Distribution and Evolution of C_4 and C_3 Eudicots:
Insights from Global and Local Analyses Using Extensive Data Sources

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"Every child should have mud pies, grasshoppers, waterbugs, tadpoles, frogs, mud turtles, elderberries, wild strawberries, acorns, chestnuts, trees to climb. Brooks to wade, water lilies, woodchucks, bats, bees, butterflies, various animals to pet, hayfields, pine-cones, rocks to roll, sand, snakes, huckleberries and hornets; and any child who has been deprived of these has been deprived of the best part of education." - Luther Burbank

Congress contribution

List of abbreviations

A	Central Desert
AICc	Akaike Information Criterion
AICc_wt	Akaike Weight
ALA	Atlas of Living Australia
approx.	Approximately
B	Central Queensland
BSC	Bundle Sheath Cells
C	Adelaide
CAM	Crassulacean Acid Metabolism
CBB-cycle	Calvin-Benson-Bassham cycle
D	Eyre Pennisula
Df	Degrees of Freedom
DL	Desert Lake
E	Nullarbor
F	Southwest Interzone
faml.	Families
G	Southwestern
GBIF	Global Biodiversity Information Facility
gen.	Genera
H	Eastern Desert
I	Western Desert
IBRA	Interim Biogeographic Regionalisation for Australia
IMCRA	Integrated Marine and Coastal Regionalisation of Australia
IPNI	The International Plant Names Index
km	Kilometers
KP	Karst Plain
MC	Mesophyll Cells
MCC	Maximum Clade Credibility
ME	Malic Enzyme
ML	Maximum Likelihood
mm	Millimeters
MTC	Mediterranean-Type Climate

Mya	Million Years Ago
PC	Principal Component
PCA	Principal Component Analysis
PEPC	Phosphoenolpyruvate Carboxylase
PEP-CK	Phosphoenolpyruvate Carboxykinase
PGA	3-Phosphoglycerate
POWO	Plants of the World Online
ppm	Parts Per Million
RADseq	Restriction Site-Associated DNA Sequencing
RL	Riverine Desert
RuBisCO	Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase
RubP2	Ribulose-1,5-Bisphosphate
s.l.	Sensu lato
s.s.	Sensu stricto
SD	Sand Desert
SP	Shield Plain
spp	Species (plural)
UV	Ultraviolet
WCVP	World Checklist of Vascular Plants

General Introduction

Since water is the lifeblood of the world's flora but often deficient, plants have developed different strategies to cope with varying levels of water availability. Central to their survival is a positive photosynthesis balance — the process by which plants, algae, and certain bacteria convert light energy into chemical energy, sustaining life on Earth. However, not all plants rely on the same approach to this essential process. This dissertation examines the distribution of certain plant groups, particularly focusing on how ecological conditions influence their prevalence and adaptation strategies. Many plants rely on C_3 photosynthesis, also referred to as the Calvin-Benson-Bassham cycle (CBB-cycle, Bassham et al., 1950). Over 85% of terrestrial plants use this pathway (Ehleringer et al., 1991; Raines, 2022), which involves a straightforward mechanism for capturing carbon dioxide. It operates through the Calvin cycle, where carbon dioxide is fixed into a three-carbon compound, 3-phosphoglycerate (Ehleringer et al., 1997). This pathway is prevalent in temperate and tropical plants, including major agricultural crops such as wheat, rice, and soybeans (Sage, 2004). C_3 photosynthesis is effective under moderate temperatures and abundant water. However, under conditions of high temperature and intense light, C_3 plants face a challenge known as photorespiration. Photorespiration occurs when the photosynthetic enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), responsible for fixing carbon dioxide, reacts with oxygen instead of carbon dioxide, leading to a loss of fixed carbon and reduced photosynthetic efficiency (Figure 1; Sage, 2004; Maroco et al., 1997). This limitation constrains the productivity of C_3 plants in hot and dry environments, where photorespiration becomes more pronounced. In response to the limitations of C_3 photosynthesis, many plants have independently evolved the C_4 photosynthetic pathway, which enhances photosynthetic efficiency in warm, high-light and arid environments (Sage and Monson, 1998). The C_4 pathway, which evolved at least 61 times within 18–19 angiosperm families over the past 60 million years, is more complex but highly efficient, enabling these angiosperms in warm, high-light and arid environments to conserve water while still producing the energy they need (Sage et al., 2011, Sage, 2017; Sage and Monson, 1998). It involves the initial fixation of carbon dioxide into a four-carbon compound, such as oxaloacetate, which is then converted

into malate or aspartate. These compounds are transported to specialised bundle sheath cells, where the Calvin cycle takes place. By concentrating carbon dioxide around RuBisCO in these specialised cells, C_4 plants reduce photorespiration and improve water-use efficiency (Figure 1; Lundgren et al., 2014). This adaptation is particularly beneficial in tropical and subtropical regions with high temperatures and intense sunlight (Rawson et al., 1977; Ehleringer et al., 1997). Notable examples of C_4 plants, such as sugarcane (*Saccharum officinarum* L.), maize (*Zea mays* L.), and sorghum (*Sorghum bicolor* (L.) Moench) — all members of the Poaceae family — exhibit remarkable productivity and resilience in arid environments (Long, 1999; Sage, 2001). Remarkably, about 80% of C_4 species are monocots, of which 61% belong to the Poaceae family (Sage, 2017). While most research on C_4 plants focuses on monocots, C_4 eudicots exhibit a much broader structural and ecological diversity, spanning seven orders and 15 families. This dissertation aims to deepen the understanding of C_4 eudicots by identifying global centres of species richness, analysing their climatic preferences, and exploring functional photosynthesis-related traits, such as succulence and salt tolerance. These findings are then compared to C_4 grasses (Chapter 1).

While C_4 photosynthesis has evolved as an adaptation to hot, arid environments, enabling plants to optimise water use, other plant lineages — particularly in Australia's arid landscapes — presumably perform the C_3 photosynthetic pathway (Sage et al., 2018). Despite lacking the biochemical advantages of C_4 photosynthesis, C_3 species such as *Acacia* (Fabaceae), *Eucalyptus* (Myrtaceae), *Eremophila* R.Br. (Scrophulariaceae), *Ptilotus* R.Br. (Amaranthaceae), and species of the Australian Camphorosmeae tribe have successfully diversified and adapted to Australia’s arid and semi-arid ecosystems (Bowman and Cook, 2002; Sage et al., 2007; Kattge et al., 2011). Research on the distribution of Australian *Neurachne* (Poaceae) has shown that several C_3 species, such as *Neurachne tenuifolia* S.T.Blake, thrive in arid and semi-arid zones, indicating that some C_3 plants can survive in these challenging environments (Prendergast and Hattersley, 1985, Lauterbach et al., 2024). Crassulacean Acid Metabolism (CAM) is another known photosynthetic mechanism, where plants open their stomata at night to fix carbon dioxide, reducing water loss in arid conditions (Figure 1; Yang et al., 2015). The genus *Calandrinia* (Caryophyllales) spans much of the Australian continent and provides an excellent case study for

examining the evolution of photosynthetic diversity. Many species within this genus exhibit a facultative form of CAM, where CAM is up-regulated during drought conditions, as evidenced by changes in carbon isotope ($\delta^{13}C$) values that reveal changes in their photosynthetic pathways (Hancock et al., 2019). This facultative CAM suggests a flexible adaptation mechanism, allowing these species to balance C_3 photosynthesis with CAM as environmental conditions fluctuate. Understanding the interplay between C_3 and CAM photosynthesis in these plants' sheds light on the complex strategies that contribute to plant survival in Australia's diverse and challenging climates (Hancock et al., 2019).

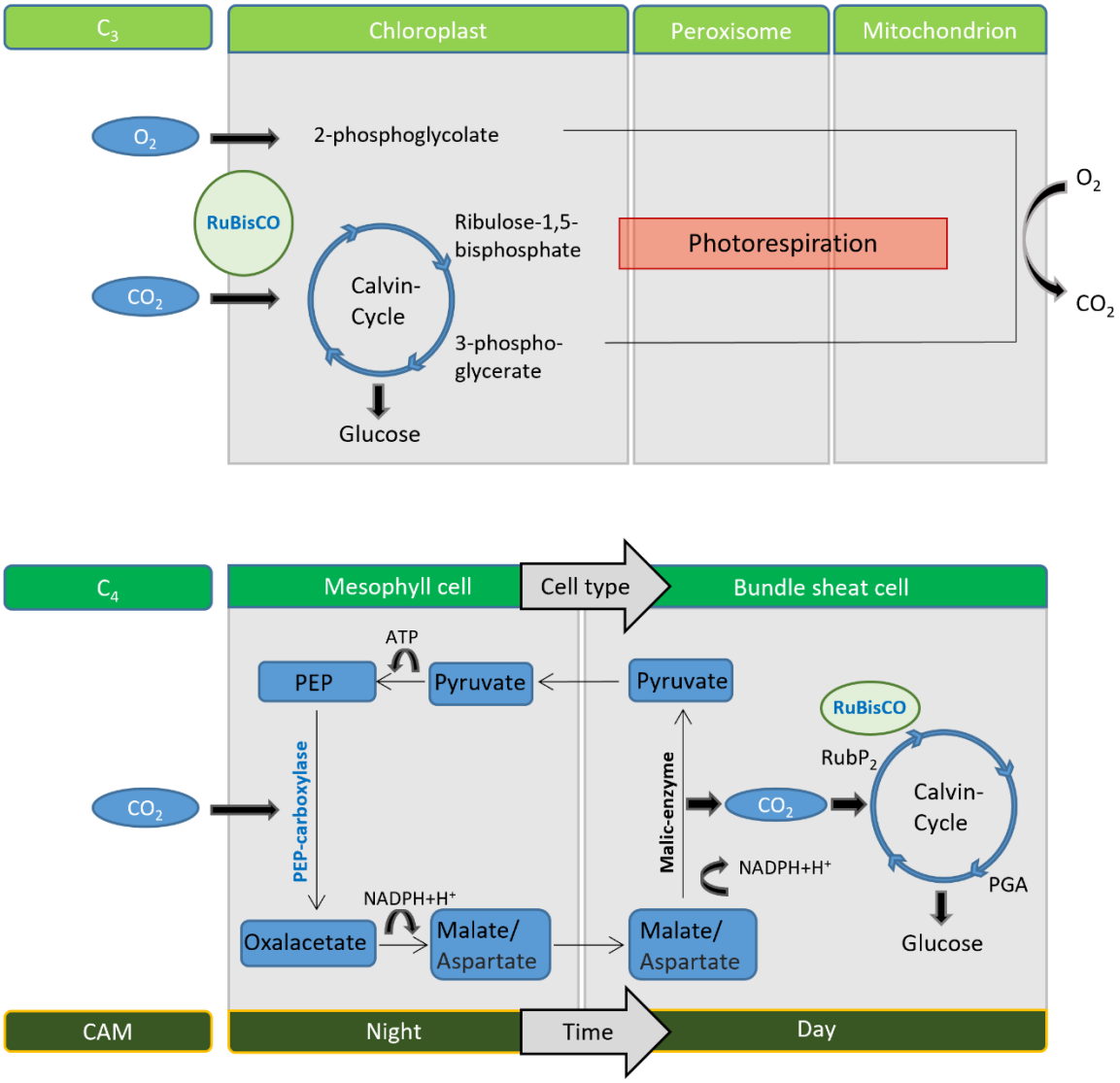

Figure 1: Simplified schematic representation of the C_3 photosynthesis pathway, as well as the C_4 photosynthesis pathway and CAM. PEP = Phosphoenolpyruvate; RuBisCO = Ribulose-1,5-bisphosphate carboxylase/oxygenase; RubP2 = Ribulose-1,5-bisphosphate; PGA = 3-phosphoglycerate.

Both functional plant traits and climatic factors play a central role in plant evolution and diversification, with climatic conditions influencing trait evolution and driving correlations between plant traits and adaptive strategies, ultimately facilitating species diversification. Changes in climate, such as shifts in temperature, precipitation and drought, have historically shaped speciation and the adaptations required to survive in different environments. For example, periods of climatic instability, such as droughts or glaciations, can increase diversification as species adapt to new conditions (Feeley et al., 2020).

On a broader scale, global climate patterns influence the evolution of functional traits, such as those related to photosynthesis, which are crucial for plant survival and reproduction (Díaz et al., 2016; Ahrens et al., 2020). These traits often evolve in response to the specific climatic conditions of a region, resulting in a diversity of strategies for coping with environmental stress. The relationship between climate and evolution is dynamic, with climate acting both as a driver of diversification and as a filter, determining which species survive based on their traits (Cooney et al., 2016; Tosso et al., 2019). Adaptation is a process by which species evolve traits that improve survival and reproduction in specific environments. In extreme conditions, such as arid or saline regions, plants develop specialised morphological and physiological traits. These adaptations may include succulent leaves and stems for water conservation, deep root systems to access water, and mechanisms for tolerating high soil salinity and aridity. The Australian Camphorosmeae, for example, are highly adapted to challenging conditions, like saline soils and arid areas (Kadereit et al., 2005). Their adaptations include reduced leaf size, succulence, and hairy or scaly surfaces that help minimise water loss (Kadereit and Freitag, 2011). Understanding these adaptations offers insights into how species have historically responded to environmental challenges and how they may respond to future climate changes. Molecular phylogenetics is a powerful approach for reconstructing the evolutionary history of plant species (Smith et al., 2020). By analysing genetic data, researchers can infer the relationships between species, trace diversification events, and identify the evolutionary origins of key adaptations. Phylogenetic trees constructed from molecular data provide a visual representation of these relationships, showing how lineages have evolved and diversified over time. Such studies often aim to uncover the biogeographic history of species, revealing how lineages have dispersed across

regions and adapted to different environmental conditions. Additionally, molecular phylogenetics can track the evolution of specific traits, showing how these traits have evolved in response to climatic shifts. By providing insights into evolutionary processes, this method enhances our understanding of biodiversity and the historical factors influencing species distribution.

To particularly understand the spatial distribution of different plant species, as well as the ecological and evolutionary processes that shape these patterns, requires a biogeographical approach. By mapping species distributions and analysing the factors influencing their spread, biogeographical studies offer valuable insights into the drivers of biodiversity. While global analyses often focus on species distributions across various climates and regions, more localised studies might examine the distribution within specific environments, such as arid or saline regions. The biogeography and diversification of the Australian Camphorosmeae are another focus of this dissertation (Chapter 2). This chapter investigates how this group has adapted to various habitat types in Australia's arid regions. It underscores the importance of geographic isolation, historical climatic events, and ecological niches in determining the current distribution of species. Understanding these patterns is crucial for predicting how species might respond to future environmental changes and for informing conservation efforts. By exploring and deepening the evolutionary history and biogeographical patterns of these putative C_3 plants, this study provides new insights into their diversification, distribution and persistence in landscapes that are also widely inhabited by C_4 species, such as the cosmopolitan *Atriplex* (with around 60 species).

The exploration and understanding of evolutionary adaptations and global plant distribution requires extensive datasets encompassing physiological plant traits, environmental conditions, and species distributions (Ackerly, 2003). With the rise of digital technologies, biodiversity databases like the Global Biodiversity Information Facility (GBIF) and the Atlas of Living Australia (ALA) — both of which are utilised in this dissertation — have become invaluable resources for researchers (Nelson and Ellis, 2019; Belbin and Williams, 2016). These large-scale repositories of georeferenced occurrence data enable global and regional analyses of plant diversity and ecological trends (Smith et al., 2016). However, managing and interpreting these big datasets presents significant challenges (Feeley, 2015). GBIF, a global network aggregating biodiversity data from numerous sources, including herbarium specimens,

field observations, and literature (Meyer et al., 2016), offers millions of occurrence records that span a broad range of taxa and geographic regions. This allows researchers to conduct large-scale studies on plant diversity, distribution patterns, and ecological interactions, as demonstrated in Chapter 1. Similarly, the ALA offers a comprehensive database focusing on Australian's flora and fauna, aggregating data from a variety of sources, including field observations, herbarium specimens, and ecological surveys (Belbin and Williams, 2016), allowing for detailed insights into plant distributions, climatic preferences, and habitat types, especially on a local scale as used in Chapter 2. However, both GBIF and ALA datasets require numerous data cleaning and validation due to issues such inaccurate georeferencing, outdated taxonomy, and variability in data reporting standards (Beck et al., 2014; Zizka et al., 2020).

The heterogeneity of biodiversity datasets complicates data management and analysis. Differences in data standards and practices between sources can result in issues like inaccurate georeferencing, outdated taxonomy, and incomplete records (Meyer et al., 2016). To address these challenges, researchers often rely often on a combination of automated tools and manual validation processes (Zizka et al., 2019; Jin and Yang, 2020). Tools like the "CoordinateCleaner" package, help filter out erroneous records based on predefined criteria, while manual validation ensures taxonomic accuracy (Zizka et al., 2019). However, interpretation of the results requires careful consideration of the underlying data quality and potential limitations. The need for detailed, reliable analyses must be balanced with an understanding of the inherent limitations of large-scale biodiversity data (Beck et al., 2014). Consequently, the challenge of managing and interpreting these large datasets remains central to the success of plant science research, as highlighted in Chapters 1 and 2.

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RESEARCH ARTICLE

Global distribution, climatic preferences and photosynthesis-related traits of C_4 eudicots and how they differ from those of C_4 grasses

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Abstract

C_4 is one of three known photosynthetic processes of carbon fixation in flowering plants. It evolved independently more than 61 times in multiple angiosperm lineages and consists of a series of anatomical and biochemical modifications to the ancestral C_3 pathway increasing plant productivity under warm and light-rich conditions. The C_4 lineages of eudicots belong to seven orders and 15 families, are phylogenetically less constrained than those of monocots and entail an enormous structural and ecological diversity. Eudicot C_4 lineages likely evolved the C_4 syndrome along different evolutionary paths. Therefore, a better understanding of this diversity is key to understanding the evolution of this complex trait as a whole. By compiling 1207 recognised C_4 eudicots species described in the literature and presenting trait data among these species, we identify global centres of species richness and of high phylogenetic diversity. Furthermore, we discuss climatic preferences in the context of plant functional traits. We identify two hotspots of C_4 eudicot diversity: arid regions of Mexico/Southern United States and Australia, which show a similarly high number of different C_4 eudicot genera but differ in the number of C_4 lineages that evolved in situ. Further eudicot C_4 hotspots with many different families and genera are in South Africa, West Africa, Patagonia, Central Asia and the Mediterranean. In general, C_4 eudicots are diverse in deserts and xeric shrublands, tropical and subtropical grasslands, savannas and shrublands. We found C_4 eudicots to occur in areas with less annual precipitation than C_4 grasses which can be explained by frequently associated adaptations to drought stress such as among others succulence and salt tolerance. The data indicate that C_4 eudicot lineages utilising the NAD-ME decarboxylating enzyme grow in drier areas than those using the NADP-ME decarboxylating enzyme indicating biochemical restrictions of the later system in higher temperatures. We conclude that in most eudicot lineages, C_4 evolved in ancestrally already drought-adapted clades and enabled these to further spread in these habitats and colonise even drier areas.

KEYWORDS
biome, C_4 photosynthesis, climatic preferences, desert, GBIF, salt tolerance, succulence

TAXONOMY CLASSIFICATION
Biodiversity ecology, Biogeography, Botany

1 | INTRODUCTION

By the early 1950s, it was widely assumed that all plants use the same C_3 carbon fixation pathway, the Calvin-Benson-Bassham cycle (CBB-cycle; Bassham et al., 1950). Shortly after a brief note on the discovery of a four-carbon CO_2 fixation pathway in sugarcane – now known as C_4 photosynthesis – published in the 1954 Annual Report of the Hawaiian Sugar Planters Association Experiment Station (Burr et al., 1957; Hatch, 2005), researchers set out to investigate this unexplored photosynthetic pathway. They found that the CBB cycle and RuBisCO were restricted to bundle sheath cells (BSC) and that in the mesophyll cells (MC) an auxiliary carbon fixing pathway with phosphoenolpyruvate carboxylase (PEPC) as the key enzyme generated C_4 molecules that are transported into the BSC and fuel the CBB-cycle. Since its discovery, understanding C_4 photosynthesis has become a vibrant research discipline, integrating the fields of biochemistry, physiology, organismic biology, ecology and evolution (Langdale, 2011). Evolving knowledge about the C_4 pathways has been published in various reviews (see Furbank and Kelly, 2021; Niklaus and Kelly, 2019; Sage et al., 2018; Schlüter and Weber, 2020 for four recent ones on different aspects of C_4 photosynthesis) and special issues (e.g., JXB special issue: C_4 Photosynthesis – 50 years of discovery and innovation – Von Caemmerer et al., 2017).

C_4 photosynthesis evolved in at least 18 angiosperm families and more than 60 times independently (Sage, 2017; Sage et al., 2018; Figure 1a). Around 80% of the C_4 species are found in the Poales with 5044 C_4 species in Poaceae and 1322 C_4 species in Cyperaceae. With two C_4 species in Hydrocharitaceae (Alismatales), this adds up to 6368 C_4 species in 339 genera in monocots, opposed to around 1777 eudicot C_4 species in 79 genera (Sage, 2017 and ref. therein) (Figure 1b). Interestingly, the eudicot C_4 lineages are phylogenetically more equally distributed and occur in three rosid and four asterid families belonging to six different orders. Nevertheless, the C_4 species-richest eudicot clades are restricted mostly to eight families of the Caryophyllales (Figure 1a, based on Sage, 2017: table 3). Amaranthaceae s.l. (Caryophyllales) is by far the most species-rich

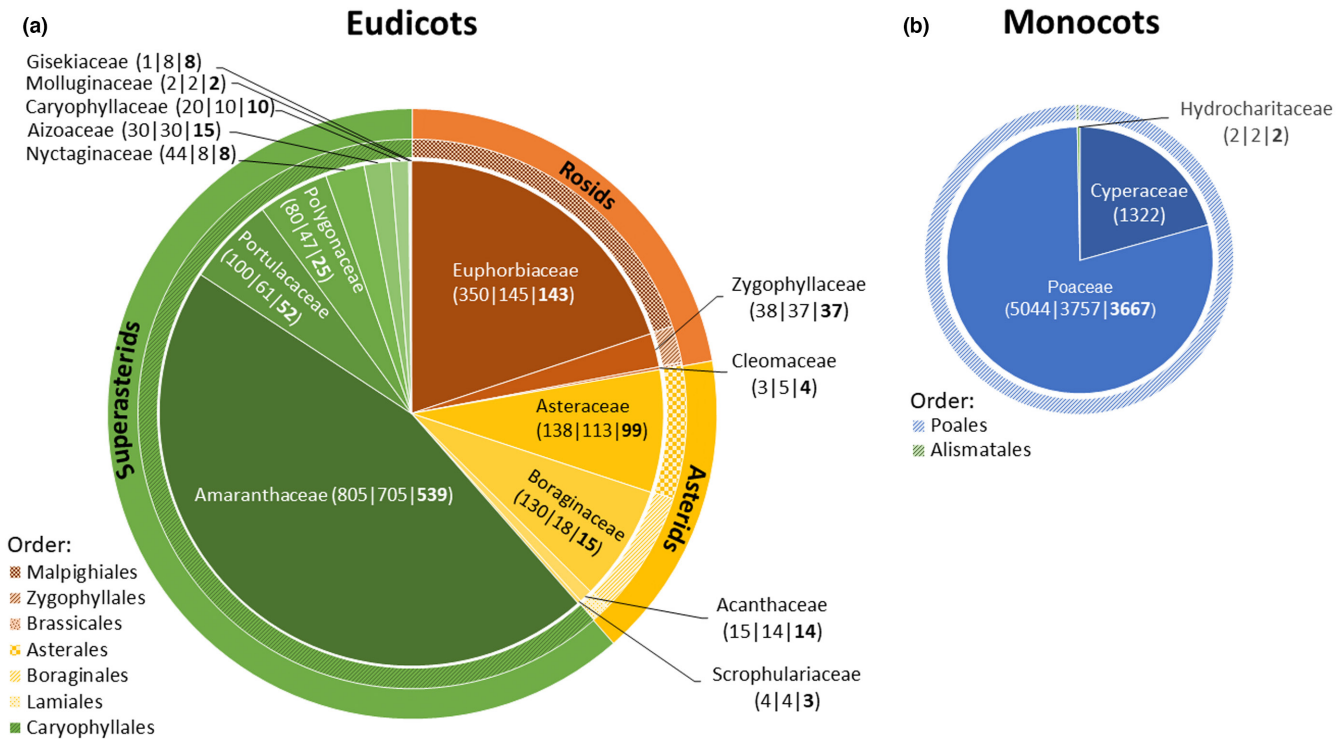

FIGURE 1 Angiosperm families in eudicots (a) and monocots (b) including C_4 species. The first number represents the total number of C_4 species per family according to Sage (2017) the second number represents the number of C_4 species with verified information about C_4 photosynthesis performance (Table S1) the number in bold shows the remaining number of C_4 species after various data cleaning steps (Table 1). Proportions shown in the diagrams and mentioned in the text were calculated according to species numbers in Sage (2017) but do not change much when based on the other two reduced numbers.

C_4 eudicot family, followed by Euphorbiaceae (Malpighiales, rosids), Asteraceae and Boraginaceae (Asterales and Lamiales, respectively, asterids; Figure 1a). Although C_4 photosynthesis in eudicots is phylogenetically more widespread and ecologically and structurally more diverse than in monocots (e.g., Muhaidat et al., 2007; Rudov et al., 2020), the latter have received more attention (mainly in the Poaceae). This is partly due to C_4 grasses, such as maize and sugarcane, being initial model species of C_4 research, thus making their close relatives the focus of C_4 research even today (Hatch, 2005). Furthermore, research focus on grasses can be attributed to their great economic and ecological importance (Linder et al., 2018). However, understanding the diversity of the C_4 syndrome in eudicots is key to understand the evolution of this complex trait (Heyduk et al., 2019) because C_4 eudicot lineages evolved the C_4 syndromes along different evolutionary paths (e.g., Bohley et al., 2015; Kadereit et al., 2012; Lauterbach et al., 2019).

C_4 photosynthesis, which includes an auxiliary pathway to reduce photorespiration, likely arose in hot, dry and/or saline regions where C_3 photosynthesis performance is reduced (Sage et al., 2018). This is achieved by the fixative enzyme, phosphoenolpyruvate-carboxylase (PEPC), and by generating a local high CO_2 concentration around the key enzyme RuBisCO, to reduce its oxygenase activity (Sage et al., 2012). C_4 photosynthesis is usually associated with warm habitats with high evapotranspiration. Yet, the distribution of C_4 plants cannot be explained entirely by individual environmental factors (Christin & Osborne, 2014) because C_4 species occur in a variety of habitats, for example on nutrient-poor or fertile soils, in the tropics, in deserts or in the boreal zone, on open grasslands or forest undergrowth (Collins & Jones, 1986; Mahdavi & Bergmeier, 2018; Rudov et al., 2020). This diversity results from the multifaceted evolutionary history of the C_4 pathway (Christin & Osborne, 2014; Sage et al., 2011, 2018). However, studies linking the evolution of adaptive traits and ecological niches in C_4 lineages are still insufficient. Lundgren et al. (2015) for example showed that C_4 photosynthesis does not initially lead to a shift of the ancestral niche in *Alloteropsis semialata* J. Presl (Poaceae), but rather expands its niche to cover a wider range of conditions that include the ancestral ones. This improves the success of occasional long-range dispersal events and thus increases the geographical range (Lundgren et al., 2015). As C_4 photosynthesis is a complex syndrome that increases the efficiency of plants to use available water and nitrogen, C_4 might be advantageous under various environmental conditions, but evolved predominantly in the tropics and subtropics (Griffiths et al., 2013; Sage et al., 2018).

1.1 | Global expansion of C_4 grasses

C_4 plants account for one-quarter of the earth's primary terrestrial production, and almost a quarter of the Earth's surface is dominated by C_4 grasslands and savannas (Barbehenn et al., 2004; Grace et al., 2006; Sage et al., 2018). C_4 grasses likely intruded into C_3 grasslands and forests from open biomes of warm regions, subsequently replaced them during the late Miocene to the Pliocene (3–8 Mya) and expanded worldwide into drier biomes (Edwards & Smith, 2010; Ehleringer et al., 1997; Osborne & Freckleton, 2009). However, molecular research suggested that C_4 photosynthesis in several grass lineages evolved earlier, around 18–30 Mya (mid/late-Oligocene) presumably in warm, arid locations where water limitation was the main selective force to increase photorespiration (Christin et al., 2011; Zhou et al., 2018). As the atmospheric CO_2 in the late Miocene fell below ~300 ppm (Royer, 2006), a small number of hyperdominant C_4 grass species that were able to outcompete C_3 and C_4 relatives became dominant due to their advantage of a low CO_2 compensation point (Christin & Osborne, 2014; Lehmann et al., 2019). South America seems to be the major hotspot for the origin of C_4 grasses (Sage et al., 2011), and today, C_4 grasses are confined mostly to tropical and subtropical areas (Shoko et al., 2016; Woodward et al., 2004; Woodward & Lomas, 2004). Climatic patterns and the distribution of C_4 grasses in North America suggest that high minimum temperatures during the growing season favour C_4 grasses at regional scale (Teeri & Stowe, 1976). Yet, at the local scale, topographic and edaphic variables may exert more influence (Yan & de Beurs, 2016).

1.2 | Evolutionary and ecological diversity of C_4 photosynthesis in eudicots

Individual C_4 lineages had originated independently from the Oligocene into the Quaternary (Christin et al., 2011; Niklaus & Kelly, 2019). In Amaranthaceae s.l., which includes the largest number of C_4 lineages in eudicots, the earliest assumed origins of C_4 date back to the Oligocene and are roughly as old as the oldest C_4 grass subfamily Chloridoideae, which evolved around 32–25 Mya. This implies that C_4 eudicots are per se not younger than C_4 monocots (Christin et al., 2008, 2011; Kadereit et al., 2012). However, many C_4 lineages within eudicots (as well as in the monocots) originated in the Miocene when the climate got increasingly drier (Kadereit et al., 2003, 2010). In addition, there are many evolutionary young C_4 lineages in eudicots, for instance in *Flaveria* (Asteraceae), *Sesuvium* (Aizoaceae) and *Tecticornia* (Chenopodiaceae s.s.) that arose approximately 5–1 Mya (Christin et al., 2011; Kadereit et al., 2012; Sage et al., 2012). Since the range size of C_4 lineages as well as the physiological refinement of the C_4 syndrome is highly dependent on time, the age of the respective C_4 lineage needs to be taken into account when lineages are compared to each other (Niklaus & Kelly, 2019).

Although South America seems to be the hotspot of origins of the nowadays cosmopolitan C_4 grasses, six geographic regions were highlighted as potential ancestral areas for C_4 eudicot lineages. For most C_4 eudicot lineages Central Asia, North America, South Africa, northeast Africa and Arabia count as centres of origin (Kadereit & Freitag, 2011; Sage, 2016; Sage et al., 2011), an assessment based mainly on current distribution that still awaits the review of detailed phylogenetic, biogeographical studies in the individual eudicot lineages (e.g., Lauterbach et al., 2019). Due to the diverse nature of C_4

eudicots, no list of the global distribution of C_4 eudicots has been compiled thus far.

1.3 | Functional plant traits in C_4 eudicots

Several traits such as succulence, salt tolerance, fast seed germination and longevity seem to be associated with C_4 photosynthesis in eudicots (e.g., Kadereit et al., 2012, 2017). While succulence is generally rare among grasses (only in *Spinifex littoreus* (Burm.f.) Merr.; Ho et al., 2019), this trait results in an unmatched leaf and stem anatomical diversity in C_4 eudicots (Bohley et al., 2015; Kadereit et al., 2003; Muhaidat et al., 2007, 2018; Voznesenskaya et al., 2017). Grasses usually show a classical Kranz anatomy (similar to an atriplicoid leaf anatomy), whereas eudicots exhibit a broad variety of succulent and non-succulent C_4 leaf types in addition to the atriplicoid type (Edwards & Voznesenskaya, 2011). Among the succulent C_4 leaf types, the annual *Suaeda aralocaspica* (Bunge) Freitag (= *Borszczowia aralocaspica*; Amaranthaceae s.l.), *Bienertia cycloptera* Bunge ex Boiss, *B. sinuspersici* Akhani and *B. kavirense* Akhani are particularly noteworthy because their C_4 photosynthesis is carried out within a single photosynthetic cell and without the supposedly mandatory C_4 Kranz anatomy (Akhani et al., 2005, 2012; Freitag & Stichler, 2000; Sharpe et al., 2020; Voznesenskaya et al., 2002, 2003). Although many different examples of anatomical diversity in C_4 eudicots have been found, there are still many representatives of the other C_4 eudicot lineages that are not well characterised and might contribute to the ecological and morphological diversity (Muhaidat et al., 2007).

Most succulent C_4 species tolerate elevated salinity, suggesting that their succulence is primarily an evolutionary response to (physiological) drought. While in grasses a repeated gain and loss of salt tolerance throughout the history of the family prevails and halophytic grass species are isolated at the tips of the phylogeny (Bromham & Bennett, 2014), there are multiple evolutionary older halophytic lineages among eudicots that additionally acquired C_4 photosynthesis. This is particularly the case for C_4 lineages of Amaranthaceae (Kadereit et al., 2012, 2017; Piirainen et al., 2017) but also for Gisekiaceae (Bissinger et al., 2014), Sesuvioideae-Aizoaceae (Bohley et al., 2015) and Euphorbiaceae (Ghazanfar et al., 2014; Rudov et al., 2020). Some eudicot lineages acquired even further alternative carbon fixation pathways. The widespread succulent annual *Portulaca oleracea* L. is a halophytic C_4 species that is able to conduct both C_4 and CAM photosynthesis depending on the environmental conditions (Ferrari et al., 2020, 2022).

