

Canopy structure in Late Cretaceous and Paleocene forests as reconstructed from carbon isotope analyses of fossil leaves

Heather V. Graham^{1,2}, Fabiany Herrera³, Carlos Jaramillo^{4,5}, Scott L. Wing⁶ and Katherine H. Freeman²

¹NASA Goddard Spaceflight Center, Code 691, Greenbelt, Maryland 20771, USA

²Department of Geosciences, The Pennsylvania State University, University Park, Pennsylvania 16802, USA

³Chicago Botanic Garden, 1000 Lake Cook Road, Glencoe, Illinois 60022, USA

⁴Smithsonian Tropical Research Institute, Box 0843-03092, Balboa, Panama

⁵ISEM, Université de Montpellier, CNRS, EPHE, IRD, 34090 Montpellier, France

⁶Department of Paleobiology, National Museum of Natural History, Smithsonian Institution, P.O. Box 37012, Washington, D.C. 20013, USA

ABSTRACT

While modern forests have their origin in the diversification and expansion of angiosperms in the Late Cretaceous and early Cenozoic, it is unclear whether the rise of closed-canopy tropical rainforests preceded or followed the end-Cretaceous extinction. The “canopy effect” is a strong vertical gradient in the carbon isotope ($\delta^{13}\text{C}$) composition of leaves in modern closed-canopy forests that could serve as a proxy signature for canopy structure in ancient forests. To test this, we report measurements of the carbon isotope composition of nearly 200 fossil angiosperm leaves from two localities in the Paleocene Cerrejón Formation and one locality in the Maastrichtian Guaduas Formation of Colombia. Leaves from one Cerrejón fossil assemblage deposited in a small fluvial channel exhibited a 6.3‰ range in $\delta^{13}\text{C}$, consistent with a closed-canopy forest. Carbon isotope values from lacustrine sediments in the Cerrejón Formation had a range of 3.3‰, consistent with vegetation along a lake edge. An even-narrower range of $\delta^{13}\text{C}$ values (2.7‰) was observed for a leaf assemblage recovered from the Cretaceous Guaduas Formation, and suggests vegetation with an open canopy structure. Carbon isotope fractionation by Late Cretaceous and early Paleogene leaves was in all cases similar to that by modern relatives, consistent with estimates of low atmospheric CO_2 during this time period. This study confirms other lines of evidence suggesting that closed-canopy forests in tropical South America existed by the late Paleocene, and fails to find isotopic evidence for a closed-canopy forest in the Cretaceous.

INTRODUCTION

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

It is not well understood when angiosperm-dominated closed-canopy tropical forests first

developed, and estimates of their origin range from the mid-Cretaceous to the early Paleogene (Burnham and Johnson, 2004). Time-calibrated molecular phylogenetic trees constructed for extant angiosperms place the modern tropical rainforest lineages as far back as 100 Ma and could indicate that angiosperm-dominated, closed-canopy forests have been present since the mid-Cretaceous (Soltis and Soltis, 2004; Davis et al., 2005), except that fossils documenting the morphological and ecological traits common to canopy-forming angiosperms are rare until the Paleocene (Bruun and Ten Brink, 2008; Herrera et al., 2014). Further, leaf features that indicate dense canopy can reflect multiple drivers, leaving few empirical tools that can be used to assess ancient forest structure (Beerling and Royer, 2002; Feild et al., 2011; Carins Murphy et al., 2014). In modern forests, it has been

observed that the stable carbon isotope composition of leaves ($\delta^{13}\text{C}_{\text{leaf}}$) declines strongly downward from upper canopy to understory (Vogel, 1978). This “canopy effect” provides a promising approach that could be applied to relatively common leaf compression fossils. If this isotope gradient is preserved in fossils, it would allow canopy placement to be estimated for fossil leaves and leaf fragments.

Three major mechanisms contribute to the canopy effect. (1) High rates of respiration by soil biota combined with restricted atmospheric mixing create elevated CO_2 concentrations and ^{13}C -depleted CO_2 ($\delta^{13}\text{C}_{\text{atm}}$) in the understory (Brooks et al., 1997; Medina and Minchin, 1980). (2) Higher humidity lower in the understory permits stomata to remain open without loss of leaf water, resulting in a fuller expression of ^{13}C fractionation during enzymatic carbon fixation (Δ_{leaf} ; Ehleringer et al., 1986; Madhavan et al., 1991). (3) High light in the upper canopy increases the rate of photosynthesis up to four times that of leaves in the understory, and leads to less ^{13}C discrimination (Zimmerman and Ehleringer, 1990; Hanba et al., 1997). As a result of these pronounced gradients in CO_2 , water, and light, closed-canopy forest $\delta^{13}\text{C}_{\text{leaf}}$ values range as much as 10‰ from the sun-lit canopy top to the dark and humid understory.

