

# Insect herbivory and herbivores of *Ficus* species along a rainforest elevational gradient in Papua New Guinea

by Sam, K., Koane, B., Sam, L., Mrazova, A., Segar, S., Volf, M., Moos, M., Simek, P., Sisol, M. and Novotny, V.

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| Complete List of Authors:     | Sam, Katerina; Biology Centre CAS, Institute of Entomology; University of South Bohemia, Faculty of Sciences<br>Koane, Bonny; The New Guinea Binatang Research Centre<br>Sam, Legi; Biology Centre CAS, Institute of Entomology<br>Mrazova, Anna; Biology Centre CAS, Institute of Entomology; University of South Bohemia, Faculty of Sciences<br>Segar, Simon; Biology Centre of Czech Academy of Sciences, Institute of Entomology Branisovska 31 Ceske Budejovice, CZ 37005, Tropical Ecology; University of South Bohemia, Faculty of Sciences; Harper Adams University, Department of Crop and Environment Sciences<br>Volf, Martin; Biology Centre CAS, Institute of Entomology; University of South Bohemia, Faculty of Sciences; German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig<br>Moos, Martin; Biology Centre CAS, Institute of Entomology<br>Simek, Petr; Biology Centre CAS, Institute of Entomology<br>Sisol, Mentap; The New Guinea Binatang Research Centre<br>Novotny, Vojtech; Institute of Entomology; University of South Bohemia, Faculty of Sciences |
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Manuscripts

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3 **Insect herbivory and herbivores of *Ficus* species along a rainforest**  
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17 K. Sam<sup>\* a,b</sup>, B. Koane<sup>c</sup>, L. Sam<sup>a</sup>, A. Mrazova<sup>a,b</sup>, S. Segar<sup>a,b,d</sup>, M. Volf<sup>a,b,e</sup>, M. Moos<sup>a</sup>, P. Simek<sup>a</sup>,  
18  
19 M. Sisol<sup>e</sup>, V. Novotny<sup>a,b</sup>  
20  
21  
22

23  
24 <sup>a</sup> Biology Centre of Czech Academy of Sciences, Institute of Entomology, Ceske Budejovice,  
25  
26  
27 Czech Republic  
28

29 <sup>b</sup> Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic  
30  
31

32 <sup>c</sup> The New Guinea Binatang Research Center, Madang, Papua New Guinea  
33

34 <sup>d</sup> Department of Crop and Environment Sciences, Harper Adams University, UK  
35

36  
37 <sup>e</sup> Molecular Interaction Ecology Group, German Centre for Integrative Biodiversity Research  
38  
39 (iDiv) Halle-Jena-Leipzig, Leipzig, Germany  
40  
41

42  
43  
44  
45  
46  
47 \* Corresponding author Katerina Sam: katerina.sam.cz@gmail.com  
48

49 Orcid ID of Katerina Sam: [orcid.org/0000-0002-3436-0579](https://orcid.org/0000-0002-3436-0579)  
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## Abstract

Classic research on elevational gradients in plant-herbivore interactions holds that insect herbivore pressure is stronger under warmer climates of low elevations. However, recent work has questioned this paradigm, arguing that it oversimplifies the ecological complexity in which plant-insect herbivore interactions are embedded. Knowledge of antagonistic networks of plants and herbivores is however crucial for understanding the mechanisms that govern ecosystem functioning. We examined herbivore damage and insect herbivores of eight species of genus *Ficus* (105 saplings) and plant constitutive defensive traits of two of these species, along a rainforest elevational gradient of Mt. Wilhelm (200 – 2700 m a.s.l.), in tropical Papua New Guinea. We report overall herbivore damage 2.4% of leaf area, ranging from 0.03% in *Ficus endochaete* at 1700 m a.s.l. to 6.1% in *F. hombroniana* at 700 m a.s.l. Herbivore damage and herbivore abundances varied significantly with elevation, as well as among the tree species, and between the wet and dry season. Community-wide herbivore damage followed a hump-shaped pattern with the peak between 700 and 1200 m a.s.l. and this pattern corresponded with abundance of herbivores. For two tree species surveyed in detail, we observed decreasing and hump-shaped patterns in herbivory, in general matching the trends found in the set of plant defences measured here. Our results imply that vegetation growing at mid-elevations of the elevational gradient, i.e. at the climatically most favourable elevations where water is abundant, and temperatures still relatively warm, suffers the maximum amount of herbivorous damage which changes seasonally, reflecting the water availability.

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3 **Key-words** leaf damage, leaf-chewers, defensive traits, defoliating insects, phytophagy,  
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5 plant-herbivore interactions, trophic interactions, altitudinal gradient, top-down  
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8 interactions.  
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## Introduction

Arthropods are a major component of ecosystems, in terms of both their biomass and the variety of functional roles they play. Insect herbivores, as the most important primary consumers in tropical forests, consume up to ~70 % of total leaf area (Coley & Barone 1996). In tropical Asia, insect herbivory accounted for over 98 % of the leaf loss, and 93 % of the insect herbivory was due to chewing insects alone (Adams *et al.* 2009). Other studies report that, on average, insect herbivores consume 18 percent of the biomass produced annually in terrestrial ecosystems (Cyr & Face 1993), and these losses roughly correspond to the energy invested by plants into reproduction (Schoonhoven *et al.* 2005). The relationships between plants and herbivores are therefore among the most intensively studied biotic interactions (Moles *et al.* 2011a, Moles *et al.* 2011b, Jamieson *et al.* 2012, Kozlov *et al.* 2015, Anstett *et al.* 2016). Yet we lack a clear understanding of how arthropod abundances change along environmental gradients and what functions they play along them.

Due to arthropod's enormous diversity, it is difficult to examine patterns of species richness across all arthropods and most studies tend to focus on one (e.g. Niemela *et al.* 1992, Brehm *et al.* 2003, Sanders *et al.* 2007) or several taxonomic groups (e.g. Peters *et al.* 2016), rather than on whole feeding guilds or on patterns of total arthropod abundances.

There is a general assumption in the literature that herbivory (more exactly, folivory, because plant damage by sap-feeders and root-feeders is usually not considered) decreases with increasing elevation. Hypothesised mechanism for this pattern include greater diversity of herbivores (e.g. Hodkinson 2005, Beck *et al.* 2011), more specialist consumers (Rodríguez-Castañeda *et al.* 2010) and their higher consumption per capita (Scheidel & Bruelheide 2001) at lower elevations; elevational difference in host-plant quality (Hodkinson 2005) and reduced abundances of insects at higher elevations owing to lower temperatures

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2  
3 (Hodkinson 2005). The relative costs for compensating for herbivory also differ with  
4  
5 elevation. A plant can more easily compensate for the damage when it has plentiful  
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7 resources. So it may not be overall herbivory but rather the relative costs, which drives plant  
8  
9 defences along the elevational gradients (e.g. Pellissier *et al.* 2016).  
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13         There is a growing evidence that such generalities are not consistent across space,  
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15 time and interacting resource gradients. Various patterns of arthropod abundance have been  
16  
17 observed along elevational gradients. Some studies report highest arthropod abundance at  
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19 low elevations (e.g. Collins 1980, Wolda 1987), while others show highest summer arthropod  
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21 abundance at mid-elevations (Janzen 1973, Ghosh-Harihar 2013).  
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25         Similarly, studies along elevational gradients report a variety of patterns including  
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27 decreasing (e.g. Louda 1982, Galen 1990, Kelly 1998, Alonso 1999, Poveda *et al.* 2012, Suzuki  
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29 *et al.* 2013, Metcalfe *et al.* 2014, Hülber *et al.* 2015), increasing (e.g. Koptur 1985, Erelli *et al.*  
30  
31 1998, Hagen *et al.* 2007, Bito *et al.* 2011, Altmann & Claros 2015, Matías & Jump 2015, Zhang  
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33 *et al.* 2015) or constant herbivory (Hódar & Zamora 2004, Bito *et al.* 2011, Lay *et al.* 2013).  
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37         Generally, conclusions about arthropod herbivory along elevational gradients are far from  
38  
39 definitive, and there is growing evidence that results are not consistent across time and  
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41 space (Andrew *et al.* 2012, Moreira *et al.* 2018). Recent review called not only for critical re-  
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43 evaluation of the evidence for geographic gradients in plant-insect herbivore interactions,  
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45 but also for development of a new mechanistic framework for predicting the patterns that  
46  
47 do exist (Moreira *et al.* 2018).  
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52         A major issue with assessing arthropod herbivory along environmental gradients is  
53  
54 the variation among plant species sampled within a gradient. Species replacement along  
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56 large-scale gradients preclude comparisons of intra- and inter-specific patterns at the same  
57  
58 scale (Johnson & Rasmann 2011, Anstett *et al.* 2016). There is usually a complete turnover of  
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3 plant species along long elevational gradients, from lowlands to alpine areas. Restricting  
4  
5 analysis to a single widespread host plant species is only a partial solution to this problem  
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8 (e.g. Zhang *et al.* 2015), as it will limit the gradient length available for the study. In some  
9  
10 studies, a plant community was studied for leaf herbivory (Bito *et al.* 2011) and mixed  
11  
12 approach (i.e. community-wide survey and closer focus on a species with long range) was  
13  
14 used in others (Weissflog *et al.* 2017). The observed patterns differed in the two types of  
15  
16 studies. While a species-focused studies reported usually more complex patterns (Moreira *et*  
17  
18 *al.* 2018, Weissflog *et al.* 2017), the community-wide studies reported decreasing patterns  
19  
20 more often (Moreira *et al.* 2018). Some species occupy long elevational gradients, but still  
21  
22 have peak abundances within a particular elevation. This peak might lead to increased  
23  
24 herbivory due to the increased apparency of the plant species, or decreased herbivory due to  
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26 increased selection for plant defence (Moreira *et al.* 2018, Weissflog *et al.* 2017). Local  
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28 adaptations and changes in plant defences along the gradient might therefore be quite  
29  
30 important, adding complexity to the pattern.  
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38 The higher herbivory rates at lower elevations were often detected even though  
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40 lowland tropical leaves are less nutritious and better defended (Coley & Aide 1991, Pellissier  
41  
42 *et al.* 2012, Pellissier *et al.* 2016, Galmán *et al.* 2018). Mature lowland tropical leaves have  
43  
44 significantly lower foliar nitrogen and water, and higher concentrations of tannins (Coley &  
45  
46 Aide 1991) than those from higher elevations. Tropical young leaves have further several  
47  
48 novel types of defences (delaying greening, extrafloral nectaries (Coley & Kursor 1996)).  
49  
50 Since both mature and young leaves of lowland tropical species are better defended, the  
51  
52 higher rates of herbivory in tropical lowlands were hypothesised to reflect overall herbivore  
53  
54 pressure (Coley 1998). This hypothesis was confirmed by Garibaldi *et al.* (2011) in South  
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56 America but not by Bito *et al.* (2011) in Australia.  
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3 Specific leaf area (SLA) and trichome density is often considered to be a surrogate for  
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5 leaf thickness and toughness, which is also frequently correlated with water content (Groom  
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7 & Lamont 1999), as parameters of leaf morphology with a possible impact on leaf-chewing  
8  
9 insects. Low SLA is believed to be an adaptation to harsh abiotic conditions. having high  
10  
11 SLA values where resources are plentiful, a plant can escape competition for light  
12  
13 (Kergunteuil *et al.* 2018). Specific leaf area was previously shown to decrease with increasing  
14  
15 elevation and correlate positively with herbivore damage (Garibaldi *et al.* 2011, Pellissier *et*  
16  
17 *al.* 2012). Leaf chemistry and physiological traits can substantially change along the gradient  
18  
19 even among conspecific plants (Maschinski 1989, Renwick *et al.* 2001, Külheim *et al.* 2011).  
20  
21 High concentrations of phenolic compounds may deter insect feeding, reduce insect growth  
22  
23 and survivorship, and reduce herbivore densities and herbivore species richness (Rossiter *et*  
24  
25 *al.* 1988, Forkner *et al.* 2004). However, the changes in plant quality with respect to elevation  
26  
27 and importance of individual aspects of plant quality are species-specific and still largely  
28  
29 unknown. Further, leaf chemistry and physiological traits can substantially change along the  
30  
31 gradient even among conspecific plants (Maschinski 1989, Renwick *et al.* 2001, Külheim *et al.*  
32  
33 2011).

