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Major roads have important negative effects on insectivorous bat activity

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FC, YB, SJP, BA and CK designed the study. FC and CK implemented the methodology. FC collected the data and digitalized environmental data. FC and CK analysed the data with the assistance of JP and led manuscript writing. All authors critically contributed to manuscript drafts and gave final approval for publication.

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Data accessibility

Acoustic data was delivered to the citizen science program “VigieChiro” (<https://vigiechiro.herokuapp.com/>)

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1 Major roads have important negative effects on insectivorous bat activity

2

3 **Running title:** Roads and bat activity

4

5 **Highlights**

- 6 • Major roads affect activity in five of the thirteen studied taxa.
- 7 • Low-flying species are more affected than those that fly in the open.
- 8 • Road impacts may be due to road kills, traffic disturbance and ruptured connectivity.

9

10 **Abstract** (241/250)

11 The development of transportation infrastructure has been identified as one of the main
12 pressures on biodiversity. The effects of transport infrastructure are more documented for
13 terrestrial mammals, birds and amphibians than for bats. To assess the impacts of roads on bat
14 activity, we carried out full-night acoustic recordings of bat calls at 306 sampling points at
15 different distances from a major road at three study sites in France. To assess the relationship
16 between bat activity and the distance to the major road, we performed generalized linear
17 mixed model analyses for thirteen different species or groups and additionally explored the
18 non-linear effect with generalized additive mixed models. Our results showed that low-flying
19 species are more affected than high-flying species. Indeed, we found a significant negative
20 effect of major roads on bat activity for the ‘clutter-adapted’ species, *Eptesicus serotinus*,
21 *Myotis* spp., *Pipistrellus pipistrellus* and *Rhinolophus hipposideros*. These results demonstrate
22 that the road-effect zone of major roads extends up to five kilometres. Extrapolating those
23 road-effects zones to the major roads in the European Union, we estimated that 35% of the
24 European Union is potentially negatively impacted. Finally, it seems urgent to consider these
25 road effects with the cumulative effects of other roads by improving habitat connectivity and

26 foraging areas in land use policies. Additionally, to implement drastic conservation practices
27 for species of conservation concern in environmental impact assessment studies, efficient
28 mitigation and offset measures implemented should be sized proportionally to the disturbance
29 caused.

30

31 **Keywords**

32 Chiroptera, barrier effect, bat activity, habitat loss, habitat degradation, road-effect zone

33

34 **1 Introduction**

35 Transportation has been identified as one of the ten main pressures on biodiversity (Maxwell
36 et al., 2016) because it contributes to habitat destruction, habitat degradation and barrier
37 effects, fragmentation, light and noise disturbance, chemical pollution and direct mortality by
38 collision with vehicles (i.e., road kills) (Forman and Alexander, 1998; Forman and Deblinger,
39 2000; Trombulak and Frissell, 2000). These dramatic changes in landscape configurations
40 have consequences on the overall functionality of ecosystems, from individual behaviour all
41 the way up to population dynamics (Krauss et al., 2010; Quinn and Harrison, 1988; Saunders
42 et al., 1991). Indeed, the cumulative ecological effect of roads on biodiversity at the landscape
43 scale (i.e., the road effect zone) can extend to several kilometres away depending on the type
44 of road, the traffic volume and the habitat crossed by the road (Benítez-López et al., 2010;
45 Forman, 2000; Forman and Deblinger, 2000).

46 By 2050, the global road infrastructure is expected to have increased by approximately
47 60% compared to the 2010 levels (Dulac, 2013). In this context, several studies have been
48 carried out in recent years with the aim of documenting the road effects on biodiversity and
49 ecosystem functioning [i.e., road ecology (Forman, 1998)]. Studies currently cover a variety
50 of taxa, terrestrial mammals, amphibians and birds (Benítez-López et al., 2010; Fahrig et al.,

51 1995). Surprisingly, little is comparatively known about the impact of roads on bat activity
52 (Bennett et al., 2013; Berthinussen and Altringham, 2012a; Kitzes and Merenlender, 2014;
53 Medinas et al., 2019; Pourshoushtari et al., 2018). Pourshoushtari et al. (2018) found that
54 activity was higher when the road crossed a forest and lower along major roads as well as
55 roads within open areas. Bennett et al. (2013), who studied the impact of roads in the vicinity
56 of roost maternity, found that roads with two lanes had little or no effect on bat movement,
57 while roads with four lanes had more effect on bat movement. However, Medinas et al.
58 (2019) found that roads with low-medium traffic can impact bat activity for ‘clutter-adapted’
59 and ‘open-adapted’ species within 0 to 300 m of roads in woodlands and within up to 500 m
60 of roads in open fields. These results are congruent with Kitzes & Merenlender (2014), who
61 also found a negative effect of roads on bat activity within 300 m (corresponding to the
62 maximum distance considered) for four common bat species in California (USA), *Tadarida*
63 *brasilensis*, *Eptesicus fuscus*, *Lasiurus cinereus* and *Lasionycteris noctivagans*. Berthinussen
64 & Altringham (2012) reported correlations between bat activity and the distance to a major
65 road. They found a decline in activity for a common bat species, *P. pipistrellus*, to a distance
66 of at least 1.6 km (i.e., the maximum distance considered) on both sides of a road in Cumbria
67 (United Kingdom). They also found a decline in species diversity in the proximity of roads.