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

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

MATERIALS AND METHODS

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

The Cerrejón Formation is a coal-bearing fluvial unit widely exposed in terraces of the Cerrejón Mine on the La Guajira Peninsula. Palynofloral assemblages indicate a middle to late Paleocene age, ca. 58–60 Ma (Jaramillo et al., 2007). The formation consists of a variety of lithologies (sandstones, mudstones, and coals) deposited in a mosaic of fluvial and lacustrine settings typical of an estuarine coastal plain. Leaf margin and size analyses indicate a mean annual precipitation (MAP) of 2.3–4.6 m/yr and mean annual temperature (MAT) of 24–31°C (Wing et al., 2009). The two Cerrejón localities (Site 0315 at 11°8'6"N, 72°34'12"W; Site 0318 at 11°7'41"N, 72°33'18"W) were separated by <2 km laterally and 100 m stratigraphically. Fossils from both localities were collected from small areas (4–6 m²) that represent distinct terrestrial settings. Leaves from site 0315 were deposited in heterolithic sediments suggestive of a low- to medium-energy channel. Sampled leaves

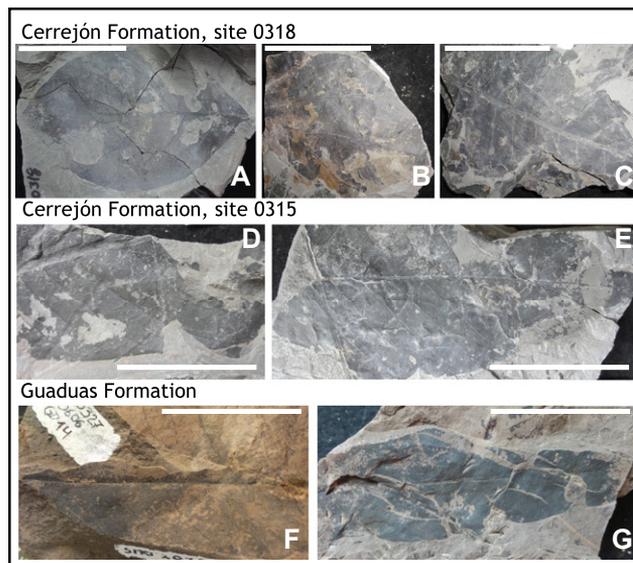


Figure 1. Photos of mega-fossils representing preservation of sampled specimens (see Wing et al. [2009] for Cerrejón Formation, Columbia, fossils) from all three fossil localities, Cerrejón (sites 0318 and 0315) and Guaduas Formations, Colombia. Morphotype identifier and best-guess family are given for each. Scale bars: 5 cm. (A) Euphorbiaceae, CJ10. (B) Menispermaceae, *Menispermites cerrejonensis*, CJ6. (C) Sapotaceae, CJ8. (D) Sapotaceae, CJ8. (E) Fabaceae, CJ1. (F) Indeterminate dicot. (G) Dicot, GD14.

were associated with ten morphotypes from nine families, as well as a selection of taxonomically indeterminate non-monocot (magnoliid or eudicot) angiosperm leaves. At site 0318, leaves were collected from a laterally extensive, thinly bedded, flat-laminated siltstone interpreted as a shallow lake deposit. Sampled leaves included 19 morphotypes from 10 families as well as a selection of indeterminate non-monocot leaves. Herrera et al. (2008) and Wing et al. (2009) described family identification and morphotype assignment.

Late Cretaceous fossils were collected from the middle Guaduas Formation of Boyacá Department (5°55'45"N, 72°47'43"W). Palynoflora indicate an age of ca. 68–66 Ma (Muller et al., 1987). Leaf margin and size analyses

indicate an estimated MAT of $22.1 \pm 3.4^\circ\text{C}$ and MAP of ~ 2.4 m/yr (Gutierrez and Jaramillo, 2007). At the time of deposition, this location was a coastal plain similar to that of the Cerrejón Formation (Gutierrez and Jaramillo, 2007). Fossil leaves from the Guaduas Formation were taken from laminated and massive mudstones with sandstone interbeds above fine-grained sand beds intercalated with coal seams (Guerrero, 2002). Most angiosperm leaves from the Guaduas flora can only be described as indeterminate dicots coexisting with abundant gymnosperms in a community that has no modern analog. One leaf for this study could be assigned to a family, and nine others could be assigned to one of five morphotypes (Gutierrez and Jaramillo, 2007).