34  
35 Results of our colleagues (Volf *et al.* 2018) suggest that it is mainly the diversity of  
36  
37 secondary metabolites that drove insect community structure on *Ficus* plants but not their  
38  
39 total content. Some other studies also reported that while the total content of phenolics had  
40  
41 little effect on larval leaf-chewers, polyphenol oxidative activity (which represents  
42  
43 polyphenol capacity to cause oxidative stress to insects) affected whole communities of  
44  
45 larval leaf-chewers (Dyer *et al.* 2003, Salminen & Karonen 2011).

46  
47 Our objective was to quantify the effects of elevation on herbivorous damage, and to  
48  
49 find out whether the herbivory damage correlates with the host defences, and if abundances  
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3 of herbivorous insects correlated directly with herbivorous damage. If there is a clear  
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5 correlation between herbivory and plant defences, it would be a strong argument that  
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7 absolute values of the damage drive the defences. If somewhat differential pattern is found  
8  
9 in both (e.g. herbivory damage shows hump-shaped distribution, but defences don't), it  
10  
11 would suggest that abiotic factors play a role.  
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### 18 **Materials and methods**

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20 We performed the study along the Mt. Wilhelm elevational gradient in the Central Range of  
21  
22 Papua New Guinea. The surveyed part of the transect, ca. 25 km long, comprises six study  
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24 sites spaced regularly at 500 elevational metre intervals, from the lowest study site located  
25  
26 within the lowland floodplains of the Ramu River at 200 m a.s.l. (05° 44' S, 145°20' E) to the  
27  
28 elevational limit of *Ficus* distribution at 2700 m a.s.l. (05°48' S, 145°09' E). Mean annual  
29  
30 temperature (measured by data loggers Comet R3120 placed in forest understorey)  
31  
32 decreases from 27.4°C at the lowland site to 8.37°C at the tree line at a constant rate of 0.54°C  
33  
34 per 100 elevational metres (see Sam *et al.* 2019 for more detail). Air humidity measured by  
35  
36 the same dataloggers ranged between 90 and 100% at all elevations, however the rainfall  
37  
38 was not evenly distributed during the year (see Sam *et al.* 2019 for more detail). Average  
39  
40 annual precipitation is 3,288 mm (measured at local meteorological station, data provided  
41  
42 by Phil Shearman) in the lowlands, rising to 4,400 mm at 2,700 m a.s.l., with a distinct  
43  
44 condensation zone between 2,500 and 2,700 m a.s.l. (Sam *et al.* in prep). Mean monthly  
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46 precipitation along the gradient is 315 mm, and mean monthly precipitation between the  
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48 two survey (i.e. wet season) periods was 398 mm. The elevational gradient, further  
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50 description of study sites, climatic and habitat characteristics are published elsewhere  
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60 (Tvardikova 2013, Sam & Koane 2014, Sam *et al.* 2015, Sam *et al.* 2019).

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3 Unfortunately, no tree genus was distributed along the entire elevational gradient.  
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6 Therefore, we selected species from the widely distributed genus *Ficus* (Moraceae) as a  
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8 model system, which allowed us to work from the lowlands up to 2,700 m a.s.l. (05° 48' S,  
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10 145°09' E, Table 1). *Ficus* is an exceptionally species-rich genus, with New Guinea as the  
11  
12 center of its diversity (Ronsted *et al.* 2008). *Ficus* also has high diversity (at least 75 species)  
13  
14 and abundance (typically >5% of stems with DBH  $\geq$ 1 cm) along the Mt. Wilhelm elevational  
15  
16 gradient (L. Sam, unpubl. data). *Ficus* has an upper elevational limit at 2,900 m a.s.l at Mt.  
17  
18 Wilhelm, as well as elsewhere (Berg & Corner 2005). Some species of *Ficus* (e.g. *F. hahliana*  
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20 Diels, 1935) have particularly wide elevational ranges (Berg & Corner 2005).  
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25 We selected and tagged experimental saplings belonging to 4 – 6 evergreen *Ficus*  
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27 species at each elevational study site (Table 2). As far as possible, we selected the locally  
28  
29 most common species which had also broad elevational ranges. The selected plant species  
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31 did not produce any exudates or sugar droplets attracting ants. We selected between 14 and  
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33 20 saplings per elevational study site, i.e. 3 – 5 per plant species and elevation or 105  
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35 saplings along the gradient (Table 2). For statistical independence, we allowed at least 80 m  
36  
37 between any pair of individuals. We visually assessed saplings of the focal species and  
38  
39 selected individuals that looked similar, had approximately 500 leaves growing within a  
40  
41 well-developed crown 2.5 – 4 m above the ground. The saplings did not have any ant nests  
42  
43 and did not have any abnormally high herbivory or fungal damage. Average leaf-sizes of  
44  
45 the selected species ranged from 16.31 to 154.10 cm<sup>2</sup>, and two species (*Ficus arfakensis* King,  
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47 1888 and *F. endochaete* Summerhayes, 1941) had significantly different sized leaves at some  
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49 elevations (Table 2).  
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57 At the beginning of the experiment, at the end of the dry season between 31-Aug-  
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59 2014 and 3-Nov-2014 (Table 1), we first collected all arthropods (described below). Then we  
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3 counted all leaves present at the sapling (to be able to estimate the total leaf area of the  
4  
5 sapling) and further we proceeded with collection of leaves for herbivory measurement. To  
6  
7 assess herbivore damage, we randomly selected two branches (with ca. 30 leaves each;  $57 \pm$   
8  
9  $5.6$  (mean  $\pm$  SE) in total per sapling, i.e. up to ca. 15% of standing foliage) per sapling. We  
10  
11 clipped these two branches and collected all leaves from them. This survey period denotes  
12  
13 the first point measurement ( $t = 0$ ) of herbivore damage. A branch clipping (i.e. simulation of  
14  
15 an artificial herbivory by a vertebrate herbivore) from a grown sapling should have only  
16  
17 short-term and non-significant effect on subsequent herbivory measurement and plant  
18  
19 growth in our experiment (Strauss *et al.* 1996, Seldal *et al.* 2017).  
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26 Using a  $50 \times 50$  cm<sup>2</sup> white backgrounds, we took photographs of all collected and  
27  
28 flattened leaves from each sapling (i.e. we took as many photos as needed to photograph all  
29  
30 collected leaves). Using Adobe Photoshop CS6 (Adobe Systems Inc., USA). We first outlined  
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32 the missing edges on the photographed leaves based on the expected shape and whitened or  
33  
34 blackened various damages or holes on the leaves. Using various guides, google image  
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36 searches, and our previous expertise, we carefully distinguished leaf damage caused by  
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38 chewing and mining herbivores (which was whitened), while we did not consider fungal  
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40 damage or mechanical leaf damage (which we blackened). We then turned the photos to  
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42 black and white pictures. We used ImageJ version 1.47 (National Institute of Health, USA) to  
43  
44 calculate the remaining leaf area ( $a$ , in cm<sup>2</sup>), the extrapolated leaf area without any herbivore  
45  
46 damage ( $b$ ), and the area lost to herbivory ( $c = b - a$ ). We then estimated the percentage of  
47  
48 leaf-area loss as  $c/b \times 100$ . We calculated the herbivory loss as loss in % of are per leaf and in  
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50 cm<sup>2</sup> per 100cm<sup>2</sup>. These two values were highly correlated, so we used only % of herbivory  
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52 per leaf in analyses and figures.  
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3 We conducted a second survey of herbivore damage, on the very same saplings as  
4 used in the first survey, approximately six months after the first survey (between 21-Mar-  
5 2015 and 11-May-2015, Table 1) at the end of the rainy season of 2014/2015. Similarly, to the  
6 first survey, we conducted the arthropod collection first. Then we collected all leaves from  
7 each sapling into a bag. We randomly selected 55-60 of leaves (to match the samples size of  
8 the first survey), photographed them on the same white background as earlier, and analysed  
9 them in the same way as in the first survey.  
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20 After each survey, we obtained values for  $57 \pm 5.6$  (mean  $\pm$  SE, min = 50, max = 95)  
21 leaves per sapling. We weighed all scanned and the remaining leaves to calculate the total  
22 leaf area for each sapling from the second survey. We used measured mean leaf size per  
23 sapling multiplied by the calculated number of leaves on sapling to obtain total leaf area of  
24 the sapling the beginning of the experiment (first survey) The amount of herbivore damage  
25 was assessed blindly; viz. the field collector (BK) and the research assistant (AM) handling  
26 the leaves did not know that the study aimed to measure leaf damage, preventing any bias  
27 in leaf selection.  
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40 We measured herbivory on at least three saplings of each plant species at each  
41 elevational study site (Table 2) and in average of 438 leaves per plant species and elevation  
42 (or  $114 \pm 11.8$  leaves per sapling), which is a number recommended by previous studies  
43 (Zhang *et al.* 2015, Kozlov & Zvereva 2017). By analysis of 11,448 leaves, our study belongs  
44 to those larger (Bito *et al.* 2011, Metcalfe *et al.* 2014, Kozlov & Zvereva 2017). The number of  
45 individual saplings sampled per species and elevational study site seemed to be sufficient as  
46 their herbivory between individuals did not differ significantly in majority of cases (Table  
47 S1) and the model with tree number as a random effect differed only marginally from model  
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3 without random effect (AIC = 2.12). We therefore decided to use mean herbivory per species  
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5 at each elevation and survey in further analyses.  
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### 10 *Arthropod survey*

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12 Arthropod censuses were performed destructively during both surveys at the same  
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14 individuals that were used for herbivory measurements, just before we started  
15  
16 measurements of herbivorous damage and leaf collections. We slowly lowered the trunk of  
17  
18 the sapling above a mosquito net, wrapped it to the mosquito net and sprayed with fast  
19  
20 knock-down insecticide (Mortein®). After a while, we shook foliage firmly, opened the net  
21  
22 and collected all arthropods (>1mm) and preserved them in vials filled with DNA grade  
23  
24 ethanol. In laboratory, arthropods were counted, identified into three feeding groups (i.e.  
25  
26 “chewing herbivores”, “predators”, “other arthropods” who have no relationship to  
27  
28 herbivorous damage as we measured it and do not act as mesopredators – e.g. adult flies,  
29  
30 pupae, adult Lepidoptera etc., sap sucking herbivores). Abundances of chewing herbivorous  
31  
32 arthropods was then calculated as number of individuals in feeding guild per m<sup>2</sup> of leaf  
33  
34 area.  
35  
36  
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43  
44

