Plant herbivory responses through changes in leaf quality have no effect on subsequent leaf-litter decomposition in a neotropical rain forest tree community

Rafael E. Cárdenas[1,2,3], Stephan Hättenschwiler[4], Renato Valencia[5], Adriana Argoti[1] and Olivier Dangles[2,3]

[1]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; [2]Institut de Recherche pour le Développement (IRD), UR 072, LEGS-CNRS, UPR 9034, CNRS, Gif-sur-Yvette, Cedex 91198, France; [3]Université Paris-Sud 11, Orsay, Cedex 91405, France; [4]Centre d'Écologie Fonctionnelle et Évolutive (CEFE UMR 5175 – Université de Montpellier – Université Paul-Valéry Montpellier – EPHE), 1919 route de Mende, 34293 Montpellier Cedex 5, France; [5]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

Author for correspondence:
Rafael E. Cárdenas
Tel: +593 2 2991 700 ext 1802
Email: recardenasm@yahoo.com

Summary

- It is commonly accepted that plant responses to foliar herbivory (e.g. plant defenses) can influence subsequent leaf-litter decomposability in soil. While several studies have assessed the herbivory–decomposability relationship among different plant species, experimental tests at the intra-specific level are rare, although critical for a mechanistic understanding of how herbivores affect decomposition and its consequences at the ecosystem scale.
- Using 17 tree species from the Yasuní National Park, Ecuadorian Amazonia, and applying three different herbivore damage treatments, we experimentally tested whether the plant intra-specific responses to herbivory, through changes in leaf quality, affect subsequent leaf-litter decomposition in soil.
- We found no effects of herbivore damage on the subsequent decomposition of leaf litter within any of the species tested. Our results suggest that leaf traits affecting herbivory are different from those influencing decomposition. Herbivore damage showed much higher intra-specific than inter-specific variability, while we observed the opposite for decomposition.
- Our findings support the idea that interactions between consumers and their resources are controlled by different factors for the green and the brown food-webs in tropical forests, where herbivory may not necessarily generate any direct positive or negative feedbacks for nutrient cycling.

Introduction

Biotic interactions at the interface between the above- and belowground compartments of terrestrial ecosystems play a fundamental role in regulating their structure and functioning, such as nutrient cycling (Bardgett & Wardle, 2010). For example, the decomposition of dead organic matter (OM) of aboveground origin is a key process influenced by both aboveground (e.g. the quality of decaying litter; Kaspari *et al.*, 2008; Hättenschwiler *et al.*, 2011) and belowground factors (e.g. the diversity of decomposer organisms; Pramanik *et al.*, 2001; Gessner *et al.*, 2010). In addition, decomposition can be modified by biotic interactions aboveground, such as herbivory, through changes in energy fluxes with feedbacks on soil fauna biomass distribution (Mulder *et al.*, 2008), and the quality and quantity of OM input (Grime *et al.*, 1996; Wardle *et al.*, 2004; van Dam & Heil, 2011). Such herbivore effects can have important consequences for nutrient availability and plant productivity (reviewed by Vitousek & Sanford, 1986; Hunter, 2001; Cebrián & Lartigue,

2004). Herbivory can show either negative or positive effects on decomposition depending on the context and the specific type of ecosystem, but also depending on the temporal scale at which the herbivore impact is evaluated. According to the fertility and productivity of an ecosystem, herbivory has different consequences for plants, leading to distinct impacts on nutrient cycling (Buckland & Grime, 2000; Wardle *et al.*, 2004). For example, in the short term, and in infertile ecosystems, herbivores may negatively affect nutrient cycling and plant productivity through selective foraging on plants that have relatively nutrient rich and less defended tissues compared with co-occurring plants. At the ecosystem scale this leads then to overall poorer quality leaves that subsequently turn into poor quality litter with comparatively slow decomposition rates (Bardgett & Wardle, 2010). As a consequence, in the long term, well-defended plants that also produce recalcitrant litter will be selected and ultimately dominate the community (Ritchie *et al.*, 1998), leading to further reduced rates of nutrient cycling (Bardgett & Wardle, 2010). A similar, but positive feedback loop can occur in fertile and productive

ecosystems (Bardgett & Wardle, 2010). Accordingly, we would expect a negative feedback loop for nutrient-impoverished tropical rainforests (Irion, 1978), but the relationship between herbivory and litter decomposition in tropical forests remains controversial. Several studies have suggested a weak association between the two processes (Didham, 1998; Kurokawa & Nakashizuka, 2008; Cárdenas & Dangles, 2012).

High herbivore diversity and herbivory pressure in some tropical rain forests (Novotny *et al.*, 2006; Salazar & Marquis, 2012) have led to large variations in tree strategies to avoid herbivory, such as phenological defenses (e.g. growth rates, leaf production, synchronized flushing and leaf turnover; Aide, 1988, 1993; Coley, 1988; Cárdenas *et al.*, 2014), indirect defenses by attracting enemies of herbivores (extra-floral nectaries/glands on the twigs; Kessler & Heil, 2011), physical defenses (leaf toughness, presence of hairs and spines; Lowell *et al.*, 1991; Choong *et al.*, 1992; Hanley *et al.*, 2007; Cárdenas *et al.*, 2014) and chemical defenses (reviewed by Coley & Barone, 1996; Fine *et al.*, 2013). This wide diversity of plant defense strategies combined with the structural diversity of tropical forests results in a high spatial and temporal heterogeneity in leaf and leaf-litter quality and subsequent litter decomposition.

It is often assumed that intra-specific variability is negligible compared with inter-specific variability (Garnier *et al.*, 2001; Westoby *et al.*, 2002), and hence most studies have typically analyzed the herbivory–decomposition relationship by comparing different plant species (i.e. comparing averaged trait values among species), but ignoring the potential effect of intra-specific variability (Asplund & Wardle, 2014). Within-species differences in herbivore pressure and plant defense status may result in considerable differences in plant responses to herbivore damage and hence in leaf-litter quality among individuals of a given species, and consequently in the activity of decomposers exploiting them. Intra-specific differences in leaf quality and the degree of herbivore damage have resulted in significant differences in the decomposability of leaf litter in stream systems (e.g. LeRoy *et al.*, 2007; Lecerf & Chauvet, 2008). To our knowledge, this has not been tested in plant species-rich terrestrial ecosystems. However, even in these ecosystems, such as the tropical rainforests, intra-specific differences in leaf herbivory can be large (Coley, 1983a; Boege & Dirzo, 2004; Brenes-Arguedas *et al.*, 2008). Variability in the percentage of damaged leaf area of up to 100-fold has been documented within species of tropical trees (Lowman, 1984; Cárdenas *et al.*, 2014). It is largely unknown how common such high intra-specific variability in leaf damage is among different species in these species-rich ecosystems, and how plant responses to herbivory affect subsequent leaf-litter decomposition.

Here, we aimed to evaluate whether the intra-specific responses to herbivory, through changes in leaf quality, affect the subsequent leaf-litter decomposition of 17 different tree species growing in the highly diverse neotropical rainforest of the Yasuní National Park in Ecuador. For this purpose we experimentally tested the relationship between herbivore damage and decomposition. Taking into account the plant phylogenetic relatedness of the studied species, we assessed whether variability in herbivore damage affects litter decomposability, asking the following specific questions. (1) Does leaf litter previously damaged by canopy herbivores decompose at a different rate compared with undamaged litter? (2) Does the action of canopy herbivores over the leaf lifetime affect the quality of leaf litter? (3) Can the variability in decomposition of damaged vs undamaged leaf litter be explained by the same suite of initial litter quality traits?

Materials and Methods

Using litterbags to assess decomposition and statistical regression analyses, we addressed our first question by testing whether there exists a relationship between the susceptibility of green leaves to herbivory and leaf-litter decomposition across 17 common tree species in the study area. To answer our second question, we compared the covariance of a suite of physical and chemical traits of undamaged green leaves with damaged and undamaged leaf litter across tree species. Finally, our third question was tackled by exploring which physical and chemical traits better explained variability in decomposition of herbivore-damaged and undamaged leaf litter using phylogenetic generalized least square and simple linear regression analyses.

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 (*c.* 17.7% of the Ecuadorian territory; Valencia *et al.*, 2004) harboring 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. Our study area was located in the vicinity of the Yasuní Research Station (YRS) of the Pontificia Universidad Católica del Ecuador (00°40′16.7″S, 07°24′1.8″W) in a *c.* 4300-m^2 *terra firme* forest floor plot composed of slope- and valley-type habitats (see Valencia *et al.*, 2004 for a detailed description of micro-habitats). Valleys occasionally flood, but only for brief periods. Soils are mostly clayey, udult ultisols, with an average pH of 4.6 (John *et al.*, 2007) and a texture dominated by silt (Tuomisto *et al.*, 2003). Tree canopy height at our study site varies between 15 and 30 m, with some emergent trees reaching 40–50 m (Valencia *et al.*, 2004). Rainfall and temperature are aseasonal; that is, there are no clear patterns of dry and wet or warm and cool seasons. During 53 months of existing meteorological records at the research station, the longest period without rain was 3 wk, the mean annual rainfall was 2826 mm and none of the 12 calendar months had < 100 mm of rainfall (Valencia *et al.*, 2004). Mean air temperature is $24.9 \pm 3.9°C$ (daily averages ranging from 22.0 to 32.0°C) and the mean relative air humidity is $88.4 \pm 13.9\%$ (data obtained from YRS meteorological station; http://www.yasuni.ec).

