Agriculture, Ecosystems and Environment 411 (2026) 110621

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

Trophic ecology of lizards in vineyards: Diet composition and implications for pest control

Giulia Simbula[a,c,*], Ana Ramos[a,b], Sara F. Nunes[a,c,d], Miguel A. Carretero[a,c,d], Ricardo J. Lopes[e,**,1], Catarina Rato[f,**,1]

[a] BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Vairão, Portugal
[b] ICBAS – Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal
[c] CIBIO Associate Laboratory, Research Centre in Biodiversity and Genetic Resources, University of Porto, Vairão, Portugal
[d] Department of Biology, Faculty of Sciences, University of Porto, Porto, Portugal
[e] CE3C, Center for Ecology, Evolution and Environmental Changes & CHANGE, Global Change and Sustainability Institute, Departamento de Biologia, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal
[f] APH, Associação Portuguesa de Herpetologia, Porto, Portugal

ARTICLE INFO

Keywords:
Agriculture management
Metabarcoding
Stable Isotopes
Reptiles

ABSTRACT

Promoting pest management strategies, based on pest consumption by natural enemies rather than pesticide applications, represents a promising and more sustainable pathway in agriculture. Despite being considered mid-level consumers, "farmers' friends", present in agroecosystems, lizards are still largely overlooked as potential biological control agents in agricultural landscapes. In this study, we combined DNA metabarcoding and stable isotopes approaches to assess the trophic role of the wall lizard *Podarcis bocagei*, and specifically their contribution to pest consumption. We sampled seven vineyards from NW Portugal, across a gradient of pesticide application regimes. Overall, 316 arthropod prey species, from 95 families, 25 orders and 7 classes were detected in the lizard pellets. *Agrotis* sp. (Lepidoptera) was the only vineyard pest identified, and it occurred at relatively low frequencies across populations. Both DNA metabarcoding and isotopic approaches recovered consistent patterns in trophic structure across lizard populations: generalist diets, high overlap in prey composition among populations, and only limited divergence in niche breadth, regardless of local differences in prey availability and pesticide exposure. Although pest consumption by *P. bocagei* was limited, our study highlights that wall lizards in agroenvironments remain flexible enough to consume a wide array of arthropod groups and, expectably, other pests eventually emerging. The complexity of trophic interactions under agricultural intensification indicates a need for integrative, multi-seasonal, and multi-method approaches to fully understand reptile-mediated ecosystem services.

1. Introduction

Ecosystems are increasingly recognized as providers of essential services to support human life and well-being (Millennium Ecosystem Assessment Program., 2005). Within this framework, humans are recognised as both beneficiaries of ecosystem services and key drivers of ecological change. Agro-environments offer a clear example of this duality, with biodiversity providing important essential ecosystem services (i.e., food, plant materials) in and around farms (Brook et al., 2008), but at the same time, still relying on external intervention, such as chemical application, to maintain their functionality. This reliance has often led to the intensification of agricultural practices through mechanisation, removal of natural vegetation, fertilisation, and the widespread use of pesticides (Benton et al., 2021). As a paradigmatic case, in many South European countries, the recent reforms under the EU Common Agricultural Policy are driving rapid and profound changes in agricultural landscapes (Bastian et al., 2007). Because these shifts are both recent and fast, it remains difficult to disentangle the agricultural practices that are truly sustainable in the long-term, from those that merely exploit short-term biodiversity resilience.

---

* Corresponding author at: BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Vairão, Portugal.
** Corresponding authors.
E-mail addresses: giulia.simbula@gmail.com (G. Simbula), rjlopes@ciencias.ulisboa.pt (R.J. Lopes), catirato@gmail.com (C. Rato).

[1] These two authors equally contributed to the work.

https://doi.org/10.1016/j.agee.2026.110621
Received 29 July 2025; Received in revised form 22 June 2026; Accepted 25 June 2026

One of the challenges emerging from these rapid agricultural modifications is the increased vulnerability of crops to pest outbreaks (Sharma et al., 2017; Deutsch et al., 2018). Pesticide application is one of the main strategies adopted to control crop pests, with new formulations constantly emerging to meet global agricultural demand (Khan et al., 2023). However, growing evidence highlights the negative impacts of pesticide use on human health, biodiversity, and the environment (Ali et al., 2021). Moreover, excessive reliance on these chemicals can, over time, trigger negative feedback loops in crop production by accelerating the development of pesticide resistance among the target pest species (Liang et al., 2025), disrupting ecological networks, and ultimately leading to pest outbreaks due to the decline of natural enemies (Serrão et al., 2022). As such, promoting pest management strategies that strengthen ecological interactions through natural enemies represents a promising and more sustainable pathway (Stemmelen et al., 2025).

While considerable attention has been given to natural pest regulators as specialized insect predators and parasitoids (Angon et al., 2023), as well as to some insectivorous vertebrates such as birds and mammals (Baroja et al., 2019; Garcia et al., 2020), other potential biological control agents, such as reptiles, have been largely overlooked (Ghosh, 2025).

Many reptiles (i.e., lizards, geckos, snakes) have been considered “farmers’ friends” because of their roles in seed dispersal, crop health improvement due to reduction of pest populations (Keasar et al., 2023), and pollination enhancement (Olesen and Valido, 2003; Ghosh, 2025). They are important generalist and opportunistic predators with a wide-ranging prey base, including various arthropod orders, some of which are severe crop pests (Ghosh et al., 2024). In particular, lacertid lizards are widely abundant in agroecosystems across southern Europe (Băncilă et al., 2023), forming a notable component of the biodiversity in these environments. They utilize agricultural structures (e.g., stone walls, field margins and even crops themselves) for thermoregulation and shelter, and actively hunt invertebrates, which make up the core of their diet. Furthermore, lizards occupy a unique position in the trophic network as mid-level consumers, acting as both predators of primary and secondary consumers and prey base for higher-order carnivores (Carretero, 2004).

Despite these ecological features, very few studies have addressed the functional role of lizards as predators in agroecosystems (Borkhataria et al., 2006, 2012; Monagan et al., 2017; Pringle et al., 2019). Moreover, their high presence in agricultural environments also makes them particularly vulnerable to the impacts of agricultural intensification, even more so than other vertebrate taxa (Simbula et al., 2021b). Agrochemicals and other management practices can impose significant fitness costs on lizards, affecting their physiology (Amaral et al., 2012a; Simbula et al., 2021c; Llanos-Garrido et al., 2023; Limnios et al., 2026), reproduction (Simbula et al., 2021a), and body condition (Mingo et al., 2017). If widespread, such effects may already be compromising the potential ecological role of lizards, as suggested by documented population declines in some areas (Gibbons et al., 2000). However, predicting the repercussions of agricultural practices on reptiles remains difficult because of limited ecotoxicological data and the low reliability of extrapolating findings from other vertebrate groups (Ortiz-Santaliestra et al., 2018). Evidence on how agricultural activities disturb these trade-offs and their repercussions for the crops is still lacking. This knowledge gap hinders our ability to understand the ecological role of lizards and harness the potential ecosystem services they may offer in an era of rapid landscape transformation.

In the present study, we investigated the potential pest-control role and diet of the wall lizard *Podarcis bocagei* (Seoane, 1885) in agricultural areas (i.e., vineyards) from northwestern Portugal, subjected to different management practices. This lizard species is native to northwest Iberia, stands quite abundant in agricultural systems, and has a generalist insectivorous diet (Galán, 2015). Although *P. bocagei* is considered ubiquitous, its ecology in agricultural landscapes has not been thoroughly investigated (Galán, 2015). Furthermore, there is evidence of the negative effects of agrochemicals on the health status and fitness of the species (Amaral et al., 2012a, 2012b, 2012c). This study aimed to determine whether: (i) lizards actively consume vineyard pests and in which proportion; (ii) management practices influence prey diversity/availability; and (iii) the richness and/or composition of the lizard’s trophic niche vary across distinct agricultural management regimes. We hypothesized that i) *P. bocagei,* as a generalist predator, consumes a broad range of vineyard pests, especially in low management areas. ii) Moreover, intensive agricultural practices would reduce the overall prey richness due to habitat simplification, although not necessarily trophic niche width, as lizards may broaden their prey spectrum in impoverished environments (Carretero, 2004). iii) Finally, we expected that reduced prey availability would lead to increased dietary divergence among individuals, even if not reflected in broader patterns of trophic niche differentiation. To address these objectives, we used two complementary dietary analysis techniques: DNA metabarcoding and stable isotopes. These methods give detailed information on prey consumption, allowing reliable prey identification and quantification across large sample sizes. Specifically, metabarcoding captures a snapshot of recent dietary intake based on non-invasive samples (e.g. scats), but maximizes taxonomic resolution, and the detection of rare events and soft, small or invisible prey items, ultimately decreasing the biases of traditional methods (Carretero and Llorente, 2001; Pompanon et al., 2012; Roslin and Majaneva, 2016; Nielsen et al., 2018; Sow et al., 2020). In contrast, stable isotopes (e.g. tail tips) provide an integrated view of the overall diet over extended periods, offering quantitative measures of trophic level, niche breadth, and niche overlap (Layman et al., 2012). Although separately, both techniques have already been successfully applied to dietary studies in lizards (Pereira et al., 2019; Neves et al., 2022; Nunes et al., 2022; Rato et al., 2022).

2. Materials and methods

2.1. Study area and diversity of pest species

Fieldwork was carried out across seven vineyard sites in Northwest Portugal, within the “Vinhos Verdes” region, one of the fourteen officially designated Portuguese wine regions (Fig. 1). This area stands out as one of the oldest and largest Demarcated Region in the country, covering approximately 21,000 ha of vineyards, primarily located along major river valleys (Costa et al., 2021). The region is characterized by a temperate oceanic sub-mediterranean climate type, with mild summers average temperatures ranging from 18 to 22 ºC and consistently high

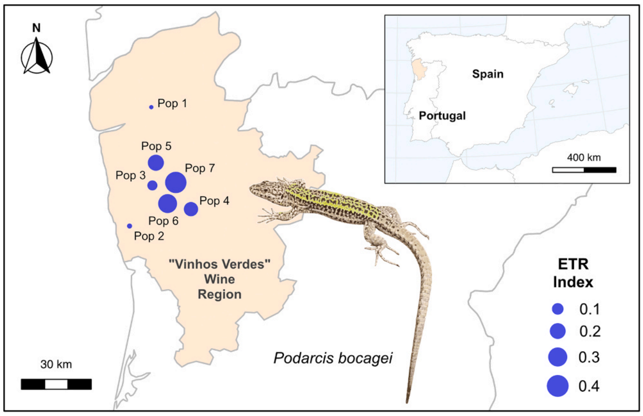

Fig. 1. Location of the seven sampling sites (Pop1-Pop7; blue points) in northwestern Portugal, within the “Vinhos Verdes” wine region. The size of each point is proportional to the ETR (Exposure-Toxicity Ratio) index, with larger circles indicating a higher ETR index, and consequently a greater potential ecological risk.

relative humidity (Macome, 2022). The seven vineyard sites (hereafter referred to as populations, Pop) were separated by a minimum distance of 10 km and were therefore considered spatially independent sampling units, given the limited dispersal range of lizards and ground-dwelling arthropods sampled by pitfall traps.

