

Are trait-scaling relationships invariant across contrasting elevations in the widely distributed treeline species *Nothofagus pumilio*?¹

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PREMISE OF THE STUDY: The study of scaling examines the relative dimensions of diverse organismal traits. Understanding whether global scaling patterns are paralleled within species is key to identify causal factors of universal scaling. I examined whether the foliage–stem (Corner's rules), the leaf size–number, and the leaf mass–leaf area scaling relationships remained invariant and isometric with elevation in a wide-distributed treeline species in the southern Chilean Andes.

METHODS: Mean leaf area, leaf mass, leafing intensity, and twig cross-sectional area were determined for 1–2 twigs of 8–15 *Nothofagus pumilio* individuals across four elevations (including treeline elevation) and four locations (from central Chile at 36°S to Tierra del Fuego at 54°S). Mixed effects models were fitted to test whether the interaction term between traits and elevation was nonsignificant (invariant).

KEY RESULTS: The leaf–twig cross-sectional area and the leaf mass–leaf area scaling relationships were isometric (slope = 1) and remained invariant with elevation, whereas the leaf size–number (i.e., leafing intensity) scaling was allometric (slope \neq -1) and showed no variation with elevation. Leaf area and leaf number were consistently negatively correlated across elevation.

CONCLUSIONS: The scaling relationships examined in the current study parallel those seen across species. It is plausible that the explanation of intraspecific scaling relationships, as trait combinations favored by natural selection, is the same as those invoked to explain across species patterns. Thus, it is very likely that the global interspecific Corner's rules and other leaf–leaf scaling relationships emerge as the aggregate of largely parallel intraspecific patterns.

KEY WORDS Corner's rules; leaf area; leafing intensity; LMA; Nothofagaceae; Patagonia; plant height; plant development; scaling

The study of scaling in biology examines the relative dimensions of diverse organismal traits (Brown et al., 2000). It has long been recognized as important in the evolutionary biology of animal design (e.g., Harvey and Pagel, 1991; Brown et al., 2000) and in recent decades has gained similar attention in plants through universal metabolic scaling (e.g., Niklas, 1994; Enquist et al., 1999) and from other perspectives (see Poorter et al., 2015). Many decades ago, however, E. J. H. Corner (1949) had already suggested a spectrum of tropical tree forms based on his qualitative impressions of leaf–stem relationships. He suggested a spectrum from species with large leaves, thick twigs, and few branches to species with small

leaves, thin twigs, and many branches (Corner, 1949). These leaf–stem relationships have come to be called one of Corner's rules (Hallé et al., 1978; White, 1983; Brouat et al., 1998; Olson et al., 2009). According to Brouat et al. (1998) and Olson et al. (2009), these scaling relationships reflect the fact that, in most cases, natural selection has resulted in the impossibility of one organ evolving independently of another, and some coordination of the functional requirements involved in the scaling of different organs that contribute to the same function must exist; these scaling relationships can be considered part of the integral coordination between organs in plants (Pigliucci, 2004). This coordination in plant traits represents a situation in which none of the traits is at a value of maximal performance, but the combination of traits is one in which maximal performance is achieved (Stearns, 1989; Li et al., 2015). Thus, it is implicit that evolutionary changes in a given trait unavoidably involve changes in other traits in the plant. For example, on the

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basis of an isometric relationship between the cross-sectional area of the twig and the foliage area borne on it, vascular supply is proposed to be directly proportional to the requirements of leaves supported by the stem, and likewise, that stem demands are directly proportional to the metabolic support provided to them from the leaves, e.g., the Huber value (Tyree and Ewers, 1991; Olson et al., 2009).

Further investigating this leaf–stem relationship, recent studies have also shown the pervasive occurrence of a parallel trait–trait coordination continuum across species between the size (or area) of leaves and the number of leaves for a given unit of supporting shoot tissue, i.e., a fundamental interspecific leaf size–number trade-off (e.g., Kleiman and Aarssen, 2007; Yang et al., 2008; Whitman and Aarssen, 2010; Scott and Aarssen, 2012). For a given leaf biomass allocation or total leaf area displayed, plants may have either few large or many small leaves. This is very similar to other frequently observed size–number trade-offs in plants. Some examples include the seed size–number trade-off (Moles and Westoby, 2004)—and the related tolerance–fecundity trade-off based on seed size (Muller-Landau, 2010) that may even explain species coexistence (Adler et al., 2013)—and the plant size–fecundity trade-off (Aarssen et al., 2006), that may explain species distributions. According to Dombroskie and Aarssen (2010), most angiosperms have relatively small individual leaf mass. A strong and isometrically negative relationship between leaf size and leafing intensity—the number of leaves produced per unit of supporting nonleaf dry mass or per unit plant body size (Kleiman and Aarssen, 2007)—has been found in many studies (Scott and Aarssen, 2012). Species producing smaller and lighter leaves can produce proportionally (i.e., isometric) more leaves per unit of supporting shoot or plant body size than species with larger, heavier leaves. Related to the previous relationship, Niinemets et al. (2007) found a disproportionate (i.e., allometric) overall support investment associated with leaf area, i.e., a positive scaling relationship between leaf area and costs involved in leaf construction (e.g., midribs and petioles). This means that leaf mass should scale positively with leaf area because more mechanical support would be needed (Grubb, 1998; Milla and Reich, 2007). Yet, others have postulated that the leaf mass–leaf area scaling relationship should be negative given that the production of larger leaves would minimize the investment in support per leaf area due to a reduction in the number of leaves per shoot to support a given leaf area displayed (Givnish, 1979; Reich et al., 2004). It is more costly—and probably less efficient—to manufacture larger leaves (Sack et al., 2012). So far, empirical studies have found more support for a positive, rather than a negative, leaf mass–leaf area scaling relationship (Milla and Reich, 2007; Pan et al., 2013). In spite of this knowledge, we are not yet certain about the adaptive consequences and selection mechanisms associated with covariation patterns between stem and leaf at several scales, including variation of individuals within species across environmental gradients.

