

## LETTER

# Herbivory makes major contributions to ecosystem carbon and nutrient cycling in tropical forests

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### Abstract

The functional role of herbivores in tropical rainforests remains poorly understood. We quantified the magnitude of, and underlying controls on, carbon, nitrogen and phosphorus cycled by invertebrate herbivory along a 2800 m elevational gradient in the tropical Andes spanning 12°C mean annual temperature. We find, firstly, that leaf area loss is greater at warmer sites with lower foliar phosphorus, and secondly, that the estimated herbivore-mediated flux of foliar nitrogen and phosphorus from plants to soil via leaf area loss is similar to, or greater than, other major sources of these nutrients in tropical forests. Finally, we estimate that herbivores consume a significant portion of plant carbon, potentially causing major shifts in the pattern of plant and soil carbon cycling. We conclude that future shifts in herbivore abundance and activity as a result of environmental change could have major impacts on soil fertility and ecosystem carbon sequestration in tropical forests.

### Keywords

Climate change, ecosystem biogeochemistry, net primary productivity, nitrogen cycle, plant–soil feedbacks, soil phosphorus, montane rainforest.

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## INTRODUCTION

Tropical rainforests play a key role in the global carbon (C) cycle (Cramer *et al.* 2004) and are home to a large portion of the world's terrestrial species (Dirzo & Raven 2003), many of which depend on leaves as their primary food source. These herbivores potentially affect the diversity and structure of tropical plant communities (Coley & Barone 1996) in part by influencing cycles of C, nitrogen (N) and phosphorus (P) (Huntly 1991; Feeley & Terborgh 2005) in these nutrient limited ecosystems (Vitousek 1984). The pathways through which herbivores affect C and nutrient cycles have mostly been elucidated in temperate or boreal forests, grassland or tundra ecosystems and for large-bodied mammalian consumers (McNaughton *et al.* 1989). By comparison, little is known about the role of invertebrate herbivores in tropical forest canopies on ecosystem biogeochemistry, despite evidence that leaf damage rates in tropical forests are relatively high and that tropical herbivory is dominated by insects (Coley & Barone 1996).

A range of herbivore-mediated processes can exert both positive and negative effects over the quantity and quality of organic material transferred to the soil, and/or plant species composition, with variable net consequences for soil processes depending on the herbivore/s and ecosystem in question (Bardgett & Wardle 2003; Hartley & Jones 2004). Available

studies on tropical invertebrate herbivores have focused on the effects of various deposits (excreta, bodies, unconsumed leaf fragments) from particular herbivore groups on soil and plant processes. In general, these deposits were relatively labile: decomposing more rapidly than plant litter (Fonte & Schowalter 2004), increasing levels of soil N and P, and driving a variety of shifts on soil organic matter cycling (Fonte & Schowalter 2005; Schowalter *et al.* 2011). These results are broadly consistent with studies on invertebrate herbivores in temperate and boreal forests (Bardgett & Wardle 2003; Hartley & Jones 2004), and on vertebrate herbivores in tropical forests (Wardle *et al.* 2001; Feeley & Terborgh 2005). Therefore, herbivory potentially represents an important missing link in the tropical forest nutrient cycle, but the sensitivity of this component to environmental change remains poorly understood compared to other ecosystem processes.

The biotic and abiotic factors regulating herbivory are still debated. While support exists for effects of climate (Coley 1998), natural enemies (Elton 1973), resource quantity (McNaughton *et al.* 1989) and quality (Coley *et al.* 1985), a number of recent global syntheses find surprisingly few clear relationships between plant community properties (productivity, foliar structure or chemistry) or climate and regional patterns of herbivory (Endara & Coley 2011; Moles *et al.* 2011). Therefore, the drivers of ecosystem-level herbivory have yet to be conclusively identified.

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Elevational gradients provide useful perspectives on this debate by serving as natural experiments to test general hypotheses about the links between plant traits, temperature and consumers at the scale of whole ecosystems (Sundqvist *et al.* 2013). Shifts in temperature with elevation are likely to exert a direct influence over the metabolism of ectothermic herbivores, imposing fundamental limits on ectotherm activity and abundance in cold conditions (Bale *et al.* 2002; Deutsch *et al.* 2008). Indirectly, temperature is a key driver of a broad suite of elevational shifts in soil and plant properties, with warmer conditions often linked to more rapid soil nutrient mineralisation and dominance of fast growing species producing nutrient rich foliage (Sundqvist *et al.* 2013). These changes should together result in a decline in herbivory with increasing elevation, though the complicating influence of natural enemies and other factors (e.g. precipitation) could confound this pattern. Empirical tests of this hypothesis are rare, and available studies provide only mixed support (Andrew *et al.* 2012). Only two studies attempt to further quantify the ecosystem impacts of invertebrate herbivory with elevation, from the same temperate deciduous forest gradient (Reynolds & Hunter 2001; Hunter *et al.* 2003), and neither detect any clear elevational change in various herbivore deposits (excreta, unconsumed leaf fragments, leachates in throughfall) despite large shifts in leaf damage from the same system (Reynolds & Crossley 1997). Hence, the effects of elevation-associated shifts in temperature and other variables on herbivory rates and herbivore-mediated plant–soil feedbacks remain largely unknown. In tropical rainforests, the potential consequences of this lack of knowledge are particularly serious because of the abundance and diversity of herbivores and the likely sensitivity of both herbivores (Coley 1998; Bale *et al.* 2002; Deutsch *et al.* 2008) and forest ecosystems (Clark *et al.* 2003; Feeley *et al.* 2007) to global warming.

