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Organic farming reduces pesticide load in a bird of prey

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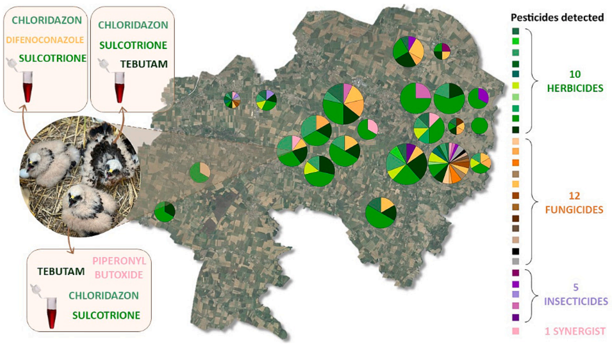
HIGHLIGHTS

- No study exists on inter- and intra-brood variability of non-persistent pesticides.
- 28 compounds were detected in blood of 55 *Circus pygargus* chicks from 22 nests.
- There is a high variability of contamination levels within and among nests.
- More organic farming around nests reduces the number of pesticides in chicks' blood.
- Neither sex nor its interaction with hatching order influences chicks' contamination.

GRAPHICAL ABSTRACT



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ABSTRACT

Human activities have led to the contamination of all environmental compartments worldwide, including bird species. In birds, both the environment and maternal transfer lead to high inter-brood variability in contamination levels of pollutants, whereas intra-brood variability is generally low. However, most existing studies focused on heavy metals or persistent compounds and none, to our knowledge, addressed the variability in contamination levels of multiple pesticides and the factors influencing it. In this study, the number of pesticides detected (of 104 compounds searched) and the sum of their concentrations in the blood of 55 Montagu's harrier (*Circus pygargus*) nestlings from 22 nests sampled in 2021 were used as metrics of contamination levels. We investigated the effect of organic farming at the size of male's home range (i.e., 14 km^2) and chicks' sex and hatching order on contamination levels. We did not find a difference between inter-brood and intra-brood variability in pesticide contamination levels, suggesting a different exposure of siblings through food items. While chicks' sex or rank did not affect their contamination level, we found that the percentage of organic farming around the nests significantly decreased the number of pesticides detected, although it did not decrease the total concentrations. This finding highlights the potential role of organic farming in reducing the exposure of birds to a pesticide cocktail.

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1. Introduction

Anthropogenic pollution associated with industrialisation, urbanization, and agricultural intensification has led to the contamination of multiple environmental compartments (i.e., biotic and abiotic elements), notably wildlife (Mañosa et al., 2001; Perugini et al., 2011). For decades, studies have investigated the factors influencing the exposure of marine bird species to contaminants *in natura*, mostly heavy metals and Persistent Organic Pollutants (POPs) including perfluoroalkyl and polyfluoroalkyl substances and organochlorine pesticides such as DDT (Becker, 1992; Hario et al., 2000; Janssens et al., 2002; Custer et al., 2010; Jouanneau et al., 2021). Various contamination pathways for these pollutants have been identified; contamination may occur through direct contact with contaminated environments, ingestion, and maternal transfer (Becker and Sperveslage, 1989; Jouanneau et al., 2021). Direct contact or ingestion of contaminated food is supposed to be reduced with the increasing distance to the pollution source. For instance, levels of lead in feathers of house sparrows showed an urbanization gradient, that is, higher levels of contamination closer to heavily urbanized areas (Bichet et al., 2013). Moreover, geographical factors influence the spatial and temporal trends in contamination levels (Devalloir et al., 2023). In addition, the food brought by adults to the nest may show variation in contaminant burden; thus, variation in prey selection and foraging habitat preferences of parents may explain the differences in chick contamination between broods (Goutner et al., 2001). Thus, in general for heavy metals and POPs, inter-brood variability in contamination patterns is attributable to both direct environmental factors associated with the nest location and food provisioning by adults.

In species such as the peregrine falcon (*Falco peregrinus*; Newton et al., 1989) and common tern (*Sterna hirundo*; Power et al., 2021), eggs from the same clutch have been reported to show similar contamination levels; however, some other species show great variation within a clutch according to the egg laying sequence (e.g., American avocets *Recurvirostra americana*, black-necked stilts *Himantopus mexicanus*, and Forster's terns *Sterna forsteri*; Ackerman et al., 2016). In general, maternal transfer of contaminants increases with the laying order, with the last laid egg being the most contaminated, because egg laying may be used as a detoxification mechanism in females (for instance in herring gulls *Larus argentatus*; Mineau, 1982; Robinson et al., 2012). However, some studies have reported that the first laid egg is the most contaminated one, indicating a reversed detoxification pattern of females (in herring gulls and common terns; Becker et al., 1994; blue tits *Cyanistes caeruleus*; Van den Steen et al., 2009; Audouin's gull *Larus audouinii*; Vicente et al., 2015). However, these findings vary not only with species but also with the type of contaminant studied (Janssens et al., 2002; see Table 1 in Van den Steen et al., 2009). Similarly to eggs, hatchlings display contamination patterns that reflect the contamination levels of the breeding female and the detoxification pattern in egg laying sequence, that is, the highest contamination levels may be observed in either the first-hatched or the last-hatched offspring (Lemmetyinen et al., 1982; Becker and Sperveslage, 1989; Becker et al., 1993). Nevertheless, the contamination pattern of chicks also depends on food (Becker and Sperveslage, 1989), and one may expect siblings to display the same contamination levels, as they share the same environment and food. However, access to prey items may vary within the same clutch owing to variations in the morphological, physiological, and behavioural traits of chicks and the feeding strategy of parents (Viñuela, 1999), as hatching asynchrony generally leads to competitive and developmental hierarchies among chicks (Love et al., 2003). For instance, in the brown falcon, *Falco berigora*, the last-hatched chicks displayed lower growth rates and body condition and a higher probability of mortality than chicks hatched earlier (McDonald et al., 2005). Nonetheless, these differences are not observed in all species exhibiting hatching asynchrony (Krebs, 1999). Consequently, it is difficult to predict the direction of eggs and nestlings' contamination levels based on their laying and hatching order, respectively. Intra-brood variability may also occur because of sex-related differences among chicks. This is especially the case in dimorphic bird species, in which one of the sexes exhibits larger body size. The larger and thus faster-growing chicks may benefit from a "dilution effect" as the higher tissue growth rate is greater than the rate of contaminant deposition in them (Stewart et al., 1997; Goutner et al., 2001; Ackerman et al., 2011). Moreover, food provisioning leads to sibling competition, with usually the larger sex being favoured (Anderson et al., 1993). In Montagu's harriers, *Circus pygargus*, first-laid eggs are more likely to produce females, the larger sex, which are produced in higher numbers than males especially in years of high food abundance (Leroux and Bretagnolle, 1996; Arroyo, 2002). Consequently, sex and hatching order are key characteristics leading to dominant–subordinate hierarchies among nestlings during feeding events, as larger individuals might outcompete the smaller ones and gain better access to prey items (Anderson et al., 1993). Thus, intra-brood variability in contamination patterns may be attributable to both rank and sex of the chicks, which set their initial contamination level and affect their ingestion of contaminants.