Quantitative data on current and historical land management and agricultural practices (i.e., chemical application) of the selected sites were obtained by consulting the landowners and were used to classify the sites according to a management gradient (from low to high pesticide values) using an exposure-toxicity (ETR) approach (Reus et al., 2002; Feola et al., 2011). This method is based upon the ratio between environmental exposure and toxicity for relevant organisms (Linders et al., 1994). Specifically, the exposure-toxicity ratio (ETR) for each field (j) was calculated as follows:

$$ETR = \sum \frac{PEC_{ij}}{Toxicity_i}$$

where PEC_{ij} represents the predicted environmental concentration in soil of substance i in field j, and $Toxicity_i$ is expressed as the no-observed-adverse-effect-level (NOAEL) values from rats' food intake (reference from EFSA conclusions for each substance). A higher ETR suggests a greater potential ecological risk.

An extensive review of pest databases (e.g., EPPO Global Database - EPPO (2025) EPPO Global Database. https://gd.eppo.int [12–02–2025]) was conducted to identify the main pest species associated with vineyard ecosystems in Portugal and other EU countries, and to compile a tailored reference database for our model system. Limiting the taxonomic assignments to pest species known to inhabit the study area enhances both the accuracy and interpretability of the results (Pompanon et al., 2012).

2.2. Sample collection

Lizard sampling was carried out from May to June 2023. Overall, 159 adult lizards were captured by hand or noose (Sillero and García-Muñoz, 2010), sexed using sexual secondary characters (Galán, 2015), measured from snout to vent (SVL) with a calliper (precision 0.01 mm), and weighted (Digital scale, DIPSE TP 2000, precision 0.1 g). Each lizard's tail tips (max. 2 cm) were collected and stored dry at -20°C for stable isotope analysis. There is already evidence that tail tip removal has minimal repercussions on lizard behaviour and habitat use (García-Muñoz et al., 2011). A total of 126 faecal pellets were collected and preserved in 96% ethanol and frozen until processed in the laboratory. Afterwards, individuals were released in the same site where they were captured.

In order to assess prey availability at each study site, pitfall traps were placed along a linear transect following the main vineyard walls at 40-meter intervals. Because vineyard walls differed in length among sites, the number of traps deployed varied accordingly (Pop1 = 4, Pop2 = 5, Pop3 = 6, Pop4 = 5, Pop5 = 6, Pop6 = 5, Pop7 = 5). The traps consisted of plastic containers (10 cm diameter and 16 cm height), inserted in the soil so that its edge was just at the ground level, and filled with 150 ml of preservative fluid (ratio of 50% propylene glycol to 50% water; Hoekman et al., 2017; Nakamura et al., 2020). A non-transparent plastic lid was placed above each trap to prevent rainwater from flooding the traps, and to reduce evaporation of the preservative solution. In each field, traps were emptied and replaced once every 15 days from April to June, and their contents pooled together. Captured arthropods were preserved in 100% ethanol, and later identified at order taxonomic level in the laboratory.

2.3. Metabarcoding laboratory protocol

DNA extraction from approximately 200 mg of each faecal sample was carried out using the Stool DNA Isolation Kit (Norgen Biotek Corporation) following the manufacturer's protocol. Samples were extracted in batches of 23 plus a negative control in which no faecal material was added. Two 100 μL elutions from each pellet, including controls, were obtained and stored at −20°C in 96-well plates until amplification.

Two sets of markers designed for invertebrates' DNA amplification (specifically arthropods) were chosen in order to assess the lizards' diet: i) a modified version of IN16STK (Kartzinel and Pringle, 2015) targeting a portion of the 16S rRNA region (~110 bp), following da Silva et al. (2019); and ii) a short fragment (157 bp) of the mitochondrial cytochrome c oxidase subunit I (COI) ZBJ-ArtF1c/ZBJ-ArtR2c (Zeale et al., 2011; da Silva et al., 2019; Mata et al., 2021) (detailed information is available in Table S1). For each species listed as potential pest, it was verified whether it was already barcoded for any of the gene regions, and also the primers' amplification affinity to their mtDNA, using the software Geneious Prime v. 2024.0.2 (Biomatters). All primers were modified to contain Illumina adapters at the 5′ end of the sequence. The PCR reactions were carried out in volumes of 10 μl, comprising 5 μl of QIAGEN Multiplex PCR Master Mix, 0.3 μl of each 10 mM primer, 3.4 μl of ultra-pure water, and 1 μl of DNA extract. Cycling conditions used initial denaturation at 95°C for 15 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 45°C for 30 s and extension at 72°C for 30 s, with a final extension at 72°C for 10 min. The success of all amplifications was checked by running the PCR products in 2% agarose gels.

Library preparation followed two rounds of PCR: an initial clean-up PCR and an indexing PCR to assign unique dual indices to each sample. All ZBJ PCR products were diluted with 5 μL of water prior to indexing, while IN16STK products, which showed no primer dimers, were used undiluted. Clean-up of ZBJ PCR products was performed using AMPure XP beads (Beckman Coulter, USA), following standard ethanol wash steps and elution in EB buffer. Indexing PCR was performed using 7 μL of KAPA HiFi HotStart ReadyMix (Roche, KAPA Biosystems, Switzerland), 0.7 μL of each P5 and P7 index (7 bp unique barcode, differing by ≥3 bp), and 5.6 μL of cleaned ZBJ PCR product or 2.8 μL of uncleaned IN16STK PCR product and 2.8 μL of ultra-pure water. Cycling conditions were: initial denaturation at 95°C for 3 min; 10 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s; final extension at 72°C for 5 min. Following indexing, a second bead-based clean-up was performed for IN16STK only. Indexed PCR products for ZBJ showed strong primer dimer overlapping with the target fragment, which led to the decision to omit the bead clean-up, and instead run a gel purification (Macherey-Nagel™ NucleoSpin™ Gel and PCR Clean-up Kit) to isolate the fragment of interest. DNA quantification was performed using an Epoch microplate spectrophotometer (Agilent Technologies, USA). ZBJ libraries were normalized to 130 nM and IN16STK to 50 nM, and samples were pooled per marker. Quality control of pooled libraries was assessed using the TapeStation (Agilent Technologies), confirming fragment size and the presence/absence of primer dimer. Libraries were validated by qPCR using the KAPA Library Quant Kit (Bio-Rad iCycler) and diluted to 4 nM. Final pools were sent to Biomarker Technologies (BMK) for sequencing on an Illumina NovaSeq X PE150 using Partial Lane Sequencing (4 Gb/pool).

2.4. Metabarcoding bioinformatics analyses

Sequencing reads were trimmed using *cutadapt* v4.0 (Martin, 2011) to remove primers and isolate the fragment of interest. Reads were merged and error-corrected with FLASH, and clustered into Molecular Operational Taxonomic Units (MOTUs), which were refined using LULU R package (>84% default minimum threshold of sequence similarity) to eliminate residual errors (Frøslev, 2025). The 16S MOTUs were assigned taxonomically using BLASTn against NCBI. When multiple matches occurred, we prioritized taxa known from Portugal or nearby regions. The COI MOTUs were identified using BOLDigger2 (Buchner and Leese, 2020), following similarity thresholds: < 85% (class), 90–95% (family), 95–97% (genus), > 97% (species), as in Deso et al. (2024). MOTUs present in the blank samples, for each batch of 23 samples, were considered contaminants and removed. For unresolved MOTUs, neighbor-joining trees were built in Geneious Prime v. 2024.0.2 (Biomatters), and checked for patterns of genetic similarity (~98%) in order to cluster them into distinct taxa (e.g., Carabidae 1, Carabidae 2, and so on). Non-dietary taxa (e.g., mammals, nonvascular plants, nematodes) were excluded. Dietary MOTUs representing less than 1% of the total dietary reads of each sample were not included in order to reduce false positives associated with lab and sequencing cross-contamination. Then, samples with fewer than 100 dietary reads were considered to have failed and were discarded. To resolve taxonomic overlaps, avoid redundancy and enhance complementarity, both markers were merged following the Python script from da Silva et al. (2019).

2.5. *Stable isotopes laboratory protocol*

Lizard muscle tissues (tail samples) were delipidified by immersion in Chloroform–Methanol (2:1), centrifuged (4000 r.p.m., 2 min), dried at 60 °C from 24 to 48 h and ground to a fine powder. Samples were loaded into tin capsules and isotopic ratios of carbon (13 C/12 C) and nitrogen (15 N/14 N) were determined by continuous flow isotope mass spectrometry (CF-IRMS) (Preston and Owens, 1983), on a Sercon Hydra 20–22 (Sercon, UK) stable isotope ratio mass spectrometer, coupled to a EuroEA (EuroVector, Italy) elemental analyser for online sample preparation by Dumas-combustion. The δ^{13}C and δ^{15}N values were calculated relative to Vienna Pee-Dee Belemnite (VPDB) and atmospheric N_2 standards, respectively, using the following formula: δ^{13}C or δ^{15}N = [(Rsample - Rstandard) -1] *1000, where R represents the isotope ratio of the heavier and the lighter isotope (i.e., 13C/12 C or 15 N/14 N). Uncertainty of the isotope ratio analysis, calculated using values from 6 to 9 replicates of laboratory standards, interspersed among samples in every batch of analysis, was $\leq 0.1‰$. The major mass signals of N and C were used to calculate total N and C abundances, using Protein Standard OAS (Elemental Microanalysis, UK, with 13.32%N, 46.5%C) as elemental composition reference material. All laboratory procedures were performed at the “Laboratório de Isótopos Estáveis LIE” - Stable Isotopes Analysis Facility, at the Faculdade de Ciências, Universidade de Lisboa, Portugal.

2.6. *Animal data and prey availability statistical analyses*

All statistical analysis were carried out in R 4.4.2 (R Core Team, 2024). Statistical significance was considered for p-values ≤ 0.05.

