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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10

Abstract

11

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 46 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 & Lõhmus 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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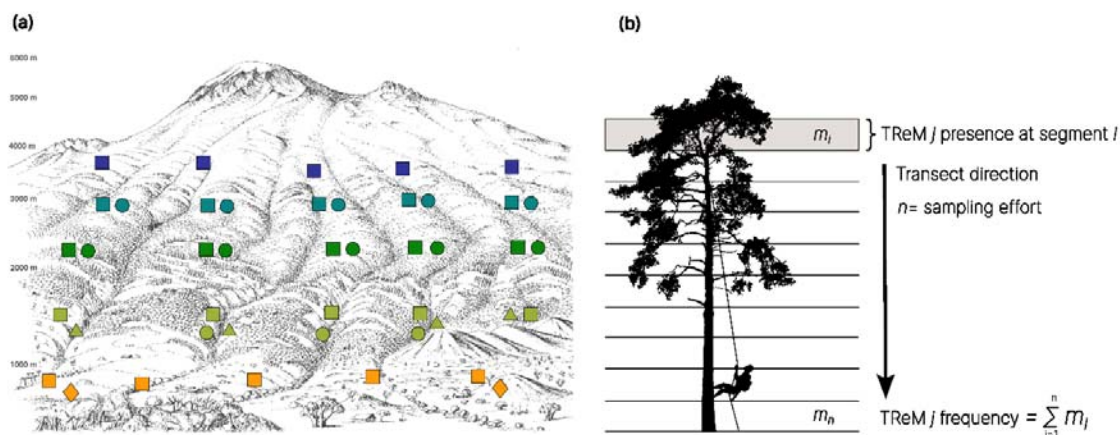
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110 **Figure 1. a) The study system on Kilimanjaro.** The diagram portraits the southern slope of

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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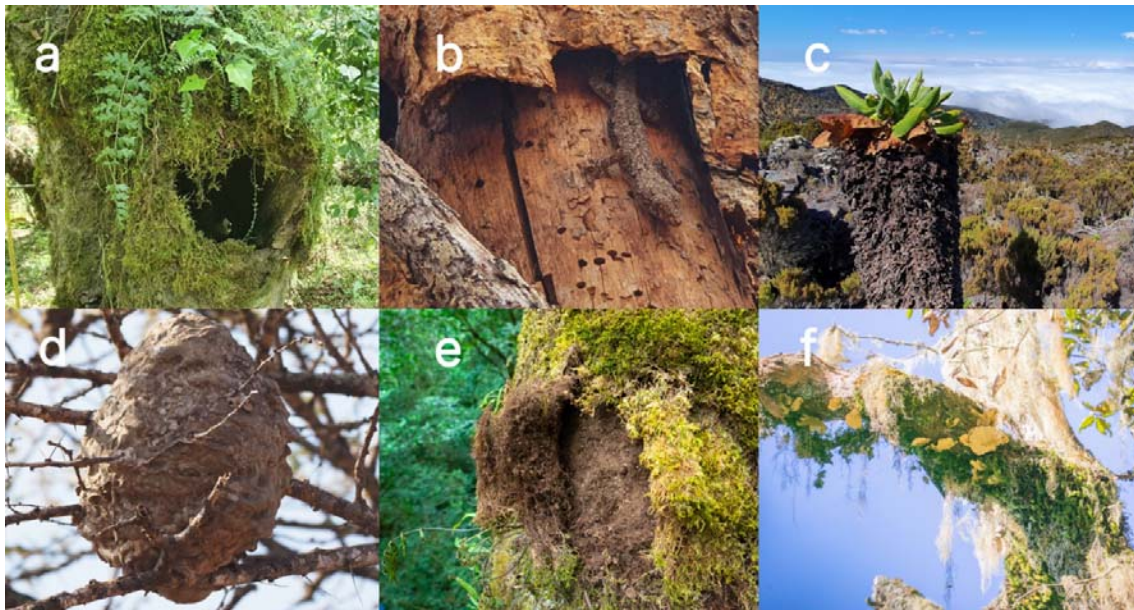
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138   **Figure 2. Some of the sampled TReMs on Kilimanjaro.** A) Trunk rot-hole. B) Bark shelter, with gecko.

