Nitrogen isotopes reveal independent origins of N$_2$-fixing symbiosis in extant cycad lineages

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Cycads are ancient seed plants (gymnosperms) that emerged by the early Permian. Although they were common understory flora and food for dinosaurs in the Mesozoic, their abundance declined markedly in the Cenozoic. Extant cycads persist in restricted populations in tropical and subtropical habitats and, with their conserved morphology, are often called "living fossils." All surviving taxa receive nitrogen from symbiotic N$_2$-fixing cyanobacteria living in modified roots, suggesting an ancestral origin of this symbiosis. However, such an ancient acquisition is discordant with the abundance of cycads in Mesozoic fossil assemblages, as modern N$_2$-fixing symbioses typically occur only in nutrient-poor habitats where advantageous for survival. Here we use foliar nitrogen isotope ratios – a proxy for N$_2$ fixation in modern plants – to probe the antiquity of the cycad-cyanobacterial symbiosis. We find that fossilized cycad leaves from two Cenozoic representatives of extant genera have nitrogen isotopic compositions consistent with microbial N$_2$ fixation. In contrast, all extinct cycad genera have nitrogen isotope ratios that are indistinguishable from coexisting non-cycad plants and generally inconsistent with microbial N$_2$ fixation, pointing to nitrogen assimilation from soils and not through symbiosis. This pattern indicates that, rather than being ancestral within cycads, N$_2$-fixing symbiosis arose independently in the lineages leading to living cycads during or after the Jurassic. The preferential survival of these lineages may therefore reflect the impacts of competition with angiosperms and Cenozoic climatic change.

49   Nitrogen is an essential nutrient and plays an important role in regulating the productivity and
50   trophic structure of ecosystems[1]. However, despite its abundance at Earth's surface as atmospheric
51   N_2, bioavailable nitrogen is scarce in many habitats. This is because only some prokaryotic
52   microbes (~15% of phyla[2]) – and no eukaryotes – possess the metabolic capacity for splitting the
53   N_2 molecule and forming bioavailable nitrogen (*i.e.*, "N_2 fixation"). Thus, almost the entire supply
54   of nitrogen to the biosphere flows through these N_2-fixing prokaryotes.

56   Most plants obtain their nitrogen from nitrate (NO_3^-), ammonium (NH_4^+), or organic-bound
57   nitrogen that is available for uptake from soil either directly via roots or through associations with
58   mycorrhizal fungi. Importantly, these nitrogen compounds all require prior activity of prokaryotes
59   that fix atmospheric N_2 into bioavailable form. In contrast, a few plant groups have forged
60   symbiotic associations with bacteria that are capable of N_2 fixation. These include the nodulating
61   legumes, actinorhizal taxa (*e.g.*, in the Betulaceae, Rosaceae), some liverworts and hornworts, and
62   cycads, among others[3,4]. The ability of these plants to obtain nitrogen directly from atmospheric
63   N_2 via their symbionts allows them to persist in low-nutrient soils,[5] or in the midst of other species
64   that might otherwise outcompete them for fixed nitrogen[6].

66   Given the ability of nitrogen to regulate biological productivity and influence ecosystem structure,
67   it follows that access to bioavailable nitrogen may have played an important role in major
68   evolutionary or ecological events in Earth's history. While these dynamics have been elucidated
69   in the context of the marine biosphere[7], we currently lack a record of the role of nitrogen in
70   terrestrial biospheric evolution. An important example of such an event may be the ecological
71   decline and subsequent rapid speciation of cycads in the Cenozoic.

73   Several traits suggest that symbiotic N_2 fixation played an important role in the evolutionary
74   ecology of the cycad lineage (Cycadales). First, modern cycads harbor cyanobacteria (*e.g., Nostoc*
75   and *Calothrix*[8]) as their symbionts, as opposed to the rhizobia hosted by legumes or *Frankia* hosted
76   by actinorhizal plants[4]. Additionally, these symbiotic cyanobacteria reside directly within unique,
77   subaerial to shallow subterranean coralloid roots[9,10] which are swollen, dichotomously branching
78   and upwards-growing, distinct from the deep root nodules characteristic of other plant-microbe
79   symbioses[4]. Further, nitrogen is transported from these endophytic cyanobacterial symbionts to
80   cycad hosts in the form of amino acids (glutamine and citrulline[11]), in contrast to ammonia (NH_3),
81   which is the nitrogen transport substrate in other symbioses[4]. Lastly, symbiosis with N_2-fixing
82   cyanobacteria is observed in all extant cycad species[9,12,13], unlike the patchy distributions in other
83   plant clades, such as the legumes[4].

85   Today's occurrence of cycads in nutrient-poor soils, where their capacity for N_2 fixation enables
86   their persistence[5], stands in stark contrast to their wide geographic, climatic and habitat distribution
87   during the Mesozoic[14]. This begs the question: is the cycad-cyanobacterial N_2-fixing symbiosis
88   ancestral, established in the lineage since at least the Triassic, when major lineages of extant cycads
89   diverged[15–17]? The universal occurrence of N_2-fixing symbioses in extant cycads implies such a
90   scenario[13]; however, the ubiquity of cycads in Mesozoic floras contradicts the observation that in
91   modern ecosystems, N_2 fixation is a costly process only undertaken when necessary for survival
92   in nitrogen-poor soils[3,4]. On the other hand, if cycads did not forge symbioses with cyanobacteria
93   (or other N_2-fixing prokaryotes) during their early evolution, did their unique mode of N_2-fixing
94   symbiosis arise independently in extant lineages? Phylogenetic analyses of fossil and modern

95   cycads suggest that, although modern species diversity was not generated until the Miocene[18,19],
96   most extant genera diverged evolutionarily in the Mesozoic[15–17]. Thus, a late Mesozoic or
97   Cenozoic appearance of N_2-fixing symbiosis in cycads would imply large-scale convergent
98   evolution across the clade. Finally, what environmental or ecological changes could have
99   promoted this strategy to be adopted by cycads, whether through a one-time acquisition in the
100   ancestor of crown-group cycads, or through later, convergent evolution across the clade?
101
102   *A nitrogen isotope record of symbiotic N_2 fixation*
103
104   We investigated the antiquity of N_2 fixation in fossil cycads by utilizing a proxy that is well-studied
105   in modern plants: foliar nitrogen isotope ratios ($^{15}N/^{14}N$, expressed in delta notation as $\delta^{15}N$). This
106   proxy is derived from the observation that the foliage of plants obtaining nitrogen from N_2-fixing
107   symbionts tends to be distinct from that of plants assimilating nitrogen from soil[20]. Specifically,
108   plants with N_2-fixing symbionts mostly have foliar $\delta^{15}N$ values near 0‰ (***Fig. 1***), which is the
109   value of atmospheric N_2, because the process of microbial N_2 fixation only slightly fractionates
110   nitrogen isotopes (typically <2‰ relative to the N_2 source[21,7]). In contrast, bioavailable nitrogen
111   in soils tends to be isotopically variable due to redox transformations occurring in the soil
112   environment, and is often fractionated relative to atmospheric N_2 by more than a few permil[20,21]
113   (*i.e.*, $\delta^{15}N \neq 0$‰; ***Fig. 1***). Thus, in a given habitat, N_2-fixing plants can be distinguished from non-
114   N_2-fixing plants if the former have foliar $\delta^{15}N$ values near 0‰, while the latter have $\delta^{15}N$ values
115   $\neq$ 0‰ (ref. [20]).
116
117   While built on sound logic, this proxy has limitations. First, isotopic fractionation during transport
118   of nitrogen from symbiont to host can lead to elevated foliar $\delta^{15}N$ values despite active N_2
119   fixation[22]. However, such a process is not observed in cycads[23], perhaps due to their transport of
120   nitrogen as amino acids rather than NH_3, which is volatile and thus prone to leakage from the plant-
121   symbiont system with an accompanying isotopic fractionation[7].
122
123   Second, the facultative nature of microbial N_2 fixation means that plants may only periodically
124   receive nitrogen from their symbionts, whereas at other times they assimilate nitrogen from soil[3].
125   Such fluctuations would cause foliar $\delta^{15}N$ values to integrate the isotopic composition of the two
126   pools (soil and atmospheric N_2), perhaps leading to non-zero $\delta^{15}N$ values despite active N_2 fixation.
127   All modern cycads studied to-date have $\delta^{15}N$ values that fall within the range generated by
128   microbial N_2 fixation[23–26], consistent with cycads receiving most of their nitrogen from their
129   symbionts. However, if aiming to identify N_2 fixation in deep time, it must be recognized that the
130   $\delta^{15}N$ proxy is specifically tracking the physiological and ecological expression of nitrogen uptake,
131   not simply the capacity for N_2 fixation (*i.e.*, phenotype rather than genotype).
132
133   Lastly, if a habitat has soil $\delta^{15}N$ values close to the atmospheric value (~0‰), then plants
134   assimilating nitrogen from those soils will have similar $\delta^{15}N$ values to plants that are receiving
135   nitrogen from N_2-fixing symbionts[27]. Such data do not preclude the possibility of active N_2
136   fixation, but rather make the $\delta^{15}N$ proxy inconclusive in those cases, as cycads and non-N_2-fixing
137   plants would have similar $\delta^{15}N$ values[23].
138
139   With these limitations in mind, we applied this proxy to fossilized cycads to search for the presence
140   of N_2 fixation through their evolutionary history. While stable carbon isotopes of carbonaceous

141 compression fossils have been investigated as a palaeoecological proxy[28], nitrogen isotope ratios
142 in fossilized foliage remain unexplored. This is largely due to the low nitrogen content of
143 carbonaceous compression fossils and recalcitrant nature of fossil organic matter, which together
144 make isotopic analysis difficult. We adapted methods designed for nitrogen recovery from
145 nitrogen-poor Precambrian rocks[7] to enable us to measure nitrogen stable isotope ratios in
146 fossilized plant foliage (**Fig. 2**; see **Methods**).

