Holocene climate, fire and ecosystem change on Kangaroo Island (Karti), South Australia

A thesis submitted in fulfilment of the requirements for the degree of Master of Philosophy

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Table of Contents

Chapter five: Lake sedimentary ancient DNA reveals ecosystem response to fire and climate on Kangaroo Island (Karti), Australia

Abstract

The Earth system – a complex interplay of climate, landscapes, fire, ecosystems, and people – is on the precipice of a geologically significant crisis. Examining Earth systems interactions in the past offers important context for contemporary changes and helps to predict and plan for the future.

In this thesis I present a multi-proxy reconstruction of the past 7,000 years of climate, fire and ecosystem change from the lake sediments of Lashmars Lagoon, Kangaroo Island (Karti), South Australia. Kangaroo Island (Karti) is uniquely positioned to fill a regional gap in Holocene palaeoenvironmental reconstructions and has a singular history of human habitation ideal for investigating the long-term implications of losing Aboriginal land stewardship.

A robust chronology combining ^{14}C dating with ^{210}Pb analyses and Pu isotopic profiling was established for the site from a new ~7.5-metre-long lake sedimentary record. Changes in catchment processes and hydrology were inferred using X-ray fluorescence elemental scanning combined with mineralogical analysis through X-ray diffraction and bulk organic geochemistry. Past fire and vegetation history was reconstructed using macroscopic charcoal, which augmented microscopic charcoal and pollen data analysed by another researcher. Finally, ecosystem responses to fire – with particular focus on the Viridiplantae (green plants) – were inferred from the analysis of sedimentary ancient DNA (*sed*aDNA) using shotgun metagenomics, in one of the first studies of this kind in Australia.

In this thesis, fire activity was inferred to have increased around Lashmars Lagoon at ~3.3 ka, providing improved chronological precision for a similar finding in a previous study. The validity of this result was reinforced by the absence of a clear correspondence with changes in depositional processes previously implied to have biased the palaeofire record at this site. Pollen and *sed*aDNA analyses provided ecological context for the fire regime change. The pollen record shows an increased diversity of mid-story shrubs at ~3.3 ka. The *sed*aDNA record also records compositional changes at this point, as well as increased relative abundance of Viridiplantae DNA after ~3.3 ka.

All evidence considered, it appears that the change to more frequent and or bigger fires on Kangaroo Island (Karti) at ~3.3 ka was the combined result of a change in land use and climate. The loss of Aboriginal land stewardship sometime after ~4.3 ka may have led to the development of a dense shrubby mid-story landscape, priming it for more fire with increased fuel loads. Ultimately, late Holocene climate change – namely increased aridification linked to an apparent intensification of the El Niño Southern Oscillation (ENSO) – may have tipped the balance of an unmanaged system into a novel fire regime.

Critically, the palaeoenvironmental record from Lashmars Lagoon may provide a rare analogue to contemporary fire regime changes on mainland Australia – and thus an opportunity to understand the potential long-term consequences of current events. This thesis paves the way for future palaeoenvironmental research on Kangaroo Island (Karti), encouraging a more detailed assessment of the human occupation history of the island and additional work to distinguish between more informative aspects of past fire regimes including fire intensity, frequency and biomass burned.

Originality declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

I acknowledge that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University’s digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Lucinda Duxbury

Personal and academic acknowledgments

For the Islanders, past and present – I hope this is of use for the future.

A big thank you to my dream team of supervisors – Jonathan Tyler, Linda Armbrecht and Haidee Cadd – all brilliant people as much as they are brilliant scientists. I also had the fortune to be supported by an AINSE Ltd. Postgraduate Research Award during my candidature. A special thanks also to the guidance and academic advice from Alex Francke, Boone Law, Stefan Loehr, Simon Haberle and Vilma Pérez Godoy along the way. To all the Mawson building staff and students, as well as the friendly Mawson ghost who kept me company when I stayed late at the Uni. To my office mates and lab mates – thanks for keeping me accountable #winetimeworks. Thanks, of course, to all my beautiful friends and family. Especially Luce, Eleanor, Alex and my brother Jack – thanks for keeping me happy and for keeping up with wherever my brain bounced. For my uncle Ken and for Jacob Stengle – two people with whom I wish I could have shared the outcomes of this research.

My heartfelt thanks to the KI locals who have been so generous with their time and warm with their welcome whenever I have visited. I want to especially thank Bronwyn, Colin and the Lashmar family, Boone, Matt, James, Mark Agnew, the Kangaroo Island Landscape Board and the Kangaroo Island branch of the Department of Environment and Water.

The work in this thesis could not have been done if it were not for the work of Robin Clark, a pioneering woman in the Earth Sciences, who thought about her research with a depth akin to the timescales she studied. Robin quotes the poet TS Eliot on *time* as the epigraph to her Honours thesis:

Time present and time past
Are both perhaps present in time future.
And time future contained in time past.
If all time is eternally present
All time is unredeemable.
What might have been is an abstraction
Remaining a perpetual possibility
Only in a world of speculation.
What might have been and what has been
Point to one end, which is always present.

To that, I respond with Eliot on *place*:

And the end of all our exploring
Will be to arrive where we started
And know the place for the first time.

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It also feels wholly appropriate to quote Douglas Adams on *deadlines:*
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I love the whooshing noise they make as they go by.

And *cats*:

If you try and take a cat apart to see how it works, the first thing you have on your hands is a non-working cat.

Sorry about the cat.

Acknowledgement of Country and Indigenous knowledges

I pay my respects to elders past, present and emerging on whose land this research has been conducted and on which sovereignty was never ceded. Always was, always will be.

I acknowledge that knowledge of Aboriginal land stewardship and cultural burning practices in Australia stems first and foremost from First Nations people themselves. Australian Indigenous knowledges have been maintained through millennia by cultural practice, oral traditions, and deep and ancient spiritual connections to the land.

Western science and scientists have failed to recognise the legitimacy of these knowledges and have caused deep hurt for Aboriginal people since colonisation. For this, I am truly sorry.

In this thesis, I was limited by the constraints of this Western framework in capturing the full breadth and depth of Indigenous knowledge. Consequently, I want to clearly recognise here that I believe Indigenous knowledges are equally valid to knowledge I have produced in this thesis within a Western scientific framework, especially pertaining to cultural burning practices and history of Aboriginal people on Kangaroo Island (Karti).

Further, I feel it is important to note that while on the balance of present evidence it appears to me that Aboriginal people were not living permanently on Kangaroo Island (Karti) when Europeans first arrived in the area, the island has never been abandoned in a cultural and spiritual sense. Kangaroo Island (Karti) is an important place for living Ngarrindjeri, Kaurna, Narungga, Nhawu and Barngarla people today, as well as for the Tasmanian Aboriginal women who were brought there by sealers in the nineteenth century, whose descendants are Islanders today.

Note on the Aboriginal (Ngarrindjeri) name used for Kangaroo Island in this thesis

Tindale and Maegraith (1931) and Tindale (1937) were the first to assign the name ‘Kartan’ to the archaeological industry found on Kangaroo Island. Ronald Lampert followed suit in his epic work ‘The great Kartan mystery' (1981), recording that 'Karta' was the name given to the island by the Ramindjeri, a western group of the Ngarrindjeri. Despite this, the origins of this name are unclear. The Ngarrindjeri dictionary compiled by Mary-Anne Gale and Ngarrindjeri Elders and community in 2009 makes three references to Kangaroo Island:

> **Kukakungarr (p. 26) |** *Noun.* Kangaroo Island. *Written source:* M= Ku:kakungarr.
>
> **karti (p. 14) |** *Noun*. low thick scrub; useless land; island. Written source: M= karte 'low thick scrub, useless land': T= karte 'island': Kin BI= ka:rti 'island'. Note: Teichelmann and Schurmann recorded in 1840 the name of Kangaroo Island as Karta, which was presumably a Kaurna word, but very similar to the word karti.
>
> **powongko (p. 113) |** *Noun.* spirit; soul of life. *Written source:* Tn= powoIJko 'spirit, soul of life - that part of a man which after death goes away to the north west to Karta on Kangaroo Island' (Tindale ms Milerum)'. *Note:* According to Milerum this is 'that part of a man which after death goes away to the north west to Karta on Kangaroo Island'.

Here, I have decided to use *Karti* in reference to Kangaroo Island, to reflect what is recorded in the recently published Ngarrindjeri dictionary and maintain a sense of continuity with previous literature. This does not reflect an official naming of the island; it is a judgement I have made with the available information. In doing so, I hope to encourage conversation to determine an officially recognised dual naming convention for Kangaroo Island from one or more of South Australia's Aboriginal languages.

Notes on pronunciation of *Karti*:

'K' is pronounced halfway between the English 'k' and 'g' as in 'Kaurna', the people of the Adelaide Plains

'a' is as in the English words: mama, papa, visa

'rt' is one sound which involves curling your tongue back in your mouth while pronouncing an English 't' – similar to the 'rt' in cart with a strong North American accent

'i' as in the English words: bit, pit, sit

I recommend that readers consult with Ngarrindjeri Elders and speakers to practise pronunciation and note that the language remains the intellectual property of the Ngarrindjeri community.

References

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1

Introduction

Global context: fire, climate, and ecosystem change

The Earth system is in the midst of unprecedented upheaval that threatens our very survival as a species. Since industrialisation, widespread anthropogenic disruption of the complex equilibrium between climate, landscapes, fire, ecosystems, and people has caused accelerated global warming and the widespread loss of biodiversity and ecosystem services (IPCC, 2022). Many have called for the definition of a new geological era – the Anthropocene – to reflect the magnitude of the influence that humans have had on global systems in this short time (Lewis and Maslin, 2015).

Regional context: southern Australia

In southern Australia, climate change is leading to lower rainfall, higher temperatures and increased dangerous fire weather (Figure 1; CSIRO and Bureau of Meteorology, 2020). In recent decades, climate change combined with land use modifications since British colonisation – like shrub encroachment and biomass build up in the absence of Aboriginal stewardship – has resulted in drastic changes to fire regimes[1] (Abram et al., 2021, Mariani et al., 2022, Bowman et al., 2020). Fire regimes in southern Australia are trending towards catastrophic megafires that are projected to become more frequent, more intense and burn over longer fire seasons (Hennessy et al., 2022). Clearly both climate and fire regime changes are having detrimental impacts on biodiversity in southern Australia (Murphy and van Leeuwen, 2021, Dickman, 2021). However, the long-term impacts of changes in climate and fire on Australian biodiversity is uncertain because of a paucity of data relating to long-term responses of ecosystems.

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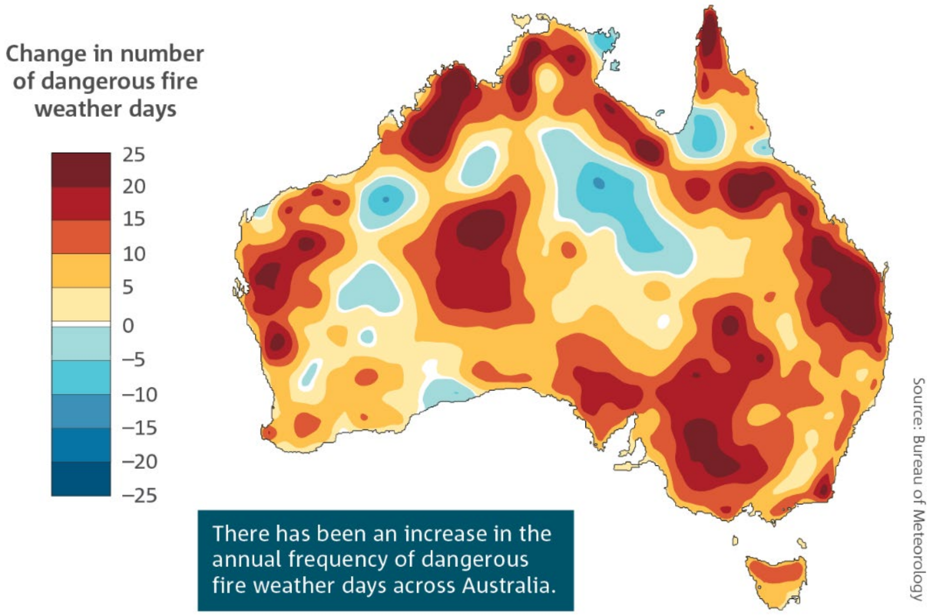
[1] The term fire regime is a broad term that incorporates many different aspects of fire, including fire frequency, intensity, severity, size, fuel type and burn season. For a detailed discussion of the term see: KREBS, P., PEZZATTI, G. B., MAZZOLENI, S., TALBOT, L. M. & CONEDERA, M. 2010. Fire regime: history and definition of a key concept in disturbance ecology. *Theory in Biosciences,* 129**,** 53-69.



There has been an increase in the annual frequency of dangerous fire weather days across Australia.

Source: Bureau of Meteorology

Figure 1. *Change in number of dangerous fire weather days (days where the Forest Fire Danger Index exceeds its 90th percentile) from 1950-85 to 1985-2020. Reproduced with permission from CSIRO and Bureau of Meteorology (2020).*

Contemporary fire and fire management

The recent Black Summer of the 2019-2020 austral summer was the worst fire season since western historical records of bushfires began (Davey and Sarre, 2020). The fires started in July 2019 and continued until the end of March 2020, claiming 33 human lives, over 3,000 homes, causing over $10 billion of economic losses, burning over 8 million hectares of native forest and affecting vertebrates and invertebrates in the order of their billions and trillions (Binskin et al., 2020, Davey and Sarre, 2020). As Black Summer unfolded, a review of prescribed burning in south-eastern Australia that was already underway was coming to the (perhaps redundant) conclusion that present fire management practices in Australia are not equipped to deal with current and projected fire regimes (Morgan et al., 2020). Indeed, the fire season of 2019-2020 saw emergency services completely overwhelmed.

As fire regimes change, fire management strategies and policies must also adapt (Lindenmayer and Taylor, 2020). The Royal Commission into National Natural Disaster Arrangements in the wake of the Black Summer fires highlighted the need to use local knowledge in the development of fire management plans, citing Aboriginal land stewardship as an example of how local knowledge can be successfully applied to mitigate against uncontrolled bushfires (Binskin et al., 2020). Similarly, the recently published Australian State of the Environment Report 2021 calls for nationwide consideration of Indigenous fire management (Metcalfe and Costello, 2021), echoing global momentum (Hoffman et al., 2021).

The role of Aboriginal land stewardship in mitigating catastrophic bushfires

Western science has shown that people have lived in Australia for at least 50,000 years (Tobler et al., 2017, Clarkson et al., 2017), adapting to survive on one of the most fire-prone continents in the world (Bradstock et al., 2012). Aboriginal fire management, once widespread before British invasion commenced in the late eighteenth century, has been suggested to mitigate against catastrophic bushfires (Bird and Nimmo, 2018, Fletcher et al., 2021, Mariani et al., 2022). Aboriginal land stewardship, the framework under which Aboriginal fire management and cultural burning falls, ceased in many parts of Australia when Aboriginal people were forcibly displaced by incoming Europeans. Despite this, these practices and knowledge systems have survived in some places and are being steadily reintroduced in others (Neale et al., 2019, Bird et al., 2008, Steffensen, 2020, McKemey et al., 2020, McKemey et al., 2022).

Indigenous fire management harnesses fire as a tool to make the landscape more amenable to human needs, including protecting people and landscapes against large, uncontrolled bushfires by burning in strategic mosaics and reducing fuel loads (Steffensen, 2020, Bird et al., 2008, Bird et al., 2012). The deep cultural and ecological knowledge of these practices that Aboriginal people hold implies the importance of humans as drivers of fire in Australia (Steffensen, 2020, Yunkaporta, 2019). Often this knowledge is transmitted orally and safeguarded in formats that are foreign to western scientists. As such, there is a gap in the scientific literature when it comes to understanding these practices from the perspective of the Indigenous peoples who practice them.

In addition to evidence from Indigenous knowledge holders, work within western scientific frameworks since the 1970s has steadily begun to acknowledge the important cultural and ecological application of fire by Indigenous Australians. In 1969, Jones popularised the term ‘fire-stick farming’, acknowledging deliberate use of fire to manage ecosystem resources via the creation of habitat mosaics that increased diversity on scales convenient to humans. He suggested that fire acts as an intermediate disturbance and maximises the diversity by creating patches at different stages of ecological succession. More recently, historian Bill Gammage detailed Aboriginal fire management practices in his influential book, ‘The Biggest Estate on Earth’ (Gammage, 2013). Gammage used historical accounts from European ‘newcomers’, to illustrate the intentional nature of Aboriginal burning to make resources ‘abundant, convenient and predictable’ in the landscape. Notably, he compared accounts of vegetation types and densities at the time of European invasion to the present day. Consistently, grasslands and open woodlands were lost in favour of dense revegetation, a finding corroborated by pollen and charcoal studies from lake sediment cores (Mariani et al., 2022, Fletcher et al., 2020). The increase in dense vegetation makes forests more conducive to bigger, uncontrolled bushfires. Indeed, historical bushfires have been found to map almost exactly to Aboriginal massacre sites, implying that loss of indigenous management leads to increased bushfires (Fletcher et al., 2021). A contemporary quantitative test of the ‘fire-stick farming’ hypothesis was carried out in an arid desert region in Western Australia under the stewardship of Martu people (Bird et al., 2008). Compared with the control – an adjacent unmanaged landscape – Bird et al. (2008) found higher vegetational diversity in regions within the regular range of the Martu people, and that this diversity returned higher yields for the hunting of the economically important monitor lizard. Subsequent studies also found that Aboriginal burning can have a protective effect on small-mammal populations as it safeguards against large fires (Bird et al., 2013). While current fire management is starting to recognise the importance of local and Aboriginal knowledge, scientific understanding of how Aboriginal fire management interacted with long-term climate and ecosystem changes in different Australian landscapes is still poorly understood by western science.

Records of past environments can address gaps in contemporary fire management

Holistic long-term reconstructions of past environmental change are necessary to inform contemporary management and to protect human lives and ecosystems into the future. Reconstructions of past fire occurrence, in response to changing climates and anthropogenic activity, can provide precolonial baselines against which current fire behaviour can be contextualised and long-term causes and consequences investigated. The Black Summer fires were certainly unprecedented in modern times – but how far from ‘normal’ are summers like these over the long term? Have devastating fires happened on these scales before? And, importantly, how resilient can we expect ecosystems to be in the face of changing fire regimes? Lake sediments are important archives of past environmental change that allow these questions to be addressed. Over time, lake sediments accumulate, often leaving near-continuous records of past (1) fires, often in the form of fossil charcoal, (2) climates, inferred from geochemical signals and microfossil analysis, and (3) terrestrial and aquatic biodiversity, often gleaned from identification of pollen grains and aquatic microfossils (Last and Smol, 2002b, Last and Smol, 2002a, Smol et al., 2002, Smol et al., 2001).

The influence of climate and people on fire in the Holocene

Most commonly in Australia, past fire is studied from fossil charcoal found in sediments (Mooney and Tinner, 2011), yet it is difficult to discern whether the charcoal derives from ignition by humans or natural sources, such as lightning. Unsurprisingly, the relative contribution of people and climate to Holocene fire regimes in southern Australia is hotly debated (Clark, 1983b, Kershaw et al., 2002, Mooney et al., 2011, Gammage, 2013, Fletcher et al., 2020, Fletcher et al., 2021, Mariani et al., 2022).

An established body of literature discusses the effects of climate on fire regimes in Australia. Mooney et al. (2011) compiled 223 palaeofire reconstructions over the Late Quaternary and found a correlation between climate variables and fire regime change. Indeed, Mooney et al. (2020) and McWethy et al. (2017) find a positive correlation between higher incidence of fire and intensification of El Niño Southern Oscillation (ENSO) in the Holocene. The link between climate and fire is also reflected in the recent past: Indian Ocean Dipole events, which cause reduced rainfall in south-eastern Australia, have been predictable precursors to bad fire years (Dey et al., 2019). Many have also made the link between anthropogenic climate change and the increase in bushfires (Marlon, 2020, Bowman et al., 2020). Increasing temperatures and decreasing precipitation in the south of Australia, both linked to anthropogenic climate change, laid the foundations for the terrible fire conditions of 2019 (Abram et al., 2021).

Problematically, there seems to be a tendency in Australian palaeoenvironmental research to consider human agency in controlling fire regimes only in the absence of a climatic or environmental explanation. This default assumption belies reciprocal interactions between people and environment and the ubiquity of complex, integrated systems of land stewardship practiced by Aboriginal people. An illustration of the problem is evidenced in a study by Bird et al. (2016), who found that anthropogenic fire ignition rates covary with climate change. In other words, people adapt to climate change by altering fire management strategies – evoking a fire regime change triggered by climate but mediated by human behaviour – demonstrating thus how causation and correlation could be confounded if only climate is considered. Furthermore, contemporary Aboriginal stewardship has also been shown to buffer the effects of climate on fire regimes (Bird et al., 2012) – a circumstance that would leave no discernible change in the fire record. In this example, Martu hunting fires in spinifex grasslands have been shown to buffer the effects of climate change on fire regimes by maintaining spatial heterogeneity and keeping biomass low (Bird et al., 2012).

Despite the challenges of studying human influence on Holocene palaeofire records, there are some exceptions which seek to integrate multiple lines of evidence to infer the role of humans in maintaining or changing fire regimes. Examples of this include the work I discuss in this thesis by Gammage (2013), Fletcher et al. (2020) (2021), and Mariani et al. (2022). Additionally, palaeoenvironmental records from Australia's coastal islands – isolated from the mainland during Holocene sea level rise to the point of becoming inhospitable – offer a unique opportunity to untangle the confounding influence of climate and people on fire regimes when compared to more continuously inhabited mainland areas. This approach was pioneered by Clark (1983a) on the sediments of Lashmars Lagoon, Kangaroo Island (Karti; hereby abbreviated to as KI), South Australia and has since been applied elsewhere in southern Australia (Hope, 1999, Mooney et al., 2020, Adeleye et al., 2021).

In summary, disentangling the interconnected interactions between fire, climate, and people is challenging, especially across different temporal and spatial scales. It is evident that both humans and climate drive fire, but the extent of their respective contributions is dependent on local context. While climate variables might be predominant in some places, human management may be more important in others. By the same token, at the same place, over a different timescale, the balance can shift, and one can become more influential than the other. On broader continental spatial scales, synthesis studies seem to show that climate is more influential (Mooney et al., 2011). However, human influence may be more predictive on smaller, more local scales (Bird et al., 2008). Thus, local and national fire management strategies must account for the context-dependent and emergent effects of Aboriginal stewardship and climate change.

Vegetation and fire in the Holocene

Australian ecosystems have coexisted with fire for millennia in one of the most fire-prone places in the world (Bradstock et al., 2012). However, the evolutionary extent of this relationship is contentious (Dodson, 2001, Keeley et al., 2011, Bowman et al., 2012). In southern Australia, research into past fire and ecosystem interactions in the Holocene has focused on the relationship between vegetation and fire histories reconstructed from fossil pollen and charcoal preserved in lake sediments (e.g., (Clark, 1983a, Bickford and Gell, 2005).

Presently, much of southern Australia is populated by sclerophyllous open forests, woodlands and shrublands – plant communities often found in Mediterranean-type climates and adapted to hot and dry conditions (Dodson, 2001). Many plants in these ecosystems also have fire-adapted traits; for example, flowering in grass trees is stimulated by fire (Ward and Lamont, 2000), heat from fires opens banksia fruits and cones (He et al., 2011) and bark in eucalyptus trees contains fire-promoting oils (Steinbauer, 2010). The presence of these traits forms the basis of an elegant theory in which fire and certain vegetation types may have coevolved with specific fire regimes to be mutually beneficial (Mutch, 1970). For example, Fletcher et al. (2020) used a pollen and charcoal record form the Surrey Hills, Tasmania, to infer the precolonial maintenance of open grassland ecosystems in favour of rainforests by Indigenous-managed fire regimes. However, it is not always clear if fire-adapted traits evolved specifically to promote a particular fire regime or whether they are flammable for other reasons in an evolutionary process dubbed ‘exaptation’ (Gould and Vrba, 1982). For example, traits that appear adapted for fire might have also arisen from herbivory pressure, drought, or low soil fertility, all real stressors on the Australian continent (Keeley et al., 2011).

Studying the Holocene palaeoenvironmental record offers important insight into the evolution of Australian vegetation in concert with fire regimes and can help resolve key evolutionary questions on longer timescales, helping to predict the key responses of Australian vegetation to the widespread fire regime changes occurring today. While studies of this nature across the southern Australian region are numerous in this active field of research (e.g., Adeleye et al., 2021, Beck et al., 2017, Mariani and Fletcher, 2017, Mariani et al., 2019, Romanin et al., 2016, Thomas et al., 2022), an updated synthesis of the literature is overdue.

Methodological biases and limitations of palaeoenvironmental reconstructions

Charcoal and pollen analyses have made a hugely important contribution to our understanding of Australian Holocene palaeoenvironments over the past 50 years. However, worrying biases and limitations associated with these standard methodologies are being increasingly recognised in recent years.

Charcoal – produced by the incomplete combustion of organic materials in fires – is a widely used proxy for fire, applied to lake and peat sediments across the globe (Hawthorne et al., 2018) and in Australia since the 1980s (Singh et al., 1981). Despite its long history of use, no single, standardised method prevails (Tsakiridou et al., 2020). Although iterations are innumerable, methods typically involve the following key steps: 1) deflocculation, 2) bleaching, 3) wet sieving into size fractions and 4) counting by microscopy or image analysis to determine the total particle count and area (Stevenson and Haberle, 2005). Recently, there has been rising concern that current methods can degrade charcoal particles; Constantine IV and Mooney (2021) found that charcoal produced in cooler fires (<400 °C) is preferentially lost with standard bleaching treatments. Furthermore, many common methods are optimised for Northern Hemisphere sediments, where very different fire regimes prevail compared to those in Australia. For this reason, there is also considerable uncertainty in applying charcoal morphology methods – used as proxies for fuel type (Vachula et al., 2021) – to Australian contexts and vegetation.

Pollen based vegetation reconstructions are also plagued by problems due to pollen production and dispersal biases in different landscapes. Models have been developed to account for these biases to produce quantitative reconstructions of past plant-cover (Sugita, 2007), however there is still the problem that low-pollen producers or insect dispersed pollen types may not even be represented in the sediment record. These models have only recently been adapted and applied to Australian landscapes (Mariani et al., 2017, Mariani et al., 2022) and are not yet widespread. It is also important to remember that fires affect not just vegetation but a sweeping gamut of Australian ecosystems and processes (Dickman, 2021). For example, mass mortality of fish and invertebrates in aquatic freshwater and estuarine environments was reported after the Black Summer fires of 2019-2020 up to 70 km downstream from burnt areas (Silva et al., 2020). This was most likely a consequence of increased delivery of sediment and ash along with other toxic elements to waterways, followed by subsequent anoxia of the aquatic environment. In this context, there is increasing recognition – both nationally and worldwide – of the need to understand the impact of fire on traditionally overlooked aspects of ecosystems. For instance, soil microbes – crucial for the cycling of nutrients in ecosystems – have recently been shown to respond to fire severity on modern timescales in North America (Nelson et al., 2022, Brown et al., 2019). In Australia, particularly, we lack a holistic understanding of broader ecosystem interactions with fire and climate – especially on Holocene timescales.

Lake sedimentary ancient DNA, a novel method for holistic ecosystem reconstructions

Ancient DNA preserved for thousands of years in lake sediments (*sed*aDNA) has the potential to provide new ecosystem-wide insights into palaeoecology (Capo et al., 2021). Extraction of lake *sed*aDNA allows both terrestrial and aquatic community composition to be reconstructed at high taxonomic resolution and can detect organisms beyond the limits of microscopic identification (Capo and Monchamp, 2022). The relatively new *sed*aDNA proxy represents a growing field of interest, with publications increasing each year thanks to the exponential growth of next generation sequencing technologies and increasing affordability of sample processing costs (Parducci et al., 2017).

Optimal preservation of ancient DNA is assumed to be achieved in small, deep and cold lakes with anoxic sediment-water interfaces (Parducci et al., 2018). Lakes like these are commonly found at high altitudes but are rare in the relatively flat landscapes and warm, dry climates of southern Australia. This may explain why only a handful of attempts have so far been made to extract ancient DNA from non-marine sediment cores in Australia (Thomas et al., 2022, Foster, 2021).

*Sed*aDNA methods have enormous potential to provide much-needed information and evolutionary insights about ecosystem-wide interactions with climate and fire over Holocene timescales in Australia. Additionally, *sed*aDNA analysis of plant communities can be combined with traditional pollen records to provide a complementary picture of past vegetation (Parducci et al., 2019, Parducci et al., 2013, Parducci et al., 2015). For example, Willerslev et al. (2014) detected insect-pollinated taxa with *sed*aDNA, helping to account for biases in pollen records from Arctic permafrost sediments. While the *sed*aDNA technique offers promise in Australia, the limits of *sed*aDNA preservation in the sub-optimal conditions of Australian lakes (e.g., warm, shallow, periodically dry and oxygenated lakes subject to high UV irradiance) remain undocumented.

Thesis outline and aims

The Black Summer fires of 2019-2020 shrouded my Christmas and New Years in a haze of smoke. When I started this project seven months later in August 2020, fire was cemented in my mind as a timely tenet of this research. My thesis explores the long-term causes and consequences of fire on Kangaroo Island (Karti) through the analysis of sediment cores from Lashmars Lagoon, contextualising the extreme fire events like Black Summer that we face in Australia today. Fire does not exist in a vacuum, so along with fire history, I have simultaneously reconstructed aspects of past climate, landscape, and ecosystem change, and considered the role humans have played in maintaining fire regimes since time immemorial.

Kangaroo Island (Karti), South Australia, is ideally placed to investigate the complex interplay of climate, ecosystem, fire and people over the Holocene. The last undisputed archaeological evidence for precolonial human activity on the once-inhabited island dates to ~4.3 ka (Lampert, 1981, Walshe, 2005), potentially offering the tantalising opportunity to interrogate the long-term effects of losing Aboriginal land stewardship on a southern Australian landscape.

The study location also fills a regional gap in Holocene palaeoenvironmental reconstructions and as such will help to answer pertinent questions about the long-term spatial variability and consequences of contemporary regional climate influences like the ENSO and the Southern Westerly Winds (SWW).

Chapter two: site description

Here, I provide an in-depth site description and review previous research from Lashmars Lagoon.

Chapter three: sedimentology and chronology

This chapter establishes a high-resolution chronology and detailed geochemical characterisation of the new sediment cores taken from Lashmars Lagoon. Itrax scanning was used to reconstruct elemental changes throughout the record at 1 mm resolution. X-ray diffraction (XRD) and organic geochemistry were then applied to further understand elemental variation in our record and infer climate and catchment processes. These findings were then assessed in the context of a growing body of literature concerning Holocene climate dynamics across southern Australia. This study addresses previously unresolved questions about this site, thus providing a reliable foundation from which to interpret the ecosystem and fire reconstructions in the coming chapters.

Chapter four: fire and vegetation history

The fourth chapter reconstructs fire and vegetation history from fossil charcoal and pollen in the Lashmars Lagoon sediments. Given recent developments in charcoal methods and debate of a previous charcoal record at this site, a macrocharcoal method that removed potential charcoal biases was employed. The method used involves a gentle pre-treatment of samples to avoid the potentially destructive effects of bleaching and the quantification of charcoal particles under a live microscope stream using the CharTool plugin in the software ImageJ, which also allowed the collection of several informative morphological characteristics of charcoal particles such as length: width ratios. In this chapter, these data are used to interpret the role of vegetation in shaping fire regimes and vice versa. Finally, the results are compared to regional fire history and climate reconstructions, developing a theoretical framework that aims to untangle the long-term influences of climate, people, and vegetation on fire regimes across southern Australia over the last 7,000 years.

Chapter five: sedimentary ancient DNA record

Chapter five explores a new method – sedimentary ancient DNA (*sed*aDNA) – in a region and setting where the potential of this technique is largely uncharted. In this study, shotgun metagenomics was applied to sequence genetic material preserved in lake sediments and identify taxa across all three domains of life – the Archaea, Bacteria and Eukaryota. Given the importance of vegetation to the central aims of this thesis, special attention is paid to the Viridiplantae signal, and the results are compared to the pollen study in chapter four. While our study highlights some issues with the use of *sed*aDNA that need to be prioritised in future research, the data also offer unprecedented insight into ecosystem-wide responses to fire and climate in Australia and should encourage the further application of *sed*aDNA analyses in Australian terrestrial palaeoecology.

Chapter six: discussion

The final chapter of this thesis summarises my major findings, considers the limitations and emergent patterns from all three data chapters and recommends directions for future research.

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2

Site description

Overview

The study site that is the focus of this thesis is Lashmars Lagoon (35°48'16.0"S, 138°03'50.5"E), located on the Dudley Peninsula in the far east of Kangaroo Island (Karti; hereby abbreviated to KI) (Figure 1). KI covers an area of 4430 km^2, making it Australia's third largest island (Robinson and Armstrong, 1999). The island is within eyesight of the South Australian mainland, separated from the Yorke and Fleurieu peninsulas by the Investigator Strait and Backstairs Passage, and is a significant place for local Aboriginal people on the adjacent mainland (Figure A1 for the AIATSIS map of Indigenous Australia). KI is 14 km from the mainland at its closest and is estimated to have been last connected to the mainland at ~8.9 ka (Belperio and Flint, 1999) by a land bridge that flooded as sea levels rose after the last glacial maximum and eventually stabilised at modern levels around 7.5 – 8 ka (Lewis et al., 2013, Williams et al., 2018). This notable sea level rise has been recorded in oral traditions of neighbouring Aboriginal groups (Nunn and Reid, 2016).

Human history

KI is a significant place for Aboriginal people. For example, the Ngarrindjeri have a dreaming story that recounts how the ancestor Ngurunderi chased his two wives along the Fleurieu Peninsula (Bell, 2008, Nunn and Reid, 2016). As he caught sight of them crossing the land bridge that once connected KI to the mainland, he flooded the Backstairs Passage in rage – the women becoming the smaller islands known as Meralang or The Pages. More recently, Tasmanian Aboriginal women, along with Kaurna and Ngarrindjeri mainland women, were also kidnapped and taken to KI to be wives of white sealers in the early 19th century (Taylor, 2008). The island played an important part in the history of early colonial South Australia, and was originally intended to be the site of the first city before water scarcity forced colonisers to go elsewhere (Taylor, 2008).

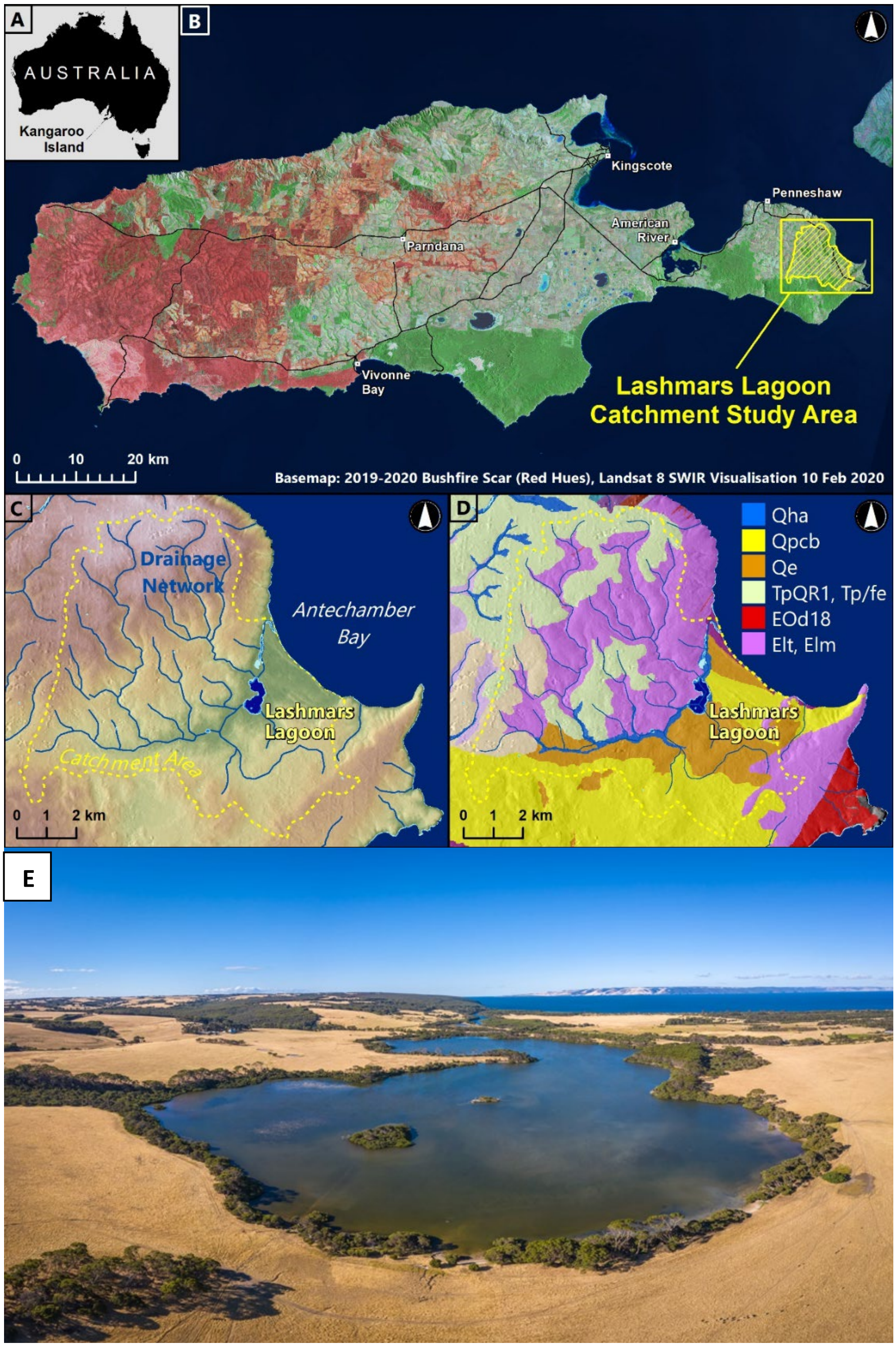

Figure 1. *Map of Kangaroo Island (Karti) and the Lashmars Lagoon drainage network and catchment. (A) Kangaroo Island (Karti) in relation to the Australian mainland. (B) Kangaroo Island (Karti) overlayed with the fire scar from 2019-2020. (C) Lashmars Lagoon catchment and drainage network. (D) Surface geology of the Lashmars Lagoon catchment (sourced from the South Australian Resources Information Gateway (SARIG) online: https://map.sarig.sa.gov.au/; Table 1 for detailed description). (E) Drone image of Lashmars Lagoon viewed from the south, looking across Backstairs Passage to the Fleurieu Peninsula on the Australian mainland (image courtesy of Quentin Chester)*

Today, the island is called home by a population of 4,702 (Australian Bureau of Statistics, 2016) and even in a pandemic attracted over 150,000 domestic tourists from March 2020 to March 2021 (South Australian Tourism Commission, 2021). However, it has been suggested that the island may not have been inhabited by people on a permanent basis for thousands of years prior to the British invasion of Australia (Lampert, 1981). Both Flinders and Baudin, British and French captains who navigated Australian waters around KI in the early 1800s, noted that the vegetation was denser than on the adjacent mainland (Flinders, 1814, Baudin, 1803); open grasslands, so common on the mainland, were not a feature of the island's vegetation (Clark, 1983b). Flinders (1814) also noted a conspicuous lack of campfire smoke emanating from the island, normally a common sign of people on the mainland. Additionally, animals on the island, especially the kangaroos and wallabies, were noted to be tame, hinting at a lack of human predation (Flinders, 1814).

Despite the apparent absence of people on the island at the turn of the 19th century, the archaeological record attests to human activity for at least several thousand years after the isolation of KI at ~8.9 ka (Belperio and Flint, 1999). The last undisputed record of human occupation from the archaeological record on KI dates to ~4.3 ka (Lampert, 1981, Walshe, 2005). However, radiocarbon dating of charcoal layers associated with stone tools have returned dates as young as 400 ± 50 years BP (Draper, 1991), apparently suggesting people were still present at this time or visited the island intermittently. In addition to archaeological data, a charcoal record from the sediments of Lashmars Lagoon has been used to infer the latest date for human disappearance from KI (Clark, 1983a). This record showed an abrupt increase in charcoal concentrations at ~2.5 ka, which was interpreted to reflect the loss of Aboriginal stewardship and the use of cultural burning to mitigate against uncontrolled bushfires. To better constrain the timing of human habitation on the island, more archaeological work is needed as a matter of priority.

Climatic context

The study of sediment cores from Lashmars Lagoon provides insight into the past climate of KI. Clark (1983a) suggested that the change in the pollen record from *Casuarina* to *Eucalyptus* dominance at ~5 ka marked a transition from wetter to drier conditions. A diatom microfossil study also showed increasing lake salinity from the mid Holocene to present day, which has been interpreted to reflect late Holocene aridification at Lashmars Lagoon (Illman, 1998), although these data are confounded by the question of the influence of the sea on lagoon salinity (Clark, 1976).

Presently, KI has a Mediterranean-type climate with warm dry summers and cool wet winters (Clark, 1983a). There is a significant rainfall gradient across the island, ranging from an annual average of ~800 mL in the west to ~500 mL in the east (Clark, 1976). While winter temperatures are comparable to the adjacent mainland, summer temperatures – and thus also evaporation – are lower (Clark, 1976). The Cape Willoughby lighthouse (7 km southeast of the lagoon) is the closest weather station to the study site, Lashmars Lagoon. For the last 30 years (1991 – 2020), the lighthouse recorded annual average minimum and maximum temperatures of 13.1 °C and 18.4 °C and a mean annual rainfall of 513.5 mm (Bureau of Meteorology, 2022b).

