

1 Canopy Structure in Late Cretaceous and Paleocene Forests as
2 Reconstructed from Carbon Isotope Analyses of Fossil Leaves.

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11 **ABSTRACT**

12 While modern forests have their origin in the diversification and expansion of angiosperms in the
13 late Cretaceous and early Cenozoic, it is unclear if the rise of closed-canopy tropical rainforests
14 preceded or followed the end-Cretaceous extinction. The “canopy effect” is a strong vertical
15 gradients in the carbon isotope ($\delta^{13}\text{C}$) composition of leaves in modern closed-canopy forests that
16 could serve as a proxy signature for canopy structure in ancient forests. To test this, we report
17 measurements of the carbon isotope composition of nearly 200 fossil angiosperm leaves from
18 two localities in the Paleocene Cerrejón Formation and one locality in the Maastrichtian Guaduas
19 Formation. Leaves from one Cerrejón fossil assemblage deposited in a small fluvial channel
20 exhibited a 6.3‰ range in $\delta^{13}\text{C}$, consistent with a closed-canopy forest. Carbon isotope values
21 from lacustrine sediments in the Cerrejón Fm. had a range of 3.3‰, consistent with vegetation
22 along a lake edge. An even narrower range of $\delta^{13}\text{C}$ values (2.7‰) was observed for a leaf

23 assemblage recovered from the Cretaceous Guaduas Fm., and suggests vegetation with an open
24 canopy structure. Carbon isotope fractionation by late Cretaceous and early Paleogene leaves
25 was in all cases similar to modern relatives, consistent with estimates of low atmospheric CO₂
26 during this time period. This study confirms other lines of evidence suggesting closed-canopy
27 forests in tropical South America existed by the late Paleocene, and fails to find isotopic evidence
28 for a closed-canopy forest in the Cretaceous.

29 **INTRODUCTION**

30 Closed-canopy tropical forests are the most diverse modern biome and can drive water, carbon,
31 and climate dynamics at continental and global scales (Burnham & Johnson, 2004). Although
32 tropical rainforests comprise only ~12% of the Earth's surface, they account for ~45% of the carbon
33 in terrestrial biomass (IPCC, 2000; Malhi et al., 2002). These forests help maintain consistent
34 temperatures and the wet conditions (mean annual precipitation ≥ 2000 mm/yr) to which they are
35 adapted via their low albedo and massive movement of transpired water across continents, both
36 of which influence large-scale atmospheric circulation and temperatures (Bastable et al., 1993;
37 Betts, 1999; Bonan, 2008; Boyce et al., 2010).

38 It is not well understood when angiosperm-dominated closed-canopy tropical forests first
39 developed and estimates of their origin range from the mid-Cretaceous to the early Paleogene
40 (Burnham & Johnson, 2004). Time-calibrated molecular phylogenetic trees constructed for
41 extant angiosperms place the modern tropical rainforest lineages as far back as 100 Ma and
42 could indicate that angiosperm-dominated, closed-canopy forests have been present since the
43 mid-Cretaceous (Soltis & Soltis, 2004; Davis et al., 2005), except that fossils documenting the
44 morphological and ecological traits common to canopy-forming angiosperms are rare until the
45 Paleocene (Bruun & Ten Brink, 2008; Herrera et al., 2014). Further, leaf features that indicate
46 dense canopy can reflect multiple drivers, leaving few empirical tools that can

47 assess ancient forest structure (Beerling & Royer, 2002; Feild et al., 2011; Carins Murphy, 2014).
48 In modern forests it has been observed that the stable carbon isotope composition of
49 leaves ($\delta^{13}\text{C}_{\text{leaf}}$) declines strongly downward from upper canopy to understory (Vogel, 1978).
50 This “canopy effect” provides a promising approach that could be applied to relatively common
51 leaf compression fossils. If this isotope gradient is preserved in fossils, it would allow canopy
52 placement to be estimated for fossil leaves and leaf fragments.

53 Three major mechanisms contribute to the canopy effect. High rates of respiration by soil
54 biota combined with restricted atmospheric mixing create elevated CO_2 concentrations and ^{13}C -
55 depleted CO_2 ($\delta^{13}\text{C}_{\text{atm}}$) in the understory (Brooks et al., 1997; Medina & Minchin, 1980). Higher
56 humidity lower in the understory permits stomata to remain open without loss of leaf-water
57 resulting in a fuller expression of ^{13}C fractionation during enzymatic carbon fixation (Δ_{leaf} ;
58 Ehleringer et al., 1986; Madhavan et al., 1991). Finally, high light in the upper canopy increases
59 the rate of photosynthesis up to four times that of leaves in the understory, and leads to less ^{13}C
60 discrimination (Zimmerman & Ehleringer, 1990; Hanbe et al., 1997). As a result of these
61 pronounced gradients in CO_2 , water, and light, closed-canopy forest $\delta^{13}\text{C}_{\text{leaf}}$ values range as
62 much as 10‰ from the sun-lit canopy top to the dark and humid understory.

