Bats actively track and prey on grape pest populations

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ABSTRACT

There is growing evidence about the role of insectivorous bats against agricultural pests in various crops. Nevertheless, little research addressed the aggregational and functional responses of bat assemblages to changes in pest availability across a spatio-temporal scale. Therefore, we examined the activity and diet habits of different bat species using DNA metabarcoding by simultaneously monitoring the relative abundance of two major pests (the European grapevine moth, *Lobesia botrana*, and the leaf rolling tortrix, *Sparganothis pilleriana*) through the grape growing season, in a vineyard region of the Iberian Peninsula. During pest major irruptions, we found the highest bat activity levels and frequencies of grape pests in the diet of bats, although not all bat species contributed equally to pest suppression. Bats of different foraging guilds positively responded to pest abundances, indicating distinct bat species may synergistically play a role at suppressing agricultural pests at broad scales of the aerospace. For instance, narrow space foragers exploiting major irruptions in grape interior, edge space foragers hampering pest dispersion at local scale, and open space foragers preventing infestations of new grapevine patches at broader scales. Yet, our study exposed the current methodological constraints regarding pest dispersion dynamics, acoustic monitoring of bats' foraging activity or the unfeasibility of metabarcoding to reliably quantify prey abundance in bats diet, and thus further improvement in these issues is required in order to gain insight on the agroecological interactions between bats and pests.

1. Introduction

Insect pests are responsible for substantial annual losses in crop production (16–26%; Oerke, 2006; Culliney, 2014). Currently, more sustainable pest suppression solutions to conventional chemical pesticide use are in demand by consumers, and as a measure to tackle the biodiversity crisis caused by intensive farming. Those alternatives stand primarily on the spraying of the *Bacillus thuringiensis* toxin (Ifoulis and Savopoulou-Soultani, 2004), pheromone-based mating disruption techniques (Louis and Schirra, 2001; Louis et al., 2002), the introduction of egg parasitoids (Moreau et al., 2009) and on identifying, preserving and promoting the contribution of local natural enemies (Pickett et al., 1998; Begg et al., 2017). Unlike specialist predators, generalists that show temporal persistence can be efficient biocontrol agents in these agroecosystems since, as opportunistic feeders, they may shift between different pest outbreaks feeding on alternative prey while main pest species are scarce or rare (Ehler, 1998; Symondson et al., 2002). Besides, natural enemies of pests to be effective in agroecosystems should have,

1) a rapid colonizing and dispersal ability to track sudden spatio-temporal pest invasions (Ehler and Miller, 1978); 2) opportunistic feeding behaviour that elicits the exploitation of highly changeable pest outbreaks, and 3) an aggregative response to pest availability (Symondson et al., 2002). The ecological features of some insectivorous bats not only comply with these requirements but bats have regularly been documented to display an aggregative and functional response to pest bursts (McCracken et al, 2012; Charbonnier et al., 2014; Puig-Montserrat et al., 2015; Korine et al., 2020).

Note that monoculture crops surrounded by simplified landscape cover the vast majority of current agricultural fields. Thereby, a substantial part of the prey source of bats in these crop systems often comes from insect pests (Symondson et al., 2002; Segoli and Rosenheim, 2012). Assuming that predators' distribution, abundance and activity depend on the spatial dispersion, patchiness and variation of food accessibility (Resource Dispersion Hypothesis; Carr and MacDonald, 1986), we should expect predator–prey interactions to be directly influenced by the aggregational and functional responses of predators to prey density

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(Abrams and Ginzburg, 2000; Bayliss and Choquenot, 2002). The aggregational response implies that predators' density and activity patterns are coupled with prey numbers in patches with high food supply (Hassell and May, 1974), whereas the functional response asserts that predators adjust their intake rate to prey abundance (Goss-Custard et al., 2006). Large populations of prey are spatio-temporally structured, meaning that successful predators should be able to reach bountiful feeding patches. Bats can forage over many kilometres in a single night (Müller et al., 2012), but they also eavesdrop on successful foragers, resulting in an increasing number of bats that feed rapidly on newly available resources (Gillam, 2007; Cvikel et al., 2015). However, because flying is energetically expensive, some bats may have limited commuting range, which can limit their choice of foraging areas. For instance, while bats such as *Tadarida brasiliensis* or *Miniopterus schreibersii* can commute nightly to foraging sites located at up to tens of kilometres away from the day roost (Best and Geluso, 2003; Vincent et al., 2010), others (e.g. *Pipistrellus pipistrellus* or *Myotis lucifugus*) show limited nightly mobility (Henry et al., 2002; Davidson-Watts and Jones, 2006) and consequently, these species may not encounter potentially profitable but distant foraging sites.

The advent of DNA metabarcoding has extended our ability to detect pest species among bats' prey revealing that bats frequently consume pest arthropods (Aizpurua et al., 2018; Krauel et al., 2018; Baroja et al., 2019a). In most cases, bats attack adult aerial arthropods, avoiding them from laying eggs and preventing subsequent growth of larvae, which usually are responsible for crop damage (Russo et al., 2018). Pest exploitation by bats, though, does not necessarily imply bats performing a pest regulation function. To guarantee control over insect populations, bats should track pest abundance by increasing both their activity and intake rate as a response to sudden pest irruptions (Russo et al., 2018). So far, few works have addressed these issues, though, and some revealed that bats positively respond to pest abundances (McCracken et al., 2012; Charbonnier et al., 2014; Puig-Montserrat et al., 2015; Korine et al., 2020). Yet, some critical issues have been overlooked. For example, previous research either tended to focus on the bat's diet or on activity levels to assess bat-pest interactions, but both features are fairly important. Despite this, very few studies have simultaneously studied bats' diet, activity patterns and pest dynamics throughout the whole active period of any insect pest (but see Charbonnier et al 2021).

Grapevine crops, covering 7.4 million hectares, account for 15% of agricultural lands worldwide (OIV, 2019), and are thus considered one of the fruit crops with the highest economic importance (Vivier and Pretorius, 2002). They are regularly attacked by arthropod pests, resulting in huge yield reduction and consequent economic losses (Ioriatti et al., 2012; Thiéry et al., 2014; 2018;; Delbac and Thiéry, 2016). The European grapevine moth (*Lobesia botrana*: Lepidoptera, Tortricidae; hereafter "LB") and the leaf rolling tortrix (*Sparganothis pilleriana*: Lepidoptera, Tortricidae; hereafter "SP") are among the most destructive insects of grapevine in the Palearctic. Production losses are caused by direct damage to the plant as well as by the subsequent infection by fungi, such as *Botrytis cinerea* and *Aspergillus* sp., which cause grape cluster rotting (Moschos, 2005, 2006; Ioriatti et al., 2012). Besides, the species are rapidly responding to climate change by prolonging their active periods and spreading northward (Martín-Vertedor et al., 2010; Gutierrez et al., 2018). With this in mind, and in light of the potential pest regulatory function of insectivorous bats, the present paper aims to determine the bat-pest agroecological interactions in a vineyard system by analysing the aggregative and functional responses of bats to changes in pests' populations. To test this, we studied the spatio-temporal activity and diet habits of different bat species by simultaneously monitoring the relative abundance of the pests within a vineyard region in the northern Iberian Peninsula (Southwestern Europe). We hypothesized that bat activity and pest consumption patterns are associated with seasonal changes in LB and SP imagoes' activity.