68 Moreover, the effects of roads on bats are more numerous, including habitat loss,
69 reduced habitat quality and mortality by collision (Abbott et al., 2015; Bennett and Zurcher,
70 2013; Bontadina et al., 2002; Fensome and Mathews, 2016; Frey-Ehrenbold et al., 2013;
71 Lodé, 2000; Luo et al., 2015; Medinas et al., 2013; Møller et al., 2016; Zurcher et al., 2010).
72 The cumulative effects of these factors could be deleterious to bat populations (Altringham
73 and Kerth, 2016). Furthermore, major roads act as barriers for bat movement because they
74 disconnect existing flight paths along linear features (e.g., hedges) and interrupt bat
75 commuting movements between roosts and foraging areas (Bennett et al., 2013; Bennett and

76 Zurcher, 2013; Berthinussen and Altringham, 2012a; Kerth and Melber, 2009; Kitzes and
77 Merenlender, 2014).

78 Most bat species use linear elements, such as hedges, to commute nightly (Frey-
79 Ehrenbold et al., 2013) partly because a majority of them are reluctant to fly in the open or are
80 avoiding light (Azam et al., 2018). Moreover, even small gaps in linear elements can
81 drastically affect the probability of bats crossing. Indeed, in Indiana (USA), gaps of 5 m in
82 tree or shrub cover along flight routes have been shown to significantly impact bat commuting
83 movements (Bennett and Zurcher, 2013). A study undertaken in the United Kingdom
84 demonstrated that a gap of only 10 m may disturb bat commuting (Entwistle et al., 2001).
85 Furthermore, Pinaud et al. (2018) demonstrated that bat movements were significantly
86 affected by gap width: the probability of crossing a gap dropped below 0.5 for gaps larger
87 than 38 m, which corresponds to a width similar to the gap caused by major roads. With the
88 addition of traffic, this effect could likely be even greater (Zurcher et al., 2010). Moreover,
89 Hale et al. (2012) demonstrated that bat foraging and commuting activity in a habitat patch
90 increased with the patch's degree of connectivity to the surrounding landscape. This is of
91 utmost importance because, for a majority of bat species, individuals travel far from their
92 roosts to their foraging areas (Dietz et al., 2013; Encarnacao et al., 2005; Flanders and Jones,
93 2009; Nardone et al., 2015; Szentkuti et al., 2013). The necessity for bats to travel long
94 distances implies a high probability for them to be impacted by the network of roads within
95 their home range.

96 Many European bats are endangered throughout much of their range, and numerous
97 causes have been identified, including habitat loss and degradation and road kills, which can
98 be caused by roads (Temple and Terry, 2007). According to their life cycle (i.e., low
99 fecundity, late maturation), adult mortality by road collision is expected to have significant
100 negative impacts on populations (Medinas et al., 2013). All bats are legally protected in

101 European countries through national or European laws (Council Directive, 1992; Convention
102 on Migratory Species, 1985–2008; and Agreement on the Conservation of Populations of
103 European Bats). Among these protections, some require that a development project evaluate
104 its effect on biodiversity, and any negative effect must be limited and/or compensated through
105 mitigation hierarchy (avoiding, reducing, restoring, and offsetting effects) with the aim to
106 achieve a zero net loss of biodiversity or a net environment (Regnery et al., 2013). Although
107 bats benefit from a strict protection status in many countries and negative road impacts appear
108 important for bats, surprisingly, in Europe, approximately half of the countries implemented
109 bat mitigation and compensation measures (Elmeros et al., 2016). Moreover, most road
110 mitigation measures dedicated to bats are more focused on restoring connectivity via, for
111 example, bat overpasses but rarely consider compensation for habitat loss (Møller et al.,
112 2016).

113 According to bat home range size and the importance of landscape connectivity for bat
114 daily movements, we hypothesize that roads may affect bat activity at greater distances. To
115 evaluate this, we conducted acoustic surveys at three sites of 100 km², each centred on a
116 major road. We used a confidence threshold of species identification and tested the effect of
117 the distance to major roads on the activity of several bat taxa while controlling for the habitat,
118 including interactions with hedges and wetlands. Then, we estimated the road effect zone of
119 major roads in Europe.

120 **2 Materials and methods**

121 *2.1 Study sites*

122 The study was carried out in France, which experienced an increase in roads of 12% between
123 1995 and 2015 (MEEM, 2017). We selected three sites located in rural areas in western
124 France, including for each site, at a central position, a highway. Each study site is a 100 km²
125 square with different land uses (Fig. 1). The size of the area was selected to study the potential
126 impact of the road at a scale that compares to a majority of bat home ranges (Dietz et al.,
127 2013; Flanders and Jones, 2009; Szentkuti et al., 2013). The first site was surrounded by
128 intensive farming, located near Niort (46°24'N, 0°35'W) and centred on highway A83
129 (operational since 2001; road with tarmac; 4 lanes with shoulders; speed limit: 130 km/h,
130 2015 average daily traffic: 16218 vehicles). The second site was mainly surrounded by
131 woodlands and grasslands, located near La Rochelle (45°50'N, 0°37'W) and centred on
132 highway A10 (operational since 1994; same features as A83; 2015 average daily traffic:
133 27377 vehicles). The last site was mainly surrounded by woodlands and grasslands, located
134 near Rennes (48°2'N, 14°57'W) and centred on the national road N24 (operational since 1981;
135 road with tarmac; 4 lanes without shoulders; speed limit: 110 km/h; 2015 average daily
136 traffic: 33800 vehicles). Finally, there are no road lights on the highway portions studied,
137 except along a service station for A10 (800 m on both sides of the road).

138

139 *2.2 Sampling design*

140 To assess the influence of major roads on bat activity, we performed acoustic recordings of
141 bat activity at each site: 100 points for A83, 94 points for A10 and, 112 points for N24. We
142 sampled five main categories of habitats at each site (wetlands, woodlands, agricultural lands,
143 urban areas and hedgerows) at different distances from the road (from 26 to 5420 metres).
144 Each main category of habitat was sampled equitably on average 61 ± 5 times, spread across

145 several distances from the road (Figs A.1, A.2). The five main categories of habitats were
146 simultaneously sampled on one night along a gradient of distances to the major road by
147 several acoustic recorders. As acoustic surveys were performed on successive nights (see next
148 section), we sampled new points while maintaining a gradient of distances to the major road.
149 This sampling plan allowed us to avoid correlations between night conditions and variables
150 tested (habitats and distance to the major road) (Table A.3, Fig. A.4).

151

152 *2.3 Acoustic surveys*

153 Fieldwork was carried out during the seasonal peak of bat activity between the 28th of May
154 and the 17th of August 2016. Recordings were conducted during nine successive nights for
155 A83 (in May-June), eight successive nights for A10 (in July) and ten successive nights for
156 N24 (in August). Recordings were also conducted under favourable meteorological night
157 conditions as follows: temperatures (A83: \bar{x} , 14.37 °C; SE, 0.47; A10: \bar{x} , 19.51 °C, SE, 3.31;
158 N24: \bar{x} , 15.76 °C, SE, 2.63), precipitation (A83: \bar{x} , 0.13 mm; SE, 0.34; A10: \bar{x} , 0; N24: \bar{x} , 0.05
159 mm; SE, 0.21), and speed wind (A83: \bar{x} , 8.04 km/h, SE, 3.31; A10: \bar{x} , 9.69 km/h, SE, 2.43;
160 N24: \bar{x} , 8.1 km/k; SE, 1.72).

