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Agriculture shapes the trophic niche of a bat preying on multiple pest arthropods across Europe: Evidence from DNA metabarcoding

Ostaizka Aizpurua[1] | Ivana Budinski[2] | Panagiotis Georgiakakis[3] | Shyam Gopalakrishnan[1] | Carlos Ibañez[4] | Vanessa Mata[5] | Hugo Rebelo[5] | Danilo Russo[6] | Farkas Szodoray-Parádi[7] | Violeta Zhelyazkova[8] | Vida Zrncic[9] | M. Thomas P. Gilbert[1,10] | Antton Alberdi[1]

[1]Section for Evolutionary Genomics, Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark

[2]Department of Genetic Research, Institute for Biological Research "Siniša Stanković", University of Belgrade, Belgrade, Serbia

[3]Department of Biology School of Sciences and Engineering, University of Crete, Irakleio, Greece

[4]Evolutionary Ecology Department, Estacion Biologica de Doñana (CSIC), Seville, Spain

[5]CIBIO-InBIO, Centro de Investigação em Biodiversidade Recursos e Genéticos, Universidade do Porto, Vairão, Portugal

[6]Wildlife Research Unit, Dipartimento di Agraria, Università degli Studi di Napoli Federico II, Portici, Italy

[7]Romanian Bat Protection Association, Satu-Mare, Romania

[8]National Museum of Natural History, Sofia, Bulgaria

[9]Geonatura d.o.o., Zagreb, Croatia

[10]NTNU University Museum, Trondheim, Norway

Correspondence
Antton Alberdi, Section for Evolutionary Genomics, Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark.
Email: antton.alberdi@snm.ku.dk

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Abstract

The interaction between agricultural production and wildlife can shape, and even condition, the functioning of both systems. In this study, we i) explored the degree to which a widespread European bat, namely the common bent-wing bat *Miniopterus schreibersii*, consumes crop-damaging insects at a continental scale, and ii) tested whether its dietary niche is shaped by the extension and type of agricultural fields. We employed a dual-primer DNA metabarcoding approach to characterize arthropod 16S and COI DNA sequences within bat faecal pellets collected across 16 Southern European localities, to first characterize the bat species' dietary niche, second measure the incidence of agricultural pests across their ranges and third assess whether geographical dietary variation responds to climatic, landscape diversity, agriculture type and vegetation productivity factors. We detected 12 arthropod orders, among which lepidopterans were predominant. We identified >200 species, 44 of which are known to cause agricultural damage. Pest species were detected at all but one sampling site and in 94% of the analysed samples. Furthermore, the dietary diversity of *M. schreibersii* exhibited a negative linear relation with the area of intensive agricultural fields, thus suggesting crops restrict the dietary niche of bats to prey taxa associated with agricultural production within their foraging range. Overall, our results imply that *M. schreibersii* might be a valuable asset for biological pest suppression in a variety of agricultural productions and highlight the dynamic interplay between wildlife and agricultural systems.

KEYWORDS
agriculture, Chiroptera, eDNA, Invertebrates, *Miniopterus schreibersii*, pest suppression, predator–prey interactions

1 | INTRODUCTION

Deciphering the dynamic interplay between agricultural activities and wildlife is essential for not only increasing the productivity and quality of crops (Savary, Ficke, Aubertot, & Hollier, 2012), but also improving our understanding of the biology of many animal species (Federico et al., 2008). One such interaction that is often argued to be of considerable significance is the consumption of the arthropod pests of crops by insectivorous animals (Kunz, Braun de Torrez, Bauer, Lobova, & Fleming, 2011; Wenny et al., 2011). In fact, the annual crop destruction caused by herbivorous arthropods (mainly lepidopteran larvae) has been estimated to be *ca*. 10% globally (Oerke, 2006). As the regulation of pesticides is becoming increasingly strict, many pests are rapidly developing resistance to such treatments and there is a growing consumer demand for organically produced products (Jensen, Karlsson, Sarrocco, & Vannacci, 2016). Thus, there is ever growing attention towards the importance of biological suppression of pest species (Naranjo, Ellsworth, & Frisvold, 2015; Zehnder et al., 2007). Due to their dietary habits, insectivorous bats (Order Chiroptera) are argued to be one of the most promising biological suppressors of the arthropods that both harm crops (Kunz, Whitaker, & Wadanoli, 1995; Kurta, Bell, Nagy, & Kunz, 1989; O'Farrell, Studier, & Ewing, 1971) and affect free-ranging livestock (Ancillotto et al., 2017). Indeed, recent estimates suggest that their use as natural pest suppressors might lead to savings in the order of billions of US dollars per year (Boyles, Cryan, McCracken, & Kunz, 2011; Maine & Boyles, 2015).

Several previous studies that aimed to estimate the impact of bats on crop pests used species-specific primers to detect crop-damaging arthropod species in DNA extracts from bat faeces, either through quantitative PCR (qPCR) or Sanger sequencing (Brown, de Torrez, & McCracken, 2015; McCracken et al., 2012; Puig-Montserrat et al., 2015). Such approaches are useful for assessing the bats' consumption of a few well-characterized prey taxa in geographically restricted areas. However, many pest species are specific to a certain type of crop, and different pests can affect identical agricultural productions in different regions. One solution is to use DNA metabarcoding of bat faecal pellets, whereby mini-barcoding PCRs are coupled with high-throughput sequencing (HTS) technology to assess the arthropod diversity of a sample (Bohmann et al., 2011). This approach has been successfully used to study sexual and seasonal variation in bat diet (Mata et al., *2016;* Vesterinen et al., 2016), predator–prey interactions (Clare, Fraser, Braid, Fenton, & Hebert, 2009; Dodd, Chapman, Harwood, Lacki, & Rieske, 2012) and resource partitioning (Bohmann et al., 2011; Razgour et al., 2011), among others. Although geographically localized DNA metabarcoding studies have revealed some bat populations occasionally (e.g., Razgour et al., 2011; Vesterinen et al., 2016) or regularly (e.g., Krauel, Brown, Westbrook, & McCracken, 2017) consume crop pest species, no previous study has shown the incidence of pest species in the dietary niche of a species across a whole continent.

