



Interspecific and seasonal variations in crop pest consumption by bats in a bocage landscape

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Abstract

Bats are important natural enemies of agricultural pests, yet their role in heterogeneous landscapes like the European bocage remains understudied. These landscapes host diverse bat communities that may enhance pest suppression in crops. However, their heterogeneity could also hamper pest consumption due to greater alternative prey availability. Understanding dietary variation among bat species and across seasons is key to assessing their contribution to pest control. We conducted a dietary DNA-metabarcoding study in a bocage landscape in Asturias, northern Spain, to investigate pest consumption by three common European bat species: *Pipistrellus pipistrellus*, *Rhinolophus ferrumequinum*, and *Rhinolophus hipposideros*. Our study focused on identifying agricultural pest species in their faecal contents and on examining variation in pest consumption among species and across seasons. We identified 191 pest species in bat diets potentially affecting a wide range of crops. Overall, bats consistently consumed agricultural pests throughout the year, though we observed some seasonal variation in pest richness and composition. Although our analyses revealed substantial overlap in pest consumption among the three bat species, they also showed clear differences in the specific pest taxa consumed and the frequency of consumption. These results highlight both complementarity and partial functional overlap among bat species in their consumption of agricultural pests, underscoring their collective role in maintaining resilient pest control services across time and crop types within bocage landscapes.

Keywords Agroecosystems · DNA barcoding and metabarcoding · Ecosystem service · Diet molecular analysis · Pest control · *Pipistrellus pipistrellus* · *Rhinolophus ferrumequinum* · *Rhinolophus hipposideros*

Key message

- The three bat species exhibited broad diets, mainly consisting of lepidopterans and dipterans.
- All bat species consumed a wide range of pests affecting various crops.
- Bats consumed pests across seasons, though the composition of consumed pests varied temporally.
- Pest consumption analysis suggests functional complementarity and redundancy within the bat community.
- The results emphasize the need to conserve all bat species throughout the year.

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Introduction

Agricultural pests cause significant economic losses by reducing crop yields worldwide, thus threatening food security (Oerke 2006; Savary et al. 2019). Regulating agricultural pests is, therefore, a major global challenge. While biological control by predatory arthropods has been extensively studied (Symondson et al. 2002; Cock et al. 2016), the role of vertebrates, such as birds and bats, as natural pest suppressors has received comparatively less attention until recently. As important arthropod predators, insectivorous bats provide essential ecosystem services by reducing

populations of agricultural pests, forest pests, and disease vectors affecting humans and livestock (Ramírez-Francel et al. 2022; Tuneu-Corral et al. 2023). Specifically, bats are known to actively prey on at least 760 insect species considered crop pests worldwide (Tuneu-Corral et al. 2023). The ability of bats to exploit diverse agricultural landscapes (Aizpurua et al. 2018; Aihartza et al. 2023) makes them, particularly, valuable agents for integrated pest management strategies (Boyles et al. 2011; Tuneu-Corral et al. 2023) and for ensuring food security (Wanger et al. 2014).

Numerous studies have examined bat-mediated pest suppression in monoculture, homogeneous landscapes, such as those of cornfields (Maine and Boyles 2015), rice paddies (Tuneu-Corral et al. 2024), vineyards (Rodríguez-San Pedro et al. 2020), and cotton farms (Kolkert et al. 2021). The economic value of insectivorous bats in these agroecosystems is considerable, reaching, for example, 8€/ha/year in corn (Maine and Boyles 2015), 21–56€/ha/year in rice (Puig-Montserrat et al. 2015; Tuneu-Corral et al. 2024), 190–250€/ha/year in vineyards (Rodríguez-San Pedro et al. 2020), or 100–360€/ha/year in cotton (Kolkert et al. 2021). The overall value of bats to the agricultural industry in the USA has been estimated at roughly 22.9 billion USD per year (Boyles et al. 2011). Moreover, the impact of bats on pest suppression extends beyond the savings from reduced pesticide use, also providing indirect health benefits associated with lower pesticide exposure (Frank 2024).

Relatively little is known, however, about the contribution of bats to pest control in heterogeneous agricultural landscapes, such as the bocage (but see, for instance, Liu et al. 2024, for another example of smallholder farming systems). The bocage is a traditional agricultural system found in parts of Western Europe, characterized by a fine-grained mosaic of pastures and crops interspersed with seminatural woody habitats such as hedgerows and woodlands (Baudry et al. 2000; Thenail and Baudry 2004). These features promote habitat heterogeneity, which in turn may enhance the coexistence of multiple bat species (Mendes et al. 2017; Lacoëuilhe et al. 2018; Miñarro and García, 2025). Increased species richness could lead to functional complementarity or redundancy among bats, thereby strengthening natural pest control services (Tortosa et al. 2023; Augusto et al. 2024). However, an alternative hypothesis suggests that the prevalence of agricultural pests in bat diets may actually decline in highly heterogeneous landscapes, as seminatural woody habitats offer a wide range of alternative prey and may concentrate bat activity away from crop fields (Aizpurua et al. 2018). This could lead bats to exploit the greater availability of alternative prey in such landscapes, potentially reducing the relative proportion of crop pests in their diet compared to the more homogeneous conditions of large-scale monocultures. Overall, the extent to which bats contribute to pest regulation in bocage landscapes remains poorly understood.

One critical aspect of understanding the role of bats in pest suppression is assessing the variability in dietary composition among species and across seasons (Baroja et al. 2021; Hughes et al. 2021; Ancillotto et al. 2022). Many bat species exhibit broad and flexible diets, which can result in complementary roles in insect pest control through trophic niche segregation and resource partitioning (Hughes et al. 2021; Mata et al. 2021; Augusto et al. 2024). At the same time, dietary generalism among species often leads to trophic overlap, promoting functional redundancy and thereby increasing the resilience of pest control services (Letourneau et al., 2009; Maas et al. 2016; Salinas-Ramos et al. 2020). Bats may also respond to seasonal fluctuations in prey availability by adjusting their diets accordingly (Cohen et al. 2020; Baroja et al. 2021; Aihartza et al. 2023). Different agricultural pests, even within the same crop, often exhibit distinct phenological patterns. For instance, in apple orchards, aphids typically emerge early in the season, whereas pests such as the codling moth (*Cydia pomonella*) may be present from spring through autumn (Blommers 1994). This temporal variation in pest occurrence underscores the importance of maintaining continuous pest control coverage throughout the entire period of bat activity. Understanding both interspecific dietary differences and phenological shifts in pest consumption is essential for assessing how bat assemblages function and persist in agricultural ecosystems, as well as how bat conservation efforts can maximize their pest control benefits.

DNA metabarcoding was employed in this study to investigate pest consumption by three common European bat species—the common pipistrelle (*Pipistrellus pipistrellus*), the greater horseshoe bat (*Rhinolophus ferrumequinum*), and the lesser horseshoe bat (*Rhinolophus hipposideros*)—occurring in a bocage landscape in Asturias, northern Spain. Specifically, we aim to address the following questions: (1) Do these three bat species consume agricultural pests, and if so, which pest species are targeted and in what quantities? (2) To what extent do their pest consumption patterns differ in terms of pest species richness and composition? (3) How do seasonal factors influence the consumption of pest species by these bats? By answering these questions, we aim to enhance our understanding of the ecological role of bats in heterogeneous agricultural landscapes, provide insights into their conservation and potential for sustainable pest management strategies, and support decision-making in agroecosystems.

