Received: 25 September 2025 | Accepted: 20 April 2026

DOI: 10.1111/een.70096

ORIGINAL ARTICLE

Ecological Entomology

Nutritional composition of pollen stores in managed bees across European agro-ecosystems reveals species-specific differences but limited pesticide effects

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Funding information
Fonds De La Recherche Scientifique - FNRS; Horizon 2020 Framework Programme, Grant/Award Number: 773921

Associate Editor: James Gilbert

Abstract

1. Nutritional ecology is a key determinant of bee health, yet species-specific preferences and how bees respond nutritionally to real-world pesticide exposure remain unclear.
2. We collected pollen stores from three managed bee species in 128 sites across two widespread agro-ecosystems (i.e., oilseed rape fields and apple orchards) in eight European countries. We measured protein content, lipid content, protein-to-lipid ratio as well as pesticide residues in pollen stores.
3. We provide the first evidence of species-specific macronutritional patterns in the pollen stores of three managed bee species. The buff-tailed bumble bee stored pollen with significantly lower lipid content (mean: 44.1 μg/mg) than the European honey bee (mean: 57.2 μg/mg) and the red mason bee (mean: 54 μg/mg). This reduced lipid content translated into a higher protein-to-lipid (P:L) ratio in the buff-tailed bumble bee (mean: 8.53) when compared to the European honey bee (mean: 5.85) and the red mason bee (mean: 5.60).

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4. Pesticide risk, measured as toxicity-weighted concentrations in pollen stores, did not influence P:L ratios in any species. However, increasing pesticide risk was associated with reduced protein content and lipid content in buff-tailed bumble bee stores, potentially leading to suboptimal colony development, whereas it was associated with increased protein content in red mason bee stores.
5. Our findings suggest that bees maintain consistent macronutritional profiles in their pollen stores under pesticide exposure, at least in terms of P:L ratios, suggesting limited capacity to adapt their macronutritional dietary choices to mitigate agrochemical stress. These findings highlight a critical need to balance floral enhancements with a reduction in pesticide use to safeguard pollinators in agricultural landscapes.

KEYWORDS
agrochemical, *Apis mellifera*, *Bombus terrestris*, ecotoxicology, foraging, *Osmia bicornis*, plasticity

INTRODUCTION

Adequate nutrient intake is fundamental to the survival and reproductive success of heterotrophic organisms. However, anthropogenic disturbances increasingly challenge access of organisms to nutritionally optimal diets (Birnie-Gauvin et al., 2017). In agro-ecosystems, intensive land-use resulting in habitat fragmentation and landscape homogenisation significantly limits the availability and diversity of suitable food resources. This concern is particularly significant for pollinators such as bees (Lau et al., 2023). In addition, exposure to pesticides has been shown to impair cognitive functions in bees (Siviter et al., 2018), further diminishing their foraging efficiency and ability to locate and exploit floral resources (O'Reilly & Stanley, 2023; Stanley et al., 2016). Recently, field exposure to pesticides has been linked to reduced colony development in bumble bees, yet it remains unclear whether these effects are mediated by disruptions in optimal foraging behaviour (Nicholson et al., 2024).

Bees are known to selectively forage on pollen sources in a non-random manner to meet their specific nutritional needs (Leonhardt & Blüthgen, 2012; Ruedenauer et al., 2016; Wood et al., 2021). Consequently, experiments conducted under controlled laboratory conditions have shown that both solitary and social bee species exhibit delayed development when restricted to nutritionally inadequate pollen diets (Lawson et al., 2020; Schwarz et al., 2024; Vanderplanck et al., 2018). In recent years, the protein-to-lipid ratio (P:L) of pollen has been proposed as a meaningful metric to evaluate the macronutritional needs of bees (Vaudo et al., 2020). Under laboratory conditions, the diet of bees follows species-specific P:L ratios. The European honey bee (*Apis mellifera*), the common Eastern bumble bee (*Bombus impatiens*), and the Japanese orchard bee (*Osmia cornifrons*) have been shown to regulate their diets toward specific P:L ratios of approximately 1:1, 12:1, and 3:1, respectively (Crone et al., 2023; Stabler et al., 2021; Vaudo et al., 2016). Although laboratory data are increasing, field data remain scarce (Vaudo et al., 2020, 2024), and the macronutritional needs of bees under natural conditions are virtually unexplored. Such field-based data are crucial for a better understanding of the dietary needs and associated health consequences of both managed and wild bee populations in agro-ecosystems, as mass-flowering crops may fail to meet species-specific nutritional needs, whereas semi-natural floral resources could be essential to support adequate diets (Ammann et al., 2024; Tourbez et al., 2025).

Bees are known to shift their nutritional patterns in response to stressors, as illustrated by self-medicative behaviours in which bees preferentially collect specialised metabolites that mitigate parasite infection (Baracchi et al., 2015; Gherman et al., 2014). Similarly, pollen diets have been shown to influence bee resilience to pesticide exposure (Barascou et al., 2021; Knauer et al., 2022; Wintermantel et al., 2022). For instance, when exposed to the insecticide chlorpyrifos, European honey bees exhibited higher survival rates when consuming pollen with a lower P:L ratio compared to those fed on pollen with higher P:L ratios (Crone & Grozinger, 2021). Likewise, honey bees exposed to the acaricide spirodiclofen collected more lipid-rich pollen, likely in response to pesticide-induced lipid depletion (Deeter et al., 2023). These findings suggest that bees may dynamically adjust the P:L ratio of their pollen provisions to alleviate the toxic effects caused by agrochemicals, although the occurrence of such behaviours has never been investigated. Similarly, while pesticide exposure is known to impair foraging behaviour and reduce the ability of bees to collect food efficiently (O'Reilly & Stanley, 2023; Stanley et al., 2016), it remains unclear whether pesticide exposure hinders the bees' ability to access or select optimal P:L ratios. This knowledge gap is particularly concerning given the widespread promotion of floral enhancements to support bee populations in agro-ecosystems, despite little understanding of how bees actively modulate their macronutritional foraging choices in response to pesticide exposure (Rundlöf et al., 2022).

