

1 **Changes in the diversity and composition of tree-related microhabitats across climate and human**  
2 **impact gradients on a tropical mountain**

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11 **Abstract**

12 Tree-related microhabitats (TReMs) have been proposed as important indicators of biodiversity to  
13 guide forest management. However, their application has been limited mostly to temperate  
14 ecosystems, and it is largely unknown how the diversity of TReMs varies along environmental  
15 gradients. In this study, we assessed the diversity of TReMs on 180 individual trees and 44 plots  
16 alongside a large environmental gradient on Kilimanjaro, Tanzania. We used a typology adjusted to  
17 tropical ecosystems and a tree-climbing protocol to obtain quantitative information on TReMs on  
18 large trees and dense canopies. We computed the diversity of TReMs for each individual tree and  
19 plot and tested how TReM diversity was associated with properties of individual trees and  
20 environmental conditions in terms of climate and human impact. We further used non-metric  
21 multidimensional scaling (NMDS) to investigate the composition of TReM assemblages alongside the  
22 environmental gradients. We found that diameter at breast height (DBH) and height of the first  
23 branch were the most important determinants of TReM diversity on individual trees, with higher  
24 DBH and lower first branch height promoting TReM diversity. At the plot level, we found that TReM  
25 diversity increased with mean annual temperature and decreased with human impact. The  
26 composition of TReMs showed high turnover across ecosystem types, with a stark difference  
27 between forest and non-forest ecosystems. Climate and the intensity of human impact were  
28 associated with TReM composition. Our study is a first test of how TReM diversity and composition  
29 vary along environmental gradients in tropical ecosystems. The importance of tree size and  
30 architecture in fostering microhabitat diversity underlines the importance of large veteran trees in  
31 tropical ecosystems. Because diversity and composition of TReMs are sensitive to climate and land-  
32 use effects, our study suggests that TReMs can be used to efficiently monitor consequences of global  
33 change for tropical biodiversity.

34

## 35 1. Introduction

36 Forest ecosystems are home to two-thirds of the world's terrestrial biodiversity (Pillay *et al.* 2022)  
37 and are among the most threatened ecosystems from anthropogenic land-use change, with the  
38 majority of forest ecosystems being degraded to some extent (Watson *et al.* 2018). This makes  
39 monitoring forest biodiversity a priority amidst the global biodiversity crisis. However, monitoring  
40 biodiversity in forests is time consuming and often difficult, especially for understudied taxa  
41 (Hochkirch *et al.* 2021) and in highly diverse ecosystems (Schmeller *et al.* 2017). To address this  
42 shortfall, several cost-effective indicators have been devised to indirectly estimate forest biodiversity.  
43 Most of these indicators, such as the vertical stratification of vegetation or deadwood availability,  
44 aim to describe the capacity of a forest to provide different microhabitats to species (Gao *et al.*  
45 2014). In line with this, the habitat heterogeneity hypothesis states that heterogeneity in biotic and  
46 abiotic environmental conditions promotes a higher niche dimensionality, fosters species  
47 coexistence, and increases biodiversity (Stein *et al.* 2014). Such patterns appear to hold at different  
48 spatial scales (Stein *et al.*, 2014) and have been observed for various taxa (Freemark & Merriam  
49 1986; Price *et al.* 2010), although different organism groups may differ in how they respond to  
50 heterogeneity (Heidrich *et al.* 2020).

51 One of the more recently devised indicator systems of forest biodiversity are tree-related  
52 microhabitats (TReMs). TReMs are defined as all structures occurring on a living or dead standing  
53 tree, which provide niches to specific organisms at some point of their life cycle. They include  
54 structural features of trees like cavities (Fig. 2), bark pockets and damaged branches, but also  
55 epiphytic organisms like ferns, mosses, and lichens (Larrieu *et al.* 2018). Given their relevance for  
56 many organism groups, TReMs provide a suitable indicator system to quantify the potential  
57 occurrence of multiple species simultaneously, they are relatively cost-effective and can be  
58 monitored all year round (Asbeck *et al.*, 2021). First evidence of TReMs being a reliable predictor of

59 biodiversity comes from studies on bat and insect diversity (Basile *et al.* 2020) and bird diversity  
60 (Paillet *et al.* 2018), but these studies have been restricted to temperate forests.

61 Understanding the determinants of TReM abundance and diversity can help identify important  
62 attributes of forest ecosystems and evaluate their role as indicators of environmental change. Studies  
63 carried out in temperate forests have concluded that large trees provide more microhabitats (Asbeck  
64 *et al.*, 2021). In addition, tree architecture can influence the formation rate of some microhabitats,  
65 e.g., by creating weak spots in the crown (Larrieu *et al.* 2022). On a larger scale, climate is known to  
66 affect the presence of epiphytes (Benzing 1998) and cavity formation (Remm & Löhmus 2011) and  
67 managed forests generally host fewer TReMs than old-growth forests (Asbeck *et al.*, 2021). So far,  
68 most assessments of TReMs have been conducted in temperate and boreal forests, mostly in North  
69 America and Europe (Martin *et al.* 2022) and, thus, were restricted to a relatively small pool of tree  
70 species (Mamadashvili *et al.* 2023) and a limited range of environmental conditions. This limits our  
71 understanding of how TReMs are shaped by climatic factors and human impacts (Martin *et al.* 2022).  
72 Studying TReMs along environmental gradients is, thus, essential to understanding how climate and  
73 human impact affect TReM diversity and composition.