The dominant driver of rainfall on KI today is the delivery of cold frontal systems controlled by the Southern Westerly Winds (SWW) (Ray, 2022a). The latitudinal position of the SWW, indexed by the Southern Annular Mode (SAM), varies from decadal to millennial and glacial to interglacial timescales (Abram et al., 2014, Gillett et al., 2006). The El Niño Southern Oscillation (ENSO) and the Indian Ocean Dipole also influence contemporary climate on KI (Ray, 2022a).

The SAM has been progressing southwards away from KI since the 1940s as a result of climate change (Abram et al., 2014). This trend is predicted to continue, moving winter cold fronts, and therefore rainfall, away from the island. The Australian Bureau of Meteorology has downscaled Global Climate Models (GMCs) to create high-resolution regional models (RCMs) at ~5 km grid scale (Bureau of Meteorology, 2022a); these models predict that, if global emissions reductions efforts continue in line with current action (IPCC RCP4.5), rainfall on KI will decrease by 15 – 20% in 2090 from 2000 – 2019 averages (Figure 2, Ray, 2022b). In summary, changes in the climate system will mean that KI will become drier overall, although the less frequent rain events will be more intense (Ray, 2022a).

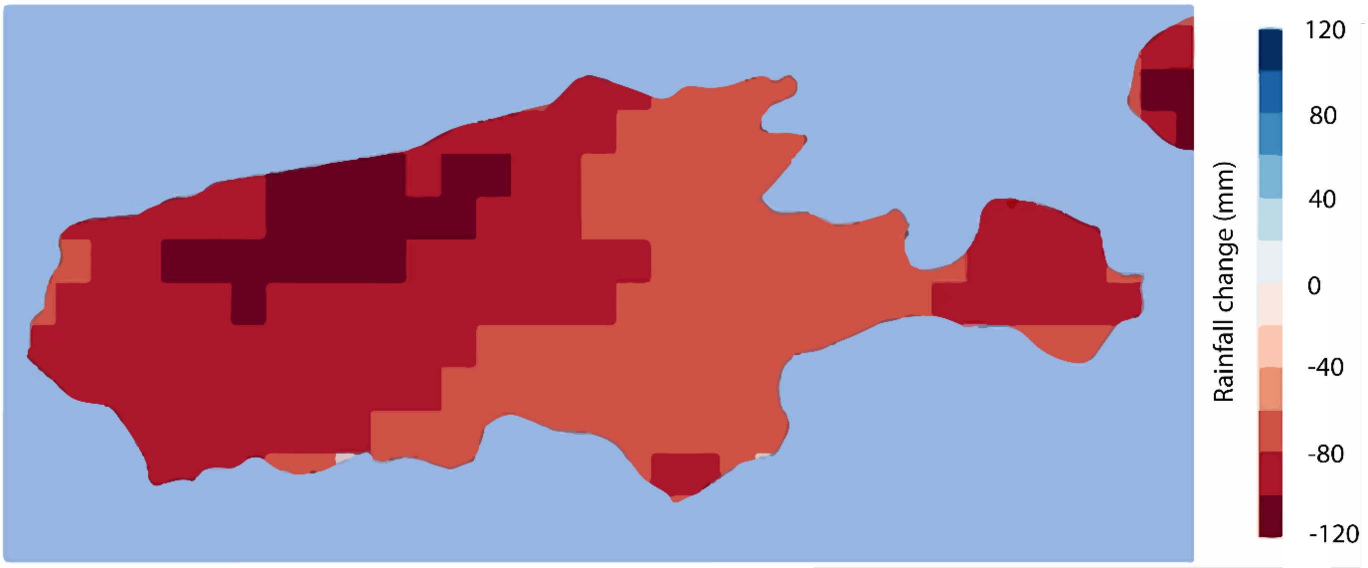

Figure 2. *Projected winter rainfall change (April to October) for 2090 under the RCP4.5 scenario on Kangaroo Island (Karti). Changed relative to 2000 – 2019 average rainfall. Source: (Ray, 2022a)*

Fire context

The 2019–20 fires on KI devastated much of the remnant vegetation on the island, heavily impacting the island's two main industries: agriculture and tourism. These were the biggest fires in KI's recorded European history and burnt nearly half of the island's area, causing widespread ecological damage (Figure 3, Bonney et al., 2020). The total area burnt exceeded 200,000 ha and a perimeter of 528.36 km. In 2007, another uncontrolled fire burnt approximately 20% of the island's vegetation (Peace and Mills, 2012). Other bushfires recorded on the island since satellite imagery records began in 1988 do not approach the scale and intensity of these two recent bushfires (Bonney et al., 2020).

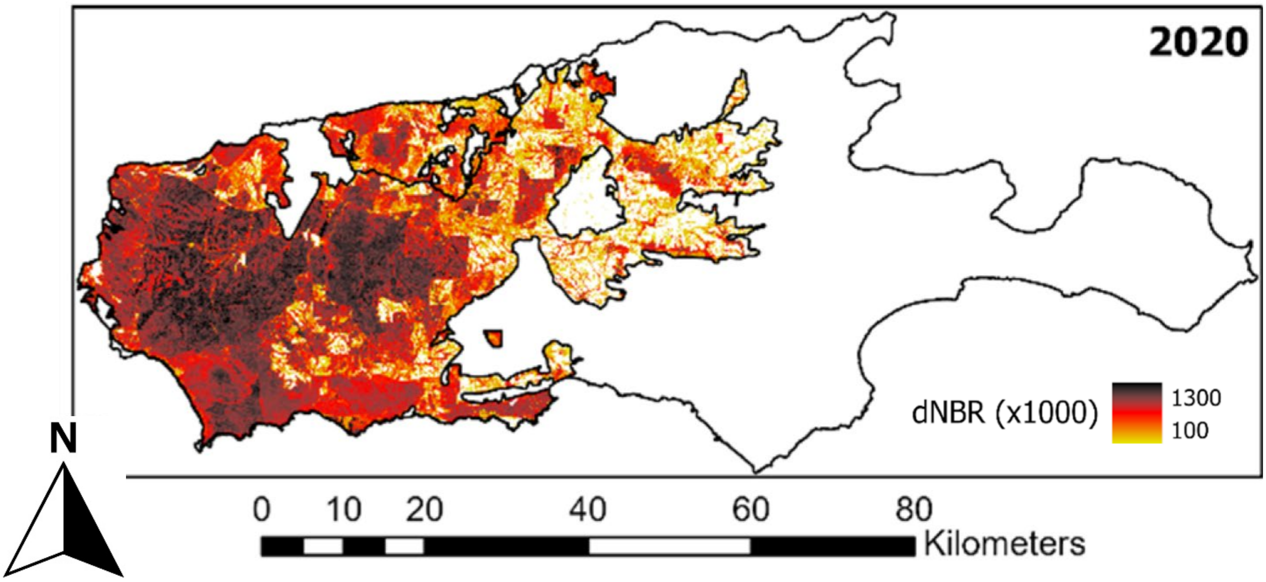

Figure 3. *Burn severity (dNBR) of the 2020 Black Summer fires on Kangaroo Island (Karti). Source: Bonney et al. (2020).*

Prior to instrumental records, little is known for certain about fire occurrence on KI. To improve modern day fire predictability and management, deeper time contexts for fire regimes are crucial. This is especially relevant to KI because 'natural' unmanaged fire regimes, likely to have been the case on KI for several millennia, are thought to operate on longer decadal to centennial timescales. For example, Hassell and Dodson (2002) found that in areas rarely frequented by people in south west Australia, intervals between major fires were in the range of 30 to over 100 years, whereas fire intervals were in the range of 1 – 10 years in areas fully occupied by Aboriginal people.

A charcoal record spanning most of the Holocene from Lashmars Lagoon provides a longer time context for burning on the island (Figure 4; Clark, 1983a). This charcoal record was interpreted to show an abrupt shift from small but frequent fire events in the early Holocene to large but infrequent fires after ~2.5 ka. This change in fire regime was used to infer the cessation of 'fire-stick farming' and therefore the cessation of Aboriginal occupation on KI. On the contrary, Illman (1998) suggested that this apparent shift in fire regime corresponds to a shift in sedimentology, suggesting that the increased charcoal counts might instead reflect more reducing lake conditions leading to the production of pyrite, a mineral potentially mistaken for charcoal. The original fire record by Clark (1983a) has also been disputed by Illman (1998) and Gammage (2013) on the grounds that such a dramatic shift in fire regime should be linked to a change in vegetation of a similar magnitude, which the pollen record suggests was not the case (Clark, 1983a).

Ecological context

Today, KI is well-known for its natural landscapes, and has retained native vegetation over nearly half of its area, mostly concentrated in the west (Kangaroo Island Landscape Board, 2021). KI, like many islands, has a unique ecology with many endemic species, thus has been spared the environmental disasters resulting from the introduction of common mainland invasive species like foxes and rabbits (Robinson and Armstrong, 1999). A biological survey of the island found 901 native plants (45 of which are endemic), 25 species of non-marine mammals, 252 native bird species, 18 reptile species and 6 species of amphibians (Robinson and Armstrong, 1999). Tourism and agriculture (predominately sheep farming), the two staple industries of the island's economy both rely heavily on the natural resources of KI (Australian Bureau of Statistics, 2016).

The area around the Lashmars Lagoon catchment has been progressively cleared for livestock grazing since 1874 (Taylor, 2008). Farmland currently makes up 60% of the Lashmars catchment, and the system is in an overall degraded state with eutrophic lake conditions and a sparse macroinvertebrate community (Environmental Protection Agency, 2013). About three kilometres upstream of Lashmars Lagoon along the Chapman River, the same environmental monitoring study also revealed nitrogen enrichment, salinisation, fine sediment deposition and an abundance of introduced weeds and grasses in the riparian zone. Livestock have direct access to the creek, expediating erosional processes and eutrophication. Despite this, Lashmars Lagoon is fringed by native remnant riparian zone vegetation, dominated by South Australian swamp paperbarks (*Melaleuca halmaturorum*) over samphire and sedges (Clark, 1983a, Clark, 1976). The lagoon also hosts abundant aquatic vegetation, such as *Myriophyllum, Ruppia* and *Characeae*. The lagoon supports a diverse birdlife; 56 bird species been recorded visiting the area, of which at least 35 have been documented breeding (Lashmar, 1935).

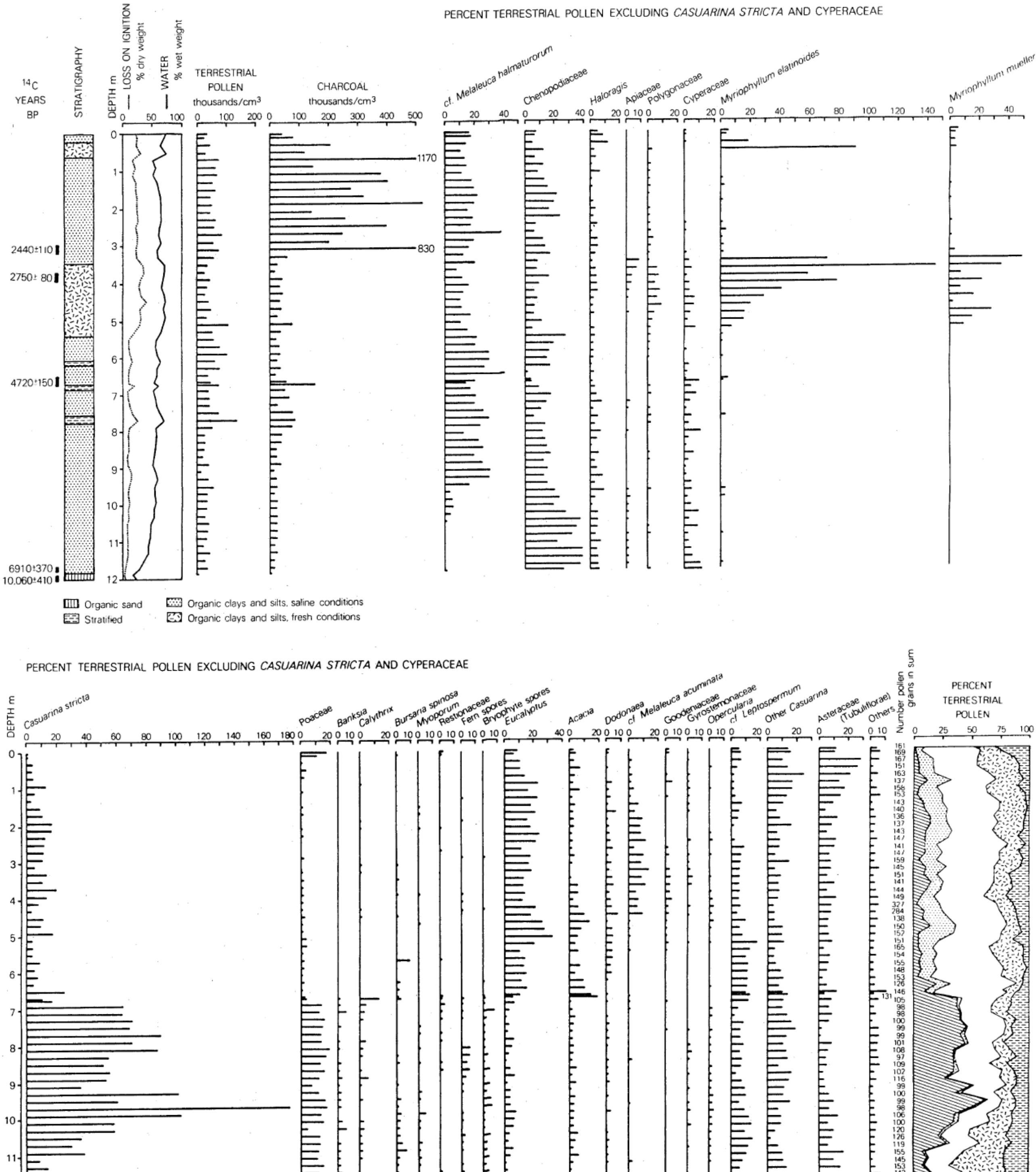

Figure 4. *The microcharcoal and pollen record from Lashmars Lagoon compiled by Robin Clark (1983) in her PhD thesis 'Fire history from fossil charcoal in lake and swamp sediments', showing '^{14}C dates, sediment stratigraphy, loss on ignition and water content, pollen and charcoal concentrations and abundances of aquatic, [wetland and dry land taxa and fern and bryophyte spores] taxa expressed as percentages of total terrestrial pollen grains excluding* Casuarina stricta *and* Cyperaceae'.

The Dudley Peninsula presently has six Major Vegetation Sub-groups that include woodlands, mallee heath and shrublands (Table 1) (Department of Environment and Water, 2020). Of these, two have been identified as having particularly considerable conservation value: Kangaroo Island Narrow-leaved Mallee (*Eucalyptus cneorifolia*) woodland and Sheoak (*Allocasuarina verticiliata*) grassy low woodland.

Table 1: *Major vegetation subgroups present on the Dudley Peninsula and associated likely maximum fire fuel hazard rating. Source: Adapted from the Dudley Peninsula Fire Management Plan (Department of Environment and Water, 2020).*

Major Vegetation Sub-group	**Likely maximum overall fuel hazard**
Eucalyptus woodlands with a shrubby understory	Extreme
Other Acacia tall open shrublands and shrublands	Very high
Casuarina and Allocasuarina forests and woodlands	High
Mallee heath and shrublands	Extreme
Eucalyptus open woodlands with shrubby understory	Extreme
Melaleuca shrublands and open shrublands	High

Hydrology of Lashmars Lagoon

Lashmars Lagoon consists of a north and south basin that combined cover an area of about 500 m^2 in total. The lagoon is characterised by shallow, brackish and alkaline water (in September 2020: 90 cm depth, PSU = 7.40, pH = 9.9, reported in Chapter 3). When full, the lake can reach depths of about two metres (Lashmar, 1935) but contracts significantly through the summer months (Cooter, personal communication, 2021). Occasionally, the lake has been known to dry out completely over summer, although even then the surface sediments remained wet in the centre of the basins (Lashmar, 1935, Clark, 1976, Cooter, personal communication, 2021). The lagoon drains a catchment of approximately 60 km^2, including several permanent and ephemeral creeks. The three main creeks that flow into the lagoon comprise the Chapman River in the southwest, the biggest of the streams, another smaller but more permanent creek in the north-west and a winter creek in the southeast. The lagoon discharges via the downstream Chapman River estuary to the northeast, which connects Lashmars Lagoon to the sea, meandering for about three kilometres before reaching a beach berm that is rarely breached (Clark, 1976).

Catchment geology

Lashmars Lagoon intersects three distinct geologies: the Ballaparudda Subgroup, the Bridgewater Formation and Quaternary aeolian sediments (Figure 1D). The Ballaparudda Subgroup, the predominant geology of the Lashmars catchment, is Cambrian in age and is composed of greywacke to siltstone cycles, sandstone carbonaceous, sulphidic or calcareous siltstone and shale. The

Bridgewater Formation dates from the Pleistocene and consists of calcrete aeolianite. The Bridgewater Formation is not too dissimilar to the Quaternary aeolian sediments which are sands from inland dune fields and include alluvial and regolith materials, including calcrete, in interdunal areas.

Appendix

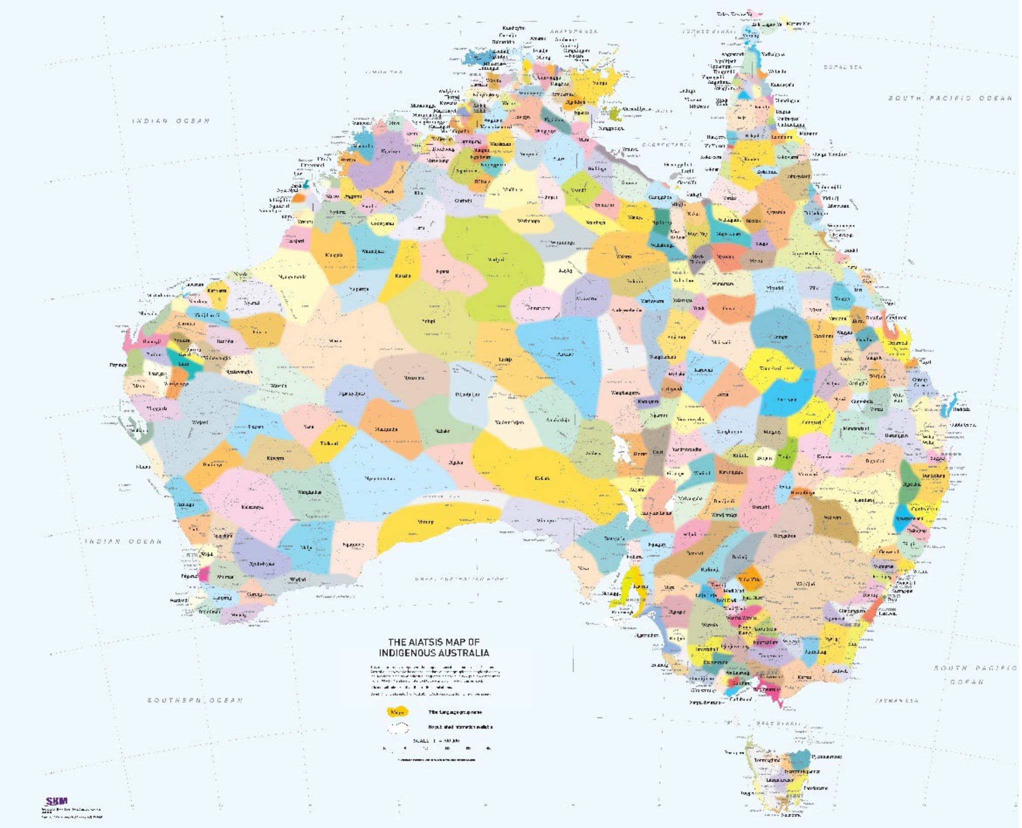

Figure A1. *AIATSIS map of Indigenous Australia, placing the study site in the in the broader context of Aboriginal Australia and the many mainland Aboriginal groups that are adjacent to Kangaroo Island (Karti). This map attempts to represent the language, social or nation groups of Aboriginal Australia. It shows only the general locations of larger groupings of people which may include clans, dialects or individual languages in a group. It used published resources from the eighteenth century-1994 and is not intended to be exact, nor the boundaries fixed. It is not suitable for native title or other land claims. David R Horton (creator), © AIATSIS, 1996. No reproduction without permission. To purchase a print version visit: https://shop.aiatsis.gov.au/*

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Principal Author

Name of Principal Author (Candidate)	Lucinda Duxbury		
Contribution to the Paper	Worked on the study design, undertook field and lab work, data analysis and interpretation, and wrote the first draft of the manuscript		
Overall percentage (%)	75 %		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	17/10/2022

Co-author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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3

Holocene climate and landscape change inferred from the geochemistry of Lashmars Lagoon, Kangaroo Island (Karti), southern Australia

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Abstract

Rapidly changing climates are placing extraordinary stress on ecosystems and societies in already harsh Australian landscapes. Lake sediments provide windows into the long-term dynamics of climate changes within the Earth system. As such, these records shape our understanding of the present and increase our ability to predict an uncertain future. Here, we investigate a 7000-year sedimentary record from Lashmars Lagoon, Kangaroo Island (Karti) to infer climate and catchment change in a region that has been heavily impacted by drought and bushfires in recent decades. We use high-resolution X-ray fluorescence (XRF) core scanning, complemented by X-ray diffraction (XRD) and bulk organic geochemical analyses of catchment soil and lake sediments to constrain the interpretation of our record. Al/K ratios reflect changes in the kaolinite to illite clay proportions, which we interpret to indicate the relative amount of chemical to physical weathering. Periods of high Al/K from 5.9 ka – 5.6 ka and 4.2 ka – 2.5 ka are therefore inferred to reflect wetter phases with more chemically weathered landscapes. These two periods coincided with high sediment organic matter inferred from Br and total organic carbon concentrations, indicating high lake productivity and lake water freshening during these times. These proxies are inversely correlated with Ca, which reflects the concentration of calcite from fossil molluscs, ostracods, and foraminifera in the sediments. The fossil taxa are predominantly associated with brackish conditions – implying a link to drier climates consistent with a published diatom record or possibly periods of marine influence. We find moderate to high calcite from 6.8 ka to 4.2 ka, followed by a period of low calcite between 4.2-2.5 ka. The co-occurrence of calcite and evaporitic gypsum after 2.5 ka suggests an even drier climate prevailed from this time to present. Our data indicate that Lashmars Lagoon and its surrounding catchment underwent significant hydrological changes over the past ~ 7,000 years, in concert with regional hydroclimatic changes, possibly indicating temporal variability in the Southern Westerly Winds (SWW) and the late Holocene intensification of the El Niño Southern Oscillation (ENSO). Importantly, our data provide climate and environmental context for the complex human and ecological history of Kangaroo Island (Karti).

Key words

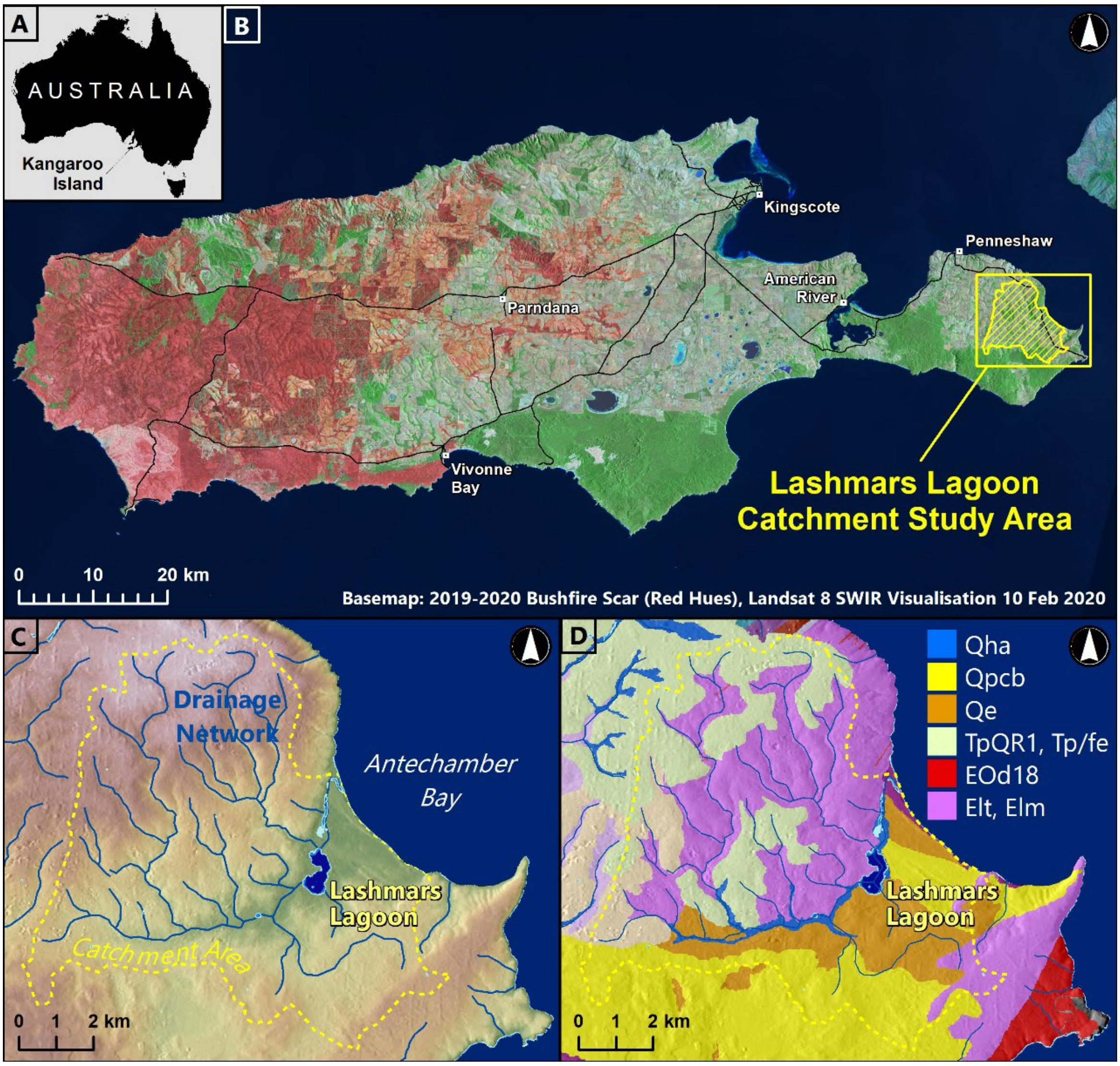
Lake sediment; Itrax core scanning; X-Ray Diffraction (XRD); weathering, El Niño Southern Oscillation (ENSO); Southern Westerly Winds (SWW)

Introduction

Southern Australia is becoming increasingly inhospitable. Over the coming decades, winter rainfall is predicted to further decline, while temperatures, extreme fire danger and droughts are expected to rise – posing a threat to both the environment and the economy (CSIRO and Bureau of Meteorology, 2020, Hennessy et al., 2022). For example, the Millennium Drought – which lasted for about a decade across southeast Australia before ending in 2010 (Van Dijk et al., 2013) – wreaked environmental havoc in Murray Darling Basin ecosystems (Bond et al., 2008) and reduced agricultural total factor productivity in Australia by 18% (Sheng and Xu, 2019). More recently, the region faced its worst recorded bushfire season in 2019-2020 (Davey and Sarre, 2020). The fires claimed 33 human lives and over 3,000 homes and caused over $10 billion of economic losses and immeasurable environmental devastation (Binskin et al., 2020).

To manage and adapt to these changes, it is crucial to understand the region's natural range of climatic and environmental variability. However, despite the clear need to establish long-term baselines that contextualise current patterns, past climate reconstructions that extend beyond the last century of instrumental observations are not well constrained. This is due in part to the scarcity of high quality and long-lived tree ring, speleothem, or lake sediment archives that record past environmental changes in high temporal resolution – a problem which is exaggerated in the dry state of South Australia.

Lashmars Lagoon on Kangaroo Island (Karti; hereby abbreviated to KI; Figure 1) is a rare example of a fast-accumulating continuous Holocene lake sediment record in South Australia (Clark, 1983a) and is well placed geographically to investigate the temporal and spatial variability of climate influences across southern Australia. The island's climate is heavily influenced by the position and strength of the Southern Westerly Winds (SWW), which dominates precipitation across the southern portion of the Australian continent (Gillett et al., 2006). The island is also impacted by the El Niño Southern Oscillation (ENSO), which normally predominately affects eastern Australia (Bureau of Meteorology, 2022a). This study location is thus ideally positioned to help constrain long term hydroclimate variability in places like the Murray Darling Basin – hence informing water security in a critical ecological and agricultural region of Australia (Ho et al., 2014).



Basemap: 2019-2020 Bushfire Scar (Red Hues), Landsat 8 SWIR Visualisation 10 Feb 2020

Figure 1. *Map of Kangaroo Island (Karti) and the Lashmars Lagoon drainage network and catchment. (A) Kangaroo Island (Karti) in relation to the Australian mainland. (B) Kangaroo Island (Karti) overlayed with the fire scar from 2019-2020. (C) Lashmars Lagoon catchment and drainage network. (D) Surface geology of the Lashmars Lagoon catchment (sourced from the South Australian Resources Information Gateway (SARIG) online: https://map.sarig.sa.gov.au/; Table 1 for detailed description)*

The sediments from Lashmars Lagoon, KI, were the focus of pioneering research into past fire and vegetation variability in Australia (Clark, 1983a, Clark, 1983b, Singh et al., 1981). These early studies were well-cited in foundational literature that sought to elucidate long term complex interactions between climate, fire, ecosystems and people in Australia (Bowman, 1998, Dodson, 2001, Kershaw et al., 2002, Lampert, 1981, Horton, 1982, Flannery, 1990, Shulmeister et al., 2004, Kohen, 1995, Kershaw et al., 1991, Head, 1989). The original study (Clark, 1983a) presented pollen and microscopic charcoal (microcharcoal) records that revealed (1) a change from *Casuarina* to *Eucalyptus* woodland at ~ 5 ka – interpreted as a climate drying signal and (2) a marked increase of charcoal concentrations at ~ 2.5 ka. Clark (1983b) hypothesised this shift might have been caused by the disappearance of people from the island and the subsequent lack of fire management practices by Aboriginal people on KI. The hypothesis posits that the absence of people would have allowed biomass density to increase, thus leading to bigger fires. Further, a subsequent study on a core from Lashmars Lagoon used diatom microfossil analysis to infer increasingly saline lake conditions from the mid-Holocene, suggesting that late Holocene aridity may have contributed to cessation of permanent habitation on the island (Illman, 1998).

Now forty years on from the original study, the record from Lashmars Lagoon provides an outstanding opportunity to revisit this iconic site with modern techniques providing high-resolution, multi-proxy records with tight chronological constraint. Since its publication, the original microcharcoal record from Lashmars Lagoon has drawn criticism for various reasons (Head, 1989, Illman, 1998, Gammage, 2013). A major shift in sedimentology – from organic to clay rich sediments – occurs at approximately the same time charcoal concentrations increase. It was therefore suggested that the increase in charcoal at ~2.5 ka was an artefact of the misidentification of pyrite (FeS_2) for charcoal, pyrite being a dark mineral produced in reducing lake conditions (Illman, 1998). It has also been discussed that there is no corresponding shift in vegetation from the pollen record, as would be presumed to accompany such a distinct change in fire regime (Illman, 1998, Gammage, 2013). Another point of contention concerns controls on lake salinity at Lashmars Lagoon. The proximity of the lagoon to the ocean and presence of marine gastropods in the sediments suggest that marine incursions may have influenced lake salinity in the past (Clark, 1976). However, the sediment diatom composition is dominated by non-marine taxa, implying instead that saline phases were driven by lake evaporation (Illman, 1998).

In summary, the Lashmars Lagoon sediment record is important for understanding long-term climate variability in a region vulnerable to drought – as well as providing a unique case study to consider broader questions about how climate interacts with fire, vegetation, and human history in Australia. The limitations of previous studies, however, emphasise the need to first resolve the depositional history of the sediments and update the chronology, applying modern methods at a higher resolution. To these ends, we present a detailed sedimentological study of new core material from Lashmars Lagoon, underpinned by a detailed radiometric chronology, using a combination of radiocarbon dates, ^{210}Pb and ^{239}Pu analyses. We combine high-resolution X-ray fluorescence (XRF) core scanning with mineralogical and organic geochemical analysis of lake and catchment sediment samples to untangle the potential confounding influences of local versus regional climate and environmental change over the past ~7,000 years. We contextualise our findings within the broader patterns of regional climate change, testing 1) whether previously identified changes in charcoal abundance are influenced by increased pyrite formation during periods of more reducing lake conditions and 2) the link between sedimentology and changes in salinity at Lashmars Lagoon, and how these relate to climatic processes versus marine intrusions.

Site information

KI is Australia's third largest island, covering an area of 4430 km^2 (Figure 1, Robinson and Armstrong, 1999). The area was disconnected from the mainland at about 9 ka when rising sea severed the land bridge to the mainland (Belperio and Flint, 1999). The island is culturally significant for Aboriginal groups on the adjacent mainland, holds historical importance for the establishment of the state of South Australia, and is lauded for its unique biodiversity and conservation areas. The climate on KI can be described as 'Mediterranean' in nature, with warm dry summers and cool wet winters (Clark, 1983a). The dominant driver of rainfall today is the delivery of cold frontal systems controlled by the South Westerly Winds (SWW) (Ray, 2022). ENSO and the Indian Ocean Dipole also influence contemporary climate on KI.

Lashmars Lagoon is located on the Dudley Peninsula, in the far east of the island (35°48'16.0"S 138°03'50.5"E; Figure 1). The Cape Willoughby Lighthouse weather station, 7 km southeast of the lagoon, recorded annual average minimum and maximum temperatures of 13.1 °C and 18.4 °C, and an average annual rainfall of 513.5 mm over the 30-year period ending in 2020 (Bureau of Meteorology, 2022b). The lagoon is almost permanently wet, although its surface area and water depth decline significantly in summer (Cooter, personal communication, 2021). When full, the lagoon covers about half a square kilometre. Occasionally, the lagoon has been known to dry out completely during very dry summers, like in 1914 and 2009, but even then, the surface sediments remained wet in the deepest parts (Clark, 1976, Cooter, personal communication, 2021). In its present state, the lagoon is shallow, brackish (Illman, 1998) and has suffered eutrophication in recent times from the influence of livestock and farming in the area (Environmental Protection Agency, 2013).

The Lashmars Lagoon catchment drains an area of approximately 60 km^2, the majority of which has been cleared, a process that occurred progressively on the Dudley Peninsula and surrounds since the early 1820s, first with the arrival of convicts, sealers and their Aboriginal wives and then more intensively since the 'Hundred of Dudley' was officially proclaimed by the governor in 1874 (Taylor, 2008). As of 2013, 60% of the Lashmars catchment was farmland, although the lagoon itself is still fringed by remnant native vegetation (Environmental Protection Agency, 2013). Three main creeks flow into the lagoon. In order of most to least permanent, they are: (1) a small unnamed creek in the northwest (2) the comparatively large Chapman River in the southwest and (3) an ephemeral winter creek in the southeast. The lagoon discharges via the downstream Chapman River to the northeast, which reaches the sea after three kilometres at a beach berm that is rarely breached, even after intense rainfall or storms; in 1976, Alan Lashmar recollected that the berm had only been breached twice in the previous 50 years (Clark, 1976). Lashmars Lagoon sits at the intersection of three distinct surface geologies (Figure 1D; Table 1). Qpcd and Qe are both Quaternary aeolian sand deposits (henceforth referred to as the Quaternary calcreted aeolianite and aeolian sands respectively), while El comprises Cambrian aged interbedded greywacke and siltstone, as well as carbonaceous, sulphidic or calcareous siltstone and shale (referred to from this point as Cambrian greywacke, siltstone, and shale).

Table 1. *Descriptions of surface geologies present in the Lashmars catchment. Sourced from the South Australian Resources Information Gateway (SARIG) online: https://map.sarig.sa.gov.au/.*

Map unit	Description
El	Greywacke to siltstone cycles, sandstone, carbonaceous, sulphidic or calcareous siltstone and shale
EOd4	Granitoid rock of the Delamerian Orogeny
Qe	Aeolian sand of inland dune fields; includes associated alluvial and regolith materials (including calcrete) in interdunal areas
TQr1, *Tp/fe*	Early Tertiary to Pleistocene dissected ferruginous duricrust and ferruginous gravel, sand, silt and clay
Qpcb	Calcreted aeolianite

Materials and methods

Sediment coring and field work

Sediment cores were collected from Lashmars Lagoon (Figure 1; 35°48.289'S, 138°03.853'E) in September 2020 using a Bolivia piston corer (Wright, 1967). Cores were collected in ~1 m increments at three locations (LAS20-2, LAS20-3 and LAS20-4) in the deepest part of the lake, located within a ~5 m radius, one series of which was started at 0.5 m to ensure sediment core overlap. A ~0.3 m long gravity core (LAS20-1) was also collected using a Pylonex HTH corer (Renberg and Hansson, 2008), prior to Bolivia coring, to recover undisturbed surface sediments. The gravity core was subsampled in the field at 2.5 mm intervals. All Bolivia cores and gravity core subsamples were stored at 4°C in the dark until further processing at the University of Adelaide, South Australia.

Lake water pH and salinity (PSU) was measured using a HI98194 multiparameter water quality meter (Hanna instruments, Australia). Modern soil and surface sediment samples were collected in November and December 2021 to characterise mineralogical composition. Samples were taken from the lagoon, surrounding catchment, and the streams that flow in and out of the lagoon. These samples were stored at 4°C until further analysis.

Sediment stratigraphy

In the lab, the Bolivia sediment core sections were split lengthways and described for colour, grain-size, structure, consistency, and macrofossil content. Subsequent core correlation was informed by these core log descriptions, complemented by Itrax XRF data (described below) using the software packages CORRELATOR (cse.umn.edu/csd/correlator) and CORELYZER (cse.umn.edu/csd/corelyzer) according to the workflow set out by Francke (2017) to construct a composite depth scale.

Chronology

Nine samples for radiocarbon dating were taken along the composite profile (Table S1C). A total of six plant macrofossils and one shell were picked for dating after visual inspection of the sediments. Additionally, two pollen samples were prepared for dating. The plant macrofossils were pre-treated using the Acid-Base-Acid method, combusted to form CO_2 and graphitised (Hua et al., 2001). All macrofossil and a shell sample (pre-treated at ANSTO) were analysed at the Australian Nuclear Science and Technology Organisation (ANSTO), Lucas Heights. The two smallest samples were run on the ANTARES particle accelerator while the remaining five samples were run on the VEGA particle accelerator (Fink et al., 2004). Pollen concentrates were extracted from bulk sediment samples at the Chronos 14-Carbon cycle facility, University of New South Wales according to Cadd et al. (2022). Pretreated pollen samples were graphitised with an AGE3 system and ^{14}C measurements were undertaken using a MICADAS (Ionplus, Switzerland) AMS (Wacker et al., 2010).

A total of 15 samples were dated using ^{210}Pb analysis by alpha spectrometry. Seven samples, each 0.25 cm thick, were taken from the entire length of the gravity core of the surface sediments at 0 cm, 2 cm, 3 cm, 6 cm, and then at 5 cm intervals from 10 cm to 30 cm. To reach natural background levels of ^{210}Pb concentration, a further eight samples of 1 cm thickness were taken from even intervals between 5 and 70 cm from the composite depth profile. To independently validate the ^{210}Pb chronology, 14 one-centimetre-thick samples between 1 and 40 cm were analysed for Pu isotopes. The samples were processed and analysed at ANSTO (Eakins and Morrison, 1978).

A sedimentary age-depth model for the composite core profile was generated using the *rPlum* package in R which uses Bayesian statistics to combine ^{210}Pb and radiocarbon dates (Aquino-López et al., 2018). The model was anchored using the coring date of September 2020 and the 1964 timestamp from the Pu isotopic analysis. Radiocarbon ages were calibrated against the SHCal20 calibration curve and are reported in calibrated years before present (BP), where ‘present’ is CE 1950.

Itrax µXRF core scanning

Sediment cores were scanned on the Itrax µXRF core scanner located at ANSTO. All ~1 m sections from three cores (LAS20-2, -3 and -4) were scanned at 1 mm resolution with a 10-second exposure. Optical images for these cores were also obtained (Figure S1). LAS20-1, as it was extruded at 2.5 mm intervals in the field, was scanned as discrete dry powdered samples also with a 10-second exposure time.

Mineralogical and organic geochemical analyses

Organic geochemical and mineralogical analyses were performed on ten samples of ~2 cm^3 from LAS20-4. Samples were selected to target to significant changes in the Itrax data, as well as to maintain a reasonably even spread of samples down core. Further mineralogical analyses were conducted on an additional 14 subsamples to target more specific changes in the Itrax data, as well as on the catchment samples collected on field work in 2021. Mineralogical and geochemical analyses were conducted at the Mawson Analytical Spectrometry Services (MASS) at The University of Adelaide.

In preparation for mineralogical analysis, samples were first freeze dried and crushed using an agate pestle and mortar. The fourteen additional mineralogical samples to characterise specific changes were also further micronized so as not to preclude the possibility of more quantitative analysis in the future. Mineralogy was determined by semi-quantitative XRD analysis with a Bruker D8 ADVANCE Powder X-ray Diffractometer with a Cu-radiation source operating at 40 KV and 40 amps, scanning 2 theta from 5 to 65 degrees with sample rotation of 30 rotations per minute. Data was processed using the software package DIFFRAC.EVA (Bruker, Karlsruhe, Germany) and Crystallography Open Database (www.crystallography.net/cod/) reference patterns for identifying mineral phases. Semi-quantitative estimates of mineral composition were calculated in the program DIFFRAC.TOPAS 4.2 (Bruker, Karlsruhe, Germany).