### 45 *Plant defensive trait measurements*

46  
47 We measured plant defensive traits for two plant species (out of the 8 surveyed for herbivory).  
48  
49 For each of these two species at each elevation, we collected leaf discs from five individuals.  
50  
51 Where possible, the same individuals were used for herbivory and insect survey and trait  
52  
53 measurements, but up to two individuals per plant species and elevation were sampled from  
54  
55 different individual. These were usually individuals with smaller amount of leaves than was  
56  
57 acceptable for the herbivory and herbivore survey. We collected two 4.5 cm<sup>2</sup> leaf discs per leaf  
58  
59  
60

1  
2  
3 from 20 young, but fully expanded leaves for each individual, avoiding the central vein (1 g  
4  
5 of dry weight in total on average). The discs were air-dried and stored in silica gel. Half of  
6  
7 them were used for the analysis of triterpenes and half of them for measurement of physical  
8  
9 traits.  
10  
11

12  
13 Physical Traits: We measured trichome density and specific leaf area (SLA). Trichome  
14  
15 density and SLA are parameters of leaf morphology with a possible impact on leaf-chewing  
16  
17 insects. The total number of trichomes per 10 mm<sup>2</sup> and their average length was measured on  
18  
19 five leaf discs per individual using ImageJ (ver.1.48). Values for dorsal and ventral sides of  
20  
21 the discs were combined. Specific leaf area (SLA) was calculated as the area per unit mass of  
22  
23 five dried leaf discs collected from five leaves of known diameter for each individual.  
24  
25  
26

27  
28 Triterpene analysis: Dried powdered leaf tissue samples were ground with methanol  
29  
30 in TissueLyser. Then they were centrifugated and aliquot was saved for further use.  
31  
32 Terpenoids in aliquot were measured on a Dionex Ultimate 3000 LC system equipped with  
33  
34 an Open XRS autosampler and coupled to a Q Exactive Plus Orbitrap mass spectrometer  
35  
36 (Thermo Fisher Scientific, San Jose, CA, USA). A reversed phase Kinetex was used for  
37  
38 separating the analytes. For further measurement details, refer to the Methods S1 in  
39  
40 Supplementary information.  
41  
42  
43  
44  
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47

#### 48 Data analysis

49  
50 We calculated the herbivory as (a) loss in % of are per individual leaf and in (b) cm<sup>2</sup> per  
51  
52 100cm<sup>2</sup>. Actual leaf area damaged (in cm<sup>2</sup> per 100 cm<sup>2</sup>, log transformed) should have  
53  
54 statistically higher power than arcsine-transformed proportional data (Warton & Hui 2011),  
55  
56 and were previously recommended for analyses of herbivore damage, while most of the  
57  
58 authors usually report percentage of the area damaged. The results for herbivory measured  
59  
60



1  
2  
3 as leaf area (in cm<sup>2</sup> denominated by leaf size, binomial distribution, logit link function)  
4  
5 showed the same patterns (Fig. S1 and Table S1) and results (Study sites: Dev. = 2.54, df = 5,  
6  
7  $\chi^2 < 0.001$ ; Species: Dev. = 24.87, df = 13,  $\chi^2 < 0.001$ ; Survey: Dev. = 0.51, df = 1,  $\chi^2 < 0.081$ ) as  
8  
9 the herbivory measured in %. Because the results did not differ significantly, and we  
10  
11 observed no overdispersion in our data on herbivore damage measured in % and arcsine-  
12  
13 transformed, we decided to report only results for percentages of herbivore damage from  
14  
15 here onwards; which is common practice for most authors. The arcsine square root  
16  
17 transformed percentages of the leaf area damaged by arthropod herbivores, met the  
18  
19 normality assumption.  
20  
21  
22  
23  
24

25 To investigate the main effects (elevation, season and plant species) along the  
26  
27 elevational gradient, we ran a Generalized Linear Mixed-effect Model (GLMM; with  
28  
29 Gaussian family and identity link function for arcsine-transformed percentage data and with  
30  
31 binomial family and logit link function for actual damaged area data; McCullagh and Nelder  
32  
33 1989) with elevation, plant species and season as the explanatory variables, tree number as a  
34  
35 random effect, and herbivory as the response variable. The GLMM model using percentage  
36  
37 data did not suffer from over-dispersion as might sometimes happen (Warton & Hui 2011).  
38  
39 We ran Tukey Posthoc-tests ('glht' function, 'multcomp' package) to compare individual  
40  
41 species or elevational study sites. Pearson's correlations were run to correlate the data from  
42  
43 herbivory, plant traits or arthropod abundances ('cor.test' function). We deposited all data  
44  
45 with Dryad: <https://doi.org/10.5061/dryad.kwh70rz06>.  
46  
47  
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49  
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52  
53

## 54 55 **Results**

56  
57 We analysed herbivore damage on 11,448 leaves from 105 saplings belonging to 8 *Ficus*  
58  
59 species (Table 2, Table S1) occurring naturally between 200 and 2,700 m a.s.l. of the  
60

1  
2  
3 elevational gradient of Mt. Wilhelm. The overall proportion of consumed leaf area during  
4  
5 the first survey of herbivory was  $2.22 \pm 0.14$  % (mean  $\pm$  SE) and  $2.62 \pm 0.12$  % during the  
6  
7 second survey. This corresponds approximately to  $1.4 \pm 0.06$  cm<sup>2</sup> per one leaf and  $2.44 \pm 0.09$   
8  
9 cm<sup>2</sup> per 100 cm<sup>2</sup> of leaf area (mean  $\pm$  SE, Fig. S1). We found significant variation in herbivore  
10  
11 damage (measured as % of leaf area lost, arcsine-transformed) among the six elevational  
12  
13 study sites (Dev. = 1.14, df = 5,  $\chi^2 < 0.001$ ) and among tree species (Dev. = 3.57, df = 13,  $\chi^2 <$   
14  
15 0.001). Herbivory differed marginally significantly between the two surveys conducted six  
16  
17 months apart (Dev. = 0.09, df = 1,  $\chi^2 = 0.052$ ; Fig. 2, Fig. S1 and Table S1).  
18  
19  
20  
21  
22

23 The mean (weighted mean herbivory per plant species across the both surveys)  
24  
25 proportional foliar losses to arthropods measured was highest at 700 m a.s.l. ( $3.73 \pm 0.53\%$ ),  
26  
27 followed by 1200 m a.s.l. ( $3.08 \pm 0.38\%$ ; Fig. 2b), 1700 m a.s.l. ( $2.48 \pm 0.19\%$ ) and by 200 m  
28  
29 a.s.l. ( $2.25 \pm 0.17\%$ ).  
30  
31  
32

33 Losses of plant foliage varied significantly among plant species within 200 – 1700 m  
34  
35 a.s.l. ( $\chi^2 = 21.09 - 9.56$ ,  $P = 0.048 - < 0.001$ ) study sites along the elevational gradient of Mt.  
36  
37 Wilhelm. As an example, we present herbivore damage on four plant species at 200 m a.s.l.  
38  
39 (Fig. 3,  $\chi^2 = 9.56$ ,  $P = 0.048$ ). In Kausi, the leaf sizes of the *Ficus* trees studied ranged between  
40  
41 38 – 180 cm<sup>2</sup>.  
42  
43  
44

45 We found some variation in the elevational trends in herbivory among plant species  
46  
47 occurring at more than one elevation (Fig. 4, *F. hahliana* vs. *F. trichocerasa*  $P = 0.04$ ; *F.*  
48  
49 *iodotricha* vs. *F. hahliana*  $P = 0.06$ , vs. *F. saccate*  $P = 0.04$ ). Herbivory damage of *F. hahliana*, the  
50  
51 species with the longest elevational distribution (between 200 and 2700 m a.s.l.), was  
52  
53 significantly higher at 200 – 700 m a.s.l. compared to 1200 – 2700 m a.s.l. (Fig. 4). Generally,  
54  
55 herbivore damage on species occurring at low elevations peaked at 700 m or 200 m a.s.l. and  
56  
57 then decreased. Herbivory decreased with increasing elevation in the majority of species  
58  
59  
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1  
2  
3 occurring at elevations above 1700 m. Herbivore damage did not change significantly  
4  
5 between elevational study sites in several species (Fig. 4), while the non-overlapping  
6  
7 elevational ranges did-not allow comparisons of patterns between some plant species.  
8  
9

### 13 *Herbivorous damage vs. abundances of herbivores*

14  
15 Mean abundances of arthropods on saplings of individual plant species did not differ  
16  
17 significantly but were slightly higher (1.25 times) at the end of the rainy season than at the  
18  
19 end of dry season (Table 1, Figure 2) and this was true at all elevational study sites (Survey:  
20  
21  $F_{1,183} = 12.09$ ,  $P < 0.001$ ; Survey\*Plant species:  $F_{8,183} = 0.64$ ,  $P = 0.791$ , Survey\*Site:  $F_{5,183} = 0.21$ ,  
22  
23  $P = 0.791$ ). Plant species in interaction with elevational study site, but not elevational study  
24  
25 site alone, had significant effect on the observed abundances of herbivorous arthropods  
26  
27 (Plant species\*Site:  $F_{12,183} = 3.687$ ,  $P = 0.049$ , Site:  $F_{5,183} = 0.759$ ,  $P = 0.58$ ). The positive  
28  
29 relationship between the mean values for herbivorous damage and abundance of  
30  
31 herbivorous arthropods for the plant species on specific elevations were significant (Survey  
32  
33 1:  $r = 0.490$ ,  $p = 0.005$ , Survey 2:  $r = 0.506$ ,  $p = 0.004$ ;  $N = 27$  for each survey). However,  
34  
35 abundances of herbivorous arthropods and herbivorous damage at individual saplings did  
36  
37 not correlate significantly (Survey 1:  $r = 0.013$ ,  $p = 0.898$ ; Survey 2:  $r = 0.043$ ,  $p = 0.763$ ;  $N = 105$   
38  
39 for each survey).  
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### 50 *Herbivorous damage vs. plant defences*

51  
52 Most host-plant defences measured for the two *Ficus* species showed non-linear correlations  
53  
54 with elevation and had a hump-shaped distribution along the gradient (Fig. 5). However,  
55  
56 specific elevational trends in the defensive traits studied here differed between *Ficus hahliana*  
57  
58 and *F. arfakensis*. In the case of both species, triterpene diversity correlated negatively  
59  
60

1  
2  
3 significantly with herbivorous damage (Table S2). Trichome density correlated significantly  
4  
5 positively with herbivorous damage of *F. arfakensis* but negatively with herbivory of *F.*  
6  
7 *hahliana*. The triterpene content of *F. arfakensis* decreased non-linearly towards higher  
8  
9 elevations with the maximum at 700 m and was positively correlated with herbivory.  
10  
11  
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## 15 Discussion