2. Material and methods

2.1. Study region

The study was conducted in the Rioja wine region (42° 32′ N, 2° 34′ W), one of the most important wine-growing areas in Southwestern Europe (Fig. 1). The region has a continental Mediterranean climate with average annual temperatures around 13 °C and low mean annual rainfall (500 mm/year). The land use is mainly devoted to agriculture where vineyards predominate (52% of the total area). In 2017, during the grape-growing season from late April to the end of September, we simultaneously surveyed bats' activity, diet as well as the flight dynamics of LB and SP within the region.

2.2. Insect pest surveys

We deployed delta traps baited with synthetic sex pheromone (ECONEX) in the interior of eight randomly selected grapevine patches, to lure adult male LB and SP moths. Traps were placed one meter above the ground, and they were checked and sticky inserts replaced once every two weeks. We monthly substituted pheromone lures following the manufacturer's instructions. In our analysis, we also analysed data from additional LB traps deployed and surveyed at a regional scale by a public agency. Overall, we obtained 35 and eight data points for LB and SP, respectively, in each sampling time (Fig. 1).

2.3. Bat surveys

We placed eight automatic D500X ultrasound detectors (Pettersson Elektronik AB) alongside the moth traps (Fig. 1) to passively record the first four hours of bat activity after sunset (4896 recording hours in total). We set the detectors to record 3-second-long sequences at 300 kHz sampling rate and very high sensitivity. Location of detectors was stationary throughout the study period. We measured the number of bats passes for every two weeks and site (bat activity), to indicate relative bat activity (Walsh et al., 2004). We scanned recorded files with filtering software (Kaleidoscope v. 4.5.4, Wildlife Acoustics), discarding files with ≤2 bat calls, insect noise and non-biological sounds such as rain (Rydell et al., 2017). Then, we visually identified bat calls to the species level using BatSound v. 4.0.3. (Pettersson Electronic, AB) based on identification criteria by Barataud, (2015), Russ (2012) and Russo and Jones (2002), and grouped species according to foraging guilds following Denzinger and Schnitzler (2013): open space foragers (bats that exploit airborne insects flying far from background, e.g. *Tadarida teniotis*, *Nyctalus* spp., *Eptesicus serotinus*), edge space foragers (bats that exploit the airborne prey near the edges of vegetation, above the ground and water surfaces, e.g. *Barbastella barbastellus*, *Miniopterus schreibersii*, *Hypsugo savii* and *Pipistrellus* spp.) and narrow space foragers (bats that prey upon insects positioned on or close to vegetation or the ground, e.g. *Rhinolophus* spp., *Plecotus* spp. and *Myotis* spp.). Several bats, such as some *Myotis* spp. and *Plecotus* spp., could not be reliably identified to species and thus, they were classified at the genus level. Likewise, *Pipistrellus kuhlii* and *P. nathusii* exhibit similar call structure and overlap in spectrogram shape and sound frequency. Nevertheless, the former is relatively common and abundant, whereas the latter has only been recorded on very few occasions in the region (Aihartza and Garin, 2002). Therefore, these calls were assigned to *P. kuhlii*. Similarly, identifying calls of *Nyctalus leisleri* and *N. noctula* was not always feasible, thereby some bat passes were identified as *Nyctalus* spp. Finally, given the overlap in call structure and frequency of maximum energy between *Pipistrellus pygmaeus* and *M. schreibersii* as well as between *N. leisleri* and *E. serotinus* (Papadatou et al., 2008; Russo and Papadatou, 2014), several passes were classified as sonotypes *P. pygmaeus-M. schreibersii* and *N. leisleri-E. serotinus*. We also counted the "feeding buzzes" (calls emitted by bats before tackling the prey) as a measure of bat foraging activity (Gillam, 2007).
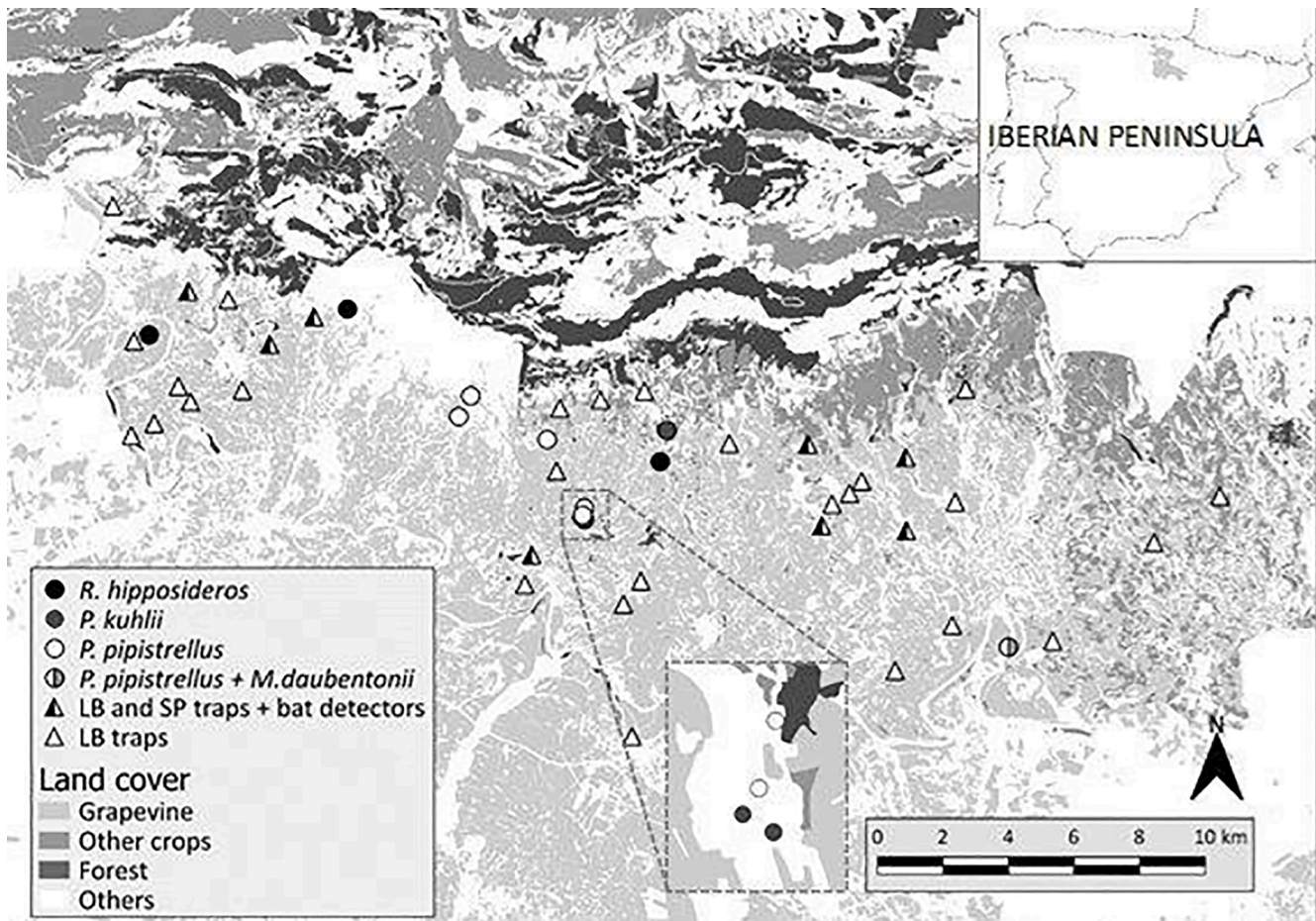
Fig. 1. Map of the study area showing land cover and locations of bats roosts, detectors and pheromone traps within the Rioja wine region (Southwestern Europe). LB = *Lobesia botrana*; SP = *Sparganothis pilleriana*.