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 (Ferger *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 "estimateD", 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).

234

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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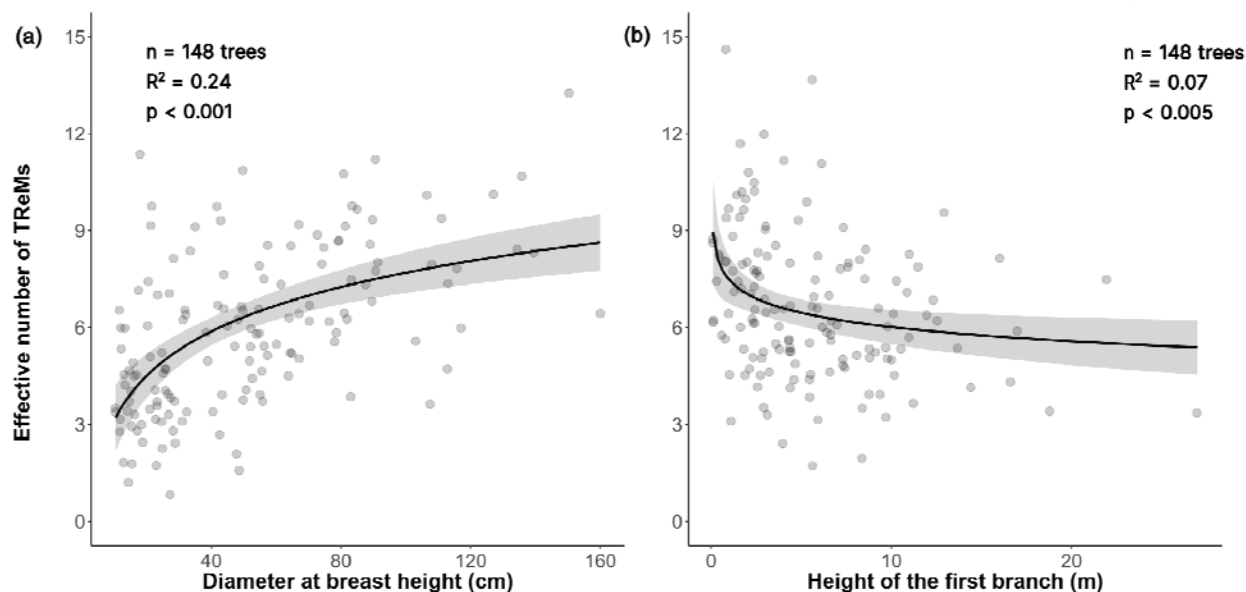
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252   **Figure 3.** *Partial residual plot showing the associations of TReM diversity with DBH and height of the*

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.

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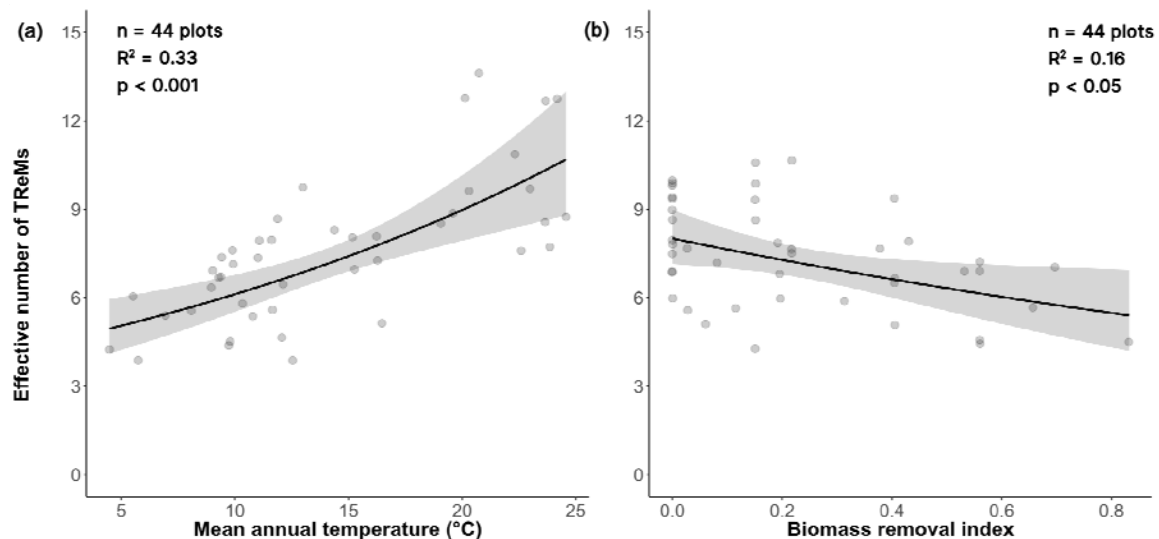
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267   **Figure 4** *Partial residual plots of the effect of environment on TReM diversity*. The Y axis shows the