Lizards’ body size (SVL) and weight (BW), which can influence diet composition (Costa et al., 2008; Kaliontzopoulou et al., 2012), were tested for differences across the fixed factors (sexes and populations). SVL was tested using an analysis of variance (ANOVA), while BW was analysed using an analysis of covariance (ANCOVA), with SVL included as a covariate to control for body size. In both models, the interaction between sex and population was also tested. All analyses were performed with the *aov* function. Pairwise comparisons (post-hoc Tukey test) were carried out using the *emmeans* package 1.10.5 (Lenth, 2024). For simplicity, only SVL was used in the subsequent diet analyses, as it provides a more consistent and reliable measure of body size in organisms such as lizards, where tail loss and regeneration can cause significant fluctuations in body mass (Meiri, 2008, 2010).

Prey availability abundance was calculated for each population by pooling trap contents collected on the same date and was standardised to account for differences in pitfalls sampling effort among populations by dividing prey counts by the fixed number of pitfall traps deployed per population (e.g. six traps). This approach partially controls for differences in sampling effort among populations. Differences among populations were analysed with a permutational multivariate analysis of variance (PERMANOVA) with adonis2 function from the vegan 2.6–8 package (Oksanen et al., 2024) based on a Bray-Curtis dissimilarity index using the *vegdist* function (vegan package). Homogeneity of dispersion (function *betadisper*) was checked to make sure that the differences observed with PERMANOVA were not due to unequally dispersed values across the different groups, as it evaluates the homogeneity of multivariate dispersions. Pairwise comparison was conducted with the *pairwise.adonis2* function (*pairwiseAdonis* package, Martinez Arbizu, 2020). Finally, a rank abundance plot was created to visualise the overall top 10 most abundant prey items collected in the traps across populations; prey abundance was corrected by dividing raw counts by the total number of traps deployed at each population across the entire sampling season (e.g. six traps sampled on four occasions, total = 24 traps).

2.7. *Metabarcoding statistical analyses*

The frequency of occurrence (FO) of each prey item was calculated as the number of occurrences of the diet item (limited to a maximum of 1 occurrence per sample) divided by the total number of sequenced lizard faecal samples.

Differences in dietary descriptors (i.e., diet richness and composition) were compared across three taxonomic levels: MOTU (the highest taxonomic resolution), family, and order. The rank abundance plot (n of occurrences), however, was created only at the order level to visualise the top 10 most frequent prey items across populations, allowing for direct comparison with prey availability data. Additionally, we performed a Spearman correlation (function *cor*) between the rank abundance values from pitfall traps and diet to assess the overall correspondence in prey use and environmental availability. In order to assess prey selection, we also estimated the selectivity index (*D*) of Ivlev (1961) modified by Jacobs (1974) using the *ivlev* function from the *selectapref* package (Richardson, 2020). This index is widely used in studies of trophic preferences (Martín et al., 2023), and it ranges from −1 (total avoidance), to 0 (no or random selection) to + 1 (maximum positive selection). For each population, the index was calculated by comparing the relative availability of each prey item to its relative consumption. To test the statistical significance of prey selectivity, we performed χ^2 tests comparing the observed frequency of each prey type in the diet with the expected frequencies based on environmental availability.

A Generalized Linear Model (GLM) was implemented to investigate the effects of Pop and SVL in both sexes (Kaliontzopoulou et al., 2008, 2012) on diet richness (i.e., the number of different prey items detected per faecal sample), assuming a Poisson distribution (Shapiro-Wilk test, all $p < 0.0001$). Significance was tested with the *anova* function (*car* package Fox and Weisberg, 2019), and pairwise differences explored with *emmeans*. Marginal means of richness for each Pop and SVL were estimated with the *effects* package in R (Fox, 2003) and visualized using *ggplot2* (Wickham, 2016).

Richness among Pop and sexes was analysed by means of rarefaction and extrapolation curves using the iNEXT v. 3.0.1 package (Chao et al., 2014; Hsieh et al., 2024; confidence level at 84% MacGregor-Fors and Payton, 2013; and 1000 bootstraps). The estimated richness was compared considering completeness (i.e., sample coverage) instead of sample size (i.e., number of samples), to avoid biases of communities with different levels of richness requiring different sampling efforts to be sufficiently characterised (Chao and Jost, 2012).

Diet composition differences were tested using PERMANOVA with Pop, sex, SVL as fixed factors and their interactions, based on a Jaccard dissimilarity index and checking for homogeneity of dispersion.

2.8. *Stable isotopes statistical analyses*

To assess isotopic niche variation among lizard populations, we analysed δ^{13}C and δ^{15}N values using the SIBER package (Jackson and Parnell, 2023). This method is based on the calculation of standard ellipse area (SEA) which presents the bivariate equivalent of standard deviations. Values of SEA were corrected for small sample size (SEA_C) to estimate niche overlap and generate Bayesian estimates of SEA (SEA_B) for testing differences in trophic niches based on 95% credible limits (Jackson et al., 2011). Biplots of $\delta^{13}C$ and $\delta^{15}N$ values were generated with standard ellipses to visualize isotopic space occupied by each population. Differences in $\delta^{13}C$ and $\delta^{15}N$ values were tested using GLM with Pop and sex as fixed factors, and their interactions. Pairwise comparisons were performed to assess differences between populations.

3. Results

SVL and BW data were normally distributed (Shapiro-Wilk test, SVL: $p = 0.93$; BW: $p = 0.09$). The analysis of variance indicated that size changed among populations and between sexes but not in their interaction. For body mass, Pop, sex and SVL were significant, with males bigger and heavier than females in all populations (Table S2, Figure S1). The pairwise comparisons for SVL and BW among Pop, detected significant differences only between a few population pairs (Table S2).

3.1. Prey availability

Rank-abundance of prey diversity caught in the pitfalls revealed distinct community structures across the seven populations (Fig. 2a), with a consistent pattern of a few dominant taxa. In most populations, the first four to five arthropod orders accounted for the majority of the total abundance, indicating a high level of dominance within the communities. Coleoptera was the dominant order across most populations, representing up to 40–45% of total items in some cases. Isopoda comprised approximately 35–40% of the recorded prey counts. Araneae and Hymenoptera were consistently among the top four ranks in several populations, contributing between 10–25% of total prey items collected depending on the population. Rare taxa, such as Scutigeromorpha, Opiliones, and Lithobiomorpha, consistently occupied lower positions in the rank-abundance curves and each represented less than 5% of all prey items collected. Generally, populations exposed to lower pesticide levels (e.g., Pop1) tend to show more even distribution of prey taxa among orders. In contrast, sites with higher values of ETR (e.g., Pop7) were dominated by one or two taxa, with steep curve slopes indicating low diversity. Although the PERMANOVA revealed a significant overall effect among populations ($F_{6,19} = 4.98$, $p = 0.001$), with no evidence of heterogeneity in dispersion ($p = 0.164$), post hoc pairwise comparisons did not detect any significant differences between individual group pairs (all $p > 0.05$).

3.2. Metabarcoding

The number of paired-end reads per sample averaged 139,962 for IN16STK and 105,562 for ZBJ. After applying negative controls, removing singletons, replicates, and filtering taxa, the final dataset of *P. bocagei* diet consisted of 316 MOTUs (including 105 MOTUs that were coincident between the two sets of primers), belonging to 95 families, 25 orders and 7 classes (Table S3). The use of two sets of primers increased the MOTU coverage with 140 MOTUs unique to IN16STK and 71 MOTUs unique to ZBJ. From a total of 26 species that were listed as potential agricultural pests (Table 1), prey items from the genus *Agrotis* (Lepidoptera) were the sole crop pests, found in only 5 faecal samples collected in Pop1, Pop3, Pop5, and Pop7.

Overall, among the prey items, Coleoptera (61.11%) was the most prevalent order, followed by Hemiptera (57.14%), Hymenoptera (51.59%), and Araneae (49.21%) (Fig. 2b). The rank of prey abundance observed in the lizards' diet was significantly correlated with the prey availability in most of the populations for both sexes (Table S4). The patterns of prey selection or avoidance varied markedly across populations and taxa, indicating population-specific foraging behaviour (Table S4). For instance, Diptera and Hemiptera were positively selected in most populations and both sexes, whereas Hymenoptera showed a similar pattern, albeit in only two populations. Lepidoptera, although significant in only one population (Pop4) and in both sexes, was strongly positively selected. On the contrary, Coleoptera and Isopoda showed negative selectivity values for both females and males, suggesting avoidance, whereas Araneae showed mixed responses (Table S4). However, these patterns should be interpreted with caution, as the relatively small sample sizes within each population-sex combination may limit the statistical power of these comparisons.

The GLM analysis showed statistically significant differences in diet richness (Table 2, Fig. 3a-c). At the MOTU taxonomic level, diet richness differed significantly among populations in both sexes. At the family level, a significant population effect was detected in males, whereas in females diet richness was significantly associated only with SVL. At the order level, a significant population effect was observed in males, while no effects at all were detected in females. Pairwise comparisons between populations are reported in Table S5.

The analysis based on sampling completeness indicated a total niche overlap among populations at both MOTU (Fig. 4a) and family (Fig. 4b) levels, while at the order level, only Pop3 appeared to have a slightly lower niche breadth (Fig. 4c). Regarding the difference between sexes, at the MOTU level, females seem to have a slightly larger niche breadth than males, although there is a marginally overlap on the extrapolation portion of the curve (Figure S2a). On the contrary, a complete overlap between sexes was found at both family and order levels (Figure S2b-c).

The diet composition analysis (PERMANOVA) showed a significant effect only for the interactions between populations and sexes at the MOTU ($F_{6,110} = 1.138$, $p = 0.009$) and family ($F_{6,110} = 2.421$, $p = 0.001$) levels, while at the order level no significant effects were observed ($p > 0.05$). However, in both MOTU and family cases, there was a significant difference in multivariate dispersion (MOTU: $F_{3,112} = 3.04$, $p < 0.001$; family: $F_{3,112} = 1.898$, $p = 0.04$), indicating unequal within-group variability.

3.3. Stable isotopes

Isotopic analyses were conducted for 158 tail tips. A considerable overlap in the isotopic niche for all populations was observed, despite some variation along the $\delta^{13}C$ axis for Pop5, which showed a broader niche width (Figure S3). Significant differences for $\delta^{13}C$ were observed only among populations ($F_{6,151} = 39.081$, $p < 0.001$), while for $\delta^{15}N$, both populations ($F_{6,151} = 7.360$, $p < 0.001$), and the interaction population*sex ($F_{6,1446144} = 2.515$, $p = 0.024$) were significant. Pairwise comparisons among populations are reported in Table S6. While for the $\delta^{13}C$ the difference between pairs of populations seems not to be connected with the pesticide index classification, a slightly trend for the $\delta^{15}N$ was observed with populations with higher pesticide index showing enriched $\delta^{15}N$ signatures (Figure S4).

