


## REVIEW

# Conservation Blind Spots: Scenarios for Assessing the Exposure Risk of Brazilian Mammals to Pesticides

Érica Fernanda Gonçalves Gomes-de-Sá<sup>1,2</sup>  | Gabriela Fernanda da Silva Ferreira<sup>2</sup> | Anna Carolina Figueiredo de Albuquerque<sup>2</sup> | Vinícius Araújo Costa<sup>2</sup> | Henrique Villas Boas Concone<sup>3</sup> | Natan Diego Alves de Freitas<sup>1,2</sup> | Mayara Guimarães Beltrão<sup>4</sup> | Patrício Adriano da Rocha<sup>1,2</sup> | Pedro Cordeiro-Estrela<sup>1,2</sup>

<sup>1</sup>Programa de Pós-graduação em Ciências Biológicas, CCEN, Universidade Federal da Paraíba, João Pessoa, Brazil | <sup>2</sup>Departamento de Sistemática e Ecologia, Centro de Ciências Exatas e da Natureza, Laboratório de Mamíferos, Universidade Federal da Paraíba, João Pessoa, Brazil | <sup>3</sup>Instituto Pró Carnívoros, Atibaia, São Paulo, Brazil | <sup>4</sup>Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil

**Correspondence:** Érica Fernanda Gonçalves Gomes-de-Sá ([gomesdesa.ERICA@gmail.com](mailto:gomesdesa.ERICA@gmail.com)) | Pedro Cordeiro-Estrela ([estrela@dse.ufpb.br](mailto:estrela@dse.ufpb.br))

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## ABSTRACT

1. Brazil is a large agricultural producer and a megadiverse country. In this context, the use of pesticides poses risks to non-target species, including wild mammals.
2. Environmental Risk Assessment (ERA) for pesticides has been adopted by the European Food Safety Authority (EFSA) and the United States Environmental Protection Agency (EPA). Brazil has yet to present a pesticide risk assessment for vertebrates.
3. To design an ERA for Brazilian mammals, data is needed on the occurrence and distribution of species within and outside crops and agroecosystem types, their biological characteristics and life history traits.
4. We analyse a comprehensive dataset of mammal occurrences in Brazilian agroecosystems. We identify the main crops studied, review if pesticides were listed as threats for mammals endangered of extinction and discuss mammal traits that lead to pesticide exposure across agroecosystems.
5. We show that 54% of terrestrial mammals in Brazil occur in agroecosystems (319/716), with 64.3% (205/319) of these found in crop. Most studies registered mammals in large-scale monocultures, such as annual croplands, tree plantations and pasture grazing. Small farming emerges as an important knowledge gap. We found 25 species threatened with extinction (Critically Endangered, Endangered, and Vulnerable) occurring in crop in Brazilian agroecosystems.
6. Concerning ERA for Brazilian agricultural scenarios, in the screening tier process, it is suggested to use an indicator model species (IMS) with the following traits: terrestrial, crepuscular, and large body mass for pasture-grazing, tree plantations and annual croplands. Conversely, in agroforestry and perennial crop agroecosystems, we recommend considering at least one IMS with arboreal habits and a frugivorous and/or nectarivorous diet. Furthermore, in Tier 1, we recommend that a generic model species (GMS) encompassing carnivorous and herbivorous mammals should be considered in pasture-grazing systems, tree plantations and annual cropland. In agroforests and perennial croplands, GMS that represent the diet of frugivorous and nectarivorous mammals should be prioritised.

## RESUMO

1. Brasil é um grande produtor agrícola e um país megadiverso. Nesse contexto, o uso de agrotóxicos apresenta riscos para espécies não-alvo, incluindo mamíferos silvestres.

2. A Avaliação de Risco Ambiental (ARA) para pesticidas foi adotada pela Autoridade Europeia para a Segurança dos Alimentos (EFSA) e pela Agência de Proteção Ambiental dos Estados Unidos (EPA). Enquanto o Brasil ainda não apresenta uma avaliação de risco de pesticidas para vertebrados.
3. Para elaborar uma ARA para os mamíferos brasileiros, são necessários dados sobre a ocorrência e distribuição das espécies dentro e fora das culturas e tipos de agroecossistemas, suas características biológicas e traços de história de vida.
4. Analisamos um conjunto abrangente de dados de ocorrências de mamíferos em agroecossistemas brasileiros. Identificamos as principais culturas estudadas, revisamos se os pesticidas foram listados como ameaças para mamíferos ameaçados de extinção e discutimos as características dos mamíferos que levam à exposição a pesticidas nos agroecossistemas.
5. Mostramos que 54% dos mamíferos terrestres no Brasil ocorrem em agroecossistemas (319/716), sendo 64,3% (205/319) destes encontrados em lavouras. A maioria dos estudos registrou mamíferos em monoculturas de larga escala, como lavouras anuais, plantações de árvores e pastagens. A pequena agricultura surge como uma importante lacuna de conhecimento. Encontramos 25 espécies ameaçadas de extinção (Criticamente em Perigo, Em Perigo e Vulnerável) ocorrendo em lavouras nos agroecossistemas brasileiros.
6. Em relação à ARA para cenários agrícolas brasileiros, no processo de triagem, sugere-se o uso de uma espécie modelo indicadora (IMS) com as seguintes características: terrestre, crepuscular e grande massa corporal para pastagens, plantações de árvores e culturas anuais. Por outro lado, nos agroecossistemas agrofloretais e de culturas perenes, recomendamos que seja considerada pelo menos uma espécie-modelo indicadora com hábitos arbóreos e uma dieta frugívora e/ou nectarívora. Além disso, no nível 1, recomendamos uma espécie modelo genérica (GMS) que englobe mamíferos carnívoros e herbívoros seja considerada em sistemas de pastagem, plantações de árvores e terras de cultivo anuais. Em agroflorestas e terras de cultivo perenes, deve ser dada prioridade a GMS que representem a dieta de mamíferos frugívoros e nectarívoros.

## 1 | Introduction

After the suppression of native vegetation, most agricultural management practices maintain chronic detrimental impacts on biodiversity through soil erosion, water depletion (Sud 2020) and the use of fertilisers and pesticides. Both are most consistently recognised as detrimental to biodiversity (Berny 2007; López-Bao and Mateo-Tomás 2022). Pesticides have been blamed for the world's insect decline and a predicted collapse of pollination systems (Wagner et al. 2021). Other indirect ecosystem services have yet to be assessed for pesticide-induced disruptions. Adverse effects have also affected the human population, with clear links to teratogenicity, mutagenicity, carcinogenicity and hormonal alteration (Lushchak et al. 2018; Oliveira et al. 2021). However, these adverse effects on wildlife are mostly noted through acute effects such as massive deaths, population crashes and embryonic malformation (Gibbons, Morrissey, and Mineau 2015; Hoshi 2021; Zúñiga-Venegas et al. 2022) and thoroughly documented when affecting endangered species (Vicente and Guedes 2021). Despite this large body of evidence, pesticides are seldom identified as threats to wildlife (Ducatez and Shine 2017), unlike other threats such as genomic erosion, diseases, climate change or invasive species (Diez-Del-Molino et al. 2018; Dueñas et al. 2021; Plowright et al. 2021; Jia et al. 2022). The chronic effects of pesticides can include a wide range of alterations that will often impact population dynamics in the long run such as immunotoxicity, endocrine system disruption, decrease in reproductive success and alteration of behavioural patterns (Berny 2007).