This intraspecific variation is important because it should shed light on the causes of global cross-species relationships such as Corner's rules. One of the best contexts in which to find substantial intraspecific variation is along environmental gradients. All of the mechanistic hypotheses proposed to explain global patterns of trait covariation across species should in principle apply within species as well. For example, selection favoring coordination of leaf area and stem hydraulic characteristics has been repeatedly proposed as underlying Corner's rules (White, 1983; Ackerly and Donoghue, 1998).

There is no reason to expect that selection would not favor such coordination across variation in leaf size within species as well, e.g., larger leaves require greater stem mechanical support and water conductive area regardless of whether the variation in leaf size is within or across species. Any global pattern, moreover, must be overlain on such intraspecific variation (e.g., Cheverud, 1982; Pélabon et al., 2013). However, the degree to which Corner's rules apply within a species has been little examined, so I turned here to variation across a marked environmental gradient that is ideal for provoking similarly marked intraspecific variation in the traits of interest.

It is well known that the magnitude, dispersion, and covariation of traits changes across environmental gradients. One conspicuous environmental gradient is driven by decreasing temperatures with increasing elevation in mountain systems (Körner, 2007). Previous work has proposed that trees are more strongly affected by low temperatures at high elevations than smaller-stature plants due to tree architecture and the associated aerodynamics (Körner, 2012; Fajardo and Piper, 2014). Thus, all of the tree species that grow at the treeline elevation have considerably reduced height and growth rates—forming crooked-like trees—when compared with conspecifics growing at lower elevations (e.g., Fajardo and McIntire, 2012). Although plant architecture is recognized as being commonly influenced by ecological factors, little is known about how decreasing temperature directly and indirectly affects scaling relationships in tree species. Knowing that leaf size, leaf mass, and stem mechanical properties like wood density change with elevation (e.g., Fajardo and Piper, 2011; Milla and Reich, 2011), one would tend to think that this variation should influence the leaf–stem and leaf–leaf scaling relationships at the intra- and interspecific levels. The scaling relationships may vary depending on the relative limitations of the environment on the physiological activity of the different organs (Poorter et al., 2015).

Considering that low temperatures generally limit tree development, particularly at high elevations (e.g., Körner, 1998; Fajardo and McIntire, 2012), in this study I assessed how the elevational gradient affects (1) the universal scaling between leaves and stems as a mechanism of Corner's rules and the coordination of both, (2) the leaf size–number trade-off, i.e., leafing intensity, and (3) the leaf mass–leaf area scaling relationship on a single tree species, *Nothofagus pumilio*, in the southern Andes of Chile. With most studies of Corner's rules focusing on the comparative level (e.g., Sun et al., 2006; Olson et al., 2009), the assumption that different trait combinations along the Corner's rules spectrum are possible within a species has remained largely unexamined (but see Osada et al., 2015). Since intraspecific variation is a central assumption within the explanation of Corner's rules, it is essential to examine this level of variation. The fact that trait relationships at the intraspecific level vary predictably would necessarily complement our knowledge on the leaf–stem coordination across species. To do so, I compared the covariation of functional traits in *Nothofagus pumilio*, a wide-distributed treeline species, across contrasting climatic conditions, covering a latitudinal gradient of almost 20 degrees in the southern Andes, ranging from the Mediterranean-like climate in central Chile (36°S) to the southernmost tip of the continent in Tierra del Fuego (54°S).

Taking into account the reduction in tree height and the documented decrease in leaf size with elevation (Barrera et al., 2000; Westoby et al., 2002; Sun et al., 2006; Fajardo and Piper, 2011), the question is, will the leaf–stem and leaf–leaf scaling relationships remain invariant with elevation at the within-species level? In other