148 We analyzed a large sample set ($n = 178$) of carbonaceous compression fossils from 12 localities,
149 including both cycads and other plants from the same strata. The study sites span much of the
150 evolutionary history of cycads: *Antarcticycas* from the Early Triassic Fremouw Formation of
151 Antarctica[15], *Taeniopteris* and *Bjuvia* from the Middle Triassic flora of Thale, Germany[29],
152 *Nilssonia* from the Late Triassic flora of Lunz, Austria[30], *Pseudoctenis, Doratophyllum and*
153 *Nilssonia* from the end-Triassic to Early Jurassic Primulaelv Formation of East Greenland[31],
154 *Nilssonia* from the Middle Jurassic Cloughton Formation (Yorkshire flora) of England[32],
155 *Pseudoctenis* from the Late Cretaceous Comox Formation of Vancouver Island, British Columbia,
156 Canada[33], *Dioonopsis* from the earliest Palaeocene Denver Formation of Colorado, USA[34],
157 *Bowenia* from the Eocene Macquarie Harbor Formation of Tasmania[35], *Dioonopsis* from the
158 Eocene Chuckanut Formation of Washington, USA[36], *Zamia* from the Late Eocene Gatuncillo
159 Formation of Panama[37], *Ceratozamia* from the Miocene Most Formation of Bohemia[38], and
160 *Pseudodioon* from the Miocene Soma of Turkey[39]. We targeted sites that had been the focus of
161 previous studies, allowing us to utilize robustly-identified cycad foliage. Additionally, we
162 analyzed non-cycad foliage from the same lithological units – representing other vascular plants
163 living in the same habitat as cycads – to discern whether cycads were assimilating nitrogen from
164 soil, or whether they relied on symbiotic microbial N_2 fixation.

166 We found (**Fig. 3**) that cycad fossils from two extant genera have foliar $\delta^{15}N$ values that are similar
167 to those of modern cycads[23–26] and consistent with symbiotic N_2 fixation. These include
168 *Ceratozamia* from the Most Formation (~20 Ma) and *Zamia* from the Gatuncillo Formation (~35
169 Ma). Furthermore, at these sites a dichotomy between cycad and non-cycad $\delta^{15}N$ values suggests
170 that non-cycads were assimilating nitrogen from an isotopically-fractionated soil reservoir. At a
171 third site with fossil representatives of an extant genus (*Bowenia* from the Macquarie Harbor
172 Formation, ~50 Ma), a similarity in cycad and non-cycad $\delta^{15}N$, as well as values at the upper
173 threshold of what is observed in modern cycads, may reflect a lack of symbiosis. Importantly, at
174 the sites with isotopic differences between cycads and non-cycads, the C/N ratios of analyzed
175 cycad and non-cycad material is similar, and not elevated relative to that of modern plants,
176 suggesting that these trends are unlikely to be influenced by poor preservation (**Extended Data**
177 **Fig. 1**). We therefore conclude that while the *Bowenia* data are somewhat ambiguous, symbiotic
178 N_2 fixation is the most parsimonious explanation for the *Zamia* and *Ceratozamia* data.

180 In contrast to the representatives of extant genera, all extinct cycad genera (*Antarcticycas,*
181 *Taeniopteris, Bjuvia, Nilssonia, Pseudoctenis, Doratophyllum, Dioonopsis, Pseudodioon*) show
182 foliar $\delta^{15}N$ values that are indistinguishable from other plants growing alongside, and most (8 of
183 9 sites) are fractionated relative to atmospheric N_2 (**Fig. 3**). The Chuckanut Fm. (~50 Ma) data are
184 an exception, with cycads (*Dioonopsis*) resembling modern $\delta^{15}N$ values; however, the overlap with
185 non-cycad data makes this signal ambiguous as to N_2 fixation status. The other eight units range
186 from Early Triassic (~245 Ma) to Miocene (~20 Ma) in age. These isotopic trends are also unlikely

187 to be artifacts of poor preservation, as the cycad fossils from all sites have foliar C/N ratios that
188 fall within the range of modern plants and are similar to those of the co-occurring non-cycad
189 foliage analyzed (***Extended Data Fig. 1***). The most parsimonious interpretation of these data is
190 that cycads at these sites (which include forested riverbank and floodplain understory
191 environments[15,30,31,33,34]) predominantly assimilated nitrogen from the same soil pools as other
192 plants growing in their midst. Hence, cycads at these sites seem not to have relied on a microbial
193 N_2-fixing symbiosis.

195 We leveraged these isotopic constraints to conduct an ancestral state reconstruction (ASR) of N_2
196 fixation in cycads (see ***Methods***). The ASR strongly suggests at least two independent origins of
197 N_2 fixation within the Cycadales. These are reconstructed as occurring within the genus *Cycas* and
198 at the base of the crown group Zamiaceae (***Fig. 4a***). Dating of the tree suggests that these
199 acquisitions occurred by the Jurassic and Late Cretaceous in the Zamiaceae and Cycadaceae,
200 respectively. Both the ancestral state reconstructions and dates are highly sensitive to the
201 phylogenetic distribution of fossils with isotopic data. Within Zamiaceae, the distribution of extant
202 and recent fossil taxa, which are all N_2-fixing (or ambiguous), suggests that N_2 fixation was
203 acquired at the base of this crown group. However, there is a ~150 Myr gap between this node and
204 the oldest unambiguous isotopic evidence for N_2 fixation in the group (*Zamia nelliae*, ~35 Ma).
205 This gap includes many fossil taxa for which we were unable to acquire isotopic data, and thus it
206 is possible that additional data from these taxa would reveal a more complicated history of the
207 acquisition of N_2 fixation in the Zamiaceae. Indeed, there is evidence to suggest that *Antarcticycas*,
208 which our isotopic data constrain as lacking N_2 fixation, had affinities to taxa nested within the
209 Zamiaceae[40]. While *Antarcticycas* was not included in the recently-published phylogeny[17] used in
210 our reconstructions, its inclusion would likely yield an inference of both more numerous and more
211 recent independent origins of N_2-fixing symbiosis. Further isotopic and phylogenetic work is
212 therefore needed to resolve these details.

214 While the $\delta^{15}N$ values and ASR indicate a shift in nutrient acquisition strategy of cycads between
215 the Mesozoic and Cenozoic, some inherent limitations of the $\delta^{15}N$ proxy cloud our understanding
216 of these transitions. For instance, it is uncertain if the symbionts providing nitrogen to fossil *Zamia*
217 and *Ceratozamia* were cyanobacteria or other prokaryotic taxa. Modern coralloid root microbial
218 communities can contain several non-cyanobacterial taxa[41], and it is possible that similar variation
219 was present in fossil taxa. Nevertheless, our data from the Eocene Gatuncillo Formation mark the
220 oldest geochemical evidence of a terrestrial plant-prokaryote symbiosis in the geologic record.

222 Additionally, it is unclear whether any of these cycads facultatively used symbiotic N_2 fixation to
223 supplement uptake from soil nitrogen pools. Quantifying this balance using foliar $\delta^{15}N$ values is
224 uncertain even in modern settings[20,27], and nearly impossible in palaeoenvironments. It is possible
225 that *Bowenia* in the Macquarie Harbor Formation and *Dioonopsis* in the Chuckanut Formation
226 received some nitrogen from symbionts. But while ambiguous at those sites, our data provide a
227 robust indication of significant nitrogen supply from symbiotic microbial N_2 fixation in extant
228 cycad lineages by 35 Ma, with no clear evidence for such an association in extinct genera.