We deployed managed bee species, namely the European honey bee (*Apis mellifera*), the buff-tailed bumble bee (*Bombus terrestris*) and the red mason bee (*Osmia bicornis*), across 128 sites situated in two mass-flowering entomophilous crops in bloom, namely oilseed rape fields and apple orchards, spanning eight European countries (Hodge et al., 2022) (Figure 1). We collected samples of stored pollen from all three bee species and analysed their protein and lipid concentrations to calculate P:L ratios. Given that species-specific macronutritional needs remain poorly

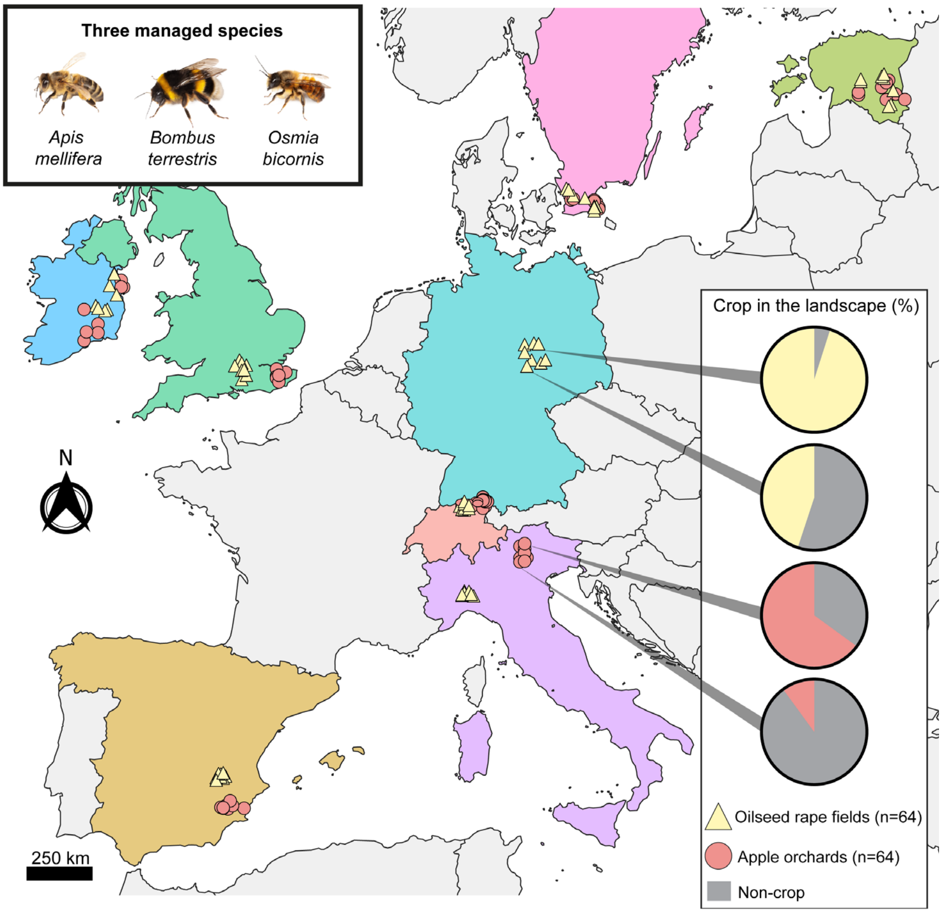

FIGURE 1 Map depicting the three managed bee species deployed in 128 agro-ecosystems (oilseed rape crops and apple orchards) in eight European countries, with eight sites per crop per country. Sites were chosen along a gradient of cropland proportion in the landscape. The pie charts shown for four selected sites are illustrative examples only and do not represent all sites along the gradient.

understood under field conditions, we first examined whether the protein content, lipid content and P:L ratios of pollen stores differed among the three bee species. Because the influence of pesticide exposure on bees' nutritional resilience is largely unknown, we further investigated whether the macronutritional composition of pollen stores varied with the pesticide risk (i.e., summed toxicity-weighted pesticide concentrations) measured in the pollen stores of each species. We hypothesise that (i) pollen stores would exhibit species-specific macronutritional profiles reflecting differences in nutritional preferences (i.e., fundamental ecological question) and that (ii) these macronutritional profiles would vary with pesticide risk, consistent with potential alterations in foraging behaviour or potential nutritional plasticity to better face chemical stress (i.e., applied ecotoxicological question).

METHODS

Biological model and implementation of field experiments

We considered three generalist bee species, namely the European honey bee *Apis mellifera* L., the buff-tailed bumble bee *Bombus terrestris* L. and the red mason bee *Osmia bicornis* L. (Michez et al., 2019). We considered eight European countries, namely Estonia, Germany, Ireland, Italy, the United Kingdom (UK), Spain, Sweden and Switzerland. In each country, we selected eight apple orchards (*Malus domestica* Borkh.) and eight oilseed rape fields (*Brassica napus* L.) along a gradient in the proportion of cropland (Appendix S1) within a 1-km radius (total number of sites across Europe = 128) with sites at least 3 km apart (Hodge et al., 2022). At each site, three locally sourced European honey bee hives, three commercially produced 'Standard' buff-tailed bumble bee colonies (*B. terrestris terrestris* for continental Europe and *B. t. audax* for Ireland and the UK; produced by Biobest Group, Westerlo, Belgium; with about 80 workers each) and three red mason bee trap nests, each seeded with about 100 commercially produced cocoons, equally divided between male and female cocoons (produced by Wildbiene & Partner AG, Zurich, Switzerland) were placed along a linear field boundary for around 6 weeks. All hives, colonies and nests were placed at least five meters apart. The European honey bee hives were placed from 1 to 2 weeks before the crop flowering started, while the buff-tailed bumble bee colonies and red mason bee nests were placed from 3 to 7 days before the crop flowering started. Note that red mason bee nests were not deployed in Ireland and the UK since this species is not naturally found in these countries. Bee subspecies used in each country are listed in Appendix S2.

