Using novel biochemical and modelling techniques to reveal the past extent of native food plants in Victoria, southeast Australia.

Thesis Submitted for the Degree

Master of Research (Science) Geography

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“I said I liked being half educated;
you were so much more surprised at everything
when you were ignorant.”

“The Return” – My Family and Other Animals, Gerald Durrell

Abstract

Under the compounded stresses of anthropogenic climate change, biodiversity losses, and a global agricultural system reliant on a small number of crops, agricultural productivity is projected to increase its risk of collapse as humanity progresses through the 21st century. Within this prescient modern context, archaeological and palaeoecological sciences can play an important role in shaping humanity's future by drawing upon its past. The exploration of ancient and Indigenous food systems globally has been suggested as offering new pathways for sustainable agricultural practices in the future. However, studying ancient food histories using palaeoecological methods is inhibited broadly by taxonomic resolution issues, which limit researchers' abilities to explore ancient food plant histories quantitatively. Palynology, a discipline of palaeoecological study in the Quaternary sciences, as the study of ancient pollen and spores, has operated within these constraints since its conception in 1916.

In this thesis, I have employed novel chemotaxonomic (classification of species based on their biochemistry) techniques which can improve the taxonomic resolution of modern pollen to a species level and have extended these methods to the sub-fossil record. This method relies on obtaining vibrational spectra of pollen sporopollenin (the wall material of pollen) using microscopic Fourier transform infrared spectroscopy (FTIR) analyses and classifying the data using the Random Forest (RF) machine learning algorithm. Additionally, I have applied the mechanistic Regional Estimates of VEgetation from Large Sites (REVEALS) model to quantify vegetation extent from sub-fossil pollen.

My results indicate that chemotaxonomy is a viable pathway to improve taxonomic fidelity in palaeoecological records and can be combined with vegetation reconstruction techniques. I find that interspecific signals are present within pollen vibrational spectra and can be used to reconstruct ancient and Indigenous food plant histories. Thus, I present the first quantitative reconstruction of an Indigenous staple food plant, a first for Australia. Furthermore, I present the world's first test of quantified vegetation extent reconstructions of taxa identified by chemotaxonomy.

Preface

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Declaration

I hereby declare that the research conducted in this investigation is original and a result of my own endeavours (and copious amounts of caffeine), with the support and guidance of those acknowledged.

Content Warning

Indigenous peoples are respectfully advised this research contains accounts, images and references of persons who have died and past cultural practices. Furthermore, colonist quotes are generally kept in their original written context, thus they may contain phrases now considered culturally inappropriate. This research uses the term Indigenous as an umbrella term, embracing the many diverse Aboriginal and Torres Strait Islander peoples and their respective cultures.

Acknowledgement of the Traditional Owners of Country

I acknowledge and celebrate the First Nations Owners' past, present and future of the land upon which this research was undertaken. I acknowledge all First Nations peoples in Australia and the Torres Strait who have suffered historical injustices due to European colonial invasion.

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Glossary of Key and Abbreviated Terms

- calBP: Calibrated Years Before Present, where the 1st of January 1950 is considered ‘‘Present’’.
- AD: Anno Domini in the Gregorian Calendar, used for modern dates after calBP reaches 0, 1949 = 1AD.
- FTIR: Fourier transform infrared spectroscopy; a microspectroscopy scale is present in this study.
- RF: Random Forest classification algorithm.
- MLA: Machine Learning Algorithm.
- LRA: Land Reconstruction Algorithm.
- REVEALS: Regional Estimates of VEgetation At Large Sites model.
- Cryptic pollen: pollen that cannot be identified to a species/genus level using traditional morphological identification methods due to a shared pollen grain morphology at lower taxonomic ranks, e.g., family level.
- TR, SH, and HG: The plant structural groups Trees, Shrubs, Herbs and Grasses.
- Interspecific: Variation found between species.
- Murnong: Culturally significant Indigenous staple food plants for south-eastern Australia. Refer to Plant Profile (S1) and Chapter 2.
- Country: Refers to more than just geographical space, a concept linked to Indigenous spiritual beliefs merging the physical landscape and culture as a physical entity. Somewhat comparable to the idea of cultural landscapes, a landscape shaped by long-standing human interactions and spirituality.

Chapter 1: Introduction and Aims

1.1 Introduction

The need for rethinking conventional agriculture is now, with the global convergence of biodiversity loss (Cardinale et al., 2012), climate change (Calvin et al., 2023), and an environmentally destructive monoculture diet (Jägermeyr et al., 2021). These issues are pressing environmental scientists to explore concepts and practices surrounding alternative food plants and cultivation methods. Recent scientific approaches are beginning to incorporate Indigenous (in a global sense) knowledge of sustainable ancient food systems that were once standard practice with staple foods, unlike those found in modernised diets (Antonelli, 2023). Indigenous-managed Australia is a unique case study for the complex interactions of food plant cultivation outside typical farm structures (e.g., fenced plots) alongside landscape-level management through cultural burning practices (Gammage, 2011; Pyne, 1991). Perhaps due to these complexities, they have been overlooked by traditional agroeconomic systems focused on yield and profit. To understand opportunities in sustainable ancient food systems, we can look to Indigenous Australians, who have managed country for tens of thousands of years, making them some of the world's oldest cultivating peoples (Gammage, 2011; Pascoe, 2014).

Indigenous people arrived in Australia around ~65,000 years ago (Bowler et al., 1970; Malaspinas et al., 2016); for southeast Australia, the earliest evidence of human inhabitation begins at ~40,000 years ago (Matchan et al., 2020). Throughout the occupation of Australia, Indigenous people ensured food security as part of complex land management systems. The practice of cultural burning maintained openness in woodlands by reducing the shrub cover of the landscape while also promoting the growth of an herbaceous understory. This openness improved yields from hunting activities while reducing the effect of uncontrolled wildfire activity.

Ethnographic accounts from colonists describe complex food plant practices across south-eastern Australia. However, contemporary perceptions of Indigenous food culture often fail to recognise complexities within Indigenous food plant management, taking a perspective rooted in the biases of many of Australia's early European colonists. Whereby deliberate practices undertaken by Indigenous peoples to improve food plant yields do not indicate cultivation practices. Archaeological records of plant food usage are available in Australia but are limited to rare Digging Stick and millstone occurrences. Therefore, a primary avenue to investigate food plant histories in Australia is through Palynology, the study of

pollen, to explore shifting patterns in food plants around sedimentary archives of pollen like lakes and wetlands.

To date, palaeoecological research that attempts to quantify the extent of native food plants in Australia is limited in both the quantity and the quality of research, e.g., Macphail and Woodward (2017) are limited by a taxonomic resolution problem preventing native food plant identification. Following colonisation in 1788, native food plant practices declined, pressured by introduced sheep grazing and the cessation of landscape management systems, including cultural burning. Moreover, poor taxonomic resolution, using standard palynological techniques, makes identifying many native food plant pollen difficult or impossible (Macphail and Woodward, 2017; Mander and Punyasena, 2014). Cichorioideae native food plants have cryptic pollen morphology, sharing the same grain structure as all non-food and non-native taxa. Thus, most native food-plant taxa cannot be identified within the pollen record.

Methods that analyse pollen based on differences in pollen sporopollenin (the highly resistant protein that forms the outer wall structure of pollen and spores) biochemistry (chemotaxonomy) have allowed species-level classification of pollen grains using Fourier-transform infrared (FTIR) spectroscopy (e.g., Jardine et al., 2019; Julier et al., 2016; Zimmermann, 2018). Despite chemotaxonomic classifications providing a possible pathway to improve taxonomic resolution in palaeoecological reconstructions, limited quantitative information can be gained by applying chemotaxonomy to qualitative palaeoecological reconstructions (Hellman et al., 2008). Thus, chemotaxonomy warrants application with landcover reconstruction models like REVEALS (e.g., Sugita, 2007a) to uncover the extent of cryptic vegetation systems.

Therefore, this research intends to uncover the extent of a key cryptic Indigenous staple food plant group in southeast Australia, Murnong, the tuberous Yam Daises (*Microseris* spp, Cichorioideae subfamily, Asteraceae family). By quantifying ancient Murnong extent, assumptions about the nature of Indigenous food plant practices can begin to be explored. Moreover, this research features a first-ever attempt at combining chemotaxonomic classifications with landcover reconstruction models (REVEALS) to develop a method to uncover the extent of ancient Australian subsistence systems, potentially the world's oldest.

1.2 Aims

I. Setting a palaeoclimatic scene for future investigations

Although not the primary focus of this thesis, shedding light on the palaeoclimatic conditions of south-eastern Australia will aid in future understandings of Indigenous food plant distributions and extent changes through time. The palaeoclimate of south-eastern Australia is an underexplored but important dimension for elucidating past spatial distributions of Indigenous food plants like Murnong. Studying the palaeoclimate of the region sets the scene for concluding broader vegetation changes occurring in ancient south-eastern Australia. Thus, this thesis aims to examine the relationships between palaeoclimate and the other palaeoecological records explored in this thesis and future works studying Indigenous food plants at a landscape level.

ii. Reconstructing vegetation from pollen quantitatively in south-eastern Australia

This research aims to explore changes to vegetation extent in south-eastern Australia inferred from sub-fossil pollen records from a large lake in western Victoria. The work seeks to examine the complex interplays between vegetation extent changes inferred from lake pollen records and other human-climatic drivers. Quantitative vegetation reconstruction models will allow insight into shifts in vegetation extent before colonial invasion. Thus, this study aims to analyse these different environmental signals' interplay over time. For example, interpreting the transition from an Indigenous-managed landscape to a post-invasion landscape and identifying if the onset of colonial activity is visible in palaeoecological records for the region.

iii. Creation of a chemotaxonomic classification model able to identify Murnong in the fossil record

Chemotaxonomy is the classification of species based on the biochemical compounds of an organism. However, its application in palynology for ancient pollen identification is yet to be applied. This thesis aims to address this by analysing the vibrational spectra of pollen sporopollenin to achieve a species-level classification of sub-fossil pollen from lake records. Native Australian Cichorioideae (subfamily) pollen is selected for this research for three main reasons: it is limited by the number of native species, Cichorioideae pollen is easily recognisable but not to a species level, and the subfamily has historical significance

as a group containing key Indigenous food plant in south-eastern Australia. This study employs machine learning algorithms to analyse the data and aims to provide a new level of taxonomic detail that has long been lacking in palynology.

iv. Quantitative reconstructions of Murnong

This investigation's key and final aim is to provide a first-of-its-kind quantitative regional reconstruction of Murnong extent, setting the stage for future palaeoecological and archaeological inquiries into Indigenous food plant histories in Australia. To date, no quantitative palaeoecological reconstructions of Indigenous food plants exist for Australia. This thesis aims to establish a proof of concept that working at the fringes of traditional disciplines, such as Palynology, can yield transformative results. Specifically, this study aims to use vibrational spectra and machine learning classification to quantitatively reconstruct morphologically cryptic sub-fossil pollen.

Chapter 2: Background Literature

2.1 Country

i. Setting the scene: cultural burning and the "Australian Estate"

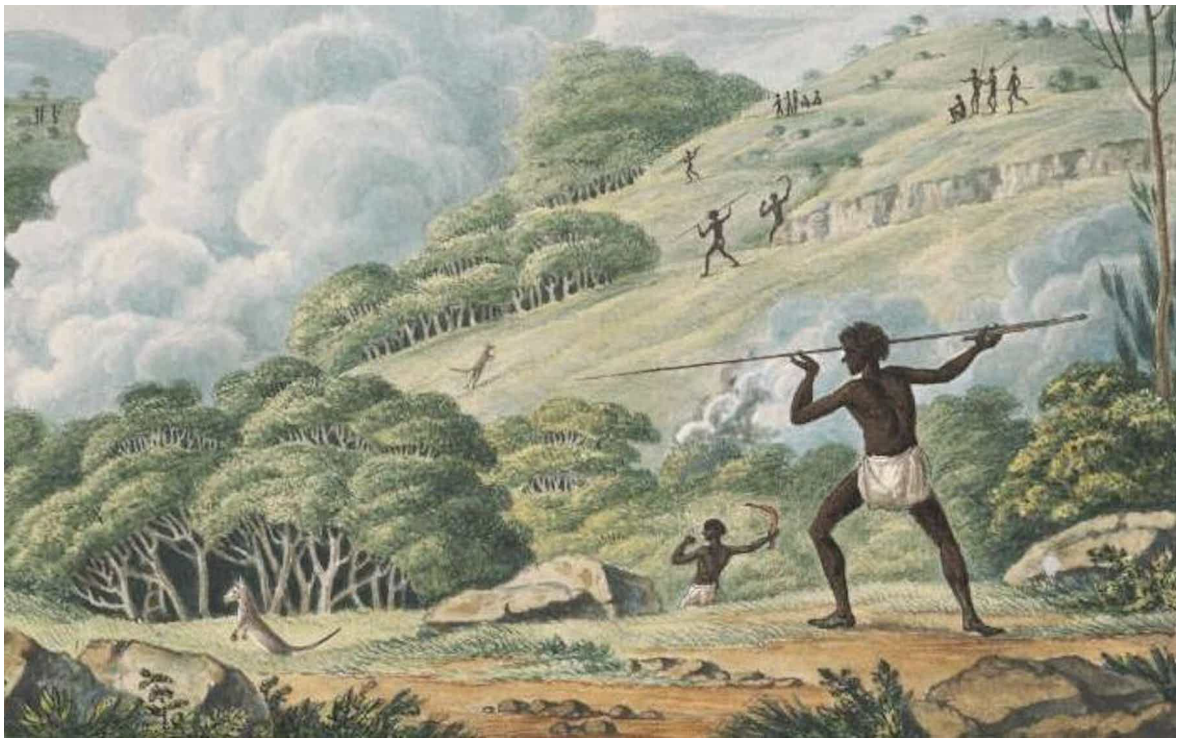


Figure 1 Indigenous People using fire to hunt kangaroos (Lycett, 1817). Famously, the cover of The Biggest Estate on Earth: how Aborigines made Australia (Gammage, 2011)

Cultural burning or cool burning (and in some cases fire-stick farming) is a form of controlled burning where country is deliberately burnt for various health, ecological, and hunting benefits. It is well-established that Indigenous peoples used fire in many ways to hunt and manage country (Bowman, 1998; Gammage, 2011; Jones, 2012). Evidence from the Western Desert suggests fire was also used as a tool for cooking, warmth, illumination, ceremony, ritualistic ordeals, felling of trees, clearing camps, signalling, driving game, regenerating senescent vegetation, smoking animals from burrows and asphyxiating bats in caves (Gould, 2010). Controlled burns were also used to increase the abundance of food plants and increase hunting yields (Bowman, 1998); in such cases, fire-stick farming can be used to describe these practices (Jones, 2012).

Recent palaeoecological study has quantified regional levels of trees, shrubs, and herbaceous vegetation cover through past millennia, covering pre- and post-invasion periods across south-eastern Australia. Therein, the encroachment of shrubs (woody fuels) and the decline of native herbaceous vegetation after colonial invasion in 1788 is

described, which is interpreted as part-causal for the catastrophic wildfires which are seen today in drought periods across southeast Australia (Mariani et al., 2022). While Indigenous landscape management has been quantified regarding woody fuels for past millennia in southeast Australia, no quantified evidence of fire-stick farming practices and plant cultivational practices exist.

The first recorded evidence of an Indigenous-managed Australian landscape comes from the diaries and journals of European settlers and explorers. In 1770, onboard the HMS Endeavour Cook described a view of Australia's eastern coastline (and with subsequent inland expeditions), a landscape which had the potential to be cultivated "without cutting down a single tree" as trees were so distanced, and peculiarly there was "no underwood" of any kind (Parkin, 2020, p. 189). Cook summates the landscape of Australia's east coast at the end of his expedition.

> *"The mountains or hills are chequered with woods and lawns. Some of the hills are wholly covered with flourishing trees; others but thinly, and the few that are on them are small and the spots of lawns or Savannahs are rocky and barren" (Parkin, 2020, p. 448).*

After the establishment of Botany Bay (Sydney) in 1788, marked as a clear date of colonial invasion, settlers are shown to repeat Cook's sentiments (Gammage, 2011). The described landscape mosaic was a curiosity of many early settlers. It was noted that natural woodland-scrubland transition zones (ecotones) appeared abrupt, lacking "underwood". These features are commonly described in colonist accounts as "Belts", "Edges", "Clumps", and "Clearings" (Gammage, 2011, chap. 7). Perhaps most intriguingly, the terms "gentleman's park" or "estate" were used to describe the Australian landscape, initially written by Sydney Parkinson in 1770 (Gammage, 2011, chap. 1; Parkinson, 1984, p. 134).

Colonist depictions of the landscape were also echoed through artwork. The landscapes depicted by Von Guérard and Joesph Lycett portray the openness of the landscape, with trees being spaced and lacking dense shrub cover (Fig. 1-3). Moreover, comparing the scenery recorded by colonist artworks to modern-day images of the same sites reveals a general increase in woody vegetation and a reduction in openness (Fig. 4-5).



Figure 2 Top of Mount Lofty, near Adelaide (Von Guérard, 1855). Above the hunters the openness of the understory is apparent, this is followed along the crest of the peak into the distance. On the right of the hillcrest there is an openness without vegetation.



Figure 3 View on the Wingeecarrabee River, New South Wales (Lycett, 1824). In the close foreground there is a lack of dense shrub understory, which immediately transitions into floodplain grassland. Further into the scene to the centre you can see Indigenous people hunting a Kangaroo. On the opposite side of the river a lack of understory is shown in the woodland edge as tree trunks are prominent all the way to the ground.



Figure 4 View of Moroit [i.e Koroit] or Tower Hill, extinct volcano between Lady Bay & Port Fairy in Australia Felix (Von Guérard, 1860). In the foreground there is an open glade with Indigenous people with their huts. Similar openness is shown in the other side of the river, which is surrounded by dense woodland on all sides; openness is shown to the hillslope on the far left of the scene and over the horizon beyond the central mountains/hills.



Figure 5 Google Earth Image of Tower Hill from the Von Guérard lookout taken in 2022 (Bene, 2022).

ii. Victorian country

The modern landscape of the Victorian region is characterised by a mixed temperate environment with broadleaf and mixed forests, montane grasslands and shrublands, savannah, and mallee and sclerophyll forests (Dinerstein et al., 2017). Native *Eucalyptus* forests are a significant proportion (77%) of Australia's forests, covering 94% of the native forests in Victoria, of which 40% are medium open forests (ABARES, 2018).

The Basaltic Plains of south-western Victoria is the region focussed on this study (Fig. 6). The Basaltic Plains is the third largest volcanic plain in the world and is considered dormant or extinct (Johnson, 2009). Indigenous oral histories describe witnessing eruptions in the Basaltic Plains (Birch, 2000). Today, the Basaltic Plains is categorised by grasslands and agricultural activity with minimal forest cover. Landscape openness has likely remained high throughout the Holocene due to the general aridity of and the clay soils of the region; such soils inhibit tree and shrub establishment as they are susceptible to cracking and desiccation (D'Costa et al., 1989; Mills et al., 2013). However, another element which may have affected shrub establishment through the Holocene is the use of cultural burning by Indigenous peoples (Gammage, 2011).

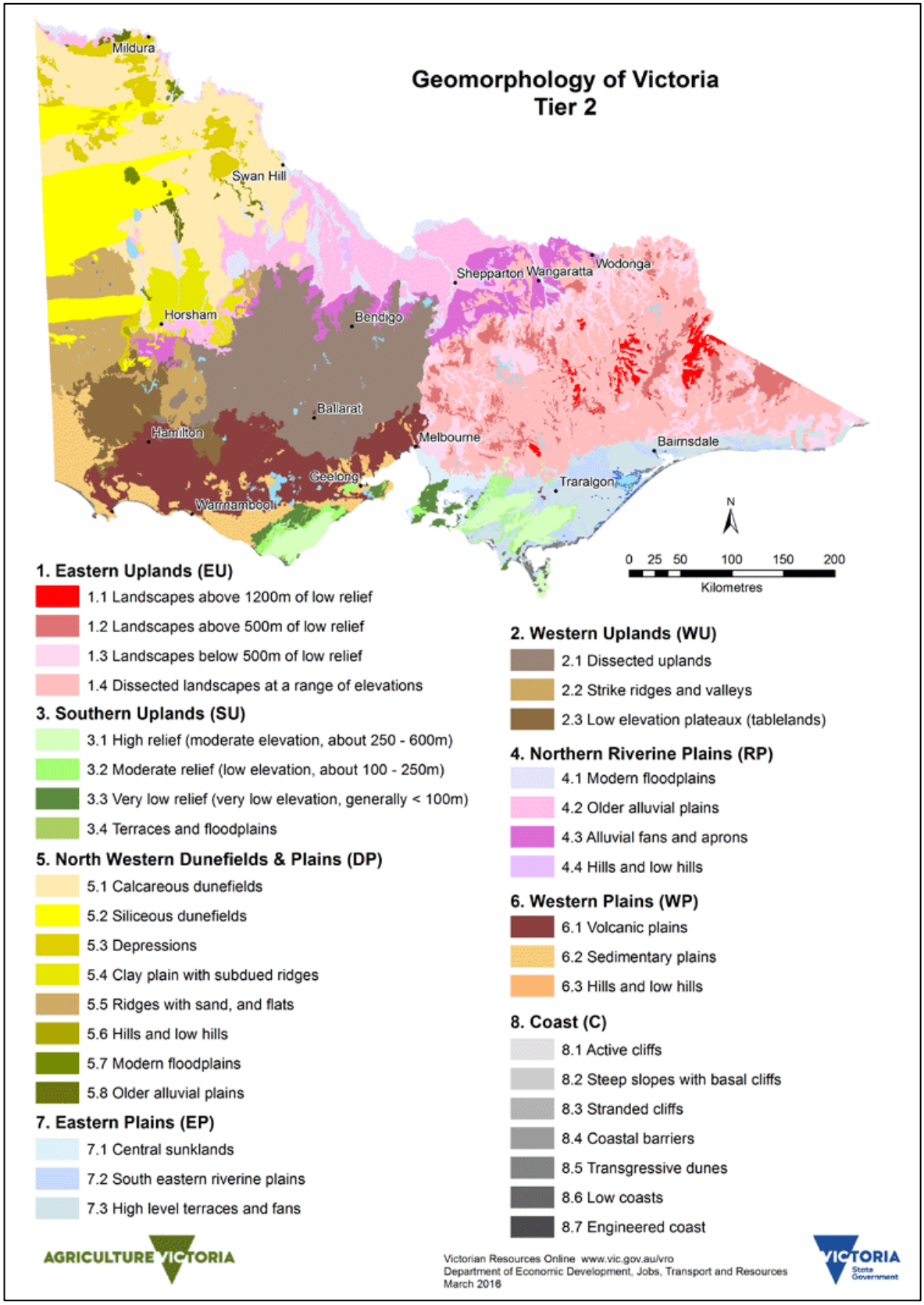


Figure 6 Geological map of Victoria, highlighting the extent of the Basaltic Plains (6.1) (Agriculture Victoria, 2020).

In Victoria, it was noted by Banks that tree cover was present on the lower slopes of hills and mountains but “were not very large and stood separate from each other without the least under-wood” (Gammage, 2011, p. 34). Near Omeo (Victoria), in 1843, Henry Haygarth extensively describes the curious landscapes:

> *“the gloomy forest had opened, and about two miles before, or rather beneath us – for the ground, thinly dotted with trees, sloped gently downwards – lay a plain about seven miles in breadth. Its centre was occupied by a lagoon… on either side of this plain, for some distance, was as level as a bowling-green, until it was met by the forest, which shelved picturesquely down towards it, gradually decreasing in its vast masses until they ended in a single tree. In the vicinity of the forest the ground was varied by gentle undulations, which, as they intersected each other, formed innumerable grassy creeks and open flats, occasionally adorned with native honeysuckles and acacias… Two remarkable conical hills, perfectly free from timber rose in the middle of the largest plain… The whole, as far as the eye could reach, was clothed in a thick coat of grass, rich and luxuriant, as if the drought, so destructive elsewhere, had never reached this favourable spot.” (Haygarth, 1848, p. 136).*

Furthermore, in this region, George Russel describes how burning regimes created great open plains in the landscape, that looked almost like pastureland.

> *“[The plains had a] great extent of rich soil, many hundreds of acres being almost without a tree… covered in a rich sward of Kangaroo Grass. The country around had all been burnt by bush-fires during the previous summer, and the grass that was now growing in the ground was as green and luxuriant as if it had been a field of grain” (Russell and Brown, 1935, p. 190).*

The rich black soils that Russel refers to are the basaltic soils across Victoria. Generally, the nutrient-rich Basaltic Plains refers to the land dotted with volcanic crater lakes between Melbourne to the east and the Grampian Mountains to the west. Within the Basalt Plains, Granville Stapylton exclaims.

> *“Here we have undulating ground clear of timber except occasional picturesque clumps of Trees Mould of the finest and richest black soil of great depth, and grass and herbage so verdant and thick that the*

ground is literally matted with it" (Stapylton and Andrews, 1986, pp. 180–1).

George Haydon in southwest Victoria refers to "Beautiful plains with nothing on them but a luxuriant herbage, gentle rises with scarcely a tree, and all that park-like country" (Haydon, 1846, p. 15). Near Mt. Alexander Victoria, Mossman and Banister continue that the bush…

"was typical of a great proportion of pastoral lands of Victoria. It consisted of undulating open forest-land, which has often been compared, without exaggeration, to the ordinary park-scenery of an English domain; the only difference which strikes the eye being the dead half-burnt trees lying about. To bring it home to the comprehension of a Londoner, these open forest-lands have very much the appearance of Hyde Park and Kensington Gardens, presenting natural open glades like the east end of the former" (Mossman and Banister, 1853, p. 62)

Again, in 1839 William Russel describes the agricultural potential of the Victorian landscape, noting the…

"rich alluvial soil [of the Basaltic Plains near Shelford Victoria] and without bush or tree and all ready for the plough"(Russell and Brown, 1935, p. 190).

From Cook's first accounts of Australia's landscape, a vegetation mosaic is described, lacking a cohesive understory; however, Cook does not attempt to suggest if this landscape was natural or a product of indigenous management, a sentiment which has been pervasive in past ethnographic perspectives of Australia, e.g., *Terra nullius*. For example, the Geographer Griffith Taylor suggested that the interior of Australia was totally "uninhabitable" and "almost useless", propelling notions of environmental determinism (Pawson, 2009, sec. Austral(as)ia, idea of).

2.2 Agriculture or Cultivation?

i. Definition and origins of agriculture

Agriculture can be most straightforwardly defined through farming, which is "the land-based, human-managed production of food and fibre by the transformation of seed into crops and/or the raising of livestock" (Guthman, 2009, sec. Farming). Domestication is considered a key tenet of farming. It refers to the process by which humans artificially

select plants with desirable traits for cultivation, over time leading to genetic modifications forming a “crop” (Whatmore, 2009, sec. Domestication). Indigenous Australian food plants are not considered domesticated, as it is unknown whether Indigenous food plants have been artificially selected for desirable traits leading to genetic changes. Under this combined definition, Indigenous Australians were not agriculturalists. However, it is recognised that Indigenous peoples extensively managed environments to increase food availability (Gammage, 2011; McNiven et al., 2015; Smith, 2001, 1998) and practised cultivation of certain food plants (Goth, 2008; Gott, 1983, 1982).

In Australia, ethnohistorical and archaeological evidence of milling and cultivation suggests ancient Indigenous peoples can perhaps be described as “hunter-gatherer-cultivators” (Keen, 2004, p. 94). More broadly, cultivation can be used as a term to describe Indigenous food-landscape management practices, applied as an umbrella term capturing aquaculture (e.g., Budj Bim Cultural Landscape in western Victoria) and the tillage and re-planting of plants, Yams in particular (Keen, 2006) (see Fig 7). All activities that have been recorded as employed by Indigenous peoples in ethnohistorical, archaeological and colonist records.



Figure 7 Sketch of Indigenous women digging for Murnong using a Yam Digging Stick (Wedge, 1835).

Thus, following a traditional definition of agriculture and excluding fire-stick farming and Indigenous Australian cultivation, agriculture can be suggested to originate (albeit spatially mixed globally) around the Levant “Fertile Cresent” in the Neolithic Revolution ~11 ka. Under the assumption that Indigenous Australians have practised some level of fire-stick

farming since initial occupation ~65 ka, becoming more prominent in charcoal records in the last ~10 ka (Constantine et al., 2023), there is a provocative argument to suggest while they could be among the world's oldest cultivators, they were not agriculturalists. Positioning this in a global context, the major events in ancient human plant cultivation alongside key dates in ancient Australian history are given below for reference (Tab 1).

Table 1 Globally important cultivation events in positioning the possible onset of Australian cultivation practices. Adapted from World History Encyclopedia (2023). Other sources: (Marean et al., 2007; McNiven, 2017; Mercader, 2009; Snir et al., 2015).

Approximated Ages (ka)	Globally Important Cultivation Events in Positioning the Onset of Australian Cultivation
200	Anatomically modern humans appear in Africa
105	Evidence of seed grinding and consumption of wild grains in Africa
65	**The first people arrive in Australia**
48	The first people arrive in Europe (for reference)
40	Indigenous Australians occupy south-eastern Australia
23	**Small-scale cultivation of cereals in modern-day Israel**
12	Nascent moves towards agriculture begin in the Middle East
11	**Cereal cultivation in the Fertile Crescent**
9.6	**Cereal domestication in the Fertile Crescent**
8.9	Cultivation in North America
8.4	Pea cultivation in the Eastern Mediterranean
7.9	Manual Irrigation emerges, and Neolithic Greek agriculture
6.9	Farming in Mesopotamia and Egypt, terraced rice farms in China, pea

cultivation in Europe

6.6 Eel traps at Budj Bim heritage area show Indigenous Australian aquaculture

6.4 Invention of the plough

5.4 Farming spreads across Europe

4.9 Potato farming in the Andes

4.6 Corn cultivation in Mesoamerica

ii. Sedentism

Sedentism is often described as an integral part of agricultural transitions, a product of increased food security brought through farming and domestication. It is noted that the Indigenous peoples of western Victoria show some of the highest levels of sedentism across Australia (McNiven, 1998; McNiven et al., 2017; Sutton and Walshe, 2021, p. 125). In the Corangamite basin where Lake Colac (study lake) is found, it is suggested that Indigenous peoples settled around the lakes in Summer-Autumn when the wider landscape became dry, relying on fish caught in Lake Colac. In Winter-Spring, larger communities split into smaller groups occupying the wider landscape with more readily available water, transitioning to Murnong-based diets, gathering other food plants and hunting land animals (Bride, 1898; Frankel, 2017). Archaeological transects around Lake Colac reveal it as a key gathering point in the Corangamite Basin and relate this to its low salinity relative to the other Corangamite Lakes (McNiven, 1998).

2.3 Murnong (*Microseris* spp.) – a key Indigenous food plant in south-eastern Australia.



Figure 8 Microseris flowers from the left: M. scapigera, M. walteri, and M. lanceolata (approx. 2-5cm wide) (EditorAndrew, 2021a, 2020a, 2020b)



Figure 9 Microseris tubers from the left: M. scapigera, M. walteri, and M. lanceolata (Brown, 2021; EditorAndrew, 2021b, 2021c)

Microseris spp. is a genus of flowering plants within the tribe Cichorieae found native to Australia, New Zealand, and North America (Figs. 8-9). In south-eastern Australia, the genus comprises three native species: *M. lanceolata*, *M. scapigera*, and *M. walteri*. Murnong likely describes all three species of the genus *Microseris* spp. native to Australia, as it is unknown whether Indigenous peoples were aware of the three congeners, which all have edible and tolerably flavoured tubers (Fig 9). Murnong is described in colonial and Indigenous ethnographic records as a food plant by peoples in temperate south-eastern Australia outside of the arid millstone-grain zone (Fig. 10) (Smith, 2013; Tindale, 1974) crudely referred to as a “grain belt” (Fig. 10B) (Pascoe, 2014). It is recorded that the tubers of the three species were cultivated and considered a staple food for First Nations Peoples in south-eastern Australia. It is suggested that Murnong requires human intervention to proliferate, specifically Indigenous cultural burning and digging practices (Gott, 1983, 1982), often employing a ‘Yam Digging Stick’ (Figs. 7,11). The introduction of sheep grazing and cattle trampling and competition with exotic Cichorioideaen plant introductions after colonial invasion in 1788 significantly reduced the range of Murnong, particularly in south-eastern Australia. For a more comprehensive profile of Murnong, refer to (S1).

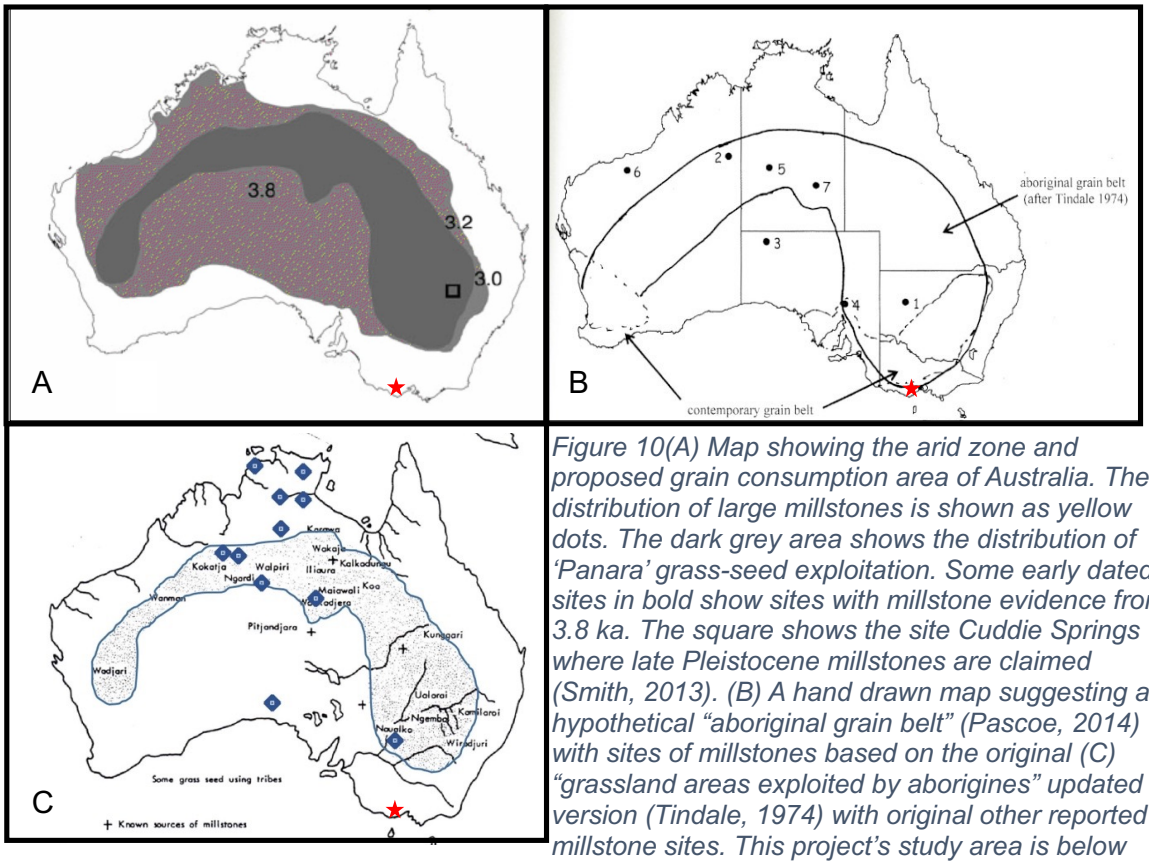


Figure 10(A) Map showing the arid zone and proposed grain consumption area of Australia. The distribution of large millstones is shown as yellow dots. The dark grey area shows the distribution of ‘Panara’ grass-seed exploitation. Some early dated sites in bold show sites with millstone evidence from 3.8 ka. The square shows the site Cuddie Springs where late Pleistocene millstones are claimed (Smith, 2013). (B) A hand drawn map suggesting a hypothetical “aboriginal grain belt” (Pascoe, 2014) with sites of millstones based on the original (C) “grassland areas exploited by aborigines” updated version (Tindale, 1974) with original other reported millstone sites. This project’s study area is below the grain belt and is denoted by red stars.