While C_4 trees and large shrubs are generally rare, several eudicot C_4 species can be woody and/or perennial such as *Anabasis* from Eurasian steppes and semi-deserts (Lauterbach et al., 2019); the saxaul (*Haloxylon ammodendron* (C.A. Mey.) Bunge ex Fenzl), which dominates continental deserts of Asia (Pyankov et al., 1999) and the Hawaiian C_4 trees *Euphorbia olowaluana* Sherff and *E. herbstii* (W.L. Wagner) Oudejans (Pearcy & Troughton, 1975; Young et al., 2020).

Despite anatomical and ecological differences, the biochemical forms that exist in C_4 photosynthesis are similar in grasses and eudicots. There are three biochemical subtypes in both grasses and eudicots, which are usually constant in a C_4 lineage, but may vary within and between plant families: NADP-malic enzyme (ME; e.g., in Caryophyllaceae; Sage et al., 2011), NAD-ME (e.g., C_4 species in Boraginaceae, Cleomaceae; Muhaidat et al., 2007), and the third further decarboxylating enzyme, PEP-CK that is more common in C_4 monocots (Wang et al., 2014). Due to the high number of fast growing and highly productive C_4 grasses many of which are interesting biofuel crops and phytoremediation plants (such as *Miscanthus*; Pidlisnyuk et al., 2014), one might assume that C_4 grasses are more competitive than C_4 eudicots given the right growing conditions. However, interestingly the species with the fastest CO_2 assimilation rates of 80 $\mu mol\, m^{-2} s^{-1}$ at 325 $\mu mol\, mol^{-1}$ is not a grass species but *Amaranthus palmeri* S. Watson (Amaranthaceae, Ehleringer, 1983; Sage, 2017).

1.4 | Scope and aims

C_4 grasses entail the majority of C_4 species and dominate in biomass production, yet the anatomical, physiological and ecological diversity of C_4 syndromes seems larger in C_4 eudicots. While shifts to C_4 physiology in grasses probably represent a pre-adaptation to open and arid subtropical habitats (Edwards & Smith, 2010; Osborne & Freckleton, 2009), the evolution of the C_4 pathway in eudicots, e.g., Amaranthaceae s.l. (incl. Chenopodiaceae), Nyctaginaceae and Sesuvioideae, is more likely a post-adaptation to the selection pressure in dry, saline and coastal environments that enabled survival in these habitats (Bohley et al., 2015; Kadereit et al., 2012; Khoshravesh et al., 2020). Already, Stowe and Teeri (1978) suggested that C_4 eudicots do not follow the climate preferences that have been reported for C_4 grasses and therefore might have followed a different evolutionary pathway to C_4.

In this study, we aimed to characterise the global occurrence of C_4 eudicots, identify diversity hotspots and climatic preferences, and assign these to functional traits such as succulence, salt tolerance, biochemical subtype and anatomical leaf type. We hypothesised that the phylogenetic and structural diversity of C_4 eudicots is reflected in their colonisation of a wide range of climatic regions and environments and that the combination of C_4 photosynthesis with other traits enabled C_4 eudicots to invade areas not or less frequently colonised by C_4 grasses. To test this hypothesis, we compare the biogeographic patterns found in eudicots with those of the more species-rich C_4 grasses. Moreover, we link the geographical origins of C_4 photosynthesis to the diversity hotspots discovered in those C_4 eudicot lineages examined within a phylogenetic framework.

2 | MATERIALS AND METHODS

We compiled an initial dataset of C_4 eudicots according to Sage (2017). This list consisted of 16 eudicot families with indication of lineages and the number of C_4 species per lineage

(Table S1). In order to list each C_4 species, literature research was conducted. If trait data were available, we recorded leaf anatomy, biochemical subtypes (NAD-ME, NADP-ME), succulence, woodiness, salt tolerance and life form (perennial, annual) from floras, revisions, reports, databases and online sources (see sources in Table S1). To reduce the artificial increase in species numbers and distribution areas due to synonymisation, we cross-checked for synonyms using plantsoftheworldonline.org (POWO, 2020). In grasses, there are over 60,000 published scientific names corresponding to approximately 11,313 accepted species (Clayton et al., 2002 onwards; Osborne et al., 2014). Our list of C_4 eudicot species includes members of 15 families (Chenopodiaceae included in Amaranthaceae) with 1207 accepted species and a total of around 3969 synonyms. These 1207 species have verified information about C_4 photosynthesis performance and the literature and/or online resources provided detailed information about the traits discussed above. For comparison with C_4 grasses, we compiled a list of 309 genera following Osborne et al. (2014) (Table S1), as all species of these genera are assumed to perform C_4 photosynthesis.

2.1 | Occurrence data

We extracted georeferenced occurrence data of the 1207 C_4 eudicot species from the Global Biodiversity Information Facility (gbif.org) using the "rgbif" v3.2.0 package (Chamberlain et al., 2020) in R (for DOIs see GBIF-References; GBIF.org, 2020a, 2020b, 2020c, 2020d, 2020e, 2020f, 2020g, 2020h, 2020i, 2020j, 2020k, 2020l, 2020m, 2020n, 2020o). We downloaded occurrence records at species level for C_4 eudicots (categorised by family) and at genus level for C_4 grasses (categorised by family/subfamily) due to the aforementioned assumption (GBIF.org, 2020p, 2020q, 2021r, 2020s, 2020t). Records describing fossilised specimens, records based on literature alone and records of unknown origin were excluded (R code available at https://docs.ropensci.org/rgbif/articles/rgbif.html). GBIF records originated from a variety of sources, including human and machine observation (e.g., photograph), living and preserved specimens. For 208 C_4 eudicot species (17%) included in our list of verified C_4 species, no reliable occurrence data were available in GBIF.

2.2 | Data cleaning

Since georeferenced occurrence records from public datasets such as gbif.org are error-prone (Maldonado et al., 2015; Zizka et al., 2020), automated data cleaning of the C_4 eudicot and C_4 grasses coordinate datasets was performed with the "CoordinateCleaner" v2.0-18 package in R (Zizka et al., 2019) using the default options. Following the process outlined in Zizka et al. (2019), erroneous records within 1000 m of country and/or province centroids and within 10,000 m of countries' capitals, within urban areas, records with locations as zeros, identical values, near GBIF headquarters, near biodiversity institutions and records on an ocean surface were removed. In addition to the "CoordinateCleaner," the dataset was manually checked for incorrect synonymisation relying on plantsoftheworldonline.org as the taxonomic backbones of the GBIF are not always following the currently accepted taxonomic treatments by "The International Plant Names Index" (IPNI, 2020) and "World Checklist of Vascular Plants" (WCVP, Govaerts et al., 2021). Besides, duplicated coordinates, based on species name and coordinates, were removed. Likewise, the taxonomic reliability using the distribution information of plantsoftheworldonline.org was checked and occurrence points considered incorrect based on their distribution outside the native ranges of species were excluded. As a result of all cleaning steps, the number of coordinates was reduced from 1,012,557 to 247,205 occurrence points.

The cleaning of 2,296,101 occurrence records of C_4 grasses was also carried out with the "CoordinateCleaner" package. Additionally, the occurrence points outside the native ranges and duplicate coordinates per species were excluded. Manual cleaning of the incorrectly synonymised species was not carried out here, as we focussed on the genus level only.

2.3 | Analyses

We used 100 × 100 km grid cells to infer geographic patterns of C_4 species richness, with an equal area Behrmann projection. Species richness maps for each C_4 eudicot family for the uncleaned and cleaned GBIF dataset were generated using the package "speciesgeocodeR" v2.0-10 (Töpel et al., 2017; Figures S1–S15). Grids with species numbers were calculated using *RichnessGrid*. In addition to the individual species richness maps for each C_4 eudicot family, total species richness maps for C_4 eudicots and C_4 grasses were created with the cleaned datasets. These maps provide information on the total distribution (showing outstanding regions of C_4 species richness) of both groups. Grids showing richness above 50 species were defined to be C_4 species hotspots of high diversity.

Since data obtained from GBIF may be biased by unequal sampling in different areas (Hughes et al., 2021; Zizka et al., 2021), we additionally obtained data on species occurrences on the world geographic scheme for recording plant species Level-3 (TDWG, 2001) from the World Checklist of Vascular Plants (WCVP, Govaerts et al., 2021) as comparison. The WCVP contains a complete (to the best of current knowledge) list of all vascular plant species and hence is less biased by differences in sampling (but in exchange for lower spatial resolution and unequally sized sample areas; Antonelli et al., 2023; Schellenberger Costa et al., 2023). We obtained the distribution of C_4 eudicots from WCVP using the rWCVP package (Brown et al., 2023). First, we matched the names of our species list with WCVP using the wcvp_match_names function, with subsequent manual resolution of multiple matches (Table S2) and then obtained all natural, present,

not doubtful occurrences of these species. For C_4 grasses, we first matched the list of our genera with WCVP (Table S2) with sub-sequential manual resolution of all multiple matches and then obtained the natural, present and undoubtful distributions of all species in these genera.

To compare the importance of individual regions between C_4 eudicots and C_4 grasses, we calculated the rank difference for each region, by first ranking each region by the number of C_4 eudicot and C_4 grass species (most species = 1, fewest species = 354) with a mean tie-breaker (Figure 2g). We only included botanical countries with at least one C_4 grass and at least one C_4 eudicot.

Two bioclimatic variables (Bio1 – Annual Mean Temperature (°C*10); Bio12 – Annual Mean Precipitation (mm)) were extracted from WorldClim v.2 with a spatial resolution of 10 min (~340 km^2) (Fick & Hijmans, 2017). We plotted annual mean temperature and precipitation values using ggplot2 to compare the distribution of C_4 eudicots and C_4 grasses along these two climatic variables (Wickham et al., 2016). Since the focus here is mainly on comparing these two groups and there is probably no systematic difference in bias between them, the cleaned GBIF dataset was used for this analysis. We are aware that this approach does not integrate biogeographical history which is beyond the scope of this paper. Likewise, boxplots for each family of C_4 eudicots, the C_4 grasses and all C_4 eudicots together were calculated in relation to the climate variables by first calculating the mean values of each species for each climate variable. Statistical analyses were conducted in R v4.0.2 (R Core Team, 2020) using RStudio v1.2.5042 (RStudio, Inc., 2009–2020) and R Commander (Fox, 2005). We used a Kruskal–Wallis test (for between C_4 eudicot families), followed by a post-hoc Tukey–Kramer test to determine where the differences were, with a *p*-value equal to or <.05 being considered statistically significant and a Mann–Whitney *U* test (between C_4 eudicots and C_4 grasses) to determine significance according to annual mean precipitation and annual mean temperature. A one-way ANOVA test was performed to determine whether eudicot C_4 species with NAD-ME as the primary decarboxylating enzyme are distributed in areas with significantly lower annual precipitation than eudicot C_4 species of the NADP-ME subtype.

To display the distribution areas of each C_4 eudicot family at the biome level (Olson et al., 2001), a table in Figure 4a was created. It shows the number of family species within a biome (Terrestrial ecoregions of the world; sensu Olson et al., 2001). Only plant families with the highest number of species in that study according to Figure 1a were selected for this table.

2.4 | Literature survey

To place our findings on the diversity hotspots of C_4 eudicots in a spatiotemporal framework, we conducted a literature review of phylogenetic and biogeographical studies incorporating C_4 eudicot lineages in order to reveal the current understanding of the C_4 photosynthesis origin in these groups. The aim was to assess whether a lineage developed C_4 photosynthesis in a particular area (in situ origin) or developed C_4 before colonising a particular area (ex situ origin).

3 | RESULTS

3.1 | The impact of data cleaning

We included only species for which direct evidence of C_4 photosynthesis (such as C_4-like $\delta^{13}C$ values or C_4 leaf anatomy) is documented in the literature. This was the case for 1207 species of the approximately 1777 C_4 eudicots according to Sage (2017). Since Sage (2017) estimates the number of C_4 species per lineage, our refined C_4 eudicot species list is substantially shorter (Table S1). For 208 of these 1207 C_4 eudicot species, no occurrence points were documented in GBIF. Therefore, the final list of C_4 eudicots analysed here included 999 species.

Performing the necessary cleaning steps reduced the raw C_4 eudicot occurrence points dataset, which originally contained more than 1 million records, to less than a quarter (Table 1). After the use of the "CoordinateCleaner" package, 280,935 C_4 eudicot records (27.75%) of 1,012,557 were removed (Table 1). A manual check for erroneous synonymisation removed 153,236 occurrence records from the remaining dataset. In the next step, 155,481 duplicate coordinates were removed. Notable is the additional reduction of 175,700 distribution points, after filtering out the outliers. After all these cleaning steps, we retained 963 species with a total of 247,205 occurrences. 620 species were represented by more than or equal to 10 records, whereas 343 species were represented by <10 records. Altogether, 75.59% of the occurrences for the C_4 eudicots were excluded (Table 1).

In the raw C_4 grasses dataset, which contained 2,296,101 distribution points for 271 C_4 grass genera (GBIF.org, 2020p, 2020q, 2021r, 2020s, 2020t), around 382,595 points (16.66%) were removed after applying the "CoordinateCleaner" package. Excluding occurrence points outside the original distribution areas resulted in the elimination of 375,560 distribution points. Duplicate coordinates per species were removed, resulting in an additional 464,424 occurrence points being excluded. After all cleaning steps, approximately 53.25% distribution points were removed from the raw C_4 grasses dataset, leaving 1,073,522 distribution points. Manual cleanup of incorrectly synonymised species was not performed, as only the genus level was considered.

Intermediate analyses with uncleaned or only partly cleaned data showed that these datasets would have led to different results (Figures S1–S15 illustrate this). The false occurrence data are prevalent to the extent that they blur any meaningful result of the clean data. The usability and consequently the sustainable success of large data repositories such as GBIF will thus in the future largely depend on the effort put into the curation of the data. Currently, these data should only be used with caution (Zizka et al., 2020), and a meaningful dataset can only be extracted via several filtering steps, as seen in this study.

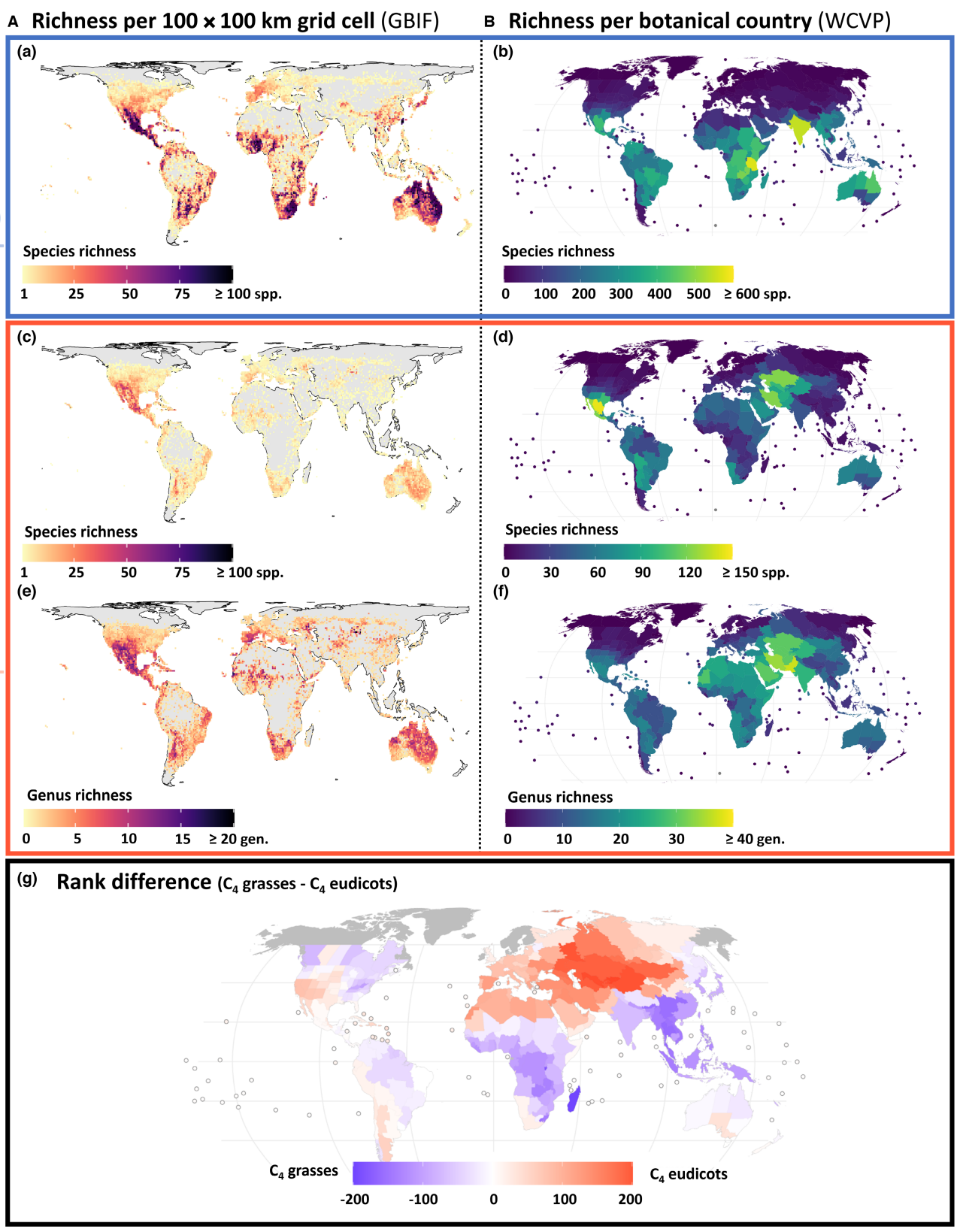

FIGURE 2 Global maps of total C_4 grasses and C_4 eudicots. A. Richness per 100 × 100 km grid cell. B. Richness per botanical country. (a) and (b) Species richness of C_4 grasses. (c) and (d) Species richness of C_4 eudicots. (e) and (f) Genus richness of C_4 eudicots. (g) Rank difference (botanical countries ranked by the number of respective species) C_4 grasses – C_4 eudicots. Blue indicates botanical countries more important for C_4 grasses, and red indicates botanical countries more important for C_4 eudicots.

3.2 | C_4 eudicot and C_4 grasses comparison

Species richness is a commonly used measure of biodiversity (Albrecht et al., 2021; Gould, 2000). Richness maps are used to explore patterns of richness and help to investigate the processes that shape those patterns. The species richness maps of C_4 grasses and C_4 eudicots show the generally higher species diversity of C_4 grasses (Figure 2a-d).

C_4 eudicots and C_4 grasses considered together, two regions stood out with a high C_4 species richness: Mexico/Southern United States and Australia (Figure 2a,c). Additionally, when considering species richness per botanical country, South America can also be identified as a region rich in C_4 plant species (Figure 2b,d). For the C_4 eudicots, the hotspot of a high diversity at generic and family rank was in Mexico and extended further north into the United States, where deserts and xeric shrublands prevail, and the Australian hotspot lied in the deserts and xeric shrublands of Central Australia, but extended also into the (sub)tropical region in the north and the Mediterranean region in the west (Figures 2e,f and 4c).

C_4 grasses showed four diversity hotspots: (1) the tropical and subtropical open coniferous forests, as well as the adjacent deserts of thorn scrubs with fleshy plants and pastures at slightly higher elevations of Mexico, where temperate to semi-arid climate prevails; (2) the tropical and subtropical grasslands and shrublands of Queensland, Australia; (3) South America; and (4) Africa in tropical and subtropical grasslands, savannas and shrublands (Figure 2a,b). Since India is not divided into provinces like the other tropical countries, it stands out due to its size and the associated high number of species. Looking at the more detailed species richness map with a resolution of 100 × 100 km grid cells, India as a region does not seem to have a high C_4 species richness (Figure 2a,b). This emphasises the importance of considering different scales and resolutions when analysing ecological patterns.

The rank difference of C_4 eudicots versus C_4 grass species occurrence revealed the particular importance of deserts and xeric shrublands (e.g., Africa, Arabian Desert, Asia) and the temperate northern hemisphere (e.g., Mediterranean) for C_4 eudicots; and the Afrotropical realm (particular Madagascar) and the tropical zone of South America, Southeast Asia and Australia for C_4 grasses (Figure 2g).

Both, C_4 grasses as well as C_4 eudicots, occurred in a wide range of annual mean temperatures from 1 to 31.2°C and 6 to 30.5°C (95% interval), respectively (Figure 3). The median temperature was 19.0°C for C_4 grasses and 17.4°C for C_4 eudicots. C_4 grasses, in addition to increasing in the range of 15–18°C, had a second steep increase in occurrence records ranging between 27 and 30°C, dominated by the subfamilies Chloridoideae and Panicoideae. That last peak could not be observed in the C_4 eudicots. Occurrence points of C_4 grasses were found in a broad niche of annual mean precipitation profiles, from 0 to approx. 2000 mm (95% interval). However, the predominant occurrence of C_4 grasses tended to be in the semi-wet areas. An increase in occurrence points was seen in the range between 600 and 900 mm, with the median of 772 mm. C_4 eudicots, on the other hand, occurred in distinctly less precipitation areas, with a median of 394 mm (Figure 3). An increase of C_4 eudicot occurrence points was observed in regions with approx. 300–600 mm precipitation/year (Figure 3). On a per-continent basis, the occurrence of C_4 grasses and C_4 eudicots differed most prominently in Europe and Africa (Figure S16). In Africa, C4 eudicots show a higher density in areas with <500 mm precipitation, especially in regions with cooler temperatures. In Europe, C_4 eudicots show a higher density in areas with <400 mm precipitation and warm temperature.

Overall, the diversity and abundance of C_4 plants increased with increasing annual mean temperature and dry season and decreased with increasing cold temperatures and rainfall. For C_4 grasses, there was a trend to wetter areas than in C_4 eudicots. Areas with cool and dry conditions are primarily colonised by C_4 eudicots.

3.3 | Diversity of C_4 eudicots

Mapping C_4 occurrence points at family level revealed many C_4 eudicots hotspots of high taxonomic diversity at higher ranks with C_4 species from greater than or equal to seven families occurring in the same area (Figure 4c). These hotspots were Mexico/Southern United States, Australia, South and West Africa, and South America. In South America, the hotspot was located in the montane grasslands and shrublands of Argentinian Patagonia, whereas the Australian hotspot at the family level expanded into the tropical and subtropical grasslands and shrublands.

At the genus richness of C_4 eudicots, additional diversity hotspots were retrieved: in Asia the temperate grasslands, savannas and shrublands and the Altai-Sayan mountain range and in Europe in the Mediterranean shrublands (Figure 2e,f).

Amaranthaceae, Asteraceae, Euphorbiaceae, Portulacaceae and Zygophyllaceae are the five eudicot families with the highest numbers of C_4 species (Figure 4a). While C_4 Amaranthaceae showed high species richness in many different biomes, the major biomes of C_4 eudicots were tropical and subtropical moist and dry broadleaf forests (Biome 1 & 2), tropical, subtropical and temperate grasslands, savannas and shrublands (Biome 7 & 8), and deserts and xeric shrublands (Biome 13).

We found statistically significant differences in precipitation and temperature between C_4 eudicot families (Kruskal–Wallis: for annual mean precipitation – chi-squared = 231.34, df = 14, p-value <2.2e-16; for annual mean temperature – chi-squared = 161.73, df = 14, p-value <2.2e-16) and between C_4 eudicots and C_4 grasses (Mann–Whitney U test: for annual mean precipitation – p-value <2.2e-16; for annual mean temperature – p-value <2.2e-16). These results point towards a wide adaptation range to diverse environmental conditions among the families and both groups (Figure 5).

Focusing on the five families (Amaranthaceae, Asteraceae, Euphorbiaceae, Portulacaceae and Zygophyllaceae) considered as

TABLE 1 The impact of filtering on the raw occurrence datasets of the various eudicots plant families containing C_4 species (including the number of occurrence points at each step). Beginning with the raw record list.

	Occurrences after downloading from GBIF[a]	Occurrences after applying CoordinateCleaner[b]	Occurrences after manual cleaning[c]	Occurrences after removing duplicates	Occurrences after removing outliers[d]
Acanthaceae	1380	1335	1335	988	855
Aizoaceae	4894	4009	4009	3135	2632
Amaranthaceae	645,813	463,332	336,492	242,485	144,100
Asteraceae	14,369	12,772	12,767	10,889	9562
Boraginaceae	5261	4782	4253	3156	3003
Caryophyllaceae	9338	8644	8606	6389	3224
Cleomaceae	2093	1735	1563	1163	925
Euphorbiaceae	122,123	85,559	82,052	61,509	45,285
Gisekiaceae	563	525	399	315	312
Molluginaceae	1347	1263	1240	1031	528
Nyctaginaceae	21,248	18,395	16,530	14,271	13,645
Polygonaceae	765	719	719	391	210
Portulacaceae	155,597	106,501	86,490	61,308	12,197
Scrophulariaceae	239	216	204	171	169
Zygophyllaceae	27,527	21,835	21,727	17,504	10,558
C4 eudicots	1,012,557	731,622	578,386	422,905	247,205

[a]See references for DOI number.

[b]CoordinateCleaner v2.0-18 (Zizka et al., 2019).

[c]Manual cleaning: Nomenclatural and taxonomic checking including [the correction of] wrong synonymisations.

[d]Deleting outliers: after checking whether the native distribution information of plantsoftheworldonline.org matches the distribution country (Country Code) of each distribution point from GBIF.

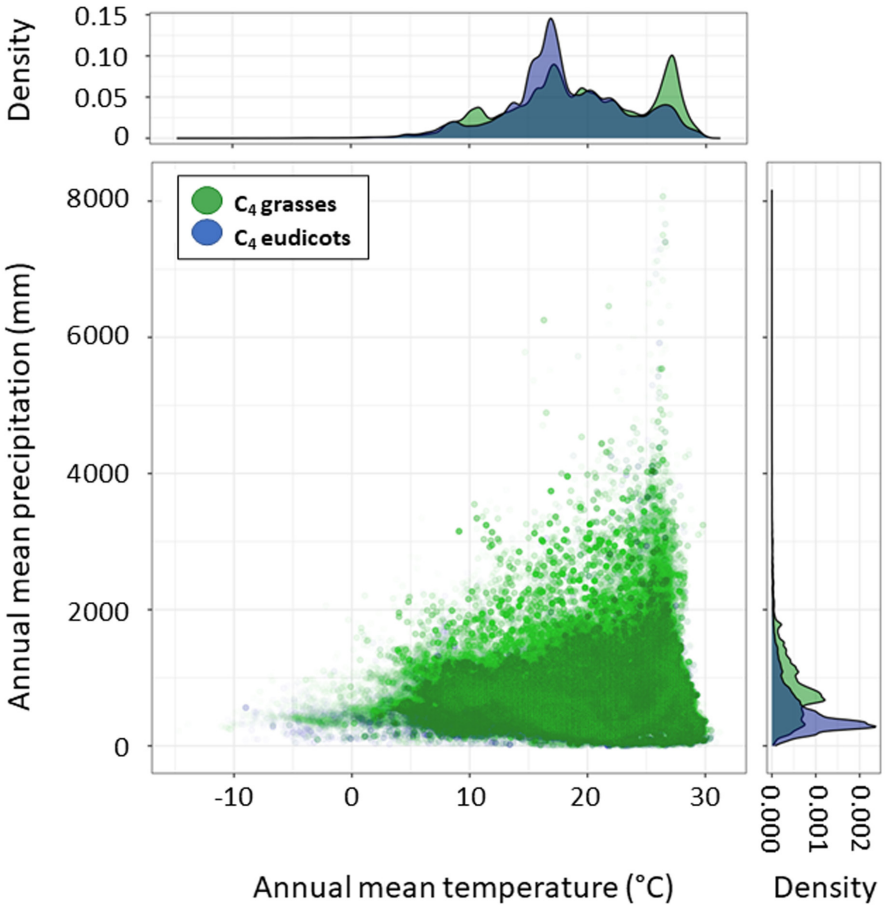

FIGURE 3 Scatterplot showing annual mean temperature (°C) and annual mean precipitation (mm) data of C_4 grasses (green) and C_4 eudicot (blue) occurrence points.

species-rich per grid in Figure 4, we noticed that Amaranthaceae differed significantly (Post-hoc Tukey test, *p*-value of <.01) in the annual mean temperature and precipitation range from the other four families, except when comparing Amaranthaceae and Zygophyllaceae for the precipitation range (Post-hoc Tukey test, *p*-value of 1). Further multiple comparisons between the four other plant families showed no significant difference in terms of temperature (Post-hoc Tukey test, *p*-value of >.06). In terms of precipitation range, the comparisons between Zygophyllaceae-Asteraceae, Zygophyllaceae-Euphorbiaceae and Zygophyllaceae-Portulacaceae showed a significant difference (Post-hoc Tukey test, *p*-value of <.01). Amaranthaceae had the lowest mean value of 17.77°C of these five families. Furthermore, the mean value of annual precipitation in Amaranthaceae was 333 mm. The mean value of the preferred annual mean temperature for Asteraceae was 22.91°C, and annual precipitation of 929 mm, occurring in wetter and warmer areas compared to the other four families. C_4 species of Euphorbiaceae preferred rather wet areas (mean = 797 mm) with a wide interquartile range and temperatures that intersect with the preferred areas of Amaranthaceae and Asteraceae (mean = 20.62°C). A similar pattern was retrieved for C_4 species of the Portulacaceae. Their preferred temperature lied between the values of Amaranthaceae and Asteraceae (mean = 20.69°C) and the C_4 species of Portulacaceae occurred in areas with an annual precipitation of 878 mm. Zygophyllaceae together with Asteraceae preferred the warmest areas among these five families (Zygophyllaceae: mean = 22.71°C), and the mean value of the preferred annual precipitation for Zygophyllaceae was second lowest at 459 mm.

Families with only one C_4 lineage (genus) stood out among the others. In Polygonaceae, a single C_4 genus *Calligonum* that occurs in the cold deserts of Central Asia was particularly conspicuous in climatic preferences with the lowest mean value of 11.48°C and a precipitation preference in the very dry range (mean = 136 mm). A different picture was observed in C_4 species of the family Caryophyllaceae which also contains only one C_4 genus, *Polycarpaea*. These C_4 species preferred comparatively warmer (mean = 26.72°C) and wetter regions (mean = 895 mm).

3.4 | Traits

A total of 394 (39% of total) succulent C_4 species were recorded in eight eudicot families (Aizoaceae, Amaranthaceae, Caryophyllaceae, Gisekiaceae, Polygonaceae, Portulacaceae, Molluginaceae, Zygophyllaceae). These are distributed around a mean value of annual precipitation of 444 mm, which is higher than the mean for all C_4 eudicots. Salt tolerance is documented in seven families with about 485 (49%) species, 333 (33%) of which are succulents from five

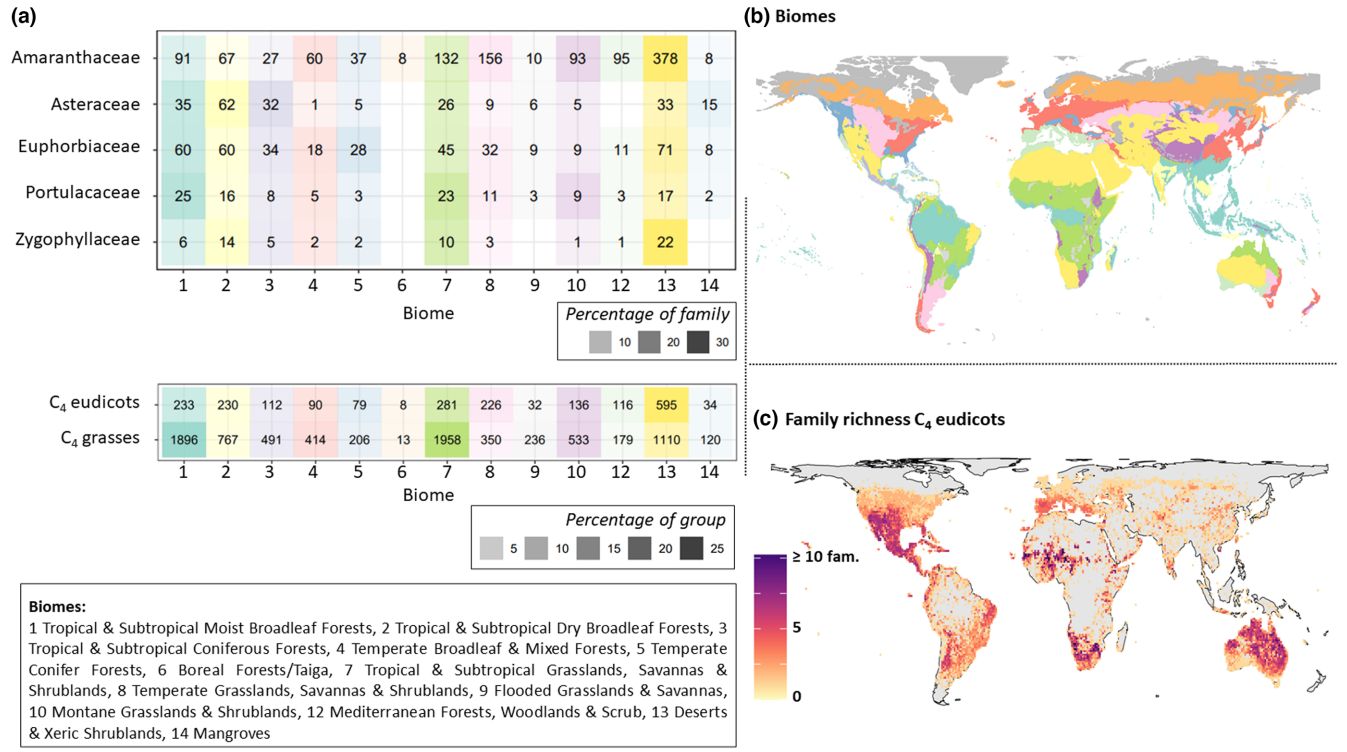

FIGURE 4 (a) The table shows the number of species of a family within a biome (Terrestrial ecoregions of the world (Olson et al., 2001)). Species are considered present in a biome if at least 5% of the distribution points are in that biome. The five C_4 eudicot families with the highest number of species in this study are shown (Figure 1a), as well as C_4 eudicots and C_4 grasses in general. Colours correspond to the biomes shown in (b). Saturation shows the percentage of species per family or per group (C_4 eudicots, C_4 grasses) in each biome. (b) Terrestrial biomes of the world (Olson et al., 2001). (c) Global richness map of C_4 eudicot families, showing the number of families occurring in each grid (100 × 100 km).

families (Aizoaceae, Amaranthaceae, Polygonaceae, Portulacaceae, Zygophyllaceae). The biochemical subtypes were also examined. 459 (46%) investigated species within 11 families were retrieved to have the NAD-malate enzyme as the predominant decarboxylase. In contrast, 559 (56%) species within ten families exhibited the NADP-ME biochemical subtypes.

