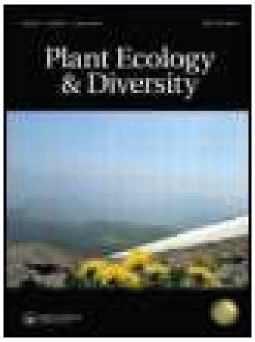

Plant Ecology & Diversity

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tped20

Minimum temperature drives community leaf trait variation in secondary montane forests along a 3000-m elevation gradient in the tropical Andes

Melissa Llerena-Zambrano, Jenny C. Ordoñez, Luis D. Llambí, Masha van der Sande, Esteban Pinto, Laura Salazar & Francisco Cuesta

To cite this article: Melissa Llerena-Zambrano, Jenny C. Ordoñez, Luis D. Llambí, Masha van der Sande, Esteban Pinto, Laura Salazar & Francisco Cuesta (2021): Minimum temperature drives community leaf trait variation in secondary montane forests along a 3000-m elevation gradient in the tropical Andes, Plant Ecology & Diversity, DOI: 10.1080/17550874.2021.1903604

To link to this article: https://doi.org/10.1080/17550874.2021.1903604

PLANT ECOLOGY & DIVERSITY
https://doi.org/10.1080/17550874.2021.1903604

Taylor & Francis
Taylor & Francis Group

ARTICLE

Minimum temperature drives community leaf trait variation in secondary montane forests along a 3000-m elevation gradient in the tropical Andes

Melissa Llerena-Zambrano[a], Jenny C. Ordoñez[b], Luis D. Llambí[c], Masha van der Sande[d], Esteban Pinto[e], Laura Salazar[f] and Francisco Cuesta[a]

[a]Grupo de Investigación en Biodiversidad, Medio Ambiente y Salud –BIOMAS- Universidad de Las Américas (UDLA), Quito, Ecuador; [b]Facultad de Ingeniería Agroindustrial, Universidad de Las Américas, Quito, Ecuador; [c]Instituto de Ciencias Ambientales y Ecológicas (ICAE), Universidad de Los Andes, Mérida, Venezuela; [d]Forest Ecology and Forest Management, Wageningen University, The Netherlands; [e]Columbus State University, University System of Georgia, Atlanta, USA; [f]Centro de Investigación de la Biodiversidad y Cambio Climático, (BioCamb) e Ingeniería en Biodiversidad y Recursos Genéticos, Facultad de Ciencias del Medio Ambiente, Universidad Tecnológica Indoamérica, Quito, Ecuador

ABSTRACT

Background: Leaf functional traits (LFT) influence resource acquisition and are important for understanding ecosystem processes. Climate and land use are key filters of community composition and LFT, however, how the relative importance of these filter changes with elevation has been little studied in the Andes.

Aims: To gain insight into the functional response of Andean forests to climate and disturbance in naturally regenerated forest stands.

Methods: We measured leaf blade thickness (LBT), leaf area (LA), specific leaf area (SLA), and leaf dry matter content (LDMC) from 13 secondary forest communities, along a 3000-m elevation transect. We derived basal area-weighted mean community (CWM) trait values to assess the effect of climate and disturbance on the functional structure of regenerating tree communities.

Results: Community LFT progressively shifted along the elevation gradient driven by changes in temperature and successional stages towards communities with thicker leaves with low SLA. Reduction in LDMC with elevation suggested that both succulence and sclerophylly were important strategies in these forests.

Conclusions: Our findings reinforce the validity of LFT as a powerful predictor to explore the ecological strategies of tree species in climate scenarios. Warmer conditions could result in a shift from slower to faster resource acquisition strategies at higher elevations.

ARTICLE HISTORY
Received 19 September 2020
Accepted 11 March 2021

KEYWORDS
Climate; disturbance; functional diversity; leaf morphology; mountains; trees; redundancy analysis; tropical Andes

Introduction

The seminal work of Tilman and co-workers (Tilman and Downing 1994; Tilman et al. 2006) on plant strategies and vegetation dynamics was key in promoting the study of the relationship between species diversity and ecosystem functioning (Wright et al. 2010; Lefcheck et al. 2015; Zhu et al. 2016). Analysing functional traits, in addition to taxonomic diversity is essential for understanding the relationship between biodiversity and ecosystem processes and dynamics (Cornelissen et al. 2003; Tilman et al. 2014; Poorter et al. 2017). Hence, the study of functional trait diversity has become a key dimension of community ecology, particularly in the analysis of the relationships between plant community structure and the maintenance of key ecosystem processes and services such the regulation of water, carbon and nutrient cycles (Kattge et al. 2011; Poorter et al. 2015). Studies on functional diversity relate plant functional traits to the distribution and abundance of species along environmental and spatial gradients, the variations in the structure of plant communities (i.e., patterns of species abundance) and how in turn these changes in functional community structure influence key ecosystem processes (Wright et al. 2004; Cornwell and Ackerly 2009). Moreover, plant functional types have been key for the development of mechanistic vegetation dynamic models e.g. Harper et al. (2018).

Plant functional traits include a wide range of morpho-physio-phenological characteristics of stem, leaves, roots, and reproductive structures that reflect plant ecological strategies and impact plant fitness (Reich 2014). Within this group of traits, leaf traits are the most accessible and cost-effective to measure for large numbers of species (Pérez-Harguindeguy et al. 2013). Some of the most studied leaf traits include leaf blade thickness (LBT),

CONTACT Francisco Cuesta ✉ francisco.cuesta@udla.edu.ec
Supplemental data for this article can be accessed here

leaf area (LA), specific leaf area (SLA), leaf dry matter content (LDMC) and leaf nutrient concentrations. SLA and LDMC are related to plant growth and litter decomposition (Bakker et al. 2011), carbon uptake (Van Der Sande et al. 2017), resource use strategies, i.e., acquisition vs. conservation (Diaz et al. 2004; Wright et al. 2004), and response to gradients of nutrient and water availability (Garnier et al. 2004; Ordoñez et al. 2009; Lohbeck et al. 2012). Species with a short leaf lifespan tend to have a large SLA (i.e., efficient deployment of leaf area per unit biomass invested), high nitrogen (N) concentration, and consequently, high rates of assimilation and respiration. Conversely, species with a long leaf lifespan show low SLA and slow metabolic rates (Llambi et al. 2003; Poorter and Bongers 2006). LBT and LA are primarily related with light capture and adaptation to dry or less fertile environments (Vaieretti et al. 2007). Further, trees growing on soils with low P and N availability tend to have thick leaves, with well-developed mesophyll and cuticles (Grubb 1977b, 1986).

Traits from individual species can also be used for studying community and ecosystem properties based on the biomass ratio hypothesis *sensu* Grime (1998) that suggests that primary productivity is largely determined by the traits of dominant plants and is therefore relatively insensitive to the abundance of locally rare species (Lavorel and Garnier 2002; Poorter et al. 2017). Hence, evaluating leaf trait variation of the dominant species can serve as a proxy to scale up the values of functional traits from individuals to communities by using community-weighted means (CWM) (Diaz et al. 2004; Ackerly and Cornwell 2007).

Tree community assemblages and ecosystem processes (e.g., productivity) in tropical Andean forests (Andean forest hereafter) are constrained by strong environmental gradients (Malizia et al. 2020). The decrease in temperature of ca. 6 K km^{-1} elevation, annual rainfall and air humidity (Körner 2003; Jarvis and Mulligan 2011), together with increasing solar and UV radiation at higher elevations (Cavelier 1996; Halladay et al. 2012; Fyllas et al. 2017) shape diversity and leaf characteristics in mountain forests. Trees at higher elevations are smaller in size and show smaller and thicker leaves than species at lower elevations (Kappelle and Leal 1996; Bruijnzeel et al. 2010; Van Der Sande et al. 2020). These adaptations reflect increases in LBT and reductions in SLA and LA as a response to higher UV radiation coupled with drier and/or colder conditions (Velázquez-Rosas et al. 2002; Van De Weg et al. 2009; Fyllas et al. 2017). Such LFT syndromes probably reflect more conservative, stress tolerant, strategies at higher elevations *sensu* Grime (1977). The response of LDMC along elevation gradients has not been extensively studied in the Andes. There are some mixed results considering tissue density, with some studies showing an increase and others a decrease in the ratio of leaf dry mass to fresh mass along environmental gradients (Van De Weg et al. 2009), so that LDMC patterns appear to depend on whether sclerophylly or succulence are the main strategies shaping community responses (Vendramini et al. 2002).

Plant functional traits are also related to disturbance that changes growth conditions. Tree species growing in younger secondary forests are often characterised by traits that favour rapid assimilation and tree growth (high SLA and low LDMC) whereas forest species of advanced successional stages tend to have traits related to resource conservation – high LDMC and low SLA – (Diaz et al. 2004; Bakker et al. 2011). Forests have a long legacy of anthropogenic disturbances in many regions of the Andes (Mathez-Stiefel et al. 2017). For example, on the western versant of the Ecuadorian Andes, forests have been subject to timber extraction and conversion to cattle pasture since the middle of the twentieth century (e.g., Pinto et al. 2018). Past and current anthropogenic disturbances, together with natural disturbances owing to the inherent instability of steep topographies, have created a highly heterogeneous mosaic of different successional stages throughout these forests (Sylvester et al. 2017).

Given the increasing pressures in recent decades on these ecosystems due to combined effects of climate change, land use and land use change, understanding the recovery dynamics of such secondary tropical montane ecosystems is of great importance, given their outstanding levels of biodiversity and contribution to global biogeochemical cycles (Aide et al. 2019; Fadrique et al. 2018). The use of functional traits could become a way to characterise how tree community composition and species abundance patterns in Andean forests are related to environmental gradients and how they deviate in response to human disturbance and thus can be used as indicators of ecosystem recovery (Chazdon et al. 2007, 2017; Lohbeck et al. 2012). Moreover, since the response in forest community structure and composition to environmental changes can be linked to changes in the morphological and physiological traits of trees, understanding how tree species change their functional traits along elevation gradients may be used as a space-for-time substitution to project potential global warming impacts on the dynamic changes of tree communities and their effect on ecosystem functions.

