary koala browse trees in harvesting prescriptions appear to be the most beneficial for habitat nutritional quality. Our key findings are outlined in more detail below for each of the stated project objectives.

Nutritional composition of eucalypt species and sites

Surveyed sites differed substantially in their average concentrations of digestible N, FPCs and UBFs, and thus their habitat nutritional quality for koalas. Despite variation in nutritional quality within species, differences in site quality were primarily driven by differences in tree species composition. Sampled eucalypts that are currently considered to be primary or secondary browse species for koalas in northern NSW timber production forests (*E. micorcorys*, *E. robusta*, *E. tereticornis*, *E. propinqua*, *E. saligna* and *E. andrewsii*; NSW Environment Protection Authority 2020) contained higher average concentrations of digestible N than most other eucalypt species. An additional unlisted species, *E. grandis*, also contained high digestible N concentrations on average. The suitability of this species as browse for koalas in the region could be determined with captive feeding studies or by diet analysis of wild koalas in areas in which they encounter *E. grandis*. Certainly, koalas are documented to use *E. grandis* extensively more broadly across NSW (NSW Office of Environment and Heritage 2018).

Sites with higher average concentrations of digestible N are generally more valuable habitat for koalas. For example, Au (2018) found a strong relationship between average site digestible N concentration and koala densities across Australia, although the average concentrations of FPCs and UBFs also moderated these effects. The effect of digestible N is likely explained by the fact that koalas must obtain sufficient protein to meet their daily requirements (Cork 1986), which is not always possible when eucalypts contain low digestible N concentrations. Furthermore, DeGabriel et al. (2009) showed that common brushtail possums (*Trichosurus vulpecula*) living in eucalypt forests with higher concentrations of digestible N had higher reproductive success, and the effects are probably similar for koalas. In forests within the NSW North Coast regeneration harvesting zone, sites with the highest mean digestible N concentrations had high proportions of koala browse species, low proportions of blackbutt, or both.

Most of the identified koala browse species that we sampled were from the symphyomyrtle subgenus, and thus contained variable concentrations of FPCs. As a consequence, sites with higher proportions of browse species were predicted to have higher average FPC concentrations. Although higher average concentrations of FPCs generally correspond with lower koala densities at a given concentration of digestible N (Au 2018), FPCs only deter koalas from feeding when they occur above

a certain concentration. For example, Victorian koalas were not deterred by FPC concentrations below 20 mg.g⁻¹ dry matter (DM) in several local eucalypt species (Moore et al. 2005; Marsh et al. 2007). The threshold for deterrence has not been determined for koalas and eucalypt species from northern NSW, but it is worth noting that almost half of the individual trees classified as koala browse species contained concentrations less than 20 mg.g⁻¹ DM. This suggests that, even at sites with high average FPC concentrations, koalas would likely find many individual trees with lower concentrations from which they could feed.

In contrast to FPCs, UBFs, which are found in trees from the monocalypt subgenus, can deter feeding by koalas at relatively low concentrations (e.g. 6 mg/g; Marsh et al. 2021). Less than 10 % of monocalypt individuals sampled in NSW North Coast forests had concentrations below this amount, suggesting that monocalypts are likely to be used sparingly as food by koalas. The high average UBF concentrations and low average concentrations of digestible N in blackbutt support current views that it is not a primary or secondary browse species in North Coast timber production forests. In contrast, *E. andrewsii*, another monocalypt species, is listed as a secondary koala browse tree in Coastal IFOA protocols (NSW Environment Protection Authority 2020). We encountered *E. andrewsii* at only one survey site, and thus sampled only four individual trees. However, these four trees contained very high concentrations of UBFs, which likely outweigh any benefits of the high digestible N concentrations the foliage also contained. If UBF concentrations are consistently high in this species it may explain why *E. andrewsii* is used irregularly by koalas more broadly across NSW (NSW Office of Environment and Heritage 2018). A combination of captive feeding studies, faecal dietary analysis from wild koalas, and/or nutritional analysis of additional samples could be used to re-evaluate whether *E. andrewsii* should be considered a koala browse tree under Coastal IFOA protocols.

Several studies have found that koalas prefer bigger trees, at least during daylight hours when they are resting (e.g. Moore et al. 2010; Marsh et al. 2014). Our findings support previous suggestions (e.g. Marsh et al. 2014) that this preference is not related to their nutritional requirements. The largest tree size class had slightly less total nitrogen than other tree size classes, but none of the other measured foliar chemical constituents differed between size classes. The fact that nutritional deterrents are not specifically associated with trees of a particular size class in the study area suggests that, provided koalas are able to meet requirements for shelter and thermoregulation, and as long as suitable browse (quality and quantity) is present, there is no nutritional requirement for large trees. Thus, the average nutritional value of a forest site dominated by a small size class of trees could be expected to be similar to one dominated by a larger size class of the same species composition.

For the most part, measured environmental variables had little to no influence on nutritional quality. The exception was for elevation. The digestible N and UBF concentrations of blackbutt and the FPC concentration of a primary koala browse species, tallowwood (*E. microcorys*), were higher at higher elevations. Moore et al. (2004) likewise found that tallowwood trees growing at higher elevations had higher concentrations of a specific FPC, sideroxylonal. The likely consequence of these findings is that forest sites at higher elevations on the NSW North Coast provide poorer quality food for koalas than those at lower elevations with the same species composition. This may help to explain why Moore et al. (2004) found fewer koala faecal pellets under tallowwood trees at higher elevations, and why a widespread acoustic survey for koalas on the NSW North Coast found that sites at higher elevations were less likely to be occupied (Law et al. 2018).

Effect of harvesting and regeneration on habitat nutritional quality

Because tree size had little effect on the concentrations of nutritional constituents in sampled eucalypt foliage, sites that regenerate with the same eucalypt species composition as pre-harvest conditions are likely to maintain their nutritional quality, even when trees are as small as 10 cm DBH. In contrast, habitat nutritional quality may either improve or be reduced if there are changes in tree species composition after regeneration. The number and species of eucalypts that regrow after harvesting are influenced by a combination of natural and anthropogenic factors, including the initial species present, harvesting pattern, trees retained, treatment of the site (e.g. post-harvest burning), and whether regrowth occurs from seeds or lignotubers (King 1985; Florence 1996; Nicholson 1999; Kinny et al. 2012). For example, blackbutt seedlings are less tolerant of shade than many co-occurring eucalypt species, including many koala browse species, and also tend to regrow from seed rather than lignotubers (Florence 1996). Selective harvesting can therefore favour the regrowth of more competitive species at the expense of blackbutt, while intensive harvesting practices that generate larger clearings can allow blackbutt to regenerate at higher rates (Florence 1996).

In the absence of specific data on how new koala browse tree retention prescriptions affect the final species composition of regenerating forests, we focussed on two specific potential outcomes that are likely to be the most relevant to koalas. These were: 1) maintenance or a change in the proportion of koala browse trees, and 2) maintenance or a change in the proportion of blackbutt. Our findings suggest that increasing the proportion of koala browse species at a given site would increase the mean digestible N and FPC concentrations and reduce the mean concentration of UBFs. Overall, this suggests an improvement in habitat nutritional quality for koalas. Increasing the proportion of blackbutt had the opposite effect (i.e. reduced digestible N and FPC concentrations, and increased concentration of UBFs), but the likely biological significance of this effect differed between forest types. For example, sites already dominated by blackbutt tended to be of poor nutritional quality, and there was a relatively small difference in digestible N concentrations between simulated low and high levels of replacement of standing trees with blackbutt. Thus, in these landscapes, blackbutt proportions could increase with little detrimental effect to overall site nutritional quality, especially when coupled with the preferential retention of a portion of the available koala browse trees. At sites with lower initial proportions of blackbutt, the most effective means to conserve nutritional quality was to limit any increase in the proportion of blackbutt and to retain koala browse trees.

Predicted koala densities under harvesting and regeneration scenarios.

The nutritional composition of eucalypts measured in this study were at the lower end of those observed in forests occupied by koalas in other locations across the range of the koala (e.g. Figure 1; Youngentob 2015; Au 2018). As a consequence, we would not expect high koala densities (i.e. no greater than 0.25 koalas per hectare, and most likely substantially lower) at any of the sites we surveyed. However, due to differences in the way in which samples were analysed between studies, as well as the lack of incorporation of UBF effects into the initial koala density model, we chose to calculate a koala density index from the nutritional data rather than absolute koala densities. This density index allowed us to explore relative differences between sites and regeneration scenarios.

The koala density index, which assumes that the effect of UBFs on koala densities is negligible, was influenced most strongly by the proportion of blackbutt at a site. Thus, sites with the lowest proportions of blackbutt were predicted to support the highest densities of koalas, regardless of the relative proportions of koala browse or other eucalypt species. Likewise, sites that regenerated with proportions of blackbutt that were similar to initial conditions were predicted to support similar densities of koalas, while those with lower or higher proportions of blackbutt were predicted to

support higher or lower koala densities, respectively. As with the nutritional data, these latter findings were also moderated by the initial forest type. Specifically, the koala density index was reasonably stable until a high rate of replacement with blackbutt at blackbutt-dominated sites, whereas it declined more sharply in mixed forests with low initial proportions of blackbutt. In both cases, selective retention of koala browse trees assisted with maintaining koala densities, and/or slowing the rate of decline in the koala density index as replacement by blackbutt increased.

The above findings should only be interpreted in conjunction with an understanding of the likely impacts of UBF concentrations on the koala density index. For example, in a study by Au (2018), koala densities were highly variable at sites with low mean UBF concentrations, but were uniformly low at sites with higher mean UBF concentrations (Figure 3). It is therefore probable that the koala density index would decline in a more linear fashion with increasing proportions of blackbutt if the effects of UBFs were incorporated. Likewise, we would expect to see a larger increase in the koala density index with increasing proportions of koala browse species when accounting for the effects of UBFs.

Strategies to minimise long-term impacts of forestry on koala populations

The simulations undertaken in this study provide an overview of how changes in eucalypt species composition in any direction could affect habitat nutritional quality and the capacity to support koalas. Where the goal is to maintain or increase koala numbers (e.g. at sites that are currently of high nutritional quality), mitigation strategies should aim to preserve or increase the proportion of koala browse species, and/or preserve or reduce the proportion of blackbutt.

