Tree size, microhabitat diversity and landscape structure determine the value of isolated trees for bats in farmland

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Author contributions

1 # Tree size, microhabitat diversity and landscape structure determine

2 # the value of isolated trees for bats in farmland

3

4

5 **Abstract**

6 Isolated trees are increasingly recognised as playing a vital role in supporting biodiversity in agricultural

7 landscapes, yet their occurrence has declined substantially in recent decades. Most bats in Europe are

8 tree-dependent species that rely on woody elements in order to persist in farmlands. However, isolated

9 trees are rarely considered in conservation programs and landscape planning. Further investigations are

10 therefore urgently required to identify which trees – based on both their intrinsic characteristics and

11 their location in the landscape – are particularly important for bats. We acoustically surveyed 57 isolated

12 trees for bats to determine the relative and interactive effects of size, tree-related microhabitat (TreM)

13 diversity and surrounding landscape context on bat activity. Tall trees with large diameter at breast

14 height and crown area positively influenced the activity of *Pipistrellus pipistrellus* and small *Myotis*

15 bats (*Myotis* spp.) while smaller and thinner trees favoured *M. myotis* activity. The diversity of TreMs

16 that can be used as roosts had a positive effect on (i) *Barbastella barbastellus* activity only when trees

17 were relatively close (<50 m) to woody patches, (ii) *Pipistrellus nathusii/kuhlii* activity only in the most

18 heterogeneous landscapes, and (iii) *Myotis* spp. activity only in the most forested environment (>10%

19 within 100 radius scale). The potential benefits of isolated trees for bats result from ecological

20 mechanisms operating at both tree and landscape scales, underlining the crucial need for implementing

21 a multi-scale approach in conservation programs. Maintaining the largest and most TreM-diversified

22 trees located in the most heterogeneous agricultural landscapes will provide the greatest benefits.

23

27

1. Introduction

Isolated trees are prominent features of farmlands worldwide (Prevedello et al., 2018). The key defining feature of isolated trees is the dispersed spatial distribution of the trees throughout the landscape matrix (Dunn, 2000; Manning et al., 2006). These dispersed elements are also referred to as scattered trees (Manning et al., 2006), pasture trees (Poltz & Zotz, 2011), paddock trees (Fischer & Lindenmayer, 2002), or remnant trees (Barth et al., 2015). Despite the relatively recent interest in isolated trees for both ecosystem function and conservation issues, their importance as "keystone ecological structures" has been widely recognised (Manning et al., 2006; Gibbons et al., 2008). They provide disproportionately diverse ecological functions for biodiversity relative to their small spatial extent, including food resources, shelters or nest sites for a large range of vertebrate, arthropod and plant taxa (Prevedello et al., 2018). The presence of scattered trees is especially beneficial to wildlife in agricultural landscapes, increasing both species abundance and richness (Dunn, 2000; DeMars et al., 2010). Moreover, scattered trees provide multiple ecosystem services that benefit farmers and crop production, such as soil maintenance, pollination of crops, shading for cattle, regulation of nitrogen dynamics, carbon sequestration, and wood provision (Barton et al., 2016; Cuni Sanchez & Lindsell, 2017; Hartel et al., 2017).

Isolated trees also increase spatial heterogeneity and forest connectivity allowing enhanced species dispersal by acting as stepping stones for mobile and tree-dependent species such as bats (Fischer & Lindenmayer, 2002; Manning et al., 2009, Rocha et al., 2021). Most bat species are highly dependent on woody elements such as forest edges, woodlands, hedgerows and scattered trees which confer navigational reference during commuting, and also provide shelter from wind and protection from predators within the agricultural matrix (Frey-Ehrenbold et al., 2013; Fuentes-Montemayor et al., 2013; Kalda et al., 2015; Lacoeuilhe et al., 2018; Froidevaux et al., 2019). Tall trees with large canopies may act as better acoustic landmarks than small ones for bats in farmlands (Frey-Ehrenbold et al., 2013). At the local scale, tree-related microhabitats (TreMs) may favour bats either through enhancing the abundance of insect prey or by providing suitable diurnal and maternity roosting sites – even though

54 the influence of TreMs on bats has so far only been assessed in forests (Regnery et al., 2013; Paillet et

55 al., 2018; Larrieu et al., 2019; Basile et al., 2020).

56   At the landscape level, the diversity of habitat types in the surrounding matrix irrespective of

57 their spatial arrangement (i.e. landscape compositional heterogeneity) and the spatial arrangement of

58 patches irrespective of habitat types (i.e. landscape configurational heterogeneity) influence bat activity

59 and species richness in farmland through several processes including landscape

60 complementation (Ancillotto et al., 2017; Monck-Whipp et al., 2018; Rodríguez-San Pedro et al., 2019,

61 Laforge et al., 2021). In contrast to less human-altered landscapes, intensively managed farmland is

62 relatively homogeneous, so improving landscape compositional and/or configurational heterogeneity at

63 different spatial scales through farming systems and management practices represents a great

64 opportunity for biodiversity conservation (Tscharntke et al., 2021). Furthermore, bat activity may

65 increase with habitat connectivity and matrix quality in the surrounding landscape (Hale et al., 2012),

66 and this relationship is largely scale-dependent (Mendes et al., 2017). For instance, Le Roux et al. (2018)

67 demonstrated that the number of bat species and their activity around scattered trees were significantly

68 reduced in urban areas compared to semi-natural landscapes. Therefore accounting for matrix

69 composition is critical to understand how bats respond to the distribution of isolated trees in farmland.