Carbon and nitrogen elemental and isotope ratio analyses were performed on 100 mg aliquots of acidified and non-acidified samples. 2 mL of 10% AnalaR HCl was added to the acidified samples for 20 minutes to remove inorganic carbon, they were then rinsed three times with Milli-Q water, freeze dried and re-ground. Total C and N for acidified and non-acidified samples were analysed by elemental analysis using a Perkin Elmer 2400 series II CHNS/O Elemental Analyzer in CHN configuration. Samples were analysed in duplicate with results calibrated to the organic analytical standard acetanilide (PerkinElmer, United States) with known abundances of C (71.09%), H (6.71%) and N (10.37%). Total Organic Carbon (TOC) of the acidified samples was verified by source rock analysis of the non-acidified samples using a Weatherford Source Rock Analyser™. An analysis blank was run with the sample batch and the blank data was automatically subtracted from all analyses. A standard was run at the start of each batch to check instrument status, with additional standards run every 10 samples. Acidified samples were analysed for $^{13}C/^{12}C$ isotope ratios, reported as $\delta^{13}C$ (relative to the standard Vienna Pee Dee Belemnite), using a continuous flow isotope ratio mass spectrometer (Nu Horizon, Wrexham, UK) equipped with an elemental analyser (EA3000, EuroVector, Pavia, Italy). All samples were corrected for instrument drift and normalised according to reference values using in-house standards (n=25; glycine, glutamic acid and triphenylamine), which were calibrated against USGS and IAEA certified reference materials (USGS40, USGS 41, IAEA-2). Analytical reproducibility was < 0.3‰ for all $\delta^{13}C$ analyses.

Data processing and statistical analysis

XRF elemental data was first trimmed at the top and bottom of each core to remove data points not representative of sediment geochemical composition. Raw XRF elemental data was then processed in R by filtering elements that passed three statistical tests (runs test, percent zero counts, signal to noise ratio). The remaining elements were then plotted as raw counts, normalised against counts per seconds (cps) and log normalised to assess the effect of different normalisations on the data, as this has been shown to affect results (Kemp et al., 2020). Pearson's correlation test (using R with the package *vegan*) was used to compare these three methods of data normalisation. Principal Components Analysis was performed in R on the elements that passed all three tests, to examine the common patterns between elements and identify major determinants of variability in the record. A Pearson's correlation test was used to select elements strongly correlated ($-0.8 > r > 0.8$) with the first two principal component axes (PCs).

Pearson correlation tests were further used to determine statistical correlation between Itrax elemental data, mineralogy, and organic geochemical analyses. Cluster analysis was applied to the semi-quantitative mineral percentage XRD data from the catchment soil and surface sediment samples using classical cluster multivariate analysis in the software *PAST 4.03* (Hammer et al., 2001).

Results

Sediment coring and field work

In total 11 cores, each ~1 m in length, were recovered from Lashmars Lagoon from four discrete core locations (Table 2). The deepest core extended to a composite depth of 7.49 m. On the date of coring (September 2020), the lagoon was brackish (7.40 PSU and 12,790 µmS/cm) and alkaline (pH 9.9) and had a maximum water depth of ~90 cm.

***Table 2**. Sediment cores retrieved from Lashmars Lagoon, Kangaroo Island (Karti) in September 2020.*

Core name	**Coring method**	**Field depths (cm)**	**Composite depths (to the nearest cm)**
LAS20-1	Gravity corer, extruded at 0.25 cm intervals in the field	Surface – 30 cm	0 – 30 cm
LAS20-2	65 mm Bolivia corer fitted with 1 m long PVC pipes	0 cm – 400 cm	15 – 384 cm
LAS20-3		0 cm – 100 cm	19 – 120 cm
LAS20-4		50 cm – 739 cm	56 – 749 cm

Sediment lithology and chronology

Lashmars Lagoon's deposits consisted of mainly homogenous, fine grained, organic and clay rich sediments that ranged in colour from greys and black to browns and olives (Figure 2B). There were some fine laminae throughout the cores, particularly in the 5.5 - 6.5 m section from LAS20-4 with grey-white bands interspersed in a brown to black silty clayey matrix (optical images Figure S1J). Shell fragments and occasionally intact shells were scattered throughout the cores, as were small twigs and root hairs. We found no visual indications of hiatuses or erosional surfaces. The lithological units were further refined below using the XRF data.

Radiocarbon analysis on samples from between 94 cm to 728 cm displayed stratigraphic consistency throughout most of the core (Figure 2A; Table S1C). ^{210}Pb measurements from the upper sediments returned unsupported ^{210}Pb concentrations that decreased with depth (Figure S2; Table S1B). Pu isotopic profiling indicates that the 1964 bomb peak occurred in our sediments at 13.5 cm in the composite depth profile (Figure S3; Table S1A).

The Bayesian *Plum* model, combining all three dating methods, returned a modelled basal median age of 6770 cal. years BP to the nearest decade at 728 cm (Figure 2A). The radiocarbon age of an unidentified shell at 97 cm was an outlier from the model and returned an age range of 1,258 – 1,058 cal. years BP, ~300 years offset from its paired macrofossil date at 100 cm (922 – 791 cal. years BP), and consistent with a reservoir effect (Jull et al., 2013). The inferred sediment accumulation rate was mostly constant for the long section of the record that was dated with radiocarbon, despite considerable variation in sedimentology (~1.1 mm/year from ~ 6,770 to 730 cal. years BP (750 - 90 cm)). The accumulation rate was higher for the upper 70 cm, which was dated with the Pu-validated ^{210}Pb model (~2.6 mm/year). Between 70 cm and 90 cm there is a period of low accumulation (~0.05 mm/year). It is possible that this period represents a hiatus in the record, however no changes in sedimentology occur during this phase, suggesting this discrepancy reflects a small discordance between the radiocarbon and ^{210}Pb chronologies.

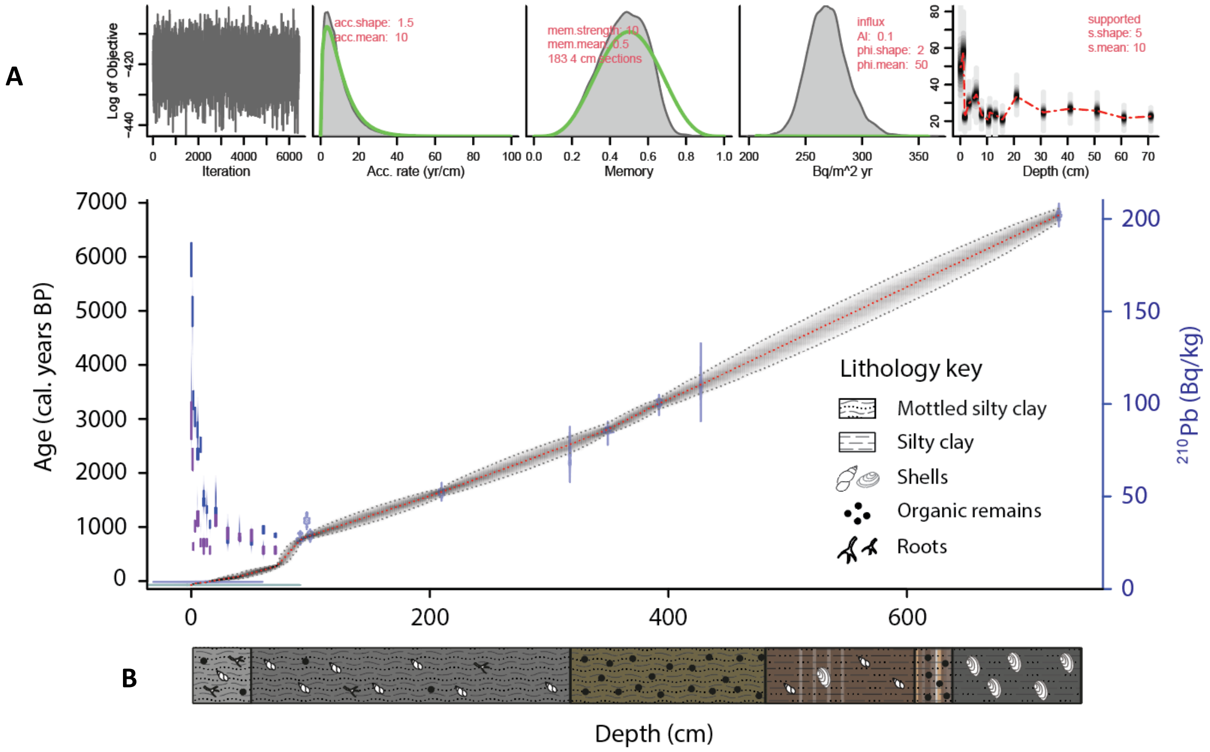


Figure 2. *(A) Bayesian age depth model for the complete 749 cm core, constructed using the package Plum in R. The model incorporates radiocarbon and ^{210}Pb dating. The section of the model that relies on ^{210}Pb dating (0 – 60 cm) is displayed in Figure S2. The model is anchored with the coring date (September 2020) at 0 cm and the Pu-isotope inferred bomb peak at 1964 at 13.5 cm. (B) Sediment lithology based on visual core description and refined using the XRF data (see Figure 5 and Table 4 below for more detail). Colour reflects colour of sediments.*

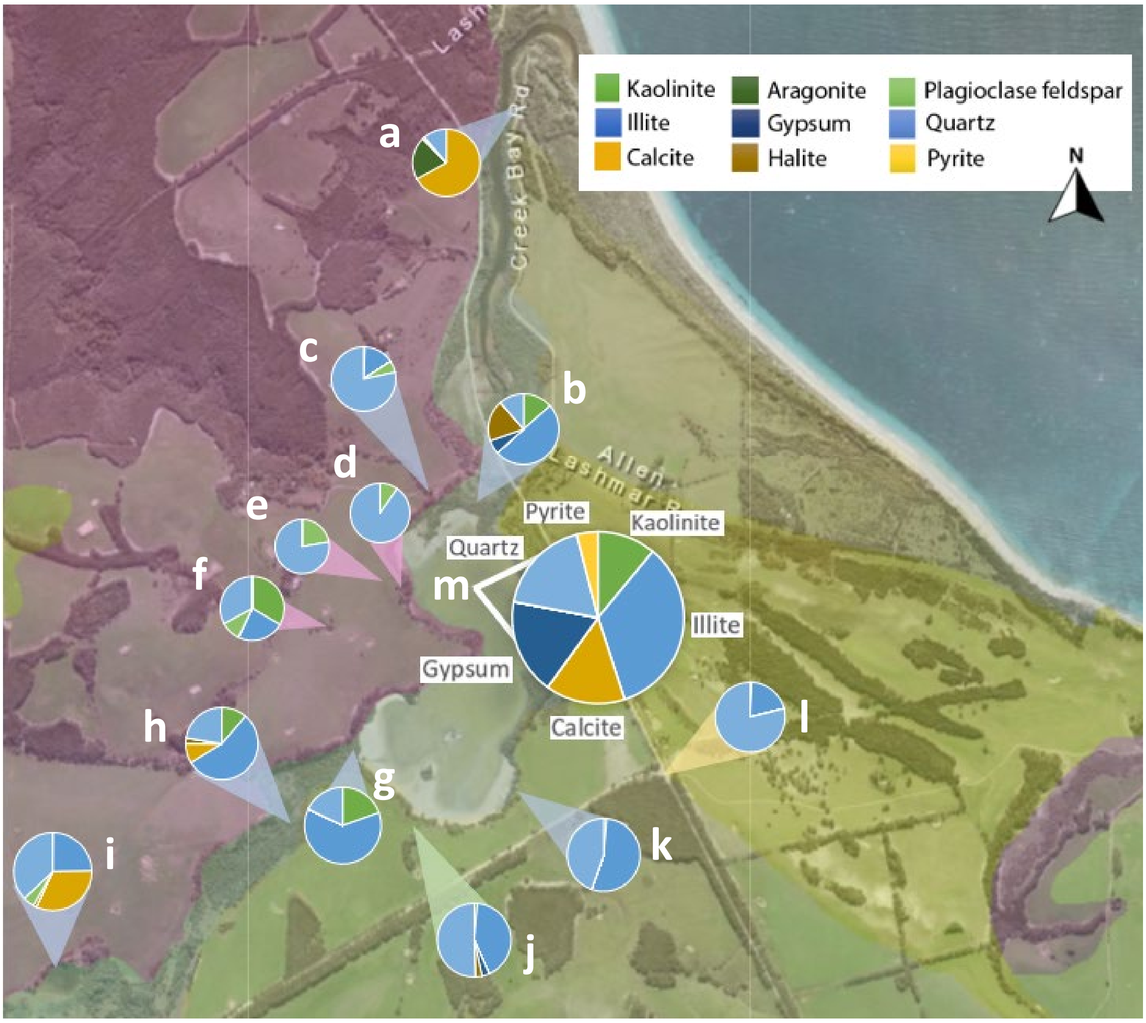
Lake sediment and catchment mineralogy

XRD analysis revealed the presence of eight minerals in the sediments of Lashmars Lagoon and surrounding catchment: calcite, halite, pyrite, kaolinite, illite, gypsum, quartz, and plagioclase feldspar (Table 3).

Table 3. *Minerals identified in Lashmars Lagoon sediments and catchment sediments and soils from XRD analysis*

	Chemical formula	**Description**
Calcite	$CaCO_3$ or $(Ca,Sr)CO_3$	Inorganic or organic origin; in its organic form it is the main constituent of shells; in its inorganic form it precipitates as a result of increased calcium ions in the water column from processes like evaporation or possibly from marine incursions
Halite	$NaCl$	Evaporitic mineral; this is likely an artefact from freeze-drying. Solubility of halite is very high, would not form at unless lagoon became hyper saline, and then would dissolve with subsequent freshening
Pyrite	FeS_2	Produced authigenically in reducing lake conditions
Kaolinite	$Al_2Si_2O_5(OH)_4$	Clay mineral; normally a product of more chemical vs physical weathering which is typically associated with wetter and warmer climates
Illite	Typically $(K,H_3O)(Al,Mg,Fe)_2(Si,Al)_4O_{10}[(OH)_2\cdot(H2O)]$	Clay mineral; commonly considered a product of more physical vs chemical weathering; rubidium can commonly replace potassium
Gypsum	$CaSO_4\cdot 2H_2O$	Evaporitic mineral
Quartz	SiO_2	Widely occuring mineral, highly resistant to weathering
Plagioclase feldspar	Ranges from pure (end-member) albite, $Na(AlSi_3O_8)$, to pure anorthite, $Ca(Al_2Si_2O_8)$	Produced during crystallisation of the melt to form igneous rocks; possibly sourced from the granite in the Lashmars Lagoon catchment (Figure 1); highly susceptible to weathering

Mineralogical analysis of soil samples from around Lashmars Lagoon allowed us to characterise the three surface geologies that immediately surround the lake (Figure 3). Three soil samples (d, e and f) were taken from the Cambrian greywacke, siltstone and shale surface geology (represented by the pink shaded area west of the lake in Figure 3). The samples were taken along a transect of increasing elevation and distance from the lake. The two samples closest to the lake (d and e) were both dominated by quartz and contained smaller amounts of plagioclase feldspar. The sample at the top of the slope and furthest from the lake (f) also contained quartz and some plagioclase feldspar but was predominately composed of the clay minerals kaolinite and illite. Catchment samples to the east of the lake (j, k and l) came from the Quaternary aeolian sands and Quaternary calcreted aeolianite, two very similar surface geologies that we consider together as a single sediment source (represented respectively by the green and yellow overlay in Figure 3). These samples were characterised by high amounts of quartz and illite. The Quaternary aeolian sands sample also contained trace amounts of the evaporitic minerals gypsum and halite.



	Sample description	Sample type
a	Chapman River downstream 1	Wet river sediment
b	Chapman River floodplain	Dry floodplain sediment
c	Northwest creek	Wet river sediment
d	Cambrian greywacke, siltstone and shale 1	Catchment soil
e	Cambrian greywacke, siltstone and shale 2	Catchment soil
f	Cambrian greywacke, siltstone and shale 3	Catchment soil
g	Chapman River upstream 1	Wet river sediment
h	Chapman River upstream 2	Wet river sediment
i	Little Lashmars Lagoon	Wet lagoon sediment
j	Quaternary aeolian sands	Catchment soil
k	Southeast winter creekbed	Dry river sediments
l	Quaternary calcreted aeolianite	Catchment soil
m	Lashmars Lagoon surface	Wet lagoon sediment

Figure 3. *Mineralogy of catchment samples and Lashmars Lagoon surface sediments. Surface geology from Figure 1 is overlayed: pink = Cambrian greywacke, siltstone and shale (El); green = Quaternary aeolian sands (Qe); yellow = Quaternary calcreted aeolianite (Qpcb).*

Additionally, we characterised the mineralogy of Lashmars Lagoon's modern and ancient sediments. In general, the inorganic portion of the lake sediment is clay-rich, with illite and kaolinite composing on average nearly 60% of each sample (Figure S4). We compared the mineralogy of the lake sediments to catchment soils and inflow sediments to assess possible contemporary and past lake sediment sources. The lake sediments are comprised of varying concentrations of 1) illite, 2) quartz, 3) gypsum, 4) calcite, 5) kaolinite, 6) pyrite, 7) plagioclase feldspars and 8) halite (Figure S4). We identified potential sources for each of these minerals from the catchment samples. 1) Illite was present in high concentrations in most of the surrounding catchment and inflows to the lagoon; however, it was absent from the downstream Chapman River sample (sample a) and from two of the three Cambrian greywacke, siltstone and shale samples (d and e). 2) Quartz was also found in all samples in high quantities except the downstream Chapman River sample (a). 3) Gypsum was present only in small quantities in the Chapman River floodplain (b) and the Quaternary aeolian sands sample (j). 4) Calcite was found downstream in the Chapman River (a), in one of the upstream Chapman River samples (h), and in Little Lashmars Lagoon (i). 5) Kaolinite was identified in the Chapman River floodplain (b), upstream Chapman River samples (g and h) and in one of the western catchment soil samples (f). 6) Pyrite was not identified in any of the surrounding catchment soils or sediments. 7) Plagioclase feldspars were present in the northwest creek (c), western catchment soils samples from the Cambrian greywacke, siltstone and shale surface geology samples (d, e and f), and Little Lashmars Lagoon (i). 8) Halite was constrained to the Chapman River floodplain (b), and trace amounts in the Quaternary aeolian sands soil sample (k).

Cluster analysis (Figure 4) shows that the Lashmars modern surface sediments are most similar to the Chapman River floodplain sample (b) and the upstream Chapman River samples (g and h), consistent with an important Chapman River sediment source to Lashmars (blue box, Figure 4A). The Chapman River downstream sample (a), however, was the most different from, and formed its own sister clade to, the other samples. We also considered the relationship between the catchment samples and the down-core sediment samples, evaluating whether the source of sediment to Lashmars Lagoon changed over time (Figure 4B). We found a similar pattern of relatedness to the surface sediments: only the upstream Chapman River sediments and floodplain cluster with the Lashmars Lagoon sediment samples, suggesting that the source of sediments to Lashmars Lagoon has not changed significantly over the past ~7,000 years.

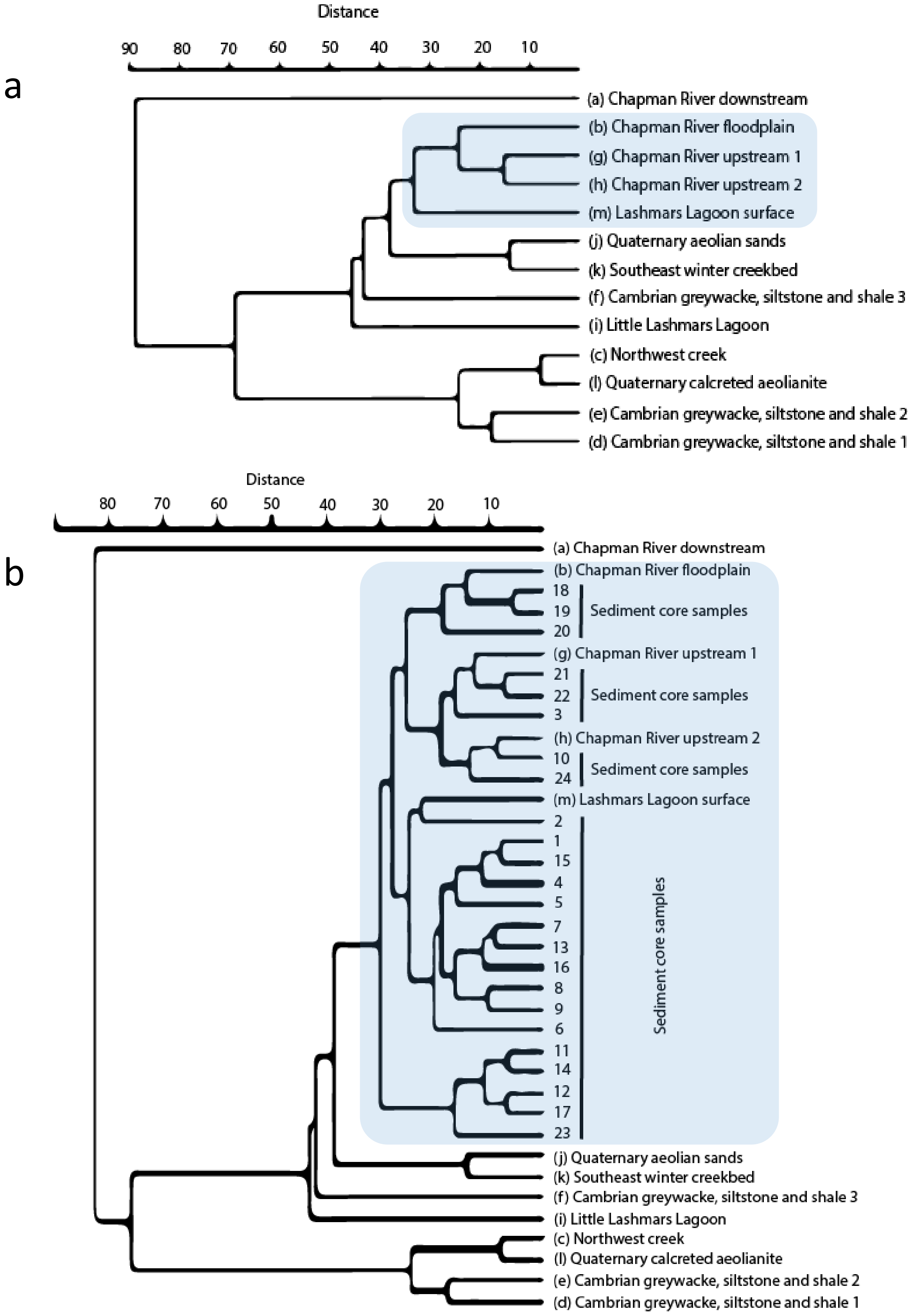


Figure 4. *Classical paired group cluster analysis of (A) catchment soils and sediments and Lashmars Lagoon surface sediments and (B) catchment soils and sediments, and modern (surface) and ancient (sediment core) lake sediments.*

Organic geochemistry

Organic geochemical proxies like total organic carbon (TOC), total organic carbon to nitrogen (C/N) and $\delta^{13}C$ isotopic ratios can be combined to determine changes in organic matter provenance over time (Meyers and Lallier-Vergès, 1999). Total Organic Carbon (TOC) values were variable throughout the record, ranging from 3.3 – 13.5% (Figure S5A), suggesting notable changes in the catchment and or climate around Lashmars Lagoon over the past ~7,000 years. C/N values ranged from 10.79 – 15.10 and were relatively stable throughout the record except for two comparatively low values at 5.06 ka and 4.27 ka (10.79 and 11.64 respectively) (Figure S5C). These values fall between those typical for algae and terrestrial plants, thus indicating mixed sources of sediment organic matter at Lashmars Lagoon (Meyers, 1994). $\delta^{13}C$ values, which can be used to infer photosynthetic processes in plants and algae (e.g., Heyng et al., 2012), were between -21.71 and -25.44‰ and are relatively low prior to 3.60 ka and relatively high after this point (Figure S5B).

Elemental geochemistry

The filtered XRF data returned 32 detectable elements of the initial 40 captured in the raw data. All three normalisation methods (raw counts, cps normalised, and log normalised) were strongly positively correlated with each other (Table 4) and did not majorly change trends in the elements of interest. For the sake of simplicity, we chose to use raw counts per second (cps) of the elements for subsequent analysis.

Table 4. *Coefficient of determination (R^2 values) to two decimal places between the normalisation methods (raw counts, cps normalised, and log normalised) for the XRF elements of interest.*

	Br	***K***	***Fe***	***Rb***	***Ti***	***Ca***	***Sr***	***Sb***	***S***
Raw vs cps normalised	0.94	0.96	0.91	0.91	0.93	0.99	0.98	0.99	0.97
Raw vs log normalised	0.97	0.94	0.96	0.96	0.95	0.79	0.90	0.99	0.83
Cps vs log normalised	0.93	0.91	0.88	0.88	0.90	0.77	0.87	0.59	0.76

The first three principal component (PC) axes of the analysis of all 32 elements explained 52.6% of the overall variance (31.5%, 15.6% and 5.2% respectively). We found eight elements strongly correlated with the first two PCs: bromine (Br), calcium (Ca), strontium (Sr), antimony (Sb), potassium (K), titanium (Ti), iron (Fe) and rubidium (Rb) (Table S4). Br and the group of lithic elements containing K, Ti, Rb and Fe were anticorrelated and together explained most of the variation along PC1 (Figure 5). The elements Ca, Sr and Sb were strongly correlated with PC axis 2.

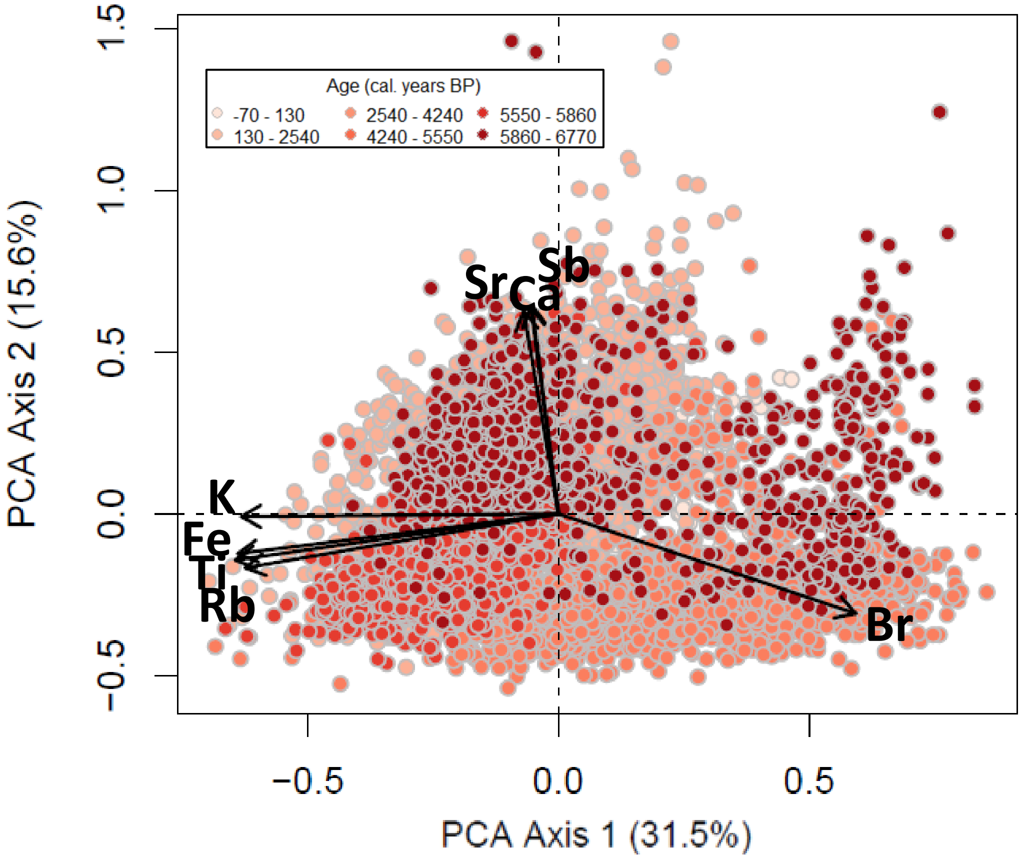


Figure 5. *Principal Components Analysis (PCA) of the filtered XRF elements using raw counts. XRF elements strongly correlated with the PCA axis 1 (PC1) and PCA axis 2 (PC2) are displayed. Bromine (Br) is strongly positively correlated with PC1, while the group including potassium (K), iron (Fe), titanium (Ti) and rubidium (Rb) is strongly negatively correlated with PC1. Strontium (Sr), calcium (Ca) and antimony (Sb) are strongly positively correlated with PC2. Points are coloured by according to defined stratigraphic units (Table 5), dark red to light reds represent older through younger sediments.*

A summary of the temporal variation in sediment geochemistry was visualised (Figure 6) by plotting the significant XRF elements with selected statistically correlated mineralogical and organic geochemical analyses (Table 6). The full suite of mineralogical and organic geochemical analyses completed in this study were plotted in Figure S4 and S5.

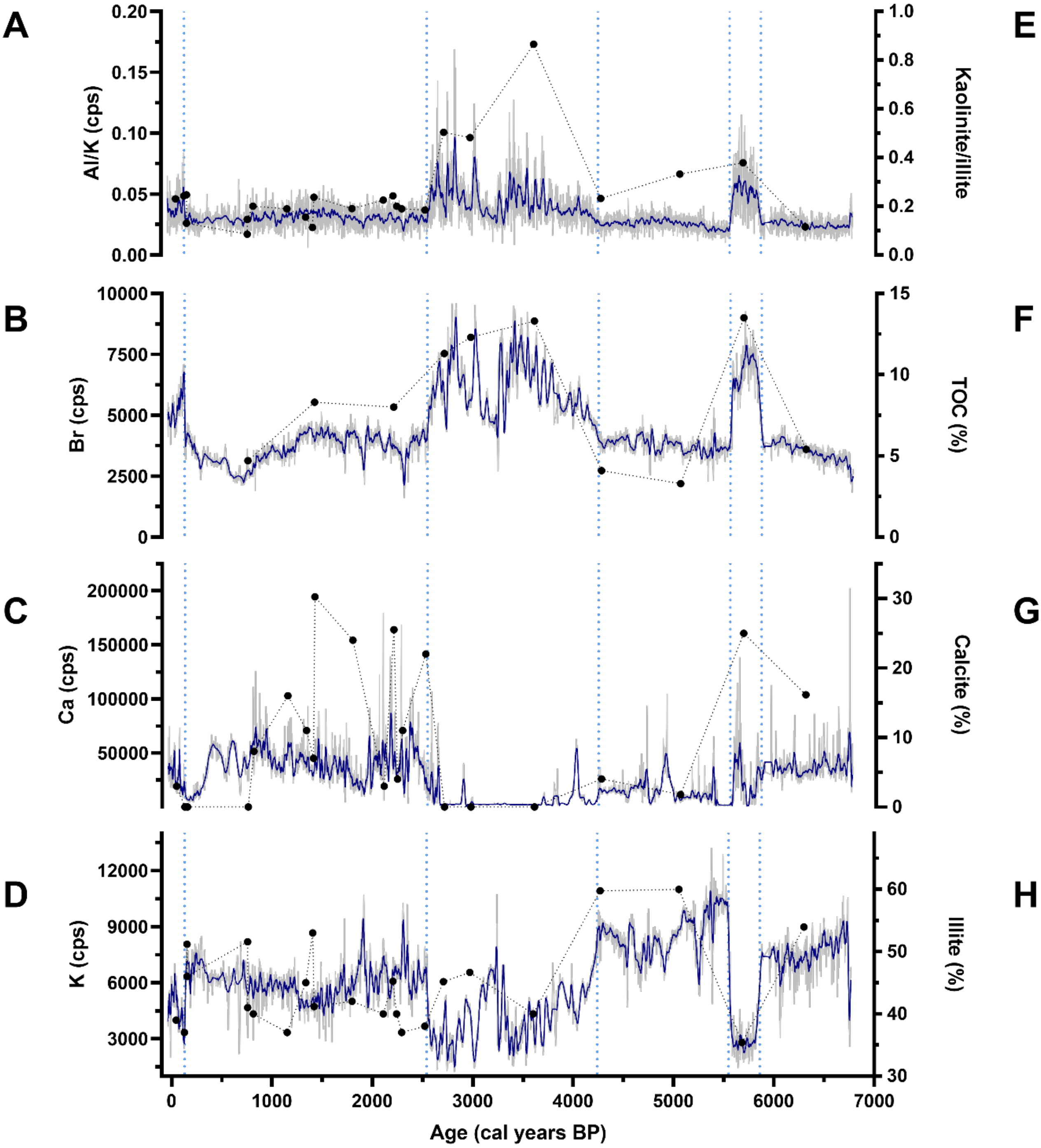


Figure 6. *Left y axes: high resolution (1 mm) XRF elemental scanning against age (grey lines with dark blue line showing weighted moving average of 40 data points). Right y axes: calibration with organic geochemistry and mineralology (grey dotted lines with black dots). (A) Al/K ratio overlayed with (E) kaolinite/illite ratio, (B) Br overlayed with (F) low resolution percent total organic carbon (TOC), (C) Ca (representative of Sr, Sb) overlayed with (G) percent calcite of the inorganic sediment, (D) K (representative of Fe, Ti, Rb) overlayed with (H) percent illite of inorganic sediment. Blue dotted lines show stratigraphic boundaries at 0.13, 2.54, 4.24, 5.55 and 5.86 ka (Table 5; Figure 2B)*

The variation in the significant XRF elements combined with the visual core descriptions informed the determination of six distinct stratigraphic units: unit 1 (6.77 – 5.86 ka), unit 2 (5.86 – 5.55 ka), unit 3 (5.55 – 4.24 ka), unit 4 (4.24 – 2.54 ka), unit 5 (2.54 – 0.13 ka) and unit 6 (0.13 ka – 2020 CE) (Table 5).

Table 5. *Description of stratigraphic units for the Lashmars Lagoon cores, in order of oldest unit (1) through to youngest unit (6).*

Composite depth and age	**Visual sediment descriptions and XRF-inferred geochemistry**
Unit 1 *727 – 640 cm* *(6.77 – 5.86 ka)*	***Colour, grain size:*** Very dark grey silty clay ***Composition:*** Homogenous sediments with large, intact bivalves and smaller fragments scattered throughout ***Geochemistry:*** High Ca (statistically linked to calcite), low Br (TOC), high K (illite), low Al/K (kaolinite/illite)
Unit 2 *640 – 610 cm* *(5.86 – 5.55 ka)*	***Colour, grain size:*** Light grey, brown, dark brown and black silty clays ***Composition:*** Organic rich, crumbly sediments with some shell fragments and fine laminations throughout ***Geochemistry:*** Variable Ca (calcite), high Br (TOC), low K (illite), high Al/K (kaolinite/illite)
Unit 3 *610 – 485 cm* *(5.55 – 4.24 ka)*	***Colour, grain size:*** Dark brown to black silty clay with some thin grey horizons ***Composition:*** Clay rich sediments with fragments of bigger shells scattered throughout, a mostly intact bivalve found near the middle of the unit, some shelly layers ***Geochemistry:*** Moderate Ca (calcite), low Br (TOC), very high K (illite), low Al/K (kaolinite/illite)
Unit 4 *485 – 319 cm* *(4.24 – 2.54 ka)*	***Colour, grain size:*** Mottled dark olive silty clay, some darker banding ***Composition:*** Crumbly, soft, highly organic sediment, very few small shell fragments scattered throughout ***Geochemistry:*** Low Ca (calcite), mostly high Br (TOC), mostly high K (illite), mostly high Al/K (kaolinite/illite)
Unit 5 *319 – 47 cm* *(2.54 – 0.13 ka)*	***Colour, grain size:*** Black to very dark grey mottled silty clay, scattered at irregular intervals throughout this section are thin grey horizons, shelly layers, and bands of shiny black very fine clays ***Composition:*** Clay rich sediments with fragmented and intact shells present throughout, small amounts of fibrous plant material, small twigs and root hairs ***Geochemistry:*** High Ca (calcite), low Br (TOC), high K (illite), low Al/K (kaolinite/illite), S intensity highly variable, compared to stable background
Unit 6 *47 – 0 cm* *(2020 CE – 0.13 ka)*	***Colour, grain size:*** Mottled dark grey silty clay ***Composition:*** Highly organic sediments with plant fibres obviously visible, intact and fragmented shells visible throughout; Strange cone-shaped artefact at the lower boundary of this unit, assumed to be an artefact of coring due to the forced convergence of two layers of different densities ***Geochemistry:*** Overall variable geochemistry: high Ca (calcite), at 47 cm abrupt peaks in Br (TOC), S, Fe and pyrite, abrupt drop in K (illite) and pyrite

Br showed considerable variation throughout the core; low Br counts were measured in the oldest part of the core from 6.77 – 5.86 ka (Table 5; unit 1), a brief period of high counts until 5.55 ka (Table 5; unit 2), an extended low period again until 4.24 ka (Table 5; unit 3), an extended high period across the middle of the record until 2.54 ka (Table 5; unit 4), another long low period until 0.13 cal. years BP (Table 5; unit 5) and finally a recent relatively high period until modern day (Table 5; unit 6). Br correlated most strongly with TOC % (r=0.91, p=0.0006; Table 6, Figure 6b), consistent with previous work indicating that Br can be a proxy for TOC (Seki et al., 2019, Ziegler et al., 2008). Br also showed a significant positive relationship with halite, kaolinite, and pyrite (Table 6).

K, Ti, Fe and Rb all correlate with each other, thus only K is shown in Figure 6 as a representative element. These elements, along with Si, are commonly associated with lithogenic detritus in lake sediments (Davies et al., 2015). K and Rb are likely hosted by illite and feldspars, Ti and Si in detrital silicates and rutile, and Fe in oxyhydroxides. However, all the detrital elements showed significant positive correlations with concentration of the clay illite (Table 6). Of these, K showed the strongest and most significant correlation with illite (r=0.66, p=0.0001) (Table 6). Additionally, Fe was significantly positively correlated with gypsum (r=0.68, p=0.0002) (Table 6). The inorganic detrital group is inversely proportional to Br through time (Figure 6b and 6d).

Ca, Sr, and Sb varied considerably through the core, following a somewhat similar trend to the group represented by K (Figure 6c). This group were all positively correlated with calcite and quartz (Table 6). Ca and Sr are hosted together in the carbonates calcite and aragonite most likely sourced from calcium carbonate producing organisms like charophyte oospores, foraminifera, and molluscs, all of which are known to occur in the sediments of Lashmars Lagoon (Figure S7). On the other hand, Sb can be incorporated in quartz (Monnier et al., 2021). Calcite was most strongly and significantly correlated with Ca (r=0.55, p=0.0066), while quartz was strongly correlated with Sb (r=0.57, p=0.0038). There are moderate to high counts of Ca, Sr, and Sb from 6.77 – 4.24 ka (units 1-3), followed by a phase of very low counts in the middle of the record from 4.24 – 2.54 ka (unit 4), and finally, a period of high counts from 2.54 ka to present day (units 5-6).

In addition to the significant XRF elements, we considered the elemental ratio Al/K, which has been linked to kaolinite/illite ratios, thus shedding light on weathering processes (Wei et al., 2006, Burnett et al., 2011). The Al/K elemental ratio follows the same trends as Br and is positively and significantly correlated with the kaolinite/illite ratio (r=0.62, p=0.0012; Figure 6a and e).

Table 6. *Correlations between significant XRF elements and minerals and geochemical proxies identified in the sediments. Three elements that were not identified as 'significant elements' in XRF data filtering but are components the identified minerals are also included: silicon (Si), sulphur (S) and chlorine (Cl). Only significant positive correlations (p < 0.05) from a Pearson correlation are shown. R-values are given with their corresponding p-value in brackets. The most significant XRF element for each mineral or geochemical measure is highlighted in green. C/N and δ 13C values (not displayed) did not correlate significantly and positively with any XRF element in the table. See supplement Figure S4 for a discussion on the presence of halite in the core.*

	Calcite	Halite	Quartz	Kaolinite	Illite	Pyrite	Gypsum	TOC%
Br		0.83 (p=0.0001)		0.67 (p=0.0006)		0.44 (p=0.03)		0.91 (p=0.0006)
Ca	0.52 (p=0.0098)		0.53 (p=0.0067)					
Sr	0.55 (p=0.0066)		0.54 (p=0.0065)					
Sb	0.46 (p=0.0229)		0.57 (p=0.0038)					
K					0.66 (p=0.0001)			
Ti					0.59 (p=0.0023)			
Fe					0.45 (p=0.0273)		0.68 (p=0.0002)	
Rb					0.55 (p=0.0038)			
Si					0.66 (p=0.0006)			
S			0.52 (p=0.007)					
Cl		0.71 (p=0.0001)						0.92 (p=0.013)

We also investigated temporal variations in geochemical proxies linked to pyrite (FeS_2) and its formation, the mineral implicated in the potential misinterpretation of the original Lashmars Lagoon charcoal record (Figure 7, Clark, 1983a, Illman, 1998). Pyrite concentrations were generally below 10% and show little variation throughout the record. S and Fe are the two elemental components of pyrite. As described above, Fe follows largely the same trends as K. S concentration is stable through most of the record, expect for the period from 2.54 – 0.13 ka (unit 5), where there are several distinct S peaks. There are peaks in both Fe and S coincident with the highest pyrite concentrations at ~ 0.13 ka. However, neither Fe nor S showed a consistently significant relationship with pyrite over the length of the record (Table 6). Instead, pyrite showed a significant but weak positive correlation with Br (r=0.44, p=0.03) and a stronger correlation with Mn/Fe (r=0.56, p=0.0042), a common proxy for reducing conditions (Makri et al., 2021). This is consistent with the conditions under which pyrite is likely to form as bacterial sulphate and Fe reduction are required, both fuelled by degradation and oxidation of organic matter.