Here, we quantify the combined impacts of leaf chewing invertebrate herbivores on cycling of C, N and P, and explore potential underlying mechanisms driving these patterns, in six mature Amazonian and Andean rainforests along a 2800 m elevational transect spanning  $\sim 12^\circ\text{C}$  mean annual temperature (MAT) (Table S1). We relate leaf area loss to data on ecosystem-level C cycling, foliar traits and climate across all six plots. Further, we use literature estimates of insect energetics and foliar elemental resorption to estimate herbivore-mediated fluxes of C, N and P (in excreta, moults, bodies and unconsumed leaf fragments) from plants to the soil. Our general hypothesis is that herbivory rate will decrease with elevation but, more specifically, we ask: (1) Which abiotic (e.g. climate) and biotic (e.g. resource quality and quantity) factors account for any observed change in herbivory rate with elevation and, (2) How do estimated herbivore transfers of C, N and P from trees to the soil in the lowland forests compare to other major ecosystem inputs of these elements?

## MATERIAL AND METHODS

### Study site

The plots surveyed are part of the RAINFOR-GEM network (<http://gem.tropicalforests.ox.ac.uk/>), and are located along

the eastern slope of the Peruvian Andes. Eight permanent sampling plots (1 ha projected area) were established in 2005 in closed-canopy, mature forest with no clear signs of human disturbance, within homogenous stands (for plot details see Table S1). Tree species richness along the gradient is around 100, decreasing with elevation from  $\sim 170$  species  $\text{ha}^{-1} \geq 10$  cm diameter at breast height (DBH, 1.3 m) at 300 m above sea level to  $\sim 40$  species  $\text{ha}^{-1} \geq 10$  cm DBH at 3000 m above sea level (Jankowski *et al.* 2013). There is high tree species turnover along the elevational gradient, with a mean tree species elevational range size of 250 m, and no single tree species occurring in all plots (Jankowski *et al.* 2013). Weather stations have been established at or near the plots to record temperature and precipitation since 2007. In the case of the upper and lower plot pairs (ESP-01, WAY-01, TAM-06, TAM-05), the weather stations are located in open areas  $< 2$  km from the plots, while at the mid-elevation plots (SPD-01, SPD-02) the sensors are located in the centre of each plot and extend approximately 2 m above the emergent canopy.

### Foliar trait measurements

Detailed methodologies for foliar trait data collection and analyses are presented elsewhere (Asner & Martin 2011) and laboratory protocols are downloadable from the Carnegie spectranomics website (<http://spectranomics.ciw.edu>). Given the exceptional taxonomic and structural diversity at the plots, it was not possible to sample all tree species at multiple canopy positions. Therefore, at each plot, only 30 trees with full sunlight canopies were randomly selected. A voucher specimen was collected from each tree selected. Vouchers were matched by local expert taxonomists to type specimens kept at the National Agrarian University La Molina Herbarium in Peru and the Missouri Botanical Garden, USA. We also matched genus names to information provided by Kew Botanic Gardens, UK. Trees selected represented 14–20 different families and 20–30 different species per plot. These species represented  $\sim 20\%$  and  $\sim 45\%$  of the total tree species pools on the lowland (TAM-06, TAM-05) and mid-/upper-elevation plots (ESP-01, WAY-01, SPD-01, SPD-02) respectively. Leaf collections were conducted using tree climbing techniques. For each tree, two fully sunlit branches at the top of the canopy were selected and cut, sealed in large polyethylene bags to maintain moisture, stored on ice in coolers, and transported to a local site for processing within 3 h, and usually less than 30 min. A subset of fully expanded, undamaged leaves was randomly selected from the branches for scanning to determine fresh leaf area, and weighing to record fresh and dry weight. Values of fresh leaf area were divided by dry weight to determine leaf mass per unit area (LMA).

Dried leaves were ground and analysed for a variety of elements. Total element concentrations of calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P) and zinc (Zn) were determined by inductively coupled plasma spectroscopy (ICPOES; Thermo Jarrel-Ash, IRIS Advantage, MA, USA) after microwave digestion with concentrated ( $\sim 70\%$ ) nitric acid solution (CEM MARSXpress; Matthews, NC, USA). C fractions (cellulose and lignin) were determined using sequential

digestion of increasing acidity in an Ankom fiber analyser (Ankom Technology, Macedon, NY, USA) and are presented on an ash-free dry mass basis. A subset of the ground material was processed to determine total C and N concentration by combustion-reduction elemental analysis (Costec Analytical Technologies Inc. Valencia, CA, USA).