Materials and methods

Study area

The study was conducted in Asturias, a region located in the northern coast of Spain (Fig. 1). Asturias is characterized by

a temperate oceanic and hyperoceanic climate, with annual precipitation typically exceeding 1100 mm, distributed relatively evenly throughout the year (Álvarez Santacoloma et al. 2022). The region's topography is highly variable, ranging from a narrow coastline to mountainous terrain inland, with altitudes ranging from sea level to 2500 m a.s.l. This results in high heterogeneity in agricultural patterns and historical land use. Asturias has a highly diversified landscape, characterized by a fine-grain mosaic of small plots of pastures, agricultural fields, fruit plantations, eucalyptus plantations, and patches of natural woody vegetation (Fig. 1C). As an example of plot sizes, apple orchards in the study area ranged from 0.5 to 20.6 ha. The agricultural landscape is thus composed of small, fragmented plots limited by hedgerows, a defining feature of the “bocage” landscape typical of the Atlantic fringe, in contrast to more expansive, open landscapes (Baudry et al. 2000; Somoano et al. 2022).

Asturian farming is multifaceted, with livestock farming playing a central role in the regional rural economy. The numerous pastures and corn (*Zea mays*) plantations are

essential for livestock feed. Fruit cultivation, particularly apples (*Malus x domestica*), is another defining feature of the agricultural landscape, as Asturias is the leading region for cider apple production in Spain. Blueberries (*Vaccinium corymbosum* and *Vaccinium ashei*), kiwifruits (*Actinidia deliciosa*), and numerous domestic gardens, which feature a wide variety of fruit trees and horticultural crops, also contribute to shaping the region's rural landscape. In the study area, the landscape was dominated by pastures (42.8% cover), followed by seminatural woody habitats (23.4%), urbanized land (14.5%), eucalyptus plantations (8.4%), fruit plantations (8.4%), and other cover types (2.5%) (Miñarro and García, 2025).

This bocage landscape is associated with high animal biodiversity, including rich communities of arthropods (Martínez-Sastre et al. 2020; Miñarro et al. 2023), birds (García et al. 2024; Jiménez-Albarral et al. 2025), and bats (Miñarro and García, 2025). The bat assemblage in the study area is numerically dominated by *Pipistrellus pipistrellus* (67% of total bat activity detected by acoustic

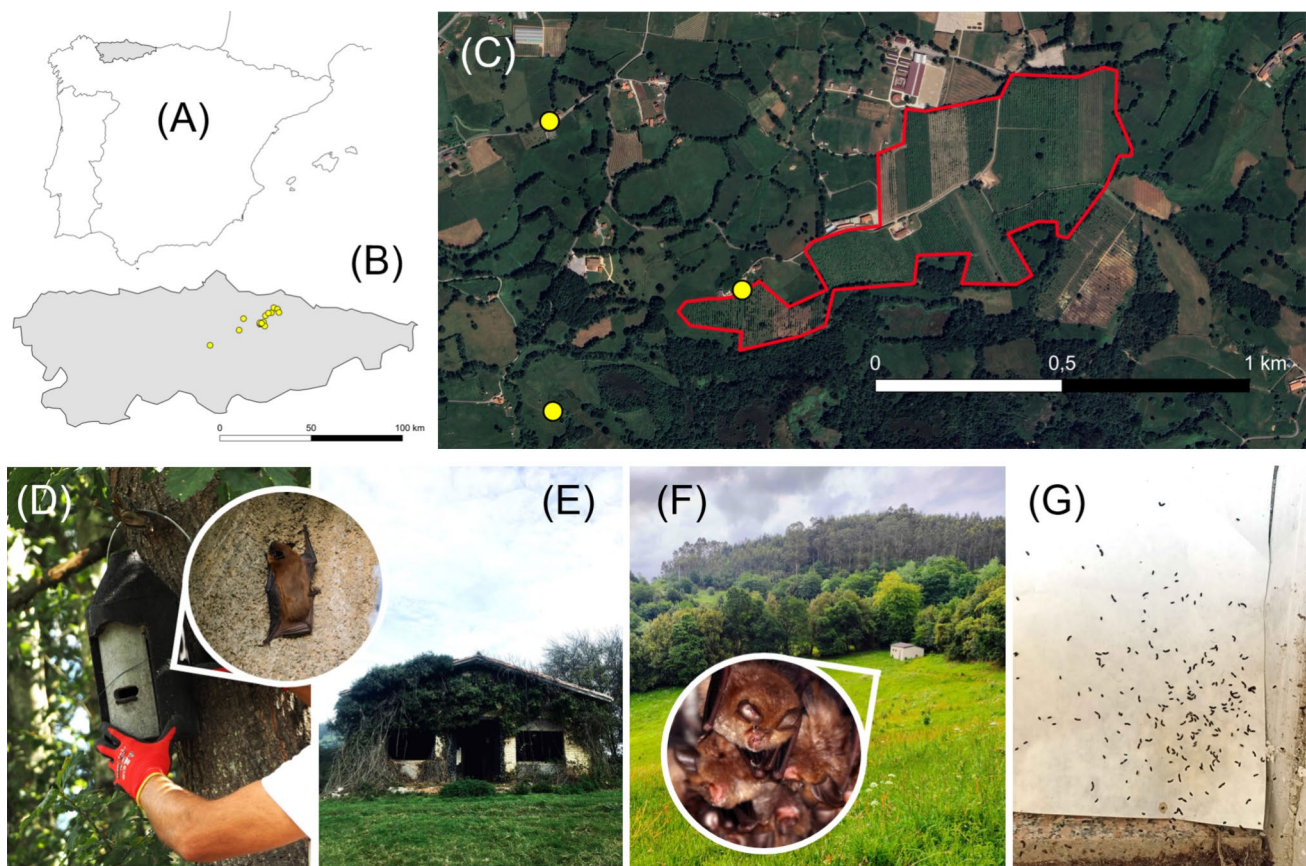


Fig. 1 Schematic representation of the study sites: **A** Study area, with Asturias region highlighted in grey within the Iberian Peninsula; **B** Locations in the map of Asturias of the fifteen identified roosts; **C** Orthophoto detail depicting the bocage landscape, with an apple plantation (red) and three roosts (yellow) indicated; **D** Bat box,

including an enlarged view of *Pipistrellus pipistrellus*; **E** Abandoned house used as a roost; **F** Stable with a detailed view of a *Rhinolophus hipposideros* colony; and **G** Sheet of paper containing bat faecal pellets

recorders; Miñarro and García, 2025). Other species detected include *Eptesicus serotinus*, *Nyctalus leisleri*, *Barbastella barbastella*, *Pipistrellus kuhlii*, *Pipistrellus nathusii*, *Plecotus austriacus*, *Plecotus auritus*, *Myotis myotis*, *Miniopterus schreibersii*, *Hypsugo savii*, *Tadarida teniotis*, *Rhinolophus ferrumequinum*, and *Rhinolophus hipposideros* (Miñarro and García, 2025).