The common bent-wing bat, *Miniopterus schreibersii*, is one European bat species that holds considerable promise as a natural consumer of pest insects. This species can form colonies of thousands of individuals (Hutson, Aulagnier, Karataş, J, & Paunović, 2008), and thanks to its fast flight (45 km/hr), it can cover large foraging ranges (estimated up to 223 km^2), with nightly moves of up to 30 km between the roost and the feeding grounds (Vincent, Nemoz, & Aulagnier, 2010). Previous morphology-based analyses of its diet have reported contrasting results: some found that its dietary niche is overwhelmingly dominated by lepidopterans (Lugon, 2006; Presetnik & Aulagnier, 2013), which encompass the most damaging crop pest species, yet others reported wider dietary niche breadths that include large consumption of coleopterans, orthopterans, hemipterans and lepidopterans (Whitaker & Karataş, 2009).

Although we are beginning to improve our understanding of the prey species consumed, one facet of bat–pest interaction that remains less well studied is how agricultural activities modify this interaction (Maine & Boyles, 2015). Given some bat species have been documented to considerably modify their dietary habits in response to prey availability (Almenar, Aihartza, Goiti, Salsamendi, & Garin, 2012; Fenton & Morris, 2011; Gonsalves, Law, Webb, & Monamy, 2013; McCracken et al., 2012), we hypothesized that the intensive agricultural processes that trigger local population blooms of specific arthropod taxa could shape the feeding habits of *M. schreibersii* by restricting their dietary niches to prey taxa associated to crops within their foraging range. To test this, we conducted a study aimed at improving our understanding of the ecological relation between *M. schreibersii*, agricultural crops and their associated pest arthropods, through metabarcoding the dietary DNA content of their faeces. Samples were collected from individual bats across 16 localities in eight southern European countries, which encompass a range of different agricultural crops, and cover most of the geographical distribution of the species (Figure 1). We subsequently (i) characterized *M. schreibersii*'s dietary niche throughout the sampled area, including the incidence of pest species within its diet, (ii) tested whether its dietary niche exhibits geographical differences and (iii) identified the main biotic and abiotic factors driving such variation, with special emphasis on agricultural factors. We specifically tested whether the extension of intensive agricultural production areas reduces the dietary niche dimension and whether the resulting diet is specialized on crop pest arthropods associated with nearby crops. In the light of the results, we discuss the potential role of *M. schreibersii* as a natural biological suppressor of crop pests.

2 | MATERIALS AND METHODS

2.1 | Sampling and sample storage

We collected droppings from 79 individual *M. schreibersii* bats caught in 16 caves (4.9 ± 0.25 bats/cave) distributed across Southern Europe between May and September 2015–2016 (Figure 1). Bats were captured in or near cave entrances using harp-traps and/or mist-nets

FIGURE 1 (a) Geographical distribution of the caves where *Miniopterus schreibersii* bats were captured to collect faecal samples. The shaded area represents the species' European range according to IUCN (Hutson et al., 2008). (b) Location of sampling sites in the climatic space of *M. schreibersii*'s geographical distribution. The principal components were generated for sampling (n = 16; large coloured dots) and random localities (n = 500; small dots) using four climatic predictors, namely annual mean temperature and seasonality, and annual total precipitation and seasonality [Colour figure can be viewed at wileyonlinelibrary.com]

when returning from foraging (1am–7am), which ensured fast defecation. To avoid sample cross-contamination, each bat was kept separately in a clean, single-use, UV-radiation sterilized cotton bag for 15–20 min, then identified, sexed and aged before releasing it back into the cave. Faecal pellets were collected from the bags and stored in 1.5-ml collection tubes filled with silica gel granules (Chameleon® C 1–3 mm, VWR). Samples were kept dried and refrigerated (4–8°C) until they were transported to the laboratory, after which they were stored at −20°C until DNA extraction. All captures were authorized according to the laws of the countries where they were carried out (Table S1).

2.2 | DNA extraction, qPCR screening and amplification

DNA was extracted from 1 to 5 bat droppings (dry weight 15-20 mg) per bat using the PowerSoil® DNA Isolation Kit (MoBio, CA, USA). The manufacturer's protocol was used with the modifications detailed in Alberdi, Aizpurua, Gilbert, & Bohmann (2017). Each extraction round included 15 bat faecal samples and one negative control. We used two primer sets covering different mitochondrial markers to reduce primer-specific taxonomic bias and thereby optimize taxonomic diversity retrieval (Alberdi et al., 2017). The ZBJ-ArtF1c/ZBJ-ArtR2c primers (Zeale, Butlin, Barker, Lees, & Jones, 2011) amplify a cytochrome oxidase I (COI) marker within the traditional barcode region and the Coleop_16Sc/Coleop_16Sd (Epp et al., 2012) primers target a 16S rRNA marker. The primer sets are hereafter referred to as Zeale and Epp primers, respectively. Both primer sets were 5′ nucleotide tagged with 6- to 7-bp tags to create a set of 60 tagged forward and 60 tagged reverse primer for each (Binladen et al., 2007). qPCR with SYBR green chemistry was carried out on a subset of samples and negative controls to optimize the following metabarcoding PCR amplifications (Murray, Coghlan, & Bunce, 2015). Specifically, for each primer pair, the amplification dynamics of different template volumes of 3 μl, 2 μl and 1 μl neat DNA extract, and dilutions at 1:1, 1:5 and 1:10 were assessed. qPCRs were carried out on an Agilent Technologies Stratagene Mx3005P qPCR Thermocycler (Agilent Technologies, Santa Clara, CA, USA). For full details, see Alberdi et al. (2017).