Elevation trends in leaf plant morphology are well documented in mountain forests worldwide (Cavelier and Goldstein 1989; Leal and Kappelle 1994; Cavelier 1996; Kappelle and Leal 1996; Ledo et al. 2009; Bruijnzeel et al. 2010). However, the relationship between environmental and disturbance variables and how these interactions shape tree species and the functional structure of forest communities in the Andes has been studied in few localities. Here we report the gradual changes in CWM leaf functional traits (CWM-LFT) in an Andean forest along a 3000-m elevation gradient, and the effect disturbance has on the expected changes over the leaf traits studied in a new forest transect located in the western equatorial Andes. We hypothesised that: (1) CWM-LBT and CWM-LDMC values would increase, while CWM-LA, and CWM-SLA would decrease along elevation and temperature gradients due to the effect of increasingly harsh climate conditions at higher elevations favouring conservative, stress tolerant, strategies; (2) Tree communities in an advanced successional stage would be characterised by lower CWM-SLA and CWM-LA values and higher CWM-LDMC and CWM-LBT values due to the differences in the resource-use strategies between mid-successional (acquisitive) and late-successional (conservative) species.

Materials and methods

Study site

All samples were collected from the ‘Pichincha long-term forest dynamics and carbon monitoring transect’, situated on the north-western slope of the Ecuadorian Andes (Figure 1; Teunissen van Manen et al. (2019)). The transect is composed of 17 permanent plots of 60 m x 60 m where environmental conditions vary gradually with increasing elevation (Pinto and Cuesta 2019). Mean annual air temperature diminishes from 21.6°C at 653 m a.s.l. to 7.2°C at 3507 m a.s.l., whereas minimum annual air temperature (minMAT hereafter), averaged from absolute monthly minimum temperatures, varies from 16.8°C at the lower end of the transect (653 m a.s.l), decreasing ca. 0.56°C per 100 m a.s.l., reaching values of 0.7°C at the upper end (3507 m a.s.l.; Table 1).

Forests in the study area are humid evergreen ecosystems with limited or no seasonality; thus,

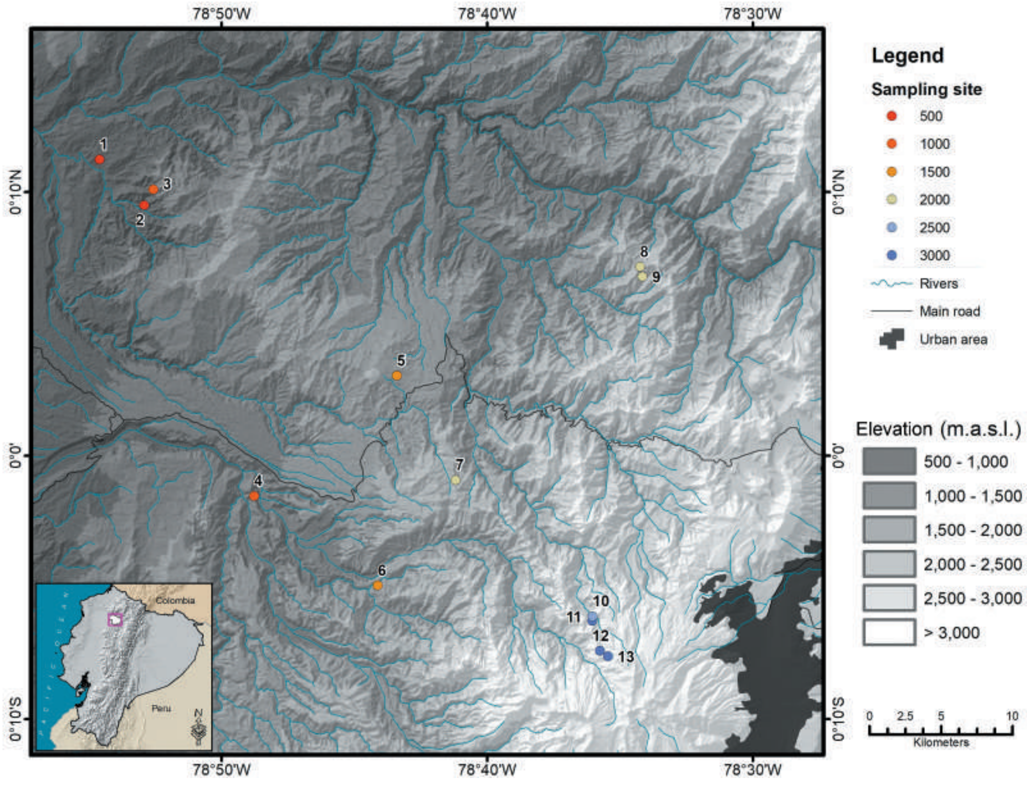

Figure 1. Location of the 13 permanent plots studied along an elevation gradient on the western slope of the equatorial Andes. Dots and numbers refer to sites in Table 1, ordered by elevation. Grey shading indicates 500-m elevation belts.

Table 1. Environmental variables and community features of 13 permanent plots along an elevation gradient on the western versant of the equatorial Andes (hereinafter in 13 locations along an elevation gradient): elevation, minimum annual temperature (minMAT), relative humidity (RH), annual precipitation (AP), net above-ground biomass productivity (n_{AGB}), wood density (WD), and above ground biomass (AGB). Mapi = Mashpisahungo/Pambiliño; Malo = Mashi Lodge; Mind = Sacha Urcu; Ribr = Río Bravo; Inti = Intillacta; Becl = Bellavista Cloud Forest; Cedr = El Cedral Ecolodge; Verd = Verdecocha; Yana = Yanacocha.

Plot number	Plot code	Elevation (m a.s.l.)	minMAT (°C)	RH (%)	AP (mm y^{-1})	n_{AGB} (Mg ha^{-1} y^{-1})	WD (g cm^{-3})	AGB (Mg ha^{-1})
1	Mapi_01	653	16.8	81.5	2075	3.3	0.523	188
2	Malo_01	827	16.2	66.0	2225	1.7	0.548	179
3	Malo_02	1018	16.4	71.6	2253	2.4	0.600	317
4	Mind_01	1277	12.2	67.1	2704	1.4	0.498	153
5	Ribr_01	1640	11.0	42.7	2347	0.8	0.561	110
6	Inti_02	1829	9.6	46.8	1939	1.2	0.518	128
7	Cedr_03	2212	7.3	56.7	1595	−2.3	0.528	123
8	Becl_01	2313	7.9	40.1	1471	3.3	0.581	120
9	Cedr_01	2492	7.9	25.2	1351	−0.5	0.533	114
10	Verd_02	2932	2.9	45.3	1251	0.0	0.544	82
11	Verd_03	3109	2.9	22.4	1251	−0.2	0.529	80
12	Verd_01	3421	3.7	15.8	1271	0.2	0.582	105
13	Yana_01	3507	0.7	31.1	1377	0.4	0.598	120

tree species do not lose their leaves throughout the year. We selected 13 out of the 17 permanent plots and considered each of them as representative of the tree community at the site. The selected communities were separated from each other by at least 150 m of elevation (Figure 1, Table 1) and corresponded to early (i.e., secondary) and late successional forests affected by human activities of burning and timber extraction in the 1980s and 1990s (i.e., secondary forests *sensu lato*, given that no complete forest clearance has occurred in these plots). As such, the successional stage of the stands was assumed to have affected their species composition and community leaf trait scores. To quantify it, we included disturbance proxies. Currently, these forests are located within private conservation reserves, which has allowed between 30 and 40 years of ongoing recovery, evidenced in the forest composition, structure, and canopy configuration (Teunissen van Manen et al., 2019).

Species selection and leaf trait measurements

Species selection was made based on the biomass ratio hypothesis (Grime 1998; Van Der Sande et al. 2017). For each plot, we measured leaf traits for species that contributed 32–76% (average 62% ± 15%) of the total basal area of each plot (Table 2). This selection criterion resulted in a total of 119 tree species being sampled (5–29 species per plot), excluding palms and tree ferns, due to technical constraints to accurately estimate leaf traits in the field for these growth forms. We acknowledge that, due to the species selection procedure, two out the 13 plots have a low percentage (<40%) of the total basal area of each sampled area (Malo_01 and Cedr_01) represented in the samples. It could be

Table 2. Community-weighted mean values and standard deviation (in brackets) of four-leaf functional traits from 13 permanent plots (communities) along an elevation gradient on the western slope of the equatorial Andes. Leaf blade thickness (LBT), leaf area (LA), leaf dry matter content (LDMC) and specific leaf area (SLA). For each community, the table includes total species richness; number of species collected; number of individual trees and leaves (ca. 10-15 per individual tree) collected and absolute and relative basal area sampled.

Plot code	Basal area per plot (m^2)	Species richness	Species collected	Basal area (%)	Trees (n)	Leaves (n)	LBT (mm)	LA (cm^2)	SLA (cm^2 g^{-1})	LDMC (mg g^{-1})
Mapi_01	9.69	74	32	54.2	58	1063	0.266 ± 0.07	243.531 ± 254.46	224.41 ± 65.16	273.92 ± 82.64
Malo_01	10.75	106	17	38.8	24	453	0.233 ± 0.06	230.568 ± 154.06	150.59 ± 64.82	307.60 ± 76. 93
Malo_02	13.82	103	17	42.0	25	618	0.216 ± 0.04	208.211 ± 101.63	170.93 ±52.44	334.79 ± 69.90
Mind_01	11.63	75	19	46.0	42	599	0.244 ± 0.07	123.857 ± 55.78	175.43 ± 44.75	329.23 ± 80.90
Ribr_01	9.54	96	18	66.2	35	515	0.303 ± 0.06	123.996 ± 121.90	131.71 ± 40.91	293.17 ± 77.52
Inti_02	13.82	79	30	46.7	31	581	0.24 ± 0.05	190.262 ± 110.50	210.43 ± 105.29	262.66 ± 101.09
Cedr_03	7.76	53	18	49.5	33	686	0.298 ± 0.09	93.602 ± 85.93	206.33 ± 86.47	216.82 ± 80.83
Becl_01	10.44	47	16	64.1	51	889	0.314 ± 0.09	150.084 ± 96.91	157.09 ± 57.65	302.35 ± 108.89
Cedr_01	11.14	66	12	32.2	38	569	0.373 ± 0.11	78.497 ± 112.73	126.57 ± 111.23	280.23 ± 79.01
Verd_02	7.54	57	7	52.1	21	296	0.404 ± 0.11	125.311 ± 113.49	142.20 ± 104.27	215.79 ± 73.75
Verd_03	8.59	41	8	55.9	27	375	0.429 ± 0.10	105.547 ± 91.05	143.34 ± 62.82	196.28 ± 90.72
Verd_01	10.56	39	11	49.0	22	307	0.389 ± 0.08	36.581 ± 99.54	105.640 ± 90.16	286.81 ± 74.29
Yana_01	8.30	22	6	75.5	20	264	0.403 ± 0.06	64.657 ± 71.02	156.17 ± 53.56	249.03 ± 58.13

possible that the species leaf trait values, averaged at the community level (i.e., the basal area-weighted mean), might differ from the values estimated in this research if a higher number of species were included for those two plots. To account for this potential bias, we made all analyses with and without the two plots with low basal area representation, to evaluate if the results were robust enough despite this limitation (Tables S3-S6).