230 Overall, this dataset is consistent with the absence of fossilized coralloid roots in well-preserved
231 Permian-Cretaceous cycad fossils (*e.g., Antarcticycas*[42]). The implication of this analysis is that
232 either the unique coralloid roots evolved convergently in extant cycad lineages or, perhaps more

likely, that predecessors of coralloid roots, the genetic machinery to effect N_2 fixation via cyanobacterial symbiosis, or both, were present in most or all cycads but served a different purpose, or were at least not widely used for N_2 fixation[10]. To test these hypotheses, the poorly known genetic mechanisms involved in the cycad-cyanobacterial symbiosis[10] should be further explored.

Drivers of acquisition of N_2 fixation

What drove this stark shift in cycad ecology? By far the most conspicuous change in terrestrial flora during the Mesozoic and into the Cenozoic was the rise of angiosperms to ecological dominance[14]. From their origins in or before the earliest Cretaceous[43], angiosperms came to dominate most biomes in terms of diversity and biomass by the Late Cretaceous[44]. Alongside this rise of angiosperms was a pronounced decline in the abundance of gymnosperms, and of cycads in particular[44], in fossil assemblages from the Cretaceous to present (***Fig. 4b,c***). While multiple factors are likely to have influenced this re-structuring of terrestrial ecosystems[45] – including extrinsic drivers such as terrestrial biomass deposition and associated changes in weathering rates[46], the K-Pg bolide impact[47], or the Cenozoic trend of declining CO_2 levels and global cooling[48] – direct competition between angiosperms and gymnosperms is often invoked as playing an important mechanistic role[45,48].
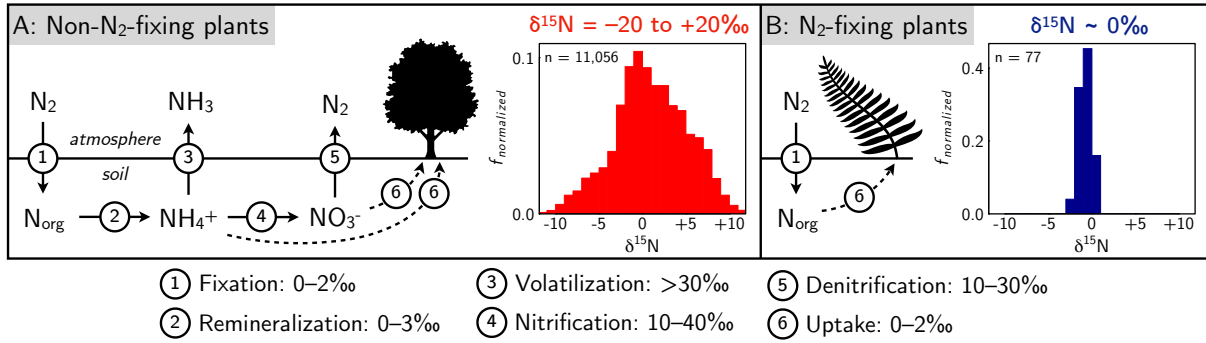
In modern habitats, angiosperms commonly outcompete gymnosperms for access to essential nutrients, including nitrogen[6,49]. It has traditionally been assumed that this competition is the reason why living cycads employ the costly strategy of forging a symbiosis with N_2-fixing prokaryotes[3,6,24]. In contrast, early Mesozoic cycads were widespread, ecologically diverse, and abundant in a broad range of habitats[50,51], indicating that they were not always limited by such competition for nutrients. Our data support the idea of a switch in ecological strategy, by implying that two or more disparate cycad lineages independently became reliant on symbioses with N_2-fixing prokaryotes between the late Mesozoic and early Cenozoic. This may have been a response to the expansion of angiosperms into habitats where cycads previously thrived. While many characteristic Mesozoic plant lineages went extinct during the Cenozoic[14], the cycads survived, albeit in limited numbers. With the isotopic signature of N_2 fixation only observed in fossil representatives of extant genera (***Fig. 3***), our data might therefore suggest that the capacity of cycads to harbor N_2-fixing symbionts played a critical role in their survival through Cenozoic time.

Conclusion

Cycad evolutionary history is marked by the loss of large swaths of diversity since the Jurassic[17,19]. Our results offer a mechanistic explanation for the preferential survival of certain lineages, namely those in which symbiotic N_2 fixation had been acquired. In contrast, lineages lacking this ecological strategy perished, perhaps as a result of competition with angiosperms and Cenozoic climate change. We therefore propose that the universal occurrence of N_2 fixation across extant cycads reflects a selective pressure for survival in an angiosperm-dominated world, thus reconciling the early history of cycads as abundant understory flora with their modern niche as rare, N_2-fixing plants.

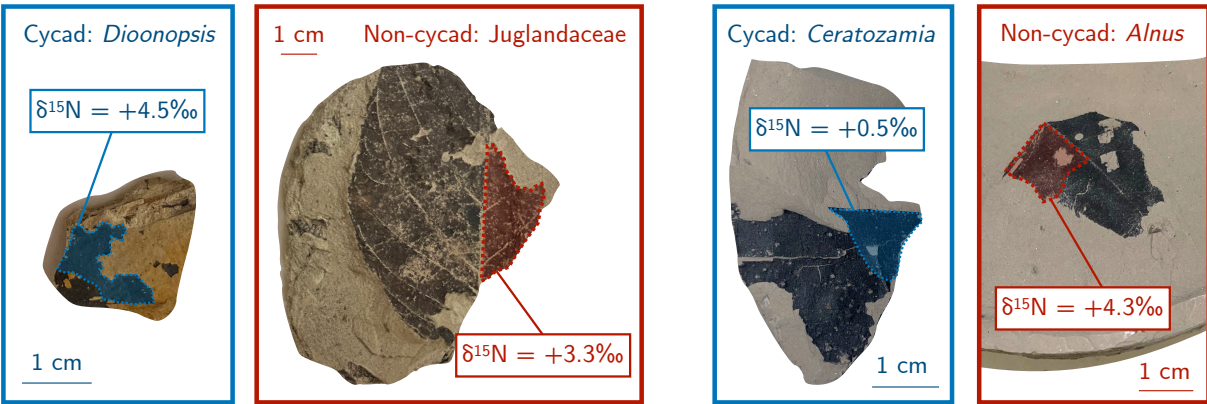A: Non-N₂-fixing plants

$\delta^{15}N = -20$ to $+20‰$

N₂   NH₃   N₂

atmosphere

soil

N_org → ② → NH₄⁺ → ④ → NO₃⁻

n = 11,056

B: N₂-fixing plants

$\delta^{15}N \sim 0‰$

N₂

N_org

n = 77

① Fixation: 0–2‰   ③ Volatilization: >30‰   ⑤ Denitrification: 10–30‰

② Remineralization: 0–3‰   ④ Nitrification: 10–40‰   ⑥ Uptake: 0–2‰

Figure 1. Nitrogen isotope fractionation in the terrestrial nitrogen cycle. Isotopic fractionations compiled from ref. [7], non-N₂-fixer foliar $\delta^{15}N$ data from TRY database (see *Methods*), N₂-fixer data from modern cycads[23]. Fluxes and plants are not drawn to scale.

Figure 2. Nitrogen isotopic analyses of select fossil samples. Cycad and non-cycad foliage from the Denver Formation (Castle Rock flora) and Most Formation are depicted. Dashed lines and shading denote portions of fossils that were sampled for isotopic analysis.