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).
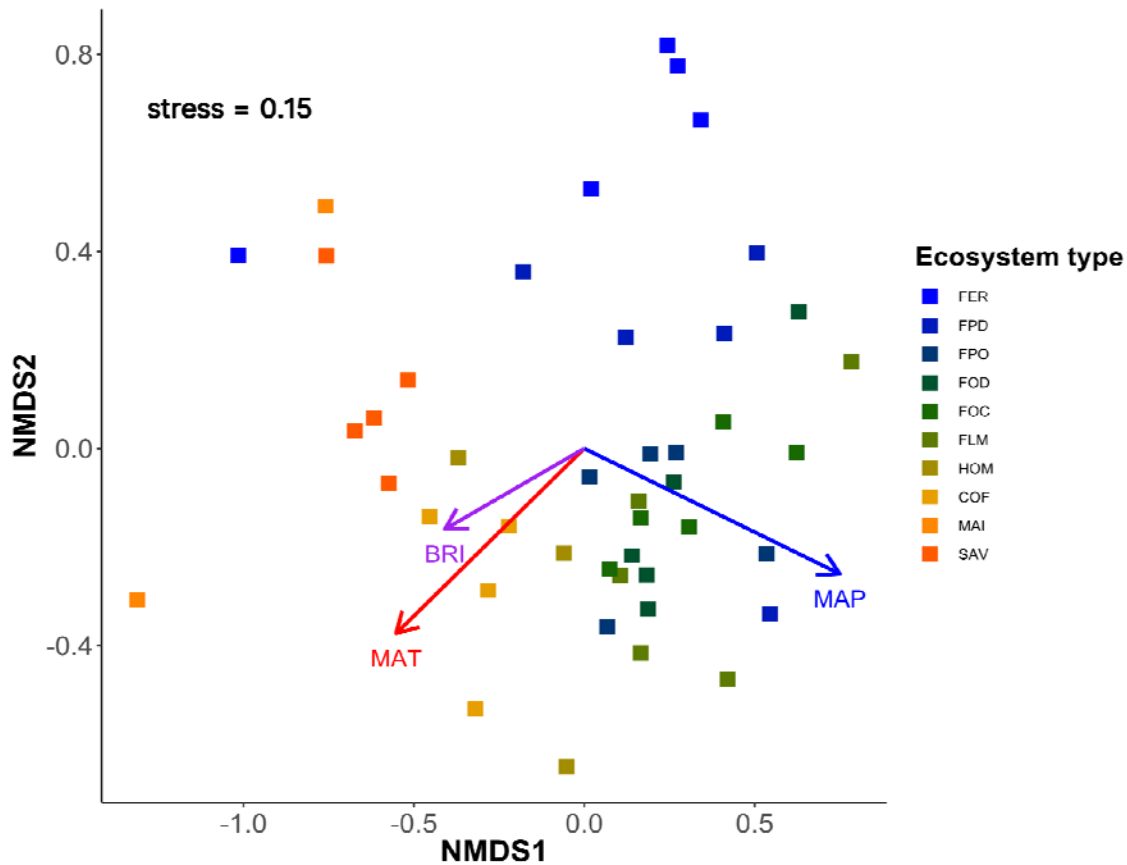
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291   **Figure 5 Differences in TReM composition across ecosystem types**. Each coloured square represents

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.

