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## TITLE

**Landscape context matters for attractiveness and effective use of road underpasses by vulnerable bat species**

## AUTHORS

Alexis Laforge<sup>a,b\*</sup>, Frédéric Archaux<sup>c</sup>, Yves Bas<sup>d,e</sup>, Nicolas Gouix<sup>a</sup>, François Calatayud<sup>b</sup>, Thomas Latgé<sup>a,b</sup>, Luc Barbaro<sup>b,d</sup>

## AUTHOR'S INSTITUTIONAL AFFILIATIONS

<sup>a</sup> Conservatoire d'Espaces Naturels Midi-Pyrénées, 75 voie du TOEC, BP 57611, 31076 Toulouse, France

<sup>b</sup> Dynafor, INRA-INPT, Univ. Toulouse, Auzeville, France

<sup>c</sup> Irstea, UR EFNO, Domaine des Barres, 45290 Nogent-sur-Vernisson, France

<sup>d</sup> Centre d'Ecologie et des Sciences de la Conservation, Museum national d'Histoire naturelle, CNRS, Sorbonne-Univ., Paris, France

<sup>e</sup> CNRS, PSL Research University, EPHE, UM, SupAgro, IRD, INRA, UMR 5175 CEFE, F-34293 Montpellier,

\*Correspondence: Laforge Alexis, INRA, UMR Dynafor 24 Chemin de Borde Rouge - Auzeville, F-31326 Castanet Tolosan, France. Email: alexis.laforge@inra.fr – Phone: +33(0)561285258

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### **DATA ACCESSIBILITY**

The acoustic data are hosted in the citizen science program “VigieChiro” site

(<https://vigiechiro.herokuapp.com/>).

1 **TITLE**

2 **Landscape context matters for attractiveness and effective use of road underpasses by**  
3 **bats**

4 **ABSTRACT**

5 The worldwide expansion of road networks is a major concern in biological conservation because of  
6 its predominantly negative effects on terrestrial fauna. Roads also affect bats, acting as barriers to  
7 movements and causing direct mortality by collisions with vehicles. Among wildlife crossing  
8 structures existing to maintain landscape connectivity, road underpasses are considered as one of the  
9 most effective conservation measure for bats. While a few studies assessed the effects of underpass  
10 attributes on bat use, none to date has assessed the impact of landscape context on underpass use and  
11 attractiveness. To address this knowledge gap, we monitored bat activity during three consecutive  
12 nights around 24 underpasses selected along a gradient of forest cover. We compared bat activity  
13 below and above underpasses (i.e., underpass use), at road sections with and without underpasses and  
14 at habitats adjacent to roads (i.e., underpass attractiveness). We found a significant positive effect of  
15 forest cover on both underpass use and attractiveness for *Myotis* spp and *Barbastella barbastellus*, and  
16 significant negative effects of distance to the nearest forest patch for *Rhinolophus* spp and hedgerow  
17 length for *Myotis* spp. Our study highlights the key influence of landscape context on road underpass  
18 efficiency to maintain landscape connectivity for bats. We advocate incorporating a landscape-scale  
19 approach in the decision-making process of underpass location during road project planning to  
20 enhance efficiency of such costly crossing structures.

21 **KEYWORDS**

22 Chiroptera; wildlife crossing structures; landscape connectivity; landscape permeability; conservation  
23 measure improvement

24

25

26

27

## 28 1. INTRODUCTION

29 Road network have expanded worldwide by 12 million km since 2000, and 25 million km of  
30 additional roads are expected by 2050 (Laurance et al., 2014). In line with the recent rise of road  
31 ecology issues in the field of biological conservation, there is now widespread evidence for overall  
32 negative effects of roads on biodiversity. These effects include direct mortality caused by vehicle  
33 collisions (Clevenger et al., 2003; Santos et al., 2011), habitat loss and fragmentation reducing  
34 landscape permeability and increasing barriers or filters to animal movement (Tucker et al., 2018).  
35 Consequently, individual animals may not be able to access critical food resources, breeding grounds  
36 or hibernacula. This may reduce population size and movements between populations, leading to a  
37 stronger inbreeding (Brehme et al., 2013; de Oliveira et al., 2011; Seidler et al., 2015; Tucker et al.,  
38 2018). Studies have evidenced that animal abundance and diversity are generally lower on both sides  
39 of roads where environmental conditions are strongly affected by the road (Clarke et al., 2013; Forman  
40 and Deblinger, 2000; Martin et al., 2018; Nafus et al., 2013; Northrup et al., 2012). Survival, foraging  
41 and breeding success of various animal taxa are also influenced by the proximity to roads (Halfwerk et  
42 al., 2011; Lukanov et al., 2014; Luo et al., 2015; Meillère et al., 2015; Tennessen et al., 2014).  
43 Increasing the density of road networks can therefore isolate populations, disconnect vital resources,  
44 cause severe habitat loss and ultimately impede population persistence for a wide range of taxa,  
45 including mammals, amphibians, reptiles, birds and invertebrates (Bennett, 2017; Ward et al., 2015).

46 In their seminal review, Fahrig and Rytwinski (2009) found that species with a particular  
47 combination of life traits, i.e., low reproductive rate, long life-span, high daily mobility and a  
48 sensitivity to noise/light pollution are the most vulnerable to landscape fragmentation and mortal  
49 vehicle casualties associated with roads. Bats typically share all these traits and attributes, so that they  
50 are likely to be particularly impacted by road network. Several factors explain why bat activity and  
51 species richness are lower on, or close to, roads (Berthinussen and Altringham, 2012a; Kitzes and  
52 Merenlender, 2014; Medinas et al., 2019) : (i) collisions with motor vehicles may significantly  
53 contribute to overall bat mortality (Fensome and Mathews, 2016; O'Shea et al., 2016); (ii) food  
54 resources of bats may be depleted along roads (Martin et al., 2018); and (iii) road traffic noise masks  
55 echolocation calls of species such as *Myotis myotis* and *Myotis daubentonii*, which reduces their

56 foraging efficiency and forces them to avoid roadside habitats when foraging (Luo et al., 2015; Schaub  
57 et al., 2008). Finally, artificial light is another road-related pollution which can negatively impact bat  
58 occurrence and activity (Azam et al., 2016; Hale et al., 2012), alter movement behavior - bats avoiding  
59 the illuminated areas - and hence decrease overall landscape connectivity (Hale et al., 2015; Kuijper et  
60 al., 2008; Laforge et al., 2019; Stone et al., 2009). Taken together, these impacts make roads an  
61 important driver of landscape fragmentation for bats, which can impact population viability and thus  
62 deserve routine consideration during road construction (Fahrig and Rytwinski, 2009).

63 To reduce impacts of roads and maintain functional landscape connectivity, several conservation  
64 measures aiming at crossing roads in safe conditions are already implemented worldwide. These can  
65 be divided in two categories: (i) underpasses such as bridges and culverts and; (ii) overpasses such as  
66 bat gantries and hop-overs (Claireau et al., 2018; Glista et al., 2009; Møller et al., 2016). Overpasses  
67 are specifically built to facilitate bat movements, while underpasses are generally set for drainage or  
68 human activities (minor roads, agricultural tracks or hiking paths for instance) but are favorable to a  
69 many taxa including bats. Underpasses have been shown to be effective at maintaining bat commuting  
70 routes and road permeability (Dekker et al., 2016; Møller et al., 2016) and seem more effective than  
71 overpasses (Abbott et al., 2012a, 2012b; Bach et al., 2004; Claireau et al., 2018).

72 Most previous works on underpasses compared bat activity above and below the roads to quantify  
73 bat underpass use. Underpasses are more used by bat species flying at low heights with high  
74 maneuverability, such as clutter-adapted and foliage-gleaning species by opposition to hawkers, open  
75 aerial foragers and edge-adapted species (Abbott et al., 2012a; Bhardwaj et al., 2017). However, this is  
76 not always true and underpasses close to hedgerows and natural corridors can be used by virtually all  
77 bat guilds (Abbott et al., 2012a; Berthinussen and Altringham, 2012b). So far, experiments carried out  
78 on underpass use by bats mainly focused on how attributes of road underpasses could influence their  
79 efficiency. Hence, among underpasses, bridges seem to be more efficient than culverts (Abbott et al.,  
80 2012a; Bhardwaj et al., 2017), and underpass width and height, but not length, are important for  
81 determining their use by bats (Abbott et al., 2012a; Bhardwaj et al., 2017; Boonman, 2011).

82 It is not enough to evaluate the effectiveness of underpasses solely through their use but also  
83 through their attractiveness, which is a key element often missing in previous assessments. For

84 example, an underpass can be effective in reducing road collision risk but only for a negligible part of  
85 a given bat population crossing roads daily. In addition, an underpass could be attractive but not  
86 actually used, and could thus act as a potential ecological trap by attracting more bats in the vicinity of  
87 the roads and increasing the collision risk. Only Abbott et al., (2012a) compared bat activity at roads  
88 with and without underpasses, showing that underpasses can have a certain attractiveness, i.e., bat  
89 activity was higher at roads with underpasses than at roads without underpasses.

90 Most studies that investigated the use of underpasses by bats only considered local factors such as  
91 dimensions, type of structure or presence of corridors without considering variation in landscape  
92 context (Bennett, 2017). This is a major concern for bat conservation, since the efficiency of an  
93 underpass to facilitate individual movements and overall landscape permeability is expected to depend  
94 not only on its local characteristics, but also on its location in a given landscape (Abbott et al., 2012a;  
95 Bennett, 2017). Landscape context is known as a key factor influencing the spatial variations of bat  
96 casualties by vehicles (Gaisler et al., 2009; Lesiński, 2008, 2007, Lesiński et al., 2011a, 2011b;  
97 Medinas et al., 2013), and of bat activity at road verges (Medinas et al., 2019). Furthermore, bat  
98 activity and consequently road collision risk is higher when multiple roads cross high quality habitats  
99 such as forests, wetlands, streams or hedgerows (Roemer, 2018). Bat community composition, activity  
100 and species diversity are expected to change with the proportion of key wooded habitats for bat  
101 roosting, foraging and commuting, such as forests and hedgerows, occurring at the landscape scale  
102 (Arroyo-Rodríguez et al., 2016; Charbonnier et al., 2016; Fuentes-Montemayor et al., 2017; Heim et  
103 al., 2015).

104 Finally, the relationships between bats and landscape contexts are species/guilds dependent  
105 (Ducci et al., 2015; Mendes et al., 2016) which is also the case between bats and their response to  
106 roads and underpasses (Abbott et al., 2012a, 2012b; Bhardwaj et al., 2017; Kerth and Melber, 2009;  
107 Medinas et al., 2019, 2013). Thus, it is expected that landscape context will influence differently the  
108 use and attractiveness of underpasses depending on the species or guilds.

109 The main objective of our study was thus to evaluate how the landscape context, and especially  
110 the amount of forests and hedgerows in the surrounding landscape, change underpass use and  
111 attractiveness for bats, irrespective of local underpass attributes. More precisely, we tested the

112 predictions that (i) road underpass attractiveness for bats would decrease with increasing forest cover  
113 and total hedgerow length because a more wooded landscape would reduce the need for bats to cross  
114 roads when commuting between roosting and foraging sites; (ii) bat use of road underpasses would  
115 increase with surrounding forest cover and total hedgerow length because of higher abundance of  
116 clutter-adapted and gleaning bat species, which are more reluctant to cross roads in open air; and (iii)  
117 underpass attractiveness and use would be more influenced by landscape attributes for species that are  
118 highly dependent on tree elements to commute because of their low and slow flights (i.e., gleaners and  
119 clutter-adapted species) while local features would be more influential for aerial hawkers. We expect,  
120 by conducting analyses per species and group of species, to precisely evaluate which landscape  
121 features help the most species to use underpasses, therefore improving the success in future  
122 conservation and land-management plans.

123

## 124 **2. MATERIALS AND METHODS**

### 125 2.1 Study area

126 We conducted our study in the Occitanie region (N 43.70, E 1.56), south-western France. The  
127 study area has a predominantly temperate climate with diverse influences (Atlantic, Continental,  
128 Mediterranean and mountainous). We monitored bat activity along four main roads: N124 (average  
129 daily traffic in 2015: 12,128; heavy vehicles: 8.1%), N21 (7,923; 10.15%), N126 (10,957; 9.35%),  
130 A68 (24,470, 8%) and N88 (20,933; 8.5%; see Fig. 1 and Table 1). Information on the average daily  
131 traffic was obtained from the database of “Direction Interdépartementale des Routes du Sud-Ouest”  
132 (DIRSO), a government agency in charge of monitoring and maintenance road structures  
133 (<http://www.dir.sud-ouest.developpement-durable.gouv.fr>). The roads are surrounded by mosaic  
134 agricultural landscapes (crops, vineyards, pastures) including patches of grasslands and woodlands of  
135 various sizes, tree-lined hedgerows, rivers, minor roads, scattered rural housing and small-medium  
136 conurbations with an elevation of <400m a.s.l. (Fig. 1). In comparison with previous studies on  
137 underpass use by bats in Northern Europe (Abbott et al., 2012a, 2012b; Bach et al., 2004; Boonman,  
138 2011), our study area has a less dense road network with more preserved landscapes and hosts a higher  
139 bat diversity with 27 bat species recorded at the regional scale (Bodin et al., 2011).



