New Phytologist

Research

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

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Received: *25 September 2014*
Accepted: *12 February 2015*

New Phytologist (2015) **207**: 817–829
doi: 10.1111/nph.13368

Key words: above–belowground interaction, canopy, decomposers, detritivores, Ecuador, folivory, soil ecology, Yasuní National Park.

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

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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 ± 3.9°C (daily averages ranging from 22.0 to 32.0°C) and the mean relative air humidity is 88.4 ± 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

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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 (± SD) litter-fall traps collected leaves from *c.* 7 (± 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 × 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

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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

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variability in herbivory and decomposition was compared with the coefficient of variation (% CV = (σ/μ) × 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

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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

	Blomberg's K phylogenetic signal		PGLS estimates	
Variable	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: $r = -0.384$; $P > 0.05$; $n_{\text{litter-fall traps}} = 2–31$; $n_{\text{litterbags}} = 16–20$). 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

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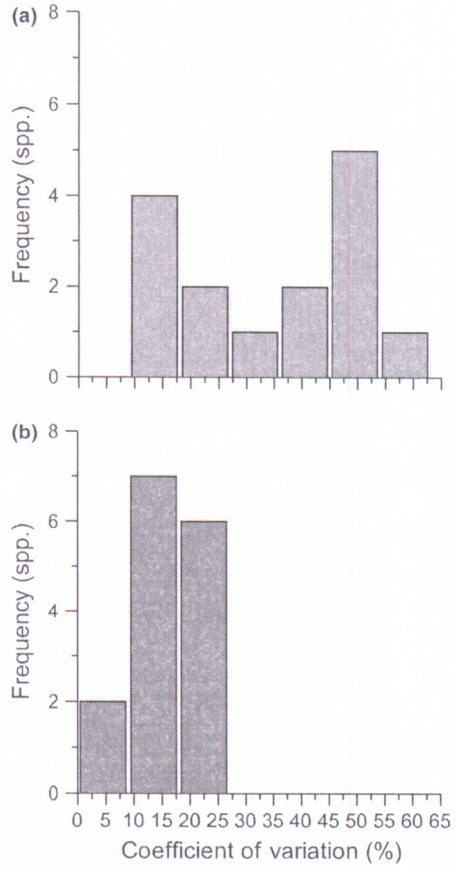


Fig. 1 Frequency distribution of coefficients of variation (CVs) of (a) herbivore damage (% lamina area lost; data from Cárdenas *et al.*, 2014) and (b) decomposition (% mass loss). CVs were calculated across 15 of the 17 studied tree species using species-specific mean values from litter-fall traps 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.

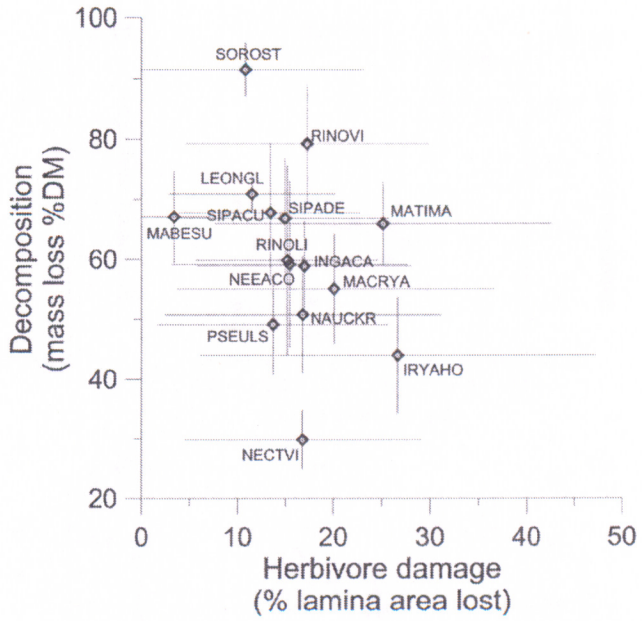


Fig. 2 Scatter-plot showing the average percentage of herbivore damage in fresh senescent leaves ($n = 2$–31 litter-fall traps) determined at our study site previously (Cárdenas *et al.*, 2014), and average decomposition (undamaged and damaged leaves averaged within each species; total $n = 16$–20 litterbags) 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