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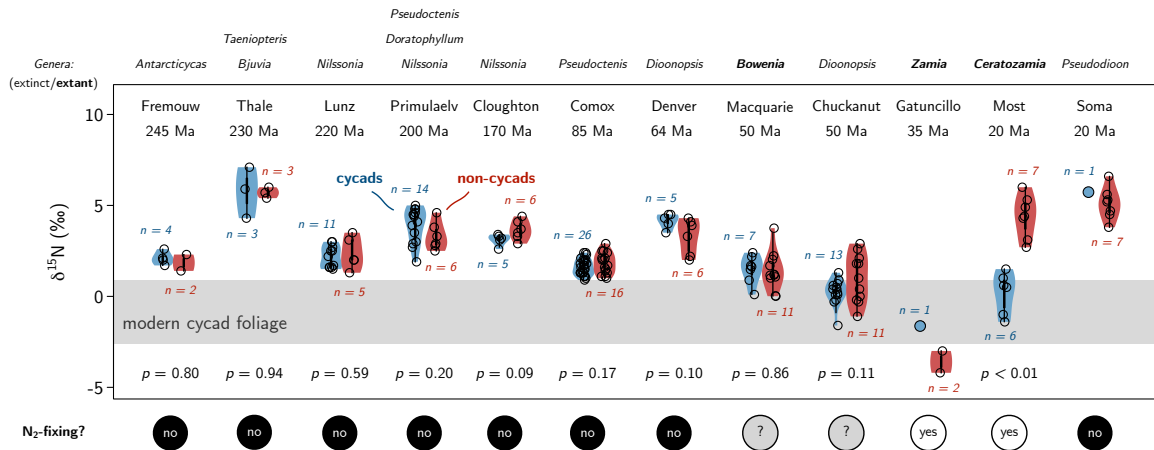
Figure 3. Foliar nitrogen isotope data from cycad (blue) and non-cycad (red) fossils across the last ~250 million years. Fossil-bearing units listed with approximate ages. Extinct cycad genera have foliar $\delta^{15}N$ values that are indistinguishable from other fossil plants in the same units and typically elevated relative to atmospheric nitrogen. These observations are most parsimoniously interpreted as reflecting nitrogen uptake from the same soil nitrogen pools by cycads and co-existing non-cycads. In contrast, Cenozoic fossil representatives of two extant genera (*Zamia, Ceratozamia*) show foliar $\delta^{15}N$ patterns that are consistent with symbiotic N_2 fixation (near-zero in cycads, fractionated in other plants). This suggests that cycads did not rely on symbiotic N_2 fixation until late in their evolutionary history, and that this metabolic capacity may have played an important role in their survival and speciation through the Cenozoic.
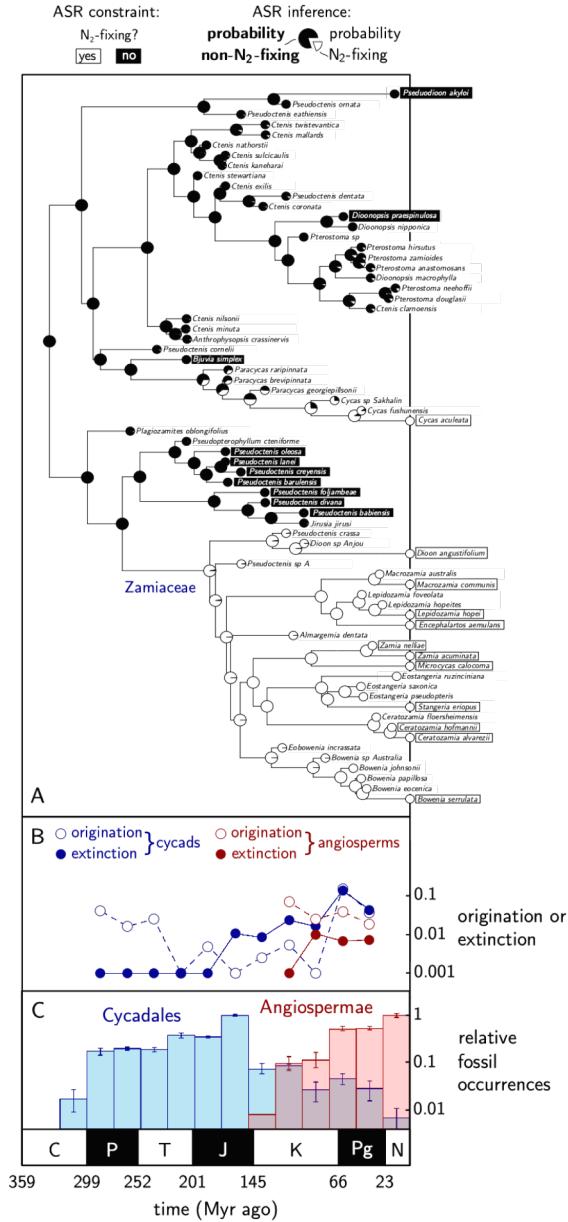
Figure 4. Evolutionary ecology of cycads. (A) Ancestral state reconstruction (ASR) of N_2-fixing capacity, (B) origination and extinction rates of Cycadales and Angiosperms, (C) relative abundance of cycad and angiosperm fossils through time. See ***Methods*** for details of all calculations. In (A), white shading in pie charts denotes probability of N_2 fixation (100% in all modern taxa; inferred from ASR in fossil taxa); black shading in pie charts marks lack of N_2 fixation. Taxa highlighted in grey are constrained as non-N_2-fixing via isotopic data; taxa highlighted in green show isotopic evidence of N_2 fixation. Fossil flora trends in (B) and (C) are from the Paleobiology Database (https://paleobiodb.org/#/). Relative fossil occurrence rates are plotted as bootstrap resampled means. The ASR implies a lack of N_2 fixation in fossil taxa prior to ~200 Ma, indicating that reliance on N_2 fixation is not ancestral to cycads, and requiring that N_2 fixation emerged more than once in the clade. The emergence of this trait coincides roughly with an increase in extinction rate in the Cycadales, as well as a decline in cycad abundance relative to angiosperms.

Acknowledgments

Author Contributions

MAK, EES, CAES, and RB designed the study. CAES, VMA, BE, RSH, KRJ, JK, JCM, IMM, MS, and VV provided fossil specimens. MAK and EES conducted the isotopic measurements. MAK, EES, CAES, and RB analyzed the data. WHB conducted the ancestral state reconstruction, with input from MAK, EES, CAES, and RB. MAK wrote the manuscript, with input from all authors.

Materials & Correspondence

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Competing Interests

The authors declare no competing interests.

Methods

Recovery of organic leaf residues

Organic residues were removed from the background rock matrix using one of two methods. In the majority of cases, the carbonaceous film was separated from the matrix using a stainless steel razor blade. Blades were wiped with methanol between all samples and air-dried to avoid cross-contamination. In some samples that were not amenable to handling with a razor blade, an ultra-fine drill was used to abrade a thin layer of organic matter from the fossil.

Under both protocols, the same method was applied to a portion of the rock matrix adjacent to the fossil in which no carbonaceous fossil remains were evident. This allowed a quantification of the background carbon and nitrogen content that could potentially be contaminating the signal obtained from the recovered fossil material. The concentration of nitrogen (TN, total nitrogen) in the isolated fossil material was consistently higher than in the background matrix, on average by a factor of 9.1. Similarly, carbon concentrations (TC, total carbon) were higher in isolated fossil material than the matrix by a factor of 20.3 on average. Furthermore, the isotopic composition of nitrogen does not co-vary with the difference in TN (***Extended Data Fig. 2***). These observations lead us to conclude that the methods used for isolating fossilized organic matter were indeed capturing the signature of the discrete fossilized specimens and not disseminated organic matter in the matrix or nitrogen bound in clay minerals. If anything, it is more likely that the nitrogen in the adjacent sediment matrix was contributed in part by degradation of the leaf specimens, as has been observed in studies of animal degradation in soil environments[52].

Isotopic analysis

The concentration and isotopic composition of carbon and nitrogen in powders isolated from fossil and matrix material were measured on a Costech™ ECS 4010 Elemental Analyzer coupled to a Thermo Finnigan™ MAT253 continuous flow isotope ratio mass spectrometer in IsoLab at the University of Washington following published protocols[23]. Combustion was carried out at 1000°C with a 20 mL pulse of O_2. The resulting gases were then passed through a reduced copper column held at 700°C to reduce NO_x species to N_2 and scrub excess O_2 from the gas stream. A magnesium perchlorate trap was then used to remove water from the gas stream, after which the gases were separated via gas chromatography and fed into the mass spectrometer via a Thermo Finnigan Conflo III.

Raw isotopic data were corrected using a two-point calibration[53] with three in-house standards: two glutamic acids (GA1, TC = 40.8%, TN = 9.5%, $\delta^{13}C$ = -28.3‰, $\delta^{15}N$ = -4.6‰; GA2, TC = 40.8%, TN = 9.5, $\delta^{13}C$ = -13.7‰, $\delta^{15}N$ = -5.7‰) and dried salmon (SA, TC = 45.7%, TN = 11.8%, $\delta^{13}C$ = -21.3‰, $\delta^{15}N$ = +11.3‰), which are calibrated against international reference materials USGS-40 and USGS-41. Each in-house standard was analyzed four times per analytical sequence. Analytical blanks resulting from combustion were measured and subtracted from nitrogen data; analytical blanks were below detection limits for carbon.