### Leaf area loss measurements

Mesh traps (25 per plot, 1 m above the ground, 1 m<sup>2</sup> in area) captured abscised leaves falling from the canopy over 2 week periods. Leaves were collected from all plots in this way every 2–3 months and photographed, over a full seasonal cycle in 2009/2010. Non-angiosperm plant material constituted a small proportion of total leaf litterfall at the plots and was excluded from the subsequent analyses. Each photograph was analysed with software (ImageJ, NIH, MD, USA) to calculate leaf area including damage ( $A_h$ , cm<sup>2</sup>), then manually adjusted to fill in missing leaf portions, to estimate leaf area prior to damage ( $A_{nh}$ , cm<sup>2</sup>). The difference between  $A_{nh}$  and  $A_h$  was divided by  $A_{nh}$  to calculate proportional leaf area loss ( $H$ ).

In total, 18 428 leaves (~ 3000 per plot) were analysed in this way. Mean values of  $H$  for all leaves collected per trap were calculated, and plot-level standard errors were calculated from the 25 mean trap values. Leaves were not identified by species so  $H$  values represent plot-level means reflecting the weighted foliar traits of the local plant community. We assumed that the hole:leaf area ratio, and therefore  $H$ , remained approximately constant with leaf expansion. We found that cases of severe leaf area loss were extremely rare (see Fig. S1) suggesting that complete consumption was not common enough to cause a strong underestimate in our results. However, we acknowledge that we do not capture the contributions of larger bodied herbivores which can remove entire leaves and branches instantaneously. A portion of the leaf area removed may have occurred post-abscission, but any additional damage was likely minimal given that leaves were collected every two weeks and that the material was of relatively poor quality compared to live leaves. In addition, we do not account for other forms of leaf damage incurred by organisms (e.g. galls, rolls), or forms of damage not resulting in tissue loss (e.g. browning associated with leaf miners, leachates from leaf wounds and sapsuckers).

### Herbivory calculations

After leaves were photographed, they were dried at 70 °C until constant mass and weighed to estimate total foliar litterfall dry mass ( $F_l$ , Mg ha<sup>-1</sup> year<sup>-1</sup>). In a steady state system, as expected in these mature forests,  $F_l$  represents total foliar production minus the portion removed by herbivores. Therefore, we estimated total foliar biomass production ( $F_p$ , Mg ha<sup>-1</sup> year<sup>-1</sup>) by dividing  $F_l$  by  $1-H$ .  $F_l$  and  $F_p$  were converted to elemental fluxes  $F_{lc}$  and  $F_{pc}$  (Mg ha<sup>-1</sup> yr<sup>-1</sup>) respectively, by multiplying dry biomass by live foliar C, N and P concentrations (g g<sup>-1</sup>). We then multiplied  $F_{pc}$  by  $H$  to calculate mean plot  $F_{pc}$  removed by herbivores ( $F_{he}$ , Mg ha<sup>-1</sup> year<sup>-1</sup>). Using only abscised leaves, we measured  $H$  accumulated over the entire lifespan of leaves which could be

directly combined with the mass of total foliar litterfall, to provide an unbiased estimate of  $F_{he}$  (Sand-Jensen *et al.* 1994). Our approach assumes that measured leaf area loss is entirely attributable to herbivory, rather than damage by pathogens and other small animal activities (gall forming, leaf mining and rolling). This is a reasonable approximation as available evidence shows that the large majority of leaf area loss in tropical forests is caused directly or indirectly by leaf chewing herbivores (García-Guzmán & Dirzo 2004; Pontes Ribeiro & Basset 2007). Assuming steady state, where outputs balance inputs, we take  $F_{he}$  to be approximately equal to the quantities of the same elements released by herbivores to the ground via excreta, bodies, moults and unconsumed leaf fragments. This steady state assumption is valid if (1) the total biomass of herbivores remains stable and (2) the measurements are performed over sufficiently long time scales and large spatial scales. The first assumption seems reasonable in these mature forests with no apparent anthropogenic disturbance where the quantity of primary production should also be stable over time. In addition, the plots are surrounded on all sides by a matrix of similar forests, and so are unlikely to be a net source of, or sink for, migratory herbivores. The second assumption appears reasonable since herbivory was estimated over five sampling periods covering a full seasonal cycle across a hectare of forest at each plot.

In the case of C, some portion of consumed C would be respired by herbivores. To estimate this respiratory flux, we used available literature on insect herbivore energetics (summarised in Wiegert & Petersen 1983; Table S2) to estimate the mean proportions of ingested C excreted, respired or used to grow herbivore body tissue. Most studies were conducted in temperate grasslands but, given the extreme paucity of studies on tropical insect herbivore energetics, we present these values as a useful first approximation of invertebrate herbivore C partitioning in our study. We assumed for this particular analysis that all leaf area loss was attributable to consumption by insect herbivores, though evidence suggests that other organisms account for a substantial minority of total herbivory (~ 25%, Coley & Barone 1996).