Faecal sample collection

We collected faeces for diet analysis from bat roosts. We identified 15 roosts in the study area, including inhabited and abandoned houses, stables, sheds, churches, and bat boxes placed in fruit orchards (Fig. 1D–F). All roosts were located within a maximum distance of 1 km from fruit orchards (apple or blueberry) with a distance between roosts ranging from 0.5 km to 41.4 km. We focused on studying three species (*P. pipistrellus*, *R. ferrumequinum*, and *R. hipposideros*) because these were the species for which we successfully obtained faecal samples. Five roosts (including the bat boxes) were inhabited by *P. pipistrellus*, one by *R. ferrumequinum*, four by *R. hipposideros*, and five by both rhinolophid species. The roosts were sampled from spring 2022 to autumn 2023, although not all roosts were sampled throughout the study period because some were discovered by us or colonized by bats (e.g. bat boxes) after the study began, or they did not consistently produce faecal samples. In all roosts except for the bat boxes, we placed collecting sheets beneath the bat colonies to gather faecal samples (Fig. 1G). These sheets were replaced each time samples were collected to ensure that the faeces were recent. Similarly, the bottoms of the bat boxes were cleaned after each sample collection. We visited most roosts approximately every two weeks. Prior to sample collection at a given roost, after several weeks without sampling, a new sheet of paper was placed the week before to ensure that the collected samples corresponded to recent faeces. During each visit, we collected up to 10 samples per roost site, each consisting of 1–14 pellets (mean: 3.1, SE = 0.1), depending on pellet availability, using sterile forceps. In total, we collected 748 faecal samples, which were stored at -20°C until DNA extraction. No animal ethics clearance was required for the study, as the samples were passively collected without involving bat manipulation.

Based on bat biology in temperate climates, we divided the samples temporally into three activity seasons: spring (from May to mid-June, before pups are born), summer (from mid-June to mid-August, when pups are raised), and autumn (from mid-August to November, when juveniles are weaned and adults disperse to hibernacula) (Andriollo et al. 2021).

Molecular procedures

DNA extraction and bat species identification

We extracted DNA from faecal samples using a silica solid-phase protocol, with modifications based on the methods described by Longmire et al. (1997) and Rohland et al. (2010). Briefly, samples were incubated overnight at 37°C with Longmire (BE) and MixPK buffers. The following day, samples were centrifuged for 2 min at 9600 g, and the supernatant was transferred to clean tubes. DNA was adsorbed onto 80 μl of silica per sample using a high-concentration guanidine thiocyanate buffer. Low pH conditions were maintained by adding 90 μl of 3 M sodium acetate. DNA-bound silica was thoroughly washed with Wash Buffer (50% ethanol, 0.01 M Tris–HCl pH 8, 0.001 M EDTA pH 8, and 0.125 M NaCl). The samples were then loaded onto empty spin columns, and DNA was eluted by centrifugation at 16,000 g using 60 μl of TE buffer.

Bat species identification was confirmed using a DNA barcoding approach through Sanger sequencing. Because we expected to get low-quality and low-quantity DNA from faecal samples, we used DNA mini-barcode primers specifically designed for Chiropteran species. We amplified a region of the Cytochrome Oxidase subunit I (COI) gene using the primers SFF_145f (5' GTHACHGCYCA YGCHTTYGTAATAAT 3') and SFF_351r (5' CTCCWGCRTGDGCWAGRTTTC 3') for all faecal samples (Walker et al. 2016). Polymerase chain reactions (PCR) were set up in a total volume of 10 μl , including 5 μl of 2 \times QIAGEN Multiplex PCR Master Mix, 0.5 μl (10 mM) of each primer and 1.5 μl of genomic DNA. PCRs were performed under the following conditions: initial hot-start denaturation at 95°C for 15 min; followed by 40 cycles of denaturation at 95°C for 60 s, annealing at 56°C for 30 s, and extension at 72°C for 30 s; concluding with a final extension at 60°C for 30 min. All PCRs included two negative controls. PCR products were visualized on 1.5% agarose gels stained with GelRed®. Sequencing of the PCR products was performed using the PerkinElmer BigDye terminator (v. 3.1), with primer SFF_145f. Sequences were aligned manually using BioEdit version 7.01 (Hall 1999) and Chromas Lite (<https://technelysium.com.au/wp/chromas/>). We used both NCBI's BLAST and BOLD Identification System tool to identify the bat species. A few samples from roosts inhabited by more than one species could not be confidently assigned to a single species, presumably because the pellets originated from different bat species, and were therefore excluded from the diet analysis. Ultimately, we selected a subset of 365 individual samples for molecular analysis, representing different roosts and seasons for three bat species: *P. pipistrellus* (101 samples), *R. ferrumequinum* (112), and *R. hipposideros* (152).

Library preparation and sequencing

To detect arthropod DNA from individual faecal samples through PCR amplification, we utilized the primer pair LepF1 (5' ATT CAA CCA ATC ATA AAG ATA TTG G 3') (Hebert et al. 2004) and ZBJ-ArtR2c-deg (5' WAC TAA TCA ATT WCC AAA HCC HCC 3') (Shutt et al. 2020) to amplify a 225 bp region of the mitochondrial COI gene in two separate reactions. These primers also incorporated Illumina sequencing primer sequences at their 5' ends. We verified the library size by running the libraries on 3% agarose gels stained with GreenSafe (NZYTech) and imaging them under UV light. Subsequently, we purified the libraries using Mag-Bind RXNPure Plus magnetic beads (Omega Bio-tek), following the manufacturer's instructions. The final libraries were pooled in equimolar amounts, as determined by a Qubit dsDNA HS Assay (Thermo Fisher Scientific) quantification. The pooled library was then sequenced on a fraction of a NovaSeq PE250 flow cell (Illumina), aiming for a total output of 18 gigabases.

Processing of sequencing data and taxonomic assignment

We assessed the quality of the FASTQ files using FastQC (Andrews 2010) and summarized the output with MultiQC (Ewels et al. 2016). We employed Cutadapt v3.5 to trim non-biological DNA sequences, including primers, indices, and sequencing adapters. The processed amplicon reads were then analyzed using DADA2 (Callahan et al. 2016), implemented within QIIME 2 (Bolyen et al. 2019). The first step in the DADA2 pipeline involved trimming and filtering the data to remove amplification primers and eliminate low-quality bases. Based on the quality profiles of the reads, forward and reverse reads were truncated at position 125. Error rates were learned from the dataset to perform denoising using the parametric error model in DADA2. The dataset was dereplicated, and the dereplicated forward and reverse reads were used to infer Amplicon Sequence Variants (ASVs) using the core sample inference algorithm (Callahan et al. 2016). Following this, corresponding R1 and R2 reads were merged into pairs with a minimum overlap of 12 identical base pairs. Finally, PCR and sequencing artefacts, such as chimeras, were removed.

We performed taxonomic assignment of each ASV using a pre-trained classifier from the COInr reference database (Meglecz 2022; version 2023–05–03). To compare each ASV sequence to the reference database and obtain taxonomic assignments, we used the VSEARCH algorithm (Rognes et al. 2016), implemented in QIIME 2 via the feature-classifier approach, classify-consensus-vsearch (Bokulich et al. 2018). A minimum per cent identity of 90% was set for the comparison. After algorithmic assignment, we applied the following steps for filtering of potentially

spurious assignments, due to methodological or biological constraints: 1) singleton ASVs (those containing only a single sequence in the entire dataset) and unassigned ASVs were excluded; 2) ASVs with a frequency of <0.1% in each sample were discarded to avoid including accidental or secondary consumption records (Deagle et al. 2019); 3) ASVs assigned to two closely related species, one present and one non-present in the study region, were reassigned to the present species; 4) ASVs assigned to species non-present in the study region were discarded, considering that these automatic assignments were likely due to a lack of specific sequence data in public repositories; and 5) only taxa within the classes Arachnida and Insecta were retained for further analysis. Library preparation, sequencing, and taxonomic assignment were performed by AllGenetics & Biology SL (www.allgenetics.eu).