398   All nitrogen isotopic data are reported in delta notation relative to air. The external reproducibility
399   (1σ) of isotopic measurements, as determined by replicate analyses of in-house standard UW-
400   McRae ($\delta^{15}N$ = +5.6‰), was ±0.2‰. Average precision (relative error) of concentration
401   measurements was ±1.7% for TN and ±1.4% for TC.
402
403

404   *Statistical analyses of isotopic data*
405

406   For all sites with multiple cycad and non-cycad specimens, we statistically compared their isotopic
407   compositions. We first used the Shapiro-Wilk normality test to determine whether data adhered to
408   a normal distribution. In cases when the null hypothesis was rejected, we proceeded with a non-
409   parametric (Wilcoxon) test; in the rest of cases, t tests were used to compare the means of
410   populations. The resulting *p* values are presented in ***Fig. 3***.
411
412

413   *Ancestral state reconstruction*
414

415   To evaluate number of acquisitions of N_2 fixation within the Cycadales, as well as their timing, we
416   ran ancestral state reconstructions employing the recently published, time calibrated phylogeny of
417   Coiro et al.[17]. All taxa included in the phylogeny were coded for the presence or absence of N_2
418   fixation. Where no data were available, taxa were assumed to have equal probability of possessing
419   either trait, and their status as N_2-fixers was inferred by the model. Ancestral states were estimated
420   using stochastic character mapping with 1000 simulations under an "all rates different" model of
421   character evolution, which allows for the gain and loss of N_2 fixation to occur at different rates.
422   Akaike information criterion (AIC) supported this over a simpler "equal rates" model. Analysis
423   was completed using the phytools[54] package (v. 1.0-1) in the R software environment[55] (v. 4.1.2).
424

425   Members of seven fossil genera for which we obtained isotope data were included in Coiro et
426   al[17].'s phylogeny. Three of our sampled fossils (*Ceratozamia hofmannii*, *Pseudodioon akyoli*, and
427   *Zamia nelliae*) were identified to the species level, while the remainder could only be identified to
428   the genus level. Isotope data for the latter were coded as follows. *Bjuvia simplex* was coded as
429   non-N_2-fixing, although fossils were only identified to genus, this is the only included member of
430   *Bjuvia* in the phylogeny. The N_2 fixation states of all fossil *Bowenia* were treated as uncertain due
431   to the equivocal isotope data for this taxon. Isotopic data from *Dioonopsis* fossils show clear
432   absence of N_2 fixation in specimens of the Denver Fm., while more recent Chuckanut fossils are
433   ambiguous. As such, we coded *Dioonopsis praespinulosa*, the closest geographic and temporal
434   match for the Denver Fm. fossils, as lacking N_2 fixation and left the remaining species ambiguous.
435   Coiro et al.[17] demonstrated that the genus *Pseudoctenis* is highly polyphyletic. For our analyses,
436   we treated all species of *Pseudoctenis* that fell within the clade that included the majority of
437   Laurasian species as non-N_2-fixing. This excluded the Gondwanan fossils assigned to this genus,
438   the early Late Triassic *P. cornelii*, and two Late Jurassic taxa (*Pseudoctenis eathiensis*,
439   *Pseudoctenis sp A*). While not monophyletic, the remaining taxa are closely related (***Fig. 4a***), and
440   their geographic and temporal distribution suggests this is likely the clade sampled in our isotope
441   dataset (see ***Geologic Context***). Attributing our isotope data to specific species within this clade is
442   not feasible, so we therefore make the assumption that all "*Pseudoctenis*" within this clade lacked

443   N_2 fixation. The strong signal rejecting N_2 fixation in the two sampled members, separated by >
444   100 Ma suggests this assumption may be justified.
445
446

447   *Quantitative analyses of the fossil record*

448

449   Temporal trends in the abundance and diversity of cycads and angiosperms were assessed using
450   data compiled in the Paleobiology Database. Data were downloaded via the paleobioDB package[56]
451   (v 0.7.0) in the R software environment on March 30, 2023 by separately querying all fossil
452   occurrences of Cycadales and Angiospermae. As the fossil record of cycad data is complicated by
453   misdiagnoses (typically *Bennettitales*), the downloaded data were filtered to only include taxa of
454   unambiguous cycad affinity. This reduced the number of fossil genera from 51 to 34
455   (**Supplementary Information Table S2**). We further filtered the occurrence data to only include
456   fossil taxa that represent foliar features (*i.e.,* excluding taxa ascribed to other organs such as stems
457   or cones). This further reduced the number of fossil genera from 34 to 24 (**Supplementary
458   Information Table S2**), though we note this filtering step did not substantively change the
459   observed trends. The final, filtered dataset contained 2,285 unique cycad fossil occurrences. The
460   angiosperm dataset contained 36,206 fossil occurrences.

461

462   Three types of information were gleaned from these fossil occurrence compilations: 1) abundance,
463   2) diversity, and 3) origination/extinction rate. For each of these calculations, data were divided
464   into 25 Myr bins to allow comparison of intervals of equal length. Fossil occurrence ages were
465   taken as the midpoint of the maximum and minimum ages in the Paleobiology Database. While
466   some ages have large (>10 Myr) uncertainty, using only maximum or minimum ages does not
467   significantly impact the observed trends.

468

469   Fossil abundance trends were assessed in two ways. First, the raw abundance was calculated as
470   the number of fossil occurrences (Cycadales or Angiospermae) per time interval. Second, the
471   cycad and angiosperm datasets were bootstrap resampled with replacement (1000 iterations, each
472   time sampling up to 1000 occurrences), and the mean number of occurrences per interval was
473   reported with a 95% confidence interval (2σ). All occurrence data are plotted as relative abundance
474   (*i.e.*, scaled to the highest interval) to simplify comparison across clades of different size
475   (Cycadales vs. Angiospermae).

476

477   Fossil diversity trends were also assessed via multiple methods. First, raw genus richness was
478   calculated as the number of genera (within Cycadales or Angiospermae) observed per time
479   interval. In this and subsequent genus richness calculations, rangethrough genus richness was
480   calculated by adding to each interval genera that are observed in time bins both before and after.
481   Second, the cycad and angiosperm datasets were bootstrap resampled as above, and the mean
482   genus richness per interval was reported with a 95% confidence interval (2σ). Third, the bootstrap
483   resampled datasets were used to estimate "true" genus richness using two extrapolation methods:
484   TRiPS[57] and Chao1[58]. These approaches aim to provide more robust estimates of "true" richness
485   by leveraging the abundance of rare taxa and making statistical assumptions about the distribution
486   of occurrence data. Both methods have merits and shortcomings[59]. Here we aimed to compare
487   these approaches and raw genus richness estimates to determine whether coherent trends in cycad
488   and angiosperm diversity can be identified. All inferred genus richness estimates are plotted as

489 relative genus richness (*i.e.*, scaled to the highest interval) to simplify comparison across clades of
490 different size (Cycadales vs. Angiospermae). We find that all approaches broadly agree that cycad
491 genus richness shows no clear secular trend, whereas angiosperm genus richness increases in
492 tandem with abundance from the Cretaceous to present (***Extended Data Figure S3***).
493
494 Lastly, the cycad and angiosperm occurrence data were used to estimate origination and extinction
495 rates following the approach of Foote[60]. Rates were calculated for each time bin besides the first
496 and last, where available data are insufficient.
497
498
499 *TRY database*
500
501 Nitrogen isotope data from foliage of non-N_2-fixing plants was downloaded from the TRY
502 database[61], resulting in 11,056 isotopic data that are plotted in ***Fig. 1a***.
503
504
505 *Geologic context*
506
507 *Soma Formation*
508
509 The Soma Formation in western Turkey is a sedimentary unit that overlies clastic sediments and
510 carbonates of the Izmir-Ankara zone and conformably underlies the Denis Formation[62]. The Soma
511 Formation is comprised of basal conglomerates overlain by sandstones, mudstones, marls and coal
512 beds. Nonmarine deposition has been interpreted for the lignite-bearing horizons and intervening
513 marls that contain leaf fossils[63]. The flora is dominated by Fagacae, with a notable presence of
514 Lauraceae and the gymnosperms *Pinus* and *Glytpostrobus*, among other taxa[39,64]. The unit has
515 been assigned an early to middle Miocene age on the basis of palynological observations[64–66] and
516 radiometric ages of associated volcanic rocks[67–69].
517
518 Carbonaceous cycad and non-cycad fossils from the Soma Formation were obtained from the
519 Hungarian Natural History Museum in Budapest. The cycad material was identified as
520 *Pseudodioon akyoli*[39] on the basis of cuticular and macroscopic morphological characteristics.
521 Non-cycad specimens analyzed include representatives of Fagaceae, Cupressaceae, Rosaceae, and
522 *Daphnogene*.
523
524 *Most Formation*
525
526 The Most Formation is a sedimentary unit in northern Czech Republic that overlies Proterozoic
527 gnessic basement and conformably underlies the Strezov Formation. Collectively the Most and
528 Strezov Formations comprise the sedimentary fill of the Most Basin, which contains ~0.5 km of
529 siliciclastic material thought to have been deposited in fluvial, deltaic, and lacustrine
530 environments[70]. Coal-bearing horizons in the Most Formation have been extensively explored and
531 contain rich fossil plant assemblages[71,72]. The timing of deposition is constrained to be Burdigalian
532 (early Miocene, 16-21 Ma) on the basis of mammalian biostratigraphy[73] and palaeofloristic
533 correlation[71,72], as well as palaeomagnetostratigraphy and cyclostratigraphy[70].
534

Carbonaceous cycad and non-cycad fossils from the Most Formation were obtained from the National Museum in Prague, Czech Republic. Cycad material was previously identified as *Ceratozamia hofmannii* on the basis of stomatal and macroscopic morphology[38]. The non-cycad material analyzed here includes several angiosperms identified to the genus or species level. All analyzed specimens were recovered from the Lom Member (~16.5 Ma[74]) of the Most Formation in drillcore material from Osek, Czech Republic.