The exposure of animals to pesticides occurs through inhalation, dermal contact and the consumption of contaminated food, water or soil (US EPA 2017). Exposure levels often differ inter- and intra-species, as modulated by behavioural traits, dietary habits or habitat occupancy and preference

(US EPA 2017). Even forest-dwelling species can be exposed to pesticides through direct contact with overspray (i.e., from aerial spraying) (Freemark 1995). Therefore, species occurring within crop fields (in crop henceforth) are more likely to be exposed, particularly in fields that are treated more frequently and/or receive larger amounts of pesticides (European Food Safety Authority [EFSA] et al. 2023), such as large-scale monocultures. Mobile species with large home ranges are also exposed through contact with contaminated vegetation or through the trophic chain when their prey are exposed to treated crops (Freemark 1995; USEPA 2017). Due to the adverse effects on human health and wildlife, Environmental Risk Assessment (ERA) for pesticide registration has been adopted by the European Food Safety Authority (EFSA et al. 2023) or the USA—United States Environmental Protection Agency (US EPA 2004, 2017). Both agencies assess the potential exposure of non-target organisms and include the use of exposure models to predict environmental concentrations (exposure), in order to avoid approving compounds that may pose unacceptable risks to the environment or human health (Vryzas, Ramwell, and Sans 2020).

Conversely, Brazil faces significant challenges in implementing environmental risk assessment to pesticides for vertebrates due to its high biodiversity and a diverse landscape configuration (dos Santos et al. 2021; Convention on Biological Diversity 2024). Moreover, the diversity of crops, agricultural practices and environmental conditions ranging from tropical to subtropical climates, with humid, monsoonal and semi-arid variants, make way for numerous scenarios. In addition, Brazil was the largest user of pesticides in the world in 2021, with 720,000 tons of pesticide applications for agricultural use (FAO 2023), with a market reaching USD \$20 billion in 2022–23 (Kynetec 2024). With more than 400 active ingredients in 2022, sales reached 800,652 tons, an increase of

about 11% compared to 2021 (Ibama/MMA 2022). Nowadays, in Brazil, ERA has been under specific regulation since 2017 (IN IBAMA no. 02/2017), resulting in a pesticide risk manual for bees (Cham et al. 2017), and a pesticide risk assessment for vertebrates underway. The Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) opted for the EFSA approach to environmental risk assessment (Portaria IBAMA no. 84/1996, Decreto no. 4.074/2002, IBAMA/MMA 2023—[www.gov.br/ibama](http://www.gov.br/ibama)).

The Environmental Risk Assessment (ERA) for pesticide registration at the EU and member state levels requires the assessment of the effect of pesticide (toxicity data) and an exposure assessment. Exposure scenarios are based ‘on the consumption of pesticide-contaminated food items foraged in crops’ (EFSA et al. 2023). In other words, the exposure to pesticide in this model is trophic. Exposure can be assessed in up to four steps (tiers) of increasing complexity and realism. The assessment begins at the screening level, or screening tier. This stage requires data on species occurrence within and outside agricultural fields, crops species, agroecosystem types, species biological characteristics and life history traits. Afterwards, these exposure scenarios are used for different exposure assessment tiers. The screening tier uses the Indicator Model Species (IMS), which is not a real species but a model species that theoretically has a high exposure to pesticides because of its model characteristics. This species model eats only crops treated by pesticides therefore maximising exposure and has a high food intake. Because of its high exposure it is therefore protective for all real species that are exposed to pesticides in crop. It is protective since real species would not eat only crops exposed to pesticides. If the risk of exposure of IMS to the pesticide is too high in the screening tier, the risk analysis is carried in Tier1. Tier 1 also employs a non-real species model, the Generic Model Species (GMS), considered a ‘realistic worst case’ of exposure, in that it represents a species of specific feeding guild and feeding stratum (EFSA et al. 2023). Risk assessment supposes that the GMS will, for example be frugivorous and arboreal, thus restricting exposure to more realistic conditions. Thus, the effective implementation of exposure assessment for Brazilian mammals initially requires data on the occurrence, richness and functional characteristics of mammals in agroecosystems in Brazil.

In this paper, we provide an extensive background about the occurrence records of mammals in Brazilian agroecosystems based on a systematic literature review. More specifically, we aimed to answer the following questions: (1) In which crops and in which agroecosystem types do mammals occur? (2) Does the taxonomic distribution differ between the occurrences of mammals registered off crop and in crop? (3) How many species of threatened mammals occur in crop? Are pesticides identified as threats to these species? (4) What are the top 10 crops with the highest number of mammal occurrences in crop? Additionally, which mammal species occur in these top 10 crops and how frequently do they occur? (5) What are the functional traits of mammals in these top 10 crops of mammalian occurrences, and how can the functional space be used to discuss exposure risks in agroecosystem types? Finally, we discuss the risk of mammal exposure in agroecosystems within the context of Brazilian agriculture.

## 2 | Methods

### 2.1 | Bibliographic Search

We conducted our literature search following PRISMA (Preferred reporting items for systematic reviews and meta-analysis) guidelines (Page et al. 2021) across Google Scholar, Web of Science and SCOPUS databases. We did not restrict our search using the year of publication and collected references up to, and including, August 2022. We searched for the following keywords: ‘didelphimorphia’ OR ‘cingulata’ OR ‘pilosa’ OR ‘primates’ OR ‘lagomorpha’ OR ‘rodentia’ OR ‘chiroptera’ OR ‘carnivor\*’ OR ‘perissodactyla’ OR ‘artiodactyla’ OR ‘mammal\*’ OR ‘bat\*’ OR ‘small mammal\*’ OR ‘medium and large sized mammal\*’ OR ‘carnivore\*’ OR ‘rodent\*’ OR ‘marsupial\*’ AND ‘human modified landscapes’ OR ‘pastures’ OR ‘farm’ OR ‘mosaic’ OR ‘matrix’ OR ‘agriculture’ OR ‘agro\*’ OR ‘crop\*’ OR ‘monoculture’ OR ‘land use’ OR ‘defaunation’ AND ‘brazil\*’ OR ‘atlantic forest’ OR ‘pantanal’ OR ‘pampa’ OR ‘campos sulinos’ OR ‘amazon\*’ OR ‘cerrado’ OR ‘caatinga’. The terms were searched in the title, abstract, keywords and main text. Additionally, we searched in grey literature studies on the national database ‘Brazilian Digital Library of Theses and Dissertations’ (<https://bdtd.ibict.br/vufind/>) using the same terms. The studies were excluded if: (1) the study was not carried out in Brazil; (2) the species that were recorded in a specific agroecosystem or crop type were not specified; and (3) the study was not based on primary empirical observational or experimental research. Our literature review retrieved 200 studies. Our selection process and dataset are presented in Appendices S1 and S2.

### 2.2 | Data Compilation

#### 2.2.1 | General Information

We summarised the occurrences of the 200 studies that reported wild mammals in Brazilian agroecosystems. Nomenclature and taxonomy followed Abreu et al. (2021), and the vernacular names were consulted in Emmons and Feer (1990) and Paglia et al. (2012). We assigned each species of mammal to one or more crop types, depending on whether its spatial occurrence was specifically associated with a single crop or with multiple crops in a study. Consequently, we quantified mammal occurrences by crop types. This step was necessary since many studies described the occurrence at the agroecosystem landscape level, which encompasses multiple crops and/or crop rotation, a pervasive practice in the tropics. For instance, when a mammal was sighted in an area with two or more cultivated species (such as sugarcane and pasture), the occurrence was attributed to both crops.

We grouped crops into five agroecosystem types: (1) Agroforestry: tree and shrub crops shaded by native or exotic trees (e.g., coffee, cocoa); (2) Tree plantation: arborescent plantations allocated to the production of wood, pulp and other timber products species (e.g., Eucalyptus, Pine, Oil Palm); (3) Perennial cropland: crops that are cultivated and live longer than 2 years without the need of being replanted each year, including perennial monocultures (e.g., coconut,

banana); (4) Annual cropland: monoculture or polyculture lasting over a single or a few harvests within a year (e.g., soybean, corn) (Ferreira et al. 2018); and (5) Pasture-grazing systems: continuous grazing, simple rotational grazing, and intensive rotational grazing, or general classifications cited by the studies as grazing/pasture or livestock/cattle ranching. When the occurrence was reported in the landscape without information on the specific spatial occurrence, we classified it as off crop to be conservative. On the other hand, when a study identified a mammal within one or more crops, we classified this record as in crop. The terms ‘off crop’ and ‘in crop’ are usually used by the working groups in the environmental risk assessment. In crop refers to the area within the field where pesticides are applied, while off crop denotes occurrences outside the field, which may be in the agricultural landscape (Alix et al. 2012; Cham et al. 2017).

