


Patterns of leaf trait variation underlie ecological differences among sympatric tree species of *Damburneya* in a tropical rainforest

Laura J. Giraldo-Kalil^{1,2} | Julio Campo³ | Horacio Paz⁴ | Juan Núñez-Farfán¹ 

¹Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México (UNAM), Ciudad de México, México

²Posgrado en Ciencias Biológicas, Unidad de Posgrado, Circuito de Posgrados, Universidad Nacional Autónoma de México (UNAM), Coyoacán, C. P. 04510, Ciudad de México, México

³Departamento de Ecología Funcional, Instituto de Ecología, Universidad Nacional Autónoma de México (UNAM), Ciudad de México, México

⁴Instituto de Investigaciones en Ecosistemas y Sustentabilidad, Universidad Nacional Autónoma de México (UNAM), Morelia, México

Correspondence

Juan Núñez-Farfán, Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México (UNAM), Ciudad de México, México.

Email: farfan@unam.mx

Abstract

Premise: Although ecological differentiation driven by altitude and soil is hypothesized to promote coexistence of sympatric tree species of *Damburneya* (Lauraceae), the mechanistic role of leaf functional variation on ecological differentiation among co-occurring species remains unexplored. We aimed to determine whether the patterns of leaf trait variation reflect ecological differences among sympatric *Damburneya* species. We tested whether trait correlations underlying functional strategies and average species traits vary in response to local soil heterogeneity along an altitudinal gradient, potentially affecting species distributions.

Methods: At two contrasting altitudes (100, 1100 m a.s.l.) in a Mexican tropical rainforest, we characterized soil chemical and physical properties and sampled four *Damburneya* species to quantify five leaf functional traits. We used linear models to analyze paired and multivariate trait correlations, spatial and interspecific effects on trait variation, and trait response to local soil heterogeneity. Relative contributions of intra- and interspecific variation to local trait variability were quantified with an ANOVA.

Results: Soil nutrient availability was higher at low altitude, but all species had a high leaf N:P ratio across altitudes suggesting a limited P supply for plants. Species distribution differed altitudinally, with some species constrained to low or high altitude, potentially reflecting soil nutrient availability. Leaf traits responded to altitude and local soil properties, suggesting interspecific differences in functional strategies according to the leaf economics spectrum (conservative vs. acquisitive).

Conclusions: The interspecific divergence in functional strategies in response to local environmental conditions suggests that trait variation could underlie ecological differentiation among *Damburneya* sympatric species.

KEYWORDS

closely related species, ecological differentiation, environmental gradient, Lauraceae, leaf economics spectrum, Mexico, plant functional traits, soil heterogeneity, topographic effects, tropical rainforest,

Resumen en Español

Premisa: Aunque se ha propuesto que la diferenciación ecológica impulsada por la variación del suelo y la altitud ha promovido la coexistencia de especies arbóreas simpátricas de *Damburneya* (Lauraceae), el papel de la variación de funcional foliar como mecanismo para explicar las diferencias ecológicas entre especies que coocurren sigue sin explorarse. Nuestro objetivo fue determinar si los patrones de variación de los rasgos funcionales foliares reflejan diferencias ecológicas entre especies simpátricas de *Damburneya*. Hipotetizamos que, tanto las correlaciones entre los rasgos que determinan

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2022 The Authors. *American Journal of Botany* published by Wiley Periodicals LLC on behalf of Botanical Society of America.

las estrategias funcionales, como los valores promedio de los rasgos de las especies, varían en respuesta a la heterogeneidad local del suelo a lo largo de un gradiente altitudinal, afectando potencialmente la distribución de las especies.

Método: En dos altitudes contrastantes (100, 1000 m s.n.m) en una selva húmeda tropical mexicana caracterizamos propiedades físicas y químicas del suelo y muestreamos cuatro especies de *Damburneya* para cuantificar cinco rasgos funcionales foliares. Usamos modelos lineales para analizar las correlaciones pareadas y multivariadas entre rasgos, los efectos espaciales e interespecíficos en la variación de rasgos, y la respuesta de los rasgos a la heterogeneidad local del suelo. Además, cuantificamos la contribución relativa de la variación intra- e interespecífica en la varianza local de los rasgos con un análisis de varianza.

Resultados: La disponibilidad de nutrientes en el suelo fue mayor en la zona baja, pero todas las especies tuvieron valores altos de N:P foliar en ambas altitudes, lo que sugiere un suministro limitado de P para las plantas. La distribución de las especies difirió altitudinalmente y algunas de ellas se restringieron a zonas altas o bajas, reflejando potencialmente la disponibilidad de nutrientes en el suelo. Los rasgos foliares respondieron a la altitud y a las propiedades edáficas locales, sugiriendo diferencias interespecíficas en las estrategias funcionales según el espectro de economía foliar (conservativa vs. adquisitiva).

Conclusiones: La divergencia interespecífica en las estrategias funcionales en respuesta a las condiciones ambientales locales sugiere que la variación de rasgos podría explicar algunas diferencias ecológicas entre las especies simpátricas de *Damburneya*.

PALABRAS CLAVE

diferenciación ecológica, efectos topográficos, especies cercanamente emparentadas, espectro de economía foliar, gradiente ambiental, heterogeneidad del suelo, Lauraceae, México, rasgos funcionales foliares, selva húmeda tropical

Closely related species are expected to have similar phenotypes and conserved ecological niches (Wiens and Graham, 2005); consequently, the ecological differences between them can greatly impact their performance, coexistence within communities, and evolution (MacArthur and Levins, 1967; Chesson, 2000). Several differences in species' niches, ecological strategies, and other factors promoting plant species coexistence can be robustly assessed by analyzing the environmental effects on functional trait variation across species (Reich et al., 1999; McGill et al., 2006; Violle and Jiang 2009; Adler et al., 2013; Carmona et al., 2016). Functional traits are physiological or morphological features that affect the individuals' survival, growth, or reproduction and determine their responses to the environment (Violle et al., 2007). Variation in leaf functional traits can drive ecological niche differentiation whenever it favors differential performance among plant species along environmental gradients. Trait-driven ecological differentiation can be promoted by mechanisms that affect species abundance, distribution and competition at a local scale such as spatial and temporal heterogeneity, resource partitioning, and natural enemy attack (Adler et al., 2013).

Functional trait correlations reflect trade-offs underlying plant strategies that vary across species depending on resource availability (Reich et al., 2003; Wright et al., 2004; Shipley et al., 2006; Reich, 2014; Maynard et al., 2022). A key example is the worldwide leaf economics spectrum

(LES) that describes global patterns in leaf trait correlations and variation across species encompassing plant functional strategies from slow to fast resource uptake, use, and return along environmental gradients. The LES is based on trade-offs between light capture, photosynthetic carbon-gain rates, nutrient use, leaf longevity and turnover, and resource investment in tissue construction (Reich et al., 1999; Wright et al., 2004; Shipley et al., 2006, Díaz et al., 2016; Onoda et al., 2017).

Briefly, species with an "acquisitive" strategy have a fast return of biomass and nutrient investment. They are expected to have high leaf nutrient concentrations and photosynthetic rates, with low investments in leaf mass per area and leaf lifespan that allow high rates of leaf turnover. In contrast, "conservative" species with slow return have increased construction costs due to high mass investment and long lifespan. They usually have low nutrient concentrations and carbon-gain rates, and reduced vulnerability to physical damage and herbivory. The patterns of leaf trait correlations underlying these functional strategies can be widely affected by the simultaneous effect of several environmental factors like soil properties (Koerselman and Meuleman, 1996; Aerts and Chapin, 2000; Campo et al., 2014; Roa-Fuentes et al., 2015a), herbivory, climate, and water and light availability (Campo and Dirzo, 2003; Wright et al., 2004; Hodgson et al., 2011).

Nevertheless, several studies report that trait relationships predicted by the LES at a global scale do not

necessarily hold at local scales, in part because the LES does not consider local drivers of trait variation like intraspecific variation (Violle et al., 2012; Niinemets, 2015; Messier et al., 2017; Anderegg et al., 2018; Fajardo and Siefert, 2018). Furthermore, the LES predictions can also fail to hold between closely related species owing to environmental variation, and the effects of natural selection on particular trait combinations within and between species (Donovan et al., 2011; Mason and Donovan, 2015; Muir et al., 2017; Agrawal, 2020; Ji et al., 2020). Thus, the assessment of intraspecific variation, and the contrast of local trait correlations with those expected according to the LES could be a helpful approach to disentangle the constraints to functional variation in sympatric closely related plant species.

Our research is focused on a group of species from the genus *Damburneya* Raf. (Lauraceae) that diversified in tropical rainforests of southern Mexico and northern Central America (Rohwer, 1993). Some of these species are locally abundant (Popma et al., 1988) and constitute genetic reservoirs for tropical rainforest regeneration (Arroyo-Rodríguez et al., 2009; Sánchez-Gallen et al., 2010). Their fruits are an important food source for birds, rodents, and insects, and the trees are used locally for timber (Rohwer, 1993). As with other Lauraceae in Mexico, *Damburneya* trees are threatened by deforestation (Lorea-Hernández, 2002, 2011; de Kok, 2020a, 2020b, 2020c). Despite this threat, ecological studies of most species of this genus (and even of the Lauraceae more broadly) are still scarce due to complex taxonomy and difficulty in collecting samples from tall trees. Distinguishing species in the field is challenging because identification often requires the examination of flowers or fruits, which are produced at intervals of several years and thus not always available (Rohwer, 1993; van der Werff and Richter, 1996; Lorea-Hernández, 2002).

Although all *Damburneya* species have ornithochorous seed dispersal (i.e., by birds), sympatric species usually differ in their local abundance and distribution, suggesting that ecotypic differentiation has a key role in their speciation (Rohwer and Kubitzki, 1993). This divergence process likely occurred by edaphic specialization, altitudinal sorting, and other factors such as phenological isolation (Rohwer and Kubitzki, 1993). Elevation and soil nutrient variation are local environmental drivers of plant distribution and ecological differentiation in other Lauraceae (van der Werff, 1992; Sri-Ngernyuang et al., 2003; Srinivas and Krishnamurthy, 2019). Ecological divergence between sympatric *Damburneya* in response to such environmental variation seems to be common (Rohwer and Kubitzki, 1993); however, the role of leaf functional trait variation on ecological differentiation has been unexplored.

Here we aimed to determine whether sympatric species of *Damburneya*, co-occurring in the tropical rainforest at Los Tuxtlas Biosphere Reserve, display ecological differences in leaf functional traits and to describe how traits vary in relation to soil heterogeneity along an altitudinal gradient. Specifically, we hypothesized that *Damburneya*

species differ in (1) leaf trait correlation patterns and consequently, in functional strategies, and in (2) leaf trait variation in response to the soil heterogeneity at high and low altitudes. Furthermore, we aimed to analyze the effects of functional strategies and soil physical and chemical variation on local patterns of abundance and distribution along the altitudinal gradient. To this end, we characterized the distribution of four *Damburneya* species co-occurring in the studied area and analyzed the correlations between five leaf functional traits. Moreover, we assessed how leaf trait variation responds to soil heterogeneity and the extent to which it is determined locally by inter- and intraspecific variation. Finally, we discuss our findings in light of the drivers of leaf functional trait variation and its potential implications considering the current threats to *Damburneya* species.