70   Scattered woody features are disappearing from many agricultural landscapes worldwide

71 (Gibbons & Boak, 2002; Orłowski & Nowak, 2007; Fischer et al., 2010b; Lindenmayer, 2017), often

72 being perceived as incompatible with current farming practices and intensification/mechanization

73 (Gibbons et al., 2008). Meanwhile, isolated trees are rarely considered in conservation programs and

74 landscape planning (Prevedello *et al.,* 2018; Wintle *et al.,* 2019). There is an urgent need to help retain

75 isolated trees in agricultural landscapes, since agricultural intensification accompanied with a loss of

76 established trees in farmland is extremely detrimental to bat populations (Azam et al., 2016). It is

77 therefore critical to know which isolated trees, based on both their intrinsic characteristics and their

78 location in the landscape, should be prioritised for retention when conflicts of interest between

79 biodiversity conservation and food productivity occur (Le Roux et al., 2018). To fulfil this need, we

80 investigated how the local tree characteristics and their surrounding landscapes influence insectivorous

81 bats at isolated trees in farmlands.

82   The main objectives of our study were twofold: (i) to evaluate how tree size, tree-related

83   microhabitat (TreM) diversity and surrounding landscape context influence bat activity around isolated

84   trees in farmlands; and (ii) to assess the interactive effects of TreM diversity and landscape-level

85   variables on bats. More precisely, we tested the predictions that (i) larger tree size and higher TreM

86   diversity increase bat activity at isolated trees; (ii) bat use of isolated trees with high microhabitat

87   diversity decreases with distance to woodlands and hedgerows, and in less heterogeneous landscape;

88   and (iii) edge density positively influences edge specialist bats while other bat species benefit mostly

89   from increasing habitat diversity in the surrounding landscape.

90

91   ## 2. Material and methods

92   ### 2.1. Study area and sampling design

93   The study was conducted in the east of France, in Doubs district (Bourgogne Franche-Comté

94   region; Fig. 1). We selected a subunit of the first plateau of the Jura mountains (c. 47°12′N, 6°32′E),

95   namely the "plateaux d'Aissey / Sancey / Feule et d'Orsans à Terre-de-Chaux" (407 km², 389-859 m

96   a.s.l.). The landscape of this rural area consists of a mosaic of patchily distributed habitats including

97   native forests (49%), grassland (45%), arable land (5%), small urban areas (1%, the largest village has

98   < 1500 inhabitants), and wetlands (<1%).

99   Using Google Earth 2017, we identified all isolated trees that were present in the study area.

100   We defined for this study an isolated tree as a single tree located at least 20 m from any other woody

101   habitat patch such as other isolated trees, hedgerows, orchards, and forests. We used this 20 m threshold

102   to minimise recording bats from other habitats and because it represents for many bat species a distance

103   after which the probability of crossing a gap between woody patches substantially decreases (Hale et

104   al., 2015; Pinaud et al., 2019). We then randomly selected trees that were situated at least 1 km apart

105   (to minimize the likelihood of counting the same bats at different sites), 50 m from asphalt roads (to

106   avoid any road edge effect) and located in farmland. We ground checked all selected trees and identified

107   the main agricultural land type surrounding the trees (grassland vs. cropland). As most of the trees

108   selected were in cattle-grazed pastures and meadows, trees in croplands were disregarded.

109   We classified trees into three categories, namely oak trees (pedunculate oak, *Quercus robur*),

110   fruit trees and other trees. Fruit trees mainly consisted of cherry (*Prunus avium*) and apple (*Malus*

111   *domestica*) while other trees comprised ash (*Fraxinus excelsior*), beech (*Fagus sylvatica*), maple (*Acer*

112   *pseudoplatanus* and *A. campestre*), and linden (*Tilia* spp.). We applied a final tree selection by

113   considering diameter at 1.30 m (hereafter referred to as "diameter at breast height - DBH") and distance

114   between trees and the nearest woody habitat patches to obtain similar gradients of tree size and habitat

115   connectivity among tree categories. This resulted in a selection of 57 single, isolated trees in grassland,

116   comprising 21 oak trees, 16 fruit trees and 20 other trees (Fig. 1).

117

118   **2.2. Tree assessment**

119   We assessed tree size by using measurements of DBH, tree height, and tree crown area. Tree

120   height was measured with a laser distance meter (Tacklife LDM03; Shenzen Temie Technology Co.,

121   Shenzen, China). To calculate tree crown area, we measured the distance between the edge of the tree

122   crown and the tree trunk at the four cardinal points (i.e. north, east, south, west) and four intercardinal

123   points (i.e. NE, SE, SW, NW). We then we derived the coordinates of these eight sampling points and

124   used ArcGIS Desktop v10 (ESRI, Redlands, CA, USA) to create a polygon around the tree and calculate

125   its area.

126   We surveyed tree-related microhabitats (TreMs) at each tree following the typology of Larrieu

127   et al. (2018). We only considered TreMs that could potentially enhance bat prey abundance and/or

128   provide suitable summer roosts for bats based on expert knowledge and existing literature (Regnery et

129   al., 2013; Larrieu et al., 2018). Thus, we inventoried the presence/absence of 12 TreM types (Appendix

130   S1). All observations were performed by the same surveyor. The number of tree-related microhabitat

131   types are subsequently referred to as TreM diversity. We counted the number of microhabitat types that

132   could potentially enhance prey abundance ("TreM-prey diversity"), and the number of microhabitat

133   types that can be used as roosting sites ("TreM-roost diversity", see Appendix S1).