We selected three to five individuals with ≥5 m in height and ≥10 cm DBH for each species. Following standardised protocols (Pérez-Harguindeguy et al. 2013) a sample of about 10–15 fully expanded, mature leaves (mostly sun leaves) were taken from each individual. As many as possible of the collected leaves were collected from trees with a sunlit position and leaves had the least amount of herbivory or other damage (fungal attack, necrosis, or diseases). Sample collection was carried out between April and September 2018, starting from the lower end of the transect. Samples were tagged, bagged, and placed inside plastic coolers. Each field trip lasted no more than 3–4 days to preserve the integrity of the collected leaves before laboratory processing. On the last day of each field trip, all samples were transported and cold-stored (4°C) until further analysis at the Universidad de las Americas, Quito, Ecuador.

Before processing, all samples were rehydrated until saturation in water for 24 to 48 h in the laboratory (Garnier et al. 2001). LA was measured on fresh leaves without petiole, placing them on a white background with a 6-cm-long reference line. They were then covered with a transparent glass of 100 cm x 70 cm and 4 mm thick to extend the leaves. A photograph of each leaf was taken with a Canon Eos rebel T3 digital camera, set on a support 1 m above the ground. Subsequently, we imported each photographic image into the Image J software (Image J version 1.50i, NIH, USA), and established a size reference measure. For this purpose, we followed these sequential steps: Analyze> Set scale> Known distance: 6, Length unit: cm, Global scale. Later, the images were processed using: Image> Type> 16 bits; Adjust> Threshold. Finally, we selected each leaf individually using the 'wand' tool and then estimated the area of each leaf using the Analyze> Measure commands. To determine LBT, we averaged three different measures taken at three different sections of each leave, avoiding veins as much as possible, with a high precision digital micrometre (0.001 mm, Starret-series 3732). Fresh weight of the water-saturated leaves and dry leaf weight (oven-dried at 70°C until reaching a constant weight) were obtained using a high precision analytical balance (± 0.002, OHAUS PA153). We estimated SLA as the fresh leaf area (cm^2) divided by its dry mass (g). Finally, we derived LDMC by dividing the dry leaf mass by the fresh leaf mass (mg/g) (Garnier et al. 2001).

Species mean trait values per site were estimated by averaging trait values obtained from all leaves collected per species. Between 30 and 75 leaves, obtained from 3 to 5 selected individuals following Pérez-Harguindeguy et al. (2013). We excluded the upper and lower 2.5% quantiles of the leaf data before calculating means (Table S7). Quantiles were used rather than the absolute maximum and minimums to reduce the influence of outliers potentially caused by errors in the laboratory or at digitising (Cuesta et al. 2020).

To assess differences in community-level leaf traits among the 13 plots, basal area-weighted mean (also known as the community-weighted mean, CWM) trait values were calculated for the 4 leaf traits, following Cornwell and Ackerly (2009).

$$\mathrm{CWM} = \frac{\sum_{i=1}^{s} aij * tij}{\sum_{i=i}^{s} aij} \tag{1}$$

Where S is the total number of species considered, *aij* corresponds to the abundance (i.e. basal area) of the *i*-th species on the plot *j*, and *tij* is the value of the trait in the *i*-th species in plot *j*. Species trait values used for the estimation of community weighted means were $\log_{10}$ transformed to satisfy normality assumptions (Sokal and Rohlf 2011).

Environmental and disturbance data

Mean minimum annual air temperature (minMAT) and Relative Humidity (RH hereafter) data were registered at each plot between 2016 and 2018, with HOBO Pro Temp/RH data loggers (U23-001; Onset, USA) at one-hour intervals and 1 m above ground level within the forest understory (Teunissen van Manen et al. 2019). Temperature loggers were placed below the standard meteorological convention of 2 m height to prevent the direct incidence of sunlight on the data logger (as loggers have no UV-shield) and damage from branch and tree falls which are frequent in successional Andean forests. For estimated annual precipitation (hereafter 'precipitation') we used information from the CHELSA dataset at 1 km^2

resolution (Karger et al. 2017). In total, the environmental gradients across the transect spanned 7.2– 21.6°C for mean air temperature and 1251–2771 mm for annual precipitation from the upper end to the lower end.

To assess the effect of past human disturbance on CWM leaf traits we used three proxies: (1) Net above ground biomass productivity (n_{AGB}) (Finegan et al. 2015); (2) community weighted mean wood density (WD) of each plot, derived from the individual wood density values of each tree (Slik et al. 2010), retrieved from an existing database (see below); and (3) the above-ground biomass (AGB) of each plot (Aide et al. 2000; Van Der Sande et al. 2017). Although tree WD is considered a functional trait, we used it as an indicator of human disturbance as WD is closely linked to forest successional stage. Trees that have softer wood are likely to represent early successional, light-demanding, fast-growing tree species, whereas trees that have denser wood are likely to come from a population of slow-growing, shade-tolerant, late-successional species. Thus, we assumed that the community weighted mean WD of coexisting tree species could be used as a proxy of the successional stage of the sampled forest stand (Slik et al. 2008). As elevation related changes in climate could also affect WD in a correlated fashion, we tested such a relationship and found no significant correlation ($r^2 = 0.09$; $P = 0.3$). Therefore, we kept this variable as a proxy of disturbance. To estimate WD (g cm^{-3}), we assigned the WD values to each species found in each plot using the *getWoodDensity* function from the BIOMASS package (Réjou-Méchain et al. 2017). For unidentified individuals, we used the average WD value of all other individuals in the plot.

Annual net above ground productivity (n_{AGB}) was derived from two censuses carried out in all plots during 2015 and 2017, in all trees with DBH ≥ 5 cm (Cuesta et al. unpublished). To estimate n_{AGB} for each plot, first we estimated the above-ground biomass (AGB) of each tree using the allometric equation model developed by Chave et al. (2014), defined as: $AGB = 0.0673 \times (WD \times DBH^2 \times H)^{0.976}$; where AGB (kg) is the estimated above-ground biomass, WD (g cm^{-3}) is the tree trunk wood density, DBH (cm) is the diameter of the tree at breast height (1.3 m), and H (m) is the estimated total height. Tree height (H) was estimated using a hypsometer. The AGB per hectare was then determined by converting kg to Mg, summing the values for all trees in a plot, and extrapolating to 1 ha. Subsequently, AGB net change (n_{AGB}; Mg ha^{-1} $year^{-1}$) was derived from the annualised difference between AGB productivity and tree mortality (Valencia et al. 2009). AGB productivity was obtained by estimating AGB change due to recruitment and growth between 2015 and 2017, following Brienen et al. (2015).

Data analyses

To explore the relationships between CWM traits vs. environmental and disturbance variables, we carried out a redundancy analysis (RDA) (Legendre and Legendre 2012) after excluding strongly correlated (Pearson correlation, $r < 0.6$) explanatory variables. The explanatory variables retained included (1) minimum temperature (minMAT), (2) annual precipitation, (3) CWM-WD and (4) AGB. Subsequently, the CWM traits were $\log_{10}$ transformed and all variables were normalised using a z-score to account for data that is not dimensionally homogeneous (Anderson et al. 2008). We then constructed a between-plots similarity matrix using Euclidean distance for the CWM traits, to be used in the RDA. The RDA analysis was carried out in Permanova in PRIMER 6 (Clarke and Gorley 2006).

To relate individual environmental factors to observed CWM traits, we used simple bivariate linear regressions taking as independent variables: elevation, minMAT, RH, and precipitation. Normality of the data was checked using a Shapiro–Wilk test (Sokal and Rohlf 2011). All analyses were carried out in JMP 8.0 (SAS 2008). Elevation was used in addition to minMAT to explore the possible effects of other environmental drivers that covary with it e.g., CO_2 and O_2 partial pressures, UV radiation incidence, among others (Körner 2007).

Lastly, we assessed the combined effect of multiple environmental and disturbance variables in explaining CWM trait patterns by a multiple regression analysis using standard least-squares for fitting a model with each CWM-LFT as the dependent variable (Sokal and Rohlf 2013). Given the high correlation between minMAT, elevation, RH, and precipitation we performed a Principal Component Analysis (PCA) to summarise environmental effects in uncorrelated explanatory axes. PCA1 explained 89.2% of the total variance of the temperature variables and had high loading from minMAT and

Elevation. PCA2 explained 6.3% of the total variance and had high loading of RH and precipitation (Table S1). Therefore, we only used the PCA1 and PCA2 as explanatory variables in the subsequent regression models to represent an overall climate gradient of temperature (PCA1) and precipitation (PCA2). Although there is potential loss of information, the use of PCAs instead of individual variables minimises multicollinearity and better captures the multidimensional environmental gradient of our study locations (Báez et al. 2015). Mean WD and AGB were included as response variables representing successional stage in the regression models. We excluded n_{AGC} because a high correlation (i.e., $r > 0.6$) with PCA1 and AGB. We selected the best model for each leaf functional by minimising the residual mean square and maximising r^2 (Table S2; Sokal and Rohlf 2013).

Results

The RDA showed a clear segregation of plots along elevation in terms of their functional leaf trait community assemblages (Figure 2). The first ordination axis accounted for 79.7% of the fitted variation in functional community similarity, segregating tree communities along a thermal and precipitation gradient (Table 3). Communities from low elevations (with thinner and broader leaves) were located on the right-hand side of the diagram, and communities from the upper section of the gradient on the left (trees with thicker and smaller leaves; Figure 2, Table 3). The second axis accounted for 18.9% of total variation and organised tree communities along a successional gradient not related to elevation (Table 3). Particularly for communities from low elevations the WD gradient clearly divided communities with high levels of disturbance (Inti_02, Mapi_01, Mind_01) characterised by trees with somewhat higher CWM-SLA and low CWM-LDMC from communities of mature forests at similar elevations (Malo_01 and Malo_02, Ribr_01, Figure 2, Table 2). For communities at high elevations the pattern linked with disturbance was less clear and differences in WD were less marked. A similar ordination resulted from the analysis when the two plots under-sampled in terms of basal area were excluded (Table S3; Figure S1).

