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

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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 (δ13C) 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 δ13C, 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 δ13C 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 CO₂ 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 (Solis and Solis, 2004; Davis et al., 2005), except that fossils documenting the morphological and ecological traits common to canopy-forming angiosperms are rare until the Paleocene (Bruan 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 (δ13Cleaf) 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 CO₂ concentrations and 13C-depleted CO₂ (δ13Cair) 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 13C discrimination (2.7‰) than in the canopy. (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 13C discrimination (Zimmerman and Ehleringer, 1980; Madhavan et al., 1991). (4) High light increases the rate of photosynthesis up to four times that of leaves in the understory, and leads to less 13C discrimination (Zimmerman and Ehleringer, 1980; Madhavan et al., 1991). As a result of these pronounced gradients in CO₂ water, and light, closed-canopy forest δ13Cleaf 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 δ13Cleaf 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 δ13Cleaf to estimate canopy structure in leaf fossil assemblages from the Maastrichtian Guaduas Formation and Paleocene Cerrejón Formation of Colombia. We also use fossil δ13Cleaf data in...
combination with the predicted $\delta^{13}C$ values of paleoatmospheric CO$_2$ to determine if photosynthetic fractionation ($\Delta_{leaf}$) differs greatly between these leaves and their modern descendants. $\delta^{13}C_{leaf}$ 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 $\Delta_{leaf}$ 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 $\delta^{13}C_{leaf}$ from three fossil assemblages with that of leaves from modern forests to determine if the $\delta^{13}C_{leaf}$ 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 forest (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 (Gutierrez and Jaramillo, 2007). These assemblages were selected in order to compare reconstructed canopy isotopic 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 an estimated MAT of 22.1 $\pm$ 3.4°C and MAP of $\sim$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 1. Photos of megafossils representing preservation of sampled specimens (see Wing et al. [2009] for Cerrejón Formation, Colombia, 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, CJ6. (E) Fabaceae, CJI. (F) Indeterminate dicot. (G) Dicot, GD14.

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 δ¹³C_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 δ¹³C_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 Peedee belemnite (VPDB). Instrument precision based on reproductibility of standards was 0.09‰ (n = 76), and accuracy (average difference between measured and true δ¹³C 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 δ¹³C_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 δ¹³C_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 δ¹³C_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 −24.7‰ to −27.4‰, (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 habitat (see the GSA Data Repository).

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. 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-CO₂ Concentrations

Fossil δ¹³C_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 CO₂ levels, and taxonomic affiliation on δ¹³C_leaf expression. Further, δ¹³C_leaf...
measured values for individual leaves ranged several percentage units (reflecting Δ_{leaf} variability; Fig. 4), particularly for the closed-canopy flora. Thus, palaeoclimate 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 δ^{13}C_{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 event (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 pCO_{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 palaeoclimate applications, including temperature and CO_{2} proxy studies.

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