Tree species

Leaves are the most important component of annual litter-fall in Amazonian forests (Chave *et al.*, 2010), with strong angiosperm

tree domination (Kurokawa & Nakashizuka, 2008). We selected 17 angiosperm tree species that are common at our study site (Valencia *et al.*, 2004). In addition to commonness, the species were also chosen in order to represent a wide range of taxa, covering a total of 11 different families and nine orders that are abundant at our study site (Table 1).

Assessment of herbivore damage proportions

We took the herbivory-induced leaf damage data from Cárdenas *et al.* (2014). Briefly, in this previous study, herbivore damage was quantified as the proportion of lost lamina area of pressed, dried and scanned senesced leaves using the IMAGEJ open source image processor (http://rsb.info.nih.gov/ij/; Abràmoff *et al.*, 2004). Measurements were performed on fallen leaves collected in 100 litter-fall traps installed under the forest canopy (separated by *c.* 20 m in a 600×400 m^2 area; see appendix S1 in Cárdenas *et al.*, 2014) and covering a range of different individuals of each tree species over an 11-month period between February 2011 and January 2012. The leaves collected in the traps corresponded to the litter fall of the last 15 continuous days of each month, whereupon traps were emptied. Overall, 4–40% of the litter-fall traps collected leaves of the 17 studied species covering the whole study area. Although impossible to quantify precisely, we calculated that on average ($\pm$ SD) litter-fall traps collected leaves from *c.* 7 ($\pm$ 5) individual trees per species (Supporting Information Table S1; and see fig. 7d in Cárdenas *et al.*, 2014). All leaves intercepted in the litter-fall traps were included (e.g. leaves from all parts of the tree canopy including sun and shade leaves). After scanning, leaf images were cleaned (to erase shadows, fill scratches and eliminate the petiole) and binary-transformed (Cárdenas *et al.*, 2014). This herbivore damage metric captures primarily the action of leaf chewers (e.g. Orthopterans, Lepidopteran larvae, Molluscs, and Coleopterans such as Chrysomelids). Piercing/sucking damage (e.g. by Cicadellids, Curculionids, and Cercopids) was not taken into account as it was impossible to measure the damage of such

type of herbivory using our sampling methodology. Additionally, leaves with mines, galls, necrotic areas, and scraped, scratched, ripped or torn surfaces were excluded from the analysis (< 20% of the total leaf collection; R. E. Cárdenas, pers. obs.). For comparison purposes within this study, herbivory data were averaged at the litter-fall trap sample unit (see later section 'Data analyses').

Measuring herbivory on senesced leaves allows integration of the accumulated damage by herbivores over the course of the entire leaf life span (Brenes-Arguedas *et al.*, 2008; Schuldt *et al.*, 2012). At the same time, the herbivore damage proportions determined on senescent leaves are representative for decomposing leaves, as opposed to methods based on the quantification of herbivory rates at different phenological stages of canopy leaves (e.g., Kurokawa & Nakashizuka, 2008).

Experimental study: herbivory–decomposability relationship

A potential approach for understanding herbivory–decomposition relationships is to compare the decomposability of senescent leaves with different degrees of herbivore damage (Cárdenas & Dangles, 2012). For this, in April 2011, freshly fallen senescent leaves were collected from three to five individuals of sub-adult and adult trees of the 17 above-mentioned common tree species by shaking the trunk or individual branches five to 20 times. Falling leaves were intercepted in white cotton sheets (1.5 m $\times$ 3.5 m) that were tied at 1 m above the forest floor. Leaves that were too young (typically presenting bright green colors), too old (i.e. rotten or presenting large amounts of necrosis), or presenting obvious fungal infection or insect galleries or galls were discarded (*c.* 5–20% of the total leaves collected per species). Leaves were then dried at 40°C for up to 72 h.

All leaves were visually sorted into 'undamaged' leaves (range within 0–10% of herbivore damage) and 'damaged' leaves (range within 30–60% of herbivore damage). These two classes were defined based on previous experiments in tropical forests

Table 1 List of the 17 common species used in the decomposition experiment along with taxonomical information (species names in apostrophes are temporary)

Code	Tree species name	Family	Order
DUROHI	*Duroia hirsuta* (Poepp.) K. Schum.	Rubiaceae	Gentianales
INGACA	*Inga capitata* Desv.	Fabaceae	Fabales
IRYAHO	*Iryanthera hostmannii* (Benth.) Warb.	Myristicaceae	Magnoliales
LEONGL	*Leonia glycycarpa* Ruiz & Pav.	Violaceae	Malpighiliales
MABESU	*Mabea* 'superbrondu' [nomen nudum]	Euphorbiaceae	Malpighiliales
MACRYA	*Macrolobium* 'yasuni' [nomen nudum]	Fabaceae	Fabales
MATIMA	*Matisia malacocalyx* (A. Robyns & S. Nilsson) W.S. Alverson	Malvaceae	Malvales
MICOPU	*Miconia* 'purpono' [nomen nudum]	Melastomataceae	Myrtales
NAUCKR	*Naucleopsis krukovii* (Standl.) C.C. Berg	Moraceae	Rosales
NECTVI	*Nectandra viburnoides* Meisn.	Lauraceae	Laurales
NEEACO	*Neea* 'comun' [nomen nudum]	Nyctaginaceae	Caryophyllales
PSEULS	*Pseudolmedia laevis* (Ruiz & Pav.) J.F. Macbr.	Moraceae	Rosales
RINOLI	*Rinorea lindeniana* (Tul.) Kuntze	Violaceae	Malpighiliales
RINOVI	*Rinorea viridifolia* Rusby	Violaceae	Malpighiliales
SIPACU	*Siparuna cuspidata* (Tul.) A. DC.	Siparunaceae	Laurales
SIPADE	*Siparuna decipiens* (Tul.) A. DC.	Siparunaceae	Laurales
SOROST	*Sorocea steinbachii* C.C. Berg	Moraceae	Rosales

where damaged proportions were measured (e.g. Lowman, 1984; Landsberg & Ohmart, 1989; Sterck *et al.*, 1992; Brenes-Arguedas *et al.*, 2008; Cárdenas *et al.*, 2014). For each species, the collected leaves were then grouped into three batches: leaves that were damaged by herbivores; undamaged leaves; and a subgroup of undamaged leaves that were afterwards punched artificially. Punched leaves were included to account for purely physical effects of herbivore damage (as opposed to the potential chemical effects of herbivory events), that is, increased leaf-edge accessibility for decomposers. Punching was done in such a way as to represent the natural inter- and intra-specific variability of herbivore damage determined in the first batch of herbivore-damaged leaves across individual trees. Leaves were punched with a 14-mm-diameter iron cork borer. As leaf size differed within and among species, the number of holes per leaf necessary to achieve damage of c. 30–60% differed.

Litterbags were constructed using two plastic mesh disks (20 cm in diameter), slightly 'U' bent, and sown together around the edges. The resulting oval-shaped litterbags allowed the enclosed litter to retain its natural three-dimensional structure without flattening the litter, as is the case with the traditionally flat litterbags. This considerably facilitates access by detritivorous arthropods (e.g. cockroaches or thysanurans which present tigmotactism). Because soil fauna plays an important role in decomposition, particularly in tropical rainforests (Coq *et al.*, 2010), the top side had holes of 900 mm^2 to allow free access for soil micro-, meso-, macro- and mega-fauna. By contrast, we used mesh with holes of 100 mm^2 for the soil-facing bottom side of the litterbags in order to limit gravimetric loss of litter material while still allowing access to micro-, meso- and macro-fauna (see Swift *et al.*, 1979 for detritivore size classification). Each litterbag was filled with two to seven leaves (petioles removed) depending on its size. This resulted in 2.91 ± 1.57 g (mean ± SD) of leaf-litter material per bag. In total, we constructed 510 litterbags (17 species × 3 treatments × 10 litterbag replicates). Litterbags were divided into 85 lots (3 treatments × 2 out of 17 species chosen randomly) and placed directly on the soil surface, in the same area where we collected the leaves. Because of the particular topography of the study area (ridges, slopes and valleys; Valencia *et al.*, 2004), the maximum distance between lots was c. 172 m and the minimum was c. 5 m (in a grid of 17 × 5 lots) in order to ensure comparable biotic and geomorphologic site conditions. After 100 d of exposure in the field, all litterbags were collected for analyses. In the laboratory, leaves were gently cleaned in order to remove soil particles, adhering debris, and invertebrates, and then dried at 40°C for up to 96 h, and weighed.

Leaf-litter trait analyses

For the 17 studied tree species, a subset of 10–30 undamaged and damaged leaves per species collected in April 2011 were separated from the rest of the collected leaves into three to five subsamples corresponding to individual trees for subsequent leaf quality analyses. All leaves were dried at 40°C for up to 96 h, ground in a coffee grinder and kept in dry conditions until analysis.