words, is the scaling conserved across elevation when individuals of the same species are considered? Invariant scaling would mean there would be no changes in the scaling slope of the traits' relationships and would thus be in line with an integral coordination of leaf and stem traits manifested into the Corner's rules at the within-species level. Alternatively, finding that the slope or intercepts can change with elevation would help to map out the extent to which these relationships can be altered in ontogeny and help to identify the causes (e.g., temperature) of these differences. For example, it could be that krummholz trees located at the treeline elevation will have more and smaller leaves per unit of supporting shoot tissue than their counterparts located at lower elevations. That is, under stressful site conditions, like the low temperatures that exist at the treeline elevation, natural selection would favor large leaf number (Kleiman and Aarssen, 2007). This result would be in line with some recent studies (Whitman and Aarssen, 2010; Scott and Aarssen, 2012; Yan et al., 2012) that have found that smaller plants (like crooked-like trees—krummholz—at treeline elevation) produce smaller and more abundant leaves for a given shoot body size (i.e., higher leafing intensity). Finally, based on previous studies (Milla and Reich, 2007; in particular Pan et al., 2013), I expected to find a positive allometric scaling relationship between individual leaf mass and leaf area that nonetheless would not be conserved with elevation (significantly different slopes) given that the higher the elevation the lower the leaf area and the higher the leaf mass (higher leaf mass per area, LMA).

MATERIALS AND METHODS

Species and research site descriptions—*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 potential mechanisms responsible for trait variation under contrasting climates while controlling for phylogenetic effects (Fajardo and Piper, 2011). Here, I selected one important environmental driver for capturing the trait variations of *N. pumilio*, a short-scale locally influenced climatic gradient that covers a decreasing temperature trend with elevation. I replicated this study in four sites, covering 18 degrees of latitude, from Mediterranean-like to cold temperate climatic conditions (Fig. 1). The first site was in Termas de Chillán ($36^{\circ}54'S$, $71^{\circ}24'W$), where the treeline occurs at 2080 m a.s.l. The annual precipitation is 1520 mm (Las Trancas weather station, Dirección General de Aguas, 2005–2014, 1250 m a.s.l.). The second site was in the Antillanca area within the Puyehue National Park ($40^{\circ}47'S$, $72^{\circ}11'W$), where the treeline occurs at 1350 m a.s.l. The annual precipitation is 3660 mm (Daniels and Veblen, 2004; Aguas Calientes weather station, 1991–1997, 475 m a.s.l.). In these two sites, most precipitation falls as snow from May to September, and the soils are derived from andesitic rocks of volcanic origin. The third site was in the Coyhaique National Reserve ($45^{\circ}31'S$, $72^{\circ}02'W$), hereafter called Coyhaique, where the treeline occurs at 1230 m a.s.l. The annual precipitation here is 890 mm (Coyhaique National Reserve weather station, Dirección General de Aguas, 2004–2013, 400 m a.s.l.). The soil is derived from aeolian volcanic ash deposits. The fourth site was in the Karukinka Private Reserve in Tierra del Fuego ($54^{\circ}06'S$, $69^{\circ}21'W$), hereafter called Karukinka, where the treeline occurs at 630 m a.s.l. The annual

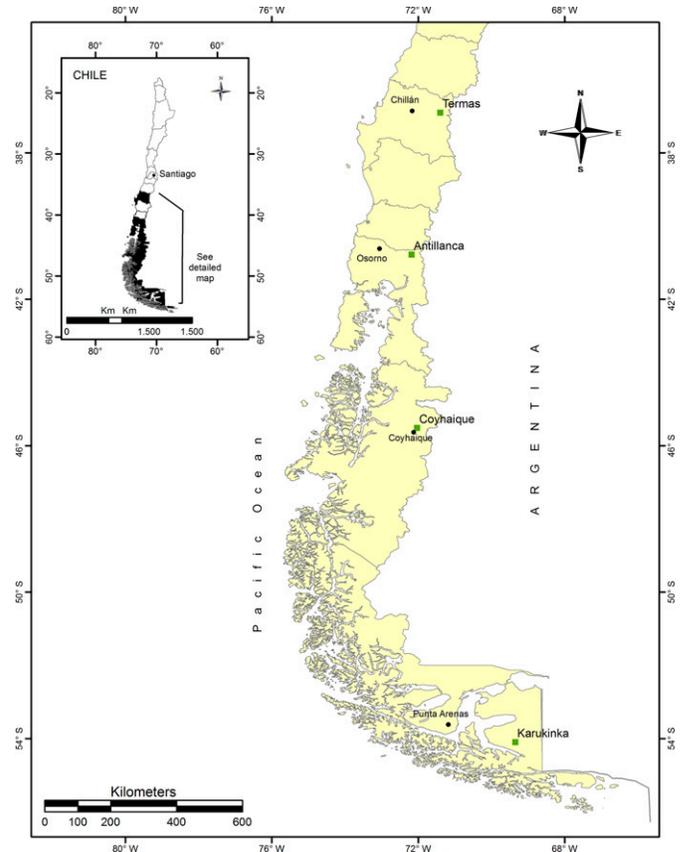


FIGURE 1 Map of the southern Andes, where study locations appear as small squares: Termas de Chillán ($36^{\circ}54'S$, $71^{\circ}24'W$, treeline elevation at 2080 m a.s.l.) in central Chile where there is a Mediterranean influence; Antillanca ($40^{\circ}47'S$, $72^{\circ}11'W$, treeline elevation at 1350 m a.s.l.) in southern Chile with humid climatic conditions; Coyhaique ($45^{\circ}31'S$, $72^{\circ}02'W$, treeline elevation at 1230 m a.s.l.) in central Patagonia; and Karukinka in Tierra del Fuego ($54^{\circ}06'S$, $69^{\circ}21'W$, treeline elevation at 630 m a.s.l.). The last two sites have a cold-humid climate.

precipitation is 565 mm (Lago Deseado weather station, Dirección General de Aguas, 2006–2013, 400 m a.s.l.).