Although studies have examined the inter- and intra-brood variability in contamination levels in birds for decades, few have focused on non-persistent pesticide contaminants (i.e., excluding POPs), and none—to our knowledge—have investigated multiple pesticides other than chlorinated pesticides (Custer et al., 2010; Bustnes et al., 2015). A first reason was likely the absence of technical means to simultaneously detect and quantify multiple molecules. However, suitable analytical methods are now available (Rodrigues et al., 2023); therefore, pesticide contaminants should be investigated. Agricultural intensification has led to the extensive use of pesticides and widespread contamination of agroecosystems; consequently, synthetic fertilizers and pesticides are found in both abiotic and biotic environmental compartments (Székács et al., 2015; Wintermantel et al., 2020; Fritsch et al., 2022; Fuentes et al., 2023a). Organic farming is a production system that excludes the use of synthetic farm inputs (Lockeretz, 2007). Therefore, the application of organic farming practices is expected to reduce contamination levels in the environment and consequently in wildlife. In an analysis of topsoil samples collected across Europe, samples from organic farms showed significantly fewer pesticide residues and in lower concentrations than those from conventional farms (16 vs. 5 residues/sample and 70 to 90 % lower concentrations; Geissen et al., 2021). Pesticide mixtures have also been reported to be lower in both soils and earthworms sampled in organic farms than in those from conventional farms (Pelosi et al., 2021); however, some studies report contradictory findings, with no effect of the practices on the contamination levels in small mammals (Fritsch et al., 2022). This may result from unforeseen contamination of organic fields from either persistence of molecules or large-scale drifts of pesticide particles during spraying or as dust or runoff waters (Humann-Guilleminot et al., 2019; Wintermantel et al., 2020). Still, a larger proportion of organic farms (i.e., lower proportion of conventional farms applying pesticides) in the landscape should reduce the unintentional pesticide contamination of untreated fields and thus the global exposure of wildlife to pesticides.

The Montagu's harrier is a declining bird of prey species, specialist in agroecosystems, nesting on the ground, generally of cereal fields. Consequently, their eggs and chicks are directly exposed to local pesticide contamination throughout their growth period, through direct spraying on eggs, contact with contaminants remaining on the soil and on the crop, and through feeding on contaminated prey. Montagu's harriers mainly prey on voles and feed upon orthopterans and passerine birds as alternative prey (Salamolard et al., 2000). Males are the main sex involved in providing food resources, hunting at large distances from the nest (5.8 ± 4.1 km; Guixé and Arroyo, 2011); females also provide food from the surroundings (<1 km), mainly before fledging, especially in years of low food abundance (Garcia and Arroyo, 2005; Wieringa et al., 2019). The species is sexually dimorphic; females are ~19 % heavier than males, and this difference becomes detectable during the second part of the rearing period (Millon and Bretagnolle, 2005), which may lead to sibling competition for food. Nevertheless, because chicks of the same brood share the same parents, environment, and food, they are expected to show similar contamination patterns those eventually differ from the ones observed in chicks from another brood.

The present study aimed at investigating the inter- and intra-brood variability in pesticide contamination found in Montagu's harrier chicks and the factors influencing this variability. We used the number of pesticides detected and the total sum of concentrations of pesticides in chick blood as proxies of contamination levels. Indeed, these metrics have been commonly used in ecotoxicology studies to describe individual or environmental contamination loads (Tartu et al., 2014; Fritsch et al., 2022; Zaller et al., 2022). Assuming an additive effect of pesticides, a higher number of compounds and/or higher total concentrations are expected to increase the number of potential interactions among mixture components and thus the risk of cocktail effects (Hernández et al., 2017; Zaller et al., 2022). We first investigated the relative inter-brood vs. intra-brood variability in contamination levels in chicks. Then, we tested the effects of the proportion of organic farming at the scale of male's home range (i.e., 14 km^2, Salamolard, 1997, see below for details). Lastly, we tested the effects of sex and hatching order of chicks on contamination levels. We expected to find a higher inter-brood variability than intra-brood variability and lower contamination levels with a higher proportion of organic farming. We also expected to identify a relationship between the sex and hatching rank of chicks and the contamination levels, with females of rank 1 being less contaminated because of a "dilution effect" and a higher pesticide load in last-laid eggs.

2. Materials and methods

2.1. Study site and model species

The study site is a 450 km^2 area located in southwestern France (46°11′N, 0°28′W) in the Long-Term Social-Ecological Research platform, Zone Atelier Plaine & Val de Sèvre (LTSER ZAPVS). Every year, crop identification is performed in this area, allowing mapping of organic crop plots in detail (Bretagnolle et al., 2018, see also Fuentes et al., 2023b; Fig. 1). In France, organic farming complies with the European legislation on organic production, and thus synthetic pesticides and fertilizers to grow crops are banned. In the ZAPVS, Montagu's harrier nests have been monitored since 1994 (Bretagnolle et al., 2018). These birds nest on the ground in cereal crops and lay up to eight eggs (Arroyo et al., 1998). Eggs are incubated by females for 29 days, and chicks are reared in the nest for 30–35 days (Arroyo et al., 2007). At this site, the mean productivity is reported to be 2.05 fledglings per breeding attempt (Arroyo et al., 2004), breeding success depending mainly on the availability of its main prey, the common vole (*Microtus arvalis*; Salamolard et al., 2000). In years of poor vole availability, Montagu's harriers also feed on orthopterans (Butet and Leroux, 2001). Nestling food is