We selected a total of nine physical and chemical traits that have been shown to correlate with decomposition previously (Pérez & Jeffries, 1992; Cornelissen *et al.*, 2003; Duarte *et al.*, 2008; Kurokawa & Nakashizuka, 2008; Hättenschwiler *et al.*, 2011; Kaspari *et al.*, 2014). Thickness was measured, avoiding primary and secondary veins, using an analog 0–25-mm micrometer caliper at 0.005 mm precision (Amico Corp., Richmond Hill, ON, Canada) (Cornelissen *et al.*, 2003). Chemical traits were measured at the Colorado State University (Fort Collins, CO, USA) Soil, Water and Plant Testing Laboratory (http://www.soiltestinglab.colostate.edu/). Nitrogen (N) and carbon (C) were measured with a CN analyzer (Leco® TruSpec Micro CN analyzer; Leco Corp., St Joseph, MI, USA). Concentrations of lignin and cellulose were determined gravimetrically using acid detergent fiber (ADF) and acid detergent lignin (ADL) methodology (Möller, 2009). Condensed tannins were measured using the Butanol-HCl method and expressed as leucocyanidin equivalent (% DM) following Porter *et al.* (1986). Manganese (Mn), copper (Cu) and sodium (Na) concentrations were measured using the inductively coupled plasma atomic emission spectroscopy (ICP-AES) methodology (Boumans, 1987). Finally, ash content, considered as a measure of anti-herbivore defenses such as silica-based phytoliths and calcium oxalates (Moles *et al.*, 2013), corresponded to the leaf mass remaining after combustion of ADL samples at 550°C for 2 h (Möller, 2009).

Green leaf trait analyses

Green leaf trait measurements were taken from Cárdenas *et al.* (2014). Briefly, in August 2012 undamaged green foliar material was collected from the same set of selected individual trees located near YRS-PUCE trails used for decomposition experiments. Individuals that showed unusually heavy impact of herbivores or that lacked sufficient recently produced, fully expanded new leaves were rejected (Cornelissen *et al.*, 2003). Typically, the targeted leaves were the youngest of the leaves on the tree, exhibiting bright green coloration relative to older (mature) leaves. A range of traits were selected, both physical and chemical, that have previously been shown to correlate with herbivory or anti-herbivory properties (Cornelissen *et al.*, 2003; Hanley *et al.*, 2007; Kurokawa & Nakashizuka, 2008; Moles *et al.*, 2013). Among these traits, leaf thickness and the concentrations of N, C, condensed tannins, cellulose, lignin and ash of 10 species (i.e. those coinciding with the species of this study for which we had leaf trait measurements: *Inga capitata*, *Leonia glycycarpa*, *Matisia malacocalyx*, *Naucleopsis krukovii*, *Neea* 'comun', *Pseudolmedia laevis*, *Rinorea viridifolia*, *Siparuna cuspidata*, *Siparuna decipiens* and *Sorocea steinbachii*) were used to assess the effect of herbivory on leaf-litter quality. Green leaf trait quantification methods were the same as those used for leaf litter.

Data analyses

Intra-specific variability of herbivory proportions and litter decomposition For 15 of the 17 studied species, intra-specific

variability in herbivory and decomposition was compared with the coefficient of variation (% $CV = (\sigma/\mu) \times 100$). Herbivory CV was calculated from the damage proportion measurements of fallen leaves collected in litter-fall traps over an 11-month period (data from Cárdenas *et al.*, 2014). For this, mean values of herbivory for each species were first calculated at the level of individual litter-fall traps (with no less than three leaves per trap when available) and averaged (with no less than two traps per species when available). *Duroia hirsuta* and *M.* 'purpono' were not included in this analysis because of insufficient leaf litter collected from the traps. For four species (*Iryanthera hostmannii*, *Mabea* 'superbrondu', *Macrolobium* 'yasuni' and *Rinorea lindeniana*), herbivory data from nearby traps (at a distance of no more than 50 m) were combined into one single 'trap' to meet the criteria of having at least three leaves per trap and two traps per species for comparisons. Decomposition CV was calculated from mass loss data among individual litterbags of the damaged and undamaged leaf litter (i.e. 16–20 litterbags per species). The two CV frequency distributions were additionally compared in terms of their skewness (g_1) and kurtosis (g_2), and using the Kolmogorov–Smirnov goodness of fit test as herbivory data did not adjust to normality (Shapiro–Wilk test; $P < 0.05$) (Gotelli & Ellison, 2004). Additionally, inter- and intra-specific variability of herbivory and decomposition were, respectively, analyzed using an F-test of the averaged herbivory and decomposition data, and a t-test for the standard deviations means of each species (Shapiro–Wilk tests; $P > 0.05$ for these data sets). The F-test was used to assess whether inter-specific herbivory and decomposition values had different variances, and the t-test was used to assess whether intra-specific averaged standard deviations had different means. These analyses were performed using Past software v.2.17 (Hammer *et al.*, 2001).

Herbivory and decomposition patterns at the tree community level In order to compare our data with the existing literature on the relationship between herbivore damage and decomposition, which for tropical forests is currently restricted to inter-specific comparisons, we also evaluated interspecific variability of herbivore damage and decomposition for our study species. To do so, we compared the average percentage of litter mass loss after 100 d of each species with the average herbivore damage for the same species sampled at the same location previously (Cárdenas *et al.*, 2014). The significance of the relationship was assessed with simple regression models and Pearson correlation using Table Curve 2D software v.5.01 and Past software v.2.17 (Hammer *et al.*, 2001) respectively.

For exploring the potential effect of herbivory on the quality of leaf litter (plant responses to damage), the relationship in leaf trait values between senescent (both damaged and undamaged) and green leaves (data taken from Cárdenas *et al.*, 2014) of 10 of the 17 tree species (i.e. those coinciding with the species of this study for which we had leaf trait measurements: *I. capitata*, *L. glycycarpa*, *M. malacocalyx*, *N. krukovii*, *N.* 'comun', *P. laevis*, *R. viridifolia*, *S. cuspidata*, *S. decipiens* and *S. steinbachii*) were fitted to linear regressions, and slopes of regression lines were compared using an analysis of covariance (ANCOVA; see Gotelli & Ellison, 2004). Analyses were performed using Table Curve 2D software v.5.01 and Past software v.3.04 (Hammer *et al.*, 2001). Comparisons of the quality of undamaged green leaves vs the quality of damaged/undamaged senescent leaves were necessary to separate the effects of senescence and herbivory on leaf-litter quality, while revealing the potential strategies used by plants after herbivore attack, such as nutrient resorption or tissue lignification. ANCOVA was also used to compare damaged vs undamaged leaf-litter quality.

Mechanical damage effect on decomposition The effect of mechanical damage on leaves among the three treatments (undamaged, undamaged-punched and damaged) was assessed by comparing the percentage of mass loss of undamaged vs damaged leaves, and undamaged vs punched leaves, against the line of the 1 : 1 relationship along which decomposition rates are identical. To assess the potential effect of mechanical damage on leaf decomposition, decomposition rate bi-plots of both undamaged and punched leaves were fitted to linear regressions, the slopes of which were compared using an ANCOVA as described above.

Phylogenetic signal and decomposition predictors As phylogenetic nonindependence (sensu Felsenstein, 1985) can inflate measures of correlation among traits explaining herbivory and those explaining decomposition, we tested for phylogenetic signal based on a molecular phylogenetic tree constructed for the 17 studied species (details in Methods S1; Fig. S1). Phylogenetic signal, a measure of the statistical dependence among species trait values attributable to their phylogenetic relationships (Revell *et al.*, 2008), was assessed 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 order to measure the extent to which correlations in traits reflect their shared evolutionary history (as approximated by Brownian motion), we additionally tested for the full model Pagel's lambda phylogenetic signal (Pagel, 1999) using the package 'Caper' in the R software (R Development Core Team, 2013).

Because full models analyzing Pagel's lambda phylogenetic signal were high for both damaged and undamaged treatments ($\lambda_{Und.} = 0.80$ and $\lambda_{Dam.} = 1.00$), and cellulose content showed significant K phylogenetic signal ($P = 0.032$; Table 2), 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 decomposition predictors while accounting for phylogenetic relationships among species. PGLS was performed using the package 'Caper' in the R software (R Development Core Team, 2013), based on the above-mentioned phylogenetic tree for the 17 studied species (Methods S1; Fig. S1). This analysis was used to test the effects of plant traits on decomposition rate constants (k). As the inclusion of the nine continuous traits (and their interactions) would have resulted in an over-parameterization of the model, before the PGLS analysis we used principal components analysis (PCA) to reduce the number of variables to test (Jongman *et al.*, 1995). Variable reduction by PCA allows extraction

Table 2 Estimates from the phylogenetic generalized least square (PGLS) analysis of undamaged and damaged senescent leaf decomposition rates k (a^{-1}) of 17 plant species against four of nine plant functional traits chosen after principal components analysis (PCA) variable reduction

Variable	Blomberg's K phylogenetic signal		PGLS estimates	
	$P (K_{Und.})$	$P (K_{Dam.})$	Und.	Dam.
k rate	0.662	0.760	–	–
Mn : Cu	0.274	0.170	**0.173*** **	**0.199*** **
Thickness	0.334	0.520	−0.065	−0.247
CT	0.548	0.858	**−0.215** **	**−0.173** **
Cellulose	**0.032**	0.124	−0.057	−0.001

P-values of Blomberg's K phylogenetic signal for all the traits and decomposition k rates in both treatments are shown. Und., undamaged estimate; Dam., damaged estimate. Mn : Cu, manganese : copper ratio; CT, condensed tannins. Significant relationships are indicated in bold where: **, $P < 0.01$; ***, $P < 0.001$. Full model Pagel's lambda phylogenetic signal: $\lambda_{Und.} = 0.80$ and $\lambda_{Dam.} = 1.00$; both PGLS models showed $R^2 > 0.84$ and $P < 0.001$.

of a set of uncorrelated principal components (PCs) which represent a large fraction of the variability of the original variables in reduced dimensionality (Legendre & Legendre, 1998). PCA was performed with normalized data where C : N, Mn : Cu and lignin : N ratios were added to the model. PCA results showed that Mn : Cu, thickness, condensed tannins and cellulose were the factors that better explained the variation in the model in the first four axes (PC1–PC4) which accounted for 79% of the total model variation. The Spearman correlation matrix showed that these variables were uncorrelated. PCA of the remaining unselected variables showed that they were not significantly related to decomposition rates except for Mn and Cu single variables for undamaged and damaged treatments, respectively (results not shown). PCA and Spearman correlation analyses were performed using PAST software v.2.17 (Hammer *et al.*, 2001). For PGLS analysis, k (a constant that characterizes the decomposition rate based on an exponential litter matter mass loss) was calculated following Levins (1968) as $k = -(\log_e(L_t/L_0)/t)$, where L_t is the litter mass at time t, and L_0 is the litter mass at time 0. k values were calculated for the mass loss at t of 365 d and were expressed as k (a^{-1}).