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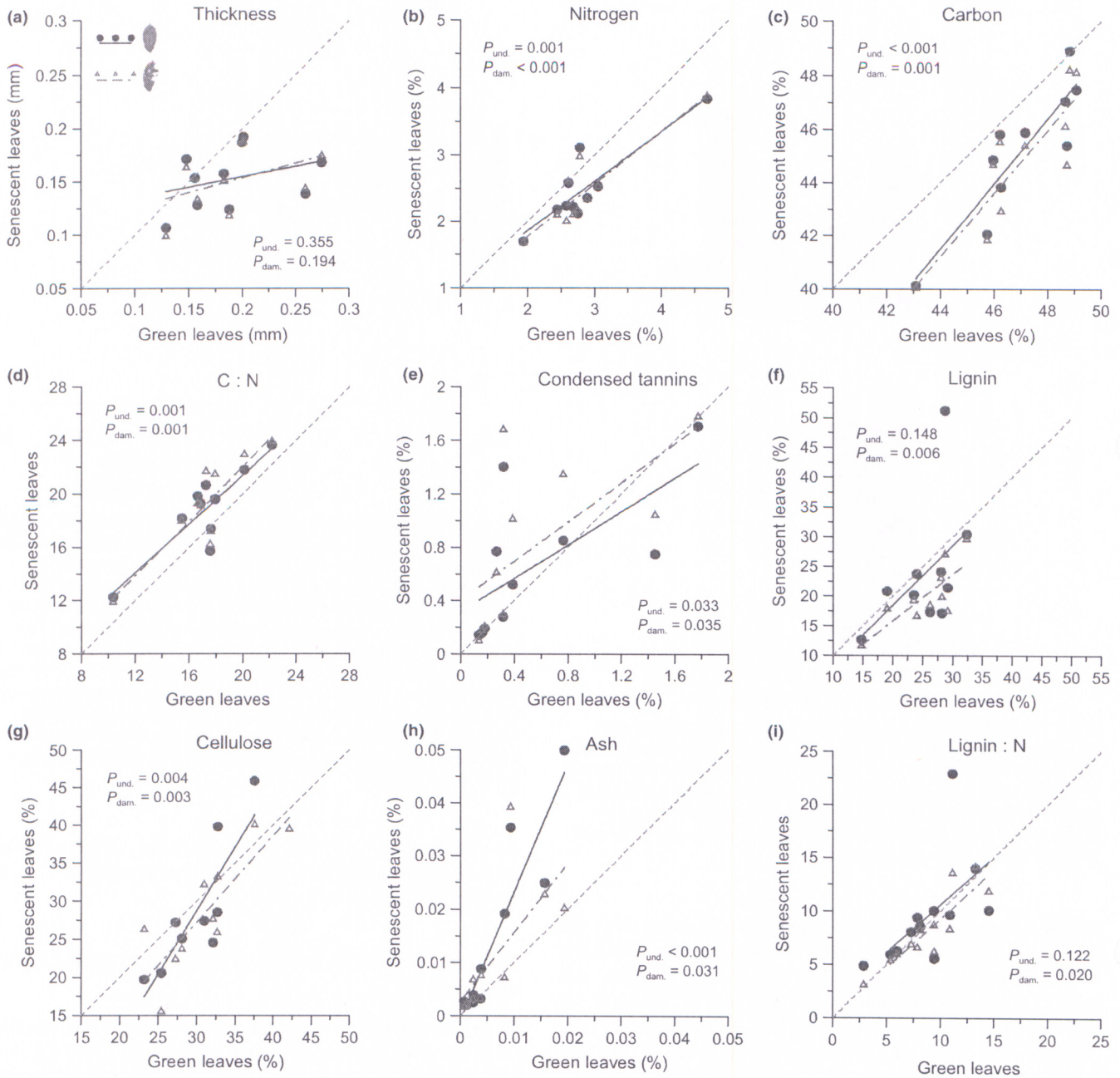


Fig. 3 Leaf trait relationships between undamaged green and senescent leaves of 10 species represented by closed blue circles (undamaged senescent leaves; und.) and open brown triangles (damaged senescent leaves; dam.). Blue solid and brown dot-dashed lines represent simple linear regressions of undamaged 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 $\text{cellulose}_{und.}$

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

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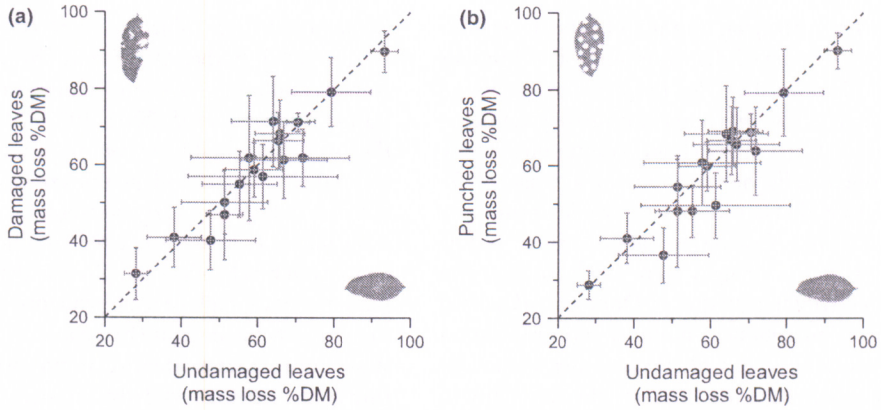


Fig. 4 Mass loss of (a) damaged and (b) punched leaf litter as a function of mass loss of undamaged leaf litter (black dots and error bars represent species mean and ± SD, respectively; $n = 8$–10). The light gray dashed diagonal line represents a 1 : 1 relationship. DM, dry matter.

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 subgroup 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

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homogeneous for leaf litter decaying on the forest floor. Accordingly, Donoso *et al.* (2010) showed that common soil arthropods were homogeneously distributed in the Barro Colorado Island forest floor (BCI, Panamá). This was also 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 in Yasuní [Correction added after online publication 13 March 2015: changes were made in the two preceding sentences to clarify the citation of Donoso *et al.*'s 2010 study]. 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

We thank the staff of the Yasuní Research Station, in particular the administrative personnel, David Lasso, Pablo Jarrín, Carlos