16  
17 Insect herbivory is an interaction with main consequences for ecosystem functioning. Direct  
18  
19 effects of herbivory on ecosystem are via nutrient cycling, indirect effects involve effects on  
20  
21 plant species composition including succession, on plant resource allocation and on food  
22  
23 web interactions. We aimed to quantify the effects of elevation on herbivorous damage, and  
24  
25 to find out whether the herbivory damage correlates with the host defences, and if  
26  
27 abundances of herbivorous insects correlated directly with herbivorous damage. In  
28  
29 concordance with our predictions, we found correlation between abundance of herbivorous  
30  
31 arthropods and herbivory damage at plant species and site level, but not at the level of  
32  
33 individual saplings. We assume that this is because while arthropods are mobile, and  
34  
35 collected at one point in time, herbivorous damage accumulated in time reflects their  
36  
37 presence in past. For the two plant species studied in detail, we observed rather tight  
38  
39 correlations between plant defences and herbivory damage, which would mean that  
40  
41 absolute values of the damage drive the defences.  
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50 In contrast to previous studies of herbivory, we found much lower herbivory damage,  
51  
52 2.4% of leaf area, than reported in previous studies. Our value is more than 10 times lower  
53  
54 than the average values reported for tropical regions in several highly cited review papers  
55  
56 (from 11.1 to 48.0%, measured usually at one time point (Coley & Aide 1991, Cyr & Face  
57  
58 1993, Coley & Kursor 1996) but see (Suzuki *et al.* 2013; reporting 1.6-8.7% herbivory from  
59  
60

1  
2  
3 Borneo)). Even along the same elevational gradient, some species suffer considerably higher  
4 herbivory than other. e.g. Plowman *et al.* (2017) 20 – 40% damage per individual leaf. Kozlov  
5  
6 *et al.* (2014) found that blind measurements (i.e. where assistants/researchers naïve to the  
7  
8 purpose of the study or to treatment conduct the measurements) of herbivore damage  
9  
10 demonstrated that woody plants in Brazil lose, on average, 1.11% of their leaf area to  
11  
12 defoliating arthropods. Zava & Cianciaruso (2014), working with numerous plant species in  
13  
14 Emas National Park in Brazil, also reported that community-wide losses of woody plants to  
15  
16 arthropod were as low as 1.35%, i.e., close to our value (2.4%) and to Kozlov *et al.* (2014)  
17  
18 1.1% as obtained by a blind method. We support the urge for caution (Kozlov *et al.* 2014,  
19  
20 Zvereva & Kozlov 2014) in obtaining values of herbivory in non-blind experiments, often  
21  
22 used as rough estimates, and in obtaining community-wise characteristics from the results of  
23  
24 single-species studies.  
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33 Along a tropical elevational gradient, we found a peak in herbivory at lower mid-  
34  
35 elevations and a decrease towards the lowlands and higher elevations. This trend of  
36  
37 decreasing herbivory seems to continue even to higher elevations (up to 3700 m) if we  
38  
39 consider non-*Ficus* species, which we measured by exactly the same methods, above the  
40  
41 elevational distributional limits of *Ficus* at Mt. Wilhelm gradient (Figure S1). Herbivory as a  
42  
43 subject has been studied along elevational gradients at least 17 times already, with the  
44  
45 majority of research coming from the temperate regions and usually from short elevational  
46  
47 gradients, where the mean length was 700 elevational m (min = 500, max = 4025 m). The  
48  
49 majority (63%) of previously published studies on trends in herbivory along elevational  
50  
51 gradients reported an overall decreasing trend with increasing elevation, while 25%  
52  
53 reported increasing trends and the rest found no trend in herbivory (Zvereva & Kozlov  
54  
55 2014). Two studies most closely comparable to ours showed (reporting chewing herbivory  
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3 along a tropical gradient of similar length) that herbivory increased with elevation (Koptur  
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5  
6 1985, Zhang *et al.* 2015). Similarly to other studies, we focused solely on the damage caused  
7  
8 by chewing arthropods, as one of the herbivore guilds which generally causes the highest  
9  
10 damage to plants (Schoonhoven *et al.* 2005). This was also largely because mines and galls  
11  
12 were relatively rare at our study sites, and generally hard to find at the low forest strata  
13  
14 where we measured herbivory. We report a unimodal elevational trend in herbivory with a  
15  
16 peak at 700 m a.s.l. at the plant community level, but several different patterns at the plant  
17  
18 species level.  
19  
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22  
23 When considering previously known chemistry and our own data, we might conclude  
24  
25 that none of the eight species included is especially well protected in comparison to some  
26  
27 other species of *Ficus* (Xiang & Chen 2004). For example, some sympatric New Guinean  
28  
29 species contain latex with higher proteolytic activity and higher concentrations of triterpenes  
30  
31 than the species studied here (e.g. *F. subtrinervia* and *F. rubrivestimenta*) (Volf *et al.* 2018).  
32  
33 However, we should keep in mind, that they might rely on other on different forms of  
34  
35 defences. In particular, *F. trachypison* and *F. concocephalifolia* have low protease activity and a  
36  
37 low concentration of triterpenes compared to these species (Volf *et al.* 2018). For example, *F.*  
38  
39 *hahliana* has significantly higher herbivory at 200 – 1200 m (Fig. 4) but appears to be better  
40  
41 defended at higher elevations as leaves have a higher density of trichomes, higher triterpene  
42  
43 diversity and lower specific leaf area at elevations above 1500 m (Fig. 5). The surprising  
44  
45 hump-shaped pattern in herbivory seen in *F. arfakensis* also corresponds to leaves with lower  
46  
47 triterpene diversity and higher specific leaf area, but trichome density is highest at the  
48  
49 middle elevations in this species. This shows that individual defensive traits show varying  
50  
51 responses to elevation and their relative importance in terms of anti-herbivore protection  
52  
53 may differ between elevations (Pellissier *et al.* 2016). Further, our results confirm that  
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3 different plant species have different defensive strategies against herbivory at different  
4  
5 elevations, but the causality is difficult to assign in some cases. We still do not know  
6  
7 whether variation in defensive traits is driven by herbivory or whether herbivory is related  
8  
9 to defensive traits that are responding to the environment. Furthermore, there are clearly  
10  
11 trade-offs between some traits (e.g. triterpene diversity and triterpene content) which  
12  
13 require further investigation.  
14  
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17  
18 We found only weakly positive relationship between the herbivorous damage and  
19  
20 abundance of herbivorous arthropods for the plant species on specific elevations. However,  
21  
22 herbivory and abundances of herbivores did not correlate when we focused on individual  
23  
24 saplings. In a different study, levels of herbivory were not related to beetle abundance,  
25  
26 whereas it was significantly negatively related to beetle species richness (Bito *et al.* 2011).  
27  
28 Overall, the abundances of herbivorous insect tend to peak at mid-elevations of the gradient,  
29  
30 where most favourable conditions are seen. Water availability (measured as air humidity  
31  
32 and rainfall) is high there due to condensation zone, and temperatures still relatively high  
33  
34 and suitable for growth of dense and tall vegetation (Sam *et al.* 2019). The observed hump-  
35  
36 shaped pattern in abundances of herbivorous insect might be also partly driven by high  
37  
38 predation at low elevations (Sam *et al.* 2015) and unfavourable climatic conditions at high  
39  
40 elevations. Similarly to herbivorous damage, abundances of insect were higher during the  
41  
42 second survey, which corresponded with higher herbivorous damage reported during that  
43  
44 survey too. This might imply, that climate (especially water availability in case of our  
45  
46 gradient) might have significant effect on changes in herbivory and herbivore abundances. If  
47  
48 a climate change happens, insect herbivores are likely to react faster than plants (Robinet &  
49  
50 Roques 2010), which might lead to a movement of herbivores to higher elevations, where  
51  
52 plants have different (lower) defences or where it will be more difficult for them to  
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2  
3 compensate for herbivorous damage. Colwell *et al.* (2008) showed that extinction due to  
4  
5 upland shifts can be true for tropical insects. Using data collected for 1,902 species of insects  
6  
7 and plants along an altitudinal transect in Costa Rica, they showed that a high proportion of  
8  
9 tropical species analysed ( $\approx 53\%$ ) may be faced with range-shift gaps and might face  
10  
11 extinction with a 1000m range shift in isotherms. Based on our data, which elevational  
12  
13 distributions of several *Ficus* species, would surely support this finding. Many arthropod  
14  
15 species dependent on specific *Ficus* species would disappear, if they are pushed to different  
16  
17 locations due to climate change.  
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23 While this study was conducted in 2014, a strong El Niño event occurred in 2015 and  
24  
25 affected mostly lowland sites (200 and 700 m a.s.l.) of Mt. Wilhelm gradient by severe  
26  
27 droughts and extremely high temperatures ( $2.1\text{ }^{\circ}\text{C}$  higher than average for those months).  
28  
29 During the El Niño, we observed significant drop in abundances of insect and insectivorous  
30  
31 birds at these lowland sites (Sam *et al.* in prep.). The drop was at the lower range of the drop  
32  
33 observed in Puerto Rican forest, where arthropod biomass fell 10 to 60 times after  
34  
35 temperatures increased by  $2.0\text{ }^{\circ}\text{C}$  (Lister and Garcia 2018). For many insect herbivores,  
36  
37 synchronization to plant phenology is crucial. We can only guess whether the insect  
38  
39 changed their phenology temporarily or move quickly to higher elevations.  
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45 Our study suffers from a lack of information on leaf longevity and the lack of  
46  
47 information about the fate of individual leaves (without tagging and following individual  
48  
49 leaves, we miss information about the leaves that disappear completely). The importance of  
50  
51 leaf longevity for the interpretation of single-point measurements of herbivory depends on  
52  
53 the way the herbivore damage accumulates during the lifetime of the leaf. At one extreme, it  
54  
55 is believed that, almost all damage is acquired when the leaf is young so that the subsequent  
56  
57 leaf life span is irrelevant. This might be because the aging leaves accumulate chemical  
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3 compounds and strengthen mechanical defences (e.g. Kouki *et al.* 2002, Read *et al.* 2003). At  
4  
5 the other extreme, herbivory might be accumulated steadily throughout the entire leaf life  
6  
7 span and is therefore proportional to lifespan (Sand-Jensen *et al.* 1994). The data from  
8  
9 tropical forests suggest the former scenario is a more realistic one (e.g. Kursar & Coley 2003).  
10  
11 We therefore believe that our point measurements of standing damage report overall  
12  
13 herbivory relatively precisely. Along elevational gradients in tropical areas, leaf-longevity  
14  
15 has been shown to increase with elevation (Kikuzawa 1996). Another study showed that  
16  
17 species native to Amazonian forests had average leaf life spans that differed according to  
18  
19 forest structure, e.g. of 3.2 years in shaded understory, 1.9 years in small gaps, and 1.6 years  
20  
21 in sunlit mature canopy trees (Reich *et al.* 2004). We should also keep in mind that some  
22  
23 plants drop damaged leaves earlier than undamaged leaves (Faeth *et al.* 1981), and very little  
24  
25 is known about this phenomenon in *Ficus*.  
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33 The ecological and evolutionary importance of plant–herbivore interactions is widely  
34  
35 appreciated. However, is herbivory as low as 1-2% still significant? In an experiment in  
36  
37 temperate region, the annual removal of 2, 4, 8 and 16% of the leaf area from naturally  
38  
39 growing mountain birch *Betula pubescens* saplings during a seven-year period resulted in a  
40  
41 pronounced reduction of plant vertical growth (–30, –34, –45 and –78%, respectively;  
42  
43 Zvereva *et al.* 2012). Leaf size decreased first, followed by a considerable decrease in the  
44  
45 length of long shoots in all treatments (Zvereva *et al.* 2012). Marquis (1992) found that 10%  
46  
47 experimental defoliation of an understory shrub, *Piper arieianum*, reduced growth and seed  
48  
49 production, delayed flowering, and decreased seed viability. Annual survivorship was 85%  
50  
51 for undamaged seedlings of *Dipteryx panamensis* and 0% for seedlings with 8% of their leaf  
52  
53 area missing (Clark & Clark 1985). Sixty-five percent of the species surveyed in the  
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3 understory of the Los Tuxtlas tropical rain forest had an average leaf area damage per plant  
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5 of less than 1% (range: 0.25-20.52%).  
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7

