

# Plant traits predict inter- and intraspecific variation in susceptibility to herbivory in a hyperdiverse Neotropical rain forest tree community

Rafael E. Cárdenas<sup>1,2,3\*</sup>, Renato Valencia<sup>4</sup>, Nathan J. B. Kraft<sup>5</sup>, Adriana Argoti<sup>1</sup> and Olivier Dangles<sup>1,2,3,6</sup>

<sup>1</sup>Museo de Zoología QCAZ, Laboratorio de Entomología, Escuela de Ciencias Biológicas, Pontificia Universidad Católica del Ecuador, Av. 12 de octubre 1076 y Roca, Apdo. 17-01-2184, Quito, Ecuador; <sup>2</sup>Institut de Recherche pour le Développement (IRD), UR 072, LEGS-CNRS, UPR 9034, CNRS, Gif-sur-Yvette, Cedex 91198, France; <sup>3</sup>Université Paris-Sud 11, Orsay, Cedex 91405, France; <sup>4</sup>Herbario QCA, Laboratorio de Ecología de Plantas, Escuela de Ciencias Biológicas, Pontificia Universidad Católica del Ecuador, Av. 12 de octubre 1076 y Roca, Apdo. 17-01-2184, Quito, Ecuador; <sup>5</sup>Department of Biology University of Maryland, College Park, MD 20742, USA; and <sup>6</sup>Instituto de Ecología, Universidad Mayor San Andrés, Calle 27, Casilla 10077 - Correo Central, Cota Cota, La Paz, Bolivia

## Summary

**1.** A key issue in plant/herbivore interaction research is to understand which plant traits drive differences in herbivore damage. Variation in chemical, physical or phenological traits of plants may all modulate the degree of herbivore damage among species and individuals, yet the relative importance of these factors is still subject to debate, particularly in species-rich systems such as tropical rain forests.

**2.** To address this issue, we quantified leaf herbivore damage in 28 common tree species of the Yasuní forest dynamic plot (YFDP) in the Ecuadorian Amazon over 11 months. Census data from the YFDP allowed us to quantify several aspects of tree ecology potentially affecting herbivory including leaf turnover and spatial distribution patterns. We measured six chemical, eight physical and four ecological traits of the focal species. Using a combination of multivariate analyses and phylogenetic generalized linear regression model (PGLS), we assessed trade-offs between physical and chemical traits and the relative effect of all these traits on leaf herbivore damage.

**3.** Herbivore damage was highly variable among species and individuals, with leaves on average displaying damage over 13.4% (2.5–29.5%) of their area. We found no significant trade-off between physical and chemical defences for the 28 studied tree species. Overall, leaf size, shearing resistance, cellulose, ash content and leaf size × ash were the best predictors of herbivore damage. Surprisingly, condensed tannins and latex did not significantly correlate with herbivore damage. In addition, we found no relationships between herbivory and local tree density. However, we did find a weak effect of tree clustering and strong effect of tree leaf turnover rates on herbivore damage.

**4. *Synthesis.*** In the western Amazon, leaves are defended against herbivores through a combination of physical (toughness), chemical (toughness-related elements), and phenological (rapid leaf replacement) characteristics that do not appear to be subject to obvious trade-offs. Conventional strategies, such as condensed tannins or latex, do not seem to be strongly involved as a defence against herbivores in this community.

**Key-words:** Amazonia, Ecuador, forest dynamics plot, Janzen–Connell hypothesis, leaf turnover, plant-chewing–herbivore interactions, species defence functional traits, tree clustering, Yasuní National Park

## Introduction

Plant/herbivore interaction has been a topic of intense investigation over the past 40 years with significant contributions from empirical, experimental and theoretical research, and meta-analyses (Price *et al.* 1980; Loeuille, Loreau & Ferriere

\*Correspondence author. E-mail: recardenasm@yahoo.com

Correction added on 16 June, after first online publication: Fig. 2 legend amended to clarify that bars represent upper and lower deciles, and not standard deviations.

2002; Ohgushi 2005; Endara & Coley 2011; Johnson *et al.* 2012). A number of studies have shown that the extent of herbivore damage on leaves is mainly driven by plant physical, chemical and phenological traits (Coley & Barone 1996; Carmona, Lajeunesse & Johnson 2011; Loranger *et al.* 2013). For example, high nitrogen content and specific leaf area (SLA) can make plants more palatable to herbivores (Mattson 1980; Choong *et al.* 1992; Hanley *et al.* 2007), while the presence of secondary metabolites and structural traits is known to increase plant resistance to herbivory (Agrawal 2007; Hanley *et al.* 2007). Phenological aspects of plant life history, such as synchronous leaf production and rapid leaf expansion rates, have also been shown to be negatively correlated with herbivory (Aide 1988, 1992, 1993; Coley & Barone 1996).

There is no consensus in the literature about what determines intra- and interspecific variation in herbivory among co-occurring tropical trees. Several causes have been proposed to explain high herbivory variation in the tropics. First, plant defence strategies may rely on a suite of multiple traits (syndromes) that may covary across species as a consequence of shared evolutionary ancestry or because of adaptive convergence (Agrawal & Fishbein 2006). Plants inhabiting a given environment may have – convergently or not – evolved a set of particular defence traits as a function of local pathogens pressures (Agrawal 2007). Second, these traits may trade-off against each other because of physiological constraints suggesting that trees may fall along an escape–defence continuum (Kursar & Coley 2003). On the one hand, extreme ‘escape’ species are predicted to have few chemical defences, but rapid synchronous leaf expansion that minimizes the window of vulnerability, and low leaf nutritional quality during expansion making leaves less palatable. On the other hand, extreme ‘defence’ species may have high chemical defence, low nutritional quality, and asynchronous leaf expansion (Kursar & Coley 2003). Based on existing literature, Agrawal & Fishbein (2006) proposed the so-called ‘defence syndrome triangle’ hypothesis that includes the most important defence categories: ‘low nutritional quality’, ‘nutrition and defence’ and ‘tolerance/escape’ defence syndromes in an ‘edibility/digestibility’ and ‘toxicity/barrier to feeding’ two-dimensional array.

Although the investment in plant defences is conceived as a survival strategy to guarantee tissue development, the energy necessary for this purpose may interfere with growth rates (Coley 1987). This plant dilemma of ‘to grow or defend’ has been of interest to ecologists for decades (Herms & Mattson 1992). The widely accepted ‘resource availability hypothesis’ predicts that the optimal level of defence will vary with plant growth rate (Coley, Bryant & Chapin 1985). In unproductive habitats, such as some tropical forests, investment in defences would cause slower growth rates to plants due to the impossibility of allocating energy simultaneously to both growth and defence (Coley 1987). Moreover, limited resources constrain the ability of plants to compensate for herbivory because of the cost of replacing lost tissue (Janzen 1974).

An additional factor that may explain the variability in herbivore damage is the relative plant abundance and spatial distribution patterns (Huntly 1991). The Janzen–Connell hypothesis

(Janzen 1970; Connell 1971) states that if adult trees serve as reservoirs for natural enemies, conspecifics of the surroundings may increase such effect (e.g. attracting herbivores), thus, for example, presenting higher levels of herbivore damage. Herbivore pressure, plants growth rate declining and mortality are therefore predicted to increase along with conspecific plant density (negative density dependence effect; Janzen 1974; Sullivan 2003). Species having a strong negative impact on themselves typically occur at lower abundances (Comita *et al.* 2010). For example, species susceptible to herbivory may be more sensible to biotic interactions that inhibit their own regeneration such as high levels of conspecific density or clustering (Gilbert, Hubbell & Foster 1994; Bell, Freckleton & Lewis 2006). Yet, different perspectives suggest that negative density dependence (NDD) could also be generated by intraspecific resource competition making individuals more prone to attack by pathogens, so pathogens may be a symptom rather than a cause of density dependence (Dobson & Crawley 1994).

In this study, we test for the relative importance of intrinsic plant traits (e.g., leaf quality) and ecological factors (e.g., leaf turnover, spatial distribution) in driving variation in leaf herbivore damage within and among species in a hyperdiverse tree community located in the Ecuadorian Amazon. To do this, we first quantified variability in leaf herbivore damage for the 28 most abundant tree species in our study plot over the course of 1 year. Second, we analysed the effects of several key leaf functional traits on herbivore damage and also tested for a trade-off between physical and chemical defences among species (Moles *et al.* 2013). The assumption that defences are costly (Bazzaz *et al.* 1987) leads to the prediction of a physical/chemical trade-off because plants have a finite pool of resources to invest in defence (Twigg & Socha 1996; Read *et al.* 2009). Tests focusing on univariate measures have rarely revealed trade-offs. A more integrative perspective suggests that consideration of the sets of traits that make up syndromes are more likely to exhibit trade-offs because multiple defensive traits are likely needed to defend against a range of potential herbivores (Agrawal 2007). In this study, we assessed whether sets of traits associated with resistance and defence against herbivory exhibit trade-offs with sets of traits associated with tolerance and escape. Third, we used the ecological census data of the forest dynamics plot to test three hypotheses that may explain interspecific variation in herbivore damage: (i) fast-growing tree species have lower amounts of constitutive defences than slow-growing species (resource availability hypothesis, Coley, Bryant & Chapin 1985); (ii) species that produce better quality leaves may be more tolerant to herbivory and may present a higher leaf turnover rate (total number of discarded leaves per individual per species in 1 year) as an escape strategy for deterring herbivores (the ‘tolerance/escape’ syndrome, Agrawal & Fishbein 2006); (iii) herbivore damage is associated with spatial clustering of conspecific adult individuals (based on the Janzen/Connell’s concept and prediction, Janzen 1970; Connell 1971) where the most clustered species may suffer more herbivore damage than the less clustered ones. Finally, we addressed a fourth hypothesis related to intraspecific variation in damage,

following on the Janzen/Connell hypothesis concept: (iv) a tree surrounded by a higher number of conspecifics in its neighbourhood exhibits higher herbivore damage than trees isolated from their conspecifics.

## Material and methods

### STUDY SITE

The Yasuní National Park (YNP) and the adjacent Waorani Indigenous territory cover 1.6 million ha of forest (1.8 times the Yellowstone National Park in the USA) and form the largest protected area in Amazonian Ecuador (~17.7% of the Ecuadorian Territory; Valencia *et al.* 2004a), harbouring the world's most diverse tropical forests (Bass *et al.* 2010). YNP is an evergreen lowland wet forest ranging in altitude from 200 to 300 m above sea level. It has a 15–30 m canopy with some emergent trees reaching 50 m (Dangles, Carpio & Woodward 2012). Rainfall and temperature are aseasonal, with a mean annual rainfall of 2826 mm (none of the 12 calendar months averaging < 100 mm) and a mean monthly temperature ranging from 22 to 32 °C (min: 16.9; max: 38.9 °C) (see Valencia *et al.* 2004a for more details; data obtained from YRS meteorological station, <http://www.yasuni.ec>).