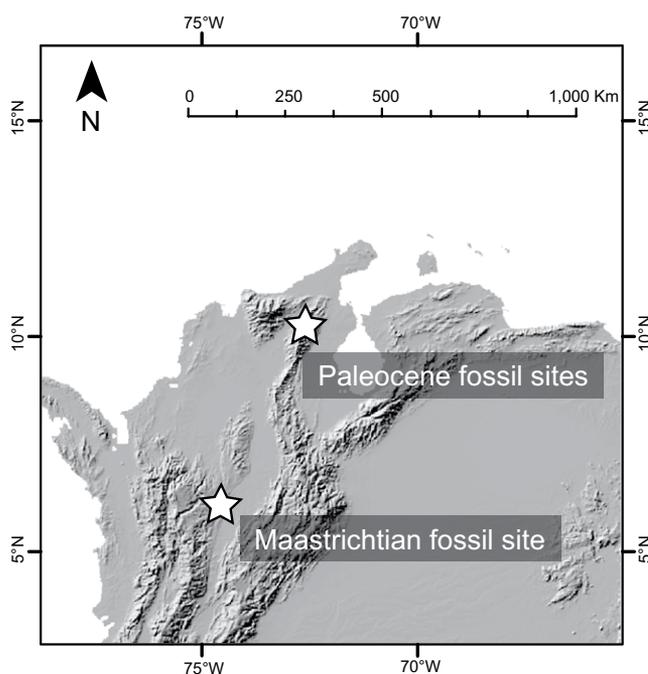


Figure 2. Location of Paleocene Cerrejón Formation fossil flora and Maastrichtian Guaduas Formation fossil flora, Colombia. Digital elevation model by Amante and Eakins (2009).

Analytical Methods

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

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

RESULTS

The $\delta^{13}\text{C}_{\text{leaf}}$ range threshold that indicates canopy-closure signature varies with the size of a sample population (see boxes in Fig. 3). For the number of leaves from the Guaduas assemblage,

the range threshold to indicate canopy closure was 4.9‰, while for the slightly larger sample sets from the Cerrejón, the canopy closure range thresholds were 5.2‰ for site 0318 and 5.4‰ for site 0315.

For the Cerrejón channel deposit assemblage (site 0315), the observed $\delta^{13}\text{C}_{\text{leaf}}$ range of 6.3‰ (Fig. 3), from -22.7‰ to -29.0‰ , is well above the threshold to be interpreted as representing a closed-canopy forest. Leaves from site 0318 expressed a narrow isotopic range of 3.3‰, from -26.9‰ to -23.6‰ (Fig. 3). This is consistent with an open-canopy forest, as would be anticipated for leaves from a lake-edge environment. Edges of forests experience greater sun and wind exposure, leading to mean $\delta^{13}\text{C}_{\text{leaf}}$ values that are as much as 2‰ higher than observed for leaves from a forest center (Kapos et al., 1993). The Guaduas flora expressed the narrowest range, 2.7‰, from -27.5‰ to -24.7‰ , (Fig. 3), well below the threshold value for a closed canopy. This narrow range indicates that the non-monocot angiosperm taxa of the Guaduas Formation were unlikely to have derived from a closed-canopy forest habit (see the GSA Data Repository¹).

INTERPRETATION AND DISCUSSION

Canopy Closure Estimation

Results from this study indicate that the carbon isotope range preserved in fossil leaves can serve as a proxy for canopy structure in ancient angiosperm-dominated forests, provided this evidence is consistent with the geographic,

sedimentological, and taxonomic context. The lacustrine environment suggested by lithology at Cerrejón site 0318 supports the edge-forest interpretation of the narrow isotopic range found in the fossil leaves (3.3‰). Site 0318 also includes four times more fossil Menispermaceae leaves. These are interpreted as lianas, a plant habit that thrives in gaps and forest edges (Doria et al., 2008; Herrera et al., 2011). Because fossil leaves are generally best preserved in lake and river sediments, we anticipated that isotope ranges indicating forest edges would more likely be the rule than the exception. Thus, the strikingly wide isotope range observed at Cerrejón site 0315—interpreted as a closed-canopy signal—is a remarkable find and consistent with the depositional environment of a small stream in a forest interior. Isotope evidence for forest canopy structure is also consistent with vein density data indicating that Cerrejón leaves were from an angiosperm-dominated closed canopy, while the vein density of Guaduas leaves is not the same as for extant tropical forests (Crifò et al., 2014).