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Padilla and Karla Rivadeneira, for constant support during our stays at the Station. We are grateful to Pablo Alvia who provided invaluable expertise on plant taxonomy in the field. Patricio Andino, Rodrigo Espinosa, Mayra Buenaño, Fernanda Samaniego and Pamela Arias helped with field work and in the laboratory. This study would have been logistically impossible to carry out without them. Jim Self provided comprehensive guidance and support for leaf analyses at CSU. We thank Philippe Belmont, Claire Capdevielle, Stéphane Dupas and Nathan Kraft for their assistance with PGLS analysis. We are grateful to the ECB of the Pontificia Universidad Católica del Ecuador (PUCE), in particular to its dean Hugo Navarrete, for constant support. We thank three anonymous reviewers for very constructive comments that improved earlier versions of the manuscript. The PUCE (project codes H13164 and J13041) and the French Institute for Research and Development (IRD) supported this study through research grants. R.E.C. was partly financed by SENESCYT/Prometeo Project (grant no. 20140548BP) during the preparation of the manuscript. This study was endorsed by the Ministerio de Ambiente del Ecuador (permits MAE: 09-FLO-MA-DPO-PNY and 06-2011-FAU-DPAP-MA).

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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.

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

t = total number of litter-fall traps that collected leaves for each of the 15 studied species. i=total number of individual trees (trunk with diameter at breast height $\geq$ 1 cm) at a distance of 20 m from the litter-fall traps t. i/t represents the potential number of individual trees that contributed with leaves for the herbivore damage assessment. SD = standard deviation.

species	t	i	i/t
Inga capitata	40	188	5
Iryanthera hostmannii	4	7	2
Leonia glycycarpa	11	56	5
Matisia malacocalyx	47	317	7
Mabea 'superbrondu'	5	6	1
Macrolobium 'yasuni'	6	11	2
Naucleopsis krukovii	8	126	16
Neea 'comun'	15	221	15
Nectandra viburnoides	6	24	4
Pseudolmedia laevis	31	370	12
Rinorea lindeniana	4	6	2
Rinorea viridifolia	16	65	4
Siparuna cuspidata	17	145	9
Siparuna decipiens	17	125	7
Sorocea steinbachii	37	287	8
mean	**18**	**130**	**6.6**
SD	**13.8**	**118.4**	**4.5**

Methods S1. Plant species phylogenetic analysis

A phylogenetic tree was built using *rbc*L chloroplastic gene sequences (Gielly & Taberlet 1994) obtained from GenBank. When available, we used the sequence of the same species analysed. When missing, we used the sequence of a sister species from the same genus that is distributed in Yasuní or the Amazonian region. In the case of two species belonging to the same genus for which we did not find their own sequences, we assigned the same sister species to both. Sequences were aligned manually with MacClade 4.07 (Maddison & Maddison 2000) and phylogenetic analyses were performed using 1276 bp for all the species. Original loci accessions obtained from the GenBank in parentheses in the figure below.

The analysis with Bayesian inference was done using MrBayes v3.12 (Ronquist & Huelsenbeck 2003). The best-fit model of evolution for our dataset was determined by using the Akaike Information Criterion (AIC), as implemented in Modeltest v3.7 (Posada & Crandall 1998). The run consisted of two independent analyses with the following settings: four Markov chains of five million generations, random starting trees, default priors, and trees sampled every 100 generations (branch lengths were also saved). A burn-in period of one million generations was used. Support of nodes for the analysis was provided by clade posterior probability (CPP) estimates. The phylogenetic tree was finally ultrametricized using Grafen's method (Grafen 1989) using the R packages 'Caper' and 'Ape' (R Development Core Team 2013).

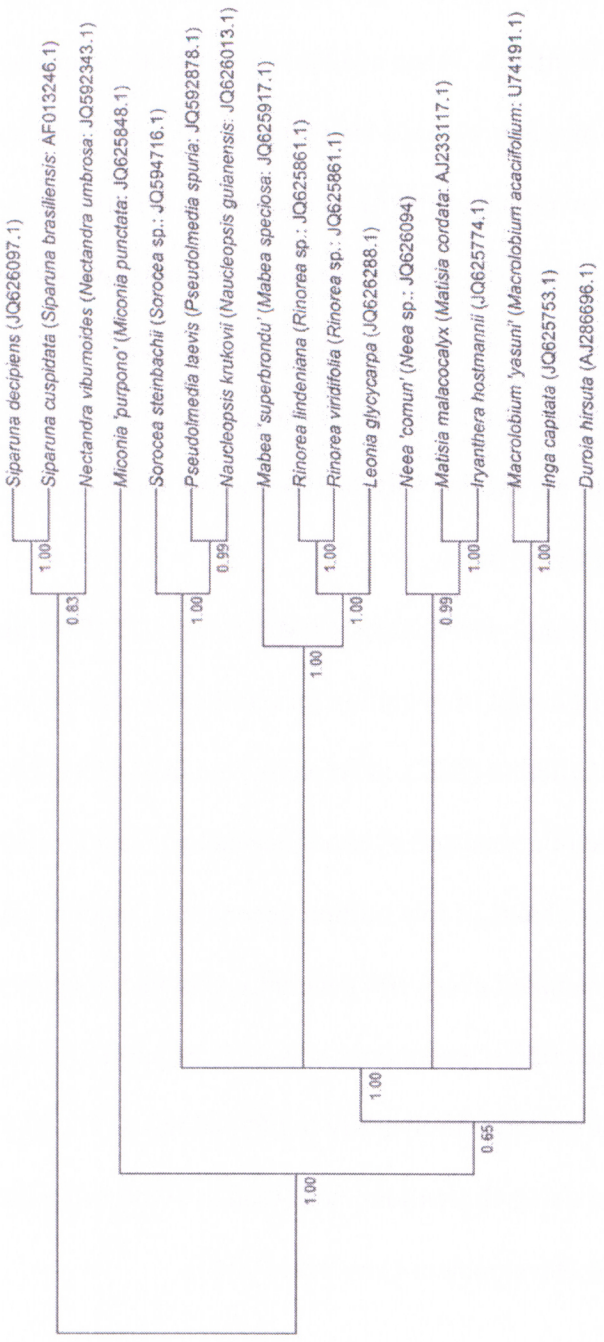


Fig. S1. Phylogenetic tree using *rbc*L chloroplastic gene sequences. Original loci accession from GenBank in parentheses. Numbers in nodes correspond to bootstrap support values.