Sampling, tissue collection, and processing—At each site, I selected trees at four elevations, covering the last 200 m of elevation of the treeline ecotone. For clarity and brevity, I hereafter refer to elevations by their short names: E1, closed, tall, lowest elevation forest; E2, intermediate stand located between E1 and timberline; E3, timberline; and E4, treeline. This classification is in accordance with previous studies on the *N. pumilio* treeline (e.g., Fajardo and Piper, 2011; Fajardo et al., 2011), where the main criterion has always been to cover the complete treeline ecotone, from tall, erect trees in the lowland up to crooked-shape trees at the treeline elevation (Fig. 2). Although the four treeline sites occurred at different elevations, I was certain that the temperature gradient with elevation was of comparable magnitude because elevational treelines occur at analogous isotherms—treelines worldwide share a similar mean growing season temperature of $6.4^{\circ}C$, including the *Nothofagus pumilio* treeline (Hoch and Körner, 2012; Fajardo and Piper, 2014). In these treeline ecotones, trees of comparable age may differ from up to 7-fold in height between the treeline (~ 3 m) and the E1 (~ 21 m, Fig. 2).



FIGURE 2 Significant difference in form and height between *Nothofagus pumilio* individuals growing at treeline elevation (E4 ~1300 m a.s.l. in Coyhaique) and conspecifics growing at lower elevation (E1 ~1050 m a.s.l.). The treeline, krummholz-like tree in the left panel is less than 3 m tall, whereas trees in the right panel (closed forest, E1) are ca. 15 m tall. Lower temperature seems to be the main driver provoking a significant decrease in tree stature.

Sampling was conducted in late January 2013 for the Termas de Chillán and the Antillanca sites, and in early February 2014 for the Coyhaique and the Karukinka sites, when leaf expansion and shoot growth were complete. The collection of branches was conducted between 10:00 and 16:00 hours. At each of the four elevations, for tissue collection I selected 8–15 individuals that were at least 30 m apart and that were always at the edge of a patch; i.e., no shade leaves were collected, particularly at low elevations. I thus restricted the sampling to adult, unshaded trees without browsing or other damage. For each individual tree, I identified and cut a couple of terminal, ca. 1 m long, fully expanded sun-exposed branch using a 5.6 m telescoping pole (ARS Corp., Sakai, Japan). Branches consisted of a terminal set of internodes and the leaves upon them. From each of these branches, a 3-yr-old twig with no apparent leaf area loss was selected and cut for measurement. Annual nodes were easily identifiable by bud scars (Puntieri et al., 1999), differently colored shoot epidermis, and different shoot diameter. Branches were cut about one cm from the older node. Two twig diameter measurements (d_1 and d_2) were taken immediately above the cut using a Vernier caliper set to 0.1 mm of accuracy. The twig-cross-sectional area was then calculated from the diameters as $\text{area} = (\pi d_1 d_2)/4$. Branches were labeled and placed in a cooler for transportation. For each individual tree surveyed, I also measured diameter at breast height (DBH, 1.35 m) and tree height using a clinometer (Suunto, Vantaa, Finland). In the laboratory, I placed the branches in water to minimize leaf dehydration. Then, all of the leaves on the twig were taken off to measure leaf area and mass. For LMA determination, 20 leaves were randomly separated from the lot and separately laid flat and photographed with a reference square of known area using a Nikon Coolpix 5000 digital camera (Nikon, Tokyo, Japan). The total projected leaf area was then calculated using the image-processing software SIGMAPROC (Systat Software, Richmond, California, USA). A mean individual leaf area was calculated as the total projected leaf area divided by the 20 leaves. All of the leaves were then dried in a forced-air stove (Memmert GmbH, Schwabach, Germany) at 70°C for 72 h, and the 20 photographed leaves were subsequently weighed with a scale calibrated to 0.0001 g of precision to determine LMA. The rest of collected leaves were also weighed for total leaf area determination. I computed LMA as the oven-dried mass of the 20 leaves divided by their total foliar area and total leaf area per twig as the total leaf mass multiplied by the

inverse of the LMA of that twig. Finally, leafing intensity was determined as the number of leaves borne on a twig divided by the twig mass (Yang et al., 2008), which can be used to reflect functional trade-offs between leaf size and leaf number per unit of twig size (Kleiman and Aarssen, 2007).