Model Constraints

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

Canopy Effect Representation in Other Fossil Reservoirs

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

Photosynthetic Fractionation and Paleo- CO_2 Concentrations

Fossil $\delta^{13}\text{C}_{\text{leaf}}$ can be used to reconstruct fractionation during photosynthetic carbon fixation by ancient trees. These reconstructions can help our understanding of the potential influences of water, atmospheric CO_2 levels, and taxonomic affiliation on $\delta^{13}\text{C}_{\text{leaf}}$ expression. Further, $\delta^{13}\text{C}_{\text{leaf}}$

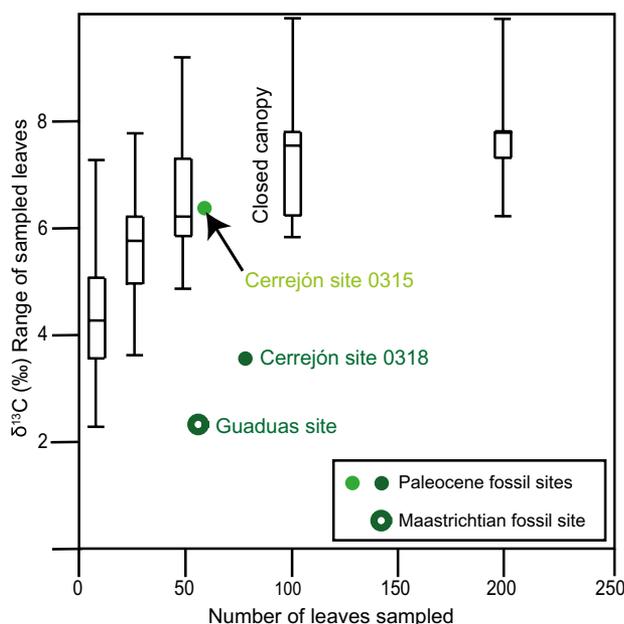


Figure 3. $\delta^{13}\text{C}_{\text{leaf}}$ range for each fossil leaf locality (circles) compared with predicted isotopic ranges (box plot showing median, maximum, minimum, and upper and lower quartiles) for closed-canopy leaf litter based on sample size (from Graham et al., 2014).

¹GSA Data Repository item 2019344, Table DR1 (Cerrejón 0318 paleoflora), is available online at <http://www.geosociety.org/datarepository/2019/>, or on request from editing@geosociety.org.

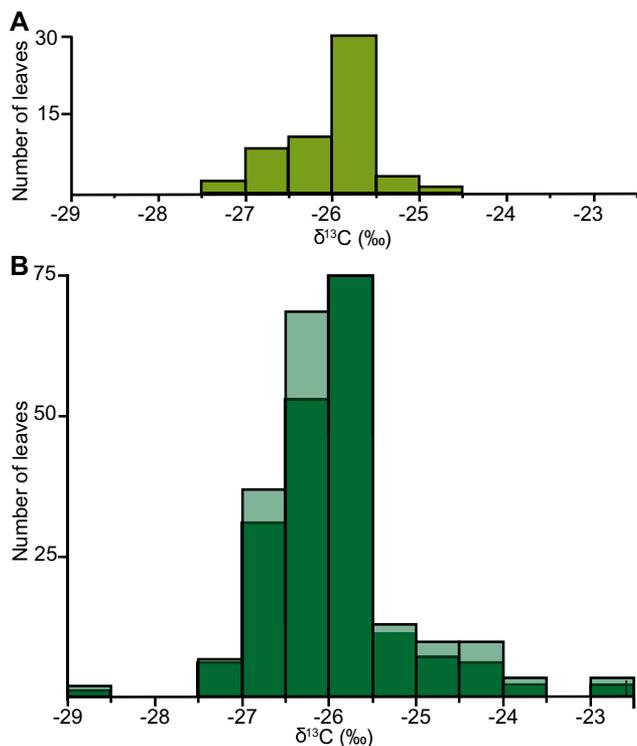


Figure 4. Histograms of measured $^{13}\text{C}_{\text{leaf}}$ range for fossil leaves show frequency with which $^{13}\text{C}_{\text{leaf}}$ values are expressed in fossil leaves and rarity of ^{13}C -depleted understory leaves. (A) Leaves from Cretaceous Guaduas Formation locality, Colombia. (B) Cerrejón Formation (Colombia) leaves, where light green represents site 0315 and dark green represents site 0318. Right skew in data is likely due to bias in fossil assemblages toward preservation of ^{13}C -enriched upper-canopy leaves (see Spicer, 1981).

measurements can strengthen reconstructed atmospheric CO_2 levels based on stomatal data (Franks et al., 2013, 2018). Fractionation by plants [$\Delta_{\text{leaf}} = (^{13}\text{C}_{\text{atm}} - ^{13}\text{C}_{\text{leaf}})/(1 + ^{13}\text{C}_{\text{leaf}}/1000)$] reflects the supply of carbon dioxide from the atmosphere (C_a), relative to assimilated carbon and internal CO_2 concentrations (C_i) and rates of photorespiration. The stomatal conductance of CO_2 (C_i/C_a) can be predicted by MAP and is regulated by plants via the size and density of stomata (Farquhar and Sharkey, 1982; Franks et al., 2018). These modifications that maintain a concentration gradient for CO_2 uptake can persist on generational time scales (Bernier, 1998; Franks and Beerling, 2009; Porter et al., 2019).