The linear regressions showed a significant trend for three of the four traits along the elevation gradient (Figure 3). CWM-LBT showed a significant positive relationship with elevation ($r^2 = 0.84$; $P < 0.0001$) while CWM-LA ($r^2 = 0.77$; $P < 0.0001$) and CWM-SLA ($r^2 = 0.46$; $P < 0.01$) showed a negative relationship. Tree communities at the two lowest elevations had thinner mean ± SE (0.25 ± 0.02 mm) and larger (237.1 ± 9.2 cm^2) leaves than leaves from the three highest elevations where were nearly twice as thick (0.41 ± 0.02 mm) and much smaller (68.9 ± 34.7 cm^2). Leaves with the largest SLA also decreased monotonically from 224.4 cm^2 g^{-1}

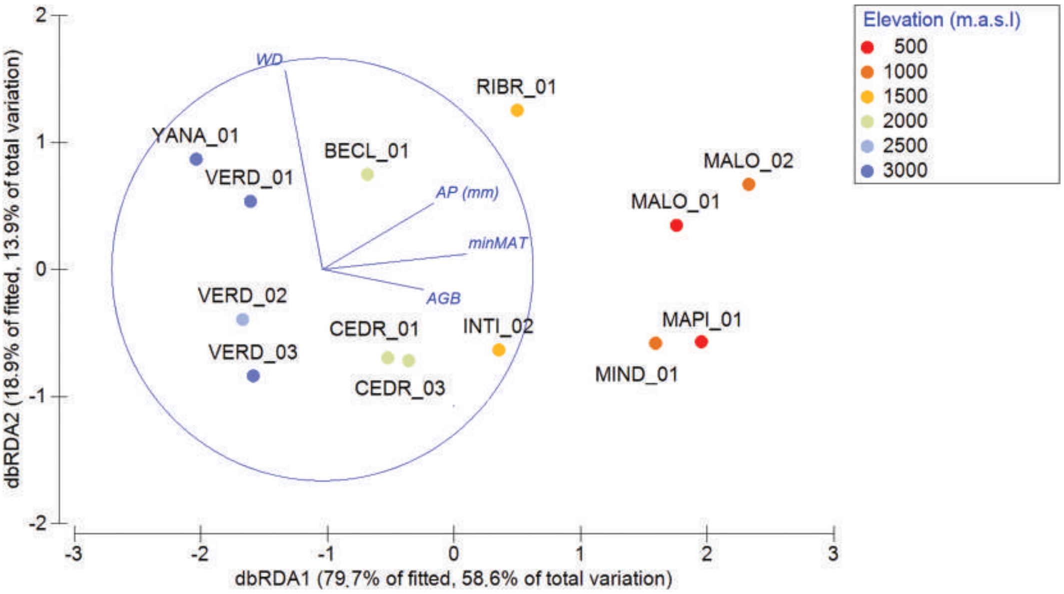

Figure 2. Distance-based redundancy analysis (dbRDA) for 13 locations (communities) along an elevation gradient on the western versant of the equatorial Andes, based on four community-weighted mean leaf functional traits (species data) vs. minimum air temperature (minMAT), annual precipitation (AP), above-ground biomass (AGB), and wood density (CWM-WD), as explanatory variables. Dots with identical colour combination indicate plots within the same elevation range.

Table 3. Correlation coefficients between environmental variables and axis scores from a distance-based Redundancy Analysis (dbRDA) based on four functional leaf traits recorded in 13 locations along an elevation gradient on the western slope of the equatorial Andes. The first axis accounted for the 79.7% of total variation, while the second axis accounted for 18.9% of total variation. Correlations ≥ 0.5 are represented in bold. Signs reflect arbitrary selection of gradient direction by Primer 6.0. for minimum air temperature (minMAT), annual precipitation (AP), community-weighted mean wood density (WD) and above-ground biomass (AGB).

Variable	RDA1	RDA2	RDA3	RDA4
minMAT (°C)	**0.680**	0.072	**0.727**	−0.058
AP (mm)	**0.525**	0.312	−0.471	**0.636**
WD (g cm^{-3})	−0.177	**0.942**	0.050	−0.279
AGB (Mg ha^{-1})	0.480	−0.096	−0.496	−0.717

at the lowest elevation to a mean value of 130.9 cm^2 g^{-1} ±35.7 at the highest elevation sites (Table 2). CWM-LDMC, although showed a tendency to decrease along the elevation gradient, this relation was not statistically significant (r^2 = 0.182; P = 0.081). The results were similar when the two outlier plots were excluded from the analyses (Table S4).

The results of the linear regressions between CWM traits and environmental variables suggested that minimum temperature was the single strongest environmental predictor among our variables of CWM trait changes along the elevation gradient (Figure 3). CWM-LBT had a significant negative relationship with minMAT (r^2 = 0.818; $P < 0.0001$), RH (r^2 = 0.689; $P < 0.001$) and precipitation (r^2 = 0.722; $P < 0.001$), whereas CWM-LA had a significant positive relationship with minMAT (r^2 = 0.671; $P < 0.001$), RH (r^2 = 0.656; $P < 0.001$) and precipitation (r^2 = 0.414; $P < 0.05$). CWM-SLA had a positive relationship with minMAT (r^2 = 0.322; $P < 0.05$) and RH (r^2 = 0.608; $P < 0.01$) but no significant relationship with precipitation. Lastly, CWM-LDMC had a significant positive relationship with precipitation (r^2 = 0.303; $P < 0.05$) and minMAT (r^2 = 0.294; $P < 0.05$), but not with RH (r2 = 0.035; P = 0.26) (Figure 3). The exclusion of the two plots with low basal area (Malo_01 and Cedr_01) produced similar relationships between the CWM leaf traits and the environmental variables (Table S5).

Multiple regression analyses showed that variation in the four CWM-LFT was related differently to the thermal/elevation gradient (PCA1) and past human disturbances (i.e., WD, AGB; Table 4). Changes along the elevation gradient in CWM-LBT and CWM-LA were only driven by climatic variables (PCA1, PCA2) and disturbance effects were not significant. On the contrary, the variation of CWM-SLA was related primarily to disturbance (Table 4). CWM-SLA decreased in communities with lower AGB and high WD (Table 4). Lastly, variation in CWM-LDMC was related to an interaction of a thermal/elevation gradient (PCA1) combined with community mean WD (Table 4). LDMC decreased with a reduction in air temperature along the elevation gradient and decreased in communities composed of trees with low WD (Table 2). The regression models that excluded the two outlier plots returned similar results (Table S6).

Discussion

We assessed how community-weighted mean (CWM) leaf traits change along elevation, and how these changes can be explained by environmental variables related to climate and disturbance. Our findings agreed with the elevation trend in leaf functional traits previously described in the Andes and other tropical locations (for references see Table 5) and to our understanding of the relationship between environmental and disturbance drivers in affecting tree species and in shaping the functional structure of and communities along elevation gradients. We first discuss how CWM traits varied along the elevation gradient and then evaluate what we think drives spatial variation in CWM leaf traits. Finally, we discuss the value of using CWM traits as a tool for improving our understanding on how tree communities in Andean forests respond to environmental gradients and human disturbances using CWM traits as indicators of ecosystem responses.

CWM trait changes along elevation

We expected that CWM-LBT and CWM-LDMC values would increase, while CWM-LA, and CWM-SLA would decrease along the elevation gradient due to increasingly colder temperatures favouring conservative, stress tolerant, strategies. Consistent with our first hypothesis, CWM-LFT changed as expected, reflecting plant adaptation to harsher conditions as elevation increases. CWM-LBT, CWM-LA and CWM-SLA were significantly correlated with elevation while CWM-LDMC was not. Leaf thickness increased and leaf area together with SLA decreased with elevation. These results agree with studies elsewhere that have reported a positive effect of resource availability on the abundance of species with acquisitive strategies (Poorter and Bongers 2006; Poorter et al. 2009; Conti and Díaz