MATERIALS AND METHODS

Study site

The study was conducted in August 2018 in well-preserved areas of mature tropical rainforest within the core zone of Los Tuxtlas Biosphere Reserve at the San Martín Volcano. The altitudinal gradient ranges from sea level to 1700 m a.s.l. and impacts the local temperature and the precipitation regimes (Gutiérrez-García and Ricker, 2011). The regional climate is perhumid (based on the Lang aridity index, the annual ratio of precipitation to temperature is greater than 100). With increasing altitude, the mean annual temperature decreases (from 23–24°C at 1100 m a.s.l. to 20–21°C at 100 m a.s.l.) and the mean annual precipitation increases (from 4000–5000 mm at 100 m a.s.l. to 6000–7000 mm at 1100 m a.s.l.). The precipitation varies through the year and is concentrated in the rainy season (June to February), while monthly precipitation can drop below 100 mm during the dry season (March to May; Gutiérrez-García and Ricker, 2011). In addition, the forest canopy is more open at higher altitudes allowing more light entrance in comparison to lower altitudes (maximum illuminance: 8331.34 lx at 1100 m a.s.l. to 5941.73 lx at 150 m a.s.l., L. Giraldo-Kalil, unpublished data).

The mountain ranges in this region originated from volcanic activity; as a result, soils are young, and their physical and biological properties are spatially variable (Flores-Delgado et al., 1999; Krasilnikov et al., 2013). Soils in low altitudes are entisols, including typic ustorthents and lithic ustorthents (Tobón et al., 2011), and at high altitudes are predominantly andisols (Sommer-Cervantes et al., 2003). Soil drainage is good at high altitudes and moderate at low altitudes (Siebe et al., 1996).

The Los Tuxtlas region sustains more than 40% of the floristic richness of the tropical rainforest of Mexico (Villaseñor et al., 2018). However, changes in land cover and land-use for livestock have caused soil chemical, physical and biological degradation (Figuroa et al., 2020)

and the loss of approximately 60% of the original vegetation cover (von Thaden et al., 2020).

Study system

This study focused on the four *Damburneya* species reported for the state of Veracruz and for Los Tuxtlas Biosphere Reserve (Ibarra-Manríquez and Sinaca-Colín, 1995; Lorea-Hernández, 2002): *Damburneya ambigens* (S.F. Blake) Trofimov, *Damburneya colorata* (Lundell) Trofimov, *Damburneya gentlei* (Lundell) Trofimov, and *Damburneya salicifolia* (Kunth) Trofimov. These four tree species were formerly included in the genus *Nectandra* Rol. ex Rottb. and were recently reinstated in the genus *Damburneya* Raf. by Trofimov et al. (2016).

The species differ in altitudinal range, distribution, and stature. *Damburneya ambigens* and *D. gentlei* are distributed in lowland tropical rainforests from southern Mexico to Honduras (Rohwer, 1993) and are locally used for timber (Ibarra-Manríquez and Sinaca-Colín, 1995). Generally, *D. ambigens* occurs from sea level up to 1100 m a.s.l. and is abundant in the upper canopy of forests in the study area (Bongers et al., 1988; Dirzo et al., 1997). In contrast, *D. gentlei* is usually restricted to altitudes below 300 m a.s.l. (Rohwer, 1993). Both species are large trees, reaching 35 m or more in height. Unlike most Lauraceae species, *D. salicifolia* can resist disturbed conditions (Lorea-Hernández, 2002); it occurs in soils with different fertility (Rohwer, 1993) at a wide altitudinal range from sea level up to 2300 m a.s.l., and is distributed from Mexico to Nicaragua (Rohwer, 1993). The distribution and variety of habitats where this species occurs suggest high ecological plasticity (Rohwer and Kubitzki, 1993). The trees of *D. salicifolia* range from 10 to 20 m in height, and the wood is used for construction (Rohwer, 1993; Ibarra-Manríquez et al., 1997). Moreover, *D. salicifolia* has been described as a species complex because its phenotypic variation has led to several local forms from which very similar *Damburneya* species originated, making them difficult to distinguish (Rohwer, 1993). Indeed, *D. colorata* is part of the *D. salicifolia* complex, but contrary to that species, it is rarely found and reports of it in the study area and through its entire distribution have been very scant. The trees of *D. colorata* can reach 25 m in height and are used as firewood (Rohwer, 1993). The species is found in forests at altitudes close to 650 m a.s.l. in southern Mexico, Belize, and Guatemala (Rohwer, 1993).

Sampling design

We established six 1-ha rectangular plots (200 × 50 m) in a mature tropical rainforest to characterize the distribution of *Damburneya* trees and soil variation in the study area. The plots were located in two zones with contrasting altitudes to encompass the variation in soil properties and species distribution and abundance. Three plots were established at

low altitude below 300 m a.s.l. and three at high altitude between 675 and 1100 m a.s.l. The plots were located between 18°32'N and 18°35'N, and 95°4'W. To characterize soil properties, we collected 10 soil samples per plot at two depths (0–10 and 10–20 cm); we used a soil auger to sample along a diagonal line covering the soil variation across the plot. The soil samples were homogenized, sifted (2-mm mesh), and refrigerated in plastic bags until processed in the laboratory.

All trees of *Damburneya* in each plot with a diameter at breast height (DBH) > 1 cm were tagged, sampled, and their geographic coordinates recorded with a portable GPS (Garmin Oregon 550; Garmin, Olathe, KS, USA). We collected one to three branchlets and up to 25 mature fully expanded green leaves per tree, including shade and sun leaves, and avoided leaves damaged by pathogens and/or herbivores. Leaf samples were stored in black plastic bags with water to keep them hydrated and avoid transpiration until processing in the laboratory. Fresh leaves with petioles were photographed beside a millimeter scale, then oven-dried and individually weighed to record the dry mass. A branchlet sample per tree was preserved as a reference voucher to confirm species identity with the help of Dr. Francisco Lorea, a specialist in the Lauraceae. The vouchers of 26 representative specimens of the four species were deposited in three herbaria (Appendix S1).

Soil physicochemical, physical, and chemical properties

We quantified the following soil properties from air-dried subsamples: soil pH, soil texture, and soil organic carbon (SOC), total nitrogen (STN), ammonium (NH₄), nitrate (NO₃), total and available phosphorus (STP and SAP, respectively) concentrations, and the C:N and NO₃:NH₄ ratios.

The pH of the soil was measured in a water suspension with a pH meter. Soil texture was characterized by determining the clay, sand, and silt contents with a density analysis (Elliott et al., 1999) and classified with the texture triangle. Soil organic C was analyzed in an automated C-analyzer (SHIMADZU 5005 A) after an air-dried subsample (5 g) was ground and passed through a 100-mesh screen. The STN and STP in a soil subsample (1 g) were determined after Kjeldahl acidic digestion in concentrated H₂SO₄ (Anderson and Ingram, 1993), and the concentrations of N and P were quantified with an NP elemental analyzer (Technicon Autoanalyzer III, methods G-188-97 and G-189-97, respectively). To determine mineral N concentrations, NO₃ and NH₄, were extracted from a subsample (15 g) of each composite soil sample in 100 mL 2 M KCl (Sollins et al., 1999) by shaking for 1 h and allowing the sample to settle overnight. A 20-mL aliquot of the supernatant was then transferred into vials and frozen for later analysis. All KCl extracts were analyzed colorimetrically using the NP autoanalyzer system to determine

$\text{NO}_3\text{-N} + \text{NO}_2\text{-N}$ (method G-109-94), which were reported as NO_3 concentration. The salicylate–hypochlorite procedure (method G-102-93) was employed to determine NH_4 concentration.

Soil available P was extracted from a soil subsample (0.5 g) in 30 mL of 0.5 N NaHCO_3 by shaking for 16 h. The sample was centrifuged at 10,000 rpm at 0°C for 10 min, then digested with ammonium persulfate (Anderson and Ingram, 1993), and later analyzed on a NP autoanalyzer system (method G-304-04) by the molybdate method after ascorbic acid reduction (Murphy and Riley, 1962) to determine available P concentration. The NaHCO_3 extract provides a measure of relative labile and plant-available P, including readily solubilized inorganic P and mineralized organic P (Lajtha et al., 1999). We calculated the $\text{NO}_3\text{:NH}_4$ and the C:N ratios (using SOC and STN concentrations) to assess soil N availability and quality of soil organic matter. Briefly, high $\text{NO}_3\text{:NH}_4$ ratios indicate high NO_3 availability in the system due to high nitrification. Additionally, low C:N ratios (close to 10) indicate a high quality of soil organic matter (Chapin et al., 2011).

Leaf functional traits

We measured five traits involved in the LES that are associated with plant productivity and competitive ability (specific leaf area, SLA; leaf nitrogen concentration, LNmass; and leaf phosphorus concentration, LPmass), with drought tolerance and resistance to physical damage (leaf dry matter content, LDMC), and with soil nutrient supply (Leaf N:P ratio).

Specific leaf area (cm^2/g), which accounts for the investment of leaf area for light capture per unit of biomass, was calculated as the leaf area divided by leaf dry mass. Leaf area was quantified from leaf photographs with ImageJ (Schneider et al., 2012). Leaf dry matter content (mg/g), which reflects biomass investment on the leaves, was calculated as leaf dry mass divided by fresh mass, including leaf petioles (Pérez-Harguindeguy et al., 2013). These morphological traits reflect the trade-off between carbon gain and leaf longevity (Wright et al., 2004).

The leaves from each tree were dried and powdered to determine leaf nutrient concentration (i.e., LNmass and LPmass; mg/g) using the Kjeldahl acidic digestion method (Anderson and Ingram, 1993). We calculated the leaf N:P (per mass), which reflects nutrient limitation to plant growth and productivity according to threshold values. Low values ($\text{N:P} < 14$) are indicative of limited soil N availability for plant demand, high values ($\text{N:P} > 16$) indicate limited availability of P, and intermediate values indicate a limited availability of both (i.e., N and P; Koerselman and Meuleman, 1996).

Data analyses

R version 3.6.1 (R Core Team, 2021) and the averaged trait values per tree were used for all statistical analyses

and graphs. The packages and functions are described for each analysis.

Functional strategies

Leaf trait variations

Descriptive statistical analyses were used to characterize leaf trait variation. Additionally, multivariate patterns of variation for all leaf traits were explored using standardized values in a principal component analysis (PCA) in the FactoMineR package (Lê et al., 2008). The leaf N:P ratio was excluded from these analyses because leaf nutrient variation is already accounted for by including LNmass and LPmass.

Trait correlations

Scaled bivariate correlations between leaf metrics were calculated to analyze whether there were differences in trait variation or in trade-offs that could account for differences in the functional strategies among sympatric *Damburneya* species. For that, standardized major axis regressions were performed using the smatr package and log-transformed axes (Warton et al., 2012). We tested whether there was a common slope among species using a likelihood ratio test.