Metabarcoding was carried out on all sample extracts and all negative extraction controls. We only used primers carrying matching tag combinations when performing the metabarcoding PCRs (F1-R1, F2-R2, etc.), which allowed us to build 60 PCR replicates into each Illumina library while avoiding that tag jumps caused false assignments of sequences to samples (Schnell, Bohmann, & Gilbert, 2015). Each sample was PCR amplified in three replicates to balance diversity detection in the faecal samples while minimizing the effects of PCR stochasticity and primer biases (Alberdi et al., 2017). Amplification of the three PCR replicates from each sample was undertaken using different tag combinations to minimize potential effects of tag bias (Berry, Ben Mahfoudh, Wagner, & Loy, 2011). All PCRs were set up in a dedicated pre-PCR laboratory to minimize the risk of contamination. A PCR negative control was included for every 14 reactions.

2.3 | Amplicon pooling, library preparation and sequencing

PCR products with different tags were pooled at volumes determined by gel band strengths, which produced three amplicon pools for each of the two markers. The six amplicon pools were subsequently bead-purified using Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA) at a 1:2 amplicon pool:bead ratio. Purified amplicon pools were built into Illumina sequencing libraries using the single-tube "BEST" library build protocol (Carøe et al., 2017). The libraries were indexed using PCR (see Alberdi et al., 2017 for further information), bead-purified, quantified on an Agilent 2100 Bioanalyzer and pooled at equal molarities before sequencing on an Illumina MiSeq spiked with 15% PhiX using 250 bp paired-end chemistry.

2.4 | Bioinformatics processing

Reads were demultiplexed based on the library indices, after which paired reads were merged and quality-filtered using AdapterRemoval 2.1.7 with standard parameters (Lindgreen, 2012). Quality statistics of the sequences before and after quality filtering were generated using FastQC 0.11.5 (Andrews, 2010). The quality-filtered sequences were sorted by primers and tags, and only sequences that were represented by at least two copies and that appeared in at least two of the three PCR replicates were retained using a modified version of the DAMe (Zepeda-Mendoza, Bohmann, Carmona Baez, & Gilbert, 2016) toolkit (https://github.com/shyamsg/DAMe). Sequences in samples that were identical to those detected in the negative extraction control of the corresponding extraction round were removed using a custom shell script (available from the authors on request). Sumaclust (Mercier, Boyer, Bonin, & Coissac, 2013) was used to cluster OTUs at a 98% similarity threshold following Alberdi et al., 2017; and the *tabulateSumaclust.py* script from the modified DAMe toolkit was used to generate the OTU tables and normalize the number of sequences per sample to ensure comparability. OTU rarefaction curves and curvature indices of all samples were generated using the R package DivE 1.0, and samples that neither reached the rarefaction plateau, nor showed a curvature index below 0.85, were discarded. The BOLD and GenBank nt databases were used to assign taxonomy, using Bold Retriever 1.0.0 (Vesterinen et al., 2016) and the Blast+ 2.5.0 suite (Camacho et al., 2009). From each of the two databases, we retrieved the best 50 matches per OTU sequence. Order-level taxonomy was assigned at >95% identity values, family-level taxonomy at >96.5%, and species-level taxonomy was assigned when the identity values between the query and reference sequences were above 98%, following Alberdi et al., 2017;. Species-level identification was only performed using the Zeale data set due to the low reliability of Epp sequences for species-level taxonomic identification (Alberdi et al., 2017; Kaunisto, Roslin, Sääksjärvi, & Vesterinen, 2017). All identified species were manually checked, and species-level assignment was restricted only to species known to be present at each sampling region. When multiple species shared the highest matching score or when both databases yielded different taxa, we assigned the taxonomy of the species present in the geographical area, and if the uncertainty persisted, identification was downgraded to the highest common taxonomic level. Family-level taxonomic diversity detected by the two primer sets was visualized as a multilayered pie chart using Krona 2.7 (Ondov, Bergman, & Phillippy, 2011).

2.5 | Spatial analyses

As lepidopteran assemblages are shaped by biotic and abiotic conditions, we also explored whether the dietary variation observed among sampling sites responded to habitat structure, vegetation productivity or climatic conditions (Summerville & Crist, 2004; Wilson et al., 2005). To assess the potential foraging habitat available at each sampling site, we consulted Copernicus (www.copernicus.eu) to retrieve land use data from the Corine Land Cover European seamless 100 m raster database (version 18.5). The land cover categories were reclassified into five classes: urban, intensive agriculture, extensive agriculture, natural landscape and wetland (details in Table S2). Habitat types that *M. schreibersii* does not use for foraging, such as sea, glacial areas or salines, were excluded from the calculation of the total useful area (Vincent et al., 2010). As sampling was undertaken at different times to account for local seasonal effects, the Normalized Difference Vegetation Index (NDVI) of the total useful area and the intensive crops was also analysed. Weekly NDVI data between 1/1/2014 and 31/12/2016 with 250-m resolution were retrieved from the website of the Institute of Surveying, Remote Sensing and Land Information (IVFL) of the University of Natural Resources and Applied Life Sciences (BOKU), Vienna (http://ivfl-info.boku.ac.at/). Absolute NDVI values for each locality were calculated by averaging the 2–5 weeks prior the sampling, to account for the uncertainty of the life cycle rhythms of the consumed arthropods (Alford, 2014; Bailey, 2007), given that the NDVI of interest is when larvae were feeding rather than when adults are flying. To calculate relative NDVI values, the annual 95% and 5% NDVI percentiles (to exclude extreme events) of each cell using the 3-year data were calculated, and the absolute NDVI values were normalized to 0–1 scale. Based on the reported home range of *M. schreibersii* (Vincent et al., 2010), spatial analyses were restricted to areas within a 30-km radius around each sampling location. Climatic data were retrieved from WorldClim Database (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005). All GIS analyses were performed in ArcGis 10.1 (ESRI, Redlands, CA, USA).