Most C_4 eudicot species showed the classical atriplicoid leaf anatomy without or only little accompanied water storage tissue (Figure 6c). This anatomy with minor differences was also predominant in C_4 grasses (Edwards & Voznesenskaya, 2011). In cases of succulence, there was often a deviation from atriplicoid anatomy. A high diversity in the succulent leaf anatomy of C_4 eudicots was observed, with most C_4 leaf types occurring only in a few species (Figure 6). However, the salsoloid leaf anatomy was clearly the most common leaf type among succulent C_4 eudicots, not only in the Amaranthaceae but also in Aizoaceae and Polygonaceae (Table S1). Among the succulent species, ca. 3% were stem succulents, while the rest were leaf succulents. The diversity of the leaf anatomy was closely linked to succulence and non-succulent leaves were usually atriplicoid. Eudicot C_4 species with NAD-ME as the primary decarboxylating enzyme are distributed in areas with significantly lower annual precipitation than NADP-ME subtype eudicot C_4 species ($F = 26{,}936$, p-value <2.2e-16; annual mean precipitation of 405.83 mm versus 698.39 mm).

3.5 | Results of the literature survey on geographical origin of C_4 photosynthesis

Seventeen genera out of eight C_4 eudicot families were distributed in the diversity hotspot of Mexico and Southern United States (Table 2). Five of these genera encompassed more than ten C_4 species found in the area, with six C_4 genera originated in the area. Out of these in situ C_4 genera two, *Euphorbia* and *Pectis*, are species-rich.

Sixteen genera out of ten C_4 eudicots families were distributed in the diversity hotspot Australia (Table 2). Two of these contained more than ten C_4 species found in the area, with only one species-poor C_4 lineage (C_4 *Tecticornia*) unequivocally originating in the area. *Atriplex* and *Gomphrena* were the species richest C_4 genera in Australia. While C_4 Australian *Atriplex* originated ex situ the area of origin of C_4 in *Gomphrena* is currently unknown (Table 2 and citation therein). Overall, in 14 cases the area of origin of C_4 photosynthesis is still insufficiently investigated (Table 2).

4 | DISCUSSION

We characterised the global occurrence of C_4 eudicots, identified diversity hotspots and climatic preferences, assigned these to specific functional plant traits and conducted literature research on

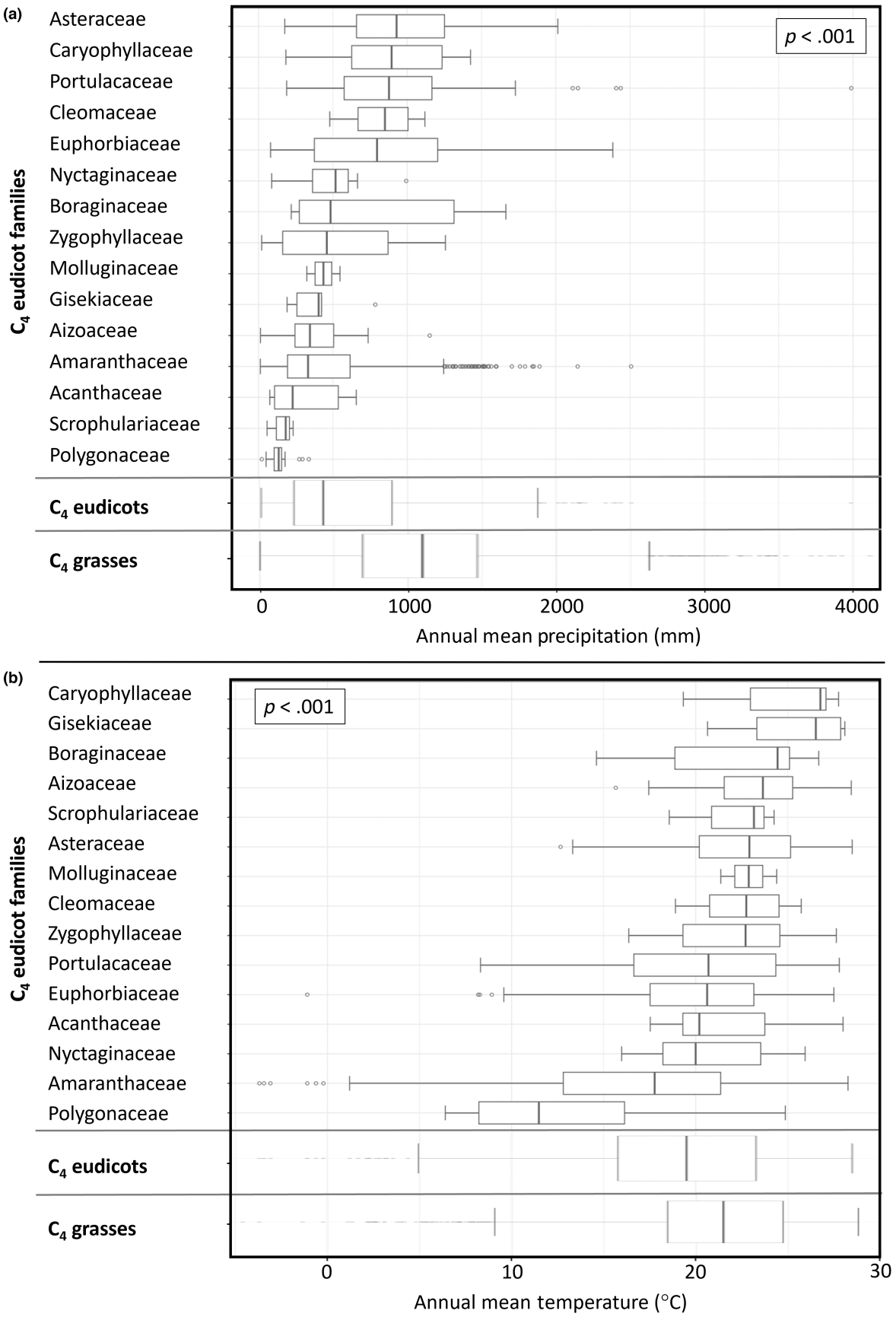

FIGURE 5 Differences in climatic preferences of the C_4 species in eudicot families, and C_4 eudicots and C_4 grasses in general. A Kruskal–Wallis test was used over C_4 eudicot families: $p < .001$ statistically significant. (a) Annual mean precipitation (b) and annual mean temperature.

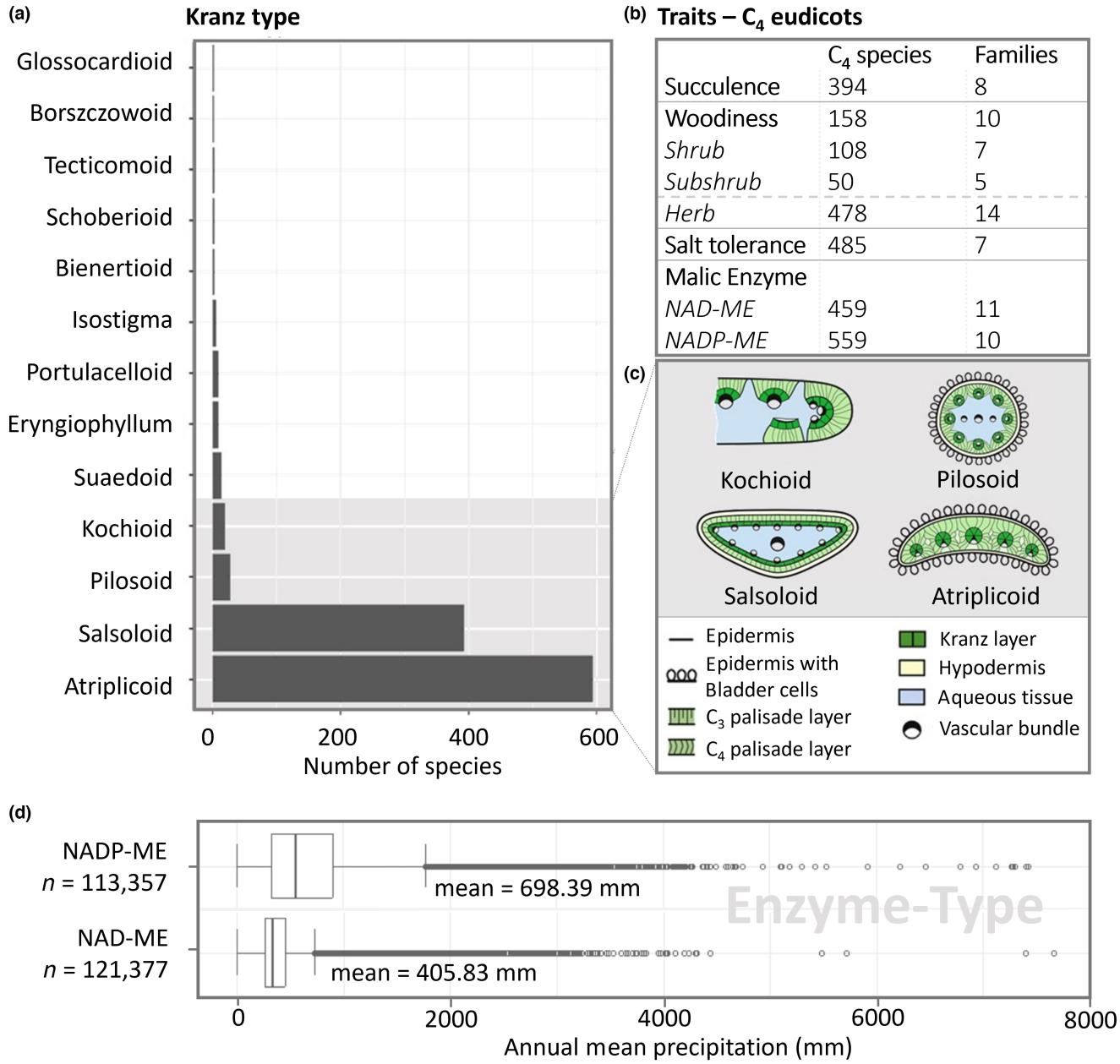

FIGURE 6 (a) Distribution of Kranz types present in C_4 eudicots. (b) Distribution of the traits summarised within the C_4 eudicots. Separated into the number of C_4 species and the number of families in which the traits occur. (c) Schematic illustration of the four common C_4 leaf types (Schematic drawings for illustration of leaf anatomical types are adapted from Bohley et al., 2015). (d) Range and mean of annual precipitation of species with NADP-ME and NAD-ME as primary decarboxylating enzymes.

spatiotemporal histories of C_4 lineages. The database attached to this paper (Table S1) includes the current knowledge of physiological and morphological traits underlying large-scale patterns for C_4 eudicots. Our approach combines the thus far known worldwide distribution of C_4 eudicots, the evolution of photosynthesis and associated traits and climatic preferences of the individual lineages.

So-called "big data," as in our case with many distribution points of many different species from GBIF, are valuable resources for wide-scale analyses and can provide novel insights. However, accurate and elaborate cleaning of the data is essential to obtain meaningful results (Zizka et al., 2019). Another challenge is that in a project with the scope presented here a verification of all the identifications is unrealistic if not close to impossible. Also, we have to take into consideration the sampling density bias of Europe, North America and Australia over large areas of poorly sampled areas of Africa and Asia and thus have to interpret our findings applying to these regions with caution, which is why we additionally obtained species richness maps with the use of WCVP. While WCVP data are less affected by sampling differences, it comes with its own limitations, such as lower spatial resolution and unevenly sized sample areas (e.g., India sticks out as particular species rich in C_4 eudicots, particularly due to its size). Despite these challenges, using data from both sources allows

TABLE 2 List of eudicots C_4 genera occurring in the diversity hotspots Mexico/Southern United States and Australia according to our occurrence points and of C_4 genera with molecular evidence of C_4 in situ origin within the remaining diversity hotspots. Genera that are species-rich (>10 species) in the respective region are indicated in bold (**x**). Genera that likely originated in the respective region are marked with *.

Family	Genus	Mexico/south US	Australia	C_4 origin	References
Acanthaceae	*Blepharis*			Africa	Fisher et al. (2015)
Aizoaceae	*Sesuvium*			Africa	Bohley et al. (2015)
	Trianthema	x	x	Africa	Bohley et al. (2015)
	Zaleya		x	Africa	Bohley et al. (2015)
Amaranthaceae	*Alternanthera*	x	x	More likely origin: South American tropics	Sage et al. (2007), Sánchez-del Pino et al. (2012)
	Amaranthus	**x**	x	Origin unknown	
	Atriplex	**x**	**x**	Continental Asia	Žerdoner Čalasan et al. (2022)
	Froelichia	x		Origin unknown	
	Gomphrena	x	**x**	Origin unknown	
	Guilleminea	x		Origin unknown	
	Suaeda	x		Origin unknown	
	Tecticornia		x*	Australia	Shepherd et al. (2005), Voznesenskaya et al. (2008)
	Tidestromia	x*		Mexico/South US	Sánchez-del Pino and Motley (2010)
Asteraceae	*Flaveria*	x*		Mexico/South US	Morales-Briones and Kadereit (2023)
	Glossocardia		x	Origin unknown	
	Pectis	**x***		Mexico/South US	Hansen et al. (2016)
Boraginaceae	*Euploca*	x	x	Origin unknown	
Caryophyllaceae	*Polycarpaea*		x	Origin unknown	
Cleomaceae	*Cleome*		x	Origin unknown	
Euphorbiaceae	*Euphorbia*	**x***	x	Mexico/South US (*Euphorbia* subg. *Chamaesyce* sect. *Anisophyllum* subsect. *Hypericifoliae)*	Yang and Berry (2011), Horn et al. (2014)
Gisekiaceae	*Gisekia*			Africa	Bissinger et al. (2014)
Nyctaginaceae	*Allionia*	x*		Mexico/South US	Douglas and Manos (2007)
	Boerhavia	x*	x	Mexico/South US	Douglas and Manos (2007)
Portulacaceae	*Portulaca*	**x**	x	Origin unknown	
Scrophulariaceae	*Anticharis*			Africa	Khoshravesh et al. (2012)
Zygophyllaceae	*Kallstroemia*	x		Origin unknown	
	Tribulopis		x(*)	Less clear origin: Australia	Lauterbach et al. (2019)
	Tribulus		x	Origin unknown	
	Zygophyllum			Africa (*Z. simplex*)	Bellstedt et al. (2012)

for a more comprehensive and cautious analysis, taking into account the strengths and limitations of each dataset. These facts additionally underline the general need of well-curated data in our biodiversity repositories and collecting efforts to fill the sampling gaps if we want them to be used by a broad community of researchers and to provide useful data for wide-scale analyses.

4.1 | Similarities and differences in the global distribution of C_4 grasses and C_4 eudicots and their climatic and biome preferences

Both, C_4 monocots and C_4 eudicots, include multiple C_4 lineages. While the C_4 monocots are distinctly richer in species and all except two belong to the order Poales, the C_4 eudicots belong to seven angiosperm orders (spanning across basal eudicots, rosids and asterids; Figure 1). Nevertheless, this is not indicative of differences in evolutionary age of C_4 photosynthesis. In both, C_4 grasses and C_4 eudicots, the age of C_4 origins spans from the Oligocene to the Pleistocene with most origins dating back to the Late Miocene (see Sage, 2017 for a summary), an era of global expansion of C_4 vegetation due to declining atmospheric CO_2 combined with global cooling and increase in climate seasonality and aridity (Cerling et al., 1997; Wen et al., 2023).

The global maps of C_4 species richness (Figure 2a-c) reveal two shared hotspots for C_4 grasses and C_4 eudicots: one in Mexico/Southern United States and one in Australia. In these two regions, C_4 lineages seem to have diversified more intensively than in other parts of the world, and in case of the C_4 eudicots, this diversity has been recruited independently from multiple families and even multiple times within one family (Figures 2e,f and 4c; Table 2). Two additional hotspot regions for the C_4 grasses are found in South and West Africa. However, since Africa is in many areas poorly-sampled, these two regions might appear as C_4 grasses hotspots due to being proportionally more densely sampled than other regions in Africa. Additionally, looking at the richness of C_4 grasses per botanical country, East (Tanzania) and Central Africa also count as C_4 species-rich (Figure 2b). The richness maps showing the diversity at the genus (Figure 2e,f) and family level (Figure 4c), however, indicate possible further smaller hotspots for the C_4 eudicots, in Africa (as in grasses), as well as in Patagonia, Central Asia and the Mediterranean. In these regions, diversification within genera is less prominent, but multiple C_4 eudicot lineages evidently colonised these areas as well (Figures 2e,f and 4c).

Whether these regions of high diversity of C_4 lineages represent areas of C_4 origin or were just preferably colonised by already existing C_4 lineages or both need to be evaluated for each C_4 lineage and region in a phylogenetic and biogeographical context (see below).

Generally, both C_4 grasses and C_4 eudicots showed broad climatic ranges with a large overlap. On average C_4 grasses occurred in only slightly warmer but distinctly wetter areas than C_4 eudicots (Figure 3). The occurrence of C_4 grasses increases in regions with an annual rainfall of around 800 mm (Figure 3) and warm temperatures coupled with high insolation – conditions common in the southern hemisphere (Still et al., 2014). Where these climatic conditions are met C_4 grasses not only tend to show a high species diversity but often also dominate the vegetation, especially when fires occur regularly (Hoetzel et al., 2013; Sage, 2004; Still et al., 2014). Here are three examples where this is the case: (i) C_4 grasslands in the highveld of southern Africa, dominated by *Hyparrhenia hirta* (L.) Stapf (Panicoideae) and *Sporobolus pyramidalis* P.Beauv. (Chloridoideae) receive an annual mean precipitation between 400 and 900 mm mainly during the warm summer months (Bond, 2008; Low & Rebelo, 1996; Mills & Cowling, 2006); (ii) species-rich C_4 grasslands at the tropical Sudanian savanna near the Volta-, Benue- and Niger-River experience a peak of summer precipitation of 600 mm in the north and 1000 mm in the south and the West African monsoons that occur between June and August result in warmer and wetter summers that support C_4 vegetation (Olusegun et al., 2018); (iii) species-rich C_4 grasslands in north-east Queensland and the Northern Territory, Australia, are associated with the tropical to subtropical climate along the coastal strip, the warm and wet summer months (December–February) and the Australian monsoon bringing up to 1300 mm rainfall (Ondei et al., 2016).

C_4 eudicots colonise predominantly dry to very dry areas with 80% of the occurrences in areas with <800 mm precipitation. One prominent example of an area where C_4 eudicots show a higher diversity is the cold deserts of Eurasia, with temperatures below the freezing point for an extensive period of time throughout the year (Johnston, 1996; Rudov et al., 2020; Winter, 1981). These are dominated by woody (sub)shrubs *Haloxylon persicum* and *H. ammodendron* as well as *Calligonum aphyllum*, *C. mongolicum* and *Anabasis brevifolia*. All five C_4 species represent an integral part of the cold desert vegetation and no closely related C_3 relatives are known from either of these genera (Kürschner, 2004; Lauterbach et al., 2019; Wu et al., 1994–2013). Less prominent C_4 floral elements of cold Central Asian deserts include species-poor genera such as *Horaninovia*, *Iljinia*, *Nanophyton*, *Piptoptera*, *Pyankovia*, *Turania* and *Xylosalsola*. These are together with *Anabasis* and *Haloxylon* all members of an evolutionary old C_4 lineage within Salsoloideae (Amaranthaceae) that likely spread into the cold desert areas several times independently (Akhani et al., 2007; Kadereit et al., 2012). The biogeographically most comprehensively studied genus among these is *Anabasis*, which revealed the adjacent hot deserts of the Irano-Turanian Provinces as source areas for the species occurring in the cold deserts of the Mongolian Province (Lauterbach et al., 2019). Another example of vegetation with high diversity of C_4 eudicots is the hot deserts of Central Australia where certain species of the large genus *Atriplex* such as *A. holocarpa*, *A. lindleyi* and *A. vesicaria* are highly abundant (Wilson, 1984).

In terms of preferred biomes, our analyses revealed that C_4 grasses are most common in (sub)tropical grasslands, savannas and shrublands, whereas the highest species diversity of C_4 eudicots was recorded from deserts and xeric shrublands (Figure 4). Both, C_4 grasses and C_4 eudicots, are fairly common even in (sub) tropical moist broadleaf forests. All these biomes, except the latter,

are demarcated by scarcity and/or seasonality of precipitation. For example, one part of C_4 grasses occurs in the rainforests of the Australasian realm such as tropical and Central Range montane rainforests of Queensland. Another part occurs in the Indomalayan realm, such as Borneo lowland rainforests, Kayah-Karen and Sri Lanka montane rainforests and South Taiwan monsoon rainforests. Expectedly, both C_4 groups are scarcer in water-rich and cooler biomes probably because the C_4 syndrome is less advantageous and C_3 species are more competitive. In regions where C_3 trees dominate, the C_4 syndrome might be a disadvantage due to the limitations of the higher ATP-demand of this pathway in shady habitats (Ehleringer & Björkman, 1977; Sage & McKown, 2006). Within C_4 eudicots, Amaranthaceae are ecologically the most diverse and are the only C_4 eudicot clade found at higher latitudes in boreal forests.

4.2 | Did C_4 lineages originate in the diversity hotspots or did C_4 lineages colonise these areas?

4.2.1 | C_4 hotspot Mexico/southern United States

The C_4 hotspot in Mexico and the Southern United States encompasses mostly deserts and xeric shrublands with different climatic regimes. This southern tip of the Nearctic realm comprises warm deserts such as Mojave Desert, Sonoran Desert, Chihuahuan Desert as well as cold deserts of the Great Basin, and large adjacent and equally diverse semi-desert areas (Laity, 2008). This climate and habitat diversity probably promoted speciation, making these areas particularly species-rich. About two-thirds of the flora is endemic to this region and the most common plant families represented in this flora are Cactaceae, Asteraceae and Boraginaceae (Villarreal-Quintanilla et al., 2017). The majority of endemic species and also the most widely distributed species that typify these landscapes such as *Ambrosia monogyra*, *Artemisia filifolia* and *Flourensia cernua* (Asteraceae), *Ephedra torreyana* (Ephedraceae), *Larrea tridentata* (Zygophyllaceae), *Penstemon thurberi* (Plantaginaceae), *Poliomintha incana* (Lamiaceae), *Prosopis glandulosa* and *Psorothamnus scoparius* (Fabaceae) and *Yucca elata* (Asparagaceae, Shreve, 1939), however, do not perform C_4 photosynthesis. Nevertheless, here we recorded many C_4 species from 17 eudicot genera belonging to eight different families (Table 2), supporting the findings of Sage et al. (2011) that Mexico and the Southern United States are a hotspot of C_4 lineage diversity. For at least six of these lineages, the current molecular phylogenies deliver sufficient evidence for an in situ origin of C_4 photosynthesis within this area, with two of these being species-rich (Table 2).

One is the neotropical genus *Pectis* (Tageteae, Asteraceae) which includes approximately 90 C_4 species and is represented by about 47 species in Mexico and the Southern United States (Hansen et al., 2016). The sister genus *Porophyllum* performs C_3 photosynthesis and is also distributed in tropical and subtropical America. Hansen et al. (2016) show that the transition to C_4 photosynthesis occurred most likely during the Late Miocene in the stem lineage of *Pectis* which was probably distributed in North/Central Mexico. Within the mega-diverse family Euphorbiaceae the evolution of carbon concentrating mechanisms led to diversification bursts (Horn et al., 2014). Here we find the second example of in situ origin of C_4 photosynthesis in Mexico/Southern United States with subsequent diversification (Horn et al., 2014; Yang & Berry, 2011). The C_4 pathway evolved only once within the subgenus *Chamaesyce* at the stem of section *Anisophyllum* subsection *Hypericifoliae* during the Mid-Miocene and gave rise to approximately 350 C_4 species, which constitutes the largest eudicot C_4 lineage known thus far. For the well-known C_4 model *Flaveria* (Asteraceae; Monson & Moore, 1989; Sage et al., 2014) the phylogenetic tree topology suggests that the C_4 pathway originated in this area, likely during the Pliocene (Morales-Briones & Kadereit, 2023). The genus comprises 21 species, mainly distributed in Southern North America, with few species occurring in the Caribbean and South America (Powell, 1978). The C_4 genera of Nyctaginaceae, *Boerhavia* and *Allionia*, belong to the "North American xerophytic clade" of the family (Douglas & Manos, 2007; Khoshravesh et al., 2020). This clade likely diversified in the deserts of the southwestern United States and northwestern Mexico because all genera are either confined to or represented in the area (Douglas & Manos, 2007). *Boerhavia* subsequently spread and diversified in subtropical regions worldwide. The situation in the species-rich *Euploca* (Boraginaceae) is challenging to assess as the published molecular phylogeny lacks support along the backbone (Frohlich et al., 2022). Nevertheless, an in situ origin of the North American C_4 species seems likely.

Amaranthaceae s.l. are well-represented in Mexico and the Southern United States with eight genera containing native C_4 species (Table 2), however, only for *Tidestromia* which consists entirely of C_4 species that are all but one endemic to the region, an in situ origin seems likely (Sánchez-del Pino & Motley, 2010). *Froelichia*, *Guilleminea* and *Gomphrena* belong to a species-rich and widespread C_4 clade that probably started to diversify during the Mid-Miocene (Limarino & Borsch, 2020). However, due to insufficient sampling, it is currently impossible to infer whether the C_4 pathway originated in tropical South America or in subtropical southern North America. Insufficient phylogenetic information also prevents us from inferring the origin of North American *Amaranthus* species (Waselkov et al., 2018). However, since the entire genus exhibits C_4 and probably originated in South America, *Amaranthus* seems to be a migratory C_4 lineage in Mexico and the Southern United States. Other migratory C_4 lineages are *Trianthema* which spread into the area from Africa (Bohley et al., 2015), *Atriplex* which arrived from South America (Žerdoner Čalasan et al., 2022), *Portulaca* (Ocampo & Columbus, 2012; Tamboli et al., 2022) and *Suaeda* (Schütze et al., 2003). The biogeography of the C_4 genus *Kallstroemia* which is distributed from Central and Southern North America to tropical and subtropical South America remains unclear due to limited phylogenetic support (Lauterbach et al., 2019).

Nowadays, the C_4 hotspot in Mexico and the Southern United States receives a limited amount of rain, ranging from around 50–250 mm per year (Pearcy & Ehleringer, 1984), due to rain shadow

casted by the mountain ranges of Sierra Madre Occidental and Sierra Nevada, and Sierra Madre Oriental on either side, which are of Late Mesozoic and Early Cenozoic age (Dickinson, 2004). The earliest evidence of desertification in this area dates to the Middle Miocene and corresponds with the diversification events of arid-adapted lineages (Eronen et al., 2012; Hyland et al., 2019; Said Gutiérrez-Ortega et al., 2018; Vásquez-Cruz & Sosa, 2020). This refers also to some of the in situ originated C_4 lineages, such as *Pectis*, *Flaveria* (both Asteraceae) and *Allionia* and *Boerhavia* (both Nyctaginaceae) which have originated and spread since the Mid to Late Miocene (Table 2). We suggest that the overall high diversity of ancestral C_3 lineages in the area adapted to arid conditions in addition to the high selective pressure in favour of the evolution of a carbon concentration mechanism is responsible for the exceptionally high diversity of C_4 lineages that originated in Mexico and the Southern United States. In addition to these in situ C_4 lineages a high number of migratory C_4 lineages occur finding suitable growing conditions in the area.

4.2.2 | C_4 hotspot Australia

Within Australia, two regions of high C_4 plant diversity with different precipitation profiles are observed. The first one constitutes deserts and xeric shrublands of the Eremaean floristic region (sensu Ebach et al., 2015), rich in C_4 *Atriplex* (about 60 species) but also in C_3 Camphorosmeae (about 150 species) and *Chenopodium* (about 50 species; Kadereit et al., 2005). Most other Australian C_4 eudicots are restricted to the northern parts of the continent where tropical and subtropical grasslands, savannas and shrublands prevail. Here the biggest C_4 genera are *Gomphrena* (Amaranthaceae) with 30 C_4 species, followed by *Euphorbia* (Euphorbiaceae) with seven and *Portulaca* (Portulacaceae) and *Polycarpaea* (Caryophyllaceae) with five C_4 species each.

While there are many different C_4 plant lineages known from these areas, the majority of them did not evolve in situ (Table 2), albeit little direct evidence is available, due to the lack of robust phylogenies. Some migratory C_4 lineages diversified in Australia. C_4 *Atriplex* lineages, for example, reached Australia at least two times independently – once from the Mediterranean/Pontic region at the end of the Miocene and once from Central Asia at the end of the Pliocene, with the latter, younger one, going through an extensive diversification after reaching the continent (Žerdoner Čalasan et al., 2022). A similar pattern can be inferred from Australian C_4 Aizoaceae (*Trianthema* and *Zaleya*) with Africa as their source area (Bohley et al., 2015). The spatial and temporal aspects of other C_4 representatives of Australian flora (*Euploca*, *Glossocardia*, *Gomphrena*, *Polycarpaea*, *Portulaca* and *Tribulus*) remain unclear. Limited data, however, point towards ex situ evolution of the C_4 syndrome in these genera.

The only clear C_4 in situ origin is currently known from *Tecticornia* (Shepherd et al., 2005; Voznesenskaya et al., 2008). This taxon is adapted to hyper-saline conditions and builds extensive vegetation stands along the edges of Australian inland salt lakes (Shepherd et al., 2004). This genus comprises about 60 species, out of which only a clade of five taxa is known to perform C_4 photosynthesis. While many C_3 species have rather restricted distribution areas (which may or may not be a result of lack of surveys in poorly accessible Australian outback), one of the two C_4 species, *Tecticornia indica*, exhibits a wide distribution range along saline lake shores across the whole continent (Wilson, 1984). Another, albeit less clear example is the small Australian genus *Tribulopis*. Conflicting phylogenetic signals between the nuclear and chloroplast-encoded genes point towards a complex evolutionary history of this taxon (Lauterbach et al., 2019). Contrarily to *Tecticornia*, here the C_3 representatives show a wider distribution range, whereas the C_4 species tend to be geographically restricted (Wilson, 1984). While reasons for this peculiar distribution remain unknown, this example clearly indicates that the factors promoting the evolution of C_4 photosynthesis are multifold and that each individual C_4 lineage has its own unique evolutionary history. Both taxa arrived to Australia post-Miocene (Piirainen et al., 2017; Wu et al., 2018), which coincides with several geological and climate features that initiated and promoted aridification of this continent. These include the northward drift towards the equator, expansion of the Antarctic ice cap, and the formation of the circum-Antarctic Ocean current and subtropical high-pressure system (Fujioka & Chappell, 2010; Kemp, 1978).

4.2.3 | Other C_4 eudicot hotspots

The scarce dataset revealed three regions in Africa that seem to favour C_4 eudicots. The first includes the tropical and subtropical grasslands, savannas and shrublands of Africa. The second C_4-rich region is located in south-east Africa. Here we should mention that the Drakensberg Mountain Centre located in this region is a known biodiversity hotspot, which may or may not influence the number of C_4 species (Carbutt, 2019; Popp & Kalwij, 2021). The third region is in southwestern Africa (Fisher et al., 2015; Schulze & Schulze, 1976; Vogel & Seely, 1977).