Pollen store sampling

In each hive, colony and nest, ~5 g of provisions from pollen stores (i.e., beebread for honey bees) were sampled using a stainless steel spatula. For red mason bees, ~10 tubes were sampled. For European honey bees and buff-tailed bumble bees, sampling occurred once toward the end of the flowering period, while for mason bees sampling occurred once at the peak of crop bloom activity. Although pollen composition may vary over time due to processes such as bacterial fermentation, pollen was collected once at each site for practical reasons. We are however confident that the validity of our results was not affected, as pollen collection occurred during or right after the blooming period. Moreover, the broad spatial scale of the study, encompassing 128 sites distributed across multiple biogeographical regions with distinct plant communities (Hodge et al., 2022), captures a degree of variability comparable to seasonal fluctuations. This spatial heterogeneity therefore mitigates potential biases associated with single-time-point sampling and supports the interpretation that observed patterns in pollen composition reflect consistent ecological differences. Samples were pooled per bee species per site and homogenised, and each sample was divided into three sub-samples for palynological, nutritional content and pesticide residue analyses. Sub-samples were stored at −20°C, and pesticide residues were directly analysed (see Section 2.5). Other sub-samples were sent on dry ice for palynological analyses (see Section 2.3) and for nutritional content analyses (see Section 2.4).

Palynological analyses

Palynological analyses were performed according to the standard method described in Von Der Ohe et al. (2004). From each pollen sub-sample, 1 g of the homogenised pollen was collected using a stainless-steel spatula and then dissolved in 20 mL of distilled water. A drop was spread on a slide covering an area of about 18 mm × 18 mm. The slides were dried, and the pollen was fixed using glycerine jelly. Pollen grains were identified under a microscope (magnification 400–1000×), and the abundance of each morphotype was quantified by counting the pollen grains along equidistant parallel lines until 500 pollen grains per slide were identified. Recognition of pollen type was based on comparing the observed pollen forms with those present in the CREA collection of reference slides (a database with more than 1000 thermophilous species developed using anthers of identified plant species). These palynological data, previously explored in Tourbez et al. (2025), were used here to measure the proportion of crop pollen in pollen stores and to test for collinearity with pesticide risk (see Section 2.7).

Nutritional content analyses

Protein concentration in pollen stores was evaluated using a Bradford assay following Vaudo et al. (2020). Briefly, ~1 mg of pollen was mixed with 1.5 mL of 0.1 M NaOH. We conducted the Bradford assay with the Bio-Rad Protein Assay Kit microassay 300 μL microplate protocol using bovine γ-globulin as the protein standard (Bio-Rad Laboratories, Inc., Hercules, CA). We used three technical replicates for each biological replicate and measured absorbance at 595 nm using a SpectraMax 190 spectrophotometer (Molecular Devices, LLC, Sunnyvale, CA). Protein concentrations were calculated using linear regression analysis from the protein standards.

Lipid concentration in pollen stores was evaluated using a Folch assay following Van Handel and Day (1988). Briefly, in 2-mL microcentrifuge tubes, we added 200 μL 2% sodium sulfate and 1.6 mL chloroform/methanol to ~1 mg of each pollen sample before a 5 min centrifugation. Supernatant was transferred to a clean glass tube with 600 μL deionised water and centrifuged for 5 min. We separated the top carbohydrate/water/methanol fraction, and the remaining chloroform fraction was used for lipid analysis. The lipid/chloroform fraction was left overnight in a fume hood to fully evaporate the solvent. We added 200 μL sulfuric acid to the sample and heated it at 100°C for 10 min. Then, 5 mL of vanillin/phosphoric acid reagent was added. We used three 300 μL technical replicates for each biological replicate and measured absorbance at 525 nm using a VersaMax microplate spectrophotometer (Molecular Devices, LLC, Sunnyvale, CA). Lipid concentrations were calculated using linear regression analysis from vegetable oil standards.

Pesticide residue analyses

We used 0.3 g of homogenised pollen store samples for the analysis of 267 compounds, including isomers and metabolites, according to the methodology that has undergone validation following the SANTE/12682/2019 guidelines and is accredited in compliance with the ISO 17025 standard (Kiljanek et al., 2021). In the initial step, a sample underwent extraction with a 1 mL solution containing 5% formic acid in acetonitrile, followed by the addition of ammonium formate salt. The resulting extract underwent purification through freezing and a two-step dispersive solid phase extraction using Supel QuE Verde sorbents. Following the first step, dispersive solid phase extraction (dSPE), a segment of the extract underwent analysis via a liquid chromatography tandem mass spectrometry system (Agilent 1260 HPLC coupled with an AB Sciex QTRAP 6500 mass spectrometer) targeting 200 pesticide residues. The remaining extract underwent a second step dSPE clean-up using another Supel QuE Verde. Subsequently, after concentration and solvent exchange, this extract was analysed via gas chromatography tandem mass spectrometry (Agilent GC 7890 A+ coupled with a 7000B mass spectrometer) targeting an additional 61 pesticides and 6 ndl-PCB residues. The calibration process utilised procedural standard calibration, with reagent blanks and blank samples included in each analytical batch. Recovery checks were conducted in each batch using samples spiked with pesticides at the limit of quantification (LOQ) levels, ensuring compliance with SANTE/12682/2019 criteria. All values lower than the LOQ were recorded as zero.