Statistical analyses—All statistical analyses were conducted in the program R version 2.15.3 (R Core Team, 2013). In the analysis, I first normalized all of the data with \log_{10} transformations. I fitted linear mixed-effect regression models (LMM) using the nlme package (Pinheiro et al.,

2009) in R to analyze the three scaling relationships, i.e., (1) foliage area and twig cross-sectional area, (2) leaf size and leafing intensity, and (3) leaf mass and leaf area. I chose to use LMM instead of a standardized major axes (SMA) analysis (Warton et al., 2006), which is traditionally used for scaling relationships, because I was interested in accounting for the hierarchical structure of the sampling design and because the use of one model to handle all the data at once is more convenient. In the modeling, the twig cross-sectional area (or leafing intensity, or leaf area) and elevation (4 levels) were considered the fixed factors, with random effects for sites and trees nested in each site; the former to take into account the among-site variation and the latter was considered because tree individuals within each site were more likely to resemble one another than tree individuals from other sites. Random effects and error terms were considered mutually independent random variables of normal distribution, zero mean, and common unknown variance (Goldstein, 2003). Following Olson et al. (2009), I used the interaction term of the models to test for similar foliage area–twig cross-sectional area, leaf size–leafing intensity, and leaf mass–leaf area scaling relationships across elevations. A significant interaction term between twig cross-sectional area (or leafing intensity, or leaf area) and elevation would indicate differing scaling slopes among elevations, i.e., not showing constant scaling relationship between traits and elevation. To determine whether scaling relationships were isometric ($b = 1$) or allometric ($b \neq 1$), I examined the slope coefficients of the numerical factor (twig cross-sectional area or leafing intensity) in the models, and their confidence intervals; if they included 1 or -1 the scaling relationship was isometric, otherwise it was allometric. Finally, I also fitted LMM to determine whether elevation had any effect on tree height, DBH, leaf size and LMA. When elevation was significant, I conducted post hoc multiple comparisons among elevations to discern which elevation was different. For this I used the multcomp package (Hothorn et al., 2008) in R.

RESULTS

Functional trait variation with elevation—Across four different treeline ecotone forests of *Nothofagus pumilio*, which covered 18 degrees of latitude in southern Chile, individual leaf area decreased significantly with elevation ($F_{3, 139} = 3.64$, $P = 0.014$); from

3.79 cm² at E1 to 2.94 cm² at E4, a decrease of 22.43%. The LMA, however, did not vary with elevation ($F_{3,139} = 0.20, P = 0.899$) since the difference was comparatively negligible among elevations; a maximum difference of 2.5 g m⁻² (2.34%) between E2 and E1 was found (Table 1). Leafing intensity, a measure of leaf density, significantly increased with elevation ($F_{3,139} = 4.46, P = 0.005$), from 25.93 to 32.11 leaves per dry-gram of nonleaf twig tissue from E1 to E4. Tree height ($F_{3,139} = 62.51, P < 0.001$) and DBH ($F_{3,139} = 23.84, P < 0.001$) decreased significantly with elevation, from trees with more than 15 m of height (maximum = 23 m) and more than 25 cm of DBH (maximum = 50.3) at the lowest elevations down to 3.5 m of height (minimum = 2.8) and 10.8 cm of DBH (minimum = 6.8) at the treeline elevation (Table 1, Fig. 2).

Scaling relationships across elevation—The twig cross-sectional area was strongly and positively correlated with the total leaf area displayed by the twig (Table 2, Fig. 3A). The twig cross-sectional area and elevation interaction term of the model were not significant ($P > 0.05$), which means that the scaling relationship was invariant in relation to elevation based on the scaling slope. The slope of this scaling relationship was isometric, with a value of 1.096 (95% CI = 0.784–1.408), not being different from 1. Thus, the higher the twig cross-sectional area, the higher the total leaf area displayed. This scaling was proportional or isometric and, most importantly, was not altered by changes in elevation (i.e., temperature) (Fig. 3A). As an aside, I also computed the relationship between twig mass (or nonleaf tissue mass) and the total leaf area displayed. The twig mass appeared to behave very similarly to the twig cross-sectional area, where the interaction term was not significant, and the slope of the relationship did not differ from 1 (Table 2).

I found a strong and significantly negative correlation between individual leaf area and the leafing intensity (i.e., the number of leaves for a given mass of nonleaf tissue) (Table 2, Fig. 3B). The slope of the scaling relationship proved to be significantly different from -1, with a value of -0.758 (95% CI: -0.998 to -0.518, Table 2), i.e., an allometric scaling relationship. The interaction term between leafing intensity and elevation was not significant ($P = 0.122$). Lastly, the scaling relationship between individual leaf area and leaf mass was significantly positive and isometric (slope = 0.929, 95% CI: 0.754–1.103) and remained constant across elevation (i.e., the interaction term was not significant, $P = 0.708$) (Table 2,

Fig. 3C). Larger leaves tended to also have higher mass, and therefore LMA remained constant with leaf area in this tree species. In this scaling relationship, elevation proved to be nonsignificant ($P = 0.275$).