2.6 | Statistical analyses

All statistical analyses were performed in the R statistical environment (R Development Core Team 2008). Rarefaction and extrapolation analyses were conducted using the library *iNEXT* (Chao et al., 2014), and figures were plotted using *ggplot2*. Shannon diversity indices were computed for each sample based on OTU tables using the *diversity* function included in the library *vegan*. For taxonomic analyses, OTUs were aggregated at different taxonomic levels using the *aggregate* function included in the library *data.table*. For species-level analyses, only the Zeale data set was employed due to the low discrimination capacity of the Epp marker (Alberdi et al., 2017), while family- and order-level analyses included averaged values of both Zeale and Epp data sets. Statistical differences of species-level and order-level taxonomic composition between localities were tested using nonparametric permutational multivariate analysis of variance implemented in the function *adonis* with locality information as a single explanatory variable. Taxonomic differences between localities were computed with the function *vegdist* using Jaccard distances applied to both species-level and order-level taxonomy tables aggregated by locality. The resulting distance matrices were visualized as pairwise species-level and order-level similarities between localities using the library corrplot. Correlation between dietary composition and geographical distance among sites was tested using Mantel

statistic based on Pearson's product-moment correlation. The relation between dietary diversity (Shannon Index) and climate (temperature, precipitation), habitat (habitat diversity) and productivity (total NDVI, crop NDVI) was tested using linear regression as implemented in the R function *lm*.

2.7 | Pest species characterization

Information about the dietary niche and economic impact of all species detected was gathered from multiple sources (Alford, 2012, 2014; Bailey, 2007; Carter, 1984; Hill, 2002). Using this information, species were classified in three groups: (i) Innocuous: species that do not regularly affect agricultural activities; (ii) Minor pests: species that regularly affect agricultural productivity yet without high economical impact; and (iii) Major pests: species that regularly affect agricultural productivity and have a high economical impact.

3 | RESULTS

We generated a total of 11.3 and 11.1 million paired-end reads from the Zeale and Epp libraries respectively. The data sets were reduced