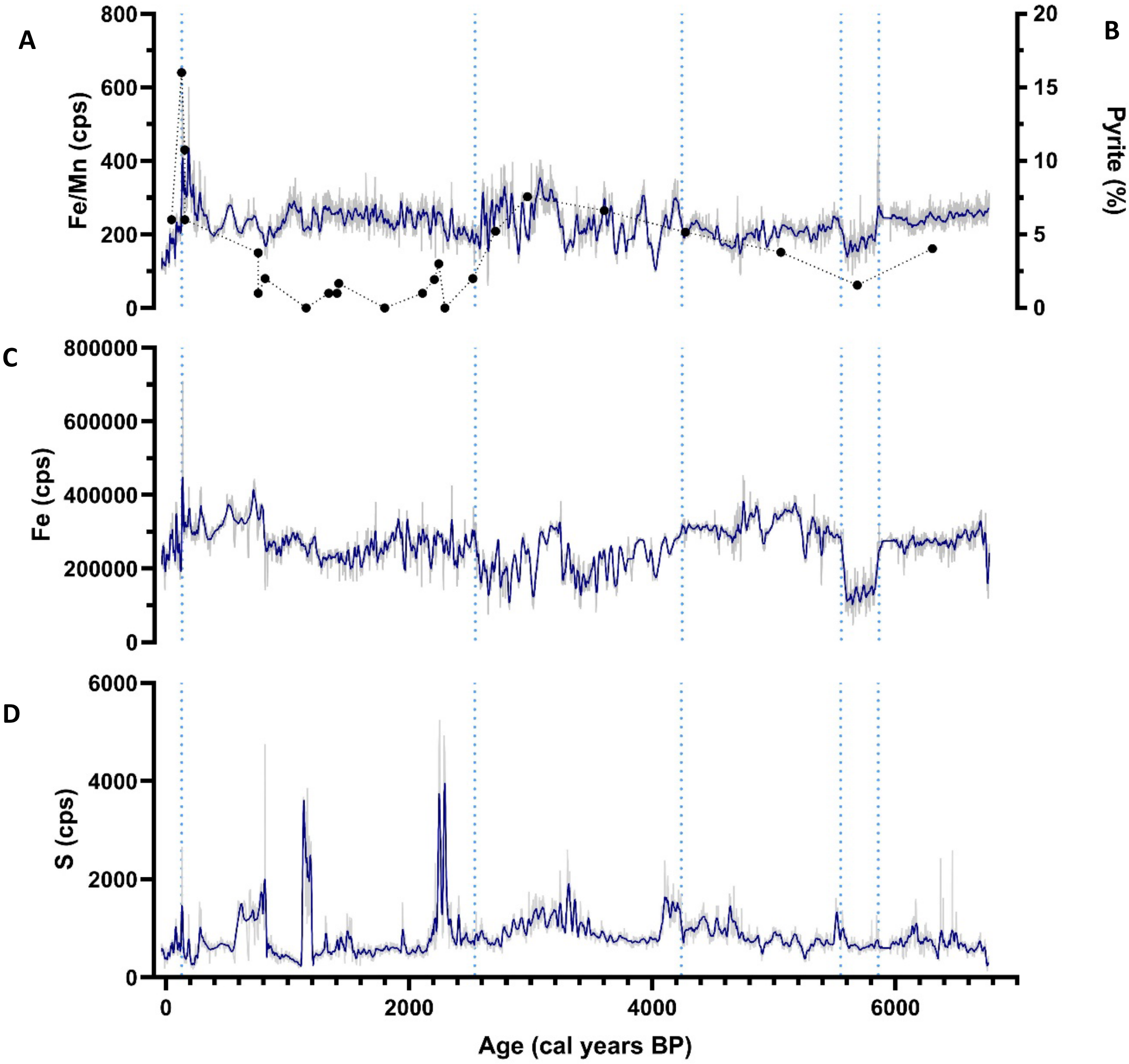


Figure 7. *Temporal variation in geochemical proxies associated with pyrite (FeS_2) and its formation. (A) Pyrite overlayed with (B) Fe/Mn ratio. (C) Iron, Fe. (D) Sulphur, S.*

Discussion

Past and present sediment delivery to Lashmars Lagoon

Characterisation of catchment soil and inflow and outflow sediments using XRD can be used to infer changes in sediment delivery to lakes over time (Morlock et al., 2019). Analysis of catchment and lake surface sediments revealed that the upstream Chapman River sediments clustered with the Lashmars surface and deeper core sediments, suggesting that the inflowing Chapman River has been an important sediment delivery source to Lashmars Lagoon for at least the last ~7,000 years (Figure

4). The Chapman River continues its passage to the sea through an intermittently connected northeast outflow, through which seawater has been known to back up to lake during storm surges as described in a personal communication by Allen Lashmar (Clark, 1976). The bulk of the mineralogy in the Chapman River estuary sample was composed of calcite, presumably of biogenic origins as is commonplace in estuarine environments, and it was thus hypothesised that these backwashing events may have been the source of the calcite in Lashmars Lagoon. However, while the floodplain that facilitates the connection between lake and sea clustered with the lake sediments and the upstream Chapman River samples, the mineralogy of the downstream Chapman River estuary distinctly formed a separate clade to all other samples, suggesting that flow between the lake and the sea is either slow or largely absent and thus does not transport much sediment to Lashmars Lagoon. Further, the smaller Little Lashmars Lagoon, upstream on the Chapman River and far from the influence of the sea, had higher concentrations of calcite than Lashmars Lagoon, suggesting that a connection to the sea is not necessary for the presence of calcite in the sediments. This was supported by the observation of intact and presumed in situ calcite bearing organisms including charophyte oospores, foraminifera, and molluscs. However, the transport of calcite from the downstream Chapman River estuary cannot be wholly discounted given the presence of fragmented shells and discrete layers of sandy, shelly, beach-like material scattered throughout the record in unit 5 and 3, that suggest transport from a marine or estuarine source. This is consistent with the identification of uniquely marine molluscs in the lagoon sediments in a previous study (Clark, 1976), although not much more than this can be inferred due to difficulties aligning our record to the broad lithological descriptions and limited geochemical data from Clark's study.

Geochemical signals in the Lashmars Lagoon sediments

Changes in sediment geochemistry, given careful contextual consideration, can be interpreted as evidence for changing climate and catchment processes (Davies et al., 2015). Itrax core scanning provides detailed assessment of chemical composition, which can be used to infer mineralogy and thus changes in the lake and catchment. We found three main modes of variation in our Itrax data: Br, a cluster containing Sb and the carbonate elements Sr and Ca, and the inorganic detrital elements (K, Fe, Ti and Rb). Variation in these elements in lacustrine sediments is known to reflect several different processes (Table 7). However, Itrax scanning can only provide semi-quantitative estimates of elemental composition, so it is important to ground interpretations of these data with other geochemical proxies (Moreno et al., 2007, Morlock et al., 2019). Our study aimed primarily at inferring information about wetter or drier climates, lake water salinity, and pyrite formation. Here, we critically evaluate the extent to which our data can elucidate these questions.

Table 7. *Significant XRF core scanning elements at Lashmars Lagoon and possible interpretations according to the review of the literature by Davies et al. (2015).*

Element	**Possible interpretations from the literature**	**Proxy at Lashmars Lagoon**
Br	Increased biological productivity/organic content; Sea spray/increased storminess	Percent total organic carbon (TOC)
K	Increased detrital input; Flood layers; Drier conditions (physical > chemical weathering); Clay rich layers in varved sediments; Fine-grained detrital inputs; Tephra	Detrital input
Ti	Increased run-off/rainfall; Increased detrital input; Detrital input (glacier advance)	
Fe	Clay rich layers in varved sediment; detrital inputs, redox conditions (non-stationarity); Fine silt, clay or volcanic origin; Tephra	
Rb	Detrital inputs; Fine-grained detrital inputs; Tephra	
Sr	In-lake SrCO3 precipitation; Erosion of granodiorite; Tephra	Sediment calcite concentration, reflects carbonate fossils putatively linked to increased evaporation and or increased marine influence
Ca	Increased calcite precipitation/evaporative concentration; Endogenic calcite production + detrital carbonates; Increased primary productivity; Increased marine influence; Increased allochthonous lithoclastic material; Tephra	
Sb	NA	Linked to quartz occurrence, possibly a proxy for grain size and therefore related to proximity to lake shoreline

The ratio of Al/K has been applied as a weathering proxy in previous studies (Wei et al., 2006, Burnett et al., 2011). We find that Al/K correlates with the ratio of kaolinite to illite, supporting a similar interpretation of this ratio at Lashmars Lagoon. When chemical weathering of feldspar minerals occurs, K is progressively leached, forming intermediate weathering products such as illite, and finally the end-member kaolinite when no K remains at all. Thus, the more intense the chemical weathering, the more kaolinite there will be in relation to illite. As more chemical weathering normally occurs in warmer and wetter conditions, we tentatively interpret high kaolinite to illite (Al/K) ratios to be a qualitative tracer of higher past rainfall and temperature. Chemical weathering may also be promoted by soil-root interaction of large trees which are more abundant in warmer and wetter climates in Australia (Francke et al., 2022). The Al/K ratio follows a very similar trend to Br. We found that Br strongly correlated with TOC, consistent with other studies that show Br can be used as a proxy for organic matter and supporting this interpretation at Lashmars Lagoon (Woodward and Gadd, 2019, Guevara et al., 2019). The C/N ratios (10.79 – 15.10) and δ^{13}C values (-21.71 and -25.44‰) indicated that sediment organic matter included mixed contributions from aquatic macrophytes, algae and land plants, albeit weighted towards a predominant algal source

(Meyers, 1994). We suggest that wetter and less saline conditions would be most conducive to producing high sediment organic matter as these conditions would promote growth of terrestrial plants and are more amenable to aquatic macrophytes and algae productivity. We therefore interpret the periods of higher Br, and higher Al/K to reflect periods of higher rainfall in the Lashmars Lagoon catchment.

K, Ti, Fe and Rb – elements linked in previous studies to increased detrital input (Table 7) – correlated with the clay illite and were anticorrelated with Br and the Al/K ratio. Consistent with the inference that increased Br and Al/K ratios reflect wetter climates, illite is an intermediate product of the incomplete chemical weathering of feldspars and is retained in landscapes when chemical weathering is supressed in drier conditions (Francke et al., 2020). Ca and Sr, both common elements in carbonates, were correlated with calcite in the sediments of Lashmars Lagoon, suggesting this mineral is where these elements are mainly contained in the Lashmars Lagoon sediments. Calcite in the Lashmars Lagoon sediments is presumed to be of biogenic origin due to the documented presence of carbonate bearing organisms such as charophyte oospores, foraminifera, and molluscs in the sediment. We envisage that increased sediment calcite reflects periods of higher lake salinity with enhanced concentrations of bioavailable calcium and carbonate ions supporting a diversity of calcium carbonate producing organisms. Saline lake conditions could be induced by increased evaporation in drier climates or from an enhanced hydrological connectivity to the sea via the Chapman River estuary. It is also possible that calcite was sourced from the calcite-rich sediments of the Chapman River estuary, although our analysis of sediment delivery to Lashmars Lagoon revealed that this was unlikely to have been a common occurrence. Additionally, calcite, Ca and Sr were correlated with Sb and quartz. We expect that the presence of Sb in the sediments is linked to quartz, as Sb is known to occur in quartz veins (Monnier et al., 2021). Quartz grains are often associated with coarser sands and silts that require more energy to be transported than finer clays. Thus, they are usually more concentrated closer to the edge of lakes or in shallower lakes where wave action has a more important influence on the sediment transportation and resuspension. As a result, fluctuations in grain size and quartz delivery to the coring location can reflect changes in lake depth or width over time (Bowler and Hamada, 1971, Wilkins et al., 2013, Aufgebauer et al., 2012). Where there is more quartz in the sediment, particularly in relation to finer clays, the shoreline distance of the coring location might have been shorter, and the lake may have been smaller and or shallower. The co-occurrence of elements associated with calcite (Ca and Sr) and quartz (Sb) is therefore parsimoniously explained by drier conditions that would have increased evaporation, simultaneously increasing ionic concentrations and decreasing lake size. Adding weight to the hypothesis of a dominant climate control on Ca, Sr and Sb concentrations is that these elements are roughly anticorrelated with Br and Al/K which were inferred to be associated with wetter climates. However, the imperfect nature of the anticorrelation suggests that other factors also exert some control over sediment calcite and quartz. Gypsum ($CaSO_4{\cdot}2H_2O$), for example, is an evaporitic mineral that hosts Ca that was also detected in the record (Figure 8). The presence of gypsum in lake sediments is an unequivocal indication of increased evaporation and therefore drier conditions (Burn and Palmer, 2014).

Climate and catchment variability over the last ~7,000 years at Lashmars Lagoon

From this evidence, we can infer the broad trends in climate at Lashmars Lagoon over the past 7,000 years (Table 5, Figure 8).

Unit 1 | 6.77 – 5.86 ka

This unit is characterised by relatively high calcite (Ca and Sr) and quartz (Sb), low organic matter (Br), and low inferred chemical weathering (Al/K), therefore suggesting a period of drier and shallower conditions, possibly with marine influence.

Unit 2 | 5.86 – 5.55 ka

Unit 2 has high organic content (Br) and an elevated kaolinite to illite (Al/K) ratio indicating relatively high chemical weathering at this time, pointing to a relatively wet and fresh period between 5.86 – 5.55 ka. However, the calcite (Ca) concentrations throughout this period are variable, which may be linked to deposition of calcite from the sediments of the Chapman River estuary facilitated by marine intrusions, e.g., during storm surges.

Unit 3 | 5.55 – 4.24 ka

The period from 5.55 – 4.24 ka heralded a return to an extended period of drier, more saline and shallower conditions, demonstrated by the combination of increased calcite (Ca) and quartz (Sb) concentrations, low organic content (Br) and high illite (K) and reduced chemical weathering (Al/K). There is evidence for possible marine intrusions during this period with pronounced sandy and shelly layers scattered throughout this layer.

Unit 4 | 4.24 – 2.54 ka

From 4.24 ka – 2.54 ka, Lashmars Lagoon apparently experienced an extended overall wetter period that resulted in fresher and deeper lake conditions – which was interrupted by a fleeting dry episode from ~3.3 - 3.0 ka, possibly alluding to more variable climate conditions in this phase. We infer this through the mostly high organic content (Br) in these sediments, coupled with mostly low calcite

(Ca) and quartz (Sb), high K (illite), mostly high Al/K (kaolinite/illite). These trends reversed momentarily during the inferred short dry period.

Unit 5 | 2.54 – 0.13 ka

Unit 5 marked the first occurrence of the evaporitic mineral gypsum in the record, putatively partially explaining the S peaks diagnostic of this period, and clearly defining this period as one of accentuated evaporation and thus particularly dry conditions. This is supported by the other geochemical indices in this phase: we detected very high calcite (Ca), low organic content (Br), high K (illite), low chemical weathering (Al/K). The existence of bands of shelly, sandy material hints at the possibility of transport of marine or estuarine material from marine intrusions.

Unit 6 | 0.13 ka – 2020 CE

The youngest stratigraphic unit, from 130 cal. years BP (~1820 CE) to 2020 CE is almost too short to observe long-term trends against background variability. Nevertheless, the weathering proxy Al/K appears to indicate a brief return to wet conditions before potentially trending towards drier conditions again. However, it is quite possible that the geochemistry of this period is biased by the land use changes brought about by the first arrival of sealers, convicts, and their Tasmanian wives on the Dudley Peninsula. In fact, the start of this designated sedimentary unit at ~1820 CE coincides well with the documented recommencement of human habitation on KI in 1824, early in the commencement of the colonial period in South Australia (Taylor, 2008). Interpretating geochemically inferred climatic and catchment processes in this section of the Lashmars Lagoon record demands a suite of contextual considerations beyond the scope of this study.

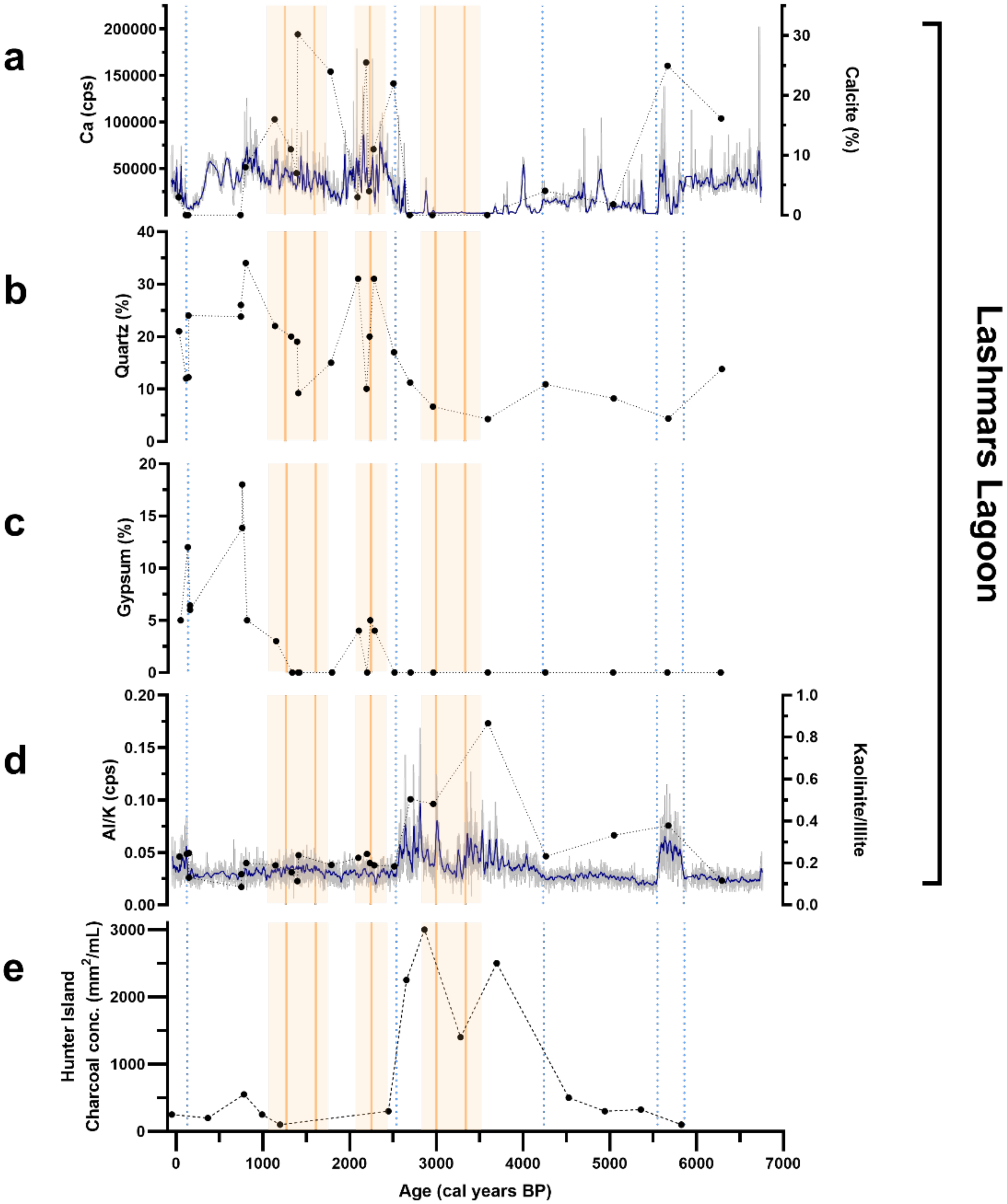


Figure 8. *(A-D) Summary of the key geochemical signatures of the Lashmars Lagoon record that reflect important catchment and climate processes: (A) Ca and calcite, (B) Quartz, (C) Gypsum and (D) Al/K and Kaolinite/Illite. (E) Regional comparison to the charcoal concentration record at Hunter Island (Hope, 1999). Orange lines and shaded area are the arid period and their age uncertainty inferred from radiocarbon dated desiccation horizons in Coorong lakes, directly adjacent to KI (Ahmad, 1996).*

Regional climate comparison

Our ~ 7,000-year palaeoclimate record from KI fills an important gap in Holocene palaeoclimate archives from southern Australia.

From ~6.8 – 4.2 ka our record suggests predominately drier conditions at Lashmars Lagoon, an inference that is somewhat at odds with other regional records from across southern Australia which record relatively wet climates throughout this interval (De Deckker, 2022, Gouramanis et al., 2013, Wilkins et al., 2013, Fletcher and Moreno, 2012). Indeed, marine sediment cores offshore from KI show that reconstructed sea surface temperatures (SST) were high during this same period (De Deckker et al., 2020), conceivably implying that precipitation should have increased over Lashmars Lagoon. We did in fact find evidence for a brief wet phase from 5.8 – 5.6 ka within this broader time period that overlaps with what has been recently defined as the Holocene hypsithermal (De Deckker, 2022), referring specifically to the unusually warm and wet period that occurred in Australian from 8.2 – 5.5 ka in Australia. While our interpretation of the geochemistry at Lashmars Lagoon suggested mostly dry conditions during this interval, this may be explained by warmer conditions leading to more evaporative lake conditions or the possibility of a marine influence in the system prior to 5.8 ka that may have obscured the climate signal in the sediment geochemistry.

We inferred that the climate was relatively wet at Lashmars Lagoon between ~4.2 – 2.4 ka. Interestingly, this section bears striking similarity to a charcoal record from Stockyard Swamp on Hunter Island, a small island in the Bass Strait (Figure 8E, Hope, 1999). When Lashmars Lagoon is wet, charcoal concentrations on Hunter Island are high, which we hypothesise may be a response to regional climate. We expect that fire on Hunter Island, which is covered mostly by low health, is limited by biomass. As such, wetter phases would increase biomass and therefore fuel more fires.

Broadly speaking, our record shows increasingly dry conditions throughout the Late Holocene from ~2.4 ka. The continual decline of SST from the nearby offshore marine sediment cores from about ~4.5 ka until present day may provide a possible mechanism to explain decreased precipitation on KI (De Deckker et al., 2020), although this is difficult to reconcile with our previous comparison of this record to climate at Lashmars Lagoon during the interval from 6.8 – 4.2 ka. The timing of the shift to drier climates at Lashmars Lagoon at ~2.4 ka is somewhat coherent with evidence from radiocarbon dated lake desiccation horizons in the Coorong region which is adjacent to KI on the mainland which record a dry phase at ~2.5 ka (Ahmad, 1996). The Coorong study also picks up evidence from earlier dry phases at 3.3 and 3.0 ka. Interestingly, these two dates constrain a short inferred dry phase amidst the longer wetter phase from ~ 4.2 – 2.4 ka at Lashmars Lagoon. There is evidence on the mainland too of substantial climatic change around this time. For example, at 3.2 ka, Tasmanian devils (*Sarcophilis harrisii*) and the Tasmanian tiger (*Thylacinus cynocephalus*) suffered a synchronous extinction on mainland Australia, a major ecological shift putatively linked to climate change (White et al., 2018). The Late Holocene drying pattern at Lashmars Lagoon also fits well with other regional syntheses that conclude this trend occurred across southern Australia (Gouramanis et al., 2013, Gouramanis et al., 2012, Wilkins et al., 2013, Fletcher and Moreno, 2012). Previous studies have suggested that this period was associated with an intensification of ENSO-like variability (Barr et al., 2019, Perner et al., 2018) and thus more frequent El Niño-like dry conditions. Of particular relevance to this study is the foraminifera in the marine record from offshore KI that record changes in the Leeuwin Current linked to the SWW (De Deckker et al., 2020). After ~3 ka, there is evidence for the absence of the Leeuwin Current off the coast of South Australia, which is thought to be associated with the southern migration of the SWW which increases El Niño occurrence and decreases seasonal variation in rainfall on KI (drier winters and wetter summers).

In summary, our reconstruction of palaeoclimate from the geochemistry of Lashmars Lagoon bears some resemblance to regional climate records but highlights important differences and contradictions that warrant further investigation. For the time being, we await a more nuanced understanding of the temporal and spatial variation of Holocene climate across Holocene southern Australia to better contextualise the complex KI climate record.

Returning to an old question with new methods: pyrite formation and the interpretation of the charcoal record

A pertinent unresolved question at Lashmars Lagoon relates to the suggestion that pyrite, formed under reducing lake conditions, may have been misidentified as charcoal (Illman, 1998). Thus, an earlier study that documented an increase in fire biomass burning at ~2.5 ka (Clark, 1983a) may have been biased. While we show that pyrite formation occurs in reducing conditions, as it significantly correlates with the Fe/Mn ratio, an established proxy for lake reducing conditions (Makri et al., 2021), this trend bore no resemblance to the charcoal record. Instead, pyrite showed a significant correlation with Br, a proxy for organic matter. This finding is consistent with the oxidation of organic matter fuelling various reducing reactions and coherent with similar findings in other studies (Moreno et al., 2007).

It was also suggested that the change in charcoal concentrations at Lashmars Lagoon may have been affected by depositional bias given the change in the charcoal record was roughly coincident with a change in sedimentology (Illman, 1996). However, we find little evidence of changes in lake sediment sources over time from our modern catchment study, suggesting that the delivery of charcoal to the lake would have been reasonably consistent through time and unaffected by depositional changes.

In summary, our data suggest that pyrite was not misidentified, nor the charcoal affected by sediment source depositional biases, thus lending support to the interpretation of the charcoal record as a reliable palaeofire proxy.

Future directions

Our ~7,000-year high-resolution record of catchment and climate processes encourages the retrieval of more Holocene records in South Australia to resolve regional uncertainties and paves the way for future work on this important lake archive from Kangaroo Island (Karti). The analysis of palaeoecological and palaeofire records from Lashmars Lagoon should be priorities to build a holistic understanding of interactions between climate, landscapes, fire, and ecosystems. Scientists also need to place better constraints on the regional climate history and history of Aboriginal people on the island from both archaeological and Indigenous knowledge systems perspectives to understand the role of Aboriginal stewardship in Earth system dynamics in southern Australia.

Author contributions

LD worked on the study design, undertook field and lab work, data analysis and interpretation, and wrote the first draft of the manuscript; JT, WL, AF assisted with field work; JT, HC, AF and LJ-M helped with the study design and interpretation; LJ-M processed the first batch of XRD samples and the organic geochemistry; SL and GB helped with interpretation; BL made Figure 1; All authors contributed to the editing of the manuscript.

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In Australia, we work and live on Aboriginal land. This research was developed and conducted on Kaurna Yerta (Kaurna land) at The University of Adelaide and on Karti (Kangaroo Island), a place of deep cultural and spiritual significance for Aboriginal people in South Australia, including for Ngarrindjeri, Kaurna, Narungga, Nhawu and Barngarla people. I pay my respects to elders past, present, and emerging. I acknowledge that sovereignty was never ceded.

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Supplementary information

Figure S1. *Optical images of the sediment cores from Itrax core scanning. (A-C) LAS20-2 sections: (a) 0-1m, (b) 1-2m and (c) 2-3m and 3-4m. (D) LAS20-3 0-1m. (E-K) LAS20-4 sections: (e) 0.5-1.5m, (f) 1.5-2.5m, (g) 2.5-3.5m, (h) 3.5-4.5m, (i) 4.5-5.5m, (j) 5.5-6.5m and (k) 6.5-7.5m.*

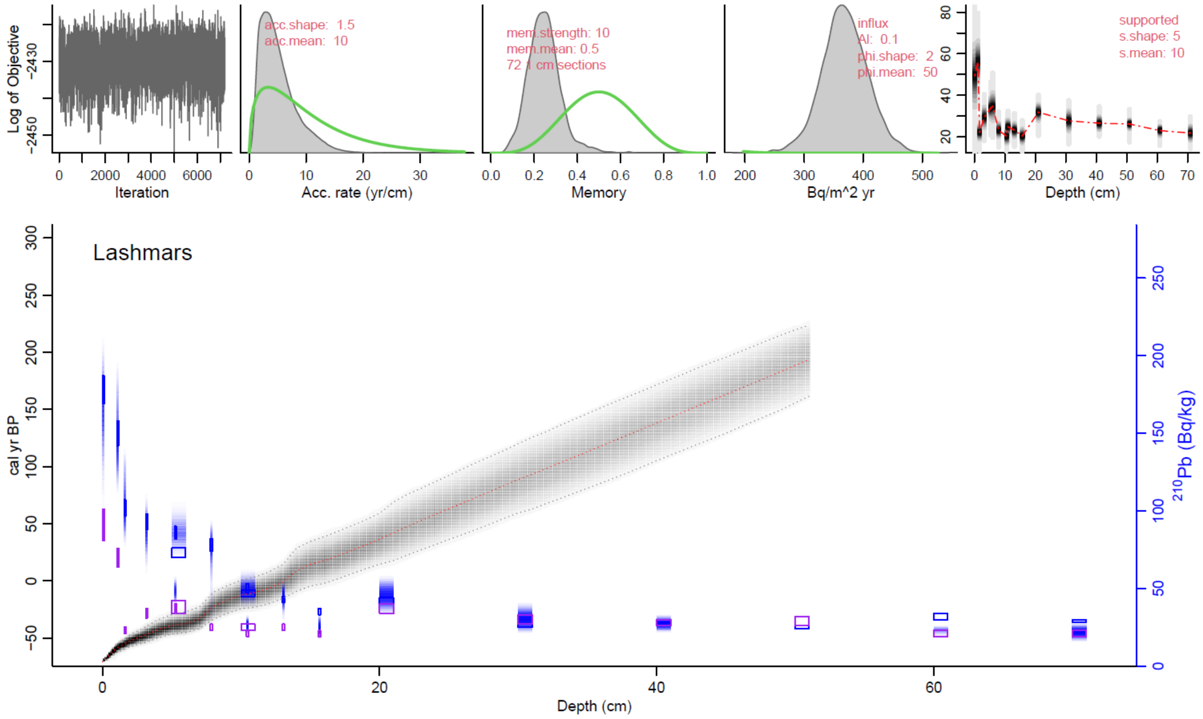

Figure S2. *^{210}Pb Chronology. Anchored with the coring date (September 2020) at 0 cm and the 1964-timestamp at 13.5 cm from the bomb peak in the Pu isotopic profile (Figure S3)*

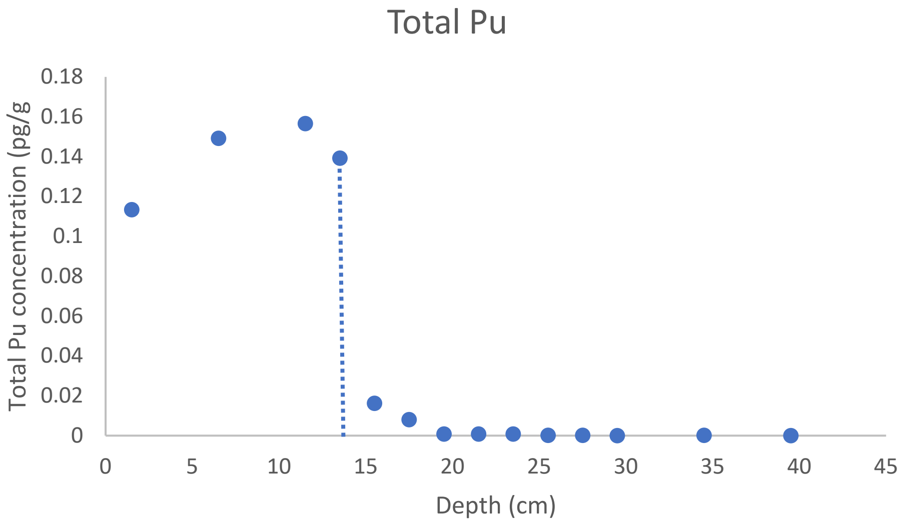

Figure S3. *Total Pu. Dotted line denotes the 1964 bomb peak.*

Table S1. *Raw age model data from (A) Pu, (B) $_{210}$Pb, (C) radiocarbon techniques.*

A

ANSTO code	**Depth start (cm)**	**Depth end (cm)**	**Mean depth (cm)**	**Total Pu conc (pg/g)**	**+/- err**
10396	1	2	1.5	0.113357	0.002473
10397	6	7	6.5	0.149189	0.003199
10398	11	12	11.5	0.156499	0.003342
10399	13	14	13.5	0.139236	0.002944
10400	15	16	15.5	0.016248	0.00042
10401	17	18	17.5	0.00808	0.000235
10402	19	20	19.5	0.000809	4.82E-05
10403	21	22	21.5	0.000777	4.96E-05
10404	23	24	23.5	0.000786	4.53E-05
10405	25	26	25.5	0.000146	2.18E-05
10406	27	28	27.5	8.06E-05	1.68E-05
10407	29	30	29.5	6.17E-05	1.86E-05
10408	34	35	34.5	0.000107	4.18E-05
10409	39	40	39.5	2.96E-05	1.35E-05

B

ANSTO code	**Depth (cm)**	**Density (g/cm^3)**	**^{210}Pb (Bq/kg)**	**Sd (^{210}Pb)**	**Thickness (cm)**	**^{226}Ra (Bq/kg)**	**Sd (^{226}Ra)**
X138	0.13	0.49	178	9	0.13	91	10
X139	1.17	0.23	150	8	0.13	70	6
X335	1.69	0.23	102	5	0.13	23	2
X140	3.25	0.2	93	5	0.13	34	3
X141	5.33	0.2	86	4	0.13	37	3
X319	6	0.43	73	3	1	38	4
X142	7.93	0.24	78	4	0.13	25	2
X143	10.53	0.24	50	3	0.13	21	2
X320	11	0.45	47	2	1	25	2
X336	13.13	0.24	43	2	0.13	25	2
X144	15.73	0.23	35	2	0.13	21	2
X321	21	0.48	42	2	1	37	3
X322	31	0.44	29	4	1	30	3
X323	41	0.39	27	1	1	28	2
X324	51	0.33	25	1	1	29	3
X325	61	0.54	32	2	1	21	2
X326	71	0.65	29	1	1	21	2

ANSTO sample* C *code	**Sample depth interval (cm)**	**Estimated Δ ($_{13}$C) (per mil)**	**% Modern Carbon (pMC)**	**Uncalibrated age (years BP)**	**Description**
OZAE45	89-94	-25	89.12 ± 0.23	925 ± 25	relatively long elongate and twig-like, quite confident this is not of marine origin
OZAE46	99-100	-25	88.52 ± 0.24	980 ± 25	relatively long elongate and twig-like, quite confident this is not of marine origin
OZAE49	210-210	-25	80.24 ± 0.22	1770 ± 25	clearly woody, a small stick
OZAE50	317-317	-25	74.53 ± 0.77	2360 ± 90	potentially seagrass/of marine or aquatic origin/might also be a thin strip of bark
OZAE52	392-392	-25	67.68 ± 0.16	3135 ± 20	look like charred seeds, only the 2 seed-like structure were dated
OZAE53	427-427	-25	65.41 ± 0.89	3410 ± 110	small, looks like a bit of fluff, quite possibly seagrass/of marine or aquatic origin
OZAE54	97-97	0	85.57 ± 0.24	1250 ± 25	small, spiral, conical shells

	Sample depth interval (cm)	**F14C**	**F14C error**
CHRONOS pollen sample 1	349-350	0.7149	0.00138
CHRONOS pollen sample 2	727-728	0.4748	0.0012528

Table S2. *Pearson correlation coefficients (r values) to two decimal places between the normalisation methods (raw counts, cps normalised, and log normalised) for the XRF elements of interest.*

	Br	**K**	**Fe**	**Rb**	**Ti**	**Ca**	**Sr**	**Sb**	**S**
Raw – cps normalised	0.97	0.98	0.95	0.95	0.96	0.99	0.99	1.00	0.99
Raw – log normalised	0.99	0.97	0.98	0.98	0.98	0.89	0.95	1.00	0.91
Cps – log normalised	0.96	0.96	0.94	0.94	0.95	0.87	0.93	0.77	0.87

Table S3. *Coefficient of determination (R^2 values) to two decimal places between the normalisation methods (raw counts, cps normalised, and log normalised) for the XRF elements of interest.*

	Br	***K***	***Fe***	***Rb***	***Ti***	***Ca***	***Sr***	***Sb***	***S***
Raw – cps normalised	0.94	0.96	0.91	0.91	0.93	0.99	0.98	0.99	0.97
Raw – log normalised	0.97	0.94	0.96	0.96	0.95	0.79	0.90	0.99	0.83
Cps – log normalised	0.93	0.91	0.88	0.88	0.90	0.77	0.87	0.59	0.76

Table S4. *XRF elements with strongly correlated Pearson correlation coefficients (-0.8 > r > 0.8) to PC1 and PC2.*

	Br	**Ca**	**Sr**	**Sb**	**K**	**Ti**	**Fe**	**Rb**
PC1	0.85	-0.08	-0.08	-0.10	-0.90	-0.92	-0.91	-0.89
PC2	-0.44	0.92	0.91	0.90	-0.01	-0.20	-0.18	-0.24

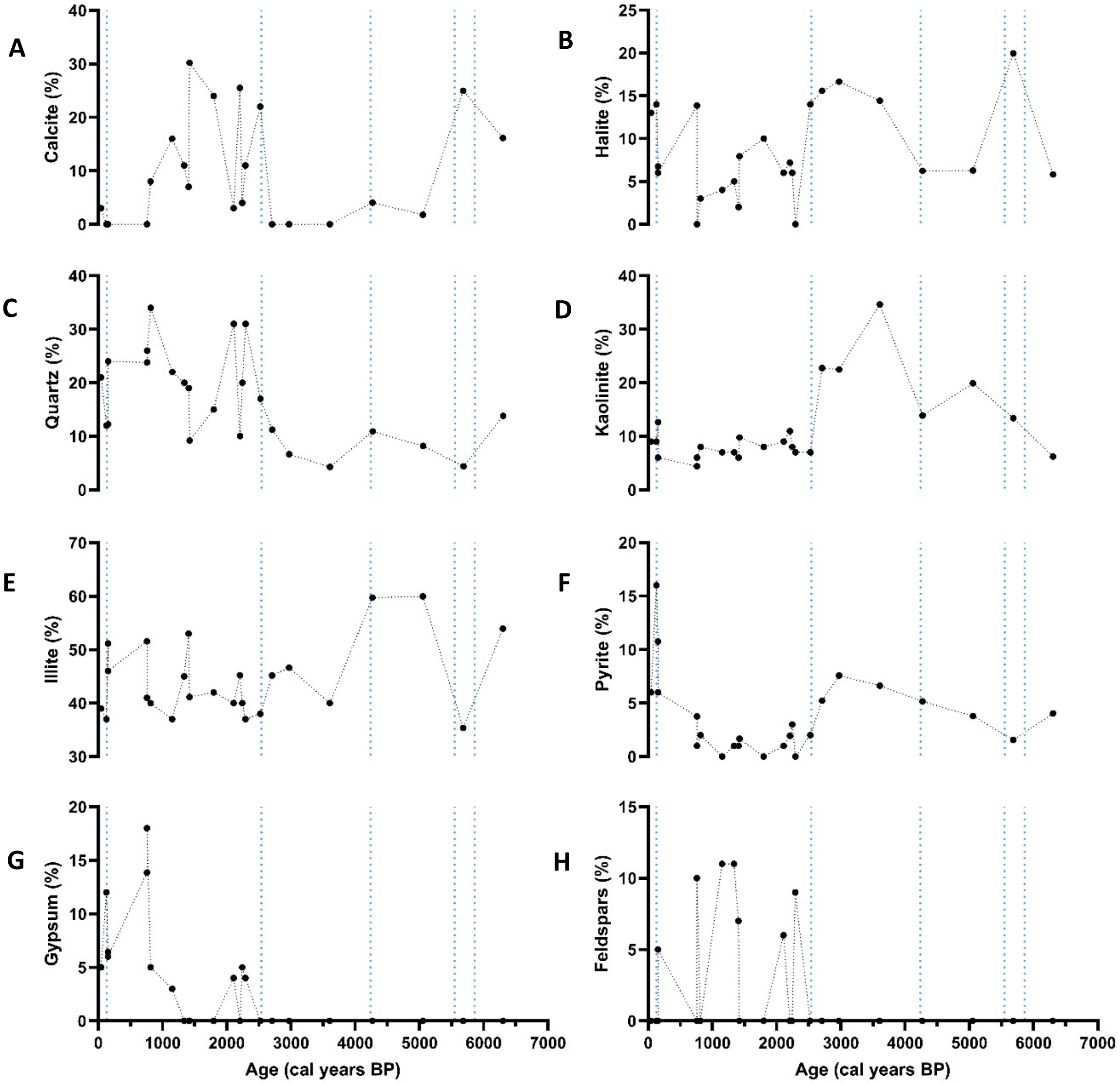

Figure S4. *XRD minerals against age. (A) Calcite, (B) halite, (C) quartz, (D) kaolinite, (E) illite, (F) pyrite, (G) gypsum and (H) feldspars.*

Note S1. *Discussion on the presence of halite:*

Halite (NaCl) is another mineral that normally precipitates in evaporitic conditions and significantly correlates with chlorine in our record. Paradoxically, we found that halite and chlorine trended not with calcite, but with organic matter. We expect that this was an artefact of halite precipitation from the evaporation of core pore water in the lab during subsampling. The organic layers are more porous and therefore precipitate more halite. Indeed, inspection of the cores showed evidence of halite precipitation at organic-rich sections.