Not all of this  $F_{he}$  would have been returned anyway to the soil via litterfall, because herbivores remove foliar material prior to resorption of C, N and P associated with leaf senescence. This material either falls directly as unconsumed leaf fragments or is converted to invertebrate excreta, bodies and moults which is transferred to the soil more slowly. To estimate this additional flux, we therefore estimated the pathways for C, N and P invested in  $F_{pe}$  with and without herbivory. In the absence of herbivory, elements retained in the canopy via resorption ( $R_{nh}$ , Mg ha<sup>-1</sup> year<sup>-1</sup>) were estimated by multiplying  $F_{pe}$  by the mean proportions of foliar C, N and P resorption for tropical evergreen angiosperms ( $Q$ ) (Vergutz *et al.* 2012), while the portion transferred as dead leaves ( $L_{nh}$ , Mg ha<sup>-1</sup> year<sup>-1</sup>) was calculated as follows:

$$L_{nh} = F_{pe} \times (1 - Q)$$

With herbivores present, elemental canopy resorption ( $R_h$ , Mg ha<sup>-1</sup> year<sup>-1</sup>) was calculated by multiplying  $F_{lc}$  by  $Q$ , and litter transfer ( $L_h$ , Mg ha<sup>-1</sup> year<sup>-1</sup>) was calculated as follows:

$$L_h = F_{ic} \times (1 - Q)$$

Given the paucity of foliar resorption estimates from tropical montane forests, we restrict this analysis to our lowland plots (TAM-5, TAM-6) where the ecosystem-level mean resorption estimates for tropical evergreen angiosperms in Vergutz *et al.* (2012) are more likely to be representative. Note that the sums of fluxes without ( $R_{nh} + L_{nh}$ ) and with ( $R_h + L_h + F_{he}$ ) herbivory are equal but the sums of fluxes to soil are different. The herbivore-mediated change in C, N and P transferred to the soil ( $I$ , Mg ha<sup>-1</sup> year<sup>-1</sup>) was calculated as follows:

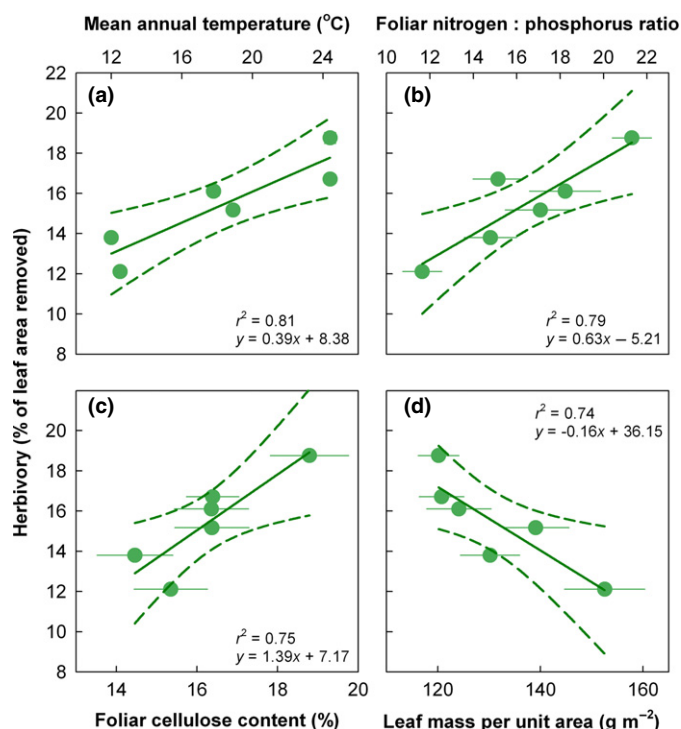
$$I = L_h + F_{he} - L_{nh}$$

### Data analysis

Errors were propagated by taking the square root of the sum of squared absolute errors for addition and subtraction, and squared relative errors for division and multiplication (Taylor 1997). This assumes that the errors are independent and normally distributed. Statistical analyses were conducted with PASW Statistics 18 for Windows (IBM, NY, USA). Variables were transformed to conform to the assumptions of parametric analysis where necessary. To identify associations between mean plot leaf area loss rates and a range of plot factors we ran a Spearman's rank correlation with these variables included (Table S3). These correlations were used to identify a subset of factors for formal inclusion in a regression model (Table S4). To explore the potential to simplify the regression model, we used collinearity statistics to prioritise the factors accounting for most unique variation in mean plot leaf area loss.

### RESULTS

As hypothesised, leaf area loss decreases significantly with elevation-associated temperature decline (Fig. 1a). Leaf area loss rates along the elevational gradient are primarily driven by climate and physico-chemical properties of the food resource, rather than the total amount of food resource available. Thus, rates of leaf damage are not significantly associated with total canopy C uptake, mass, productivity or turnover (Table S3), but are significantly greater at plots with higher mean MAT ( $r^2 = 0.81$ ), higher average foliar cellulose content ( $r^2 = 0.75$ ), higher N : P ratio ( $r^2 = 0.79$ ), and lower LMA ( $r^2 = 0.74$ ) (Fig. 1, Table S3). The link between leaf area loss and foliar N : P ratio is driven more by P ( $r^2 = 0.62$ ) than N ( $r^2 = 0.31$ ) (Table S3). After accounting for the general elevational trend in leaf area loss driven by MAT, average plot foliar P is the only parameter tested that explains a significant portion of the residual variation in herbivory among plots (Table S3, Spearman's rank correlation:  $r^2 = 0.46$ , coefficient =  $-0.812$ ,  $P = 0.050$ ). Together, MAT and foliar P explain 94% of the observed variation in mean plot leaf area loss rate (Table S4, Linear regression:  $F = 22.34$ ,  $P = 0.016$ ). Of the total variance in leaf area loss explained by plot MAT and foliar P, 31% is shared by both factors because they partly covary with each other. However, the majority of variance explained (69%) is uniquely attributable to each factor in isolation (Table S4) sug-