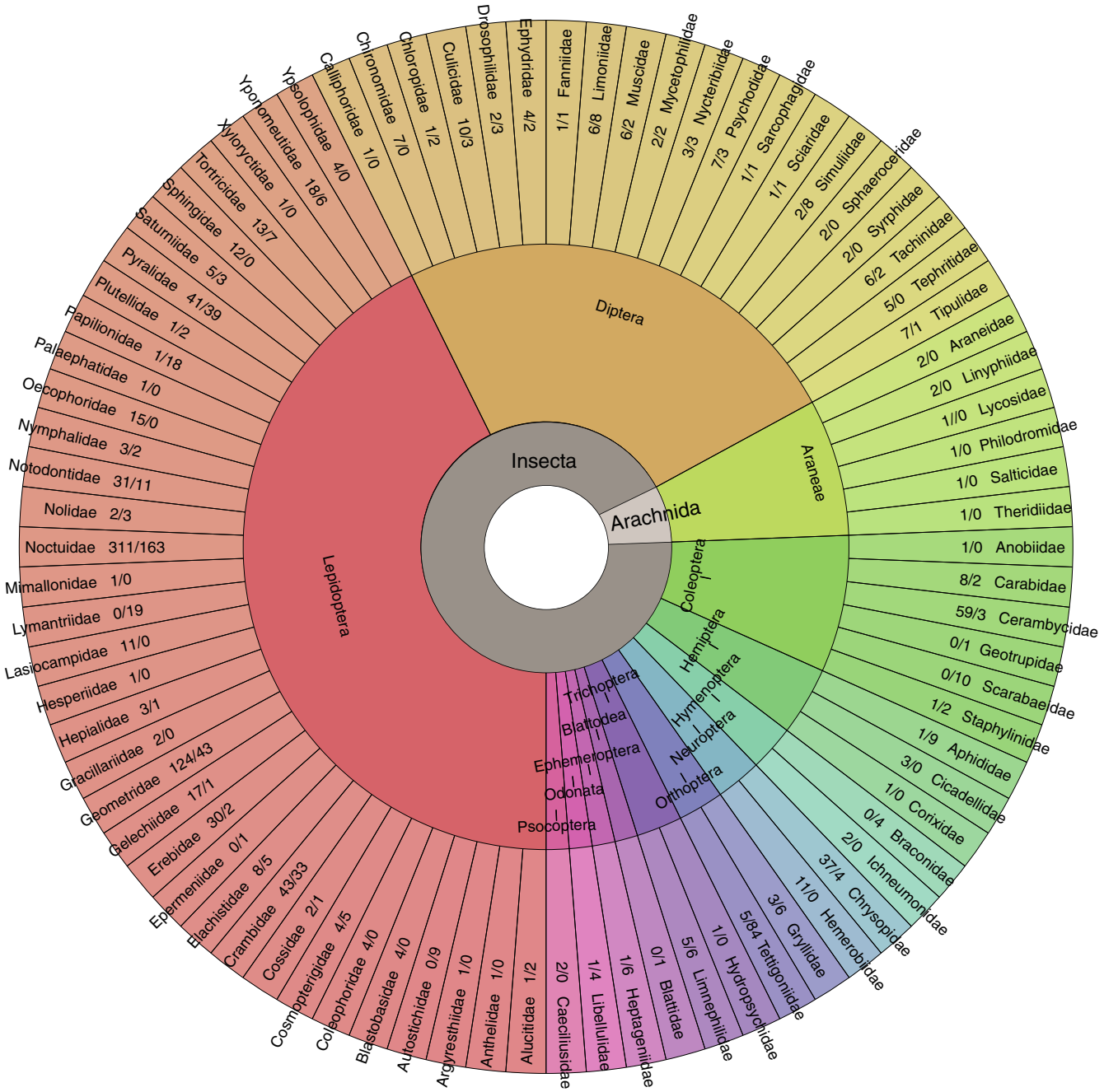

FIGURE 2 Family-level taxonomic diversity detected in the diet of *Miniopterus schreibersii*. The numbers next to the taxonomic names indicate the number of OTUs identified using the Zeale primers/Epp primers [Colour figure can be viewed at wileyonlinelibrary.com]

to 4.7 and 4.6 million (*ca.* 20,000 sequences per PCR replicate per sample), after merging, trimming, quality filtering, sorting by individual tags, removing singletons and filtering by PCR replicate. Zeale sequences were clustered into 1,204 OTUs, of which 966 (80%) were identified to order level and assigned to 10 taxa, and 566 (47%) of them were identified to species level and assigned to 276 taxa (Figure 2). Epp sequences were clustered into 677 OTUs, of which 426 (63%) were assigned to 11 arthropod orders. The orders Araneae and Psocoptera were detected only with the Zeale primers, while the orders Blattodea and Odonata were only detected with the Epp primers (Figure S1). Using the Zeale primers, the average number of OTUs detected and species identified per individual was 24.3 ± 19.2 and 8.4 ± 5.6, respectively. The mean species-level Shannon diversity was 0.63 ± 0.53. The dietary spectrum was clearly dominated by Lepidoptera, which accounted for 75% of the taxonomy-assigned OTUs. Moths were consumed by all but two individuals (Figure 3a). Within lepidopterans, Geometridae and Noctuidae were the most represented families (Figure 3b). Dipterans were the second most consumed prey. Several taxa of Coleoptera, Neuroptera, Orthoptera and Trichoptera were also recorded, although at a much lower frequency. Lastly, single representative taxa from Araneae, Blattodea, Hemiptera, Hymenoptera and Psocoptera were also found in the diet of *M. schreibersii*.

We detected 44 agricultural pest species in the diet of *M. schreibersii*, 22 of which are considered of special concern (Table 1). Pest species were detected at all but one sampling sites and were consumed by 92% of the analysed bats. Minor pests were detected in 68.1% of the samples, while major pests appeared in the diet of 72.7% of individual bats (Figure S2). In terms of the number of sequences, 57.2% of the sequences clustered into OTUs with species-level taxonomic assignment belong to pest species. Among minor pests, the most recurrent were *Noctua pronuba*, *Autographa gamma* and *Anarta trifolii*. Among major pests, *Agrotis ipsilon*, *A. segetum*, *Peridroma saucia*, *Thaumetopoea pityocampa*, *Peribatodes rhomboidaria* and *Prays citri* were found at highest incidence.

The species-level taxonomic composition was different across sampling sites (F = 1.19, df = 15,76, r^2 = .26, p-value <.001), with all pairwise Jaccard similarity values below 0.5 (Figure 4a). Compositional differences were nonexistent at the order level (F = 1.15, df = 15,76, r^2 = .22, p-value = .211), since all sites but two exhibited pairwise Jaccard similarity values above 0.5 (Figure 4a). Lepidopterans were overwhelmingly dominant in all sites except two, where coleopterans and dipterans dominated. Rarefaction and extrapolation analyses showed that a single sampling location would cover, on average, 7.9% and 14.2% of the estimated OTUs and species, respectively, and over 50 localities would need to be sampled under our sampling strategy in order to capture 90% of the total estimated OTU diversity. In each sampling site, we detected 34 ± 8.5% of the total OTU diversity estimated through rarefaction extrapolation, and we estimated that 33 ± 12 individuals per site would be needed in average to cover 90% of the total OTU diversity. Mantel tests showed a significant association between the species-level dietary composition and geographical distance of the studied localities (r = .263, p-value = .005). Neither dietary composition nor diversity showed any association with habitat diversity, habitat productivity and climatic variables. However, dietary diversity showed a negative linear relation with the area of intensive agricultural fields (F = 5.078, df = 1,13, p-value = .040) (Figure 4b).

4 | DISCUSSION

Although insectivorous bats are considered to be the main predators of arthropod pests (reviewed in Riccucci & Lanza, 2014), actual measures of the incidence of crop pest insects within the diet of bat species have been seldom studied (but see Krauel et al., 2017), principally because the molecular techniques that allow species-level