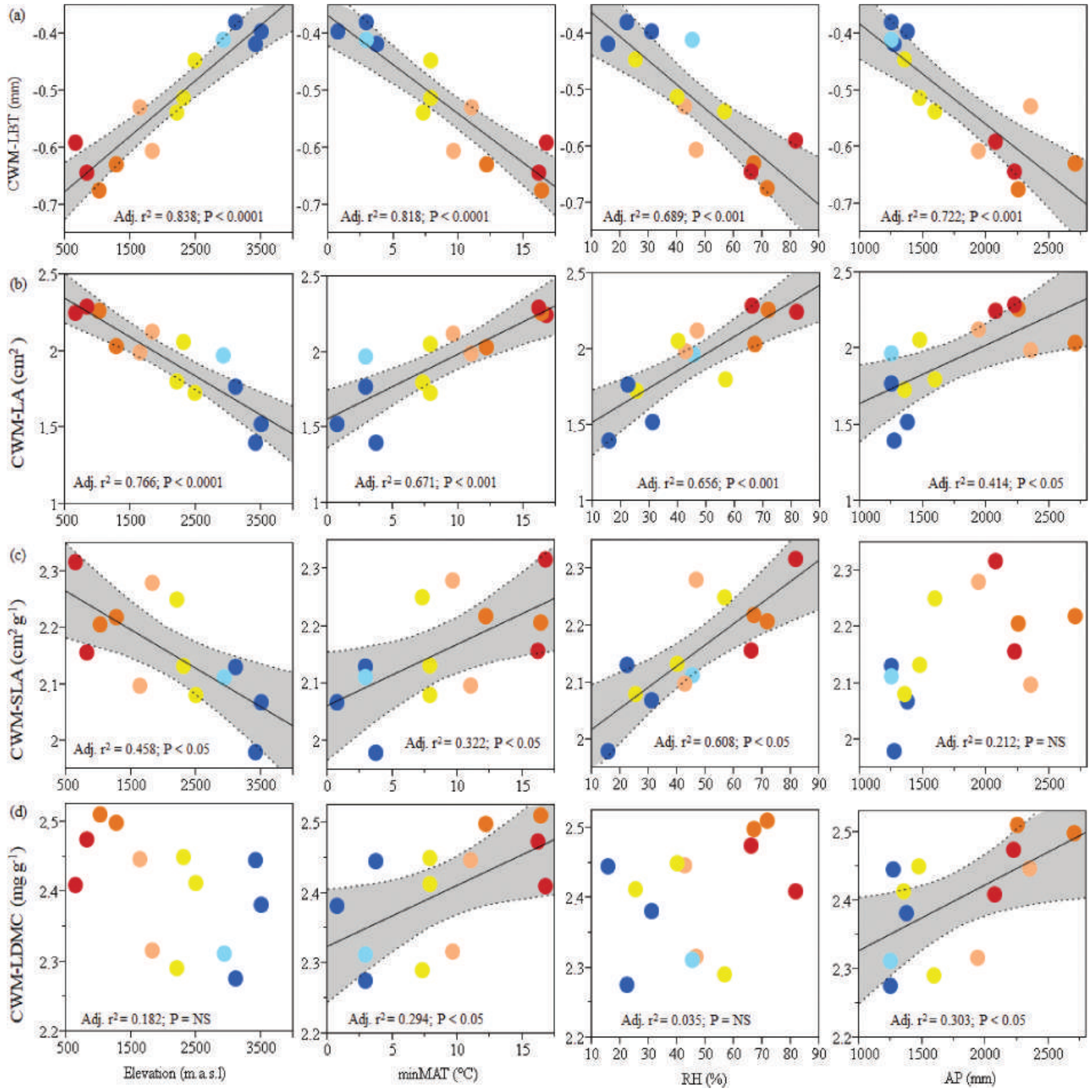

Figure 3. Bivariate linear regressions between four community-weighted mean leaf functional trait values vs. elevation and environmental variables: minMAT (°C), RH (%), and AP (mm) along an elevation gradient in Tropical Mountain Forest of the equatorial Andes: (a) leaf blade thickness (LBT, mm); (b) leaf area (LA, cm^2); (c) specific leaf area (SLA, cm^2 g^{-1}); and (d) leaf dry matter content (LDMC, mg g^{-1}). Model r^2 and *P* values are presented for each trait-elevation combination. Dots with identical colour combination indicate plots within the same elevation range.

2013; Finegan et al. 2015). As elevation increases, plant communities shift towards more conservative resource use strategies and are characterised by thick and small leaves with low SLA typical of tropical high mountain ecosystems (Llambi et al. 2003). In general, our observed leaf trait patterns agreed with those inferred from the comparison of numerous sites distributed over a range of elevations in different locations of tropical forests (Grubb 1977b; Tanner and Kapos 1982; Givnish 1984; Leal and Kappelle 1994; Tang and Ohsawa 1999; Velázquez-Rosas et al. 2002; Poorter and Bongers 2006; Poorter et al. 2008; Wright et al. 2010; Van Der Sande et al. 2017) as well as from studies along elevation transects (Enquist et al. 2017; Van Der Sande et al. 2020), and a recent global meta-analysis (Midolo et al. 2019; Table 5).

The increasing dominance of species with conservative resource-use strategies at higher elevation may be caused by the marked reduction in the average annual minimum air temperature from 16°C at 640 m to 0.7°C at 3503 m (Velázquez-Rosas et al. 2002; Moser et al. 2007; Van De Weg et al. 2009). Furthermore, the observed changes in leaf traits are probably a combined response conferring tolerance to reduce water loss due to high

Table 4. Best-fit models for Leaf blade thickness (LBT), leaf area (LA), leaf dry matter content (LDMC) and specific leaf area (SLA) vs. environmental and disturbance variables along an elevation gradient on the western slope of the equatorial Andes. The r^2adj coefficient is the proportion of the CWM leaf functional trait variance explained by the model, and the RMSE shows the sum of all regression errors. We selected the best model for each LFT based on the following criteria: minimise the residual mean square and maximise the multiple correlation values, r^2. Significant relations are represented with an asterisk (*).

	Estimate	Std Error	t ratio	Prob>|t|	Summary of fit
LBT					
Intercept	-0.521	0.010	-50.55	<0.0001*	
PCA1	0.051	0.006	9.02	<0.0001*	
					r^2 adj = 0.87 P < 0,0001* RSME = 0.037
LA					
Intercept	1.937	0.040	48.15	<0.0001*	
PCA1	-0.127	0.022	-5.73	0.00019*	
PCA2	0.116	0.083	1.384	0.196	
					r^2 adj = 0.732 P < 0.0006* RSME = 0.145
SLA					
Intercept	2.778	0.323	8.6	<0.0001*	
WD	-2.088	0.574	-3.640	0.005*	
AGB	0.0001	0.0003	3.141	0.0105*	
					r^2 adj = 0.58 P = 0.0055* RSME = 0.062
LDMC					
Intercept	1.679	0.266	6.31	0.0001*	
WD	1.315	0.484	2.72	0.024*	
PCA1	-0.029	0.008	-3.49	0.007*	
PCA2	-0.059	0.023	-1.97	0.080	
					r2 adj = 0.58 P = 0.013* RSME = 0.052

radiation coupled with low RH and low-temperature stress (Niinemets 2001; Vendramini et al. 2002), which may explain the marked increase in CWM-LBT and reduction in CWM-LA and CWM-SLA. This finding agrees with the general principle espoused by Parkhurst and Loucks (1972) that states that leaf morphology adjusts to maximise water use efficiency and to avoid excessive water loss for each unit of CO_2 acquired under increasingly harsher environmental conditions. A progressive reduction in CWM-SLA describes the decrease of leaf area relative to leaf biomass and may decrease water loss relative to carbon gain within a plant canopy (Van De Weg et al. 2009; Liu et al. 2013; Van Der Sande et al. 2017). Besides, it is possible that small and tough leaves have a longer lifespan, withstand strong wind, and suffer less damage from intense radiation.

We expected CWM-LDMC to increase along elevation as a higher LDMC is usually associated with more conservative strategies' characteristic of stress-tolerant species at high elevations. However, we found that CWM-LDMC slightly decreased along the thermal/elevation gradient. The observed pattern in CWM-LDMC differs from previous global analyses, which show that low SLA is accompanied by high LDMC in communities where sclerophylly dominates plant responses (Wilson and Lee 2000). In our transect, lower elevation plots, particularly those from earlier successional stages, were characterised by large leaves with high SLA and high LDMC, probably due to plant investments in support tissues (thick rachis) in species that maintain large leaves (Niinemets et al. 2006). As elevation increased and conditions become colder and drier, CWM-LDMC slightly decreased, in opposition to our expectations. Such patterns suggest high elevation forest communities in this transect presented anatomical and morphological adaptations that seemed to emulate succulence more than sclerophylly. It has been observed that for woody perennials in tropical montane forests an increase in leaf thickness with elevation is a function of increases in air and water volumes (as well as CO_2 assimilation) rather than increases in dry matter contents as a response to high radiation and drier conditions (Tanner and Kapos 1982; Cavelier 1996; Nadal et al. 2018). Stone (2018) has found a similar pattern, with increases in succulence across the elevation gradient associated with changes in the relationship in leaf resource investment on an area basis (i.e., smaller and thicker leaves with increased investment in air or water space). This finding argues in favour of a variety of leaf trait strategies

Table 5. Leaf functional trait values reported from neotropical secondary forests.

				LTB (mm)			LA (cm^2)			SLA (cm^2 g^{-1})			LDMC (mg g^{-1})		
Publication	Location	Vegetation	Elevation (m)	Mean±SD	Min.	Max.	Mean±SD	Min.	Max.	Mean±SD	Min.	Max.	Mean±SD	Min.	Max.
Species scale															
This study	Ecuador	Evergreen	640–3503	0.29 ± 0.10	0.1	0.644	153.2 ±144.3	8.3	1410.6	181 ± 77.8	62.7	496.1	268.1 ± 89.3	91.8	522.6
Velázquez-Rosas et al. (2002)	Mexico	Evergreen	1220	0.19											
			1830	0.24											
			2010	0.24											
			2260	0.24											
			2430	0.24											
			2560	0.32											
Williams-Linera (2020)	Mexico	Mesic* (Xalapa)	1250				47.7 ± 2.1	18.2	50.5	164 ± 4.2					
		Evergreen (Xalapa)	1250				47.2 ± 2.7	31.6	69.9	148.3 ± 5.1					
		Mesic (Banderilla)	1470				36.2 ± 2.0	11.7	73.7	177.8 ± 3.6					
		Evergreen (Banderilla)	1470				58.2 ± 2.7	36.5	70.1	126.8 ± 4.0					
Moser et al. (2007)	Ecuador	Evergreen	1050							87.9 ± 1.5					
			1540							67.8 ± 1.4					
			1890							58.5 ± 1.8					
			2340							52.7 ± 1.8					
			3060							61.1 ± 2.4					
Kapelle and Leal (1996)	Costa Rica	Evergreen	2950				55.5 ± 31.8	24.3	107.4						
Tanner (1982)	Jamaica	Evergreen	1500				31.6 ± 22.6	4.8	135.7	81.4 ± 3	36	160	0.2 ± 0.1	0.1	0.7
McWilliam (1993)	Brazil	Evergreen								90					
CWM Scale															
This study	Ecuador	Everghreen	640–3503	0.3 ± 0.1	0.2	0.4	136.5 ± 64.7	36.6	24.5	161.6 ± 35	105.6	224.4	0.3 ± 0.0	0.2	0.3
Finegan et al. (2015)	Bolivia	Mesic	250							12.63	9.84	17.1	407.58	299.0	489.8
	Brazil	Evergreen	50–100												
	Costa Rica	Mesic	235–345												
Conti and Díaz (2013)	Argentina	Mesic								103.2 ± 14.5			477.9 ± 25.5		

*Vegetation growing in conditions of medium water supply: *mesic plants.*

employed by tropical tree species to cope with harsh environmental conditions on high mountains.