74 In this study, we present a first comprehensive assessment of TReMs along a large environmental  
75 gradient comprising ten ecosystem types studied along an elevational gradient of more than 3000  
76 meters on Kilimanjaro, Tanzania. We devised a protocol for assessing TReM diversity which allows to  
77 record accurate information on TReM presence and abundance even in complex rainforest canopies.  
78 Based on this assessment, we analysed the diversity and composition of TReMs on 180 individual  
79 trees growing on 44 plots that varied widely in climatic conditions and intensity of human impact.  
80 With these data, we tested the following hypotheses:

81 A) Larger tree size and more complex tree architecture promote TReM diversity (Larrieu *et al.*  
82 2022).

- 83 B) TReM diversity at the plot level increases with increasing rainfall and temperature, while it  
84 decreases with higher levels of human impact (Asbeck et al., 2021).
- 85 C) Ecosystems characterized by similar environmental conditions are expected to host similar  
86 assemblages of TReMs (Asbeck *et al.* 2019).

## 87 **2. Methods**

### 88 **2.1 Study Area**

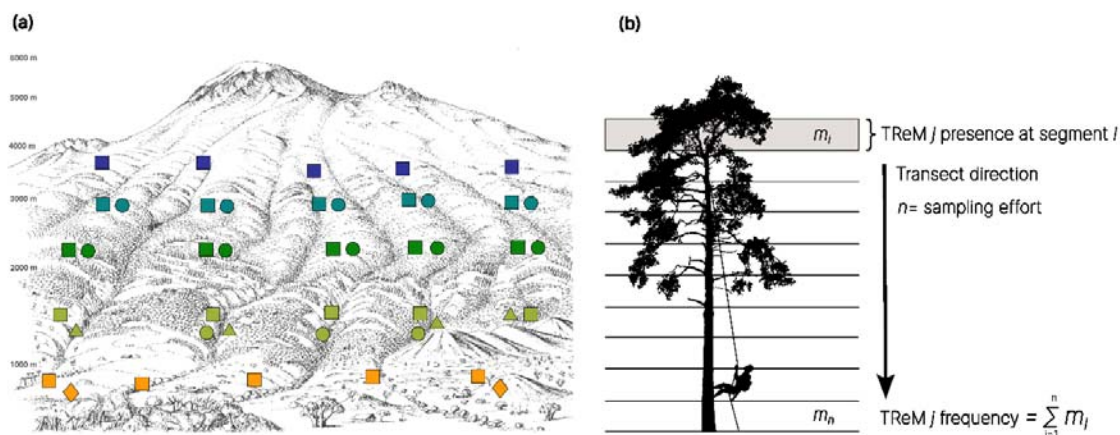
89 Kilimanjaro is a free-standing dormant volcano located in Tanzania (2°45' – 3° 25' S, 37° 00' - 37 43'  
90 E) rising from a plateau at 700m above mean sea level (AMSL) to its summit at 5895m AMSL.  
91 Alongside this elevational gradient, the mountain hosts a variety of ecosystems which vary widely in  
92 climatic conditions and in intensity of human impacts. Below 1800m AMSL, maize plantations,  
93 managed grasslands, coffee plantations and traditional homegardens are the ecosystems with the  
94 strongest human footprint, while lowland savannas and lower montane forest are mostly natural  
95 ecosystems. Above 1800m, lower montane forest is replaced by *Ocotea* forest, and the latter is then  
96 replaced by *Podocarpus* and *Erica* forests at higher elevations. Some *Ocotea* forests have historically  
97 experienced selective logging, while *Podocarpus* and *Erica* forests can be damaged by human-caused  
98 fires (Hemp 2006).

### 99 **2.2 Study design**

100 We conducted our TReM surveys across three field seasons that took place between February-April  
101 2022, August-November 2022, and January-April 2023. We assessed TReM diversity across 44 plots  
102 (50x50 m in size), distributed alongside five elevational transects and representing the ten ecosystem  
103 types which harbor trees on the mountain (Figure 1a). These plots were established within the KiLi  
104 (FOR 1246) and the Kili-SES (FOR 5064) DFG research units. In these plots, we selected the five  
105 largest trees that were deemed safest to climb. Where several options were available, we prioritised  
106 sampling trees of different species.