140

## 141 2.2 Underpass selection procedure

142 To select underpasses, we used the DIRSO database encompassing all geolocated underpasses in  
143 the study area (n=551). We then defined a ‘landscape’ as the area within a 1 km radius around each  
144 underpass. We used this spatial extent based on a trade-off between mean daily movements of bat  
145 species occurring in the study area (about 1–3 km) and the need to maximize the landscape gradient  
146 we targeted to sample (Bodin et al., 2011; Ethier and Fahrig, 2011; Pasher et al., 2013). Furthermore,  
147 it has been shown on other taxa that the cumulative ecological effect of the road system on  
148 biodiversity at landscape scale (i.e., the road effect-zone) can extend 800 m (Forman, 2000; Forman  
149 and Deblinger, 2000). To eliminate potential landscape-scale confounding factors, we reduced the  
150 candidate set of underpasses by selecting landscapes which had small areas of impervious surface  
151 (<20%; buildings and paved areas such as roads, sidewalks, driveways and parking lots) and of water  
152 surface (<10%) (Ethier and Fahrig, 2011). This step reduced the set of underpasses (N=213), for which  
153 we calculated forest cover for each landscape (as predictor of interest used to stratify our sample of  
154 underpasses) and categorized this predictor into three classes: 0-15%, 15-30%, 30-45% (Fig.1). In the  
155 study area, interior and edges of forests provide the most important foraging and commuting areas for  
156 both gleaner/clutter-adapted bat species (*Myotis* spp., *Plecotus* spp., *Rhinolophus* spp.) and for edge-  
157 adapted species (*Pipistrellus* spp., *Eptesicus serotinus*, *Barbastella barbastellus*). They also represent  
158 tree roosting areas for many aerial hawking species (Cel’uch and Kropil, 2008; Müller et al., 2013;  
159 Plank et al., 2012). In addition, we further carried out field validation of the selected underpass  
160 dimensions and entrance cluttering (which reduced the set of underpasses to N=68). To ensure that all  
161 sampled underpasses could be used by the whole bat community, we selected underpasses with  
162 entrance dimensions of at least two meters in width and height, following previous studies (Abbott et  
163 al., 2012a, 2012b; Bach et al., 2004; Berthinussen and Altringham, 2012b; Bhardwaj et al., 2017;  
164 Boonman, 2011). For each underpass, we calculated 11 environmental predictors: 4 local predictors  
165 (habitat within underpasses, width and height of the underpasses and presence or absence of  
166 hedgerows at least at one entrance of the underpasses), one road-related predictor (number of lanes on  
167 the road above the underpasses) and 6 landscape predictors (forest proportion, forest contrast as the

168 difference of forest cover between the two demi-buffers at both sides of the roads above the  
169 underpasses, hedgerow length, hedgerow contrast as the difference in hedgerow length between the  
170 two demi-buffers at both sides of the roads above the underpasses, distance between underpass and the  
171 nearest water body and distance between underpass and the nearest forest patch; see Table 1). This  
172 selection procedure finally reduced the set of candidate underpasses to 24 (mean distance between  
173 sites:  $84.4 \pm 55.3$  km; Fig. 1). Mostly because we excluded urban landscapes during underpass  
174 selection procedure, none of our studied underpasses had street lights above, around or inside. We  
175 found bat roosting inside only one studied underpass ( $\approx 15$  *Pipistrellus* spp), that was consequently  
176 deleted from the data set to avoid bias in estimating underpass use for the *Pipistrellus* spp. All the  
177 landscape predictors were calculated with ArcGis 10 (ESRI, Redlands, CA) based on land use data  
178 from the French National Institute for Geographic and Forestry Information (<http://www.ign.fr/>). The  
179 land use data were obtained by photo-interpretation to an accuracy ranging from 1 to 30 m spatial  
180 resolution depending on the type of habitat sampled.

181

### 182 2.3 Sampling design

183 To test our two predictions (i.e., forest cover and hedgerow length would decrease underpass  
184 attractiveness but increase underpass use), we first assessed if the presence of an underpass attracted  
185 bats in comparison with neighboring road sections without an underpass. As the attractiveness of an  
186 underpass could also depend on the habitat crossed by the underpass, we compared bat activity within  
187 underpasses with bat activity in the same habitat adjacent to the road. Moreover, a higher  
188 attractiveness for a given underpass does not necessarily mean a more frequent use by bats, so that we  
189 built a sampling design and calculated ad hoc metrics to take this into account (Fig. 2).

190 Bat activity was recorded with autonomous ultrasound recorders (Batlogger A, Elekon AG,  
191 Lucerne, Switzerland). Each recorder was calibrated to automatically trigger in reaction to any sound  
192 whose frequency was between 8 and 192 kHz and with a signal-to-noise-ratio level above 6 dB  
193 (Claireau et al., 2019a). Five recorders were deployed at four different locations in the landscape for  
194 each underpass (Fig. 2). To assess the attractiveness, two recording points were settled 200 m away  
195 from each other and from studied underpasses to avoid recordings of same bat calls at multiple

196 recorders: one recording point on the road edge without underpass and one in the same habitat present  
197 within underpass (Fig. 2). Three recording points were dedicated to evaluating the underpass use: one  
198 within the road structure in the middle of the underpasses and two on both road edges above the  
199 underpasses (Fig. 2). As Batloggers are omnidirectional recorders and therefore cannot distinguish  
200 between bats effectively crossing roads and bats simply active in the vicinity of the roads, our  
201 sampling design provided the proportion of bats using the underpasses from those in proximity to the  
202 underpasses (real road-crossing above underpasses or not).

203

#### 204 2.4 Bat recording and identification

205 We simultaneously surveyed three underpasses from at least two different forest classes  
206 (i.e., 15 deployed detectors) for three consecutive full nights. Each recorder was programmed to start  
207 recording ultrasound calls half an hour before sunset and to end half an hour after sunrise. The  
208 sampling was carried out between 1 July and 13 August 2018, corresponding to (i) the seasonal peak  
209 of bat species activity in the study region, as recommended by the French national bat-monitoring  
210 program ‘Vigie-Chiro’ (<http://www.vigienature.mnhn.fr/>), (ii) the parturition time which is an  
211 important period for conservation of bats and (iii) one of the seasonal peaks in bat mortality from  
212 vehicle collisions (Fensome and Mathews, 2016; Medinas et al., 2013). Recordings were only  
213 performed when there was no rain, the wind was below 30 km/h and the ambient temperature above  
214 12 °C. If we had at least one night with more than two hours of rain, we started again the three days of  
215 recording.

216 We identified bat call sequences to species using an acoustic automatic classifier software  
217 *Tadarida* (Bas et al., 2017). Then, we used Syrinx software version 2.6 (Burt, 2006) to manually check  
218 the identification of the bat passes from the classifier at the genus level. To make identification as  
219 unambiguous as possible and to make error risk negligible, we considered groups of similar species,  
220 mostly based on their echolocation call types, which greatly limits potential misidentification issues  
221 (Millon et al., 2015). *Rhinolophus* spp, *Plecotus* spp and *Myotis* spp are considered as gleaners  
222 foraging in clutter environments (Arlettaz et al., 2001). Despite the different genus, we opted for  
223 creating a composite group of *Pipistrellus/Miniopterus* spp whose echolocation calls are very similar

224 (Medinas et al., 2019). *Pipistrellus/Miniopterus* spp are considered as aerial hawkers foraging mostly  
225 on flying preys in open spaces (Dietz et al., 2009; Holderied and von Helversen, 2003). We conducted  
226 analyses at the species level only for *Barbastella barbastellus*, a typical edge-foraging species which  
227 produces very distinctive calls (Obrist et al., 2004).

228 For each group of species, we defined the response variable as the number of bat passes during  
229 three consecutive nights (named ‘bat activity’), where one bat pass was defined by one or several  
230 echolocation calls during a 5 second interval (see e.g., Azam et al., 2016). This time interval is  
231 considered as a good compromise in regard to the mean duration of all bat species passes (Kerbiriou et  
232 al., 2018).

233 Using Syrinx software version 2.6 (Burt, 2006), we also checked and evidenced that very few  
234 echolocation calls were simultaneously detected above and within the underpasses (<0.02% of the  
235 total bat passes for a given group of species) and that this negligible overlap only concerned  
236 *Rhinolophus* spp and *Barbastella barbastellus*.

237

## 238 2.5 Data analysis

239 We modelled the activity for each bat group separately, using Generalized Linear Mixed Models  
240 (GLMMs). Models were fitted using a negative binomial error distribution with a log link function to  
241 take into account the over-dispersion of our data (Zuur et al., 2009). Underpass attributes, road  
242 characteristics and landscape predictors were included in these models as fixed factors while  
243 ‘underpass’ was included as a random effect to account for the non-independence of the five spatial  
244 replicates among each underpass. To assess differences in bat activity between locations of recording  
245 points, we used ‘location’ as an extra predictor: H (Habitat), UI (Underpass Inferior), US (Underpass  
246 Superior), R (Road), see Fig. 2. In the models, we assigned two different weights depending on the  
247 location of the recording points: 1 for H, UI, R (always one recording point of each location in the  
248 landscapes) and 0.5 for US (always two recording points of this location in the landscape, see Fig. 2).  
249 Among our 11 predictors (Table 1), all the continuous variables were centered and rescaled (with a  
250 mean of 0 and a standard deviation of 1) to achieve a set of unit-free predictors that are directly  
251 comparable and to aid model fitting (Ashrafi et al., 2013). Then we used a forward stepwise procedure

252 to identify the subsets of predictors with the highest performance, and further select the best final  
 253 model according to its best fit to the data using the Akaike Information Criterion corrected for small  
 254 samples (AICc) (Mac Nally et al., 2018). We assessed the quality of our best models by comparing it  
 255 to the null model using AICc and the likelihood-ratio chi-squared test (Mac Nally et al., 2018).

256 To assess underpass use and attractiveness for each group of species, we compared bat activity  
 257 levels between the different locations of recording points in the landscape. Hence, at each step of the  
 258 model selection procedure, we ran the models twice: (1) once with the predictors in interaction with  
 259 the factorial variable “location” and (2) then again with all the same predictors in addition to  
 260 “location” as follows:

261 (1) *Bat activity ~ location \* environmental predictors + 1|site*

262 (2) *Bat activity ~ location + environmental predictors + 1|site*

263 This also allowed us to dissociate the predictors that influence bat activity differently between  
 264 locations (interactive effect) from the predictors that affect bat activity similarly between locations  
 265 (additive effect). To avoid multicollinearity of predictors in the models, we evaluated the correlations  
 266 between variables using Pearson’s coefficient to detect obvious correlations (Zuur et al., 2009). Only  
 267 variables with correlation coefficients between -0.70 and 0.70 were included simultaneously in the  
 268 models (Dormann et al., 2013). All the statistical analyses were carried out in R (v3.5.1; R  
 269 Development Core Team, 2011) using packages “glmmTMB” and “DHARMA”.

270 To assess the use and attractiveness of underpasses, we applied two different ratios (symmetric around  
 271 0) adapted to our sampling design:

$$272 \quad \text{Underpass use} = \frac{p_{UI} - p_{US}}{p_{UI} + p_{US}}$$

273

$$274 \quad \text{Underpass attractiveness} = \frac{\frac{p_{UI} + p_{US}}{2} - p_{H \text{ (or } p_R)}}{\frac{p_{UI} + p_{US}}{2} + p_{H \text{ (or } p_R)}}$$

275 where  $p$  is the predicted activity of bats from models for each locations (UI; US; H or R). The  $\pm$   
 276 95 % confidence intervals of the ratios have been calculated from 100,000 bootstrap iterations (Manly,  
 277 1997), based on the values of the estimates and the variance-covariance matrix of the GLMMs.

278 Differences in bat activity among location of recording points and the response curves of the  
279 predicted activity for each location used to calculate the ratios are presented in Appendix A and B,  
280 respectively (see Table 2 for ratio interpretations). A threshold of 10% was used to define the range of  
281 values considered as ecologically irrelevant (i.e., indicating no significant difference in bat activity;  
282 see Abbott et al 2012a).