8 Besides the direct effect on saplings, herbivory can also cause very important indirect  
9  
10 damage via pathogens. A survey showed that 43% of the leaves were damaged by  
11  
12 herbivores and pathogens concurrently, 16% showed damage by insect herbivory alone, and  
13  
14 only 1.4% of the sampled leaves showed damage by pathogens alone (García-Guzmán &  
15  
16 Dirzo 2001). This mean that pathogens transmitted by herbivores represent serious problem  
17  
18 even for plants with little herbivore damage. Hence, herbivory may have real and  
19  
20 substantial direct and indirect impacts on the growth and survival of plants, especially in  
21  
22 their younger stages. This impact might be more substantial in the tropics than in the  
23  
24 temperate zone (García-Guzmán & Dirzo 2001), as 68% of lifetime herbivory of tropical  
25  
26 shade-tolerant species occurs during the small window of leaf expansion (a 1-3 week long  
27  
28 period).  
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35 We found the community-wide herbivorous damage to peak in lower mid-elevations  
36  
37 of the 2700 m long elevational gradient, and we found correlation between species-specific  
38  
39 herbivorous damage at each elevation and abundance of herbivorous insect. Further, we  
40  
41 found correlations between herbivorous damage and species-specific plant defences. We  
42  
43 conclude, that the plant traits, communities of herbivores and herbivory are likely to be  
44  
45 affected by climatic conditions at specific elevations. Along our tropical elevational gradient,  
46  
47 where temperature is generally high, decreasing linearly, the water availability seemed to  
48  
49 shape the relationships between plants and arthropods. In lowlands, where more distinct  
50  
51 dry and wet seasons occur, the total abundances of insect were lower than at middle  
52  
53 elevations, where temperatures are still relatively high but humidity higher and dry seasons  
54  
55 not so distinct. At highest elevations (above 2200 m a.s.l.) the low temperatures seem to  
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3 cause low abundances and diversity of insects (Sam *et al.* 2019). and After the rainy season,  
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5 the herbivory was significantly higher at upper three study sites only but not at lower three  
6  
7 study sites than during the first, dry-season, survey.  
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### 35 **DATA AVAILABILITY**

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38 Data available from the Dryad Digital Repository:

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41 <https://doi.org/10.5061/dryad.kwh70rz06> (Sam *et al.* 2019).  
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55 chronic herbivory substantially reduces birch growth. *Oikos* **121**: 2036-2043.  
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3 **Figure 1.** Location of (a) the study area in Papua New Guinea, and (b) the six study sites of  
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5 the Mt. Wilhelm elevational gradient in Bismarck Range, Madang Province. An example of  
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7 the original leaf photos (c) and leaf photos processed in Photoshop, ready to be analysed in  
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9 ImageJ (d).

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15 **Figure 2.** Mean ( $\pm$  S.E.) herbivore damage (a; in percentages of individual leaves) and  
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17 herbivorous arthropod abundances (b) of all species studied at each elevation combined at  
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19 six study sites along the elevational gradient of Mt. Wilhelm in Papua New Guinea.  
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21 Herbivore damage at each elevational site was measured at the end of dry season (Survey 1)  
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23 and six months later, at the end of the wet season (Survey 2).

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30 **Figure 3.** Mean ( $\pm$  S.E.) herbivore damage (in percentages of individual leaves) of four plant  
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32 species surveyed at the 200 m a.s.l. study site (Kausi). ARF = *Ficus arfakensis*, CON = *F.*  
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34 *conocephalifolia*, HAH = *F. hahliana*, and TRI = *F. trichocerasa*. Same letters (lowercase letters  
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36 for Survey A, capital letters for Survey A) mark non-significant results of the Unequal N  
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38 HSD post-hoc test. \*\* mark significant difference ( $p \leq 0.01$ ) within the species between the  
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40 two surveys.  
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48 **Figure 4.** Mean ( $\pm$  S.E.) herbivore damage (in percentages of individual leaves) on eight *Ficus*  
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50 species surveyed along the Mt. Wilhelm elevational gradient. The values marked with  
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52 different letters differ significantly from each other (Tukey test,  $P = 0.05$ ). n.s. = non-  
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54 significant differences in herbivory between elevational study sites for given species. Means  
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56 are based on the average across the both surveys.  
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**Figure 5.** Scatter plots of trait values for *Ficus arfakensis* (a) and *F. hahliana* (b) fitted with second order polynomial curves. For each plant species, following characteristics are fitted: Upper left: trichome density per 10mm<sup>2</sup> (average of both sides of the leaf lamina), upper right: Shannon diversity of triterpenes, lower left: total triterpene content (total sum of peak areas/mg) and lower right: specific leaf area.

**Table 1. Characteristics of study sites.** Location, elevation and mean annual temperature at six elevational study sites where the research was conducted. The first herbivory measurement was made at the end of the dry season 2014, and the second measurement was made at the end of the rainy season of 2014/2015 (mean monthly precipitation during the study period was 378 mm). Mean abundances of herbivorous arthropods (individuals/m<sup>2</sup>) during both surveys did not differ significantly between the seasons.

| Locality      | Latitude   | Longitude   | Elevation (±50m a.s.l) | Temperature (mean 2014, °C) | First survey (all in 2014) | Second survey (all in 2015) | Days between surveys | Herb. Abund. First Survey | Herb. Abund. Second Survey |
|---------------|------------|-------------|------------------------|-----------------------------|----------------------------|-----------------------------|----------------------|---------------------------|----------------------------|
| Kausi         | 05°44'33"S | 145°20'01"E | 200                    | 24.5                        | 1-4 Nov                    | 13-17 Apr                   | 163                  | 3.76±0.67                 | 5.66±0.43                  |
| Numba         | 05°44'14"S | 145°16'12"E | 700                    | 22.3                        | 27-30 Oct                  | 7-9 Apr                     | 162                  | 4.59±0.42                 | 5.91±0.52                  |
| Memeku        | 05°43'18"S | 145°16'17"E | 1200                   | 19.5                        | 21-25 Oct                  | 30 Mar -4 Apr               | 160                  | 6.03±0.61                 | 7.25±0.55                  |
| Bananumbu     | 05°45'21"S | 145°14'11"E | 1700                   | 18.4                        | 25-28 Oct                  | 8-11 Apr                    | 165                  | 7.05±0.96                 | 7.49±0.77                  |
| Sinopass      | 05°45'34"S | 145°10'49"E | 2100                   | 15.7                        | 6-10 Oct                   | 14-20 Mar                   | 159                  | 3.83±0.83                 | 5.02±0.75                  |
| Bruno Sawmill | 05°48'57"S | 145°09'02"E | 2700                   | 13.2                        | 8-10 Oct                   | 19-23 Mar                   | 162                  | 3.05±0.73                 | 4.04±0.50                  |

**Table 2.** Number of saplings (represented by  $114 \pm 11.8$  leaves each) of individual species studied at each elevational study site, their overall mean leaf sizes and mean leaf sizes in cases where there were significant differences between leaf sizes at individual elevational study sites. n.s. = non-significant differences in leaf size between elevations.

|                               | 200 | 700 | 1200 | 1700 | 2200 | 2700 | Mean Leaf size | Leaf sizes dif.      |
|-------------------------------|-----|-----|------|------|------|------|----------------|----------------------|
| <i>Ficus arfakensis</i>       | 3   | 3   |      | 3    |      |      | 46.97          | 36.7 (67.7;<br>59.6) |
| <i>Ficus conocephalifolia</i> | 3   | 3   |      |      |      |      | 154.10         | n.s.                 |
| <i>Ficus endochaete</i>       |     |     | 5    | 3    | 3    | 5    | 23.70          | 23.6 (31.7)<br>22.3  |
| <i>Ficus hahliana</i> *       | 4   | 4   | 5    | 4    | 4    | 5    | 63.76          | n.s.                 |
| <i>Ficus hombroniana</i> †    |     |     | 5    | 4    | 4    |      | 34.36          | n.s.                 |
| <i>Ficus iodotricha</i>       |     |     |      | 3    | 3    | 5    | 69.11          | n.s.                 |
| <i>Ficus saccata</i>          |     |     |      |      | 3    | 5    | 101.99         | n.s.                 |
| <i>Ficus trichocerasa</i> •   | 4   | 4   | 5    | 3    |      |      | 62.33          | n.s.                 |
| Number of species             | 4   | 4   | 4    | 6    | 5    | 4    |                |                      |



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6 \**F. hahliana* is confirmed as a good species from 200-1200m of our elevational gradient. After  
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8 this (1700m-2700m) a close relative/sister species occurs. However, this potential split was  
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10 discussed only recently based on molecular differences. We were not able to distinguish the  
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12 two species in the time of our experiment. See discussion in Segar et al. in revision.  
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15 ‡*F. hombroniana* is found between 200-1200m. There are a few individuals at 1,700m but most  
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17 individuals classified as *F. hombroniana* here are probably (and at 2,200m) the closely related  
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19 *F. ihuensis*.  
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23 •*F. trichocerasa* has two sub-species along the elevational gradient. *F. trichocerasa* subsp.  
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25 *trichocerasa* occurs between 200-1700m and *F. trichocerasa* subsp. *pleioclada* occurs between  
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27 1700m and 2200m. They co-occur at 1700m, and both subspecies were included in our study  
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29 as they are difficult to distinguish in the field at 1700m.  
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## SUPPORTING INFORMATION

**Insect herbivory and herbivores of *Ficus* species along a rainforest elevational gradient in Papua New Guinea**

K. Sam<sup>\*a,b</sup>, B. Koane<sup>c</sup>, L. Sam<sup>a</sup>, A. Mrazova<sup>a,b</sup>, S. Segar<sup>a,b,d</sup>, M. Volf<sup>a,b,e</sup>, M. Moos<sup>a</sup>, P. Simek<sup>a</sup>,  
M. Sisol<sup>c</sup>, V. Novotny<sup>a,b</sup>

<sup>a</sup> Biology Centre of Czech Academy of Sciences, Institute of Entomology, Ceske Budejovice, Czech Republic

<sup>b</sup> Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic

<sup>c</sup> The New Guinea Binatang Research Center, Madang, Papua New Guinea

<sup>d</sup> Department of Crop and Environment Sciences, Harper Adams University, UK

<sup>e</sup> Molecular Interaction Ecology Group, German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