The study plot was located in the vicinity of Yasuní Research Station of the *Pontificia Universidad Católica del Ecuador* (YRS-PUCE; 76°24'1.8" W; 00°40'16.7" S). As part of a global network of permanent forest dynamics plots the YRS-PUCE, in collaboration with the Smithsonian Tropical Research Institute and Aarhus University, created in 1995 the Yasuní 50-ha plot (500 × 1000 m; 76° 24' W; 00°41' S; see [http://www.puce.edu.ec/portal/content/Dinámica del Bosque Yasuní](http://www.puce.edu.ec/portal/content/Dinámica%20del%20Bosque%20Yasuní)). The primary objective of the project is to describe the long-term demography of thousands of plant species and explain their dynamics with ecological theories (e.g. Kraft, Valencia & Ackerly 2008; Romero-Saltos 2011). To achieve this goal, all trees with trunk ≥ 1 cm d.b.h. (diameter at breast height) have been tagged, mapped and identified to the species level (census methodology described in detail in Condit 1998 and Valencia *et al.* 2004b). The plot lies at 230 m above sea level, and contains three large ridges and intervening valleys (see Appendix S1 in Supporting Information) that include small streams and a small swamp. There is a 33.5 m difference between altitude extreme points in the plot which presents an average slope of 13% (Valencia *et al.* 2004a). Forest dynamics plots such as the Yasuní 50 ha plot offer a unique opportunity to test the factors driving herbivory patterns in the tropics. Observational studies have the advantage of providing natural conditions and fully intact assemblages of animals and plants with natural interactions that are not reproducible in short-term experiments.

### LEAF LITTER COLLECTION

To measure herbivore damage in the study plot, leaf litter was collected from 100 litter-fall traps (mesh of 0.71 m<sup>2</sup> fixed on 0.8 m high plastic tubes) located in a Western 650 × 400 m portion of the forest plot (see Appendix S1). Traps were censused monthly between February 2011 and January 2012 (except May 2011). The leaf biomass collected in the traps corresponded to the litter fall of the last 15 continuous days of each month only (we excluded the leaf litter fall of the first 15 continuous days) and included leaves of all kinds: sun and shade leaves, coming from adults, subadults and seedlings. We targeted leaves of the 75 most abundant freestanding woody plants tree species of the plot (accounting for > 36 % of the total number of

individuals and belonging to 26 plant families). Leaves were identified in the field with the expertise of a parataxonomist, and species identities were confirmed using voucher material deposited at the YRS-PUCE. Tree relative abundance was assessed using the 2009 census of the 50-ha plot (R. Valencia, unpublished data).

### LEAF HERBIVORE DAMAGE QUANTIFICATION

Of the 75 most abundant tree species in the plot that we targeted for collection, we captured leaf material from 53 species in the litter-fall traps. Leaves were pressed, dried at 60 °C for 48 h, weighted and scanned at 2300 × 3300 pixels resolution for the quantification of herbivore damage. We focused analyses on the 28 species (from 13 families, Table S1) for which we were able to collect more than 30 leaves and did not consider the 25 remaining species further. We defined herbivore damage as the proportion of leaf area eaten over the leaf life span. This represents the cumulative percentage of leaf area lost (Schuldt *et al.* 2012) including herbivory events from previous seasons (see Brenes-Arguedas, Coley & Kursar 2008). Leaves totally eaten (i.e. with only the petiole remaining) were not taken into account for herbivore damage estimations. Our herbivore damage metrics therefore primarily capture the action of leaf chewers (e.g. Orthopterans, Lepidopteran larvae, Coleopterans like Chrysomelids, Molluscs). Piercing/sucking damage (e.g. Cicadellids, Curculionids, Cercopids) was not taken into account as it was impossible to measure its damage impact using our sampling methodology. Additionally, leaves with mines, galls, necrotic areas, and scraped, scratched, ripped or torn surfaces were excluded from analysis (< 20 % of the total leaf collection, RC personal observation).

Leaf damage was quantified using ImageJ (<http://rsb.info.nih.gov/ij/>; Abràmoff, Magalhães & Ram 2004). For this, leaf images were cleaned (i.e. erase shadows, fill small scratches, eliminate the petiole) and binary-transformed. We then quantified total and damaged areas and then calculated the proportion of herbivore damage (eaten area divided by total area). In the case of leaf-margin damage, we cloned the missing edge from the opposite side of the same leaf or from similar size-shaped leaves of the same species to infer the initial total area in Photoshop CS (Adobe Systems Incorporated, San José, CA, USA).

### SAMPLING FOR LEAF TRAIT MEASUREMENTS

To quantify leaf physicochemical characteristics, foliar material was collected from randomly selected individual trees located nearby YRS-PUCE trails, rejecting individuals that showed generalized heavy impact of herbivores, or that lacked sufficient recently produced, fully expanded leaves (Cornelissen *et al.* 2003). We targeted both understorey and outer canopy young (new) green leaves from adults, subadults and (rarely) seedlings in the 28–276 mm diameter at breast height size class (Valencia *et al.* 2004b) that could be readily accessed from the ground. Our sampling was designed to reflect the diversity of leaves found in the leaf litter-fall traps. Typically, the targeted leaves were the youngest of the leaves on the tree, exhibiting bright green coloration relative to older (mature) leaves. Literature has demonstrated that approximately 60–80% of the herbivore damage in shade-tolerant tropical species occurs during the first weeks, while the leaf is young and expanding (Coley & Aide 1991; Kursar & Coley 1992; Coley & Barone 1996). However, we chose to perform the leaf trait measurement on recently full-expanded young green leaves as (i) quantitative and representative data on young expanding-leaves are exceedingly difficult to obtain (Coley & Aide 1991). Soft expanding

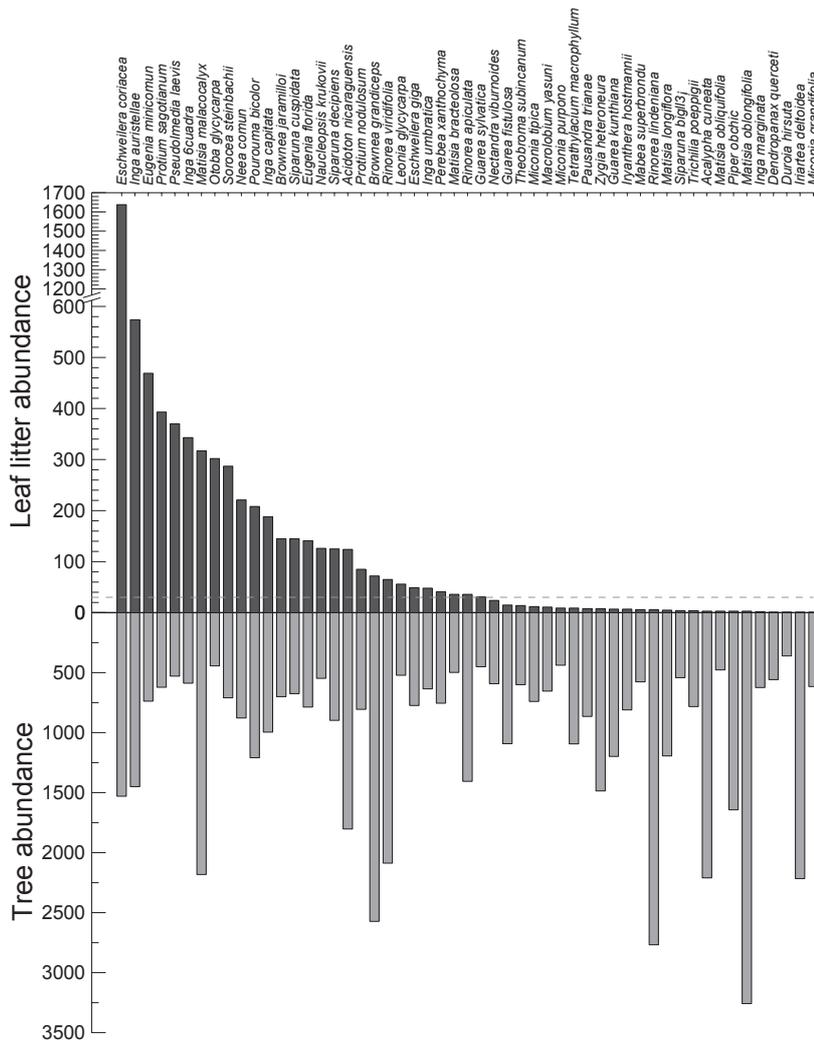
young leaves are very rare in the plot (RC personal observation) and new leaf flushes are unpredictable for most species in aseasonal forests like Yasuní. (ii) 65% of our studied species showed some kind of chromatic expanding-leaf defence as a strategy to deter herbivores (Kursar & Coley 1992). Recently, Queenborough *et al.* (2013) have confirmed that chromatic defences in tropical species significantly lowered mortality in seedlings and trees in Yasuní National Park (Ecuador) and Barro Colorado Island (Panamá). Hence, we assumed most of our studied species were well defended during leaf expansion. (iii) Herbivory events may have also occurred when leaves were fully expanded but still young (probably not so far from ‘age zero’, see Fig. 1 in Kursar & Coley 2003). At this stage, leaves are still palatable for herbivores as they are not completely tough, nutritious and are depressed in some chemical defences such as condensed tannins and other phenolic compounds (comparing to mature leaves, see Coley & Aide 1991; Kursar & Coley 2003). It was therefore relevant to correlate our targeted leaves quality with herbivore damage amounts.

In total, 40 to 60 young, fully expanded green leaves were sampled from 5 to 6 individuals from the 28 tree species. Leaves designated for chemical analyses (10–25 depending on its size and tangible biomass) were dried at 45 °C for 48–96 h, separated into five groups, homogenized by grinding, and kept in dry conditions until analyses.

LEAF TRAIT MEASUREMENTS

We selected a range of vegetative functional traits, both physical and chemical, that have been shown to be correlated with herbivory or anti-herbivory properties (Cornelissen *et al.* 2003; Hanley *et al.* 2007; Kurokawa & Nakashizuka 2008; Carmona, Lajeunesse & Johnson 2011; Moles *et al.* 2013). We first characterized the ‘structural’ and ‘indirect defences’ of each species (Hanley *et al.* 2007; Carmona, Lajeunesse & Johnson 2011; Kessler & Heil 2011) by determining the presence/absence of hairs/scales on the leaves and the presence/absence of extra-floral nectaries/glands on the twigs. Physical traits – or resistance to physical damage traits (Carmona, Lajeunesse & Johnson 2011) – corresponded to leaf thickness, and necessary force for punching, shearing and tearing (Onoda *et al.* 2011). Tests were performed over 28–30 leaves per species, except for tearing tests where only 15–18 leaves were used for three species (*Inga auristellae*, *Eugenia florida*, *Eugenia ‘minicomun’*).