Most C_4 eudicot species in these regions belong to Acanthaceae (*Blepharis* sect. *Acanthodium*), Aizoaceae, Gisekiaceae (genus *Gisekia*), Scrophulariaceae (genus *Anticharis*) and Zygophyllaceae (Table 2). The estimated centre of in situ origin for C_4 photosynthesis, with molecular phylogenetic evidence, in *Blepharis* (Fisher et al., 2015), *Anticharis* (Khoshravesh et al., 2012), *Gisekia* (Bissinger et al., 2014), Sesuvioideae (*Sesuvium/Trianthema/Zaleya*; Bohley et al., 2015) and the species *Zygophyllum simplex* (Zygophyllaceae; Bellstedt et al., 2012) appears to be the so-called "Horn of Africa," which defines the south of the Sahara-Sindian region, and the arid southwestern Africa (Bellstedt et al., 2012 and references therein).

In South America, two centres of C_4 eudicot biodiversity are recognised, both under strong influence of arid desert or steppe climate with a pronounced dry period. These two centres of C_4 diversity are most evident in *Euploca* (Boraginaceae) and *Portulaca*

(Portulacaceae). However, they are also found in other eudicotyledons such as Amaranthaceae, Asteraceae and Euphorbiaceae. *Portulaca* has the highest C_4 species diversity in north-eastern Brazil. C_4 *Portulaca* clade started to radiate in the late Miocene/Pliocene (Ocampo & Columbus, 2012), following the expansion of C_4 vegetation due to decreased CO_2 levels and increased aridity (Salzmann et al., 2008; Strömberg, 2011). Nevertheless, the north-eastern portion of Brazil became consistently arid very recently in geological history – at the end of Younger Dryas (Auler et al., 2004). This leads us to believe that the high C_4 diversity of *Portulaca* in that region is of refugial origin, as continuous wetter interglacial cycles prior to that diminished any advantage of C_4 species over their C_3 congeners. Poorly resolved phylogeny and lack of time divergence estimation preclude us from discussing potential stages in the evolutionary history of *Euploca* (Frohlich et al., 2022). For C_4 *Atriplex* lineages molecular phylogenetic studies show two long-distance dispersal events to reach South America – one possibly from continental Asia and one from North America (Žerdoner Čalasan et al., 2022).

It is important to mention that there are also regions, such as the Central Asian Deserts, that are not a main area of origin for C_4 lineages, but have a high C_4 lineage diversity due to a lot of migration. However, while the knowledge on the geological history of this region increased dramatically in recent years (Barbolini et al., 2020; Hurka et al., 2019), the evolutionary history of its flora remains largely unknown (Seidl et al., 2021; Žerdoner Čalasan et al., 2021).

4.3 | Functional traits lead to ecological diversity in C_4 eudicots

Precipitation and temperature preference among the C_4 eudicot families differ significantly (Figure 5). This supports the well-known fact that C_4 is advantageous under different environmental conditions. Various phylogenetic analyses (cited in Table 2) indicate that different evolutionary context dependencies together with the respective ancestral anatomical and physiological phenotypes are likely to have influenced the evolution of C_4 and with it associated traits of the plant group. Whether C_4 evolution improved fitness and evolutionary persistence of a lineage or as a key factor enhanced diversification by opening up new niches is lineage-specific and depends on C_4-associated and other lineage-specific traits. Universal C_4-associated traits, such as Kranz anatomy, therefore represent one of several notable examples of phenotypic convergence across a wide range of functional, biochemical and phylogenetic diversity. While it remains poorly understood how Kranz anatomy was initiated and how it arose in C_4 plants (Schlüter & Weber, 2020), it required a variety of complex developmental changes and thus possibly represent a bottleneck to the C_4 origin (Lauterbach et al., 2019). Hence, the high number of independent C_4 origins and their morphological, ecological and physiological diversity in eudicot lineages is unexpected.

Kranz anatomy is an important unifying trait for almost all C_4 species, and the majority of species show a similar (so-called atriplicoid) C_4 anatomy with Kranz cells (or bundle sheath cells) surrounding the vascular bundle and an outer ring of specialised mesophyll cells (Figure 6c). However, many eudicot C_4 lineages deviate from this common anatomical type and show additional anatomical specialisations related mostly to leaf or stem succulence. All lineages that combine succulence and C_4 photosynthesis seem to be derived from ancestrally succulent C_3 lineages (e.g., *Tetraena simplex* in Zygophyllaceae (Lauterbach et al., 2016), C_4 *Sesuvium* in Aizoaceae (Bohley et al., 2015), C_4 *Tecticornia* in Amaranthaceae (Shepherd et al., 2005), and C_4 lineages of Salsoleae, Camphorosmeae and Suaedeae (Kadereit et al., 2012). The annual mean precipitation for occurrence points of C_4 eudicot succulents is 444.47 mm which does not indicate that the C_4 succulents are confined to particularly dry areas. Non-succulent C_4 eudicots such as Scrophulariaceae and Acanthaceae can be found in comparably dry (but not in saline) habitats. Sometimes succulence is lost within a C_4 clade as found in *Bassia* (Kadereit & Freitag, 2011). In the salt-tolerant C_4 grass *Spinifex littoreus*, however, C_4 evolution preceded that of succulence and salt tolerance (Ho et al., 2019; Morrone et al., 2012).

Together with salt tolerance known from most of the succulent C_4 eudicots (Santos et al., 2016), this combination of traits provides solutions to a broad variety of unfavourable climatic and edaphic conditions, which may explain the ecological diversity of C_4 eudicots. In some succulent C_4 eudicots, the C_4 pathway is combined with weak CAM which might provide a rescue mechanism under severe drought. The best-studied example is *Portulaca oleracea* (Portulacaceae; Moreno-Villena et al., 2022), but a co-occurrence of CAM and C_4 was also found in species of Sesuvioideae in Aizoaceae (Winter et al., 2021: *Trianthema portulacastrum*; Siadjeu and Kadereit, under review.: *Sesuvium sesuvioides*) and might be more common than initially thought. As these studies require living specimens and a multiplexed experimental design to detect the potential co-occurrence of CAM and C_4 the low number of reported cases might be due to lack of data.

According to our results, C_4 eudicots with NADP-ME tend to be more prevalent in areas with significantly higher rainfall (mean value = 698.39 mm) than those utilising the NAD-ME as the decarboxylating enzyme (mean value = 405.83 mm; Figure 6d). This has been thus far supported by studies that comparatively investigated the physiology of NADP-ME and NAD-ME C_4 grasses. While species with the NAD-ME subtype seem to perform better under drier conditions (Carmo-Silva et al., 2009; Ghannoum et al., 2002), NADP-ME species tend to have a more effective CO_2-capturing system by keeping stomata open longer under wetter conditions and can more efficiently utilise nitrogen (Pinto et al., 2016; Schulze et al., 1996; Taub, 2000). Within C_4 eudicots the NAD-ME subtype seems more common than in C_4 grasses where the NADP-ME pathway prevails (Sage et al., 2011). This might also explain why C_4 eudicots managed to successfully occupy even the most arid regions around the globe, whereas C_4 grasses are on average found in regions with more rainfall (Figure 5a).

4.4 | A brief summarising family perspective of C_4 evolution in eudicots

The morphological, physiological and ecological diversity within C_4 eudicots is immense. The most diverse plant family is Amaranthaceae, in which C_4 photosynthesis developed several times independently in different environmental conditions (Kadereit et al., 2003, 2012; Sage et al., 2007). While in Salicornioideae (incl. Salsoloideae, Camphorosmoideae and Suaedoideae) and Chenopodioideae, C_4 photosynthesis arose in ancestrally subtropical and temperate arid, and predominantly succulent and salt-tolerant lineages, C_4 clades within Amaranthoideae more likely originated from tropical ancestors. Both NAD-ME as well as NADP-ME pathways are present in multiple lineages of this family and its C_4 representatives can be found in most biomes, including the C_4 less-favourable cool boreal zones. A number of rare features in the context of C_4 photosynthesis are found only in Amaranthaceae. For example, the occurrence of single cell C_4 in terrestrial angiosperms occurs only in the genus *Bienertia* and in *Suaeda aralocaspica* (both taxa belong to the tribe Suaedeae; Voznesenskaya et al., 2002). Furthermore, the shift from C_3 photosynthesis in cotyledons to C_4 in adult leaves has been so far only reported from various species of Salsoleae (Lauterbach et al., 2017). Two species of *Tecticornia* represent the only known stem-succulent C_4 species with window cells in their mesophyll (Marchesini et al., 2014; Moir-Barnetson, 2014), and the *Salsola divaricata* agg. represents the first C_4 lineages that was shown to have arisen from ancestral hybridisation events between a C_4 and a C_3 lineage within Salsoleae (Morales-Briones & Kadereit, 2023).

Amaranthaceae are in terms of the C_4 species rich followed by Euphorbiaceae and Asteraceae (Figure 1). In the former predominantly tropical plant family, the C_4 syndrome evolved only once resulting in a clade of about 150 species found for the most part, but not exclusively, in seasonally dry and arid zones. In Hawaii, *Euphorbia* species occur in habitats ranging from arid coastal beaches to rainforests. One example is *Euphorbia clusiifolia* Hook. & Arn. (formerly *E. forbesii* Sherff, not accepted by Govaerts et al., 2000), a tree species that grows up to 13 m high and is endemic to the cool, mesic, subtropical forests in the geographically young Hawaiian Islands (Pearcy & Ehleringer, 1984). Further C_4 trees include *Euphorbia olowaluana* and *E. remyi*, which occur in dry open and subalpine, and humid forests, respectively (Young et al., 2020). These examples strongly indicate that the C_4 pathway is not limited to herbaceous or shrubby life forms but may also occur in trees, albeit these examples evolved woodiness secondarily from herbaceous ancestors on the island (Zizka et al., 2022). This link is particularly interesting since the secondary evolution of woodiness may in turn be linked to drought adaptation in other lineages (Dória et al., 2018; Hooft van Huysduynen et al., 2021). Many other C_4 *Euphorbia* species occur in the understory rainforests of Hawaii, where the precipitation is rather high with an average range of 1200 mm to 1800 mm annually (Pearcy & Ehleringer, 1984) – conditions, in which C_4 photosynthesis does not seem to have a physiological advantage.

In Asteraceae, the C_4 syndrome originated several times resulting in about 100 species that often sympatrically occur with the C_3 congeners in dry and arid habitats. Due to the presence of many C_3–C_4 intermediate species, *Flaveria* became a model of C_4 evolution (Monson & Moore, 1989; Sage et al., 2014). The species of the genus *Isostigma*, native to Bolivia, Brazil and North Argentina, show Kranz anatomy in the stems and two different types of Kranz anatomy in leaves (Peter, 2009). The *Eryngiophyllum* type, common in hot and arid conditions, has one Kranz unit per leaf and sclerenchyma tissue, whereas the *Isostigma* type, more common in places with higher precipitation, shows more than one Kranz unit per leaf and no sclerenchyma tissue (Peter & Katinas, 2003). C_4 representatives of Euphorbiaceae and Asteraceae rely solely on the NADP-ME pathway with the exception of *Euphorbia mongolica*, which can be found in highly seasonal temperate East Asia (Zang et al., 2021) and exhibits the NAD-ME pathway.

Preliminary data suggest that C_4 photosynthesis evolved only once in Caryophyllaceae in the genus *Polycarpaea* somewhen in the Pliocene (Kool, 2012). Apart from one widely distributed C_4 species, all the other C_4 species are restricted predominantly to either subtropical forests and (semi)arid zones of Australia or to the western Africa and adjacent regions. The highest species diversity of the C_3 representatives is the more mesic Canary Islands. Low number of extremely xeric species might be explained by the presence of the NADP-ME mechanism, which seems less efficient under extremely arid conditions, at least in grasses (Rao & Dixon, 2016). In Boraginaceae, the C_4 photosynthesis arose probably at least twice independently and while genera *Euploca* and *Heliotropium* are distributed worldwide, the C_4 representatives of either genus are found predominantly in the seasonally dry and (semi)arid habitats around the globe. A similar pattern is observed also in C_4 representatives of Cleomaceae, Molluginaceae and Nyctaginaceae, in all three of which the diversity of C_4 species is markedly lower than that of C_3 species.

While the majority of Aizoaceae rely on the CAM carbon fixation pathway, there are about 30 species that perform C_4 photosynthesis. The details of anatomy and biochemistry vary greatly among closely related species with several origins of both NAD-ME and NADP-ME pathways (Bohley et al., 2015). The most widely distributed C_4 species in this group under current taxonomic treatments is *Trianthema portulacastrum*, which can carry out CAM and C_4 (Winter et al., 2021). While this physiological plasticity possibly facilitated its wide distribution, other widely distributed non-C_4 (but also weak CAM; Winter et al., 2019) species such as *Sesuvium portulacastrum* show that C_4, albeit beneficial, cannot solely explain species' wide distribution. In monogeneric Portulacaceae about two-thirds of currently known species from the genus *Portulaca* exhibit C_4 anatomical features using NAD-ME and NADP-ME carbon fixation pathways in closely related lineages (Ocampo et al., 2013). Furthermore, many species also show reversible physiological signs of an effective CAM carbon fixation pathway under extreme drought stress (Holtum et al., 2017; Moreno-Villena et al., 2022). While this physiological plasticity may again explain the wide distribution range of *Portulaca*

oleracea complex, its taxonomic uncertainty hampers our understanding of its evolutionary history and its true distribution extent (Ferrari et al., 2020).

While majority of the C_4 eudicots occur in regions with a moderate drought period, Polygonaceae, Scrophulariaceae and Zygophyllaceae have the highest C_4 species diversity in much drier arid and semi-arid regions. The shrubby species of *Calligonum* (Polygonaceae) possess only the NAD-ME mechanism for carbon fixation (more effective in drier regions), have a salsoloid Kranz type and are found only in cold and dry deserts of Eurasia and Africa (Sage et al., 2011; Sage, 2017; Pyankov et al., 2000, 2010; Muhaidat et al., 2012; Figure S12b; Table S1). In Africa, it shares its distribution range with the only C_4 taxon from Scrophulariaceae, *Anticharis*, whose four species are restricted to this warm region and exhibit NAD-ME mechanism for carbon fixation as well. In Zygophyllaceae, the C_4 photosynthesis developed several times independently and majority of the representatives are found in hot deserts around the world (Lauterbach et al., 2019). Interestingly, apart from one species (*Tetraena* (=*Zygophyllum*) *simplex*) all C_4 species of Tribuloideae seem to possess the NADP-ME subtype. While it remains unclear why this is the case, *Tetraena simplex* is also the only species whose C_4 anatomy resembles the kochioid Kranz type, while the rest of the taxa show atriplicoid Kranz type. This is not the case in other taxa, where NADP-ME and NAD-ME are not restricted to a particular Kranz anatomy.

C_4 representatives of Acanthaceae and Gisekiaceae have a comparable distribution area and are found across seasonally dry to arid habitats of (sub)tropical Africa and southwestern Asia. In both families the C_4 syndrome arose probably only once (Bissinger et al., 2014; Fisher et al., 2015). While *Blepharis* (Acanthaceae) includes C_3 representatives as well, and both NAD-ME and NADP-ME pathways are known from its C_4 representatives, in *Gisekia* (monogeneric Gisekiaceae) all currently known species perform NAD-ME-based C_4 photosynthesis. Despite this restriction, both taxa are found along a wide range of open and often disturbed arid to mesic habitats (Bissinger et al., 2014).

5 | CONCLUSION/OUTLOOK

Although the climatic ranges of C_4 grasses and C_4 eudicots mostly overlap, our findings suggest that C_4 eudicots tend to inhabit substantially drier regions than their C_4 monocot counterparts. This could be due to the numerous phylogenetically less constrained morphological, ecological and physiological adaptations to harsh environments present in C_4 eudicots. C_4 eudicot lineages employing the NAD-ME decarboxylating enzyme inhabit notably drier regions in comparison to those utilising the NADP-ME decarboxylating enzyme. We conclude that C_4 evolution in most eudicot lineages occurred in ancestral drought-adapted clades, thereby facilitating the expansion of such plants in these habitats and allowing them to colonise even drier areas. We identified primary hotspots of C_4 eudicots that are corroborated by phylogenetic studies as source and sink areas of C_4 diversity, respectively: the arid regions in Mexico/Southern United States, Australia and Central Asia, where multiple C_4 eudicot lineages diversified independently possibly due to increasingly drier environmental conditions. Our literature review on evolutionary histories of individual taxa in these regions, albeit scarce, indicates that evolutionary history of C_4 elements in these regions differs greatly. Mexico and the Southern United States exhibit a high number of C_4 lineages that originated in situ, whereas high C_4 diversity in Australia and Central Asian deserts results primarily from the secondary migration of C_4 lineages into these areas.

AUTHOR CONTRIBUTIONS

Jessica A. Berasategui: Conceptualization (equal); data curation (lead); formal analysis (lead); visualization (lead); writing – original draft (lead); writing – review and editing (lead). **Anže Žerdoner Čalasan:** Writing – original draft (supporting); writing – review and editing (supporting). **Alexander Zizka:** Conceptualization (supporting); formal analysis (supporting); visualization (equal); writing – review and editing (supporting). **Gudrun Kadereit:** Conceptualization (equal); formal analysis (supporting); writing – original draft (supporting); writing – review and editing (supporting).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Publication I - Supplement

Figures S1-S15: Species richness maps showing the impact of data cleaning of all 15 families. (a) Map showing the uncleaned distribution points of the families. (b) Distribution points of the families after data cleaning.

Figure S16: Scatterplot showing annual mean temperature (°C) and annual mean precipitation (mm) data of C_4 grasses (green) and C_4 eudicot (blue) occurrence points per continent.

Table S1 (Pendrive): Dataset of all C_4 species occurring here in the study, arranged by family (15 eudicot families) and by C_4 grasses including their occurrences, synonyms, trait data and the corresponding sources. The first sheet gives the references and the reference number.

Table S2 (Pendrive): List of matching names of our species (C_4 eudicot) or genera (C_4 grasses) list with WCVP.

Figure S1 - Acanthaceae

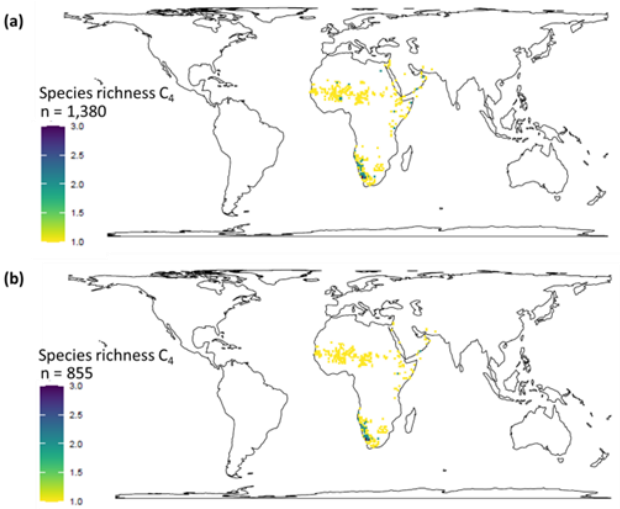


Figure S2 - Aizoaceae

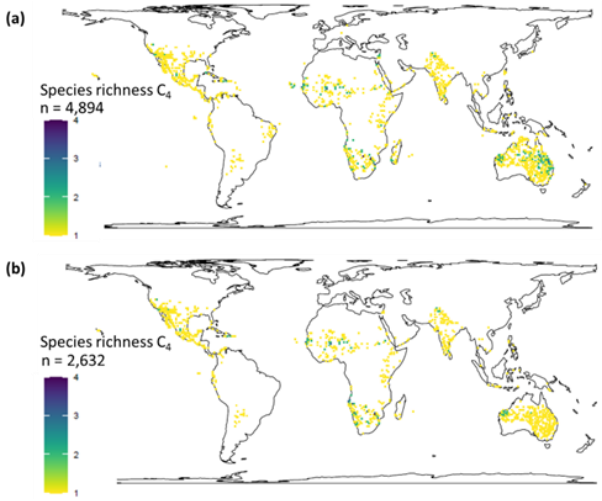
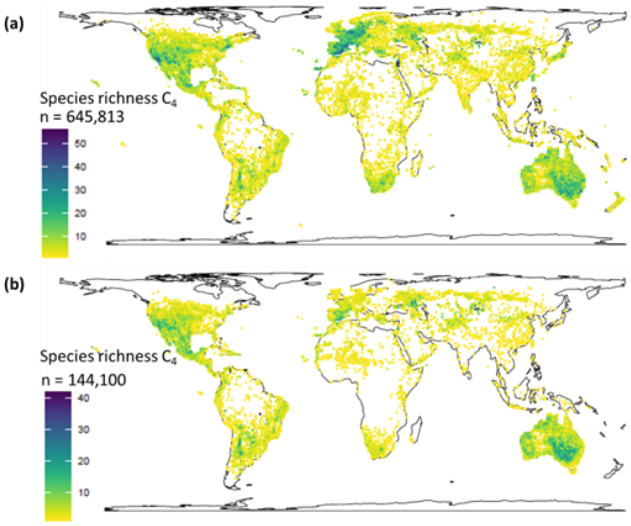


Figure S3 - Amaranthaceae



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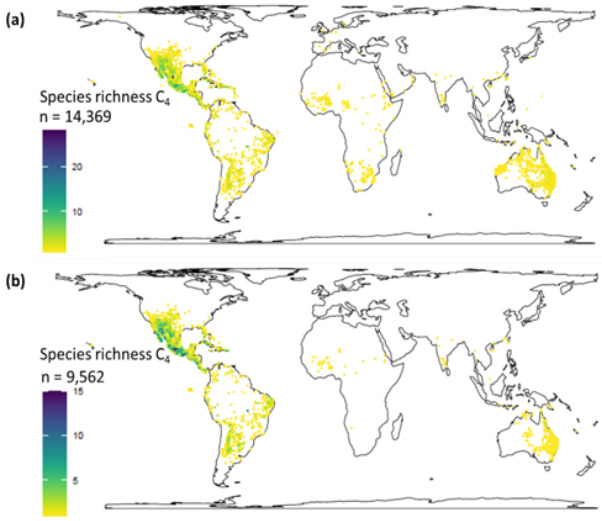


Figure S5 - Boraginaceae

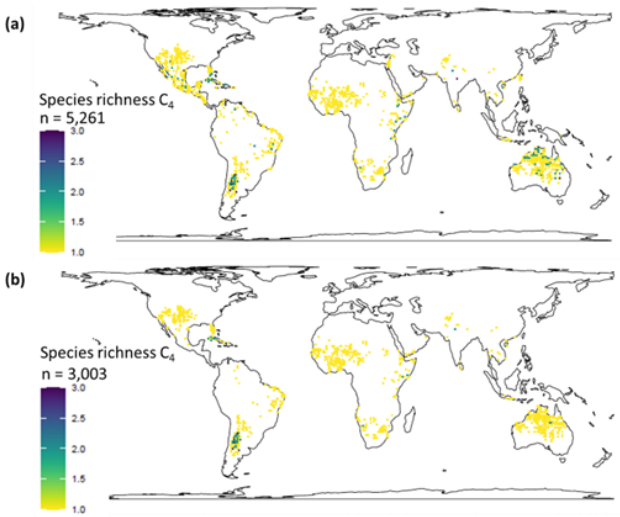


Figure S6 - Caryophyllaceae

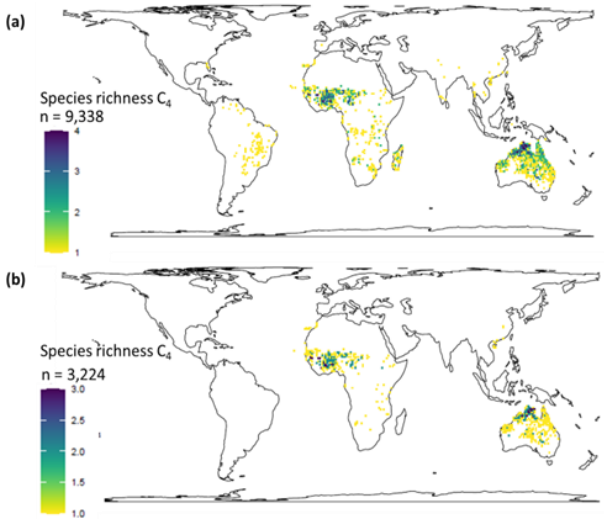


Figure S7 - Cleomaceae

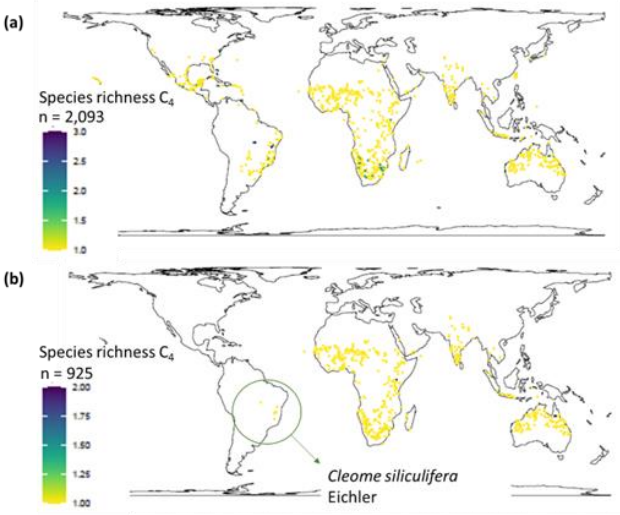


Figure S8 - Euphorbiaceae

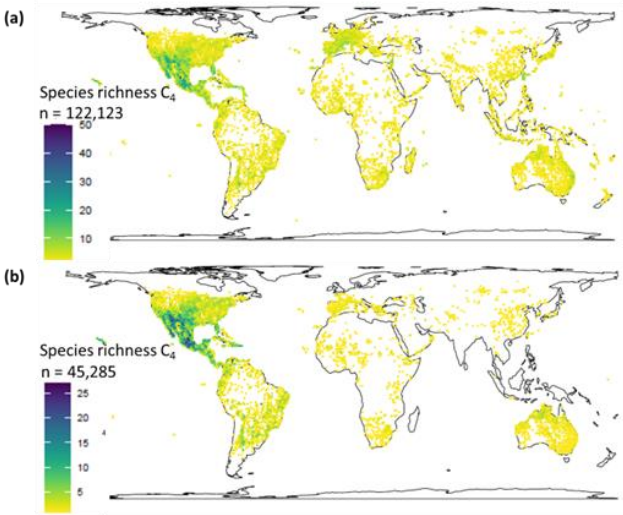
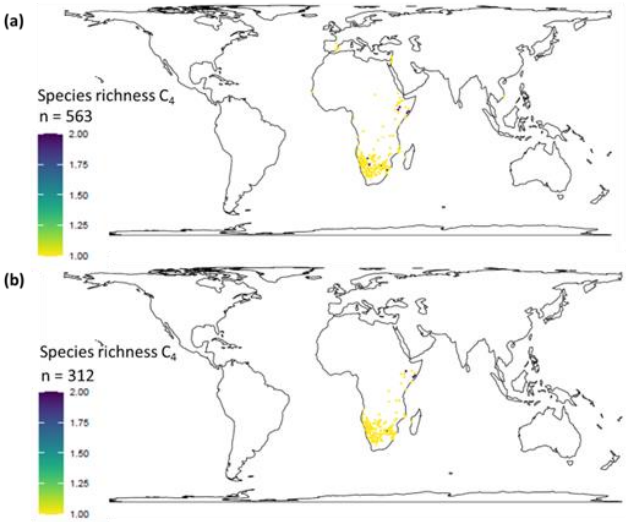


Figure S9 - Gisekiaceae



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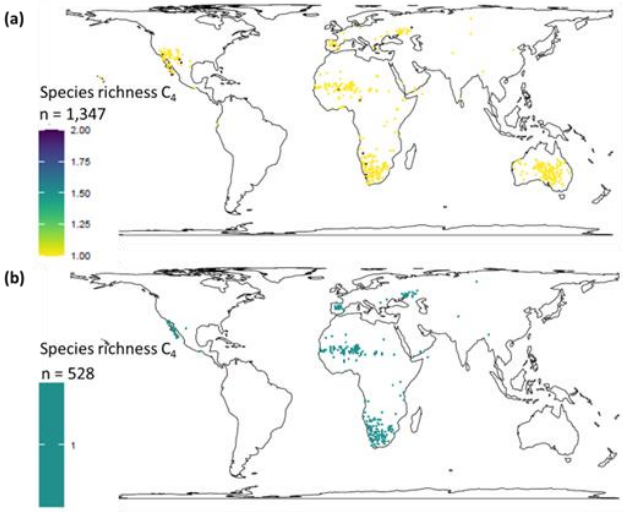

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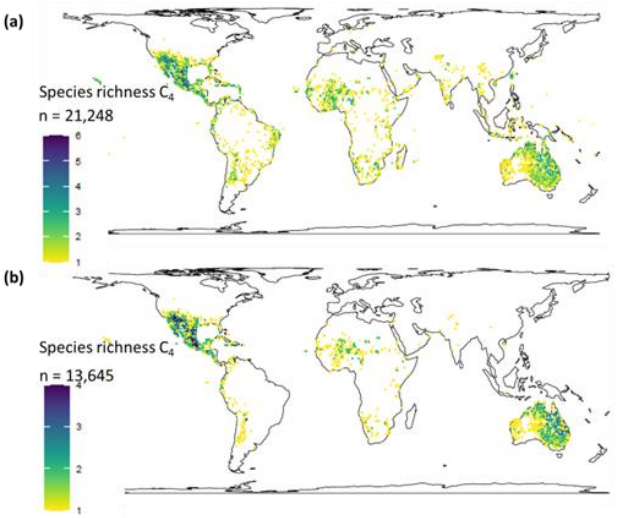

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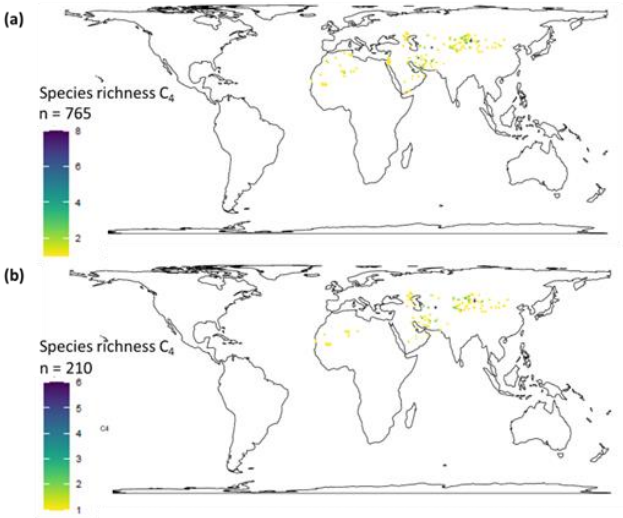

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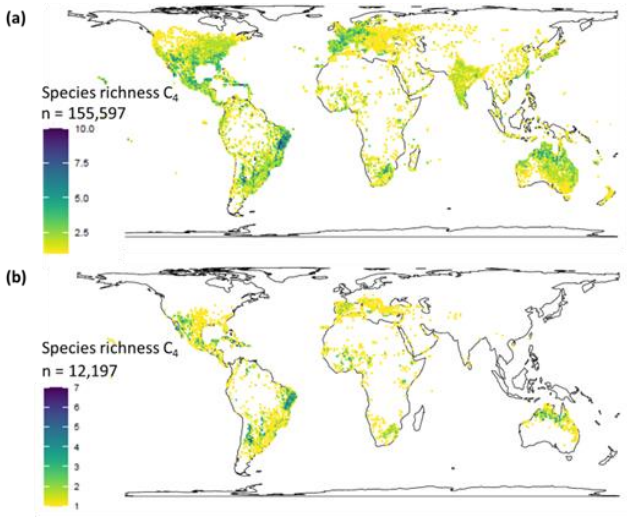

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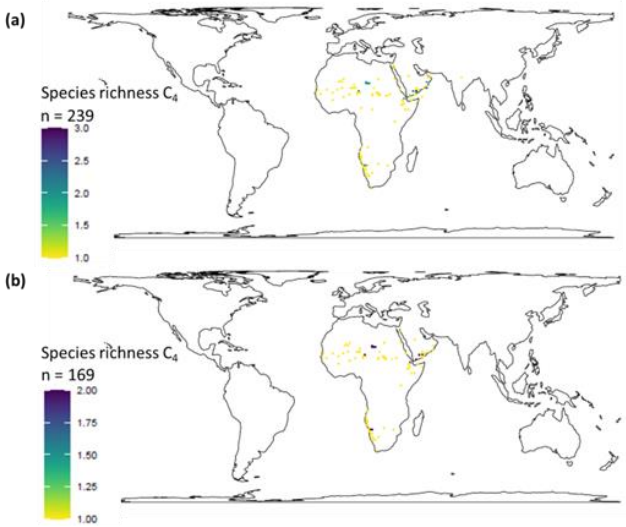


