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Major roads have important negative effects on insectivorous bat activity

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Authors' contributions

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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Major roads have important negative effects on insectivorous bat activity

Running title: Roads and bat activity

Highlights

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

Abstract (241/250)

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

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

Keywords

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

1 Introduction

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

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

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

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

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

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

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

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

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

2 Materials and methods

2.1 Study sites

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

2.2 Sampling design

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

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

2.3 Acoustic surveys

Fieldwork was carried out during the seasonal peak of bat activity between the 28th of May and the 17th of August 2016. Recordings were conducted during nine successive nights for A83 (in May-June), eight successive nights for A10 (in July) and ten successive nights for N24 (in August). Recordings were also conducted under favourable meteorological night conditions as follows: temperatures (A83: \bar{x} , 14.37 °C; SE, 0.47; A10: \bar{x} , 19.51 °C, SE, 3.31; 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 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; N24: \bar{x} , 8.1 km/k; SE, 1.72).

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

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

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

2.4 Species identification

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

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

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

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

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

2.5 Environmental variables

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

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

2.6 Bat activity modelling

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

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

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

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

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

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

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

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

2.7 Road-effect zone

Following the Forman & Deblinger (2000) approach, we assessed the potential extent of the "road-effect zone" at the scale of the European Union. Drawing on the results obtained in our models by species (i.e., the distance impact found and the linear or non-linear effect), we created a buffer around all roads considered major roads in Europe [based on the E-Road 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.

3 Results

3.1 Bat monitoring

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

3.2 Impact of major roads on bat activity

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

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

4 Discussion

4.1 Road effects

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

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

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

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

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

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

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

4.2 Limitations and robustness of results

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

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

4.3 Road-effect zone

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

4.4 Recommendations

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

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

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

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

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

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Major roads have important negative effects on insectivorous bat activity

Tables and figures

Table 1 Total bat passes, occurrence, number of points with recorded bat passes (%) and mean with standard error of bat passes per sample point for each sample point at 0.5 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