Gatuncillo Formation

The Gatuncillo Formation comprises several hundred meters of terrestrial and marine sedimentary deposits that outcrop across Panama[75,76]. The unit rests atop Cretaceous basement and deposition is estimated as late Eocene to early Oligocene on the basis of U-Pb detrital zircon geochronology[77], Sr isotope chemostratigraphy[78], and foraminiferal biostratigraphy[79–81]. The depositional environment is thought to have been a series of volcanic islands surrounded by mangrove swamps and limestone reefs[82], and represents an interval of localized terrestrial sedimentation prior to the complete closing of the Isthmus of Panama in the Pliocene[83].

A cycad specimen identified as *Zamia nelliae* was collected from a carbonaceous sandstone bed in the Gatuncillo Formation near Buena Vista[37]. The bed containing the cycad specimen has since been destroyed by quarrying activity, but contained various other plant taxa and overlay a marine mudstone succession estimated as Bartonian-Priabonian and Priabonian-Rupelian on the basis of nannoplankton and foraminiferal biostratigraphy, respectively[37]. Conformable deposition of the cycad-bearing unit atop the marine mudstone horizon suggests that the age of the cycad specimen is Priabonian (37.7-33.9 Ma) or Rupelian (33.9-27.8 Ma). Fragments of *Zamia nelliae* and of adjacent non-cycad carbonaceous material were provided by the Florida Museum of Natural History.

Chuckanut Formation

The Chuckanut Formation is a several-km thick sedimentary package[84] in northwestern Washington State, USA. The unit was first named by McLellan[85], with type sections later characterized by Glover[86] and Weaver[87], who identified deposition of the unit in a coastal plain setting. The present view is that deposition occurred in several small fault-bounded basins prior to the uplift of the Cascade Range[88–90], though some contend that deposition occurred as large, sheet-like deposits spanning the Formation[91] that were later dissected by strike-slip faulting[92]. In either case, deposition occurred in a coastal plain prior to mid-Tertiary Cascade uplift.

Johnson[84] divided the Chuckanut stratigraphy into two periods of deposition. The earlier period is represented by the lower units, the Bellingham Bay (~3.3 km thick) and Slide (~1.9 km thick) Members, which include alternating beds and arkose sandstone and siltstone with minor conglomerate and coal[84]. The upper units identified by Johnson[84] were the Padden Member (~3.0 km thick) and the minor Governor's Point, Maple Falls, Warnick and Bald Mountain Members, which are comprised of massive arkose sandstone and conglomerate with alternating mudstone and siltstone. Palaeontological data suggest a significant climatic change between deposition of the lower and upper Chuckanut Formation, from subtropical flora in the lower units to warm temperate in the upper units[92,93].

581
582   The Chuckanut Formation originally was mistakenly correlated with the Upper Cretaceous
583   Nanaimo Group[85,93,94], but more recent work has demonstrated an Eocene age for the former unit.
584   Palynological data are consistent with late Paleocene to early Oligocene deposition[95]. This is
585   corroborated by geochronological work, including a 49.9 ± 1.2 Ma zircon fission track age from a
586   tuff bed of the lower Chuckanut[84], a 44.5 ± 4.5 Ma zircon fission track age from a bentonite bed
587   in the upper Chuckanut[90], K-Ar ages of 40.5 ± 5 Ma and 36.8 ± 9.2 Ma on volcanic rocks overlying
588   the Chuckanut[96], and zircon fission track ages of 52.7 ± 2.5 Ma[97] and 52.5 ± 4.8 Ma[90] from a
589   rhyolite flow and tuff, respectively in the lower Chuckanut. We therefore follow these studies in
590   inferring a ~50 Ma age for deposition of the lower Chuckanut Formation, noting that uncertainty
591   on this age estimate does not influence the conclusions of this study.
592
593   Carbonaceous compression fossils from the Chuckanut Formation were obtained from the Burke
594   Museum of Natural History and Culture. Cycad specimens utilized in this study were previously[36]
595   referred to the extant genus *Dioon*. However, our closer analysis of the specimens here reveals a
596   more likely assignment of *Dioonopsis*, on the basis of anastomosing or dichotomizing veins,
597   insertion of the leaflets onto the rachis, and the overall shape of the leaflets. Non-cycads were
598   identified to varying taxonomic levels as allowed by specimen morphology. All specimens were
599   collected from the lower Chuckanut Formation in the Bellingham Bay Member.
600
601   *Macquarie Harbor Formation*
602
603   The Macquarie Harbor Formation in southwest Tasmania comprises a thick sequence of
604   fossiliferous mudstones filling a rift basin[98]. The flora is thought to represent estuarine vegetation
605   growing in a warm and humid climate[99,100]. Mangroves[101], seed ferns[102], rainforest
606   angiosperms[103,104], and conifers (including Araucariaceae, Cupressaceae, and
607   Podocarpaceae[105,106]) are present in the assemblage. The age of the sediments is constrained to 53-
608   50 Ma on the basis of palynological and marine biostratigraphic records[107].
609
610   Carbonaceous compression fossils were obtained from the Lowana Road and Regatta Point sites
611   within the Macquarie Harbor Formation via University of Adelaide. The cycad material studied
612   here was identified as a member of the extant genus *Bowenia* on the basis of stomatal and
613   macroscopic morphology[35]. The non-cycad material analyzed includes several angiosperms and
614   one conifer (*Araucarioides*).
615
616   *Castle Rock flora, Denver Formation*
617
618   The Castle Rock flora is an exceptionally diverse fossilized forest floor deposit first discovered in
619   1994[34,108,109]. The fossil site occurs within the D1 sequence of the upper Denver Formation in the
620   Denver Basin in Colorado, USA[110]. The depositional age of the site is constrained to 63.8 ± 0.1
621   Ma based on five zircon U-Pb analyses from a stratigraphically proximal ash layer[111].
622
623   In contrast to other Paleogene fossiliferous deposits, the Castle Rock flora evidently records
624   autochthonous or parautochthonous burial of a forest floor, inferred to have occurred during
625   multiple flooding events[108]. In addition to this unusual depositional style, the flora is markedly
626   diverse, and exhibits leaf morphology and plant diversity indicative of modern rainforests[34,108],

627 indicating that in at least some habitats, terrestrial biodiversity had rebounded substantially within
628 ~2 Myr of the Cretaceous-Paleogene mass extinction[34]. The flora is dominated by angiosperms
629 (94% dicots[108]), and an exceptionally well-preserved cycad individual and isolated leaves were
630 also recovered[34,112].

632 Carbonaceous compression fossils from the Castle Rock flora were obtained from the Denver
633 Museum of Nature & Science. Cycad specimens utilized in this study were previously
634 characterized[112] to the genus level (*Dioonopsis*) and may derive from a single individual; non-
635 cycads were identified to at least the family level (Lauraceae, Platanaceae, Juglandaceae). All
636 specimens were collected from the Castle Rock site excavated by the Denver Museum of Nature
637 & Science.

639 *Comox Formation, Nanaimo Group*

641 The Comox Formation is the lowermost unit of the Upper Cretaceous Nanaimo Group on
642 Vancouver Island, British Columbia. The Nanaimo Group is comprised of several km of
643 nonmarine, shallow marine and deep marine facies that are thought to have been deposited in a
644 forearc or peripheral foreland basin[113–115] known as the Georgia Basin, which to the south and east
645 contains Tertiary deposits including the Eocene Chuckanut Formation described above. The age
646 of the Comox Formation is estimated as Santonian (ca. 86-83 Ma) based on marine
647 biostratigraphy[113] and a U-Pb zircon age[116] of 82.5 ± 1 Ma (earliest Campanian) from a tuff in the
648 Dunsmuir Member in the upper Comox Formation.

650 Cycads were identified in the Comox Formation near the town of Nanaimo in the late 19th
651 century[117] and have been studied ever since[33,36]. In a recent study, Jonsson and Hebda[33] noted that
652 while angiosperms dominate the Nanaimo Group flora overall, cycads in the Saanich Member of
653 the Comox Formation were disproportionately found in gymnosperm-rich deposits that were
654 floristically distinct from other angiosperm-dominated sites, the latter of which tend to be more
655 species-rich. This overall pattern is consistent with the inference of widespread ecological
656 dominance of angiosperms by the Santonian stage[44], and perhaps suggests that the cycad-rich sites
657 represent refugia that mimic earlier Mesozoic ecological conditions.