CWM trait changes and the influence of human disturbance

We expected that spatial variation in CWM-LFT would partly be explained by past human land use (as indicated by AGB, CWM-WD). Specifically, early successional forests would be expected to be characterised by species with higher SLA, LA and lower LDMC. The RDA results and the multiple linear regression analyses supported our second hypothesis (i.e., the influence of past human disturbances in explaining spatial changes in CWM traits, particularly at low and mid elevation where disturbance effects were larger than in locations at the upper section of the transect). Changes in AGB and community mean WD (RDA axis 2) seemed to influence CWM traits. These two proxies of successional stage of the plots were important in explaining variations in CWM-LDMC and CWM-SLA. Results from the regression analyses indicated that younger successional forest communities (<40 years of recovery) with low WD and high AGB, were dominated by species with rapid resource acquisition strategies (i.e., high SLA, low LDMC). These early successional communities (e.g., the plots Mapi_01, Inti_02 and Mind_01, Table 2) were in fact, dominated by fast-growing acquisitive species such as *Aegiphila alba, Castilla elastica* and *Elaeagia utilis* (Table S7), with low WD and very high SLA, that drove the CWM-SLA upwards in comparison with older forests at similar elevations. The results of Slik et al. (2008) have shown the relevance of WD to assess disturbance levels across spatial and temporal scales as a mean to assess forest regeneration. Likewise, Lohbeck et al. (2013) have found that SLA and LBT were the only traits that consistently showed community-level changes during succession in both dry and evergreen forests in Mexico. The relationship between WD and SLA at the community level found in our study reinforces the role of both as key traits for assessing secondary succession along environmental gradients in Andean forests.

CWM traits a key tool for improving our knowledge on dynamics of Andean forests

Our results indicate that species functional traits scaled up to the community level could depend on (i) changes in climate drivers along elevation and (ii) disturbance (Lavorel and Garnier 2002; Chapin 2003). Overall, the response to past disturbance appears less significant than the decrease in minimum air temperature and precipitation with increasing elevation in these tropical Andean forests. Nevertheless, the successional gradient assessed in this study is not extensive (i.e., 20 years in time difference between plots since disturbance), and hence a stronger effect of the assessed disturbance proxies could not be expected. Future research should focus in expanding the disturbance gradient, including plots from early succession (<10-20 years) and late succession (>40 years). This would provide a larger successional gradient of similar importance to the wide elevation gradient covered by the current forest transect.

The strong effect of minimum temperature on CWM leaf traits suggests that ongoing climate change could strongly alter tree communities, resulting in species range shifts (Duque et al. 2015; Fadrique et al. 2018). This could be further accentuated by differential dispersal abilities of tropical mountain tree species along elevation gradients, although further studies are required to evaluate this (Holl et al. 2000; Chapman et al. 2016). Future research should assess changes in CWM traits through time via repeated censuses in these permanent plots which are part of the Andean Forest Network for long-term monitoring, see Malizia et al. (2020). This research line offers a unique opportunity to monitor temporal changes in functional composition and link the functional characteristics of forest communities with species diversity and ecosystem dynamics (Van Der Sande et al. 2016). As such, the link between functional traits at species and community scales with ecosystem dynamics hold great promise and could constitute a powerful quantitative tool to describe and predict future trends in tropical mountain ecosystems.

Acknowledgements

This study was carried out under permit No. N029-2019 IC-FLO-DNB/MA of the Ministry of Environment, Ecuador. We would like to express our gratitude to the owners and administrators of the private reserves where the forest dynamics permanent plots are established: Alejandro Solano, María Emilia Arcos and Oliver Torres from Mashpi Shungo and Pambiliño; Mateo Roldán and Carolina Proaño from Mashpi Lodge/Fundación Futuro, Sonia Saltos from Río Bravo, Juan Diego Ortíz and Sol Acosta from Sacha Urcu, Inti Arcos and Nina Duarte from Intyllacta, Richard Parson and Favio Fernández from Bellavista Cloud Forest, Germán Toasa from El Cedral Ecolodge, María Elisa Vizuete and Enrique Maldonado from Verdecocha and the Jocotoco foundation from Yanacocha Reserve. We thank the Universidad de las Americas for financially supporting this research (Grant No. FGE.FCC.19.15). F.C., M.LL-Z. and E.

P. received additional funding from the EcoAndes Project of CONDESAN and UNEP GEF (Grant No. 4750) and the Andean Forest Program of the Swiss Agency for Development and Cooperation (Grant No. 81028631). MvdS was supported by the Veni research programme with project number VI. Veni.192.027, of the Netherlands Organization for Scientific Research (NWO).

Disclosure statement

The authors declare that no competing interests exist.

Funding

This work was supported by the Swiss Agency for Development and Cooperation (SDC) [81028631]; Global Environment Fund [4750]; Universidad de Las Americas [FGE.FCC.19.15]; Swiss Agency for Development and Cooperation (SDC).

Notes on contributors

Mellisa Llerena-Zambrano is an environmental biologist specialised in the analysis of leaf functional traits in tropical mountain ecosystems.

Jenny Ordoñez is an interdisciplinary scientist working in understanding the relationship of plant diversity in natural and agricultural systems to ecosystem services provision.

Luis D. Llambí is a Professor and specialises in plant community dynamics and plant–plant interactions in tropical mountain ecosystems.

Masha van der Sande is a scientist interested in understanding the drivers of resilience of tropical forests to droughts, fires, and human disturbances, from local to continental spatial scales.

*Esteban Pinto*is an ecologist interested in plant and carbon dynamics and the drivers that influence different patterns in mountainous ecosystems in the tropical Andes.

Laura Salazar is a plant ecologist interested in the patterns of diversity and distribution with a special focus in tropical plants along environmental gradients.

Francisco Cuesta is a scientist interested in study plant community dynamics and ecosystem function in tropical ecosystems.

ORCID

Jenny C. Ordoñez http://orcid.org/0000-0003-0558-7229
Luis D. Llambí http://orcid.org/0000-0002-2031-810X
Masha van der Sande http://orcid.org/0000-0002-6845-2308
Esteban Pinto http://orcid.org/0000-0002-3844-1181
Laura Salazar http://orcid.org/0000-0001-7179-5822
Francisco Cuesta http://orcid.org/0000-0002-5150-073X

Data availability statement

Species trait data are available from the Dryad Digital Repository: Species trait data are available upon request to francisco.cuesta@udla.edu.ec.

Authors' contributions

F.C. funding acquisition, conceived the idea and designed the methodology; M.LL-Z., E.P. and F.C. collected the trait and environmental data; M.LL-Z., F.C., J.O. and L.D.LL. analyzed the data; F.C., M.LL-Z. and J.O. led the writing of the manuscript. M.v.S. and L.S. reviewed & edited the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

References

Ackerly DD, Cornwell WK. 2007. A trait-based approach to community assembly: partitioning of species trait values into within- and among-community components. Ecol Lett. 10 (2):135–145. doi:10.1111/j.1461-0248.2006.01006.x.

Aide TM, Grau HR, Graesser J, Andrade-Nuñez MJ, Aráoz E, Barros AP, Campos-Cerqueira M, Chacon-Moreno E, Cuesta F, Espinoza R. 2019. Woody vegetation dynamics in the tropical and subtropical Andes from 2001 to 2014: satellite image interpretation and expert validation. Glob Chang Biol. 25(6):2112–2126. doi:10.1111/gcb.14618.

Aide TM, Zimmerman JK, Pascarella JB, Rivera L, Marcano-Vega H. 2000. Forest regeneration in a chronosequence of Tropical abandoned pastures: implications for restoration ecology. Restoration Ecology. 8(4):328–338. doi:10.1046/j.1526-100x.2000.80048.x.

Anderson MJ, Gorley RN, C K. 2008. PERMANOVA+ for PRIMER: guide to software and statistical methods. UK: PRIMER-E Ltd Plymouth.

Báez S, Malizia A, Carilla J, Blundo C, Aguilar M, Aguirre N, Aquirre Z, Álvarez E, Cuesta F, Duque Á, et al. 2015. Large-scale patterns of turnover and basal area change in Andean forests. PLoS ONE. 10(5):e0126594. doi:10.1371/journal.pone.0126594.

Bakker MA, Carreño-Rocabado G, Poorter L. 2011. Leaf economics traits predict litter decomposition of tropical plants and differ among land use types. Funct Ecol. 25 (3):473–483. doi:10.1111/j.1365-2435.2010.01802.x.

Brienen RJ, Phillips OL, Feldpausch TR, Gloor E, Baker TR, Lloyd J, Lopez-Gonzalez G, Monteagudo-Mendoza A, Malhi Y, Lewis SL. 2015. Long-term decline of the Amazon carbon sink. Nature. 519(7543):344–348. doi:10.1038/nature14283.

Bruijnzeel L, Kappelle M, Mulligan M, Scatena F. 2010. 72. Tropical montane cloud forests: state of knowledge and sustainability perspectives in a changing world. In: Bruijnzeel LA, Scatena FN, Hamilton LS, editors. p. 691–740. Cambridge University Press.

Cavelier J. 1996. Environmental factors and ecophysiological processes along altitudinal gradients in Wet Tropical Mountains. In: Mulkey SS, Chazdon RL, Smith AP, editors. Tropical Forest Plant Ecophysiology. Boston (MA): Springer US; p. 399–439.

Cavelier J, Goldstein G. 1989. Leaf anatomy and water relations in tropical elfin cloud forest tree species. In: Structural and functional responses to environmental stresses. The Hague (The Netherlands): Academic Publishing; p. 243–253.

Chapin FS III. 2003. Effects of plant traits on ecosystem and regional processes: a conceptual framework for predicting the consequences of global change. Ann Bot. 91 (4):455–463. doi:10.1093/aob/mcg041.

Chapman H, Cordeiro NJ, Dutton P, Wenny D, Kitamura S, Kaplin B, Melo FP, Lawes MJ. 2016. Seed-dispersal ecology of tropical montane forests. Journal of Tropical Ecology. 32 (5):437–454. doi:10.1017/S0266467416000389.

Chave J, Réjou-Méchain M, Búrquez A, Chidumayo E, Colgan MS, Delitti WB, Duque A, Eid T, Fearnside PM, Goodman RC. 2014. Improved allometric models to estimate the aboveground biomass of tropical trees. Glob Chang Biol. 8(10):3177–3190. doi:10.1111/gcb.12629.

Chazdon R, Brancalion P, Lamb D, Laestadius L, Calmon M, Kumar C. 2017. A policy-driven knowledge agenda for global forest and landscape restoration. Conservation Letters. 10(1):125–132. doi:10.1111/conl.12220.

