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Remote sensing and threatened species surveys to better understand risks of forest fragmentation from the Narrabri Gas Project

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Contents

I Ackno	owledgei	ments	. 11
II Execu	utive sur	nmary	. 12
1	Introdu	ction	16
	1.1	Background	16
	1.2	Aims of GISERA project B.8	. 19
	1.3	Report outline	20
2	Genera	l methods	22
	2.1	Study area	22
	2.2	Project timeline	. 23
	2.3	Climate	. 25
	2.4	Wildfire	. 25
3	Assessn	nent of fragmentation in the NGP area	28
	3.1	Introduction	. 28
	3.2	Methods	. 29
	3.3	Results and discussion	31
4	Threate	ened species – vertebrates	34
	4.1	Introduction	34
	4.2	Methods	36
	4.3	Results and discussion	38
5	Threate	ened species – plants	41
	5.1	Introduction	41
	5.2	Methods	43
	5.3	Results and discussion	45
6	LiDAR-c	lerived structural attributes of the habitat	47
	6.1	Introduction	47
	6.2	Methods	48
	6.3	Results and discussion	53
7	Species	occupancy and habitat relationships	55

	7.1	Introduction	55
	7.2	Methods	55
	7.3	Results	59
	7.4	Discussion	74
8	Potenti	al to upscale monitoring	76
	8.1	Background	76
	8.2	Approach	76
	8.3	Results	78
	8.4	Conclusion	
9	Mycorrhizal communities		
	9.1	Introduction to mycorrhizal biology	
	9.2	Methods	
	9.3	Results and discussion	91
	9.4	Conclusions	
10	Conclus	sions	
11	Referer	nces	

Figures

Figure 1.1 Location of the NGP in eastern Australia (from Sreekanth et al., 2018, Figure 1)
Figure 1.2 Map of the study area showing the outline of the NGP (red)
Figure 2.1 Project area (horizontal grey lines) within the state forest areas of the Pilliga Forest
Figure 2.2 Map of the Pilliga Forest showing the extent and severity of the wildfire that occurred in December 2023. Blue dots are the project's threatened vertebrate survey sites. (NSW Government SEED website https://www.seed.nsw.gov.au/)
Figure 3.1 Classes of the FAD metric
Figure 3.2 Map of the Pilliga Forest region showing the NGP area (enclosed by the red line) and the distribution of FAD classes
Figure 3.3 Map of the Pilliga Forest region showing the NGP area (enclosed by the red line) and the distribution of MSPA classes
Figure 4.1 A. Eastern pygmy-possum; B. black-striped wallaby; C. pale-headed snake; D. squirrel glider (Photo credit, all images: Stewart Macdonald)
Figure 4.2 A sample survey transect: starting on the road at site 27, one observer walked north-east and recorded the pink track, while the second observer walked south-west and recorded the green track
Figure 4.3 The number of sites (out of 30) where each species was detected over the course of the four survey periods. Only one species (the eastern spiny-tailed gecko, <i>Strophurus williamsi</i>) was detected at all 30 sites
Figure 5.1 Images of the five focal plant species: (a) and (b) Androcalva procumbens; (c) Vincetoxicum forsteri; (d) and (e) coolabah bertya; (f) and (g) winged peppercress; and (h) spiny peppercress. (Photo credit, all images: Bruce Murray)
Figure 6.1 Location of UAV-LiDAR survey sites within the study region
Figure 6.2 Example of the forward-nadir-backward scan pattern adopted by the VUX-120 laser scanner, showing how it is optimised for data collection of vertical surfaces (adapted from the RIEGL VUX-120 manual by RIEGL Laser Measurement Systems GmbH, 2021)
Figure 6.3 Example of a canopy height model derived from a UAV-LiDAR survey. Cross- hatch lines: north-south/east-west flight patterns; grey lines: processed flight line data; red lines: discarded lower quality data collected in turns and in transit
Figure 6.4 Cross-section view through a 60 m transect of high-density UAV-LiDAR point cloud (8,000 points/m ²)

Figure 6.5 Overview of the structural variability within and between research plots. The left-hand component of the violin plots represents foliage projected cover (FPC), and the right-hand component represents canopy height (CHM)
Figure 7.1 FAD values for each of the 30 vertebrate monitoring sites; higher values represent more intact (i.e. less fragmented) habitat
Figure 7.2 Two commonly observed species of lizard: eastern spiny-tailed gecko (on left); eastern stone gecko (on right) (Photo credit: Stewart McDonald)
Figure 7.3 The likelihood of occupancy for the eastern spiny-tailed gecko (<i>Strophurus williamsi</i>) as a function of FAD. The species occurred at all sites, so the model is uninformative. Black dots represent the actual FAD values for sites where the species was detected
Figure 7.4 The likelihood of occupancy for the eastern stone gecko (<i>Diplodactylus vittatus</i>) as a function of FAD. The species occurred at most sites and across a wide range of FAD values, so the model is uninformative. The grey shaded area represents confidence intervals (and spans the entire likelihood range of 0–1). Black dots represent the actual FAD values for occupied sites
Figure 7.5 The effect of maximum daily temperature on the detectability of the eastern stone gecko (<i>Diplodactylus vittatus</i>). Detectability increases with increasing temperature. The grey shaded area represents confidence intervals. Black dots represent the actual temperature for surveys in which this species was detected
Figure 7.6 The effect of maximum daily temperature on the detectability of <i>Gehyra</i> <i>versicolor</i> . Detectability increases with increasing temperature. The grey shaded area represents confidence intervals. Black dots represent the actual temperature for surveys in which this species was detected
Figure 7.7 Modelled likelihood of occupancy of the eastern pygmy-possum (<i>C. nanus</i>) as a function of FAD (upper graph). Modelled detectability as a function of daily maximum temperature (lower graph). The grey shaded area represents confidence intervals 67
Figure 7.8 Modelled likelihood of occupancy of the eastern pygmy-possum (<i>C. nanus</i>) versus (upper graph)) rumple and (lower graph) VCI. The grey shaded area represents confidence intervals, which span the entire range from 0–1, indicating a very poor model fit
Figure 7.9 Cluster analysis using multidimensional scaling for eight sites where the eastern pygmy-possum (<i>C. nanus</i>) is present (green circles) or absent (red circles)71
Figure 7.10 Occupancy of coolabah bertya (<i>B. opponens</i>) as a function of FAD value. The grey shaded area represents confidence intervals. Black dots represent results from survey data
Figure 7.11 Occupancy of <i>A. procumbens</i> as a function of the CRR. The grey shaded area represents confidence intervals. Black dots represent results from survey data

Figure 8.1 Location of the 15 UAV-LiDAR field sites (green squares) within the broader Pilliga Forest landscape
Figure 8.2 Sentinel-1 median composite image (Jul–Sept 2023); RGB (red, blue, green) rendered on dB scale (R = VV, G = VH, B = VV:VH)
Figure 8.3 Sentinel-1 median composite image (Jul–Sept 2024); RGB (red, green. Blue) rendered on dB scale (R = VV, G = VH, B = VV:VH)
Figure 8.4 Relationship between Sentinel-1 backscatter intensity variables and UAV- LiDAR derived LAI: (a) VV; (b) VH; and (c) VV:VH. Kernel density overlay indicates the percentile distribution of data
Figure 8.5 Relationship between Sentinel-1 backscatter intensity variables and UAV- LiDAR derived LAI: (a) VV; (b) VH; and (c) VV:VH. Kernel density overlay indicates the percentile distribution of data
Figure 8.6 The relationship between Sentinel-1 VH backscatter intensity variables and UAV-LiDAR derived height percentiles (20 m pixels): (a) 50 th percentile; (b) 75 th percentile; and (c) 95 th percentile. Kernel density overlay indicates the percentile distribution of data
Figure 8.7 Spatial predictions of habitat structural metrics from Sentinel-1 radar imagery, trained with UAV-LiDAR data: (a) seasonal composite (July–September 2024) of VH backscatter; (b) LAI; (c) canopy density; and (d) 75 th canopy height percentile. Note the prominence of the fire scar in the south-western quadrat of the images
Figure 8.8 Observed vs predicted results from Random Forest modelling of LAI as a function of Sentinel-1 backscatter variables (VV:VH). Model trained with UAV-LiDAR data. Kernel density overlay indicates the percentile distribution of data
Figure 9.1 Examples of ectomycorrhizal root tips (photos courtesy of Marc-André Selosse)
Figure 9.2 Examples of sporocarps: (a) a typical mushroom form; (b) bracket fungi; (c) a puffball/truffle form that fruits below ground; (d) a clathrate (net-like) fruiting body; (e) an earth star
Figure 9.3 Collection sites for <i>A. procumbens</i>
Figure 9.4 Collection sites for <i>B. opponens</i>
Figure 9.5 Collection sites for <i>L. aschersonii</i> and <i>L. monoplocoides</i>
Figure 9.6 Collection sites for <i>V. forsteri</i>
Figure 9.7 Micrographs of various plant roots, magnification 200×: (a) <i>B. opponens</i> ; (b) <i>L. monoplocoides</i> ; (c) <i>V. forsteri</i> ; (d) <i>A. procumbens</i> . Inset: <i>B. opponens</i> ectomycorrhizal root tips
Figure 9.8 OTUs from the study site associated with <i>B. opponens</i> were related to <i>R. inquinata</i> , a rare (potentially endangered) fungus, from New Zealand's South Island

(photo reproduced with permission; photo by Shirley Kerr,	
https://www.kaimaibush.co.nz/index.html)	99
Figure 9.9 Phylogram depicting the relationships between <i>Russula</i> species detected on <i>opponens</i> in the present study (OTUs 7, 161 and 600) and their closest described	В.
relatives, R. inquinata, R. atramentosa and R. denissima	99
Figure 9.10 Phylogram depicting the relationships between <i>Tomentella</i> species detecte on <i>B. opponens</i> in the present study (OTUs 19 and 31) and their closest described	d
relatives, T. tedersooi, T. stipitobasidia, T. longisterigmata and T. stuposa1	101

Tables

Table 3.1 Pixel classification used for MSPA, resulting in eight distinct classes
Table 4.1 Details of the survey sites including the FAD value 36
Table 5.1 Details of belt transects and number of seedling, flowering and fruiting <i>B. opponens</i> in the study area. Site numbers correspond to vertebrate survey sites
Table 7.1 List of sites used in occupancy modelling. Sites 101–103 are separate sitessurveyed for A. procumbens. 'UAV-LiDAR' indicates that UAV-LiDAR structural habitatdata were collected.56
Table 7.2 Detection histories for vertebrate species from four survey periods across 30sites61
Table 7.3 Summary of eastern pygmy-possum (<i>C. nanus</i>) sightings during the surveys 66
Table 7.4 Values of the five LiDAR-derived structural metrics across 15 sites
Table 7.5 Results for the occupancy model for the eastern pygmy-possum (C. nanus)using LiDAR-derived metrics69
Table 9.1 Plant root and soil samples obtained91
Table 9.2 Statistical differences in composition of fungal communities by plant species. Values shown are p-values from post-hoc contrasts after PERMANOVA analyses. p-values less than 0.05 are considered to be significant
Table 9.3 Top 20 species from <i>B. opponens</i> roots and rhizosphere soils. Each row shows an OTU (~species), its nearest match in the GenBank database. The DNA-DNA identity (%) is shown for each OTU and its match, as is the mean relative abundance of that taxon, along with the number of roots and rhizosphere soils from which this taxon was observed
Table 9.4 Top 20 species from <i>A. procumbens</i> roots and rhizosphere soils. Each row shows an OTU (~species), its nearest match in the GenBank database. The DNA-DNA identity (%) is shown for each OTU and its match, as is the mean relative abundance of that taxon, along with the number of roots and rhizosphere soils from which this taxon was observed.
Table 9.5 Top 20 species from <i>L. monoplocoides</i> roots and rhizosphere soils. Each row shows an OTU (~species), its nearest match in the GenBank database. The DNA-DNA identity (%) is shown for each OTU and its match, as is the mean relative abundance of that taxon, along with the number of roots and rhizosphere soils from which this taxon was observed
Table 9.6 Top 20 species from <i>V. forsteri</i> roots and rhizosphere soils. Each row shows an OTU (~species), its nearest match in the GenBank database. The DNA-DNA identity (%) is

OTU (~species), its nearest match in the GenBank database. The DNA-DNA identity (%) is shown for each OTU and its match, as is the mean relative abundance of that taxon,

along with the number of roots and rhizosphere soils from which this taxon was	
observed	104

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II Executive summary

This project (B.8) was developed and carried out to provide scientific understanding that addresses a key community concern related to the potential impact of the Narrabri Gas Project (NGP) on the intactness of ecosystems and their component species in the Pilliga Forest. The research characterises the level of fragmentation in the NGP area and examines whether fragmentation resulting from the NGP will impact biodiversity, including threatened animals and plants and mycorrhizal communities.

The main components of the research comprise:

- 1. remotely sensed quantification of fragmentation and connectivity metrics across the entire NGP area
- 2. on-ground surveys for occurrence of a subset of threatened plants (five species) and vertebrates (four species)
- 3. on-ground surveys for mycorrhizal symbionts of the threatened plants and subsequent lab-based assessment of diversity
- 4. LiDAR-derived quantification of structural habitat metrics at a subset of survey sites where selected threatened species were present (or absent)
- 5. occupancy modelling of species occurrence based on fragmentation metrics and LiDARderived site variables
- 6. consideration of approaches and feasibility of upscaling monitoring to enable remote assessment of environmental variables.

Fragmentation and connectivity metrics

Fragmentation and connectivity were quantified using metrics that have been developed to harness the potential of earth observation science to measure these processes at large spatial scales.

- Fragmentation was measured with the Foreground Area Density (FAD) approach, which describes the density of a foreground class of interest. It is designed to provide a pixel-level measure of fragmentation.
- Connectivity was measured with Morphological Spatial Pattern Analysis (MSPA). This
 approach uses mathematical morphometry techniques to classify binary patterns into
 discrete classes including cores, edges and bridges that can be used to assist in
 identifying important habitat patches and components of the landscape that connect
 core habitats to one another.

The fragmentation map of the NGP shows that the southern two-thirds of the region, dominated by woody vegetation, is relatively unfragmented consisting of FAD classes 4–6

(i.e. 'dominant', 'interior' and 'intact'). Large patches of 'intact' vegetation (i.e. FAD value = 100%) are present in the north and east of the region.

The connectivity map of the NGP confirms that the woody vegetation in the region is mostly unfragmented with the vast majority classified as 'core', meaning that it is continuous. Components of the landscape that connect core habitats to one another, such as 'branch', 'bridge' and 'loop', are absent or very rare.

On-ground surveys for threatened species

Fieldwork was undertaken from October 2023 to October 2024, a period of moderate rainfall that included a large wildfire in December 2023 that burnt 129,000 ha of the study area. Such wildfires occur periodically in the Pilliga Forest.

Four threatened species of vertebrates (eastern pygmy-possum, *Cercartetus nanus*; squirrel glider, *Petaurus norfolcensis*; black-striped wallaby, *Macropus dorsalis*; and pale-headed snake, *Hoplocephalus bitorquatus*) were surveyed across a range of sites including a network of 30 sites that were repeat sampled (3–4 times each) during nocturnal walk transects. Two of the four target species (eastern pygmy-possum and black-striped wallaby) were located but only the eastern pygmy-possum was present in the network of 30 repeat sampled sites.

Five threatened species of plants (coolabah bertya, *Bertya opponens*; spiny peppercress, *Lepidium aschersonii*; winged peppercress, *Lepidium monoplocoides*; *Androcalva procumbens*; and *Vincetoxicum forsteri*) were surveyed using a range of methods. Some of the survey sites overlapped with those used for the vertebrate surveys but most were standalone. Each of the five species was detected during field surveys. Soil and root samples were taken from individuals of each species for laboratory analysis of mycorrhizal symbiont diversity.

LiDAR-derived quantification of structural habitat metrics

A light-detection-and-ranging (LiDAR) remote sensing approach was adopted to quantify the structural attributes of a subset (15) of vertebrate and plant survey sites. This was done with an Acecore Noa hexacopter fitted with a RIEGL survey-grade VUX-120 laser scanner. Each unoccupied aerial vehicle (UAV) flight covered an area of 15 ha, flown at an altitude of 80 m above ground level using a cross-hatched east-west/north-south pattern at a speed of 5.3 m s⁻¹ and with a line spacing of 60 m. Total flight times ranged from 24 to 28 mins. Flight logs were downloaded and processed, with the final LiDAR datasets consisting of plot-level structural metrics capturing key characteristics of structural diversity. These were calculated using the R statistical modelling environment (v. 4.3.2) with the lidR package and its companion package, lidRmetrics. A total of 115 metrics were obtained, grouped into descriptive statistics, canopy arrangement metrics, canopy heterogeneity metrics and canopy cover metrics.

Occupancy modelling of species occurrence based on fragmentation and LiDAR-derived site metrics

An occupancy modelling approach was used to assess the relationship between site occupancy (presence or absence) and environmental attributes for a range of species. For vertebrate species where repeat transects were carried out, a repeated detection function that accounts for imperfect detectability of the species was added. The analyses included two species of threatened plants (*B. opponens* and *A. procumbens*), one threatened vertebrate (eastern pygmy-possum) and several additional species of non-threatened vertebrates that were frequently detected during surveys. The fragmentation metric used was the FAD value, which ranged from 72% to 96% across the survey sites. Five of the LiDAR-derived habitat structural variables were chosen from the 115 variables available (canopy relief ratio, mean leaf area density, vertical complexity index, rugosity and rumple). No statistically meaningful relationship between the presence/absence of the focal (or additional) species and the FAD or any of the structural attributes measured was found.

The original intention had been to combine information on the fragmentation and connectivity indices of the landscapes in which each threatened species was recorded with the LiDAR-derived data on structural attributes of each species' habitat to make predictions on their likely population-level response to additional forest fragmentation resulting from the NGP. However, this work was not undertaken in the way it had originally been developed. The impact of the extensive December 2023 wildfire precluded undertaking surveys in mature habitat for each species and the structural attributes of habitat could not be assessed in mature habitat. In addition, the degree of fragmentation of the Pilliga Forest was low, as represented by the typically very high FAD values of most sites. This was unexpected and was not an ideal design for testing the role of fragmentation on species occupancy. It is possible that a fragmentation response would emerge if sites had been available at lower levels of FAD values and if a larger sample of sites had been examined or the species chosen were more sensitive to fragmentation.