\* Corresponding author Katerina Sam: katerina.sam.cz@gmail.com

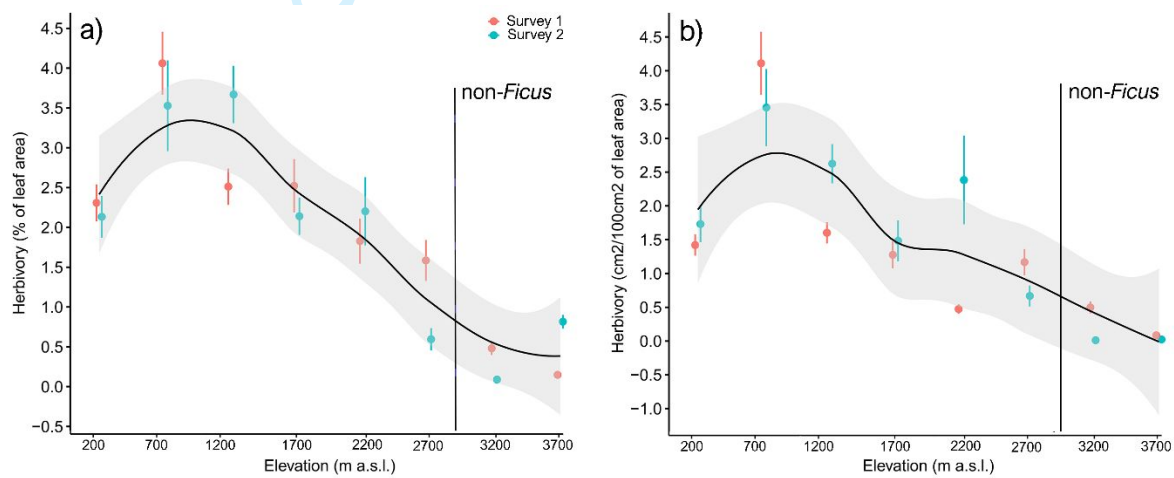
**Table S1.** Mean herbivory ( $\pm$  S.D. and S.E.) for all plant species at each of the elevational study site. Results of tests comparing variability between individual saplings within each of the two surveys, and between the two surveys when measurements from all 15,194 leaves. Significant differences are marked in bold. *Ficus arfakensis* = ARF, *Ficus conocephalifolia* = CON, *Ficus endochaete* = END, *Ficus hahliana* = HAH, *Ficus hombroniana* = HOM, *Ficus iodotricha* = IOD, *Ficus saccata* = SAC, *Ficus trichocerasa* = TRI.

| Species | Site | Mean herbivory | S.D.   | S.E.  | Differences between individual trees |                  | Difference between the two surveys |
|---------|------|----------------|--------|-------|--------------------------------------|------------------|------------------------------------|
|         |      |                |        |       | Survey 1                             | Survey 2         |                                    |
| ARF     | 200  | 1.818          | 4.556  | 0.223 | <b>&lt;0.001</b>                     | <b>0.002</b>     | 0.122                              |
| ARF     | 700  | 2.995          | 6.492  | 0.628 | <b>&lt;0.001</b>                     | 0.341            | 0.142                              |
| ARF     | 1700 | 2.139          | 5.245  | 0.437 | 0.406                                | 0.415            | 0.211                              |
| CON     | 200  | 1.493          | 4.336  | 0.970 | 0.554                                | 0.032            | <b>&lt;0.001</b>                   |
| CON     | 700  | 1.672          | 3.331  | 0.666 | 0.035                                | 0.357            | 0.616                              |
| END     | 1200 | 2.188          | 5.494  | 0.596 | 0.128                                | 0.364            | <b>0.013</b>                       |
| END     | 1700 | 0.265          | 0.403  | 0.058 | 0.471                                | 0.985            | 0.753                              |
| END     | 2200 | 3.149          | 8.527  | 0.972 | 0.277                                | 0.409            | <b>&lt;0.001</b>                   |
| END     | 2700 | 1.522          | 6.150  | 0.446 | 0.496                                | 0.207            | <b>0.002</b>                       |
| HAH     | 200  | 3.058          | 6.220  | 0.451 | <b>&lt;0.001</b>                     | <b>&lt;0.001</b> | 0.059                              |
| HAH     | 700  | 1.370          | 2.349  | 0.266 | 0.316                                | 0.143            | 0.805                              |
| HAH     | 1200 | 1.566          | 5.732  | 0.662 | 0.529                                | 0.409            | 0.789                              |
| HAH     | 1700 | 2.149          | 6.191  | 0.424 | 0.087                                | 0.918            | <b>&lt;0.001</b>                   |
| HAH     | 2200 | 1.333          | 3.436  | 0.353 | 0.249                                | 0.109            | 0.226                              |
| HAH     | 2700 | 0.510          | 1.794  | 0.119 | 0.174                                | 0.062            | 0.059                              |
| HOM     | 1200 | 3.091          | 7.509  | 0.219 | <b>&lt;0.001</b>                     | 0.898            | <b>&lt;0.001</b>                   |
| HOM     | 1700 | 1.489          | 4.555  | 0.328 | <b>0.001</b>                         | <b>0.008</b>     | <b>&lt;0.001</b>                   |
| HOM     | 2200 | 1.761          | 6.044  | 0.395 | 0.309                                | 0.527            | 0.077                              |
| IOD     | 1700 | 3.739          | 10.882 | 1.301 | 0.606                                | 0.307            | <b>0.001</b>                       |
| IOD     | 2200 | 2.489          | 6.816  | 0.501 | <b>0.017</b>                         | 0.319            | <b>0.014</b>                       |
| IOD     | 2700 | 1.498          | 4.122  | 0.556 | 0.111                                | 0.501            | <b>&lt;0.001</b>                   |
| SAC     | 2200 | 0.725          | 2.264  | 0.281 | 0.069                                | 0.095            | <b>&lt;0.001</b>                   |
| SAC     | 2700 | 1.695          | 5.562  | 0.355 | 0.211                                | 0.757            | 0.023                              |
| TRI     | 200  | 2.257          | 5.228  | 0.763 | 0.778                                | 0.356            | 0.114                              |
| TRI     | 700  | 3.277          | 6.466  | 0.611 | <b>&lt;0.001</b>                     | <b>&lt;0.001</b> | <b>0.011</b>                       |
| TRI     | 1200 | 1.505          | 3.109  | 0.440 | 0.064                                | 0.319            | 0.319                              |
| TRI     | 1700 | 2.628          | 4.727  | 0.893 | 0.615                                | 0.414            | 0.975                              |

**Table S1.** Results of Unequal N HSD post-hoc tests (P values) for herbivore damage among the elevational study sites of the Mt. Wilhelm elevational gradient (see Figure 2). Results for first (\_1) and second (\_2) survey are presented. Results for herbivory measured in % under diagonal and results for herbivory measured as actual area damaged in cm<sup>2</sup> above diagonal.

|        | 1_200  | 1_700  | 1_1200 | 1_1700 | 1_2200 | 1_2700 | 2_200  | 2_700  | 2_1200 | 2_1700 | 2_2200 | 2_2700 |
|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 1_200  |        | <0.001 | 0.169  | 0.223  | 0.037  | <0.001 | 0.993  | <0.001 | <0.001 | 0.971  | 0.470  | <0.001 |
| 1_700  | <0.001 |        | <0.001 | <0.001 | <0.001 | <0.001 | 0.013  | 0.971  | <0.001 | <0.001 | <0.001 | <0.001 |
| 1_1200 | 0.169  | <0.001 |        | 0.466  | <0.001 | <0.001 | 0.013  | 1.000  | <0.001 | 0.235  | 0.235  | <0.001 |
| 1_1700 | 0.223  | <0.001 | 0.169  |        | <0.001 | <0.001 | 0.969  | 0.003  | 0.002  | 0.986  | 0.391  | <0.001 |
| 1_2200 | 0.037  | <0.001 | <0.001 | <0.001 |        | 0.466  | 0.019  | 0.007  | 0.021  | <0.001 | 0.005  | 0.516  |
| 1_2700 | <0.001 | <0.001 | <0.001 | <0.001 | 0.466  |        | <0.001 | <0.001 | <0.001 | <0.001 | 0.953  | <0.001 |
| 2_200  | 0.993  | <0.001 | 0.466  | 0.969  | 0.019  | 0.013  |        | <0.001 | <0.001 | 0.470  | 0.696  | <0.001 |
| 2_700  | <0.001 | 0.986  | <0.001 | 0.003  | 0.007  | <0.001 | <0.001 |        | 1.000  | 0.007  | 0.007  | <0.001 |
| 2_1200 | <0.001 | <0.001 | <0.001 | 0.002  | 0.021  | <0.001 | <0.001 | 1.000  |        | 0.021  | <0.001 | <0.001 |
| 2_1700 | 0.971  | <0.001 | 0.235  | 0.891  | 0.007  | <0.001 | 0.470  | 0.007  | 0.021  |        | 0.989  | <0.001 |
| 2_2200 | 0.470  | <0.001 | 0.235  | 0.470  | 0.005  | 0.470  | 0.696  | 0.007  | <0.001 | 0.989  |        | <0.001 |
| 2_2700 | <0.001 | <0.001 | <0.001 | <0.001 | 0.516  | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |        |

**Figure S1.** Mean ( $\pm$  S.E.) herbivory damage in percentages of leaf area (a) and in  $\text{cm}^2$  of leaf area (b) of all species studied at each elevation combined at six study sites along the elevational gradient of Mt. Wilhelm in Papua New Guinea. Herbivore damage at each elevational site was measured at the end of dry season (Survey 1) and six months later, at the end of the wet season (Survey 2). Species surveyed at 3200 and 3700 m a.s.l. were *Macaranga melanosticta*, *Myrsine papuana*, *Myrsine womersleyi*, *Pittosporum berberidoides*. The data on higher elevations are not published yet, but we collected them the exact same way as we collected the *Ficus* species in current study.



**Table S2.** Correlations between plant defences and herbivory of two plant species

|                      | <i>F. arfakensis</i> |             | <i>F. hahliana</i> |             |
|----------------------|----------------------|-------------|--------------------|-------------|
|                      | r                    | p           | r                  | p           |
| Triterpene content   | 0.02                 | 0.98        | 0.73               | <b>0.05</b> |
| Triterpene diversity | -0.74                | <b>0.05</b> | -0.77              | <b>0.04</b> |
| C:N ratio            | 0.68                 | 0.52        | -0.6               | 0.13        |
| Trichome density     | 0.99                 | <b>0.04</b> | -0.87              | <b>0.02</b> |
| Trichome length      | 0.18                 | 0.88        | 0.2                | 0.72        |
| SLA                  | -0.06                | 0.82        | 0.33               | 0.72        |
| Insect abundance     | 0.96                 | 0.16        | -0.27              | 0.44        |

## Methods S1

### Physical Traits

We measured trichome density and specific leaf area (SLA), a surrogate for leaf thickness and toughness, which is also frequently correlated with water content (Groom & Lamont 1999), as parameters of leaf morphology with a possible impact on leaf-chewing insects. The physical traits were measured using leaf discs. Leaf discs were cut (avoiding the central vein) and air dried to constant weight. The total number of trichomes per 10 mm<sup>2</sup> and their average length was measured on five leaf discs per individual using ImageJ (ver.1.48). Values for dorsal and ventral sides of the discs were combined. Specific leaf area (SLA) was calculated as the area per unit mass of five dried leaf disc of known diameter for each individual.

### Terpenoids

Chemicals: Deionized water (NANOpure Barnstead water purification system, Watrex, Prague, Czech Republic), LC/MS-grade methanol and 2-propanol, Fisher Scientific (Pardubice, Czech Republic), formic acid (Sigma-Aldrich, Prague, Czech Republic) were used.

Sample preparation: Approximately 50 mg of dried powdered sample was ground with 1 ml methanol in a TissueLyser LT (DyneX Technologies, Bustehrad, Czech Republic) at 30 Hz for 2 min. After centrifugation (10000 rpm) at 8°C for 10 min, a 100 µl of the supernatant's aliquot was mixed with 200 µl of methanol containing 0.1% formic acid.