Finally, the relationships between leaf decomposition and further interacting traits (not considered in the PGLS, *sensu stricto*) that had previously been used in the literature (e.g. Melillo *et al.*, 1982; Prescott, 2010) were evaluated by simple linear and three nonlinear regression analyses (log, power and hyperbolic). The significance of the relationship was assessed with an ANOVA using TABLE CURVE 2D software v.5.01.

Results

Variability in leaf herbivory and decomposability

Both herbivory and decomposition showed a high intra-specific variability (mean CV = 33.7% and 16.1%, respectively; Figs 1, S2). Coefficient of variation distributions of herbivory and decomposition were significantly different (Kolmogorov–Smirnov goodness of fit $P < 0.01$; Fig. 1). The herbivory CV showed a skewness of $g_1 = 1.02$ (i.e. right skewed), and the decomposition CV a skewness of $g_1 = -0.09$ (i.e. normally distributed).

Compared to a normal distribution, CV kurtosis showed that the herbivory CV distribution was more expanded to the tails ($g_2 = 1.50$) compared with decomposition, which was more centered ($g_2 = -0.36$).

At the inter-specific level, herbivory and decomposition showed significantly different variances (F-test; $P < 0.001$), and at the intra-specific level both processes showed significantly different standard deviation means (t-test; $P = 0.02$). Furthermore, we found that intra-specific variation was higher than inter-specific variation in herbivory (means of standard deviations of herbivory damage were $SD_h = 12.24$ and $SD_{\bar{h}} = 5.40$ for intra- and inter-specific variation, respectively) and, conversely, intra-specific variation was lower than inter-specific variation in decomposition (means of standard deviations of decomposition averages were $SD_d = 8.83$ and $SD_{\bar{d}} = 14.28$ for intra- and inter-specific variation, respectively).

Relationships between herbivory and decomposition at the community level

Our exploratory analyses showed no significant relationships (either linear or curvilinear) or correlation between Cárdenas *et al.*'s (2014) herbivore damage (averaged) data and decomposability for 15 of the 17 tree species studied (Fig. 2; simple linear regression model: $R^2 = 0.148$; $F = 2.251$; $P = 0.157$; Pearson correlation test: $r = -0.384$; $P > 0.05$; $n_{litter-fall\ traps} = 2–31$; $n_{litterbags} = 16–20$). We found nearly all types of relationship between herbivory and decomposability: low herbivory/high decomposability (e.g. *S. steinbachii*, *M. superbrondu*, *L. glycycarpa* and *S. cuspidata*), high herbivory/low decomposability (e.g. *I. hostmannii* and *N. viburnoides*), high herbivory/high decomposability (e.g. *M. malacocalyx* and *M.* 'yasuni'), and moderate herbivory/moderate decomposability (e.g. *N. krukovii* and *P. laevis*).

Herbivory effect on leaf-litter quality

In order to assess the potential effects of canopy herbivores on leaf-litter quality, we compared the slopes of the relationships between green and senescent leaf quality metrics. Undamaged
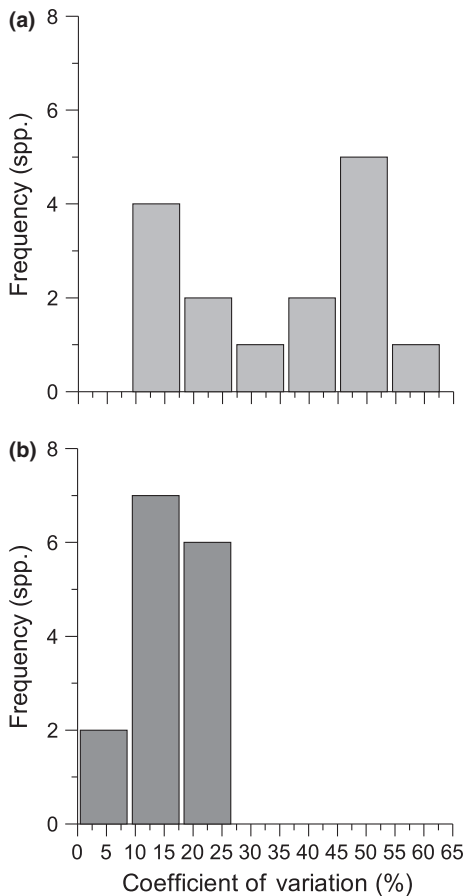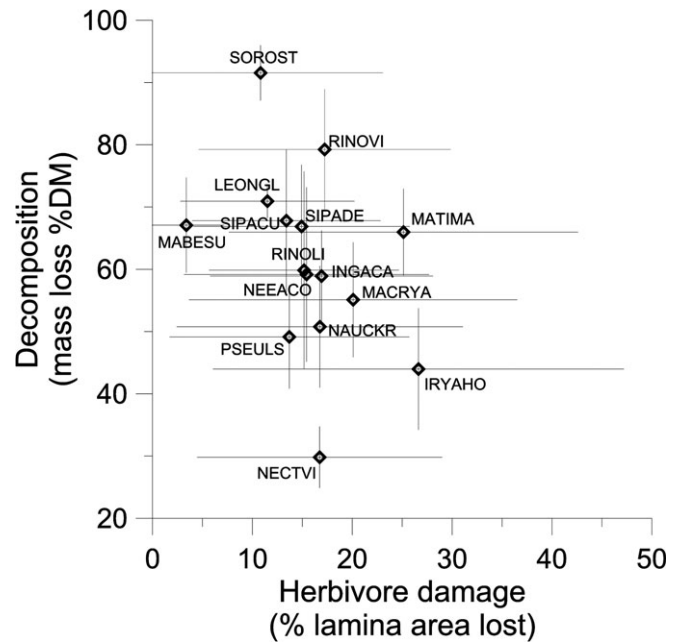
Fig. 1 Frequency distribution of coefficients of variation (CVs) of (a) herbivore damage (% lamina area lost; data from Cárdenas *et al.*, 2014) and (b) decomposition (% mass loss). CVs were calculated across 15 of the 17 studied tree species using species-specific mean values from litter-fall traps for herbivory, and litterbags for decomposition. CVs of *Duroia hirsuta* and *Miconia* 'purpono' were not included in the analysis because of insufficient herbivore damage data.

Fig. 2 Scatter-plot showing the average percentage of herbivore damage in fresh senescent leaves ($n = 2$–31 litter-fall traps) determined at our study site previously (Cárdenas *et al.*, 2014), and average decomposition (undamaged and damaged leaves averaged within each species; total $n = 16$–20 litterbags) of leaf litter from the same species at the same site for 15 of the 17 tree species studied in the Yasuní Forest Dynamic Plot. Neither linear nor curvilinear trend regressions showed significant fits (simple linear regression model; $R^2 = 0.148$; $F = 2.251$; $P = 0.157$; Pearson correlation test; $P > 0.05$). *Duroia hirsuta* and *Miconia* 'purpono' were not included in the analysis because of insufficient herbivore damage data. Refer to Table 1 for full names of species. DM, dry matter. Horizontal and vertical error bars correspond to herbivory and decomposition standard deviations, respectively.

and/or damaged leaves showed significant differences from the 1 : 1 relationship line for two of the quality traits: thickness (ANCOVA; $P_{undamaged} = 0.004$; $P_{damaged} = 0.006$; Fig. 3a), and ash ($P_{undamaged} = 0.008$; Fig. 3h). In contrast, N, C, the C : N ratio, condensed tannins, lignin, cellulose and the lignin : N ratio did not show significant differences between undamaged and damaged leaves or from the 1 : 1 relationship line (ANCOVAs: $P > 0.05$; Fig. 3b–g, i).

Decomposition experiment with damaged, undamaged and punched leaves

Damaged, undamaged and punched leaves showed high variability in k, with a 7.2-fold difference in the mean values among species across the three treatments (k_{min}: 1.21; k_{max}: 10.02; Table S2). *Sorocea steinbachi* was the species with the fastest rates of decomposition, whereas *N. viburnoides* showed the slowest rates, regardless of the state of damage. In fact, our results showed that undamaged, damaged and punched leaf litter did not decompose at significantly different rates (Fig. 4; Table S2). ANCOVAs

showed P-values > 0.05 for all comparisons: undamaged/damaged vs undamaged/punched; undamaged/damaged vs 1 : 1; and undamaged/punched vs 1 : 1.