Thickness was measured, taking care to avoid primary and secondary veins, using an analogue 0–25 mm micrometre caliper with 0.005 mm precision (Amico Corporation, Ontario, Canada). For punching, shearing and tearing quantification, we used a digital dual-range force sensor (Vernier Software & Technology, 2010, Beaverton, Oregon, USA) fixed to a handmade steel instrument that allowed the performance of standard movements for all the three tests. Forces



**Fig. 1.** Abundance distribution of trees and leaf litter input in soil. Y<sub>1</sub>: Leaf litter abundance in traps; Y<sub>2</sub>: Tree abundance in the study area. Shapiro–Wilk normality test showed no-normality distribution for both data set ( $W_{\text{leaves}} = 0.514$  and  $P < 0.001$ ;  $W_{\text{trees}} = 0.804$  and  $P < 0.001$ ). Tree abundance and leaf litter abundance in the traps were not correlated (Spearman correlation coefficient  $\rho = 0.01$ ; d.f. = 51;  $P = 0.934$ ). Dashed-grey line corresponds to 30 leaves criterion used to discriminate species for analyses. The following correspond to morphospecies names (*nomen nudum*: waiting for a formal description): *Eugenia ‘minicomun’*, *Inga ‘6cuadra’*, *Mabea ‘superbrondu’*, *Miconia ‘purpono’*, *Miconia ‘tipica’*, *Neea ‘comun’*, *Siparuna ‘bigl3i’* and *Piper ‘obchic’*.

were measured in Newtons (N) at 0.01 or 0.05 N precision for resistances  $\pm 10$  N or  $\pm 50$  N, respectively. For the punching test, we screwed on the force sensor a 1.68-mm-diameter aluminium flat-ended rivet (area: 2.22 mm<sup>2</sup>) and measured the maximum force per unit of fracture ( $N_{\max}/\text{mm}^2$ ) to perpendicularly punch the leaf avoiding primary and secondary veins (Onoda *et al.* 2011).

For the shearing test, we screwed on the force sensor a peg-like folded steel sheet that supported a razor blade (Procter & Gamble Co., Rio de Janeiro, Brazil). The leaf was fixed between two pressure plates leaving a space for shearing. Blades were replaced every 28–30 measurements (i.e. one blade per species). Measures were recorded as the force per unit of time ( $s \times N$ ; the area under the curve) and normalized to force per unit of fracture ( $s \times N/\text{mm}$ ; Onoda *et al.* 2011).

For tearing tests, we cut a leaf segment from the central part of the leaf, in parallel to its main axis and avoiding the midrib. Length and width were measured (mm) using a digital caliper (Fowler Tools of Canada, Ontario, Canada), and the leaf strip was fixed between two under-pressure plaques –one fixed, the other mobile– leaving a space for tearing. The force sensor was attached to an arm that performed a horizontal movement. A rubber bumper directly screwed to the sensor pushed the mobile plaque until the leaf strip was ripped. As tearing tests measured the maximum force to tear a leaf strip, we normalized the data per unit of fracture ( $N_{\max}/\text{mm}$ ; Onoda *et al.* 2011). Leaf size (cm<sup>2</sup>) and specific leaf area (SLA, defined as the ratio of fresh leaf area in cm<sup>2</sup> to dry weight) of the 28 tree species were taken from Kraft, Valencia & Ackerly (2008) and Kraft & Ackerly (2010, and unpublished data). Finally, the presence/absence of latex was considered as a physical defence following Moles *et al.* (2013).

For the 28 tree species, we quantified the following chemical traits: nitrogen, carbon, lignin, cellulose, ash and condensed tannins. Nitrogen and carbon values were taken from Kraft & Ackerly (2010 and unpublished data). All other traits tests were measured at Colorado State University (Fort Collins, CO, USA) Soil, Water and Plant Testing Laboratory (<http://www.soiltestinglab.colostate.edu/>). Lignin and cellulose contents followed the gravimetric determination of acid detergent fibre (ADF) and acid detergent lignin (ADL) methodology (Möller 2009). Ash content corresponded to the leaf mass remaining after combustion of ADL samples at 550 °C for 2 h (Möller 2009). Condensed tannins were measured using the Butanol-HCl method and expressed as leucocyanidin equivalent (% DM) following Porter, Hrstich & Chan (1986).

## STATISTICAL ANALYSES

### Plant trait relationships

We first explored, at the interspecific level, the relationships among all plant traits simultaneously using a categorical principal component analysis (CATPCA; Gifi 1991) in SPSS 15.0 (SPSS Inc., Chicago, IL, USA). CATPCA allows for the analysis of categorical and numerical variables, and for nonlinear relationships between traits (Ellis *et al.* 2006; Costantini, Linting & Porzio 2009). Binary traits were treated as ordinal (nectaries, hairs, latex), while continuous traits were treated as numerical. We used the ‘symmetrical normalization’ option as it is optimal for testing the relation between variables.

We then tested for potential trade-offs between chemical and physical defence trait groups. We used the first axes eigenvalues of the CATPCA run on physical and chemical defence traits (see Moles *et al.* 2013). SLA, leaf size, thickness, tearing, punching, shearing and leaf turnover numerical traits, as well as extra-floral nectaries, hairs and latex ordinal traits, were included in the model as physical

factors (Moles *et al.* 2013). Lignin, C, N, C:N, tannins, cellulose and ash were included in the model as chemical factors. A simple linear fit was used to characterize the general form of the relationship between the defence groups.

### Phylogenetic signal

As phylogenetic nonindependence (*sensu* Felsenstein 1985) can inflate measures of correlation among traits, we tested for phylogenetic signal in 13 traits in addition to total herbivore damage. We first constructed a molecular phylogeny (details in Appendix S3) and then assessed signal with Blomberg’s *K* index using the ‘Picante’ package in R (R Development Core Team 2013), with statistical significance assessed using 999 replicates of a tip-swap null model. In addition, we test for six traits and interactions Pagel’s lambda phylogenetic signal (Pagel 1999) using the package ‘Caper’ in R software (R Development Core Team 2013). This test is used to measure the extent to which correlations in traits reflect their shared evolutionary history (as approximated by Brownian motion).

### Herbivore damage predictors

Because leaf thickness, C:N ratio, condensed tannins and ash content showed significant *K* phylogenetic signal ( $P = 0.006$ ,  $P = 0.004$ ,  $P = 0.050$  and  $P = 0.006$ , respectively, but  $\lambda = 0$  in all cases except for leaf size  $\times$  ash interaction where model  $\lambda = 0.498$ , Table 1), we decided to use phylogenetic generalized least square (PGLS) linear regression analyses to be conservative (Felsenstein 1985; Grafen 1989). Using PGLS, we tested for the best herbivore damage predictors while accounting for phylogenetic relationships among species. PGLS was performed using the package ‘Caper’ in R software (R Development Core Team 2013), based on a phylogenetic tree for the 28 studied species (Appendix S3). Analysis was used to test the effects of plant traits on herbivore damage. As the inclusion of the 13 plant continuous traits (and their interactions) would have resulted in an over-parameterization on the model, we only used traits that were the best linear predictors of herbivore damage. We selected six plant traits (thickness, leaf size, shearing, C:N, cellulose and ash contents) which were the strongest predictors of leaf damage linear regressions ( $P \leq 0.14$ ). A parallel stepwise multiple regression analyses including all plant traits confirmed that these six traits were the best predictors of herbivore damage. To test for potential multicollinearity among selected plant traits, we built a pairwise regression matrix, which showed that none of the predictors included in the PGLS were autocorrelated ( $r < 0.60$ ).

### Herbivore susceptibility vs. tree growth rates, leaf turnover and spatial distribution

We considered three factors related to the concentration of leaf resources for herbivores. First, we evaluated the relationship between species inherent growth rates averages with leaf physicochemical eigenvalues obtained from CATPCA analysis (axes 1 and 2). Growth rates and physicochemical leaf traits are intrinsic to a species and could last over time reflecting herbivory events from the past (Tuomi *et al.* 1984) that in turn may have affected plants growth. Growth data rates were obtained measuring the diameter of the trunk of every tree  $\geq 1$  cm d.b.h. over a period of 5 years (2002 and 2007). Second, we confronted herbivore damage to the leaf turnover of each of the 28 selected tree species. This was measured in two ways: the number of leaves collected in the litter-fall traps per individual per species,

**Table 1.** Results of the phylogenetic signal analyses and the PGLS of seven leaf plant traits on herbivore damage. In the PGLS adjusted  $R^2 > 0.4$  and  $P \leq 0.035$  for every model. Significant  $P$  values of both analyses indicated in bold. Quotation marks mean the same terms included in the model

Effect	Phylogenetic signal			PGLS					
	$\lambda$	$K$	$P$	Terms included in the initial model	Estimate	Standard error	$t$ -value	$P$ -value	
Thickness	0.000	0.28	<b>0.006</b>	Thickness, leaf size, shearing, C:N, cellulose, ash	42.96	22.98	1.87	0.076	
Leaf size	0.000	0.17	0.240	Thickness, leaf size, shearing, C:N, cellulose, ash	0.027	0.012	2.231	<b>0.037</b>	
Shearing	0.000	0.17	0.216	Thickness, leaf size, shearing, C:N, cellulose, ash	-855.1	264.3	-3.235	<b>0.004</b>	
C:N	0.000	0.24	<b>0.004</b>	Thickness, leaf size, shearing, C:N, cellulose, ash	0.218	0.212	1.028	0.316	
Condensed tannins	0.000	0.19	<b>0.050</b>	–	–	–	–	–	
Cellulose	0.000	0.12	0.794	Thickness, leaf size, shearing, C:N, cellulose, ash	0.519	0.176	2.953	<b>0.008</b>	
Ash	0.000	0.29	<b>0.006</b>	Thickness, leaf size, shearing, C:N, cellulose, ash	-392.2	181.7	-2.159	<b>0.043</b>	
Leaf size $\times$ ash	0.498	–	–	Thickness, leaf size, shearing, C:N, cellulose, ash + interactions	-10.83	3.502	-3.092	<b>0.007</b>	

and the number of leaves collected per unit of species biomass. Biomass was inferred from the trunk basal width mean data obtained from the last plot census. The assumption behind this is that a biggest tree may contain a largest number of leaves. Third, we explored the relationship between leaf damage and tree clustering in the forest plot. We analysed the degree of tree clustering using the Ripley's  $K$  statistics (Ripley 1981) and the associated  $L(r)$  function (normalized with the complete spatial randomness function). For each tree species, a tree clustering index (TCI) was defined as the area under the curve of the  $L(r)$  function. Aggregation is indicated when  $TCI > 0$ , whereas  $TCI < 1$  indicates spacing at some scale, and  $TCI = 0$  a random distribution. Fourth, we assessed the potential local effect of tree clustering on herbivore damage by testing for a correlation between herbivore damage of a focal tree species (from one to dozens of individuals) near a focal litter-fall trap and the density nearby conspecifics. This analysis could only be performed for tree species *Eschweilera coriacea*, for which we collected a total of 1503 leaves in 53 litter-fall traps (traps with  $< 3$  leaves were excluded from the analysis). Using *E. coriacea* tree locations acquired during the 2009 census, we were able to count the number of *E. coriacea* trees within a 20 m radius around each of 100 traps. We then compared herbivore damage (averaged using all leaves in each trap) with tree densities around the trap to test the prediction that individual trees experience greater herbivory when their local neighbourhood includes many conspecific individuals. All spatial distribution analyses were performed using the 'SpatStat' package in R (R Development Core Team 2013).