659 Carbonaceous compression fossils from the Comox Formation were obtained from the Royal BC
660 Museum. Cycad specimens utilized in this study were previously characterized to the genus level
661 (*Pseudoctenis*); non-cycads were identified to varying taxonomic levels as allowed by specimen
662 morphology. Specimens were collected from three sites within the Comox Formation: BR-1 and
663 BR-2 on the Saanich Peninsula, and the #8 Mine near Courtenay.

665 *Yorkshire flora, Cloughton Formation*

667 The Yorkshire flora of England is a long-studied palaeobotanical archive[32,118–120]. Mesozoic
668 deposition in the Cleveland Basin created a thick stratigraphic package, of which the Cloughton
669 Formation in the Ravenscar Group is largest unit[121]. The Cloughton Formation comprises the
670 Sycarham, Lebberston and Gristhorpe Members, with the Sycarham and Gristhorpe being non-
671 marine and fossiliferous[119,121,122]. Biostratigraphic constraints place Cloughton Formation
672 deposition in the Lower Bajocian stage[119]. Early work by Harris[32] noted the presence of cycads in

the Cloughton Formation, and to-date more than 300 taxa have been identified, including bennettitaleans, sphenophytes, and an abundance of ferns[119,120,122].

Carbonaceous cycad and non-cycad specimens from the Yorkshire flora were obtained from the Yale Peabody Museum. All cycad (*Nilssonia*) and non-cycad (*Cladophlebis, Phlebopteris, Sagenopteris, Nilssoniopteris*) specimens were identified to the genus level.

Primulaelv Formation, Kap Stewart Group

The Kap Stewart Group in eastern Greenland is comprised of sedimentary rocks spanning the Triassic–Jurassic boundary. The Group is divided into the Innakajik, Primulaelv, and Rhaetelv Formations, which transition from conglomerate and sandstone dominated alluvial plain deposition, to mixed sandstone-shale deposition in a delta plain, to mixed sandstone and shale deposition in a lacustrine setting, respectively[123–125]. The age of the Kap Stewart Group was initially constrained biostratigraphically by Harris[126], who noted a Rhaetian (209-201 Ma) flora characterized by *Lepidopteris* that gave way to a Hettangian (201-199 Ma) assemblage characterized by *Thaumopteris*. An >80% species turnover between the assemblages led Harris[126] to locate the Triassic-Jurassic boundary (~201 Ma), reflecting the mass extinction of terrestrial flora[125].

The flora of the Kap Stewart Group is gymnosperm-dominated, containing an abundance of cycads, ginkgos, conifers, and ferns[31,127,128], similar to other Triassic assemblages in Europe (*e.g.*, the Lunz and Thale flora described below). For this study, carbonaceous compression fossils from the Primulaelv Formation at Astartekløft were obtained from the Field Museum. The studied cycad specimens were previously characterized[31] to the genus level (*Nilssonia, Doratophyllum, Pseudoctenis*); as were non-cycads (all *Bennettitales*: *Pterophyllum, Cycadolepis* and *Anomozamites*), on the basis of stomatal morphology.

Lunz Formation

The successions of the Lunz Formation of Austria are comprised of shallow marine marls and terrestrial sandstones, shales and coal[129,130]. Marine biostratigraphy from marine successions bracketing the continental plant-bearing beds[131,132] and palynology[133] indicate a Carnian (237–227 Ma) age for the Formation. Sedimentological, palaeogeographic and palaeoecological study has suggested that deposition of the Lunz Formation occurred in a lowland swamp to deltaic environment[130].

A rich fossil flora has been studied in the Lunz Formation for over a century[134,135]. The assemblage is dominated by ferns, cycads and bennettitaleans[129,130,136]. For this study, carbonaceous compression fossils from the Lunz Formation were obtained from the Swedish Museum of Natural History. The studied cycad specimens were previously characterized[30,137] to the genus level (*Nilssonia*), as were non-cycads[138,139] (all Bennettitales: *Pterophyllum* and *Nilssoniopteris*), on the basis of stomatal morphology.

Thale flora, Lower Keuper

719   The Thale flora is a rich fossil assemblage in the vicinity of the village of Thale in central Germany.
720   The existing specimens were collected by a school teacher in the early 19th century and
721   posthumously deposited in German and Swedish museums[140,141]. The plant-bearing successions
722   were later dated to early Keuper[140,141], giving them a Ladinian (242–273 Ma) age.

724   A detailed and comprehensive study of both macroflora and palynoflora revealed that a lush
725   vegetation grew at the site. The flora is interpreted as autochthonous, most likely representing
726   growth on a floodplain. The macroflora is dominated by ferns, horsetails, and cycads while the
727   palynological assemblages are dominated by bisaccate pollen grains typical of conifers[142]. For this
728   study, carbonaceous compression fossils from the Thale flora were obtained from the Swedish
729   Museum of Natural History. The studied cycad specimens were previously characterized[29] to the
730   genus level (*Bjuvia, Taeniopteris*), as were non-cycads (here all representing the seed fern
731   *Scytophyllum*).

733   *Fremouw Formation*

735   The Triassic Fremouw Formation is located in the central Transantarctic Mountains and consists
736   of ~750 m of sandstones, carbonaceous shales, and volcaniclastic deposits[143]. The upper member
737   of the Fremouw Formation contains a larger proportion of carbonaceous shale, including a
738   silicified horizon representing permineralized peat[143]. Deposition is inferred to have occurred via
739   braided streams in a flood plain setting[143,144]. Palynology and vertebrate fossils suggest an Anisian
740   (247–242 Ma) age for the unit[145,146].

742   The floral assemblage of the Fremouw Formation reflects a high-latitude forest ecosystem
743   characterized by the *Dicroidium* foliage morphotype[144]. Permineralized cycad fossils have also
744   been recognized and studied in the Fremouw Formation since the 1980's[15,40,42,147,148]. For this
745   study, silicified cycad stems and *Dicroidium* leaf layers from the Fremouw Formation were
746   obtained from the Natural History Museum at University of Kansas. The studied cycad specimens
747   were previously characterized[15,42] to the genus level (*Antarcticycas*), and all analyzed non-cycad
748   specimens were of *Dicroidium* affinity.