Environmental effects on leaf trait variation

Mixed models

We performed linear mixed models to analyze the effect of spatial and interspecific variation on leaf trait. The models were built for standardized trait values and included the sampling plot, which accounts for local soil variation, and the species as effects with random intercepts using the lmer function in the lme4 package (Bates et al., 2015). Altitude (i.e., low and high altitudes) was included as a fixed effect. Since the four species did not occur in all plots, we did not include the species \times plot interaction. REML-likelihood ratio tests were performed to determine whether the random effects significantly affected the variance explained by the models (Luke, 2017); we used the ranova function in the lmerTest package, which indicates whether the model becomes significantly worse when each random effect is dropped. The denominator degrees of freedom of the fixed effects were approximated with the Satterthwaite method (Kuznetsova et al., 2017). Furthermore, we quantified the percentage of variance explained by random effects.

Environment–trait regressions and variance partition

We performed linear regression models to understand how soil heterogeneity affects local trait variation in sympatric *Damburneya* species. All measured soil properties were modeled as independent variables. Abundance-weighted average trait values per plot (i.e., community weighted mean trait values) were included as response variables (further calculation details are as described by Kichenin et al., 2013).

Furthermore, we quantified the relative contribution of intra- and interspecific variation to local trait variance in response to soil variables. An analysis of variance was performed for linear regression models with the flexanova function proposed by Lepš et al. (2011). According to the approach of Kichenin et al. (2013), the total sum of squares of the abundance-weighted plot average trait variance was decomposed as $SS_{\text{specific}} = SS_{\text{fixed}} + SS_{\text{intraspecific}} + SS_{\text{covariation}}$, where SS_{specific} refers to the species average trait values in the plot and SS_{fixed} is the site independent trait value calculated using the mean trait values for the species in all plots and weighted by the species relative abundance. $SS_{\text{intraspecific}}$ accounts for intraspecific variation and is calculated as the difference between the last two terms. $SS_{\text{covariation}}$ is the effect of the covariation between intra- and interspecific variation in response to the soil variables calculated as $SS_{\text{specific}} - (SS_{\text{intraspecific}} + SS_{\text{fixed}})$.

RESULTS

Soil variation and species distribution

Soil properties varied with altitude, indicating a greater content of organic nutrient sources and a lower mineralization rate at high altitude, and consequently, lower nutrient availability than at low altitude (Appendix S2). The soils at low altitude had a loamy and sandy loam texture, with higher clay content and available nutrient concentrations (NH_4 , NO_3 , and available P), and more basic pH than the soils of higher altitude sites. In contrast, at high altitude, the predominant soil textures are loamy sand and sandy loam, and the sand content, organic C, total P, and total N concentrations, C:N and $\text{NO}_3:\text{NH}_4$ ratios were higher in the soils at high altitude than at low altitude.

We censused a total of 135 trees, with 62 *D. ambigens*, 45 *D. salicifolia*, 16 *D. colorata*, and 12 *D. gentlei*. The spatial distribution differed among species: *D. gentlei* occurred only at low altitude (12 trees); in contrast, *D. salicifolia* was present only at high altitude (45 trees). *Damburneya ambigens* occurred at both low and high altitudes with similar abundance (28 and 34 trees, respectively); *D. colorata* was also present at both altitudes but more abundant at low than at high altitude (12 and 4 trees, respectively).

Functional strategies

Leaf trait variation

There were differences in trait variation patterns that suggest both diverging functional strategies among species and differences on trait variation across altitudes (Figure 1, Table 1; Appendix S3). Leaf nutrient concentrations were the most variable traits across species (coefficients of variation, $\text{CV} > 20\%$) and were higher at low than at high

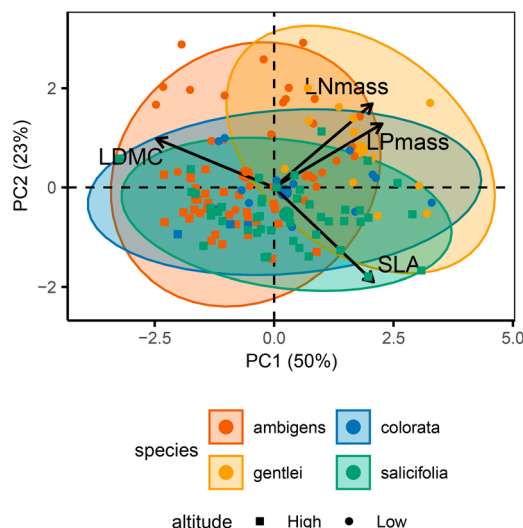


FIGURE 1 Principal component analysis of the variance of leaf functional traits of four sympatric *Damburneya* species (*D. ambigens*, *D. colorata*, *D. gentlei*, *D. salicifolia*) at Los Tuxtlas. SLA = specific leaf area, LDMC = leaf dry matter content, LNmass = leaf nitrogen concentration, LPmass = leaf phosphorus concentration. Squares: samples from high altitudes; circles: samples from low altitudes.

TABLE 1 Eigenvalues, cumulative percentage of variation and eigenvectors of the first three principal components analysis for four leaf traits that best predicted the functional leaf trait spectrum of four sympatric *Damburneya* species at Los Tuxtlas Biosphere Reserve.

	PC1	PC2	PC3
Eigenvalue	2.00	0.92	0.61
Cumulative % of variance	49.97	72.92	88.14
Eigenvectors			
SLA	0.66***	-0.60***	0.25**
LDMC	-0.78***	0.31***	0.21*
LNmass	0.65***	0.53***	0.52***
LPmass	0.72***	0.40***	-0.48***

Notes: The lower panel of the table shows trait correlations with the principal components. SLA = specific leaf area, LDMC = leaf dry matter content, LNmass = leaf nitrogen concentration, LPmass = leaf phosphorus concentration. Significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

altitudes (Appendix S3); morphological traits were the least variable ($\text{CV} < 20\%$). In addition, mean leaf N:P ratio of all species ranged from 21 to 42 across altitudes, suggesting a limited soil P supply for plant demand (Appendix S3). On the other hand, the PCA showed differences in multivariate trait variation among species. Most of the trait variance (72.9%) was explained by the first two principal components (50.0% and 22.9%, respectively), and all traits contributed significantly to PC1 and PC2 (Figure 1, Table 1).

The first axis reflects the LES and shows the positive correlation of leaf nutrient concentrations with SLA against a negative correlation with LDMC. Clearly, all species

spanned the continuum from quick to slow return on investments of biomass and nutrients, with *D. ambigens* at the resource conservative end and *D. gentlei* at the resource acquisitive end. These two species occupied different zones of the multivariate trait space, suggesting functional differences between them and the other two species. (Figure 1; see Appendix S3). *Damburneya salicifolia* and *D. colorata* showed a wide intraspecific variation across the slow-fast tradeoff (PCA 1), suggesting high trait plasticity.

The second axis shows differences in trait variation according to altitude among and within species; leaf nutrient concentrations decreased and SLA increased as altitude increased. Contrasting leaf trait variation occurred between two species segregated by altitude: *D. gentlei*, a low-altitude species with high leaf nutrient concentration, and *D. salicifolia*, a high-altitude species with high SLA (Figure 1). Similarly, the intraspecific trait variation of *D. ambigens* encompassed individuals at low altitude that had higher leaf nutrient concentrations and lower SLA than those at high altitude.

Trait scaling and correlations

Leaf morphological traits were correlated with nutrient concentration within species; however, the slope and direction (i.e., slope sign) differed among species, indicating differences in nutrient investment on leaf construction (Appendix S4). In *D. salicifolia* and *D. colorata*, LNmass was positively correlated with SLA (*D. salicifolia*: slope = 4.63, $r^2 = 0.29$, $P < 0.001$; *D. colorata*: slope = 3.40, $r^2 = 0.22$, $P = 0.064$) and negatively with LDMC (*D. salicifolia*: slope = -5.11, $r^2 = 0.29$, $P = 0.005$, slope = -6.96; *D. colorata*: $r^2 = 0.24$, $P = 0.056$), suggesting that N concentration increases in leaves with low construction costs. In contrast, these two traits were negatively correlated in *D. ambigens*, indicating a high nutrient investment on leaves with high

construction costs and supporting a functional conservative strategy (slope = -3.25, $r^2 = 0.13$, $P = 0.004$), but had no significant correlation in *D. gentlei* (slope = -1.22, $r^2 = 0.10$, $P = 0.326$). Leaf N:P ratio was negatively correlated with SLA in *D. ambigens* and *D. gentlei* (*D. ambigens*: slope = -3.24, $r^2 = 0.27$, $P < 0.001$; *D. gentlei*: slope = -1.52, $r^2 = 0.43$, $P = 0.021$) but positively in *D. salicifolia* (slope = 5.32, $r^2 = 0.08$, $P = 0.058$), suggesting that *D. salicifolia* allocates less P to leaves that have higher area per unit biomass.

In contrast, some traits had the same correlation patterns in all the species. For instance, LDMC decreased as SLA increased, and LPmass was negatively correlated with LDMC, but positively with SLA (Appendix S4). In general, the slopes showed an allometric relationship among traits. The only exception was the isometric scaling between nutrients (i.e., LPmass and LNmass) in *D. ambigens* and *D. colorata* (slope ≈ 1 , $P < 0.05$). Furthermore, the scaling between foliar nutrients was also isometric in *D. gentlei* and close to 2/3 for *D. salicifolia*, but these relationships were not significant (Appendix S4).

Environmental effects on leaf traits variation

The mixed models showed that spatial variation and interspecific differences explained the variation in leaf traits. Altitude only significantly affected LPmass variation (slope = 0.948, $P = 0.054$; Appendix S5). On the other hand, differences among plots significantly explained the variation of all leaf traits except in the case of LPmass ($P = 0.082$). Interspecific differences contributed significantly to the variation of SLA, LDMC, and LNmass, accounting for more than 20% of the variation of these traits, and notably explaining 43% in LDMC (Figure 2; Appendix S6). Conversely, less than 10% of variation of LPmass and leaf N:P ratio was determined by interspecific differences. The

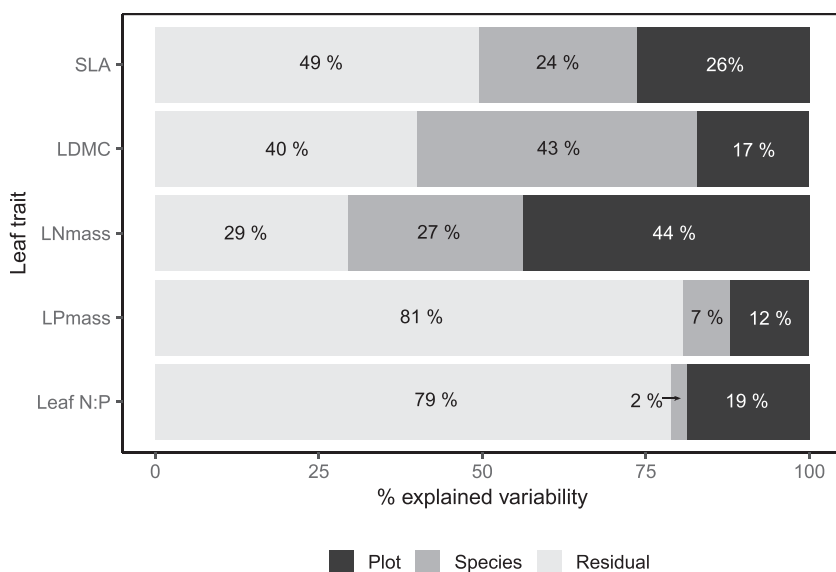


FIGURE 2 Variance structure of leaf functional traits in four sympatric *Damburneya* species at Los Tuxtlas according to mixed linear models. The models were built with REML considering altitude as fixed effect, the species and sampling plot were modeled as effects with random intercepts. SLA = specific leaf area, LDMC = leaf dry matter content, LNmass = leaf nitrogen concentration, LPmass = leaf phosphorus concentration, Leaf N:P = leaf nitrogen to phosphorus ratio.

variance of all traits, except LDMC, was mostly explained by plot variation (i.e., local environmental variation) rather than by interspecific differences. Both factors explained less than 25% of the variance of LPmass and leaf N:P ratio (Figure 2).