Figure S15 - Zygophyllaceae

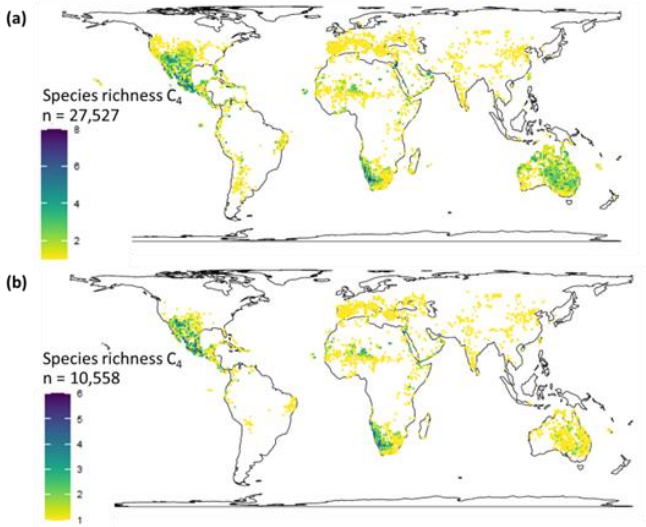
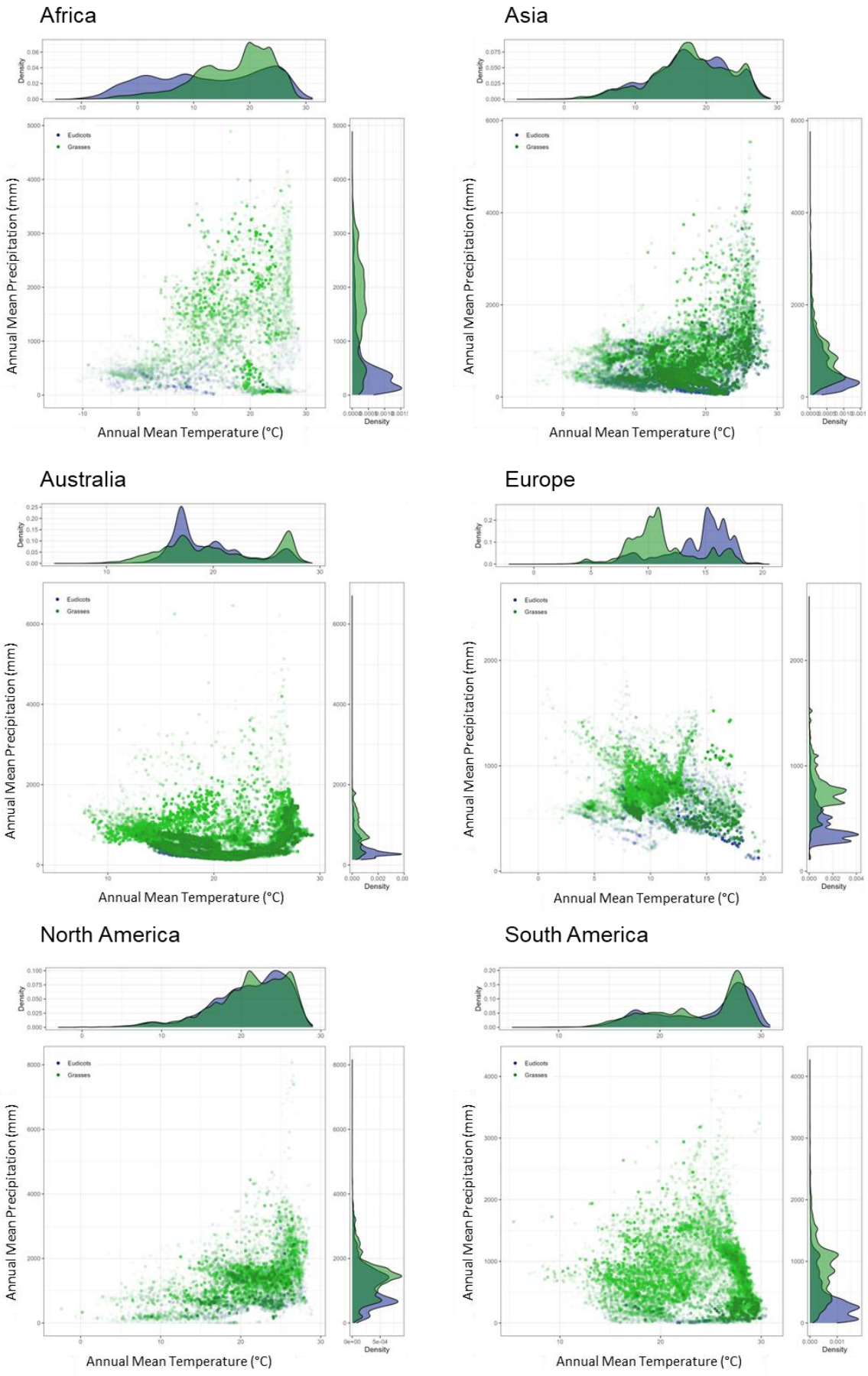


Figure S16


Africa
Asia
Australia
Europe
North America
South America
Eudicots
Grasses
Density
Annual Mean Precipitation (mm)
Annual Mean Temperature (°C)

Chapter II - PUBLICATION II

Ecology and Evolution

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RESEARCH ARTICLE OPEN ACCESS

Biogeography of Australian Camphorosmeae and Diversification in Climatic Space and Across Arid Habitat Types

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ABSTRACT

This study investigates the biogeography of the Australian Camphorosmeae (Amaranthaceae *s.l.*) lineage and how it relates to shifts in climatic niche and habitat types. Building on previous research and data resources, we integrate molecular phylogenetics, bioclimatic data and biogeographical models to deepen our understanding of the diversification and adaptation of this group across Australia's diverse landscapes in relation to palaeoclimatic changes. For 159 species representing 12 genera, georeferenced distribution points were used to define the most informative bioclimatic variables using principal component analysis. Evolutionary shifts in climatic niches and habitat types were analysed, revealing clade-specific shifts and adaptations to different habitats and climatic conditions. Biogeographical analyses allowed us to infer ancestral areas of Camphorosmeae in Australia and relate their expansion over evolutionary time to habitat shifts. Preadaptation of this group to warm and dry habitats coupled with key periods of aridification in Australia, particularly during the Late Miocene to Pliocene, were critical in driving its diversification through migration and local adaptation to varied habitats of arid Australia. Our analyses suggest that the 'Riverine Desert' habitat offered suitable conditions for ancestral Australian Camphorosmeae and facilitated their early widespread dispersal in the Western and Eastern Desert. We hypothesise that early diverging lineages such as *Roycea* adapted to the later emerging 'Desert Lake' habitat when it spread in Western Australia during the Early Pliocene. Further, habitat type shifts occurred from 'Riverine Desert' to 'Shield Plain', 'Karst Plain' and to 'Sand Desert' also during the Pliocene and Pleistocene once these habitat types emerged. This study illustrates the complex interplay between ecological flexibility and niche conservatism in shaping the biodiversity of Australian Camphorosmeae.

1 | Introduction

Australia is characterised by diverse landforms, a wide variety of biogeographical regions and unique fauna and flora. While the northern regions of the continent experience tropical savannah climate with high humidity and a pronounced wet summer and dry winter season, the humidity, temperature and seasonality gradient decline along the eastern coast, which experiences a more moderate oceanic climate (Hadwen et al. 2011). On the contrary, southern and southwestern regions are under strong Mediterranean influence with high summer temperatures and a rainfall period during the winter months. The largest central portion of the continent, however, experiences a hot semi-arid and arid climate, with annual precipitation below 500 mm and

250 mm, respectively (Hadwen et al. 2011; Pearce et al. 2010). One of the most noteworthy palaeoclimatic events in Australia's history with substantial effects on flora and fauna occurred around 33 million years ago, during the Eocene to Oligocene transition. During this period, the continent experienced significant climate cooling and increased seasonality, followed by a later phase of aridification (Byrne et al. 2008; Crisp and Cook 2013; Feakins, Warny, and DeConto 2014; Nge et al. 2020). These climatic shifts led to the spread of arid environments across much of Australia, influencing the dispersal and diversification of sclerophyllous plant species, especially those which were already preadapted to nutrient-poor and drought-prone conditions (Martin 2006; Crisp and Cook 2013). Around 30 million years ago, Australia had separated from Antarctica and continued to drift northwards with increased aridification leading to the transformation of previously abundant rainforest vegetation into sclerophyll woodlands. In these woodlands, the flora became dominated (in terms of biomass and impact on the environment) by a variety of plant families, including Proteaceae (*Banksia* L.f., *Grevillea* R. Br. ex Knight, and *Hakea* Schrad. & J. C. Wendl.), Myrtaceae (*Eucalyptus* L'Hér.), Fabaceae (*Acacia* Mill) and Asteraceae (*Olearia* Moench) (Dettmann and Jarzen 1998; Ladiges, Udovicic, and Nelson 2003; Crisp and Cook 2013; Jansen, Michaels, and Palmer 1991).

Australia's diverse habitats and ecosystems, spanning from the Gondwanan remnant rainforests to the much younger outback deserts, are categorised into distinct biogeographical regions. This classification guides conservation efforts, ecological research, land management and environmental policies. By identifying areas of high biodiversity, researchers can prioritise conservation efforts, study species distribution patterns and develop environmental policies. In essence, the organisation of Australia's ecosystems into biogeographical regions is a practical tool for understanding, conserving and managing the country's remarkable biodiversity and natural resources. However, this led to multiple bioregionalisations in Australia, with areas being synonymised or overlapped (Ebach 2012).

One of the most common classifications is the *Interim Biogeographic Regionalisation for Australia* (Thackway and Cresswell 1995; IBRA7), which divides Australia into 89 bioregions and 419 subregions. This classification system is based on shared characteristics such as climate, geology, landforms, vegetation and species distributions (Thackway and Cresswell 1995). Another well-known bioregionalisation is the *Integrated Marine and Coastal Regionalisation of Australia* (IMCRA, Commonwealth of Australia 2006), which classifies marine and coastal environments into several bioregions based on oceanographic data, biological distributions and habitat types for marine conservation. The definition of bioregions or subregions, in general, is fundamental to understanding the distribution patterns of biodiversity. By defining regions based on shared ecological characteristics and species distribution patterns, these classifications enable targeted interventions to conserve biodiversity hotspots. (Ebach 2012; Ebach et al. 2015) suggested a taxonomic convention for defining and naming the phytogeographical areas of Australia. They identified several broad phytogeographical regions and subregions across the continent based on the distribution patterns of specific taxonomic groups rather than also considering geology and landforms as used in the IBRA system. Each of these regions or subregions is characterised by different ecological traits, biodiversity and geological characteristics and contributes to the overall diversity of Australia's landscapes and ecosystems. Overall, the subregions are divided into 20 phytogeographical regions, including the western, central and eastern deserts, the Great Sandy Desert Interzone, Central and Eastern Queensland, the Nullarbor, Eyre Peninsula and Adelaide areas.

Around 18% of Australia's central landscape is classified as desert, with each desert landscape having its characteristics and attributes (Geoscience Australia 1994). Additionally, there are other areas that experience desert-like conditions due to low rainfall and high temperatures. Mabbutt (1988) described and mapped eight habitat types across arid Australia based on landforms and corresponding vegetation types (e.g., Desert Uplands, Shield Plains, Stony Desert, Karst Plain, Sand Desert, Riverine Desert, Desert Clay Plains and Desert Lakes). McDonald (2020) introduced two additional habitat types following his study of Australian chenopods (i.e., formerly classified under Chenopodiaceae, now part of the Amaranthaceae) across different arid landscapes and adjacent temperate, subtropical and coastal zones (e.g., Mesic Plain, Range, and Coast). Due to the diversity of Australia's ecosystems coupled with high isolation of the whole continent, high number of species as well as high levels of endemism are observed in contemporary Australian angiosperms. The most species-rich Australian plant families with notable endemic lineages include Asteraceae, Cyperaceae, Ericaceae (Epacridoideae), Euphorbiaceae, Fabaceae (Mimosoideae), Goodeniaceae, Myrtaceae, and Orchidaceae, Poaceae, Proteaceae and Rutaceae, with Amaranthaceae Juss. sensu lato present in high numbers in Australian arid regions (Beadle 1981; Morley and Toelken 1983; Hnatiuk 1990; Crisp and Cook 2013; Ladiges, Udovicic, and Nelson 2003; Funk 2009; Walker et al. 2018; Morales-Briones et al. 2021).

One of the most species-rich Australian tribes within Amaranthaceae sensu lato is Camphorosmeae Moq. It comprises around 160 Australian species placed in 12 genera (according to Plants of the World Online; POWO 2024), with the two biggest genera being *Sclerolaena* R.Br. (79 spp.) and *Maireana* Moq. (58 spp.). Australian Camphorosmeae are widely distributed across the continent, and are found in different habitats, from coastal dunes to inland deserts. While Australia harbours the highest species diversity of this tribe, there are approximately 35 species across eight genera that occur outside of Australia. These non-Australian species are distributed in various arid and semi-arid regions, including the Canary Islands, parts of Eurasia (mainly in the Old World Desert Belt stretching from the Middle East to Central Asia), North America (e.g., *Neokochia* (Ulbr.) G. L. Chu and S. C. Sand.) and South Africa. These species typically inhabit arid, semi-arid and coastal regions, often characterised by saline or sandy soils and low precipitation (Kadereit, Gotzek, and Freitag 2005; Kadereit and Freitag 2011; Hühn et al. 2024). Biogeographical and molecular evidence suggests that the non-Australian species represent early divergent lineages of the tribe Camphorosmeae, particularly within Eurasia. The genus *Grubovia* Freitag and G. Kadereit is sister to the Australian Camphorosmeae and native to Central Asia (Kadereit and Freitag 2011; Kadereit et al. 2014). Camphorosmeae likely originated in the Old World during the Late Eocene to Early Oligocene

(Kadereit and Freitag 2011). The earliest divergent lineages, such as the *Chenolea* Thunb. clade, which includes species from Eurasia, western North America and southern Africa, are thought to be remnants of older evolutionary branches that have undergone significant extinctions (Kadereit and Freitag 2011).

The Australian Camphorosmeae are remarkably well adapted to extreme environmental conditions, including saline soils and arid areas (Kadereit, Gotzek, and Freitag 2005), while presumably performing exclusively C_3 photosynthesis (Carolin 1975; Jacobs 2001; Freitag and Kadereit 2014). These adaptations include morphological traits such as reduced leaf size, succulence and hairy or scaly surfaces, which minimise water loss (Kadereit and Freitag 2011). This lack of specialised C_4 photosynthesis is also common in many other species-rich Australian desert plants, such as *Acacia* (Fabaceae), *Eucalyptus* (Myrtaceae), *Eremophila* R.Br. (Scrophulariaceae) and *Ptilotus* R.Br. (Amaranthaceae), which indicates that C_4 photosynthesis alone cannot explain the evolutionary success of some C_4 lineages in water-scarce conditions (Bowman and Cook 2002; Sage et al. 2007; Kattge et al. 2011). Understanding the evolutionary history and biogeographical patterns of Camphorosmeae helps to elucidate the potential mechanisms underlying the diversification and adaptation of lineages in arid ecological niches.

Molecular phylogenetic research has become an important tool to improve the understanding of evolutionary relationships and genetic diversity within this tribe, albeit initially with limited sampling and low support. The taxonomic position of the Camphorosmeae has thus changed several times (Freitag and Kadereit 2014). Cabrera, Jacobs, and Kadereit (2009) conducted the first molecular phylogenetic study for this tribe, including 71 species from all recognised Camphorosmeae genera. Cabrera's study concluded that although some morphological characters such as fruiting perianth were relevant, the molecular results did not fully support the existing taxonomy, attributing this discrepancy to incomplete lineage sorting and ongoing hybridisation within the Australian Camphorosmeae group. Subsequent biogeographical analyses suggested that the Australian Camphorosmeae migration started in the southwest of Australia via a single long-distance dispersal event from continental Asia during the Miocene, from where this lineage expanded eastwards and northwards over time (Cabrera, Jacobs, and Kadereit 2011). An enhanced diversification took place during the Pliocene, possibly driven by increasing aridity, together with inland migration along palaeodrainage systems especially pronounced in species adapted to coastal conditions (Cabrera, Jacobs, and Kadereit 2011; Kadereit and Freitag 2011).

McDonald (2020) subsequently examined the evolution of chenopods in Australia, proposing several key hypotheses regarding their diversification and migration. He hypothesised that the primary evolutionary spaces for the range expansion and diversification of most chenopod taxa (including Camphorosmeae) were the Yilgarn and Eyre-Murray centres. These regions, which contain 97% of all Australian chenopod species, are not restricted to the arid zone but represent critical centres for chenopod evolution. He noted that these centres share 43% of all species, indicating widespread initial colonisation from coastlines followed by multiple migrations across these regions. He furthermore identified the inland province of Sandland South (Great Victoria Desert) as an important migration link between the Yilgarn and Eyre-Murray centres, rather than the coastal Nullarbor Plain, dominated by chenopod vegetation today (albeit with low species diversity). Finally, he also noted that the chenopod abundance in Riverine Deserts and Desert Lakes underscores the influence of niche conservatism, particularly regarding salinity and flooding in the main inland habitat of chenopods.

Despite these valuable insights, both studies suffered from unresolved phylogenies with low levels of support and overall limited taxon sampling. A recent study by Hühn et al. (2024) re-evaluated the diversification of the Australian Camphorosmeae using an adapted RADseq approach. Sequence data were generated for 104 species from all 12 Australian genera. A modified NGS-based methodology was used to improve the phylogenetic resolution, identify statistically supported clades and place the phylogeny into a temporal frame. Seventeen statistically well-supported clades were identified, with their habitat preference demonstrating the influence of landscape change and the emergence of new habitats in arid Australia since the late Miocene, with migration likely following a west-to-east pattern of aridification. The Camphorosmeae arrival coincided with significant palaeoclimatic, landscape and biotic changes. Possibly aided by preadaptation of their progenitors to coastal (i.e., saline) environments, Hühn et al. (2024) suggested that Camphorosmeae migrated inland along Riverine Desert landscapes (a habitat type defined by Mabbutt (1988)) formed by changes in palaeodrainage systems in southern and western Australia (Hühn et al. 2024).

Those three primary research hypotheses, proposed by Cabrera, Jacobs, and Kadereit (2011), McDonald (2020) and Hühn et al. (2024), offer significant insights into the evolutionary history of Camphorosmeae in Australia. While sharing common elements, these hypotheses exhibit unique differences that highlight the complex interplay of diverse mechanisms of dispersal, colonisation and adaptation. All of them concur that the ancestors of Australian Camphorosmeae arrived via a long-distance dispersal event from continental Eurasia during the Middle to Late Miocene. This period marks the beginning of significant climatic shifts, leading to the aridification of Australia. The adaptation of Camphorosmeae to arid and saline environments is a central topic across these hypotheses, suggesting that these plants were pre-adapted to such conditions before they arrived in Australia. Hühn et al. (2024) and Cabrera, Jacobs, and Kadereit (2011) share similar results, particularly in their biogeographical patterns and diversification trends. Both propose that initial colonisation occurred in the south and west of Australia, followed by expansion across the continent. They also emphasise the Late Miocene and Pliocene as periods of significant diversification, driven by the intensifying aridification. However, they note a slowdown in speciation during the Pleistocene, likely due to habitat contraction and climatic oscillations. Nevertheless, it is important to consider the alternative explanation proposed by Cusimano and Renner (2010), who suggest that such slowdowns in diversification rates may be artefacts of incomplete sampling rather than genuine biological phenomena. This raises the possibility that the observed slowdown in speciation during the Pleistocene in both studies could be influenced

by gaps in phylogenetic or fossil data. McDonald's hypothesis introduces the littoral connection hypothesis (Burbidge 1960; Shmida 1985), positing that chenopods initially arrived in the coastal regions, from where they expanded towards inland. This hypothesis underscores niche conservatism, where species retain ancestral ecological characteristics, influencing their migration patterns and habitat preferences. McDonald also explores two scenarios—fragmentation and amalgamation—to explain the development of chenopod diversity in Australia, providing a unique perspective on range expansion and evolutionary centres. The fragmentation scenario involves an initial colonisation and establishment phase with coastal bridgeheads, followed by a landscape spread where chenopods extend their range, particularly towards the west and south coasts, linked through the ancestral Great Victoria Desert. This is followed by two diversification stages, where speciation increases and distinct lineages form in the western Yilgarn and central-eastern Eyre-Murray centres of diversity. Conversely, the amalgamation scenario begins similarly with coastal bridgehead formation and expansion but follows with separate provincial expansions and early diversification in the west and east. As aridity intensifies, these centres merge, facilitating species exchange and forming the subcontinental Arid-Mediterranean group through further range expansion (McDonald 2020).

Each of the three approaches has its methodological considerations and challenges. Cabrera, Jacobs, and Kadereit (2011), for example, highlighted the reliance of molecular data for age estimates in the absence of direct fossil evidence, noting that these estimates are consistent with the fossil record of chenopod-like pollen in Australia. The oldest chenopod-like pollen fossils were discovered at the boundary between the Oligocene and Miocene and are the oldest known in Australia (Martin 1981; Christophel 1989; Cabrera, Jacobs, and Kadereit 2011). However, the study suffered from poor resolution of the phylogenetic tree. McDonald (2020), on the other hand, drew most of his conclusions based on the ecology of the species and the landform evolution of individual habitats on palaeoenvironmental and geological evidence but did not fully resolve the phylogenetic history of the taxon in question. This was succeeded only by Hühn et al. (2024), by using a modified RADseq protocol for sequencing. Their study revealed new clades and at least partially overcame the issue of poor phylogenetic resolution, despite the challenges posed by the high proportion of missing data.

Building on these hypotheses, our research aims to integrate their strengths and unique aspects through a phylogenetically based ancestral analysis. Rather than testing each hypothesis individually, we focus on synthesising the insights from these studies to place the evolutionary history of Camphorosmeae into a spatial context and infer its ancestral ecological niche. By using a well-sampled and resolved phylogeny, we aim to explore the roles of coastal and inland pathways in the dispersal and diversification of Camphorosmeae, emphasising the impact of climatic fluctuations and ecological niche conservatism. To validate these hypotheses regarding the climatic niches, habitat types, evolutionary dynamics and biogeography of Australian Camphorosmeae, mapping habitat type occupations or bioclimatic variables onto the well-resolved dated phylogenetic tree of Hühn et al. (2024) will reveal clade-specific shifts, providing insight into how different Camphorosmeae species have adapted to various habitats or responded to climate change. Additionally, biogeographical analyses will help unravel the complex interplay between evolutionary history, geographical distribution and diversification events over time.

Overall, this study aims to deepen the understanding of the evolutionary history of Australian Camphorosmeae by integrating phylogenetic analyses with biogeographical models, climate data and habitat preferences, offering new insights into their distribution and diversification across Australia's diverse landscapes.

2 | Materials and Methods

2.1 | Taxon Sampling and Occurrence Data

For this study, the georeferenced distribution points of 159 species, representing all 12 genera of the Camphorosmeae, were extracted from Australia's online species database, the Atlas of Living Australia (ALA), a repository of biodiversity data including records from lodged herbarium specimens (Belbin and Williams 2016; Belbin et al. 2021).

Before analysis, manual cleaning steps were required, which included removing records without coordinates, distribution points outside Australia and around botanical gardens, duplicate coordinates per species and records categorised under taxonomic ranks such as 'genus' or 'family'. To ensure that only naturally occurring distributions were included, we conducted an additional filtering step to exclude any records flagged as 'cultivated' in the dataset. Additionally, we cross-checked the records for incorrect synonymisation relying on plantsoftheworldonline.org (POWO 2024). To relate the present analyses to the most recent dated phylogeny of the Australian Camphorosmeae by Hühn et al. (2024), distribution points of 103 Australian Camphorosmeae species included in the phylogeny, except for *Sclerolaena* sp. *yeltacowie* ineditus due to missing coordinates, were selected (Table S1).

2.2 | Bioclimatic Variables and Principal Component Analysis

All 19 bioclimatic variables were extracted from WorldClim v.2 (Fick and Hijmans 2017) at a spatial resolution of 2.5 min (~4.5 km at the equator), to perform Principal Component Analysis (PCA) aimed at determining the optimal bioclimatic variables for reconstructing their ancestral state based on the dated Australian Camphorosmeae phylogeny. The extent() and crop() methods in R v4.0.2 (R Core Team, 2020), using RStudio v1.2.5042 (RStudio Inc. 2009–2020), were used to obtain bioclimatic data specifically for the Australian habitat. To achieve this, the extent for Australia was defined as, $x\text{min} = 112$, $x\text{max} = 154$, $y\text{min} = -44$ and $y\text{max} = -10$. Bioclimatic variable values were extracted for each presence point of the 103 Australian Camphorosmeae. With this dataset, the PCA was carried out to obtain the best bioclimatic variables for further analyses.

To evaluate the proportion of the climatic niche occupied by Camphorosmeae in Australia, we quantified the climatic space of Australia using 406,372 climatic data points. These data points represent the entire climatic space of Australia at a resolution of 2.5 arc minutes. We then performed a PCA to capture the entire range of climatic variation across the continent, allowing us to assess the distribution of Camphorosmeae within this climatic space. After conducting PCA to identify the major axes of variation, we projected the locations of all occurrences from 103 Australian Camphorosmeae species onto the principal component (PC) space. This transformation enabled us to observe and analyse the distribution patterns of Camphorosmeae in Australia within this modified space. In addition, PCA was conducted on the climatic data of the Australian Camphorosmeae. The principal components, capturing the most significant variation, were identified (PC1 and PC2) for the variable correlation plot, with variables exhibiting high loadings used to determine the orientation of the axes. We selected the variables with the highest loading value for PC1 and PC2, respectively, essentially choosing the variables that contribute the most to the variability captured by PC1 and PC2, respectively. Since they were capturing different sources of variability, they were less likely to be highly correlated with each other. Additionally, a pairwise Pearson's correlation coefficient test was done to evaluate collinearity among these variables.

2.3 | Ancestral State Reconstruction With Key Climatic Variables and Ancestral Habitat Type Reconstruction

To investigate the evolutionary history and ecological adaptations of Australian Camphorosmeae two different reconstructions were made: Ancestral Bioclimatic Reconstruction with the two most relevant environmental bioclimatic variables (Bio05—Maximum Temperature of Warmest Month; and Bio13—Precipitation in the Wettest Month) and Ancestral Habitat Type Reconstruction based on defined habitat types. The dated maximum clade credibility (MCC) tree by Hühn et al. (2024) was used. For the Ancestral Bioclimatic Reconstruction, the mean value of each bioclimatic variable for 103 species was calculated. We treated these characters as continuous and used the ML-based 'ace' function from the 'ape' package in R (Joy et al. 2016; Paradis and Schliep 2019) to perform the ancestral state reconstruction. The results were visualised using the 'contMap' function from the 'phytools' package in R (Revell 2024), which allowed for a more accurate depiction of the evolutionary changes of these continuous traits over time.

Following the publication by Hühn et al. (2024), which inferred the ancestral status of habitat types based on chronological order, this study conducted a formal analysis using 'Ancestral Habitat Type Reconstruction' to test their assumptions. To do so, 10 habitat types defined by Mabbutt (1988) and McDonald (2020) were used. The specific habitat types corresponding to the individual species were derived from the work of Hühn et al. (2024). Since habitat types represent discrete categorical variables, the maximum parsimony method was considered appropriate for this ancestral reconstruction. Accordingly, we followed the topology of Hühn et al. (2024) and implemented the reconstruction using Mesquite (v3.81, www.mesquiteproject.org).

2.4 | Biogeographical Analysis

To investigate the biogeographical history of Australian Camphorosmeae, again, the dated MCC tree by Hühn et al. (2024) was used in the R package 'BioGeoBEARS' v1.1.2 (Matzke, 2013) and its dependencies. BioGeoBEARS presents a versatile, likelihood-based system, designed to characterise the dynamic shifts of branches within phylogenies across discrete biomes over evolutionary time. These biome shifts encompass both anagenetic events, occurring within a single branch, such as dispersal and extinction processes (DEC; Ree and Smith 2008), and cladogenetic events, which transpire at branching points and involve sympatric (BAYAREALIKE; Landis et al. 2013), vicariant (DIVALIKE; Ronquist 1997) and founder-event speciation processes (DEC + J, BAYAREALIKE+J, DIVALIKE+J; Matzke 2014, Van Dam and Matzke 2016).

A large-scale analysis based on an almost complete sampling by Hühn et al. (2024) was performed. For the distribution data, 20 Australian phytogeographical subregions were selected following the ranges identified by Ebach et al. (2015). Subregions were chosen for each species only if more than 10% of their occurrence points were present within those areas. Any subregion with ≤ 10% of occurrence points per species was treated as insignificant. This enables the main occurrences of the respective species to be analysed and avoids potential identification biases. The required subregion shapefile from Ebach et al. (2015) was downloaded for this purpose. However, since some species in Camphorosmeae occur very close to the coast, adjustments to the shapefile were necessary. The edges of the shapefile did not always align precisely with the coast, leading to the exclusion of some coastal occurrence points in the initial attempt. Consequently, the shapefile was overlaid on an Australian landscape map via QGIS v3.30 (QGIS, 2024) and the coastal boundaries were extended accordingly. This adaptation allowed the inclusion of many distribution points near the coast in the biogeographical analysis for coastal species such as *Threlkeldia diffusa* R.Br., *Maireana oppositifolia* (F. Muell.) Paul G. Wilson, *M. brevifolia* (R. Br.) Paul G. Wilson, *Sclerolaena uniflora* R. Br. and *Dissocarpus biflorus* (R. Br.) F. Muell.

As a result, there were one to a maximum of four main subregions for most of the species. A table showing this reduction can be found in the supplement (Table S2). Nine subregions in total for 103 species were selected: Central Desert, Central Queensland, Adelaide, Eyre Peninsula, Nullarbor, Southwest Interzone, Southwestern, Eastern Desert and Western Desert. The maximum range size was set to four, as the most widely distributed Australian Camphorosmeae species covered a maximum of four subregions. Six models have been tested (DEC, BAYAREALIKE and DIVALIKE and their +J alternatives). The best-fit model was chosen by comparing the corrected Akaike information criterion (AICc) and Akaike weight (AICc_wt).

3 | Results

3.1 | Data Compilation and Cleaning

A total of 228,875 occurrence points from ALA were compiled (Atlas of Living Australia, 2021; Atlas of Living Australia occurrence download at https://doi.org/10.26197/ala.a4134452-6171-4ad7-8da0-46682199a2d8. Accessed 30 May 2023). Manual data cleaning reduced the total number of records to 195,758. Distribution points of 103 Australian Camphorosmeae species included in the most recently dated phylogeny by Hühn et al. (2024) were specifically selected for the analyses (Table S1). This results in 178,355 included distribution points.

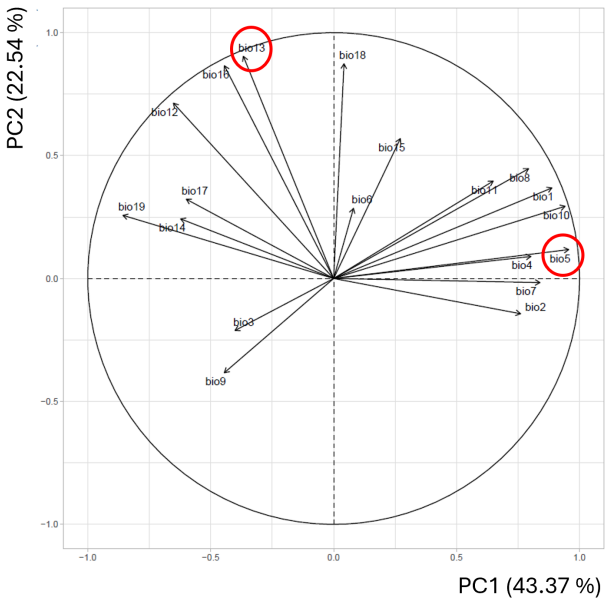
3.2 | Bioclimatic Characterisation and Niche Occupancy

This analysis aimed to identify the bioclimatic variables driving the distribution patterns of Australian Camphorosmeae and to assess the proportion of climatic niches occupied by the species within the Australian habitat. Through the Principal Component Analyses (PCA) of 19 bioclimatic variables, significant axes of climatic variation could be identified. The PCA was conducted with all distribution points of 103 Australian Camphorosmeae species together (Figure 1).

The resulting correlation circle, displayed in Figure 1, represented the distribution of 19 environmental variables (Bio01 to Bio19) across the first two principal components, PC1 and PC2, and revealed strong relationships between those variables based on their distance from the centre and orientation relative to each other. Bioclimatic variables located far from the centre and close to each other exhibited strong positive correlations (r-value close to 1), while those far from the centre and orthogonal were not correlated (r-value close to 0). Variables positioned on opposite sides of the centre demonstrated significant negative correlations (r close to −1).

PCA analysis of 103 species together, explained a substantial portion of the variability among all climatic variables, with both PC axes accounting for 65.91% of the total variance (Figure 1). PC1 and PC2 explained 43.37% and 22.54% of the total variance, respectively. Interpretation of the loading values highlighted the dominant climatic axes captured by each principal component. PC1, primarily reflecting the temperature axis, was strongly influenced by variables such as Bio05 (Maximum Temperature of the Warmest Month; loading value of 0.333) and Bio10 (Mean Temperature of the Warmest Quarter; loading value of 0.328). On the other hand, PC2, representing the precipitation axis, was mainly driven by variables including Bio13 (Precipitation in the Wettest Month; loading value of 0.437) and Bio18 (Precipitation in the Warmest Quarter; loading value of 0.422), indicating their substantial contribution to this component. In addition to the correlation circle, the Pearson correlation coefficient test also showed strong correlations between the variables Bio05 and Bio10, and between Bio13 and Bio18. Therefore, only the variable with the highest loading value per PC was selected for further analysis (Bio05 and Bio13). Initially, they were used to project the distributions of Australian Camphorosmeae species onto their PC space (Figure 2).

