

ASSOCIATION OF FUNCTIONAL TRAIT VARIATION OF *QUERCUS CASTANEA* WITH TEMPERATURE AND WATER AVAILABILITY GRADIENTS AT THE LANDSCAPE LEVEL

RELACIÓN DE LA VARIACIÓN DE ATRIBUTOS FUNCIONALES DE *QUERCUS CASTANEA* CON GRADIENTES DE TEMPERATURA Y DISPONIBILIDAD DE AGUA A NIVEL DE PAISAJE

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Abstract

Background: Phenotypic variability of tree species is often associated to environmental factors. *Quercus castanea* is a Mexican red oak with a wide geographical and altitudinal distribution along contrasting environments. It is the most abundant oak species in the Cuitzeo basin, which is characterized by highly heterogeneous environmental conditions.

Hypothesis: We hypothesized that gradients in temperature, precipitation and soil characteristics across the distribution of *Q. castanea* within the Cuitzeo basin promote variability in functional traits related to the adjustment to differential water availability at a landscape level.

Studied species: *Quercus castanea* Née (Fagaceae).

Study site and years of study: Cuitzeo basin in Central Mexico. 2015-2016.

Methods: We quantified leaf chlorophyll concentration (CC), leaf area (LA), leaf thickness (LT), leaf mass per area (LMA) and the Huber value (HV) in 10 individuals from 22 populations of *Q. castanea* throughout the basin.

Results: Despite the relatively small geographical area (4,000 km²), our results revealed significant differentiation among populations in the studied functional traits. The strongest variation found was in LT, which was negatively correlated with precipitation seasonality. This pattern is opposite to previous reports on Mediterranean oaks but similar to tropical oaks and suggests that the combination with other traits such as leaf phenology is important in the response to water availability.

Conclusions: Significant functional differences exist among populations of *Q. castanea* separated by a few kilometers in the heterogeneous landscape of the Cuitzeo basin. This species shows clearly sclerophyllous leaves, but leaf thickness varies to a considerable degree across populations.

Key words: climate gradient, leaf economic spectrum, plant morpho-physiological variation, *Quercus*.

Resumen

Antecedentes: La variación fenotípica de especies arbóreas con frecuencia se asocia a factores ambientales. *Quercus castanea*, un encino rojo mexicano de amplia distribución geográfica y altitudinal en ambientes contrastantes es el encino más abundante en la cuenca de Cuitzeo, caracterizada por presentar condiciones ambientales heterogéneas.

Hipótesis: Los gradientes de temperatura, precipitación y características del suelo en la cuenca de Cuitzeo promueven la variación en los atributos funcionales de *Q. castanea* relacionados con el ajuste a la disponibilidad de agua a nivel de paisaje.

Especie en estudio: *Quercus castanea* Née (Fagaceae).

Sitio de estudio y fechas: Cuenca de Cuitzeo, centro de México. 2015-2016.

Métodos: Cuantificamos la concentración de clorofila de la hoja (CC), el área foliar (LA), el grosor foliar (LT), la masa por área foliar (LMA) y el valor de Huber (HV) en 10 individuos de 22 poblaciones de *Q. castanea* distribuidas en la cuenca.

Resultados: A pesar del área geográfica pequeña (4,000 km²), nuestros resultados mostraron una diferenciación significativa en los atributos funcionales entre las poblaciones. La variación más marcada fue en LT, que se correlacionó negativamente con la estacionalidad de la precipitación. Este patrón es opuesto a resultados previos en encinos mediterráneos pero similar a encinos tropicales y sugiere que la combinación con otros atributos, como la fenología foliar, es importante en la respuesta a la disponibilidad de agua.

Conclusiones: Existen diferencias funcionales entre las poblaciones de *Q. castanea* separadas unos pocos kilómetros dentro de la cuenca. En particular el grosor de la hoja varía considerablemente.

Palabras clave: espectro económico foliar, gradiente climático, *Quercus*, variación en atributos funcionales.

Variation in phenotypic traits is a fundamental attribute of natural populations and often reflects the linking of traits with geographical and climate variables across the range of a species (McKnow *et al.* 2014). In tree species, variation among populations in several fundamental functional traits is considerably influenced by water availability, which in turn depends on the amount and distribution of rainfall, soil physical properties, and the relationship between evaporation and transpiration (Souto *et al.* 2009, Ramírez-Valiente *et al.* 2010, Cooper *et al.* 2018). Therefore, differences in water availability along the distribution of a species are expected to set contrasting selection pressures leading to local adaptation of populations, as has been shown in many tree species (Ramírez-Valiente *et al.* 2010, Aranda *et al.* 2015, Lind *et al.* 2017). However, the extent of local adaptation at fine spatial scales in long-lived tree species characterized by high rates of gene flow has not been sufficiently studied yet (Cavender-Bares & Ramírez-Valiente 2017). Alternatively, such species may face environmental challenges by maintaining high levels of within-population genetic variation or through phenotypic plasticity, particularly when single individuals may experience a range of conditions throughout the course of a long lifespan (Cavender-Bares & Ramírez-Valiente 2017, Meireles *et al.* 2017).

The genus *Quercus* (oak species) is a very diverse group of tree species distributed in temperate, subtropical and tropical regions of the northern hemisphere, where it is considered one of the most important taxa of trees (Cavender-Bares 2016). This genus, along with others in the Fagaceae family are emerging as non-classical models to study adaptive variation, integrating ecology and evolution (Petit *et al.* 2013, Cavender-Bares 2019). Mexico is an important diversification center for the oaks, with about 161 species in total (32-40 % of the diversity in the world) and more than 100 endemics (Valencia-A 2004). Oaks constitute a very important group for understanding the factors that determine phenotypic variability, due to their particularly high levels of variation at different levels, that is, among species, among populations, within populations and within individuals (Uribe-Salas *et al.* 2008, Hernández-Calderón *et al.* 2014, García-Nogales *et al.* 2016).

Plant functional traits are measurable characteristics that influence performance or fitness and are assumed to reflect evolutionary responses to external conditions and can frequently refer to ecological factors changing along a gradient (Lavorel *et al.* 2007). Research on different oak species has revealed functional variation related to leaf traits, phenology, and growth rates associated to altitudinal, latitudinal, water availability or temperature gradients (Bruschi 2010, Hernández-Calderón *et al.* 2013, Ramírez-Valiente *et al.* 2017). This variation has been explained as resulting from strategies to avoid or tolerate different types

of environmental stress, allowing oak species to develop under a wide range of environmental conditions and resource availability. However, most of these studies have been conducted at broad geographical scales, and little is known about how functional traits vary among tree populations at finer spatial scales such as the landscape level.