Figure 11 Pointed end of a Yam Digging Stick (Nugent, 2006).

All studied native Cichorioideae taxa for Victoria, including non-food plants, have been compiled from the Compositae database (Gostel and Bonifacino, 2020) and are listed below (Tab 2).

Table 2 Native Cichorioideae taxa for south-eastern Australia, separated by the category of food or non-food.

Taxon Name (genus and species)	Category
Microseris lanceolata	Food
Microseris scapigera	Food
Microseris walteri	Food
Acites megalocarpus	Non-food
Cymbonotus lawsonianus	Non-food
Picris angustifolia	Non-food
Sonchus hydrophylous	Non-food
Taraxacum aristum	Non-food
Picris angustifolia subsp. angus	Non-food

i. A recorded story of Murnong

Evidence of Murnong consumption, harvesting and cultivation through means such as fire are found in the accounts of Australia's early European colonists. Murnong is said to have been highly abundant in the Plains of Victoria. Gammage (2011) notes that Murnong grew in dry places, high country, and open forests. However, Murnong grew most abundant in the open plains from Melbourne to Mt. Gambier, a region known as the Basaltic Plains. Famously describing the Basaltic Plains, Augustus Robbinson records another settler's description that there…

"were millions of murnong or yam, all over the plain" (Clark, 2014, p. 156).

Thomas Mitchell documents an abundance of Murnong in the Basaltic Plains.

"open grassy country, extending for as we could see, hills round and smooth, meadows broad, and either green as an emerald, or of a rich golden colour from the abundance, as we soon afterwards found, of a little ranunculus-like flower [Murnong]" (Mitchell, 1839, p. 564).

Soon after, he finds a sole Indigenous woman digging the Murnong "of this splendid valley, resembling a nobleman's park on a gigantic scale" (Mitchell, 1839, p. 566). George Angas similarly witnesses Indigenous women digging for yams and roots, finding…

"a woman, and a mother, wandering in search of roots, with her digging-stick in her hand" carrying "a heavy load at her back" (Angas, 1847, p. 75).

Importantly, Murnong cannot proliferate to an extent where there are "millions" of individuals without some level of human intervention (Gott, 1983, 1982). This concept is contextualised through another description of Robbinson, who recorded that…

"Today the native women were spread out over the plain as far as I could see them, collecting punimim, murnong…" noting that they "burn the grass to better see the roots"(Clark, 2014, p. 326).

It is unknown to what extent the burning of grass to expose Murnong and other food herbage provided some nutrition benefitting the growth of new Murnong and if the Indigenous women were aware of this. Despite this, it is well documented that cultural burning was a tool for hunting and, in the context of this investigation, a tool to assist plant foraging, improving yields. Without frequent low-intensity fires, *Acacia* and Myrtaceous shrubs encroach and smother herb and tuber foods (Jones, 2012). Gammage (2011, p. 158) suggests that "suitably timed fires can trigger species to regenerate", sprout, flower, germinate, and seed. Edward Curr noted that Indigenous people near him lived…

"principally on wild roots and animals" and "tilled his land and cultivated his pastures with fire" (Curr, 1883, p. 189).

Pyne (1991) notes that in southeast Australia, the range of Murnong can be increased with suitable firing techniques. Pyne (1991) quotes George Angas, who observed the deliberate burning of shrubs as part of Murnong harvesting…

"In order to get more easily at the roots in the underwood and scrub", the Indigenous peoples "set fire to the 'bush' in many places; when the fire is extinguished, they dig up the roots." (Angas, 1847, p. 232).

Like many others who witnessed cultural burning activities, Angas frequently describes the "luxuriance" of the new herbage that sprouts from the burnt landscape. Fire was both a precious and dangerous tool for cultivating and harvesting roots (including Murnong), running the risk of destroying the aboveground plant and making root harvesting and re-planting difficult. It is documented that Indigenous peoples were acutely aware of this and often smothered fires, preventing food landscapes from indiscriminate fire (Pyne, 1991).

Like using fire to locate and clear space for abundant Murnong growth, knowledge of careful tillage and re-planting is documented for the Indigenous women who harvested Murnong (Keen, 2006). For example, on a gentle ridge at Sunbury Asylum, Melbourne Issac Batey extensively notes…

"The soil is a rich basaltic clay, evidently well fitted for the production of myrnongs. On the spot are numerous mounds with short spaces between each, and as all these are at right angles to the ridge's slope it is conclusive evidence that they were the work of human hands extending over a long series of years. This uprooting of the soil, to apply the best term, was accidental gardening, still it is reasonable to assume that the aboriginals were quite aware of the fact that turning the earth over in search of yams, instead of diminishing that form of food supply, would have a tendency to increase it." (Frankel, 1982, pp. 43–45).

In this self-contradictory statement, Batey first describes the deliberate human intervention with a non-random layout of plants and soil tillage around the plants, not to uproot them to preserve and increase the yields of Murnong. Then perhaps a result of his colonially biased perceptions of Indigenous peoples suggests this form of "gardening" (cultivation) was accidental, despite it being the product of "a long series of years" of deliberate human intervention.

Murnong is described elsewhere in Victoria with varying levels of abundance. In Melbourne, it is suggested that Murnong was apparent on the flats north of the gentle slopes of Batman's Hill (now Collin's Street). The hill itself appeared "cultivated… and that a crop of grain had been sown on it" (Russell and Brown, 1935, p. 80). Above the now relict Yarra Falls (a part of the Yarra River near The Koorie Heritage Trust and Arts Centre

Melbourne buildings) existed an abundance of Murnong, particularly on the banks of streams within the Yarra swamp complex north of the falls where “Wallaby and other grasses, herbs and murnong swathed the riverbank” (Gammage, 2011, p. 304). For example, at ‘Blue Lake’ (now part of the Yellow Gum Park), McCrae explains that Murnong was so abundant that…

> *“the whole air was heavy with the mingled odours of the golden myrnong flowers and purple-fringed lilies” (McCrae, 1912, p. 117).*

It is known that Murnong was consumed raw but also cooked in earth ovens (Gott, 1983). The practice of preparing earth ovens for Murnong (and other foods) is described by James Dawson.

> *“Ovens are made outside the dwellings by digging holes in the ground, plastering them with mud, and keeping a fire in them till quite hot, then withdrawing the embers and lining the holes with wet grass. The flesh, fish, or roots are put into baskets, which are placed in the oven and covered with more wet grass, gravel, hot stones, and earth, and kept covered till they are cooked” (Dawson, 1881, p. 17).*

Further to recorded evidence of Murnong consumption, there is perhaps a biological signature of Murnong consumption through an understanding of modern Indigenous health. It is said that the taste of *M. lanceolata* and *M. scapigera* is tolerably bitter, whereas *M. walteri* has a sweet taste (Walsh, 2016). The tubers of *M. lanceolata* contain a high proportion of fructans and are similar to commercial crops in their proportion of stored carbohydrates (Incoll et al., 1989). The high retention of fructans and carbohydrates in Murnong and other Indigenous roots is thus interesting, considering modern Indigenous People’s susceptibility to insulin-resistant diabetes when following urban western diets (O’Dea, 1991).

European colonists also consumed Murnong on some occasions, mostly when provisions ran out. At a Camp on Indented Head (Bellarine Peninsula, Melbourne), John Batman’s expedition crew ate Murnong to avoid starvation, William Todd of the expedition notes…

> *“We have commenced eating Roots the same as the Natives do” (Dawson, 1881, p. 17)*

Similarly, after escaping captivity as a convict near Sorrento, Victoria, William Buckley lived with the Wathaurong people for an extended period and details consuming Murnong describing…

"a particular kind of root the natives call Murning — in shape, and size, and flavour, very much resembling the radish" (Morgan, 1980, p. 91).

The nutritional value of Murnong is recognised in 'The Select Committee On the Condition of The Aborigines – 1845' in New South Wales by James Malcolm noting…

"There is a nutritious root which [Indigenous peoples] eat and are fond of; and that, I think, has greatly diminished, from the grazing of sheep and cattle over the land, because I have not seen so many of the flowers of it in the spring as I used to see. It bears a beautiful yellow flower. The native name of this root is 'murnong'" (Moller, 1845, p. 13).

Interestingly, Malcom presents he has witnessed a decline in Murnong that he attributes to cattle and sheep grazing/trampling. Within the same committee meeting, Frederick Almond Powlett attests also that the Indigenous peoples of Victoria's…

"ordinary means of subsistence has diminished in the open located parts of the district, as game generally leaves the country where stock is depastured, and the sheep by clipping the grass make it more difficult to discover the yams; but the natives on the banks of rivers like the Goulburn and Murray have much the same means of subsistence as formerly…" (Moller, 1845, p. 42).

Elsewhere, Mitchell notes a cattle and sheep-driven decline of Murnong, stating…

"the cattle are very fond of the leaves of this plant, and seem to thrive upon it" (Mitchell, 1839, p. 676).

While Murnong or Yam Daisies are explicitly referred to in many colonist accounts, there are more numerous accounts of settlers marvelling at the herbage around Melbourne and Victoria, which likely included several food plants, including Murnong. For example, John Fawkner claimed his men were excited to land at Victoria from Tasmania, finding…

"the beauty of the country. The velvet-like grass carpet, decked with flowers of the most lively hues, most liberally spread over the land" (Bonwick, 1856, p. 56).

Fawkner scribes another settler's recordings who notes…

"a continuation of good land with plenty of good grass and herbage [at Port Phillip – Melbourne]" (Greig, 1928, p. 113).

However, being unable to elucidate what genera were growing as part of these common herbages means little can be said about their usage as potential plant food meadows.

ii. Reconstructing Murnong

Colonists' accounts allude to a dramatic decline of Murnong due to cattle and sheep introductions. Using density analysis to map known records of Murnong (Fig. 12), dated from 1770-present, from the Living Atlas of Australia (ALAS, 2023). Shows that in areas like the Basaltic Planes where Murnong was potentially abundant, it is almost unrecorded, potentially as land has been converted to European-style agricultural lands and an increased recording of the abundances of Murnong elsewhere driving density analyses, e.g., in the past swamps of northern Melbourne. While the ALAS records and colonial accounts of Murnong are helpful in indicating the recent past to the current presence of Murnong, they cannot be used to quantify Murnong's presence or provide insight into the species before colonial invasion.

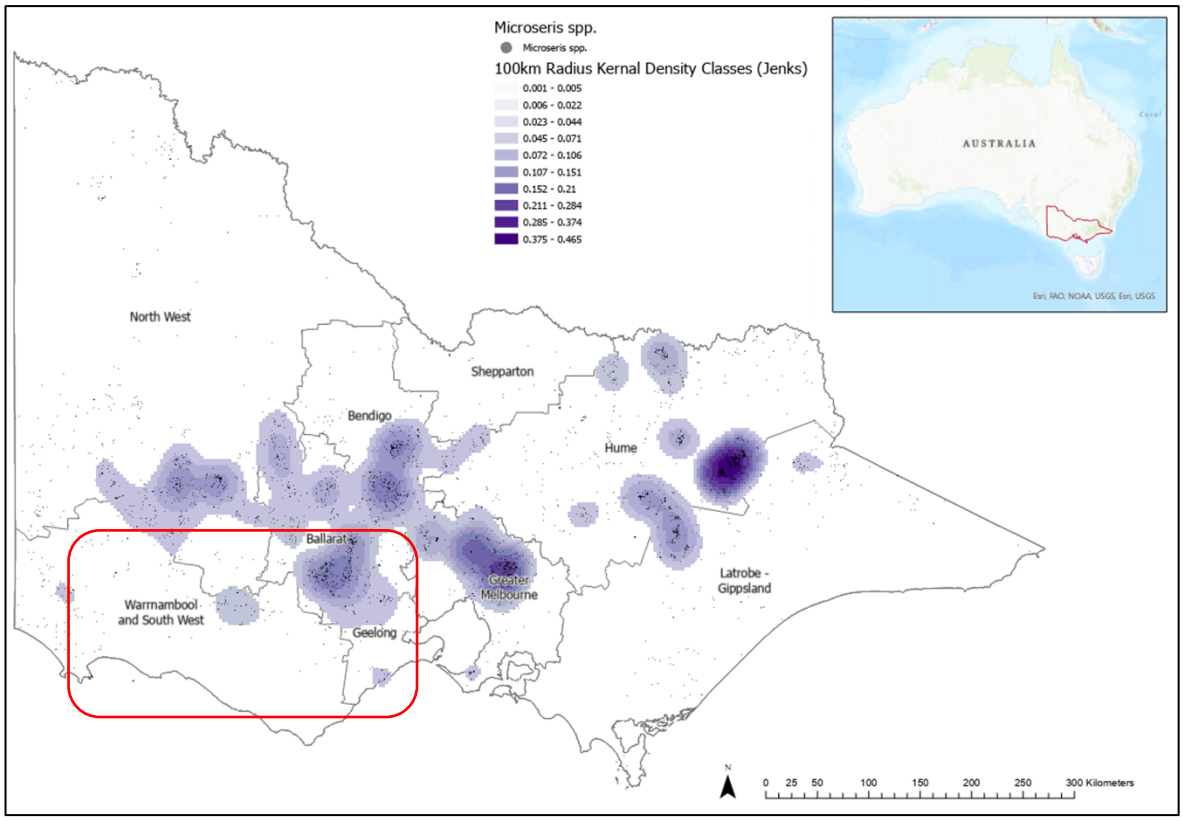


Figure 12 Murnong density mapping for Victoria using all available Microseris records on the Australian Living Atlas database (ALAS, 2023). The broad Basaltic Plains region falls within the red outline.

An attempt to use palynological methods to reconstruct the history of Murnong has been attempted for Tasmania (where Murnong was also consumed). However, it is limited to a sub-family level of identification of fenestrate pollen as Cichorioideae (Macphail and Woodward, 2017). Other studies have begun to classify the morphology of fenestrate pollen, including *Microseris* spp., to start to discriminate species (Feuer and Tomb, 1977; Wenger, unpub). However, this research is both limited and unpublished, respectively. Moreover, the fine-scale morphological features of fenestrate pollen are often degraded when found in sedimentary records.

Within the multifaceted context of Murnong, an important Indigenous food plant, harvested, cultivated with fire, and cooked by women across south-eastern Australia and once found in open grass pastures and swamp-floodplains proliferated by landscape-level cultural burning, I intend to provide a novel method to reconstruct its ancient extent using pollen analysis and chemotaxonomy.

2.4 Palynology

Palynology is the study of organic-walled microfossils between 1-500µm (micrometres) and has been studied in its most basic form since the creation of the microscope. The interpretation of pollen and other sporomorphs to give meaningful insights into the pollen source environment is relatively new (Faegri et al., 2007; Halbritter et al., 2018). Pollen analysis, specifically regarding vegetation reconstruction, has been studied since 1916 (Ibid., 2007; 2018). Following Tobler's First Rule of Geography (Hepple, 2009; Tobler, 1970), pollen analysis assumes that pollen counts primarily reflect vegetation cover local to the source site of the pollen assemblage. Pollen analysis has its roots in the rise of microscopy, but the concept of pollination was potentially known by the Assyrians long before the invention of the microscope (Halbritter et al., 2018). Modern pollen analysis is studied regarding ecology, plant taxonomy and speciation processes, animal behaviour, particulate dispersal mechanisms, allergies, analogues for palaeoecology, and even criminology (Faegri et al., 2007; Halbritter et al., 2018; Kapp et al., 2000). However, for this research, only ancient pollen and how it informs us in palaeoecological and archaeological contexts are of concern. Hereafter, references to pollen analysis in this research refer to the reconstruction of ancient vegetation.

Pollen is the vessel that carries the male gamete of seed-producing plants. Pollination is essential as it is the vessel for gene flow between most populations of plant taxa (Sork and Smouse, 2006). Pollen is well studied due to its exceptional preservation, resulting

from the resistant sporopollenin wall material of pollen grains. Pollen is transported via zoophilous (animal) and anemophilous (wind) methods, with grain structures that generally represent the selected dispersal method (Chisholm, 1911; Faegri et al., 2007; Tonsor, 1985). Generally, in arboreal parts of the northern hemisphere, anemophilous taxa are more prevalent than zoophilous taxa in sedimentary stores (Faegri et al., 2007). However, the nature of each pollen record shifts the balance of zoophilous and anemophilous presence. For example, honey (as an archive) is dominated by zoophilous taxa (Faegri et al., 2007; Halbritter et al., 2018). Similarly, it would be common to see a high presence of aquatic dispersing taxa for sedimentary records in wetland areas. For Australia (including Tasmania), the pollen dispersal regime has a high proportion of zoophilous selected dispersers (Mariani et al., 2022, 2017, 2016).

Lennart von Post created the first pollen diagram in 1916 (Manten, 1967). This seminal research presented ancient arboreal pollen from Swedish-Danish peat bogs in a stratigraphic style diagram (pollen diagram) (Fries, 1967). As a principal aim of Palynology since its inception was the quantified reconstruction of vegetation through time, no other single advancement in the discipline had much of an effect on pollen analysis as it is studied today. In the subsequent ~100 years following the creation of the pollen diagram, the aim of quantifying outputs of sedimentary pollen research slowed, compounded by biases in the interpretation of sub-fossil pollen assemblages (Edwards et al., 2017). It is important to understand that many of the forcings (Fig. 13) that affect the interpretation of the sub-fossil pollen record are interrelated. For example, phenological pollen releases interact with seasonal atmospheric conditions and sedimentation rates, thus altering pollen dispersal into records and the pollen assemblage.

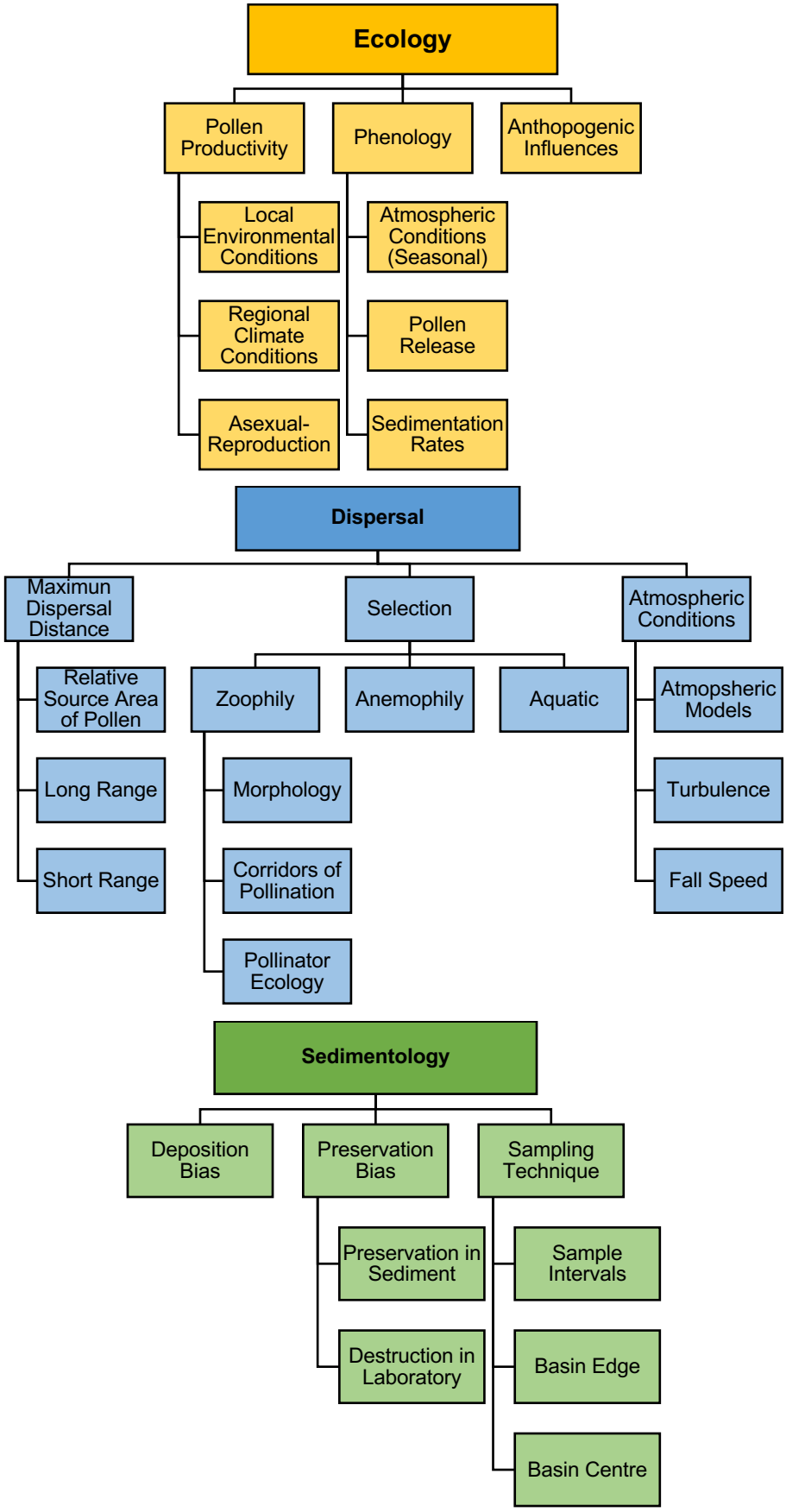


Figure 13 Key points to address for quantitative pollen analysis under Ecology, Dispersal and Sedimentology categories. Many nodes can be overlapped across categories; here, they have been represented in as few instances as possible for brevity.

2.5 A taxonomic resolution problem

Despite the many parameters that reconstruction models can account for, another key obstacle exists more broadly within Palynology: poor pollen taxonomic resolution. Pollen analysis is usually undertaken using standard tools: a biological microscope with a magnification of 400x-600x; a modern reference collection of pollen images e.g., PalDat (PalDat, 2000), ALAS (Australian National University, 2023) and The Global Pollen Project (Martin and Harvey, 2017); and where available, a key for regional pollen morphologies e.g., The Textbook of Pollen Analysis (Faegri et al., 2007) or the Pollen and Spores handbook (Kapp et al., 2000). This itinerary is standard for most Palynologists; however, it limits the confident identification of many pollens. It is common for most palynological studies to identify most taxa at only a genus-family level. In many cases, this is more than appropriate to understand ancient vegetation where a community-level identification of pollen alongside the identification of other indicator taxa can provide a means for interpreting pollen assemblages. The concept of niche overlap, where multiple taxa share similar environmental preferences, alongside using specific indicator taxa, has been combined to infer ancient agricultural landscapes (Faegri et al., 2007).

The cause of poor taxonomic resolution within the pollen record is due to the indistinguishable and shared morphologies of many pollen grains. Moreover, pollen morphology often does not coincide with plant taxonomies (Faegri et al., 2007). The term cryptic can be applied to taxa like *Microseris* spp., where the pollen grain morphology is indistinguishable from other species within the same genus or family using standard palynological tools (e.g., Julier et al., 2016).

i. Image-based methods of cryptic pollen identification

Several methods have attempted to distinguish cryptic pollen. Still, they are unsuitable for vegetation reconstruction, being either time-consuming and expensive or not providing a high enough morphological resolution to identify cryptics consistently. Methods like bright field, dark field, phase contrast, and Scanning Electron Microscopy (SEM) allow the visualisation of pollen and features not readily visible under standard light microscope. However, these methods rely upon the visual identification of pollen by the counter using known pollen references, much like traditional pollen microscopy, unless used alongside other image analyses (e.g., Sevillano et al., 2020). Novel machine learning image-based classification methods like Convolutional Neural Network based pollen identification (see

Table 1 in Sevillano et al. (2020) for an overview of all classifier types), are rapid and accurate for pollen counting (Polling et al., 2021; Sevillano et al., 2020).

ii. Non-image methods of cryptic pollen identification

Alternative methods can be used to identify pollen, which are not restrained by cryptic pollen morphologies. DNA analyses are applicable for pollen species identification where the genetic information has not degraded (Baksay et al., 2020; Bell et al., 2017; Parducci et al., 2005). For palaeoecological investigations, most of the genetic information of pollen has degraded and isolating single grains for sedimentary deposits is unfeasible. Recently, methods that analyse pollen luminescence properties under different light fluorescence conditions can be used to identify pollen. Fluorescence-lifetime imaging microscopy has been used to identify acetolysed pollen of Poaceae (cryptic pollen) based on the decay rate of sporopollenin fluorophores, suggesting differences in fluorophore decay result from interspecific variation rather than phenological factors (Hu et al., 2023). Elsewhere, the differences in the blue-green spectra of bulk pollen images taken with fluorescence microscope filters have also been considered to provide interspecific signals and may improve the taxonomic resolution of cryptic pollen (Mitsumoto et al., 2009). However, the most widely researched spectral method that aims to identify cryptic pollen is Fourier Transform Infrared (FTIR) and Raman spectroscopy.

2.6 FTIR

FTIR is a technique used to identify the molecular structure of materials. In FTIR transmission scans, organic chemical bonds of materials are identified by analysing the vibrational patterns of different bonds when excited at varying infrared wavenumbers. Each wavenumber forms an individual “scan” of the material; when each scan is compiled by the mean absorbance value into a composite, it forms a spectrum, allowing spectral analyses (Griffiths et al., 2007).

FTIR analyses have been used across the palaeosciences (Chen et al., 2015). FTIR spectra of biological materials have been applied across climatological, archaeological and geoscience studies. In a palaeoclimatological context, the FTIR spectra of ancient sporomorphs have been used as a proxy for paleo UV-B flux by interpreting changes to the aromatic (C=C) peak at the wavelengths~1510 and 1520 cm^{-1} (Bell et al., 2018; Chen et al., 2015; Fraser et al., 2014; Jardine et al., 2016; Lomax et al., 2008). Similarly, FTIR spectra can be used to estimate total organic carbon and biogenic silica from lacustrine

sediments (Liu et al., 2013). FTIR has been used in archaeological contexts to distinguish ancient natural and anthropogenic burning regimes from phytoliths (Esteban et al., 2023; Rodríguez-Cintas and Cabanes, 2017). In Australia, FTIR has been suggested as a possible tool to reconstruct palaeofire intensities and the onset of cultural burning activity (Constantine et al., 2023; Lu et al., 2022). Additionally, FTIR has been used to glean information on ancient cultivational practices through analyses of plant residues on Palaeolithic stone tools (Longo et al., 2022; Monnier et al., 2017). In human osteoarchaeology, bones have been characterised using FTIR spectral data, particularly concerning ancient cultural body cooking or heat-stress mortuary practices, like cremation (Legan et al., 2020).

i. Spectroscopic chemotaxonomic classification using machine learning

Chemotaxonomy is the classification of biological material into taxonomies by identifying the material's biochemical compounds. Chemotaxonomy as a concept provides an altogether alternative pathway for the taxonomic ranking and classification of biological material. Chemotaxonomies for ancient pollen provide an alternative that does not rely on traditional morphological identification approaches (Bell et al., 2017; Kraaijeveld et al., 2015). Attempts to characterise pollen using spectroscopic chemotaxonomy have developed in the past two decades (Pappas et al., 2003). Pollen spectral characterisation and classification has been tested on both bulk (Bağcıoğlu et al., 2015; Dell'Anna et al., 2009; Gottardini et al., 2007; Muthreich et al., 2020; Wolkers and Hoekstra, 1997, 1995) and single grains (e.g., Bağcıoğlu et al., 2017; Depciuch et al., 2018; Diehn et al., 2020; Jardine et al., 2021, 2019; Woutersen et al., 2018; Zimmerman et al., 2016; Zimmermann, 2018).

The most promising method to classify pollen involves predictions of FTIR spectral data using Machine Learning Algorithms (MLAs). MLAs generally work by observing patterns within a training dataset of known values. Training a MLA on the patterns and relationships from an input dataset allows a MLA to make predictions on unknown values that are closely related to the known training data. Thus, it is not possible for a MLA to make accurate predictions on unknown values that are non-similar to the input training dataset (Hastie et al., 2009). Two common MLAs that can make predictions are *k*-nn (*k* – Nearest Neighbours) and RF (Random Forest) algorithm. *K*-nn tests how many neighbours (*k*) are in the prediction data compared to the training dataset. The value of *k* can be manually adjusted and represent the number of neighbours that will be used to assign an unknown data point to a known category (Fig. 14) (Cover and Hart, 1967;

Hastie et al., 2009). The RF algorithm's process is slightly different and relies on decision-making through several prediction trees. First, the training data is randomly sampled and split into subsets, each subset as its own decision-making tree with the same number of nodes in each tree, matching the training dataset (there can be slight variation in the number of nodes in each tree, due to the random selection of training data). Effectively, each node in each subset tree is a different attribute used to make decisions in categorising an unknown. When unknown prediction data is passed through an RF model, it passes through each subset tree separately. After each tree has the unknown data filtered through, the majority outcome of all the RF trees is selected as the category for the unknown (Fig. 15) (Breiman, 2001; Hastie et al., 2009; Tin Kam Ho, 1995). Random forest models outperform decision tree supervised learning as they are less susceptible to overfitting the training data (Hastie et al., 2009).

K-nn was the first MLA used in modern pollen FTIR classification by Dell'Anna et al. (2009); RF models have also been applied, for example Diehn et al. (2020). Other classification models, like linear discriminant analysis using canonical powered partial least squares, have also been explored for the classification of pollen based on vibrational spectra (Muthreich et al., 2020).

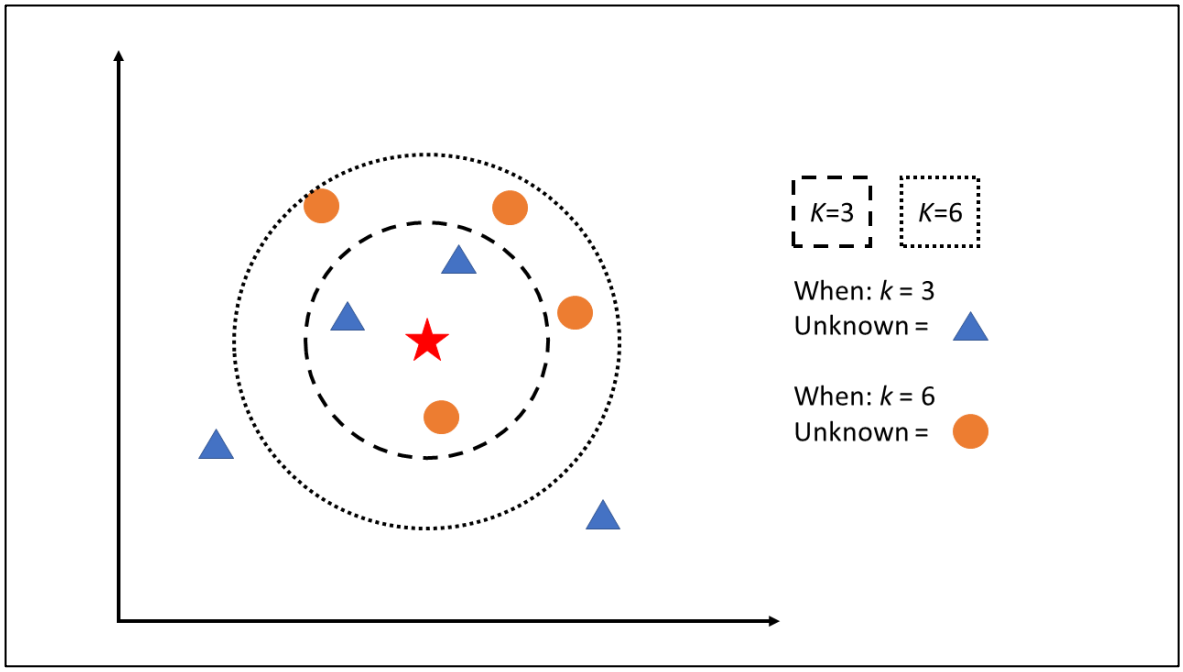


Figure 14 Basic diagram outlining the process of unknown assignment in the k-nn algorithm. The unknown sample is represented as a star, the two know categories are triangles and circles. The k-nn model sorts unknowns into categories based on the Euclidean distance of an unknown to the number of its nearest neighbours (knowns).

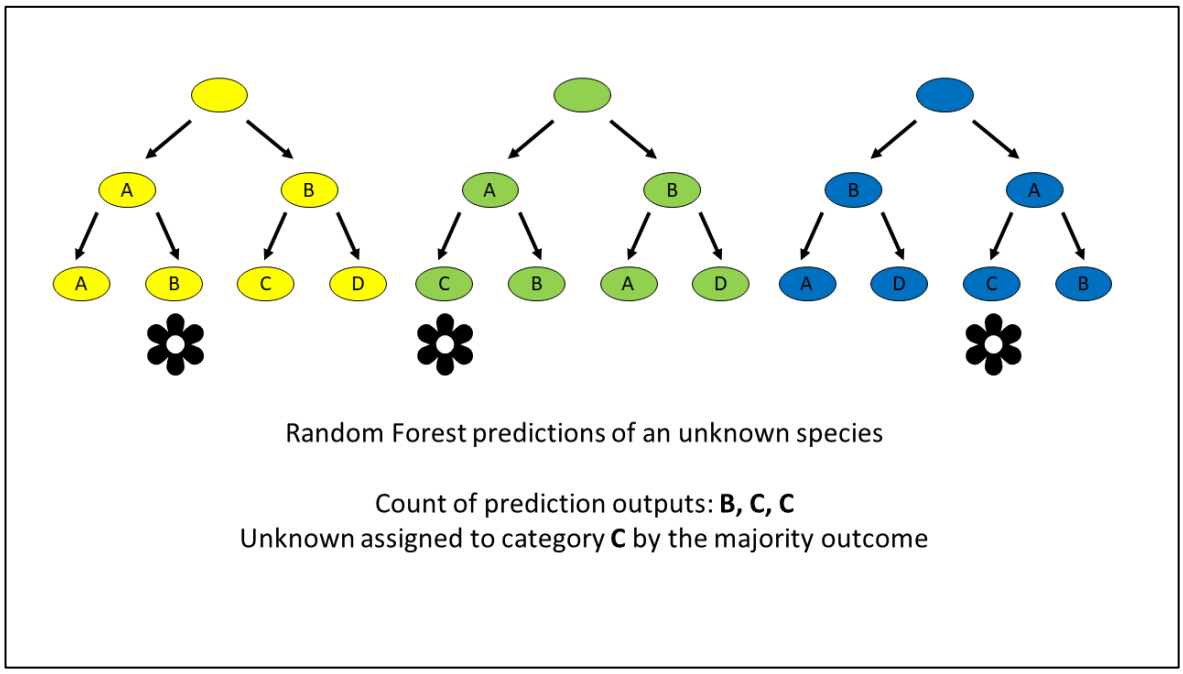


Figure 15 Basic diagram outlining the process of unknown assignment in the Random Forest algorithm. Each node (ABCD) represents a random category selected from the training dataset for which a decision is made in the sorting of an unknown.