63 A Monte Carlo style leaf resampling model from closed-canopy forest litter has shown that the
64 wide diagnostic range of $\delta^{13}\text{C}_{\text{leaf}}$ values unique to the closed-canopy forest can be found by
65 carbon isotope measurements from as few as 50 leaves (Graham et al., 2014). Here we use
66 $\delta^{13}\text{C}_{\text{leaf}}$ to estimate canopy structure in leaf fossil assemblages from the Maastrichtian Guaduas
67 Fm. and Paleocene Cerrejón Fm. of Colombia. We also use fossil $\delta^{13}\text{C}_{\text{leaf}}$ data in combination

68 with the predicted $\delta^{13}\text{C}$ values of paleoatmospheric CO_2 to determine if photosynthetic
69 fractionation (Δ_{leaf}) differs greatly between these leaves and their modern descendants. $\delta^{13}\text{C}_{\text{leaf}}$
70 values reflect the source CO_2 composition as well as the carbon isotope discrimination occurring
71 during photosynthesis. Fractionation is subject to environmental influences and genetic factors
72 that affect isotopic expression trends (Hubick et al., 1990). Comparison of the Δ_{leaf} values from
73 modern plants with their fossil ancestors will indicate how conserved these fractionation trends
74 are within plant families.

75 MATERIALS AND METHODS

76 This study compares the $\delta^{13}\text{C}_{\text{leaf}}$ from three fossil assemblages with leaves from modern
77 forests to determine if the $\delta^{13}\text{C}_{\text{leaf}}$ range preserved in the fossil cuticles is consistent with a closed
78 canopy (See representative specimens in Fig. 1). All three fossil assemblages were collected in
79 Colombia (Fig. 2); two from the Paleocene Cerrejón Fm. and one from the Late Cretaceous
80 (Maastrichtian) Guaduas Fm. Both Cerrejón assemblages include many of the families dominant
81 families in modern closed-canopy forests of the Neotropics (e.g., Fabaceae, Arecaceae,
82 Lauraceae), and physiognomic leaf features – size, entire margins, vein density – that indicate a
83 closed-canopy, multi-layered rainforest (Wing et al., 2009; Herrera et al., 2011). In contrast, the
84 Guaduas Fm. paleoflora was neither physiognomically similar to contemporary closed-canopy
85 communities nor does it include taxa assigned to extant families dominant in Neotropical
86 rainforests (Guierrez & Jaramillo, 2007). These assemblages were selected in order to compare
87 reconstructed canopy isotope gradients before and after the events of the K-Pg mass extinction.

88 The Cerrejón Fm. is a coal-bearing fluvial unit widely exposed in terraces of the Cerrejón
89 Mine (La Guajira Peninsula, at 11° 5' 60" N, 72° 30' 0" W). Palynofloral assemblages indicate a
90 middle-to-late Paleocene age, approximately 58-60 Ma (Jaramillo et al., 2007). The formation

91 consists of a variety of lithologies (sandstones, mudstones, and coals) deposited in a mosaic of
92 fluvial and lacustrine settings typical of an estuarine coastal plain. Leaf margin and size analyses
93 a MAP of 2.3-4.6 m/yr and MAT of 24-31°C/yr (Wing et al., 2009).

94 The two Cerrejón localities were separated by less than two km laterally and 100 m
95 stratigraphically. Fossils from both localities were collected from small areas (4-6 m²) that
96 represent distinct terrestrial settings. Leaves from Site 0315 were deposited in heterolithic
97 sediments suggestive of a low-to-medium energy channel. Sampled leaves were associated with
98 ten morphotypes from nine families, as well as a selection of taxonomically indeterminate non-
99 monocot (magnoliid or eudicot) angiosperm leaves. At Cerrejón Site 0318, leaves were collected
100 from a laterally extensive, thinly bedded, flat-laminated siltstone interpreted as a shallow lake
101 deposit. Sampled leaves included nineteen morphotypes from ten families as well as a selection of
102 indeterminate non-monocot leaves. Herrera et al. (2008) and Wing et al. (2009) describe family
103 identification and morphotype assignment.