### 2.2.2 | Occurrences of Mammals in Crop

A subset of our database comprises only in crop occurrences [reported in 98 studies]. We assessed national and international conservation status (ICMBio/MMA 2022; IUCN 2023) of the in crop species occurrences and reviewed their assessments for pesticides and/or contaminants identified as threats. We considered a mammal species threatened by pesticides if its threat category “9.3.3. Herbicides and pesticides” was listed in the IUCN assessment (IUCN Threats Classification Scheme Version 3.2; Available at: <https://www.iucnredlist.org/resources/threat-classification-scheme>). Similarly, we searched for pesticide threats in the National Action Plans (PANs) for the Conservation of Endangered Species in Brazil (Available at: <https://www.gov.br/icmbio/pt-br/assuntos/biodiversidade/pan>). Finally, we compiled a list of the top 10 crops with the highest occurrences of mammals in crop, categorising these crops into one of the five agroecosystem types and discussing exposure scenarios based on mammal functional space. We collected information on four natural history and behavioural traits (Wilman et al. 2014) that can increase or decrease pesticide exposure (EFSA et al. 2023): body mass, feeding guild, daily activity and foraging class (Appendices S3 and S7).

## 2.3 | Data Analysis

We mapped the agricultural land in Brazil based on the Mapbiomas collection 8.0 (MapBiomas Project 2023). We calculated the area covered by each crop in each of the Brazilian biomes (through `mapbiomas-user-toolkit-lulc.js`): Atlantic Forest, Amazon, Cerrado, Pantanal, Caatinga and Pampa. We removed natural land classes using the `tidyterra` (Hernangómez 2024) and `terra` (Hijmans 2024) packages in R (R Core Team 2024, <https://www.r-project.org/>), and then created the layout, maintaining only crop classes (with packages `geobr`: R package `geobr` version 1.8.2 2024, `sf`: Pebesma and Bivand 2023 and `ggspatial`: Dunnington 2023).

Species accumulation curve was calculated to extrapolate the number of unobserved species based on the number of species present in each of the five agroecosystem types, with 999 random subsampling iterations in the `vegan`

package (Oksanen 2022). We used the non-parametric bootstrap method with a 95% confidence interval to estimate species richness. A Mann–Whitney *U*-test was used to statistically evaluate the difference between the observed (Sobs) and bootstrap estimated (Sexp) richness.

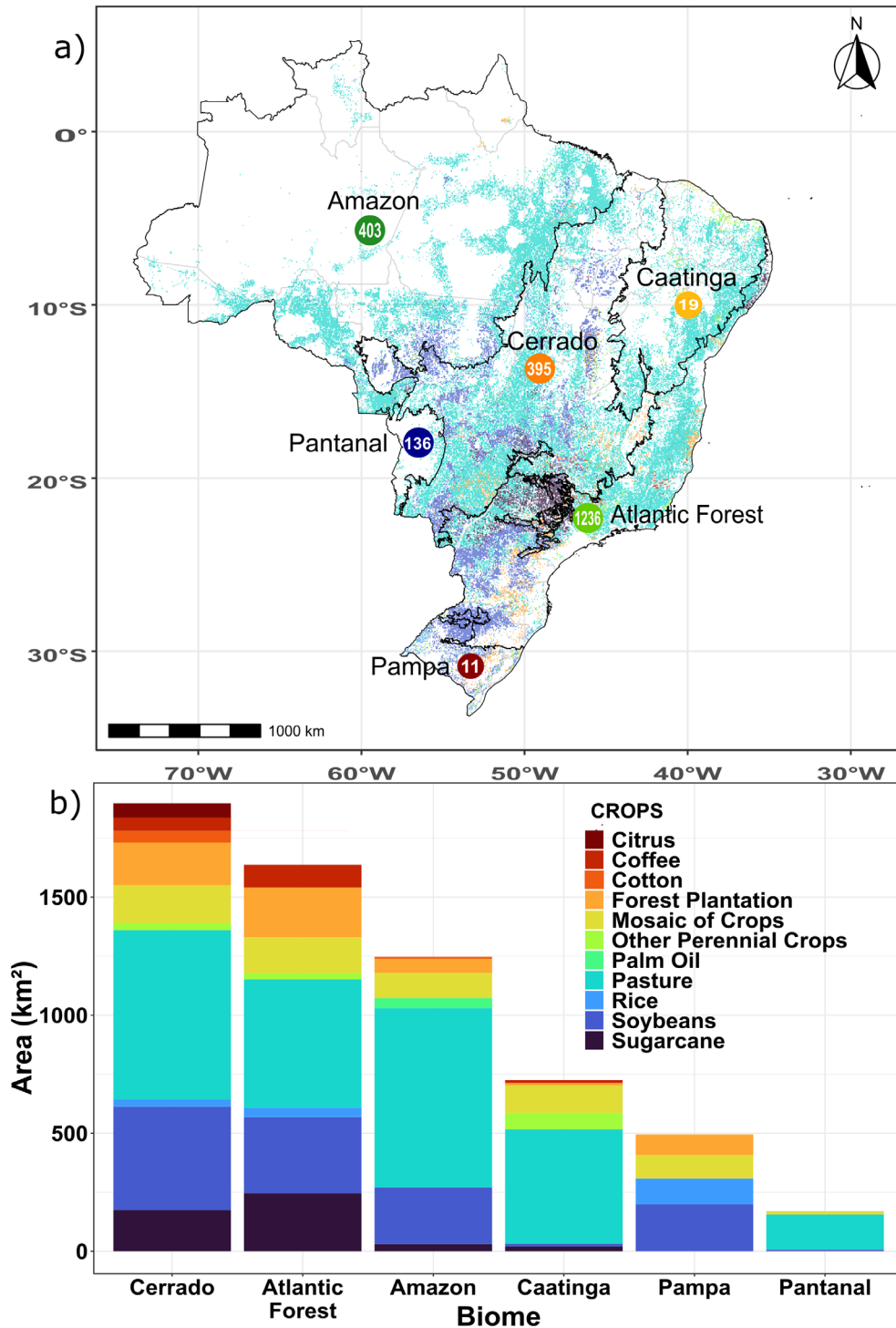
### 2.3.1 | Top 10 Crops and Exposure Trait Group for Mammals

Exposure to pesticides is currently mainly estimated through the ingestion of crops treated by pesticides. Dermal and inhalation exposure will likely be incorporated into risk analysis in the future. As species/crop occurrences are currently insufficient to model risk at this level of detail, and because ERA uses functional characteristics of species and not real species, we suggest an analysis of functional traits for each crop type. Then, we build a typology of the most represented functional groups of mammals pre-crop type. We log-converted body mass and z-transformed all traits to converge to data normality (Villéger, Mason, and Mouillot 2008; Cox, Gardner, and Gaston 2021). Then we carried a PCA of the sum of trait values of each locality for each agroecosystem type. We then estimated the space of functional characteristics by agroecosystem type. The probability of occurrence of trait combinations within the functional space was estimated with the `funspace` library (Carmona, Pavanetto, and Puglielli 2024) through multivariate kernel probability density. We evaluated the probability density functional quantiles as a proxy for functional exposure probability through diet, locomotion and body mass. In this context, areas within the highest probability quantiles represent the most represented set of traits for each agroecosystem type. Trait loadings represent the functional exposure routes within the total functional space of the agroecosystem type. In this context, we chose to evaluate only groups of occurrences with quantiles > 0.5, 50% probability of occurrence of trait combinations. We then built a categorical typology of exposure based on the loadings of the traits in the functional space and their correlation by agroecosystem type. For this step, the density hotspots were ranked in decreasing order according to the probability gradient (A>B>C). For each high probability density hotspot, we evaluated where occurrences were grouped and the combination of traits with higher loadings which resulted in Exposure Traits Groups (ETG).