## Results

### PATTERNS OF LEAF LITTER FALL AND HERBIVORE DAMAGE

Over 11 months, a total of 6802 leaves from 53 of the 75 targeted most common tree species in the plot were collected from the 100 litter-fall traps (Fig. 1). This means that leaves of some 22 common species in the plot were never collected in our leaf litter survey. We found no significant relationship between tree abundance in the plot and leaf litter abundance in the traps (Spearman correlation,  $\rho = 0.01$ ; d.f. = 51;  $P = 0.934$ ). For example, the third most abundant tree species in the plot, *Brownea grandiceps* ( $n = 2573$ ), contributed with only 1.06% of the total number of fallen leaves. Contrastingly,

*E. coriacea* ( $n = 1530$ ), ranked in the 10th position of tree abundance in the plot represented 24.08% of the total fallen leaves, the highest leaf litter contribution in the plot. Temporal trends over 1 year of litter fall for the 28 species are presented in Appendix S2.

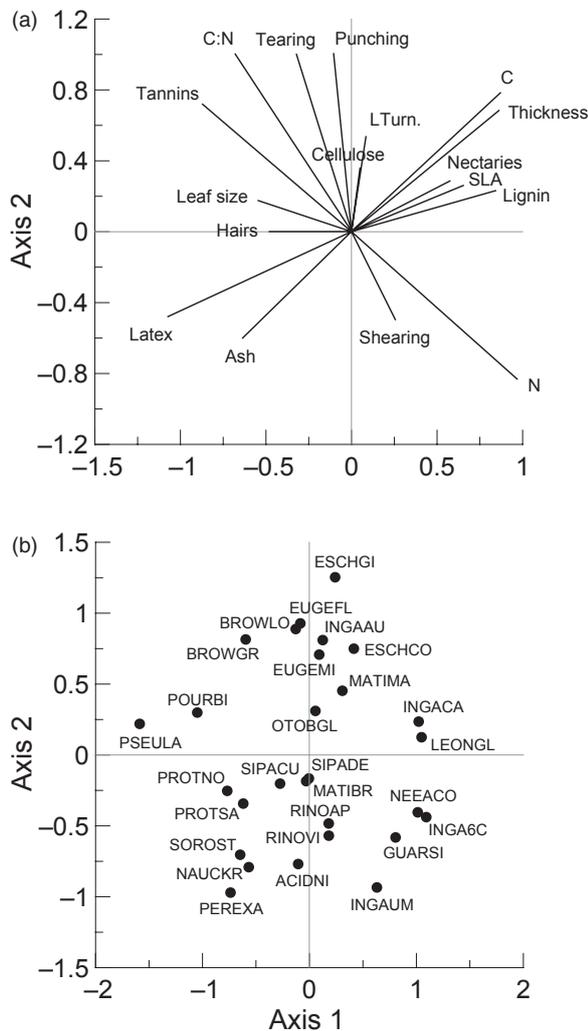
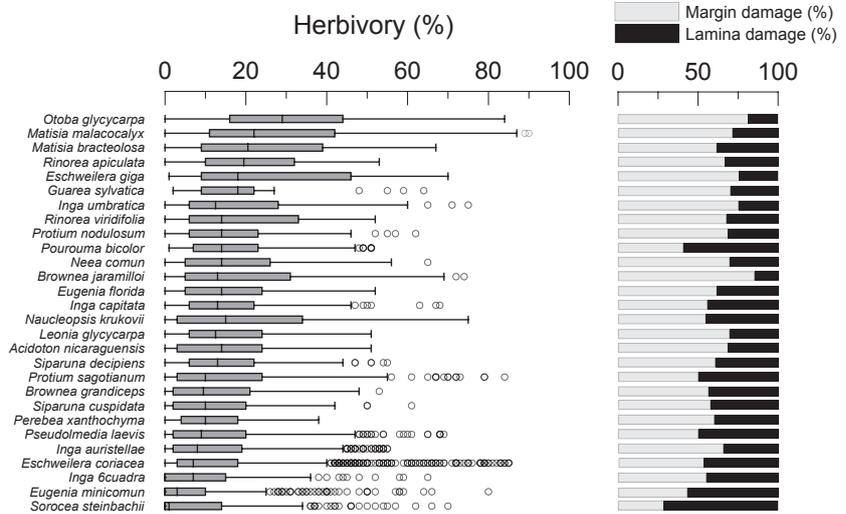
For the 28 most common tree species, mean herbivore damage was of  $13.4\% \pm 5.9$  (min: 2.49%; max: 29.46%). There was a high herbivore damage variability within and between tree species (Fig. 2). Average standard deviation of the full data set was 15.64% (min: 10.72; max: 22.69). *Otobalglycyarpa*, *Matisia malacocalyx*, *Matisia bracteolosa*, and *Rinorea apiculata* showed the highest proportions of herbivore damage,  $> 20\%$  on average. On the opposite end, *Inga '6cuadra'*, *E. 'minicomun'* and *Sorocea steinbachii* presented the lowest proportions of herbivore damage,  $< 5\%$  on average. For most species (82.1%), we found that herbivore damage occurred mostly ( $> 50\%$ ) on the leaf edge rather than on the lamina (Fig. 2). *Brownea jaramilloi* was the species that presented the highest proportion of herbivore damage on the edges (85.1%), while *S. steinbachii* presented the highest herbivore damage on the lamina (71.6%).

### RELATIONSHIPS AMONG PLANT TRAITS

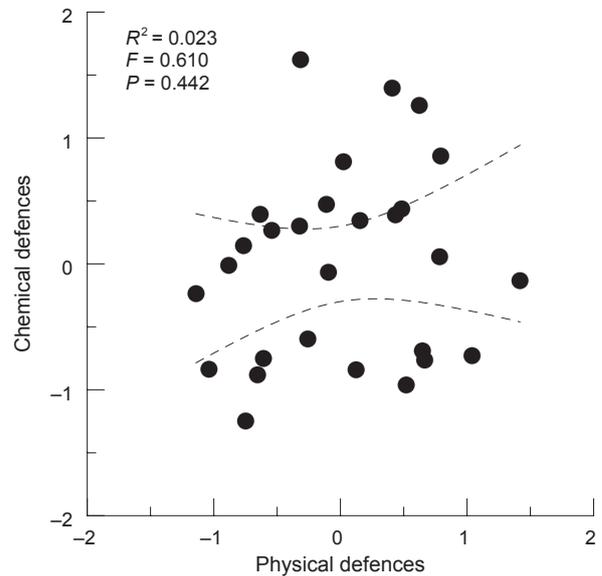
The categorical PCA used to assess multivariate relationships among all plant traits explained 18.89% of the total variance on the first axis, which separated species with high values of N, C and thickness from those with high values of tannins and latex (Fig. 3). The second axis explained 15.94% of the variation and was best explained by species with high values of punching, C:N ratio and tearing. The third axis explained 11.26% and separated species with high values of hairs from those with high values of lignin. The force to shear was best explained by the fourth axis (9.23%). The fifth axis (8.69%) separated those species with high values of leaf turnover and ash content from those with high values of cellulose. Leaf size was better explained by the sixth axis (7.04%), SLA by the seventh (5.90%) and nectaries by the eighth (5.18%).

The relationship between indexes of overall physical and chemical traits was positive (slope = 0.17, Fig. 4) though not

**Fig. 2.** On the left, herbivore damage (%) on fully expanded green leaves from adult trees of 28 common species in Yasuni National Park. On the right, herbivore damage (%) differentiated by margin (grey) vs. lamina damage (black).



**Fig. 3.** Indirect, mechanical, phenological and resin-type defence traits of the 28 more common spp in Yasuni National Park (fully expanded young green leaves). First and second axes explained by 18.89% and 15.94%, respectively. Biplot of species coordinates arrayed on the first two axes of the CATPCA. LTurn. refers to leaf turnover. Species codes are detailed in Table S1.



**Fig. 4.** Relationship of physical (X) and chemical defences (Y) from CATPCA eigenvalues representing the factor loadings for traits on the first axes. Upper and lower dash-lines represent 5% and 95% confidence intervals of a linear regression (ANOVA statistics showed):  $y = 0.17x - 1.2 \times 10^{-17}$ .

significant ( $R^2 = 0.023$ ,  $P = 0.442$ ). This means that some species with high physical defences may also present high chemical defences (e.g. *Inga capitata* and *Neea 'comun'*), and vice versa (e.g. *Pseudolmedia laevis* and *Protium nodulosum*).

PLANT TRAITS AFFECTING HERBIVORE DAMAGE

PGLS analysis showed significant relationships between herbivore damage and several physicochemical traits and one interaction (Table 1). When considering factors without interactions leaf size, shearing, cellulose and ash best explained differences in herbivore damage (Table 1 and Fig. 5). Contrastingly, thickness and C:N were not significant predictors of herbivore damage ( $P = 0.076$ ;  $P = 0.316$ , respectively,