Quercus castanea is a Mexican red oak with a wide geographical and altitudinal distribution that occupies contrasting environments, with populations in the Sierra Madre Occidental, the Central Plateau, the Trans-Mexican Volcanic Belt, and the Sierra Madre del Sur (Valencia-A 2004). It is a dominant element in temperate forests and mountain cloud forests and is frequently found in perturbed areas with a xerophytic shrub type of vegetation (Valencia-Cuevas *et al.* 2015). Therefore, *Q. castanea* represents a suitable species to study patterns of phenotypic variation in response to environmental factors at a landscape scale, despite potentially very high gene flow even in a fragmentation scenario (Herrera-Arroyo *et al.* 2013, Oyama *et al.* 2017).

In this study, we hypothesized that gradients in temperature, precipitation and soil characteristics across the distribution of *Q. castanea* within the Cuitzeo basin promote variability in functional traits related to the adjustment to differential water availability. We focused on leaf traits that have been considered easy and inexpensive to measure in a standardized manner (leaf chlorophyll concentration, leaf area, leaf mass per area, leaf thickness and the Huber value) but that capture the general patterns of plant functional responses to environmental gradients (Wright *et al.* 2004, Lavorel *et al.* 2007). According to these previous generalizations, we expected *Q. castanea* individuals to have a higher Huber value and smaller, thicker leaves with a higher mass per area in the parts of the basin with higher temperature and lower precipitation. Particularly, the objectives were: i) to evaluate the amount and patterns of variation in this set of functional traits across *Q. castanea* populations at a landscape level and ii) to determine significant associations between functional traits and environmental variables.

Materials and methods

Study site and sampling procedure. This study was conducted in the basin of Lake Cuitzeo. It has an area of 4,026 km² and is located at 19° 30' - 20° 05' N and 100° 35' - 101° 30' W in the Trans-Mexican Volcanic Belt in the northern part of Michoacan state and the southern part of Guanajuato state (Figure 1). Climate in the basin is temperate with a marked rainy season during summer months (June to September). There is a significant climate gradient, with precipitation increasing and temperature decreasing from north to south and with altitude (Mendoza *et al.* 2006). The topography and soils of the study area are

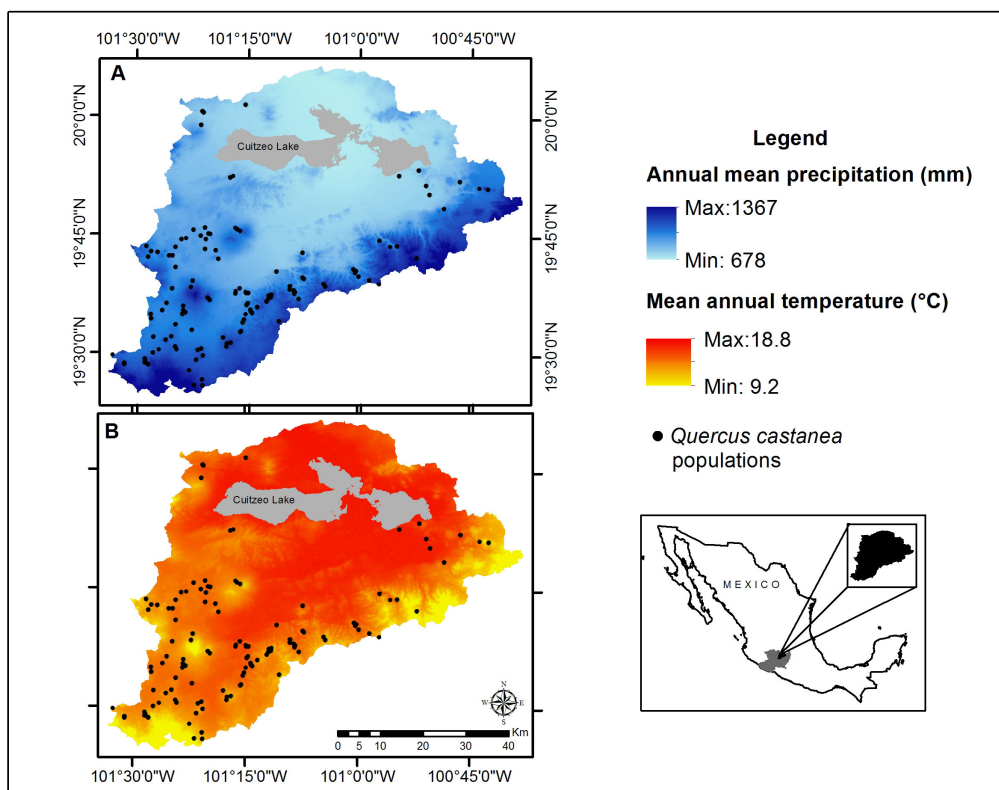


Figure 1. Climate gradients in the Cuitzeo lake basin and the distribution of the *Q. castanea* populations; a) annual mean temperature, b) annual mean precipitation.

product of the volcanic activity of the Quaternary. The dominant soil groups are vertisols, luvisols, leptosols, acrisols and andosols (Mendoza *et al.* 2006, Chávez-Vergara *et al.* 2014).

Quercus castanea grows throughout the Cuitzeo basin, except in the northeastern portion (Aguilar-Romero *et al.* 2016). In the study region, populations can be found between 2,000 and 2,800 m (Herrera-Arroyo *et al.* 2013). Twenty-two populations were chosen for sampling, mostly covering the distribution of the species within the Cuitzeo basin (Table S1, Figure 2). At each population, 10 randomly chosen adult trees separated by at least 20 m from each other were sampled by taking four haphazardly selected sun-exposed branches, with terminal twigs with at least 10 mature leaves with no visible damage, at heights between 2 and 5 m. Only in population 10, fewer trees were collected (6) because of their low availability.

Functional traits. In each individual tree, we measured the following functional traits: leaf chlorophyll concentration (CC), leaf area (LA), leaf mass per area (LMA), leaf thickness (LT) and Huber value (HV, the sapwood cross section area divided by the leaf area distal to the stem) (Tyree & Ewers 1991). Studying these functional traits is relevant because they are adaptive and vary along

environmental gradients in many plant species (Auger & Shipley 2013, Rosbakh *et al.* 2015, Cochrane *et al.* 2016). CC directly influences the photosynthetic capacity of plants (Croft *et al.* 2017). LA is important in the balance of water and energy of the plant and usually smaller leaves are associated with high radiation, heat-, cold- or drought-stress (Cornelissen *et al.* 2003). LMA is positively related to the investment in structural defenses and with leaf lifespan and usually higher values of LMA occur in environments with higher resources stress. LT is associated with photosynthetic rates per unit of leaf area and is usually correlated positively with mean temperature and with solar radiation (Niinemets 2001). Finally, HV is higher in more arid sites (Carter & White 2009).