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

	<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

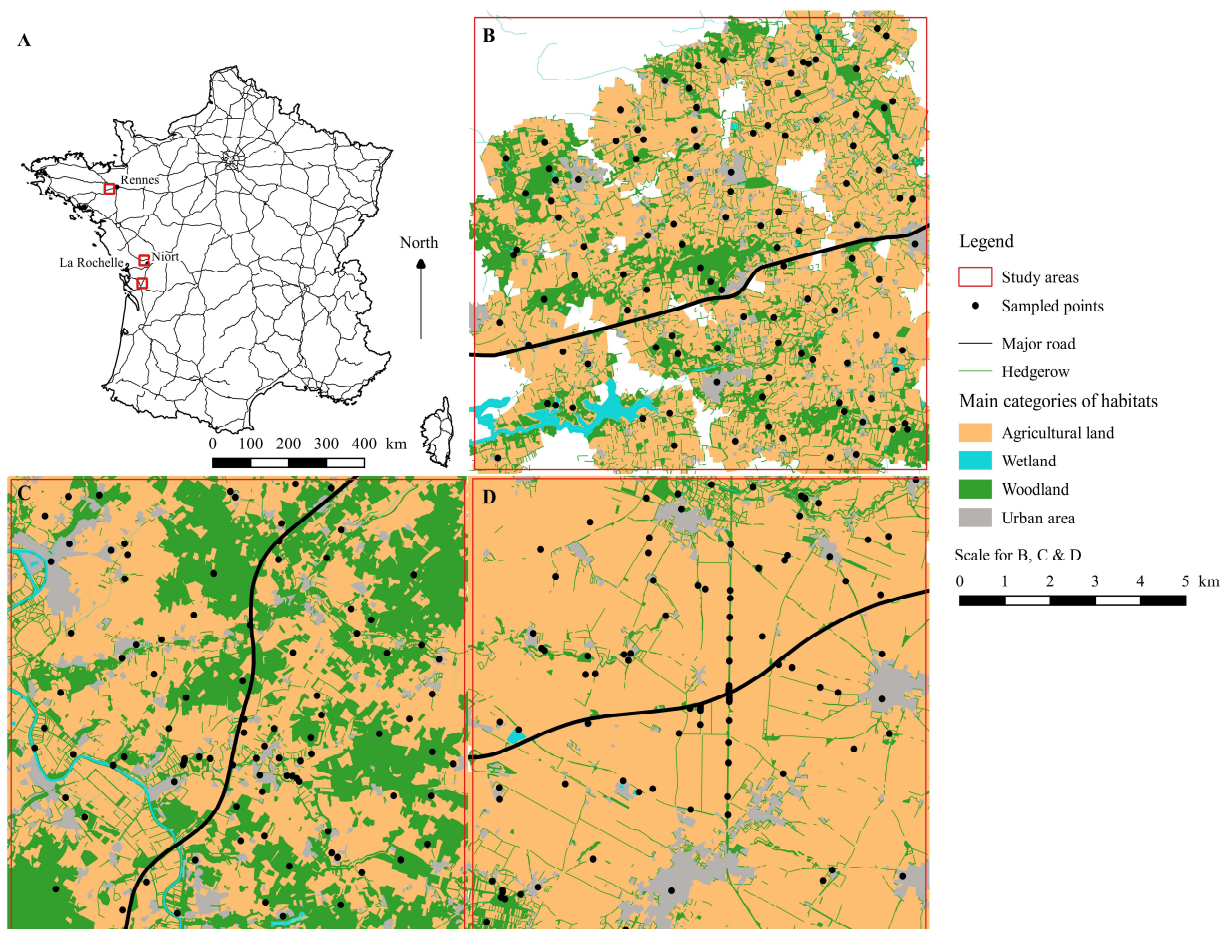
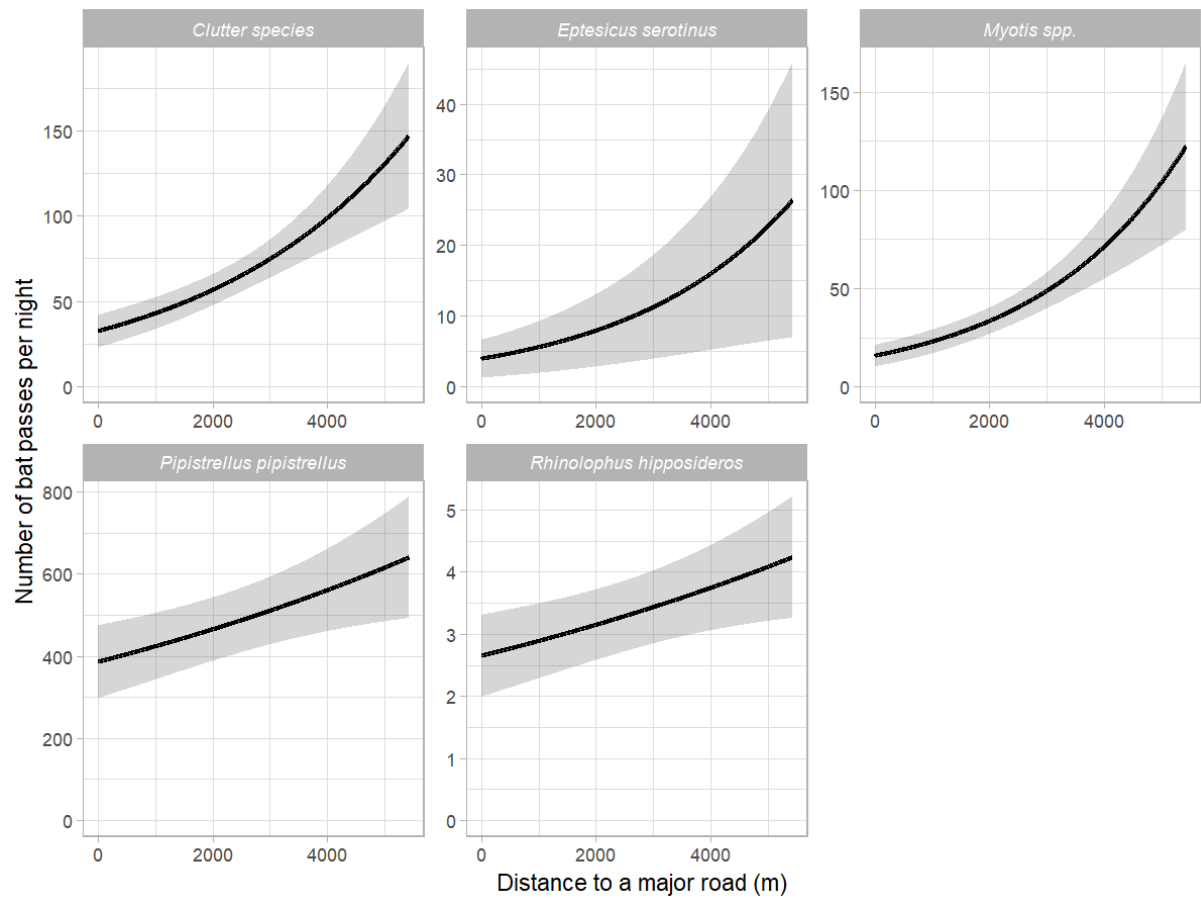