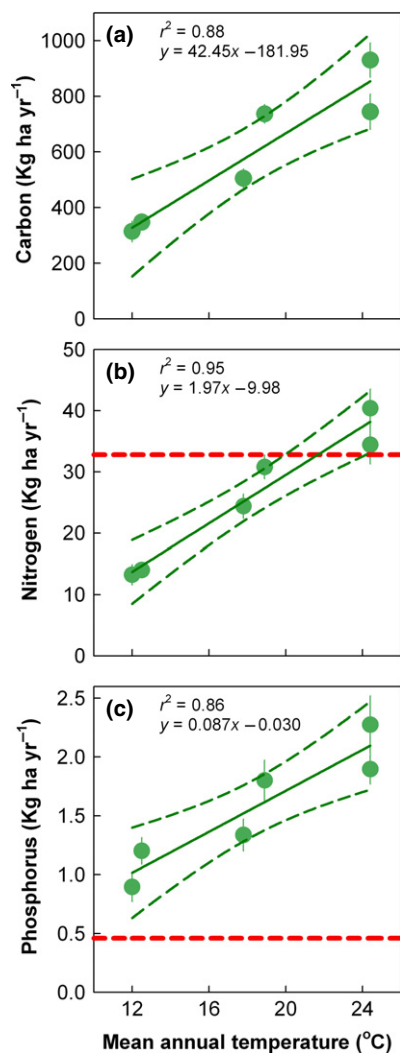


**Fig. 1** Relationship between mean plot leaf area loss and (a) annual air temperature, (b) foliar nitrogen : phosphorus ratio, (c) foliar cellulose content, and (d) leaf mass per unit area. Herbivory is quantified as the percentage of leaf area removed from recently abscised leaves. Circles indicate plot means, error bars denote standard errors ( $n = 25$  for herbivory,  $n = 30$  for foliar traits). Green solid and dashed lines represent linear regressions fitted through the plot data and 95% confidence intervals around the regressed means respectively.  $r^2$  values and equations apply to the linear regression fits in each panel.

gesting that, to a great degree, MAT and foliar P exert independent controls over patterns of leaf area loss among plots.

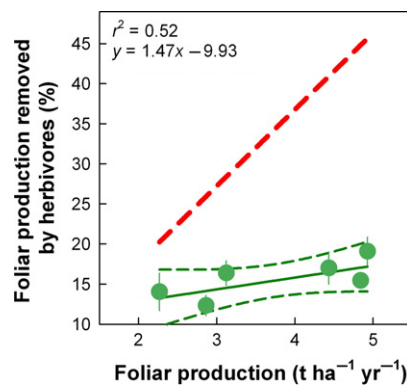
The estimated amount of foliar C lost via herbivory across the gradient ranges from 0.3 to 0.9 Mg C ha<sup>-1</sup> year<sup>-1</sup> (Fig. 2a), equivalent to 12–19% of estimated foliar productivity (Fig. 3) and ~7% of above-ground production, which is typical for tropical forests (Table 1). Predicted herbivory for our plots based upon a global data set (McNaughton *et al.* 1989), was 20–46% of foliar productivity (See Online Supporting Information), with the discrepancy between observed and predicted herbivory increasing sharply with plot productivity (Fig. 3). Using available data on insect herbivore energetics (Wiegert & Petersen 1983) (Table S2), we estimate that  $79 \pm 3\%$  of the foliar tissue consumed by invertebrates is subsequently transferred to the soil as herbivore excreta, moults and bodies, while  $21 \pm 3\%$  is released to the atmosphere as respired CO<sub>2</sub> (Fig. 4). We estimate that foliar herbivory results in the loss of 13–40 kg N ha<sup>-1</sup> year<sup>-1</sup> (Fig. 2b) and 0.9–2.3 kg P ha<sup>-1</sup> year<sup>-1</sup> (Fig. 2c) from trees across the plots surveyed.

Not all of this C, N and P lost from plants via herbivory would have been returned to the soil anyway in the form of litterfall, because to some extent these elements are resorbed from leaves prior to abscission but not from the live leaf



**Fig. 2** Relationship between plot mean annual air temperature and mean plot fluxes of (a) carbon, (b) nitrogen and (c) phosphorus from herbivore removal of live leaf material. Closed circles represent plot means, error bars denote standard errors ( $n = 25$ ). Green solid and dashed lines represent linear regressions fitted through the plot data and 95% confidence intervals around the regressed means respectively.  $r^2$  values and equations apply to the linear regression fits in each panel. For comparison with key ecosystem fluxes of the same elements in lowland forests, red lines denote the estimated magnitude of (b) total N inputs from atmospheric deposition and biological fixation (Phoenix *et al.* 2006; Table S5) and (c) total P inputs from atmospheric deposition and weathering of bedrock (Lewis *et al.* 1987; Ramirez & Andara 1993; Mahowald *et al.* 2008) in lowland tropical forests.