Current Coastal IFOA conditions for intensive harvesting require 5 or 10 koala browse trees to be retained per hectare where koala browse prescriptions apply (NSW Environment Protection Authority 2020). In the short term, these trees are likely to play an important role in supporting resident koalas until regrowth has reached an appropriate size. However, there is no information on how the retention of different numbers of koala browse trees affects the species composition after longer term site regeneration. Without this data, it is not possible to determine whether either strategy effectively mitigates the impacts of forest harvesting on the capacity of production forests to support koala populations into the future. However, our findings show that, in order to maintain or improve the density of current koala populations, the post-regeneration composition of sites that are subjected to koala browse prescriptions should contain at least the same proportion of koala browse species to pre-harvest conditions.

Our findings also suggest that intensive harvesting operations that favour the regrowth of blackbutt could be undertaken at sites of low nutritional quality with relatively minor effects on the capacity to support koala populations into the future. Such sites include those where blackbutt comprises at least 50 % of trees greater than 10 cm DBH. Based on their nutritional composition, blackbutt-dominated sites are currently expected to support very low densities of koalas, and the mean site nutritional quality and density index show little change with further increases in blackbutt proportion, especially when koala browse species are preferentially retained.

Other considerations and future directions

Currently, statistical models that predict how changes in eucalypt species composition affect koala densities do not include the effect of UBFs. We are working to incorporate these effects into koala density models. This will significantly improve our capacity to determine how changes in the proportions of monocalypt species, including blackbutt, affect the koala density index.

Koala habitat harvesting prescriptions on the NSW North Coast depend on the mapped potential quality of the landscape for koalas. The eucalypt species composition is an important parameter in determining this quality. The nutritional measurements for each eucalypt species could be used to evaluate and refine the current mapping parameters.

There is currently no information about how the post-regeneration composition of sites subjected to koala browse retention strategies (i.e. leave 5 or 10 koala browse trees per hectare) compares to pre-harvest composition. Longer term monitoring projects should prioritise collecting this information to determine whether these protocols are sufficient to achieve the goal of maintaining or improving habitat nutritional quality and the koala density index.

References

- Andrew RL, Peakall R, Wallis IR, Wood JT, Knight EJ, Foley WJ (2005) Marker-based quantitative genetics in the wild?: The heritability and genetic correlation of chemical defenses in *Eucalyptus*. *Genetics* 171:1989-1998. doi: 10.1534/genetics.105.042952
- Au J (2018) Multi-scale effects of nutrition on an arboreal folivore. PhD thesis, The Australian National University
- Au J, Clark RG, Allen C, Marsh KJ, Foley WJ, Youngentob KN (2019) A nutritional mechanism underpinning folivore occurrence in disturbed forests. *Forest Ecol Manag* 453:1-8
- Au J, Marsh KJ, Wallis IR, Foley WJ (2013) Whole-body protein turnover reveals the cost of detoxification of secondary metabolites in a vertebrate browser. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology* 183:993-1003. doi: 10.1007/s00360-013-0754-3
- Au J, Youngentob KN, Foley WJ, Moore BD, Fearn T (2020) Sample selection, calibration and validation of models developed from a large dataset of near infrared spectra of tree leaves. *Journal of Near Infrared Spectroscopy* 28:186-203
- Cork SJ (1986) Foliage of *Eucalyptus punctata* and the maintenance nitrogen requirements of koalas, *Phascolarctos cinereus*. *Aust J Zool* 34:17-23. doi: 10.1071/zo9860017
- DeGabriel JL, Moore BD, Foley WJ, Johnson CN (2009) The effects of plant defensive chemistry on nutrient availability predict reproductive success in a mammal. *Ecology* 90:711-719
- DeGabriel JL, Wallis IR, Moore BD, Foley WJ (2008) A simple, integrative assay to quantify nutritional quality of browses for herbivores. *Oecologia* 156:107-116. doi: 10.1007/s00442-008-0960-y
- Florence RG (1996) *Ecology and Silviculture of Eucalypt Forests*. CSIRO Publishing, Collingwood, Victoria, Australia
- Forestry Commission of New South Wales (1989) *Research Note No. 17: Forest Types in New South Wales*, Sydney, Australia
- Horne R (1994) *Technical Paper No. 54: Thinning schedules for blackbutt regrowth forests*. State Forests of New South Wales, Sydney, Australia
- King G (1985) Natural regeneration in wet sclerophyll forest with an overstorey of *Eucalyptus microcorys*, *E. saligna* and *Lophostemon confertus*. *Australian Forestry* 48:54-62. doi: 10.1080/00049158.1985.10674423
- Kinny M, McElhinny C, Smith G (2012) The effect of gap size on growth and species composition of 15-year-old regrowth in mixed blackbutt forests. *Australian Forestry* 75:3-15

Law BS, Brassil T, Gonsalves L, Roe P, Truskinger A, McConville A (2018) Passive acoustics and sound recognition provide new insights on status and resilience of an iconic endangered marsupial (koala *Phascolarctos cinereus*) to timber harvesting. PLoS One 13:e0205075. doi: 10.1371/journal.pone.0205075

Marsh KJ, Blyton MDJ, Foley WJ, Moore BD (2021) Fundamental dietary specialisation explains differential use of resources within a koala population. Oecologia:doi: 10.1007/s00442-00021-04962-00443. doi: 10.1007/s00442-021-04962-3

Marsh KJ, Moore BD, Wallis IR, Foley WJ (2014) Feeding rates of a mammalian browser confirm the predictions of a 'foodscape' model of its habitat. Oecologia 174:873-882. doi: 10.1007/s00442-013-2808-3

Marsh KJ, Saraf I, Hocart CH, Youngentob KN, Singh IP, Foley WJ (2019) Occurrence and distribution of unsubstituted B-ring flavanones in *Eucalyptus* foliage. Phytochemistry 160:31-39. doi: 10.1016/j.phytochem.2019.01.005

Marsh KJ, Wallis IR, Foley WJ (2007) Behavioural contributions to the regulated intake of plant secondary metabolites in koalas. Oecologia 154:283-290. doi: 10.1007/s00442-007-0828-6

Marsh KJ, Wallis IR, Kulheim C, Clark R, Nicolle D, Foley WJ, Salminen JP (2020) New approaches to tannin analysis of leaves can be used to explain in vitro biological activities associated with herbivore defence. New Phytol 225:488-498. doi: 10.1111/nph.16117

Moore BD, Foley WJ, Wallis IR, Cowling A, Handasyde KA (2005) *Eucalyptus* foliar chemistry explains selective feeding by koalas. Biol Lett 1:64-67. doi: 10.1098/rsbl.2004.0255

Moore BD, Lawler IR, Wallis IR, Beale CM, Foley WJ (2010) Palatability mapping: a koala's eye view of spatial variation in habitat quality. Ecology 91:3165-3176

Moore BD, Wallis IR, Wood JT, Foley WJ (2004) Foliar nutrition, site quality, and temperature influence foliar chemistry of tallowwood (*Eucalyptus microcorys*). Ecol Monogr 74:553-568. doi: 10.1890/03-4038

Nicholson E (1999) Winds of change for silvicultural practice in NSW native forests. Australian Forestry 62:223-235. doi: 10.1080/00049158.1999.10674787

NSW Environment Protection Authority (2020) Coastal Integrated Forestry Operations Approval - Protocols, Paramatta, NSW, Australia

NSW Office of Environment and Heritage (2018) A review of koala tree use across New South Wales, Sydney, NSW, Australia

Tucker DJ, Wallis IR, Bolton JM, Marsh KJ, Rosser AA, Brereton IM, Nicolle D, Foley WJ (2010) A metabolomic approach to identifying chemical mediators of mammal-plant interactions. J Chem Ecol 36:727-735. doi: 10.1007/s10886-010-9803-5

Wallis IR, Nicolle D, Foley WJ (2010) Available and not total nitrogen in leaves explains key chemical differences between the eucalypt subgenera. *Forest Ecol Manag* 260:814-821. doi: 10.1016/j.foreco.2010.05.040

Wallis IR, Watson ML, Foley WJ (2002) Secondary metabolites in *Eucalyptus melliodora*: field distribution and laboratory feeding choices by a generalist herbivore, the common brushtail possum. *Aust J Zool* 50:507-519. doi: 10.1071/ZO02029

Youngentob KN (2015) Charting forage quality for koala conservation Emerging Priorities Final Report

Youngentob KN, Zdenek C, van Gorsel E (2016) A simple and effective method to collect leaves and seeds from tall trees. *Methods Ecol Evol* 7:1119-1123. doi: 10.1111/2041-210X.12554

Addendum: Resampling eucalypts after fire

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December 2021



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Summary

After the completion of leaf sampling in 2019 for the main component of the project, a subset of sites burnt in the 2019/2020 “Black Summer” bushfires. This provided a unique opportunity to investigate whether fire altered the nutritional composition of eucalypt forests for koalas. In February 2021, we resampled leaves from 150 trees that formed part of the original study. These trees came from six sites that burnt in 2019 and six paired sites that did not burn.

Mature leaves collected in 2021 contained about 3 % more water than mature leaves collected from the same trees 17 to 20 months earlier. This is likely because there was substantially higher rainfall in 2020/2021 than in 2019. Comparing 2021 samples to samples collected in 2019, mature leaves from species from the symphyomyrtle and alveolata subgenera of eucalypts also had slightly higher concentrations of digestible N, while mature leaves from monocalypt species had lower concentrations of UBFs. The differences may be due to small changes in chemical production between seasons and/or between drought and non-drought conditions, but this would require further testing to resolve. Fire did not affect constituent concentrations in mature leaves in the timeframe of the study, as evidenced by the similar results in sites that burnt or did not burn.

Epicormic leaves produced after fire had a higher water content than mature leaves collected pre fire from the same trees. In addition, epicormic leaves from symphyomyrtle species had higher digestible N concentrations, while monocalypt epicormic leaves were lower in both digestible N and UBF concentrations than mature leaves. As a consequence, sites with high proportions of blackbutt (a monocalypt) and/or other non-browse species were predicted to have lower mean site digestible N concentrations after fire, while those with high proportions of koala browse species (typically symphyomyrtles) were predicted to be higher in mean digestible N. This suggests that the effects of fire on habitat quality for koalas depends on the site species composition. Specifically, sites that are of poor nutritional quality due to a low abundance of koala browse species are likely to decline further where epicormic leaves predominate after fire. In contrast, post-fire epicormic regrowth may further enhance the nutritional quality of sites with a high abundance of koala browse species on the NSW North Coast.