Leaf traits controlling undamaged and damaged leaf-litter decomposition

The PGLS showed that undamaged and damaged leaf decomposition rates correlated significantly with the Mn : Cu ratio ($P_{und.} = 0.0004$ and $P_{dam.} = 0.0030$) and condensed tannins ($P_{und.} = 0.0006$ and $P_{dam.} = 0.0031$; Table 2), but not with any other leaf-litter traits chosen for analysis after the PCA (Table 2). Moreover, simple linear and nonlinear best regression models of trait interactions were fitted and are plotted in Fig. S3 (and see Notes S1), where lignin × condensed tannins (CT) fitted a logarithmic model ($R^2 = 0.43$; $F = 11.322$; $P = 0.004$), lignin : N ratio fitted a power model ($R^2 = 0.41$; $F = 10.563$; $P = 0.005$), CT : N ratio fitted a linear model ($R^2 = 0.38$; $F = 9.004$; $P = 0.009$), CT : Mn ratio fitted a power model ($R^2 = 0.56$; $F = 18.940$; $P < 0.001$), CT × thickness fitted a linear model ($R^2 = 0.28$; $F = 5.716$; $P = 0.03$), Mn : Cu ratio fitted a linear model ($R^2 = 0.57$; $F = 19.816$; $P < 0.001$), (Mn : Cu) × Na fitted a power model ($R^2 = 0.67$; $F = 30.662$; $P < 0.001$) and Na : lignin
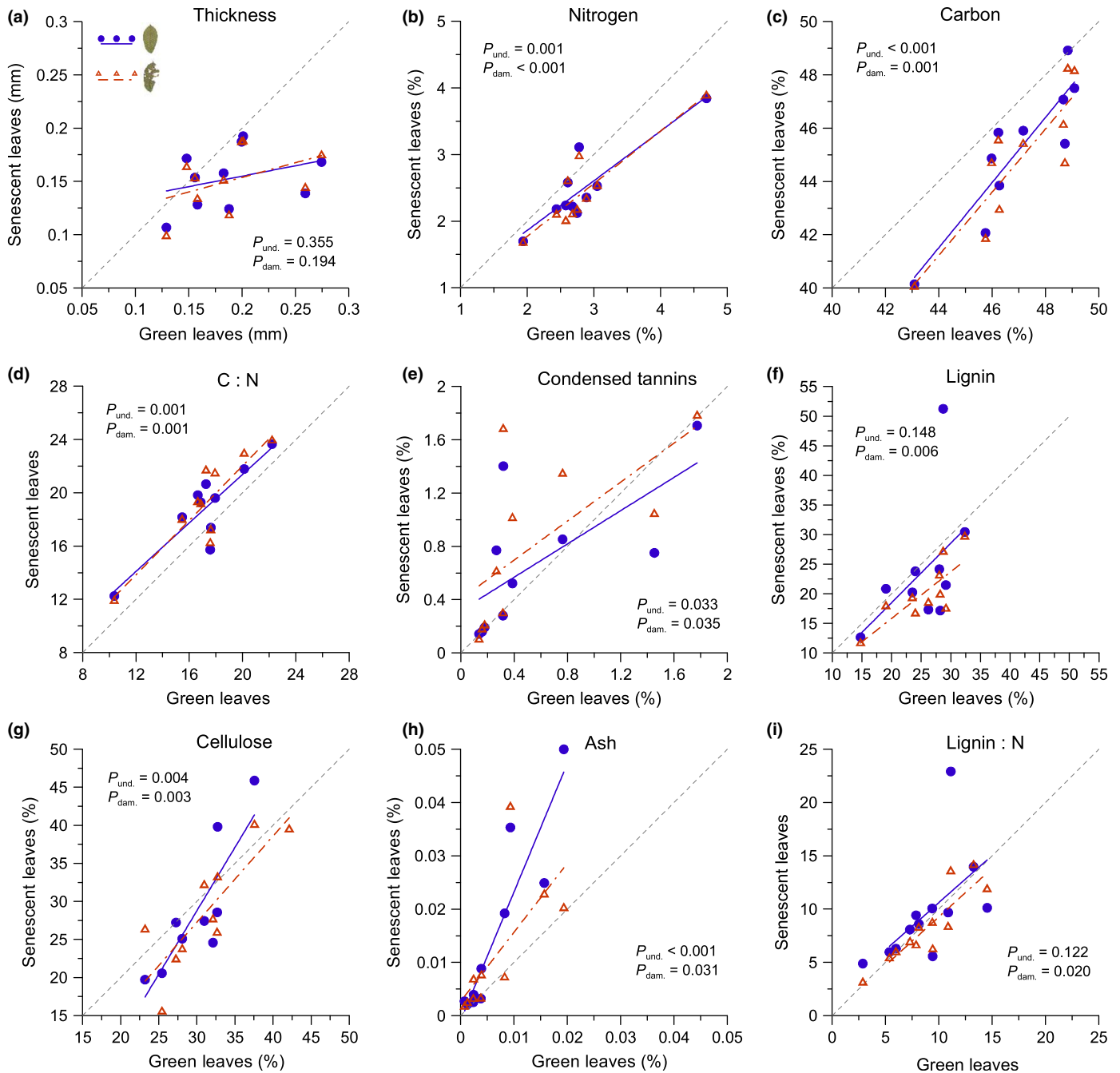
Fig. 3 Leaf trait relationships between undamaged green and senescent leaves of 10 species represented by closed blue circles (undamaged senescent leaves; und.) and open brown triangles (damaged senescent leaves; dam.). Blue solid and brown dot-dashed lines represent simple linear regressions of undamaged and damaged leaves, respectively. The light gray dashed diagonal line represents a 1 : 1 relationship. P-values of simple linear regressions are given; R^2 and F statistics are presented in Supporting Information Notes S2 and Table S3. In (g) $n = 9$ for cellulose$_{und.}$

ratio fitted a linear model ($R^2 = 0.56$; $F = 18.743$; $P < 0.001$) (Notes S1; Fig. S3).

Discussion

Assessment of the herbivory effect on leaf-litter quality

It has previously been shown that herbivory can induce considerable changes in leaf physico-chemical properties (reviewed by Coley & Barone, 1996; Ohgushi, 2005). However, our results indicated no significant effects of the action of canopy herbivores on the quality of senescent leaves. More generally, there was also little difference between green leaf quality and leaf-litter quality, irrespective of herbivore attack. Compared with senescent leaves (both damaged and undamaged), green leaves were thicker and had a significantly lower ash content (compared with undamaged senescent leaves only). Regardless of herbivore attack, decreasing leaf thickness during leaf senescence is a logical consequence of
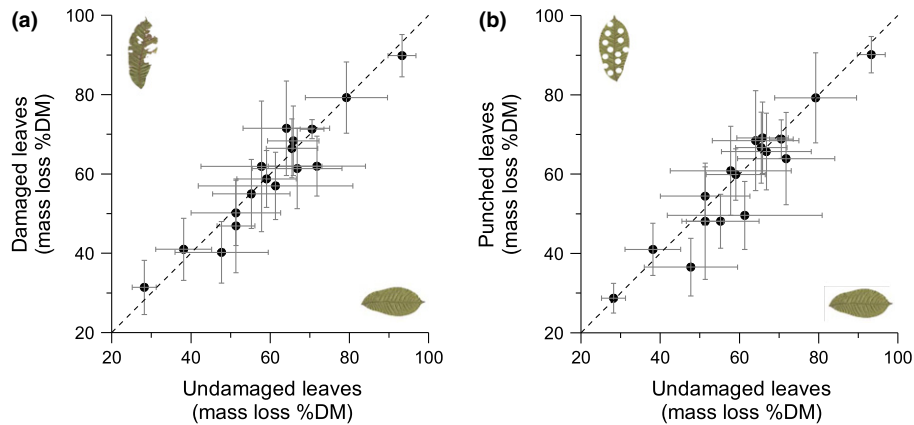
Fig. 4 Mass loss of (a) damaged and (b) punched leaf litter as a function of mass loss of undamaged leaf litter (black dots and error bars represent species mean and $\pm$ SD, respectively; $n = 8$–10). The light gray dashed diagonal line represents a 1 : 1 relationship. DM, dry matter.

loss of turgor of dying cells. A significantly lower ash concentration in green leaves compared with undamaged senescent leaves may be a consequence of changes in other, more mobile components, such as nonstructural carbohydrates, during senescence, rather than changes in the absolute amount of minerals in the remaining ash. Interestingly, when the *N. krukovii* outlier was removed from the analysis in Fig. 3(h) (upper triangle), significant differences were found between damaged and undamaged senescent leaves (ANCOVA: $P = 0.003$; results not shown). This may be explained in two ways. The first and most likely explanation is that, because damaged leaves chosen for the experiment were mostly eaten at the leaf margins (i.e. the parts where the quality of nutrients, including minerals, is high), the remaining structural parts closer to the leaf center, such as the lignin-rich mid-rib, may passively produce a decrease in the ash concentration in senescent herbivore-damaged leaves without any changes in its absolute content. Secondly, and perhaps rather speculatively, the difference between damaged and undamaged senescent leaves may indicate some kind of resorption and reallocation of ash-related elements, such as Ca, to prevent losses after herbivory events (Genua & Hillson, 1985; Chapin *et al.*, 2002). In particular, there is evidence that, under conditions of Ca shortage, Ca can be resorbed by plants from calcium oxalate stored in leaves (Genua & Hillson, 1985; Prychid & Rudall, 1999 and references therein). Ca resorption from Ca oxalate is a potential calcium-conservation strategy, which has never been tested in forests such as Yasuní, where the low soil pH value (< 5) significantly reduces the availability of this element (John *et al.*, 2007). Further and more specific analyses would be required to test whether Ca could account for the differences observed here between the ash concentrations in damaged and undamaged senescent leaves.