Dell'Anna et al. (2009) initially presented the use of machine learning algorithms to identify FTIR-derived pollen spectral data as a powerful tool for characterising and classifying allergenic pollen. Since their seminal paper, attempts to refine and standardise the methodology of FTIR pollen classification have been explored. FTIR pollen characterisation has been completed on bulk samples of modern pollen (e.g., Muthreich et al., 2020) and single modern pollen grains (e.g., Bağcıoğlu et al., 2015; Dell'Anna et al., 2009; Diehn et al., 2020; Jardine et al., 2019; Woutersen et al., 2018). Both single and bulk sample analyses have revealed the accuracy of machine learning model predictions. However, it is noted that multi-grain/bulk samples provide a higher level of classification success when compared to single-grain classification success (Zimmermann, 2018).

Individual pollen grain spectra can be highly variable and not reflective of bulk sample spectra of the same species. This effect results from strong Mie beam scattering occurring largely but not exclusively at the edge of pollen grains (Lukacs et al., 2015), and the orientation of the grain when scanned (Zimmermann, 2018). Studies have attempted to reduce beam scattering by embedding pollen in paraffin, but require complicating spectra pre-processing to reduce a paraffin signal (Diehn et al., 2020; Zimmerman et al., 2016).

ii. FTIR characterisation of pollen in Quaternary sediments

As the biopolymer sporopollenin is highly resistant and is usually the only remains present in fossil and sub-fossil forms of pollen, it is thus possible for chemical spectra to be derived from ancient pollen grains using FTIR (Jardine et al., 2019). For example, the biochemistry of fossilised *Nitraria* spp. pollen dated to the Miocene Epoch has been studied in comparison to extant samples of the same genus. Therein, it is noted that standardisation of pollen preparation treatments in modern training sets and unknown fossil sample sets is key to begin classifying ancient unknown pollen grains to a species level (Jardine et al., 2021).

Pollen retrieved from sedimentary archives is commonly digested with 10% HCl, 10% KOH, and Acetolysis procedures to improve visual identification in traditional pollen analysis (Erdtman, 1960; Faegri et al., 2007; Halbritter et al., 2018). Recent studies have debated how these treatments can alter the spectra of pollen. Acetolysis is suggested to strongly alter spectral peaks, HCL and KOH treatments less so (Wang et al., 2023). Despite this, many elements of sporopollenin biomacromolecule are stable after exposure to acetolysis treatments (Jardine et al., 2019, 2017, 2016, 2015). This is more so when acetolysis treatment occurs for shorter periods and at lower temperatures (Jardine et al., 2015). Treating modern and sedimentary samples with the same treatment (Katsi et al., in review a) offers a pathway to classify ancient pollen using chemotaxonomy, which will be expanded here. Under the assumption the strength of spectral peaks caused by treatments (treatment peaks) is the same in training and prediction datasets, it should not drive classifications, especially if the same peaks are observed in untreated pollen (Katsi et al., in review a). Additionally, acetolysis may improve classification accuracy by reducing Mie scattering (Katsi et al., in review b). Additionally, the RF model allows insight into variable (wavenumber for spectral data) importance in decision-making (Breiman, 2001), which can be used to identify if “treatment peaks” are driving pollen classifications.

2.7 Aspects of pollen analysis quantification

A critical obstacle in quantitative vegetation reconstruction from sedimentary records is the non-linear relationship between stored pollen and the vegetation from which it was sourced. This is a factor of numerous issues, primarily taxonomic differences in pollen dispersal, pollen productivity and preservation that lead to the over and under-representation of different taxa. When studying pollen assemblages in their totality, i.e., examining the trends of all major taxa through time, it is suggested that many of the broad uncertainties of pollen analysis (e.g., Fig 12) are negligible (Faegri et al., 2007; Sugita, 2007a).

i. Using pollen sums and abundances

The pollen sum and percentage transformations are core elements of traditional palynology. The pollen sum is the sum of different taxa investigated within an assemblage, which is crucial for transforming absolute counts into percentages (Faegri et al., 2007). However, as a means of simple quantification, the pollen sum is an essential control on vegetation reconstruction, and even complex reconstruction algorithms must consider the pollen sum (Bunting et al., 2018; Sugita, 2007a). The selection of the pollen sum can be gleaned from the scope of each study. For example, if an archaeological study wished only to reconstruct vegetation dynamics in open arable land, a simple way to portray this would be to exclude arboreal pollen from the pollen sum. Doing such would remove the noise of non-focus taxa from the pollen diagram, and the percentage transformed data would be proportional only to the in-focus taxa (Sugita, 2007a). Similarly, taxa may be sorted into Plant Functional Types (PFTs) for studies investigating broader vegetation changes at larger scales; using PFTs is a key component of the Biomization quantification method (Prentice et al., 1996). While a convenient and straightforward solution for tidying the pollen assemblage for specific inferences, specifying the pollen sum and using abundance data fails to account for many of the key biases in sedimentary palynology.

ii. Correcting for production and dispersal biases

Pollen abundances are not linearly related to the abundance of plants surrounding the pollen store and vary with the number of taxa counted. This non-linear relationship is called the Fagerlind Effect (Fagerlind, 1952). Davis' (1963) R-value marks an attempted movement of pollen analysis from qualitative towards quantitative, which may account for the Fagerlind Effect (Equation 1). R-values are ratios of pollen to the vegetation surrounding the assemblage specified to individual taxa; R-values can be used to interpret ancient pollen as vegetation cover percentages (Parsons and Prentice, 1981). However, it is broadly accepted that the standalone usefulness of R-values in interpreting pollen vegetation relationships is limited. Moreover, quantifying ancient vegetation through R-value calculations ignores all modifying factors influencing pollen assemblages from initial plant flowering onwards (Faegri et al., 2007, p. 118).

$$R_m = \frac{P_m}{V_m}$$

$$V_f = \frac{P_f}{R_m}$$

Equation 1 Basic R-value equation for the interpretation of fossil pollen records. m = modern, f = fossil. R = Representation value (>1 pollen overrepresentation, <1 pollen underrepresentation) P = Percentage of pollen taxa within the assemblage, V_m = Percentage of corresponding modern taxa within surveyed vegetation, V_f = percentage reconstruction of ancient vegetation.

An alternative to the R-value is the coefficient of similarity. Initially introduced by Jaccard (1908). The coefficient of similarity uses the intersection of two sets of data and calculates the cardinality of shared elements between the sets. Extended R-values (ERVs) develop the concept of the R-value and begin to account for some of the obstacles that impede a quantitative reconstruction of vegetation from pollen data (Bunting et al., 2004; Parsons and Prentice, 1981; Prentice and Parsons, 1983; Sugita, 1994). There are three common ERV sub-models which reflect different assumptions about the background pollen component. The ERV is used to estimate Pollen Productivity Estimates (PPEs), which begin to account for taxonomic production bias that affects the abundances of taxa counted within fossil pollen assemblages (Bunting et al., 2013; Prentice and Webb III, 1986). The ERV is a core element of Sugita's (2007a, 2007b) Land Reconstruction Algorithm (LRA), which is used to quantify ancient vegetation extent from wetland pollen records.

Pollen found in sedimentary deposits can be either from local or regional “background” sources. The Prentice–Sugita pollen dispersal and deposition model defines the distance where the background pollen load begins, called the Relative Source Area of Pollen (RSAP). The RSAP is defined where adding vegetation data from a greater distance from the pollen site does not improve the goodness of fit between the Prentice-Sugita model to pollen count and vegetation abundance data for the study region, effectively the distance at which background pollen loads are constant between sites (Bunting et al., 2013; Hellman et al., 2008; Sugita, 2007b, 1994).

2.8 Quantified landcover reconstructions, the REVEALS model, and its alternatives

i. The REVEALS model

The REVEALS model published in 2007 was a key element of the PAGES LandCover6k working group's understanding of Holocene vegetation extent. The model was developed to quantify ancient vegetation abundances by analysing wetland sedimentary deposits at regional scales. The REVEALS model is one half of the LRA, which also encompasses the LOcal Vegetation Estimates (LOVE) model (Sugita, 2007a, 2007b). The REVEALS model is best described through the sum of its parts, particularly the parameters it accounts for (Tab 3). Therein, each part addresses a methodological obstacle. Such obstacles prevented a truly quantitative pollen-based vegetation reconstruction after Von Post's original pollen diagram.

Table 3 Key accounted parameters of the REVEALS model, the causal 'Obstacle' for the parameters, and the parameters that can be defined within the model (Sugita, 2007a; Theuerkauf et al., 2016).

Accounted Parameters	**Obstacle**	**Model Parameters**
Pollen productivity	Taxonomic differences in the productivity of pollen are reflected in sedimentary records. Where some taxa are overrepresented and others underrepresented in raw and percentage counts of pollen, presenting bias in vegetation reconstructions.	Pollen Productivity Estimates are inputted into the model for each modelled taxon and are assumed constant for the region and time periods with the same climatic regime.
Basin size	With reducing the size of the sedimentary basin (lake or bog), the chance for overrepresentation of local taxa increases. Stochasticity of the pollen assemblage increases with increasing basin size; however, a minimum basin size needs to be defined. Doing such ensures preferential deposition of local taxa in the basin is negated, regional pollen	Minimum basin size is defined as 300 meters of shortest radius and is inputted into the model.

	rain is captured in the record, and the model is repeatable.	
Taxonomic differences in pollen fall speed	Differences in the fall speed of pollen affect the deposition potential of different taxa. Not accounting for the fall speed of different taxa introduces taxa bias into the record.	Pollen fall speeds are estimated and used for each modelled taxon.
Maximum extent of vegetation reconstructions	Effected by both the ability of the record to capture long-range and short-range dispersing taxa (a component of basin size and pollen dispersal). The maximum spatial extent of the reconstruction needs to be included in the model to allow inference into the geographic nature of the results.	The model assumes the maximum spatial extent of reconstructed vegetation is a 100km radius from the lake centre.
Atmospheric conditions	Atmospheric conditions like turbulence affect the deposition of taxa into the sedimentary record. Long-range dispersers like pollen with air sacs may be underrepresented in periods of high turbulence being blown further afar but may originate from outside the records pollen source area.	Two general models of atmospheric conditions have been used in the model: the Gaussian Plume Model and the Lagrangian Stochastic Model.

REVEALS has a clear set of assumptions built into the model: 1) when large lakes (≥300m radius) are sampled, any pollen source plant distribution biases are negligible. 2) Most pollen sources are within 50-100km of the sample site. 3) The regional vegetation structure around a lake is homogeneous; if multiple REVEALS reconstructions are used, the model will capture no gradient changes between sites. 4) The sedimentary basin is circular with no canopy cover. 5) Anemophilous transport is the only vector for pollen dispersal. 6) PPEs are regionally and temporally constant and specific for an individual taxon. 7) Wind speed and atmospheric conditions are constant. 8) Pollen is well mixed in surface water before deposition and sedimentation. Critically, the REVEALS model assumes that pollen deposition into a large wetland site equates to the mean percentage

cover of the taxa at a regional scale multiplied by its pollen productivity and the taxa pollen dispersal-deposition coefficient (K). The REVEALS model effectively is the inverse of this assumption and calculates regional vegetation percentage cover from pollen (Theuerkauf et al., 2016). With this clear set of assumptions built into the model, it is repeatable, and any error associated with it is consistent (Sugita, 2007a, 2007b; Theuerkauf et al., 2016a, 2016b). The REVEALS model has since been revised and is part of the DISQOVER R package, where the Lagrangian Stochastic Model (LSM) can be used instead of the Gaussian Plume Model (LSM) to more accurately account for turbulent air conditions and the regional pollen influx it causes (Kuparinen, 2006; Theuerkauf et al., 2013). The general REVEALS equation is expressed below (Eq 3).

$$V_i = 100 \times \frac{n_i / PPE_i K_i}{\sum_{j=1}^{m} n_j / PPE_i K_j}$$

Equation 3 REVEALS model equation (Theuerkauf et al., 2016). Where V_i = abundance of taxon i, n_i = pollen count of taxon i, PPE_i = pollen productivity estimate for taxon i, K_i = 'pollen dispersal-deposition coefficient' for i and m = total number taxa to be modelled (Theuerkauf et al., 2016).

The REVEALS model was initially validated on pollen data for ten large lakes in Sweden; it is noted that the most successful application of the REVEALS model is the reconstruction of structural/functional groups of taxa, e.g., trees, shrubs, and herbs-grasses (Hellman et al., 2008). The REVEALS model has since been applied globally, for example in Europe (e.g., Fyfe et al., 2013; Hoevers et al., 2022; Mazier et al., 2012; Nielsen et al., 2012; Trondman et al., 2016), America (e.g., Sugita et al., 2010) Africa (e.g., Tabares et al., 2021) and China (e.g., Wan et al., 2022). REVEALS has also been extended to multiple uniform small sites rather than using the secondary LOVE model (Fyfe et al., 2013; Mazier et al., 2012; Trondman et al., 2016).

The REVEALS model has been validated in Australia and Tasmania (Mariani et al., 2022, 2017). Australia is dominated by zoophilous selected taxa, which are low pollen producers (relative to anemophilous dispersers). Despite this, PPEs acquired for south-eastern Australia and Tasmania perform well in the REVEALS modelling of vegetation (Mariani et al., 2022, 2017, 2016). Moreover, it is suggested in zoophilous dispersal dominated environments, the LSM performs better than the GPM of dispersal for the reconstruction of zoophilous taxa. LSM accounts for both long-range pollen dispersal (>10km relative source area of pollen) and the dispersal of heavier, generally zoophyl selected, taxa which display secondary anemophilous dispersal under turbulent atmospheric conditions

(Mariani et al., 2016; Theuerkauf et al., 2016, 2013). While the REVEALS model has had broad application at a global scale for its ability to quantifiably reconstruct ancient regional vegetation, it is poignant to understand there are several alternative methods to reconstruct past vegetation, each with individual drawbacks and assumptions.

ii. Other reconstruction methods

While the LRA is one method to achieve quantitative vegetation reconstruction, other techniques can be used to reconstruct past vegetation cover (Edwards et al., 2015). For example, the Modern Analogue Technique (MAT) is a technique that can be applied to pollen reconstructions. Where a database of modern pollen-vegetation data is compared to samples of an ancient pollen record, it assumes modern vegetation is comparable to ancient vegetation cover. When a comparable modern pollen analogue is found for an ancient sample, the vegetation data from the modern pollen-vegetation survey is used as the reconstructed vegetation (Jackson and Williams, 2004; Overpeck et al., 1985). The Biomization method categorises taxa into PFTs based on traits like leaf form, phenology, and environmental adaptations. Modern PFT analogues define pollen-to-biome relationships; thus, Biomization enables the reconstruction of ancient biomes based on the observed pollen assemblage and can be used to map ancient vegetation with multi-site large-scale datasets (Prentice et al., 1996).

Mechanistic approaches like the LRA include the Multiple Scenario Approach (MSA). Like Biomization, the MSA uses Palynological Equivalents (Pes) that are effectively PFTs but can also be an individual taxon. The MSA incorporates spatial analysis using Geographic Information System (GIS) landscape constraints, e.g., topography. The MSA calculates the many possible distributions of past plant communities based on inputted spatial constraints. It then compares the simulated reconstruction against modern pollen-vegetation data for the same location to select the most probable past vegetation mosaic. Like the MAT, the MSA is limited by the availability of modern pollen-vegetation data for the study area. Like the LRA, the Prentice-Sugita mechanism underpins the MSA for accounting for pollen dispersal and deposition biases (Bunting et al., 2018; Bunting and Farrell, 2018; Bunting and Middleton, 2009).

The Extended Downscaling Approach (EDA) is a newly developed mechanistic model for pollen-based vegetation reconstructions. The EDA provides spatially explicit reconstructions of individual species independent from ecological variables, where taxa with different ecological demands may co-exist and overlap within a given space or

landscape gradient. The EDA is not reliant on the assemblage and archive type, allowing a spatially explicit reconstruction from pollen from various wetland sites like forest hollows and lakes in conjunction with other pollen and charcoal data, e.g., archaeological charcoal (Abraham et al., 2023; Theuerkauf et al., 2014; Theuerkauf and Couwenberg, 2017; Theuerkauf and Joosten, 2009).

Given the rich Indigenous oral histories and colonist recordings of Indigenous plant usage, another possible avenue of vegetation reconstruction would be a non-traditional reconstruction based on ethnographic records (e.g., Richer and Gearey, 2018).

Chapter 3: Methods

i. Outline

To achieve the aims of this investigation, two general workflows were followed. Firstly, pollen counted from a large (regional pollen influx capturing) lake record was modelled as part of the REVEALS model. PPEs and pollen fall speeds required for the REVEALS model were obtained from a prior publication which has validated the REVEALS model for mainland Australia's taxa (Mariani et al., 2022). Secondarily, a chemotaxonomic library of acetolysed Cichorioideae pollen FTIR spectra was created, which was used to make predictions on unknown Cichorioideae pollen of the lake sediment. This forked approach is visualised below (Fig. 16) before constituent parts of the methodology are described.

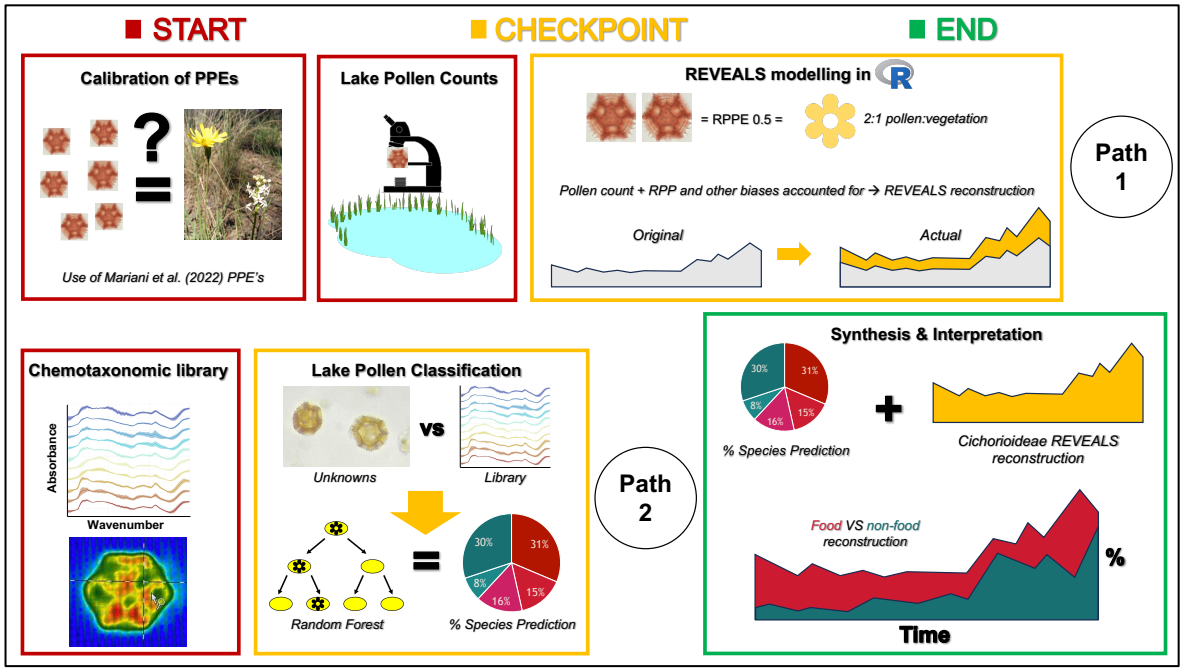


Figure 16 Generalised project workflow separated into two paths, top three nodes represent Path 1, bottom two nodes represent Path 2, the final green node represents the merging of the two workflows.

Data analysis and plotting were performed in R (R Core Team, 2013); stratigraphic pollen diagrams were produced using riojaPlot (Juggins, 2023), and all other plots, including time series diagrams were produced using ggplot2 (Wickham, 2016). Statistical smoothing applied to plots using Generalised Additive Models (GAMs) and Locally Estimated Scatterplot Smoothing (LOESS) statistical smoothing methods were produced using the mgcv, gam (Wood, 2017) and the R stats, loess (R Core Team, 2013) functions. Principal Component Analyses were performed using the prcomp function (R Core Team, 2013). Maps were produced using ArcGIS Pro (ESRI, 2023).

3.1 Study area

i. Lake Colac

Lake Colac's sediment record is studied as part of this research (Fig. 17). Lake Colac is situated within the Basaltic Plains region west of Melbourne. The Plains formed in the late Pleistocene through numerous eruption points, creating large flows of Olivine basalts (Khan, 2003a). Lake Colac is a large Volcanic Depression lake situated at the east of the Corangomite Basin (Mills et al., 2013) within the wider region where Murnong extent has been described in colonist accounts (e.g., Clark, 2014). Today, the region is broadly used for agricultural purposes, largely pastureland.

Lake levels and salinity are primarily functions of the regional evaporation-precipitation controls, a result of the semi-closed nature of the lake and the climate regime of warm-dry summers and cool-wet winters (Winstanley, 2002), with outflow only occurring at times of exceptional periods of rainfall (Frankel, 2017; McNiven, 1998). However, it is noted that groundwater recharge also plays an influence in determining lake levels in the region (Khan, 2003a, 2003b). The lake is relatively shallow and has a maximum water depth of 2.4m. During sampling in 2008, the lake was considered dry. Despite its shallowness and semi-closed nature, Lake Colac is the least saline of the major lakes within the Corangamite basin (Frankel, 2017; McNiven, 1998). Extensive archaeological activity is recorded in the Corangamite Basin, which is centralised on lacustrine sites (Frankel, 2017). Archaeological surveys found Lake Colac exhibited high concentrations of archaeological sites within 100m of the lake edge. Moreover, a relationship between lake salinity and archaeological site density is found within the Corangomite basin, with Lake Colac exhibiting the highest density of archaeological activity within its vicinity out of all the Basin's lakes (McNiven, 1998). Historically, fish were generally obtainable from inflowing rivers feeding the Basin; in the summer, a reliance on Murnong for food is suggested for the peoples of the Corangamite Basin (Bride, 1898; Frankel, 2017). The vegetation type around Lake Colac is suggested to have remained open throughout the Holocene with marginal woodland patches (D'Costa et al., 1989; Mills et al., 2013).



Figure 17 Location of Lake Colac on a (A) regional and (B) national scale. (C) A short axis diameter measurement of the lake.

The lake material was initially presented in 2011 to the Australian Government as part of a government-funded research project and has since been re-published, see Mills et al. (2013). Preliminary investigations suggested Lake Colac was viable for FTIR and vegetation reconstruction analyses of Murnong, providing the first regional background estimates of Murnong extent.

ii. Radiometric chronology

The following description of Lake Colac's radiometric chronology is based on the comments of Mills et al. (2013). ^{210}Pb ages were originally calibrated using the Constant Rate of Supply (CRS) model (Appleby and Oldfield, 1978), which places 1964 as the peak year for nuclear weapons fallout across the southern hemisphere. ^{210}Pb concentrations were determined by gamma emissions at 46.5keV. ^{210}Pb fallout is considerably lower in the southern hemisphere, so sampling for ^{210}Pb is restricted to the most recent sediments of the cores, where detection is most likely. Mills et al. (2013) suggest ^{210}Pb dates are limited ~300 years before present for Lake Colac. More generally, ^{210}Pb is assumed to be limited to 150-200 years (Appleby, 1998; Aquino-López et al., 2018). ^{210}Pb dates extracted are in the depth range of 0-9.2cm; all ^{210}Pb concentrations below this depth are below or near the limit of detection.

Radiocarbon (^{14}C) dates were acquired from bulk organic sediments in the absence of terrestrial macrofossils. Bulk organic sediment dating is less preferred to macrofossil dating as bulk samples may be affected by old carbon, which can mix with newer sediment before deposition, creating a dating unconformity in the stratigraphy. Seven ^{14}C dates were obtained from the lake, all taken below the appearance of *Pinus* pollen in the record. Five of the seven dates are in order of superposition. Two dates (35 and 60cm) were submitted two years after the initial dating of the lake and have likely been subjected to contamination in the laboratory. These were excluded from Mills et al. (2013) and are excluded in this research. ^{137}Cs concentrations like ^{210}Pb are low; no subsurface peak in ^{137}Cs concentrations could be paired with an early 1960s "bomb" event. The highest concentration of ^{137}Cs occurred in the uppermost sample of sediment.

Radiometric dates have been recalibrated to create a new chronology for Lake Colac within the lake sediment (see Chapter 4.1). Radiocarbon ^{14}C dates have been recalibrated using the current calibration curve for the southern hemisphere, SHCal20 (Hogg et al., 2020). Radiometric ^{210}Pb values were also recalibrated using the five basal samples as the marker for unsupported levels of ^{210}Pb. A modelled age-depth curve was created using Bayesian methods using the rplum package (Aquino-López et al., 2018) to estimate ages of depths between dated material by reconstructing sediment accumulation rates, providing a more robust and statistically defined age estimation in compared to older models, e.g., CRS.

iii. Relative chronologies

The available radiometric dates are supplemented with relative dating techniques. The occurrence of exotic pollen taxa in the record acts as a relative timer for the colonisation of Australia in 1788 (Mariani et al., 2022). While radiometric dating supersedes pollen as a dating method, relative pollen dating remains useful where radiometric dates are questionable or absent (Davis, 1984).

Here, Pinaceae (family) pollen is used as a relative dating method for the onset of colonial settlement in Australia. Pinaceae is considered a key taxon for the identification of the colonial invasion of Australia, as there are no "true" pines (Pinaceae) native to Australia. The presence of Pinaceae in a single sample is not alone enough to warrant the onset of colonial activity as Pinaceae is a long-range pollen disperser with air sacs increasing the dispersal range (Faegri et al., 2007). For example, relatively local dispersers like Chenopods are found in Antarctic ice cores (e.g., Burckle et al., 1988). Therefore, an

approach was taken to count the presence from the oldest samples to the youngest ones; if >1 Pinaceae pollen grain is observed in a count of >200 pollen grains per sample, that sample is classed as post-invasion. If only a single grain is counted and another is present within the adjacent sample, they are both considered post-invasion. The first presence of Pinaceae is reported separately in the time series if only a single grain is counted, and adject sample presence is reported if this is the case.

3.3 Vegetation reconstruction

i. Justification for the REVEALS model

The REVEALS model is selected for this study; several key factors drive this decision. Firstly, the research aims to understand the regional extent of a single taxon, Murnong (*Microseris* spp.). Thus, a model designed for autecological reconstructions, e.g., the LRA, MSA and EDA, are preferred to the models with a synecological focus, e.g., Biomization. Secondly, this investigation is preliminary, and thus, it attempts to test the efficacy of a novel method that identifies ancient pollen with cryptic pollen morphology using a testing method of landscape reconstruction. While the REVEALS model reconstruction is coarse for single sites and other multi-site methods may provide higher resolution, e.g., the MSA (Bunting and Middleton, 2009), using a model which can be applied to a single test site for a preliminary investigation is warranted. Finally, the only model that has been tested for its efficacy in Australia's zoophilous dispersal dominated environment for pollen-based vegetation reconstruction is the REVEALS model (Mariani et al., 2022, p. 202, 2017, 2016). Before attempting to quantify a single genus using other models, comparing other techniques to the LRA in Australia may be appropriate.

Lake Colac has a short axis diameter of ~4000 meters, a size appropriate for regional reconstructions using the REVEALS model (Fig. 17C), where the recommended minimum lake radius is ~300 meters (Sugita, 2007a). REVEALS is also suitable for Lake Colac as many smaller volcanic crater lakes surround the lake, which may be incorporated into a full landscape reconstruction as part of LRA's secondary LOVE model. Moreover, it is suggested that the vegetation across the Basaltic Plains has remained open throughout the Holocene and thus conforms to the REVEALS model assumption of regional uniformity – as a homogenous extent of open patchy vegetation (D'Costa et al., 1989; Mills et al., 2013; Sugita, 2007a).

ii. Model parameters

REVEALS modelling was conducted with the REVEALSinR function, using a Lagrangian Stochastic Model (LSM) of dispersal (Kuparinen, 2006) in the DISQOVER package (Theuerkauf et al., 2016). PPEs and pollen fall speeds were sourced from Mariani et al. (2022), the PPEs are relative to the reference taxa *Eucalyptus*. The model was set to run at the default 1000 times per sample level (Theuerkauf et al., 2016).

iii. Pollen counting procedure

Pollen was counted using a SWIFT SW380T trinocular microscope at 400x magnification. Only the RPP taxa (Mariani et al., 2022) and Pinaceae were counted; Cichorioideae (Liguliflorae) was counted separately from Asteroideae (Tubuliflorae) and were summed to create an Asteraceae count for REVEALS modelling. Pollen identification was aided by the Macphail Tasmanian Pollen Reference Collection (Macphail, unpublished) and the Australian Pollen and Spore Atlas (Australian National University, 2023). A minimum of 200 RPP pollen grains were counted per sample (and to the end of the sample, which surpassed 200), not including Pinaceae. A count of over 200 grains ensures the “200 grains rule” was achieved, allowing greater than 75-85% accuracy (Pearsall, 1989, p. 280). Thus, the pollen counted is representative of the entire sample. Slides were counted from the top leftmost corner of the slide and followed a transect across to the right. When the transect ended, the stage would be moved down so that none of the observed material in the first transect was visible; then, the transect would move against the direction of the prior transect. If <200 grains were counted on a single slide, another slide was prepared, and counts were added to the prior slide total. In some cases (n = 2), slides contained no pollen (after two slides were prepared) and were excluded.

3.3. Pollen treatments

Herbarium samples of native Cichorioideae were provided by the National Herbarium of Victoria, Melbourne and the Australian National Herbarium, Canberra (see S2). Treated sedimentary samples were provided by Emeritus Professor Peter Kershaw (see Mills et al. (2013) for the first usage of these materials). Sedimentary material was dispersed in 10% $Na_4P_2O_7$ as a deflocculant, treated with 10% KOH for 15 minutes at ~80°C and then sieved, then treated with 5ml of 10% HCL, acetolysis ~10 mins (Erdtman, 1960), and HF overnight. Liquid residues were evaporated with ethanol G_2H_5O before the samples were mounted with glycerol. Herbarium samples were treated with 10% KOH at ~80°C for 15

minutes, 5ml of 10% HCl, and then acetolysis for ~10 minutes before ethanol evaporation and glycerol mounting (Erdtman, 1960; Faegri et al., 2007; Halbritter et al., 2018; University of Nottingham, 2021).

i. FTIR subsampling

For FTIR analysis, only pre-invasion sediment samples were selected under the assumption that they contain only native Cichorioideaen pollen, as the reference library contains only native Cichorioideae pollen and is not trained to classify exotic Cichorioideae pollen. Sediment pucks from treated samples were subsampled with a pipette and stored in acetone. These FTIR samples were disturbed for 3 minutes, separating the sediment and glycerol, then centrifuged at 4000rpm for 3 minutes. The supernatant was decanted to remove glycerol, and this was repeated until no glycerol residue remained. Samples were then stored in acetone until FTIR analyses.

3.2 FTIR analyses

i. FTIR microspectroscopy collection procedure

FTIR spectral analysis was performed using an Agilent Cary 670 FTIR spectrometer with a KBr beamsplitter mounted to a Cary 610 FTIR microscope with a liquid nitrogen cooled focal plane array detector (School of Biosciences, University of Nottingham). All isolated pollen grains were scanned 64 times per replicate at a resolution of 4 cm-1 in transmission mode; background scans were taken at 128 times per replicate. The pollen was mounted on 0.5mm CaF_2 slides, and the slides were cleaned with acetone between sample mounting. Slide background spectra were collected at 128 scans per replicate before each new sample/species and whenever appropriate within a sample. A chemical spectra reference library for each species is produced using ~40 replicates (after outlier removal). Classification only includes the “fingerprint region” (1800 to ~ 948.81cm^{-1}), which holds the most interspecific spectral variability in pollen. As sediment samples are likely to not be fully isolated, only pollen grains greater than 75% isolated from other materials were considered. In such instances, non-pollen material which overlapped was excluded from the scanning window (70x70µm) to ensure only relevant transmission data were derived.

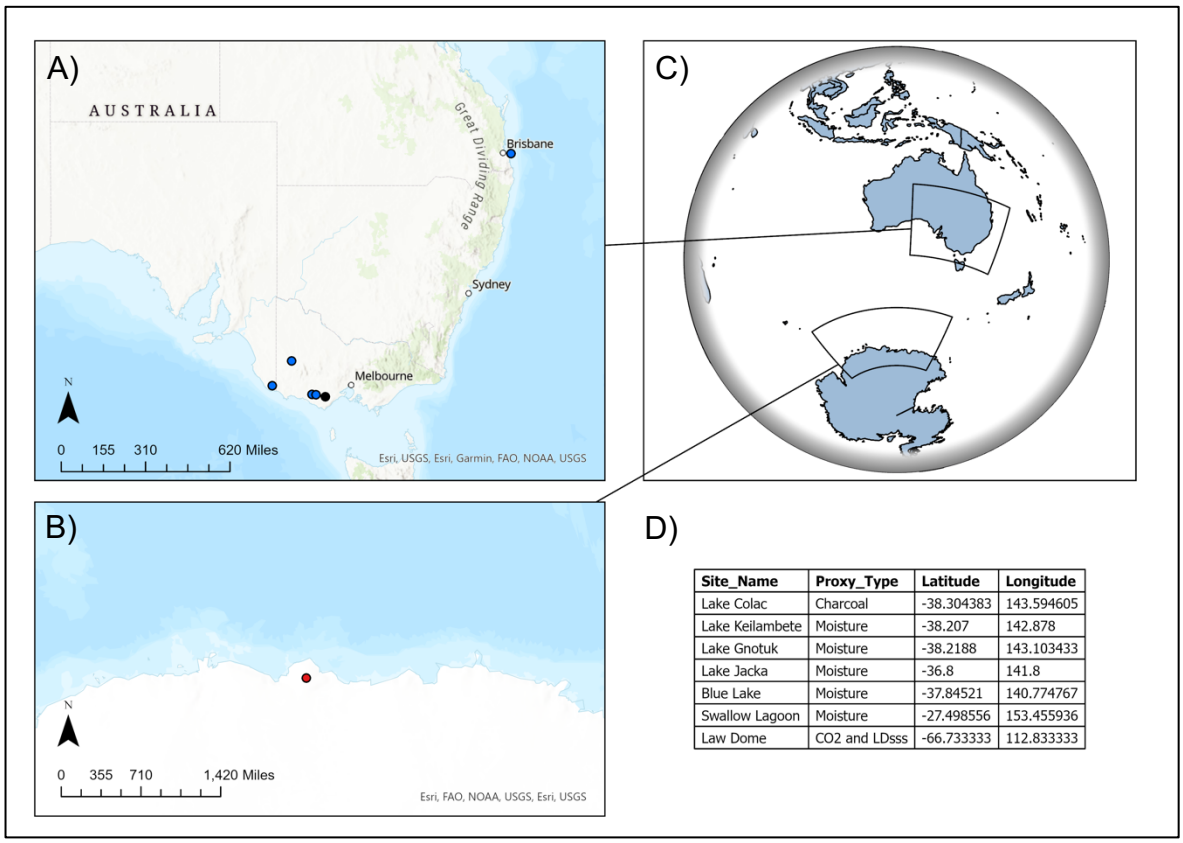
ii. Spectral processing and MLA parameters

Spectral analyses were performed in the Specsplorer software (Kent et al., in prep). All spectra were treated with EMSC to reduce the influence of Mie scattering, which is common in isolated grain analyses (Lukacs et al., 2015; Zimmermann, 2018). Outliers were removed from the herbarium dataset using PCA analyses of spectra within the fingerprint region (1800 to ~ 948.81cm^{-1}). The Specsplorer software allows live interactive viewing of individual spectra while removing outliers, allowing judgement of when to end the removal of outliers, as observed outlier clusters may be a species with a unique chemical spectrum. After outlier removal, each species contained at least 30 replicates in the library.