Soil-leaf trait relationships

Linear regressions between average plot leaf traits and soil variables indicate that the edaphic properties influence local leaf trait variation. Leaf nutrient concentrations were sensitive to several soil properties: LPmass decreased with increasing SOC and STP concentrations and C:N ratio (Appendix S7) but increased with increasing soil pH and SAP concentration (Figure 3). The same applied to LNmass, which increased with soil clay content (Figure 3), decreased with STN and NO₃ concentrations, and had no significant relationship with NH₄ concentration in soil (Figure 4). Also,

Leaf N:P ratio had a negative relationship with SOC, STN, NO₃, and STP concentrations (Figure 3; Appendix S7). Leaf morphological traits were only significantly affected by soil N availability (Figure 4; Appendices S7 and S8). Specific leaf area was negatively correlated with NH₄ concentrations, and LDMC had a positive significant relationship with the concentrations of NO₃ and NH₄ (Figure 4).

The variance partitioning analysis showed that interspecific differences significantly affected the average plot trait variation in response to local soil properties. Conversely, intraspecific variation and its covariance with interspecific variation explained less than 10% of the average plot trait variance and did not affect it significantly. Intraspecific variation better explained the variance of morphological traits rather than that of leaf nutrients. Additionally, soil properties mostly affected leaf nutrients and, in contrast, explained less than 30% of morphological trait variation (Appendix S9).

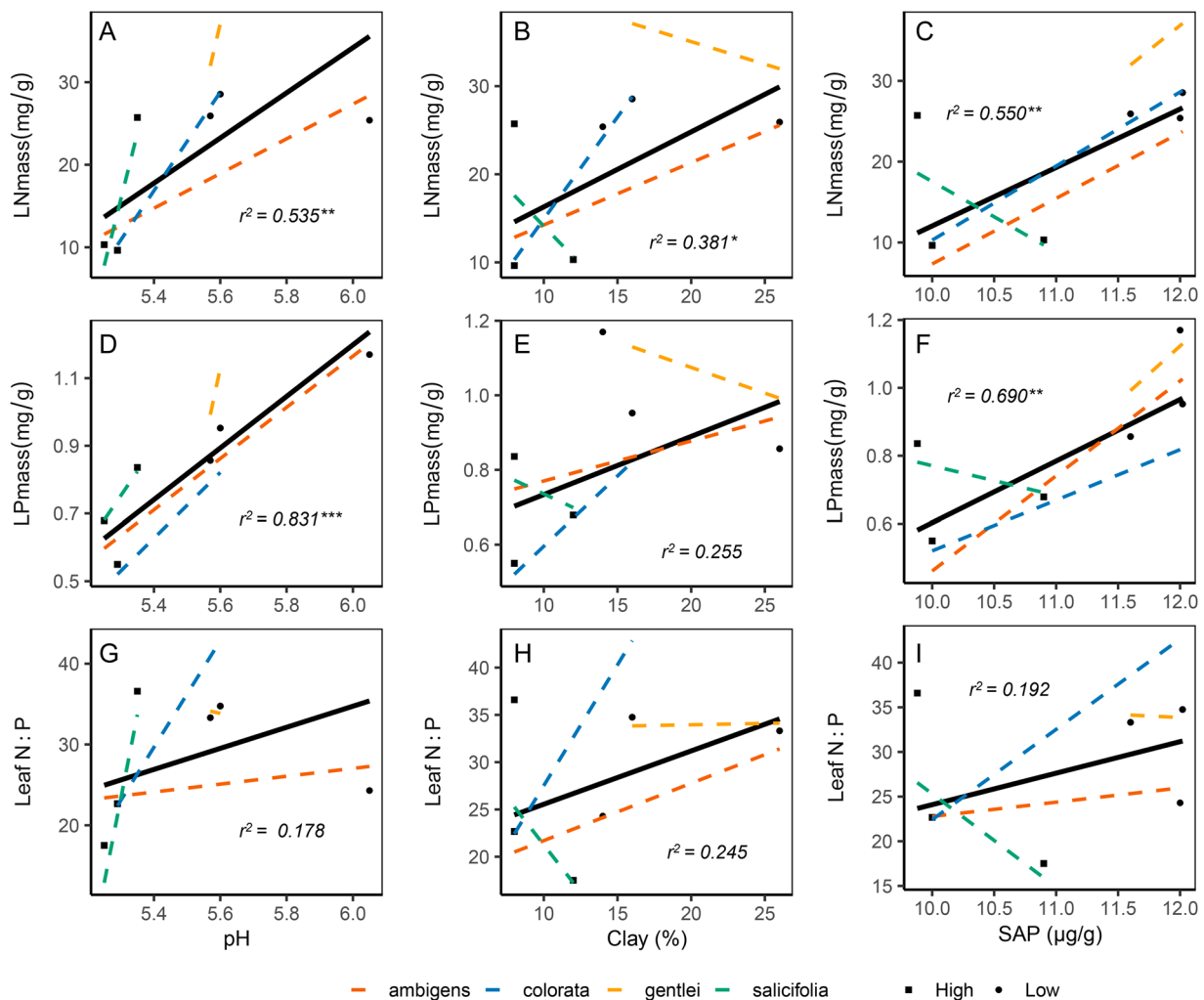


FIGURE 3 Relationships of the plot average leaf nutrient concentrations of four sympatric *Damburneya* species (*D. ambigens*, *D. colorata*, *D. gentlei*, *D. salicifolia*) at Los Tuxtlas with pH, clay content, and soil available phosphorus (SAP). LNmass: leaf nitrogen concentration (A–C), LPmass: leaf phosphorus concentration (D–F) and leaf N:P (G–I). Points represent the abundance-weighted average plot trait values, and the shape symbolizes the altitude. Plot trait variation in response to soil properties is represented by solid black regression lines, and the regression coefficient (r^2) and significance are provided for these values. Significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Colored dashed lines represent each species trends of variation.

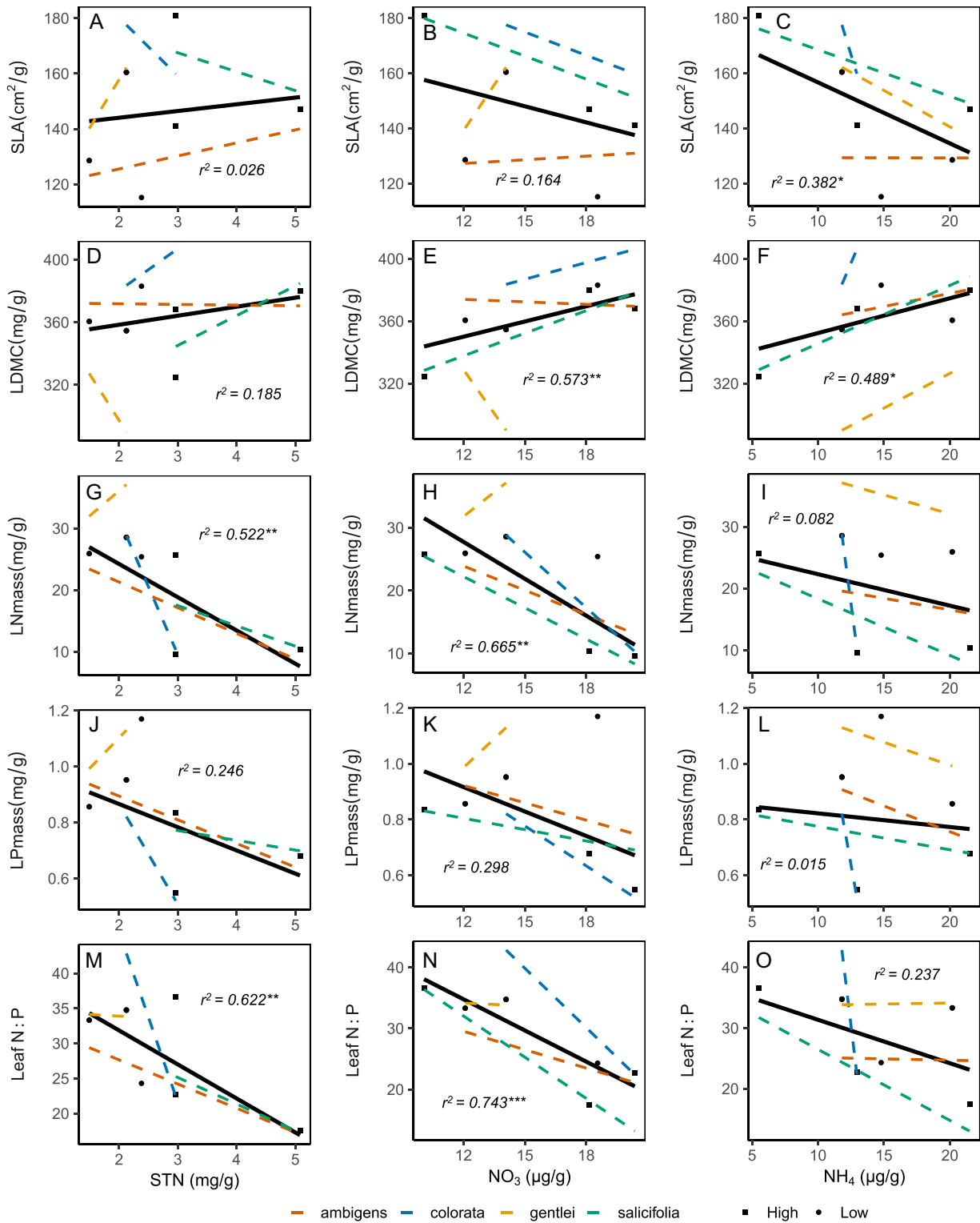


FIGURE 4 Relationships of the plot average leaf functional traits of four sympatric *Damburneya* species (*D. ambigens*, *D. colorata*, *D. gentlei*, *D. salicifolia*) at Los Tuxtlas with soil total nitrogen (STN), nitrate (NO_3), and ammonium (NH_4). SLA = Specific leaf area (A–C), LDMC = leaf dry matter content (D–F), LNmass = leaf nitrogen concentration (G–I), LPmass = leaf phosphorus concentration (J–L), and leaf N:P (M–O). Points represent the abundance-weighted average plot trait values, and the shape symbolizes the altitude. Plot trait variation in response to soil properties is represented by solid black regression lines, and the regression coefficient (r^2) and significance are provided for these values. Significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Colored dashed lines represent each species trends of variation.