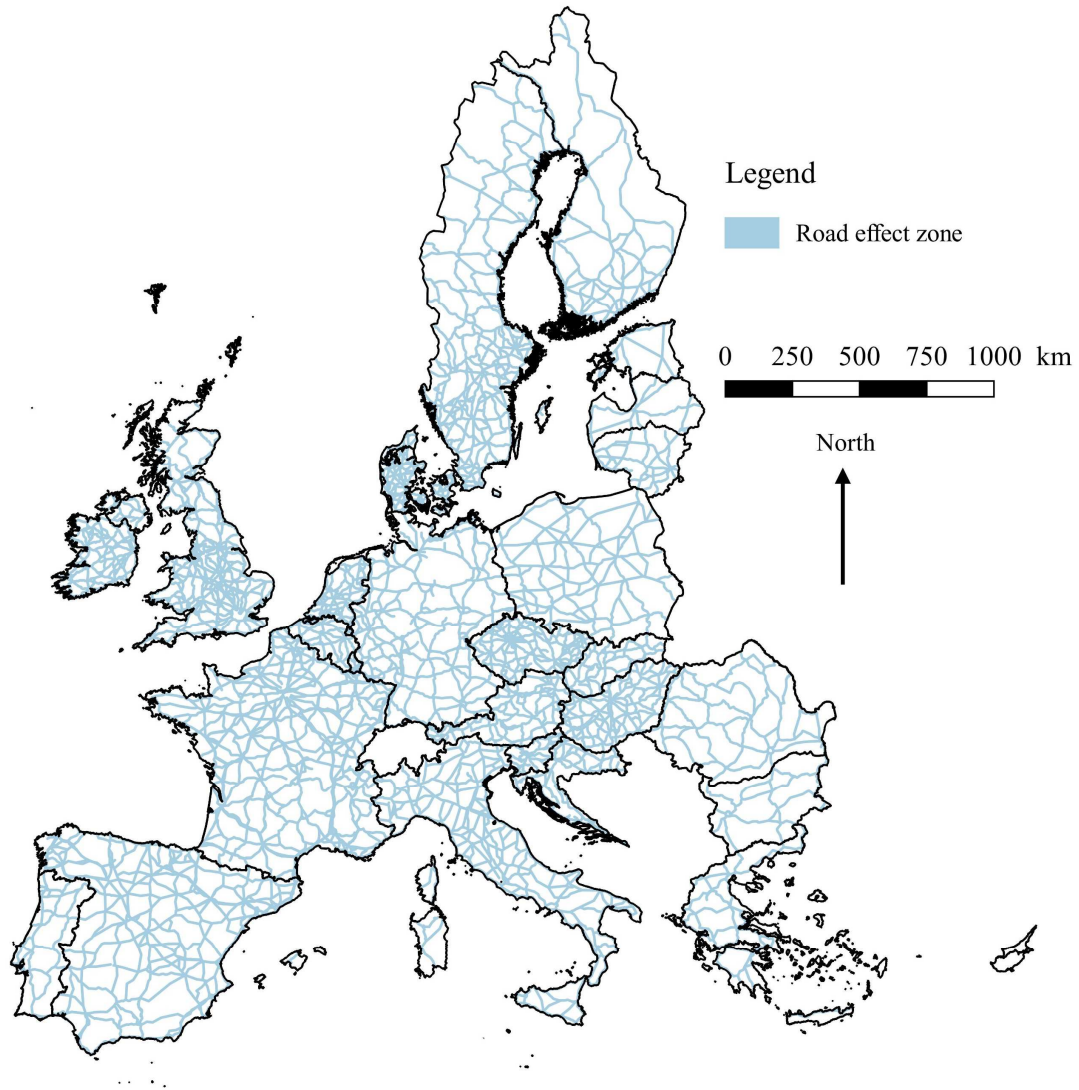
Figure 1 Study sites: N24 (B), A10 (C) and A83 (D). Manual mapping of land use in five main categories of habitat. Black points represent sample points of recordings.



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.