161 Bat activity was assessed by recording bat calls using Song Meter SM2Bat+ devices
162 (Wildlife Acoustics Inc., Concord, MA, USA) fitted with SMX-US omnidirectional ultrasonic
163 microphones (Wildlife Acoustics Inc., Concord, MA, USA) placed 1 m above the ground
164 attached to a small wooden stake. We systematically tested microphone sensitivity with a
165 same source when we installed and removed each device. During these tests, the signal-to-
166 noise-ratio (SNR) on new microphones was always between 15 to 20 dB. If, *a posteriori*, the
167 sensitivity was below 15 dB, the sampled point was removed and not included in our
168 analyses. Recordings were performed during the whole night (from 30 min before civil sunset
169 to 30 min after civil sunrise). Moreover, an acoustic recorder can detect bats at an average

170 distance of 25 m for common species, such as *Pipistrellus* spp. (Barataud, 2015). This
171 detection distance was taken into account for the placement of acoustic recorders in the
172 sampling to avoid recording and hence counting the same bat with two acoustic recorders (\bar{x} ,
173 493 m).

174 With such passive acoustic recordings, the detectors automatically recorded all sounds
175 in full spectrum with a sample rate of 384 kHz. We used a trigger level threshold of 6 dB
176 SNR for frequencies and a trigger window of 2 seconds, following the protocol of the French
177 Bat Monitoring Programme (FBMP): <http://vigienature.mnhn.fr/page/protocole-point-fixe>

178

179 *2.4 Species identification*

180 We analysed the ultrasound recordings with the software *Tadarida* in its latest version [(Bas
181 et al., 2017), online repository: <https://github.com/YvesBas>]. This software automatically
182 detects and extracts sound parameters of recorded echolocation calls and classifies them into
183 known classes according to a confidence index value that a call is from a specific group/bat
184 species using a random forest algorithm (Breiman, 2001).

185 To assess the influence of identification uncertainty on the results, we followed the
186 Barré et al. (2018) approach, proposing a cautious method to account for identification errors
187 in acoustic surveys without fully checking recordings. This first consisted of modelling the
188 error rate in automated identification, performing logistic regressions between manual checks
189 (i.e., success/failure in automatic species assignment) and confidence indexes provided by the
190 automated identification software (i.e., 0 to 1). Manual checks were performed on 8405
191 independent bat passes recorded throughout France as part of the FBMP. This allowed the
192 computation of the minimum confidence index used to ensure the error rate was below the
193 chosen thresholds (methodology detailed in appendix B). Then, we filtered out bat passes
194 having smaller confidence indexes than required to ensure the two targeted maximum error

195 rates (i.e., 0.5 and 0.1) at which the analyses were performed. Each maximum error rates (i.e.,
196 threshold) involves different caveat. Indeed, a threshold that is too cautious could lead to high
197 generated false negative rates (i.e., by discarding a large proportion of data containing true
198 positives below a given confidence score), which could result in a lack of statistical power. In
199 contrast, a threshold that is not cautious enough could lead to high false positive rates (i.e.,
200 fails in automated identifications), particularly through the inclusion of records of species
201 which are most similar acoustically, which involve statistical noise. Therefore, filtering the
202 data with two different thresholds allowed us to check for consistency of results and ensure
203 limited biases occurred in the dataset (i.e., false positive rate) in relation to tested variables
204 (Barré et al., 2018).

205 First, we studied the activity of all species together and then the activity of two groups
206 of species based on their flying and foraging strategies. The ‘open-adapted’ species group is
207 composed of five species that are medium- to high-altitude fast-flying species: *Eptesicus*
208 *serotinus*, *Nyctalus leisleri*, *N. noctula*, *Pipistrellus kuhlii* and *P. pipistrellus* (Blake et al.,
209 1994; Roemer et al., 2017). The ‘clutter-adapted’ species group is composed of low-altitude
210 slow-flying species that generally forage in cluttered vegetation: *Barbastella barbastellus*, *R.*
211 *ferrumequinum* and *R. hipposideros* and two genera: *Myotis* spp. and *Plecotus* spp., which
212 cannot be identified at the species level with certainty (Obrist et al., 2004). In addition, we
213 conducted a separate analysis for each of the eight species and the two genera.

214 Finally, to measure bat activity for each species, we retained one bat pass per five-
215 second interval, which is the mean duration of all bat species passes such as recommended by
216 Millon et al. (2015) and Kerbiriou et al. (2018b).

217 2.5 Environmental variables

218 To assess the effect of the distance to major roads on bat activity while accounting for the
219 surrounding environment at each sample point, we extracted 57 variables that correspond to
220 habitat/configuration variables that have been identified to influence bat activity in several
221 studies (Boughey et al., 2011a; Frey-Ehrenbold et al., 2013; Kaňuch et al., 2008; Kelm et al.,
222 2014; Lacoeuilhe et al., 2016; Rainho and Palmeirim, 2011; Russo and Jones, 2003; Verboom
223 and Huitema, 1997) (Tables A.1 A.2, step 1 in Fig. A.3). These variables are either distances
224 between the sampled point to an environmental variable (e.g., to the major road, to hedges) or
225 the proportion of area of each habitat (wetlands, woodlands, agricultural lands, urban areas
226 and hedgerows) calculated for different buffer sizes (50, 200 and 500 m). We tested different
227 buffer sizes because, depending on the taxa and the landscape variable considered, bat activity
228 may be affected at a different spatial scale (Table A.1) (Bellamy et al., 2013; Grindal and
229 Brigham, 1999; Kerbiriou et al., 2018a; Lacoeuilhe et al., 2016). As hedgerows are linear
230 elements, we also computed the density of hedgerows within each buffer (Table A.1).

231 Landscape data were obtained through manual digitization by photointerpretation
232 (Fig. 1), and distances, lengths and proportions were calculated using QGIS 2.18.14 (QGIS
233 Development Team, 2017).