Approaches and feasibility of upscaling monitoring to enable remote assessment of environmental variables

Given the large extent of the NGP area, there is scope to consider the upscaling of UAV-LiDAR structural metrics to larger areas using Synthetic Aperture Radar (SAR) for measuring and monitoring the structure of forest habitat. The analysis made use of C-band Sentinel-1 data (10 m spatial resolution, 10–15 day revisit time), which are sensitive to vegetation structural elements. The Sentinel-1 based analyses were trained and validated with the UAV-LiDAR data collected for habitat structural assessments. Sentinel-1 backscatter intensity showed varying degrees of correlation with structural metrics derived from our UAV-LiDAR surveys. Bimodal clustering was evident in most relationships, indicating that the C-band (5.6 cm) wavelength of Sentinel-1 is sensitive to high and low values of the structural metrics but has limited ability to distinguish more subtle variations or changes in these metrics. The Sentinel-1 imagery tested was typically able to account for at least 50% of the variation in the UAV-LiDAR metrics. Given that Sentinel-1 data are openly available for the entire Australian continent, these results are encouraging for the use of satellite data in modelling habitat structure over larger areas.

Mycorrhizal communities

The mycorrhizal status of four of the threatened plants (*A. procumbens, B. opponens, L. monoplocoides* and *V. forsteri*) was assessed to determine whether the fungal partners of each (should they exist) were widespread in the region. Roots from individuals of each species were collected, along with loose rhizosphere soil (as an indication of fungal communities immediately adjacent to the plant). The samples were examined in pairs (root:soil) by species using a DNA-based approach (next-generation sequencing of the internal transcribed spacer region).

A total of 5,669 fungal OTUs (operational taxonomic units \approx species) was observed across the 54 samples examined. All plants were significantly different from each other in terms of fungal communities in their roots and rhizosphere soils. In most cases, roots and soils were markedly different in their fungal communities, though for some individual plants this was less clear. *B. opponens* was shown to form ectomycorrhizas, the first evidence of such associations in an Australian Euphorbiaceae species. The mycorrhizal partners (*Russula* and *Tomentella* species) are common, suggesting that dispersal is unlikely to be an issue for the plant. None of the other three plant species examined was convincingly mycorrhizal.

Summary

Our research revealed relatively low levels of fragmentation and typically high connectivity across the landscapes of the Pilliga. At the species level, the study did not find evidence that vertebrates or threatened plants persisting in the Pilliga are impacted by current levels of fragmentation. However, it did confirm several known and imminent threats that are of high concern for biodiversity conservation. These pressures include the ongoing effects of the loss of large, hollow-bearing trees and the effects of large-scale wildfires. There should be a focus on ensuring that these threats are not exacerbated through activities associated with the development of the NGP, particularly within the Pilliga Forest. The NGP offers an opportunity to invest in improving the environmental condition of the Pilliga Forest.

1 Introduction

In this section we present the context for our research project from both the environmental and community perspective, describe the gaps in scientific understanding that created the need for the research and set out the aims of the report.

1.1 Background

1.1.1 The Narrabri Gas Project

The NGP is located in the Gunnedah Basin, approximately 600 km north-west of Sydney, and immediately south/south-west of the town of Narrabri within the Northern Inland Catchments IBRA region (Figure 1.1). The project area of 95,000 ha is in the north-east of the Pilliga Forest, with approximately 60% of the area on state land in a section of the Pilliga State Forest (Figure 1.2) set aside by the NSW Government for uses including logging and extractive industries. The Australian energy company Santos is the proponent.

The NGP will involve the production of natural gas through the progressive development of up to 850 wells on 425 sites in the project area connected into processing facilities south of Narrabri at Leewood and Bibblewindi. The NGP will also include the construction of a comprehensive interconnected network of access roads and linear infrastructure linking well pads to transport infrastructure in addition to the construction of the Bibblewindi to Leewood corridor. Operations will be located on around 1,000 ha of the project area. The project has been approved and in February 2023 Phase 1 commenced, which included the drilling of a series of water monitoring bores. The gas field has an estimated life of 25 years.

Environmental concerns regarding the NGP were raised during the approvals process for the project. The environmental impact assessment (EIA) for the NGP generated 23,000 submissions to the NSW Government (the highest number for any development in NSW history). The Independent Planning Commission of NSW approved the project on 30 September 2020; however, its report from the further public hearing into the NGP (State of NSW, 2020) specifically listed public concerns over forest fragmentation and the negative impacts of edge effects on biodiversity. The public raised concerns over:

"... fragmentation impacts resulting in detrimental edge effects and increased invasion of native vegetation communities by introduced predators and other pest animals and weeds".

Fragmentation is foremost among the major impacts requiring mitigation and management identified by the EIA process (Appendix J1 of Environmental Impact Assessment, pp 127–9).

Activities undertaken during the NGP will increase the amount of forest fragmentation, creating more patches and increasing the degree of isolation of some patches. The number of patches in the study area covered by the EIA is predicted to increase from 387 to 721. An increase in linear transport infrastructure and habitat clearing potentially threatens the biodiversity of the region. However, development is taking place in a landscape that is already fragmented from other land uses, particularly activities associated with timber harvesting for forestry.



Figure 1.1 Location of the NGP in eastern Australia (from Sreekanth et al., 2018, Figure 1)

1.1.2 The Pilliga Forest

The Pilliga Forest is approximately 535,000 ha in area and constitutes the largest inland plains forest and woodland in eastern Australia. Approximately 50% is managed by the Forestry Corporation of NSW for timber production (Gonsalves et al., 2018), while the remainder is managed for conservation by the National Parks and Wildlife Service (much of this area was also previously managed for timber production). The Pilliga Forest has been important for timber production since the 1800s, especially for the cutting of ironbark trees for railway sleepers and the logging of cypress trees (Law et al., 2018).

The Pilliga Forest is considered to have originally consisted mostly of open woodland with a similar plant species composition to today. The dominant tree species were white cypress (*Callitris glaucophylla*) and narrow-leaved ironbark (*Eucalyptus crebra*) (Binns & Beckers, 2001). An increasing tree density, particularly of white cypress pine, following European settlement of the area appears to have resulted from a combination of changed fire regimes, invasion by the introduced European rabbit (*Oryctolagus cuniculus*) and interactions with drought and flood. The vegetation today is dominated by dense stands of white cypress as well as black cypress (*C. endlicheri*) and buloke (*Allocasuarina luehmannii*), with *Acacia* spp. and narrow-leaved ironbarks scattered throughout the forest. Silvicultural treatment to maximise tree growth for timber production has developed a forest with a range of management histories, including logging and thinning of various ages (Gonsalves et al., 2018; Waters et al., 2018).

Surveys within the NGP have identified 807 species of terrestrial plants (691 of which are native), 22 distinct plant communities and 289 species of terrestrial vertebrates. Of the plants, 10 species were classified as threatened either under the NSW *Threatened Species Conservation Act* and/or the Commonwealth *Environment Protection and Biodiversity Conservation Act* at the time this project commenced (in 2023). Of the vertebrate animals, 27 species were classified as threatened under the *NSW Threatened Species Conservation Act* and four species as threatened under the *Environment Protection and Biodiversity Conservation Act*.



Figure 1.2 Map of the study area showing the outline of the NGP (red)

1.2 Aims of GISERA project B.8

GISERA project B.8 was developed and carried out to provide scientific understanding that addresses the key community concern related to maintaining the intactness of ecosystems and their component species in the Pilliga Forest. Specifically, the research examines whether fragmentation resulting from the NGP will add to the existing impacts of fragmentation on biodiversity, especially the persistence of threatened animals and plants, in the forest. The project also included a component that assessed the diversity of ectomycorrhizal and arbuscular mycorrhizal fungi. This component was included because these organisms are essential for ecosystem functioning in Australian woodlands, and measures of their diversity provide a broader ecological context than the presence of threatened species alone. Most plant species rely on specific associations with fungi called mycorrhiza. The availability of suitable mycorrhizal hosts is important for the distribution of certain species. Fungi produce subsurface (hypogeous) fruiting bodies that are a key food source for ground-dwelling mammals, including threatened species such as bettongs.

The project carried out the following tasks:

- assessed the existing levels of fragmentation of woody vegetation in the NGP area using remote sensing and associated metrics that are based on large datasets and are designed to harness the potential of earth observation science to measure fragmentation at large spatial scales
- undertook on-ground surveys for a subset of threatened animal and plant species and the mycorrhizal symbionts of the plants. The animal species targeted were the paleheaded snake (Hoplocephalus bitorquatus), squirrel glider (Petaurus norfolcensis), eastern pygmy-possum (Cercartetus nanus) and black-striped wallaby (Macropus dorsalis). The plant species targeted were coolabah bertya (Bertya opponens), spiny peppercress (Lepidium aschersonii), winged peppercress (Lepidium monoplocoides), Androcalva procumbens and Vincetoxicum forsteri
- 3. assessed the diversity of ectomycorrhizal and arbuscular mycorrhizal communities, based on soil samples taken during plant surveys, using a DNA-based approach (next-generation sequencing of the internal transcribed spacer region)
- 4. quantified structural attributes of habitat at survey sites at which selected threatened species were present (or absent) using LiDAR from a drone-based platform
- 5. assessed the presence of threatened species in relation to fragmentation metrics and site variables under an occupancy modelling framework
- 6. explored approaches to upscale monitoring to enable remote assessment of environmental variables during the life of the NGP.

1.3 Report outline

The next section consists of an introduction to the study area. It is followed by a section for each of the research components and a final section that presents conclusions, sets out the limitations of the research project and suggests next steps.

The project components are heavily interrelated. The fragmentation and connectivity metrics were used to decide the location of the fauna survey sites. Soil and root samples taken at the plant survey sites were used to assess the presence and diversity of mycorrhizal symbionts. The results of the threatened species surveys dictated the choice of sites for the

LiDAR-derived quantification of structural habitat metrics that in turn were used for the occupancy modelling.

Despite this interdependency, each component is presented as a standalone section in this report with a brief introduction, a detailed method and often a combined results and discussion. Some sections go into more detail and for those components, the results of which form part of a subsequent component (e.g. the LiDAR-derived quantification of structural habitat metrics, section 6), the standalone results are not presented. However, these data are available for use by the research community and more broadly through CSIRO's Data Access Portal (DAP). Likewise, the satellite-based fragmentation and connectivity metrics (section 3) cover the entire project area of the NGP and are potentially valuable for a range of uses well beyond this project. All of these datasets are available for subsequent use through CSIRO's DAP.

2 General methods

In this section we provide a background to the study area, the project timelines (both procedural and in terms of fieldwork completion), the historical climate and the climate conditions experienced during the life of the project. The specifics of the methods undertaken during each of the individual project components are provided in the respective sections.

2.1 Study area

The project was undertaken entirely within the NGP in northern NSW. The project area of 95,000 ha consists mostly of state forest (60%), private land and a small area of state conservation reserve (see section 1.1).

A brief summary of the geographical coverage of the NGP area for each project component is provided here. Refer to Figure 1.2 for the location of these sites.

- Quantification of fragmentation and connectivity metrics of woody vegetation based on remotely sensed data was undertaken for the entire NGP and also considered a wider spatial extent (see section 3).
- Surveys of threatened vertebrates were mostly focused on state forest within the NGP area. The 30 survey sites that underwent repeat surveys were located in Jacks Creek, Bibblewindi and Pilliga East state forests in the south and south-east of the NGP (see section 4). Other surveys were undertaken in brigalow (*Acacia harpophylla*) vegetation to search for black-striped wallabies in the vicinity of Brigalow State Conservation Area and Brigalow Nature Reserve in the north-west of the NGP area. A limited amount of additional surveys were undertaken in and around Yarrie Lake Flora and Fauna Reserve (in the extreme north-west of the NGP, 28 km west of Narrabri). This is a Crown Reserve that is managed by a volunteer Trust Board. The Yarrie Lake fieldwork involved spotlighting (3 November 2023) and night drive transects (18 December 2023).
- Surveys for threatened plants took place in state forest (Jacks Creek, Bibblewindi and Pilliga East state forests), in and around Brigalow State Conservation Area and Brigalow Nature Reserve (for *L. aschersonii*) and along roadside areas elsewhere within the NGP (see section 5). Assessment of ectomycorrhizal and arbuscular mycorrhizal communities was based on soil and root samples taken during the plant surveys (see section 9).
- The assessment of structural attributes of species habitat using LiDAR took place at fauna and flora survey sites located within state forest (see section 7).

2.2 Project timeline

2.2.1 Approvals

Project B.8 was approved by GISERA's NSW Research Advisory Committee (RAC) in December 2022. The project commenced in early 2023, with a period of 6 months set aside to obtain ethics and access approvals and permissions. The date on which approval was granted from each entity and the date of submission of each application are given below. This information is provided for transparency and to show how administrative requirements influenced the timeline of fieldwork.

- Animal Ethics Approval (2023–23) was granted on 23 October 2023 (application submitted on 4 July 2023) by the CSIRO Wildlife, Large and Laboratory Animals Animal Ethics Committee (CWLLA AEC). This approval covered the surveys for threatened vertebrates.
- The Scientific Licence (SL102835) from the NSW Department of Climate Change, Energy, the Environment and Water was approved on 21 February 2024 (application submitted on 11 August 2023). This permit covered research on all threatened species, access to the National Park estate (Brigalow State Conservation Area, Brigalow Nature Reserve, Pilliga East CCA Zone 3 State Conservation Area) and collecting and exporting (to Queensland) soil samples.
- The Forest Permit Research (permit to enter state forests for flora and fauna survey activities) from the Forestry Corporation of NSW for wildlife surveys was approved on 22 September 2023 (application submitted on 4 September 2023). The approval was for Jacks Creek, Bibblewindi and Pilliga East state forests (Figure 2.1). This permit included all on-ground aspects of the research including wildlife surveys and drone flights.

2.2.2 Fieldwork

A preliminary visit to scope potential field sites was undertaken from 21 to 25 August 2023. Fieldwork commenced on 1 November 2023 and continued until 4 October 2024. Seven separate field trips were undertaken during this time.



Figure 2.1 Project area (horizontal grey lines) within the state forest areas of the Pilliga Forest

2.3 Climate

2.3.1 Climate history

Rainfall totals are available from Narrabri Airport (BOM station number: 54038, 30.32° S, 149.83° E) for the period 2001–2024. Annual rainfall during this period averaged 580.6 mm with extremes of 206.2 mm (in 2019) and 896.0 mm (in 2021). This rainfall is similar to that for a much longer term weather station at Narrabri – Mollee (BOM station number: 053026, 30.26° S, 149.68° E) that is 16 km to the west-north-west of Narrabri Airport AWS (further from the study area). Annual average rainfall over a 99-year period (1926–2024) at Narrabri – Mollee is 605.7 mm.

Rain falls across the year with a moderate summer bias. Most rain (58%, based on monthly rainfall averages) falls in the five months from November to March. The wettest months (mean monthly rainfall at Narrabri Airport AWS) are November (76.6 mm), December (68.3 mm), January (66.6 mm), March (65.4 mm) and February (58.3 mm).

The annual mean temperature range at Narrabri Airport AWS for the period 2001–2024 was 12.2–26.9°C. The hottest month is January with a mean maximum of 35.0°C and the coldest month is July with a mean minimum of 4.1°C.

2.3.2 Climate during the project

The four years leading up to the project included the record low rainfall year of 2019 (206.2 mm) followed by well-above average rainfall years in 2020 (771.2 mm), 2021 (896.0 mm) and 2022 (806.4 mm). The project took place over two years that were close to average in terms of rainfall: 511.6 mm (2023) and 639.4 mm (2024).

2.4 Wildfire

2.4.1 Fire history

The Pilliga Forest periodically experiences large-scale wildfires and has done so since at least 1950. Prior to this, for many years Indigenous peoples followed a process of controlled burns, before Europeans arrived in the 1830s and began to manage the land and reduce the incidence of fire. From the 1830s to the 1880s fires were completely excluded and grazing was increased and became extreme during droughts, while perennial grasses declined and eucalypts, cypress pine and shrub regeneration increased (Date et al., 2002). Logging also commenced during this period. The 1890s through to the 1940s was a period that experienced the effects of grazing and digging by a high density of introduced European

rabbits. Simultaneously, regeneration was reduced (except in the south-east Pilliga), grazing by stock decreased and logging increased in frequency, intensity and area (Date et al., 2002).

Date et al. (2002) noted that the state forests of the eastern Pilliga experienced two to six summer wildfires and one to three fuel reduction burns from 1950 to 1991. Since 1950, notable large wildfires have been recorded in the Pilliga Forest in November 1951 (Norris et al., 1991), late 1982/early 1983 when 120,000 ha burnt (Law et al., 2018), December 1997/early 1998 when 144,000 ha burnt (Law et al., 2018), January 2002 (Date et al., 2002) and November to December 2006 when over 120,000 ha burnt (Law et al., 2018).

The 2006 fire was probably sparked by a lightning strike on 28 November and was exacerbated by extremely dry conditions and strong winds the following day. It burnt a large portion of forest in and around the south-west of the Pilliga. Most of the damage was done within the first day of the fire; however, the forest continued to burn for over a month due to two consecutive nights of electrical storms in mid-December (Law et al., 2018).

2.4.2 Wildfire in December 2023

The current study was seriously impacted by an extensive wildfire in December 2023, which commenced on or around 7 December when a lightning strike sparked three fires within the Pilliga Forest. Two fires were contained but the third (now known as the 'Duck Creek fire') continued until at least 23 December and burnt approximately 129,000 ha. The fire burnt extensive areas of the Pilliga East and Bibblewindi state forests (Figure 2.2).

The effects of this wildfire on the project were threefold:

- The project team was in the Pilliga at the time and a short-term issue was that the threatened species could not be fully completed. Only 24 of the 30 sites that were repeatedly sampled during the project were surveyed in December 2023.
- The majority of the project's threatened vertebrate survey sites were burnt in the fire (Figure 2.2). A total of 18 sites were affected. The rationale of our sampling design was to survey in mature vegetation so this became an important issue.
- Public access to the state forests of the Pilliga (including Pilliga East, Bibblewindi and Jacks Creek) was not possible until 23 February 2024. Therefore, the project had an enforced hiatus before the team could revisit and assess the situation on the ground.

A compounding issue was that the wildfire was so extensive that alternative unburnt habitat in locations we were not already sampling was difficult to find. As a consequence, we decided to continue surveys at our existing sites.



Figure 2.2 Map of the Pilliga Forest showing the extent and severity of the wildfire that occurred in December 2023. Blue dots are the project's threatened vertebrate survey sites. (NSW Government SEED website https://www.seed.nsw.gov.au/)

3 Assessment of fragmentation in the NGP area

3.1 Introduction

Fragmentation and connectivity are important topics in conservation science. Fragmentation of habitat can lead to changes in the persistence of species. Forest fragmentation can be a threat to biodiversity. It is a process that involves both habitat loss and a change in the configuration of the remaining habitat. Fragmentation often alters the microenvironment at the edge of the fragment and can result in increased light levels, higher daytime temperatures, greater wind speeds and lower humidity. Each of these edge effects can have a significant impact on the vitality and composition of the species in the fragment (Primack & Morrison, 2013). Species that are sensitive to humidity, such as amphibians, some insects, and herbaceous plants, may be eliminated from fragments.