104 Late Cretaceous fossils were collected from the middle Guaduas Fm. of Boyacá
105 Department (5°55'45"N, 72°47'43"W). Palynoflora indicate an age of ca. 68-66 Ma (Muller et
106 al., 1987). Leaf margin and size analyses estimate an MAT of 22.1 +/- 3.4°C/yr and MAP of ~2.4
107 m/yr (Gutierrez & Jaramillo, 2007). At the time of deposition this location was a coastal plain
108 similar to the Cerrejón Fm. (Gutierrez & Jaramillo, 2007). Fossil leaves from the Guaduas Fm.
109 were taken from laminated and massive mudstones with sandstone interbeds above fine-grained
110 sand beds intercalated with coal seams (Guerrero, 2002). Most angiosperm leaves from the Guaduas flora
111 can only be described as indeterminate dicots co-existing with abundant gymnosperms in a community
112 that has no modern analog. One leaf for this study could be assigned to a family and nine others
113 could be assigned to one of five morphotypes (Gutierrez & Jaramillo, 2007).

114 **Analytical Methods**

115 To determine the minimum number of leaves required to recover the $\delta^{13}\text{C}_{\text{leaf}}$ range
116 characteristic of a closed-canopy Graham et al., (2014) used leaf properties and litter flux data from
117 modern canopies and performed statistical resampling with replacement. Given an isotope variance
118 similar to than in modern tropical closed-canopy forests, a minimum of 50 randomly selected fossil leaves
119 is required for the $\delta^{13}\text{C}_{\text{leaf}}$ range to reflect a robust signal of canopy closure (Fig. 3; overlapping with open
120 boxes) We therefore sampled and analyzed 53 fossil leaves from Guaduas, 68 from Cerrejón Site
121 0315, and 78 for Cerrejón Site 0318.

122 Briefly, well-preserved fossilized leaf mesophyll tissue (Fig. 1) was mechanically removed from
123 rock either by dental drill or scalpel, ground, treated in 6N HCl, lyophilized, and then analyzed by a
124 Costech Elemental Analyzer coupled to a Thermo Finnigan Delta XP isotope ratio mass spectrometer
125 Values were calibrated by IAEA and USGS standards, corrected for sample size, and reported relative to
126 Vienna Pee Dee Belemnite (VPDB). Instrument precision based on reproducibility of standards was
127 0.09‰ (n=76) and accuracy (average difference between measured and true $\delta^{13}\text{C}$ value) is 0.02‰
128 (n=96). Duplicate analyses were performed for half of leaves and triplicate analyses were performed on
129 leaves outside the 95% of the observed isotopic range because of their role in defining the isotopic range.

130 RESULTS

131 The $\delta^{13}\text{C}_{\text{leaf}}$ range threshold that indicates canopy closure signature varies with the size of
132 a sample population (see boxes in Fig. 3). For the number of leaves from the Guaduas assemblage,
133 the threshold to indicate canopy closure was 4.9‰, while for the slightly larger sample sets from the
134 Cerrejón, the canopy closure range thresholds were 5.2‰ for Site 0318 and 5.4‰ for Site 0315.

135 For the Cerrejón channel deposit assemblage (Site 0315), the observed $\delta^{13}\text{C}_{\text{leaf}}$ range of
136 6.3‰ (Fig. 3, light closed circle), from -22.7 to -29.0‰, is well above the threshold to be
137 interpreted as a closed-canopy forest. Leaves from Site 0318 expressed a narrow isotopic range

138 of 3.3‰, from -26.9 to -23.6‰ (Fig. 3, dark closed circle). This is consistent with an open-
139 canopy forest, as would be anticipated for leaves from a lake-edge environment. Edges of
140 forests experience greater sun and wind exposure, leading to mean $\delta^{13}\text{C}_{\text{leaf}}$ values that are as
141 much as 2‰ higher than observed for leaves from a forest center (Kapos et al., 1993). The
142 Guaduas flora expressed the narrowest range, 2.7‰, from -27.5 to -24.7‰, (Fig. 3, open circle),
143 well below the threshold value for a closed canopy. This narrow range indicates that the non-
144 monocot angiosperm taxa of the Guaduas Fm. were unlikely to have derived from a closed-
145 canopy forest habit.