MLA predictions were completed using the RF algorithm, with the model set to a seed of 1234, with 200 model runs. 80% of the reference library was used for training, and 20% was used as a test set to build the prediction model (Hastie et al., 2009). For spectral pre-treatments, only EMSC was applied to reduce the effect of Mie scattering. While Savitzky Golay (SG) smoothing with higher window sizes and predictions based on derivative spectral data often produces higher classification accuracy (Bağcıoğlu et al., 2017; Jardine et al., 2021, 2019; Woutersen et al., 2018), using the most basic spectral treatments in predictions is appropriate for this preliminary test, allowing more straightforward interpretations of what may drive RF model classifications.

3.4 Paleoclimate data

This research uses available paleoclimate moisture records that cover the Holocene epoch with the future aim of extending pollen FTIR works further than initially presented here. Palaeoclimate data will be interpreted as precipitation-evaporation indicators, fire histories, and southern hemisphere CO_2 emissions. Statistical smoothing methods are applied using LOESS and GAM methods to identify shifts in signals throughout the study period. Palaeoclimate data sources are presented below (Fig. 18) and in the following sections.



Site_Name	Proxy_Type	Latitude	Longitude
Lake Colac	Charcoal	-38.304383	143.594605
Lake Keilambete	Moisture	-38.207	142.878
Lake Gnotuk	Moisture	-38.2188	143.103433
Lake Jacka	Moisture	-36.8	141.8
Blue Lake	Moisture	-37.84521	140.774767
Swallow Lagoon	Moisture	-27.498556	153.455936
Law Dome	CO2 and LDsss	-66.733333	112.833333

Figure 18 Map panel of palaeoclimatic data (non-inclusive of regional charcoal records (Global Palaeofire Working Group, 2023)). (A) Regional view of terrestrial palaeoclimate records in Australia, (B) Location of the Law Dome in Antarctica, (C) Globe view of the extent that maps (A-B) cover, (D) Table of palaeoclimate data types and coordinates. Data sources are described in the following text.

i. Terrestrial palaeomoisture

Lake salinity and level are direct proxies of evaporation-precipitation fluctuations in the regional climate. As many of the lakes in the Basaltic Plains have high average salinity levels, isotopic records of precipitation are more difficult to interpret as the usual ostracod-based reconstructions of these proxies are determined by the presence of salinity-tolerant ostracod species. Therefore, it is more straightforward to interpret evaporation and precipitation fluctuations from sediment mineral salinity measures and lake depth reconstructions. Salinity reconstructions taken from Lakes Keilambete, Elingamite, Blue, and Jacka are considered in this investigation (Barr et al., 2014; Gouramanis et al., 2010; Kemp et al., 2012; Wilkins et al., 2013). Lake depth records from Lake Gnotuk (Wilkins et al., 2013) and Lake Keilambete (Kemp et al., 2012; Wilkins et al., 2013) are considered in this investigation as long-term records of evaporation-precipitation for the study region. A rainfall reconstruction spanning the Holocene is included from Swallow Lagoon (Barr et al., 2019). Records signals interpreted inversely have had the reciprocal calculated prior

to site level Z-score calculation and baseline corrections, creating a composite of terrestrial palaeomoisture.

ii. Fire histories

Local and regional fire histories have been reconstructed from the Lake Colac charcoal concentration record (Mills et al., 2013) and the 190 Australian charcoal records from the Global Charcoal Database and Neotoma (Global Palaeofire Working Group, 2023; Williams et al., 2018). Charcoal concentrations were converted to charcoal accumulation rates (influx) by modelling accumulation rates using chronological information using the tapas R package (Finsinger and Iago-Lito, 2022). Charcoal influx provides a more realistic analysis of historical fire peaks from lacustrine records compared to traditional charcoal concentrations (Higuera et al., 2009).

iii. Proxy records outside of terrestrial Australia

While terrestrial moisture records are preferred to ice and oceanic reconstructions for Australia (Ho et al., 2014). Australia is considered a dust source for some Antarctic ice domes. For example, the EPICA Dome C ice core composite traces some of its dust chemistry to the Murray-Darling basin in south-eastern Australia, linking these two records (De Deckker and Norman, 2010; Lambert et al., 2008; Revel-Rolland et al., 2006). In the absence of more terrestrial moisture records, Antarctic records can be seen as a useful proxy for ancient Australia and complementary to more accurate terrestrial proxies. Here, the Law Dome CO_2 and summer sea salt (LDsss) reconstructions are studied due to their linkages with terrestrial proxies of south-eastern Australia (Curran et al., 2022; MacFarling Meure et al., 2006; Rubino et al., 2013; Vance et al., 2013). To supplement these reconstructions, a 1 ka temperature anomaly ensemble reconstruction for Australasia is presented (Gergis et al., 2016).

Chapter 4: Results

4.1 Chronology

Radiometric date recalibration reveals a somewhat anomalous 55.5cm radiocarbon date, likely due to a hiatus in the core (Fig. 19). This is because the position of the core within the lake is within the littoral zone (which is greatly extended for this lake), whereby in periods of drought, large extents of the lake may not be submerged, slowing depositional processes (Mills et al., 2013). The anomalous 55.5cm sample was thus included as an accepted limitation of Lake Colac's ^{14}C chronology. Despite its inclusion into the model, Rplum shows it has little weighting on the outputted chronology using default settings, automatically excluding it as an outlier (Aquino-López et al., 2018).

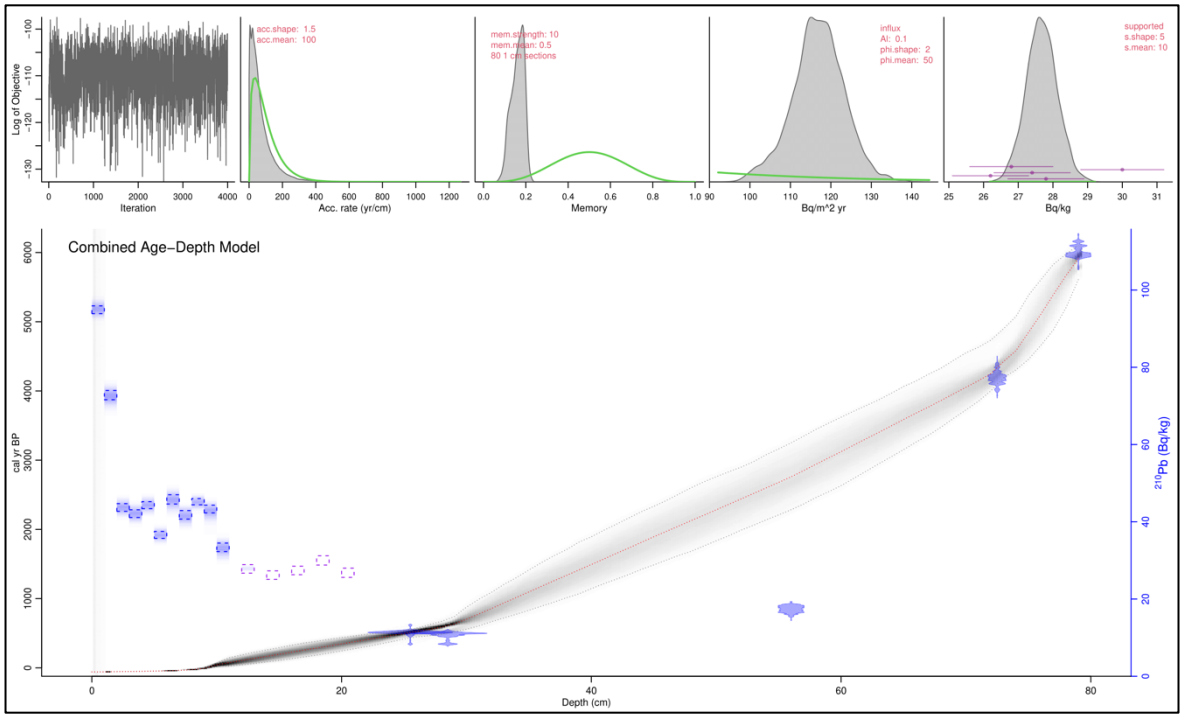


Figure 19 Age-depth model for Lake Colac.

The model reveals through the Log of Objective iteration plots that the Markov Chain Monte Carlo algorithm has converged into a stationary position. Thus, the sampled ages are likely from the target distribution, fitting the available information well. The memory mean of 0.5 and strength of 10 suggest a moderate level of autocorrelation between adjacent samples across the 1cm sections in determining the age of a given depth. The Bacerels/m^2/yr for ^{210}Pb plot shows a generally low level of activity (green line), common across Australian lake deposits (Mills et al., 2013). Ages estimated from using rplum agree with prior conclusions for Lake Colac (Ibid).

Radiometric date recalibration shows good agreement with exotic Pinaceae occurrence in the record. Pinaceae is recorded concurrently in samples from 17.7cm, estimated to have an age of 1694.2 AD (or 255.8 calBP), which is 93.8 years before 1788 AD (colonial invasion). Considering that the youngest samples of radiocarbon can produce ages a few hundred years older than expected (Bowman, 1990), likely due to sediment reworking, both relative and radiometric dates broadly agree on a period of colonial activity onset within the region (Mills et al., 2013). With the inclusion of relative dating techniques to the radiometric chronology of Lake Colac, a robust estimation of the colonial onset period is determined between 14-17cm (Tab 4).

Table 4 Recalibrated radiometric chronology for Lake Colac based on ^{210}Pb and ^{14}C concentrations. Pollen material was retrieved from depths up to 53cm. Positive calendar ages are AD, and negative ages are BC.

Depth (cm)	Median Age (calBP)	Calendar Age (BCAD)	Mean Accumulation Rate (cm/yr)
0	-58.5	2008.5	0.352043879
1	-57.9	2007.9	0.532681331
2	-55.1	2005.1	0.416642363
3	-53.2	2003.2	0.249512204
4	-50.8	2000.8	0.34719992
5	-46.7	1996.7	0.127904389
6	-43.9	1993.9	0.111308498
7	-36	1986	0.052001674
8	-27.1	1977.1	0.019456275
9	-7.7	1957.7	0.037465462
10	43.6	1906.4	0.03691072
11	71	1879	0.03938652
12	102.8	1847.2	0.039789201
13	133.2	1816.8	0.040534324
14	164.6	1785.4	0.040435693
15	194.7	1755.3	0.03908968
16	225.5	1724.5	0.039434748
17	255.8	1694.2	0.039959002
18	285.2	1664.8	0.038333789

19	316.3	1633.7	0.037819771
20	348.2	1601.8	0.039051441
21	379.3	1570.7	0.039567763
22	409.7	1540.3	0.039386624
23	440.3	1509.7	0.039882985
24	470.7	1479.3	0.041018578
25	498.8	1451.2	0.038685768
26	528.8	1421.2	0.03420324
27	559.7	1390.3	0.025366675
28	594.1	1355.9	0.017250347
29	638.5	1311.5	0.015237167
30	699.2	1250.8	0.015016629
31	770	1180	0.015160648
32	847.7	1102.3	0.0149136
33	926.4	1023.6	0.015261096
34	1007	943	0.015478666
35	1086.5	863.5	0.015265137
36	1163.9	786.1	0.016025926
37	1246	704	0.015673232
38	1325.8	624.2	0.015110627
39	1405.9	544.1	0.015281235
40	1485.8	464.2	0.014678286
41	1565	385	0.014635044
42	1644.4	305.6	0.015245681
43	1723.3	226.7	0.015443063
44	1804.9	145.1	0.015362517
45	1884.5	65.5	0.015108493
46	1963.7	-13.7	0.014778697
47	2043.1	-93.1	0.014981083
48	2122.4	-172.4	0.015169754
49	2199.5	-249.5	0.015068824
50	2279.8	-329.8	0.014699516
51	2357.9	-407.9	0.015076215
52	2436.3	-486.3	0.015887087
53	2513.1	-563.1	0.014983688

Modelled sedimentation accumulation rates (Fig. 20) are in general agreement with those in Mills et al. (2013). Several broad regimes of sedimentation are recognised throughout the record. As pollen material is only available until 2513.1 calBP, sedimentation rates change at ~28cm (594.1 calBP) and ~8cm (-27.1 calBP), marking three general phases of sedimentation rates across the record above the 53cm depth.

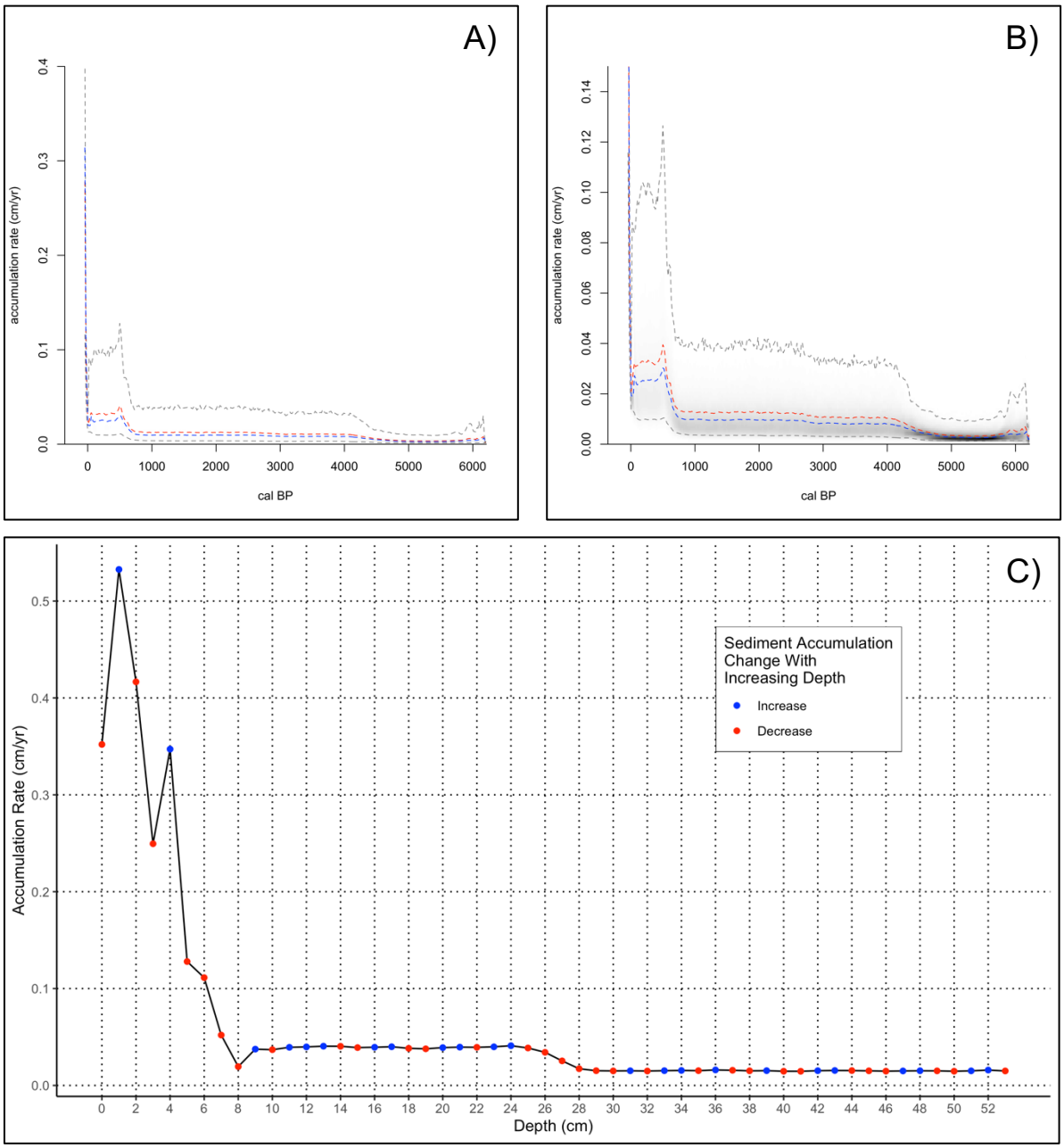


Figure 20 Sediment accumulation rates for the entire chronology by (A) ages and (B) with ages with y-axis cropping of 0.15 cm/yr for better visibility of changes, model iterations are shaded in (B) only where they are more visible. Grey dashed lines = modelled maximum and minimum accumulation rates, red dashed line = median accumulation, and blue dashed line = mean accumulation. (C) Shows median sedimentation rates and changes from the prior depth for depths where pollen material is available <2513.1 calBP at 53cm.

4.2 REVEALS

i. Structural group level reconstruction

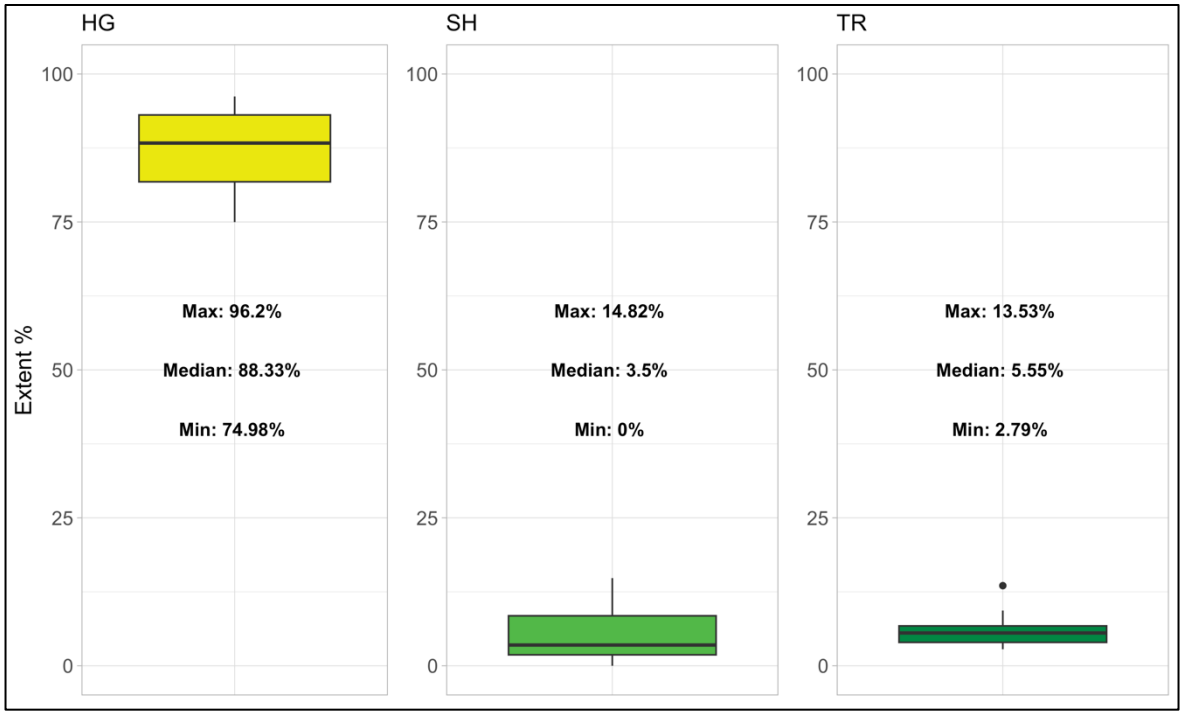


Figure 21 Boxplot of reconstructed structural types for Lake Colac.

Reconstructing the structural groups Trees (TR), Shrubs (SH), and Herbs and Grasses (HG) using the REVEALS model for Lake Colac conforms to the assumption that the western plains region has remained open throughout the past ~2500 years (Figs. 21-22). Openness, interpreted as HG extent, is high across the record; HG levels show a minimum of 74.98%, a maximum of 96.2%, and a median of 88.33% extent. Woody vegetation (TR and SH) is markedly lower than HG extent at all depths. Across the record, SH levels show a minimum of 0%, a maximum of 14.82% and a median of 3.5%; and TR levels show a minimum of 2.79%, a maximum of 13.53%, and a median of 5.55% extent.

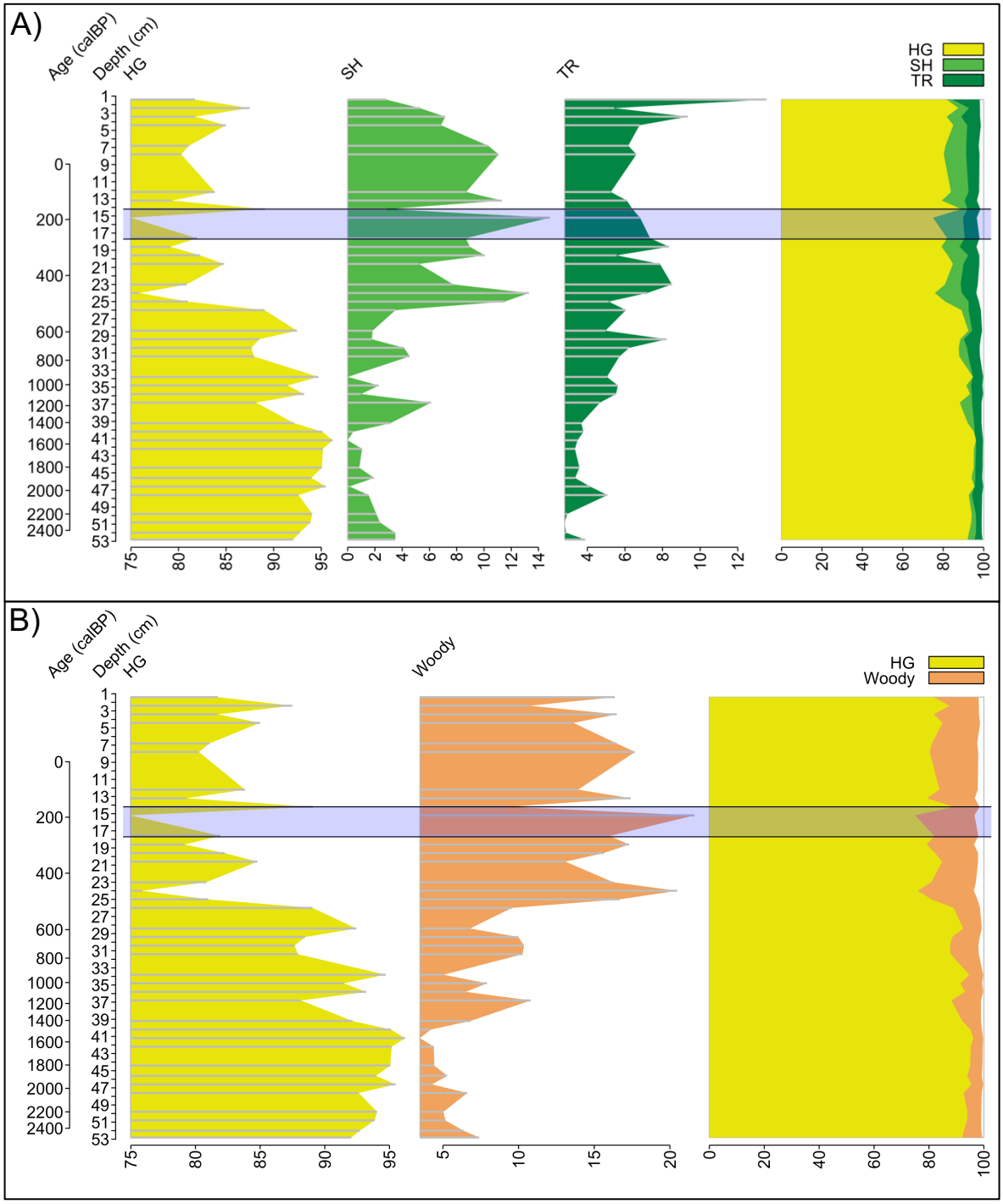


Figure 22 Stratigraphic diagrams of vegetation extent to (A) a structural level, (B) herbaceous and summed tree and shrub levels as woody vegetation. X-axes show % vegetation extent.

At the deepest samples of the record, HG levels are high at >90% and remain high until ~1400 calBP, where levels fluctuate between >85% to 95% until 528.8 calBP (Fig. 22). HG extent dramatically reduces from 26cm to 24cm (528.8 calBP until 470.7calBP), where levels fall to 75.9%, the second lowest HG extent. Afterwards, HG extent rises to 84.3% at 20.6cm, then fluctuates between 75% to 85% extent until the surface. Broadly, SH levels are inversely related to HG levels. Basal SH levels are 3.2% at 52.8cm, which falls to 0%

extent at 41.2cm. Afterwards, levels fluctuate inverse to HG levels, ranging from 0% to 6.1% until 528.8 calBP. Like HG extent, a dramatic change is recorded at this period as SH levels expand to 11.1% at 25cm and remain high. SH extent then varies from 2.7% to 13.9% until the surface extent of 2.8%. Basal extents of TR are low and begin a sustained increase throughout the pre-colonial period, like SH levels. At 17.7cm (the first recorded evidence of Pinaceae), TR extent increases curtail and decreases to 5.7% at 12.2cm. After this minimum in the post-colonial period, TR extent gradually increases before a sharp extent increase at the surface. TR extent appears largely independent from HG and SH extent; apparent shifts in TR relative to marked HG and SH fluxes are found at 1.4cm and 2.4cm, with a decrease and then a sharp increase until the maximum extent of 13.8% is recorded.

ii. Species level reconstruction

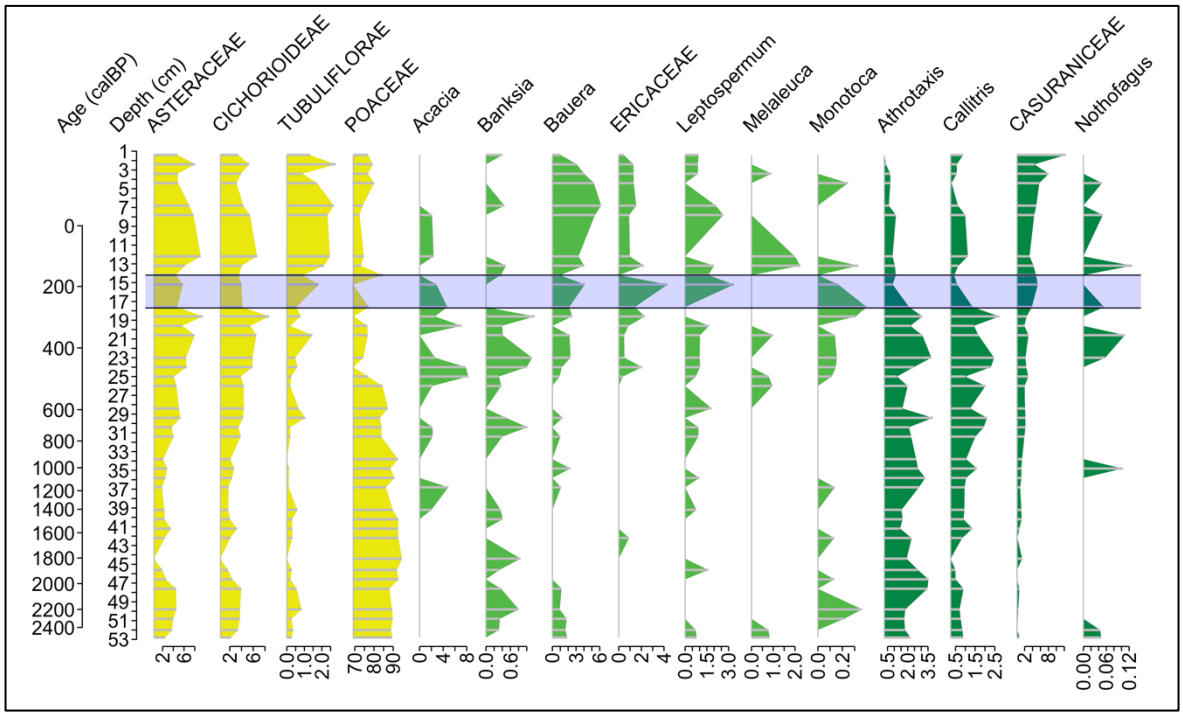


Figure 23 Stratiagraphic diagram of vegetation extent to a taxon level. Only PPE taxa are included, except for Asteraceae, which is the sum of Cichorioideae and Tubuliflorae taxa. The first recorded evidence of Pinance is the lower limit of the blue shaded band, and the first radiometric date post-1788 is the upper limit of the shaded blue band. Due to plotting limitations in riojaPlot, italicising species separate from family/subfamily levels is impossible; family and subfamily names are capitalised for clarity. X-axes show vegetation extent.

Interestingly, SH and TR increases alongside relative HG decline predate the period of colonial onset obtained through relative and radiometric dating (Fig. 23). HG levels show two main compositional phases relative to SH and TR extent increases. Below 25cm at 498.8 calBP, Poaceae shows a median extent of 89.2%; above 25cm, median Poaceae

levels fall to 71.8%. Cichorioideae levels were low before colonial invasion, despite a pre-invasion peak of 1.38% at 20.6cm, 384.2 calBP; median Cichorioideae levels before 17.7cm are 0.27%. Above 17.7cm, the median extent rises to 1.73%, a slight but apparent 1.42% median value increase. In the post-colonial period, Cichorioideae peaks at 2.73% at 2.4cm, with the age -57.9 calBP (2005 AD). The rise of Asteraceae in the post-colonial period perhaps reflects the increasing presence of exotic Asteraceae in the record, and weed increases with increased western agriculturalism in the region.

However, compositional changes within the HG structural type alone do not account for the relative ~10% decline in Poaceae before and sustained beyond the colonial period to the present day. Exploring the SH and TR extent reconstruction elucidates what other taxa may drive relative pre-colonial extent decreases in HG. Prior to colonial invasion, *Acacia*, *Banksia*, *Baurea*, Ericaceae, *Leptospermum* and, to a lesser extent, *Monotoca* drive SH extent in a mixed composition. However, a significant increase in SH cover is recorded at ~26cm (528.8 calBP). Around this depth (± 2cm), all SH RPP taxa are recorded. *Acacia* peaks to its highest extent at 7.9% at 25cm and *Banksia* at 1.23% at 23cm. At 26cm, *Leptospermum* begins a largely sustained increase, peaking at 6.04% at 6.8cm (1993 AD) before falling to 1.09% at 1.4cm. *Melaleuca* shares similar patterns in extent changes to *Leptospermum* after 26cm to the uppermost samples. *Melaleuca* subsurface peaks at 15.2cm within the colonial invasion period (radiometrically dated to 1755 AD but after the first occurrence of Pinaceae 17.7cm). Similarly, *Monotoca* peaks alongside the first recorded evidence of Pinaceae at 0.34%, an extent similar to 49.8cm (2199.5 calBP).

TR extent excluding *Nothofagus* (*N. cunninghamii* type) conforms to the ~17.7cm placing of colonial invasion in the record where a sustained reduction in Casuarinaceae, a timber selected for colonial buildings, occurs above this depth. Prior to colonial invasion, Casuarinaceae exhibited a median extent of 2.18%, peaking at 3.94% at 29.4cm (638.5 calBP); after invasion, the median extent falls to 0.77% with a minimum extent of 0.25% at 1.4cm. Prior to invasion, Casuarinaceae extent is variable and shows no sustained increasing trend. In contrast, *Eucalyptus* extent shows a general prolonged increase from basal samples to 18.6cm. Interestingly, *Eucalyptus* shares the same general trends as Casuarinaceae despite being a less favourable timber (Cox et al., 1969). Before colonial invasion, its median extent was 2.5%; after 17.7cm, *Eucalyptus* extent falls to a median of 0.29%; in the topmost sample, (1.4cm) *Eucalyptus* begins an extent expansion. *Nothofagus* extent is low and variable throughout the record; however, at 23cm, *Nothofagus* presence becomes more stable with a maximum of 1.2% extent and remains intermittently present after colonial invasion. *Callitris* type (Cupressaceae) increases in

extent almost consistently from the bottommost to topmost samples of the record, with a median of 2.09% extent, reaching its greatest extent of 12.74% at 1.4cm. While *Eucalyptus* and Casuarinaceae extent falls around colonial invasion, *Callitris* continues to increase, becoming a key TR taxon within the post-invasion period.

4.3 Palaeoclimatic background

i. Palaeoclimate indices

The palaeoclimate data I have compiled is presented below (Fig. 24). The palaeomoisture record I compiled is fitted with a LOESS smooth, using a deliberately low span value of 0.15 (a degree of freedom, where 15% of the data along the x-axis is used to formulate a smooth), designed to capture smaller – or noisy – moisture fluctuations for south-eastern Australia. Despite this, the LOESS smoothing reveals generally stable moisture conditions from ~2500 calBP until ~750 calBP. The record highlights dry fluxes at ~2050, ~1350, ~1100, and ~800 calBP before a significant reversal to wetting is recorded after ~750 calBP.

Comparing the results of the terrestrial palaeomoisture index to the Law Dome summer sea salt (LDsss) record finds the terrestrial moisture composite shares the overall observed trends in rainfall changes. Between ~1750-750 calBP, three moisture and two aridity pulses are identified within the LDsss, matched by reductions in CO_2; these pulses closely match the pulses observed within the terrestrial palaeomoisture composite. From ~750 calBP to present, the distinct shift to wetter conditions is not recorded in the LDsss record. However, the overall trend of increased aridity after ~250 calBP is recorded in both moisture records. The compilation of palaeoclimate data for south-eastern Australia reveals a generally stable period from 2000-750 calBP for the study region with a gradual increase in aridity.

Increases in CO2 emissions are captured in the Law Dome ice core composite (MacFarling Meure et al., 2006). A peak is recorded at ~1700 calBP before troughing at ~1450 calBP. Emissions then form a plateau between ~1250-1000 calBP with CO2 concentrations of ~280 ppm. A peak in concentrations is noted at ~1600 calBP before decreasing towards ~600 calBP. A minor emission increase appears to form at ~400 calBP before a sharp reduction at ~250 calBP. After this point, CO_2 concentrations exponentially increase to a maximum of 350 ppm, likely reflecting the global intensification of agriculture and industry.