Although underappreciated, there is a considerable amount of published research that shows that habitat fragmentation can also have positive effects on biodiversity (Fahrig, 2003; 2017; Fahrig et al., 2018). These positive effects include increased functional connectivity resulting from either a larger number of small patches with smaller distances between them and/or a higher edge density facilitating the movement of species that preferentially move along edges; increased landscape complementation with increased fragmentation; and positive edge effects (Fahrig, 2003; 2017). Positive edge effects occur when edges are more productive and structurally diverse than interiors, thus providing higher food availability and lower predation risk. If a species is favoured by edges, then it is favoured by fragmentation because a fragmented landscape contains more edges.

The response of individual species to habitat fragmentation can be difficult to predict because fragmentation is an aggregate process that involves both a decline in the area of habitat and alteration of its spatial configuration (Yeager et al., 2016; Pavey et al., 2021). When considering changes in spatial configuration, a range of attributes have been shown to be important, including patch size (the area of a fragment of potential habitat), patch isolation, the number of patches, matrix (the area between the remaining fragments) quality and edge characteristics.

The connectivity of landscapes provides a measure of the impact of fragmentation. Connectivity describes the degree to which habitats are connected, enabling the movement of species and flow of ecological processes across landscapes.

Multiple approaches have been developed to measure and describe habitat fragmentation. Most approaches are designed to operate at a landscape or patch scale; an example is the FRAGSTATS software program (McGarigal et al., 2023). More recent approaches have sought to develop metrics that harness the potential of earth observation science to measure fragmentation at large scales. FAD is designed to provide a pixel-level measure of fragmentation. It describes the density of a foreground class of interest and was developed to track changes in forest fragmentation in the USA (Riitters & Wickham, 2012). We used FAD to assess fragmentation of the NGP area.

Similarly, there are multiple methods and metrics for describing connectivity and most common approaches are designed to operate at the landscape or patch scale. More recent approaches are aligned with opportunities to harness larger datasets. We used MSPA for this task. It provides a general approach to characterising structure in binary patterns (Vogt et al., 2007; Soille & Vogt, 2009). It has been proposed for several applications, such as landscape analyses of habitat connectivity, segmenting circuit board designs and classifying structural components in medical imagery. MSPA uses mathematical morphometry techniques such as erosion, geodesic dilation, reconstruction by dilation and anchored skeletonisation to classify binary patterns into discrete classes. These classes include cores, edges and bridges that can be used to assist in identifying important habitat patches, patch characteristics and components of the landscape that connect core habitats to one another. The classes that are typically segmented using MSPA are shown in Table 3.1 in section 3.2.3.

3.2 Methods

In this section we outline the establishment of a machine learning model for predicting woody vegetation from satellite data within the study area.

3.2.1 Woody cover mapping

To generate woody cover maps, we used a Random Forest machine learning approach. The model was trained and tested on woody vegetation datasets derived from height and cover metrics generated using all available Global Ecosystem Dynamics Investigation (GEDI) 2A and 2B product observations. These datasets were accessed and processed using Google Earth Engine (Gorelick et al., 2017). Points were rasterised and designated as woody cover if vegetation height (rh98) exceeded 2 m and cover exceeded 10%. Other points were defined as non-woody cover to generate a binary map.

As predictors, 11 bands from the Sentinel-2 multispectral Instrument (MSI) 2A\2B datasets were considered. Winter median composites with a resolution of 10 m were generated for June to August 2022 to mitigate seasonal trends in woody cover. Imagery extraction was conducted for the study region, and cloud removal was performed using the CSIRO's Earth Analytics Science and Innovation (EASI hub) platform. To construct the model's training data, GEDI woody vegetation and satellite datasets were extracted to randomly generated points (N = 10,000), evenly distributed between woody and non-woody classes.

3.2.2 Random Forest modelling

The Random Forest model was trained on 70% of the dataset, with the remaining 30% used for validation. Model hyperparameters were fine-tuned through a grid search involving 20 unique combinations of 'mtry' (number of trees in the forest) and 'min_n' (minimum number of data points in a terminal node). Implementation of models was carried out in R using the 'tidymodels' package, and hyperparameters were tuned using the 'tune' package.

The performance of the final optimised model was evaluated using independent ten-fold validation, achieving an overall accuracy of 0.82, a sensitivity of 0.86 and a specificity of 0.81. This optimised model was used to generate binary maps distinguishing between woody and non-woody cover, forming the foundational data for subsequent fragmentation assessments. Water bodies were masked from the final woody cover maps using a threshold-based approach applied to the Normalised Difference Water Index (NDWI; McFeeters, 1996), which is calculated using the green (G) and near-infrared bands (Equation 1). Derived from the underlying Sentinel imagery and calculated using equation 1, the NDWI classified areas with values of 0 or greater as water (Xu, 2006):

(1)
$$NDWI = \frac{G - NIR}{G + NIR}$$

3.2.3 Fragmentation and connectivity metrics

Fragmentation and connectivity analyses were conducted using the GuidosToolbox (Guidos) Workbench, a Linux-based module (Vogt & Riitters, 2017; Vogt et al. 2022), along with the GuidosToolbox (GTB) desktop application (Vogt & Riitters, 2017), which enabled additional post-processing of network components. The FAD, employed for fragmentation assessment, evaluated the proportion of foreground pixels within a moving window and categorised them on a scale of 1–6 (Figure 3.1). The MSPA, used for connectivity assessment, classified structural patches based on their properties using mathematical morphometry techniques, designating them as background, branch, bridge, core, loop, perforation or islet (Table 3.1).

FAD 6-class	Color	FAD range
1-Rare		FAD < 10%
2-Patchy		10% ≤ FAD < 40%
3-Transitional		40% ≤ FAD < 60%
4-Dominant		60% ≤ FAD < 90%
5-Interior		90% ≤ FAD < 100%
6-Intact		FAD = 100%

Class	Description
Background	Background
Branch	Connected at one end to edge, perforation, bridge or loop
Bridge	Connected to different core areas
Core	Interior area excluding perimeter
Edge	External object perimeter
Islet	Disjoint and too small to contain core
Loop	Connected to the same core area
Perforation	Internal object perimeter

Table 3.1 Pixel classification used for MSPA, resulting in eight distinct classes

3.3 Results and discussion

The map of the project area classified using the FAD metric for fragmentation (Figure 3.2) shows that the northern third of the region contains little woody vegetation (i.e. it is primarily the 'background' FAD class). This land is primarily used for agriculture. The remaining two-thirds of the region is dominated by woody vegetation. The map shows that this vegetation is relatively unfragmented consisting of FAD classes 4–6 (i.e. 'dominant', 'interior' and 'intact'). The FAD value is >60% for all of these classes (Figure 3.1). Large patches of 'intact' vegetation (i.e. FAD value = 100%) are present in the north and east of the region.

The map of the region classified using the MSPA metric for connectivity (Figure 3.3) confirms that the woody vegetation in the study area is mostly unfragmented. The vast majority of the woody vegetation is classified as 'core', meaning that it is continuous. Therefore, the components of the landscape that connect core habitats to one another, such as 'branch', 'bridge' and 'loop', are absent or very rare. These components become more common in the south-west corner of the woody vegetation (Figure 3.3), which corresponds to an area where the FAD is classified as 'dominant' (60–90% FAD); that is, it is not 'intact' (100% FAD) or 'interior' (>90% but <100%).



Figure 3.2 Map of the Pilliga Forest region showing the NGP area (enclosed by the red line) and the distribution of FAD classes



Figure 3.3 Map of the Pilliga Forest region showing the NGP area (enclosed by the red line) and the distribution of MSPA classes

4 Threatened species – vertebrates

4.1 Introduction

Surveys within the NGP have recorded 289 species of terrestrial vertebrates. Of these, 27 were classified as threatened under the NSW *Threatened Species Conservation Act* and four were classified as threatened under the Commonwealth *Environment Protection and Biodiversity Conservation Act* at the time this project commenced (in 2023). Four species were selected as focal species on which to base this component of the study. The species were chosen because they were: (1) considered potentially detectable within the study area during the life of the project; (2) potentially impacted by fragmentation and other threatening processes that may increase during the NGP, including inappropriate fire regimes and weed invasion; and (3) not the focus of existing research. The koala, *Phascolarctos cinereus*, was not selected because of the latter reason.

Each species is briefly introduced below and shown in Figure 4.1.

- The eastern pygmy-possum (*Cercartetus nanus*) is a very small possum reaching a maximum body mass of 43 g. The species occurs in eastern and southern Australia (NSW to the border with Queensland, Victoria, the extreme south-east of South Australia, and Tasmania). It is listed as vulnerable in NSW. It occupies a wide range of vegetation including rainforest, open forest and heath. Eastern pygmy-possums are nocturnal, feeding on nectar in blossoms, fruit and invertebrates. They shelter in tree hollows or build their own nests.
- The black-striped wallaby (*Macropus* (*Notamacropus*) *dorsalis*) is a medium-sized macropod, with the males (body mass 8.7–21 kg) much larger and heavier than the females (5.2–7.6 kg). It is widespread in eastern and central Queensland and occupies a small area of north-eastern NSW. Although common in Queensland it is listed as endangered in NSW. The species occupies forest and woodland areas, including rainforest margins and regrowth, typically with a dense shrub layer. Active at night, it feeds on a range of grasses and forbs.
- The squirrel glider (*Petaurus norfolcensis*) is a gliding possum found in eastern Australia (Queensland, NSW and Victoria) with a body mass up to 300 g. It is listed as vulnerable in NSW. Squirrel gliders live mainly in open forest and woodland. They feed at night on nectar, pollen, plant exudates (sap), invertebrates and honeydew (produced by scale insects). During the day they rest in hollows in trees, especially gums.
- The pale-headed snake (*Hoplocephalus bitorquatus*) is a species of elapid (venomous) snake up to 80 cm in length. It occurs in coastal and interior regions of eastern Queensland and NSW and is listed as vulnerable in NSW. Open forest and woodland areas west of the coastal ranges appear to be core habitat for the species. It shelters

under loose bark or in tree hollows during the day. Active at night, it feeds on frogs and other vertebrates.



Figure 4.1 A. Eastern pygmy-possum; B. black-striped wallaby; C. pale-headed snake; D. squirrel glider (Photo credit, all images: Stewart Macdonald)

4.2 Methods

4.2.1 Survey design

A variety of survey approaches were used. These included night drive transects, night walk transects and active searches during the day.

We chose 30 survey sites within state forests to carry out repeat nocturnal surveys. The sites were selected using the FAD metric that was quantified for the NGP area (see section 3). The 30 sites were selected to encompass as broad a range of FAD values as possible while still covering a manageable area and placing our sites in areas of suitable habitat (e.g. a cleared paddock has a low FAD value but would not be considered suitable habitat for our target fauna species). FAD values across the entire NGP project area ranged from 48% to 100% (note that lower FAD values represent more-fragmented habitat). Our sites spanned FAD values of 72% to 96% (Table 4.1).

Our aim was to survey all 30 sites during each of the four survey periods, which were spread out across seasons. The sampling was influenced by the wildfire of December 2023 (see section 2). As a consequence, the four survey periods were in November 2023, December 2023, May 2024 and June 2024. Only 24 of the 30 sites were surveyed in December 2023. Details of the sites and sampling are provided in Table 4.1.

4.2.2 Survey methods

Surveys were conducted by two people walking along a road-based transect, using headtorches and thermal scopes to detect animals. From a starting point on the road, the observers walked in opposite directions for 15 mins, then returned to the starting location over the course of another 15 mins (Figure 4.2). This gave a transect duration of 30 mins for each observer, for a total of one person-hour per site per survey. The first transect started just after sunset each night, at approximately 20:00 hours during summer and 19:00 hours during winter. Five to six transects were completed each night.

Site number	FAD value	Location		Notes
1	92.70	-30.453102	149.753539	
2	89.00	-30 478970	149 768345	
2	03.00	20.514046	140.000000	
3	84.98	-30.514046	149.830083	
4	90.23	-30.504208	149.783023	
5	92.21	-30.467580	149.748456	
6	85.79	-30.495973	149.815958	

Table 4.1 Details of the survey sites including the FAD value
7	87.91	-30.531678	149.796385	
8	95.63	-30.522110	149.781193	
9	88.94	-30.494633	149.789538	
10	86.47	-30.539561	149.812632	
11	94.38	-30.530521	149.776997	
12	87.45	-30.533556	149.830310	
13	88.84	-30.714063	149.609233	Not surveyed in December 2023
14				Site not used
15	79.46	-30.709711	149.643612	Not surveyed in December 2023
16	85.36	-30.714337	149.622094	Not surveyed in December 2023
17	85.08	-30.692005	149.604281	Not surveyed in December 2023
18	87.42	-30.712154	149.585270	
19	92.65	-30.696076	149.752782	
20	72.23	-30.684712	149.752694	
21	77.51	-30.663435	149.707817	
22	81.77	-30.621459	149.687888	
23	79.16	-30.645724	149.673816	
24	90.24	-30.666146	149.682684	
25	85.91	-30.719584	149.696061	
26	79.49	-30.695209	149.704202	
27	77.54	-30.662480	149.644444	
28	88.94	-30.526609	149.826956	
29	78.83	-30.681568	149.627923	Not surveyed in December 2023
30	81.31	-30.725190	149.657714	Not surveyed in December 2023
31	81.46	-30.697775	149.797582	



Figure 4.2 A sample survey transect: starting on the road at site 27, one observer walked north-east and recorded the pink track, while the second observer walked south-west and recorded the green track.

Headtorches worn by experienced observers allow the user to detect animals by 'eyeshine' – the bright reflection of the light bouncing off the animal's eyes and back to the observer. This technique is very effective for detecting nocturnal mammals such as possums, gliders and wallabies; nocturnal birds such as owls and nightjars; and nocturnal reptiles and amphibians such as geckos and frogs. The following data were recorded using mobile devices during the surveys: transect start and stop times; transect GPS track; and timestamp, location and species for all vertebrates detected.

4.3 Results and discussion

In this section we examine the results of the surveys for the four focal vertebrate species. We also describe the occurrence of all vertebrate species during the repeat sampling of the 30 survey sites. Analysis of the data within an occupancy modelling framework is covered in section 7.

4.3.1 Focal vertebrate species

During the project, two of the four target species were located. However, only one of these – the eastern pygmy-possum – was present on the sites that were repeatedly sampled. The black-striped wallaby was not detected at any of the 30 sites that were repeatedly sampled.

Multiple individual, black-striped wallabies were recorded at Brigalow Nature Reserve during a spotlight walk transect on 3 November 2023. The species was also observed further west within the NGP area in the vicinity of Yarrie Lake during a nocturnal drive transect on 1 December 2023. A road-killed individual was also seen that night. The remaining brigalow (*Acacia harpophylla*) vegetation that dominates the remnant patches in the north-west of the NGP area represents ideal habitat for the black-striped wallaby.

Eastern pygmy-possums were observed within the 30-site survey network at sites 3, 19, 20 and 30. Individuals were seen only during the November 2023 and June 2024 survey periods.

A large number of gliders in the genus *Petaurus* were seen across nine of the repeat survey sites. Each individual was carefully examined if possible, given the viewing conditions, and identified to species. In each case where the animal was seen well enough, the identification was as a member of the sugar glider (*Petaurus breviceps*) complex, most likely Krefft's glider (*Petaurus notatus*). No squirrel gliders were seen during the surveys.

The pale-headed snake was not recorded during the surveys.

4.3.2 Other species

During the repeat surveys at our 30 sites we identified 93 species, including outside of sampling times and while driving between sites. In total, 43 species were detected during the actual surveys and deemed 'of interest' (i.e. species such as resting diurnal species were not considered further). The number of sites at which each species was detected (combined for the four survey periods) varied dramatically across species (Figure 4.3).

Only one species, the eastern spiny-tailed gecko (*Strophurus williamsi*), was recorded at all survey sites. Six other species were observed at more than 50% of the survey sites: two more geckoes (eastern stone gecko, *Diplodactylus vittatus*; and *Gehyra dubia*), two nocturnal birds (Australian owlet-nightjar, *Aegotheles cristatus*; and tawny frogmouth, *Podargus strigoides*), one frog (Sudell's frog, *Neobatrachus sudelli*) and one mammal (eastern grey kangaroo, *Macropus giganteus*).



Figure 4.3 The number of sites (out of 30) where each species was detected over the course of the four survey periods. Only one species (the eastern spiny-tailed gecko, *Strophurus williamsi*) was detected at all 30 sites.

5 Threatened species – plants

5.1 Introduction

A total of 10 species of plants that occur within the Pilliga Forest were classified as threatened under either the NSW *Threatened Species Conservation Act* and/or the Commonwealth *Environment Protection and Biodiversity Conservation Act* when this project was developed in 2022. On-ground surveys were undertaken for five of these species during the project. The species were coolabah bertya (*B. opponens*), spiny peppercress (*L. aschersonii*), winged peppercress (*L. monoplocoides*), *A. procumbens* and *V. forsteri*. The species were chosen because they were: (1) considered likely to be detectable within the study area during the life of the project; (2) listed as threatened at both the state and national level; and (3) potentially impacted by fragmentation and other threatening processes that may increase during the life of the NGP, including inappropriate fire regimes and weed invasion. Each species is briefly introduced below.

B. opponens (Euphorbiaceae) is a slender shrub or small tree that grows up to 4 m high and occurs in eastern NSW and Queensland (Figure 5.1). It is listed as vulnerable both nationally (*Environment Protection and Biodiversity Conservation Act*) and in NSW (*Threatened Species Conservation Act*). It is a fire-sensitive species (i.e. it is killed by fire) that is an obligate seeder. It germinates following other forms of disturbance such as road clearing. It is threatened by inappropriate fire regimes, habitat clearance and browsing by goats. A large population occurs in Jacks Creek State Forest within the NGP area.

L. aschersonii (Brassicaceae) is a small, perennial herb growing to 30 cm that occurs in NSW, Victoria and Western Australia (Figure 5.1). It is listed as vulnerable both nationally (*Environment Protection and Biodiversity Conservation Act*) and in NSW (*Threatened Species Conservation Act*). It grows on heavy clay soils in areas that are seasonally flooded or prone to waterlogging, such as gilgai depressions and the margins of freshwater and saline marshes and shallow lakes. It is usually associated with brigalow vegetation. It is threatened by weed invasion, grazing, altered hydrology and habitat clearance (Carter, 2010). The Brigalow Park Nature Reserve within the NGP has a large – and potentially the largest known – population of this species (Carter, 2010).