146 **INTERPRETATION AND DISCUSSION**

147 **Canopy Closure Estimation**

148 Results from this study indicate the carbon isotope range preserved in fossil leaves can
149 serve as a proxy for canopy structure in ancient angiosperm-dominated forests, provided this
150 evidence is consistent with the geographic, sedimentological, and taxonomic context. The
151 lacustrine environment suggested by lithology at Cerrejón Site 0318 supports the edge-forest
152 interpretation of the narrow isotopic range found in the fossil leaves (3.3‰). This fossil
153 assemblage also includes four times as many Menispermaceae leaves interpreted as lianas, a
154 plant habit that thrives in gaps and forest edges (Doria et al., 2008; Herrera et al., 2011).
155 Because fossil leaves are generally best preserved in lake and river sediments, we anticipated
156 isotope ranges indicating forest edges would more likely to be the rule than the exception. Thus,
157 the strikingly wide isotope range observed at Cerrejón Site 0315 - interpreted as a closed-canopy
158 signal - is a remarkable find and consistent with the depositional environment of a small stream
159 in a forest interior. Isotope evidence for forest canopy structure is also consistent with vein
160 density data indicating that Cerrejón leaves were from an angiosperm-dominated closed-canopy
161 while the vein density of Guaduas leaves is not the same as extant tropical forests (Crifò et al., 2014).

162 ***Model Constraints***

163 The expected $\delta^{13}\text{C}_{\text{leaf}}$ ranges for closed-canopy forests were established using data from
164 modern leaves sampled from the full range of light environments in the interior of a forest and
165 represent a random sampling of leaves delivered directly to an allochthonous litter bed (Graham
166 et al., 2014). This model collection did not include forest edge leaves commonly observed in
167 fossil assemblages (Spicer, 1981) and contrasts with Guaduas and Cerrejón fossil leaves which
168 were transported at least a modest distance before preservation (Gutierrez & Jaramillo, 2007;
169 Wing et al., 2009).

170 ***Canopy Effect Representation In Other Fossil Reservoirs***

171 Sun-lit upper canopy leaves in modern tree canopies are replaced more frequently than
172 shaded leaves (Reich et al., 1991). Hence, upper canopy leaves have a high representation in sediments
173 and dominate the litter of many modern forests (Spicer, 1981) and this is reflected in the litter flux data
174 used in leaf sample model (Graham et al., 2014). Thus, the mean enriched carbon isotope values for fallen
175 fallen leaves, litter and ultimately soil organic matter reflect the conditions of the upper canopy, including
176 regional moisture patterns and community composition (Diefendorf et al., 2010; Graham et al., 2014).

177 ***Photosynthetic Fractionation and Paleo CO₂ Concentrations***

178 Fossil $\delta^{13}\text{C}_{\text{leaf}}$ can be used to reconstruct fractionation during photosynthetic carbon
179 fixation by ancient trees. These reconstructions can help our understanding of the potential
180 influences of water, atmospheric CO₂ levels, and taxonomic affiliation on $\delta^{13}\text{C}_{\text{leaf}}$ expression.

181 Further, $\delta^{13}\text{C}_{\text{leaf}}$ measurements can strengthen reconstructed atmospheric CO₂ levels based on
182 stomatal data (Franks et al., 2014; 2017). Fractionation by plants ($\Delta_{\text{leaf}} = (C_{\text{atm}}^{13} - C_{\text{leaf}}^{13}) / (1 +$
183 $C_{\text{leaf}}^{13} / 1000)$), reflects the supply of carbon dioxide from the atmosphere (C_a), relative to

184 assimilated carbon and internal CO₂ concentrations (C_i) and rates of photorespiration. The
185 stomatal conductance of CO₂ (C_i/C_a) can be predicted by MAP and is regulated by plants via the
186 size and density of stomata (Farquhar & Sharkey, 1982; Franks et al., 2017). These
187 modifications that maintain a concentration gradient for CO₂ uptake can persist on generational
188 time scales (Berner, 1998; Franks & Beerling, 2009; Porter et al., 2019).

189 Based on organic and carbonate archives, the ¹³C_{atm} CO₂ during the late Cretaceous and
190 early Paleogene was -4.9‰ and -4.4‰ or 3.3 to 3.8‰ higher than in 2010 (-8.2‰) (Tippie et al.,
191 2010; Scripps Global CO₂, 2012). Using these estimates, the calculated Δ_{leaf} values for the fossil
192 leaves range from 18.3 to 24.6‰ for the Paleocene and 20.3 to 23.2‰ for the Cretaceous. These
193 ranges are typical of modern warm and wet environments, where humid conditions enable plant
194 fractionation to approach maximum values (Farquhar et al., 1989). These Δ_{leaf} values are similar
195 to those observed in modern Neotropical forests (Diefendorf et al., 2010) . A vertical survey of leaves
196 from a modern forest in Panamá expressed fractionation values ranging from 17.5 to 25.5‰ (Graham et
197 al., 2014). The smaller Δ_{leaf} values were observed in the sun-lit leaves, given the decreased
198 fractionation that accompanies water stress and the higher Δ_{leaf} values are more typical of leaves
199 in the humid understory.