134

135   **2.3. Bat echolocation call recording and identification**

136   We sampled bats acoustically at all 57 trees between June and August 2017 using Song Meter

137   SM2BAT+ recorders (sampling rate: 384 kHz) connected to SMX-U1 omnidirectional ultrasonic

138   microphones (Wildlife Acoustics, Concord, USA). At each tree, we attached the recorder around the

139   bare tree trunk or a branch at 1.8 m height and installed a data logger (RC-5; accuracy: 0.5°C; Elitech,

140   London, UK) to monitor temperature at night every 15 min. Recording was triggered automatically

141   when sounds in the frequency range 12–192 kHz with a signal-to-noise-ratio level ≥12 dB were

142   detected, and continued for 15 s. Sampling took place during one full night, from 30 min before sunset

143   to 30 min after sunrise, and only when meteorological conditions were suitable for bats to forage, i.e.,

144   dry and calm nights with wind speed <30 km/h and temperature at sunset > 12 °C. Before each sampling

145   night, we randomly selected between one and six trees to sample simultaneously depending on logistical

146   constraints and landowner permission. We used the number of bat passes per night as a measure of bat

147   foraging and commuting activity. Each bat pass (i.e. a series of minimum two echolocation calls with

148   interpulse intervals <1 s) present within each 15 s recording was identified using a semi-automatic

149   approach. In brief, we used BatScope 3.2 (Obrist & Boesch, 2018) to detect and sort recordings

150   containing bat calls and automatically extract call parameters. We then manually identified each bat

151   pass to the lowest taxonomic level (see full details in Appendix S2).

152

153   **2.4. Landscape analysis**

154   We extracted landscape characteristics around each isolated tree at different spatial scales. We

155   created five buffers (100, 250, 500, 1000, 2000 m radii) around the trees using ArcGIS Desktop v10.

156   Larger spatial scales were not considered to minimise the likelihood of landscapes overlapping as most

157   trees were located < 2 km apart (mean: 1.6 km; range: 1.0 – 3.1 km), even though overlapping

158   landscapes do not themselves represent a violation of independence (Zuckerberg et al., 2020). While

159   the smaller spatial scales allow us to describe the environment close to the trees, the larger ones

160   represent the mean daily foraging movement of many bat species present in the study area (Laforge et

161   al., 2021). Using land use data compiled at 10 m spatial resolution for a previous study (Tournant et al.,

162   2013), we quantified within each buffer the (i) landscape composition using the proportion of forest

163   (which was negatively correlated with the proportion of grassland across the five spatial scales, $|r|$ >

164   0.7), (ii) landscape configuration using the edge density (i.e. total length of all edge segments between

165   multiple habitat patches in the buffer divided by the buffer area, m/ha) because many bat species use

166   habitat patch edges to forage and commute, and (iii) landscape compositional heterogeneity using

167   Shannon's diversity index calculated across all the reclassified habitat types (n = 10; Appendix S3).

168   These variables were calculated with the "landscapemetrics" R-package (Appendix S4). We finally

169   calculated the distance between trees and the nearest woody habitat patch (i.e. forests and hedgerows

170   only) and used this metric as a proxy of habitat connectivity that is easily interpretable by land managers

171   (Frey-Ehrenbold et al. 2013).

172

173   **2.5. Statistical analysis**

174   We conducted a series of generalized linear mixed-effect models (GLMMs; "glmmTMB″

175   package) to assess the effects of tree and landscape attributes on bat taxon-specific activity. The

176   response variable was the number of bat passes per night for species or group of species. Because bat

177   activity is count data, GLMMs were fitted with a negative binomial error distribution due to over-

178   dispersion and coupled with a logit link function.

179   Variables related to tree characteristics, tree connectivity, and landscape context were included

180   as fixed effects and tree category was considered as random effect. We also added mean temperature at

181   night and Julian day as covariates. All fixed variables were continuous and scaled (mean = 1; SD = 0)

182   to enable direct comparisons. All analyses were conducted in R version 3.4.3 (R Development Core

183   Team 2017) and references of packages used are presented in Appendix S5.

184   We took a multi-step approach before building our final full models that included (i) a Principal

185   Component Analysis (PCA) on DBH, tree height, and tree crown area, (ii) a selection of the best "scale

186   of effect" for each landscape variable, and (iii) a selection of most relevant interaction between TreM

187   diversity and landscape variables (see Appendix S6 for full details). Thus, for each species or group of

188   species the full model included as fixed effects (i) the first PCA axis summarizing information on tree

189   size, (ii) TreM type diversity, (iii) the interaction between TreM type diversity (either TreM-prey or

190   TreM-roost) and one landscape variable (at its most relevant scale for buffer-dependent metric), (iv) the

191   three area-based landscape variables at their most relevant scales, (v) the distance to the nearest woody

7

habitat, and (vi) two covariates (i.e. mean temperature at night and Julian day). We examined collinearity among predictors present within the full models using the Spearman's correlation coefficient and the variance inflation factors (VIFs) and results did not highlight collinearity issues ($|r|$ < 0.7, VIF < 3).

We conducted model selection based on the full models. We generated all possible models ("MuMIn" package) and ranked the most parsimonious ones based on *AICc* (Burnham & Anderson, 2002). We conducted a model-averaged procedure of most parsimonious models (i.e. those with *ΔAICc* < 2), accounting for model selection uncertainties (Grueber et al., 2011) and report the full model average estimates. The significance of the effects was evaluated using 95% confidence intervals (Nakagawa & Cuthill, 2007). When a significant interaction between TreM type diversity and landscape variable was found, we undertook a spotlight analysis to explore the nature of the interaction effect using the "emmeans" package. In this analysis, we investigated the effects of TreM type diversity on bat activity at specific values (mean, mean - SD, mean + SD) of the selected landscape metric. Details on model validation can be found in Appendix S7.