234

235 2.6 Bat activity modelling

236 We assessed whether bat activity (i.e., our response variable is the number of bat passes) was
237 influenced by the distance to the road using a generalized linear mixed model (GLMM) with
238 the *glmmTMB* function [R package *glmmTMB* v0.2.1.0 (Brooks et al., 2017)]. Due to the
239 nature of the response variable (i.e., count data with overdispersion), we used a negative
240 binomial distribution with a log link (Zuur et al., 2009). For species occurring at less than
241 50% of the overall sampling points for the three study sites, we conducted the models with a

242 zero-inflation parameter (Tables C.3, C.5). According to the relatively well-balanced
243 sampling design (i.e., simultaneous recordings of bat activity on the same night in different
244 habitats at different distance classes in successive nights), we included a two-level random
245 effect: night, nested within site, to take into account the spatial structure as recommended in
246 Bates *et al.* 2014. Moreover, no correlation >0.7 was detected between environmental
247 variables and meteorological conditions (see Table A.3, Fig. A.4).

248 To assess the effect of road distance as a continuous variable on bat activity while
249 accounting for surrounding habitat influence, we included the distance to the road and
250 landscape co-variables as fixed effect in the models. We also explored interactions between
251 the distance to the road and two key habitats in agricultural land well represented among the
252 three sites: hedges [distance or density (Boughey *et al.*, 2011b; Fonderflick *et al.*, 2015)] and
253 wetlands [proportion or distance (Rainho and Palmeirim, 2011)]. All fixed effects were scaled
254 so that the regression coefficients were comparable in magnitude and their effects were
255 biologically comparable (Schielzeth, 2010). To avoid over-parametrization, we selected the
256 best scale of covariates (i.e., 50, 200 or 500 m) before including them in the full model, using
257 hierarchical partitions (step 2 in Fig. A.3) [R package *hier.part* v 1.1-4 (Walsh and Mac
258 Nally, 2013)]. This selection process led us to choose the 5 best covariates among the 56
259 included in the full model. Thus, our full models included 8 environmental covariates (6
260 simple effects and 2 interactions) and were structured in the following way (step 3 in Fig.
261 A.3): Bat activity \sim Distance to major road + Hedges + Wetlands + Agricultural lands +
262 Woodlands + Urban areas + Distance to major road:Hedges + Distance to major
263 road:Wetlands + 1|Site/Night.

264 To avoid potential multicollinearity problems, we systematically evaluated the
265 correlations among explanatory variables using Spearman's rho, and no correlation >0.7 was
266 detected (Dormann *et al.*, 2013). In addition, we assessed the variance inflation factor (VIF).

267 Following the approaches of Chatterjee & Bose (2000) and Zuur, Ieno & Elphick (2010), as
268 all variables showed a VIF value <3 and the mean VIF values was <2 , there was no evidence
269 of multicollinearity. Then, we checked the spatial autocorrelation of residuals of each selected
270 model using Moran's I test [R package *ape* v5.1 (Paradis and et al, 2018)]. If spatial
271 correlation was detected, we corrected our models with the *autocov_dist* function (R package,
272 *spdep*). Model validation was carried out by visual inspection of the patterns of the model
273 residuals (Zuur et al., 2009).

274 From the full model, we performed a backward selection based on Akaike's
275 information criterion (AIC) (step 4 in Fig. A.3). Furthermore, we evaluated the quality of our
276 models by comparing them to the null model (including only the random effects) using
277 Akaike's information criterion (AIC) (Burnham et al., 2011; Mac Nally et al., 2017).

278 To assess the robustness of our results in relation to the level of identification
279 uncertainty, we performed analyses sorting out data with a 0.5 maximum error rate (see 2.4).
280 Then, we confirmed p-values and estimates produced on a more restrictive threshold of 0.1.

281 Finally, the potential non-linear effect of the distance to the road was checked by
282 visual inspection of the plot from the generalized additive mixed models [GAMM, R package
283 *mgcv* v1.8-23 (Wood, 2018)].

284

285 *2.7 Road-effect zone*

286 Following the Forman & Deblinger (2000) approach, we assessed the potential extent of the
287 "road-effect zone" at the scale of the European Union. Drawing on the results obtained in our
288 models by species (i.e., the distance impact found and the linear or non-linear effect), we
289 created a buffer around all roads considered major roads in Europe [based on the E-Road
290 Traffic Census 2005 (http://www.unece.org/trans/main/wp6/e-roads_census_2005.html)]

291 using QGIS 2.18.14 (QGIS Development Team, 2017). Then, we calculated the proportion of
292 area throughout which bat activity could be impacted by major roads in the European Union.

293 **3 Results**

294 *3.1 Bat monitoring*

295 In the dataset allowing for a maximum error rate of 0.5, there was a total of 223601 bat passes
296 for ten species or species groups across the three study sites. Bat activity levels for ‘open-
297 adapted’ species ($n=200072$ bat passes; 89.4%) were higher than for ‘clutter-adapted’ species
298 ($n=23729$ bat passes; 10.6%). The most abundant genera were *Pipistrellus* spp. ($n=191546$ bat
299 passes; 85.6%) and *Myotis* spp. ($n=18282$ bat passes; 8.2%), and the least abundant genera
300 were *Nyctalus* spp. ($n=3383$ bat passes; 1.5%), *Rhinolophus* spp. ($n=1354$ bat passes; 0.6%)
301 and *Plecotus* spp. ($n=1257$ bat passes; 0.5%) (Tables 1, C.5).

302

303 *3.2 Impact of major roads on bat activity*

304 Our results showed a significant negative effect of major roads on bat activity for the four
305 species or species group among the ten studied, i.e., for them, bat activity increased with
306 distance to the major road. These species or species groups were *E. serotinus* ($P = 0.03$),
307 *Myotis* spp. ($P < 0.001$), *P. pipistrellus* ($P = 0.02$) and *R. hipposideros* ($P < 0.01$) (Table 2 &
308 C.1). Moreover, we found a significant negative effect of major roads only for the activity of
309 ‘clutter-adapted’ species ($P < 0.001$) (Table 2). Note that all selected models had a lower AIC
310 value than their respective null models ($\Delta > 2$) (Table C.3).