2.4. Faecal sample collection

We identified 13 maternity roost sites exclusively inhabited by one of the following bat species: *Rhinolophus hipposideros* (three roosts), *P. kuhlii* (three), *P. pipistrellus* (six) and *Myotis daubentonii* (one). We placed collecting sheets beneath bat colonies and gathered faecal droppings every two-weeks during the roost occupancy period through the grape growing-season (Appendix A: Table A1). Collecting sheets were replaced after every two-weeks. Bat droppings were kept in 15 ml tubes, dried at 40 °C and then stored frozen at −80 °C until processed. For analysis, an average of 24.2 (±5) pellets was pooled per colony and two-week period in each sample, but we included more samples in those periods where pest outbreaks occurred, gathering 439 samples in total (Appendix A: Table A1). Finally, we homogenised each sample in a buffer solution before DNA extraction. No animal ethics clearance was required for this study because samples were passively and non-invasively collected, not involving manipulation of endangered or legally protected species.

2.5. DNA extraction, library preparation and sequencing

DNA was extracted from samples using the Dneasy PowerSoil Kit (Qiagen) following the manufacturer's protocol with a few modifications (see Baroja et al., 2019a; 2019b). We included extraction blanks in every extraction round. We used a combination of two cytochrome oxidase I gene (COI) primer sets to PCR amplify DNA from each sample to reduce primer-specific taxonomic bias (Elbrecht and Leese, 2015; Aldasoro et al., 2019). The first primer set, hereafter called "Zeale",

targeted arthropod prey DNA (Zeale et al., 2011). The second, henceforth called "Gillet", amplified both bat and prey DNA sequences (Gillet et al., 2015). For the amplification process we followed the Qiagen 2X kit protocol using 12.5 μL Qiagen Multiplex PCR kit 2x, 1.25 μL forward primer (10 μM), 1.25 μL reverse primer (10 μM), 8 μL H2O and 2 μL DNA for a total volume of 25 μL for each sample and primer set. Each primer set was subjected to different PCR cycling conditions (Appendix A: Table A2). Then, PCR products were migrated in agarose gel electrophoresis to test the efficiency and homogeneity of amplification. PCR negative controls were included in every amplification round and all blank extractions were checked for contamination. Amplicons were bead-purified with CleanPCR kit (CleanNA, PH Waddinxveen, The Netherlands). A second PCR reaction was performed to assign a dual unique combination of tags and Illumina sequencing adapters to the amplicons, using the Nextera XT Index Kit, following the guide for metagenomics sequencing library by Illumina (Illumina, 2013) with some modifications. Once indexed and adapters attached, samples were bead-purified, fluorometrically quantified and pooled at equal molarities to finally sequence in an Illumina MiSeq with 5%.

DNA library construction and sequencing processes were done at the Genomics and Proteomics General Service (SGIker) of the University of the Basque Country.

2.6. Metabarcoding bioinformatic procedures

After sequencing, paired-end reads were merged and quality-filtered and primers removed using Usearch v.10 (Edgar, 2010) and Cutadapt (Martin, 2011). Sequences in samples that were identical to those in the

corresponding extraction blanks were removed and the remaining sequences clustered into haplotypes using USEARCH's *-fastx_uniques* command. Singletons and chimaeras were discarded, and the remaining haplotypes collapsed into zero-radius operational taxonomic units (ZOTUs), an amplicon sequencing error-correction method used to infer accurate biological template sequences (Edgar, 2016). We manually assigned taxon to ZOTUs by comparing them against the reference Barcode of Life Database, BOLD (Ratnasingham and Hebert, 2007). We used the taxonomic assignment criteria of Razgour et al. (2011) with slight modifications as follows: a) when query sequences matched to a single reference species above 98.5% similarity value, we assigned it to such species; b) when the haplotype coincided with more than one species (>98.5%) belonging to the same genus, we ascribed it to the genus-level, and c) when it matched to several species of different genera (>98.5%), we only included those present in the Iberian Peninsula. Only arthropod DNA sequences were considered as potential prey items. Afterwards, for each sampling period and bat species, we calculated the % frequency of occurrences (%FOO) and percentage of occurrences (POO) of diet content. The former refers to the number of samples that contain a given food item divided by the total number of samples multiplied by 100. The latter is %FOO, rescaled so that the sum across all food items is 100% (Deagle et al., 2019).

2.7. Data analysis

2.7.1. Spatio-temporal dispersion patterns of pests

On the one hand, we evaluated the distribution patterns of pests via the Morisita's index and the Standardized Morisita index proposed by Morisita (1959) and Smith-Gill (1975), which estimates the degree of spatial dispersion and intraspecific aggregation level of populations. Hence, we studied clumpiness of SP and LB populations, for which we used *dispindmorisita ()* function of the *vegan* package in R (Oksanen et al., 2007). The index values ranged from −1 to 1 (<-0.5 = uniform; −0.5≤ ≤0.5 = random; > 0.5 = aggregated). On the other hand, given the spatio-temporal variation of insect populations (Hassell et al., 1991), we estimated the recommended sample size (number of insect traps; from Krebs, 1999, equation 7.17) in every two weeks under different levels of precision (defined as the closeness of repeated measurements to the same item, Krebs, 1999) for the densities of LB in the study area. We did not perform the sample size estimation analysis for SP due to the low number of traps.

2.7.2. Distance buffers

We created distance buffers around each recording station (0 km – "local"–, 2 km – "short"–, 5 km – "medium"–, 10 km – "long" – and whole study area – "regional"–) and bat roost (5 km – "medium"–, 10 km – "long"– and whole study area – "regional"–) using QGIS version 3.0.3 (QGIS Development Team, 2018). These buffers were used to estimate the relative mean abundance of LB and SP within the area surrounding each monitoring location, to decipher the bat response to grape pest abundances at different spatial scales. The scales analysed were limited by the number of traps within each buffer.