During the 2021 sampling period, young leaves (i.e. adult phase leaves that were not yet fully expanded) were also present on many trees. We opportunistically sampled these leaves and found that they had a higher water content than mature leaves from the same trees, and higher concentrations of FPCs or UBFs on average. Monocalypt species also had lower concentrations of digestible N, potentially exacerbating the effects of higher UBF concentrations. Together, the findings suggest that both the eucalypt species composition and the type of leaves available (mature, young or epicormic) can influence habitat nutritional quality for koalas, although the effect of leaf type is likely to be transient as the young leaves mature and the trees stop producing epicormic growth.

Background

Fire can be incredibly damaging to ecosystems in the short term, but it plays an essential role in shaping vegetation composition and biodiversity in the Australian landscape (Burrows 2008). A plant's response to fire can be classified in one of two ways; 'seeders' perish after fire and new individuals emerge via seed, while 'resprouters' regenerate from existing buds (Burrows 2013). Resprouters can be further divided into 'basal' (regeneration from buds at or below ground level and through structures such as lignotubers) or 'epicormic' (regeneration above ground on branches and stems) (Burrows 2013). Epicormic resprouting allows quicker recovery in fire-prone landscapes, which in turn assists in the recovery of ecosystem functioning (Pausas and Keeley 2017).

As a group, eucalypts (species of *Angophora*, *Corymbia* and *Eucalyptus*) are considered to be the most proficient and successful epicormic resprouters (Burrows 2013), potentially resulting in only short-term loss of food for koalas and other eucalypt folivores (Matthews et al. 2007). However, the recovery of eucalypts post-fire is dependent on a number of factors, including forest composition, seasonal conditions before and after the fire, and the patchiness and intensity of the fire (Martin and Handasyde 1999). It is also not clear whether the nutritional value of epicormic regrowth differs from mature canopy foliage. Fire could have substantial impacts on the nutritional quality of available browse for koalas because eucalypts likely allocate resources differently to epicormic regrowth than to mature canopy. This may mean that some previously palatable eucalypt species become unpalatable after fire, or vice versa, and that the quality of habitat after fire cannot be assumed from pre-fire values. There is currently limited information about how the nutritional composition of post-fire epicormic regrowth in eucalypt species differs from mature canopy, and whether it is beneficial or detrimental to koalas.

The extensive "Black Summer" bushfires of 2019/20 burnt through 12.6 million hectares on the eastern side of Australia between August 2019 and March 2020 (NSW DPIE 2020). Over 5 million hectares burnt in NSW alone, encompassing 25 % (over 3.5 million hectares) of the State's suitable koala habitat (NSW DPIE 2020). More than 1.9 million hectares of this was classified as high or very high suitability habitat for koalas (NSW DPIE 2020). With fires expected to become more intense and regular, it is critical to understand the nutritional quality of epicormic regrowth to determine whether burnt habitat contains sufficient food resources to support the persistence and recovery of koala populations, and for identifying areas that are most likely to act as nutritional refugia.

A subset of the sites sampled in 2019 for determining the effects of forest harvesting on habitat nutritional quality for koalas burnt during the Black Summer fires. This provided a unique opportunity to analyse the nutritional composition of epicormic regrowth from individual eucalypt trees for which we had already collected pre-fire nutritional data. The additional work complements and extends the original project by providing new information on how fire affects the nutritional landscape for koalas on the NSW north coast.

Objective

Determine whether the nutritional quality of trees and sites is affected by fire.

We compared the nutritional composition of trees sampled more than one year post fire to their pre-fire values. To account for any natural changes in nutritional quality that were unrelated to the effects of fire (such as recovery from drought), we also measured the nutritional composition of leaves collected during the same sampling periods from unburnt sites, matched by forest type to burnt sites.

Methods

Post-fire sample collection

In February 2021, we revisited six sites that burnt during bushfires in the second half of 2019, and six sites that did not burn (Figure 15). The burnt and unburnt sites were paired by forest type so that similar eucalypt species were sampled within each treatment. At each site, we collected leaves from every tree that was sampled during the original collection, unless 1) it was not possible to identify the original tree (e.g. the flagging tape identifying the tree was lost in the fire and there were multiple trees of the same size and species within the vicinity of the recorded GPS location), or 2) the tree was dead. We recorded the appearance of the canopy at each site (e.g. full canopy loss, full to partial intact canopy with epicormic regrowth, or unburnt). Where more than one leaf type was being produced by a tree (e.g. mature canopy and juvenile epicormic growth), we collected separate samples of each. Leaves were weighed immediately after collection and then frozen.

Preparation and analysis of leaf samples

We prepared and analysed leaf samples using the methods outlined in the main study.

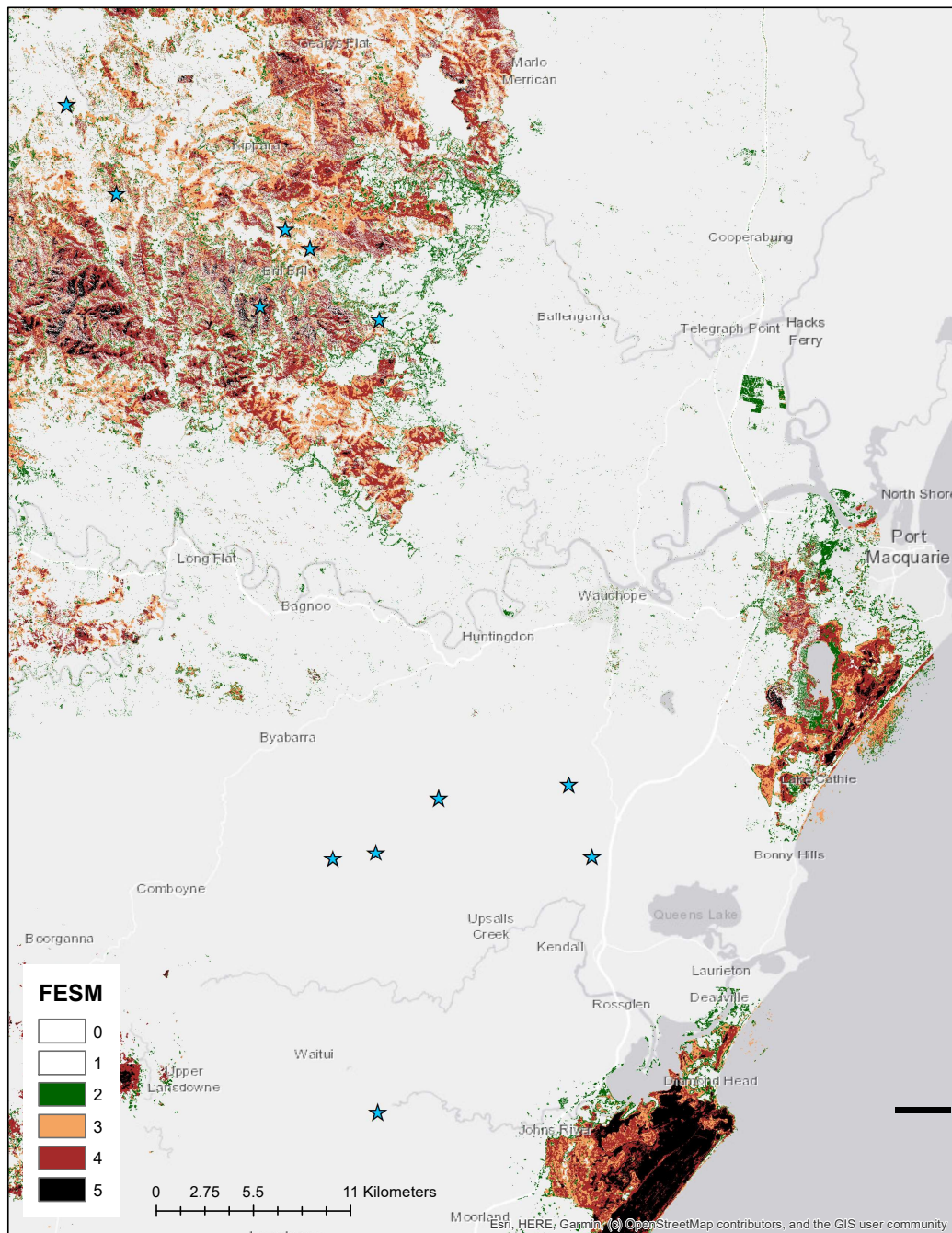


Figure 15. Location of sites sampled post fire (blue stars) relative to fire extent and severity (FESM; 2 = lowest severity, 5 = highest severity; State Government of NSW and Department of Planning Industry and Environment 2020).

Comparison of samples collected before and after fire

Linear regressions were conducted to model differences in nutritional constituents between burnt and unburnt areas, and between pre-fire (original samples collected in 2019) and post-fire (collected in 2021) measurements, noting that “post fire” samples refers to all samples collected in 2021 regardless of whether a site burnt or not. The dependent variables were:

- i. The differences between the post-fire and pre-fire values of nutritional variables (% dry matter, total N, digestible N, UBFs and FPCs) measured in mature leaves from the same trees;
- ii. The differences between nutritional variables (% dry matter, total N, digestible N, UBFs and FPCs) measured in post-fire epicormic leaves and pre-fire mature leaves from the same trees (in burnt areas only);
- iii. The differences between nutritional variables (% dry matter, total N, digestible N, UBFs and FPCs) measured in post-fire mature leaves and post-fire young leaves from the same trees.

The analysis was a paired analysis as the dependent variables are all differences in measures taken from the same trees. Only trees where both of the relevant variables were measured were used in each analysis. The independent variables were:

- Subgenus (monocalypts vs symphyomyrtles, where *E. microcorys* was classified as a symphyomyrtle because, like symphyomyrtles, it makes FPCs). Subgenus was not used for UBF and FPC models, as these variables are only non-zero for one subgenus;
- Burnt (whether a site had burnt at any level of severity). This was not used for dependent variables (ii) above, as epicormic leaves only occurred in burnt areas.
- Interaction between subgenus and burnt (where both independent variables were used in the model).

This represents a total of 15 linear regression models as there are three dependent variables each for % dry matter, total N, digestible N, UBFs and FPCs. All models and calculations were implemented in the R Statistical Environment.

