

Intraspecific trait variation and covariation in a widespread tree species (*Nothofagus pumilio*) in southern Chile

Alex Fajardo and Frida I. Piper

Centro de Investigación en Ecosistemas de la Patagonia, Bilbao 449, Coyhaique, Chile

Summary

Author for correspondence:

Alex Fajardo

Tel: +56 67 244503

Email: alex.fajardo@ciep.cl

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Key words: altitudinal gradient, leaf area per mass, *Nothofagus pumilio*, plant functional traits, standardized major axes, variance partitioning, wood density.

- The focus of the trait-based approach to study community ecology has mostly been on trait comparisons at the interspecific level. Here we quantified intraspecific variation and covariation of leaf mass per area (LMA) and wood density (WD) in monospecific forests of the widespread tree species *Nothofagus pumilio* to determine its magnitude and whether it is related to environmental conditions and ontogeny. We also discuss probable mechanisms controlling the trait variation found.

- We collected leaf and stem woody tissues from 30–50 trees of different ages (ontogeny) from each of four populations at differing elevations (i.e. temperatures) and placed at each of three locations differing in soil moisture.

- The total variation in LMA (coefficient of variation (CV) = 21.14%) was twice that of WD (CV = 10.52%). The total variation in traits was never less than 23% when compared with interspecific studies. Differences in elevation (temperature) for the most part explained variation in LMA, while differences in soil moisture and ontogeny explained the variation in WD. Traits covaried similarly in the altitudinal gradient only.

- Functional traits of *N. pumilio* exhibited nonnegligible variation; LMA varied for the most part with temperature, while WD mostly varied with moisture and ontogeny. We demonstrate that environmental variation can cause important trait variation without species turnover.

Introduction

Knowledge of plant functional traits and diversity offers a promising path to understanding and predicting how vegetation properties and composition change along geographical gradients (Díaz & Cabido, 2001; McGill *et al.*, 2006; Westoby & Wright, 2006). Aspects such as plant morphology, function and diversity of a community can be partially assessed by determining variation in plant traits (Weiher *et al.*, 1999; Westoby *et al.*, 2002; Preston *et al.*, 2006; Díaz *et al.*, 2007). The focus has correspondingly been on functional trait comparisons at the interspecific level, while intraspecific trait variation has received remarkably little attention (Alonso & Herrera, 2001; Hulshof & Swenson, 2010); that is, traits are commonly pooled together around a community and only an average trait value for species is considered, ignoring intraspecific variation (Cianciaruso

et al., 2009; Lake & Ostling, 2009; Albert *et al.*, 2010). This does not mean, however, that intraspecific trait variation is either negligible or not important, but it is recognized as being lower, particularly in places where plant diversity is high. Notably, when cross-species studies are conducted, it is acknowledged that trait values are the result of both phylogenetic inertia and natural selection of the environment (Felsenstein, 1985). One special case, however, where phylogenetic inertia is absent and not artificially ruled out (i.e. most of the variation is the result of selective forces from the environment) is when trait comparisons are done at the within-species level.

Within the functional trait paradigm, intraspecific trait variation across environmental gradients has, in the main, been disregarded and consequently less studied than interspecific variation, for several reasons. First, in most of the cases, species composition changes substantially along

environmental gradients and thus trends in traits are largely the result of species turnover rather than intraspecific variation (Ackerly & Cornwell, 2007; Cornwell & Ackerly, 2009). Secondly, it has been stated and traditionally accepted, albeit with little empirical support, that intraspecific trait variation is significantly smaller than interspecific trait variation (Kraft *et al.*, 2008). Thirdly, for the purpose of comprehending ecological processes at the community level, it is more convenient that traits vary more among than within species (Cornelissen *et al.*, 2003; Cavender-Bares *et al.*, 2004; Chave *et al.*, 2006; McGill *et al.*, 2006). Finally, in functional ecology, mean trait values have been commonly employed and proved to be sufficient (i.e. no need of intraspecific variation) when comparing related species. However, intraspecific variation for some functional traits does occur even at short environmental gradients (Cordell *et al.*, 1998; Alonso & Herrera, 2001; Premoli *et al.*, 2007; Cianciaruso *et al.*, 2009; Albert *et al.*, 2010; Hulshof & Swenson, 2010), because of either local adaptation or phenotypic plasticity (Hulshof & Swenson, 2010). Recent studies have demonstrated that when intraspecific variation is considered, relationships between trait values and species variation along environmental gradients may be considerably modified (Cianciaruso *et al.*, 2009; Hulshof & Swenson, 2010; Messier *et al.*, 2010). Further, intraspecific trait variation has also been proved to have significant effects on certain functions in communities and ecosystems (Poorter *et al.*, 2009) such as nutrient and carbon cycles (Díaz *et al.*, 2007).

As with plant traits, correlations between traits can be affected by taxonomic biases when compared across an environmental gradient (Falsenstein, 1985; Wright *et al.*, 2007), and thus studies with a single species may be more appropriate to distinguish environmental effects on trait correlations. Patterns of correlation, and in particular of covariation of functional traits, along environmental gradients may imply potential trade-offs (Westoby & Wright, 2006) that operate within or between environments. Covariation of functional traits may mean that traits share genetic control or their expressions have similar functional roles, showing correlated responses to environmental variability (Schlichting, 1989; Wright *et al.*, 2007). The absence of covariation in traits (orthogonal relationship) is also meaningful since the traits under scrutiny may convey independent information about plant strategies (Ackerly, 2004). Nonetheless, the patterns and causes of morphological traits covariation in plants are still not clear. The study of covariation patterns between leaf mass per area (LMA) and wood density (WD), two carbon investment-related traits, within and among populations can provide complementary evidence on the processes (evolutionary forces) operating on character divergence and speciation at a geographical scale. LMA and WD are two ecologically relevant traits that are under strong selective pressure as they are important to the fitness of a species in its environment (Falster, 2006; King *et al.*, 2006). These two traits share common tradeoffs as they involve construction costs (Wright *et al.*, 2004; Swenson

& Enquist, 2007) and yet it is not clear whether such construction costs vary with environmental changes. It is expected, for example, that at the interspecific level LMA and WD will be positively related as they both reflect the continuum from fast-growing, light-demanding pioneer species (low LMA and WD) to slow-growing, shade-tolerant climax species (high LMA and WD). A recent study mostly found no correlation between these traits in seven Neotropical forests (Wright *et al.*, 2007), but others did (Bucci *et al.*, 2004; Ishida *et al.*, 2008). These mixed results at the interspecific level suggest a rather complex relationship between both traits (Chave *et al.*, 2006) where intraspecific variation may be the key. Likewise, the variation of these traits along with ontogeny and environmental conditions (e.g. light, temperature) is not well understood, but is recognized as important (Lake & Ostling, 2009). For example, increasing WD with age has been explained both as an adaptation to avoid cavitation in tall trees (where WD increases as a consequence of thinner xylematic conduits) (Preston *et al.*, 2006), and as a result of limited growth rate caused by increasing hydraulic constraints in older trees (Zhang *et al.*, 2009). Following both arguments it would be expected to find steeper increase of WD with age at drier sites. However, the increasing of WD and LMA values with plant height (i.e. age) is not universal (Wright *et al.*, 2007) and deserves more investigation.