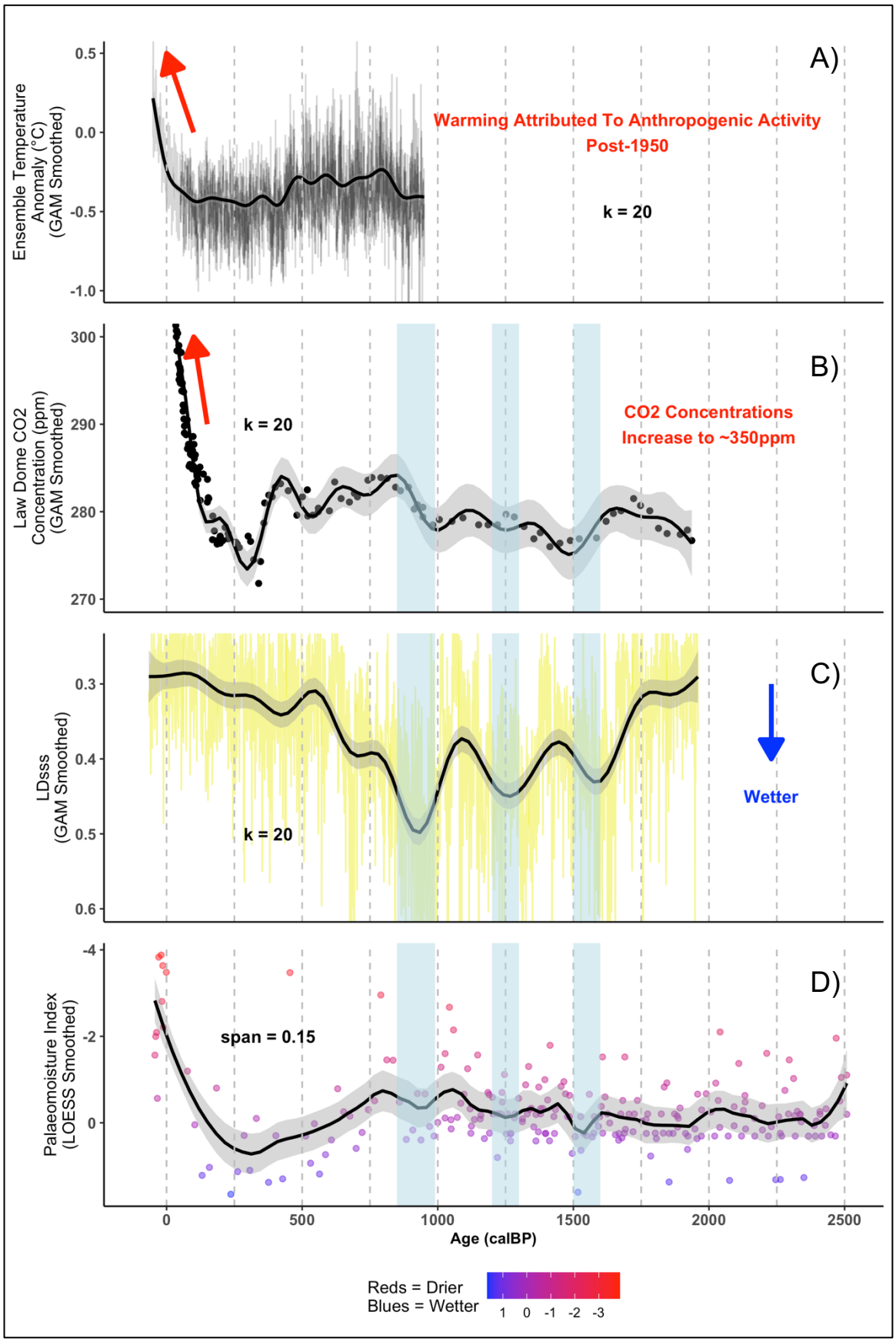


Figure 24 Palaeoclimatic records. (A) 1 ka temperature anomaly ensemble for Australasia with a GAM smooth with a smoothing parameter of k = 100, (B) 2 ka Law Dome CO_2 reconstruction with a GAM smooth with a smoothing parameter of k = 100, (C) 2ka Law Dome summer sea salt rainfall

proxy for Australasia with a GAM smooth with a smoothing parameter of k = 100, (D) Palaeomoisture Z-Scores with a LOESS smooth with a smoothing parameter of span = 0.15.

CO_2 concentrations appear tentatively linked to the moisture record of south-eastern Australia, though not perfectly synchronous. A slight CO_2 "plateau" between ~1250-1000 calBP matches with a gradual and shallow wetting pulse ~1400-1100 calBP, within a period of general long-term aridity increase until ~750 calBP.

From ~750 calBP, all records other than the LDsss show a related change with decreasing CO_2 concentrations, temperatures, and increased moisture to ~250 calBP before reversing to the highest levels seen in a “hockey stick” style of trend. The breakdown in the relationship between the LDsss record and the terrestrial palaeomoisture suggests that ice core records require circumspection when used to interpret terrestrial moisture conditions (Ho et al., 2014).

ii. Moisture and fire

Locally, drier conditions are present at the start of the record that, albeit variably, gradually increase until ~750 calBP. Moisture and fire for Lake Colac do not appear straightforwardly linked; the offset between moisture pulses and burning suggests a complex interplay between moisture events followed by biomass accumulation and then followed by burning events, e.g., the three highlighted moisture pulses (Fig. 25). Regional fire histories binned to 200-year intervals reveal similar trends to the local trends of Lake Colac’s charcoal record between ~2000-250 calBP with a gradual increase in fire activity. Regionally, major fire events are recorded in synergy with moisture pulses at ~1250 calBP. However, the distinctly high fire activity before ~2000 calBP for Lake Colac is not represented within the regional fire history composite, likely representing local-scale cultural burning or just natural ignition wildfires after a shift to wetter conditions from the drier conditions found at the start of the record (~2513 calBP). With the dramatic shift of regional moisture to more arid conditions after ~250 calBP, the relationship between increasing moisture (likely causing biomass accumulation) and increased fire is emphasised.

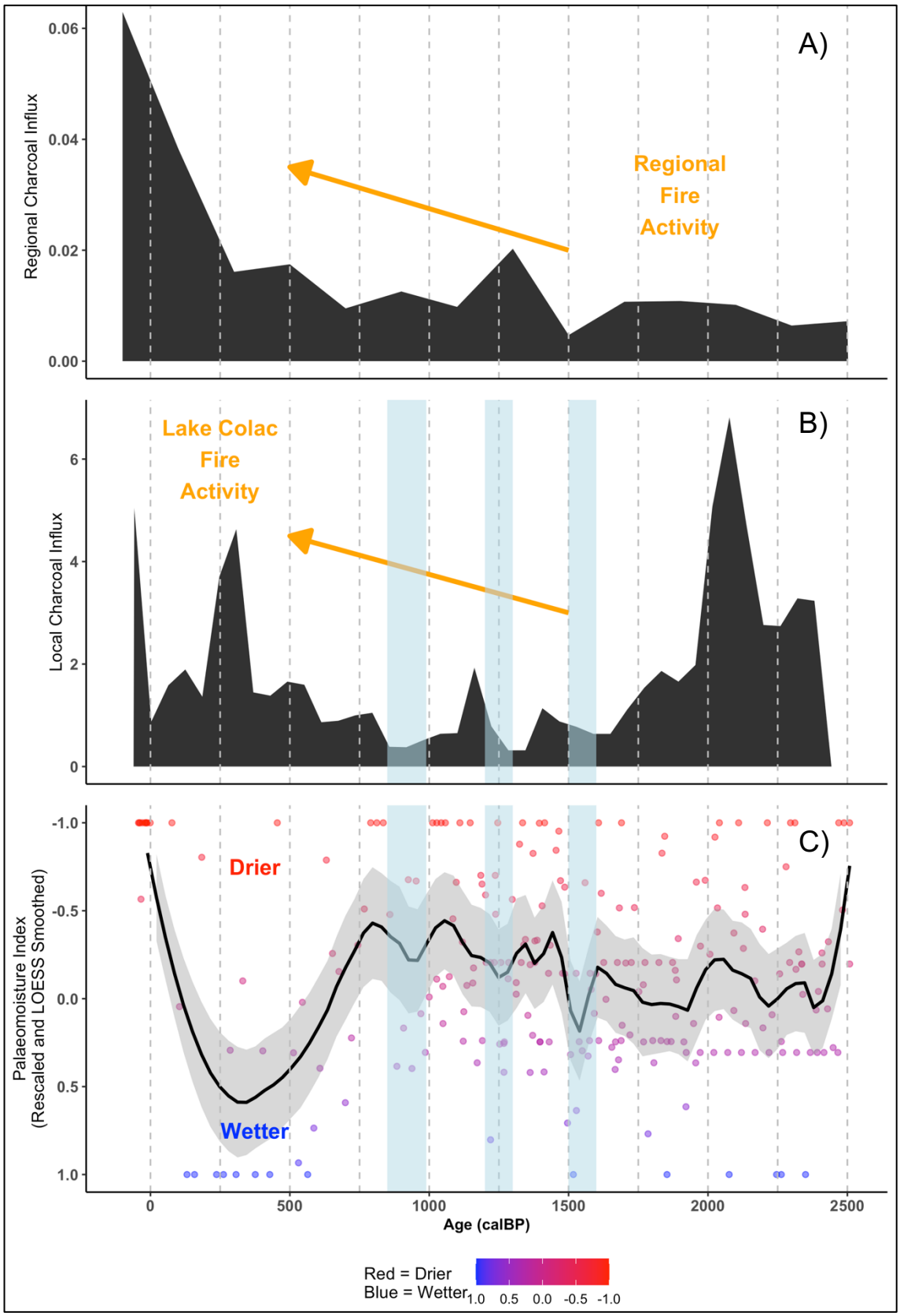


Figure 25 Palaeoclimate indices for the Lake Colac. (A) Reconstructed regional fire history record (charcoal influx) calculated from The Global Palaeofire Database (Global Palaeofire Working Group, 2023).(B) Reconstructed local (Lake Colac) fire history record (charcoal influx) calculated from Mills et al. (2013) charcoal concentrations cm3. (C) Rescaled palaeomoisture index compiled from various lake records interpreted as evaporation-precipitation and aridity fluctuations.

4.4 FTIR spectra and machine learning predictions

i. Herbarium pollen spectra

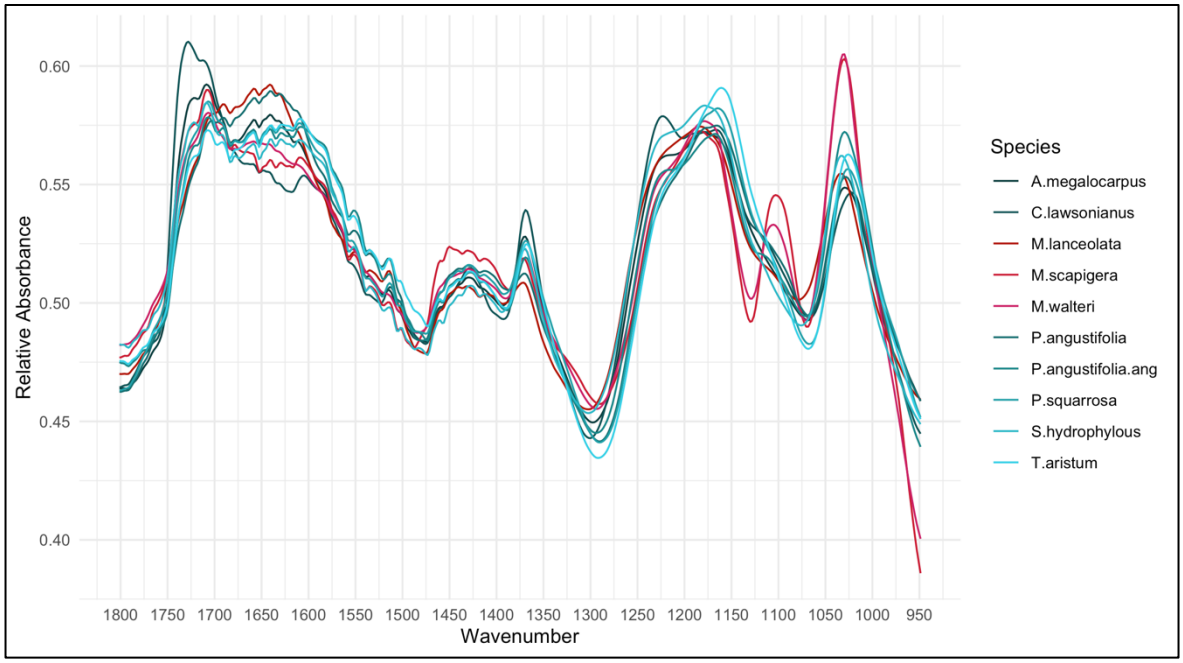


Figure 26 Composite of mean acetolysed herbarium pollen spectra.

Viewing the herbarium pollen spectra (Figs. 26-27) highlights broad chemical similarities of the 10 Cichoraceous species studied. Peaks previously attributed to aromatics in pollen are recorded at 1030cm^{-1}, 1165cm^{-1}, 1237cm^{-1}, 1370cm^{-1}, and 1515cm^{-1}, and a single lipid peak at 1710cm^{-1} (Bağcıoğlu et al., 2015; Lutzke et al., 2020). These peaks are highlighted in future spectral plots for reference (Figs. 27, 29, 32-33). Significant differences are found at ~1000-1050cm^{-1} and ~1110cm$^{-1,}$ where *M. scapigera* and *M. walteri* have pronounced peaks in regions characterised by C-O stretching (Domínguez et al., 1998; Sigma-Aldrich, 2023). At ~1125cm^{-1}, *M. scapigera* and *M. walteri* trough, and at ~1237cm^{-1}, a heightened peak is recorded for *C. lawsonianus* regions both characterised by C-O stretching (Bağcıoğlu et al., 2015; Lutzke et al., 2020; Sigma-Aldrich, 2023). At ~1400-1425cm^{-1}, a region characterised by O-H bending *M. scapigera* shows a pronouncement relative to other species (Sigma-Aldrich, 2023). Spectra appear to show heightened interspecific variation at ~1600-1700cm^{-1}.

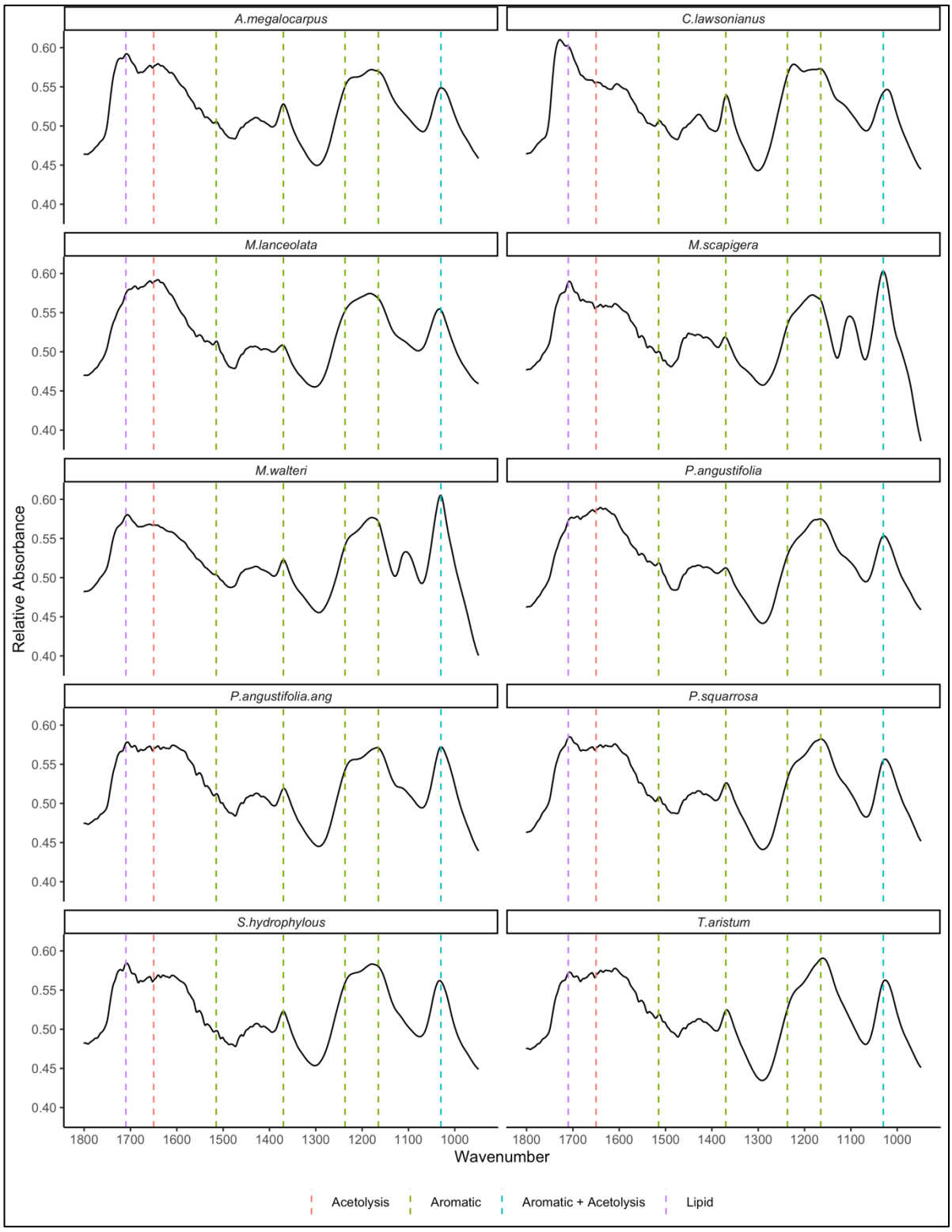


Figure 27 Mean acetolysed herbarium pollen spectra species stacked. Key aromatic and lipid peaks, along with potential treatment peaks, are annotated.

The removal of lipids via acid digestion removes a peak at ~1740cm^{-1} found in fresh pollen samples but not in sub-fossil specimens (Wang et al., 2023). The wavenumber region below ~1250cm^{-1} has been previously attributed to acetolysis driven derivatisation

within the pollen macromolecule through the creation of new C-O bonds, resulting in spectral deformations when compared to untreated pollen (Domínguez et al., 1998). Thus, the ~1030cm^{-1} peak found in these spectra can potentially attributed to acetolysis effects. Similarly, the wavenumber 1650cm^{-1} is debatably affected by acetolysis treatment (Jardine et al., 2017), although this may result from proteins within this region (Lutzke et al., 2020), elsewhere it has been found resistant to acid digestion (Domínguez et al., 1998). The spectral peaks at ~1030cm^{-1} and ~1650cm^{-1} (Figs. 26-27) found within the herbarium spectra also appear to affect PCA ordination present in PC2 (Figs. 28-29).

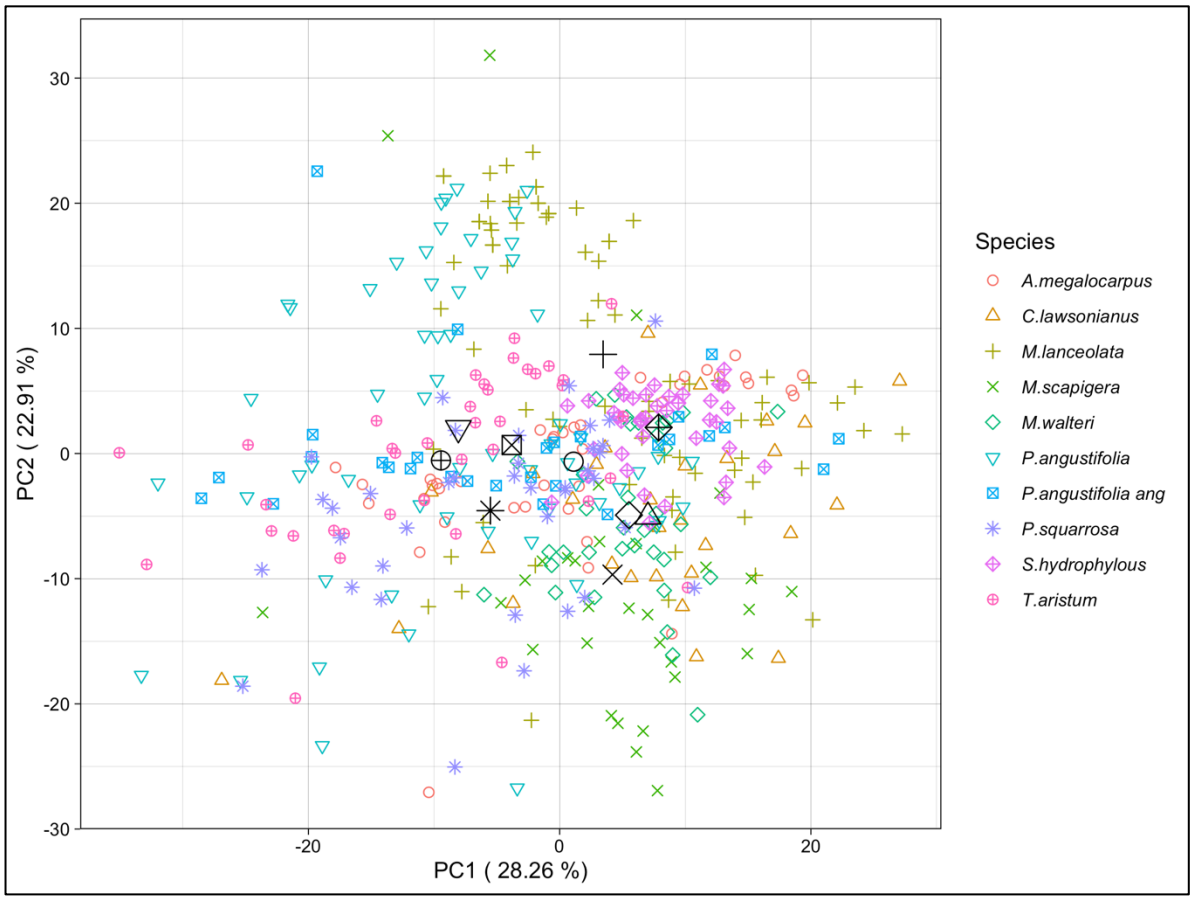


Figure 28 Unconstrained PCA ordination plot of herbaria spectra symbolised at a species level, mean centroids for the species are plotted black for the corresponding species symbols.

A PCA ordination of herbarium spectra (Fig. 28) shows evidence of species-specific clustering in Euclidean space. Most taxa clustering is somewhat apparent at a species level; however, there is a broad chemical similarity of native Cichoraceous taxa. Weakened clustering may be a product of the low number of scans per species (30-40), preventing more apparent clustering formations. Viewing the mean centroids for the species, a general clustering of points towards the origin is found. Centroids for *M. walteri* and *C. lawsonianus* are closely related, sharing similar mean principal component values.

Despite this, a general level of separability is present. PC1 explains 28.26% of the variance and PC2 explains 22.91% of the variance, cumulatively 51.17% of the variance is explained. Suggesting a level of linear separability when data is ordinated at a species level in line with the separability of the centroids.

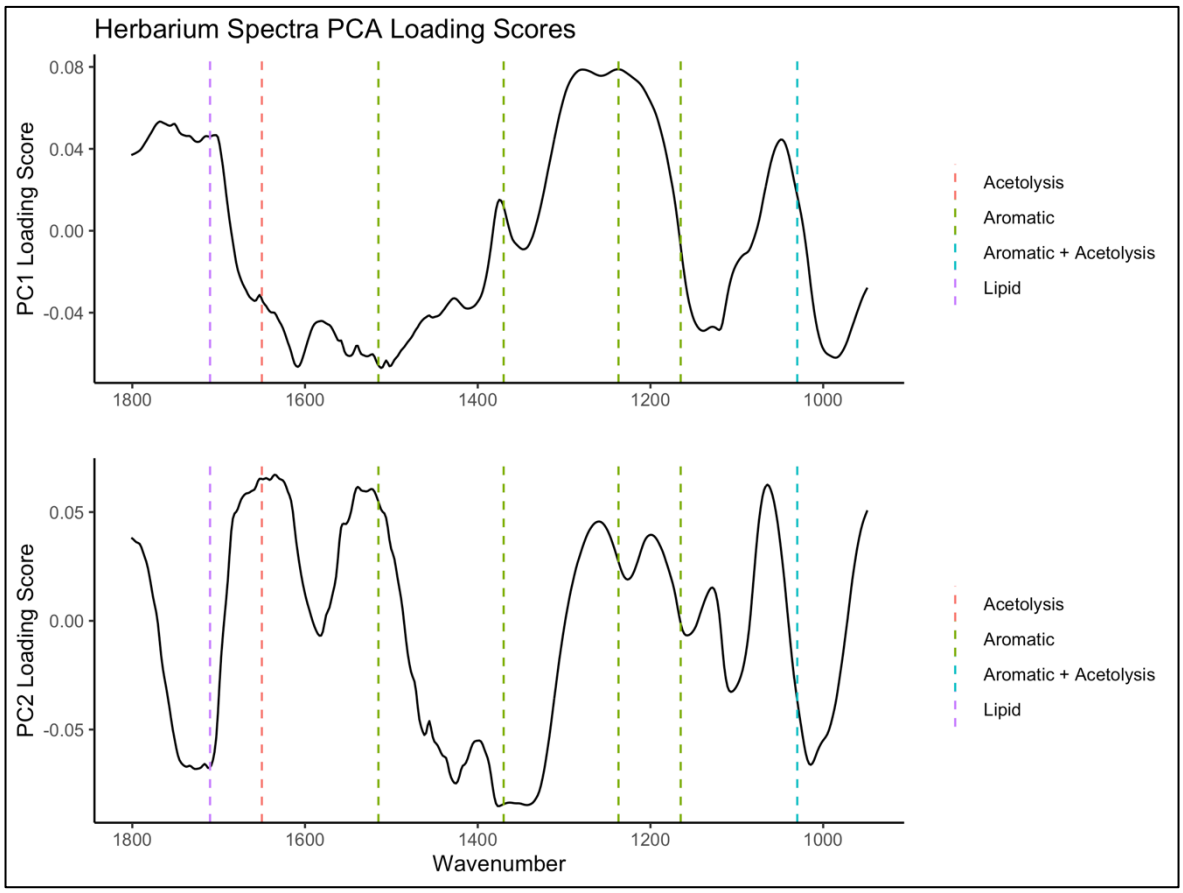


Figure 29 PC1 and PC2 loadings for herbarium spectra PCA (Fig. 28). Key aromatic and lipid peaks, along with potential treatment peaks, are annotated.

Exploring the PCA loading value (Fig. 29) demonstrates the highest important loading wavenumbers for PC1 appear in the wavenumber regions ~1300-1190cm^{-1} characterised by S=O, C-N and C-O stretching, ~1800-1700cm^{-1} characterised by C=O stretching, and between ~1180-1040cm^{-1} characterised by C-O and S=O stretching. Negative loading regions for PC1 are found at ~1650-1400cm^{-1} predominantly characterised by C=C and C=O stretching, 1160-1120cm^{-1} characterised by C-O stretching, and ~1000-960cm^{-1} characterised by C=C bending (Sigma-Aldrich, 2023).

PC2 is most positively loaded by the wavenumber regions ~1650-1620cm^{-1}, predominately characterised by C=C bending stretching, and ~1540-1520cm^{-1} characterised by N-O stretching. At ~1270-1240cm^{-1} and around ~1200cm^{-1},

characterised by C-O and C-N stretching, and around ~1060cm^{-1}, characterised by C-O and S=O stretching. High PC2 loading values are found at the two ends of the fingerprint region at ~1801.2cm^{-1} and ~948.8cm^{-1}, characterised by C=O and C=C bending, respectively. Negative loading is found in three regions ~1750-1710cm^{-1} characterised by C=O stretching, ~1450-1375cm^{-1} characterised by O-H bending and S=O stretching, and ~1025-1010cm^{-1} which is currently uncategorised (Sigma-Aldrich, 2023).

ii. RF classification model

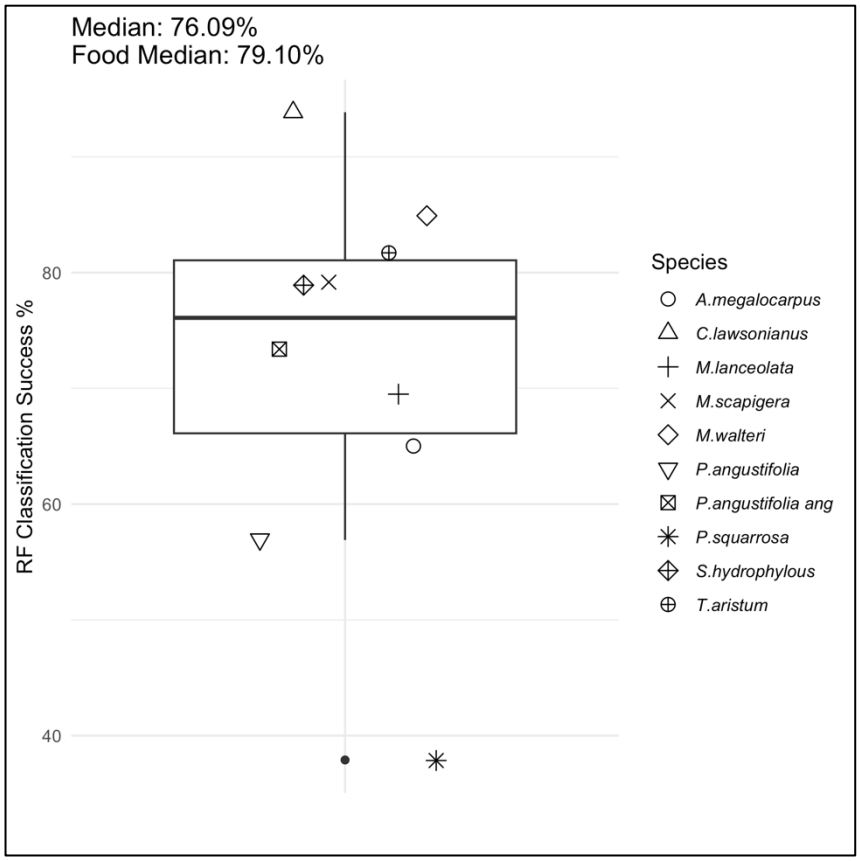


Figure 30 Boxplot of herbarium RF test matrix successful species classification.

Classification success is the metric of the positive classification of species in the RF test model. Running the RF classification model on herbarium pollen FTIR spectra produces a median classification success of 76.09% for all species; for food plants, median classification success is higher at 79.1%. Classification success is least random for *A. megaolcarpus* (64.97%), *C. lawsonianus* (93.86%), *M. lanceolata* (69.56%), *M. scapigera* (79.10%), *M. walteri* (84.96%), *P. angustifolia ang* (73.35%), *S. hydrophylous* (78.84%), *and T. aristum* (81.71%) all achieving classification successes >60%. The most random

classifying species are *P. angustifolia* and *P. squarrosa,* classifying at 56.9% and 37.89%, respectively (Fig. 30).

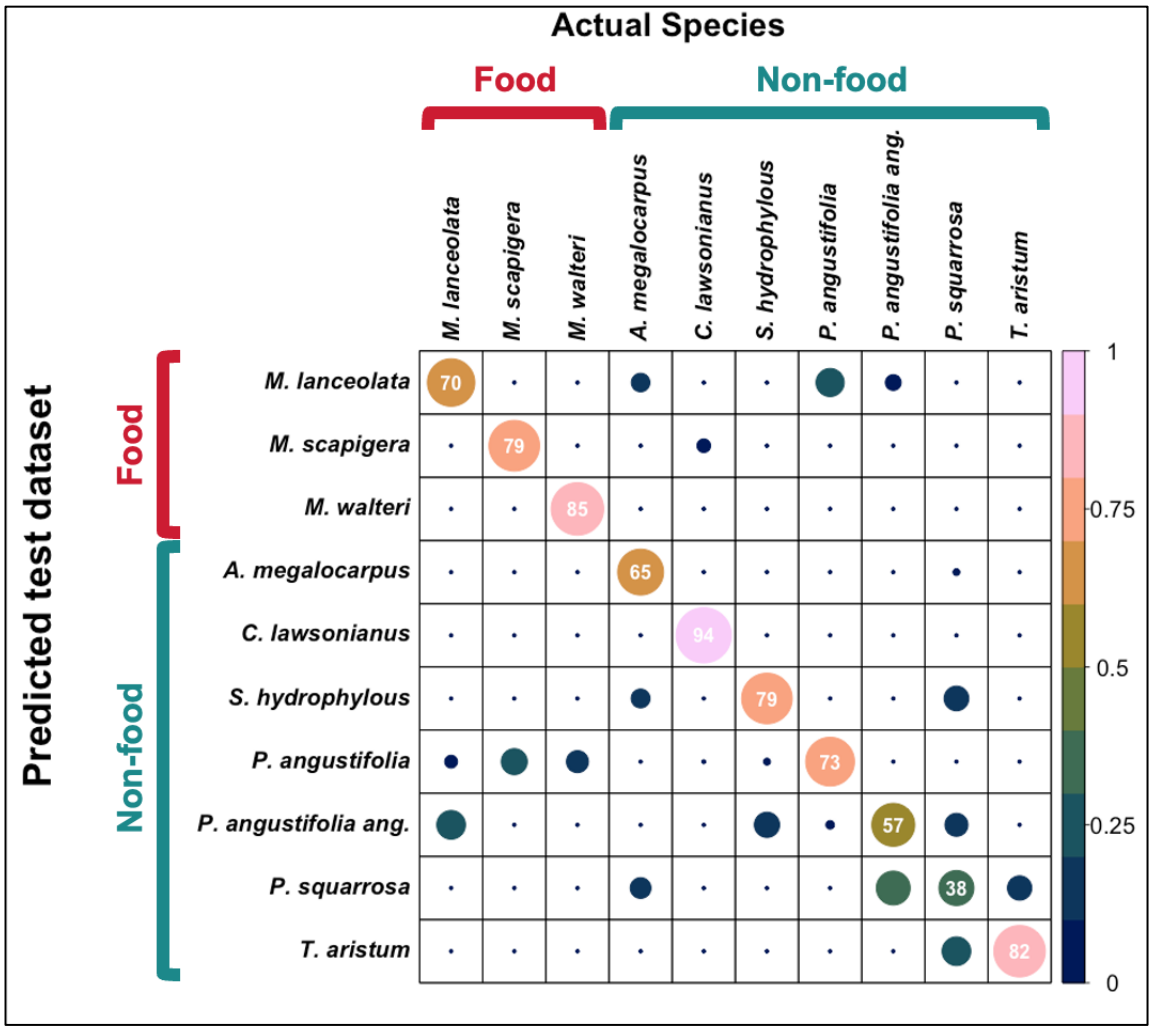


Figure 31 Correlation matrix of herbarium spectra, node colour, represents classification %, nodes of successful classification (diagonal direction) are also annotated with their classification success value. The size of the node represents the number of individual pollen grains classified.

Species misclassifications are present across all species to varying degrees (Fig. 31). *Microseris* spp. most commonly misclassified with *P.* angustifolia and its subspecies *P. angustifolia ang* (~25%), minor misclassification occurred with *C. lawsonianus.* Misclassification is absent between *Microseris* spp. and *P. squarrosa,* which presented the worst classification success across all studied species. *P. squarrosa* misclassified strongly with *P. angustifolia ang* and *T. aristum*, with other misclassifications between *S. hydrophylous* and *A. megaolcarpus*. High levels of overall median classification success (76.09%) and at a course "food plants" level (79.1%) are promising for a food plant versus non-food plant reconstruction using FTIR chemotaxonomy.