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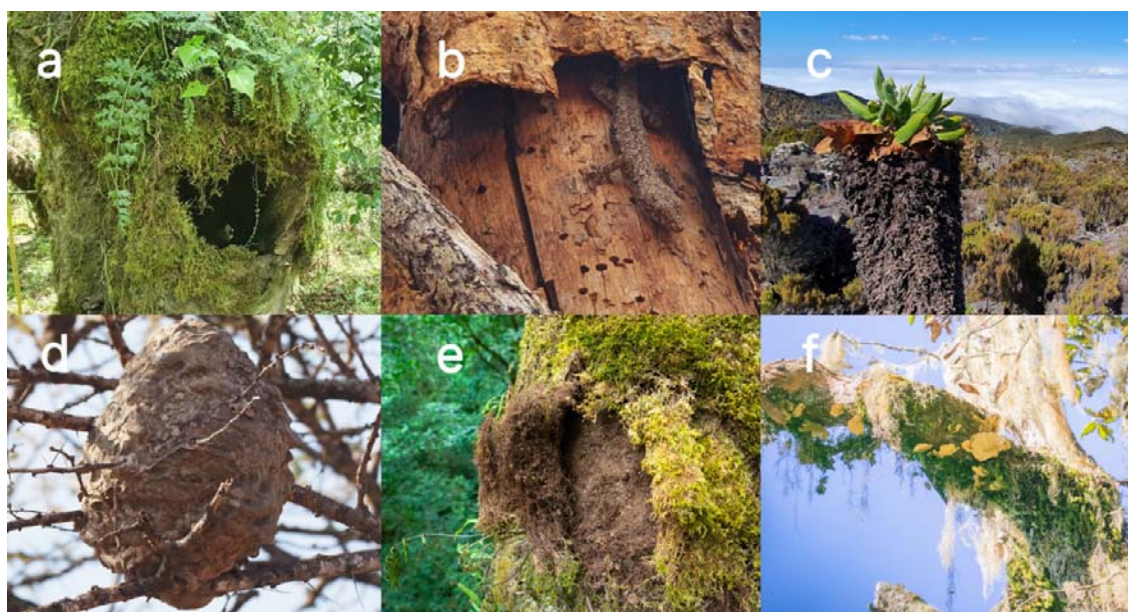
110 **Figure 1. a) The study system on Kilimanjaro.** The diagram portrays the southern slope of  
111 Kilimanjaro and the spatial distribution of the studied plots ( $n = 44$ ). Orange squares represent  
112 natural savanna plots; orange diamonds represent cultivated maize plots. Green circles represent  
113 homegardens; green triangles represent coffee plantations; green squares represent lower montane  
114 forest. Dark green squares represent *Ocotea* forest and circles disturbed *Ocotea* forest. Aquamarine  
115 squares represent *Podocarpus* forest, circles represent disturbed *Podocarpus* forest. Blue squares  
116 represent *Erica* forest. Drawing by A. Hemp. **b) Scheme of the tree-climbing survey of TReMs.** The  
117 scheme depicts the climber doing the survey descending from the highest anchor point in the tree  
118 canopy. The climber records the presence of all 44 TReM types at every one-meter segment. The  
119 frequency of each TReM type is given by the sum of the presences for that tree. The sampling effort  
120 equals the number of segments surveyed on each tree.

### 121 2.3 TReM survey

122 We adapted the hierarchical typology for tree related microhabitats developed by Larrieu et al.  
123 (2018) for tropical forests (Nußer et al. 2024). To this end, we merged the three kinds of woodpecker  
124 breeding cavities into one single TReM type, which encompasses the breeding cavities of

125 woodpeckers, barbets and tinkerbirds (van der Hoek *et al.* 2017). We added epiphytic orchids as an  
126 additional TReM type within the epiphyte TReM form because they are known to be fundamental for  
127 the life cycle of several pollinator species (Spicer & Woods 2022). We grouped epiphytic vascular  
128 plants, which were not orchids, ferns or vines, in the TReM type “other epiphyte”. We further  
129 described a new TReM type, named “dead leaves frill”. On Kilimanjaro, this TReM type is specific to  
130 plants of the *Dendrosenecio* genus, as these plants build an insulating layer of leaves that protect  
131 them from frost and provide shelter to invertebrates (Beck 1986; Tomlinson 1985). A similar  
132 structure is also found on plants of the *Espeletia* and *Puya* genera in the Neotropics (Smith 1979).  
133 The typology used in this study comprises 44 TReM types corresponding to 6 TReM forms (Cavities,  
134 Injuries, Exudates, Deadwood, Fungi and Epiphytes & Epiphytic structures; see Figure 2 for some  
135 examples).

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137

138 **Figure 2. Some of the sampled TReMs on Kilimanjaro.** A) Trunk rot-hole. B) Bark shelter, with gecko.  
139 C) Dead leaves frill found on *Dendrosenecio* spp. D) Invertebrate nest. E) Bark microsoil forming  
140 beneath epiphytic mosses. F) Polypore growing on a dead limb.

141 For trees taller than five meters, we employed rope-based techniques to access the tree canopy. This  
142 was necessary because the tall and complex canopies of tropical forests are difficult to assess from  
143 the forest floor. To this end, we set up a throwline on a suitable anchor point from the ground using a  
144 catapult (BigShot catapult by Notch Equipment) and throwbags (10oz and 12oz). The throwline was  
145 then used to hoist a climbing rope (Courant Rebel, 11mm) for single rope technique up to the  
146 anchor point. The tree climber secured himself to the rope via a Petzl ID's belay/rappel device and  
147 proceeded to ascend the tree until the anchor point. If the situation demanded it, once the anchor  
148 point was reached, the tree climber set a second line (Tree Runner 12 mm) to continue ascension  
149 with Double Rope Technique (Anderson *et al.* 2015). Once at the highest reachable anchor point, the  
150 tree climber proceeded to descend the entirety of the tree. During the descent, the climber (G.  
151 Bianco) paused at one-meter intervals to assess the presence of each of the 44 TReM types (Fig. 1b).  
152 This survey resulted in an incidence matrix of TReM types for each surveyed tree, where rows  
153 represent the one-meter segments screened for the 44 TReM types. Based on these data, we were  
154 able to calculate the frequency of each TReM type on each sampled tree and plot.

## 155 **2.4 Tree measurements**

156 Within each plot, all trees with a diameter at breast height (DBH) greater than ten cm were identified  
157 to the species level, and individually marked with aluminium tags (Ensslin *et al.* 2015).  
158 Measurements of the DBH, height, and height of the first branch were recorded for every tagged  
159 individual. DBH was measured with a diameter tape (Forestry Suppliers, USA) at 1.3 m for normally  
160 shaped trees and 20 cm below or above when branches or irregular shapes impeded measurement  
161 at that height. Tree height was measured from the highest ground level around the stem to  
162 standardize measurements taken on slopes. For trees which were strongly buttressed or too big to  
163 measure by hand, a laser dendrometer (Criterion RD 1000 with TruPulse 200/200, Centennial, USA)  
164 was used to measure the tree above the buttresses and at 1.3 m. Height of the highest visible leaf  
165 and height of the first branch were measured using an ultra-sonic hypsometer (Vertex IV



166 Hypsometer, Haglöf, Langsele, Sweden) or a laser rangefinder (TruPulse 200/200). We obtained  
167 measurement of the wood density for each tagged species by Ensslin *et al.* (2015), as wood density  
168 may be linked to the frequency and type of damage that a tree experiences in its life history (King *et*  
169 *al.* 2006).