The significance of the “burnt” variable was assessed for each model that included this independent variable. The marginal means of each variable were also estimated, and their significance assessed, for each dependent variable, broken down by subgenus. This was done by marginalising over the other independent variables using the emmeans package in R. A significant result would be evidence that the mean difference is not zero. For example, for dependent variables (i) above, this would indicate a difference between pre-fire and post-fire values in mature leaves, marginalising over burnt and unburnt areas and subgenera.

Results

Samples collected

In February 2021, leaf samples were collected from 150 trees that had previously been sampled between May and September 2019. Of these trees, 89 were in sites that burnt in late 2019 and 61 were in sites that did not burn (Table 4). Regardless of whether a specific site burnt, in the subsequent paragraphs, samples collected in 2019 are referred to as the “pre-fire” collection and those collected in 2021 are referred to as the “post-fire” collection.

Table 4. Number of trees sampled in burnt and unburnt areas during post fire surveys. Subgenus M = *Monocalypt*, S = *Symphyomyrtle*, A = *Alveolata*.

Species	Subgenus	Burnt area	Unburnt area
<i>E. pilularis</i>	M	22	14
<i>E. microcorys</i>	A	20	16
<i>E. saligna</i>	S	14	3
<i>E. acmenoides</i>	M	8	0
<i>E. carnea</i>	M	6	3
<i>E. propinqua</i>	S	5	5
<i>E. siderophloia</i>	S	7	1
<i>E. andrewsii</i>	M	3	0
<i>E. agglomerata</i>	M	1	0
<i>E. globoidea</i>	M	1	3
<i>E. resinifera</i>	S	1	11
<i>E. umbra</i>	M	1	1
<i>E. grandis</i>	S	0	4

Nutritional quality of leaves after fire

The nutritional composition of mature leaves collected post fire did not differ between sites that burnt or did not burn ($p > 0.05$ for all constituents; Table A 3). However, mature leaves collected post fire contained about 3 % less dry matter (i.e. 3 % more water) than mature leaves collected from the same trees pre fire ($p < 0.001$ for both the monocalypt and symphyomyrtle subgenera; Table 5; Figure 16). Post-fire mature leaves from both subgenera also contained higher concentrations of total N (mean increase of 0.11 for monocalypts and 0.15 for symphyomyrtles; $p < 0.001$ for both; Table 5). The digestible N concentration of mature leaves was higher in symphyomyrtles post fire than pre fire ($p < 0.001$; Table 5), but did not differ for monocalypts ($p = 0.551$; Table 5). Monocalypts, however, had lower concentrations of UBFs in post fire compared to pre fire (mean difference of 7 mg.g⁻¹ DM; $p < 0.001$; Table 5). There was no difference in the concentration of FPCs in mature leaves collected from the same trees in the two sampling periods ($p = 0.212$; Table 5; Figure 16).

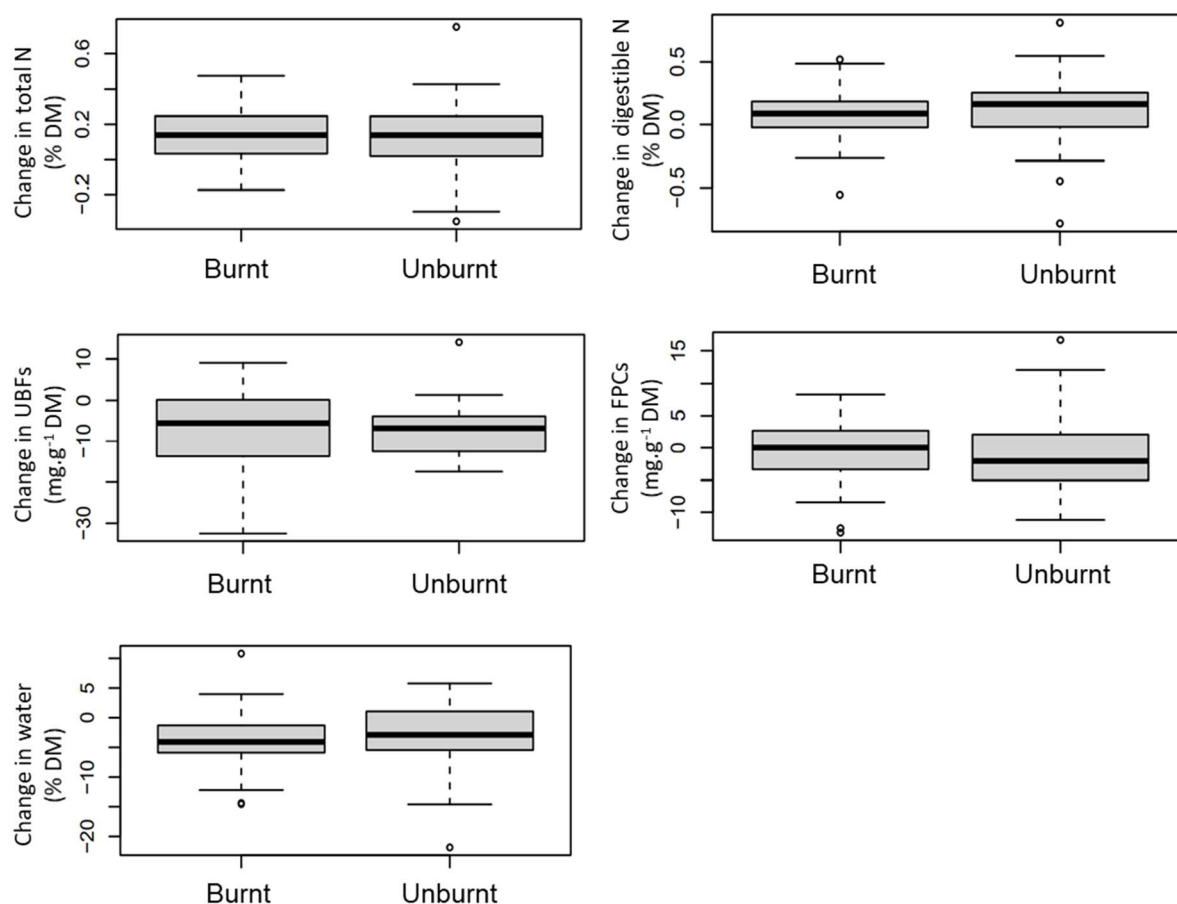


Figure 16. The difference in concentrations of a) total N, b) digestible N, c) UBFs, d) FPCs and e) dry matter between mature leaf samples collected post fire (2021) and mature leaves from the same trees collected pre fire (2019) from areas that did or did not burn.

Table 5. Summary of mean differences in constituent concentrations between mature leaves collected post fire and mature leaves collected pre fire from the same trees. A negative value indicates that the constituent measurements were higher in mature leaves collected pre-fire. Subgenus M includes species from the monocalypt subgenus, while S includes species from the symphyomyrtle and alveolata subgenera. CL = confidence limit. A p-value <0.05 (highlighted in bold) indicates that the difference between the two collection periods is significantly different to 0.

Constituent (units)	Subgenus	mean	SE	df	Lower CL	Upper CL	t-ratio	p-value
Dry matter (%)	M	-3.31	0.91	83	-5.12	-1.49	-3.619	0.001
Dry matter (%)	S	-3.41	0.70	83	-4.80	-2.02	-4.877	0.000
Total N (% DM)	M	0.11	0.02	118	0.06	0.15	4.575	0.000
Total N (% DM)	S	0.15	0.02	118	0.11	0.19	8.095	0.000
Digestible N (% DM)	M	0.02	0.03	118	-0.05	0.08	0.598	0.551
Digestible N (% DM)	S	0.16	0.03	118	0.11	0.21	6.124	0.000
FPCs (mg.g ⁻¹ DM)	S	-0.8	0.67	72	-2.2	0.5	-1.260	0.212
UBFs (mg.g ⁻¹ DM)	M	-6.9	1.27	44	-9.4	-4.3	-5.420	0.000

The water content of epicormic leaves collected post fire from burnt sites was 10 to 11 percentage units higher than the water content of mature leaves collected from the same trees pre fire ($p < 0.001$; Table 6), with no significant difference between the subgenera (Table A 4). Although epicormic leaves from both subgenera had a higher concentration of total N than pre-fire mature leaves (average of 0.16 % higher for monocalypt species and 0.35 for symphyomyrtles; $p < 0.001$; Table 6), this was not always the case for digestible N. Epicormic leaves from symphyomyrtle species contained higher concentrations of digestible N than pre-fire mature leaves (0.26 % higher on average; Table 6), but the concentrations of digestible N in monocalypt epicormic leaves were 0.14 % lower on average than pre-fire mature leaves (Table 6). The FPC concentration in epicormic leaves of symphyomyrtle species was similar to the concentration in pre-fire mature leaves ($p = 0.64$; Table 6), while the UBF concentration in monocalypt species was about 10 mg.g⁻¹ lower in epicormic leaves ($p < 0.001$; Table 6).

Table 6. Summary of mean differences in constituent concentrations between epicormic leaves collected post fire and mature leaves collected pre fire from the same trees. A negative value indicates that the constituent measurements were higher in mature leaves. Subgenus M includes species from the monocalypt subgenus, while S includes species from the symphyomyrtle and alveolata subgenera. CL = confidence limit. A p-value < 0.05 (highlighted in bold) indicates that the difference between epicormic and mature leaves is significantly different to 0.

Constituent (unit)	Subgenus	mean	SE	df	Lower CL	Upper CL	t-ratio	p-value
Dry matter (%)	M	-10.9	1.34	22	-13.70	-8.17	-8.192	0.000
Dry matter (%)	S	-10.2	1.45	22	-13.18	-7.16	-7.005	0.000
Total N (% DM)	M	0.16	0.04	31	0.08	0.24	4.031	0.000
Total N (% DM)	S	0.35	0.05	31	0.26	0.45	7.551	0.000
Digestible N (%DM)	M	-0.14	0.06	31	-0.25	-0.02	-2.466	0.019
Digestible N (% DM)	S	0.26	0.06	31	0.13	0.39	4.097	0.000
FPCs (mg.g ⁻¹ DM)	S	1.8	3.74	12	-6.4	10.0	0.480	0.640
UBFs (mg.g ⁻¹ DM)	M	-10.0	2.12	18	-14.4	-5.5	-4.718	0.000

Nutritional quality of young leaves

The concentrations of nutritional constituents in young leaves (i.e. adult phase leaves that were not yet fully expanded) collected post fire did not differ between sites that burnt or did not burn for all compounds measured ($p > 0.05$ for all; Table A 5). However, the water content of young leaves was, on average, 9 to 11 % higher than the water content of mature leaves growing on the same tree at the same time ($p < 0.001$; Table 7). In symphyomyrtle species, young leaves had higher concentrations of total N (mean of 0.20 % higher; $p < 0.001$) and FPCs (8 mg.g⁻¹ DM higher on average; $p < 0.001$) than mature leaves, but did not differ in digestible N concentration (Table 7). In monocalypt species, young leaves had lower concentrations of digestible N (mean of 0.14 % lower) and higher concentrations of UBFs (8 mg.g⁻¹ DM higher on average; $p = 0.001$) than mature leaves (Table 7).