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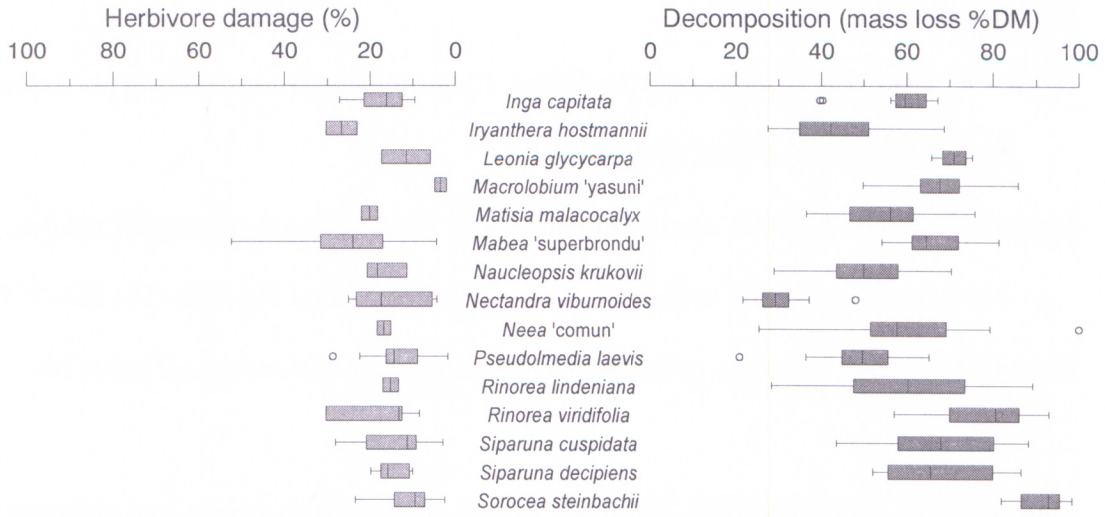


Fig. S2. Canopy leaf herbivore damage vs. soil leaf-litter mass loss. Observed proportions of leaf herbivore damage (left panel), and leaf litter mass loss (right panel) of 15 of the 17 studied tree species in the Yasuní National Park. *D. hirsuta* and *M.* 'purpono' were not included in the analysis because of insufficient herbivore damage data. Herbivore damage variation corresponds to measurements performed in 11 months census (data from Cárdenas *et al.*, 2014) among individual litter-fall traps (n = 2–31). Mass loss data represents the variation among individual litterbags of the damaged and undamaged leaf litter (n = 8–10).

Table S2. Annual decomposition *k* rates

Annual decomposition *k* rates, mass loss during the 100 days of experimentation and calculated annual mass loss for undamaged (Und.) and damaged (Dam.) leaves. Kolmogorov-Smirnov test was used for comparing the annual *k* rates between the three treatments (the whole data set did not adjust to normality). *t*-test was used for comparing both mass loss results (data adjusted to normality). No significant differences were found between the three treatments for any of the decomposition parameters ($P > 0.05$). Annual *k*-rates data should be taken with caution as they rely in two points only (at $t = 0$ and $t = 100$ days).

species	*k* rates (a^{-1})			Mass $loss_{100}$ (%DM)			Mass loss(a^{-1}) (%DM)		
	Und.	Punched	Dam.	Und.	Punched	Dam.	Und.	Punched	Dam.
Duroia hirsuta	1.83	1.86	1.91	38.16	41.02	40.99	83.95	84.48	85.22
Inga capitata	3.24	3.30	3.22	59.07	59.91	58.75	96.10	96.30	96.01
Iryanthera hostmannii	2.35	1.68	1.91	47.72	36.56	40.23	90.51	81.32	85.18
Leonia glycycarpa	4.44	4.13	4.53	70.56	68.85	71.30	98.82	98.38	98.92
Matisia malacocalyx	3.92	3.89	4.06	65.50	66.68	66.44	98.02	97.95	98.27
Mabea 'superbrondu'	3.90	4.38	4.20	65.84	69.16	68.35	97.98	98.75	98.50
Macrolobium 'yasuni'	2.89	2.41	2.87	55.24	48.13	54.97	94.45	91.01	94.33
Miconia 'purpono'	4.19	4.09	3.56	66.81	65.69	61.37	98.49	98.33	97.15
Naucleopsis krukovii	2.64	2.84	2.51	51.35	54.45	50.18	92.90	94.15	91.87
Neea 'comun'	3.68	2.47	3.12	61.34	49.59	56.99	97.49	91.58	95.56
Nectandra viridifolia	1.21	1.23	1.34	28.22	28.70	31.40	70.30	70.87	73.86
Pseudolmedia laevis	2.62	2.36	2.38	51.36	48.10	46.91	92.76	90.58	90.74
Rinorea lindeniana	3.22	3.36	3.44	57.80	60.86	61.91	96.00	96.54	96.79
Rinorea viridifolia	5.71	5.72	5.79	79.24	79.24	79.25	99.67	99.67	99.69
Siparuna cuspidata	3.77	4.38	4.41	64.10	68.45	71.51	97.70	98.75	98.78
Siparuna decipiens	4.55	3.79	3.52	71.79	63.91	61.96	98.95	97.75	97.05
Sorocea steinbachii	10.02	8.62	8.41	93.28	90.14	89.83	100.00	99.98	99.98