In this study, we determined the existence, magnitude and spatial structure of intraspecific variation and covariation of LMA and WD in *Nothofagus pumilio* (Nothofagaceae) across locations with contrasting climatic conditions (latitude), populations within locations (different elevations) and individual trees (different ages) within populations, and related this variation to abiotic and ontogenetic factors. Thus, we studied variation in these traits at two climate-influenced scales (altitudinal and latitudinal), where mean temperature strongly decreases with elevation (Körner, 2007) and soil moisture differs markedly among locations. We worked with *N. pumilio* because it mostly forms monospecific forests (Fajardo & de Graaf, 2004) and it extends through a wide latitudinal and altitudinal distribution from a Mediterranean-influenced climate in central Chile at 35°S to a humid climate in Tierra del Fuego at the southernmost point of the continent at 56°S, and from mid-elevation forests to treeline. This can therefore be considered a special case where there is an important environmental variability but only one tree species persists, that is, there is no species turnover. It is valuable then to ask the following questions: can intraspecific trait variability be considered negligible when we examine one single species across a large geographic area where the range of interspecific trait values is expected to be high? Can it still be considered negligible when compared with other studies focusing on interspecific trait variation? How is intraspecific variability of LMA and WD structured spatially (among individuals, sub-populations and populations)? How do LMA and WD and their covariation respond to environmental conditions

(temperature, moisture) and tree age? We were interested in uncovering the existence and magnitude of intraspecific variation in LMA and WD, discerning the importance of environmental conditions (moisture, temperature) and ontogeny in trait variation, and quantifying the pattern of covariation between LMA and WD and its relationship with environmental gradients and ontogeny. We discuss the most probable mechanisms driving intraspecific variation at the different scales under study.

Materials and Methods

Study species and locations

Nothofagus pumilio (Poepp. et Endl.) Krasser (Nothofagaceae) is a deciduous broadleaf light-demanding tree species that extends through a wide latitudinal and altitudinal range

in the southern Andes of Chile and Argentina. Its ample distribution provides a unique opportunity to study probable mechanisms responsible for trait variation under contrasting climates while controlling for phylogenetic effects. This feature of *N. pumilio* has already been used in some studies that have found genetic and morphological variation (Barrera *et al.*, 2000; Premoli, 2004; Premoli *et al.*, 2007) across abiotic gradients, although these previous studies have not dealt with LMA and WD (but see Barrera *et al.*, 2000), reported magnitude of variation or been framed into the functional trait paradigm. Here, we selected two environmental drivers for capturing trait variation of *N. pumilio*: a large-scale climatic (latitudinal) moisture difference that covers three locations, from Mediterranean to cold temperate climatic conditions; and a short-scale local influenced climatic (altitudinal) gradient that covers, at each location, a decreasing temperature trend with altitude.

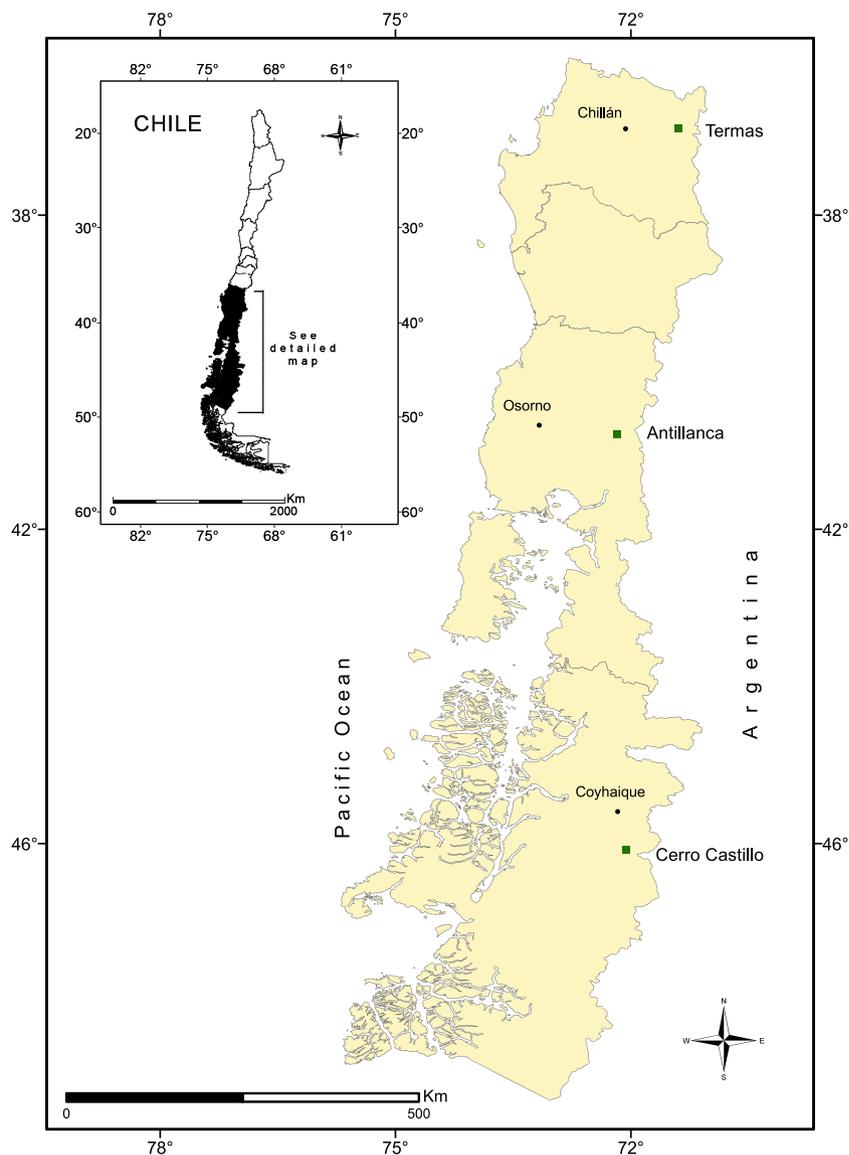


Fig. 1 Map of South–Central Chile, where study locations appear as small squares: Termas (36°54'S, 71°24'W, treeline altitude at 2080 m above sea level (asl)) in central Chile where there is a Mediterranean climatic influence; Antillanca (40°47'S, 72°11'W, treeline altitude at 1350 m asl) in southern Chile with humid climatic conditions (annual precipitation = 4000 mm); and Cerro Castillo (46°04'S, 72°03'W, treeline altitude at 1310 m asl) in central Patagonia with a cold-humid climate.