Variation in pest consumption across species and seasons

We compiled information on the pest status of all prey taxa detected in the faecal samples from various bibliographic sources (Table S1). To evaluate the variability in pest consumption across species and seasons, we used two complementary approaches. The first one was based on pest species richness. Given the interspecific differences in sample sizes, we first assessed the reliability of our sampling in representing the pest richness consumed by each bat species. To do this, we built rarefaction curves based on Hill numbers, a unified family of diversity indices that quantify diversity as the effective number of prey taxa while accounting for both richness and evenness (Chao et al. 2014). We then calculated the Chao2 estimator for each bat species to allow robust comparisons of dietary diversity among species using the R package iNEXT (Hsieh et al. 2016). Next, we assessed the co-occurrence of pest species between bat species and across seasons, represented by Venn diagrams. Additionally, interspecific and temporal differences in pest richness (number of pests per faecal sample) were tested using a generalized linear mixed model (GLMM) considering a negative binomial distribution (log link) of pest richness. We considered a model with two fixed factors (species, season, and their interaction) and a random factor (roost). The model was fitted using the R package lme4 (Bates et al. 2015), and model adequacy was checked by visual diagnosis (residuals vs. fitted values plot and quantile–quantile plot). Marginal R^2 (variance explained by fixed effects) and conditional R^2 (variance explained by both fixed and random effects) were calculated using the R package MuMIn (Barton 2022).

The second approach was based on the composition of pest consumed by bats. For that we calculated the weighted per cent of occurrence (wPOO), which represents the frequency of occurrence of the pests in the bat diet. For a

given pest species present in a faecal sample, the wPOO was calculated as one (indicating the species' presence, or zero when absent) divided by the total number of pest species detected in that sample (Deagle et al. 2019). The same procedure was applied to calculate the wPOO based on all prey taxa (not just pests), in order to represent the overall diet of the three bat species. To quantify and visualize the differences in the composition of pest consumption across species and seasons, we conducted two nonmetric multidimensional scaling ordination analyses (NMDS; Quinn and Keough 2002). The first NMDS was based on a matrix of pest wPOO of individual faecal samples of each bat species. Using Bray–Curtis distance measures for dissimilarity, we built a two-dimensional (NMDS1 and NMDS2) compositional space to plot the position of samples for each bat species. Representing the area of this compositional space occupied by the samples of the different bat species may inform about the ecological overlap (i.e. redundancy) between bats foraging on different pests. We then performed an ANOSIM (Analysis of Similarities) on the same pest-bat species matrix to test whether dissimilarity in pest composition was greater among bat species than within the same species. The value of the R statistic in an ANOSIM analysis ranges from -1 to 1 . When $R > 0$, the differences between groups are greater than the differences within each group. Following the same procedure, we conducted a second NMDS and ANOSIM based on a matrix of individual faecal samples for each season. All analyses were performed using the R package *vegan* (Oksanen et al. 2022). The NMDS1 and NMDS2 scores of each faecal sample were considered as proxies of pest composition. As for pest richness, interspecific and seasonal differences in NMDS1 and NMDS2 were tested using two independent GLMMs, one for each NMDS score. In both models, we considered a Gaussian distribution (identity link) for the response variable (NMDS1 or NMDS2) as well as species

and season (and their interaction) as fixed factors and roost as a random factor.

Results

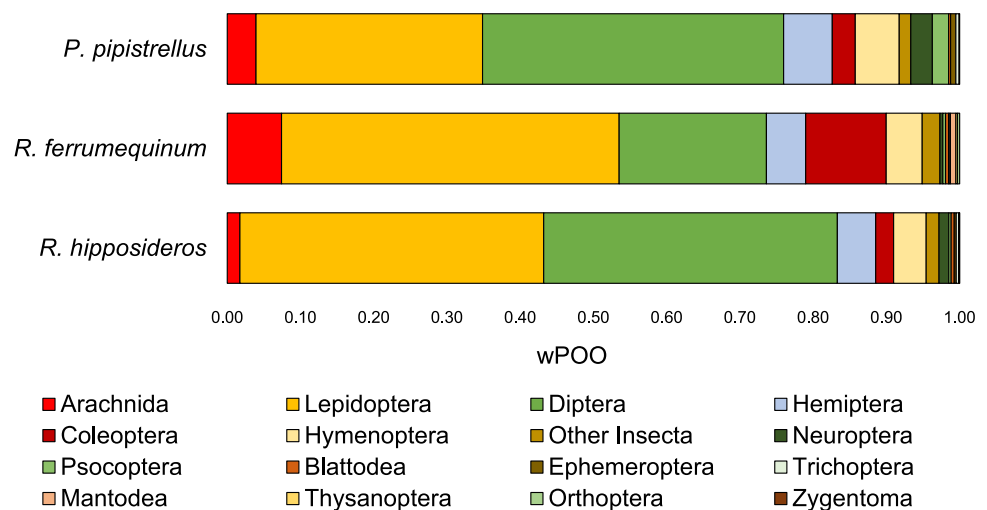
Overall diet description

A total of 1293 prey taxa were identified in the faecal samples of the three bat species. In total, 78.7% of them were taxonomically identified to the species level, while the remaining were classified at the genus (14.4%), family (5.6%), order (1.2%) or class (0.1%) level. Among all prey taxa, 758 were detected in *P. pipistrellus* samples, 359 in *R. ferrumequinum*, and 848 in *R. hipposideros*, with an average of 29.7 (SE = 1.4), 11.0 (SE = 0.6), and 30.9 (SE = 1.3) prey taxa per sample, respectively. The majority of the prey belonged to the class Insecta (96.1%, 92.6%, and 98.3% for *P. pipistrellus*, *R. ferrumequinum*, and *R. hipposideros*, respectively), with the remaining percentage consisting of Arachnida (Fig. 2). The diet of *P. pipistrellus* was dominated by Diptera (41.1%) and Lepidoptera (30.9%), followed at a distance by Hemiptera (6.6%) and Hymenoptera (6.0%). In the case of *R. ferrumequinum*, lepidopterans (46.1%) doubled the proportion of dipterans (20.1%), being coleopterans (11.0%) the third most frequent order. For *R. hipposideros*, Lepidoptera (41.5%) and Diptera (40.1%) were also the most abundant prey, followed by Hemiptera (5.2%) and Hymenoptera (4.5%).