283

### 284 3. RESULTS

#### 285 3.1 Total bat activity and species recorded

286 We recorded 114,618 bat passes at 111 recording points (9 recording points failed) spread over 24  
287 landscapes/underpasses. The group *Pipistrellus/Miniopterus* spp represented 90.6% of the total bat  
288 passes and was recorded at 100% of recording points, these values were respectively 6.1% and 89.0%  
289 for *Myotis* spp, 1.0% and 57.6% for *Barbastella barbastellus*, 0.4% and 54.9% for *Rhinolophus* spp  
290 and 0.3% and 60% for *Plecotus* spp.

#### 291 3.2 *Myotis* spp

292 Forest cover and hedgerow length had significant negative effects on the attractiveness of  
293 underpasses in comparison with adjacent habitats for *Myotis* spp: along the gradients, the ratios  
294 decreased respectively from 0.6 to -0.2 for forest cover and from 0.9 to -0.7 for hedgerow length (Fig.  
295 3). From 3% to 28% of forest cover in the surrounding landscape, *Myotis* spp activity was significantly  
296 higher at roads with underpasses than at adjacent habitats. However, their activity reversely became  
297 higher at adjacent habitats than at underpasses from 38% of forest cover onwards (Fig. 3). By contrast,  
298 *Myotis* spp were more active at underpasses only when landscapes included between 6 and 15 km of  
299 hedgerows and became more active at adjacent habitats beyond 17 km of hedgerows (Fig. 3).

300 For *Myotis* spp, road sections with underpasses were always more attractive (i.e., roads with  
301 underpasses displayed more activity) than adjacent road sections without underpasses (i.e., R points)  
302 along the gradients of forest cover and hedgerow length (Fig. 3). While the attractiveness of road  
303 sections with an underpass increased with forest cover (from 55% to 85% of bat passes recorded at  
304 road sections with an underpass), it decreased with hedgerow length from 90% to 55% (Table 3 and  
305 Fig. 3).

306 *Myotis* spp were always significantly more active inside than in the vicinity of underpasses along  
307 the respective gradients of forest cover and hedgerow length (Fig. 3). Landscape-scale forest cover  
308 increased underpass use by *Myotis* spp (from 50% to 70%) but hedgerow length decreased it (from  
309 80% to 20%; Fig. 3). For *Myotis* spp, underpass use and attractiveness were not affected by local  
310 predictors. However, the habitat within the underpasses impacted significantly the total activity (all  
311 recorded points combined) of *Myotis* spp, which ranged from the highest to lowest in the following  
312 order: river under bridge > river under culvert > track > road (Fig. 5).

### 313 3.3 *Rhinolophus* spp

314 For horseshoe bat, distance between underpass and the nearest forest patch decreased the  
315 attractiveness in comparison with adjacent habitats but did not affect attractiveness in comparison with  
316 road sections without underpass (Fig. 3). Underpasses height did not affect attractiveness in  
317 comparison with adjacent habitats but slightly increase attractiveness in comparison with adjacent road  
318 sections without underpasses (Fig. 4).

319 Distance to the nearest forest patch strongly decreased underpass use by *Rhinolophus* spp (from  
320 0.9 to -1.0), which moreover used preferentially underpasses when located at a distance not exceeding  
321 260 m from the nearest forest patch (Table 3 and Fig. 3). Between 260 m and 600 m from the nearest  
322 forest patch, horseshoe bat activity was higher around underpasses than within it (Fig. 3).

323 At local scale, underpass height had a significant negative effect on their use by horseshoe bats  
324 (the predicted ratio decreasing from 0.8 to -0.4; Fig. 4). It is predicted that most horseshoe bats would  
325 use underpasses preferentially when their height lies between 2.5 and 4.7 m (Fig. 4). As for *Myotis*  
326 spp, the habitat within the underpasses impacted significantly the total activity of *Rhinolophus* spp  
327 which is the highest when there was a river under a bridge (Fig. 5).

### 328 3.4 *Barbastella barbastellus*

329 For barbastelle bat, forest cover significantly increased underpass attractiveness in comparison  
330 with adjacent habitats (from -0.4 to 0.4; Table 3 and Fig. 3). Barbastelle bat activity was higher at  
331 adjacent habitats when forest cover was between 3 and 28% while the reverse was found in landscapes  
332 with more than 32% of forest (Fig. 3).

333 Landscape-scale forest cover also increased underpass attractiveness in comparison with road  
334 sections without underpass (from 0.0 to 0.6; Table 3 and Fig. 3). We did not find a significant  
335 ecologically relevant difference in barbastelle bat activity between road sections with or without  
336 underpass, in landscapes with forest proportion ranging between 3 and 18%. Beyond 18% of forest in  
337 the surrounding landscape, barbastelle bats were more active around underpasses than at road sections  
338 without underpass.

339 Landscape-scale forest cover increased underpass use by *B. barbastellus* (from -0.85 to 0.5; Fig.  
340 3), which only used preferentially underpasses in landscapes with more than 30% of forest (Fig. 3).  
341 For this species, underpass use and attractiveness were not affected by local predictors.

### 342 3.5 *Plecotus* spp

343 Landscape context did not affect underpass use and attractiveness for *Plecotus* spp, whose total  
344 activity decreased significantly with distance to the nearest water body. Long-eared bat activity also  
345 increased significantly with the contrast in forest cover between the two sides of the underpasses/roads  
346 (Appendix C).

347 At local scale, the number of road lanes above the underpasses did not significantly influence  
348 underpass attractiveness for long-eared bats when compared with both adjacent habitats and road  
349 sections without underpasses (Fig. 4). By contrast, long-eared bats used underpass more when the  
350 number of lanes was higher (ratio increasing from -0.9 to 0.7): they tended to avoid underpass interiors  
351 below four road lanes and used preferentially underpasses beyond four lanes (Fig. 4).

### 352 3.6 *Pipistrellus/Miniopterus* spp

353 Landscape context significantly influenced neither underpass use nor attractiveness for the  
354 *Pipistrellus/Miniopterus* group (Table 3). However, the habitat within the underpasses impacted  
355 significantly the total activity of this group of species: it was highest when there was a river under a  
356 bridge (no significant difference with tracks) and the lowest when there was a road (bridge and culvert  
357 combined) or a river under a culvert (Fig. 5).

358 For all the relationships detailed above, the AICc value of the best model was at least 14.8 points  
359 lower than the corresponding null model and likelihood-ratio chi-squared tests between the best and  
360 null models were systematically significant (Table 3).



361

## 362 4. DISCUSSION

### 363 4.1. Landscape context matters to determine underpass use and attractiveness for bats

364 Our study validates our predictions that taking into account the landscape context greatly  
365 enhances the road underpass use and attractiveness for bats, as previously shown for other road-  
366 sensitive taxa (Carr et al., 2002; Clewenger and Waltho, 1999). More precisely, the present study  
367 showed that landscape contexts conditioned the locations of bat commuting routes along and across a  
368 given road. This main result is consistent because landscape configuration is a key driver of bat  
369 movements through the ‘landscape complementation’ process allowing bats to access different non-  
370 substitutable critical resources across different habitats within the landscape mosaic (Dunning et al.,  
371 1992; Ethier & Fahrig, 2011). As expected, we found that the effects of landscape context on  
372 underpass use and attractiveness for bats are species and guild specific. More specifically, we found  
373 significant effects for gleaners and clutter-adapted species such as *Rhinolophus* spp and *Myotis* spp, as  
374 well as for *Barbastella barbastellus*, but not for *Plecotus* spp and *Pipistrellus/Miniopterus* spp. Forest  
375 cover, hedgerow length and distance to the nearest forest patch were the major landscape attributes  
376 significantly influencing bat attractiveness and use of underpasses, likely because wooded elements  
377 are often used by bats for commuting between roosting and foraging sites (Boughey et al., 2011;  
378 Downs and Racey, 2006; Entwistle et al., 1996; Nicholls and Racey, 2006; Russ and Montgomery,  
379 2002; Verboom and Huitema, 1997). Wooded landscape elements are also known to provide acoustic  
380 navigational landmarks for species with limited perceptual range using echolocation (Holderied et al.,  
381 2006; Jensen et al., 2005; Verboom and Spoelstra, 1999) but also important food resources (i.e.,  
382 insects), shelter from wind, roosting sites and protection against avian predators (Ekman and de Jong,  
383 1996). Forests are therefore key roosting and foraging habitats for most European bat species and  
384 greatly influence presence, abundance and diversity of bat communities at the landscape scale  
385 (Arroyo-Rodríguez et al., 2016; Charbonnier et al., 2016; Fuentes-Montemayor et al., 2017; Heim et  
386 al., 2015).

#### 387 4.1.1. Underpass attractiveness

388 Contrary to our prediction that road underpass attractiveness for bats would decrease with  
389 increasing forest cover and total hedgerow length, we found that forest cover increased underpass  
390 attractiveness in comparison with road sections without underpasses for *Myotis* spp and *B.*  
391 *barbastellus*. On the contrary, hedgerow length reduced this attractiveness for *Myotis* spp which is in  
392 line with our initial predictions. We thus suggest that increasing hedgerow density at the landscape  
393 scale provides more potential commuting routes for bats to cross a given road, limiting the  
394 attractiveness of underpasses. In addition, it is likely that changing commuting routes for safer  
395 crossing roads using new underpasses is less costly for bats in a forest-dominated matrix than in an  
396 agricultural matrix (Abbott et al., 2012b; Kerth and Melber, 2009; Krull et al., 1991).

397 Our finding that forest cover decreased road underpass attractiveness in comparison with adjacent  
398 habitats for *Myotis* spp but increased it for *B. barbastellus* is supported by a radio-tracking study by  
399 Kerth and Melber (2009). In a forest context, they found that a smaller proportion of *Myotis*  
400 *bechsteinii* effectively crossed the motorway in comparison with *barbastelle* bats. They thus suggested  
401 that in a forest habitat context, roads acted more as a barrier to foraging movements for *Myotis*  
402 *bechsteinii* than for *B. barbastellus*. Furthermore, previous findings also indicated that, in less forested  
403 landscapes including small forest patches, most *Myotis* individuals forage regularly outside forests,  
404 thus increasing their home range size and the probability of crossing roads during their nightly  
405 movements (Kerth et al., 2002). Other studies have shown that gleaning bat activity and species  
406 richness decreased when approaching roads (Berthinussen and Altringham, 2012a; Claireau et al.,  
407 2019; Kitzes and Merenlender, 2014; Zurcher et al., 2010). This seems particularly true in woodland  
408 habitats because, as interior forest foragers, gleaning bats tend to avoid the vicinity of roads with lower  
409 tree cover and lower prey abundance than in the adjacent habitats (Fensome and Mathews, 2016;  
410 Medinas et al., 2019). Thus, we assume that these specific *Myotis* activity patterns resulted from a  
411 combination of different ecological mechanisms, i.e., smaller home-ranges in forest contexts, road-  
412 related barrier effects, increased activity with distance to roads and higher prey abundance in adjacent  
413 habitats, all leading to fewer road crossing events for *Myotis* spp in a forest context. The decrease in  
414 underpass attractiveness in comparison with adjacent habitats with the increase in total hedgerow  
415 length for *Myotis* spp could also result from the same ecological mechanisms, since hedgerows are

416 known to be very important landscape features for foraging and commuting individual movements for  
417 these species (Dietz et al., 2009, 2013; Duvergé and Jones, 1994; Fonderflick et al., 2015; Froidevaux  
418 et al., 2017). In addition, in agricultural landscapes with few hedgerows left, roadside verges are often  
419 the only remnants of suitable semi-natural habitats providing corridors and foraging opportunities for a  
420 large number of taxa in the immediate roadside vicinity (Augusto et al., 2016; Davies and Pullin,  
421 2007; Penone et al., 2012; de Redon et al., 2015).

#### 422 4.1.2. Underpass use

423 In accordance with our prediction that bat use of road underpasses would increase with  
424 surrounding forest cover and total hedgerow length, we found that forest cover significantly increased  
425 the use of underpasses by *Myotis* spp and *B. barbastellus*. In landscapes with low forest cover, wooded  
426 elements are reduced to small patches or tree lines, where bats depend on edge effects between trees  
427 and open habitat matrix (and potentially so in the vicinity of underpasses) for foraging or commuting  
428 without the need of crossing a road, contrarily to forest landscapes with sharp edges acting as corridors  
429 (Roemer, 2018). Previous works studying the effect of landscape context on bat mortality (Lesiński,  
430 2008, 2007, Lesiński et al., 2011a, 2011b; Medinas et al., 2013), on bat collision risk related to vehicle  
431 collisions (Roemer, 2018) or on bat activity at road verges (Berthinussen and Altringham, 2012a;  
432 Medinas et al., 2019) have shown that bats are much more likely to cross roads (and therefore to use  
433 underpasses) in forest-dominated landscapes. These landscapes actually host more specialist bat  
434 species such as *Myotis* spp or *B. barbastellus*, both for roosting and foraging (Charbonnier et al., 2016;  
435 Dietz et al., 2009; Lesiński, 2007). Thus, even if the proportion of individuals flying in the vicinity of  
436 underpasses does not vary among different landscapes, an increase in forest cover would augment the  
437 proportion of individuals flying through road underpasses (Kerth and Melber, 2009; Lesiński, 2007).  
438 We also found that along the gradient of forest cover, and unlike *B. barbastellus*, the proportion of  
439 *Myotis* individuals crossing roads through underpasses was always higher than around underpasses,  
440 which was congruent with previous studies (Abbott et al., 2012a, 2012b). Most of the *Myotis* spp  
441 actually forage at low flight heights in forest understory whereas *B. barbastellus* is more an edge  
442 specialist (Dietz et al., 2009), for which it is likely that landscape contexts will modulate flight height  
443 in a greater extent than for *Myotis* spp (Müller et al., 2013; Roemer et al., 2017).