DISCUSSION

In this study, the foliage–twig and the leaf mass–leaf area scaling relationships appear to be unresponsive to an environmental and ecological gradient, such as elevation, known to influence local community composition, abundance, and, definitively, average plant size and shape (Körner, 2007) (Fig. 2). Thus, the scaling relationship between the cross-sectional area of the twig and the foliage area borne on it remained positive and invariant across elevation for the *Nothofagus pumilio* tree species in four forest sites across 18 degrees of latitude, no matter where the tree was located along the elevational gradient and, regardless of whether the tree was a 3-m krummholz or an erect 20-m tree. This relationship is fully consistent with Corner's rules and with what Olson et al. (2009) found when comparing conspecifics growing in strikingly different environments (sheltered and salt-pruned trees) in Veracruz, Mexico. How can an invariant foliage–stem scaling relationship with elevation be explained in a place where leaf area and tree height decrease significantly with elevation? Regardless of leaf area variation with elevation, twigs may experience phenotypic accommodation (West-Eberhard, 2003), which relates to the plasticity of particular traits between contrasting environments, but with the maintenance of crucial relationships between them (Olson et al., 2009), i.e., a coordination of functional roles (Pigliucci, 2004). Thus, although leaf area and leafing intensity vary with elevation, the twig cross-sectional area also varies accordingly, maintaining a constant vascular supply to the foliage, where leaves provide proportional metabolic support to twig demands. The underlying mechanism explaining the foliage–stem-scaling invariance in regards to elevation should thus be a universal scaling metabolism between the leaves and the stem (West et al., 1999; Olson et al., 2009). Although individuals of *N. pumilio* differed greatly in tree height and individual leaf area, tight adjustments between foliage and stem tissues seem to result in a given amount of foliage area requiring a determined amount of stem conductive, mechanical, and storage tissue; a given amount of woody tissue also requires a corresponding amount of photosynthates provided by a certain amount of foliage area.

A similar invariant and positively significant isometric scaling with elevation was found for the leaf mass–leaf area relationship. Regardless of elevation, larger leaves had higher mass; therefore, LMA remains constant (in addition, LMA did not vary with elevation, Table 1). Thus, contrary to my initial expectation, larger leaves may not need higher specific support per unit of leaf area (e.g., more investment in petioles, midribs, or venation stiffness) than smaller leaves at the intraspecific level, as was previously assumed by some researchers making interspecific comparisons (e.g., Howland, 1962; Grubb, 1998; Niinemets and Kull, 1999). My results are in accordance with those of Milla and Reich (2007), who found empirical support

TABLE 1. Mean and standard error (± 1 SE) of individual leaf area, leaf mass per area (LMA), leafing intensity, tree height, and tree diameter at breast height (DBH, 1.35 m aboveground) of *Nothofagus pumilio* individuals along different elevations (E1: closed forest; E2: intermediate; E3: timberline; and E4: treeline) across four treeline sites in the southern Andes of Chile covering 18° of latitude (from central Chile to Tierra del Fuego). Data were pooled across sites. Different letters stand for significant differences ($P < 0.05$) in trait values among elevations.

| Elevation | Leaf area (cm ²) | LMA (g·m ⁻²) | Leafing intensity (no. leaves/g nonleaf tissue) | Height (m) | DBH (cm) |
|-----------|------------------------------|--------------------------|---|----------------|----------------|
| E1 | 3.79 a (0.27) | 104.11 (2.98) | 25.93 a (1.44) | 16.38 a (0.50) | 26.21 a (1.16) |
| E2 | 3.50 ab (0.23) | 106.61 (2.98) | 24.97 a (1.24) | 11.34 b (0.35) | 20.65 b (0.66) |
| E3 | 3.14 ab (0.21) | 104.42 (2.36) | 28.92 ab (1.45) | 6.99 c (0.26) | 15.19 c (0.36) |
| E4 | 2.94 b (0.18) | 104.38 (2.51) | 32.11 b (1.66) | 3.48 d (0.47) | 10.83 d (0.44) |
| Mean | 3.32 | 104.85 | 28.24 | 9.17 | 17.75 |
| Median | 3.03 | 106.39 | 26.77 | 7.75 | 16.20 |
| Range | 1.07–8.27 | 67.29–154.63 | 12.52–61.12 | 2.8–23 | 6.8–50.3 |
| SD | 1.41 | 16.91 | 9.73 | 2.12 | 4.04 |

TABLE 2. Estimated linear mixed model coefficients and statistics describing the relationships between total leaf area (mm^2) and twig cross-sectional area (mm^2), mean individual leaf area (mm^2) and leafing intensity (number of leaves given a certain nonfoliar structure mass, n g^{-1}), and between mean individual leaf size (mm^2) and leaf mass per area (LMA, g m^{-2}) for *Nothofagus pumilio* individuals along elevational gradients across four treeline sites in the southern Andes of Chile covering 18 degrees of latitude (from central Chile to Tierra del Fuego). Only fixed effects statistics are depicted.