751   **Data Availability**

753   All data that form the basis of these interpretations are available in the Supplementary Information.

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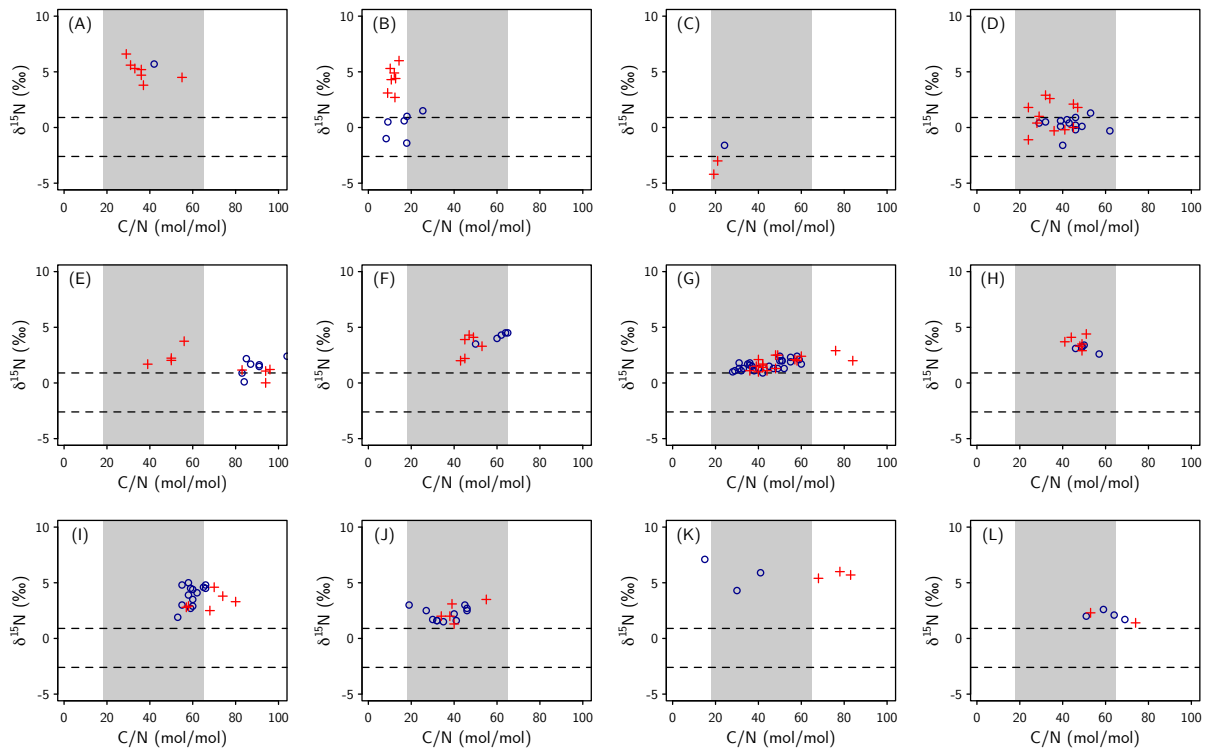
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1113 **Extended Data Figure 1. δ¹⁵N vs. C/N for all studied units.** (A) Soma flora, (B) Most Formation,
1114 (C) Gatuncillo Formation, (D) Chuckanut Formation, (E) Macquarie Harbor Formation, (F) Castle
1115 Rock flora, Denver Formation, (G) Comox Formation, Nanaimo Group, (H) Yorkshire flora,
1116 Cloughton Formation, (I) Primulaelv Formation, Kap Stewart Group, (J) Lunz flora, Lunz
1117 Formation, (K) Thale flora, Lower Keuper (L) Fremouw Formation. Cycad data shown as blue
1118 circles; non-cycad data as red crosses. Grey bands denote range of C/N ratios observed in modern
1119 cycads; dashed lines denote range of δ¹⁵N values observed in modern cycads. Fossil cycad foliage
1120 predominantly falls within the range of C/N ratios observed in modern plants. Fossil cycad foliage
1121 also overwhelmingly overlaps with the C/N ratios of other analyzed plants, with one stark
1122 exception (Thale flora, panel K). In that case, the lack of δ¹⁵N vs. C/N correlation within either
1123 cycads or non-cycads suggests that diagenetic processes (which would impart a δ¹⁵N vs. C/N
1124 correlation) did not appreciably alter the isotopic composition of either group, or create a post-
1125 depositional isotopic offset between the two groups. Overall, the C/N data suggest that post-
1126 depositional alteration is unlikely to have imparted or obscured the isotopic trends observed across
1127 units.
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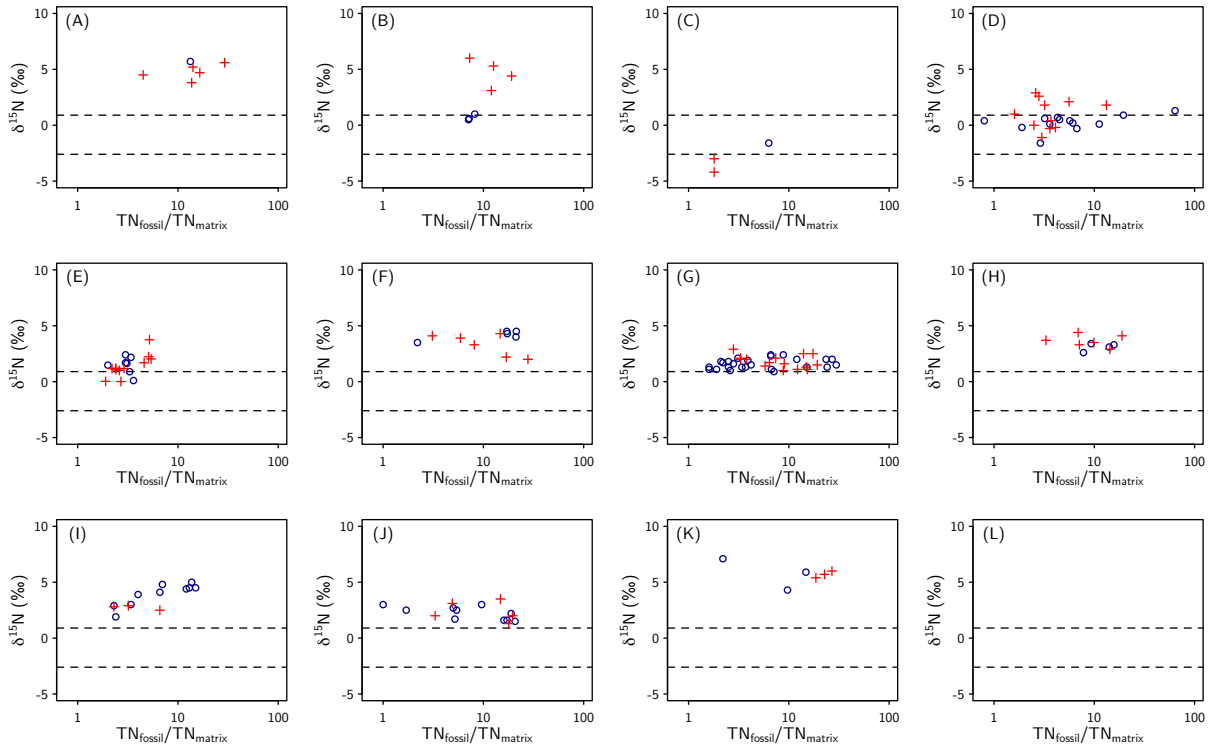
Extended Data Figure 2. δ¹⁵N vs. TN content of fossil over matrix. (A) Soma flora, (B) Most Formation, (C) Gatuncillo Formation, (D) Chuckanut Formation, (E) Macquarie Habor Formation, (F) Castle Rock flora, Denver Formation, (G) Comox Formation, Nanaimo Group, (H) Yorkshire flora, Cloughton Formation, (I) Primulaelv Formation, Kap Stewart Group, (J) Lunz flora, Lunz Formation, (K) Thale flora, Lower Keuper, (L) Fremouw Formation. Fremouw Formation samples were permineralized and thus did not allow a separate characterization of carbonaceous compression fossil versus matrix. Cycad data shown as blue circles; non-cycad data as red crosses. Dashed lines denote range of δ¹⁵N values observed in modern cycads. Isotopic trends within and between units are not correlated with the N concentration of recovered foliage. Recovered fossil material has on average an order of magnitude more nitrogen than the background matrix, indicating that the isotopic signatures derive from the foliage and not soil organic matter.
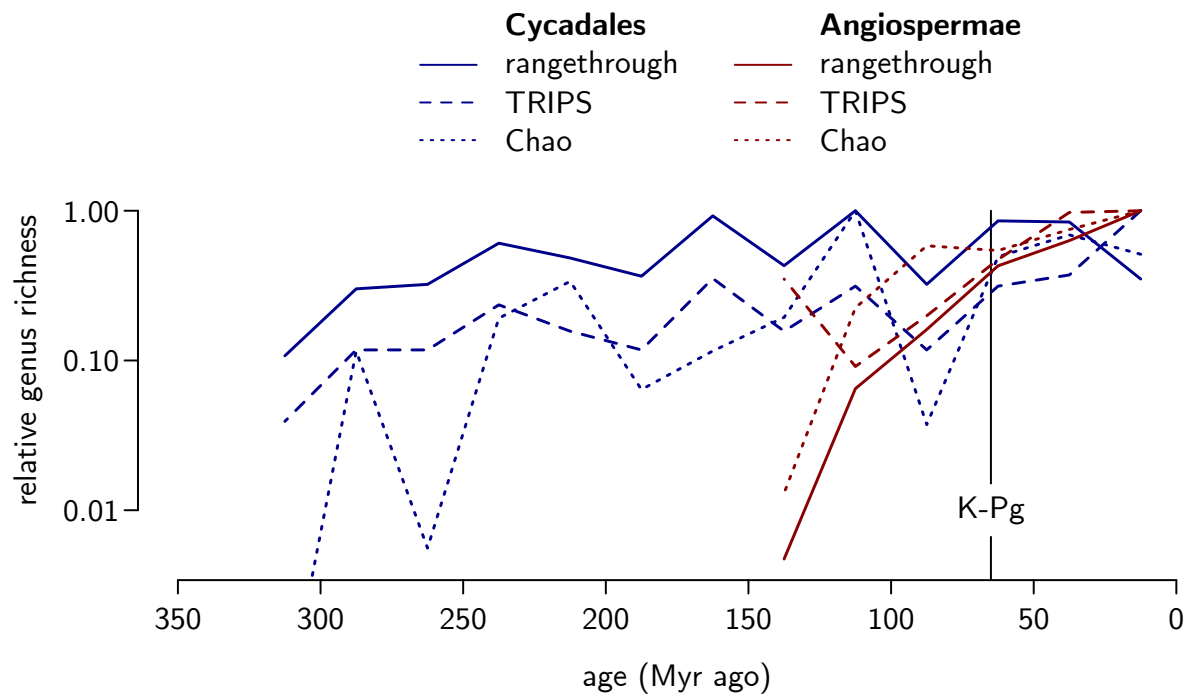
Extended Data Figure 3. Relative genus richness of Cycadales and Angiospermae. Lines separately denote genus richness estimated via bootstrap-resampled rangethrough genus richness, TRiPS estimated genus richness, and Chao1 estimated genus richness. Calculations are described in ***Methods***.