Leaf chlorophyll concentration was measured in the field using the Minolta Soil Plant Analysis Development (SPAD-502) chlorophyll meter (Markwell *et al.* 1995) in three randomly chosen leaves per individual. For the other traits, fully developed leaves without damage were dried at 55 °C, weighed in an analytical balance, and lamina thickness measured in a portion of the leaf without veins using a Mitutoyo Absolute digital caliper (model 500-172-20) with a 0.01 mm precision. Leaves were then imaged with a flatbed high resolution scanner (Epson V700), and the area of each leaf was determined

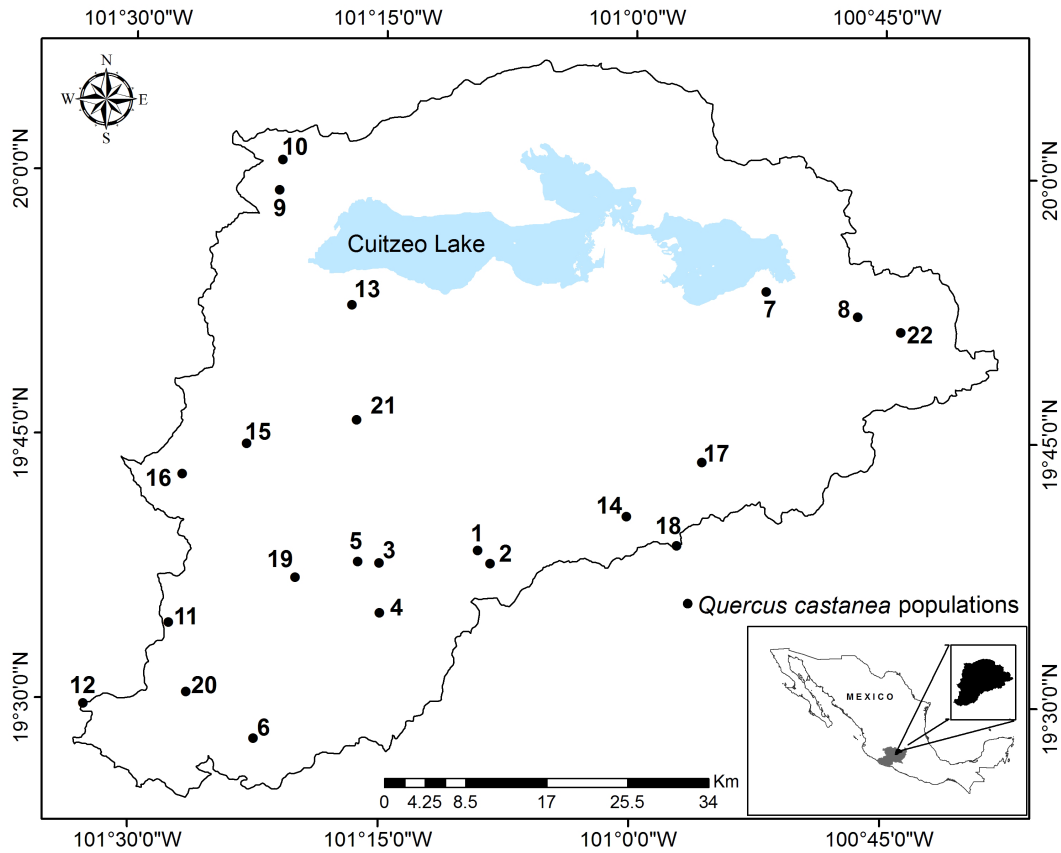


Figure 2. Cuitzeo lake basin and the distribution of the 22 *Q. castanea* populations sampled.

using the ImageJ software (Rasband 2010). Leaf mass per area was calculated as the ratio between dry mass and leaf area values. These traits were measured in ten randomly chosen leaves per tree. For the Huber value, three twigs per tree were selected. The leaf area was determined by digitalizing all the twig leaves with the high-resolution flatbed scanner and summing their individual areas. The area of the sapwood was determined from the diameter of cross sections of the leafless twigs measured with the digital caliper after removing the bark. This trait was determined for three twigs per individual.

Geographic and environmental variables. Spatial coordinates and altitude of each sampling population were recorded using a global positioning system (GPS) unit (Table 1S). Soil type for each population was extracted from a soil surface (Cabrera-González et al. 2010) using GIS Arc View ver. 3.3 (ESRI 1999). To determine soil water holding capacity, three soil samples were taken in different random directions (keeping a minimum angle of 70° between samples) one meter away from the main trunk of each oak tree sampled using a soil core sampler. In the laboratory, soil samples were dried in an oven at 70 °C for 3 days.

Subsequently, 10 g of dry soil from each sample were weighted, then wetted to field capacity and weighted again. Soil water holding capacity (WHC) was calculated as follows:

$$\text{WHC} = (\text{weight of wet soil} - \text{weight of dry soil} / \text{weight of dry soil}) \times 100$$

This variable was determined as an average of values of the three soil samples per oak tree and then an average value per population was obtained.

To characterize the climate at each population, 19 bioclimatic variables derived from monthly precipitation and temperature values (period 1910-2009) were extracted at 30 arc seconds and downscaled using a digital elevation model at 30 m of resolution (Cuervo-Robayo et al. 2014, Correa-Ayram et al. 2017). Nineteen climatic variables were finally obtained for each population using GIS Arc View v3.3 (ESRI 1999).

Data Analysis. The significance of the differences among populations for each functional trait were evaluated through one-way analyses of variance (ANOVA) using average values of individual trees. To determine how the total

variance for each functional trait is partitioned among populations, among trees within populations, and within trees, we estimated the variance components using the restricted maximum likelihood (REML) method. Residual variation was considered to correspond to differences among leaves within trees. In this analysis the full database with individual leaf values was used. A stepwise canonical discriminant analysis (CDA) using individual tree averages for each functional trait was performed to further determine which functional traits have the highest variation among the populations sampled. Finally, to evaluate the degree of association among the different functional traits, a pairwise correlation analysis was performed. These analyses were carried out in JMP 8 (SAS Institute, Cary, North Carolina).

