

THE EVOLUTION OF BARK MECHANICS AND STORAGE ACROSS HABITATS IN A CLADE OF TROPICAL TREES¹

JULIETA A. ROSELL^{2,4,5} AND MARK E. OLSON³

²Department of Biological Sciences, Macquarie University, North Ryde, New South Wales 2109 Australia; and ³Instituto de Biología, Universidad Nacional Autónoma de México, Tercer Circuito s/n de Ciudad Universitaria, México DF 04510 Mexico

- *Premise of the study:* Bark functional strategies vary conspicuously within communities. As a result, predicting most community-level bark traits based on environment often reveals little association. To complement this community-based view, we took a clade-based approach to study potentially adaptive differences in bark water storage and biomechanics across habitats and examined ontogenetic mechanisms that lead to these differences.
- *Methods:* We studied the branches of nine species in the *simaruba* clade of *Bursera* in dry to wet, fire-free neotropical forests. We measured mechanical properties from branch tips to bases, as well as the relative area and water content of bark. Using raw data and phylogenetically independent contrasts, we then tested predictions regarding trait associations with environment and mapped branch tip-to-base ontogenetic changes.
- *Key results:* Across our wet–dry gradient, bark water storage was greater in drier habitats, whereas bark tissue mechanical rigidity was greater in the taller species of moister forests. Bark was the principal mechanical tissue in branch tips and an important contributor even in branches 3 m long. Within species, bark contributions to mechanical support and water storage came mostly through a tip-to-base increase in bark quantity rather than alterations in tissue properties. Quantitative developmental alterations in proportions of bark to wood led to habit differences.
- *Conclusions:* Our clade-based approach shows that, in marked contrast to most community-based results, environment can strongly predict bark functional traits across species in ways that seem plausibly adaptive.

Key words: adaptation; bark; bark thickness; biomechanics; *Bursera*; dry forest; ecological strategies; trade-offs; trait ecology; water storage.

Bark is a very morphologically diverse and functionally important part of stems (Romero, 2013). Ranging from smooth to rough, scaly, spiny, furrowed, and tessellated, bark is known to play roles in protection from desiccation, pathogens, and fire (Srivastava, 1964; Uhl and Kauffman, 1990; Pinard and Huffman, 1997; Romero and Bolker, 2008; Lawes et al., 2011b). Bark is also involved in photosynthate transport (Jensen et al., 2012), photosynthesis (Pfanzen et al., 2002; Cernusak and Hutley, 2011), storage (Scholz et al., 2007), and stem mechanical support (Niklas, 1999), and it is even likely to intervene in repair of xylem embolism (Nardini et al., 2011).

Various comparative approaches can be applied to study the origin and functional significance of bark diversity across species. One approach involves sampling a wide array of plant lineages, within or across communities (Westoby et al., 2002). This community-based approach has allowed the discovery of fundamental trade-offs and functional coordination within the bark and with other parts of the plant (Poorter et al., 2013; Rosell et al., 2014). The main advantage of this approach is that it provides generality, with patterns potentially stretching across

the woody plants at large. Another approach is a clade-centered one, which focuses on the descendants of a common ancestor. Because all of the species are closely related and share ancestry, any divergence in form or function between them has accrued since divergence from the same ancestral state (Martins, 2000). This approach therefore helps to identify features that potentially represent adaptive changes (Romero et al., 2009; Losos, 2011). It also allows for detailed comparisons of ontogeny to identify the developmental mechanisms underlying morphological and functional divergence between species (Raff, 2000). Here, we use a clade-centered approach to study the evolution of diversity in bark function across a clade of tropical trees with a high diversity in habitat, habit, and bark features. In particular, we focus on the roles of bark in the mechanical support of the stem, and in stem water storage, two potentially vital functions often neglected in favor of study of other bark functions such as fire protection.

We chose as our study system nine species within the *simaruba* clade of the neotropical genus *Bursera* (De-Nova et al., 2012). This clade has as its namesake the gumbo-limbo tree *Bursera simaruba*. With its large stature (to over 30 m tall) and its brilliant red bark, the gumbo-limbo is one of the most conspicuous trees in the lowland (sub)tropics of Florida, the West Indies, Mexico, and Central America. Other members of the clade range from small, water-storing trees of very dry tropical deciduous forests (Figs. 1A, B), to tall species of moister habitats (Rosell et al., 2010). The clade also includes two species of unusual habits, *B. instabilis*, a tree with long, lianescent branches in Mexican tropical dry forests (Fig. 1C), and *B. standleyana*, a primary hemiepiphyte (Zotz, 2013) growing high in rainforest canopies in Costa Rica. The *simaruba* clade is known for its “peeling bark” (Fig. 1E–G). However, only the outermost layer of bark, the phellem (Fig. 1D), peels. Produced

¹Manuscript received 13 March 2014; revision accepted 1 April 2014.

J.A.R. acknowledges CONACYT for funding; F. Sánchez-Sesma and J. López for advice on the mechanics of the materials; C. Villegas for help in building the testing device; R. Coates, J. Vega, and F. Morales for assistance during fieldwork, and R. Medina for help with species identification and fieldwork. The authors are thankful for the support of CONACYT project 132404 and comments of the anonymous reviewers and Associate Editor.

⁴Author for correspondence: (e-mail: julieta.rosell@gmail.com)

⁵Current address: Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 70-275, México Distrito Federal 04510, Mexico

by the cork cambium (Gómez-Vazquez and Engleman, 1984), the phellem comprises just 1–2% of the total bark thickness in main trunks. Although bark in the clade is always very thick, made up of mostly secondary phloem and phloem rays (Fig. 1D), there is wide variation in thickness across species (Fig. 1H, I). The extensive environmental and morphological range in the *simaruba* clade, all derived from a single common ancestor, allowed us to examine both the way that the environment is associated with bark functional variation and the developmental differences leading to functional diversity in the bark of these species. Moreover, the neotropical dry and wet forests that the *simaruba* clade inhabits do not experience natural fire as a significant selective factor. In these sites, fire is absent or returns at such wide intervals that it has never been recorded (Janzen, 1988; Murphy and Lugo, 1995; Maas et al., 2002; Pennington et al., 2009; Cantarello et al., 2011; Sánchez-Azofeifa and Portillo-Quintero, 2011). No species in these forests exhibits fire-adaptive traits (Pennington et al., 2009) such as serotiny, and there is no evidence of burning in the vegetation (e.g., burned stems, resprouting stumps; Maas et al., 2002; Rodríguez Trejo, 2008). Furthermore, the genus *Bursera* has been shown to have radiated within seasonally dry tropical forests, the same fire-free habitats they occupy today (De-Nova et al., 2012), ruling out the possibility that bark traits in the *simaruba* clade have been shaped evolutionarily by fire. As a result, we can in effect factor out fire as an important selective pressure on the bark of the *simaruba* clade, allowing us to focus on the role of mechanics and storage functions in the wide variation in bark traits of the clade.

We started by examining patterns in bark mechanics and water storage across habitats. Most explanations for variation in bark traits in the current ecological literature appeal to adaptation to environmental conditions. For example, variation in bark thickness is usually explained by differences in fire regime (Gill and Ashton, 1968; Catry et al., 2010; Lawes et al., 2011a). Although bark can be thick in large trees of areas that burn frequently, such as savannas, recent cross-community comparative studies have revealed that bark traits generally vary more within than across communities (Rosell et al., 2014). This diversity reflects the mixture of ecological strategies of the different lineages that make up any given community (Falster and Westoby, 2003). In contrast, members of a single lineage are likely to respond to selection through a similar set of strategies. In our case, all species of the *simaruba* clade have bark of generally similar structure, but very different proportions across species. For this reason, they represent an ideal system to examine the degree to which variation in bark amount and other bark functional traits might reflect adaptation to environmental conditions.

Here, we tested whether environmental variables predict bark traits associated with storage and mechanics, supporting the idea that environmental selective pressures do mold bark diversity across habitats within the *simaruba* clade. Stem water storage in dry forest species seems crucial to buffer daily and seasonal changes in water availability (Scholz et al., 2011) and to fuel leaf and fruit production (Borchert, 1994; Chapotín et al., 2006). Water stored in bark has been found to contribute in varying degrees to stem capacitance (Zweifel et al., 2000; Zweifel and Häslér, 2001; Scholz et al., 2007; Díaz-Espejo et al., 2013). Given the large volume of water stored in the bark of the *simaruba* clade species, we predicted bark amount to be associated negatively with precipitation and positively with seasonality. Higher water storage has been shown to trade off with mechanical rigidity in wood (Pratt et al., 2007; Chave et al., 2009).

We predicted an analogous trade-off in bark. As a result, we expected a positive association between the rigidity of bark and wood with environmental variables reflecting greater water availability, and a negative one with seasonality variables. We tested these hypotheses with raw data and also taking into account phylogenetic relationships.

Bark plays a vital role in mechanical support and water storage (Romero, 2013), but it is not clear whether differences in performance reflect differences in tissue quantity, tissue performance, or both. As a tree grows, the resistance to bending of the wood in its trunk increases as a result of both its increase in girth (change in tissue quantity, Niklas, 1992), and the production of stiffer wood (change in tissue quality, Van Gelder et al., 2006). In contrast, previous studies have suggested that despite wide variation in bark tissue properties across species, an increase in mechanical performance in bark is achieved mainly by higher amounts of bark, and not so much by changes in tissue properties (Rosell et al., 2014). Here, we investigated the quality–quantity issue in bark storage and mechanics across the species of the *simaruba* clade. We also took an ontogenetic perspective on the issue, testing whether the mechanical and storage contribution of bark changed along branches via bark quantity, or whether tissue properties, as measured by bark Young's modulus and bark water content, also affected these contributions.

Bark must perform its mechanical support role not as a disarticulated tissue, but as a functionally and structurally integrated part of the stem, together with wood (Niklas, 1992). It has been shown that bark and wood covary strongly regarding density and water content (Poorter et al., 2013; Rosell et al., 2014), but it is unclear what this covariation implies for the mechanics of the branch. We compared the contribution of bark and wood to the stiffness of branches across species and habitats and also along branches within species. The *simaruba* clade is a particularly useful system for addressing the mechanical role of bark. Its wide range in environment and size, in addition to its growth habit, including lianescent and hemiephytic, allow us to examine how developmental differences in bark and wood traits can lead to morphological and functional diversity (Speck and Rowe, 2001; Rowe et al., 2006; Rosell et al., 2012). Together with the strong bark–environment associations across species, these developmental data illustrate how our clade-based approach provides an essential complement to community-based studies.

MATERIALS AND METHODS

Localities and sampling—We sampled nine species in eight localities with very different precipitation and temperature regimes ranging from very dry, tropical deciduous forest to rainforest (Table 1, Appendix 1). All collections were performed during the May–October rainy season.