Finally, as it became apparent that TreM-roost and tree size were key factors driving bat activity around isolated trees, we built a generalized linear mixed-effect model to investigate the relationship between TreM-roost diversity (response variable) and tree size (explanatory variable). We used the same random structure as described above and model was fitted with a Poisson distribution since no overdispersion was detected.

3. Results

We recorded 4,091 bat passes around 57 isolated trees in agricultural grasslands (Appendix S8). The bat assemblage was dominated by *Pipistrellus pipistrellus* with 2,305 bat passes (56.3% of the total bat activity), followed by small *Myotis* bats (12.1%, *Myotis* spp.), nyctaloids (11.5%, *Eptesicus/Nyctalus/Vespertilio* spp.), *Barbastella barbastellus* (5.1%), *Pipistrellus nathusii/kuhlii* (4.5%), *Myotis myotis* and *Rhinolophus hipposideros* (both 2.2%), *Plecotus* spp. (1.4%), *Miniopterus schreibersii* (0.6%), and *Rhinolophus ferrumequinum* (<0.1%; Appendix S8). We could not attribute

219 with certainty <5% of the bat passes to a species or species/genus group. We were able to model the

220 activity of *P. pipistrellus, P. nathusii/kuhlii, B. barbastellus, M. myotis, Myotis* spp., *Plecotus* spp., *R.*

221 *hipposideros* and nyctaloids with a total variance explained ranging from 42 to 79% (i.e. R^2 marginal:

222 proportion of variance explained by the fixed effects; Table 1).

223

224 **3.1. Effects of isolated tree characteristics on bats**

225 The effect of tree size on TreM-roost diversity was significant (estimate ± SE: 0.41 ± 0.15,

226 lower 95% confidence interval: 0.12, higher 95% CI: 0.70). The diversity of TreM-roost increased with

227 increasing tree height, crown area and DBH.

228 Our analyses confirmed that the size (DBH, tree height, and tree crown area) of isolated trees

229 significantly influence bat activity. More precisely, we found that tall trees with large DBH and crown

230 area significantly enhanced the activity of *P. pipistrellus* and *Myotis* spp. while the activity of *M. myotis*

231 was positively associated with smaller trees (Table 1, Fig. 2). The diversity of TreM-roost was selected

232 in our best models for six out eight taxa while TreM-prey diversity was only retained in the most

233 parsimonious models on *R. hipposideros* (Table 1). TreM-roost diversity had only a significant positive

234 effect on *M. myotis* activity (Table 1) and the effects of TreM diversity on the other taxa largely

235 depended on the landscape context.

236

237 **3.2. Interactive effects of TreMs and landscape structure**

238 Our models revealed significant interactions between TreM-roost diversity and different

239 landscape features (Table 1). Thus, the positive effect of TreM-roost diversity was significant on (i) the

240 activity of *B. barbastellus* only when isolated trees were relatively close (<50 m) to a woody habitat

241 patch (i.e. forest or hedgerow), (ii) *P. nathusii/kuhlii* spp. activity only in the most heterogeneous

242 landscapes, and (iii) on *Myotis* spp. activity only in the most forested environment (Fig. 3). TreM-prey

243 diversity was positively associated with *R. hipposideros* activity only when isolated trees were located

244 away (>100 m) from a woody feature (Fig. 3).

245

246 **3.3. Influence of landscape composition and configuration on bats**

247   At the landscape level, significant effects of edge density and landscape compositional

248   heterogeneity (i.e., Shannon diversity of habitats) were always positively associated with bat activity.

249   Edge density at 1 km radius scale positively influenced *P. pipistrellus* activity, while Shannon diversity

250   of habitats at 2 km radius scale had a positive effect on the activity of nyctaloids, *M. myotis* and *B.*

251   *barbastellus* (Table 1). The proportion of forest surrounding isolated trees had a more contrasting effect

252   on bats, whose responses were taxon-specific: the activity of nyctaloids and *R. hipposideros* increased

253   with forest cover while the opposite was true for *M. myotis*. Finally, we also found that the activity of

254   *Plecotus* spp. decreased significantly with the distance to the nearest woody habitat (Table 1).

255

4. Discussion

257   Disregarded for a long time, the potential importance of isolated trees for wildlife has recently

258   gained more attention (see meta-analysis of Prevedello et al., 2018). Yet, it can be difficult to identify

259   the key mechanisms underlying the attractiveness of such isolated habitats within the agricultural

260   matrix. Here, we demonstrate that the ecological importance of isolated trees for bats is determined by

261   tree-related microhabitat diversity, tree size and landscape heterogeneity. More specifically, we found

262   additive and interactive effects of local (i.e. tree characteristics) and landscape (i.e. both composition

263   and configuration) factors on bats recorded around isolated trees. While retaining isolated trees is

264   extremely important and should be urgently included in conservation planning (Le Roux et al., 2018)

265   (e.g. through agri-environment schemes), our results shine a light on the isolated tree attributes and

266   landscape contexts likely to contribute the most to bat conservation in farmland mosaic landscapes.

267

4.1. Species and guild-specific responses of bats to tree size

269   In line with our predictions, tall trees with large DBH and crown area enhanced the activity of

270   edge-specialist (here *P. pipistrellus*) and clutter-adapted (here *Myotis* spp.) bat species but did not

271   influence open-space forager activity (here nyctaloids). These three taxa accounted for more than 80%

272   of the total bat activity recorded in the study area. However, contrary to our expectation, our results also

273   revealed a negative relationship between tree size and *M. myotis* activity. Overall, our results do not

corroborate the findings of Le Roux et al., (2018) who found no effect of tree size on insectivorous bats in Australia, probably because their studies included very contrasting and diverse landscapes with features that may have masked any effects of tree size on bat activity. Nevertheless, Polyakov et al., (2019) found similar results to ours around isolated trees located in Californian vineyards, with larger trees increasing the activity of edge-specialist bats.