The associations between the population mean values of each functional trait and the environmental variables at each sampling population were evaluated with Spearman's correlation analyses. Before conducting the correlation analyses, redundancy was reduced among the environmental variables by assessing pairwise correlations and discarding the more specific variable in each pair of highly correlated variables ($r \geq 0.8$). The included environmental variables were soil water holding capacity (WHC), annual mean temperature (B1), mean diurnal range (B2), isothermality (B3), minimum temperature of the coldest month (B6), temperature annual range (B7), annual precipitation (B12), precipitation of the driest month (B14), precipitation seasonality (B15) and precipitation of the driest quarter (B17). The JMP 8 program was used in these analyses (SAS Institute, Cary, North Carolina).

To separate the effects of spatial distance and environmental variables on phenotypic variation we performed a Redundancy Analysis (RDA) (Van den Wollenberg 1977), which is a constrained ordination method analogous to linear regression for datasets with multiple dependent and multiple independent variables. Three separate RDAs were conducted: (1) a full model with environmental and geographic variables (latitude and longitude of the populations) as explanatory variables; (2) a partial RDA (pRDA1) where we removed geographic variables (*i.e.*, a pure environmental model) and (3) a partial RDA (pRDA2) where we removed environmental variables (*i.e.*, a pure geographic model). We then used variance partitioning to calculate the proportion of variation explained by the independent contributions of environmental and geographic variables and their joint effect (Borcard *et al.* 1992, Peres-Neto *et al.* 2006). The significance of each RDA model was calculated using a permutation test with 1,000 permutations. We calculated the adjusted coefficient of multiple determination (R^2_{adj}) for all models (Peres-Neto *et al.* 2006). Data of the five functional traits were used as the response variables in the three RDAs.

The five variables were \log_{10} -transformed to correct for skew, and then centered and standardized before the RDA analysis. The explanatory variables included eight of the environmental variables described above, but we excluded isothermality (B3) because centering and standardizing this variable resulted mostly into zero values. Explanatory variables also included spatial variables defined using principal coordinates of neighborhood matrices (PCNM), which were calculated from the geographic coordinates in decimal degrees (Dray *et al.* 2006). We retained half of the PCNM variables with positive eigenvalues, as has been suggested by some authors (Manel *et al.* 2012, Fitzpatrick & Keller 2015), which were seven in our case. Calculation of PCNM, RDA, test for the significance and adjusted coefficient of multiple determination of the models were performed in R (R Core Team 2016), using the 'vegan' 2.3-0 package (Oksanen *et al.* 2015).

Results

One-way ANOVAs indicated highly significant differences among populations for the five functional traits evaluated (Table 1). However, total variance was partitioned differently depending on the trait. LT showed the largest variation among populations (43.9 %) while for the other four traits variation among trees within populations and leaves within trees accounted for the largest proportion of the variance (Table 2).

Table 1. Results of the one-way ANOVAs for the comparison of five functional traits among 22 populations of *Q. castanea* in the Cuitzeo basin, Michoacan state, Mexico.

Trait	$F_{21,186}$	P
Chlorophyll concentration	5.33	< 0.0001
Leaf area	6.08	< 0.0001
Leaf mass per area	3.4	< 0.0001
Leaf thickness	13.94	< 0.0001
Huber value	4.44	< 0.0001

The stepwise canonical discriminant analysis indicated that the variable that contributes more importantly to differentiate populations is LT, followed by LMA, LA, CC and HV. The two first canonical discriminant functions allowed highly significant discrimination among populations (Wilks' lambda = 0.048; $P < 0.0001$) and explained 71.2 and 16.13 % of the variation, respectively (Table 3). The variables that contributed more strongly to the first canonical function were LT and LMA, while LA, CC and HV contributed to the second canonical function.

We found moderate but significant positive correlations of leaf mass per area with leaf thickness and with the Huber

value (Table 4). Chlorophyll content had positive weak correlations with leaf mass per area and the Huber value. In contrast, negative correlations were observed between leaf mass per area and leaf area, between chlorophyll content and leaf thickness and between the Huber value and leaf area (Table 4).

According to the Spearman's correlation analyses, LMA had a significant negative correlation with annual mean temperature ($r_s = -0.5$; $P = 0.017$) and a positive correlation with isothermality ($r_s = 0.5$; $P = 0.016$). CC and HV were

also positively related to isothermality ($r_s = 0.48$; $P = 0.02$ and $r_s = 0.43$; $P = 0.04$, respectively), while LT was negatively correlated with precipitation seasonality ($r_s = -0.6$; $P = 0.003$).

The full RDA model for the combined effect of geographic and climatic explanatory variables on functional trait variation was significant ($P = 0.001$). The pRDA1 for the association between environmental variables and functional trait variation while controlling for geographic effects was also significant ($P = 0.001$, $R^2_{\text{adj}} = 13.9\%$).

Table 2. Restricted maximum likelihood (REML) variance components (as percentage of total variation) for five functional traits for *Q. castanea* in the Cuitzeo basin. Variation among populations, among individuals within populations and within individuals (residual variance) was considered.

Trait	Population	Individuals within populations	Residual
Chlorophyll concentration	24.25	43.74	32
Leaf area	20.7	34.73	44.56
Leaf mass per area	7.2	25.8	67
Leaf thickness	43.9	27.98	28.11
Huber value	16.4	30.53	53.06
Average	22.49	32.56	44.95

Table 3. Results of the stepwise canonical discriminant analysis for functional traits in *Q. castanea*.

	Canonical 1	Canonical 2
Eigenvalue	4.36	0.99
Percent (%)	71.2	16.13
Cumulative percent (%)	71.2	87.33
Functional trait	Scoring coefficients	
CC	0.02	0.14
LMA	767.15	-57.96
LA	0.07	-0.167
LT	-63.5	9.86
HV	-0.05	0.152

CC = chlorophyll concentration, LMA = leaf mass per area, LA = leaf area, LT = leaf thickness, HV = Huber value

Table 4. Pairwise correlations among functional trait variables.

Variables	CC	LA	LT	HV	LMA
CC	1.0000				
LA	-0.1232	1.0000			
LT	-0.1362*	0.0520	1.0000		
HV	0.1944**	-0.4651**	0.0368	1.000	
LMA	0.1570*	-0.2934**	0.5229**	0.4640**	1.0000

CC = chlorophyll concentration, LA = leaf area, LT = leaf thickness, LMA = leaf mass per area, HV = Huber value * $P < 0.05$; ** $P < 0.01$

(Table 5). In this analysis, the most important variables were annual precipitation (B12) and precipitation of the driest quarter (B17), followed by minimum temperature of the coldest month (B6), annual mean temperature (B1), mean diurnal range (B2), temperature annual range (B7) and precipitation of the driest month (B14) (Table S2). The pRDA2 indicated that geography alone explained a slightly higher proportion of functional trait variation ($P = 0.001$, $R^2_{\text{adj}} = 18.4\%$). Finally, joint climate and geography accounted for 71.9 % of the variation (Table 5).