## 170 **2.5 Environmental variables**

171 We selected key variables measured at the plot level to describe the variability in climatic conditions  
172 and human impacts on Kilimanjaro (Albrecht *et al.* 2021; Peters *et al.* 2016). We selected mean  
173 annual temperature (MAT) and mean annual precipitation (MAP) as the main descriptors of the  
174 climatic conditions on the mountain. Air temperature was measured in every plot via a sensor placed  
175 at a height of about two meters above ground. MAT was calculated from two years of measurements  
176 taken at 5 minutes intervals (Peters *et al.* 2016). MAP was interpolated for every plot, using data  
177 collected with a network of 70 rain gauges over a time span of 15 years (Hemp 2006).

178 We obtained metrics of biomass removal (BRI) and vegetation structure (VSI), both associated with  
179 the intensity of human impact from Peters *et al.* (2019). BRI is a measure of direct human impact on  
180 a study plot in terms of plant biomass removed by humans via mowing, cattle grazing, fire events,  
181 logging, and firewood collection. Estimates of the percentage of plant biomass removed were taken  
182 multiple times per plot and cross-checked with information on land use provided by the local  
183 landowners (Peters *et al.* 2019). BRI was calculated as the mean of these estimates so that a BRI  
184 value of zero indicates a pristine ecosystem without human impact (i.e., no biomass removal), while  
185 a value of one indicates that the entirety of plant biomass was removed. VSI quantifies human  
186 modification of the vegetation structure relative to the natural conditions at that elevation. To  
187 estimate VSI, canopy closure, canopy height and vegetation heterogeneity (expressed as the  
188 Shannon-Wiener diversity of canopy cover at heights interval of 1, 2, 4, 16, 32, and 64 meters) were  
189 measured at nine points on each plot and averaged (Ferber *et al.* 2014). The VSI value of each plot  
190 was then computed as the mean Euclidian dissimilarity of each plot's vegetation structure relative to

191 the vegetation structure of plots with undisturbed vegetation at that elevation (Peters *et al.*, 2019).  
192 The four environmental variables (MAT, MAP, BRI and VSI) were only weakly correlated across the 44  
193 plots ( $r < 0.5$  in all cases), except for a moderate positive relationship between MAT and BRI ( $n = 44$   
194 plots,  $r = 0.64$ ,  $p < 0.05$ ).

## 195 **2.6 TReM diversity**

196 We calculated TReM diversity at the level of individual trees and for each plot. At the tree level, we  
197 calculated the frequency of each TReM type on every surveyed tree by summing the occurrences of  
198 TReMs across each of the one-meter segments of the vertical transect. This resulted in a matrix  
199 containing the frequency of all 44 TReM types on every sampled tree.

200 Based on this frequency matrix, we computed the Shannon diversity index controlling for sampling  
201 effort. To this end, we used the number of segments of the vertical transects as a measure of  
202 sampling effort (Fig. 1b) and calculated a rarefied estimate of Shannon diversity of microhabitats for  
203 every single tree (R package iNEXT, function “estimatedD”, sampling coverage = 0.85) (Hsieh *et al.*  
204 2016). At the plot level, we summed TReM occurrences for all the surveyed trees within that plot.  
205 Based on these data, we computed a rarefied Shannon diversity using the total number of transect  
206 segments as a measure of sampling effort for each plot. Rarefaction was performed so that we would  
207 not systematically estimate a higher diversity of habitats in plots with taller trees or a lower diversity  
208 on plots on which less than five trees could be sampled, as we always surveyed a maximum of five  
209 trees per plot.

## 210 **2.7 Statistical analysis**

211 We tested the hypothesis that larger and architecturally more complex trees hosted a higher TReM  
212 diversity by fitting a linear mixed model that related tree-level, rarefied Shannon diversity to each  
213 tree’s DBH, height of the first branch, and to species-level wood density. We included plot identity  
214 and tree species identity as random factors, because multiple trees were sampled on each plot.

215 While we surveyed a total of 180 individual trees on the field, this analysis uses data from 148 trees,  
216 as not all trait measurements were available for all tree individuals.

217 To test the hypothesis that climate and human impact affect TReM diversity at the plot level, we  
218 fitted a linear model that related the plot-level, rarefied Shannon diversity to MAT, MAP, BRI, and VSI.  
219 For this analysis we were able to use data from all 180 surveyed trees. For both models, we  
220 performed model selection across all possible combination of predictor variables (main effects only)  
221 using the function “dredge” from R package MuMIn (Bartoń 2022) using the second order Akaike’s  
222 Information Criterion (AICc) to select the best model. For the model addressing the second  
223 hypothesis, it was not possible to choose a single best model as three models had an AICc difference  
224 lower than two units. In this case, we performed model averaging across all the models that were  
225 within a range of two AICc units relative to the best model.

226 To test whether the composition of TReM assemblages depended on the environmental conditions  
227 on each plot, we employed non-metric multidimensional scaling (NMDS) to quantify changes in  
228 TReM composition among plots. We used the R package “vegan” (Oksanen *et al.* 2022) to calculate  
229 the Bray-Curtis dissimilarity between all pairs of plots based on TReM frequencies, which were  
230 standardized via a Wisconsin double standardization, and then applied NMDS scaling to two axes  
231 (function “metaMDS”). We tested how microhabitat composition was related to MAT, MAP, BRI and  
232 VSI by performing an environmental vector fit onto the NMDS ordination (n=10.000 permutations,  
233 function “envfit”, R package “vegan”) (Oksanen *et al.* 2022).