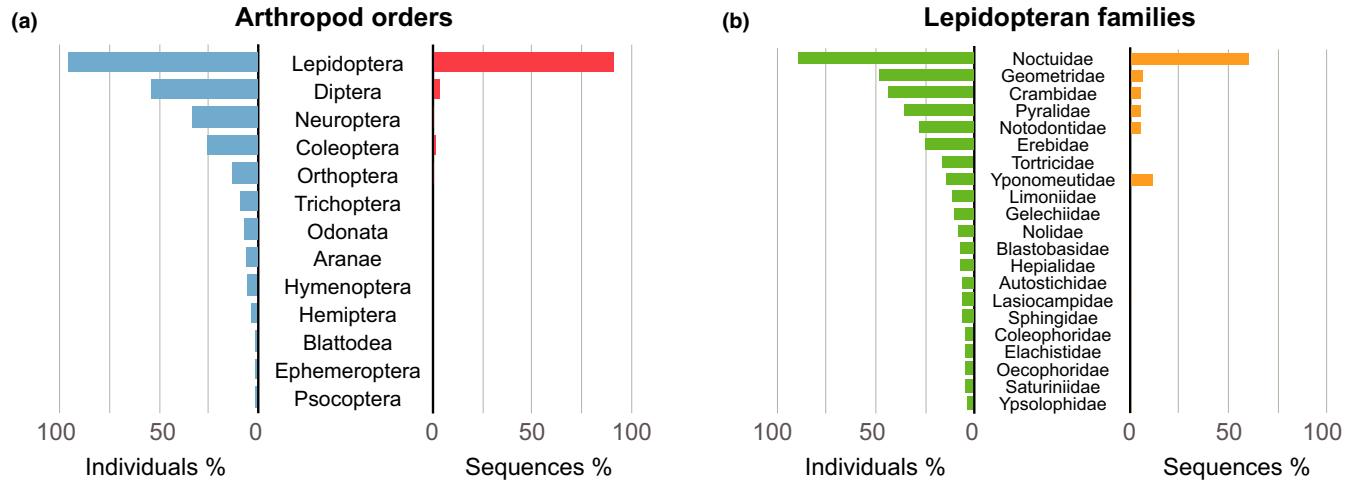

FIGURE 3 Relative representation of (a) arthropod orders and (b) Lepidoptera families in the diet of *Miniopterus schreibersii* expressed, respectively, as percentage of occurrence (i.e., relative number of individuals in which the taxon was detected) and sequence percentage (i.e., relative number of sequences clustered into OTUs assigned to the taxon) using both primers [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 List of arthropod pest species preyed upon by *Miniopterus schreibersii*, crop types affected by each species, and their frequency of occurrence in *M. schreibersii*'s diet. Category 1 refers to minor pests, while category 2 corresponds to major pests

Species	Family	Affected plants	Category	# samples	% samples	# sites	% sites
Acleris variegana	Tortricidae	Rose family (apple, pear, apricot. . .)	2	1	1.4	1	6.3
Acronicta tridens	Noctuidae	Rose family (apple, pear, apricot. . .)	1	1	1.4	1	6.3
Agrotis ipsilon	Noctuidae	Crops and pastures	2	15	21.4	10	62.5
Agrotis munda	Noctuidae	Crops and pastures	2	2	2.9	2	12.5
Agrotis segetum	Noctuidae	Root vegetables and cereals	2	13	18.6	8	50.0
Anarta trifolii	Noctuidae	Woody and herbaceous plants	1	8	11.4	6	37.5
Autographa gamma	Noctuidae	Leguminous cultures	1	9	12.9	7	43.8
Cadra figulilella	Pyralidae	Drying or dried fruits (figs, clusters of grapes on vines. . .)	1	1	1.4	1	6.3
Campaea margaritaria	Geometridae	Fruit trees (apple)	1	1	1.4	1	6.3
Ectomyelois ceratoniae	Pyralidae	High-value nut and fruit (dates, almonds, pistachios. . .)	2	1	1.4	1	6.3
Ephestia elutella	Pyralidae	Dry plants (cocoa, beans, tobacco), cereals and dried fruit and nuts	2	1	1.4	1	6.3
Etiella zinckenella	Pyralidae	Leguminous crops (soya beans,	2	2	2.9	2	12.5
Galleria mellonella	Pyralidae	Honeycombs	2	3	4.3	3	18.8
Gymnoscelis rufifasciata	Geometridae	Citrus and olives	1	5	7.1	3	18.8
Helicoverpa armigera	Noctuidae	Polyphagous (tomato, cotton, chickpea, rice, sorghum, cowpea. . .)	2	1	1.4	1	6.3
Homoeosoma nebulella	Pyralidae	Sunflowers	2	2	2.9	2	12.5
Hypena rostralis	Noctuidae	Hop	1	1	1.4	1	6.3
Hypsopygia costalis	Pyralidae	Clover hay	1	1	1.4	1	6.3
Loxostege sticticalis	Pyralidae	Sugar beet and tobacco	1	4	5.7	2	12.5
Mimas tiliae	Sphingidae	Fruit trees	1	1	1.4	1	6.3
Mythimna loreyi	Noctuidae	Cereals (wheat, barley, rice, corn. . .)	2	8	11.4	5	31.3
Mythimna separata	Noctuidae	Cereals (wheat, maize, rice, corn. . .)	2	3	4.3	2	12.5
Noctua comes	Noctuidae	Grape and tobacco	1	3	4.3	3	18.8
Noctua pronuba	Noctuidae	Strawberry, potato, grasses. . .	1	19	27.1	10	62.5
Ostrinia nubilalis	Crambidae	Corn	2	3	4.3	2	12.5
Ostrinia scapulalis	Crambidae	Hop	1	1	1.4	1	6.3
Pandemis heparana	Tortricidae	Trees and shrubs (apple, pear, apricot, cherry. . .)	1	2	2.9	2	12.5
Peribatodes rhomboidaria	Geometridae	Grapevine, fruit trees	1	8	11.4	5	31.3
Peridroma saucia	Noctuidae	Crops, trees, Shrubs	2	13	18.6	8	50.0
Plutella xylostella	Plutellidae	Cruciferous crops	2	3	4.3	3	18.8
Prays citri	Yponomeutidae	Citrus crops	2	8	11.4	2	12.5
Pyralis farinalis	Pyralidae	Stored food (milled plant products)	2	1	1.4	1	6.3
Sesamia nonagrioides	Noctuidae	Maize	2	4	5.7	3	18.8
Sitotroga cerealella	Gelechiidae	Cereal crop (wheat, barley, corn, rice, sorghum, millet)	2	1	1.4	1	6.3
Spilarctia luteum	Erebidae	Blackberry, raspberry, strawberry, apple	1	1	1.4	1	6.3
Spodoptera exigua	Noctuidae	Vegetable, field and flower crops (asparagus, cabbage, pepper, tomato, lettuce, celery, strawberry)	2	7	10.0	5	31.3
Thaumetopoea pityocampa	Notodontidae	Pine tree	2	10	14.3	3	18.8
Tipula oleracea	Tipulidae	Fruit crops (cane fruit, strawberry, hop)	1	3	4.3	3	18.8
Trichiura crataegi	Lasiocampidae	Rosaceous fruit trees (apple, plum)	1	3	4.3	3	18.8
Trichoplusia ni	Noctuidae	Cereal crop (wheat, barley, corn, rice, sorghum, millet)	2	1	1.4	1	6.3
Udea ferrugalis	Crambidae	Plum, gooseberry	1	4	5.7	2	12.5