2.7.3. Statistical analysis

All the statistical analysis were carried out in R Studio v.1.2.5042 and R v.3.6.1. (R Core Team, 2019). Modelling the responses of predators to prey density fluctuations over spatio-temporal scales entails methodological issues, such as spatial and/or temporal dependency structures that must rigorously be regarded (Zuur et al., 2017). Not accounting for spatio-temporal dependencies results in biased parameter estimates and p-values (Zuur et al., 2017), which may lead to erroneous conclusions (Carroll and Pearson, 2000). Current statistical tools to deal with spatial and temporal correlation structures of binary or count data are rather limited in a frequentist setting (but see *glmmPQL* and *GLMM* functions of MASS [Ripley et al., 2013] and lme4 [Bates et al., 2018] packages, respectively). To overcome these issues, we

modelled the spatio-temporal dependency of our data through a Bayesian approach based on the Integrated Nested Laplace Approximation (INLA, Rue et al., 2009). We used *R-INLA* package in R (Bakka et al., 2018), which has proven very useful as multiple tools capable of handling with dependencies are available for spatio-temporal models.

We carried out the analyses on the four species that constitute the bulk of the bat activity in the study area (i.e. *P. kuhlii*, *P. pipistrellus*, *Hypsugo savii* and *T. teniotis*, totalling 89% of bat calls). We tested for the associations between the measured bat species' activity and relative mean abundance of pests (i.e. LB and SP) at the different distance ranges (0 km, 2 km, 5 km, 10 km and overall region). We checked different plausible distribution models for count data (Poisson and negative binomial) and selected them through DIC and WAIC values (Zuur et al., 2017), overdispersion check and model residuals' graphical plots for visual comparison of distributions. We included "site" (vineyard plot) as random effect and temporal correlation was modelled as a function of sampling date, adopting a random walk term of the first order, to account for spatial and temporal dependencies, and modelled the bat activity pattern with a negative binomial distribution, a log link and diffuse or uninformative priors. We further tested for spatial and temporal autocorrelation in the residuals of the selected models using *variogram()* and *acf()* functions of the respective *gstat* and *stats* R packages (Pebesma and Heuvelink, 2016; R Core Team, 2019). Concerning the relationship between the abundance of pests at each distance category (5 km, 10 km and overall region) and their consumption frequency by bats, we included "site" (bat roost) as a random effect and modelled the relationship with a binomial distribution, a logit link and diffuse priors. Nonetheless, some ordinary binomial models had an excess number of zeros (e.g. presence of LB in the diet of *R. hipposideros*) and in such cases, zero-altered (or hurdle) binomial (ZAB) models were implemented (Yee, 2015). Hurdle models are based on the assumption that zero counts are generated from a different process (binary) than are positive counts (Hilbe, 2011). The binary component is generally estimated using a Bernoulli distribution model on the presence and absence data whereas the positive count component is estimated using a zero-truncated binomial distribution model on the non-zero data (Hilbe, 2011; Zuur et al., 2017). For the rest, we checked for spatio-temporal dependencies as described above. In line with the Bayesian framework, we assessed the support for each parameter in the models by examining the 95% credible intervals. Precisely, we considered as important or significant the covariates whose coefficient density distributions did not contain zero between quantiles 0.025 and 0.975 in their intervals.

3. Results

3.1. Bat activity

We recorded 9440 passes of at least 17 species (Table 1) and 400 feeding buzzes. Most of the recordings belonged to open and edge space foragers such as *P. kuhlii* (42%), *P. pipistrellus* (32%), *H. savii* (11%) and *T. teniotis* (4%), which showed the highest foraging activity levels (Table 1). To a lesser extent, we also recorded *Nyctalus lasiopterus*, *N. leisleri*, *N. noctula*, *E. serotinus*, *M. schreibersii*, *P. pygmaeus*, *B. barbastellus*, *R. ferrumequinum*, *R. euryale* and *R. hipposideros*, as well as some bats from *Myotis* and *Plecotus* genera. The highest bat activity levels occurred during June and July, along with the pests' major outbreaks (Fig. 2). For the rest of months, bat activity remained at lower levels, except for *P. pipistrellus*, with an activity peak during LB third generation in August.

3.2. Diets of bats

Overall, 5550 ZOTUs were generated from DNA extracted and successfully amplified belonging to 436 faecal samples. Out of them, 2724 corresponded to 969 arthropod species from 14 orders (Table S1). The dietary spectrum of the four bat species was dominated by Lepidoptera

Table 1

Overall bat activity (number of bat calls) and number of feeding buzzes in the study area. Numbers in brackets correspond to the percentage of the total bat activity.

Bat taxa	Bat calls (%)	Feeding buzzes
Open space foragers:		
H. savii	1021 (11)	30
Nyctalus spp.*	198 (2)	1
T. teniotis	342 (4)	6
Edge space foragers:		
P. kuhlii	3941 (42)	179
P. pipistrellus	3047 (32)	163
P. pygmaeus	59 (<1)	2
Others	55 (<1)	1
Narrow space foragers:		
Myotis spp.	99 (<1)	0
Plecotus spp.	178 (2)	0
Rhinolophus hipposideros	20 (<1)	0
Other *Rhinolophus*	4 (<1)	0
Sonotypes:		
P. pygmaeus-M. schreibersii	479 (5)	18
Total bat activity	9440	400

*We included the sonotype *N. leisleri-E. serotinus*.

and Diptera, which accounted for most of the identified ZOTUs and their occurrences (Fig. 3). Among the most regularly consumed species *Emmelina monodactyla, Aproaerema anthyllidela, S. pilleriana, Metzneria hilarella, Cydia fagiglandana* or *Agrotix trux* prevailed within Lepidoptera whereas *Culex pipiens* and *Psychoda albipennis* predominated amongst Diptera (Appendix A: Figs. A1, A2, A3 and A4). Ephemeropteran species diversity was poor but a few species, such as *Choroterpes picteti, Ephoron virgo, Caenis luctuosa* or *C. pusilla* were rather frequent (Appendix A: Figs. A1, A2, A3 and A4), primarily in the diet of *M. daubentonii*, whose second most consumed prey were ephemeropterans (Fig. 3). The rest of orders were recorded at a much lower frequency (<10%) but yet, again, some species were frequently preyed upon by some of the bat species —*Hydropsyche exocellata, Lepidostoma hirtum* (Trichoptera), *Ophonus ardosiacus* (Coleoptera) or *Psammotettix confinis* (Hemiptera) (Appendix A: Figs. A1, A2, A3 and A4). We did not find hymenopterans in *M. daubentonii* nor blattodeans in *P. kuhlii* and some other taxa were only detected in a single bat species' diet: for instance, Thysanoptera was only consumed by *P. kuhlii;* and Dermaptera solely by *P. pipistrellus*. In addition, during LB and SP main outbreak period (June-July) some non-pest arthropod species appeared frequently in the diet of bats (Appendix A: Figs. A5, A6, A7 and A8). A few samples contained non-prey DNA

traces —including human (*Homo sapiens*), mouse (*Apodemus sp.*), rat (*Rattus* sp.), fungi (Eurotiales, Mucorales, Pythiales), bacteria (Rickettsiales, Xanthomonadales) and mite (Mesostigmata, Sarcoptiformes, Trombidiformes) —, which were considered environmental pollution. Sequences in blank samples corresponded mainly to potential prey taxa (Table S1).