Consumption of crop pests

Of the 1293 prey taxa consumed by the three bat species, 191 are considered crop pests (Table S1). The majority of those pests belong to Lepidoptera (77.0% of the species), followed by Hemiptera (12.0%), Diptera (6.8%), Coleoptera

Fig. 2 Proportion (weighted per cent of occurrence; wPOO) of Arachnida and Insecta prey (N = 1293) detected in faecal samples of *Pipistrellus pipistrellus* (101 samples), *Rhinolophus ferrumequinum* (112 samples), and *Rhinolophus hipposideros* (152 samples) in Asturias, Spain. Data for Insecta are shown classified by taxonomical order



(3.7%), and Hymenoptera (0.5%). These pests potentially affect numerous crops, including fruit crops, such as the apple tree (up to 56 pests detected; e.g. *Cydia pomonella*, *Pandemis heparana*), hazelnut (37; e.g. *Pandemis corylana*), chestnut (28; e.g. *Curculio elephas*, *Cydia splendana*), or plum tree (19; e.g. *Grapholita funebrana*); soft fruits like blueberry (33; e.g. *Drosophila suzukii*), blackberry (16; e.g. *D. suzukii*) or strawberries (11; e.g. *D. suzukii*); cereals (16; e.g. *Mythimna unipunctata*); forage crops like lucerne (10; e.g. *Adephocoris seticornis*); or vegetables like cabbage (7; e.g. *Plutella xylostella*) or broad beans (6; e.g. *Aphis fabae*). It is important to note that some pest species can affect multiple crops (e.g. *D. suzukii* or *Tipula oleracea*; Table S1).

The list also includes invasive pests detected in the bat's diet, such as the spotted wing drosophila (*D. suzukii*), the chestnut gall wasp (*Dryocosmus kuriphilus*), or the tomato leaf miner (*Tuta absoluta*).

Comparison of pest consumption among bats

Almost all the faecal samples (95.1% for *P. pipistrellus*, 98.2% for *R. ferrumequinum* and 99.3% for *R. hipposideros*) contained pests. Pest remains were detected in the faecal samples collected from all roosts, indicating that their consumption is widespread across the species' habitats. The samples from *P. pipistrellus* included 113 (59.2%) of the 191 pest species, *R. ferrumequinum* 68 (35.6%) and *R. hipposideros* 140 (73.3%) (Table S1). The percentage of the total prey taxa that were pests was 14.9% for *P. pipistrellus*, 18.9% for *R. ferrumequinum*, and 16.5% for *R. hipposideros*.

Chao2 extrapolation from species accumulation curves estimates the potential pest richness in the diet of *P. pipistrellus* (143 species), *R. ferrumequinum* (89 species), and *R. hipposideros* (217 species). These estimates indicate that our data represent 64% (*R. hipposideros*)–79% (*P. pipistrellus*) of the pest species potentially consumed by these bat species. The lack of overlap in the 95% confidence intervals of the species accumulation curve for *R. ferrumequinum* with those of the other species indicates that this bat species is consuming a lower number of pest species (Fig. 3A). This result was confirmed by the GLMM analysis on the number of pest species per faecal sample, which showed that *P. pipistrellus* (5.24; SE=0.34) and *R. hipposideros* (6.68; SE=0.29) did not differ significantly from each other, while *R. ferrumequinum* had significantly fewer pest species per sample (3.15; SE=0.16) (Table 1; Fig. 3B).

At the order level, all three bat species predominantly consumed lepidopteran pests (75–82%), followed by dipteran and hemipteran pests. However, at the family level, the differences in pest consumption among bat species were particularly pronounced. For instance, *R. ferrumequinum* consumed roughly twice the proportion of Noctuidae, Hepialidae, and Blastobasidae moths (51.0% in total) compared to the other two bat species (20.8% for *P. pipistrellus* and 26.4% for *R. hipposideros*), which primarily consumed tortricids (21.3% and 33.6%, respectively, vs 10.2% in *R. ferrumequinum*) (Fig. 4A). Similarly, among the dipterans, both *Rhinolophus* species consumed a significantly higher proportion of Tipulidae compared to *P. pipistrellus*, which predominantly consumed drosophilid flies (Fig. 4B). These

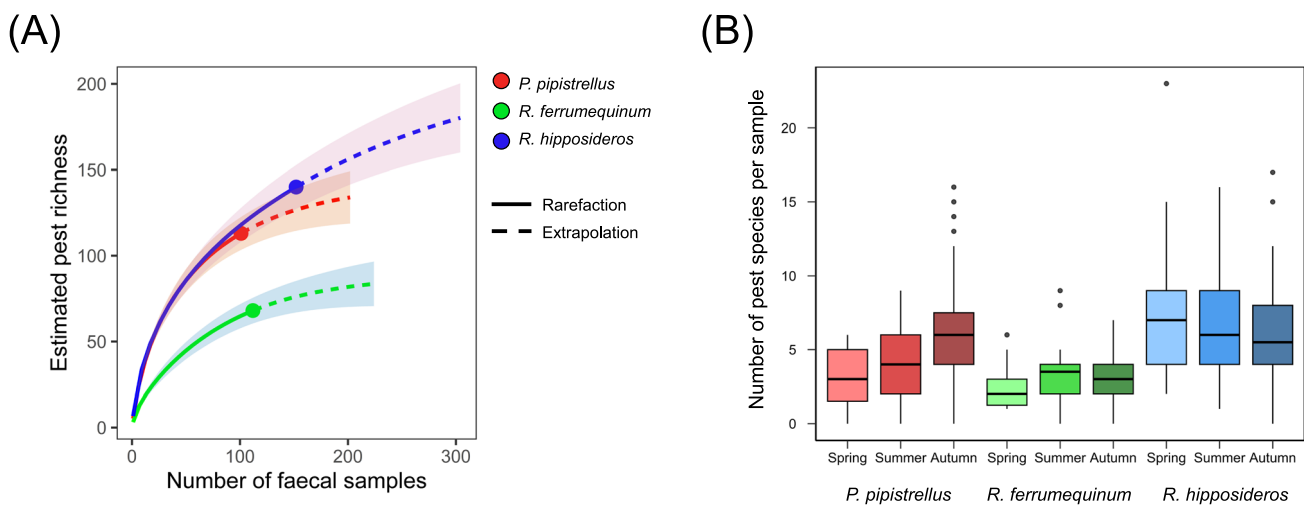


Fig. 3 Pest richness in faecal samples of *Pipistrellus pipistrellus*, *Rhinolophus ferrumequinum* and *Rhinolophus hipposideros* in Asturias, Spain. **A** Species accumulation curves for the three bat species. Solid lines represent interpolated richness based on observed data, while dashed lines indicate extrapolated richness beyond the observed sample size. Shaded areas correspond to 95% confidence inter-

vals. **B** Number of pest species per sample for the three bat species across the seasons. Boxplots indicate 25–75% quartiles (box boundaries), median (horizontal line inside the box), the largest and smallest observed values within 1.5 times the interquartile range (vertical lines) and outliers (small circles)

Table 1 Results of the Generalized Linear Mixed Model (GLMM) evaluating the effects of bat species and season (and their interaction) on the number of pest species per faecal sample and on pest species composition (nonmetric multidimensional scaling ordination (NMDS) dimensions 1 and 2) in Asturias, Spain. The reference categories used for comparisons were *Pipistrellus pipistrellus* for bat species and autumn for season. Details of the family of distribution and link function used (in parentheses), values of marginal and conditional (in parentheses) R^2 , values of z (for negative binomial distributions) and t (for Gaussian) statistics, as well as variance (SD) estimates for roost, considered as random factor, are also shown