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### 235 **3 Results**

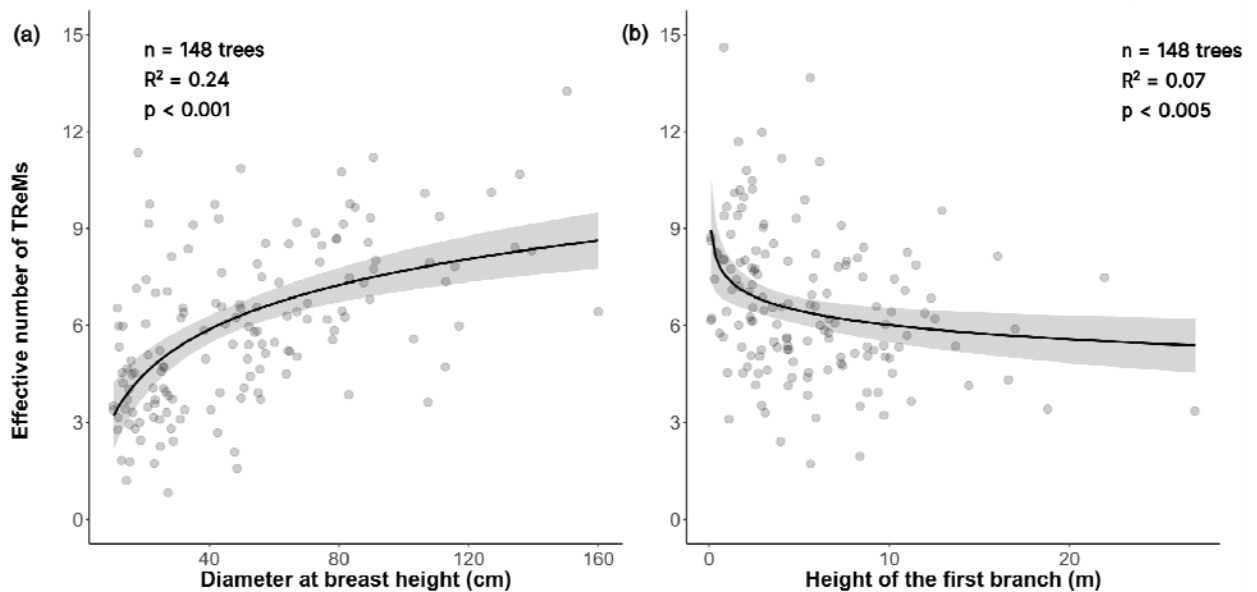
236 We surveyed a total of 180 trees belonging to 41 plant species distributed over 29 plant families (see  
237 the plant species list in **Table S1**). We encountered 43 out of the 44 TReM types from our catalogue.  
238 The most abundant TReMs were moss patches (1580 occurrences), lichen patches (1026 occurrences)

239 and bark microsoil (759 occurrences). The rarest TreMs were the witch's broom (1 occurrence), semi-  
240 open trunk rot-holes (2 occurrences) and bird foraging excavations (3 occurrences).

### 241 3.1 TReM diversity at the tree level

242 At the individual tree level, diameter at breast height and height of the first branch were the most  
243 important predictors of TReM diversity, whereas wood density was not significantly associated with  
244 TReM diversity (Table 1a). TReM diversity increased strongly with DBH, showing a saturating trend  
245 with increasing DBH (Fig. 2a). Our model predicted that trees with a DBH of 40 cm on average  
246 harboured an effective diversity of six TReMs, while trees with a DBH of 80 cm harboured more than  
247 seven TReMs (Fig. 2a). Conversely, the height of the first branch was negatively correlated with TReM  
248 diversity (Fig. 2b). Our model shows that trees with a first branch at a height of two meters hosted on  
249 average eight TReMs, while trees branching at 20 m hosted about five TReMs.

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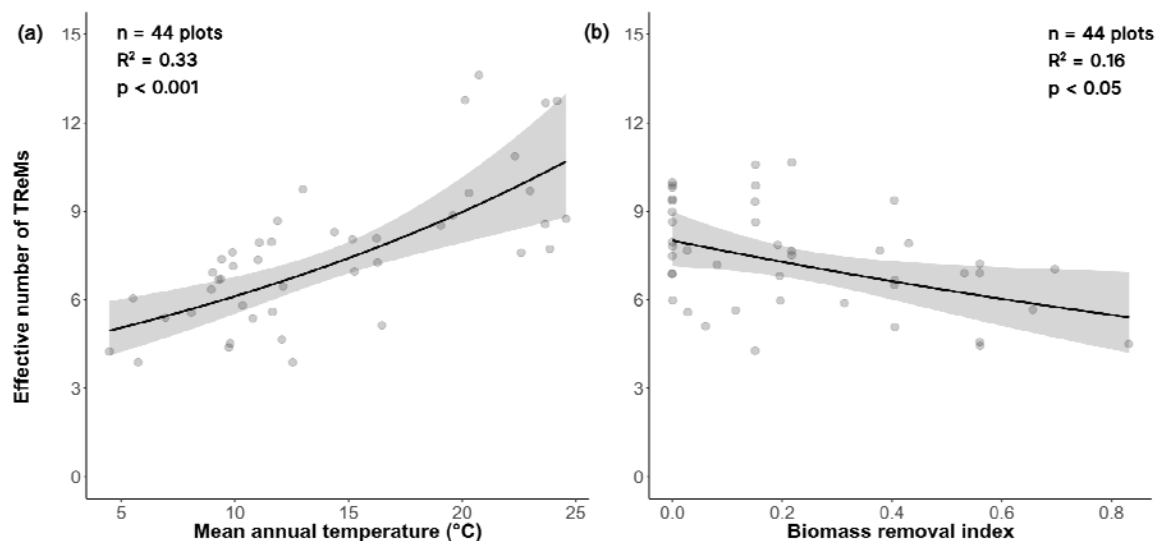
252 **Figure 3.** Partial residual plot showing the associations of TReM diversity with DBH and height of the  
253 first branch. The Y axis shows the effective number of TReMs in relationship to (a) the diameter at  
254 breast height and (b) the height of the first branch. The grey shading represents the 95% confidence