4. Discussion

In this study we combined DNA metabarcoding and stable isotopes approaches to assess the trophic functional role of *P. bocagei*, specifically in relation to pest consumption in vineyards, under a varying gradient of pesticide management. Specifically, prey availability and metabarcoding data were directly linked through correlation and selectivity analyses, allowing us to relate environmental prey communities to realized diet, whereas stable isotope analyses provided an independent, integrative perspective on trophic position and resource use. Our results provide insights into the dietary patterns of this lizard species described by the metabarcoding technique for the first time, and its potential role in the ecological functioning of agricultural systems.

The combination of two sets of primers clearly increased the number of MOTUs retrieved, since only 33% of the MOTUs were identified by both sets. On the other hand, the taxonomic resolution of each set may differ due to the availability of reference sequences, resulting in more MOTUs not identified to the genus/species level. Concerning the target

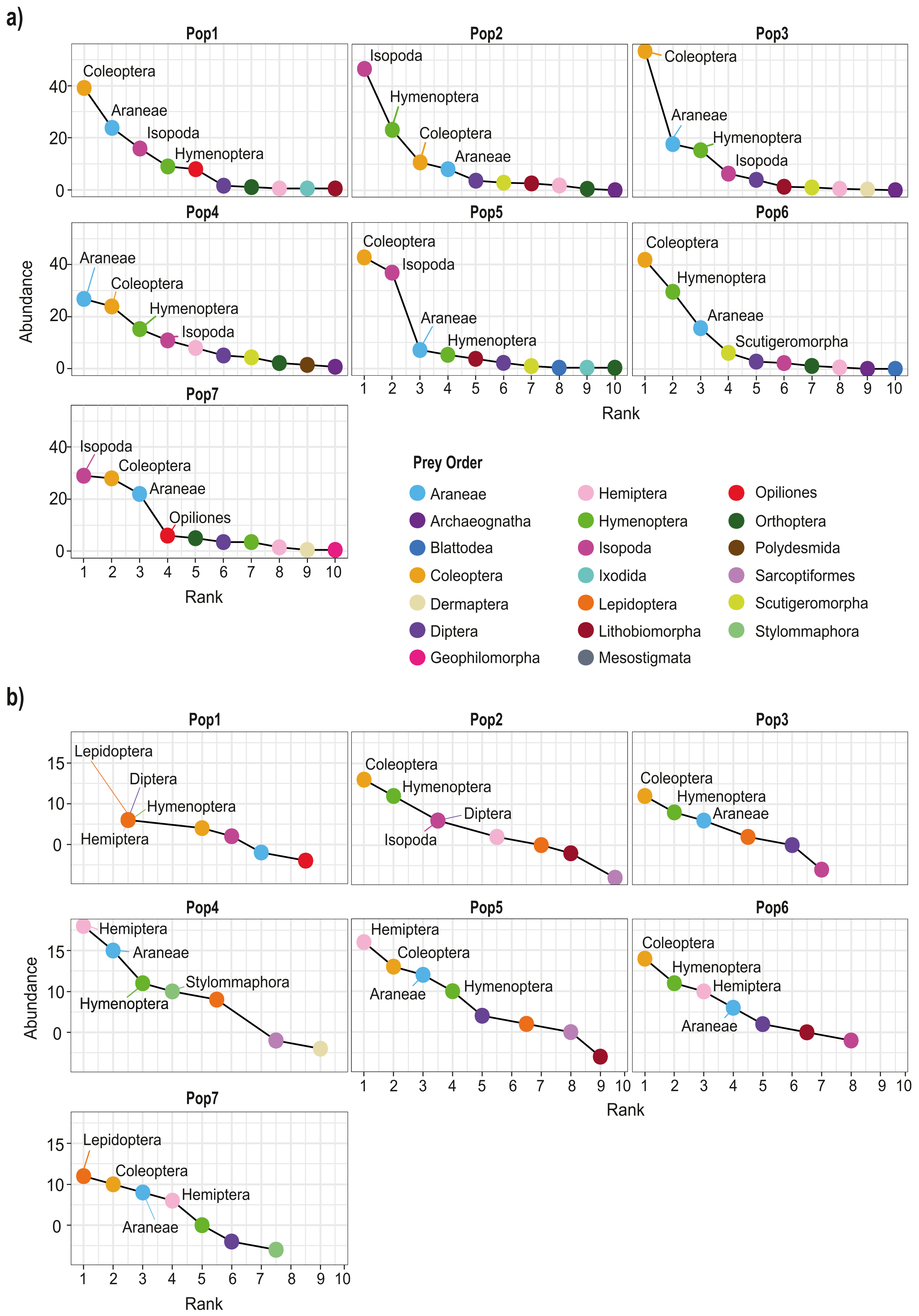

Fig. 2. Rank-abundance of prey taxa caught in the pitfalls (a) and diet (b) in each population, identified at the order level. Populations are ordered based on the pesticide index (ETR) from the lowest value (Pop1) to the highest (Pop7).

Table 1
List of the main pest species associated with vineyard ecosystems in Portugal and other EU countries.

Order	Family	Species
Lepidoptera	Noctuidae	*Agrotis* spp.
Diptera	Tephritidae	*Ceratitis capitata*
Diptera	Drosophilidae	*Drosophila suzukii*
Hemiptera	Cicadellidae	*Empoasca decipiens*
Hemiptera	Cicadellidae	*Empoasca solani*
Hemiptera	Cicadellidae	*Empoasca vitis*
Hemiptera	Cicadellidae	*Erasmoneura vulnerata*
Lepidoptera	Tortricidae	*Eupoecilia ambiguella*
Hemiptera	Cicadellidae	*Jacobiasca lybica*
Lepidoptera	Tortricidae	*Lobesia botrana*
Lepidoptera	Noctuidae	*Noctua* spp.
Coleoptera	Othiorinchus	*Othyorinchus sulcatus*
Acarida	Tetranychidae	*Panonychus ulmi*
Hemiptera	Coccidae	*Parthenolecanium corni*
Hemiptera	Coccidae	*Parthenolecanium persicae*
Hemiptera	Pseudococcidae	*Planococcus ficus*
Hemiptera	Pseudococcidae	*Planococcus citri*
Coleoptera	Scarabaeidae	*Popillia japonica*
Hemiptera	Coccidae	*Pulvinaria vitis*
Hemiptera	Cicadellidae	*Scaphoideus titanus*
Hemiptera	Cicadellidae	*Sophonia orientalis*
Lepidoptera	Noctuidae	*Spodoptera exigua*
Acarida	Tetranychidae	*Tetranychus urticae*
Hemiptera	Phylloxeridae	*Viteus vitifoliae*
Coleoptera	Cerambycidae	*Xylotrechus arvicola*
Hemiptera	Cicadellidae	*Zygina rhamni*

Table 2
Summary results of GLMs to investigate the effects of population (Pop) and SVL in both sexes on diet richness at the three taxonomic levels. Significant results are reported in bold.

	Males			*Females*		
	Deviance	Resid. Dev.	P-value	Deviance	Resid. Dev.	P-value
MOTUs						
Pop	52.611	140.96	**< 0.001**	12.962	73.104	**0.044**
SVL	2.122	138.84	0.145	3.129	69.975	0.076
Family						
Pop	33.067	91.509	**< 0.001**	10.556	54.692	0.103
SVL	1.919	89.591	0.166	4.280	50.411	**0.039**
Order						
Pop	15.212	70.347	**0.019**	7.004	31.359	0.320
SVL	0.388	69.959	0.533	1.696	29.663	0.193

pest species, metabarcoding results detected a single pest taxon, *Agrotis* sp. (Lepidoptera), as being consumed by *P. bocagei*. This prey item was identified in multiple populations, including those with low and high pesticide exposure. It was also identified by both sets of primers. Nevertheless, only 5 of the 126 faecal samples analysed (4%), contained this agricultural pest species, with occurrences scattered across populations at relatively low frequencies. This limited evidence of pest consumption may reflect both the trophic behaviour of *P. bocagei* and the ecological context of the study area, although reduced pest availability due to pesticide efficacy cannot be completely excluded.

Wall lizards are known to forage preferentially in structurally complex microhabitats, such as stone walls, hedgerows, or field margins (Avery, 1978; Ouboter, 1981), which may offer shelter and thermoregulatory advantages. However, this behaviour may limit them, even by short distances, from accessing vineyard rows, where pest outbreaks typically occur. Consequently, their spatial foraging patterns may reduce encounters with pest species concentrated inside the vineyards. Moreover, the generalist behaviour of *P. bocagei* may further reduce its specificity for crop pests. As generalist predators, lacertid lizards exhibit broad dietary and habitat spectra, they can buffer spatiotemporal variations in trophic resources, and can adjust their diet based on prey availability (Carretero, 2004), which may lead them to feed opportunistically on non-pest arthropods. In such cases, the overall impact of reptiles on pest regulation may be neutral or even negative, especially if lizards consume predatory arthropods (e.g., spiders, beetles) that may contribute to pest suppression (Perez-Alvarez et al., 2019; Ghosh et al., 2024). Therefore, trophic interactions may interfere with pest regulation (Ghosh and Basu, 2023). However, several studies suggest that lizards positively select nutritionally valuable prey even when these are rare, implying that pest consumption could still occur if such species provide significant nutritional benefits (see Carretero, 2004, and references therein).

Furthermore, the temporal range may also constrain the ability to detect pest consumption. For instance, the Erhard's Wall Lizards (*Podarcis erhardii*) has been shown to be more effective in suppressing pests of a particular size as summer approaches (Lisiecki, 2019). Since faecal samples in our study were collected during a single season (late spring), the resulting dietary data represent only a narrow temporal snapshot. Such limited sampling may have missed seasonal variation in pest abundance. To obtain a more comprehensive understanding of trophic interactions and potential pest control services, further studies should consider repeated sampling across different phenological stages of both crops and pests to offer a more comprehensive view of trophic interactions.

Finally, other factors, such as a general low pest abundance in the fields or the efficacy of pesticides, may have also contributed to the limited detection of pests. However, although prey availability was assessed, the sampling methods used were not specifically designed to effectively capture agricultural pest taxa, and therefore do not allow a reliable evaluation of their actual abundance in the field. In addition, pesticide effectiveness was not directly assessed in our work. Future studies integrating metabarcoding with sampling methods more targeted to agricultural pests, and site-specific chemical pressure analysis could help clarify these aspects.