The first location is in Termas de Chillán (36°54'S, 71°24'W, Fig. 1), hereafter called *Termas*, where treeline occurs at an altitude of 2080 m. This area belongs to the meso-Mediterranean belt with humid climatic conditions and with a predominant drought period during summer (Amigo & Ramírez, 1998). The annual precipitation is 1950 mm most of which falls as snow from May to September. The soils are derived from andesitic rocks of volcanic origin. The second location is in the Antillanca area within the Puyehue National Park (40°47'S, 72°11'W), hereafter called *Antillanca*, where treeline occurs at an altitude of 1350 m. This area belongs to the meso-Temperate belt with supra-Humid climatic conditions (Amigo & Ramírez, 1998). The annual precipitation is *c.* 4000 mm (Aguas Calientes weather station 1980–2000), most of which falls as snow from May to September. The soils are derived from andesitic rocks of volcanic origin. The third location is in Cerro Castillo National Reserve (46°04'S, 72°03'W), hereafter called *Cerro Castillo*, with the treeline at an altitude of 1310 m. This area belongs to the supra-Temperate belt with humid climatic conditions (Amigo & Ramírez, 1998). Mean precipitation for 1992 to 2007 was 416 mm for the five warmest months (November to March). The soil is derived from aeolian volcanic ash deposits. Soil moisture measurements (mean \pm SE of volumetric water content of soil) recorded during two growing seasons were $0.0855 \pm 0.0029 \text{ m}^3 \text{ m}^{-3}$ for Termas, $0.193 \pm 0.0034 \text{ m}^3 \text{ m}^{-3}$ for Antillanca, and of $0.1659 \pm 0.0030 \text{ m}^3 \text{ m}^{-3}$ for Cerro Castillo (HOBO Micro station, Echo probes, F. I. Piper, unpublished).

Field sampling and traits determination

Two traits were examined: LMA (g m^{-2}), which measures the leaf dry-mass investment per unit of leaf area deployed (Lambers *et al.*, 1998; Wright *et al.*, 2004), and WD (g cm^{-3}), also referred to as wood specific gravity (Swenson & Enquist, 2007), defined here as the oven-dry mass divided by green volume (Chave *et al.*, 2009). At each location, *N. pumilio* trees were studied at four altitudes, covering the last *c.* 300 m of altitude of the treeline ecotone (Table 1). For clarity and brevity, we hereafter refer to altitudes by their short names: CF, closed and mature lowest altitude forest; IN, intermediate stand between closed forest and timberline; TB, timberline; and TL, treeline (Table 1). Although the three treelines occur at different altitudes, we were certain that the temperature gradient with elevation was of comparable magnitude as treelines occur at analogous isotherms (treelines worldwide share a similar mean growing season temperature of 6.7°C, Körner & Paulsen, 2004), and the different elevations were similarly equidistant to each other across locations. At each altitude, we set a sampling line perpendicular to the slope and every 30 m along this line four to five trees belonging to different height

Table 1 Structural stand characteristics for the three *Nothofagus pumilio* study locations (latitudes) differing in soil moisture: Termas (36°54'S, 71°24'W), Antillanca (40°47'S, 72°11'W) and Cerro Castillo (46°04'S, 72°03'W). At each location, four populations (altitudes) were sampled: CF, closed forest; IN, intermediate stand; TB, timberline; TL, treeline

	<i>N</i>	Altitude (m asl)	DBH (cm)	Tree age	Height (m)
Termas					
CF	40	1750	1.5–75.0	14–344	2.0–24.9
IN	37	1860	1.5–77.0	6–355	2.0–19.6
TB	37	1950	1.8–52.0	48–206	2.0–11.0
TL	35	2080	1.5–20.5	15–122	2.0–4.5
Antillanca					
CF	30	1140	1.5–68.2	22–168	2.5–21.2
IN	30	1200	1.5–50.1	23–201	2.5–17.8
TB	31	1290	1.5–28.4	23–112	2.3–11.5
TL	30	1350	1.0–18.5	18–94	2.0–4.0
Cerro Castillo					
CF	50	1080	0.5–94.8	6–350	1.5–19.4
IN	50	1180	1.2–56.1	4–315	1.5–16.3
TB	50	1250	1.5–30.6	45–164	2.0–12.6
TL	50	1310	2.0–21.6	19–127	1.8–4.5

asl, above sea level; *N*, number of trees sampled.

Values shown for diameter at breast height (DBH); tree age and height refer to ranges.

classes (i.e. different ages) were selected for sampling. A total of 30–50 trees were selected at each altitude. Individuals with extensive browsing or other damage were excluded. Only unshaded individuals were sampled. Height and stem diameter at breast height (DBH, 1.35 m) were recorded on the selected trees, spanning the whole range of dimensions to enable us to estimate a wide range of potential ages. Tree ages for Termas and Cerro Castillo were estimated using exponential models fitted at the age–DBH relationship for each altitude determined from a data subset of tree cores collected at each altitude ($N = 60–80$) (A. Fajardo, unpublished). Tree ages for Antillanca were all directly determined (i.e. all trees were cored for age determination). Tree cores for age determination were extracted at *c.* 30 cm height and placed dried in labelled plastic straws. Once in the laboratory, cores were mounted in grooved wooden sticks and sanded with successively finer grades of sandpaper to reveal annual rings. Rings were read using a microscope mounted on a dendrochronometer with a Velmex sliding stage and Accurite measuring system. Cross-dating accuracy was checked using COFECHA v6.06P (Holmes, 2001).