Dependent variable and factors in the models	Estimate	SE/SD	z/t	P	R^2
Pest richness (Negative binomial, log)					0.315 (0.358)
Intercept	1.840	0.085	21.54	<0.001	
<i>R. ferrumequinum</i>	-0.660	0.119	-5.53	<0.001	
<i>R. hipposideros</i>	-0.006	0.111	-0.05	0.960	
Spring	-0.639	0.213	-2.99	0.003	
Summer	-0.378	0.118	-3.21	0.001	
<i>R. ferrumequinum</i> -Spring	0.350	0.274	1.28	0.201	
<i>R. hipposideros</i> -Spring	0.804	0.235	3.42	<0.001	
<i>R. ferrumequinum</i> -Summer	0.439	0.201	2.19	0.029	
<i>R. hipposideros</i> -Summer	0.509	0.158	3.216	0.001	
Roost (random factor)	0.005	0.068			
Pest composition NMDS1 (Gaussian, identity)					0.259 (0.259)
Intercept	0.406	0.078	5.18	<0.001	
<i>R. ferrumequinum</i>	-0.733	0.101	-7.23	<0.001	
<i>R. hipposideros</i>	-0.448	0.100	-4.49	<0.001	
Spring	-0.458	0.202	-2.27	0.024	
Summer	0.071	0.116	0.61	0.540	
<i>R. ferrumequinum</i> -Spring	0.207	0.240	0.86	0.389	
<i>R. hipposideros</i> -Spring	0.554	0.226	2.45	0.015	
<i>R. ferrumequinum</i> -Summer	-0.092	0.184	-0.50	0.618	
<i>R. hipposideros</i> -Summer	0.110	0.157	0.701	0.484	
Roost (random factor)	0.277	0.526			
Pest composition NMDS2 (Gaussian, identity)					0.240 (0.350)
Intercept	0.131	0.164	0.80	0.426	
<i>R. ferrumequinum</i>	-0.002	0.185	-0.01	0.993	
<i>R. hipposideros</i>	0.343	0.170	2.02	0.045	
Spring	-0.297	0.171	-1.74	0.083	
Summer	-0.240	0.170	-1.41	0.158	
<i>R. ferrumequinum</i> -Spring	0.131	0.223	0.59	0.558	
<i>R. hipposideros</i> -Spring	0.153	0.196	0.78	0.435	
<i>R. ferrumequinum</i> -Summer	-0.211	0.203	-1.04	0.298	
<i>R. hipposideros</i> -Summer	-0.273	0.190	-1.442	0.150	
Roost (random factor)	0.029	0.172			

differences in pest consumption become more evident when examining the prey shared at the pest species level. Of the 191 observed pests, only 33 (17.3%) were consumed by all three species (Fig. 4C). These shared prey species included 16 of the 20 most frequent pests identified in the study as well as key pests such as *C. pomonella*, *C. splendana*, *Plutella xylostella* and *D. kuriphilus* (Table S1). On the contrary, between one-fifth and one-third of the pests were found exclusively in the diet of a single bat species. Specifically, 19.9% of the pests were found exclusively in *P. pipistrellus*, 23.5% in *R. ferrumequinum* and 35.7% in *R. hipposideros* (Fig. 4C).

The NMDS analysis on the occurrence of consumed pests (stress = 0.194) evidenced a widespread distribution of faecal samples of the different bat species in the compositional space of NMDS1 and NMS2 axes (Fig. 5A). The

three bat species shared a central core area with numerous and less differentiated samples, representing interspecific overlap in the identity of consumed pests, but also divergent samples indicating specificity in pest consumption by the different bats. In this sense, *R. hipposideros* showed a compositional space of pest consumption largely nested in those of the other two bat species, whereas *R. ferrumequinum* and, especially, *P. pipistrellus*, showed wider compositional spaces encompassing highly differentiated samples, suggesting non-overlapping diets. An ANOSIM confirmed that the dissimilarity in pest composition among samples was greater between species than within species ($R=0.140$; $p=0.001$; 999 permutations). The significant but relatively low value of R indicates that, although there are differences in pest consumption between species, these differences are not extremely large. The higher median dissimilarity of P .

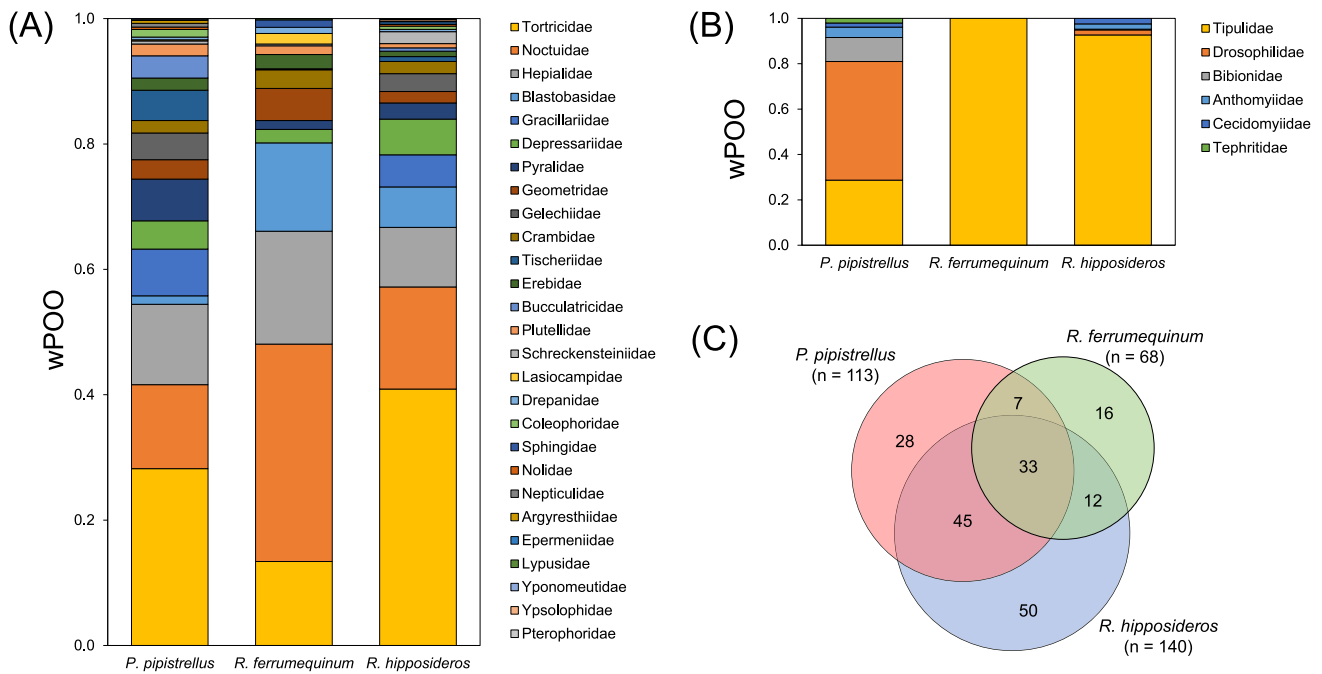


Fig. 4 Proportion (wPOO) of crop pests consumed by *Pipistrellus pipistrellus*, *Rhinolophus ferrumequinum* and *Rhinolophus hipposideros* belonging to the families **A** Lepidoptera and **B** Diptera in Astu-

rias, Spain. **C** Venn diagram representing the number of shared or specific crop pests detected for the three bat species (n represents the total number of pests recorded for each species)

pipistrellus (Figure S1A) suggests that the samples within this species exhibit considerable variability in pest consumption, particularly when compared to *R. ferrumequinum*. The GLMM analysis on the NMDS dimensions confirmed interspecific differences in the composition of consumed pest, especially pronounced across the NMDS1 axis (Table 1; Figure S2).