As observed with other woody features embedded within the agricultural matrix such as hedgerows (Froidevaux et al., 2019), several mechanisms may explain the positive effects of tree size on edge-specialist and clutter-adapted species. First, tall trees with large canopy may act as better acoustic landmarks than smaller ones, especially for these species that mainly rely on woody features for commuting (Frey-Ehrenbold et al., 2013). Thus, large isolated trees may serve as stepping stones between forests and hedgerows and ultimately increase landscape connectivity (Saura et al., 2014). Second, bats may benefit from greater foraging opportunities around large trees. While we did not assess prey abundance along a tree size gradient, larger trees are likely to provide more shelter for bat insect prey in temperate farmland (Merckx et al., 2010). Prey aggregations around large trees are more likely to benefit relatively manoeuvrable edge-specialist and clutter-adapted bats (Denzinger & Schnitzler, 2013). Third, larger trees may offer more roosting sites for tree-dwelling bat species compared to smaller ones (Tillon et al., 2016). Diameter at breast height is one of the main driver of TreM diversity (Larrieu et al., 2014; Kozák et al., 2018; Asbeck et al., 2019) and TreM formation accelerates as trees grows (Courbaud et al., 2017). This is also confirmed for isolated trees by the positive and significant relationship we found between tree size and the diversity of TreM-roosts.

4.2. Effects of microhabitat diversity on bats depend on the landscape context

The level of habitat use by bats may vary depending on a range of local and landscape factors (Mendes et al., 2017). We found that the activity of *B. barbastellus* increased with increasing TreM-roost diversity only around trees close to (<50 m) woody habitat patches. *Barbastella barbastellus* is a tree-dwelling species and this pattern most likely describes spillover effects of individuals from adjacent woody habitat such as forests and hedgerows to isolated trees when the latter are of higher quality (i.e. with high diversity of potential suitable roosts). Moreover, our findings suggest that *P. nathusii/kuhlii*

11

302   and *Myotis* spp. were more active at trees bearing a high diversity of TreM-roost only in the most

303   heterogeneous and forest-covered landscapes, respectively. These significant interactions were found

304   at the smallest landscape scale considered in this study (i.e. 100 m), which represents the landscape

305   context at the vicinity of the trees. Landscape compositional heterogeneity at such scales may promote

306   the activity of *P. nathusii/kuhlii* through increasing overall landscape permeability as these edge-

307   specialist species may better access to trees that are isolated in the agricultural matrix when this matrix

308   is diverse. Similarly, as many *Myotis* spp. are clutter-adapted species, an increase of forest cover at the

309   vicinity of the isolated trees may enhance the potential access to isolated trees. Depending on both the

310   landscape context and the ecological requirements of the different *Myotis* bat species occurring in the

311   study area, isolated trees bearing a relative high diversity of TreMs may be considered as both roosting

312   habitats and secondary foraging habitats. We hypothesize that *Myotis* bats which mainly forage in

313   forests may use isolated trees as foraging grounds in response to (i) higher inter or intra-specific

314   competition in forests (Lewanzik et al., 2019), and (ii) potential asynchrony in the nightly emergence

315   of insects among different habitats (e.g. between forest and isolated trees) (Ruczyński et al., 2020)

316   arising from less buffered microclimatic conditions around trees. Finally, only *R. hipposideros*

317   responded to the diversity of TreM-prey, with more activity detected around the most TreM-diversified

318   trees that are located further away (>100 m) from woody habitat patches. This slow-flying forager

319   highly relies on woody features for commuting, and crossing an open area can represent a potential risk

320   (e.g. predation). We therefore stipulate that the cost-benefit ratio of crossing an open habitat to access

321   isolated foraging sites is only advantageous for this species when the latter are of high quality (i.e. prey-

322   rich patches).

323   Interestingly, *M. myotis* activity was enhanced by TreM-roost diversity. Although our models

324   on TreM-prey were not retained, this attic-dwelling species may not benefit from roosting opportunities

325   but by enhanced prey resources around isolated trees.

326

327   **4.3. Influence of the surrounding environment**

328   Landscape compositional heterogeneity is known to enhance bat activity and species richness

329   in farmland (Ancillotto et al., 2017; Monck-Whipp et al., 2018; Rodríguez-San Pedro et al., 2019). Our

study corroborates these findings as we found that landscape heterogeneity (here defined as the Shannon diversity of habitats) positively influenced the activity of three taxa (nyctaloids, *M. myotis* and *B. barbastellus*). Landscape compositional heterogeneity is likely to benefit bats through an enhanced process of non-substitutable resource complementation (Dunning et al., 1992; Monck-Whipp et al., 2018). In line with other studies (Mendes et al., 2017; Rodríguez-San Pedro et al., 2019), our results further suggested that edge density between multiple habitat patches (i.e. a proxy of landscape configuration) at a 1 km radius scale positively influenced the activity of the most abundant edge-specialist species *P. pipistrellus*. Finally, we found that bat responses to forest cover at the landscape scale were species-specific, depending on roosting and foraging habitat preferences. Bat species that rely on forests for roosting or foraging (such as *Nyctalus* spp. and *R. hipposideros*, respectively) were positively associated with forest cover whereas *M. myotis,* an attic-dwelling species known to mainly forage above meadows, was negatively affected.