L. monoplocoides (Brassicaceae) is a small, annual herb growing to 20 cm that occurs in inland south-eastern Australia (Victoria and NSW) (Figure 5.1). It is listed as endangered both nationally (*Environment Protection and Biodiversity Conservation Act*) and in NSW (*Threatened Species Conservation Act*). It typically grows in open woodland with a tussock grass understorey on heavy clay or clay-loam soils, usually in areas that are seasonally flooded or prone to waterlogging. It is threatened by weed invasion, grazing and altered

hydrology (Mavromihalis, 2010). A small number of individuals were recorded in the NGP during the EIA process (Appendix J2 of Environmental Impact Assessment).



Figure 5.1 Images of the five focal plant species: (a) and (b) Androcalva procumbens; (c) Vincetoxicum forsteri; (d) and (e) coolabah bertya; (f) and (g) winged peppercress; and (h) spiny peppercress. (Photo credit, all images: Bruce Murray) A. procumbens (formerly Commersonia procumbens) (Malvaceae) is a prostrate shrub with slender trailing stems (Figure 5.1). It is endemic to NSW. It is listed as vulnerable both nationally (*Environment Protection and Biodiversity Conservation Act*) and in NSW (*Threatened Species Conservation Act*). It is threatened by inappropriate fire regimes, habitat clearance and invasion by woody shrubs. In the NGP area, populations occur in the Pilliga Nature Reserve and Pilliga East State Forest.

V. forsteri (formerly *Tylophora linearis*) (Apocynaceae) is a herbaceous climber that grows to about 2 m long (Figure 5.1). It occurs throughout inland central NSW and into southern Queensland. It is listed as endangered nationally (*Environment Protection and Biodiversity Conservation Act*) and as vulnerable in NSW (*Threatened Species Conservation Act*). It grows in open forest and woodland at altitudes of 300–400 m above sea level. Threats include weed invasion, grazing and inappropriate fire regimes. The species is widespread within the NGP area.

5.2 Methods

Surveys of threatened plants were undertaken during two time periods, November 2023 (pre-fire) and October 2024 (post-fire). In addition, opportunistic surveys for *V. forsteri* were carried out in June 2024 during vertebrate fauna surveys.

Threatened plant surveys included both quick searches of areas where focal species had previously been recorded and more rigorous random walk surveys within 100 x 100 m plots at sites corresponding to the fauna survey sites. These random walk surveys were generally longer than 900 m.

At each site, the following information was collected:

- GPS coordinates
- dominant species in the tallest stratum
- vegetation community (based on the Specht classification)
- soil colour.

The survey methods varied with species and these are outlined below.

5.2.1 B. opponens

Preliminary searches for *B. opponens* were undertaken in locations within the study area where the species had previously been recorded that were also vertebrate survey sites (sites 3, 4, 6, 8, 10, 12 and 28).

Belt transects were set up at sites where the species was located and individuals were counted in assigned categories (seedling, flowering adult or non-flowering adult). At two of the sites (12 and 28), three 100 m x 2 m belt transects were set up on each site. At site 3, a

901 x 2 m random walk belt transect was used to provide a better representation of the site as a whole. This was because the density was lower and patchier than at the other two sites so using three belt transects seemed unlikely to provide a true representation of abundance. Visual inspection of the extent of the population was undertaken from the road to facilitate an estimation of the population size for this species in the area.

5.2.2 L. monoplocoides

Preliminary searches for *L. monoplocoides* were undertaken along roadsides in locations within the study area where the species had previously been recorded. Once the species was located, all individuals in an area were counted in assigned categories (seedling, flowering adult or non-flowering adult). Searches were terminated when no new individuals could be found within at least 50 m of a *L. monoplocoides* individual. Given that the population was growing along a roadside and next to private property (where the land use was cropping), the search was only bidirectional.

Many of the locations where the species has been recorded previously within the NGP area are on private property. These sites were not surveyed during this project.

5.2.3 L. aschersonii

A total of four targeted random walk transects were undertaken in November 2023 at sites where the species had previously been located. One transect (4,488 m in length) was in Brigalow State Conservation Area, two transects (4,344 m and 2,394 m in length) were in Brigalow Park Nature Reserve and another transect (579 m in length) was adjacent to the highway. Searches were undertaken again for the species in Brigalow State Conservation Area and Brigalow Nature Reserve in October 2024.

5.2.4 A. procumbens

Rapid searches were carried out at all four sites within the NGP area where *A. procumbens* had previously been recorded (based on records in the *Atlas of Living Australia*). Two sites were in open forest or woodland along roadsides. Once the species was located, all individuals in an area were counted in assigned categories (seedling, flowering adult or non-flowering adult). Searches continued for up to 50 m from where the last individual was recorded until no further individuals were located.

GPS points were recorded for each individual or group of individuals (a group being defined as individuals within 5 m of each other).

5.2.5 V. forsteri

Numerous rapid searches were carried out at locations within the NGP area where *V. forsteri* had previously been recorded (based on records in the *Atlas of Living Australia*). These were mostly undertaken in November 2023 but two additional surveys were completed in October 2024. In addition, random walk transects were carried out at several fauna survey sites (14, 22 and 24) in November 2024 corresponding to areas where *V. forsteri* had been previously identified. At these sites, random walk surveys of at least 900 m were carried out within a 100 × 100 m² plot.

5.3 Results and discussion

During the course of the project, all five target species were located. Below we detail the results of the surveys for each species.

5.3.1 B. opponens

The species was located at three of the seven fauna survey sites that were searched (sites 3, 12 and 28). A total of seven belt transects were completed (Table 5.1), and seedlings and flowering adults were present on all transects. The total density (seedlings and adults) ranged from 0.2 to 1.92 individuals/m² across the transects.

Variable	Site 3		Site 12		Site 28				
		1	2	3	1	2	3		
Transect length (m)	901	100	100	100	100	100	100		
Transect width (m)	2	2	2	2	2	2	2		
Transect area (m ²)	1802	200	200	200	200	200	200		
Number of seedlings	249	100	48	87	100	48	0		
Number of flowering adults	102	56	63	120	31	77	79		
Number of non-fruiting/flowering adults	10	0	0	0	0	0	0		
Total number of individuals	361	383	245	225	131	125	166		
Total density of individuals (/m ²)	0.20	1.92	1.23	1.13	0.66	063	0.83		

Table 5.1 Details of belt transects and number of seedling, flowering and fruiting *B. opponens* in the study area. Site numbers correspond to vertebrate survey sites.

5.3.2 L. monoplocoides

The species was found at a single location along the roadside at a known site, Wilga Power Station.

5.3.3 L. aschersonii

No individuals were found during targeted surveys in November 2023. This absence was unexpected given that it is a perennial and the large population size in Brigalow Nature Reserve reported previously. Two contrasting explanations might account for its absence during this period. Firstly, conditions were relatively dry at the time of survey so individuals might have perished. However, the previous 3 years had well above average rainfall (see section 3). Secondly, it is possible that flooding during this period resulted in its absence, as there is anecdotal evidence that the species can disappear for extended periods following inundation by floodwater.

By comparison, *L. aschersonii* was abundant in Brigalow State Conservation Area and Brigalow Nature Reserve in October 2024.

5.3.4 A. procumbens

A. procumbens was located at three of the four sites where it had previously been recorded, including two roadside locations. None of these sites correspond to the vertebrate survey sites. Seedlings, flowering adults and non-reproductive adults were seen at the site with the largest number of plants (83 individuals were detected here), but only two individuals were found at the second site and only four were found at the third site. While most individuals were identified along roadsides, numerous individuals were observed at greater distances from the road while undertaking UAV-LiDAR surveys in October 2024. It is likely that these individuals were able to capitalise on the increase in bare ground resulting from the extensive wildfire in December 2023.

5.3.5 V. forsteri

No *V. forsteri* individuals were identified during rapid searches or transect surveys in November 2023 and October 2024. However, the species was located during opportunistic surveys in June 2024.

6 LiDAR-derived structural attributes of the habitat

6.1 Introduction

We adopted a LiDAR remote sensing approach to quantify the structural attributes of a subset of survey sites within the Pilliga study area. LiDAR uses laser pulses to measure the distance of objects from the laser instrument. It is analogous to radar but uses laser light rather than radio waves. The advent of laser scanning has enabled examination of the three-dimensional (3D) structure of vegetation, meaning we can estimate important parameters including forest height, stand volume and biomass (Dubayah & Drake, 2000).

As well as ecosystem-scale understanding, LiDAR approaches are used to assess habitat and environmental relationships of single species, both plants and animals. Assessment of the 3D structure of vegetation represents a fundamental physical attribute of wildlife habitat. LiDAR can supply detailed information about the vertical and horizontal structure of vegetation elements and their spatial variability. As a consequence, LiDAR has become prominent in wildlife habitat assessment (Lefsky et al., 2002).

Broadly, there are three approaches to using LiDAR to obtain information on vegetation structure (for a recent summary, see Cosgrove et al., 2024):

- Ground-based LiDAR consists of mobile laser scanning and terrestrial laser scanning. Terrestrial laser scanning provides the highest resolution LiDAR data for use in wildliferelated research. However, it can only be used over a limited scale, generally not more than several hectares (Calders et al., 2020). Calders et al. (2020) describe the two main approaches to analysing the data produced: (1) gap probability methods, which are used to estimate plant area index or leaf area index of forest stands; and (2) geometrical modelling, which allows reconstruction of individual tree structure.
- Airborne laser scanning increases the scale over which high-resolution data can be obtained. Until recently, airborne laser scanning was undertaken from crewed aircraft (airplanes, helicopters); however, increasingly flights are now undertaken by drones (UAV-LiDAR). The structural attributes of sites can be quantified using UAV-LiDAR scanning with a survey-grade multi-angle UAV-LiDAR sensor, enabling this method to approach terrestrial laser scanning in terms of structural detail but operating over larger spatial scales (Reckziegel et al., 2025).
- Space-based laser scanning can scan transects over massive areas of the globe but it typically captures relatively low structural detail.

We used UAV-LiDAR to acquire data on vegetation structure at 15 sites (Figure 6.1) from June to October 2024.



Figure 6.1 Location of UAV-LiDAR survey sites within the study region

6.2 Methods

In this section we describe the site selection and outline the approaches taken to acquire and process UAV-LiDAR data in the Pilliga Forest.

6.2.1 Site selection

The 15 sites were chosen to provide high-quality data for the occupancy modelling of wildlife (see section 7). All the vertebrate survey sites where the eastern pygmy-possum was observed (sites 3, 19, 20 and 30) were included, as were a series of eight vertebrate survey sites where no focal species was detected (sites 6, 8, 10, 12, 15, 25, 26 and 28). In addition,

two sites where *A. procumbens* was recorded and an additional site where surveys confirmed that it was absent were sampled.

The UAV-LiDAR data were collected from June to October 2024. This timing meant that flights took place after the large wildfire in December 2023 (see section 2). As most vertebrate and plant sampling sites had been burnt in the fire, the availability of relatively intact sites from which to select was limited. This factor influenced site selection and the number of sites that were flown.

6.2.2 Data acquisition

UAV-LiDAR data were acquired using an Acecore Noa hexacopter fitted with a RIEGL surveygrade VUX-120 laser scanner (Figure 6.2). The VUX-120 has a ranging precision of 5 mm. It uses a forward-nadir-backward scan pattern, alternating between scanning at -10° from the surface directly below the sensor (nadir), the nadir and $+10^{\circ}$ from the nadir, which increases the probability of receiving laser returns from vertical surfaces, as shown in Figure 6.2. The VUX-120 sensor was operated at a scanning rate of 1,200 kHz with a line speed of 291 lines per second.

Each UAV flight covered an area of 15 ha, flown at an altitude of 80 m above ground level using a cross-hatched east-west/north-south pattern (Figure 6.3). Surveys were conducted at a speed of 5.3 ms⁻¹ and with a line spacing of 60 m (Figure 6.3), with total flight times ranging between 24 and 28 mins. A field base station, consisting of a Leica GS16 Global Navigation Satellite System (GNSS) receiver mounted on a tripod, logged data over a 2-hour period, including the flight duration.



Figure 6.2 Example of the forward-nadir-backward scan pattern adopted by the VUX-120 laser scanner, showing how it is optimised for data collection of vertical surfaces (adapted from the RIEGL VUX-120 manual by RIEGL Laser Measurement Systems GmbH, 2021)



Figure 6.3 Example of a canopy height model derived from a UAV-LiDAR survey. Cross-hatch lines: north-south/east-west flight patterns; grey lines: processed flight line data; red lines: discarded lower quality data collected in turns and in transit.

6.2.3 Data processing

The GNSS logs collected by the base station were processed using Geoscience Australia's online GPS processing service, AUSPOS (version 2.4). AUSPOS uses up to 15 nearby reference stations from the International GNSS Service and the Asia-Pacific Reference Frame Network to perform double differencing. This method calculates the difference between signals received from two satellites at two different receivers, reducing the influence of clock and atmospheric errors and ensuring high coordinate precision.

These precise coordinates were then used to refine the raw flight path data collected by the APX-20 Inertial Measurement Unit and GPS onboard the UAV. This refinement was performed using the Trimble Applanix POSPac UAV Suite 9.1 software, which specialises in accurately processing and adjusting UAV trajectories.

Raw UAV-LS flight lines were processed along with corrected trajectory data using RIEGL's RiPROCESS software suite. First, we clipped the flight lines using the RXP cutter tool to

remove lower quality data collected during flight turns or while transiting to the planned mission (shown in red in Figure 6.3). Next, we performed strip alignment using RIEGL's RiPrecision tool to reduce systematic errors and biases in overlapping flight lines, which may arise from variations in GNSS signal quality and flight conditions. The adjusted flight lines were then exported, and their alignment was fine-tuned using BayesStripAlign 2.24 software, which uses Bayesian inference using errors as Bayesian parameters to increase data accuracy.

To produce the final LiDAR datasets, we performed ground detection to classify points representing the underlying ground. This classification allowed us to normalise the point clouds to reflect their height above the ground. We then removed any points that were either below ground level or above 40 m to eliminate errors and irrelevant data, which may arise from interactions with moisture in the atmosphere or birds in flight.

6.2.4 Point cloud analysis

Plot-level structural metrics capturing key characteristics of structural diversity were calculated using the R statistical modelling environment (v. 4.3.2) with the lidR package (Roussel et al., 2020) and its companion package, lidRmetrics (Tompalski, 2024). These included:

- Descriptive statistics to assess the vertical height patterns, including the mean, maximum, minimum, standard deviation, coefficient of variation, kurtosis and skewness. Height percentiles were calculated (1st, 5th–95th in 5% increments, and 99th), along with the percentage of points exceeding specific height thresholds (mean, 2m and 5m). Additionally, we considered cumulative height metrics, which were calculated by dividing heights into 10 equal intervals and determining the cumulative proportion of returns for each interval (1–10), as described by Woods et al. (2008).
- Canopy arrangement metrics to assess the position and distribution of physical structures within the canopy. These included the canopy relief ratio, interquartile range, entropy, vertical complexity index, and mean absolute deviations from the mean and median, and leaf area density (LAD) profile metrics (LAD mean, maximum, minimum, sum and coefficient of variation). We also calculated volume-based metrics using 1 m voxels (3D rectangular prisms equivalent to a pixel). These included the ratio of filled to non-filled voxels, the ratio of filled to non-filled voxels relative to the height of the canopy, and metrics such as vertical surface roughness or rumple, vertical standard deviation and vertical coefficient of variation.
- **Canopy heterogeneity metrics** to capture canopy surface variability. These included the top rugosity, which assesses variations in surface height, and the rumple index, which

assesses surface roughness using the ratio between the projected area and the ground, and Jenness's algorithm (Jenness, 2004).

Canopy cover metrics to describe the distribution of canopy cover, defined as the projected area of the ground covered by the canopy. We calculated cover at increasing height thresholds (1–25 m in 5 m increments) using a canopy height model generated at 0.5 m resolution.

6.3 Results and discussion

Figure 6.4 shows the level of detail available in a typical cross-section from one of the transects flown in this study. This figure uses the viridis colour scale, which is designed to improve readability for users with common forms of colour blindness and/or colour vision deficiency. It clearly differentiates the height strata in the forest.



Figure 6.4 Cross-section view through a 60 m transect of high-density UAV-LiDAR point cloud (8,000 points/m²)

A comparison of foliage projected cover and canopy height across 12 of the 15 sites (Figure 6.5) shows considerable variation.



Figure 6.5 Overview of the structural variability within and between research plots. The left-hand component of the violin plots represents foliage projected cover (FPC), and the right-hand component represents canopy height (CHM).

More than 100 plot-level structural metrics were calculated for each of the 15 sites using this approach. We discuss these data in section 7.

7 Species occupancy and habitat relationships

7.1 Introduction

In this section we explore the relationship between the occurrence of the target vertebrate and plant species and a range of environmental attributes. Specifically, we examine the effect of environmental variables on the occupancy of species. We analyse the data generated in sections 3–6 with the overall aim of identifying any species that may experience a population-level response to additional forest fragmentation resulting from the NGP.

7.2 Methods

In this section we discuss the methods used to carry out occupancy modelling, including the selection of habitat structural variables to include in the modelling. The methods related to each of the preceding components of the project – fragmentation metrics, vertebrate and plant surveys, and LiDAR-derived structural variables – are covered in sections 3–6.

7.2.1 Habitat structural characterisation

We used two broad types of environmental attributes:

- Survey-level attributes vary over time at the same location (e.g. weather, observer, survey method). Statistical modelling is used to determine the effect that these attributes have on the detectability of the focal species. For example, reptiles may be more detectable in warmer weather.
- Site-level attributes do not vary over the timescale of the surveys (e.g. habitat structure variables, such as fragmentation). Modelling is used to determine the effect of these attributes on the occupancy of a site by the focal species. For example, a species that shelters under fallen woody debris is more likely to present at a site with high levels of structural complexity on the ground.

Survey-level attributes

Weather data were obtained from the Bureau of Meteorology. In an effort to assess seasonal effects on detectability, maximum daily temperature was used as a survey-level covariate in occupancy modelling.

Site-level attributes

The project used remote sensing technology (i.e. satellite imagery and drone-based sensors) to determine the environmental characteristics of the study sites. This was done at two spatial scales:

- At the landscape-scale, the FAD (a habitat fragmentation index) was calculated using satellite imagery.
- At the site-scale, UAV-LiDAR was used to calculate 3D habitat structural metrics. LiDAR data were not collected from all sites. After completing the wildlife survey work, a subset of sites was selected for structural attributes to be assessed by UAV-LiDAR (Table 7.1).