3.3. Spatio-temporal distribution of pests and sample size estimation

The spatial distribution of LB and SP showed some degree of aggregation (Morisita index > 1 with p < 0.0001 and Standardized Morisita index > 0.5; Appendix A: Table A3), with the south-eastern corner of the study region as the main spot of pest abundance. Nevertheless, the locations of moth hotspots partially changed over time (Appendix A: Figs. A9, A10, A11, A12 and A13). The sample size estimation analysis revealed that the number of traps required for estimating the population density of LB varied with time and, as expected, increased abruptly at greater precision levels (Appendix A: Fig. A14). The power analysis suggested that the 35 traps used across all study sites encompassed pest densities with precision values between 30% and 50%, respectively. About 92 to 282 traps would be needed for a precision of 20% depending on mean and variance data for every two weeks (Appendix A: Fig. A14).

3.4. The response of bats to grapevine pests

3.4.1. Aggregational response

The aggregational response of the bats to LB and SP abundance measured over the various distance buffers from detectors was generally weak (Appendix A: Table A4). Greater SP densities triggered a significant increase in the activity of *P. kuhlii* (95% CI: 0.003, 0.045) and *H. savii* (95% CI: 0.004, 0047) at the local scale. For the rest of the bats, we did not find any effect within any distance range (Appendix A: Table A4). Besides, *P. kuhlii* activity increased when LB abundances raised over two, five and ten kilometres (95% CI: 0.004, 0.027; 95% CI: 0.005, 0.031; and 95% CI: 0.003, 0.029, respectively) but not at the local (95% CI: −0.003, 0.022) and regional scales (95% CI −0.008, 0.068). Similarly, *H. savii* and *T. teniotis* exhibited positive activity responses at low, medium and long scales (*H. savii* at 5 km, 95% CI: 0.002, 0.030; 10 km, 95% CI: 0.004, 0.029; and *T. teniotis* at 2 km, 95% CI: 0.004, 0.036; 5 km, 95% CI: 0.008, 0.05; 10 km, 95% CI: 0.002, 0.049) but not at the local (*H. savii*, 95% CI: −0.002, 0.029; *T. teniotis*, 95% CI: −0.011, 0.035), low (only *H. savii,* 95% CI: −0.001, 0.021) and regional scales (*H. savii,* 95% CI: −0.017, 0.031; *T. teniotis*, 95% CI: −0.093, 0113). By
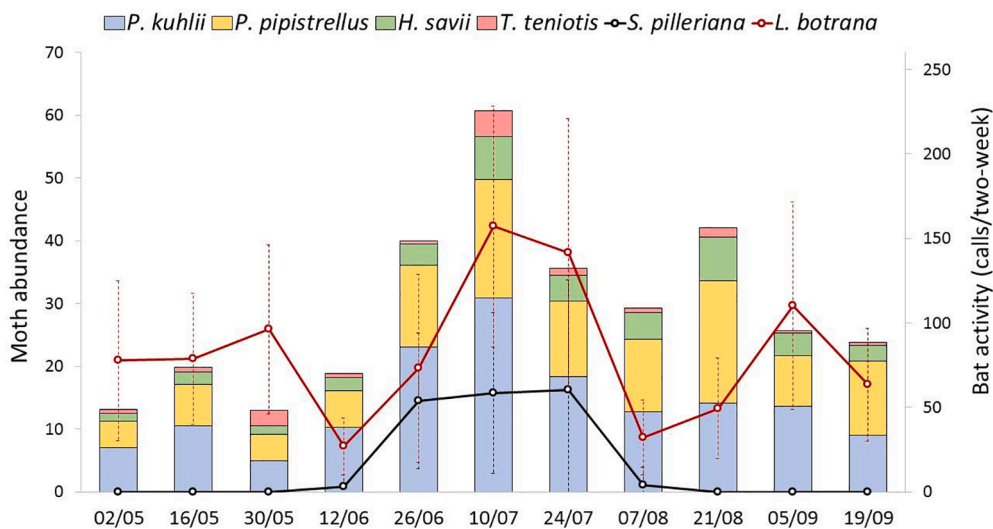

Fig. 2. Two-week mean and standard deviation (SD) of abundance values of *L. botrana* (three generations) and *S. pilleriana* (single generation, July) (left Y-axis) and mean bat activity (right Y-axis) from May to September 2017.
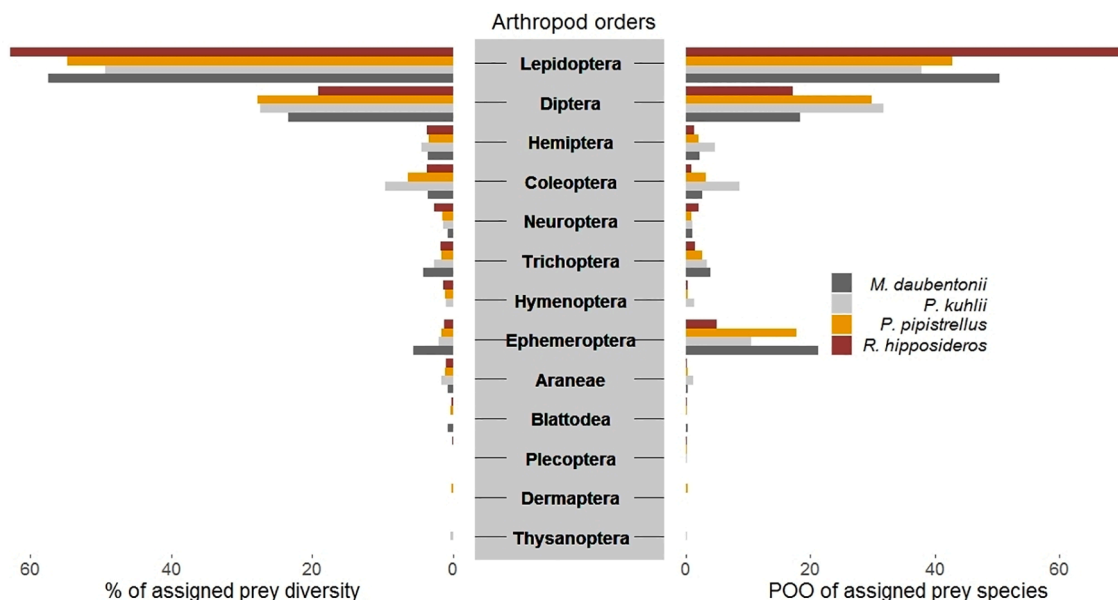
Fig. 3. Percentage of assigned prey diversity and percentage of occurrence (POO) of prey orders in the diet of each bat species.

contrast, we could not find such effect within any distance range in the activity of *P. pipistrellus*. Plots of models with significant effects of pest densities are shown in Fig. 4.