Based on organic and carbonate archives, the $^{13}\text{C}_{\text{atm}}$ CO_2 during the Late Cretaceous and early Paleogene was -4.9‰ and -4.4‰ , respectively, or 3.3‰ – 3.8‰ higher than in A.D. 2010 (-8.2‰) (Tippie et al., 2010; Scripps Institution of Oceanography Global CO_2 Program, <http://scrippsco2.ucsd.edu>). Using these estimates, the calculated Δ_{leaf} values for the fossil leaves range from 18.3‰ to 24.6‰ for the Paleocene and 20.3‰ to 23.2‰ for the Cretaceous. These ranges are typical of modern warm and wet environments, where humid conditions enable plant fractionation to approach maximum values (Farquhar et al., 1989). These Δ_{leaf} values are similar to those observed in modern Neotropical forests (Diefendorf et al., 2010). A vertical survey of leaves from a modern forest in Panamá expressed fractionation values ranging from 17.5‰ to 25.5‰ (Graham et al., 2014). The smaller Δ_{leaf} values were observed in the sun-lit leaves, given the decreased fractionation that

accompanies water stress, and the larger Δ_{leaf} values are more typical of leaves in the humid understory.

Many, though not all, proxy-based reconstructions of Late Cretaceous and Paleocene $p\text{CO}_2$ levels are below ~ 500 ppmV (Royer et al., 2001; Foster et al., 2017; Porter et al., 2019). The nearly constant maximum leaf-level discrimination in Cretaceous, Paleocene, and modern leaves suggests that if there was an effect of $p\text{CO}_2$ on discrimination (Schubert and Jahren, 2012), it is not detectable amidst other factors that influence discrimination. Although mean Δ_{leaf} values for leaf assemblages were consistent, Δ_{leaf} values for individual leaves ranged several per mil (reflecting δ_{leaf} variability; Fig. 4), particularly for the closed-canopy flora. Thus, paleoclimate and plant development studies that employ plant fractionation and stomata analyses will be strengthened by using leaf assemblages large enough to account for canopy influences.

CONCLUSION

The $\delta^{13}\text{C}_{\text{leaf}}$ range from individual fossil leaves indicates that open-canopy conditions prevailed during deposition of the Maastrichtian Guaduas Formation and reveals closed-canopy conditions in one of two Paleocene floras. While this study does not rule out the possibility that closed-canopy environments were present in the Cretaceous, the isotopic results do confirm the floristic, physiognomic, ecological, and vein-density analysis data that indicate that closed-canopy forests were present in northern South America after the end-Cretaceous extinction (Wing et al., 2009).

Average Δ_{leaf} values calculated for fossil leaves were similar for Maastrichtian and Paleocene forests, independent of structure and taxonomic composition, and similar to those of modern tropical rainforests. Fossil data indicate that isotope discrimination during the globally warmer Maastrichtian–Paleocene essentially did not differ from modern expressions of fractionation. Our findings agree with recent models suggesting that any changes in leaf-level carbon isotope discrimination caused by $p\text{CO}_2$ over geologic time would be hard to distinguish from the broad variation in discrimination caused by light environment, precipitation, and other factors (Franks et al., 2018). Carbon isotope gradients, when properly contextualized, offer a potentially powerful approach to forest canopy structure reconstruction for the geologic past, and can provide a useful context for leaves used in other paleoclimate applications, including temperature and CO_2 proxy studies.

ACKNOWLEDGMENTS

This project was completed with support from the Smithsonian Tropical Research Institute as well as the Committee for Institutional Cooperation, the Anders Foundation and Oak Spring Garden Foundation, and the U.S. National Science Foundation (grant EAR 1829299).