444 Contrarily to our initial predictions, we found that increasing the total length of hedgerows in the  
445 surrounding landscape decreased the use of underpasses for *Myotis* spp. Although counterintuitive,  
446 this observation could result from the fact that in presence of hedgerows, *Myotis* bats would fly higher  
447 and therefore use less frequently underpasses to cross roads. The configuration of adjacent matrix  
448 habitats, including the presence of hedgerows or small woods, actually influences bats' tendency to fly  
449 across open grounds such as roads (Abbott et al., 2012a). As expected, the distance between  
450 underpasses and the nearest forest patch also decreased significantly underpass use by horseshoe bats.  
451 This result is consistent with the fact that horseshoe bats are reluctant to fly in open fields most of the  
452 time (Dietz et al., 2009). It is also congruent with previous studies suggesting that increasing distance  
453 to surrounding woody elements decreased bat crossing frequency (Abbott et al., 2012a; Bennett and  
454 Zurcher, 2013; Roemer, 2018; Russell et al., 2009).

455

#### 456 4.2. Local road underpass attributes can influence their use and attractiveness for bats

457 Underpass use by *Rhinolophus* spp decreased with entrance height, in accordance with Abbott  
458 et al., (2012b). Horseshoe bats habitually mate or roost in caves and other underground structures  
459 during various parts of their life cycle and are thus presumably more confident to predation risk in  
460 narrow environments. They also display specific adaptations to use small underpasses in regard to  
461 their specific flight maneuverability and echolocation call types (Abbott et al., 2012b; Glover and  
462 Altringham, 2008; Mcaney, 1999). While underpass height had no effect on attractiveness compared  
463 to adjacent habitats for horseshoe bats, it slightly increased attractiveness in comparison with road  
464 sections without underpasses. This suggests that the largest underpasses concentrated more horseshoe  
465 bat individuals without improving the safety of road-crossings.

466 Long-eared bats used underpasses according to the number of road lanes above the structure,  
467 especially when roads included four lanes or more. Considering that the number of lanes on roads is a  
468 good proxy of traffic volume, such a result could indicate a negative response to an increasing traffic  
469 volume which is known to greatly change bat flight behavior (Zurcher et al., 2010). Bats actually  
470 avoid the zones with the high collision risk probably because they recognize the danger triggered by  
471 vehicles (Roemer, 2018). For instance, Zurcher et al., (2010) found that 60% of bats approaching a

472 road reversed their flight in the presence of a vehicle. Such a result could also come from a direct  
473 effect of road width: the wider a road is and the longer the time needed to cross and the higher the risk  
474 of vehicle collision or predation for low flying species such as *Plecotus* spp. The absence of similar  
475 activity patterns for other gleaning/low-flying species may be explained by *Plecotus* spp being able to  
476 cross more often roads without using an underpass in a greater extent of road dimension, while they  
477 would preferably use underpasses only at high traffic volume (Abbott et al., 2012b). Thus, our results  
478 tend to confirm that road avoidance behavior is bat guild-specific (Medinas et al., 2013).

479 Although the type of habitat within the underpasses did not influence their use and attractiveness,  
480 this environmental predictor had a significant effect on the total activity (all locations of recording  
481 points combined) of *Myotis*, *Rhinolophus* and *Pipistrellus/Miniopterus* species. We found that, for  
482 these three groups of species, the activity was highest when there was a river within the underpasses,  
483 an expected result since that wetlands are known to provide important food resources for bats (Korine  
484 et al., 2016; Straka et al., 2016). We also recorded more activity for these groups when there was an  
485 agricultural track rather than a minor road within the underpasses. We explain this by the fact that  
486 agricultural tracks provide more food resources through grassy track borders than roads. In addition,  
487 roads negatively impact bat activity through different effects (i.e., direct mortality, higher collision risk  
488 with vehicles, disturbance related to light and noise pollution, habitat and food resource loss) making  
489 them much more unsuitable than tracks.

490

#### 491 4.3. Implications for bat conservation

492 According to our results, we suggest that taking into consideration habitat composition and  
493 configuration at landscape scale should be done systematically in the earliest stages during road  
494 construction planning to identify the best locations to set up underpasses and optimize their efficiency  
495 for bat conservation purposes (Thorne et al., 2009; van der Ree et al., 2011).

496 Underpasses are the most attractive and used in the most forested landscapes for *B. barbastellus*  
497 and *Myotis* spp (except for the comparison with adjacent habitats for the latter group), in the  
498 agricultural landscapes with the lower hedgerow density for *Myotis* spp, and when the underpasses are  
499 the closest to a forest patch for *Rhinolophus* spp. Underpasses are therefore useful for bat conservation

500 not only in natural, forest-dominated landscapes, but also in human-transformed landscapes dominated  
501 by intensive agriculture. This has important consequences for bat conservation in the current context  
502 of worldwide biodiversity loss with agricultural intensification (Newbold et al., 2015). We especially  
503 showed that when underpasses are accurately located in landscapes, they provide a useful tool for  
504 maintaining landscape connectivity and reducing road collisions for threatened bat populations (Voigt  
505 and Kingston, 2016). As the underpasses studied here were already settled for at least four years (see  
506 Table 1), and bats are long-lived mammals with very good knowledge of their home-ranges (Dietz et  
507 al., 2009), we suggest that the patterns of bat underpass use and attractiveness observed during our  
508 study actually resulted both from a previous behavioral adaptation of individuals living around roads,  
509 as well as from previous mortality events occurring during road early establishment. Yet, bat  
510 individuals may need a sufficient time lapse to learn which commuting routes are the safest to cross a  
511 new road when established, especially in forest-dominated landscapes where bat species pool,  
512 including threatened species such as *Myotis* spp and *B. barbastellus*, is the richest compared to  
513 agricultural habitats (Abbott et al., 2012b, Charbonnier et al. 2016). In other words, it is not because  
514 underpasses in forest landscapes seem to be more efficient to enhance local bat mobility through road  
515 network, that the existing populations in these landscapes have not experienced a previous decline  
516 related to road construction (Zimmermann Teixeira et al., 2017). Furthermore, Roemer (2018) and  
517 Lesiński (2007) respectively showed that the proportion of bat low-height flights synonym of high  
518 collision risk with vehicles, and the incidence of road casualties were higher in the more forested  
519 contexts for most bat species. Thus, it should be preferable to limit road construction across  
520 continuous forests as much as possible, but if it is inevitable, the creation of underpasses should be  
521 systematic. Finally, the best conservation measure to improve connectivity for most bats as well as  
522 other taxa is to vary the dimensions of underpasses for a given road in different landscape contexts,  
523 with a particular attention to high-traffic road sections in large forests (Abbott et al., 2012b; Carr et al.,  
524 2002; Bhardwaj et al., 2017).

525 Previous studies have shown that roads and their verges can become ecological traps increasing  
526 the risk of road kills for some species (Bernes et al., 2016), leading some authors to recommend to  
527 create suitable habitats for bats away from roads (Berthinussen and Altringham, 2012b; Medinas et al.,

528 2019). According to our results, we could recommend to conserve hedgerows only when occurring  
529 close to underpasses in agricultural landscapes. Yet, it is likely that underpasses could act as an  
530 ecological trap or, at least, could increase the ecological trap effect of a road, especially in particular  
531 landscape contexts. As demonstrated in this study, underpasses can be largely attractive for bats, thus  
532 questioning the fact that, by enhancing landscape connectivity for bat movements, bat mortality would  
533 be systematically and significantly reduced. Furthermore, our results have shown that the same  
534 landscape or local features may have opposite effects on underpasses attractiveness and use.

535       Actually, the attractiveness of underpasses in comparison with adjacent habitats can depend on  
536 either a local effect of resource accumulation (Villemey et al., 2018), a barrier effect, or a convergence  
537 effect to use underpasses to access required habitats. Further studies including e.g., BACI experiments  
538 and long-term temporal monitoring of bat mortality, landscape genetics and acoustic trajectography,  
539 are now needed to assess the potential ecological trap effect created by the attractiveness of  
540 underpasses for bats (Carr et al., 2002; Claireau et al., 2019a), especially to assess the spatial extent at  
541 which underpass attractiveness acts. Despite using only two recording points per landscape to assess  
542 attractiveness, we already found significant effects which make our results likely conservative. In  
543 addition, the effectiveness of underpasses to reduce population-level road impacts and ensure their  
544 viability is still unknown but remains one of the fundamental measures of mitigation success,  
545 irrespective of how many individuals actually cross a road through an underpass (Carr et al., 2002; van  
546 der Ree et al., 2011, 2007). To improve conservation recommendations for road establishment and  
547 landscape planners, further empirical studies are also needed to quantify the separate effects of traffic  
548 mortality and landscape connectivity loss on population persistence (Carr et al., 2002). For instance,  
549 Jackson and Fahrig (2011) found, by simulations, that mitigation measures that minimize mortality on  
550 roads (such as fences) should more effectively promote genetic diversity and so population viability  
551 than measures that attempt to promote connectivity (such as underpasses).

552

## 553 **5. CONCLUSIONS**

554       Our study is the first to demonstrate the preponderant influence of landscape context on  
555 underpass efficiency to maintain landscape connectivity for bats, for a wide range of road dimensions.

556 By disentangling underpass use from attractiveness, we provide detailed knowledge on how building  
557 an underpass can impact bat activity and mobility in space, thus highlighting the importance of  
558 incorporating a landscape-scale approach in the decision-making process of underpass location during  
559 road project planning. We finally suggest that underpasses should be established in a wide range of  
560 landscapes with a particular attention on forest and intensive agricultural landscapes where they seem  
561 to be the most efficient to safely facilitate bat movements. Wooded elements such as forest patches  
562 and hedgerows are the key components of the landscape to be taken into account for road location  
563 planning scheme. Although underpasses are rarely built primarily for animal conservation, we  
564 advocate for searching the best trade-off between human and wildlife needs when selecting their  
565 optimal establishment in mosaic landscapes.



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## Table captions

**Table 1.** Summary of sampled underpasses, road attributes and landscape variables. Variable names in bold are predictors tested in the models. Hab\_under: habitat within the underpass ; Wide and Height refer to the dimensions of underpass entrances; Hedge: presence or absence of an hedgerow at one or two entrance(s) of the underpass; Forest contrast: difference in forest cover between the two demi-buffers at both sides of the road above the underpass; Hedgerow contrast: difference in hedgerow length between the two demi-buffers at both sides of the road above the underpass; Distance to water: distance between underpass and the nearest water body; Distance to forest: distance between underpass and the nearest forest patch.