## 3 | Results

### 3.1 | Occurrence of Mammals in Brazilian Agroecosystems

The 200 studies selected were published between 1998 and 2022, with 60% published after 2015. We found a total of 3085 records of mammals, documented in all six Brazilian biomes: Atlantic Forest (39.61%), Amazon (13.06%), Cerrado (12.8%), Pantanal (4.41%), Caatinga (0.62%) and Pampa (0.36%) (Figure 1). Moreover, 26.86% of these records were found in ecotones, which are transitional areas between biomes. The crop species planted in the agroecosystem were identified in 3038 records (98.48%), summing 39 species/genus distributed among the five agroecosystem types.



**FIGURE 1** | (a) Number of mammal occurrences in agroecosystems in Brazilian biomes from 200 studies between 1998 and 2022 and current planted area by crops. State borders in light grey; (b) planted area in km<sup>2</sup> in each biome stratified by crops extracted from MapBiomias Project (2023).

A total of 319 mammal species were recorded among the 716 non-strictly aquatic continental mammals in Brazil. Just over half of the recorded species (140 spp.; 43.8%) had at least five occurrence records in the agroecosystem ( $\geq 5$ ). Conversely, mammal species with more than 20 occurrence records represented 13.48% (43 species) of the total mammal records (Appendix S4). All orders of terrestrial mammals were found within Brazilian agroecosystems. The most recorded orders were Chiroptera (102 spp.; 31.97%) and Rodentia

(93 spp.; 29.15%), followed by Primates (41 spp.; 12.85%), Didelphimorphia (33 spp.; 10.34%) and Carnivora (25 spp.; 7.8%). The least represented orders were: Cetartiodactyla [Artiodactyla] (9 spp.; 2.82%), Cingulata (8 spp.; 2.50%), Pilosa (5 spp.; 1.56%), Lagomorpha (2 spp.; 0.62%) and Perissodactyla (1 spp.; 0.31%) as expected from their relative diversity. The agroecosystem types were ranked by their mammalian richness, with pasture-grazing systems having the highest species count of 260 spp., followed by tree plantations (221 spp.),

annual croplands (163 spp.), agroforestry (150 spp.) and perennial croplands (84 spp.).

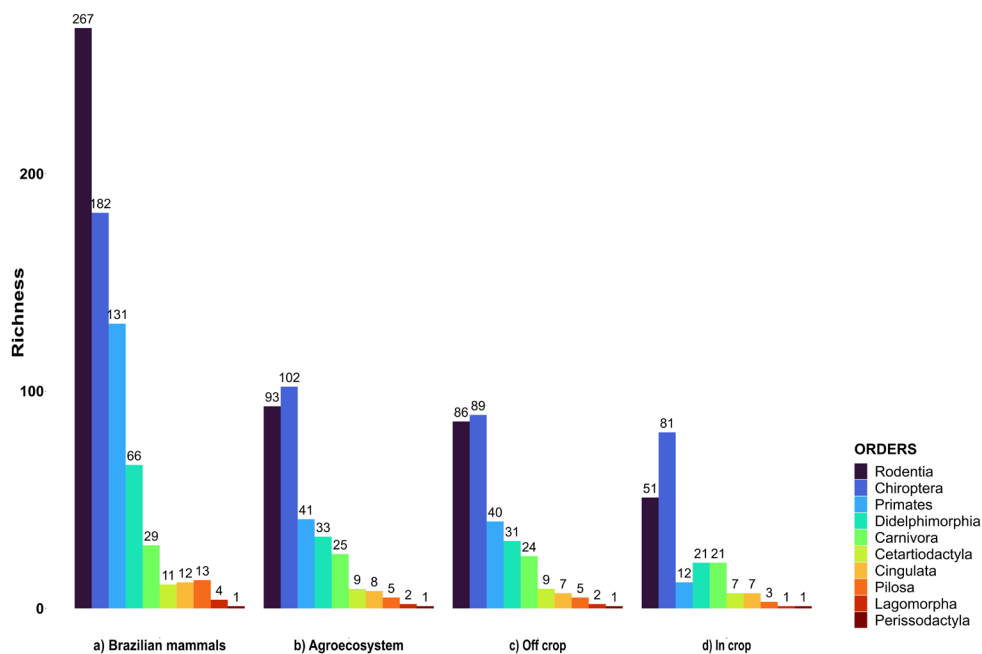
The accumulation curves revealed significant differences between observed (incidence-based data) and expected (bootstrap) mammal richness within pasture-grazing system (Sobs = 260; Sexp = 305 ( $\pm 5.7$ );  $p < 0.01$ ), tree plantation (Sobs = 224; Sexp = 268.24 ( $\pm 7.24$ );  $p < 0.01$ ), agroforestry (Sobs = 151; Sexp = 173.71 ( $\pm 6.68$ );  $p < 0.01$ ) and annual cropland (Sobs = 167; Sexp = 196 ( $\pm 3.89$ );  $p < 0.01$ ). In summary, there were less mammal species detected in these agroecosystem types than expected, whereas we found no differences in observed and expected richness in perennial croplands (Sobs = 84; Sexp = 107.26 ( $\pm 5.52$ );  $p = 0.16$ ) (Appendix S5). In terms of fine-scale spatial occurrence, all mammalian orders are found in both off crop and in crop. But we found significantly more records of mammals off crop ( $n = 1931$ ; 62.59%) than in crop ( $n = 1154$ ; 37.41%;  $W = 66,923$ ;  $p$ -value  $< 0.01$ ) as would be expected. Moreover, there was a significant reduction in richness in crop records ( $W = 65,076$ ;  $p < 0.01$ ), particularly for primates (−70%) and rodents (−50%) (Figure 2).

### 3.2 | Conservation Status and Threats to Mammals Occurring in Crop

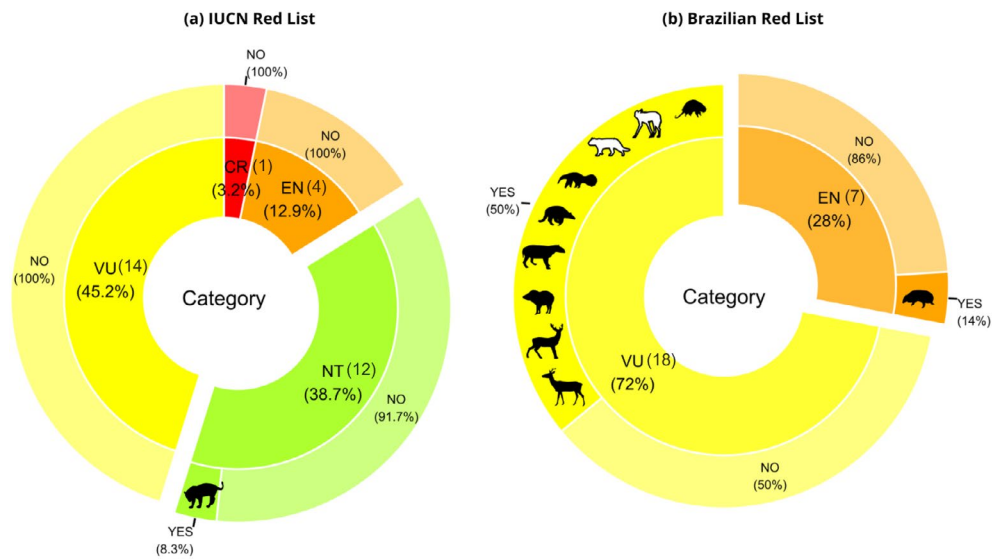
Of the 205 mammals occurring in crop in Brazil, 19 species (9.2% of in crop mammals) are listed as threatened (Critically Endangered—CR, Endangered—EN, Vulnerable—VU) in the IUCN Red List (IUCN 2023). The Golden-Bellied Capuchin (*Sapajus xanthosternus*) is the only Critically Endangered (CR) species registered in crop, in cocoa plantations in the Atlantic Forest of Bahia state (Cassano, Barlow, and Pardini 2012, 2014; de Almeida-Rocha et al. 2020; Suscke, Presotto, and Izar 2021).