Mechanical traits, percentage areas, and water contents of bark and wood—We collected 7–14 straight leading branches with few side branches from 2–4 trees per species and divided them into 2–8 segments. Branches varied in length from 112 to 383 cm in self-supporting species, and up to 603 cm in the lianescent species. Segments ranged in diameter from more than 5 cm in the oldest ones tested to around 0.5 cm in the segments closer to the tips of branches. Leaves on the sampled branches were restricted to the current year's growth. We measured the distance from the midpoint of each segment to the tip of the branch (hereafter “distance from the tip”) and performed three-point bending tests using a digital micrometer to measure the deflection of the segment caused by adding weights to a bucket suspended at the segment midpoint. Segments were tested within the same day of collection. To minimize shear, segments had 1:20 diameter to length ratios (Vincent, 1992). For each

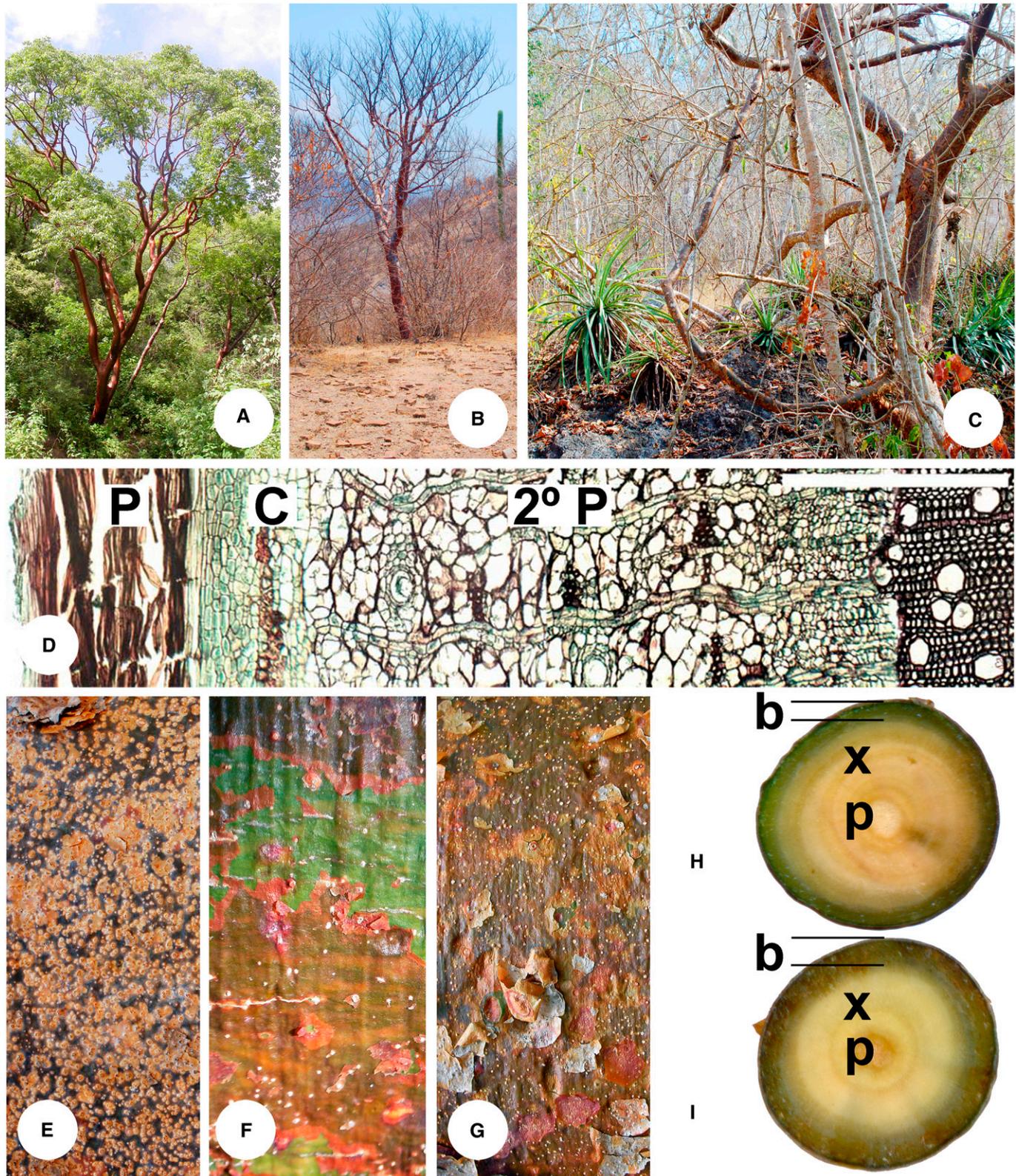


Fig. 1. Habit, habitat, and bark diversity in the *simaruba* clade of *Bursera*. (A) *B. cinerea*, a tree of dry tropical forests, in the wet season. (B) *B. longipes*, a small dry forest tree, leafless in the dry season. (C) *B. instabilis* in Pacific coast dry forest. Note the branches that do not support their own weight, coming to rest on the ground before snaking into the canopy. (D) Light micrograph of a paraffin transection of bark and secondary xylem of a young twig of *B. instabilis*, outer surface at left, secondary xylem at right, to show the structural plan common to the bark of all *simaruba* clade species. Stained with safranin and alcian blue. P = phellem; in older stems the phellem is always very thin relative to the secondary phloem. C = "secondary cortex"; between P

segment, we calculated the flexural stiffness of the whole structure (EI_{struct}) with Eq. 1:

$$EI = \frac{L^3}{48m}, \tag{1}$$

where L is the length of the tested segment between the supports, and m is the slope of the initial linear (elastic or Hookean) portion of the maximum deflection vs. force curve. We debarked the segment and calculated the flexural stiffness of the wood (EI_{wood}) repeating the same procedure. Bark flexural stiffness (EI_{bark}) was inferred from the difference between EI_{struct} and EI_{wood} (Niklas, 1999). We computed second moments of area for the whole branch (I_{struct}), the bark (I_{bark}), and the wood (I_{wood}) using Eqs. 2 to 4 for hollow, circular sections. We used hollow, circular sections assuming that bark is a ring with wood and pith in the center. The same applies to wood and bark + wood, both of which have pith in the middle. That the outer bark in the species of the clade is only a few cells thick obviated the need to distinguish between outer and inner bark in mechanical calculations.

$$I_{\text{struct}} = \frac{\pi}{4}(a_{\text{stem}}^4 - a_{\text{pith}}^4) \tag{2}$$

$$I_{\text{wood}} = \frac{\pi}{4}(a_{\text{wood}}^4 - a_{\text{pith}}^4) \tag{3}$$

$$I_{\text{bark}} = \frac{\pi}{4}(a_{\text{stem}}^4 - a_{\text{wood}}^4), \tag{4}$$

where a represents the radius of the whole branch, the wood, or the pith (Pisarenko et al., 1979). In a very few cases, segment cross sections were elliptical. For these cases, we used Eqs. 5 to 7 to calculate the second moments of area:

$$I_{\text{struct}} = \frac{\pi}{4}(a_{\text{stem}}^3 b_{\text{stem}} - a_{\text{pith}}^3 b_{\text{pith}}) \tag{5}$$

$$I_{\text{wood}} = \frac{\pi}{4}(a_{\text{wood}}^3 b_{\text{wood}} - a_{\text{pith}}^3 b_{\text{pith}}) \tag{6}$$

$$I_{\text{bark}} = \frac{\pi}{4}(a_{\text{stem}}^3 b_{\text{stem}} - a_{\text{wood}}^3 b_{\text{wood}}), \tag{7}$$

where a and b represent the minor and major radii of an ellipse (Pisarenko et al., 1979). In addition to reflecting tissue quantity, these moments of area reflect how well the different tissues are deployed geometrically to resist bending. We measured the major and minor diameters of the whole branch, of the wood, and of the pith with digital calipers, and averaged diameters measured at the apex and the base of each segment. Computation of the Young's modulus, an index of the capacity of a material to resist bending, of the whole branch (E_{struct}), the bark (E_{bark}), and the wood (E_{wood}) was straightforward after measuring EI and I . Given that plant tissues are composite anisotropic materials (i.e., their mechanical properties change in different directions), the E calculated here through bending tests will likely differ from the E calculated in other directions (Niklas, 1992). However, longitudinal bending performance is indisputably a feature of critical adaptive importance in biological cantilevers such as branches and therefore of greatest relevance here.

We estimated the percentage mechanical contribution of bark to the stiffness of the branch (%BMC) using Eq. 8. We calculated the contribution of wood in a similar way.

$$\%BMC = \frac{EI_{\text{bark}}}{EI_{\text{struct}}} \times 100 \tag{8}$$

For each segment tested mechanically, we calculated bark thickness (BT) and the percentage area occupied by bark (bark % area), wood (wood % area), and pith (pith % area) in cross section based on the average of apical and basal diameters of these tissues. Bark thickness was equal to the difference between outer and wood diameter divided by two. We preferred percentage over raw areas to reflect the relative investment of the branch in each tissue. Moreover, species showed marked differences in branch allometry, with species from dry environments having shorter and thicker branches than those of moister habitats, making comparison of raw areas difficult for a given distance from the tip.

We used percentage water content in the bark (%BWC) and the wood (%WWC) to reflect water storage capacity. Percentage water content reflects the amount of water per unit of dry mass (Domec and Gartner, 2002) and has been found to covary closely with water storage physiological traits such as capacitance (Ogburn and Edwards, 2012). Water content traits were measured in the laboratory from samples fixed in 70% ethanol taken from the basal portion of the branches tested mechanically. We rehydrated the samples for 2 h and weighed them after removing excess water. We then dried the samples at 100°C until constant mass was reached (Williamson and Wiemann, 2010). We calculated percentage bark water content using Eq. 9.

$$\%BWC = \frac{\text{saturated mass} - \text{dry mass}}{\text{dry mass}} \times 100 \tag{9}$$

We calculated percentage wood water content (%WWC) in a similar way. Water contents were not available for *B. standleyana*.

Three data sets were generated. The data set including the trait values of all the segments measured, hereafter referred to as the “ontogenetic” data set, was used to analyze the change of bark and wood traits along branches and across species. From this large data set, we selected segments between 100 and 140 cm from the tip to reflect the traits of mature bark and wood and assembled the second data set, hereafter referred to as the “mature segments” data set. We selected this range of distance from the tip to maximize the number of mature segments available across species. This data set was used to test correlation hypotheses of bark and wood traits. The final data set, named “species means”, included the mean trait values per species of the segments in the “mature segments” data set. Species means were used to test hypotheses of trait–environment associations.

Correlations between bark and wood traits and the environment—To estimate trait–environment associations, we extracted 19 environmental variables from the database WorldClim v.1.4 (Hijmans et al., 2005) using the coordinates of sampled localities (Appendix S1, see Supplemental Data with the online version of this article). Given that functional traits are likely to change with distance from the tip, we estimated Pearson correlations between traits and environmental variables based on the “species means” data set. Precipitation variables were \log_{10} -transformed to achieve linear relationships.

To take into account phylogenetic nonindependence in correlation estimations potentially reflecting adaptation, we calculated phylogenetically independent contrasts (PIC) for traits and environmental variables (Felsenstein, 1985). We used the phylogeny of Rosell et al. (2012) and the R package ape (Paradis et al., 2004) to calculate contrasts. We performed all analyses in R v.3.02 (R Development Core Team, 2013).