REFERENCES CITED

- Amante, C., and Eakins, B.W., 2009, ETOPO1 1 arc-minute global relief model: Procedures, data sources and analysis: U.S. National Oceanic and Atmospheric Administration Technical Memorandum NESDIS NGDC-24, 19 p.
- Bastale, H.G., Shuttleworth, W.J., Dallarosa, R.L.G., Fisch, G., and Nobre, C.A., 1993, Observations of climate, albedo, and surface radiation over cleared and undisturbed Amazonian forest: *International Journal of Climatology*, v. 13, p. 783–796, <https://doi.org/10.1002/joc.3370130706>.
- Beerling, D.J., and Royer, D.L., 2002, Fossil plants as indicators of the Phanerozoic global carbon cycle: *Annual Review of Earth and Planetary Sciences*, v. 30, p. 527–556, <https://doi.org/10.1146/annurev.earth.30.091201.141413>.
- Berner, R.A., 1998, The carbon cycle and CO_2 over Phanerozoic time: The role of land plants: *Philosophical Transactions of the Royal Society of London: Series B, Biological Sciences*, v. 353, p. 75–82, <https://doi.org/10.1098/rstb.1998.0192>.
- Betts, R.A., 1999, Self-beneficial effects of vegetation on climate in an ocean-atmosphere general circulation model: *Geophysical Research Letters*, v. 26, p. 1457–1460, <https://doi.org/10.1029/1999GL900283>.
- Bonan, G.B., 2008, Forests and climate change: Forcings, feedbacks, and the climate benefits of forests: *Science*, v. 320, p. 1444–1449, <https://doi.org/10.1126/science.1155121>.
- Boyce, C.K., Lee, J.-E., Feild, T.S., Brodrribb, T.J., and Zwieniecki, M.A., 2010, Angiosperms helped put the rain in the rainforests: The impact of plant physiological evolution on tropical biodiversity: *Annals of the Missouri Botanical Garden*, v. 97, p. 527–540, <https://doi.org/10.3417/2009143>.
- Brooks, J.R., Flanagan, L.B., Varney, G.T., and Ehleringer, J.R., 1997, Vertical gradients in photosynthetic gas exchange characteristics and