Discussion

Our analysis at a landscape scale showed significant variation among populations of *Q. castanea* in the functional traits studied. Furthermore, we identified the environmental variables with the largest influence on these functional traits at this spatial scale. It is widely acknowledged that leaf area, leaf mass per area, leaf thickness and Huber value are adaptive and vary according to the environment. These traits are related to water use efficiency and tolerance to temperature and water stress (Westoby *et al.* 2002, Wright *et al.* 2004, Lohbeck *et al.* 2013), and leaf mass per area and leaf thickness are functional traits within the leaf economic spectrum (LES) (De La Riva *et al.* 2016).

Variation among populations. In comparison to other oak species studied across the world, we found that *Q. castanea* showed a high leaf mass per area (range 134-183 g m⁻²), relatively low leaf areas (9.38-18.62 cm²) and intermediate leaf thickness (0.11-0.24 mm) (Table S3). The value of leaf mass per area is situated at the higher end of values reported for oaks, and comparable to values found in evergreen Mediterranean and evergreen arid species (Gil-Pelegrín *et al.* 2017). According to Flexas *et al.* (2014) a leaf mass per area value higher than 120 g m⁻² indicates a true sclerophyllous species. Leaf thickness was within the range of mean values reported for deciduous temperate and evergreen arid oak species. In turn, leaf area was higher than for Mediterranean and arid evergreen species, but

lower than for tropical and temperate evergreen and deciduous species (Gil-Pelegrín *et al.* 2017). These three traits (leaf area, leaf thickness, leaf mass per area) showed highly significant variation among the sampled populations of *Q. castanea* even though, except for leaf thickness, variation among trees within populations was higher, suggesting the importance of microenvironmental factors (or other unaccounted factors) in determining intraspecific functional trait variation.

Leaf mass per area has been considered a key leaf functional trait for plants (Wright *et al.* 2004, Poorter *et al.* 2009). In *Q. castanea* populations, leaf mass per area showed a positive correlation with leaf thickness and a negative correlation with leaf area (Table 4). However, leaf thickness was the trait that showed the lower intra-individual and intra-population variation and the higher among-population variation (Table 2), suggesting its importance in the adjustment of *Q. castanea* to the environment.

In a previous study within the Cuitzeo basin, Aguilar-Romero *et al.* (2017) had compared water-use strategies among nine oak species (including *Q. castanea*) distributed along an aridity gradient. In that study, species from more arid areas had more deciduousness and a higher instantaneous water-use efficiency, while their more humid counterparts had less deciduousness and a xylem that was more resistant to embolisms, suggesting a trade-off between the xylem vulnerability to embolism and deciduousness. In that study, *Q. castanea* was characterized as occupying an intermediate position along the aridity gradient, with a brevideciduous phenology and also intermediate values (in relation to the other eight species) of wood density, water use efficiency, Huber value and xylem resistance to embolism (Aguilar-Romero *et al.* 2017). However, the breadth of the environmental niche of each species and its correspondence with the degree of intraspecific variability in functional traits was not considered. As we have seen in this study, such evaluation is critical, since some traits may exhibit even higher intraspecific than interspecific variation. For example, both in greenhouse grown seedlings and in

Table 5. Results of redundancy analysis (RDA) for the association between functional trait variation, geography and climate variables for *Quercus castanea* populations. Two partial analysis are shown pRDA1 (effect of climatic variables while controlling for geographic effects) and pRDA2 (effect of geographic distribution while controlling for climatic variation). The proportion of variance explained by both geographic and climate effects is also indicated (joint climate/geography). Proportion constrained corresponds to the partitioned variance relative to the constrained variance of the full RDA model.

	Inertia	Proportion	R^2_{adj}	P
Full model: geography and climate (constrained variance)	5.000	1	0.28	0.001
Pure climate (pRDA1)	0.5917	0.1183	0.13	0.001
Pure geography (pRDA2)	0.8136	0.1627	0.18	0.001
Joint climate/geography	3.5947	0.7190	-0.04	NA

adult trees from the field, [Ramírez-Valiente et al. \(2015\)](#) found higher differences among populations of the tropical oak *Q. oleoides* than among species within the same clade (*Virentes*, the live oaks), for various functional traits in response to drought. Therefore, the extent and ratio of intra- versus interspecific variation, and their relationship with the niche breadth of the species and their coexistence within communities is still an open question ([Cavender-Bares et al. 2004](#)).

Association with environmental variables. Even at the relatively fine spatial scale of the Cuitzeo basin, we were able to define several significant associations of the traits measured with environmental variables. The strongest pairwise association was a negative correlation between leaf thickness and precipitation seasonality, meaning that leaves are thinner at populations where precipitation is more seasonal (i.e. there are more marked dry and wet periods). In turn, leaf mass per area had a positive correlation with isothermality and negative correlation with annual mean temperature, and the Huber value had a positive correlation with isothermality. Higher Huber values mean that the tree produces more wood per unit of leaf area, a characteristic that suggests an improvement in its water and nutrient storage capacity ([Callaway et al. 1994](#)) and that reduces the vulnerability to embolism of the xylem ([Tyree & Dixon 1986](#)).

In turn, the multivariate RDA identified annual precipitation (B12) and precipitation of the driest quarter (B17) as the most important environmental variables influencing functional trait variation of *Q. castanea* in the Cuitzeo basin, with also a considerable contribution of precipitation of the driest month (B14). Overall, these results indicate that leaves of *Q. castanea* are more sclerophyllous (i.e. have higher leaf mass per area) in sites that are cooler and less seasonal in precipitation and temperature variation, and less sclerophyllous in more seasonal and warmer sites. Previously, sclerophylly has been considered an adaptation to arid environments across biomes ([Niinemets 2001](#)). At the intraspecific level, leaf mass per area has been shown to increase with aridity in Mediterranean oaks such as *Q. ilex* and *Q. coccifera* ([Peguero-Pina et al. 2016](#)). However, the opposite pattern has been shown in populations of *Q. oleoides* that tended to have more sclerophyllous leaves in more mesic areas ([Ramírez-Valiente et al. 2015, 2017](#)). The authors suggested that this counterintuitive result is due to phenological differences: populations from mesic populations maintain leaves for longer time during the dry season and therefore maintain function with increasing water stress ([Cavender-Bares & Ramírez-Valiente 2017](#)). Therefore, selection has favored an increase in deciduousness (and less investment in tissue construction) with increasing dry season severity in

this tropical oak species, contrasting with Mediterranean species that are limited by both cold winters and dry summers ([Cavender-Bares & Ramírez-Valiente 2017](#)). Therefore, *Q. castanea* follows a pattern resembling more a tropical than a Mediterranean species. We hypothesize that phenological patterns (i.e. degree of deciduousness) in this species should be negatively associated with leaf mass per area along populations in the Cuitzeo basin, but this should be evaluated in further studies.