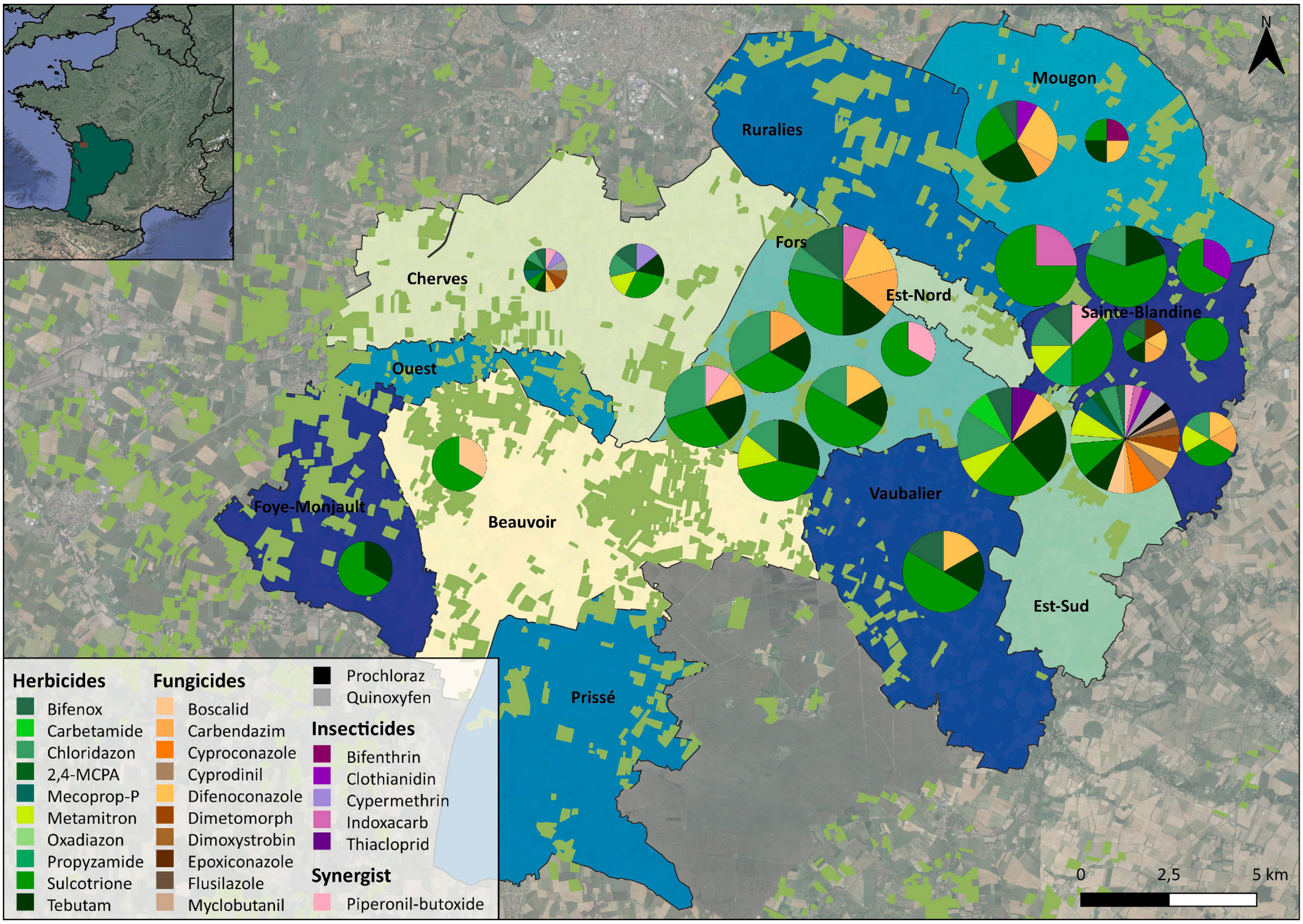

Fig. 1. Pesticide contamination of Montagu's harrier chicks from the Zone Atelier Plaine & Val de Sèvre (ZAPVS) in 2021. For each nest, a pie chart represents the proportion of each molecule detected in the chicks from the corresponding nest. Pesticide molecules are classified according to their target group: insecticides, herbicides, fungicides and synergist. The size of the pie chart reflects the brood size—that is, the larger the size of the pie chart, the higher the number of chicks were sampled—and its localisation corresponds to the approximate nest localisation across the sectors of the study area. In total, 22 nests corresponding to 55 nestlings are represented. Organic crop plots in 2021 are depicted in light green.

mainly provided by males, and females contribute more towards the end of the rearing period, usually by hunting close to the nest (Garcia and Arroyo, 2005). During the breeding season, home ranges and hunting distances vary according not only to sex and breeding phase (incubation, nestling, fledgling), but also to the study area and the year's food abundance (Wieringa et al., 2019; Krupiński et al., 2020; Berger-Geiger et al., 2022). In the study area, the home range of males is ~14 km^2 (Salamolard, 1997). To cover their home range, we considered zones within a radius of 2200 m around nests as foraging areas thus corresponding to an area of ~15 km^2.

2.2. Sampling procedure

Between May 2021 and early August 2021, professional ornithologists located and visited all nests in the study area, which were then mapped using global positioning system (GPS) coordinates. Nest coordinate data were stored on a geographical information system (GIS; QUANTUMGIS 3.16.5; QGIS Development Team, 2023; Fig. 1). The nests were visited twice before eggs hatched and every week subsequently (lag time of 7 ± 2 days between visits). During the hatching visit, chicks were head-marked (using water-based coloured pens) allowing their individual identification. Once chicks were 15 days old, they were sexed according to the colour of their iris (brown for females and grey for males; Leroux and Bretagnolle, 1996) and banded with a unique coded aluminium ring provided by the Museum National d'Histoire Naturelle de Paris (France). When nestlings were 26 ± 2 days old, they were caught, carefully handled in the shortest time possible to collect blood samples and released at their nest. For each chick, 50 μL of blood was collected by puncturing the brachial vein using a sterile needle and heparinised capillary tubes. Samples were placed in plastic microcentrifuge tubes and stored and transported in a cooler (0–5 °C) until they were brought to the laboratory, where they were stored at −20 °C for further analyses.