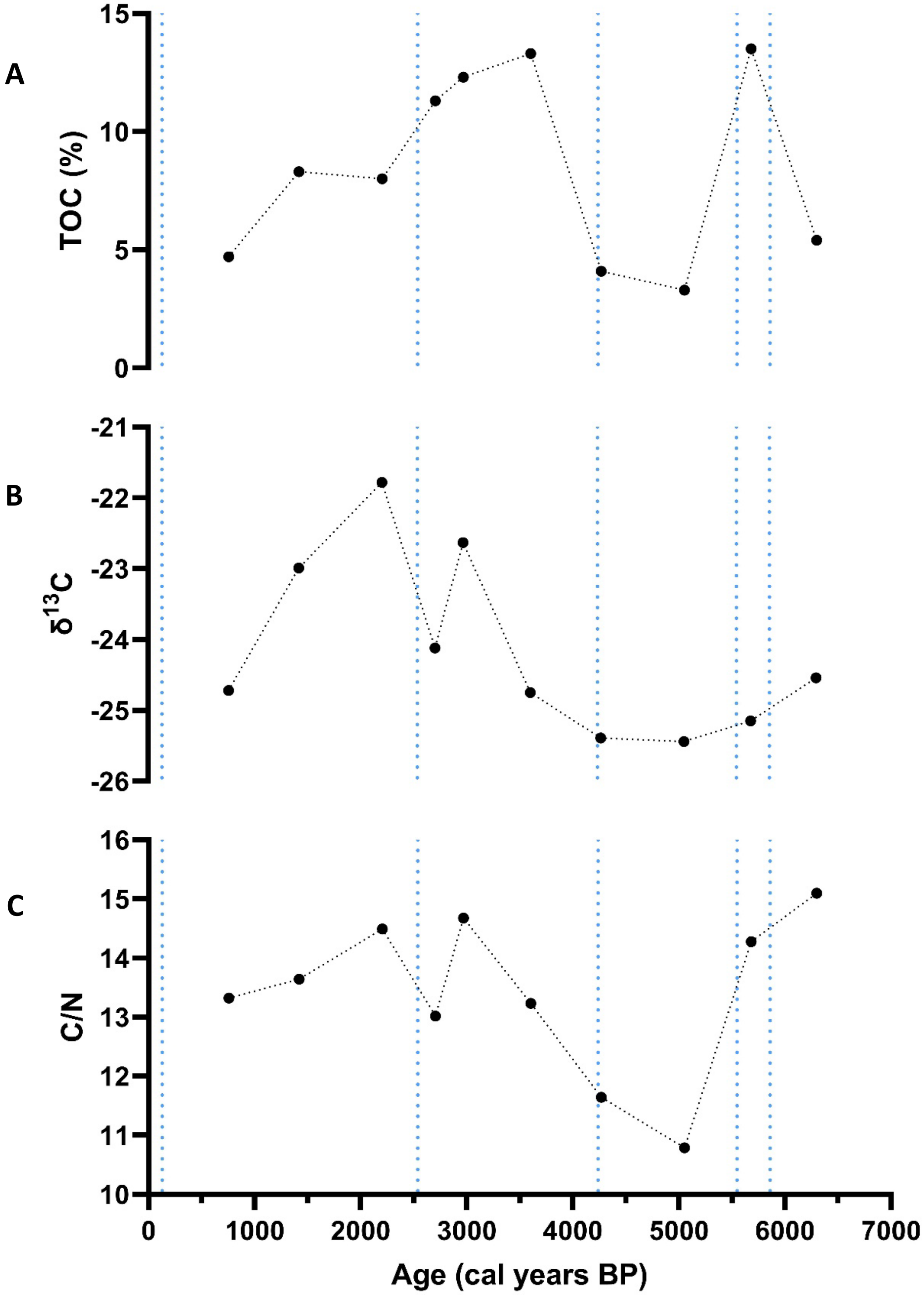

Figure S5. *Organic geochemical proxies against age. (A) Total Organic Carbon (TOC). (B) δ13C. (C) Carbon to nitrogen ratio (C/N).*

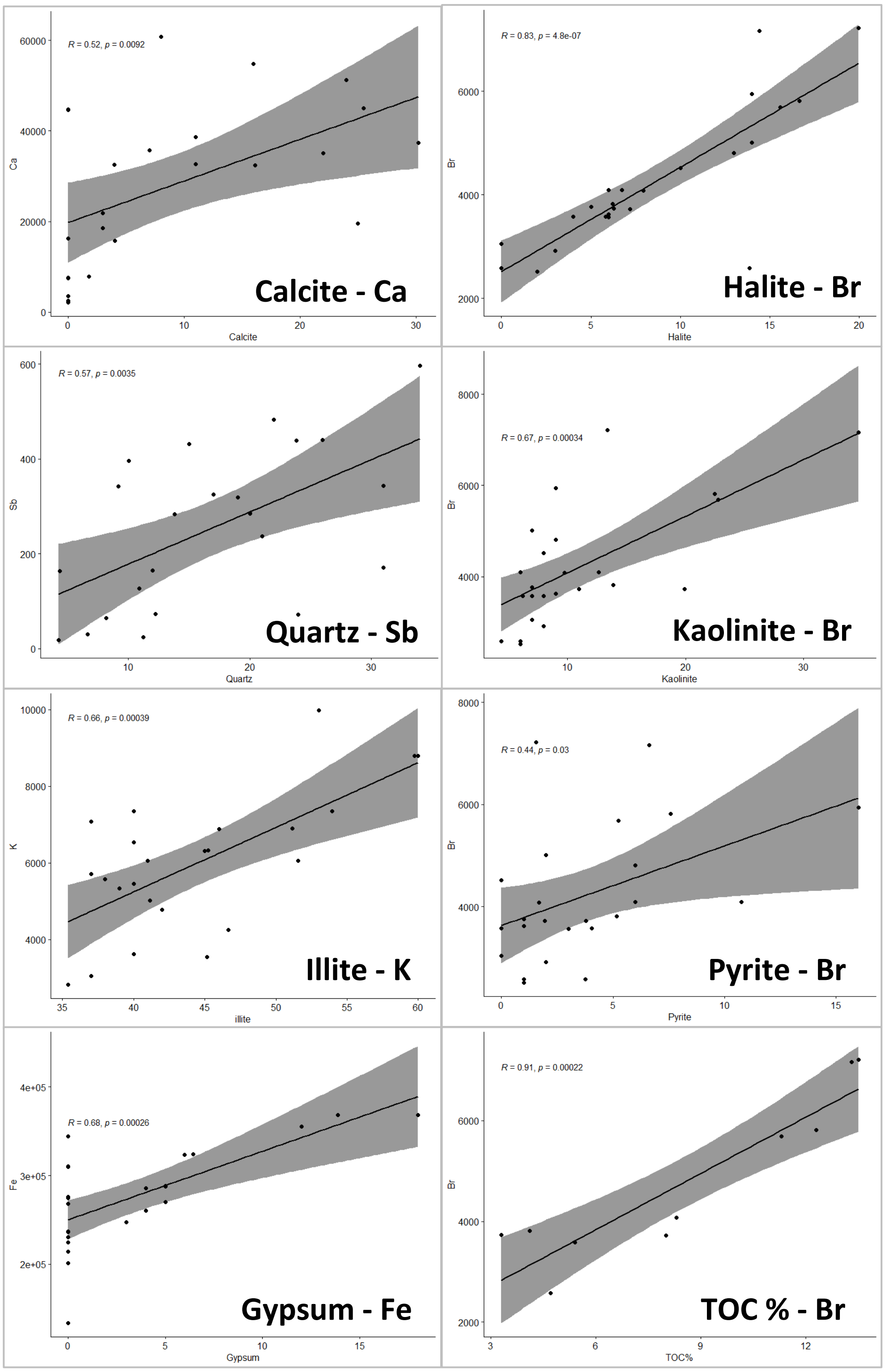

Figure S6: *Mineral and geochemical proxies and their most significantly correlated Itrax micro-XRF element.*

Table S5. *Sediment core XRD sample no. to composite depth reference table.*

Sample no.	**Composite depth**
1	29 cm
2	48 cm
3	51 cm
4	51 cm
5	91 cm
6	91 cm
7	96 cm
8	142 cm
9	167 cm
10	176 cm
11	178 cm
12	228 cm
13	266 cm
14	278 cm
15	282 cm
16	288 cm
17	317 cm
18	338 cm
19	364 cm
20	424 cm
21	488 cm
22	563 cm
23	623 cm
24	682 cm

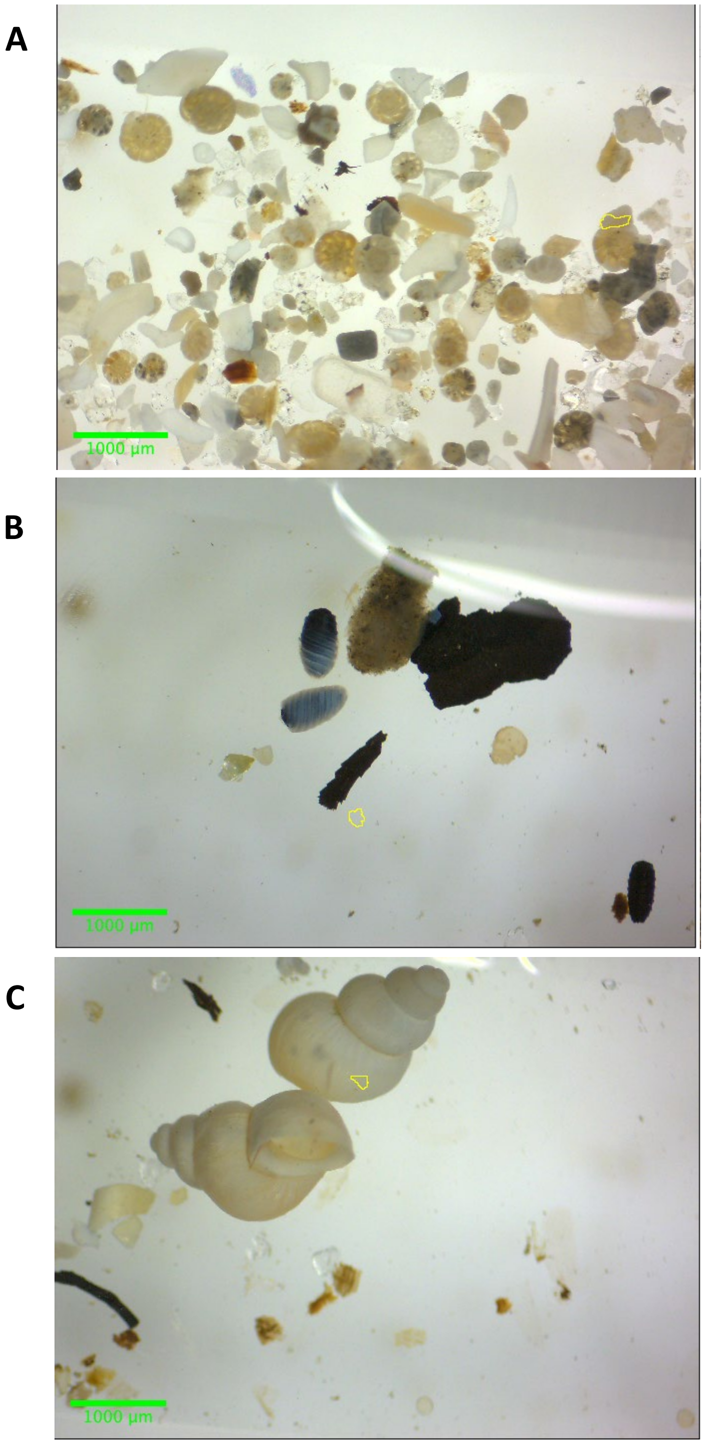

Figure S7. *Microscope images showing charaphyte oospores, foramnifera and molluscs in the sediments of Lashmars Lagoon.*

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ii. permission is granted for the candidate in include the publication in the thesis; and

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7,000 years of fire and vegetation change inferred from the sediments of Lashmars Lagoon, Kangaroo Island (Karti), Australia

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Abstract

Fire regimes in Australia are shifting towards dangerous extremes, threatening human lives, property, and biodiversity. However, projections of future fire trajectories and impacts across southern Australia are unresolved. This is in part due to the scarcity of palaeofire records that reveal long-term causes and consequences of fire in a dynamic Earth system where climate, vegetation, landscape processes and humans all interact. Here we present a ~7,000-year reconstruction of fire and vegetation change inferred from charcoal and pollen from the sediments of Lashmars Lagoon, Kangaroo Island (Karti), southern Australia. We compare our results to climate and catchment reconstructions based on core scanning X-ray fluorescence, X-ray diffraction and to microcharcoal analyses from previous studies on the same site. Our findings are discussed in the context of the island's archaeology, and regional reconstructions of fire, climate, and vegetation. Our new record documents an abrupt shift from low to high macrocharcoal accumulation rates at ~3.3 ka, indicating a change in the fire regime from low- to high-biomass burning and or fire frequency that occurred more than 1 ka earlier than previously estimated at the same site. Macrocharcoal accumulation rate increased in magnitude towards the present, reaching a maximum at ~800 cal. years BP. This suggests a link between fire and climate drying due to the coincident intensification of the El Niño Southern Oscillation and southern migration of the Southern Westerly Winds. We also observe a general decrease in the length to width ratio of macrocharcoal particles towards present, suggesting a change from fine herbaceous fuels to more woody sources. Two major changes are evident in the pollen record; the first – at ~5.5 ka – is characterised by a switch from Casuarinaceae- to *Eucalyptus*-dominated open woodland and is consistent with regional vegetation responses to late Holocene drying. The second change in vegetation – after ~3.3 ka – reflects an increase in shrub taxa, possibly indicating a transition to a denser and more diverse shrubby mid-story, and consistent with the transition from finer to more woody fuel types inferred from the macrocharcoal length to width ratios. This change coincides with the abrupt increase in charcoal accumulation rates and occurred at a time when Aboriginal people were believed to no longer be living permanently on the island. We conclude that the combined and interconnected impacts of human management and climate changes drove major shifts in vegetation and fire on Kangaroo Island (Karti) in the Holocene. Our data suggest that Kangaroo Island (Karti), having experienced a transition to either more frequent or larger bushfires at ~3.3 ka, could be an important analogue for current widespread changes in fire regimes across southern Australia. Furthermore, the complex interplay between humans, climate, fire, and ecosystems revealed from our record offers insights relevant to regional bushfire mitigation and recovery programs today. Our study suggests that the characteristically dense native vegetation of Kangaroo Island (Karti) is likely to burn in large and or frequent fires in dry climates, such as those projected for Kangaroo Island (Karti) in the coming decades. This study thus underlines the importance of careful and considered management in these landscapes to mitigate the risk of catastrophic bushfires looming on the horizon.

Key words

Charcoal; pollen; charcoal length to width ratio; X-ray fluorescence core scanning; X-ray diffraction; Aboriginal fire management

Introduction

Fire has long been an important part of Australian landscapes (Pyne, 1991, Mooney et al., 2011, Bowman, 1998). However, fire regimes in southern Australia have undergone unprecedented changes in recent decades (Mariani et al., 2022, Bowman et al., 2020). The Black Summer fires in 2019-2020, devastated nearly two times the area burned by bushfires in any fire season since 1851 (Morgan et al., 2020, Davey and Sarre, 2020) and devastated human lives, homes and biodiversity (Dickman, 2021, Davey and Sarre, 2020). On Kangaroo Island (Karti; hereby abbreviated to KI), two people lost their lives as about half of Australia's third largest island ignited (Taylor, 2020, Bonney et al., 2020).

Across southern Australia, fire regimes are shifting because of combined effects of anthropogenic climate change and changes in land use following British colonisation. Since the beginning of instrumental records in Australia, regional average annual rainfall has decreased, while temperatures have increased (CSIRO and Bureau of Meteorology, 2020). Into the future, these trends are projected to accelerate to produce longer fire seasons and more days of extreme fire weather (Dey et al., 2019). The 2019-2020 Black Summer fires in southeast Australia followed the hottest and driest year on record, associated with recorded positive modes of the El Niño Southern Oscillation (ENSO) and Indian Ocean Dipole (IOD) and a northwards migration of the Southern Westerly Winds, as indicated by a negative phase of the Southern Annular Mode (SAM) (Abram et al., 2021). The loss of Aboriginal fire management across much of the region, a practice that was widespread prior to European colonisation (Gammage, 2013), has also led to shrub encroachment of forests, further promoting catastrophic bushfires (Gammage, 2013, Mariani et al., 2022).

Long-term palaeofire records, inferred from charcoal deposited in lake sediments, offer insight into the long-term variability of fire regimes (Whitlock and Larsen, 2002), helping to contextualise present day fire regimes and better predict the future. In Australia, several decades of palaeoenvironmental research has focused on untangling the complex interplay between climate, people, ecosystems and fire (Singh et al., 1981, Kershaw et al., 2002, Mooney et al., 2011, Bowman et al., 2012, Mariani et al., 2017, Fletcher et al., 2020, Adeleye et al., 2021). However, the relative contribution and response of each variable to fire is highly debated. Further, very few Holocene palaeofire records exist in South Australia (see the Global Palaeofire Database, Power et al., 2010), adding to uncertainty in the study region.

KI fills a regional gap in Holocene palaeoenvironmental records for southern Australia and is ideally placed to resolve debates relating to the importance of anthropogenic and climatic long-term drivers and ecological consequences of fire. The island has several lakes that store lacustrine palaeoenvironmental archives, one of which, Lashmars Lagoon (Figure 1), has a record known to stretch back through most of the Holocene (Clark, 1983a). KI is distinct from the mainland owing to a unique history of human habitation, similar to islands in the Bass Strait that have been studied for similar reasons (Ladd et al., 1992, Adeleye et al., 2021). Archaeological evidence implies that Aboriginal people had ceased permanent occupation of KI thousands of years before British invasion (Walshe, 2005, McDowell et al., 2015, Lampert, 1981). Thus, the island serves as a natural laboratory to investigate the impact of the loss of Aboriginal management on ecosystems and fire regimes over the long term.

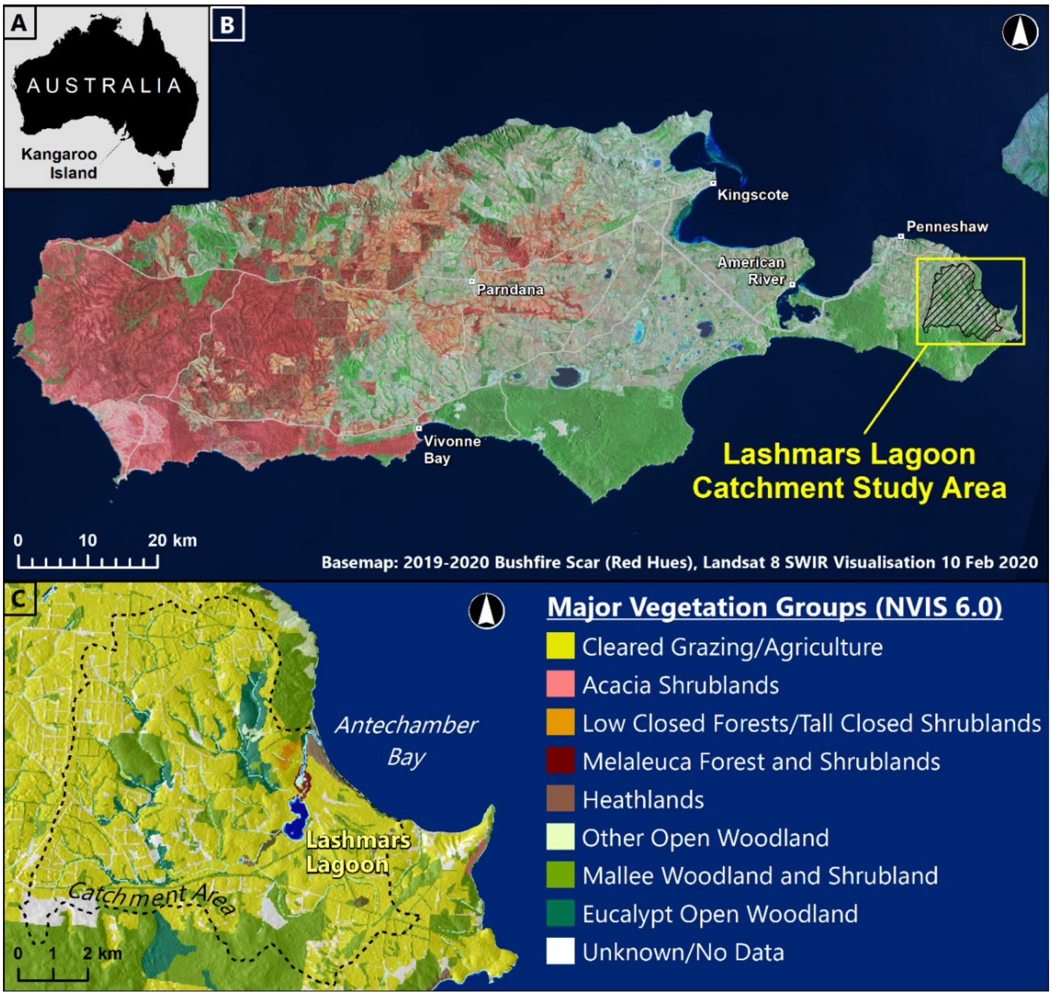

Figure 1. *Map of the study site. (A) Kangaroo Island (Karti) in relation to the Australian mainland. (B) The location of the study area, Lashmars Lagoon, in relation to Kangaroo Island (Karti). The base map is the 2019-2020 Bushfire Scar (Red Hues) Landsat 8 SWIR Visualisation, February 10, 2020. (C) The Major Vegetation Groups (NVIS 6.0) presently surrounding Lashmars Lagoon.*

South Australia is also an interesting region to investigate the long-term variability and dynamics of two important non-seasonal Australian climate influences on temperature and rainfall – the SAM and ENSO – and assess how these might affect vegetation and fire regimes in both managed and unmanaged landscapes. KI sits at the easternmost margin of ENSO influence in Australia and in a region where the effect of SAM varies markedly between summer and winter (Bureau of Meteorology, 2022). In other parts of southern Australia, a positive SAM results in a year-round wetter climate, however in the region in which KI is located this specifically means wetter climate in winter but a drier climate in summer (Figure S1). The sediments from Lashmars Lagoon therefore offer the opportunity to interrogate the effects of this increased seasonal divergence on fire activity in the region. A positive SAM, for example, may conceivably supress catastrophic fire risk in regions where year-round wetter climates prevail. However, how does a positive SAM effect fire in places like KI where growth-promoting wet winters are followed by drier summers?

Previous studies from Lashmars Lagoon, KI, pioneered the use of charcoal and pollen in lake sediments as proxies for fire and vegetation in Australia (Clark, 1983a, Clark, 1983b, Singh et al., 1981, Clark, 1976). The original microscopic charcoal record from Lashmars Lagoon showed a conspicuous change from low charcoal levels to high levels, dated to have occurred at ~2.5 ka (Clark, 1983a), albeit with significant uncertainty due to low sampling resolution and limited chronological constraint. This change was inferred to reflect the loss of 'firestick farming' by Aboriginal people in the form of small-scale, low-intensity mosaic burning. Clark (1983a) argues that this loss was followed by an abrupt transition to uncontrolled, catastrophic bushfires, thus providing a timestamp for human departure from the island. However, in intervening years, this interpretation has been challenged. For example, a synthesis of late Quaternary charcoal records from Australasia found no correlation between biomass burning and human activity (recorded in the archaeological record) (Mooney et al., 2011). More directly, the charcoal record from Lashmars Lagoon itself has been questioned. As yet, there is no unambiguous archaeological evidence that indicates cultural burning land management practices were employed by ancient Islanders, and it has been suggested that the increased charcoal counts reflect accumulation or depositional bias, given the change in charcoal counts occurs in proximity to a change in sediment composition (Illman, 1998). Illman (1998) argues that the inferred change in fire regime at ~2.5 ka may have been coincident with a change to reducing lake conditions which are conducive to the formation of pyrite, a dark mineral putatively confused for microscopic charcoal under microscope identification. This theory was countered by the sedimentological analysis in this thesis (Chapter 3), which found no correlation between pyrite and charcoal concentration. The original study at Lashmars Lagoon has also been criticised because the accompanying pollen record showed no evidence of vegetation change (Gammage, 2013, Illman, 1998), a finding at odds with an extensive body of literature that has established a tight coupling between vegetation change and fire (Lynch et al., 2007).

Nearly forty years have passed since the original study at this site, and it is worth considering some major developments in charcoal processing procedures over the past decades. It is now standard practice to partition charcoal fragments into different size fractions, reflecting the higher likelihood that smaller particles are transported to the site of sedimentation from further away (Whitlock and Larsen, 2002). Thus, microscopic charcoal (microcharcoal; typically < 100-125 µm), like the charcoal counted in the study by Clark (1983b), is inferred to reflect more regional fires, while macroscopic charcoal (macrocharcoal; typically > 100-125 µm) is used to reconstruct more local fire history. More recently, traditional methods are being increasingly challenged and reinvented, with significant ramifications for palaeofire literature (Tsakiridou et al., 2020, Vachula et al., 2022, Constantine IV and Mooney, 2021). The use of sodium hypochlorite or ‘bleach’, normally part of a standard charcoal sample pre-treatment, has been shown to preferentially destroy charcoal particles produced in fires less than 400 °C (Constantine IV and Mooney, 2021). Technological advancement in image processing, microscopy and machine learning has automated aspects of charcoal quantification, increasing processing efficiency and the statistical power of analyses (Snitker, 2020, Rehn et al., 2019). These new methods also allow measurement of the shape and size of charcoal particles, and thus the ability to make inferences about past fire regimes beyond the simple metric of charcoal concentration based on particle counts developed by Clark (1982) and applied in Clark (1983a). Although measuring charcoal concentration is still a popular method today, contemporary charcoal analysis extends to inferring fuel type through fragment morphometry (Vachula et al., 2021) and reconstructing past fire intensity though the use of Fourier-transform infrared (FTIR) spectroscopy to reconstruct past fire intensity (Constantine IV et al., 2021). Furthermore, open access databases, like the Global Palaeofire Database (GPD), facilitate comparisons and syntheses of multiple palaeofire reconstructions (Power et al., 2010). With this in mind, a revision of the original Lashmars Lagoon charcoal record is overdue, particularly given the importance of the site and region for understanding fire regimes across southern Australia.

Here, we aim to reconstruct the past 7,000 years of fire and vegetation change using fossil charcoal and pollen from the sediments of Lashmars Lagoon, Kangaroo Island (Karti), southern Australia. We couple our study with a detailed interrogation of sediment composition to resolve past site-specific uncertainties related to the production of pyrite. Furthermore, we consider our findings within the broader context of climatic and landscape processes and assess the implications for debates around the relative contributions of people, climate, and ecosystems to shaping the long-term variability of fire regimes in southern Australia.

Site information

A detailed site description for Lashmars Lagoon, is given in Chapter 2 of this thesis. Description of core collection and dating is given in Chapter 3.

Materials and methods

Macrocharcoal analysis

We subsampled the Bolivia cores using 'double-L (LL) section' longitudinal sampling using 1 x 1 x 100 cm aluminium angle, following Nakagawa et al. (2021). The LL sections were subsequently sampled at 1 cm intervals to obtain volumetric 1 cm^3 samples using a centi-slicer (Nakagawa et al., 2021). Macrocharcoal analysis was performed on 51 of these subsamples taken at 5 cm intervals for the first metre and then on average every 25 cm along the composite profile.

We applied a variation of standard macrocharcoal analyses (Tsakiridou et al., 2020) which was designed to reflect recent advances in charcoal processing methods (Vachula et al., 2021, Snitker, 2020, Constantine IV and Mooney, 2021) and address potential shortcomings of an earlier charcoal study at this site (Clark, 1983b, Illman, 1998). Specifically, it was important to incorporate the following into our method: 1) a live stream microscope set up to quantify charcoal fragments, allowing for real-time detailed inspection of the sample and the use of image capture to document and externally review the charcoal identification process, 2) the collection of charcoal fragment area and morphometric data, which can provide further information about fire regimes (e.g. inferring fuel type using fragment length to width ratios) and 3) the avoidance of bleaching during sample pre-treatment so as to preserve charcoal produced in cooler burns of < 400°C (Constantine IV and Mooney, 2021), often associated with Aboriginal fire management or cooler winter burns.

To deflocculate the sediment, samples were freeze-dried in 50 mL centrifuge tubes and then pressed with a metal spatula to gently break apart the sediment. The samples were then left in 10 mL 5% sodium hexametaphosphate for five days, with sample tubes gently inverted three times daily. Next, the samples were sieved using a 125-µm stainless steel mesh. The larger fraction (> 125 µm) was kept for macrocharcoal analysis, and the smaller fraction (< 125 µm) was kept for pollen and microcharcoal analysis. Initially we attempted to further partition the samples to include a third, > 250 µm, fraction, but this yielded very little material, so this size fraction was amalgamated into the > 125 µm portion. Finally, samples were transferred wet into Bogorov chambers (purchased from Dr. Thomas Bishop, University of Manchester) for image analysis. Image analysis was completed using a Zeiss Stemi 508 microscope connected to an Axiocam ERc 5s camera that was run through the imaging software imageJ. The plugin, CharTool (Snitker, 2020), was used to collect a suite of morphometric parameters including our variables of interest (total count, area, fragment length, length to width ratio) for each individual charcoal particle identified under the live microscope. The line of the Bogorov chamber was followed systematically from top to bottom with the live microscope field of view and a needle was used to manipulate the sample to separate charcoal from other sedimentary detritus where necessary. Representative still images were saved from each sample to document and review the process.

Microcharcoal and pollen analysis

The < 125 µm sediment fraction was processed for pollen, spore and microscopic charcoal using a modified version of Faegri and Iverson (1989). Initial treatment with 10% potassium hydroxide was followed by a rinse through a 100 µm sieve. 1-2 lycopodium spores were added to each sample and dissolved in 10% hydrochloric acid. Silicates were separated using sodium polytungstate with a specific density of 1.9 – 2.0 g/cm. Float material was then subject to acetolysis treatment, being heated in a 9:1 mixture of acetic anhydride and sulfuric acid, before samples were mounted in glycerol and sealed with nail varnish. Identification and counting of pollen, microscopic charcoal and spores was undertaken on a light microscope at 400X magnification.

Mineralogical interrogation of peaks in sulphur (S) intensity

Given preliminary inspection of the macrocharcoal data revealed that some peaks in macrocharcoal coincide with peaks in S intensity (> 1000 counts per second (cps)) from the core scanning X-ray fluorescence (XRF) data, we judged it necessary to assess the mineralogy of these sulphur peaks in greater detail to determine the phase in which S was contained in these layers using the X-ray diffraction (XRD) dataset presented in Chapter 3. In addition to this, we analysed an additional four samples using XRD, from four other sulphur peaks, to augment this comparison.

Since we identified the precipitate gypsum as one such sulphur-hosting mineral in these samples, we sought to verify that the detected gypsum was in-situ and had not precipitated from saline sediment pore water when the sample was dried during XRD preparation. Additional undisturbed sediment material was recovered from wet cores to identify possible grains resembling the crystal structure of gypsum. This analysis was completed using material from the same depth horizons of two samples where gypsum and high S concentrations were detected (samples S6 and S7; Table 1). We then identified the mineral phase of six individual grains under a 25-µm beam using a Bruker Tornado M4 µ-XRF instrument. Operating conditions were 50kV/600uA at 20 mbar pressure, and spectra were acquired for 30 seconds.

Data pipelines and statistical analysis

The summarised macrocharcoal metrics were compiled and plotted using the R-code pipeline developed by Snitker (2020) and available from https://github.com/gsnitker/CharTool. Charcoal concentrations (counts and area) were combined with the sediment accumulation rate and converted to charcoal accumulation rate (CHAR) (no. cm^2 yr^{-1} and mm^2 cm^2 yr^{-1}). A Pearson's correlation test was performed in R using the package *vegan* to test the relationship between charcoal counts and area.

Pollen and microcharcoal analyses were undertaken by Dr. Haidee Cadd, University of Wollongong. A total of 300 pollen grains of terrestrial origin were identified from each sample depth and form the base pollen sum. Percentages of aquatic taxa are based on a sum including both aquatic and terrestrial pollen taxa. A combined sum of all taxa forms the basis for percentages of algal species. Concentration was calculated for all pollen taxa using counts of exotic marker grains of lycopodium, sediment subsample volume and pollen counts. Zonation of the terrestrial pollen concentrations was conducted using a COnstrained Incremental Sum of Squares (CONISS) cluster analysis in the *rioja* package in R. Detrended Correspondence Analysis (DCA) was conducted on the terrestrial pollen spectra to summarise the main patterns of change also using the *rioja* package in R.

Supplementary analyses

Sediment stratigraphy, geochemistry, chronology, and mineralogy are detailed in Chapter 3.

Results

Charcoal record

Macrocharcoal counts and area CHAR were strongly and significantly positively correlated ($R = 0.96$, p-value $< 2.2e-16$; Figure S2). Macrocharcoal counts are presented in Figure 2C. We note that 20 of the macrocharcoal samples from the top metre of the core were affected by a calibration scaling error which affected the morphometric measurements of area and fragment length but not the size-independent measures of counts or length to width ratios. The affected samples' area and fragment length measurements were therefore discounted from further analysis. A full list of the affected samples is provided in the supplementary information (Note S1).

There is a major change in macroscopic CHAR at ~3.3 ka from consistently low concentrations with a mean of 2.2 counts cm^2 yr^{-1} to a regime with large peaks generally > 10 counts cm^2 yr^{-1} superimposed on a modestly elevated background of around 5.5 counts cm^2 yr^{-1}. We observed six distinct peaks against the background macrocharcoal levels at 3.29 ka, 2.33 ka, 1.88 ka, 1.23 ka, 780 cal. years BP and 1980 CE. These peaks generally increase in magnitude through time, except for the most recent peak at 1980 CE. Mean macrocharcoal fragment length (Figure 2D) is relatively constant through time but is skewed towards greater lengths within the charcoal peaks. Macrocharcoal length to width ratios are generally high at 3.2 on average from ~6.8 ka to 3.3 ka and then relatively low with an average of 2.5 for the remainder of the record. This trend is consistent across the record aside from two recent peaks, at 1840-1850 and 1910 CE respectively, which have length to width ratios of 3.5 and 3.3 respectively (Figure 2E).

A similar abrupt transition from low to high charcoal is observed in our microcharcoal record (Figure 2B). However, the change in our microcharcoal record at ~2.5 ka occurs significantly later than the change recorded in the macrocharcoal at ~3.3 ka. We note, nonetheless, a small microcharcoal peak in our microcharcoal record that corresponds to the first macrocharcoal peak at ~3.3 ka. An outlier, 11.5 times the inter-quartile range, in our microcharcoal record at ~4.2 ka was discounted as this sample had unexpectedly low counts of the lycopodium pollen standard used to normalise the charcoal concentrations, probably suggesting uncertainties in pollen recovery for this sample (red dot in Figure 2B).

Peaks in S intensity from the XRF data were coincident with peaks in macrocharcoal at 2.33 ka, 1.23 ka and 780 cal. years BP (S6-7, S3 and S2, Table 1; Figure 2F). Whilst pyrite was present in some samples where S peaked, gypsum was the main hosting phase of S in the peaks that coincided with high charcoal counts. Micro-beam XRF microscopy of putative gypsum grains, sampled directly from the original wet core material, confirmed the gypsum was part of the original sediment composition, not the result of precipitation from saline sediment pore water during desiccation in XRD sample preparation (Figure S3).

Table 1. *Characterisation of the sulphur peaks. Colours represent samples mineral concentrations (green = highest concentrations to red = lowest concentrations).*

Sample	**S peak ranking (largest to smallest)**	**S (cps)**	**Gypsum (%) ($CaSO_4 \cdot 2H_2O$)**	**Pyrite (%) (FeS_2)**
S7 (see Fig 2E)	1	3774.81	4	0
S6 (see Fig 2E)	2	3442.952	5	3
S3 (see Fig 2E)	3	2447.742	3	0
S4	4	1744.194	0	1
S2 (see Fig 2E)	5	1618.774	5	2
S9	6	1486.976	0	2
S5	7	1272.171	4	1
S1 (see Fig 2E)	8	1202.381	12	16
S8	9	1069.927	0	0

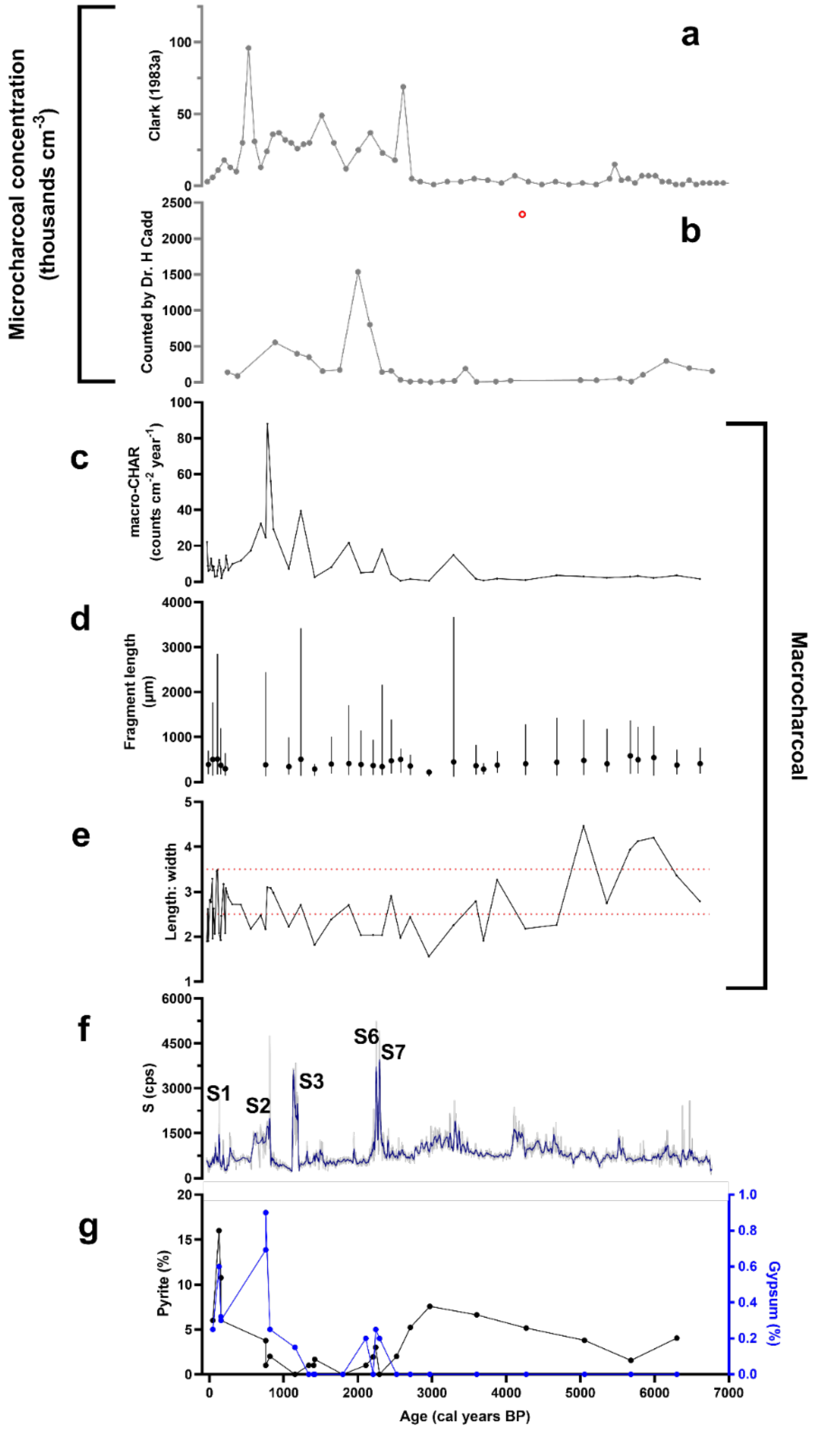

Figure 2. *Charcoal records from Lashmars Lagoon, Kangaroo Island (Karti) and associated datasets. (A-B) Microcharcoal records: (A) 1983 microcharcoal record from the site (Clark, 1983a), data sourced from the GPD (Power et al., 2010) and (B) microcharcoal counted from pollen slides by Dr. Haidee Cadd, University of Wollongong (red dot = discounted outlier). (C-E) Macrocharcoal metrics: (C) Macro-CHAR, (D) fragment length (dots = average, lines extend from minimum to maximum value) and (E) fragment length to width ratio. (F) Sulphur (S) intensities (Chapter 3). (G) Pyrite (black) and gypsum (blue) concentrations (Chapter 3).*

Pollen inferred vegetation

Identification of spore and pollen grains was predominantly restricted to family or genus level using a combination of online, print and reference material, including the Australasian Pollen and Spore Atlas (Figure 3). Pollen preservation was good throughout the record; however, terrestrial pollen concentration was higher in the top of the core from 300 – 0 cm. One sample was excluded from the concentration calculations due to anomalously low lycopodium counts (13 total lycopodium spores counted, compared to a mean 413).

The terrestrial pollen spectrum was dominated by arboreal taxa from Casuarinaceae (7 - 62%) and *Eucalyptus* (7 - 36%), with low, but consistent inputs from Leptospermum (average of 12%) and Myrtaceae (average of 11%). Mid and understory taxa are dominated by Asteraceae (4 - 19%) and Chenopodiaceae (2 - 29%). Poaceae values are less than 6 % throughout the entire record but are highest at the bottom (~6.8 - 5.8 ka) and middle (~4.1 - 2.7 ka) of the core.

Aquatic pollen and spore spectra were dominated by *Botryococcus* (0 - 72%) and *Myriophyllum* (0 – 51%). There are two phases with increased abundance of aquatic taxa in the record: from ~5.8 - 5.5 ka and ~4.2 - 2.6 ka. The aquatic taxa show a possible successional pattern through the latter period, initially starting with the appearance of emergent reed taxa from *Cyperaceae* and *Juncaceae* represented alongside aquatic macrophytes and green algae before a transition at ~3.3 ka to particularly strong representation from *Myriophyllum*, a submerged aquatic macrophyte, and *Botryococcus*, a colonial green alga.

The DCA of terrestrial pollen spectra revealed the major compositional changes in the terrestrial pollen record. The biggest change occurred at ~5.5 ka characterised by a decline in Casuarinaceae along with Chenopodiaceae and Poaceae, and an increase in *Eucalyptus* and *Melaleuca*. The next biggest change occurred at ~3.3 ka with increase in the representation of shrubby mid-story taxa like Asteraceae, Chenopodiaceae, Myrtaceae and *Amperea*.