We conclude that, while previous studies have considered functional variation in oak species at a whole-range level, we have shown that significant functional differences exist among populations of *Q. castanea* separated by a few kilometers in the heterogeneous landscape of the Cuitzeo basin. This species shows clearly sclerophyllous leaves but leaf thickness varies to a considerable degree across populations in association with precipitation seasonality, indicating that the temporality of water availability represents a significant environmental pressure for this oak species. Whether the observed functional variation is due to local adaptation, plasticity or a combination of both will be clarified in ongoing common garden and landscape genomics studies.

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Supplemental data

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Literature Cited

Aguilar-Romero R, García-Oliva F, Pineda-García F, Torres I, Peña-Vega E, Ghilardi A, Oyama K. 2016. Patterns of distribution of nine *Quercus* species along an environmental gradient in a fragmented landscape in central Mexico. *Botanical Sciences* **94**: 471-482. DOI: <https://doi.org/10.17129/botsci.620>

- Aguilar-Romero R, Pineda-García F, Paz H, González-Rodríguez A, Oyama K. 2017. Differentiation in the water-use strategies among oak species from central Mexico. *Tree Physiology* **37**: 915-925. DOI: <https://doi.org/10.1093/treephys/tpx033>
- Aranda I, Cano FJ, Gascó A, Cochard H, Nardini A, Mancha JA, López R, Sánchez-Gómez D. 2015. Variation in photosynthetic performance and hydraulic architecture across European beech (*Fagus sylvatica* L.) populations supports the case for local adaptation to water stress. *Tree Physiology* **35**: 34-46. DOI: <https://doi.org/10.1093/treephys/tpu101>
- Auger S, Shipley B. 2013. Inter-specific and intra-specific trait variation along short environmental gradients in an old-growth temperate forest. *Journal of Vegetation Science* **24**: 419-428. DOI: <https://doi.org/10.1111/j.1654-1103.2012.01473.x>
- Borcard D, Legendre P, Drapeau P. 1992. Partialling out the spatial component of ecological variation. *Ecology* **73**: 1045-1055. DOI: <https://doi.org/10.2307/1940179>
- Bruschi P. 2010. Geographical variation in morphology of *Quercus petraea* (Matt.) Liebl. as related to drought stress. *Plant Biosystems* **144**: 298-307. DOI: <https://doi.org/10.1080/11263501003672462>
- Cabrera-González A, Medina-Orozco LE, Sánchez-Espinosa F, Alcalá-de Jesús M, Ayala-Gómez JM. 2010. Los suelos de la Cuenca de Cuitzeo. In: Cram S, Israde I, Mendoza M, Sommer I, Galicia L, eds. *Atlas de la Cuenca del Lago Cuitzeo: un análisis de la geografía del lago y su entorno socioambiental*. Universidad Nacional Autónoma de México y Universidad Michoacana de San Nicolás de Hidalgo, pp 44-47. ISBN: 978-607-02-1830-9
- Callaway RM, DeLucia EH, Schlesinger WH. 1994. Biomass allocation of montane and desert ponderosa pine: an analog for response to climate change. *Ecology* **75**: 1474-1481. DOI: <https://doi.org/10.2307/1937470>
- Carter JL, White DA. 2009. Plasticity in the Huber value contributes to homeostasis in leaf water relations of a mallee Eucalypt with variation to groundwater depth. *Tree Physiology* **29**: 1407-1418. DOI: <https://doi.org/10.1093/treephys/tpu076>
- Cavender-Bares J. 2016. Diversity, distribution and ecosystem services of the North American oaks. *International Oaks* **27**: 37-49.
- Cavender-Bares J. 2019. Diversification, adaptation, and community assembly of the American oaks (*Quercus*), a model clade for integrating ecology and evolution. *New Phytologist* **221**: 669-692. DOI: <https://doi.org/10.1111/nph.15450>
- Cavender-Bares J, Kitajima K, Bazzaz FA. 2004. Multiple trait associations in relation to habitat differentiation among 17 Floridian oak species. *Ecological Monographs* **74**: 635-662. DOI: <https://doi.org/10.1890/03-4007>
- Cavender-Bares J, Ramírez-Valiente JA. 2017. Physiological evidence from common garden experiments for local adaptation and adaptive plasticity to climate in American Live Oaks (*Quercus* Section Virentes): Implications for conservation under global change. In: Gil-Peregrin E, Peguero-Pina JJ, Sancho-Knapik D, eds. *Oaks Physiological Ecology. Exploring the Functional Diversity of Genus Quercus L. Tree Physiology*. eISBN 978-3-319-69099-5
- Chávez-Vergara B, Merino A, Vázquez-Marrufo G, García-Oliva F. 2014. Organic matter dynamics and microbial activity during decomposition of forest floor under two native neotropical oak species in a temperate deciduous forest in Mexico. *Geoderma* **235**: 133-145. DOI: <https://doi.org/10.1016/j.geoderma.2014.07.005>
- Cochrane A, Hoyle GL, Yates CJ, Neeman T, Nicotra AB. 2016. Variation in plant functional traits across and within four species of Western Australian Banksia (Proteaceae) along a natural climate gradient. *Austral Ecology* **41**: 886-896. DOI: <https://doi.org/10.1111/aec.12381>
- Cooper CE, Vogel JG, Muir JP, Moore GW. 2018. Leaf functional trait responses to changes in water status differ among three oak (*Quercus*) species. *Plant Ecology* **219**: 1463-1479. DOI: <https://doi.org/10.1007/s12558-018-0894-3>
- Cornelissen JHC, Lavorel S, Garnier E, Díaz S, Buchmann N, Gurvich DE, Reich PB, Steege HT, Morgan HD, Van Der Heijden MGA, Pausas JG, Poorter. 2003. A handbook of protocols for standardized and easy measurement of plant functional traits worldwide. *Australian Journal of Botany* **51**: 335-380. DOI: <https://doi.org/10.1071/BT02124>
- Correa-Ayram CA, Mendoza ME, Etter A, Pérez-Salicrup DR. 2017. Potential distribution of Mountain Cloud Forest in Michoacán, México: Priorization for conservation in the context of landscape connectivity. *Environmental Management* **60**: 86-103. DOI: <https://doi.org/10.1007/s00267-017-0871-y>
- Croft H, Chen JM, Luo X, Barlett P, Chen B, Staebler RM. 2017. Leaf chlorophyll content as a proxy for leaf photosynthetic capacity. *Global Change Biology* **23**: 3513-3524. DOI: <https://doi.org/10.1111/gcb.13599>
- Cuervo-Robayo AP, Téllez-Valdés O, Gómez-Albores MA, Venegas-Barrera CS, Manjarrez J, Martínez-Meyer E.