Overall, both DNA metabarcoding and isotopic approaches identified consistent patterns in trophic structure across populations: generalist diets, high overlap on prey composition among populations, and only limited divergence in niche breadth, regardless of local differences in prey availability and pesticide exposure. Although prey communities captured via pitfall traps showed variation in composition and evenness within-populations, lizards exhibited broadly similar diet composition across populations, with high taxonomic overlap. Furthermore, diet richness was significantly distinct among populations at all three taxonomic levels. This pattern confirms the degree of dietary broadness typical of many lacertid lizards, suggesting that their diet does not solely mirror prey availability but is also driven by active prey choice (Carretero, 2004; Vitt and Pianka, 2007; Simbula et al., 2022). The high overlap in stable isotope niches among populations further supports these outcomes. Nevertheless, in some populations, certain taxa such as Coleoptera, despite being the most abundant prey, showed negative values of Jacobs' D index. This indicates that despite their high abundance, these prey were consumed less than expected based on availability, highlighting how high consumption does not necessarily imply a positive selection (Kaliontzopoulou et al., 2012).

Although prey availability patterns (e.g., lower evenness, stronger dominance by a few taxa) in high-pesticide sites suggest some community-level simplification, this was not mirrored by a corresponding reduction in dietary richness or isotopic niche breadth, as initially expected. For instance, contrary to our expectations, the effect of pesticides on trophic interactions appeared to be not severe enough to significantly alter lizard trophic niche than anticipated, appearing instead subtle or context-dependent. These results may also reflect a resilience and dietary plasticity of *P. bocagei*, allowing lizards to maintain relatively stable diets by selecting among available prey taxa (Carretero, 2004). The observed patterns appear to be driven by context-dependent prey selection, which can modulate lizard diets even when environmental prey availability is altered. This pattern is

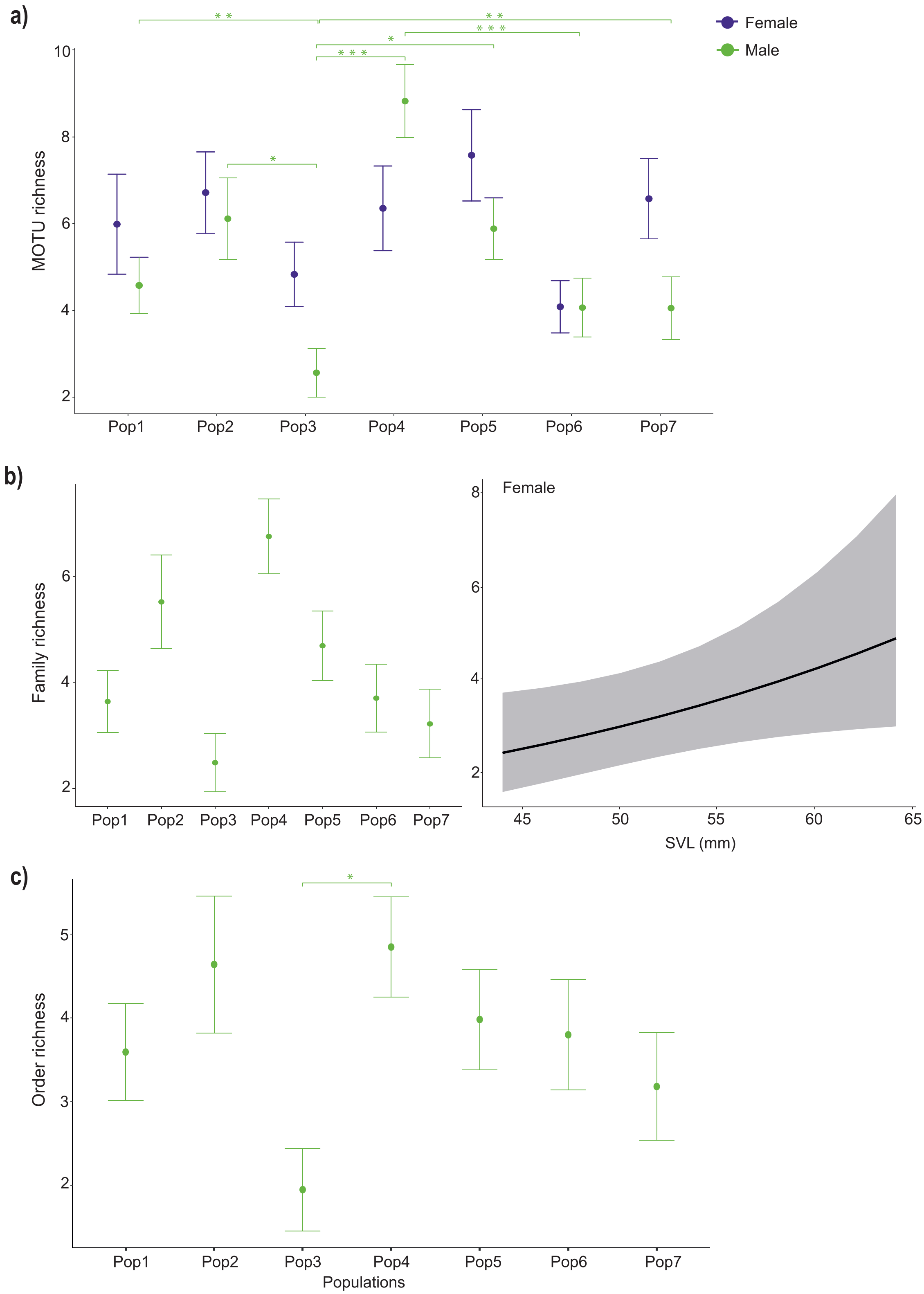

Fig. 3. Graphical representation of the General Linear Models results denoting: the statistically significant effects of: a) populations on MOTU richness in both sexes; b) population (for males) and SVL (for females) on family richness; c) populations on order richness in males. The y-axis represents the average of the estimated fixed effect coefficient. Shaded areas represent 95% confidence intervals. Asterisk symbols indicate pairwise significance: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

consistent with other lizard studies, where the species selected prey according to local management or habitat conditions, maintaining a diet less variable in time or space than the environmental prey availability (Mella et al., 2010; Simbula et al., 2022). Nevertheless, a note of caution is necessary since small sample size and high within-population variability, as also reflected by differences in multivariate dispersion, might prevent us from drawing definitive conclusions about pesticide effects on diet composition or prey availability.

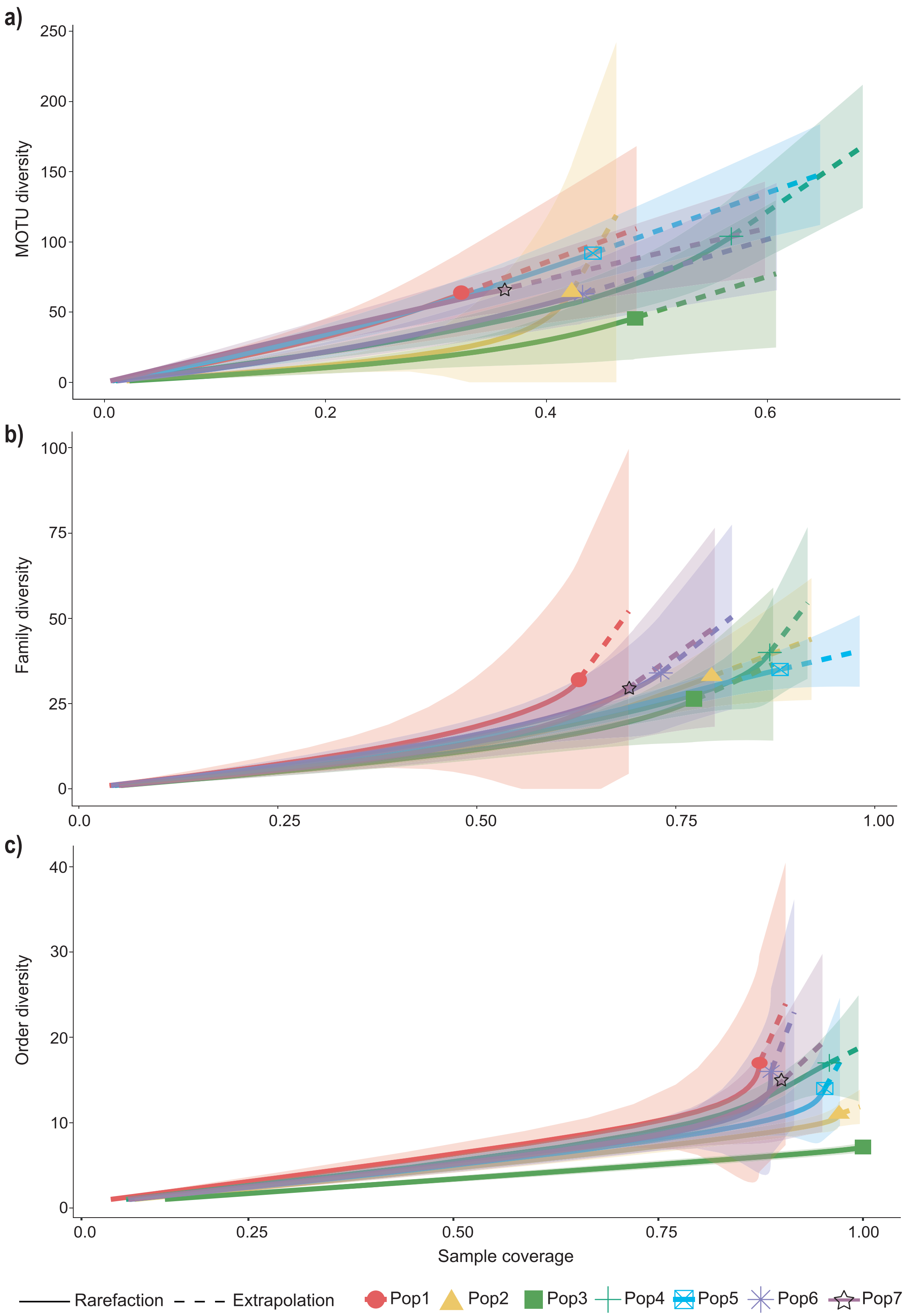

Fig. 4. Rarefaction curves for each population at the MOTU (a), family (b), and order (c) levels, showing the observed (full line) and estimated (dashed line) richness, until double the reference sample size, and respective 84% confidence interval by sample.