3.4.2. Functional response

DNA from SP was found in all surveyed bat species and it was detected in 33% of all bat species samples: in 10% of *P. kuhlii*, in 18% of *P. pipistrellus* and *M. daubentonii*, and even in 56% of *R. hipposideros*, where SP reached one of the highest frequency occurrences of all prey taxa in its diet (Fig. S1). Further, during the SP outbreak (July 2017) we found the highest frequency occurrences of SP in the bats' diet: for instance, 95% of R. *hipposideros* faecal samples contained the pest, 36% of *M. daubentonii*, 22% of *P. pipistrellus* and 20% of *P. kuhlii*. In some instances, bats also consumed SP when low or null abundances of it were recorded in traps (Fig. 5). All but *M. daubentonii* consumed LB, but only 10% of overall samples contained it: FOO ranged from 3.7% (*P. pipistrellus)* to 13% (*P. kuhlii*) and 18% (*R. hipposideros*). Amid the first generation of LB, we did not detect it in bat faeces, but during the second generation (Fig. 5) FOO of LB raised to 27% in *R. hipposideros*, 17% in *P. kuhlii* and 5% in *P. pipistrellus*. Lastly, during the third-generation consumption rates dropped to 13% in *R. hipposideros*, 6% in *P. pipistrellus* and zero in *P. kuhlii*.

We observed a strong association between the abundance of SP and its FOO in the diets of *R. hipposideros* (95% CI: 0.22, 0.37) and *M. daubentonii* (95% CI: 0.01, 0.33), but not in *P. kuhlii* (95% CI: −0.10, 0.66) and *P. pipistrellus* (95% CI: −0.01, 0.10). Further, the zero-truncated binomial distribution model showed an important association between the occurrence of LB in the *R. hipposideros* diet and its abundance at the medium (95% CI: 0.04, 0.15) and long scales (95% CI: 0.04, 0.17) but not, though, at the regional scale (95% CI: −0.02, 0.07). Nevertheless, the probability of presence of LB in the diet increased when greater numbers of LB were measured at regional scale (95% CI: 0.02, 0.17). On the contrary, we found no important effect of LB availability in *P. pipistrellus* diet within any distance scale (medium, 95% CI: −0.03, 0.07; long, 95% CI: −0.04, 0.07; regional, 95% CI: −0.05, 0.07). Finally, the model on *P. kuhlii* did not fit the data, probably due to limited sample size. Plots of models with significant effects of pest abundances are shown in Fig. 4.

4. Discussion

We provide proof of different predation rates and responses by a bat ensemble on two economically important pests of grape through their seasonal fluctuations. The observed pattern suggests that not all bat species contribute equally to that response. Based on our findings, *R. hipposideros* were the primary bats that prey upon of SP and LB moths. Their diet showed a much greater frequency of pest moths than any other diet of the bat species inspected. Moreover, the consumption rates of both grape pests were positively associated with their abundances in vineyards, indicating that *R. hipposideros* tracked SP and LB densities. Besides, other insectivorous bats such as *P. kuhlii, H. savii, T. teniotis* and *M. daubentonii* were also responsive to at least one of the grape pests, and they are likely to exert predation pressure on the adult stages of moths.

4.1. Spatio-temporal insect dynamics

Our results indicated that population dynamics of LB and SP within the study area showed an aggregated distribution like previously pointed by Sciarretta et al., (2008) and Peláez et al., (2006), with the south-eastern corner of the study region showing the highest density hotspots. These spatially structured patterns may likely answer to habitat suitability, due to slight local variations in temperature, humidity and wind strength or direction, which have a direct influence on the larval development and adult emergence (Weiss et al., 1993; Rank et al., 2020). In fact, the clumped pattern of pest populations leads to an exponential decrease in the provided level of precision and a concomitant increase in the required sample size (Subramanyam and Harein, 1990). The number of traps used in our study did not enable precise population density estimates. As many as 282 traps would be needed for accurate population calculations at the regional scale. The relatively low detection ranges of pheromone traps, together with the spatial heterogeneity of LB populations, highlight the need to augment the monitoring efforts by wine producers and public agencies. Uncertain spatiotemporal estimates of pest populations may lead to the application of misleading pest control treatments, resulting in the loss of human and economic resources. For our research purposes, though, such high degree of certainty was not critically necessary, especially considering that the number of traps used appropriately matched the number of expected generations of pests and their corresponding peak and slack periods with previous observations in the region (Ortega-Lopez et al., 2014).
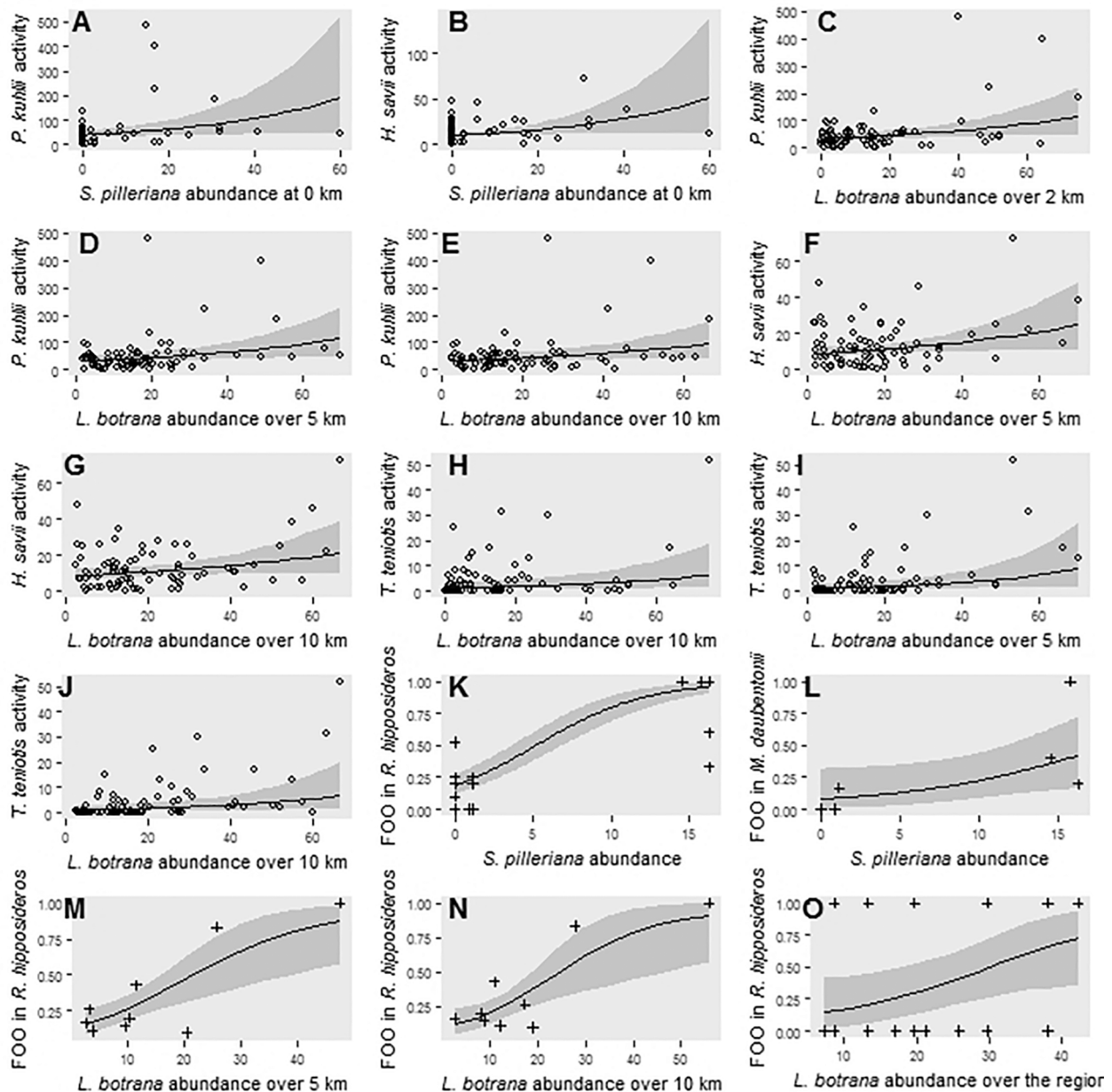
Fig. 4. Relationships between the density of *L. botrana* and *S. pilleriana* over different distance buffers and the activity of bats (number of calls/two-week, A-J) as well as pest abundances against their frequency of occurrence (FOO) in the diet of *R. hipposideros* and *M. daubentonii* (K-O).