In total, 70 chicks from 24 nests hatched during the study period, of which 12 died before fledging and 3 fledged but were not sampled, representing a total of 55 chicks from 22 nests sampled. Of the 22 nests studied, 13 had >2 fledglings sampled, and 4 nests had up to 4 fledglings at the last visit. The ranking procedure that consisted in attributing the rank 1 to the oldest chick (i.e., the first-hatched) accounted thus for missing siblings that died before the last visit in these broods (7 chicks), using data from previous visits so that the ranking matched the hatching order. For 85 % of chicks, hatching order was determined during the hatching visit through head-marking of chicks. For the remaining 15 % of chicks, *a posteriori* determination of age was made using a morphometric estimation of age at last visit (function using wing length measure [Arroyo, 1995]; calculator available at https://busards.com/index.php/Outils/biometry). In 2021, the time between two hatching events within broods was on average 1.69 days (± standard deviation: 0.94).

No sex ratio bias was observed among the 55 chicks sampled during the study period (29 females and 26 males; binomial test $p = 0.53$) or among each rank class (binomial test at rank 1 $p = 0.50$ ($n = 20$), rank 2 $p = 0.23$ ($n = 18$), rank 3 $p = 0.06$ ($n = 14$) and rank 4 $p = 1$ ($n = 3$)).

2.3. Pesticide analysis

Pesticide extraction was conducted following the method developed by Rodrigues et al. (2023). In brief, blood samples were concentrated and subjected to multiresidue analysis using liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) and automated thermal desorption gas chromatography coupled to tandem mass spectrometry (ATD-GC–MS/MS) (see Rodrigues et al., 2023 for the complete methodology). Inter and intra-day variability in precision is taken into account through the injection of a standard at the start of each series of analyses, to check if the analytical equipment is giving the expected results. A total of 104 compounds were searched, including herbicides, fungicides, insecticides, a synergist and a safener (see Rodrigues et al., 2023 for the complete list of compounds searched). The method used for each compound detected and limits of detection (LOD) and quantification (LOQ) values are provided in Supplementary material (Table S1).

2.4. Statistical analyses

The contamination levels of chicks were determined using two metrics, the number of pesticides detected and the sum of the concentrations of pesticides in chick's blood. All analyses were performed using R v.4.2.2 software (R Core Team, 2022). Both response variables (number of pesticides and sum of concentrations) data did not meet the normality and homoscedasticity assumptions; therefore, before model implementation, sum of concentrations were log-transformed and the normal distribution fit was visually checked using the fitdist function from the "fitdistrplus" package (Delignette-Muller and Dutang, 2015). For the number of pesticides variable, the distribution with the best fit—Poisson or negative binomial distribution—was selected using the goodness-of-fit criteria from the gofstat function of the same package. The "lme4" package was used to run the linear mixed-effects model (LMM) for the sum of concentrations (log-transformed) and the generalized LMM fitted with a negative binomial distribution (NBGLMM) for the number of pesticides (Bates et al., 2015). For each model detailed hereafter, residuals were checked using the "DHARMa" package (Hartig, 2022) and compared using likelihood ratio-based χ^2-statistics.

To investigate whether the intra-brood variability in contamination levels was lower than the inter-brood variability (i.e., whether chicks from the same nests had similar contamination levels in comparison with chicks from different nests), we assessed repeatability using the intraclass correlation (ICC) coefficient, which is the ratio of between-groups variance to total variance (the sum of between-groups and within-groups variance) (Nakagawa and Schielzeth, 2010; Nakagawa et al., 2017; Carrasco, 2022a), the group being the nest identity in our case. Thus, the value of this metric lies between 0 and 1: if it equals 1, the total variance is attributable to inter-brood variability, and if it is 0, the total variance is attributable to intra-brood variability. Contamination level repeatability of chicks within nests was obtained using the rpt function from the "rptR" package v.0.9.22 (Stoffel et al., 2017), with the log-transformed sum of concentrations as the outcome and the nest identity as grouping factor (the random factor of the LMM) and using the icc_counts function from the "iccCounts" package v.1.1.1 (Carrasco, 2022b), with the number of molecules as outcome, the nest identity as subjects (the random factor of the GLMM), and negative binomial distribution family.

To test the effect of organic farming in local foraging areas on the level of contamination of chicks, the log-transformed sum of concentrations was used as response variable in an LMM using the percentage of organic farming (log-transformed) as a fixed-effect and similarly for the number of pesticides detected, used as response variable in an NBGLMM. To avoid pseudo-replication, nest identity was included as a random effect (as chicks can belong to the same nest) (Bolker et al., 2009). As we wanted to investigate the effect of organic farming on the level of contamination of chicks at a local scale, we tested the percentage of organic farming around the nests for 20 buffer sizes corresponding to radii ranging from 100 to 2200 m, with an increment of 100 m, as described in Fuentes et al. (2023b). Thus, we ran 20 LMMs and 20 NBGLMMs (one per buffer size) and used the Akaike Information Criterion corrected for small sample size (AICc from "AICcmodavg" package, Mazerolle, 2023) to select the best model (i.e., having the lowest AICc), assuming that models with a difference in AICc <2 are similarly supported and cannot be distinguished from each other (Burnham and Anderson, 2004). The direction and significance of the effect of organic farming was determined using effect size (estimate) and its bootstrapped 95 % confidence interval (CI; see Fuentes et al., 2023b for further details). The factors influencing the intra-brood variability in contamination levels were assessed using the total pesticide concentrations (log-transformed) and the number of pesticides detected as the response variable in LMM and NBGLMM, respectively, and chick sex and hatching order and their interaction as fixed effects, including nest identity as a random effect.

3. Results

3.1. Pesticide load overview and variability

A total of 28 pesticides of the 104 searched were detected above the LOD in Montagu's harrier chicks: 10 herbicides, 12 fungicides, 5 insecticides and 1 synergist (see Supplementary material Table S2), among which 26 were quantified (i.e., above LOQ). The geographical distribution among the nests is depicted in Fig. 1. All chicks sampled ($n = 55$) were found to be contaminated with at least one pesticide, and the maximum number of pesticides detected per chick was 16 (mean ± standard deviation = 3.2 ± 3.1; Table 1). Total concentrations ranged from 24.41 to 7265.52 pg.mg^{-1} (1538.00 ± 1279.40 pg.mg^{-1}; Table 2). The relationship between the number of pesticides and the sum of concentrations is depicted in Fig. 2, the two metrics were not correlated to each other (Spearman's rank correlation test, $\rho > 0.21$, 95 % CI = [−0.07; 0.48], $n = 55$) even when considering females ($\rho > 0.26$, 95 % CI = [−0.19; 0.64], $n = 29$) and males ($\rho > 0.17$, 95 % CI = [−0.07; 0.48], $n = 26$) separately.