Pesticide risk calculations

We use toxicity-weighted concentrations (TWC) as a basis for indicating pesticide risk for bees (Knapp et al., 2023; Nicholson et al., 2024), where the TWC of each compound ($\mathrm{TWC_i}$) is the ratio between the concentration (μg kg^{-1}; c_i) of a detected compound in bee pollen stores and its respective acute toxicity endpoint ($\mathrm{LD_{50i}}$, the median lethal dose). Then, following a concentration addition approach, the recommended default for mixture environmental risk assessment (Bopp et al., 2018), we summed the TWCs to calculate the additive toxicity-weighted concentration of all compounds within a sample per site and bee species (TWC_{mix}) and refer to this metric as 'pesticide risk':

$$TWC_{\mathrm{mix}} = \sum_{i=1}^{n} \frac{c_i}{\mathrm{LD}_{50i}}$$

We used an average of the acute oral and contact lethal doses $\mathrm{LD_{50}}$ for each compound sourced from the Pesticide Properties DataBase (Lewis et al., 2016) to provide an overall indicator of toxicity, reflective of how bees encounter pesticides in the landscape and their multiple exposure routes. We used the $\mathrm{LD_{50}}$ values for adult European honey bees because there are still incomplete toxicity data for the remaining bee species. However, when data are available, $\mathrm{LD_{50}}$ values for other bee species correlate with the corresponding European honey bee $\mathrm{LD_{50}}$ values (Arena & Sgolastra, 2014; DiBartolomeis et al., 2019). Consequently, we did not apply any safety factor (Adriaanse et al., 2023), as our objective was to derive a biologically interpretable index of pesticide risk rather than implementing a worst-case regulatory scenario. We rounded $\mathrm{LD_{50}}$ values down when based on limit tests (i.e., $\mathrm{LD_{50}}$ is greater than 100 μg/bee; typically for fungicides and herbicides) and expressed as 'greater than' (OECD, 1998a, 1998b).

Statistical analyses

We tested the effects of bee species and pesticide risk (TWC_{mix}) on protein content, lipid content and P:L ratio using linear mixed-effects models (LMMs). Bee species, pesticide risk and their interaction as well as crop type were included as explanatory variables, while site nested within country was added as a random intercept. Although differences between crop types were not the primary focus of this study, to control for crop-dependent heterogeneity, crop type was retained as a fixed effect (results in Appendix S3) rather than a random effect as it comprised only two levels (Silk et al., 2020). Given a strong right skew, pesticide risk values were log-transformed ($\ln(x + 0.1)$) to ensure the normal distribution and the homoscedasticity of the residuals. To compare bee species, we performed *post hoc* pairwise comparisons based on tests of estimated marginal means, with false discovery rate (FDR) correction for multiple testing. Similarly, to assess whether pesticide risk had a significant effect within each bee species (i.e., whether the slope differed from zero), we used *post hoc* tests of estimated marginal means for linear trends with FDR correction.

To avoid potential bias arising from causal inference, we verified that pesticide risk was not correlated with the proportion of crop pollen in pollen stores, as both variables could independently influence pollen nutritional content. A Pearson correlation test confirmed no significant relationship between pesticide risk and the proportion of crop pollen ($t = 0.28$, df $= 307$, $p = 0.783$).

Models were fitted using the *lme4* packages (Bates et al., 2021), and *post-hoc* tests were run using the *emmeans* package (Lenth, 2022). We checked deviance residual diagnostics to ensure goodness of fit of the models using the *DHARMa* (Hartig, 2021) and *performance* packages (Lüdecke et al., 2021). Marginal predictions from mixed-effects models were plotted using the *ggplot2* (Wickham et al., 2020), *ggeffects* (Lüdecke, 2018) and *ggpubr* (Kassambara, 2020) packages. We used $\alpha = 0.05$ as a conventional reference threshold; yet *p*-values below this threshold were interpreted within a language-of-evidence framework (Muff et al., 2022). The dataset and R script are publicly available online on Zenodo (Gekière, 2026).

RESULTS

We assessed whether there were significant differences in protein content, lipid content and P:L ratio in pollen stores among managed bee species. We found moderate evidence for differences in protein content in pollen stores among bee species ($\chi^2 = 6.33$, df $= 2$, $p = 0.042$); yet these differences were small in magnitude and were not supported by pairwise comparisons (Figure 2a; honey bee: mean ± SD = 295 ± 45 μg/mg; bumble bee: mean ± SD = 298 ± 73 μg/mg; mason bee: mean ± SD = 277 ± 99 μg/mg). By contrast, there was very strong evidence for differences in lipid content in pollen stores among bee species ($\chi^2 = 52.45$, df $= 2$, $p < 0.001$; Figure 2b). Pairwise comparisons showed a lower lipid content in the pollen stores of bumble bee colonies (mean ± SD = 44.1 ± 22.7 μg/mg) when compared to the pollen stores of the two other bee species, whereas lipid content did not differ between pollen stores collected in honey bee hives and mason bee nests (honey bee: mean ± SD = 57.2 ± 19.1 μg/mg; mason bee: mean ± SD = 54 ± 19 μg/mg). Accordingly, there was very strong evidence for differences in P:L ratios in pollen stores among bee species ($\chi^2 = 91.80$, df $= 2$, $p < 0.001$, Figure 2c), with pairwise comparisons showing a higher P:L ratio in the pollen stores of bumble bee colonies (mean ± SD = 8.53 ± 4.20) when compared to the pollen stores of the two other bee species (honey bee: mean ± SD = 5.85 ± 2.14; mason bee: mean ± SD = 5.60 ± 2.22).