Site number	FAD value	Vertebrate survey	Plant survey	UAV-LIDAR	Other name
1	92.70	V			
2	89.00	V			
3	84.98	V	٧	٧	
4	90.23	V	٧		
5	92.21	V			
6	85.79	V	٧	٧	
7	87.91	V			
8	95.63	V	٧	٧	
9	88.94	V			
10	86.47	V	٧	٧	
11	94.38	V			
12	87.45	V	٧	٧	
13	88.84	V			
15	79.46	V		٧	
16	85.36	V			
17	85.08	V			
18	87.42	V			
19	92.65	V		V	
20	72.23	V		٧	

Table 7.1 List of sites used in occupancy modelling. Sites 101–103 are separate sites surveyed for *A. procumbens*.

21	77.51	V			
22	81.77	٧			
23	79.16	٧			
24	90.24	V			
25	85.91	V		V	
26	79.49	V		V	
27	77.54	V			
28	88.94	V	V	V	
29	78.83	V			
30	81.31	V		V	
31	81.46	V			
101	81.57		V	V	C. pro pres 1
102	83.31		V	V	C. pro pres 2
103	83.46		V	٧	C. pro abs 1

'UAV-LiDAR' indicates that UAV-LiDAR structural habitat data were collected.

7.2.2 Occupancy modelling — threatened vertebrates

Our aim was to use remotely-sensed habitat metrics (satellite-based and drone-based) to predict whether a site was likely to be occupied by our focal species of threatened vertebrates.

We took an occupancy modelling approach using the *unmarked* package (Fiske & Chandler, 2011; Kellner et al., 2023) in the R statistical programming environment (R Core Team 2021). Occupancy modelling is essentially a generalised linear model that relates environmental variables to the presence or absence of a focal species, with an additional detection function that accounts for imperfect detectability of the species. Occupancy modelling uses site-based attributes to model occupancy and survey-based attributes to model detectability. The site-based attributes were the satellite-derived FAD value and the multiple metrics derived from UAV-LiDAR. The survey-based attributes were maximum daily temperature for the date on which the site was surveyed. Both groups of covariates were scaled (such that their mean was 0 and their standard deviation was 1) to better help the occupancy model converge.

Repeated surveys at the same site are a core component of determining detectability. The intention was to survey each site four times over the course of approximately 8 months (from November 2023 to June 2024) to achieve seasonal coverage. One of the key assumptions of occupancy modelling is that the study area is a closed system. This means

that a species' occupancy of a site does not change over the course of the project, even if it is not detected on all surveys. For example, if a species is not detected at a site on any surveys, we assume that it does not occur at that site. If a species is detected at a site on the first survey, but is not detected on any subsequent surveys, we assume that it was present the entire time but that it went undetected after the initial survey. A site is deemed 'occupied' by a species if it is detected on any survey of that site. A species that was detected at a site on all four surveys has a higher detectability than a species that was detected at a site on only one survey and went undetected on the other three surveys.

7.2.3 Occupancy modelling — threatened plants

Because structured repeat surveys were not conducted for the focal plant species, we took a more traditional approach and fitted generalised linear models to assess the relationship between site occupancy and environment for two of the five species: *B. opponens* and *A. procumbens*. The environmental variables used were the FAD value and five LiDAR-derived covariates. Note that this approach does not assess detectability.

7.2.4 Selection of LiDAR-derived variables

The post-processing pipeline produced 115 variables from the raw LiDAR data (section 6). A subset of only five was chosen for use in occupancy modelling. The selection process was based on both preliminary exploratory analysis and the expert opinion of the authors in relation to the biology of the focal species. The first three variables are canopy arrangement metrics and the final two are canopy heterogeneity metrics:

- **Canopy relief ratio (CRR)** measures the vertical variation or roughness of a forest canopy. It describes the degree to which canopy surfaces are in the upper (CRR >0.5) or lower (CRR <0.5) portions of the height range.
- Mean leaf area density (lad_mean) is the mean of leaf area density across all voxels (a voxel is a 3D grid of the canopy).
- Vertical complexity index (VCI) is complex and is defined as a fixed normalisation of entropy across user-defined height bins. Understory complexity approaches 1.0 as vegetation structure becomes more homogeneously distributed throughout the forest strata, and approaches 0.0 as it becomes more heterogeneous, or stratified.
- **Rugosity** is the standard deviation of rasterised maximum canopy height (derived from the canopy height model).
- **Rumple** is the ratio of the outer canopy surface to the ground surface (i.e. a measure of 3D canopy roughness).

7.3 Results

7.3.1 Habitat fragmentation index

The satellite-derived FAD values across the entire NGP area ranged from 48% to 100%. The 30 survey sites were at the higher end of this range spanning FAD value from 72% to 96% (Figure 7.1).



Foreground Area Density (FAD) values for fauna monitoring sites

Figure 7.1 FAD values for each of the 30 vertebrate monitoring sites; higher values represent more intact (i.e. less fragmented) habitat

7.3.2 Occupancy modelling — vertebrates

The detection histories for these 30 vertebrate species of interest are shown in Table 7.2. Detection histories are used to determine the detectability of a species. If a species was never detected at a site on any of the four surveys, its detection history will be 0000. If it was found at the site on all four surveys, its detection history will be 1111. Those sites that went unsurveyed due to fires in the second survey period will have an x in position two to indicate no data (e.g. 1x01).

One species of gecko, the eastern spiny-tailed gecko (*Strophurus williamsi*) (Figure 7.2), was detected at every site and was seen on three or four out of four surveys (excluding site 29, which was not surveyed in December 2023). Given that it was detected at every site, occupancy modelling for this species is of little value. The graph for likelihood of occupancy against FAD value shows a flat line — all levels of FAD are conducive to occupancy for this species (Figure 7.3).

The next most common species was another gecko, the eastern stone gecko (*Diplodactylus vittatus*) (Figure 7.2). While this species was not detected at every site, it was detected at most sites and those sites spanned a wide range of FAD values. As such, the graph for likelihood of occupancy against FAD value is, similarly, not very informative (Figure 7.4). However, as a reptile (with activity levels that are likely to be linked to temperature), it is interesting to look at how environmental temperatures might influence its detectability. Figure 7.5 illustrates the likelihood of detection against the maximum daily temperature and shows a clear increase in detectability with increasing temperature. A similar detectability pattern is seen with another gecko, *Gehyra versicolor* (Figure 7.6).

	Site																													
Species	01	02	03	04	05	06	07	08	09	10	11	12	13	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Aegotheles cristatus	1000	0110	0000	0111	1110	1000	1111	0011	0111	1011	0011	1100	0x10	0x00	0x00	0x01	0100	1100	0000	0100	0001	0001	0100	0000	0100	0000	1110	0x00	0x01	1101
Antechinus flavipes	0010	0100	0100	0000	0110	0000	0000	0100	0000	0000	0000	0000	0x10	0x00	1x00	0x00	0010	0000	0000	0000	0000	0000	0010	0000	0000	0000	0010	0x00	0x00	0000
Cercartetus nanus	0000	0000	1000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0x00	0x00	0x00	0x00	0000	1001	1001	0000	0000	0000	0000	0000	0000	0000	0000	0x00	1x00	0000
Diplodactylus vittatus	1110	1100	1100	1110	1000	1010	1100	1110	0010	0110	1110	1100	1x10	0x10	1x10	0x00	1110	1110	0111	1110	1110	0000	0000	1100	0100	0010	1000	1x10	0x10	0111
Felis catus	0000	0000	0000	0000	0000	0000	0100	0000	0000	0000	0000	0000	0x00	0x00	0x00	0x00	0000	0000	0000	0100	0000	0000	0000	0000	0000	0000	0000	0x01	0x00	0000
Gehyra dubia	1010	1000	0000	0100	1000	1000	1100	1100	1100	1100	0100	0100	1x00	1x00	1x00	1x00	0100	0000	0100	0000	0100	1100	0000	1000	1110	1001	0000	0x00	1x00	0000
Heteronotia binoei	0000	0000	1000	0000	0000	0000	0000	0000	0000	0100	0000	0000	0x00	0x00	0x00	0x00	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0100	0x00	1x00	0000
Lepus capensis	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0x00	0x00	0x00	0x00	0001	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0x00	0x00	0000
Limnodynastes dumerilii	0000	0000	0000	0000	1000	0000	0000	0000	0000	0000	0000	0000	0x00	0x00	0x00	0x00	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0x00	0x00	0000
Limnodynastes grayi	0010	0000	0010	0010	0010	0010	0000	0000	0000	0000	0000	0000	0x00	0x00	0x00	0x00	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0x00	0x00	0000
Limno. terraereginae	0000	0010	0000	0010	0000	0010	0000	0010	0010	0000	0000	0000	0x10	0x00	0x00	0x00	0000	0000	0010	0000	0000	0000	0000	0000	0000	0000	0010	0x00	0x00	0000
Litoria caerulea	1000	0000	0000	0000	1000	0000	0100	0000	0000	0000	1100	0000	0x00	0x00	1x00	0x00	1000	0000	0000	0000	0100	0100	0000	1010	0000	0000	0000	1x00	0x00	0000
Macropus giganteus	0100	0010	0000	0001	0000	0000	0000	0001	0000	0100	0000	0000	0x01	0x00	0x11	0x10	1000	0000	0000	0010	0001	0011	0000	0000	0000	0100	0000	0x10	0x00	0011
Macropus robustus	0000	0000	0000	0000	0000	0100	0000	0000	0000	0000	0000	0000	0x00	0x00	0x00	0x00	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0x00	0x00	0000
Macropus rufogriseus	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0x00	0x00	0x00	0x00	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0x00	0x00	0001
Neobatrachus sudelli	0000	0010	0000	0010	0010	0010	0010	0010	0010	0000	0010	0000	0x10	0x00	0x00	0x01	0000	0010	0011	0000	0010	0000	0000	0010	0010	0000	0000	0x11	0x00	0000
Ninox novaeseelandiae	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0001	0000	0x00	0x00	0x00	0x00	0000	0000	0100	0000	0000	0000	0000	0000	0000	0000	0000	0x00	0x00	0000
Oedura monilis	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0x00	0x00	0x00	0x00	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0x00	0x00	0100
Petaurus notatus	0000	0100	0000	0000	0000	1110	0010	0001	0000	0000	0000	0000	0x00	0x00	0x00	0x00	0111	0000	0000	0000	1010	0000	0000	0000	0000	1110	0000	0x00	1x00	1000
Platyplectrum omatum	1000	1000	0100	0000	0000	0000	0010	0000	0000	0000	0000	0000	0x10	0x00	0x00	0x00	0000	0000	0100	0000	0010	0000	0000	0000	0000	0000	0000	0x00	1x10	0000
Podargus strigoides	1000	1011	0010	0000	1000	0000	0010	1010	0000	1010	1110	0100	0x00	1x00	1x01	1x01	0000	0001	0000	0000	0010	1100	0000	0000	0000	0000	0100	0x00	0x00	0000
Pseudechis guttatus	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0x00	0x00	0x00	0x00	0000	0000	0000	0000	0100	0000	0000	0000	0000	0000	0000	0x00	0x00	0000
Pseudomys pilligaensis	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0x11	0x11	0x11	0x00	0000	0000	0010	0000	0000	0010	0000	0000	0000	0000	0000	0x10	0x01	0010
Saccolaimus flaviventris	1000	0000	0000	0000	1000	0000	0000	0000	0000	0000	1000	0000	0x00	0x00	0x00	0x00	0000	0000	0000	0000	0000	1000	0000	0000	0000	0000	0000	0x00	0x00	0100
Sminthopsis murina	0000	0000	0000	0000	0000	0000	0000	0000	0010	0000	0000	0000	0x00	0x00	0x00	0x00	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0x00	0x00	0000
Strophurus williamsi	1111	1111	1111	1111	1110	1111	1111	1111	1111	1111	1111	1111	1x11	1x11	1x11	1x11	1110	1111	1111	1111	1110	1110	1111	1111	1111	1110	1111	1x10	1x11	1111
Tachyglossus aculeatus	0000	0000	0000	0000	0000	0000	0000	0000	0001	0000	0000	0000	0x00	0x00	0x00	0x00	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0x00	0x00	0001
Trichosurus vulpecula	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0x00	0x00	0x00	0x00	0000	0000	0000	0000	0000	0000	0000	0000	0000	0010	0000	0x00	0x00	0000
Turnix varius	0000	0000	0000	0000	0000	0000	0000	0000	0100	0000	0000	0000	0x00	0x00	0x00	0x00	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0x00	0x00	0000
Wallabia bicolor	0000	0000	0000	0000	0000	0000	0000	0000	0000	0100	0000	0000	0x00	0x01	0x00	0x00	0000	0000	0000	0000	0000	0000	0000	0001	0000	0000	0000	0x00	0x00	0000

Table 7.2 Detection histories for vertebrate species from four survey periods across 30 sites

A '1' in the first position means the species was detected at that site during the first survey (and so on). A '0' in the second position means the species was not detected on the second survey (and so on). An 'x' in the second position means that site was not surveyed in the December 2023 period due to intense wildfire. Shades of green represent the number of surveys on which the species was detected at that site: white for never detected, up to dark green for detection on all four surveys.



Figure 7.2 Two commonly observed species of lizard: eastern spiny-tailed gecko (on left); eastern stone gecko (on right) (Photo credit: Stewart McDonald)



Figure 7.3 The likelihood of occupancy for the eastern spiny-tailed gecko (*Strophurus williamsi*) as a function of FAD. The species occurred at all sites, so the model is uninformative. Black dots represent the actual FAD values for sites where the species was detected.



Figure 7.4 The likelihood of occupancy for the eastern stone gecko (*Diplodactylus vittatus*) as a function of FAD. The species occurred at most sites and across a wide range of FAD values, so the model is uninformative. The grey shaded area represents confidence intervals (and spans the entire likelihood range of 0–1). Black dots represent the actual FAD values for occupied sites.



Figure 7.5 The effect of maximum daily temperature on the detectability of the eastern stone gecko (*Diplodactylus vittatus*). Detectability increases with increasing temperature. The grey shaded area represents confidence intervals. Black dots represent the actual temperature for surveys in which this species was detected.



Figure 7.6 The effect of maximum daily temperature on the detectability of *Gehyra versicolor*. Detectability increases with increasing temperature. The grey shaded area represents confidence intervals. Black dots represent the actual temperature for surveys in which this species was detected.

7.3.3 Occupancy modelling for focal vertebrates

Of the focal vertebrates, only the eastern pygmy-possum (*C. nanus*) was recorded during the repeat surveys of the 30 sites (Table 7.3). It was detected during two survey periods (November 2023 and June 2024) and at four sites (3, 19, 20 and 30).

Survey period	Site number	Time stamp
November 2023	20	2023-11-02 21:14:08
November 2023	19	2023-11-02 22:23:00
November 2023	30	2023-11-04 20:12:32
November 2023	03	2023-11-07 22:18:25
June 2024	20	2024-06-26 19:24:58
June 2024	19	2024-06-26 20:00:37

Table 7.3 Summary of eastern pygmy-possum (C. nanus) sightings during the surveys

We used the four eastern pygmy-possum 'presence' sites and the remaining 26 'absence' sites to model occupancy as a function of FAD, and to model detectability as a function of maximum temperature on the day on which the survey was conducted. While this modelling shows a trend towards lower occupancy at higher FAD values (Figure 7.7), the confidence intervals are so wide that it is unwise to put any faith in this trend. Likewise, while there is a trend for detectability to increase with increasing temperature (Figure 7.7), the model fit is too poor to be of use.



Figure 7.7 Modelled likelihood of occupancy of the eastern pygmy-possum (*C. nanus*) as a function of FAD (upper graph). Modelled detectability as a function of daily maximum temperature (lower graph). The grey shaded area represents confidence intervals.

A second occupancy model was fitted, looking at the relationship between the occupancy of the eastern pygmy-possum and the five LIDAR variables for the 12 vertebrate survey sites from which they were collected (Table 7.4). The data for each variable across the 12 sites are given in Table 7.4. While all five variables were included in a single model (Table 7.5), the variables are plotted on separate graphs (Figure 7.8). Once again, maximum daily temperature was included as a detection covariate. Modelling showed no clear effect for any of the LIDAR-derived metrics on occupancy for the eastern pygmy-possum (Table 7.5). While estimates were obtained from the model, the very high p-values (p >0.84 for all variables, whereas statistical significance would be indicated by p <0.05) indicate that the model had low explanatory power.

	Species	presence/a	absence		L	IDAR metrics		
Site number	Cer nan	Com pro	Ber opp	VCI	CRR	Mean LAD	Rugosity	Rumple
3	1	0	1	0.64	0.16	0.06	3.81	3.94
6	0			0.78	0.15	0.04	5.01	4.79
8		0	0	0.80	0.10	0.04	4.97	5.30
10		0	0	0.75	0.16	0.04	6.26	6.17
12				0.67	0.10	0.04	4.18	4.43
15	0			0.44	0.05	0.01	5.47	4.24
19	1	0	1	0.65	0.09	0.03	4.33	4.03
20	1			0.49	0.04	0.01	3.25	3.47
25	0			0.45	0.06	0.03	2.16	3.87
26	0			0.60	0.06	0.02	4.97	4.67
28		0	1	0.69	0.20	0.08	3.84	3.95
30	1			0.72	0.12	0.03	5.81	4.85
101		1	0	0.71	0.10	0.03	4.70	5.12
102		1	0	0.48	0.03	0.01	2.96	3.32
103		0	0	0.69	0.10	0.03	4.44	5.15

Table 7.4 Values of the five LiDAR-derived structural metrics across 15 sites

1 = site was surveyed and species was detected; 0 = site was surveyed but species was not detected.

Cer nan = eastern pygmy-possum (*C. nanus*); *Com pro* = *A. procumbens*; *Ber opp* = coolabah bertya (*B. opponens*)

Table 7.5 Results for the occupancy model for the eastern pygmy-possum (*C. nanus*) using LiDAR-derived metrics

Variable	Estimate	Std Err	Z	р
(Intercept)	86.67	438	0.19788	0.843
VCI	-7.47	772	-0.00968	0.992
CRR	-26.14	2789	-0.00937	0.993
Mean LAD	-14.87	4916	-0.00302	0.998
Rugosity	16.85	108	0.15655	0.876
Rumple	-35.57	191	-0.18600	0.852



Figure 7.8 Modelled likelihood of occupancy of the eastern pygmy-possum (*C. nanus*) versus (upper graph)) rumple and (lower graph) VCI. The grey shaded area represents confidence intervals, which span the entire range from 0–1, indicating a very poor model fit.

7.3.4 Cluster analysis for focal vertebrates

Multidimensional scaling was used to assess the similarity of the LIDAR-derived metrics for the sites where the eastern pygmy-possum was present or absent (Figure 7.9). Site similarity is based on all 115 LIDAR-derived habitat structural metrics. Based on this limited sample size, there is a slight trend for the present sites (green circles) to be clustered together more centrally, with most absent sites (red circles) grouped to the top-left of the present sites in ordination space.