Intra- and inter-specific variability in herbivory and decomposition

Our results showed that herbivore damage in tree canopies was significantly more variable than litter decomposition at the intra-specific level. This observation may be explained in various ways. First, differences in herbivore damage among leaves may in part reflect a greater heterogeneity in green leaf quality as a result of differences in the leaf ontogeny or plant defense status of individual leaves (Tuomi *et al.*, 1984; Quintero & Bowers, 2011). On the one hand, herbivores specialized to consume highly defended low-quality leaves, in addition to sun, shade, young, mature, and/or senescent leaves (Coley & Barone, 1996; Dominy *et al.*, 2003; Boege & Marquis, 2005), may increase herbivore damage heterogeneity. On the other hand, it is known that trees may respond to local herbivory pressures such as herbivore clustering or outbreak events at small spatial scales (Miler & Straile, 2010; Salazar & Marquis, 2012). The positive skewness in the observed distribution pattern of herbivore damage may also indicate a large sub-group of individual trees selected for lower defenses and only a few individuals in the extreme upper tail with strong selection for defenses (Coley, 1983b). Secondly, growing evidence suggests that the defense response can be either limited to the site of attack (local induction) or expressed in remote undamaged plant parts (systemic induction), ranging from structural defenses to toxic chemical compounds (Bezemer & van Dam, 2005), but the existence of any of these strategies in Amazonian plants still remains largely unknown (Miler & Straile, 2010; Warman *et al.*, 2011). Tropical plants may invest in defenses such as nutrient value reduction and/or herbivore chemical pathway inhibition, at the leaflet, leaf, branch, or whole-tree level, but chemical plant defenses could be nonuniformly distributed even at the leaf level (Shroff *et al.*, 2008). All this information is important in the sense that the more the plant responses are localized within the canopy and the more the degree of response reflects the intensity of herbivore attack, the more heterogeneity of leaf quality at the individual, population and community levels would be expected. For example, Edwards *et al.* (1993) showed for two individual trees of *Eucalyptus sideroxylon* a large difference in defoliation of between 15% and 95% that was associated with inversely proportional concentrations of the antiherbivore compound cineole. Such highly dynamic plant responses to herbivores at multiple spatial and temporal scales may explain in part the high variability in herbivore damage at the intra-specific level found in our study.

While environmental conditions (e.g. light availability, water vapor deficit, and temperature) for individual leaves within tree

canopies, and thus their susceptibility to herbivores, can differ substantially, environmental conditions appear to be more homogeneous for leaf litter decaying on the forest floor. Accordingly, Donoso *et al.* (2010) showed that common soil arthropods were homogeneously distributed in the Yasuní forest floor. This was confirmed by Cárdenas (2013), who showed that soil invertebrate communities were taxonomically and functionally homogenous in a range of 0–1200 m distance in a study that considered the whole community of detritivores. These differences in habitat conditions and distribution patterns for herbivores compared with decomposer organisms may contribute to the observed lower intra-specific variability in litter decomposition compared with herbivore damage, with soil communities (invertebrate detritivores, bacteria, and fungi) adapted to efficiently transform all dead OM of variable quality (Lavelle, 2002; Madritch & Lindroth, 2011). In a macro-ecological analysis, Makkonen *et al.* (2012) indeed showed that decomposer communities present 'little specialization and high metabolic flexibility in processing plant litter, irrespective of litter origin'. However, larger inter-specific variability in decomposition (compared with herbivory) may be associated with the fact that different species decompose at different rates as a consequence of intrinsic differences in traits such as the Mn : Cu ratio and the concentrations of condensed tannins. For example, *S. steinbachii*, the species with the fastest decomposition rate, had a Mn : Cu ratio > 500-fold higher than that of *N. viburnoides*, the species with the slowest decomposition rate. Conversely, *N. viburnoides* had a > 10-fold higher concentration of condensed tannins compared with *S. steinbachii*.

No association between herbivory and decomposability shown by inter-specific analyses

Tree species shown to be more prone to herbivore damage did not necessarily show higher levels of decomposability, and vice versa. Moreover, the relationship between decomposability and herbivore damage was negative, in contrast to predictions (Grime *et al.*, 1996) and the reported positive correlation between decomposability and herbivory found in previous studies (Stark *et al.*, 2000; Wardle *et al.*, 2002, 2004; Chapman *et al.*, 2003, 2006). In another tropical study in Asia, Kurokawa & Nakashizuka (2008) reported a weak but positive correlation between decomposition and herbivory across 40 tree species. In line with our results, they concluded that, with the exception of some particular species, 'better defended' leaves (or leaves that are less susceptible to herbivore damage) may not necessarily turn into less decomposable litter. Leaf traits controlling decomposition may thus differ from those controlling herbivory. In agreement with other studies in tropical ecosystems, we found that condensed tannins correlated negatively with leaf-litter mass loss (Kurokawa & Nakashizuka, 2008; Coq *et al.*, 2010; Hättenschwiler & Bracht Jørgensen, 2010) but not with herbivore damage (Cárdenas *et al.*, 2014). Numerous canopy herbivores might be adapted to deal with complex compounds (Carmona *et al.*, 2011). Although host plants frequently form discrete patches of suitable habitat for some invertebrate herbivores that are subject to

dispersal limitations (Gripenberg & Roslin, 2005; Barber & Marquis, 2011), species of herbivores in tropical rainforests have shown little richness turnover and specialization (Novotny *et al.*, 2002, 2007, 2010). This may suggest that the invertebrate herbivore communities in the tropics present generalized physiological adaptations to many types of plant chemical defenses such as secondary metabolites. For example, the ability of some insects to tolerate ingested tannins appears to originate from a variety of biochemical and physical defenses in their guts, including surfactants, high pH, antioxidants, and a peritrophic envelope that protects the midgut (Barbehenn & Constabel, 2011). Recent studies reveal that condensed tannins may be less effective in deterring herbivores than oxidative ellagitannins or vescalagin hydrolysable tannin (Roslin & Salminen, 2008; Salminen & Karonen, 2011); future studies should consider analyzing such secondary metabolites in more detail.

Given the overall null effect of herbivore damage on decomposition, it is not surprising that the artificially punched leaf litter decomposed at the same rate as intact leaf litter. Overall, decomposition rates did not differ significantly among undamaged, damaged and punched leaves. The absence of any effects of physical damage on decomposition suggests that the edge to surface area of decomposing leaves has little importance for decomposition (see also Cárdenas & Dangles, 2012), despite the fact that fragmentation (e.g. by herbivores) creates fresh surfaces for decomposer organism access (Chapin *et al.*, 2002).

Conclusions

Collectively, our data provide evidence that leaf herbivory has no direct consequences for leaf-litter decomposition in our study system. First, our analyses on the inter-specific variation in defenses showed that none of the analyzed quality traits differed between damaged and undamaged leaves. This may indicate that leaves recover an important and comparable amount of nutrients from senescing leaves, regardless of herbivore damage. Secondly, the quality traits controlling decomposition and herbivory differed at the community level, which is in agreement with previous findings (Kurokawa & Nakashizuka, 2008). Finally, our study provides additional support for the idea that interactions between consumers and their resources are controlled by different factors for the green and the brown food-webs in tropical forests (Kurokawa & Nakashizuka, 2008). Trophic interactions in green food-webs may actually be top-down controlled, in contrast to trophic interactions in brown food-webs that have been shown to be bottom-up controlled (reviewed by Mulder *et al.*, 2013). However, data are still too limited to allow general conclusions to be drawn about whether and why (i.e. concerning mechanisms) the relationships between green leaves and herbivores, on the one hand, and between leaf litter and decomposers, on the other hand, differ in tropical forests compared with temperate ecosystems.

Acknowledgements

References

Abràmoff MD, Magalhães PJ, Ram SJ. 2004. Image processing with ImageJ. *Biophotonics International* 11: 36–42.

Aide TM. 1988. Herbivory as a selective agent on the timing of leaf production in a tropical understory community. *Nature* 336: 574–575.

Aide TM. 1993. Patterns of leaf development and herbivory in a tropical understory community. *Ecology* 74: 455–466.

Asplund J, Wardle DA. 2014. Within-species variability is the main driver of community-level responses of traits of epiphytes across a long-term chronosequence. *Functional Ecology* 28: 1513–1522.

Barbehenn RV, Constabel PC. 2011. Tannins in plant–herbivore interactions. *Phytochemistry* 72: 1551–1565.

Barber NA, Marquis RJ. 2011. Leaf quality, predators, and stochastic processes in the assembly of a diverse herbivore community. *Ecology* 92: 699–708.