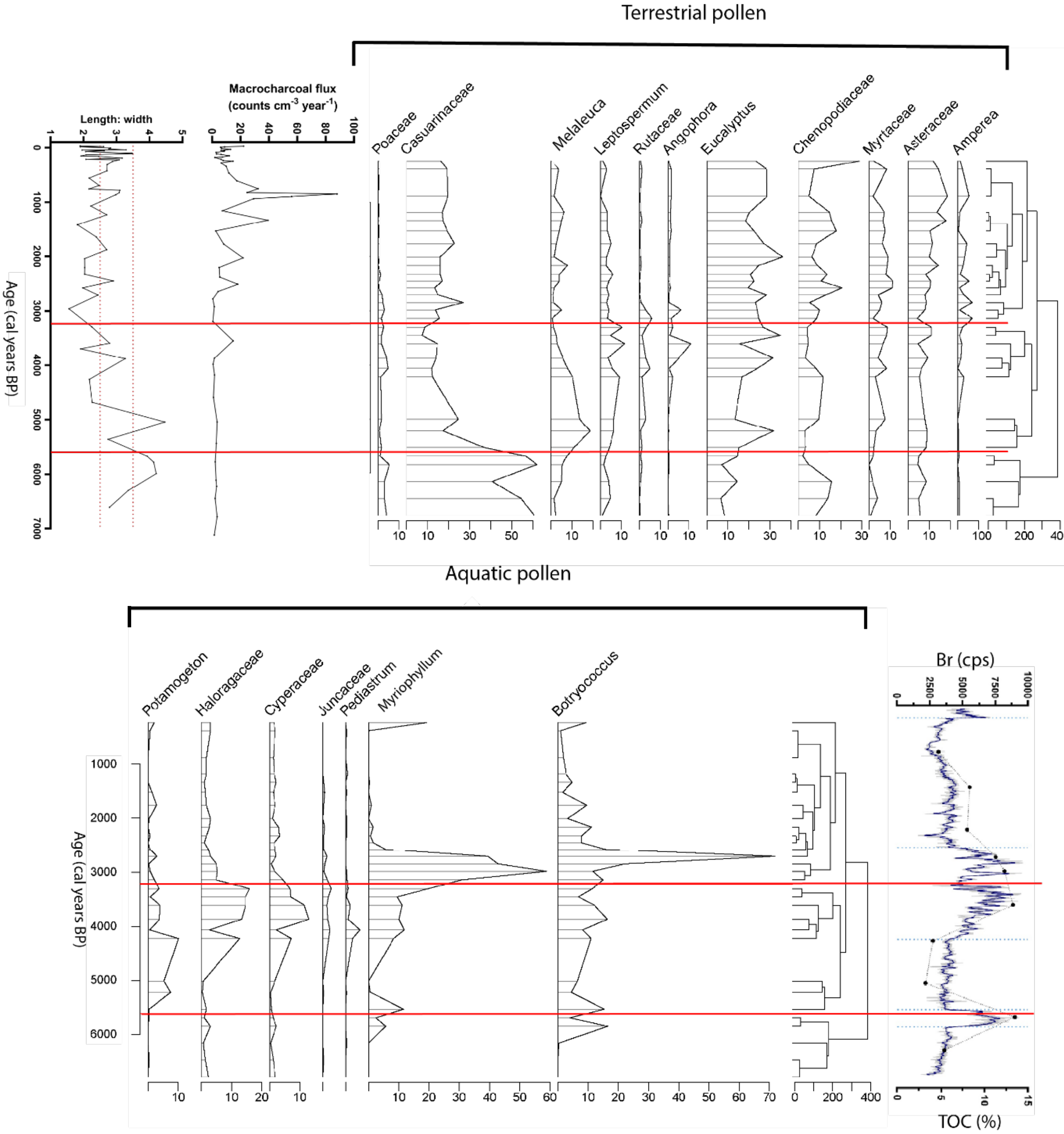

Figure 3. *Terrestrial and aquatic pollen diagrams associated analyses from Lashmars Lagoon, Kangaroo Island (Karti). Percent terrestrial pollen with DCA (top right). (B) Macrocharcoal length to width ratio and macro-CHAR (top left). Percent aquatic pollen with DCA (bottom left). Bottom right: Br (blue solid line) and TOC percent (dotted black line with points) (Chapter 3). Red lines designate the two major splits in the pollen record inferred from the DCA.*

Discussion

Addressing previous uncertainties around the pollen and charcoal records at Lashmars Lagoon: preservation bias and misidentification

Our study resolves long-standing uncertainties concerning the pollen and charcoal record at Lashmars Lagoon and confirms its interpretation as a reliable record of past vegetation and fire. The pollen and micro- and macrocharcoal record indicate distinct past vegetation and fire change (Figure 2 and 3), building on work from Chapter 3 by paying special attention to depositional processes that may have influenced the results (Table 1).

Our macrocharcoal data, for which we (a) took care to avoid lab-induced biases, and (b) identified and characterised the morphometry of individual charcoal pieces under a live microscope during counting, exhibit an increase in CHAR at ~3.3 ka (Figure 2C). Different sized charcoal particles are interpreted to record fire history at different spatial scales; larger macrocharcoal particles are inferred to reflect local fires and smaller microcharcoal is thought to reflect a more regional fire signal (Whitlock and Larsen, 2002). The large macrocharcoal peak recorded at ~3.3 ka corresponds to only a small microcharcoal peak (Figure 2B), preceding microcharcoal peaks that are clearly distinguishable from background charcoal levels by ~1 ka years. The timing of the first microcharcoal peak at ~2.2 ka, which occurs *after* the first peak in the macrocharcoal is consistent with the timing of the first peak in Clark's microcharcoal record (Clark, 1983a; originally reported as ~2.5 ka and revised more recently in the GPD (Power et al., 2010) to ~2.6 ka).

The discrepancy between the micro- and macrocharcoal records at ~3.3 ka suggests that this event could have been a highly localised fire that would have produced more macrocharcoal than microcharcoal. Since charcoal produced in cooler burns (< 400°C) may have been destroyed in the comparatively harsh treatment to which pollen samples, the source of microscopic charcoal counts, are subject (Constantine IV and Mooney, 2021), another possibility is that this event was the result of a cooler burn. Such a burn may have occurred in the wetter winter season or as the result of deliberate Aboriginal fire management. The peak in the charcoal records at 3.3 ka, nevertheless, marks the onset of changing fire conditions around Lashmars Lagoon from a period of low micro- and macrocharcoal to a period where charcoal peaks are clearly distinguishable from background levels. Since the sampling resolution of our record (~ every 200-300 years) is relatively low, we cannot confidently distinguish between the metrics of fire frequency and biomass burned. The increase in charcoal observed at Lashmars Lagoon after ~3.3 ka could therefore represent either increasing biomass burning or fire frequency or a combination of both.

Statistical analysis of our pollen data identified a shift in terrestrial vegetation composition at ~3.3 ka, concurrent with our macrocharcoal record, suggesting a link between fire and vegetation (Figure 3). Further, we used the length to width ratio of charcoal particles to infer changes in fire fuel types, which can imply changes in the availability of vegetation types that will burn in fires (Figure 2e). Typically, charcoal with a length to width ratio < 2.5 is considered of woody origins, while values > 3.5 are associated with more herbaceous taxa (Vachula et al., 2021). In general, we found that length to width ratios were higher (mean 3.2) when charcoal was lower from ~ 6.8 – 3.3 ka, and lower (mean 2.5) from 3.3 ka to present. This suggests that the fire regime shifted from finer fuels, putatively linked to herbaceous fuels, to more woody fuels, potentially linked to the burning of trees and shrubs – providing further evidence for a link between vegetation and fire at Lashmars Lagoon. However, these conclusions remain tentative until this proxy is calibrated with Australian vegetation types. Clark (1983a), who compared microcharcoal data to a pollen record, did not find a link between fire and vegetation, a theoretical inconsistency that cast doubt on the legitimacy of the record as a whole (Illman, 1998, Gammage, 2013). In light of our new macro- and microcharcoal records, it seems the initial pollen and charcoal study of Lashmars Lagoon failed to detect a fire-vegetation relationship because only microcharcoal was considered. The lack of correspondence between the vegetation and fire records in Clark (1983a) may reflect a spatial mismatch between the microcharcoal and pollen records or a degradation of charcoal induced in the harsh processing treatment of micro- compared to macrocharcoal. Additionally, the coincident changes in vegetation composition and fire regime at ~3.3 ka that we observe in our new pollen record from Lashmars Lagoon was the result of subtle compositional shifts revealed by statistical analysis, techniques which were not applied forty years ago by Clark (1983a).

Interestingly, three macrocharcoal peaks (at 2.33 ka, 1.23 ka and 780 cal. years BP) corresponded with peaks in S intensity as inferred from XRF core scanning reported in Chapter 3 (Figure 2F). As S is a key component of pyrite (FeS_2), implicated by Illman (1998) as potentially being misidentified for microcharcoal, XRD analyses were completed on S peaks to ensure pyrite was not responsible for inflating charcoal counts at these depths. Importantly, we found that pyrite does not consistently explain high S values associated with these high charcoal counts. Instead, gypsum ($CaSO_4 \cdot 2H_2O$), which is not readily misidentified for charcoal, was the most important S-bearing mineral detected at these depths. Consequently, we conclude that it is unlikely that Clark (1983a) misidentified pyrite as charcoal.

In summary, the pollen and charcoal records, coupled with our detailed investigation of the depositional processes at Lashmars Lagoon (further explored in Chapter 3), largely corroborate the findings from the first palaeoenvironmental study of Lashmars Lagoon (Clark, 1983a) and highlights the importance of considering both macro- and microcharcoal. We conclude that our new, multi-metric macrocharcoal record is more reflective of local fire changes and unaffected by the potentially destructive effect of bleach pre-treatment.

Fire and vegetation over the past ~7,000 years at Lashmars Lagoon, in the context of local climate, vegetation and human activity

Based on the vegetation (pollen) and fire (macrocharcoal) records inferred from this study, we define four separate palaeoenvironmental phases at Lashmars Lagoon over the past ~7,000 years.

Phase 1 (~6.8 ka – 4.2 ka) | Open Casuarinaceae- then *Eucalyptus*- dominated woodland with low biomass and or less frequent grassy fires | Drier climate with brief interluding wet phase and archaeological evidence of human habitation on KI

The local fire record inferred from macrocharcoal during this phase suggests a period of low fire occurrence (low macro-CHAR), with mostly fine, elongated fuel types (high length to width ratio) (Figure 2B and 2C). During this period, our pollen data record a statistically significant change from open Casuarinaceae woodland with a grassy understory of Poaceae and Chenopodiaceae to an open *Eucalyptus* woodland at ~5.5 ka (Figure 3A). This change in vegetation was also observed at ~4.8 ka in a pollen record from the same site (Clark, 1983a). The timing mismatch between the two records likely reflects inconsistencies between the two age models combined with uncertainties associated with sampling resolution. A similar transition from Casuarinaceae to *Eucalyptus* dominated woodland is also observed sometime between ~5.2 - 4.5 ka at the nearby Boat Harbour Swamp, Fleurieu Peninsula, on the adjacent mainland (Bickford and Gell, 2005). Again, the discrepancy in age estimate between these two sites that are only 25 km apart is assumed to reflect chronological uncertainty and coarse temporal resolution of sampling. Nevertheless, the rough similarity in the timing of the switch from Casuarinaceae to *Eucalyptus* between these two records hints at a regional response to climate change. Indeed, at Lashmars Lagoon the change occurs just following an abrupt climate change recorded in the lake sediment geochemistry from inferred wet to dry conditions (Chapter 3), suggesting that *Eucalyptus* was more equipped than Casuarinaceae to prosper under drier climates. Alternatively, this change might also reflect an enhanced ability of Casuarinaceae to tolerate water saturated soils.

At Lashmars Lagoon, relatively wet versus dry climates are indicated by the Al/K ratio, discussed in detail in Chapter 3. The proxy is based on the relative concentrations of kaolinite and illite, two clay minerals that are present in different ratios depending on the intensity of chemical to physical weathering, a process that is controlled in large part by climate. When Al/K ratio is high, so too is chemical weathering intensity. At Lashmars Lagoon, the Al/K ratio correlates strongly with Br, a proxy for organic matter that indicates fresh lake conditions and enhanced vegetation growth. Taken together, the high Al/K and Br values indicate overall wetter periods (Figure 4). Apart from the brief wetter conditions between ~5.9 - 5.6 ka, we inferred relatively dry conditions for the most part of this period that is characterised by low macrocharcoal (Chapter 3).

Archaeological evidence for human activity implies that people were living on the island for the most part of this period. The KI archaeological record extends from as far back as 16,110 ± 100 BP at Seton Rockshelter to a date of 4310 ± 90 BP ka at Sand Quarry (Lampert, 1981). Thus, a potential explanation for the low biomass burning and or fire frequency and relatively open vegetation in this period might feasibly be the use of Aboriginal burning practices to manage the woodland under-story and mid-story.

Phase 2 (~4.2 - 2.5 ka) | Closing *Eucalyptus* woodland with fire regime transitioning to higher-biomass and or more frequent woody fires | Wetter overall climate and no undisputed archaeological evidence of people

At Lashmars Lagoon, we observe a change in vegetation and fire at ~3.3 ka (Figure 3). The pollen record shows a transition to a denser, more diverse shrubby midstory and corresponds to a charcoal peak (14 counts cm^{-2} year^{-1}) indicating a large fire event, or events, dominated by woody fuel (low length to width ratio). Aside from this large peak, charcoal concentrations are low throughout the phase. Evidence from Al/K ratios (Figure 4B) and organic-rich sediment layers (high Br), which were interpreted to reflect an increase in chemical weathering and high lake levels (Chapter 3), suggests that these fire and vegetation conditions occurred in a relatively wet phase. The pollen record for this period is consistent with this inference, displaying a larger relative amount of aquatic pollen, such as *Myriophyllum*, suggesting deeper and less-saline lake conditions. The possible successional pattern in aquatic pollen from reeds to submerged aquatic macrophytes can also be interpreted to reflect a transition to a deeper or wider lake during this time. However, nestled within the overall wetter climate conditions of this phase is a brief return to dry conditions for a few hundred years from ~3.3 - 3.0 ka.

Regionally drier conditions at this time are also recorded in two calcrete desiccation horizons dated to 3.3 ka and 3.0 ka from lakes in the Coorong region on the adjacent mainland, according well with evidence from Lashmars Lagoon (Ahmad, 1996). The macro-CHAR peak at 3.3 ka at the commencement of the short dry phase possibly indicates that while fire activity may have been supressed by immediately preceding wet conditions, these wetter conditions presumably also favoured vegetation growth and may have primed the landscape with fuel loads highly susceptible to promote fire following the abrupt change to drier climate.

Clark (1983a) suggests that commencement of the change in fire regime, which she attributes to a loss of Aboriginal stewardship, timestamps the latest possible date of human habitation on KI to ~2.5 ka. By this same logic, our new macrocharcoal data revises this date to ~3.3 ka. Since it seems unlikely that there would have been insurmountable environmental pressures on human islander populations during relatively wet climates and fresh lake conditions (~4.2 - 3.3 ka), we tentatively further infer that people were already no longer permanently living on the island prior to ~4.2 ka. This inference is concordant with the last reliable evidence of human occupation on KI just before the start of this period around ~4.3 ka (Lampert, 1981, Walshe, 2005, McDowell et al., 2015). It is perhaps feasible that the extended period of dry conditions in phase 1 (~6.8 – 4.2 ka) made sustaining a genetically viable population on a small, isolated island difficult, if not impossible – providing a possible explanation for the disappearance of people from KI. However, it should be noted that if people persisted into phase 2, that the climate-linked increase in fire at 3.3 ka could have ultimately been what made the island eventually uninhabitable. Until more is known for certain about the history of human habitation on KI in the Holocene, these statements remain hypothetical conjectures.

Phase 3 (~2.5 ka – 1820 CE) | *Eucalyptus* dominated woodland with denser shrubby mid-story and increasingly large and or frequent woody fires | Very dry climate and absence of evidence of human activity in the archaeological record

In this period there is evidence for increased biomass burning and or fire frequency in macro-CHAR peaks observed at 2.33 ka (18 counts cm^{-2} $year^{-1}$), 1.88 ka (22 counts cm^{-2} $year^{-1}$), 1.23 ka (39 counts cm^{-2} $year^{-1}$) and 780 cal. years BP (88 counts cm^{-2} $year^{-1}$). The pollen record indicates that this fire regime occurred in *Eucalyptus* woodland vegetation, possibly with a denser mid story, which is consistent with evidence for woodier fuels indicated by lower macrocharcoal length to width ratios. Other sources corroborate the dense nature of the vegetation on KI, at least at the conclusion of this period. Reports by British and French captains Matthew Flinders and Nicolas Baudin, who visited the island in the early 1800s, echo this conclusion (Tindale and Maegraith, 1931).

Linguistic evidence further hints at the thick state of the vegetation. In the Ngarrindjeri dictionary (Gale and Ngarrindjeri Elders and Community, 2009) the word for Kangaroo Island, *karti*, is also defined as 'low thick scrub' according to an original written source by Meyer (1843). Geochemical evidence from Lashmars Lagoon during phase 3 reveals a transition to very dry conditions, as indicated by an increase in carbonate fossils and the first occurrence of gypsum in the sediments (Figure 4A; Chapter 3). Lakes in the Coorong region also show evidence of regional aridity with three desiccation horizons at 2.25 ka, 1.61 ka and 1.27 ka (Ahmad, 1996) (Figure 4), coherent with a scenario of climate drying where vegetation is more primed for bigger and or more frequent fires. Throughout this period, there is no undisputed archaeological evidence of human activity on the island (Walshe, 2005). Further, historical sources suggest that people were absent from the island at the first arrival of European people in the early 1800s (Tindale and Maegraith, 1931). It follows that if people were absent from KI during this period, then Aboriginal fire management would also have been lacking, thus promoting shrub encroachment of open vegetation types (Mariani et al., 2022, Adeleye et al., 2022). We note that this conjecture assumes that Aboriginal fire management strategies were employed by Islander populations during earlier parts of the Holocene, and that more empirical evidence is needed within a western scientific framework to increase the certainty with which this can be argued.

A point of curiosity in this phase is the S intensity peaks from the XRF data (Chapter 3). These S peaks also show concordance with some of the macrocharcoal peaks – 2.33 ka, 1.23 ka and 780 cal. years BP – suggesting a relationship with either fire and or climate (Figure 2). More detailed mineralogical analysis of these peaks in this study found that S in mineral form was mainly contained in the evaporite mineral gypsum (Table 1). It is possible that particularly dry conditions at these points, also conducive to fire, led to higher evaporation of lake water and the eventual precipitation of gypsum at the coring site. However, given the low overall concentrations of gypsum in comparison to the magnitude of the S intensity peaks, there may be other contributing factors. For example, high S might also be explained by higher delivery of mobile elemental S contained in ash from fires, a link that has been previously established in speleothem archives (Bian et al., 2019, Nagra et al., 2016). Alternatively, this could also reflect an increased marine influence on the lagoon when freshwater influx is low in dry periods, as seawater is highly enriched in S in the form of sulphate.

Phase 4 (~1820 – 2020 CE) | Increasing clearance of land for agriculture and relatively high fires and or frequency with evidence for woody and grassy burns | Variable climate and European farming practices

Macro-CHAR throughout this period is relatively high (average 9 counts cm^{-2} $year^{-1}$) but is dwarfed in comparison to the preceding macro-CHAR peak at 780 cal. years BP (88 counts cm^{-2} $year^{-1}$). The most recent sample (~1980 CE) had the largest recorded macro-CHAR of the period (22 counts cm^{-2} $year^{-1}$) and a low length to width ratio (1.9). This may well be from a 1980 bushfire that burnt all of the nearby Lesueur Conservation Park, an area equating to 3,789 ha, in the largest recorded bushfire on the Dudley Peninsula since 1940 (Department of Environment and Water, 2020). Regrettably, the pollen record does not extend into this period. While the generally low macrocharcoal length to width ratio suggest mainly woody fuels, there are some notable instances of high length to width ratios indicative of finer, grassy fuel types. These high ratios perhaps reflect the burning of the more open grass-dominated farmland, characteristic of the European period. However, other historical evidence can help to reconstruct vegetation through this period. Historical sources suggest that the area was progressively cleared for farming since a population of convicts, sealers and Aboriginal women began living in the surrounding area from as early as the 1820s, and more intensively since the ‘Hundred of Dudley’ was proclaimed in 1874 (Taylor, 2008). Climate inferences from lake sediment geochemistry from this period are uncertain given the introduction of European farming practices disturbing natural processes (Chapter 3). A more detailed investigation of the changes that occur in this period is warranted but beyond the scope of this study.

Comparison to local fire records

The two charcoal records from Lashmars Lagoon show low correspondence with fire records from the adjacent mainland. A two metre > 8 ka sedimentary sequence retrieved from Boat Harbour Creek swamp, a shallow peatland located 290 m above sea level on the Fleurieu Peninsula, is the closest fire record to Lashmars Lagoon (Bickford and Gell, 2005). The Boat Harbour macrocharcoal record was attributed to localised and deliberate Aboriginal wetland burning to promote germination of the economically important food source *Typha*, and thus has limited scope to reflect broad scale regional fire patterns. Microcharcoal concentrations at Boat Harbour Creek swamp are generally high before ~4.2 ka (where Lashmars Lagoon charcoal is low), before transitioning to relatively low values until the European era (where Lashmars Lagoon charcoal is high). Thus, the microcharcoal record from this site exhibits an opposing trend to the Lashmars Lagoon macrocharcoal, potentially alluding to the role of mainland human populations in supressing the effects of climate change on fire regimes. However, we caution that this interpretation is presently tentative as the Boat Harbour Creek swamp record is limited by chronological uncertainties (5 radiocarbon dates, no age model) and coarse sampling resolution (23 samples for > 8ka).

Another regional charcoal record comes from 100 km away in the Mount Lofty Ranges at Wilson Bog where a swamp sediment infill sequence was exposed after a 2005 flood (Buckman et al., 2009). The charcoal record from this site (Figure 4D) extends from ~1 - 7 ka over about two metres and is highly resolved (95 samples) but somewhat poorly chronologically constrained record (5 radiocarbon dates, 2 optically stimulated luminescence dates). At Wilson Bog, there is some evidence for more frequent, larger fires in the Mid Holocene compared to the Late Holocene, a similar pattern to the microcharcoal record from Boat Harbour and opposite to the pattern recorded at Lashmars Lagoon. In the Wilson Bog study, charcoal particles > 840 µm were separated from sediment and dried to give ‘% weight per 100 g of sediment’. The unusually large size of charcoal particles processed in this study (presumably reflecting ultra-local fire activity) and non-standard method of sampling perhaps diminish the relevance of the comparison of Wilson Bog to Lashmars Lagoon.

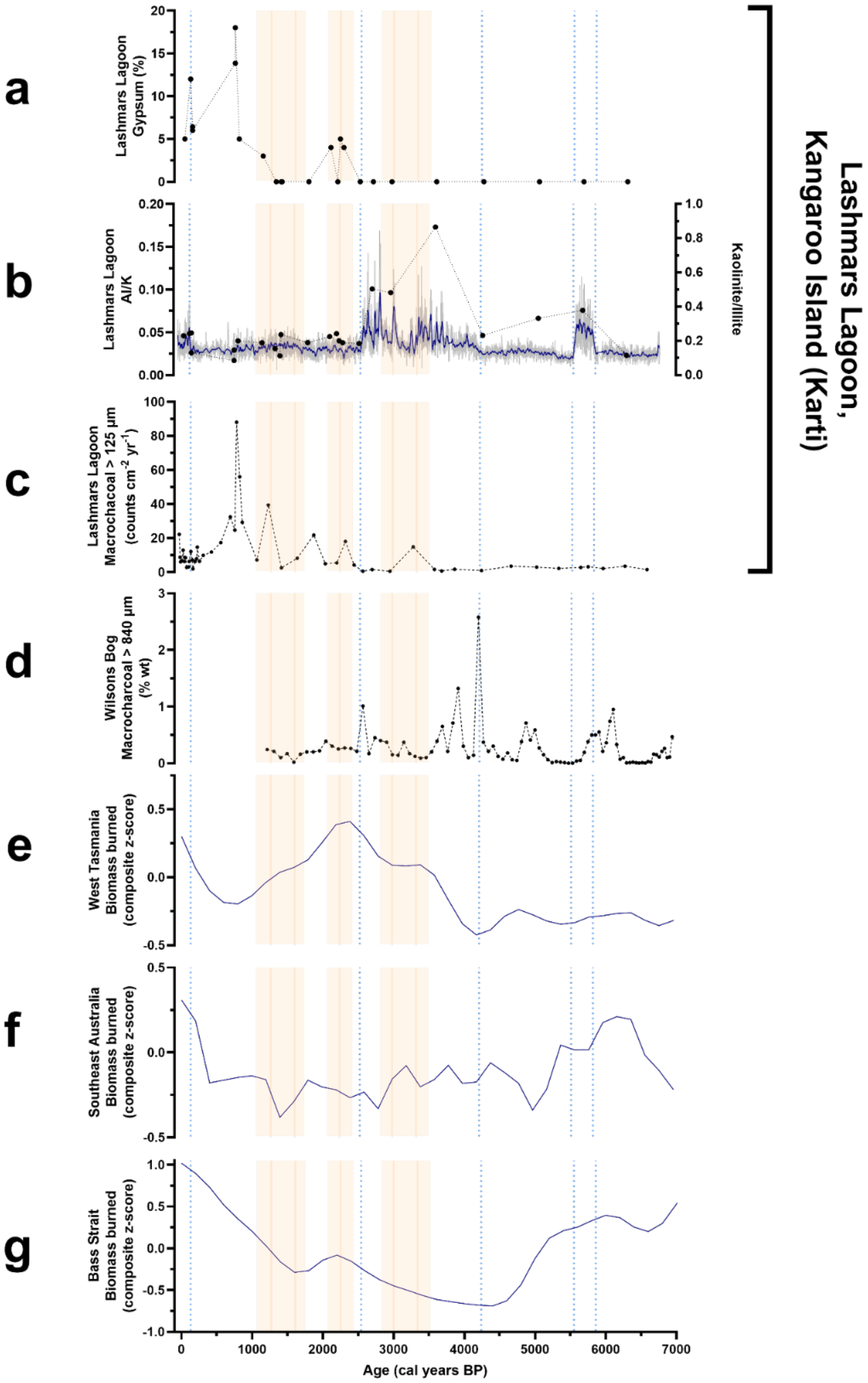

Figure 4. *Regional fire summary. (A) Gypsum in Lashmars Lagoon sediments (Chapter 3). (B) Lashmars Lagoon Al/K ratios in blue and kaolinite/illite ratio with dashed black line (Chapter 3). (C) Lashmars Lagoon macrocharcoal record. (D) Wilson Bog, Mount Lofty Ranges charcoal (Buckman et al., 2009) accessed through the GPD (www.paleofire.org; (Power et al., 2010)). (E-G) Regional*

charcoal synthesis for biomass burning (Adeleye et al., 2021): (E) western Tasmania, (F) southeasten Australia and (G) Bass Strait. Orange lines are the desiccation horizons with orange shaded error recorded in Coorong lakes (Ahmad, 1996).

Comparison to regional charcoal records

We compared the Lashmars Lagoon macrocharcoal record to fire records from across the southern Australian region (Figure 4). From ~7 ka – ~2 ka, the trend in our record that is characterised by a transition from low charcoal in the Mid Holocene to high charcoal in the Late Holocene (Figure 4C), is most similar to the western Tasmanian biomass burning synthesis record (Figure 4E, Adeleye et al., 2021). Although western Tasmania is more distant to KI than southeast Australia (Figure 4F) and the Bass Strait (Figure 4G), this link suggests that fire regimes in these two regions were driven by similar climate influences during this period. This may well be attributed to the particularly strong influence of the SAM in both regions (Mariani and Fletcher, 2016, Ray, 2022). However, after ~2 ka these two records diverge. On KI macrocharcoal accumulation continues to increase until ~800 cal. years BP in accordance with local climate drying signals (Chapter 3) and evidence for increased prevalence of El Niño events in the Late Holocene inferred from marine sediment cores from offshore southern Australia (Perner et al., 2018, De Deckker, 2022). Conversely, in western Tasmania, biomass burning decreases until ~800 cal. years BP. The difference between these records might then reasonably be explained by the hypothesis that while people were living in western Tasmania at this time, Aboriginal people were not using cultural burning practices to manage the environment on KI. This fits well with evidence from Romano and Fletcher (2018) who found that climate and fire variability at a site in northwest Tasmania became decoupled when population growth peaked between 1.7 ka and 900 cal. years BP. The role of Aboriginal stewardship in maintaining the stability of fire regimes in the face of climate variability through cultural burning practices has also been demonstrated in a contemporary study of the Martu peoples in the Western Desert (Bird et al., 2012).

Future directions

Future work should focus on better constraining the history of Holocene human habitation on KI and the exact nature of Aboriginal fire management in the ancient past. Emphasis should be on continued archaeological work and incorporating Indigenous ways of knowing into knowledge creation processes in science. Further characterisation of the fire record is also recommended: (1) macrocharcoal analysis should be completed contiguously at high resolution to untangle the metrics of biomass burned and fire frequency and (2) fire intensity could be reconstructed with FTIR spectroscopy (Rehn et al., 2021, Constantine IV et al., 2021). A more complete picture of the changes in vegetation at Lashmars Lagoon through quantitative pollen models (e.g., Mariani et al., 2016) would enrich our understanding of long-term interactions between vegetation biomass, fire, climate and people. Finally, novel techniques such as the analysis of sedimentary ancient DNA could provide a more holistic overview of the palaeoecology of KI in the Holocene.

Conclusion

This study examines macrocharcoal, microcharcoal and pollen in a ~7,000-year lake sediment record from Lashmars Lagoon to reconstruct past fire and vegetation change on Kangaroo Island (Karti) in the context of climate change and the likely loss of Aboriginal stewardship. We conclude that it was likely the combined effect of climate change and a loss of Aboriginal stewardship that ultimately produced the conditions to shift the fire regime from low to high biomass burning and or fire frequency at ~3.3 ka. During changed climate conditions, the landscape unmanaged by Aboriginal people, led to the unchecked build-up of the shrubby understory during relatively wet periods, ultimately creating the conditions necessary to spark catastrophic fires. Our study is a warning that while wetter phases supressed fire occurrence for their duration, if vegetation builds up unchecked in these periods, fire risk is ultimately increased as soon as the climate becomes dry again. Our study suggests that drier climates, with no management, leads to more fires. However, our study also bears a message of optimism – pointing to the potential for human management to buffer the risk of catastrophic fires under the contemporary threat of climate change. The projected drier climates in the future call for careful human management of Kangaroo Island (Karti)'s landscape, especially in areas where remnant dense vegetation remains.

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In Australia, we work and live on Aboriginal land. This research was developed and conducted on Kaurna Yerta (Kaurna land) at The University of Adelaide and on Karti (Kangaroo Island), a place of deep cultural and spiritual significance for Aboriginal people in South Australia, including for Ngarrindjeri, Kaurna, Narungga, Nhawu and Barngarla people. I pay my respects to elders past, present, and emerging. I acknowledge that sovereignty was never ceded.

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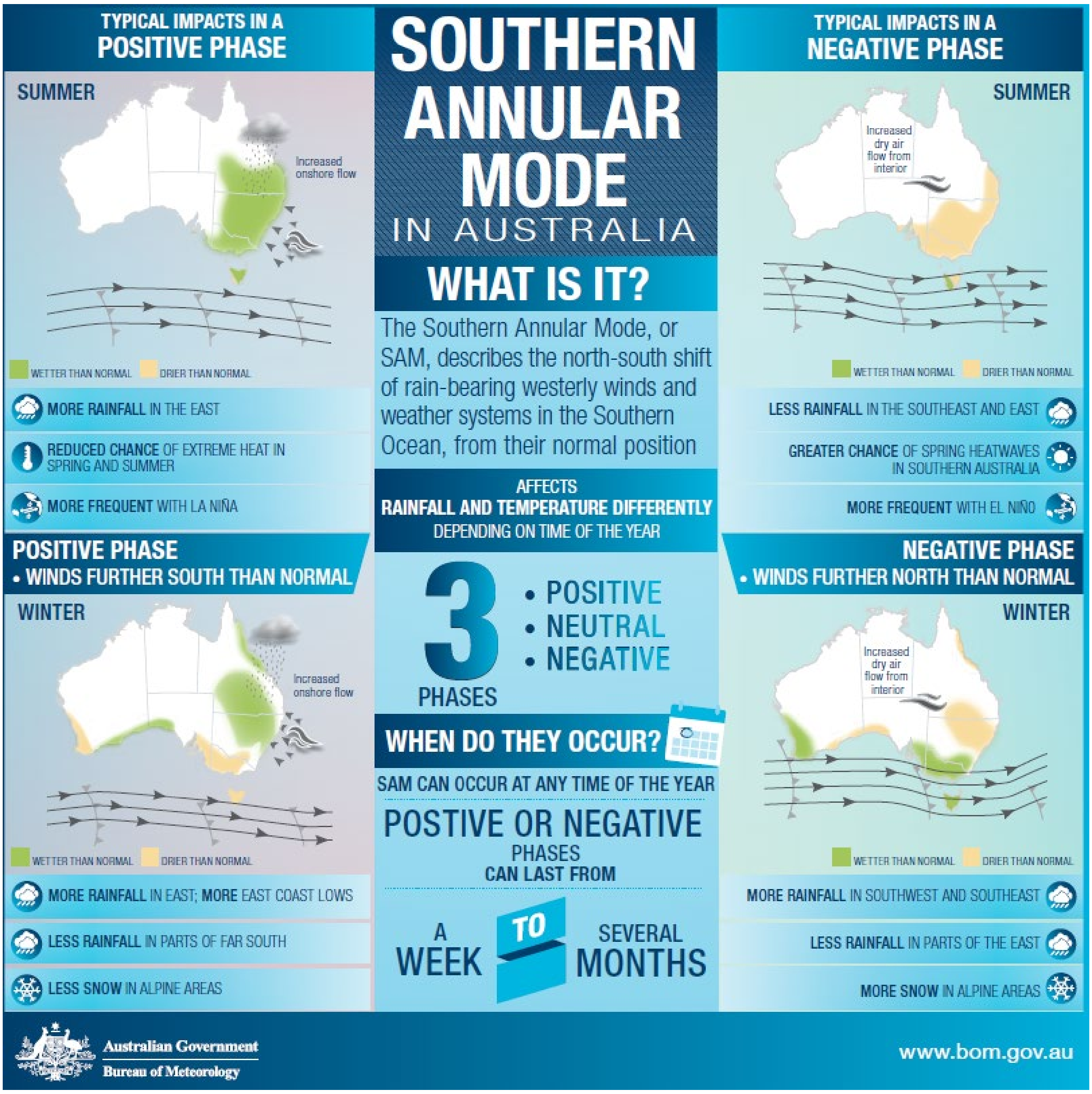
Author contributions

LD – Conceptualisation, field work, investigation, formal analysis, visualisation, writing – original draft; HC – Supervision, conceptualisation, investigation (pollen identification), formal analysis, writing – review and editing; SL – Supervision, investigation (XRD), writing – review and editing; LJ-M – Investigation (XRD), writing – review and editing; AF – Conceptualisation, field work, writing – review and editing; WL – Field work, visualisation, writing – review and editing; LA – Supervision, writing – review and editing; JT – Conceptualisation, supervision, field work, investigation, writing – review and editing.

Supplementary information



SOUTHERN ANNULAR MODE IN AUSTRALIA

TYPICAL IMPACTS IN A POSITIVE PHASE

SUMMER

Increased onshore flow

🟩 WETTER THAN NORMAL 🟨 DRIER THAN NORMAL

MORE RAINFALL IN THE EAST

REDUCED CHANCE OF EXTREME HEAT IN SPRING AND SUMMER

MORE FREQUENT WITH LA NIÑA

POSITIVE PHASE

- WINDS FURTHER SOUTH THAN NORMAL

WINTER

Increased onshore flow

🟩 WETTER THAN NORMAL 🟨 DRIER THAN NORMAL

MORE RAINFALL IN EAST; **MORE** EAST COAST LOWS

LESS RAINFALL IN PARTS OF FAR SOUTH

LESS SNOW IN ALPINE AREAS

WHAT IS IT?

The Southern Annular Mode, or SAM, describes the north-south shift of rain-bearing westerly winds and weather systems in the Southern Ocean, from their normal position

AFFECTS

RAINFALL AND TEMPERATURE DIFFERENTLY

DEPENDING ON TIME OF THE YEAR

3 PHASES

- POSITIVE
- NEUTRAL
- NEGATIVE

WHEN DO THEY OCCUR?

SAM CAN OCCUR AT ANY TIME OF THE YEAR

POSTIVE OR NEGATIVE PHASES CAN LAST FROM

A WEEK TO SEVERAL MONTHS

TYPICAL IMPACTS IN A NEGATIVE PHASE

SUMMER

Increased dry air flow from interior

🟩 WETTER THAN NORMAL 🟨 DRIER THAN NORMAL

LESS RAINFALL IN THE SOUTHEAST AND EAST

GREATER CHANCE OF SPRING HEATWAVES IN SOUTHERN AUSTRALIA

MORE FREQUENT WITH EL NIÑO

NEGATIVE PHASE

- WINDS FURTHER NORTH THAN NORMAL

WINTER

Increased dry air flow from interior

🟩 WETTER THAN NORMAL 🟨 DRIER THAN NORMAL

MORE RAINFALL IN SOUTHWEST AND SOUTHEAST

LESS RAINFALL IN PARTS OF THE EAST

MORE SNOW IN ALPINE AREAS

Australian Government
Bureau of Meteorology

www.bom.gov.au

Figure S1. *The seasonal effects of the Southern Annular Mode (SAM). Source: http://www.bom.gov.au/*

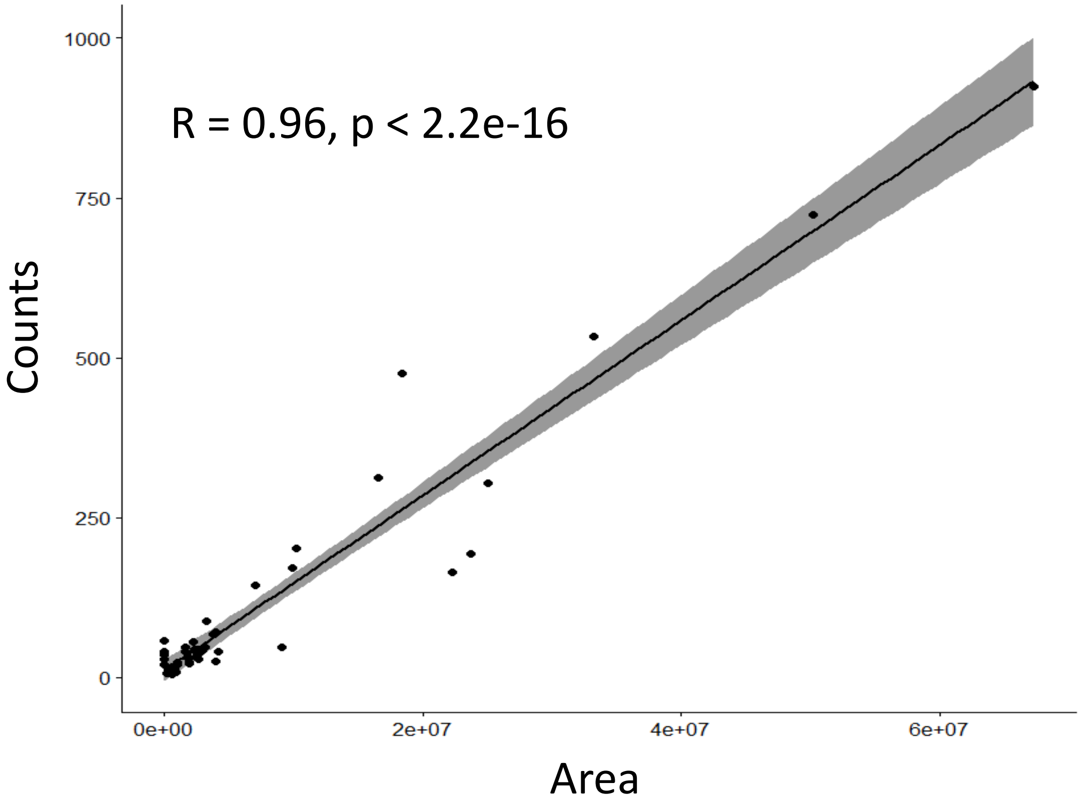


Figure S2 *The relationship between macrocharcoal counts and area (Pearson's correlation). Samples with calibration error were discounted (see Note S1).*

Note S1. *List of samples discounted from analysis (composite profile age, rounded to the nearest year): -33, -20, 6, 23, 41, 56, 72, 102, 131, 162, 193, 224, 255, 309, 424, 557, 693, 781, 827 and 863 cal. years BP.*

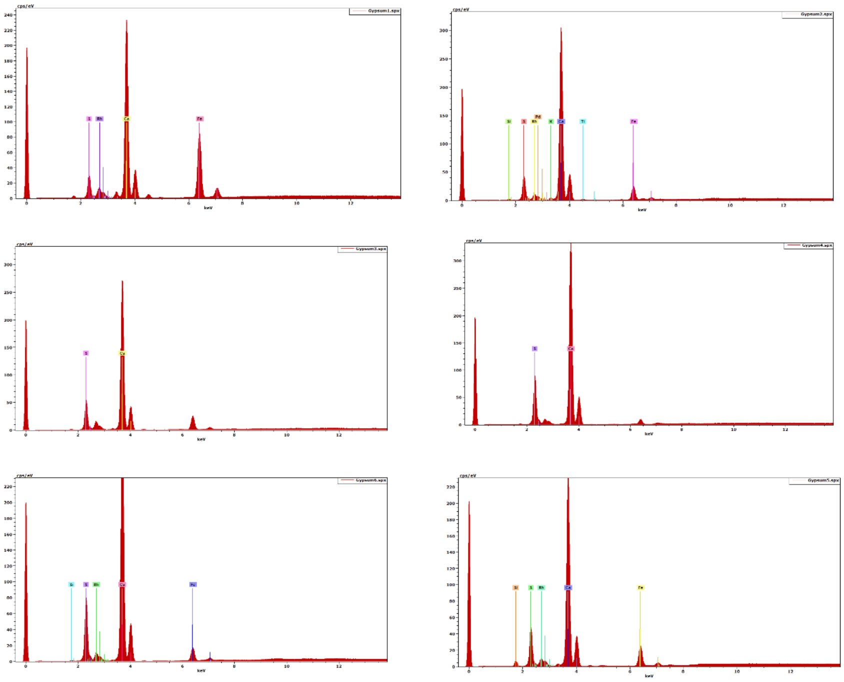


Figure S3. *Micro-beam XRF microscopy of putative gypsum grains in S6 and S7*

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5

Lake sedimentary ancient DNA reveals ecosystem response to fire and climate on Kangaroo Island (Karti), Australia

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Abstract

Records of historical climate, fire, and ecosystem change – particularly those that extend beyond instrumental records – are rare across many parts of the Southern Hemisphere, and especially in southern Australia. Furthermore, studies into long-term links between ecological change and environmental drivers (like fire and climate) are often limited to the analysis of pollen in sedimentary records, which are restricted in taxonomic scope and subject to systematic biases. Ancient DNA from lake sediments (*sed*aDNA) represents a powerful new proxy to reconstruct past ecosystems in their full ecological breadth. This study focuses on Kangaroo Island (Karti), where bushfires decimated the island's unique ecosystems in 2019-2020. We use shotgun metagenomics to study aquatic and terrestrial biodiversity over the last ~7,000 years from the sediments of Lashmars Lagoon, Kangaroo Island (Karti). We compared this *sed*aDNA record to charcoal-inferred fire history and geochemical proxies for climate and landscape change from the same record. Using multivariate statistics, we reveal the role of climate and fire in shaping ecological communities across the three domains of life (Eukaryota, Bacteria and Archeaea). We focused our analyses on the Eukaryota group of green plants – the Viridiplantae – comparing these to an existing pollen record to garner new insights into plant community responses to climate and fire. We found compositional changes statistically linked to fire history and climate change. Specifically, sediment calcite content (linked to drier climates) significantly explained changes in composition within all three domains (Archaea, Bacteria and Eukaryota) and the Viridiplantae. Statistical analysis further revealed that the major compositional change within the Viridiplantae, including a decrease in the Fabaceae family (which includes the genus *Acacia*), coincided with an inferred increase in fire activity at ~3.3 ka. Importantly, we also found evidence for increased amounts of plant DNA during this period of increased biomass burning and or more frequent fires, alluding to the role of fuel loads and vegetation density in controlling fire regimes. Overall, our study sheds new light on the way climate and fire have shaped biodiversity on Kangaroo Island (Karti). These data help to contextualise the complex human history of Kangaroo Island (Karti) and contribute to understanding how the putative mid-Holocene cessation of Aboriginal land management on the island may have impacted the island's ecology. Lastly, this study demonstrates the potential for the preservation of *sed*aDNA dating back to at least ~7,000 years in Australian lake sediment and encourages the application of this novel proxy to investigate ecological responses to fire and climate change across the region on millennial timescales.