255 interval, while the dots represent the individual trees (n=148). Partial residual plots show the  
256 association between response and predictor variable controlling for the effect of other predictors  
257 included in the statistical model (see Table 1a for all model coefficients). Shown relationships are  
258 non-linear because the values shown are the exponential of the Shannon diversity, which  
259 corresponds to the effective number of TReM types on each individual tree (Jost 2006).

### 260 3.2 TReM diversity at the plot level

261 MAT and BRI were the most important environmental variables driving TReM diversity at the plot  
262 level. TReM diversity increased with increasing temperature (Fig. 3a). BRI was negatively associated  
263 with TReM diversity so that human impacts decreased TReM diversity at the plot level (Fig. 3b). VSI  
264 and MAP did not show significant relationships with TReM diversity.

265



266

267 **Figure 4** Partial residual plots of the effect of environment on TReM diversity. The Y axis shows the  
268 effective number of TReMs in relationship to (a) MAT and (b) BRI measured at the plot level. The light  
269 grey shading represents the 95% confidence interval and the dots represent plots. Partial residual  
270 plots show the association between response and predictor variable controlling for the effect of  
271 other predictors included in the statistical model (see Table 1a for all model coefficients). Shown

272 relationships are non-linear because the values shown are the exponential of the Shannon diversity,  
273 which corresponds to the effective number of TRem types on each plot (Jost 2006).

274

275

276 **Table 1. Associations between the diversity of TRem types (a) at tree level and (b) plot level.** Given  
277 are the results of the linear models relating TRem Shannon diversity to variables describing (a) tree-  
278 level and (b) plot-level variability. Parameter estimates are shown in the second column, followed by  
279 lower and upper confidence intervals in the third and fourth columns. Test statistics and p-values are  
280 given in the last two columns. The tree-level model included random-intercept effects of plot and  
281 tree species identity. The plot-level model is based on model averaging across the three best models.

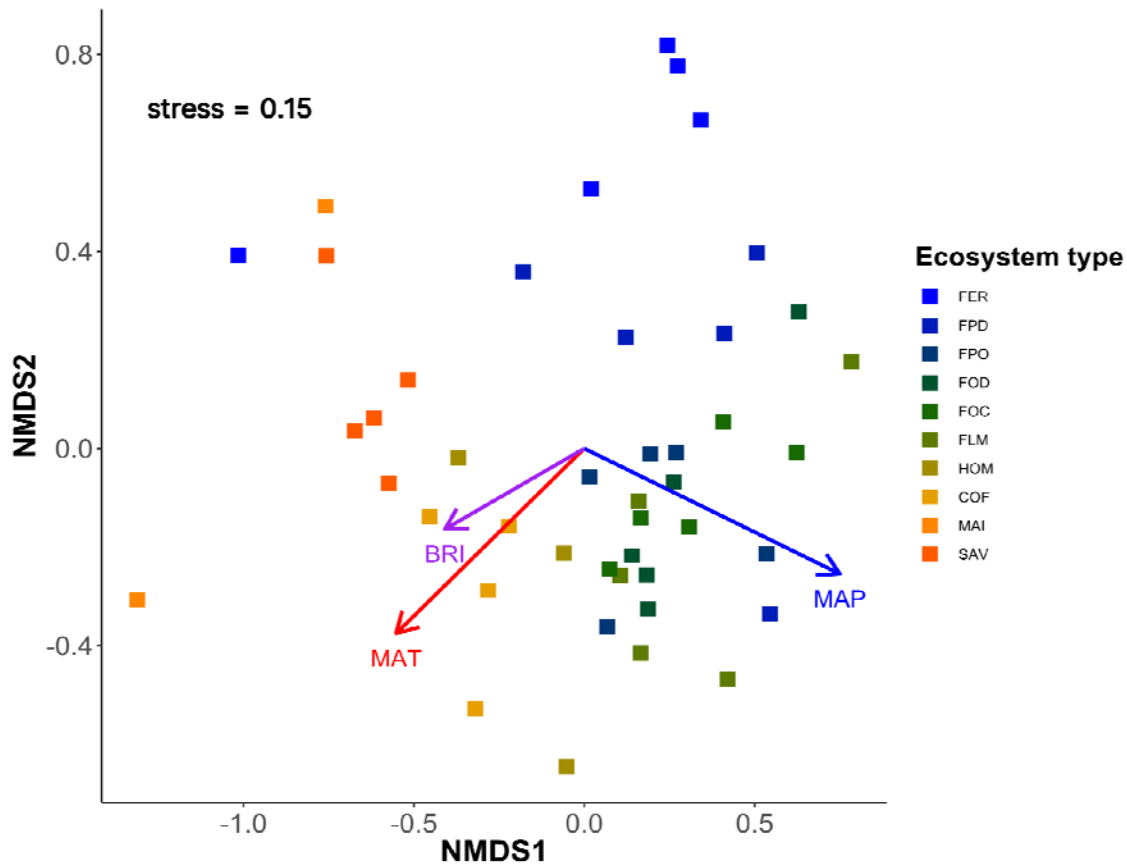
(a) Tree level model	Estimate	CI low	CI high	t	p
Intercept	1.74	1.68	1.81	53.04	<0.001
DBH	0.23	0.16	0.3	6.07	<0.001
Height of first branch	-0.11	-0.18	-0.04	-2.99	<0.005
Wood density	0.01	-0.05	0.08	0.46	0.64
(b) Plot level model	Estimate	CI low	CI high	z	p
Intercept	1.97	1.9	2.04	53.79	<0.001
BRI	-0.11	-0.21	-0.01	2.2	<0.05
MAT	0.22	0.12	0.33	4.05	<0.001
VSI	0.06	0	0.16	1.31	0.19
MAP	0.05	-0.02	0.18	0.85	0.39