DISCUSSION

Our results suggest that the patterns of leaf trait variation along the altitudinal and edaphic gradient partially explained ecological differences among species and support the study of trait variation as a promising tool to assess the performance of *Damburneya* species in different environments.

Soil variation and species distribution

Besides their high biodiversity, tropical rainforests have a high soil biogeochemical heterogeneity at multiple scales (Townsend et al., 2008). The tropical rainforest at Los Tuxtlas is not the exception, as evidenced by the altitudinal variation of soil pH, texture, and nutrient availability, which is likely driven by the interaction of soil processes and climate. For example, high nutrient concentrations at low altitudes could be promoted by higher temperature and better soil moisture for microbial activity as expected by high water retention associated with a greater clay content (Siebe et al., 1996; Benner et al., 2011; Weil and Brady, 2017) and nutrient transport from the upper to the lower slope (Campo-Alves, 2003). In contrast, at higher altitude, the greater precipitation can promote higher rates of soil weathering and nutrient leaching (Campo et al., 2001; Santiago et al., 2004), while the lower temperature slows decomposition rates, as evidenced by the higher organic matter content (Benner et al., 2011).

The distribution and abundance of the four studied species of *Damburneya* differed between altitudes and are consistent with previous reports (Rohwer, 1993; Ibarra-Manríquez and Sinaca-Colín, 1995; Dirzo et al., 1997; Lorea-Hernández, 2002). *Damburneya ambigens* spanned the altitudinal gradient; it was the most abundant species and occurred in almost all the plots. *Damburneya colorata* was less abundant and occurred at high and low altitude plots, below 650 m a.s.l. In contrast, *D. gentlei* occurred only at low altitude and *D. salicifolia* was abundant at high altitudes and absent from low altitude plots. However, we have seen several trees of *D. salicifolia* in the study area along roadsides and forest edges and fragments, from sea level up to 1100 m a.s.l. (L. Giraldo-Kalil, unpublished data); these observations support the tolerance of this species to disturbance (Lorea-Hernández, 2002). The absence of *D. salicifolia* at low altitude in well-preserved areas of mature tropical rainforest may be due to other unmeasured biophysical factors (e.g., light availability, competition, limited seed dispersal) and decreased plant survival and recruitment in undisturbed compared to open disturbed areas. Although few trees of *D. gentlei* and *D. colorata* were recorded, our study accurately reflects the distribution of both species considering the limited reports within the study area (GBIF.org, 2021a, 2021b). We stress that our reports of *D. colorata* are among the most complete so far, as this rare species has not been well sampled throughout its

distribution and is poorly represented in herbaria (GBIF.org, 2021a). It is difficult to establish whether the very few reports of this species are due to small population sizes or to a lack of records or erroneous determination in herbaria because of its notorious similarity with *D. salicifolia* (Rohwer, 1993); in any case, further studies of the natural populations of this species are necessary.

Effects of interspecific differences and environment on leaf trait variation

We hypothesized that leaf trait variations will differ among species according to soil heterogeneity at low and high altitudes. In support of this hypothesis, the variation of SLA, LDMC, and LNmass differed among *Damburneya* species and was determined by altitude and soil spatial variation among plots (Figure 2; Appendices S3, S5, and S6). It is noteworthy that the leaf morphological traits were less sensitive than leaf nutrient concentrations to soil variation and were only significantly affected by soil N availability (Figure 4; Appendices S3 and S8). For example, SLA decreased with increasing soil NH₄ concentration, while LDMC increased with NO₃ and NH₄, suggesting that soil N availability could favor the construction of thick and/or dense leaves.

Morphological traits could be influenced by herbivory and altitudinal variation of climate and light, in addition to nutrient availability. Thick and dense leaves with high LDMC increase mechanical resistance against herbivores (Westoby et al., 2002; Onoda et al., 2011) and heat tolerance in tropical forests (Slot et al., 2021); hence, they usually occur in habitats with high temperatures (Niinemets, 2001; Wright et al., 2004) like the low altitude zone. Conversely, SLA usually correlates positively with precipitation amount and temperature but negatively with light availability (Wright et al., 2004; Poorter et al., 2009; Gong and Gao, 2019). The finding of high SLA values at higher altitudes in our study (Figure 1) suggests that the signal of low light in lower altitude overrides other factors and that leaf traits are shaped by several interacting environmental factors rather than simple relationships with physical environmental variables.

Leaf nutrients vary depending on soil nutrient availability and the factors affecting it (Aerts and Chapin, 2000; Hodgson et al., 2011). Leaf P varied significantly with altitude (Appendix S5), likely because P uptake by plants increases at pH close to six (Schachtman et al., 1998; Marschner, 2012), which explains the increase in LPmass with SAP and pH at lower altitudes (Figure 3). In addition, unlike other traits, LPmass did not vary significantly among species (Appendix S6), suggesting that *Damburneya* species share similar P requirements (Dalling et al., 2016) and/or limitations. When plants experience P deficiency, they increase P absorption at the expense of N absorption, resulting in higher leaf N:P values, suggesting a pervasive P limitation at our study site (Chapin, 1991; Koerselman and

Meuleman, 1996; Güsewell, 2004; Chapin et al., 2011). This finding is consistent with the argument that the tropical rainforest of Los Tuxtlas is a P-limited ecosystem (Tobón et al., 2011), as in many lowland tropical rainforests (Vitousek, 1984; Tanner et al., 1998; Turner et al., 2018) and other tropical ecosystems (Augusto et al., 2017; Hou et al., 2021). This hypothesis could be verified with a study of primary production at the ecosystem level, analyzing how P-limited forests respond to a release of such a limitation (Harrington et al., 2001; Turner et al., 2018).

Interestingly, LN_{mass} and Leaf N:P ratio were negatively correlated with soil NO₃ and STN concentrations and were not significantly related to NH₄ concentration in the soil, suggesting a decoupling between N supply and plant demand. This decoupling could be due to reduced N uptake by the roots under excess soil N (Chapin et al., 2011) and/or low P supply (Chapin, 1991) and is concordant with the hypothesized P limitation of *Damburneya* species at Los Tuxtlas. Moreover, besides soil N availability, LN_{mass} could be determined by plant demand and regulatory mechanisms affecting nutrient accumulation and metabolism in plant organs (Marschner, 2012; Lambers and Oliveira, 2019).

On the other hand, the response of leaf functional traits to local soil heterogeneity was determined by interspecific variation (Appendix S9), suggesting that species vary in the way they adjust to environmental heterogeneity (Chesson, 2000; Adler et al., 2013; Schulze et al., 2019). Moreover, the intraspecific variation in *Damburneya* had a higher influence on morphological traits than on leaf nutrient concentrations, likely due to the high variability of plant nutrient contents (Cárdenas and Campo, 2007; Lepš et al., 2011; Kichenin et al., 2013; Fajardo and Siefert, 2018).

Functional strategies differ among species

As predicted, our results show diverging patterns of trait variation that suggest interspecific differences in functional strategies involved in plant productivity and light and nutrient demands among species. Why leaf strategies involved in leaf resource gain and use differ among coexisting species of *Damburneya* is unclear. However, the observed patterns of trait variation could arise in response to simultaneous environmental pressures like herbivory (Givnish, 1988; Kitajima, 1994; Valladares and Niinemets, 2008; Poorter, 2009; Westbrook et al., 2011; Onoda et al., 2017) and to contrasting gradients of resource availability, as occur in sites with richer soils and lower light availability (Baltzer and Thomas, 2007; Coomes et al., 2009; Dent and Burslem, 2016; Fajardo and Siefert, 2018).

In our study, the analysis of intraspecific variation shows that some species (*D. colorata* and *D. salicifolia*) express the patterns of trait correlations expected by the leaf economic spectrum, such as a positive association between SLA and LN_{mass} or a negative association between LDMC and LN_{mass}; but strikingly, other species do not. For example, *D. ambigens* has high nutrient content and LDMC in

nutrient-rich soils at low altitude (Figure 1; Appendices S3 and S4). The high nutrient allocation to leaves and the high carbon investment in leaf construction (Onoda et al., 2017) likely favor CO₂ fixation when light is limited (Valladares and Niinemets, 2008; Schlesinger and Bernhardt, 2020) and/or tissue is lost due to high herbivory (Valladares and Niinemets, 2008) as expected in tropical rainforests (Kitajima, 1994; Poorter, 2009; Westbrook et al., 2011). Previous studies analyzing responses to simultaneous nutrient and light gradients in other forests have shown that the number of coexisting functional strategies to deal with shade may increase in nutrient-rich soils (Givnish, 1988; Combes and Grubb, 2000; Coomes et al., 2009; Mason et al., 2012; Dent and Burslem, 2016). Whether the relaxation of one factor of resource limitation allows for the expression of alternative functional strategies to deal with other environmental factors, and if such may drive departures from the LES expectations, are very interesting hypotheses worth to be investigated for *Damburneya* species.

Interestingly, *D. colorata* and *D. salicifolia* exhibit functional overlap, probably due to their high morphological similarity (Figure 1). The wide intraspecific trait variation of these species encompasses both conservative and acquisitive strategies, suggesting wide ecological plasticity (Figure 1). Such intraspecific trait variation could explain the adaptability of *D. salicifolia* to divergent habitats and environmental pressures (Rohwer and Kubitzki, 1993; Lorea-Hernández, 2002). Significant positive correlations between SLA and leaf N:P ratio suggest that *D. salicifolia* could require lower P investment for leaf construction compared to the other species (Appendix S4). The high SLA values of *D. salicifolia* suggest that this species can optimize carbon gain in the environment with higher light availability. These trait relationships suggest a differential efficiency in resource-use between *D. salicifolia* and *D. colorata* trees, but further research is needed to test this possibility. In addition, the functional divergence between species could also lead to divergent herbivory rates. For example, *D. salicifolia* and *D. colorata* likely face lower costs of leaf construction and a faster replacement of lost tissue than *D. ambigens*, at expenses of higher vulnerability to the attack of natural enemies (Poorter, 2009) and shorter leaf longevity (Wright et al., 2004).

Perspectives on ecological differentiation of *Damburneya* species

According to Rohwer and Kubitzki (1993), landscape elevation and soil variation play a critical role in ecological differentiation among *Damburneya* species and explain differences of local distribution and abundance in sympatric species. Here, we showed the effects of soil properties on leaf functional trait variation for four *Damburneya* species. The interspecific divergence of leaf traits relationships in our study are potentially good indicators of the differential

effects of soil heterogeneity on sympatric *Damburneya* and could explain differences in distribution and abundance among species. For example, *D. gentlei* was restricted to low altitude, likely due to a high nutrient demand (i.e., an acquisitive strategy). In addition, *D. salicifolia*, which seems to use P more efficiently, was the most abundant species at high altitudes, where soil P availability is lower.