For Termas and Cerro Castillo, leaf and wood samples were collected in late February–early March 2007, once leaves had completed their development and reached their final size. For Antillanca, the collection of tissue was done in late February 2008. Sampling and measurements followed methodological protocols dictated by Cornelissen *et al.* (2003). In each sampled tree, we randomly collected a

fully expanded, sun-exposed branch from which 15–30 leaves were selected. Stem sapwood xylem cores were extracted perpendicular to the bark at *c.* 50 cm height from the same tree bole using a 5.15-mm-increment bore (Hagl f, L ngsele, Sweden). Water displacement measurement of WD value was not possible at the field locations, so we carefully cut the ends of each core section perpendicularly to the sides, measured its length with a calliper (10^{-5} m precision), avoiding pressure of the calliper blades on the wood, and calculated fresh volume by multiplying the length by the cross-sectional area. All cores were *c.* 10 cm long, except when saplings were considered. Tissue samples were bag-labelled and stored in a cool box for transportation. Back in the laboratory, leaves were laid flat separately and photographed with a reference square of known area using a Nikon Coolpix 5000 digital camera (Nikon Corporation, Tokyo, Japan). Total projected leaf area was calculated using the image-processing software SIGMAPROC (Systat Software Inc., Richmond, CA, USA). Leaves and cores were then oven-dried at 70 C to constant weight, and subsequently were weighed with a scale to a precision of 0.0001 g for determination of LMA (as oven-dry weights of the 15–30 leaves divided by their total leaf surface) and WD (as oven-dry weights of cores divided by the core fresh volume). We determined LMA and WD for a total of 470 trees. Our method to determine WD might differ from other more elaborate methods (Williamson & Wiemann, 2010); however, our procedure proved to be the best at hand to have a rapid, nondestructive sampling of trees in remote sites having few local facilities.

Statistical analysis

Wood density was normally distributed but LMA was right-skewed (Kolmogorov–Smirnov test for goodness-of-fit), and it was therefore \log_{10} -transformed before analysis. First, to determine how variance of both traits was partitioned across latitude, elevation and individuals' age, we used a nested analysis of variance (ANOVA) with random effects (Messier *et al.*, 2010). In this analysis, each level (e.g. latitude, elevation and tree age) was considered a nested level. As tree age was determined for each individual (i.e. a continuous variable), we categorized for this sole analysis, based on tree age frequency distribution, a variable called 'age class' into four groups: I, trees \leq 50 yr old; II, trees of 51–100 yr; III, trees of 101–200 yr; IV, trees $>$ 201 yr. Thus a variable component analysis was conducted on the mixed effects nested ANOVA model using the 'varcomp' function, 'ape' and 'nlme' (Pinheiro *et al.*, 2009) packages of R (R-Development, 2009). The variances computed here represent the variance around the mean for each level (e.g. sites, altitudes, age classes). We used a bootstrapping procedure to compute 95% confidence intervals (CIs) around the variance estimation.

We also computed coefficient of variation (CV) for sites and populations within sites as a complementary index to interpret intraspecific variation. Additionally, we compared the intraspecific trait variation of our study with trait variation values (SD) reported from a nonexhaustive list of other studies (Wright & Westoby, 2002; Muller-Landau, 2004; Wright *et al.*, 2004; Chave *et al.*, 2006; He *et al.*, 2006; Chao *et al.*, 2008; Ishida *et al.*, 2008; Yang *et al.*, 2008; Hallik *et al.*, 2009; Souto *et al.*, 2009) that mostly dealt with interspecific variation across broad scales.

To test whether there is any significant covariation between LMA and WD across locations, populations or individuals (within populations), we analysed our data using the Model Type II regression method (Wright *et al.*, 2007), where the regression slope was computed as standardized major axes (SMAs). Relationship strength was quantified using the coefficient of correlation (r^2 and P -values) as well as CIs of both the slope and intercept. This slope-fitting technique was also considered appropriate, as there was error associated with both dependent and independent variables (i.e. the residual variance is minimized in both x and y dimensions). Additionally, as we were also interested in testing the potential differences in LMA and WD across latitude and elevation, and the relationships between both variables and tree age, we used differences in the SMA slopes (Falster & Westoby, 2005), which were tested following Warton & Weber's (2002) method using SMA tests and routines (Falster *et al.*, 2003). Thus, when common slopes across latitude and elevation were demonstrated (homogeneity of slopes) and calculated, differences in the y -intercept of the regression slope across latitude and elevation were tested using an ANCOVA-like procedure (ANOVA following transformation of the data to remove any correlation between, for example, LMA and tree age) (Falster *et al.*, 2003). The statistical software (s)MATR (Falster *et al.*, 2003) was used for all the analyses. Tukey's HSD post-hoc test was applied whenever a significant difference among locations was found.

Table 2 Variance partitioning of the full nested ANOVA with random effects model on leaf mass per area (LMA, g m^{-2}) and wood density (WD, g cm^{-3}) across three nested ecological scales: three sites (latitudes); four plots at each site (comparable altitudes); and four age classes. Parentheses represent the 95% CIs, which were computed by bootstrapping (500 runs with n randomly sampled data points with replacement)

Scale	% variance of trait (95% CI)	
	log LMA ($n = 433$)	WD ($n = 470$)
Tree and error	52.40 (39.03–65.51)	57.27 (40.29–69.55)
Age class	0.03 (0–0.09)	13.04 (10.87–26.34)
Altitude	38.31 (28.30–47.85)	6.80 (1.47–13.25)
Site	9.26 (4.19–19.78)	22.89 (13.01–31.55)

Results

Intraspecific trait variation

The total intraspecific variation for LMA (CV = 21.14%) was significantly higher ($t = 6.233$, $P = 0.011$) than that of WD (CV = 10.52%). The variance partitioning strongly differed depending on the scale and the trait being considered; except for the variance not explained by the factors under scrutiny (> 50%), altitude accounted for the largest total variation in LMA, whereas sites (latitude) and age classes accounted for the largest variation in WD (23 and 13%, respectively, Table 2). The ontogeny for LMA and altitude for WD accounted for the smaller total variation. When compared with other across-species studies, the intraspecific

variation we found was lower, though not negligible. Intraspecific trait variation of *N. pumilio* was not less than 26% in LMA (except when compared with Wright *et al.*'s (2004) worldwide study) and 23% in WD of the interspecific trait variation across several biomes. The total variation found in the current study even reached 95% of the interspecific variation found by Hallik *et al.* (2009) for LMA in a set of northern hemisphere deciduous broad-leaved tree species, and 42% for WD in one tropical forest location in Amazonia, Brazil (Muller-Landau, 2004). When compared with the only other study reporting trait variation at the intraspecific level, the total variation found for LMA in *N. pumilio* was 64% of the variation in *Embothrium coccineum*, another widespread tree species in the southern Andes (Souto *et al.*, 2009) (Table 3).