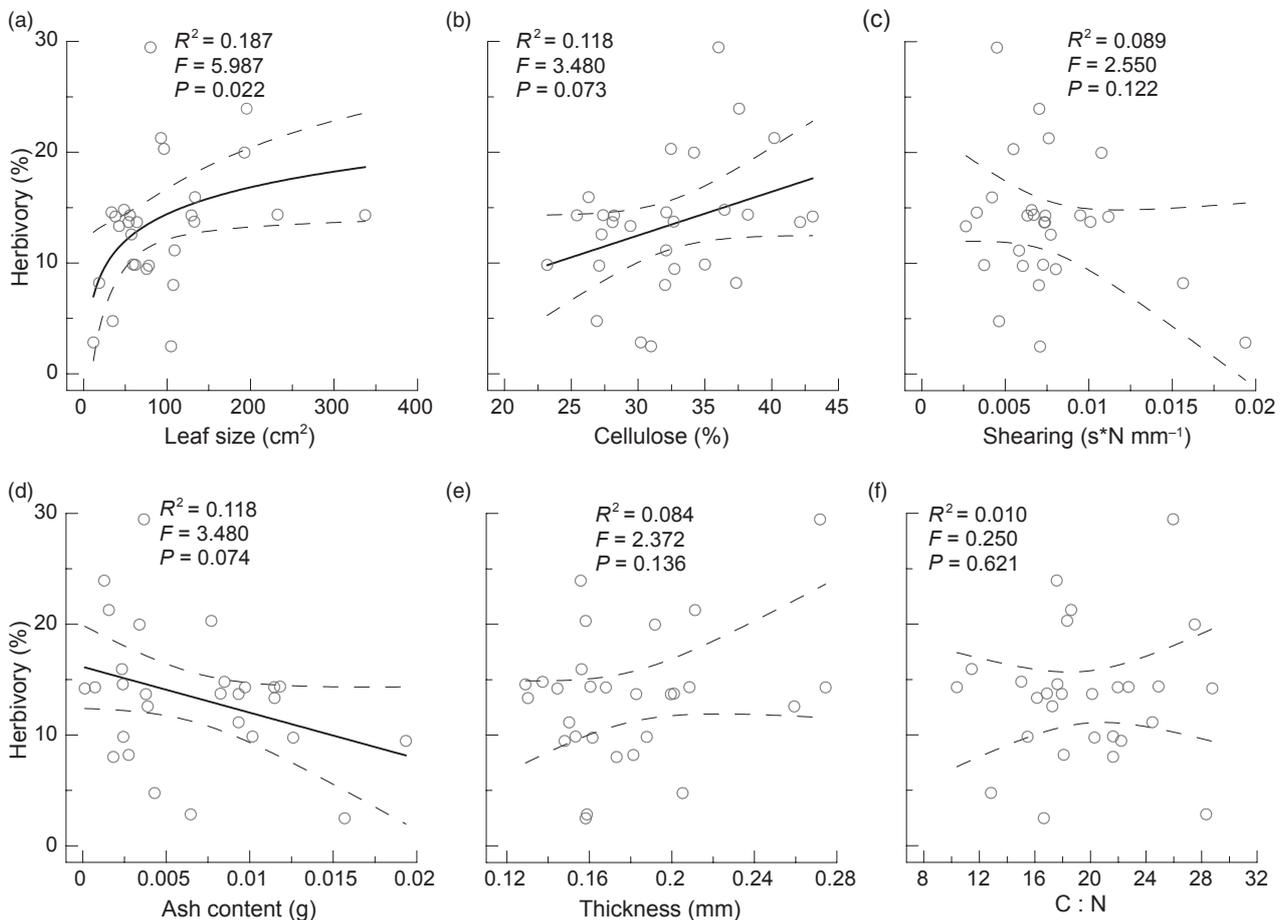


Fig. 5. Herbivory vs. physical and chemical traits of 28 common species in Yasuní National Park (full-expanded young green leaves). The solid lines represent the trend of the relationship (for  $P$  values  $\leq 0.1$ ). Upper and lower dash-lines represent 5% and 95% confidence intervals.

Table 1) but showed a general positive relationship (Fig. 5e, f). The same PGLS model including interaction showed that the term leaf size  $\times$  ash was significant predictor of herbivore damage ( $P = 0.007$ , Table 1).

#### HERBIVORE SUSCEPTIBILITY VS. TREE GROWTH RATES, LEAF TURNOVER AND SPATIAL DISTRIBUTION

Species growth rates showed no significant relationships with physicochemical defences (eigenvalues from axes 1 and 2) (Fig. 6, linear regressions  $P > 0.05$  in both cases). Contrary to the prediction higher physicochemical defended species (e.g. *I. capitata*, *O. glycyarpa*) did not necessarily present the lowest growth rate, or *vice versa* (e.g. *B. grandiceps*, *Acidoton nicaraguensis*).

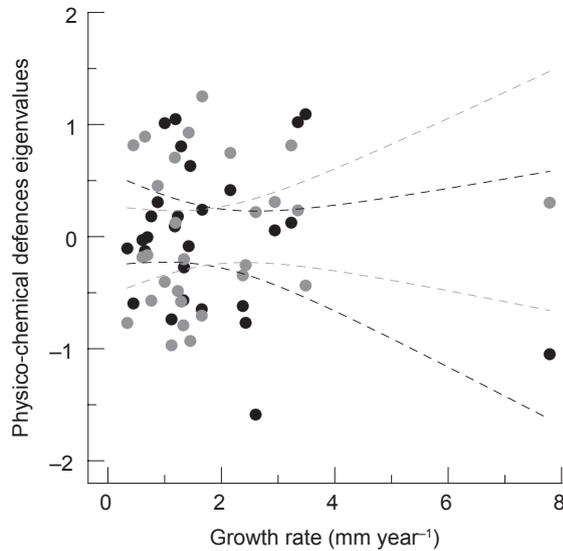
The relationship between herbivore damage and leaf turnover resulted in a marginally significant fit to a logarithmic regression (Fig. 7a;  $P = 0.073$ ). However, the relationship became highly significant when the fit excluded one outlier species *O. glycyarpa* ( $P = 0.001$ ;  $y = 7.32 - 2.96 \ln x$ , results not shown). This same trend was conserved when analysing the amount of herbivore damage against the leaf turnover per unit of biomass per species (Fig. 7b;  $P = 0.038$ ). Species with low leaf turnover ( $LT_{\text{dbh}}$ , Table S2) seemed to be more susceptible

to herbivory than species with mid/high leaf turnover. This was confirmed when comparing the five more 'productive' species in terms of the number of leaves per unit of biomass (*E. coriacea*, *A. nicaraguensis*, *I. auristellae*, *E. 'minicomun'*, *Protium sagotianum*) vs. the less productive species (*Guarea sylvatica*, *M. bracteolosa*, *R. apiculata*, *Perebea xanthochyma*, *Eschweilera giga*), against herbivore damage. We found the first group in average was two times less damaged comparing to the second group (Table S2).

Herbivore damage was not significantly correlated with the spatial distribution of tree species ( $P = 0.816$ , Fig. 7c). Here again, the relationship was strongly affected by one species, *R. apiculata* as, when it was removed from the fit, a significant logarithmic regression could be adjusted to the data ( $P = 0.031$ ;  $y = 12.37 - 0.92 \ln x$ , results not shown). Finally, we found that tree density of *E. coriacea* at a given location in the plot did not affect local herbivore damage ( $P = 0.191$ , Fig. 7d).

#### Discussion

This study examined the factors that determine inter- and intraspecific variation in susceptibility to herbivory at the community level in a hyperdiverse tropical rain forest in the

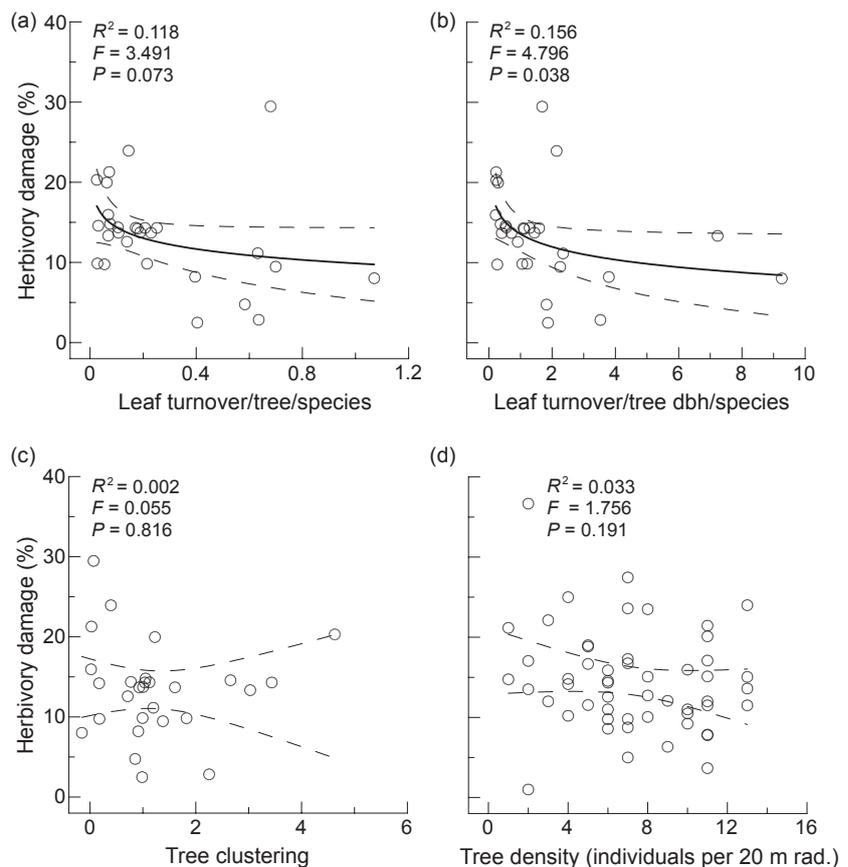


**Fig. 6.** Relationship of species growth rates with physicochemical eigenvalues. Black dots represent axis 1 eigenvalues, and grey dots represent axis 2 eigenvalues. Upper and lower dash-lines represent 5% and 95% respective confidence intervals of linear regressions:  $y_{Axis1} = -0.09x + 0.16$ ;  $y_{Axis2} = 0.07x - 0.12$ ; ( $P > 0.05$  in both cases).

Ecuadorian Amazon. We first explored whether sets of defensive traits trade-off among each other. We then tested if species traits (e.g. leaf quality) and phenological or ecological factors (e.g. leaf turnover, spatial distribution) predicted variation in leaf herbivore damage.

#### GENERAL CONSIDERATIONS: LEAF HERBIVORY PATTERNS IN A SPECIES-RICH TROPICAL FOREST

Data on leaf damage levels in rain forests are not common, and very few of them correspond to the Amazonian region. The overall range of herbivore damage for the 28 common species in our study was of 13.4% (2.49–29.46%), within the range of those observed in previous studies. Landsberg & Ohmart's (1989) review suggests that defoliation levels of tropical rain forests are between 5 and 15%. Lowman (1984) found an average of 14.6% of damage level in Australian subtropical rain forest canopies while Sterck, van der Meer & Bongers (1992) found that overall damage was  $> 5\%$  (0.8–12.8% in range) in French Guiana. More recently, Brenes-Arguedas, Coley & Kursar (2008) found ranges between 25% and 40% (32% in average) for *Inga* species in Panamá (BCI) and Ecuador (Yasuní). Note that we did not measure leaves that were completely eaten or subject to galls or mining damage, which therefore underestimates the total amount of herbivore damage occurring in Yasuní (e.g. Lowman 1984 found 6.3% underestimation in subtropical forests). However, studies that have compared single measurements to rates derived from repeated measurements of marked leaves underestimated 50% of herbivore damage in average (Coley & Barone 1996). Measurements made in our study represented the cumulative percentage of leaf area lost (Schuldt *et al.* 2012) including herbivory events from previous seasons, and more importantly, of the whole leaf life span (Coley & Barone 1996; Brenes-Arguedas, Coley & Kursar 2008). Factors such as



**Fig. 7.** Relationship of herbivore damage (%) with (a) leaf turnover/tree abundance, (b) leaf turnover/tree biomass estimation, and (c) tree clustering (from 28 common species in the study area). (d) Herbivore damage (%) and *E. coriacea* tree density (number of trees in a 20 m circle radius around the litter-fall traps) relationship. Empty circles in grey correspond to excluded data from the regressions that led to adjustments with  $P$  values  $< 0.05$  in both (a) and (c). The solid lines represent the trend of the relationship (for  $P$  values  $\leq 0.1$ ). Upper and lower dash-lines represent 5% and 95% confidence intervals.

local environment, microhabitat (i.e. canopy, understorey and gaps), temporality (i.e. dry vs. wet season) and the species of plants involved, may be explaining results variability worldwide (Landsberg & Ohmart 1989; Coley & Barone 1996).