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Underpasses (n = 24)								Roads				Landscape variables (Buffer 1 km)					
Site	Type	<b>Hab_under</b>	<b>Wide (m)</b>	<b>Height (m)</b>	Length (m)	<b>Hedge</b>	<i>Year of construction</i>	Road	<b>Wide (m)</b>	<b>Lanes</b>	Speed limit (km/h)	<b>Forest (%)</b>	<b>Forest contrast (%)</b>	<b>Hedgerow length (m)</b>	<b>Hedgerow contrast (m)</b>	<b>Distance to water (m)</b>	<b>Distance to forest (m)</b>
11	Culvert	<b>road</b>	<b>10</b>	<b>5</b>	35	<b>Yes</b>	<i>2001</i>	N124	20	<b>4</b>	110	<b>7.5</b>	<b>8.7</b>	<b>10792.1</b>	<b>2367.8</b>	<b>1046.0</b>	<b>118.0</b>
58	Culvert	<b>road</b>	<b>6</b>	<b>4.5</b>	15	<b>Yes</b>	<i>1992</i>	N126	14	<b>3</b>	80	<b>3.3</b>	<b>2.0</b>	<b>11913.5</b>	<b>704.8</b>	<b>134.0</b>	<b>265.0</b>
74	Culvert	<b>track</b>	<b>3.5</b>	<b>2.5</b>	40	<b>Yes</b>	<i>1995</i>	N126	12	<b>2</b>	80	<b>17.0</b>	<b>14.4</b>	<b>11815.9</b>	<b>2148.4</b>	<b>594.0</b>	<b>246.0</b>
110	Culvert	<b>river</b>	<b>4.5</b>	<b>3</b>	50	<b>Yes</b>	<i>2007</i>	N124	20	<b>4</b>	110	<b>11.7</b>	<b>9.2</b>	<b>21959.4</b>	<b>515.4</b>	<b>920.0</b>	<b>150.0</b>
120	Culvert	<b>river</b>	<b>7</b>	<b>4</b>	30	<b>Yes</b>	<i>1987</i>	A68	25	<b>4</b>	130	<b>12.5</b>	<b>4.6</b>	<b>10083.0</b>	<b>696.0</b>	<b>297.0</b>	<b>205.0</b>
123	Culvert	<b>river</b>	<b>4</b>	<b>2.5</b>	46	<b>Yes</b>	<i>1987</i>	A68	40	<b>5</b>	130	<b>27.1</b>	<b>5.9</b>	<b>7826.0</b>	<b>1814.6</b>	<b>690.0</b>	<b>583.0</b>
126	Culvert	<b>road</b>	<b>6.5</b>	<b>5</b>	40	<b>Yes</b>	<i>1991</i>	A68	35	<b>4</b>	130	<b>30.8</b>	<b>12.5</b>	<b>9865.8</b>	<b>300.4</b>	<b>315.0</b>	<b>327.0</b>
164	Culvert	<b>road</b>	<b>5</b>	<b>5.5</b>	20	<b>Yes</b>	<i>1999</i>	N124	12	<b>2</b>	80	<b>16.2</b>	<b>1.9</b>	<b>20329.6</b>	<b>4065.1</b>	<b>908.0</b>	<b>210.0</b>
176	Bridge	<b>river</b>	<b>6</b>	<b>3</b>	12	<b>Yes</b>	<i>1900</i>	N124	9	<b>2</b>	80	<b>12.0</b>	<b>2.1</b>	<b>19129.6</b>	<b>4483.3</b>	<b>954.0</b>	<b>365.0</b>
196	Bridge	<b>river</b>	<b>4</b>	<b>3</b>	18	<b>Yes</b>	<i>1846</i>	N21	8	<b>2</b>	80	<b>9.9</b>	<b>12.7</b>	<b>18207.7</b>	<b>1303.1</b>	<b>815.0</b>	<b>72.0</b>
201	Culvert	<b>road</b>	<b>8</b>	<b>4.5</b>	25	<b>No</b>	<i>1995</i>	N124	20	<b>4</b>	90	<b>30.7</b>	<b>25.6</b>	<b>6388.9</b>	<b>3673.2</b>	<b>1467.0</b>	<b>140.0</b>
218	Bridge	<b>river</b>	<b>3</b>	<b>3</b>	30	<b>Yes</b>	<i>1900</i>	N21	10	<b>2</b>	80	<b>11.9</b>	<b>4.8</b>	<b>12503.3</b>	<b>1342.8</b>	<b>1060.0</b>	<b>272.0</b>
225	Bridge	<b>river</b>	<b>4</b>	<b>5</b>	5	<b>Yes</b>	<i>1900</i>	N124	9	<b>2</b>	80	<b>18.7</b>	<b>14.8</b>	<b>14545.4</b>	<b>1393.5</b>	<b>1466.0</b>	<b>46.0</b>
311	Bridge	<b>river</b>	<b>15</b>	<b>6</b>	10	<b>Yes</b>	<i>1900</i>	N21	10	<b>2</b>	80	<b>33.5</b>	<b>16.1</b>	<b>10222.0</b>	<b>1665.5</b>	<b>1311.0</b>	<b>85.0</b>
464	Culvert	<b>track</b>	<b>8</b>	<b>5</b>	30	<b>No</b>	<i>2014</i>	N88	30	<b>4</b>	110	<b>9.9</b>	<b>4.7</b>	<b>15537.5</b>	<b>6416.8</b>	<b>1503.0</b>	<b>190.0</b>
471	Culvert	<b>track</b>	<b>6</b>	<b>4</b>	30	<b>No</b>	<i>2014</i>	N88	30	<b>4</b>	110	<b>4.3</b>	<b>7.7</b>	<b>16419.8</b>	<b>2244.9</b>	<b>310.0</b>	<b>224.0</b>
476	Culvert	<b>track</b>	<b>8</b>	<b>4</b>	38	<b>No</b>	<i>2014</i>	N88	30	<b>4</b>	110	<b>5.4</b>	<b>6.3</b>	<b>11903.2</b>	<b>4240.7</b>	<b>897.0</b>	<b>457.0</b>
485	Culvert	<b>track</b>	<b>5</b>	<b>4</b>	30	<b>Yes</b>	<i>2011</i>	N88	30	<b>4</b>	110	<b>16.2</b>	<b>19.3</b>	<b>12800.4</b>	<b>3608.0</b>	<b>1131.0</b>	<b>138.0</b>
487	Culvert	<b>river</b>	<b>5</b>	<b>3.5</b>	92	<b>Yes</b>	<i>2003</i>	N88	30	<b>4</b>	110	<b>17.2</b>	<b>10.9</b>	<b>10120.0</b>	<b>30.0</b>	<b>1013.0</b>	<b>112.0</b>
489	Culvert	<b>river</b>	<b>2.5</b>	<b>2.5</b>	100	<b>Yes</b>	<i>2003</i>	N88	30	<b>4</b>	110	<b>20.2</b>	<b>25.1</b>	<b>10950.6</b>	<b>420.3</b>	<b>1098.0</b>	<b>100.0</b>
494	Culvert	<b>river</b>	<b>3</b>	<b>3</b>	100	<b>Yes</b>	<i>2007</i>	N88	30	<b>4</b>	110	<b>14.1</b>	<b>10.8</b>	<b>12430.2</b>	<b>329.8</b>	<b>1532.0</b>	<b>140.0</b>
501	Culvert	<b>track</b>	<b>8</b>	<b>4</b>	32	<b>Yes</b>	<i>2003</i>	A68	25	<b>3</b>	110	<b>41.0</b>	<b>12.2</b>	<b>15813.8</b>	<b>5333.8</b>	<b>1539.0</b>	<b>150.0</b>
514	Culvert	<b>road</b>	<b>11</b>	<b>5</b>	30	<b>No</b>	<i>2009</i>	N124	20	<b>4</b>	110	<b>13.7</b>	<b>12.8</b>	<b>10807.1</b>	<b>2709.2</b>	<b>2787.0</b>	<b>83.0</b>
530	Culvert	<b>river</b>	<b>10</b>	<b>3</b>	34	<b>Yes</b>	<i>2007</i>	N126	15	<b>2</b>	80	<b>24.4</b>	<b>20.1</b>	<b>22044.6</b>	<b>2650.0</b>	<b>1869.0</b>	<b>208.0</b>
Mean	-	-	<b>6.4</b>	<b>3.9</b>	37.2	-	1979	-	21.4	<b>3.3</b>	100.4	<b>17.0</b>	<b>11.1</b>	<b>13517.0</b>	<b>2269.1</b>	<b>1069.0</b>	<b>203.6</b>
SD	-	-	<b>3.0</b>	<b>1.0</b>	25.7	-	48.5	-	9.6	<b>1.0</b>	18.1	<b>9.6</b>	<b>6.8</b>	<b>4301.7</b>	<b>1743.7</b>	<b>580.0</b>	<b>127.8</b>
Min	-	-	<b>2.5</b>	<b>2.5</b>	5.0	-	1846	-	8.0	<b>2.0</b>	80.0	<b>3.3</b>	<b>1.9</b>	<b>6388.9</b>	<b>30.0</b>	<b>134.0</b>	<b>46.0</b>
Max	-	-	<b>15.0</b>	<b>6.0</b>	100.0	-	2014	-	40.0	<b>5.0</b>	130.0	<b>41.0</b>	<b>25.6</b>	<b>22044.6</b>	<b>6416.8</b>	<b>2787.0</b>	<b>583.0</b>

**Table 2.** Interpretation of ratio values used to assess underpass use and attractiveness for bats.

<b>Ratio values</b>	<b>Use</b>	<b>Attractiveness compared to adjacent habitat</b>	<b>Attractiveness compared to unequipped road sections</b>
<b>1</b>	100% of individuals were recorded within underpasses but none above it	100% of individuals were recorded in the vicinity of underpasses but none in the adjacent habitat	100% of individuals were recorded at road sections with an underpass but none at unequipped road sections
<b>Between 0.1 and 1</b>	Significant bat preference for using underpasses	Significant underpass attractiveness for bats	Significant underpass attractiveness for bats
<b>Between -0.1 and 0.1</b>	No significant preference	No significant attractiveness	No significant attractiveness
<b>Between -0.1 and -1</b>	Significant bat avoidance of underpass interiors	Significant bat preference for adjacent habitat	Significant bat preference for unequipped road sections
<b>-1</b>	100% of individuals were recorded above underpasses but none within it	100% of individuals were recorded in the adjacent habitat but none close to underpass	100% of individuals were recorded at unequipped road sections but none at road sections with underpass

**Table 3.** Estimates  $\pm$  SE and  $\chi^2$  values from AIC-based selection of best models for each bat species groups and *Barbastella barbastellus* (\* =  $P < 0.05$ ; \*\* =  $P < 0.01$ ; \*\*\* =  $P < 0.001$ ). Estimates and standard errors were obtained from a multiple comparison test (ANOVA) and  $\chi^2$  values from a Wald test.

Scale	Predictors	<i>Myotis</i> spp		<i>Plecotus</i> spp		<i>Pipistrellus/</i> <i>Miniopterus</i> spp		<i>Rhinolophus</i> spp		<i>Barbastella</i> <i>barbastellus</i>	
		Estim. $\pm$ SE	Chisq	Estim. $\pm$ SE	Chisq	Estim. $\pm$ SE	Chisq	Estim. $\pm$ SE	Chisq	Estim. $\pm$ SE	Chisq
<b>Local</b>	Location of recording point	UI 5.66 $\pm$ 0.55***	49.14***	US 1.18 $\pm$ 0.54**	0.32	UI 7.65 $\pm$ 0.46***	27.90***	UI 2.52 $\pm$ 0.68***	18.69***	H 1.78 $\pm$ 0.49***	7.9*
	Habitat within underp.	RB 4.40 $\pm$ 0.41***	24.69***	-	-	RB 7.43 $\pm$ 0.36***	7.44*	RB 1.53 $\pm$ 0.44***	19.52***	-	-
	Height	-	-	-	-	-	-	-	10.02**	-	-
	Lanes	-	-	-	1.09	-	-	-	-	-	-
<b>Landscapes</b>	Hedgerow length	-	4.80*	-	-	-	-	-	-	-	-
	Hedgerow contrast	-	-	0.42 $\pm$ 0.19*	4.5*	-	-	-	-	-	-
	Forest cover	-	0.87	-	-	-	-	-	-	-	3.30*
	Dist. to forest	-	-	-	-	-	-	-	0.09	-	-
	Dist. to water	-	-	-0.73 $\pm$ 0.22***	11.04***	-	-	-	-	-	-
<b>Interaction</b>	location:Lanes	-	-	UI 1.60 $\pm$ 0.79*	9.18*	-	-	-	-	-	-
	location:Height	-	-	-	-	-	-	H -0.81 $\pm$ 0.44* UI -1.20 $\pm$ 0.68* R -1.16 $\pm$ 0.71*	6.76*	-	-
	location:Forest cover	H 0.68 $\pm$ 0.26**	7.9*	-	-	-	-	-	-	UI 1.54 $\pm$ 0.73*	5.07
	location:Dist. to forest	-	-	-	-	-	-	UI -1.97 $\pm$ 0.86**	12.60**	-	-
	location:Hedgerow length	H 0.42 $\pm$ 0.27* UI -1.06 $\pm$ 0.47**	16.14**	-	-	-	-	-	-	-	-
	$\Delta$ AICc	40.8		14.8		23.9		21.5		28.7	
	Chisq	95.5***		26.1**		34.1***		47.6***		36.8***	
	R <sup>2</sup> m (R <sup>2</sup> c)	0.68 (0.75)		0.63 (0.70)		0.23 (0.37)		0.70 (0.76)		0.26 (0.89)	

Bat activity recorded at adjacent habitat (H), at road sections without underpass, within (UI) and above underpasses (US). RB means the modality "River under Bridge".  $\Delta$  AICc = difference of AICc values between the best and the null models; Chisq = likelihood-ratio chi-squared test between the best and the null models; R<sup>2</sup>m = marginal coefficient of determination; R<sup>2</sup>c = conditional coefficient of determination.

## Figure captions

Fig. 1 Land use map of the study area, indicating the location of sampled roads and underpasses.