Bark quantity vs. quality in the contribution to mechanics and water storage—We asked whether variation in bark mechanics across species was accounted for by variation in E_{bark} , a tissue level property, or whether it was mostly attributable to variation in bark quantity. To do so, we compared the

← and C is the phellogen and a few layers of phelloderm. 2°P = secondary phloem; active phloem is made up of the smaller cells close to the xylem/cambium. Two phloem rays reach the outer secondary phloem; they become sinuous because of the death and crushing of the phloem. A gum canal can be seen in the outer part of the secondary phloem. Scale bar = 500 μm. (E–G) External views of bark of (E) *B. attenuata* and (F, G) *B. simaruba*; the axial direction is left to right. (H, I) Photographs showing the relative bark proportions in branches of (H) *B. arborea* and (I) *B. instabilis* in branchlets 1.5 cm in diameter; b = bark, x = xylem, p = pith.

TABLE 1. Localities (coordinates), vegetation type, and traits measured for the nine sampled species; for each trait, the mean value and range are reported based on segments between 100 and 140 cm from the tip of branches.

Trait	Abbreviation (unit)	<i>B. arborea</i>	<i>B. attenuata</i>	<i>B. cinerea</i>	<i>B. grandifolia</i>	<i>B. instabilis</i>	<i>B. longipes</i>	<i>B. roseana</i>	<i>B. simaruba</i>	<i>B. standleyana</i>
Locality	—	19.51 N 105.04 W	21.52 N 104.97 W	17.45 N 96.81 W	18.74 N 99.19 W	19.49 N 105.04 W	18.00 N 99.54 W	19.27 N 102.08 W	18.58 N 95.07 W	9.68 N 84.4 W
Vegetation	—	TDF	TSDF	TDF	TDF	TDF	TDF	TSDF	RF	RF
Branch diameter	D (cm)	1.65 (1.22–2.12)	2.38 (1.77–3.55)	2.23 (1.45–3.04)	2.27 (2.17–2.4)	1.58 (1.16–1.92)	1.94 (1.69–2.3)	2.08 (1.43–2.61)	2.12 (1.42–3.25)	1.09 (0.72–1.36)
Bark thickness	BT (mm)	1.7 (1.3–2.3)	2.9 (1.8–4.1)	2.9 (1.9–4.3)	3.5 (3.2–3.7)	1.3 (0.5–2.1)	2.5 (1.8–3)	2.3 (1.7–2.8)	1.7 (1.2–2.1)	0.8 (0.5–1.1)
Bark percent area	%BA (%)	37.4 (30.2–47.1)	42.9 (29.7–50.5)	45.6 (39–48.9)	51.5 (48.3–54)	30.6 (12.4–45.1)	45.4 (37.1–52)	40.4 (36.9–44.1)	30.2 (21–49.7)	28.5 (23.5–33.1)
Wood percent area	%WA (%)	61.4 (51.8–69.1)	54.2 (45.2–68.8)	53.2 (48.5–60.1)	46.4 (42.8–50.1)	68.4 (54.2–87.1)	53.6 (47.2–61.7)	56.3 (51.0–60.3)	65.9 (45.0–77.3)	68.7 (61.1–74.8)
Bark Young's modulus	E_{bark} (GN/m ²)	0.45 (0.05–0.88)	0.49 (0.22–1.23)	0.22 (0.05–0.43)	0.13 (0.06–0.25)	0.61 (0.12–0.97)	0.23 (0.04–0.38)	0.45 (0.19–0.66)	0.49 (0.28–0.84)	0.7 (0.32–1.29)
Structural Young's modulus	E_{struct} (GN/m ²)	1.51 (0.8–3.69)	1.45 (0.81–2.4)	0.89 (0.57–1.62)	0.42 (0.27–0.57)	0.96 (0.4–1.58)	0.69 (0.08–1.56)	1.25 (1.09–1.51)	2.45 (1.55–3.92)	3.1 (1.66–4.43)
Wood Young's modulus	E_{wood} (GN/m ²)	3.55 (1.88–7.55)	3.68 (2.59–5.53)	2.5 (1.66–4.07)	1.34 (0.85–1.95)	1.42 (0.78–2.81)	2.08 (1.33–3.38)	2.7 (1.95–3.27)	4.24 (2.19–5.79)	4.94 (3.1–5.64)
Bark mechanical contribution	%BMC (%)	14.5 (3.5–27)	20.6 (9–33.4)	16.8 (4.3–31.5)	25.1 (8.1–48.7)	31.4 (20.5–63.1)	28.1 (14.6–43.4)	23.3 (10.6–39.4)	10.8 (7.2–18.8)	10.7 (6.1–17.5)
Bark water content	%BWC (%)	294.5 (226.5–351)	453.7 (381.3–548.1)	401.3 (314.4–469.1)	353.7 (318.1–400)	342.3 (270–435.7)	184.5 (150.2–211.8)	358.2 (273.9–489.7)	317.3 (270.2–370.8)	—
Wood water content	%WWC (%)	182.3 (172.6–192.9)	228.4 (204–242.7)	190.6 (143.6–229.2)	160.6 (151.9–169)	216.4 (181.7–234.6)	157.6 (134.7–177.5)	204.3 (142.8–260.4)	217.2 (157.3–262.5)	—

Notes: TDF = tropical dry forest, TSDF = tropical subdeciduous forest, RF = rainforest.

correlations of the mechanical contribution of bark with traits reflecting bark amount, such as bark thickness, bark % area, and E_{bark} , vs. the correlations with E_{bark} . We based all these correlations on the “mature segments” data set. This data set was also the basis for testing for the presence of a mechanics–storage trade-off in bark.

We also examined the effect of quantity vs. quality from an ontogenetic point of view. We calculated correlations of traits reflecting bark amount (see above), and traits reflecting bark tissue mechanics (E_{bark}) and storage capacity (%BWC) with distance from the tip. We calculated these correlations with all species pooled, but also within each species. Variables were \log_{10} -transformed to achieve linearity.

Bark vs. wood contributions to branch stiffness—After having examined the way that bark mechanical behavior changes across species and along branches, we then turned to the relative contributions of bark and wood to branch stiffness. Given the importance of tissue quantity for mechanical performance, we started by comparing the relative investment of bark vs. wood across species. We calculated the distance from the tip of branches where bark and wood had the same percentage area (“area inversion point”). This distance pinpointed the part of the branch at which the relative abundance of bark and wood was inverted. Older portions from this point had more wood, whereas younger portions of branches had more bark. We used linear models per species fitted on \log_{10} -transformed variables to find this distance per species. We quantified the relative contribution of bark and wood in a similar way. We fitted linear models based on \log_{10} -transformed data to calculate the distance for which both tissues had an equal contribution to flexural stiffness of the branch. We called this distance the “mechanical contribution inversion point.” Again, this point divides the branch in two portions, an older one, in which wood is the main contributor to structural flexural stiffness, and a younger one, in which bark has a larger contribution. We compared the area and mechanical contribution inversion points across species. Finally, we examined the correlation between bark and wood traits across species to shed light on how bark and wood change in a parallel fashion.

RESULTS

We measured traits in 447 segments from 94 branches in nine species, making up the “ontogenetic” data set used for the analyses of trait change along branches. Branches ranged from 112 to 383 cm long in the self-supporting species and up to 603 cm in *B. instabilis*, the lianescent species. This variation in length resulted from the markedly different allometries across species (Rosell et al., 2012). In the species with shorter, thicker branches, older, consequently thicker, segments could not be tested due to limitations of our testing apparatus. Segments between 100 and 140 cm from the tip were grouped in the “mature segments” data set, which had 68 points and for which the descriptive statistics are reported in Table 1.

Correlations between bark and wood traits and the environment—Environmental variables indicated a wide ecological range in the habitats of the *simaruba* clade. For example, annual precipitation ranged from 680 in very dry, tropical forests to 3600 mm in the rainforest. Temperature seasonality also varied markedly, with annual temperature ranges covering from 25°C in a tropical, dry forest with markedly different cooler wet and hot, dry seasons, to 14°C in a relatively uniform rainforest (Appendix S1).

Bark and wood traits were closely associated with environmental variables, as suggested by correlations based on the “species means” data set. As predicted, traits reflecting the amount of bark, such as bark thickness and bark % area, were tightly and positively associated with temperature seasonality variables, and negatively with precipitation ones (Table 2; Appendix S2, see online Supplemental Data), suggesting greater allocation to water storage in dry areas. Bark thickness increased with the annual ($r = 0.91$, $r_{\text{PIC}} = 0.89$, $P < 0.001$, Fig. 2A) and

TABLE 2. Pearson correlations between bark and wood traits and environmental variables based on mean traits for nine species of *Bursera* (see Table 1).

Variable	BT	%BA	E_{bark}	E_{struct}	E_{wood}	%BMC	%BWC	%WWC
Ppt coldest qtr ^a	-0.63	-0.71*	0.63	0.94***	0.82**	-0.72*	0.10	0.55
Ppt warmest qtr ^a	-0.56	-0.71*	0.74*	0.75*	0.68*	-0.48	0.28	0.73*
Ppt driest qtr ^a	-0.49	-0.58	0.34	0.77*	0.64	-0.69*	-0.15	0.21
Ppt wettest qtr ^a	-0.60	-0.73*	0.73*	0.86***	0.73*	-0.51	0.14	0.69
Ppt seasonality ^a	0.51	0.58	-0.35	-0.8*	-0.67*	0.73*	0.16	-0.17
Ppt driest month ^a	-0.32	-0.48	0.33	0.77*	0.70*	-0.76*	0.23	0.43
Ppt wettest month ^a	-0.62	-0.76*	0.76*	0.86***	0.74*	-0.53	0.15	0.71*
Annual ppt ^a	-0.60	-0.71*	0.64	0.9***	0.76*	-0.62	0.06	0.57
Mean T coldest qtr	-0.67*	-0.5	0.36	0.31	0.12	0.00	-0.82*	-0.35
Mean T warmest qtr	-0.44	-0.3	0.13	0.1	-0.05	0.13	-0.82*	-0.4
Mean T driest qtr	-0.58	-0.47	0.26	0.32	0.1	-0.05	-0.76*	-0.34
Mean T wettest qtr	-0.5	-0.43	0.28	0.15	0.02	0.07	-0.60	-0.14
Annual range in T	0.91***	0.95***	-0.83**	-0.90***	-0.75*	0.64	0.08	-0.57
Min T coldest month	-0.83*	-0.76*	0.58	0.67*	0.47	-0.35	-0.68	-0.03
Max T warmest month	0.1	0.28	-0.38	-0.35	-0.44	0.45	-0.79*	-0.77*
T seasonality	0.73*	0.57	-0.57	-0.54	-0.34	0.24	0.42	0.15
Isothermality	-0.43	-0.23	0.41	0.16	0.05	0.05	0.03	-0.21
Mean diurnal range	0.79*	0.89***	-0.72*	-0.86***	-0.75*	0.66*	0.09	-0.57
Mean annual T	-0.59	-0.45	0.29	0.23	0.05	0.05	-0.8*	-0.34

Notes: Ppt = precipitation, qtr = quarter, T = temperature, Max = maximum, Min = minimum; other abbreviations as in Table 1; *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; see Appendix S2 for the equivalent correlations based on phylogenetically independent contrasts.