282

### 283 3.3 TRem composition

284 TRem composition changed along climatic gradients and in response to human impact (Fig. 4). TRem  
285 composition was generally more similar within than among ecosystem types ( $R^2 = 0.69$ ,  $p < 0.001$ ) and

286 within forested than non-forested ecosystems ( $R^2 = 0.36$ ,  $p < 0.001$ ) (Fig. 4). This turnover in TReM  
287 composition was associated with differences in rainfall ( $R^2 = 0.69$ ,  $p < 0.001$ ) and temperature ( $R^2 =$   
288  $0.49$ ,  $p < 0.001$ ), while biomass removal was significantly, but more weakly associated with  
289 microhabitat composition ( $R^2 = 0.2$ ,  $p < 0.01$ ).



290

291 **Figure 5 Differences in TReM composition across ecosystem types.** Each coloured square represents  
292 a plot belonging to one of the ten ecosystem types in which trees were surveyed. Ecosystem types  
293 are indicated by the following abbreviations: FER, *Erica* forest; FPD, disturbed *Podocarpus* forest;  
294 FPO, *Podocarpus* forest; FOD, disturbed *Ocotea* forest; FOC, *Ocotea* forest; FLM, lower montane  
295 forest; HOM, traditional homegarden; COF, coffee plantation; MAI, Maize plantation; SAV, savanna.  
296 Closer squares are more similar in TReM composition than squares farther apart. Arrows show the  
297 direction of change of the environmental variables that are significantly related to TReM turnover.

298

## 299 **4. Discussion**

300 This study is the first comprehensive assessment of TReM diversity along a large environmental  
301 gradient in the tropics. We found that large trees were particularly important for fostering TReM  
302 diversity. Along the studied environmental gradient, MAT had a positive effect on TReM diversity,  
303 while human impact had a negative impact. TReM composition was systematically related to both  
304 climatic differences and human impact. This work shows that TReMs can be used as sensitive  
305 indicators of microhabitat diversity and composition both at the level of individual trees and across  
306 large environmental gradients.

### 307 **4.1 TReM diversity at tree level**

308 At the tree level, the strongest predictor of microhabitat diversity was diameter at breast height. This  
309 finding is in accordance with patterns observed in temperate forests (Asbeck *et al.* 2021). Diameter  
310 at breast height is one of the most widely used measurements of tree size in forestry, because it is  
311 easy to record in a standardised way (Blanchard *et al.* 2016). Trees with higher DBH are generally  
312 taller and have a larger crown area, and these allometric relationships have been confirmed across  
313 multiple tropical forests (Blanchard *et al.*, 2016). This indicates that taller trees with a broader  
314 canopy constitute a larger habitat patch for the formation of TReMs. In addition, trees with larger  
315 DBH are generally older, which means that they are more likely to show structural changes and  
316 damages, such as limb and stem breakages, cracks and bark loss. Moreover, older trees likely host  
317 standing deadwood such as dead branches, or rotten areas where cavities are more likely to form  
318 naturally (Larrieu *et al.* 2018). This has important implications in terms of conservation and forestry  
319 management, as it underlines the importance of large veteran trees as hotspots of biodiversity  
320 (Kozák *et al.* 2023; Lindenmayer 2017). Our findings demonstrate that DBH is a cost-effective  
321 indicator to measure TReM diversity in forests. In this study, we did not assess TReM diversity on  
322 snags, because climbing such trees safely is challenging and requires additional safety measures  
323 (Anderson *et al.* 2015). Snags are, however, known to be important in providing TReMs (Paillet *et al.*



324 2017), and future studies should attempt to include information on how they contribute to  
325 microhabitat diversity in tropical forests.

326 In addition to tree size, we found that the height of the first branch was negatively associated with  
327 TReM diversity, indicating that trees with lower first branches tend to have more TReMs. The height  
328 of the first branch is generally considered an indicator of a tree's growth strategy. Trees that branch  
329 out at greater heights are likely large leaved trees which show a low degree of ramification (King  
330 1998). These species can be expected to have a less complex canopy architecture which support a  
331 lower number of TReMs. While the tree growth form has not been the focus of previous TReM  
332 studies, mostly because the diversity of growth forms is lower in temperate than tropical forests, it  
333 might be interesting to study such relationships in more detail in tropical ecosystems that are  
334 characterised by a high number of tree species and a wide variety of growth strategies.