Overall, we found a high variance in leaf herbivore damage at both inter- and intraspecific levels, which is congruent with other studies in both tropical and subtropical ecosystems (Kurokawa & Nakashizuka 2008; Schuldt *et al.* 2012). Coley (1983) found that intraspecific variance in leaf damage was rather similar among tree species showing different life histories, defensive characteristics and levels of leaf damage. Our results fully support these findings and further revealed that herbivore damage was also highly variable in space and time (results not shown), without any obvious patterns.

#### DO TROPICAL TREE SPECIES TRADE-OFF INVESTMENT ON PHYSICOCHEMICAL ANTIHERBIVORE DEFENCE?

Because in unproductive habitats energy investment and allocation in growth and defence should be carefully dosed, we expected to find a trade-off between physical and chemical defence investment given prior research and the fact that our study was performed in the relatively infertile soils of the Amazonian Region (Irion 1978; Fine *et al.* 2006). Despite this prediction, 4 of the 28 studied species showed either low physical and low chemical defences (e.g. *P. laevis*, *P. nodulosum*) or high physical and high chemical defences (e.g. *I. capitata*, *N. 'comun'*), which does not support a general trade-off at this site. Trade-offs among defences of most of the common species in this community may, however, fall along a tolerance/escape/defence syndromes continuum (Kursar & Coley 2003; Agrawal & Fishbein 2006) because not all combinations of multiple co-varying traits are possible due to physiological constraints (Kursar & Coley 2003). For example, five species (*P. sagotianum*, *P. laevis*, *M. malacocalyx*, *N. 'comun'* and *I. capitata*) found along a continuum of physicochemical defence syndromes, showed contrasting patterns of leaf turnover (Table S2). The most the investment on representative PCA physicochemical defences (e.g. thickness and tannins) the less the investment on leaf turnover. Regardless to the type of syndrome used by these plant species, with the exception of *M. malacocalyx*, their amount of damage caused by herbivores was comparable: 11.15%, 9.47%, 14.30% and 13.73%, respectively (Table S2).

Our results presenting single and interacting physical and chemical traits as deterrents of herbivores, agree with a pluralistic approach in the study of plant defences that considers a wide spectrum of defence traits and other ecological correlates (Agrawal 2011).

#### PHYSICOCHEMICAL LEAF TRAITS AND PLANT TOLERANCE/ESCAPE STRATEGIES TO DEAL WITH HERBIVORY IN THE AMAZON

Suites of covarying leaf traits are thought to reflect alternative strategies for coping with high herbivore pressure in aseasonal tropical forests. However, the relative importance of putative

defences remains unclear. Of the 14 plant traits we analysed, three physical and three chemical traits were strongly correlated with herbivore damage (Table 1), though, perhaps surprisingly, tannins and latex were not. PGLS analysis showed that leaf size, shearing resistance, cellulose and ash content were the best factors explaining herbivore damage. Positive correlation with leaf size supports the idea that larger leaves may attract more herbivores (Garibaldi, Kitzberger & Ruggiero 2011). Our results reveal that small leaves experience low herbivory as well. This could be explained, at least in part, through biomechanical constraints as very small or highly divided and dissected leaves, which may reduce herbivore feeding efficiency (Brown, Lawton & Grubb 1991). Interestingly, *E. 'minicomun'* and *I. auristellae*, both possessing the smallest leaves of the whole data set, also present nectaries as indirect defences while *Pourouma bicolor*, *P. nodulosum* and *M. malacocalyx* possessing the largest leaves lack this kind of indirect defences. The presence of extra-floral nectaries has proven to be an effective defence that use hymenopterans (ants particularly) that might be contributing to protect the plants against herbivore damage (Rosumek *et al.* 2009; Kessler & Heil 2011).

The force to shear (a measure of toughness) showed a significant negative relationship with herbivore damage, confirming that toughness is an efficient anti-herbivore defence (Lowell, Markham & Mann 1991; Choong *et al.* 1992; Coley & Barone 1996). This result makes sense biologically if we consider that most herbivore damage in the tropics are caused by invertebrates (~75%, Coley & Barone 1996) comparing with mammals (< 20%, Coley & Barone 1996) and that invertebrate radulas and mandibles act mainly as shears.

Cellulose showed to be positively related to herbivory in both simple linear regression and PGLS, contradicting the prediction that multiple carbon-based structural compounds are contributing factors improving leaf resistance to mechanical damage (Coley & Barone 1996; Schuldt *et al.* 2012). This may be related to the fact that we performed our measurements in young expanded leaves. Compared with mature leaves, young leaves may be more attractive and digestible to herbivores as they have less abundant cellulose, lignin and other cell wall compounds (Kursar & Coley 2003).

Ash content is a measure of defences such as calcium oxalates and silica-based phytoliths (the latter helps increasing toughness of plant tissues, Massey, Ennos & Hartley 2007; Moles *et al.* 2013), two components that strongly reduce herbivory (Korth *et al.* 2006; Massey, Ennos & Hartley 2006). Our results corroborate these findings showing a negative relationship between herbivore damage and ash content. Furthermore, PGLS analysis showed a significant interaction between ash and leaf size suggesting a generalized physicochemical defence strategy in our studied plant community respecting these functional traits. This suggests that from the physiological perspective, these strategies are compatible, not mutually exclusive, and are consequences of particular habitat selection pressures (Kursar & Coley 2003; Agrawal 2007).

Surprisingly, we found no support that tannins and latex represent effective antiherbivore defences. Our condensed tannins results agree with other large-scale analyses that found weak or no relationships between these phenolic compounds and herbivore damage (Coley 1983; Kurokawa & Nakashizuka 2008; Carmona, Lajeunesse & Johnson 2011). On the other hand, in spite that in the Amazon 20–35% of the plants are laticiferous –compared with 8.9% of the rest of the world– our results did not confirm the presence of latex as a predictor of lower herbivore damage in this ecosystem (Farrell, Dussourd & Mitter 1991; Lewinsohn 1991; Konno 2011). Tannins and latex may instead have a secondary role in defending plants from herbivores relative to morphology and physical resistance traits (Carmona, Lajeunesse & Johnson 2011; Schuldt *et al.* 2012). The presence of these compounds in high quantities may support the view that some plants contain accumulated, largely inactive secondary compounds resulting from past selection pressures (Jones, Firn & Malcolm 1991; Berenbaum 1995).

The defensive strategy chosen by any species may depend on the particular herbivore pressure each species is submitted to and the microenvironment where it grows (Janzen 1974; Fine *et al.* 2006). This confirms that the underlying factors and mechanisms behind defence syndromes and its continuums is a complex mosaic of action/reaction between plants and herbivores, especially in extreme biodiverse ecosystems such as tropical rain forests where there is an enormous variation of herbivory kinds and consequently plant responses.

#### EFFECT OF TREE GROWTH, LEAF TURNOVER AND SPATIAL DISTRIBUTION ON HERBIVORE SUSCEPTIBILITY

In a recent review, Endara & Coley (2011) evaluated the resource availability hypothesis (Coley, Bryant & Chapin 1985) that states in part that ‘for fast-growing species the optimal defence level is low, even though this leads to high rates of herbivory, and for slow-growing species, the optimal defence level is high, even though this cost further reduces the realized growth rate’ (Endara & Coley 2011). Our results did not support this prediction, as species with higher growth rates did not exhibit lower levels of defences and species with lower growth rates did not exhibit higher levels of defences. This is in contrast to Fine *et al.*'s (2006) study that showed a trade-off between growth rate and defence investment in 20 common species growing in two contrasting microhabitats in South-western Amazonia. Additional analyses where we compared growth rates with physical defences, chemical defences, cellulose, leaf size and ash, as well as some traits interactions (leaf size:ash, shearing:(C:N) ratios and shearing × ash), did not support this prediction either (results not shown). Other factors not measured in our study, such as root-feeding herbivory, may be contributing on the control of plants defence/growth strategies, as different kinds of herbivory (e.g. piercing) have different effects on the response of the plant and its consequent fitness (Strauss & Agrawal 1999). Finally, for pre-

cisely testing whether tree growth and herbivory are actually related, both parameters should be measured on individual trees and in the same time interval, as growth responses can be correlated with soil nutrients (Baribault, Kobe & Finley 2012) and light availability (Rüger *et al.* 2011).

A novel finding of our analysis was that trees producing fewer leaves were more susceptible to herbivore damage than more productive species. Our results strongly suggest that high frequency of leaf replacement may help plant species to deter herbivores more efficiently. Literature has shown that leaf production patterns are clearly associated with herbivory where, for example, synchronous flushing (new leaf production/emergence) is one strategy used by species to satiate specialist herbivores (Aide 1993; Coley & Barone 1996). Part of herbivore damage variability in these ecosystems may actually be explained by the fact that some individuals are heavily defoliated, while others are left untouched as a consequence of herbivore satiation (Lamarre *et al.* 2012). Unfortunately, we are not currently able to identify which of the species exhibit continuous or synchronous leafing, at what season were produced and what is its life span (see Aide 1993; Coley & Barone 1996 for other phenological traits implications on herbivory rates). Continuous or synchronous leafing patterns may not be necessarily reflected in the leaf litter-fall traps in part because some tropical tree species shed most or all of their leaves during the dry season to reduce water stress (Singh & Kushwaha 2005). Moreover, we did not find differences in herbivore damage between continuous and synchronous-shedding species in our study (*t*-test:  $F = 2.29$ ;  $P = 0.323$ ; results not shown).