^aLog₁₀-transformed.

diurnal ranges in temperature ($r = 0.79$, $r_{PIC} = 0.75$, $P < 0.05$), as well as bark % area ($r = 0.95$ and 0.89 , $r_{PIC} = 0.92$ and 0.85 , $P < 0.01$). Bark % area decreased with precipitation variables such as the precipitation of the wettest month ($r = -0.76$, $r_{PIC} = -0.74$, $P < 0.05$, Fig. 2B) or annual precipitation ($r = -0.71$, $P < 0.05$; $r_{PIC} = -0.55$, $P > 0.05$).

Mechanical traits also changed across environments as predicted. The Young’s modulus of the whole branch, the bark, and the wood increased with precipitation of the warmest and wettest quarters of the year ($0.68 \leq r \leq 0.86$, $0.70 \leq r_{PIC} \leq 0.87$, $P < 0.05$) and decreased with annual and diurnal ranges in temperature ($-0.90 \leq r \leq -0.72$, $-0.85 \leq r_{PIC} \leq -0.72$, $P < 0.05$, Fig. 2C). The mechanical contribution of bark decreased in wetter sites (with precipitation of the driest month, $r = -0.76$, $P < 0.05$; $r_{PIC} = -0.52$, $P > 0.05$, Fig. 2D) but increased in more seasonal sites (with mean diurnal range $r = 0.66$, $P < 0.05$; $r_{PIC} = 0.35$, $P > 0.05$).

Contrary to our expectations, bark tended to have higher water content in sites with lower mean temperatures (with mean temperature of the coldest quarter, $r = -0.82$, $r_{PIC} = -0.79$, $P < 0.05$), and this content was not associated with precipitation (Table 2). Also in disagreement with our predictions, wood tended to have more water in conditions of higher precipitation (with precipitation in the warmest quarter and wettest month, $r \geq 0.71$, $P < 0.05$; $r_{PIC} \geq 0.66$, $P > 0.05$), and less water in warmer weather (with maximum temperature of the warmest month, $r = -0.77$, $P < 0.05$; $r_{PIC} = -0.64$, $P > 0.05$).

Bark quantity vs. quality in bark contribution to mechanics and water storage—Bark traits reflecting bark quantity, such as bark thickness and % area, were closely associated with one another ($r = 0.81$, $P < 0.001$, Table 3), and also with I_{bark} , a trait also reflecting the amount of bark from a mechanical perspective ($r_{I_{bark}-BT} = 0.88$, $r_{I_{bark}-Bark\%area} = 0.44$, $P < 0.001$). The quantity of bark was negatively associated with bark mechanical quality ($r_{E_{bark}-BT} = -0.70$, Fig. 3, and $r_{E_{bark}-I_{bark}} = -0.59$, $P < 0.001$).

Across species, bark achieved a higher contribution to branch stiffness more through changes in bark quality than bark quan-

tity. Across species, stiffness (quality) was more closely correlated with higher Young’s modulus ($r_{E_{bark}-\%BMC} = 0.26$, $P > 0.05$) than with the amount (thickness) of bark or its moment of area ($r_{\%BMC-BT} = 0.14$, $r_{\%BMC-I_{bark}} = 0.24$, $P > 0.05$, Table 3). In contrast to this across-species pattern, along the ontogeny of a branch, bark’s contribution to stiffness depended more on bark quantity and not quality. The amount of bark (quantity) changed along branches, with bark thickness increasing and bark % area decreasing toward the older portions of the branch. Based on data for all species, bark thickness and distance from the tip were positively associated ($r = 0.47$, $P < 0.001$), whereas bark % area decreased with distance from the tip ($r = -0.60$, $P < 0.001$). These trends were stronger within species, with correlations ranging from 0.61 to 0.85 for bark thickness and -0.57 to -0.83 for bark % area (Table 4). The only exceptions were *B. instabilis*, the lianescent species, and *B. longipes*, a small species of very dry tropical forest, which showed no directional change in bark % area along branches. In contrast with bark quantity (thickness), bark Young’s modulus (quality) did not show an ontogenetic increase with distance from the tip, with the exception of three of the stiffer-branched species (Table 4). In the case of water content (the quality trait reflecting water holding capacities of bark tissue), species had divergent behaviors. Bark water content did not covary with distance in most species; in three species, the water content decreased, whereas in another species this content increased (Table 4). In a majority of species, in both mechanics and water storage, ontogenetically the quantity of bark produced higher contributions than variation in bark quality.

Regarding the predicted mechanics–storage trade-off in bark, we found that more water per unit of dry tissue did not seem to affect E_{bark} ($r = 0.18$, $P > 0.05$). In fact, the water content of bark did not show any significant association with other bark traits. The mechanics–storage trade-off was observed in wood (Table 3). Although wood water content was associated positively with E_{wood} across species ($r = 0.30$, $P > 0.05$), within species these traits had a negative association (correlations per species not shown).

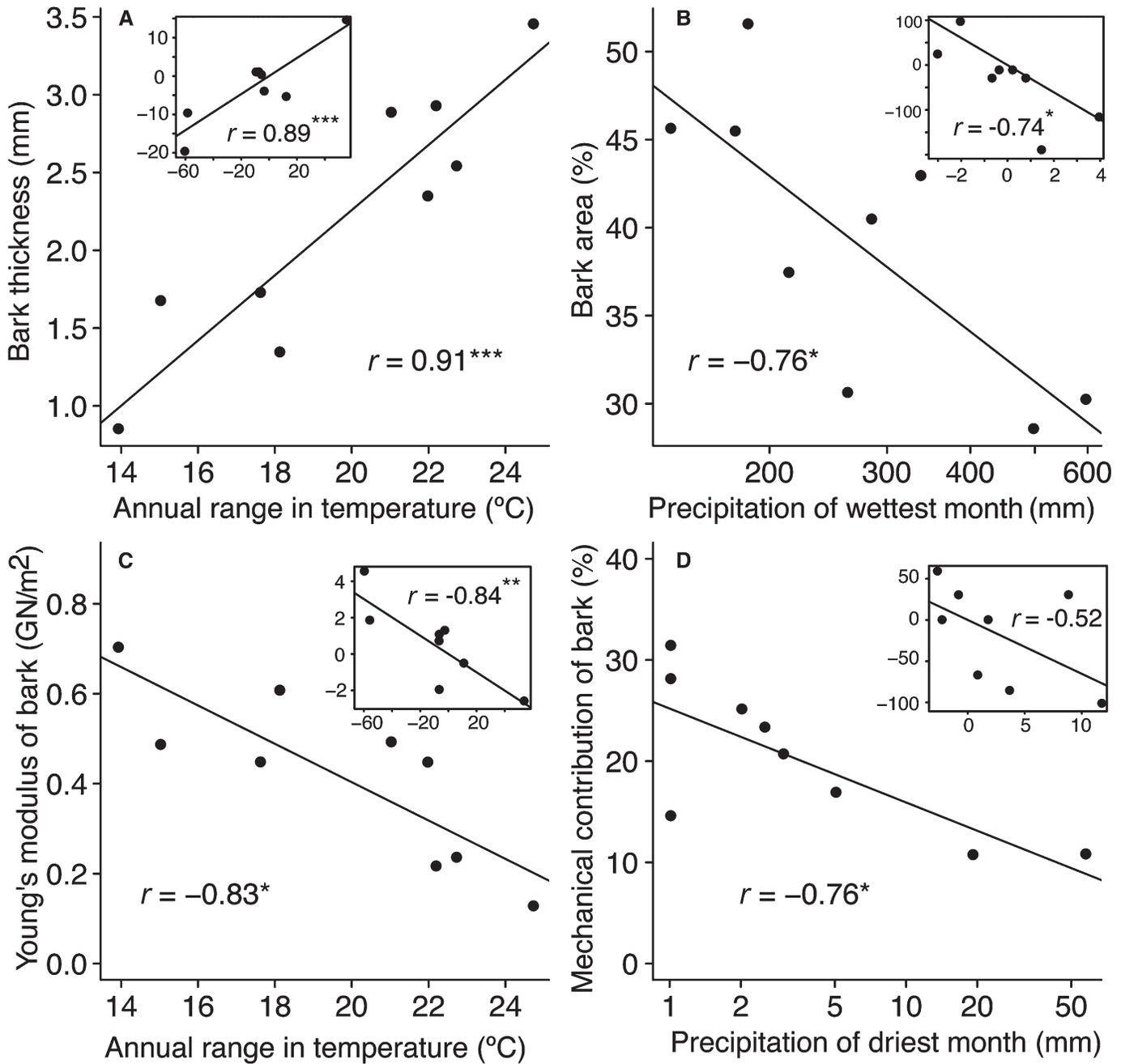


Fig. 2. Correlations between bark traits and environmental variables. Correlations based on phylogenetically independent contrasts (PIC) are shown in insets; *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

Bark vs. wood contributions to branch stiffness—The *simaruba* clade had a very significant investment in bark as indicated by the large percentages of cross-sectional area occupied by this tissue. Percentage bark area ranged across species mean values from 30% to 52% at the base of branches 100–140 cm long (Table 1). This percentage decreased with distance ($r = -0.60$, $P < 0.001$, Table 4), indicating that although bark thickened as branches aged ($r_{BT-Distance} = 0.47$, $P < 0.001$), less bark was produced relative to wood. Figure 4 illustrates the two general ontogenetic trends in percentage areas of bark, wood, and pith observed in the clade. The first

trend is exemplified by *B. cinerea*, a species of very dry sites with abundant bark representing 40–60% of transectional area along the first 3 m of branches (Fig. 4A). In this species, bark was the most abundant tissue up to 80 cm from the tip (Table 4). In contrast, thinner-barked rainforest species *B. simaruba* had more drastic ontogenetic changes in percentage tissue areas. Up to 40 cm from the tip, bark was the most abundant tissue, decreasing to 20% at 3 m from the tip (Fig. 4C). Although pith was abundant in the first 20 cm from the tip in all species, its relative area decreased quickly thereafter.

TABLE 3. Pearson correlations between bark and wood traits based on segments between 100 and 140 cm from the tip.

Variable	BT ^a	%BA	%WA	E_{bark}	E_{struct}	E_{wood}	I_{bark}^a	I_{wood}^a	%BMC ^a	%BWC
%BA	0.81***									
%WA	-0.77***	-0.99***								
E_{bark}	-0.70***	-0.66***	0.61***							
E_{struct}	-0.60***	-0.62***	0.56***	0.66***						
E_{wood}	-0.30*	-0.32*	0.27*	0.36**	0.88***					
I_{bark}^a	0.88***	0.44***	-0.40***	-0.59***	-0.45**	-0.25				
I_{wood}^a	0.61***	0.04	0.01	-0.40**	-0.24	-0.14	0.91***			
%BMC ^a	0.14	0.24	-0.25	0.26*	-0.38**	-0.51***	0.05	-0.04		
%BWC	0.15	0.06	-0.10	0.18	0.02	0.03	0.15	0.11	0.14	
%WWC	-0.26	-0.27	0.19	0.47*	0.39**	0.30*	-0.12	0.01	0.03	0.24

Notes: Abbreviations as in Table 1; *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; $N = 47\text{--}68$ segments depending on trait, due to missing data in some variables.