On the other hand, our data suggest that leaf traits and species distribution could vary in response to herbivory or abiotic drivers of ecological differentiation (e.g., climate and the availability of light) that vary along the altitudinal gradient. For example, differences in leaf mechanical resistance underlying divergent herbivory rates could explain the wide distribution of *D. ambigens* in contrast to the restricted distribution of *D. gentlei*, and higher light availability at higher altitudes could favor the establishment of *D. salicifolia*. Nevertheless, studies regarding the impact of leaf trait variation on plant fitness and other factors impacting species abundance and distribution like recruitment, demography, phenology, competition, and even timber extraction, are still needed.

We do not know to what extent leaf functional trait variation reflects phylogenetic signal or evolutionary processes like local adaptation, nor how much of this variation is genetically or environmentally determined. Common garden experiments could be used to test how species respond to the same ranges of environmental variation (i.e., light, soils, elevation) and whether patterns of trait correlation exhibit phenotypic plasticity or are maintained under changing environments (Reich et al., 2003).

Like most Lauraceae species in Mexico, the *Damburneya* species studied here are threatened by habitat loss and deforestation (de Kok, 2020a, 2020b, 2020c) and are vulnerable to disturbance because they usually do not thrive in secondary vegetation (Lorea-Hernández, 2002). Among the natural protected areas in Mexico, the Los Tuxtlas Biosphere Reserve harbors the greatest number of Lauraceae species (Lorea-Hernández, 2002). During the last decades, this reserve has suffered deforestation and fragmentation (Dirzo et al., 2007; von Thaden et al., 2018), causing soil deterioration and nutrient loss (Tobón et al., 2011; Roa-Fuentes et al., 2015b) that could be especially harmful to *Damburneya* species with small population sizes.

The variation in leaf traits and distribution among species of *Damburneya* can inform future management and ecological restoration projects of tropical rainforests in the region. We provided evidence that the *Damburneya* species are sensitive to local environmental variation and exhibit divergent functional strategies. Based on our results, we would expect these species to be differentially affected by environmental changes, including the collateral effects of deforestation (i.e., changes in light incidence, water, and soil nutrient availability) and global climate change (in precipitation, temperature, evapotranspiration, and soil moisture; IPCC, 2021). Hence, the species of *Damburneya* may require different management strategies; we encourage

future research on this topic and highlight the need for monitoring natural populations.

CONCLUSIONS

Here we explored the potential role of leaf trait variation as a mechanistic basis of ecological divergence among sympatric, closely related species of *Damburneya* in a tropical rainforest. The results suggest that interspecific differences in trait correlations and variation in response to soil heterogeneity along the altitudinal gradient could explain some ecological differences among species. Our research highlights the role of soil variation as a potential driver of functional divergence within the genus. However, we acknowledge that other unmeasured factors (i.e., climate, light, and herbivory) could be key potential drivers of ecological differentiation among *Damburneya*. Species of *Damburneya* exhibited divergent functional strategies, but all appear to be limited by soil phosphorus supply. Altogether, the patterns of trait variation likely contribute to explain local differences in species abundance and distribution and are a promising tool to compare species performance across environmental gradients. Given the impact of land-use change in the tropical rainforest biome and the need for deeper knowledge of forest tree species for effective management and ecological restoration of this threatened ecosystem, the topic of the present study warrants further investigation.

AUTHOR CONTRIBUTIONS

L.J.G.K. and J.N.F. planned and designed the research, conducted the fieldwork, collected the samples, and recorded the data. J.C. gave advice on fieldwork sampling and supervised laboratory work. J.C. and H.P. gave advice on research planning and data analysis. L.J.G.K. compiled and analyzed the data and wrote the first draft of the manuscript. All authors contributed to data interpretation and helped with revising the manuscript.

ACKNOWLEDGMENTS

The authors thank Santiago Sinaca Colín for help and guidance on fieldwork and Fernando López Ordáz, Brenda Hernández, Valeria Flores, Daniel Matías, Ana Lucía Castrejón and Pilar Suárez, who helped with sample collection and processing. Also, we acknowledge Enrique Solís Villalpando for help and guidance with soil analysis, Francisco Lorea-Hernández (INCOL-Xalapa) for the taxonomic determination of collected specimens, Rosalinda Tapia-López for logistical support during planning and execution of the project, and the staff at the Estación de Biología Tropical Los Tuxtlas, especially the Director, Rosamond Coates-Lutes for ensuring several facilities for our research team in the field. We thank Miguel Martínez Ramos for his valuable comments and advice and the anonymous reviewers and Associate Editor for detailed suggestions on our previous drafts. This paper is part of the

doctoral research of L.J.G.K., who acknowledges the Posgrado en Ciencias Biológicas at the Universidad Nacional Autónoma de México for its support and the Consejo Nacional de Ciencia y Tecnología (CONACYT) of Mexico for the scholarship no. 463686. This article constitutes a prerequisite for the Ph.D. in Science degree in the Programa de Posgrado en Ciencias Biológicas. The project was funded by SEP-CONACYT Ciencia Básica grant to J.N.F. (no. 284692).

CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare.

OPEN DATA BADGE



This article has earned an Open Data Badge for making publicly available the digitally shareable data necessary to reproduce the reported results. The data are available at <https://doi.org/10.6084/m9.figshare.20452599.v4>. Learn more about the Open Practices badges from the Center for Open Science: https://github.com/ljgiraldok/ecological_differentiation_Damburneya.

DATA AVAILABILITY STATEMENT

The data are available at Figshare at <https://doi.org/10.6084/m9.figshare.20452599.v4> (Giraldo-Kalil and Núñez-Farfán, 2022). Scripts employed for statistical analyses and figures are available at GitHub at https://github.com/ljgiraldok/ecological_differentiation_Damburneya.

ORCID

Juan Núñez-Farfán  <http://orcid.org/0000-0001-5829-8338>

REFERENCES

- Adler, P. B., A. Fajardo, A. R. Kleinhesselink, and N. J. B. Kraft. 2013. Trait-based tests of coexistence mechanisms. *Ecology Letters* 16: 1294–1306.
- Aerts, R., and F. S. I. Chapin. 2000. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research* 30: 1–67.
- Agrawal, A. A. 2020. A scale-dependent framework for trade-offs, syndromes, and specialization in organismal biology. *Ecology* 101: e02924.
- Anderegg, L. D. L., L. T. Berner, G. Badgley, M. L. Sethi, B. E. Law, and J. HilleRisLambers. 2018. Within-species patterns challenge our understanding of the leaf economics spectrum. *Ecology Letters* 21: 734–744.
- Anderson, J. M., and J. S. I. Ingram. 1993. Tropical soil biology and fertility: a handbook of methods, 2nd ed. CAB International, Wallingford, UK.
- Arroyo-Rodríguez, V., J. C. Dunn, J. Benítez-Malvido, and S. Mandujano. 2009. Angiosperms, Los Tuxtlas Biosphere Reserve, Veracruz, Mexico. *Check List* 5: 787–799.
- Augusto, L., D. L. Achat, M. Jonard, D. Vidal, and B. Ringeval. 2017. Soil parent material—A major driver of plant nutrient limitations in terrestrial ecosystems. *Global Change Biology* 23: 3808–3824.
- Baltzer, J. L., and S. C. Thomas. 2007. Determinants of whole-plant light requirements in Bornean rain forest tree saplings. *Journal of Ecology* 95: 1208–1221.
- Bates, D., M. Mächler, B. M. Bolker, and S. C. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67: 1–51.
- Benner, J., P. M. Vitousek, and R. Ostertag. 2011. Nutrient cycling and nutrient limitation in tropical montane cloud forests. In L. Bruijnzeel, F. Scatena, and L. Hamilton [eds.], Tropical montane cloud forests: science for conservation and management (International Hydrology Series), 90–100. Cambridge University Press, Cambridge, MA, USA.
- Bongers, F., Popma, J., Meave del Castillo, J. and Carabias, J. 1988. Structure and floristic composition of the lowland rain forest of Los Tuxtlas, Mexico. *Vegetatio* 74: 55–80.
- Campo, J., and R. Dirzo. 2003. Leaf quality and herbivory responses to soil nutrient addition in secondary tropical dry forests of Yucatán, Mexico. *Journal of Tropical Ecology* 19: 525–530.
- Campo, J., J. F. Gallardo, and G. Hernández. 2014. Leaf and litter nitrogen and phosphorus in three forests with low P supply. *European Journal of Forest Research* 133: 121–129.
- Campo, J., M. Maass, and L. de Pablo. 2001. Weathering in a tropical dry forest of Mexico. *Agrociencia* 35: 245–254.
- Campo-Alves, J. 2003. Nutrient availability and fluxes along a toposequence with tropical dry forest in México. *Agrociencia* 37: 211–219.
- Cárdenas, I., and J. Campo. 2007. Foliar nitrogen and phosphorus resorption and decomposition in the nitrogen-fixing tree *Lysiloma microphyllum* in primary and secondary seasonally tropical dry forests in Mexico. *Journal of Tropical Ecology* 23: 107–113.
- Carmona, C. P., F. de Bello, N. W. H. Mason, and J. Lepš. 2016. Traits without borders: integrating functional diversity across scales. *Trends in Ecology & Evolution* 31: 382–394.
- Chapin, F. S. I. 1991. Effects of multiple environmental stresses on nutrient availability and use. In H. A. Mooney, W. E. Winner, E. J. Pell, and E. Chu [eds.], Response of plants to multiple stresses, 67–88. Academic Press, San Diego, CA, USA.
- Chapin, F. S. I., P. A. Matson, and P. M. Vitousek. 2011. Principles of terrestrial ecosystem ecology, 2nd ed. Springer, NY, NY, USA.
- Chesson, P. 2000. Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics* 31: 343–366.
- Coomes, D. A., G. Kunstler, C. D. Canham, and E. Wright. 2009. A greater range of shade-tolerance niches in nutrient-rich forests: An explanation for positive richness-productivity relationships? *Journal of Ecology* 97: 705–717.
- Dalling, J. W., K. Heineman, O. R. Lopez, S. J. Wright, and B. L. Turner. 2016. Nutrient availability in tropical rain forests: the paradigm of phosphorus limitation. In G. Goldstein and L. Santiago [eds.], Tropical tree physiology, 6, 261–273. Springer, Cham, Switzerland.
- Dent, D. H., and D. F. R. P. Burslem. 2016. Leaf traits of dipterocarp species with contrasting distributions across a gradient of nutrient and light availability. *Plant Ecology and Diversity* 9: 521–533.
- Díaz, S., J. Kattge, J. H. C. Cornelissen, I. J. Wright, S. Lavorel, S. Dray, B. Reu, et al. 2016. The global spectrum of plant form and function. *Nature* 529: 167–171.
- Dirzo, R., G. Ibarra-Manríquez, and C. M. Sánchez-Garduño. 1997. *Nectandra ambigens*. In E. González-Soriano, R. Dirzo, and R. C. Vogt [eds.], Historia natural de Los Tuxtlas, 124–129. Universidad Nacional Autónoma de México, CONABIO, Distrito Federal, México.
- Dirzo, R., E. Mendoza, and P. Ortiz. 2007. Size-related differential seed predation in a heavily defaunated neotropical rain forest. *Biotropica* 39: 355–362.
- Donovan, L. A., H. Maherali, C. M. Caruso, H. Huber, and H. de Kroon. 2011. The evolution of the worldwide leaf economics spectrum. *Trends in Ecology and Evolution* 26: 88–95.
- Elliott, E. T., J. W. Heil, E. F. Kelly, and M. H. Curtis. 1999. Soil structural and other physical properties. In G. P. Robertson, D. C. Coleman, C. S. Bledsoe, and P. Sollins [eds.], Standard soil methods for long-term ecological research, 74–85. Oxford University Press, NY, NY, USA.
- Fajardo, A., and A. Siefert. 2018. Intraspecific trait variation and the leaf economics spectrum across resource gradients and levels of organization. *Ecology* 99: 1024–1030.
- Figueroa, D., P. Ortega-Fernández, T. F. Abbruzzini, A. Rivero-Villar, F. Galindo, B. Chavez-Vergara, J. D. Etchevers, and J. Campo. 2020. Effects of land use change from natural forest to livestock on soil C, N