The estimated repeatability for the number of compounds (ICC mean ± standard error = 0.32 ± 0.16, 95 % CI = [−0.02; 0.60]) and the log-transformed sum of concentrations ($R = 0.08 \pm 0.12$, 95 % CI = [0; 0.40]) were low and not significant, indicating that there was no statistical difference of within and between nest variation (see Supplementary material Figs. S1 and S2).

3.2. Effects of organic farming

None of the buffer sizes was appropriate to analyse the effect of organic farming (log-transformed) on the total concentrations (log-transformed) as almost all models had a difference of AICc <2 (Fig. S3). The model with a buffer size of 300 m was best supported for the effect of the organic farming on the number of molecules, as it had the lowest AICc and no other model had an AICc difference < 2 (Fig. S4). In a zone with a radius of 300 m around the nests, a higher percentage of organic farming (log-transformed) significantly reduced the number of pesticides detected in chick's blood (effect size = −0.38; 95 % CI = [−0.66; −0.13]; Tables 3 and S3; Fig. 3). A higher proportion of organic farming around nests significantly decreased the number of pesticides in Montagu's harrier chicks both at the scale of the crop plot (300–500 m) and at a larger scale (1100–2200 m, see Fig. S4 in Supplementary material).

3.3. Effects of the sex and rank of chicks

The summary statistics of sex and rank factors are provided in Table 1 for the number of pesticides detected and in Table 2 for total pesticide concentrations. Rank 4 female chicks showed the highest number of pesticides detected with an average of 7.5 ± 9.2 compounds ($n = 2$), whereas rank 1 males showed the lowest mean with 2.5 ± 1.8 compounds ($n = 8$). Similarly for total pesticide concentrations, females of fourth rank had the highest average with 3876.52 ± 4792.75 pg.mg^{-1} and males of rank 2 the lowest with 896.58 ± 635.15 pg.mg^{-1} ($n = 6$). Although a tendency of increased contamination levels was observed for last-hatched chicks (Figs. 4b, 5b), there was no significant effect of rank neither on the number of pesticides detected nor on the log-transformed sum of concentrations (Table 3 and Tables S3, S4). Neither sex nor its interaction with the rank influenced the contamination levels of chicks (Table 3 and Tables S3, S4; Figs. 4 and 5).

4. Discussion

In a previous study, we investigated the potential of Montagu's harrier chicks for the biomonitoring of non-persistent pesticides, describing contamination patterns and the routes of exposure (Fuentes et al., 2024) while the present study aimed at characterizing the variation of pesticide contamination level of Montagu's harrier chicks among and within nests, and investigating the link with the composition of the surroundings in organic crops and with chicks' sex and rank. Thus, for further details concerning the description of compounds detected refer to Tables S1 and S2 in Supplementary material.

4.1. Pesticide load overview and variability

Pesticide loads in Montagu's harrier chicks were variable, some chicks being strongly contaminated (up to 16 pesticides and 7265.51 pg.mg^{-1}) and others having low contamination levels (1 compound and 24.41 pg.mg^{-1}). However, our finding concerning the intra-brood vs. inter-brood variability was unexpected as the intra-brood variability was similar to the inter-brood variability in pesticide load (Nakagawa and Schielzeth, 2010). In other words, chicks from the same nest did not differ in their contamination levels from chicks of other nests. This result contrasts with findings in the Squacco heron (*Ardeola ralloides*) where the variability in mercury levels in chicks' feathers was mostly attributable to inter-brood differences rather than intra-brood differences (Goutner et al., 2001). Subsequently, some studies suggested that sampling a random egg from a clutch would provide an appropriate estimation of the contamination of the whole clutch (Bustnes et al., 2015; Power et al., 2021). However, in the present study, it appears that sampling blood from a randomly chosen chick would have yielded an imprecise estimation of the nest's contamination. For instance, the number of compounds detected in blood samples from four siblings from the same nest was 6, 5, 2, and 1, and total concentrations were 2113.18, 426.06, 1081.94, and 1364.10 pg.mg^{-1}, respectively; thus, random sampling would have led to an overestimation or underestimation of the nest's contamination level. In that respect, caution should be exercised when choosing a sampling strategy (random or systematic) in

Table 1
Summary of the number of pesticides detected in Montagu's harrier chicks sampled in 2021. Ranks of chicks correspond to the hatching order in clutches (R1 being the first-hatched chicks, i.e., the oldest). Sample size (n), range (min–max), mean, and standard deviation (SD) are given for each sex and rank class.

	R1	R2	R3	R4	Total
Males					
n	8	6	11	1	26
Range	1–6	1–5	1–16	–	1–16
mean ± SD	2.5 ± 1.8	2.8 ± 1.7	3.6 ± 4.2	6.0	3.19 ± 3.06
Females					
n	12	12	3	2	29
Range	1–12	1–4	1–6	1–14	1–14
mean ± SD	3.2 ± 3.3	2.7 ± 1.1	3.0 ± 2.6	7.5 ± 9.2	3.24 ± 3.11
Total					
n	20	18	14	3	55
Range	1–12	1–5	1–16	1–14	1–16
mean ± SD	2.90 ± 2.77	2.72 ± 1.27	3.50 ± 3.88	7.00 ± 6.56	3.22 ± 3.10

Table 2
Summary of the sum of concentrations of pesticides in Montagu's harrier chicks sampled in 2021. Ranks of chicks correspond to the hatching order in clutches (R1 being the first-hatched chicks, i.e., the oldest). Sample size (n), range (min–max), mean, and standard deviation (SD) are given for each sex and rank class.