Four species registered in crop are classified as Endangered: two primates (*Leontopithecus chrysomelas* and *Sapajus robustus*), one lagomorph (*Sylvilagus brasiliensis*) and one bat (*Lonchophylla dekeyseri*). Fourteen species registered in crop are classified as Vulnerable: three primates (*Callithrix kuhlii*, *Saguinus ursula* and *Sapajus cay*), two cats (*Leopardus gutullus* and *Leopardus tigrinus*), the marsh deer (*Blastocerus dichotomus*), the Atlantic forest maned sloth (*Bradypus torquatus*), the bristle-spined porcupine (*Chaetomys subspinosus*), the giant anteater (*Myrmecophaga tridactyla*), the giant armadillo (*Priodontes maximus*), the tapir (*Tapirus terrestris*), the white-lipped Peccary (*Tayassu pecari*), the three-banded armadillo (*Tolypeutes tricinctus*) and one bat (*Chiroptes albinasus*). Making it the category with the highest number of species threatened with extinction registered in crop in Brazil. Although not a threatened category, mammals listed as Near threatened had a significant number of species (38.7%,  $N = 12$ ). Furthermore, our search in the IUCN Red List showed that pesticides are listed as a threat only for the jaguar (*Panthera onca*) NT (Quigley et al. 2017) (Appendix S6; Figure 3a).

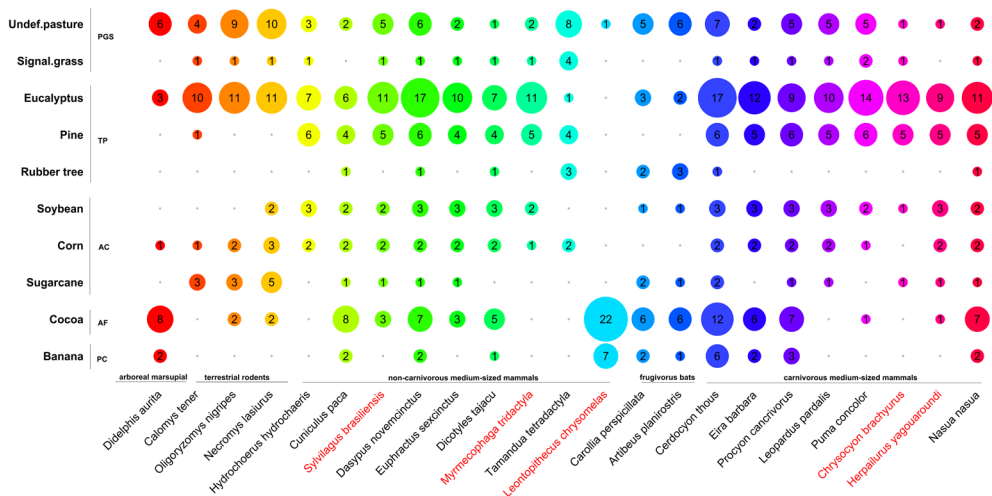
In the list of threatened mammals of Brazil (MMA 2022), of the 205 mammals occurring in crop in Brazil 25 are listed as Threatened (12.2% of in crop mammals), seven species are classified as Endangered (3.4% of in crop mammals) and 18 species are classified as Vulnerable (8.7%). In the National Action Plans for the Conservation of Endangered Mammals (PANs), pesticide threats and/or contamination (even heavy metals) is a concern for 50% ( $N = 9$ ) of species listed in the vulnerable category. These species were the bristle-spined porcupine (*Chaetomys subspinosus*), the giant anteater (*Myrmecophaga tridactyla*), the giant armadillo (*Priodontes maximus*), the tapir (*Tapirus terrestris*), the white-lipped peccary (*Tayassu pecari*), the marsh deer (*Blastocerus dichotomus*) and the pampas deer



**FIGURE 2** | Number of mammal species recorded by spatial category from 200 studies between 1998 and 2022 in Brazil. (a) List of Brazilian Mammals ( $N = 716$  Continental Mammals—Non-strictly aquatic, Abreu et al. 2021), (b) total agroecosystem ( $N = 319$  spp.), (c) off crop ( $N = 294$  spp.), (d) in crop ( $N = 205$  spp.). The colours represent the orders of mammals recorded in each category.



**FIGURE 3** | The proportion of mammal species for which pesticides, either directly (black figures) or through bioaccumulation (white figures), is considered a threat (YES) for each threat category. Results are presented for the (a) IUCN Red List (2023); (b) Brazilian Red List (MMA 2022). The proportion of species which are threatened by pesticides is shown in the external donut chart and the corresponding threat criteria (indicated by letters) in the central pie chart. CR, critically endangered (red); EN, endangered (orange); NT, near threatened (green); VU, vulnerable (yellow). For clarity, the panels do not include the Not Applicable (NA), Data Deficient (DD) and Least Concern (LC) categories (Appendix S6).



**FIGURE 4** | Frequency of occurrence of mammal species ( $\geq 20$  in crop records) in each of the top 10 crops. Agroecosystem types: AC—annual cropland, AF—agroforestry, PC—perennial cropland, PGS—pasture grazing systems, TP—tree plantation. Threatened species are marked in red (MMA 2022).

(*Ozotocerus bezoarticus*). For the maned wolf (*Chrysocyon brachyurus*) and Geoffroy’s cat (*Leopardus geoffroyi*) assessments did not clearly associate pesticides to threats but indicated potential bioaccumulation threats. Among the mammals in the endangered category, only the three-banded armadillo (*Tolypeutes tricinctus*) had pesticides included as a possible threat in its assessment (Appendix S6; Figure 3b).

### 3.3 | Top 10 Crops, Mammal Richness and Exposure Trait Group

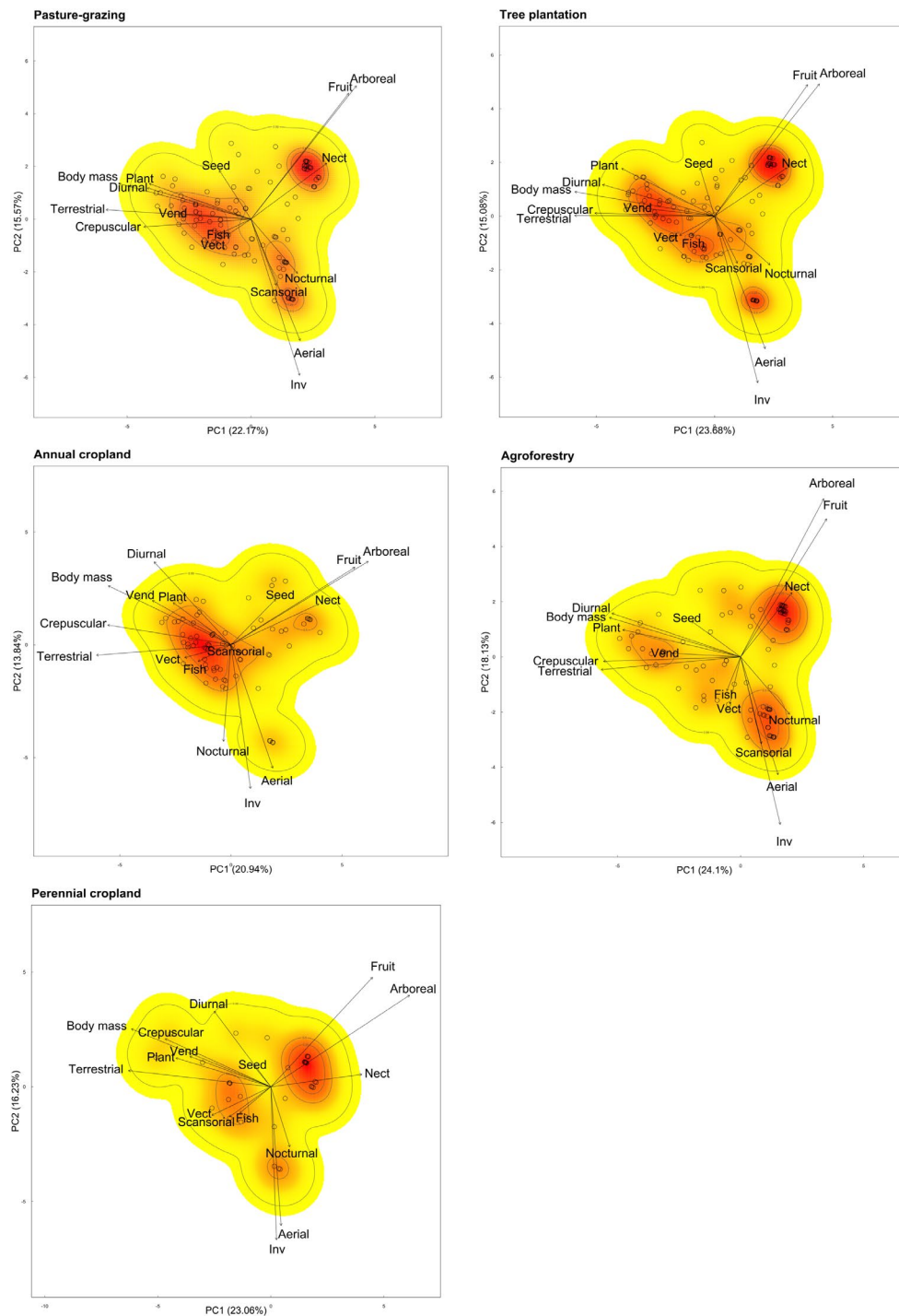
The top 10 crops with the highest number of mammal occurrences and their respective agroecosystem type were by decreasing numbers: eucalyptus (*Eucalyptus* spp.), pine (*Pinus* spp.) and