- refixation of respired CO₂ within boreal forest canopies: *Tree Physiology*, v. 17, p. 1–12, <https://doi.org/10.1093/treephys/17.1.1>.
- Bruun, H.H., and Ten Brink, D.J., 2008, Recruitment advantage of large seeds is greater in shaded habitats: *Ecoscience*, v. 15, p. 498–507, <https://doi.org/10.2980/15-4-3147>.
- Burnham, R.J., and Johnson, K.R., 2004, South American palaeobotany and the origins of neotropical rainforests: *Philosophical Transactions of the Royal Society of London: Series B, Biological Sciences*, v. 359, p. 1595–1610, <https://doi.org/10.1098/rstb.2004.1531>.
- Carins Murphy, M.R., Jordan, G.J., and Brodribb, T.J., 2014, Acclimation to humidity modifies the link between leaf size and the density of veins and stomata: *Plant, Cell & Environment*, v. 37, p. 124–131, <https://doi.org/10.1111/pce.12136>.
- Crifo, C., Curran, E.D., Baresch, A., and Jaramillo, C., 2014, Variation in angiosperm leaf vein density have implications for interpreting life form in the fossil record: *Geology*, v. 42, p. 919–922, <https://doi.org/10.1130/G35828.1>.
- Davis, C.C., Webb, C.O., Wurdack, K.J., Jaramillo, C.A., and Donoghue, M.J., 2005, Explosive radiation of Malpighiales supports a mid-Cretaceous origin of modern tropical rain forests: *American Naturalist*, v. 165, p. E36–E65, <https://doi.org/10.1086/428296>.
- Diefendorf, A.F., Mueller, K.E., Wing, S.L., Koch, P.L., and Freeman, K.H., 2010, Global patterns in leaf ¹³C discrimination and implications for studies of past and future climate: *Proceedings of the National Academy of Sciences of the United States of America*, v. 107, p. 5738–5743, <https://doi.org/10.1073/pnas.0910513107>.
- Doria, G., Jaramillo, C.A., and Herrera, F., 2008, Menispermaceae from the Cerrejón Formation, middle to late Paleocene, Colombia: *American Journal of Botany*, v. 95, p. 954–973, <https://doi.org/10.1007/ajb.2007216>.
- Ehleringer, J.R., Field, C.B., Lin, Z.-f., and Kuo, C.-y., 1986, Leaf carbon isotope and mineral composition in subtropical plants along an irradiance cline: *Oecologia*, v. 70, p. 520–526, <https://doi.org/10.1007/BF00379898>.
- Farquhar, G.D., and Sharkey, T.D., 1982, Stomatal conductance and photosynthesis: *Annual Review of Plant Physiology*, v. 33, p. 317–345, <https://doi.org/10.1146/annurev.pp.33.060182.001533>.
- Farquhar, G.D., Ehleringer, J.R., and Hubick, K.T., 1989, Carbon isotope discrimination and photosynthesis: *Annual Review of Plant Physiology and Plant Molecular Biology*, v. 40, p. 503–537, <https://doi.org/10.1146/annurev.pp.40.060189.002443>.
- Feild, T.S., et al., 2011, Fossil evidence for Cretaceous escalation in angiosperm leaf vein evolution: *Proceedings of the National Academy of Sciences of the United States of America*, v. 108, p. 8363–8366, <https://doi.org/10.1073/pnas.1014456108>.
- Foster, G.L., Royer, D.L., and Lunt, D.J., 2017, Future climate forcing potentially without precedent in the last 420 million years: *Nature Communications*, v. 8, 14845, <https://doi.org/10.1038/ncomms14845>.
- Franks, P.J., and Beerling, D.J., 2009, Maximum leaf conductance driven by CO₂ effects on stomatal size and density over geologic time: *Proceedings of the National Academy of Sciences of the United States of America*, v. 106, p. 10,343–10,347, <https://doi.org/10.1073/pnas.0904209106>.
- Franks, P.J., et al., 2013, Sensitivity of plants to changing atmospheric CO₂ concentration: From the geological past to the next century: *New Phytologist*, v. 197, p. 1077–1094, <https://doi.org/10.1111/nph.12104>.
- Franks, P.J., Bonan, G.B., Berry, J.A., Lombardozi, D.L., Holbrook, N.M., Herold, N., and Oleson, K.W., 2018, Comparing optimal and empirical stomatal conductance models for application in Earth system models: *Global Change Biology*, v. 24, p. 5708–5723, <https://doi.org/10.1111/gcb.14445>.
- Graham, H.V., Patzkowsky, M.E., Wing, S.L., Parker, G.G., Fogel, M.L., and Freeman, K.H., 2014, Isotopic characteristics of canopies in simulated leaf assemblages: *Geochimica et Cosmochimica Acta*, v. 144, p. 82–95, <https://doi.org/10.1016/j.gca.2014.08.032>.
- Guerrero, J., 2002, A proposal on the classification of systems tracts: Application to the allostratigraphy and sequence stratigraphy of the Cretaceous Colombian Basin. Part 2: Barremian to Maastrichtian: *Geología Colombiana*, v. 27, p. 27–49.
- Gutiérrez, M., and Jaramillo, C., 2007, Maastrichtian paleotemperature and paleoprecipitation from the Guaduas Formation, Colombia: *Palynology*, v. 32, p. 260.
- Hanba, Y.T., Mori, S., Lei, T.T., Koike, T., and Wada, E., 1997, Variations in leaf δ¹³C along a vertical profile of irradiance in a temperate Japanese forest: *Oecologia*, v. 110, p. 253–261, <https://doi.org/10.1007/s004420050158>.
- Herrera, F.A., Jaramillo, C.A., Dilcher, D.L., Wing, S.L., and Gómez-N., C., 2008, Fossil Araceae from a Paleocene Neotropical rainforest in Colombia: *American Journal of Botany*, v. 95, p. 1569–1583, <https://doi.org/10.3732/ajb.0800172>.