	R1	R2	R3	R4	Total
Males					
n	8	6	11	1	26
Range	734.75–3184.67	24.41–1641.10	583.33–4565.70	–	24.41–4565.70
mean ± SD	1323.74 ± 804.90	896.58 ± 635.15	1498.04 ± 1074.64	2113.18	1329.27 ± 897.82
Females					
n	12	12	3	2	29
Range	426.05–5501.34	634.65–4337.48	606.30–1144.86	487.53–7265.51	426.05–7265.51
mean ± SD	1788.35 ± 1334.14	1504.84 ± 1024.65	919.22 ± 279.69	3876.52 ± 4792.75	1725.14 ± 1536.36
Total					
n	20	18	14	3	55
Range	426.05–5501.34	24.41–4337.48	583.33–4565.70	487.53–7265.51	24.41–7265.51
mean ± SD	1602.51 ± 1150.52	1302.09 ± 940.78	1374.01 ± 980.37	3288.74 ± 3538.60	1538.00 ± 1279.40

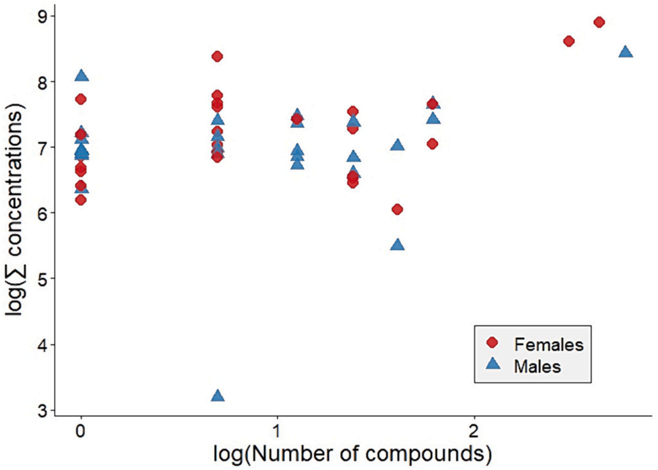

Fig. 2. Relationship between the number of pesticides detected and the sum of concentrations of pesticides in the blood of Montagu's harrier chicks. Both metrics were log-transformed for better visualization. Red dots represent females (n = 29) and blue triangles stand for males (n = 26).

Table 3
Summary of the effects of organic farming (in a zone with a radius of 300 m around the nest) on the number of pesticides detected (Nb. pesticides) and effects of sex and rank of Montagu's harrier chicks on Nb. pesticides and on the log-transformed sum of concentrations [log(∑ concentrations)], analysed using linear mixed-effects model (LMM) and generalized LMM fitted with a negative binomial distribution. Chi-squared statistics χ^2, df = degrees of freedom, and p = p-value, are given for each model. Estimates, standard errors, statistic values, and significance of each effect are provided in Supplementary material, Table S3.

Parameters	χ^2	df	p
Nb. pesticides			
% Organic farming	8.91	1	<0.01
Nb. pesticides			
Sex	0.00	1	0.98
Rank	4.77	3	0.19
Sex × Rank	2.36	3	0.50
log(∑ concentrations)			
Sex	1.99	1	0.16
Rank	3.75	3	0.29
Sex × Rank	4.48	3	0.21

biomonitoring schemes, as using an unsuitable method may lead to erroneous conclusions about the contamination levels.

4.2. Effects of organic farming

Despite the absence of effect of organic farming on the total concentrations of pesticides in chick's blood, we have showed that a higher proportion of organic farming around nests decreased the number of pesticides both at the field scale (300–500 m) and at a larger scale (1100–2200 m). This result is consistent with expectations; as organic farming practices exclude the use of synthetic pesticides, increasing the number of organic farming crop plots correspondingly reduces the pesticides found in the environment. In fact, in the present study, we detected some pesticides common to those reported in a recent study sampling small mammals in the study area (Fritsch et al., 2022), which is consistent with a dietary exposure route (17 out of the 28 compounds found in the present study; for more details see Fuentes et al., 2024). The lower number of compounds found in chicks from nests surrounded by higher proportions of organic farming at the field and larger scales, suggests that not only the direct environment of nests (soil and vegetation) is less contaminated but also that the prey hunted by parents in the close vicinity and brought to chicks is less contaminated. One way to properly investigate this hypothesis would be to quantify contamination levels in food pellets collected at nests. Another important habitat features in the nests' surroundings to consider would be hedgerows, as these semi-natural elements are known to increase small mammal abundance (Gelling et al., 2007) and to act as a natural barrier to pesticide drift (Langenbach et al., 2022). Thus, one may expect that higher proportions of hedgerows around nests may decrease the contamination levels linked to the reduction of pesticide drift.

Although chicks from a nest surrounded by a higher proportion of organic farms had less pesticides in their blood, the variability in pesticide contamination among nests was similar to that within nests (see previous section). In blackbirds (*Turdus merula*), habitat-related differences in metal contamination levels were attributable to both environmental/dietary contamination levels and foraging behaviours of parents (Fritsch et al., 2012). Montagu's harrier females generally bring small prey items, especially insects, to nestlings by the end of the rearing period, whereas males keep bringing most of the prey—generally voles—and hunt kilometres away from their nests, selecting their foraging area according to prey availability (Garcia and Arroyo, 2005; Krupiński et al., 2020). Thus, the chicks' diet includes prey not only from local foraging areas (≤2200 m) but also from larger distances. Moreover, dietary composition has been suggested to be the main driver of contaminant transfer, as some food taxa exhibit higher levels of contaminants but also differences in quality (i.e., levels of essential elements or proteins), which affects the uptake of contaminants during digestion and/or their metabolization (Fritsch et al., 2012). Insects and voles differ in their nutritional composition as voles are energy rich, whereas insects are richer in carotenoids, an antioxidant provided only by diet (Sternalski et al., 2010). As these antioxidants are involved in the detoxification processes, a higher uptake of insects, thus of antioxidants, may lead to a higher detoxification of pesticide compounds, therefore reducing the number and concentration of pesticides (Møller et al.,