At the interspecific level, tree clustering (when excluding *R. apiculata*) showed significant negative correlation with herbivore damage, such that species with clumped spatial distributions tended to have low herbivore damage. In two experimental studies Fine, Mesones & Coley (2004), and Fine *et al.* (2006) showed that species grew best on their home soil type (habitat specialization) in the presence of herbivores. This suggests that species at the limit of their habitat tolerances may be more susceptible to or less capable to recover from attack (Comita *et al.* 2010). Accordingly, soil properties and microtopography have been shown to shape species distributions in Yasuní (Valencia *et al.* 2004a; Endara & Jaramillo 2011). Clustering may thus reflect habitat specialization (Svenning 1999) and may imply specialized species may inhabit only very specific microenvironments, which are closer to its optimal. Our five most spatially aggregated species (*R. apiculata*, *B. jaramilloi*, *A. nicaraguensis*, *Rinorea viridifolia* and *E. 'minicomun'*) tended to show high investment in defence and, with the exception of *R. apiculata*, good effectiveness in deterring herbivores. Our results may contradict Janzen (1974) who suggested that clustered species adapted to resource-rich soils may better tolerate herbivory and invest less in defence relative to species on resource poor soils. If we hypothesize that the studied species grow in optimal soil environment (e.g. Endara & Jaramillo 2011), they should be able to tolerate local clustering by profusely investing in defences to counteract the synergistic effect of host density on herbivory events.

In high diversity tropical forests, individuals with many neighbouring conspecifics may have reduced survival due to increased competition for shared resources or elevated pressure from natural enemies (Janzen 1970; Connell 1971; Queenborough *et al.* 2007). Such negative density dependence (NDD) has important consequences for species coexistence and patterns of commonness and rarity (e.g. Comita *et al.* 2010). The mechanisms behind NDD are commonly thought to result from density-dependent host-specific natural enemies (e.g. soil pathogens, Freckleton & Lewis 2006). At the intraspecific level, we found, however, that *E. coriacea* tree density was not related to the amount of herbivory. Data on this particular species suggest that individuals are probably susceptible to herbivore damage because of relatively low physicochemical defences, and its commonness (i.e. abundance in the plot) may rely in part on efficient strategies to deter pathogens at relatively low energetic cost (e.g. high leaf turnover; extreme right angle circle, Fig. 7a).

## Acknowledgements

We thank the staff of the Yasuní Research Station, in particular the administrative personnel, David Lasso, Pablo Jarrín, Carlos Padilla and Karla Rivadeneira for constant support during the stays at the Station. Milton Zambrano and Pablo Alvia provide invaluable expertise on plant taxonomy in the field. This study would have been logistically impossible to carry out without them. We are grateful to Pablo Alvia, Anelio Looor, Gabriela Torrens and Álvaro Pérez for their help in plant measurements. Jim Self provided guidance and full support for leaf analyses at Soil, Water & Plant Testing Laboratory at CSU. We warmly thank to Philippe Belmont, Claire Capdevielle, and Stéphane Dupas for technical assistance on PGLS analysis. Simon Queenborough kindly allowed us to use unpublished data on young leaves colours of several species. Special acknowledgements to Nathan Swenson and three anonymous reviewers for insightful and critical comments that significantly helped improving this manuscript. We are grateful to the School of Biological Sciences of the PUCE, in particular to its dean Hugo Navarrete for constant support. The PUCE (project codes H13164 and J13041) and the French Institute for Research and Development (IRD) supported this study through research grants. RV thanks the program LTREB (award # 1122634) and Smithsonian Institution (Carbon Dynamics Project). This study was endorsed by the *Ministerio de Ambiente del Ecuador* permits MAE: 09-FLO-MA-DPO-PNY and 06-2011-FAU-DPAP-MA.

## References

- Abràmoff, M.D., Magalhães, P.J. & Ram, S.J. (2004) Image processing with ImageJ. *Biophotonics International*, **11**, 36–42.
- Agrawal, A.A. (2007) Macroevolution of plant defence strategies. *Trends in Ecology and Evolution*, **22**, 103–109.
- Agrawal, A.A. (2011) Current trends in the evolutionary ecology of plant defence. *Functional Ecology*, **25**, 420–432.
- Agrawal, A.A. & Fishbein, M. (2006) Plant defense syndromes. *Ecology*, **87**, S132–S149.
- Aide, T.M. (1988) Herbivory as a selective agent on the timing of leaf production in a tropical understory community. *Nature*, **336**, 574–575.
- Aide, T.M. (1992) Dry season leaf production: an escape from herbivory. *Biotropica*, **24**, 532–537.
- Aide, T.M. (1993) Patterns of leaf development and herbivory in a tropical understory community. *Ecology*, **74**, 455–466.
- Baribault, T.W., Kobe, R.K. & Finley, A.O. (2012) Tropical tree growth is correlated with soil phosphorus, potassium, and calcium, though not for legumes. *Ecological Monographs*, **82**, 189–203.
- Bass, M.S., Finer, M., Jenkins, C.N., Kreft, H., Cisneros-Heredia, D.F., McCracken, S.F. *et al.* (2010) Global conservation significance of Ecuador's Yasuní National Park. *PLoS ONE*, **5**, e8767.
- Bazzaz, F.A., Chiariello, N.R., Coley, P.D. & Pitelka, L.F. (1987) Allocating resources to reproduction and defense. *BioScience*, **37**, 58–67.
- Bell, T., Freckleton, R.P. & Lewis, O.T. (2006) Plant pathogens drive density-dependent seedling mortality in a tropical tree. *Ecology Letters*, **9**, 569–574.
- Berenbaum, M.R. (1995) The chemistry of defense: theory and practice. *Proceedings of the National Academy of Sciences*, **92**, 2–8.
- Brenes-Arquedas, T., Coley, P.D. & Kursar, T.A. (2008) Divergence and diversity in the defensive ecology of *Inga* at two Neotropical sites. *Journal of Ecology*, **96**, 127–135.
- Brown, V.K., Lawton, J.H. & Grubb, P.J. (1991) Herbivory and the evolution of leaf size and shape [and discussion]. *Philosophical Transactions of the Royal Society B*, **333**, 265–272.
- Carmona, D., Lajeunesse, M.J. & Johnson, M.T. (2011) Plant traits that predict resistance to herbivores. *Functional Ecology*, **25**, 358–367.
- Choong, M.F., Lucas, P.W., Ong, J.S.Y., Pereira, B., Tan, H.T.W. & Turner, I.M. (1992) Leaf fracture toughness and sclerophylly: their correlations and ecological implications. *New Phytologist*, **121**, 597–610.
- Coley, P.D. (1983) Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecological monographs*, **53**, 209–234.
- Coley, P.D. (1987) Interspecific variation in plant anti-herbivore properties: the role of habitat quality and rate of disturbance. *New Phytologist*, **106**, 251–256.
- Coley, P.D. & Aide, T.M. (1991) Comparison of herbivory and plant defenses in temperate and tropical broad-leaved forests. *Plant-Animal Interactions: Evolutionary Ecology in Tropical and Temperate Regions* (eds P.W. Price, T.M. Lewinsohn, G.W. Fernandes & W.W. Benson), pp. 25–49, Wiley, New York.
- Coley, P.D. & Barone, J.A. (1996) Herbivory and plant defenses in tropical forests. *Annual Review of Ecology and Systematics*, **27**, 305–335.
- Coley, P.D., Bryant, J.P. & Chapin, F.S. (1985) Resource availability and plant antiherbivore defense. *Science*, **230**, 895–899.
- Comita, L.S., Muller-Landau, H.C., Aguilar, S. & Hubbell, S.P. (2010) Asymmetric density dependence shapes species abundances in a tropical tree community. *Science*, **329**, 330–332.
- Condit, R. (1998) *Tropical Forest Census Plots: Methods and Results from Barro Colorado Island, Panama and a Comparison with Other Plots*. Springer-Verlag, Berlin.
- Connell, J.H. 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and rain forest trees. *Dynamics of Numbers in Populations*. (eds P.J. den Boer & G.R. Gradwell), pp. 298–312. Proceedings of the Advanced Study Institute, Osterbeek, 1970. Centre for Agricultural Publication and Documentation, Wageningen.
- Cornelissen, J.H.C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D.E., Reich, P.B., ter Steege, H., Morgan, H.D., van der Heijden, M.G.A., Pausas, J.G. & Poorter, H. (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany*, **51**, 335–380.
- Costantini, P., Linting, M. & Porzio, G.C. (2009) Mining performance data through nonlinear PCA with optimal scaling. *Applied Stochastic Models in Business and Industry*, **26**, 85–101.
- Dangles, O., Carpio, F. & Woodward, G. (2012) Size-dependent species removal impairs ecosystem functioning in a large-scale tropical field experiment. *Ecology*, **93**, 2615–2625.
- Dobson, A. & Crawley, M. (1994) Pathogens and the structure of plant communities. *Trends in Ecology & Evolution*, **9**, 393–398.
- Ellis, R.N., Kroonenberg, P.M., Harch, B.D. & Basford, K.E. (2006) Non-linear principal component analysis: an alternative method for finding patterns in environmental data. *Environmetrics*, **17**, 1–11.
- Endara, M.J. & Coley, P.D. (2011) The resource availability hypothesis revisited: a meta-analysis. *Functional Ecology*, **25**, 389–398.
- Endara, M.J. & Jaramillo, J.J. (2011) The influence of microtopography and soil properties on the distribution of the speciose genus of trees, *Inga* (Fabaceae: Mimosoidea), in Ecuadorian Amazonia. *Biotropica*, **43**, 157–164.
- Farrell, B.D., Dussourd, D.E. & Mitter, C. (1991) Escalation of plant defense: do latex and resin canals spur plant diversification? *The American Naturalist*, **138**, 881–900.
- Felsenstein, J. (1985) Phylogenies and the comparative method. *American Naturalist*, **125**, 1–15.
- Fine, P.V., Mesones, I. & Coley, P.D. (2004) Herbivores promote habitat specialization by trees in Amazonian forests. *Science*, **305**, 663–665.
- Fine, P.V., Miller, Z.J., Mesones, I., Irazuzta, S., Appel, H.M., Stevens, M.H.H., S. ksj rvi, I., Schultz, J.C. & Coley, P.D. (2006) The growth-defense trade-off and habitat specialization by plants in Amazonian forests. *Ecology*, **87**, S150–S162.
- Freckleton, R.P. & Lewis, O.T. (2006) Pathogens, density dependence and the coexistence of tropical trees. *Proceedings of the Royal Society B: Biological Sciences*, **273**, 2909–2916.