Table 7. Summary of mean differences in constituent concentrations between mature leaves collected post fire and young leaves collected post fire from the same trees. A negative value indicates that the constituent measurements were higher in young leaves. Subgenus M includes species from the monocalypt subgenus, while S includes species from the symphyomyrtle and alveolata subgenera. CL = confidence limit. A p-value <0.05 (highlighted in bold) indicates that the difference between young and mature leaves is significantly different to 0.

Constituent (unit)	Subgenus	mean	SE	df	Lower CL	Upper CL	t-ratio	p-value
Dry matter (%)	M	8.60	1.16	42	6.26	10.94	7.405	0.000
Dry matter (%)	S	11.37	0.83	42	9.70	13.03	13.743	0.000
Total N (% DM)	M	-0.08	0.05	60	-0.17	0.01	-1.719	0.091
Total N (% DM)	S	-0.20	0.04	60	-0.27	-0.12	-5.433	0.000
Digestible N (% DM)	M	0.14	0.06	60	0.02	0.26	2.324	0.024
Digestible N (% DM)	S	-0.01	0.05	60	-0.11	0.08	-0.259	0.797
FPCs (mg.g ⁻¹ DM)	S	-8.4	1.0	38	-10.4	-6.4	-8.522	0.000
UBFs (mg.g ⁻¹ DM)	M	-8.2	2.0	23	-12.4	-4.0	-4.032	0.001

The nutritional quality of sites can be influenced by the type of leaves available in addition to the combination of species. When considering only mature leaves, sites with high proportions of koala browse species were predicted to have higher mean digestible N concentrations in the post-fire sampling period than in the pre-fire period (Figure 17a, b). In contrast, there was little difference in mean digestible N concentrations at sites with high proportions of blackbutt in between the two periods (Figure 17a, b). Compared to the findings with mature leaves, when trees are producing young leaves, mean digestible N concentrations were predicted to be substantially higher at sites with high proportions of koala browse trees, but lower when sites contained low proportions of these species (Figure 17c). The predictions with epicormic leaves were similar – sites with low proportions of koala browse species had lower mean site digestible N concentrations than when mature leaves were present pre fire. Conversely, sites with a high proportion of koala browse species had higher mean digestible N post fire when epicormic growth was present compared to pre-fire mature growth (Figure 17d).

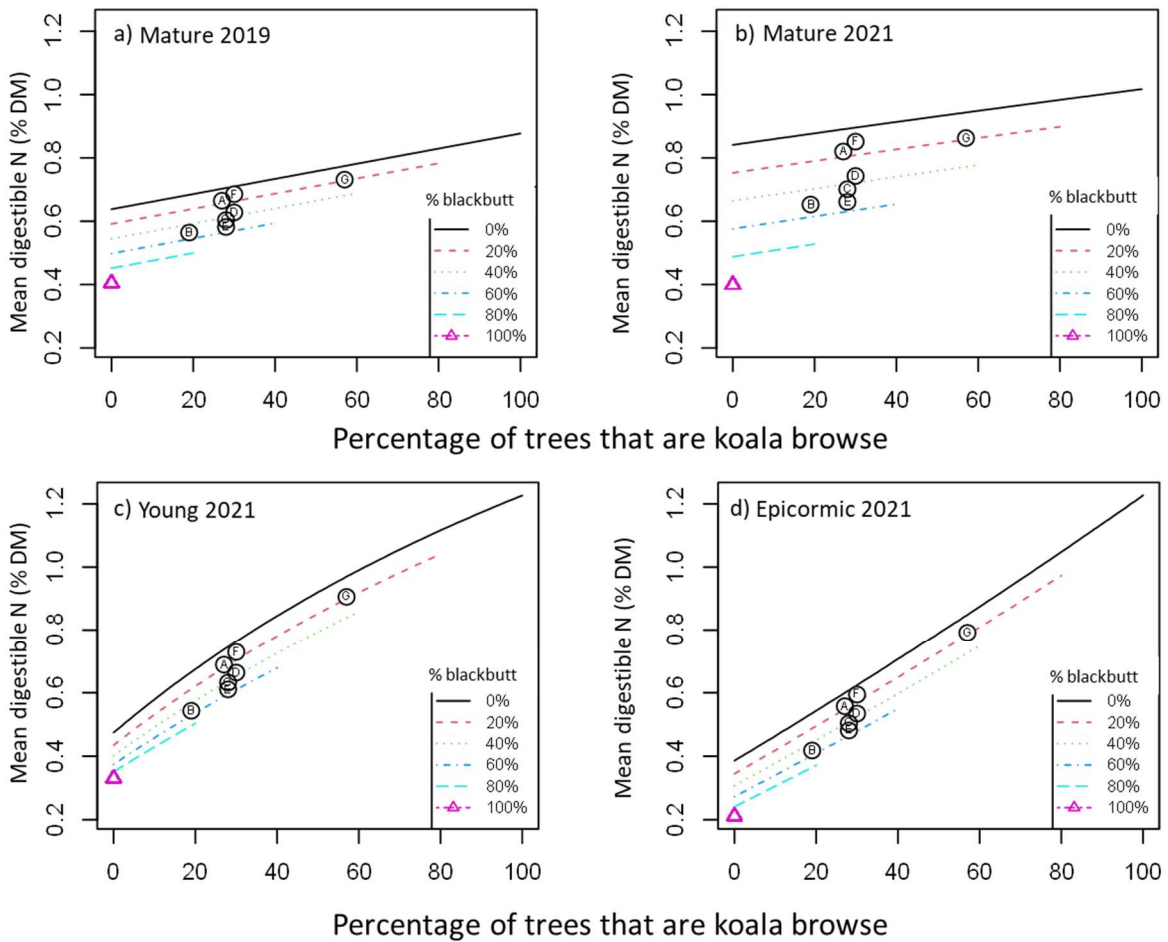


Figure 17. A comparison of the mean digestible N composition of sites with different proportions of koala browse species, blackbutt and other eucalypt species depending on the type of leaves present. a) sites containing mature leaves surveyed in 2019 (pre-fire), b) sites containing mature leaves surveyed in 2021, regardless of whether or not they burnt, c) sites containing young leaves in 2021 (assuming mature leaves absent), regardless of whether or not they burnt, and d) sites sampled in 2021 (post fire) with only epicormic regrowth available.

Discussion

The concentrations of several nutritional constituents differed slightly but significantly between mature leaves collected in 2019 and mature leaves collected from the same trees in 2021 after some sites had burnt. In particular, mature leaves collected in 2021 contained approximately 3 % more water, while symphyomyrtle species had higher concentrations of total and digestible N and monocalypts had lower concentrations of UBFs. The same effects were found in both the burnt and unburnt (control) sites, indicating that the changes are unlikely to be related to fire. Further study would be required to determine the cause of this variation, but some possibilities include natural fluctuations or seasonal effects (e.g. 2019 samples were collected during winter and spring, while 2021 samples were collected during summer) or recovery of the landscape from drought. Previous studies have similarly found small changes in total and digestible N concentrations between sampling periods within a year, with little change in FPC concentrations (Alexander 2013). There

have been no previous investigations of whether UBF concentrations in leaves vary with time, but our findings suggest that investigating this further would be worthwhile. If UBF concentrations do vary with season, monocalypt species may be more likely to be eaten by koalas at certain times of year.

On average, epicormic leaves from symphyomyrtle species on the NSW north coast are likely to be more palatable than mature leaves from the same trees to koalas, at least at the time of sampling for the study (i.e. more than 12 months after fire). At this point in time, symphyomyrtle leaves contained higher concentrations of digestible N and similar FPC concentrations to pre-fire mature leaves from the same trees. Higher digestible N concentrations are known to enhance FPC tolerance and hence intake of leaves containing these compounds (Marsh et al. 2014; Marsh et al. 2018). It is worth noting, however, that the effects may vary between individual trees and eucalypt folivores have been shown to only prefer young leaves with higher digestible N concentrations when FPC concentrations are low (Marsh et al. 2018). Nevertheless, the findings suggest that koalas could obtain more dietary protein by browsing epicormic growth rather than mature foliage from symphyomyrtles. Dietary protein plays important roles in the growth, health and reproduction of individuals and populations (e.g. DeGabriel et al. 2009; McArt et al. 2009), and its higher availability in the epicormic growth of some recognised koala browse species may be beneficial to koalas after fire.

Epicormic leaves from monocalypt species had lower concentrations of UBFs than pre-fire mature leaves on average, and also had lower concentrations of digestible N. It should be noted that the difference in measured UBF concentrations between mature and epicormic leaves (i.e. $10 \text{ mg.g}^{-1} \text{ DM}$) is likely to be less than it appears. This is because mature leaves in the post-fire sampling period also had 7 mg.g^{-1} lower UBF concentrations on average compared to pre-fire values. Because very few trees were producing both epicormic and mature leaves at the same time, we were unable to directly compare the two leaf types collected from the same trees at the same time, and instead compared the two different time points. Although a reduction in UBF concentrations is likely to be beneficial to koalas to a certain extent, if the UBF concentration in epicormic growth on a tree still exceeds koala tolerance levels, that tree would remain unsuitable as food. For example, in a recent feeding study in the NSW Monaro region, captive koalas chose to eat mature leaves from a range of monocalypt and symphyomyrtle species in preference to epicormic leaves from monocalypts (M. Lane and K. Marsh, unpublished data). Without specific investigation of trees on the NSW north coast, it is difficult to know how the combination of reduced UBFs and digestible N would influence feeding by koalas. It is clear, however, that koalas would obtain less dietary protein were they to feed on epicormic leaves from monocalypt species relative to mature leaves.