HPLC-HRMS equipment: Triterpenes were measured on a Dionex Ultimate 3000 LC system equipped with an Open XRS autosampler and coupled to a Q Exactive Plus Orbitrap mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). A reversed phase Kinetex C18 100AHPLC column, 150mm x 2.1 mm i.d., 2.6µm (Phenomenex, Torrance, CA, USA) was used for separation of analytes. A positive atmospheric pressure ionization mode (APCI)

and a combined full scan mass range (250 – 625 Da) and a data dependent tandem MS<sup>2</sup> scan modes were used. Acquired data were processed by an Xcalibur 2.1 Software (Thermo Fisher Scientific) and the triterpene data were further mined by means of an in-house developed Metabolite Mapper platform.

#### HPLC-HRMS analysis (Liquid chromatography - high resolution mass spectrometry)

Analytical conditions: Flow rate, 200  $\mu$ L/min; injection volume of 5  $\mu$ L; mobile phase, A = 0.1% formic acid in 2-propanol, B = 0.1% formic acid in 1% 2-propanol and methanol, C = 0.1% formic acid in water. Gradient elution, 0 min, 0 % A + 85 % B + 15 % C; 12.0 min, 29 % A + 70 % B + 1% C; 18.5 min, 29 % A + 70 % B + 1% C; 19.0 min, 0 % A + 85 % B + 18 % C; 25.0 min, 0 % A + 85 % B + 15 %; total analysis time, 30 min.

HRMS analytical conditions: Full HRMS scanning: resolving power, 70 000; scan rate,  $\pm$  3Hz; automatic gain control (AGC) target,  $3 \times 10^6$ ; maximum ion injection time (IT), 100 ms. The data dependent MS<sup>2</sup> scanning: resolving power, 17 500; automatic gain control (AGC) target,  $2 \times 10^5$ ; maximum ion injection time (IT), 100 ms; isolation window; 3 Da, and normalized collision energy, 32, TopN 1. Ion source: spray voltage, 3.0 kV; capillary temperature, 250°C; sheath gas, 25 au; aux gas, 5 au; auxiliary gas, 1 au; auxiliary gas heater temperature, 250°C; S-Lense, 60 au; lock mass, hexakis(2,2-difluoroethoxy)phosphazene, 621.0211 Da.

Data Processing: A list of presumed terpenoid metabolites was compiled reviewing literature, Table S1. For data processing, only metabolites that were detected in at least 50 % of the sample pool were used for statistical processing.

A list of the presumed terpenoid metabolites involved in this study:

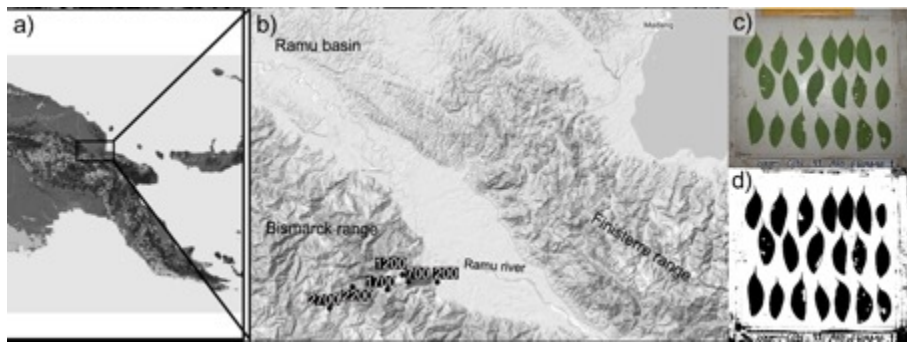
| Compound name  | Composition                                    | [M+H] <sup>+</sup> | [M-H <sub>2</sub> O+H] <sup>+</sup> |
|--|--|--------------------|-------------------------------------|
| Stigmasterol   | C <sub>29</sub> H <sub>48</sub> O              | 413.3778           | 395.3672                            |
| Lupeol   | C <sub>30</sub> H <sub>50</sub> O              | 427.3935           | 409.3829                            |
| 8,26-cyclo-urs-21-en-3 $\beta$ ,20 $\beta$ -diol (ursane type) | C <sub>30</sub> H <sub>49</sub> O <sub>2</sub> | 442.3806           | 424.3700                            |
| 3 $\beta$ -acetoxy-8,26-cyclo-ursan-20 $\beta$ -ol             | C <sub>32</sub> H <sub>52</sub> O <sub>3</sub> | 485.3989           | 467.3883                            |
| 3-friedelanone   | C <sub>30</sub> H <sub>50</sub> O              | 427.3935           | 409.3829                            |
| Oleanolic acid   | C <sub>30</sub> H <sub>48</sub> O <sub>3</sub> | 457.3676           | 439.3570                            |
| betulinic acid   | C <sub>30</sub> H <sub>48</sub> O <sub>3</sub> | 457.3676           | 439.3570                            |
| lupeol acetate   | C <sub>32</sub> H <sub>52</sub> O <sub>2</sub> | 469.4040           | 451.3934                            |
| $\alpha$ - and $\beta$ -amyrine                                | C <sub>30</sub> H <sub>50</sub> O              | 427.3935           | 409.3829                            |
| 3,5,7,4-tetrahydroxyflavane                                    | C <sub>15</sub> H <sub>14</sub> O <sub>5</sub> | 275.0914           | 257.0808                            |
| 3,5,7,3,4-pentahydroxyflavane                                  | C <sub>15</sub> H <sub>14</sub> O <sub>6</sub> | 291.0863           | 273.0757                            |
| 27-nor-3 $\beta$ -hydroxy-25-oxocycloartane                    | C <sub>29</sub> H <sub>48</sub> O <sub>2</sub> | 429.3727           | 411.3621                            |
| (22E)-25,26,27-trinor-3 $\beta$ -hydroxycycloart-22-en-24-al   | C <sub>27</sub> H <sub>42</sub> O <sub>2</sub> | 399.3258           | 381.3152                            |

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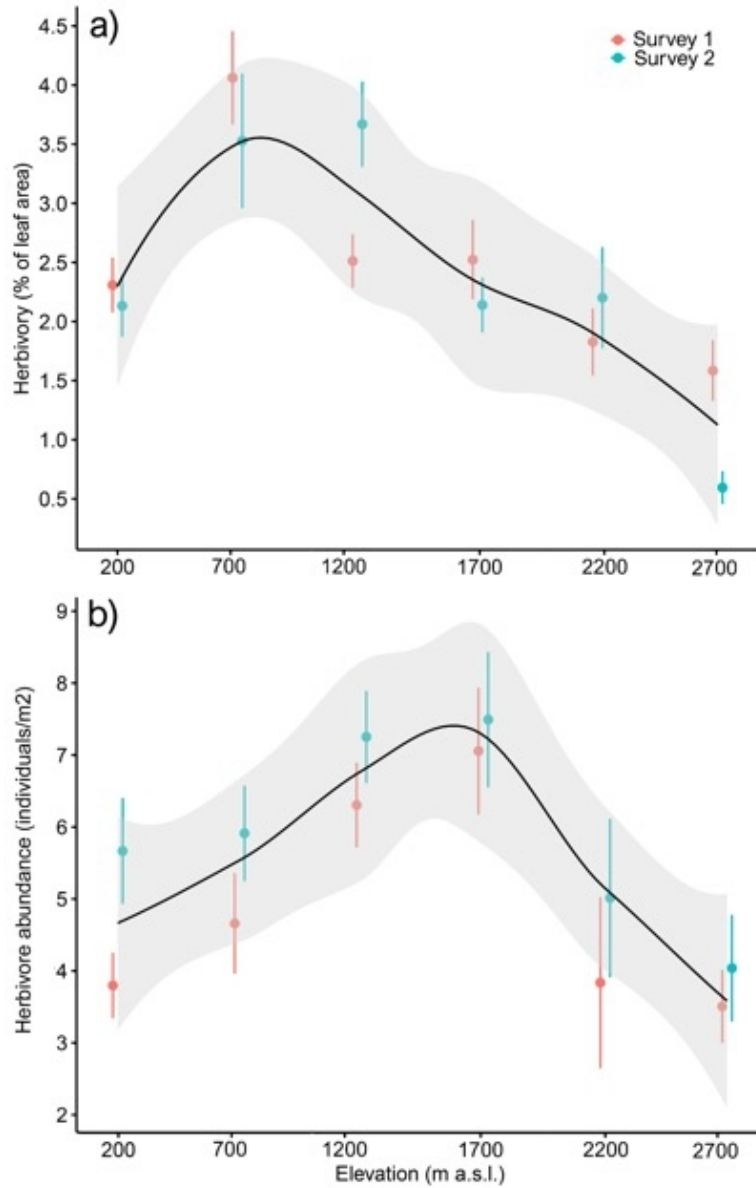
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| 3b -acetoxy-15a-hydroxy-13,27-cyclours-11-ene                  | C <sub>32</sub> H <sub>50</sub> O <sub>3</sub> | 483.3833 | 465.3727 |
| 3b-acetoxy-12a-formyloxy-13,27-cycloursan-11a-ol               | C <sub>33</sub> H <sub>52</sub> O <sub>5</sub> | 529.3888 | 511.3782 |
| 3b-acetoxy-12,19-dioxo-13(18)-oleanene                         | C <sub>32</sub> H <sub>48</sub> O <sub>4</sub> | 497.3626 | 479.3520 |
| 3b-acetoxy-19(29)-taraxasten-20a-ol                            | C <sub>32</sub> H <sub>52</sub> O <sub>3</sub> | 485.3989 | 467.3883 |
| 3b-acetoxy-21a,22a-epoxytaraxastan-20a-ol                      | C <sub>32</sub> H <sub>52</sub> O <sub>4</sub> | 501.3939 | 483.3833 |
| 3,22-dioxo-20-taraxastene                                      | C <sub>30</sub> H <sub>46</sub> O <sub>2</sub> | 439.3571 | 421.3465 |
| 3b-acetoxy-11a,12a-epoxy-16-oxo-14-taraxerene                  | C <sub>32</sub> H <sub>48</sub> O <sub>4</sub> | 497.3626 | 479.3520 |
| 3b-acetoxy-25-methoxylanosta-8,23-diene                        | C <sub>33</sub> H <sub>54</sub> O <sub>3</sub> | 499.4146 | 481.4040 |
| oleanolic acid   | C <sub>30</sub> H <sub>48</sub> O <sub>3</sub> | 457.3676 | 439.3570 |
| acetylbetulonic acid   | C <sub>32</sub> H <sub>50</sub> O <sub>4</sub> | 499.3782 | 481.3676 |
| betulonic acid   | C <sub>30</sub> H <sub>46</sub> O <sub>3</sub> | 545.3520 | 527.3414 |
| acetylursolic acid   | C <sub>32</sub> H <sub>50</sub> O <sub>4</sub> | 499.3782 | 481.3676 |
| ursonic acid   | C <sub>30</sub> H <sub>46</sub> O <sub>3</sub> | 455.3520 | 437.3414 |
| ursolic acid   | C <sub>30</sub> H <sub>48</sub> O <sub>3</sub> | 457.3676 | 439.3570 |
| 3-oxofriedelan-28-oic acid                                     | C <sub>30</sub> H <sub>48</sub> O <sub>3</sub> | 457.3676 | 439.3570 |
| acetate-a-amyrin   | C <sub>32</sub> H <sub>52</sub> O <sub>2</sub> | 469.4040 | 451.3934 |
| acetate-b-amyrin   | C <sub>32</sub> H <sub>52</sub> O <sub>2</sub> | 469.4040 | 451.3934 |
| 3b -acetoxy-22,23,24,25,26,27-hexanordammaran-20-one           | C <sub>26</sub> H <sub>42</sub> O <sub>3</sub> | 403.3207 | 385.3101 |
| 3b -acetoxy-20,21,22,23,24,25,26,27-octanordammaran-17b -ol    | C <sub>24</sub> H <sub>40</sub> O <sub>3</sub> | 377.3050 | 359.2944 |
| 3b -acetoxy-(20R,22E,24RS)-20,24-dimethoxydammaran-22-en-25-ol | C <sub>34</sub> H <sub>58</sub> O <sub>5</sub> | 547.4357 | 529.4251 |
| 3b -acetoxy-(20S,22E,24RS)-20,24-dimethoxydammaran-22-en-25-ol | C <sub>34</sub> H <sub>58</sub> O <sub>5</sub> | 547.4357 | 529.4251 |
| 29(20-19)abeolupane-3,20-dione                                 | C <sub>30</sub> H <sub>48</sub> O <sub>2</sub> | 441.3727 | 423.3621 |
| 19,20-secoursane-3,19,20-trione                                | C <sub>30</sub> H <sub>48</sub> O <sub>3</sub> | 457.3676 | 439.3570 |
| lupenone   | C <sub>30</sub> H <sub>48</sub> O              | 425.3778 | 407.3672 |
| a-amyrone  | C <sub>30</sub> H <sub>48</sub> O              | 425.3778 | 407.3672 |
| 20(30)-taraxastene-3b ,21a-diol                                | C <sub>30</sub> H <sub>50</sub> O <sub>2</sub> | 443.3884 | 425.3778 |
| 20a,21a-epoxytaraxastan-3b -ol                                 | C <sub>30</sub> H <sub>50</sub> O <sub>2</sub> | 443.3884 | 425.3778 |
| 20-taraxastene-3b ,22b -diol                                   | C <sub>30</sub> H <sub>50</sub> O <sub>2</sub> | 443.3884 | 425.3778 |
| and 3b -acetoxy-20-taraxasten-22-                              | C <sub>32</sub> H <sub>50</sub> O <sub>3</sub> | 483.3833 | 465.3727 |
| 20-taraxasten-3b -ol (pseudo-Taraxasterol)                     | C <sub>30</sub> H <sub>50</sub> O              | 427.3935 | 409.3829 |
| 3b -acetoxy-11a-methoxy-12-ursene                              | C <sub>33</sub> H <sub>54</sub> O <sub>3</sub> | 499.4146 | 481.4040 |
| 3b -acetoxy-11a-ethoxy-12-ursene                               | C <sub>34</sub> H <sub>56</sub> O <sub>3</sub> | 513.4302 | 495.4196 |
| 3b -acetoxy-11a-hydroperoxy-12-ursene                          | C <sub>32</sub> H <sub>52</sub> O <sub>4</sub> | 501.3939 | 483.3833 |
| 3b -hydroxy-11a-hydroperoxy-12-ursene                          | C <sub>30</sub> H <sub>50</sub> O <sub>3</sub> | 459.3833 | 441.3727 |
| 3b -acetoxy-11a-ethoxy-12-oleanene                             | C <sub>34</sub> H <sub>56</sub> O <sub>3</sub> | 513.4302 | 495.4196 |
| 3b -acetoxy-11a-hydroperoxy-12-oleanene                        | C <sub>32</sub> H <sub>52</sub> O <sub>4</sub> | 501.3933 | 483.3827 |