The climatic niche occupied by the Camphorosmeae species in Australia was evaluated by conducting a PCA on the entire climatic range of Australia. Figure 2 shows the PCA of the climatic range across Australia, based on 406,372 location points



Variable	Loading value (PC1)	Loading value (PC2)
Bio01 - Annual Mean Temperature	0.309	0.178
Bio02 - Mean Diurnal Range	0.265	−0.069
Bio03 - Isothermality	−0.140	−0.102
Bio04 - Temperature Seasonality	0.279	0.043
Bio05 - Max Temperature of Warmest Month	**0.333**	0.057
Bio06 - Min Temperature of Coldest Month	0.028	0.138
Bio07 - Temperature Annual Range	0.292	−0.008
Bio08 - Mean Temperature of Wettest Quarter	0.276	0.216
Bio09 - Mean Temperature of Driest Quarter	−0.155	−0.185
Bio10 - Mean Temperature of Warmest Quarter	0.328	0.142
Bio11 - Mean Temperature of Coldest Quarter	0.226	0.191
Bio12 - Annual Precipitation	−0.227	0.345
Bio13 - Precipitation of Wettest Month	−0.128	**0.437**
Bio14 - Precipitation of Driest Month	−0.217	0.117
Bio15 - Precipitation Seasonality	0.094	0.275
Bio16 - Precipitation of Wettest Quarter	−0.155	0.418
Bio17 - Precipitation of Driest Quarter	−0.209	0.156
Bio18 - Precipitation of Warmest Quarter	0.015	0.422
Bio19 - Precipitation of Coldest Quarter	−0.299	0.124

FIGURE 1 | Principal component analysis (PCA) Biplot and Loadings for 19 bioclimatic variables conducted with all distribution points of 103 Australian Camphorosmeae species together. The biplot shows the first principal component (PC1) on the x-axis and the second principal component (PC2) on the y-axis. Each vector represents a bioclimatic variable, with the direction and length indicating its contribution to the principal components. Variables Bio05 and Bio13 are highlighted on the biplot as they have significant contributions to PC1 and PC2, respectively. The table on the right lists the loading values of each variable on PC1 and PC2. The bold values indicate the variables with the highest loadings on each principal component. PCA loadings can be found in Table S3.

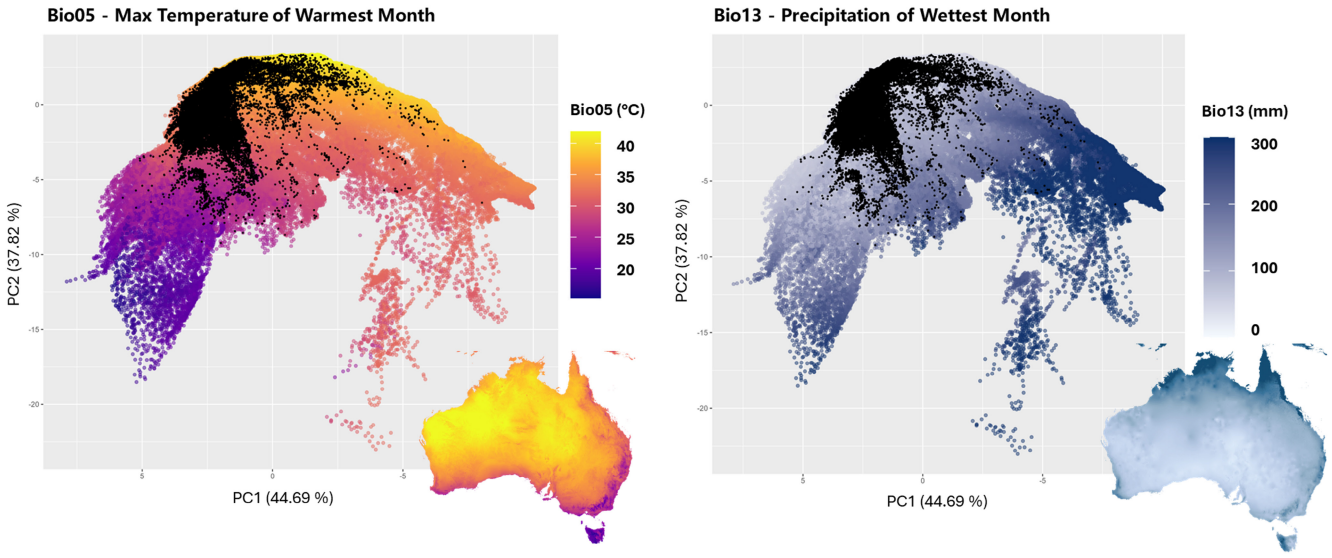


FIGURE 2 | PCA biplots for Bio05 (Maximum Temperature of Warmest Month) and Bio13 (Precipitation of Wettest Month) over Australia's climatic space. Each point represents one of 406,372 locations across Australia, derived from climatic data at a 2.5 arc-minute resolution. For Bio05 (maximum temperature of the warmest month, °C), points are coloured according to temperature, with a gradient ranging from purple (cooler temperatures) to yellow (warmer temperatures). For Bio13, points are coloured according to the precipitation of the wettest month in millimetres (mm). The colour gradient ranges from light blue (lower precipitation) to dark blue (higher precipitation). Inset maps show the spatial distribution of these bioclimatic variables across Australia. Black dots indicate the distribution points of 103 Australian Camphorosmeae species.

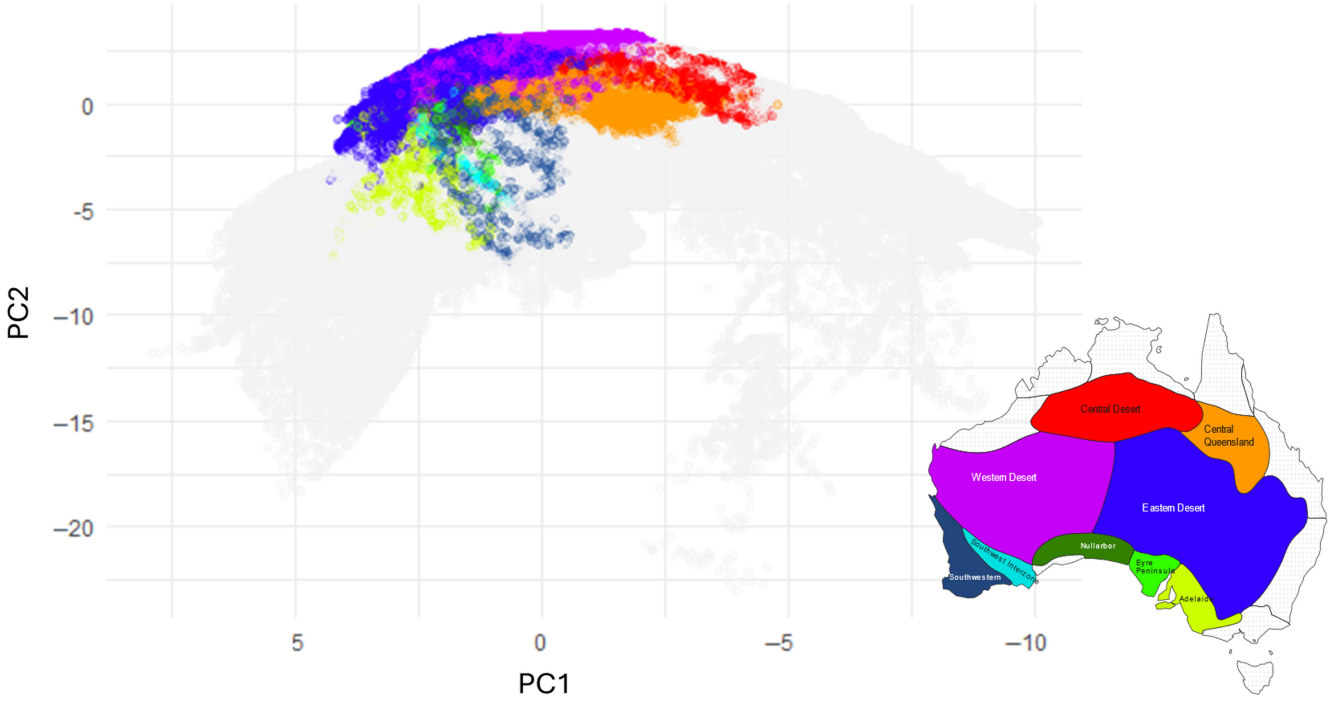


FIGURE 3 | PCA of climate range across Australia. Each point represents one of 406,372 locations across Australia, derived from climatic data at a 2.5 arc-minute resolution. Coloured dots represent the subregions with more than 10% of the occurrence points per species. Subregions are shown on the map. PCA loadings can be found in Table S3.

for each of the two bioclimatic variables. The PC space, with PC1 and PC2 accounting for 44.69% and 37.82% of the total variance, respectively, highlights the significant contributions of these principal components. These components were used to project the occurrence of 103 Australian Camphorosmeae species onto the PC space. The Camphorosmeae species predominantly occupy areas with high maximum temperatures, as indicated by the clustering of occurrence points in the warmer regions of the climatic space. The species occurrences are concentrated in areas with low to moderate rainfall, with a notable presence in regions experiencing lower precipitation.

Figure 3 represents the principal component (PC) space, with colours representing the subregions of Australia as defined by

Ebach et al. (2015). Only subregions with more than 10% of occurrence points per species are displayed. These visualisations provide insights into the main axes of climatic variation and the spatial distribution of climatic conditions across the continent. PC1 explains a significant portion of the variance, capturing a broad climatic gradient across Australia. PC2 represents another important climatic gradient, orthogonal to PC1.

3.3 | Ancestral Estimation and Transitions

The bioclimatic niche evolution of those two selected bioclimatic variables (Bio05 and Bio13) is shown in Figure 4, and the Ancestral Habitat Type Reconstruction in Figure 5. To facilitate the evaluation and discussion of these patterns and results, the same clade numbers as assigned by Hühn et al. (2024) were utilised.

Considering the influence of the two bioclimatic variables, a discernible pattern emerges. Firstly, it is apparent that the ancestor of Australian Camphorosmeae and the ancestors of clades 1–6, 9, as well as *Maireana oppositifolia, M. sedifolia* (F. Muell.) Paul G. Wilson and *M. enchylaenoides* (F. Muell.) Paul G. Wilson preferred lower peak temperatures in the warmest month (Bio05) of under 34°C. Subsequently, a multiple transition in numerous clades towards slightly warmer Maximal temperatures of the warmest month is observed in the descendants, namely Clades 7–8, 10–17, *M. cannonii* (J. M. Black) Paul G. Wilson, and *Neobassia proceriflora* (F. Muell.) A. J. Scott. Interestingly, a subsequent return to lower temperatures occurs in some of these species.

Looking at the precipitation of the wettest month (Bio13), the ancestor of Australian Camphorosmeae likely preferred at least 40 mm of precipitation during the wettest month. Ancestors of clades 1–3 and *M. enchylaenoides* were more likely to be found in slightly wetter areas with precipitation values more than 40 mm during the wettest month, transitioning into marginally drier conditions (≤38 mm). Multiple shifts back to areas with wetter conditions can be observed on at least 10 terminal nodes (Figure 4).

The reconstruction of the ancestral habitat types shown in Figure 5 reveals three main ancestral types, with Riverine Desert being the most prominent and spanning the entire ancestral backbone. For clades 1–3, Riverine Desert and Desert Lake can be recognised as ancestral habitats. Riverine Desert and Karst Plain for *M. enchylaenoides* and *M. sedifolia* and Riverine Desert for all other clades (Figure 5).

3.4 | Biogeographical History

For the BioGeoBEARS analyses using a maximum range of four areas, BAYAREALIKE was the best-fit model (AICc = 822.7; AICc_wt = 0.68), followed by BAYAREALIKE+J (AICc = 824.5; AICc_wt = 0.28). Both models indicate low rates of dispersal ($d = 0.013$) but notably higher extinction ($e = 0.13$) values.

The biogeography analysis highlights two main combinations of subregions within the phylogenetic backbone as potential ancestral areas (Combination 1 and 2). There is a 47.75% probability that the ancestor of clades 1–6 and 9, along with species *Maireana oppositifolia, M. enchylaenoides* and *M. sedifolia*, inhabited Adelaide, Eastern Desert and Western Desert (C + H + I, Figure 6). During the end of the Late Miocene, the ancestors of Clade 3 as well as of Clades 7, 8 and 10–17, along with species *M. cannonii* and *Neobassia proceriflora*, show a significant presence in the Eastern and Western Desert (H + I, Figure 6) regions, with a probability range of 19%–23% for Clade 3 and 60.32% for the rest. Dispersal events to the Eyre Peninsula (D) occurred for clades 2 and 4. These events are represented by the C + D + H + I combination (3) with probabilities of 34.05% and 55.28% at the crown, respectively, contemporaneously during the end of the Late Miocene.

Only during the Pliocene and Pleistocene areas such as the Southwestern (G) in combination with the Western Desert (I) were inhabited. This is evident for Clade 1 at the beginning of the Pliocene. In addition, the Southwestern Interzone (F) was occupied more recently by some species like *Roycea divaricata* Paul G. Wilson, *M. eriosphaera* Paul G. Wilson, *M. carnosa* (Moq.) Paul G. Wilson, *Enchylaena lanata* Paul G. Wilson, *M. suaedifolia* (Paul G. Wilson) Paul G. Wilson, *M. marginata* (Benth.) Paul G. Wilson, *M. amoena* (Diels) Paul G. Wilson, *Didymanthus roei* Endl., *Sclerolaena eurotioides* (F. Muell.) A.J. Scott, *S. fusiformis* Paul. G. Wilson and *S. drummondii* (Benth.) Domin. Additionally, several young species (*Sclerolaena obliquicuspis* (R. H. Anderson) Ulbr, *S. brevifolia* (Ising) A. J. Scott, *S. uniflora*, *S. holtiana* (Ising) A. J. Scott and *S. diacantha* (Nees) Benth.) from clade 17 migrated into the Adelaide region (C + H, Figure 6) during the early Pleistocene.

4 | Discussion

Australia's complex palaeoclimatic history has profoundly influenced the evolution and distribution of its flora, including the Camphorosmeae. The evolutionary history of Camphorosmeae in Australia has been the subject of extensive research over the past few decades, with particular emphasis on the integration of morphological, molecular and biogeographical data. The main hypotheses put forward by Cabrera, Jacobs, and Kadereit (2011), McDonald (2020) and Hühn et al. (2024) offer complementary yet distinct perspectives on the biogeographical patterns and processes driving the diversification and distribution of this group. Building on this previous work and integrating biogeographical analyses, climatic niche evolution and habitat type shifts, we reached a more elaborate understanding of the evolution of Camphorosmeae in Australia.

4.1 | Climatic Influence on Evolution and Distribution of Australian Camphorosmeae

Our study shows significant environmental gradients influencing the occurrence of Camphorosmeae species (Figure 1). The first principal component, driven by Maximum Temperature of Warmest Month (Bio05) and Mean Temperature of the Warmest Quarter (Bio10), explains 43.37% of the variance. This suggests that temperature extremes are crucial in determining and

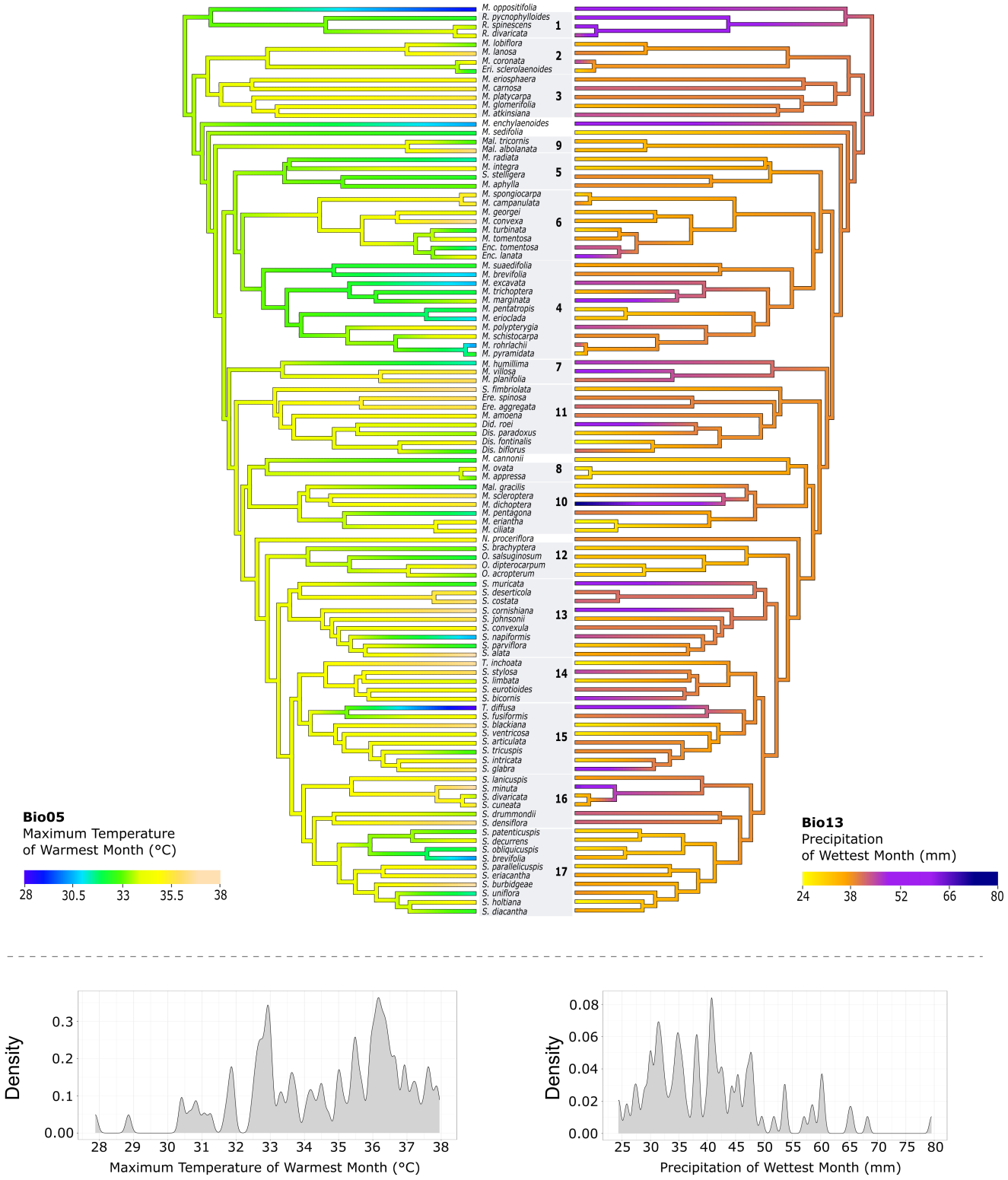


FIGURE 4 | Phylogenetic visualisation of bioclimatic niche reconstruction at ancestral nodes for two environmental variables in the phylogenetic MCC tree of Australian Camphorosmeae. Density plots show the distribution frequency across all clades. Bio05: Branches are coloured by temperature (°C). Bio13: Branches are coloured by precipitation (mm).

limiting the distribution of these species. The second principal component, linked to Precipitation in the Wettest Month (Bio13) and Precipitation in the Warmest Quarter (Bio18), accounts for 22.54% of the variance, indicating the importance of precipitation minimum and seasonality.

The Camphorosmeae species occupy predominantly areas with high maximum temperatures during the warmest month, as indicated by the clustering of occurrence points in the warmer regions of the climatic space. The maximum temperature in the warmest month for the Australian Camphorosmeae ranges from 27.9°C to

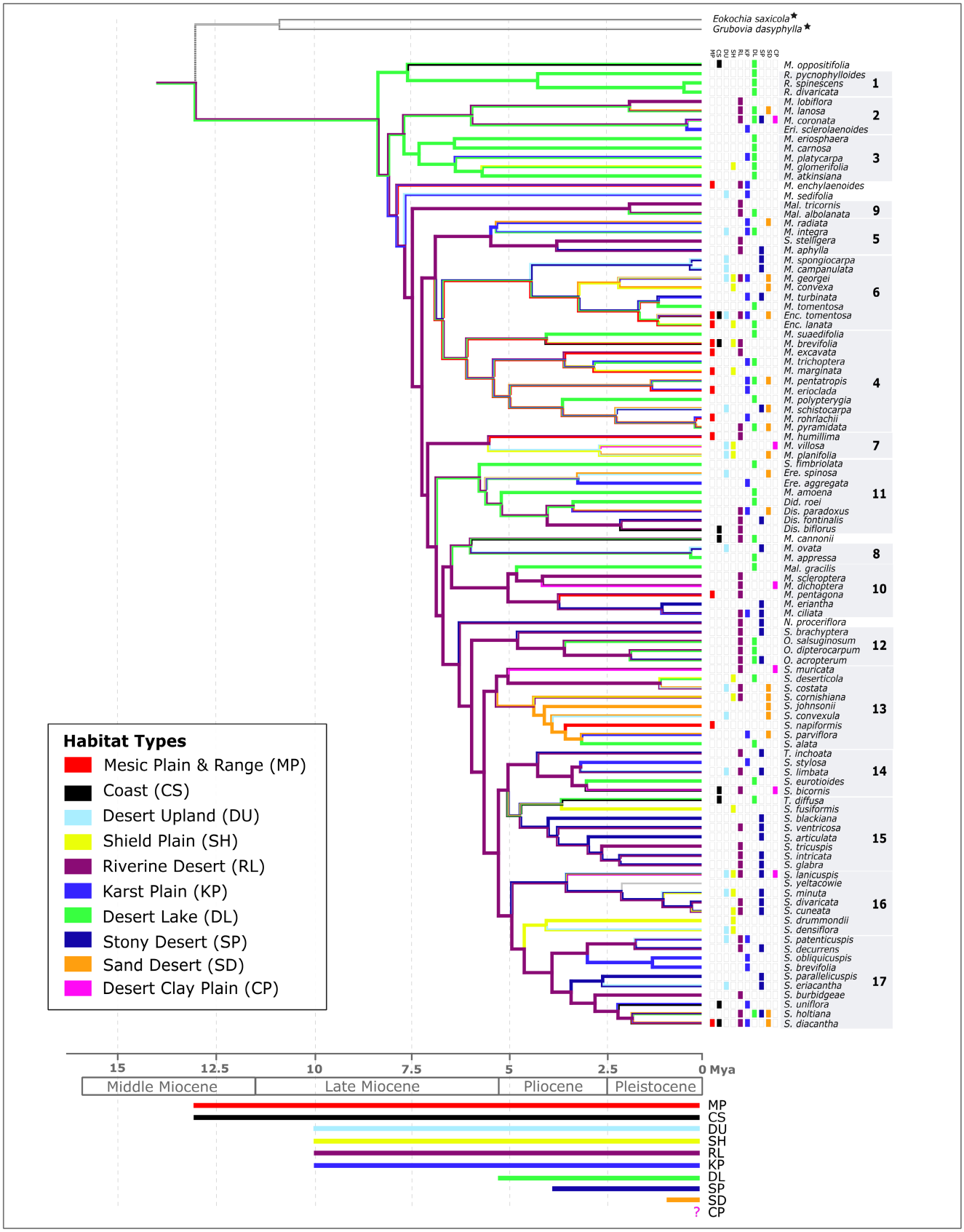


FIGURE 5 | Legend on next page.

38.0°C, emphasising the adaptation to high temperatures. These temperatures fall within the range observed in the semi-arid and desert biomes of Australia, where temperatures frequently exceed 35°C during summer, though the upper range of 40°C or more is more characteristic of the central desert regions (Geoscience Australia 1994). Additionally, their distribution in areas of low to

FIGURE 5 | Ancestral Habitat Type Reconstruction for the Australian Camphorosmeae based on the dated MCC tree. It represents the evolutionary relationships among 104 (*Sclerolaena* sp. *yeltacowie* included) Australian Camphorosmeae species with 10 habitat types defined by Mabbutt (1988) and McDonald (2020). Branch colours indicate each node's most likely ancestral habitat type, inferred from habitat reconstruction analyses using Mesquite's maximum parsimony method. The right side of the tree shows the current habitat type distributions for each species based on McDonald (2020). Major clades are labelled from 1 to 17 based on Hühn et al. (2024). The phylogeny is calibrated with that time scale at the bottom, divided into the Middle Miocene, Late Miocene, Pliocene and Pleistocene epochs. The horizontal bars below the time scale show the different habitat types over geological periods based on McDonald (2020). Outgroup species are marked with a star.

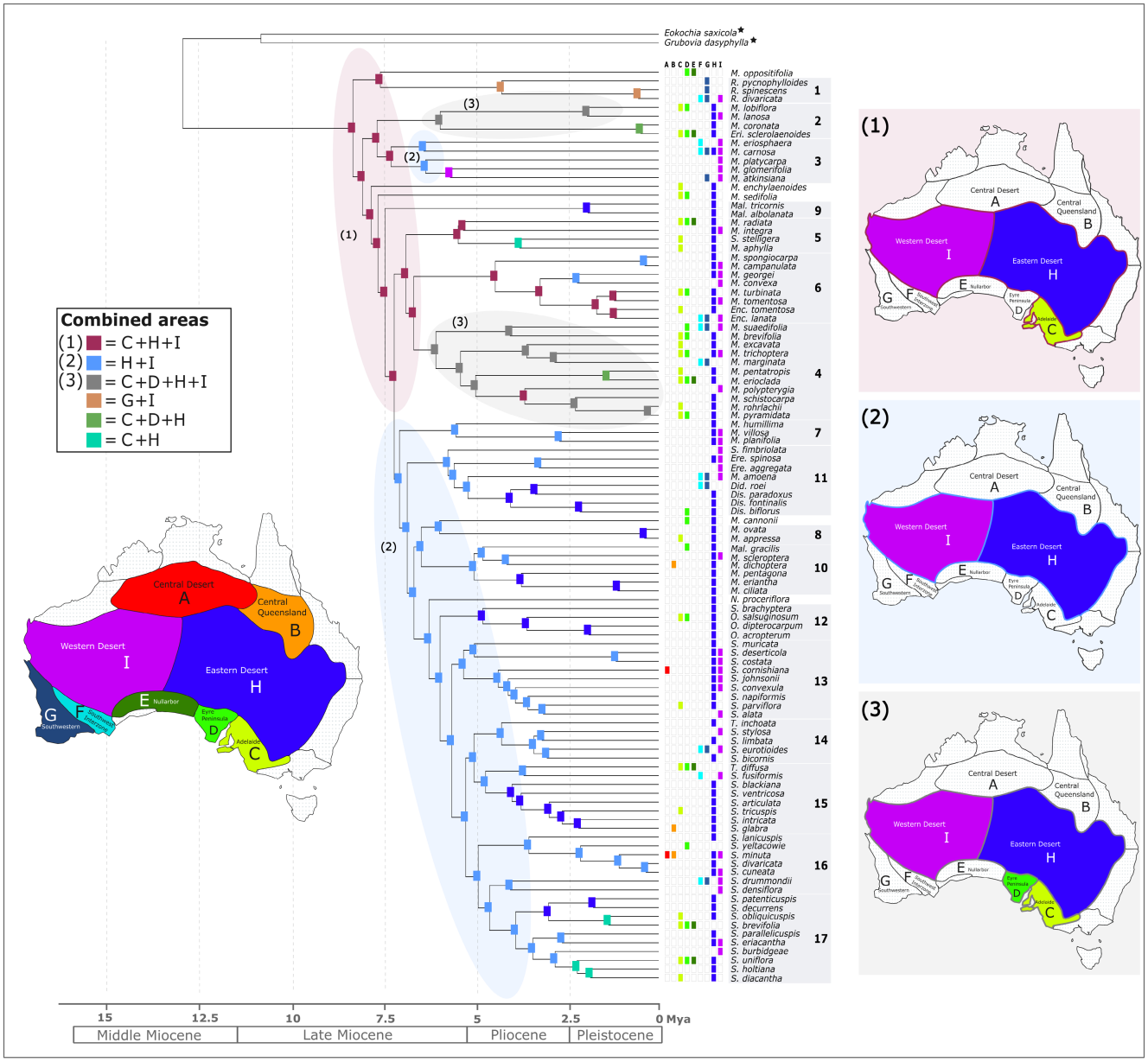


FIGURE 6 | Biogeography analysis on the time-calibrated phylogenetic MCC tree showing the distribution of Australian Camphorosmeae species across various subregions in Australia over time. Biogeographical analysis was conducted in BioGeoBEARS v1.1.2 with a maximum range size of four. The colour-coded matrix next to the species names corresponds with the current species distributions. Subregions were chosen only if more than 10% of each species' occurrence points were present within those areas. Squares at nodes represent the ancestral range with the highest probability from the BAYAREALIKE analysis. Colours represent the areas as indicated on the map or combinations of them. The Australian subregion map is based on Ebach et al. 2015. Combined area maps on the right: (1) shows the combination of the subregions C, H and I in dark red, (2) shows the combination of H and I in light blue and (3) shows the combination of C, D, H and I in grey. Outgroup species are marked with a star.

moderate precipitation during the wettest month (24.3–79.5 mm) highlights their adaptability to different levels of aridity, with a notable presence in regions experiencing moderate rainfall (37–52 mm). It is important to note that these precipitation levels refer specifically to the wettest month within the distribution range of Camphorosmeae, not to the wettest month in Australia

overall. This precipitation range is consistent with the definition of the semi-arid biome in Australia, where annual rainfall is less than 250–500 mm, and monthly wet season precipitation typically varies within this range (Geoscience Australia 1994; Huang et al. 2016). The exclusively annual genus *Grubovia*, exhibits distinct climatic preferences that closely match those of its Australian relatives. Our analysis of the distribution of *Grubovia* species reveals a preferred maximum temperature in the warmest month (Bio05) ranging from 23.2°C to 27.9°C. This range is lower than the range of the Australian Camphorosmeae, but still overlaps, indicating an adaptation to relatively high temperatures. Regarding precipitation during the wettest month (Bio13), *Grubovia* species thrive in regions with 42.0 mm to 53.3 mm of precipitation. This range is within the wider range of 24.3 mm to 79.5 mm observed for Australian Camphorosmeae. It corresponds to the moderate precipitation conditions of 30–50 mm where the Australian species are most abundant. Thus, while *Grubovia* occupies a slightly cooler and more consistently moderate rainfall niche compared to Australian Camphorosmeae, both clades show strong adaptability to environments characterised by high temperatures and variable rainfall, particularly moderate conditions.

Looking at the ancestral climatic preferences of the Australian Camphorosmeae, we see that they favoured warmest month temperatures up to 34°C and areas with precipitation during the wettest month of at least 52 mm. This suggests that the ancestors of these two sister lineages were already adapted to this type of precipitation regime. The milder and lower the temperatures, similar to those preferred by *Grubovia*, furthermore suggest that the ancestors initially thrived in cooler climates. However, it is important to acknowledge that reconstructing ancestral climatic preferences based on extant taxa is controversial in the literature (Cunningham, Omland, and Oakley 1998; Cunningham 1999; Vanderpoorten and Goffinet 2006), especially given the significant climatic changes since the Pliocene, including cycles of re-wetting and dry/wet, hot/cold periods during the Pleistocene. Despite this uncertainty, it appears that over time, the lineage that became the Australian Camphorosmeae adapted to the changing climate within Australia since the end of the Late Miocene.

4.2 | Habitat Evolution

Reconstruction of ancestral habitat types may reveal critical ecological transitions that facilitated the adaptation and diversification of Camphorosmeae species. Our analysis shows distinct clusters of points in principal component (PC) space, indicating unique climatic conditions in different subregions (Figure 3). This reflects the ecological versatility and/or specialisation of these species, suggesting that they have evolved to occupy different climatic niches.