^aLog₁₀-transformed.

Regarding mechanical traits, we found that both the rigidity of bark and wood at the tissue level, as reflected by Young’s modulus E and their relative contributions to branch stiffness, as reflected by the proportion that each tissue contributed to total segment flexural stiffness EI , changed with ontogeny. Young’s modulus for wood was greater in older segments (based on all data, $r_{E_{\text{wood-Distance}}} = 0.41$, $P < 0.001$; within species, $0.34 \leq r_{E_{\text{wood-Distance}}} \leq 0.76$, $P < 0.05$). In contrast, bark did not become stiffer with ontogeny, with the exception of three species (Table 4, Fig. 5A). In turn, the mechanical contribution of bark to branch stiffness usually decreased with ontogeny ($r = -0.46$, $P < 0.001$, Table 4), with wood quickly taking over the support of branches with stem age. The contribution of bark was larger than that of wood in just the first few centimeters of branches (up to 10 cm from the tip, Table 4), after which wood took over as the main contributor to branch stiffness. However, this does not mean that the mechanical contribution of bark was negligible later in ontogeny. For example, in *B. cinerea*, the contribution of bark was 40% in younger branches to 15% in older segments 3 m from the tip (Fig. 4B). In *B. simaruba*, the contribution of bark started at 30%, decreasing to around 10% in older segments (Fig. 4D). In the lianescent *B. instabilis*, percentage bark area and mechanical contribution varied widely (Fig. 4E, F). Mechanical contribution in this species ranged from 10% to 60%, with a mean of 27% and no steady decrease with ontogeny.

As was the case with mechanical traits, the content of water in bark tended to increase with distance from the tip when all species were pooled ($r = 0.25$, $P < 0.05$), but contrasting trends were observed across species. Water content did not change in four species (Fig. 5B), decreased in three species, and tended to increase in one. Similarly divergent trends were also observed in the water content of wood (Table 4).

Bark traits covaried with some wood traits (Table 3). E_{bark} was positively associated with E_{wood} , meaning that species with more flexible wood also had more flexible bark ($r = 0.36$, $P < 0.01$). Water content of bark and wood tended to be correlated, but not significantly ($r = 0.24$, $P > 0.05$). In contrast, bark and wood covaried strongly in their second moment of area (I , $r = 0.91$, $P < 0.001$) and in their % areas ($r = -0.99$, $P < 0.001$).

DISCUSSION

Our results highlight the complementary nature of the community- and clade-based approaches to study the significance

of bark functional diversity. Community-based approaches have tended to highlight the variable nature of bark and, in general, seem to indicate that environment only weakly predicts important bark functional variables (Paine et al., 2010; Rosell et al., 2014). These perspectives highlight the diversity of bark functional strategies that can coexist in any single locality (Gignoux et al., 1997; Dantas and Pausas, 2013). The clade-based approach taken here, in contrast, reveals bark variation that is predicted very well by environmental variables, with stiffer bark appearing to be favored in the tall species of moister sites, and bark water storage being favored in drier habitats (Table 2, Fig. 2). We first discuss these coarse-scale, across-environment associations before turning to our developmental analyses.

Across the *simaruba* clade, the associations between bark traits and climate seemed congruent with the importance of bark as a site of water storage (Srivastava, 1964; Borchert, 1994; Scholz et al., 2007; Gillison, 2013). With both raw data and PICs, the absolute amount and the percentage of bark was

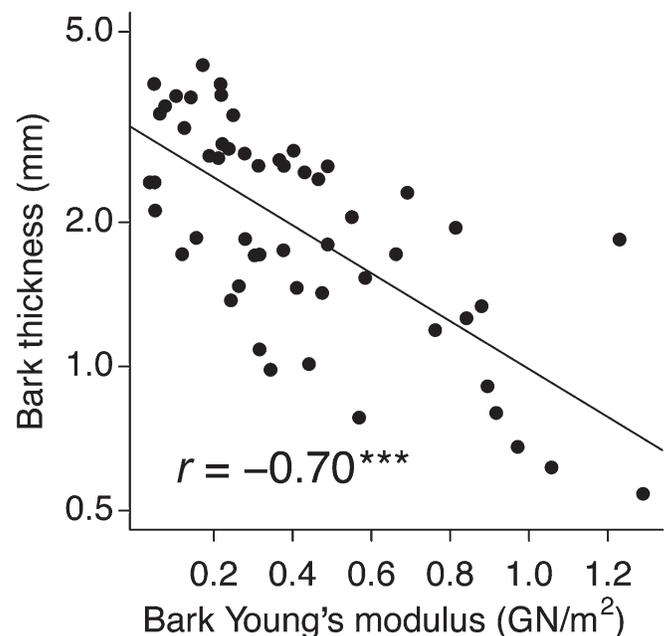


Fig. 3. Correlation between Young’s modulus of bark (E_{bark}) and the amount of bark as reflected by bark thickness. *** $P < 0.001$.

TABLE 4. Pearson correlations between distance to the tip and bark and wood traits (\log_{10} -transformed) to evaluate the ontogenetic change in these traits, and the distance from the tip of the branch where the percentage areas and mechanical contributions of bark and wood were the same (area and mechanical inversion points).

Taxon	N	BT	%BA	%WA	E_{bark}	E_{struct}	E_{wood}	%BMC	%BWC	%WWC	Area inversion point	Mech. inversion point
All <i>Bursera</i> spp.	250–363	0.47***	-0.60***	0.73***	0.24***	0.57***	0.41***	-0.46***	0.25***	-0.08	—	—
<i>B. arborea</i>	16–37	0.85***	-0.73***	0.79***	0.52**	0.68***	0.40*	-0.04	-0.28	-0.70***	39.8	No crossing
<i>B. attenuata</i>	40–50	0.74***	-0.69***	0.84***	0.53***	0.85***	0.76***	-0.62***	-0.38*	0.34*	67.7	1.6
<i>B. cinerea</i>	47–52	0.78***	-0.57***	0.82***	0.04	0.54***	0.34*	-0.46***	0.61***	-0.37**	77.7	0.6
<i>B. grandifolia</i>	9–14	0.61*	-0.72**	0.81***	-0.27	0.4	0.12	-0.61*	0.43	-0.98***	157.2	10.4
<i>B. instabilis</i>	27–38	0.79***	0.19	-0.06	-0.16	-0.25	-0.16	0.05	0.22	0.06	No crossing	No crossing
<i>B. longipes</i>	29–35	0.81***	-0.24	0.38*	0.01	0.3	0.37*	-0.35*	0.05	-0.66***	110.5	7.4
<i>B. roseana</i>	41–49	0.76***	-0.80***	0.83***	0.16	0.74***	0.61***	-0.58***	-0.34*	-0.1	55.8	7.4
<i>B. simaruba</i>	41–48	0.72***	-0.83***	0.89***	0.35*	0.76***	0.56***	-0.63***	-0.40**	-0.22	40.3	0.4
<i>B. standleyana</i>	37–40	0.61***	-0.69***	0.78***	-0.16	0.69***	0.55***	-0.62***	—	—	11.5	1.2

Notes: Abbreviations as in Table 1; *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