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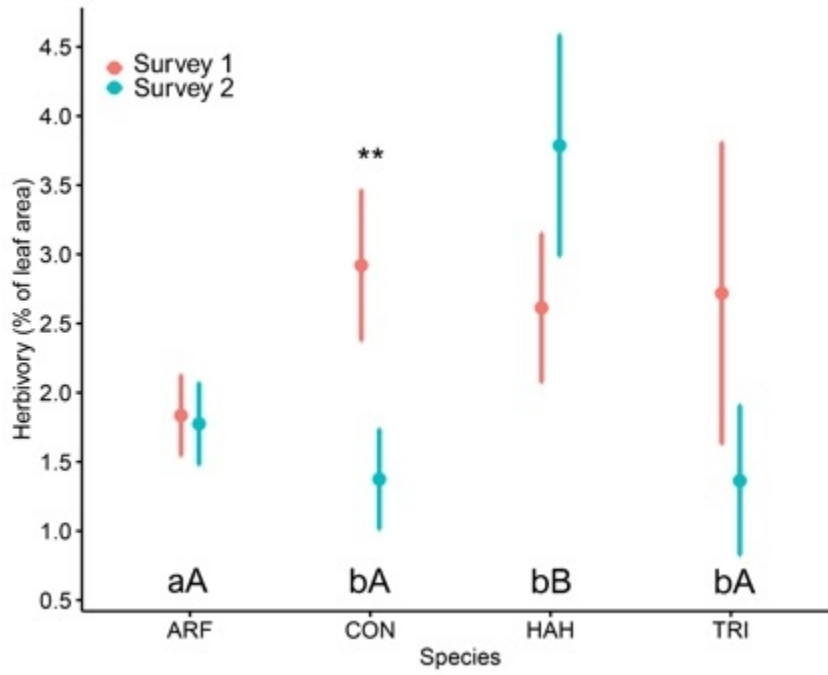


159x58mm (72 x 72 DPI)

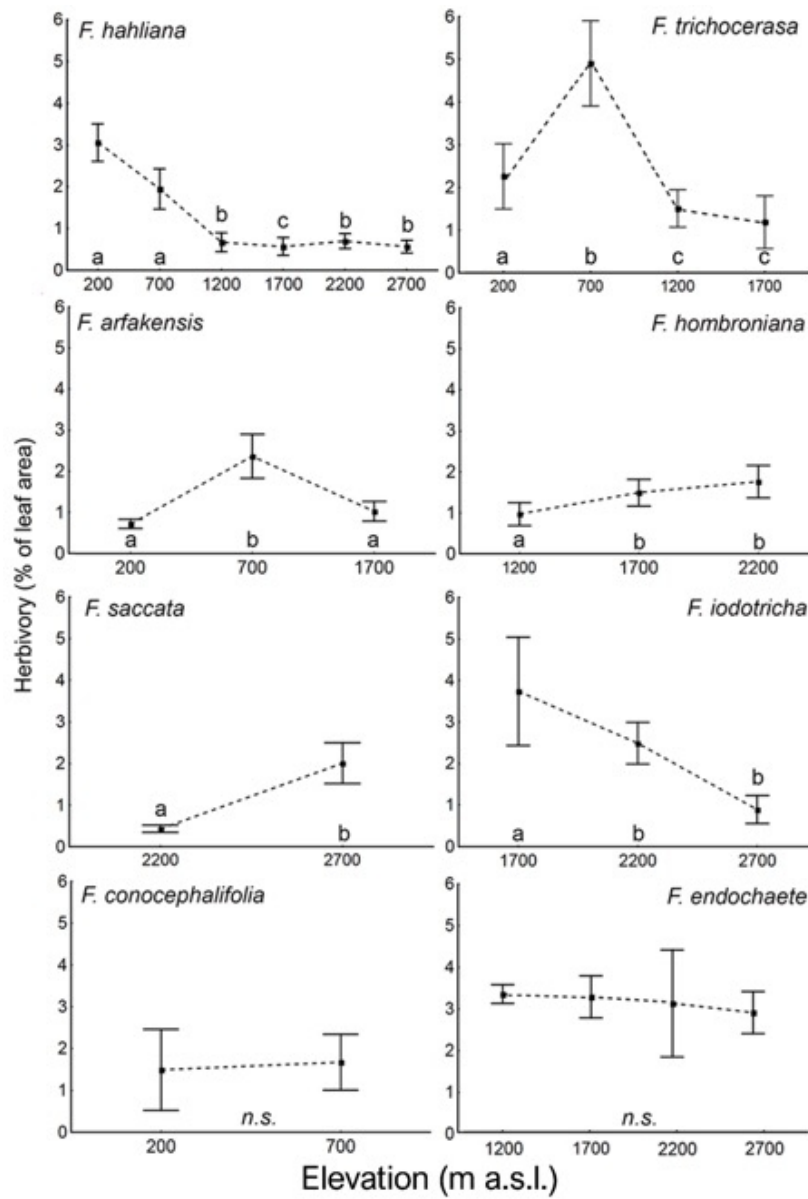


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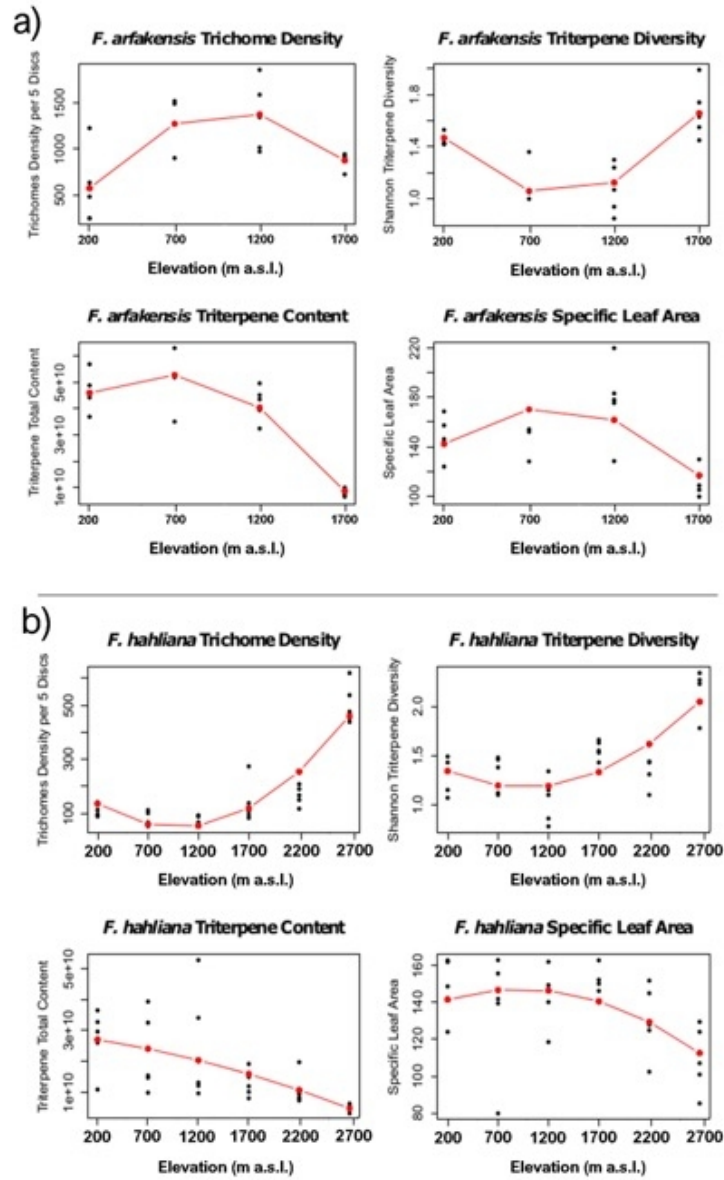
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158x120mm (72 x 72 DPI)



159x232mm (72 x 72 DPI)



146x233mm (72 x 72 DPI)

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