rubberwood (*Hevea brasiliensis*)—tree plantations [577]; undeveloped pasture and signal grass (*Urochloa* spp.)—pasture-grazing system [285]; soybean (*Glycine max*), maize (*Zea mays*) and sugarcane (*Saccharum officinarum*)—annual cropland [222]; cocoa (*Theobroma cacao*)—agroforestry [283]; banana (*Musa paradisiaca*)—perennial crop [61]. Upon filtering the number of occurrences of each mammal, only 23 species had at least 20 occurrences (Figure 4). These results are important for future studies selecting focal species in each crop.

Concerning functional groupings, all agroecosystem types presented three high-density mammal functional groups, except annual cropland which presented only two functional groups. For most functional spaces, the functional groups are defined by a correlated set of traits. Functional groups that are present over all

the agroecosystem types are defined by the following combination of functional traits: (i) terrestrial carnivores and herbivores with a large body mass (in pasture-grazing system, tree plantation and annual cropland); (ii) arboreal frugivores and nectarivores (in all agroecosystem types); (iii) aerial with invertebrate diet (in all agroecosystem types except annual croplands) (Figure 5). The

main functional vectors of exposure for the pasture system, tree plantations and annual crops were terrestrial locomotion, crepuscular activity and large body mass. On the other hand, the main vectors for agroforestry and perennial crops were arboreal locomotion, fruit and nectar diet. The exposure trait groups by agroecosystem type are summarised in Table 1.



**FIGURE 5** | Principal components of the functional space of occurrences of mammals in Brazilian agroecosystems (dots) by agroecosystem type. The colour gradient stands for the probability density distribution of occurrences (red = high probability, yellow = low probability). The trait loadings in the functional trait space are figured as arrows proportional to the loading. Contour lines represent the 0.99, 0.50 and 0.25 quantiles of the probability distribution. The percentage of variance explained by each component is figured in the axes. Foraging stratum: Terrestrial (including semi-aquatic foraging); Scansorial; Arboreal; Aerial. Activity period: Crepuscular; Diurnal; Nocturnal. Diet: Fruit (fruits, drupes); Inv (invertebrates, insects, worms, gastropods, etc.); Nect (nectar, pollen, plant exudates); Plant (grass, leaves, sprouts); Seed (seed, maize, grains); Vect (vertebrates ectotherms, reptiles, amphibians); Vend (vertebrates endotherms, mammals, birds); Fish.

**TABLE 1** | Mammal functional space by agroecosystem type showing exposure trait groups defined by the set of functional traits (main functional vectors of exposure) and by diet (exposure route by diet).

Agroecosystem type	Number of mammal functional groups	Exposure trait group <sup>a</sup>	Main functional vectors of exposure	Exposure route by diet
Pasture-grazing	3	A	Terrestrial, Crepuscular, Body mass	Vertebrates (Vend, Vect), Plant
		B	Arboreal, Fruit	Fruit, Nectar
		C	Invertebrates, Aerial	Invertebrates
Tree plantation	3	A	Terrestrial, Crepuscular, Body mass	Vertebrates (Vend, Vect), Plant
		B	Arboreal, Fruit	Fruit, Nectar
		C	Invertebrates, Aerial	Invertebrates
Annual cropland	2	A	Terrestrial, Crepuscular, Body mass	Vertebrates (Vend, Vect), Plant
		B	Arboreal, Fruit, Nectar, Seed	Fruit, Nectar, Seed
Agroforestry	3	A	Arboreal, Fruit, Nectar	Fruit, Nectar
		B	Aerial, Invertebrates, Scansorial	Invertebrates
		C	Terrestrial, Crepuscular, Body mass	Plant, Endothermic vertebrates
Perennial cropland	3	A	Arboreal, Fruit, Nectar	Fruit, Nectar
		B	Ectotherms vertebrates, Fish, Scansorial	Ectotherms vertebrates, Fish
		C	Invertebrates, Aerial	Invertebrates

<sup>a</sup>Trait groups of mammals within the quantiles with a probability > 50% (0.50 quantiles).

## 4 | Discussion

We found that almost half of the species (44.55%) and all the orders of continental mammals (non-strictly aquatic) listed for Brazil occur across the five different agroecosystem types. Here we discuss biases and future directions of research of mammals in agroecosystems, as well as the impact of our occurrence patterns for Environmental Risk Analysis (ERA).

### 4.1 | Consequences for ERA of Pesticides for Mammals

The taxonomic diversity of mammals recorded in agroecosystems reflects the species richness observed in Brazil, with bats, small mammals (rodents and marsupials), carnivores and primates being the most frequently documented groups. However, the orders Primates and Rodentia showed a significant reduction, likely due to the environmental filtering exerted by agroecosystems on mammal communities (Suárez-Tangil and Rodríguez 2023). Croplands are confirmed as a major threat for primate population (Estrada, Raboy, and Oliveira 2012) because of their dependence on forest environments. Worldwide studies show that surviving primate species may exhibit dietary flexibility in forest-agricultural mosaics, with crops providing them with fallback foods (de Freitas et al. 2008; Lins and Ferreira 2019;

Bryson-Morrison et al. 2020). For instance, capuchin monkeys (*Sapajus* spp.) are known to occupy landscapes dominated by intensively managed crops within São Paulo state, where native forest remnants are immersed in sugarcane plantations. In these areas, there are reports of *Sapajus* spp. directly feeding on sugarcane (de Freitas et al. 2008). Similarly, the blonde capuchin (*Sapajus flavius*) registered in our data inhabits the highly deforested Pernambuco Endemism Center (Feijó et al. 2023), with frequent reports of animals raiding plantations, such as sugarcane (Lins and Ferreira 2019). ERA for pesticide exposure should be particularly aware of these fallback foods for primates in Brazil, which harbours the largest primate diversity and pesticide use in the world. This is most important since no primate extinction assessment, at the national or international levels, listed pesticides as a threat.