Although DNA metabarcoding did not detect clear differences in the consumption of higher trophic-level prey (e.g., Araneae) across pesticide gradients, stable isotope analysis showed elevated δ^{15}N values in lizards inhabiting vineyards with higher pesticide exposure. Several factors should be considered when interpreting these contrasting patterns. DNA metabarcoding is quite effective for recovering qualitative presence–absence diversity data. However, it does not accurately reflect the information on biomass of prey consumed (Stapleton et al., 2022). Thus, it is possible that prey such as Araneae, although consumed in similar frequency across sites, contributed more substantially to the diet (e.g., through spiders' larger size or higher intake by lizards) in higher-exposure areas, as reflected by the isotopic values. On the other hand, other prey groups can act as mesopredators (e.g., Hymenoptera and Hemiptera), thus contributing to elevated δ^{15}N values. Moreover, the seasonal availability dependence of the different prey taxa (e.g., Hymenoptera, Lepidoptera or Diptera) compared to the year-round consumption of spiders may influence the isotopic signature independently of pesticide exposure.

Alternatively, the contrasting results between isotopic signatures and prey composition may suggest that the observed isotopic differences are not driven by dietary changes, but rather by environmental baseline shifts in the isotopic values of primary producers (Tiunov, 2007). Agricultural intensification, particularly through the repeated use of synthetic pesticides and fertilizers, can alter soil nutrient cycling and microbial processes (Drinkwater et al., 1998). This often leads to δ^{15}N enrichment at the base of the food web, which can be transferred through herbivorous arthropods to higher trophic levels, ultimately resulting in elevated δ^{15}N levels in predator tissues (i.e., lizards), even in the absence of a detectable change in diet composition (Birkhofer et al., 2011). In contrast, the δ^{13}C values differed among sites but did not correlate with pesticide usage. Even if all sampling sites were vineyards, this unexpected pattern between some populations might reflect local differences in baseline carbon sources, microhabitat use, or foraging in adjacent non-crop habitats. Lizards may be feeding on prey not directly linked to vineyard vegetation (e.g., hedge-associated or detrital food webs), as a response to prey diversity and/or habitat structures, rather than to a lack of food within the fields (Simão et al., 2015), which could shift δ^{13}C values independently of vineyard management intensity. Moreover, differences in ground vegetation, irrigation, or surrounding landscape features may still drive local variability in δ^{13}C through shifts in primary production pathways (Choi et al., 2005; Spangenberg and Zufferey, 2023). All together, these findings emphasize the importance of integrating DNA metabarcoding and stable isotope analyses. While the former offers high-resolution taxonomic insight into recent feeding events, the latter captures integrative signals of trophic assimilation and can reveal environmental effects not directly evident in dietary inventories.

In conclusion, understanding the role of reptiles in agroecosystems remains a significant challenge, particularly in the context of their potential contribution to pest control and their vulnerability to agricultural intensification (Ghosh et al., 2024). Our work suggests that while *P. bocagei* is a resilient and functionally consistent predator in vineyard systems, and can consume pests, its role in direct pest suppression seems limited at least under the conditions of this study. Rather than acting as a specialized biocontrol agent, it appears to serve as a flexible, mid-level generalist predator whose trophic role may contribute more to food web stability than to pest regulation per se. However, this does not diminish its ecological importance. On the contrary, reptiles are integral components of agroecosystems, and their persistence across varying management intensities may reflect a buffering role in ecological networks.

CRediT authorship contribution statement

Catarina Rato: Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Ricardo J. Lopes:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Sara F. Nunes:** Formal analysis. **Miguel A. Carretero:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Giulia Simbula:** Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation. **Ana Ramos:** Writing – review & editing, Investigation, Formal analysis.

Declaration of Competing Interest

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

Acknowledgements

This work was supported by Fundação para a Ciência e Tecnologia (FCT, Portugal) through the project Agrolizards + (2022.03391.PTDC). All applicable international, national and/or institutional guidelines for the care and use of animals were followed. Collecting permits (no. 749–752/2023/CAPT) were provided by the Institute for Nature Conservation and Forests (ICNF, Portugal). We thank Prof. A. Aguiar from the Agriculture Department of University of Porto for her invaluable help in coordinating with landlords and gathering pesticide-related information; Prof. R. Rebelo from the University of Lisbon for his assistance in the isotopic analysis; and A. Limnios, P. Oliveira, L.B. Sreelatha, and P. Aguilar for their support with the field work. We also thank the landowners for granting access to their vineyards and for providing information on pesticide application practices.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2026.110621.

Data availability

Data will be made available on request.

References

Ali, S., Ullah, M., Sajjad, A., Shakeel, Q., Hussain, A., 2021. Environmental and Health Effects of Pesticide Residues. SARV 48: Pesticide occurrence, analysis and remediation vol. 2 analysis. Springer International Publishing, Cham, pp. 311–336. https://doi.org/10.1007/978-3-030-54719-6_8.

Amaral, M.J., Bicho, R.C., Carretero, M.A., Sanchez-Hernandez, J.C., Faustino, A.M., Soares, A.M., Mann, R.M., 2012a. The use of a lacertid lizard as a model for reptile ecotoxicology studies: part 2–Biomarkers of exposure and toxicity among pesticide exposed lizards. Chemosphere 87, 765–774. https://doi.org/10.1016/j.chemosphere.2012.01.048.

Amaral, M.J., Carretero, M.A., Bicho, R.C., Soares, A.M., Mann, R.M., 2012b. The use of a lacertid lizard as a model for reptile ecotoxicology studies-Part 1 Field demographics and morphology. Chemosphere 87, 757–764. https://doi.org/10.1016/j.chemosphere.2011.12.075.

Amaral, M.J., Sanchez-Hernandez, J.C., Bicho, R.C., Carretero, M.A., Valente, R., Faustino, A.M., Soares, A.M., Mann, R.M., 2012c. Biomarkers of exposure and effect in a lacertid lizard (*Podarcis bocagei* Seoane) exposed to chlorpyrifos. Environ. Toxicol. Chem. 31, 2345–2353. https://doi.org/10.1002/etc.1955.

Angon, P.B., Mondal, S., Jahan, I., Datto, M., Antu, U.B., Ayshi, F.J., Islam, Md.S., 2023. Integrated pest management (IPM) in agriculture and its role in maintaining ecological balance and biodiversity. Adv. Agric., 5546373 https://doi.org/10.1155/2023/5546373.

Avery, R.A., 1978. Activity patterns, thermoregulation and food consumption in two sympatric lizard species (*Podarcis muralis* and *P. sicula*) from central Italy. J. Anim. Ecol. 143–158. https://doi.org/10.2307/3928.

Băncilă, R.I., Lattuada, M., Sillero, N., 2023. Distribution of amphibians and reptiles in agricultural landscape across Europe. Landsc. Ecol. 38, 861–874. https://doi.org/10.1007/s10980-022-01583-w.

Baroja, U., Garin, I., Aihartza, J., Arrizabalaga-Escudero, A., Vallejo, N., Aldasoro, M., Goiti, U., 2019. Pest consumption in a vineyard system by the lesser horseshoe bat (*Rhinolophus hipposideros*). PLOS ONE 14, e0219265. https://doi.org/10.1371/journal.pone.0219265.

Bastian, O., Corti, C., Lebboroni, M., 2007. Determining environmental minimum requirements for functions provided by agro-ecosystems. Agron. Sustain. Dev. 27, 279–291. https://doi.org/10.1051/agro:2007027.

Benton, T.G., Bieg, C., Harwatt, H., Pudasaini, R., Wellesley, L., 2021. Food system impacts on biodiversity loss. Three levers for food system transformation in support of nature. Chatham House Lond. 02–03.

Birkhofer, K., Fließbach, A., Wise, D.H., Scheu, S., 2011. Arthropod food webs in organic and conventional wheat farming systems of an agricultural long-term experiment: a stable isotope approach. Agric. For. Entomol. 13, 197–204. https://doi.org/10.1111/j.1461-9563.2010.00511.x.

Borkhataria, R.R., Collazo, J.A., Groom, M.J., 2006. Additive effects of vertebrate predators on insects in a puerto rican coffee plantation. Ecol. Appl. 16, 696–703. https://doi.org/10.1890/1051-0761(2006)016%255B0696:AEOVPO%255D2.0.CO;2.

Borkhataria, R.R., Collazo, J.A., Groom, M.J., Jordan-Garcia, A., 2012. Shade-grown coffee in Puerto Rico: opportunities to preserve biodiversity while reinvigorating a struggling agricultural commodity. Agric. Ecosyst. Environ. 149, 164–170. https://doi.org/10.1016/j.agee.2010.12.023.

Brook, B., Sodhi, N., Bradshaw, C., 2008. Synergies among extinction drivers under global change. Trends Ecol. Evol. 23, 453–460. https://doi.org/10.1016/j.tree.2008.03.011.

Buchner, D., Leese, F., 2020. BOLDigger–a Python package to identify and organise sequences with the Barcode of Life Data systems. Metabarcod. Metagenom 4, e53535. https://doi.org/10.3897/mbmg.4.53535.

Carretero, M.A., 2004. From set menu to *a la carte*. Linking issues in trophic ecology of Mediterranean lacertids. Ital. J. Zool. 71, 121–133. https://doi.org/10.1080/11250000409356621.

Carretero, M.A., Llorente, G.A., 2001. What are they really eating? Stomach versus intestine as sources of diet information in lacertids. Mediterranean basin lacertid lizards: a biological approach. ICN Lisbon 105–112.

Chao, A., Jost, L., 2012. Coverage-based rarefaction and extrapolation: standardizing samples by completeness rather than size. Ecology 93, 2533–2547. https://doi.org/10.1890/11-1952.1.

Chao, A., Gotelli, N.J., Hsieh, T.C., Sander, E.L., Ma, K.H., Colwell, R.K., Ellison, A.M., 2014. Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. Ecol. Monogr. 84, 45–67. https://doi.org/10.1890/13-0133.1.

Choi, W.-J., Chang, S.X., Allen, H.L., Kelting, D.L., Ro, H.-M., 2005. Irrigation and fertilization effects on foliar and soil carbon and nitrogen isotope ratios in a loblolly pine stand. For. Ecol. Manag. 213, 90–101. https://doi.org/10.1016/j.foreco.2005.03.016.

R. Core Team, 2024. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Costa, A.M., Monteiro, B., Silva, S., 2021. Wine tourism and digital marketing strategies in the vinho verde region. Int. Conf. Tour. Res. 119–126.

Costa, G.C., Vitt, L.J., Pianka, E.R., Mesquita, D.O., Colli, G.R., 2008. Optimal foraging constrains macroecological patterns: body size and dietary niche breadth in lizards. Glob. Ecol. Biogeogr. 17, 670–677. https://doi.org/10.1111/j.1466-8238.2008.00405.x.

da Silva, L.P., Mata, V.A., Lopes, P.B., Pereira, P., Jarman, S.N., Lopes, R.J., Beja, P., 2019. Advancing the integration of multi-marker metabarcoding data in dietary analysis of trophic generalists. Mol. Ecol. Resour. 19, 1420–1432. https://doi.org/10.1111/1755-0998.13060.