- and P dynamics along a rainfall gradient in Mexico. *Sustainability* 12: 8656.
- Flores-Delgado, L., I. Sommer-Cervantes, J. R. Alcalá-Martínez, and J. Álvarez-Sánchez. 1999. Estudio morfológico de algunos suelos de la región de Los Tuxtlas, Veracruz, México. *Revista Mexicana de Ciencias Geológicas* 16: 81–88.
- GBIF.org. 2021a. GBIF occurrence download. Global Biodiversity Information Facility, Copenhagen, Denmark. Website: <https://doi.org/10.15468/dl.tz2eh7>. [accessed 8 April 2021].
- GBIF.org. 2021b. GBIF occurrence download. Global Biodiversity Information Facility, Copenhagen, Denmark. Website: <https://doi.org/10.15468/dl.bqscrw>. [accessed 8 April 2021].
- Giraldo-Kalil, L. J., and J. Núñez-Farfán. 2022. Patterns of leaf trait variation underlie ecological differences among sympatric tree species of *Damburneya* in a tropical rainforest. Data set at figshare. <https://doi.org/10.6084/m9.figshare.20452599.v4>
- Givnish, T. J. 1988. Adaptation to sun and shade: a whole-plant perspective. *Australian Journal of Plant Physiology* 15: 63–92.
- Gong, H., and J. Gao. 2019. Soil and climatic drivers of plant SLA (specific leaf area). *Global Ecology and Conservation* 20: e00696.
- Güsewell, S. 2004. N:P ratios in terrestrial plants: variation and functional significance. *New Phytologist* 164: 243–266.
- Gutiérrez-García, G., and M. Ricker. 2011. Climate and climate change in the region of Los Tuxtlas (Veracruz, Mexico): a statistical analysis. *Atmósfera* 24: 347–373.
- Harrington, R. A., J. H. Fownes, and P. M. Vitousek. 2001. Production and resource use efficiencies in N- and P-limited tropical forests: a comparison of responses to long-term fertilization. *Ecosystems* 4: 646–657.
- Hodgson, J. G., G. Montserrat-Martí, M. Charles, G. Jones, P. Wilson, B. Shipley, M. Sharafi, et al. 2011. Is leaf dry matter content a better predictor of soil fertility than specific leaf area? *Annals of Botany* 108: 1337–1345.
- Hou, E., D. Wen, L. Jiang, X. Luo, Y. Kuang, X. Lu, C. Chen, et al. 2021. Latitudinal patterns of terrestrial phosphorus limitation over the globe. *Ecology Letters* 24: 1420–1431.
- Ibarra-Manríquez, G., and S. Sinaca-Colín. 1995. Lista florística comentada de la Estación de Biología Tropical “Los Tuxtlas”, Veracruz, México. *Revista de Biología Tropical* 43: 75–115.
- Ibarra-Manríquez, G., M. Ricker, G. Angeles, S. Sinaca-Colín, and M. A. Sinaca-Colín. 1997. Useful plants of the Los Tuxtlas rain forest (Veracruz, Mexico): considerations of their market potential. *Economic Botany* 51: 362–376.
- IPCC [Intergovernmental Panel on Climate Change]. 2021. Summary for policymakers. In V. Masson-Delmotte, P. Zhai, A. Pirani, S. L. Connors, C. Péan, S. Berger, N. Caud, et al. [eds.], *Climate change 2021: The physical science basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*, 3–32. Cambridge University Press, Cambridge, UK and NY, USA. <https://doi.org/10.1017/9781009157896.001>
- Ji, W., S. E. LaZerte, M. J. Waterway, and M. J. Lechowicz. 2020. Functional ecology of congeneric variation in the leaf economics spectrum. *New Phytologist* 225: 196–208.
- Kichenin, E., D. A. Wardle, D. A. Peltzer, C. W. Morse, and G. T. Freschet. 2013. Contrasting effects of plant inter- and intraspecific variation on community-level trait measures along an environmental gradient. *Functional Ecology* 27: 1254–1261.
- Kitajima, K. 1994. Relative importance of photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 tropical trees. *Oecologia* 98: 419–428.
- Koerselman, W., and A. F. M. Meuleman. 1996. The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology* 33: 1441–1450.
- de Kok, R. 2020a. *Damburneya ambigens*. The IUCN red list of threatened species 2020: e.T146610634A146651525. Website: <https://doi.org/10.2305/IUCN.UK.2020-1.RLTS.T146610634A146651525.en>. [accessed 21 January 2021].
- de Kok, R. 2020b. *Damburneya gentlei*. The IUCN red list of threatened species 2020: e.T146611071A146651540. Website: <https://doi.org/10.2305/IUCN.UK.2020-1.RLTS.T146611071A146651540.en>. [accessed 21 January 2021].
- de Kok, R. 2020c. *Damburneya salicifolia*. The IUCN red list of threatened species 2020: e.T146611340A146651565. Website: <https://doi.org/10.2305/IUCN.UK.2020-1.RLTS.T146611340A146651565.en>. [accessed 21 January 2021].
- Krasilnikov, P., M. del C. Gutiérrez-Castorena, R. J. Ahrens, C. O. Cruz-Gaistardo, S. Sedov, and E. Solleiro-Rebolledo. 2013. *Soils of Mexico*. Springer, NY, NY, USA.
- Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2017. lmerTest package: tests in linear mixed effects models. *Journal of Statistical Software* 82: 1–26.
- Lambers, H., and R. S. Oliveira. 2019. *Plant physiological ecology*, 3rd ed. Springer, NY, NY, USA.
- Lajtha, K., C. T. Driscoll, W. M. Jarell and E. T. Elliott. 1999. Soil phosphorus: characterization and total element analysis. In G. P. Robertson, D. C. Coleman, C. S. Bledsoe, and P. Sollins [eds.], *Standard soil methods for long-term ecological research*, 115–142, Oxford University Press, NY, NY, USA.
- Lê, S., J. Josse, and F. Husson. 2008. FactoMineR: an R package for multivariate analysis. *Journal of Statistical Software* 25: 1–18.
- Lepš, J., F. de Bello, P. Šmilauer, and J. Doležal. 2011. Community trait response to environment: disentangling species turnover vs intra-specific trait variability effects. *Ecography* 34: 856–863.
- Lorea-Hernández, F. G. 2002. La familia Lauraceae en el sur de México: diversidad, distribución y estado de conservación. *Boletín de la Sociedad Botánica de México* 71: 59–70.
- Lorea-Hernández, F. G. 2011. Aguacatillos y especies afines (Lauraceae). In Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO) [ed.], *La biodiversidad en Veracruz: Estudio de estado*, vol. 2, 141–148. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO); Gobierno del Estado de Veracruz, Universidad Veracruzana, Instituto de Ecología A.C., México.
- Luke, S. G. 2017. Evaluating significance in linear mixed-effects models in R. *Behavior Research Methods* 49: 1494–1502.
- MacArthur, R., and R. Levins. 1967. The limiting similarity, convergence, and divergence of coexisting species. *American Naturalist* 101: 377–385.
- Marschner, P. 2012. *Marschner's mineral nutrition of higher plants*, 3rd ed. Elsevier, San Diego, CA, USA.
- Mason, C. M., and L. A. Donovan. 2015. Evolution of the leaf economics spectrum in herbs: evidence from environmental divergences in leaf physiology across *Helianthus* (Asteraceae). *Evolution* 69: 2705–2720.
- Mason, N. W. H., S. J. Richardson, D. A. Peltzer, F. de Bello, D. A. Wardle, and R. B. Allen. 2012. Changes in coexistence mechanisms along a long-term soil chronosequence revealed by functional trait diversity. *Journal of Ecology* 100: 678–689.
- Maynard, D. S., L. Bialic-Murphy, C. M. Zohner, C. Averill, J. van den Hoogen, H. Ma, L. Mo, et al. 2022. Global relationships in tree functional traits. *Nature Communications* 13: 3185.
- McGill, B. J., B. J. Enquist, E. Weiher, and M. Westoby. 2006. Rebuilding community ecology from functional traits. *Trends in Ecology and Evolution* 21: 179–185.
- Messier, J., B. J. McGill, B. J. Enquist, and M. J. Lechowicz. 2017. Trait variation and integration across scales: Is the leaf economic spectrum present at local scales? *Ecography* 40: 685–697.
- Muir, C. D., M. Conesa, E. J. Roldán, A. Molins, and J. Galmés. 2017. Weak coordination between leaf structure and function among closely related tomato species. *New Phytologist* 213: 1642–1653.
- Murphy, J., and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* 27: 31–36.
- Niinemets, Ü. 2001. Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. *Ecology* 82: 453–469.
- Niinemets, Ü. 2015. Is there a species spectrum within the world-wide leaf economics spectrum? Major variations in leaf functional traits in the Mediterranean sclerophyll *Quercus ilex*. *New Phytologist* 205: 79–96.