404   **6. Acknowledgments**

411   **6. References**

412   Albrecht, J., Peters, M.K., Becker, J.N., Behler, C., Classen, A., Ensslin, A., *et al.* (2021). Species

413   richness is more important for ecosystem functioning than species turnover along an

414   elevational gradient. *Nat. Ecol. Evol.*, 5, 1582–1593.

415   Anderson, D.L., Koomjian, W., French, B., Altenhoff, S.R. & Luce, J. (2015). Review of rope-based

416   access methods for the forest canopy: Safe and unsafe practices in published information

417   sources and a summary of current methods. *Methods Ecol. Evol.*, 6, 865–872.

418   Asbeck, T., Großmann, J., Paillet, Y., Winiger, N. & Bauhus, J. (2021). The Use of Tree-Related

419   Microhabitats as Forest Biodiversity Indicators and to Guide Integrated Forest Management.

420   *Curr. For. Reports.*

421   Asbeck, T., Pyttel, P., Frey, J. & Bauhus, J. (2019). Predicting abundance and diversity of tree-related

422   microhabitats in Central European montane forests from common forest attributes. *For. Ecol.*

423   *Manage.*, 432, 400–408.

424   Barlow, J., França, F., Gardner, T.A., Hicks, C.C., Lennox, G.D., Berenguer, E., *et al.* (2018). The future

425   of hyperdiverse tropical ecosystems. *Nature*, 559, 517–526.

426   Bartoń, K. (2022). MuMIn: Multi-modal inference. *Cran.*

427   Basile, M., Asbeck, T., Jonker, M., Knuff, A.K., Bauhus, J., Braunisch, V., *et al.* (2020). What do tree-

428   related microhabitats tell us about the abundance of forest-dwelling bats, birds, and insects? *J.*

429   *Environ. Manage.*, 264, 110401.

430   Beck, E. (1986). Biology of Afroalpine Dendrosenecio (Asteraceae). *Plant Syst. Evol.*, 152, 123–131.

431   Benzing, D.H. (1998). Vulnerabilities of tropical forests to climate change: The significance of

432   resident epiphytes. *Clim. Change*, 39, 519–540.

433   Blanchard, E., Birnbaum, P., Ibanez, T., Boutreux, T., Antin, C., Ploton, P., *et al.* (2016). Contrasted

434   allometries between stem diameter, crown area, and tree height in five tropical biogeographic

435   areas. *Trees*, 30, 1953–1968.

436 Ensslin, A., Rutten, G., Pommer, U., Zimmermann, R., Hemp, A. & Fischer, M. (2015). Effects of

437   elevation and land use on the biomass of trees, shrubs and herbs at Mount Kilimanjaro.

438   *Ecosphere*, 6, 1–15.

439 Ferger, S.W., Schleuning, M., Hemp, A., Howell, K.M. & Böhning-Gaese, K. (2014). Food resources

440   and vegetation structure mediate climatic effects on species richness of birds. *Glob. Ecol.*

441   *Biogeogr.*, 23, 541–549.

442 Freemark, K.E. & Merriam, H.G. (1986). Importance of area and habitat heterogeneity to bird

443   assemblages in temperate forest fragments. *Biol. Conserv.*, 36, 115–141.

444 Gao, T., Hedblom, M., Emilsson, T. & Nielsen, A.B. (2014). The role of forest stand structure as

445   biodiversity indicator. *For. Ecol. Manage.*, 330, 82–93.

446 Heidrich, L., Bae, S., Levick, S., Seibold, S., Weisser, W., Krzystek, P., *et al.* (2020). Heterogeneity–

447   diversity relationships differ between and within trophic levels in temperate forests. *Nat. Ecol.*

448   *Evol.*, 4, 1204–1212.