Considering the three bee species together, pesticide risk had no significant effect on protein content ($\chi^2 = 0.25$, df $= 1$, $p = 0.617$),

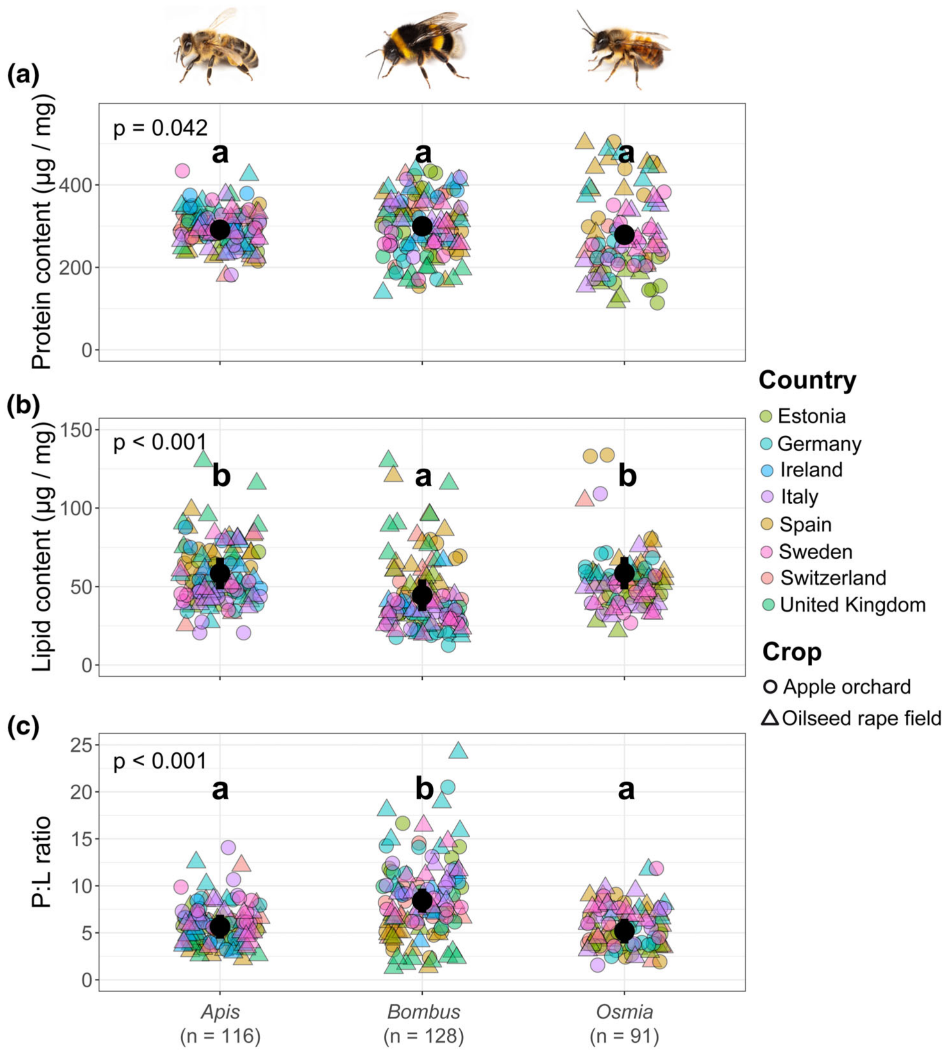

FIGURE 2 Inter-specific nutritional profiles in the pollen stores of the three studied bee species. (a) Protein content (µg protein/mg pollen). (b) Lipid content (µg lipid/mg pollen). (c) Protein-to-lipid (P:L) ratio. Means and standard deviations are plotted. *p*-values are retrieved from linear mixed-effect models. Species not sharing the same letter show significant differences in their nutritional profiles (contrasts between estimated marginal means).

lipid content ($\chi^2 = 1.06$, df = 1, $p = 0.303$) or the P:L ratio ($\chi^2 = 0.10$, df = 1, $p = 0.755$). However, we found strong evidence for an interaction between bee species and pesticide risk for protein content ($\chi^2 = 24.73$, df = 2, $p < 0.001$) and moderate evidence for lipid content ($\chi^2 = 8.22$, df = 2, $p = 0.016$), whereas no such interaction was detected for the P:L ratio ($\chi^2 = 2.28$, df = 2, $p = 0.319$). We therefore performed tests of estimated marginal means for linear trends to examine the effect of pesticide risk within each species separately.

For the honey bee, pesticide risk did not influence protein content ($t = -0.50$, df = 299, $p = 0.614$, Figure 3a) or lipid content ($t = 0.53$, df = 297, $p = 0.600$, Figure 3b). Accordingly, pesticide risk did not influence the P:L ratio in the pollen stores of honey bee hives ($t = -0.52$, df = 299, $p = 0.602$, Figure 4a). For the bumble bee, there was strong evidence that increased pesticide risk was negatively associated with protein content ($t = -2.96$, df = 299, $p = 0.003$, Figure 3c) and negatively associated with lipid content ($t = -2.61$, df = 293, $p = 0.009$, Figure 3d). Accordingly, as both protein and lipid content were reduced with increased pesticide risk, pesticide risk did not influence the P:L ratio in the pollen stores of buff-tailed bumble bee colonies ($t = -0.08$, df = 295, $p = 0.933$, Figure 4b). For the mason bee, there was very strong evidence that increased pesticide risk was positively associated with protein content ($t = 3.47$, df = 299, $p < 0.001$, Figure 3e), but pesticide risk did not influence lipid content ($t = 0.86$, df = 297, $p = 0.391$, Figure 3f). Similarly, pesticide risk did not influence the P:L ratio in the pollen stores of mason bee nests ($t = 1.33$, df = 299, $p = 0.186$, Figure 4c).