At a site level, habitats with high proportions of blackbutt and/or other non-browse species were predicted to have lower mean digestible N concentrations when producing epicormic leaves after fire compared to pre-fire mature leaves. In contrast, habitats with high proportions of koala browse species were predicted to have higher mean digestible N concentrations when epicormic leaves were present. The contrast between the two subgenera is interesting, and suggests that the nutritional quality of koala habitat could temporarily shift either up or down after fire depending on the eucalypt species composition of a site.

One caveat on the findings of the post-fire work is that epicormic leaves were sampled up to 18 months after fire. It is not known whether epicormic leaves at an earlier stage of regrowth would differ in nutritional composition from those reported here. This could be determined in the future by sampling leaves at different intervals post-fire. It is clear, however, that when a fire promotes eucalypt epicormic buds to sprout, the effects on the quality of available food for koalas can last for

more than a year. In contrast, when the canopy remains intact after fire, food quality for koalas is similar to unburnt sites.

Conclusions and future directions

This study demonstrates that fire alters the nutritional quality of eucalypt forests for koalas on the north coast of NSW when trees are producing epicormic regrowth. The magnitude and direction of changes in the nutritional quality of available browse was strongly influenced by the site-specific tree species and eucalypt subgenus composition. Digestible nitrogen in eucalypt regrowth tended to decrease in leaves from the monocalypt subgenus and increase in the symphyomyrtle subgenus compared to mature leaves collected pre fire.

When epicormic regrowth is prevalent after fire, areas dominated by symphyomyrtle species on the north coast of NSW are likely to provide better nutritional quality browse for the koala than areas dominated by monocalypt species. More research is needed to determine whether this pattern is consistent across a wider range of eucalypt species, because it may aid in predicting koala population recovery rates after fire in different landscapes. Given that eucalypt leaf chemistry is the primary determinant of koala food choice, koalas probably adjust their diet when trees are producing epicormic regrowth after fire to make use of the highest nutritional quality browse and avoid species that become less palatable. Some eucalypt species, and the areas in which they occur, may therefore become more valuable to koalas after fire, and these tree species may differ from ones that are typically considered the most preferred browse when mature leaf is available. For example, forest harvesting prescriptions consider *E. saligna* and *E. propinqua* to be secondary browse species, but they had some of the highest nutritional quality browse after fire. Similarly, tree species that were typically lower quality browse based on nutritional quality data became even worse when epicormic growth was the dominant leaf type available (e.g. *E. pilularis*, *E. acmenoides*). In a separate study on the southern tablelands of NSW, we found that the most preferred local browse species was largely ignored when only epicormic growth was available (Marsh et al. unpublished). This type of information is key for developing appropriate forest management strategies that not only support koalas during normal times, but also after stochastic disturbance events that influence the type of browse available to the animals. Understanding how fire affects the nutritional quality of tree species across the range of the koala would also assist with planning revegetation programs that can support koala population persistence in landscapes that are fire prone.

This study collected epicormic regrowth several months post-fire. Data on epicormic regrowth immediately after fire could not be collected due to COVID related travel restrictions. It is possible that eucalypt leaf chemistry changes over time as leaves grow from early epicormic sprouts to expanded epicormic regrowth. Future research should investigate how eucalypt leaf chemistry changes as epicormic leaves age. There are likely to be differences between, for example, the first flush of epicormic leaves and those that are retained on trees to maturity, which were not captured in this study. This additional data would provide a more complete understanding of the nutritional resources available to koalas, particularly immediately after fire, and whether interventions such as providing supplementary leaf would be beneficial in some circumstances. In addition, future research should more fully explore the influences of drought and season on eucalypt leaf nutritional composition and foliar moisture, given that droughts and heat waves are expected to occur more frequently under climate change scenarios. This information would also assist with refining future habitat suitability models and climate-ready revegetation strategies.

References

Alexander R (2013) Quantifying the temporal variation in plant secondary metabolites – a case study in the Koala Coast Honours thesis, University of Queensland

Au J (2018) Multi-scale effects of nutrition on an arboreal folivore. PhD thesis, The Australian National University

Burrows GE (2013) Buds, bushfires and resprouting in the eucalypts. *Aust J Bot* 61:331. doi: 10.1071/bt13072

Burrows ND (2008) Linking fire ecology and fire management in south-west Australian forest landscapes. *Forest Ecol Manag* 255:2394-2406. doi: 10.1016/j.foreco.2008.01.009

DeGabriel JL, Moore BD, Foley WJ, Johnson CN (2009) The effects of plant defensive chemistry on nutrient availability predict reproductive success in a mammal. *Ecology* 90:711-719

Marsh KJ, Moore BD, Wallis IR, Foley WJ (2014) Feeding rates of a mammalian browser confirm the predictions of a 'foodscape' model of its habitat. *Oecologia* 174:873-882. doi: 10.1007/s00442-013-2808-3

Marsh KJ, Ward J, Wallis IR, Foley WJ (2018) Intraspecific variation in nutritional composition affects the leaf age preferences of a mammalian herbivore. *J Chem Ecol* 44:62-71. doi: 10.1007/s10886-017-0911-3

Martin R, Handasyde K (1999) The koala: natural history, conservation and management. UNSW Press

Matthews A, Lunney D, Gresser S, Maitz W (2007) Tree use by koalas (*Phascolarctos cinereus*) after fire in remnant coastal forest. *Wildlife Res* 34:84-93. doi: 10.1071/WR06075

McArt SH, Spalinger DE, Collins WB, Schoen ER, Stevenson T, Bucho M (2009) Summer dietary nitrogen availability as a potential bottom-up constraint on moose in south-central Alaska. *Ecology* 90:1400-1411. doi: 10.1890/08-1435.1

NSW DPIE (2020) NSW Fire and the Environment 2019–20 Summary, Sydney, NSW, Australia

Pausas JG, Keeley JE (2017) Epicormic Resprouting in Fire-Prone Ecosystems. *Trends in Plant Science* 22:1008-1015. doi: 10.1016/j.tplants.2017.08.010

State Government of NSW and Department of Planning Industry and Environment (2020) Fire Extent and Severity Mapping 2019/20

Appendix

Table A 1. RN17 forest types used in site selection for the study.

RN17 number	Type	Description
36	Moist blackbutt	Wet sclerophyll forest dominated by blackbutt (usually more than 50%) with an understorey of shrubs and herbs
37	Dry blackbutt	Dry sclerophyll forest dominated by blackbutt (usually more than 50%) with an open understorey
48	Flooded gum	Tall wet sclerophyll forest dominated by flooded gum with rainforest understorey
53	Brush box	Tall wet sclerophyll forest comprising more than 50% brush box associated with various eucalypt species and rainforest understorey
60	Narrow leaved white mahogany – red mahogany – grey ironbark – grey gum	Wet sclerophyll forest of mixed eucalypt species with a dense understorey
62	Grey gum – grey ironbark – white mahogany	Dry sclerophyll forest of mixed eucalypt species with a sparse understorey
74	Spotted gum – ironbark/grey gum	Dry sclerophyll forest of mixed eucalypt species
163	New England blackbutt	Dry to wet sclerophyll forest dominated by New England blackbutt

Table A 2. Sites at which leaf samples were collected.

Site	State Forest	Zone	Last harvest ^a	RN17 forest type	latitude	longitude	elevation	% browse species	% blackbutt	% other species
112_628	Nana Creek	north	2	60	-30.213877	152.992526	251	22	22	56
158_1	Bril Bril	mid	3	53	-31.31499	152.593127	227	8	12	80
181_23	Burrawan	south	2	60	-31.547536	152.752426	66	31	41	28
184_49	Broken Bago	south	3	37	-31.527949	152.678751	96	48	32	19
21_226	Kangaroo River	north	1	163	-29.968242	153.145561	525	72	0	28
24_12	Candole	north	1	62	-29.708121	153.207297	116	0	0	100
24_17	Candole	north	1	74	-29.697815	153.20698	184	16	6	78
24_19	Candole	north	2	36	-29.766957	153.216545	45	9	16	75
284_243	Middle Brother	south	3	60	-31.678715	152.737103	56	34	66	0
29_590	Lower Bucca	north	2	37	-30.194343	153.065439	238	16	50	34
29_594	Lower Bucca	north	2	37	-30.174419	153.075913	62	38	9	53
29_595	Lower Bucca	north	1	60	-30.16914	153.090842	109	19	31	50
291_178	Lansdowne	south	1	60	-31.745896	152.570534	198	72	22	6
291_182	Lansdowne	south	3	36	-31.719966	152.590475	70	22	9	69
291_195	Lansdowne	south	1	48	-31.76738	152.580518	65	22	19	59
30_774	Bagawa	north	3	74	-30.13896	152.933083	341	38	0	63
31_8	Kalateenee	mid	1	37	-31.1305	152.821523	88	22	6	72
31_10	Kalateenee	mid	1	37	-31.135492	152.812979	72	38	25	38
360_739	Wedding Bells	north	1	48	-30.147869	153.107996	106	28	56	16
469_31	Maria River	mid	1	74	-31.136554	152.741381	117	22	3	75
487_271	Newry	mid	2	60	-30.521116	152.9237	23	3	88	9
487_272	Newry	mid	1	37	-30.542093	152.921196	283	41	38	22
487_277	Newry	mid	3	48	-30.53347	152.943332	12	6	63	31
487_279	Newry	mid	3	60	-30.54076	152.95089	33	9	13	78
487_281	Newry	mid	2	48	-30.5408	152.951629	36	25	22	53