Table 3 Comparison (proportion of variation) of leaf mass per area (LMA, g m⁻²) and wood density (WD, g cm⁻³) general statistics of the current study with other cross-species studies. The selection of studies is not exhaustive

Study	LMA		WD		%
	Mean	SD	Mean	SD	
Our study					
Southern Andes	85.190	18.01	0.521	0.055	
Chao <i>et al.</i> (2008)					
NW Amazonia			0.63	0.15	37
NE Amazonia			0.71	0.14	39
Chave <i>et al.</i> (2006)					
Lowland wet tropical forest			0.65	0.18	31
Montane tropical forest			0.59	0.18	31
Hallik <i>et al.</i> (2009)					
Deciduous broadleaved	79	19			94.79
Evergreen broadleaved	144	33			54.56
Evergreen conifers	247	80.7			22.32
He <i>et al.</i> (2006)					
Tibetan Plateau, China	89.2	29.47			61
Ishida <i>et al.</i> (2008)					
Northern Pacific	147	57.33	0.6	0.22	31; 25
Muller-Landau (2004)					
La Selva			0.48	0.14	39
BC Island			0.52	0.16	34
Cocha Coshu			0.61	0.14	39
Km 41			0.75	0.13	42
Souto <i>et al.</i> (2009) ^a					
Southern Andes	106.38	28.2			63.86
Wright & Westoby (2002)					
Eastern Australia	165.1	66.8			26.96
Wright <i>et al.</i> (2004)					
Global	185.6	160.97			11
Yang <i>et al.</i> (2008)					
Deciduous mix lowland			0.59	0.23	23.91
Evergreen mix lowland			0.53	0.14	39.29
Deciduous mix upland			0.68	0.20	27.5

Chao *et al.* (2008), NW Amazonia (northern Peru), NE Amazonia (eastern Venezuela); Chave *et al.* (2006), tropical regions of the Americas; Hallik *et al.* (2009), using a wide range of tree species in the Northern Hemisphere; He *et al.* (2006), shrubs of the Tibetan Plateau, China; Ishida *et al.* (2008), Bonin Islands, Japan; Muller-Landau (2004), La Selva (Costa Rica), Barro Colorado (Panama), Cocha Coshu (Peru), km 41 (Brasil); Souto *et al.* (2009), *Embothrium coccineum* in southern Chile and Argentina (c. 20° of latitude), they reported mean and CV for specific leaf area (SLA = 1/LMA) which were transformed for comparison purposes; Wright *et al.* (2004), contrasting precipitation and soil nutrient woody communities in eastern Australia; Wright *et al.* (2004), shrubs worldwide; Yang *et al.* (2008), Gongga Mountain, south-west China.

^aThe only study working at the intraspecific level.

The effect of soil moisture, temperature and ontogeny on LMA and WD

According to variance partitioning magnitudes and depending on the trait and the spatial scale (see the 'Intraspecific trait variation' section in the Results), both LMA and WD mean values varied significantly across locations (moisture); values of LMA were significantly different according to elevation (temperature), while WD differed only according to ontogeny. First, there were significant differences across locations with varying soil moisture content in the mean values of LMA ($F = 4.577$, $P = 0.011$) and WD ($F = 60.903$, $P < 0.0001$, Fig. 2), where the maximum value of each trait was found at different locations; the maximum value of LMA was in Antillanca (112.035 g m^{-2} , moister location) and that of WD was in Termas (0.557 g cm^{-3} , drier location). Secondly, in the main, LMA increased significantly with elevation (Table 4); individuals at higher elevations had thicker and/or denser leaves than individuals at lower elevations. Finally, at each elevation, our results generally indicated that LMA remains constant across different ages of trees, while WD decreases with tree age (Table 5). WD varied negatively with tree age even at the krummholz belt (treeline in Termas) where the range of ages was notably narrower (Table 1).

Covariation between LMA and WD

Values of LMA correlated positively and significantly with WD at each location (Fig. 3, Termas, $P = 0.0062$; Antillanca, $P = 0.0186$; Cerro Castillo, $P = 0.0007$). The trend remained consistent across locations (Fig. 3; slopes of each location did not differ one to another, $P > 0.05$). When covariation patterns were scrutinized among individuals within each elevation, we did not find a significant covariation between both traits, except in Termas at the CF altitude (Table 6), where both traits covaried positively. Thus, at the within-population level (same elevation), individual trees that have similar LMA values do not tend to have similar WD values. All this suggests the existence of a similar covariation pattern between both traits relative to temperature only; that is, the environmental variation across elevation is a decreasing pattern of temperature with altitude.

Discussion

Intraspecific trait variation is not negligible

Using a widely distributed tree species located in different and contrasting environmental conditions (from Mediterranean-like to humid conditions), we observed a substantial amount of intraspecific variation, particularly for LMA (CV = 21.14%). Also notable was the fact that the variation of WD was half that of LMA, suggesting the conserved character of