449 Hemp, A. (2001). Ecology of the pteridophytes on the southern slopes of Mt. Kilimanjaro. Part II:

450   Habitat selection. *Plant Biol.*, 3, 493–523.

451 Hemp, A. (2006). Vegetation of Kilimanjaro: hidden endemics and missing bamboo. *Most*, 305–328.

452 Hemp, A. (2011). Altitudinal zonation and diversity patterns in the forests of Mount Kilimanjaro,

453   Tanzania. In: *Tropical Montane Cloud Forests*. Cambridge University Press, pp. 134–141.

454 Hochkirch, A., Samways, M.J., Gerlach, J., Böhm, M., Williams, P., Cardoso, P., *et al.* (2021). A

455   strategy for the next decade to address data deficiency in neglected biodiversity. *Conserv. Biol.*,

456   35, 502–509.

457   van der Hoek, Y., Gaona, G. V. & Martin, K. (2017). The diversity, distribution and conservation status

458   of the tree-cavity-nesting birds of the world. *Divers. Distrib.*, 23, 1120–1131.

459   Hsieh, T.C., Ma, K.H. & Chao, A. (2016). iNEXT: an R package for rarefaction and extrapolation of

460   species diversity (Hill numbers). *Methods Ecol. Evol.*, 7, 1451–1456.

461   King, D.A. (1998). Influence of leaf size on tree architecture: first branch height and crown

462   dimensions in tropical rain forest trees. *Trees*, 12, 438.

463   KING, D.A., DAVIES, S.J., TAN, S. & NOOR, N.S.M. (2006). The role of wood density and stem support

464   costs in the growth and mortality of tropical trees. *J. Ecol.*, 94, 670–680.

465   Kozák, D., Svitok, M., Zemlerová, V., Mikoláš, M., Lachat, T., Larrieu, L., *et al.* (2023). Importance of

466   conserving large and old trees to continuity of tree-related microhabitats. *Conserv. Biol.*, 37, 1–

467   12.

468   Krasylenko, Y., Rydlo, K., Atamas, N., Sosnovsky, Y., Horielov, O., Maceček, I., *et al.* (2023). Druid

469   Drone—A portable unmanned aerial vehicle with a multifunctional manipulator for forest

470   canopy and mistletoe research and management. *Methods Ecol. Evol.*, 14, 1416–1423.

471   Larrieu, L., Courbaud, B., Drénou, C., Goulard, M., Bütler, R., Kozák, D., *et al.* (2022). Key factors

472   determining the presence of Tree-related Microhabitats: A synthesis of potential factors at site,

473   stand and tree scales, with perspectives for further research. *For. Ecol. Manage.*, 515.

474   Larrieu, L., Paillet, Y., Winter, S., Bütler, R., Kraus, D., Krumm, F., *et al.* (2018). Tree related

475   microhabitats in temperate and Mediterranean European forests: A hierarchical typology for

476   inventory standardization. *Ecol. Indic.*, 84, 194–207.

477   Lindenmayer, D.B. (2017). Conserving large old trees as small natural features. *Biol. Conserv.*, 211,

478   51–59.

479   Lindenmayer, D.B., Cunningham, R.B., Donnelly, C.F., Tanton, M.T. & Nix, H.A. (1993). The

480   abundance and development of cavities in Eucalyptus trees: a case study in the montane

481   forests of Victoria, southeastern Australia. *For. Ecol. Manage.*, 60, 77–104.

482   Madden, J.C., Brisson-Curadeau, É., Gillung, J.P., Bird, D.M. & Elliott, K.H. (2022). Optimal settings

483   and advantages of drones as a tool for canopy arthropod collection. *Sci. Rep.*, 12, 18008.

484   Mamadashvili, G., Brin, A., Bässler, C., Chumak, V., Chumak, M., Deidus, V., *et al.* (2023). Drivers of

485   tree-related microhabitat profiles in European and Oriental beech forests. *Biol. Conserv.*, 285,

486   110245.

487   Martin, M., Paillet, Y., Larrieu, L., Kern, C.C., Raymond, P., Drapeau, P., *et al.* (2022). Tree-Related

488   Microhabitats Are Promising Yet Underused Tools for Biodiversity and Nature Conservation: A

489   Systematic Review for International Perspectives. *Front. For. Glob. Chang.*, 5, 1–18.

490   van der Meer, P.J. & Bongers, F. (1996). Patterns of Tree-Fall and Branch-Fall in a Tropical Rain Forest

491   in French Guiana. *J. Ecol.*, 84, 19.