Site	State Forest	Zone	Last harvest ^a	RN17 forest type	latitude	longitude	elevation	% browse species	% blackbutt	% other species
487_295	Newry	mid	1	48	-30.533961	153.008056	40	31	9	59
524_13	Bellangry	mid	1	36	-31.278188	152.552129	423	22	66	13
524_17	Bellangry	mid	1	53	-31.307538	152.522444	381	19	81	0
524_20	Bellangry	mid	1	60	-31.268196	152.537511	527	31	13	56
526_49	Tamban	mid	2	53	-30.948618	152.818158	69	16	53	31
526_64	Tamban	mid	2	62	-30.902618	152.879622	53	26	32	42
526_65	Tamban	mid	2	37	-30.889324	152.860123	66	4	52	43
53_141	Upsalls Creek	south	2	48	-31.632608	152.528198	318	25	53	22
535_618	Orara West	north	3	36	-30.241636	152.987402	394	28	56	16
535_619	Orara West	north	3	163	-30.218589	152.98112	394	39	32	29
535_637	Orara West	north	1	53	-30.332103	152.982684	206	48	19	32
535_638	Orara West	north	2	53	-31.631023	152.76561	204	38	63	0
535_796	Orara West	north	3	53	-30.222895	152.930548	635	0	88	13
536_565	Orara East	north	2	74	-30.20242	153.129247	126	44	31	25
536_583	Orara East	north	2	163	-30.207318	153.057362	212	19	44	38
54_114	Kerewong	south	1	53	-31.587095	152.589764	315	47	53	0
54_117	Kerewong	south	2	36	-31.590276	152.564185	414	13	88	0
54_138	Kerewong	south	3	62	-31.589726	152.718899	49	24	34	41
543_313	Nambucca	mid	3	37	-30.631567	152.986505	38	16	56	28
543_319	Nambucca	mid	3	36	-30.66142	152.953228	50	13	72	16
58_85	Lorne	south	2	53	-31.559631	152.627458	237	41	50	9
58_90	Lorne	south	3	53	-31.603474	152.631796	142	75	25	0
59_62	Cowarra	south	1	37	-31.466543	152.787179	74	23	67	10
59_64	Cowarra	south	2	62	-31.474966	152.795909	41	25	25	50
59_65	Cowarra	south	1	62	-31.49411	152.789157	52	75	0	25
60_60	Bulls Ground	south	2	37	-31.55302	152.705212	45	11	52	37
62_73	Kew	south	1	36	-31.598214	152.699389	65	23	23	53

Site	State Forest	Zone	Last harvest^a	RN17 forest type	latitude	longitude	elevation	% browse species	% blackbutt	% other species
698_154	Comboyne	south	3	48	-31.658212	152.490576	374	94	6	0
827_404	Newfoundland	north	1	36	-29.944943	153.153428	70	16	56	28
827_405	Newfoundland	north	2	62	-29.968156	153.145584	99	19	6	75
827_406	Newfoundland	north	3	37	-29.953546	153.121912	94	50	44	6
910_324	Mount Boss	mid	3	36	-31.249863	152.436961	577	22	72	6
910_56	Mount Boss	mid	1	163	-31.204451	152.407907	812	100	0	0

^a 1 = before 2000; 2 = between 2000 and 2008; 3 = between 2009 and 2018

Model A1: The modelled relationship between koala density and site nutritional quality at 75 sites across the range of the koala. Model from Au (2018).

$$\log(\text{density}_{ij}+0.05) = -4.953 + 2.388*N_d - 0.038*FPC + 1.911*(\text{proportion of symphyomyrtus}>0) + u_i + e_{ij}$$

Where N_d is the mean digestible N concentration at a site, FPCs is the mean site FPC concentration when considering species from the *Symphyomyrtus* and *Alveolata* subgenera only, and the terms u and e take regional differences in climate and environment into account. For region i , area j , where u_i are normal with expected value zero and variance 0.749, and e_{ij} are normal with expected value zero and variance 0.451.

Model A2: Adapted model of the relationship between koala density and site nutritional quality used in the current study.

$$\begin{aligned} \log(\text{density}+0.05) &= -4.953 + 2.388*N_d - 0.038*FPC + 1.911*P[\text{proportion of symphyomyrtus}>0 \text{ from a} \\ &\text{random sample of 30 trees}] + u_i + e_{ij} \\ &= -4.953 + 2.388*N_d - 0.038*FPC + 1.911*[1-(1-P_{ij})^{30}] + u_i + e_{ij} \end{aligned}$$

Where P_{ij} is the proportion of symphyomyrtle species in the area.

Model A3: Calculation for obtaining the expected density of koalas from Model A2.

$$\text{Expected density} = \exp(-4.953 + 2.388*N_d - 0.038*FPC + 1.911*[1-(1-P_{ij})^{30}] + 0.5*(0.749+0.451)) - 0.05.$$

Where “exp” is the exponential function.

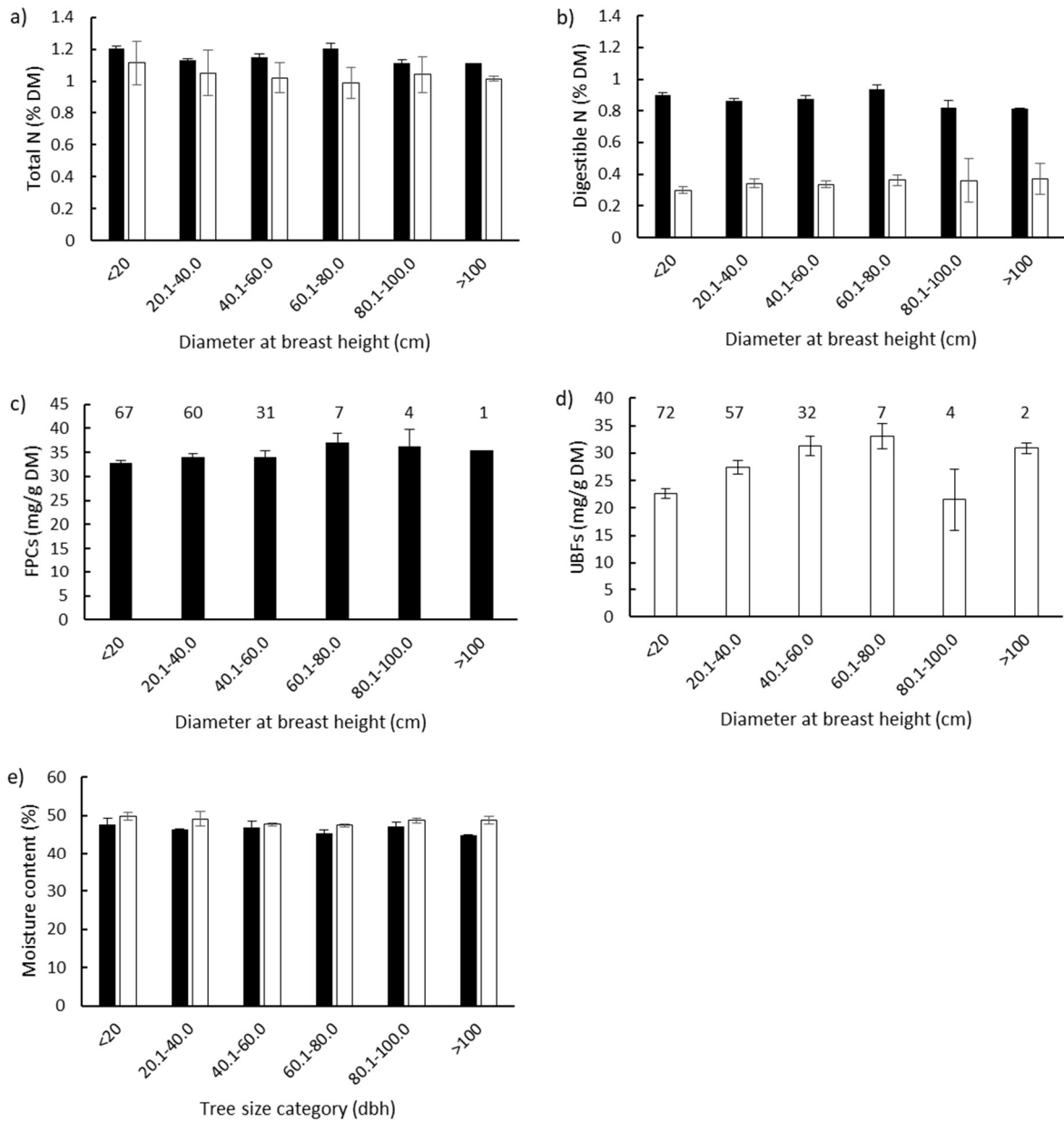
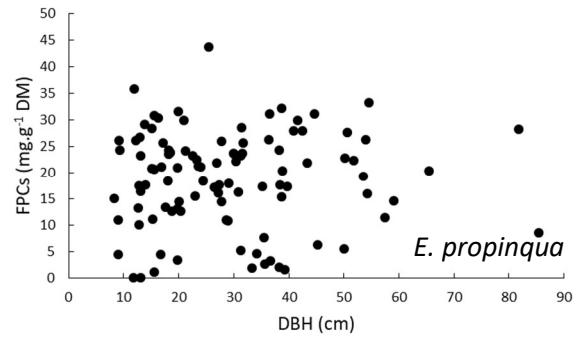
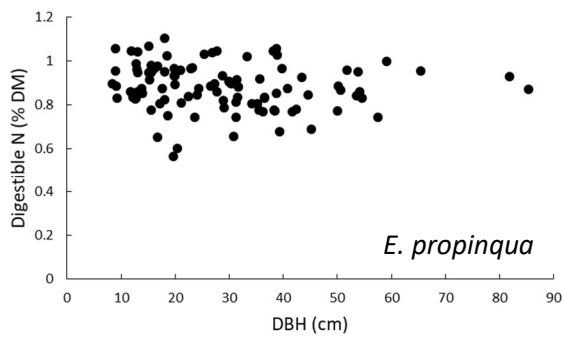
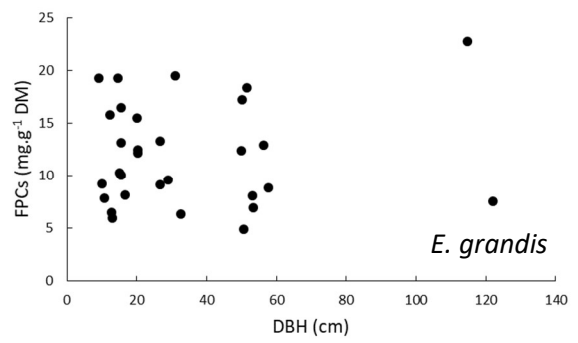
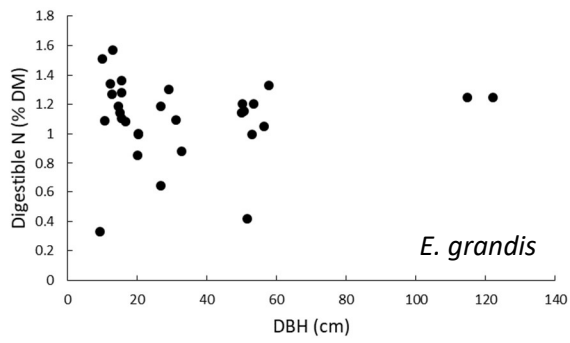
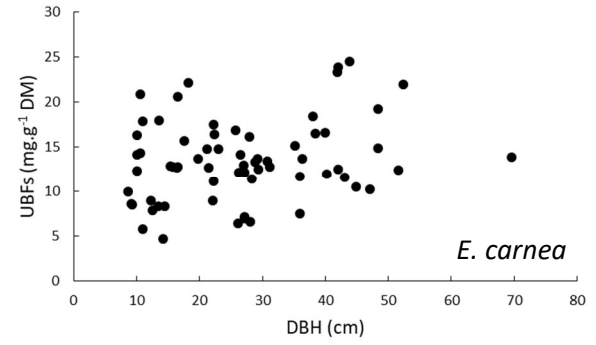
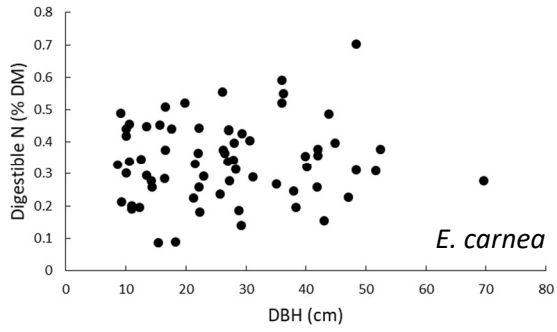
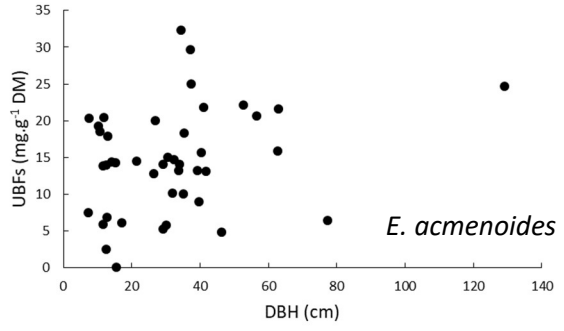
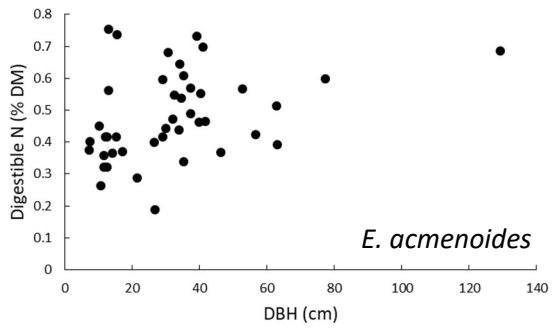