Our results indicate that the diverse range of habitats occupied by Australian Camphorosmeae highlights significant ecological diversity within the group. The habitat type 'Riverine Desert' spans the entire backbone of the phylogeny, strongly suggesting it to be the ancestral habitat type of the Australian Camphorosmeae. This habitat type, characterised by open vegetation and arboreal growth along floodplains and river channels with mild to moderate salinity and variable soil types, likely emerged in the Middle to Late Miocene when palaeovalleys ceased to exist (Figure 7; Van de Graaff et al. 1977; McDonald 2020). Riverine Lake habitats have been present in Western Australia since the Miocene gradually shifted with the progressive cessation of the palaeodrainage systems (Kershaw, Martin, and Mason 1994; Hühn et al. 2024). As these systems slowly disappeared, the river systems transformed into desert river channels, and the floodplains began to dry out, creating the 'Riverine Desert' habitat. This habitat type, which is well suited to species adapted to high temperatures and arid to semi-arid regions, expanded eastwards due to the west-to-east gradient of declining precipitation and increasing aridity that occurred during the Late Miocene and Pliocene (Martin 2006). It is consistent with the adaptations observed in many Australian Camphorosmeae species. This demonstrates that 'Riverine Desert' landscapes played a significant role as a migration corridor, further facilitating the spread and diversification of these species, as suggested by McDonald (2020) and Hühn et al. (2024) and is supported by our analysis. Certain clades strongly associate with other specific habitats, such as the 'Shield Plain' for clades 6 and 16, 'Desert Lake' for clades 1–3, 8 and 11 and 'Sand Desert' habitats for clade 13. This suggests that habitat specialisation has played a crucial role in the evolutionary divergence of the lineage. The adaptability of Camphorosmeae to semi-arid and arid environments prevalent in Australia is reflected by the strong association between specific clades and particular habitats, indicating that habitat shift might have been one key driver of diversification within this group (Figure 5). The 'Shield Plain' habitats are non-saline and contain a variety of xerophytic plant species (McDonald 2020). *Sclerolaena drummondii* and *S. densiflora* (W. Fitzg.) A. J. Scott are two examples of a habitat shift from the regularly inundated 'Riverine Desert' to the highly exposed rocky 'Shield Plain' habitat. The 'Desert Lake' habitat is characterised by a strong salinity gradient, ranging from the bare salt crust to the slightly salty edges with halophytic vegetation (Mabbutt 1988). The species present there (e.g., most *Maireana* species), have a high-temperature tolerance, allowing them to thrive in this environment, while those adapted to moderate amounts of precipitation are likely to be found on the less saline edges where conditions are less extreme. The very young 'Sand Desert' habitat, which formed less than a million years ago during the Late Pleistocene (Fujioka and Chappell 2010), is characterised by hummock grasslands, tall shrublands or open woodlands, and generally lacks salinity. This is the case except in areas where thin sand sheets cover older landscapes inhabited by glycophytes that are more competitive in the absence of salinity (McDonald 2020; Flowers, Galal, and Bromham 2010; Waisel 1972). It is important to mention that these habitat types are flexible in time and location and are very similar in their overall climatic conditions. They have changed continuously with changes in geography and climate, and the boundaries of the arid and semi-arid areas have changed since the end of the Miocene in response to fluctuations in temperatures (Smith 2013).

4.3 | Biogeography and Historical Processes

The conducted large-scale biogeographical analysis identifies key dispersal and vicariance events that have shaped the current distribution of Camphorosmeae species. The overlap and separation of habitat types and occupied subregions highlight the diversity of climatic niches occupied by these species, reflecting historical biogeographical processes (Figures 3, 5, 6 and 7). These findings provide insights into the evolutionary pressures

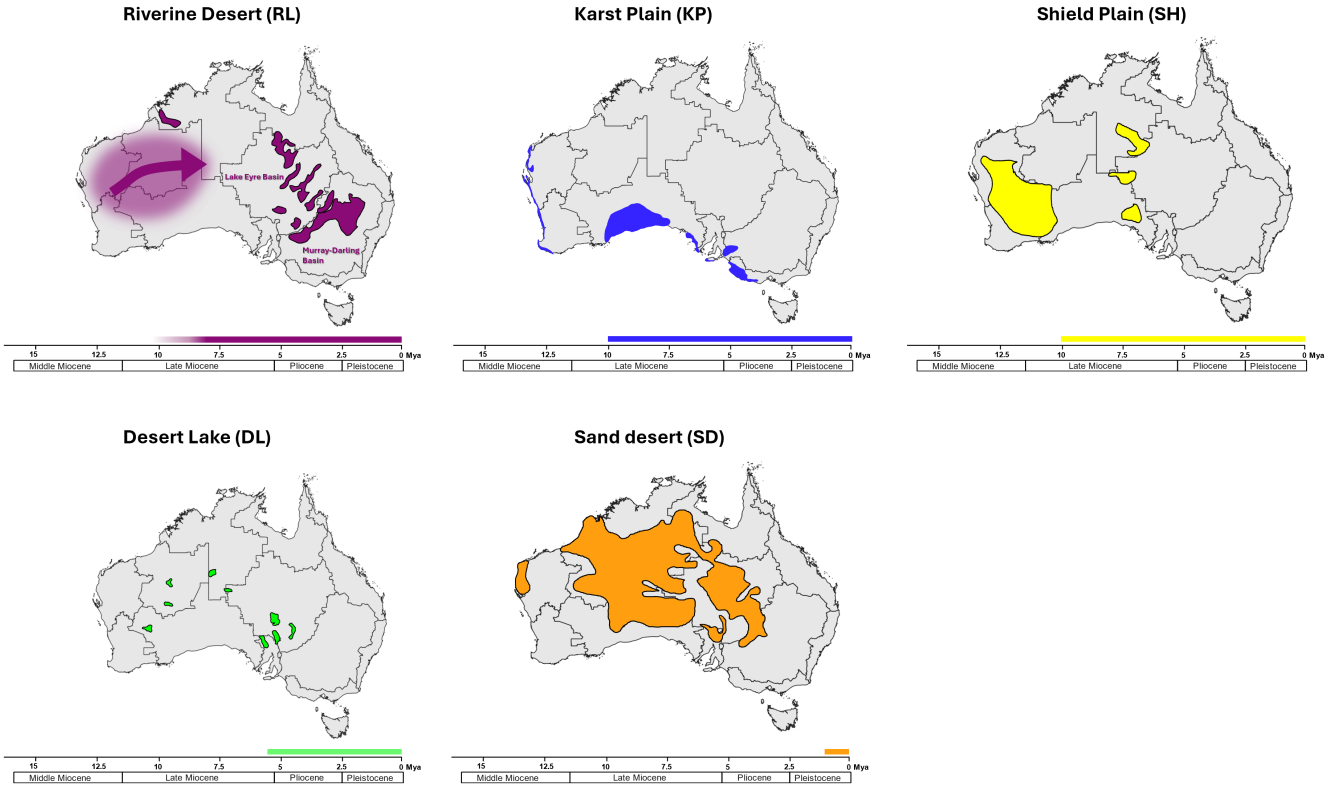


FIGURE 7 | The five important habitat types of the Australian Camphorosmeae. Riverine Desert (RL), Karst Plain (KP), Shield Plain (SH), Desert Lake (DL) and Sand Desert (SD). Each map shows the geographical extent of a particular habitat type and its evolution over geological periods from the Middle Miocene to the Pleistocene based on Mabbutt (1988) and McDonald (2020). The arrow in the Riverine Desert map indicates the distribution through time. Coloured habitat types are based on Mabbutt (1988) and Webb, Grimes, and Osborne (2003).

and ecological dynamics that have influenced the distribution patterns of the Australian Camphorosmeae.

The combination of the Western Desert, Eastern Desert and Adelaide geographical subregions (sensu Ebach et al. 2015) is identified as the ancestral range of Australian Camphorosmeae. Throughout the evolutionary timeline, particularly from the Late Miocene to the Pliocene, this region served as the core habitat for many Australian Camphorosmeae. The combination of Western and Eastern Desert, the so-called outback, occurs at several points in the phylogenetic tree. In particular, it is retrieved as the ancestral region for clades 3, 7–8 and 10–17. The persistence of this combination may highlight the climatic stability and continuity of these deserts throughout the Late Neogene. Alternatively, the role of the age and gradual expansion of the arid/semi-arid biome could be considered. The arid climate initially developed in northern and central Australia and progressively expanded southwards as the continent drifted northwards. This implies that the Western and Eastern Desert regions might have been among the first arid habitats to form, serving as key refuge areas for Camphorosmeae. The later expansion into the Eyre Peninsula, observed in clades 2 and 4, could reflect this gradual southwards spread of arid conditions. The Eyre Peninsula's open vegetation during the Early to Middle Pliocene, as reported by Kershaw, Martin, and Mason (1994), supports this scenario of biome expansion rather than just climatic stability.

Southwestern Australia has been identified as a significant source of lineages that dispersed into arid regions, particularly the Eremaean zone. This region has acted as a centre of diversification for several other plant groups. The asymmetric dispersal of *Calytrix* from southwestern Australia to the Eremaean region highlights the role of southwestern Australia as a key source area for arid-adapted flora (Nge et al. 2022). Similarly, Goodeniaceae species repeatedly dispersed from southwestern Australia into the arid central and southeastern regions of Australia, especially after the Miocene aridification (Jabaily et al. 2014). In previous studies, it was assumed that the southwest part of Australia could be the area of origin of the Camphorosmeae (Cabrera, Jacobs, and Kadereit 2011). However, more recent analyses, including those by Hühn et al. (2024), challenged this view. Lamont and He (2017) proposed that the Mediterranean-type climate (MTC), which currently exists in southwestern Australia, likely originated in northwestern Australia around 30 Ma. As the Australian continent drifted northwards, the MTC shifted southwards towards its current location. This climatic shift suggests that the ancestors of Australian Camphorosmeae may not have initially dispersed directly to the southwest but rather into northwestern Australia, taking advantage of more uniform precipitation patterns at the time. As the continent aridified and the MTC established further south, the lineage likely followed this climatic gradient, dispersing progressively towards the southwest. This challenges previous assumptions about the migration of Camphorosmeae, suggesting a more complex dispersal history driven by large-scale climate shifts. Similar counterarguments to the southwest origin hypothesis have been presented in studies on other Australian plant groups. For example, McLay, Bayly, and Ladiges (2016) argues that for taxa such as *Hakea*, the

southwestern region may not represent a true centre of origin but could instead reflect an artefact due to a methodological bias arising from phylogenetic analyses. Their work questions whether divergence patterns attributed to southwestern Australia could be more accurately explained by broader continental processes rather than regional isolation. Nge et al. (2024) further demonstrated that in groups like *Cryptandra* (Rhamnaceae), spine evolution and the diversification of these taxa occurred in response to environmental changes across multiple regions, not just in the southwest. In their earlier work, Nge et al. (2022) also found that *Calytrix* species showed biogeographical disjunctions between eastern and southwestern Australia, indicating that these plants underwent complex evolutionary processes far beyond the southwestern region alone. Thus, while the littoral connection hypothesis still provides a plausible explanation for the observed distribution of chenopods (McDonald 2020), suggesting that they maintained their ecological preferences over time and directed their spread from coastal regions into the interior deserts, it is likely that this process involved multiple dispersal phases. Beginning in the north and moving southwards in conjunction with the shifting climatic zones, this process seems to align more closely with continental-scale dispersal mechanisms observed in other Australian plant taxa. Ultimately, the idea of southwestern Australia as a singular centre of origin must be reconsidered in light of these broader biogeographical studies that illustrate more complex evolutionary and dispersal histories across the Australian continent.

The recovery of the 'Riverine Desert' as an ancestral habitat type adds another layer of complexity. While this habitat type supports the idea of a Western origin due to the presence of certain taxa, it appears to contradict the broader biogeographical patterns observed (Figure 7). The data suggests that while there may be taxa in the western part of the Western Desert, the main ancestral range remains more centrally located between the Western and Eastern Deserts and Adelaide, due to the presence of many Late Miocene/Early Pliocene lineages and the greatest diversity of taxa (Figures 5–7). The dynamic environmental conditions described by Lamont and He (2017), particularly the southward migration of the MTC, may have played a crucial role in shaping these distribution patterns, influencing both the dispersal and adaptation of Camphorosmeae species across different arid and semi-arid areas of Australia.

4.4 | Migration and Local Adaptation

This complex dispersal history of the Camphorosmeae mirrors broader patterns of plant migration and adaptation observed across Australia's arid zones, where changing climates have shaped biodiversity in significant ways (Crisp and Cook 2013; Weston, Jordan, and Keith 2017). Plants in arid regions of Australia have developed a variety of complex evolutionary strategies to survive, avoid and persist in hot and water-limited environments (Norton, Malinowski, and Volaire 2016). These strategies include processes of adaptation, in which plants change their structures or functions to better suit their environment (Dörken, Ladd, and Parsons 2020), and migration, in which they disperse seeds to new areas that offer suitable conditions. The evolution of plant groups in Australia's Eremaean Zone involves vicariance, pre-adapted immigration and in situ adaptation (Cauz-Santos et al. 2024). For example, *Eucalyptus* and *Calytrix*, both from the Myrtaceae family, are examples of vicariance (Cauz-Santos et al. 2024; Nge et al. 2022; Martin 2006). An example of pre-adapted immigration, however, is the genus *Ptilotus*, which was already adapted to thrive in arid environments (Hammer et al. 2021). In situ adaptation illustrates the evolution of traits specialised for arid conditions within the Eremaean zone. An example of in situ adaptation in Australia is the persistence of *Banksia* (Proteaceae) species in response to changing climatic conditions, facilitated by adaptive genetic variation within populations (He et al. 2016). The Australasian grass flora reflects similar processes. The migration and adaptation of grasses is influenced by factors such as climate stability and the suitability of new environments. The spread of grasses to Australia was facilitated by their pre-adaptation to dry and high light that is, UV conditions, allowing them to outcompete native species (Bryceson et al. 2023). This migration was enhanced by broad land bridges that existed during the Last Glacial Maxima due to lower sea levels (Bryceson and Morgan 2022). Dispersal and vicariance have also driven diversification in other plant lineages across Australia's arid zones. *Acacia* (Fabaceae), for instance, demonstrates a wide range of habitat adaptations within the arid zone. Research by Ladiges, Ariati, and Murphy (2006) and Renner et al. (2021) reveals that *Acacia* species exhibit diverse adaptations to varying climatic conditions. Analysis by Ladiges, Ariati, and Murphy (2006) indicates that the differentiation of *Acacia* species among regions, such as Arnhem and the northwest semi-arid regions, suggests ancient biogeographical divisions that potentially date back to the Cenozoic era. The study highlights how *Acacia* species have radiated into different habitats within the arid zone, reflecting a complex interplay between migration and local adaptation. It demonstrates the range of habitats that *Acacia* has colonised and adapted to, contrasting with the more specialised habitat preferences observed in Camphorosmeae. *Cryptandra* (Rhamnaceae) diversified through several vicariant events, followed by in situ diversification with little exchange between regions, since the diversification of this genus was negatively affected by the spread of aridity (Nge et al. 2024). This contrasts with *Hakea* (Proteaceae), which underwent frequent biome shifts, showing a similar dispersal pattern to Camphorosmeae, by diversifying in southern Australia and dispersing into other biomes across the Australian continent, including arid regions (Cardillo et al. 2017). Similarly, *Callitris* (Cupressaceae) diversified in response to increasing aridity from the Oligocene onwards, exhibiting adaptations to extreme aridity, such as high embolism resistance (Larter et al. 2017). These patterns suggest that ecological pressures, such as those faced by Camphorosmeae in saline and xeric environments, play a key role in shaping the flora of Australia's arid zone.

In our study, we observe several transitions from arid ecologies to areas with higher precipitation during the wettest month. Consequently, new traits are required for species to successfully invade these habitats. According to the dated phylogeny, these in situ transitions are recent and involve comparatively young species and clades. This pattern of habitat transition is not unique to our study; for example, research by Hammer et al. (2021) on *Ptilotus* also documents a similar shift from arid to wetter environments in some clades, highlighting a broader trend of adaptive trait evolution associated with changing

precipitation patterns. Additionally, it is important to note that our study shows also examples of recent dispersal events into arid zones, which should be considered in the context of these findings.

The two species-rich genera of the Australian Camphorosmeae, *Maireana* and *Sclerolaena* are known to prefer environments such as lake systems and saline areas. These species are likely to be influenced not only by edaphic factors such as soil type but also by the hydrology of the area, which is highly variable. There have been significant changes during the Pliocene, particularly around the Lake Eyre system, affecting the extent of lakes and saline environments (Habeck-Fardy and Nanson 2014).

Our formal analyses clarify and detail patterns previously hypothesised, revealing both migration and local adaptation in different species. For example, the *Roycea* group in clade 1, which is one of the early divergent lineages, is nowadays widespread in western Australia, mostly in the subregions Southwestern (*R. pycnophylloides*, *R. spinescens* and *R. divaricata*), Western Desert and Southwest Interzone (*R. divaricata*), with Western Desert (I), the Eastern Desert (H) and Adelaide (C) as the ancestral areas (Figure 6). The ancestors of these three species are likely to have originated in the Riverine Desert habitat and remained in the area while the habitat shifted eastwards and other habitats arose or changed (Figure 7). The Desert Lake habitat, for example, began to form in the Pliocene, of western Australia. The age of the current habitats in which *Roycea* occurs do not match the age of the lineage. This suggests that *Roycea* did not migrate with the Riverine Desert habitat, which originated in the west of Australia (Cabrera 2007) and shifted eastwards but instead adapted to the Desert Lake habitat in west Australia (Figures 5 and 7). Another example is the two species *Sclerolaena drummondii* and *S. densiflora* that occur in the Shield Plain habitat. That habitat originated in the Yilgran Block in Western Australia and central Australia in the Arunta Block (Mabbutt 1988) during the Late Miocene. Both species adapted to the Shield Plain habitat in place, while the Riverine Desert habitat shifted eastwards.

Previous hypotheses suggested that the Southwestern Interzone (F) and the Southwestern (G) habitat types might be ancestral, but this analysis indicates otherwise. Focusing on the Riverine Desert, discussing its age, distribution and role as a connecting habitat is crucial. These rivers around the Lake Eyre and Murray-Darling Basin, which are ephemeral and flow for only 3–4 months following seasonal rainfall, follow biogeographical patterns. This demonstrates a balance between niche conservatism and ecological flexibility. Species that retained the Riverine Desert niche migrated east with the habitat, while others that either were ecologically broader and not dependent on the Riverine Deserts, or managed to adapt to the new habitats could remain in the west.

Travelling further east from the arid centre, seasonality becomes more pronounced, and precipitation decreases. In the far southeast, the Great Dividing Range, including the Australian Alps, creates a significant rain shadow, reducing rainfall further inland. This geographical barrier has shaped the climate across the region, limiting moisture in areas to the west of the range. Ancestral lineages that migrated southeast, such as the clades 9 and 12, as indicated by branches showing precipitation values under and equal to 38 mm during the wettest month (Figure 4), adapted to these increasingly arid conditions. The Riverine Desert has long been considered a connecting habitat, and our findings confirm this notion. Hühn et al. (2024) initially hypothesised this and our formal analysis, using ancestral character state reconstruction, supports and extends the conclusions by Hühn et al. (2024). Some species that migrated south or towards the coast of Australia like *Maireana enchylaenoides*, *M. marginata*, *Enchylaena lanata*, *Didymanthus roei* or *Threlkeldia diffusa* have branches showing precipitation equal to or greater than 52 mm during the wettest month (Figure 4). Although the ancestors were adapted to survive in areas with precipitation equal to or greater than 52 mm, the descendant species occurred in areas with less than 52 mm precipitation during the wettest month. These mentioned species are presumably more competitive in regions with a precipitation of 52 mm or more in the wettest month.

In examining the interplay between migration and adaptation in Australian Camphorosmeae in Australia's dynamic habitats, it becomes clear that distinguishing between the two processes is complex by nature. Habitats and distribution areas of species are both changing constantly, making it difficult to clearly separate migration from local adaptation. In most cases, a mixture of both processes is at work, with one occasionally dominating the other. This observation aligns with the evolutionary histories of many other Australian plant lineages, where aridification, biome shifts and convergent evolution have shaped the flora of Australia's arid zones. Consequently, it is reasonable to question the need for a clear distinction between migration and adaptation especially in areas that are connected.

4.5 | Challenges and Further Work

Studying the general biology of the Australian Camphorosmeae is challenging due to several factors, including Australia's wide and often remote landscapes, which can hinder fieldwork and sample collection, resulting in sampling gaps. Additionally, the complex geological history of the Australian continent, characterised by tectonic activity, climatic fluctuations and sea level changes, has influenced the distribution and diversification of plant species over millions of years (Dettmann 1994). Furthermore, the Camphorosmeae tribe comprises species with different ecological requirements and dispersal abilities (Cabrera 2007), further complicating biogeographical analyses.

The phylogenetic analysis also presents challenges with temporal constraints, especially concerning the Sand Desert habitat. The discrepancy between the estimated age of the sand desert and the migration patterns of Camphorosmeae species, particularly in clade 13, which inhabits the Sand Desert, is shown in Figure 5. This suggests either an error in estimating the age of the habitat, or multiple migrations into the Sand Desert over time. These taxa might have had different ecologies and only recently migrate into the Sand Desert. Evidence, particularly from groups such as *Triodia* spp., suggests that the Sand Desert habitats are relatively recent (Mabbutt 1988; Grigg 2009). The branch length extending back 5 to 4 million years complicates the interpretation, as introducing temporal constraints into the biogeographical model may not significantly affect the state shift estimates. The habitat types,

such as Karst Plain, Shield Plain or Desert Lake span multiple geographical regions, making it difficult to perform precise biogeographical reconstructions, a problem exacerbated by the constant shifting of those regions. Similarly, clades 1–3 and the species *M. oppositifolia* show Desert Lake as an ancestral habitat dated to the Late Miocene, whereas the estimated age of these habitats based on palaeogeographical evidence appears to be the Early Pliocene in Western Australia and Late Pliocene in Central Australia (McDonald 2020). If we accept these estimated habitat ages, only the Riverine Desert could be the ancestral habitat due to its age. However, this raises questions about the true ancestral status given the unresolved backbone of the phylogeny. In addition to these biogeographical uncertainties, it is worth noting that inferring ancestral climatic preferences from extant taxa presents additional complexities (Cunningham, Omland, and Oakley 1998; Cunningham 1999; Vanderpoorten and Goffinet 2006). The evolutionary trajectory of these lineages has been shaped undoubtedly by significant climatic changes since the Pliocene, including periods of warming, cooling and fluctuating precipitation patterns during the Pleistocene. These environmental changes would have affected the ecological niches of ancestral taxa, suggesting that caution is required when interpreting their past climatic adaptations. Thus, while the preferences of current species can provide clues, they may not fully represent the conditions experienced by their ancestors. The diverse range of habitats occupied by Camphorosmeae species, particularly their association with saline environments and fluctuating hydrological conditions (Cabrera, Jacobs, and Kadereit 2011; Hühn et al. 2024), suggests that edaphic factors and hydrology may influence their distribution greatly. This variability, particularly around systems such as Lake Eyre, highlights the need for finer scale analyses and potentially more nuanced biogeographical coding to capture the dynamic environmental changes over the past 8 million years.

Data limitations are another challenge. The partly unresolved backbone and the absence of 56 species (36 *Sclerolaena*, 15 *Maireana*, one *Dissocarpus*, one *Malacocera*, one *Neobassia* and two *Osteocarpum* species) from the phylogeny limit the scope of the analysis. Although the current phylogeny represents an improvement over previous datasets and does not exhibit significant sampling bias, including these species in future analyses could refine our understanding of their evolutionary and biogeographical patterns. After conducting an additional analysis to account for the missing 56 species by filtering the most important subregions (i.e., areas with more than 10% of occurrences per species), three further subregions (Eastern Queensland, Great Sandy Desert Interzone and Southeastern) were identified to be important (Table S2, Figure S1). It may be that the inclusion of ecologically highly specialised species such as *Sclerolaena hostilis* (Diels) Domin would be a valuable contribution to a more comprehensive phylogenetic framework, as this species only occurs in the Great Sandy Desert Interzone with a 100% occurrence rate. In future work, we additionally aim to further explore the role of soil variation in shaping diversification rates within Camphorosmeae, particularly by incorporating more precise clade-specific sampling probabilities, additional soil quality parameters and methods such as target enrichment for improved genetic sampling. While preliminary analyses have not yielded conclusive results, more refined data and approaches may help to clarify the influence of soil characteristics on diversification patterns.

All these challenges highlight the complexity of studying the evolutionary and biogeographical history of Camphorosmeae species in Australia. Future research should address these limitations by improving phylogenetic resolution through methods such as target enrichment or genome skimming, conducting finer scale habitat and biogeographical analyses and including currently missing species to provide a complete picture of their evolutionary dynamics. The distinction between migration and adaptation can be effectively studied through the combined use of population genetics and ecological niche modelling. These methods provide complementary insights into the processes that drive species distribution and evolution.

5 | Conclusion

The study of Camphorosmeae in Australia reveals a vibrant evolutionary and biogeographical history shaped by climate change and habitat diversification. The combination of morphological, molecular and biogeographical data has provided insights into the evolutionary dynamics of this lineage, highlighting the influence of temperature and precipitation on species distributions. The ancestral adaptation to warm and moderately humid climates allowed the migration and local adaptation of Camphorosmeae to different habitats, in particular, the Riverine Desert, which played a crucial role in their diversification. Despite advances in understanding, challenges such as incomplete sampling and complex geological histories remain, requiring further research to refine phylogenetic frameworks and biogeographical models. Overall, this research highlights the interplay between ecological flexibility and niche conservatism in shaping the biodiversity of Australian Camphorosmeae.

Author Contributions

Jessica A. Berasategui: conceptualization (lead), data curation (lead), formal analysis (lead), visualization (lead), writing – original draft (lead), writing – review and editing (lead). **Anže Žerdoner Čalasan:** conceptualization (supporting), writing – original draft (supporting), writing – review and editing (supporting). **Gudrun Kadereit:** conceptualization (supporting), formal analysis (supporting), funding acquisition (lead), project administration (lead), writing – original draft (supporting), writing – review and editing (supporting).

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are uploaded as Supporting Information.

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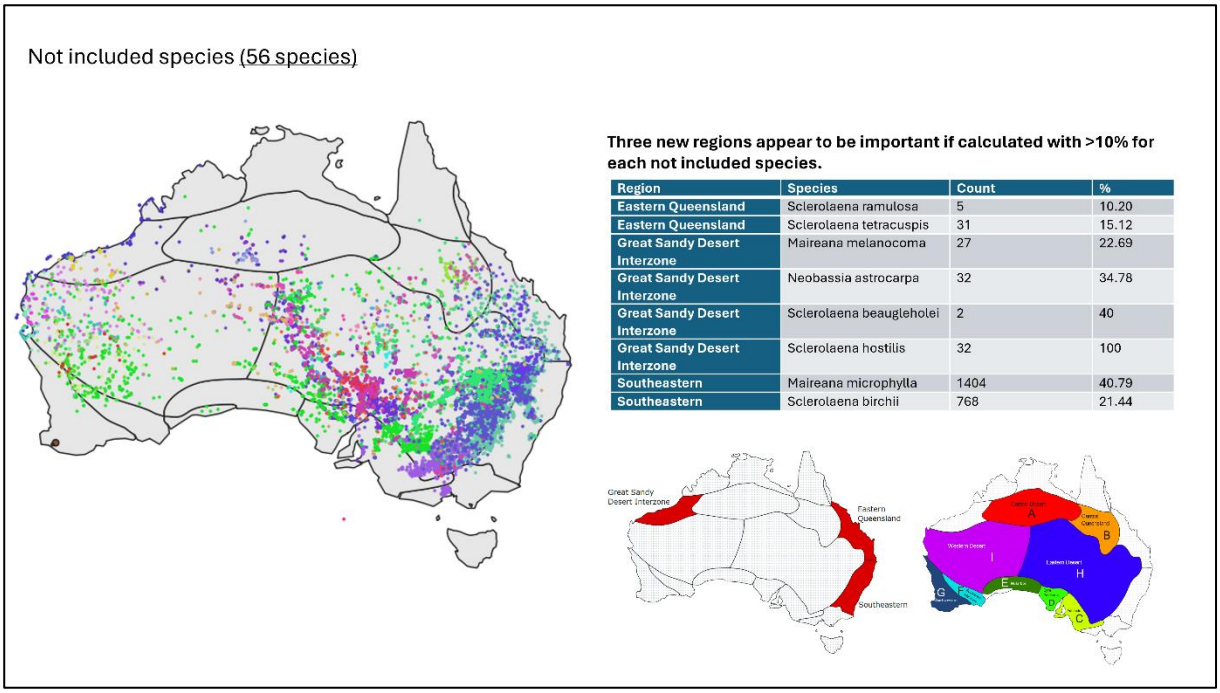
Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Publication II - Supplement

Figure S1: Occurrence of excluded species.



Region	Species	Count	%
Eastern Queensland	Sclerolaena ramulosa	5	10.20
Eastern Queensland	Sclerolaena tetracuspis	31	15.12
Great Sandy Desert Interzone	Maireana melanocoma	27	22.69
Great Sandy Desert Interzone	Neobassia astrocarpa	32	34.78
Great Sandy Desert Interzone	Sclerolaena beaugleholei	2	40
Great Sandy Desert Interzone	Sclerolaena hostilis	32	100
Southeastern	Maireana microphylla	1404	40.79
Southeastern	Sclerolaena birchii	768	21.44

Table S1 (pendrive): Coordinates of all species downloaded from ALA.

Table S2 (pendrive): Percentage of occurrence points per species across subregions.

Table S3 (pendrive): PCA loadings.

General Discussion

The overarching aim of this dissertation was to contribute to the understanding of the distributions and evolutionary adaptations of C_4 eudicots and C_3 Camphorosmeae (Amaranthaceae), focusing on the role of climatic preferences, functional traits and geographic isolation. Through a combination of global (Chapter 1) and local (Chapter 2 — Australia) analyses, the dissertation presents key findings that reveal complex relationships between species distributions, photosynthetic pathways and evolutionary adaptations in response to climate. These results provide important insights into how environmental factors shape evolutionary trajectories while also addressing methodological challenges. This discussion highlights the broader implications of the results and suggests directions for future research.

By integrating large datasets of occurrence data, climatic data and phylogenetic inference, the dissertation identifies diversity hotspots and trace the origins of certain lineages. However, while the use of big data sources provides a wealth of information, it also presents several challenges. One of the most significant obstacles is the quality and completeness of public datasets. While public repositories such as GBIF provide a vast amount of georeferenced occurrence data, they often contain erroneous or incomplete records. The analyses in both chapters showed that data cleaning is essential to remove inaccuracies, such as duplicates, correcting synonymisation errors, and verifying geolocation accuracy, which could otherwise obscure meaningful results (Chapter 1, Table1; Supplementary Figures S1–S15). These challenges highlight the importance of data curation and refinement before meaningful meta-analyses can be conducted. Despite these difficulties, meta-analyses remain a powerful tool for synthesising findings across studies. Meta-analyses can help to address many unanswered questions, but their success depends on well-formulated research questions and clean, accurate data. In Chapter 1, the phylogenetic approach allowed the identification of regions of high C_4 diversity, highlighting the different environmental conditions that may have driven the evolution of these traits. Similarly, the formal analysis of Australian Camphorosmeae in Chapter 2, based on a relatively well-resolved phylogeny by Hühn et al. (2024), represents a pioneering effort to

conduct a formal analysis with descriptive metadata on this group, and to understand the diversification of this lineage across arid habitats. These results highlight the value of meta-analyses when applied with a clear research framework and clean data, providing new insights where individual studies may have been limited. Looking ahead, the potential of large datasets lies in expanding the integration of different data types, such as genomic data, bioclimatic data, and fossil records, into meta-analyses to provide more robust evolutionary and ecological insights. The incorporation of machine learning can further enhance the predictive power of such datasets. In addition, deeper integration of palaeoclimatic data and improved taxonomic resolution can help refine biogeographic models and trace more precise lineage histories, revealing evolutionary patterns that remain ambiguous today (Chapter 2). In summary, while big data sources offer incredible opportunities to advance our understanding of biodiversity and evolution, limitations in data quality and the challenges of integrating disparate datasets must be carefully managed. Automated data curation built into repositories from the outset can ensure that datasets are regularly checked and cleaned, reducing errors such as duplicates, outdated taxonomic names, and inaccurate georeferencing. This proactive curation helps prevent metadata from becoming outdated and unusable, thereby increasing the longevity and reliability of these resources. With cleaner data and well-structured analyses, as demonstrated in the studies presented, meta-analyses can make a significant contribution to addressing many outstanding questions in evolutionary biology and biogeography. However, further methodological advances are needed to realise the full potential of these data sources.

This potential is highlighted in Chapter 1, which examines the distribution of C_4 eudicots relative to C_4 grasses on a global scale. It explores the environmental factors, particularly climatic variables, that promote the diversification of C_4 eudicots, emphasising the role of drought tolerance, succulence, and salinity tolerance in the expansion of these lineages into arid and semi-arid regions. It also compares the diversity, geographic distribution and functional traits of C_4 eudicots with those of C_4 grasses, revealing clear differences in their ecological preferences. C_4 eudicots are shown to inhabit drier regions than C_4 grasses and often possess additional traits, such as succulence, that enable them to thrive in extreme environments. The findings in Chapter 1 highlight the significant influence of global climatic conditions, with a focus on C_4 species-rich regions of high biodiversity, known as hotspots. These include areas such as Mexico, the southern United States, and large regions of Australia, which have

played a role in the diversification of C_4 lineages in grasses and eudicots. Six distinct C_4 eudicot lineages are likely to have originated in situ in the Mexico/Southern United States region, including *Tidestromia* (Amaranthaceae); *Flaveria* and *Pectis* (Asteraceae); *Euphorbia* (Euphorbiaceae); *Allionia* and *Boerhavia* (Nyctaginaceae). In contrast, only one C_4 lineage, *Tecticornia* (Amaranthaceae), is known to have originated in Australia. This highlights regional differences in evolutionary pathways. Furthermore, C_4 eudicots, whether succulent or not, are generally more likely to inhabit drier regions than C_4 grasses, supporting the hypothesis that certain adaptive traits, such as the use of the NAD-ME decarboxylating enzyme, have enabled them to thrive better in drier environments than their NADP-ME relatives. In C_4 eudicots, the NAD-ME subtype appears to be more widespread than in C_4 grasses, where the NADP-ME pathway predominates.