Key words

*sed*aDNA; Holocene; shotgun metagenomics; Viridiplantae; pollen; charcoal; XRF core scanning

Introduction

Humanity is in the midst of a self-inflicted climate and biodiversity crisis of geological significance (Lewis and Maslin, 2015). In southern Australia, decreased rainfall and increased temperatures combined with drastic land use changes following British colonisation are driving fire regimes to dangerous extremes (Abram et al., 2021, Mariani et al., 2021). The 2019-2020 fire season in Australia, now known as the 'Black Summer', devastated local communities and ecosystems alike (Davey and Sarre, 2020). Kangaroo Island (Karti; henceforth abbreviated to KI), lauded for its unique biodiversity, was particularly hard hit as fires burned uncontrolled for 11 days across a total of 2,100 km^2 – almost half the island (Figure 1A; Bonney et al., 2020, Taylor, 2020). Nationwide, the fires burned more than eight million hectares of vegetation, affected at least half the range or population of over 800 species of vascular plants (Godfree et al., 2021), and were estimated to have impacted vertebrates and invertebrates in their millions and trillions, respectively (Dickman, 2021).

Our understanding of how ecosystems respond to major shifts in climate and fire regimes often relies on evidence from the past, either through direct analogues, or indirectly, through the development and validation of ecological models (Fordham et al., 2020). Lake sediments are important palaeoarchives that accumulate over time, preserving characteristic signatures of terrestrial and aquatic environments from the past (Smol et al., 2002, Smol et al., 2001). These palaeoenvironmental archives are important because they document the natural variability of ecosystems, providing long-term ecological baselines to inform the management of biodiversity and ecosystem services in today's rapidly changing earth system.

However, as discussed in the first chapter of this thesis, despite half a century of efforts to study past climate, fire and ecosystem change in Australia with lake records, several major uncertainties remain around these connections, thus limiting our ability to manage post fire recovery (Dickman, 2021). One major limitation is that most terrestrial palaeoecological records rely on the identification of either pollen grains, or much less abundant plant macrofossils, preserved in lake sediments to reconstruct past vegetation. Not only does this limit palaeoecological studies to a subset of aquatic and terrestrial biodiversity, but pollen records are subject to biases from the unequal production and delivery of pollen by plants in the landscape (Mariani et al., 2016, Kershaw and Strickland, 1990). In Australia for example, pollen percentages have been shown to underestimate landscape openness (Mariani et al., 2017). Morphological similarities between the pollen from different species can also compromise the level of taxonomic precision, a problem particularly pronounced for Myrtaceae (which includes *Eucalyptus*), a very common family in Australian vegetation (Pickett and Newsome, 1997).

Lake sedimentary ancient DNA (*sed*aDNA) is a new approach to palaeoecological reconstructions (Capo and Monchamp et al., 2022) that provides a complementary tool to reconstruct past vegetation and biodiversity. *Sed*aDNA has the power to reveal diversity in both aquatic and terrestrial taxa across all three domains of life – Archaea, Bacteria, Eukaryota – capturing vestiges of organisms not normally detectable by more traditional palaeoecological methods (Bixby et al., 2015). Importantly, microorganisms from soil, sediments and the water column, often the building blocks of entire ecosystems and crucial for the cycling of key nutrients, can be detected from their DNA (Thomas et al., 2022). Metabarcoding techniques, which amplify specific target genes in ancient DNA extracts, have been shown to complement pollen studies when it comes to reconstructing plant communities (Parducci et al., 2017, Parducci et al., 2012, Parducci et al., 2013, Parducci et al., 2015, Parducci et al., 2019, Pedersen et al., 2013). *Sed*aDNA approaches also have the power to account for biases in pollen records by documenting low-pollen producers, such as the detection of insect-pollinated taxa in permafrost (Willerslev et al., 2014). More recently, shotgun metagenomics – a more robust technique to study old and fragmented DNA, with less inherent bias in DNA sequencing – has been shown to complement pollen studies to reconstruct past plant communities (Courtin et al., 2022). To date, most *sed*aDNA studies have focused on deeper lakes in alpine and or high-altitude regions, where DNA is known to preserve well due to cooler temperatures, minimal bioturbation and anoxic conditions at the sediment-water interface (Capo and Monchamp et al., 2022). However, much less is understood about the preservation of DNA in shallow lakes with well-oxygenated water columns, in moderate to warm climates – like on KI, Australia.

Here, we apply shotgun metagenomics to the sediments of Lashmars Lagoon, KI, Australia, an important site for investigating past climate, fire, and ecosystem change, in the context of unique changes in human management. This thesis builds on previous work from this site (Clark, 1983b, Singh et al., 1981, Illman, 1998, Clark, 1983a). In Chapter 3 of this thesis, a new chronology for the sediments was presented and climate and catchment processes were inferred from a detailed interrogation of the sediment geochemistry, and in Chapter 4, fire and ecological change was inferred from macroscopic charcoal and pollen records. Notably, these data indicate a change to larger and or more frequent fires around 3.3 ka, which we suggested could reflect the compounding effects of climate change coupled with a loss of Aboriginal fire management and resultant shrub encroachment (Chapter 4). As such, KI may provide an important analogue for the widespread catastrophic fire conditions that are facing the Australian mainland today. Given the limitations of traditional pollen records, questions have been raised regarding the impact of fire on vegetation and ecosystem changes at Lashmars Lagoon (Clark, 1983a, Singh et al., 1981, Illman, 1998, Gammage, 2013)

In this study, we present a ~7,000-year-old lake *sed*aDNA record from Lashmars Lagoon, KI, illustrating the promise of shotgun metagenomics in Australian palaeoenvironmental research. Here, we focus on the Viridiplantae *sed*aDNA signal to reconstruct fluctuations in past terrestrial and aquatic plant communities, complementing an existing pollen record from the site, and addressing pertinent and timely questions about long term ecological responses to fire and climate change in Australia.

Site information

See Chapter 2 of this thesis for a detailed site description for Lashmars Lagoon and KI.

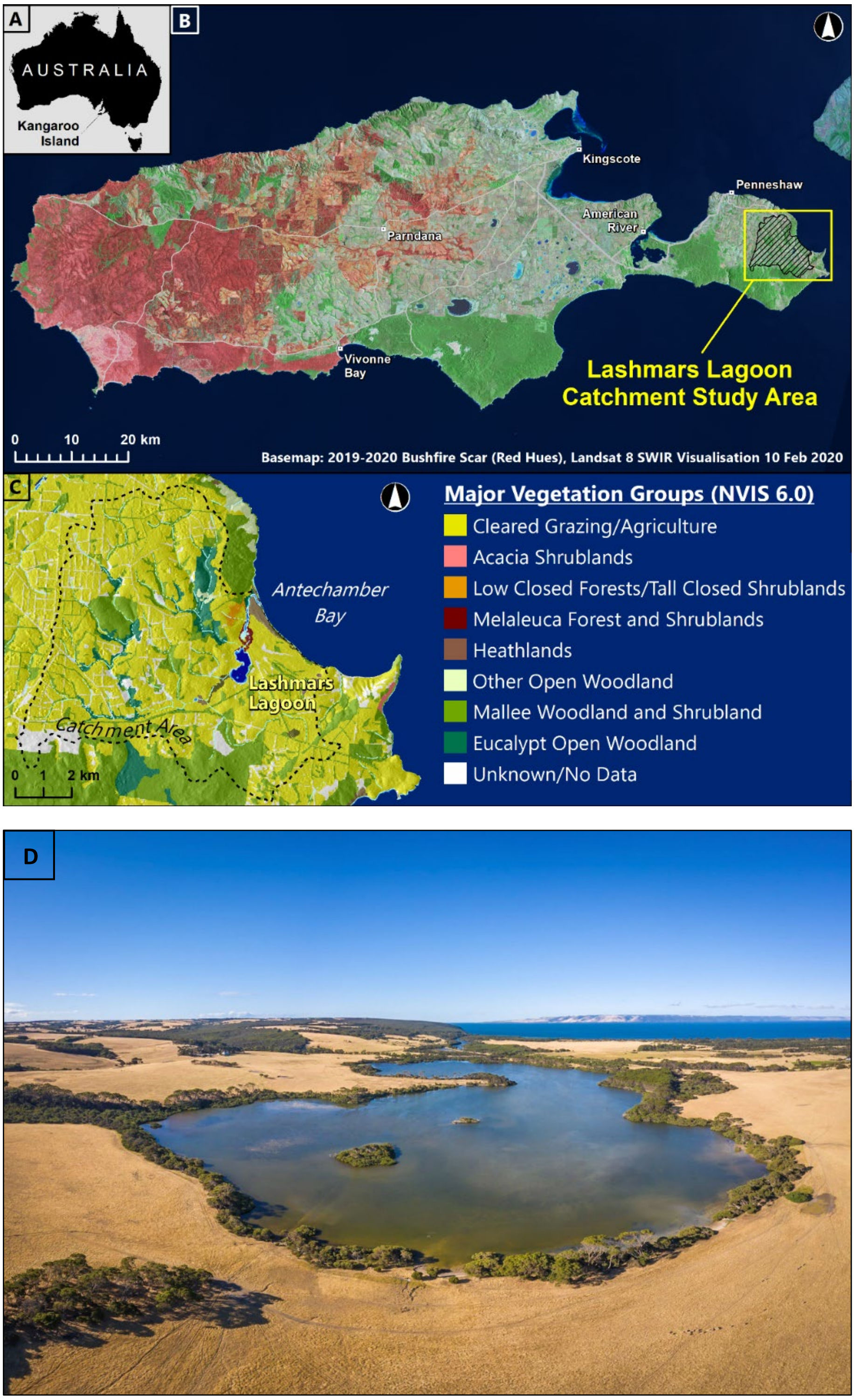

Figure 1. *Map of the study site. (A) Kangaroo Island (Karti) in relation to the Australian mainland. (B) The location of the study area, Lashmars Lagoon, in relation to Kangaroo Island (Karti). The base map is the 2019-2020 Bushfire Scar (Red Hues) Landsat 8 SWIR Visualisation, February 10, 2020. (C) The Major Vegetation Groups (NVIS 6.0) presently surrounding Lashmars Lagoon. (D) Drone image (courtesy of photographer Q. Chester) of Lashmars Lagoon looking NE across Backstairs Passage to the Australian mainland.*

Materials and methods

Sediment coring

In September 2020, a 100 cm and 739 cm sediment core (LAS20-3 and LAS20-4) were collected from the northern basin of Lashmars Lagoon, KI (Figure 1; S 35°48.289’ E 138°03.853’), in a water depth of ~90 cm, using a 65 mm diameter Bolivia corer (Wright, 1967). Cores were taken in ~100 cm sections, using 65 mm PVC core tubes. Coring equipment and tubes were pre-sterilized with sodium hypochlorite (bleach, 3%) and ethanol (70%). The coring tubes were then sealed in plastic prior to coring. To minimize the risk of contamination in the field, sealed core tubes were opened immediately prior to coring and the coring equipment was cleaned with soapy water and 70% ethanol between each core drive. During sediment coring, masks and long-sleeved clothes were worn, hair was tied-back, skin was covered where possible, and gloves changed immediately when contaminated. Sealed cores were transported to The University of Adelaide, Australia, where they were stored in the dark at 4° C.

Core cutting, opening, and subsampling for *sed*aDNA

Cores were cut, opened, and subsampled in February and March 2021 in pre-cleaned and sterilised sediment processing labs (physically separated from any molecular genetics labs) at The University of Adelaide. To avoid cross-contamination, each core section was processed individually. During core opening and subsampling, protective clothing was worn, including gloves (changed when contaminated and between cores; and 2 pairs during subsampling), hairnets, long-sleeved clothing, face masks, lab coats (during initial core opening) or disposable coveralls (during subsampling) and safety glasses. In the following, ‘sterilisation’ refers to decontamination using 3% bleach and then 70% ethanol.

We cut the plastic core tubing on a bench in the main sediment core lab that was sterilised between processing core sections. For each core, we removed the plastic wrap, and sterilised the core tubing. The plastic tubing was then cut lengthways from bottom to top with an oscillating saw attached to a hand-held 3D-printed jig. We sterilised the blade of the saw and the jig between processing different core sections. Finally, we sterilised the outside of the core tubing before proceeding to the next step. To open and subsample the cores, we moved each core, tube casing cut but core still closed, from the sediment core lab to an adjacent dedicated clean room (sterilised between subsampling each core section). Sterilised fishing wire was used to split the sediment core in half by pulling the wire lengthways through the core from bottom to top. Prior to sampling, we scraped away the top few millimetres of surface sediment with a sterilised metal scraper, working always from bottom to top.

We then used sterilised metal spatulas to remove two to three cubic centimetres of sediment from one-centimetre-thick intervals from the centre of the core section at five-centimetre intervals from bottom to top along the core section. Control samples were taken in the subsampling lab to track environmental contamination during the subsampling: two air controls (the tube was uncapped and swirled around the ambient air for about one minute), one blank swab control (a clean cotton tip) and one sampling swab control (a cotton tip swab of a glass slide placed on the bench next to the core while subsampling). All samples were immediately frozen at –20°C.

*Sed*aDNA extraction, library preparation and sequencing

*Sed*aDNA was analysed from 26 samples and six controls in June 2021 at the Australian Centre for Ancient DNA's ultraclean facilities at The University of Adelaide. The samples (n=26; Table 1) covered sedimentological changes, while also maintaining relatively even spacing between samples. The six controls included the four environmental contamination controls (see previous section) and two extraction blanks controls (empty tubes that underwent the same extraction protocols). We processed these samples in two batches of 16 samples (each contained one of the extraction blanks). Samples and environmental controls were assigned randomly to a batch.

We extracted *sed*aDNA according to Armbrecht et al. (2020), a method optimized for the extraction of ancient eukaryote DNA from aquatic sedimentary environments. The method uses ~0.25 g of wet sediment as starting material and then combines an overnight incubation step of the sediments in 0.5M EDTA followed by bead-beating (3 × 20 sec with 5 min breaks, FastPrep FP120, Thermo Savant, USA) and DNA purification via the DNeasy PowerLyzer PowerSoil Kit (Qiagen) with a DNA binding step in QG Buffer (Qiagen) with dissolved SiO_2.

Metagenomic shotgun library preparation was also undertaken following Armbrecht et al. (2020). This protocol largely follows Meyer and Kircher (2010) and includes the ligation of unique 7-mer barcodes (P5 and P7 adapters), an initial amplification using primers IS7 and IS8 (22 cycles), DNA purification (MinElute Reaction Cleanup Kit, Qiagen), a second amplification using primers IS4 and a GAII_Index (Index 9, 14 cycles), and another round of DNA purification. Each amplification was performed with the samples divided equally into eight replicates of 25 µL to reduce the effect of stochastic amplification bias. Next, we selected for a DNA fragment length of < 500 base pairs (bp) using magnetic beads (AxyPrep, Axygen) and repeated the bead-based purification until no primer-dimer was present.

The samples were pooled ('multiplexed') at an equimolar concentration (4.88 nmol/L) and sent to the Australian Genome Research Facility (Adelaide) for shotgun sequencing on the 2 x 150 bp Illumina NovaSeq platform.

***Table 1**. SedaDNA sample metadata (n=26). Dates are from the age model presented in Chapter 3 and are reported to the nearest decade.*

Sample no.	**Sample code**	**Composite depth range (cm)**	**Midpoint median age (ka)**
1	LAS20-3_0-1m_10-11cm	13.5-14.5	-0.10 (1960 CE)
2	LAS20-3_0-1m_40-41cm	43.5-44.5	0.11 (1840 CE)
3	LAS20-4_0.5-1.5m_10-11cm	50.9-51.9	0.15 (1800 CE)
4	LAS20-4_0.5-1.5m_50-51cm	90.9-91.9	0.76
5	LAS20-4_0.5-1.5m_90-91cm	130.9-131.9	1.07
6	LAS20-4_1.5-2.5m_10-11cm	152.7-153.7	1.23
7	LAS20-4_1.5-2.5m_35-36cm	177.7-178.7	1.42
8	LAS20-4_1.5-2.5m_65-66cm	207.7-208.7	1.64
9	LAS20-4_1.5-2.5m_95-96cm	237.7-238.7	1.88
10	LAS20-4_2.5-3.5m_15-16cm	257.4-257.4	2.04
11	LAS20-4_2.5-3.5m_35-36cm	277.4-277.4	2.21
12	LAS20-4_2.5-3.5m_65-66cm	307.4-307.4	2.45
13	LAS20-4_2.5-3.5m_95-96cm	337.4-337.4	2.71
14	LAS20-4_3.5-4.5m_20-21cm	363.7-364.7	2.97
15	LAS20-4_3.5-4.5m_50-51cm	393.7-394.7	3.30
16	LAS20-4_3.5-4.5m_80-81cm	423.7-424.7	3.60
17	LAS20-4_4.5-5.5m_10-11cm	452.4-452.4	3.90
18	LAS20-4_4.5-5.5m_45-46cm	487.4-487.4	4.27
19	LAS20-4_4.5-5.5m_85-86cm	527.4-527.4	4.69
20	LAS20-4_5.5-6.5m_20-21cm	562.4-562.4	5.06
21	LAS20-4_5.5-6.5m_50-51cm	592.4-592.4	5.37
22	LAS20-4_5.5-6.5m_80-81cm	622.4-622.4	5.68
23	LAS20-4_5.5-6.5m_90-91cm	632.4-632.4	5.79
24	LAS20-4_6.5-7.5m_10-11cm	652.4-652.4	6.00
25	LAS20-4_6.5-7.5m_40-41cm	682.4-682.4	6.31
26	LAS20-4_6.5-7.5m_70-71cm	712.4-712.4	6.62

Bioinformatics pipeline

We used the Phoenix High Performance Computer at The University of Adelaide to process our raw sequences according to the bioinformatics pipeline described in Armbrecht et al. (2020). The pipeline first performs quality control (fastQC, Brabraham Bioinformatics, Andrews (2010)) on the raw reads, then uses AdapterRemoval2 (Schubert et al., 2016) for demultiplexing, adapter-removal and read collapsing. Next, a fastQC (Brabraham Bioinformatics) and a multiQC (Ewels et al., 2016) is performed on the collapsed reads before removal of low complexity reads (Komplexity; Clarke et al. (2019); threshold = 0.55) and the removal of duplicate sequences (Dedupe function in BBMap;

BBMap/36.62-intel-2017.01-Java-1.8.0_121). A final fastQC and multiQC was performed on the filtered reads. To assign our reads to taxa, we used MALT (malt/0_4_1, Herbig et al. (2016), parameters: semiglobal alignment, minimum percent identity = 95%, LCA = 80) to align our reads against the SILVA SSU rRNA database (version 132, arb-silva.de/documentation/release-132). Next, we converted the '.blastn' files to '.rma6' files (blast2rma tool in MEGAN6, Huson et al. (2016); parameters: mpi=95%, me=50, sup=0 'off'). Finally, we imported the rma6 files in MEGAN6 CE and subtracted species identified in the controls from the samples (list of identified contaminant taxa provided in Table S1).

Ancient DNA authentication

To verify the ancient signal of our samples, we performed *sed*aDNA damage analysis using Heuristic Operations for Pathogen Screening (HOPS, v0.33-2, Hübler et al. (2019)). HOPS has previously been used to assess *sed*aDNA authenticity based on DNA damage profiles of key taxonomic groups, for which at least 50 reads (minimum required input, Hübler et al. (2019)) are available (Armbrecht et al., 2021). We applied the 'MALTExtract' and 'postprocessing' tools of HOPS, using the previously generated rma6 files as input and the default configurations with the following modifications: topMaltEx=0.10, minPIdent=95, meganSummary=1, and destackingOff=1. We ran the program searching for damage patterns in a) Eukaryota, b) Viridiplantae, c) Bacteria and d) Archaea. Thus, we had four input species lists: a) a list that contained only the term 'Eukaryota' (providing results for all eukaryotic taxa detected), b) 'Viridiplantae' (to investigate whether this group showed damage patterns similar to the broader group of Eukaryota, or less damage, which may be a sign for contamination; see Results), c) 'Bacteria' and d) 'Archaea'. HOPS 'def_anc' mode categorised reads into reads that passed stringent filtering criteria ('default') and reads with at least one damage lesion in their first 5 bases from either the 5' or 3' end ('ancient'). This output was used to calculate the sum of ancient and default reads per sample, converted to '% *sed*aDNA damage' i.e., proportion of ancient reads per sample for each of the four groups (Armbrecht et al., 2021).

Data analysis

To visualise relative composition of organisms derived from *sed*aDNA data, we first exported the following data from MEGAN6 CE: a) reads assigned to all taxa at domain level, b) reads assigned to Eukaryota at phylum level, c) reads assigned to Viridiplantae at family and genus level. The data (text files) were processed in R using specifically developed functions that convert read counts to taxon relative abundance within each sample and then group rare taxa (defined here as taxa that do not occur at over 1% in at least one sample) into a single group named 'rare taxa'. Next, the tables were inspected, and any ostensibly similar groups of taxa were grouped manually (e.g., 'unclassified

Chlorophyceae' and 'Chlorophyceae'). Finally, we created relative abundance barplots using the package *ggplot2*. The full R code is available in the supplementary information (Note S1).

We ran a multivariate canonical correspondence analysis (CCA) using the *vegan* package in R to investigate relationships between the *seda*DNA record and key proxies for climate, catchment, and fire regime changes that were determined from the same lake sedimentary sequence in Chapter 3 and 4 (Table 2). Then we tested for statistical significance of the environmental variables in explaining variation in the *seda*DNA data using ANOVAs. We did this for the following subgroups at genus level: a) Archaea, b) Bacteria, c) Eukaryota, d) Viridiplantae, e) Streptophyta and f) Chlorophyta.

Table 2. *Key explanatory environmental variables from Chapter 3 and 4 that were compared to the sedaDNA record*

Environmental variable	Source	Inferred to be a proxy for
Calcium (Ca) intensity from core scanning XRF	Chapter 3	Sediment calcite concentration, reflects carbonate fossils putatively linked to increased evaporation and or increased marine influence
Titanium (Ti) intensity from core scanning XRF	Chapter 3	Detrital input
Bromine (Br) intensity from core scanning XRF	Chapter 3	Sediment organic matter, correlates well with Al/K ratio (a proxy for chemical weathering intensity, periods of high Br are inferred to reflect wetter climates with increased lake productivity and increased intensity of catchment chemical weathering
Macrocharcoal accumulation rate (CHAR)	Chapter 4	Biomass burned and or fire frequency
Macrochacoal length: width	Chapter 4	Fuel type (high values = finer fuels, likely more herbaceous fires; low values = more likely woody fires)

Additionally, we performed a COnstrained Incremental Sum of Squares (CONISS) cluster analysis in the *rioja* package in R to investigate the responses of plants to climate, catchment and fire history (using the Viridiplantae portion of the *seda*DNA record as input 'plant' data).

Results

Sequencing data quantity, quality, and ancient signal authentication

We were able to recover ancient DNA from all 26 samples of the 7,000-year-old Lashmars Lagoon lake record. In total, 1,298,881,402 raw reads were sequenced. After filtering and alignment to the SSU database, a total of 272,819 reads were assigned in the samples, and 127 in the controls. Overall, 21 species (15 Bacteria and six Eukaryota) were identified in the controls and were therefore considered contaminants; 11 were identified in the subsampling controls and 10 in the extraction blank controls (Table S1).

To authenticate the ancient origins of our signal, we analysed the percentage of DNA damage in different taxonomic subgroups. All groups exhibited a general increase in ancient signal with sample age, characteristic of ancient DNA degradation over time (Figure S1). On average across all samples the Viridiplantae DNA was the most degraded (19% ± 5% damage), followed by Eukaryota (18% ± 5%), Archaea (17% ± 7%) and Bacteria (16% ± 6%) (Table S2).

Relative taxonomic composition derived from *sed*aDNA

The relative taxonomic composition of Eukaryota, Bacteria and Archaea changed with depth (and therefore age) throughout the sediment core (Figure 2A). Prokaryotes (Bacteria and Archaea) accounted for most of the genetic signal in the samples (89% on average across all samples). Eukaryota sporadically contributed large portions to the composition of certain samples (e.g., up to 46% at ~1840 CE, based on the chronology presented in Chapter 3). While the relative abundances of Bacteria and Archaea were anticorrelated, the relative abundance of Eukaryota varied independently. There was an obvious shift in the contribution of each of the three domains at ~3.3 ka: 1) the relative abundance of Eukaryota increased from an average of 4% to an average of 17%, 2) the Bacteria begin a gradual increase and 3) the Archaea begin a gradual decrease (Figure 2A).

The green plant (Viridiplantae) phyla dominated the Eukaryota signal (Figure 2B). The Viridiplantae kingdom was composed of the Chlorophyta (green algae) and the Streptophyta (land plants and Charophyte green algae). The Viridiplantae kingdom made up an average of 58% of the Eukaryota signal per sample but explained 98% of the overall signal variance (Pearson's correlation coefficient $R^2=0.98$; Figure S2). Five samples were noteworthy for their conspicuously high Streptophyta relative abundance: 2.97 ka (78%), 2.71 ka (92%) and the youngest three samples: 1800 CE (76%), 1840 CE (92%) and 1960 CE (69%). Chlorophyta exhibited a period of relative dominance between 2.45 ka and 0.76 ka, making up 31% of the Eukaryota signal per sample in this period.

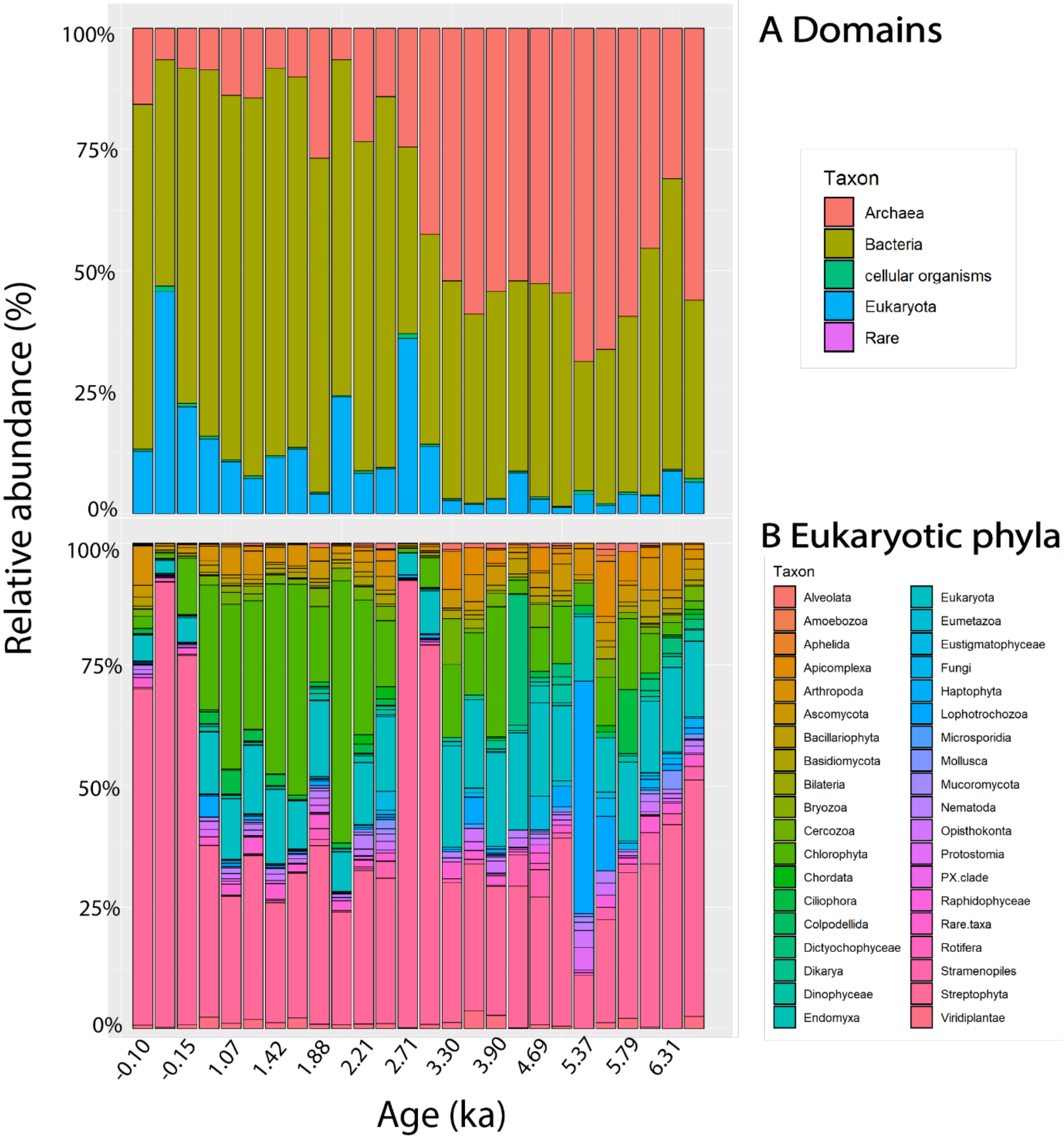

Figure 2. *Relative abundance of major taxonomic groups at Lashmars Lagoon over the past ~7 thousand years (ka) of A) all cellular organisms at domain level and B) Eukaryota at phylum level. The group 'Rare.taxa' summarises all taxa that did not occur at over 1% in at least one sample.*

Given the importance of Viridiplantae in the Eukaryota signal, we explored the composition within this kingdom at higher taxonomic resolution (Figure 3). CONISS analysis of the Viridiplantae genera revealed that the most significant composition change occurred between samples dated 3.30 ka and 2.71 ka. Another pronounced change occurred between 0.76 ka and 1800 CE. The class of flowering plants Magnoliopsida dominated the Viridiplantae signal, contributing 68% on average across all samples. However, inspection of this clade at species level returned high yields of exotic taxa such as *Citrus maxima* (pomelo), and to a lesser degree, *Ammopiptanthus mongolicus, Nicotiana attenuata* (tobacco), *Daucus carota* (wild carrot), *Myriophyllum spicatum* (Eurasian water milfoil), *Gossypium arboreum* (tree cotton), *Nelumbo nucifera* (Indian lotus) and *Capsicum annuum* (capsicum) (Figure S3). This reflected a broader trend of exotic taxa identification in the Viridiplantae. For example, nine out of the 10 most abundant species within the phylum Streptophyta were of exotic origin and are likely misassignments. Other observed taxa were of less dubious origin, for example, *Dunaliella salina*, an ecologically informative algae species commonly found in saline lakes in Australia, and *Myriophyllum*, a genus of freshwater aquatic plant known to occur in KI lakes (Clark, 1976). We also report detection of taxa from two genera of marine angiosperms (seagrasses) known to occur nearby, *Posidonia* and *Zostera*.

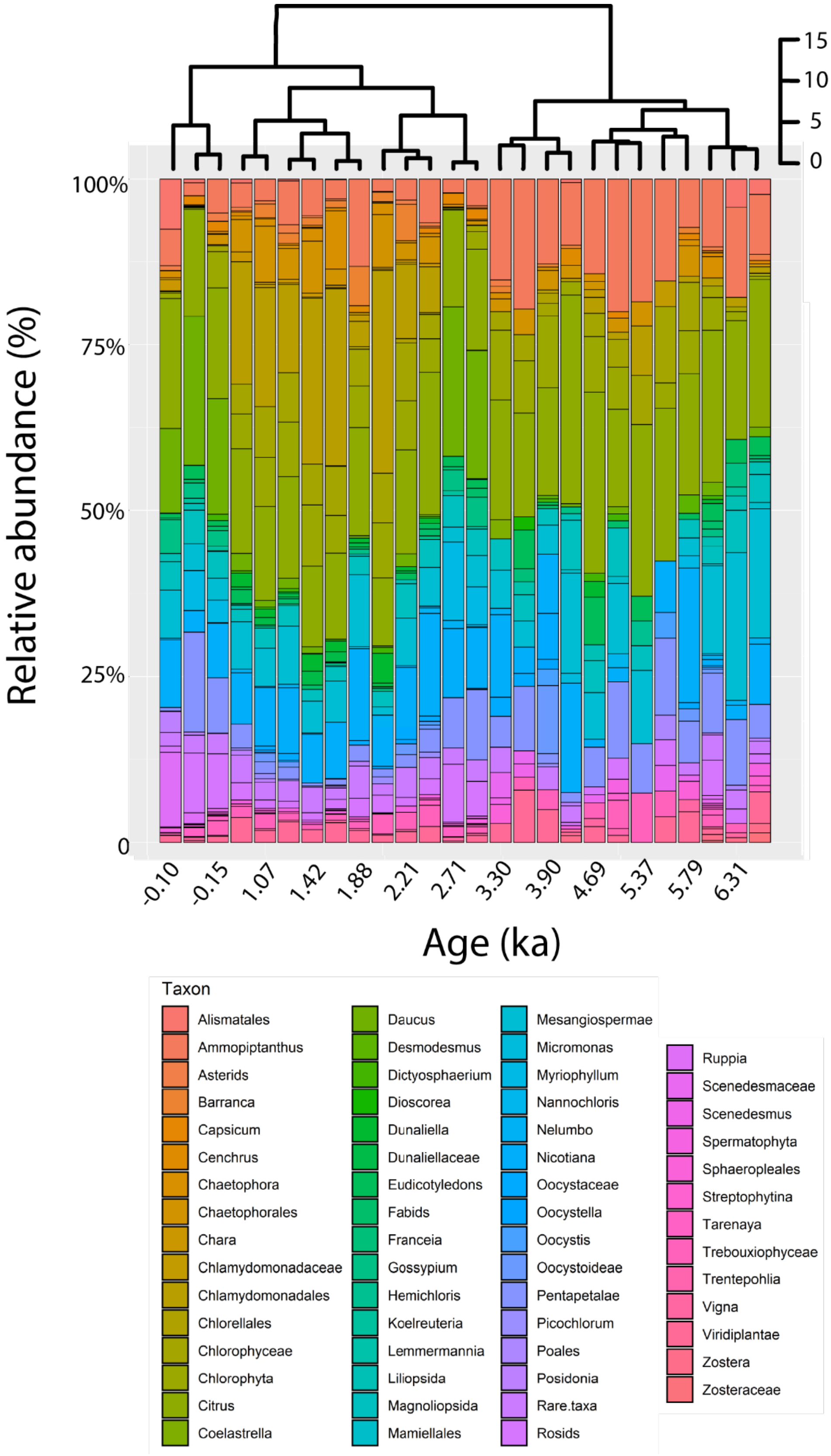

Figure 3. *Relative abundance and CONISS analysis of Viridiplantae genera at Lashmars Lagoon over the past ~7 thousand years (ka). The group 'Rare.taxa' summarises relative abundance of taxa that do not occur at over 1% in at least one sample.*

Multivariate statistical analysis of *seda*DNA and key environmental variables

We found that three of the five key environmental proxies (Ca, Br and charcoal length: width ratios) determined from the Lashmars Lagoon sediments in Chapter 3 and 4 were significant predictors (p-values < 0.05) of variation within the six taxonomic groupings we tested (Table 3). Ca, a proxy for sediment calcite concentration linked to relatively dry climate (Chapter 3), significantly explained the variance in all groupings. Br, a proxy for sediment organic matter and linked with relatively wetter conditions (Chapter 3), explained variation within the Archaea. Finally, charcoal length: width ratios, which was inferred in Chapter 4 to be a tentative proxy for fire fuel type, was a significant explanatory variable for the Eukaryota, Viridiplantae and Streptophyta.

Table 3. *Summary of p-values from the ANOVAs performed on the CCAs of different subsets of the sedaDNA data using genus level taxonomic resolution. ANOVA permutations = 999. Significant values (p < 0.05) are shown in bold font.*

	Ca (sediment calcite concentration)	**Br (sediment organic matter)**	**Ti (detrital input)**	**CHAR (function of biomass burned and fire frequency)**	**Charcoal length: width (fire fuel type)**
Archaea	**0.001**	**0.001**	0.176	0.073	0.059
Bacteria	**0.001**	0.652	0.705	0.443	0.061
Eukaryota	**0.010**	0.101	0.592	0.798	**0.006**
Viridiplantae	**0.002**	0.283	0.533	0.767	**0.043**
Streptophyta	**0.026**	0.160	0.264	0.783	**0.006**
Chlorophyta	**0.003**	0.216	0.858	0.867	0.392

Discussion

Preservation of *seda*DNA at Lashmars Lagoon

Lake *seda*DNA is thought to preserve best in cold stratified lakes with anoxic sediments (Parducci et al., 2017). Lashmars Lagoon, however, has a shallow, well-mixed water column that has been known to occasionally dry out over hot, dry Australian summers. As such, Lashmars Lagoon met few of the criteria for optimal DNA preservation in lake sediments (Parducci et al., 2018). Despite this, we successfully extracted high yields of *seda*DNA with shotgun metagenomics from a ~7,000-year-old sequence, encouraging further application of the technique to other challenging environments.

Recently, the effects of pH and conductivity have also been explored by Jia et al. (2022) who found optimal DNA preservation in cool water lakes with conductivities of 100 – 500 µS/cm (freshwater) and pH 7 – 9 (neutral to slightly alkaline lakes). The alkaline nature of Lashmars Lagoon (pH 9.9, Chapter 3) may therefore assist the persistence of DNA in the sediment. On the other hand, the brackish and relatively warm lake water, resulting in conductivity of 12,790 µS/cm at the time of sampling in Spring 2020 (Chapter 3), is less favourable for DNA preservation.

The *sed*aDNA signal was dominated by Prokaryota (Bacteria and Archaea). However, we also report a relatively high proportion of Eukaryotic reads (up to 46%, and on average 11%) compared to what has previously been detected in Australian environments with the same method (< 2%), albeit in marine settings (Armbrecht et al., 2020). We demonstrated increasing *sed*aDNA damage with age across all three domains (Eukaryota, Bacteria, Archaea) and the Viridiplantae, authenticating the ancient origins of our data.

Ecosystem change in response to fire and climate at Lashmars Lagoon, Australia, over the last ~7,000 years

The lake sediments from Lashmars Lagoon record a change from low to high macrocharcoal accumulation rates – a function of either biomass burned or fire frequency – at ~3.3 ka (Chapter 4). The change was hypothesised to be the result of a culmination of the loss of Aboriginal stewardship, regional aridification and a putatively denser shrubby midstory vegetation increasing fuel loads (Chapter 4). In the *sed*aDNA record presented in this study, we observed broadscale ecological shifts coincident with the fire regime transition: 1) an abrupt increase in the Eukaryota signal, and 2) the onset of a more gradual shift towards Bacterial over Archaeal dominance within the Prokaryota that stabilised around 2.45 ka.