| Model components | Coefficient | SE | 95% CI | P |
|---|-------------|-------|------------------|--------|
| Ln total leaf area vs. Ln twig cross-sectional area | | | | |
| y-Intercept | 0.179 | 0.142 | -0.098 to 0.456 | 0.209 |
| Ln twig area | 1.096 | 0.160 | 0.784 to 1.408 | <0.001 |
| Elevation | -0.077 | 0.029 | -0.139 to -0.015 | 0.021 |
| Ln twig area: elevation | -0.060 | 0.059 | -0.175 to 0.055 | 0.309 |
| Ln total leaf area vs. Ln twig mass | | | | |
| y-Intercept | 0.043 | 0.103 | -0.157 to 0.244 | 0.675 |
| Ln twig mass | 0.862 | 0.093 | 0.682 to 1.043 | <0.001 |
| Elevation | -0.023 | 0.019 | -0.065 to 0.019 | 0.255 |
| Ln twig area: elevation | -0.008 | 0.037 | -0.079 to 0.062 | 0.821 |
| Ln leaf size vs. Ln leafing intensity | | | | |
| y-Intercept | 0.121 | 0.149 | -0.169 to 0.411 | 0.416 |
| Ln leafing intensity | -0.758 | 0.124 | -0.998 to -0.518 | <0.001 |
| Elevation | -0.059 | 0.025 | -0.112 to -0.005 | 0.037 |
| Ln twig area: elevation | 0.064 | 0.041 | -0.016 to 0.144 | 0.122 |
| Ln leaf area vs. Ln leaf mass | | | | |
| y-Intercept | 0.032 | 0.074 | -0.112 to 0.176 | 0.668 |
| Ln leaf mass | 0.929 | 0.090 | 0.754 to 1.103 | <0.001 |
| Elevation | -0.018 | 0.015 | -0.050 to 0.015 | 0.275 |
| Ln twig area: elevation | -0.012 | 0.031 | -0.072 to 0.049 | 0.708 |

for a negative leaf size–SLA (i.e., positive leaf size–LMA) scaling relationship for most of the 157 species they studied. However, they did not work along a stressful elevational gradient. Pan et al. (2013) did work in an elevational gradient and similarly found a positive leaf mass–leaf area relationship when working with 121 vascular plants along an elevational gradient in a subtropical area of eastern China. They, however, found that elevation exerted a significant effect on the leaf mass–leaf area relationship; species growing at higher elevations had larger leaves allocating proportionately more biomass to support tissues than smaller leaves. It is known that

species, here I examined the less but still considerable variation observed within species across an elevational gradient.

Across contrasting elevations and at the intraspecific level, an evident trade-off was found between leaf area and the number of leaves attached to a unit of given dry-mass twig. In general, this trade-off is in accordance with Corner's rules (1949) and with what Kleiman and Aarssen (2007) proposed. Contrary to the previous two scaling relationships, I found that the leaf area–leafing intensity scaling was allometric ($b \neq 1$). This means that the leafing intensity downscaled disproportionately faster than leaf area, making this

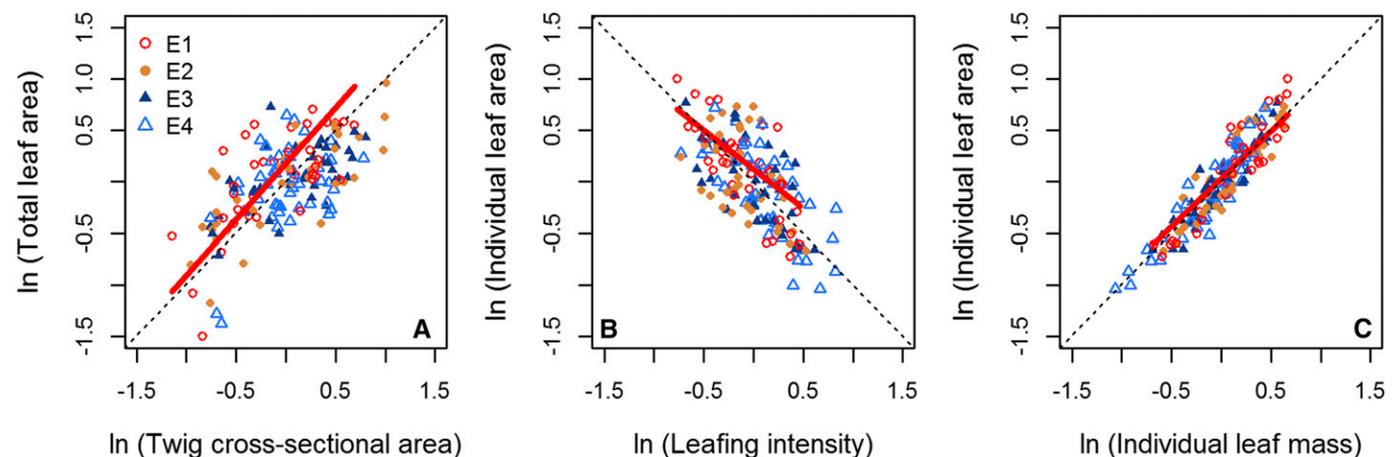


FIGURE 3 Scaling relationships between (A) twig cross-sectional area (mm^2) and total leaf area (mm^2), (B) leafing intensity (number of leaves given a certain nonfoliar structure mass, n g^{-1}) and mean individual leaf size (mm^2), and (C) individual leaf mass per area (g) and mean individual leaf size (mm^2) for *Nothofagus pumilio* individuals along elevational gradients across four treeline ecotones in the southern Andes of Chile covering 18° of latitude (from central Chile to Tierra del Fuego). Elevations included are treeline (E4, blue triangles and dot-dashed line), timberline (E3, solid triangles, dotted line), intermediate (E2, solid circles, dashed lines), and closed forest (E1, red circles, solid line). Values were log-transformed and centered around the mean.