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

Leaf traits controlling decomposition – new considerations

While nitrogen, carbon, sodium, and thickness alone appear to do not influence decomposition of leaf litter in Yasuní (Fig. S3a–c, f), lignin, condensed tannins, manganese and copper did significantly (Fig. S3d, e, g, h). Leaves with the higher levels of lignin, condensed tannins and copper, and low levels of manganese decomposed more slowly. To simplify, the relationships of these single traits with decomposition are discussed based on the results obtained of the interacting variables.

The 'lignin × CT' and lignin : N ratio (Fig. S3i, j) showed a significant negative correlation with decomposition (but not with herbivory; Kurokawa & Nakashizuka, 2008). 'Lignin × CT' shows an additive effect of both complex molecules where there appears to be a minimum amount of both elements combination that significantly slows down the leaf-litter decomposition rates. The lignin : N ratio has often been identified as a good predictor of decomposition in a wide range of terrestrial ecosystems (Melillo *et al.*, 1982; Taylor *et al.*, 1989; Moore *et al.*, 1999; Kurokawa & Nakashizuka, 2008; Wieder *et al.*, 2009), although Hättenschwiler *et al.*, (2011) found no significant effect of this ratio on decomposition in the Guyana forest. Our results suggest that this negative relationship seems to be driven by the lignin effect on detritivory as nitrogen alone has a –not significant– positive trend with respect to decomposition (Fig. S3a, d). Considering the effect of condensed tannins, some other interactions have shown to predict well the leaf-litter decomposition: CT : N, CT : Mn and CT × thickness (Fig. S3k, l, m). Here, the highest the occurrence of CT, the slowest the decomposition rates (negative relationship). This is in agreement with other studies showing that metabolites such as polyphenolics (e.g. condensed tannins) can play an important role in terrestrial

nutrient cycling (Hättenschwiler & Vitousek, 2000) by reducing decomposition rates (Findlay *et al.*, 1996; Coq *et al.*, 2010) nitrification (Baldwin *et al.*, 1983), nitrogen mineralization (Northup *et al.*, 1995) or nitrogen fixation (Schimel *et al.*, 1998).

Mn : Cu ratio showed a significant positive relationship with decomposition rates (see Fig. S3l). It is known that Mn content has direct implications for lignin degradation as it is essential for the production and activity of Mn-peroxidase, a lignin-degrading enzyme (Pérez & Jeffries, 1992; Berg, 2000) and is involved in the regulation of other lignolytic enzymes, including laccase (Archibald & Roy, 1992) and lignin peroxidase (Pérez & Jeffries 1992). Besides, Cu is considered an inhibitor of soil microbial respiration (Doelman & Haanstra, 1984). High concentrations of this element in leaves would make them poorly palatable for detritivores. Interacting factors significantly correlated to decomposition as well.

Finally, relationships such as (Mn:Cu) × Na, and Na : lignin (Fig. S3o, p) showed a significant positive relationship as well (in spite that Na alone did not). Na is essential to the metabolism of plant consumers, both decomposers and herbivores, as it maintains homeostasis, and gradients of cell solutes concentration and membranes voltage (Dudley *et al.*, 2012). Kaspari *et al.*, (2009) showed that adding NaCl solution to the leaf litter in an Amazonian rainforest, enhanced litter mass loss by 41% in no more than 20 days. Kaspari *et al.*, (2014) showed that decomposition rates after adding NaCl were consistently higher by up to 70% for cellulose paper, and 78%, 68%, and 29% for three woods of increasing lignin contents. They finally found that density of termite workers averaged 17-fold higher on NaCl plots. The role of micronutrients on decomposition is still poorly understood, more detailed analyses are necessary to understand its role and its relationship with detritivore and decomposer communities physiological needs.