200 Many, though not all, proxy-based reconstructions of Late Cretaceous and Paleocene
201 pCO₂ levels are below ~500 ppmV (Royer et al., 2001; Foster et al., 2017; Porter et al., 2019).
202 The nearly constant maximum leaf-level discrimination in Cretaceous, Paleocene and modern
203 leaves suggest that if there was an effect of pCO₂ on discrimination (Schubert & Jahren, 2012),
204 it is not detectable amidst other factors that influence discrimination. Although mean Δ_{leaf} values
205 assemblages were consistent, Δ_{leaf} values for individual leaves ranged several per mil (reflecting
206 δ_{leaf} variability; Fig. 4), particularly for the closed-canopy flora. Thus, paleoclimate and plant

207 development studies that employ plant fractionation and stomata analyses will be strengthened
208 by using leaf assemblages large enough to account for canopy influences.

209 **CONCLUSION**

210 The $\delta^{13}\text{C}_{\text{leaf}}$ range from individual fossil leaves indicates that open canopy conditions
211 prevailed during deposition of the Maastrichtian Guaduas Fm. and revealed closed-canopy
212 conditions in one of two Paleocene floras. While this study does not rule out the possibility that
213 closed-canopy environments were present in the Cretaceous, the isotopic results do confirm the
214 floristic, physiognomic, ecological, and vein-density analysis data that indicate closed-canopy forests
215 were present in northern South America after the Cretaceous extinction (Wing et al., 2009).
216 Average Δ_{leaf} values calculated for fossil leaves were similar for Maastrichtian and
217 Paleocene forests, independent of structure and taxonomic composition, and similar to modern
218 tropical rainforests. Fossil data indicate that isotope discrimination during the globally warmer
219 Maastrichtian-Paleocene essentially did not differ from modern expressions of fractionation. Our findings
220 agree with recent models suggesting that any changes in leaf-level carbon isotope discrimination caused
221 by $p\text{CO}_2$ over geologic time will be hard to distinguish from the broad variation in discrimination
222 caused by light environment, precipitation, and other factors (Franks et al., 2017). Carbon
223 isotope gradients, when properly contextualized, offer a potentially powerful approach to
224 forest canopy structure reconstruction in the geologic past, and can provide a useful context for
225 leaves used in other paleoclimate applications, including temperature and CO_2 proxy studies.

226

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355 **FIGURE CAPTIONS**

356 Fig 1. Morphotype identifier and best guess family for megafossils representing preservation of
357 sampled specimens (see Wing *et al.*, 2009 for Cerrejón fossils) from all three fossil
358 localities. Scale bar: 5 cm. (A) Euphorbiaceae, CJ10 (B) Menispermaceae, *Menispermites*
359 *cerrejonensis*, CJ6 (C) Sapotaceae, CJ8 (D) Sapotaceae, CJ8 (E) Fabaceae, CJ1 (F)
360 indeterminate dicot (G) dicot GD14.

361 Fig 2. Location of Paleocene Cerrejón fossil flora and Maastrichtian Guaduas fossil flora. Digital
362 elevation model by Amante & Eakins (2009).

363 Fig 3. Circles represent $^{13}\text{C}_{\text{leaf}}$ range for each fossil leaf locality compared with the predicted
364 isotopic range (a box plot showing the median, maximum, minimum and upper and lower
365 quartiles) for closed-canopy leaf litter based on sample size (from Graham *et al.*, 2014).

366 Fig 4. Histograms of measured $^{13}\text{C}_{\text{leaf}}$ range for fossil leaves show the frequency with which
367 $^{13}\text{C}_{\text{leaf}}$ values are expressed in fossil leaves and the rarity of ^{13}C -depleted understory leaves.
368 (A) is data for leaves from the Cretaceous Guaduas locality. (B) is data for Cerrejón leaves
369 where the light green represents locality 0315 and the dark green represents locality 0318.
370 The right skew in the data is likely due to the bias in fossil assemblages towards
371 preservation of ^{13}C -enriched upper canopy leaves (See Spicer, 1981).