311 We also found a significant negative effect of the interaction between the distance to
312 major roads and the distance to hedges for *Myotis* spp., *P. pipistrellus* ($P = 0.01$) and ‘clutter-
313 adapted’ species ($P < 0.01$). Moreover, a significant negative effect of the interaction between
314 the distance to major roads and the density of hedges in a buffer of 200 m was found for
315 *E. serotinus* ($P = 0.05$) (Table 2 & C.1). Overall, the interactions showed that *Myotis* spp., *P.*
316 *pipistrellus*, ‘clutter-adapted’ species and *E. serotinus* exhibited a relatively greater activity
317 around hedges in the vicinity of a major road (Fig. C1).

318

319 *3.3 Additional analyses with GAMM*

320 Among the species whose activity was affected by the distance to major roads, we only
321 detected a non-linear effect for the clutter-adapted species group and the genera *Myotis* spp.
322 For both, major roads had a much stronger negative effect at distances shorter than one km to
323 the road (Fig. 2, Fig. C.2).

324

325 *3.4 Influence of error rate*

326 To ensure that our results were robust independent of the level of identification uncertainty,
327 we ran the analyses with a more restrictive tolerance of a 0.1 maximum error rate (i.e.,
328 minimizing false positives) (Table C.4). We found qualitatively similar results for all taxa,
329 except for the effect of distance to major road on *P. pipistrellus*, which lost significance when
330 filtering with the 0.1 maximum error rate.

331

332 *3.5 Road-effect zone*

333 We assessed the "road-effect zone" detected previously in our results, which highlighted the
334 impact of major roads on bat activity at up to five km for five taxa. We applied it at the scale
335 of the European Union and found that in 35 % of the European Union, bat activity is
336 potentially negatively influenced by the proximity of major roads (Fig. 3), suggesting
337 important but hidden habitat loss.

338 **4 Discussion**

339 *4.1 Road effects*

340 Among the thirteen bat species or species groups studied, five were negatively impacted by
341 major roads, and none were positively affected. The species or species groups impacted
342 included the ‘clutter-adapted’ and *Myotis* spp. groups, and the following three species: *R.*
343 *hipposideros*, *E. serotinus* and *P. pipistrellus*. Our results showed an avoidance of major roads
344 by bats up to five km away from the road without noticeable slope change according to the
345 distance to the major road [except for the clutter-adapted species and *Myotis* spp. where major
346 roads had a much stronger negative effect at distances shorter than 1 km to the road
347 (Fig. C.2)]. Moreover, our results show a greater impact than previously identified. Indeed,
348 the effect of major roads was not limited to a few metres as in Kitzes & Merenlender (2014)
349 or in Medinas et al. (2019) but had an impact at the landscape scale, highlighting possible
350 impacts at population scales. Such impacts at the landscape scale are congruent with the
351 Berthinussen and Altringham (2012a) study, which showed that bat activity was 3.5 times
352 higher at a distance of 1600 m away from roads than at major roads. These studies, excluding
353 Medinas et al. (2019), could not detect a greater (in terms of distance) impact of major roads
354 on bat activity as negative impacts were still detected at the maximum distance under
355 investigation. In addition, according to our sampling plan (i.e., acoustic surveys within five
356 kilometres in the vicinity of the major road), we cannot know if the impact is greater.
357 However, we hypothesise that the impact of major roads on bat activity occurs at distances
358 even greater than five kilometres given that the effect detected is linear across all investigated
359 distances.

360 Moreover, our results are congruent with those of Fensome & Mathews (2016) who
361 found, in Europe, that low-flying species are more prone to road kills than high-flying species.
362 This can be explained by the ecology of the species. Indeed, ‘clutter-adapted’ species are

363 gleaners more so than 'open-adapted' species and thus forage more in woodlands and fly less
364 in open space. Furthermore, in France, *Myotis* spp. are considered the species with the most
365 road fatalities (Capo et al., 2006). Hence one hypothesis would be that the mortality induced
366 from road kills decreases local populations densities, implying lower activity. We also found a
367 significant interaction between the distance to major roads and hedges for *E. serotinus*, *Myotis*
368 spp., *P. pipistrellus* and 'clutter-adapted' species. Although there is an imperfect distribution
369 of points along the gradient of the interaction, these groups appeared to use more hedges
370 when they fly closer to a major road. It is suggested that this is a possible behavioural
371 response when exposed to a situation perceived as risky (i.e., bats seek refuge in hedges).
372 Other interactions should also be explored, such as that of the temperature and woodlands
373 (Berthinussen and Altringham, 2012a; Kitzes and Merenlender, 2014).

374 Another non-exclusive hypothesis to explain the effect of major roads on bat activity
375 could be that bats avoid areas with light and noise from traffic. First, it is known that bats
376 avoid traffic noise itself because they are averse to it. Noise disturbs the movement of bats
377 (Bennett and Zurcher, 2013; Bosen et al., 2015; Schaub et al., 2008; Siemers and Schaub,
378 2011) and reduces their foraging performance, and can adversely affect even those species
379 that do not rely on sounds to find prey with direct fitness effects (Luo et al., 2015). This
380 impact of noise can also depend on the habitat context, and more research is needed to better
381 understand the extent of its impact (Luo et al., 2015). Second, vehicle headlights impact
382 biodiversity (Gaston and Holt, 2018) and especially bats that can be impacted while
383 commuting or foraging at different spatial scales (i.e., 50 m to landscape scale) (Azam et al.,
384 2016; Hale et al., 2015; Stone et al., 2009). Furthermore, artificial light at night can induce
385 habitat loss (Azam et al., 2018).

386 A last but non-mutually exclusive hypothesis to explain bats' avoidance of the vicinity
387 of major roads is the rupture of habitat connectivity by bisecting the bat commuting routes,

388 such as linear features (e.g., hedges, watercourses) and non-linear habitats (e.g., woodlands).
389 Although bats are able to cross large roads of up to 100 m (Abbott et al., 2012; Claireau et al.,
390 2019b), major roads create a barrier effect, and the probability of crossing the road gap
391 decreases with an increase in gap width (Bennett and Zurcher, 2013; Entwistle et al., 2001;
392 Pinaud et al., 2018). The decrease of the accessibility of foraging areas caused by the barrier
393 effect can have more consequences on species' survival than when direct habitat loss is
394 considered alone (Eigenbrod et al., 2008), reducing the reproductive success in proximity to a
395 major road (Kerth and Melber, 2009) and decreasing home range quality, thus potentially
396 affecting their fitness and population dynamics (Froidevaux et al., 2017). Overall, there is a
397 consensus regarding the importance of preserving connected linear features to facilitate bat
398 commuting within the landscape (Hale et al., 2012).