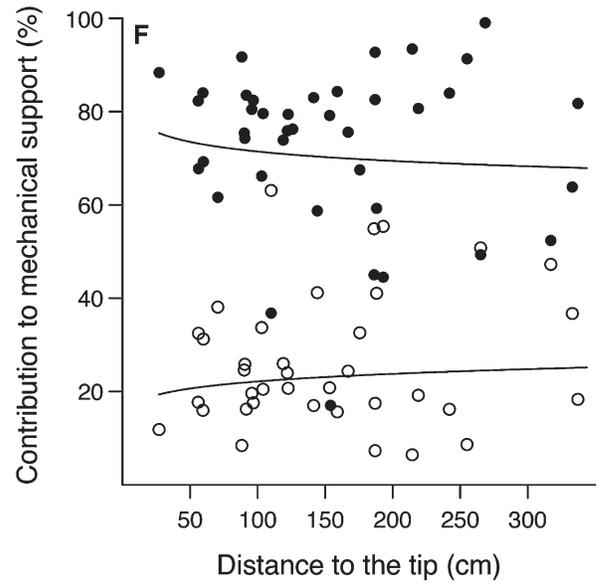
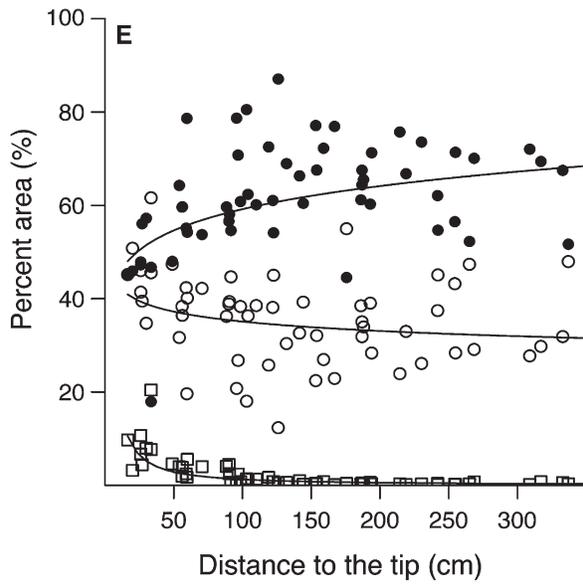
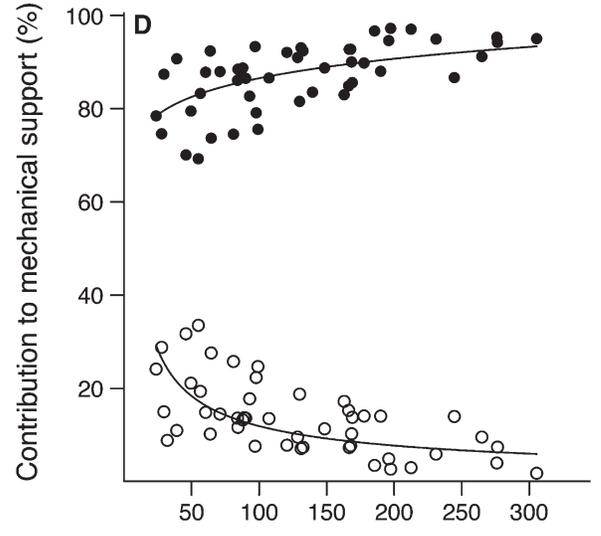
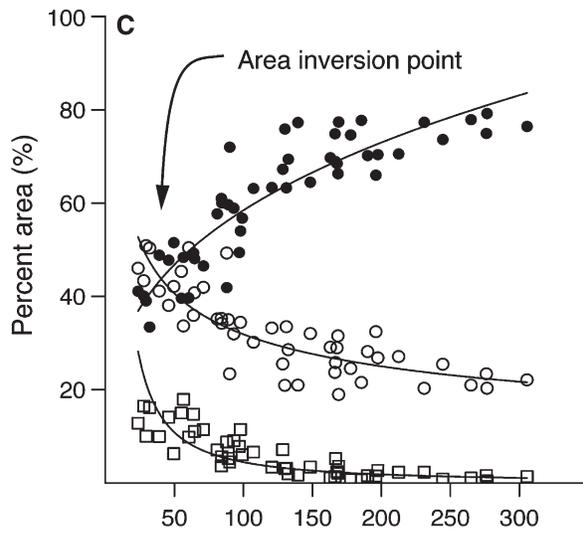
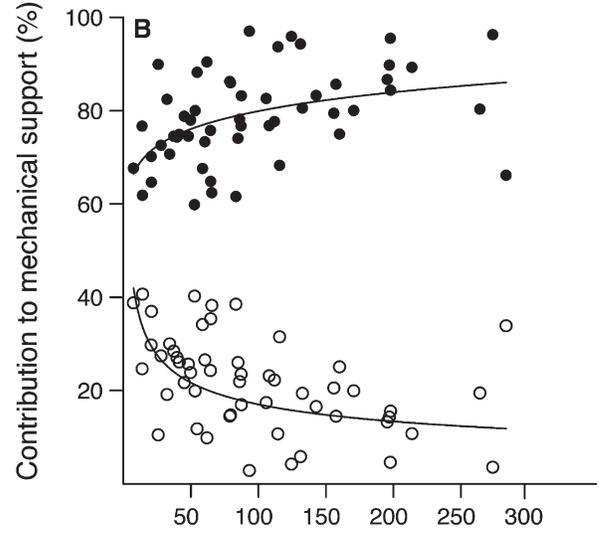
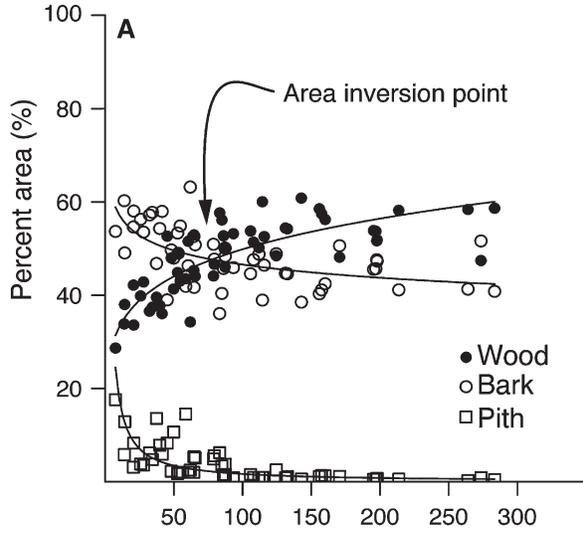
negatively associated with moisture availability, as reflected not only by precipitation variables but also those indicating seasonality (Table 2). For example, annual temperature oscillation is much higher in the seasonally dry, hot, tropical forests, as opposed to lowland rainforests, which have more even temperatures and rainfall throughout the year (Appendix S1). The observed bark–environment correlations suggest that, compared with those of wetter areas, the species of drier areas with more seasonal precipitation had thicker bark and also a higher investment in bark as compared with wood (Fig. 2A,B). The species of dry areas store conspicuous amounts of water and also starch both in the abundant living fibers and ray cells in the xylem, as well as in the bark (Roth, 1981; Gómez-Vazquez and Engleman, 1984). Greater amounts of water-storing bark in dry areas is congruent with the notion that selection favors greater allocation to storage in areas with long dry seasons (see Boyce and Leslie, 2012). We found complementary results with regard to branch mechanics. The species of tall, moist forest tended to have stiffer bark tissue (higher E_{bark}), though the total mechanical contribution of bark was lower in these species than in dry forest species with their very thick bark (Table 1, Fig. 4B, D). The role of other bark functions such as photosynthesis or protection against nonfire agents in variation in bark amount and other bark traits remains to be understood for the *simaruba* clade and for plants in general (Paine et al., 2010). However, the patterns recovered here seem congruent with adaptive variation in water storage and mechanical support along a wet–dry environmental gradient.

The vegetations inhabited by the *simaruba* clade and the evolutionary history of this group allowed us to focus on the functions of water storage and mechanical support, factoring out fire as a relevant selective factor shaping bark traits. Seasonally dry tropical forests, the vegetation in which most of the species of the clade occur, do not experience natural fire (Murphy and Lugo, 1995; Maas et al., 2002; Pennington et al., 2009; Rodríguez Trejo, 2008). Although seasonally dry, these forests lack the grassy understory characterizing seasonal fire-prone environments such as savannas (Mooney et al., 1995). Furthermore,

tropical dry forests completely lack species with fire-adaptive traits such as serotiny or requiring fire for seed germination. Moreover, they usually include fire-sensitive succulents such as columnar cacti or stem-photosynthetic bottle trees (Pennington et al., 2009), whose long-lived epidermis always lack the conspicuous scars they would bear if burned. As a result of this lack of fire adaptation, anthropogenic fire is considered a serious threat to tropical dry forests, given the large-scale damage it can produce (Cantarello et al., 2011; Sánchez-Azofeifa and Portillo-Quintero, 2011). The evolutionary history of *Bursera* unequivocally suggests that radiation of its species occurred within seasonally dry tropical forests, very similar to the ones the species of the clade inhabit today (De-Nova et al., 2012). The species in the genus have not been able to invade fire-prone habitats, but some of them have invaded wetter environments (e.g., *B. simaruba* and *B. standleyana*) and deserts (*B. microphylla*, *B. hindsiana*), areas that also do not experience natural fire on evolutionarily relevant intervals. The studied clade offers an excellent system to begin unraveling the associations between bark traits and environmental conditions distinct from fire. Our clade-based approach also allows for detection of ontogenetic differences of functional significance between species.

Ontogenetic studies confirmed that bark amount is a crucial aspect influencing bark performance across species, including not only mechanics but also storage. In six species, E_{bark} remained constant along the length of branches, even as E_{wood} and I_{bark} increased (Fig. 5A, Table 4). The trend for constant E_{bark} indicates that, although the absolute contribution of bark to branch mechanical support increased with ontogeny, it did so mainly via quantity—the addition of more bark—and not quality (increase in E_{bark}). In the other three species, E_{bark} did increase along the branch length (Table 4). These were all tall wet forest species, which leads us to our cross-species comparisons. Across species, the mechanical contribution of bark was slightly more associated with bark quality than quantity (Table 3). Stiffer barks appear to be associated with moister habitats, where the trees are larger and mechanical support needs presumably

Fig. 4. Ontogenetic change in tissue amount and bark and wood contribution to branch stiffness. (A) Change in the percentage area of bark, wood, and pith, and in (B) the mechanical contribution of bark and wood along the branches of *Bursera cinerea*, a stem succulent of dry sites; change in (C) percentage area of bark and (D) mechanical contributions of the rainforest species *B. simaruba*; change in (E) percentage area of bark and (F) mechanical contributions of linescent *B. instabilis*. Linear regressions based on \log_{10} -transformed variables were fitted to each tissue and are plotted on untransformed axes.



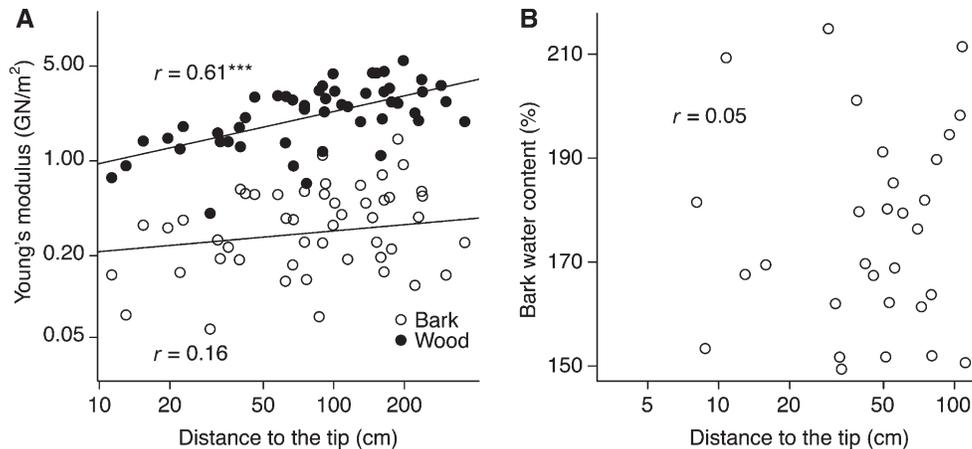


Fig. 5. Ontogenetic change in bark traits. (A) Change in mechanical traits of bark and wood in *Bursera roseana*. Wood becomes stiffer with distance from the tip, but bark does not change significantly and has a much lower Young's modulus than wood throughout the branch. (B) Lack of change in bark water content in *B. longipes*.

greater. With regard to bark water storage, bark water content increased in some species, decreased in others, and was constant along the branch in still others. Our results depict a situation in which both tissue quality and quantity vary. There are predictable increases in bark rigidity with increasing moisture and stature across species, but within species, along the branch the increase in bark contribution to mechanical support is largely due to more bark rather than bark of increasing rigidity.

Even though the mechanical properties of bark tissue vary relatively little in ontogeny, the relative mechanical contribution of bark to the total mechanical stiffness of a branch varies drastically, because the relative proportions of bark vs. wood vary from the stem tip to the base. Bark has a much lower Young's modulus than wood does (see Table 1; Onoda et al., 2010; Paine et al., 2010), so as secondary growth ensues and wood cross-sectional area increases, the proportional contribution of the wood to branch mechanical support increases and surpasses bark, even when I_{bark} is still greater than I_{wood} . However, the vital terminal organs of trees—leaves, flowers, and fruits—are likely generally supported, at least in the terminal 1–10 cm, mainly by bark. Even though it is surpassed in importance by wood after this point, its contribution can still be very important. In some species, bark relative contribution to branch mechanical support is 40% at 3 m from the branch tip. We were not able to measure rupture resistance of bark with our field-testing device. However, bark is known to resist tension and torsion well (Romero, 2013). For this reason, it would be expected that, in addition to contributing to the bending resistance of branches, bark would have an important contribution to the resistance to rupture. Bark is almost never considered an important mechanical tissue (Niklas, 1999), but our results underscore the ecological importance of bark in forest dynamics (Romero, 2013). They also highlight the role of bark ontogenetic changes potentially associated with habit diversification.