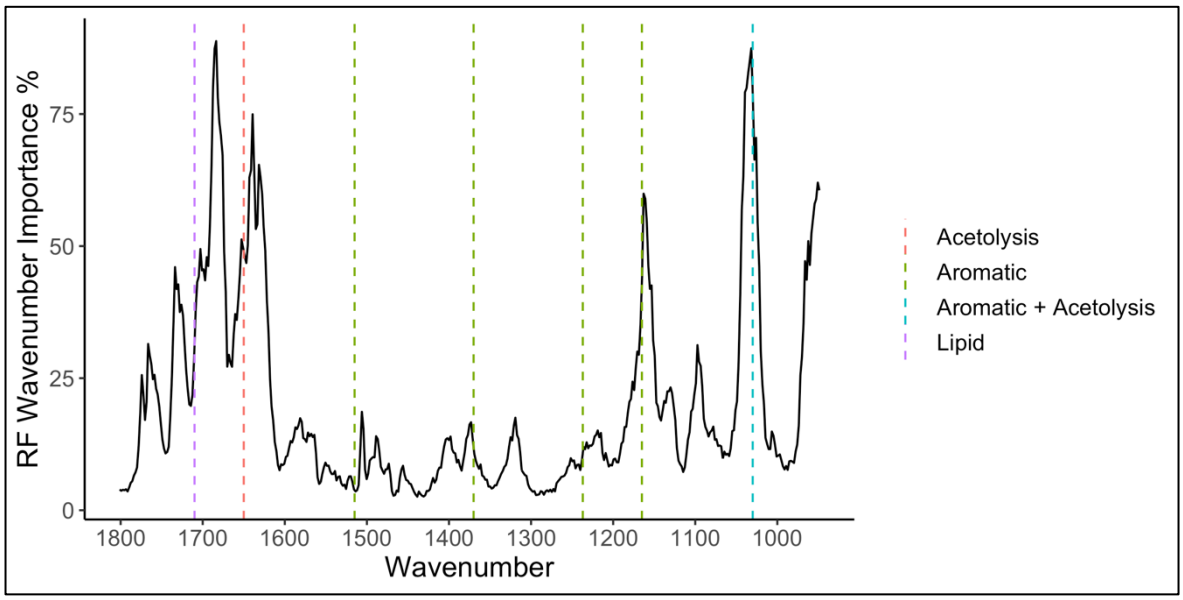


Figure 32 Wavenumber importance for the RF classification model. Key aromatic and lipid peaks, along with potential treatment peaks, are annotated.

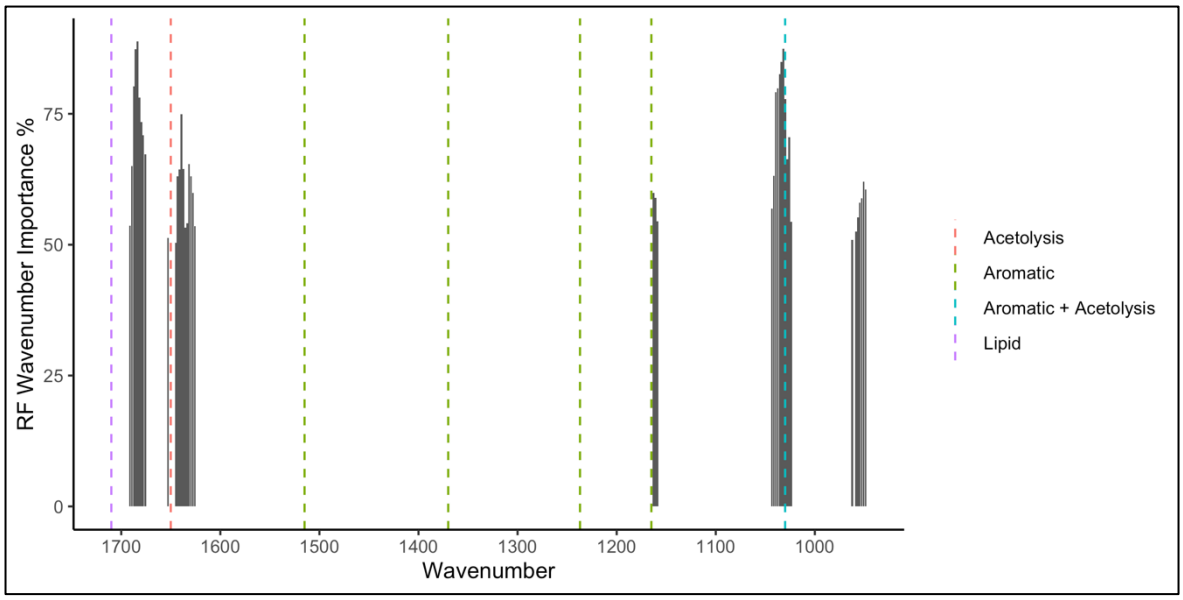


Figure 33 Wavenumber importance for the RF classification model cropped to >50% importance. Key aromatic and lipid peaks, along with potential treatment peaks, are annotated.

RF classification models allow the exploration of variables of importance in the decision-making trees of the algorithm; for chemical spectra, this is expressed as wavenumber importance (Figs. 32-33). RF wavenumber importance suggests that ~5 key wavenumber regions drive the classification model with an importance value >50%. The wavenumber region ~1030cm^{-1} drives classification with an importance of ~80%, and ~1650cm^{-1} drives classification with an importance of ~60%. Both regions may be susceptible to alteration through acetolysis (Jardine et al., 2017; Wang et al., 2023).

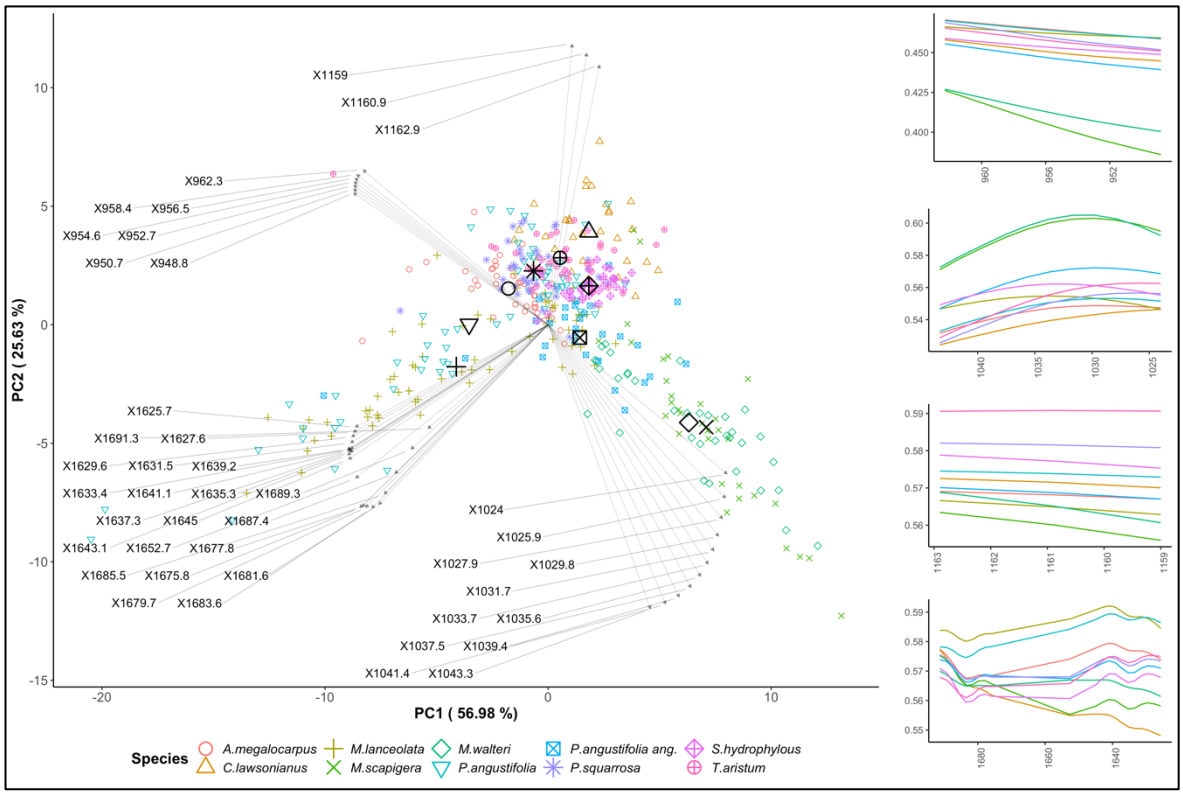


Figure 34 Unconstrained PCA ordination of herbarium spectra cropped to wavenumber regions with >50% classification importance. Mean centroids for the species are plotted in black for the corresponding species symbols. PCA loadings are represented by grey arrows and annotated with their corresponding wavenumber as X(…). Miniature spectra plots represent the four key loading regions ~950cm^{-1}, ~1030cm^{-1}, ~1160cm^{-1}, and ~1660cm^{-1}.

Cropping the herbarium spectra to regions that are >50% important for RF classification and performing another unconstrained PCA (Fig. 34) reveals four prominent loading clusters around the regions ~950cm^{-1}, ~1030cm^{-1}, ~1160cm^{-1}, and ~1660cm^{-1}. Species specific clustering of cropped spectra appears clearer for all species when compared to a PCA of un-cropped herbarium spectra (Fig. 32). For food plants viewing the clusters alongside the miniature plots, which highlight the spectra of the four main loading regions for the cropped PCA, finds high separability in *M. scapigera* and *M. walteri* from other species in both PC1 and PC2 at ~1030cm^{-1}, whereas *M. lanceolata* appears loaded by ~1650cm^{-1} to a lesser degree, showing closer similarities to the other non-food species. PC1 and PC2 account for 82.61% of the variance; thus, combined with apparent species level clustering PCA analysis of the cropped spectra suggests that RF classification is likely selecting regions high in interspecific signals as important in classification.

iii. Fossil pollen classification

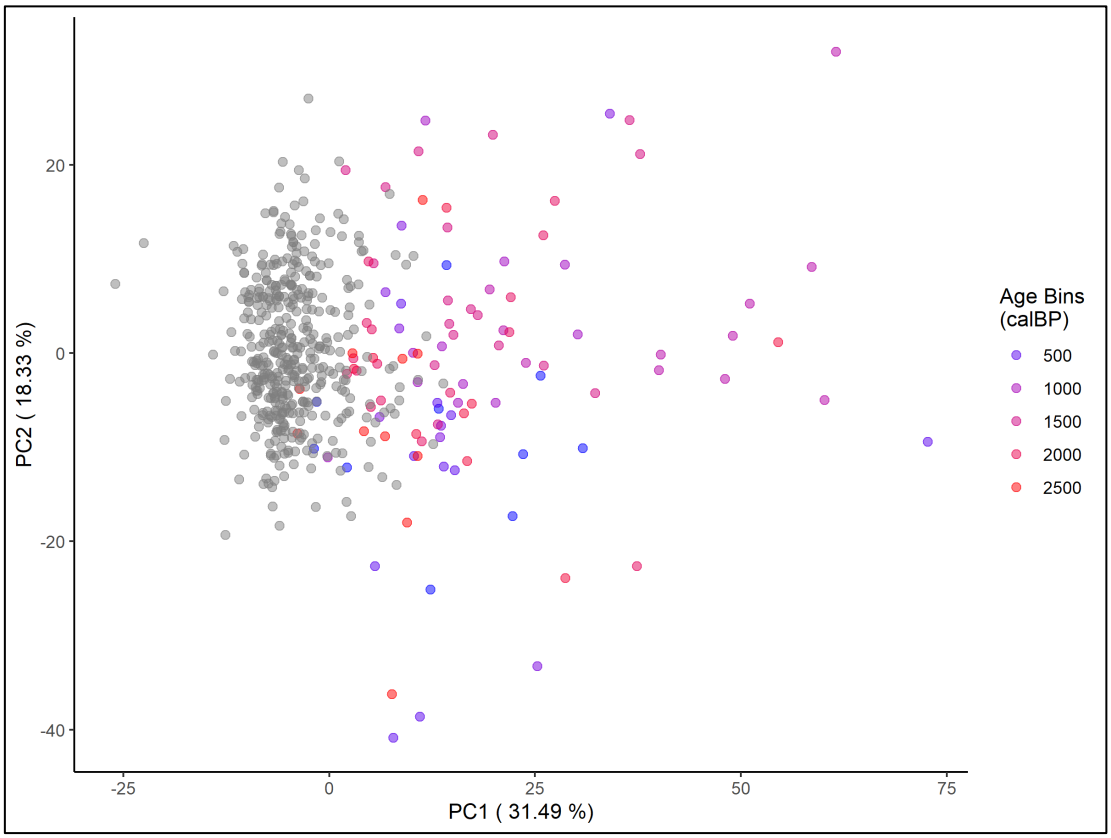


Figure 35 Unconstrained PCA ordination plot of herbaria and fossil pollen spectra, (grey) points are herbarium pollen spectra.

Comparing the PCA ordination of all herbaria spectra against sub-fossil pollen acquired from Lake Colac (Fig. 35) suggests a non-ideal overlap between the two pollen types. However, this provides a biologically meaningful result where the differences between the herbaria and sub-fossil pollen measured across the fingerprint region at first appear clearly different and warrant further investigation. Despite this, there is some evidence that many of the lake pollen spectra show some level of overlap with herbaria spectra. PC1 accounts for 31.93% of the explained variance, and PC2 accounts for 18.33% of the explained variance within the data. The first two principal components account for 50.26% of the variation within the complete spectral data. Comparing the ages of the sub-fossil pollen spectra in comparison to the herbarium pollen demonstrates that a simple linear relationship between pollen age and spectral similarities to the herbarium training is not present. It is likely that more complex spectral altering forces are in effect within pollen derived from lake sediments.

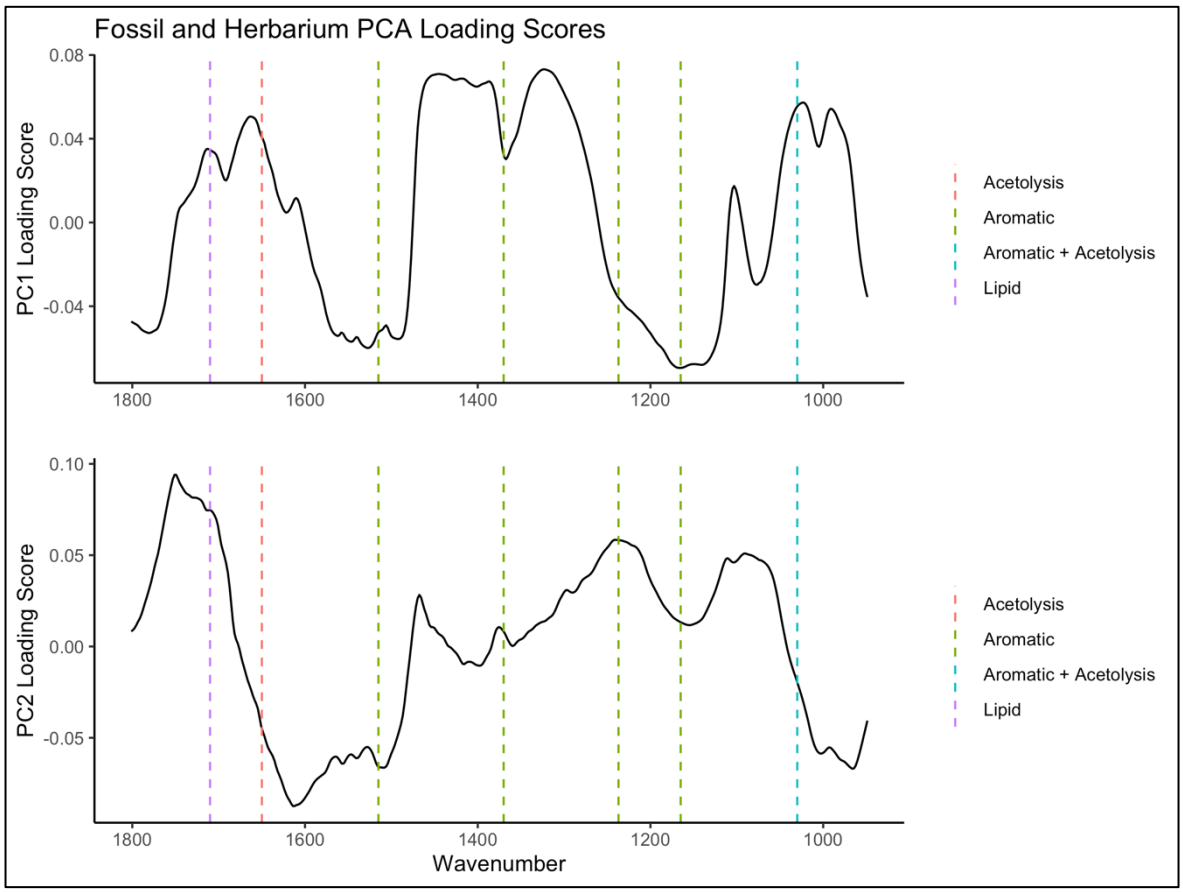


Figure 36 PC1 and PC2 loadings for herbarium and sub-fossil pollen spectra (Fig. 35). Key aromatic and lipid peaks, along with potential treatment peaks, are annotated.

PCA loading values (Fig. 36) show the highest positive loading wavenumbers for PC1 appear in the regions ~1720-1700cm^{-1} characterised by C=O stretching, ~1670-1650cm^{-1} characterised by C=C and C=O stretching, ~1460-1400cm^{-1} characterised by C-H and O-H stretching, and S=O bending, ~1340-1300cm^{-1} characterised by S=O and C-N stretching, and around ~1030cm^{-1}. Negative loading of PC1 occurs at ~1560-1500cm^{-1}, characterised by N-O stretching and C-H bending, and at ~1230-1120cm^{-1}, characterised by C-N and C-O stretching. Low loading values are found at both ends of the fingerprint region at ~1801.2cm^{-1} and ~948.8cm^{-1}, characterised by C=O and C=C bending, respectively (Sigma-Aldrich, 2023).

PC2 is most positively loaded in three regions ~1760-1720cm^{-1}, characterised by C=O stretching, around the aromatic peak at ~1237cm^{-1}, and at ~1120-1100cm^{-1}, characterised by C-O and C-N stretching. Negative loading on PC2 occurs at ~980-960cm^{-1}, a wavenumber region currently uncategorised, and at ~1630-1500cm^{-1}, characterised by C=C and N-O stretching and N-N bending (Sigma-Aldrich, 2023).

Perhaps the non-ideal overlap of sub-fossil and herbarium pollen may, in part, result from the additional treatments of 10% KOH and 10% HCL used on the sub-fossil pollen. It has been suggested that these two digestion treatments affect pollen FTIR spectra much less than acetolysis (Wang et al., 2023). Although untreated spectra for sub-fossil specimens are not available to assess the influence of these treatments, potentially affected regions are not visualised in spectral plots like acetolysis is. FTIR spectral analyses of the sediment depth where pollen was extracted may elucidate the non-clustering of herbarium and sub-fossil pollen (Fig. 35). Here, PCA is utilised as a visual tool to explore the general comparability of the data across the fingerprint region. The RF model employed in classification selects variables of importance in classification independent from PCA loading values, which are unrelated to classification.

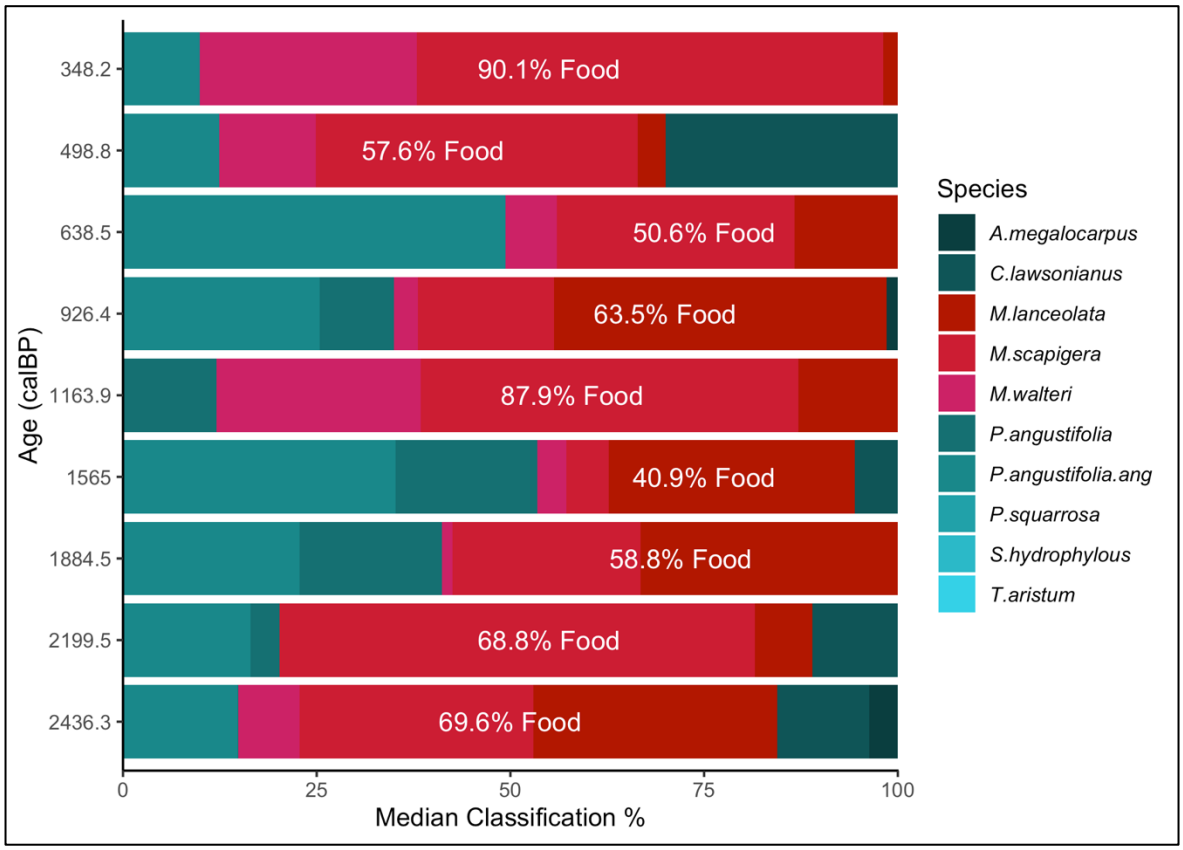


Figure 37 Lake Colac's Cichoraceous pollen predictions using RF model.

Applying the RF classification model to predict unknown Cichorioideae pollen from Lake Colac outputs a time series generally dominated by food plant Cichorioideaens (Fig. 37). Across the studied time periods, food plant prediction outputs a median of 63.5%, a maximum of 90.1% (348.2 calBP) and a minimum of 40.9% (1565 calBP). From the oldest studied sample at 2436.3 calBP, food plant levels are 69.6% and decline to minimum levels at 1565 calBP. Food plant levels then fluctuate for ~900 years, peaking at 87.9% at

1163.9 calBP, a reduction occurs at 638.5 calBP to 50.6%, and afterwards an increasing trend occurs, ending at 90.1% food plants at 348.2 calBP. *P. squarrosa*, *S. hydrophylous*, and *T. aristum* were not predicted in any of the studied samples. *A. megalocarpus* is only present at 2436.3 calBP with 3.66% and at 926.4 calBP with 1.45%; these two samples are the most species rich for Cichorioideaens based on RF predictions. *C. lawsonianus* is present in four of the nine depths, achieving its highest prediction level of 30% at 498.8 calBP. Concerning food plant predictions, *M. walteri* is absent at 2199.5 calBP, achieving its highest prediction level of 28% at 348.2 calBP, with a median 6.6% prediction across the record. *M. lanceolata* is present throughout the record, finding a maximum prediction of 42.9% at 926.4 calBP and a median prediction of 13.3%. *M. scapigera* finds a maximum prediction of 61.33% at 2199.5 calBP and a median prediction of 30.7%.

4.5 Synthesis of results

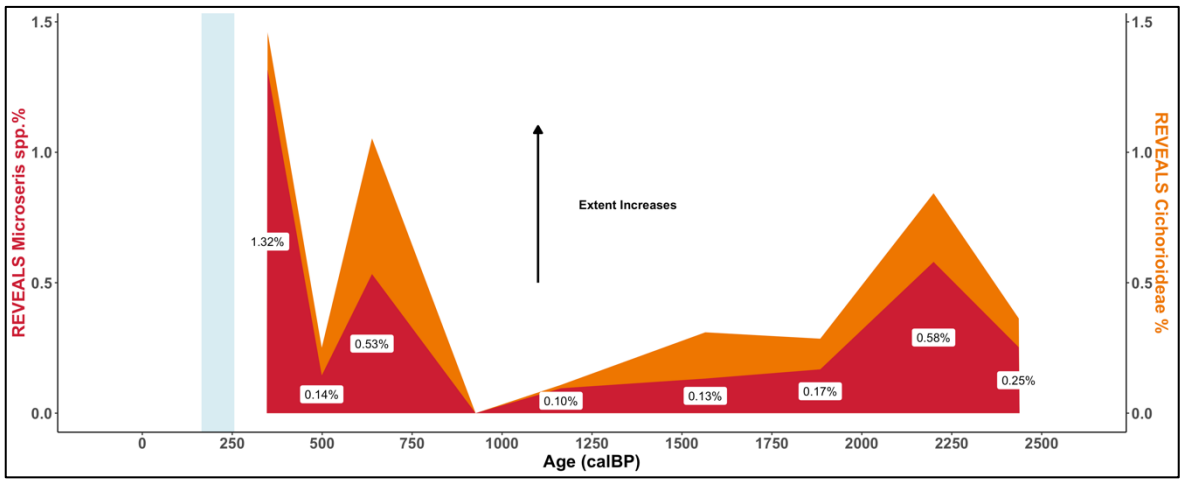


Figure 38 REVEALS reconstruction of Cichorioideae subdivided by food plants (Microseris spp.). The shaded blue area marks the colonial invasion period.

Synthesising the two key outputs of this investigation, 1) a quantified REVEALS reconstruction of Cichorioideae vegetation (Fig. 37) and 2) FTIR and machine learning predictions of unknown Cichorioideae pollen, reveals a median 0.21% extent of food plant Cichorioideaens across the record (Fig. 38). While the extent of reconstructed Cichorioideae taxa is low across the record, the percentage of the Cichorioideae reconstruction derived from the three food plant species is generally >50% (Fig. 37). Three peaks in food plant extent are identified at 2199.5 calBP at 0.58%, 638.5 calBP at 0.53%, and 348.2 calBP at 1.32% (the highest extent recorded throughout the studied depths). Food plant extent appears linearly related to the extent of Cichorioideae except for the sample at 1565 calBP, where a reduction in food plants occurs relative to Cichorioideae extent increases.

Chapter 5: Discussion

In this thesis, I have compiled a new composite record of palaeomoisture for south-eastern Australia to set the climate scene and allow comparison to regional palaeoclimate, fire and vegetation reconstructions for south-eastern Australia. Palaeoclimatic records include southern hemisphere CO_2 concentrations (MacFarling Meure et al., 2006) and Sea Salt records (Curran et al., 2022) from Law Dome ice core composites, an ensemble record of Australasian temperature anomalies for the last millennia (Gergis et al., 2016), and a regional charcoal influx composite for south-eastern Australia (Global Palaeofire Working Group, 2023). Additionally, a REVEALS vegetation reconstruction was performed on pollen from Lake Colac, a large volcanic depressional lake west of Melbourne, in the Basaltic Plains region. This reconstruction method overcomes traditional pollen biases, e.g., pollen productivity, that inhibit quantitative estimates of vegetation extent from traditional sub-fossil pollen analysis. Combined with regional and local fire histories, alongside palaeoclimatic records, new insights into the drivers of landscape change around Lake Colac are inferred, revealing linkages like woody vegetation and CO_2 concentrations. Finally, chemotaxonomic classification of pollen using FTIR spectra has been extended to sub-fossil Cichorioideae pollen in the pre-invasion period. This classification has allowed a first quantification of the culturally important Indigenous food plant group Murnong for Australia.

A synthesis composite plot (Fig. 39) is provided on the next page relating to discussions occurring across this chapter for reference; the constituent components of this composite can be found in (S3). Some individual plots have been reproduced from Chapter 4 for clarity in the discussion.

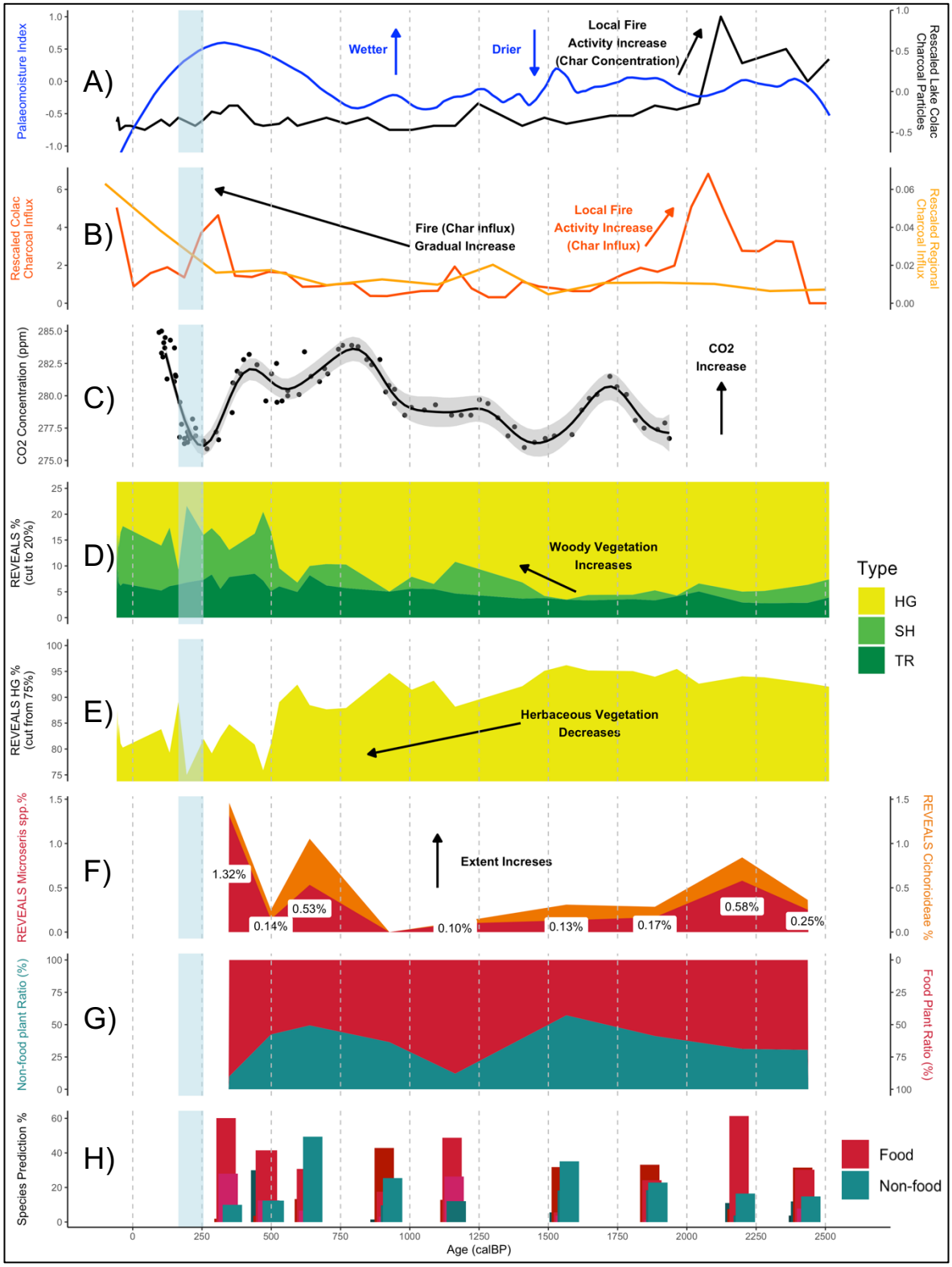


Figure 39 Composite plot. (A) Rescaled terrestrial palaeomoisture index and charcoal concentrations for Lake Colac. (B) Rescaled charcoal influx from Lake Colac and across southeast Australia (190 records Global Palaeofire Database). (C) CO_2 concentrations from the Law Dome ice core composite cropped to 285ppm. (D) Structural level REVEALS reconstruction for Lake Colac cropped to 25%. (E) HG REVEALS reconstruction for Lake Colac cropped from 75%. (F) Quantitative vegetation reconstruction of Cichorioideae and Microseris spp. at the nine FTIR analysed sample depths from Lake Colac. (G) Food plant to non-food plant ratios from the nine FTIR analysed sample depths from Lake Colac. (H) Species-level classification of Cichorioideae

taxa at the nine FTIR analysed depths for Lake Colac. CO_2 was not subscripted due to plotting limitations. The species-level key for this plot is the same as that in (Fig. 37).

5.1 Palaeoclimatic insights into south-eastern Australia

i. Setting the scene

While a palaeoclimatic reconstruction was not the key aim of this thesis, understanding the environmental context of south-eastern Australia is crucial to understanding the past extents and spatiality of Murnong and other Indigenous food plants. For context in the Corangomite Basin, it is understood that Indigenous people expanded into country, transitioning to Murnong-based diets in winter and centralised around rivers and lakes in the summer, transitioning to majority fish diets (Bride, 1898; Frankel, 2017). In south-eastern Australia's broadly temperate climate, root availability may increase with moisture availability in the winter. Other studies have found that the genetic diversity of Murnong increases with latitude, yet most fragmented populations remain close to the overall genetic makeup of the species (Prober et al., 1998; Vijverberg et al., 2000), the relation of potential latitudinal climate/environment effects on population distributions and genetic diversity are unfortunately unknown. Antithetical to Australian accounts of Murnong (Bride, 1898; Frankel, 2017), studies outside of Australia have suggested that Cichorioideae taxa may proliferate in relatively drier compared to wetter conditions in arid regions (Florenzano et al., 2015; Homami Totmaj et al., 2021; Leroy et al., 2013). Underscoring a need to relate environmental and climatic conditions to Indigenous food plant abundances.

This thesis identifies that a composite of existing terrestrial moisture data for south-eastern Australia (Barr et al., 2014; Gouramanis et al., 2010; Kemp et al., 2012; Wilkins et al., 2013) is linked with LDsss moisture records from Antarctica, regional and local scale fire activity, and CO_2 concentrations from the Law Dome ice core composite (Curran et al., 2022; Global Palaeofire Working Group, 2023; MacFarling Meure et al., 2006; Mills et al., 2013; Rubino et al., 2013; Vance et al., 2013) (Fig. 40).