492   Mollel, N.P., Fischer, M. & Hemp, A. (2017). Usable wild plant species in relation to elevation and

493   land use at Mount Kilimanjaro, Tanzania. *Alp. Bot.*, 127, 145–154.

494   Nußer, R., Bianco, G., Kraus, D., Larrieu, L., Feldhaar, H., Schleuning, M., *et al.* (2024). An adapted

495   typology of tree-related microhabitats including tropical forests. *bioRxiv*, 2024.04.20.590405.

496   Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., *et al.* (2022).

497   Package "Vegan" - Community Ecology Package. *Cran*.

498   Paillet, Y., Archaux, F., Boulanger, V., Debaive, N., Fuhr, M., Gilg, O., *et al.* (2017). Snags and large

499   trees drive higher tree microhabitat densities in strict forest reserves. *For. Ecol. Manage.*, 389,

500   176–186.

501   Paillet, Y., Archaux, F., du Puy, S., Bouget, C., Boulanger, V., Debaive, N., *et al.* (2018). The indicator

502   side of tree microhabitats: A multi-taxon approach based on bats, birds and saproxylic beetles.

503   *J. Appl. Ecol.*, 55, 2147–2159.

504   Paillet, Y., Coutadeur, P., Vuidot, A., Archaux, F. & Gosselin, F. (2015). Strong observer effect on tree

505   microhabitats inventories: A case study in a French lowland forest. *Ecol. Indic.*, 49, 14–23.

506   Peters, M.K., Hemp, A., Appelhans, T., Becker, J.N., Behler, C., Classen, A., *et al.* (2019). Climate–

507   land-use interactions shape tropical mountain biodiversity and ecosystem functions. *Nature*,

508   568, 88–92.

509   Peters, M.K., Hemp, A., Appelhans, T., Behler, C., Classen, A., Detsch, F., *et al.* (2016). Predictors of

510   elevational biodiversity gradients change from single taxa to the multi-taxa community level.

511   *Nat. Commun.*, 7, 13736.

512   Pillay, R., Venter, M., Aragon-Osejo, J., González-del-Pliego, P., Hansen, A.J., Watson, J.E.M., *et al.*

513   (2022). Tropical forests are home to over half of the world's vertebrate species. *Front. Ecol.*

514   *Environ.*, 20, 10–15.

515   Price, B., Kutt, A.S. & McAlpine, C.A. (2010). The importance of fine-scale savanna heterogeneity for

516   reptiles and small mammals. *Biol. Conserv.*, 143, 2504–2513.

517   Remm, J. & Lõhmus, A. (2011). Tree cavities in forests – The broad distribution pattern of a keystone

518   structure for biodiversity. *For. Ecol. Manage.*, 262, 579–585.

519   Schmeller, D.S., Böhm, M., Arvanitidis, C., Barber-Meyer, S., Brummitt, N., Chandler, M., *et al.*

520   (2017). Building capacity in biodiversity monitoring at the global scale. *Biodivers. Conserv.*, 26,

521   2765–2790.

522   Smith, A.P. (1979). Function of Dead Leaves in Espeletia schultzii (Compositae), and Andean

523   Caulescent Rosette Species. *Biotropica*, 11, 43.

524   Spicer, M.E. & Woods, C.L. (2022). A case for studying biotic interactions in epiphyte ecology and

525   evolution. *Perspect. Plant Ecol. Evol. Syst.*, 54, 125658.

526   Stein, A., Gerstner, K. & Kreft, H. (2014). Environmental heterogeneity as a universal driver of species

527   richness across taxa, biomes and spatial scales. *Ecol. Lett.*, 17, 866–880.

528   Tomlinson, R. (1985). Observations on the giant groundsels of upper Kilimanjaro. *Biol. Conserv.*, 31,

529   303–316.

530   Watson, J.E.M., Evans, T., Venter, O., Williams, B., Tulloch, A., Stewart, C., *et al.* (2018). The

531   exceptional value of intact forest ecosystems. *Nat. Ecol. Evol.*, 2, 599–610.

532