For rodents, greater agricultural intensification may result in a decrease in rodent richness because of habitat conversion, small home ranges, specific niches (Maestri et al. 2017) and restricted distributions (do Prado and Percequillo 2013). Competition may also be a determinant in rodent diversity reduction since the food supply in agricultural fields tends to lead to an increase in the abundance of generalist species (Gentili, Sigura, and Bonesi 2014; Gomes de Sá, Silva, and Cordeiro-Estrela 2024), making them more common and abundant worldwide (Sullivan and Sullivan 2006; Caudill, Vaast, and Husband 2014;

Verdade et al. 2020). ERA for pesticide exposure is thus crucial for rodent species because most can be herbivorous, feed in treated crops or seeds and be sensitive to local effects because of the characteristics above. Bats did not exhibit greater differences between off crop and in crop assemblies; they remained the richest group in terms of species within crops (Figure 2). We encourage researchers to detail the specific occurrences within the agroecosystem (e.g., outside the crop, at the edge, or inside the crop) as this information was generally lacking, highlighting existing gaps in studies on biodiversity in agroecosystems (Prosser et al. 2016; Ferreira et al. 2018).

## 4.2 | Exposure to Pesticides as Threats in the Assessment of Risk of Extinction

We show that 25 species threatened with extinction (Critically Endangered, Endangered, and Vulnerable) occur in crop in Brazilian agroecosystems. Of these, six species occur within the top 10 crops with more mammal occurrence records: *Sylvilagus brasiliensis*, the cotton-tail; *Myrmecophaga tridactyla*, the giant anteater; *Chrysocyon brachyurus*, the maned-wolf; *Leontopithecus chrysomelas*, the golden-headed lion Tamarin (GHLT); *Puma concolor*, the puma; and *Herpailurus yagouaroundi*, the jaguarundi. As the endangered cotton-tail was restricted to the Northern Atlantic forest under the taxon *Sylvilagus brasiliensis* sensu stricto, most of the records here do not apply to the endangered taxon (Ruedas et al. 2017). For the other five species, pesticides are direct threats only to the giant anteater in the PAN Anteaters and Armadillos. For the maned-wolf (PAN Canids), pesticides are listed as potential threats of bioaccumulation due to contamination by heavy metals and pesticides. For the other species pesticides are not listed as threats. Our data shows potential exposure for the GHLT in agroforestry and perennial crops.

In the IUCN Red List, the only species for which pesticides were included as a threat was the Near Threatened jaguar (Quigley et al. 2017), which is listed as Vulnerable for Brazil but without mention of pesticides as a threat in the PAN Large Felids. Furthermore, no threats of bioaccumulation or presence of heavy metals were listed in the jaguar PAN, even though metal contamination has already been recorded for the jaguar in Brazilian Pantanal (May Júnior et al. 2018) and Amazon forest (Lopes et al. 2020). Recently, Medici et al. (2021) found tapirs contaminated by organophosphates, carbamates, pyrethroids and metals in the Brazilian Cerrado, and pesticides were included as a threat in the PAN Ungulates. The need for other quantification studies is crucial for conservation (e.g., Brait, Antoniosi Filho, and Furtado 2009; May Júnior et al. 2018; Lopes et al. 2020; Soresini et al. 2021) since for many threatened carnivores occurring in Brazil, like the small cats (e.g., *Herpailurus yagouaroundi*, *Leopardus guttulus*, *L. wiedii*, *L. tigrinus*), pesticides are not mentioned in national action plans (PAN Small Felids). Thus, given that there is little data available on the effects and exposure for most endangered species, relying on ecotoxicology studies to fill this gap is one of the priorities for advancing risk assessment and coverage of endangered species in ERA. Non-lethal, non-invasive or minimally invasive biomarkers of exposure (blood, hair, spines, faeces, skin and/or fat biopsies, or carcasses) may be suitable for investigating exposure

to pollutants (Rogival et al. 2006; Wang et al. 2020; Medici et al. 2021; van As et al. 2022; García-Muñoz et al. 2023) which should be complemented by research on exposure pathways and impacts on threatened species (Smith et al. 2007). Paired off crop and in crop designs on the quantification of pesticides in biological samples are important for use in ERA.

## 4.3 | Top 10 Crops, Mammal Richness and Exposure Trait Group

The top 10 crops with the highest number of mammal occurrences enabled us to discuss focal species (actual species that might be exposed to pesticides) by crop and explore the functional traits that led to mammal exposure in agroecosystem types. Focal species are ideally selected for their ecological relevance, occurrence and distribution in agricultural fields, sensitivity to potential stressors (different groups of pesticides) and ease of sampling (Vallon et al. 2018). In-depth knowledge of the ecotoxicology and exposure of these species can therefore provide reliable information on the effects on many other species (EFSA Scientific Committee 2016; Vallon et al. 2018). According to our data, 23 mammals had a frequency  $\geq 20$  in the top 10 crops, and these could be potential focal species (Figure 4). The rodents of family Cricetidae, subfamily Sigmodontinae—*Necomys lasiurus*, *Oligoryzomys nigripes* and *Calomys tener*—can be focal species in pasture grazing systems and tree plantation (*Eucalyptus*). The high diversity and ample distribution of these cricetid rodents make them suitable for assessing pesticide exposure in realistic agricultural field scenarios (do Prado and Percequillo 2013; Fritsch et al. 2022). Additionally, as the main prey of several predators, they are important components of the food chain (Sousa and Bager 2008).

Insectivorous small mammals and rodents are also of particular focus in post-registration surveys to assess pesticide impacts on mammals, being largely represented in the list of ‘mammal indicator species’, ‘generic focal species’ in the first levels of the ERA (EFSA et al. 2023). *Didelphis aurita* was the only marsupial among the most frequent mammals, mainly in pastures and cocoa fields and might forage in sugarcane fields (de Camargo et al. 2022). Didelphimorph marsupials are unique components of the Neotropical fauna, exhibiting great diversity of feeding habits and locomotor patterns (Bonvicino et al. 2022). Furthermore, due to their ancient divergence from placental mammals, known model organisms (mainly rodents such as *Mus musculus* and *Rattus* spp.) (EFSA et al. 2023) are likely inadequate models to understand the pesticide exposure risks faced by marsupials. Their presence in crops should be of concern, as they may be intensely exposed to pesticides and the consequences of exposure are not covered by current models.

Concerning primates, the endangered golden-headed lion tamarin (*Leontopithecus chrysomelas*) emerges as a potential focal species for primates in cabucas, where it occupies landscapes shaded by agroforestry cocoa (Flesher 2015; Oliveira et al. 2011; de Almeida-Rocha et al. 2020) and rubber plantations (De Vleeschouwer and Oliveira 2017), as well as causing damage to forest plantations (Mikich and Liebsch 2014). Many carnivores showed higher frequency of occurrence in the top 10 crops,

among them *Chrysocyon brachyurus* and *Herpailurus yagouaroundi* were listed as vulnerable, potential focal species in tree plantation. Indeed, eucalyptus, pine and palm oil plantations are the most frequently used by carnivores (Ferreira et al. 2018), but foraging also occurs in annual crops because of the presence of abundant prey (Courtalón and Busch 2010; Gheler-Costa et al. 2012; Gomes de Sá, Silva, and Cordeiro-Estrela 2024). The most frequent mammal in the top 10 crops is the Crab-eating fox (*Cerdocyon thous*) which could be a potential focal species to cover other carnivores in Brazil, due to its generalist habits and large distribution.

Finally, two bat species (*Carollia perspicillata* and *Artibeus planirostris*) were among the most frequent species. Bats exhibit an array of feeding adaptations (Kunz et al. 2011), using tree plantations like rubber trees as stopover sites or foraging habitats (Heer et al. 2015). Frugivorous and nectarivores species feed on fruit plants both in the canopy and in the herbaceous layers in shade plantations (Estrada and Coates-Estrada 2002) and are frequently recorded in agroforestry, like cocoa cultivation in Brazil (Faria et al. 2006; Faria and Baumgarten 2007). Due to the provision of ecosystem services as pollination, seed dispersal and pest control bats should be considered in risk assessment with regard to the protection goals of ecosystem services in the ERA assessment (Kunz et al. 2011; Ramírez-Francel et al. 2022; Buxton et al. 2022).