Seasonal patterns in pest consumption

All three bat species consumed pests across the three studied seasons, with Lepidoptera being the predominant prey group throughout (Figure S3). However, the proportion of lepidopteran pests in the diet showed a decrease in spring for *P. pipistrellus* and *R. ferrumequinum*. When analyzed at the pest species level, the number of consumed pests varied across seasons: accumulated pest richness increased in autumn and decreased in spring for the three species, although it is important to note that sample sizes varied considerably across the different seasons (Figure S4). The GLMM analysis on the number of pest species per faecal sample confirmed seasonal differences, with pest richness being significantly higher in autumn (Table 1; Fig. 3B). In addition, significant interactions between species and season were also detected. For example, while the trend of increased pest richness in autumn was evident for *P. pipistrellus*, this pattern was not observed for *R. hipposideros*,

which showed an average of 7.3, 7.1, and 6.1 pest species per sample in spring, summer, and autumn, respectively (Fig. 3B).

The proportion of pest species shared across spring, summer, and autumn was only 8.4% for *P. pipistrellus*, 10.3% for *R. ferrumequinum*, and 24.3% for *R. hipposideros* (Figure S4). In contrast, the proportion of pest species consumed in only one of the three seasons was 54.9%, 63.2%, and 55.6% for the respective species (Figure S4).

The NMDS ordination also revealed differences in pest composition among samples from different seasons (Fig. 5B). Also in this case, there were samples belonging to the three seasons placed in a core position, indicating similarity and temporal overlap in the occurrence of pest species in the diet of bats, but also divergent positions, especially in autumn and summer, suggesting temporal differentiation. ANOSIM confirmed that dissimilarity in pest composition was significantly greater between seasons than within seasons ($R = 0.225$, $p = 0.001$, 999 permutations). The high dissimilarity ranges between and within seasons (Fig. 5B and Figure S1B) highlight that bats exhibit considerable flexibility in pest consumption throughout the year. A seasonal pattern of pest composition was further supported by the GLMM analysis of NMDS dimensions, which revealed significant differences between spring and autumn in NMDS1 (Table 1; Figure S2A). No seasonal effect was, however, detected for NMDS2 (Table 1; Figure S2B).

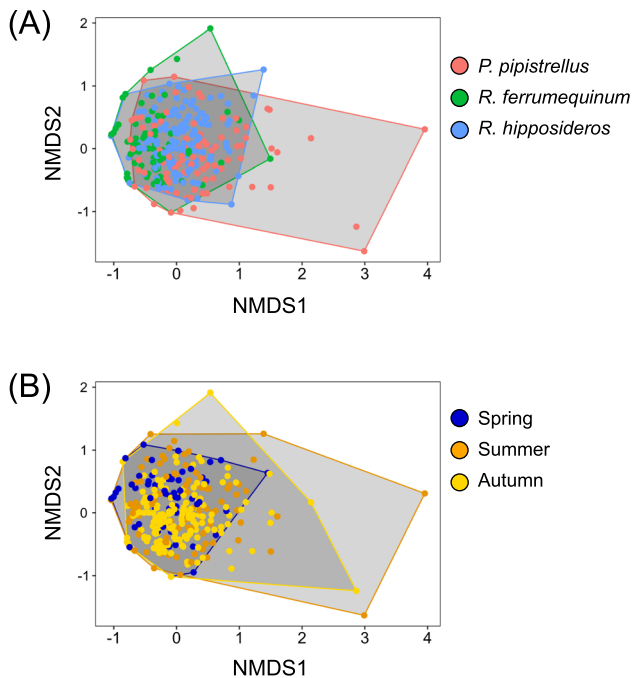


Fig. 5 Nonmetric multidimensional scaling (NMDS) analysis of pest composition in the diet of *Pipistrellus pipistrellus*, *Rhinolophus ferrumequinum* and *Rhinolophus hipposideros* **A**, and NMDS for the same samples across different seasons in Asturias, Spain **B**. Dots closer to each other correspond to samples with a more similar pest composition. Polygons group all dots of each species (**A**) or season (**B**)

Discussion

Our study revealed that three common European bat species—*Pipistrellus pipistrellus*, *Rhinolophus ferrumequinum*, and *R. hipposideros*—inhabiting a bocage landscape in Asturias, Spain, feed primarily on Lepidoptera and Diptera, including a broad range of pests affecting various crops. Bats consistently fed on pests across seasons, with some temporal variation in the richness and composition of pest species consumed, as well as moderate dietary overlap among species. These findings highlight the complementarity and redundancy of these bat species in their consumption of agricultural pests, underscoring their collective role in maintaining resilient pest control services within bocage landscapes.

Pest consumption across bat species

The results of our study clearly demonstrate that all three bat species consume a wide variety of agricultural pests, particularly lepidopteran species, even in a bocage landscape rich in seminatural habitats and where crops are

not the dominant land cover type. We recorded a higher number of crop pest species in the diet of the bat species studied than those previously reported (Tuneu-Corral et al. 2023). Previous studies on bat predation of agricultural pests have been mostly conducted in homogeneous habitats, such as monocultures (e.g. Kolkert et al. 2020; Whitby et al. 2020; Baroja et al. 2021), where bats are likely exposed to fewer pest species compared to more complex landscapes, which include a variety of crops. In fact, dietary diversity tends to be lower in areas dominated by intensive agricultural fields, suggesting that monoculture crops restrict the dietary niche of bats (Aizpurua et al. 2018).

Many agricultural pests detected in the bat faeces in our study pose a serious threat to a wide range of crops, including economically important local crops such as apple, blueberry, and corn. Notably, we identified several key invasive pest species in the bats' diet, such as the spotted wing drosophila (*D. suzukii*), which feeds directly on soft fruits causing significant economic losses in berry farming (Tait et al. 2021); the chestnut gall wasp (*D. kuriphilus*), which affects chestnut trees by forming galls on the branches, stunting tree growth and reducing fruit production (Avtzis et al. 2019); or the tomato leaf miner (*T. absoluta*), a pest that has become a significant threat to tomato crops in Europe (Desneux et al. 2022). These findings underscore not only the economic importance of bats as natural pest regulators in agricultural landscapes (Ramírez-Fráncel et al. 2022; Tuneu-Corral et al. 2023), but also their ecological significance as consumers, and even as detectors (Maslo et al. 2017), of invasive insect species. However, it is important to note that DNA metabarcoding provides information on diet composition and relative prey occurrence, but does not allow quantifying consumption intensity or biomass intake. Therefore, assessing the population-level impact of bat predation on pest species would require complementary approaches, such as bat exclusion experiments (e.g. Rodríguez-San Pedro et al. 2020; Tuneu-Corral et al. 2024).

In terms of both pest richness and composition, our analyses revealed overlap in pest consumption among the three bat species, together with specific differentiation in the types of pests consumed and the frequency of consumption of specific taxa. These differences are already noticeable at the family level and become even more pronounced at the species level, with 20–35% of the pest species in the diet of each bat species being exclusive to that species. On the other hand, only 17% of the total pest species detected were shared in the diet of all three bat species, and many of these were among the pest taxa most frequently consumed by bats in our study (see Andriollo et al. (2021) for similar proportions in three sympatric *Plecotus* species). This likely reflects their prevalence across the bocage landscape and the region, making them

easier for the bats to encounter and capture, although this would need to be confirmed with insect abundance data (Wray et al., 2021).