Bardgett RD, Wardle DA. 2010. *Aboveground-belowground linkages: biotic interactions, ecosystem processes, and global change.* Oxford, UK: Oxford Series in Ecology and Evolution, Oxford University Press.

Bass MS, Finer M, Jenkins CN, Kreft H, Cisneros-Heredia DF, McCracken SF, Pitman NCA, English PH, Swing K, Villa G et al. 2010. Global conservation significance of Ecuador's Yasuní National Park. *PLoS One* 5: e8767.

Bezemer TM, van Dam NM. 2005. Linking aboveground and belowground interactions via induced plant defenses. *Trends in Ecology & Evolution* 20: 617–624.

Boege K, Dirzo R. 2004. Intraspecific variation in growth, defense and herbivory in *Dialium guianense* (Caesalpiniaceae) mediated by edaphic heterogeneity. *Plant Ecology* 175: 15–69.

Boege K, Marquis RJ. 2005. Facing herbivory as you grow up: the ontogeny of resistance in plants. *Trends in Ecology & Evolution* 20: 441–448.

Boumans PWJM. 1987. *Inductively coupled plasma emission spectroscopy. Part II: applications and fundamentals, vol. 2.* New York, NY, USA: John Wiley & Sons.

Brenes-Arguedas T, Coley PD, Kursar TA. 2008. Divergence and diversity in the defensive ecology of Inga at two Neotropical sites. *Journal of Ecology* 96: 127–135.

Buckland SM, Grime JP. 2000. The effects of trophic structure and soil fertility on the assembly of plant communities: a microcosm experiment. *Oikos* 91: 336–352.

Cárdenas RE. 2013. *Plant and fauna functional trait diversity affecting leaf herbivory and decomposability in a Neotropical rainforest (Yasuní National Park – Ecuador).* PhD thesis, Pierre and Marie Curie University (Paris 6), France.

Cárdenas RE, Dangles O. 2012. Do canopy herbivores mechanically facilitate subsequent litter decomposition in soil? A pilot study from a Neotropical cloud forest. *Ecological Research* 27: 975–981.

Cárdenas RE, Valencia R, Kraft NJ, Argoti A, Dangles O. 2014. Plant traits predict inter- and intraspecific variation in susceptibility to herbivory in a hyperdiverse Neotropical rainforest tree community. *Journal of Ecology* 102: 939–952.

Carmona D, Lajeunesse MJ, Johnson MT. 2011. Plant traits that predict resistance to herbivores. *Functional Ecology* 25: 358–367.

Cebrián J, Lartigue J. 2004. Patterns of herbivory and decomposition in aquatic and terrestrial ecosystems. *Ecological Monographs* 74: 237–259.

Chapin FS, Matson PA, Mooney HA. 2002. *Principles of terrestrial ecosystem ecology.* New York, NY, USA: Springer Verlag.

Chapman SK, Hart SC, Cobb NS, Whitham TG, Koch GW. 2003. Insect herbivory increases litter quality and decomposition: an extension of the acceleration hypothesis. *Ecology* 84: 2867–2876.

Chapman SK, Schweitzer JA, Whitham TG. 2006. Herbivory differentially alters plant litter dynamics of evergreen and deciduous trees. *Oikos* 114: 566–574.

Chave J, Navarrete D, Almeida S, Alvarez E, Aragão LE, Bonal D, Châtelet P, Silva-Espejo JE, Goret JY, von Hildebrand P et al. 2010. Regional and seasonal patterns of litterfall in tropical South America. *Biogeosciences* 7: 43–55.

Choong MF, Lucas PW, Ong JSY, Pereira B, Tan HTW, Turner IM. 1992. Leaf fracture toughness and sclerophylly: their correlations and ecological implications. *New Phytologist* 121: 597–610.

Coley PD. 1983a. Intraspecific variation in herbivory on two tropical tree species. *Ecology* 64: 426–433.

Coley PD. 1983b. Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecological Monographs* 53: 209–234.

Coley PD. 1988. Effects of plant growth rate and leaf lifetime on the amount and type of anti-herbivore defense. *Oecologia* 74: 531–536.

Coley PD, Barone JA. 1996. Herbivory and plant defenses in tropical forests. *Annual Review of Ecology and Systematics* 27: 305–335.

Coq S, Souquet JM, Meudec E, Cheynier V, Hättenschwiler S. 2010. Inter-specific variation in leaf-litter tannins drives decomposition in a tropical rainforest of French Guiana. *Ecology* 91: 2080–2091.

Cornelissen JHC, Lavorel S, Garnier E, Díaz S, Buchmann N, Gurvich DE, Reich PB, ter Steege H, Morgan HD, van der Heijden MGA et al. 2003. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany* 51: 335–380.

van Dam NM, Heil M. 2011. Multitrophic interactions below and above ground: *en route* to the next level. *Journal of Ecology* 99: 77–88.

Didham RK. 1998. Altered leaf-litter decomposition rates in tropical forest fragments. *Oecologia* 116: 397–406.

Dominy NJ, Lucas PW, Wright SJ. 2003. Mechanics and chemistry of rain forest leaves: canopy and understorey compared. *Journal of Experimental Botany* 54: 2007–2014.

Donoso DA, Johnston MK, Kaspari M. 2010. Trees as templates for tropical litter arthropod diversity. *Oecologia* 164: 201–211.

Duarte S, Pascoal C, Alves A, Correia A, Cassio F. 2008. Copper and zinc mixtures induce shifts in microbial communities and reduce leaf-litter decomposition in streams. *Freshwater Biology* 53: 91–101.

Edwards PB, Wanjura WJ, Brown WV. 1993. Selective herbivory by Christmas beetles in response to intraspecific variation in *Eucalyptus* terpenoids. *Oecologia* 95: 551–557.

Felsenstein J. 1985. Phylogenies and the comparative method. *American Naturalist* 125: 1–15.

Fine PV, Metz MR, Lokvam J, Mesones I, Ayarza Zuñiga JM, Lamarre GPA, Vásquez Pilco M, Baraloto C. 2013. Insect herbivores, chemical innovation and the evolution of habitat specialization in Amazonian trees. *Ecology* 94: 1764–1775.

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: 69–83.

Genua JM, Hillson CJ. 1985. The occurrence, type and location of calcium oxalate crystals in the leaves of fourteen species of Araceae. *Annals of Botany* 56: 351–361.

Gessner MO, Swan CM, Dang CK, McKie BG, Bardgett RD, Wall DH, Hättenschwiler S. 2010. Diversity meets decomposition. *Trends in Ecology & Evolution* 25: 372–380.

Gotelli NJ, Ellison AM. 2004. *A primer of ecological statistics*. Sunderland, MA, USA: Sinauer Associates.

Grafen A. 1989. The phylogenetic regression. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 326: 119–157.

Grime JP, Cornelissen JHC, Thompson K, Hodgson JG. 1996. Evidence of a causal connection between anti-herbivore defence and the decomposition rate of leaves. *Oikos* 77: 489–494.

Gripenberg S, Roslin T. 2005. Host plants as islands: resource quality and spatial setting as determinants of insect distribution. *Annales Zoologici Fennici* 42: 335–345.

Hammer Ø, Harper DAT, Ryan PD. 2001. PAST: Palaeontological Statistics software package for education and data analysis. *Palaeontologica Electronica* 4: 1–9.

Hanley ME, Lamont BB, Fairbanks MM, Rafferty CM. 2007. Plant structural traits and their role in anti-herbivore defence. *Perspectives in Plant Ecology, Evolution and Systematics* 8: 157–178.

Hättenschwiler S, Bracht Jørgensen H. 2010. Carbon quality rather than stoichiometry controls litter decomposition in a tropical rain forest. *Journal of Ecology* 98: 754–763.

Hättenschwiler S, Coq S, Barantal S, Handa IT. 2011. Leaf traits and decomposition in tropical rainforests: revisiting some commonly held views and towards a new hypothesis. *New Phytologist* 189: 950–965.

Hunter MD. 2001. Insect population dynamics meets ecosystem ecology: effects of herbivory on soil nutrient dynamics. *Agricultural and Forest Entomology* 3: 77–84.

Irion G. 1978. Soil infertility in the Amazonian rain forest. *Naturwissenschaften* 65: 515–519.

John R, Dalling JW, Harms K, Yavitt JB, Stallard RF, Mirabello M, Hubbell SP, Valencia R, Navarrete H, Vallejo M *et al.* 2007. Soil nutrients influence spatial distributions of tropical tree species. *Proceedings of the National Academy of Sciences, USA* 104: 864–869.

Jongman RHG, ter Braak CJF, van Tongeren OFR, eds. 1995. *Data analysis in community and landscape ecology*. Cambridge, UK: Cambridge University Press.

Kaspari M, Clay NA, Donoso DA, Yanoviak SP. 2014. Sodium fertilization increases termites and enhances decomposition in an Amazonian forest. *Ecology* 95: 795–800.

Kaspari M, Garcia MN, Harms KE, Santana M, Wright SJ, Yavitt JB. 2008. Multiple nutrients limit litterfall and decomposition in a tropical forest. *Ecology Letters* 11: 35–43.

Kessler A, Heil M. 2011. The multiple faces of indirect defences and their agents of natural selection. *Functional Ecology* 25: 348–357.

Kurokawa H, Nakashizuka T. 2008. Leaf herbivory and decomposability in a Malaysian tropical rain forest. *Ecology* 89: 2645–2656.