- Onoda, Y., M. Westoby, P. B. Adler, A. M. F. Choong, F. J. Clissold, J. H. C. Cornelissen, S. Díaz, et al. 2011. Global patterns of leaf mechanical properties. *Ecology Letters* 14: 301–312.
- Onoda, Y., I. J. Wright, J. R. Evans, K. Hikosaka, K. Kitajima, Ü. Niinemets, H. Poorter, et al. 2017. Physiological and structural tradeoffs underlying the leaf economics spectrum. *New Phytologist* 214: 1447–1463.
- Pérez-Harguindeguy, N., S. Díaz, E. Garnier, S. Lavorel, H. Poorter, P. Jaureguiberry, M. S. Bret-Harte, et al. 2013. New handbook for standardized measurement of plant functional traits worldwide. *Australian Journal of Botany* 61: 167–234.
- Poorter, L. 2009. Leaf traits show different relationships with shade tolerance in moist versus dry tropical forests. *New Phytologist* 181: 890–900.
- Poorter, H., Ü. Niinemets, L. Poorter, I. J. Wright, and R. Villar. 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist* 182: 565–588.
- Popma, J., F. Bongers, and J. Meave del Castillo. 1988. Patterns in the vertical structure of the tropical lowland rain forest of Los Tuxtlas, Mexico. *Vegetatio* 74: 81–91.
- R Core Team. 2021. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Website: <https://www.R-project.org/>
- Reich, P. B. 2014. The world-wide 'fast-slow' plant economics spectrum: a traits manifesto. *Journal of Ecology* 102: 275–301.
- Reich, P. B., D. S. Ellsworth, M. B. Walters, J. M. Vose, C. Gresham, J. C. Volin, and W. D. Bowman. 1999. Generality of leaf trait relationships: a test across six biomes. *Ecology* 80: 1955–1969.
- Reich, P. B., I. J. Wright, J. Cavender-Bares, J. M. Craine, J. Oleksyn, M. Westoby, and M. B. Walters. 2003. The evolution of plant functional variation: traits, spectra, and strategies. *International Journal of Plant Sciences* 164: S143–S164.
- Roa-Fuentes, L. L., C. Martínez-Garza, J. Etchevers, and J. Campo. 2015a. Recovery of Soil C and N in a tropical pasture: passive and active restoration. *Land Degradation and Development* 26: 201–210.
- Roa-Fuentes, L. L., P. H. Templer, and J. Campo. 2015b. Effects of precipitation regime and soil nitrogen on leaf traits in seasonally dry tropical forests of the Yucatan Peninsula, Mexico. *Oecologia* 179: 585–597.
- Rohwer, J. G. 1993. Lauraceae: *Nectandra*. Flora neotropica, 1–332. New York Botanical Garden Press, Bronx, NY, USA.
- Rohwer, J. G., and K. Kubitzki. 1993. Ecogeographical differentiation in *Nectandra* (Lauraceae), and its historical implications. *Botanica Acta* 106: 88–99.
- Sánchez-Gallen, I., F. J. Álvarez-Sánchez, and J. Benítez-Malvido. 2010. Structure of the advanced regeneration community in tropical rain forest fragments of Los Tuxtlas, Mexico. *Biological Conservation* 143: 2111–2118.
- Santiago, L. S., K. Kitajima, S. J. Wright, and S. S. Mulkey. 2004. Coordinated changes in photosynthesis, water relations and leaf nutritional traits of canopy trees along a precipitation gradient in lowland tropical forest. *Oecologia* 139: 495–502.
- Schachtman, D. P., R. J. Reid, and S. M. Ayling. 1998. Phosphorus uptake by plants: from soil to cell. *Plant Physiology* 116: 447–453.
- Schlesinger, W. H., and E. S. Bernhardt. 2020. Biogeochemistry: an analysis of global change, 4th ed. Academic Press, San Diego, CA, USA.
- Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9: 671–675.
- Schulze, E. D., E. Beck, N. Buchmann, K. Müller-Hohenstein, and M. Scherer-Lorenzen. 2019. Plant ecology, 2nd ed. Springer, NY, NY, USA.
- Shipley, B., M. J. Lechowicz, I. J. Wright, and P. B. Reich. 2006. Fundamental trade-offs generating the worldwide leaf economics spectrum. *Ecology* 87: 535–541.
- Siebe, C., R. Jahn, and K. Stahr. 1996. Manual para la descripción y evaluación ecológica de suelos en el campo. Publicación especial 4. Sociedad Mexicana de la Ciencia del Suelo, A. C., Chapingo, México.
- Slot, M., D. Cala, J. Aranda, A. Virgo, S. T. Michaletz, and K. Winter. 2021. Leaf heat tolerance of 147 tropical forest species varies with elevation and leaf functional traits, but not with phylogeny. *Plant Cell and Environment* 44: 2414–2427.
- Sollins, P., C. Glassman, E. A. Paul, C. Swanston, K. Lajtha, J. W. Heil, and E. T. Elliott. 1999. Soil carbon and nitrogen: pools and fractions. In G. P. Robertson, D. C. Coleman, C. S. Bledsoe, and P. Sollins [eds.], Standard soil methods for long-term ecological research, 89–105. Oxford University Press, NY, NY, USA.
- Sommer-Cervantes, I., L. Flores-Delgadillo, and M. Gutiérrez-Ruiz. 2003. Caracterización de los suelos de la Estación de Biología Tropical Los Tuxtlas. In J. Álvarez-Sánchez and E. Naranjo-García [eds.], Ecología del suelo en la selva tropical húmeda de México, 17–69. Instituto de Ecología, A. C., Instituto de Biología, Facultad de Ciencias, UNAM., Xalapa, Mexico.
- Sri-Ngernyuan, K., M. Kanzaki, T. Mizuno, H. Noguchi, S. Teenjuntuk, C. Sungpalee, M. Hara, et al. 2003. Habitat differentiation of Lauraceae species in a tropical lower montane forest in northern Thailand. *Ecological Research* 18: 1–14.
- Srinivas, S. G., and Y. L. Krishnamurthy. 2019. Altitude and ecological distribution of genus *Litsea* (Lauraceae) in Western Ghats of Karnataka, India. *Journal of Tropical Forestry and Environment* 9: 108–119.
- Tanner, E. V. J., P. M. Vitousek, and E. Cuevas. 1998. Experimental investigation of nutrient limitation of forest growth on wet tropical mountains. *Ecology* 79: 10–22.
- Tobón, W., C. Martínez-Garza, and J. Campo. 2011. Soil responses to restoration of a tropical pasture in Veracruz, south-eastern Mexico. *Journal of Tropical Forest Science* 23: 338–344.
- Townsend, A. R., G. P. Asner, and C. C. Cleveland. 2008. The biogeochemical heterogeneity of tropical forests. *Trends in Ecology and Evolution* 23: 424–431.
- Trofimov, D., B. Rudolph, and J. G. Rohwer. 2016. Phylogenetic study of the genus *Nectandra* (Lauraceae), and reinstatement of *Damburneya*. *Taxon* 65: 980–996.
- Turner, B. L., T. Brenes-Arguedas, and R. Condit. 2018. Pervasive phosphorus limitation of tree species but not communities in tropical forests. *Nature* 555: 367–370.
- Valladares, F., and Ü. Niinemets. 2008. Shade tolerance, a key plant feature of complex nature and consequences. *Annual Review of Ecology, Evolution, and Systematics* 39: 237–257.
- van der Werff, H. 1992. Substrate preference of Lauraceae and ferns in the Iquitos area, Peru. *Candollea* 47: 11–20.
- van der Werff, H., and H. G. Richter. 1996. Toward an improved classification of Lauraceae. *Annals of the Missouri Botanical Garden* 83: 409–418.
- Villaseñor, J. L., E. Ortiz, and A. Campos-Villanueva. 2018. High richness of vascular plants in the tropical Los Tuxtlas Region, Mexico. *Tropical Conservation Science* 11: 1–12.
- Violle, C., B. J. Enquist, B. J. McGill, L. Jiang, C. H. Albert, C. Hulshof, V. Jung, and J. Messier. 2012. The return of the variance: intraspecific variability in community ecology. *Trends in Ecology and Evolution* 27: 244–252.
- Violle, C., and L. Jiang. 2009. Towards a trait-based quantification of species niche. *Journal of Plant Ecology* 2: 87–93.
- Violle, C., M. L. Navas, D. Vile, E. Kazakou, C. Fortunel, I. Hummel, and E. Garnier. 2007. Let the concept of trait be functional! *Oikos* 116: 882–892.
- Vitousek, P. M. 1984. Litterfall, nutrient cycling, and nutrient limitation in tropical forests. *Ecology* 65: 285–298.
- von Thaden, J. J., J. Laborde, S. Guevara, and P. Mokondoko-Delgadillo. 2020. Dinámica de los cambios en el uso del suelo y cobertura vegetal en la Reserva de la Biosfera Los Tuxtlas (2006–2016) dynamics. *Revista Mexicana de Biodiversidad* 91: e913190.
- von Thaden, J. J., J. Laborde, S. Guevara, and C. S. Venegas-Barrera. 2018. Forest cover change in the Los Tuxtlas Biosphere Reserve and its future: the contribution of the 1998 protected natural area decree. *Land Use Policy* 72: 443–450.
- Warton, D. I., R. A. Duursma, D. S. Falster, and S. Taskinen. 2012. SMATR3—an R package for estimation and inference about allometric lines. *Methods in Ecology and Evolution* 3: 257–259.
- Weil, R. R., and N. C. Brady. 2017. The nature and properties of soils, 15th ed. Pearson Education, Boston, MA, USA.

- Westbrook, J. W., K. Kitajima, J. G. Burleigh, W. J. Kress, D. L. Erickson, and S. J. Wright. 2011. What makes a leaf tough? Patterns of correlated evolution between leaf toughness traits and demographic rates among 197 shade-tolerant woody species in a neotropical forest. *American Naturalist* 177: 800–811.
- 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.
- Wiens, J. J., and C. H. Graham. 2005. Niche conservatism: integrating evolution, ecology, and conservation biology. *Annual Review of Ecology, Evolution, and Systematics* 36: 519–539.
- Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, et al. 2004. The worldwide leaf economics spectrum. *Nature* 428: 821–827.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Vouchers of representative specimens of four species of *Damburneya* collected in the tropical rainforest in Los Tuxtlas Biosphere Reserve.

Appendix S2. Soil physical, physicochemical, and chemical properties at low and high altitude in Los Tuxtlas Biosphere Reserve.

Appendix S3. Leaf functional trait variation in four sympatric *Damburneya* species (*D. ambigens*, *D. colorata*, *D. gentlei*, *D. salicifolia*) at Los Tuxtlas Biosphere Reserve according to altitude (high vs. low).

Appendix S4. Bivariate relationships among leaf functional traits of four sympatric species of *Damburneya* at Los Tuxtlas Biosphere Reserve calculated with standardized major axis regressions.

Appendix S5. Effects of altitude on leaf functional trait variation of four sympatric *Damburneya* species at Los Tuxtlas Biosphere Reserve according to linear mixed models.

Appendix S6. Likelihood ratio test and significance of the random effects of mixed linear models for leaf functional traits of four sympatric *Damburneya* species (*D. ambigens*, *D. colorata*, *D. gentlei*, *D. salicifolia*) at Los Tuxtlas Biosphere Reserve, according to species, altitude, and sampling plot.

Appendix S7. Relationships of the plot average leaf functional traits of four sympatric *Damburneya* species (*D. ambigens*, *D. colorata*, *D. gentlei*, *D. salicifolia*) at Los Tuxtlas with soil organic carbon (SOC), C:N ratio, and soil total phosphorus (STP).

Appendix S8. Relationships of the plot average morphological leaf traits of four sympatric *Damburneya* species (*D. ambigens*, *D. colorata*, *D. gentlei*, *D. salicifolia*) at Los Tuxtlas with soil available phosphorus (SAP), pH, and clay content.

Appendix S9. Decomposition of the variation of intra- and interspecific effects of abundance weighted average plot trait values of four sympatric species of *Damburneya* (*D. ambigens*, *D. colorata*, *D. gentlei*, *D. salicifolia*) at Los Tuxtlas tropical rainforest in response to soil nutrients.

How to cite this article: Giraldo-Kalil, L. J., J. Campo, H. Paz, and J. Núñez-Farfán. 2022. Patterns of leaf trait variation underlie ecological differences among sympatric tree species of *Damburneya* in a tropical rainforest. *American Journal of Botany* 109(9): 1394–1409. <https://doi.org/10.1002/ajb2.16056>