The dissimilarities in the composition of the pest consumed can be attributed in part to trait matching between the different bat species and their prey, as bat traits such as size, echolocation patterns, and hunting behaviours directly influence their ability to capture and consume different types of pests (Hughes et al. 2021; Divoll et al. 2022; Liu et al. 2024). For instance, the weight of *R. ferrumequinum* is four times that of *R. hipposideros* and *P. pipistrellus*, likely enabling it to capture larger, more robust pests. This is reflected, for example, in the comparably higher frequency of pest moths from families Noctuidae and Hepialidae in its diet, which often include species much larger than Tortricidae (Manley 2021), the family preferred by *R. hipposideros* and *P. pipistrellus*. Accordingly, no microlepidopteran pests from the families Gelechiidae and Gracillariidae were detected in the diet of *R. ferrumequinum*. Furthermore, the feeding strategies differ among the three bat species, allowing them to forage in different microhabitats and access to different types of prey. The rhinolophids are considered narrow-space foragers, able to prey on arthropods positioned on or near to vegetation or the ground, whereas *P. pipistrellus* is classified as edge-space forager, typically hunting airborne prey near the edges of vegetation (Schnitzler et al. 2003). In sum, differences in body size, foraging behaviour, habitat use, prey selection, and the abundance of particular pest species can drive dietary specialization among bat species, potentially reducing interspecific competition (Novella-Fernández et al. 2020; Andriollo et al. 2021; Liu et al. 2024), with implications for their pest control services (Hughes et al. 2021; Kolkert et al. 2021; Augusto et al. 2024).

Variation in pest occurrences and seasonal foraging patterns among bat species may enable trophic niche segregation and resource partitioning, as suggested by the species-specific pest proportion in the diet of each bat and the significant interspecific differences in pest composition observed in the NMDS analyses. These dietary differences across species may be interpreted as functional complementarity that could enhance the overall magnitude of pest control by ensuring that a wider array of pest species is consumed across time and space (Mata et al. 2021; Liu et al. 2024). At the same time, the observed dietary overlap among species suggests the existence of functional redundancy within the bat assemblages. Such redundancy may confer resilience to ecosystem services like pest suppression, as the loss or decline of one species could be compensated by others with similar feeding habits (Biggs et al., 2020; Salinas-Ramos et al. 2020). The combination of niche partitioning and redundancy highlights the ecological value of maintaining diverse bat assemblages, not only to maximize the effectiveness of pest control, but also to ensure the stability of this

service under changing environmental conditions (Baroja et al. 2021; Augusto et al. 2024; Liu et al. 2024).

An ecological network analysis based on the diet of 19 bat species in a Mediterranean landscape revealed a modular structure with six sets of bat and pest species that are more likely to interact with each other (Mata et al. 2021). Interestingly, *P. pipistrellus*, *R. ferrumequinum*, and *R. hipposideros* were classified into three distinct modules. The fact that each bat species targets different subsets of pests underscores the need to preserve diverse bat populations in agroecosystems, as each species may play a unique role in regulating particular groups of pest species (Mata et al. 2021). Importantly, our results echo previous findings for birds in the same bocage system (García et al. 2024), suggesting a common pattern in crop pest control, regardless of vertebrate group involved.

Seasonal variation in pest consumption

The analysis of seasonal variation in pest consumption revealed that bats consumed certain pest species consistently across seasons, while others were consumed depending on the time of year. Only a small proportion of pest species (8.4–24.3% per bat species) was shared across seasons, whereas over 50% of pest taxa were detected exclusively in a single season for each bat species. Notably, considering the percentage of pest species exclusive to each bat species (20–35%) or to individual seasons (55–63%), along with the ANOSIM R values (0.140 for species and 0.225 for seasons), temporal variation in pest consumption appears to exceed interspecific variation, as previously observed for other sympatric bat species (Andriollo et al. 2021).

These seasonal patterns suggest a combination of temporal consistency and season-specific shifts in pest consumption. The consistent detection of certain pest species across all seasons may reflect the non-selective consumption of abundant pests with broad phenologies—that is, species that are present in the environment throughout the bat activity period. For example, *M. unipuncta*, *Triodia sylvina*, and *T. oleracea* were abundantly detected year-round in all or most bat species. In contrast, the seasonal occurrence of other pest taxa in the diet may result either from passive tracking of prey availability (neutral processes; e.g. Baroja et al. 2021; Charbonnier et al. 2021) or from active prey selection in response to seasonal energetic demands related to breeding or fat accumulation prior to hibernation (deterministic processes; e.g. Levin et al. 2013; Wray et al., 2021).

Overall, the observed seasonal turnover in pest taxa, together with the consistent consumption of some species across seasons, highlights the dietary flexibility of bats and their potential to contribute to pest suppression across the growing season (Weier et al. 2019; McCracken et al. 2012; Aihartza et al. 2023). However, a more robust understanding

of the drivers of temporal dietary variation—particularly distinguishing between availability-driven and preference-driven patterns—requires complementary data on pest abundance and phenology in the landscape (Wray et al., 2021).

Implications for pest control

The widespread occurrence of crop pests in the diets of all three bat species suggests that bats may play a significant role in pest control within bocage landscapes. The presence of not only key crop pests, such as the codling moth, tomato leaf miner, and spotted wing drosophila, but also numerous minor pests underscores the broad contribution of bats in reducing pest damage to various fruit and vegetable crops. Notably, the three species studied here, along with *Miniopterus schreibersii* (also present in the study area; Miñarro and García, 2025), have been identified as the four key targets for bat conservation in Iberian agroecosystems, as they are major pest consumers and collectively cover a wide spectrum of pest taxa (Mata et al. 2021). The consistent consumption of key pests across seasons indicates that bats provide a continuous background level of predation that complements other pest-control strategies. This makes bat activity highly relevant for Integrated Pest Management (IPM) frameworks and for environmentally friendly pest-control incentive schemes (Tuneu-Corral et al. 2023). For farmers, sustaining bat activity can reduce the need for chemical treatments, lower production costs and help minimize pesticide residues on fruits. In regions such as Asturias—characterized by small, closely juxtaposed plots of fruit orchards, maize and horticultural crops—this year-round, multi-species pest suppression is especially advantageous. Promoting bat conservation through habitat protection and restoration is therefore essential, but the limited evidence on its effectiveness needs to be addressed through broad collaboration between scientists and practitioners, tailored to the local context, to better integrate research into land-use policy (Tuneu-Corral et al. 2023). Safeguarding roost sites, providing water sources, and preserving foraging habitats can enhance both the magnitude and resilience of natural pest control services by bats, thereby reducing reliance on chemical pest control. This approach not only supports agricultural productivity but also contributes to broader biodiversity conservation efforts, fostering a more sustainable balance between farming and wildlife.

Author contributions

Conceptualization: MM, DG. Data curation: MM. Formal analysis: DG, MM. Investigation: All authors. Methodology: MM, DG, JCI. Visualization: MM. Writing—original

draft: MM. Writing—review & editing: All authors. Funding acquisition: MM, DG. Project administration: MM.

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Data availability Data will be made available on request.

Declarations

Conflict of interest The authors declare no competing interests.

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