The increase in the Eukaryota signal was primarily explained by an increase in the relative abundances of the Viridiplantae kingdom, comprised of phyla Streptophyta and Chlorophyta (Figure 5). This increase suggests a link between increased vegetation and higher amounts and or frequency of biomass burning, lending support to the hypothesis developed in Chapter 4 that the change in fire regime was coincident with an increase in denser, mid-story vegetation. Furthermore, the major compositional shift within the Viridiplantae occurred at ~3.3 ka, highlighting the link between vegetation and fire at Lashmars Lagoon. Similarly, statistical analysis of pollen data also revealed a change in composition at this point, although the shift at 5.5 ka from Casuarinaceae to *Eucalyptus,* putatively linked to climate, was more significant in the pollen data (Chapter 4). This change was characterised by a switch in arboreal taxa from Casuarinaceae to *Eucalyptus*. In our *sed*aDNA record at ~3.3 ka, we found a sudden decrease in the relative abundance of the genus *Ammopiptanthus* (Fabaceae family) in the Streptophyta, apparently linked to the increase in charcoal abundance (Figure 5). *Ammopiptanthus* is endemic to the central Asian desert and thus may be a misidentification of *Acacia*, the closest relative identified in the Lashmars Lagoon pollen record (Table 4). Some *Acacia* species are resilient to fire in Australia (Gordon et al., 2017), however, changes in fire regimes and climate conditions at this time may have been incompatible with the persistence of *Acacia* species at Lashmars Lagoon. We also found that charcoal length: width, a metric putatively linked to fire fuel type in Chapter 4, was a significant explanatory variable for the

Streptophyta (predominantly land plants) but not for the Chlorophyta (green algae), suggesting that changes in terrestrial vegetation coverage influenced the change in fire regimes and the type of fuel burnt.

While the link between the Viridiplantae and fire from the *sed*aDNA builds on decades of research into the relationship between fire and vegetation in Australia (e.g., Singh et al., 1981, Kershaw et al., 2002, Bowman et al., 2012, Mariani et al., 2022), mechanisms linking changes in the Prokaryota to fire are more elusive and warrant further investigation beyond the scope of this proof-of-concept study. Some avenues that could be explored in future research include: 1) the effects of fire on catchment soil microbes, recently investigated in the United States forests on short-term, modern timescales (Nelson et al., 2022) and or 2) the effects of changing lake chemistry from ash and other pyrogenic debris on lake and sediment microbiomes (Pereira et al., 2012, Korsman and Segerstrom, 1998). It is also possible that the shift after 3.3 ka from Bacteria to Archaea dominance in the prokaryotic signal is not related to the changes in fire regime but is rather an artefact of changes in lake depth or the vertical stratification of extant microbes in the sediment. This phenomenon is known to occur in other lakes (Rissanen et al., 2019, Wurzbacher et al., 2017), albeit these examples span much shallower sediment profiles than the ~7.5 m of sediment analysed from Lashmars Lagoon. That said, we can be confident of the authenticity of the prokaryotic *sed*aDNA signal, for which we specifically selected DNA fragments in the target range for ancient DNA. Subsequent damage analysis further revealed: 1) increasing DNA damage with depth for the Prokaryota, consistent with DNA of an ancient origin, and 2) average DNA damage for Bacteria (16%) and Archaea (17%) was slightly lower but comparable to the more indisputably ancient taxonomic grouping of Viridiplantae (19%).

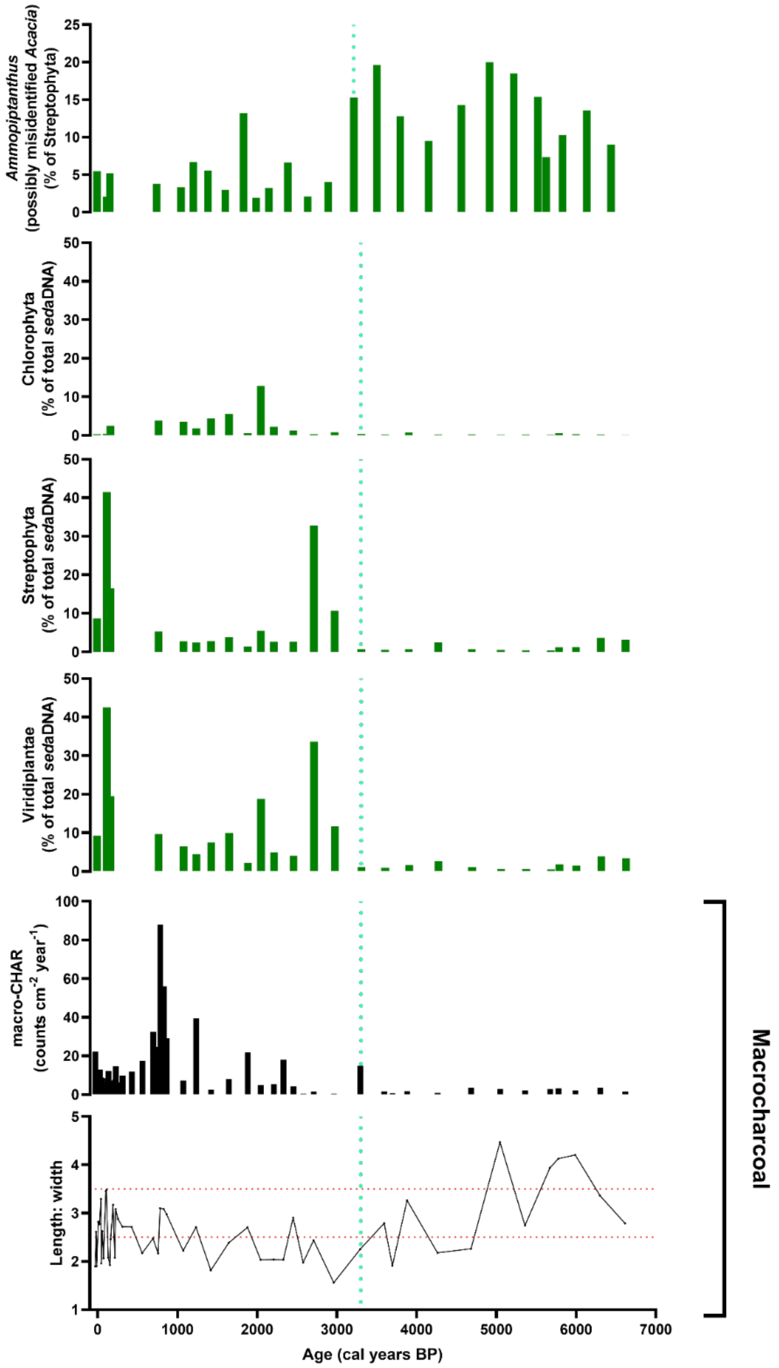

Figure 5. *Summary of vegetation and fire at Lashmars Lagoon. (A) Relative abundance of the genus Ammopiptanthus (Fabaceae family, possibly misidentified Acacia) as a portion of the Streptophyta. (B-D) As a portion of the total sedaDNA signal key taxa: (B) Chlorophyta, (C) Streptophyta and (D) Viridiplantae. (E-F) Key fire metrics from Chapter 4: (E) macrocharcoal accumulation rate (macro-CHAR), a function of biomass burning or fire frequency and (F) Length to width ratio of charcoal particles (higher values = finer, grassier, fuel types). Green dotted line signifies the change in fire regime at 3,300 years ago.*

In addition to the relationship between fire and ecology, we also considered the impact of climate through correlating the *sed*aDNA data with geochemical proxy data determined in Chapter 3. Ca was a significant environmental determinant of compositional variation in all the subgroups analysed (Bacteria, Archaea, Eukaryota, Viridiplantae, Streptophyta and Chlorophyta). We determined Ca to be more prevalent in drier climates, thus suggesting a level of climatic control across all five taxonomic subgroups.

Ca primarily correlated with sediment calcite content, the production of which was inferred, in the context of corroborating evidence, to have been controlled primarily by climate (Chapter 3). Calcite is suggested to have been produced in higher quantities by biogenic sources in drier climates when lake water salinity increases through evaporation, and through the increased contribution of seawater to the lake via possible marine incursions. Thus, Ca reflects both sediment properties and, more indirectly, climate and catchment processes. It is therefore difficult to disentangle the primary driver of Ca over the whole record. We posit that the Streptophyta, predominantly a terrestrial group, was more likely influenced by climate factors that acted to control Ca, than changes in lake chemistry that came about from climate shifts. On the other hand, the community composition of the Chlorophyta, which are predominately aquatic, would more likely be responding to changes in lake chemistry such as salinity, which can be a direct result of climate or marine influences at Lashmars Lagoon. It is also prudent to reiterate that these proxies are all related to key sedimentological properties that may exert some control over the presence or preservation of some taxa. A further consideration is the effect that the inferred shallowing of Lashmars Lagoon in the Late Holocene had on the accumulation and preservation of the *sed*aDNA record, as Ca correlated with quartz, a proxy for lake depth (Chapter 3). To fully assess the impact of climate on the *sed*aDNA composition at Lashmars Lagoon, independent climate or hydrological proxies, such as oxygen isotopes (e.g., (Chamberlayne et al., 2022), should be integrated into this analysis. Finally, considering the ecological context of the *sed*aDNA data may also assist to disentangle the variables (e.g., specifically targeting taxa with known climatic and environmental tolerances).

Comparison of *sed*aDNA and pollen records from Lashmars Lagoon, Australia

Pollen and *sed*aDNA data can be integrated to create more holistic illustrations of past vegetation (Parducci et al., 2013, Parducci et al., 2015). The CONISS analysis of both pollen and Viridiplantae *sed*aDNA records from Lashmars Lagoon revealed significant shifts in plant composition coincident with the shift in fire regime at ~3.3 ka, corroborating the importance of this change. This change was more prominent in the *sed*aDNA, while a change at ~5.5 ka, postulated to be related to climate, was important in the pollen but insignificant in the *sed*aDNA. The discrepancy might be attributed to the fact that the two techniques capture biological information on different scales; pollen is known to travel vast distances to be deposited in lake sediments and can be more reflective of regional vegetation, while *sed*aDNA is more reflective of local communities (Boessenkool et al., 2014). At Lashmars Lagoon, the regional pollen signal, which perhaps includes the nearby mainland, appears thus to be more strongly controlled by climate, while local vegetation changes on KI were more fire dependent.

Comparison of pollen and *sed*aDNA data led us to conclude that the Viridiplantae data is most appropriately analysed at family level, as higher taxonomic resolutions were plagued with problems of reference database bias that favour exotic taxa (Table 4). Some temporal trends in the prominent *sed*aDNA families echo those observed in the pollen (e.g., Haloragaceae*,* Fabaceae, Apiaceae), in essence cross-referencing the identification of these individual taxa. However, other groupings are less coherent across the two different methods and demand more detailed interrogation (e.g., Rutaceae, Malvaceae, Solanceae).

Nearly all the ten most common Streptophyta genera detected in the *sed*aDNA data were identified to at least family level in the pollen data, except for the lotus-lily family, Nelumbonaceae, which was only represented in the pollen at the order level (Table 4). However, these taxa were comparatively poorly represented in the pollen data, a finding that is not uncommon in other studies that compare pollen and plant *sed*aDNA (Matisoo-Smith et al., 2008, Parducci et al., 2019, Parducci et al., 2013, Parducci et al., 2015, Pedersen et al., 2013, Wilmshurst et al., 2014, Courtin et al., 2022). Importantly, sequences assigned to the exotic genus *Ammopiptanthus* (Fabaceae family) were the second most abundant genera of Streptophyta in the *sed*aDNA record, but *Acacia,* its most closely related representative in the pollen record, constituted an average of just 2% in each sample. In this regard, our findings mirror a study of Eastern Siberian permafrost deposits where palynology failed to detect Fabaceae pollen, while both shotgun and metabarcoding *sed*aDNA analyses succeeded in detecting Fabaceae presence (Courtin et al., 2022). This is a significant finding, as *Acacia* pollen is typically underrepresented in Australian pollen records due to poor pollen dispersal (Francke et al., 2022, Mariani et al., 2022), and thus its long-term fire responses are poorly understood. Surprisingly, we also noticed that the two most abundant pollen taxa, the quintessentially Australian *Eucalyptus* and Casuarinaceae, were conspicuously absent from the *sed*aDNA record. This may be due to a pollen-production bias, differences in taphonomic pathways between *sed*aDNA and pollen, or could be a further demonstration of a northern hemisphere bias in the DNA reference database.

Table 4. *The ten most prevalent species of the phylum Streptophyta (which include the land plants) detected in the Lashmars Lagoon sedaDNA record, their closest representatives in the pollen record (Chapter 4) and their closest extant native relatives. Species are presented in order of most to least prevalent. Closest extant native relatives were determined with the curated online database 'syzygium.xyz/saplants' and a species list of flora found in Lashmar Conservation Park (CP) downloaded from the NatureMaps website curated by the Department of Environment and Water, South Australia.*

Species identified from *seda*DNA	**Closest related pollen taxon or taxa (Chapter 4)**	**Closest extant native relatives**
***Citrus maxima,* Rutaceae**	Rutaceae (represents an average of 1% of total terrestrial pollen counted)	Many Rutaceae species on KI but most likely *Boronia pariflora*, a wetland species common around lagoons on KI
***Ammopiptanthus mongolicus,* Fabaceae**	*Acacia* (2%), Fabaceae (0.3%), Caesalpinioideae (0.02%)	Pollen suggests could be one of the 18 species of *Acacia* native to KI
***Nicotiana attenuate,* Solanceae**	Solanceae (0.3%)	Might be *Nicotiana maritima,* an erect herb that often grows near the coast or *Solanum simile* (known to occur in the nearby Lashmar CP)
***Daucus carota,* Apianceae**	Apiaceae (2%)	Potentially *Daucus glochidiatus* (native carrot) that has been recorded in Lashmar CP but many species of Apiaceae are found on KI
***Myriophyllum spicatum,* Haloragaceae**	*Myriophyllum* (9%), Haloragaceae (4%), *Myriophyllum muelleri* (2%)	Could be *Myriophyllum salsugineum* or *M. muelleri* (identified from the pollen record by Clark (1983a)) or possibly could be one of the other Halorgaceae species recorded in taxonomic surveys of the area
***Gossypium arboretum,* Malvaceae**	Malvaceae (0.6%)	Might be one of the three *Lawrencia spp.*, small salt tolerant herbs or the shrub *Malva weinmanniana*
***Ruppia maritima*,* Potamogetonaceae**	Potamogetonaceae (2%)	Could be a valid identification but may also be one of the other Potamogetonaceae species known to occur on KI
***Nelumbo nucifera,* Nelumbonaceae**	Not identified in pollen but the family Proteaceae was identified (*Banksia* (0.1%) and Proteaceae (0.01%))	Nelumbonaceae are not native to KI, more likely to be a species from the Proteales order like *Banksia spp.*
***Capsicum annuum,* Solanceae**	Solanceae (0.3%)	A handful of species in the Solanceae family are native to KI

**Not of definite exotic origin*

Future directions

Our dataset has enormous potential for further exploration. However, the problem of reference bias first needs to be addressed. Next, future work should concentrate on examining the trends in the prokaryotic signal and understudied taxa. New techniques can also be applied to build on the existing dataset. For example, reads of focus taxonomic groups like the Viridiplantae could be amplified through the application of hybridisation capture (Horn, 2012). There is potential too to answer evolutionary questions through the reconstruction of complete genomes (Lammers et al., 2021), so that one day we may be able to understand the genetic basis for adaptations in *Acacia* on KI in response to changes in fire regime. We also acknowledge the importance of co-designing research questions in this space with local land managers, especially on KI where the question of ecosystem – fire dynamics is incredibly pertinent for management concerns. The creation of scientific knowledge in this space should align closely with their priorities.

Conclusions

Our study used shotgun metagenomics to unearth molecular vestiges of ancient terrestrial and aquatic ecosystems preserved in a lake sedimentary ancient DNA archive at Lashmars Lagoon, Kangaroo Island (Karti), Australia. We confirmed the authenticity of our ~7,000-year-old ancient signal with DNA damage analysis. As such, this study represents the oldest lake *sed*aDNA record in Australia, a significant finding given the common assumption that warm, dry climates are not suitable for preserving ancient environmental DNA.

Here, we provide new biological insights from an important palaeoenvironmental site with a complex climatic, cultural, ecological and fire history. We found Ca, a proxy associated with climate drying, was significantly linked with ecosystem wide changes over the last 7,000 years. We also found significant relationships between changes in fire regime and the composition of the Viridiplantae, particularly within the phylum Streptophyta, which includes the land plants, but faced challenges of taxonomic bias when interpreting temporal trends of individual taxa within the group. Nevertheless, we augmented our understanding of past vegetation change at Lashmars Lagoon by comparing our *sed*aDNA record to native extant local taxa and a pollen record from the same site. For example, we detected high relative abundances of what is likely to be *Acacia* in the *sed*aDNA, a fire adapted genus typically underrepresented in Australian palynology due to poor pollen dispersal. However, more work is needed to completely untangle discrepancies between pollen and *sed*aDNA data to distinguish between real complementary palaeoecological insights gleaned from the methods and misleading artefacts of the taxonomic biases in reference databases.

Finally, our study demonstrates both the feasibility and value of extracting ancient DNA from lake sediments in Australia and invites others to consider the untapped potential of this novel technique in challenging environments.

Author contributions

LD – Conceptualisation, field work, investigation, formal analysis, visualisation, writing – original draft; LA – Conceptualisation, supervision, investigation, formal analysis, writing – review and editing; VPG – Supervision, formal analysis; HC – Supervision, writing – review and editing; JT – Conceptualisation, field work supervision, formal analysis, writing – review and editing; AF – Field work, investigation, writing – review and editing; WL – Supervision, field work, visualisation.

Acknowledgements

In Australia, we work and live on Aboriginal land. This research was developed and conducted on Kaurna Yerta (Kaurna land) at The University of Adelaide and on Karti (Kangaroo Island), a place of deep cultural and spiritual significance for Aboriginal people in South Australia, including for Ngarrindjeri, Kaurna, Narungga, Nhawu and Barngarla people. I pay my respects to elders past, present, and emerging. I acknowledge that sovereignty was never ceded.

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Supplementary information

Table S1. *List of contaminants identified in the controls.*

	Air control 1	Blank swab	Air control 2	Bench swap	EBC 1	EBC 2
Euphorbia tirucalli	0	0	0	0	1	0
Bacteroidia	0	2	0	0	0	0
Thecofilosea	3	1	3	0	0	0
Teleostei	0	0	0	0	0	1
Basidiomycota	0	0	0	0	2	0
Kabatiella microsticta	0	0	2	0	0	0
Pycnora sorophora	0	0	0	0	1	0
Streptococcus pneumoniae	0	0	0	0	1	0
Lactobacillus	1	0	0	0	0	0
Lactiplantibacillus plantarum	0	0	0	0	1	0
Bacillales	0	0	0	0	1	0
Microbacterium	0	0	0	0	4	0
Luna-1 subcluster	0	1	0	0	0	0
Paracoccus	0	0	0	0	1	0
Acetobacteraceae	1	0	0	0	0	0
Rickettsiales	0	1	0	0	0	0
Sphingomonas	0	0	0	0	1	0
Enterobacterales	0	2	0	0	0	0
Acinetobacter lwoffii	0	1	0	0	0	0
Perlucidibaca	0	5	0	0	0	0
Pseudomonas	0	2	1	0	0	0

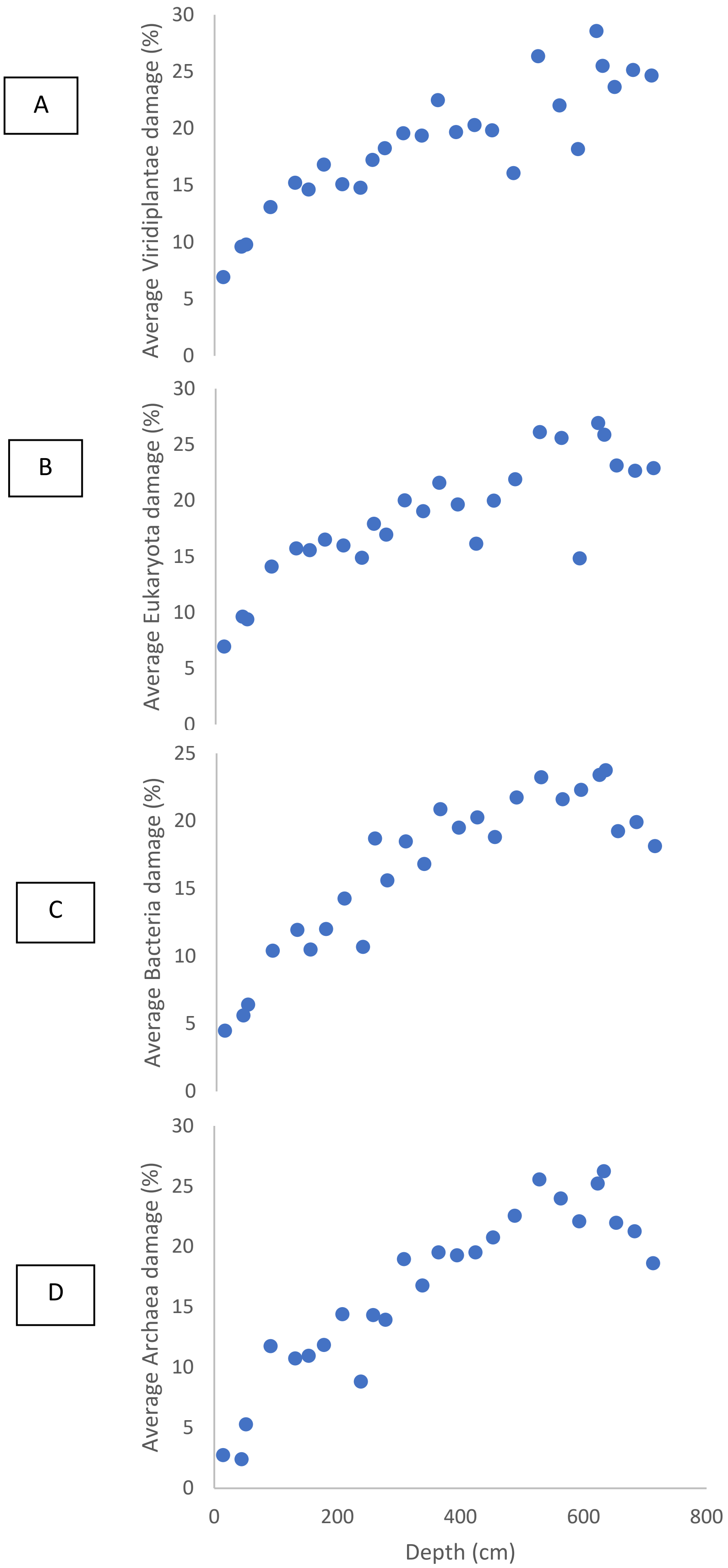

Figure S1. *SedaDNA damage analysis through the depth profile. Average damage (%) for (A) Viridiplantae, (B) Eukaryota, (C) Bacteria and (D) Archaea. Percent damage calculated from the* *ancient portion of the total reads for each group (Viridiplantae and Eukaryota) in HOPS.*

Table S2. *Average, minimum and maximum sedaDNA damage of all 26 samples across the three domains of life (Eukaryota, Bacteria, Archaea) and within the kingdom Viridiplantae.*

	Eukaryota	Viridiplantae	Bacteria	Archaea
Ave % damage	18%	19%	16%	17%
Min % damage	7%	7%	4%	2%
Max % damage	27%	29%	24%	26%

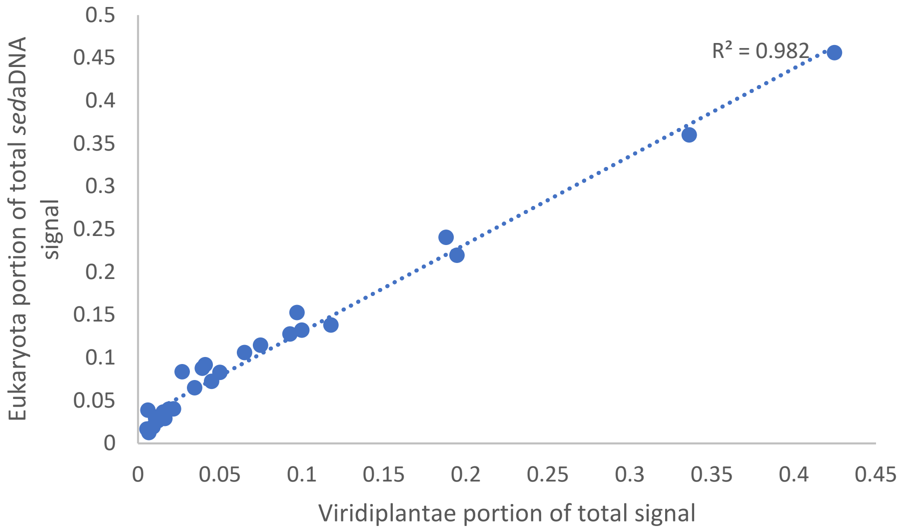

Figure S2. *Pearson's correlation of Eukaryota and Viridiplantae portions of the total sedaDNA signal.*

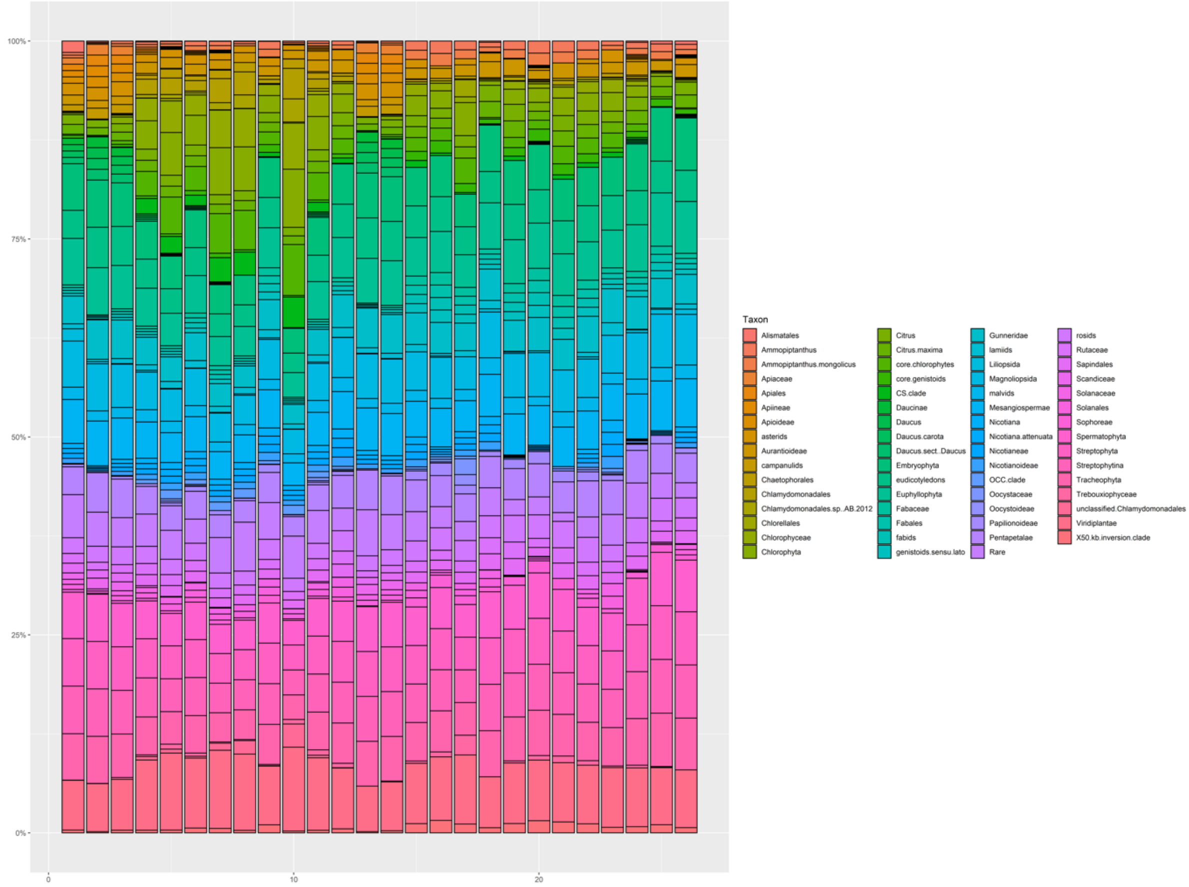

Figure S3. *Relative abundance of Viridiplantae species at Lashmars Lagoon over the past ~7 ka. The group 'Rare taxa' summarises relative abundance of taxa that do not occur at over 1% in at least one sample.*

Note S1. *R-code for data visualisation*

```
#Filtering and visualising data from raw MEGAN csv's

#MEGAN files should be exported as csv's selecting
  #data to export: 'taxonName_to_count'
  #count to use: 'assigned'
  #separator to use: 'comma'

#file structure should be:
    #MEGAN filters and figures - MAKE THIS THE WORKING DIRECTORY
      #put all the raw text files exported from MEGAN here
       #I suggest naming them 'Group_resolution' e.g. 'Fungi_class'
      #put the a file called 'sample_no.csv' here - it must be a csv
        #should be a list with depth information or the order of samples
      #Filtered_tables
        #output of the 'rarefilter' function will appear here
      #Figures
        #output of the 'makeplot' function will appear here

#load required packages
library(readxl)
```

```
library(dplyr)
library(writexl)
library(tidyr)
library(janitor)
library(ggplot2)
library(cowplot)

#set wd as 'MEGAN filters and figures' folder and name it
#wd <- setwd("./MEGAN filters and figures")
wd <- setwd("C:/…")

#read in a list of the raw data files you want to process and their names
files <- list.files(path="./", pattern = ".txt+")
file_list <- as.data.frame(files)
names <- gsub(".txt","",as.character(files))
file_names <- as.data.frame(names)

#define the functions
  #function 1 is 'rarefilter':
    #discards all groups with 0 total counts
    #converts counts to relative composition
    #filters data to group rare taxa
      #rare taxa are defined as not > a given threshold (1% default) in a given number of samples (1 is the default))
    #produces a csv of the filtered data table
rarefilter <- function(filename) {
  data <- read.delim(file = filename, sep=",")
  data_t <- t(data)
  data_rowToNames <- row_to_names(data_t, row_number = 1)
  data_df <- as.data.frame(data_rowToNames)
  data_df[] <- lapply(data_df, as.numeric)
  data_clean <- data_df[,colSums(data_df) > 0]
  data_clean_percent <- prop.table(data.matrix(data_clean), 1)
  data_clean_percent_binary <- prop.table(data.matrix(data_clean), 1)
  data_clean_percent_binary[data_clean_percent_binary>0.01]=1 #change me to change the threshold for rare taxa
  data_clean_percent_binary[data_clean_percent_binary<0.01]=0 #change me to change the threshold for rare taxa
  data_clean_percent_binary_df <- as.data.frame(data_clean_percent_binary)
  data_naOmit <- na.omit(data_clean_percent_binary_df) #gets rid of rows (samples) that were all zeros
  rare_taxa_names <- colnames(data_naOmit[,colSums(data_naOmit) < 1]) # change me to change the number of samples for the rare taxa filter
  data_rare_taxa_percent <- data_clean_percent[, rare_taxa_names]
  Rare <- rowSums(data_rare_taxa_percent) #change 'Rare' to what you want to name the group e.g. 'Taxa < 1%'
  data_clean_percent_df <- as.data.frame(data_clean_percent)
  data_clean_percent_notRare <- select(data_clean_percent_df, -(rare_taxa_names))
  sample_no <- read.csv("./sample_no.csv")
  pt_input <- cbind(sample_no,data_clean_percent_notRare,Rare)
}
```

```
 #function 2 is 'makeplot'
  #makes a relative abundance barchart
makeplot <- function(filename){
 ggplot(filename, aes(fill=Taxon, y=RelativeAbundance, x=SampleNo)) +
  geom_bar(position="fill", stat="identity") +
  ylab("") +
  xlab("") +
  geom_col(colour = "black", position = "fill") +
  scale_y_continuous(labels = scales::percent)
}

#run both functions and export filtered data table and figure
list_length <- nrow(file_list)
for(i in 1:list_length){
 x <- file_list[i,1]
 filtered_data <- rarefilter(x)
 write.csv(filtered_data, paste0(wd,"/Filtered_tables/",file_names[i,1],"_filtered",".csv"))
 plotme <- data.frame(filtered_data %>%
                 pivot_longer(-SampleNo, names_to = "Taxon", values_to = "RelativeAbundance"))
 mybarplot <- makeplot(plotme)
 png(filename = paste0(wd,"/Figures/",file_names[i,1],".png"), width = 16, height = 10, units = 'in',
res = 300)
 print(mybarplot)
 dev.off()
}
```

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6

Discussion

In this MPhil thesis, I present a study of a unique sedimentary lake archive from southern Australia. I attempt to untangle the complex interplay of climate, landscape, fire, ecosystems, and humans on millennial timescales – providing long term baselines that contextualise current environmental changes in Australia.

To these ends, I have used a multi-proxy approach to reconstruct Earth system processes at Lashmars Lagoon, Kangaroo Island (Karti), southern Australia, using a ~7,000-year-old lake sediment record. These proxies included geochemical, charcoal, pollen, and sedimentary ancient DNA (*sed*aDNA) analysis. I then compared my data to regional records of climate, vegetation and fire and used available archaeological and historical sources to consider the environmental implications of the putative precolonial loss of Aboriginal stewardship from the island, previously suggested to have caused a change in fire regime at ~2.5 ka (Clark, 1983).

The aims of this research project were achieved in the chapters of this thesis, specifically through:

1. **Establishing a high-resolution updated chronology for new sediment cores from Lashmars Lagoon (Chapter 3).** I refined the chronology using high resolution Bayesian methods, allowing my record to be compared reliably to contemporary palaeoenvironmental reconstructions. I found mostly continuous sedimentation for my ~7,000-year-old record. This refines the timing of the onset of the change in fire regime from ~2.5 ka (Clark, 1983) to ~3.3 ka.
2. **A detailed geochemical study of the sediments to understand catchment dynamics, provide indirect climate proxy data and resolve uncertainties from previous studies (Chapter 3).** I found major sedimentological changes throughout the record. I constrained the timing of these major changes to shifts at 5.86 ka, 5.55 ka, 4.24 ka, 2.54 ka and 0.13 ka using proxies inferred to reflect salinity, sediment organic matter and chemical to physical weathering. The six stratigraphic units that were defined are summarised below:

   a. Unit 1 | 6.77 – 5.86 ka: inferred drier climate with low organic matter, high inferred salinity, and lower chemical compared to physical weathering
   b. Unit 2 | 5.86 – 5.55 ka: inferred brief wetter climate phase with high organic matter and high chemical compared to physical weathering
   c. Unit 3 | 5.55 – 4.24 ka: inferred somewhat drier climate with low organic matter, moderate inferred salinity, and low chemical to physical weathering ratio
   d. Unit 4 | 4.24 – 2.54 ka: inferred sustained wetter phase with high organic matter, low inferred salinity, and high chemical compared to physical weathering
   e. Unit 5 | 2.54 – 0.13 ka: inferred pronounced dry phase with low organic matter, high inferred salinity, and low chemical compared to physical weathering
   f. Unit 6 | 0.13 ka – 2020 CE: climatically ambiguous variable phase with high organic matter, high inferred salinity, and variable weathering – potentially reflecting change in catchment land use associated the introduction of European land use

   Importantly, through XRD analysis of modern catchment soils and inflow sediments, I found no evidence of change in sediment source across these different zones.

3. **Reconstructing vegetation and fire history using traditional palaeolimnological techniques of pollen and charcoal analysis (Chapter 4).** Macrocharcoal analysis revealed a change in fire regime at ~3.3 ka from lower to higher fire frequency and or biomass burning. Statistical analysis of the pollen record showed two major changes in vegetation composition linked to climate and fire. The first change at ~5.5 ka reflected a change from *Eucalyptus* to Casuarinaceae dominated woodland, and the change at ~3.3 ka represented a diversifying shrubby midstory. Crucially, I found no correlation between pyrite and periods of high charcoal concentration, dispelling a key criticism of an earlier fire and vegetation study by Clark (1983): that pyrite formation biased the record.
4. **Applying a new palaeoecological tool – *sed*aDNA– to reconstruct broad scale ecological changes, paying particular attention to the Viridiplantae (the 'green plants') signal to complement pollen analysis (Chapter 5).** I successfully reconstructed a sedimentary ancient DNA record using shotgun metagenomics, capturing a larger breadth of the ecological spectrum in an environment typically considered suboptimal for DNA preservation. I found evidence for ecosystem responses to climate and fire across and within the three domains of life. Most obviously, the change in fire regime at ~3.3 ka was accompanied by a major compositional shift in the Viridiplantae and marked the beginning of a more gradual shift from Archaea to Bacteria dominance in the Prokaryota. The Viridiplantae DNA complemented the pollen record as a more local record of vegetation change, helping to

mitigate inherent biases in pollen methods, supporting taxonomic classifications and creating a more complete picture of past vegetation. Notably, large amounts of the family Fabaceae (putatively *Acacia*) were detected in *sed*aDNA record while only trace amounts were found in the pollen record. The Fabaceae in the *sed*aDNA record showed a clear response to fire, abruptly declining after the increase in fire activity at ~3.3 ka, exemplifying how this new technique can shed light on the effects of fire on typically underrepresented taxa in Australian palaeoecology.

5. **Considering these new data in the context of recent regional palaeo-reconstructions and the unique precolonial human history of Kangaroo Island (Karti) (Considered throughout this thesis).** The timing of changes in this record shows correspondence with records from western Tasmania, suggesting that the Southern Westerly Winds were also influential on Kangaroo Island (Karti) throughout the mid to late Holocene. I also noted some parallels between Kangaroo Island (Karti) and the Bass Strait Islands in the response of fire to putative human disappearance after interglacial sea level rise made the land inhospitable to humans, alluding to the influence of Aboriginal stewardship on controlling fire regimes.

Recommendations for future research

This thesis lays important groundwork for future research, both on Lashmars Lagoon and across the southern Australian region more broadly.

Most urgently, better constraint of the history of Aboriginal people on Kangaroo Island is overdue (Karti). Within a western scientific lens, more archaeological work on the island is needed. There is also a tremendous opportunity to engage with and learn from Indigenous knowledge and explore the space where these knowledges intersect with western science. Scientific knowledge about past fire, climate and ecosystem change might also represent an opportunity to repatriate knowledge about Country to Traditional Owners, especially in places where cultural disruption through colonisation has been greatest.

Another priority is the analysis of more palaeoenvironmental records from the central southern Australian region, of which there are few, to better understand the spatial variability of climate influences like ENSO and SAM on Holocene timescales. This is important for future climate projections in the region as it will help to resolve a disconcerting discord between two methods of Holocene palaeoclimate reconstructions – those based on proxy records and those produced from hindcasts of the physical models of the Earth system used for future climate projections (Ray, personal communication, 2022). Independent climate proxies that are not affected by sediment depositional processes – such as stable isotopes – are also necessary for more robust interpretations of records like Lashmars Lagoon. Further on this theme, a high-resolution record of climatic variability from the tightly chronologically constrained recent sediments of Lashmars Lagoon could be compared with the local historical instrumental record from Bureau of Meteorology archives to calibrate a deeper time climate record.

The sediments from Lashmars Lagoon, Kangaroo Island (Karti) record a shift to more frequent and or bigger bushfires – quite possibly the combined result of climate and anthropogenic land use changes – thousands of years before the widespread catastrophic fire conditions we see occurring on the mainland today. For these reasons, Kangaroo Island (Karti) may be a useful past analogue to the present. However, first the precise nature of the fire regime change should be exacted. To this end, the following research should be undertaken:

1. The low resolution macrocharcoal record should be filled in at contiguous 1 cm resolution to better disentangle fire frequency and the amount of biomass burned
2. Charcoal morphometric analyses (e.g., length to width ratios) should be calibrated with modern vegetation types common on Kangaroo Island (Karti) to provide a locally appropriate interpretive framework for our existing record
3. Fourier Transform Infrared (FTIR) spectral analysis should be applied to the macrocharcoal as a proxy for fire intensity (e.g., as in Constantine IV et al. (2021))
4. To assess the spatial variability of fire regimes on Kangaroo Island (Karti), comparative data from sites in central and western locations on the island are necessary
5. To further understand fire regimes, other types of records should also be explored to corroborate lake sedimentary archives with independent lines of evidence. For example, ash can change dripwater chemistry in the formation of speleothems (Nagra et al., 2016) and fire scars from individual fire events can be detected with dendrochronology (O'Donnell et al., 2010, Mooney and Maltby, 2006).

To ensure outcomes of palaeofire research are applicable to end users – such as natural resources managers on Kangaroo Island (Karti) and policy makers – research in this space should emphasise codesign of scientific studies to maximise tangible outcomes that address pressing local problems. In this context, local environmental knowledge has untapped potential to provide invaluable insights when interpreting data. Preliminary consultation with land managers on Kangaroo Island (Karti) has indicated that a site on the west of the island should also be studied with a matter of urgency – as this is where the fire danger is concentrated in the present day.

There is exciting potential for the novel proxy of sedimentary ancient DNA (*sed*aDNA) to be explored in Australian lake environments. However, first there must be considerable work to address severe issues with reference database bias. Given the unique history of Kangaroo Island (Karti), future studies should consider using genomic evolutionary techniques to answer timely questions about evolutionary adaptation and selection processes of key taxa (e.g., Fabaceae) in response to changes in fire, climate, and land management. Here, we also only assessed the green plant group Viridiplantae in detail. In-depth exploration of other groups, for example within the Prokaryota, is therefore another priority. *Sed*aDNA analyses also have enormous potential, when considered with other proxy data, to augment holistic interpretations of Earth systems processes. For example, the presence of halophilic taxa, like the green algae *Dunaliella salina* in the record could be used to strengthen the interpretation of the calcium record, a geochemical proxy for salinity. The detection of the DNA of marine taxa should also be considered to further understand the influence of marine incursions through time at Lashmars Lagoon. Organic rich layers can also be further characterised with DNA analysis, augmenting organic geochemical methods.

Overall, this research highlights the importance of using multiple lines of evidence to gain a holistic understanding of the Earth system and a nuanced interpretation of the long-term causes and consequences of fire in southern Australia.

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