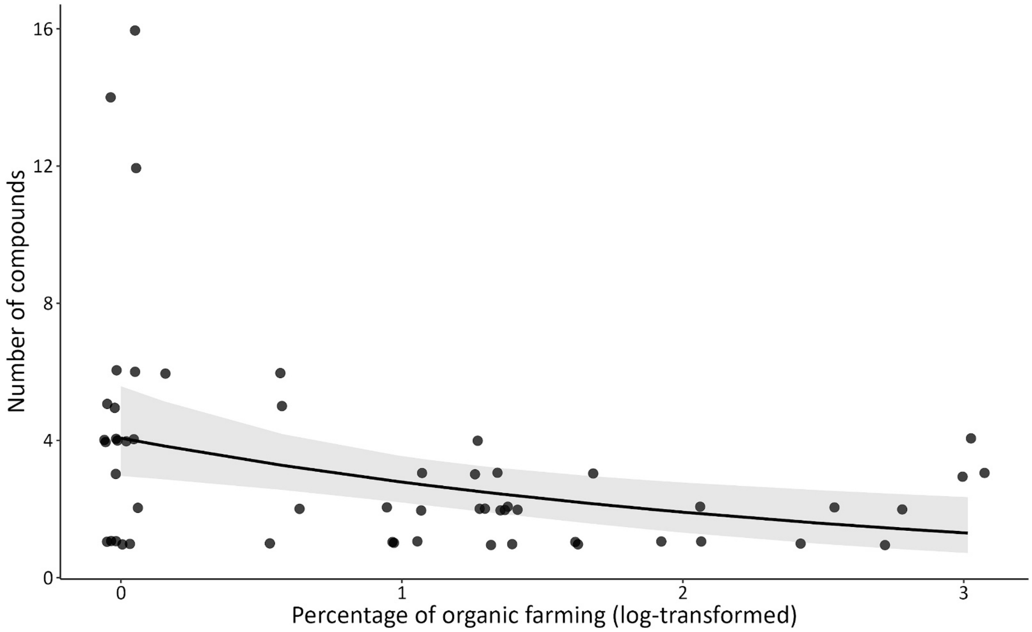

Fig. 3. Effect of organic farming (in a zone with a radius of 300 m around nests) on the number of pesticide compounds detected in Montagu's harrier chicks. The percentage of organic farming was log-transformed prior to model implementation. Dots were plotted with a small degree of random variation to the location of each point so that most chicks' values can be visualized. The line and the shading represent the predicted values and 95 % confidence interval from the generalized linear mixed-effects model fitted with a negative binomial distribution.

2000). Consequently, prey types may add contamination variability among broods depending on parents' hunting strategies and within the brood depending on which chick monopolizes the prey.

4.3. *Effects of the sex and rank of chicks*

In Montagu's harrier, females—being the larger sex—have an advantage over males in competition for larger food items within the brood (Arroyo, 2002). However, contrary to expectations, sex did not influence the number or the total concentrations of pesticides. Similarly for chick rank, we did not find an effect on contamination levels even in interaction with chick sex. However, our sample size for rank 4 chicks was limited to 3, mainly owing to the mortality of the latest hatched chicks, especially in years of low vole abundance, such as 2021 (V.B., unpublished data). Thus, our model lacks statistical power, which may explain why chick's rank did not have a significant effect despite the tendency observed. In fact, only half of the nests had more than two chicks at fledging (13 of 22 nests), whereas most had more than two laid eggs (20 of 22 nests), which indicates that the later-laid eggs and/or later-hatched chicks did not survive until sampling. For instance, in one of the nests monitored, only the first-hatched chick of the 5 laid eggs survived up to fledging. Smaller Montagu's harrier males that hatch later may have a higher probability to die from starvation (Arroyo, 2002). During the 2021 breeding season, low amounts of food resources were available, as reflected by the lower reproductive success in 2021 than in other years monitored in the study area (see Fuentes et al., 2023b for an overview of brood sizes in previous years). Thus, we cannot exclude the possibility that males that hatched later died before being sampled. Another explanation for the higher likelihood of death in younger chicks would be their pesticide load, assuming that there is a tendency of higher contamination levels in the last ranks. Indeed, the teratogenic effects of pesticides have been poorly investigated in wild birds, but some studies indicate that they reduce clutch sizes and increase chick mortality (Hernández et al., 2008; Lopez-Antia et al., 2015; Ortiz-Santaliestra et al., 2020). Thus, a contamination bias towards last eggs/younger chicks would lead to their hatching failure/premature death. In the present study, molecules showing teratogenic effects *in natura* were not tested for (organochlorines and polychlorinated biphenyls, Hernández et al., 2008; thiram, Lopez-Antia et al., 2015) or detected (tebuconazole and 2.4-D, Ortiz-Santaliestra et al., 2020) in fledglings. However, future studies should sample and analyse the pesticide content of unhatched eggs and younger chicks. Moreover, pesticides can lead to endocrine disruption with diverse consequences depending on the sex and on the cocktail of molecules involved such that males and females may differ in their sensitivity to pesticide accumulation (see Moreau et al., 2022 for review). An early death of males that hatched later in a brood may thus be attributable to both poor food conditions, their low ability to compete for food, and a potentially higher contamination load. If so, there should be a difference in the number of later-hatched females and males, biased towards females; however, we did not find a sex ratio bias in any of the rank classes. This contradicts an increased death rate of later-hatched males. However, differences in sex distribution among ranks should be properly investigated using, for example, DNA sexing of unhatched eggs and hatchlings because chicks cannot be visually sexed before they are 15 days old. Together with the quantification of pesticide loads of unhatched eggs, this would also constitute a promising approach to investigate teratogenic effects in farmland birds. An alternative explanation for the lack of effect of sex and rank is linked to the dominant–subordinate interactions among siblings. In fact, brood competition for food items may lead to unequal ingestion of pesticides among chicks belonging to the same nest. Therefore, we hypothesize that the most vigorous and aggressive chick, irrespective of its rank or sex, is the one monopolizing more and larger prey and thus displays the higher pesticide load. The role of brood competition in this context can be investigated through the examination of chick behaviour, either directly from video recordings or from behavioural metrics (e.g., the rate of beak attacks) taken during chicks' handling.

4.4. *Limits and prospects*

To our knowledge, this is the first study to report a high variability in the level of pesticide exposure among different chicks from the same nest. High intra-brood variability in contamination levels have been