399 Further studies should be carried out to assess the relative importance of these
400 different mechanisms explaining the observed decrease in bat activity in the area surrounding
401 roads. Ideally, these studies should also consider the habitat type (Berthinussen and
402 Altringham, 2012a; Pourshoushtari et al., 2018).

403

404 *4.2 Limitations and robustness of results*

405 Our results are robust regardless of the level of error rate considered, except for those of
406 *P. pipistrellus*, where the influence of distance to major roads became not significant when we
407 considered a 0.1 maximum error rate. Using a 0.5 maximum error rate threshold seems to be a
408 satisfying trade-off to retain a good quantity of data while limiting the number of false
409 positives. More restrictive thresholds aiming to reduce the false positives rate can also
410 generate false negatives by discarding true positives. This constitutes a possible explanation
411 for the loss of significance detected for *P. pipistrellus*.

412 Our sampling design (3 sites, 306 points sampled, high proportion of simultaneous
413 sampling) provides a powerful analysis. Hence, we hypothesize that if there is an effect of
414 major roads on the other tested species, it should be weak for species with large amounts of
415 data. For species with very few data, such as *R. ferrumequinum* and *Plecotus* spp., which are
416 ecologically similar to *R. hipposideros* ('clutter-adapted' species, use of linear element), we
417 did not detect an effect of major roads on their activity. For *R. ferrumequinum*, this can be
418 explained by the fact that many data from the A10 site (91 %) were influenced by the colony
419 of Annepont close to the highway [740 m; (Pinaud et al., 2018)], which also might bias the
420 results for this species (Table C.2). However, for one 'clutter-adapted' species with sufficient
421 data, *B. barbastellus*, we did not find an effect of major roads on their activity. This absence
422 of an effect requires further investigation with more replications.

423

424 *4.3 Road-effect zone*

425 This study shows that roads should be considered a major pressure on bats because 35 % of
426 the European Union, by extrapolation of our results, is potentially impacted by major roads.
427 This calculations do not consider the potential cumulative road-effect zone of other roads in
428 Europe (Medinas et al., 2019). From now on, road construction must take into account the
429 road effects for bats. Considering that other taxa are also impacted by roads, e.g., Forman
430 (2000) found a road-effect zone covering approximately one-fifth of the USA land area for
431 bird species, it seems urgent to consider the road-effect zone in land use policies and to
432 implement drastic conservation practices for species of conservation concern.

433

434 *4.4 Recommendations*

435 This study highlights a major effect often neglected in mitigation hierarchy (Bigard et al.,
436 2017). It is necessary to develop strategies to limit the impact of roads on bats through, for

437 example, upgrading old roads instead of building new roads in habitats of good quality for
438 bats or looking to radically anew transport strategies as proposed by Lawrence (2018). The
439 effect of roads must be considered at the landscape level to efficiently preserve habitats and
440 commuting routes, especially in areas of particular importance for conservation such as
441 Natura 2000 areas.

442 If avoidance of road impacts is impossible (i.e., major roads impact bat foraging
443 areas), it is necessary to improve habitat connectivity. Many mitigation measures have been
444 proposed to restore habitat connectivity, such as the implementation of overpasses (e.g.,
445 wildlife crossings), underpasses (e.g., viaducts), speed reduction, deterrence and diversion
446 (e.g., planting hedges), and habitat improvement (Møller et al., 2016). Recent studies have
447 suggested that green bridges and underpasses could be the best solution to restore ecological
448 continuity, whereas bat overpasses seem to be less effective because green bridges and
449 underpasses offer greater protection to cross the roads (i.e., reduce light and noise
450 disturbances and allow bats to cross the roads safely) (Abbott et al., 2015, 2012; Berthinussen
451 and Altringham, 2015, 2012b; Claireau et al., 2019b; Møller et al., 2016).

452 Furthermore, mitigation measures are not sufficient to improve the habitat loss
453 induced by major roads. It is necessary to reduce the impacts of major roads, such as light and
454 noise disturbance, by planting hedges, for example. Moreover, it is necessary to propose
455 offset measures by improving the quality of habitat in the wider areas surrounding major
456 roads. These measures must be considered in the planning state of the road project (i.e.,
457 developers must anticipate the purchase of land to compensate for habitat loss).

458 Finally, it is imperative to know if these measures have been proven to be efficient
459 (Quétier and Lavorel, 2011) thanks to before-after and control-impact (BACI) studies
460 (Claireau et al., 2019a; Roedenbeck et al., 2007).

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722

1 **Major roads have important negative effects on insectivorous bat activity**

2

3 **Tables and figures**

4

5 **Table 1** Total bat passes, occurrence, number of points with recorded bat passes (%) and

6 mean with standard error of bat passes per sample point for each sample point at 0.5

7 maximum error risk tolerance.

Species	Total bat passes	Occurrence on 306 sample points	Occurrence (%)	Mean of bat passes per sample point	SE of bat passes per sample point
<i>B. barbastellus</i>	2 836	181	59.15	9.27	1.46
<i>E. serotinus</i>	5 143	167	54.58	16.81	3.78
<i>Myotis</i> spp.	18 282	244	79.74	59.75	12.53
<i>N. leisleri</i>	1 726	111	36.27	5.64	1.25
<i>N. noctula</i>	1 657	69	22.55	5.42	1.80
<i>P. kuhlii</i>	29 090	222	72.55	95.07	17.74
<i>P. pipistrellus</i>	162 456	299	97.71	530.90	53.75
<i>Plecotus</i> spp.	1 257	141	46.08	4.11	0.80
<i>R. ferrumequinum</i>	319	53	17.32	1.04	0.30
<i>R. hipposideros</i>	1 035	105	34.31	3.38	1.25

8

9 **Table 2** Estimates, standard errors and p-values of the distance from the major road variable
 10 in the best model for all bats, the two guilds, the two species group and the eight species
 11 studied according a maximum error in species identification risk of 0.5. Legend: *, spatial-
 12 correlation in the model subsists even if we added the autocov_function; X, distance from the
 13 major road, not selected in the best model. Complete results of other covariates can be found
 14 in Table C.1.