material targeted by herbivores. Due to low estimated rates of foliar C resorption (Vergutz *et al.* 2012), and the portion of foliar C consumed by herbivores which is subsequently respired, we estimate that the presence of herbivores results in little net change in the magnitude of C fluxes from the canopy to the soil (Fig. 4). By comparison, N and P resorption rates in tropical evergreen angiosperms are generally higher (Vergutz *et al.* 2012), meaning that the presence of leaf herbivores results in an estimated additional flux of  $18.5 \pm 5.4 \text{ kg N ha}^{-1} \text{ year}^{-1}$  and  $1.2 \pm 1.1 \text{ kg P ha}^{-1} \text{ year}^{-1}$  from plants to the soil in the lowland plots surveyed in this



**Fig. 3** Relationship between plot foliar production and herbivory. Closed circles represent plot means, error bars denote standard errors ( $n = 25$ ). Green solid and dashed lines represent a linear regression fit through the plot data and 95% confidence intervals around the regressed means respectively. The  $r^2$  value and equation apply to the linear regression fit. The red line denotes the relationship predicted for our plots, based on their foliar production rate, from Fig. 2 in the McNaughton *et al.* (1989) synthesis.

**Table 1** Rates of herbivory in major terrestrial habitat types (Cebrian 2004) and in our study

	Herbivory (% of above-ground NPP)	$n$
Tundra shrublands and grasslands	$1.8 \pm 0.6$	8
Temperate/tropical shrublands and forests	$7.9 \pm 1.5$	52
Our study	$7.0 \pm 0.5$	6
Temperate/tropical grasslands	$34.1 \pm 5.3$	19
Freshwater and marine marshes	$16.8 \pm 5.0$	16
Mangroves	$3.5 \pm 0.5$	11

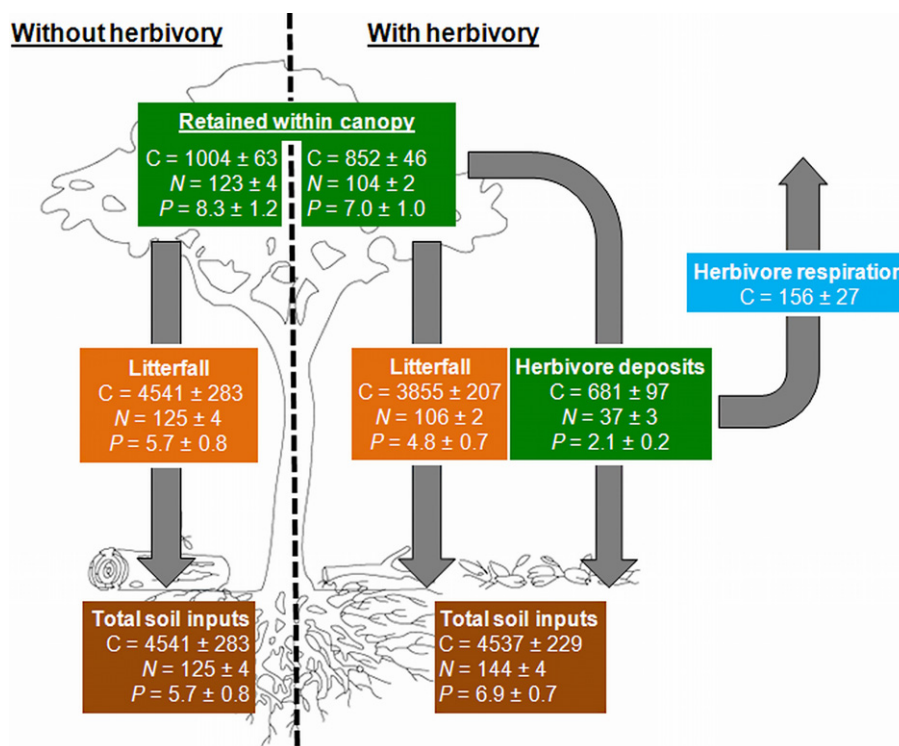
All values represent means  $\pm$  SE.

study (Table 2, Fig. 4). This herbivore-mediated additional flux of nutrients to the soil (Fig. 4) equates to  $\sim 56\%$  of ecosystem N inputs via atmospheric deposition and biological fixation, and  $\sim 260\%$  of P inputs from atmospheric deposition and bedrock weathering (Table 2).

## DISCUSSION

### Drivers of herbivory along elevational gradients

Given the potential for both positive and negative interactions among herbivores (Van der Putten *et al.* 2001), multi-species assemblages of herbivores in nature may have quite different effects upon plant communities and ecosystem function than would be inferred from single-species studies. We present the first estimate of the net consequences of entire invertebrate folivorous herbivore communities on biogeochemical cycling in tropical forests. We find, firstly, evidence for separate roles of temperature and plant community properties, foliar P in particular, in controlling patterns of invertebrate herbivory (Fig. 1, Table S4) and, secondly, that herbivores supply a remarkably large quantity of nutrients to these ecosystems



**Fig. 4** Carbon, nitrogen and phosphorus fluxes of lowland Amazon forest with and without invertebrate herbivory. Values represent means  $\pm$  SE ( $n = 2$ ), in units of  $\text{kg ha}^{-1} \text{ year}^{-1}$ , calculated from the two lowland forest plots in this study. Herbivore deposits represent the sum of excreta, moults, bodies and unconsumed leaf fragments. Resorption efficiencies are means for tropical evergreen angiosperms from a global synthesis,  $C = 18.1\%$ ,  $N = 49.5\%$  and  $P = 59.3\%$  (Vergutz *et al.* 2012). The diagram assumes that no resorption has occurred on leaves consumed by herbivores, which is reasonable given that herbivores tend to prefer young leaves (Coley & Barone 1996). Partitioning of consumed C to herbivore deposits and respiration is estimated from a synthesis of insect energetics (Wiegert & Petersen 1983, Table S2).