DISCUSSION

Across 128 sites spanning European agro-ecosystems, our study provides the first empirical evidence that managed bee species differ in

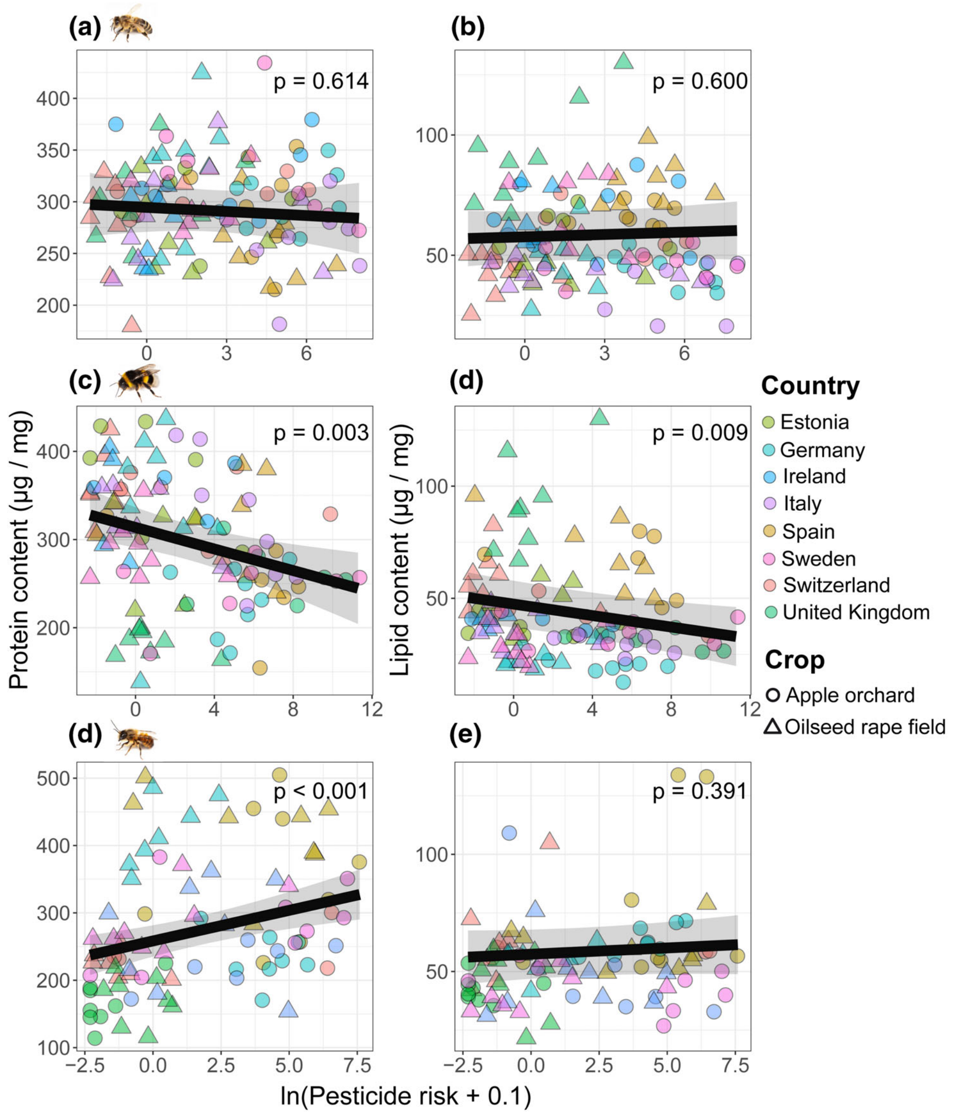

FIGURE 3 Intra-specific variation in pollen stores according to pesticide risk for the three studied bee species. (a, c, e) Protein content. (b, d, f) Lipid content. (a, b) The European honey bee. (c, d) Buff-tailed bumble bee. (e, f) The red mason bee. Marginal predictions and 95% confidence intervals from the models are plotted. *p*-values are retrieved from tests of estimated marginal means for linear trends.

their macronutritional needs under field conditions, with the buff-tailed bumble bee storing pollen with a higher protein-to-lipid (P:L) ratio compared to the European honey bee and the red mason bee. Crucially, we also show that pesticide risk, quantified as the summed toxicity-weighted concentrations of pesticides in pollen stores, does not influence the P:L ratios of the pollen stores these species accumulate. Together, these findings reveal species-specific macronutritional needs in real-world agricultural landscapes and suggest that managed bees exhibit rather constant macronutritional profiles in their pollen stores, at least in terms of P:L ratios, across gradients of pesticide risk.

Our investigation revealed clear differences in P:L ratios among species, with the pollen stores of the buff-tailed bumble bee characterised by a higher ratio (around 8.5:1) than those of the European honey bee (around 5.5:1) and the red mason bee (around 5.5:1). Hence, bumble bees exhibited an approximately 1.5-fold higher P:L ratio compared to both honey bees and mason bees. These findings partially align with a previous field study reporting a threefold higher P:L ratio in corbicular pollen loads of the common Eastern bumble bee (around 4:1 P:L) compared to pollen loads of the European honey bee (around 1.5:1 P:L) (Vaudo et al., 2020). However, our findings do not align with an intermediate P:L ratio (around 3:1 P:L) of the horn-faced mason bee, another mason bee species investigated in Vaudo et al. (2020), which lies between those of the common Eastern bumble bee and the European honey bee.