Fig. 2 Scheme of sampling design indicating the locations of the different recorders for each studied underpass. We installed one recorder under the underpasses (UI) and two recorders at each road edges above the underpasses (US) to assess bat use. We also installed two recorders at 200 m from the underpasses: one in the adjacent habitat (H) and one at a road section without underpass (R).

Fig. 3 Ratios of road underpass use and attractiveness for bats in response to landscape context calculated from the predictions of Generalized Linear Mixed Models. The  $\pm 95\%$  confidence intervals of the ratios have been calculated from 100,000 bootstrap iterations based on the values of the estimates and the variance-covariance matrix of the GLMMs. The two parallel dotted lines represent the range of values considered as ecologically irrelevant.





Fig. 4 Ratios of road underpass use and attractiveness for bats in response to local road underpass attributes calculated from the predictions of Generalized Linear Mixed Models. The  $\pm 95\%$  confidence intervals of the ratios have been calculated from 100,000 bootstrap iterations based on the values of the estimates and the variance-covariance matrix of the GLMMs. The two parallel dotted lines represent the range of values considered as ecologically irrelevant.

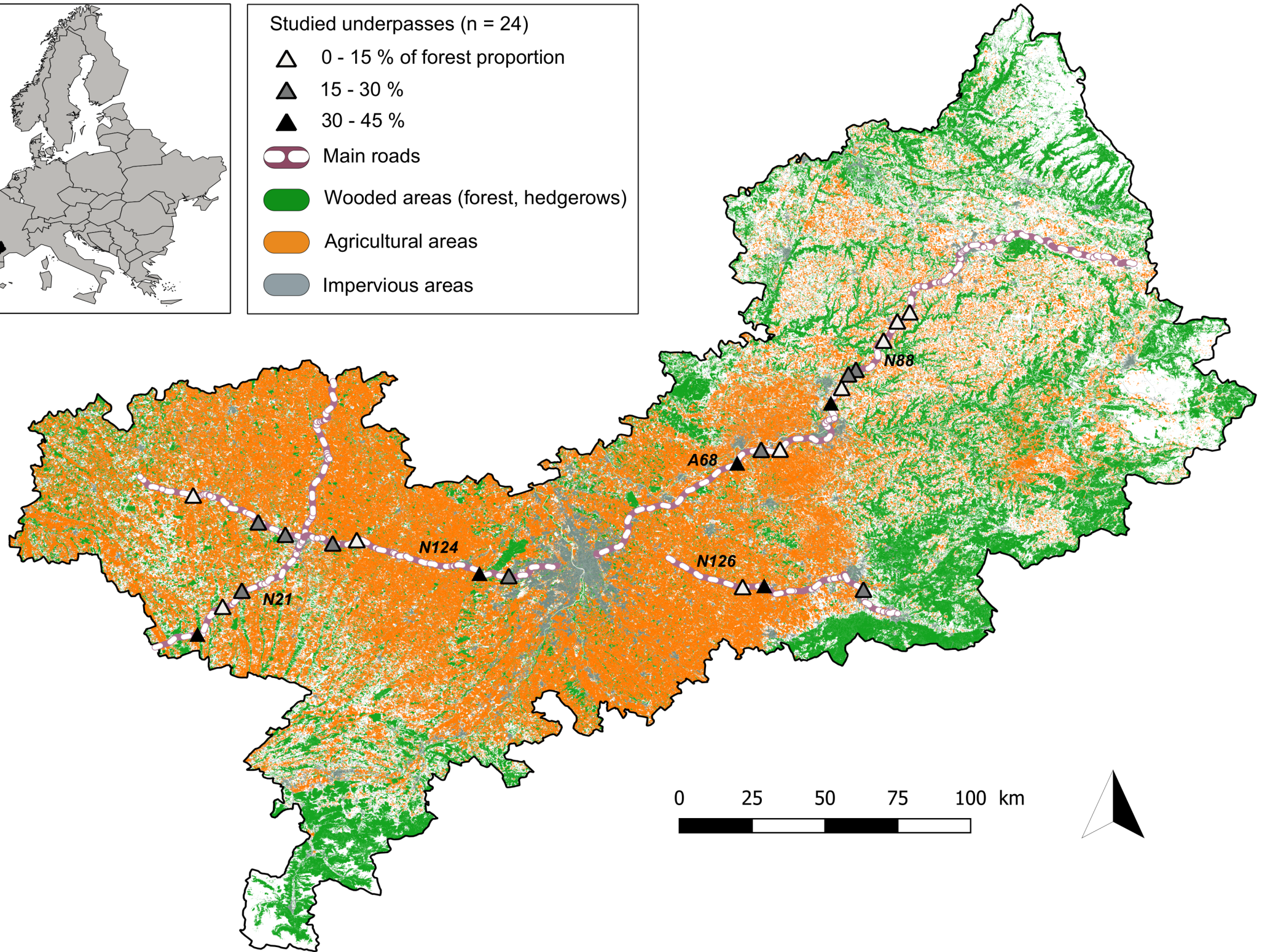
Fig. 5 Effect of local habitat within underpasses on total bat activity (all locations of recording points combined) for three groups of species: *Myotis* spp and *Rhinolophus* spp and *Pipistrellus/Miniopterus* spp. Predictions were obtained from Generalized Linear Mixed Models (GLMMs) fitted with a negative binomial error distribution and a log link function. Dots represent the means and the error bars show 95% confidence intervals. Bat activity at rivers under bridges were used as the reference (i.e., intercept) in each model ( $***P < 0.001$ ;  $**P < 0.01$ ;  $*P < 0.05$ ).

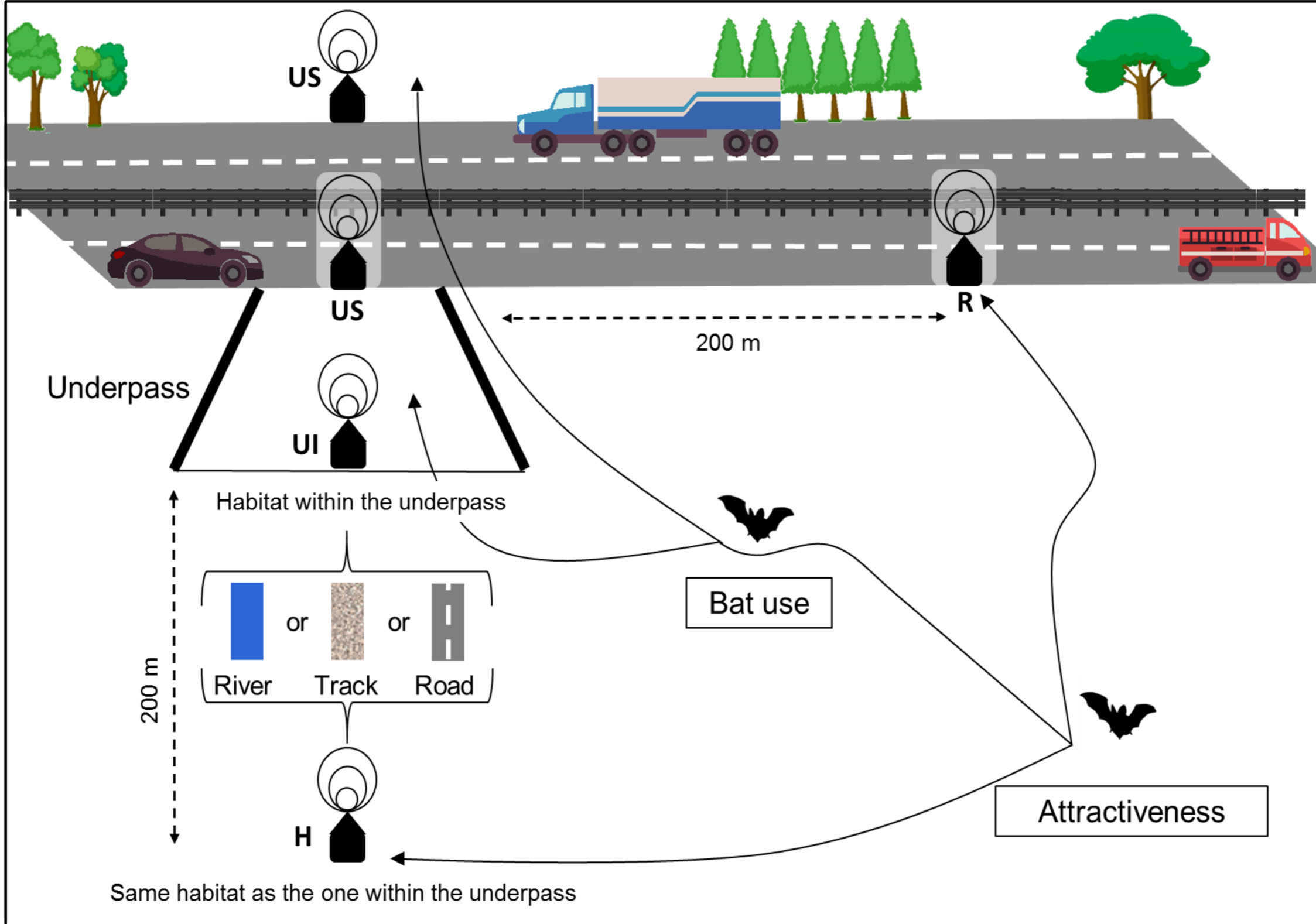




Studied underpasses (n = 24)

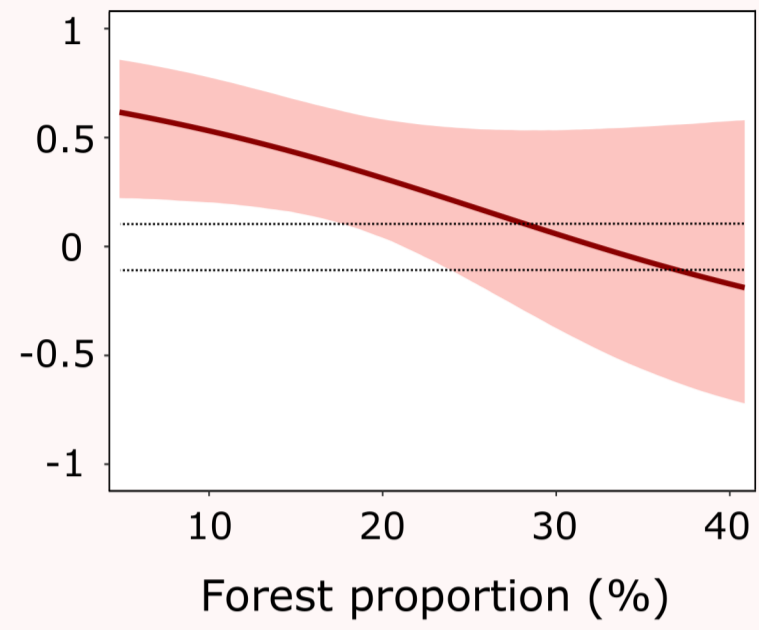
- △ 0 - 15 % of forest proportion
- ▲ 15 - 30 %
- ▲ 30 - 45 %
-  Main roads
-  Wooded areas (forest, hedgerows)
-  Agricultural areas
-  Impervious areas



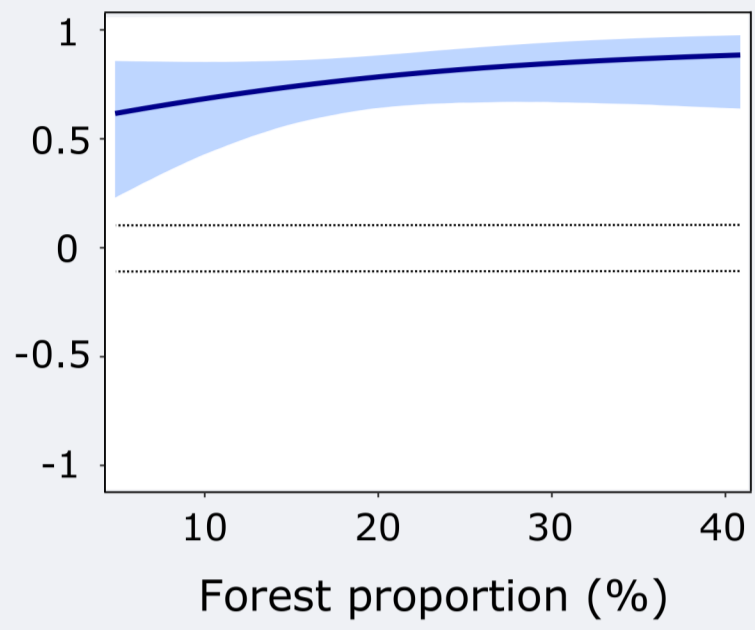


**Ratios**  
(2.5 in materials and methods)

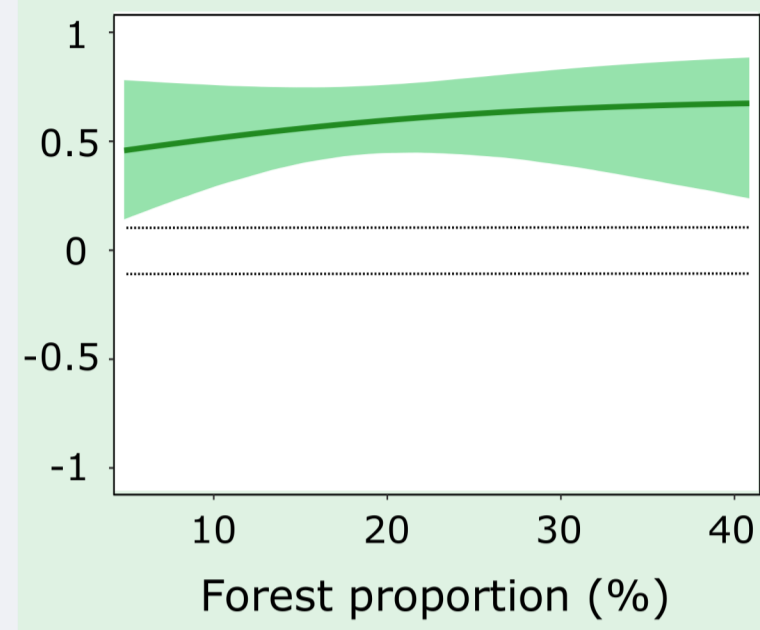
**Underpass attractiveness vs adjacent habitat**