4.4. Implications for conservation and recommendations for management practices

Widely recognized as a keystone ecological structure (Manning et al., 2006) due to the disproportionate roles they provide for many species (Fischer et al., 2010a), isolated trees also represent an important landscape feature for bats in farmland (Le Roux et al., 2018; Polyakov et al., 2019). In accordance with other studies (Gibbons et al., 2008; Manning et al., 2009; Prevedello et al., 2018), our findings highlight the crucial need of maintaining a diversity of isolated trees within the agricultural matrix for both biodiversity conservation and ecosystem functioning. The contrasting response of bats to tree size implies that maintaining isolated trees with varying tree size should benefit the whole bat community (Wood et al., 2017). Our results also highlight the importance of maintaining a wide diversity of tree species in farmland to hinder near-future massive tree losses (Fischer et al., 2010b) and to preserve a large range of tree shapes and structures, as well as continuous amount of dead and decaying trees within grassland at various locations in the wider landscape. While we advocate the preservation of isolated trees for the benefit of the entire bat community, our results also provide relevant information to be applied for species-specific conservation actions. For instance, the preservation of large trees in close vicinity of hedgerows and forest edges appears to be important for

13

358   the conservation of the near-threatened barbastelle bat. Nevertheless, integrating the retention of such

359   small features in conservation planning within farmlands might be challenging as their inventory have

360   been neglected in many countries. Fortunately, remote sensing technologies offer substantial

361   opportunities for both inventorying and monitoring trees in farmland (Malkoç et al., 2021).

362   The potential benefits of isolated trees to bats result from mechanisms that operate at both tree

363   and landscape scales, and our results suggest that trees located in heterogeneous landscapes (in terms

364   of both composition and configuration) will provide the greatest benefits. Finally, as isolated trees are

365   often perceived as incompatible with most current farming practices (Gibbons et al., 2008), it would

366   also be of great value to assess the potential of these trees in enhancing the biodiversity benefits for

367   farmers. Such benefits include suppression of pest insects by bats in farmland (Russo et al., 2018), but

368   also the various ecological functions fulfilled by isolated trees as well as the cultural ecosystem services

369   they provide to agricultural landscapes.

Data availability

Data collected for this study will be available to download on Figshare.