- Herrera, F., Manchester, S.R., Hoot, S.B., Wefferling, K.M., Carvalho, M.R., and Jaramillo, C., 2011, Phytogeographic implications of fossil endocarps of Menispermaceae: *American Journal of Botany*, v. 98, p. 2004–2017, <https://doi.org/10.3732/ajb.1000461>.
- Herrera, F., Manchester, S.R., Carvalho, M.R., Jaramillo, C., and Wing, S.L., 2014, Paleocene wind-dispersed fruits and seeds from Colombia and their implications for early Neotropical rainforests: *Acta Palaeobotanica*, v. 54, p. 197–229, <https://doi.org/10.2478/acpa-2014-0008>.
- Hubick, K.T., Hammer, G.L., Farquhar, G.D., Wade, L.J., von Caemmerer, S., and Henderson, S.A., 1990, Carbon isotope discrimination varies genetically in C₄ species: *Plant Physiology*, v. 92, p. 534–537, <https://doi.org/10.1104/pp.92.2.534>.
- Jaramillo, C.A., Bayona, G., Pardo-Trujillo, A., Rueda, M., Torres, V., Harrington, G.J., and Mora, G., 2007, The palynology of the Cerrejón Formation (Upper Paleocene) of northern Colombia: *Palynology*, v. 31, p. 153–189, <https://doi.org/10.1080/01916122.2007.9989641>.
- Kapos, V., Ganade, G., Matsui, E., and Victoria, R.L., 1993, δ¹³C as an indicator of edge effects in tropical rainforest reserves: *Journal of Ecology*, v. 81, p. 425–432, <https://doi.org/10.2307/2261521>.
- Madhavan, S., Treichel, I., and O'Leary, M.H., 1991, Effect of relative humidity on carbon isotope fractionation in plants: *Botanica Acta*, v. 104, p. 292–294, <https://doi.org/10.1111/j.1438-8677.1991.tb00232.x>.
- Malhi, Y., Meir, P., and Brown, S., 2002, Forests, carbon and global climate: *Philosophical Transactions of the Royal Society: Series A, Mathematical, Physical and Engineering Sciences*, v. 360, p. 1567–1591, <https://doi.org/10.1098/rsta.2002.1020>.
- Medina, E., and Minchin, P., 1980, Stratification of δ¹³C values of leaves in Amazonian rain forests: *Oecologia*, v. 45, p. 377–378, <https://doi.org/10.1007/BF00540209>.
- Muller, J.E., Di Giacomo, E., and Van Erve, A.W., 1987, A Palynological Zonation for the Cretaceous, Tertiary, and Quaternary of Northern South America: *American Association of Stratigraphic Palynologists Contribution* 19, 76 p.
- Porter, A.S., Evans-Fitz Gerald, C., Yirotis, C., Montañez, I.P., and McElwain, J.C., 2019, Testing the accuracy of new paleoatmospheric CO₂ proxies based on plant stable carbon isotopic composition and stomatal traits in a range of simulated paleoatmospheric O₂:CO₂ ratios: *Geochimica et Cosmochimica Acta*, v. 259, p. 69–90, <https://doi.org/10.1016/j.gca.2019.05.037>.
- Reich, P.B., Uhl, C., Walters, M.B., and Ellsworth, D.S., 1991, Leaf lifespan as a determinant of leaf structure and function among 23 Amazonian tree species: *Oecologia*, v. 86, p. 16–24, <https://doi.org/10.1007/BF00317383>.
- Royer, D.L., Wing, S.L., Beerling, D.J., Jolley, D.W., Koch, P.L., Hickey, L.J., and Berner, R.A., 2001, Paleobotanical evidence for near present-day levels of atmospheric CO₂ during part of the Tertiary: *Science*, v. 292, p. 2310–2313, <https://doi.org/10.1126/science.292.5525.2310>.
- Schubert, B.A., and Jahren, A.H., 2012, The effect of atmospheric CO₂ concentration on carbon isotope fractionation in C₃ land plants: *Geochimica et Cosmochimica Acta*, v. 96, p. 29–43, <https://doi.org/10.1016/j.gca.2012.08.003>.
- Soltis, P.S., and Soltis, D.E., 2004, The origin and diversification of angiosperms: *American Journal of Botany*, v. 91, p. 1614–1626, <https://doi.org/10.3732/ajb.91.10.1614>.
- Spicer, R.A., 1981, The sorting and deposition of allochthonous plant material in a modern environment at Sillwood Lake, Sillwood Park, Berkshire, England: *U.S. Geological Survey Professional Paper* 1143, 77 p., <https://doi.org/10.3133/pp1143>.
- Tipple, B.J., Meyers, S.R., and Pagani, M., 2010, Carbon isotope ratio of Cenozoic CO₂: A comparative evaluation of available geochemical proxies: *Paleoceanography*, v. 25, PA302, <https://doi.org/10.1029/2009PA001851>.
- Vogel, J.C., 1978, Recycling of carbon in a forest environment: *Oecologia Plantarum*, v. 13, p. 89–94.
- Watson, R.T., Noble, I.R., Bolin, B., Ravindranath, N.H., Verardo, D.J., and Dokken, D.J., eds., 2000, *Land use, land-use change, and forestry*: Cambridge, UK, Cambridge University Press, Intergovernmental Panel on Climate Change Special Report, 875 p.
- Wing, S.L., Herrera, F., Jaramillo, C.A., Gómez-Navarro, C., Wilf, P., and Labandeira, C.C., 2009, Late Paleocene fossils from the Cerrejón Formation, Colombia, are the earliest record of Neotropical rainforest: *Proceedings of the National Academy of Sciences of the United States of America*, v. 106, p. 18,627–18,632, <https://doi.org/10.1073/pnas.0905130106>.
- Zimmerman, J.K., and Ehleringer, J.R., 1990, Carbon isotope ratios are correlated with irradiance levels in the Panamanian orchid *Catasetum viridiflavum*: *Oecologia*, v. 83, p. 247–249, <https://doi.org/10.1007/BF00317759>.

Printed in USA