Deso, G., Reynier, T., Rato, C., 2024. Unsuspected pollinator potential? First DNA dietary analysis in *Euleptes europaea* (Gené, 1839) and confirmation of an omnivorous diet using video surveillance. Amphib. Reptil. 45, 455–463. https://doi.org/10.1163/15685381-bja10201.

Deutsch, C.A., Tewksbury, J.J., Tigchelaar, M., Battisti, D.S., Merrill, S.C., Huey, R.B., Naylor, R.L., 2018. Increase in crop losses to insect pests in a warming climate. Science 361, 916–919. https://doi.org/10.1126/science.aat3466.

Drinkwater, L.E., Wagoner, P., Sarrantonio, M., 1998. Legume-based cropping systems have reduced carbon and nitrogen losses. Nature 396, 262–265. https://doi.org/10.1038/24376.

Feola, G., Rahn, E., Binder, C.R., 2011. Suitability of pesticide risk indicators for Less Developed Countries: a comparison. Agric. Ecosyst. Environ. 142, 238–245. https://doi.org/10.1016/j.agee.2011.05.014.

Fox, J., 2003. Effect displays in R for generalised linear models. J. Stat. Softw. 8, 1–27.

Fox, J., Weisberg, S., 2019. Third. ed. An R Companion to Applied Regression. Sage, Thousand Oaks CA.

Frøslev, T.G., 2025. lulu: Post Clustering Curation of Amplicon Data for Reliable Biodiversity Metrics.

Galán, P., 2015. Lagartija de Bocage - Podarcis bocagei. Enciclopedia Virtual de los Vertebrados Españoles. Museo Nacional de Ciencias Naturales, Madrid. 〈http://www.vertebradosibericos.org/〉.

García-Muñoz, E., Ceacero, F., Pedrajas, L., Kaliontzopoulou, A., Carretero, M.Á., 2011. Tail tip removal for tissue sampling has no short-term effects on microhabitat selection by P*odarcis bocagei,* but induced autotomy does. Acta Herpetol. 6, 223–227. https://doi.org/10.13128/Acta_Herpetol-9814.

Garcia, K., Olimpi, E.M., Karp, D.S., Gonthier, D.J., 2020. The good, the bad, and the risky: can birds be incorporated as biological control agents into integrated pest management programs? J. Integr. Pest. Manag. 11, 11. https://doi.org/10.1093/jipm/pmaa009.

Ghosh, D., 2025. Giving the cold shoulder: why and how do we conserve farmland herpetofauna in India? Environ. Conserv. 1–6. https://doi.org/10.1017/S0376892924000316.

Ghosh, D., Basu, P., 2023. Does agricultural intensification impact pest regulation service by frogs in a natural multi-trophic system? Perspect. Ecol. Conserv. 21, 216–223. https://doi.org/10.1016/j.pecon.2023.06.002.

Ghosh, D., Aditya, V., Aravind, N.A., 2024. Farmland Herpetofauna: What Do They Do for Us? J. Herpetol. 58. https://doi.org/10.1670/23-026.

Gibbons, J.W., Scott, D.E., Ryan, T.J., Buhlmann, K.A., Tuberville, T.D., Metts, B.S., Greene, J.L., Mills, T., Leiden, Y., Poppy, S., Winne, C.T., 2000. The Global Decline of Reptiles, Déjà Vu Amphibians: Reptile species are declining on a global scale. Six significant threats to reptile populations are habitat loss and degradation, introduced invasive species, environmental pollution, disease, unsustainable use, and global climate change. BioScience 50, 653–666. https://doi.org/10.1641/0006-3568(2000)050%255B0653:TGDORD%255D2.0.CO;2.

Hoekman, D., LeVan, K.E., Gibson, C., Ball, G.E., Browne, R.A., Davidson, R.L., Erwin, T.L., Knisley, C.B., LaBonte, J.R., Lundgren, J., Maddison, D.R., Moore, W., Niemelä, J., Ober, K.A., Pearson, D.L., Spence, J.R., Will, K., Work, T., 2017. Design for ground beetle abundance and diversity sampling within the National Ecological Observatory Network. Ecosphere 8, e01744. https://doi.org/10.1002/ecs2.1744.

Hsieh, T.C., Ma, K.H., Chao, A., 2024. iNEXT: Interpolation and Extrapolation for Species Diversity.

Ivlev, V.S., 1961. Experimental ecology of the feeding of fishes. Connecticut. Yale University Press.

Jackson, A., Parnell, A., 2023. SIBER: Stable Isotope Bayesian Ellipses in R.

Jackson, A.L., Inger, R., Parnell, A.C., Bearhop, S., 2011. Comparing isotopic niche widths among and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. J. Anim. Ecol. 80, 595–602. https://doi.org/10.1111/j.1365-2656.2011.01806.x.

Jacobs, J., 1974. Quantitative measurement of food selection. Oecologia 14, 413–417. https://doi.org/10.1007/BF00384581.

Kaliontzopoulou, A., Carretero, M.A., Llorente, G.A., 2008. Head shape allometry and proximate causes of head sexual dimorphism in *Podarcis* lizards: joining linear and geometric morphometrics. Biol. J. Linn. Soc. 93, 111–124. https://doi.org/10.1111/j.1095-8312.2007.00921.x.

Kaliontzopoulou, A., Adams, D.C., van der Meijden, A., Perera, A., Carretero, M.A., 2012. Relationships between head morphology, bite performance and ecology in two species of *Podarcis* wall lizards. Evol. Ecol. 26, 825–845. https://doi.org/10.1007/s10682-011-9538-y.

Kartzinel, T.R., Pringle, R.M., 2015. Molecular detection of invertebrate prey in vertebrate diets: trophic ecology of Caribbean island lizards. Mol. Ecol. Resour. 15, 903–914. https://doi.org/10.1111/1755-0998.12366.

Keasar, T., Wajnberg, E., Heimpel, G., Hardy, I.C.W., Harpaz, L.S., Gottlieb, D., van Nouhuys, S., 2023. Dynamic economic thresholds for insecticide applications against agricultural pests: importance of pest and natural enemy migration. J. Econ. Entomol. 116, 321–330. https://doi.org/10.1093/jee/toad019.

Khan, B.A., Nadeem, M.A., Nawaz, H., Amin, M.M., Abbasi, G.H., Nadeem, M., Ali, M., Ameen, M., Javaid, M.M., Maqbool, R., Ikram, M., Ayub, M.A., 2023. Pesticides: Impacts on Agriculture Productivity, Environment, and Management Strategies. In: Aftab, T. (Ed.), Emerging Contaminants and Plants: Interactions, Adaptations and Remediation Technologies. Springer International Publishing, Cham, pp. 109–134. https://doi.org/10.1007/978-3-031-22269-6_5.

Layman, C.A., Araujo, M.S., Boucek, R., Hammerschlag-Peyer, C.M., Harrison, E., Jud, Z.R., Matich, P., Rosenblatt, A.E., Vaudo, J.J., Yeager, L.A., Post, D.M., Bearhop, S., 2012. Applying stable isotopes to examine food-web structure: an overview of analytical tools. Biol. Rev. 87, 545–562. https://doi.org/10.1111/j.1469-185X.2011.00208.x.

Lenth, R.V., 2024. emmeans: Estimated Marginal Means, aka Least-Squares Means.

Liang, J., Xiao, F., Ojo, J., Chao, W.H., Ahmad, B., Alam, A., Abbas, S., Abdelhafez, M.M., Rahman, N., Khan, K.A., Ghramh, H.A., Ali, J., Chen, R., 2025. Insect resistance to insecticides: causes, mechanisms, and exploring potential solutions. Arch. Insect Biochem. Physiol. 118, e70045. https://doi.org/10.1002/arch.70045.

Limnios, A., Boratyński, Z., Enriquez-Urzelai, U., Pafilis, P., Charles, S., Simbula, G., Carretero, M.A., 2026. Combined effects of glyphosate and ambient temperature on the energy management of a lizard: evidence from a controlled exposure study. Environ. Res., 124415 https://doi.org/10.1016/j.envres.2026.124415.

Linders, J., Jansma, J., Mensink, B., Otermann, K., 1994. Pesticides: Benefaction or Pandora's Box? A synopsis of the environmental aspects of 243 pesticides. Report No. 679101014. RIVM, Bilthoven, The Netherlands.

Lisiecki, C., 2019. Efficacy of the Aegean wall lizard (Podarcis erhardii) as a potential biological control agent in Mediterranean agroecosystems. (Doctoral dissertation).

Llanos-Garrido, A., Santos, T., Díaz, J.A., 2023. Negative effects of the spatial clumping of thermal resources on lizard thermoregulation in a fragmented habitat. J. Therm. Biol. 115, 103604. https://doi.org/10.1016/j.jtherbio.2023.103604.

MacGregor-Fors, I., Payton, M.E., 2013. Contrasting diversity values: statistical inferences based on overlapping confidence intervals. PLOS ONE 8, e56794. https://doi.org/10.1371/journal.pone.0056794.

Macome, M.C.Z., 2022. Life Cycle Assessment (LCA): Environmental impacts of wine production in a company in the Vinho Verde Region in Portugal. (Master's thesis, Instituto Politecnico de Braganca (Portugal)).

Martín, J., Ortega, J., García-Roa, R., Rodríguez-Ruiz, G., Pérez-Cembranos, A., Pérez-Mellado, V., 2023. Coping with drought? Effects of extended drought conditions on soil invertebrate prey and diet selection by a fossorial amphisbaenian reptile. Curr. Zool. 69, 367–376. https://doi.org/10.1093/cz/zoac056.

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet. J. 17, 10–12. https://doi.org/10.14806/ej.17.1.200.

Martinez Arbizu, P., 2020. pairwiseAdonis: Pairwise multilevel comparison using adonis. R package. Version 0.4. Available on URL: 〈https://github. com/pmartinezarbizu/pairwiseAdonis〉.

Mata, V.A., Da Silva, L.P., Veríssimo, J., Horta, P., Raposeira, H., McCracken, G.F., Rebelo, H., Beja, P., 2021. Combining DNA metabarcoding and ecological networks to inform conservation biocontrol by small vertebrate predators. Ecol. Appl. 31, e02457. https://doi.org/10.1002/eap.2457.

Meiri, S., 2008. Evolution and ecology of lizard body sizes. Glob. Ecol. Biogeogr. 17, 724–734. https://doi.org/10.1111/j.1466-8238.2008.00414.x.