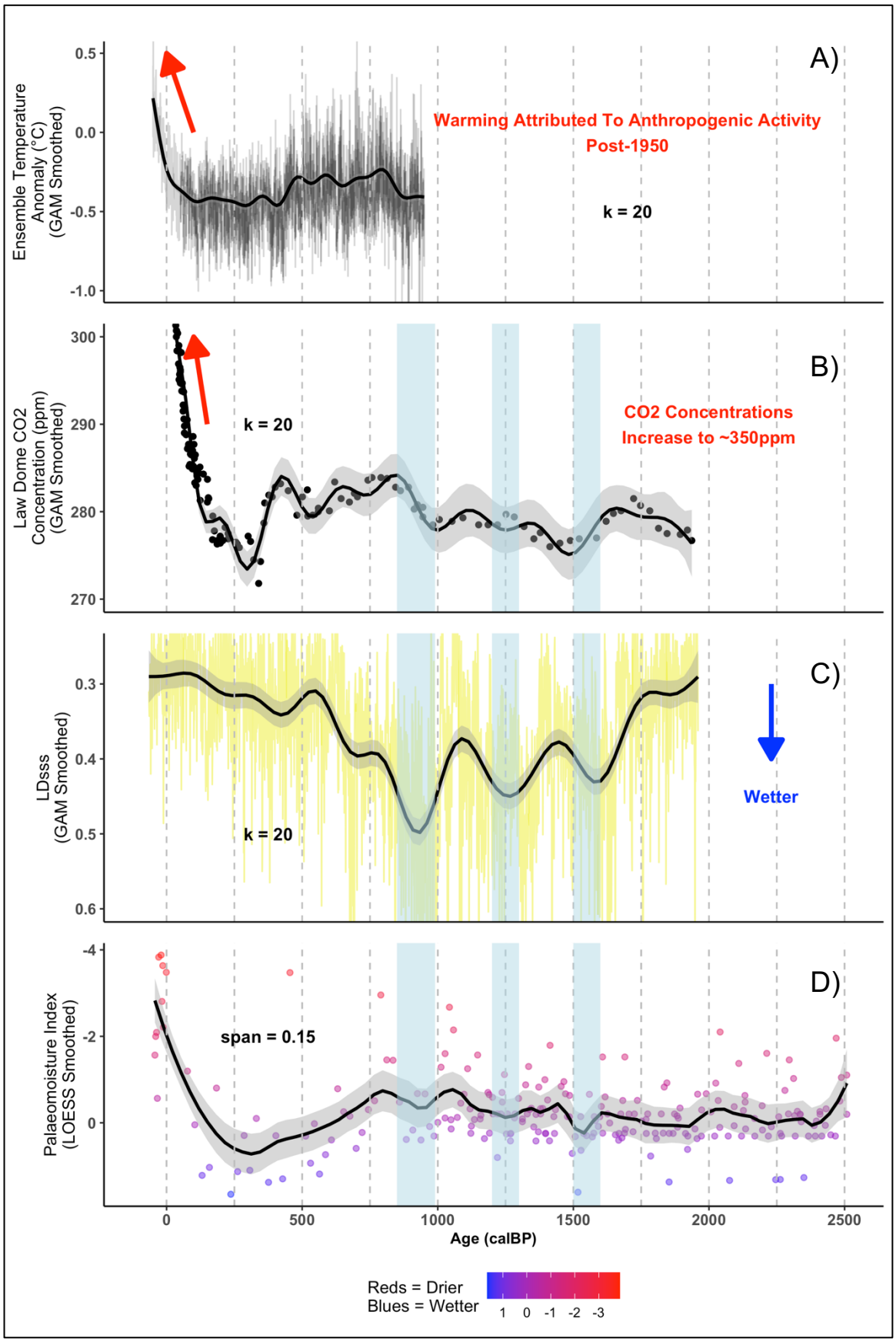


Figure 40 Palaeoclimatic records. (A) 1 ka temperature anomaly ensemble for Australasia with a GAM smooth with a smoothing parameter of k = 100, (B) 2 ka Law Dome CO_2 reconstruction with a GAM smooth with a smoothing parameter of k = 100, (C) 2ka Law Dome summer sea salt rainfall

proxy for Australasia with a GAM smooth with a smoothing parameter of k = 100, (D) Palaeomoisture Z-Scores with a LOESS smooth with a smoothing parameter of span = 0.15.

This study finds that the Law Dome ice core's CO_2 and LDsss records are linked to south-eastern Australia's composite palaeomoisture and woody vegetation records. The LDsss record has previously been correlated with south-eastern Australian instrumental precipitation records and is used to interpret regional moisture conditions beyond the instrumental period ~1950 AD (Gergis et al., 2012; Vance et al., 2013). Here I find that, when compared to terrestrial moisture proxy compilation extended far beyond 1950, the LDsss record shows comparability to terrestrial moisture conditions of south-eastern Australia. Future analysis of salt chemistry found within the LDsss may reveal a chemical origination of salts to the Murray-Darling Basin, as De Deckker and Norman (2010) observed for the EPICA Dome C ice cores.

It is recognised lacustrine climate records across Australasia are linked to ice core records from Antarctica (e.g., De Deckker and Norman, 2010; Vance et al., 2013). For example, three punctuated moisture events are represented across moisture (and CO_2) records from Australia and Antarctica between ~1750-750 calBP. The relationship found between a composite of available terrestrial palaeomoisture and Antarctic palaeomoisture between ~1750-750 calBP suggests the adequacy of composite terrestrial moisture records for future analysis. Thus, the available data should not be mistaken for an "absence of evidence", as while not expansive, it provides new insights into south-eastern Australia's moisture history, which may be critical for future Indigenous food plant studies.

Unfortunately, the length of Law Dome records are limited to ~2 ka, preventing a comparative analysis of older Holocene-spanning terrestrial moisture reconstructions. Moreover, the relationship with the LDsss and terrestrial palaeomoisture composite from ~750-present appears to break down or at least weaken. This relationship weakening between Antarctica and Australia may represent multiple complex climate teleconnections. However, for the terrestrial moisture record of south-eastern Australia, it is critical to recognise that human activities have likely impacted Australian wetland environments for the entire Holocene (Dixon et al., 2017) and thus the recorded signals. This, in part, may also present itself as the weakening of the relationship between the terrestrial and Antarctic LDsss palaeomoisture records, as at the time of colonial invasion, Australia's landscape appeared almost totally managed by Indigenous landscape activities (Gammage, 2011).

Furthermore, environmental conditions like moisture can now be integrated into the quantitative vegetation reconstruction technique, the EDA, which has yet to be tested in Australia and Tasmania's zoophilous dispersal-dominated environment. Including environmental parameters in this model will no doubt improve spatial understanding of past vegetation distributions, particularly elusive taxa like Murnong, whose suggested ethnohistorical distributions contradict its contemporary relict extent (Prober et al., 1998). For example, the usage of fire is considered an essential aspect of Murnong cultivation (Angas, 1847; Pyne, 1991); if burning for Murnong was applied at a landscape level where Murnong was most abundant then linkages between moisture and fire should be considered a key parameter in spatial reconstructions of Murnong.

5.2 Vegetation and fire histories

Elaborating the pollen counts for Lake Colac using the REVEALS model has allowed the quantification of vegetation extents of up to ~100km^2 around the lake to a taxon and plant structural level (HG, SH, TR). This thesis finds for the past ~2.5 ka, evidence from plant structural group extents conforms to the prior consensus that the Basaltic Plains has remained open (high HG extent), likely throughout the entire Holocene (D'Costa et al., 1989; Mariani et al., 2022; Mills et al., 2013), until ~500 calBP. Minor expansions in woody vegetation (SH and TR) occurred between ~1500-500 calBP but were not maintained as the landscape returned to an open state. However, at ~500 calBP, woody vegetation expansion occurred and remained high following colonial invasion (~250 calBP) despite colonially driven declines in two major TR taxa, *Eucalyptus* and Casuarinaceae. This broad trend conforms to previous conclusions that colonially driven shrub encroachment occurred across south-eastern Australia. Despite a pre-invasion onset of woody vegetation expansion around Lake Colac, returns to openness (e.g., ~1500-500 calBP) were likely inhibited by Indigenous cultural burning activities as the wider landscape was transformed for European-style agriculture (Mariani et al., 2022). *References to the extent of Cichorioideae are described after FTIR analyses see Chapter 5.3.*

i. Shrubs and their relationship to fire, moisture, and CO_2

Before ~500 calBP, this thesis finds a nuanced relationship between CO_2 concentrations, woody vegetation expansion (particularly SH) and moisture conditions between ~2000-500 calBP. During this period, shrub extent temporarily increases from a baseline of ~5% to ~10% in two phases around ~1250 and ~750 calBP, matching two broad moments of CO_2 concentration increases. Moreover, during this period, three punctuated wetting-to-

drying events occur. These moisture pulses are likely co-responsible for SH extent expansion alongside increased atmospheric CO_2 availability. While elevated CO_2 levels appear to contribute to the overall expansion of woody vegetation, fire histories show peak charcoal influx is more closely related to fluctuations in moisture than CO_2, with peak burning occurring in arid phases following periods of increased wetness.

Moreover, given that Indigenous cultural burning practices targeted SH understories and have occurred in the region since its initial Indigenous occupation (~40 ka), and that microscopic charcoal is mostly the product of the incomplete combustion of mostly woody plants (Mariani et al., 2022; Pearsall, 1989). It is likely that the fire events shown here represent Indigenous cultural burning activities, inasmuch as them also being a product of underlying increases in moisture and CO_2 availability, allowing woody vegetation expansion.

Whether anthropogenic or natural, fire is considered the dominant and an ecologically important driver of global vegetation changes (Bond et al., 2005). Moreover, the interactions of fire, moisture, people and vegetation are complex and dependent on each other (Bowman et al., 2009). In Australia, since Indigenous occupation ~65,000 years ago (Clarkson et al., 2017), the dominant fire ignition method shifted from lighting ignition to anthropogenic ignitions through controlled cultural burns, which prevented the expansion of woody vegetation and the maintenance of open herbaceous landscapes (Gammage, 2011; Gott, 2005; Mariani et al., 2022). Therefore, despite the lack of reconstructions of fire intensity, which would separate cool cultural burns from hotter wildfires in charcoal records (Constantine et al., 2023), cultural burns are likely captured in the fire histories presented here.

ii. Changing taxon extent is indicative of colonial invasion

Despite some uncertainties in the radiometric dating of Lake Colac (Mills et al., 2013), this thesis finds only a ~90-year disagreement with the first occurrence of Pinaceae (17.7cm) and the first modelled date after colonial invasion. Here, using colonial indicators in the pollen record allowed a robust estimation of the onset of colonial activity within the region.

Demarcations of colonial onset are revealed through a step change decrease in Casuarinaceae and *Eucalyptus* extent after 17.7cm; both extent declines likely represent a selective clearing for use as timber in early colonist buildings (Cox et al., 1969). Given

the modern agricultural landscape that typifies the Basaltic Plains, it is equally possible both declines suggest clearing for the conversion of land into European-style pastures. Casuarinaceae and *Eucalyptus* extent do not recover after the initial colonial invasion period to pre-invasion extent, suggesting an inability to re-establish due to the aridity and desiccation-prone clay soils of the Basaltic Plains (D'Costa et al., 1989; Mills et al., 2013).

Converse to Casuarinaceae and *Eucalyptus* extent declines, *Callitris* is found to expand throughout the record. Previous studies have questioned the reliability of reconstructing this taxon discretely from introduced Cupressaceae across western Victoria due to similar pollen grain morphologies (Kershaw et al., 1994; Luly, 1997) and a tendency for Cupressaceae pollen to be poorly preserved (Spieksma et al., 1994). Introduced Cupressaceae were commonly used as windbreaks and shelter belts, common in agricultural pasturelands, which are found across the Basaltic Plains today (Luly, 1997). Therefore, it is almost certain that the majority of *Callitris* type vegetation extent expansion after colonial invasion is driven by introduced Cupressaceae coinciding with landscape conversion to agricultural pastures rather than an expansion of native *Callitris* vegetation.

Similarly, *Nothofagus* is found native to south-eastern Australia and Tasmania. The variable presence of *Nothofagus* throughout the record may suggest a long-range dispersal from the old-stand woodlands, most likely from the Otway Ranges (within the ~100km^2 regional catchment assumption of the REVEALS model). A more stable presence of *Nothofagus* persisting through the colonial period may indicate a reduction in Indigenous cultural burning activities within the Otway Ranges area, allowing marginally increased proliferation of *Nothofagus* (Busby, 1986).

iii. Dramatic shifts in the last ~500 years

Around 500 calBP, a systemic ecological shift occurred, marked by a step-change increase in shrub levels that stabilised at approximately 15-20%. This shift was preceded by a trend toward increasing moisture conditions that began around 750 calBP and peaked between 500 and 250 calBP. Strikingly, a rapid transition to peak aridity conditions followed 250 calBP. This arid phase was synchronised with exponential increases in CO_2 levels and a consistent rise in both regional and local fire activity. These CO_2 surges occurred with the global advent of industrialisation in the 18th century and the colonisation of Australia. Notably, these environmental shifts were already in motion before pronounced warming impacts become clearly recognisable post-1950 AD (Gergis et al., 2016). The early onset of dramatic moisture shifts around 750 calBP may also point to

localised geochemical changes, potentially linked to indigenous activities. Regardless of whether climatic or human factors drove these shifts, they suggest that significant environmental transformations occurred in south-eastern Australia before global industrialisation and colonial invasion in Australia. Combined with the sudden cessation of Indigenous cultural burning practices post-invasion (Mariani et al., 2022), the shift to high aridity levels after a dramatic wet pulse allowing biomass accumulation has created a volatile environmental context, exacerbating the current risk of Australia's catastrophic wildfires.

5.3 Pollen FTIR chemotaxonomy

In this thesis, I have expanded on the rapidly developing field of pollen chemotaxonomy using the FTIR vibrational spectra of sporopollenin. Presenting a world's first quantified reconstruction of ancient sub-fossil pollen based on a machine learning prediction algorithm's (RF) ability to classify ancient sub-fossil pollen using their FTIR spectra.

i. Potential for species discrimination – herbarium pollen spectra

The ten herbarium Cichoraceous pollen species studied in this investigation show a broad similarity from observations of their Fourier transform infrared absorption spectra. Aromatic and lipid peaks previously identified in sporopollenin FTIR studies are present in the herbarium data at 1030cm^{-1}, 1165cm^{-1}, 1237cm^{-1}, 1370cm^{-1}, and 1515cm^{-1} (aromatics), and 1710cm^{-1} (lipids) (Bağcıoğlu et al., 2015; Lutzke et al., 2020). However, this thesis highlights wavenumber regions of increased chemical interspecificity at ~1050-1000cm^{-1} where *M. scapigera* and *M. walteri* peak, and at ~1125cm^{-1} where they trough relative to the other eight species. Furthermore, at ~1400-1425cm^{-1}, the *M. scapigera* peak is pronounced relative to other species, and at ~1237cm^{-1}, *C. lawsonianus* peaks in a similar regard. The wavenumber region ~1700-1600cm^{-1} is a region of increased interspecificity for all species and is also found important in RF classifications. While the spectra of the Cichoraceous pollen species are largely comparable throughout the "fingerprint region", distinct interspecific signals at the above (and other) wavenumber regions indicate the potential for accurate species discrimination of pollen using their biochemistry alone.

Conceptually, using the standard pollen digestion treatments 10% HOH, 10% HCL and acetolysis on herbarium pollen makes the chemistry of known herbarium reference pollen comparable to the chemistry of unknown sub-fossil pollen (Jardine et al., 2021).

Integrating these digestion treatments into FTIR spectral analyses lays the foundation for a single homogenous pollen processing protocol, where techniques like FTIR that analyse pollen biochemistry may not require separate laboratory processes. Alternative pollen extraction techniques proposed for FTIR analyses that lack digestion pose additional challenges for the discrimination of sub-fossil pollen (Wang et al., 2023). Labile peaks found in untreated modern pollen, e.g., lipid peaks at ~1740cm^{-1}, are not found in sub-fossil and to varying degrees older untreated herbarium samples, due to varying levels of pollen deterioration and oxidisation (Wang et al., 2023). Chemical digestion protocols neutralise these differences, eliminating labile and protein peaks along with other extraneous material, thereby streamlining the analytical processes. Allowing sub-fossil pollen extracted from different depths and potentially various environmental settings to be compared to a single modern reference library that has undergone the same chemical digestion treatments.

However, using common palynological extraction techniques can introduce or modify existing spectral peaks. Among the treatments used in this investigation, acetolysis is regarded to modify pollen spectra more than other chemical treatments when compared to untreated pollen spectra (Wang et al., 2023). This thesis notes that acetolysis could affect the spectral regions of ~1650cm^{-1} and ~1030cm^{-1}. However, the extent of this effect is unknown as the untreated pollen spectra were not captured prior to chemical digestion treatments due to the time limitations caused by the novelty of this work, preventing comparison between untreated and treated spectral data.

Pseudoreplication is another issue apparent within the herbarium spectral library. As only a single plant was sampled for pollen for each species (two of *M. lanceolata and P. angustifolia*), due to herbarium limitations, there is the chance the individual scans of pollen represent an individual specific (single plant from a species) chemical profile rather than a species level. Unfortunately, when classifying unknown sub-fossil pollen, an individual level rather than a species level (multiple plants from the same species) classification model is preferred. This is because the modern reference spectra of an individual-level library may not represent the typical species-level chemical profile of the sub-fossil pollen, where the pollen record captures many individual plant pollens. Thus, future analyses of Murnong using FTIR classification will improve with the introduction of more plant individuals grown in laboratory conditions from which pollen can be sampled and used to create a more robust herbarium spectral reference library.

ii. Training a classification model using herbarium pollen spectra

In agreement with previous studies, the mean classification success of machine learning prediction models of modern (herbarium) samples is high at 76.09%. Achieving high mean classification accuracy despite the potentially suboptimal use of spectra pre-treated with only EMSC, lacking SG smoothing, and classification using derivative spectra, this approach is promising for future applications in predicting unknown sub-fossil pollen. All species other than *P. angustifolia* and *P. squarrosa* classify with success values >60%, but misclassifications are found for all species. Food plants are misclassified most strongly with *P. angustifolia* and its subspecies *P. angustifolia ang*, and to a lesser extent, *A. megalocarpus* and *C. lawsonianus*. Despite this, food plant classification success gave a median value of 79.1%. The misclassification of the food plants to (predominantly) the *Picris* genus could suggest one of two things: either food plants are being underrepresented and classified as *Picris* or overrepresented for the same reason to a degree of ~20%. Regarding *P. squarrosa,* the RF classification model found it held the lowest classification accuracy at 38% misclassifying with *A. megalocarpus, P. angustifolia ang*, *T. aristum*, and *S. hydrophylous*. This result no doubt limits the ability to confirm whether it is actually present in any of the samples. However, as it did not directly misclassify with any of the food plants (*Microseris* spp.), this has little effect on the reconstruction of the food plants.

One of the regions ~1030cm^{-1} that is likely influenced by the acetolysis treatment is found to give >50% importance in RF classification. Future investigations into untreated pollen of the same species are necessary to gauge the effect of chemical digestion on these taxa spectra. Comparing the variable importance of RF classification between untreated and treated pollen could also determine whether this wavenumber region is inherently important for classifying the taxa selected in this study without alteration through chemical digestion.

To date, no other tried method comes close to discriminating sub-fossil pollen with cryptic morphologies with a level of accuracy than the machine learning analyses of pollen vibrational spectra. Recent characterisations of modern pollen using photon decay rates (Hu et al., 2023) and autofluorescence colour spectra (Mitsumoto et al., 2009), image-based analyses of pollen using CNNs (Bourel et al., 2020; Punyasena et al., 2022; Romero et al., 2020), and ancient pollen DNA (Baksay et al., 2020; Bell et al., 2017; Parducci et al., 2005) may offer alternate pathways. For example, CNN image identification provides the most promising alternative to pollen vibrational spectra-based

pollen identification. However, it is limited for fossil pollen application at a species level of identification where species have cryptic pollen morphology (Romero et al., 2020). High classification success of cryptic sub-fossil pollen using CNNs can be achieved where discrimination occurs between a small number of (n=3) species (Kong et al., 2016). However, as CNN predictions are image-based, the deterioration of pollen features in a sedimentary context impedes accurate species classification of morphologically cryptic pollen unless high-resolution surface texture analyses are undertaken (Punyasena et al., 2022). The deterioration of pollen morphological features impeding visual analyses stands adjunct to chemical-based studies where sporopollenin biomacromolecules remain stable in sedimentary settings in Quaternary sediments (Jardine et al., 2021).

These alternative methods (and others yet to be developed) may come to contend with pollen vibrational spectra studies of cryptic pollen. Future studies may use a mixture of sub-fossil pollen classification methods to benchmark cryptic pollen classification using vibrational spectra identification.

iii. Herbarium pollen FTIR spectra: harmonising supervised and unsupervised analyses

Unsupervised analyses of herbarium pollen spectra using unconstrained PCA ordinations find that PC1 and PC2 account for 51% of the variation in the spectra. This indicates a level of linear separability at the species level, as confirmed by the observed clustering of pollen species. However, it's noteworthy that the mean centroids of species, particularly those of M. walteri and C. lawsonianus, are nearly overlapping. Despite this limitation, the general pattern in the PCA ordination further suggests the capability to discriminate and classify pollen species using their FTIR spectra. Examining the principal component loadings, it is evident that the wavenumber region below ~1300cm-1 is positively associated with PC1 and PC2, implying a significant contribution to spectral variation.

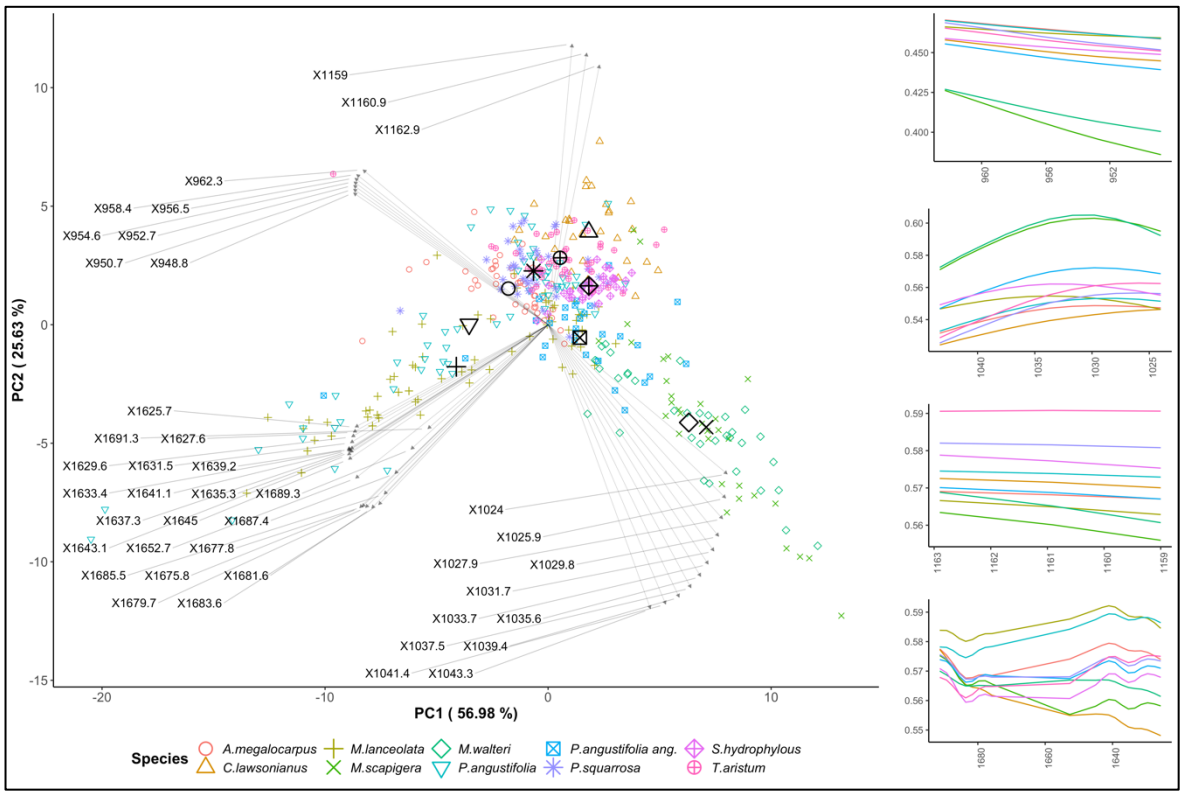


Figure 41 Unconstrained PCA ordination of herbarium spectra cropped to wavenumber regions with >50% classification importance. Mean centroids for the species are plotted in black for the corresponding species symbols. PCA loadings are represented by grey arrows and annotated with their corresponding wavenumber as X(…). Miniature spectra plots represent the four key loading regions ~950cm^{-1}, ~1030cm^{-1}, ~1160cm^{-1}, and ~1660cm^{-1}.

Further exploration of the relationship between unsupervised (PCA) and supervised (RF) methods corroborates this finding (Fig. 41). Performing a PCA on spectral regions cropped to >50% importance in RF models shows that three of the four major loading zones lie in wavenumber regions below ~1300cm-1. Despite the overall spectral similarity within the Cichorioideae subfamily, the distinction of species through clusters and mean spectra (miniature line plots) are evident in this cropped dataset. This leads to the conclusion that the regions identified as "important" by RF are most likely areas of high interspecific variation, accounting for 82.61% of explained PCA variance within the cropped spectral data.

Future studies may find semi-supervised methods (e.g., more advanced constrained PCAs) examined alongside decision-making variables of supervised prediction methods (variable importance of RF here) may reveal the relationships between pollen spectra interspecificity and machine learning decision-making in a more profound manner than explored here.

iv. Comparing sub-fossil and herbarium data

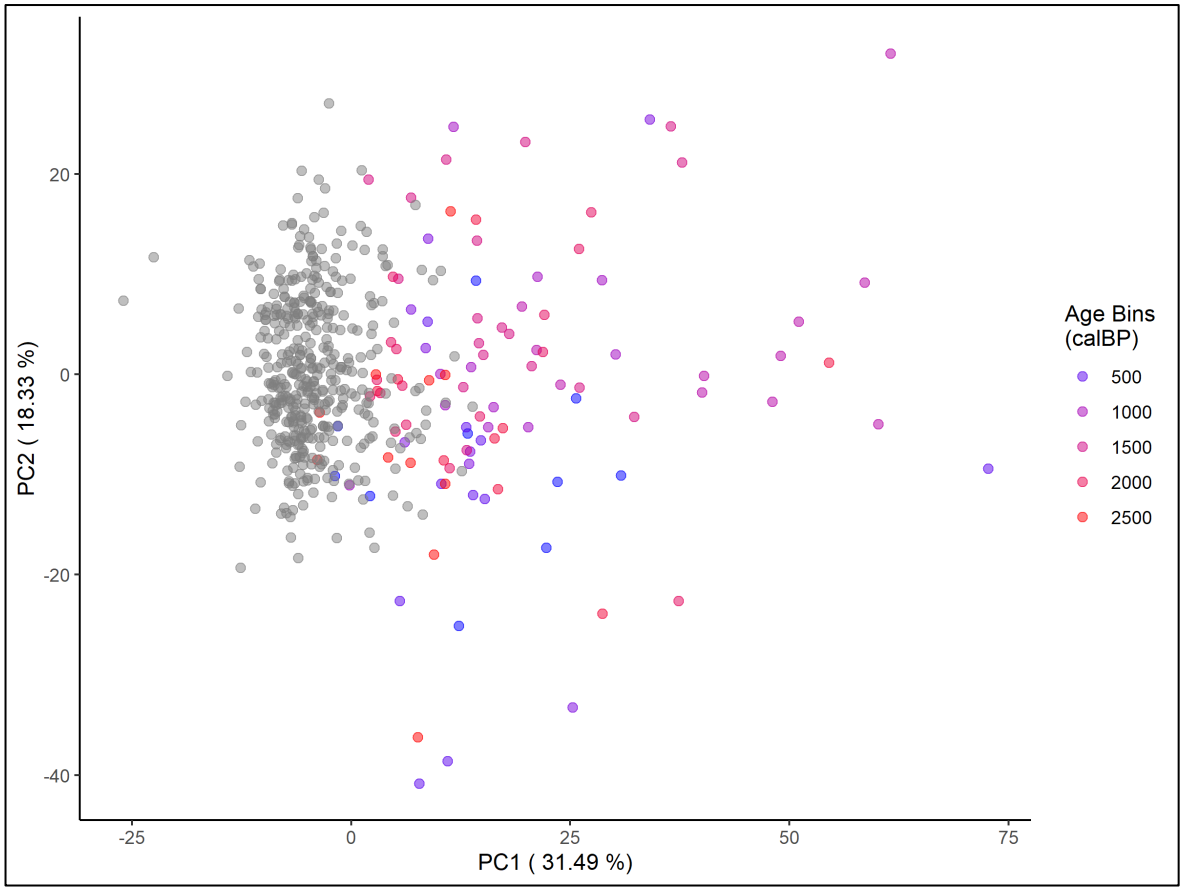


Figure 42 Unconstrained PCA ordination plot of herbaria and fossil pollen spectra, (grey) points are herbarium pollen spectra.

Introducing unknown sub-fossil spectra into PCA analyses results in a non-ideal overlap with herbarium spectra and poor clustering of sub-fossil spectra (Fig. 42), indicating the presence of modifying factors unique to sub-fossil specimens. Moreover, a lack of clustering by age is apparent; this is converse to what would be assumed true with pollen being affected by burial and oxidisation effects with increasing time of burial. These findings underscore a critical need for research into the environmental, depositional, and oxidational properties affecting the vibrational spectra of pollen extracted from lake sediments.

The RF classification model, which is solely built on herbarium data, assigns low importance to many wavenumber regions, which are found to be variation drivers in PCA ordinations. For example, the loading scores of PC1 and PC2 for herbarium and sub-fossil pollen highlight the wavenumber region of ~1460-1300cm^{-1} as positively associated with PC1 and PC2. Compared to herbarium-only PCA loadings, this region appears negatively

associated in both PC1 and PC2. This may suggest that the RF classification can filter out regions of poor comparability of variation drivers between the two sets by excluding regions of variability that are inconsistent between modern and sub-fossil spectra. In these regions, variability may be the result of forcings from the depositional environment. Consequently, potential environmental effects on pollen chemistry in this region may not significantly impact the prediction of unknown spectra. However, a caveat is that when considered cumulatively, low-importance regions could still greatly influence pollen classification with unknown spectra. Combined, perhaps classification using the entire "fingerprint region" as it is currently practised for modern pollen is too broad for the classification of sub-fossil pollen and should cropped to specific wavenumbers. After screening the data using PCA and RF models in cohesion regions possibly suscept to environmental conditions causing variance, to only the sub-fossil data are excluded.

However, an important concept to address is that RF classification models built on the entire spectral fingerprint region are likely overfitting unimportant – containing non-collinear (separable) wavenumber regions that do not hold species-specific information – data. This is due to the high number of data inputted across the fingerprint region into the RF model. Methods that eliminate collinearity across entire spectral regions exist (e.g., Luo et al., 2022); however, the complete elimination of collinearity is not the solution. Moreso, the screening for regions of multiple spectra collinearity across the fingerprint region should be the purpose of these analyses. Therefore, first screening the fingerprint region using methods that analyse the collinearity of spectra to find wavenumber regions that are separable may be more appropriate to gather regions of "importance" rather than using decision tree (RF) methods to retrieve this data. These methods are niche, and thus, this approach has not been applied to spectral data for the purposes of classification. Additionally, RF models classifying from a reduced wavenumber region would be faster and have fewer decision-making trees. This is important as RF classification tends to overfit results with an increasing number of decision trees (Hastie et al., 2009).

Based on the above discussions, I propose a broad conceptual classification workflow for future studies aiming to predict unknown pollen using RF classification of vibrational spectra:

1. Screen the entire fingerprint region of herbarium training data to identify regions of importance for classification purposes. This should be undertaken with models independent from RF classification models so that decision-making trees are not created in

excess to filter non-focus and non-interspecific signals, thus overfitting the RF classification to non-interspecific data.

2. Perform ordinations of the wavenumber regions characterised as important. Assess whether these selected regions provide interspecific differentiation identified by species-level clustering.

3. Introduce unknown (sub-fossil) spectra into the PCA ordinations, focusing on the identified important wavenumber regions. Evaluate the level of comparability between the known (herbarium/training) and unknown (subfossil/environmental) samples.

4. Construct a final RF classification model that classifies spectra based on the wavenumber regions identified as containing only high interspecific variation in the herbarium dataset.

This conceptual workflow could optimise classification accuracy by focusing on regions with high interspecific variation in the training dataset while also excluding regions in both modern and sub-fossil datasets that may be producing false-positive interspecific signals derived from chemical forcings of unknown chronology effects, the chemistry of the pollen source environment, and pollen oxidation levels. However, significant consideration needs to be placed on the potential overfitting of classification success using this methodology and a substantial loss of data from removing large areas of the fingerprint region. However, this workflow may improve the generality of the RF classification model when new spectra are introduced to it.

5.3 Quantitative reconstructions of Murnong

The key and final aim of this investigation was to provide a quantitative regional estimation of ancient Murnong extent, setting the context for future investigations into Murnong extent at various scales, e.g., small-scale archaeological to regional spatial reconstructions. This study finds that food plants comprise >50% of the Cichorioideaens in eight of the nine studied sampled depths (Fig. 43) and that Murnong can then be quantified using vegetation reconstruction models (Fig. 44).