2014. An update of high-resolution monthly climate surfaces for Mexico. *International Journal of Climatology* **34**: 2427-2437. DOI: <https://doi.org/10.1002/joc.3848>
- De la Riva EG, Olmo M, Poorter H, Ubera JL, Villar R. 2016. Leaf mass per area (LMA) and its relationship with leaf structure and anatomy in 34 Mediterranean woody species along a water availability gradient. *PLOS ONE* **11**: e0148788. DOI: <https://doi.org/10.1371/journal.pone.0148788>
- Dray S, Legendre P, Peres-Neto PR. 2006. Spatial-modelling: a comprehensive framework for principal coordinate analysis of neighbor matrices (PCNM). *Ecological Modelling* **196**: 483-493. DOI: <https://doi.org/10.1016/j.ecolmodel.2006.02.015>
- ESRI [Environmental Systems Research Institute]. 1999. Arcview GIS 3.3. Environmental Systems Research Institute, Redlands.
- Fitzpatrick MC, Keller SR. 2015. Ecological genomics meets community-level modelling of biodiversity: Mapping the genomic landscape of current and future environmental adaptation. *Ecology Letters* **18**: 1-16. DOI: <https://doi.org/10.1111/ele.12376>
- Flexas J, Díaz-Espejo A, Gago J, Gallé A, Galmés J, Gulías J, Medrano H. 2014. Photosynthetic limitations in Mediterranean plants: A review. *Environmental and Experimental Botany* **103**: 12-23. DOI: <https://doi.org/10.1016/j.envexpbot.2013.09.002>
- García-Nogales A, Linares JC, Laureano RG, Seco JI, Merino J. 2016. Range-wide variation in life-history phenotypes: spatiotemporal plasticity across the latitudinal gradient of the evergreen oak *Quercus ilex*. *Journal of Biogeography* **43**: 2366-2379. DOI: <https://doi.org/10.1111/jbi.12849>
- Gil-Pelegrín E, Saz MA, Cuadrat JM, Peguero-Pina JJ, Sancho-Knapik D. 2017. Oaks under Mediterranean-type climates: functional response to summer aridity. In: Gil-Peregrin E, Peguero-Pina JJ, Sancho-Knapik D, eds. *Oaks Physiological Ecology: Exploring the functional diversity of genus Quercus L.* eISBN 978-3-319-69099-5
- Hernández-Calderón E, González-Rodríguez A, Méndez-Alonzo R, Vega-Peña E, Oyama K. 2013. Contrasting leaf phenology in two white oaks, *Quercus magnoliifolia* and *Quercus resinosa*, along an altitudinal gradient in Mexico. *Canadian Journal of Forest Research* **43**: 208-213. DOI: <https://doi.org/10.1139/cjfr-2012-0406>
- Hernández-Calderón E, Méndez-Alonzo R, Martínez-Cruz J, González-Rodríguez A, Oyama K. 2014. Altitudinal changes in tree leaf and stem functional diversity in a semi-tropical mountain. *Journal of Vegetation Science* **25**: 955-966. DOI: <https://doi.org/10.1111/jvs.12158>
- Herrera-Arroyo ML, Sork VL, González-Rodríguez A, Rocha-Ramírez V, Vega E, Oyama K. 2013. Seed-mediated connectivity among fragmented populations of *Quercus castanea* (Fagaceae) in a Mexican Landscape. *American Journal of Botany* **100**: 1663-1671. DOI: <https://doi.org/10.3732/ajb.1200396>
- Lavorel S, Diaz S, Cornelisse JHC, Urcelay C. 2007. Plant functional types: are we getting any closer to the Holy Grail? In: Canadell J, Pitelka LF, Pataki D, eds. *Terrestrial Ecosystems in a Changing World*. Berlin: Springer-Verlag. pp 149-164. DOI: https://doi.org/10.1007/978-3-540-32730-1_13; eISBN 978-3-540-32730-1
- Lind BM, Friedline CJ, Wegrzyn JL, Maloney PE, Vogler DR, Neale DB, Eckert AJ. 2017. Water availability drives signatures of local adaptation in whitebark pine (*Pinus albicaulis* Engelm.) across fine spatial scales of the Lake Tahoe Basin, USA. *Molecular Ecology* **26**: 3168-3185. DOI: <https://doi.org/10.1111/mec.14106>
- Lohbeck M, Poorter L, Lebrija-Trejos E, Martínez-Ramos M, Meave JA, Paz H, Pérez-García EA, Romero-Pérez IE, Tauro A, Bongers F. 2013. Successional changes in functional composition contrast for dry and wet tropical forest. *Ecology* **94**: 1211-1216. DOI: <https://doi.org/10.1890/12-1850.1>
- Manel S, Gugerli F, Thuiller W, Alvarez N, Legendre P, Holderegger R, Gielly L, Taberlet P, IntraBioDiv Consortium. 2012. Broad-scale adaptive genetic variation in alpine plants is driven by temperature and precipitation. *Molecular Ecology* **21**: 3729-3738. DOI: <https://doi.org/10.1111/j.1365-294X.2012.05656.x>
- Markwell J, Osterman JC, Mitchell JL. 1995. Calibration of the Minolta SPAD-502 leaf chlorophyll meter. *Photosynthesis Research* **46**: 467-472. DOI: <https://doi.org/10.1007/BF00032301>
- McKnow AD, Guy RD, Klápšte J, Gerald A, Friedmann M, Cronk QCB, El-Kassaby YA, Mansfield SD, Douglas CJ. 2014. Geographical and environmental gradients shape phenotypic trait variation and genetic structure in *Populus trichocarpa*. *New Phytologist* **201**: 1263-1276. DOI: <https://doi.org/10.1111/nph.12601>
- Meireles JE, Beulke A, Borkowski DS, Romero-Severson J, Cavender-Bares J. 2017. Balancing selection maintains diversity in a cold tolerance gene in broadly distributed live oaks. *Genome* **60**: 762-769. DOI: <https://doi.org/10.1139/gen-2016-0208>
- Mendoza ME, Bocco G, Bravo M, López-Granados E, Osterkamp WR. 2006. Predicting water-surface fluctuation of continental lakes: ARS and GIS based