Chazdon RL, Letcher SG, Van Breugel M, Martínez-Ramos M, Bongers F, Finegan B. 2007. Rates of change in tree communities of secondary Neotropical forests following major disturbances. Philosophical Transactions of the Royal Society B: Biological Sciences. 362(1478):273–289. doi:10.1098/rstb.2006.1990.

Clarke K, Gorley R. 2006. PRIMER v6 user manual and program. Plymouth: PRIMER-E Ltd.

Conti G, Díaz S. 2013. Plant functional diversity and carbon storage–an empirical test in semi-arid forest ecosystems. Journal of Ecology. 101(1):18–28. doi:10.1111/1365-2745.12012.

Cornelissen JHC, Lavorel S, Garnier E, Az S, Buchmann N, Gurvich DE, Reich PB, Ter Steege H, Morgan HD, Heijden MGAVD, et al. 2003. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. Australian Journal of Botany. 51 (4):335–380. doi:10.1071/BT02124.

Cornwell WK, Ackerly DD. 2009. Community assembly and shifts in plant trait distributions across an environmental gradient in coastal California. Ecol Monogr. 79 (1):109–126. doi:10.1890/07-1134.1.

Cuesta F, Tovar C, Llambí LD, Gosling WD, Halloy S, Carilla J, Muriel P, Meneses RI, Beck S, Ulloa Ulloa C. 2020. Thermal niche traits of high alpine plant species and communities across the tropical Andes and their vulnerability to global warming. J Biogeogr. 47 (2):408–420. doi:10.1111/jbi.13759.

Diaz S, Hodgson J, Thompson K, Cabido M, Cornelissen JH, Jalili A, Montserrat-Marti G, Grime J, Zarrinkamar F, Asri Y. 2004. The plant traits that drive ecosystems: evidence from three continents. Journal of Vegetation Science. 15(3):295–304. doi:10.1658/1100-9233(2004)015[0295:TPTTDE]2.0.CO;2.

Duque A, Stevenson PR, Feeley KJ. 2015. Thermophilization of adult and juvenile tree communities in the northern tropical Andes. Proceedings of the National Academy of Sciences. 112(34):10744–10749. doi:10.1073/pnas.1506570112.

Enquist, B. J., L. P. Bentley, A. Shenkin, B. Maitner, V. Savage, S. Michaletz, B. Blonder, V. Buzzard, T. E. B. Espinoza, and W. Farfan-Rios. 2017. Assessing trait-based scaling theory in tropical forests spanning a broad temperature gradient. Global Ecology and Biogeography 26:1357–1373.

Fadrique B, Báez S, Duque Á, Malizia A, Blundo C, Carilla J, Osinaga-Acosta O, Malizia L, Silman M, Farfán-Ríos W, et al. 2018. Widespread but heterogeneous responses of Andean forests to climate change. Nature. 564 (7735):207–212. doi:10.1038/s41586-018-0715-9.

Finegan B, Peña-Claros M, De Oliveira A, Ascarrunz N, Bret-Harte MS, Carreño-Rocabado G, Casanoves F, Díaz S, Eguiguren Velepucha P, Fernandez F, et al. 2015. Does functional trait diversity predict above-ground biomass and productivity of tropical forests? Testing three alternative hypotheses. Journal of Ecology. 103(1):191–201. doi:10.1111/1365-2745.12346.

Fyllas NM, Bentley LP, Shenkin A, Asner GP, Atkin OK, Díaz S, Enquist BJ, Farfan-Rios W, Gloor E, Guerrieri R. 2017. Solar radiation and functional traits explain the decline of forest primary productivity along a tropical elevation gradient. Ecol Lett. 15(6):730–740. doi:10.1111/ele.12771.

Garnier E, Cortez J, Billès G, Navas M-L, Roumet C, Debussche M, Laurent G, Blanchard A, Aubry D, Bellmann A. 2004. Plant functional markers capture ecosystem properties during secondary succession. Ecology. 85(9):2630–2637. doi:10.1890/03-0799.

Garnier E, Laurent G, Bellmann A, Debain S, Berthelier P, Ducout B, Roumet C, Navas ML. 2001. Consistency of species ranking based on functional leaf traits. New Phytologist. 152(1):69–83. doi:10.1046/j.0028-646x.2001.00239.x.

Givnish TJ. 1984. Leaf and canopy adaptations in Tropical Forests. In: Medina E, Mooney HA, Vázquez-Yánes C, editors. *Physiological ecology of plants of the wet tropics: proceedings of an international symposium held in Oxatepec and Los* Tuxtlas, *Mexico, June 29 to July 6, 1983*. Dordrecht: Springer Netherlands; p. 51–84.

Grime J. 1998. Benefits of plant diversity to ecosystems: immediate, filter and founder effects. Journal of Ecology. 86(6):902–910. doi:10.1046/j.1365-2745.1998.00306.x.

Grime JP. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. Am Nat. 111(982):1169–1194. doi:10.1086/283244.

Grubb P. 1986. Sclerophylls, pachyphylls, and pycnophylls: the nature and significance of hard leaf surface. Insects and the Plant Surface. Eds BE Juniper, TRE Southwood. Edward Arnold: London. pp 137–150.

Grubb PJ. 1977b. Control of forest growth and distribution on wet Tropical Mountains: with special reference to mineral nutrition. Annu Rev Ecol Syst. 8(1):83–107. doi:10.1146/annurev.es.08.110177.000503.

Halladay K, Malhi Y, New M. 2012. Cloud frequency climatology at the Andes/Amazon transition: 1. Seasonal and diurnal cycles. Journal of Geophysical Research. 117, D23102. doi:110.1029/2012JD017770.

Harper AB, Wiltshire AJ, Cox PM, Friedlingstein P, Jones CD, Mercado LM, Sitch S, Williams K, Duran-Rojas C. 2018. Vegetation distribution and terrestrial carbon cycle in a carbon cycle configuration of JULES4. 6 with new plant functional types. Geoscientific Model Development. 11 (7):2857–2873. doi:10.5194/gmd-11-2857-2018.

Holl KD, Loik ME, Lin EH, Samuels IA. 2000. Tropical montane forest restoration in Costa Rica: overcoming barriers to dispersal and establishment. Restoration Ecology. 8(4):339–349. doi:10.1046/j.1526-100x.2000.80049.x.

Jarvis A, Mulligan M. 2011. The climate of cloud forests. Hydrol Process. 25(3):327–343. doi:10.1002/hyp.7847.

Kappelle M, Leal ME. 1996. Changes in leaf morphology and foliar nutrient status along a successional gradient in a Costa Rican upper Montane Quercus Forest. Biotropica. 28(3):331–344. doi:10.2307/2389197.

Karger DN, Conrad O, Böhner J, Kawohl T, Kreft H, Soria-Auza RW, Zimmermann NE, Linder HP, Kessler M. 2017. Climatologies at high resolution for the earth's land surface areas. Scientific Data. 4(1):170122. doi:10.1038/sdata.2017.122.

Kattge J, Diaz S, Lavorel S, Prentice IC, Leadley P, Bönisch G, Garnier E, Westoby M, Reich PB, Wright IJ. 2011. TRY–a global database of plant traits. Glob Chang Biol. 17:2905–2935.

Körner C. 2003. Alpine plant life: functional plant ecology of high mountain ecosystems; with 47 tables. 2nd edn. ed. Berlin: Springer Science & Business Media.

Körner C. 2007. The use of 'altitude' in ecological research. Trends Ecol Evol. 22(11):569–574. doi:10.1016/j.tree.2007.09.006.

Lavorel S, Garnier E. 2002. Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. Funct Ecol. 16 (5):545–556. doi:10.1046/j.1365-2435.2002.00664.x.

Leal ME, Kappelle M. 1994. Leaf anatomy of a secondary montane Quercus forest in Costa Rica. Revista de Biología Tropical. 3:473–478.

Ledo A, Montes F, Condes S. 2009. Species dynamics in a montane cloud forest: identifying factors involved in changes in tree diversity and functional characteristics. Forest Ecology and Management. 258:S75–S84. doi:10.1016/j.foreco.2009.07.055.

Lefcheck JS, Byrnes JEK, Isbell F, Gamfeldt L, Griffin JN, Eisenhauer N, Hensel MJS, Hector A, Cardinale BJ, Duffy JE. 2015. Biodiversity enhances ecosystem multifunctionality across trophic levels and habitats. Nat Commun. 6(1):6936. doi:10.1038/ncomms7936.

Legendre P, Legendre LF. 2012. Numerical ecology. Elsevier.

Liu C, Wang X, Wu X, Dai S, He J-S, Yin W. 2013. Relative effects of phylogeny, biological characters and environments on leaf traits in shrub biomes across central Inner Mongolia, China. Journal of Plant Ecology. 6:220–231.

Llambi L, Fontaine M, Rada F, Saugier B, Sarmiento L. 2003. Ecophysiology of dominant plant species during old-field succession in a high tropical Andean ecosystem. Arctic, Antarctic, and Alpine Research. 35(4):447–453. doi:10.1657/1523-0430(2003)035[0447:EODPSD]2.0.CO;2.

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(6):1211–1216. doi:10.1890/12-1850.1.

Lohbeck M, Poorter L, Paz H, Pla L, Van Breugel M, Martínez-Ramos M, Bongers F. 2012. Functional diversity changes during tropical forest succession. Perspectives in Plant Ecology, Evolution and Systematics. 14(2):89–96. doi:10.1016/j.ppees.2011.10.002.

Malizia A, Blundo C, Carilla J, Osinaga Acosta O, Cuesta F, Duque A, Aguirre N, Aguirre Z, Ataroff M, Baez S. 2020. Elevation and latitude drives structure and tree species composition in Andean forests: results from a large-scale plot network. PloS One. 15(4):e0231553. doi:10.1371/journal.pone.0231553.

Mathez-Stiefel S-L, Peralvo M, Báez S, Rist S, Buytaert W, Cuesta F, Fadrique B, Feeley KJ, Groth AAP, Homeier J, et al. 2017. Research priorities for the conservation and sustainable governance of Andean forest landscapes. Mt Res Dev. 37(3):323–339. doi:10.1659/MRD-JOURNAL-D-16-00093.1.