While our findings are consistent with previous research showing that bumble bees preferentially collect pollen with higher P:L ratios (Vaudo et al., 2020, 2024; Wood et al., 2021), they are somewhat unexpected in light of a parallel study reporting that both honey bees and bumble bees rely heavily on the same crop pollen, which constitutes 31%–53% of the pollen stored in their colonies or hives,

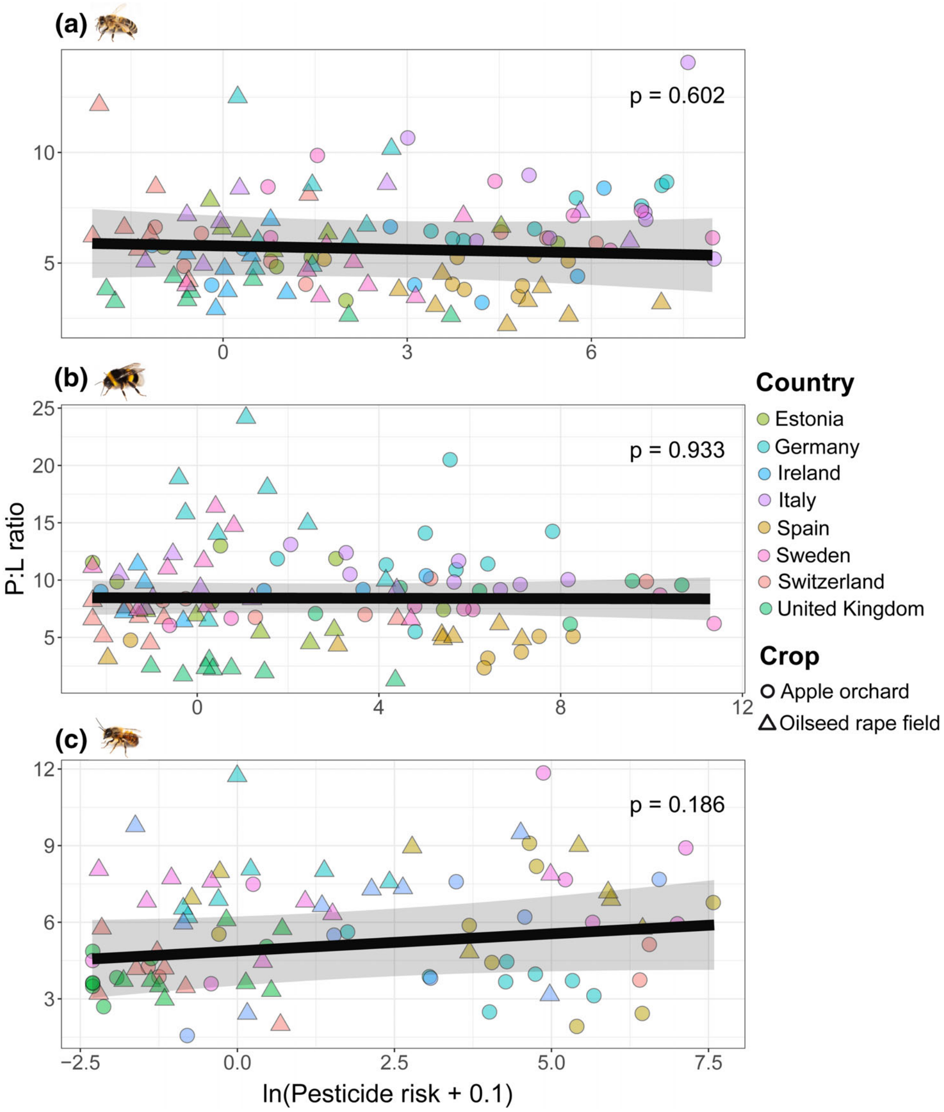

FIGURE 4 Intra-specific variation in the protein-to-lipid (P:L) ratio in pollen stores according to pesticide risk for the three studied bee species. (a) European honey bee. (b) Buff-tailed bumble bee. (c) Red mason bee. Marginal predictions and 95% confidence intervals from the models are plotted. *p*-values are retrieved from tests of estimated marginal means for linear trends.

suggesting they may collect similar P:L ratios (Tourbez et al., 2025) (but see Bertrand et al., 2019). One possible explanation lies in the different use of non-crop floral resources, since bumble bees appear to forage more frequently on Fabaceae species, with Fabaceae pollen comprising 10%–11% of their stores, compared to only 4%–5% in honey bee stores (Tourbez et al., 2025). Given that Fabaceae pollen is particularly rich in protein relative to lipids (Vaudo et al., 2020, 2024), this preference may partially explain the higher P:L ratios observed in bumble bee pollen stores.

Dietary differences during the larval development stages may contribute to the observed variations among bumble bees and honey bees. While honey bee larvae receive highly processed food with a constant P:L ratio (around 2.5:1 P:L) regardless of the nutritional profiles in pollen stores, bumble bee larvae are nourished with unprocessed pollen (Carnell et al., 2020). Consequently, the nutritional quality of pollen stores in bumble bees is anticipated to align more closely with the specific nutritional demands of their larvae. Another reason underlying the discrepancy between bumble bee and honey bee nutritional needs may be their colony size. Because bumble bee colonies only comprise a few hundred individuals, they may exhibit a more selective foraging strategy by prioritising quality over quantity (i.e., focussing on plants with specific macronutritional profiles), whereas honey bee hives, with tens of thousands of individuals, may tend to prioritise quantity over quality (i.e., foraging on all available plants; Avni et al., 2014; Hung et al., 2019; Leonhardt & Blüthgen, 2012). Importantly, these hypotheses remain speculative, and field experiments controlling for colony size and brood presence would help clarify the potential influence of colony size and brood-dependent nutritional demands on macronutritional preferences.

Our results revealed that the P:L ratio found in the nest provisions of the red mason bee (i.e., 5.6:1) is substantially higher than the P:L ratio reported for the horned-face mason bee (i.e., 3:1) (Crone et al., 2023; Vaudo et al., 2020). This disparity may be attributed to the especially broad spectrum of species-specific floral choices within the mason bee clade (Eckerter et al., 2022; Sedivy et al., 2011; Vaudo et al., 2024). Unlike managed social bees, the solitary red mason bee exhibits a marked preference for non-crop plants in agro-ecosystems, with Fagaceae pollen comprising 30%–51% of their pollen stores (Bertrand et al., 2019; Knapp et al., 2023; Tourbez et al., 2025). This key finding suggests that mason bees are more inclined to diversify their pollen sources in agro-ecosystems compared to their social counterparts (Knapp et al., 2023; Knauer et al., 2024; Tourbez et al., 2025). Such foraging flexibility may account for the discrepancies in P:L ratios reported across studies focusing on mason bees, as mason bees appear highly responsive to the non-crop floral landscape composition. To confirm this, future studies should employ multivariate models to investigate how the botanical sources of pollen influence the macronutrient profiles of pollen stores across different bee species.