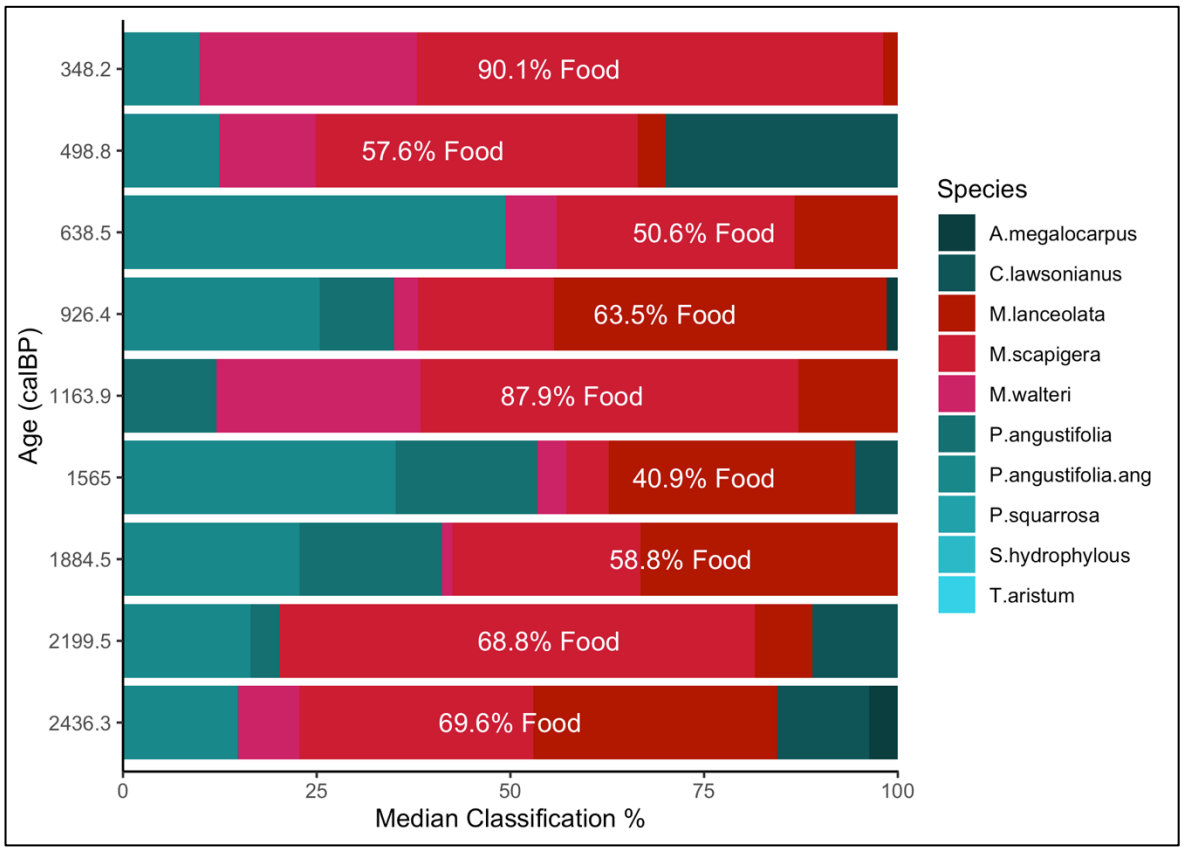


Figure 43 Lake Colac's Cichoraceous pollen predictions using RF model.

i. Quantification of Cichorioideae extent

Interestingly, despite ethnohistorical records of Murnong extent reductions after colonial invasion, a relative increase in Cichorioideae extent is observed. However, post-invasion increases in Cichoraceous taxa likely represent the invasion of exotic taxa introduced by colonists and the establishment of agricultural weeds. Future studies may elucidate changing compositions of post-colonial Cichorioideae pollen through the introduction of exotic taxa to reference spectral libraries, allowing predictions of post-invasion Cichorioideae extent. The discrimination of Cichorioideae to a food plant versus non-food and exotic versus native taxa during colonial invasion will provide a practical pathway to begin to understand the effects of colonialism on Indigenous food plant extent and cultivational practices, alongside a reconstruction of European-style agricultural expansion after the initial colonial invasion.

ii. Using chemotaxonomy to quantify Murnong extent

Using the RF classification model to predict unknown sub-fossil Cichorioideae pollen to a species level revealed a dominance of food plants (summed *M. lanceolata, M. scapigera,* and *M. walteri*) at all but one sample dated to 1565 calBP. A median classification of 63.5% attests that Murnong was a key foodplant in the region surrounding Lake Colac, despite foods only representing three of the ten species that could be classified from sub-fossil pollen within the region. Across the record, *M. walteri* finds a median prediction of 6.6%, *M. lanceolata* a median prediction of 13.3%, and *M. scapigera* a median prediction of 30.7%. *M. walteri*. Due to the low number of FTIR analysed depths (n = 9), it is not possible to infer changes between food plant predictions and other vegetation-climatic reconstructions. Interestingly, *P. squarrosa*, *S. hydrophylous*, and *T. aristum* were not predicted in any of the studied samples, suggesting these species may be absent from the entire record.

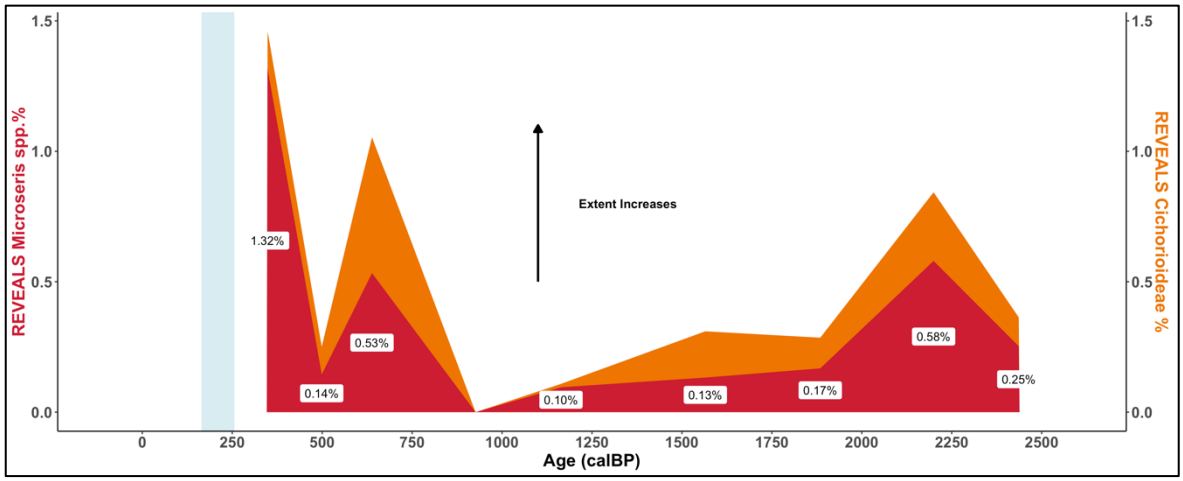


Figure 44 REVEALS reconstruction of Cichorioideae subdivided by food plants (Microseris spp.). The shaded blue area marks the colonial invasion period.

Dividing the REVEALS reconstruction of Cichorioideae vegetation by food to non-food FTIR prediction by the sample depths finds a median of 0.21% and a maximum of 1.32% Murnong vegetation cover around Lake Colac for the studied samples. This result contradicts extensive colonist depictions of south-eastern Australia. However, it should be noted that colonist descriptions are not directly focussed on the study site, Lake Colac. Rather, Lake Colac falls within a broader regional context where Murnong was described (Basaltic Plains), which may explain the lower extent observed. In this regard, low quantified extents may be expected, thus setting the scene for future localised study at sites with clearer ethnohistorical linkages to Murnong presence and cultivation.

Regardless, this low extent poses several considerations and questions for future analyses into this food plant taxa.

iii. Considerations for the quantitative reconstruction of Murnong

Firstly, zoophilous dispersing taxa like Murnong are probably underrepresented in REVEALS-based vegetation reconstructions (Duffin and Bunting, 2008), despite the validation of the REVEALS model for reconstructing vegetation extent in zoophily dominated environments using the LSM of atmospheric dispersal (Mariani et al., 2022, 2016). Moreover, the level of uncertainty when a single morphological (Cichorioideae type) taxon is reconstructed using the anemophily biased REVEALS model (Hellman et al., 2008) is compounded by the established fact that zoophilous dispersers are broadly underrepresented in large lake sediments (Faegri et al., 2007). This is part-expressed in the assumption of the REVEALS model that anemophilous transport is the only vector for pollen dispersal in the model (Sugita, 2007a). Therefore, reconstructions of a single taxon of primarily zoophilous dispersal may be reconstructed as only a fraction of its actual regional vegetation extent. Thus, a key consideration of future Murnong reconstructions using mechanistic models should be addressing the biases of zoophilous, local (lakeside), and anemophilous deposition of Cichorioideae pollen into large lake records. Potentially, estimates of the frequency of high atmospheric turbulence conditions using instrumental data around the record being studied may help improve confidence that lake records are not underrepresenting zoophilous dispersing taxa. Where available, ethnographic, archaeological, and sedimentary DNA evidence along lake margins may assist in recognising the effect of lakeside deposition of pollen.

Secondly, the lack of modern Cichorioideae vegetation in unmanaged country presents a challenge for accurate Murnong extent reconstructions using the LRA. This is due to the lack of calculated PPEs for this subfamily in Australia. This thesis's approach assumes that Cichorioideae PPEs are near identical to Tubuliflorae PPEs. Existing studies from Europe and Asia suggest there are minor differences between Cichorioideae and Tubuliflorae pollen productivity (Broström et al., 2004; Kuneš et al., 2019; Li et al., 2017). For solutions to this, transferring PPEs from these continents to the Australian context is unfeasible due to regional and climatic variances in pollen productivity. Thus, the only potential solution is targeted pollen collection in areas rich in Cichorioideae vegetation, like Indigenous-maintained Murnong gardens (privately owned). More broadly, collaboration with Indigenous owners of country is not just the responsible but also the most beneficial pathway for this genre of research (Antonelli, 2023). Considering this

study finds Murnong the dominant Cichorioideaen in sub-fossil records, this approach could provide accurate PPEs for the entire Cichorioideae subfamily, which is not overly biased to Murnong as this study indicates it was the dominant Cichorioideae taxa before colonial invasion, improving reconstruction accuracy.

Finally, this study is the first to attempt a novel combination of pollen biochemistry and vegetation modelling. Due to the embedded novelty and related challenges, in the time available, I could only analyse nine sample depths for Murnong presence using FTIR methods. The data, therefore, is far too granular to make conclusions about changes to Murnong's extent throughout the Holocene. Moreover, the lack of exotic introduced Cichorioideae pollen within the herbarium spectra library means uncovering the ethnographically recorded colonially driven declines of Murnong extent is not possible within the scope of this study. Future research involving higher resolution pollen records and including estimations of Murnong extent after colonial invasion is essential for a more nuanced understanding.

Concluding Remarks

This thesis represents a first step towards truly quantitative vegetation reconstructions of taxa limited by pollen morphological resolution, often food plants utilised by ancient human communities. Through this study's focus on Murnong, a key Indigenous food plant that was cultivated likely for as long as south-eastern Australia has been inhabited, this research adds to an underrepresented intersection of disciplines and ideas in the Quaternary Sciences and Archaeology. This intersection bridges modelled quantitative landscape reconstruction methods, emerging chemotaxonomic classification workflows, and ethnographical resources of a culturally significant Indigenous food plant. Doing such, this thesis begins to uncover new possibilities available to scientists by working at the fringes of disciplines steeped in tradition, e.g., Palynology. This study provides the first quantitative reconstruction of Murnong, a staple Indigenous food plant, and the first quantitative reconstruction of morphologically cryptic sub-fossil pollen using vibrational spectra and a machine learning classification model.

This thesis presents a tentative proof of concept that cryptic sub-fossil pollen can be classified to a species level using machine learning classification models and sporopollenin vibrational spectra in reference to herbarium analogues. However, several methodological questions are raised, for example, how to test if chemotaxonomic

classification models built on modern reference material are classifying unknown sub-fossil pollen using comparable spectra regions only. While this work cannot answer all the questions it creates, it nonetheless positively works towards addressing the challenge of poor pollen taxonomic resolution. In pollen-based vegetation reconstructions, alongside the need for more accurate non-structural level quantification, poor taxonomic resolution is a fundamental limiting factor in all future studies. This thesis suggests chemotaxonomy provides a very promising pathway to address this, a first of its kind in the context of Australia.

While the theme of this study was broadly technical, combining novel pollen discrimination methods using chemical spectra (FTIR and machine learning) in conjunction with recently validated vegetation reconstruction methods for Australia (REVEALS), it is important not to dissociate the studied plant group Murnong from the socio-cultural context it relates to. Australia's post-invasion landscape was an attempted erasure of Indigenous persons and Indigenous practices. In the following centuries after colonisation, the prevailing notion that ancient Indigenous Australians were primitive hunter-gatherers has persisted. However, recent studies have revealed that Indigenous people were cultivators and aqua-culturalists, adding to the compelling notion that pre-invasion Indigenous activities were much more complex than they may have appeared to the first colonists and explorers. This study builds the methodological foundations to ask the question of: to what extent and where were they cultivating food plants.

Moreover, written colonist accounts attest to the idea that the collection and cultivation of Murnong were primarily roles undertaken by Indigenous women. This adds another layer of importance to the study of Murnong, highlighting the instrumental role that ancient women have played in food acquisition and, consequently, the health of ancient Indigenous communities. Colonist accounts acknowledge a botanical skilfulness of Indigenous women related to many food plants, but especially Murnong, being the primary participants in its ancient cultivation. Therefore, any discussion about Murnong would be incomplete without recognising the essential role that Indigenous women have played in its past ecology.

Going forward, analysing multiple high-resolution records for FTIR analyses across varying site sizes and types (e.g., non-wetland). Utilising the LRAs secondary LOVE model for small sites and testing the newly emerged EDA approach for spatially explicit vegetation reconstructions are key to understanding ancient Murnong extent and spatiality. Given the rich Indigenous oral histories and colonist recordings of Indigenous

plant usage, another possible avenue would be a non-traditional vegetation reconstruction based on ethnographic records alone (e.g., Richer and Gearey, 2018).

Crucially, however, spatial reconstructions using the EDA could be expanded to include archaeological and riparian zones where Murnong has been attested to in colonist accounts. Human activity – specifically, the gathering and estimated transportation distance of Murnong from hotspots, e.g., rivers and small lakes to settlements by Indigenous peoples – can serve as an alternate non-mechanistic metric for pollen dispersal allowing spatially explicit reconstructions of human-managed plants (Abraham et al., 2023). These techniques focussing on ancient Indigenous activity could contribute to modern Indigenous-led Murnong conservation efforts and concepts of reconciliation around pre-invasion Indigenous land usage. These concepts, among others, could offer a more holistic view of Murnong's historical extent and usage. These lines of inquiry have the potential to deepen not only our understanding of the ecological history of Murnong and its integral role in pre-invasion Indigenous food systems but also the exploration of sustainable foods and cultivation practices as we move into uncertain agricultural futures (Antonelli, 2023). These questions developing from the first test method and concepts presented here set the stage for further multidisciplinary inquiry into this underrepresented genre of research and have the potential to reshape our understanding of the ecological history of Murnong and the complexities of Indigenous Australia.

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Supplementary Information

S1 Genus profile for native Australian *Microseris* spp.

Indigenous names: ‘Murnong’ (Koorie, Victoria), ‘Garngeg’ or ‘Nyamin’ (New South Wales), also known as ‘Yam Daisy’.
Clade: Asterids
Order: Asterales
Family: Asteraceae
Subfamily: Cichorioideae
Tribe: Cichorieae (also known as Lactuceae)
Genus: *Microseris*
Species (Native to Australia): *M. lanceolata, M. scapigera, M. walteri*

Microseris spp. is a genus of flowering plants within the tribe Cichorieae found native to Australia, New Zealand, and North America. In south-eastern Australia the genus is comprised of three native species *M. lanceolata*, *M. scapigera*, and *M. walteri*. It is recorded that the tubers of these three species were cultivated and considered a staple food for First Nations Peoples in south-eastern Australia. It is suggested that *Microseris* spp requires human intervention to proliferate, specifically Indigenous cultural burning and digging practices (Gott, 1983, 1982), often employing a ‘Yam Digging Stick’ (Figs. 1-2). The introduction of sheep and cattle grazing along with exotic Cichorioideaen plants after colonisation in 1788 significantly reduced the range of *Microseris* particularly in south-eastern Australia.



Figure 1 Pointed end of a Yam Digging Stick (Nugent, 2006).



Figure 2 Sketch of Indigenous women digging for Murnong using a Yam Digging Stick (Wedge, 1835).

It is noted further disambiguation between the cultivation uses of each individual species is needed to clarify if cultivational usage varied at a species level (Walsh, 2016). Furthermore, *M. walteri* was only recently resurrected and clarified as the third congener of *Microseris* in Australia (Walsh, 2016). Here we assume the cultivational usage of *Microseris* occurred at the genus level.

Flowers and Seeds



Figure 3 Microseris flowers from the left: M. scapigera, M. walteri, and M. lanceolata (approx. 5cm wide) (EditorAndrew, 2020; EditorAndrew, 2020, 2021a).

A single yellow flower is produced on the top of each stem of *Microseris* plants, visually similar to Dandelion flowers (*Taraxacum* genus). From the available resources on *Microseris* ecology it is suggested that capitula of *M. lanceolata* remains open for a ‘day or more’ with variable seed production, compared to the shorter fertilisation window of *M.*

scapigera were the capitula remain open for a few hours, suggesting cleistogamy (Walsh, 2016).



Figure 4 Microseris seeds from the left: M. scapigera, M. walteri, and M. lanceolata (EditorAndrew, 2021b).



Figure 5 Microseris seed clock from the left: M. scapigera, M. walteri, and M. lanceolata (EditorAndrew, 2019, 2021c; Vanderveck, 2011).

Tubers



Figure 6 Microseris tubers from the left: M. scapigera, M. walteri, and M. lanceolata (Brown, 2021; EditorAndrew, 2021d, 2021e).

Microseris tubers are variable in size and shape but are generally milky coloured and fleshy. For *M. scapigera* and *M. lanceolata* tubers are generally cylindrical and long, branching below the leaves and just below ground level respectively. *M. walteri* forms a single long fleshy tuber. The taste of the *M. lanceolata* and *M. scapigera* is said to be tolerably bitter where *M. walteri* has a sweet taste. The tubers of *M. lanceolata* contain a high proportion of fructans and is similar to commercial crops in its proportion of stored of carbohydrates (Incoll et al., 1989). The high retention of fructans and carbohydrates in *Microseris* and other Indigenous foods are of particular interest considering modern Indigenous Peoples susceptibility to insulin-resistant diabetes following urban western diets (O'Dea, 1991)

Habitat

Microseris is found in the ecologically temperate geopolitical regions of Victoria, New South Wales, South Australia, and Tasmania. *Microseris* is generally found across the grassy plains of New South Wales and Victoria where it is present in the Basaltic Plains west of Melbourne. *M. lanceolata* and *M. scapigera* are also found in alpine to sub-alpine localities like the Grampian Mountains. See the Spatial Portal of the Atlas of Living Australia (2023) for more geographical distribution information.

Pollen

Microseris pollen is characteristic of most fenestrate (echinocolpate) Cichorieae (tribe) pollen; where the sexine (exine outer layer) is raised into a ridge pattern (lophae) with a single layer of spines (echinae), surrounding large fenestrate apetures (lacunae) (Macphail and Woodward, 2017). *Microseris* pollen can thus be classified as cryptic, such as it is difficult to discriminate the genus from other members of the Cichorioideae subfamily. This is particularly apparent when pollen is sampled from sedimentary archives subject to mechanical and biological alteration, and thus studied using standard light microscope techniques. The three *Microseris* species are described from the limited available literature below (Tab 1) (Feuer and Tomb, 1977; Wenger, unpub), which highlights the necessity of further morphological study of *Microseris* spp, particularly *M. walteri* pollen grains.

*Table 1 Morphological characteristics of Microseris pollen based on morphological studies *Fueer and Tomb (1977), **Wenger (unpub).*

Morphological characteristics	*M. scapigera*	*M. walteri*	*M. lanceolata*
Morphological type	Fenestrate (echinolophate)	Fenestrate (echinolophate)	Fenestrate (echinolophate)
Average equatorial size (µm)	*45.5	No data	**52
Average polar size (µm)	*38.4	No data	**44.4
Average number of polar spines	No data	No data	**9
Exine thickness (µm)	No data	No data	**8.4
Notes	*Lacunae not clearly defined. Variable pollen shape susceptible to shape distortion	-	-

Comparing herbarium *M. lanceolata* pollen to the most common non-food species pollen in our chemotaxonomic classification, *Picris angustifolia* highlights the morphological uniformity of pollen of the Cichorieae tribe (Figs. 7-8). The micrographs of Figures 7-8 are from modern samples collected directly from anther and have been treated with 10%KOH and acetolysis. Modern samples should be considered pristine and do not represent likely distorting affects e.g., crumpling and mixing with other material, present when analysing unknown sub-fossil pollen grains.

Plate Key

a. Polar view in high focus
b. Polar view in lower focus
c. Polar view in optical section, focus on equatorial ridges
d. Equatorial view showing equatorial ridge, with paraporal lacunae above and below
e. Equatorial view showing aperture, with inter-lacunar gaps and abporal lacunae
f. Equatorial view in optical section

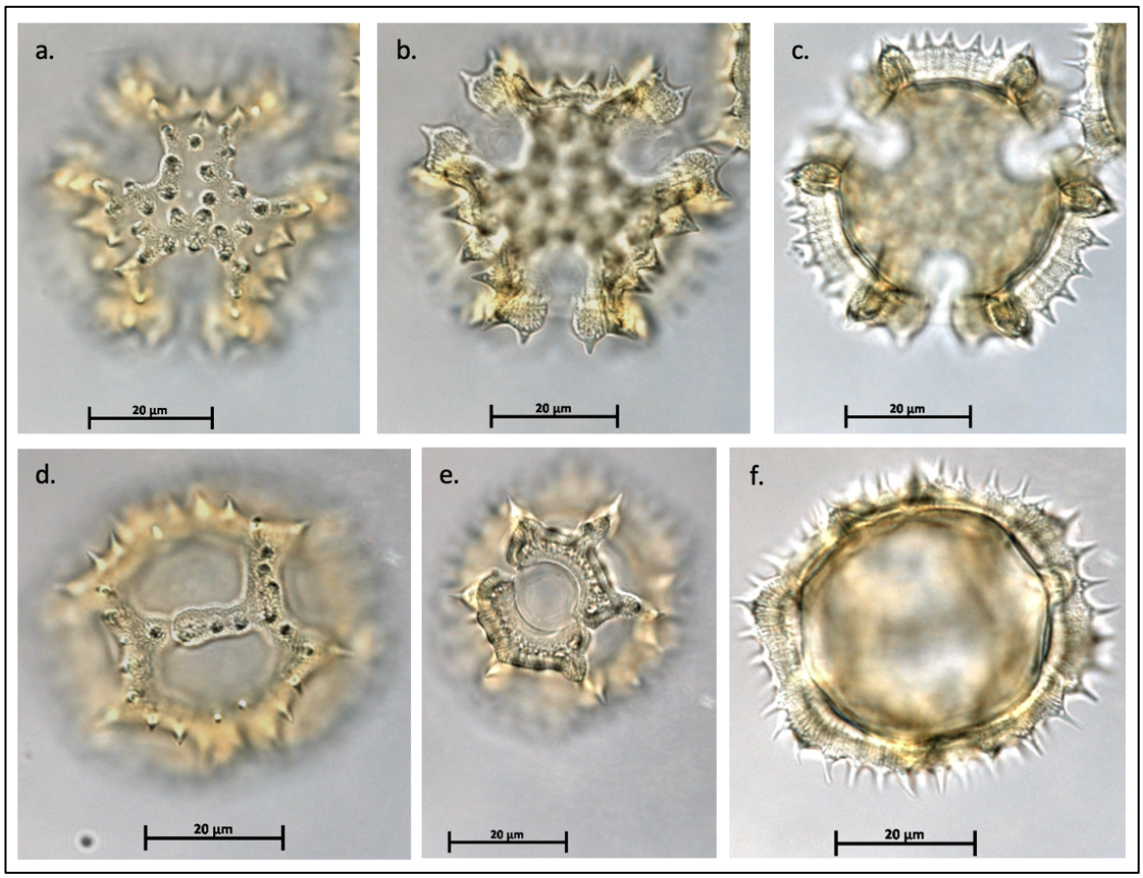


Figure 7 Plate of M. lanceolata pollen (Wenger, unpub).

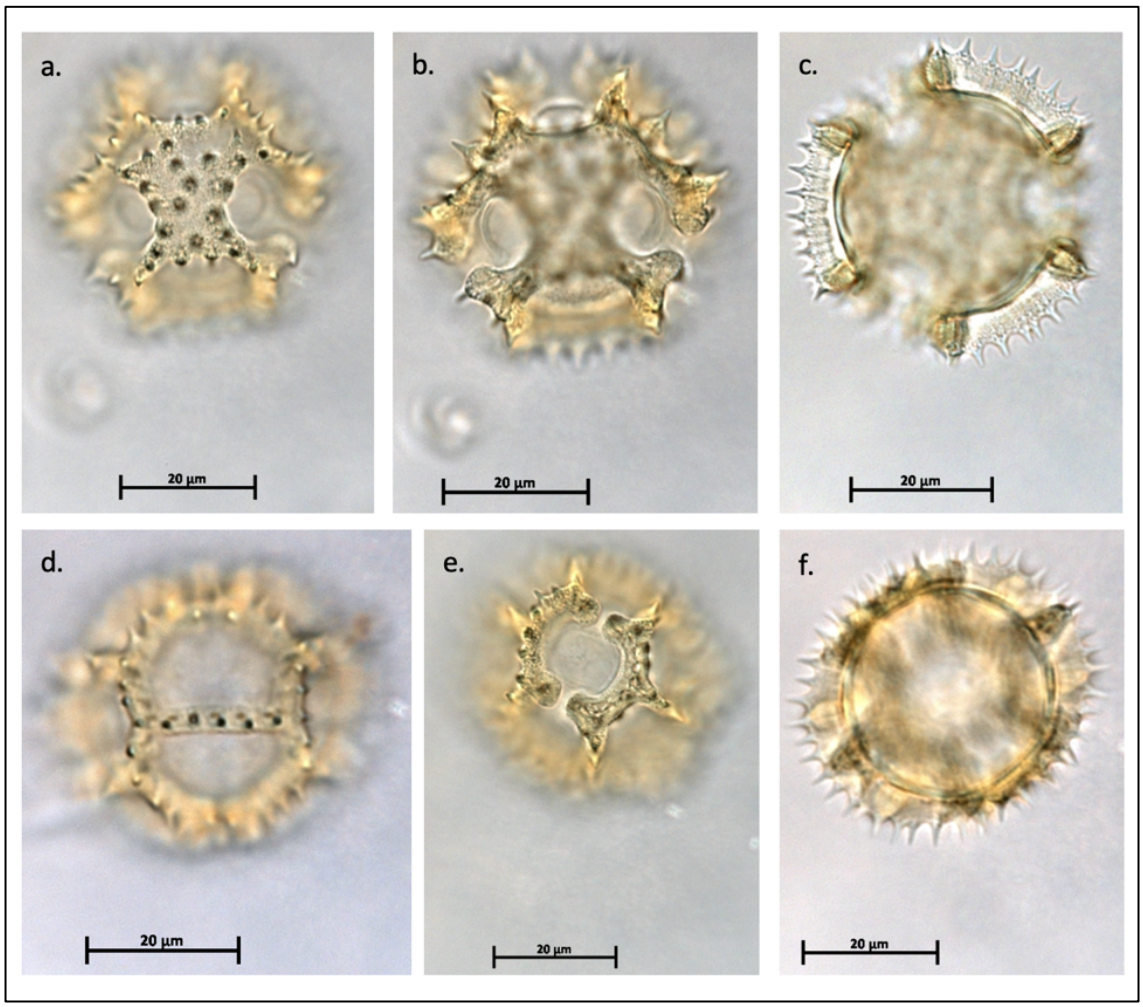


Figure 8 Plate of Picris angustifolia pollen (Wenger, unpub).

S2. Herbarium pollen metadata

Two herbarium samples for *M. lanceolata* and *P. angustifolia* were merged to provide a total count.

Taxon	Herbarium	Herbarium code	Location	Number of scanned grains
Acites megalocarpus	National Herbarium of Victoria	MELUID 116638a	Melbourne	40
Cymbonotus lawsonianus	National Herbarium of Victoria	MEL238303	Melbourne	36
Microseris lanceolata	Australian National Herbarium	CANB 908802	Canberra	20
Microseris lanceolata	National Herbarium of Victoria	MEL2040366	Melbourne	20
Microseris scapigera	National Herbarium of Victoria	MEL2447418	Melbourne	39
Microseris walteri	National Herbarium of Victoria	MEL2377579A	Melbourne	40
Picris angustifolia	National Herbarium of Victoria	C. Anderson 22 Nov 1979	Melbourne	20
Picris angustifolia	Australian National Herbarium	CANB 892401	Canberra	20
Sonchus hydrophylous	National Herbarium of Victoria	Sheet 1- Neville Scarlett April-May 1972	Melbourne	40

Taraxacum arisтum	National Herbarium of Victoria	MELUID19339	Melbourne	40
Picris angustifolia subsp. angus	National Herbarium of Victoria	S. Clinton Dec 1918	Melbourne	31

S3. Synthesis plot figures

Select elements of the final composite plot are re-printed below in a slightly larger format for the readers convenience. All plots have the period of colonial onset appear as a shaded blue region.

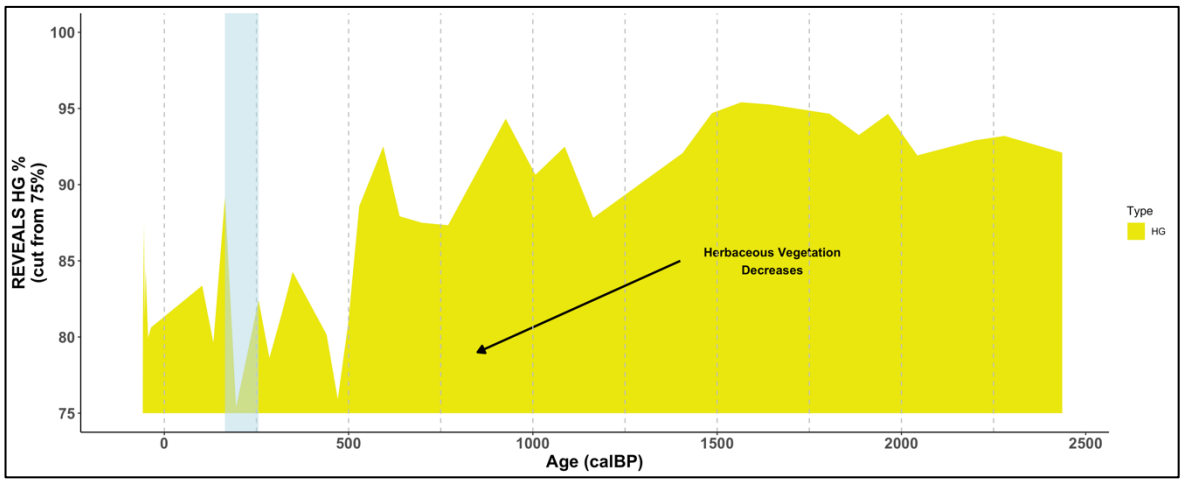


Figure 1 Herbaceous REVEALS vegetation reconstruction cut from 75%.

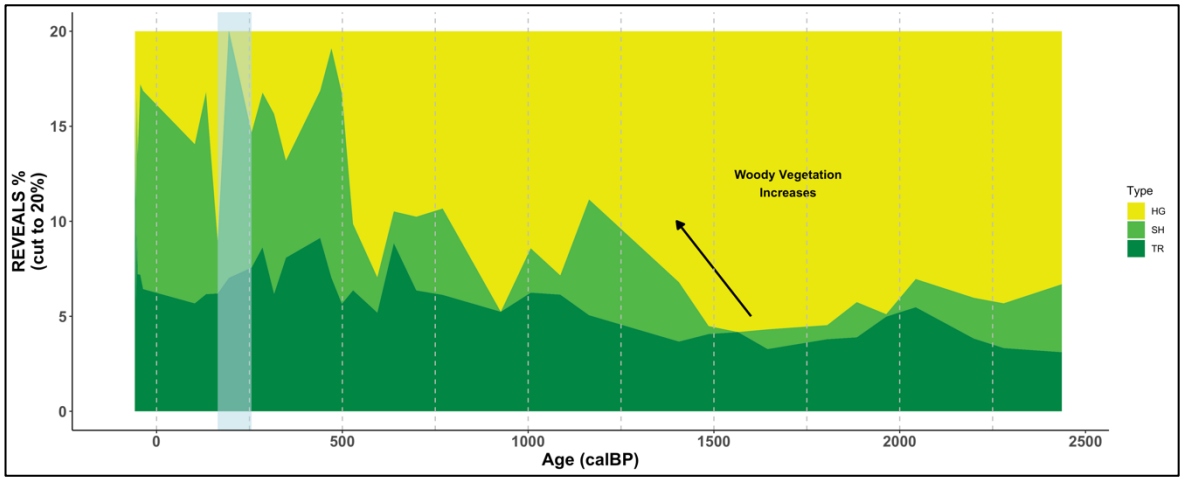


Figure 2 Structural group REVEALS vegetation reconstruction cut to 20%.

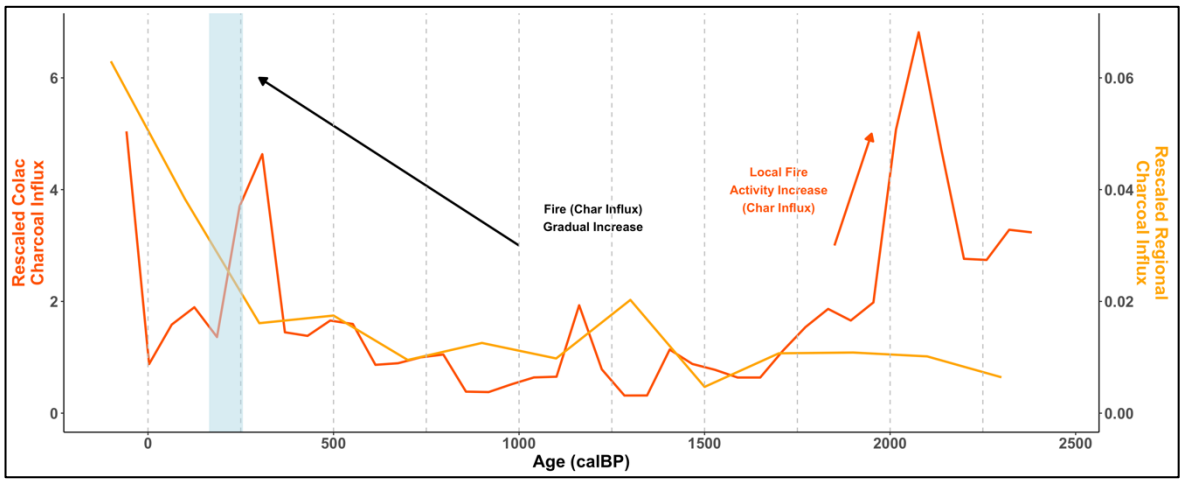


Figure 3 Overlayed regional and local (Lake Colac) charcoal influx.

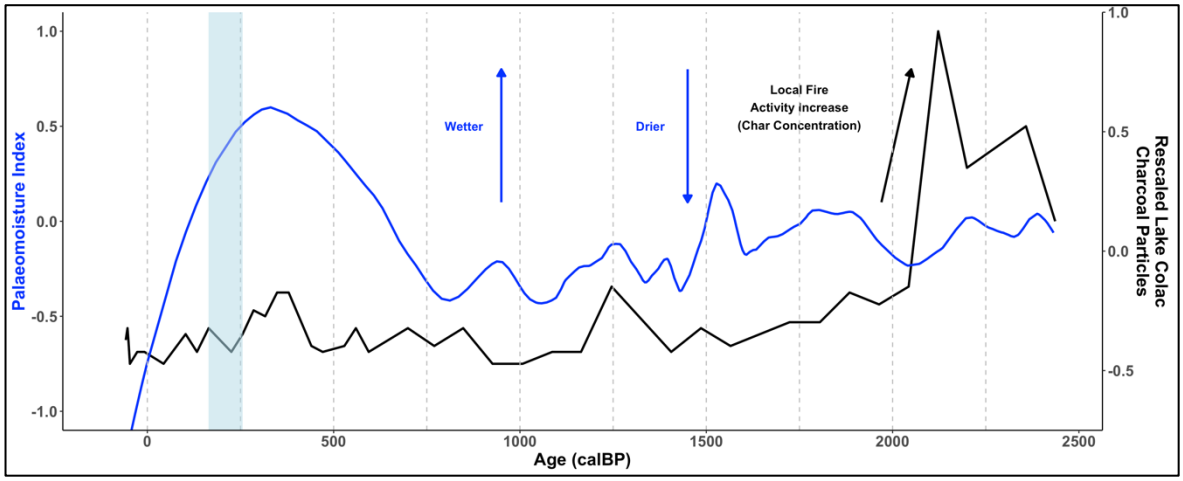


Figure 4 Overlayed terrestrial palaeomoisture index and Lake Colac charcoal concentrations.