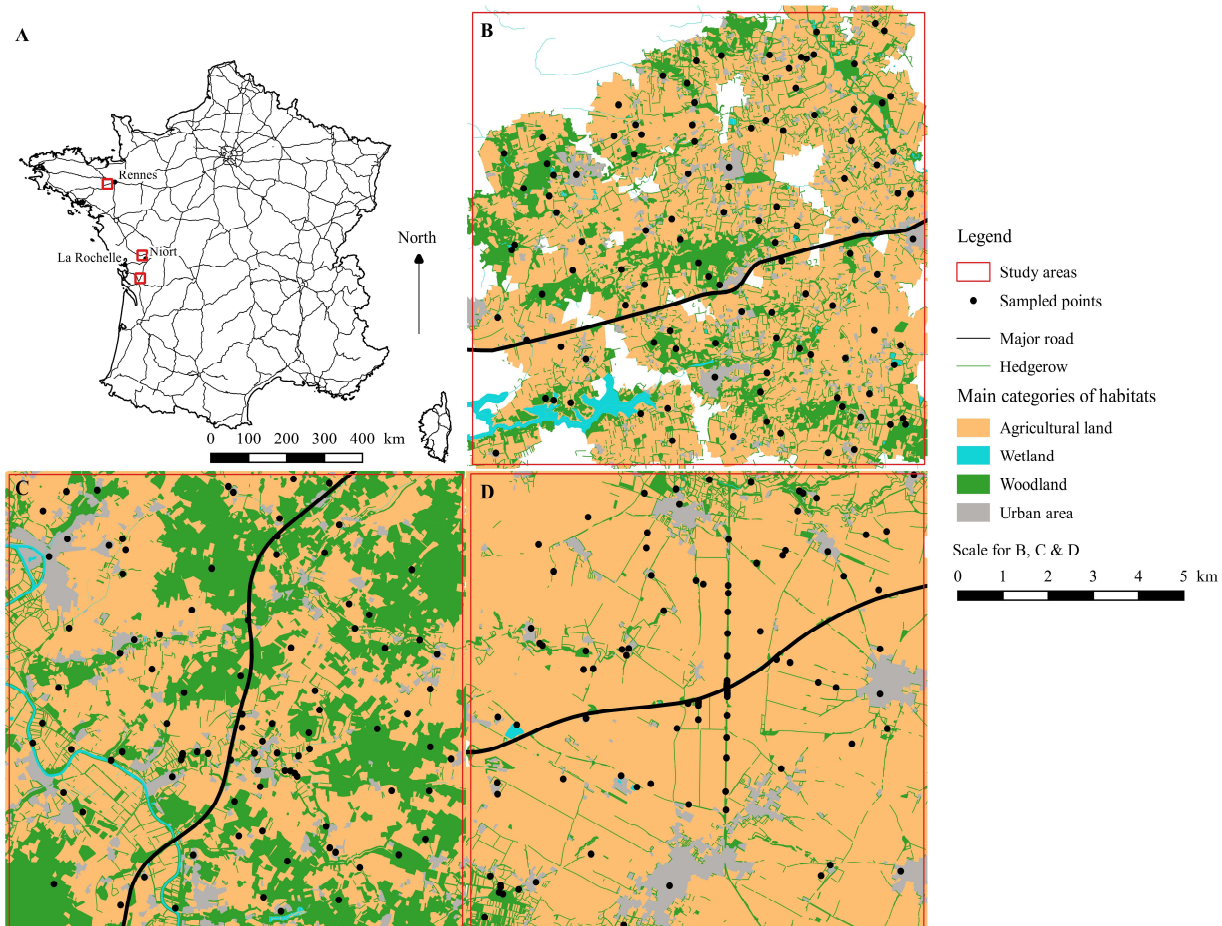
	All bats	Aerial species	Clutter species	<i>B. barbastellus</i>	<i>E. serotinus</i>	<i>Myotis</i> spp.	<i>N. leisleri</i>
β	0.13212	0.10848	0.28870	-0.11880	0.34200	0.41421	X
SE	0.07095	0.07703	0.08566	0.13490	0.15910	0.09659	X
p-value	0.06260	0.15900	0.00075	0.37853	0.03160	0.00002	X

15

	<i>N. noctula</i>	<i>P. kuhlii</i>	<i>P. pipistrellus</i>	<i>Plecotus</i> spp.	<i>R. ferrumequinum</i> *	<i>R. hipposideros</i>
β	X	0.03277	0.18830	X	-0.02274	0.47630
SE	X	0.13775	0.08159	X	0.22713	0.20250
p-value	X	0.81190	0.02100	X	0.92030	0.01870