Figure 7.9 Cluster analysis using multidimensional scaling for eight sites where the eastern pygmypossum (*C. nanus*) is present (green circles) or absent (red circles)

7.3.5 Occupancy modelling — focal plants

Generalised linear models were fitted for the two target flora species that were found at or near our standard fauna survey sites: *B. opponens* and *A. procumbens*. For both species, the models

assessed presence/absence as a function of habitat fragmentation (FAD value) or habitat structure (VCI, CRR, mean LAD, rugosity, rumple).

As with the vertebrates, the models were uninformative for both *B. opponens* (Figure 7.10) and *A. procumbens* (Figure 7.11).



Figure 7.10 Occupancy of coolabah bertya (*B. opponens*) as a function of FAD value. The grey shaded area represents confidence intervals. Black dots represent results from survey data.


Figure 7.11 Occupancy of *A. procumbens* as a function of the CRR. The grey shaded area represents confidence intervals. Black dots represent results from survey data.

7.4 Discussion

A key aim of the project was to see whether habitat fragmentation (measured with the FAD metric) and structural characteristics of vegetation measured with satellite imagery and UAV-LiDAR can be used to predict the presence of focal plant and animal species. For the purposes of our project, the focal species were all classified as threatened at either the state or national level (or both). The species consisted of five plants and four vertebrates. In addition, we considered an additional 29 vertebrate species (none listed as threatened) in our analysis. These species were regularly recorded during our repeat nocturnal surveys at 30 sites.

The analyses we undertook did not find statistically meaningful relationships between the presence/absence of the focal (or additional) species and the FAD value or any of the structural attributes measured. There are several potential explanations that could account for the lack of a fragmentation effect from our data.

One is that FAD has no effect on the occupancy of these species (i.e. the metric may not accurately represent habitat fragmentation from the perspective of the focal species). Potentially a different habitat-fragmentation metric may give different results.

Another is that the quality and quantity of our threatened wildlife data were not sufficient to find such a signal, irrespective of the structural detail obtained. There were certainly weaknesses with our sampling intensity and the number of records obtained. Specifically, for the vertebrates it is debatable whether the 'absence' sites used in occupancy modelling were definitely not occupied by those species. More intense sampling with the addition of camera and box/pitfall trapping could increase confidence in the 'absence' of eastern pygmy-possums from sites. A positive in terms of sampling effectiveness is that our nocturnal walking transects incorporated the use of thermal scopes and were carried out by observers experienced in their use. Most sightings of pygmy-possums were by thermal scope.

An additional issue, entirely unexpected at the outset of the study, was the influence of the massive wildfire (the Duck Creek fire) in December 2023. This occurred in the middle of our field campaign. The fire covered approximately 129,000 ha and burnt most of our survey sites and a significant amount of the Pilliga Forest as a whole (~25%). The effect of the fire on occupancy of sites and the quality of habitat available at most sites and in surrounding areas is expected to be significant. This factor is likely to have confounded our results.

However, probably the most important explanation for the absence of a fragmentation effect is that, from a biological perspective, the wildlife present in the Pilliga Forest are species that can cope with the level of fragmentation that has occurred. The species that persist must be able to cope with the changes in habitat availability and structure that have taken place since European settlement. It is also important to state that the level of fragmentation in the Pilliga Forest is relatively low on an absolute scale. For the first time, this study has quantified fragmentation (and connectivity) across the entire Pilliga Forest (and NGP area and beyond) using remote sensing in combination with specific analysis tools designed for use with very large datasets. The quantification of fragmentation using the FAD metric showed that across the NGP area, values

ranged from 48% to 100% (with lower FAD values representing more-fragmented vegetation) and across the 30 survey sites FAD values ranged from 72% to 96%.

Our survey results do show an effect of temperature on the detectability of various gecko species, but not on any mammal species (including the eastern pygmy-possum). This is not surprising, as the activity levels of ectotherms (cold-blooded animals, such as reptiles) are known to be influenced by temperature. Eastern pygmy-possums are a mammal (an endotherm) with a very small body mass (up to 43 g). During times of environmental stress, including extremes of cold and heat, food shortages and during fires, mammals are known to enter a state of torpor, when activity is stopped and metabolic rate and body temperature are reduced (Geiser, 2021).

8 Potential to upscale monitoring

8.1 Background

UAV-LiDAR is an emerging tool for local-scale characterisation of habitat structure and dynamics, providing structural metrics and insights that cannot be measured through traditional fieldwork. However, given the large extent of the NGP area, there is scope to consider upscaling the UAV-LiDAR structural metrics to larger areas using Synthetic Aperture Radar (SAR) for measuring and monitoring the structure of forest habitat. This section explores this possibility. The analysis made use of C-band Sentinel-1 data (10 m spatial resolution, 10–15-day revisit time), which is sensitive to vegetation structural elements. Plans to also use L-band data from NASA's NISAR mission did not eventuate as the launch date for that mission was delayed until February/March 2025. The Sentinel-1 based analyses were trained and validated with the UAV-LiDAR data collected for the habitat structural assessments (see section 6).

8.2 Approach

High-resolution UAV-LiDAR data was acquired at 15 field sites within the broader Pilliga study region in July and September 2024 (Figure 8.1). The collection and processing parameters are detailed in section 6. The UAV surveys spanned a diverse range of vegetation structures and included locations that were both affected by the large wildfire in December 2023.

Sentinel-1 images that intersected the broader study region were downloaded from the Copernicus Australasia Regional Data Hub and processed within CSIRO's Earth Analytics and Innovation Platform (EASI). Images were processed through to Radiometrically Terrain Corrected (RTC) backscatter values (Gamma naught) at 20 m spatial resolution, for the VV and VH polarisations (VV = vertical transmit and receive; VH = vertical transmit and horizontal receive). The Copernicus 30 m Digital Elevation Model (DEM) was used for the underlying terrain correction. In total 72 scenes intersected the study region for the 2023–2024 calendar years. Given that the UAV-LiDAR surveys were conducted from July to September 2024, we processed the SAR images collected during these months for our analyses. We also processed the 2023 images for these same months to enable pre- and post-fire comparison under similar phenological conditions. The median composites (July/August/September) for 2023 (Figure 8.2) and 2024 (Figure 8.3) show very clear delineation of forested and cleared/cultivated landscapes, with the fire scars from the December 2023 wildfire prominent in the southern extents of the forest in the 2024 composite.



Figure 8.1 Location of the 15 UAV-LiDAR field sites (green squares) within the broader Pilliga Forest landscape



Figure 8.2 Sentinel-1 median composite image (Jul–Sept 2023); RGB (red, blue, green) rendered on dB scale (R = VV, G = VH, B = VV:VH).



Figure 8.3 Sentinel-1 median composite image (Jul–Sept 2024); RGB (red, green. Blue) rendered on dB scale (R = VV, G = VH, B = VV:VH).

Our aim was to understand how well complex structural metrics, obtained from high-resolution UAV-LiDAR, could be represented by readily available and open access SAR imagery. As such, we generated the suite of structural metrics covered in section 6, for each pixel of the Sentinel-1 SAR imagery that intersected our UAV-LiDAR flights. For each of the UAV-LiDAR derived structural metrics, we assessed the pattern and strength of correlation with the VV and VH backscatter intensities, and the VV:VH ratio.

8.3 Results

The results showed that while certain structural metrics were clearly positively correlated with VV and VH backscatter, there was a lot of spread in the data and the relationships were often strongly bimodal, with high and low clusters forming. Using Leaf Area Index (LAI) as an example (Figure 8.4), we observe that while LAI generally increases with the strength of VV and VH backscatter intensity, there are two prominent clusters at ~0.4 and ~1.25 LAI. We see a similar pattern in the relationship between backscatter intensity and canopy density (Figure 8.5). This indicates that while the C-band of Sentinel-1 (5.6 cm wavelength) is useful for distinguishing between areas of dense and sparse canopy, it might struggle to differentiate among more subtle variations in leaf area or canopy density.



Figure 8.4 Relationship between Sentinel-1 backscatter intensity variables and UAV-LiDAR derived LAI: (a) VV; (b) VH; and (c) VV:VH. Kernel density overlay indicates the percentile distribution of data.



Figure 8.5 Relationship between Sentinel-1 backscatter intensity variables and UAV-LiDAR derived LAI: (a) VV; (b) VH; and (c) VV:VH. Kernel density overlay indicates the percentile distribution of data.

Vegetation canopy height is one of the more commonly used vegetation structural metrics and is important from both forestry and ecological perspectives. At the 20 m spatial scale of our analysis, we found that Sentinel-1 showed sensitivity to the 50th and 75th height percentiles (Figure 8.6a and Figure 8.6b), but the signal becomes quite saturated by the 95th height percentile (Figure 8.6c).

Relationships between UAV-LiDAR metrics and Sentinel-1 backscatter varied slightly among the different polaritons (VV, VH and VV:VH). Given that these relationships were not simply linear, we used a Random Forest machine learning approach to model the relationship between each UAV-LiDAR metric and the combined effects of VV, VH and VV:VH. The modelling was conducted on CSIRO's EASI cloud computing platform, using the scikit-learn Python package. A 70/30 test-train split was used for model building and evaluation.



Figure 8.6 The relationship between Sentinel-1 VH backscatter intensity variables and UAV-LiDAR derived height percentiles (20 m pixels): (a) 50th percentile; (b) 75th percentile; and (c) 95th percentile. Kernel density overlay indicates the percentile distribution of data.

Spatial outputs from the Random Forest models provide an example of how UAV-LiDAR, which is high in structural detail but low in spatial coverage, can be used to inform the modelling of structural metrics over larger areas with satellite imagery (Figure 8.7). Statistical comparison of the

observed (UAV-LiDAR derived) versus predicted (Sentinel-1 modelled) metrics used was based on 30% of the data that were not used in the model training. Using LAI as an example, the Sentinel-1 data were able to account for 56% of the variation in the UAV-LiDAR metric, with an RMSE of 0.33 (Figure 8.8). Comparison of the model trendline (red) and the 1:1 line (black) indicates overprediction of lower-end values and underprediction at the upper-end of the value range.



Figure 8.7 Spatial predictions of habitat structural metrics from Sentinel-1 radar imagery, trained with UAV-LiDAR data: (a) seasonal composite (July–September 2024) of VH backscatter; (b) LAI; (c) canopy density; and (d) 75th canopy height percentile. Note the prominence of the fire scar in the south-western quadrat of the images.



Figure 8.8 Observed vs predicted results from Random Forest modelling of LAI as a function of Sentinel-1 backscatter variables (VV:VH). Model trained with UAV-LiDAR data. Kernel density overlay indicates the percentile distribution of data.

8.4 Conclusion

Sentinel-1 backscatter intensity showed varying degrees of correlation with structural metrics derived from our UAV-LiDAR surveys. The bimodal clustering evident in most relationships indicates that the C-band (5.6 cm) wavelength of Sentinel-1 is sensitive to high and low values of the structural metrics but has limited ability to distinguish more subtle variations or changes in these metrics. Random Forest modelling was effective for mapping spatial predictions of the UAV-LiDAR derived metrics over the extent of the Sentinel-1 imagery, but tended to overpredict at the lower end of the metric range and underpredict at the upper end. The Sentinel-1 imagery tested was typically able to account for at least 50% of the variation in the UAV-LiDAR metrics that we explored. Given that Sentinel-1 data are openly available for the entire Australian continent, these results are very encouraging for the use of satellite data in modelling habitat structure over larger areas.

Important to note is that we only tested the use of backscatter intensity in this study. More complex processing of the radar signal, such as polarimetric decomposition and temporal coherence, might further improve these results, as would including additional satellite data (e.g. optical reflectance from Landsat-8/-9 or Sentinel-2) and ancillary data (e.g. terrain metrics). We were not able to test the use of NASA's NISAR L-band (24 cm wavelength) as initially planned due to launch delays. However, if this system is successful, it will provide open access to L-band data over the whole country. The longer wavelength of L-band can penetrate more deeply into forests than C-band and is more sensitive to larger vegetative elements such as tree trunks. The fusion of

C-band and L-band radar should provide a more holistic representation of forest structure than what either technology can achieve independently.

The use of Sentinel-1 data in biodiversity research is gaining momentum but has seen limited use in Australian ecosystems. Foundational work by Bae et al. (2019) showed that Sentinel-1 C-band data could be used to predict the diversity of 12 taxa in German forested ecosystems. A critical component of their study was the availability of expansive on-ground biodiversity data. Our work under this component indicates that Sentinel-1 imagery can be used to estimate complex vegetation structural metrics over landscape scales. Uncertainty in these predictions can be reduced through the inclusion of larger area training data, and through the integration of additional data sources in the modelling process. However, direct links between Sentinel-1 and faunal/botanical diversity will always be challenging to establish due to the limitation of sample sizes in most on-ground biodiversity studies. The use of airborne or UAV-LiDAR as an intermediate tool for capturing high-resolution vegetation structural detail will continue to be critical for bridging these scales.

9 Mycorrhizal communities

9.1 Introduction to mycorrhizal biology

Mycorrhizas are the symbiotic association between fungi and plant roots that play a vital role in the nutrient uptake of plants. The term 'mycorrhiza' is derived from the Greek words *mycos* (fungus) and *rhiza* (root).

Mycorrhizal symbiosis generally facilitates the transfer of nutrients (particularly phosphorus and nitrogen) from the soil to the host plant by the fungus in exchange for plant photosynthate (sugars) (Bücking, 2012). These associations are generally very advantageous to the plant, and much of the phosphorus and nitrogen in Australian soils is held in organic forms that are otherwise inaccessible to plants.

The formation of mycorrhizas is critical to plant health, enhancing nutrient absorption and protecting plants from pathogens and environmental stresses.

There are several major types of mycorrhiza, each characterised by distinct modes of interaction with plant roots. We focus on ectomycorrhizas and arbuscular mycorrhizas, but also introduce the other types of mycorrhiza.

9.1.1 Ectomycorrhizas

Ectomycorrhizas form a sheath around the exterior of roots, known as the mantle, and extend hyphal networks into the soil, facilitating nutrient absorption (Tedersoo et al., 2010) (Figure 9.1). Many common tree species in the Narrabri region (e.g. *Eucalyptus, Angophora, Callitris* and *Casuarina*) can form ectomycorrhizal associations. The fungal partners in ectomycorrhizal associations are mostly basidiomycetes (members of the fungal phylum, Basidiomycota), although some other phyla (e.g. Ascomycota) may be involved.



Figure 9.1 Examples of ectomycorrhizal root tips (photos courtesy of Marc-André Selosse)

9.1.2 Arbuscular mycorrhizas

Arbuscular mycorrhizas (also known as AM or the more general name, endomycorrhiza) are the most common type of mycorrhiza (occurring in a vast number of plant families). They penetrate the root cell walls, but not the cell membrane, and form highly branched structures called arbuscules and circular structures called vesicles (Smith & Read, 2008). Fungi that form arbuscular mycorrhizas are exclusively from the Glomeromycota. Like ectomycorrhizas, arbuscular mycorrhiza associations enhance plant nutrition and may play other roles in disease prevention and avoiding water stress (Wahab et al., 2023).

9.1.3 Other types of mycorrhiza

The other major types of mycorrhiza, including ericoid mycorrhizas and orchid mycorrhizas, tend to be limited to specific groups of plants.

For ericoid mycorrhizas, this association is mostly limited to plants in the family Ericaceae. Species from this family are widespread in the study region. For example, reports on flora from the Pilliga Nature Reserve on the southern boundary of the NGP include various *Leucopogon* species, urn heath (*Melichrus urceolatus*) and pink five-corners (*Styphelia trifloral*) (Office of Environment and Heritage, 2015), though no threatened ericaceous species are known from the region.¹ Fungi that form ericoid mycorrhizas with Australian Ericaceae are distinct from those found in the northern hemisphere (Cairney & Ashford, 2002; Midgley et al., 2016; 2017), with the most common species in Australia being *Gamarada debralockiae* (Midgley et al., 2018). The formation of ericoid

¹ NSW Office of Environment and Heritage. Threatened Species found in Pilliga IBRA sub-region https://threatenedspecies.bionet.nsw.gov.au/cmaSearchResults?SubCmaId=260

mycorrhizas is regarded as critical for the success of the family in edaphically stressful conditions (Cairney & Meharg, 2003).

Unlike other mycorrhizas, orchid mycorrhizas (which only occur in the family Orchidaceae) function very differently. Typically these partnerships are less mutualistic, with the orchid receiving organic carbon from the fungus (the reverse situation from all other mycorrhizas), and there is generally less evidence that the orchid provides the fungus with significant advantage in return (Dearnaley, 2007). Typical orchid mycorrhizal fungi in Australia include a range of *Ceratobasidium/Rhizoctonia* species (Perkins et al., 1995; Batty et al., 2002). Like ericaceous plants, no threatened orchid species are known in the region.

9.1.4 Mycorrhizal complexity

While it is tempting to simplify mycorrhizal associations as being associated with one plant group (e.g. trees) or plant family (in the case of the Ericaceae or Orchidaceae), the reality is more complex. Plants can associate with multiple fungi in multiple ways. For example, single plants are known to associate with diverse species of fungi within the same mycorrhizal type (e.g. Midgley et al., 2002; 2004; Bahram et al., 2011) or even with multiple mycorrhizal types (e.g. Chen et al., 2000). Furthermore, plants known to engage in one type of association sometimes appear to engage in relationships with unexpected groups. For example, McGee (1986) demonstrated that at least one ericaceous plant (an *Astroloma* species) in a semi-arid woodland in South Australia formed ectomycorrhizas along with several plants that had vesicles (a structure formed by arbuscular mycorrhizas) in plant species that were thought to be free of mycorrhizas. Conversely, Chambers et al. (2008) demonstrated that ericoid mycorrhizal fungal species associated with a range of non-ericaceous host plants.

It should be noted that for some species, the mycorrhizal associates of individual plants are highly specific, while at other times these are highly variable – and probably serendipitous – relationships.

9.1.5 Mycorrhizal fungal and non-mycorrhizal fungal fruiting bodies as food

Mycophagy, 'the eating of fungi', is common in the environment (Santamaria et al., 2023). It can refer to consumption of mycelium (the tube-like structures of which most fungi are comprised), but in this study we specifically focus on consumption of fungal 'fruiting bodies' called sporocarps. These are the 'mushroom' like structures that many will be familiar with, although sporocarps come in a range of 'less-mushroomy' forms (Figure 9.2).