The inclusion of a lianescent species, *B. instabilis*, pointed to potential structural and developmental differences between self- and non-self-supporting plants (Speck and Rowe, 2001). In contrast to the divergent behavior of *B. instabilis*, the branches of the hemiepiphyte *B. standleyana* were entirely in line with the behavior of the other species with self-supporting branches. *Bursera standleyana* has long lianescent roots that reach to the

forest floor, but stiff, self-supporting branches (Rosell et al., 2010). In the self-supporting species of the *simaruba* clade, the distance from the branch tip predicted percentage bark area and relative contribution of bark to mechanical support very well (Fig. 4C, D). In contrast, along the long and flexible stems of *B. instabilis*, distance from the branch tip was a poor predictor of these aspects (Table 4, Fig. 4E, F). In self-supporting plants, the base of a tall stem will be subject to greater moments (the tendency to produce rotation about an axis, proportional to the load applied and the distance between the load and the axis) than a small one, and the loads on the bark will be proportionately larger (Gere and Timoshenko, 1999). As a result, selection should favor greater mechanical stiffness and resistance to breakage in both wood and bark. The greater moments at the base of longer stems explain why stem length predicts mechanical properties so well in self-supporting plants (Rosell and Olson, 2007). However, non-self-supporting plants rest on other plants. As a result, neither distance from the branch tip nor branch diameter predicts the moments to which the branches are subjected in *B. instabilis*. This lack of clear mechanical change with distance from the tip is coupled with marked fluctuation in the relative amount of bark and wood along the branch (Fig. 4E). To our knowledge, this is the first time that the mechanical contribution of bark has been assessed in a non-self-supporting plant. It has been shown that ontogenetic changes in whole stem E are closely associated with diversification in plant habit (Speck and Rowe, 2001; Rowe et al., 2004). Given that bark tends to be very abundant in lianas and other non-self-supporting habits, and thus has a strong effect in whole stem E , the role of bark in plant habit diversification deserves further examination (see Rosell et al., 2012 for the role of E_{wood}).

Functional studies of bark also highlight that the emergent mechanical behavior and the storage capacity of a stem is the result of the interaction between bark and wood and that neither of these tissues ever functions alone (Niklas, 1999). Natural selection should act on the mechanical and storage performance of stems as a whole, making understanding the coordination of bark and wood essential (Rosell and Olson, 2007). Congruent with this prediction, we found tissue quality to covary between bark and wood in terms of the Young's modulus of the tissue; the water storage capacity of the bark also showed correlation

in the expected direction with wood water storage but was not significant (Table 3). It has been previously shown that traits affecting these two functions such as density and water content per unit of dry tissue are closely associated between bark and wood (Poorter et al., 2013; Rosell et al., 2014). An unexpected result of the present study was the absence of a storage–mechanics trade-off within bark as reflected by nonsignificant covariation between E_{bark} and bark water content ($r = 0.18$, $P > 0.05$). This trade-off has been previously observed across species in bark (Rosell et al., 2014) and wood (Pratt et al., 2007; Poorter et al., 2010), and even in the wood of the *simaruba* clade (Rosell et al., 2012). It is possible that the range of %BWC and E_{bark} across the species studied was insufficient to detect the trade-off but that it would emerge with wider sampling.

Finally, our results raise the issue of the causes of variation in bark thickness. In the *simaruba* clade, both branch mechanical support and water storage increased with tissue amount. As a result, selection favoring increased bark contribution to branch mechanical support, as well as to bark water storage, would lead to increased bark thickness. For example, the highest bark transectional areas and the lowest Young's modulus for wood in the *simaruba* clade branches were found in the dryland species. These species grow in tropical dry forests that have relatively low rainfall (as low as 679 mm annual ppt), falling in just 4 mo of the year, leaving the landscape hot and dry the rest of the time. In addition to increasing water storage, selection could increase bark amount in these species by favoring an increase in mechanical support of low stiffness-wooded branches, especially in terminal portions. In general, variation in bark thickness has mainly been explained as an adaptive response to fire, and indeed, in tropical savannas, frequent fire seems to be a major selective factor favoring thick bark (Lawes et al., 2013). That mechanics and storage are likely important drivers of bark thickness in the fire-free habitats of the *simaruba* clade suggests that these factors might be also important even in highly seasonal fire-prone habitats. This idea underscores the multitasking nature of bark, with functions such as pathogen and fire protection as well as photosynthesis, water and photosynthate storage, and mechanical support, all representing likely targets of natural selection. A challenge for future studies of bark diversity across habitats is therefore to tie together this multiplicity.

Conclusion—Our study shows the complementarity of the clade- and community-based approaches for studying bark diversity. Taken together, these approaches suggest that the diversity within communities potentially reflects adaptive differences within the structural plans of each lineage. Bark structural plans, understood as the relative arrangements of tissue types and typical developmental patterns, tend to vary relatively little within clades (Roth, 1981). At any given site, variation in bark morphology likely represents to an extent the qualitative and potentially synapomorphic features of the various major lineages that make up the community (e.g., the abundant phloem fibers of Malvaceae; Crivellaro and Schweingruber, 2013). However, though these “plans” often vary relatively little in their qualitative features, their quantitative features are subject to marked variation, as is illustrated by the *simaruba* clade. In this group, variation seems of plausible functional significance, especially variation in bark thickness in the context of water storage and mechanical support. The fact that multiple factors may be involved in the response of bark thickness to natural selection means that attempts to explain bark thickness as a sole response to fire are almost certainly overlooking crucial bark functions.

There is no solution for this but to study multiple bark attributes simultaneously, within clades as well as across communities, to be able to disentangle the often parallel vectors of selection acting on bark as a multitasking structure.

LITERATURE CITED

- BORCHERT, R. 1994. Soil and stem water storage determine phenology and distribution of tropical dry forest trees. *Ecology* 75: 1437–1449.
- BOYCE, C. K., AND A. B. LESLIE. 2012. The paleontological context of angiosperm vegetative evolution. *International Journal of Plant Sciences* 173: 561–568.
- CANTARELLO, E., A. C. NEWTON, R. A. HILL, N. TEJEDOR-GARAVITO, G. WILLIAMS-LINERAB, F. LÓPEZ-BARRERA, R. H. MANON, ET AL. 2011. Simulating the potential for ecological restoration of dryland forests in Mexico under different disturbance regimes. *Ecological Modelling* 222: 1112–1128.
- CATRY, F. X., F. REGO, F. MOREIRA, P. M. FERNANDES, AND J. G. PAUSAS. 2010. Post-fire tree mortality in mixed forests of central Portugal. *Forest Ecology and Management* 260: 1184–1192.
- CERNUSAK, L. A., AND L. B. HUTLEY. 2011. Stable isotopes reveal the contribution of cortical photosynthesis to growth in branches of *Eucalyptus miniata*. *Plant Physiology* 155: 515–523.
- CHAPOTIN, S. M., J. H. RAZANAMEHARIZAKA, AND N. M. HOLBROOK. 2006. Baobab trees (*Adansonia*) in Madagascar use stored water to flush new leaves but not to support stomatal opening before the rainy season. *New Phytologist* 169: 549–559.
- CHAVE, J., D. COOMES, S. JANSEN, S. L. LEWIS, N. G. SWENSON, AND A. E. ZANNE. 2009. Towards a worldwide wood economics spectrum. *Ecology Letters* 12: 351–366.
- CRIVELLARO, A., AND F. SCHWEINGRUBER. 2013. Atlas of wood, bark and pith anatomy of eastern Mediterranean trees and shrubs. Springer, Berlin, Germany.
- DANTAS, V. L., AND J. G. PAUSAS. 2013. The lanky and the corky: Fire-escape strategies in savanna woody species. *Journal of Ecology*.
- DE-NOVA, J. A., R. MEDINA, J. C. MONTERO, A. WEEKS, J. A. ROSELL, M. E. OLSON, L. E. EGUIARTE, ET AL. 2012. Insights into the historical construction of species-rich Mesoamerican seasonally dry tropical forests: The diversification of *Bursera* (Burseraceae, Sapindales). *New Phytologist* 193: 276–287.
- DÍAZ-ESPEJO, A., E. NICOLÁS, P. NORTES, C. M. RODRIGUEZ-DOMINGUEZ, M. V. CUEVAS, A. PEREZ-MARTIN, AND J. M. TORRES-RUIZ. 2013. Xylem functioning and water relations of the elastic living tissue of the bark: New insights about their coordination. *Acta Horticulturae* 991: 163–169.
- DOMEC, J. C., AND B. L. GARTNER. 2002. How do water transport and water storage differ in coniferous earlywood and latewood? *Journal of Experimental Botany* 53: 2369–2379.
- FALSTER, D. S., AND M. WESTOBY. 2003. Plant height and evolutionary games. *Trends in Ecology & Evolution* 18: 337–343.
- FELSENSTEIN, J. 1985. Phylogenies and the comparative method. *American Naturalist* 125: 1–15.
- GERE, J. M., AND S. P. TIMOSHENKO. 1999. Mechanics of materials, 4th SI ed. Nelson Thornes, Cheltenham, UK.
- GIGNOUX, J., J. CLOBERT, AND J.-C. MENAUT. 1997. Alternative fire resistance strategies in savanna trees. *Oecologia* 110: 576–583.
- GILL, A. M., AND D. H. ASHTON. 1968. The role of bark type in relative tolerance to fire of three central Victorian Eucalypts. *Australian Journal of Botany* 16: 491–498.
- GILLISON, A. N. 2013. Plant functional types and traits at the community, ecosystem and world level. In E. van der Maarel and J. Franklin [eds.], *Vegetation ecology*, 347–386. Wiley-Blackwell, Chichester, UK.
- GÓMEZ-VAZQUEZ, B. G., AND E. M. ENGLEMAN. 1984. Bark anatomy of *Bursera longipes* (Rose) Standley and *Bursera copallifera* (Sessé & Moc.) Bullock. *Iawa Bulletin* 5: 335–340.
- HIJMANS, R. J., S. E. CAMERON, J. L. PARRA, P. G. JONES, AND A. JARVIS. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1965–1978.
- JANZEN, D. H. 1988. Management of habitat fragments in a tropical dry forest: Growth. *Annals of the Missouri Botanical Garden* 75: 105–116.