Moreover, as expected, we show that the analysis of functional traits across agroecosystem types cannot be summarised into a single exposure trait group, given the high complexity of the functional space of mammals. Among mammals, there are considerable variations in behaviour and functional traits that determine the occupation of specific niches (Cox, Gardner, and Gaston 2021) that influence its potential for exposure to contaminants. Therefore, the use of model organisms in risk assessment is limited and poses a challenge (Smith et al. 2007). The ERA scheme assumes that food exposure is the main route of exposure and considers a single diet type in the screening tier of the exposure assessment by the use of an Indicator Model Species (IMS). Although not a true species, IMS is considered to have a higher internal exposure than all wild species and is therefore sufficiently protective for mammals in a conservative exposure scenario (EFSA et al. 2023). Given the diversity of crops and mammals found in this study, we do not consider that a single model indicator species approach is appropriate in the Brazilian screening tier. Our evaluation of exposure trait groups showed that at least three groups of species should be considered among agroecosystem types. The exception is annual cropland, where we found two exposure trait groups.

It's worth mentioning that for the three types of agroecosystems (pasture-grazing, tree plantation and annual cropland), the primary functional vectors of exposure in group A were: terrestrial, crepuscular and larger body mass, with vertebrates as their main diet. This suggested that for these agroecosystems, a single indicator model species might suffice to cover focal species at the screening tier level. Conversely, in agroforestry and perennial crop agroecosystems, we recommend considering at least one indicator model species with arboreal habits and a frugivorous and/or nectarivorous diet.

The Tier 1 (steps in ERA) exposure assessment in the subsequent step relies on oral exposure to generic model species (GMS). It is believed that GMS, which are identified by their distinct feeding guilds, serve as a representative sample for all focal mammal species within crop groupings characterised by comparable growth patterns (EFSA et al. 2023). Thus, it is assumed that in the pasture-grazing system, tree plantations and annual cropland should be considered as exposure routes for carnivorous and herbivorous mammals. In agroforests and perennial croplands, GMS that represent the diet of frugivorous and nectarivorous mammals should be prioritised. Furthermore, in three of the five agroecosystem types (pasture, tree plantation and perennial crop), the primary functional vectors of exposure were Invertebrates and Aerial. Although these traits were clustered together in group C due to the low probability distribution of the trait combinations in the functional space, we recommend the inclusion of aerial insectivorous bats as a third GMS in Tier 1, especially because the abundance of prey in agricultural areas serves as a source of food for bats (Wickramasinghe et al. 2004) and insectivorous bats are considered more susceptible to pesticide contamination (Bennett and Thies 2007).

#### 4.4 | Biases and Future Directions

Mammal occurrences were registered mainly in large-scale monoculture agroecosystems, including pasture-grazing (notably managed pasture but also unidentified management systems), tree plantations (especially eucalyptus and its subspecies) and annual croplands (mainly sugarcane, soybeans and corn). Nevertheless, although there are occurrences in all five Brazilian biomes, the Atlantic Forest from the southeastern region is overrepresented, as for most scientific data, because of the higher concentration of universities and research investments in this region (Geocapes 2022). This is a clear bias because of the lack of research focusing on small family farming properties that occupy 23% of Brazil's territory with 80.9 million hectares (IBGE 2017) and produce important crops, strategic to food security, like beans, cassava, sweet potato, bananas, pineapples and coffee (IBGE 2017). Except for coffee and bananas, the other crops were poorly represented in our data. Other important crops are underrepresented in studies, especially cotton, one of Brazil's five main crops, with 2.60 million hectares (MapBiomass Project 2023) which is barely mentioned in the literature.

Data availability and agroecosystem descriptors are lacking. Significant omissions of agroecosystem descriptions in the study areas caused exclusion of articles from our review, specifically for the species of crop in the study area. The absence of standardised information on agroecosystem descriptors may have introduced biases and gaps in our analysis—refer to Box 1 for detailed crop descriptors crucial for accurate exposure assessment.

The threat of mammal exposure in agroecosystems can manifest as acute or chronic impacts. The main research challenge in this regard lies in identifying the chronic effects of the exposure to pesticides applied to crops, which are contingent upon the presence and duration of mammal exposure within the crop, as well as the potential biodynamics and exposure routes of the

Data	Description
<b>Mammal occurrence</b>	<ul style="list-style-type: none"> <li>· Outside the treated area, within remnants of natural vegetation (off crop)                             <ul style="list-style-type: none"> <li>· At the edge of the field (edge - in crop)</li> <li>· Treated area (in crop)</li> </ul> </li> </ul>
<b>Crop</b>	<ul style="list-style-type: none"> <li>· Scientific name</li> <li>· Plant cultivar or variety</li> <li>· first and last harvest times</li> <li>· Phenological stages of crop at the time of study</li> <li>· Agricultural area (ha)</li> </ul>
<b>Landscape</b>	<ul style="list-style-type: none"> <li>· Information about agroecosystem types and landscape detail, if:                             <ul style="list-style-type: none"> <li>· planted pasture</li> <li>· perennial or annual agricultural land                                     <ul style="list-style-type: none"> <li>· agroforestry</li> <li>· tree plantation</li> <li>· or other types</li> </ul> </li> </ul> </li> </ul>
<b>Pesticides</b>	<ul style="list-style-type: none"> <li>· When feasible, collecting information on the types of pesticides, class of use, mode of application, and time of application in the field at the time of study implementation is valuable</li> </ul>

pesticide. Here, we outline several future directions for investigating mammal exposure: (i) field validation—Carrying out field studies to validate the effectiveness of the model and generic model species in predicting exposure in Brazilian agricultural scenarios, including incorporating more detailed ecological data and refining trait-based approaches; (ii) develop studies in biomes with gaps—according to our review, the knowledge gaps are more pronounced in Caatinga and Pampa; (iii) pesticides as a threat to endangered species—Beyond agroecosystems, exposure to pesticides is probably the most neglected potential threat in the National Action Plans for the Conservation of Endangered Species in Brazil. But since there are very few quantifications of pesticides or research of occurrences of mammals in agroecosystems, pesticides are a blindspot in threat evaluation. We advocate for a comprehensive examination of pesticides as threats to these species and the initiation of research projects aimed at quantifying pesticide exposure in endangered species; (iv) development of quantification and monitoring protocols to accurately assess exposure to pesticides in wild mammals is essential to track changes in mammal populations related to exposure patterns over time; (v) create a database—establish a comprehensive database on the occurrence of wild mammals Brazilian crops.

## 5 | Conclusions

Our review addresses a pioneering topic on the occurrence and exposure of mammals in Brazilian agroecosystems and provides important information, applicable to research and the environmental regulatory sector, on the environmental risk assessment

of pesticides for non-target organisms. We demonstrated that at least 44% of Brazilian terrestrial mammals occur in agricultural landscapes, and more than half of these species (64.26%) are found in crops. The presence of mammals in crops is an unequivocal indication of the need to consider pesticide threats in endangered species assessments. Furthermore, it is important to carry out more studies that focus on the gaps in our review, such as family farming and the Caatinga and Pampa biomes.

Finally, our functional traits approach revealed the main exposure traits by agroecosystem type, indicating that more than one indicator model species is needed for mammals in the Neotropical region. We emphasise that pesticide quantification and monitoring studies, especially on endangered species, should be carried out to fill these conservation blind spots.

### Author Contributions

**Érica Fernanda Gonçalves Gomes-de-Sá:** conceptualization, investigation, writing – original draft, methodology, formal analysis, data curation. **Gabriela Fernanda da Silva Ferreira:** investigation, methodology, writing – review and editing, data curation. **Anna Carolina Figueiredo de Albuquerque:** investigation, methodology, writing – review and editing, data curation. **Vinicius Araújo Costa:** data curation, methodology, writing – review and editing, investigation. **Henrique Villas Boas Concione:** investigation, writing – review and editing, data curation, methodology. **Mayara Guimarães Beltrão:** investigation, writing – review and editing, data curation. **Patrício Adriano da Rocha:** investigation, writing – review and editing, data curation. **Pedro Cordeiro-Estrela:** conceptualization, investigation, writing – review and editing, methodology, supervision, formal analysis

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## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

Data will be made available on request.

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### Supporting Information

Additional supporting information can be found online in the Supporting Information section.