Numerous fauna depend on fungi as a primary or supplementary food resource, thereby influencing fungal reproduction and ecological interactions. That fungal fruiting bodies form important food sources for some mammals is well established. For a detailed review, refer to Elliot et al. (2022). Mycophagy encompasses both epigeous fungi, which form fruiting bodies above ground, and hypogeous fungi, which form the fruiting bodies below ground.



Figure 9.2 Examples of sporocarps: (a) a typical mushroom form; (b) bracket fungi; (c) a puffball/truffle form that fruits below ground; (d) a clathrate (net-like) fruiting body; (e) an earth star

Numerous Australian mammals are known to use fungal sporocarps as food, including the longnosed potoroo (*Potorous tridactylus*), southern brown bandicoot (*Isoodon obesulus*), Pilliga mouse (*Pseudomys pilligaensis*) and southern bush rat (*Rattus fuscipes*) (Claridge & Cork, 1994; Tory et al., 1997; Nguyen, 2000; Claridge & Trappe, 2004; Tokushima & Jarman, 2010). Many (but not all) of these fungi are mycorrhizal. For example, the Pilliga mouse has been shown to feed on fungal spores from arbuscular mycorrhizas (Tokushima & Jarman, 2010).

9.1.6 Mycorrhizas in target plant species

In our study, the target plant species were *A. procumbens* (Malvaceae), *B. opponens* (Euphorbiaceae), *L. ascherosonii*, *L. monoplocoides* (Brassicaceae) and *V. forsteri* (Apocynaceae). The mycorrhizal associates of these individual species, if they occur, are unknown.

Androcalva – family Malvaceae

There do not appear to be any reports of mycorrhizas in the genus *Androcalva*. The Malvaceae family are known to form arbuscular mycorrhizas (Peterson et al., 1985; Brundrett et al., 1996; O'Connor et al., 2001).

Bertya – family Euphorbiaceae

There are no mycorrhizal records for family *Bertya*, though in Australia the Euphorbiaceae is mostly recorded as forming arbuscular mycorrhizas (Peterson et al. 1985). While Warcup (1980) reported ectomycorrhizal formation by Australian Euphorbiaceae, this plant (*Poranthera microphylla*) was later moved into a different family (the Phyllanthaceae). Warcup's discussion of Euphorbiaceae also reports *Uapaca togoensis* as ectomycorrhizal, but like *Poranthera* this genus was later moved to the Phyllanthaceae.

Lepidium – family Brassicaceae

The Brassicaceae are perhaps the most 'accepted' non-mycorrhizal family of plants. This is discussed at some length in Trautwig et al. (2023), including increasing reports of the plant species within this family forming mycorrhizas. Relatively few *Lepidium* species have been examined in Australia and the one that has, *L. virginicum* (on Heron Island, Great Barrier Reef), appears to form arbuscular mycorrhizas (Peterson et al., 1985). Other *Lepidium* species including *L. bonariense*, *L. bidentatum* and *L. didymium* were also shown to form these associations, although these plants were not in Australia (Koske et al., 1992; Massenssini et al., 2014; Yaseen et al., 2020).

Vincetoxicum - family Apocynaceae

Vincetoxicum species in Australia have received little attention, with no species having been examined in Australia. In contrast, other non-Australian *Vincetoxicum* species have been determined to form arbuscular mycorrhizas (Smith et al., 2008).

9.2 Methods

9.2.1 Samples

As part of the flora sampling for this project, plant roots from selected species were collected for mycorrhizal examination. In addition, loose rhizosphere soil was collected as an indication of fungal communities immediately adjacent to the plant, and washed roots were also collected. The

samples were examined in pairs, by species. For example, for *V. forsteri*, five individual plants were sampled (numbered 1–5), each with a corresponding soil sample.

All samples were collected in a preservation solution containing dimethyl sulfoxide (DMSO), disodium EDTA and saturated NaCl (abbreviated to DESS) to preserve microbial DNA for various molecular analyses (Seutin et al., 1991; Yoder et al., 2006). This solution kills microbes in the sample without lysing their cell walls, thereby protecting the DNA from degradation. Additionally, use of DESS simplified collection, allowing samples to be stored at room temperature, shipped by freight or carried.

For each pair, approximately 5 g of soil was sampled using sanitised utensils into 15 mL centrifuge tubes, overlayed with DESS (~10 mL) and mixed thoroughly. Plant roots were collected using sanitised utensils into 15 mL centrifuge tubes, overlayed with DESS (~10 mL) and mixed thoroughly.

9.2.2 Microscopy of roots

Root examination followed Brundrett et al. (1996). Roots were first examined for ectomycorrhizal root tips using a dissecting microscope (Optico ASZ-400T trinocular stereo zoom microscope). After this inspection, fine roots were cleared using a solution of 10% KOH at 60°C. Clearing time varied by plant and root diameter, but in general ranged from 1 to 4 hours. Roots were then stained using Chlorazol Black E² in a lactoglycerol solution (Brundrett et al., 1984), which consisted of a 1:1:1 mixture of 80% lactic acid, glycerol and deionised H₂O. Cleared and stained roots were then mounted onto slides and viewed at 100–400x under a Zeiss Axio Imager A1 microscope.

9.2.3 DNA extraction from soil and root samples

DNA was extracted from roots using the DNeasy PowerSoil[®] kit (Qiagen Cat No./ID: 12855), following the manufacturer's method. Soil extractions were carried out on 0.25 g of soil which was added directly to the bead beating tubes using a sterilised spatula. For plant root extractions, plant material was cut into approximately 1 cm pieces with a sterile blade and approximately 0.25 g of material was added directly to the bead beating tubes using a sterilised spatula. The bead beating tubes were then processed as per the manufacturer's instructions using a FastPrep 24 5G instrument (MP Biomedical).

9.2.4 PCR

DNA was then subject to PCR using the fungal specific primer pairITS1f (CTTGGTCATTTAGAGGAAGTAA; Gardes & Bruns, 1993) and ITS2 (GCTGCGTTCTTCATCGATGC; White et al., 1990), following the methods recommended by the Earth Microbiome Project.³

 $[\]label{eq:chlorazol} ^{2} Chlorazol Black E = 4-Amino-3-[[4'-[(2,4-diaminophenyl]azo][1,1'-biphenyl]-4-yl]azo]-5-hydroxy-6-(phenylazo)-2,7-naphthalenedisulfonic acid acid between the second second$

³ Earth Microbiome Project, https://earthmicrobiome.org/protocols-and-standards/its/

9.2.5 DNA sequencing

All DNA sequencing conducted used Illumina 250bp PE sequences. Sequencing was conducted by Molecular Research LP (MR) DNA Laboratories in Texas, USA.

9.2.6 Bioinformatics

ITS amplicon datasets were processed using the Greenfield Hybrid Amplicon Pipeline (GHAP; https://researchdata.edu.au/greenfield-hybrid-analysis-pipeline-ghap/981523). Analyses for ITS followed the typical workflow: in brief, data were demultiplexed, subjected to quality control prior to merging, clustered into operational taxonomic units (OTUs) and mapped back to the resultant OTU table. For ease of understanding an OTU can be considered the same as a microbial species, although in practice the two differ.

Sample contamination was addressed by an in-house method that determines the level at which a given OTU signal is untrustworthy.

9.2.7 Statistics

Various statistical approaches were used including correlations, PERMANOVA and SIMPER. The statistical ordinations used included principal component analysis and non-metric multidimensional scaling.

PERMANOVA

PERMANOVA (permutational multivariate analysis of variance) is a statistical test used to assess whether there are significant differences between groups based on multivariate data (Anderson, 2017). It is a non-parametric method that compares the dissimilarity between samples within and between groups by permuting the data to generate a distribution of test statistics under the null hypothesis.

The technique is particularly useful when the data involve multiple variables or measurements (such as community composition, species abundance) and the assumptions of traditional ANOVA (such as normality) are not met. PERMANOVA is undertaken on a distance matrix (a dataset that records how similar different samples are). The distance matrix commonly used for ecological data is the Bray-Curtis measure, which is a mathematical approach for comparing the number of shared and different species between communities (Bray & Curtis, 1957; Beals, 1984).

SIMPER

When a significant difference between communities is detected using PERMANOVA, the SIMPER (SIMilarity PERcentage) method is used to determine which species are most responsible for this difference (Clarke, 1993).

9.2.8 Other software

Analyses were conducted in a range of programs including Microsoft Excel and Past 3 (Hammer et al., 2001), as well as customised analysis and visualisations in Python 3.7.3 and R languages (R Core Team, 2017). Most visualisations were conducted in Python using Matplotlib (Hunter, 2007), with Pandas (McKinney, 2010) and, in some cases, Seaborn (Waskom, 2021). Statistics in Python were undertaken using SciPy and Scikit-learn (Pedregosa et al., 2011, Virtanen et al., 2020). For R, the vegan module (Oksanen et al., 2017) was also used for some ecological statistics. All phylograms were created using the phylogeny.fr website, with the default settings (Dereeper et al., 2008).

9.3 Results and discussion

Across this study, 10 paired root:soil samples were obtained for both *A. procumbens* and *B. opponens*; five paired root:soil samples were obtained from *V. forsteri*; and just two root:soil pairs were recovered *L. monoplocoides* (Table 9.1 and Figures 9.3–9.6).

Plant (number collected)	Root samples	Soil samples
A. procumbens (10)	10	10
B. opponens (10)	10	10
L. monoplocoides (2)	2	2
V. forsteri (5)	5	5

Table 9.1 Plant root and soil samples obtained



Figure 9.3 Collection sites for A. procumbens



Figure 9.4 Collection sites for *B. opponens*



Figure 9.5 Collection sites for L. aschersonii and L. monoplocoides



Figure 9.6 Collection sites for V. forsteri

From these plants and soils, a total of 5,669 fungal OTUs (≈ species) were observed across the 54 samples examined. In all cases, both soil and roots resulted in a positive PCR and sequence data (Table 9.2).

	L. monoplocoides	B. opponens	A. procumbens	V. forsteri
L. monoplocoides	1			
B. opponens	0.0202	1		
A. procumbens	0.0018	0.0001	1	
V. forsteri	0.0105	0.0004	0.0001	1

Table 9.2 Statistical differences in composition of fungal communities by plant species.

Values shown are p-values from post-hoc contrasts after PERMANOVA analyses. p-values less than 0.05 are considered to be significant.

All plants were significantly different from each other in terms of the fungal communities in their roots and rhizosphere soils. In most cases, the roots and soils were markedly different in their fungal communities, although this was less clear for some individual plants. These root–soil differences for individual plant species were generally statistically significant with p-values of 0.0001, 0.0002 and 0.0086 for *A. procumbens, B. opponens* and *V. forsteri*, respectively. The same difference may be present in *L. monoplocoides*, although there was insufficient statistical power from the two plant/soils collected for this species.

It should be noted that while significant differences were observed between plant species, the samples were taken in different locations and it may be that the differences are indicative of the edaphic conditions or other physical aspects (e.g. soil type, aspect, moisture) of the regions in which they were collected rather than the plants themselves.

Of the 5,669 taxa detected, most fungi were from the Ascomycota (2,458|43.4%) or Basidiomycota (1,417|25.0%). Small numbers of Glomeromycota (49|0.9%) and Chytridiomycota (61|1.1%) were also detected. The primers used in this study tend to favour Ascomycota or Basidiomycota and exhibit some bias against Glomeromycota (Bellemain et al., 2010). As such, these two minor groups are almost certainly underrepresented in the dataset. In addition, some taxa that failed to be identified were almost certainly novel lineages within this group. For example, OTU106 was unidentified in the study using the default bioinformatic pipeline; alternative methods suggest this may be a Glomeromycota OTU.

Along with taxa from defined phyla many unclassified sequences were also detected. Some of these sequences were quite commonly recovered. For example, OTU28 was recovered almost 140,000 times, mostly in association with *A. procumbens*. Its identity is unknown but it is most closely related (perhaps at the family level or above) to sequences recovered by Chen and Cairney (2002) from Australian dry sclerophyll woodlands (near Sydney) that had been subject to woodland fires. Experience with other data from the region suggests that these taxa may be deeply divergent Ascomycota or Basidiomycota, novel chytrids, Cryptomycota species or a range of non-fungal sequences from other basal eukaryotes (e.g. oomycetes, ciliates and other taxa).

Across the fungal species observed, two fairly obvious trends are present in the data. Single plants (if they associated with mycorrhizal fungi) tended to be dominated by a single ectomycorrhizal species. These species were often highly abundant, but in few samples. In contrast, other abundant taxa were present in most soils examined in this study. These tended to be those taxa that produce many air-dispersed spores, and most were moulds including those from *Aspergillus*, *Penicillium* or similar genera. These species are not known to have key roles in plant-growth promotion and are not fungi typically recognised as foods for mycophagous mammals. For this reason, these taxa are not discussed at length in this report, although they are present in the molecular data and some of the summary tables. For completeness, some brief notes are provided below.

Of these air-dispersed sporulating moulds, some obvious examples include the most abundant fungus observed in soils and roots associated with *A. procumbens* (OTU3; Table 9.4). This taxon was a strain of *As. felis*. Along with *As. felis*, the taxon is over 99% identical in its DNA sequence to *As. siamensis, As. udagawae, As. viridinutans, As. duricaulis* and *As. brevipes*. While these taxa are currently all separately described species, extensive molecular work from multiple genes outside of ITS sequencing are required to distinguish them (Samson et al., 2014). One simpler explanation is that these represent divergent strains of a single species. This is important as *As. felis* is a noted feline pathogen, but other species in the group are not, and it is impossible to know from the current data which of these species OTU3 represents. Like OTU3, OTU9 was also abundant, and was commonly detected in multiple plant and soil samples for *A. procumbens, B. opponens* and *V. forsteri*. This was a species of *Talaromyces* related to *T. popeae* and *T. flavus*, the latter of which is known to be the most common *Talaromyces* species in nature (Pitt, 2014).

In terms of mycorrhizal status, it is important to consider not only the molecular data from each plant (Tables 9.3–9.6) but also microscopic analyses of the roots from these plants.

From these analyses it is clear that *B. opponens* forms ectomycorrhizas and its most common mycorrhizal associates were novel *Russula* species, which were present on plants 2, 3, 4, 5, 7 and 9. Sample roots exhibited the formation of clear ectomycorrhizal root tips with mantles (Figure 9.7; inset). The species they form ectomycorrhizas with (OTUs 161, 7 and 600) were related to, but distinct from, *R. inquinata*.



Figure 9.7 Micrographs of various plant roots, magnification 200×: (a) *B. opponens*; (b) *L. monoplocoides*; (c) *V. forsteri*; (d) *A. procumbens*. Inset: *B. opponens* ectomycorrhizal root tips.

Interestingly, another common mycorrhizal association with *B. opponens* was a species related to *Gamarada debralockiae* (OTU27; Table 9.3), the most common ericoid mycorrhizal species in Australia (Midgley et al., 2018) and a species also known to associate with non-Ericaceous plants (Chambers et al., 2008).

R. inquinata is a native *Russula* with a very restricted distribution on the South Island of New Zealand (McNabb, 1973; Figure 9.8). Data from an another GISERA project (GISERA W27: Chemical and microbial baseline studies and biodegradation experiments of chemical compounds used in coal seam gas activities in the Narrabri region) suggest that OTU7 is common across the region occurring at many other locations in the Pilliga woodlands. OTU161, however, is much more restricted, being only detected (to date) in association with *B. opponens*. It is important to note that OTU7 and OTU161 were both related to *R. inquinata*, and more distantly to other *Russula* species (Figure 9.9). It is also important to note that while the OTUs from this study were not closely related to each other (~93–95% ITS identity), they were more closely related to each other than to other, non-Australian species (Figure 9.9).



Figure 9.8 OTUs from the study site associated with *B. opponens* were related to *R. inquinata*, a rare (potentially endangered) fungus, from New Zealand's South Island (photo reproduced with permission; photo by Shirley Kerr, https://www.kaimaibush.co.nz/index.html)



Figure 9.9 Phylogram depicting the relationships between *Russula* species detected on *B. opponens* in the present study (OTUs 7, 161 and 600) and their closest described relatives, *R. inquinata*, *R. atramentosa* and *R. denissima*

Phylogram was constructed at Phylogeny.fr using default settings.

Table 9.3 Top 20 species from *B. opponens* roots and rhizosphere soils.

ΟΤυ	Best species	Identity %	Mean abundance*	Roots	Soils
OTU161	Russula inquinata (MW683785)	93.6	10.7	3	3
OTU7	Russula inquinata (MW683785)	95.7	10.6	2	3
OTU17	Russula chloroides (UDB011192)	86.6	6.3	1	1
OTU10	Gymnopus subpruinosus (DQ450025)	89.1	4.5	1	0
OTU600	Russula inquinata (MW683785)	91.3	3.9	1	1
OTU8	Clavulina cinerea (UDB001532)	85.2	3.3	0	1
OTU15	Penicillium simplicissimum (NR 138290)	99.1	2.7	7	9
OTU126	Mycena pura (JF908472)	89.4	2.5	1	0
OTU27	Gamarada debralockiae (AY230776)	96.7	2.1	7	3
OTU243	Hydropus murinus (PQ498249)	90.4	2.1	1	0
OTU19	Tomentella alpina (NR 121330)	92.3	2.0	1	1
OTU45	Falciformispora senegalensis (KX381125)	97.8	1.9	3	1
OTU3730	Coprinopsis afrocinerea (NR 159796)	87.6	1.8	1	1
OTU16	Inocybe pluviosilvestris (NR 152366)	92.8	1.8	1	1
ОТU30	Setchelliogaster australiensis (AF325628)	95.5	1.6	2	2
OTU31	Tomentella tedersooi (NR 121359)	93.4	1.5	1	1
OTU46	Orbiliales sp. (LS985578)	91.0	1.4	1	2
OTU55	Unknown fungal sequence	-	1.4	1	0
OTU61	Cortinarius xenosmatoides (NR 157920)	94.9	1.3	0	1
OTU9	Talaromyces verruculosus (HQ608070)	100	1.3	5	4

Each row shows an OTU (~species), its nearest match in the GenBank database. The DNA-DNA identity (%) is shown for each OTU and its match, as is the mean relative abundance of that taxon, along with the number of roots and rhizosphere soils from which this taxon was observed.

*Relative abundance.

Interestingly, other ectomycorrhizal species were also detected in molecular analysis of roots from the *B. opponens* plants. Indeed, roots from plant 7 had significant *Russula*-related sequences and yielded significant numbers of sequences that matched to another ectomycorrhizal genus: *Tomentella*. While plant 7's roots had both *Russula* and *Tomentella* species, plant 10's appeared to lack sequences from *Russula* and most recovered sequences were from *Tomentella*-related species. Like those OTUs with affinities to *Russula*, recovered sequences were distinct from known species, with both OTUs 19 and 31 representing novel species (Figure 9.10).