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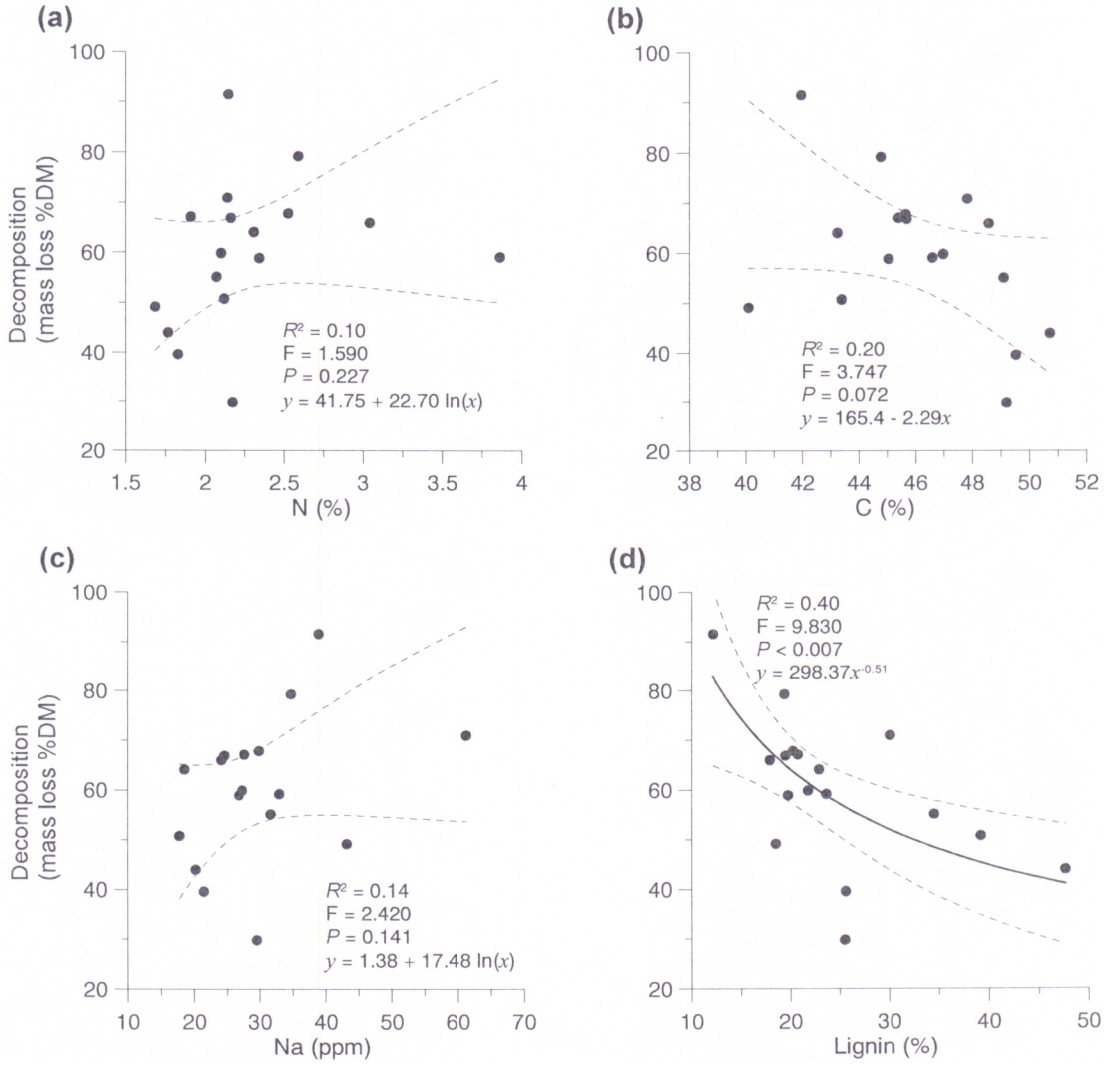


Fig. S3. Continued.