4.2. Bat-pest interactions

The highest overall bat activity was found during mid-summer (July), co-occurring with the peaks of SP and LB (second generation) moth emergence. This increase in the bat activity, along with the positive aggregational response of some species (e.g. *P. kuhlii*, *H. savii*, and *T. teniotis*) to pest abundances, suggests bat species of different ecological and morphological features plausibly track abundances of SP and LB moths. Nevertheless, we did not observe strong species-specific responses and therefore, we claim that distinct bat species may synergistically play a role at suppressing grape pests, rather than the action of specific-species alone. The diet confirmed the consumption of both grape pests by at least some of the bat species, which primarily consumed them during pest irruptions, and thereby strengthened the role of bats as pest suppressors. Within the bat assemblage that we investigated, our results confirm *R. hipposideros* as the major candidate pest suppressor (Baroja et al., 2019a), primarily because pest

consumption frequencies were greater than for the other bat species, and there was a strong association between the abundances of SP and/or LB and their frequency of occurrences in its diet. *R. hipposideros*, a narrow space hunter, typically flies close to the vegetation in cluttered environments, which suggests that SP and LB encounters may preferentially occur on the crop canopy, or between grapevine rows at or close to ground level. Conversely, despite the increased activity levels of *P. kuhlii* during grape pest irruptions, it showed low consumption frequencies of grapevine pests. Previous work defined *P. kuhlii* as an opportunistic predator, feeding upon a vast array of prey taxa (Goiti et al., 2003; Cohen et al., 2020). As a consequence, prey and pests other than SP and LB that are also plentiful in agroecosystems (e.g. *Culex* spp. mosquitoes, Puig-Montserrat et al., 2020) may contribute significantly to this bat's diet.

Pipistrellus pipistrellus was the second most active bat in vineyards overall, but we neither observed an elevated activity during pest irruptions nor a responsive behaviour to their abundance. Accordingly,
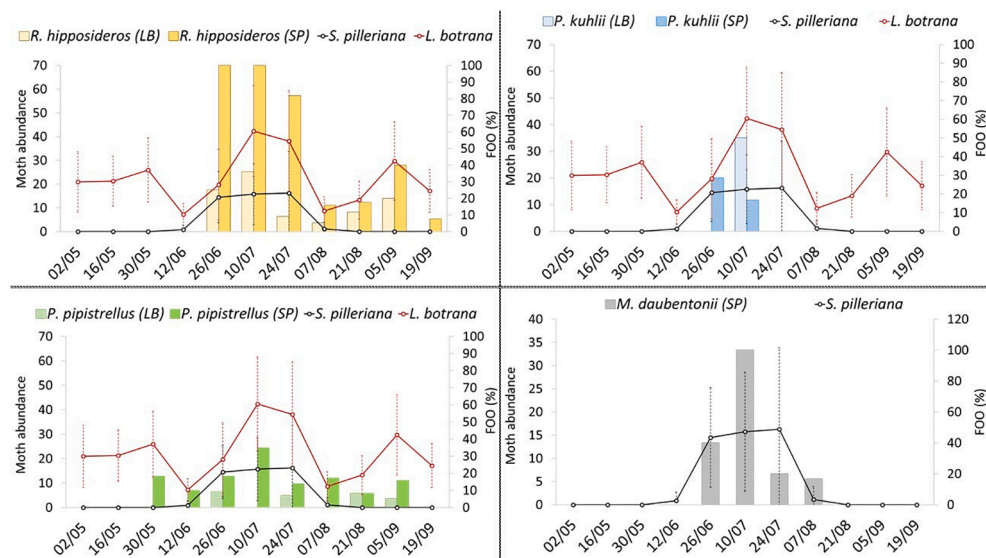
Fig. 5. Two-week mean and standard deviation (SD) of abundance values of *L. botrana* and *S. pilleriana* (left Y-axis) and their % frequency of occurrence in the bats' diet (right Y-axis) from May to September 2017. Consumed pest is shown in brackets.

the species did not functionally respond to grape pests. This may relate to various factors: firstly, *P. pipistrellus* is a synanthropic bat that forages in a wide variety of habitat types (Russ and Montgomery, 2002), including urban areas, treelines and forest and park edges, some of which were rather abundant just around the roosts; secondly, the species forages over relatively short distance ranges (<3 km) from roosts (Davidson-Watts and Jones, 2006). Further, we found aggregated dispersion of grape moths and, thus, *P. pipistrellus* populations might not reach pest-abundant but distant areas. Besides, our findings on its diet composition revealed some ephemeropterans (e.g. *Choroterpes picteti, Ephoron virgo, Caenis luctuosa* and *Caenis pusilla*) and dipterans (e.g. *Psychoda albipennis* and *Paramormia ustulata*) being frequent prey, especially during the LB and SP outbreak period from July. Finally, we found DNA traces of SP in *P. pipistrellus* faeces 2–4 weeks earlier and after the peak of the pest recorded by our traps, which suggests that the number of traps used to monitor SP did not entirely capture neither the temporal nor the spatial dynamics of the regional population.

Myotis daubentonii predominantly forages in riparian habitats or in the nearby (Swift and Racey, 1983), generally not farther than a few kilometres away from the roost (Nardone et al., 2015). Previous research found mostly Diptera, but also Trichoptera and Lepidoptera as their prevailing food items (Vesterinen et al, 2013; 2016). Thus, given the fondness for riparian environments and the feeding habits of *M. daubentonii*, we would expect low or null encounter rates with detrimental insects flying over grape clusters. However, the species exhibited a functional response to SP densities and consumed the pest during the moth outbreak (mid-summer). Given there were grapevines nearby the roost surrounded by the Ebro river, *M. daubentonii* would likely prey upon SP over water or in the vineyards adjacent to water bodies during its burst. *Tadarida teniotis* also exhibited pest density-responsive behaviour. Although we could not survey its diet, *T. teniotis* is a typical open space hunter that forages in open areas. While females primarily consumed large migratory moths that fly at high altitudes, males fed on smaller but more abundant sedentary moths flying closer to the ground than females (Mata et al., 2019) where encounters with swarms of LB are more likely to occur. Even if the intense narrowband and low-frequency echolocation calls of *T. teniotis* appear to be a specialization for long-range detection of relatively large insects (Rydell and Arlettaz, 1994), they can also detect swarms of small-size insects (e. g. *L. botrana*) from long distances (Boonman et al., 2019).