**Table 2** Key ecosystem sources of nitrogen and phosphorus in lowland tropical forests, compared with herbivory fluxes from the two lowland study plots

	Nitrogen	Phosphorus	Source
Herbivore consumption	37.4 (34.4–40.4)	2.1 (1.9–2.3)	This study
Herbivore-mediated input*	18.5 (17.0–20.0)	1.2 (1.1–1.3)	This study
Atmospheric deposition	4.8 (0.3–9.3)	0.05 (0.01–0.10)	Phoenix <i>et al.</i> 2006; Mahowald <i>et al.</i> 2008;
Weathering	—	0.41 (0.31–50)	Ramirez & Andara 1993; Lewis <i>et al.</i> 1987.
Biological fixation	28 (11–91)	—	This study, Table S5

All values are in units of  $\text{kg ha}^{-1} \text{ year}^{-1}$  and represent means (minimum–maximum). \*The additional flux of N and P from plants to soil caused by herbivores removing green leaf material before resorption of these elements which would usually occur before leaf drop, assuming typical values of foliar N and P resorption for tropical evergreen angiosperms (Vergutz *et al.* 2012).

compared to other, more well known, sources (Fig. 2, Table 2). Given the mounting evidence for a narrow thermal range of ectotherms in the tropics, the presence of some direct temperature regulation on herbivore population activity and/or abundance appears highly plausible (Bale *et al.* 2002; Deutsch *et al.* 2008). Therefore, ongoing efforts to integrate plant–soil interactions into global vegetation models (Ostle *et al.* 2009) to more accurately predict the consequences of future warming on terrestrial ecosystems should incorporate the impacts of consumers and their climatic sensitivities.

The observed change in plant traits, and their effects on herbivory, along the gradient are consistent with previous findings (Coley *et al.* 1985; Sundqvist *et al.* 2013) showing

that plant communities in harsh conditions tend to be dominated by slow-growing plant species, producing poor quality leaves that are generally less palatable to herbivores. The observed shifts in foliar traits were associated with shifts in plant species community composition along the gradient, rather than intraspecific variation or soil properties, since no single tree species occurred across the entire gradient (Jankowski *et al.* 2013) and elevational shifts in soil nutrients were relatively modest or nonlinear (peaking at mid-elevations) (Table S1). Insectivorous bird diversity also strongly declines with increasing elevation along the same gradient (Jankowski *et al.* 2013), providing evidence for coordinated responses of producers and consumers across multiple trophic level to abiotic shifts associated with elevation.

### Links between ecosystem production and herbivory

In contrast with established theory, we find no significant link between foliar productivity and our direct measure of herbivore consumption – leaf area loss (McNaughton *et al.* 1989) (Table S3). The relationship between foliar production and herbivory developed by McNaughton *et al.* (1989) predicts that consumption at our most productive, lowland forest plots should approach 40–50% of foliar production, whereas we estimate values nearer to 20% (Fig. 3). Thus, the large estimated nutrient fluxes resulting from invertebrate herbivory at our study plots (Fig. 2) occur despite a relatively low herbivory pressure by global standards (Table 1). The most productive systems included in the McNaughton *et al.* (1989) synthesis are predominantly African savannahs with intact assemblages of megafauna. Thus, we suggest that the lower overall consumption levels and the weaker coupling between producers and consumers observed in the present study could represent a distinctive facet of tropical forest functioning and/or of herbivore communities dominated by invertebrates. This is consistent with available data on herbivory across major terrestrial habitats (Table 1), suggesting that theories predicting that the tropics should generally be characterised by more, and more intense, biotic interactions (Schemske *et al.* 2009) may be oversimplified. One reason for the disparity between observed and predicted herbivory at our plots (Fig. 3) could be the inaccessibility of tropical forest foliar production to herbivores compared to savannahs because the leaves are relatively well-defended and/or are physically out of reach from ground-based herbivores. In addition, smaller-bodied ectothermic herbivores, which often dominate in tropical forests (Coley & Barone 1996), will tend to have both lower energetic requirements and faster gut passage times (Parra 1978), reducing their utilisation of available foliar resources, compared to the communities of large-bodied mammalian herbivores in savannahs. Another possibility is that Amazon plant communities have not yet fully equilibrated to the extinctions of large-bodied herbivores in the Pleistocene (Doughty *et al.* 2013) in terms of C uptake and allocation. This hypothesis could be readily tested by comparing ecosystem productivity and herbivory in rainforests with more abundant megafauna (e.g. in Africa and Asia), and across gradients of megafaunal abundance.