Meiri, S., 2010. Length–weight allometries in lizards. J. Zool. 281, 218–226. https://doi.org/10.1111/j.1469-7998.2010.00696.x.

Mella, J., Tirado, C., Cortés, A., Carretero, M., 2010. Seasonal variation of prey consumption by *Liolaemus barbarae*, a highland lizard endemic to Northern Chile. Anim. Biol. 60, 413–421. https://doi.org/10.1163/157075610X523288.

Millennium Ecosystem Assessment (Program), 2005. Ecosystems and human well-being: synthesis (Ed.). Island Press, Washington, DC.

Mingo, V., Lötters, S., Wagner, N., 2017. The impact of land use intensity and associated pesticide applications on fitness and enzymatic activity in reptiles—a field study. Sci. Total. Environ. 590, 114–124. https://doi.org/10.1016/j.scitotenv.2017.02.178.

Monagan Jr., I.V., Morris, J.R., Davis Rabosky, A.R., Perfecto, I., Vandermeer, J., 2017. Anolis lizards as biocontrol agents in mainland and island agroecosystems. Ecol. Evol. 7, 2193–2203. https://doi.org/10.1002/ece3.2806.

Nakamura, S., Tamura, S., Taki, H., Shoda-Kagaya, E., 2020. Propylene glycol: a promising preservative for insects, comparable to ethanol, from trapping to DNA analysis. Entomol. Exp. Appl. 168, 158–165. https://doi.org/10.1111/eea.12876.

Neves, V., Rund, D., Pinho, C.J., Vasconcelos, R., Bustamante, P., Quillfeldt, P., 2022. Diet of the exotic Madeiran wall lizard: first insights into trophic interactions in an Atlantic seabird sanctuary. Herpetozoa 35, 107–113. https://doi.org/10.3897/herpetozoa.35.e82096.

Nielsen, J.M., Clare, E.L., Hayden, B., Brett, M.T., Kratina, P., 2018. Diet tracing in ecology: Method comparison and selection. Methods Ecol. Evol. 9, 278–291. https://doi.org/10.1111/2041-210X.12869.

Nunes, S.F., Mota-Ferreira, M., Sampaio, M., Andrade, J., Oliveira, N., Rebelo, R., Rocha, R., 2022. Trophic niche changes associated with the eradication of invasive mammals in an insular lizard: an assessment using isotopes. Curr. Zool. 68, 211–219. https://doi.org/10.1093/cz/zoab038.

Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Caceres, M.D., Durand, S., Evangelista, H.B.A., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M.O., Lahti, L., McGlinn, D., Ouellette, M.-H., Cunha, E.R., Smith, T., Stier, A., Braak, C.J.F.T., Weedon, J., 2024. vegan Community Ecol. Package.

Olesen, J.M., Valido, A., 2003. Lizards as pollinators and seed dispersers: an island phenomenon. rends Ecol. Evol. 18, 177–181. https://doi.org/10.1016/S0169-5347(03)00004-1.

Ortiz-Santaliestra, M.E., Maia, J.P., Egea-Serrano, A., Lopes, I., 2018. Validity of fish, birds and mammals as surrogates for amphibians and reptiles in pesticide toxicity assessment. Ecotoxicology 27, 819–833. https://doi.org/10.1007/s10646-018-1911-y.

Ouboter, P.E., 1981. The ecology of the island-lizard *Podarcis sicula salfii*. Amphib.-Reptil. 2, 243–257. https://doi.org/10.1163/156853881X00078.

Pereira, A., Xavier, R., Perera, A., Salvi, D., Harris, D.J., 2019. DNA metabarcoding to assess diet partitioning and feeding strategies in generalist vertebrate predators: a case study on three syntopic lacertid lizards from Morocco. Biol. J. Linn. Soc. 127, 800–809. https://doi.org/10.1093/biolinnean/blz061.

Perez-Alvarez, R., Nault, B.A., Poveda, K., 2019. Effectiveness of augmentative biological control depends on landscape context. Sci. Rep. 9, 8664. https://doi.org/10.1038/s41598-019-45041-1.

Pompanon, F., Deagle, B.E., Symondson, W.O.C., Brown, D.S., Jarman, S.N., Taberlet, P., 2012. Who is eating what: diet assessment using next generation sequencing. Mol. Ecol. 21, 1931–1950. https://doi.org/10.1111/j.1365-294X.2011.05403.x.

Preston, T., Owens, P., N.J, 1983. Interfacing an automatic elemental analyser with an isotope ratio mass sepectrometer: the potential for fully automated total nitrogen and nitrogen-15 analysis. Analyst 108, 971–977. https://doi.org/10.1039/AN9830800971.

Pringle, R.M., Kartzinel, T.R., Palmer, T.M., Thurman, T.J., Fox-Dobbs, K., Xu, C.C.Y., Hutchinson, M.C., Coverdale, T.C., Daskin, J.H., Evangelista, D.A., Gotanda, K.M., A. Man in 't Veld, N., Wegener, J.E., Kolbe, J.J., Schoener, T.W., Spiller, D.A., Losos, J. B., Barrett, R.D.H., 2019. Predator-induced collapse of niche structure and species coexistence. Nature 570, 58–64. https://doi.org/10.1038/s41586-019-1264-6.

Rato, C., Dellinger, T., Carretero, M.A., 2022. Dietary variation is driven by landscape heterogeneity in an insular omnivorous endemic lizard, revealed by DNA metabarcoding. Diversity 14, 1078. https://doi.org/10.3390/d14121078.

Reus, J., Leendertse, P., Bockstaller, C., Fomsgaard, I., Gutsche, V., Lewis, K., Nilsson, C., Pussemier, L., Trevisan, M., van der Werf, H., Alfarroba, F., Blümel, S., Isart, J., McGrath, D., Seppälä, T., 2002. Comparison and evaluation of eight pesticide environmental risk indicators developed in Europe and recommendations for future use. Agric. Ecosyst. Environ. 90, 177–187. https://doi.org/10.1016/S0167-8809(01)00197-9.

Richardson, J., 2020. selectapref: Analysis of Field and Laboratory Foraging.

Roslin, T., Majaneva, S., 2016. The use of DNA barcodes in food web construction—terrestrial and aquatic ecologists unite! Genome 59, 603–628. https://doi.org/10.1139/gen-2015-0229.

Seoane, V.L., 1885. Identidad de *Lacerta schreiberi* (Bedriaga) y *Lacerta viridis var. gardovii* (Boulenger) e investigationes herpetologicas de Galicia. La Coruña 28, 1–19.

Serrão, J.E., Plata-Rueda, A., Martínez, L.C., Zanuncio, J.C., 2022. Side-effects of pesticides on non-target insects in agriculture: a mini-review. Sci. Nat. 109, 17. https://doi.org/10.1007/s00114-022-01788-8.

Sharma, S., Kooner, R., Arora, R., 2017. Insect Pests and Crop Losses. In: Arora, R., Sandhu, S. (Eds.), Breeding Insect Resistant Crops for Sustainable Agriculture. Springer, Singapore, pp. 45–66. https://doi.org/10.1007/978-981-10-6056-4_2.

Sillero, N., García-Muñoz, E., 2010. Two new types of noose for capturing herps. Acta Herpetol. 5 (2), 259–263, 2010.

Simão, F.C.P., Carretero, M.A., Amaral, M.J., Soares, A., Mateos, E., 2015. Composition and seasonal variation of epigeic arthropods in field margins of NW Portugal. Turk. J. Zool. 39, 404–411. https://doi.org/10.3906/zoo-1401-69.

Simbula, G., Vignoli, L., Carretero, M.A., Kaliontzopoulou, A., 2021c. Fluctuating asymmetry as biomarker of pesticides exposure in the Italian wall lizards (*Podarcis siculus*). Zoology 147, 125928. https://doi.org/10.1016/j.zool.2021.125928.

Simbula, G., Macale, D., Gomes, V., Vignoli, L., Carretero, M.A., 2021a. Effects of pesticides on eggs and hatchlings of the Italian wall lizard (*Podarcis siculus*) exposed via maternal route. Zool. Anz. 293, 149–155. https://doi.org/10.1016/j.jcz.2021.06.001.

Simbula, G., Moltedo, G., Catalano, B., Martuccio, G., Sebbio, C., Onorati, F., Stellati, L., Bissattini, A.M., Vignoli, L., 2021b. Biological responses in pesticide exposed lizards (*Podarcis siculus*). Ecotoxicology 30, 1017–1028.

Simbula, G., Bissattini, A.M., Vignoli, L., 2022. Linking agricultural practices to lizard trophic behaviour: An ecological approach. Sci. Total. Environ. 830, 154822. https://doi.org/10.1016/j.scitotenv.2022.154822.

Sow, A., Haran, J., Benoit, L., Galan, M., Brévault, T., 2020. DNA Metabarcoding as a Tool for Disentangling Food Webs in Agroecosystems. Insects 11, 294. https://doi.org/10.3390/insects11050294.

Spangenberg, J.E., Zufferey, V., 2023. Soil management affects carbon and nitrogen concentrations and stable isotope ratios in vine products. Sci. Total. Environ. 873, 162410. https://doi.org/10.1016/j.scitotenv.2023.162410.

Stapleton, T.E., Weinstein, S.B., Greenhalgh, R., Dearing, M.D., 2022. Successes and limitations of quantitative diet metabarcoding in a small, herbivorous mammal. Mol. Ecol. Resour. 22, 2573–2586. https://doi.org/10.1111/1755-0998.13643.

Stemmelen, A., Le Provost, G., Giffard, B., Rusch, A., 2025. Pesticide use and large patch size reduce natural pest control potential in vineyards. J. Appl. Ecol. 62, 1509–1519. https://doi.org/10.1111/1365-2664.70058.

Tiunov, A.V., 2007. Stable isotopes of carbon and nitrogen in soil ecological studies. Biol. Bull. Russ. Acad. Sci. 34, 395–407. https://doi.org/10.1134/S1062359007040127.

Vitt, L.J., Pianka, E.R., 2007. Feeding ecology in the natural world. Lizard Ecol. Evolut. Conséq. foraging mode 141–172. https://doi.org/10.1017/CBO9780511752438.007.

Wickham, H., 2016. Toolbox. Ggplot2: Elegant Graphics for Data Analysis. Springer, pp. 33–74.

Zeale, M.R., Butlin, R.K., Barker, G.L., Lees, D.C., Jones, G., 2011. Taxon-specific PCR for DNA barcoding arthropod prey in bat faeces. Mol. Ecol. Resour. 11, 236–244. https://doi.org/10.1111/j.1755-0998.2010.02920.x.