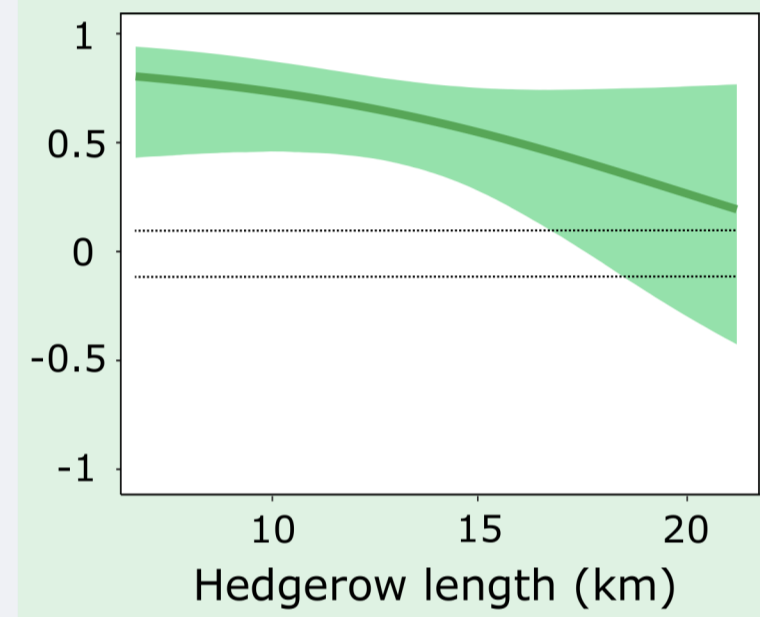
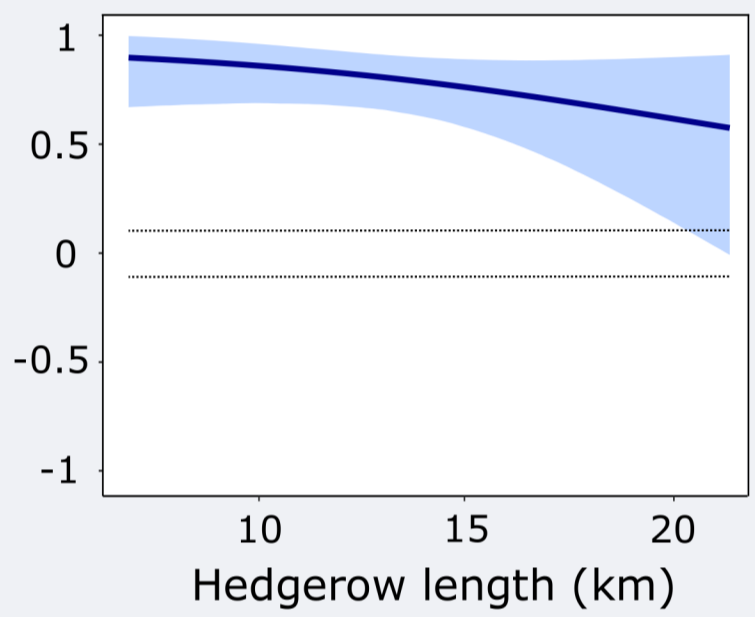
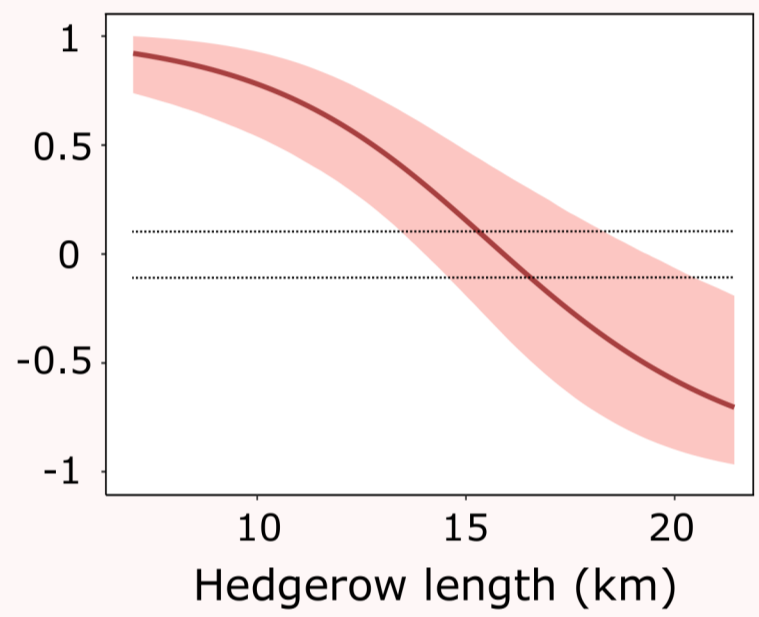
**Underpass attractiveness vs adjacent road**



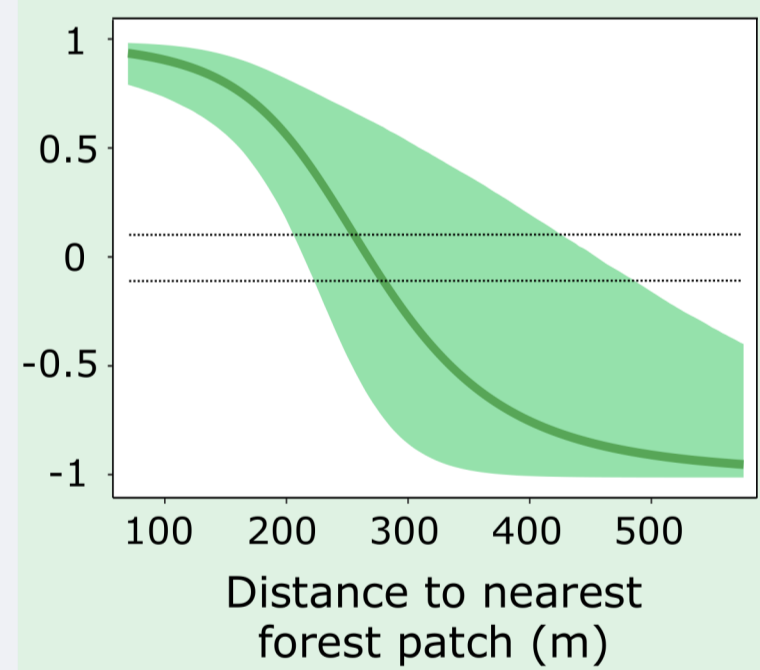
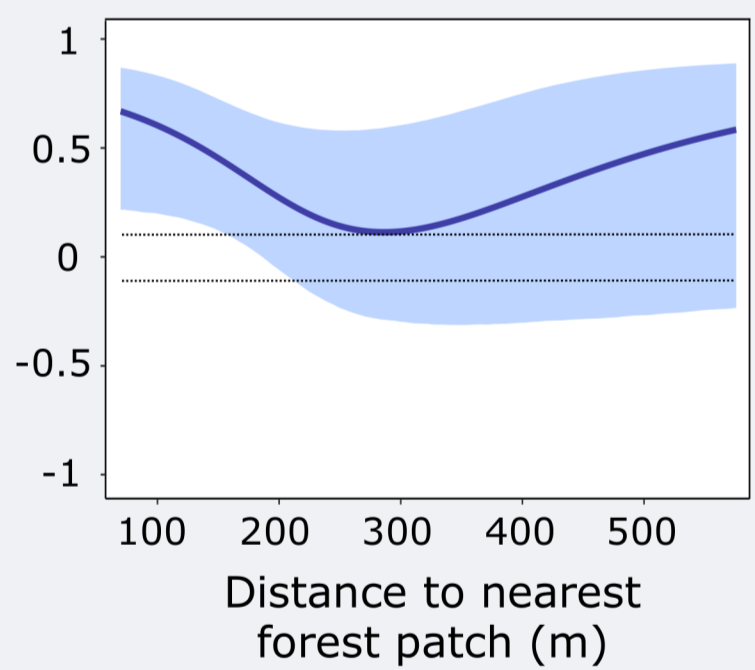
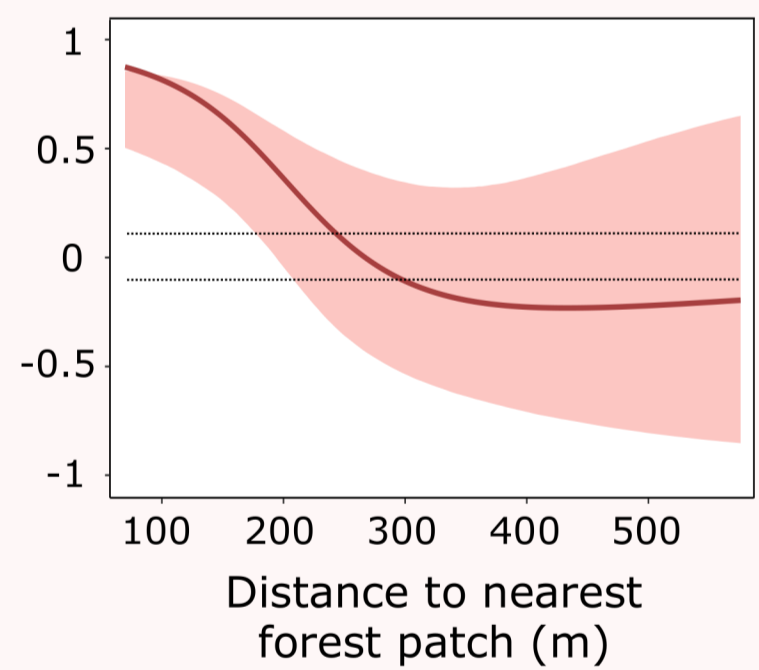
**Underpass use vs road above**