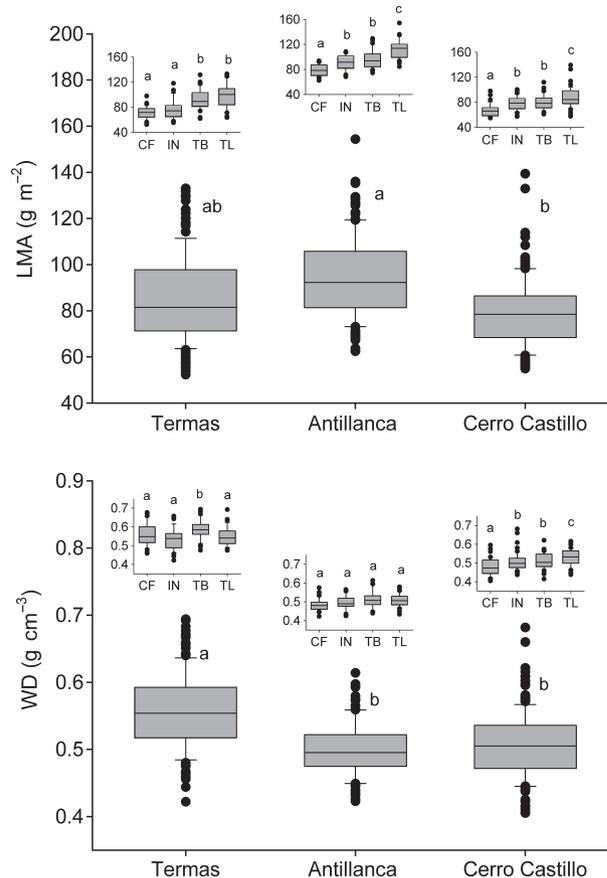


Fig. 2 Box-plots of leaf mass per area (LMA, g m^{-2}) and wood density (WD, g cm^{-3}) of the tree species *Nothofagus pumilio* growing in three different locations (different moisture contents) – Termas de Chillán ($36^{\circ}54'S$, $71^{\circ}24'W$), Antillanca ($40^{\circ}47'S$, $72^{\circ}11'W$) and Cerro Castillo ($46^{\circ}04'S$, $72^{\circ}03'W$), Chile – and at four elevations (different temperatures) at each location. The median values are represented by the horizontal line, quartiles (25 and 75% percentiles) by boxes with error bars. Extreme data values are plotted with individual markers. Comparisons among locations and elevations within each location were done using univariate ANCOVA-like procedures (ANCOVA following transformation of the data to remove any correlation between LMA and WD, with tree age) with standardized major axes (SMA) analysis. Individual SMA slopes did not differ ($P\text{-value}_{\text{LMA}} = 0.124$; $P\text{-value}_{\text{WD}} = 0.251$) among populations and therefore intercepts could be compared. With a common slope of -0.318 (95% CI = -0.291 to -0.348) and of -0.000772 (95% CI = -0.000712 to -0.000838), at a given tree age, populations (intercepts) significantly differ in LMA ($P = 0.011$) and WD ($P < 0.0001$), respectively. Different letters indicate a statistically significant difference among locations and among elevations within locations ($P < 0.05$, least significant difference). LMA was \log_{10} -transformed to achieve normality.

WD, which is in agreement with previous evidence that WD is less variable within species than expected by chance (Chave *et al.*, 2006; Swenson & Enquist, 2007). The intraspecific variation we found for *N. pumilio* was, for both traits, not less than 23% of the entire interspecific variation found in other cross-species studies (some of them including

Table 4 Leaf mass per area (LMA, g m⁻²) and wood density (WD, g cm⁻³) statistics in the tree species *Nothofagus pumilio* from three study locations (latitudes) – Termas (36°54'S, 71°24'W), Antillanca (40°47'S, 72°11'W) and Cerro Castillo (46°04'S, 72°03'W), Chile – and from four altitudes at each location

Altitudes (m asl)	LMA					WD				
	Mean	N	Range	SE	CV	Mean	N	Range	SE	CV
Termas										
CF (1750)	71.53a	35	52.28–97.80	1.64	0.14	0.56a	40	0.46–0.68	0.009	0.11
IN (1860)	76.04a	33	55.27–118.18	2.60	0.20	0.54a	37	0.42–0.66	0.009	0.11
TB (1950)	92.65b	37	61.96–131.70	2.62	0.17	0.59b	36	0.48–0.69	0.008	0.09
TL (2080)	98.78b	34	63.72–132.98	3.22	0.19	0.55a	35	0.48–0.69	0.009	0.09
Total	84.89	139	52.28–132.98	1.59	0.22	0.56	148	0.42–0.69	0.005	0.10
<i>F</i> (<i>P</i> -value)	25.597 (< 0.001)					7.94 (< 0.001)				
Antillanca										
CF (1140)	79.08a	30	62.43–93.58	1.66	0.12	0.49a	30	0.42–0.57	0.006	0.07
IN (1200)	90.86b	30	68.46–108.33	2.28	0.14	0.50a	30	0.43–0.57	0.007	0.07
TB (1290)	96.17b	31	74.45–129.31	2.77	0.16	0.52a	31	0.44–0.61	0.008	0.09
TL (1350)	112.04c	30	84.71–154.36	2.80	0.14	0.51a	30	0.43–0.58	0.006	0.07
Total	94.55	121	62.43–154.36	1.61	0.19	0.50	121	0.42–0.61	0.004	0.08
<i>F</i> (<i>P</i> -value)	21.51 (< 0.001)					1.95 (> 0.05)				
Cerro Castillo										
CF (1080)	67.06a	38	54.89–97.92	1.66	0.15	0.48a	49	0.41–0.60	0.006	0.09
IN (1180)	78.33b	39	57.29–100.42	1.77	0.14	0.51b	50	0.44–0.68	0.007	0.10
TB (1250)	79.91b	49	61.23–111.99	1.66	0.15	0.51b	50	0.42–0.62	0.006	0.08
TL (1310)	87.85c	47	57.73–139.38	2.35	0.18	0.53c	50	0.44–0.62	0.006	0.08
Total	78.89	173	54.89–139.38	1.10	0.18	0.51	199	0.41–0.68	0.003	0.10
<i>F</i> (<i>P</i> -value)	20.94 (< 0.001)					9.679 (< 0.001)				

N, number of trees sampled; SE, standard error; CV, coefficient of variation; CF, closed forest; IN, intermediate stand; TB, timberline; TL, tree-line; asl, above sea level.

F- and *P*-values refer to univariate ANCOVAs (tree age as the covariable) testing for differences among altitude means within each population. Different letters in the same column indicate a statistically significant difference among altitudes ($P < 0.05$, least significant difference).

multiple woody species and complete biomes). Similarly, in a recent study, Messier *et al.* (2010), working with LMA and leaf dry matter content in tropical forests in Panama, found that the total amount of intraspecific variation was comparable to the amount of interspecific variation; thus, intraspecific variation can be important (not negligible), at least for some clades (Chave *et al.*, 2006; Swenson & Enquist, 2007). It should be noted that in our comparative analysis with other studies reporting the magnitude of trait variation, the comparison was done in absolute terms of variation and did not consider whether the other studies were conducted under similar conditions (i.e. similar environmental conditions). As such, only one of the studies scrutinized (Souto *et al.*, 2009) was conducted along a latitudinal gradient with one tree species (*Embothrium coccineum*), while the others considered biomes or precipitation gradients as the comparative pattern. With this rather simplistic comparative analysis, we have shown that, in most cases, intraspecific variation of *N. pumilio* represented at least a quarter of the total interspecific variation across similar spatial scales. This finding may imply two things: our tree species is unusual in that it varies in a way that other species cannot vary, since they cannot tolerate different abiotic conditions, and thus are less competitive and less persistent; and other woody species being part of community assemblies could potentially have similar and

important trait variations (Hulshof & Swenson, 2010; Messier *et al.*, 2010), which have not traditionally been accounted for. When this is the case (coexisting species having important intraspecific trait variation), it could be argued that these species may be functionally redundant; that is, their overlap may be larger than the difference between their mean trait values (Messier *et al.*, 2010). It would be very illustrative to compute intraspecific trait variation along a species-rich gradient and to test whether this variation changes and ultimately depends on biotic interactions (Gross *et al.*, 2009). In a similar vein, Cornwell & Ackerly (2010) found a significant relationship between abundance of species and trait values at the community scale (e.g. the higher the LMA value, the higher the abundance of those species), but not at the landscape scale. *N. pumilio* constitutes a forest community where the abundance may be close to 100%; does the other small fraction of woody species have radically different trait values to those exhibited by *N. pumilio*?