among some species individual leaf area tends to decrease with increased environmental stress (Westoby et al., 2002), while leaf mass tends to increase, which leads to disproportionately LMA increases with leaf area increases (Pan et al., 2013). According to the current study, however, this interspecific trend is not occurring at the intraspecific level. A positive leaf mass–leaf area scaling relationship certainly implies higher leaf construction and maintenance costs that may constrain the maximum individual leaf area displayed, i.e., the “diminishing support” on mass investment with increasing leaf area, as coined by Niklas et al. (2007). The patterns observed here within species parallel those seen across species. Likewise, the plausible explanation of intraspecific patterns, as trait combinations favored by natural selection, are the same as those invoked to explain across species patterns. As a result, it is very likely that the global interspecific Corner's rules pattern emerges as the aggregate of largely parallel intraspecific patterns. Whereas most studies of Corner's rules examine the very wide variation in traits such as leaf size across

change relatively smoother for higher elevation trees (Fig. 3). For instance, if leafing intensity doubled at the treeline elevation (E4), the leaf area would decrease by 33.2% [leaf area = $e^{(-0.061)} \times \text{leafing intensity}^{(-0.582)}$], while the same increment at the lowest-elevation forest (E4) would render a decrease in leaf area of 41.2% [leaf area = $e^{(0.005)} \times \text{leafing intensity}^{(-0.766)}$]. In other words, cold temperatures at the treeline elevation not only make leaves smaller, but also force twigs to be disproportionately denser in terms of number of leaves per twig mass than at lower elevations, i.e., an allometric decline of leafing intensity with leaf area and with elevation occurs. Thus, individuals located at lower elevations have larger leaves that are more widely spaced than those with small leaves at the treeline elevation. This result is in accordance with what White (1983) found across deciduous species in eastern North America, without considering an environmental gradient per se. In addition, Hernández-Calderón et al. (2014), working in an elevational gradient of a 2000 m in Mexico and at the interspecific level, found that the Huber value (sapwood to leaf area ratio) increased significantly with elevation, which could imply that lower elevations have stems that are able to better supply hydraulically larger leaves than at high elevation. Although I was careful to collect distal, sun-exposed branches, it may be possible that short-stature trees at the treeline elevation, which have fewer layers of foliage, also have less between-branch competition for harvesting light than multilayered, tall trees at lower elevations. Treeline trees then would have less self-shading, which may be the reason for a high leafing intensity. According to Horn (1971), the amount of leaf area per unit of crown area increases with increasing plant size; thus, it is indeed more likely to find higher self-shading in the foliage of taller trees than in short-stature trees. Therefore, the fact that at the treeline elevation small leaves are found on twigs with a denser-than-expected number of leaves may be a matter of tree stature rather than a reaction to cold temperatures. An ontogenetic comparison of the leaf size–leafing intensity scaling under similar environmental conditions will help answer this query. Another more proximal explanation may be that a short growing season at the treeline elevation leads to limited time for a twig to enlarge its internodes, resulting in short twigs with a similar or even higher number of buds.

Although other empirical studies have also found a negative relationship between leaf area and leafing intensity (Ogawa, 2008; Yang et al., 2008; Milla, 2009), this study is the first to find an allometric, negative scaling in a deciduous tree species. Milla (2009), in a multiple-species assessment of the trade-off across elevation, found that deciduous species showed a very accurate isometric relationship, also found by Ogawa (2008). In Milla's study, evergreen species showed an allometric leaf area–number relationship. These contrasting results may be explained in terms of shoot longevity: Milla (2009) only collected 1-yr-old twigs, whereas I collected older twigs.

CONCLUSIONS

In the current study, a major environmental and ecological gradient such as elevation, with an accompanying temperature decrease, did not alter fundamental scaling relationships such as between foliage area and twig cross-sectional area and between leaf mass and leaf area. Olson et al. (2009) proposed that one way to explain the pervasiveness of foliage–stem covariation in nature, i.e., one of Corner's rules, is by universal scaling in plants. I have here adhered to this

proposition, and the evidence from these two scaling relationships proved to be isometric and invariant between contrasting elevations in one treeline species with contrasting statures across almost 20° of latitudinal distribution. However, elevation did alter the leaf area–leafing intensity scaling relationship, which may be explained by temperature-associated tree stature, rather than temperature alone. Lastly, it remains to be uncovered whether these foliage–stem and leaf area scaling relationships across environmental gradients constitute a valid connection with the leaf economics (Wright et al., 2004) and the wood economics spectra (Chave et al., 2009), and in particular with previous efforts studying the coordination of leaf and stem traits, e.g., LMA–wood density (Baraloto et al., 2010; Méndez-Alonzo et al., 2012; Li et al., 2015).

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