Midolo G, De Frenne P, Hölzel N, Wellstein C. 2019. Global patterns of intraspecific leaf trait responses to elevation. Glob Chang Biol. 25(7):2485–2498. doi:10.1111/gcb.14646.

Moser G, Hertel D, Leuschner C. 2007. Altitudinal change in LAI and stand leaf biomass in Tropical Montane Forests: a transect study in Ecuador and a pan-Tropical meta-analysis. Ecosystems. 10(6):924–935. doi:10.1007/s10021-007-9063-6.

Nadal M, Flexas J, Gulías J, Penuelas J. 2018. Possible link between photosynthesis and leaf modulus of elasticity among vascular plants: a new player in leaf traits relationships? Ecol Lett. 21(9):1372–1379. doi:10.1111/ele.13103.

Niinemets Ü. 2001. Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. Ecology. 82(2):453–469. doi:10.1890/0012-9658(2001)082[0453:GSCCOL]2.0.CO;2.

Niinemets Ü, Portsmuth A, Tobias M. 2006. Leaf size modifies support biomass distribution among stems, petioles and mid-ribs in temperate plants. New Phytologist. 171 (1):91–104. doi:10.1111/j.1469-8137.2006.01741.x.

Ordoñez JC, Van Bodegom PM, Witte JPM, Wright IJ, Reich PB, Aerts R. 2009. A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. Global Ecology and Biogeography. 18 (2):137–149. doi:10.1111/j.1466-8238.2008.00441.x.

Parkhurst DF, Loucks O. 1972. Optimal leaf size in relation to environment. J Ecol. 60(2):505–537. doi:10.2307/2258359.

Pérez-Harguindeguy N, Díaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-Harte M, Cornwell W, Craine J, Gurvich D. 2013. New handbook for standardised measurement of plant functional traits worldwide. Australian Journal of Botany. 61:167–234.

Pinto E, Cuesta F. 2019. Monitoreo de Biodiversidad, Contenidos de Carbono, Productividad y Rasgos Funcionales en Bosques Montanos. Ecuador: CONDESAN, Quito.

Pinto E, Pérez AJ, Ulloa-Ulloa C, Cuesta F. 2018. Árboles representativos de los bosques montanos del noroccidente de Pichincha, Ecuador. Ecuador: CONDESAN, Quito.

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 (3):565–588. doi:10.1111/j.1469-8137.2009.02830.x.

Poorter L, Bongers F. 2006. Leaf traits are good predictors of plant performance across 53 rain forest species. Ecology. 87(7):1733–1743. doi:10.1890/0012-9658(2006)87[1733:LTAGPO]2.0.CO;2.

Poorter L, Van Der Sande MT, Arets EJ, Ascarrunz N, Enquist BJ, Finegan B, Licona JC, Martínez-Ramos M, Mazzei L, Meave JA. 2017. Biodiversity and climate determine the functioning of Neotropical forests. Global Ecology and Biogeography. 26(12):1423–1434. doi:10.1111/geb.12668.

Poorter L, Van Der Sande MT, Thompson J, Arets EJMM, Alarcón A, Álvarez-Sánchez J, Ascarrunz N, Balvanera P, Barajas-Guzmán G, Boit A, et al. 2015. Diversity enhances carbon storage in tropical forests. Global Ecology and Biogeography. 24(11):1314–1328. doi:10.1111/geb.12364.

Poorter L, Wright SJ, Paz H, Ackerly DD, Condit R, Ibarra-Manríquez G, Harms KE, Licona J, Martinez-Ramos M, Mazer S. 2008. Are functional traits good predictors of demographic rates? Evidence from five neotropical forests. Ecology. 89(7):1908–1920. doi:10.1890/07-0207.1.

Reich PB, Cornelissen H. 2014. The world-wide 'fast–slow'-plant economics spectrum: a traits manifesto. Journal of Ecology. 102(2):275–301. doi:10.1111/1365-2745.12211.

Réjou-Méchain M, Tanguy A, Piponiot C, Chave J, Hérault B, Goslee S. 2017. biomass: an r package for estimating above-ground biomass and its uncertainty in tropical forests. Methods in Ecology and Evolution. 8 (9):1163–1167. doi:10.1111/2041-210X.12753.

SAS. 2008. JMP®, version 8. Cary (NC): SAS Institute Inc.; p. 1989–2008.

Slik J, Aiba SI, Brearley FQ, Cannon CH, Forshed O, Kitayama K, Nagamasu H, Nilus R, Payne J, Paoli G. 2010. Environmental correlates of tree biomass, basal area, wood specific gravity and stem density gradients in Borneo's tropical forests. Global Ecology and Biogeography. 19(1):50–60. doi:10.1111/j.1466-8238.2009.00489.x.

Slik JF, Bernard CS, Breman FC, Van Beek M, Salim A, Sheil D. 2008. Wood density as a conservation tool: quantification of disturbance and identification of conservation-priority areas in tropical forests. Conservation Biology. 22(5):1299–1308. doi:10.1111/j.1523-1739.2008.00986.x.

Sokal R, Rohlf F. 2013. Biometry: the principles and practice of statistics in biological research. In: RR Sokal, Rohlf FJ, editors. SERBIULA (sistema Librum 2.0).

Sokal RR, Rohlf FJ. 2011. Biometry: the principles and practice of statistics in biological research 4th edition. 4 edn. ed. New York (USA): Freeman & Company, W&H.

Stone PMR. 2018. Response of rainforest trees to climate warming along an elevational gradient in the Peruvian Andes. Edinburgh: University of Edinburgh.

Sylvester SP, Heitkamp F, Sylvester MD, Jungkunst HF, Sipman HJ, Toivonen JM, Inca CAG, Ospina JC, Kessler M. 2017. Relict high-Andean ecosystems challenge our concepts of naturalness and human impact. Sci Rep. 7(1):1–13. doi:10.1038/s41598-017-03500-7.

Tang CQ, Ohsawa M. 1999. Altitudinal distribution of evergreen broad-leaved trees and their leaf-size pattern on a humid subtropical mountain, Mt. Emei, Sichuan, China. Plant Ecology. 145(2):221–233. doi:10.1023/A:1009856020744.

Tanner EVJ, Kapos V. 1982. Leaf structure of Jamaican upper montane rain-forest trees. Biotropica. 14 (1):16–24. doi:10.2307/2387756.

Teunissen VM, Jansen ML, Cuesta B, León-Yánez F, Gosling WD. 2019. Leaf wax n-alkane patterns of six tropical montane tree species show species-specific environmental response. Ecol Evol. 9(16):9120–9128. doi:10.1002/ece3.5458.

Tilman D, Downing JA. 1994. Biodiversity and stability in grasslands. Nature. 367(6461):363. doi:10.1038/367363a0.

Tilman D, Isbell F, Cowles JM. 2014. Biodiversity and ecosystem functioning. Annu Rev Ecol Evol Syst. 45(1):471–493. doi:10.1146/annurev-ecolsys-120213-091917.

Tilman D, Reich PB, Knops JMH. 2006. Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature. 441(7093):629–632. doi:10.1038/nature04742.

Vaieretti MV, Díaz S, Vile D, Garnier E. 2007. Two measurement methods of leaf dry matter content produce similar results in a broad range of species. Ann Bot. 99 (5):955–958. doi:10.1093/aob/mcm022.

Valencia R, Condit R, Muller-Landau HC, Hernandez C, Navarrete H. 2009. Dissecting biomass dynamics in a large Amazonian forest plot. Journal of Tropical Ecology. 25 (5):473–482. doi:10.1017/S0266467409990095.

Van De Weg MJ, Meir P, Grace J, Atkin OK. 2009. Altitudinal variation in leaf mass per unit area, leaf tissue density and foliar nitrogen and phosphorus content along an Amazon-Andes gradient in Peru. Plant Ecol Divers. 2 (3):243–254. doi:10.1080/17550870903518045.

Van Der Sande MT, Arets EJMM, Peña-Claros M, De Avila AL, Roopsind A, Mazzei L, Ascarrunz N, Finegan B, Alarcón A, Cáceres-Siani Y, et al. 2016. Old-growth Neotropical forests are shifting in species and trait composition. Ecol Monogr. 86 (2):228–243. doi:10.1890/15-1815.1.

Van Der Sande MT, Bush MB, Urrego DH, Silman M, Farfan-Rios W, García Cabrera K, Shenkin A, Malhi Y, McMichael CH, Gosling W. 2020. Modern pollen rain predicts shifts in plant trait composition but not plant diversity along the Andes–Amazon elevational gradient. Journal of Vegetation Science. n/a: e12925.

Van Der Sande MT, Poorter L, Kooistra L, Balvanera P, Thonicke K, Thompson J, Arets EJ, Garcia Alaniz N, Jones L, Mora F. 2017. Biodiversity in species, traits, and structure determines carbon stocks and uptake in tropical forests. Biotropica. 49(5):593–603. doi:10.1111/btp.12453.

Velázquez-Rosas N, Meave J, Vízquez-Santana S. 2002. Elevational variation of leaf traits in montane rain forest tree species at La Chinantla, Southern México1.

Biotropica. 34(4):534–546. doi:10.1646/0006-3606(2002)034[0534:EVOLTI]2.0.CO;2.

Vendramini F, Díaz S, Gurvich DE, Wilson PJ, Thompson K, Hodgson JG. 2002. Leaf traits as indicators of resource-use strategy in floras with succulent species. New Phytologist. 154(1):147–157. doi:10.1046/j.1469-8137.2002.00357.x.

Wilson JB, Lee WG. 2000. C-S-R triangle theory: community-level predictions, tests, evaluation of criticisms, and relation to other theories. Oikos. 91(1):77–96. doi:10.1034/j.1600-0706.2000.910107.x.

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JH, Diemer M. 2004. The worldwide leaf economics spectrum. Nature. 428(6985):821–827. doi:10.1038/nature02403.

Wright SJ, Kitajima K, Kraft NJ, Reich PB, Wright IJ, Bunker DE, Condit R, Dalling JW, Davies SJ, Díaz S. 2010. Functional traits and the growth–mortality trade-off in tropical trees. Ecology. 91(12):3664–3674. doi:10.1890/09-2335.1.

Zhu J, Jiang L, Zhang Y. 2016. Relationships between functional diversity and aboveground biomass production in the Northern Tibetan alpine grasslands. Sci Rep. 6(1):34105. doi:10.1038/srep34105.