Environmental conditions and ontogeny affect LMA and WD variation differently: probable mechanisms

Although, half the variation was not accounted for by any of the drivers proposed (residuals), our results indicate that

Table 5 Type II regression relationships between leaf mass per area (LMA, g m^{-2}) and wood density (WD, g cm^{-3}) values with their respective tree age across four elevations (CF, closed forest; IN, intermediate stand; TB, timberline; TL, treeline) of the tree species *Nothofagus pumilio* growing at three locations (latitudes) in southern Chile: Termas ($36^{\circ}54'S$, $71^{\circ}24'W$), Antillanca ($40^{\circ}47'S$, $72^{\circ}11'W$) and Cerro Castillo ($46^{\circ}04'S$, $72^{\circ}03'W$)

	Termas		Antillanca		Cerro Castillo	
	LMA	WD	LMA	WD	LMA	WD
CF						
<i>b</i>	-0.139	-0.00073	0.221	-0.000812	-0.120	-0.00043
CIA	0.098	0.00042	0.173	0.0006	0.081	0.00019
r^2	0.011	0.232	0.004	0.087	0.003	0.431
<i>P</i> -value	0.553	0.0017	0.7307	0.1135	0.7610	0.0001
IN						
<i>b</i>	-0.180	-0.000595	-0.266	-0.000771	-0.132	-0.00049
CIA	0.131	0.000363	0.186	0.000597	0.082	0.000251
r^2	0.002	0.209	0.189	0.001	0.136	0.210
<i>P</i> -value	0.792	0.0044	0.0163	0.8712	0.0211	0.0008
TB						
<i>b</i>	-0.553	0.002178	0.799	-0.002414	0.417	-0.00151
CIA	0.366	0.001518	0.603	0.001702	0.245	0.000814
r^2	0.071	0.001	0.011	0.139	0.001	0.134
<i>P</i> -value	0.111	0.888	0.5685	0.0387	0.8967	0.0088
TL						
<i>b</i>	-0.699	-0.001831	0.870	-0.001936	0.532	-0.00144
CIA	0.501	0.001204	0.673	0.001495	0.317	0.000828
r^2	0.009	0.139	0.001	0.005	0.020	0.019
<i>P</i> -value	0.590	0.0274	0.9002	0.6992	0.3409	0.3363

b, slope; CIA, 95% CIs of the amplitude of the slopes; r^2 , correlation coefficient.

P-values refer to correlation analyses following standardized major axes (SMA) procedures (see the Material and Methods section).

P < 0.05, in bold refers to a significant correlation between the particular trait and tree age.

LMA was \log_{10} -transformed to achieve normality.

Sample size (*N*) corresponds to Table 2.

the most important environmental conditions affecting LMA and WD were elevation (temperature) and location (moisture), respectively. WD and, in particular, LMA values increased with altitude for *N. pumilio* (covaried similarly, see later). An increase of LMA with altitude is a common pattern found in many other species, particularly evergreens (Cordell *et al.*, 1998; Hovenden & Vander Schoor, 2004), and it was also found for *N. pumilio* in an altitudinal gradient in Tierra del Fuego (Barrera *et al.*, 2000). A higher LMA is related to thicker laminae or higher tissue density, or combinations of these (Niinemets, 1999). This may suggest the existence of more expensive-to-construct leaves at higher elevations, where to tolerate stress (e.g. low temperatures) may be more important than having high rates of photosynthesis. However, Premoli & Brewer (2007) found that under both field and common garden conditions, net photosynthesis of *N. pumilio* was higher in individuals of upper than of lower elevations. This finding, along with the results of the present study, suggest that LMA may be positively correlated with net photosynthesis in *N. pumilio* and that expected trade-offs between leaf functional traits and physiological performance (Wright *et al.*, 2004) might not apply in this species. Even though altitude only explained 6.8% of the total variation of WD,

the increasing trend with altitude may be explained in terms of a decreasing growth rate as a result of environmental stress (e.g. Termas and Cerro Castillo).

Site variation accounted for 23% of total variation in WD. The highest values were found at the location exhibiting drought (Termas), which is in accordance with other studies (e.g. Chave *et al.*, 2006; Preston *et al.*, 2006). Higher WD may be an adaptation to drought stress (Hacke *et al.*, 2001) as it is associated with low stem vulnerability to cavitation (Tyree & Sperry, 1989; Preston *et al.*, 2006), or it may be explained in terms of drought-induced growth limitations (Zhang *et al.*, 2009). Site variation only accounted for 9% of total variation in LMA, which is why this variable was not positively related to moisture conditions. Similarly, Wright *et al.* (2004) emphasize that LMA and rainfall are not related in deciduous species; however, LMA does relate positively with leaf longevity which has been found to be shorter at colder locations, as a result of the shorter growing season (Wright *et al.*, 2004). Although Wright *et al.*'s (2004) dealt with interspecific comparisons, the selective force of environment might act similarly within species (Cornwell & Ackerly, 2009), and thus the lower LMA in Cerro Castillo (the southernmost location) might be associated with the shorter growing season, which precludes

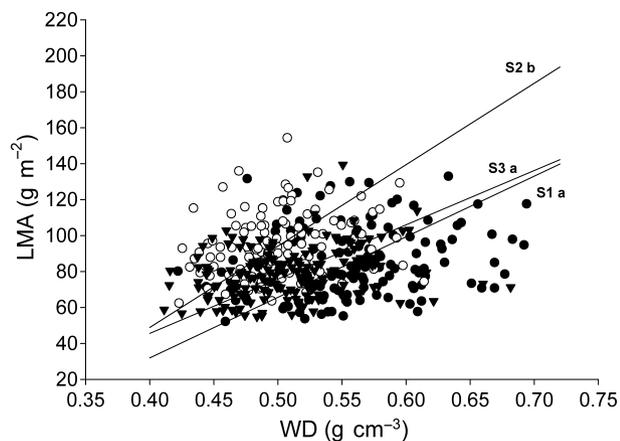


Fig. 3 Type II regression relationships (covariation) of leaf mass per area (LMA, g m^{-2}) and wood density (WD, g cm^{-3}) values collected from the same individual for populations (altitudes: CF, closed forest; IN, intermediate stand; TB, timberline; TL, treeline) across locations in southern Chile (latitudes: Termas de Chillán ($36^{\circ}54'S$, $71^{\circ}24'W$), Antillanca ($40^{\circ}47'S$, $72^{\circ}11'W$) and Cerro Castillo ($46^{\circ}04'S$, $72^{\circ}03'W$)) of the tree species *Nothofagus pumilio*. Correlations were: $\text{LMA} = 1.68 (1.43\text{--}1.98) \times \text{WD} + 0.979$ for Termas; $\text{LMA} = 2.05 (1.71\text{--}2.44) \times \text{WD} + 0.945$ for Antillanca; and $\text{LMA} = 1.61 (1.39\text{--}1.86) \times \text{WD} + 1.069$ for Cerro Castillo (values in parentheses correspond to 95% CIs around the slope). Solid lines indicate SMA slopes. Correlation significances are given for the relationship between both traits: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Individual slopes were homogeneous ($P = 0.113$).

leaves from complete development and anticipates leaf senescence.