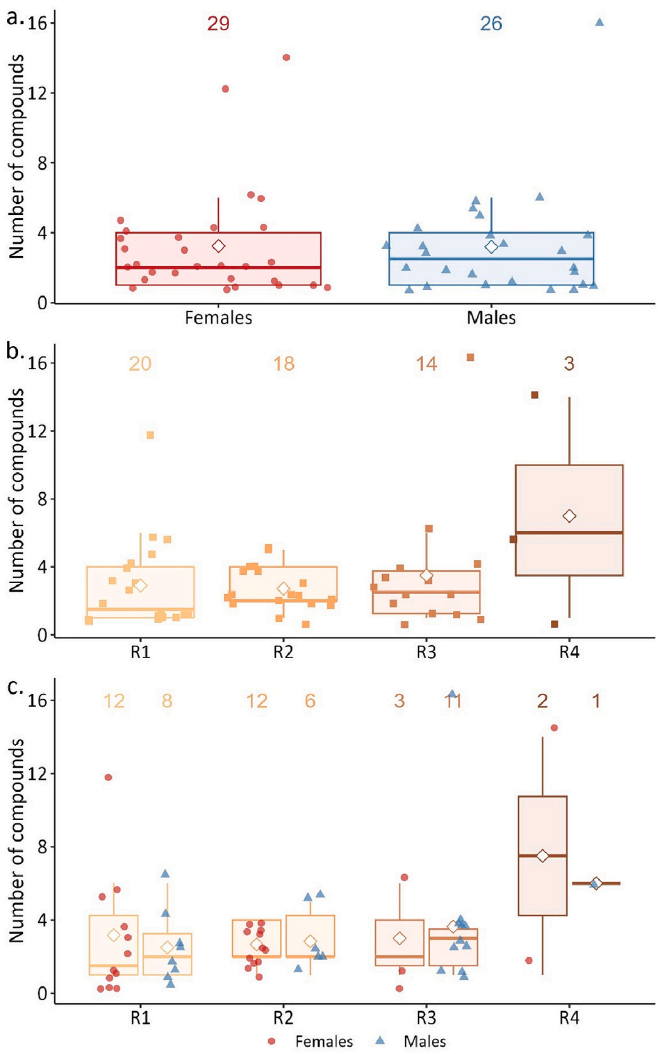

Fig. 4. Number of pesticides detected in Montagu's harrier chicks according to their sex (a), rank (b), and their interaction (c). The bottom and top lines of the boxes correspond to the first and third quartiles, middle line corresponds to the median, and whiskers correspond to the lower and higher values included in the 95 % confidence interval. All values were plotted with a small degree of random variation to the location of each point; therefore, they do not correspond to a round number. Blue triangles stand for males and red circles for females. Blank diamonds correspond to the mean; sample sizes are specified above the boxes.

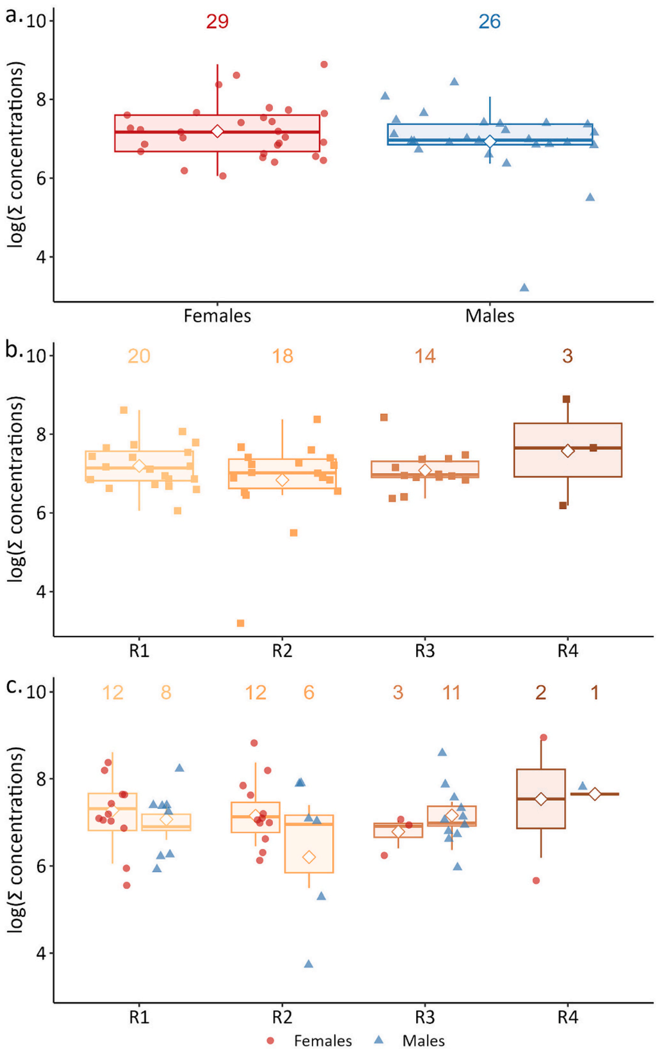

Fig. 5. Sum of concentrations of pesticides (log-transformed) in Montagu's harrier chicks according to their sex (a), rank (b), and their interaction (c). For legend details, see Fig. 4.

previously reported for heavy metals with contrasting results depending on the species and the contaminant studied (Janssens et al., 2002; Hofer et al., 2010). In addition, these investigations generally focused on eggs or feathers as biological matrices. Distribution of contaminants in biological tissues depends on their chemical properties and affinity with the tissue; thus, detection levels from different matrices are difficult to compare (Espín et al., 2016). Considering the present findings, biomonitoring schemes should carefully consider the model species, the contaminants involved, and the matrix sampled, to avoid misleading calculations of contamination levels. Moreover, it should be noted that the contamination levels reported here depend on the detection limits, which are method-dependent. Analytical methods differ in their sensitivity—a lower LOD would have increased the probability of detection, likely leading to a higher number of compounds detected. This should be considered whenever comparing contamination level variability among studies.

In the present study, we did not find the same pattern for the effect of organic farming on the two measures of contamination levels of chicks. Whereas the negative relationship between the proportion of organic farming and the number of compounds was clear, the link between organic farming and total concentrations was not statistically supported. This mismatch might arise from the fact that some nestlings with few pesticides had them in large concentrations. Moreover, high total concentrations do not necessarily indicate high toxicity to organisms as this will depend on the relative toxicity of each compound and on the presence of other compounds with potentially synergistic or antagonistic effects (Hernández et al., 2017). As no alternative to consider the complexity of such effects is available to date, the use of concentration addition is still widely applied, but results should be considered with caution.

5. Conclusions

Because the Montagu's harrier is at the top of the trophic chain and a specialist predator species of agricultural lands, studying its contamination with pesticides is particularly relevant as an indicator of larger contamination of the environment (*One Health* concept). The present study reveals that organic farming reduces the number of pesticides in Montagu's harrier chicks, which may have a beneficial effect on its population, as chemical inputs have been shown to drive farmland bird population decline across Europe (Rigal et al., 2023). Nevertheless, future work should investigate how pesticide cocktails are linked to the life-history traits of birds and affect population dynamics. Deciphering the effects of pesticides on birds' health status could potentially clarify the underlying mechanisms linking pesticide inputs and decline of farmland birds.

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CRediT authorship contribution statement

Elva Fuentes: Writing – original draft, Formal analysis, Conceptualization. **Jérôme Moreau:** Writing – review & editing, Supervision, Conceptualization. **Maurice Millet:** Writing – review & editing, Formal analysis. **Vincent Bretagnolle:** Writing – review & editing, Conceptualization. **Karine Monceau:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.172778.

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