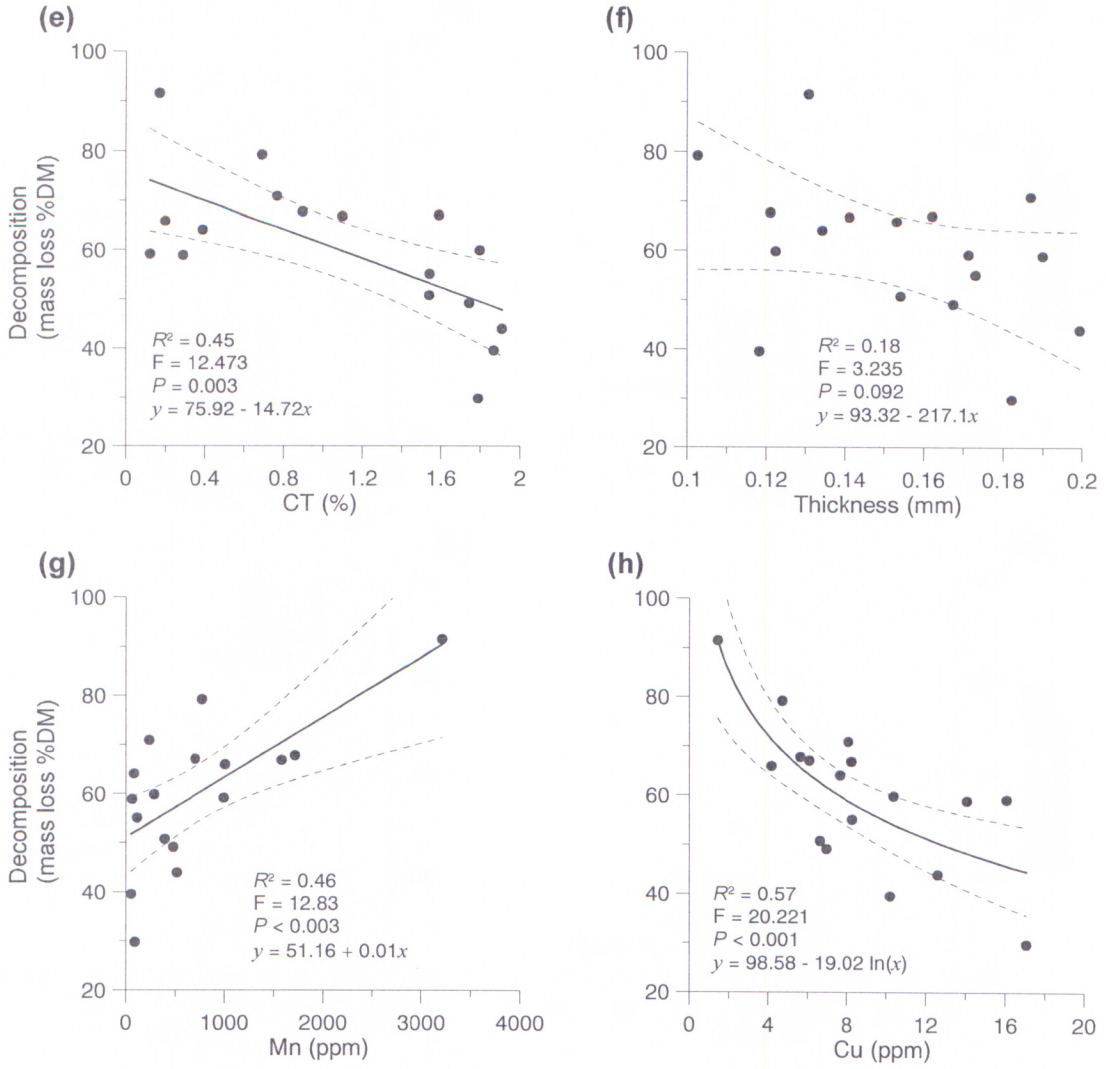


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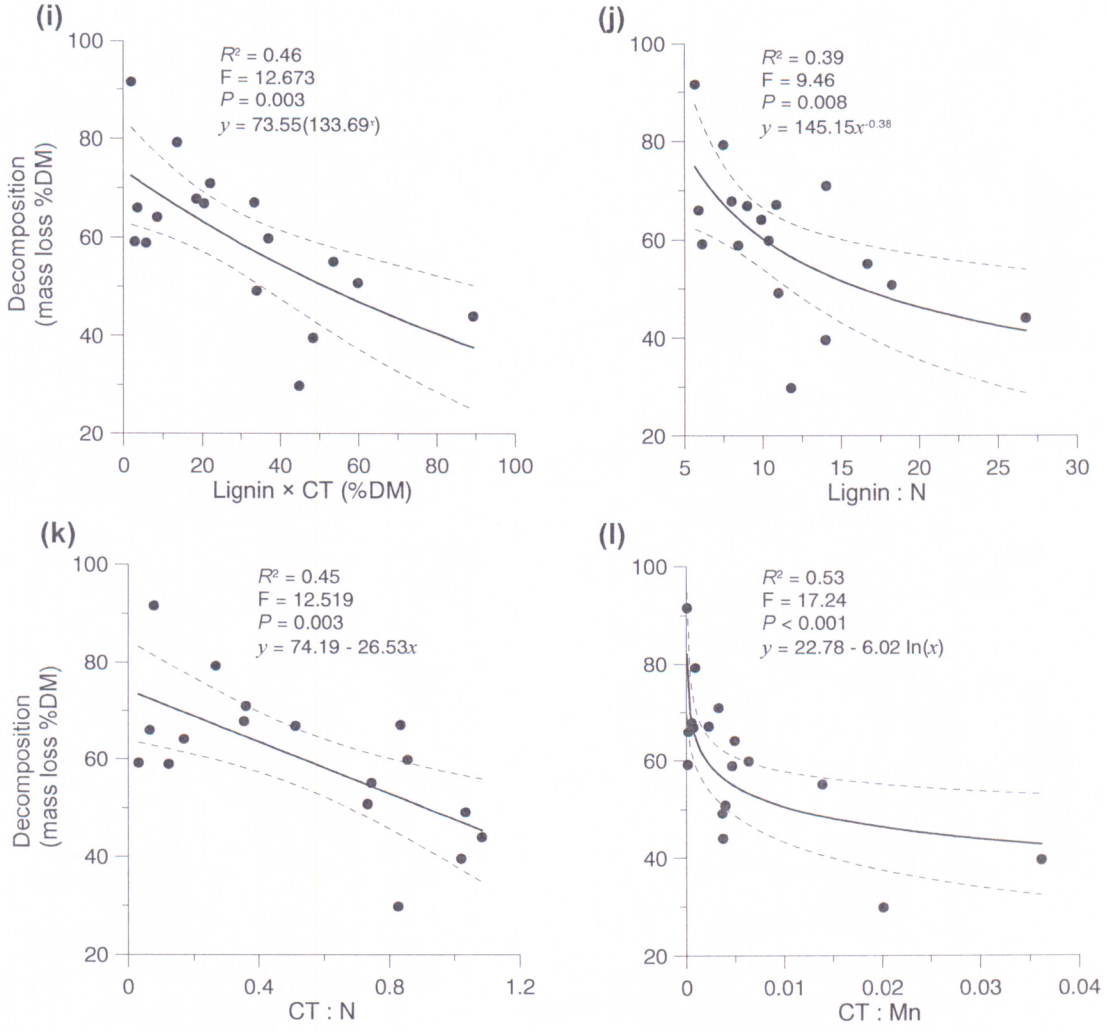