Ontogeny affected very differently for both traits, not accounting for any variation in LMA. In general, LMA has been found to be lower in younger than in older trees (Day *et al.*, 2001; Thomas & Winner, 2002; Zhang *et al.*, 2009), and this is probably the result of the different light environments. Our results, however, indicate that leaves of *N. pumilio* do not vary their construction costs along with ontogeny. This is the opposite of what has been found for other deciduous species (Thomas & Winner, 2002). As we controlled the light environment by collecting sun-exposed leaves, our result is in line with previous studies pointing out that light exposure is the main driver of LMA variation with ontogeny (Rozendaal *et al.*, 2006; Poorter, 2007). Regarding WD, an increase is expected when growth is restricted with ontogeny (Zhang *et al.*, 2009). We nonetheless found the opposite: WD declined with ontogeny across two locations (for Antillanca the trend was not significant). Discarding the fact that we collected sapwood WD (see the Materials and Methods section), we have no clear explanation for this trend, but it evidently indicates that decreasing WD with ontogeny cannot be interpreted as a consequence of declining efficiency in growth, as reported, for example, by Zhang *et al.* (2009). One potential explanation is that denser wood in younger trees could be related to frequent water stress affecting the superficial root zone of shorter

Table 6 Type II regression relationships (covariation) of leaf mass per area (LMA, g m^{-2}) and wood density (WD, g cm^{-3}) values of the tree species *Nothofagus pumilio* for individuals within each altitude (CF, closed forest; IN, intermediate stand; TB, timberline; TL, treeline) at three locations in southern Chile: Termas ($36^{\circ}54'S$, $71^{\circ}24'W$), Antillanca ($40^{\circ}47'S$, $72^{\circ}11'W$) and Cerro Castillo ($46^{\circ}04'S$, $72^{\circ}03'W$)

	Termas	Antillanca	Cerro Castillo
CF			
<i>b</i>	1.06	1.52	1.47
CI	0.77–1.46	1.04–2.20	1.05–2.06
r^2	0.153	0.031	0.010
<i>P</i> -value	0.020	0.351	0.554
IN			
<i>b</i>	1.48	1.71	1.48
CI	1.04–2.12	1.18–2.46	1.11–1.98
r^2	0.009	0.060	0.018
<i>P</i> -value	0.590	0.193	0.363
TB			
<i>b</i>	1.49	1.44	1.48
CI	1.06–2.10	1.00–2.09	1.11–1.97
r^2	0.002	0.001	0.010
<i>P</i> -value	0.784	0.930	0.490
TL			
<i>b</i>	1.68	1.72	1.77
CI	1.20–2.35	1.18–2.50	1.33–2.37
r^2	0.084	0.009	0.036
<i>P</i> -value	0.097	0.621	0.204

b, slope; CI, 95% CIs of the slopes; r^2 , correlation coefficient. *P*-values refer to correlation analyses following standardized major axes (SMA) procedures (see the Materials and Methods section). $P < 0.05$, in bold refers to a significant correlation between the particular trait and tree age. LMA was \log_{10} -transformed to achieve normality. Sample size (*N*) corresponds to Table 2.

trees. In the driest site (Termas), taller trees may escape from water stress by accessing deeper sources of water. Yet another potential explanation for this pattern is that increasing vessel area with tree height may act as a compensation mechanism for increased hydraulic resistance (Becker *et al.*, 2000). This may account for the negative relationship between WD and tree height, even when it might be somewhat counterbalanced by a reduction in vessel density.

Relationships between plant trait variation

At the interspecific level, Cornwell & Ackerly (2009) found that WD and LMA responded similarly to environmental gradients, suggesting covariation of the two traits and common tradeoffs across locations. A similar positive relationship between WD and LMA was found by Bucci *et al.* (2004) and Ishida *et al.* (2008) working in multi-species tropical forests. On the other hand, Wright *et al.* (2007) found that these two traits were not related to each other among seven tropical species. In particular, the highest consensus so far on the relationship between the environment

and LMA and WD is that their values increase with dry conditions (Wright *et al.*, 2004; Preston *et al.*, 2006; Cornwell & Ackerly, 2009). We found a stronger control of temperature, rather than moisture and ontogeny, on the covariation of these traits (i.e. they covary across a temperature but not across a moisture gradient (or dry conditions)), suggesting that the effect of environmental drivers on the covariation between LMA and WD differ at the intraspecific level. Basically, we did not find any relationship between traits and ontogeny because LMA did not vary with tree age while WD did; nor was there a relationship with moisture, as WD mostly varied with it, whereas LMA's variation with moisture was small. The fact that both traits covaried predictably with temperature represents crucial information to be considered for future incorporation into vegetation-climatic models (Wright *et al.*, 2005).

Conclusions

Leaf mass per area and WD are part of a whole suite of interconnected traits that together shape the performance of plants (Westoby & Wright, 2006). So far, much importance has been placed on their interspecific variation. Here we quantified trait variation of both LMA and WD attributed to moisture, temperature and tree ontogeny while controlling for phylogeny. We conclude, first, that trait variation at the intraspecific level is relevant, particularly when compared with interspecific variation values reported by other studies; second, that LMA and WD varied differently depending on the environmental driver and ontogeny, for which we offered explanations on the most probable mechanisms driving these variations; and third, that LMA and WD covaried positively with temperature only. Additionally, in spite of the existence of an increasing understanding of local adaptation and acclimation to environmental conditions at the within-species level, our study, as far as we know, is the first one attempting to place this intraspecific variation into the context of community ecology and assembly processes at large scale. Intraspecific variation should be recognized and considered when interspecific trait variation studies are conducted, particularly for LMA with temperature and for WD with moisture, because environmental variation is indeed causing important trait variations but with no effects on species turnover. Widespread species forming monospecific forests, and thus having maximal abundance in the forest community, may constitute a valid exception to take into account and a key system for testing assumptions and consequences of the functional trait paradigm.

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