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535   pseudoreplication or a red herring in landscape ecology? *Current Landscape Ecology Reports*,
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Table 1. Standardized, model-averaged parameter estimates with associated standards errors (SE) and 95% confidence intervals of the best GLMMs ($\Delta AICc$ < 2) relating the effects of tree and landscape attributes on bat taxon-specific activity. Variables in bold represent influential variables for which 95% CI did not overlap zero. Marginal R^2 (variance explained by the fixed effects only) of the full models is given.
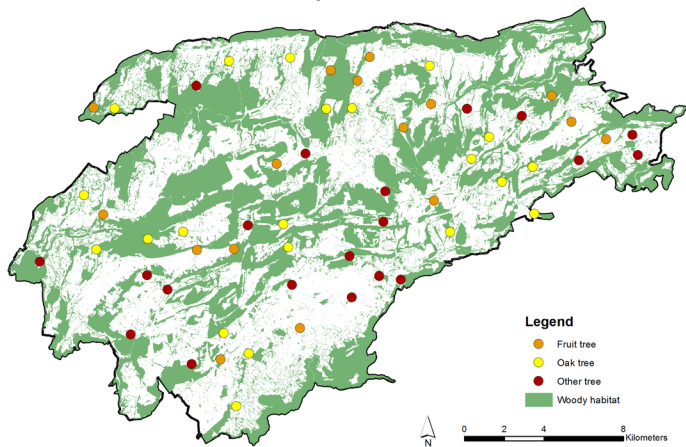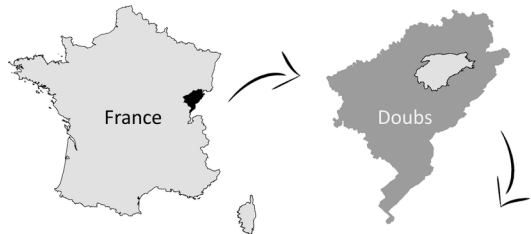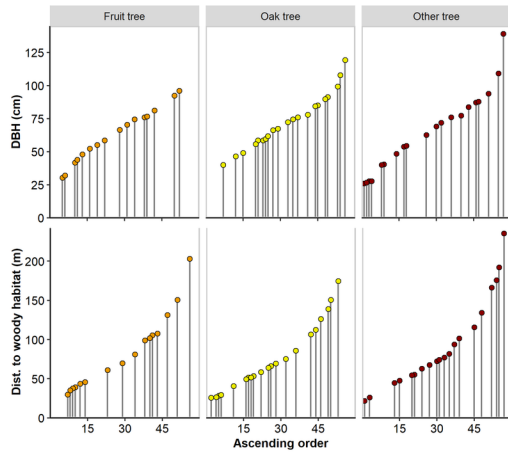
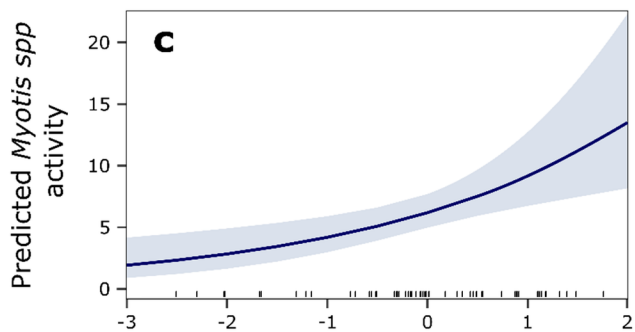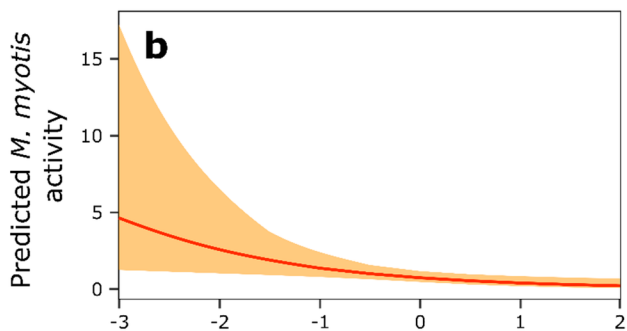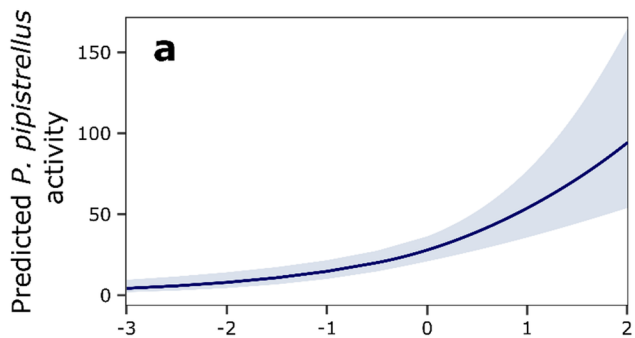| Variables	*Barbastella barbastellus*	Nyctaloids	*Pipistrellus pipistrellus*	*Pipistrellus nathusii/kuhlii*	*Myotis myotis*	Small *Myotis* spp.	*Plecotus* spp.	*Rhinolophus hipposideros*
Julian day	/	-0.25 ± 0.16 (-0.56, 0.05)	**0.35 ± 0.15 (0.04, 0.65)**	0.17 ± 0.17 (-0.16, 0.50)	/	**0.34 ± 0.11 (0.12, 0.55)**	0.18 ± 0.20 (-0.21, 0.57)	-0.37 ± 0.25 (-0.86, 0.12)
Temperature	0.50 ± 0.30 (-0.08, 1.08)	**0.28 ± 0.12 (0.03, 0.52)**	0.19 ± 0.17 (-0.14, 0.52)	/	**0.75 ± 0.23 (0.29, 1.21)**	**0.41 ± 0.12 (0.17, 0.65)**	0.16 ± 0.24 (-0.31, 0.62)	/
Tree size	0.37 ± 0.29 (-0.19, 0.92)	/	**0.60 ± 0.13 (0.34, 0.86)**	/	**-0.60 ± 0.23 (-1.06, -0.14)**	**0.39 ± 0.12 (0.15, 0.63)**	/	/
TreM-prey	/	/	/	/	/	/	/	0.15 ± 0.25 (-0.34, 0.63)
TreM-roost	-0.11 ± 0.30 (-0.71, 0.48)	0.23 ± 0.14 (-0.06, 0.51)	0.21 ± 0.16 (-0.09, 0.52)	0.19 ± 0.20 (-0.20, 0.58)	**0.84 ± 0.21 (0.43, 1.26)**	0.15 ± 0.12 (-0.08, 0.39)	/	/
Dist. woody edge	-0.78 ± 0.41 (-1.58, 0.02)	/	/	/	0.36 ± 0.26 (-0.14, 0.87)	/	**-0.63 ± 0.31 (-1.24, -0.03)**	-0.15 ± 0.29 (-0.72, 0.42)
Edge density	-0.60 ± 0.31 (-1.21, 0.01)[4]	/	**0.53 ± 0.19 (0.14, 0.92)**[4]	0.25 ± 0.23 (-0.19, 0.70)[1]	/	/	/	-0.49 ± 0.26 (-0.99, 0.02)[3]
% of forest	-0.36 ± 0.29 (-0.93, 0.21)[1]	**0.30 ± 0.14 (0.03, 0.57)**[1]	/	/	**-0.40 ± 0.22 (-0.82, 0.03)**[2]	-0.17 ± 0.18 (-0.52, 0.17)[1]	0.20 ± 0.28 (-0.35, 0.45)[1]	**0.48 ± 0.23 (0.04, 0.93)**[1]
SHDI	**0.95 ± 0.37 (0.22, 1.67)**[5]	**0.45 ± 0.16 (0.14, 0.76)**[5]	0.30 ± 0.20 (-0.10, 0.69)[4]	0.33 ± 0.21 (-0.08, 0.74)[1]	**0.72 ± 0.23 (0.28, 1.17)**[5]	0.21 ± 0.17 (-0.13, 0.55)[1]	0.41 ± 0.32 (-0.22, 1.04)[1]	-0.40 ± 0.27 (-0.92, 0.13)[2]
TreM:Dist. woody edge	**-1.23 ± 0.41 (-2.03, -0.43)**	/	/	/	/	/	/	**0.58 ± 0.24 (0.11, 1.05)**
TreM:Edge density	/	/	/	/	/	/	/	/
TreM:% of forest	/	0.24 ± 0.18 (-0.12, 0.60)	/	/	/	**0.53 ± 0.16 (0.22, 0.84)**[1]	/	/
TreM:SHDI	/	/	/	**0.65 ± 0.21 (0.23, 1.07)**[1]	/	/	/	/
R² marginal	0.79	0.50	0.55	0.48	0.68	0.63	0.42	0.43

Spatial scales: [1] 100 m radius scale, [2] 250 m radius scale, [3] 500 m radius scale, [4] 1000 m radius scale, [5] 2000 m radius scale.
TreM: tree-related microhabitats. SHDI: Shannon's diversity index calculated across all the reclassified habitat types.