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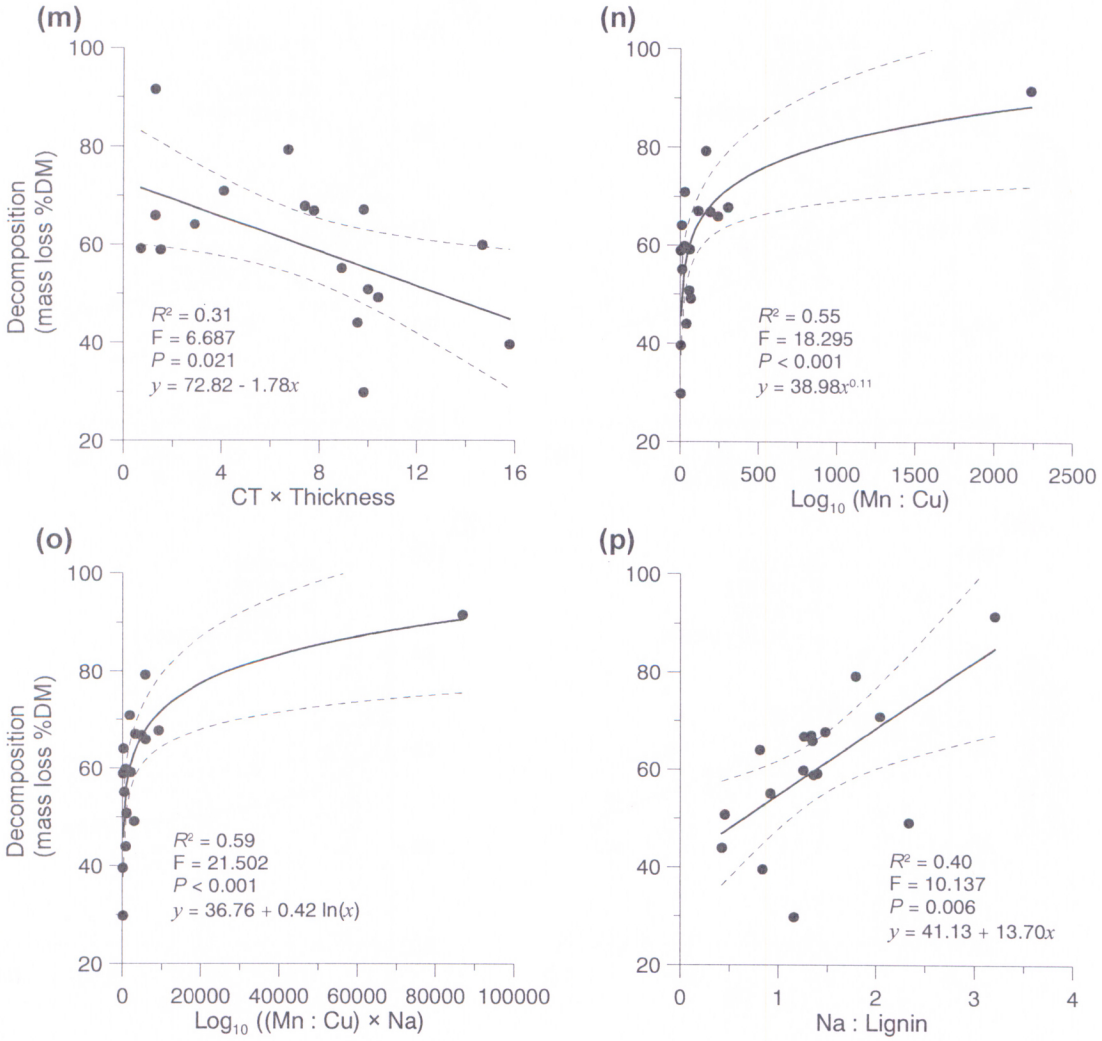


Fig. S3. Decomposition of undamaged and damaged leaf litter (mass loss percentage of dry matter averaged) as a function of single and interacting physico-chemical traits. Values of R^2, F and P are given for linear, log, hyperbolic or exponential regressions (solid lines drawn for significant regressions only). Dashed lines correspond to ±95% confidence intervals. DM = Dry matter.

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

Immediate effects of attacking herbivores on the plant defense status (hormone-regulated) have been shown for herbaceous and woody plants in temperate ecosystems (Agrawal *et al.*, 2012; Giron *et al.*, 2013). There is evidence indicating that under natural conditions such responses may be relaxed in woody plants 3 to 4 years after the attack by herbivores (Tuomi *et al.*, 1984), suggesting a mid- term "after-herbivory effect" on leaf quality. Direct effect of herbivory on leaf quality was not observed in our study site probably because trees may be still responding to past herbivory events across several years.

Table S3. ANOVA tests of the simple linear regression models of the relationship between senescent undamaged and damaged leaves decomposition rates with plant leaf traits. (C : N = carbon : nitrogen ratio; CT = condensed tannins). n = 10 species for all regressions except for undamaged cellulose leaves analysis where n = 9.

leaf trait	**undamaged leaves**			**damaged leaves**		
	R^2	*F*	*P*	R^2	*F*	*P*
thickness	0.107	0.961	0.355	0.201	2.009	0.194
nitrogen	0.789	29.696	< 0.001	0.821	36.748	< 0.001
carbon	0.799	31.718	< 0.001	0.757	24.896	0.001
C : N	0.759	25.208	0.001	0.766	26.253	< 0.001
CT	0.454	6.654	0.033	0.444	6.393	0.035
lignin	0.242	2.561	0.148	0.626	13.334	0.006
cellulose	0.721	18.057	0.004	0.699	18.539	0.003
ash	0.846	44.384	< 0.001	0.460	6.802	0.031

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