Figure 9.10 Phylogram depicting the relationships between *Tomentella* species detected on *B. opponens* in the present study (OTUs 19 and 31) and their closest described relatives, *T. tedersooi*, *T. stipitobasidia*, *T. longisterigmata* and *T. stuposa*

Note that OTU31 was most closely related to two sequences recovered from woodland soils in Sydney's western suburbs (KC222706 and HQ829321). In contrast, OTU19 was approximately equidistant from a sequence recovered from dry tropical forest soils in Costa Rica (KX499291) and *T. stuposa*. Phylogram was constructed at Phylogeny.fr using default settings.

In contrast, for the other plants, no obvious mycorrhizal structures were observed. The roots from one of the *A. procumbens* plants did show some circular structures (Figure 9.7) but these were probably non-mycorrhizal. This was somewhat surprising as *A. procumbens*, and potentially *V. forsteri*, are probably mycorrhizal plants.

Molecular data from *A. procumbens* suggest that the roots and rhizosphere are colonised by a range of saprotrophic fungi, with *Aspergillus* and *Coniothyrium* species being the most abundant (Table 9.4). OTU9, a strain of *T. verruculosus*, was found in multiple plant species in this study and was also reported in GISERA W27. *T. verruculosus* has been reported as a fungal endophyte able to produce plant growth-promoting phytohormones (Wani et al., 2016). Some potentially mycorrhizal genera (*Sebacina* and *Inocybe*) were observed in the molecular data, but there were no obvious ectomycorrhizal root tips for this species.

Table 9.4 Top 20 species from *A. procumbens* roots and rhizosphere soils.

ΟΤυ	Best species	Identity %	Mean abundance*	Roots	Soils
ОТИЗ	Aspergillus felis CBS 130245 (NR 138341)	100	10.0	6	8
OTU6	Coniothyrium hakeae (NR 154839)	92.4	6.7	4	7
ОТU22	Sebacina flagelliformis (NR 138387)	93	3.5	1	1
OTU14	Rickenella sp. (MT537051)	92.9	3.2	1	2
OTU57	Inocybe spp.	-	2.9	1	0
ОТU37	Aspergillus brasiliensis ATCC MYA-4553 (NR 111414)	100	2.3	4	5
OTU23	Phialomyces macrosporus CBS 430.64 (NR 160098)	95	2.3	0	7
OTU28	Uncultured fungus (AF461577)	89.9	2.1	1	4
OTU9	Talaromyces verruculosus (HQ608070)	100	2.1	5	9
ОТU20	Aureobasidium insectorum KCL139 (NR 189919)	100	1.7	4	5
OTU15	Penicillium simplicissimum (NR 138290)	99.1	1.7	2	1
OTU414	Coprinellus curtus (AY461824)	93.4	1.6	1	1
OTU101	Uncultured fungus (AF461577)	90.6	1.5	0	3
OTU214	Heteropsathyrella macrocystidia (NR 173319)	90.6	1.4	0	1
OTU18	Unknown <i>Venturiales</i> sp.	-	1.4	1	8
OTU29	Cryptococcus sp AL V(JN255470)	99.5	1.3	2	3
OTU47	Scytalidium circinatum CBS 654.89 (NR 160180)	92.4	1.3	1	6
OTU25	Tolypocladium inegoense	100	1.3	2	3
OTU191	Uncultured fungus (AB986403)	94.2	1.2	1	3
OTU63	Phaeoacremonium italicum (NR 136114)	99.6	1.1	1	0

Each row shows an OTU (~species), its nearest match in the GenBank database. The DNA-DNA identity (%) is shown for each OTU and its match, as is the mean relative abundance of that taxon, along with the number of roots and rhizosphere soils from which this taxon was observed.

*Relative abundance.

Despite the presence of arbuscular mycorrhizas in other *Lepidium* species (Peterson et al. 1985), no arbuscular mycorrhizas were detected in the roots of *L. monoplocoides* in the present study (Table 9.5). Unusually, a range of poorly characterised fungi (OTUs 41, 68, 201 and 11579; likely novel species) and chytrids (OTUs 51, 111, 596 and 7412) were commonly observed on the roots or rhizosphere soils of this species (Table 9.5). The high relative abundance of chytrids on roots was surprising, as chytrids are typically relatively uncommon (or at least at low prevalence) in soils of the region. Most chytrids are saprobes and their presence on *Lepidium* roots and in the rhizosphere may be related to root exudates, or that some of the collected roots were senescing.

Table 9.5 Top 20 species from *L. monoplocoides* roots and rhizosphere soils.

ΟΤυ	Best species	Identity %	Mean abundance*	Roots	Soils
OTU13	Ceratobasidium sp. AG S(HQ269819)	91.2	22.8	1	0
OTU51	Spizellomycetales sp.	-	5.1	1	1
OTU41	Unknown fungus	-	4.8	1	0
OTU7412	Unknown chytrid	-	4.3	1	0
OTU68	Unknown fungus	-	3.9	0	1
OTU111	Unknown chytrid	-	3.0	1	0
OTU114	Coniella paracastaneicola (NR 154823)	100	2.8	0	1
OTU11579	Unknown fungus	-	2.6	1	0
OTU183	Unknown Russulales	-	1.9	1	0
OTU152	Didymella rosea BRIP 50788 (NR 136125)	96.2	1.8	1	2
OTU2357	Talaromyces californicus NRRL 58168 (NR 165527)	99.5	1.7	0	2
OTU201	Unknown fungus	-	1.7	0	1
OTU13028	Lepidium virginicum (LC090012)	99.1	1.6	1	0
OTU596	Undescribed chytrid (MW215611)	99.7	1.6	0	1
OTU297	Neocatenulostroma castaneae (NR 176757)	94.1	1.5	0	1
OTU2689	Unknown fungus	-	1.5	0	1
OTU402	Root associated Ascomycota (LT611648)	99.0	1.5	0	1
OTU360	Entoloma cremeostriatum (NR 177614)	86.1	1.4	0	1
OTU212	Phlogicylindrium mokarei CPC 29306 (NR 164412)	99.6	1.3	0	1
OTU273	Unknown fungus	_	1.3	0	2

Each row shows an OTU (~species), its nearest match in the GenBank database. The DNA-DNA identity (%) is shown for each OTU and its match, as is the mean relative abundance of that taxon, along with the number of roots and rhizosphere soils from which this taxon was observed.

*Relative abundance.

Collections of *V. forsteri* were intertwined with moss and moss rhizoids. The molecular data may include at least some of this material, though efforts were made to remove it. The presence of organisms such as *Rickenella*, known from moss, may suggest these efforts were not completely successful (Table 9.6). Interestingly, unlike for *A. procumbens* or *L. monoplocoides*, numerous ectomycorrhizal fungi were detected in the roots and rhizosphere soils of *V. forsteri*, though no ectomycorrhizal root tips were observed. These included OTU1 of *Lactifluus*, related to, but distinct from, *La. clarkae*. This species of *Lactifluus* is one of the most common ectomycorrhizal fungi in the region and was detected from sites throughout the region in the GISERA W27 project.

In addition, two other *Russula* species (OTUs 215 and 347) were observed in association with rhizosphere soils of *V. forsteri*. Furthermore, a species of novel *Tomentella* (OTU31; Table 9.3) observed on *B. opponens* was also present on *V. forsteri* roots (Table 9.6). It may be that these represent collections of extrametrical hyphae of fungi that form ectomycorrhizas with nearby trees, rather than fungi associating directly with *V. forsteri*. Alternatively, *V. forsteri* may form

ectomycorrhizas, though further work would be required to confirm this through observations on roots.

ΟΤυ	Best species	Identity %	Mean abundance*	Roots	Soils
OTU86	Rickenella sp. (MT537051)	91.0	8.3	1	1
OTU26	Latorua caligans CBS 576.65 (NR 132923)	95.3	6.2	1	0
ОТU96	Knufia victoriae MNA-CCFEE 6746 (NR 185660)	86.1	5.2	1	0
OTU1	Lactifluus (Lactarius) clarkeae (MW683784)	97.1	3.9	0	1
ОТU91	Uncultured Pezizaceae (KU353461)	96.7	3.6	0	1
OTU256	Unknown root-associated fungus (LC277138)	86.0	3.2	1	0
OTU395	Unknown fungus	-	2.8	2	2
OTU227	Gymnopilus purpureosquamulosus (AY280979)	98.9	2.2	1	1
OTU2766	Inocybe tertia (NR 153164)	88.2	2.1	1	1
OTU215	Russula echidna (NR 173171)	98.6	1.9	0	3
OTU452	Unknown fungus	-	1.8	0	1
OTU281	Uncultured fungus 'Toosoil23' (KC222803)	90.0	1.6	0	1
OTU347	Russula danksiae (NR 184926)	93.2	1.6	0	1
OTU440	Amorocoelophoma cassiae MFLUCC 17-2283 (NR 163330)	96.7	1.6	1	0
OTU31	Tomentella tedersooi (NR 121359)	93.4	1.5	1	1
OTU205	Uncultured Scleroderma 273 (KU353473)	86.5	1.5	1	1
OTU388	Eurotiales sp. KO-groupB (AB986359)	98.9	1.4	2	2
ΟΤU9	Talaromyces verruculosus (HQ608070)	100	1.4	1	1
OTU45	Falciformispora senegalensis (KX381125)	97.8	1.3	1	0
OTU248	Unknown Chaetomiaceae (HG936938)	97.5	1.3	2	3

Table 9.6 Top 20 species from *V. forsteri* roots and rhizosphere soils.

Each row shows an OTU (~species), its nearest match in the GenBank database. The DNA-DNA identity (%) is shown for each OTU and its match, as is the mean relative abundance of that taxon, along with the number of roots and rhizosphere soils from which this taxon was observed.

*Relative abundance.

In addition to the plants themselves, the current study found 56 arbuscular mycorrhizal species – mostly novel, poorly defined groups. These species were mostly Glomerales, though there were also a number of Archaeosporales. Arbuscular mycorrhizal fungi tend not to amplify well using the ITS1f-ITS2 primer pair used in this study, and individual spores can sometimes have multiple ITS sequences (Bellemain et al., 2010). These two factors decrease their observed abundance and increase their diversity, respectively. On balance, however, the absolute number of arbuscular mycorrhizal fungi is likely to be a significant underestimate.

Arbuscular mycorrhizal fungal fruiting bodies (along with other fruiting bodies) have previously been shown to be food sources for species in the region, including the Pilliga mouse (Tokushima & Jarman, 2010). Their widespread nature in the region (which is further confirmed by data from GISERA W27) suggests ready access to this food source.

9.4 Conclusions

The aims of this activity were to establish the mycorrhizal status of *A. procumbens*, *B. opponens*, L. *monoplocoides* and *V. forsteri* and to determine whether these fungal partners were widespread in the region.

Data presented here, the first time for an Australian Euphorbiaceae species, indicate that *B. opponens* forms ectomycorrhiza. In the study area it appears to associate with a small range of *Russula* and *Tomentella* species, many of which are fairly widely distributed in the region (GISERA W27). Conversely, one of the major associates of *B. opponens*, OTU161, appears to have a more limited distribution than other *Russula* species in the area (GISERA W27). Further work would be required to determine whether ectomycorrhizal associations with OTU161 provide significant advantage to *B. opponens* over *Russula* or other ectomycorrhizal species.

None of the other three plant species examined were convincingly mycorrhizal. It should be noted, however, that from *V. forsteri* roots some ectomycorrhizal DNA (from *Lactifluus, Russula* and *Tomentella* species) was detected. The absence of obvious ectomycorrhizal root tips for *V. forsteri* indicates further work may be required to determine the mycorrhizal status of this species.

Regardless, data presented here suggest that other than OTU161, which appears to have a restricted distribution, *B. opponens* probably interacts with a range of more common mycorrhizas in the region. This further suggests that availability of mycorrhizal partners is unlikely to be an issue in the dispersal and range of threatened plant species. That being so, work to clarify whether OTU161 is an important partner for *B. opponens* would be valuable. Further sampling of *B. opponens* may provide additional insights as to whether the ranges of OTU161 and *B. opponens* are linked in some fashion.

10 Conclusions

The landscapes of the Pilliga Forest have experienced intense modification since European settlement, including clearing for agricultural purposes, clearing and thinning through forestry, and grazing (Date et al., 2002). Despite this, they remain the largest contiguous inland forest in eastern Australia and continue to provide important habitat for a wide variety of native plants and animals, including species listed as of conservation concern under national and state legislation.

This project sought to provide scientific understanding to address a key community concern related to maintaining the intactness of ecosystems and their component species in the Pilliga Forest. This ambition needs to be viewed through the lens of the 200-year history of modification in the area. The project focused on fragmentation because community concerns, highlighted by the Independent Planning Commission of NSW (State of NSW, 2020), emphasised forest fragmentation and the negative impacts of edge effects on the biodiversity of the region. The community also expressed concerns related to the effects of invasive plants and invasive animals, both predators and feral herbivores, on the biodiversity of the Pilliga Forest.

Of the concerns raised by the community, forest fragmentation is the contentious one in terms of whether it is a negative influence on most species. The strength of scientific evidence currently indicates that fragmentation of habitat can have a positive effect (Fahrig, 2003; 2017; Fahrig et al., 2018). However, this is an unpopular view given the negative connotation of fragmentation in common parlance, hence there is stigma associated with research that makes this point (Riva et al., 2024).

This project used a combination of remote sensing and field-based research to address the issue of forest fragmentation and its effects. The diversity of mycorrhizal associations between threatened plants and fungi was also assessed. Up-to-date methods from earth observation science were used to assess fragmentation and connectivity across the entire NGP area. This revealed relatively low levels of fragmentation and typically high connectivity across the landscape. At the species-level, the study did not find evidence that the species targeted through the surveys are currently impacted by fragmentation.

The original intention had been to combine information on the fragmentation and connectivity indices of the landscapes in which each of the focal threatened species was recorded with the LiDAR-derived data on structural attributes of each species' habitat to make predictions on the likely population-level response of each species to additional forest fragmentation resulting from the NGP. However, the impact of the extensive December 2023 wildfire precluded undertaking surveys in mature habitat for each species, and the structural attributes of habitat could not be assessed in mature habitat in the way it was originally intended. In addition, the degree of fragmentation of the Pilliga Forest was low, as represented by the typically very high FAD values of most sites. This was unexpected and was not an ideal design for testing the role of fragmentation on species occupancy. It is possible that a fragmentation response would have emerged if sites

had been available at lower levels of FAD values and if a larger sample of sites had been examined or if the species chosen were more sensitive to fragmentation.

The Pilliga Forest currently faces several known and imminent threats that are of high concern for biodiversity conservation. These pressures include the ongoing effects of the loss of large, hollow-bearing trees through forestry and inappropriate fire regimes. There should be a focus on ensuring that these threats are not exacerbated by activities associated with the development of the NGP, particularly within the Pilliga Forest (noting that part of the NGP is located outside of the forest).

In 2002, Date et al. characterised the Pilliga Forest as a woodland with no mature cypress pine, some mature eucalypts with hollows, few medium-sized cypress pines or eucalypts with developing hollows, a mostly young overstorey of eucalypts, cypress pines and *Allocasuarina luehmannii*, and a dense understorey of small eucalypts, cypress pine, *Al. luehmannii* and shrubs. At the time, log sizes were small: 40 cm for ironbarks and 12 cm for cypress pine (Date et al., 2002).

Persistence in such an ecosystem is challenging for those species that are dependent on large trees, especially the hollows they provide. The status of two common species of arboreal marsupials in the Pilliga Forest is the most startling example of this situation. Paull and Kerle (2004) raised the issue of the disappearance of the common ringtail possum (*Pseudocheirus peregrinus*) and the rarity of the common brushtail possum (*Trichosurus vulpecula*) in the Pilliga Forest by 1999–2000, from formerly high encounter rates in 1993–1994. A shortage of suitable tree hollows was identified as a likely cause in combination with predation, especially by the introduced European red fox (*Vulpes vulpes*), as animals moved across the ground to access widely spaced resource trees. Resource availability is further compromised by the foraging activities of introduced herbivores – feral goat (*Capra hircus*), wild pig (*Sus scrofa*) and European rabbit (*Oryctolagus cuniculus*). Deer also occur in the area. Together, these herbivores alter much of the understorey and damage the soil.

The surveys carried out for this project confirmed the severe decline of the two possum species. Approximately 139 person-hours of spotlighting (walk transects) was carried out from November 2023 to June 2024 during which no common ringtail possums and only one common brushtail possum (0.01/person-hour of spotlighting) were recorded. To test whether the decline in the common brushtail possum was occurring at a regional scale (i.e. outside the Pilliga Forest), records from a visit to Yarrie Lake Flora and Fauna Reserve on the night of 3 November 2023 were examined during which a nocturnal walk transect was carried out similar to what was being undertaken on the 30 sampling sites within Pilliga Forest. Yarrie Lake is within the NGP but outside of the Pilliga Forest. Common brushtail possums were readily observed. The observation rate was 2.59/person-hour of spotlighting (walk transect). Eleven individuals were seen in total. The status of arboreal marsupials within the Pilliga Forest should be the focus of ongoing attention, as should research on methods to reverse the dramatic declines in some species.

The cumulative impact of extensive wildfires is also a concern for persistence of biodiversity in the Pilliga, especially for those species dependent on mature vegetation. This topic should be the focus of further research in the area. The December 2023 wildfire burnt 129,053 ha. It is at a minimum the fourth fire of >120,000 ha since 1982 (120,000 ha burnt in 1982–1983, 144,000 ha

burnt in 1997–1998 and >120,000 ha in November/December 2006). Such extensive fires contribute to ongoing dominance of a mostly young overstorey of eucalypts, cypress pine and *Al. luehmannii*, and a dense understorey of small eucalypts, cypress pine, *Al. luehmannii* and shrubs.

The current study has provided new scientific understanding through mapping of fragmentation and connectivity at scale and through collecting high-quality structural attribute data of forest sites with UAV-LiDAR. It has also clarified questions on the occurrence of several of the threatened plants and animals that reside in the Pilliga Forest. Importantly, the research has contributed fundamental new knowledge on the biodiversity of the area. A total of 5,669 fungal OTUs (≈ species) were observed from root and soil samples. This is new knowledge on a group of organisms that have not previously been investigated in the region but that are of fundamental importance for ecosystem functioning and, therefore, healthy ecosystems.

Furthermore, one of the threatened species, *B. opponens*, was shown to form ectomycorrhizas, the first evidence of such associations in an Australian Euphorbiaceae species. That the mycorrhizal partners (*Russula* and *Tomentella* species) are common suggests that dispersal is unlikely to be an issue for the plant. Mycorrhizal communities are a largely unexplored aspect of the region's biodiversity.
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