#### 335 **4.2 TReM diversity along the environmental gradient**

336 The diversity of TReMs was positively associated with MAT, so that more microhabitat types were  
337 found in warmer areas of the mountain. It is important to consider that several types of TReMs are  
338 constituted by living organisms, e.g., epiphytes like ferns, orchids, vines, while other TReMs are  
339 produced by living organisms, such as in the case of nests or insect galleries. These types of TReMs  
340 had lower incidences at higher elevations, likely due to environmental filtering of its associated  
341 organisms. On Kilimanjaro, like in other regions, species richness of plants and animals decreases  
342 with elevation and decreasing temperatures (Peters *et al.* 2016). This suggests that temperature is  
343 both the major driver of biodiversity and TReM diversity on the mountain.

344 TReM diversity was negatively affected by human impacts. On Kilimanjaro plant biomass is harvested  
345 primarily as a source of timber, fuel or fodder. Timber extraction, in particular, has resulted in  
346 changes in the size distribution (i.e., lower mean DBH of trees) in the disturbed plots of *Ocotea* and  
347 *Podocarpus* forests. In addition, human impact due to agricultural activities at the lower elevations,  
348 e.g., in coffee plantations and the traditional Chagga homegardens, led to a reduction in the number

349 and diversity of trees present in cultivated plots (Hemp 2006). Because large veteran trees are  
350 particularly important sources of TReMs, the logging of such trees is directly related to the lower  
351 TReM diversity in plots that have high levels of biomass removal. This is in accordance with studies  
352 carried out in temperate ecosystems, where managed forests have been found to have lower TReM  
353 diversity than old-growth forests (Asbeck *et al.* 2019). Studies of TReMs can therefore be an effective  
354 tool to quantify how human impacts shape the habitat heterogeneity provided by forest ecosystems.

### 355 **4.3 Changes in TReM composition**

356 The composition of TReM assemblages in plots was dictated primarily by climate and, to a lesser  
357 extent, by human impact. The strongest climatic determinant of TReM composition was rainfall,  
358 although it was not significantly associated with TReM diversity. According to our analysis, forested  
359 plots clustered together and were distinct from non-forested plots in their TReM composition.  
360 Foliose lichens, mosses, and bark microsoil were the most recorded TReMs in this survey and were  
361 by a large extent observed in forested plots with high rainfall. Foliose lichens and mosses are known  
362 to thrive in conditions of elevated rainfall in the tropics (Benzing 1998) and the highest abundance of  
363 ferns and other epiphytes occurs in the more rainy areas of Kilimanjaro (Hemp 2001, 2011). In  
364 addition, rainfall is known to be positively associated with the presence of TReMs like limb breakage,  
365 stem breakages and other kinds of tree structural damages in tropical forests (van der Meer &  
366 Bongers 1996). High levels of precipitation can further trigger tree falls that subsequently damage  
367 neighbouring trees and subject tree crowns to strong forces due to high water loads. Similarly, high  
368 humidity levels promote accelerated rotting of wood and foster cavity formation (Lindenmayer *et al.*  
369 1993) and are likely to contribute to the distinct TReM communities in the montane forests.  
370 Conversely, some TReMs were associated to non-forested plots: the fire damage TReM, for example,  
371 was recorded solely in savanna plots, as fire events are frequent in this dry ecosystem. Very large  
372 cavity types, like chimney trunk rot holes, and disease related TReMs like decayed cankers occurred  
373 mostly in non-forested plots like homegardens and coffee plantations. This might occur because

374 trees in these plots are pruned to harvest fodder and might be exposed to pathogen infections  
375 (Mollel *et al.* 2017).

#### 376 **4.4 TReMs as a suitable indicator system in the tropics**

377 Our findings demonstrate that TReMs are sensitive indicators of environmental gradients driven both  
378 by climatic factors and human impact. This suggests that TReMs are a valuable indicator system for  
379 tropical ecosystems, in which they might be particularly useful given the high diversity of species in  
380 these ecosystems (Barlow *et al.* 2018) and the difficulty to assess this diversity with cost-effective  
381 measures (Schmeller *et al.* 2017). We can speculate that TReMs function as useful biodiversity  
382 indicators on Kilimanjaro, because comprehensive biodiversity surveys carried out along this same  
383 gradient showed that both the diversity of vertebrate and invertebrate taxa responded similarly to  
384 the same set of climate and human impact variables (Peters *et al.*, 2016, 2019). This suggests that  
385 TReM diversity is driven by similar mechanisms as biodiversity. There is, however, a need for more  
386 studies to investigate the specific relationships between TReMs and biodiversity, especially in the  
387 tropics.

388 Our quantitative survey of the abundance of TReMs on about 200 individual trees required a high  
389 number of man hours, as ascending a 30-meter tree can require up to four hours. While TReM  
390 surveys from the ground can cover a greater number of trees faster, they might not be able to detect  
391 TReMs in the higher areas of the tree canopy, especially under complex tropical canopies (Martin *et*  
392 *al.* 2022; Paillet *et al.* 2015). For future studies, we suggest to test the use of small unmanned aerial  
393 vehicles (UAVs) with image recording capabilities that could provide similarly detailed surveys, as  
394 they have been successfully employed to monitor epiphytes or to sample arthropods from the forest  
395 canopy (Krasylenko *et al.* 2023; Madden *et al.* 2022).

#### 396 **5. Conclusions**

397 In this paper, we present the first study of TReMs along a broad climate and human impact gradient  
398 on a tropical mountain. We have shown that both the diversity and composition of TReMs are  
399 sensitive to changes in climate and human impact across the studied ecosystems. These findings  
400 demonstrate the applicability of the TReM concept as a highly sensitive monitoring tool to determine  
401 how changes in climate and human impact affect microhabitat availability and composition in the  
402 tropics. We believe that our approach to quantify TReM diversity can constitute a cost-effective  
403 monitoring tool to be employed in highly diverse ecosystems at tropical latitudes.

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