### Herbivore impacts on carbon cycling

Our results suggest that invertebrate herbivores could have considerable impacts on tropical ecosystem C cycling, through several mechanisms. Firstly, loss of photosynthetic leaf area associated with herbivory likely incurs a substantial penalty in terms of total plant C uptake, and/or the metabolic costs associated with repairing damage. Since photosynthesis is impaired in live leaf tissue for some distance around the site of damage (Zangerl *et al.* 2002), suppression of ecosystem C uptake by herbivory is probably even greater than estimates of leaf area loss would suggest. Secondly, we find that herbivores alter both the total amount and the pathways of C transferred from the canopy to the soil. Herbivory effectively reduced leaf litterfall by 12–19% across the plots. Experimental manipulations of litterfall in the tropics have demonstrated large and rapid impacts

on soil C cycling both by directly changing the amount and quality of C entering the soil, and by indirectly stimulating decomposition of previously stable soil C pools (Leff *et al.* 2012). The material removed by herbivores must have been converted into a range of deposits (excreta, bodies, moults, unconsumed leaf fragments) which would tend to more nutrient rich than litterfall because they were derived from foliar tissue prior to nutrient resorption (Vergutz *et al.* 2012). Experimental applications of invertebrate herbivore deposits to the soil, mainly of excreta in temperate systems, have found both positive and negative effects on soil nutrient and C cycling (Bardgett & Wardle 2003; Hartley & Jones 2004). This variability could reflect differences in soil properties among study systems. Specifically, decomposition of low quality organic litter is generally suppressed by fertilisation, while breakdown of nutrient rich litter is often accelerated by N addition (Knorr *et al.* 2005). Given that organic matter quality generally decreases with elevation, which, together with the effect of temperature, often leads to greater accrual of soil organic matter at higher elevations (Sundqvist *et al.* 2013), we hypothesise that differential impacts of herbivore deposits on decomposition could constitute an important feedback, reinforcing spatial patterns of soil C sequestration, along the gradient.

### Herbivore contributions to nutrient fluxes

We used literature estimates of other sources of ecosystem N and P in lowland tropical forests for comparison with our lowland plots (See Online Supporting Information). This comparison indicates that herbivore deposits (excreta, bodies, moults, unconsumed leaf fragments) likely make a major contribution to nutrient availability, particularly P, in tropical soils. Further, the nutrients in herbivore deposits are likely to be relatively labile (Bardgett & Wardle 2003; Hartley & Jones 2004) since the organic matter has been fragmented both physically (increasing the surface area susceptible to decomposition and nutrient uptake) and chemically in herbivore guts (yielding simpler, more accessible chemical compounds), and originates from live leaf material which tends to have a greater quantity of nutrients and labile C compounds than litterfall. The impacts of these nutrients in herbivore deposits on ecosystem function are difficult to predict (Bardgett & Wardle 2003; Hartley & Jones 2004), and likely to vary with soil properties (Knorr *et al.* 2005), but could be readily tested through experimental manipulations of deposits (Fonte & Schowalter 2004, 2005; Schowalter *et al.* 2011). One potentially important role of deposits could operate by influencing competition for nutrients among soil microbes and different tree species. Many tropical trees produce litter of extremely poor quality which decomposes surprisingly slowly given the near-optimal abiotic conditions (Hättenschwiler *et al.* 2011). There has been speculation that this reflects an active strategy on the part of nutrient-limited plants to starve soil microbes, generally better competitors for soil nutrients than plant roots (Kaye & Hart 1997), of the necessary energy required to take up soil nutrients (Hättenschwiler *et al.* 2011). In this context, herbivory may tip the competitive balance in tropical forests towards soil microbes, through provision of labile C necessary for microbes to effectively immobilise soil nutrients, thereby

generally exacerbating the plant nutrient limitation characterising large areas of the tropics (Vitousek 1984) and entrenching the dominance of slow-growing, resource-conserving plants in lowland forests.

## CONCLUSIONS

These results have four broad implications. Firstly, they demonstrate the general importance of invertebrate herbivore–plant interactions in tropical forests. These interactions are difficult to incorporate into terrestrial biogeochemical models but may be vital to develop an accurate predictive understanding of tropical forest processes. Secondly, they show that invertebrate herbivores liberate a surprisingly large portion of N and P from tropical trees, much of which is likely to be relatively labile, with potentially large impacts on the rates and spatial pattern of soil C sequestration across forests, and for competitive interactions among plant functional types. Thirdly, despite the apparent importance of herbivores for tropical nutrient cycling, our results indicate that Amazon forests may be characterised by an unusually weak link between primary producers and consumers compared to other major world biomes. Finally, they provide early clues about the potential impacts of changes in herbivore populations, due to extinctions, invasions or range shifts, in tropical forests on several important ecosystem functions. Further research on the biogeochemical impacts of herbivores will be essential to better understand the functioning of tropical ecosystems now and in the future.

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## AUTHOR CONTRIBUTIONS

DBM processed the leaf images, analysed the data and wrote the paper. All co-authors assisted with manuscript writing and

interpretation of results. GPA and REM coordinated collection of leaf physical and chemical traits. LC–J, FS and RT were responsible for data collection and processing of leaf physical and chemical traits. JESE, WHH, FFFA, DFGC, LDB, LPHQ, IAT, LLM, ARD, MMS, BLPV, JMLR and PCGB were responsible for collection of leaf material and leaf images for leaf area loss measurements. NSR, CAJ, CD and YM coordinated collection of leaf material and leaf images for leaf area loss measurements.

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