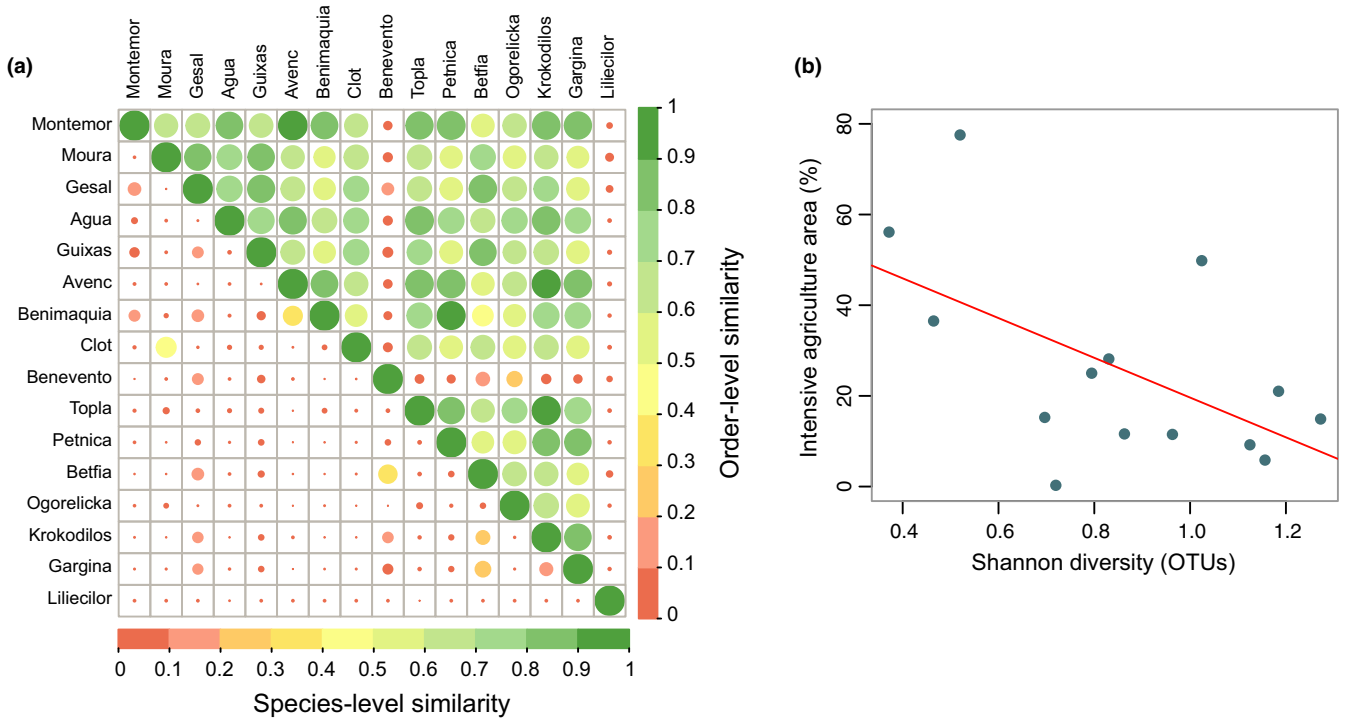

FIGURE 4 Taxonomic composition and diversity differences according to sampling sites and relative extension of intensive agriculture fields nearby. (a) Pairwise Jaccard similarity values between the average order-level (upper triangle) and species-level (lower-triangle) taxonomic composition per site. Colours and circle sizes indicate the level of similarity: red and small circles indicate low taxonomic similarity, and green and large circles indicate high taxonomic similarity. Sites are ordered according to latitude. (b) Linear model between the relative area of intensive agriculture within a 30-km radius from the bat roosting site and the averaged OTU-level Shannon diversity of the bats' diet in each site [Colour figure can be viewed at wileyonlinelibrary.com]

identification of prey items have only recently been developed (Bohmann et al., 2011). In this study, we merged DNA metabarcoding of faecal samples with spatial analyses to show that (i) the insectivorous bat *Miniopterus schreibersii* consumes a great variety of pest arthropods that affect different crops across the continent and (ii) the dietary niche of the species reflects habitat modification due to intensive farming.