Figure A 1. Mean \pm SE foliar concentrations of a) total N, b) digestible N, c) FPCs, d) UBFs, and e) moisture in different size classes of the two most widely sampled species, *E. microcorys* (black) and *E. pilularis* (white). Numbers above the bars in parts c and d show how many trees were in each size class category for each species.



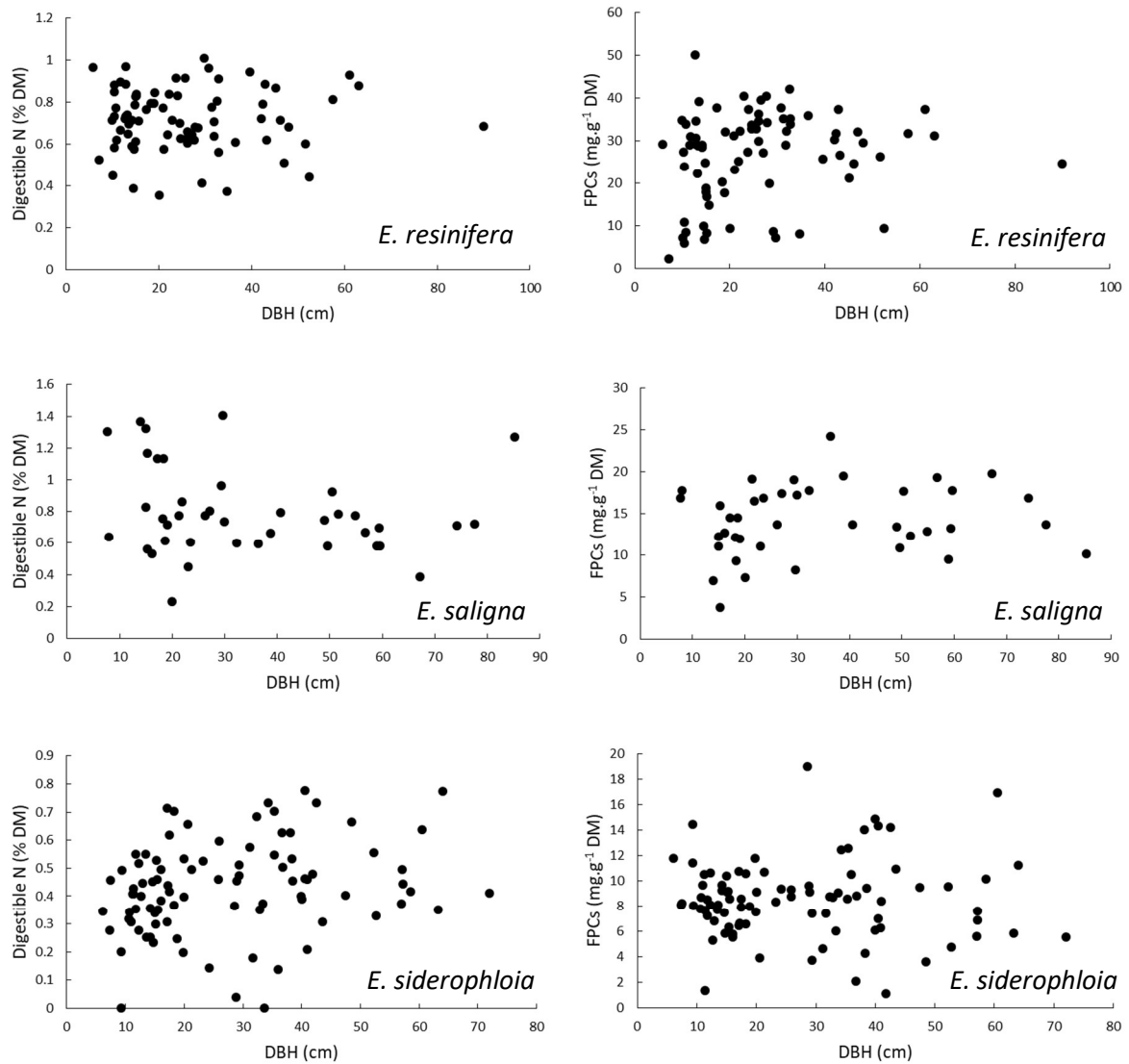


Figure A 2. Relationship between tree size and nutritional composition (concentration of digestible N and UBFs or FPCs) for the seven species of eucalypts for which more than 30 individual trees were sampled.

Table A 3. Model output for mature leaf collected post fire minus mature leaf collected pre fire.

	<i>Dependent variable:</i>				
	%DM (1)	total N (2)	avail N (3)	FPCs (4)	UBFs (5)
SubgenusS	0.247 (1.721)	0.079* (0.044)	0.143** (0.061)		
BurntB	-0.582 (1.827)	0.058 (0.047)	-0.016 (0.065)	0.081 (1.331)	0.177 (2.539)
SubgenusS:BurntB	-0.696 (2.300)	-0.076 (0.060)	-0.011 (0.083)		
Constant	-3.015** (1.422)	0.079** (0.036)	0.027 (0.049)	-0.879 (0.928)	-6.970*** (1.909)
Observations	87	122	122	74	46
R ²	0.011	0.028	0.090	0.0001	0.0001
Adjusted R ²	-0.025	0.003	0.067	-0.014	-0.023
Residual Std. Error	5.128 (df = 83)	0.160 (df = 118)	0.221 (df = 118)	5.723 (df = 72)	8.537 (df = 44)
F Statistic	0.313 (df = 3; 83)	1.119 (df = 3; 118)	3.907** (df = 3; 118)	0.004 (df = 1; 72)	0.005 (df = 1; 44)

Note:

*p<0.1; **p<0.05; ***p<0.01

Table A 4. Model output for epicormic leaves minus mature leaves collected pre fire.

	<i>Dependent variable:</i>				
	%DM (1)	total N (2)	avail N (3)	FPCs (4)	UBFs (5)
SubgenusS	0.771 (1.972)	0.191*** (0.061)	0.398*** (0.084)		
Constant	-10.936*** (1.335)	0.161*** (0.040)	-0.136** (0.055)	1.795 (3.740)	-9.986*** (2.117)
Observations	24	33	33	13	19
R ²	0.007	0.237	0.418	0.000	0.000
Adjusted R ²	-0.038	0.213	0.399	0.000	0.000
Residual Std. Error	4.813 (df = 22)	0.175 (df = 31)	0.240 (df = 31)	13.485 (df = 12)	9.227 (df = 18)
F Statistic	0.153 (df = 1; 22); 9.636*** (df = 1; 31); 22.232*** (df = 1; 31);				

Note:

*p<0.1; **p<0.05; ***p<0.01

Table A 5. Model output for mature leaves collected post fire minus young leaves collected post fire.

	<i>Dependent variable:</i>				
	%DM (1)	total N (2)	avail N (3)	FPCs (4)	UBFs (5)
SubgenusS	1.703 (1.769)	-0.156** (0.069)	-0.276*** (0.091)		
BurntB	1.667 (2.322)	0.048 (0.090)	0.0002 (0.119)	2.978 (1.973)	-2.284 (4.060)
SubgenusS:BurntB	2.127 (2.851)	0.075 (0.115)	0.252 (0.152)		
Constant	7.764*** (1.469)	-0.101* (0.054)	0.138* (0.071)	-9.897*** (1.208)	-7.042*** (2.436)
Observations	46	64	64	40	25
R ²	0.172	0.127	0.184	0.057	0.014
Adjusted R ²	0.113	0.083	0.143	0.032	-0.029
Residual Std. Error	4.406 (df = 42)	0.215 (df = 60)	0.285 (df = 60)	6.042 (df = 38)	9.743 (df = 23)
F Statistic	2.912** (df = 3; 42)	2.906** (df = 3; 60)	4.510*** (df = 3; 60)	2.277 (df = 1; 38)	0.316 (df = 1; 23)

Note:

*p<0.1; **p<0.05; ***p<0.01