- JENSEN, K. H., J. LIESCHE, T. BOHR, AND A. SCHULZ. 2012. Universality of phloem transport in seed plants. *Plant, Cell & Environment* 35: 1065–1076.
- LAWES, M. J., A. HYLTON, J. RUSSELL-SMITH, B. MURPHY, AND J. J. MIDGLEY. 2011a. How do small savanna trees avoid stem mortality by fire? The roles of stem diameter, height and bark thickness. *Ecosphere* 2: art42.
- LAWES, M. J., J. J. MIDGLEY, AND P. J. CLARKE. 2013. Costs and benefits of relative bark thickness in relation to fire damage: A savanna/forest contrast. *Journal of Ecology* 101: 517–524.
- LAWES, M. J., A. RICHARDS, J. DATHE, AND J. J. MIDGLEY. 2011b. Bark thickness determines fire resistance of selected tree species from fire-prone tropical savanna in north Australia. *Plant Ecology* 212: 2057–2069.
- LOSOS, J. B. 2011. Seein the forest for the trees: The limitations of phylogenies in comparative biology. *American Naturalist* 177: 709–727.
- MAAS, J. M., A. MARTÍNEZ-YRÍZAR, C. PATIÑO, AND J. SARUKHÁN. 2002. Distribution and annual net accumulation of above-ground dead phytomass and its influence on throughfall quality in a Mexican tropical deciduous forest ecosystem. *Journal of Tropical Ecology* 18: 821–834.
- MARTINS, E. 2000. Adaptation and the comparative method. *Trends in Ecology & Evolution* 15: 296–299.
- MOONEY, H. A., S. H. BULLOCK, AND E. MEDINA. 1995. Introduction. In S. H. Bullock, H. A. Mooney, and E. Medina [eds.], *Seasonally dry tropical forests*, 1–8. Cambridge University Press, Cambridge, UK.
- MURPHY, P. G., AND A. E. LUGO. 1995. Dry forests of Central America and the Caribbean. In S. H. Bullock, H. A. Mooney, and E. Medina [eds.], *Seasonally dry tropical forests*, 9–34. Cambridge University Press, Cambridge, UK.
- NARDINI, A., M. A. LO GULLO, AND S. SALLEO. 2011. Refilling embolized xylem conduits: Is it a matter of phloem unloading? *Plant Science* 180: 604–611.
- NIKLAS, K. J. 1992. *Plant biomechanics: An engineering approach to plant form and function*. University of Chicago Press, Chicago, Illinois, USA.
- NIKLAS, K. J. 1999. The mechanical role of bark. *American Journal of Botany* 86: 465–469.
- OGBURN, R. M., AND E. J. EDWARDS. 2012. Quantifying succulence: A rapid, physiologically meaningful metric of plant water storage. *Plant, Cell & Environment* 35: 1533–1542.
- ONODA, Y., A. E. RICHARDS, AND M. WESTOBY. 2010. The relationship between stem biomechanics and wood density is modified by rainfall in 32 Australian woody plant species. *New Phytologist* 185: 493–501.
- PAINE, C. E. T., C. STAHL, E. A. COURTOIS, S. PATIÑO, C. SARMIENTO, AND C. BARALOTO. 2010. Functional explanations for variation in bark thickness in tropical rain forest trees. *Functional Ecology* 24: 1202–1210.
- PARADIS, E., J. CLAUDE, AND K. STRIMMER. 2004. APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics (Oxford, England)* 20: 289–290.
- PENNINGTON, R. T., M. LAVIN, AND A. OLIVEIRA-FILHO. 2009. Woody plant diversity, evolution, and ecology in the tropics: perspectives from seasonally dry tropical forests. *Annual Review of Ecology Evolution and Systematics* 40: 437–457.
- PFANZ, H., G. ASCHAN, R. LANGENFELD-HEYSER, C. WITTMANN, AND M. LOOSE. 2002. Ecology and ecophysiology of tree stems: Corticular and wood photosynthesis. *Naturwissenschaften* 89: 147–162.
- PINARD, M. A., AND J. HUFFMAN. 1997. Fire resistance and bark properties of trees in a seasonally dry forest in eastern Bolivia. *Journal of Tropical Ecology* 13: 727–740.
- PISARENKO, G. S., A. P. YÁKOVLEV, AND V. V. MATVÉEV. 1979. *Manual de resistencia de materiales*. Mir Publishers, Moscow, Russia.
- POORTER, L., I. McDONALD, A. ALARCÓN, E. FICHTLER, J. LICONA, M. PEÑA-CLAROS, F. STERCK, ET AL. 2010. The importance of wood traits and hydraulic conductance for the performance and life history strategies of 42 rainforest tree species. *New Phytologist* 185: 481–492.
- POORTER, L., A. MCNEIL, V. H. HURTADO, H. PRINS, AND J. PUTZ. 2013. Bark traits and life history strategies of tropical dry- and moist forest trees. *Functional Ecology*.
- PRATT, R. B., A. L. JACOBSEN, F. W. EWERS, AND S. D. DAVIS. 2007. Relationships among xylem transport, biomechanics and storage in stems and roots of nine Rhamnaceae species of the California chaparral. *New Phytologist* 174: 787–798.
- R Development Core Team. 2013. R: A language and environment for statistical computing, version 3.0.2.
- RAFF, R. A. 2000. Evo-devo: The evolution of a new discipline. *Nature Reviews. Genetics* 1: 74–79.
- RODRÍGUEZ TREJO, D. A. 2008. Fire regimes, fire ecology, and fire management in Mexico. *Ambio* 37: 548–556.
- ROMERO, C. 2013. Bark structure and functional ecology. In A. B. Cunningham, B. M. Campbell, and M. K. Luckert [eds.], *Bark: Use, management, and commerce in Africa*, vol. 17, *Advances in economic botany*, 5–25. New York Botanical Garden Press, Bronx, New York, USA.
- ROMERO, C., AND B. M. BOLKER. 2008. Effects of stem anatomical and structural traits on responses to stem damage: An experimental study in the Bolivian Amazon. *Canadian Journal of Forest Research* 38: 611–618.
- ROMERO, C., B. M. BOLKER, AND C. E. EDWARDS. 2009. Stem responses to damage: The evolutionary ecology of *Quercus* species in contrasting fire regimes. *New Phytologist* 182: 261–271.
- ROSELL, J. A., S. M. GLEASON, R. MÉNDEZ-ALONZO, Y. CHANG, AND M. WESTOBY. 2014. Bark functional ecology: evidence for tradeoffs, functional coordination, and environment producing bark diversity. *New Phytologist* 201: 486–497.
- ROSELL, J. A., AND M. E. OLSON. 2007. Testing implicit assumptions regarding the age vs. size dependence of stem biomechanics using *Pittocaulon (Senecio) praecox* (Asteraceae). *American Journal of Botany* 94: 161–172.
- ROSELL, J. A., M. E. OLSON, R. AGUIRRE-HERNÁNDEZ, AND F. J. SÁNCHEZ-SESMA. 2012. Ontogenetic modulation of branch size, shape, and biomechanics produces diversity across habitats in the *Bursera simaruba* clade of tropical trees. *Evolution & Development* 14: 437–449.
- ROSELL, J. A., M. E. OLSON, A. WEEKS, J. A. DE-NOVA, R. M. LEMOS, J. P. CAMACHO, T. P. FERIA, ET AL. 2010. Diversification in species complexes: Tests of species origin and delimitation in the *Bursera simaruba* clade of tropical trees (Burseraceae). *Molecular Phylogenetics and Evolution* 57: 798–811.
- ROTH, I. 1981. *Structural patterns of tropical barks*. Gebruder Borntraeger, Berlin, Germany.
- ROWE, N. P., S. ISNARD, F. GALLENMÜLLER, AND T. SPECK. 2006. Diversity and mechanical architectures in climbing plants: An ecological perspective. In A. Herrel, T. Speck, and N. P. Rowe [eds.], *Ecology and biomechanics: A mechanical approach to the ecology of animals and plants*, 35–59. Taylor & Francis, Boca Raton, Florida, USA.
- ROWE, N. P., S. ISNARD, AND T. SPECK. 2004. Diversity of mechanical architectures in climbing plants: An evolutionary perspective. *Journal of Plant Growth Regulation* 23: 108–128.
- SÁNCHEZ-AZOFFEIFA, G. A., AND C. PORTILLO-QUINTERO. 2011. Extent and drivers of change of Neotropical seasonally dry tropical forests. In R. Dirzo, H. S. Young, H. A. Mooney, and G. Ceballos [eds.], *Seasonally dry tropical forests. Ecology and conservation*, 45–57. Island Press, Washington, D.C., USA.
- SCHOLZ, F., N. PHILLIPS, S. BUCCI, F. MEINZER, AND G. GOLDSTEIN. 2011. Hydraulic capacitance: Biophysics and functional significance of internal water sources in relation to tree size. In F. C. Meinzer, B. Lachenbruch, and T. E. Dawson [eds.], *Size- and age-related changes in tree structure and function*, vol. 4, *Tree Physiology*, 341–361. Springer Netherlands, Dordrecht, Netherlands.
- SCHOLZ, F. G., S. J. BUCCI, G. GOLDSTEIN, F. C. MEINZER, A. C. FRANCO, AND F. MIRALLES-WILHELM. 2007. Biophysical properties and functional significance of stem water storage tissues in Neotropical savanna trees. *Plant, Cell & Environment* 30: 236–248.
- SPECK, T., AND N. P. ROWE. 2001. Plant growth forms and biomechanics. In D. E. G. Briggs and P. R. Crowther [eds.], *Palaeobiology II*, 379–384. Blackwell Science, Oxford, UK.
- SRIVASTAVA, L. M. 1964. Anatomy, chemistry and physiology of bark. *International Review of Forestry Research* 1: 203–277.

- UHL, C., AND J. B. KAUFFMAN. 1990. Deforestation fire susceptibility and potential tree responses to fire in the eastern Amazon Brazil. *Ecology* 71: 437–449.
- VAN GELDER, H. A., L. POORTER, AND F. J. STERCK. 2006. Wood mechanics, allometry, and life-history variation in a tropical rain forest tree community. *New Phytologist* 171: 367–378.
- VINCENT, J. F. 1992. *Biomechanics—Materials: A practical approach*. Oxford University Press, Oxford, UK.
- WESTOBY, M., D. S. FALSTER, A. T. MOLES, P. A. VESK, AND I. J. WRIGHT. 2002. Plant ecological strategies: Some leading dimensions of variation between species. *Annual Review of Ecology and Systematics* 33: 125–159.
- WILLIAMSON, G. B., AND M. C. WIEMANN. 2010. Measuring wood specific gravity... Correctly. *American Journal of Botany* 97: 519–524.
- ZOTZ, G. 2013. ‘Hemiepiphyte’: A confusing term and its history. *Annals of Botany*.
- ZWEIFEL, R., AND R. HÄSLER. 2001. Dynamics of water storage in mature subalpine *Picea abies*: Temporal and spatial patterns of change in stem radius. *Tree Physiology* 21: 561–569.
- ZWEIFEL, R., H. ITEM, AND R. HÄSLER. 2000. Stem radius changes and their relation to stored water in stems of young Norway spruce trees. *Trees* 15: 50–57.

APPENDIX 1. Voucher information for the sampled species of *Bursera*.

Taxon—Collection locality, *Voucher specimen*, and abbreviation for herbarium where the voucher was deposited.

- B. arborea**—Chamela, Jalisco, Mexico, *Rosell 16*, MEXU; **B. attenuata**—Tepic, Nayarit, Mexico, *R. Medina 3412*, MEXU; **B. cinerea**—Jayacatlán, Oaxaca, Mexico, *Rosell 50*, MEXU; **B. grandifolia**—Atlacholoaya, Morelos, Mexico, *Rosell 5*, MEXU; **B. instabilis**—Chamela, Jalisco, Mexico, *Rosell 11*, MEXU; **B. longipes**—Xalitla, Guerrero, Mexico, *Cervantes 5*, MEXU; **B. roseana**—Río Cupatitzio, Michoacán, Mexico, *Rosell 25*, MEXU; **B. simaruba**—Los Tuxtlas, Veracruz, Mexico, *Rosell 44*, MEXU; **B. standleyana**—La Cangreja, San José, Costa Rica, *J.F. Morales 1944*, MO.
-