Our results depict *M. schreibersii* as a moth specialist, as lepidopterans vastly outnumbered the remaining 11 arthropod orders consumed by the species. This is in agreement with previous studies based on morphological identification of prey remains in *M. schreibersii* droppings (Lugon, 2006; Presetnik & Aulagnier, 2013), and given that the Epp data set provided a similar picture, the observed pattern is unlikely to have resulted from primer biases ascribed to the Zeale primers (Clarke, Soubrier, Weyrich, & Cooper, 2014). The 16S targeting Epp primers thus not only offered useful validation of our principal dietary niche findings, but also enabled detection of coleopterans, odonates and orthopterans that were overlooked in many samples by the Zeale primers. Although masked in general by the overall dominance of moths, the identification of nonlepidopteran taxa at relatively high incidence in some of the studied locations (e.g., Benevento and Liliecilor, Figure 4) suggests that under certain local conditions, *M. schreibersii* is able to shift its dietary niche so as to increase its intake of other arthropod orders (Whitaker & Karataş, 2009). This local variation also highlights the importance of covering a large geographical scale when characterizing the dietary niche of a species, as local studies might not reflect the species' niche broadness.

Overall, we detected high OTU and species-level diversity in the faecal samples, as a result of the extensive geographical area covered. Only a few species were detected in >50% of the sampling localities, and our rarefaction analyses estimated that each location accounted for only *ca.* 8% of the overall detected diversity. Although the diversity we detected in each locality could be probably increased by sampling more bats per locality, we believe the sampling strategy employed was the most cost-efficient approach for obtaining a global picture of the dietary niche of *M. schreibersii*, as species-level composition varied across sampling sites, and geographically related localities exhibited more similar dietary compositions.

In total, we detected 44 species that are known to cause damage to a range of agricultural productive systems, including forestry (e.g., *Thaumetopoea pityocampa*), cereal (e.g., *Agrotis segetum*) and fruit production (e.g., *Prays citri*) and apiculture (e.g., *Galleria mellonella*). Generalist pests that feed on multiple plant species, including the most commonly grown vegetables, were detected at many sites (e.g., *Noctua pronuba*, *A. segetum*, *A. ipsilon*, *Peridroma saucia*). In contrast, pest species with a narrower dietary breadth were geographically restricted, yet exhibited large local impact (e.g., *T. pityocampa*, *Prays citri*, *Mythimna loreyi*). The coniferous-specialist moth *T. pityocampa*, for example, was detected in all bats analysed in Agua, and the rice

pest *M. loreyi* in all but one bats from Montemor, a locality surrounded by extensive rice paddies. The pest moth species with the highest incidence belonged to the family Noctuidae, although pests belonging to Pyralidae, Geometridae or Yponomeutidae were also detected in multiple individuals and localities. In total, we detected over 20 lepidopteran families with different size ranges, wing morphologies, flight patterns, evasive flight ability and capacity to hear bat echolocation calls. These findings suggest that *M. schreibersii* is able to capture virtually any kind of nocturnal lepidopteran, possibly because its fast flight allows overcoming the defensive mechanisms of moths.

Although we did not analyse how the abundance of different moth species changed according to habitat and climatic factors, we found that the OTU-level dietary diversity was shaped by the relative area around the studied bat colonies that is under intensive agriculture. Increases in the extent of land under intensive agriculture reduced the dietary diversity of *M. schreibersii*, leading to a focus on the moth species that specialize on feeding on the local crop systems. For instance, the citrus tree pest *Prays citri* was the dominant species in eight of the ten individuals sampled in the Iberian Peninsula's Mediterranean area. In that region, agriculture is dominated by citrus orchards, and *P. citri* is known as one of the pest species with the highest impact on fruit production (Tena & Garcia-Marí, 2011). In the light of the high ecomorphological diversity of moths consumed by *M. schreibersii*, we argue that their frequency in the diet reflects their high abundance in the farmed landscapes where this bat hunts, rather than be a result of active prey selection. Although we were unable to capture the entire dietary diversity at each sampling site, the pattern observed across the 16 localities distributed throughout the whole continent seems improbable to be a statistical artefact. Intensive agricultural practices are known to reduce biodiversity (Benton, Vickery, & Wilson, 2003), and our results indicate that this depletion also reduces the dietary niche of predators.

5 | CONCLUSIONS

Our geographically extensive dietary analysis shows that *M. schreibersii* has a high potential to act as a biological pest suppressor, although further studies and manipulation experiments are necessary to measure whether the consumption of pest insects is large enough to limit damage to susceptible crops. Our results suggest that the conservation of *M. schreibersii* might transcend the sole goal of biodiversity preservation and could have an economic impact on the viability of important agricultural crops across Europe. A necessary measure to ensure long-term survival to *M. schreibersii* colonies is to protect their underground habitats, as surrogate roosts such as bat boxes that are effective for other bat species (Flaquer, Torre, & Ruiz-Jarillo, 2006) would not work for this strictly cave-dwelling species. In terms of conservation, the highly gregarious behaviour typical of this species makes its protection especially challenging, as the disappearance of even a single colony may have large-scale consequences (O'Shea, Cryan, Hayman, Plowright, & Streicker, 2016) and *M. schreibersii*'s high mobility requires the protection of large roost networks to ensure gene flow and support viable populations (Rodrigues, Ramos Pereira, Rainho, & Palmeirim, 2010). Generating empirical evidence about the ecosystem services this and other bat species provide will be helpful to convince agriculture and nature protection policy makers as well as farmers about the importance of conserving wild bat populations.

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DATA ACCESSIBILITY

16S and COI DNA amplicon sequences are available in the Dryad Repository, https://doi.org/10.5061/dryad.2ff46.

AUTHOR CONTRIBUTIONS

O.A., M.T.P.G. and A.A. designed the study. A.A., I.B., P.G., C.I., H.R., D.R., F.S.P., V.M. and V.Z. participated in the fieldwork. O.A. and A.A. performed the laboratory work. O.A., S.G. and A.A. analysed the data. O.A. and A.A. wrote the manuscript. All authors contributed to the final version of the manuscript.

ORCID

Antton Alberdi http://orcid.org/0000-0002-2875-6446

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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