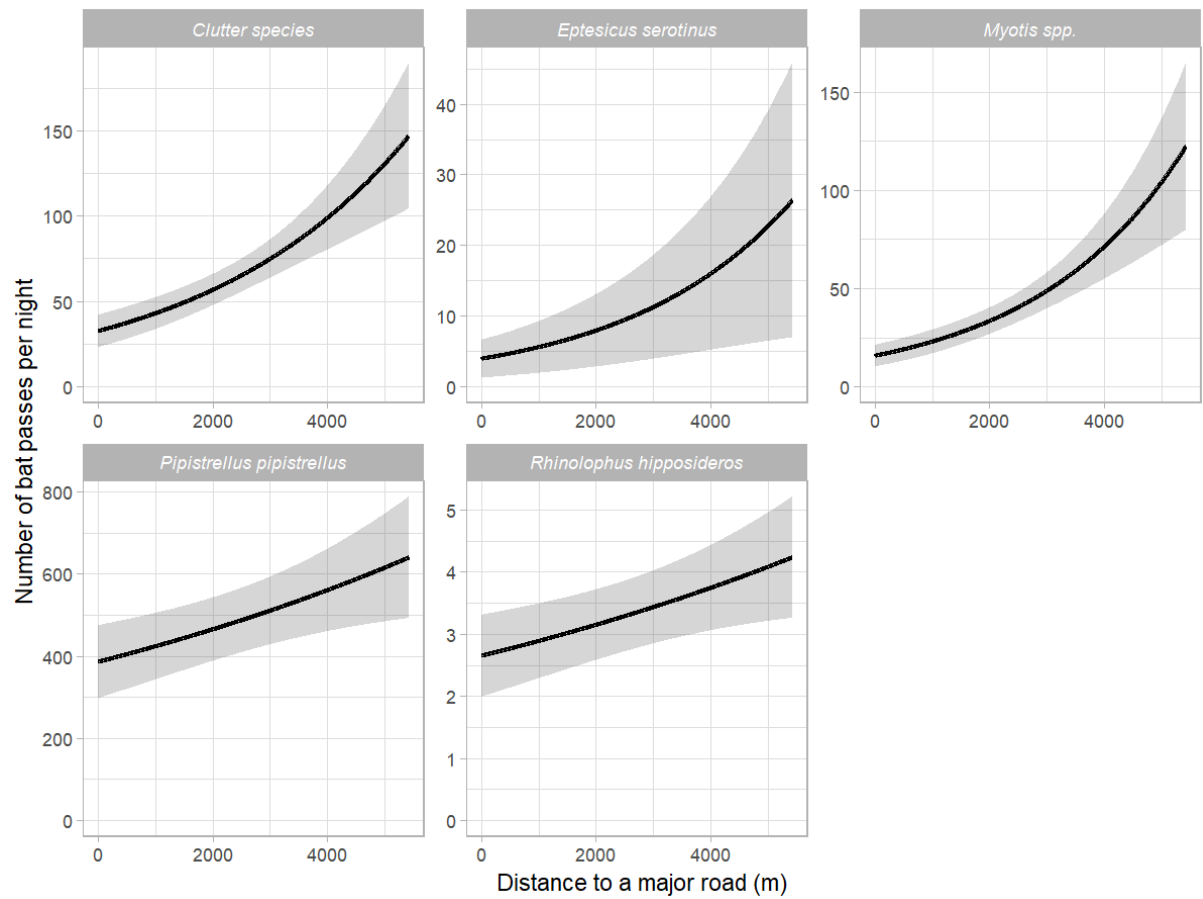
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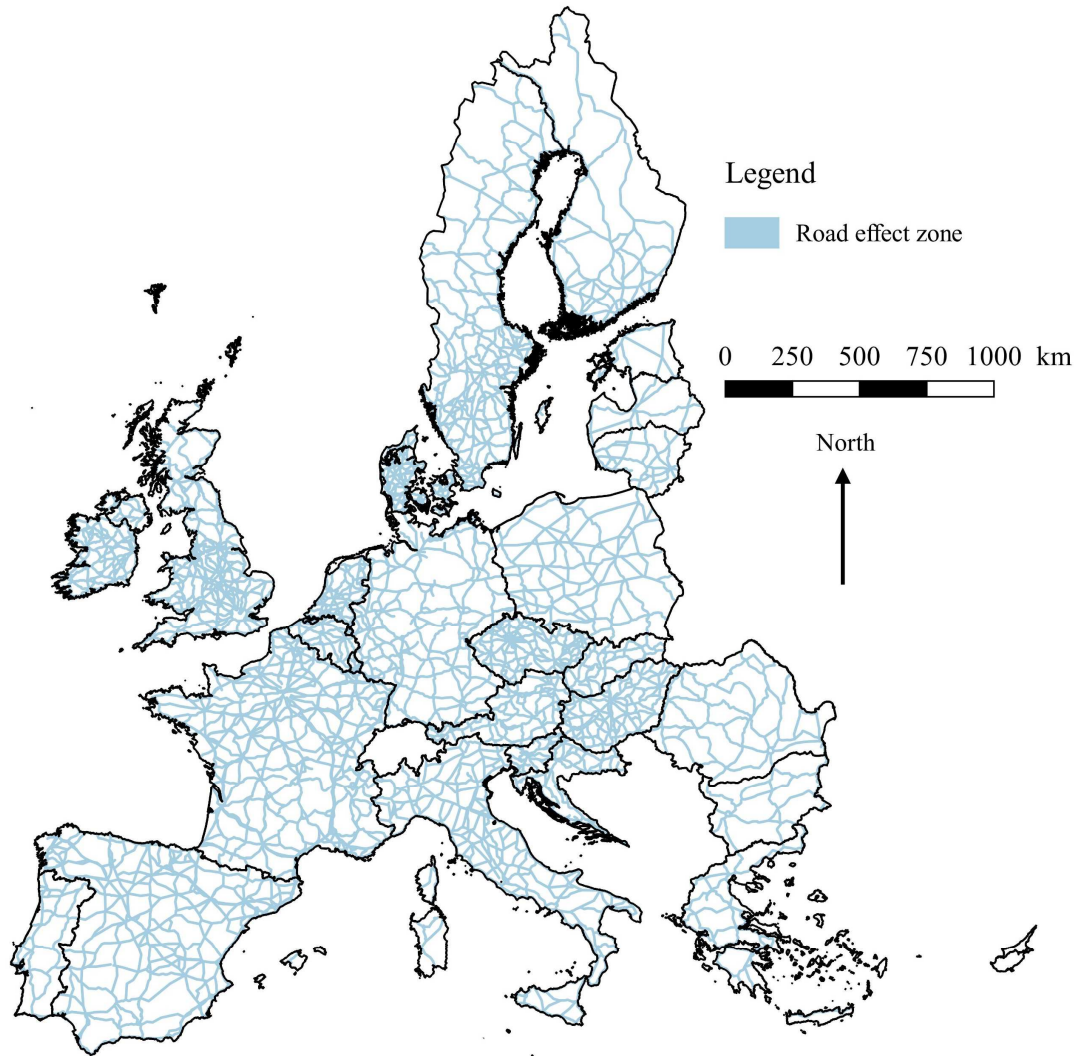
19 **Figure 1** Study sites: N24 (B), A10 (C) and A83 (D). Manual mapping of land use in five
 20 main categories of habitat. Black points represent sample points of recordings.



21

22 **Figure 2** Model prediction of the effect of the distance to a major road on the number of bat

23 passes.



24

25 **Figure 3** Map of areas impacted by major roads in Europe.