The C_4 hotspot in Mexico and the southern United States is characterised by diverse desert and semi-desert landscapes, including the Sonoran and Chihuahuan deserts (Laity, 2008). These areas have played a critical role in the evolution and diversification of C_4 plant lineages, particularly eudicots, under extreme environmental stresses such as aridity and limited rainfall. This region, part of the Nearctic realm, has a complex mix of climatic regimes that have favoured high species richness and speciation. Mexico and the southern United States are notable for the local evolution of several C_4 lineages, which likely emerged during the Miocene epoch in response to the arid conditions of the region. In addition, other C_4 lineages may have diversified within these deserts, while some may have arrived later as migratory species from other regions. The harsh environmental conditions of the region, including rain-shadow effects from surrounding mountain ranges and low annual precipitation, have created selective pressures that favoured C_4 photosynthesis, enabling plants to thrive in these arid habitats. This combination of high species diversity, evolutionary adaptations to arid environments, and the presence of both native and migratory C_4 lineages makes Mexico and the southern United States an important hub for understanding the evolutionary dynamics of C_4 photosynthesis.

Within Australia, there are two regions of high C_4 plant diversity and different rainfall profiles. Most Australian C_4 eudicots are restricted to the northern parts of the continent, where tropical and subtropical grasslands, savannas, and shrublands predominate. The second region is the desert and xeric shrublands of the Eremaean floristic region, where C_4 eudicots of the genus *Atriplex* are abundant. However, this

region also supports significant populations of C_3 species, such as the Australian tribe Camphorosmeae, highlighting a diverse range of adaptations. While C_4 photosynthesis is generally associated with arid environments due to its efficiency in water use and carbon fixation, the success of C_3 species in semi-arid and arid habitats may seem counterintuitive. C_3 photosynthesis can remain effective in arid environments under certain conditions, such as cooler temperatures or specific adaptations that minimise water loss. It's also possible that the Australian Camphorosmeae have developed alternative strategies to cope with drought, such as efficient water storage or specialised leaf structures, rather than relying on C_4 photosynthesis. For example, some species in the order Caryophyllales, such as the Australian *Calandrinia*, primarily use C_3 photosynthesis but also activate CAM (Crassulacean Acid Metabolism) under drought conditions, as shown by Hancock et al. (2019). This drought-inducible facultative CAM function suggests some plasticity in their photosynthetic pathways. Interestingly, none of the *Calandrinia* species studied by Hancock et al. (2019) showed strong CAM activity, suggesting that factors beyond CAM strength, such as life cycle constraints (e.g., their annual life cycle), may limit the evolution of more robust CAM mechanisms in these species. The evolutionary flexibility of C_3 and C_3+CAM phenotypes further suggests that CAM is not a fixed trait but can be gained or lost depending on environmental conditions. This adaptability may provide an advantage in environments with fluctuating water availability, supporting the idea that the success of some C_3 species in arid regions is not solely dependent on the evolution of CAM or C_4 pathways, but also on their ability to modify their photosynthetic strategies in response to environmental stressors (Hancock et al., 2019). Thus, the success of certain C_3 species in arid landscapes may be due to this adaptability in their photosynthetic processes, rather than a complete reliance on C_4 or CAM photosynthesis, adding complexity to our understanding of plant survival strategies in Australia's harsh climate.

Chapter 2 focuses on the diversification of the Australian Camphorosmeae C_3 lineage (Amaranthaceae *s.l.*), which is closely linked to shifts in climatic niches and habitat types, driven by palaeoclimatic changes. By integrating molecular phylogenetics, bioclimatic data and biogeographic models, the study shows that the expansion of this group across Australia was strongly influenced by preadaptation to warm, arid environments and key periods of aridification, particularly during the Late Miocene to Pliocene. Analyses of 159 species representing 12 genera reveal clade-specific

adaptations to different habitats, including shifts from the ancestral 'riverine desert' habitat, which facilitated early dispersal across the western and eastern deserts, to more specialised habitats such as 'desert lake' in Western Australia, which emerged in the Early Pliocene. Further habitat transitions from 'riverine desert' to 'shield plain', 'karst plain' and 'sand desert' occurred during the Pliocene and Pleistocene as these environments evolved. Species that retained the 'riverine desert' niche migrated east with the habitat, while others that were either more ecologically diverse and not dependent on the riverine desert, or were able to adapt to the new habitats, remained in the west. These results highlight the complex interplay between ecological flexibility and niche conservatism in shaping the evolutionary pathway of Camphorosmeae. Niche conservatism means that species tend to retain their original ecological traits even as they diversify and adapt to new environments (Wiens and Graham, 2005). This tendency may explain the persistence of certain traits, such as specific photosynthetic pathways, among species occupying similar ecological niches in different regions. Although niche conservatism provides stability in species' ecological strategies, it may also limit their ability to adapt to rapidly changing environments. This raises important questions about the resilience of species to future climate change. Habitat shifts and the timing of climate change, particularly increased aridity, have played a critical role in the diversification of this lineage across Australia's arid landscapes. Building on the findings of Chapter 1, this dissertation illustrates how climate change, habitat specialisation and preadaptation to arid environments have driven the evolutionary success of both C_3 and C_4 species across diverse ecosystems. The local focus on Australian Camphorosmeae sheds light on their adaptation to specific ecological niches within a continent shaped by significant climatic shifts. In contrast, the global analysis of C_4 eudicots provides a broader perspective on how different C_4 lineages have evolved and expanded, particularly into arid and saline habitats, enabled by distinctive physiological adaptations. This diversity of adaptations suggests that climatic extremes can accelerate evolutionary processes, leading to the rapid diversification of lineages. Together, these studies highlight the complex relationship between climate, geography, and functional traits in shaping the evolutionary trajectories of C_3 and C_4 species across regions and ecosystems.

In ongoing analyses, we are currently examining the isotope values of Australian Camphorosmeae, which may form the basis of a future chapter. This research aims to determine whether certain species exhibit characteristics associated with

Crassulacean acid metabolism (CAM), the C_2 photosynthesis, a C_3-C_4 intermediate pathway, or possibly alternative photosynthetic pathways. C_2 photosynthesis, also known as the photorespiratory carbon pump, is a pathway in which a two-celled mechanism shuttles photorespiratory glycine from mesophyll cells to bundle sheath cells. This process increases the efficiency of carbon fixation by concentrating CO_2 around RuBisCO, thereby reducing photorespiration in warm environments. It is thought to represent an early step in the evolution from C_3 to C_4 photosynthesis (Guerreiro et al., 2023). Although carbon concentration via the glycine shuttle is less effective than the C_4 cycle, it could be advantageous under hot and dry growth conditions, when photorespiration is typically high (Tefarikis et al., 2022; Schlüter et al., 2023).

Strong CAM plants, along with C_4 and C_3 plants, can be identified by the ratio of two carbon isotopes: ^{12}C and ^{13}C. Both isotopes have six protons, but ^{13}C has seven neutrons, while the ^{12}C isotope has six. Atmospheric CO_2 contains 98.9% of the ^{12}C isotope and 1.1% of the ^{13}C isotope (O'Leary, 1988). Although these isotopes have identical chemical properties, RuBisCO, the enzyme involved in carbon fixation, reacts more rapidly with the lighter $^{12}CO_2$ than with the heavier $^{13}CO_2$. This difference arises because RuBisCO binds $^{13}CO_2$ less efficiently than $^{12}CO_2$. Reasons for the faster reaction of RuBisCO with $^{12}CO_2$ include the slower diffusion of the heavier $^{13}CO_2$ within the plant and the discrimination of RuBisCO against the heavier $\delta^{13}C$ isotope. The ratio of the two isotopes is expressed as the $\delta^{13}C$ value in parts per thousand (‰). Atmospheric CO_2 has a $\delta^{13}C$ value of -8‰. When CO_2 diffuses through the stomata, this value is reduced to about -12‰ due to the disadvantage experienced by $^{13}CO_2$ (O'Leary, 1988). $\delta^{13}C$ values, which correlate linearly with the proportion of carbon assimilated in light versus dark conditions, enable differentiation among C_3, strong CAM, and C_4 plants (Winter and Holtum, 2002). Since phosophoenolpyruvate carboxylase (PEPC) does not discriminate against ^{13}C like RuBisCO does, the isotopic ratio $\delta^{13}C$ during CO_2 dark fixation in CAM plants is similar to that of C_4 plants. During light fixation from external CO_2, however, it is similar to that of C_3 plants. The distinction between the NAD-ME and NADP-ME pathways further influences these $\delta^{13}C$ values. Specifically, plants utilising the NAD-ME pathway typically have lower $\delta^{13}C$ values due to greater ^{13}C depletion during malate decarboxylation. Conversely, plants using the NADP-ME pathway generally have higher $\delta^{13}C$ values, reflecting less ^{13}C discrimination (Farquhar et al., 1989; Ehleringer and Pearcy, 1983). Under drought

stress, the proportion of dark fixation tends to increase in both plant types, leading to an accumulation of ^{13}C, especially in CAM plants. Therefore, analysis of $\delta^{13}C$ values allows inferences to be made about the plant's carbon assimilation strategy and the underlying metabolic pathways (Ting, 1985). While $\delta^{13}C$ values provide a useful indicator for strong CAM, they fall short in reliably detecting facultative and, particularly, weak CAM (Messerschmid et al., 2021). The $\delta^{13}C$ values for weak CAM species primarily fall within the Winter−Holtum zone, ranging from −23‰ to −19‰ (Males, 2018). A key question guiding our ongoing research is whether there is a relationship between the isotopic values of Australian Camphorosmeae and various climatic variables. It is hypothesised that greater drought stress, whether due to low rainfall or soil salinity, will result in less negative isotopic values, indicating plant adaptations for more efficient internal CO_2 use.

Table 1: Australian Camphorosmeae species and their distribution points with the highest carbon isotope values (‰) recorded during our ongoing research. A total of 114 species were analysed, with two to three accessions selected for each species based on different occurrences. The table displays the $\delta^{13}C$ values and corresponding occurrence coordinates for each species, indicating potential adaptations to environmental conditions that may influence their photosynthetic pathways.

Acc.	**Species**	**$\delta^{13}C$** (‰)	**Occurrence Coordinates** (Latitude; Longitude)
(1)	*Eriochiton sclerolaenoides* (F.Muell.) F.Muell. ex A.J.Scott	-22.2	-30.6181; 130.4150
(2)	*Maireana appressa* (Benth.) Paul G.Wilson	-21.6	-34.2919; 140.8581
(3)	*Maireana decalvans* (Gand.) Paul G.Wilson	-21.2	-30.0667; 145.9167
(4)	*Maireana pentatropis* (Tate) Paul G.Wilson	-21.9	-34.1609; 139.3663
(5)	*Maireana pentatropis*	-22.8	-34.6222; 139.5311
(6)	*Maireana triptera* (Benth.) Paul G.Wilson	-22.1	-27.1764; 134.6881
(7)	*Maireana triptera*	-23.0	-31.5000; 145.8333
(8)	*Neobassia proceriflora* (F.Muell.) A.J.Scott	-22.3	-30.0242; 146.3044
(9)	*Neobassia proceriflora*	-22.7	-27.5807; 135.4615
(10)	*Neobassia proceriflora*	-23.0	-30.4309; 139.4150
(11)	*Osteocarpum acropterum* (F.Muell. & Tate) Volkens	-22.8	-28.0654; 135.7840
(12)	*Sclerolaena lanicuspis* (F.Muell.) F.Muell. ex Benth.	-22.4	-32.0903; 140.9219
(13)	*Sclerolaena tricuspis* (F.Muell.) Ulbr.	-22.5	-32.3768; 137.8234

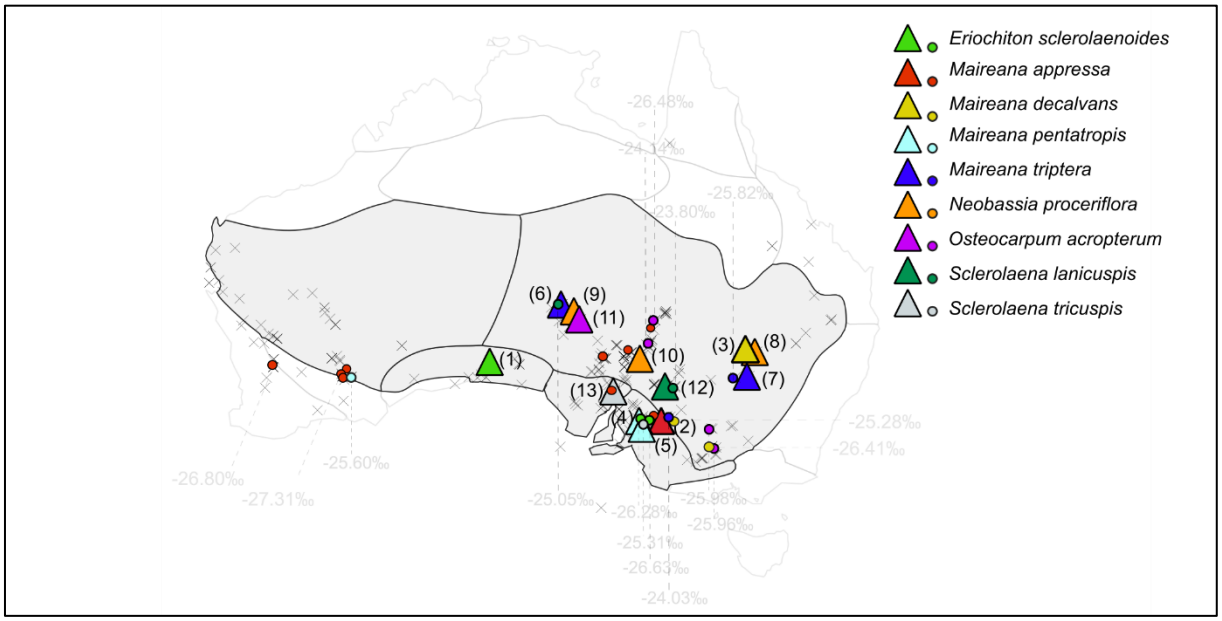


Figure 2: Map of Australia with highlighted subregions – in grey (see Figure 7 in Berasategui et al., 2024 for details of subregion definitions). The locations of accessions potentially showing facultative CAM up-regulation, based on $\delta^{13}C$ values (see Table 1 for specific values), are indicated by coloured triangles. Each triangle represents a different accession. Circles represent additional locations where the same species was sampled, but these $\delta^{13}C$ values indicate C_3 photosynthesis and some values are shown in grey. Grey crosses indicate the locations of the other 315 accessions with lower $\delta^{13}C$ values.

To date, a total of 114 Australian Camphorosmeae species (comprising 344 accessions in total) have been analysed for $\delta^{13}C$ values. Among these, 13 accessions (from nine species) exhibit values indicative of low discrimination by RuBisCO (Table 1; Figure 2). Species such as *Maireana decalvans* (-21.2‰), *M. appressa* (-22.6‰) and *M. pentatropis* (-21.9‰) show some of the highest $\delta^{13}C$ values obtained in this study. While such values could indicate limited stomatal opening and extensive internal CO_2 recycling, they could also indicate the presence of a facultative or weak CAM pathway, similar to that observed in *Clusia minor* L. (Qiu et al., 2023), or a C_2 photosynthetic pathway. To clarify these possibilities, it would be essential to investigate leaf anatomy, gas exchange, CO_2 compensation point and metabolite profiles, to determine whether the observed isotope patterns are due to internal carbon cycling rather than CAM activity. The interpretation of these new $\delta^{13}C$ values for Camphorosmeae, therefore, requires caution. The presence of a C_2 pathway is also presented as an adaptive strategy, as the C_3-C_4 intermediate pathway can increase the efficiency of carbon fixation under moderate drought and high-light conditions, without fully transitioning to CAM or C_4 mechanisms (Lundgren, 2020; Walsh et al., 2023). This intermediate photosynthetic strategy would result in $\delta^{13}C$ values that are less negative than typical C_3 values. Studies of other C_2 species, such as *Salsola divaricata* agg. ($\delta^{13}C$ values ranging from -28‰ to -21‰; Tefarikis et al., 2022), *Sedobassia sedoides* (Pall.) Freitag

& G.Kadereit (values ranging from -25.1‰ to -22.4‰; Freitag and Kadereit, 2014), *Blepharis macra* (Nees) Vollesen (values ranging from -27.1‰ to -16.4‰; Stata, 2023) and *Chenopodium* spp. (values from -32.6‰ to -26.7‰; Yorimitsu et al., 2019), illustrate the wide $\delta^{13}C$ range observed in C_3-C_4 intermediates.

In Camphorosmeae, the variation in $\delta^{13}C$ values in response to environmental factors, particularly precipitation, indicates a degree of photosynthetic flexibility and possible adaptation to local conditions, possibly through weak CAM activity. Another explanation could be the upregulation of a facultative CAM pathway under drought conditions, with these species likely relying more on C_3 photosynthesis under favourable conditions. This is supported by the observation that accessions of the same species from different locations have more negative $\delta^{13}C$ values. This suggests the possibility of a C_3-CAM or C_3-C_4 intermediate photosynthetic pathway, with these plants using the C_3 pathway under optimal conditions and switching to the CAM or C_2 pathways during periods of stress, such as drought. However, it is important to note that these $\delta^{13}C$ values discussed are accession-specific, and the same species in other locations have yielded $\delta^{13}C$ values more representative of typical C_3 plants (Figure 2). Nevertheless, the suggestion of CAM or C_2 activity in these species remains speculative. Although higher $\delta^{13}C$ values are generally associated with drought adaptation strategies, $\delta^{13}C$ values alone cannot confirm specific photosynthetic activity. Only direct physiological measurements, such as titratable acidity tests or gas exchange analyses, can provide definitive evidence of CAM or C_2 activity. Without such measurements, the hypothesis of CAM or C_2 activity in Camphorosmeae remains tentative.

Our analysis of isotope values in relation to precipitation variables further highlights the evidence for photosynthetic flexibility (Table 2). Higher precipitation levels were found to correlate with more negative isotope values. Notably, several precipitation-related variables were found to be statistically significant in the results. Annual Precipitation (Bio12) had the strongest influence, with a p-value of 0.00031 and the highest R-squared value (0.04036), indicating its substantial role in explaining isotope variability. Other significant variables included Precipitation of the Wettest Month (Bio13) ($p = 0.00191$, R-squared = 0.03006) and Precipitation of the Wettest Quarter (Bio16) ($p = 0.00066$, R-squared = 0.03612). Additionally, Precipitation of the Driest Quarter (Bio17) ($p = 0.03223$, R-squared = 0.01443), and precipitation of both the Warmest Quarter

(Bio18) and Coldest Quarter (Bio19) (p = 0.00644 and 0.00760, respectively) demonstrated moderate explanatory power with R-squared values around 0.02. In contrast, most temperature-related variables did not show significant influence on isotope values.

Table 2: Linear Regression Analysis – Influence of Bioclimatic Variables on Isotope Values. A low p-value (<0.05) indicates that the bioclimatic variable has a statistically significant effect. The multiple R-squared value represents the proportion of variance in isotope values explained by each bioclimatic variable, with higher values indicating greater explanatory power. Variables showing significance are shown in bold.

Variable	**p-value**	**Multiple R-squared**
Bio01 - Annual Mean Temperature	0.4213	0.002048
Bio02 - Mean Diurnal Range	0.09431	0.008833
Bio03 - Isothermality	0.1962	0.005282
Bio04 - Temperature Seasonality	0.8934	5.691e-05
Bio05 - Max Temperature of Warmest Month	0.5842	0.0009491
Bio06 - Min Temperature of Coldest Month	0.9817	1.659e-06
Bio07 - Temperature Annual Range	0.5871	0.0009344
Bio08 - Mean Temperature of Wettest Quarter	0.1762	0.005782
Bio09 - Mean Temperature of Driest Quarter	0.2654	0.003924
Bio10 - Mean Temperature of Warmest Quarter	0.5246	0.001283
Bio11 - Mean Temperature of Coldest Quarter	0.4369	0.001914
Bio12 - Annual Precipitation	**0.0003117**	**0.04036**
Bio13 - Precipitation of Wettest Month	**0.001914**	**0.03006**
Bio14 - Precipitation of Driest Month	0.06329	0.01087
Bio15 - Precipitation Seasonality	0.1365	0.007001
Bio16 - Precipitation of Wettest Quarter	**0.000657**	**0.03612**
Bio17 - Precipitation of Driest Quarter	**0.03223**	**0.01443**
Bio18 - Precipitation of Warmest Quarter	**0.006441**	**0.02325**
Bio19 - Precipitation of Coldest Quarter	**0.007598**	**0.02233**

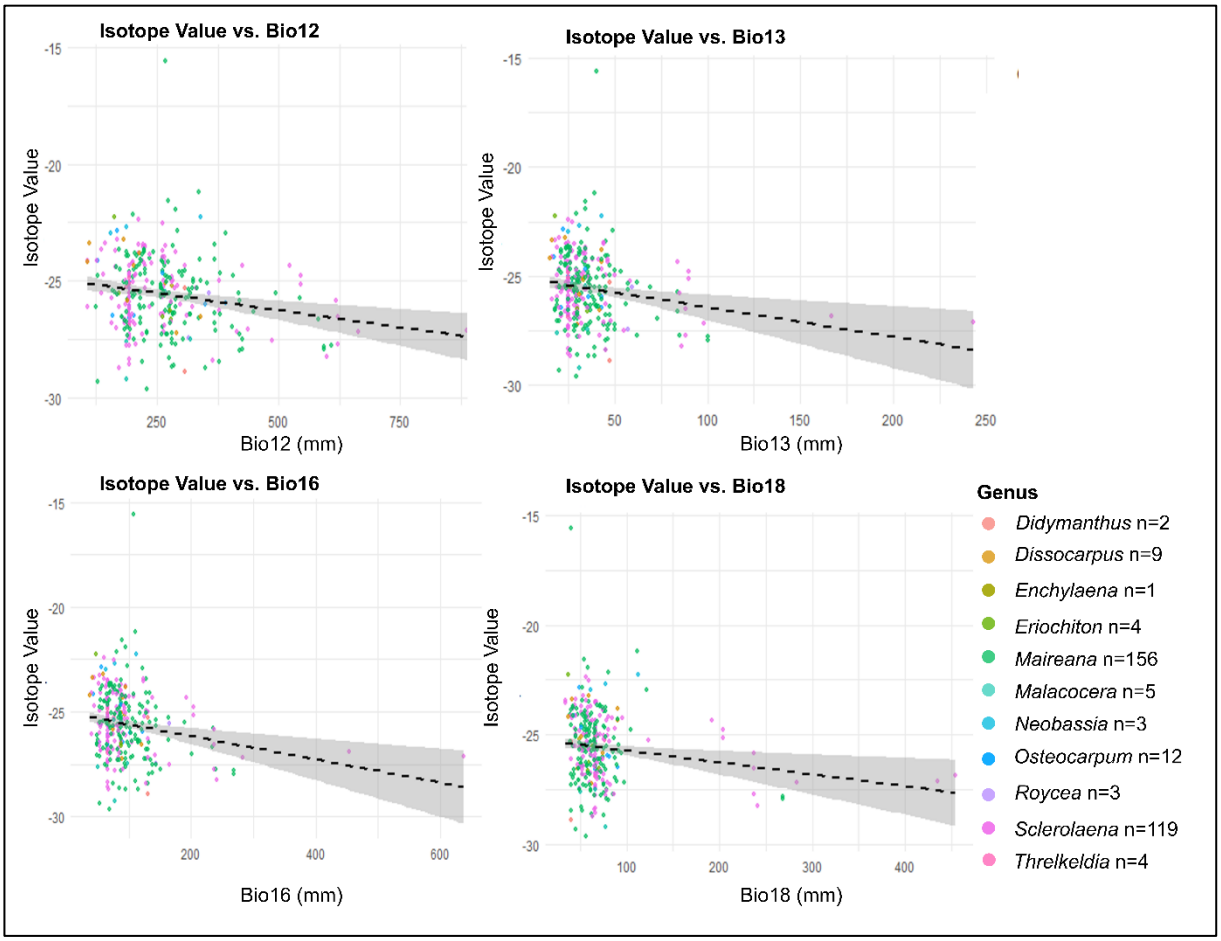


Figure 3: Scatter plots showing the relationship between isotope values (y-axis) and four bioclimatic variables (x-axis): Bio12 (Annual Precipitation), Bio13 (Precipitation of Wettest Month), Bio16 (Precipitation of Wettest Quarter), and Bio18 (Precipitation of Warmest Quarter). Each point represents an accession, with the colour indicating the genus. The black dashed line represents the linear regression trend, and the shaded area indicates the 95% confidence interval for the regression.

The *Sclerolaena* clade, including the genera *Sclerolaena* and *Maireana*, rather consistently exhibits the *Neokochia* leaf type, characterised by a central water-storage tissue, a large central vascular bundle, and numerous small peripheral vascular bundles at the boundary with a multilayered chlorenchyma (Freitag and Kadereit, 2014). These anatomical features likely reflect adaptations to arid environments, consistent with findings that higher levels of precipitation correlate with more negative isotope values. However, the low R-squared values and scatter within the data suggest that precipitation alone has modest explanatory power. In contrast, temperature related variables do not appear to have a strong influence on the observed isotope values. This hypothesis is consistent with the general observation that the drier the environment, the greater the contribution internally captured CO_2 either through CAM or C_2 photosynthesis. Thus, total precipitation appears to be more important than temperature in shaping the isotopic profiles of these plants.

Evidence from other C_3 families, such as Bromeliaceae and Orchidaceae, supports the idea that plants traditionally classified as C_3 may exhibit some degree of CAM activity (Pereira et al., 2013; Motomura et al, 2008; Messerschmid et al., 2021). For example, nocturnal increases in tissue titratable acidity and net nocturnal CO_2 uptake suggest facultative CAM activity under certain conditions (Pierce et al., 2002; Winter et al., 2015; Silvera et al., 2005). These results suggest that some species with C_3-like $\delta^{13}C$ values may still exhibit CAM activity under specific environmental conditions. In the case of Australian Camphorosmeae, further research could include testing for nocturnal CO_2 fixation or acidity fluctuations to better determine the presence of CAM, even at low levels. As CAM -if at all- would be expressed facultatively, it needs to be investigated under specific conditions, such as water deficit stress, that trigger this activity. Although CAM activity may be present in some Camphorosmeae species, C_3 photosynthesis remains the dominant pathway for carbon fixation.

In addition, the potential for C_2 photosynthesis is worth exploring in this context. *Sedobassia sedoides* (Pall.) Freitag & G.Kadereit provides a known example of a C_3-C_4 intermediate species, with isotope values ranging from -22.4‰ to -25.1‰. It is characterised by the *Sedobassia* leaf type, a C_3-C_4 intermediate structure similar to the *Neokochia* leaf type but distinguished by bundles associated with a layer of Kranz-like cells (Freitag and Kadereit, 2014). This species highlights the importance of photorespiration and cyclic electron transport in driving the evolution of C_4 photosynthesis, and its isotopic signatures underscore the variability within C_3-C_4 intermediate strategies. Considering C_2 photosynthesis as a possible mechanism in Camphorosmeae could provide new insights, especially in arid environments where adaptations to water stress are critical. Therefore, the role of total precipitation may be a more critical factor than temperature in shaping the isotopic profiles of these plants. Extending this research to include soil parameters and diversification patterns, together with isotopic data and titratable acidity tests or gas exchange analyses could shed more light on how Australian Camphorosmeae have adapted their physiological strategies in response to changing climatic and environmental stressors. This would improve our understanding of how these species manage to survive and diversify in Australia's challenging arid landscapes.

Both studies, Chapter 1 and 2, show that regions with extreme or highly variable climatic conditions, such as arid environments, are hotspots for adaptive radiation. In

these areas, species have evolved a wide variety of morphological, physiological, and biochemical traits that enable them to survive and reproduce under challenging conditions. The observed diversity of adaptations indicates that climatic extremes can accelerate evolutionary processes, leading to the rapid diversification of lineages. On a global scale, the evolution of photosynthetic traits in response to climatic conditions underscores the complex relationship between environmental factors and functional adaptation. The differentiation of C_3, C_4, and CAM and other photosynthetic pathways among plant species is a prime example of how plants have optimised their strategies for energy acquisition strategies to thrive in different climates. The ability of species to adapt their photosynthetic mechanisms to different climatic conditions has far-reaching implications for their survival and competitive success, particularly in the context of ongoing global climate change. This research deepens our understanding of the complex relationship between climate, biogeography, and plant evolution, highlighting the critical role of climate in shaping the distribution and evolution of species. It also underscores the need for careful data management and analysis in large-scale ecological and evolutionary studies. Looking forward, integrating multiple lines of evidence and improving biodiversity datasets will be essential to gain a deeper understanding of the drivers of biodiversity and to support conservation efforts in a rapidly changing world.

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Summary

The subject of this work was to explore the complex relationship between plant adaptation, climate, and biodiversity, with a focus on how plants use different photosynthetic pathways to survive in diverse environments. Photosynthesis, essential to life, varies across plant species, with adaptation to different climates playing a key role in survival.

The first study examines the global distribution and environmental preferences of C_4 eudicots compared to C_4 grasses, highlighting the independent evolution of C_4 photosynthesis in various lineages. It identifies hotspots of C_4 eudicot diversity in arid regions such as Mexico, the southern United States, and Australia. These eudicots thrive in drier, more extreme conditions, adapting through traits like succulence, salt tolerance, and the use of specific decarboxylating enzymes. The research concludes that C_4 photosynthesis in eudicots evolved as an adaptation to ancestral drought, allowing them to colonise harsher environments than C_4 grasses.

The second study focuses on the diversification of the Australian Camphorosmeae, a tribe within the Amaranthaceae family, across Australia's arid landscapes. By integrating phylogenetic and bioclimatic data, the study demonstrates that these species were pre-adapted to dry habitats, with significant diversification occurring during periods of increased aridity from the Miocene to the Pliocene.

Together, these studies highlight the complex interplay of climate in shaping the evolutionary paths of both C_3 and C_4 species. Climate is shown to be a driving force that determines species distribution and evolution. Both studies rely on large biodiversity datasets, such as those from the Global Biodiversity Information Facility and the Atlas of Living Australia. They emphasise the importance of data cleaning and validation, using automated tools and manual processes to ensure the reliability of the data before conducting analyses.

In conclusion, the work provides insights into plant adaptation to climate through different photosynthetic strategies, with implications for understanding evolutionary processes in the context of climate change. By studying both global and local scales, the research underscores how plants continuously adapt to shifting environmental conditions.

Zusammenfassung

Das Thema dieser Arbeit war die Erforschung der komplexen Beziehung zwischen Pflanzenanpassung, Klima und Biodiversität, mit einem Schwerpunkt darauf, wie Pflanzen verschiedene photosynthetische Wege nutzen, um in unterschiedlichen Umgebungen zu überleben. Die Photosynthese, die für das Leben von wesentlicher Bedeutung ist, variiert zwischen Pflanzenarten, wobei die Anpassung an verschiedene Klimabedingungen eine entscheidende Rolle für das Überleben spielt.

Die erste Studie untersucht die globale Verbreitung und Umweltpräferenzen von C_4-Eudikotylen im Vergleich zu C_4-Gräsern und hebt die unabhängige Evolution der C_4-Photosynthese in verschiedenen Abstammungslinien hervor. Sie identifiziert Hotspots der C_4-Eudikotyledonen-Diversität in trockenen Regionen wie Mexiko, dem Süden der Vereinigten Staaten und Australien. Diese Eudikotyledonen gedeihen unter trockeneren, extremeren Bedingungen und passen sich durch Merkmale wie Sukkulenz, Salztoleranz und die Nutzung spezifischer Decarboxylierungsenzyme an. Die Forschung kommt zu dem Schluss, dass die C_4-Photosynthese bei Eudikotyledonen als Anpassung an eine ursprüngliche Trockenheit entstanden ist, was ihnen ermöglichte, härtere Umgebungen als C_4-Gräser zu besiedeln.

Die zweite Studie konzentriert sich auf die Diversifizierung der australischen Camphorosmeae, einem Tribus innerhalb der Familie der Amaranthaceae, in den ariden Landschaften Australiens. Durch die Integration phylogenetischer und bioklimatischer Daten zeigt die Studie, dass diese Arten vorab an trockene Lebensräume angepasst waren und sich während der Perioden zunehmender Trockenheit vom Miozän bis zum Pliozän signifikant diversifizierten.

Beide Studien verdeutlichen zusammen das komplexe Zusammenspiel von Klima bei der Gestaltung der evolutionären Entwicklung sowohl von C_3- als auch von C_4-Arten. Das Klima erweist sich als treibende Kraft, die die Verbreitung und Evolution von Arten bestimmt. Beide Studien basieren auf großen Biodiversitätsdatensätzen, wie denen der Global Biodiversity Information Facility und dem Atlas of Living Australia. Die Arbeit betont die Bedeutung der Datenbereinigung und -validierung, wobei sowohl automatisierte Werkzeuge als auch manuelle Prozesse verwendet wurden, um die Zuverlässigkeit der Daten vor den Analysen sicherzustellen.

Abschließend liefert die Arbeit Erkenntnisse darüber, wie sich Pflanzen durch verschiedene photosynthetische Strategien an das Klima anpassen, mit Implikationen für das Verständnis evolutionärer Prozesse im Kontext des Klimawandels. Durch die Untersuchung sowohl globaler als auch lokaler Maßstäbe wird hervorgehoben, wie Pflanzen sich kontinuierlich an veränderte Umweltbedingungen anpassen.

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Curriculum Vitae