Lastly, *H. savii,* a typical representative bat of the Mediterranean, has expanded northwards its distributional range, presumably due to

temperature increase and the species' ability to settle in synanthropic environments (e.g. vineyards) (Uhrin et al., 2016; Ancillotto et al., 2018). Its ability to fly long distances in search of food, the capability to exploit locally abundant aerial prey sources and its generalist and opportunistic foraging requirements (Beck, 1995; Kipson et al., 2018), enable *H. savii* to take advantage of high concentrations of swarming insects, such as sudden pest irruptions. Accordingly, we should not disregard the potential contribution of *H. savii* as pest suppressor in vineyards. Unfortunately, we could not monitor its diet. The characteristic roosting behaviour of the species to commonly hide in rock crevices underline the intrinsic difficulties to monitor the diet of *H. savii*.

4.3. Guilds

Bats of every foraging strategy, open (*T. teniotis* and *H. savii*), edge (*P. kuhlii* and *M. daubentonii*) and narrow (*R. hipposideros*) space foragers showed a response, at least in a certain degree, to pest abundances. Although we cannot confirm the consumption of grape pests by open space foragers, these bats are known to prey upon other pests that are usually observed close to vegetation (Garin et al., 2019). The predation by different bat guilds would synergistically suppress these pests' populations, narrow space bats preying upon the moths on the canopy and at ground level in the crop interior, and edge and open space foragers hunting in the crop edges or above the grapevine canopy, likely hampering pests from spreading and interrupting the infestation of new grape patches. Further research in the vertical aerial space use by pests and the temporal diet analysis of bats from as many members of foraging guilds as possible would offer a more precise picture of the interaction between bats and pests in crops.

4.4. Distance buffers

Some bats are expected to respond to changes in prey communities at a more localised scale, while others are more likely to forage over larger hunting grounds and respond to regional-scale changes. As such, *R. hipposideros*, a highly manoeuvrable bat, positively responded to densities of LB measured up to ten kilometres from the roost. Previous research though, showed that it generally covers shorter foraging distances from the roost. However, given that their congeneric species (*R. euryale* and *R. mehelyi*; Goiti et al., 2006; Salsamendi et al., 2012) are capable of travelling long foraging distances, it is likely that *R. hipposideros* can travel longer distances in search of suitable foraging

areas than has hitherto been recorded. On the other hand, *T. teniotis, H. savii* and *P. kuhlii* exhibited responsive behaviours to SP and/or LB from local to large distance ranges, highlighting the flexible foraging behaviour of these highly mobile species to travel broad range distances in search of profitable areas (Marques et al., 2004; Uhrin et al., 2016; Ancillotto et al., 2018), potentially tracking sudden swarms of grape moths. Generally, the ability of a bat of any guild to suppress a given pest seemed to change with its spatial and foraging behaviour, particularly the distance to outbreaks and the availability of other profitable prey. For highly mobile animals like bats, foraging implies trade-off decision making between the energy gains foraging in prey-abundant areas, against the energy loss while commuting to such locations. In general terms, to promote ecosystem services provided by bats agri-environmental schemes should be oriented to multi-scale management planning (Kalda et al., 2015).

4.5. Implications and future directions

Generally, the ability of bats to aggregate and exploit grape pest irruptions but at the same time to persist and rely on diversified and alternative arthropods when pest numbers are low, confirms their opportunistic nature and highlights the pest suppression value of bats as generalist predators (Symondson et al., 2002; Snyder and Ives, 2003). Particularly, major damage levels are reached during the carpophagous phase (second and third generations of larvae) in which yield losses are especially critical (Moschos, 2006). Interestingly, bats might exert predation pressures mainly on the second generation of adult moths and therefore, they provide direct as well as indirect benefits to grapes. Estimating the economic importance of bats in vineyards is key to make policy-makers and the general public understand about the ecological, economic and social benefits of insectivorous bats. As such, Rodríguez-San Pedro et al., (2020) by exclusion field experiments, reported direct evidence of a reduction in grapevine pest infections by bats and their benefits to wine production in vineyards located in central Chile. However, given that Europe represents almost 50% of the world's vine-covered area (Eurostat, 2017), further efforts to economically quantify the contribution of bats as pest suppressors in this crop must be also addressed in this continent. Conservation measures and management strategies to promote bat communities and their ecosystem services in vineyards must be thoroughly deliberated. This may be done, for instance, conserving native forest remnants and shrubs, strengthening habitat heterogeneity, promoting artificial wetlands and increasing artificial roosting opportunities in vineyards (Stahlschmidt et al., 2012; Kelly et al., 2016; Rodríguez-San Pedro et al., 2019). Further, more intensive agricultural activities entail lower pest predation pressure by bats (Aizpurua and Alberdi, 2020) and therefore, management strategies that favour environmental sustainability may magnify the ecosystem services of bats in farmland.

4.6. Methodological constraints

In studies involving spatiotemporal pest and bat surveys, researchers find plenty of challenges and must make critical decisions that will compromise their results. It is essential researchers acknowledge them to design field and analytical approaches that optimise the methodologies used. Evaluating insect pest distribution is key to understanding questions related to pests' behaviour and ecology but also to develop forecasting systems to alert farmers about pest irruptions. The heterogeneous spatial pattern of insects though, emphasises the difficulties to survey the availability of pests at the regional scale. Furthermore, acoustic detection and monitoring of bats' activity entail intrinsic biases (see Walters et al., 2013) involving species detectability, identification and survey costs. Some species are hard to detect (e.g. *R. hipposideros*) or identify (e.g. genus *Myotis*) due to their inherent echolocation system, resulting in underestimated detection rates of such species. Further, the inability of metabarcoding to reliably quantify prey abundance in the

bats diet (Elbrecht et al., 2017), but to only provide a qualitative assessment (e.g. presence or absence) of consumed taxa, diminishes the relevance of highly consumed prey and overestimates the importance of those sporadically or occasionally consumed.

CRediT authorship contribution statement

Unai Baroja: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Writing - original draft, Visualization, Project administration. **Inazio Garin:** Conceptualization, Methodology, Software, Validation, Formal analysis, Writing - review & editing, Supervision, Project administration. **Nerea Vallejo:** Investigation, Writing - review & editing. **Joxerra Aihartza:** Conceptualization, Writing - review & editing. **Hugo Rebelo:** Formal analysis, Resources, Supervision. **Urtzi Goiti:** Conceptualization, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2021.107718.

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