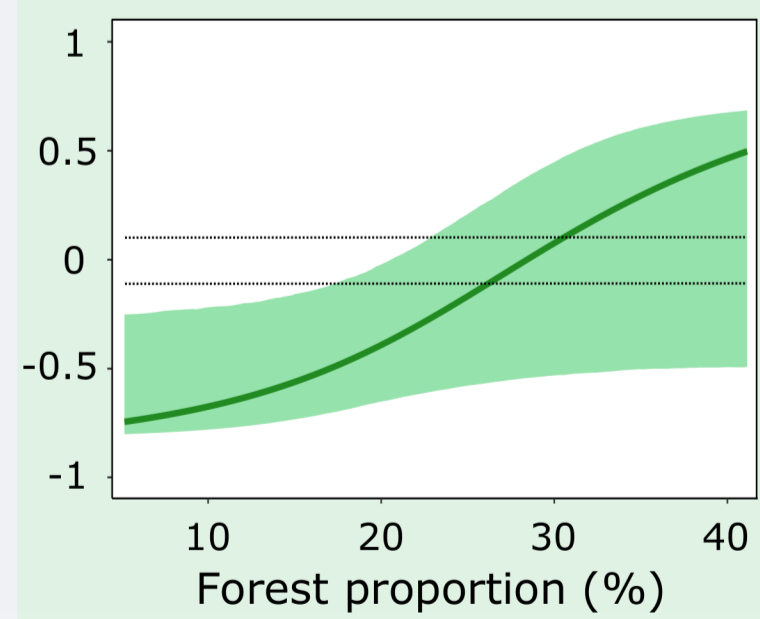
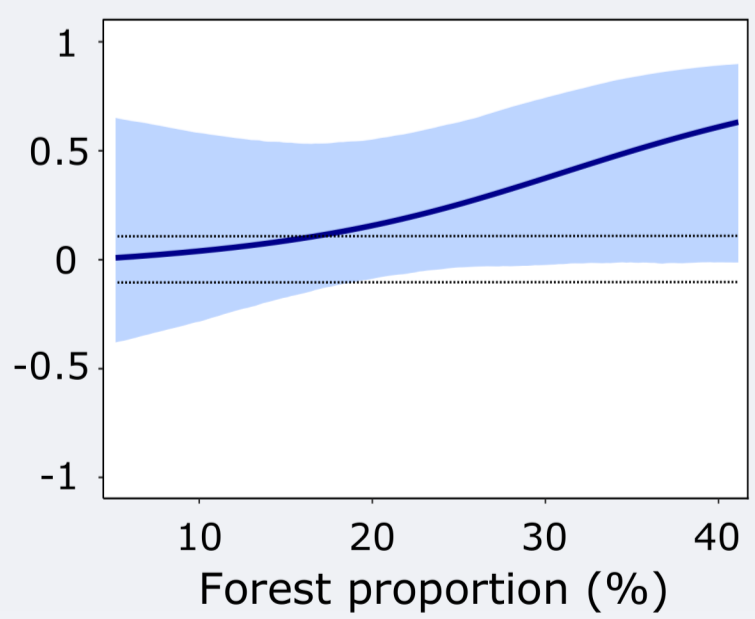
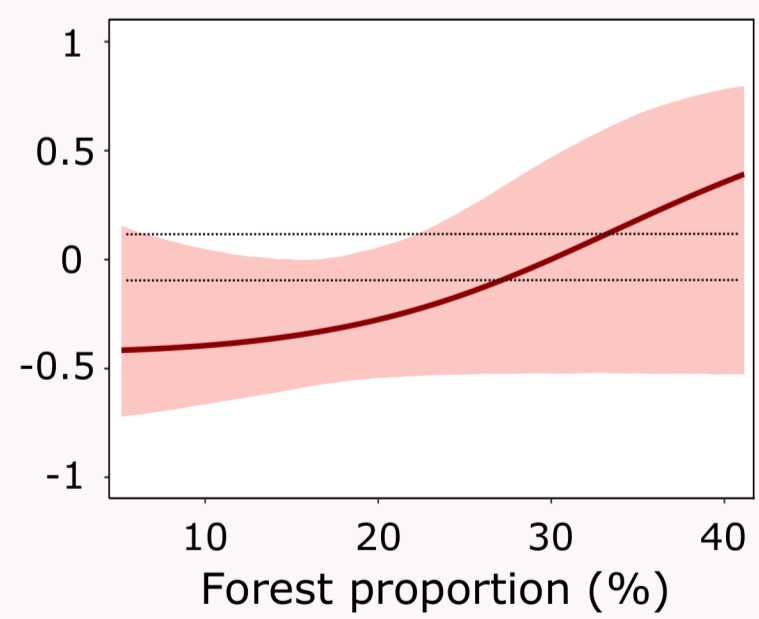
***Myotis spp***



***Myotis spp***



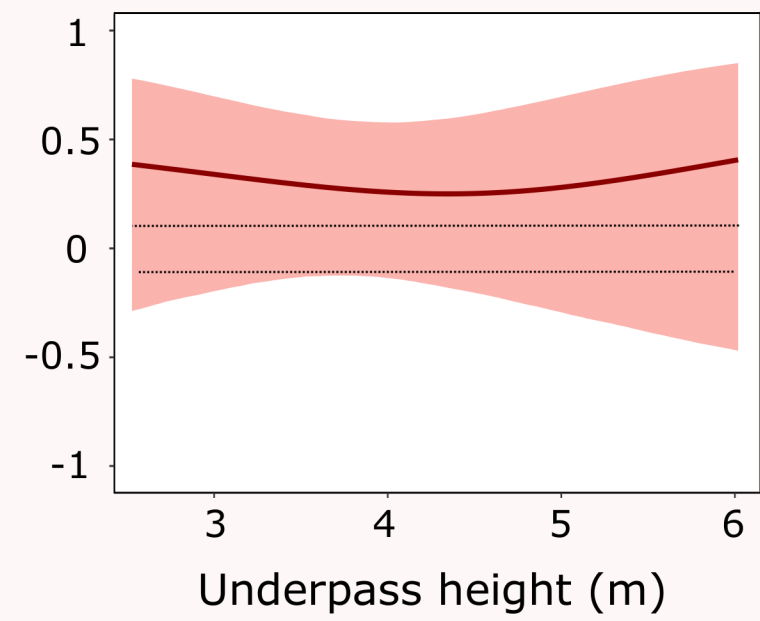
***Rhinolophus spp***



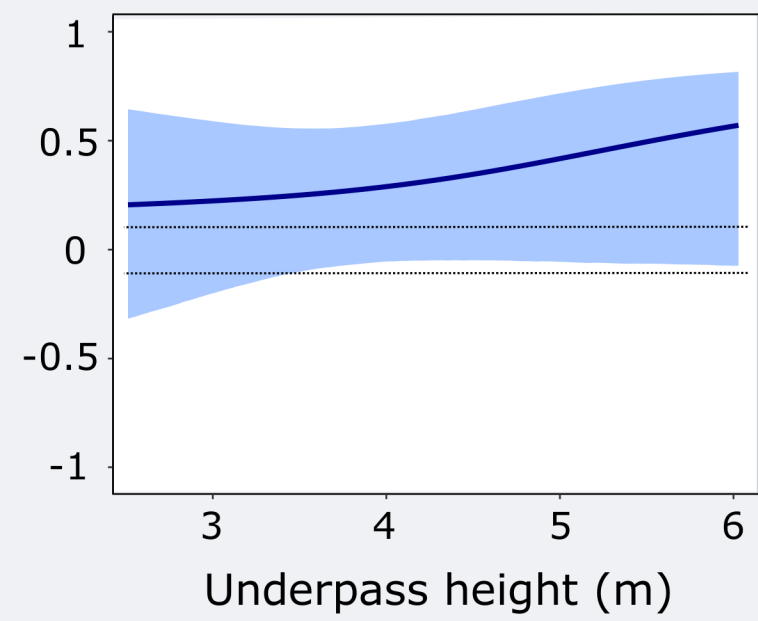
***Barbastella barbastellus***

**Ratios**  
(2.5 in materials and methods)

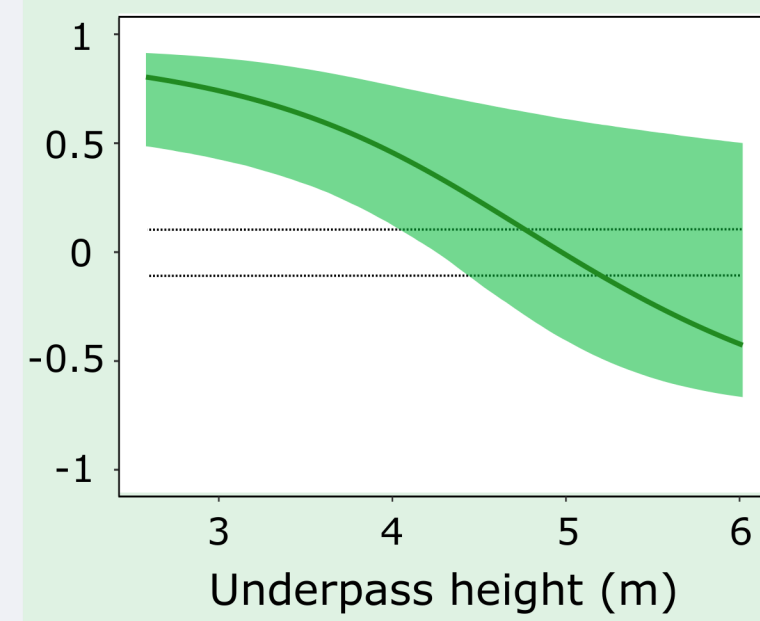
**Underpass attractiveness vs adjacent habitat**



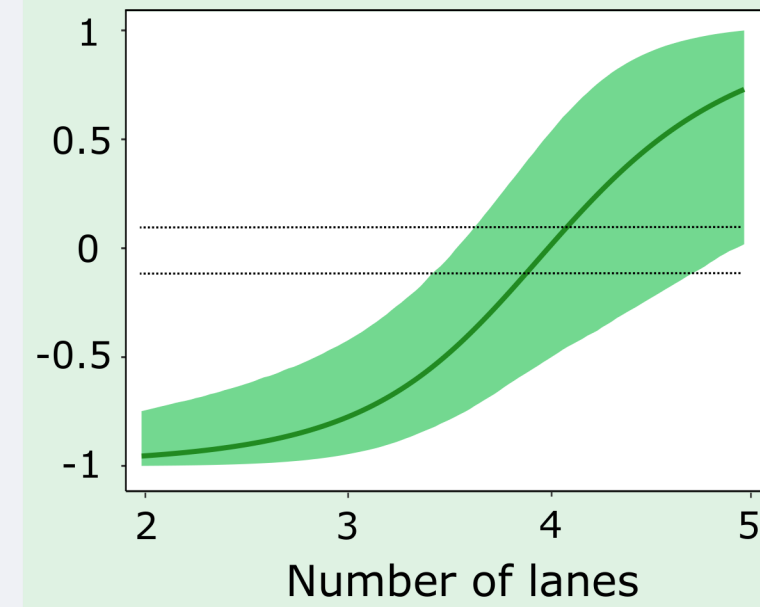
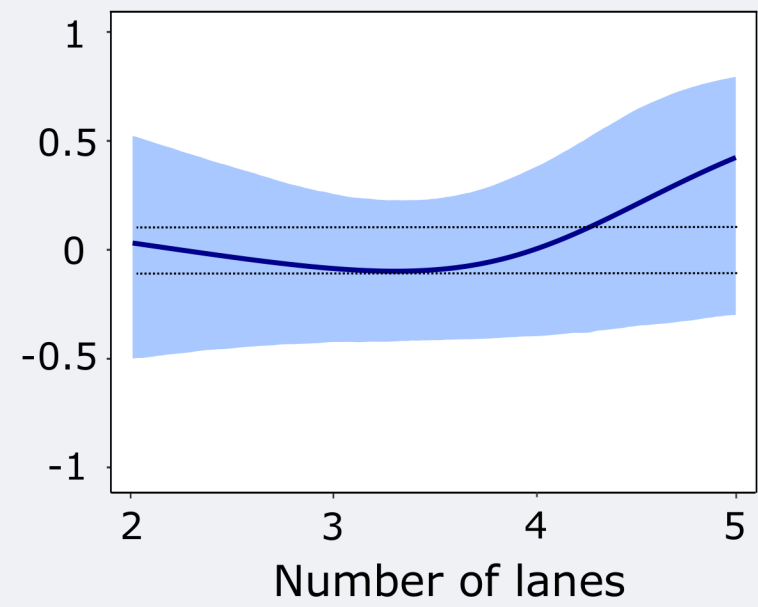
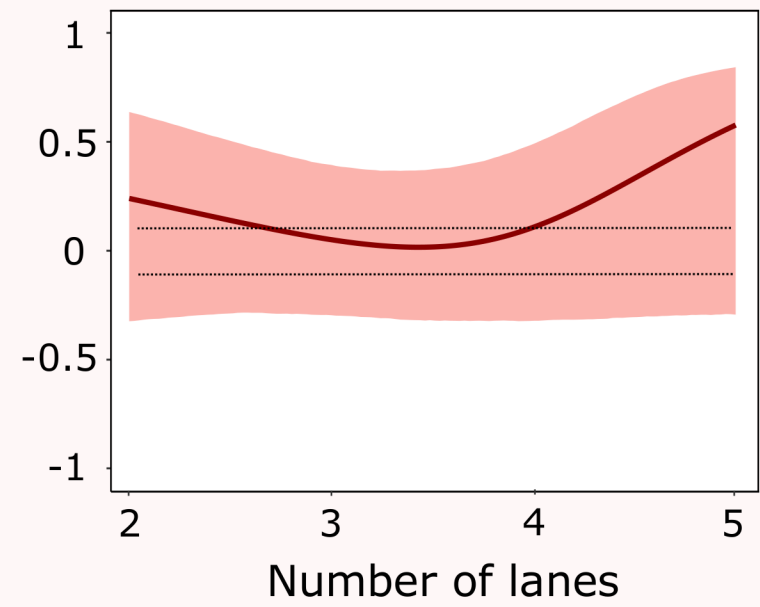
**Underpass attractiveness vs adjacent road**



**Underpass use vs road above**



***Rhinolophus* spp**



***Plecotus* spp**