Increasing pesticide risk was associated with a significant reduction in protein and lipid content of the pollen stores in bumble bee colonies. Such declines are likely to have adverse consequences for colony performance, since proteins are critical for immune function (Brunner et al., 2014) and larval development (Moerman et al., 2015), and lipids are critical structural, signalling and storing molecules (Furse et al., 2023). These findings may help elucidate the protein- and lipid-mediated mechanisms underlying the previously reported negative relationship between pesticide risk and colony growth (Nicholson et al., 2024). By contrast, although lipid-rich diets or those characterised by low P:L ratios have been shown to enhance honey bee resilience to pesticide exposure under laboratory conditions (Crone & Grozinger, 2021; Deeter et al., 2023), we did not observe an increase in the lipid content of pollen stores in honey bee hives. This lack of adjustment indicates that, in the field, honey bees did not actively alter their lipid foraging in response to pesticide exposure, highlighting a potential discrepancy between controlled findings and field observations. In addition, pesticide risk was associated with a significant increase in protein content of the pollen stores in red mason nests, raising the possibility of protein-mediated resilience in this species under pesticide exposure.

By contrast to its effect on protein content and lipid content, a gradient of pesticide risk had no detectable effect on the P:L ratios found in pollen stores across the three studied bee species, indicating consistent macronutritional profiles in terms of P:L ratios irrespective of pesticide exposure. Although our study was not explicitly designed to assess the effects of pesticides on foraging behaviour (e.g., Gill & Raine, 2014), these findings suggest that managed bees are not hindered in their ability to meet species-specific macronutritional targets under real-world pesticide exposure, at least regarding their P:L ratios. Conversely, the absence of a shift in P:L ratios also implies that bees do not adjust their foraging strategies to match specific ratios that could mitigate pesticide toxicity, contrasting with self-medicative behaviours with specialised metabolites in response to parasite stress (Baracchi et al., 2015; Gherman et al., 2014). One plausible explanation is that bees may be unable to detect pesticide residues in their food resources (Parkinson et al., 2023), thereby limiting their capacity to adjust P:L ratios in response to pesticide-induced toxicity. Alternatively, if bees are capable of sensing pesticide presence (Kessler et al., 2015), the observed stability in nutrient regulation may also reflect a consistent foraging behaviour that remains robust even under sublethal chemical stressors (Aliouane et al., 2009; Raine & Rundlöf, 2024). Another hypothesis is that bees detect pesticide residues while maintaining consistent macronutritional patterns to meet larval requirements. However, evidence from the heavy metal literature suggests that, in the presence of brood, bumble bee workers favour uncontaminated resources even when these have reduced nutritional quality, whereas workers without brood prioritise richer but contaminated solutions (Gekière et al., 2025). It is therefore unlikely that, if bees detect pesticides, they would prioritise consistent nutritional intake over avoidance of contaminated resources for their larvae.

Growing attention is being paid to the capacity of bees to exhibit behavioural plasticity in response to environmental stressors. For example, studies have demonstrated that bees can selectively forage on medicinal resources when infected with intestinal parasites (Baracchi et al., 2015; Gherman et al., 2014) or avoid pesticide-contaminated food when given a choice (Kang & Jung, 2017). However, our findings suggest that bees adhere to consistent macronutritional ratios in their pollen stores under pesticide exposure, implying a limited ability to adjust their macronutritional dietary choices in response to this particular stressor. This constraint is especially concerning in the context of global bee declines, as numerous studies advocate for the implementation of flowering strips and other floral enhancements to support pollinator populations in pesticide-intensive agro-ecosystems (Rundlöf et al., 2022). While increasing evidence suggests that bees exhibit behavioural flexibility to cope with environmental challenges (Baracchi et al., 2015; Maebe et al., 2021; Ostwald et al., 2024), our results point to a potential constraint in this flexibility, as the macronutritional ratios of pollen stores remained consistent across a gradient of pesticide risk. This absence of variation suggests that bees may have limited capacity to adjust their macronutritional foraging patterns through non-crop floral resources to mitigate the effects of pesticide exposure. However, it should be noted that our study focussed exclusively on macronutrients (i.e., primary metabolites). Future research should therefore examine whether bees alter their foraging patterns for secondary metabolites (e.g., flavonoids; Riveros & Gronenberg, 2022) along a gradient of pesticide risk (Morrison et al., 2025). These findings underscore the need for future conservation strategies not only to promote floral diversity in agricultural landscapes, but also to drastically reduce pesticide use and implement more rigorous Integrated Pest Management (IPM) approaches.

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FUNDING INFORMATION

A.G. and C.T. are supported by an F.R.S.-FNRS PhD grant “Aspirant”. The research leading to these results has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No. 773921, PoshBee Project (www.poshbee.eu).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Zenodo at (https://doi.org/10.5281/zenodo.19402214).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Proportion of cropland by country and crop type within a 1-km radius around the sites.

Appendix S2. Bee subspecies deployed in each country. APP = Apple orchards. OSR = Oilseed rape fields.

Appendix S3. Results for crop type. In our models, although we were not interested in differences between crop types, this variable was retained as a fixed factor rather than a random intercept because it only contains two levels.

How to cite this article: Gekière, A., Tourbez, C., Vanderplanck, M., Kiljanek, T., Ghisbain, G., Kardas, E. et al. (2026) Nutritional composition of pollen stores in managed bees across European agro-ecosystems reveals species-specific differences but limited pesticide effects. *Ecological Entomology*, 1–13. Available from: https://doi.org/10.1111/een.70096