- Garibaldi, L.A., Kitzberger, T. & Ruggiero, A. (2011) Latitudinal decrease in folivory within *Nothofagus pumilio* forests: dual effect of climate on insect density and leaf traits? *Global Ecology and Biogeography*, **20**, 609–619.
- Gifi, A. (1991) *Nonlinear Multivariate Analysis*. Wiley, Chichester.
- Gilbert, G.S., Hubbell, S.P. & Foster, R.B. (1994) Density and distance-to-adult effects of a canker disease of trees in a moist tropical forest. *Oecologia*, **98**, 100–108.
- Grafen, A. (1989) The phylogenetic regression. *Philosophical Transactions of the Royal Society of London, Series B*, **326**, 119–157.
- Hanley, M.E., Lamont, B.B., Fairbanks, M.M. & Rafferty, C.M. (2007) Plant structural traits and their role in anti-herbivore defence. *Perspectives in Plant Ecology, Evolution and Systematics*, **8**, 157–178.
- Herms, D.A. & Mattson, W.J. (1992) The dilemma of plants: to grow or defend. *Quarterly Review of Biology*, **67**, 283–335.
- Huntly, N. (1991) Herbivores and the dynamics of communities and ecosystems. *Annual Review of Ecology and Systematics*, **22**, 477–503.
- Irion, G. (1978) Soil infertility in the Amazonian rain forest. *Naturwissenschaften*, **65**, 515–519.
- Janzen, D.H. (1970) Herbivores and the number of tree species in tropical forests. *American Naturalist*, **104**, 501–528.
- Janzen, D.H. (1974) Tropical blackwater rivers, animals, and mast fruiting by the Dipteroocarpaceae. *Biotropica*, **6**, 69–103.
- Johnson, S.N., Clark, K.E., Hartley, S.E., Jones, T.H., McKenzie, S.W. & Krichcheva, J. (2012) Aboveground-belowground herbivore interactions: a meta-analysis. *Ecology*, **93**, 2208–2215.
- Jones, C.G., Finn, R.D. & Malcolm, S.B. (1991) On the evolution of plant secondary chemical diversity [and discussion]. *Philosophical Transactions of the Royal Society of London B*, **333**, 273–280.
- Kessler, A. & Heil, M. (2011) The multiple faces of indirect defences and their agents of natural selection. *Functional Ecology*, **25**, 348–357.
- Konno, K. (2011) Plant latex and other exudates as plant defense systems: roles of various defense chemicals and proteins contained therein. *Phytochemistry*, **72**, 1510–1530.
- Korth, K.L., Doege, S.J., Park, S.H., Goggin, F.L., Wang, Q., Gomez, S.K., Liu, G., Jia, L. & Nakata, P.A. (2006) *Medicago truncatula* mutants demonstrate the role of plant calcium oxalate crystals as an effective defence against chewing insects. *Plant Physiology*, **141**, 188–195.
- Kraft, N.J.B. & Ackerly, D.D. (2010) Functional trait and phylogenetic tests of community assembly across spatial scales in an Amazonian forest. *Ecological Monographs*, **80**, 401–422.
- Kraft, N.J.B., Valencia, R. & Ackerly, D.D. (2008) Functional traits and niche-based tree community assembly in an Amazonian forest. *Science*, **322**, 580–582.
- Kurokawa, H. & Nakashizuka, T. (2008) Leaf herbivory and decomposability in a Malaysian tropical rain forest. *Ecology*, **89**, 2645–2656.
- Kursar, T.A. & Coley, P.D. (1992) Delayed greening in tropical leaves: an anti-herbivore defense? *Biotropica*, **24**, 256–262.
- Kursar, T.A. & Coley, P.D. (2003) Convergence in defense syndromes of young leaves in tropical rainforests. *Biochemical Systematics and Ecology*, **31**, 929–949.
- Lamarre, G.P., Baraloto, C., Fortunel, C., Dávila, N., Mesones, I., Rios, J.G., Valderrama, E., Vásquez Pilco, M. & Fine, P.V. (2012) Herbivory, growth rates, and habitat specialization in tropical tree lineages: implications for Amazonian beta-diversity. *Ecology*, **93**, S195–S210.
- Landsberg, J. & Ohmart, C. (1989) Levels of insect defoliation in forests: patterns and concepts. *Trends in Ecology & Evolution*, **4**, 96–100.
- Lewinsohn, T.M. (1991) The geographical distribution of plant latex. *Chemoecology*, **2**, 64–68.
- Loeuille, N., Loreau, M. & Ferriere, R. (2002) Consequences of plant-herbivore coevolution on the dynamics and functioning of ecosystems. *Journal of Theoretical Biology*, **217**, 369–381.
- Loranger, J., Meyer, S.T., Shipley, B., Kattge, J., Loranger, H., Roscher, C., Wirth, C. & Weisser, W.W. (2013) Predicting invertebrate herbivory from plant traits: polycultures show strong nonadditive effects. *Ecology*, **94**, 1499–1509.
- Lowell, R.B., Markham, J.H. & Mann, K.H. (1991) Herbivore-like damage induces increased strength and toughness in a seaweed. *Proceedings of the Royal Society of London B*, **243**, 31–38.
- Lowman, M.D. (1984) An assessment of techniques for measuring herbivory: is rainforest defoliation more intense than we thought? *Biotropica*, **16**, 264–268.
- Massey, F.P., Ennos, A.R. & Hartley, S.E. (2006) Silica in grasses as a defence against insect herbivores: contrasting effects on folivores and a phloem feeder. *Journal of Animal Ecology*, **75**, 595–603.
- Massey, F.P., Ennos, A.R. & Hartley, S.E. (2007) Herbivore specific induction of silica-based plant defences. *Oecologia*, **152**, 677–683.
- Mattson, W.J. (1980) Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics*, **11**, 119–161.
- Moles, A.T., Peco, B., Wallis, I.R., Foley, W.J., Poore, A.G., Seabloom, E.W. *et al.* (2013) Correlations between physical and chemical defences in plants: tradeoffs, syndromes, or just many different ways to skin a herbivorous cat? *New Phytologist*, **198**, 252–263.
- Möller, J. (2009) Gravimetric determination of acid detergent fiber and lignin in feed: interlaboratory study. *Journal of AOAC International*, **92**, 74–90.
- Ohgushi, T. (2005) Indirect interaction webs: herbivore-induced effects through trait change in plants. *Annual Review of Ecology, Evolution, and Systematics*, **36**, 81–105.
- Onoda, Y., Westoby, M., Adler, P.B., Choong, A.M., Clissold, F.J., Cornelissen, J.H. *et al.* (2011) Global patterns of leaf mechanical properties. *Ecology Letters*, **14**, 301–312.
- Pagel, M. (1999) Inferring the historical patterns of biological evolution. *Nature*, **401**, 877–884.
- Porter, L.J., Hrstich, L.N. & Chan, B.G. (1986) The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry*, **25**, 223–230.
- Price, P.W., Bouton, C.E., Gross, P., McPherson, B.A., Thompson, J.N. & Weis, A.E. (1980) Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics*, **11**, 41–65.
- Queenborough, S.A., Burslem, D.F., Garwood, N.C. & Valencia, R. (2007) Neighborhood and community interactions determine the spatial pattern of tropical tree seedling survival. *Ecology*, **88**, 2248–2258.
- Queenborough, S.A., Metz, M.R., Valencia, R. & Wright, S.J. (2013) Demographic consequences of chromatic leaf defence in tropical tree communities: do red young leaves increase growth and survival? *Annals of Botany*, **112**, 677–684.
- R Development Core Team (2013) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org/>.
- Read, J., Sanson, G.D., Caldwell, E., Clissold, F.J., Chatain, A., Peeters, P., Lamont, B.B., De Garine-Wichatitsky, M., Jaffre, T. & Kerr, S. (2009) Correlations between leaf toughness and phenolics among species in contrasting environments of Australia and New Caledonia. *Annals of Botany*, **103**, 757–767.
- Ripley, B.D. (1981) *Spatial Statistics*. Wiley, New York.
- Romero-Saltoa, H.G. (2011) Community and functional ecology of lianas in the Yasuni forest dynamics plot, Amazonian Ecuador. PhD thesis, University of Miami, Miami, FL, USA.
- Rosumek, F.B., Silveira, F.A.O., Neves, F.D., Barbosa, N.P.D., Diniz, L., Oki, Y., Pezzini, F., Fernandes, G.W. & Cornelissen, T. (2009) Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. *Oecologia*, **160**, 537–549.
- Rüger, N., Berger, U., Hubbell, S.P., Vieilledent, G. & Condit, R. (2011) Growth strategies of tropical tree species: disentangling light and size effects. *PLoS ONE*, **6**, e25330.
- Schuldt, A., Bruehlheide, H., Durka, W., Eichenberg, D., Fischer, M., Kröber, W. *et al.* (2012) Plant traits affecting herbivory on tree recruits in highly diverse subtropical forests. *Ecology Letters*, **15**, 732–739.
- Singh, K.P. & Kushwaha, C.P. (2005) Emerging paradigms of tree phenology in dry tropics. *Current Science*, **89**, 964–975.
- Sterck, F., van der Meer, P. & Bongers, F. (1992) Herbivory in two rain forest canopies in French Guyana. *Biotropica*, **24**, 97–99.
- Strauss, S.Y. & Agrawal, A.A. (1999) The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology & Evolution*, **14**, 179–185.
- Sullivan, J.J. (2003) Density-dependent shoot-borer herbivory increases the age of first reproduction and mortality of neotropical tree saplings. *Oecologia*, **136**, 96–106.
- Svenning, J.C. (1999) Microhabitat specialization in a species-rich palm community in Amazonian Ecuador. *Journal of Ecology*, **87**, 55–65.
- Tuomi, J., Niemelä, P., Haukioja, E., Sirén, S. & Neuvonen, S. (1984) Nutrient stress: an explanation for plant anti-herbivore responses to defoliation. *Oecologia*, **61**, 208–210.
- Twigg, L.E. & Socha, L.V. (1996) Physical versus chemical defence mechanisms in toxic *Gastrolabium*. *Oecologia*, **108**, 21–28.
- Valencia, R., Foster, R.B., Villa, G., Condit, R.G., Svenning, J.C., Hernandez, C., Romoleroux, K., Losos, E.C., Magard, E. & Balslev, H. (2004a) Tree species distributions and local habitat variation in the Amazon: large forest plot in eastern Ecuador. *Journal of Ecology*, **92**, 214–229.
- Valencia, R., Condit, R.G., Foster, R.B., Romoleroux, K., Villa Muñoz, G., Svenning, J.C., Magard, E., Bass, M., Losos, E.C. & Balslev, H. (2004b)

Yasuní Forest Dynamics Plot, Ecuador. *Tropical Forest Diversity and Dynamism: Findings from a Large-Scale Plot Network* (eds E.C. Losos, J. Leigh & E. Giles), pp. 609–628. University of Chicago Press, Chicago.

Received 10 October 2013; accepted 31 March 2014

Handling Editor: Nathan Swenson

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Appendix S1.** Litter-fall traps distribution.

**Appendix S2.** Leaf litter abundance in traps.

**Appendix S3.** Phylogenetic analysis.

**Figure S1.** Topographic map of the YRS-PUCE subplot and the location of the 100 litter-fall traps used for this assessment (coordinates X: 50–700; Y: 100–500).

**Figure S2.** Monthly leaf litter production of 18 common species in Yasuní National Park.

**Figure S3.** Phylogenetic tree using *rbcl* chloroplastic gene sequences.

**Table S1.** List of plant species and taxonomical position.

**Table S2.** Leaf turnover estimations.