- approach in central Mexico. *Water Resources Management* **20**: 291-311. DOI: <http://dx.doi.org/10.1007/s11269-006-8199-z>
- Niinemets Ü. 2001. Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. *Ecology* **82**: 453-469. DOI: [https://doi.org/10.1890/0012-9658\(2001\)082\[0453:GSCCOL\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2001)082[0453:GSCCOL]2.0.CO;2)
- Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H. 2015. Vegan: Community Ecology Package. R package V2.2-1. http://www.pelagicos.net/MARS6910_spring2015/manuals/R_vegan.pdf
- Oyama K, Herrera-Arroyo ML, Rocha-Ramírez V, Benítez-Malvido J, Ruiz-Sánchez E, González-Rodríguez A. 2017. Gene flow interruption in a recently human-modified landscape: The value of isolated trees for the maintenance of genetic diversity in a Mexican endemic red oak. *Forest Ecology and Management* **390**: 27-35. DOI: <https://doi.org/10.1016/j.foreco.2017.01.018>
- Peguero-Pina JJ, Sisó S, Fernández-Marín B, Flexas J, Galmés J, García-Plazaola JI, Niinemets Ü, Sancho-Knapik D, Gil-Peregrin E. 2016. Leaf functional plasticity decreases the water consumption without further consequences for carbon uptake in *Quercus coccifera* L. under Mediterranean conditions. *Tree Physiology* **36**: 356-367. DOI: <https://doi.org/10.1093/treephys/tpv129>
- Peres-Neto PR, Legendre P, Dray S, Borcard D. 2006. Variation partitioning of species data matrices: Estimation and comparison of fractions. *Ecology* **87**: 2614-2625. DOI: [https://doi.org/10.1890/0012-9658\(2006\)87\[2614:VPOSDM\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2006)87[2614:VPOSDM]2.0.CO;2)
- Petit RJ, Carlson J, Curtu AL, Loustau ML, Plomion C, González-Rodríguez A, Sork V, Ducousso A. 2013. Fagaceae trees as models to integrate ecology, evolution and genomics. *New Phytologist* **197**: 369-371. DOI: <https://doi.org/10.1111/nph.12089>
- Poorter H, Niinemets Ü, Poorter L, Wright IJ, Villar R. 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist* **182**: 565-588. DOI: <https://doi.org/10.1111/j.1469-8137.2009.02830.x>
- R Core Team 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <<https://www.R-project.org/>>
- Ramírez-Valiente JA, Sánchez-Gómez D, Aranda I, Valladares F. 2010. Phenotypic plasticity and local adaptation in leaf ecophysiological traits of 13 contrasting cork oak populations under different water availabilities. *Tree Physiology* **30**: 618-627. DOI: <https://doi.org/10.1093/treephys/tpq013>
- Ramírez-Valiente JA, Valladares F, Delgado A, Nicotra AB, Aranda I. 2015. Understanding the importance of intra-population functional variability and phenotypic plasticity in *Quercus suber*. *Tree Genetics & Genomes* **11**: 35. DOI: <https://doi.org/10.1007/s11295-015-0856-z>
- Ramírez-Valiente JA, Center A, Sparks JP, Sparks KL, Etterson JR, Longwell T, Pilz G, Cavender-Bares J. 2017. Population-level differentiation in growth rates and leaf traits in seedlings of the neotropical live oak *Quercus oleoides* grown under natural and manipulated precipitation regimes. *Frontiers in Plant Science* **8**: 585. DOI: <https://doi.org/10.3389/fpls.2017.00585>
- Rasband WS. 2010. ImageJ. Bethesda, Maryland, USA. National Institutes of Health. <https://imagej.nih.gov/ij/> (Accessed April 7, 2015).
- Rosbakh S, Römermann C, Poschod P. 2015. Specific leaf area correlates with temperature: new evidence of trait variation at the population, species and community levels. *Alpine Botany* **125**: 79-86. DOI: <https://doi.org/10.1007/s00035-015-0150-6>
- Souto C, Premoli A, Reich P. 2009. Complex bioclimatic and soil gradients shape leaf trait variation in *Embothrium coccineum* (Proteaceae) among austral forest in Patagonia. *Revista Chilena de Historia Natural* **82**: 209-222. DOI: <https://doi.org/10.4067/S0716-078X2009000200004>
- Tyree MT, Dixon MA. 1986. Water stress induced cavitation and embolism in some woody plants. *Physiologia Plantarum* **66**: 397-405. DOI: <https://doi.org/10.1111/j.1399-3054.1986.tb05941.x>
- Tyree MT, Ewers FW. 1991. The hydraulic architecture of trees and other woody plants. *New Phytologist* **119**: 345-360. DOI: <https://doi.org/10.1111/j.1469-8137.1991.tb00035.x>
- Uribe-Salas D, Sáenz-Romero C, González-Rodríguez A, Téllez-Valdéz O, Oyama K. 2008. Foliar morphological variation in the white oak *Quercus rugosa* Née (Fagaceae) along a latitudinal gradient in Mexico: Potential implications for management and conservation. *Forest Ecology and Management* **256**: 2121-2126. DOI: <https://doi.org/10.1016/j.foreco.2008.08.002>
- Valencia-A S. 2004. Diversidad del género *Quercus* (Fagaceae) en México. *Boletín de la Sociedad Botánica de México* **75**: 33-53. DOI: <https://doi.org/10.17129/botsci.1692>
- Valencia-Cuevas L, Mussali-Galante P, Piñero D, Castillo-Mendoza E, Rangel-Altamirano G, Tovar-Sánchez E. 2015. Hybridization of *Quercus castanea* (Fagaceae) across a red oak species gradient in Mexico. *Plant*

- Systematics and Evolution* **301**: 1085-1097. DOI: <https://doi.org/10.1007/s00606-014-1151-4>
- Van den Wollenberg AL. 1977. Redundancy analysis: an alternative for canonical analysis. *Psychometrika* **42**: 207-219. DOI: <https://doi.org/10.1007/BF02294050>
- Westoby M, Falser DS, Moles AT, Vesk PA, Wright IJ. 2002. Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology and Systematics* **33**: 125-159. DOI: <https://doi.org/10.1146/annurev.ecolsys.33.010802.150452>
- Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley J, Navas ML, Niinemets Ü, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklas EJ, Villar R. 2004. The worldwide leaf economics spectrum. *Nature* **428**: 621-827. DOI: <https://doi.org/10.1038/nature02403>
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