Figure 1. (a) Map of the study area displaying the location of the 57 single, isolated trees in the grassland-dominated agricultural matrix. Tree species categories are represented with different colours (orange: fruit tree, yellow: oak tree, red: other tree), and woody habitat patches are displayed in green. (b) Tree species categories classed according to their diameter at breast height (DBH) and distance to the nearest woody habitat. (c) Examples of trees surveyed (left: cherry tree, centre: oak tree, right: linden tree).

Figure 2. Predicted bat responses to tree size with the 95% confidence interval (obtained from our best models): (a) predicted *P. pipistrellus* activity (i.e. number of bat passes per night); (b) predicted *M. myotis* activity; (c) predicted *Myotis* spp activity.

Figure 3. Predicted bat responses to the number of tree-related microhabitat types (TreM-roost: TreM that can be used as roosting sites; TreM-prey: TreM that can enhance prey abundance) with the 95% confidence interval within different landscape contexts: predicted (a) *Barbastella barbastellus* activity (i.e. number of bat passes per night) and (b) *Rhinolophus hipposideros* activity, at three distances between trees and the nearest woody habitat; (c) *Pipistrellus nathusii/kuhlii* activity at three levels of landscape heterogeneity calculated within 100 m radius around trees; and (d) *Myotis* spp. activity at three levels of forest proportion calculated within 100 m radius around trees. We investigated the effects of TreM type diversity on bat activity at specific values (mean, mean - SD, mean + SD) of the selected landscape metric. Bold frames represent significant slopes (i.e. 95% confidence interval not overlapping zero).

a

France — Doubs

Legend
- Fruit tree
- Oak tree
- Other tree
- Woody habitat

0 2 4 8 Kilometers

N

b

| Fruit tree | Oak tree | Other tree |

DBH (cm)

Dist. to woody habitat (m)

Ascending order

c

a

Predicted *P. pipistrellus* activity

b

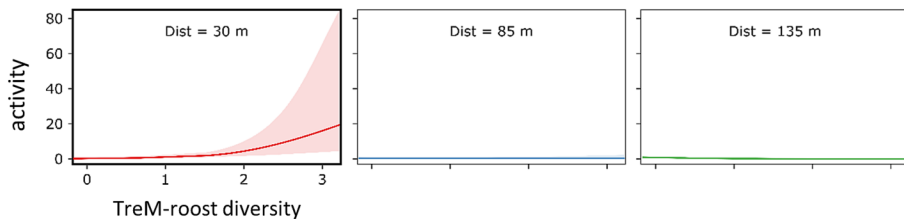Predicted *M. myotis* activity

c

Predicted *Myotis spp* activity

PCA axis

Small trees with small
DBH and crown area

Tall trees with large
DBH and crown area

Distance between trees and the nearest woody habitat (Dist.)
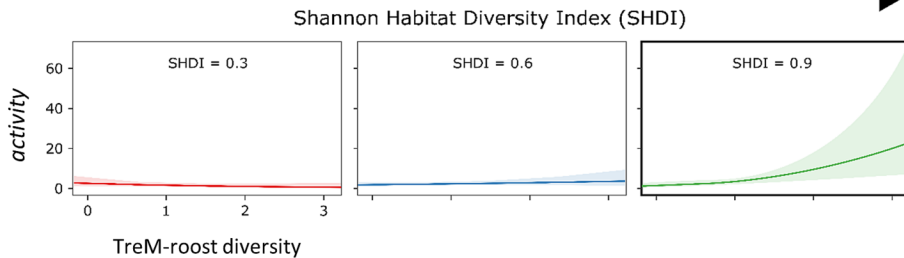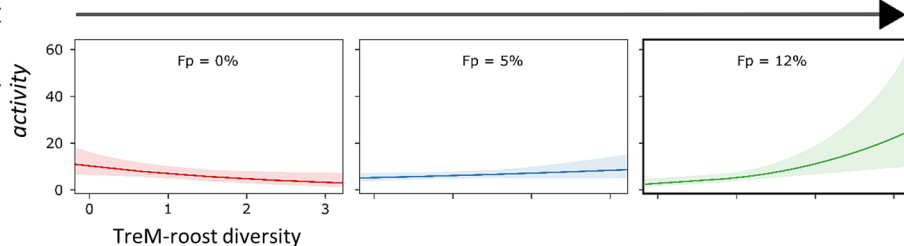
Predicted *B. barbastellus* activity

Dist = 30 m   Dist = 85 m   Dist = 135 m

TreM-roost diversity

a

Predicted *R. hipposideros* activity

TreM-prey diversity

b

Landscape compositional heterogeneity

Shannon Habitat Diversity Index (SHDI)
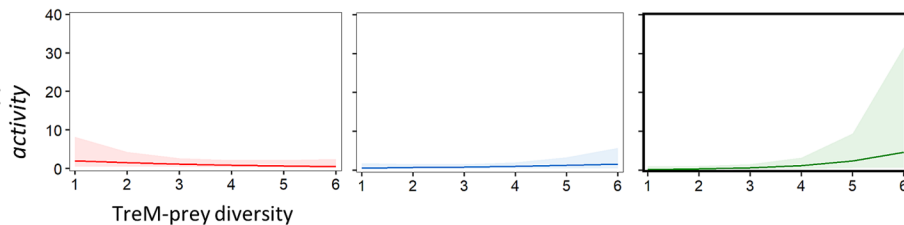
Predicted *P. nathusii/kuhlii* activity

SHDI = 0.3   SHDI = 0.6   SHDI = 0.9

TreM-roost diversity

c

Forest proportion (Fp)

Predicted *Myotis* spp. activity

Fp = 0%   Fp = 5%   Fp = 12%

TreM-roost diversity

d