Landsberg J, Ohmart C. 1989. Levels of insect defoliation in forests: patterns and concepts. *Trends in Ecology & Evolution* 4: 96–100.

Lavelle P. 2002. Functional domains in soils. *Ecological Research* 17: 441–450.

Lecerf A, Chauvet E. 2008. Intraspecific variability in leaf traits strongly affects alder leaf decomposition in a stream. *Basic and Applied Ecology* 9: 598–605.

Legendre P, Legendre N. 1998. *Numerical ecology, 2nd edn*. Amsterdam, the Netherlands: Elsevier.

LeRoy CJ, Whitham TG, Wooley SC, Marks JC. 2007. Within-species variation in foliar chemistry influences leaf-litter decomposition in a Utah river. *Journal of the North American Benthological Society* 26: 426–438.

Levins R. 1968. *Evolution in changing environments*. Princeton, NJ, USA: Princeton University Press.

Lowell RB, Markham JH, Mann KH. 1991. Herbivore-like damage induces increased toughness and toughness in a seaweed. *Proceedings of the Royal Society of London, Series B* 243: 31–38.

Lowman MD. 1984. An assessment of techniques for measuring herbivory: is rainforest defoliation more intense than we thought? *Biotropica* 16: 264–268.

Madritch MD, Lindroth RL. 2011. Soil microbial communities adapt to genetic variation in leaf litter inputs. *Oikos* 120: 1696–1704.

Makkonen M, Berg MP, Handa IT, Hättenschwiler S, Ruijven J, Bodegom PM, Aerts R. 2012. Highly consistent effects of plant litter identity and functional traits on decomposition across a latitudinal gradient. *Ecology Letters* 15: 1033–1041.

Melillo JM, Aber JD, Muratore JF. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology* 63: 621–626.

Miler O, Straile D. 2010. How to cope with a superior enemy? Plant defence strategies in response to annual herbivore outbreaks. *Journal of Ecology* 98: 900–907.

Moles AT, Peco B, Wallis IR, Foley WJ, Poore AG, Seabloom EW, Vesk PA, Bisigato AJ, Cella-Pizarro L, Clark CJ *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.

Mulder C, Ahrestani FS, Bahn M, Bohan DA, Bonkowski M, Griffiths BS, Guicharnaud RA, Kattge J, Krogh PH, Lavorel S *et al.* 2013. Connecting the green and brown worlds: allometric and stoichiometric predictability of above- and below-ground networks. In: Woodward G, Bohan D, eds. *Ecological networks in an agricultural world. Advances in Ecological Research Series*. Amsterdam, the Netherlands: Elsevier, 69–175

Mulder C, Den Hollander HA, Hendriks AJ. 2008. Aboveground herbivory shapes the biomass distribution and flux of soil invertebrates. *PLoS One* 3: e3573.

Novotny V, Basset Y, Miller SE, Weiblen GD, Bremer B, Cizek L, Drozd P. 2002. Low host specificity of herbivorous insects in a tropical forest. *Nature* 416: 841–844.

Novotny V, Drozd P, Miller SE, Kulfan M, Janda M, Basset Y, Weiblen GD. 2006. Why are there so many species of herbivorous insects in tropical rainforests? *Science* 313: 1115–1118.

Novotny V, Miller SE, Baje L, Balagawi S, Basset Y, Cizek L, Craft KJ, Dem F, Drew RAI, Hulcr J *et al.* 2010. Guild-specific patterns of species richness and host specialization in plant–herbivore food webs from a tropical forest. *Journal of Animal Ecology* 79: 1193–1203.

Novotny V, Miller SE, Hulcr J, Drew RA, Basset Y, Janda M, Setliff GP, Darrow K, Stewart AJA, Auga J *et al.* 2007. Low beta diversity of herbivorous insects in tropical forests. *Nature* 448: 692–695.

Ohgushi T. 2005. Indirect interaction webs: herbivore-induced effects through trait change in plants. *Annual Review of Ecology, Evolution, and Systematics* 36: 81–105.

Pagel M. 1999. Inferring the historical patterns of biological evolution. *Nature* 401: 877–884.

Pérez J, Jeffries TW. 1992. Roles of manganese and organic acid chelators in regulating lignin degradation and biosynthesis of peroxidases by *Phanerochaete chrysosporium*. *Applied and Environmental Microbiology* 58: 2402–2409.

Porter LJ, Hrstich LN, Chan BG. 1986. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry* 25: 223–230.

Pramanik R, Sarkar K, Joy VC. 2001. Efficiency of detritivore soil arthropods in mobilizing nutrients from leaf litter. *Tropical Ecology* 42: 51–58.

Prescott CE. 2010. Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? *Biogeochemistry* 101: 133–149.

Prychid CJ, Rudall PJ. 1999. Calcium oxalate crystals in monocotyledons: a review of their structure and systematics. *Annals of Botany* 84: 725–739.

Quintero C, Bowers MD. 2011. Plant induced defenses depend more on plant age than previous history of damage: implications for plant–herbivore interactions. *Journal of Chemical Ecology* 37: 992–1001.

R Development Core Team. 2013. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. ISBN 3-900051-07-0, [WWW document] URL http://www.R-project.org/ [accessed 17 December 2013].

Revell LJ, Harmon LJ, Collar DC. 2008. Phylogenetic signal, evolutionary process, and rate. *Systematic Biology* 57: 591–601.

Ritchie ME, Tilman D, Knops JMH. 1998. Herbivore effects on plant and nitrogen dynamics in oak savanna. *Ecology* 79: 165–177.

Roslin T, Salminen JP. 2008. Specialization pays off: contrasting effects of two types of tannins on oak specialist and generalist moth species. *Oikos* **117**: 1560–1568.

Salazar D, Marquis RJ. 2012. Herbivore pressure increases toward the equator. *Proceedings of the National Academy of Sciences, USA* **109**: 12616–12620.

Salminen JP, Karonen M. 2011. Chemical ecology of tannins and other phenolics: we need a change in approach. *Functional Ecology* **25**: 325–338.

Schuldt A, Bruelheide H, Durka W, Eichenberg D, Fischer M, Kröber W, Härdtle W, Ma K, Michalski SG, Palm W-U *et al.* 2012. Plant traits affecting herbivory on tree recruits in highly diverse subtropical forests. *Ecology Letters* **15**: 732–739.

Shroff R, Vergara F, Muck A, Svatoš A, Gershenzon J. 2008. Nonuniform distribution of glucosinolates in *Arabidopsis thaliana* leaves has important consequences for plant defense. *Proceedings of the National Academy of Sciences, USA* **105**: 6196–6201.

Stark S, Wardle DA, Ohtonen R, Helle T, Yeates GW. 2000. The effect of reindeer grazing on decomposition, mineralization and soil biota in a dry oligotrophic Scots pine forest. *Oikos* **90**: 301–310.

Sterck F, van der Meer P, Bongers F. 1992. Herbivory in two rain forest canopies in French Guyana. *Biotropica* **24**: 97–99.

Swift MJ, Heal OW, Anderson JM. 1979. *Decomposition in terrestrial ecosystems.* Oxford, UK: Blackwell Scientific Publications.

Tuomi J, Niemela P, Haukioja E, Sirén S, Neuvonen S. 1984. Nutrient stress: an explanation for plant anti-herbivore responses to defoliation. *Oecologia* **61**: 208–210.

Tuomisto H, Poulsen AD, Ruokolainen K, Moran RC, Quintana C, Celi J, Cañas G. 2003. Linking floristic patterns with soil heterogeneity and satellite imagery in Ecuadorian Amazonia. *Ecological Applications* **13**: 352–371.

Valencia R, Foster RB, Gorky V, Condit RG, Svenning JC, Hernandez C, Romoleroux K, Losos EC, Magard E, Balslev H. 2004. Tree species distributions and local habitat variation in the Amazon: large forest plot in eastern Ecuador. *Journal of Ecology* **92**: 214–229.

Vitousek PM, Sanford RL. 1986. Nutrient cycling in moist tropical forest. *Annual Review of Ecology and Systematics* **17**: 137–167.

Wardle DA, Bardgett RD, Klironomos JN, Setälä H, van der Putten WH, Wall DH. 2004. Ecological linkages between aboveground and belowground biota. *Science* **304**: 1629–1633.

Wardle DA, Bonner KI, Barker GM. 2002. Linkages between plant litter decomposition, litter quality, and vegetation responses to herbivores. *Functional Ecology* **16**: 585–595.

Warman L, Moles AT, Edwards W. 2011. Not so simple after all: searching for ecological advantages of compound leaves. *Oikos* **120**: 813–821.

Westoby M, Falster DS, Moles AT, Vesk PA, Wright IJ. 2002. Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology and Systematics* **33**: 125–159.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Phylogenetic tree using *rbc*L chloroplastic gene sequences.

Fig. S2 Canopy leaf herbivore damage vs soil leaf-litter mass loss.

Fig. S3 Leaf-litter mass loss as a function of interacting physico-chemical traits.

Table S1 Individual trees neighboring litter-fall traps in the study area

Table S2 Annual decomposition *k* rates

Table S3 Simple linear regression models of the relationship between senescent leaf decomposition and leaf traits

Methods S1 Plant species phylogenetic analysis.

Notes S1 Decomposition rates of pooled leaves as a function of single and interacting plant traits.

Notes S2 The role of herbivory in leaves and leaf litter.

Please note: Wiley Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.